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The Sigma-2 (σ_2) Receptor: A Novel Protein for the Imaging and Treatment of Cancer

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Abstract

The sigma-2 (σ_2) receptor is an important target for the development of molecular probes in oncology because of its 10-fold higher density in proliferating tumor cells than in quiescent tumor cells, and the observation that σ_2 receptor agonists are able to kill tumor cells via apoptotic and non-apoptotic mechanisms. Although recent evidence indicates the σ_2 receptor binding site is localized within the progesterone receptor membrane component 1 (PGRMC1), most information regarding this protein has been obtained using either radiolabeled or fluorescent receptor-based probes, and from biochemical analysis of the effect of σ_2 selective ligands on cells grown in culture. This article reviews the development of σ_2 receptor ligands, and presents an overview of how they have been used in vitro and in vivo to increase our understanding of the role of the σ_2 receptor in cancer and proliferation.

Keywords

Sigma-2 receptors; cell proliferation; radiotracer; fluorescent probe; positron emission tomography

INTRODUCTION

Although sigma receptors have been thoroughly studied over the past 30 years their role in cell biology is poorly understood. Sigma receptors were originally characterized as a subtype of the opiate receptor. This initial description was based on the physiological properties of ($\pm$)-SKF-10,047 (*N*-allylnormetazocine) and its structurally-related benzomorphan analogs, morphine and ketazocine, in the chronic spinal dog model.¹ In this classic study, three subtypes of the opiate receptors were proposed and named using the Greek symbol corresponding to the first letter of the drug thought to activate those receptors: μ for morphine, κ for ketazocine, and σ for ($\pm$)-SKF-10,047. Subsequent studies revealed that the opiate receptor properties of SKF-10,047 resided in the binding of the (-)-stereoisomer to μ and κ receptors. In contrast, (+)-SKF-10,047 was found to bind with high affinity to a separate protein which retained the name the " σ receptor".^{2, 3}

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The next phase in research on the σ receptors involved the identification of additional ligands and radioligands capable of binding to this protein. Initial studies by T-P Su^{4, 5} and later studies by William Tam^{6, 7} confirmed that the (+)-isomer of several benzomorphan analogs including pentazocine, dextrallorphan, and cyclazocine bound with high affinity to the σ receptor whereas the (-)-stereoisomers bound to the classical opioid receptors. Other ligands which also bind to σ receptors included the neuroleptics haloperidol, fluphenazine and chlorpromazine, the β -blocker propranolol, and the presynaptic dopamine D₂ receptor agonist (+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine, (+)-3-PPP.² However, binding to σ receptors represents an “off target” site for those ligands and their utility in the study of σ receptors is quite limited. A key breakthrough in the field of σ receptor pharmacology came with the development of [³H]*o*-ditolylguanidine (DTG) as a radioligand for in vitro binding studies of this receptor. DTG has a modest affinity for σ receptors and a low affinity for other CNS receptors. Based on the differential binding properties of (+)-[³H]SKF-10,047, (+)-[³H]3-PPP, and [³H]DTG to σ receptors found in rat PC12 adrenal tumor cells versus guinea pig brain, Hellewell and Bowen were the first to propose the existence of distinct molecular forms of the σ receptor.⁸ Subsequent radioligand binding studies and biochemical analysis have shown that there are two well characterized subtypes of σ receptors, termed σ_1 and σ_2 . Both subtypes are expressed in high density in the liver and kidneys. The σ_1 receptor is widely distributed throughout the brain, while the σ_2 receptor is associated with tumors and proliferating tissue. The σ_1 receptor is the σ receptor first described by Su et al.; it has a molecular weight of ~25 kDa; receptor-rich membranes for binding studies are often harvested from guinea pig brain homogenates. The σ_2 receptor is the receptor identified by Hellewell and Bowen in PC12 tumor cells and has a molecular weight of ~21.5 kD; receptor-rich membranes for binding studies are frequently harvested from rat liver. The radioligand [³H](+)-pentazocine is the radioligand of choice for σ_1 receptor binding assays since it has a high (~3 nM) affinity for this receptor and a low (>1,000 nM) affinity for the σ_2 receptor. Because [³H]DTG is equipotent at both σ_1 and σ_2 receptors, radioligand binding studies of σ_2 receptors typically use rat liver membrane homogenates as the tissue source and are conducted in the presence of 100 nM unlabeled (+)-pentazocine or (+)-SKF-10,047 to mask binding to σ_1 receptors.^{9, 10} The conclusive identification of endogenous ligands for these receptors has proven to be very challenging, and has limited our understanding of the functional significance of σ binding sites. Some studies have shown that neuroactive steroids bind with moderate affinity to σ_1 sites and suggest that σ_1 receptors may modulate the activity of GABA and NMDA receptors in the CNS.¹¹⁻¹³ The hallucinogen *N,N*-dimethyltryptamine (DMT) was recently shown to act as an endogenous agonist on the σ_1 receptor.¹⁴ Whether or not neuroactive steroids or other steroid-based ligands interact with σ_2 receptors is not known at this time.

The σ_1 receptor has been cloned and displays a 30% sequence homology with the enzyme C8-C7 sterol isomerase, although it lacks C8-C7 isomerase activity.^{15, 16} The gene for the σ_1 receptor contains four exons and three introns and is approximately 7 kbp long.¹⁶ The σ_1 receptor is considered to play a role in lipid compartmentalization in the endoplasmic reticulum¹⁷ and in the binding of cholesterol with subsequent remodeling of lipid rafts.¹⁸ Recently, the σ_1 receptor was classified as a receptor chaperone which forms a complex that binds to and stabilizes the inositol triphosphate receptor at the endoplasmic reticulum (ER)

membrane, thus regulating ER-mitochondrial Ca^{2+} signaling and cell survival.¹⁹ The σ_2 receptor has not been cloned, and most of what is known about the σ_2 receptor has been obtained through the use of in vitro receptor binding studies aimed at the pharmacological characterization of this receptor. The absence of the cloned gene or the purified σ_2 receptor protein has also hampered the generation of antibodies that could be used to study the subcellular localization of this receptor using immunohistochemical techniques comparable to those employed in research on the σ_1 receptor.²⁰ Recent studies have shown that the σ_2 receptor binding site may be located in the progesterone receptor membrane component 1 (PGRMC1), a protein that has been independently associated with proliferation.²¹ Despite the lack of antibodies, the development of highly selective σ_2 receptor radioligands and recent studies using fluorescent probes have increased our understanding of the subcellular distribution and intracellular trafficking of the σ_2 receptor in vivo. Fluorescence microscopy studies with σ_2 probes have detected σ_2 receptors in several organelles with the notable exception of the nucleus.^{22, 23} Fluorescent probes have also been used to compare σ_2 receptor density in human stem cells and lineage-restricted cells; a drastic reduction in the σ_2 receptor density was observed as the stem cells differentiated.²⁴

There have been a number of excellent reviews and book chapters published in recent years describing the molecular characterization of σ_1 receptors and the potential application of σ_1 agonists in the treatment of a variety of CNS disorders, including depression, cognitive disorders, and substance abuse. The chapter by Ablordepey and Glennon provides an excellent overview of the different classes of compounds which bind with high affinity and selectivity for the σ_1 receptor.²⁵ Therefore, the remainder of this Perspective will focus on: 1) the σ_2 receptor as a biomarker of tumor cell proliferation; 2) the development of σ_2 selective ligands; 3) radiolabeled probes for the σ_2 receptor 4) the subcellular distribution and identification of the putative σ_2 receptor binding site as a component of the PGRMC1 protein complex; and, 5) the use of σ_2 receptor agonists as potential therapeutics for the treatment of cancer.

1. THE σ_2 RECEPTOR AS A BIOMARKER OF THE PROLIFERATIVE STATUS OR GROWTH FRACTION OF SOLID TUMORS

As described above, the σ_2 receptor was first identified by Hellewell and Bowen through receptor binding studies in PC12 cells, a tumor cell line derived from rat adrenal pheochromocytoma cells.⁸ In a follow-up paper from this group, Vilner et al demonstrated that there was a high density of σ_2 and σ_1 receptors in a wide variety of human and murine tumor cells.²⁶ In general, the density of σ_2 receptors in the tumor cell lines was higher than that of σ_1 receptors. An exception to this was the prostate cancer cell line, LNCaP.FGC cells, and ThP-1 leukemia cells. The additional observation that [^3H]DTG-labeled σ_2 receptors are expressed in higher density in human cancer cells as compared to most normal tissues suggested that the σ_2 receptor may be a potential biomarker for imaging cancer with functional imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET). Although these studies suggest the potential utility of σ_2 receptors as a biomarker for differentiating solid tumors from the

surrounding normal tissues, the relationship between the density of σ_2 receptors and tumor cell proliferation was not addressed.

One of the key measures of proliferation in a solid tumor is the proliferative status. The proliferative status (PS) of a solid tumor is defined as the ratio of proliferating (P) cells to those driven into a non-cycling, quiescent (Q) state by nutrient deprivation and/or hypoxia:

$$\text{Proliferative status } (PS) = P/Q$$

A similar measure of cell proliferation is the growth fraction, which was initially defined by Mendelson²⁷ as the ratio of the number of P cells in a tumor to the total number of P and Q cells:

$$\text{Growth Fraction } (GF) = P/P+Q$$

Every solid tumor, whether it is a tumor growing in a cancer patient, or a transplantable tumor grown as a rodent model of cancer, contains populations of both P and Q cells.²⁷ The number of P and Q cells in a tumor is highly variable from one patient to the next, and represents a significant issue in the clinical management of cancer. For example, radiation and most chemotherapeutics kill P cells more effectively than Q cells, and clonogenic Q cells can often survive following the completion of radiotherapy and chemotherapy.²⁸ Therefore, knowledge of the GF and PS of a tumor can provide useful information to oncologists in determining an appropriate strategy for treating cancer patients by either chemotherapy or radiation therapy.^{29, 30} Tumors having a high GF and PS typically respond better to hyperfractionated radiation therapy versus conventional radiation therapy.³¹ Similarly, tumors having a high GF and PS will respond better to cell cycle specific agents such as Ara-C and gemcitabine, whereas tumors having a lower proliferative status will respond better to non-cell cycle specific agents such as cisplatin and BCNU.²⁹ Finally, GF and PS can be used to identify patients who will receive the maximum benefit from newer cancer treatment strategies (e.g., Polo-like kinase inhibitors, cyclin dependent kinase and Chk-1 inhibitors) since these drugs target various proteins that are expressed in cycling cells but which are not expressed in quiescent tumor cells.^{32, 33} (Table 1)

A number of studies have indicated that the σ_2 receptor is a useful biomarker for determining the PS and GF of solid tumors using imaging techniques such as PET and SPECT. Using the well-established diploid mouse mammary adenocarcinoma cell line 66,^{34, 35} Wheeler and colleagues demonstrated that the density of σ_2 receptors in proliferating 66P cells was ~10 times greater than the density observed in quiescent 66Q cells.³⁶ As shown in Figure 1 the density of σ_2 receptors in 3-day proliferating cells from both the diploid 66 cell line and the aneuploid 67 cell line was found to be quite high in comparison to the density measured in quiescent cells at either day 7 or day 10.³⁷ In addition, the modest σ_2 receptor density in the Q cells suggested that it still may be possible to image solid tumors having a significant Q cell fraction (i.e., low P:Q ratio) due to the lower density of σ_2 receptors in surrounding normal tissues.^{26, 38}

In a subsequent study, this group reported the upregulation and downregulation of σ_2 receptors follows the transition of mouse mammary cells between the P and Q states.³⁹ Because it takes at least three days for the downregulation of σ_2 receptors to complete once the cells make the transition from P to Q, these data suggest that this receptor is not expressed only in a single phase of the cell cycle such as the cyclin-dependent kinases and other cell-cycle specific proteins. The observation that the P:Q ratio of σ_2 receptor density in solid tumors is identical to that obtained under cell culture conditions verified that the results from cell culture conditions can be translated to solid tumors xenografts of these breast cancer cell lines.³⁹ These data demonstrate that the σ_2 receptor is a receptor-based biomarker of cell proliferation in breast tumors. Therefore, radiotracers having a high affinity and high selectivity for σ_2 receptors have the potential to assess the PS and GF of human breast tumors using noninvasive imaging techniques such as PET and SPECT. It is also likely that this approach can be extended to assess the proliferative status of other human tumors, such as head and neck, melanoma, and lung tumors, which are known to express a high density of σ_2 receptors.²⁶

The relatively high density of σ_2 receptors in Q cells also suggests that σ_2 -selective radiotracers have the potential for differentiating quiescent tumors from terminally-differentiated senescent/quiescent normal tissues. In addition, since most chemotherapeutics target P cells and not Q cells, the high density of σ_2 receptors in Q cells can also provide a method for imaging chemotherapy-resistant compartment of a solid tumor. One example where this imaging strategy was used in an animal model of breast cancer is described in greater detail below.

2. THE DEVELOPMENT OF σ_2 SELECTIVE LIGANDS

Although many different classes of compounds have been shown to bind to σ_1 and σ_2 receptors, most of these compounds bind selectively to the σ_1 receptor or have similar affinities for both σ_1 and σ_2 receptors.⁹ The development of ligands having a high selectivity for the σ_2 versus the σ_1 receptor has been challenging, and in some cases, the identification of σ_2 receptor selective ligands was an unexpected result when ligands designed for other functions were screened for cross-reactivity against a panel of CNS receptors.

2.1. Conformationally-restricted amine analogs

The first class of σ_2 selective ligands reported was the benzomorphan-7-one analogs shown in Figure 2.⁴⁰ These compounds were identified as part of a structure-activity relationship (SAR) study to improve the affinity of (-)-2-methyl-5-(3-hydroxyphenyl)morphan-7-one for μ versus κ opioid receptors.⁴¹ Incorporation of an (*E*)-benzylidene moiety into the 8-position of the ring system increased affinity for σ receptors, with the (+)-1*R*,5*R* isomer CB-64D (**1**) having a 185-fold higher selectivity for σ_2 versus σ_1 receptors whereas the (-)-1*S*,5*R* isomer, CB-64L (**2**) had a higher affinity for σ_1 than σ_2 receptors (Figure 2). The corresponding 3,4-dichloro analog, CB-184 (**3**), had an even higher affinity and selectivity for σ_2 versus σ_1 receptors whereas the corresponding (+)-isomer, CB-182 (**4**), displayed a similar affinity for σ_1 and σ_2 receptors. In vitro studies with **1** have shown an intracellular rise in Ca^{2+} levels via the release of a thapsigargin-sensitive store in the endoplasmic reticulum; this σ_2 ligand may induce cell death via a caspase-independent apoptotic pathway.^{42, 43}

Other conformationally-restricted amine analogs having a higher affinity for σ_2 versus σ_1 receptors are: 1) the hallucinogen, ibogaine (**5**), of which the neurotoxic effects have been linked to the affinity of this indole alkaloid for σ_2 receptors;^{44, 45} 2) the mixed serotonin 5-HT₃ antagonist/5-HT₄ agonist BIMU-1 (**6**);⁴⁶ 3) the tropane analog SM-21 (**7**) (a σ_2 antagonist), an acetylcholine releaser that has been utilized as an antinociceptive agent;^{47, 48} 4) the trishomocubane analog ANSTO-19 (**8**) and its 7-azabicyclo[2.2.1]heptane analog **9**^{49, 50} which have modest affinity for σ_2 receptors; and 5) the tropane analog **10** which has a modest affinity for σ_2 receptors and 20-fold selectivity for σ_2 versus σ_1 receptors was recently reported.⁵¹ It should be noted that the σ_2 -selectivity resides in the (-)-isomer of this tropane analog, whereas the (+)-enantiomer binds with near equal affinity to both σ_1 and σ_2 receptors.⁵¹

Compound **6** served as the lead compound for a series of SAR studies which have resulted in the development of many high affinity and high selectivity σ_2 receptor ligands.⁵²⁻⁵⁵ Compound **6** is an ideal lead compound for SAR studies because it provides a variety of regions where structural modifications can be made to optimize the σ_2 receptor affinity and reduce the affinity for serotonin 5-HT₃ and 5-HT₄ receptors (Figure 3). Substitution of the *N*-methyl group at the bridgehead nitrogen with a benzyl group and replacement of the urea linkage of **6** with a carbamate linkage resulted in a dramatic increase in affinity for both σ_2 and σ_1 receptors, a loss of affinity for 5-HT₃ receptors, but moderate affinity for 5-HT₄ receptors.⁵⁵ Expansion of the 8-azabicyclo[3.2.1]octan-3 β -yl ring system (i.e., tropane ring) to the corresponding 9-azabicyclo[3.3.1]nonan-3 β -yl ring system (i.e., granatane ring) did not alter the affinity for σ_1 and σ_2 receptors relative to the tropane analogs, but the ring expansion eliminated affinity for the 5-HT₄ receptor. The most interesting analog from this initial SAR study was compound **11**, which had a σ_2 receptor affinity of ~ 3 nM and a σ_1 : σ_2 selectivity of ~30 (Figure 3).⁵⁵

Compound **11** was subsequently used as a secondary lead compound for a series of studies leading to second-generation granatane analogs having an improved σ_2 receptor affinity and high σ_1 : σ_2 selectivity ratios. Substitution of the benzyl group of **11** with a 2-phenethyl group gave compound **12**, having a slightly better σ_2 receptor affinity and σ_1 : σ_2 selectivity ratio (~50) (Figure 3).⁵² Substitution of the *para* position of the benzyl moiety of **11** with a dimethylamino group (compound **14**) and the *para* position of the 2-phenethyl moiety of **12** with an amino group (compound **13**) resulted in a further increase in the selectivity for σ_2 receptors, largely by decreasing the affinity for σ_1 receptors.⁵² The amino group appears to be a preferred substituent for assuring a high affinity for σ_2 receptors and high σ_1 : σ_2 selectivity ratio based on the in vitro binding properties of the aminoalkyl analogs **15**, SV-119 (**16**), and SW-43 (**17**).⁵³ WC-26 (**14**) and **16** have shown promising results as chemotherapeutic agents in both in vitro and in vivo models of pancreatic cancer.⁵⁶⁻⁵⁸

2.2. Siramesine analogs

Siramesine (a.k.a. Lu 28-179) (**18**), a 3-(ω -aminoalkyl)-1*H*-indole analog, was originally designed as a low-efficacy serotonin 5-HT_{1A} agonist for treating depression and anxiety disorders. Subsequent studies revealed that it has a subnanomolar affinity (IC₅₀) for σ_2 receptors and a 140-fold selectivity for σ_2 versus σ_1 receptors which lead to the development

of analogs (Figure 4).^{59, 60} Replacement of the *N*-4-fluorophenyl group of **18** with a methanesulfonyl group to give **19** increased the σ_2 receptor affinity to 50 pM; however this structural change also increased the σ_1 receptor affinity so that the σ_1 : σ_2 selectivity ratio of **19** was only 26. Replacement of the methanesulfonyl group with a benzenesulfonyl group to give **20** resulted in a modest improvement in the σ_1 : σ_2 selectivity ratio and retained subnanomolar affinity for the σ_2 receptor. Interestingly, compound **21**, the tropane-based analog of **18**, displayed the best σ_1 : σ_2 selectivity ratio in this series of compounds, although its IC_{50} for σ_2 was 2.5 nM.

Although **18** displayed potent anxiolytic activity in rodent behavioral models of anxiety,⁶¹ subsequent clinical trials demonstrated it to be ineffective in the treatment of anxiety. It was abandoned as an anxiolytic by the Danish pharmaceutical company H. Lundbeck in 2002. As described in greater detail below, interest in this compound re-emerged in recent years when it was demonstrated **18** showed promise as an anticancer agent by inducing cell death via a lysosomal leakage pathway.⁶²

2.3. Conformationally-flexible amine analogs

A second series of compounds having a high affinity for σ_2 receptors are the conformationally-flexible analogs shown in Figure 5. These compounds were initially identified in SAR studies aimed at developing dopamine D_3 selective antagonists and partial agonists represented by compound **22**.⁶³ The relatively high lipophilicity of **22** ($\log P = 5.76$) indicates that the compound would not likely cross the blood-brain barrier and be active in behavioral studies. In order to reduce the lipophilicity of **22**, the 4-(2,3-dichlorophenyl)piperazine ring was replaced with other aromatic amine groups which could reduce the overall $\log P$ of the compound without altering the affinity for dopamine D_3 receptors.⁶³ This strategy resulted in a number of useful dopamine D_3 receptor ligands.^{64, 65} As an unexpected result, the replacement of the 4-phenylpiperazine moiety with a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline ring resulted in compound **23** which has a high affinity and excellent selectivity for σ_2 versus σ_1 receptors, and a dramatic reduction in affinity for dopamine receptors (Figure 5).⁶³

Additional SAR studies within this class revealed that shortening the length of the spacer group between the amide nitrogen and the nitrogen atom of the 1,2,3,4-tetrahydroisoquinoline moiety from 4 carbons to 2 carbons (i.e., compound **24**) and removing the 3-methoxy group in the benzamide aromatic ring (i.e., compound **25**) did not alter binding affinity to the σ_2 receptor. Replacement of the 5-bromo group of **25** with a methyl group (compound **26**) did not change the affinity for the σ_2 receptor, but decreased the affinity for the σ_1 receptor.⁶³ The extension of the 2-carbon spacer of **26** to the corresponding 4-carbon spacer gave RHM-1 (**27**), which still retained a high affinity for σ_2 receptors.⁶³ In a recent study, the 3-carbon spacer analogs of compounds **27** and **23** (to give compounds **28** and **29**) were shown to have a high affinity and selectivity for σ_2 versus σ_1 receptors.⁶⁶ Replacement of the benzamide moiety of the 3-carbon spacer analogs with a conformationally-restricted 1,2,3,4-tetrahydroisoquinoline-1-one, to give compound **30**, also resulted in a high σ_2 receptor affinity and good selectivity for σ_2 versus σ_1 receptors.⁶⁶

The next SAR study indicated the importance of the 6,7-dimethoxy groups in the 1,2,3,4-tetrahydroisoquinoline moiety for maintaining a high affinity for the σ_2 receptors. Removal of these two methoxy groups or replacement with methylene-, ethylene- and propylenedioxy rings (compounds **31-33**) resulted in a reduction in affinity and selectivity for σ_2 receptors (Figure 6).^{63, 67} Finally, in order to gain a better understanding of the 1,2,3,4-tetrahydroisoquinoline moiety in the affinity and selectivity for σ_2 receptors, the modification at the amine portion of this class of compounds was studied. Opening the tetrahydroisoquinoliny ring of **23** significantly decreased the affinity for σ_2 versus σ_1 receptors, as seen in compound **34**.⁶⁷ Changing the ring size of the amine-containing component fused to the aromatic ring from a 6-membered ring to 5-membered or 7-membered rings resulted in a dramatic reduction in affinity and selectivity for σ_2 receptors.⁶⁸ An exception to this trend was compound **35**, the 5-membered ring congener of **23**, which showed high affinity and selectivity for σ_2 receptors.⁶⁸ Finally, the tri-methoxy benzamide analog in which the 6,7-dimethoxy group of the tetrahydroisoquinoline ring was replaced with a 7-nitro group to give compound **36** was found to have good selectivity but modest affinity for the σ_2 receptor.⁶⁹

As discussed in greater detail below, the conformationally-flexible benzamide analogs with a 4-carbon spacer group have proven to be a very important class of σ_2 -selective compounds for the preparation of radiolabeled probes to image this receptor both in vitro and in vivo.

2.4. ($\pm$)-PB28 (**37**) analogs and other piperazine-based analogs

The *N*-cyclohexylpiperazine derivative, **37**, has been reported to be a potent ligand with subnanomolar affinity for the σ_2 receptor, but it also has a high affinity for σ_1 receptors and a σ_1 : σ_2 selectivity ratio <1 (Figure 6).^{70, 71} The utility of **37** is also limited because of its relatively high lipophilicity (log $D_{7.4}$ = 3.99). Therefore, a number of SAR studies were reported with the goals of identifying an analog having a high σ_2 affinity and improved σ_2 versus σ_1 selectivity versus **37** and a more favorable lipophilicity for in vivo studies. Replacement of the 4-nitrogen with a methane group (i.e., compound **38**) resulted in a compound with modest affinity and selectivity for σ_2 versus σ_1 receptors (Figure 7), whereas replacement of the 1-nitrogen of **37** with a methane group resulted in a potent σ_1 ligand (compound **39**). Substitution of the propyl spacer group of **37** with an amide linkage (compound **40**) resulted in enantiomers which were selective for the σ_2 versus σ_1 receptor, with (-)-(S)-**40** having a higher σ_2 affinity and selectivity relative to its (+)-(R)-isomer. This observation was in stark contrast to the in vitro binding affinities of the optical isomers of **37** for σ_1 and σ_2 receptors. That is, (-)-(R)-**37** was equipotent for σ_1 and σ_2 receptors, where the corresponding *S*-isomer had a 10-fold higher affinity for σ_1 versus σ_2 receptors.⁷² Finally, PB183 (compound **41**), the naphthyl analog of **37**, displays a subnanomolar affinity for σ_2 receptors and 13-fold higher selectivity for σ_2 versus σ_1 receptors. However, the high log P of this compound (cLog P = 4.77) and the observation that it is a substrate for P-glycoprotein (P-gp), suggest that its utility may be limited for tumor imaging and chemotherapeutic applications.⁷³ Additional SAR studies are needed in order to identify a suitable σ_2 selective ligand based on **37** and its structural congeners. A review of SAR studies on **37** was recently published.⁷⁴

The cocaine antagonist SN79 (6-acetyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)benzo[d]oxazol-2(3*H*)-one), (compound **43**) was initially reported to be a highly selective σ_2 versus σ_1 ligand.⁷⁵ However, a replication of the in vitro binding assays revealed that this compound had a much higher σ_1 affinity than initially measured, and the σ_1 : σ_2 selectivity ratio of **43** is modest at best. The *N*-2-pyridine analog, **44**, was recently reported to have a good σ_2 affinity and reasonable σ_1 : σ_2 selectivity ratio.⁷⁶ This compound represents an interesting new lead compound and could result in the development of a highly selective *N*-phenyl piperazine analog having a higher lipophilic efficiency than the other representatives shown in Figure 7.

3. RADIOLABELED PROBES FOR THE σ_2 RECEPTOR

In the absence of conventional molecular biological probes for the σ_2 receptor, the ligands described above have provided critical research tools in the development of radiolabeled probes. The following sections describe the development over the past 20+ years of radiolabeled and fluorescent probes which have provided valuable insight into the structure and in vivo distribution of the σ_2 receptor, and highlighted the potential of this receptor to function as a biomarker for imaging the proliferative status of solid tumors.

3.1. In vitro binding studies of radiolabeled σ_2 receptor ligands

Historically, [³H]DTG (**45**) has been proven to be the most useful radioligand in the study of the σ_2 receptor (Figure 8).^{9, 10} Although this ligand binds with equal affinity to both σ_1 and σ_2 receptors, the difference in binding properties between [³H]DTG and radiolabeled benzomorphan analogs such as (+)-[³H]SKF-10,047 and [³H](+)-pentazocine first led to the pharmacological characterization of the σ_2 receptor.^{9, 10} The photoaffinity analog, [³H]azido-DTG (**46**) was also instrumental in the characterization of the molecular weight of σ_1 and σ_2 receptors.^{9, 10} [³H]**46** was found to label two distinct proteins in liver membranes; these proteins had molecular weights of ~25 kDa and ~21.5 kDa. Blocking studies using 100 nM dextrallorphan to mask σ_1 binding sites indicated that the 25 kDa protein was the σ_1 receptor, whereas the 21.5 kDa was the σ_2 receptor. The 25 kDa and 21.5 kDa proteins labeled by [³H]**46** have also been identified in other tissues, including guinea pig brain and rat phenochromocytoma (PC12) cells.⁸ The accepted radioligand binding techniques for Scatchard and competition studies involve [³H](+)-pentazocine to label and quantify σ_1 receptors, and [³H]DTG in the presence of 100 nM of unlabeled (+)-pentazocine to mask σ_1 receptors and quantify σ_2 receptors. Autoradiography studies measuring the density of σ_1 and σ_2 receptors in rat and guinea pig brain have also been conducted using in vitro techniques.^{77, 78} Radioligand binding techniques have been used to establish the high σ_2 receptor density in a wide panel of murine and human tumor cells growing under cell culture conditions,²⁶ and to clarify the relationship between the σ_2 receptor density and the proliferative status of mouse mammary tumor cells.^{36, 37, 39}

Although [³H] DTG has been a very useful ligand for characterizing the σ_2 receptor, and for identifying σ_2 selective ligands, its rapid dissociation rate (i.e. k_{off}) is not ideal for in vitro binding studies. Many of the compounds shown in Figures 4 and 6 have been tritiated and show an improvement in σ_2 receptor binding properties relative to [³H] DTG. The first σ_2 selective ligand labeled with tritium was siramesine (**18**). Scatchard studies in rat and human

brain samples revealed that [³H]**18** has a K_d value of 1.1 nM.⁷⁹ Autoradiography studies of [³H]**18** showed a high density of σ_2 receptors in the motor cortex, hippocampus, and hind brain nuclei, which is consistent with previous autoradiography studies using [³H] DTG in the presence of 1 μ M dextrallorphan to mask σ_1 binding sites. Other σ_2 selective ligands that have been labeled with tritium include [³H]**27** which has the tritium label in the *o*-methoxy group,⁸⁰ and ($\pm$)-[³H]**37**.⁸¹ Cellular uptake studies showed that ($\pm$)-[³H]**37** accumulation was up to a five-fold higher in nuclear fractions than in cytosolic fractions of human SK-N-SH neuroblastoma cells and MCF7 ADR breast tumor cells, indicating that ($\pm$)-[³H]**37** is able to enter the nucleus of the cells.⁸² This is contrary to what has been reported with σ_2 selective fluorescent probes, which show no uptake in the nucleus of tumor cells.^{22, 23} A recent study presented evidence suggesting that the nuclear uptake of ($\pm$)-[³H]**37** may be attributed to its binding to the H2A/H2B histone dimer, suggesting that this ligand is not selective for σ_2 receptors.⁸² Of the tritiated σ_2 selective ligands reported to date, [³H]**27** has the highest selectivity for σ_2 versus σ_1 receptors; a key property for conducting Scatchard studies of σ_2 receptors in tumors and normal tissues. This ligand also has a low binding affinity for towards a wide panel of CNS receptor ligands, confirming its selectivity for σ_2 versus other receptor systems.⁸⁰

Iodine-125 is another radioisotope that is used frequently in receptor binding and autoradiography studies. Due to the high specific activity and short half-life relative to tritium, ¹²⁵I-labeled ligands are useful for quantifying receptors expressed in low density in tissues. The only two σ_2 selective ligands labeled with iodine-125 reported to date are the conformationally-flexible benzamide analogs [¹²⁵I]**47**⁸³ and [¹²⁵I]**48** (Figure 8).⁸⁴ As described in greater detail below, [¹²⁵I]**48** played a key role in the identification of the σ_2 receptor as a binding site within the PGRMC-1 protein complex.²¹

3.2 In vivo imaging studies of σ_2 receptors

PET Radiotracers—The high correlation between the density of σ_2 receptors and the P:Q ratio of solid tumors indicates that σ_2 selective radiotracers are likely to be for imaging the proliferative status of tumors in vivo with PET. The two classes of σ_2 -selective compounds which have been used in in vivo imaging and tumor uptake studies are the 9-azabicyclo[3.3.1]nonane (granatane) analogs and the conformationally-flexible benzamides. Of these, the conformationally-flexible benzamide analogs have demonstrated the most promise in tumor uptake and microPET imaging studies.^{54, 85} The presence of the 2-methoxy group in compounds **23**, **25**, **26**, and **27** (Figure 9) facilitated the preparation of the corresponding ¹¹C-labeled derivatives via the alkylation of the corresponding 2-hydroxy precursors with [¹¹C]methyl iodide.⁸⁶ MicroPET and tumor uptake studies were conducted with [¹¹C]**23**, [¹¹C]**25**, [¹¹C]**26**, and [¹¹C]**27**; the most promising analog proved to be [¹¹C]**27**. Although all four analogs had a high affinity for σ_2 receptors, the optimal lipophilicity of [¹¹C]**27** was responsible for the high tumor uptake and suitable target: normal tissue ratios for imaging (Figure 9). These data indicate that, in addition to receptor affinity, lipophilicity is an important property that must be considered in the design of receptor-based tumor imaging agents. The tumor: blood and tumor: fat ratios of [¹¹C]**27** in EMT-6 tumors (Figure 9) clearly show the potential of this radiotracer for imaging the σ_2 receptor status of breast tumors with PET.

Compound **37** has been ^{11}C -labeled and preliminary studies indicated that the binding of $(\pm)\text{-}[^{11}\text{C}]\mathbf{37}$ could not be blocked with the σ_2 selective ligand, $(\pm)\text{-}\mathbf{7}$.⁸⁷ These data suggests that $(\pm)\text{-}[^{11}\text{C}]\mathbf{37}$ preferentially labels σ_1 receptors in vivo since **37** has a higher affinity for σ_1 versus σ_2 receptors, and the density of σ_1 receptors in normal tissues is generally greater than that of σ_2 receptors.²⁶

Although $[^{11}\text{C}]\mathbf{27}$ provides a clear image of breast tumors in microPET imaging studies, the short half-life of carbon 11 ($t_{1/2} = 20.4$ min) is not ideal for the development of radiotracers used in clinical PET imaging studies. The relatively long half-life of fluorine-18 ($t_{1/2} = 109.8$ min) makes it a preferred radionuclide because it places fewer time constraints on tracer synthesis and allows imaging studies to be conducted up to 2 h after injection of the radiotracers. This usually results in higher tumor: normal tissue ratios for the ^{18}F -labeled radiotracers relative to their ^{11}C -congeners. Therefore, the benzamide analogs shown in Figure 5 served as lead compounds in the development of a series of ^{18}F -labeled σ_2 selective radiotracers where the 2-methoxy group in the benzamide ring was replaced with a 2-fluoroethoxy group. This is a strategy frequently used in the development of ^{18}F -labeled radiotracers.⁸⁵ The structures and σ receptor selectivity of the two most promising compounds are shown in Figure 10. MicroPET imaging studies of $[^{18}\text{F}]\mathbf{ISO}\text{-}1$ (**49**) in a rodent model of breast cancer, and of $[^{18}\text{F}]\mathbf{RHM}\text{-}4$ (**48**) in a rat model of brain cancer are also shown in Figure 10.⁸⁵ A recent phase 0 clinical trial of $[^{18}\text{F}]\mathbf{49}$ was completed in lymphoma, head & neck, and breast cancer patients.⁸⁸ The preliminary clinical data indicates that the uptake of $[^{18}\text{F}]\mathbf{49}$ correlates with the proliferative status of tumors measured by using the Ki-67 labeling score from biopsy specimens. An example of clinical imaging studies of $[^{18}\text{F}]\mathbf{49}$ in lymphoma patients is shown in Figure 11. Note the ability of $[^{18}\text{F}]\mathbf{49}$ to image the bone metastasis in patient 11.

^{18}F -labeled siramesine (**18**) has also been reported via the Ulmann arylation reaction on the corresponding des-phenyl indole precursor with $[^{18}\text{F}]\text{4-fluoriodobenzene}$.⁸⁹ However, in vivo tissue uptake studies or microPET imaging studies of $[^{18}\text{F}]\mathbf{18}$ have not been reported.

As described above, all solid tumors consist of a population of P cells and population of Q cells, with the ratio of these compartments (i.e., the P:Q ratio) being highly variable from patient to the next. Since most chemotherapeutics target cycling cells (i.e., P cells) and not Q cells (Table 1), the relatively high density of σ_2 receptors in Q cells versus normal tissue indicate that the σ_2 receptor imaging strategy has the potential to identify drug-resistant Q cells following the completion of a cycle of chemotherapy. An example of a microPET imaging study demonstrating this concept in a chemically-induced model of breast cancer is shown in Figure 12. In this model, Sprague-Dawley rats treated with the carcinogen *N*-methyl-*N*-nitrosourea (50 mg/kg, i.p.) generally develop breast tumors within 8 weeks. Tumor-bearing animals were then given bexarotene (Targretin) in their diet and the response to treatment was monitored with magnetic resonance imaging (MRI) and microPET imaging using $[^{18}\text{F}]\mathbf{49}$ as the radiotracer. Treatment with bexarotene resulted in a reduction in tumor volume which was apparent on both the MRI and microPET images. However, after 4 weeks of treatment there was a region of $[^{18}\text{F}]\mathbf{49}$ uptake in one subject which was difficult to quantify in MRI images without the benefit of the microPET imaging data (Figure 12). This region of $[^{18}\text{F}]\mathbf{49}$ uptake persisted for the next two weeks of treatment and represents a

population of treatment-resistant tumor cells. Removal of bexarotene from the diet resulted in rapid regrowth of the tumor, which was easily visualized in both the microPET and MRI images. This study demonstrates the efficacy of the σ_2 receptor imaging strategy to image treatment-resistant tumor cells over anatomical imaging techniques such as MRI or computed tomography (CT) scans.

The presence of the bromine atom in the benzamide ring of compound **23** (Figure 5) also led to the preparation of a ^{76}Br -labeled radiotracer, [^{76}Br]**23**.⁹⁰ Although the limited availability and high positron energy of bromine-76 (which degrades image resolution) restrict the utility of [^{76}Br]**23** as a potential radiotracer in clinical PET studies, the microPET imaging study of [^{76}Br]**23** clearly shows excellent visualization of the tumor in a mouse model of breast cancer (Figure 13).

SPECT radiotracers—A small number of ^{123}I -labeled radiotracers have been used in Single Photon Emission Computed Tomography (SPECT) imaging studies in breast and melanoma patients. However, since these radiotracers are selective for the σ_1 receptor and have a low affinity for σ_2 receptors, they will not be discussed here. The presence of an iodo group in compound **48** indicates that it has the potential to serve as a SPECT radiotracer. The biodistribution of [^{125}I]**48** in tumor-bearing mice has been published;⁸⁴ though SPECT imaging with [^{123}I]**48** has not been reported. The biodistribution of [^{125}I]**47** has similarly been published, but there are no reports of SPECT imaging studies.⁸³

Although the availability of technetium-99m ($^{99\text{m}}\text{Tc}$) has been limited in recent years, it is still among the most widely used radionuclides in clinical nuclear medicine. This is largely due to the convenience of the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator, its relatively long half-life ($t_{1/2} = 6.02$ h), an absence of β -emissions, and a low radiation burden to patients. A limited number of $^{99\text{m}}\text{Tc}$ -labeled σ receptors ligands have been reported to date (Figure 14). [$^{99\text{m}}\text{Tc}$]BAT-EN6, (compound **50**) has been shown to have a high level of binding in breast tumor membrane homogenates, but its affinity for σ_1 and σ_2 receptors has not been reported.⁹¹ In vivo studies with [$^{99\text{m}}\text{Tc}$]**51**, which has a high affinity for σ_2 versus σ_1 receptors,⁹² demonstrated a high uptake and clear visualization of 66 murine breast tumor xenografts in nude mice.⁹³ These data suggest that [$^{99\text{m}}\text{Tc}$]**51** may be a useful radiotracer for SPECT imaging studies of breast cancer patients.

4. PROBES FOR IMAGING THE LOCALIZATION OF σ_2 RECEPTORS IN TUMOR CELLS WITH CONFOCAL AND TWO-PHOTON MICROSCOPY

While the above data clearly show that the σ_2 receptor is a useful biomarker for imaging the proliferative status of solid tumors, the classification of the σ_2 receptor as a *molecular marker of proliferation* has been hampered by the inability to purify and sequence this protein. As previously stated, given the absence of conventional molecular biology tools (i.e., antibodies, siRNA, cDNA) to study this protein, the function and localization of the σ_2 receptors has been investigated using radiolabeled probes to measure the density of the receptor in tumor cells and normal tissues, measuring the binding of σ_2 ligands to P and Q cells growing in cell culture or as solid tumors, or by studying the effects of these ligands on the biochemical and physiological properties of tumor and normal cells. Examples of the

latter include: 1) calcium release from the endoplasmic reticulum and mitochondria;⁹⁴ 2) incorporation of [³H]palmitic acid to form [³H]ceramide;⁴³ 3) the release of cathepsins B and L from lysosomal stores;⁶² and, 4) the generation of reactive oxygen species under cell culture conditions.⁶² In vitro receptor binding studies on subcellular fractions of brain and liver tissue suggested that σ_2 receptors are localized on the endoplasmic reticulum, mitochondria, and plasma membrane.^{10, 80} However, these studies have only provided an indirect measure of potential localizations for σ_2 receptors within a cell.

Fluorescent probes permit visualization of receptors on a subcellular level and allow for kinetic measures of uptake. Compounds **16** and **17** were used in the design of the fluorescent σ_2 probes SW107 (**52**), SW116 (**53**), K05-138 (**54**) and SW120 (**55**) (Figure 15, top). These have been useful in two-photon and confocal microscopy studies of σ_2 receptors in tumor cells growing under cell culture conditions.^{22, 23}

These in vitro studies have provided a clearer picture of the localization of σ_2 receptors in breast tumor cells (Figure 16).^{22, 23} A panel of “Tracker” dyes (MitoTracker, ER-Tracker, and the nuclear marker DAPI) was used with **55** to determine which organelles and subcellular compartments express σ_2 receptors in human MDA-MB435 melanoma cells. After an incubation period, imaging of the live cells by confocal microscope showed that **55** was distributed throughout the cytoplasm of cells, but not in nucleus. Tracker co-localization studies demonstrated that **55** co-localized with MitoTracker, LysoTracker, ER Tracker and the membrane marker. This indicates that σ_2 receptors are localized in the mitochondria, lysosomes, endoplasmic reticulum and cytoplasmic membrane (Figure 16). Similar results were obtained with two-photon imaging studies using the fluorescent probes, **52** and **53**.^{22, 23} However, due to the resolution limitation of the fluorescence data, the subcellular localization of the σ_2 receptors needs to be further studied using different experimental methods such as electron microscopy.

Time-lapse confocal microscopy studies revealed a rapid uptake of **54** ($t_{1/2}$ = 16 sec), suggesting that the uptake of the fluorescent probe into the tumor cells occurs by receptor-mediated endocytosis. This was confirmed with the use of phenylarsine oxide (PAO),⁹⁵ a well-characterized endocytosis inhibitor, followed by the σ_2 selective ligand **54**. Pretreatment of MDA-MB435 cells with PAO reduced the uptake of **54** by up to 38%.²² These data demonstrate that ~40% of the σ_2 receptors were internalized by receptor-mediated endocytosis, while the remaining ~60% were internalized by another mechanism such as passive diffusion. The rapid internalization of σ_2 receptors via endocytosis suggests that σ_2 selective ligands may potentially serve as receptor-mediated probes for delivering cytotoxic agents to solid tumors. The mechanism of PGRMC1 protein endocytosis can be studied in greater detail now that the PGRMC1 complex has been identified as the putative σ_2 receptor (as described below).

A recent study revealed that **55** labels σ_2 receptors expressed in a variety of human stem cells, including bone marrow stromal, neural progenitor, hematopoietic, and embryonic stem cells in contrast to lineage restricted cells.²⁴ Internalization of the fluorescent probe was consistent with receptor-mediated endocytosis. Furthermore, the uptake of **55** in stem cells correlated with cellular markers of proliferation including EdU and Ki-67 labeling.

Differentiation of the stem cells also resulted in a complete loss of fluorescent signal of **55**. These data suggest that σ_2 receptors may be a useful biomarker for monitoring the stem cell differentiation during the course of stem cell therapy.²⁴

Fluorescent probes of ($\pm$)-**37** for visualizing σ_2 receptors using confocal microscopy have also reported (Figure 15, bottom).⁹⁶ It is interest to note that the corresponding 7-nitrobenzofurazan analog **56** had a higher potency for σ_2 versus σ_1 receptors, whereas the corresponding dansyl analog **57** had the reversed selectivity profile for σ receptors (Figure 15). The properties of **56** were found to be similar to that of the fluorescent probes based on the aza-bicyclononane analogs, **52** – **55**. That is, the internalization of this probe can be partially inhibited by Filipin III, a caveolin-mediated endocytosis inhibitor, and by PAO, a clathrin-mediated endocytosis inhibitor. These studies suggest that σ_2 receptors may be involved in caveolin- and clathrin-mediated endocytotic pathways. The co-localization of **56** with fluorescent markers of the endoplasmic reticulum and lysosome, and absence of uptake of the probe in the nucleus, confirmed the earlier reports of Zeng and colleagues regarding the subcellular distribution of σ_2 receptors.^{22, 23}

The subcellular localizations of σ_2 receptors studied by two photon and confocal microscopy are consistent with the σ_2 ligand-induced cell death mechanisms reported by several groups. Mitochondria are a key organelle to regulate the intrinsic apoptotic pathway. Apoptotic signals such as UV irradiation or chemotherapeutic agents cause the release of cytochrome c from the mitochondria and the subsequent activation of caspase-3, leading to an apoptotic cell death.⁹⁷ The subcellular localization of σ_2 ligands in mitochondria is consistent with the finding that σ_2 ligands trigger apoptosis in tumor cells by distorting mitochondria and activating caspase-3.^{56, 98} The endoplasmic reticulum (ER) serves as a dynamic Ca^{2+} storage pool.⁹⁹ Ligands that are selective for the σ_2 receptor have been reported to induce transient Ca^{2+} release from the ER, which may be responsible for σ_2 ligand-induced cell death.⁹⁴ It is possible that σ_2 receptors interact, directly or indirectly, with the Ca^{2+} release channels (InsP_3 and ryanodine receptors) and the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps resided in the ER and regulate Ca^{2+} release. Lysosomal proteases, such as cathepsins, calpains and granzymes, have been reported to contribute to apoptosis.¹⁰⁰ Under physiological conditions, these proteases are found within the lysosomes but are released into the cytoplasm upon exposure to cell damaging agents, thereby triggering a cascade of intracellular events leading to cell death. The σ_2 selective ligand siramesine has been reported to cause lysosomal leakage and induce cell death by caspase-independent mechanisms.^{62, 101} The localization of fluorescent σ_2 receptor probes in the lysosomes is consistent with the hypothesis that siramesine induces cell death in part by targeting lysosomes to cause lysosomal damage, the release of proteases, and eventually cell death. The existence of σ_2 receptors has been reported in lipid rafts¹⁰² which are largely found in the plasma membrane.¹⁰³ Lipid rafts play an important role in the signaling associated with a variety of cellular events including adhesion, motility, and membrane trafficking.^{103, 104} Lipid raft-facilitated pathways may be a target for cancer therapeutics.¹⁰⁵ The finding that the σ_2 receptor internalizes in part by caveolin- and clathrin-mediated endocytosis suggests that σ_2 ligands may be useful as chemotherapeutic agents by targeting endocytotic pathways in tumor cells.

5. MOLECULAR IDENTIFICATION OF THE σ_2 RECEPTOR

The σ_2 receptor possesses a unique ability to serve as both a diagnostic and therapeutic biomarker of solid tumors. However, the clinical application of the σ_2 receptor as a cancer biomarker has been impeded by the fact that the molecular identity of this protein was not known.

5.1. The early effort to identify the σ_2 receptor

The Bowen group was the first to report that σ_2 receptor was a 21.5 kD protein in rat liver membranes.¹⁰ This group found that photoaffinity probe [³H]**46** specifically labeled two protein bands, one at 25 kD and one at 21.5 kD. Dextrallorphan blocked the 25 kD band but not the band at 21.5 kD, suggesting that the 25 kD protein was σ_1 receptor and 21.5 kD protein was σ_2 receptor. However, these proteins were not sequenced.

In an effort to understand the nature of σ_2 receptor, Colabufo et al.¹⁰⁶ used the σ receptor ligand **37** in a **37**-coupled affinity column to trap the proteins that bound to the column. The proteins were eluted and separated by SDS-PAGE gel electrophoresis and characterized using MALDI and LC-MS analysis. This process resulted in identification of six histone binding proteins as postulated **37**-binding proteins. In the subsequent studies, [³H]**37** has been shown to bind to reconstituted histone H2A/H2B dimer.⁸² Therefore, these authors hypothesized that the σ_2 receptor is a histone-binding protein, or that σ_2 receptors bind to histone proteins as an additional site within the cell where σ_2 receptors are localized. However, the modest-selectivity of **37** for σ_1 (13 nM) versus σ_2 (0.68 nM) receptors and the lack of similarity between the known characteristics and functions of histone proteins and σ_2 receptors (as described below), as well as the absence of vigorous validation studies make this hypothesis unlikely.^{70, 107}

5.2. Identification of the PGRMC1 complex as the postulated σ_2 receptor binding site

In order to determine the molecular identity of the σ_2 receptor, Xu and colleagues developed a strategy which utilized a σ_2 selective photoaffinity probe to irreversibly label σ_2 receptors in rat liver membrane homogenates.²¹ This photoaffinity probe, WC-21 (**58**), an analog of **27**, contains an azide moiety for the photoaffinity tagging of the protein and a FITC group for protein visualization. Compound **58** has high binding affinity for σ_2 receptors ($K_i = 8.7$ nM) and relatively low binding affinity for σ_1 receptors ($K_i > 4,000$ nM). Compound **58** was incubated with rat liver membrane homogenates, and the **58**-protein complex photo-crosslinked; the proteins were then solubilized and the supernatant was separated by gel electrophoresis. Western blot analysis revealed a dominant protein band at ~24 kD that was labeled by FITC conjugated probe **58**. Labeling of this protein band with **58** could be blocked by well-characterized σ_2 receptor ligands. Proteomic studies of the protein in the ~24 kD band labeled by **58** identified it as the *progesterone receptor membrane component 1* (PGRMC1).¹⁰⁸

A review of the literature revealed a number of similarities between the characteristics and functions of PGRMC1 and σ_2 receptors which are summarized below:

- I.** Both PGRMC1 and σ_2 receptors are cancer biomarkers and therapeutic targets. PGRMC1 is a cytochrome-related protein.¹⁰⁸ In normal cells it promotes cell growth, regulates cholesterol synthesis, and is implicated in progesterone signaling. PGRMC1 is overexpressed in multiple types of cancer including breast, ovary, and lung cancer. PGRMC1 mRNA levels are increased in advanced stages of ovarian cancer.¹⁰⁹ PGRMC1 protein levels are more abundant in estrogen receptor negative breast tumors than in estrogen receptor positive breast tumors.^{110, 111} PGRMC1 protein levels were increased in squamous cell lung cancers compared to corresponding nonmalignant tissue.¹¹² PGRMC1 is also reported to promote survival in cancer cells.¹¹³ Knocking down of PGRMC1 in A549 lung cancer cells using a short hairpin RNA decreased in vivo tumor growth.¹¹³ PGRMC1 associates with EGFR and the PGRMC1-EGFR interaction is proposed to be responsible for promoting cell survival.¹¹⁴ A PGRMC1 small molecule ligand, AG-205 (**59**); (Figure 17), inhibits growth in multiple cancer cell types and destabilize EGFR.¹¹⁴ Published reports strongly support PGRMC1 as a new cancer biomarker and as therapeutic target in oncology. The σ_2 receptor has been identified as biomarker for proliferating tumor cells.^{36, 39} σ_2 Receptor ligands have been developed for the diagnostic imaging of solid tumors using PET.^{39, 84-86} The σ_2 receptor has also been reported as a potential target for treatment of cancer by multiple groups.^{42, 57, 62, 98} σ_2 Receptor ligands have been shown to induce cell death in multiple cancer types. The proposed mechanisms of cell death have includes caspase-dependent and independent apoptosis,⁴² lysosomal leakage,⁶² Ca^{2+} release,^{94, 115} oxidative stress,⁶² ceramide production,⁴³ autophagy¹⁰¹ and cell cycle impairment.⁹⁸ It is worth-noting that σ_2 receptor ligands and PGRMC1 ligand **59** induce cytotoxicity at a similar range of EC_{50} values.^{98, 112}
- II.** Both PGRMC1 and σ_2 receptors have a similar subcellular localization. PGRMC1 was first purified from porcine liver microsomal membranes.¹¹⁶ Subcellular fractionation of rat liver microsomal membranes by sucrose density centrifugation revealed a distribution for PGRMC1 identical to the 78-kDa glucose regulated protein or Binding Protein (GRP78/BiP) as a marker for membranes of the endoplasmic reticulum.¹¹⁷ Immunohistochemistry showed that PGRMC1 colocalized with endoplasmic reticular marker, GRP78/BiP or cytochrome b5, in HeLa cells.¹¹⁸ These data indicated that PGRMC1 is localized in the endoplasmic reticulum. PGRMC1 was also observed in microsomal and mitochondrial fractions of rat adrenal inner zones,¹¹⁹ suggesting that PGRMC1 localized in mitochondria. PGRMC1 in spontaneously immortalized granulosa cells was detected among the biotinylated surface proteins that were isolated by avidin affinity purification, indicating that PGRMC1 localized to the extracellular surface of the plasma membrane.¹²⁰ PGRMC1 was reported to exist in the cytoplasm and in some nuclei of OVCAR-3 cells, implying that the nuclear localization of PGRMC1 may be cell-cycle dependent.^{109, 121} High densities of the σ_2 receptor are found in rat liver and kidney microsomal membranes.¹⁰ Confocal and two-photon microscopic studies using σ_2 fluorescent probes suggest that the σ_2 receptor localizes in endoplasmic,

mitochondria, lysosome and cytoplasmic membrane.^{22, 23, 96} Therefore the subcellular localization of σ_2 receptors is largely consistent with that of PGRMC1.

III. In vitro studies have shown progesterone binding to both PGRMC1 and σ_2 receptors. Progesterone is thought to be an endogenous ligand for PGRMC1.^{116, 121} In fact, PGRMC1 was initially cloned in search of membrane receptors for progesterone.¹¹⁶ In 1988, T-P Su et al first reported that progesterone inhibited σ receptor binding and suggested steroids were endogenous ligands for σ receptors.¹²² Recently, progesterone's σ subtype specificity was determined in rat liver membranes; the binding affinities of progesterone for σ_1 and σ_2 receptors were 239 nM and 441 nM, respectively.¹²³ Moreover, σ receptor ligands have been reported to bind to progesterone binding site.¹²⁴ Binding studies using the radioligand [³H]progesterone in porcine liver membrane preparations revealed that haloperidol, a molecule which has high affinities for both σ_1 and σ_2 receptors, displaced [³H]progesterone with $K_i = 20$ nM, whereas (+)-pentazocine, a σ_1 receptor ligand, displaced the progesterone binding with only moderate affinity of $K_i = 1.13$ μ M. Taken collectively, these data suggest that progesterone binds to both PGRMC1 and σ_2 receptors.

IV. Both PGRMC1 and σ receptors are associated with cytochrome P-450 proteins. PGRMC1 contains a cytochrome b5-like domain and has been reported to bind to a number of cytochrome P-450 proteins.¹²⁵ Because some P-450 inhibitors and substrates have high σ binding affinities, σ receptors have long been hypothesized to be cytochrome P-450 family proteins.^{10, 126, 127} It is conceivable that PGRMC1 and P-450 complexes constitute the σ_2 receptor binding site.

5.3. Direct evidence supporting the σ_2 receptor as a binding site in the PGRMC1 protein complex

The similarities between PGRMC1 and σ_2 receptors support the concept that the postulated σ_2 receptor binding site is localized within the PGRMC1 protein complex. In order to provide direct evidence supporting this concept, Xu et al performed a series of experiments in HeLa cells using a variety of molecular biology techniques and in vitro binding assays.²¹ Receptor binding studies showed that the PGRMC1 ligand **59** and the known σ_2 ligands DTG (**45**) and siramesine (**18**) as well as novel ligands **16** and **14**, readily displaced σ_2 radioligand [¹²⁵I]**48** binding in HeLa cell membrane homogenates. This result suggests that the σ_2 receptor ligands and the PGRMC1 ligand share the same binding site. Knockdown of PGRMC1 using a PGRMC1-specific siRNA reduced the binding of [¹²⁵I]**48** to HeLa cells, whereas overexpression of PGRMC1 in HeLa cells increased the binding of [¹²⁵I]**48**. These data indicate that the PGRMC1 complex has binding properties similar to the σ_2 receptor. Functional assays were also conducted to examine the ability of PGRMC1 to regulate caspase-3 activation by the σ_2 receptor ligand, **14**. In HeLa cells, knocking down PGRMC1 decreased caspase-3 activation induced by **14** relative to HeLa cells treated with the non-targeting siRNA, suggesting that σ_2 ligand-induced cell death is mediated by PGRMC1. The intracellular localization of PGRMC1 and σ_2 receptors was also compared using confocal microscopy. PGRMC1 proteins were visualized with an anti-PGRMC1 antibody, whereas σ_2 receptors were visualized using the fluorescent probe **55**.²³ PGRMC1 partially colocalized

with GRP78/BiP, an endoplasmic reticulum (ER) marker, or the mitochondria marker cytochrome c oxidase subunit IV (COX-IV). The σ_2 receptor probe colocalized with fluorescent trackers specific for ER or mitochondria. These data suggest that both PGRMC1 and σ_2 receptors are co-localized within the same subcellular organelles in the cell. Taken collectively, the results of these studies demonstrate that the PGRMC1 protein complex is the putative σ_2 receptor binding site. (Figure 17) However, additional studies are clearly needed to further characterize the molecular structure of the σ_2 receptor/PGRMC1 protein complex.

Previously, using [^3H]**46**, the Bowen group showed that the molecular weight of the σ_2 receptor is 21.5 kD in rat liver membranes,¹⁰ whereas the apparent molecular weight of PGRMC1 is ~25 kD. It is not clear whether the 21.5 kD protein identified using [^3H]**46** and PGRMC1 identified using **58**²¹ are the same protein. One possibility is that they are the same protein and the discrepancy in molecular weight was due to the different protein standard sets used in the two studies.

PGRMC1 is a multi-functional protein.^{108, 128-130} PGRMC1 promotes tumor growth, at least in part, by binding epidermal growth factor receptor (EGFR).¹¹⁴ PGRMC1 regulates cholesterol biosynthesis by binding CYP51A1, a cytochrome P450 enzyme required for cholesterol biosynthesis.¹²⁵ PGRMC1 also interacts with other drug metabolism enzymes such as CYP3A4 and CYP2C8, raising the possibility that PGRMC1 may be involved in detoxification in the liver.¹³¹ PGRMC1 alone or in the complex binds to progesterone and mediates anti-apoptotic activity of progesterone.¹³² The new evidence linking the σ_2 receptor and PGRMC1 coupled with the old findings in both σ_2 receptor and PGRMC1 fields lead to a broader view of the σ_2 receptor with regard to its molecular and cellular functions in tumor and normal tissues: The σ_2 receptor/PGRMC1 promotes cell growth in tumors, regulates cholesterol synthesis in normal tissues, may regulate drug metabolism in liver, and mediates progesterone signaling. Identification of the molecular target of σ_2 ligands opens an avenue to in-depth study of the molecular mechanisms of interactions between σ_2 ligands and the σ_2 receptor/PGRMC1 complex and of the downstream signaling pathways associated with these interactions. Increased understanding of σ_2 receptors in normal and tumor cell biology will greatly facilitate clinical applications of σ_2 ligands as radiotracers for imaging cancer and as chemotherapeutic agents.

6. σ_2 RECEPTOR LIGANDS AS CHEMOTHERAPEUTICS AND CHEMOSENSITIZING AGENTS

6.1. σ_2 Ligands can act directly on cancers as chemotherapeutics

Tumors played an early and important role in the development and understanding of σ receptors. One of the first studies that successfully differentiated σ receptor binding sites from PCP-binding sites was conducted with in hybrid NCB-20 hybrid neurotumor cells using (+)-[^3H]SKF-10,047 and (+)-[^3H]3-PPP.¹³³ As previously stated, radioligand binding studies in rat PC12 adrenal tumor cells were instrumental in the identification of the σ_2 receptor.⁸ Subsequent structure-activity relationship studies led to the development of compounds with selectivity for the σ_2 receptors; multiple compounds have been used to

characterize the binding sites in other tumor cells of both human and murine origins.^{26, 134} Critical observations regarding the relationship between proliferation and σ_2 receptor expression were made in breast cancer cell lines.³⁹ These findings in tumor cell lines have inspired investigators to pursue σ_2 receptors as cancer imaging agents and subsequently as agents for therapy.

The potential anticancer effects of the ligands were not described until several years later.¹³⁵ Several observations regarding the anticancer effects of σ_2 ligands were reported with non-selective ligands and initial observations were variable, differing by cell line, σ receptor selectivity and investigator. However, it has become increasingly apparent that σ_2 selective ligands in particular have significant potential as anti-cancer therapeutics either alone or as delivery agents for other small molecules (Table 2). These σ_2 selective ligands bind to a wide spectrum of human and rodent cancers indicating that the σ_2 receptor is almost ubiquitously expressed in cancers. Numerous σ_2 selective ligands have been shown to induce cancer cell death in vitro and in vivo.^{56, 58, 135, 136} There is growing evidence that σ_2 directed therapies might play a role against a wide spectrum of cancer types.

It has been nearly 20 years since the initial observation of tumoricidal effects¹³⁵ related to σ_2 ligand administration yet the mechanism(s) by which these ligands induce tumor death remain somewhat elusive. One of the major factors obscuring the mechanism of tumor death has been the lack of understanding about the receptor for the σ_2 selective ligands. The natural ligand is not known and only recently has PGRMC1 been identified as a component of the σ_2 receptor.²¹ σ_2 Ligands are rapidly internalized upon binding and become widely distributed colocalizing with markers of multiple intracellular organelles.²² Separately the receptors have been localized to lipid rafts.¹⁰² Some σ_2 ligands have been shown to induce caspase-dependent apoptosis in pancreas cancer^{56, 58} but not in breast cancer.⁶² Several ligands induce calcium release as described in colon cancer, prostate cancer, breast cancer and neuroblastoma^{94, 137} but not always is calcium release associated with tumor death.^{94, 138} In an assessment of four σ_2 ligands including **14** and **16** investigators found differing degrees of caspase activation, inhibition of the mTOR pathway and intracellular cytoskeletal abnormalities resulting in autophagy. This study demonstrated that multiple pathways can be induced in different cell lines and that different mechanisms may underlie cell death depending on both drug and cell line.⁹⁸ Evidence is emerging that suggests membrane destabilization may be the unifying mechanism of σ_2 ligand induced cancer selective death.^{130, 139, 140} If this mechanism is correct it would partially explain some of the observed differences between ligands and cell lines in their death response. As cancer cells evade apoptosis in several ways they may also react differently in response to membrane destabilization and thus the final pathway of cell death is subsequently the result of one or another of the observed downstream pathways. For example, Hornick and colleagues examined structurally distinct σ_2 selective ligands and demonstrated that two classes of compounds both induced membrane destabilization and lysosomal well as mitochondrial membranes were differentially targeted by different ligands.¹³⁹ As a result the downstream effects varied depending on the σ_2 selective ligand utilized. It was possible to partially protect the cells from death with anti-oxidants but caspase inhibitors were effective for only one of the ligands in this model.

Despite our poor understanding of the mechanisms of anti-tumor action for these ligands, the field is building momentum towards a clinical trial. Our experience with their use in tumor-bearing animals has advanced understanding of their effects. As solo-agents σ_2 ligands inhibit tumor growth compared with controls.⁵⁶ The drug can be administered to research animal models for a short duration with effects on tumor growth and improvements in overall survival.⁵⁶ Simultaneously there have been a growing number of papers looking at σ_2 expression in an expanding panel of human tumors, most recently including ovarian and lung cancer.^{112, 141, 142} The number of review articles suggesting that σ_2 ligands will have an important role in cancer therapy is increasing.^{108, 138, 143-145} Several challenges including identification of the preferred σ_2 selective ligand, dosing, duration, and route of administration will need to be resolved at the preclinical level but there is enough excitement about this drug class that a clinical trial seems imminent.

The differential effects of σ_2 ligands on tumor cells as compared with normal cells are poorly understood. A good portion of the specificity for cancer may be based on the high expression of σ_2 receptors on proliferating cells as compared to normal cells.^{146, 147} The differential expression however does not completely explain the magnitude of the observed differences. Although ligand binding studies have reported high σ_2 receptor expression in liver and kidney, modest expression has been reported in normal cell lines and non-proliferating tissues.^{10, 26} There has been one published report describing the presence of σ_2 receptors in stem cells.¹⁴⁸ In theory, normal cells which proliferate rapidly, such as crypt cells in gut endothelium and stem cells in bone marrow, might be expected to have high levels of proliferation markers including σ_2 receptors, yet little toxicity in these tissues has been observed or reported. In our experience, high doses of σ_2 ligands can be administered to tumor-bearing animals with minimal overt toxicity as measured by weight loss, gross necropsy findings or blood chemistry.⁵⁶⁻⁵⁸ Early after administration of **16**, we observed evidence of transient pancreatitis in one model but found no clinical correlates.¹⁴⁹ It is not known why cancers would express elevated levels of σ_2 receptors, or even what advantage this expression confers to the cancer cell. We have observed inherent differences between cell lines in their sensitivity to σ_2 ligands but there are no reports of sensitive cancer cells becoming resistant to treatment with σ_2 ligands. In our experience with pancreas, ovarian and breast cancer cell lines repeated applications have not demonstrated diminishing efficacy (unpublished data).

6.2. σ_2 Ligands can act as sensitizers to standard and experimental therapeutics

In addition to acting as chemotherapeutics, σ_2 ligands would likely potentiate the effects of other more traditional anti-cancer therapies.¹³⁸ The rationale for this is the cancer selective nature of the ligands. It has been shown that cancers over-express the σ_2 receptor and that σ_2 ligands localize to cancer. We also know that selected σ_2 ligands induce cancer cell death with few systemic side effects. Therefore it makes sense to combine these ligands with other more traditional anti-cancer therapies. This is an open area in need of additional research. The current poor understanding of the mechanism of action suggests that it would be quite speculative to postulate which traditional agents might act additively or synergistically when used in combination with σ_2 ligands. What is known about the mechanism of action of σ_2 ligands suggests that cancer cells might be more sensitive to membrane destabilization than

normal cells by the nature of their rapid growth and the composition of their membranes. It is postulated that cells may alter membrane composition in pursuit of rapid growth and division. One approach has been to investigate agents with known activity in a particular cancer and examine if these drugs are augmented by the addition of σ_2 ligands. This strategy has been followed in several examples of in vitro combination therapies including radiation therapy and chemotherapy.^{42, 70, 149, 150} Recent in vivo studies combining gemcitabine chemotherapy with the σ_2 selective ligands **16** or **17** utilized lower doses of both the σ_2 selective ligands and the chemotherapeutic to demonstrate an additive clinical effect on stabilizing tumor growth (Figure 18).^{57, 58} Combination chemotherapy seems a very promising area for continued research.

A well-established functional assay for defining the agonist/antagonist behavior of σ_2 receptor ligands has not yet been developed. Many σ_2 ligands with diverse structures have been shown to induce cytotoxicity in a variety of cancer cells by triggering caspase-dependent and caspase-independent apoptosis.^{42, 62, 98} Therefore, our group uses cell viability and caspase-3 activity as functional assays to define agonist/antagonist for σ_2 receptors. Siramesine induces caspase-3 activation and cytotoxicity and is a commonly accepted σ_2 agonist. The azabicyclononane analogs (e.g. **16** and **17**) activate caspase-3 and induce cytotoxicity, and thus are categorized as agonists. The benzamide analogs (e.g. **27**) do not trigger caspase-3 activation and cytotoxicity and are thus considered as potential antagonists. Although cell lysis may be a possible factor of cytotoxicity of σ_2 ligands, the σ_2 ligand-induced caspase-3 activation is, at least in part, mediated by σ_2 receptor/PGRMC1 since knocking down PGRMC1 partially inhibited **14**-induced caspase-3 activation.²¹

6.3. σ_2 Ligands by nature of their cancer selectivity represent a platform technology for the delivery of therapeutic cargos to tumor cells

Two distinctly different approaches using σ_2 ligands as delivery agents have been reported. One approach has been the synthesis of conjugate compounds which link a σ_2 ligand to small molecule therapeutics. Spitzer et al demonstrate that multifunctional molecules based on **16** are capable of delivering short pro-apoptotic peptides and small chemotherapeutics like rapamycin. These σ_2 -conjugates are selectively internalized by cancer cells and evidence is presented showing the predicted mechanisms of action are in effect by measuring alterations in downstream cellular pathways.¹⁴⁹ This is an extremely promising area for continued research; numerous inhibitors of pathways involved with cancer have failed because they lack adequate cancer selectivity as chemotherapeutics. In addition to such research targeting drugs with existing therapeutic windows, many potentially therapeutic drugs which have unwanted off-site toxicities could also be revisited. In a second approach to cancer selective delivery of small molecules, **16** was recently conjugated to gold nanocages.¹⁵¹ This research demonstrated the feasibility of using a σ_2 ligand to assist with targeted-delivery of nanoparticles to human melanoma, prostate and breast cancer cell lines in vitro. Although hollow and porous gold nanocages have been previously explored as a mechanism for loading chemotherapeutic drugs, σ_2 ligand conjugates could prove to be useful contrast agents for in vivo imaging modalities such as photoacoustic tomography and optical coherence tomography.

7. CONCLUSION

The σ_2 receptor continues to be an important molecular target in the field of tumor biology. The high expression of this receptor in proliferating versus quiescent breast tumors indicates that the σ_2 receptor is an important clinical biomarker for determining the proliferative status of solid tumors using the functional imaging technique PET. The recent in vitro study showing that fluorescent σ_2 probes are taken up by stem cells, and that there is a decline in uptake as the cells differentiate, suggests an additional application for σ_2 receptor PET imaging studies in monitoring stem cell therapy. Since σ_2 selective ligands can kill tumors by both apoptotic and non-apoptotic pathways, it also suggests that this receptor is a potential target for the development of cancer chemotherapeutic agents. Evidence that the σ_2 receptor binding site lies within the PGRMC1 protein complex represents a key step in the identification of the functional role of this receptor in normal and tumor cell biology. The next and key step in the evolution of the σ_2 receptor is the demonstration that the promising results generated in preclinical models of cancer translate to the diagnosis and treatment of cancer patients.

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Biography

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Chenbo Zeng received her B.S. in Biochemistry from Jilin University in China in 1985 and her M.S. in Biochemistry from Jilin University in 1988. She received her Ph.D. in Biochemistry from Iowa State University (1996). She did her postdoctoral training at Washington University School of Medicine in St. Louis. Currently, Dr. Zeng is a Research Instructor in the Mallinckrodt Institute of Radiology at Washington University School of Medicine.

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Abbreviations

COX-IV	cytochrome c oxidase subunit IV
CT	computed tomography
CYP51A1	cytochrome P450, family 51, subfamily A, polypeptide 1
DMT	<i>N,N</i> -dimethyltryptamine
DTG	<i>o</i> -ditolylguanidine
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
GF	growth fraction
GRP78/BiP	78-kDa glucose regulated protein GRP78, or Binding Protein, BiP
LC-MS	Liquid chromatography–mass spectrometry
MALDI	matrix-assisted laser desorption/ionization mass spectrometry
MRI	magnetic resonance imaging
PAO	phenylarsine oxide
PET	positron emission tomography
PGRMC1	progesterone receptor membrane component 1
(+)-3-PPP	(+)-3-(3-hydroxyphenyl)- <i>N</i> -(propyl)piperidine
P	proliferating
PS	proliferative status
Q	quiescent
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SERCA	sarcoplasmic-endoplasmic reticulum Ca ²⁺ ATPase
σ₁	sigma-1
σ₂	sigma-2
SPECT	single photon emission computed tomography

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Figure 1.

Differences in the σ_2 receptor density in 3-day 66P versus 7- and 10-day 66Q cells, and in 3-day 67P versus 7- and 10-day 67Q cells. All values are mean $\pm$ SE from at least three independent experiments. P-cell experiments were run simultaneously with the corresponding Q-cell experiments.

Figure 2.

Conformationally-restricted amine analogs were among the first ligands identified as selective for σ_2 versus σ_1 receptors (K_i values).

Figure 3.
 σ_2 receptor ligands based on the BIMU-1 (K_i values).

Figure 4. The conformationally flexible amine **siramesine** and conformationally-flexible benzamide analogs. (Receptor affinity measurements are IC_{50} values).

Figure 5.
 Compounds explored in SAR studies of **22** (K_i values).

Figure 6.
Additional compounds explored in SAR studies of **23** (K_i values).

Figure 7.

Compound **37** and second generation analogs with improved selectivity for σ_2 vs. σ_1 receptors (K_i values).

Figure 8.
Radiolabeled σ_2 receptor ligands for in vitro binding studies.

Figure 9.
 ^{11}C -Labeled conformationally-flexible benzamide analogs (K_i values) and in vivo studies.

Figure 10.

The structure of ¹⁸F-labeled conformational flexible benzamide analogs (K_i values) and preclinical microPET/CT imaging studies in tumor-bearing rodents.

Figure 11.

CT, co-registered and PET images from the Phase 0 clinical trial of [¹⁸F]49. Primary tumors are indicated by a green arrow. The Ki-67 score and tumor:muscle ratio for the three lymphoma patients is also shown.

Figure 12.

Representative serial PET and MRI images of [^{18}F]**49** in a rat with chemically-induced mammary tumors. Images were acquired during the evaluation of bexarotene therapy; the table depicts the tumor volume (calculated from MRI images), the corresponding SUV in the tumor, and the tumor: muscle ratio.

Figure 13.

Structure and in vitro binding affinities (K_i values) of the ^{76}Br -labeled conformationally-flexible benzamide analog. MicroPET imaging study of $[^{76}\text{Br}]\mathbf{23}$ in an EMT-6 tumor-bearing mouse. Note the high uptake of radiotracer in the EMT-6 tumors and low uptake in the surrounding normal tissues.

Figure 14. Structures of ^{99m}Tc -labeled agents having a moderate to high affinity for σ_2 receptors. Affinity measurements are K_i values.

Figure 15.

Fluorescent sigma receptor ligands used for microscopy studies. The compounds along the top are analogs of **16** and **17** with variable (6 or 10) carbon spacers (top). Fluorescent analogs of ($\pm$)-**37** are shown below. Affinity measurements are K_i values.

Figure 16. Confocal microscopy studies with **55**. These co-localization studies reveal that σ_2 receptors are found in the mitochondria and endoplasmic reticulum but not in the nucleus.

Figure 17.

Studies which provide evidence that the σ_2 receptor is part of the PGRMC1 protein complex. Compound **58** is the photoaffinity probe which was used to purify the σ_2 receptor/PGRMC1 protein. Compound **59**, a known PGRMC1 ligand, displays σ_2 receptor pharmacology in in vitro binding assays. Expression of the cDNA of the PGRMC1 increasing the in vitro binding of [^{125}I]**48** in HeLa cells.

Figure 18.

The σ_2 ligands **16**, **17**, and **18** as chemotherapeutics.⁵⁷ (Panel A) Dose escalation curve showing in vitro efficacy of σ_2 ligands as chemotherapeutics following an 18 h treatment of mouse Panc02 pancreatic cancer cells. Viability determined relative to vehicle (DMSO). (Panel B-D) In vivo efficacy of σ_2 ligands for combination chemotherapy in the syngeneic Panc02 tumor model.

Table 1Cell cycle-specific chemotherapeutics used in the treatment of cancer.[#]

Drug	Target	Phase of Cell Cycle Affected	References
5-Fluorouracil (5-FU)	Thymidylate Synthase	S	152-154
Hydroxyurea	Ribonucleotide Reductase	S	154, 155
Methotrexate	Dihydrofolate Reductase	S	154, 156
Etoposide	Topoisomerase II	S or G2/M	154, 157, 158
Doxorubicin Epirubicin	Topoisomerase II	S or G2/M	158
Docetaxel	Tubulin	M	159
Staurosporine	CDK1	G2/M	154
SNS314 ENMD-2076 CCT137690 MK-0457/VX-680 ZM44739	Aurora Kinase A, B and C	G2/M	160-164
MLN8237 MK5108/VX-689	Aurora A	G2/M	160, 164, 165
AZD-1152	Aurora B	G2/M	163, 164
BI2536 BI6727 GSK461364 ON 01910.Na	Polo-like Kinase 1	M	33, 166-168

[#] Adapted with permission from Macmillan Publishers Ltd: British Journal of Cancer, 2009³⁰ additional references cited show structures of novel compounds in Phase I/II trials.

Table 2Expression of σ_2 Receptor in Human and Rodent Tumors

Origin	Species	Cell Lines	σ_2 ligand	Reference
Breast Cancer	Human	T47 D	^3H -DTG	26
	Human	MCF 7	^3H -DTG	26
	Mouse	EMT-6	^3H -DTG, 14, 16	85, 98, 169
Colon Cancer	Human	Primary tumor	^3H -DTG	38
Leukemia	Human	Th-P1	^3H -DTG	26
Lung	Human	NCI-H727	^3H -DTG	26
Melanoma	Human	A375	^3H -DTG	26
	Human	MDA MB-435	14, 16	98
Neurologic	Human	U-138MG	^3H -DTG	26
	Human	Primary tumor	^3H -DTG	170
	Mouse	NB41A3	^3H -DTG	26
	Mouse	N1E-115	^3H -DTG	26
	Rat	C6	^3H -DTG	171
Pancreas Cancer	Mouse	Panc-02	16	56
	Human	Panc-01	16	149
	Human	AsPc-1	16	149
	Human	CFPAC	16	149
Prostate	Human	LNCaP	^3H -DTG	26
Sarcoma	Human	Primary tumor	^3H -DTG	38