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# Comparative evaluation of 4 and 6-carbon spacer conformationally flexible tetrahydroisoquinolinyl benzamide analogues for imaging the sigma-2 receptor status of solid tumors

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ABSTRACT

*Introduction:* Nine novel analogues were synthesized including a 6-carbon spacer analogue of ISO-1 (**7**). They have moderate binding affinity for sigma-2 ($\sigma_2$) receptors and high selectivity for $\sigma_2$ receptors relative to sigma-1 ($\sigma_1$) receptors.
*Methods:* ([$^{18}$F]**7**) was synthesized and evaluated as a candidate ligand for positron emission (PET) imaging of the $\sigma_2$ receptor in tumors. Radioligand [$^{18}$F]**7** was radiolabeled with $^{18}$F *via* displacement of the corresponding mesylate precursor with [$^{18}$F]fluoride. Cellular uptake study of [$^{18}$F]**7** was performed in EMT-6 tumor cell, and *in vivo* biodistribution study of [$^{18}$F]**7** and microPET imaging study of [$^{18}$F]**3** and [$^{18}$F]**7** carried out in female Balb/c mice bearing EMT-6 tumors.
*Results:* [$^{18}$F]**7** had a respectable tumor uptake (1.55%ID/g at 60 min post-injection) and high tumor/muscle ratios at 60 and 120 min post-injection. MicroPET imaging of [$^{18}$F]**7** in tumor-bearing mice as above showed significant tumor localization and a high tumor/muscle ratio as well.
*Conclusions:* These results are similar to or better than [$^{18}$F]ISO-1 ([$^{18}$F]**3**), which indicates that [$^{18}$F]**7** has potential for imaging the $\sigma_2$ receptor status of solid tumors.



## 1. Introduction

Sigma ($\sigma$) receptors represent a class of proteins that were initially thought to be a type of opioid receptor [1]. It has been established that $\sigma$ receptors are a distinct class of receptors, largely based on *in vitro* binding and behavioral pharmacology studies [2].

$\sigma$ Receptors are present in many normal tissues including liver, kidney, endocrine glands, and the central nervous system (CNS) [3]. Two subtypes have been pharmacologically established as $\sigma_1$ and $\sigma_2$ receptors [4,5]. The $\sigma_1$ receptor has been cloned from tissues of guinea pig, rat, mouse, and man [6], and the crystal structure of the protein reveals a trimeric architecture consisting of a single trans-membrane domain in each protomer [7]. The $\sigma_2$ receptor has not been cloned yet, but our group has reported that the putative $\sigma_2$ receptor binding site resides within the PGRMC1 (progesterone receptor membrane component 1) protein complex [8]. However, recent studies using stable cell lines where the PGRMC1 had been knocked out using either a shRNA or CRISPR vector for the PGRMC1 have shown that there is no decrease in binding of [$^3$H]DTG binding in PGRMC1 knockout cells [9,10]. These data indicate that the DTG-sensitive $\sigma_2$ receptor binding site is not located within the amino acid sequence of the PGRMC1, and supports the need for further studies in the identification of the $\sigma_2$ receptor.

Although the molecular identity of the receptor is subject to debate, it is known that $\sigma_2$ receptors are overexpressed in a wide variety of human and murine tumor cell lines and solid tumors such as breast adenocarcinoma, neuroblastoma, leukemia, glioblastoma, lung carcinomas, renal carcinoma, colon carcinoma, sarcoma, urinary bladder tumor, pancreatic cancer, and prostate cancer [11–24]. The density of $\sigma_2$ receptors has been reported to be more abundant than $\sigma_1$ receptors in a wide panel of tumor cells grown under cell culture conditions [12]. In addition, the density of $\sigma_2$ receptors was found to be 8–10-fold higher in proliferating *vs.* quiescent mouse mammary adenocarcinoma cells both *in vitro* [24] and *in vivo* [23]. This correlation between $\sigma_2$ receptor density and the proliferative status of tumor cells has been reported to be independent of other factors, biological or physiological, such as species, cell types, ploidy, cell–cell contact, nutrient depletion, low pH, altered metabolic states, or tumor size [23,24]. Our group has also reported the localization of $\sigma_2$ receptors in the mitochondria, lysosomes, endoplasmic reticulum, and plasma membrane of breast cancer cells using two-photon and confocal microscopy probes [25].

In addition to being an excellent biomarker of cell proliferation for imaging studies, the $\sigma_2$ receptor has also proven to be a promising target either as a monotherapy for treating cancer or as a mechanism for delivering a therapeutic cargo to cancer cells [11,22,26–29]. Therefore, radiolabeled $\sigma_2$ receptor ligands can be used to assess the proliferative status of solid tumors with positron emission tomography (PET) and

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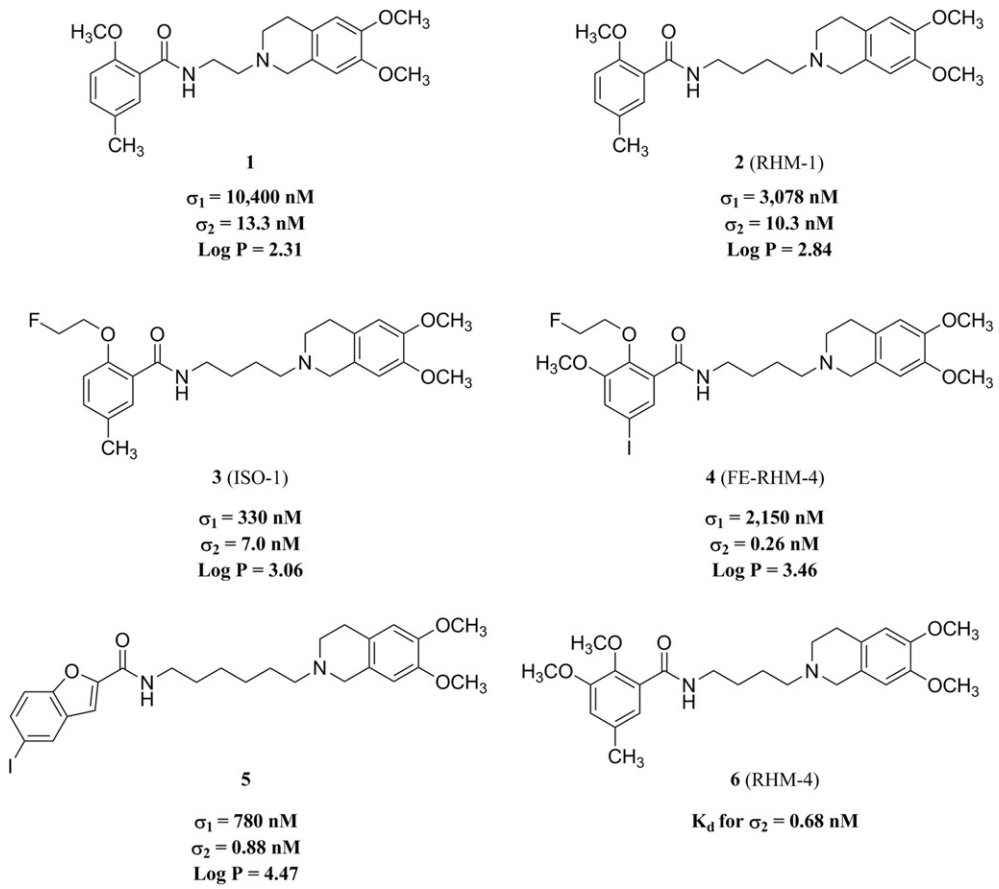


**Fig. 1.** Structures, binding affinities for $\sigma$ receptors, and *log P* of benzamide analogues.

single photon emission computed tomography (SPECT), as well as serving as a companion diagnostic for identifying tumors that would respond to $\sigma_2$ receptor targeting therapeutics [29–33].

Our group has previously radiolabeled several $\sigma_2$ receptor ligands having a high affinity for $\sigma_2$ receptors and excellent $\sigma_2$:$\sigma_1$ selectivity ratios as potential PET tracers for clinical research studies. The initial *in vitro* and *in vivo* evaluation of these radiotracers has been reported previously [34–37]. The 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline moiety is an optimal structure to increase binding affinity and selectivity for $\sigma_2$ receptors [36,38–40]. Among these compounds, $^{11}$C-radiolabeled benzamide analogues have high binding affinity and selectivity for $\sigma_2$ receptors (Fig. 1), and a good relationship between tumor uptake and lipophilicity of those compounds has been shown [38]. Although $^{11}$C-labeled benzamide analogues have a high potential for PET imaging of $\sigma_2$ receptors (compounds **1** and **2**, Fig. 1), we also developed $^{18}$F-labeled benzamide analogues (compounds **3** and **4**, Fig. 1) due to the short half-life of $^{11}$C (20.4 min) [36]. Recently, the first human imaging studies of the $\sigma_2$ receptor ligand [$^{18}$F]ISO-1 ([$^{18}$F]**3**) were accomplished and demonstrated the potential utility of this compound in imaging the proliferative status of solid tumors [41]. However, it has been reported that 1,2,3,4-tetrahydroisoquinoline with the flexible 6-carbon spacer, and benzofuran ring as the benzamide moiety (**5**, Fig. 1) has higher binding affinities and selectivity for $\sigma_2$ receptors than the 4 and 5-carbon spacer group, and the authors also suggested that the corresponding indole and benzothiophene analogues should have improved binding affinities and selectivity for $\sigma_2$ receptors [40].

In this study, we further explored this structural optimization strategy by increasing the carbon spacer length from four to six carbons for hydrophobic spacer, and replaced the benzamide ring with either an indole or benzothiophene ring (Fig. 2). We also synthesized the 6-carbon spacer analogue of ISO-1 and evaluated the compounds for their *in vitro* binding properties to the $\sigma$ receptors. The *in vivo* properties of [$^{18}$F]ISO-1 (4-carbon spacer) ([$^{18}$F]**3**), a well-established $\sigma_2$ radioligand, and [$^{18}$F] **7,** the corresponding [$^{18}$F]-labeled 6-carbon spacer analogue of ISO-1, were also compared.

## 2. Materials and methods

### 2.1. Reagents and equipment

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Alfa Aesar (Ward Hill, MA, USA). $^1$H NMR spectra were obtained using a Bruker DMX 360 (360 MHz) spectrometer (Rheinstetten, Germany), and chemical shifts ($\delta$) were reported as the ppm downfield of the internal tetramethylsilane. LCMS (Liquid Chromatography Mass Spectroscopy) and HRMS (High Resolution Mass Spectroscopy) were obtained using a Waters microMass zQ and LCT Premier XE LC/MS system (Waters Corporations, Milford, MA, USA). For purification and analysis of radioligands, HPLC analysis was conducted using the Agilent 1100 (Agilent Technologies, Santa Clara, CA, USA) equipped with a semi-preparative column (Agilent SB-C18, 5 μm, 9.4 × 100 mm) or Waters Alliance e2695 HPLC (Waters Corporations, Milford, MA, USA) equipped with an analytical column (Agilent ZORBAX Eclipse XDB-C18, 5 μm, 4.6 × 150 mm). The eluent was monitored simultaneously using UV (281 nm) and NaI(T1) radioactivity detectors. TLC was performed on Merck $F_{254}$ silica plates and analyzed on a Bioscan Mini-Scan TLC Imaging Scanner (Hopkinson, MA, USA).

[$^{18}$F]Fluoride was produced by the $^{18}$O(p,n)$^{18}$F reaction using an IBA Cyclone® 18 (Louvain-la-Neuve, Belgium) or JSW (Tokyo, Japan). Radioactivity was measured in a dose calibrator (Capintec, Ramsey, NJ, USA). Binding studies were performed using a Unifilter 96 harvester (Perkin Elmer, Boston, MA, USA), and bound radioactivity was counted