Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 2

PREPARATION OF CARBON-11 LABELLED PRAZOSIN, A POTENT AND SELECTIVE α_1-ADRENORECEPTOR ANTAGONIST

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Summary

The α_1-adrenoreceptor antagonist, Prazosin : 2-[4-(2-furoyl) piperazin-1-yl]-4-amino-6,7-dimethoxyquinazoline, has been labelled with carbon-11 for in vivo studies of α_1-adrenoreceptors using positron emission tomography. The preparation of [2-^{11}C] furoyl chloride, from cyclotron-produced [^{11}C] carbon dioxide, and its reaction with the secondary amine, 2-(piperazin-1-yl)-4-amino-6,7-dimethoxyquinazoline, provides a fast (35 min) route to carbon-11 labelled prazosin in high radiochemical yield (30-40 %, decay-corrected) with high specific activity (26-37 GBq/μmol, 0.7-1.0 Ci/μmol).

Key words : [^{11}C]Prazosin, α1-adrenoreceptor antagonist, PET.

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0362-4803/88/020177-07$05.00

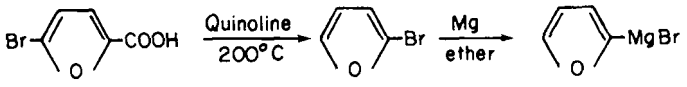
Received June 15, 1987

Introduction

Several types of receptors for neurotransmitters in living heart have been investigated by positron emission tomography (PET). The muscarinic cholinergic receptor has been characterized by using [^{11}C]MQNB (1) and the peripheral type benzodiazepine receptor by using [^{11}C]PK 11195 (2). Propranolol, practolol and CGP 12177 have also been labelled with carbon-11 for studying the beta-adrenergic receptor (3, 4, 5). For in vivo studies of α_1-adrenoreceptors using PET we chose to label the high affinity, selective antagonist, prazosin (6) with carbon-11. Our approach was based on the recently developed technique of preparing [^{11}C] acid chlorides as labelling agents (7).Here we report the preparation of [^{11}C]prazosin by the reaction of [2-^{11}C]furoyl chloride with the secondary amine, 2-(piperazin-1-yl)-4-amino-6,7-dimethoxyquinazoline [1].

Material and Methods

[^{11}C]carbon dioxide (44-55 GBq, 1.2-1.5 Ci) is produced in high radionuclidic and radiochemical purity by the ^{14}N (p, α) ^{11}C nuclear reaction on nitrogen gas. 2-Bromofuran is prepared by decarboxylation (oil bath, 200°C) of 5-bromofuroic acid (5.0 g, Aldrich Chem. Co. Ltd.) with copper chromite (1.0 g) as catalyst in freshly distilled quinoline (10 ml, Aldrich Chem. Co. Ltd.) under nitrogen (scheme 1) 2-Bromofuran is distilled out as formed (oil bath, 200°C). In order to slow decomposition (8) the 2-bromofuran is immediately dissolved in dry diethyl ether (0.29 g/10 ml) and stored over sodium wire under nitrogen at 0-4 °C. 2-Bromofuran (10 ml stock solution) is converted into 2-furoylmagnesium bromide solution (0.2 M in diethyl ether) by reaction with magnesium in a specially designed apparatus described earlier (9) and stored, after filtration through a millipore FG-filter, under nitrogen in sealed vials with teflon septa at 0-4 °C. Phthaloyl dichloride (b.p. 270°C, Aldrich Chem. Co. Ltd.) is distilled before use and stored over molecular sieve (4 A) in sealed vials with teflon septa. 2, 6-Di-t-butylpyridine was dried over molecular sieve (4 A).



Scheme 1 : Synthesis of 2 Bromofuran and 2 furoyl magnesium bromide

Synthesis of [11C]prazosin [2] (Scheme II)

The cyclotron-produced [^{11}C]carbon dioxide is trapped at a high flow rate in a stainless steel coil cooled by liquid argon. The trap is then warmed and the [^{11}C]carbon dioxide transferred with a slow flow of nitrogen (ca. 2 ml/min) into 2-furoylmagnesium bromide (0.02 mmol) in diethyl ether (1 ml) at room temperature. After 2 min the [^{11}C]carbonation is quenched by the addition of phthaloyl dichloride (2 µl, 0.014 mmol) in diethyl ether (0.3 ml). 2,6-Di-t-butylpyridine (100 µl, 0.45 mmol) in diethyl ether (0.3 ml) is then added and the diethyl ether is evaporated (oil bath, 75°C) with a flow of nitrogen (ca 10 ml/min). The generated [2-^{11}C]furoyl chloride (b.p. 174°C) is then distilled out under nitrogen flow (10-15 ml/min) by immersion of tube 1 (fig. 1) into a Wood's alloy-bath

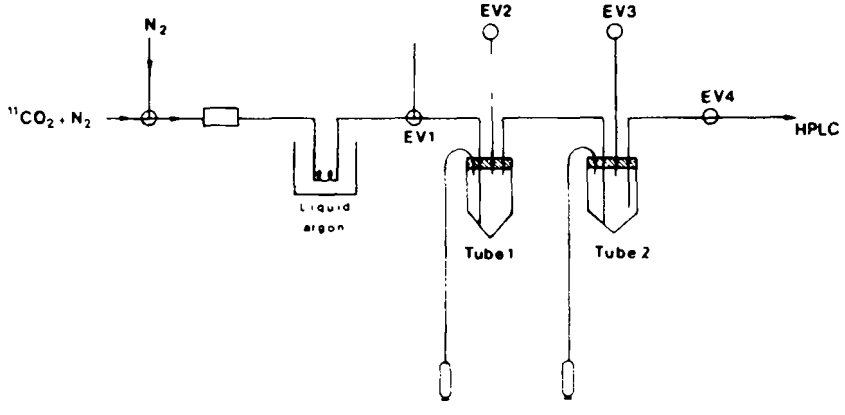
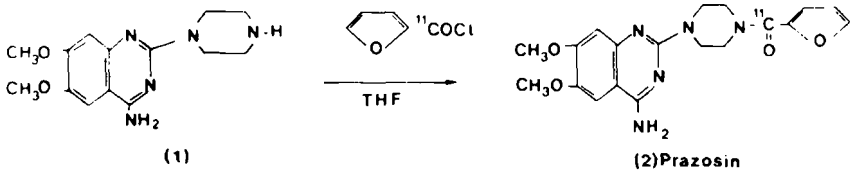


Fig. 1 : Diagram of the apparatus

(225°C) and heating the top of tube 1 and the line between tubes 1 and 2 with an electric heather (type : Leister-Chibli, pos. 3 = 350°C). The [2-^{11}C]furoyl chloride is trapped in a solution of the secondary amine (1),

2-(piperazin-1-yl)-4-amino-6,7-dimethoxy-quinazoline (2 ng, 7 µmol) in tetrahydrofuran (1 ml) kept under nitrogen at 0°C. The reaction mixture is then gently heated by immersion of the tip of tube 2 in the Wood's alloy-bath (225°C), both to promote the formation of [^{11}C]prazosin and to evaporate the solvent.



Scheme 2 : Synthesis of [^{11}C]Prazosin

The reaction vessel is cooled, and then the radioactive residue is taken up in 1.5 ml of mobile phase, dichloromethane containing 2.5 % (V/V) of solution A (ethanol : water : ethylamine 100 : 2 : 2, V/V) and injected onto a µPorasil column, 300 x 7.8 mm (Waters) which is eluted at 4 ml/min with the same mobile phase. UV-Absorbance (254 nm) and radioactivity are monitored simultaneously and [^{11}C]prazosin, which separates from other labelled and non-labelled compounds, is collected at 7.5-9 min after injection. This solution is evaporated to dryness and the residue dissolved in 600 µl of a mixture of ethanol : propylenglycol 1:1, V/V, diluted to 10 ml with sterile saline and sterilized by filtration (Millipore GS-filter, 0.22 µm).

Identification of the product

[^{11}C]Prazosin had the same capacity factor as authentic prazosin both in normal phase HPLC (described above) and reverse phase HPLC (column : µBondapak C-18 300 x 4 mm, mobile phase : acetonitrile : phosphoric acid 25 : 75 V/V, flow : 2.0 ml/min). [^{11}C]Prazosin also comigrates with authentic prazosin on two different TLC systems using plastic sheets, silica gel 60 F_{254} (Merck). System A : $CHCl_3$: MeOH : NH_4OH 18.2 : 1.6 : 0.2 V/V, rf : 0.81. System B : $CHCl_3$: EtOAC : NH_4OH 3 : 7 : 1, V/V, rf : 0.27. For further

analysis of the obtained product, the synthesis was performed under the same conditions as described above using ^{13}C-enriched carbon dioxide (90 atom %). The purified product was examined by broad-band proton-decoupled Fourier transform ^{13}C n.m.r. spectroscopy (DMSO, 22.5 MHz). The spectrum shows a single peak at -158.6 ppm in accord with the chemical shift assigned to the carbonyl carbon atom in prazosin.

Results and Discussion

For the decarboxylation of 5-bromofuroic acid, copper chromite was found to be the best catalyst because of the relatively low temperature (200°C) at which 5-bromofuroic acid was decarboxylated to 2-bromofuran (b.p. 102°C), which distilled easily at this temperature. With the initially used catalyst, copper powder (8) a higher temperature (230-235°C) was needed for the decarboxylation, and occasionally traces of quinoline (b.p. 237°C) distilled into the receiving flask, which prevented subsequent formation of the Grignard reagent (2-furoylmagnesium bromide). A solution of 2-bromofuran in diethyl ether could be stored over sodium wire at 0-4°C up to 4 weeks. In the preparation of 2-furoylmagnesium bromide it was necessary to use a small cristal of iodine as initiator and to heat the mixture to reflux to start the Grignard reagent. When the formation of the Grignard reagent was complete a black suspension was formed. After filtration (0.22 µm Millipore FG-filter) a clear yellow solution of 2-furoyl-magnesium bromide was obtained, which could be stored under nitrogen in sealed vials with teflon septa for two weeks, at 0-4°C, without loss of activity of the reagent. The amount of Grignard reagent used in the radiosynthesis has been found to be important. When we have used 0.02 mmol of 2-furoylmagnesium bromide in 1 ml diethyl ether the average entrapment of [^{11}C]carbon dioxide (in 35 preparations) has been 87 %. In attempts to decrease the amount of Grignard reagent below 0.02 mmol (in attempts to increase the specific activity of product) there was a sharp decrease in entrapment. The addition of 2 µl phthaloyl dichloride has been found to give optimal yields of [2-^{11}C]furoyl chloride and subsequently of [^{11}C]prazosin. With less phthaloyl dichloride the yield of [2-^{11}C]furoyl

chloride decreases. With more phthaloyl dichloride, physical transfer into tube 2 (see below) occures, causing a decrease in the formation of [^{11}C]prazosin by competing for the amine [1]. This competition could be reduced by adding more amine [1]. We have instead chosen to use a small amount of phthaloyl dichloride in order to conserve amine [1]. In a few experiments thionyl chloride was used instead of phthaloyl dichloride, but the yield of [^{11}C]prazosin decreased by about 50 %.

The line between tube 1 and 2 is a teflon 1/8 "o.d. tubing. With smaller i.d. tubing 1/16" o.d. or a valve between the two tubes, less than 10 % of the radioactivity is transferred from tube 1 to tube 2, as compared to 40-60 % with the teflon 1/8 "o.d. tubing. Without heating the top of tube 1 and the line between the tubes only about 10-15 % of the radioactivity distilled over to tube 2. In all preparations when we have used the teflon 1/8" o.d. tubing we have had some physical transfer of phthaloyl dichloride from tube 1 to tube 2, but this has not adversely affected the subsequent reaction.

From the end of bombardment the preparation requires only 35 min and provides an injectable solution of [^{11}C]prazosin in 30-40 % overall radiochemical yield (based on $^{11}CO_2$ used and corrected for radioactive decay). The obtained specific activity have been between 26-37 GBq/μmol (0.7 - 1.0 Ci/μmol) at end of synthesis. All tested preparations have passed independent test for apyrogenicity and sterility.

By the procedure described above sufficient amounts of [^{11}C]prazosin (4.1 - 6.7 Gbq ~ 110-180 mCi) can conveniently be produced for animal and human investigations using PET.

Acknowledgements

We thank Dr. B. Wood for producing ^{13}C n.m.r. data at the city of London Polytechnic Multinuclear Magnetic Resonance Service and Pfizer Inc. for the supply of prazosin and 2-(piperazin-1-yl)-4-amino-6,7-dimethoxy-quinazoline [1]. We also thank the SHFJ (Orsay) cyclotron team for ^{11}C-production and CEN Saclay ORIS for apyrogenicity and sterility tests.

REFERENCES

1. Syrota A., Comar D., Paillotin G., Davy J.M., Aumont M.C., Stulzaft O. and Mazière B. Proc. Natl. Acad. Sci. USA 82 : 584 (1985).

2. Charbonneau P., Syrota A., Crouzel C., Vallois J.M., Prenant C. and Crouzel M. Circulation 73 (3) : 476 (1986).

3. Berger G., Mazière M., Prenant C., Sastre J., Syrota A. and Comar D. J. Radioanal. Chem. 74 : 301 (1982).

4. Berger G., Prenant C., Sastre J., Syrota A. and Comar D. Int. J. Appl. Radiat. Isot. 34 : 1556 (1983).

5. Boullais C., Crouzel C. and Syrota A. J. Label. Compds Radiopharm. 23 : 565 (1986).

6. Cambridge D., Davey M.J. and Massingham R. Brit. J. Pharmacol. 59 : 514P (1977).

7. Luthra S.K., Pike V.W. and Brady F. J. Chem. Soc. Chem. Commun. 1423 (1985).

8. Shepard A.F., Winslow N.R. and Johnson J.R. J. Am. Chem. Soc. 52 : 2083 (1930).

9. Pike V.W., Eakins M.N., Allan R.M. and Selwyn A.P. Int. J. Appl. Radiat. Isot. 33 : 505 (1982).