SYNTHESIS OF [^{18}F]-LABELED ADENOSINE ANALOGUES AS POTENTIAL PET IMAGING AGENTS

M. M. Alauddin, J. D. Fissekis and P. S. Conti

Department of Radiology, PET Imaging Science Center, University of Southern California, 2250 Alcazar St. # 135V, Los Angeles, CA 90033, USA. E-mail: alauddin @usc.edu

Key words: Fluorine-18, Nucleoside, Adenosine, PET.

Introduction: Fluorinated analogues of adenosine are known to be potential antitumor and antiviral agents. Some 2′-deoxy-2′-fluoro-9- -D-arabinofuranosyladenines (FAA) have antitumor activity, while 3′-deoxy-3′-fluoro-9- -D-ribofuranosyladenine (FRA) is a known antiviral agent. Little information is available on the biological properties of the xylo-derivative, 3′-deoxy-3′-fluoro-9- -D-xylofuranosyladenine (FXA).

Syntheses of FAA involve the incorporation of fluorine (2-*arabino*) in the sugar followed by coupling with the purine base, or treatment of N^6,3′,5′-tritrityladenosine with (diethylamino)sulfur trifluoride (DAST). These methods are not suitable for [^{18}F]-labeled synthesis. One synthesis of [^{18}F]-FAA was reported without characterization of the product. Here we report a synthesis of [^{18}F]-FAA and [^{18}F]-FXA. This is the first ^{18}F-labeled synthesis of [^{18}F]-FXA.

Methods and Results: Adenosine **1** was reacted with methoxytrityl-chloride to give a mixture of N^6,3′,5′- and N^6,2′,5′-methoxytrityl derivatives **2** and **3**. After resolution each compound was converted to the respective triflates, **4** or **5**, which served as precursor for the syntheses of [^{18}F]-FAA or [^{18}F]-FXA.

Either compound **4** or **5** (30 mg) was dissolved in dry MeCN (1.5 mL), n-Bu_4NF (1M, 30 µL) was added and the mixture was heated for 30 min at 72-74°C. After cooling, solvent was evaporated and purified on a short silica gel column. Protected fluorinated compounds **6** and **7** were obtained in 20% and 50% yields, respectively, which on hydrolysis yielded the desired products, **8** and **9**.

Radiolabeled compounds **6** and **7** were prepared by fluorination of the respective triflates with n-$Bu_4N^{18}F$. To the dry n-$Bu_4N^{18}F$, precursor soln of either **4** or **5** (~2mg) in MeCN (0.5 mL) was added and heated for 30 min at 72-74°C. The reaction mixture was cooled and passed through a Sep-Pak cartridge (silica) and eluted with 10% methanol in dichloromethane. Compound **6** or **7** was hydrolyzed with HCl and the corresponding nucleoside **8** ([^{18}F]-FAA) or **9** ([^{18}F]-FXA) was isolated by HPLC purification using 9% MeCN/H_2O.

The radiochemical yield of this synthesis was 12-18% (d. c.) for **8** and 30-48% for **9** from the EOB in 3 runs/compound. The radiochemical purity was > 99% with an average specific activity >74 GBq/µmol. The synthesis time was 90-95 min from the EOB. In representative syntheses, 518 MBq of labeled product **8** and 3.07 GBq of **9** were obtained from 6.88 GBq and 8.54 GBq of [^{18}F]-fluoride, respectively.

Analysis of the pure product, either **8** or **9** by HPLC showed a single radioactive peak that co-eluting with an authentic unlabeled sample.

Conclusion: Syntheses of adenosine analogues, [^{18}F]-FAA and [^{18}F]-FXA have been achieved. This direct fluorination method is convenient, produces sufficient amounts of activity, and is suitable for routine production for animal and human studies with PET.

SYNTHESIS OF [^{18}F]-FBAU AND [^{18}F]-FCAU AS POTENTIAL PET IMAGING AGENTS

M.M. Alauddin, J.D. Fissekis and P.S. Conti

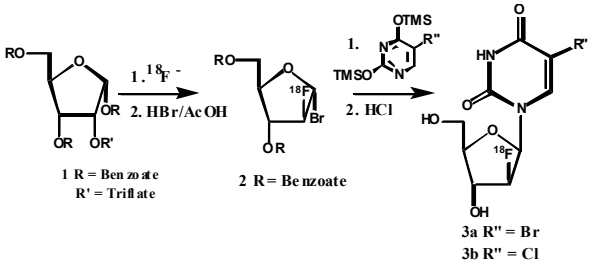
Department of Radiology, PET Imaging Science Center, University of Southern California, 2250 Alcazar St. # 135V, Los Angeles, CA 90033, USA . E-mail: alauddin@usc.edu

Key words: Fluorine-18, PET, Nucleoside, Gene Expression.

Introduction: Radiolabeled analogues of 2′-deoxy-2′-fluoro-5-(methyl, bromo and iodo)-1- -D-arabinofuranosyluracil such as [^{11}C]-FMAU, [^{76}Br]-FBAU and [^{124}I]-FIAU are potential PET imaging agents. [^{18}F]-Labeled derivatives are likely to be more advantageous given the 2h half-life of ^{18}F and its wide availability. We have reported previously a synthesis of [^{18}F]-FMAU and a few other pyrimidine nucleosides for PET imaging. We have synthesized now 2′-deoxy-2′-[^{18}F]fluoro-5-[bromo- and 5-chloro]-1- -D-arabinofuranosiluracil, [^{18}F]-FBAU and [^{18}F]-FCAU, as potential PET imaging agents.

Methods and Results: The synthetic scheme of [^{18}F]-labeled nucleosides **3a** and **3b** is shown in figure 1. Compound **2** was prepared from **1** in two steps as previously reported. Compound **2** was heated with freshly prepared 2,4-bis-O-(trimethylsilyl)uracil derivative (75-85 μmol) in 1,2-dichloroethane (0.5 mL) at ~100°C for 60 min. The reaction mixture was cooled, passed through a Sep-Pak cartridge (silica) and eluted with 10% methanol in dichloromethane (2.5 mL). After evaporation of the solvent the crude product was refluxed for 5 min with sodium methoxide (1M 0.03 mL). The reaction mixture was cooled, neutralized, diluted with HPLC solvent, and purified by HPLC using a semi-prep C-18 column and 8% MeCN in water at a flow of 4.0 mL/min. After fraction collection and solvent evaporation, the pure product was re-dissolved in saline and filtered through a 0.22 μm filter. An aliquot of the final product was analyzed by HPLC, and found to be co-eluting with the authentic unlabeled standard.

Radiochemical yield of these compounds was 8-12% (d. c.) from the EOB in six runs (3 runs/comp). The radiochemical purity was > 99% with specific activities 55-103 GBq/μmol. Synthesis time was 3.5-4.0h from the EOB. In a representative synthesis, 120 MBq of labeled product was obtained from 7520 MBq of [^{18}F]fluoride.



Conclusion: Syntheses of 2′-deoxy-2′-[^{18}F]fluoro-5-bromo- and 5- chloro-1- -D-arabinofuranosyl-uracil ([^{18}F]-FBAU and ([^{18}F]-FCAU) have been achieved. Sufficient amount of activities can be produced for animal and human studies.

5'-LABELLING WITH FLUORINE-18 OF OLIGONUCLEOTIDES

B. Kuhnast[1], R. Boisgard[2], F. Hinnen[1], B. Tavitian[2], F. Dollé[1]

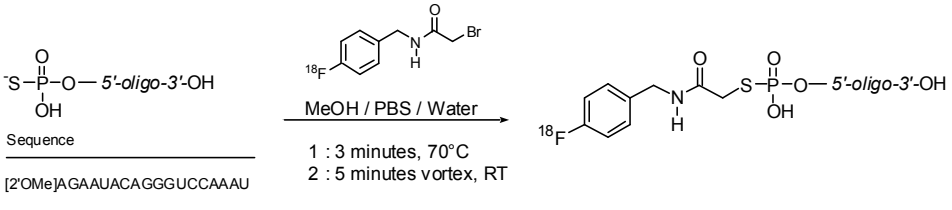
[1] Service Hospitalier Frédéric Joliot, Département de Recherche Médicale, CEA/DSV, 4 place du général Leclerc, F-91406 Orsay Cedex. [2] ERITM 0103, Unité d'Imagerie de l'Expression des Gènes, same address. E-mail contact address : kuhnast@shfj.cea.fr

Keywords : fluorine-18, oligonucleotides, positron emission tomography

Since many years, intense efforts have been devoted to the development of biological macromolecules as radiopharmaceuticals, among which oligonucleotides are promising as contrast agents to study gene expression at the nucleic acid and protein levels. We have previously established a general method to label oligonucleotides bearing phosphorothioate monoester group at their 3'-end with *N*-(4-halobenzyl)-2-bromoacetamides (1-3), based on the regiospecific conjugation of the prosthetic group to the modified 3'-end of the oligonucleotide. Regioselectivity and unicity of the conjugation of the prosthetic group were demonstrated by ^{31}P-NMR and mass spectrometry studies (1,2). We also applied this strategy to oligonucleotides differing in length, sequence and modifications of the sugar-phosphate backbone (2).

In the present study, we investigated the labelling of oligonucleotides bearing phosphorothioate monoester group at their 5'-end using the same prosthetic group *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide. We chose to label an *in vivo* stable 2'OMe-RNA modified oligonucleotide. Non-labelled reference oligonucleotide was synthesized using the methodology developed in our institution as already published (1-4) and characterized using ^{31}P-NMR and mass spectrometry.

N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was prepared as previously described (1-2). Typically, starting from a batch of 22-24 GBq of [^{18}F]fluorine, we routinely produced 2.2-2.4 GBq of HPLC-purified *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide in about 90 minutes. The conjugation of the *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was performed in a mixture of MeOH/PBS (0.1 M, pH 8.2)/water (4/1/3 : v/v/v) by (a) heating the reaction mixture at 70°C for 5 minutes without stirring ; (b) vortexing the mixture for 5 minutes at room temperature. The final radiotracer was purified by semi preparative RP-HPLC and desalted using a Sephadex cartridge. The whole synthetic procedure allows to produce up to 1 GBq of fluorine-18 labelled oligonucleotide in 160 minutes with a specific radioactivity of 37-74 GBq/µmol.



Biodistributions and pharmacokinetics performed with PET in baboons as well as plasma and urine metabolism are dramatically different than those obtained with the same oligonucleotide sequences labelled at the 3'-end (4). This study clearly demonstrates the importance of the labelling position on the oligonucleotide backbone.

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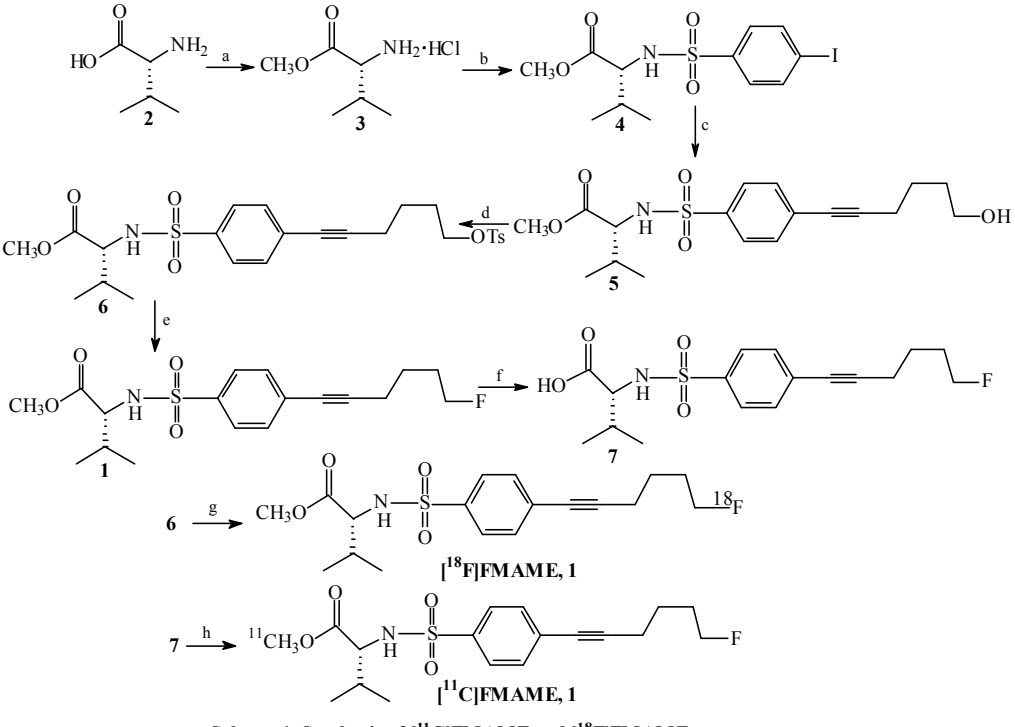
SYNTHESIS AND BIOLOGICAL EVALUATION OF POTENTIAL CANCER BIOMARKERS RADIOLABELLED MMP INHIBITORS [^{11}C]FMAME AND [^{18}F]FMAME

Q-H. Zheng[1], X. Fei[1], J-Q. Wang[1], K.L. Stone[1], T.D. Martinez[1], D.J. Gay[1], W.L. Baity[1], B.H. Mock[1], B.E. Glick-Wilson[1], M.L. Sullivan[1], K.D. Miller[2], G.W. Sledge[2], G.D. Hutchins[1]

Departments of [1]Radiology and [2]Medicine, Indiana University School of Medicine, 950 W. Walnut St., R2 E124, Indianapolis, IN 46202-5181, USA. qzheng@iupui.edu

Keywords: Matrix metalloproteinase inhibitor; cancer biomarker; positron emission tomography; [^{11}C]FMAME; [^{18}F]FMAME

(2R)-2-[[4-(6-fluorohex-1-ynyl)phenyl]sulfonylamino]-3-methylbutyric acid [^{11}C]methyl ester ([^{11}C]FMAME) and (2R)-2-[[4-(6-[^{18}F]fluorohex-1-ynyl)phenyl]sulfonylamino]-3-methylbutyric acid methyl ester ([^{18}F]FMAME) (**Scheme 1**), novel radiolabeled matrix metalloproteinase (MMP) inhibitors, have been synthesized for evaluation as new potential positron emission tomography (PET) cancer biomarkers. The appropriate acid and tosylate precursors were synthesized from amino acid (D)-valine with excellent chemical yields. The acid precursor was labelled by [^{11}C]methyl triflate through O-[^{11}C]methylation method to provide [^{11}C]FMAME. The tosylate precursor was labelled by potassium ^{18}F-fluoride/Kryptofix 2.2.2 through nucleophilic substitution to provide [^{18}F]FMAME. The biological evaluation of the tracer including *in vivo* biodistribution and micro-PET imaging was performed in breast cancer animal models MCF-7's transfected with IL-1a implanted athymic mice and MDA-MB-435 implanted athymic mice. The results show that [^{11}C]FMAME and [^{18}F]FMAME may be potential PET tracers for cancer imaging.



Scheme 1. Synthesis of [^{11}C]FMAME and [^{18}F]FMAME

a. $SOCl_2$, MeOH; b. 4-IPhSO$_2$Cl, Et_3N, CH_3CN; c. 5-Hexyne-1-ol, Pd(PPh_3)$_2Cl_2$, CuI, TEA, DMF; d. TsCl, NMM, DCM; e. tBu_4NF, THF; f. HCl/TFA; g. K^{18}F, K$_{2.2.2}$, CH_3CN; h. $^{11}CH_3OTf$, TBAH, CH_3CN

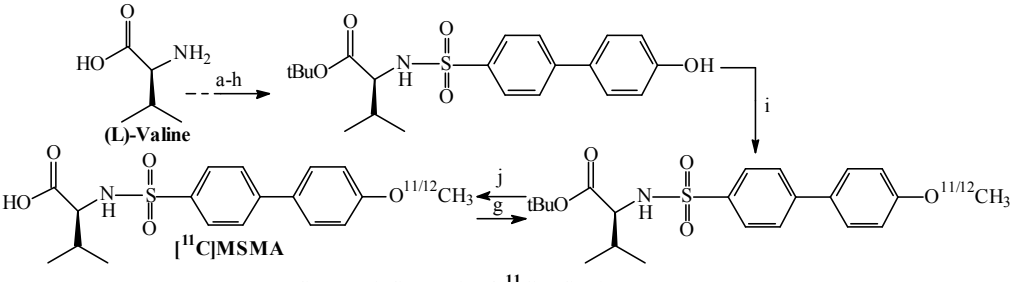
COMPARATIVE STUDIES OF POTENTIAL CANCER BIOMARKERS CARBON-11 LABELLED MMP INHIBITORS [^{11}C]MSMA AND [^{11}C]CGS 25966

Q-H. Zheng[1], X. Fei[1], X. Liu[1], J-Q. Wang[1], K.L. Stone[1], T.D. Martinez[1], D.J. Gay[1], W.L. Baity[1], K.D. Miller[2], G.W. Sledge[2], G.D. Hutchins[1]

Departments of [1]Radiology and [2]Medicine, Indiana University School of Medicine, 950 W. Walnut St., R2 E124, Indianapolis, IN 46202-5181, USA. qzheng@iupui.edu

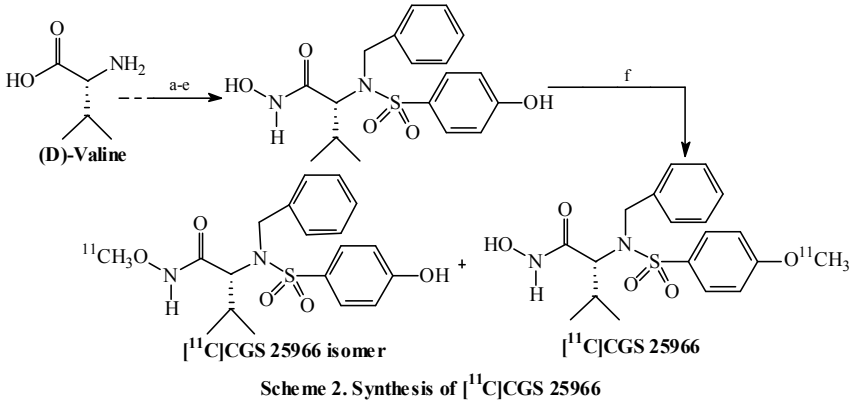
Keywords: Matrix metalloproteinase inhibitor; cancer biomarker; positron emission tomography; [^{11}C]MSMA; [^{11}C]CGS 25966

(S)-2-(4'-[^{11}C]methoxybiphenyl-4-sulfonylamino)-3-methylbutyric acid ([^{11}C]MSMA, **Scheme 1**) and N-hydroxy-(R)-2-[[(4'-[^{11}C]methoxyphenyl)sulfonyl]benzylamino]-3-methylbutanamide ([^{11}C]CGS 25966, **Scheme 2**), carbon-11 labeled matrix metalloproteinase (MMP) inhibitors, have been synthesized for evaluation as new potential positron emission tomography (PET) cancer biomarkers. The biological evaluation of [^{11}C]MSMA and [^{11}C]CGS 25966 including *in vivo* biodistribution and micro-PET imaging was performed in breast cancer animal models MCF-7's transfected with IL-1a implanted athymic mice and MDA-MB-435 implanted athymic mice. The results were compared, which show that [^{11}C]MSMA and [^{11}C]CGS 25966 might be unsuitable as PET tracers for cancer imaging.



Scheme 1. Synthesis of [^{11}C]MSMA

a. $SOCl_2$, MeOH; b. 4-IPhSO$_2$Cl, Et_3N, CH_3CN; c. 4-MeOPhB(OH)$_2$, Pd(0)(PPh$_3$)$_4$, Na_2CO_3, H_2O, C_6H_6; d. $AlCl_3$, EtSH, CH_2Cl_2, 0°C; e. BnBr (1 eq.), K_2CO_3, CH_3CN; f. NaOH, 1:3 H_2O/MeOH; g. $Cl_3CCNHtBu$, CH_2Cl_2; h. H_2, 10% Pd/C; i. $^{11}CH_3OTf$, TBAH, CH_3CN; j. HCl, CH_3CN



Scheme 2. Synthesis of [^{11}C]CGS 25966

a. $SOCl_2$, MeOH; b. 4-MeOPhSO$_2$Cl, iPr_2NEt, CH_3CN; c. BzCl, K_2CO_3, DMF; d. 1) HCl/TFA, reflux, 2) tBuONH$_2$/HCl, EDCI, HOBT, NMM, DCM; e. $AlCl_3$, EtSH, DCM; f. $^{11}CH_3OTf$, TBAH, CH_3CN

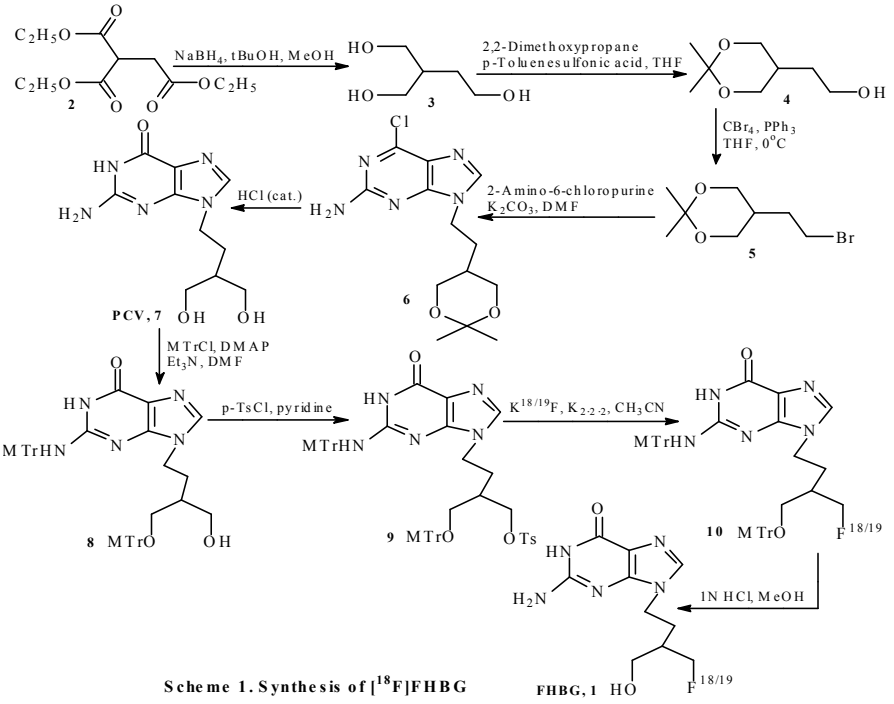
AN IMPROVED TOTAL SYNTHESIS OF [^{18}F]FHBG FOR PET IMAGING OF HSV-TK GENE EXPRESSION

Q-H. Zheng[1], J-Q. Wang[1], X. Liu[1], X. Fei[1], B.H. Mock[1], B.E. Glick-Wilson[1], M.L. Sullivan[1], S.P. Raikwar[2], T.A. Gardner[2], C. Kao[2], G.D. Hutchins[1]

Departments of [1]Radiology and [2]Urology, Indiana University School of Medicine, 950 W. Walnut St., R2 E124, Indianapolis, IN 46202-5181, USA. qzheng@iupui.edu

Keywords: HSV-tk gene; reporter probe; positron emission tomography; [^{18}F]FHBG, total synthesis

Radiolabeled penciclovir (PCV) and ganciclovir (GCV) analogues such as [^{18}F]FPCV, [^{18}F]FHBG; [^{18}F]FGCV, [^{18}F]FHPG have shown great potential as PET imaging agents to detect herpes simplex virus thymidine kinase (HSV-tk) gene expression. Considerable efforts have been devoted to the synthesis of these gene reporter probes and numerous improved syntheses were reported in the literatures, in which [^{18}F]FHBG and [^{18}F]FHPG were labelled with fluorine-18 at the side chain of PCV and GCV. However, the limited commercial availability and high costs of starting materials PCV and GCV can present an obstacle to more widespread evaluation of these intriguing agents. Wishing to study these compounds in this laboratory, we decided to make our own materials by following the literature methods. Here we report an improved total synthesis of [^{18}F]FHBG (**Scheme 1**). 9-[4-Hydroxy-3-(hydroxymethyl)butyl]guanine (PCV) was synthesized in 5 steps, starting from triethyl-1,1,2-ethanetricarboxylate and 2-amino-6-chloropurine. The unlabeled standard sample 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (FHBG) was synthesized from PCV in 4 steps. The tosylated precursor N^2-(*p*-anisyldiphenylmethyl)-9-[(4-tosyl)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine was prepared from PCV in 2 steps for radiolabeling. 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine ([^{18}F]FHBG) was synthesized by nucleophilic substitution of the precursor with potassium [^{18}F]fluoride/Kryptofix 2.2.2 followed by a quick deprotection with 1 N HCl and purification with a simplified Silica Sep-Pak extraction method.



Scheme 1. Synthesis of [^{18}F]FHBG

SYNTHESIS, BIODISTRIBUTION AND MICRO-PET IMAGING OF POTENTIAL CANCER BIOMARKERS RADIOLABELLED ANTIMITOTIC AGENTS [^{11}C]T138067 AND [^{18}F]T138067

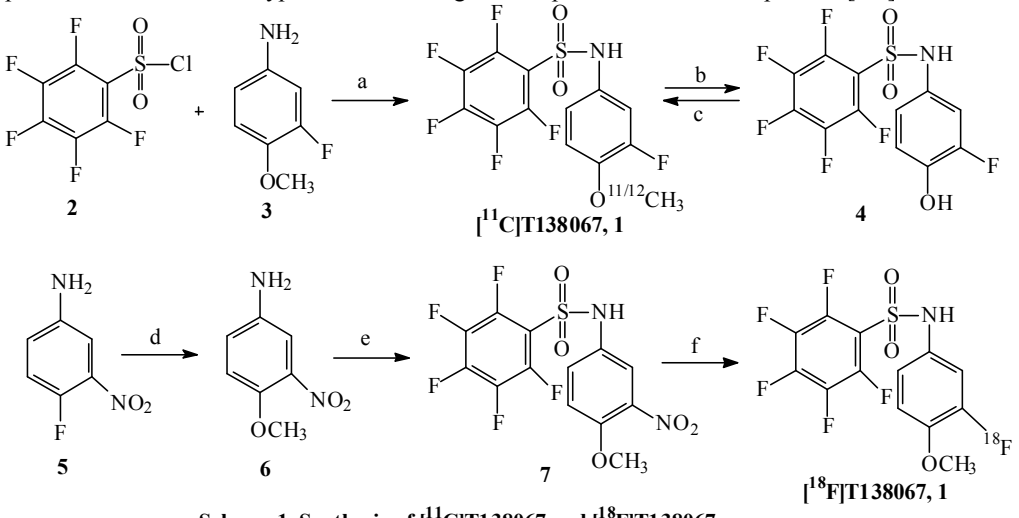
X. Fei[1], Q-H. Zheng[1], J-Q. Wang[1], K.L. Stone[1], T.D. Martinez[1], D.J. Gay[1], W.L. Baity[1], B.H. Mock[1], B.E. Glick-Wilson[1], M.L. Sullivan[1], K.D. Miller[2], G.W. Sledge[2], G.D. Hutchins[1]

Departments of [1]Radiology and [2]Medicine, Indiana University School of Medicine, 950 W. Walnut St., R2 E124, Indianapolis, IN 46202-5181, USA. qzheng@iupui.edu

Keywords: Antimitotic agent; cancer biomarker; positron emission tomography; [^{11}C]T138067; [^{18}F]T138067

Antimitotic drugs have emerged as an effective treatment for a variety of cancers. N-(3-Fluoro-4-methoxy-phenyl)-2,3,4,5,6-pentafluorobenzenesulfonamide (T138067) is a new antimitotic agent, which is currently in phase II clinical trials. N-(3-Fluoro-4-[^{11}C]methoxy-phenyl)-2,3,4,5,6-pentafluorobenzenesulfonamide ([^{11}C]T138067) and N-(3-[^{18}F]fluoro-4-methoxy-phenyl)-2,3,4,5,6-pentafluorobenzenesulfonamide ([^{18}F]T138067) (**Scheme 1**), radiolabelled antimitotic agents, have been synthesized for evaluation as new potential positron emission tomography (PET) cancer biomarkers. The biological evaluation of the tracer including *in vivo* biodistribution and micro-PET imaging was performed in breast cancer animal models MCF-7's transfected with IL-1a implanted athymic mice and MDA-MB-435 implanted athymic mice. The results show that [^{11}C]T138067 and [^{18}F]T138067 might be potential PET tracers for cancer imaging.

Unlabelled T138067 (**1**) was prepared by the reaction of pentafluorobenzenesulfonyl chloride (**2**) with 3-fluoro-4-methoxyaniline (**3**). The desmethylation of **1** gave the precursor N-(3-fluoro-4-hydroxy-phenyl)-2,3,4,5,6-pentafluorobenzenesulfonamide (**4**) for carbon-11 radiolabeling. 3-Nitro-4-fluoroaniline (**5**) was converted to 3-nitro-4-methoxyaniline (**6**), which was reacted with **2** to give the precursor N-(3-nitro-4-methoxy-phenyl)-2,3,4,5,6-pentafluorobenzenesulfonamide (**7**) for fluorine-18 radiolabeling. The phenol precursor **4** was labelled by [^{11}C]methyl triflate through O-[^{11}C]methylation method to provide [^{11}C]T138067. The nitro precursor **5** was labelled by potassium ^{18}F-fluoride/Kryptofix 2.2.2 through nucleophilic substitution to provide [^{18}F]T138067.



Scheme 1. Synthesis of [^{11}C]T138067 and [^{18}F]T138067

a. Et_3N, DCM; b. BBr_3, DCM; c. $^{11}CH_3OTf$, TBAH, CH_3CN;

d. NaOMe, MeOH; e. **2**, Et_3N, DCM; f. $K^{18}F$, $K_{2.2.2}$, CH_3CN

DETECTION OF HERPES SIMPLEX ENCEPHALITIS (HSE) WITH [^{18}F]FHPG PET

A.R. Buursma[1,3], E.F.J. de Vries[1], J. Garssen[2], D. Nanninga[1], D. Kegler[2], G.A.P. Hospers[3], W. Vaalburg[1], H.C. Klein[4]

[1]PET center, Groningen University Hospital, PO-Box 30.001, 9700 RB Groningen, the Netherlands; [2]National Institute for Public Health, Bilthoven; [3]Department of Medical Oncology, Groningen University Hospital, Groningen; [4]Centre for Mental Health Winschoten, Winschoten, the Netherlands.
E-mail: a.r.buursma@pet.azg.nl

Keywords: Herpes Simplex Virus, Encephalitis, Positron Emission Tomography, [^{18}F]FHPG

Herpes Simplex Virus (HSV-1) is a common virus that develops latency in about 90% of the human population. It typically causes infections of the oral mucosa or damaged squamous epithelial surfaces. After the primary infection, it establishes a lifelong persistence in the neural tissue serving the area of primary contact. On rare occasions, HSV-1 disseminates into the brain leading to severe Herpes Simplex Encephalitis (HSE). For effective treatment with antiviral medication early diagnosis of HSE is crucial. Currently, diagnosis is based on invasive and time consuming techniques (viral detection in spinal fluid or biopsy material). In order to facilitate the early diagnosis of HSE, a non-invasive and rapid diagnostic test would be useful. Therefore, we are studying the potential of 9-[(1-[^{18}F]fluoro-3-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG, which is selectively phosphorylated by the HSVtk enzyme) in a HSE rat model.

Rats were infected with HSV-1 by application of 1 x 10^7 pfu of the virus to the olfactory epithelium. Controls received PBS. On day 7 after inoculation, a dynamic PET-scan was acquired for 1 hour after tail injection of 15-20 MBq of [^{18}F]FHPG. In addition, regional FHPG accumulation was determined by ex vivo autoradiography after dissection of the rat brain. The [^{18}F]FHPG uptake was obtained from manually drawn ROI's.

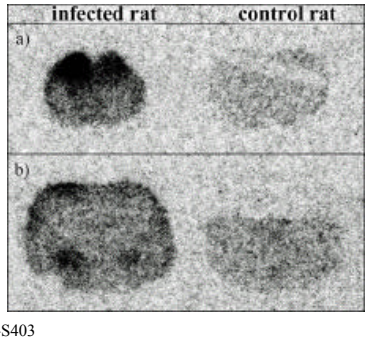
The PET images showed enhanced trapping of [^{18}F]FHPG in the infected group as compared to the control group. After 1 hour tracer uptake was 8.0 ± 2.3 x 10^{-2} percent of the injected dose per gram tissue (% ID/g) in the infected group versus –0.36 ± 1.1 x 10^{-2} % ID/g in controls ($p<0.001$). Phosphor images showed enhanced accumulation of [^{18}F]FHPG in regions known to be infected after nasal injection of HSV: the bilateral nuclei olfactorius, the motor cortex, the somatosensory cortex and the substantia nigra (Table 1, Figure 1). Furthermore, FHPG uptake seems to correlate with clinical symptoms of HSE.

This study shows that HSV can be detected non-invasively in the HSE rat brain with [^{18}F]FHPG PET. This technique could be of great value in the early diagnosis of HSE in humans and other potentially HSV related neuropsychiatric disorders.

Table 1:*[^{18}F]FHPG accumulation in specific brain regions, measured by phosphor storage imaging. Values are expressed as percentage of injected dose per gram tissue (%ID/g).*

	HSV +		Control	
	Average	SD	Average	SD
Olfactorius	0.028	0.022	0.006	0.001
Motor cortex	0.019	0.011	0.008	0.001
Somatosens.cortex	0.016	0.009	0.008	0.003
Subst.nigra	0.016	0.006	0.008	0.003

Figure 1: *[^{18}F]FHPG uptake in HSV infected and control rat brain a) the nuclei olfactorius. b) the cortex and the substantia nigra*



NOVEL EGFR IRREVERSIBLE TYROSINE KINASE INHIBITOR CANDIDATES FOR THE DIAGNOSTIC AND THERAPEUTIC TREATMENT OF CANCER

E. Mishani[1], G. Abourbeh[1], G. Ortu[1], Y. Rozen[1], I. Ben David[1], S. Froimovici[1], S. Dissoki[1], A. Gazit[2], A. Levitzki[2]

[1] Department of Nuclear Medicine, Hadassah Hebrew University Hospital, Jerusalem 91120, Israel.
[2] Department of Biological Chemistry, Hebrew University, Jerusalem 91904, Israel.

Key words: EGFR, PET, cancer

Overexpression of Epidermal Growth Factor Receptor (EGFR) is present in at least 70% of human cancers, and is the hallmark of epithelial human cancers. Thus, EGFR is an attractive target for the design and development of compounds that can specifically bind and inhibit the tyrosine kinase activity and its signal transduction pathway in cancer cells, and serve as either diagnostic or therapeutic agents. As candidates for future labeling with various isotopes, we have synthesized 4 new groups of compounds with different stability/reactivity chemical properties, based on the structure of the acrylamido quinazoline derivative ML03 (our previously labeled EGFR irreversible inhibitor (Ortu et al. 2002). Each group contains 3 compounds to afford for future labeling with various radioisotopes. In order to minimize the metabolic rate of these types of irreversible inhibitors, we reduced the chemical reactivity of the molecule. Modifications have been performed at the 6 position of the quinazoline ring. The highly chemical reactive group, the acrylamido group at the 6 position, was replaced with either a a-methoxy-acetamide (group A), a a-chloro-acetamide (group B) or a 4-dimethylamino-but-2-enoic amide (group C). In these groups, the a carbon or the ß carbon (group C) is partially positively charged and thus sufficiently reactive toward nucleophilic attack performed by the cystein moiety at the receptor binding site. However, since the energy gaps of the HOMO LUMO electronic orbitals of these reactive carbon centers are higher than the one of the ß carbon in the acryloyl group of ML03, we presumed that biological stability and bioavailability will be increased. Thus, if potency will not be dramatically affected and remains at the nano-Molar range, the inhibitor will stay at the receptor binding site long enough in-order to allow covalent bonding. To increase the solubility without affecting the chemical reactivity, the methyl-piperazine group was added at the 7 position of the quinazoline ring and the acryl-amido group at the 6 position was untouched (group D). The new compounds contain elements that allow for future radiolabeling with either C-11, F-18, Iodine isotopes or bromine isotopes. The non-labeled compounds were evaluated in-vitro in order to determine their EGFR autophosphorylation IC_{50} values by means of ELISA. The assays were performed with A431 human epidermoid (vulvar squamous cell) carcinoma cell lysate. The IC_{50}'s indicate that groups B, C and D of inhibitors possess high affinities towards EGFR. Although compounds containing the a-methoxy group (group A) are less potent than ML03, they are still good candidates to serve as drugs and when labeled as PET or SPECT bioprobes. Excluding group A, all compounds retained the irreversible binding nature to the receptor. This was demonstrated by the in-vitro experiments with intact A431 cells. Eight hours post incubation, 80% inhibition was already achieved with an inhibitor (group B) concentration of 20nM. Even with group A, which is a much more chemically stable group, partial irreversible binding was observed, although higher concentrations of inhibitors were needed. The ability of group B to form irreversible binding indicates that a chain of 4 atoms attached to position 6 in the quinazoline moiety is not essential. Structurally, a chain of 3 atoms is sufficient to achieve covalent binding at the receptor-binding pocket. In summary, we have synthesized 12 new irreversible EGFR inhibitor candidates for future labeling with various radioisotopes. These inhibitors maintain the affinity and the irreversible binding to the EGFR, are potentially more chemically stable and have higher bioavailability.

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A 4-METHYL SUBSTITUTED MIBG ANALOGUE

G. Vaidyanathan[1], D.J. Affleck[1], P. Welsh[1], K.C. Peixoto[1], S.A. Snyder[2], H.S. Friedman[3], and M.R. Zalutsky[1]

Departments of [1]Radiology, [2]Radiation Oncology-Cancer Biology, and [3]Pediatric Neuro-Oncology, Duke University Medical Center, Durham, North Carolina, USA 27710. ganesan.v@duke.edu

Key Words: MIBG, 3-Iodo-4-methylbenzylguanidine, Neuroblastoma, SK-N-SH, IMR-32

We have been seeking an MIBG analogue with prolonged tumor retention and lower nonspecific uptake in normal tissues. In previous structure-activity studies, we showed that MIBG generally accommodates introduction of substituents at its 4-position (1). To investigate the effect of a 4-methyl substituent, 3-[^{131}I]iodo-4-methylbenzylguanidine ([^{131}I]MeIBG) was synthesized at a no-carrier-added (n.c.a.) level, evaluated using SK-N-SH human neuroblastoma cells *in vitro*, and in normal mice and two neuroblastoma xenograft models *in vivo*. An unlabeled MeIBG standard was prepared from 3-iodo-4-methylbenzyl alcohol (IMBA) in two steps. To prepare the tin precursor (N^1,N^2-Bis(*tert*-butyloxycarbonyl)-N^1-(4-methyl-3-trimethylstannyl)benzylguanidine; Boc-MTMSBG), IMBA was stannylated, and the resultant derivative was converted to a protected guanidine. Boc-MTMSBG was converted to Boc-[^{131}I]MeIBG in 40% radiochemical yield, and deprotection of Boc-groups gave n.c.a. [^{131}I]MeIBG in 70% radiochemical yield (deprotection step).

The binding of [^{131}I]MeIBG (38.9 ± 3.0% of input counts) to SK-N-SH human neuroblastoma cells *in vitro* was similar to [^{125}I]MIBG (44.5 ± 3.0 %); furthermore, this uptake was blocked by several uptake-1-inhibiting conditions in a similar degree for both tracers. In a paired-label assay, SK-N-SH cells retained [^{131}I]MeIBG to a greater extent than [^{125}I]MIBG. For example, 48 h after initial binding, 62 ± 5% of [^{131}I]MeIBG remained cell associated compared with 31 ± 2% for [^{125}I]MIBG. Uptake of [^{131}I]MeIBG in normal mouse target tissues was similar to that of [^{125}I]MIBG at early time points. For example, the myocardial uptake 1 h post injection was 21.3 ± 2.8% and 19.6 ± 2.2%, respectively, for [^{125}I]MIBG and [^{131}I]MeIBG and mice pretreatment with desipramine reduced these uptakes to 43% and 52%, respectively, of control values. At 24 h the uptake in heart of [^{131}I]MeIBG was almost twice that of [^{125}I]MIBG. Uptake of [^{131}I]MeIBG in liver was 2-3-fold lower than that of [^{125}I]MIBG while its blood clearance was considerably slower. However, at 24 h, [^{131}I]MeIBG demonstrated significantly higher heart to tissue ratios for most tissues except blood.

A paired-label biodistribution was performed in athymic mice with SK-N-SH xenografts at 1 h post injection. The tumor uptake of [^{131}I]MeIBG was 1.5 ± 0.6% compared to 1.9 ± 0.8% ($p <$ 0.05) for [^{125}I]MIBG. Desipramine treatment reduced these levels by 53% and 65%, respectively. As seen in the normal mice, a substantial decrease in liver uptake and substantial increase in blood levels were seen for [^{131}I]MeIBG relative to [^{125}I]MIBG. Another paired-label tissue distribution of the two tracers was performed in mice bearing IMR32 human neuroblastoma xenografts. Both tracers showed maximum tumor uptake at 1 h post injection with [^{131}I]MeIBG having a higher uptake than [^{125}I]MIBG (1.32 ± 0.18 vs. 1.16 ± 0.2 %ID/g; $p < 0.05$). The heart uptake of [^{131}I]MeIBG was similar to that of [^{125}I]MIBG at 1 and 4h; however, it was 1.4- and 1.8-fold higher at 24 h and 48h, respectively. As seen in normal mice and in the SK-N-SH model, higher blood uptake and lower liver uptake of the 4-methyl analogue was also seen in IMR32 xenograft model. This was reflected in higher tumor-to-liver and lower tumor-to-blood ratios for [^{131}I]MeIBG. In conclusion, a 4-methyl substituted MIBG analogue has been developed. This simple modification led to improved tumor cell retention *in vitro* and lower hepatic uptake *in vivo*; however, slower blood clearance could be problematic.

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SYNTHESIS OF 2'-, 3'- AND 4'-[^{18}F]FLUOROANILINOQUINAZOLINE

P.N. Dorff, N. Vasdev, J.P. O'Neil, E. Nandanan, A.R. Gibbs and H.F. VanBrocklin*

Department of Nuclear Medicine and Functional Imaging, Lawrence Berkeley National Laboratory, Berkeley, California, 94720. *E-mail: HFVanBrocklin@lbl.gov

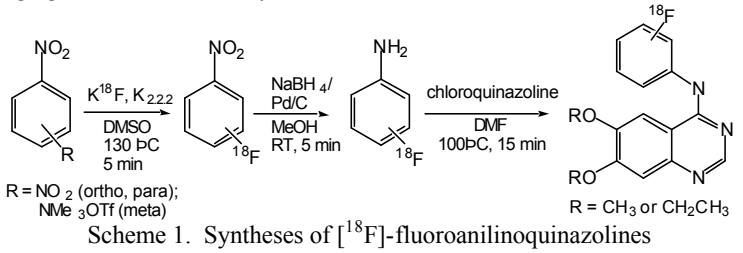
Keywords: fluorine-18, fluoronitrobenzene, fluoroaniline, quinazoline, EGFR

A variety of tumors overexpress epidermal growth factor receptors (EGFRs). For this reason EGFRs are important diagnostic biomarkers as well as therapeutic targets. The goal of the present study is to further develop fluorine-18 labelled 4-anilinoquinazoline analogs, known EGFR tyrosine kinase (tk) inhibitors, as tumor imaging agents.

The 2'-, 3'- and 4'-fluoroanilino-dialkoxyquinazolines were synthesized and shown to have high binding affinity for the EGFR tk domain.(1) Further, the methodology was developed for the preparation of the corresponding fluorine-18 compounds. All of the analogs were produced by reaction of 2-, 3-, or 4-[^{18}F]fluoroaniline (2) with the 4-chloro-dimethoxyquinazoline (Scheme 1).

Fluorine-18 labelled fluoronitrobenzenes were prepared from the corresponding *o*- and *p*-dinitrobenzenes and the *m*- from the 3-trimethylammonium-nitrobenzene triflate.(2) Decay-corrected radiochemical yields (RCY; relative to $^{18}F^-$) of fluoride incorporation onto the nitro-aromatic compounds were 81 ± 2%, 77 ± 5% and 44 ± 4% (N = 3-5) for the *o*-, *p*- and *m*-isomers, respectively. The lower RCY of 3-[^{18}F]fluoronitrobenzene was attributed to the competing reaction of fluoride with one of the methyl groups on the trimethylammonium moiety that produces volatile $CH_3{}^{18}F$. Reduction to the [^{18}F]fluoroanilines was achieved in 5 min using a modification of Feliu's procedure.(3) Following HCl quench the methanol solution was evaporated to isolate the [^{18}F]fluoroanilines. Subsequent coupling with 4-chloro-6,7-dimethoxyquinazoline gave, after HPLC purification, the *o*-, *p*- and *m*-[^{18}F]fluoroanilinoquinazolines in 31 ± 5%, 55 ± 2% and 17 ± 2% RCY, respectively. The average total synthesis time was 90 min.

An efficient synthesis of the 2-, 3-, and 4-[^{18}F]fluoroanilines with subsequent coupling to a haloquinazoline has been described with yields exceeding those previously reported by Mishani et al. for the 3'- and 4'-[^{18}F]fluoroanilino-dimethoxyquinazolines.(4) The methodology presented here will be used to develop a novel series of [^{18}F]fluoroanilino-6-acrylimidoquinazoline analogs, irreversible EGFR tk inhibitors, as well as [^{18}F]fluorohaloanilino-quinazoline analogs in search of optimal imaging agents for the EGFR system.



Scheme 1. Syntheses of [^{18}F]-fluoroanilinoquinazolines

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SYNTHESIS, RADIOLABELLING AND BIOLOGICAL EVALUATION OF DERIVATIVES OF THE P210$^{BCR-ABL}$ TYROSINE KINASE INHIBITOR AG957

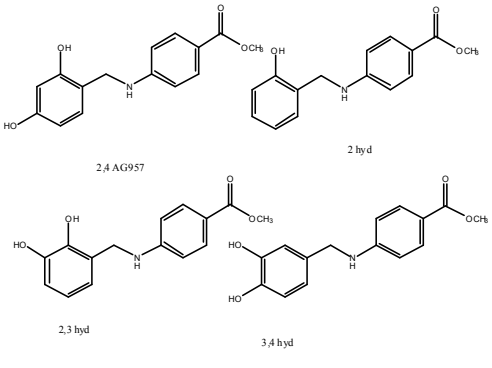
U. Ackermann[1], H.J. Tochon-Danguy[1], E. Nice[2], M. Nerrie[2], K. Young[1], J. Sachinidis[1], A.M. Scott[1,3]

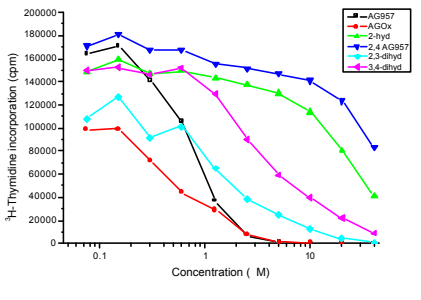
[1] Austin & Repatriation Medical Centre, Centre for PET, Studley Road, Heidelberg, VIC 3084, Australia
email: uacker@austin.unimel.edu.au
[2] Ludwig Institute for Cancer Research, PO Royal Melbourne Hospital, VIC 3050, Australia
[3] Ludwig Institute for Cancer Research, A&RMC, Studley Road, Heidelberg, VIC 3084, Australia

Keywords: Tyrphostins; AG957, kinase inhibitor, chronic myeloid leukaemia (CML); carbon-11

Tyrosine kinases are enzymes that play a crucial role in the complex mechanism of cell signalling. They mediate their effects through the phosphorylation/dephosphorylation of tyrosine residues on target proteins. Aberrant enhanced signalling emanating from tyrosine kinases results in the malfunction of cellular networks and as a consequence cancer and other diseases develop. One of those enzymes, the abnormal tyrosine kinase p210$^{bcr-abl}$ is widely regarded as the hallmark of CML, since it is found in more than 90% of all CML patients. Small molecules that inhibit p210$^{bcr-abl}$ are of potential use as anti-CML drugs and also as radioactive tracers for PET. One of those molecules, AG957, has been radiolabelled by us with the PET radioisotope carbon-11. Stability studies have shown that AG957 undergoes oxidation and readily forms its respective quinone. Biodistribution studies have also revealed that clearance of the tracer from the bloodstream is slow, which limits the use of [^{11}C]-AG957 as a radiotracer for PET.

We have therefore directed our efforts towards synthesising derivatives of AG957, which are more stable towards oxidation than the parent molecule itself, whilst retaining their biological activity. We have synthesised a set of 4 new compounds and tested for their inhibitory effect against p210$^{bcr-abl}$.





Screening of the cold compounds on FDrv210c cells has revealed, that the potential to form either the o- or p-quinone is crucial to retaining biological activity. Derivatives such as 2,4AG957 and 2 hyd, which cannot undergo oxidation readily do not show inhibition of the tyrosine kinase. This unexpected result suggests that the oxidised form may play an important role in the biological activity or may even represent the biologically active form of those tyrphostins. We have also labelled those novel inhibitors with C-11 according to the versatile procedure that was developed by us (1) and we are planning to investigate the biodistribution of those inhibitors in normal and in tumour bearing mice.

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SYNTHESIS AND RADIOLABELLING OF A TYROSINE KINASE INHIBITOR OF erbB2/neu (HER2) RECEPTORS

N. Kesarios[1,2], H.J. Tochon-Danguy[1,2], U. Ackermann[1], J. Sachinidis[1], J. Lambert[2], M. Nerrie[4], E. Nice[4], A.M. Scott[1,3]

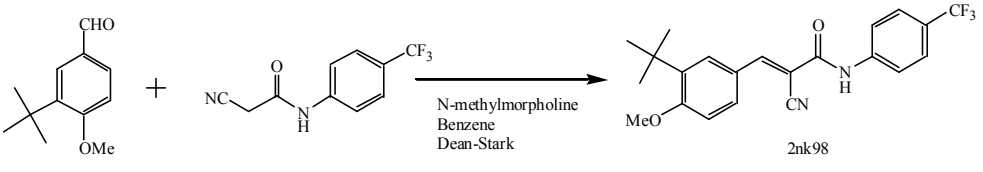
[1] Centre for PET, Austin & Repatriation Medical Centre, Studley Rd, Melbourne 3084 VIC Australia
email: htd@austin.unimelb.edu.au
[2] School of Chemistry, University of Melbourne, Parkville 3010 VIC Australia.
[3] Ludwig Institute for Cancer Research, ARMC, Studley Rd, Melbourne 3084 VIC Australia
[4] Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville 3010 VIC Australia

Keywords: PET, cancer, tyrosine kinase inhibitor, EGFR, HER2

Epidermal growth factor receptors (EGFR) are over expressed in a number of common cancers. Numerous studies have demonstrated the prognostic relevance of the erbB2 receptor, which is over expressed in 30% of human breast tumors. A suitable radiotracer directed to the erbB2/EGFR pathway would be a predictive marker for responses to various therapeutic agents. Unfortunately, to date there has been no successful radiolabelling of a compound that is an inhibitor of the erbB2 receptor.

The aim of this study was to synthesize and radiolabel for PET, a tracer specific for erbB2 receptors. Our research has looked at the synthesis of a series of compounds based on the backbone structure of AG1793, a known protein tyrosine kinase inhibitor which binds to HER2. All compounds have been tested against an EGFR inhibition assay specific for the erbB2 signalling pathway. The compounds screened showed either some level of inhibition to the erbB2 receptors, no inhibition at all, or a distinct toxic effect on the cells. The best identified compound (2nk107) showed a distinct inhibition at concentrations above 1μM, although a slight cell toxicity effect was observed for concentrations above 5μM. Radiolabelling of the compound was performed by N-methylation of the desmethyl precursor (2nk98) using [^{11}C]methyl iodide.

Synthesis of 2nk98 involved a four step reaction. First, a Vilsmeier reaction of 2-tert-butylphenol to form 3-tert-butyl-4-hydroxybenzaldehyde, followed by methylation of the hydroxyl group with dimethylsulphate and sodium hydroxide. N-[4-(trifluoromethyl)phenyl] cyanoacetamide was then synthesized by reacting trifluoromethylaniline with cyanoacetic acid. A condensation reaction of the aldehyde and cyanoacetamide, in the presence of N-methylmorpholine gave 2nk98 in 80% yield.



2nk98

Radiolabelling of the compound 2nk98 was achieved by dissolving the precursor in a mixture of potassium tert-butoxide/Kryptofix® 222, in acetonitrile. The mixture was stirred for about 5 minutes, the solvent evaporated and dried under vacuum. The complex was redissolved in dry dimethylformamide (DMF) then treated with [^{11}C]methyl iodide and heated for 5 minutes at 70°C. HPLC purification of the labelled crude product gave pure [^{11}C]2nk107 with a radiochemical yield of 25%. The biodistribution of [^{11}C]2nk107 in healthy and tumor-bearing mice is currently under evaluation. We are also pursuing an alternative route for the radiolabelling of this tyrosine kinase analogue using fluorine-18 to overcome the relatively short half-life of carbon-11.

SELECTION OF A SUITABLE THYMIDINE ANALOGUE FOR STUDY OF TUMOUR PROLIFERATION

W. Issa[1,2], H J. Tochon-Danguy[1], J. Sachinidis[1], U. Ackermann[1], F.T. Lee[3] and A. M. Scott.[1,3]

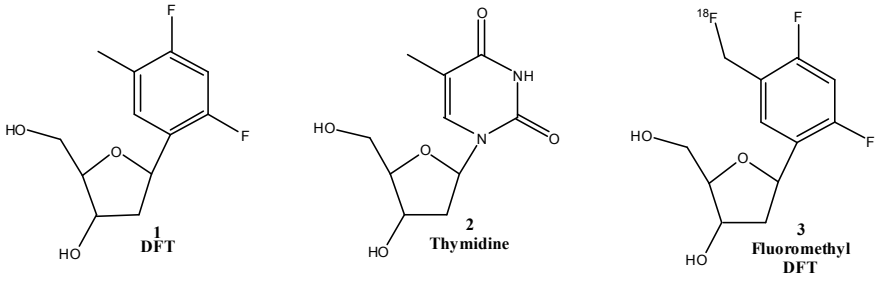
[1]Centre for PET, A&RMC Studley Rd, Melbourne 3084 VIC Australia
[2]School of Chemis try. The University of Melbourne. Parkville, 3010 VIC Australia
w.issa@pgrad.unimelb.edu.au
[3]Ludwig Institute for Cancer Research, A&RMC, Melbourne 3084 VIC Australia

Keywords: Fluorine-18, Nucleoside, DNA Proliferation, PET

The careful selection of known chemical analogues of biological molecules has provided a number of clinically valuable radiotracers for nuclear medicine. In an effort to develop a novel nucleoside based imaging agent to image DNA proliferation we have chosen to label an analogue of thymidine. First described by Kool and co-workers, difluorotoluene nucleoside **1** (DFT, log P=1.715) (1) was designed as a non-polar shape mimic for natural thymidine, **2** (log P=-0.817) (1). It has been shown that it is isosteric with thymidine as it is readily incorporated into DNA by the Klenow fragment of DNA polymerase and codes specifically and efficiently for adenine in DNA replication (2). With the expectation that DFT would display greater *in-vivo* stability than 'native' nucleosides we proposed that a derivative of DFT, fluoromethyl DFT **3**, incorporating the [^{18}F] isotope, would potentially have utility in clinical oncology.

The radiolabelling of **3** has been previously reported by our group, with the radiochemical yield being over 30% at EOS after HPLC purification. This compound showed excellent chemical stability *in vitro* when incubated in saline or human blood serum at physiological temperatures (3). Unfortunately, further analysis showed that compound **3** was being defluorinated *in vivo.* A final *in vivo* study involving BALB mice bearing SW-1222 tumour xenografts was undertaken to access the uptake of **3** into rapidly proliferating tissue. The results of this study clearly indicated that the proposed mechanism of uptake (increased rate of cell proliferation) could not compete with the rate of defluorination. Finally, in an effort to understand if more stable labelled analogues of **3** were likely to be more successful, the ability of **3** to be incorporated into DNA by cellular processes was assessed by a series of *in vitro* cell studies. These studies clearly showed that compound **3** was not able to pass beyond the cell membrane due to its physiochemical characteristics.

In the light of these results we propose that the physiochemical properties of known analogues be evaluated for their suitability prior to undertaking a long and complex chemical synthesis. We are currently investigating different analogues of thymidine using this ideology.



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SYNTHESIS AND EVALUATION OF [^{123}I]IODOEMODIN AS A POTENTIAL CANCER IMAGING AGENT

J.H. Park[1], S.D. Yang[1], S.W. Kim[1,2], M.G. Hur[1], T.S. Lee[1], H.D. Sung[1], K.S. Chun[1], C.W. Choi[1], K.H. Yu[2]

[1]Laboratory of Cyclotron Application, Department of Nuclear Medicine, Korea Institute of Radiological and Medical Science, Seoul 139-706, Korea, Seoul 139-240, Korea
Department of Chemistry, Dongguk University, Seoul 100-715, Korea[2] (alchem9333@hotmail.com)

Keywords: Tyrosine protein kinase, Emodin, Inhibitor, Tumor imaging

Objectives : Protein Tyrosine Kinases (PTKs) play important roles in signal conduction pathways that control the prolification and differentiation due to phosphorylation from ATP to the hydroxy of tyrosine. The number of the proto-oncogenes is PTK which has been frequently observed in tumors like glioma, breast cancer. Emodin (3-methyl-1,6,8-trihydroxyanthraquinone), a tyrosine kinase inhibitor, has been known that suppresses the PTKs encoded from proto-oncogene. In order to visualise a different kinds of cancers related with PTKs, [^{123}I]iodoemodin has been prepared and evaluated. **Methods** : The [^{123}I]iodoemodin was prepared by the reaction of emodin with sodium [^{123}I]iodide in the presence of peracetic acid.(Fig. 1.) 150 µl of an emodin solution in ethanol (3 mg/ml) was added to a reaction vial containing [^{123}I]NaI at pH 12, followed by 25 µl 0.5 M H_3PO_4, 50 µl peracetic acid and 50 µl ethanol. The reaction progress was monitored by radio-TLC for 30min. The reaction was completed in 5min. The unreacted $^{123}I^-$ was removed by C_{18} Sep-Pak. The [^{123}I]iodoemodin was separated and collected by HPLC. The *in vitro* study of the [^{123}I]iodoemodin was measured on glioma(9L), colon adenocarcinoma(LS174T) and breast cancer(MCF7) at 10min, 30min, 60min and 120min, respectively. **Results** : To confirm the structure, the similar reaction was performed using stable iodide. The iodoemodin has the same retention time as [^{123}I]iodoemodin. The radiochemical yield was about 62% and the radiochemical purity was over 95% after purification. The maximum celluar uptake of the [^{123}I]iodoemodin were about 0.5% at 120min for 9L and 0.45% at 120min for LS174T and 0.45% at 60min for MCF7. **Conclusion :** As a result of the cellular uptake of [^{123}I]iodoemodin, for 9L, the radioactivity was increased as a function of time. This result is given in Fig. 2. For LST174T, [^{123}I]iodoemodin showed no correlation. For MCF7, it reached maximum value at about 60min. These results suggest the possibility to use [^{123}I]iodoemodin for imaging agent for the diagnosis of cancer like a glioma. Further evaluation of [^{123}I]iodoemodin is in progress *in vivo* and *in vitro* with various cancers related with PTKs.

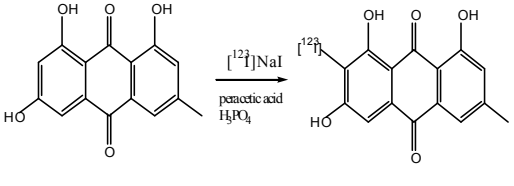


Fig 1. The preparation of [^{123}I]iodoemodin

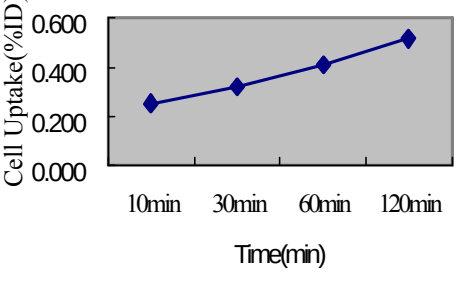


Fig 2. The results of the cellular uptake of [^{123}I]iodoemodin with 9L

NEW SYNTHETIC METHOD FOR [^{18}F]-3'-DEOXY-3'-FLUOROTHYMIDINE USING A NOSYLATE PRECURSOR IN IONIC LIQUID ([bmim][OTf])

B.S. Moon,[1,*] A.Y. Shim,[1] D.W. Kim,[2] S.W. Kim,[1] S.D. Yang,[1] D.Y. Chi[2]

[1]Laboratory of Cyclotron Application, Department of Nuclear Medicine, Korea Institute of Radiological and Medical Science, Seoul 139-706, Korea, [2]Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Korea. E-mail: bsmoon@kcch.re.kr

Keywords: FLT, Ionic liquid, Tumor imaging, ^{18}F, PET

[^{11}C]Thymidine has been used for the diagnosis of tumor or measurement of tumor's multiplication rate using PET. As [^{11}C]thymidine has some limitations to track metabolism due to rapid *in vivo* degradation and short half-life of C-11, recently 3′-[^{18}F]fluoro-3′-deoxythymidine ([^{18}F]FLT) has been developed. However, synthesis of [^{18}F]FLT gives low radiochemical yield when less than 30 mg of precursor is used.

Recently, we have developed a new method for fluorine-18 labeling using an ionic liquid as a reaction medium. Nucleophilic [^{18}F]fluorination of mesyloxyalkanes with fluoride-18 obtained from an ^{18}O(p,n)^{18}F reaction provides the corresponding [^{18}F]fluoroalkanes in excellent yields [1,2]. This new method has been applied to improve radiochemical yield of [^{18}F]FLT and to reduce the amount of nosylated precursor **1**. Optimization of labeling was performed with varying amount of precursor, kinds and equivalents of base, and amount of ionic liquid ([bmim][OTf]). The optimized labeling conditions are as follow: 5 mg of nosylate precursor **1**, 200 L of acetonitrile, 200 L of [bmim][OTf], 50 L of F^- in H_2O and 5 L of 1 M $KHCO_3$ at 120 °C for 15 min. The reaction solvent was removed with silica gel cartridge and hydrolyzed with 0.5 N HCl (300 L) at 110 °C for 5 min. Labeling yield was 61.5 ± 5% with 5 mg of precursor. Total elapsed time was about 80 min including HPLC purification. Overall radiochemical yield and purity were 30 ± 5% and >95%, respectively. We effectively decrease the amount of nosylated precursor and the method can be adapted to routine production of [^{18}F]FLT.

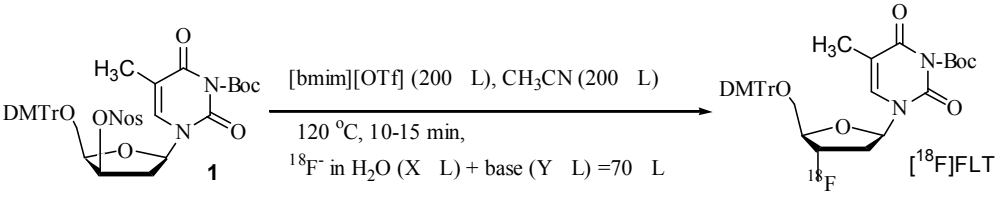


Table 1. Labeling Yields When 10 mg of **1** Is Used.

amount of carbonate	1 M $KHCO_3$	1 M Cs_2CO_3	1 M K_2CO_3
2.5 L	20.4%	34.1%	17.7%
5.0 L	**52.4%**	43.0%	33.1%
10.0 L	46.6%	18.6%	34.4%
15.0 L	34.4%	13.4%	40.1%
20.0 L	N.D.	N.D.	**51.4%**
25.0 L	N.D.	N.D.	38.4%

Table 2. Labeling Yields with various amount of **1**

1 (mg)	1 M $KHCO_3$ (5 L)	1 M K_2CO_3 (20 L)
5	**61.5%**	29.2%
10	**52.4%**	51.4%
15	24.1%	55.0%
20	20.8%	25.6%

1. Kim DW, Song CE, Chi DY. *J Am Chem Soc* 2002;124:10278-10279.
2. Kim DW, Choe YS, Chi DY. *Nucl Med Biol* 2003;30:in print

SYNTHESIS OF 5-(4-[^{18}F]FLUOROPHENYL)-10,15,20-TRIS(3-METHOXYPHENYL)PORPHYRIN AS A POTENTIAL IMAGING AGENT FOR TUMOR.

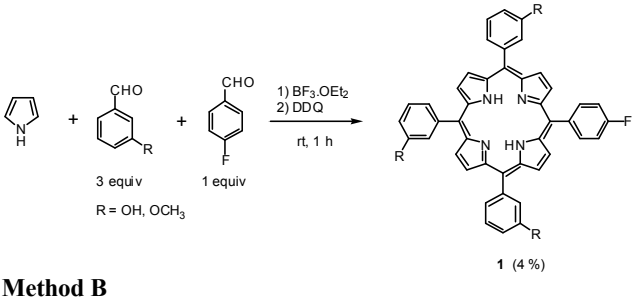
D.Y. Chi[1,*], R.R. Kavali[1], B.S. Moon[2], S.D. Yang[2], C.H. Lee[3], B.C. Lee[1]

[1]Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Korea, [2]Laboratory of Cyclotron Application, Department of Nuclear Medicine, Korea Institute of Radiological and Medical Science, Seoul 139-706, Korea, [3]Department of Chemistry, Kangwon University, 192-1 Hyoja-2-dong, Choonchon 200-701, Korea. E-mail: dychi@inha.ac.kr

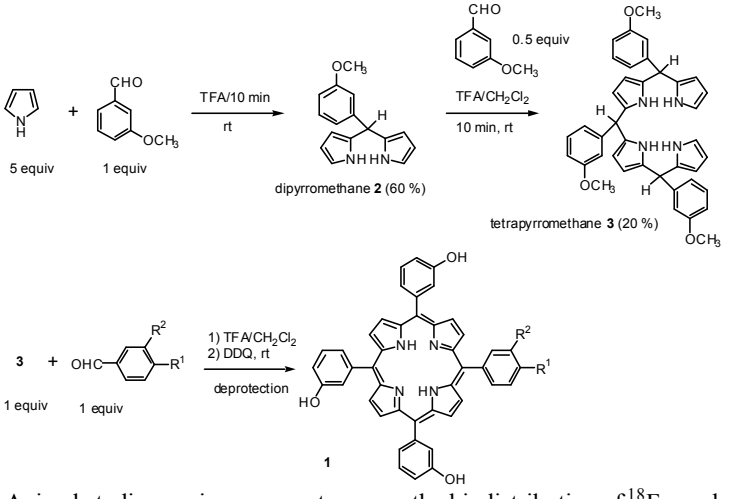
Keywords: PET, Porphyrin, ^{18}F, Tumor imaging

Therapeutic improvements are continually being made but there has been no substantial improvement in survival in some of the major cancers and the need to understand the scientific basis of the disease and develop new treatments therefore remains unabated. Photofrin is a very useful agent in photodynamic therapy (PDT) and has currently been approved for general use by licensing authorities. Recently, mesotetra(3-hydroxyphenyl)porphyrin, which is in phase III trials for brain tumor treatment, has been shown best tumor localizer and also showed a favorable tissue distribution. We have designed the synthetic routes for title compound (**1**) labeled with ^{18}F as a potential imaging agent for tumor. Synthesis of compound (**1**) has been achieved by two methods – direct synthesis (Method A) and stepwise synthesis (Method B). The comparison of two methods will be discussed. In addition, the preparation of the other target compounds such as [^{123}I]iodo or [^{18}F]fluoroalkyl groups in R^1 and OH or [^{18}F]fluoroalkoxy groups in R^2 are in progress.

Method A



Method B



Animal studies are in progress to assess the biodistribution of ^{18}F porphyrin (**1**).

SYNTHESIS OF *O*-METHYL[3-(3-[^{18}F]FLUOROPROPYL)TYROSINE (*O*-MFPT) AND BIOLOGICAL EVALUATION IN 9L GLIOMA CELL

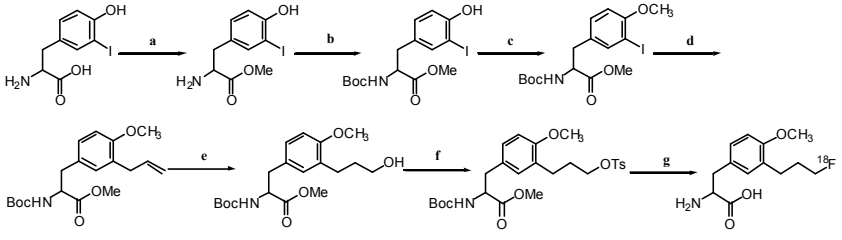
B. S. Moon,[1,*] A.Y. Shim,[1] T.S. Lee,[1] S.W. Kim,[1] D.Y. Chi,[2] S.D. Yang,[1]

[1]Laboratory of Cyclotron Application, Department of Nuclear Medicine, Korea Institute of Radiological and Medical Science, Seoul 139-706, Korea, [2]Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Korea. E-mail: bsmoon@kcch.re.kr

Keywords: Amino Acid, ^{18}F, Tumor imaging, *O*-MFPT, PET

Radiolabeled tyrosine could be used clinically in oncololy, neurology and psychiatric disorders. In many studies, various radiolabeled tyrosine derivatives have been synthesized and proven to be useful for tumor imaging agent. We hereby report the synthesis of tyrosine derivative, *O*-methyl[3-(3-[^{18}F]fluoropropyl)tyrosine. The target material has been designed based on the reported biological data of radiolabeled tyrosine derivatives. Target tyrosine derivative was prepared in 7 steps from a commercially available 3-iodotyrosine (Scheme 1). Labeling reaction was carried out by NCA nucleophilic substitution with K[^{18}F]F/K2,2,2 in acetonitrile and deprotections were achieved by hydrolysis with 4 N HCl. After hydrolysis, [^{18}F]*O*-MFPT was purified by HPLC and the collected fraction was passed through cation exchange resin for further purification. Biological properties were evaluated in 9L glioma tumor-bearing rats (n=4) after intravenous injection. The radiochemical yield of the final product, [^{18}F]*O*-MFPT, was 45% and >95% of radiochemical purity. The biodistribution and PET image of tumor bearing rat are given in Figure 1 and Figure 2. PET image of rat with 9L glioma showed localized accumulation. These results suggest the possibility to use L-[^{18}F]*O*-MFPT as a tumor imaging agent.

Scheme 1.



a) TMSCl, MeOH, rt, 24 h; b) $(Boc)_2O$, TEA, MeOH, rt, 2 h; c) NaH, MOMCl, THF, 0 °C to 70 °C, 1 h; d) allyl bromide, $Pd(PPh_3)_4$, 1,4-dioxane, 100 °C, 1 h; e) BH_3-THF complex, 4 N NaOH, 30% H_2O_2, THF, 0 °C, 2 h; f) TsCl, DMAP, CH_2Cl_2, rt, 2 h; f) i. Kryptofix 2.2.2., K_2CO_3, 110 °C, 25 min, ii. 4 N HCl.

Figure 1. Biodistribution

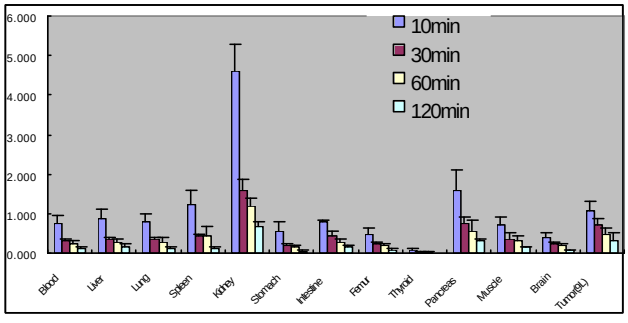
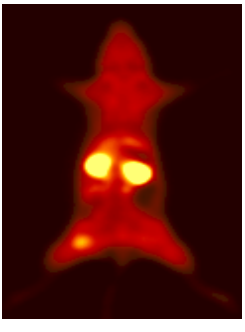


Figure 2. PET image (60 min)



SYNTHESIS OF A ^{18}F-LABELED RALOXIFENE DERIVATIVE

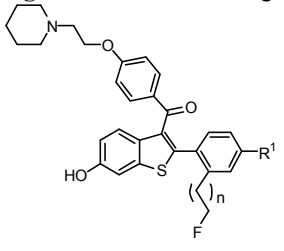
J.H. Lee,[1] B.S. Moon,[1] K.C. Lee,[2] J.A. Katzenellenbogen,[2,*] K.-H. Chung,[1] D.Y. Chi[1,*]

[1]Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Korea,
[2]Department of Chemistry, University of Illinois, Urbana, IL 61801, USA (dychi@inha.ac.kr)

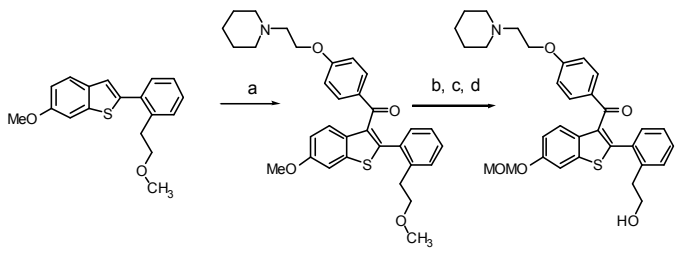
Keywords: Raloxifene, SERM, Estrogen receptor, ^{18}F, Tumor imaging

Raloxifene, [6-hydroxy-2-(4-hydroxy-phenyl)benzo[*b*]thiophen-3-yl]-[4-(2-piperidin-1-yl-ethoxy)phenyl]methanone, a tissue-selective estrogen mixed agonist/antagonist, classified as a selective estrogen receptor modulator (SERM), is currently under clinical evaluation for the prevention and treatment of postmenopausal osteoporosis, breast cancer, uterine dysfunction, and other disorders of the female reproductive system.[1,2] Based on SAR studies of substituted raloxifenes, we synthesized compounds **1**, **2**, and **3**, as shown in Figure 1. These fluoroalkylated raloxifene derivatives, modified on the phenyl ring substituent at the C2 position of benzothiophene, were prepared by a Suzuki coupling-Friedel-Crafts acylation sequence. The compounds **1**, **2**, and **3** show high relative binding affinities (RBA) to the estrogen receptor in vitro (RBA of estradiol = 100%). The RBA values for compounds **1, 2** and **3** are 89, 60, and 45%, respectively, which are all higher than that of raloxifene itself (34%).

Figure 1. Relative Binding Affinity (RBA) data of raloxifene derivatives.



1, R^1 = H, n = 1; RBA = 89 %
2, R^1 = H, n = 2; RBA = 60 %
3, R^1 = OH, n = 1; RBA = 45 %



Scheme 1. Reagent: (a) acid chloride, $AlCl_3$, CH_2Cl_2, 0 °C, 1 h; (b) EtSH, $AlCl_3$, CH_2Cl_2, 0 °C, 2 h; (c) BBr_3, CH_2Cl_2, 0 °C, 1 h; (d) MOMCl, NaH, THF, 0 °C-rt.

When the alkyl group was introduced at the ortho position of the phenyl ring at the C2 position of benzothiophene moiety, the steric bulk of the protected hydroxyalkyl group tended to interfere with Friedel-Craft acylation at the C3 position of benzothiophene moiety. We found that MOM and methyl groups were best for protecting the phenol and hydroxyalkyl side chain, and using these protective groups, we could successfully prepare the key precursor compound **1**. Compared to the other derivatives, the fluoroethyl compound **1** has the highest RBA value for the estrogen receptor. Hence, compound **1**, labeled with fluorine-18, has interesting potential as a PET imaging agent for estrogen receptor-positive tumors. The development of methods for labeling compound **2** with fluorine-18 and in vivo tissue distribution studies are in process.

1. Grese TA, Cho S, Finley DR, Godfrey AG, Jones CD, Lugar III CW, Martin MJ, Matsumoto K, Pennington LD, Winter MA, Adrian MD, Cole HW, Magee DE, Phillips DL, Rowley, ER, Short LL, Glasebrook AL, Bryant HU *J Med Chem* 1997;40:146-167.
2. Grese TA, Pennington LD, Sluka JP, Adrian MD, Cole HW, Fuson TR, Magee DE, Phillips D,L, Rowley ER, Shetler PK, Short LL, Venugopalan M, Yang NN, Sato M, Glasebrook AL, Bryant HU *J Med Chem* 1998;41:1272-1283.

SYNTHESIS OF FLUORINE-18 LABELED GLUCOSAMINO-(4-FLUOROBENZYL)-Asp-Lys-Arg-Gly-Asp-D-Phe AS A POTENTIAL TUMOR IMAGING AGENT

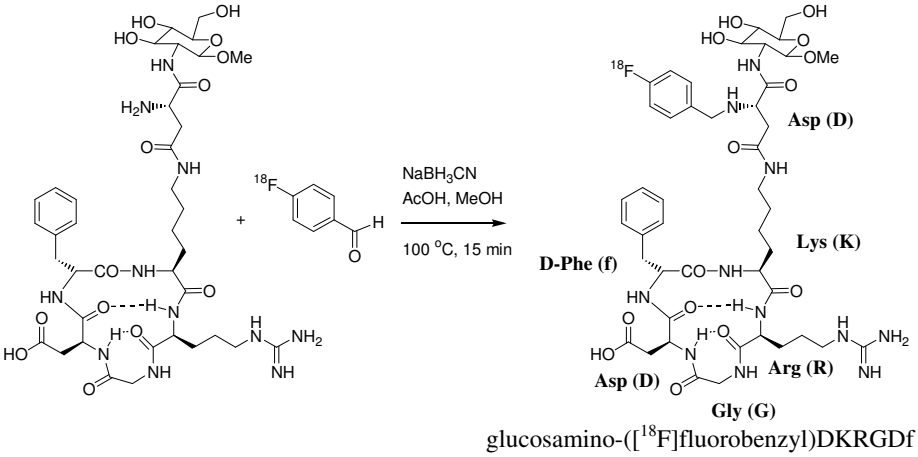
H.J. Sung,[1] B.C. Lee,[1] S.H. Song,[2] S.Y. Lee,[2] Y.S. Choe,[2] K.-H. Lee,[2] B.-T. Kim,[2] D.Y. Chi,[1]

[1]Department of Chemistry, Inha University, 253 Yonghyundong Namgu, Inchon 402-751, Korea,
[2]Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-ku, Seoul 135-230 Korea. E-mail: dychi@inha.ac.kr

Keywords: $\alpha_v\beta_3$ Integrin, Integrin binding peptide, RGD, ^{18}F, Tumor imaging

Our interest in developing new radiopharmaceuticals for in vivo visualization of angiogenesis has led us to synthesize derivatives of RGD (arginine-glycine-aspartic acid) that is known as an antagonist of $\alpha_v\beta_3$ integrins. $\alpha_v\beta_3$ Integrins is a dimeric transmembrane glycopeptide acceptor and plays as an important cell adhesion receptor involved in tumor metastasis and tumor-induced angiogenesis. As the $\alpha_v\beta_3$ integrin is expressed on proliferating endothelial cells as well as on tumor cells of various origin, tumor-induced angiogenesis could be blocked by antagonizing the $\alpha_v\beta_3$ integrins with RGD. Haubner *et al*. reported synthesis and evaluation of [^{18}F]galacto-RGD prepared by ^{18}F-labeling of RGD-containing glycopeptide with 2-[^{18}F]fluoropropionate as a prosthetic group [1].

We designed a fluorine-18 labeled RGD glycopeptide – glucosamino-*N*-fluorobenzyl-Asp-Lys-Arg-Gly-Asp-D-Phe (glucosamino-([^{18}F]fluorobenzyl)DKRGDf **1**) as a diagnostic tumor imaging agent for positron emission tomography (PET). The target peptide was prepared using a solid support coupling protocol. The precursor was obtained by deprotection of tri-*O*-benzylglucosamino-*N*-Fmoc-Asp-Lys-(*N*-pbf)-Arg-Gly-(*O*-*t*-butyl)Asp-D-Phe. Fluorine-18 labeling of the precursor underwent smoothly by reductive alkylation of 4-[^{18}F]fluorobenzaldehyde, which was obtained from the nucleophilic aromatic substitution of (4-formylphenyl)trimethylammonium triflate by [^{18}F]fluoride ion in DMSO at 90 ^{o}C within 5 min (90% yield). Glucosamino-([^{18}F]fluorobenzyl)DKRGDf was prepared in the presence of $NaBH_3CN$, AcOH and MeOH at 100 ^{o}C in 20-32% radiochemical yields and then purified by HPLC at a flow rate of 2 mL/min (0-50% CH_3CN/0.1% TFA in H_2O, 50 min). The desired fraction eluted at 36-37 min was collected and matched with cold compound. The radiolabeling conditions of the precursor are currently being optimized, and in vitro and vivo studies are in progress.



glucosamino-([^{18}F]fluorobenzyl)DKRGDf **1**

1. Haubner R, Wester H-J, Weber WA, Mang C, Zigler SI, Goodman SL, Senekowitsch-Schmidtke R, Kessler H, Schwaiger M. *Cancer Res* 2001;61:1781-1785.

INVESTIGATION OF PET NUCLEOSIDE ANALOGUES IN TUMOUR CELL LINES

J.M. Gillies[1], N. Smith[1], G. Vaidyanthan[2], J. Bailey[1], J. Zweit[1]

[1]CRUK/UMIST Radiochemical Targeting and Imaging Group, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, M20 4BX, UK., jgillies@picr.man.ac.uk & [2]Department of Radiology, Duke University Medical Center, Durham, NC, 27710, USA

PET, Nucleosides, Enzyme Substrates, Proliferation

Thymidine analogues are currently being investigated for a number of PET applications including imaging of cell proliferation and gene expression. Within the context of cell proliferation, the motivation for this is to develop an analogue that could be used without the added complications of extensive metabolite analysis and modeling as is the case with labelled [^{11}C]-thymidine. Recent work has identified FLT as a promising analogue reflecting cell proliferation despite it not being appreciably taken up by DNA.We have been interested in examining the merits of [^{124}I]-labelled IUdR and FIAU as potential *in-vivo* markers of cell proliferation. To this end we have investigated total cell uptake and nuclear incorporation of these analogues and of [^{3}H]Thymidine and [^{3}H]FLT in non-small cell lung cancer cell lines exhibiting fast and slow growing rates.

[^{124}I]IUdR was prepared by iodination of deoxyuridine (40 g, in 200 L NaH_2PO_4 at pH 7.0) using iodogen as an oxidizing agent. [^{124}I]IUdR was obtained in 69±5% overall radiochemical yield following purification with solid phase extraction. The radiotracer was produced in >95 - 99% radiochemical purity. [^{124}I]FIAU was labelled by iodination of FTAU precursor (25 g in 100 L chloroform) using a 3:1 (v/v) mixture of acetic acid:30% hydrogen peroxide as an oxidizing agent. The radiochemical yield of [^{124}I]FIAU was 80±5% obtained in >95 - 99% radiochemical purity. [^{3}H]methyl Thymidine (5.0 Ci/nmol) and [^{3}H]FLT (2.0 Ci/nmol) were obtained from Amersham and Moravek Biochemicals respetively.

Two non-small cell lung carcinoma cell lines, H460 and H596 were chosen to assess total cellular uptake and DNA incorporation of the nucleoside analogues. These cell lines exhibit a two-fold differential growth rate and we determined a doubling times of 20 hours for H460 and 50 Hours for H596. Cells were plated in a 96 well plate at between 1-2 x 10^4 cells per well and allowed to attach overnight. Incubation with 10kBq of each radiotracer was carried out in complete medium at 37°C, 5% CO2 in a humidified environment over a period of 72 hours. During the incubation period the cells remained in exponential growth and did not reach confluence. Quadruplicate samples were analysed for total cellular uptake, after PBS washing and DNA incorporation, using TCA precipitation and leeching with sodium hydroxide. Samples were counted on a microplate scintillation counter.

The total cell uptake of both [^{3}H]methyl Thymidine and [^{124}I]IUdR were higher for H460 than for H596 which is consistent with the faster growth rate of the former. After 24 hours more than 90% of the total [^{3}H]methyl Thymidine uptake was associated with DNA for H460 and >80% for H596. For [^{124}I]IUdR, the incorporation into DNA of both cell lines increased from 30% at 24 hours to a maximum of > 80% by 48 hours for H460 and > 70% for H596. Over the whole incubation period, > 80% and >50% of the total [^{124}I]FIAU uptake was nuclear bound for H460 and H596 cells respectively. In contrast, DNA incorporation levels for [^{3}H]FLT were 36% and 12% for H460 and H596 over the same period.

These preliminary results indicate that, like thymidine, the total and nuclear incorporation of [^{124}I]IUdR were greater in the faster growing cell line. The different growth rates of the two cell lines did not appear to be a factor in the uptake of [^{124}I]FIAU or [^{3}H]FLT. Work is underway to further characterize the specificity of uptake and its relation to the levels of phosphorylating enzymes.

PREPARATION OF CU-64 AND TC-99M-N2S2-VASOACTIVE INTESTINAL PEPTIDE (VIP) FOR IMAGING GENE EXPRESSION

M. Thakur[1,2], M. Aruva[1], E. Bolewska-Pedyczak[3], J. Gariepy[3]

[1] Dept. of Radiology, Thomas Jefferson University, Philadelphia, PA Email: mathew.thakur@mail.tju.edu
[2] Kimmel Cancer Center, Thomas Jefferson University Hospital, Philadelphia, PA
[3] Ontario Cancer Institute, Prince Margaret Hospital, Ontario, Canada

Introduction:

Many human cancer cells express a gene product, cell surface receptors VPAC, which have a high affinity for an endogenous 28 amino acid, growth hormone, VIP. We have labeled Tc-99m-VIP using Gly-(D)Ala-Gly-Gly as a chelating moiety that provided an N_4 configuration for strong chelation with Tc-99m (TP3654) and used it successfully to image tumors in humans. VIP-Cu-64 has also been prepared using 1,4,8,11-tetrazacyclo tetradecane-1,4,8,11-tetracetic acid (N_4) as a chelating agent.

Aim:

The goals were to 1) synthesize a protected diaminedithiol chelating moiety during a solid phase assembly of VIP, 2) use the analog for N_2S_2 chelation with Cu-64 ($^{+}$ = 19%, t½ 12.8 hr) for positron emission tomography (PET), and with Tc-99m for single photon emission tomography (SPECT), and 3) compare their tissue distribution in mice bearing human colorectal cancer LS174T.

Materials and Methods:

VIP analog with protected diaminodithiol (TP3982) was synthesized using standard Fmoc chemistry on a Wang resin. The peptide was designed to harbor a C terminus Lys residue separated from VIP-Asn_{28} by aminobutyric acid spacer. Lys was derivatized with diaminopropionic acid followed by di-benzylthioglycolic acid on the resin. HPLC purification followed characterization using MALDI mass spectrometer. Copper-64 was obtained from Mallinckrodt Institute of Radiology, St. Louis. Tc-99m labeling was facilitated using 20 g TP3982 in 0.05 M phosphate buffer pH-12 and Cu-64 using 0.2 M glycine buffer pH-9. Sn-tartrate served as a deprotecting agent. Following HPLC and ITLC analyses, preparations were injected through a lateral tail vein into four groups of five tumor bearing mice each. Four and 24 hour tissue distribution was determined.

Results:

Both Cu-64-TP3982 and Tc-99m-TP3982 were eluted as a single radio-HPLC peak. >95% radioactivity was incorporated. Colloids were <5%. Copper-64-TP3982 had a slower blood clearance than that for Tc-99m-TP3982. The Cu-64-TP3982 tissue uptake was significantly higher ($P = < 0.005$) than that of Tc-99m-TP3982 for each normal tissue and tumor, except the kidneys. However, tumor/blood (T/B) and tumor/muscle (T/M) ratios for both agents were not significantly different.

Discussion:

The T/B and T/M ratios for both Cu-64-TP3982 and Tc-99m-TP3982 were similar to those of Tc-99m-TP3654 in mice bearing LS174T tumors. Although these ratios in mice were minimal for imaging tumors, we have successfully used Tc-99m-TP3654 in humans to localize tumors of the breast, colon, bone, and high grade spindle cell carcinoma, all of which express VPAC receptors.

Conclusion:

A VIP analog (TP3982) has been prepared and labeled with Cu-64 and Tc-99m. The results warrant further work for PET and SPECT imaging of tumors with VPAC expression.

Work was supported by DOE ER63055 and NIH CA42960, R24CA86307, and HL59769.

TARGETING OF ED-B FIBRONECTIN FOR TUMOR IMAGING WITH Tc-99m AP39: RADIOLABELING OPTIMIZATION AND COMPARISON OF MONOMER/DIMER FORMATS

J.E. Cyr, D. Berndorff, D. Moosmayer, G. Malawski, M. Zerna, and L. Dinkelborg

Research Laboratories of Schering AG, Radiopharmaceuticals Research S105, 13342 Berlin, Germany, john.cyr@schering.de

Keywords: ED-B fibronectin, Tumor, Tc-99m AP39, Single chain antibody fragment, angiogenesis

ED-B fibronectin is an oncofetal antigen expressed in the stroma associated with neovasculature of solid tumors, and therefore represents a promising target for tumor imaging. AP39 is a human recombinant single chain antibody fragment (scFv) that is specific for ED-B fibronectin. This scFv has been designed to incorporate a Gly-Gly-Cys sequence at the C-terminus which is capable of forming a complex with Tc-99m.

We have devised a radiolabeling formulation for Tc-99m AP39 that is comprised of Vial 1 containing AP39 associative dimer, and Vial 2 which contains all other components needed for efficient radiolabeling. Room temperature Tc-99m radiolabeling with this formulation reliably produces Tc-99m AP39 with TLC RCP 90% and HPLC RCP 85%.

Three main forms of AP39 are encountered during its production: an associative dimer (the desired product), a covalent disulfide dimer, and a monomer. The monomer has low antigen binding compared to the dimeric species (ELISA). Most of the monomer is removed during purification steps, but small amounts may remain as an impurity in the final product. AP39 needs to be reduced to make it amenable for radiolabeling with Tc-99m, presumably because the C-terminal cysteine thiol groups need to be freed by cleavage of disulfide bonds. Reduction also converts the covalent dimer to the desired associative dimer product.

A monomeric impurity species can also be formed from the associative dimer final product at high pH. When AP39 dimer is radiolabeled at pH 10.5, Tc-99m AP39 monomer grows in, amounting to approximately 22% after 2 hours incubation at 37°C. In the optimized radiolabeling formulation (*vide supra*), Tc-99m AP39 monomer is present in small amounts (4%) as an impurity.

We were interested in comparing the biological properties of the radiolabeled monomer vs. dimer. Hence, we have compared the biodistribution properties of Tc-99m AP39 dimer vs. Tc-99m AP39 monomer in F9 murine teatocarcinoma syngenic tumor nude mice. In one study, AP39 dimer and monomer (isolated from the purification steps of the process) were directly labeled. Both formats were reduced with TCEP, radiolabeled at pH 8.6 with 30 minutes incubation at 37 °C, and then purified on a NAP5 size exclusion cartridge. The final direct-labeled products were >87% pure by HPLC. The biodistribution results showed substantially higher tumor uptake for the dimer species relative to the monomer (eg. 10.2 %ID/g vs. 2.6 %ID/g at 3 hours). In a separate study, Tc-99m AP39 monomer was generated in a pH 10.5 radiolabeling preparation, and the Tc-99m monomer and dimer were isolated by FPLC. The final FPLC-isolated products were >93% pure by HPLC. Biodistribution results in the same tumor model (F9) also showed substantially better tumor uptake for the dimer vs. the monomer (eg. 7.7 %ID/g vs. 0.9% ID/g at 3 hours).

Conclusion: Tc-99m AP39 is a single chain antibody fragment radiopharmaceutical that shows promise for tumor imaging. It can be formed in high yield at room temperature in a 2-vial radiolabeling formulation. Tc 99m AP39 monomer impurity species are present in the radiolabeling preparation, and can be derived either from direct radiolabeling of monomer impurity, or from degradation of the dimer product during radiolabeling. The Tc-99m monomer impurity species were evaluated in a tumor biodistribution model (syngenic F9 tumor implants) and observed to have inferior tumor targeting properties when compared to the Tc-99m AP39 dimer drug product.

I-124-IODOBENZOATE DOXORUBICIN DERIVATIVE FOR PET IMAGING

L. Hamblett[1], J. Gillies[1], J. Hadfield[2], J. Bailey[1], M. Ranson[3] and J. Zweit[1]

[1]CRUK/UMIST Radiochemical Targeting and Imaging Group, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, M20 4BX, UK. IHamblett@picr.man.ac.uk & [2]Department of Chemistry, University of Salford, Salford, Manchester. [3]CRUK Department of Medical Oncology, Christie Hospital NHS Trust, Wilmslow Road, Manchester, M20 4BX, UK.

Keywords: PET, I-124, doxorubicin, drug delivery

Doxorubicin (DOX) is an anticancer agent active against carcinomas and sarcomas. However, the therapeutic efficacy of the parent DOX compound is limited by cardiotoxicity and myelosuppression. One possible approach to overcome the effects of normal tissue toxicity and improve the therapeutic index of DOX is to develop novel drug delivery vehicles. The incorporation of DOX into micelles, such as polyoxypropylene-polyoxyethylene (POP-POE), is one possible approach. In order to assess the pharamacokinetics of the drug and carrier, in vivo, we have developed an I-124 analogue of DOX. [^{124}I]-DOXIB, an iodobenzoate derivative of doxorubicin, has been synthesised and purified with the aim of determining any potential improvement in tumour tissue targeting and as a PET tracer in the study of copolymer-based drug delivery systems.

We have previously developed the synthesis of a tributylstannyl precursor, DOXSN, (1). Briefly, DOXSN was synthesised from the reaction of 4-iodobenzoic acid with 2,3,5,6-tetrafluorophenol in the presence of *N*-ethyl-*N*'-diethylaminopropyl carbodiimide to give an activated ester followed by stannylation at the 4-iodo position using *tetrakis*(triphenylphosphine)palladium to give DOXSN.

A mixture of DOXSN in MeOH/AcOH 99:1, *N*-Chloro Succinamide in MeOH, PBS (1 L, 0.1M) was reacted with [^{124}I]NaI (3-10 MBq in 1-5 L for 10 min and quenched with sodium metabisulphite. RadioTLC, using a chloroform/methanol mobile phase, indicated a crude radiochemical yield of around 80%. The iodinated reaction mixture was loaded onto a pre-conditioned reverse phase C18 Sep-Pak column and eluted with water followed by 1:1 acetonitrile:water. The [^{124}I]-DOXIB containing fractions were confirmed by radioTLC and pooled. The pooled fractions were then concentrated by evaporation in a stream of nitrogen and reconstituted in 20 L acetonitrile and then purified further by direct injection onto a Phenomenex Luna 5 m reverse phase analytical (250 x 4.6 mm C18) HPLC column eluted with 100 % acetonitrile at a flow rate of 1mLmin^{-1}. The overall radiochemical yield of purified [^{124}I]-DOXIB recovered from the HPLC was approximately 55 ± 5% with a radiochemical purity of 95 ± 5%.

Both drug uptake and cytotoxicity assays have been carried out to compare the behaviour of DOX and DOXIB using MCF-7 and MCF-7 ADR cells. For the drug uptake assay, incubated cell fluorescence was measured by flow cytometry. The assay indicates that DOXIB is taken up more effectively by the MCR-7 ADR cells compared to the MCR-7 cells (ratio approx. 15:1) whilst the MDR-7 ADR cells are resistant to DOXIB uptake compared to DOX (ratio approx. 1.4:1).

Based on these results future work on the DOXIB compound will focus on radiolabelled [^{124}I]DOXIB PET biodistribution and cytotoxicity *in vitro* and *in vivo* analysis, in order to fully characterise the DOX compound prior to testing with POP-POE co-polymer drug delivery system.

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SYNTHESIS, *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF *SYN/ANTI*[^{123}I]IVACBC AS POTENTIAL TUMOR IMAGING AGENTS

W. Yu[1], J. McConathy[1], L. Martarello[1], L. Williams[1], E. Malveaux[1], V.M. Camp[1], Z. Zhang[2], J. Olson[2], M.M. Goodman[1]

[1]Division of Radiological Sciences, Emory University, Atlanta, GA 30322
[2]Department of Neurosurgery, Emory University, Atlanta, GA 30322, wyu2@emory.edu

Key words: IVACBC, iodine-123, tumor imaging

Amino acids *syn/anti*-1-amino-3-[2-iodoethenyl]-cyclobutanecarboxylic acid (*syn/anti*-IVACBC) were synthesized, [^{123}I] radiolabeled and evaluated *in vitro* and *in vivo* as potential SPECT tumor imaging agents in rat 9L gliosarcoma tumor model.

The stannylated IVACBC precursors for radiolabeling were prepared in a series of synthetic steps starting from allylbenzylether. The key intermediates, the *syn/anti*-1-[*N*-(*tert*-butoxycarbonyl)amino]-3-hydroxymethyl-cyclobutanecarboxylic acid *tert*-butyl esters were made according to the method developed by this group (Martarello L., *et al*, J. Med. Chem., 45, 2250-2259, 2002). The alcohols were transformed to the corresponding aldehydes by Swern oxidation followed by reaction with iodoform/chromium(II) chloride then hexamethylditin/palladium(0) to afford *syn/anti*-1-[*N*-(*tert*-butoxycarbonyl)amino]-3-[2-trimethylstannylethenyl]-cyclobutanecarboxylic acid *tert*-butyl esters as the radiolabeling precursors.

Radioiodination was carried out with no-carrier-added [^{123}I]NaI-H_2O_2/H^+. *Syn/anti*-[^{123}I]IVACBC were obtained by hydrolysis with trifluoroacetic acid and chromatographic purification. Radiolabeling yields were 54% (n=10, *syn*-[^{123}I]IVACBC) and 53% (n=7, *anti*-[^{123}I]IVACBC), with radiochemical purity over 99% as measured by radiometric TLC for both compounds.

The *in vitro* study was performed in rat 9L gliosarcoma cell line in amino acid-free Dulbecco's Modified Eagle's Medium incubated for 30 minutes at 37 ^{o}C to evaluate the compounds tumor cell uptake and transport mechanism. 10 mM 2-amino-bicyclo[2.2.1]-heptane-2-carboxylic acid (BCH) and 10 mM N-methyl-a-aminoisobutyric acid (MeAIB) were used as L- and A-type amino acid transporter inhibitors, respectively. The cell uptake was measured in percent CPM per million cells. The inhibition of uptake of the *syn* isomer by BCH was 97.5%, *vs.* 0 % by MeAIB. Similar results were observed for *anti* isomer. These findings suggested that *syn/anti*-IVACBC entered 9L tumor cells *in vitro* primarily *via* L-type amino acid transport.

The *in vivo* biodistribution study was performed in Fischer rats with 9L tumors implanted intracranially. The radioactivity in tumors and in normal tissues of tumor-bearing Fischer rats (n=4 at each time point) was measured at 30, 60, 120 min *p.i.* Values were reported as mean percent dose per gram tissue. The tumor and brain data is listed in Table 1. The experiment showed that IVACBC had a rapid and prolonged accumulation in tumors with a good tumor to brain ratio. Low uptake was found in heart, liver, lung, and thyroid. These results support the candidacy of *syn/anti*-[^{123}I]IVACBC as SPECT brain tumor imaging agents. Research supported by Nihon Medi-Physics Co., Ltd.

Table 1: The tumor and brain distribution of [^{123}I]IVACBC in 9L tumor-bearing Fischer rats

	30 min.	60 min.	120 min.
Syn-[^{123}I]IVACBC			
Tumor	0.877	0.830	0.711
Brain	0.156	0.113	0.094
Tumor/Brain	5.63	7.33	7.53
Anti-[^{123}I]IVACBC			
Tumor	1.401	1.427	1.474
Brain	0.269	0.220	0.201
Tumor/Brain	5.20	6.49	7.35

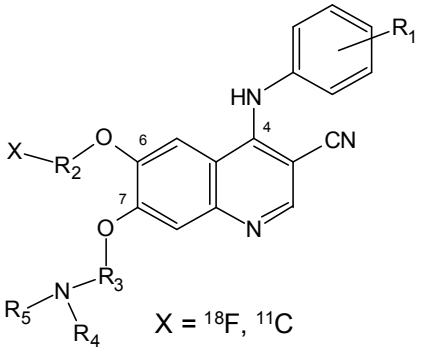
A STRATEGY FOR THE SYNTHESIS AND EVALUATION OF F-18 AND C-11 LABELED QUINOLINE DERIVATIVES AS TYROSINE KINASE INHIBITOR IMAGING AGENTS

H.C. Padgett, D. Maclean, J.P. Northrop, J.C. Walsh, and T. Ysaguirre

LA Tech Center, PETNET Pharmaceuticals, Inc., 6140 Bristol Parkway, Culver City, CA 90230, USA, henry.padgett@petnetpharmaceutical.com

Keywords: *O*-[^{18}F]Fluoroethyl-, Tyrosine Kinase, microPET, Cancer

A new generic strategy for the flexible synthesis of diverse tyrosine kinase (TK) inhibitors has been developed and used to prepare a variety of labeled compounds. A library of various quinoline-based tyrosine kinase inhibitors based on a single intermediate has been synthesized and labeled. This library of compounds was designed with an initial emphasis on inhibition potency and selectivity towards EGFR and Src kinases and is based on the work of Boschelli et al. (1). The sites on the inhibitor scaffold that were varied were the 7-position for changes in potency, and the 4-position, which involved using different aniline substituents for changes in kinase specificity. A variety of tertiary amines (-R_3-N-R_4R_5) were used, and the labeled alkyl group X-R_2-, which could be [^{18}F]fluoroethyl, [^{18}F]fluoromethyl, [^{18}F]fluorobenzyl, or [^{11}C]methyl, was attached at the 6-position phenol. This initial set of inhibitors exhibited promising IC_{50} values in enzymatic and cellular assays. A typical fluorine-18 labeling sequence involves the reaction of [^{18}F]fluoride ion with ethylene glycol di-*p*-tosylate to give the [^{18}F]fluoroethyl tosylate which was isolated by semi-prep HPLC and trapped on a Merck LiChrolute EN cartridge (2). After drying, the tosylate in DMF was released from the cartridge into a DMF solution of the precursor tetrabutylammonium phenolate salt and was heated at 120°C for 20 min. All of the desired *O*-[^{18}F]fluoroethyl quinoline derivatives were isolated by semi-prep HPLC, and were obtained in 10-20% unoptimized yields (corrected). After C18 SPE and reconstitution in saline, the labeled compounds were used in microPET mouse imaging studies. In non-tumor bearing mice, biodistribution of the *O*-[^{18}F]fluoroethyl-labeled compounds was remarkably similar, with a significant portion of the activity localizing in the gall bladder, kidneys and gut. Studies in tumor bearing nude mice again showed enhanced localization of activity in the gall bladder, kidneys, and gut, with additional low levels (<1% IDG) of activity localizing in EGF-expressing tumors. The low localization in tumors is thought to be due to poor biodistribution or active transport since these compounds are also substrates for P-glycoprotein (MDR pump 1).



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SYNTHESIS, RADIOLABELLING AND PRELIMINARY EVALUATION OF 123I-RADIOIODINATED GFRTK INHIBITORS

P. Sumner[1,2], A. Katsifis[1], F. Mattner[1], I. Greguric[1], P. Keller[2]

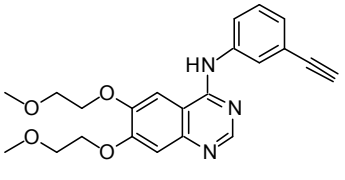
[1] Radiopharmaceuticals Division, ANSTO, New Illawarra Road, Lucas Heights, NSW, 2234, Australia. [2] Chemistry Department, University of Wollongong, Northfields Avenue, Wollongong, NSW, 2522, Australia. E-mail: psu@ansto.gov.au

Key words: Receptor tyrosine kinases, Epidermal growth factors, Anilinoquinazolines, Iodine-123, SPECT

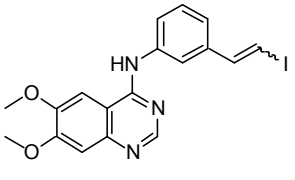
Growth Factor Receptor Tyrosine Kinases (GFRTK's) are important mediators in signal transduction processes that regulate cell division, growth and differentiation. Inhibition of these kinases block tyrosine phosphorylation and the signalling cascade leading to inhibition of the various cellular responses including tumour growth. Recent antitumour studies have therefore been directed toward the development of GFRTK inhibitors. Furthermore, the relatively high levels of growth factor expression in tumours (eg epidermal growth factor - EGF) suggest that radiolabelled tracers can be developed to assess GFRTK activity using PET and SPECT.

Derivatives of anilinoquinazolines have been identified as potent inhibitors of EGFRTK including OSI774 **[1]** (IC_{50} 2 nM). Several derivatives based on the anilinoquinazoline structure have been synthesised and radiolabelled with radioiodine. In this investigation, the *cis* and *trans* isomers of the iodovinyl derivative **[2]** have been prepared, radiolabelled with iodine-123 and evaluated *in vivo* in rats.

Free radical stannylation of the starting acetylene using tributyl tin hydride and the radical initiator AIBN gave the corresponding tributyl stannane as a mixture of *cis* and *trans* isomers in a ratio of 6:94. Both of the stannane isomers were confirmed by mass spectroscopy. Radioiodination with I-123 was carried out via iododestannylation using sodium iodide in the presence of Chloramine-T. Purification by reverse phase C-18 HPLC using acetonitrile: 0.1 M ammonium acetate (45:55) gave the corresponding *cis* and *trans* radioiodinated isomers with retention times of 18 and 24 min, respectively, in an overall radiochemical yield of 65-70%.



[1]



[2]

Preliminary *in vivo* -imaging of the *cis* and *trans* isomers of **[2]** in Fisher rats implanted with mammary adenocarcinomas indicates significant contrast in the tumours compared to normal tissue. Biodistribution studies of the *trans* isomer showed peak uptake in the tumours at 1 h (0.6-0.7 % ID/g) which was maintained for 6 h. The tumour uptake of the *cis* isomer was lower (0.3-0.4 % ID/g) and in a plateau for 3 h.

These results indicate that the iodovinyl anilinoquinazoline derivatives are promising candidates for assessing GFRTK activity and that the *trans* isomer is superior to the *cis* isomer for imaging studies.

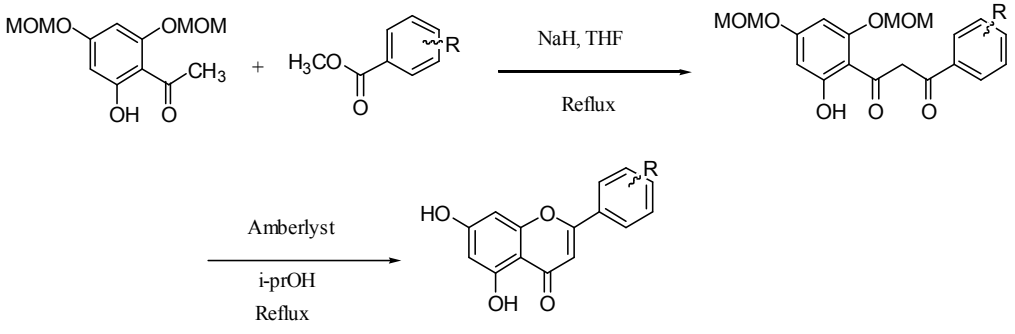
SYNTHESIS AND EVALUATION OF FLAVONOIDS AS POTENTIAL RADIOLIGANDS OF Pgp/MRP1

S. Mavel[1], B. Dikic[3], P. Emond[1], I. Greguric[3], S. Palakas[2], A. Gomez de Gracia[2], F. Mattner[3], M. Garrigos[2], J.C. Besnard[1], D. Guilloteau[1]

[1] Lab. de Biophysique Médicale et Pharmaceutique, INSERM U316, Faculté de Pharmacie, 31 av. Monge, 37200 Tours, France. [2] CEA Saclay, Dpt de Biologie Joliot Curie, Service de Biophysique des Fonctions Membranaires, CEA URA-2096, 91191 Gif sur Yvette, France. [3] Radiopharmaceuticals Division, ANSTO, New Illawarra Road Lucas Heights, NSW, 2234, Australia. email contact: mavel@univ-tours.fr

Keywords: Flavonoids, MDR, Pgp, MRP1, Cytotoxicity

Multidrug resistance (MDR) is one of the major problems affecting the treatment of cancer. This phenotype is in part mediated by the over-expression of plasma membrane transporters like the P-glycoprotein (Pgp) and the multidrug resistance associated protein (MRP1). These proteins act as efflux pumps and clear the cells of a large range of cytotoxic drugs. As this phenotype could be an intrinsic or an acquired resistance during the disease, *in vivo* visualization and quantification of MDR proteins would be of great value to better select the therapeutic strategy. In order to develop new radiopharmaceuticals to image these proteins, we anticipated that flavone-based compounds should be good candidates for development as it has been shown that flavonols interact at the ATP-binding site of Pgp *in vitro*. We report here the synthesis of a flavonoid-based series of compounds bearing halogen atom for potential development as imaging agents. These compounds have been prepared in a four-step synthesis.



R = 4-Br, 3-Br, 4-I, 4-O$(CH_2)_9$F, 4-O$(CH_2)_{10}$F

A series of *in vitro* assays were conducted on various human cell lines expressing either Pgp or MRP1 to evaluate the concentration of compound required to kill 50% of the cells (IC_{50}). IC_{50} values were found to vary from 10 to > 100 M with no significant differences between cells expressing the MDR phenotype or not. Compounds prepared here could thus be considered as weak cytotoxic agents compared to doxorubicine, colchicine or vinblastine. We are now investigating the binding selectivity of these compounds for Pgp or MRP1 through their capacity to reverse the MDR phenotype of cell lines expressing either PgP (K562/Adr) or MRP1 (GLC4/Adr). These studies would help to determine if these derivatives interact with MDR proteins by the modulatory effects of the cytotoxic agents. Hence, compounds prepared here could be radioiodinated or radiofluorinated and might form the basis for the development of imaging agents to monitor Pgp expression in tumors by SPECT or PET.

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EVALUATION OF TUMOR-DETECTING POTENTIAL OF *O*-[^{11}C]METHYL-L-TYROSINE AND *O*-[^{18}F]FLUOROMETHYL-L-TYROSINE

K. Ishiwata[1], K. Kawamura[1,2], W.F. Wang[1], S. Furumoto[3], K. Kubota[3], C. Pascali[4], A. Bogni[4] and R. Iwata[3]

[1]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi-ku, Tokyo, 173-0022, Japan; E-mail, ishiwata@pet.tmig.or.jp; [2]SHI Accelerator Service LTD., Tokyo, Japan; [3]Tohoku University, Sendai, Japan; [4]National Cancer Institute, Milan, Italy.

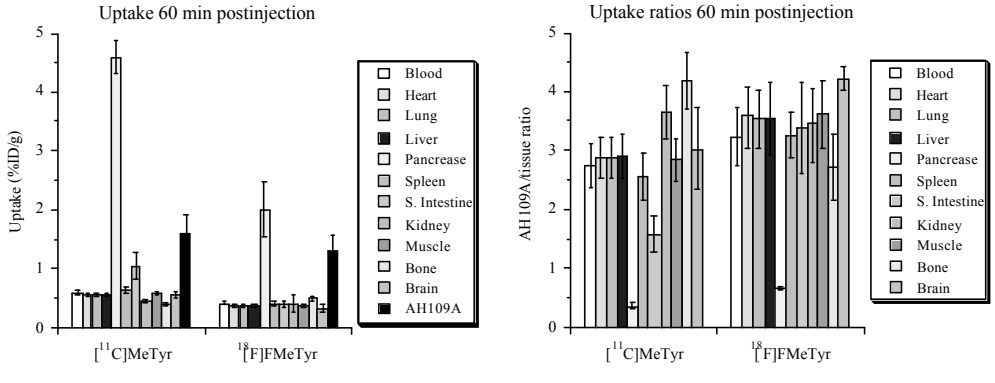
Keywords: *O*-[^{11}C]Methyl-L-tyrosine, *O*-[^{18}F]Fluoromethyl-L-tyrosine, Tumor, PET

O-[^{11}C]methyl-L-tyrosine ([^{11}C]MeTyr) and *O*-[^{18}C]fluorometyl-L-tyrosine ([^{18}F]FMeTyr) were developed by Iwata et al. as a potential PET tracers for imaging amino acid transport (1). In the present study we evaluated the potential of the two tracers for tumor detection using an animal model.

[^{11}C]MeTyr or [^{18}F]FMeTyr was injected intravenously into rats bearing AH109A hepatoma which was subcutaneously inoculated in a thigh. Tissue distribution of the radioactivity was evaluated by tissue dissection, and the uptake was measured as a percentage of the injected dose per gram of tissue (%ID/g). PET imaging was also carried out. Metabolites analysis in plasma and AH109A tissue was performed by HPLC.

Two tracers showed similar distribution patterns. A high uptake was found in the pancreas followed by AH109A. The radioactivity level in AH109A increased gradually for 60 min, while those in the other tissues decreased gradually. As shown in figure, the uptake levels for [^{11}C]MeTyr were relatively higher than those for [^{18}F]FMeTyr in all tissues, except for the bone including marrow, where the ^{18}F-level slightly increased. On the other hand, the uptake ratios of AH109A to other tissues for [^{18}F]FMeTyr were slightly larger than those for [^{11}C]MeTyr. In plasma and AH109A tissue the incorporation of the radioactivity into the macromolecules was negligible for both tracers, and no metabolite was detected by HPLC analysis. Finally PET imaging of AH109A was successfully performed in rats.

In conclusion, both tracers have the potential for tumor detection by PET. [^{18}F]FMeTyr showed slightly better properties for detection than [^{11}C]MeTyr, although a slight defluorination was suggested.



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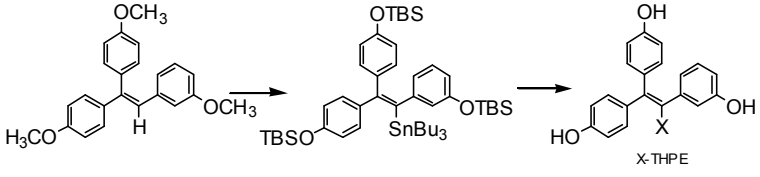
STEROIDAL AND NONSTEROIDAL APPROACHES TO ESTROGEN RECEPTOR-SEEKING RADIOPHARMACEUTICALS

R.N. Hanson[1], P.Tongcharoensirkul[1], E. McCaskill[1], A. Hughes[2], E.R. DeSombre[2]

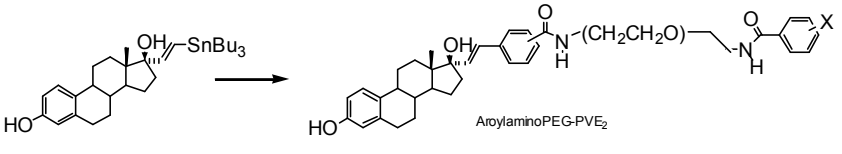
[1]Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Avenue, Boston, MA 02115
[2]The Ben May Institute for Cancer Research, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL60637

Keywords: Estrogen Receptor Ligands, Synthesis, Steroids, Triarylethylenes

As part of our program to develop estrogen receptor-seeking radiopharmaceuticals we examined two approaches. The first approach utilizes the tri-arylethylene scaffold identified by DeSombre (1) as a class of high affinity estrogenic agonists. Key to this route is the incorporation of the third phenolic hydroxyl at the meta- rather than the para-position. Although the radiolabeled compound had been reported in the literature, its synthesis has not been described.



Our second approach is based upon our efforts to develop 17 -(substituted-phenyl) vinyl estradiols as cancer chemotherapeutic agents (2,3). Those studies indicated that the receptor tolerated significant functionalization of the phenyl ring without loss of affinity or efficacy. We have modified our synthetic route to permit the introduction of a labeled group distal to the phenylvinyl moiety, in a region which docking studies suggests is tolerated by the receptor.



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3-IODO-4-HYDROXYPHENYL-CYSTEAMINE AS A HIGH-AFFINITY TUMOR-SEEKING AGENT FOR MELANIN FORMATION

R. Nishii[1], K. Kawai[2,6], M. Kobayashi[2], M. Yoshimoto[2], K. Nishimura[2], S. Kinuya[3], T. Uehara[4], S. Nagamachi[5], Y. Arano[4], N. Tonami[3] and S. Tamura[5]

(1)Department of Radiology, Fujimoto Hayasuzu Hospital, 17-1 Hayasuzu-cho, Miyakonojo, Miyazaki 885-0055, Japan, email; rnisii@sweet.ocn.ne.jp (2) School of Health Sciences, Faculty of Medicine, and (3) Graduate School of Medical Sciences, Kanazawa University, Ishikawa, Japan, (4) Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan, (5) Department of Radiology, Miyazaki Medical College, Miyazaki, Japan, (6) Biomedical Imaging Research Center, Fukui Medical University, Fukui, Japan.

Keywords: 3-Iodo-4-hydroxyphenylcysteamine, Malignant melanoma, Radiopharmaceutical, Radioiodine

PURPOSE: The aim of our study is to develop a new radiopharmaceutical labeled with radioiodine for detection and therapy of tumors, which have affinity to a characteristic metabolism in tumor. 3-Iodo-4-hydroxyphenyl-L-cysteine (I-L-PC), which we have reported previously [1], was found to have an interaction for tyrosinase, an essential and rate-limiting enzyme to melanin biosynthesis. In this study, considering higher affinity for the melanin formation, we synthesized 3-iodo-4-hydroxyphenylcysteamine (I-PCA) that was an amine derivative of I-L-PC.

METHOD/MATERIALS: 4-Hydroxyphenylcysteamine (4-PCA) was synthesized and radioiodinated in our laboratory. Synthesis of 4-PCA was confirmed by ^{1}H-NMR, mass spectrometry and elemental analysis. ^{125}I-PCA was prepared by conventional chloramine-T method under a no-carrier added condition. ^{125}I-PCA was purified by Sep-Pak-C-18 cartridge and the labeling efficiency and radiochemical purity were examined by TLC analysis. Biodistribution study of I-PCA was performed using B16 melanoma-bearing C57BL6 mice. The radioactivities of each organ were measured and % injected dose / g wet tissue was determined. Moreover, the tumor-to-blood ratio (T/B ratio) and tumor-to-muscle ratios (T/M ratio) of ^{125}I-PCA were also evaluated and were compared with ^{125}I-L-PC, ^{67}Ga-citrate, ^{125}I-L-AMT and ^{123}I-MIBG. *In vitro* accumulation study and inhibition study in B16 melanoma cells ware also performed. Inhibition studies of the membrane active transport and tyrosinase, the starting enzyme of melanin formation, were carried out using ouabain, L-tyrosine and phenylthiourea (PTU), respectively.

RESULTS: Radiosynthesis of ^{125}I-PCA was carried out conveniently and efficiently within only 15 min. A labeling efficiency of more than 73 % resulted in the labeling of 4-PCA to ^{125}I-PCA. After the simple Sep-Pak purification, no-carrier added ^{125}I-PCA with radiochemical purity greater than 90 % was obtained. The biodistribution of ^{125}I-PCA showed rapid blood clearance, renal excretion and low accumulation in normal tissue, while increase of accumulation in the tumor for 30 min. As a consequence, T/B ratio reached approximately 1.6 +/- 0.3 and T/M ratio increased up to 8.7 +/- 3.2 at 60 min after injection. It was higher than those of ^{125}I-L-PC; 6.5 +/- 0.8 and ^{67}Ga-citrate; 4.6 +/- 0.4. It indicated specific retention of the tracer in the melanoma. *In-vitro* accumulation study, there was no significant inhibition of ^{125}I-PCA with PTU in contrast to the inhibition of ^{125}I-L-PC ($p < 0.05$). It indicates that the uptake mechanism of ^{125}I-PCA to melanoma tissue was not depend on tyrosinase activity in melanoma cells. The amine structure of ^{125}I-PCA might be contributing to the further metabolic pathway in melanin formation.

CONCLUSIONS: The results suggested that I-PCA achieved the desired affinity for melanin formation. That is, I-PCA has high potentiality for diagnosis of malignant melanoma. Moreover, because I-PCA accumulated low in normal tissue and showed rapid clearance, it might be applied as a therapeutic radiopharmaceutical when labeled with I-131.

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