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Review

Potential applications for sigma receptor ligands in cancer diagnosis and therapy☆



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ABSTRACT

Sigma receptors (sigma-1 and sigma-2) represent two independent classes of proteins. Their endogenous ligands may include the hallucinogen *N,N*-dimethyltryptamine (DMT) and sphingolipid-derived amines which interact with sigma-1 receptors, besides steroid hormones (e.g., progesterone) which bind to both sigma receptor subpopulations. The sigma-1 receptor is a ligand-regulated molecular chaperone with various ion channels and G-protein-coupled membrane receptors as clients. The sigma-2 receptor was identified as the progesterone receptor membrane component 1 (PGRMC1). Although sigma receptors are over-expressed in tumors and up-regulated in rapidly dividing normal tissue, their ligands induce significant cell death only in tumor tissue. Sigma ligands may therefore be used to selectively eradicate tumors. Multiple mechanisms appear to underlie cell killing after administration of sigma ligands, and the signaling pathways are dependent both on the type of ligand and the type of tumor cell. Recent evidence suggests that the sigma-2 receptor is a potential tumor and serum biomarker for human lung cancer and an important target for inhibiting tumor invasion and cancer progression. Current radiochemical efforts are focused on the development of subtype-selective radioligands for positron emission tomography (PET) imaging. Right now, the most promising tracers are [¹⁸F]fluspidine and [¹⁸F]FTC-146 for sigma-1 receptors and [¹¹C]RHM-1 and [¹⁸F]ISO-1 for the sigma-2 subtype. Nanoparticles coupled to sigma ligands have shown considerable potential for targeted delivery of antitumor drugs in animal models of cancer, but clinical studies exploring this strategy in cancer patients have not yet been reported. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

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1. Introduction

Sigma receptors were originally described as a subtype of the opioid receptor family, but later shown to be unique proteins integrated in plasma, mitochondrial and endoplasmic reticulum membranes of several organs including liver, kidney and brain. Two subtypes of sigma receptors have been identified, termed sigma-1 and sigma-2 [1]. As discussed previously [2], the endogenous ligands for these receptors have not been identified with certainty but may include steroid hormones (particularly progesterone), sphingolipid-derived amines and *N,N*-dimethyltryptamine (DMT).

Recent evidence has been presented in support of the hypothesis that DMT, a well-known hallucinogen, may in fact be an endogenous sigma-1 agonist. DMT is a substrate for the serotonin transporter (with even higher affinity than serotonin itself) and is also a substrate for the vesicular monoamine transporter 2. These transporter proteins may allow the accumulation of DMT (and other tryptamines) in neurons to the micromolar levels needed for sigma-1 receptor activation [3]. Consistent with this hypothesis, radiolabeled DMT enters the brain of living rabbits very rapidly (10 s) and is retained there in intact form for at least 7 days, whereas the compound is cleared from the rest of the body *via* the renal route [4]. Although the authors labeled DMT with ¹³¹I and thus modified its structure, the *in vivo* behavior of the 2-iodo derivative is expected to be similar to that of the parent indolealkylamine. Persistence of DMT in the mammalian brain can be explained by the fact that DMT is stored in vesicles and thereby protected from degradation by monoamine oxidase. Using immunocytochemical techniques, indole-*N*-methyl transferase (INMT), the enzyme that converts tryptamine to DMT, was shown to be localized to postsynaptic sites of C-terminals of mouse motoneurons in close proximity to sigma-1 receptors which are enriched at these sites [5]. Moreover, DMT inhibits INMT non-competitively by binding to an allosteric site on the enzyme molecule. DMT formation may therefore be regulated *via* a negative feedback loop [6]. Interestingly, downregulation of INMT has been associated with tumor recurrence (*e.g.*, of malignant prostate and lung cancers) and *inmt* was identified as a candidate gene in the prevention of cancer progression [7].

Molecular biology techniques have indicated that sigma-1 receptors play critical roles in the mammalian nervous system. As discussed previously [2], sigma-1 knockout (KO) mice are viable and fertile and do not display any overt phenotype, although the response of such animals to painful stimuli is strongly suppressed and KO mice display depressive behavior under certain forms of stress. A more recent study has reported gender-related alterations in KO mice. Male knockouts show signs of increased anxiety in the open-field, passive avoidance and elevated plus-maze tests. They also show increased depressive-like behavior in the forced swimming test, but no memory changes. Female knockouts show deficits in spontaneous alternation or water maze learning, and avoidance escape latency. These symptoms of impaired memory appear to be related to changes in steroid tonus, since female KO mice have decreased plasma levels of 17 β -estradiol compared to wild-type mice and treatment with 17 β -estradiol reverses their memory deficits [8].

By the transduction of cultured rat hippocampal neurons with siRNA for the sigma-1 receptor and gene expression analysis using a rat genome cDNA array, knockdown of the sigma-1 receptor was shown to impair many cellular functions, including steroid biogenesis, protein ubiquitination, organization of the actin cytoskeleton and Nrf2-mediated responses to oxidative stress [9]. Several studies have shown that sigma-1 receptors play an important role in the protection of retinal cells against various forms of damage. KO mice suffer from late onset inner retinal dysfunction [10]. They show accelerated retinal ganglion cell death after optical nerve crush [11] and a more rapid loss of retinal function in diabetes [12].

In an article which preceded the current overview [2], we wrote that the identity of the sigma-2 receptor was unknown and the existence of

this subtype had only been proven pharmacologically. However, within one year a study was published [13] in which sigma-2 receptors were irreversibly labeled using WC-21, a ligand containing an azide moiety for photoaffinity tagging and a fluorescein isothiocyanate (FITC) group for visualization of the sigma-2 protein. By matrix-assisted laser desorption/ionization–mass spectrometry analysis, the membrane-bound protein which was labeled by WC-21 in rat liver was identified as progesterone receptor membrane component 1 (PGRMC1). Knockdown of the PGRMC1 protein with specific short interfering RNA (siRNA) reduced the binding of a radioiodinated sigma-2 receptor ligand in HeLa cells and of a fluorescent sigma-2 receptor ligand in human embryonic kidney 293T cells. Moreover, knockdown of PGRMC1 reduced the ability of sigma-2 agonists to induce caspase-3 activation in HeLa cells. Overexpression of PGRMC1 by transfection of HeLa cells with PGRMC1 cDNA was associated with a striking (60%) increase of the cellular binding of the radioiodinated sigma-2 ligand. Treatment of A549 lung cancer cells with a PGRMC1 ligand (AG-205) or a sigma-2 receptor ligand (WC-26) induced similar, dose-dependent upregulations of the PGRMC1 protein. Both the PGRMC1 ligand AG-205 and various sigma-2 receptor ligands (DTG, siramesine, SV119, and WC-26) displaced bound radioiodinated sigma-2 receptor ligand in tumor cell membrane homogenates in a concentration-dependent manner. Thus, these ligands appeared to bind to the same site. Confocal microscopy indicated that PGRMC1 and the sigma-2 receptor protein had the same intracellular localization, *viz.* in mitochondria and endoplasmic reticulum [13]. Based on this combined evidence, the PGRMC1 protein complex was identified as the putative sigma-2 receptor binding site.

It had already been known for a long time that sigma-2 ligands inhibit high-affinity progesterone binding to a microsomal fraction of porcine liver, suggesting that high-affinity progesterone binding sites are part of a complex including sigma receptors, if not themselves sigma receptors [14]. But the important study of Xu et al. [13] provided actual proof for the identity of the sigma-2 receptor and PGRMC1. Since PGRMC1 is a known protein, the cDNA sequence of the sigma-2 receptor gene may in fact already have been determined, both in porcine [15] and human [16] tissues.

Although the close identity of the sigma-2 receptor and PGRMC1 appears to have been established, some questions remain unanswered. First, the molecular masses of both proteins appear to be different. Values of 22 to 28 kDa have been reported for PGRMC1 [17–19] whereas a value of only 21.5 kDa has been determined for the sigma-2 receptor protein [20]. These differences may be related to post-translational processing or splice variants of a single protein. Second, inhibition of tumor cell proliferation has been reported to require the agonist action of anti-cancer drugs at sigma-2 receptors [21,22] but an antagonist action at PGRMC1 [23,24]. This contradiction may be more apparent than real, since agonist or antagonist actions at sigma receptors have not been well-defined. Compounds which were originally classified as sigma agonists may in fact be antagonists, and *vice versa* [25–27]. Caspase-3 activation by sigma-2 receptor ligands has been proposed to serve as a functional assay for differentiating sigma-2 agonists, partial agonists and antagonists [27]. Finally, PGRMC1 is known to bind to P450 resulting in the stimulation of its activity and increased cholesterol synthesis [28], but for the sigma-2 receptor such binding has not been reported. Future research in this exciting field may resolve these discrepancies and may result in further confirmation of the identity of PGRMC1 and the sigma-2 receptor protein.

Although sigma-1 and sigma-2 receptors are usually treated as members of a common sigma receptor “family” and share affinity for certain artificial ligands and steroids like progesterone, the structures of the sigma-1 and sigma-2 receptor proteins are in fact unrelated. While the sigma-2 receptor seems to belong to a progesterone receptor complex, the sigma-1 receptor has been characterized as a chaperone protein [29,30]. The function and intracellular location of this protein are altered by ligands but the protein can be active even in the absence of ligands. Thus, the notion of “agonists” and “antagonists”, which is

commonly applied to receptor ligands, may not be applicable to compounds which interact with sigma-1 receptors.

2. Receptor overexpression in tumors and tumor cell lines

Both subtypes of sigma receptors are overexpressed in rapidly proliferating normal cells and cancer cells from animal and human origins. Particularly the sigma-2 receptor is an interesting tumor imaging target since it is expressed about 10-fold more in proliferating tumor cells compared with quiescent tumor cells and because ligand binding to this receptor can result in tumor cell death both *via* apoptotic and non-apoptotic mechanisms (reviewed in [2]). A comprehensive overview of sigma receptor expression in various cell lines, bovine tumors and human tumors was presented in [2]. Here, only some recent findings will be reported.

Using a fluorescently labeled ligand (SW120), investigators from the National Institutes of Health showed that sigma-2 receptors are highly expressed in proliferating human stem cells (bone marrow stromal, neural progenitor, amniotic fluid, hematopoietic and embryonic) compared to differentiated lineage-restricted cells. These results provided additional support for the hypothesis that sigma-2 receptor expression is a biomarker of the proliferative status of cells. However, in contrast to tumor cells where sigma-2 ligands induce significant apoptosis, very little apoptosis was observed in ligand-treated stem cells [31]. Thus, sigma-2 receptor ligands may be used to selectively eliminate tumor cells.

RT-4, a human urinary bladder tumor cell line, expresses sigma-2 receptors at a very high density and sigma-1 receptors at a lower density (2108 and 279 fmol/mg protein, respectively). Membrane homogenates of this cell line can be employed for a sigma-2 receptor binding assay, using the non-subtype-selective sigma ligand [³H] ditolylguanidine (DTG) in the presence of 2 μM non-radioactive (+)-pentazocine to block sigma-1 receptors. K_i -values of sigma-2 ligands determined in that assay are in good accordance with K_i -values estimated in rat liver preparations. RT-4-derived membrane fragments are therefore an animal-friendly alternative to rat liver homogenates and can be used to determine the affinity of ligands to human sigma-2 receptors [32].

Sigma-2 receptors were found to be significantly over-expressed in 12 out of 15 samples of human small cell lung carcinoma (up to 6-fold) and were particularly elevated in poorly differentiated tumors. *In vitro* treatment of small cell lung carcinoma cells (A549, NCI-H226) with the PGRMC1/sigma-2 receptor ligand AG-205 or with siRNA for the sigma-2 receptor resulted in growth inhibition and in the case of the NCI-H226 cell line also in the loss of viability through a caspase-3- and caspase-8-independent pathway. Sigma-2 receptors were significantly over-expressed in 6 out of 15 samples of human adenocarcinomas (up to 4-fold), and sigma-2 receptor expression was inversely correlated with patient survival. Sigma-2 receptors appeared to be associated with secretory vesicles in lung cancer cells and to be secreted by these cells. Sigma-2 receptor levels in the plasma of lung cancer patients were therefore significantly elevated (up to 7-fold) compared to plasma of cancer-free individuals where these levels were very low. The authors concluded that the sigma-2 receptor is a potential tumor and serum biomarker as well as a therapeutic target for lung cancer [33].

In a subsequent study from the same group, the knockdown of the PGRMC1/sigma-2 receptor in A549 cells was found to block the expression of neutrophil gelatinase-associated lipocalin/lipocalin-2 (NGAL) and to decrease the activity of matrix metalloproteinase-9 (MMP9) in samples of the culture medium. NGAL is a secreted glycoprotein that binds to MMP9 and protects it from degradation. MMP9 activity is essential for the breakdown of the extracellular matrix and is involved in tumor invasion and survival. Transfection of cells in which the sigma-2-receptor had been knocked down with a plasmid encoding sigma-2 receptor/PGRMC1 resulted in re-expression of sigma-2 receptors and restoration of the NGAL levels. NGAL proved to be essential for the formation and growth of A549 *in vivo* tumors, since A549 cells

in which NGAL had been knocked down showed strongly impaired xenograft formation in athymic nude mice. By incubating normal cells and cells in which the sigma-2 receptor had been knocked down with inhibitors of the epidermal growth factor receptor (EGFR), protein kinase B (Akt) and extracellular signal-regulated kinases (ERK), evidence was obtained for the hypothesis that the sigma-2 receptor increases NGAL levels by activating the transcription factor NFκB *via* EGFR [34] (see Fig. 1). Since NGAL expression is dependent on the sigma-2 receptor, the latter protein may be an important target for inhibiting tumor invasion and cancer progression.

RPMI 8226, the hematopoietic cell line of human multiple myeloma, expresses sigma-1 receptors at high density (B_{max} 477 fmol/mg protein, 122,000 binding sites per cell). Fourteen different sigma-1 ligands showed similar competition with [³H]-(+)-pentazocine for binding to sigma-1 receptors in membrane fragments isolated from this cell line and the estimated K_i values were in good accordance with K_i -values assessed in guinea pig brain preparations. Thus, RPMI 8226-derived membrane fragments may be an animal-friendly alternative to guinea pig brain homogenates for assessment of the sigma-1 affinity of novel drugs [35].

Flow cytometry analysis and Western blotting indicated strong expression of the sigma-1 receptor in three different human esophageal squamous cell carcinoma (ESCC) cell lines (KYSE150, KYSE180 and, particularly, EC109). In these cell lines, further immunocytochemistry showed that the receptor protein is located mainly in the cytoplasm and the nucleus. Immunohistochemical analysis of patient samples indicated that the sigma-1 receptor is overexpressed in ESCC compared to normal epithelium. Levels of total sigma-1 receptor protein were significantly correlated with pathologic tumor, node, metastasis (TNM) classification of the tumors, and levels of nuclear sigma-1 receptor protein were correlated both with TNM classification and the presence of lymph node metastases. Thus, sigma-1 receptor expression may be a factor predicting ESCC classification and ESCC development [36].

In an animal study from our own laboratory, sigma-1 receptors were found to be overexpressed (>2-fold) in spontaneous pituitary tumors as compared to the normal pituitary. This overexpression was detected as an increase in uptake (>3-fold) and binding potential (BP_{ND} , >2-fold) of the sigma-1 receptor ligand ¹¹C-SA4503. In microPET scans of aged rats using this tracer, even very small tumors (e.g., a specimen of 17 mg) were clearly visualized. Thus, PET with a radioligand for sigma-1 receptors (like ¹¹C-SA4503) may have promise for the detection of pituitary adenomas and microadenomas. It is not yet clear whether such scans can discriminate between symptomatic, hormone-secretive and non-symptomatic, nonsecretive neoplasms [37].

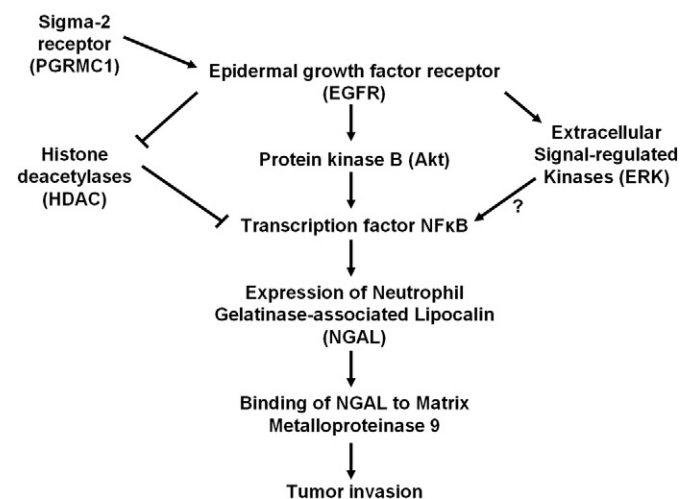


Fig. 1. Proposed signaling pathway which links the sigma-2 receptor to NGAL expression and tumor invasion. Redrawn after [34].

3. Sigma ligands for diagnostic imaging

The overexpression of sigma receptors in tumor cells motivated the development of radiolabeled sigma ligands for diagnostic imaging using positron emission tomography (PET, see Fig. 2) or single photon emission computed tomography (SPECT). An overview of the development of sigma receptor ligands for imaging purposes was provided in our previous review article [2]. At the time of writing, the most commonly applied sigma-1 receptor ligands were radioiodinated benzamides, the piperidine ^{18}F -FPS and the piperazines ^{11}C -SA4503 and ^{18}F -FESA5845. In recent years, radiochemical efforts have focused on the development of selective radioligands for each sigma receptor subtype. A comprehensive review concerning the development of sigma-2 ligands for PET, SPECT, optical imaging, photoaffinity labeling and binding assays was published in 2013 [38].

3.1. Novel sigma-1 receptor probes

A spirocyclic, radiofluorinated sigma-1 receptor ligand, 1'-benzyl-3-(2-[^{18}F]fluoroethyl)-3H-spiro[[2]benzofuran-1,4'-piperidine] or [^{18}F]fluspidine, was prepared by German investigators (see Fig. 3). Compared to ^{11}C -SA4503, this compound could offer the advantages of a very high affinity to sigma-1 receptors (K_i 0.59 nM vs 17 nM), a longer physical half life (109.8 min vs 20.4 min) and negligible affinity towards emopamil binding protein, a vertebrate sterol isomerase which is located in endoplasmic reticulum membranes and is involved in the biosynthesis of cholesterol. Biodistribution studies in female CD-1 mice and *ex vivo* phosphor storage imaging of brain slices indicated that the novel compound is a promising probe for molecular imaging of sigma-1 receptors. Tracer uptake in brain, heart, lung and spleen was significantly inhibited after the pretreatment of animals with the dopamine D2/sigma receptor ligand haloperidol (1 mg/kg), but not reduced after treatment of mice with the emopamil binding protein inhibitor tamoxifen (1 mg/kg). Specific binding appeared to be at least 75% of the total cerebral uptake of radioactivity at 60 min after injection. [^{18}F]Fluspidine showed a high brain uptake (3.9 to 4.7% ID/g), a regional distribution of radioactivity consistent with binding to sigma-1 receptors, and moderate *in vivo* metabolism. At 60 min after injection, 67% of total plasma radioactivity still represented the parent compound. Blood-brain barrier passage of radioactive metabolites was not observed [39]. In a subsequent publication from the same group, the (*R*)- and (*S*)-enantiomers of fluspidine were prepared. K_i -values of these enantiomers at sigma-1 receptors were 0.57 nM and 2.3 nM, respectively, and selectivities for the sigma-1 subtype were 2895- and 390-fold. Although incubation of the (*R*)- and (*S*)-enantiomers with rat liver microsomes led to the

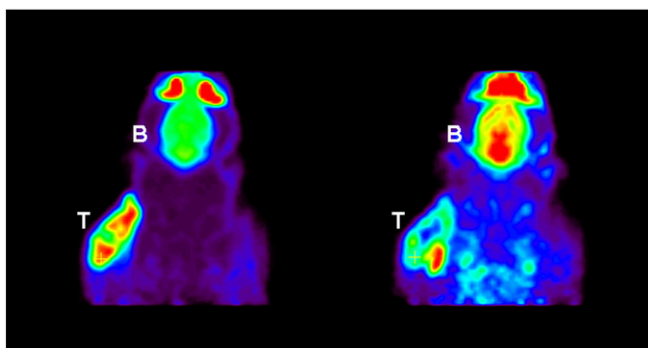
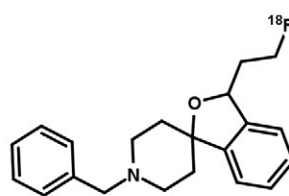
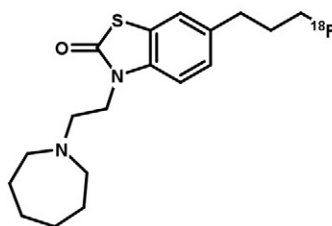


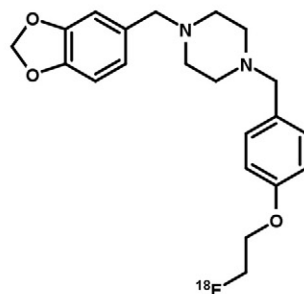
Fig. 2. PET images of the head-and-neck region of a single rat made with the glucose analog ^{18}F -FDG (left) and the sigma-1 receptor ligand ^{11}C -SA4503 (right). The animal had a C6 glioma tumor implanted in its right shoulder. Visible are the tumor (T), brain (B), Harderian gland and nasal epithelium. Note that the tumor is well-visualized by both tracers, but the areas with strong ^{18}F -FDG uptake and those with elevated binding of the sigma-1 ligand do not match.



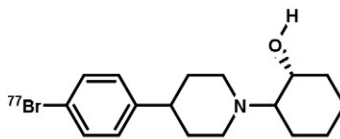
[^{18}F]Fluspidine
 $K_i \sigma_1$ 0.59 nM
 σ_1/σ_2 1,334 x



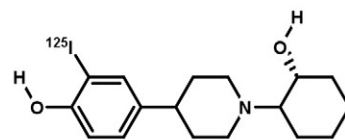
[^{18}F]FTC-146
 $K_i \sigma_1$ 2.5 pM
 σ_1/σ_2 145,000 x



[^{18}F]XW-6
 $K_i \sigma_1$ 1.85 nM
 σ_2/σ_1 157 x



[^{77}Br]pBrV
 $K_i \sigma_1$ 2.5 nM
 σ_2/σ_1 9 x



[^{125}I]-IV-OH
 $K_i \sigma_1$ 22.8 nM
 σ_2/σ_1 6 x

Fig. 3. Novel sigma-1 receptor ligands for PET imaging.

identification of seven and eight metabolites, respectively, the (*S*)-enantiomer was metabolically more stable than the eutomer [40].

A subsequent publication from the same group highlighted that small modifications of the chemical structure of a radioligand can have serious consequences for its pharmacokinetics. An analog of ^{18}F -fluspidine was prepared in which the fluoroethyl moiety was replaced by a fluoromethyl group. Although this compound had almost equally high sigma-1 receptor affinity as ^{18}F -fluspidine (K_i 0.74 nM vs 0.59 nM) and a comparable selectivity for the sigma-1 subtype (743-fold vs. 1334-fold), the fluoromethyl derivative failed as a PET tracer because of rapid metabolism, entry of two radiometabolites into the brain, low target-to-nontarget ratios in *ex vivo* autoradiography and a continuous washout from the central nervous system [41].

Investigators from Stanford University prepared a benzothiazolone sigma-1 receptor ligand, 6-(3-fluoropropyl)-3-(2-(azepan-1-yl)ethyl)benzo[d]thiazol-2(3H)-one, or [^{18}F]FTC-146 [42,43] (see Fig. 3). Pretreatment of normal BalbC mice with equal doses of three different non-radioactive sigma-1 ligands (haloperidol, FTC-146 or BD1047) reduced

the brain uptake of [^{18}F]FTC-146 by 80 to 82% at 60 min after injection. The fraction of plasma radioactivity representing the intact parent compound was 60% at 30 min and 50% at 60 min. Radioactive metabolites did not enter the brain [42]. In a later publication from the same group, [^{18}F]FTC-146 was evaluated in rats and squirrel monkeys. Biodistribution studies in control and haloperidol- or BD1047-pretreated rats indicated a large specific binding fraction in sigma-1 receptor-expression organs (brain, pancreas, spleen and liver, 85%, >80%, >65% and >70%, respectively). The regional distribution of radioactivity in the rat brain as observed by *ex vivo* autoradiography was consistent with the known distribution of sigma-1 receptors. Tracer metabolism in rats appeared to be more rapid than in mice, with about 20% of total plasma radioactivity representing the intact parent compound at 60 min after injection. However, blood–brain barrier passage of radioactive metabolites did not occur. [^{18}F]FTC-146 showed specific *in vivo* binding also in the monkey brain, and cerebral uptake of the tracer was reduced by 68% to 77% after pretreatment of monkeys with haloperidol. Metabolism in squirrel monkeys appeared to be about as equally rapid as in rats, with 15% intact parent compound remaining in monkey plasma at between 50 and 110 min [43]. [^{18}F]FTC-146 has therefore favorable characteristics for *in vivo* studies of sigma-1 receptors in the brain.

Chinese investigators prepared ^{18}F -labeled 1-(1,3-benzodioxol-5-ylmethyl)-4-(4-(2-fluoroethoxy)benzyl)piperazine (nicknamed [^{18}F]XW-6, see Fig. 3) as a potential sigma-1 receptor ligand for PET imaging. This compound shows a low nanomolar affinity to sigma-1 receptors (K_i 1.85 nM), a rather high selectivity for the sigma-1 subtype (157-fold) and an optimal log*D* (at pH 7.4) for penetration in tumors and the brain (+2.57). Favorable biodistribution data were acquired in mice and rats with >70% specific binding in the mouse brain at 60 min after injection, and sigma-1 receptor-mediated uptake in several target organs (brain, lungs, kidneys, heart and spleen) as shown by the impact of pretreatment of animals with haloperidol. Low uptake of radioactivity in bone suggested the absence of defluorination. Thus, this piperazine ligand appears to be a suitable radiotracer for *in vivo* imaging [44].

A team at Kanazawa University in Japan produced a radiobromine-labeled vesamicol analog as a sigma receptor imaging agent for PET. This compound, called (+)-[^{77}Br]pBrV, showed a nanomolar affinity for sigma-1 receptors (K_i 2.5 nM), but only 9-fold selectivity for the sigma-1 subtype (see Fig. 3). *In vitro* binding of the compound to the DU-145 prostate cancer cell line was examined and biodistribution experiments in DU-145 tumor-bearing mice were performed. Specific binding of the probe to sigma receptors was observed both *in vitro* and *in vivo*. Judged by tumor-to-blood ratios of radioactivity, >80% of the tumor uptake was blocked after pretreatment of animals with the non-radioactive sigma ligands, haloperidol and SA4503. The authors concluded that (+)-[^{76}Br]pBrV has considerable potential as a sigma receptor ligand for tumor imaging [45]. For SPECT imaging, a radio-iodinated vesamicol analog, (+)-[^{125}I]IV-OH (see Fig. 3), was also prepared and evaluated in a similar way. Tumor-to-blood ratios of that compound were 3- to 5-fold reduced after pretreatment of mice with non-radioactive haloperidol, SA4503, or (+)-pIV, and brain-to-blood ratios were reduced up to 2.5-fold, suggesting specific *in vivo* binding of (+)-[^{125}I]IV-OH to sigma receptors [46].

Analogs of prelamicol and trozamicol have also been prepared. Four compounds which were most promising showed nanomolar (1.4 to 4.0 nM) or subnanomolar (0.48 nM) affinities to sigma-1 receptors, sigma-1 subtype selectivities ranging from 1111-fold to 3627-fold, log*P* values between +2.6 and +2.8, and low affinity for the vesicular acetylcholine transporter [47]. All four can be labeled with ^{18}F or ^{11}C , but *in vivo* data for such radioligands have not yet been presented.

The well-known sigma-1 receptor ligand ^{11}C -labeled 1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)-piperazine ([^{11}C]SA4503) can not only bind to sigma-1 receptors but shows also significant *in vitro* affinity to emopamil binding protein (K_i 1.7 nM) [39,48]. For this reason, Toyohara et al. re-evaluated the *in vivo* selectivity of [^{11}C]SA4503 to sigma-1 receptors in the brain. Mice were injected with

[^{11}C]SA4503 after pretreatment with various doses of the sigma-1 receptor ligands haloperidol, ifenprodil and trifluoperidol, the emopamil binding protein blockers tamoxifen and trifluoperazine, or saline. The brain uptake of [^{11}C]SA4503 was dose-dependently decreased by sigma-1 receptor ligands, but not by inhibitors of the emopamil binding protein. Thus, [^{11}C]SA4503 shows sigma-1 selective binding in the living brain [49].

3.2. Novel sigma-2 receptor probes

In an attempt to develop novel probes for sigma-2 receptors, Abate et al. prepared benzamides which combined moieties of 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)propyl]piperazine (PB28) *N*-[4-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)butyl]-2-methoxy-5-methylbenzamide (RHM-1) in a single molecule. PB28 is a sigma ligand with sub-nM sigma-2 affinity (K_i 0.68 nM), but no subtype selectivity and a high degree of nonspecific binding, making the ^{11}C -labeled compound unsuitable for *in vivo* imaging. RHM-1 (see Fig. 4) is a sigma-2 ligand with lower affinity (K_i 8.68 nM) but strong sigma-2 subtype selectivity (248-fold); moreover, [^{11}C]RHM-1 is capable of visualizing tumor lesions in an animal model. By preparing hybrid molecules, the authors hoped to create a tracer which would combine high brain uptake with good target-to-nontarget ratios and *in vivo* selectivity for the sigma-2 subtype. Their hybrid molecules showed considerable subtype selectivity and appropriate lipophilicity, but unfortunately turned out to be substrates for the drug efflux pump P-glycoprotein which precludes their entry into the brain [50]. The most promising compound from this series was later labeled with ^{18}F and used for preclinical imaging studies. Although it had good affinity for sigma-2 receptors (K_i 9.2 nM), a high selectivity for the sigma-2 subtype (330-fold) and was lipophilic (clog*P* 3.5), it failed to enter the rat brain, probably because of P-glycoprotein-mediated efflux (EC_{50} 5.0 μM) [51].

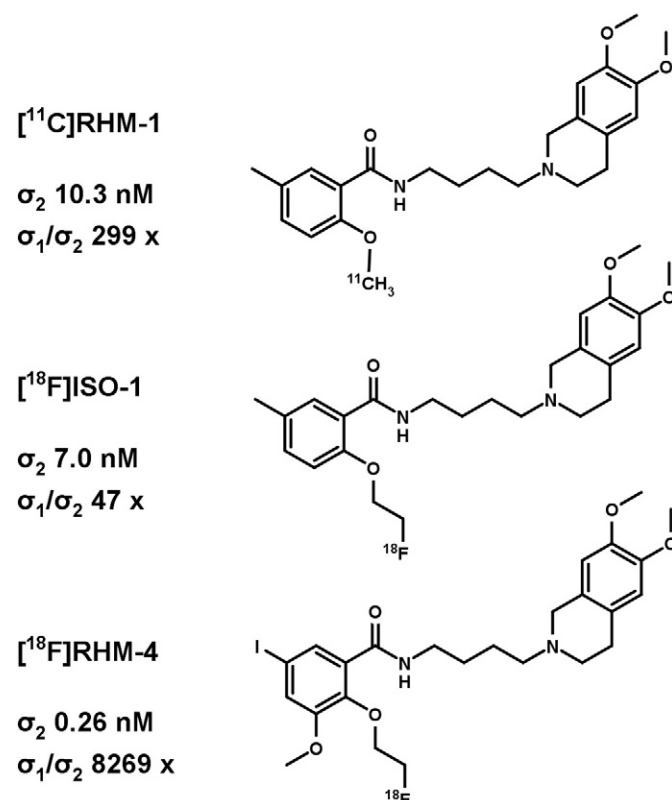


Fig. 4. Novel sigma-2 receptor ligands for PET imaging.

In another study from the same group, analogs of PB28 with reduced lipophilicity were prepared in an attempt to limit their nonspecific binding. The propylene linker and the tetralin C4 position of PB28 were modified for this purpose. However, the analogs displayed little sigma-2 subtype selectivity (maximally 2-fold) and the most promising candidate with appropriate $\log D_{7.4}$ (+2.38) turned out to be also a substrate for P-glycoprotein [52].

An Australian research team synthesized two novel phthalimido ligands for sigma-2 receptors: 2-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-5-fluoroisoindoline-1,3-dione (^{18}F -SIG343) and 2-(5-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)pentyl)-5-fluoroisoindoline-1,3-dione (^{18}F -SIG353). The compounds demonstrated high affinity (K_i values 8 and 2.4 nM) and good selectivity for the sigma-2 subtype (200- and 110-fold, respectively) in binding assays involving membrane fragments isolated from rat brain. *In vitro* blocking studies with subtype-selective nonradioactive ligands in A375 (human amelanotic melanoma) cells demonstrated that the cellular binding of ^{18}F -SIG343 was sigma-2 receptor-mediated, in contrast to ^{18}F -SIG353 which did not display any specific binding. Both tracers were evaluated in nude mice bearing A375 tumors. Particularly for ^{18}F -SIG343, specific binding was observed in sigma-receptor containing organs (brain, liver and lung) as tracer uptake was significantly reduced after pretreatment with haloperidol or non-radioactive SIG343. However, tracer uptake in the tumors was low (about 1% ID/g at 2 h post injection) and although adequate tumor-to-muscle ratios were reached (4 or 7 at 2 h post injection, respectively) and metabolic stability of the tracers was also adequate, no specific signal from sigma-2 receptors could be detected in tumor cells. Thus, in this particular animal model, [^{18}F]SIG343 and [^{18}F]SIG353 failed as probes for tumor imaging [53].

In contrast to these negative findings, very promising results were obtained with the sigma-2 receptor ligand 2-(2-[(^{18}F]fluoroethoxy)-N-(4-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)butyl)-5-methylbenzamide ([^{18}F]ISO-1, see Fig. 4). This tracer was evaluated in two different rodent models of breast cancer: female nude mice bearing tumors of murine mammary tumor 66 cells, and Sprague–Dawley rats with *N*-methyl-*N*-nitrosourea-induced tumors. Rats were subsequently treated either with bexarotene (220 mg/kg in the diet) or vorozole (1.25 mg/kg body weight by gavage). In the first model, the tumor-to-background ratio of [^{18}F]ISO-1 corresponded closely and linearly to the ratio of proliferative over quiescent cells in each tumor as determined by flow cytometry. Changes of [^{18}F]ISO-1 uptake during chemotherapy in the second model were significantly correlated with changes in tumor volume determined by repeated magnetic resonance imaging (MRI) scans. These data suggest that PET studies with a sigma-2 ligand can indicate both the proliferative status of a tumor and its growth rate. Thus, sigma-2 receptor imaging can be used in the selection and design of an appropriate treatment strategy [54]. A first human study with [^{18}F]ISO-1 in thirty cancer patients confirmed these positive expectations. In this heterogeneous group (13 patients with primary breast cancer, 10 with head and neck cancer and 7 with lymphoma), tumor-to-muscle uptake ratios and the maximum standardized uptake value of [^{18}F]ISO-1 were significantly correlated with expression of the proliferation marker Ki-67 [55].

Promising preliminary results were also obtained for a structurally similar ligand, *N*-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2-(2-fluoroethoxy)-5-iodo-3-methoxybenzamide (nicknamed RHM-4, see Fig. 4). Because of its chemical structure, this compound can be labeled either with ^{18}F (for PET), or with radioiodine (for SPECT). Both labeled molecules showed similar *in vivo* pharmacokinetics in mice bearing mammary tumors (grown from the 66 cell line). Excellent tumor-to-muscle ratios of 8 to 10 were observed at 120 min after injection [56].

A potential SPECT tracer for imaging of sigma-2 receptors in tumors has been proposed as well. Chinese investigators prepared $^{99\text{m}}\text{Tc}$ -4-(4-cyclohexylpiperazine-1-yl)-butan-1-one-1-cyclopentadienyltricarboxyl technetium for this purpose. The corresponding rhenium complex was

also prepared and was used to determine *in vitro* binding parameters of this novel ligand. It showed moderate affinity to sigma-2 receptors (K_i 64.4 nM) and moderate sigma-2 subtype selectivity (12.5-fold). The lipophilicity of the $^{99\text{m}}\text{Tc}$ -labeled ligand was about optimal for uptake into brain and in tumors ($\log D + 2.52$). *In vitro* binding experiments and biodistribution studies were performed, using C6 rat glioma cells and male ICR mice, respectively. A high tracer uptake was observed in (sigma-2 receptor-expressing) C6 cells which was reduced substantially (up to >90%) after co-incubation with haloperidol. In living mice, specific binding of the tracer was observed in receptor-expressing organs (brain, heart, lung, and spleen), but the reduction of tracer uptake after haloperidol pretreatment was not greater than 51% at 2 h post injection. Very promising results with the same tracer were obtained in C6 glioma brain tumor-bearing Sprague Dawley rats. Specific binding of the tracer in the rat brain at 2 h post injection was 67% and the tumors were well-visualized (tumor-to-brain ratio > 2). Thus, it may prove possible to develop $^{99\text{m}}\text{Tc}$ -labeled SPECT tracers for sigma-2 receptor imaging [57].

4. Sigma ligands for targeted drug delivery

Since sigma receptors (particularly the sigma-2 subtype) are overexpressed in many kinds of tumors, they have been considered as an attractive target not only for cancer diagnosis but also for anticancer drug delivery. An overview of sigma ligand-based drug targeting experiments is presented in Table 1. Chemical structures of the sigma ligands which have been employed in these experiments are depicted in Fig. 5.

Two different approaches towards sigma-ligand-based drug targeting have been explored (see Table 1). First, sigma ligands were conjugated with various nanoparticles, either *via* a polyethylene glycol (PEG) spacer or *via* direct covalent linking at the end of a long alkyl chain of the sigma ligand. Before injection into experimental animals, the nanoparticles were filled with various cytostatic or cytotoxic agents. Second, antisense oligonucleotides or antitumor peptides were conjugated with sigma ligands by direct covalent linking, and the resulting hybrid molecules were systemically administered.

Initial targeting attempts made use of anisamide and haloperidol – ligands which bind to both subtypes of sigma receptors and may in addition bind to neurotransmitter binding sites (e.g., dopamine D2 and D3 receptors). More recent attempts have employed compounds with high selectivity for the sigma-2 receptor subtype (SV119, SW43). The latter approach may result in lower brain uptake and greater uptake in the target tumors.

Conjugation of a sigma ligand to a nanoparticle (either directly or *via* a PEG spacer) may be associated with loss of affinity of the ligand to its target receptor. For this reason, most authors checked whether the tumor uptake of the targeted nanoparticles, oligonucleotides or peptides was sigma-receptor specific by comparing uptake in the presence and absence of a pharmacological dose of a sigma ligand (ditolylguanidine or haloperidol). Since uptake was strongly reduced after the pretreatment of animals with sigma ligands, the conjugates retained sufficient affinity for the intended receptor and the loss of affinity induced by conjugation appeared to be negligible (see Table 1).

Both targeted nanoparticles and antitumor peptides coupled to sigma ligands have shown considerable potential in preclinical *in vitro* and *in vivo* models of anti-tumor therapy (Table 1), but attempts to apply such strategies to the treatment of cancer patients have not yet been reported.

5. Cytotoxic effects of sigma ligands

Since both subtypes of sigma receptors are overexpressed in a large variety of tumors and can activate apoptotic pathways, sigma ligands are potentially useful as anticancer drugs either for single agent or adjuvant chemotherapy. A few comprehensive reviews on the perspectives of sigma-1 and sigma-2 receptor ligands in cancer therapy were published in recent years [74,75]. Not only drugs selective for sigma-1

Table 1
Studies employing sigma ligands for drug targeting.

Carrier and target	Payload	Outcome	Reference
Prostate cancer			
Liposomes consisting of phospholipids conjugated with anisamide via a polyethylene glycol (PEG) spacer	Doxorubicin	IC ₅₀ for toxicity to DU-145 cells reduced 8-fold, this effect is blocked by haloperidol. Targeted liposomes show greater accumulation in murine DU-145 tumors than non-targeted ones. Injection of doxorubicin-containing targeted liposomes (4 weekly doses of 7.5 mg/kg) inhibits tumor growth 2.8-fold without significant side effects. Same dose of doxorubicin alone kills mice.	[58]
Nanoparticles of hepta-guanidino-β-cyclodextrin conjugated with anisamide via PEG	siRNA for vascular endothelial growth factor	Reduction of tumor growth (2.5-fold after 21 days) in TRAMP C1 mouse model of prostate cancer. Corresponding reduction in levels of mRNA for VEGF. No significant toxic side effects.	[59]
Oligonucleotides mono- and multivalently conjugated with anisamide	Antisense oligonucleotides	Monoanisamide conjugates showed slightly higher, and trivalent anisamide conjugates about two-fold higher uptake in PC-3 cells than unconjugated oligonucleotide controls. This increase was partially blocked by haloperidol.	[60]
Lung cancer			
Liposome–protamine–heparin nanoparticles with surface-grafted PEG and anisamide	Nonapeptide mimicking the Y845 site of the epidermal growth factor receptor	Efficient peptide delivery in H460 mouse model of lung cancer; dose dependent inhibition of tumor growth (up to 8-fold after 20 days) after repeated i.v. administration (at 2 day-intervals).	[61]
Liposome–protamine–cationic lipid nanoparticles with surface-grafted PEG and anisamide	siRNA mixture (for MDM2, c-myc and VEGF) and carrier DNA	Significant reduction of lung metastases (by 70 to 80% after 17 days) in the B16F10 model of metastatic lung cancer after two consecutive low doses (0.45 mg/kg, administered 10 d after tumor cell injection). Mean survival time of animals prolonged by 30% (from 22 to 29 days). Little local or systemic immunotoxicity.	[62]
Liposome–protamine–cationic lipid nanoparticles with surface-grafted PEG and anisamide	Antisense oligonucleotide or siRNA against human survivin and carrier DNA	Dose-dependent downregulation of survivin mRNA and protein in H1299 cells, growth inhibition and induction of apoptosis. Anisamide targeting increased the delivery efficiency 4- to 7-fold.	[63]
Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating linked to the sigma ligand anisamide	siRNA for luciferase (proof of principle study)	Compared to liposome–protamine–cationic lipid nanoparticles, these novel particle structure resulted in 40-fold higher siRNA delivery to H460 lung cancer cells <i>in vitro</i> and 4-fold higher delivery to H460 tumors in living mice.	[64]
Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating linked to anisamide	siRNA mixture (for HDM2, c-myc and VEGF)	Targeting increases siRNA uptake in A549 cells 9-fold and results in efficient killing of A549 and H460 cells. Repeated i.v. injections of targeted nanoparticles in mice bearing A549 and H460 tumors significantly inhibited tumor growth (up to 4-fold after 31 days)	[65]
Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating linked to anisamide	siRNA mixture (for MDM2, c-myc and VEGF)	Repeated i.v. injections (four times 0.36 mg/kg at 2 day-intervals) of targeted nanoparticles in mice bearing B16F10 tumors significantly reduced the number of lung metastases (by 70 to 80%), prolonged the mean survival time by 27.8% and was not associated with any significant toxicity.	[66]
Breast cancer			
Cationic liposomes linked to the sigma/dopamine receptor ligand haloperidol via a PEG spacer	Plasmid DNA containing reporter or therapeutic gene	Targeted liposomes showed >10-fold greater uptake in MCF-7 cells than non-targeted liposomes. Reporter gene expression was blocked by haloperidol and ditolylguanidine (sigma ligands). Downregulation of sigma receptors with (±)-spironolactone reduced transgene expression 10-fold. Addition of serum did not impair gene delivery.	[67]
Gold nanocages conjugated to the sigma-2 ligand SV119	Chemotherapeutic agents	Sigma-2 receptor is overexpressed on the surface of breast cancer stem cells (cell type responsible for long-term tumor growth, metastasis and recurrence). Sigma-2 receptor targeted gold nanocages can be used to increase the efficiency of combination photothermal/chemotherapy	[68]
Melanoma			
Nanoparticles conjugated with anisamide and involving a guanidinium-containing cationic lipid	Small interfering RNA (siRNA) for c-myc expression	Impairment of tumor growth in MDA-MB-435 mouse model of melanoma. Sensitizes B16F10 tumor cells to paclitaxel, combination therapy with targeted nanoparticles containing c-myc siRNA and paclitaxel resulted in complete inhibition of tumor growth in mice.	[69]
Pancreatic cancer			
Various antitumor peptides covalently linked to the sigma-2 receptor ligand SV119	Bim (Bcl-2 antagonist) or CTMP-4 (Akt inhibitor) or rapamycin	Conjugates retain affinity for sigma-2 receptors and their secondary target. Dose-dependent cytotoxicity and apoptosis induction were quantified in various pancreatic tumor cell lines (Panc02, Panc-1, AsPC-1, CFPAC). The most promising conjugate (S2-Bim) was tested in mouse models of pancreatic tumor (Panc02, CFPAC). Could prevent tumor growth and prolong survival.	[70]
Ovarian cancer			
SW IV-52s covalently linked to the sigma-2 receptor ligand SW43	SW IV-52s (mimetic compound of second mitochondria-derived activator of caspase)	Conjugate retains affinity for sigma-2 receptors and about 10-fold selectivity for sigma-2 subtype. Dose-dependent cytotoxicity and apoptosis induction were quantified in various ovarian tumor cell lines (SKOV-3, CaOV-3, BG-1). SW IV-52s itself was ineffective. I.p. administration of conjugate to mouse model of ovarian cancer (SKOV3-Luc cells in SCID mice) significantly reduced tumor burden, improved overall survival (from 74 to 86.5 days) and did not have significant adverse effects.	[71,72]
Various cancers			
Liposomes consisting of PEG–dioleoyl amido aspartate covalently linked to the sigma-2 receptor ligand SV119	Doxorubicin	Targeted liposomes show significantly higher uptake than non-targeted liposomes in human prostate cancer (DU-145, PC-3), lung cancer (201T, A549) and breast cancer (MCF-7) cells, but not in normal bronchial epithelial cells. Doxorubicin-containing targeted liposomes show greater cytotoxicity in DU-145 cells than doxorubicin-containing non-targeted liposomes.	[73]

and/or sigma-2 receptors have been prepared, but also mixed action compounds combining sigma receptor interaction with binding to other targets.

Abate and co-workers described a series of 1-cyclohexyl-4-(4-arylcyclohexyl)piperazines which combine high affinity to sigma-1 and sigma-2 receptors with inhibition of human Δ_8 - Δ_7 sterol isomerase and inhibition of P-glycoprotein (P-gp), an efflux pump involved in tumor multidrug resistance [76]. The most promising compound of the series, *cis*-11, showed K_i values of 0.26, 7.92 and 7.6 nM at the first three targets. In transfected canine kidney epithelial cells overexpressing P-gp, 0.1 μ M doxorubicin alone did not have any antiproliferative effect. However, when doxorubicin was combined with 30 μ M *cis*-11, 70% cell death was observed within 24 h and a combination with 50 μ M *cis*-11 resulted even in 90% cell death. Administration of 50 μ M *cis*-11 alone caused 50% cell death in the same assay. Thus, this class of compounds is capable of inducing significant growth inhibition in P-gp overexpressing tumor cells [76].

A later study from the same group concerned the development of tetrahydroisoquinolines which combine selectivity and high affinity for the sigma-2 subtype with the capability of reversing P-gp-mediated drug resistance. The two most promising compounds from the series showed nanomolar (K_i 5.34 nM) or picomolar (K_i 0.04 nM) affinity at rodent sigma-2 receptors, and high or extremely high selectivity for the sigma-2 subtype (260-fold and >50,000-fold, respectively). They were capable of reversing P-gp-mediated resistance and re-establishing the antitumor effect of doxorubicin in P-gp-overexpressing MCF-7adr human breast cancer cells. The compound with picomolar affinity showed even stronger antiproliferative action in doxorubicin-resistant cells than in parent cells, a phenomenon that has been called "collateral sensitivity" and may be based on a P-gp-catalyzed ATP-degrading futile cycle [77]. Thus, these tetrahydroisoquinolines could be evaluated for the treatment of multidrug-resistant tumors, and be administered either alone or in combination with classic chemotherapeutic agents.

In an attempt to enhance the apoptosis-inducing ability of haloperidol without impairing its targeting ability, medicinal chemists conjugated the tertiary OH group of haloperidol with cationic lipids of varying chain lengths. Such conjugation might not affect the affinity of haloperidol to sigma-1 or sigma-2 receptors, but could increase cellular entry of the drug because of the presence of the cationic lipid chain. The most promising compound of the series, HP-C8, contained a lipid chain with 8 carbon atoms. It showed very strong cytotoxicity towards MCF-7 and MDA-MB-231 (breast cancer) cells at doses at least 100-fold lower than parent haloperidol, and at least 10-fold lower than

haloperidol conjugates with other lipid chain lengths that were tried (4, 12 and 16 carbon atoms). The toxicity of the C8 conjugate was also much higher than that of haloperidol and the C8 lipid administered in combination. Moreover, HP-C8 was 2- to 3-fold more toxic to tumor cells than normal cells (COS-1, HEK-293), and the compound induced apoptosis in cancer cells but not in normal cells. Downregulation of sigma-1 receptors in MCF-7 cells resulted in the increased viability of cells treated with HP-C8, thus, the cytotoxicity of HP-C8 appears to be sigma receptor-mediated. Five intraperitoneal injections of 7.5 mg/kg HP-C8 at 2 to 3 day intervals in mice bearing B16F10 melanoma tumors resulted in greater than 3-fold growth reduction. Experiments with HP-C8 in human umbilical vein endothelial (HUVEC) cells suggested that the compound may also be capable of suppressing angiogenesis and the formation of neovasculature in the tumor environment. Cationic lipid-modified haloperidol derivatives are therefore an interesting novel class of anticancer drugs [78].

Other modifications of haloperidol were tried as well. An Italian publication concerned the phenylbutyrate ester of metabolite II of haloperidol. This molecule, called (\pm)-MRJF4, combines inhibition of histone deacetylase with sigma receptor binding. It has affinities to sigma receptors in the 10^{-7} M range (K_i 162 and 105 nM at sigma-1 and sigma-2, respectively), but negligible affinity to dopamine D2 and D3 receptors (K_i > 5000 nM). IC_{50} values of (\pm)-MRJF4 for growth inhibition in prostate cancer cell lines (LNCaP and PC-3) are much lower (11 and 13 μ M) than those of 4-phenylbutyric acid (2324 and 2273 μ M), haloperidol metabolite II (177 and 208 μ M) or an equimolar mixture of 4-phenylbutyric acid and haloperidol (190 and 165 μ M). Blocking experiments with (+)-pentazocine and AC927 suggested that both subtypes of sigma receptors are involved in the cytotoxic effects of (\pm)-MRJF4 with a prevalence of the sigma-2 subtype [79].

A series of adamantane phenylalkylamines was tested for sigma receptor affinity and antiproliferative activity. The most interesting compound of the series bound to both sigma-1 and sigma-2 receptors with affinities in the 10^{-8} M range (IC_{50} values 48 and 85 nM, respectively). It displayed a significant antiproliferative activity to human colon (HCT-116, HCT-15), prostate (DU-145, PC-3), breast cancer (MCF-7), ovarian (OVCAR-5), brain tumor (U-251), leukemia (HL-60), pancreatic (BxPC-3) and liver cancer (SK-HEP-1) cells. Activation of caspase-3 and apoptosis was observed in addition to cell cycle arrest at the sub-G1 level. Three cycles of treatment with this adamantane (40 mg/kg doses administered on 3 consecutive days per week during a period of 3 weeks) resulted in more than 3-fold inhibition of the growth of BxPC-3 xenografts in SCID mice. A different chemotherapy

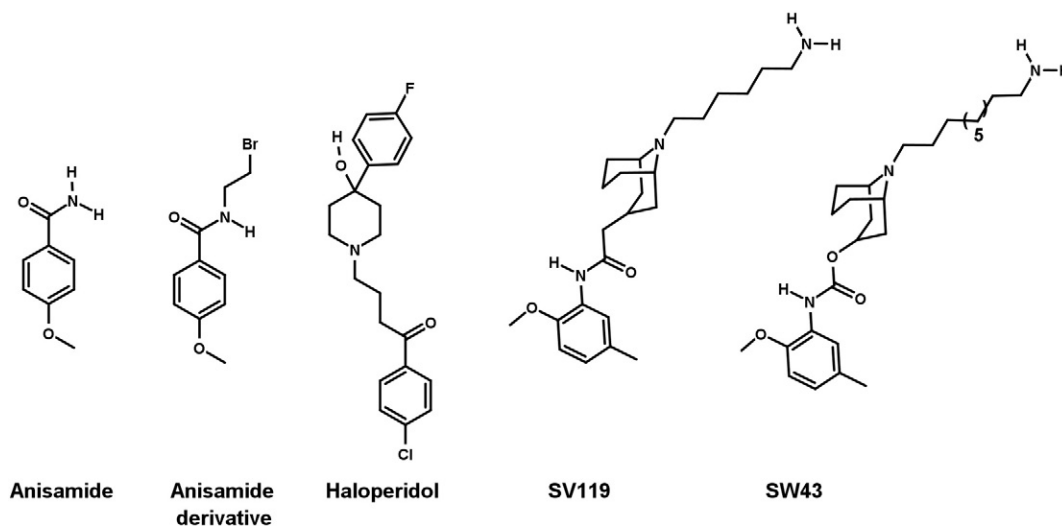


Fig. 5. Sigma ligands which have been employed for drug targeting purposes.

protocol (two cycles of treatment, 55 mg/kg doses administered on 5 consecutive days per week during a period of 3 weeks) resulted in significant but smaller growth reductions of PC-3, DU-145 and OVCAR-5 tumors [80]. These adamantane phenylalkylamines may therefore be considered as potential antitumor agents, although rather high drug doses were required for the inhibition of tumor growth.

Selective sigma-1 or sigma-2 receptor ligands may also be employed for therapeutic purposes. High affinity sigma-1 receptor ligands derived from spipethiane with sigma-1 subtype selectivities of 2630- and 29512-fold showed significant cytostatic effects in the human breast cancer cell line MCF-7/ADR [81]. A series of indole-based sigma receptor ligands was recently prepared, and the cytotoxicity of these compounds was tested in liver (HUH7), breast (MCF-7) and colon (HCT-116) cancer cell lines. The most promising ligand bound to both sigma-1 and sigma-2 receptors with affinities in the submicromolar range (K_i values 126 and 130 nM, respectively). It showed similar (MCF-7) or significantly (2- to 8-fold) greater cytotoxicity than 5-fluorouracil in the tested cell lines. Thus, indole-based sigma ligands may be useful for cancer therapy [82]. High-affinity fenpropimorph-derived sigma-1 receptor ligands [83], conformationally restricted sigma-1 receptor ligands with 7,9-diazabicyclo[4.2.2]decane scaffold [84] and hydroxyethyl substituted piperazines [85] have also shown considerable cytotoxicity in various tumor cell lines.

A publication of our own group examined the impact of daily intraperitoneal injections of rimcazole (26 mg/kg body weight) in nude mice bearing A375M human melanoma xenografts [86]. This treatment regimen resulted in a greater than 4-fold reduction of tumor weight in comparison to controls after 2 weeks. A transient increase of the tumor uptake of the glucose analog [^{18}F]FDG was observed after the first week of treatment. Rimcazole treatment was not associated with any significant adverse side effect [86]. In murine models of pancreatic cancer, sigma-2 ligands are accumulated preferentially in the tumor cells where they are internalized by both receptor-mediated and caveolin-mediated endocytosis. In preclinical studies, such ligands are capable of inducing apoptosis in the tumors with limited toxicity at non-target sites. The animal and *in vitro* data raise hope that sigma-2 ligands may be used to induce apoptosis in pancreatic tumors when resistance to traditional chemotherapies has developed [87]. At least half of the samples of human pancreatic adenocarcinoma appear to show overexpression of sigma-2 receptors.

Sigma ligands can not only kill tumor cells and inhibit tumor growth, but they can also significantly enhance the pharmacological action of other anticancer drugs. At concentrations of the individual agents having hardly any cytotoxic effects, combined administration can result in potent killing of tumor cells. In a preclinical model of pancreatic cancer (Panc02 tumors in C57BL/6 mice), combination treatment of the animals with gemcitabine and the novel sigma-2 receptor ligand SW43 was superior to single agent treatment and resulted in the stabilization of tumor volume during the treatment period with no significant adverse effects [88]. In an *in vitro* model of melanoma, the sigma ligand rimcazole synergistically enhanced the inhibition of A375M tumor cell growth induced by anti-MCSP:TRAIL [89]. During *in vitro* studies of colon and breast cancer cells, the P-glycoprotein inhibitor and sigma-1 receptor ligand MC70 strongly enhanced the effectiveness of doxorubicin [90].

6. Mechanisms underlying inhibition of tumor growth

Although it had been known for a long time that small-molecule ligands targeting sigma-1 and/or sigma-2 receptors can inhibit cancer cell proliferation, induce tumor cell death and suppress tumor growth in pre-clinical models, the cellular pathways activated by sigma ligands and the mechanisms involved in cell death had not been well-defined. Early studies in this area have been reviewed previously [2], thus only more recent and novel findings will be discussed here. Recently published reviews have focused on the interaction of sigma-1 receptors

with ion channels [91,92] and sigma-2 receptor-mediated tumor cell death [74].

Ion channels and G-protein coupled membrane receptors have been identified as the main clients for the sigma-1 chaperone protein. Protein-protein interactions are known to occur between sigma-1 receptors and voltage-gated potassium channels (Kv1.2, Kv1.4 and Kv1.5) [93–97], small conductance Ca^{2+} activated K^{+} current (SK) channels [98], voltage-gated sodium channels (Nav1.5) [99–101], voltage-gated L-type calcium channels [102], acid-sensing ion channels (ASIC1a) [103,104], volume-regulated chloride channels (VRCC) [105], the GluN1 subunit of the NMDA receptor [106], dopamine D1 receptors [107,108], dopamine D2 receptors [109], and histamine H3 receptors [107]. Moreover, the sigma-1 receptor regulates the expression of the human ether-a-gogo (HERG) channel by stimulating channel subunit biosynthesis [110]. This channel promotes the progression of many primary human cancers through the modulation of extracellular matrix adhesive interactions [110]. The mentioned protein-protein interactions and effects of sigma-1 receptors on channel subunit biosynthesis may be involved in the antitumor effects of sigma-1 ligands, since ion channels are important actors in the control of cancer growth and invasiveness [91,95,105,110–112].

American investigators noticed that treatment of tumor cell lines with certain sigma-1 ligands visibly diminished cell size. Based on this observation, they performed a study in various cell lines (T47D, MDA468 and MCF-7 human breast carcinoma, PC-3 and LNCaP prostate adenocarcinoma) which were treated with putative sigma-1 agonists (PRE084, (+) SKF-10047) and antagonists (IPAG and haloperidol). Antagonists but not agonists were found to reduce cellular protein synthesis by repressing the cap-dependent initiation of translation and phosphorylation of the translational regulator proteins p70S6K, S6 and 4E-BP1. Knockdown of sigma-1 receptors in the cells with specific siRNA had the same effect as treatment of cells with sigma-1 antagonists, confirming that the observed translational repression was indeed related to diminished sigma-1 receptor function. Thus, sigma-1 receptors appear to play a role in protein synthesis and sigma-1 ligands may be used to reversibly modulate the cellular synthetic machinery [113]. In a subsequent publication from the same group, MDA-MB-468 and T47D (breast adenocarcinoma) cells were treated with 10 μM concentrations of the previously mentioned sigma-1 agonists and antagonists. Evidence was now obtained suggesting that autophagy is involved in sigma ligand-induced decreases in cell size. Antagonists (but not agonists) induced ER stress and activated the unfolded protein response in a dose-dependent, time-dependent and reversible manner, with the extended treatment resulting in autophagy and finally in apoptosis. Knockdown of sigma-1 receptors suppressed these effects of sigma-1 antagonists, suggesting that these responses are indeed sigma-1 receptor-mediated [114].

A recent paper has indicated that the sigma-1 receptor is a key mediator of interleukin-24 (IL-24)-induced cancer-specific apoptosis. IL-24 generated from an adenovirus expressing this cytokine induces endoplasmic reticulum (ER) stress, production of reactive oxygen species and calcium mobilization resulting in the apoptosis of cancer cells. Treatment of cancer cells with the sigma-1 agonist (+)-SK-10047 blocks these responses. A direct interaction between IL-24 and sigma-1 receptors is suggested by the fact that these two molecules colocalize in immunocytochemical studies and co-immunoprecipitate after the application of either a sigma-1 receptor or an IL-24 antibody. Thus, the ER stress response, ROS production and calcium mobilization appear to be triggered by IL-24 via a sigma-1 receptor-dependent pathway and IL-24 appears to induce apoptosis through a sigma-1 receptor antagonistic mechanism [115].

The sigma ligand [^3H]PB28 was found to accumulate up to 5-fold in nuclear compared to cytosolic fractions of neuroblastoma (SK-N-SH) and breast cancer (MCF-7) cells, probably due to the fact that it binds with high affinity (0.5 nM) to histone H2A/H2B dimers [116]. Apparently, some sigma ligands can directly interact with nuclear material and may thus exert antiproliferative and cytotoxic effects.

Sigma-2 induced apoptosis can be caspase-independent and may therefore also occur in cells with caspase dysfunction [117]. SW43, a sigma-2 subtype-selective ligand, was shown to induce apoptosis in BxPC-3 and AsPC-1 pancreatic cancer cells by the permeabilization of lysosomal membranes and increasing cellular oxidative stress, but independent of caspase-3 activation, since the caspase-3 inhibitor z-DEVD-fmk did not counteract these effects and did not protect cells against the antitumor drug [118]. Siramesine, which has been classified as a sigma-2 receptor agonist, caused apoptosis and secretory granule permeabilization in mast cells also in a caspase-independent manner [119]. However, the effect of siramesine appears to be cell-type dependent. In human keratinocytes (HaCaT), cell death induced by siramesine was accompanied by caspase activation and triggered by destabilization of mitochondria rather than lysosomes. The divergent effects of siramesine in different cell lines may be due to the fact that siramesine is not a selective sigma-2 receptor ligand, but interacts with multiple molecular targets inside cells [120]. The cytotoxic and DNA-fragmenting effects of sigma-2 subtype-selective ligands other than SW43 or siramesine (WC-26, SV119 and RHM-138) were dependent on caspase-3 since they were counteracted by the caspase-3 inhibitor z-VAD-fmk. These ligands induced autophagy and decreased the activity of the mTOR (mammalian target of rapamycin) pathway [121]. The sigma-2 receptor/PGRMC1 has been shown to bind to key components of the autophagy machinery (microtubule-associated protein 1 light chain 3, UV radiation resistance associated gene) and to be required for the degradative activity of autophagy. The inhibition of PGRMC1 by RNAi or small molecule antagonists results in the accumulation of autophagy substrates and of aberrant mitochondria [122].

In summary: multiple mechanisms appear to underlie the inhibition of tumor growth and cell death after the administration of sigma ligands, and the signaling pathways are dependent both on the type of ligand and the type of tumor cell, resulting in conflicting reports in the oncological literature.

7. Conclusions

Novel, potent and subtype-selective radioligands for PET imaging of sigma receptors have become available. *In vivo* studies with these ligands may provide important information about the biology of sigma receptors and may lead to the application of PET imaging for tumor detection, tumor staging, evaluation of therapeutic strategies and anti-tumor drug development. The treatment of preclinical *in vitro* and *in vivo* models of cancer with target- and subtype-selective, nonradioactive sigma ligands may result in a greater understanding of the mechanisms underlying sigma ligand-induced cell death and may lead to clinical applications.

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