Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 12

SYNTHESIS OF ^{3}H- AND ^{14}C-CISAPRIDE

C.G.M. Janssen*, H.A.C. Lenoir, J.B.A. Thijssen, A.G. Knaeps

and J.J.P. Heykants

Department of Drug Metabolism and Pharmacokinetics,

Janssen Pharmaceutica, B-2340 Beerse, Belgium

SUMMARY

Cisapride, (±)-cis-4-amino-5-chloro-N[1-[3(4-fluorophenoxy) propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide, is a new gastrokinetic drug with a potent stimulating effect on the gastrointestinal motor activity. Metabolic studies required the synthesis of cisapride labelled at one of the three major moieties. Hence, cisapride was tritiated either in the fluorophenyl moiety by means of reductive dehalogenation, or via reductive amination in the piperidine ring.
^{14}C-Cisapride was labelled in the benzamide function. The title compounds were obtained at a specific activity of 17.0 Ci/mmol, 59.8 mCi/mmol and 7.8 mCi/mmol, respectively and with HPLC purities of > 98 %.

Key-words: ^{3}H-cisapride, ^{14}C-cisapride, gastrokinetic, reductive dehalogenation, reductive amination

INTRODUCTION

Cisapride, a new gastrokinetic drug, has a potent stimulating effect on gastrointestinal motor activity without blocking dopamine receptors or activating muscarinic cholinergic receptors and increases oesophageal, gastrointestinal and colonic motility.[1-3]

---

*To whom correspondence should be addressed.

0362-4803/87/121493-09$05.00

Received April 13, 1987

Metabolic studies necessitated the synthesis of labelled cisapride with labels at the distinctive positions. For these studies we chose to synthesize tritiated cisapride I and II and carbon-14 labelled cisapride III (Figure 1).

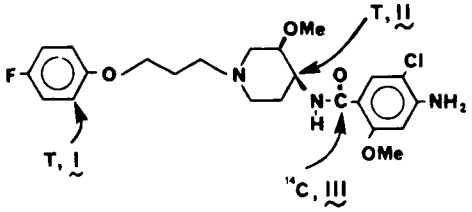
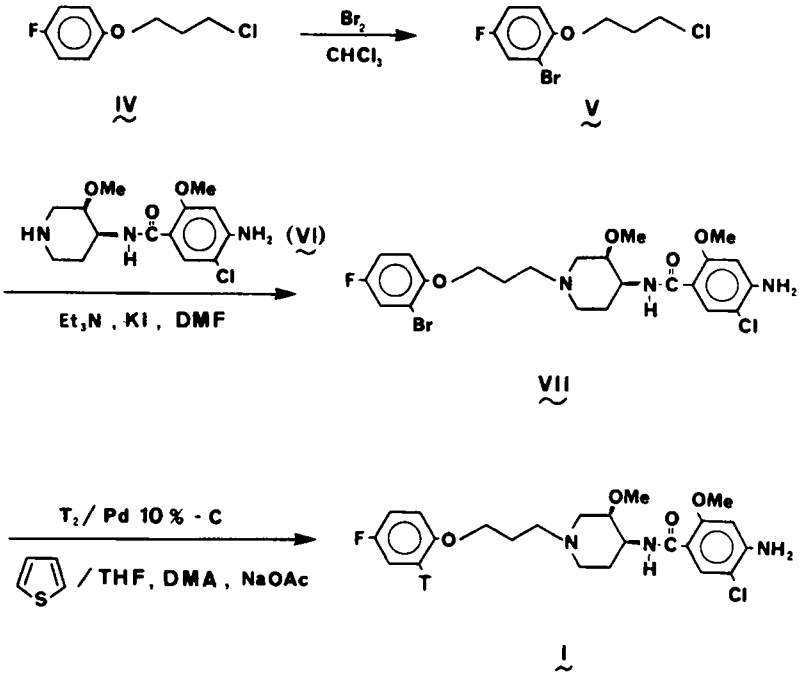


Figure 1: ^{3}H- and ^{14}C-labelled cisapride

Bromination of 1-(3-chloropropoxy)-4-fluorobenzene (IV, Scheme I) afforded 2-brominated V. Coupling with cis-4-amino-5-chloro-2-methoxy-N-(3-methoxy-4-piperidinyl) benzamide (VI)[4] gave bromo-cisapride VII which was reductively dehalogenated under tritium atmosphere to tritiated cisapride I.[5]

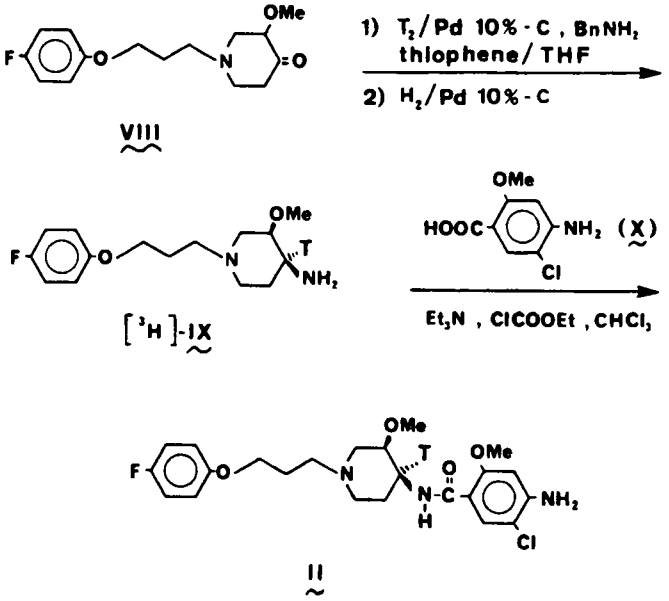
Scheme I



Tritiated cisapride II (Scheme II) was obtained by a reductive amination

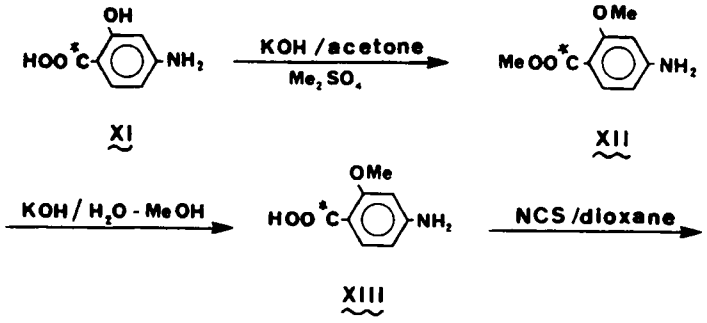
of 1-[3-(4-fluorophenoxy)-propyl]-3-methoxy-4-piperidinone (VIII)[4] with benzylamine under tritium to [^{3}H]-IX[5] followed by amidation with the mixed anhydride of 4-amino-5-chloro-2-methoxybenzoic acid X and ethyl chloroformate.

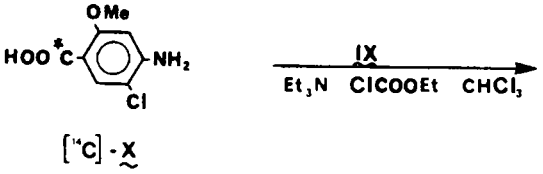
Scheme II



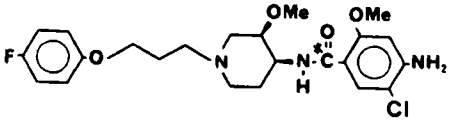
Carbon-14 labelled III (Scheme III) was prepared by amidation of unlabelled IX with [^{14}C]-marked X, which was synthesized by methylation of XI[5] to XII, hydrolysis of the ester function to XIII and a chlorination with N-chlorosuccinimide.

Scheme III





[^{14}C]-X



III

METHODS AND MATERIALS

Purification with normal-phase liquid chromatography (LC) was conducted at atmospheric pressure on columns, slurry packed with silica (Kieselgel 60, Merck art. 7734). The column sizes and solvent systems used are described in the text where appropriate. Preparative thin-layer chromatography was performed on reversed phase plates (Whatman LKC 18F) or on silica gel plates (Merck 60 F 254) with the eluate composition described where required. The radioactivity on the plates was scanned with a Berthold radiochromatogram scanner (LB 2723). Gas chromatography was performed on a Varian 3700 gas chromatograph using a 1 m OV-17 carbowax column with a linear temperature run of 26 minutes from 50° C to 310° C. The apparatus used for radioactivity measurements and for analytical HPLC has been described earlier.[6] All labelled products were HPLC identical to authentic unlabelled material.

CISAPRIDE I

2-bromo-1-(3-chloropropoxy)-4-fluorobenzene (V)

To a solution of 1-(3-chloropropoxy)-4-fluorobenzene (IV, 18.9 g, 0.1 mol) in chloroform (25 ml) was dropped bromine (18.4 g, 0.115 mol) in chloroform (15 ml). The mixture was refluxed for 3 hours, the decolorized solution was cooled and washed with water and 10 % aqueous sodium bicarbonate. The chloroform layer was dried on magnesium sulphate and evaporated to leave gas chromatographically 95 % pure V (24.0 g, 85.1 %) which was used as such in the next reaction step.

(±)-cis-4-amino-N[1-[3-(2-bromo-4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-5-chloro-2-methoxybenzamide (VII)

A solution of V (12.3 g, 46 mmol), (±)-cis-4-amino-5-chloro-2-methoxy-N-(3-methoxy-4-piperidinyl)benzamide[4] (VI, 12.5 g, 40 mmol), triethylamine (6.0 g, 60 mmol) and potassium iodide (100 mg) in N,N-dimethylformamide (DMF, 100 ml) was stirred for 18 hours at 100° C. The reaction mixture was poured into vigorously stirred water (1 litre) and the formed precipitate was filtered and chromatographed over silica [column size 200 mm x 80 mm i.d.; eluate: chloroform–methanol (90: 10 v/v)]. The fractions containing pure material (R_f = 0.58) were combined, evaporated and triturated with diisopropyl ether (250 ml) to leave a solid (13.6 g). Crystallization from 2-propanol (50 ml) afforded product (11.6 g, 54 %), mp 149.5° C. Anal. Calcd. for $C_{23}H_{28}BrClFN_3O_4$: C, 50.70; H, 5.20; N, 7.71; Cl, 6.51; F, 3.49. Found: C, 50.81; H, 5.11; N, 7.63; Cl, 6.69; F, 3.40 %.

(±)-cis-4-amino-5-chloro-N[1-[3-(4-fluoro-[2T]phenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide (^{3}H-cisapride, I)[5]

A mixture of VII (100 mg, 0.18 mmol), Pd-10 % on charcoal (100 mg), anhydrous sodium acetate (100 mg) and a 4 % thiophene solution in tetrahydrofuran (THF, 0.1 ml) was dehalogenated in N,N-dimethylacetamide (5.5 ml) with approx. 30 Ci of tritium gas for 18 hours at room temperature. The excess of tritium was adsorbed on active charcoal and the solvent was lyophilized to remove labile tritium. The residue was dissolved in chloroform–ethanol (1:1; v/v) and the catalyst was removed by Millipore filtration (Millex-SR). The filtrate was lyophilized again and the residue (1.3 Ci) was purified by preparative reversed-phase thin-layer chromatography with 0.5 M aqueous NaCl:tetrahydrofuran:methanol (50:35:15; v/v) as an eluate. The radioactive zone, corresponding to authentic cisapride was scraped off and eluted with methanol. The product, stored in methanol (73 ml), proved to be 98 % HPLC-pure cisapride I (730 mCi). It had a specific activity of 17.0 Ci/mmol (chemical and radiochemical yield 23 %).

CISAPRIDE II

(±-cis-1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-[4T]piperidinamine

([^{3}H]-IX)[5]

A mixture of 1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinone[4] (VIII, 140 mg, 0.50 mmol), benzylamine (61 mg, 0.66 mmol), Pd 10 % on charcoal (100 mg) and a 0.02 % solution of thiophene in THF (10 ml) was reacted under approx. 30 Ci of tritium gas for 3 hours at 50° C. The catalyst was filtered off and fresh palladium 10 % on charcoal (100 mg) was added. Debenzylation of the formed intermediate took place under hydrogen atmosphere for 18 hours at 50° C. The reaction mixture was filtered and evaporated under a gentle stream of nitrogen to yield 79.2 % HPLC-pure [^{3}H]-IX with a total radioactivity of 70.0 mCi (36.3 mg, 20.2 %; the reaction when carried out with hydrogen gas gave, in our hands, a 95.0 % yield). The material was used as such in the next reaction step.

(±)-cis-4-amino-5-chloro-N[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-[4T] piperidinyl]-2-methoxybenzamide (^{3}H-cisapride, II)

To a solution of 4-amino-5-chloro-2-methoxybenzoic acid (X, 190 mg, 0.95 mmol) and triethylamine (133 µl, 0.95 mmol) in chloroform (5.0 ml) was dropped ethyl chloroformate (91 µl, 0.95 mmol). The reaction mixture was stirred for 30 min at room temperature. To the formed mixed anhydride was then dropped a solution of [^{3}H]-IX diluted with unlabelled IX (36.3 mg of 79.2 % pure [^{3}H]-IX and 241 mg of unlabelled IX, representing 270 mg of pure material, 0.96 mmol, 55.4 mCi) in chloroform (5 ml). The mixture was stirred for 2 h at room temperature and then poured into water (10 ml). The organic phase was separated and the water layer was extracted with chloroform (2 x 5 ml). The combined chloroform layers were washed with water (3 x 5 ml), dried and evaporated at aspirator pressure. The residue was crystallized from methanol (4 ml) to afford product (180 mg). The filtrate was evaporated and purified with preparative thin-layer chromatography on silica gel [eluate: chloroform-methanol 90:10 v/v]. The radioactive zone corresponding with authentic cisa-

pride was scraped off, eluted with methanol, evaporated and crystallized from methanol (1 ml) to yield a second crop of product (73 mg). Cisapride II (253 mg, 0.54 mmol) was found to be 98.5 % HPLC pure, had a total radioactivity of 31.3 mCi (radiochemical yield 56.4 %) and a specific activity of 57.6 mCi/mmol.

CISAPRIDE III

Methyl-4-amino-2-methoxybenzene-[^{14}C]carboxylate (XII)

To a solution of 4-amino-2-hydroxybenzene-[^{14}C]carboxylic acid[5] ([^{14}C]-XI diluted to 1000 mg, 6.5 mmol, containing 49.3 mCi of radioactivity) in acetone (30 ml) was added potassium hydroxide (920 mg, 16.4 mmol). After stirring for 30 min, dimethyl sulphate (1950 mg, 15.5 mmol) was slowly dropped to the reaction mixture and stirring was continued for 4 hours at room temperature. The precipitate was filtered off and washed with acetone (2 x 20 ml). The filtrate was evaporated at aspirator pressure and the remaining solid was triturated with water, filtered, washed with water (2 x 10 ml) and dried to the air to yield XII (887 mg, 4.8 mmol, 36.7 mCi, 74.4 %). The product had a HPLC-purity of 94 %.

4-Amino-2-methoxybenzene-[^{14}C]carboxylic acid (XIII)

A suspension of XII (887 mg, 4.8 mmol, 36.7 mCi) in a methanol (15 ml)-water (4 ml) solution of potassium hydroxide (586 mg, 10.4 mmol) was refluxed for 3 hours. The solvent was evaporated under vacuum and the residue was dissolved in water (5 ml). Acidification to pH 5 with 1 N HCl gave white crystals which were filtered, washed with diisopropyl ether (2 x 3 ml) and dried to the air to afford 96 % pure XIII (589 mg, 3.5 mmol, 26.3 mCi, 71.7 %).

4-Amino-5-chloro-2-methoxybenzene-[^{14}C]carboxylic acid ([^{14}C]-X)

To a well-stirred solution of XIII (589 mg, 3.5 mmol, 26.3 mCi) in 1,4-dioxane (6.4 ml) was added portion-wise N-chlorosuccinimide (NCS, 512 mg, 3.8 mmol). The mixture was then refluxed for 3 hours, cooled to 80° C and

filtered in order to remove small impurities. The solvent was evaporated and the residue was partitioned between ethyl acetate (150 ml) and water (30 ml). The organic phase was separated, washed with water (2 x 10 ml), dried and evaporated at aspirator pressure. The remaining oil was triturated with methanol to yield solid product (442 mg, 2.17 mmol, 16.5 mCi, 62.7 %). According to HPLC the material was 98.5 % pure.

(±)-cis-4-amino-5-chloro-N[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl-2-methoxy-[^{14}C]benzamide ([^{14}C]-cisapride, III)

The synthesis was performed as described for II using [^{14}C]-X (442 mg, 2.17 mmol, 16.5 mCi) and triethylamine (302 µl, 2.17 mmol) in chloroform (15.0 ml) and ethyl chloroformate (208 µl, 2.17 mmol) to form the mixed anhydride. To this mixture was dropped a solution of IX (613 mg, 2.17 mmol) in chloroform (15 ml). The crude material was crystallized from methanol containing 10 % of water (10 ml) to afford III as the monohydrate (807 mg, 1.66 mmol) with a total radioactivity of 12.7 mCi (s.a. 7.6 mCi/mmol), a HPLC-purity of 98.5 % and a chemical and radiochemical yield of 76.5 and 77.0 % respectively.

ACKNOWLEDGEMENT

The authors wish to thank C. Verellen for typing this manuscript.

REFERENCES

1. Reyntjens A., Verlinden M., De Coster R., Janisch H.D., Smout A., De Cree J., Leempoels J. and Verhaegen H. - Curr. Ther. Res. 36: 1045 (1984)
2. Van Nueten J.M., Van Daele P.G.H., Reyntjens A.J., Janssen P.A.J. and Schuurkes J.A.J. - in "Gastrointestinal Motility", editor, Roman C., MTP Press Ltd., Lancaster, Boston, The Hague, Dordrecht, Kluwer Academic Publishers Group, 513 (1983)

3. Reyntjens A., Verlinden M., Schuurkes J., Van Nueten J. and Janssen P.A.J. - Curr. Ther. Res. 36: 1029 (1984)

4. Van Daele G.H.P., De Bruyn M.F.L., Sommen P.M., Janssen M., Van Nueten J.M., Schuurkes J.A.J., Niemegeers C.J.E. and Leysen J.E. - Drug Dev. Res 8: 225 (1986)

5. The tritiation steps, including the removal of labile tritium were carried out according to our directions at the National Institute for Radio-Elements (IRE), B-6220 Fleurus, Belgium. The 4-amino-2-hydroxybenzene [^{14}C]carboxylic acid (XI) with a specific activity of 18.45 mCi/mmol was obtained from ICI, Billingham, Cleveland (U.K.) and was radiochemically pure (> 98 %) according to high-performance liquid chromatography.

6. Thijssen J.B.A., Janssen C.G.M., Verluyten W.L.M. and Heykants J.J.P. - J. Labelled Comp. Pharm. 23: 223 (1986)