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Synthesis and in vitro evaluation of [¹⁸F]BMS-754807: A potential PET ligand for IGF-1R

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Abstract

Radiosynthesis and in vitro evaluation of [¹⁸F](*S*)-1-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrrolo[2,1-*f*][1,2,4]triazin-2-yl)-*N*-(6-fluoropyridin-3-yl)-2-methylpyrrolidine-2-carboxamide ([¹⁸F]BMS-754807 or [¹⁸F]**1**) a specific IGF-1R inhibitor was performed. [¹⁸F]**1** demonstrated specific binding in vitro to human cancer tissues. Synthesis of reference standard **1** and corresponding bromo derivative (**1a**), the precursor for radiolabeling were achieved from 2,4-dichloropyrrolo[2,1-*f*][1,2,4]triazine (**4**) in three steps with 50% overall yield. The radioproduct was obtained in 8% yield by reacting **1a** with [¹⁸F]TBAF in DMSO at 170 °C at high radiochemical purity and specific activity (1–2 Ci/μmol, *N* = 10). The proof of concept of IGF-1R imaging with [¹⁸F]**1** was demonstrated by in vitro autoradiography studies using pathologically identified surgically removed grade IV glioblastoma, breast cancer and pancreatic tumor tissues. These studies indicate that [¹⁸F]**1** can be a potential PET tracer for monitoring IGF-1R.

Keywords

IGF-1R; Radiotracer; PET; Phosphor imaging

Insulin-like growth factors (IGFs or IGF-I and IGF-II) are growth hormones that have high sequence homology to insulin and function as regulators of cellular proliferation, apoptosis, energy metabolism and various organ-specific functions.^{1,2} The functions of IGFs are mediated through two tyrosine kinase receptors IGF-1R, IGF-2R and IGF binding proteins (IGF-BP).^{3,4} Overexpression of IGF-1R has been found in many cancers affecting multiple aspects of malignancy and metastases. These include mesenchymal, epithelial and hematopoietic classes of a wide variety of tumors/ cancers.^{5–12} In addition, a role in brain

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development and normal brain functions, alterations of IGF-1R are also found in Alzheimer's Disease, traumatic brain injury, Amyotrophic Lateral Sclerosis, Friedreich Ataxia and aging.^{13–18} IGF-1R overexpression has also been reported in brain tumors such as glioblastoma, neuroblastoma, astrocytomas, meningiomas and medulloblastoma.^{19–22} The biological roles of IGF-1R in malignancy have been demonstrated by preclinical, epidemiological and clinical studies.^{6–8,23,24} IGF-1R is a potential therapeutic target and there are several active clinical trials evaluating anti IGF-1R effects in various diseases.^{6–8} These agents include antibodies for IGF-I, monoclonal antibodies for IGF-1R, small molecule targeting tyrosine kinase receptor inhibitors (TKRIs) and ligand binding antibodies and antisense oligonucleotides. Although clinical trials with at least six monoclonal antibody based IGF-1R therapy are still under preclinical and phases I–III clinical evaluation, many clinical trials based on antibody based IGF-1R therapy reported negative results and safety concerns.^{25–30} There is a growing interest in the development of small molecule TKRIs inhibitors of IGF-1R.^{10,24} Several small molecules based on TKRIs are in various phases of clinical investigations (phases I–III).^{7–11} The identification of noninvasive biomarkers would enable detection and quantification of IGF-1R in vivo to monitor target occupancy and treatment responses and accelerate development of medications that target IGF.

To date, five major classes of ligands have been considered for IGF-1R imaging including proteins, antibodies, peptides, affibodies and small molecule TKRIs.²³ SPECT ligands based on I¹²⁵ analogues of IGF-I were not successful in in vivo imaging IGF-I due to high protein binding and in vivo deiodination.²³ In vivo imaging of IGF-1R with ¹¹¹In-IGF1(E3R) in a mouse breast cancer xenograft tumor model shows good tumor contrast, a strong linear correlation with in vitro and in vivo IGF-1R expression level and tracer uptake.²³ R1507, a monoclonal antibody in human was radiolabeled with PET and SPECT isotopes (¹¹¹In, ⁸⁹Zr and ¹²⁵I) and tested in tumor model.³¹ Higher tumor uptake of ¹¹¹In-R1507 and ⁸⁹Zr-R1507 was observed in comparison to that of ¹²⁵I-R1507.³² ¹¹¹In-R1507 SPECT has also been utilized to predict the response to anti-IGF-1R therapy in human bone sarcoma xenografts.³³ Although antibody based imaging is successful in mice models, its translation to human study is not practical as the imaging probe requires days for equilibration, and offer poor target/nontarget contrast due to the expression of IGF-1R in normal tissues. There have been unsuccessful attempts to image IGF-1R antibodies with quantum dots using fluorescent techniques.^{23,34,35} IGF-1R based PNA has been recently radiolabeled with ⁶⁴Cu and ^{99m}Tc and tested in IGF-1R overexpressing breast cancer xenografts in mice.^{36,37} A few radioligands based on IGF-1R selected antibody have been labeled with ¹¹¹In and show modest tumor uptake and tumor to blood ratio.³⁸ Recently, [¹⁸F]FDG and [¹⁸F]FLT have been employed to assess response to IGF-1R inhibitors in preclinical and human subjects.^{39–41}

Some of the ligands listed above show promise for imaging IGF-1R, but do not cross the blood brain barrier (BBB) and hence are limited to use in imaging studies outside the brain. To quantify binding to IGF-1R in brain requires development of selective non-peptide PET ligands. High affinity, lipophilic TKRIs of IGF-1R are potential candidates for developing such imaging agents. We have screened a large number of TKRIs for this purpose and identified BMS-754807 as a small molecule dual inhibitor of IGF-1R/insulin receptor (IR), that has been clinical evaluation, as a lead ligand for imaging using PET.^{42–44} BMS-754807

(1) is an orally bioavailable, potent and reversible small molecule inhibitor of the IGF-1R/IR family kinases ($K_i < 2$ nmol/L), which is currently in phase II clinical trial for the treatment of a variety of human cancers.^{42–46} The selectivity of compound 1 over other kinases, presence of metabolically stable fluorine in the 2-substituted pyridine ring, which is amenable for radiolabeling using nucleophilic displacement with [¹⁸F]fluoride⁴⁷ and a calculated lipophilicity (clogP) 3.5, prompted us to choose it as a candidate radiotracer for imaging IGF-1R with PET.

BMS-754807 (1) and the corresponding bromo precursor (1a) for radiosynthesis, were prepared based on modification of a published procedure.⁴⁵ Preferential displacement of the C-4 chloride in compound 4 with 5-cyclopropyl-1H-pyrazol-3-amine (5) followed by displacement at C-2 position with (S)-2-methylpyrrolidine-2-carboxylic acid gave the corresponding carboxylic acid 7 in two steps with 65% yield. The carboxylic acid was then condensed with 6-bromopyridin-3-amine or 6-fluoropyridin-3-amine to yield the precursor 1a or the standard 1 in 78–70% yield (Scheme 1).⁴⁸ Radiosynthesis of [¹⁸F]BMS-754807 was initially optimized by exchange reaction with cold BMS-754807 under microwave conditions using [¹⁸F]KF/K222. However, the bromo precursor 1a did not undergo radiofluorination using [¹⁸F]KF/K222 under thermal or microwave conditions (Scheme 1). However, the radioproduct was obtained in 8% yield by treating bromo precursor 1a with [¹⁸F]TBAF in DMSO at 170 °C in high radiochemical purity (>95%) and specific activity (1–2 Ci/μmol).⁴⁹ Nucleophilic aromatic radiofluorination via the displacement of 2-bromo pyridyl system without using catalyst is widely reported in literature.⁴⁷ [¹⁸F]TBAF was in situ generated from freshly prepared tetrabutylammonium bicarbonate by azeotropic distillation with [¹⁸F]fluoride. Tetrabutylammonium bicarbonate in turn was freshly prepared by bubbling carbon dioxide from dry ice into tetrabutylammonium hydroxide (4 mL, 13% by weight in water, 0.5 M) for 4 h at room temperature. The radioproduct was stable in 10% ethanol–saline solution for over 6 h. The experimental partition coefficient (logP) of [¹⁸F]1 was measured as 2.8, indicating that the radioligand has adequate lipophilicity for brain imaging.⁵⁰

After optimizing the synthesis of [¹⁸F]1, the proof of concept of IGF-1R imaging with the radiotracer was performed in surgically removed and pathologically identified grade IV-glioblastoma, breast cancer and pancreatic tumor using phosphor imager autoradiography (Fig. 1).^{51–53} Pathologically identified frozen sections of glioblastoma IV, pancreatic tumor and breast cancer were used for phosphor image studies. The slide mounted tumor sections were brought to room temperature and incubated with [¹⁸F]1 (0.1 nM) for 1 h.⁵⁴ The adjacent sections were incubated with 1 μM of GSK1838705A, a specific IGF-1R ligand to determine nonspecific binding. After incubation, sections were washed, dried and exposed to phosphor imaging screen. As evident from Figure 1, there is specific binding of [¹⁸F]1 in the three tumor/cancer tissues tested. Grade IV glioblastoma shows higher tracer uptake and specific binding in comparison to pancreatic tumor and breast cancer tissues. The binding ratios of total versus non-specific binding obtained are 5.25 (*N* = 9), 1.92 (*N* = 4) and 1.7 (*N* = 4) for grade IV glioblastoma, pancreatic tumor and breast cancer, respectively.

In summary, we successfully synthesized [¹⁸F]1, a potential imaging agent for IGF-1R. The total time required for radiosynthesis was 60 min from EOS in 8% yield with excellent

chemical and radiochemical purities and specific activity. Autoradiography studies by phosphor imaging indicate that [^{18}F]1 binds to IGF-1R of surgically removed postmortem human glioblastoma grade IV, breast cancer and pancreatic tumor tissues. [^{18}F]BMS-754807 is the first small molecule radioligand that showed promise for imaging IGF-1R in tumor/cancer. The results indicate that [^{18}F]BMS-754807 can be a potential PET imaging agent for in vivo monitoring IGF-1R.

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BMS-754807 (**1**) and (*S*)-*N*-(6-bromopyridin-3-yl)-1-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrrolo[2,1-*f*][1,2,4]triazin-2-yl)-2-methylpyrrolidine-2-carboxamide: (*S*)-1-(4-((5-cyclopropyl-1*H*-pyrazol-3-yl)amino)pyrrolo[2,1-*f*][1,2,4]triazin-2-yl)-2-methylpyrrolidine-2-carboxylic acid (0.220 g, 0.6 mmol) in *N,N*-dimethylformamide (3 mL) was cooled to 5 °C in an ice bath. Thionyl chloride (0.057 mL, 0.78 mmol) was then added dropwise. The reaction mixture was stirred at 5–20 °C for 1 h. 6-Fluoropyridin-3-amine (0.201 mg, 1.8 mmol) or 6-bromopyridin-3-amine (0.311 g, 1.80 mmol) in 1 mL of DMF was then added in one portion and the reaction mixture was stirred at rt for 24 h. The reaction mixture was then poured into 0.5 N NaHCO₃ solution (20 mL). The resulting suspension was then stirred at rt for 1 h. The suspension was then extracted with EtOAc (3 × 50 mL), dried over MgSO₄, concentrated and column chromatographed using 50:50 EtOAc/hexane to yield **1** (192 mg, 70%) or **1a** as a colorless solid (244 mg, 78%). The spectral data for **1** were identical to that reported for reported compound⁴⁵
1a: ¹H NMR (400 MHz, CDCl₃) δ 8.98 (s, 1H), 8.27 (d, *J* = 3.1 Hz, 1H), 7.96–7.76 (m, 2H), 7.48–7.31 (m, 2H), 6.56 (ddd, *J* = 23.2, 4.8, 2.2 Hz, 2H), 6.19 (d, *J* = 3.6 Hz, 1H), 3.89–3.67 (m, 2H), 2.60 (td, *J* = 9.9, 8.5, 5.0 Hz, 1H), 2.20–1.88 (m, 2H), 1.75 (s, 3H), 1.72–1.67 (m, 1H), 1.34–1.19 (m, 1H), 1.02–0.86 (m, 2H), 0.81–0.58 (m, 2H); HRMS Calcd For C₂₃H₂₅BrN₉O (MH⁺): 522.1365. Found: 522.1376.

49.

Radiosynthesis of [¹⁸F]**1**: 1–2 mg of **1a** in 0.5 mL DMSO was added to a freshly prepared and dried [¹⁸F]TBAF. The reaction mixture was heated in a sealed vial for 30 min at 170 °C. The crude product was directly injected into a semi preparative RP-HPLC (phenomenex C18, 10 × 250 mm, 10) and eluted with acetonitrile: 0.1 M ammonium formate solution (45:55) at a flow rate of 10 mL/min. The precursor eluted after 10–11 min during the HPLC analysis. The product fraction with a retention time of 7–8 min based on γ-detector was collected, diluted with 50 mL of deionized water, and passed through a C-18 Sep-Pak[®] cartridge. Reconstitution of the product in 1 mL of absolute ethanol afforded [¹⁸F]**1** (8 ± 2% yield, based on [¹⁸F]F[−] at EOS). A portion of the ethanol solution was analyzed by analytical RP-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μ; mobile phase: acetonitrile/0.1 M ammonium formate; 45–55, flow rate: 2 mL/min, retention time: 7 min, wavelength: 254 nm) to determine the specific activity and radiochemical purity. The ethanol solution of [¹⁸F]**1** was used for further studies.

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54.

In vitro phosphor image with [¹⁸F]**1**: the in vitro experiments were performed using pathologically identified surgically removed glioblastoma grade IV, pancreatic cancer and breast cancer tissues obtained from Tissue bank of Herbert Irving Comprehensive Cancer Center, Columbia University. The frozen tumor tissues obtained were sectioned (20 μm) were collected onto glass slides. The sections were allowed to dry and stored at −80 °C until use. The phosphor image autoradiography studies were described previously^{51–53} and were used for the phosphor image studies. Four

sections each for total and nonspecific binding were assayed. The slides mounted sections were brought to room temperature and were incubated with 50 mM Tris-HCl, 5 mM of KCl and 150 mM of NaCl at pH 7.4 containing [^{18}F]**1** (0.1 nM) for 1 h. The adjacent sections were incubated with 1 μM of GSK1838705A for 1 h to determine non specific binding. After the incubation, sections were washed with ice-cold buffer containing 50 mM Tris-buffer (pH 7.4) at 4 °C and briefly dipped in ice-cold water to remove salts. Slides were quickly dried under a stream of cold air and exposed to a phosphor-imaging screen (Packard, ST screen wrapped in Mylar film) with high- and low-activity standards (American Radiolabeled Chemicals) for 60 min. Screens are scanned with a Packard Cyclone phosphorimaging system and analyzed with OptiQuant Acquisition and Analysis software (Packard).

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Figure 1.

Phosphor image of [^{18}F]**1** in surgically removed cancer tissues. Column 1: total binding of [^{18}F]**1** in glioblastoma IV sections; column 2: nonspecific binding of [^{18}F]**1** in glioblastoma IV sections; column 3: total binding of [^{18}F]**1** in pancreatic tumor sections; column 4: nonspecific binding of [^{18}F]**1** in pancreatic tumor sections; column 5: total binding of [^{18}F]**1** in breast cancer tissues; column 6: nonspecific binding of [^{18}F]**1** in breast cancer tissues.

Synthesis and radiosynthesis of BMS-754807 and [¹⁸F]BMS-754807.