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SYNTHESIS OF [^{11}C] GRANISETRON, A POSSIBLE POSITRON EMISSION TOMOGRAPHY LIGAND FOR 5-HT3 RECEPTOR STUDIES

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Summary

[^{11}C] Granisetron, a selective 5-HT3 antagonist, was synthesized by N-alkylation of the desmethyl compound using [^{11}C] methyl iodide as the labelling agent in a total synthesis time of 40 min, including semi-preparative HPLC purification. Approximately 40 mCi (1.5 GBq) of the radioligand was obtained with a specific activity of 200-250 mCi/μmol (7.4-9 GBq/μmol). Chemical (≥ 96 %) and radiochemical purity (≥ 99 %) were determined using analytical HPLC.

Key words: granisetron, 5-HT3 antagonist, carbon-11, Positron Emission Tomography

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Introduction

The serotonin receptors are widely distributed in both peripheric tissues and the central nervous system and the neurotransmitter system is involved in many physiological and pathophysiological pathways. The different subtypes of receptors, their function and pharmacology have been thoroughly studied during the past decade[1,2]. The 5-HT3 receptor, as one of those subtypes, is unique among the 5-HT receptors as it functions as a ligand-gated ion channel and not as a G-protein coupled receptor[3]. The 5-HT3 receptors are represented mainly in the peripheric system where they are found in the gastro-intestinal system (ileum) and in the cardiovascular system (heart and blood vessels), but their existence was also demonstrated in the central nervous system in 1987 by Kilpatrick *et al.*[4]. The development of selective agonists and particularly antagonist molecules for this receptor subtype opened a pathway to new *in vitro* and *in vivo* applications.

5-HT3 antagonists are well known for their anti-emetic properties in the treatment of radiation induced emesis or nausea following general anesthaesia[5]. The presence of 5-HT3 receptors in the heart does not exclude a possible role for 5-HT3 antagonists in cardiovascular disease[6]. More recent studies demonstrate the involvement of 5-HT3 antagonists in several psychiatric disorders such as anxiety[7], psychosis[8] and schizophrenia and there is some evidence indicating a role for 5-HT3 antagonists in decreasing alcohol intake[9,10] and drug withdrawal[8,11,12] (benzodiazepines and morphine). Based on these findings, a study of 5-HT3 antagonists in the psychiatric field could be interesting, especially in view of their general lack of side-effects, contrary to most psychoactive drugs. 5-HT3 receptor locations in the human brain have been studied by autoradiographic mapping[13] and high densities were found in the brainstem nuclei (50-200 fmol/mg tissue). Lower densities (4-47 fmol/ mg) were found in hippocampus, putamen, caudate and amygdala.

Until now, attempts to visualise the 5-HT3 receptors *in vivo* have been unsuccessful, due to low brain penetration[14] or substantial non-specific binding[15]. However, *in vivo* imaging of the 5-HT3 receptor using Positron Emission Tomography (PET) could be a useful tool for

psychopathological research and would allow the investigation of their role in some of the above described disorders. Hence, we describe the remote controlled synthesis of [^{11}C]-granisetron, a possible PET ligand for *in vivo* studies of 5-HT3 receptors.

Granisetron (BRL 46470A), (1-methyl-N-(endo-9-methyl-9-azabicyclo[3,3,1]-non-3-yl)-1H-indazole-3-carboxamide hydrochloride (Figure 1), is a highly selective and potent (K_i = 0.26 nM) 5-HT3 antagonist, clinically used as an anti-emetic[16].

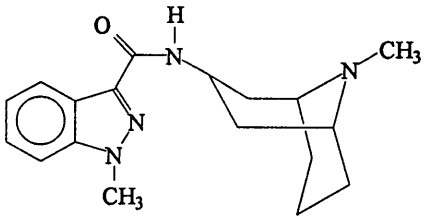

Figure 1. Granisetron

Granisetron can be easily labelled with a carbon-11 isotope by N-alkylation at the azabicyclo-structure using [^{11}C] methyl iodide in DMF.

Experimental

All chemicals were analytical grade and used without further purification.

N,N-Dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Janssen Chimica (Beerse, Belgium). $LiALH_4$ (1.0 M in THF) was purchased from Aldrich Chemie (Bornem, Belgium). DMF was distilled and kept over molecular sieves (4 A, 1-2 mm). THF was dried over sodium and distilled before use. Chromatography columns were purchased from Alltech Associates Inc. (Deerfield, IL). HPLC solvents were high-purity grade and obtained from Labscan (Co.Dublin, Ireland). Absolute ethanol was from VEL (Leuven, Belgium). ^{1}H-NMR spectra were recorded with a Bruker WP 360 (360 MHz) spectrometer. Mass spectra were recorded with a HP-5988 A mass spectrometer using electron impact ionisation (70 eV).

Synthesis of desmethylgranisetron

Granisetron free base (130 mg) was dissolved in dichloroethane (15 mL) and 4 eq. of vinylchloroformate (150 µL) were added. The mixture was refluxed for 1.5 h and evaporated to dryness under vacuum. The residue was taken up in dichloromethane and anhydrous HCl was bubbled through the solution. After evaporation of CH_2Cl_2, the residue was dissolved in methanol and gently heated. Final purification of desmethylgranisetron was achieved by flash chromatography on a silica column using ethylacetate/methanol/ TEA, 90/10/2.

^{1}H NMR ($CDCl_3$) δ (aliphatic) 1.32 (m, 3H), 1.42 (2H, d), 1.56 (1H,d), 1.74 (2H, m), 2.37 (2H,m), 3.48 (2H, d), 4.1 (3H, s, $C\underline{H_3}$), 4.29 (1H, m); (aromatic) 6.85 (1H, d), 7.28 (1H, m), 7.4 (1H, m), 8.38 (1H, d).

m/z (rel. intensity) 298 (M^+, 19), 255 (M^+ - (N-N-CH_3), 2), 159 (95), 123 (79), 96 (65) and 80 (100).

[^{11}C]Methyl Iodide

[^{11}C]Carbon dioxide was produced by irradiation of a nitrogen target using 18 MeV protons in a $^{14}N(p,\alpha)^{11}C$ reaction. After being trapped in a coil at -78°C, [^{11}C]CO_2 was transferred into a $LiAlH_4$ solution in THF to obtain $LiAl(O[^{11}C]H_3)_4$. The methanolate was hydrolysed by addition of HCl (200 µL, 6 M) and the solution heated to distil [^{11}C]methanol into 1 mL of hydroiodic acid (57 % in water), which was then heated at reflux to release [^{11}C]CH_3I[17].

[^{11}C]granisetron

[^{11}C]Methyl iodide was trapped in a cooled (-40°C) reaction vial containing desmethyl-granisetron (3 µmol) in dry DMF (200 µL) and 1µL of a 15 M NaOH aqueous solution. The vessel was sealed and heated for 6 min at 145°C. After cooling, 300 µL of mobile phase was added to the mixture. With a Valco injecting system (C6W) and a 500 µL loop, the reaction mixture was brought onto a semi-preparative Econosil Silica column (250x10 mm, 10 µ). Elution was performed with a Waters Associates Chromatography Pump (Model 6000A)

using sodium acetate buffer (150 mM, pH 4.5)/ethanol, 80/20 (v/v) at a flow rate of 5 mL/min. The effluent was monitored simultaneously by UV (Waters 440 Absorbance detector) (254 nm) and radioactivity detection (GM tube). The fraction containing [^{11}C]granisetron (t_R = 11.4 min, k' = 2.6) was collected over a 0.22 μ membrane into a sterile and pyrogen-free multidose vial. The sample (± 8 mL) was diluted with sterile, pyrogen-free water in order to obtain an injectable solution.

Quality control and determination of specific activity

Chemical and radiochemical purity of [^{11}C]granisetron was determined by straight phase analytical HPLC using a 50 μL loop on a Valco injector (C6W) and an Alltima Silica column (250 x 4.6 mm, 5 μ) with methanol/ sodium acetate buffer (50 mM, pH 5)/TEA, 80/20/2 as the mobile phase (flow rate 1 mL/min). For UV detection (254 nm) a Waters Tunable Absorbance Detector (Model 486) was used. For radioactivity detection an on-line NaI(Tl) detector (Mini Instruments, Essex, UK) was used. Peak integration was achieved using a Shimadzu C-R6A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan). The same system was used to determine the carrier amount and related specific activity by comparing the area of the UV absorbance peak of the tracer with a standard curve of granisetron.

Results and Discussion

Granisetron free base, obtained by extraction of its chloride salt using diethylether and aqueous bicarbonate, was dealkylated under mild conditions using vinyl chloroformate (VOC) in refluxing dichloroethane[18]. Semi-quantitative TLC of the VOC-intermediate (Figure 2) showed a reaction yield ≥ 85 %. Other dealkylating reagents such as ethyl chloroformate and trichloroethyl chloroformate gave lower reaction yields (50-60 %). The VOC group was easily removed by bubbling HCl gas through a desmethyl-granisetron dichloromethane solution (Figure 2). The desmethyl-compound was purified over a silica column (particle size 0.060-0.200 mm) and was obtained in a 75% overall yield as a pale yellow oil. Identification was

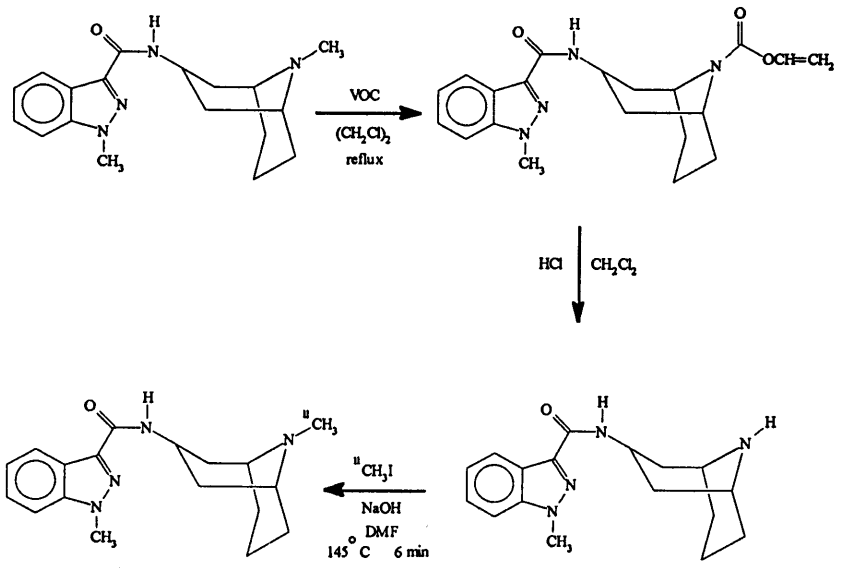

Figure 2. Synthesis of desmethyl granisetron and [^{11}C]granisetron

achieved by mass- and ^{1}H-NMR spectra and comparison with the spectra of granisetron showed that demethylation did occur at the azabicyclo-structure (δ ($CHCl_3$) 2.55 (3H, s, $\underline{CH_3}$)) and not at the methylgroup of the indazole-ring (δ ($CHCl_3$) 4.1 (3H, s, $\underline{CH_3}$)). Desmethyl granisetron was stored at 4°C, protected from light, and was dissolved in dry DMF prior to methylation. The radiochemical synthesis of [^{11}C]granisetron in a one pot reaction is shown in Figure 2. A systematic study of several influencing parameters of the N-methylation reaction (reaction time, temperature, volume, solvent, amount of starting materials) led to optimal reaction conditions resulting in a chemical reaction yield of 45%. Reaction yields were determined using analytical HPLC as described previously and are expressed as % granisetron and averaged (n =5). All experiments were performed using 1 μmol of cold methyl iodide as the limiting factor. In a first series of experiments several bases were tested at different concentrations. Best results were obtained with NaOH as a base in a small volume of water and a base/starting material ratio of 5 to 1. Larger volumes of water (≥ 5μL) decreased the

reaction yield. With *tert.* butylammonium hydroxide in methanol the reaction was not reproducible. In all further optimisation experiments NaOH was used in 1-2 µL of water in a 5 fold excess over desmethyl granisetron.

Reaction yield as a function of temperature reached a maximum at 140 °C. At higher temperatures, reaction yields were decreasing because of decomposition of the precursor, as determined by HPLC. As a function of reaction time the maximum reaction yield was obtained after 8 min and remained constant for longer reaction times.

Optimisation of reaction temperature : 1 µmol desmethyl granisetron, 250 µL DMF, 10 min

Temperature (°C)	25	40	60	80	100	120	140	160	200
Chemical reaction yield (%)	3	12	18	26	30	35	40	36	28

Optimisation of reaction time :1 µmol desmethyl granisetron, 250 µL DMF, 140 °C

Reaction time (min)	1	2	4	6	8	10	15
Chemical reaction yield (%)	14	25	29	33	42	43	43

Dry DMF was used as a solvent for the methylation reaction and good yields were obtained when using a small reaction volume (≤ 300 µL). Higher amounts of precursor gave also better yields, but when using more than 5 µmol of desmethyl granisetron, HPLC separation between starting material and methylated product became poor.

Optimisation of reaction volume :1 µmol desmethyl granisetron, 140 °C, 6 min

Reaction volume (µL)	100	200	250	300	500	1000
Chemical reaction yield (%)	44	46	45	43	38	26

Optimisation of amount of starting material: 200 µL DMF, 140 °C, 6 min

Starting material (µmol)	1	2	3	4	5	10
Chemical reaction yield (%)	30	38	44	46	45	48

When working under cold (1 µmol methyl iodide) and optimised conditions (3 µmol desmethyl granisetron in 200 µL DMF, 140 °C, 15 µmol NaOH in 1 µL water, 6 min), a chemical reaction yield of about 45 % was obtained. When performing the reaction with [^{11}C]methyl iodide, the yield of the labelling reaction was not particularly high (35-40%) which can be explained by the formation of a radioactive side product (Figure 3) making up 20-25 % of total radioactivity of the synthesis mixture. This side product was not observed or detected by UV detection when performing the reaction with cold methyl iodide.

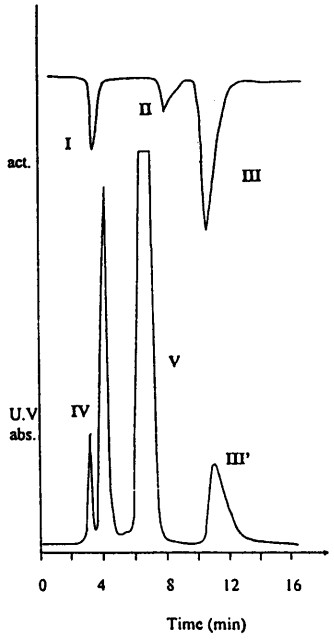

Figure 3. Semi-preparative HPLC radiochromatogram : **I.** [^{11}C] methyl iodide; **II.** [^{11}C] side product; **III.** [^{11}C] granisetron; **III'.** granisetron; **IV.** dimethylformamide (solvent); **V.** desmethylgranisetron

For purification of the radioactive product, several HPLC systems were tested to obtain a good separation between the compounds in the reaction mixture. With reversed phase HPLC, using a buffer/ethanol eluent, resolution was poor due to tailing of the peaks and /or high retention times (> 20 min). Finally, a straight phase system with an aqueous eluent gave the best resolution of [^{11}C]granisetron (t_R = 11.4 min), the side product (t_R = 8 min) and the des-methyl compound (t_R = 6.6 min) (Figure 3). With the sodium acetate/ethanol system used, the collected fraction had only to be diluted 1 to 5 (4% ethanol) to obtain a sample ready for intravenous injection.

The radiolabelled compound had the same retention time as a standard sample of granisetron. Chemical purity of the radioligand was ≥ 96 % and radiochemical purity ≥ 99 % as determined by analytical HPLC.

In a total synthesis time of 40 min some 45 mCi of injectable [^{11}C]granisetron was obtained; the specific activity was in the 200-250 mCi/μmol range.

Conclusion

The paper describes the remote controlled synthesis of [^{11}C]granisetron, using an N-alkylation reaction of the desmethyl precursor with [^{11}C] methyl iodide. Reaction parameters were optimised to obtain a radiochemical yield of 35-40 %. The radioligand was found to be both chemically and radiochemically pure with a specific activity of 200-250 mCi/μmol. Biodistribution studies directed at assessing the usefulness of this radioligand for *in vivo* studies using PET are being currently planned.

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