Nuclear Medicine and Biology 42 (2015) 399–405

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Synthesis and pharmacological evaluation of ^{11}C-labeled piperazine derivative as a PET probe for sigma-2 receptor imaging

Svetlana V. Selivanova [a,*], Annamaria Toscano [b], Carmen Abate [b,*], Francesco Berardi [b], Adrienne Müller [a], Stefanie D. Krämer [a], Roger Schibli [a], Simon M. Ametamey [a]

[a] Center for Radiopharmaceutical Sciences, ETH Zurich, Zurich, Switzerland
[b] Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari ALDO MORO, Bari, Italy

ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 26 December 2014
Accepted 29 December 2014

Keywords:
Sigma receptors
Sigma-2
PET
Imaging
Carbon-11
Cancer

ABSTRACT

Introduction: Both subtypes of sigma (σ) receptors, σ_1 and σ_2, are over-expressed in many cancers with σ_2 proposed as a biomarker of tumor proliferation. We are interested in developing a high affinity selective σ_2 radioligand for *in vivo* monitoring of proliferative status of solid tumors and response to anti-cancer therapies. 1-Cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperazine (PB28) represents one of the lead candidates in the development of σ receptor ligands for therapeutic and diagnostic applications. However, the utility of PB28 is limited due to its relatively high lipophilicity.
Methods: A more hydrophilic analogue (−)-(*S*)-**1** was radiolabeled with ^{11}C via standard *O*-alkylation. *In vitro* autoradiography with [^{11}C](−)-(*S*)-**1** was done using rat brain slices. PET imaging was performed in mice bearing EMT6, C6 or PC-3 tumors after i.v. injection of [^{11}C](−)-(*S*)-**1**.
Results: [^{11}C](−)-(*S*)-**1** was produced in 53% ± 7% isolated decay-corrected yield with radiochemical and chemical purity over 99% and specific activity greater than 100 GBq/μmol. *In vitro* autoradiography with [^{11}C(−)-(*S*)-**1** resulted in a heterogeneous binding of the tracer in the rat brain with the highest radioactivity signals in the cortex region followed by cerebellum. This binding was successfully blocked by 10 μM of either haloperidol, (+)-(*R*)-**1** or PB28. For C6 xenografts low target-to-nontarget ratio and high non-specific binding did not allow clear tumor visualization. No accumulation was visible in EMT6 tumor or in PC-3 tumor. Rat and mouse brain uptake was low and homogeneous while stronger signal was detected in the spinal cord. High accumulation of radioactivity was observed in liver and intestine suggesting hepatobiliary clearance.
Conclusions: Despite excellent *in vitro* properties, [^{11}C](−)-(*S*)-**1** did not provide high enough specific binding *in vivo* and is, therefore, not a useful PET tracer for imaging σ_2 expression in tumors.

1. Introduction

Sigma (σ) receptors were discovered in 1976 and were first believed to represent a subtype of opiate receptors, which were responsible for modulating the behavioral effect of *N*-allylnormethazocine (SKF 10,047) [1]. Later it was shown that σ receptors are distinct from opiate or phencyclidine (PCP) binding sites [2] and are expressed in the central nervous system (CNS), lung, heart, liver, spleen, kidneys, adrenal gland, and reproductive organs [3]. This family of proteins consists of two recognized σ receptor subtypes: σ_1 and σ_2, based on their opposite enantioselectivity towards benzomorphans [4].

The σ_1 receptor has a molecular mass of 25.3 kD and has been cloned in 1996 from a variety of tissues and species, including human placenta and brain [5,6]. The protein consists of 223 amino acids and locates predominantly at the endoplasmic reticulum [6]. Its amino-acid sequence shares structural homology with fungal proteins involved in sterol synthesis and does not relate to known human proteins [5]. A single transmembrane-spanning structure was first suggested for σ_1 receptor [5,6] but it is accepted now that the protein consists of two transmembrane segments with the carboxy and the amino termini on the intracellular side [7,8]. There is evidence that σ_1 receptors play a role in neuroprotection and neuroplasticity [9,10].

The structure of σ_2 receptor, its intracellular localization and mechanistic pathways are still under investigation. Recently σ_2 receptor has been identified as the progesterone receptor membrane component 1 (PGRMC1) protein [11,12]. It was shown that σ_2 receptors are over-expressed in many types of human cancer cells, including strongly metastatic breast cancer [13]. Moreover, the densities of σ_2 receptors are ten-fold higher in proliferating as opposed to quiescent or dormant cancer cells, which suggests their potential use as a biomarker of tumor proliferation [14]. Stimulation of σ_2 receptors activates multiple apoptotic pathways in cancer cell, including binding those in drug-resistant tumors [15]. σ_2 Receptors participate in CNS processes, in one instance by affecting Ca^{2+} channels in neurons [16]. σ_2 Antagonists were shown to reduce cocaine toxicity and side effects [17] and, by blocking

* Corresponding authors at: Sherbrooke Molecular Imaging Centre, CRCHUS, 3001, 12e Avenue Nord, Sherbrooke, QC, J1H 5N4, Canada. Tel.: +1 819 569 2534x16637.
E-mail addresses: svetlana.v.selivanova@usherbrooke.ca (S.V. Selivanova), abate@farmchim.uniba.it (C. Abate).

http://dx.doi.org/10.1016/j.nucmedbio.2014.12.018
0969-8051/

amyloid β oligomers binding to neurons, improve cognitive deficit in Alzheimer's disease model in mice [18]. Sigma receptors are considered as an interesting and promising therapeutic target due to their implication in cancer proliferation as well as in some other pathologies where pro-inflammatory cytokines are involved [19]. Highly potent and selective ligands for each σ subtype are sought to advance an understanding of the role, which these proteins play in evolving of the diseases, and to facilitate drug development for this therapeutic target.

1-Cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl) propyl]piperazine (PB28, Fig. 1) represents one of the lead candidates in the development of σ receptor ligands for therapeutic and diagnostic applications [20]. However, its utility is limited due to relatively high lipophilicity ($\log D_{7.4} = 3.99$) [21]. Recently, we reported several novel σ_2 receptor ligands with improved physical properties, including a more hydrophilic analogue, 2-(4-cyclohexylpiperazin-1-yl)-*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetamide (**1**) (Fig. 1, $\log D_{7.4} = 2.38$) [21]. Binding affinity (K_i) of its *S*-enantiomer, (−)-(*S*)-**1**, to σ_2 was 5.92 ± 0.52 nM with ~15-fold selectivity over σ_1 (Table 1). Due to these properties and lack of antiproliferative activity in SK-N-SH cells [21], we focused our studies on (−)-(*S*)-**1** and evaluated its potential as a tracer for imaging σ_2 receptors using positron emission tomography (PET).

2. Materials and methods

2.1. General

Chemicals were purchased from Aldrich and Acros and were used as received without further purification. Flash column chromatography was performed with 60 Å pore size silica gel as the stationary phase (1:15 w/w, 15–40 μm particle size, Merck). ^{1}H NMR spectra were recorded on a Mercury Varian 300 MHz using $CDCl_3$ as solvent. The following data were reported: chemical shift (δ) in ppm, peak shape (s = singlet, d = doublet, t = triplet, m = multiplet), integration and coupling constant(s) in hertz. Mass spectra were recorded on an Agilent 6890-5973 MSD gas chromatograph/mass spectrometer and on an Agilent 1100 series LC-MSD trap system VL mass spectrometer. Only significant *m/z* peaks, with their percentage of relative intensity in parentheses, are reported. Optical rotations were measured with a PerkinElmer 341 Polarimeter at room temperature (20 °C); concentrations are expressed as g/100 mL. Enantiomeric purity was established by HPLC using Daicel Chiralcel OD column with *n*-hexane/*i*-propylamine/diethylamine (9:1:0.1) as eluent, flow rate 0.5 mL/min, λ = 280 nm. Semi-preparative HPLC for radiolabeled product purification was carried out using an HPLC system equipped with a Merck-Hitachi L-6200A Intelligent pump, a 5 mL injection loop, a Knauer Variable Wavelength Monitor UV-detector, and an Eberline RM-14 radiodetector with a reversed-phase Waters μBondapack C18, 10 μm, 125 Å, 300 × 7.8 mm column. The mobile phase consisted of acetonitrile and 50 mM NH_4COOH buffer (pH 4.45). The gradient was: 0–10 min, isocratic 5% acetonitrile; 10–40 min, 5% → 65% acetonitrile and eluted at 4 mL/min flow rate. UV-absorbance was detected at 235 nm. Analytical HPLC was an Agilent 1100 series system equipped with a Raytest Gabi Star radiodetector. Analytical reversed-phase column was Agilent Eclipse XDB-C18, particle size 5 μm, 50 × 4.6 mm. A gradient of acetonitrile in aqueous 50 mM NH_4COOH buffer (pH 4.45) from 10% to 65% over 13 min at a flow rate of 1 mL/min was used. Purity of tested compounds was established by HPLC, confirming ≥95% purity.

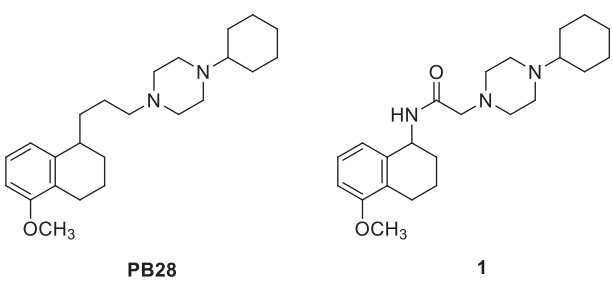

Fig. 1. Chemical structures of lead compound PB28 and novel ligand **1**.

Table 1
Binding properties and experimentally found lipophilicity values for studied compounds [20].

Compound	$\log D_{7.4}$	K_i ± SEM (nM)	
		σ_1[a]	σ_2[b]
(±)-**PB28**	3.99	0.38 ± 0.10	0.68 ± 0.20
(±)-**1**	2.38	12.6 ± 4.1	13.3 ± 2.9
(+)-(*R*)-**1**		20.4 ± 7.6	10.1 ± 2.54
(−)-(*S*)-**1**		94.6 ± 12.6	5.92 ± 0.52
Haloperidol[c]		0.90 ± 0.067	7.93 ± 0.46

[a] Guinea pig brain membranes without cerebellum;
[b] Rat liver membrains;
[c] Ref. [32], guinea pig brain homogenates.

2.2. Chemistry

2.2.1. (+)-(*R*)- and (−)-(*S*)-2-(4-cyclohexylpiperazin-1-yl)-*N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetamide (1)

Enantiomers of compound **1** were synthesized accordingly to previously published procedure [21].

(+)-(*R*)-**1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 1.00–1.35 (m, 5H, cyclohexyl), 1.55–2.05 (m, 9H, cyclohexyl, $ArCH_2CH_2CH_2$), 2.20–2.40 (m, 1H, cyclohexyl CHN), 2.55–2.78 (m, 10H, piperazine, benzyl CH_2), 3.00–3.08 (m, 2H, CH_2CO), 3.80 (s, 3H, OCH_3), 5.05–5.15 (m, 1H, NHC*H*), 6.65–7.20 (m, 3H, aromatic), 7.35 (br s, 1H, NH, D_2O exchange); LC-MS (ESI+) *m/z* 386 $[M + H]^+$. $[\alpha]_D = +48.5°$ (c = 0.75, MeOH), *ee* >99%.

(−)-(*S*)-**1**. ^{1}H NMR and LC-MS are the same as for (+)-(R)-**1**. $[\alpha]_D = -51.6°$ (c = 0.68, MeOH), *ee* >99%.

2.2.2. (*S*)-2-(4-cyclohexylpiperazin-1-yl)-*N*-(5-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetamide (2)

To a solution of compound (−)-(*S*)-**1** (1 mmol) in dry CH_2Cl_2 cooled at −78 °C, a solution of BBr_3 (1 M in CH_2Cl_2, 10 mmol, 10.0 mL) was added dropwise via syringe under argon atmosphere. After complete addition of BBr_3, the mixture was stirred at −40 °C for 5 h. The reaction mixture was poured into ice-water and basified with 1 M NaOH to reach pH 8. The reaction mixture was extracted with CH_2Cl_2 (4 × 20 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography with CH_2Cl_2/MeOH 9:1 as eluent. The title compound was obtained as a pale yellow solid in 65% yield. TLC: SiO_2, CH_2Cl_2/MeOH 8.5:1.5, $R_f = 0.60$. ^{1}H NMR (300 MHz, $CDCl_3$) δ, ppm: 1.02–1.33 (m, 5H, cyclohexyl), 1.57–1.91 (m, 7H, $ArCH_2CH_2$ and cyclohexyl), 1.98–2.07 (m, 2H, $NHCHCH_2$), 2.19–2.29 [m, 1H, $(CH_2)_2NCH$], 2.45–2.77 (m, 10H, $ArCH_2$, piperazine), 3.00–3.13 (m, 2H, $NHCOCH_2$), 5.13–5.21 (m, 1H, CONHC*H*), 6.71 (d, 1H, *J* = 7.6 Hz, aromatic), 6.78 (d, 1H, *J* = 7.6 Hz, aromatic), 7.02 (t, 1H, *J* = 7.6 Hz, aromatic), 7.41 (br s, 1H, N*H*CO); GC-MS *m/z* 371 (M^+, 1), 181 (100).

2.2.3. (*S*)-2-(4-cyclohexylpiperazin-1-yl)-*N*-[5-(2-fluoro-ethoxy)-1,2,3,4-tetrahydronaphthalen-1-yl]acetamide (2)

A solution of compound (*S*)-**2** (0.27 mmol, 0.10 g) in DMF (3 mL) was added with 2-fluoroethyl-4-methylbenzenesulfonate (0.54 mmol, 0.12 mg) and K_2CO_3 (1.1 mmol, 0.15 g). The mixture was stirred under reflux overnight. After cooling, H_2O was added (75 mL) and the mixture was extracted with ethyl acetate (4 × 50 mL). The collected organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography with CH_2Cl_2/MeOH 9:1 as eluent to