ABSTRACT

Syntheses and Bio-evaluation of Radioiodinated Organic Compounds for Application in the Nuclear Medicine Field

By

I. B. Tause

B.Sc. Ed. University of North-West

(1994)

B.Sc.Hons. University of Potchefstroom for Christian Higher Education

(1997)

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ABSTRACT

Malignant melanoma (skin cancer) is a common tumour and its frequency is increasing in the general population, the incidence varying with geographical region (John *et al.,* 1993). It is estimated that the mortality rate worldwide has doubled over the past 20 years due to this tumour. In this study radioiodine-labelled chemical compounds, [^{123}I]-ME-BA and [^{123}I]-E-BA, having different chemical structures, were prepared. These compounds were evaluated by means of their tumour uptake and compared with the control compound [^{123}I]-PAB.

The bromobenzamide derivatives were synthesized according to various standard methods. The tri-n-butyltin precursors were synthesized from the bromo compounds by a slight modification of the published procedure (Moreau *et al.*, 1998). "Cold" I-ME-BA and I-E-BA standards were prepared from their tributyltin derivatives in modest yields of 27% and 23% respectively, by reaction with NaI in the presence of chloramine-T as an oxidizing agent. I-PAB was prepared by condensing 4-iodobenzoic acid with 1-(2-aminoethyl) piperidine via its $SOCl_2$ generated benzoylchloride, and subsequent silica gel purification to afford a purified product in a modest yield (30%). [^{123}I]-ME-BA, [^{123}I]-E-BA and [^{123}I]-PAB were prepared from their tributyltin derivatives in moderate to high yields (67-88%) and high radiochemical purity (84-99%) by reaction with Na^{123}I in

the presence of chloramine-T as an oxidizing agent. Both [^{123}I]-E-BA and [^{123}I]-ME-BA bound with low affinity to sigma-receptors of the G11 cells, whereas the [^{123}I]-PAB bound with high affinity. However, [^{123}I]-E-BA bound slightly better than [^{123}I]-ME-BA.

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DEDICATION

I would like to dedicate this work to my mother who brought me up through tough times and to my brothers and sisters for their support given as this work was going on, most importantly to the almighty God who gave me strength throughout the course of this degree.

ACKNOWLEDGEMENTS

It has been a fascinating experience to prepare organic compounds and to finally test them biologically. I am indeed grateful to my supervisor Mr. Daniel Rossouw for his very useful and detailed criticisms, guidance and advice while undertaking this project. A special word of thanks and appreciation to Dr. Nico van der Walt for proof-reading this work, to Aldena Santos of the Cape Technikon for the biological testing of compounds, and to Dr. Kobus Slabbert of NAC for his assistance in doing the necessary arrangements for the testing. I am also deeply grateful for the moral support of Boikanyo Ntuane, Lesego Mogapi, and Given Mabala, for the continued financial support granted by the director of the National Accelerator Centre Prof. John F. Scharpey-Schafer, for the technical assistance of Schalk Smit, and for the tireless efforts of the MARST project committee in organizing this programme.

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INTRODUCTION

1. 1 NAC Facility Layout

The National Accelerator Centre (NAC) at Faure is a multidisciplinary scientific research laboratory and provides South Africa with centralized hi-tech equipment and expertise for:

- Training of students in basic and applied research with accelerated particle beams
- Particle radiotherapy for the treatment of cancer
- The supply of accelerator-produced radioisotopes for diagnostic nuclear medicine, cancer detection and for research.

Radioisotopes and radiopharmaceuticals are routinely manufactured at NAC using beams of protons from the Separated-Sector Cyclotron, and there is also a strong research and development programme (see Appendix 2). NAC produces a wide variety of short-lived radioisotopes such as ^{18}F, $^{81}Rb/^{81m}Kr$, ^{67}Ga, ^{123}I, ^{111}In and ^{201}Tl, as well as a variety of radioisotopically labelled compounds for medical purposes. These radiopharmaceuticals are supplied to more than 40 hospitals, clinics and research institutes

throughout the country, for use as tracers in diagnostic nuclear medicine, for cancer detection and for research. In addition, long-lived radioisotopes such as ^{22}Na, ^{55}Fe and ^{57}Co are manufactured for non-medical use, and are exported.

1. 2 Background

Melanoma is a malignant tumour of the melanocyte (Hoefnagel *et al.*, 1994) which is considered to be of neural origin. In other words, it is a disease of the skin in which cancer (malignant) cells are found in the epidermal cells that contain melanin (melanocytes), which gives the skin its colour (Physician Data Query –PDQ-1998). Thus in analogy with other neural crest tumours, melanomas are metabolically active as they have the ability to hydroxylate the amino acid tyrosine to dihydroxyphenyl-alamine (DOPA), which is subsequently converted into the pigment melanin. The primary tumour may be located in the eye or anywhere in the skin, where four types are recognized: superficial (65%) and nodular (25%) melanoma, and less frequently lentigo maligna and acral lentigous melanoma (5%) (Hoefnagel 1998).

Despite skin cancer education in the past decade, the incidence of malignant melanoma has soared dramatically between 1982 and 1989 (Michelot *et al.*, 1993). Malignant melanoma is a common tumour and its

frequency is increasing in the general population, the incidence varying with geographical region (John *et al.*, 1993). Exposure of the more susceptible white population to ultraviolet radiation is an important aetiological factor. For 1992, 32000 new cases of and 6700 deaths from melanoma were estimated for the United States and 80 cases per million inhabitants per year in Western Europe (Hoefnagel *et al.*, 1994; Hoefnagel 1998). Generally, the mortality rate worldwide has doubled over the past 20 years.

Melanoma (PDQ 1998) can spread (metastasize) quickly to other parts of the body through the lymph system or through the blood. Unless tumours are detected early and adequate surgery can be performed the prognosis of this disease is poor (Hoefnagel *et al.*, 1994 and Moreau *et al.*, 1998); in advanced cases the conventional oncological treatment modalities have had only a limited influence on survival. However, because malignant melanoma is also a neoplasia characterized by an impressive degree of malignancy, a multidisciplinary approach in both diagnosis and therapy is required (Belli *et al.*, 1989). More specifically, the multiplicity of localizations and the difficulty of diagnosis render diagnostic accuracy difficult.

In addition to standard diagnostic tools, for example physical examination, sonography, and radiography, several scintigraphic approaches have been developed for the detection of malignant melanoma and its metastases

(Brandau *et al.*, 1996). Radiolabelled monoclonal antibodies or their fragments that bind to specific melanoma antigens have been recently tried for localizing malignant melanoma. In clinical trials at multiple centers, limited success has been reported so far.

Several preclinical studies have identified radiopharmaceuticals as likely agents to localize melanomas in nuclear medicine (Michelot *et al.*, 1993). Agents with melanin affinity such as quinolines, melanin precursors such as thiouracil and nonspecific tumour localizing agents such as gallium have been evaluated and have had limited success. It has been proposed that these compounds possess some melanotropic affinity and are therefore incorporated into the melanin pigment of melanoma (John *et al.*, 1993). Although investigation in animals showed high accumulation of some of these compounds in experimental tumours, none of these derivatives has achieved clinical importance so far, mainly due to unsuitable biokinetics in humans (Brandau *et al.*, 1996 and other authors).

A new class of compounds that seems to show interesting properties is the group of benzamides (Maffioli *et al.*, 1994). These tracers have been synthesized to provide specific imaging of the structures of the brain with a high expression of dopaminergic receptors (basal ganglia) (Moreau *et al.*, 1992). The ectodermic origin of melanocytes and the presence of melanin in the substantia nigra are the theoretic bases for the application of

benzamides in the scintigraphic evaluation of melanoma in humans (Maffioli *et al.*, 1993). Iodine-123-N-isopropyl-p-iodoamphetamine (IMP), a brain imaging agent, has demonstrated metastatic melanoma in three of four patients. Cohen and co-workers reported the use of [^{123}I]IMP in the detection of primary and metastatic malignant melanoma (John *et al.*, 1992). Michelot *et al.*, (1991) described the radiopharmaceutical [^{123}I]-N-(2-diethylaminoethyl)-4-iodobenzamide (IDAB) for the detection of malignant melanoma.

Scintigraphic images of both murine B16 and human melanotic melanoma confirmed that external detection of melanoma was possible with this new iodinated radiopharmaceutical. In comparison, [^{125}I]IMP had slower blood clearance and its uptake in other organs such as liver, lung, brain and muscle was higher than that of tumour at 24 hr. Hence, good images could not be obtained because of low contrast. It was therefore concluded that IDAB was a better tracer than IMP for imaging malignant melanoma (John *et al.*, 1993). In clinical trials in Europe, [^{123}I]-DAB has been successfully used in the diagnosis of malignant melanoma. A disadvantage of [^{125}I]-DAB however, was that its biodistribution in nude mice with human melanoma xenografts indicated a high uptake of the radiopharmaceutical in nontarget organs such as the liver and lung and a slow clearance from these organs (John *et al.*, 1993).

In an attempt to prepare new radiopharmaceuticals with high tumour uptake and to understand the mechanism of uptake and retention of these benzamides, the synthesis, characterization, in vitro binding to sigma receptors and biological evaluation of N-(2-piperidinylaminoethyl)-4-iodobenzamide (IPAB) as a radiopharmaceutical for imaging malignant melanoma has been reported (John *et al.*, 1992). The radiolabelled product [^{125}I]-PAB was obtained with high yields and high specific activity using an iododestannylation reaction. In vitro pharmacological profile indicated that IPAB had a slightly higher affinity as compared to IDAB for sigma-1 sites and that it binds with affinity to melanoma cells. Its biodistribution in nude mice bearing human melanoma and imaging of nude mice with [^{131}I]-PAB suggested that [^{123}I]-PAB is a potential melanoma agent in humans.

1. 3 Rationale of Study

Certain organic compounds possess characteristic biological properties with respect to their accumulation inside the animal or human body. These properties are utilized in nuclear medicine to image specific organs inside the body after such a compound has been labelled with a radioisotope (radiopharmaceutical) and injected into the body. Radioiodine is an excellent radioisotope with which such labelling reactions can be done because of the favourable chemical properties of iodine and its

compatibility with the body. One type of radiopharmaceutical on which much research has already been done is a melanoma-seeking agent. It is well known that if a melanoma tumour can be detected early and diagnosed, the chance of survival is much better. The common types of radiopharmaceuticals for this purpose are the iodine-labelled benzamides. The influences of structure variation of these benzamides on melanoma uptake as well as melanoma: organ ratios are well documented (Hoefnagel *et al.*, 1994; John *et al.*, 1992, 1993, 1994; Maffioli *et al.*, 1994; Michelot *et al.*, 1991, 1993 and Moreau *et al.*, 1993, 1998). It was therefore envisaged to synthesize a few radioiodinated benzamide-type compounds having slightly different chemical structures from those described in literature, as well as one with well documented characteristics, and to compare their respective biological properties.

1. 4 Purpose

The purpose of this study was to prepare [^{123}I]-PAB from its already available tributyltin precursor, as well as other labelled benzamide-type organic molecules having the basic IPAB structure, but containing different substituents in the aromatic ring (see Appendix 3). A further aim was to evaluate all these compounds with respect to their tumour uptake characteristics. [^{123}I]-PAB would serve as a control compound against which the tumour uptake characteristics of the other two compounds

would be compared. The study was therefore structured as follows: (I) Preparation of synthetic precursor compounds with different chemical structures; (II) Radioiodination of the precursors using radiochemical methods and (III) Biological evaluation of the radiolabelled compounds using melanoma-specific cell line cultures.

1. 5 Hypothesis

Synthesis of a radiopharmaceutical that can be used in melanoma tumour tracing, imaging, diagnosis and detection.

Chapter 2

MATERIALS AND METHODS

2. 1 Materials

All chemicals were obtained from Aldrich Chemical Company or MERCK Chemicals. All solvents were dried with 4Å molecular sieves. The intermediate compound 5-bromo-2-ethoxy-3-methoxyethylbenzoate used for the preparation of Br-ME-B (see 2. 2. 1. 1), as well as the precursor 2-piperidinylaminoethyl)-4-tributyltinbenzamide used for the radiosynthesis of [^{123}I]-PAB (see 2. 3), have been prepared in previous unpublished work at NAC. Silica gel (0.063-0.200 mm) was used for chromatographic purification of intermediates and final compounds. Deionised water was used to prepare aqueous solutions. Radioactivity measurements were done in a Vinten radionuclide assay calibrator. Thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60 with fluorescent indicator precoated sheets, ALUGRAM® SIL G/UV$_{254}$, MACHEREY-NAGEL, Germany. TLC spots developed by various systems, were detected by illumination under a MODEL UV GL-58 UV-

254/366 nm lamp. High Performance Liquid Chromatography (HPLC) was comprised of the following components: a Phenomenex R.P. C18 column (250 x 4.60 mm); a Waters MODEL 510 pump equipped with a U6k injector; a Spectra Series UV detector and a NaI radioactivity detector (Thermo Separation Products); an ORTEC MODEL 420 ratemeter and a Hewlett Packard recorder. ^{1}H NMR spectra were recorded at 25 °C on a Varian Model VXR 300 Spectrometer in $CDCl_3$, with TMS as internal standard. [^{123}I]iodide is produced at NAC via the $^{127}I(p,5n)^{123}Xe \rightarrow {}^{123}I$ route and is recovered as a no-carrier-added product in 0.01N NaOH solution of which the volume is subsequently concentrated approximately 3 times by evaporation.

2. 2 Organic Chemistry Synthesis

2. 2. 1 Synthesis of (2-piperidinylaminoethyl)-5-iodo-2-ethoxy-3-methoxy benzamide [I-ME-BA]

2. 2. 1. 1 Preparation of 5-bromo-2-ethoxy-3-methoxybenzoic acid [Br-ME-B]

To a solution of 5-bromo-2-ethoxy-3-methoxyethylbenzoate (1.24 g, 4.1 mmol) in tetrahydrofuran [THF] (5 ml) was added NaOH (0.37 g, 9.3 mmol). The mixture was heated while stirring under reflux for approximately 1 hour and then cooled to room temperature. Another 5 ml

of THF was added and the mixture was heated again while stirred under reflux for an hour. This was followed by the addition of water (5 ml) and conc. HCl (1.7 ml). The aqueous solution was extracted with diethylether (2 x 5 ml). The combined ether extract was dried with Na_2SO_4, filtered, and the ether was evaporated under reduced pressure and 0.5 g of crude product was obtained. Product purity was assessed using the following TLC solvent systems:

- Toluene : ethanol : acetic acid = 85 : 10 : 5
- Hexane : acetone : acetic acid = 80 : 20 : 0.2

This product was used in the next step without purification.

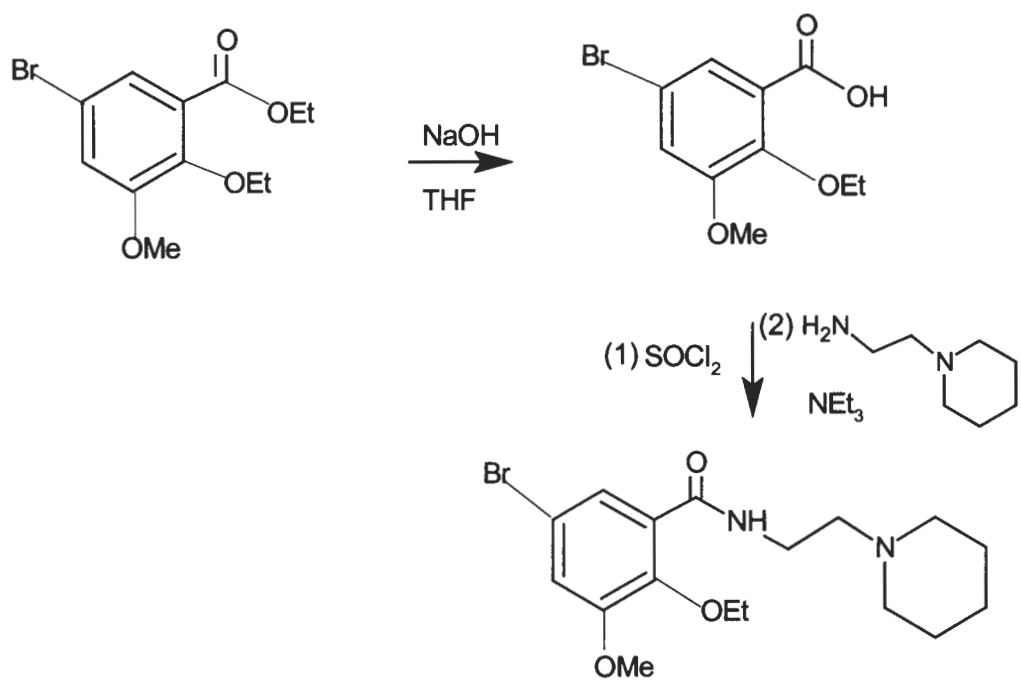
2. 2. 1. 2 Preparation of (2-piperidinylaminoethyl)-5-bromo-2-ethoxy-3-methoxybenzamide [Br-ME-BA]

A solution of $SOCl_2$ (0.6 ml, 8.22 mmol) in $CHCl_3$ (1.5 ml) was added to a solution of Br-ME-B (0.5 g, 1.82 mmol) in $CHCl_3$ (12 ml), followed by 5 drops of dimethylformamide [DMF]. The reaction mixture was stirred and heated under reflux for 1 hour, and then concentrated under reduced pressure. The residue was redissolved in $CHCl_3$ (5 ml) and the resultant solution was slowly added to a stirred solution of 1-(2-aminoethyl)-piperidine (0.25 g, 1.95 mmol) in $CHCl_3$ (4 ml). Triethylamine [TEA] (1.88 ml) was then added dropwise and the mixture was stirred at room temperature for approximately 1 hour. The reaction progress was monitored using the following TLC solvent system: diisopropylether (IPE):

MeOH: NH_4OH = 90: 10: 0.5. After completion, the reaction mixture was concentrated under reduced pressure, the residue redissolved in $CHCl_3$ (25 ml) and washed with 2% aqueous $NaHCO_3$ (2 x 12 ml). The organic phase was dried with Na_2SO_4, filtered and the $CHCl_3$ evaporated under reduced pressure affording 0.63 g of material that was used in the next step without purification.

SCHEME 2. 1

Preparation of Br-ME-BA.



2. 2. 1. 3 Preparation of (2-piperidinylaminoethyl)-5-tributyltin-2-ethoxy-3-methoxybenzamide [TBT-ME-BA]

Br-ME-BA (0.63 g, 1.63 mmol) was dissolved in dry toluene (40 ml). To this solution was added bis(tributyltin) (1.67 g, 2.88 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.15 g, 0.13 mmol). The reaction vessel was purged with nitrogen, and the reaction mixture heated under reflux while stirring. The reaction progress was monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. After completion, the reaction mixture was filtered, and the solvent evaporated under reduced pressure, leaving a black oily residue (2.1 g). This residue was subsequently chromatographed twice on 5 g silica gel with IPE: MeOH: NH_4OH = 90: 10: 0.5 affording 0.22 g of purified compound (See Scheme 2.3).

2. 2. 1. 4 Synthesis of unlabelled (2-piperidinylaminoethyl)-5-iodo-2-ethoxy-3-methoxybenzamide [I-ME-BA]

Purified TBT-ME-BA (0.20 g, 0.34 mmol) was in dissolved in methanol (3 ml). A solution of sodium iodide [NaI] (69 mg, 0.46 mmol) in H_2O (1.2 ml) was added, followed by a solution of N-chloro-4-toluenesulphonamide, sodium salt monohydrate [chloramine-T or CAT] (0.14 g, 0.49 mmol) in methanol (3 ml). The mixture was stirred at room temperature and the

reaction progress was monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. The reaction mixture was subsequently concentrated to dryness under reduced pressure to afford 0.39 g of impure I-ME-BA. This material was chromatographed on 7 g silica gel with IPE: MeOH: NH_4OH = 90: 10: 0.5 acquiring approximately 40 mg purified compound. ^1H NMR (ppm): 1.37-1.53 (br m, NCH_2, $ArOCH_2\underline{CH_3}$); 1.57-1.68 (br m, NCH_2); 2.41-2.50 (br m, NCH_2); 2.54-2.58 (t, 2 H, NCH_2); 3.55-3.64 (dt, 2 H, NCH_2); 3.84-3.95 (s, 3 H, $ArOCH_3$); 4.15-4.26 (q, 2 H, $ArO\underline{CH_2}CH_3$); 7.34 (s, 1 H, arom.); 8.1 (s, 1 H, arom.); 8.4 (br s, 1 H, NH).

2. 2. 2 Synthesis of (2-piperidinylaminoethyl)-5-iodo-2-ethoxybenzamide [I-E-BA]

2. 2. 2. 1 Preparation of 5-bromo-2-ethoxyethylbenzoate [Br-E-EB]

To a solution of 5-bromosalicylic acid (4.0 g, 18.4 mmol) in DMF (20 ml) was added anhydrous K_2CO_3 (3.8 g, 27.5 mmol) and ethyl iodide (3.7 ml, 46.3 mmol). The mixture was stirred while heating in an oil bath at 80°C. The reaction progress was monitored using the following TLC solvent system: Toluene: EtOH: Acetic acid = 85: 10: 5. After completion, the reaction mixture was filtered and the bulk of the solvent was removed under reduced pressure. The residue was subsequently diluted with 200 ml H_2O and extracted with $CHCl_3$ (2 x 50 ml). The combined $CHCl_3$ extract was washed with an aqueous solution of 10% $Na_2S_2O_3$ (50 ml)

followed by H_2O (2 x 50 ml). The $CHCl_3$ layer was dried with Na_2SO_4, filtered and evaporated to dryness affording a brownish crude product (5.2 g) which was used in the next step without purification.

2. 2. 2. 2 Preparation of 5-bromo-2-ethoxybenzoic acid [Br-E-B]

To a solution of 5-bromo-2-ethoxyethylbenzoate (4.28 g, 15.6 mmol) in THF (17 ml) was added NaOH (1.08 g, 27 mmol). The mixture was heated while stirring under reflux for an hour, then cooled to room temperature. H_2O (11 ml) and conc. HCl (3.8 ml) was added and the aqueous solution extracted with diethylether (2 x 11 ml). The combined ether extract was dried with Na_2SO_4, filtered, and the ether was evaporated under reduced pressure, delivering 5 g of crude product that was used in the next step without purification. Product purity was assessed using the following TLC solvent systems:

- Toluene: EtOH: acetic acid = 85: 10:5
- Hexane: acetone: acetic acid = 80: 20: 0.2

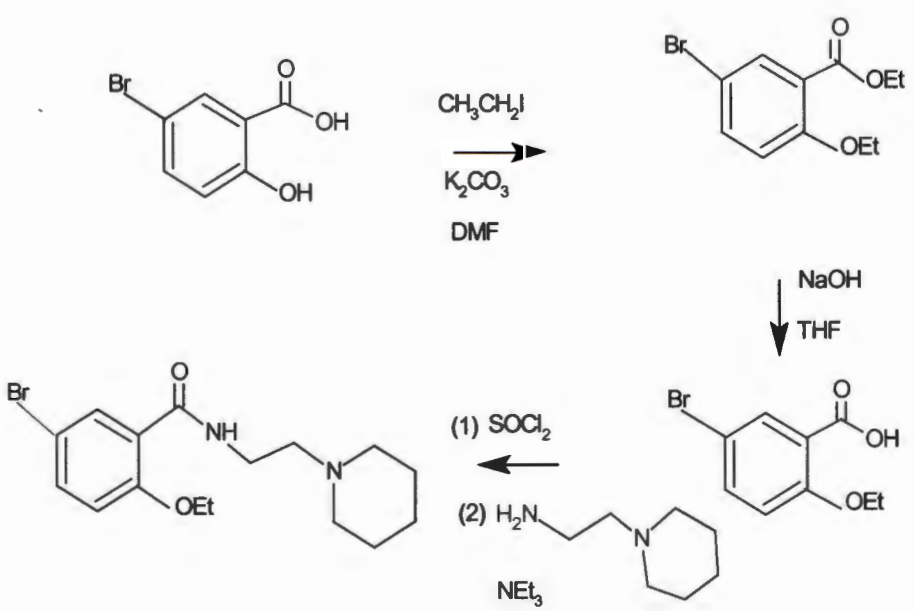
2. 2. 2. 3 Preparation of (2-piperidinylaminoethyl)-5-bromo-2-ethoxy benzamide [Br-E-BA]

A solution of $SOCl_2$ (0.67 ml, 9.22 mmol) in $CHCl_3$ (2 ml) was added to a solution of Br-E-B (0.5 g, 2.04 mmol) in $CHCl_3$ (12 ml), followed by 5 drops of dimethylformamide. The reaction mixture was stirred and heated under

reflux for an hour, and then concentrated under reduced pressure.The residue was redissolved in $CHCl_3$ (5 ml) and slowly added to a stirred solution of 1-(2-aminoethyl) piperidine (0.28 g, 2.19 mmol) in $CHCl_3$ (4.2 ml). TEA (2.1 ml) was then added dropwise and the mixture was stirred at room temperature for approximately 1 hour. The reaction progress was monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. After completion, the reaction mixture was concentrated under reduced pressure, the residue redissolved in $CHCl_3$ (25 ml) and washed with 2% aqueous $NaHCO_3$ (2 x 12 ml). The organic phase was dried (Na_2SO_4), filtered and the $CHCl_3$ evaporated under reduced pressure affording 0.58 g of a dark brown crude product, which was used in the next step without purification.

SCHEME 2. 2

Preparation of Br-E-BA.

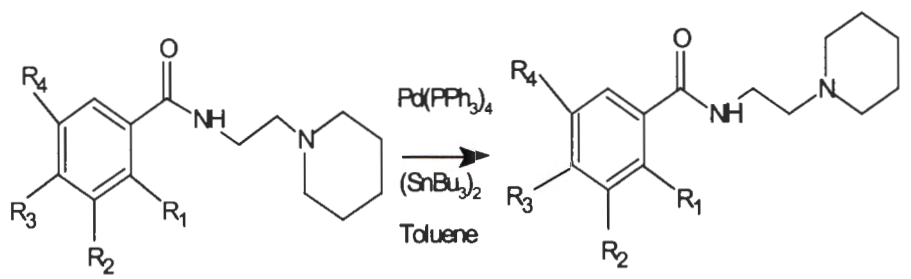


2. 2. 2. 4 Preparation of (2-piperidinylaminoethyl)-5-tributyltin-2-ethoxybenzamide [TBT-E-BA]

Br-E-BA (0.57 g, 1.62 mmol) was dissolved in dry toluene (38 ml). To this solution was added bis(tributyltin) (1.53 g, 2.64mmol) and $[Ph_3P]_4Pd$ (0.13 g, 0.12 mmol). The reaction vessel was purged with nitrogen, and the reaction mixture heated under reflux while stirring. The reaction progress was monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. After completion, the reaction mixture was filtered, and the solvent evaporated under reduced pressure, leaving a light-brown residue (2.8 g). This residue was subsequently chromatographed on 20 g silica gel with IPE: MeOH: NH_4OH = 90: 10: 0.5 delivering 0.28 g of a yellow oil.

SCHEME 2. 3

Preparation of tributyltin precursor compounds.



Br-ME-BA: R_1 = OEt, R_2 = OMe, R_3 = H
R_4 = Br

TBT-ME-BA: R_1 = OEt, R_2 = OMe, R_3 = H,
R_4 = $SnBu_3$

Br-E-BA: R_1 = OEt, R_2 = R_3 = H, R_4 = Br

TBT-E-BA: R_1 = OEt, R_2 = R_3 = H,
R_4 = $SnBu_3$

2. 2. 2. 5 Synthesis of unlabelled (2-piperidinylaminoethyl)-5-iodo-2-ethoxybenzamide [I-E-BA]

Purified TBT-E-BA (0.20 g, 0.35 mmol) was dissolved in methanol (3 ml). A solution of NaI (60 mg, 0.40 mmol) in water (1 ml) was added, followed by a solution of chloramine-T (0.11 g, 0.40 mmol) in methanol (3 ml). The mixture was stirred at room temperature and the reaction progress monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. The reaction mixture was subsequently concentrated to dryness

under reduced pressure to afford 0.38 g of impure I-E-BA. This material was chromatographed on 4 g silica gel with IPE: MeOH: NH_4OH = 90: 10: 0.5 yielding 33 mg purified compound. ^1H NMR (ppm): 1.46-1.71 (br m, NCH_2, $ArOCH_2\underline{CH_3}$); 2.41-2.50 (br m, 4 H, NCH_2); 2.52-2.61 (t, 2 H, NCH_2); 3.60-3.69 (dt, 2 H, NCH_2); 4.15-4.28 (q, 2 H, $ArO\underline{CH_2}CH_3$); 6.73-6.82 (m, arom.); 7.3-7.35 (m, arom.); 8.3 (br s, 1 H, NH); 8.56-8.58 (s, 1 H, arom.).

2. 2. 3 Synthesis of unlabelled (2-piperidinylaminoethyl)-4-iodobenzamide [IPAB]

A solution of $SOCl_2$ (0.14 ml, 1.90 mmol) in $CHCl_3$ (0.42 ml) was added to a solution of 4-iodobenzoic acid (0.1 g, 0.40 mmol) in $CHCl_3$ (2.8 ml), followed by 2 drops of DMF. The reaction mixture was heated for an hour, and then concentrated under reduced pressure. The residue was redissolved in $CHCl_3$ (1.2 ml) and slowly added to a stirred solution of 1-(2-aminoethyl) piperidine (0.07 g, 0.5 mmol) in $CHCl_3$ (1 ml). TEA (0.06 ml) was then added dropwise and the mixture was stirred at room temperature for 1 hour. The reaction progress was monitored using the following TLC solvent system: IPE: MeOH: NH_4OH = 90: 10: 0.5. After completion, the reaction mixture was concentrated under reduced pressure, the residue redissolved in $CHCl_3$ (5 ml) and washed with 2% aqueous $NaHCO_3$ (2 x 3 ml). The organic phase was dried with Na_2SO_4, filtered and the $CHCl_3$ evaporated under reduced pressure to afford 0.17 g of impure IPAB. This material was subsequently chromatographed on 3 g silica gel with IPE:

MeOH: NH_4OH = 90: 10: 0.5 affording 44 mg of a light brown solid. ^1H NMR (ppm):1.51-1.56 (br m, 2 H, NCH_2); 1.64-1.69 (br m,4 H, NCH_2); 2.5-2.55 (br m, 4 H, NCH_2); 2.61-2.66 (t, 2 H, NCH_2); 3.56-3.61 (dt, 2 H, NCH_2); 7.22 (bs, 1 H, NH); 7.58-7.68 (m, 2 H, arom.); 7.86-7.93 (m,2 H, arom.).

2. 3 Radiochemistry

Radioiodination of tributyltin precursors

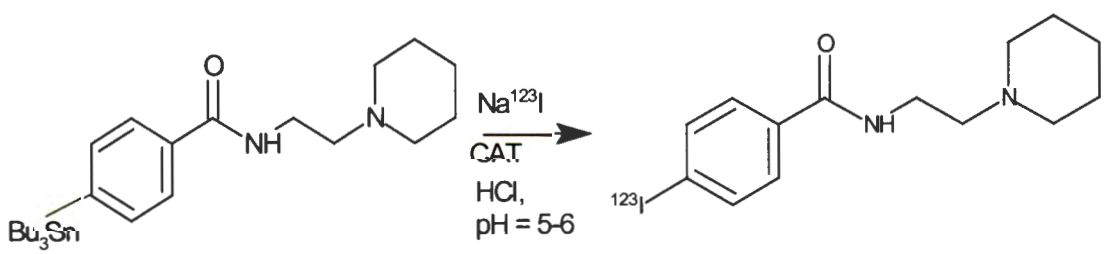
To 30 μl H_2O in a glass vial was added concentrated (32%) HCl solution (3 μl), followed by a solution of Na[^{123}I] (331 MBq, 40 μl) in approximately 0.03 N NaOH. To the resulting solution an ethanol solution of either (2-piperidinylaminoethyl)-4-tributyltinbenzamide [TBT-PAB], TBT-ME-BA or TBT-E-BA (1 mg/mL, 10 μl) was added. Finally, a freshly prepared aqueous solution of chloramine-T (1 mg/5 ml, 25 μl) was added. The solution was stirred for 15 to 20 minutes at room temperature after which the reaction was stopped by adding an aqueous solution of sodium metabisulphite [$Na_2S_2O_5$] (2 mg/ml, 10 μl). The extent of radioiodination was determined by HPLC after the first 3 to 5 minutes and after the reaction had been stopped (see Appendix 4).

After dilution with H_2O (0.5 ml), the reaction mixture was loaded onto a solid phase extraction cartridge (Waters Sep-Pak Vac 3cc, 500 mg) which

had been previously washed with approximately 2 ml of methanol followed by 2 ml of water. Free [^{123}I] iodide was removed by eluting the cartridge several times with water. In the case of [^{123}I]-ME-BA other less polar radiochemical impurities were partially removed by elution with H_2O/ethanol (60:40v/v). The radioiodinated product was then eluted with three 0.5 ml ethanol fractions. The fractions containing the bulk of the activity (approximately 1 ml) were combined, the ethanol evaporated under a stream of nitrogen with slight heating, and the remaining activity was redissolved in 0.9% NaCl (0.1-0.2 ml). The product was finally purified by filtration through a small anion exchange resin column (AG-1X8, chloride form, 10 mg) to remove residual traces of free [^{123}I]-iodide. The radiochemical purity of the product was determined by HPLC analysis using the solvent system: MeOH: H_2O: TEA = 75: 25: 0.05 at a flow rate of 1 ml/min.

SCHEME 2. 4

Radioiodination of precursor compound (TBT-PAB).



2. 4 Radiobiology

Protocol for testing of ^{123}I-labelled compounds

G11 cells (Passage number 72 and 73) in 4 x T75 flasks were washed with RPMI + 15% FCS (fetal calf serum). Trypsin was used to remove the adherent cells off the dishes. The cells were pelleted by centrifuging them at 1500 rpm. The pellet of cells was resuspended in 10 ml of medium and the number of cells was counted. There were 4.683 x 10^6 cells in 10 ml of medium.

Cells and radiolabelled product were added to tubes as follows:
250μl cells + 40 μl (300 μCi) radiolabelled compound + 10 μl medium. This was done in triplicate for each compound. Three tubes were also each filled with 250 μl cells + 60 μl medium (no activity), while a further 6 tubes were each filled with 40 μl (300 μCi) radiolabelled compound + 260 μl medium containing no cells (2 per compound). One of each of these was used to assay the amount of radioactivity added at the beginning of the experiment.

The total volume in all the tubes was 300 μl. The cells were incubated for 4 hours at 37 °C. After incubation the unbound radioactive compound was removed by washing the cells thoroughly three times as follows: 2 ml of medium was added to each tube and the cells were pelleted. The

supernatant was discarded and the pellet was resuspended in 2 ml of medium and pelleted again. This step was repeated twice. After the final wash, the supernatant was removed and the amount of radioactivity present in the tubes was assayed by means of a high purity Ge-detector coupled to a Silena multichannel analyzer.

Chapter 3

RESULTS AND DISCUSSION

In the present study, all the bromobenzoic acid derivatives were converted to their respective 2-piperidinylaminoethylbenzamides via the formation of the intermediate benzoylchloride derivative using thionyl chloride as chlorinating agent in the presence of DMF as a catalyst according to the method of John, Bowen, Saga, *et al.* (1993). The acid chloride was condensed with 1-(2-aminoethyl) piperidine. The bromobenzoic acid derivatives containing methoxy and/or ethoxy groups on the aromatic ring were obtained by simultaneous alkylation of the carboxylic and hydroxy groups of the salicylic acid derivatives with ethyl iodide, followed by saponification of the resultant ester to the corresponding benzoic acid. This whole sequence was carried out according to a modification of a method described by Fang, Mukherjee and Cooper (1993). The tri-n-butyltin precursors were synthesized by a slight modification of the published procedure (Moreau *et al.*, 1998). These debromostannylation reactions were carried out by refluxing a mixture of the bromobenzamide derivatives, bis(tributyltin) and a catalytic amount of tetrakis (triphenylphosphine) palladium in dry toluene to afford modest yields (23-30%) of tributyltin-substituted benzamide derivatives.

"Cold" I-ME-BA and I-E-BA standards were prepared from their tributyltin derivatives in modest yields of 27% and 23% respectively, by reaction with NaI in the presence of chloramine-T as an oxidizing agent (see Appendix 2). I-PAB was prepared by condensing 4-iodobenzoic acid with 1-(2-aminoethyl) piperidine via its $SOCl_2$ generated benzoylchloride, and subsequent silica gel purification to afford a purified product in a modest yield (30%).

[^{123}I]-ME-BA, [^{123}I]-E-BA and [^{123}I]-PAB were prepared from their tributyltin derivatives in oxidative electrophilic destannylation reactions, using Na[^{123}I] in the presence of chloramine-T as an oxidizing agent. Purification of the radiolabelled compounds were carried out by means of a solid phase extraction technique, followed by anion exchange chromatography. These steps allowed the isolation of products in fairly high radiochemical yields and purities (see Table 3.1), as well as high specific activities (free of precursors that are retained by the solid phase cartridge). Radiochemical purities were determined by means of HPLC-analyses as already described.

The retention times of "cold" I-ME-BA and I-E-BA were15.83 and 15.25 minutes respectively as shown in Table 3.1. The retention time for IPAB under identical conditions is 9.36 min. The difference in retention times can be ascribed to the presence of the ethoxy-and ethoxy/methoxy substituents in the aromatic ring of the benzamides for I-E-BA and I-ME-

BA respectively. The retention times of the radioiodinated compounds were very similar to their corresponding "cold" iodinated standards, thus confirming their authenticities. The radiochemical purities of [^{123}I]-E-BA and [^{123}I]-PAB were both higher than 96%, while the lower purity of [^{123}I]-ME-BA was caused by the presence of some unknown impurities that could not be quantitatively removed during the purification procedure.

In a study conducted by John, Bowen, Saga, *et al.* (1993), *in vitro* binding studies of [^{125}I]-PAB with human malignant melanoma cells (A 2058) showed that the tracer bound with high affinity. In another study (Santos *et al.*, 1997), it was shown that [^{123}I]-PAB also binds with relative high affinity to a human glioblastoma cell (G11) which has been reported to carry sigma receptors present in melanoma cells. Thus in this present study, [^{123}I]-PAB, [^{123}I]-E-BA and [^{123}I]-ME-BA were evaluated for their activity at sigma receptors of the G11, with [^{123}I]-PAB serving as a control. The check measurement of medium with activity showed that the activity of each compound that was added to cells per tube for the experiment was very close (about 23 000 counts per second) (see Table 3.2). The control experiments (number of cells equal to zero) show some remaining activity, which can be ascribed to adsorption of some of the radiolabelled compounds onto the inner walls of the tubes. These activity values were, however, not used to correct the values caused by cell binding.

Table 3. 1 Comparison of chromatographic properties, radiochemical yields and purities of the radioiodinated benzamides.

Compound	Retention Time (min)		Average Radiochemical Yields After SPP (%)	Radiochemical Purity (%)
	Radioiodo-Comp.	"cold" iodo compound		
IPAB	9. 12	9. 36	88	96 – 99
IEBA	15. 20	15. 25	79	96 – 99
IMEBA	15. 65	15. 83	67	84 – 87

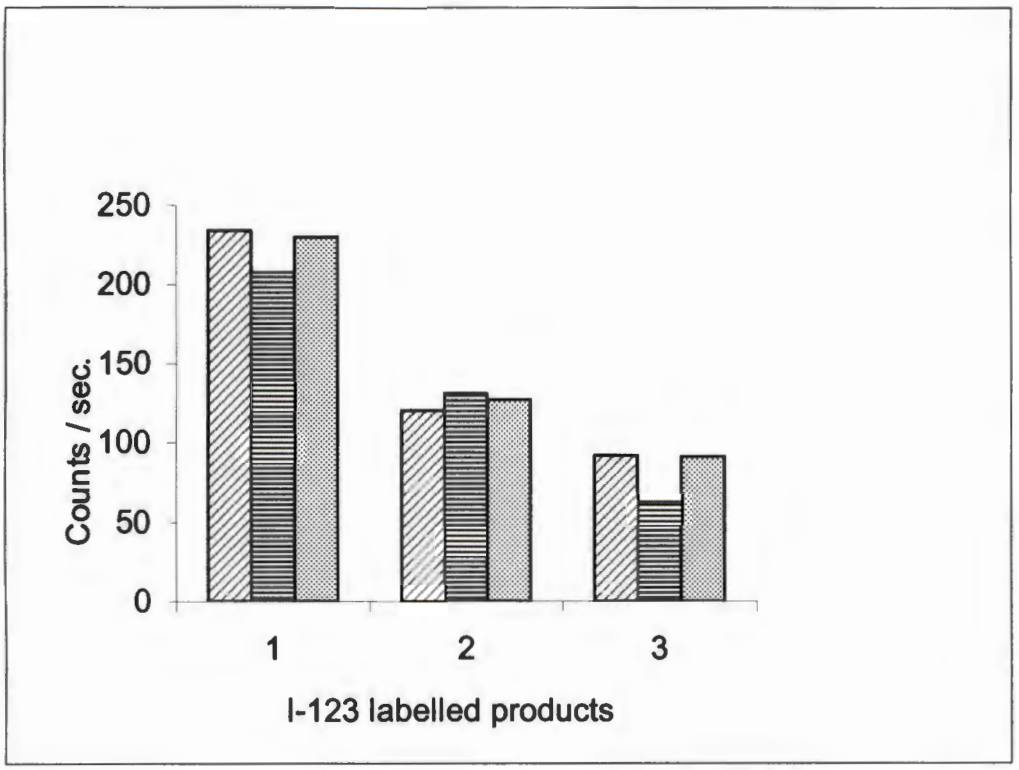
Tubes with cells only also showed some activity, which could have been due to cross contamination during the washing procedure of the cells. The uncorrected cell binding data in Table 3. 2 and Figure 3. 1 show that both [^{123}I]-E-BA and [^{123}I]-ME-BA bound with relatively low to zero (if control values are subtracted) affinity to sigma-receptors of the G11 cells, whereas the [^{123}I]-PAB bound with relative high affinity. However, [^{123}I]-E-BA bound slightly better than [^{123}I]-ME-BA. It appears as if the presence of relatively large groups close to the amide group, as in the case of [^{123}I]-ME-BA and to a lesser extent, [^{123}I]-E-BA, cause less efficient binding of

the molecule. The slightly superior binding efficiency of [^{123}I]-E-BA over [^{123}I]-ME-BA could be ascribed to the fact that the first mentioned does not contain an additional methoxy group in the vicinity of the amide group. Another compound, [^{125}I]-PMBA , a 3-iodo-4-methoxybenzamide used by John *et al.* (1999) in structure-affinity distribution studies, was being claimed to possess relatively high affinities for sigma-1 (K_i = 11.8 nM) and moderate affinities for sigma-2 receptors (K_i = 206 nM), despite the presence of a methoxy group in the aromatic ring. These relatively higher affinities exhibited by [^{125}I]-PMBA for sigma receptors could probably be explained by the bigger distance between the methoxy and the amide group in [^{125}I]-PMBA, compared to the groups in [^{123}I]-ME-BA and [^{123}I]-E-BA.

Table 3. 2 Uncorrected data showing the comparison of the binding efficiency to the G11 cells for the three I-123 labelled compounds, as expressed in counts per second (cps).

	I-123-PAB	I-123-EBA	I-123-MEBA	
40μl (μCi)	155	160	128	
Cps	22700	23100	24100	
				No. of Cells
Control (cps)	19.7	67.5	116	0
A	234	120	91.9	400000
B	208	131	63.2	400000
C	230	127	90.8	400000
Average (cps)	224	126	82.0	
Cells only (no activity) (cps)	0.67	1.05	6.6	400000

Figure 3. 1 **Binding efficiencies of Iodine-123 labelled compounds to G11 cells, expressed in counts per second, and indicated with numbers as follows: 1, [^{123}I]-PAB; 2, [^{123}I]-E-BA; 3, [^{123}I]-ME-BA.**



Chapter 4

SUMMARY, CONCLUSION AND RECOMMENDATIONS

The tracers [^{123}I]-ME-BA and [^{123}I]-E-BA were prepared. This involved the preparation of the relevant tributyltin precursors. In addition to this, the control compound [^{123}I]-PAB was also prepared from its available tributyltin precursor.

Research results from literature show that [^{123}I]-PAB is a potential melanoma-imaging agent in humans. Thus in this study these new compounds were also evaluated by means of their tumour uptake and compared with the control compound. [^{123}I]-PAB showed significantly higher affinity for the tumour cells than both new compounds. Furthermore it is important to note that the [^{123}I]-E-BA had a slightly higher affinity than [^{123}I]-ME-BA, indicating that the structure of a compound with regards to the positioning of the substituents in the aromatic ring, and probably their size, play a significant role in the binding of a compound to the cell.

It is therefore recommended that other compounds of similar nature be prepared with one or both substituents being placed further away from the

amide group of the aromatic ring. As a matter of interest all these compounds could also be biologically evaluated for detection or diagnosis in other cancer tumour types, such as brain, lung, breast and many others.

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APPENDIX 1

Abbreviations of synthesized compounds

Br- E-EB – 5-bromo-2-ethoxyethylbenzoate

Br-E-B – 5-bromo-2-ethoxybenzoic acid

Br-E-BA – (2-piperidinylaminoethyl)-5-bromo-2-ethoxybenzamide

Br-ME-B – 5-bromo-2-ethoxy-3-methoxybenzoic acid

Br-ME-BA – (2-piperidinylaminoethyl)-5-bromo-2-ethoxy-3-methoxybenz-amide

I-E-BA – (2-piperidinylaminoethyl)-5-iodo-2-ethoxybenzamide

I-ME-BA – (2-piperidinylaminoethyl)-5-iodo-2-ethoxy-3-methoxybenz amide

I-PAB – (2-piperidinylaminoethyl)-4-iodobenzamide

TBT-E-BA – (2-piperidinylaminoethyl)-5-tributyltin-2-ethoxybenzamide

TBT-ME-BA – (2-piperidinylaminoethyl)-5-tributyltin-2-ethoxy-3-methoxybenzamide

TBT-PAB – (2-piperidinylaminoethyl)-4-tributyltinbenzamide

APPENDIX 2

Definitions of Terms and Concepts

Activity

The rate of radioactive transformations (radioactive decays) in a given source or sample.

Cold

Not radioactive.

Cyclotron

A highly sophisticated facility used for the acceleration of charged particles such as protons, deuterons, ^{3}He and α-particles.

mCi and MBq

The activity of a radioactive source or sample is expressed in millicuries (1 millicurie = 3.7×10^{7} radioactive transformations or decays per second), or megabecquerel (MBq) (1 mCi = 37 MBq).

Production of Iodine-123

At the National Accelerator Centre 66 MeV protons are used to bombard a sodium iodide target to produce iodine-123. Five neutrons get knocked out when a proton hits iodine-127, resulting in xenon-123 which decays to iodine-123.

Radiochemical purity

The fraction of a specific radionuclide (isotope) that is present in the desired chemical form and in the specified molecular position.

Radioisotopes

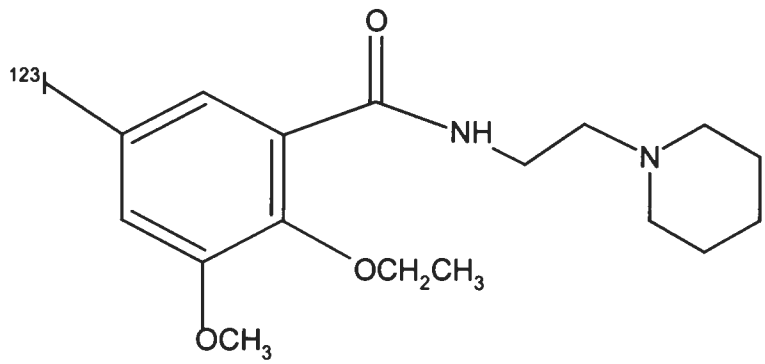
These are unstable isotopes that emit radiation due to their decay to daughter nuclides. Isotopes contain the same number of protons in the nucleus and have the same extra-nuclear electronic structure as the parent element, but differ in the number of neutrons.

Radiopharmaceutical

Medicine that contains one or more radioactive isotopes.

APPENDIX 3

Structures of the radioiodinated compounds



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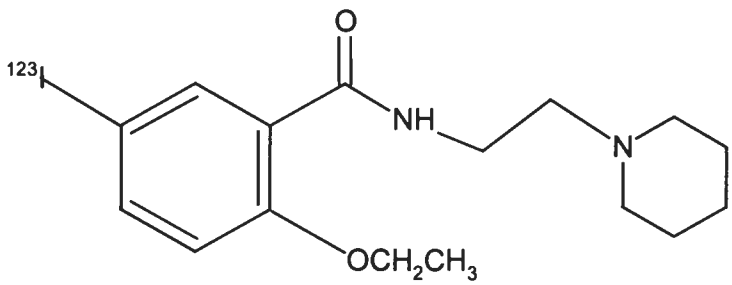

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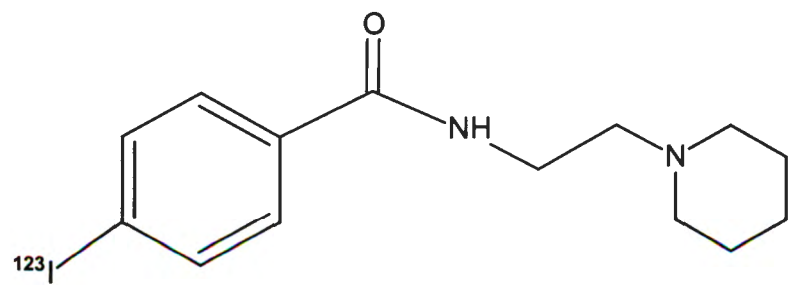
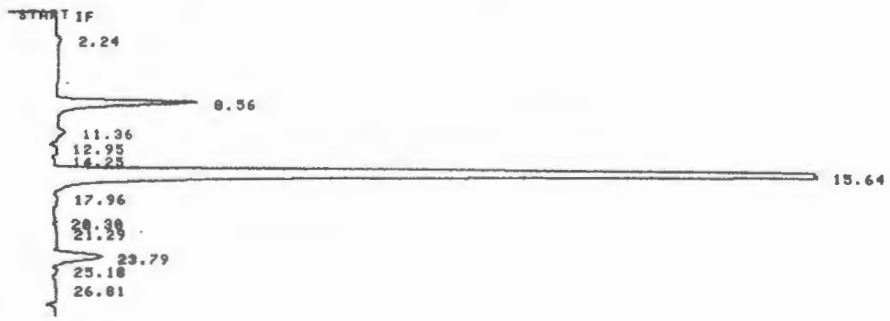


Iodine-123-N-(2-piperidinylaminoethyl)-4-iodobenzamide ([^{123}I]-PAB)

APPENDIX 4

HPLC chromatograms of radioiodinated compounds after purification by means of anion exchange resin (AG-1X8)



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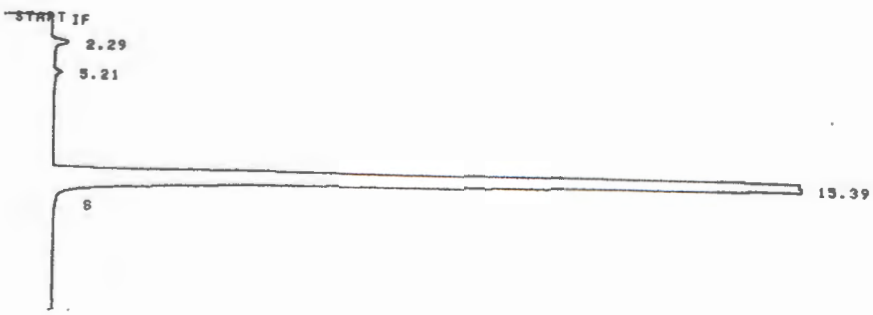

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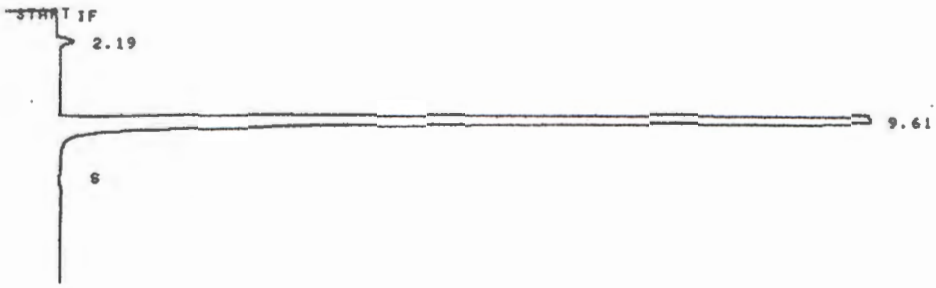

Iodine-123-N (2-piperidinylaminoethyl)-5-iodo-2-ethoxybenzamide

[^{123}I]-PAB