Bioorganic & Medicinal Chemistry Letters 20 (2010) 3482–3485

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vitro evaluation of ^{18}F labeled tyrosine derivatives as potential positron emission tomography (PET) imaging agents

Limin Wang [a], Wenchao Qu [b], Brian Lieberman [b], Karl Ploessl [b], Hank F. Kung [b,c,*]

[a] Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, United States
[b] Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, United States
[c] Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, United States

ARTICLE INFO

Article history:
Received 22 February 2010
Revised 30 April 2010
Accepted 4 May 2010
Available online 7 May 2010

Keywords:
PET
F-18
Tyrosine derivatives
Glioma

ABSTRACT

Three new ^{18}F labeled fluoroalkyl tyrosine derivatives, *O*-(2-[^{18}F]fluoroethyl)-α-methyltyrosine (FEMT, [^{18}F]**2**), *O*-(2-[^{18}F]fluoroethyl)-2-L-azatyrosine (FEAT, [^{18}F]**3**), *O*-(2-[^{18}F]fluoroethyl)-L-tyrosineamide (FETA, [^{18}F]**4**) have been synthesized and radiofluorinated with 5–34% decay-corrected yield. In vitro studies were carried out in U-138 MG human glioblastoma. Cellular uptake of new tracers was compared to clinically utilized imaging agent *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET, [^{18}F]**1**). The uptake of tracers followed the order of FET ([^{18}F]**1**) > FEAT([^{18}F]**3**) > FEMT ([^{18}F]**2**) ≈ FETA ([^{18}F]**4**).

A number of classes of metabolically based radiotracers such as radiolabeled glucose, amino acids, fatty acids and their analogs were developed for tumor imaging and have shown tremendous success in recent years. [^{18}F]fluorodeoxyglucose (FDG), which utilizes upregulated aerobic glycolysis in tumor cells,[1] is routinely used in clinics for detecting, staging and restaging of lymphomas[2] and many solid tumors.[3] However, it has certain limitations such as high uptake in the cerebral cortex and non-specific accumulation in inflammatory tissues.[4] In search of new imaging agents, a variety of radiolabeled amino acids were prepared based on increased amino acid uptake and protein metabolism in cancer cells. Numerous studies have demonstrated that amino acid based tracers show better results than FDG in detecting and delineating certain tumors, particularly in brain tumors.[5]

Currently, the most commonly used labeled amino acid in positron emission tomography (PET) is [^{11}C]methionine (MET).[6,7] Despite the success, its susceptibility to multiple metabolic pathways complicates the pharmacokinetic analysis[8,9] and its short half-life (20.3 min) limits its clinical applicability. To overcome these disadvantages, a number of ^{18}F (half-life 109.8 min) labeled amino acid analogs such as *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET, [^{18}F]**1**),[10] L-3-[^{18}F]fluoro-α-methyl tyrosine (FMT),[11] 6-[^{18}F]fluoro-L-dihydroxy phenylalanine (FDOPA),[12] and anti-1-amino-3-[^{18}F]fluorocyclobutyl-1-carboxylic acid (FACBC)[13,14] were developed. In particular, FET ([^{18}F]**1**), is a successful ^{18}F labeled amino acid tracer and it has been used in imaging brain tumors and head–neck carcinomas for diagnosis and therapy planning.[15,16]

Several close analogs of FET ([^{18}F]**1**), such as *O*-([^{18}F]fluoromethyl)-L-tyrosine,[17] *O*-(3-[^{18}F]fluoropropyl)-L-tyrosine,[18] and *O*-(3-[^{18}F]fluoropropenyl)-L-tyrosine[19] have been developed and evaluated. The uptake of these structurally similar tyrosine derivatives in tumor cells is most likely attributed to elevated amino acid transporter activity, in particular system L amino acid transporters (LATs),[5,20,21] a Na^+ independent transport system which mediates transport of branched and aromatic amino acids.[22] LAT, generally upregulated in many cancer cell lines, is a target for development of tumor imaging agents.[23–25] System L has broad substrate selectivity; substitutions on aromatic ring with fluorine, hydroxy or fluoroalkyl group as well as substitutions on α-hydrogen with methyl are generally tolerated, this enables developing a variety of structurally similar analogs as potential LATs substrates.[26]

To pursue aromatic amino acid derivatives with better tumor-avid properties than FET ([^{18}F]**1**) and exploring the structure–activity relationships (SARs) of LATs, three new ^{18}F tyrosine derivatives were synthesized and evaluated (Fig. 1): *O*-(2-[^{18}F]fluoroethyl)-α-methyl tyrosine (FEMT, [^{18}F]**2**), *O*-(2-[^{18}F]fluoroethyl)-2-L-azatyrosine (FEAT, [^{18}F]**3**), and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosineamide (FETA, [^{18}F]**4**). FEMT ([^{18}F]**2**) could be considered as fluoroalkyl derivative of well established SPECT tracer 3-[^{123}I]iodo-α-methyl-L-tyrosine (IMT).[27] We reasoned that FEAT ([^{18}F]**3**) might have specific uptake in cancer cells, because its analog 2-azatyrosine was reported to show antitumor activity and could reverse the *ras* transforming cancer cells to normal cell.[28] The amide derivative

* Corresponding author. Tel.: +1 215 662 3096.
E-mail address: kunghf@sunmac.spect.upenn.edu (H.F. Kung).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.007

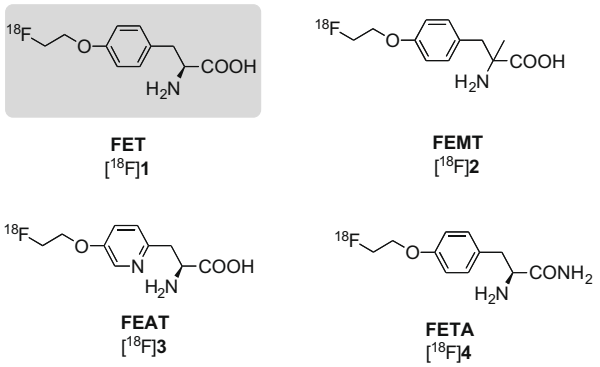


Figure 1. FET ([^{18}F]**1**) and its new analogs.

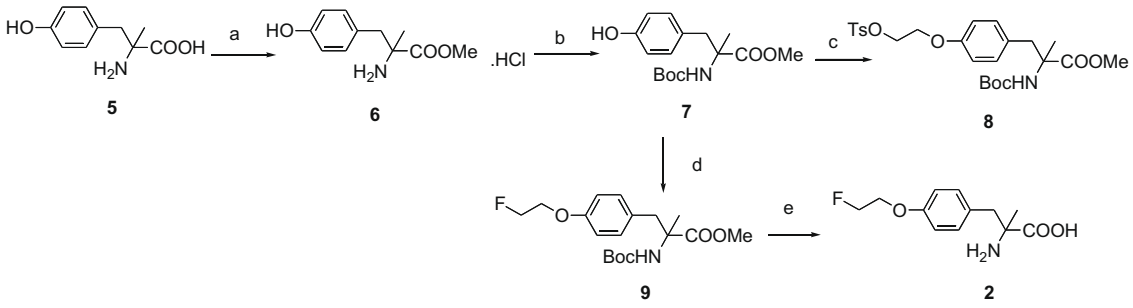
FETA ([^{18}F]**4**) was synthesized in hope that higher lipophilicity of [^{18}F]**4** may lead to improved pharmacokinetics compared to FET ([^{18}F]**1**), such as reduced excretion through urinary tract.

FEMT **2** was synthesized starting from commercially available α-methyl tyrosine **5** (Scheme 1). The initial attempt of Boc protection of amino group had low yield (<30%),[29] which might be due to steric hindrance from the α-methyl group and limited solubility of **5** in water or organic solvents. To solve this issue, we decided to protect the carboxylic acid group[29] first as shown in Scheme 1. Methylation of the carboxylic group gave α-methyl tyrosine methyl ester **6** in quantitative yield. The subsequent Boc protection produced intermediate **7** in good yield. Since direct nucleophilic radiofluorination is generally a better method than indirect labeling,[30,31] we prepared labeling precursors as protected *O*-tosylalkyl tyrosine derivatives. Tosylate precursor **8** was obtained in a yield of 46% after treating intermediate **7** with ethylene glycol ditosylate and NaH in DMF. Alkylation of **7** with 1-bromo-2-fluoroethane followed by deprotection in 6 N HCl, FEMT **2** was prepared with 23% overall yield.

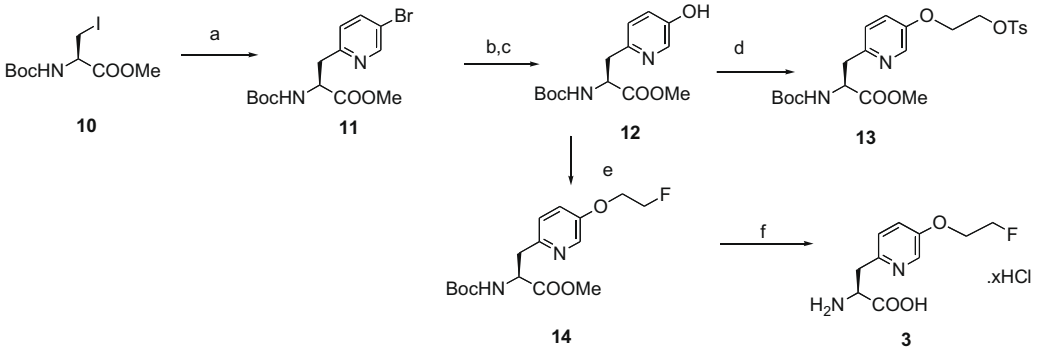
For synthesis of FEAT **3**, the important intermediate is Boc-L-2-azatyrosine methyl ester **12**, which was prepared following the same procedure reported by Germain et al.[32] via Negishi cross coupling using 2,5-dibromopyridine as precursor and subsequent hydroboration–oxidation reaction (Scheme 2). From this protected azatyrosine intermediate **12**, we could obtain labeling precursor **13** and FEAT **3** through alkylation reactions as described in FEMT synthesis with 26% and 28%, respectively, overall yield.

FETA **4**, the amide analog of FET **1**, was prepared from Boc-L-tyrosine **15** in three steps (Scheme 3)—direct alkylation on phenol, coupling reaction with 2,4,6-trimethoxy benzylamide (Tmob-NH_2) and final deprotection reaction with trifluoroacetic acid. Tosylate precursor **20** was synthesized in a similar manner, except it required selectively protecting the ethoxyl group on phenol with TBDMS and removing the protecting group with TBAF after coupling reaction with Tmob-NH_2. Overall yield of FETA was 34% and of precursor **20** was 47%.

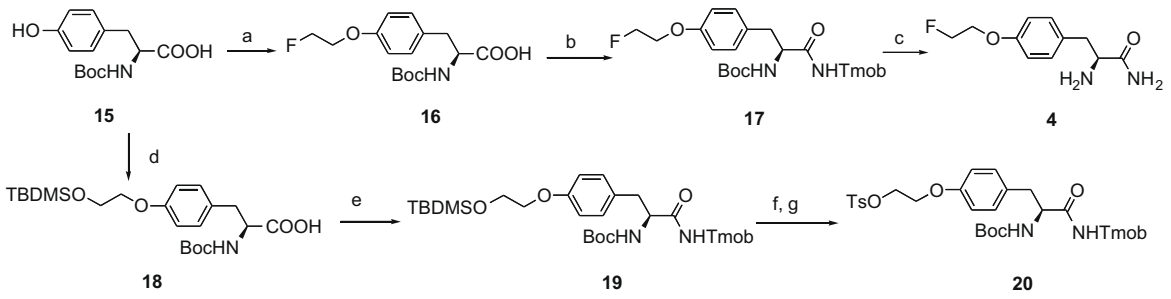
All of the ^{18}F labeled ligands, FET ([^{18}F]**1**), FEMT ([^{18}F]**2**), FEAT ([^{18}F]**3**), and FETA ([^{18}F]**4**), were prepared via nucleophilic substitution of tosylate with non-carrier-added [^{18}F]KF–$K_{2.2.2}$ complex in acetonitrile, followed by deprotection (Scheme 4). In general, fluorination reaction could reach completion within 10 min at 90 °C. Deprotection reaction conditions vary as shown in Table 1. All ligands were purified with semi-preparative HPLC and were prepared within 100 min in good radiochemical purity (RCP, >95%) determined by both analytical HPLC and TLC. Results of labeling such as decay-corrected radiochemical yield (RCY) and specific activity were summarized in Table 1.



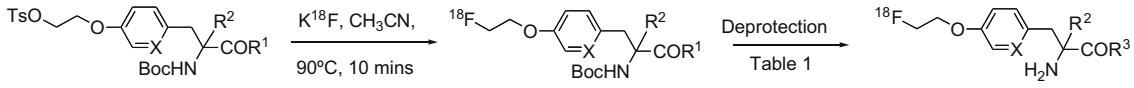
Scheme 1. Synthesis of FEMT **2**. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C to reflux, 16 h, 100%; (b) $(Boc)_2O$, Et_3N, MeOH, 50 °C, 3 d, 63%; (c) NaH, DMF, $TsOCH_2CH_2OTs$, 0 °C to 5 °C, 6 h, 46%; (d) NaH, DMF, FCH_2CH_2Br, 0 °C to rt, 6 h, 81%; (e) 6 N HCl, reflux, 2 h, 46%.



Scheme 2. Synthesis of FEAT **3**. Reagents and conditions: (a) (i) zinc dust, I_2, DMF, rt, 1 h; (ii) 2,5-dibromopyridine, $Pd(PPh_3)Cl_2$, 68 °C, 2 h, 73%; (b) bis(pinacolato) diboron, $Pd(dppf)Cl_2$, KOAc, 85 °C, 4 h; (c) H_2O_2, CH_2Cl_2, 0 °C to rt, 16 h, 76%; (d) $TsOCH_2CH_2OTs$, K_2CO_3, DMF, 70 °C, 47%; (e) FCH_2CH_2Br, K_2CO_3, DMF, 70 °C, 2 h, 67%; (f) 6 N HCl, reflux, 4 h, 75%.



Scheme 3. Synthesis of FETA **4**. Reagents and conditions: (a) NaH, FCH_2CH_2Br, DMF, 0 °C to rt, overnight, 84%; (b) Tmob-NH_2·HCl, HOBt, DIPEA, HBTU, DMF, 0 °C to rt, 2 h, 80%; (c) TFA, CH_2Cl_2, 4 h, 50%; (d) NaH, $BrCH_2CH_2OTBDMS$, DMF, 0 °C to rt, overnight, 90%; (e) Tmob-NH_2·HCl, HOBt, DIPEA, HBTU, DMF,0 °C to rt, 2 h, 77%; (f) TBAF, THF, 1 h, 78%; (g) TsCl, Et_3N, CH_2Cl_2, overnight, 87%.



22, R^1= OBut, R^2 =H, X= CH
8, R^1 = OMe, R^2 = Me, X =CH
14, R^1 = OMe, R^2 = H, X= N
21, R^1 = NHTmob, R^2 = H, X = CH

[^{18}F]23, R^1= OBut, R^2 =H, X= CH
[^{18}F]9, R^1 = OMe, R^2 = Me, X =CH
[^{18}F]15, R^1 = OMe, R^2 = H, X= N
[^{18}F]18, R^1 = NHTmob, R^2 = H, X = CH

[^{18}F]1, R^2 =H, R^3 = OH, X= CH
[^{18}F]2, R^2 = Me, R^3 = OH, X =CH
[^{18}F]3, R^2 = H, R^3 = OH, X= N
[^{18}F]4, R^2 = H, R^3 = NH_2, X= CH

Scheme 4. Radiolabeling reactions.

Table 1
Deprotection reaction conditions, average decay-corrected RCY and specific activities at end of synthesis (EOS)

Ligands	Deprotection reaction conditions	RCY[a] (%)	Specific activity[a] (GBq/μmol)
[^{18}F]**1**	TFA, 60 °C, 10 min	44 ± 8	32 ± 12
[^{18}F]**2**	(1) TFA, 60 °C, 5 min; (2) 2 N NaOH, 100 °C, 10 min	30 ± 8	13 ± 3
[^{18}F]**3**	6 M HCl, 120 °C, 10 min	37 ± 8	23 ± 12
[^{18}F]**4**	TFA, 60 °C, 10 min	5 ± 2	54 ± 12

[a] Values are means of three to six experiments, standard deviation is given in parentheses.

After successfully synthesizing ^{18}F labeled tyrosine derivatives, we carried out cell uptake studies as initial biological evaluation in U-138 MG human glioblastoma cells. U-138 MG was used as positive control in uptake of FET and IMT.[33,34] Since FET ([^{18}F]**1**), was used as reference compound, we chose U-138 MG to establish the standard for amino acid tracers' uptake. The cellular uptake of ^{18}F labeled tyrosine derivatives (Fig. 2) followed the order of FET ([^{18}F]**1**) > FEAT ([^{18}F]**3**) > FEMT ([^{18}F]**2**) ≈ FETA ([^{18}F]**4**).

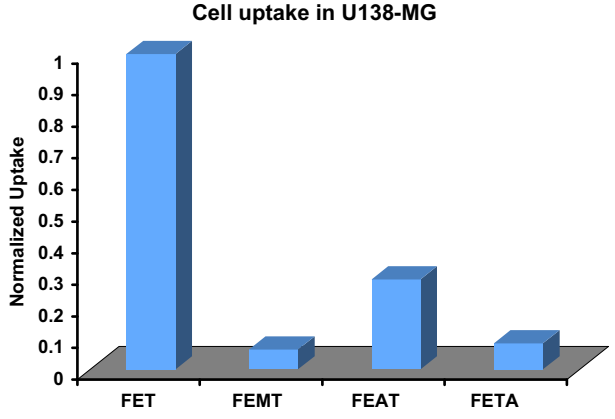


Figure 2. Uptake of new ligands in reference to FET ([^{18}F]**1**). Maximum uptake of ligands was normalized to FET's highest uptake in U-138 MG.

The uptake of the tracers depends on the ligands' binding affinity to amino acid transporters and their transport rate. From the uptake results, we could make some inference about amino acid transporters substrate recognition. The low uptake of FEMT ([^{18}F]**2**), the α-methyl substituted FET ([^{18}F]**1**), indicates *O*-fluoroalkyl α-methyl tyrosine derivatives may not be good substrates for amino acid transporters. As we know, there are four subtypes of LATs discovered by now, designated as LAT1 to LAT4. Although they have significant overlap of substrate selectivity, it is suggested that some amino acid based tracers have preference towards specific subtypes of system L, in particular, FET ([^{18}F]**1**) probably prefer to be transported via LAT2.[16] It is reported that α-methyl substitution is tolerated by LAT1 but no evidence suggest that LAT2 could accept α-methyl substitution as well,[26] this might be the reason that FEMT ([^{18}F]**2**) has significantly lower uptake than FET ([^{18}F]**1**). FEAT ([^{18}F]**3**), has a substituted pyridine side chain, which reduces its lipophilicity compared to FET ([^{18}F]**1**) and this potentially leads to its low uptake into cells. FETA ([^{18}F]**4**), which substitutes carboxylic acid with amide, is a poor substrate. In agreement, tyramine, dopamine, as well as phenylalanine methyl ester, which lack the free carboxylic acid group, are not good substrates for amino acid transporters either.[26,35] These results suggest that α-carboxylic acid might be essential amino acid transporters' substrate recognition.

In summary, three new potential PET imaging agents FEMT ([^{18}F]**2**), FEAT ([^{18}F]**3**), and FETA ([^{18}F]**4**) were synthesized, radiolabeled and evaluated via cell uptake studies in U-138 MG. New tracers exhibited lower uptake compared to clinically utilized FET ([^{18}F]**1**). Although these ligands might not be useful imaging agents, they provided insight for amino acid transporters' substrate recognition patterns.

References and notes

1. Buerkle, A.; Weber, W. A. *Cancer Metastasis Rev.* **2008**, *27*, 545.
2. Cheson, B. D.; Pfistner, B.; Juweid, M. E.; Gascoyne, R. D.; Specht, L.; Horning, S. J.; Coiffier, B.; Fisher, R. I.; Hagenbeek, A.; Zucca, E.; Rosen, S. T.; Stroobants, S.; Lister, T. A.; Hoppe, R. T.; Dreyling, M.; Tobinai, K.; Vose, J. M.; Connors, J. M.; Federico, M.; Diehl, V. *J. Clin. Oncol.* **2007**, *25*, 579.
3. Kelloff, G. J.; Hoffman, J. M.; Johnson, B.; Scher, H. I.; Siegel, B. A.; Cheng, E. Y.; Cheson, B. D.; O'Shaughnessy, J.; Guyton, K. Z.; Mankoff, D. A.; Shankar, L.;

Larson, S. M.; Sigman, C. C.; Schilsky, R. L.; Sullivan, D. C. *Clin. Cancer Res.* **2005**, *11*, 2785.
4. Paul, A. K.; Abdel-Nabi, H. *Curr. Med. Imaging Rev.* **2007**, *3*, 178.
5. McConathy, J.; Goodman Mark, M. *Cancer Metastasis Rev.* **2008**, *27*, 555.
6. Inoue, T.; Kim, E. E.; Wong, F. C.; Yang, D. J.; Bassa, P.; Wong, W. H.; Korkmaz, M.; Tansey, W.; Hicks, K.; Podoloff, D. A. *J. Nucl. Med.* **1996**, *37*, 1472.
7. Singhal, T.; Narayanan, T. K.; Jain, V.; Mukherjee, J.; Mantil, J. *Mol. Imaging Biol.* **2008**, *10*, 1.
8. Hatazawa, J.; Ishiwata, K.; Itoh, M.; Kameyama, M.; Kubota, K.; Ido, T.; Matsuzawa, T.; Yoshimoto, T.; Watanuki, S.; Seo, S. *J. Nucl. Med.* **1989**, *30*, 1809.
9. Derlon, J. M.; Bourdet, C.; Bustany, P.; Chatel, M.; Theron, J.; Darcel, F.; Syrota, A. *Neurosurgery* **1989**, *25*, 720.
10. Wester, H. J.; Herz, M.; Weber, W.; Heiss, P.; Senekowitsch-Schmidtke, R.; Schwaiger, M.; Stocklin, G. *J. Nucl. Med.* **1999**, *40*, 205.
11. Inoue, T.; Tomiyoshi, K.; Higuichi, T.; Ahmed, K.; Sarwar, M.; Aoyagi, K.; Amano, S.; Alyafei, S.; Zhang, H.; Endo, K. *J. Nucl. Med.* **1998**, *39*, 663.
12. Garnett, E. S.; Firnau, G.; Nahmias, C. *Nature* **1983**, *305*, 137.
13. Schuster, D. M.; Votaw, J. R.; Nieh, P. T.; Yu, W.; Nye, J. A.; Master, V.; Bowman, F. D.; Issa, M. M.; Goodman, M. M. *J. Nucl. Med.* **2007**, *48*, 56.
14. Schuster, D. M.; Nye, J. A.; Nieh, P. T.; Votaw, J. R.; Halkar, R. K.; Issa, M. M.; Yu, W.; Sepulveda, J.; Zeng, W.; Young, A.; Goodman, M. M. *Mol. Imaging Biol.* **2009**, *11*, 434.
15. Rau, F. C.; Weber, W. A.; Wester, H. J.; Herz, M.; Becker, I.; Kruger, A.; Schwaiger, M.; Senekowitsch-Schmidtke, R. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1039.
16. Langen, K. J.; Hamacher, K.; Weckesser, M.; Floeth, F.; Stoffels, G.; Bauer, D.; Coenen, H. H.; Pauleit, D. *Nucl. Med. Biol.* **2006**, *33*, 287.
17. Ishiwata, K.; Kawamura, K.; Wang, W. F.; Furumoto, S.; Kubota, K.; Pascali, C.; Bogni, A.; Iwata, R. *Nucl. Med. Biol.* **2004**, *31*, 191.
18. Tang, G.; Wang, M.; Tang, X.; Luo, L.; Gan, M. *Nucl. Med. Biol.* **2003**, *30*, 733.
19. Arstad, E.; Robins, E. G. WO 2007073200**,** 2007, 32.
20. Laverman, P.; Boerman, O. C.; Corstens, F. H.; Oyen, W. J. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 681.
21. Plathow, C.; Weber, W. A. *J. Nucl. Med.* **2008**, *49*, 43S.
22. Mastroberardino, L.; Spindler, B.; Pfeiffer, R.; Skelly, P. J.; Loffing, J.; Shoemaker, C. B.; Verrey, F. *Nature* **1998**, *395*, 288.
23. Yanagida, O.; Kanai, Y.; Chairoungdua, A.; Kim, D. K.; Segawa, H.; Nii, T.; Cha, S. H.; Matsuo, H.; Fukushima, J.; Fukasawa, Y.; Tani, Y.; Taketani, Y.; Uchino, H.; Kim, J. Y.; Inatomi, J.; Okayasu, I.; Miyamoto, K.; Takeda, E.; Goya, T.; Endou, H. *Biochim. Biophys. Acta* **2001**, *1514*, 291.
24. Sakata, T.; Ferdous, G.; Tsuruta, T.; Satoh, T.; Baba, S.; Muto, T.; Ueno, A.; Kanai, Y.; Endou, H.; Okayasu, I. *Pathol. Int.* **2009**, *59*, 7.
25. Fuchs, B. C.; Bode, B. P. *Semin. Cancer Biol.* **2005**, *15*, 254.
26. Uchino, H.; Kanai, Y.; Kim, D. K.; Wempe, M. F.; Chairoungdua, A.; Morimoto, E.; Anders, M. W.; Endou, H. *Mol. Pharmacol.* **2002**, *61*, 729.
27. Langen, K.-J.; Pauleit, D.; Coenen, H. H. *Nucl. Med. Biol.* **2002**, *29*, 625.
28. Monden, Y.; Hamano Takaku, F.; Shindo Okada, N.; Nishimura, S. *Ann. N.Y. Acad. Sci.* **1999**, *886*, 109.
29. Liu, W. Q.; Vidal, M.; Olszowy, C.; Million, E.; Lenoir, C.; Dhotel, H.; Garbay, C. *J. Med. Chem.* **2004**, *47*, 1223.
30. Hamacher, K.; Coenen, H. H. *Appl. Radiat. Isot.* **2002**, *57*, 853.
31. Krasikova, R. N.; Kuznetsova, O. F.; Fedorova, O. S.; Maleev, V. I.; Saveleva, T. F.; Belokon, Y. N. *Bioorg. Med. Chem.* **2008**, *16*, 4994.
32. Germain, H.; Harris, C. S.; Renaud, F.; Warin, N. *Synth. Commun.* **2009**, *39*, 523.
33. Prante, O.; Blaser, D.; Maschauer, S.; Kuwert, T. *Nucl. Med. Biol.* **2007**, *34*, 305.
34. Prante, O.; Deichen, J. T.; Hocke, C.; Kuwert, T. *Nucl. Med. Biol.* **2004**, *31*, 365.
35. Morimoto, E.; Kanai, Y.; Kim do, K.; Chairoungdua, A.; Choi, H. W.; Wempe, M. F.; Anzai, N.; Endou, H. *J. Pharmacol. Sci.* **2008**, *108*, 505.