Synthesis and Characterization of [^{125}I]-*N*-(*N*-Benzylpiperidin-4-yl)-4-iodobenzamide, a New σ Receptor Radiopharmaceutical: High-Affinity Binding to MCF-7 Breast Tumor Cells

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Breast cancer is a major cause of death for women, and estrogen receptors have been reported to play a major role in the development and growth of breast tumors. Deprivation of estrogen is one of the clinically effective methods for the treatment of breast cancer patients. Several growth factors such as insulin-like growth factor I (IGF-I), transforming growth factors (TGF-α and -β), epidermal growth factor (EGF), and platelet-derived growth factors have been shown to be involved in the growth and progression of human breast cancer cells.[1] Some growth factors such as TGF-β act as inhibitors of tumor growth. Despite the development of numerous antiestrogen and other drugs, the clinical utility of antiestrogen is limited due to resistance by the tumor cells. Therefore, the early detection of mammary tumors would enable better patient management and probably prolong the life of such patients.

σ receptors are a relatively new class of receptors that were confused earlier with opioid receptors due to the binding and actions of (±)-*N*-allylnormetazocine and structurally related benzomorphans.[2] However, the definition of σ receptors has changed in recent years as it was discovered that (−) enantiomers of benzomorphans bind to opioid receptors whereas (+) enantiomers bound preferentially to σ receptors. A variety of compounds such as the antipsychotic drugs haloperidol and fluphenazine, guanidines, aminotetralins, arylethylenediamines, and steroids bind to σ receptors.[2] Moreover, two σ subtypes, termed as σ-1 and σ-2, have been identified recently, distinguished by their molecular sizes and affinities for (+)-benzomorphans.[3,4] σ receptors are present throughout the central nervous system (CNS), as well as in peripheral tissues such as liver and kidney and endocrine glands such as ovary, adrenal, testis, and pituitary.[2,5] The biochemical and physiological functions of σ receptors are not yet completely understood. Since many antipsychotic drugs bind to σ receptor sites, it was postulated that the σ ligands may be ultimately used as antipsychotics without the extrapyramidal effects that are generally associated with D-2 dopaminergic antagonists.[6-8] A role of σ receptors in dystonia and in the regulation of motor functions has been suggested.[9-11] It has also been suggested that σ receptors may subserve an interaction between the nervous, immune, and endocrine systems.[12]

Our interest in developing new high affinity σ ligands stemmed from our recent finding that σ receptors are expressed in human melanoma cells. We have recently shown that σ-1 receptors are present in human malignant melanoma[13] C6 glioma cells[14] and non-small cell lung carcinoma cells[15] and that the σ-1-selective ligands [^{125}I]-*N*-[2-(piperidinylamino)ethyl]-4-iodobenzamide ([^{125}I]-PAB) and [^{125}I]-*N*-[2-(diethylamino)ethyl]-4-iodobenzamide ([^{125}I]DAB) were bound with high affinity (K_i = 6 and 8 nM, respectively) to human malignant melanoma cells A2058. Furthermore, we showed that σ receptors could be used as external markers for imaging malignant melanoma tumoral xenografts in nude mice model. It has also been shown by us that σ-2 binding sites are expressed in several neural and nonneural tumor-derived clonal cells.[3,16] Another independent finding by Coscia and coworkers[17,18] showed the expression of σ receptors in many human tumor (renal carcinoma, colon carcinoma, etc.) biopsied samples. It was also shown by them that the density (B_{max}) of σ receptors in the neoplastic tissue was two times greater than density of σ receptors in the normal tissues. In human clinical trials in France, [^{123}I]DAB has been successfully used to image malignant melanoma in humans.[19] Encouraged by these findings and successful results for imaging human malignant melanoma xenografts,[13,20,21] we decided to develop new high-affinity σ receptor-specific radiopharmaceuticals that could be used for noninvasive imaging of human malignancies in conjunction with single photon emission computed tomography (SPECT) and positron emission tomography (PET). A σ ligand that possessed high affinity for both σ-1 and σ-2 receptors could potentially be used to image many primary tumor and metastatic sites as well. While studying structure–affinity relationship (SAR) of iodobenzamide analogs of IPAB, we discovered a new class of structurally related compounds that possessed high affinity for both σ-1 and σ-2 sites. Herein, we report the synthesis and characterization of [^{125}I]-4-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide (4-[^{125}I]BP) and other related analogs as high-affinity σ radiopharmaceuticals and high-affinity binding of 4-[^{125}I]BP to σ receptors present on human breast carcinoma cell line MCF-7.

The halogen-substituted benzamides **Ia–c** were synthesized from the appropriately substituted iodobenzoic acids. The acids were converted to acyl chlorides in quantitative yields using thionyl chloride as the chlorinating agent and DMF as a catalyst. The acid chlorides were then condensed with 4-amino-1-benzylpiperidine in the presence of triethylamine to give high yields of benzamides. In order to obtain high specific activity of the radioiodinated [^{125}I]-*N*-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide, **III**, 4-(tributylstannyl)benzamide, **II**, precursor was synthesized from the corresponding iodo derivative, **Ic**. The reaction of 4-iodobenzamide **Ic** with bis(tributyltin) in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in triethylamine after an overnight reflux gave modest yields of 4-(tributylstannyl)-benzamide, **II**. All compounds were characterized using multinuclear NMR and mass spectroscopy and elemental analysis. The radioiodination was achieved by the oxidative iododestannylation reaction of 4-(tributylstannyl)-benzamide, **II**, and Na^{125}I (carrier free) using chloramine-T

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Table 1. Inhibition Constants (K_i = nM) for the Binding Affinities of IBP Isomers in Various Receptors

compd	σ-1 guinea pig brain [^{3}H]-(+)-pent.	σ-2 rat liver [^{3}H]DTG + DEX.	dopamine D-2 rat brain [^{3}H]-(−)-sulpiride	PCP rat brain [^{3}H]TCP	musc rat brain [^{3}H]QNB
4-IBP	1.70 ± 0.44	25.2 ± 1.28	382 ± 39	>100 000	>100 000
3-IBP	3.02 ± 1.06	84.6 ± 2.5	24.8 ± 0.02	>100 000	>100 000
2-IBP	1.64 ± 0.15	29.6 ± 0.49	63.4 ± 10.8	>100 000	>100 000

or hydrogen peroxide as an oxidizing agent using the reported method.[13,20] The radiolabeled iodobenzamide was purified using reverse-phase HPLC. The radiochemical yield ranged between 81 and 92%, and the specific activity was assumed to be 2200 Ci/mmol (theoretical specific activity of carrier free Na^{125}I). The retention time for the radiolabeled benzamide, **III**, was the same as the "cold" authentic sample of *N*-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide, **Ic**, under the same HPLC conditions.

The binding affinities of three unlabeled IBP regioisomers were studied for σ-1 receptors using the σ-1 selective ligand [^{3}H]-(+)-pentazocine[22] in guinea pig brain. Rat liver has been shown to be enriched in σ-2 receptors.[5a] σ-2 profiles were studied in this tissue using the σ subtype nonselective ligand [^{3}H]DTG[23] in the presence of dextrallorphan (DEX) to mask σ-1 sites.[5a,16,22a] Many structurally related benzamides have been reported recently to bind D-2 dopamine receptors in the CNS.[24] We therefore studied the inhibition of binding of [^{3}H]-(−)-sulpiride using IBP derivatives in the membrane homogenates of rat whole brain (minus cerebellum). The affinity for phencyclidine receptors (PCP) and muscarinic receptors were studied using [^{3}H][1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) and [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) respectively in rat brain membranes. The inhibition constants of the three IBP isomers are listed in Table 1. All three compounds showed high affinity in σ-1 assay (K_i = 1.64–3.02 nM); 2-IBP, **Ia** and 4-IBP, **Ic** showed moderate affinity for σ-2 sites (K_i = 29.6 and 25.2 nM, respectively), with 3-IBP having a moderate to low σ-2 affinity of 84.6 nM. While the 2- and 3-isomers exhibited reasonable affinity for D-2 dopamine receptors, 4-IBP showed low dopamine receptor affinity (K_i = 382 nM). All three compounds were found to have no affinity for phencyclidine and muscarinic receptor sites. The homologous displacement cell binding assay for binding of radiolabeled [^{125}I]BP to MCF-7 breast cancer cell line showed a high-affinity (K_i = 1.6 ± 0.7 nM) binding. In order to determine that the specificity of binding was related to σ receptor sites in MCF-7 cells, the inhibition of binding of [^{125}I]BP in MCF-7 cells with DTG and haloperidol (known selective and nonselective σ ligands, respectively) was studied. A high-affinity, dose-dependent decrease of specific binding was found. K_i for DTG and haloperidol were found to be 60 ± 10 and 4.6 ± 0.9 nM, respectively. The displacement curves are shown in Figure 1. Furthermore, the saturation binding (Scatchard analysis) of [^{3}H]DTG in the membrane preparations of MCF-7 cells gave K_d of 38.2 nM and a B_{max} of 3867 fmol/mg of protein, confirming the presence of σ sites in these cells.

In conclusion, the synthesis and characterization of three regioisomers of IBP and their pharmacological profiles are reported. Initial pharmacological characterization of 4-[^{125}I]BP showed that it is a new high-affinity ligand (having a high affinity in both σ-1 and σ-2 assays). The competition binding studies in MCF-7 cells indicated that σ receptors are expressed in human breast tumor cells. A high density of these receptors is present in MCF-7 cells as determined from Scatchard analysis using [^{3}H]DTG.

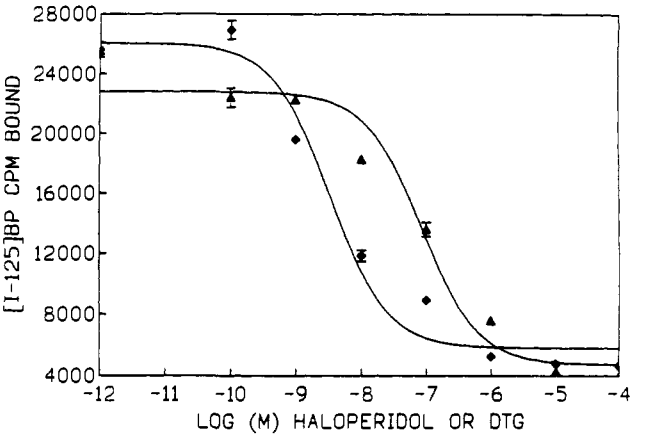
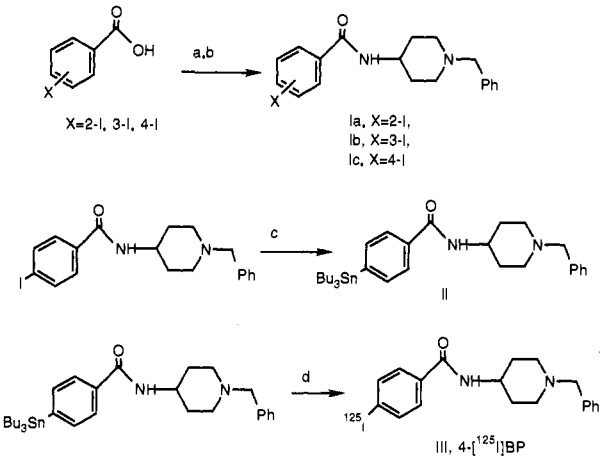


Figure 1. The competition assays for the binding of [^{125}I]BP with σ ligands in MCF-7 breast tumor cells. Solid diamonds indicate haloperidol inhibition and the solid triangles indicate DTG inhibition of [^{125}I]BP binding.

Scheme 1. Synthesis of [^{125}I]-*N*-(*N*-Benzylpiperidin-4-yl)-4-iodobenzamide (4-[^{125}I]BP)[a]



[a] (a) $SOCl_2$; (b) NEt_3, 1-benzyl-4-aminopiperidine; (c) $Sn(Bu_3)_2$, $Pd(PPh_3)_4$; (d) Na ^{125}I and chloramine-T or H_2O_2.

These results suggest that 4-[^{123}I]BP may be of potential use for noninvasive SPECT imaging of breast cancer patients. The expression of σ receptors in breast cancer cells to our best knowledge is a first-time significant finding that may have implications for the use of σ receptor ligands in the development of antitumor drugs as well. Furthermore, this ligand should be useful in quantitation of sigma receptors in CNS and in understanding the functions of σ receptors.

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Supplementary Material Available: Experimental data for the preparation of **Ia–c**, ^{1}H NMR data, and mass spectroscopic

data (14 pages). Ordering information is given on any current masthead page.

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