Sigma1 Pharmacology in the Context of Cancer

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F.J. Kim, G.W. Pasternak (eds.), *Sigma Proteins: Evolution of the Concept of Sigma Receptors*, Handbook of Experimental Pharmacology 244, DOI 10.1007/164_2017_38

Abstract

Sigma1 (also known as sigma-1 receptor, Sig1R, σ1 receptor) is a unique pharmacologically regulated integral membrane chaperone or scaffolding protein. The majority of publications on the subject have focused on the neuropharmacology of Sigma1. However, a number of publications have also suggested a role for Sigma1 in cancer. Although there is currently no clinically used anticancer drug that targets Sigma1, a growing body of evidence supports the potential of Sigma1 ligands as therapeutic agents to treat cancer. In preclinical models, compounds with affinity for Sigma1 have been reported to inhibit cancer cell proliferation and survival, cell adhesion and migration, tumor growth, to alleviate cancer-associated pain, and to have immunomodulatory properties. This review will highlight that although the literature supports a role for Sigma1 in cancer, several fundamental questions regarding drug mechanism of action and the physiological relevance of aberrant *SIGMAR1* transcript and Sigma1 protein expression in certain cancers remain unanswered or only partially answered. However, emerging lines of evidence suggest that Sigma1 is a component of the cancer cell support machinery, that it facilitates protein interaction networks, that it allosterically modulates the activity of its associated proteins, and that Sigma1 is a selectively multifunctional drug target.

Keywords

Allosteric modulation • Cancer • Cancer pain • Chaperone • Context • Drug mechanism of action • Immunomodulation • Lipid • Metabolism • Modulator • Multifunctional drug target • Protein homeostasis • Protein–protein interaction • Scaffold • Sigma1 • Sigma-1 receptor • Small molecule

1 Introduction

Sigma1 shares no significant homology with any other proteins encoded in the human genome (Hanner et al. 1996; Schmidt et al. 2016). Historically it has been considered a receptor. However, emerging evidence suggests that Sigma1 functions as a novel pharmacologically regulated integral membrane chaperone or scaffolding protein (Hayashi and Su 2007; Crottes et al. 2011, 2016; Thomas et al. 2017). Consistent with this notion, Sigma1 is involved in aspects of cellular protein homeostasis including protein synthesis, folding, trafficking, and degradation (Kim et al. 2012; Hayashi and Su 2007; Crottes et al. 2011, 2016; Schrock et al. 2013; Thomas et al. 2017).

Although most publications regarding Sigma1 describe it in the context of neuropharmacology (Cobos et al. 2008; Maurice and Su 2009), a number of publications over the years have described a potential role for Sigma1 in cancer biology. Until recently, this relationship has been largely based on two lines of

evidence: (1) reports of elevated expression levels of Sigma1 protein and *SIGMAR1* transcripts in some cancer cell lines and some tumors (reviewed in Sects. 2 and 3, below); and (2) antiproliferative and growth inhibiting effects of some small molecule inhibitors (putative antagonists) of Sigma1 on cancer cell lines (reviewed in Sect. 4, below, and Table 1). However, despite well over a hundred publications directly addressing the subject, the physiological role of Sigma1 in cancer cells remains poorly understood.

There is no compelling evidence that *SIGMAR1* is an oncogene or that Sigma1 is an oncogenic driver protein. However, several studies have demonstrated that cancer cells require functional, intact Sigma1 to grow, proliferate, and survive. Sigma1 RNAi and some small molecule inhibitors (putative antagonists) of Sigma1 have been reported to inhibit cell growth, proliferation, and cell survival. Conversely, increased Sigma1 protein levels through overexpression of recombinant Sigma1 and enhancing Sigma1 with small molecule activators (putative agonists) have been reported to promote some of these processes in cancer cells (reviewed in Sects. 4 and 6, below).

Most of our knowledge of Sigma1 comes from pharmacological studies that have implicated this protein in multiple cellular processes including control of apoptosis, cell cycle, cell growth, proliferation, endoplasmic reticulum (ER) stress, protein and lipid homeostasis, autophagy, and ion channel regulation (reviewed in Sects. 4–6, below). As it was originally identified as a receptor, small molecules with affinity for Sigma1, so-called Sigma1 ligands, have been classified as agonists and antagonists. These are evolving concepts, and in light of emerging data these definitions may not be accurate given that Sigma1 is not a bona fide receptor. We propose that the term modulator may be more appropriate for compounds with affinity for Sigma1. However, in this review we will continue to use the terms ligand/modulator, antagonist/inhibitor, and agonist/activator in order to integrate the decades of published data on the pharmacology of Sigma1 in cancer (see Sect. 4, below).

Several review articles have broadly surveyed compounds with affinity for Sigma1 and have described their effects on cancer cell lines (Abate 2012; Megalizzi et al. 2012; van Waarde et al. 2015; Brust et al. 2014). We have listed the published Sigma1 associated functional activities and binding affinities of many of these compounds in Tables 1 and 2. In this review, we will focus on a number of salient examples of how putative Sigma1 ligands have been used in cancer cell lines and what they reveal about Sigma1 biology in the context of cancer. We will review the historical classification of Sigma1 modulators as activators and inhibitors (putative agonists and antagonists), the cellular pathways and processes engaged by Sigma1 modulator compounds, the immunomodulatory effects of these compounds, and their potential as agents to treat cancer-associated comorbidities such as cancer pain as well as inhibit tumor growth (see Sects. 4–6, below). We will also review evidence from clinical trials as well as preclinical animal studies showing that the on-target effects of Sigma1 modulators do not produce adverse effects.

Table 1 Sigma ligands tested in cancer cell line studies (presented in chronological order of publication)

Reference	Compound name	Cell lines tested	Assays used	Results	MOA (proposed)
(Vilner and Bowen 1993)	Haloperidol, reduced haloperidol, fluphenazine, perphenazine, pimozide, spiperone	C6 rat glioma	Scoring of morphological changes	Loss of processes, discontinued cell division, eventual cell death	Not specified
(Vilner et al. 1995a, b)	Haloperidol, reduced haloperidol, fluphenazine, perphenazine, trifluoperazine, BD737, LR172, BD1008, SH-344, trifluperidol, thioridazine, (−)-butaclamol	C6 rat glioma, SK-N-SH, SH-SY5Y, NCB-20, NG108-15, PC12	Scoring of morphological changes, trypan blue exclusion to confirm score	Loss of processes, discontinued cell division, eventual cell death (dependent on time, dose, and pH)	Not specified
(Brent and Pang 1995)	Haloperidol, reduced haloperidol, DTG, (+)-pentazocine, (−)-pentazocine, rimcazole, (+)- and (−)-*N*-allylnormetazocine (SKF 10047)	MCF-7, LIM 1215, WIDr, melanoma (Chinnery)	MTT assay	Inhibition of cell proliferation, cell detachment, rounding of cells	Not specified
(Brent et al. 1996)	Reduced haloperidol	MCF-7, WIDr	Nuclear staining with Hoechst 33258, cellular DNA fragmentation ELISA, condensation of heterochromatin using transmission electron microscopy, FURA-2/AM calcium assay	Inhibition of cell proliferation, cell death	Induction of apoptosis, potentially through an increase in intracellular calcium
(Labit-Le Bouteiller et al. 1998)	SR31747A	Jijoye, U937, HL60, TF1, MCF-7, B9, CTLL2, M1, COS, CHO	MTT assay, gas chromatography, mass spectroscopy	Inhibition of cell proliferation, reversible by cholesterol	Inhibition of cholesterol biosynthesis via emopamil-binding protein
(John et al. 1999)	PIMBA	DU145, LNCaP, PC3	Soft agar colony formation assay	Inhibition of colony formation	Not specified
(Moody et al. 2000)	2-IBP (xenografts), IPAB (xenografts), haloperidol	NCI-H209, NCI-H345, NCI-N417 (xenografts)	MTT assay, soft agar colony formation assay, tumor xenografts	Inhibition of cell proliferation, inhibition of tumor xenograft growth	Not specified
(Crawford and Bowen 2002)	CB-64D, CB-184, haloperidol, reduced haloperidol, CB-184 in combination with doxorubicin or actinomycin D, haloperidol in combination with doxorubicin	MCF-7, T47D, MCF-7/ADR, SKBR3	CytoTox 96 kit to measure lactate dehydrogenase (LDH) release, TUNEL staining, ApoAlert annexin-V apoptosis kit	Cell death, potentiation of cytotoxicity with combination treatments	Novel p53- and caspase-independent apoptosis

(Berthois et al. 2003)	SR31747A	MCF-7 (xenografts), MDA-MB-231 (xenografts), LNCaP (xenografts), DU145 (xenografts), PC3 (xenografts), BT20	MTT assay, tumor xenografts	Inhibition of cell proliferation, inhibition of tumor xenograft growth (in combination with tamoxifen in MCF-7)	Potentially via binding to EBP or other binding site
(Barbieri et al. 2003)	Five (1α/1β-arylalkyl) quinolizidines including two thioisosteres and four spiro-[3,4-dihydro-1,2,4-benzotriazino-3,4′-(1′-substituted) piperidines]: ANS-1, ANS-2, ANS-3, ANS-4, ANS-5, FN/C-1, FN/C-2, FN/C-3, FN/C-4	MCF-7, MDA-MB-231	MTT assay	Inhibition of cell growth, cytotoxicity	Not specified
(Ferrini et al. 2003)	SR31747A	PC3, DU145, MDA-MB-231	CellTiter 96 Aqueous cell proliferation assay kit, DNA microarray, northern blot, affymetrix HC-G110 cancer gene array	Inhibition of cell proliferation	Decrease in expression of genes involved in DNA replication and progression of cell cycle, decrease in expression of 3 enzymes (dihydrofolate reductase, thymidylate synthase, thymidine kinase) involved in nucleotide synthesis
(Colabufo et al. 2004)	PB-28, NE-100, DTG, haloperidol, (+)-pentazocine	SK-N-SH, C6 rat glioma	MTT assay, CytoTox-One cytotoxicity assay (lactate dehydrogenase release), Apo-One homogenous caspase-3/7 kit	Inhibition of cell proliferation, cytotoxicity	Caspase-independent apoptosis
(Spruce et al. 2004)	Rimcazole (xenografts), IPAG (xenografts), reduced haloperidol, haloperidol (xenografts), BD1047, BD1063, *cis*-U50488 (xenografts)	MDA-MB-468 (xenografts), MDA-MB-435 (xenografts), MCF-7 (xenografts), H1299 (xenografts), PC3M (xenografts)	MTS CellTitre proliferation assay, colony formation assay, Apo-one homogeneous caspase3/7 assay, flow cytometry, FURA-2/AM calcium assay, tumor xenografts	Inhibition of cell survival, inhibition of xenograft growth	Caspase-dependent apoptosis, rise in cytosolic calcium, activation of phospholipase C, inhibition of PI3K pathway
(Renaudo et al. 2004)	(+)-Pentazocine, igmesine, DTG	NCI-H209, NCI-H146, Jurkat	Whole-cell patch-clamp, trypan blue exclusion, DEVD-pNA cleavage assay to analyze caspase activity, DNA fragmentation	Inhibition of cell growth (Jurkat, igmesine)	Inhibition of K^+ channel, accumulation of $p27^{kip1}$, not apoptosis

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Table 1 (continued)

Reference	Compound name	Cell lines tested	Assays used	Results	MOA (proposed)
(Wang et al. 2004)	Haloperidol, reduced haloperidol, progesterone, combination of reduced haloperidol + doxorubicin, vinorelbine, paclitaxel, and docetaxel	MDA-MB-231, MDA-MB-361, MDA-MB-435, MCF-7, BT20	CellTiter 96 Aqueous One cell proliferation assay	Growth inhibition, additive effect of growth inhibition with reduced haloperidol and chemotherapy combinations	Not specified
(Ostenfeld et al. 2005)	Siramesine	WEHI-S, WEHI-R4 (xenografts), MCF-7, MCF-7S1 (xenografts), MDA-MB-468, HeLa, ME-180	MTT assay, lactate dehydrogenase release, flow cytometry, caspase activity measurement, tumor xenografts	Cell death, cell shrinkage and detachment, inhibition of xenograft growth	Increase in reactive oxygen species (ROS), permeabilization of the lysosomal membrane
(Nordenberg et al. 2005)	Haloperidol, reduced-haloperidol, ifenprodil tartrate, opipramol, carbetapentane citrate, haloperidol in combination with imatinib mesylate (STI 571)	B16, SK-MEL-28	SRB colorimetric cytotoxicity assay, DNA fragmentation, flow cytometry, ELISA cell death assay, immunoblot, spectrofluorometric ATP measurement	Inhibition of cell growth (synergy with haloperidol and imatinib mesylate combination), G1 cell cycle arrest, decrease in cell viability	Apoptosis, decrease in ATP levels, decrease in cyclin D and CDK2 protein levels in cytoplasm and nucleus
(Azzariti et al. 2006)	PB-28, PB-28 in combination with doxorubicin	MCF-7, MCF-7 ADR	MTT assay, annexin-V staining, propidium iodide (PI) staining, flow cytometry, immunoblot, Apo-one homogenous caspase-3/7 kit	Inhibition of cell growth, increase in accumulation of intracellular doxorubicin, increase in cytotoxicity when in combination with doxorubicin when compared to doxorubicin alone	Increase in percent of cells in the G_0-G_1 phase, induction of caspase-independent apoptosis, decrease in P-gp expression
(Aydar et al. 2006)	(+)SKF10047, ibogaine	MCF-7, MDA-MB-231	Crystal violet staining assay, single-cell adhesion measuring apparatus	SKF10047 – inhibition of proliferation in MDA-MB-231 cells, reduction in adhesion in both cell lines Ibogaine – inhibition of cell proliferation in both cell lines, reduction in adhesion in both cell lines	Not specified
(Wei et al. 2006)	Haloperidol	PC12, N2a	MTT assay, lactate dehydrogenase release, flow cytometry, subcellular fractionation, immunoblot	Decrease in cell viability	Increase in Bcl-XS expression and translocation to mitochondria, apoptosis
(Geiger et al. 2007)	Stereoisomeric alcohols and methyl ethers from (R)- and (S)-glutamate	5637, RT-4, A-427, LCLC-103H, MCF-7	Microtiter assay with crystal violet staining	Inhibition of cell growth (methyl ethers > alcohols) and cell death (methyl ethers)	Not specified

(Kashiwagi et al. 2007)	SV119, WC-26 (tumor allografts), haloperidol	Panc-1, CFPAC-1, ASPC-1, Panc-02 (tumor allografts)	TUNEL staining, flow cytometry, tumor allografts	Cell death, decrease in tumor allograft growth, improved survival	Caspase-3/7-dependent apoptosis
(Megalizzi et al. 2007)	4-IBP	U373-MG (xenografts), C32, A549 (xenografts), PC3	Immunoblot, TUNEL staining, flow cytometry, colorimetric MTT assay, computer-assisted phase-contrast microscopy, fluorescence microscopy, scratch wound assay, tumor xenografts	Inhibition of proliferation, decrease in migration, increased sensitivity to proapoptotic (lomustin) and proautophagic (temozolomide) drugs, increased survival in vivo (U373-MG), increased therapeutic benefit of temozolomide (U373-MG) and IRI (A549) in vivo	Not apoptosis or autophagy, alteration to actin cytoskeleton organization, decrease in glucosylceramide synthase and Rho guanine nucleotide dissociation inhibitor (important for drug resistance)
(Achison et al. 2007)	Rimcazole	HCT-116 ($p53^{+/+}$ or $^{-/-}$), MDA-MB-231	Immunoblot, CellTiter 96 Aqueous one solution cell proliferation assay (MTS), PI staining, flow cytometry	Cell death	Increase in HIF-1α levels under normoxic conditions only in cancer cells (partly dependent on p53), apoptosis (more potent in $p53^{+/+}$ cells)
(Renaudo et al. 2007)	Igmesine, DTG, (+)-pentazocine, NPPB	NCI-H209, JA3, HEK-SIG (Sigma1 transfected HEK cells)	Trypan blue exclusion, immunoblot, electronic sizing for volume measurements with CASY 1 (SCARFE SYSTEM), whole cell patch clamp	Inhibition of cell proliferation, inhibition of cell cycle, delayed/eliminated regulatory volume decrease	Inhibition of volume-regulated chloride channels (VRCC), accumulation of p27, affected rate of activation of VRCC and cell volume regulation, which could protect cells from apoptosis
(Rybczynska et al. 2008)	Rimcazole, haloperidol	C6 rat glioma	Competition of ligand binding with ^{11}C-SA4503, measuring uptake of PET tracers to examine metabolic activity, trypan blue exclusion, morphology observations	Decrease in cell viability, increase in PET tracer uptake (^{18}F-FDG) or decrease in PET tracer uptake (^{18}F-FLT and ^{11}C-choline)	Very high occupancy of Sigma2 receptors
(Ostenfeld et al. 2008)	Siramesine	MCF-7 (xenografts), U2OS, WEHI-S	Immunoblot, immunocytochemistry, subcellular fractionation, acridine orange staining, lysotracker staining, measurement of cathepsin activity by zFR-AFC probe, flow cytometry, MTT assay, lactate dehydrogenase release, tumor xenografts	Cathepsin-dependent cell death, no increase in protein degradation, sensitization to cell death with combination of siramesine and autophagy inhibitor (3-MA)	Localization of siramesine in lysosomes, increase in lysosomal pH, inhibition of mTORC, acts as a lysosomotropic detergent that leads to destabilization of lysosomes, buildup of protective autophagosomes (in vivo and in vitro)

(continued)

Table 1 (continued)

Reference	Compound name	Cell lines tested	Assays used	Results	MOA (proposed)
(Megalizzi et al. 2009)	4-IBP, 4-IPAB, haloperidol, BD1008, eliprodil, donepezil, dextromethorphan, IPAG	Hs683 (xenografts), U373, T98G, U87, SW1783, A172, SW1088, U138, H4, U118	Computer-assisted phase-contrast videomicroscopy, scratch wound assay, global growth ratio calculations, tumor xenografts	Decrease in cell recolonization (4-IBP, donepezil, IPAG, dextromethorphan, BD1008, and haloperidol, U373 and T98G), cell death, decrease in cell division and increased survival in tumor xenografts (particularly with combination of donepezil and temozolomide)	Increase in cell mitosis duration, cell mitotic arrests
(Berardi et al. 2009)	Analogs of PB-28, such as piperidines **24** and **15**	SK-N-SH	MTT assay	Inhibition of cell proliferation	Not specified
(Kashiwagi et al. 2009)	SV119, SV119 in combination with gemcitabine and paclitaxel (allografts)	Panc-02 (allografts), CFPAC-1, Panc-1, ASPC-1	TUNEL staining, caspase-3 detection, flow cytometry, tumor allografts	Cell death, decreased tumor growth and increased survival in allografts (combination of SV119 and gemcitabine or paclitaxel)	Apoptosis (particularly when Sigma2 ligands in combination with chemotherapy) in vitro and in vivo
(Holl et al. 2009a, b, c)	6,8-Diazabicyclo[3.2.2]nonane derivatives, such as benzylidene derivatives **17** and benzyl ethers **11**	A-427	Microtiter crystal violet staining assay	Inhibition of cell growth	Not specified
(Holl et al. 2009)	Allyl and benzyl substituted 6,8-diazabicyclo[3.2.2]nonan-2-one derivatives **5**, ent-**5** and ent-**14**	5637	Microtiter crystal violet staining assay	Inhibition of cell growth	Not specified
(Holl et al. 2009)	6-Allyl-6,8-diazabicyclo[3.2.2] nonane derivatives, such as methyl ethers ent-**16b**, **21a,** ent-**21a**, and **21b,** and unsubstituted compounds **23a** and **23b,** and bicyclic acetal **11**	A-427	Microtiter crystal violet staining assay	Inhibition of cell growth	Not specified
(Piergentili et al. 2010)	Novel antagonists related to spipethiane, such as **4-10**	MCF-7, MCF-7 ADR	SRB assay, annexin-V staining, PI staining, flow cytometry	Inhibition of cell growth, induction of cell death of MCF-7 ADR (high expressers of Sigma1)	Inhibition of cell cycle, induction of apoptosis

(Hornick et al. 2010)	SW43, SV119, siramesine, sigma2 ligands in combination with gemcitabine	Panc-02 (allografts), MIA PaCa-2, Panc-1, BXPC3, CFPAC, ASPC-1	CellTiter-Glo assay, caspase-3 staining, annexin-V and PI staining, flow cytometry, image-iT live green reactive oxygen species detection kit, tumor allografts	Decrease in cell viability and decrease in tumor allograft growth (particularly when ligands in combination with gemcitabine)	Activation of caspase-3, increase in ROS, induction of apoptosis
(Sunnam et al. 2010)	Conformationally restricted ligands derived from a 7,9-diazabicyclo[4.2.2]decane scaffold, such as methyl ether **25b** and unsubstituted derivatives **26** and **27**	A-427, MCF-7, 5637	Microtiter crystal violet staining assay	Inhibition of cell growth (**25b**, **26**, and **27**)	Not specified
(Hajipour et al. 2010)	*N*,*N*-dialkyl (**1–3**,) or *N*-alkyl-*N*-aralkyl compounds (compounds **4–18**)	NCI-H460, SK-OV-3, DU145, MCF-7, SF-268, A549, MDA-MB-231, HT-29, HCT-15, H1299	Multiplex cytotoxicity assays by Keck-UWCCC small molecule screening facility	Inhibition of cell proliferation (**9**, **3**, **15, 19**, **20**), cytotoxicity	Not specified
(Ahmed et al. 2010)	AG-205	A549, MDA-MB-231, MDA-MB-468	MTT assay, immunoblot, flow cytometry	Inhibition of cell growth, decrease in cell viability	Inhibition of cell cycle, posttranscriptional increase in Sigma2, decrease in ERK phosphorylation
(Marrazzo et al. 2011a, b)	(−)-Methyl (1S,2R)-2-{[(3-endo)-3-(4-Chlorophenyl)-3-hydroxy-8-azabicyclo[3.2.1] oct-8-yl]methyl}-1-phenylcyclopro-panecarboxylate (9)	LNCaP, PC3	MTT assay	Inhibition of cell proliferation	Not specified
(Marrazzo et al. 2011a, b)	Phenylbutyrate ester of haloperidol metabolite II (±)-MRJF4	LNCaP, PC3	MTT assay	Decrease in cell viability	Not specified
(Yarim et al. 2011)	Indole scaffold based compounds **1a-c, 3a-b, 4a-b**	MCF-7, HUH7, HCT-116	TCA fixation and SRB staining	Cell death	Not specified
(Chu et al. 2011)	*N*-3-(4-nitrophenyl)propyl derivatives of heptylamine (2a and 2b), dodecylamine (3a and 3b)	MDA-MB-231, MCF-7, NCI-H460, A549, H1299, HCT-15, HT-29, SK-OV-3, DU145, SF-268	Calcein-AM, CellTiter-Glo assay, EthD-1	Cell death	Not specified
(Pal et al. 2011)	Haloperidol, cationic lipid-conjugated haloperidol HP-C4, HP-C8 (allografts), HP-C12, HP-C16	MCF-7, MDA-MB-231, ZR-75-1, B16F10 (allografts)	MTT assay, annexin-V and PI staining, TUNEL staining, flow cytometry, immunoblot	Inhibition of cell proliferation (particularly HP-C8, and more in cancer cells than normal cells), decrease in cell viability, inhibition of allograft tumor growth	Decrease in pAKT signaling, induction of caspase-3-mediated apoptosis

(continued)

Table 1 (continued)

Reference	Compound name	Cell lines tested	Assays used	Results	MOA (proposed)
(Abate et al. 2011)	Novel cyclohexylpiperazine derivatives *cis*-**7**, *trans*-**7**, *cis*-**8**, *trans*-**8**, *cis*-**9**, *trans*-**9**, *cis*-**10**, *trans*-**10**, *cis*-**11**, *trans*-**11**, *cis*-**12**, *trans*-**12**, *cis*-**13**, *trans*-**13**, *cis*-**14**, *trans*-**14**, **1**, **15**, *cis*-**11** in combination with doxorubicin	SK-N-SH, PC3, MDCK-MDR1	MTT assay, calcein-AM assay	Inhibition of cell proliferation (especially with *cis*-**11** in combination with doxorubicin), increased cell death, p-glycoprotein inhibition	Not specified
(Spitzer et al. 2012)	SV119, conjugates S2-CTMP-4, S2-rapamycin, and S2-Bim (allografts), S2-Bim in combination with gemcitabine and radiation	Panc-02 (allografts), Panc-1, ASPC-1, and CFPAC (xenografts)	TUNEL staining, caspase-3 staining, flow cytometry, tumor allografts and xenografts	Cell death (augmented by S2 conjugates), inhibition of allograft growth (S2-Bim > SV119), augmented cell death with combination of S2-Bim with gemcitabine and radiation in vitro	Disruption of intracellular signaling pathways (AKT for S2-CTMP-4, p70S6K for S2-rapamycin), apoptosis
(Kim et al. 2012)	IPAG, haloperidol	T47D, MCF-7, MDA-MB-468, LNCaP, PC3	Flow cytometry, trypan blue exclusion, BCA assay, m^7GTP-sepharose bead mimicking 5′ mRNA cap pull-down	Reversible decrease in cell mass, cell death with continuous treatment over time	Reversible decrease in cap-dependent translation initiation
(Hornick et al. 2012)	SW43 (xenografts), PB282 (xenografts), SV119 (xenografts), PB-28 (xenografts), derivatives of SW43 and PB282	BXPC3 (xenografts), Panc-02, ASPC-1	Flow cytometry, caspase-3 assay, microscopy, cellular protease assay, CellTiter-Glo assay, image-iT live Green reactive oxygen species detection kit, tumor xenografts	Cell death, inhibition of xenograft growth	Caspase-independent (SW43) or caspase-dependent (PB282) death after lysosomal membrane permeabilization, protease translocation, oxidative stress (SW43)
(Zeng et al. 2012)	WC-26, SV119, RHM-138, siramesine	EMT-6, MDA-MB-435	CellTiter96 Aqueous one MTS assay, lactate dehydrogenase release, TUNEL staining, flow cytometry, caspase-3 assay, immunoblot, transmission electron microscopy	Cell death	Induction of autophagy, mTOR inhibition, alteration of cell cycle progression, caspase activation, apoptosis
(Riganas et al. 2012a, b, c)	C1-substituted adamantine piperazines **2a** (xenografts), **2b, 2c, 2d, 2e**, **4**	NCI-H460, DMS 114, NCI-H69, H69AR, HL-60, MIA PaCa-2 (xenografts), BXPC3 (xenografts), SKHep1, LOX-IMVI, HCT-116, HCT-15, DU145, PC3, MCF-7, IGROV-1, OVCAR-5, SF268, SF295, U251	TCA fixation and SRB staining, annexin-V binding, caspase-3 assay, flow cytometry, PI staining, 7-AAD incorporation, tumor xenografts, formalin test	Inhibition of cell proliferation, cytotoxicity, inhibition of xenograft growth, analgesia (**2a**)	Caspase-3 activation, inhibition of cell cycle, apoptosis

(Riganas et al. 2012a, b, c)	Novel adamantane phenylalkylamines **2a**−**d**, **3a**−**c**, and **4a**−**e,** particularly **4a** (xenografts), **4a** in combination with 5-fluorouracil and gemcitabine (xenografts)	BXPC3 (xenografts), PC3 (xenografts), DU145 (xenografts), OVCAR-5 (xenografts), IGROV-1, HL-60 (xenografts), HCT-116, HCT-15, MCF-7, U251, SKHep1, MIA PaCa-2	TCA fixation and SRB staining, PI staining, annexin-V staining, flow cytometry, caspase-3 assay, tumor xenografts, observing auxiliary region and abdominal region for metastases and subsequent isolation and subculture to determine cell type origin, formalin test	Inhibition of cell proliferation, cytotoxicity, decreased tumor size in xenografts, synergistic anti-tumor effects with reference compounds in xenografts, decreased metastasis, analgesia (**4a**)	Caspase-3 activation, inhibition of cell cycle, apoptosis
(Riganas et al. 2012a, b, c)	4-(1-adamantyl)-4,4-diarylbutylamines **1**, 5-(1-adamantyl)-5,5-diarylpentylamines **2** and 6-(1-adamantyl)-6,6-diarylhexylamines **3**, **1a** (xenografts), **1a** in combination with paclitaxel (formalin test)	IGROV-1 (xenografts), HCT-116, HCT-15, Caki, DU145, PC3, MDA-MB-231, MCF-7, OVCAR-5, ADR-res NCI, SF268, U251, NCI-H460, DMS 114, HL-60 (TB), BXPC3, SKHep1, LOX-IMVI, SK-MEL-28, CCS WD6	TCA fixation and SRB staining, tumor xenografts, formalin test	Inhibition of cell proliferation, inhibition of xenograft growth, analgesia (**1a**)	Not specified
(Schrock et al. 2013)	IPAG	MDA-MB-468, T47D, MCF-7, PC3, Panc-1, HepG2	Trypan blue exclusion, PI staining, immunoblot, microscopy	Cell death	Unfolded protein response, autophagy, apoptosis
(Rybczynska et al. 2013)	Rimcazole	A375M	Tumor xenografts	Inhibition of xenograft growth, decrease in Ki67 staining	Not specified
(Niso et al. 2013)	Siramesine, PB-28, **4**, F281, **6**, **13**, **14**, **15**, **17**, **19**, **20**, **21**, **22**, **23**, **24**, **25, 15** in combination with doxorubicin, **25** in combination with doxorubicin	MCF-7, MCF-7 ADR, MDCK-MDR1	MTT assay, calcein-AM assay, bioluminescence ATP assay	Inhibition of cell proliferation, p-glycoprotein interaction, increased sensitization of MCF-7 ADR cells to doxorubicin (**15** and **25**), collateral sensitivity (siramesine and **25**)	Generation of more ROS and higher ATP consumption in MCF-7 ADR cells than parental cells
(Korpis et al. 2014)	Enantiomeric piperazines (S)-**4** and (R)-**4**	RPMI 8226	MTT assay, annexin-V and PI staining, caspase staining, flow cytometry	Inhibition of cell proliferation, cell death	Induction of incomplete autophagy, lipid peroxidation, altered mitochondrial membrane potential, caspase-independent apoptosis
(Korpis et al. 2014)	PB-28, haloperidol, novel hydroxyethyl piperazine-based sigma ligands such as (R)-**2b**	RPMI 8226, HL60, LCLC-103H, DAN-G, MCF-7, RT-4, A-427, 5637	MTT assay, crystal violet staining assay, annexin-V and PI staining	Inhibition of cell proliferation, cell death	Apoptosis ((R)-**2b** and combination of PB-28 with (R)-**2b**)
(Weber et al. 2014)	Hydroxyethyl substituted piperazines (**7c**)	RPMI 8226, 5637, A-427, MCF-7	MTT assay, annexin-V and PI staining	Inhibition of cell growth, cell death	Apoptosis

(continued)

Table 1 (continued)

Reference	Compound name	Cell lines tested	Assays used	Results	MOA (proposed)
(Garg et al. 2014)	SW IV-134 (SMAC mimetic conjugate)	SKOV3 (xenografts), OVCAR-3, HEY A8, HEY A8 MDR	Annexin-V staining, flow cytometry, immunoblot, CellTiter-Glo assay, caspase-Glo assay systems, ELISA, qRT-PCR, tumor xenografts	Cell death, decrease in tumor burden (xenografts), increase in survival (xenografts)	cIAP-1 and cIAP-2 degradation, activation of NF-κB, TNFα-dependent cell death, caspase-dependent apoptosis
(Zeng et al. 2014)	Azabicyclononane analogs SV119, SV166, WC-26, 2b, YUN245; tropane analog RHM-138; siramesine analog siramesine	EMT-6, MDA-MB-435	MTS assay, caspase-3 activation assay	Cell death	Caspase-3 activation
(Fytas et al. 2015)	Novel 1-(2-aryl-2-adamantyl) piperazine derivatives **6-15** (particularly **6** and **13**)	HeLa, MDA-MB-231, MIA PaCa-2, NCI HI975	MTT assay	Decrease in percent cell survival	Not specified
(Nicholson et al. 2015)	CM572	SK-N-SH, MCF-7, Panc-1	MTT assay, FURA-2/AM calcium assay, immunoblot	Irreversible cell death	Increase in cytosolic calcium, cleavage of Bid
(Happy et al. 2015)	Rimcazole, in combination with Ad.p53	MCF-7, T47D, MDA-MB-231, MDA-MB-157	MTT assay, annexin-V and PI staining, flow cytometry, DCFH-DA staining, immunoblot	Cell growth inhibition, cell death, synergistic anti-tumor effect with Ad.p53	Combination: Increase in ER stress, activation of the p38 MAPK pathway, increase in ROS, increase in Bax and activated caspase-3, induction of apoptosis
(Sozio et al. 2015)	(*R*)-(+)-MRJF4 and (*S*)-(−)-MRJF4	C6 rat glioma	Annexin-V and PI staining, MTT assay, transwell chamber migration assay, flow cytometry	Inhibition of cell proliferation, decrease in migration, cell death	Late apoptosis/necrosis, increase in percent of cells in S phase
(Das et al. 2016)	(+)-SKF10047	DU145, PC3, LNCaP	MTT assay, annexin-V binding assay	Reduction in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) killing	Sigma1 plays a role in activation of caspase-3 and caspase-8 after TRAIL
(Nicholson et al. 2016)	CM764	SK-N-SH, MG-63	MTT assay, CyQUANT cell proliferation assay, FURA-2/AM calcium assay, NAD^+/NADH quantification colorimetric kit, ATP colorimetric/fluorometric assay kit, DCFDA stain	Increase in MTT reduction without an increase in DNA replication or proliferation	Increase in cytosolic calcium, increase in NAD^+/NADH, increase in levels of ATP, reduction in ROS, increase in VEGF and HIF1α, potential induction of glycolysis
(Zampieri et al. 2016)	Novel 1-(4-(aryl(methyl)amino) butyl)-heterocyclic ligands such as **1a** and **1d**	SH-SY5Y	MTT assay	Cytotoxicity	Not specified
(Thomas et al. 2017)	IPAG, CT-189 (xenograft)	LAPC4, LNCaP, 22Rv1 (xenograft), VCaP, C4-2	Soft agar colony formation assay, crystal violet staining assay, trypan blue exclusion, immunoblot, tumor xenograft	Suppression of cell growth and survival, inhibition of xenograft growth	Proteasomal degradation of androgen receptor and androgen receptor splice variants

Table 2 Sigma ligand binding affinities

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
(+)-3-PPP	Sigma1			
	• $K_i = 86$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 109$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 102$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 75$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Cobos et al. 2005)
	Sigma2			
	• $K_i = 138$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 108$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
4-IBP	Sigma1			
	• $K_i = 1.7$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(John et al. 1995a, b)
	• $K_i = 2.6$ nM	Sf9 cells	[^{3}H](+)-pentazocine	(Schmidt et al. 2016)
	Sigma2			
	• $K_i = 25$ nM	Rat liver	[^{3}H]DTG	(John et al. 1995a, b)
(+)-Pentazocine	Sigma1			
	• $K_i = 3.1$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Vilner and Bowen 1993)
	• $K_i = 5.3$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 2.2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Geiger et al. 2007)
	• $K_i = 5.5$ nM	Rat cerebellum	[^{3}H](+)-pentazocine	(Ishiwata et al. 2006)
	• $K_i = 2.5$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Choi et al. 2001)
	• $K_i = 3.3$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Berardi et al. 2009)
	• $K_i = 4.2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Holl et al. 2009a, b, c)
	• $K_i = 5.6$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Sunnam et al. 2010)
	• $K_i = 2.8$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Abate et al. 2011)
	• $K_i = 3.4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Niso et al. 2013)
	• $K_i = 5.4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Weber et al. 2014)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $K_i = 36.0$ nM	RPMI 8226 cells	[^{3}H](+)-pentazocine	(Weber et al. 2014)
	• $K_i = 25.8$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 15.4$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 16.7$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 4.4$ nM	BE(2)-C cells	[^{3}H](+)-pentazocine	(Ryan-Moro et al. 1996)
	Sigma1			
	• $K_d = 5.8$ nM	DU145 cells	Saturation binding	(John et al. 1999)
	• $K_d = 23.1$ nM	SK-N-SH cells	Saturation binding	(Colabufo et al. 2004)
	• $K_d = 4.7$ nM	C6 rat glioma cells	Saturation binding	(Colabufo et al. 2004)
	• $K_d = 7.5$ nM	Rat liver	Saturation binding	(Hellewell et al. 1994)
	• $K_d = 23.3$ nM	Rat kidney	Saturation binding	(Hellewell et al. 1994)
	• $K_d = 7.1$ nM	MCF-7 cells	Saturation binding	(Azzariti et al. 2006)
	• $K_d = 3.9$ nM	MCF-7/ ADR cells	Saturation binding	(Azzariti et al. 2006)
	Sigma2			
	• $K_i = 2{,}470$ nM	Rat liver	[^{3}H]DTG	(Ishiwata et al. 2006)
	• $K_i = 1{,}923$ nM	Rat liver	[^{3}H]DTG	(Choi et al. 2001)
	• $K_i = 1{,}542$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 2{,}018$ nM	Rat liver	[^{3}H](+)-3-PPP	(Hellewell et al. 1994)
	• $K_i = 3{,}475$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 6{,}611$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
(−)-Pentazocine	Sigma1			
	• $K_i = 807$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 39$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $K_i = 41$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 40$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	Sigma2			
	• $K_i = 2{,}324$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 37$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 42$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
(+)-SKF10047	Sigma1			
	• $K_i = 597$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 54$ nM	BE(2)-C cells	[^{3}H](+)-pentazocine	(Ryan-Moro et al. 1996)
	• $K_i = 101$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 153$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 420$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	Sigma2			
	• $K_i = 39{,}740$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 11{,}170$ nM	Rat liver	[^{3}H](+)-3-PPP	(Hellewell et al. 1994)
	• $K_i = 154{,}335$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
(−)-SKF10047	Sigma1			
	• $K_i = 50{,}399$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 1{,}339$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 2{,}366$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 1{,}917$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	Sigma2			
	• $K_i = 41{,}461$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 2{,}659$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 2{,}929$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
BD737	Sigma1			
	• $K_i = 9$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 8$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 2$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	Sigma2			
	• $K_i = 68$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 96$ nM	Rat liver	[^{3}H](+)-3-PPP	(Hellewell et al. 1994)
BD1008	Sigma1			
	• $K_i = 1$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 2$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 1$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Berardi et al. 2001)
	Sigma2			
	• $K_i = 32$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 8$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 83$ nM	Rat liver	[^{3}H]DTG	(Berardi et al. 2001)
BD1047	Sigma1			
	• $K_i = 0.6$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 0.9$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Matsumoto et al. 1995)
	• $K_i = 1.9$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Entrena et al. 2009)
	• $K_i = 5.3$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Cobos et al. 2005)
	Sigma2			
	• $K_i = 47$ nM	Rat liver	[^{3}H]DTG	(Matsumoto et al. 1995)
BD1063	Sigma1			
	• $K_i = 7$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 4$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Entrena et al. 2009)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $K_i = 16$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Cobos et al. 2005)
	• $K_i = 4$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Nieto et al. 2012)
	• $K_i = 9$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Matsumoto et al. 1995)
	Sigma2			
	• $K_i = 449$ nM	Rat liver	[^{3}H]DTG	(Matsumoto et al. 1995)
CB-64D	Sigma1			
	• $K_i = 5{,}304$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 3{,}063$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Bowen et al. 1995)
	Sigma2			
	• $K_i = 61$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 17$ nM	Rat liver	[^{3}H]DTG	(Bowen et al. 1995)
CB-64L	Sigma1			
	• $K_i = 102$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 11$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Bowen et al. 1995)
	Sigma2			
	• $K_i = 759$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 154$ nM	Rat liver	[^{3}H]DTG	(Bowen et al. 1995)
CB-184	Sigma1			
	• $K_i = 7{,}436$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Bowen et al. 1995)
	Sigma2			
	• $K_i = 13$ nM	Rat liver	[^{3}H]DTG	(Bowen et al. 1995)
CM764	Sigma1			
	• $K_i = 87$ nM	Rat liver	[^{3}H](+)-pentazocine	(Nicholson et al. 2016)
	Sigma2			
	• $K_i = 4$ nM	Rat liver	[^{3}H]DTG	(Nicholson et al. 2016)
DTG	Sigma1			
	• $K_i = 203$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 60$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $K_i = 45$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 51$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 69$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011a)
	• $K_i = 71$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Zampieri et al. 2016)
	Sigma2			
	• $K_i = 58$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 13$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 22$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 23$ nM	Guinea pig brain	[^{3}H]DTG	(Marrazzo et al. 2011a)
	• $K_i = 54$ nM	Rat liver	[^{3}H]DTG	(Zampieri et al. 2016)
Haloperidol	Sigma1			
	• $K_i = 4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Vilner and Bowen 1993)
	• $K_i = 2$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Geiger et al. 2007)
	• $K_i = 3$ nM	Rat cerebellum	[^{3}H](+)-pentazocine	(Ishiwata et al. 2006)
	• $K_i = 4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Holl et al. 2009a, b, c)
	• $K_i = 6$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Sunnam et al. 2010)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011a)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011b)
	• $K_i = 7$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Weber et al. 2014)
	• $K_i = 40$ nM	RPMI 8226 cells	[^{3}H](+)-pentazocine	(Weber et al. 2014)
	• $K_i = 3$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Sozio et al. 2015)
	• $K_i = 7$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Zampieri et al. 2016)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $K_i = 1$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Choi et al. 2001)
	• $K_i = 2$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 8$ nM	Rat kidney	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 6$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	Sigma2			
	• $K_i = 78$ nM	Rat liver	[^{3}H]DTG	(Geiger et al. 2007)
	• $K_i = 167$ nM	Rat liver	[^{3}H]DTG	(Ishiwata et al. 2006)
	• $K_i = 78$ nM	Rat liver	[^{3}H]DTG	(Holl et al. 2009a, b, c)
	• $K_i = 78$ nM	Rat liver	[^{3}H]DTG	(Sunnam et al. 2010)
	• $K_i = 16$ nM	Guinea pig brain	[^{3}H]DTG	(Marrazzo et al. 2011a)
	• $K_i = 16$ nM	Guinea pig brain	[^{3}H]DTG	(Marrazzo et al. 2011b)
	• $K_i = 78$ nM	Rat liver	[^{3}H]DTG	(Weber et al. 2014)
	• $K_i = 200$ nM	RT-4 cells	[^{3}H]DTG	(Weber et al. 2014)
	• $K_i = 18$ nM	Guinea pig brain	[^{3}H]DTG	(Sozio et al. 2015)
	• $K_i = 78$ nM	Rat liver	[^{3}H]DTG	(Zampieri et al. 2016)
	• $K_i = 38$ nM	Rat liver	[^{3}H]DTG	(Choi et al. 2001)
	• $K_i = 12$ nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 18$ nM	Rat liver	[^{3}H](+)-3-PPP	(Hellewell et al. 1994)
	• $K_i = 42$ nM	Rat kidney	[^{3}H]DTG	(Hellewell et al. 1994)
	• $K_i = 221$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
Haloperidol (reduced)	Sigma1			
	• $K_i = 5$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Vilner and Bowen 1993)
	• $K_i = 3$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 47$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	Sigma2			
	• $K_i = 123$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
Haloperidol–metabolite II	Sigma1			
	• $K_i = 5$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011a)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011b)
	Sigma2			
	• $K_i = 1$ nM	Guinea pig brain	[^{3}H]DTG	(Marrazzo et al. 2011a)
	• $K_i = 1$ nM	Guinea pig brain	[^{3}H]DTG	(Marrazzo et al. 2011b)
	• $K_i = 2$ nM	Guinea pig brain	[^{3}H]DTG	(Sozio et al. 2015)
Igmesine	Sigma1			
	• $IC_{50} = 39$ nM	Rat brain	[^{3}H](+)-SKF10047	(Roman et al. 1990)
IPAG	Sigma1			
	• $K_d = 3$ nM	MDA-MB-468 cells	[^{125}I]IPAG saturation	(Schrock et al. 2013)
	• $K_d = 3$ nM	Guinea pig brain	[^{125}I]IPAG saturation	(Wilson et al. 1991)
LR172	Sigma1			
	• $K_i = 6$ nM	SK-N-SH cells	[^{3}H](+)-pentazocine	(Vilner and Bowen 2000)
	• $K_i = 1$ nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• $K_i = 0.5$ nM	C6 glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 0.4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(McCracken et al. 1999)
	Sigma2			
	• $K_i = 14$ nM	SK-N-SH cells	[^{3}H]DTG	(Vilner and Bowen 2000)
	• $K_i = 2$ nM	Rat liver	[^{3}H]DTG	(McCracken et al. 1999)
NE-100	Sigma1			
	• $K_i = 15$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Marrazzo et al. 2011a)
	• $K_i = 13$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Cobos et al. 2005)
	• $K_i = 1$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Berardi et al. 2001)
	• $K_d = 1$ nM	Guinea pig brain	[^{3}H]NE-100 saturation	(Tanaka et al. 1995)
	• $IC_{50} = 85$ nM	Guinea pig brain	[^{3}H]DTG	(Chaki et al. 1994)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• $IC_{50} = 1$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Chaki et al. 1994)
	Sigma2			
	• $K_i = 212$ nM	Rat liver	[^{3}H]DTG	(Berardi et al. 2001)
PB-28	Sigma1			
	• $K_i = 13$ nM	MCF-7 cells	[^{3}H](+)-pentazocine	(Azzariti et al. 2006)
	• $K_i = 10$ nM	MCF-7/ ADR cells	[^{3}H](+)-pentazocine	(Azzariti et al. 2006)
	• $K_i = 0.4$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Niso et al. 2013)
	• $K_i = 14$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Berardi et al. 2004)
	Sigma2			
	• $K_i = 0.28$ nM	MCF-7 cells	[^{3}H]DTG	(Azzariti et al. 2006)
	• $K_i = 0.17$ nM	MCF-7/ ADR cells	[^{3}H]DTG	(Azzariti et al. 2006)
	• $K_i = 0.68$ nM	Rat liver	[^{3}H]DTG	(Niso et al. 2013)
	• $K_i = 0.34$ nM	Rat liver	[^{3}H]DTG	(Berardi et al. 2004)
				(Lever et al. 2014)
PD-144418	Sigma1			
	• $K_i = 0.08$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Akunne et al. 1997)
	• $K_i = 0.46$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Lever et al. 2014)
	• $K_i = 4.30$ nM	Sf9 cells	[^{3}H](+)-pentazocine	(Schmidt et al. 2016)
	Sigma2			
	• $K_i = 1{,}377$ nM	NG 108–15 cells	[^{3}H]DTG	(Akunne et al. 1997)
	• $K_i = 1{,}654$ nM	Guinea pig brain	[^{3}H]DTG	(Lever et al. 2014)
PRE-084	Sigma1			
	• $IC_{50} = 44$ nM	Guinea pig brain	[^{3}H](+)-SKF10047	(Su et al. 1991)
	• $K_i = 46$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Entrena et al. 2009)
	• $K_i = 151$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Cobos et al. 2005)
	• $K_i = 53$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Garces-Ramirez et al. 2011)
	• $K_i = 9$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Brown et al. 2004)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	Sigma2			
	• $K_i = 32{,}100$ nM	Guinea pig brain	[^{3}H]DTG	(Garces-Ramirez et al. 2011)
Rimcazole	Sigma1			
	• $K_i = 406$ nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	• $K_i = 1{,}165$ nM	Guinea pig brain	[^{3}H]ne-100	(Tanaka et al. 1995)
	• $IC_{50} = 2{,}700$ nM	MDA-MB-468 cells	[^{3}H](+)-pentazocine	(Spruce et al. 2004)
	• $IC_{50} = 356$ nM	C6 rat glioma cells	[^{11}C] SA4503	(Rybczynska et al. 2008)
	• $IC_{50} = 2{,}649$ nM	Rat brain	[^{3}H](+)-SKF10047	(Roman et al. 1990)
	• $IC_{50} = 450$ nM	Guinea pig brain	[^{3}H](+)-SKF10047	(Ferris et al. 1986)
	Sigma2			
	• $K_i = 852$ nM	Rat liver	[^{3}H]DTG	(Schepmann et al. 2011)
	• $K_i = 571$ nM	RT-4 cells	[^{3}H]DTG	(Schepmann et al. 2011)
S1RA	Sigma1			
	• $K_i = 30$ nM	Mouse brain	[^{3}H](+)-pentazocine	(Nieto et al. 2012)
	• $K_i = 24$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Romero et al. 2012)
	• $K_i = 17$ nM	Not indicated	Performed by CEREP	(Romero et al. 2012)
	Sigma2			
	• $K_i = 9{,}300$ nM	Not indicated	Performed by CEREP	(Romero et al. 2012)
SA4503	Sigma1			
	• $K_i = 0.012$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Berardi et al. 2001)
	• $K_i = 4.63$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Lever et al. 2006)
	• $IC_{50} = 7$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Lever et al. 2006)
	• $IC_{50} = 17$ nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Matsuno et al. 1996a, b)
	Sigma2			
	• $K_i = 63$ nM	Guinea pig brain	[^{3}H]DTG	(Lever et al. 2006)
	• $K_i = 77$ nM	Rat liver	[^{3}H]DTG	(Berardi et al. 2001)

(continued)

Table 2 (continued)

Compound name	Binding affinity (K_i, K_d, IC_{50})	Cell lines/ tissue tested	Radioligand used	Reference
	• IC_{50} = 71 nM	Guinea pig brain	[^{3}H]DTG	(Lever et al. 2006)
	• IC_{50} = 1,784 nM	Guinea pig brain	[^{3}H]DTG	(Matsuno et al. 1996a, b)
SH-344	Sigma1			
	• K_i = 2.5 nM	Rat liver	[^{3}H](+)-pentazocine	(Hellewell et al. 1994)
	• K_i = 2.8 nM	C6 rat glioma cells	[^{3}H](+)-pentazocine	(Vilner et al. 1995a, b)
	Sigma2			
	• K_i = 43 nM	Rat liver	[^{3}H]DTG	(Hellewell et al. 1994)
Siramesine	Sigma1			
	• K_i = 10 nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Niso et al. 2013)
	• IC_{50} = 17 nM	Rat brain	[^{3}H](+)-pentazocine	(Perregaard et al. 1995)
	Sigma2			
	• K_i = 13 nM	Rat liver	[^{3}H]DTG	(Niso et al. 2013)
	• IC_{50} = 0.12 nM	Rat brain	[^{3}H]DTG	(Perregaard et al. 1995)
SR31747A	Sigma1			
	• K_i = 1 nM	MDA-MB-468 cells	[^{3}H](+)-pentazocine	(Maher et al., unpublished data)
	• K_i = 3 nM	Guinea pig brain	[^{3}H](+)-pentazocine	(Laggner et al. 2005)
	• K_d = 0.15 nM	Yeast membrane	[^{3}H] SR31747A	(Jbilo et al. 1997)

2 Sigma1 and *SIGMAR1* Expression in Tumors

Elevated expression levels of a protein or of the mRNA transcripts encoding the protein are often used to justify its relevance in cancer. In this section, we will review the literature describing the expression of *SIGMAR1* mRNA transcripts and Sigma1 protein by immunohistochemistry (IHC) and radioligand binding in tumors.

2.1 Sigma1 Protein Expression in Tumors by Immunohistochemistry

Compared to other cancer-associated proteins, there are relatively few published reports of Sigma1 immunostaining in tumors. These data are summarized in Table 3.

In one of the first reports, Casellas and colleagues performed Sigma1 IHC staining analysis of tumors from 95 breast cancer patients (Simony-Lafontaine et al. 2000). The authors found a positive correlation between Sigma1 protein and hormone receptor levels; the strongest positive correlation was with the progesterone receptor (PR) ($P = 0.01$). Interestingly, the *SIGMAR1* transcriptional promoter region contains a PR binding site (Seth et al. 1997). Together, these data suggest that Sigma1 expression may be regulated by steroid hormone feedback mechanisms. This was proposed as a rationale for considering Sigma1 as a marker to identify patients who may benefit from adjuvant hormone therapy (Simony-Lafontaine et al. 2000).

In this study, Sigma1 protein levels showed no significant positive correlation with tumor size, histological grade, nodal status, tumor proliferation (by Ki67), patient age, or whether the patients were pre- or post-menopausal. However, the absence of detectable Sigma1 was most frequently observed in tumors from post-menopausal women (Simony-Lafontaine et al. 2000).

The authors used a mouse monoclonal anti-Sigma1 antibody raised against full-length Sigma1 that was generated by the authors [first described in (Jbilo et al. 1997)]. The epitope(s) on Sigma1 was (were) not identified (Jbilo et al. 1997). An antigen retrieval step in the IHC protocol was not reported. These are important technical considerations, because depending upon the epitope against which the antibody was generated an antigen retrieval step may be needed to reveal the epitope(s) masked by formalin/formaldehyde cross-linking of the protein of interest (Leong and Leong 2007; Marchio et al. 2011). This is noteworthy because the published IHC analyses of Sigma1 in tumors, described here and below, were based on the use of different anti-Sigma1 antibodies (some without indicated epitopes) and possible variability in tissue processing and immunostaining specificity. Therefore, some of the differences in the conclusions drawn from these studies could be attributed to technical factors and may not necessarily reflect biological or clinical differences.

In a subsequent study Wang et al. performed Sigma1 IHC staining analysis of 109 tissue specimens comprising malignant breast tumors, benign breast tumors, and normal breast tissue from 58 breast cancer patients. The authors reported that Sigma1 protein was present in 60% of invasive cancers, 41% of in situ cancers, 75% of ductal hyperplasias, and 33% of normal breast tissue (Wang et al. 2004). They reported no statistically significant correlation between Sigma1 protein levels and histological grade, nodal status, and patient age. In contrast to the study by Simony-Lafontaine et al., Wang et al. found no statistically significant correlation between Sigma1 levels and estrogen receptor or progesterone receptor status. This difference may be attributable to technical factors such as different antibodies and IHC procedures as

Table 3 Immunohistochemical Analysis of Sigma1 Protein in Tumors

Reference	Cancer	Results and conclusions	Antibody used	Antigen retrieval
(Simony-Lafontaine et al. 2000)	*Breast Adenocarcinoma* (tumors from 95 breast cancer patients)	No significant correlation with tumor size, histological grade, nodal status, tumor proliferation (by Ki67), patient age, or whether the patients were pre- or post-menopausal. Significant correlation with progesterone receptor status	Mouse monoclonal anti-Sigma1 antibody against full-length Sigma1 that was generated by the authors [described in (Jbilo et al. 1997)]	An antigen retrieval step in the IHC protocol was not indicated
(Wang et al. 2004)	*Breast Adenocarcinoma* (malignant breast tumors, benign breast tumors, normal breast tissue from 58 breast cancer patients)	No significant correlation between Sigma1 protein levels and histological grade, nodal status, and patient age; no statistically significant correlation between Sigma1 levels and estrogen receptor or progesterone receptor	Goat anti-Sigma1 polyclonal antibody raised against unspecified epitope (Sigma1 L-20 antibody, Santa Cruz biotechnology, Inc.). The specificity of this antibody for Sigma1 was not confirmed	Antigen retrieval prior to IHC was performed in this study
(Xu et al. 2012)	*Esophageal Squamous Cell Carcinoma* (18 low-grade dysplasia, 8 high-grade dysplasia, 18 carcinoma, 12 non-cancerous epithelium from 18 patients)	Significant correlation with pathologic TNM classification; positive correlation with tumor size; Sigma1-positive rates generally lower in normal epithelia than in ESCC tissue	Rabbit anti-Sigma1 polyclonal antibody (Abgent). Antibody generated against a synthetic peptide, residues 47–81 of human Sigma1. The specificity of this antibody for Sigma1 was not confirmed	Antigen retrieval prior to IHC was performed in this study
(Xu et al. 2014)	*Hilar Cholangio-carcinoma (HC)* (92 HC and paired normal bile duct epithelial tissue)	Significant correlation between the percentage of tumors positive for Sigma1 immunostaining and tumor differentiation (increase in poorly differentiated tumors), lymph node metastasis, disease stage; no correlation between Sigma1 staining and tumor size or brain metastasis	Rabbit polyclonal antibody raised against an unspecified synthetic peptide derived from the C-terminal region of rat Sigma1 (ab53852; Abcam). The specificity of this antibody for Sigma1 was not confirmed	An antigen retrieval step in the IHC protocol was not indicated

well as different patient populations. However, both studies report heterogeneous expression of Sigma1 in invasive breast tumors.

The authors concluded that Sigma1 protein levels did not correlate with patient survival and were not predictive of adjuvant chemotherapy efficacy in this study. They included the caveat that their study should be considered exploratory and that it was not performed to formally evaluate prognostic value, adding that their conclusion regarding lack of statistically significant correlation may have been due to an underpowered study (Wang et al. 2004).

Xu et al. reported that Sigma1 is upregulated in esophageal squamous cell carcinoma (ESCC) and that the upregulation correlates with the pathologic tumor, node, metastasis (TNM) classification (Xu et al. 2012). The authors describe both cytoplasmic and nuclear Sigma1 immunostaining. They also report that nuclear Sigma1 has a stronger positive correlation with TNM classification and lymph node metastasis and suggest that nuclear Sigma1 may contribute to malignant progression of ESCC tumors. This group also found a significant positive correlation between Sigma1 expression and tumor size. They evaluated normal epithelium of the esophagus and compared to ESCC tissue and found that Sigma1-positive immunostaining in non-cancerous epithelium was inconsistent (33.3%, 4 of 12); however, Sigma1-positive rates were generally lower than in ESCC tissue, wherein a pattern of increasing rates of positive Sigma1 staining was observed with low-grade dysplasia (22.2%, 4 of 18) to high-grade dysplasia (61%, 11 of 18). A significant difference was observed, with 35% for low-grade dysplasia versus 60% for ESCC.

The presence or absence of Sigma1 failed to show correlation with ESCC patient survival rates; patients with high Sigma1 immunostaining had 5-year overall survival rates of 29.7% compared to 37.5% for patients with low Sigma1 immunostaining. The authors propose that Sigma1 contributes to ESCC pathogenesis and could be regarded as a novel biomarker in the prognosis of ESCC; however, they also state that their study should be regarded as exploratory (Xu et al. 2012).

Xu et al. evaluated the levels of Sigma1 in hilar cholangiocarcinoma (HC) tumors, a hepatobiliary cancer that occurs at the confluence of the right and left hepatic ducts (Xu et al. 2014). The authors performed Sigma1 IHC analysis of tissue microarrays (TMA) containing 92 HC and paired non-cancerous bile duct epithelial tissue. They report overexpression of Sigma1 in 46.7% of the HC tumors. Under their experimental conditions 53% of HC tumors presented low or no Sigma1 immunostaining, and all non-cancerous bile duct epithelial cells presented no or weak Sigma1 immunostaining. The authors report primarily cytoplasmic Sigma1 immunostaining (Xu et al. 2014).

This study found a significant positive correlation between the percentage of tumors that were positive for Sigma1 immunostaining and tumor differentiation (increased in poorly differentiated tumors), lymph node metastasis, and disease stage. However, they found no significant correlation between Sigma1 staining and tumor size or brain metastasis (Xu et al. 2014). The frequency of Sigma1 immunostaining significantly increased with disease stage, with 32.4% Sigma1 positive at TNM classification stage I/II and 56.4% at stage III/IV. They also report

that Sigma1 levels positively correlated with disease progression, poor prognosis, earlier recurrence, and diminished overall survival. HC patients with high intensity Sigma1 immunostaining presented significantly earlier recurrence (15 versus 30 months) and significantly shorter median survival duration (15 versus 42 months) compared to patients with low or no Sigma1 immunostaining. The authors report that tumor invasion, lymph node metastases, and Sigma1 immunostain intensity were independent predictive factors for tumor recurrence (Xu et al. 2014).

2.2 Sigma1 Protein Levels in Tumors Determined by Radioligand Binding

One of the first reports of the presence of sigma receptors in tumors (at the time identified as sigma binding sites) was published by Coscia and colleagues (Bowen et al. 1988; Thomas et al. 1990). The authors evaluated the density of sigma binding sites as well as opioid receptors in human brain tumors and neuroblastoma and glioma cell lines. Sigma receptor binding was performed with [^{3}H]1,3-di-o-tolylguanidine ([^{3}H]DTG) in the absence or presence of haloperidol to differentiate specific and non-specific binding. Elevated sigma binding site density was detected in 15 of 16 tumors. All brain tumor specimens were obtained from patients immediately after surgical resection. [^{3}H]DTG bound membrane preparations of meningioma with a K_d of 37–57 nM and B_{max} 683–1,260 fmol/mg protein compared to [^{3}H]DTG binding of temporal cortex tissue preps with a K_d of 60 nM and B_{max} 249 $\pm$ 105 fmol/mg protein (mean $\pm$ SE). A brain metastasis from adenocarcinoma of the lung expressed five- to tenfold greater [^{3}H]DTG than other brain tumors (Thomas et al. 1990). A caveat of this study is that haloperidol has affinity for both Sigma1 and Sigma2 binding sites; therefore, these conditions would not distinguish these two binding sites (Thomas et al. 1990).

Subsequently, this group reported increased sigma binding site density in non-neural tumors, including surgical specimens of renal and colorectal carcinoma and sarcoma (Bem et al. 1991). The freshly resected set of 9 tumors comprised 2 colon carcinoma liver metastases, 6 renal carcinomas, and 1 sarcoma metastasis. The tumors were compared to normal renal tissue and colon mucosa specimens excised from tissue adjacent to primary tumors as well as from tissue obtained during necropsy of non-cancer patients (Bem et al. 1991).

2.3 *SIGMAR1* Transcript Levels in Tumors

Systematic analyses of *SIGMAR1* gene expression, genome wide association studies, mutational analyses, or epigenetic analyses have not been reported. However, several comprehensive and well-annotated cancer focused gene expression databases are now available. These include The Cancer Genome Atlas (TCGA) (Weinstein et al. 2013) and Oncomine [https://www.oncomine.org/, first described by Chinayan and colleagues (Rhodes et al. 2004)]. These databases are a rich source

of information regarding the genomic, genetic, and epigenetic status of *SIGMAR1* in cancer that awaits data mining, analysis, and reporting. Recently, Crottès et al. reported elevated levels of *SIGMAR1* transcripts in colorectal cancers (CRC), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML) compared to their paired normal tissue based on their analysis of the Oncomine database (Crottes et al. 2016).

A few focused studies have used reverse transcriptase polymerase chain reaction (RT-PCR) based approaches to quantify *SIGMAR1* transcript levels in cancer tissue specimens. In one of the earliest such studies, Wang et al. evaluated the relative *SIGMAR1* transcript levels in 14 breast cancer specimens by quantitative real-time RT-PCR (qRT-PCR). They found that 9 of 14 (64%) of the samples had elevated *SIGMAR1* (ratio of *SIGMAR1* in cancer tissue to a pool of normal breast tissue). The ratio of *SIGMAR1* in cancer versus normal tissue ranged from 2 to 37, with a median ratio of 4 (2.85 at 25% and 17.75 at 75%). However, in 5 of 14 (36%) breast cancer samples the authors found less *SIGMAR1* compared to the reference pool of normal breast tissue, with ratios ranging from 0.8 to 0.02, with a median ratio of 0.11 (0.025 at 25% and 0.51 at 75%) (Wang et al. 2004).

Although not specifically addressed in a study of gene expression in malignant melanoma and benign melanocytic lesions by Talantov et al., a closer review of the data in this publication revealed that some malignant melanomas express extremely high levels of *SIGMAR1* transcripts compared to benign tissue controls (Talantov et al. 2005). The *SIGMAR1* gene transcript data can be found at the NCBI GEO Profile for this study (accession number GSE3189).

Skrzycki and Czeczot used semi-quantitative RT-PCR to evaluate *SIGMAR1* transcript levels in tumors from 30 CRC patients, 18 with primary CRC and 12 with liver metastatic CRC. Using this method, the authors concluded that relative *SIGMAR1* transcript levels are highest in stage III CRC based on the TNM staging system (Skrzycki and Czeczot 2013). This study also reported significantly decreased levels of *SIGMAR1* transcripts in older CRC patients. The authors conclude that increased *SIGMAR1* correlates with CRC stage and metastasis and decreases with patient age (Skrzycki and Czeczot 2013).

Analysis of *SIGMAR1* in patient tumors in the Oncomine and The Cancer Genome Atlas (TCGA) databases and survey of the literature reveals that Sigma1 is not uniformly upregulated in tumors. Interestingly, even among clinical subtypes and individual patients of each cancer, there is variability in the magnitude of enrichment of Sigma1. The significance of this variability in expression is unclear.

3 Sigma1 and *SIGMAR1* Expression in Cancer Cell Lines

3.1 Sigma1 Protein in Cancer Cell Lines Determined by Immunoblot

A number of groups have reported Sigma1 protein expression in cancer cell lines by immunoblot; a few are listed here (Vilner et al. 1995b; John et al. 1995b; Spruce et al. 2004; Aydar et al. 2006; Wang et al. 2004; Kim et al. 2010, 2012; Xu et al. 2012; Schrock et al. 2013; Thomas et al. 2017). Aydar et al. confirmed Sigma1 protein expression by immunoblot in lung (H69, H209, H510), breast (MDA-MB-361, MDA-MB-435, BT20 and MCF-7), and prostate (PC3, LNCaP) cancer cell lines (Aydar et al. 2006). Wang et al. performed immunoblots to confirm Sigma1 protein expression in MDA-MB-231, MDA-MB-361, MDA-MB-435, MCF-7, and BT20 breast cancer cell lines (Wang et al. 2004). In their hands, T47D cells did not express Sigma1. This is inconsistent with other reports (Kim et al. 2012; Schrock et al. 2013; Vilner et al. 1995b). MCF-7 cells were initially reported to be Sigma1 negative (Vilner et al. 1995b); however, a number of studies demonstrate that MCF-7 cells express Sigma1 and *SIGMAR1* and are responsive to Sigma1 ligands (Vilner et al. 1995b; John et al. 1995b; Spruce et al. 2004; Aydar et al. 2006; Wang et al. 2004; Kim et al. 2012; Schrock et al. 2013). Xu et al. reported Sigma1 protein expression in human esophageal squamous cell carcinoma (ESCC) cell lines KYSE150, KYSE180, and EC109 (Xu et al. 2012). Kim and colleagues confirmed Sigma1 protein expression by immunoblot in prostate cancer (LAPC4, LNCaP, C4-2, 22Rv1, VCaP, PC3, DU145), breast cancer (T47D, MCF-7, MDA-MB-231, MDA-MB-468, SKBR3, BT474), pancreas (Panc1), liver cancer (HepG2), and neuroblastoma (SK-N-BE(2)C) cell lines (Kim et al. 2010, 2012; Schrock et al. 2013; Thomas et al. 2017). To date, no clearly Sigma1-negative cancer cell line has been identified.

3.2 Sigma1 Binding Sites in Cancer Cell Lines Evaluated by Radioligand Binding

Most radioligand binding studies to detect and quantify Sigma1 in cancer cell lines were performed with the following three radioligands: [^{3}H](+)-pentazocine, [^{3}H](+)-SKF10047, and [^{3}H]DTG (Table 2). The pharmacological selectivity and specificity of the first two prototypic Sigma1 ligands was confirmed by a study with *SIGMAR1* homozygous knockout mice (Langa et al. 2003). In this study, [^{3}H](+)-pentazocine did not bind to brain membrane preparations from *SIGMAR1*$^{-/-}$ mice, and (+)-SKF10047 stimulation of locomotor activity was not observed in these mice (Langa et al. 2003).

High levels of Sigma1 have been quantified in a number of human and rodent cancer cell lines by radioligand binding saturation assay. These assays have been performed and Sigma1 was detected on extracted cell membrane preparations from cell lines of prostate cancer (Vilner et al. 1995b), breast cancer (Crawford and

Bowen 2002; Vilner et al. 1995b; Spruce et al. 2004; Schrock et al. 2013), colon cancer (Bem et al. 1991), melanoma (Vilner et al. 1995b), small- and non-small-cell lung carcinoma (Maneckjee and Minna 1992; John et al. 1995a; Moody et al. 2000; Vilner et al. 1995b), renal cancer (Bem et al. 1991), bladder cancer (Schepmann et al. 2011), brain tumors (Thomas et al. 1990), glioblastoma (Vilner et al. 1995b), neuroblastoma (Ryan-Moro et al. 1996; Vilner et al. 1995b), multiple myeloma (Brune et al. 2012), and sarcoma (Bem et al. 1991).

Sigma1 has been detected by radioligand binding on a number of rodent cancer cell lines as well, including C6 rat glioma (Vilner et al. 1995b), N1E-115 rat neuroblastoma (Vilner et al. 1995b), NG108-15 rat neuroblastoma x glioma hybrid (Vilner et al. 1995b), and TRAMP (transgenic adenocarcinoma mouse prostate) cells (Colabufo et al. 2008).

3.3 Accumulation of Sigma1 Radioligands in Xenografted Tumors In Vivo

Bowen and colleagues performed Sigma1 ligand biodistribution studies in nude mice xenografted with a human prostate cancer cell line (DU145). They demonstrated that radioiodinated benzamides with affinity for Sigma1 appeared to be retained in tumors compared to normal tissues. 4-[^{125}I]-PAB, [^{125}I]-PIMBA, 2-[^{125}I]-BP had tumor/blood ratios of 14, 70, and 41 at 6 h post-injection, respectively. 4-[^{125}I]PAB, [^{125}I]-PIMBA, 2-[^{125}I]-BP had tumor/muscle ratios of 57, 70, and 28 at 6 h post-injection, respectively. 2-[^{125}I]-BP had tumor/blood and tumor/muscle ratios of 35 for both at 24 h post-injection. These data suggest that Sigma1 ligands may preferentially accumulate in tumors compared to other normal tissue (John et al. 1999).

Moody et al. performed a similar biodistribution experiment with [^{125}I]-*N*-(2-(piperidino)ethyl)-2-iodobenazmide (2-IBP) in mice xenografted with NCI-N417 non-small-cell lung cancer (NSCLC) cells. In this study as well, the Sigma1 ligand was present in higher concentrations in tumors compared to normal tissue (Moody et al. 2000).

Xie et al. synthesized an ^{18}F labeled piperidine compound, 8-(4-(2-[^{18}F] fluoroethoxy)benzyl)-1,4-dioxa-8-azaspiro[4.5]decane ([^{18}F]5a), with high affinity for Sigma1 ($K_i = 5.4$ nM). The authors demonstrate specific intracellular Sigma1 binding by [^{18}F]5a in vitro in four cancer cell lines, PC3 and DU145 (prostate adenocarcinoma), MCF-7 (breast adenocarcinoma), and A375 (melanoma). Specificity of [^{18}F]5a binding to Sigma1 was confirmed with cold blocking ligands haloperidol, SA4503, and fluspidine in cellular uptake assays with all four human cancer cell lines. Consistent with the radioligand binding data, these cell lines have been reported to express different levels of *SIGMAR1* transcripts and Sigma1 by immunoblot. By autoradiography and positron emission tomography (PET) imaging, the authors demonstrate accumulation of high levels of [^{18}F]5a in subcutaneously xenografted tumors of the above cell lines in mice. Accumulation was highest in PC3 tumors > A431 > A375 > DU145. The accumulation of the [^{18}F]5a

radiotracer in PC3 and A431 xenografted tumors was significantly decreased by co-administration with haloperidol, suggesting Sigma-selective binding of this radiotracer (Xie et al. 2015).

SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride) is a high affinity Sigma1 selective small molecule ligand with negligible affinity for at least 36 other receptors and ion channels (Matsuno et al. 1996b).

A number of reports suggest that SA4503 may be a promising Sigma1 targeted tumor radiotracer (Kawamura et al. 2005; Rybczynska et al. 2009; van Waarde et al. 2004, 2006; Ye et al. 2016). Proposed advantages of [^{11}C]SA4503 are its improved selectivity for tumor cells in inflamed tissue compared to ^{18}F-fluorodeoxyglucose (^{18}F-FDG) (van Waarde et al. 2006) as well as its high tumor uptake and retention (van Waarde et al. 2004, 2006). Ramakrishnan et al. found twofold higher levels of [^{11}C]SA4503 accumulation in spontaneous pituitary tumors compared to normal pituitary tissue (Ramakrishnan et al. 2013). Van Waarde et al. evaluated [^{11}C]SA4503 as a PET ligand in rodent models. The authors reported that 1 h post-injection [^{11}C]SA4503 accumulated in C6 tumors at a tumor-to-plasma ratio of 13.4 and a tumor-to-muscle ratio of 5.0 (van Waarde et al. 2004). Kawamura et al. reported that [^{11}C]SA4503 accumulated in AH109A hepatoma xenografted tumors in rats. Uptake in this cell line decreased by carrier-loading and pre-treatment with haloperidol ([^{11}C]SA4503, 41% and 22%, respectively, at 30 min after injection), in support of Sigma1 specific binding and accumulation (Kawamura et al. 2005).

Together, these and other studies not reviewed here suggest that radiolabeled Sigma1 ligands preferentially accumulate in tumors and are promising radiotracers for tumor imaging in vivo. Interestingly, this is true even when comparing cancer cells with normal tissues that express high levels of Sigma1 protein, suggesting that Sigma1 may exist in a distinct binding conformation in cancer cells.

3.4 *SIGMAR1* Transcript Levels in Cancer Cell Lines

The availability of well-curated and publically available databases such as Cell Miner and the Cancer Cell Line Encyclopedia (CCLE), which contain the full gene expression profile of over 1,000 cancer cell lines, provides valuable reference data sets for gene expression studies. We evaluated *SIGMAR1* mRNA transcript expression levels in 1,036 cancer cell lines in the CCLE (Fig. 1). Our analysis of these databases and survey of the literature highlights that *SIGMAR1* is not uniformly upregulated in tumors and in cancer cell lines. Interestingly, even among clinical subtypes and individual patients of each cancer, there is variability in the levels of Sigma1 and *SIGMAR1* transcripts. This is reflected in the 1,036 cancer cell lines representing >20 cancers in the CCLE (Fig. 1). The significance of this variability in expression is unclear but may reflect the context-dependent functions of Sigma1, even within a cancer type.

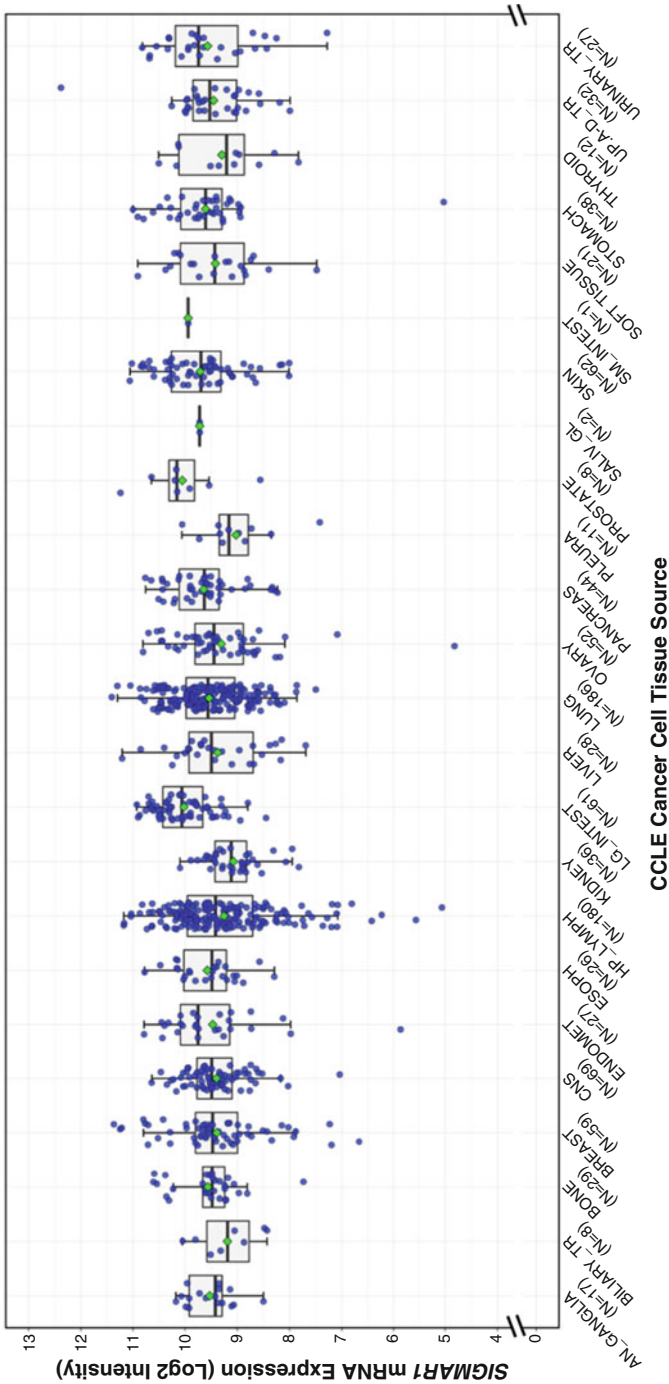

Fig. 1 *SIGMAR1* transcript levels in 1,036 cancer cell lines. Levels of *SIGMAR1* mRNA transcripts expressed as Log2 intensity values (*Y*-axis) in 1,036 cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE). Tissue of origin of the cancer cell lines indicated on the *X*-axis. N = number of cell lines in each cancer type. *Blue data* points in scatter plot represent individual cell lines. Box and whisker plots show median *SIGMAR1* Log2 intensity ± standard deviation. *Green diamond* indicates median

4 Cancer Pharmacology of Sigma1 Modulators

4.1 Sigma1 Ligands: Putative Agonists and Antagonists

Despite compelling evidence that Sigma1 is not a traditional receptor, small molecule compounds with affinity for Sigma1 continue to be described as agonists and antagonists. They were originally classified on the basis of rodent behavior assays. The synthetic Sigma1 ligands di-o-tolylguanidine (DTG) and BD1052 exacerbated cocaine-induced convulsions and locomotor activity and were classified as agonists (Matsumoto et al. 2001). In contrast, other synthetic Sigma1 ligands BD1008, BD1047, BD1063, and LR172 were defined as antagonists because they attenuated cocaine-induced convulsions, abnormal hyper-locomotor activity, and lethality in mice (McCracken et al. 1999). Consistent with pharmacological antagonists, when administered alone the Sigma1 putative antagonists produced no reported changes in behavior (Matsumoto et al. 2001).

A rodent model of memory impairment was also used to classify Sigma1 compounds as agonists and antagonists. Maurice and colleagues demonstrated that Sigma1 putative agonists (+)-pentazocine, PRE-084, and SA4503 had anti-amnesic effects in a beta-amyloid-related peptide-induced memory impairment behavior assay. Neurosteroids with affinity for Sigma1 including pregnenolone, dehydroepiandrosterone, and their sulfate esters also produced a neuroprotective effect, which was interpreted as Sigma1 agonism. Progesterone and haloperidol blocked these neuroprotective effects and were thus classified as Sigma1 antagonists in this assay. Importantly, although they blocked the beneficial effects of the Sigma1 agonists in attenuating memory impairment, these Sigma1 antagonists, when administered alone, had no effect on (i.e., did not worsen or accelerate or ameliorate) 25–35 peptide-induced symptoms (Maurice et al. 1998). A number of related studies are reviewed by Maurice and Goguadze in this volume (*Sigma-1 (σ_1) Receptor in Memory and Neurodegenerative Diseases*).

In experimental models of cancer, inhibition of cancer cell proliferation and survival are considered measures of Sigma1 inhibition (putative antagonism). Spruce et al. and Colabufo et al. were among the first to propose that Sigma1 putative antagonists/inhibitors but not agonists/activators elicit antiproliferative and cytotoxic effects on cancer cells (Spruce et al. 2004; Colabufo et al. 2004). In these seminal studies, Sigma1 antagonism/inhibition, as originally defined on the basis of behavioral endpoints, generally correlated with inhibition of cancer cell proliferation and growth, and in some cases induction of apoptosis (Colabufo et al. 2004; Spruce et al. 2004). However, this does not strictly apply. For instance, although the putative agonists/activators PRE-084 and (+)-SKF10047 do not alter cell proliferation or survival in most published studies, some putative agonists/activators such as 4-IBP [*N*-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide] have been reported to have antiproliferative properties on their own as well as the ability to sensitize cancer cells to proapoptotic and proautophagic drugs (Megalizzi et al. 2009, 2007).

To further complicate attempts at classification, most putative sigma ligands have affinity for both the Sigma1 and Sigma2 subtypes, albeit with broad differences in subtype binding affinity (Table 2). It has been proposed that the antiproliferative and proapoptotic activities of Sigma1 ligands may involve Sigma1 antagonism/inhibition combined with Sigma2 putative agonism (Zeng et al. 2014). However, the identity of Sigma2 is controversial (Pati et al. 2017; Abate et al. 2015) and the definition of Sigma2 agonism is also unclear.

If, based on the above, the physiological role of Sigma1 in cancer cell and tumor biology is to promote growth and survival, then what does it mean to activate or inhibit Sigma1? How can this be measured? To date, there is no established molecular or biochemical mechanism of action that can clearly define Sigma1 agonist/activator and antagonist/inhibitor activity. In contrast to GTPγS for G protein-coupled receptors (GPCR), kinase activity for receptor tyrosine kinases (RTKs), and ATP binding for heat shock protein 90 (HSP90), there are no established proximal signaling or enzymatic activities clearly attributable to Sigma1. A standard biochemical assay for defining compounds as Sigma1 agonists/activators and antagonists/inhibitors remains to be established.

4.2 Prototypic Small Molecule Ligands: Effects In Vitro and In Vivo

Despite the aforementioned uncertainty regarding the classification of Sigma1 ligands, much of our understanding of Sigma1 biology and pharmacology comes from studies with synthetic small molecule compounds (i.e., ligands). Compounds with affinity for Sigma1 have been reported to influence cell survival, apoptosis, cell proliferation, growth, cell adhesion, motility, migration, cell cycle progression, lipid homeostasis, and protein homeostasis pathways. In the absence of a coherent, unifying explanation for how Sigma1 pharmacology regulates these pathways and processes, thereby producing what appears to be the wide range of therapeutic opportunities, we have selected a number of prototypic Sigma1 ligands and provide a compound-centric survey of the literature to describe how they have been used to implicate Sigma1 in these cellular processes. In this section, we will review and analyze the reported properties and activities of a selected set of relatively widely published prototypic Sigma1 ligands.

4.2.1 (+)-SKF10047

Also known as (+)-*N*-allylnormetazocine, (+)-SKF10047 is a prototypic Sigma1 ligand and putative agonist/activator [see above and (Maurice et al. 1994; Hayashi and Su 2001)]. The Sigma1 selectivity of (+)-SKF10047 was confirmed by the absence of binding and activity in *SIGMAR1* knockout (KO) mice (Langa et al. 2003). Spruce et al. were among the first to delineate that putative Sigma1 antagonists/inhibitors, but not agonists/activators, inhibit tumor growth and survival

both in vitro and in vivo. They showed that some putative Sigma1 antagonists/inhibitors elicit caspase-mediated apoptosis, and that agonists/activators including (+)-SKF10047 and (+)-pentazocine block or attenuate this effect (Spruce et al. 2004).

In some cases, putative agonists/activators promote cancer cell proliferation and tumor growth. For example, in the same publication mentioned above, Spruce et al. show that (+)-SKF10047 and (+)-pentazocine both promoted in vitro proliferation of the MCF-7 breast cancer cell line, suggesting that some cancer cells can respond to agonistic signals that promote cell proliferation and survival (Spruce et al. 2004). In a later study, Happy et al. reported that (+)-SKF10047 treatment alone appeared to increase proliferation of MCF-7 and MDA-MB-231 cells (Happy et al. 2015). Consistent with the study by Spruce et al., Happy et al. reported that (+)-SKF10047 blocked the antiproliferative and proapoptotic effects of rimcazole in a panel of breast cancer cell lines (Happy et al. 2015).

Using the same approach as Happy et al., Saune and colleagues recently reported that treatment of DU145, LNCaP, and PC3 prostate cancer cell lines with (+)-SKF10047 or overexpression of recombinant Sigma1 prevented tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (Das et al. 2016). The authors proposed that higher levels of active Sigma1 render prostate cancer cells resistant to TRAIL treatment. RNAi knockdown of Sigma1 sensitized TRAIL resistant T47D, MDA-MB-157, and MDA-MB-231 breast cancer cell lines to the antiproliferative and proapoptotic effects of ectopically expressed, adenoviral vector transduced TRAIL (Das et al. 2016).

In contrast, Aydar et al. reported that (+)-SKF10047 treatment significantly inhibited cell adhesion but did not affect proliferation or migration of MDA-MB-231 and MDA-MB-468 breast cancer cell lines (Aydar et al. 2016). The authors propose that Sigma1 activation alters cell adhesion through interaction with the neonatal Nav1.5 (nNav1.5) ion channel (Aydar et al. 2016). They propose that because combining Sigma1 knockdown or (+)-SKF10047 with an nNav1.5 activity blocking polyclonal antibody (NESOpAb) had similar effects as each treatment alone, cell adhesion may be mediated through a common mechanism involving Sigma1 interaction with nNav1.5 (Aydar et al. 2016). This group also reported that (+)-SKF10047 (albeit at 100 μM) inhibited MCF-7 cell adhesion by 41% and inhibited MDA-MB-231 cell adhesion by 57%. RNAi knockdown of Sigma1 in MCF-7 and MDA-MB-231 cells also resulted in 42% and 29.76% inhibition of cell adhesion, respectively (Aydar et al. 2006). Although interesting, these observations are inconsistent with a definition of (+)-SKF10047 as an agonist/activator. Nevertheless, these data were used as evidence to suggest that Sigma1 may play a role in cancer cell metastasis (Aydar et al. 2006).

Aydar and colleagues have proposed that Sigma1 also alters cell adhesion by regulating the actions of β-integrin (Palmer et al. 2007; Aydar et al. 2006). The authors of these studies postulated that RNAi knockdown (KD) of Sigma1 and (+)-SKF10047 treatment produce effects consistent with β-integrin blockade. Although the mechanisms by which (+)-SKF10047 elicits these effects were not determined, (+)-SKF10047 treatment resulted in dissociation of Sigma1 from lipid rafts and

decreased Sigma1-β-integrin association in lipid raft fractions (Palmer et al. 2007). In this study as well, the correlation between Sigma1 KD and (+)-SKF10047 treatment is inconsistent with a definition of (+)-SKF10047 as an agonist/activator. However, this suggests that Sigma1 can contribute to cholesterol content of the surrounding lipid bilayer and possibly associated proteins, such as integrins and ion channels (Palmer et al. 2007; Aydar et al. 2002, 2004; Balasuriya et al. 2014).

Disruption of cholesterols in lipid rafts alters the functionality and composition of the signaling complexes present in these organizing and stabilizing structures (Jacobson et al. 2007; Simons and Toomre 2000). Palmer et al. have proposed that Sigma1 contains two cholesterol-binding domains (CBD) that have peripheral benzodiazepine receptor and the HIV envelope glycoprotein-like CBD motifs (Palmer et al. 2007). These CBDs are adjacent to the Sigma1 ligand-binding site (Palmer et al. 2007; Schmidt et al. 2016). The authors proposed that Sigma1 contributes to lipid raft modeling and showed that Sigma1 binding to cholesterols was inhibited by (+)-SKF10047 binding to Sigma1 (Palmer et al. 2007).

4.2.2 PRE-084

Sigma1 agonists have been reported to augment the production of immune suppressive cytokines that block the host anti-tumor immune response in the tumor microenvironment. In the first report of Sigma1 ligand-mediated suppression of anti-tumor immunity, Zhu et al. showed that Sigma1 agonists/activators enhance tumor growth in part by inducing IL-10 at the tumor site (Zhu et al. 2003). They showed that the Sigma1 putative agonists/activators PRE-084 and (+)-SKF10047 induced the extracellular secretion of IL-10, TGF-β, and PGE2, while decreasing IFN-γ at the tumor site (Zhu et al. 2003). The authors demonstrated that PRE-084 promoted tumor growth in a syngeneic lung cancer model by an IL-10 dependent mechanism (Zhu et al. 2003). In the L1C2 murine alveolar cell carcinoma syngeneic tumor model, PRE-084 (20 mg/kg, i.p) and cocaine (5 mg/kg, i.p) promoted tumor growth by >2- and 3-fold, respectively. This effect was associated with induction of IL-10 at the tumor site. The tumor growth promoting effect of PRE-084 was blocked by co-administration of BD1047 (Sigma1 putative antagonist/inhibitor, thus demonstrating that these effects were Sigma1-mediated) and by an anti-IL-10 antibody (JES-2A5, thus demonstrating that IL-10 was required for the tumor growth promoting effect). Furthermore, transplantation of lymphocytes from PRE-084 treated mice transferred the immune suppressive phenotype and promoted tumor growth (Zhu et al. 2003). However, the authors did not show whether BD1047 had immunomodulatory or tumor growth inhibiting effects when administered alone. Interestingly, in contrast to tumor bearing mice, in normal mice (i.e., in the absence of tumor) treatment with Sigma1 agonists/activators did not increase the production or secretion of TGF-β (Zhu et al. 2003). Altogether, these data suggest that Sigma1 agonists/activators induce immune suppressive cytokine production by the tumor or that they promote tumor-induced cytokine production in the mouse.

4.2.3 (+)-Pentazocine

(+)-Pentazocine is a prototypic Sigma1 ligand and putative agonist/activator that is widely accepted as a reference compound for Sigma1 specific actions. [^{3}H](+)-pentazocine binding is abolished in tissue preparations from *SIGMAR1* knockout (KO) mice, confirming that it selectively binds Sigma1 (Langa et al. 2003).

Spruce and colleagues proposed that Sigma1 functions as a "brake on apoptosis" and reported that the caspase-dependent proapoptotic actions of Sigma1 antagonists were attenuated by (+)-SKF10047 and (+)-pentazocine (Spruce et al. 2004). This group also reported that rimcazole induced hypoxia inducible factor-1alpha (HIF-1α) protein levels under normoxic conditions in colorectal (HCT-116) and mammary carcinoma (MDA-MB-231) cell lines. They concluded that induction of HIF-1α contributes to cancer cell apoptosis by rimcazole (Achison et al. 2007). (+)-pentazocine blocked induction of HIF-1α by rimcazole, supporting that this is, at least in part, a Sigma1-mediated effect. (+)-pentazocine also inhibited HIF-1α induction and response by the anoxia mimetic deferoxamine mesylate (DFX), suggesting that Sigma1 opposes HIF-1α induction in response to anoxia.

Renaudo et al. reported that sigma ligand-mediated blockade of voltage-gated K + channels inhibited proliferation of small cell lung cancer (SCLC, NCI-H209, and NCIH146) and leukemic (Jurkat) cells. They found that three putative agonists/activators, (+)-pentazocine, igmesine, and DTG, all reversibly inhibited voltage-activated K+ currents, in order of descending potency. Consistent with K+ channel blockers tetraethylammonium (TEA) and 4-aminopyridin, treatment of Jurkat and SCLC cells with these sigma ligands resulted in accumulation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ and decreased cyclin A expression and corresponding G1 cell cycle arrest (Renaudo et al. 2004). Of note, it has been reported that the IC_{50} for blockade of K+ current is 10 times higher in normal cells (Soriani et al. 1998; Lupardus et al. 2000) than in the leukemic and SCLC cell lines.

These results showing that putative Sigma1 agonists/activators can elicit cell cycle arrest and inhibit cancer cell proliferation are inconsistent with other data demonstrating the cell growth and proliferation promoting effects of Sigma1 agonists/activators (see above). It is difficult to reconcile these discrepancies. A systematic evaluation of a broad panel of Sigma1 ligands using a set of cancer cell lines should provide clarity. However, in most publications, (+)-pentazocine alone has no effect on in vitro proliferation or survival of a broad range of cancer cell lines (Labit-Labit-Le Bouteiller et al. 1998; Colabufo et al. 2004; Spruce et al. 2004; Rybczynska et al. 2008; Achison et al. 2007; Wang et al. 2004; Abate 2012; Megalizzi et al. 2012; van Waarde et al. 2015; Brust et al. 2014).

4.2.4 4-IBP

4-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP) was originally synthesized and evaluated as a radiopharmaceutical for in vivo tumor imaging. [^{125}I]-*N*-(*N*-benzylpiperidin-4-yl)-4-iodobenzamide (4-[^{125}I]BP) binds Sigma1 with high affinity, $K_d = 26$ nM. DTG and haloperidol were shown to displace 4-[^{125}I]BP with K_i values of 4.6 and 56 nM, respectively, in MCF-7 cells (John et al. 1994, 1995b). It

was later classified as an agonist or inverse agonist based on its modulation of glutamatergic responses in hippocampal neurons (Bermack and Debonnel 2005).

Mégalizzi et al. reported that 4-IBP had weak antiproliferative effects on human glioblastoma (U373-MG) and melanoma (C32) cell lines, producing $\leq$10% inhibition of proliferation after 3 days of treatment in vitro (Megalizzi et al. 2007). Human NSCLC (A549) and prostate cancer (PC3) cells were more sensitive. However, in vitro cell migration and motility of all four cell lines were suppressed by sub-micromolar concentrations of 4-IBP using live-cell phase-contrast microscopy. In this study, inhibition of U373-MG cell motility or the organization of the actin cytoskeleton after treatment with 4-IBP was not associated with changes in intracellular [Ca^{2+}] (Megalizzi et al. 2007). This contrasts with other reports that Sigma1 ligand induced changes to cancer cell cytoskeleton occur by regulating ER Ca^{2+} efflux through Sigma1 associated IP3R (Hayashi and Su 2001).

In vivo, co-administration with 4-IBP extended survival of temozolomide treated orthotopic (brain) U373-MG glioblastoma xenograft-bearing mice, suggesting that Sigma1 ligands can potentiate the therapeutic benefit of a standard of care agent in the treatment of glioblastoma (Megalizzi et al. 2007). In an A549 metastatic NSCLC orthotopic tumor xenograft model, co-administration of 4-IBP and irinotecan significantly extended survival compared to either drug alone. Tumor analysis (i.e., tumor growth inhibition or biochemical analysis of tumors) was not reported (Megalizzi et al. 2007).

Though their rationale for evaluating these processes is unclear, the authors report that 4-IBP did not induce autophagy or UPR in U373-MG glioblastoma cells; however, 4-IBP sensitized this cell line to proapoptotic (lomustin) and proautophagic (temozolomide) compounds in vitro (Megalizzi et al. 2007).

4.2.5 Adamantane Phenylalkylamines

Riganas et al. describe a series of adamantane phenylalkylamines with affinity for Sigma1 that had antiproliferative effects in vitro on cell lines representing colon cancer (HCT-116, HCT-15), androgen independent prostate cancer (DU145, PC3), hormone-sensitive breast cancer (MCF-7), ovarian cancer (OVCAR-5), brain cancer (U-251), leukemia (HL-60), pancreatic cancer (BxPC-3), and liver cancer (SK-HEP-1). These effects were associated with cell cycle arrest and in some instances, apoptosis (Riganas et al. 2012a, b, c). A particularly interesting analogue, which they named 4a, suppressed growth of xenografted pancreatic (BxPC-3), prostate (PC3, DU145), and ovarian (OVCAR-5) tumors in SCID mice (Riganas et al. 2012a, b, c). The authors report that 4a may also have antimetastatic (measured by decreased incidence of secondary tumors) and analgesic (attenuation of paclitaxel and formalin induced pain using a previously described paw-lick assay) properties (Coderre et al. 1990; Laughlin et al. 2002; Matsumoto et al. 2006; Riganas et al. 2012a, b, c).

4.2.6 Igmesine

Soriani and colleagues have published a series of studies demonstrating the involvement of Sigma1 in ion channel activity (Balasuriya et al. 2014; Crottes et al. 2016, 2011; Gueguinou et al. 2017; Renaudo et al. 2004, 2007). A number of these studies used igmesine (Gueguinou et al. 2017; Crottes et al. 2011; Renaudo et al. 2004, 2007).

Renaudo et al. showed that three Sigma1 putative agonists/activators blocked voltage-activated K+ current amplitude in SCLC (NCI-H209, NCI-H146) and leukemic (Jurkat) cells (Renaudo et al. 2004). This effect was observed with a rank order potency of igmesine > (+)-pentazocine > DTG. Igmesine reduced Jurkat cell density, in vitro, by 23.9 and 82.8% at 10 and 30 μM, respectively, after 3 days of culture. This effect was also observed with Kv1.3 channel blockers tetraethylammonium (TEA) and 4-aminopyridin. Inhibition of cell proliferation by igmesine was associated with accumulation of total cellular levels of cyclin-dependent kinase inhibitor $p27^{Kip1}$ and a decrease in cyclin A expression. However, it is unclear whether there were increased levels of $p27^{Kip1}$ in the nucleus of these cells. The authors conclude that Sigma1 ligands can inhibit cancer cell cycle progression and thus proliferation in part through inhibition of K+ channel conductance (Renaudo et al. 2004).

Pharmacological regulation of the potassium channel Kv1.3 by igmesine appears to occur through a mechanism that does not involve changes in the cellular expression or levels of Kv1.3, as igmesine does not alter cellular Kv1.3 levels, at least in chronic lymphocytic leukemia (B-CLL) cells (Szabo et al. 2015). This is consistent with a report from Soriani and colleagues that hERG levels and surface expression are not altered by igmesine in chronic myelogenous leukemia (K562) and human embryonic kidney fibroblast (HEK293) cell lines (Crottes et al. 2011).

Igmesine has been evaluated in clinical trials for depression and diarrhea (Roze et al. 1998; Volz and Stoll 2004). The compound had acceptable safety and PK properties for the depression trial and advanced to Phase III. However, it did not reach statistically significant efficacy in the larger patient population studies in Phase III (Roze et al. 1998; Volz and Stoll 2004).

4.2.7 Haloperidol

In one of the first reports of the anti-cancer cell effects of Sigma1 ligands, Vilner, Costa, and Bowen discovered that haloperidol, reduced haloperidol, BD737, BD1008, SH344, and JL-II-147 produced morphological changes consistent with cytotoxicity in human neuroblastoma cell lines SK-N-SH and SH-SY5Y in vitro (Vilner and Bowen 1993; Vilner et al. 1995a). Additionally, a number of other neuroleptic agents with affinity for Sigma1 inhibited in vitro proliferation and survival of C6 glioma cells, albeit at high concentrations, with the following rank order potency: fluphenazine = perphenazine = haloperidol = reduced haloperidol > pimozide = spiperone >>(−)-sulpiride. At the same concentrations, neuroleptic compounds without affinity for Sigma1 lacked antiproliferative or cytotoxic properties (Vilner and Bowen 1993; Vilner et al. 1995a).

Several subsequent publications confirmed the in vitro cancer cell proliferation and cell survival inhibiting effects of haloperidol. Haloperidol and reduced haloperidol inhibited in vitro cell proliferation of MDA-MB-361, MDA-MB-435, MDA-MB-231, BT20, and MCF-7 cells (Wang et al. 2004). Haloperidol had antiproliferative and anti-migratory effects on glioblastoma cells in vitro (Rybczynska et al. 2008; Megalizzi et al. 2009). It also suppressed NCI-N417 lung carcinoma cell growth and survival in proliferation and clonogenic assays in vitro (Moody et al. 2000). Haloperidol inhibited proliferation and induced apoptosis of mouse (B16) and human (SK-MEL-28) melanoma cell lines (Nordenberg et al. 2005). Furthermore, reduced haloperidol combined with doxorubicin, vinorelbine, paclitaxel, and docetaxel produced additive cytotoxic effects in vitro (Wang et al. 2004).

In one study, haloperidol had modest in vivo tumor growth inhibiting properties in xenograft experiments. Combination of haloperidol and an EGFR inhibitor (AG1478) was reported to significantly delay tumor growth in a subcutaneous U87MG glioblastoma xenograft model. At 37 days of treatment, average xenografted tumor volume with combination treatment reportedly suppressed tumor volume to 17% of vehicle treated control mice, whereas tumors in mice treated with either AG1478 or haloperidol alone had average tumor volumes of 49% and 86% of control tumors, respectively (Li et al. 2014).

4.2.8 SR31747A

SR31747A (*N*-cyclohexyl-*N*-ethyl-3-(3-chloro-4-cyclohexylphenyl)propen-2-ylamine hydrochloride) is a high affinity ($K_i = 3$ nM) Sigma1 putative antagonist/inhibitor that was initially characterized as an immune suppressive agent (Casellas et al. 2004). In murine models of acute and chronic inflammation, SR31747A elicited a dose-related inhibition of proliferative response to mitogens – including concanavalin A, allogeneic stimulation, or phorbol myristate acetate (PMA) plus interleukin-2 (IL-2) – of mouse and human lymphocytes (Casellas et al. 1994). SR31747A modulated the production of pro- and anti-inflammatory cytokines. In SR31747A-treated mice, production of the anti-inflammatory cytokine IL-10 was induced by twofold, whereas lipopolysaccharide (LPS) – or staphylococcal enterotoxin B (SEB)-induced production of pro-inflammatory cytokines IL-2, IL-4, granulocyte macrophage colony stimulating factor (GMCSF), IL-6, and TNF-α was suppressed by up to fourfold (Derocq et al. 1995; Bourrie et al. 1995, 2004). This immune suppressive effect was shown to protect mice against acute and chronic inflammatory conditions such as acute graft-versus-host reaction, SEB infection, and LPS (Casellas et al. 1994; Bourrie et al. 2004). Importantly, SR31747A modulation of cytokine production was only observed in inflammatory conditions, not basal conditions. SR31747A did not appear to directly affect humoral immune responses (Bourrie et al. 1995, 1996, 2004; Casellas et al. 1994; Derocq et al. 1995).

SR31747A has cancer cell antiproliferative as well as immune suppressive properties (Bourrie et al. 2004; Casellas et al. 2004). Casellas and colleagues published a series of papers demonstrating the anti-tumor effects of SR31747A in vitro and in vivo [reviewed in (Casellas et al. 2004)]. This group reported potent

SR31747A inhibition of cancer cell proliferation in vitro, with IC_{50} values in the nanomolar range (Labit-Le Bouteiller et al. 1998). This was surprisingly potent, particularly in these 2-D in vitro assays. These results differed from most other published data demonstrating cancer cell growth and proliferation inhibition in the micromolar drug concentration range (Casellas et al. 2004).

In vivo, the anti-tumor efficacy of SR31747A was demonstrated against xenografted human breast and prostate cancer cell lines, including MCF-7, MDA-MB-231, PC3, DU145, and LNCaP. In all of these xenografted tumor studies, SR31747A was injected intraperitoneally (i.p.) at 25 mg/kg/day into immune deficient mice. SR31747A treatment resulted in similar tumor growth inhibition (TGI) of MDA-MB-231, PC3, DU145, and LNCaP xenografted tumors with TGI values of 60%, 50%, 40%, and 45%, respectively (Berthois et al. 2003). Importantly, in all of these in vivo efficacy studies, the authors observed no weight loss of mice treated with 25 mg/kg/day SR31747A for 2–3 months compared to control mice, indicating that this drug was well tolerated at efficacious doses (Berthois et al. 2003; Labit-Le Bouteiller et al. 1998).

In light of promising developments in the field of immune oncology, it would be of interest to evaluate the dual immune modulatory and cell autonomous growth inhibiting properties of compounds such as SR31747A in relevant preclinical tumor models. However, we were unable to find any published reports of this compound in syngeneic tumor models with immune competent mice.

4.2.9 BD1047

BD1047, a prototypic Sigma1 antagonist/inhibitor, is a modest inhibitor of cell proliferation in vitro. However, it appears not to be cytotoxic (Spruce et al. 2004). BD1047 is often used to selectively block the effects of agonists and thus demonstrate Sigma1-mediated pharmacology. In vivo, BD1047 has been shown to block the tumor growth promoting effects of PRE-084 in an L1C2 murine lung carcinoma tumor model (Gardner et al. 2004). BD1047 administered alone has not been shown to alter tumor growth in vivo.

In an SEB injection model, BD1047 blocked cocaine-induced IL-10 production, but had no effect on IL-10 levels in response to SEB injection when administered alone. Further, BD1047 blocked PRE-084 induction of IL-10 mRNA expression and production of IL-10 in IL-2 treated BALB/c splenocytes (Zhu et al. 2003).

4.2.10 Rimcazole (BW234U)

Rimcazole was initially evaluated in clinical trials to treat schizophrenia but did not advance primarily due to lack of efficacy (Gilmore et al. 2004; Katz et al. 2003). Rimcazole has been classified as a Sigma1 antagonist/inhibitor in part based on its inhibition of the potentiating effects of the Sigma1 agonist/activator (+)-SKF-10047 on neurogenic contractions in the mouse vas deferens and its ability to block cocaine-induced seizures and hypermotility (Matsuno et al. 1993, 1996a; Katz et al. 2003; Gilmore et al. 2004).

Spruce and colleagues proposed this compound as a potential anti-cancer agent (Spruce et al. 2004; Achison et al. 2007). Rimcazole was among a number of prototypic putative Sigma1 antagonists/inhibitors that suppressed cell proliferation and viability in cancer cell lines, with rank order potency of IPAG > rimcazole > BD1047 > reduced haloperidol > BD1063. However, several non-transformed, non-cancer cell types such as fibroblasts, primary epithelial cells, and even cerebellar granule neurons (which express high levels of Sigma1) were insensitive to the proapoptotic effects of Sigma1 antagonists/inhibitors rimcazole and IPAG (Spruce et al. 2004). In these studies, consistent with reports from most other groups, the prototypic putative Sigma1 agonists (+)-pentazocine and (+)-SKF-10047 did not inhibit cell proliferation and were not cytotoxic. Both of these Sigma1 selective putative agonists blocked the antiproliferative and proapoptotic effects of rimcazole and IPAG, demonstrating Sigma1-mediated actions of these compounds (Spruce et al. 2004).

Spruce and colleagues also showed that in vivo tumor growth was suppressed by systemic administration of rimcazole in xenografted tumor models of hormone-insensitive breast cancer (MDA-MB-231, MDA-MB-468), hormone-sensitive and hormone-insensitive prostate cancer (LNCaP, DU145), and p53-null lung carcinoma (H1299) (Spruce et al. 2004). In a separate study by Rybczynska and colleagues, daily i.p. injection of rimcazole for 2 weeks in nude mice bearing A375M human melanoma xenografts suppressed tumor weight by fourfold compared to vehicle controls, with no observable toxic side effects (Rybczynska et al. 2013).

In a subsequent publication Spruce and colleagues showed that induction of hypoxia inducible factor-1alpha (HIF-1α) contributes to rimcazole-mediated cancer cell death, at least in some instances. They demonstrated that treatment of colorectal (HCT-116) and breast (MDA-MB-231) cancer cells with rimcazole resulted in increased HIF-1α protein levels under normoxic conditions and that this is a mediator of apoptosis in this context. Furthermore, HCT-116p53+/+ cells were more sensitive than HCT-116p53−/− cells to the proapoptotic effects of rimcazole, suggesting that p53 contributes to this mechanism of action. Co-administration of (+)-pentazocine significantly attenuated rimcazole induced HIF-1α, suggesting that these effects were Sigma1-mediated (Achison et al. 2007).

In this study, RNAi knockdown of HIF-1α attenuated rimcazole induced apoptosis to comparable extents in p53 deficient and wild type cell lines; thus, in this model HIF-1α was required for rimcazole induced apoptosis (Achison et al. 2007). Of note, (+)-pentazocine also attenuated induction of HIF-1α by the anoxia mimetic deferoxamine mesylate (DFX), suggesting that promoting Sigma1 acts to suppress proapoptotic HIF-1α activity. Rimcazole did not induce HIF-1α in non-transformed, non-cancer fibroblasts or mammary epithelial cells (Achison et al. 2007).

Consistent with the proapoptotic activities of rimcazole, de Bruyn et al. reported that co-treatment with rimcazole potentiates the proapoptotic activities of the bi-functional therapeutic fusion protein, designated anti-MCSP:TRAIL [anti-melanoma chondroitin sulfate proteoglycan (MCSP):Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL)]. Anti-MCSP:TRAIL was designed to bind

and accumulate at the cell surface of MCSP-positive melanoma cells, subsequently block MCSP-mediated growth signaling, and trigger apoptotic TRAIL-signaling (de Bruyn et al. 2010).

Although these in vitro and in vivo xenograft studies support the notion that pharmacological inhibition of Sigma1 is a valid approach to suppressing tumor growth, some of the potential off-target effects of rimcazole may render this particular compound difficult to develop as an anti-cancer agent. A concern with using doses of rimcazole that may be required for anti-tumor activity is that rimcazole is also a potent dopamine transporter (DAT) inhibitor. Rimcazole binds Sigma1 with low affinity and binds DAT with high affinity [reviewed in (Gilmore et al. 2004; Husbands et al. 1997; Katz et al. 2003)].

4.2.11 IPAG

(1-(4-Iodophenyl)-3-(2-adamantyl)guanidine), a prototypic Sigma1 antagonist/inhibitor, was synthesized as part of a series of N,N′-di-o-tolylguanidine (DTG) analogue radiotracers for positron emission tomography (Scherz et al. 1990; Wilson et al. 1991; Kimes et al. 1992). [^{125}I]-IPAG has been used to label and quantify Sigma1 binding sites in vivo, in situ in tissue samples, and in membrane preparations from cancer cell lines (Kimes et al. 1992; Whittemore et al. 1997; Schrock et al. 2013). Recently, a rapid method to radioiodinate [^{125}I]-IPAG was published that should facilitate future studies with this radioligand (Pickett et al. 2015).

The specificity of IPAG binding to Sigma1 has been demonstrated by multiple groups (Kimes et al. 1992; Whittemore et al. 1997; Spruce et al. 2004; Schrock et al. 2013). For example, RNAi knockdown of Sigma1 produces a corresponding decrease in [^{125}I]-IPAG radioligand binding (Schrock et al. 2013). And, blockade of IPAG by (+)-pentazocine and (+)-SKF10047 has been observed in functional assays with cancer cell lines (Spruce et al. 2004).

Spruce and colleagues reported that treatment of MDA-MB-468 and MCF-7 breast adenocarcinoma cell lines with IPAG produced a dose-dependent suppression of cell proliferation and induction of caspase-dependent apoptosis. IPAG treatment was reported to induce calcium-dependent activation of phospholipase C and calcium-independent inhibition of phosphatidylinositol 3-kinase (PI3K) pathway signaling. This effect was only observed in Sigma1 antagonist/inhibitor sensitive cells. Non-cancer cells, including cerebellar granule neurons (which express high levels of Sigma1) did not respond in this way to IPAG treatment, and normal mammary epithelial cells were insensitive to IPAG induced cell death (Spruce et al. 2004). The authors confirmed that these responses to IPAG were Sigma1-mediated by blocking with co-administration of (+)-SKF10047 and (+)-pentazocine (Spruce et al. 2004).

A series of more recent publications suggest that IPAG may function as a regulator of cancer cell protein homeostasis (Kim et al. 2012; Schrock et al. 2013; Thomas et al. 2017). Schrock et al. tested a panel of diverse ligands with affinity for Sigma1 and discovered that a subset of them induced the unfolded protein response (UPR) and autophagy in a number of cancer cell lines. Of these ligands, IPAG

emerged as a potent, Sigma1-selective inducer of UPR and autophagy. It does so in a dose- and time-responsive manner in a number of cancer cell lines including breast, prostate, pancreas, and liver carcinoma (Schrock et al. 2013).

Interestingly, treatment with Sigma1 antagonists/inhibitors did not activate irreversible signaling cascades toward cell death. On the contrary, Schrock et al. demonstrated that continuous, protracted antagonist/inhibitor treatment was required to produce cell death, and that the effects of IPAG were reversible. When IPAG was washed out of cell culture media, there was a sequential subsiding of autophagy followed by a return of UPR markers to basal levels. The mechanism underlying this process is unclear. However, if the basis of Sigma1 function is protein–protein interactions (PPIs), then the sequential reversal of Sigma1 antagonist/inhibitor actions upon removal of compound suggests that these effects require high Sigma1 occupancy and continuous ligand engagement to maintain the disruption of Sigma1 PPIs.

IPAG has been used in recent studies to show that Sigma1 ligands can selectively regulate the stability, trafficking, and signaling of oncogenic driver proteins in cancer cells. Thomas et al. demonstrated that these Sigma1-mediated actions could be exploited to suppress aberrant androgen receptor (AR) activity and protein levels in prostate cancer cells (Thomas et al. 2017). The dual goals of the Thomas et al. study were to better understand the role of Sigma1 with regard to the stabilization and function of an oncogenic protein, in this case AR, and to determine whether modulation of its activity may have therapeutic value (Thomas et al. 2017). The authors showed that IPAG blocked 5α-dihydrotestosterone (5α-DHT) induced nuclear translocation of AR and suppressed AR transcriptional activity. Treatment with IPAG also induced proteasomal degradation of AR, suppressing the protein levels of both full-length (AR) and constitutively active splice variant AR (ARV). Consistent with these data and with putative antagonist/inhibitor activity of IPAG, RNAi knockdown of Sigma1 also suppressed AR protein levels and transcriptional activity. Furthermore, in support of the importance of Sigma1 in prostate cancer cell growth and survival, RNAi knockdown of Sigma1 and treatment with IPAG both inhibited clonogenic growth and survival of prostate cancer cell lines (Thomas et al. 2017).

The study by Thomas et al. revealed a direct interaction between Sigma1 and the AR axis in prostate cancer and the in vivo efficacy of Sigma1 antagonists/inhibitors in suppressing prostate tumor growth through this mechanism (Thomas et al. 2017). The authors further demonstrated with co-immunoprecipitation experiments that Sigma1 physically associates with constitutively active ARVs (in this case, ARV7 and AR^{v567es}) as well as the hormone responsive full-length AR. Antagonists/inhibitors were able to suppress the transcriptional activity and protein levels of these constitutively active ARVs in metastatic castration resistant prostate cancer (mCRPC) cell lines, both in vitro and in vivo. In vivo, inhibition of Sigma1 with a drug-like analog of IPAG significantly inhibited the growth of xenografted 22Rv1 (ARV driven mCRPC cell line) tumors. Importantly, inhibition of tumor growth was associated with elimination of AR and ARV in responsive tumors, consistent with a Sigma1-AR/ARV mechanism-related response. Moreover, this Sigma1

antagonist/inhibitor produced no detectable side effects at efficacious doses; no weight loss and no behavioral abnormalities were observed under these study conditions (Thomas et al. 2017).

Interestingly, the authors observed no measurable change in glucocorticoid (GR) protein levels in response to IPAG treatment. Considering that AR and GR are closely related proteins with conserved sequences and mechanisms on action, this result was unexpected; however, it highlighted the selectivity of Sigma1 modulator actions. The properties of Sigma1 and specific mechanisms that underlie this selectivity remain to be determined.

Sigma1 also interacts with ErbB receptors, and in the study by Thomas et al., IPAG dose-responsively suppressed ErbB2/HER2 and ErbB3/HER3 protein levels in prostate cancer cells (Thomas et al. 2017). This is particularly relevant to prostate cancer disease progression and therapy as ErbB2 and 3 levels and activity have been reported to be upregulated in CRPC as an adaptive resistance mechanism engaged by malignant prostate cancer cells in response to treatment with standard of care AR-axis targeted therapies (Gao et al. 2016; Berger et al. 2006; Chen et al. 2010, 2011).

These data suggest that Sigma1 may play a role in feedback mechanisms that regulate AR-associated signaling networks and provide evidence in support of targeting Sigma1 to treat AR-driven cancers. Of particular interest, targeting Sigma1 in order to allosterically modulate AR is an intriguing approach that may bypass or prevent the adaptive resistance inherent to current AR-targeted therapies.

4.2.12 Donepezil

Although better known as an acetylcholinesterase inhibitor approved for the treatment of Alzheimer's disease, donepezil also binds Sigma1 with high affinity (Kato et al. 1999), and some of the cognitive benefits of donepezil have been associated with its affinity for Sigma1 (Maurice et al. 2006; Maurice 2016). In light of these observations, there is emerging interest in the potential use of donepezil to mitigate and treat cognitive impairment associated with radiotherapy and chemotherapy and improve the quality of life in patients being treated for cancer (Loh et al. 2016), particularly those with brain tumors (Correa et al. 2016; Shaw et al. 2006; Rapp et al. 2015). Recently, the results of a randomized, placebo-controlled pilot study to assess the ability of donepezil to improve specific measures of cognitive function in breast cancer patients was published. In this clinical trial, patients in the donepezil treatment group performed significantly better than the placebo administered control group on parameters of the Hopkins Verbal Learning Test-Revised (HVLT-R) regarding total recall and recognition discrimination (Lawrence et al. 2016). The benefit of donepezil-mediated attenuation of chemotherapy induced cognitive impairment was also observed in preclinical mouse models; this may provide experimental models to investigate the mechanisms underlying these beneficial effects (Winocur et al. 2011).

Additionally, preclinical studies have suggested that donepezil may also have anti-tumor properties. Donepezil was reported to promote caspase-dependent apoptosis in U937 human histiocytic lymphoma and HL-60 human promyelocytic leukemia cells (Ki et al. 2010). It has been reported to have antiproliferative and anti-migratory effects on glioblastoma cells in vitro (Megalizzi et al. 2009). Furthermore, treatment with a combination of donepezil and temozolomide prolonged survival of mice orthotopically grafted with Hs683 glioblastoma cells compared to temozolomide or donepezil alone (which did not prolong survival) (Megalizzi et al. 2009).

4.2.13 Endogenous Molecules That Bind Sigma1

Several endogenous molecules have been shown to bind Sigma1. These molecules include the steroid hormones didehydroepiandrosterone (DHEA), progesterone, and pregnenolone, as well as sphingolipid-derived amines (D-erythro-sphingosine) and cholesterols. Even *N*,*N*-dimethyltryptamine (DMT) has been proposed as a Sigma1 ligand [reviewed in (Maurice and Su 2009; Fontanilla et al. 2009; Narayanan et al. 2011)].

4.3 Relationship Between Sigma1/*SIGMAR1* Levels and Drug Response

Based on the current literature, it appears that *SIGMAR1* transcript and Sigma1 protein levels alone do not necessarily predict or correlate with cancer cell response to Sigma1 inhibitors.

Evaluation of rimcazole in the National Cancer Institute's NCI-60 screening panel revealed that rimcazole had growth inhibitory effects, with GI_{50} values for the 59 cell lines currently in this panel ranging from 1.9 to 38 μM (Spruce et al. 2004). Spruce and colleagues subsequently used transcript data from the NCI-60 associated Cell Miner gene expression database to show that sensitivity to rimcazole's antiproliferative and proapoptotic properties did not correlate with *SIGMAR1* transcript levels (Spruce et al. 2004). These data suggest that the mere presence of *SIGMAR1* or increased levels of *SIGMAR1* do not necessarily correlate with response to Sigma1 ligands (Spruce et al. 2004). In support of this notion, [^{3}H](+)-pentazocine radioligand binding studies confirmed that Sigma1 is present at relatively low levels on MCF-7 cells, and it is as sensitive to rimcazole treatment as MDA-MB-468 cells, which express a higher density of Sigma1 sites ($K_d = 7.7$ nM; $B_{max} = 3{,}250$ fmol/mg of protein) (Spruce et al. 2004).

In general, gene expression data and radioligand binding assay data show that normal, healthy tissues appear to express less *SIGMAR1* and Sigma1 binding sites than corresponding cancer tissue. However, some tissue/cell types intrinsically express high levels of *SIGMAR1* and Sigma1. For example, cerebellar granule neurons (CGN) (Starr and Werling 1994) and hepatocytes (Mei and Pasternak 2001) express high densities of Sigma1, greater than some cancer cell lines. However, Spruce and colleagues showed that although CGN express high levels

of Sigma1, they were not sensitive to the antiproliferative or cytotoxic effects of antagonists/inhibitors (Spruce et al. 2004). Mouse whole brains have Sigma1 density comparable to cancer cell lines with [^{3}H](+)-pentazocine radioligand binding B_{max} values in excess of 1,000 fmol/mg protein (Langa et al. 2003). Yet, neurotoxicity and hepatotoxicity have not been widely reported in animal studies with Sigma1 antagonists/inhibitors (see Sect. 4.5, below).

These observations, along with the general absence of cytotoxicity in preclinical animal studies of Sigma1 ligand efficacy and the Phase I safety assessment of selective Sigma1 antagonists/inhibitors (Abadias et al. 2013; Gris et al. 2016), altogether suggest a context-dependent response to Sigma1 ligands. In other words, it is possible that Sigma1 is being used differently in different organs/tissues as well as in normal physiological versus pathophysiological conditions.

The specific biochemical and molecular mechanisms underlying these potential context-dependent effects remain poorly understood. However, we propose that the preponderance of published data suggests that these mechanisms involve distinct, context-dependent Sigma1 protein associations. Thus, one explanation is that small molecule modulators of Sigma1 target Sigma1 protein complexes and not Sigma1 per se. The composition of distinct Sigma1 associated protein complexes may determine biochemical and cellular response to Sigma1 targeted drugs. This concept is illustrated in Figs. 2 and 3. This could explain, in part, the differential toxicity of Sigma1 inhibition in cancer versus normal cells. In this case, although Sigma1 is widely expressed, its stabilizing function is more heavily or differentially engaged in conditions such as the proteotoxic stress characteristic of metabolically stressed cancer cells. In contrast, normal cells appear to be markedly less sensitive to disruption by *SIGMAR1* knockout or Sigma1 antagonists/inhibitors and may be able to compensate or adapt to treatment.

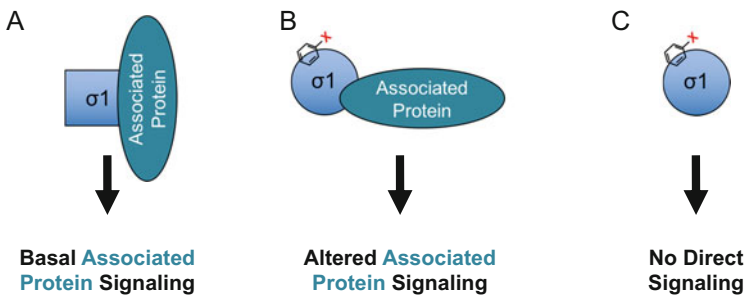

Fig. 2 Proposed model for Sigma1 ligands as allosteric modulators of protein–protein interactions. In this proposed model Sigma1 protein association, and not Sigma1 itself, determines cellular response to Sigma1 ligands. (**a**) Under basal conditions, Sigma1 binds to its associated protein(s), thus allowing for normal associated protein signaling. (**b**) Sigma1 ligand (⟨⬡⟩–x) binding to Sigma1 allosterically modulates the signaling of Sigma1 associated proteins. (**c**) Sigma1 has no known intrinsic signaling or enzymatic activity, and in the absence of associated proteins, ligand binding does not elicit direct signaling

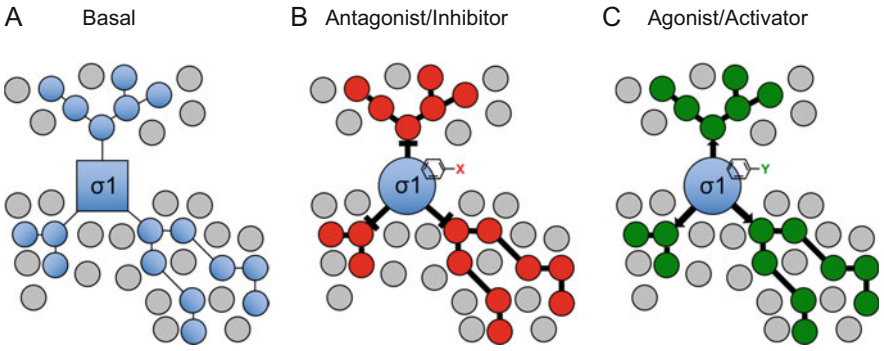

Fig. 3 Proposed model for Sigma1 as a selectively multi-functional drug target. (**a**) Under basal, steady-state conditions, Sigma1 associates with its partner proteins (●) and is surrounded by other related proteins with which it does not physically associate or regulate (○). (**b**) When a Sigma1 inhibitor/antagonist (⬡–X) binds to Sigma1, it selectively suppresses Sigma1 associated proteins and their downstream interactions and signaling pathways. (**c**) When a Sigma1 activator/agonist (⬡–Y) binds Sigma1, it promotes these associated protein pathways. Thick lines in (**b**) and (**c**) indicate increased strength of interaction. The circles directly connected to Sigma1 represent associated proteins that are physically bound to Sigma1, and indirectly connected circles represent their downstream signaling pathway components. An example of this concept is Sigma1 regulation of AR (Thomas et al. 2017)

4.4 Relationship Between Reported Ligand Binding Affinity and Functional Potency in Cell Based Assays

An important unresolved question regarding Sigma1 pharmacology in the context of cancer is how to explain apparent discrepancy between ligand binding affinity in biochemical membrane preparations and functional potency (activity) in live-cell-based functional assays. In traditional in vitro binding assays, many Sigma1 ligands bind with low nanomolar (nM) K_i/K_d whereas in cell-based functional assays, the response to Sigma1 ligands is observed at high nM to low μM concentrations. In this section, we consider a number of potential explanations.

4.4.1 High and Low Affinity Sigma1 Binding Sites

Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), along with other neurosteroids including pregnenolone and progesterone, have been proposed as endogenous modulators of Sigma1; however, their relatively low binding affinity has been the source of dispute regarding this classification. The argument assumes that only the higher affinity, low nanomolar binding sites are meaningful Sigma1 pharmacological sites. However, this has not been confirmed. Some of these "low affinity" sites may be relevant and may elucidate some of the context-dependent physiological roles of Sigma1. These distinct binding sites may reflect distinct Sigma1 conformations, multi-protein complexes, or populations. Although the physiological and pharmacological relevance of these sites remains to be

determined, there is evidence, published over several decades, of higher and lower affinity Sigma1 binding sites.

Thomas et al. performed radioligand binding saturation assays on tumors and non-cancerous tissue from patients (Thomas et al. 1990). The authors detected sigma binding sites in all nine tumors tested with [^{3}H]DTG K_d values ranging from 27 to 83 nM. Interestingly, the authors report that a two-site model fit their binding data better than a one-site model, with a high affinity binding site (K_{d1}) 18–38 nM and lower affinity binding site (K_{d2}) of 165–2,880 nM (Thomas et al. 1990).

Bowen and colleagues quantified Sigma1 binding sites with [^{3}H](+)-pentazocine in crude membrane preparations from 13 cancer cell lines including C6 glioma, N1E-115 neuroblastoma, NG108-15 neuroblastoma x glioma hybrid, human T47D breast ductal carcinoma, human NCI-H727 lung carcinoid, and human A375 melanoma (Vilner et al. 1995b). The authors identified two distinct Sigma1 binding sites in most of these cancer cell lines, high affinity (K_{d1} = 0.67–7.0 nM) with B_{max1} = 25–108 fmol/mg protein, and low affinity sites (K_{d2} = 127–600 nM) with B_{max2} = 942–5,431 fmol/mg protein. Interestingly, the low affinity site was more abundant than the high affinity site in the cancer cell lines in this study (Vilner et al. 1995b).

Wu et al. described a low affinity Sigma1 binding site in intact NCB-20 (mouse neuroblastoma x Chinese hamster brain hybrid) cells (Wu et al. 1991). This group found that [^{3}H](+)-SKF10047 binds two populations of binding sites in intact NCB-20 cells, a higher affinity binding site (K_d = 49 nM, B_{max} = 1.0 pmol/mg protein) and a lower affinity binding site (K_d = 9.6 μM, B_{max} = 69 pmol/mg protein). The rank order potencies of a number of sigma ligands at the lower affinity site correlated (using Spearman rank correlation) with the electrophysiological assay potencies both in this study and in a previously reported study using a guinea pig vas deferens assay (Vaupel and Su 1987). These data indicated that the electrophysiological responses at high and low affinity binding sites were the result of Sigma1 occupancy. The authors of this study noted that it was unclear whether the high and low affinity Sigma1 binding sites represented two separate receptors or the same receptor with two different states (Wu et al. 1991).

More recently, Safrany and colleagues described high and low affinity Sigma1 binding sites or conformations in the Sigma1-positive MDA-MB-468 breast adenocarcinoma cell line (Brimson et al. 2011). When a model assuming single-site binding was used, only the high affinity, 2.5 nM binding site was detected. However, when a multiple-site model was used, IPAG displaced [^{3}H](+)-pentazocine with a K_i of 8 μM (Brimson et al. 2011), which corresponds to concentrations at which activity is detected in cell-based assays of cancer (Spruce et al. 2004; Kim et al. 2012; Schrock et al. 2013; Thomas et al. 2017).

Spruce and colleagues noted that rimcazole displaces [^{3}H](+)-pentazocine with an IC_{50} of 2.7 ± 1.8 μM, which is close to its IC_{50} in MDA-MB-468 cell proliferation and survival assays (Spruce et al. 2004). Interestingly, this suggests that

rimcazole only binds the putative low affinity Sigma1 binding site or conformation (Spruce et al. 2004). It is noteworthy that the reported binding affinity of rimcazole to Sigma1 ranges from the high nanomolar to low micromolar range (see Table 2).

Similarly, Wilke et al. reported that iodoazidococaine (IAC), a Sigma1 photoprobe, inhibited voltage-activated potassium current (IK) in DMS-114 (small cell lung carcinoma) cells. IAC photolabeling of Sigma1 in cell homogenates was inhibited by (+)-SKF10047 with an IC_{50} of 7 μM. This was similar to the half-maximal concentration of (+)-SKF10047 that inhibited IK (14 μM) (Wilke et al. 1999).

4.4.2 Cell Penetration

One possible explanation is that the cell plasma membrane limits access to intracellular Sigma1 binding sites. Published K_d and K_i values of Sigma1 ligands are based on binding assays performed with membrane preparations or in some instances with permeabilized cells. Does facilitating compound entry increase functional potency? The availability of sufficient free compound within the cell to act on intracellular targets such as Sigma1 may also explain why the effective concentrations of many Sigma1 ligands are significantly higher in cell-based functional assays than their binding affinities – which are largely determined with biochemical membrane preparations and not intact cells.

Although the answer to this question remains unanswered, at least one report suggests that cell penetration may be a contributing factor to functional potency. Banerjee and colleagues (Pal et al. 2011) have reported that facilitating cell entry by conjugating haloperidol with cationic lipids of varying chain lengths increases the functional potency of haloperidol in in vitro cell proliferation and cytotoxicity assays. For example, HP-C8, a cationic lipid-modified haloperidol analogue with a lipid chain of 8 carbon atoms was >100-fold more potent than haloperidol in inhibiting the proliferation and survival of MCF-7 and MDA-MB-231 breast cancer cells. HP-C8 was a two- to threefold more potent inducer of apoptosis in these cancer cells compared to non-transformed COS-1 and HEK293 cells. The authors reported that HP-C8 was also efficacious in vivo. Xenografted mice bearing B16F10 melanoma tumors produced a threefold reduction in tumor growth following 5 intraperitoneal injections of 7.5 mg/kg HP-C8 at 2- to 3-day intervals (Pal et al. 2011).

4.5 Safety of Treatment with Sigma1 Ligands

Because Sigma1 is broadly expressed in tissues throughout the body, the safety of Sigma1 modulators is a common concern. However, there is little empirical or clinical evidence to support target-mediated toxicity associated with Sigma1 selective compounds. Indeed, it has been well documented in the literature that

compounds that are active at Sigma1 are generally safe (Abadias et al. 2013; Gris et al. 2016; Nieto et al. 2012; Zamanillo et al. 2013; Luedtke et al. 2012; Blasio et al. 2015; Cendan et al. 2005a; Romero et al. 2012; Maurice and Su 2009; Spruce et al. 2004; Casellas et al. 2004; Riganas et al. 2012a, b, c; Moody et al. 2000; Thomas et al. 2017).

One salient piece of evidence that Sigma1 inhibition is generally benign is that *SIGMAR1* knockout (KO) mice are viable, fertile, and do not display a phenotype overtly different from wild type mice (Langa et al. 2003), which supports the notion that inhibiting Sigma1 has minimal impact on normal tissues. This raises a separate question regarding potential compensatory mechanisms that may be engaged when *SIGMAR1* is eliminated; however, such mechanisms have not yet been identified.

Pharmacological inhibition of Sigma1 appears to be safe (benign) as well. Most recently, clinical trials of the Sigma1 antagonist/inhibitor S1RA have demonstrated lack of toxicity in humans (Abadias et al. 2013; Gris et al. 2016). S1RA (also known as E-52862) was evaluated in single- and multiple-dose phase I clinical studies and demonstrated positive safety, tolerability, and pharmacokinetic profiles in healthy human subjects (Abadias et al. 2013). Of the 175 subjects enrolled, no serious adverse events were observed, and no clinically significant changes were observed in electrocardiogram (ECG), Holter monitoring, vital signs, and laboratory assessments. This Sigma1 antagonist/inhibitor is currently in phase II clinical trials for treatment of neuropathic pain of different etiology using a daily oral dose of 400 mg (Abadias et al. 2013; Gris et al. 2016).

Consistent with this observation, in a number of published tumor xenograft studies, no adverse events (including weight loss and behavioral abnormalities) were observed at efficacious doses of Sigma1 antagonists/inhibitors (Spruce et al. 2004; Casellas et al. 2004; Riganas et al. 2012a, b, c; Moody et al. 2000; Thomas et al. 2017).

Based on their antiproliferative and cytotoxic effects on cancer cells and tumors, another common concern is whether Sigma1 antagonists/inhibitors have the potential to promote neurodegeneration (Tsai et al. 2014). As with the general safety concerns, there is little empirical or clinical evidence demonstrating that Sigma1 selective antagonists/inhibitors promote neurodegeneration or exacerbate symptoms of neurodegenerative disease. At the cellular level, cerebellar granule neurons, which express higher levels of Sigma1 than many cancer cells, were not sensitive to the antiproliferative or cytotoxic effects of Sigma1 antagonists in at least one report (Spruce et al. 2004). In behavioral models focusing on cognitive deficits, Sigma1 antagonists/inhibitors did *not* worsen symptoms, and did *not* promote symptoms (Matsumoto et al. 1995; Maurice et al. 1994, 1998). In most published studies, antagonists were used to block the effects of agonists and demonstrate their Sigma1-mediated actions. However, when administered alone, the antagonists generally manifested no effect in rodent models of behaviors associated with Alzheimer's disease. This has been demonstrated in a number of studies (Wang et al. 2003; Espallergues et al. 2007; Villard et al. 2009; Yang et al. 2012; Maurice 2016).

5 Sigma1: Receptor, Chaperone, or Scaffold?

It is becoming increasingly clear that Sigma1 is not a traditional receptor. Although it remains unclear whether Sigma1 should be defined as a chaperone or scaffolding protein in cancer cells, the absence of clear enzymatic or signaling activity of Sigma1 along with its association with and modulation of diverse signaling molecules are evidence in support of Sigma1 as a scaffolding protein. Scaffolds have no enzymatic or signaling activity; however, they physically interact with other proteins to assemble, localize, and regulate signaling complexes. They coordinate the organization of signaling or chaperone molecules into discrete complexes to facilitate efficient and specific activity (Good et al. 2011; Bauer and Pelkmans 2006). Scaffolding proteins can allosterically modulate signaling or enzymatic activity as well as coordinate the activity of chaperones such as HSP70 and HSP90 (Cesa et al. 2015; Good et al. 2011). Scaffolds can also be inhibitory by blocking protein–protein and protein–lipid interactions (Good et al. 2011; Bauer and Pelkmans 2006). They are flexible platforms that can form multiple oligomeric conformations that comprise combinatorial assemblies of protein interaction domains that enable regulation of diverse biological processes. Consistent with recently published reports, our data suggest that Sigma1 is present as oligomers (Gromek et al. 2014; Schmidt et al. 2016). These oligomeric structures may also be a determinant of how Sigma1 forms multi-protein complexes. As a potential membrane bound scaffolding protein, Sigma1 is reminiscent of caveolins and tetraspanins (Bauer and Pelkmans 2006; Patel et al. 2008; Hemler 2014).

We propose that Sigma1 is a ligand-regulated scaffolding protein that engages in selective protein interactions. We have found that Sigma1 physically and functionally interacts with AR and ErbB-2 and -3 receptors and that these receptors are regulated by Sigma1 ligands (Thomas et al. 2017). Our data, along with published reports from other groups, suggest that Sigma1 engages in a number of multi-protein complexes, and the composition of these protein complexes appears to be context-dependent. It remains to be determined whether Sigma1 modulators directly alter PPIs or the intracellular transport and localization of Sigma1-associated protein complexes. The biochemical mechanisms and protein determinants that dictate Sigma1 PPIs have not yet been clearly defined. Therefore, the mechanistic basis of Sigma1 partner and client protein selectivity is unknown. This is a crucial missing link to understanding the complex pharmacology of Sigma1.

6 Sigma1 as a Multifunctional Drug Target

Whether Sigma1 is eventually classified as a scaffolding protein or chaperone, it is already clear that it engages in a range of heterogeneous but selective functional protein interactions (illustrated in Fig. 3). Sigma1 modulators alter multiple processes and systems in cancer cells by targeting distinct Sigma1 associated protein complexes that appear to assemble in a context-dependent manner. The known

biochemical properties and cellular activities of Sigma1 are consistent with a role as a component of the cancer cell support machinery [concept reviewed in (Dobbelstein and Moll 2014)]. Importantly, Sigma1 inhibitors are not pleiotropic, and they suppress or alter oncogenic proteins and pathways by a mechanism distinct from other drugs that target the cancer cell support machinery (Thomas et al. 2017). With respect to Sigma1 drug discovery and pharmacology, a key challenge is to understand how to harness the selective multifunctionality of Sigma1 as a drug target.

6.1 Cell Intrinsic Signaling and Activities

Multifunctional drug targets such as Sigma1 can have a number of advantages over single target therapies in regulating cell intrinsic signaling and processes. Specific targeted therapies such as tyrosine kinase inhibitors, selective receptor antagonists, and targeted monoclonal antibodies are prone to adaptive, acquired drug resistance (Komarova and Wodarz 2005; Bozic et al. 2012; Pao et al. 2005; Schwartz et al. 2015). In contrast, Sigma1 modulators used alone or in combination with targeted therapeutic agents may delay or even bypass such resistance.

In the case of prostate cancer, the inevitable resistance to androgen receptor (AR)–targeting agents is associated with reactivation of the AR axis through induction of intratumoral steroidogenesis, increased expression of AR, gain-of-function mutant AR, and constitutively active AR splice variants (Mostaghel et al. 2014; Knudsen and Kelly 2011; Attard et al. 2016; Ferraldeschi et al. 2015; Bambury and Scher 2015). This is further complicated by compensatory upregulation or feedback regulation of associated pathways such as ErbB receptor upregulation and PI3K (phosphatidyl inositol-3-kinase) activation in PTEN (*p*hosphatase and *ten*sin homolog) deficient prostate cancers (Gao et al. 2016; Carver et al. 2011). For prostate cancer, these examples demonstrate the importance of discovering and developing novel approaches to co-targeting the AR axis and the networks on which it depends.

Recently, Thomas et al. showed that three CRPC lines (C4-2, VCaP, and 22Rv1) evaluated were all responsive to small molecule Sigma1 inhibition. AR levels were suppressed in C4-2 cells and AR and ARV levels were suppressed in the AR splice variant driven VCaP and 22Rv1 cell lines. In vitro colony formation of all three lines was dose-responsively suppressed by treatment with IPAG (Thomas et al. 2017). IPAG also reduced ErbB2/HER2 and ErbB3/HER3 protein levels (Thomas et al. 2017), thus abrogating the compensatory upregulation of ErbB2/HER2 and ErbB3/HER3 that occurs in response to AR-targeted therapies (Carver et al. 2011; Mostaghel et al. 2014; Gao et al. 2016).

PTEN deficiency, by mutation or loss of PTEN, has a significant impact on prostate cancer progression. Indeed, over 50% of advanced prostate cancers are PTEN deficient (Li et al. 1997; Mulholland et al. 2011; Carver et al. 2011). Small

molecule Sigma1 inhibitors suppress growth pathway signaling in PTEN mutant LNCaP and C4-2 and PTEN null PC3 cells (Kim et al. 2012; Thomas et al. 2017). These data suggest that Sigma1 inhibitors can engage mechanisms downstream of PTEN or mechanisms that cooperate with but are distinct from canonical PI3K/Akt growth and survival signaling pathways. The ability to suppress growth signaling in PTEN deficient cancers (Kim et al. 2012; Schrock et al. 2013; Thomas et al. 2017) as well as the ability to suppress compensatory mechanisms that emerge in response to AR-targeted inhibition demonstrates that Sigma1 ligands may provide a way to bypass or suppress the redundancies and complex feedback mechanisms that render current therapeutic approaches to target growth regulatory pathways susceptible to resistance (She et al. 2010; Carver et al. 2011; Zhang and Yu 2010; Hsieh et al. 2011; Mostaghel et al. 2014; Gao et al. 2016).

Thus, the ability to pharmacologically modulate multifunctional targets such as Sigma1 is advantageous in cancer, as it imposes a barrier to compensatory response mechanisms to targeted therapies without the broad and often toxic effects of chemotherapy.

6.2 Immunomodulation

The multifunctionality of Sigma1 as a drug target may extend beyond cell intrinsic signaling and regulation of oncogenic driver proteins and pathways. For example, a series of papers in the late 1990s and early 2000s have reported immunomodulatory effects of Sigma1 ligands (Bourrie et al. 1995, 1996, 2002, 2004; Carayon et al. 1995, 1996; Derocq et al. 1995; Gardner et al. 2004; Zhu et al. 2003). These papers, which describe the cytokine modulating effects of SR31747A, PRE-084, and (+)-SKF10047, are discussed in Sect. 4, above. In summary, this work demonstrates that Sigma1 agonists/activators promote tumor growth, in part by suppressing anti-tumor immunity. However, these studies stopped short of evaluating the ability of Sigma1 antagonists/inhibitors to promote anti-tumor immunity. Although prototypic Sigma1 antagonists/inhibitors were used to block the immune suppressive and tumor promoting effects of Sigma1 agonists/activators, the direct effects of Sigma1 antagonists/inhibitors on anti-tumor immunity were not determined.

Recently, we discovered that the Sigma1 agonist/activator (SA4503) and antagonist/inhibitor (IPAG) differentially regulate the stability, trafficking, and activity of the checkpoint molecule programmed death-ligand 1 (PD-L1, also known as B7-H1 and CD274). We found that IPAG induced autophagic degradation of PD-L1 in androgen independent prostate cancer (PC3) and triple negative breast cancer (MDA-MB-231) cell lines. This resulted in decreased functional PD-L1 at the surface of these cancer cells. Consistent with this effect, RNAi knockdown of Sigma1 resulted in decreased PD-L1 levels. Conversely, treatment with SA4503 blocked these IPAG-mediated effects, and SA4503 alone promoted increased cell surface PD-L1 levels (Maher et al., unpublished data).

Taken together, these data suggest that pharmacological modulation of Sigma1 can regulate PD-L1 production and activity via immune response-induced

cytokine-mediated extracellular feedback loops as well as directly, via cell intrinsic mechanisms. Thus, Sigma1 ligands may be used as regulators of the tumor microenvironment.

6.3 Cancer-Associated Pain

Sigma1 has been extensively investigated in pain. For recent, detailed reviews of the subject see the chapters in this volume by Pasternak (*Allosteric Modulation of Opioid G-Protein Coupled Receptors by Sigma$_1$ Receptors*) and by Merlos et al. (*Sigma1 Receptor and Pain*). A number of studies over several decades have demonstrated that Sigma1 antagonists/inhibitors, but not agonists/activators, can potentiate opioid analgesia, and some Sigma1 antagonists/inhibitors produce analgesia on their own. The precise biochemical mechanism by which Sigma1 antagonists/inhibitors produce analgesia remains unclear. However, consistent with the antinociceptive effects of pharmacological inhibition, *SIGMAR1* KO mice (Langa et al. 2003) have demonstrated a decreased sensitivity to neuropathic pain in preclinical murine models (Cendan et al. 2005a, b; Entrena et al. 2009; de la Puente et al. 2009; Tejada et al. 2014). A potent and safe Sigma1 antagonist/inhibitor, S1RA (also known as E-52862), is currently in phase II clinical trials as a non-opioid analgesic, providing clinical proof of concept of safety and efficacy (Abadias et al. 2013; Gris et al. 2016; Zamanillo et al. 2013; Romero et al. 2016) (also see Sect. 4.5, above).

Sigma1 pharmacology has not been well studied in the context of cancer pain. However, a few preliminary reports suggest that Sigma1 antagonists/inhibitors may be effective analgesics to treat neuropathic pain associated with cancer (Nieto et al. 2012, 2014; Zamanillo et al. 2013). Cancer-associated pain can be mechanical, caused by pressure of a growing tumor on nerves, bone, and other tissue (Glare et al. 2014). It also can be caused by damage to nerves that can occur with treatments such as chemotherapy, radiotherapy, and surgery.

Nieto et al. compared the ability of paclitaxel to induce cold and mechanical allodynia in *SIGMAR1* KO and wild type (WT) *SIGMAR1* mice. They demonstrated that whereas cold and mechanical allodynia were similar in KO and WT mice, treatment with paclitaxel only produced these forms of allodynia in WT mice. Consistent with the absence of paclitaxel-induced neuropathy in *SIGMAR1* KO mice, administration of the Sigma1 antagonists/inhibitors BD1063 and S1RA prior to paclitaxel prevented both cold and mechanical allodynia in *SIGMAR1* WT mice. Furthermore, administration of BD1063 and S1RA after the onset of allodynia reversed paclitaxel-induced neuropathic pain (Nieto et al. 2012, 2014).

Pain associated with bone metastatic tumors is particularly problematic with myelomas and with lung, prostate, and breast cancers (Lozano-Ondoua et al. 2013; Suva et al. 2011; Roodman 2004; Mundy 2002). To evaluate the potential analgesic properties of Sigma1 antagonists/inhibitors, Zhu et al. implanted Walker 256 rat mammary carcinoma cells into the tibia of Sprague–Dawley rats to induce bone cancer pain. Administration of BD1047 attenuated mechanical allodynia.

Interestingly, Sigma1 expression in the spinal cord was elevated in tumor bearing rats compared to control (sham) rats (Zhu et al. 2015a). The Walker 256 rat mammary carcinoma cell bone pain model is reviewed elsewhere (Shenoy et al. 2016; Zhu et al. 2015b; Slosky et al. 2015).

These data raise the question, can antineoplastic small molecule Sigma1 antagonists/inhibitors also be analgesic in the context of cancer-associated pain? A compound that integrates these properties of Sigma1 pharmacology has yet to be reported.

7 Conclusions and Perspectives

A principal take-away message of this review is that the pharmacology of Sigma1 is complex, and there is still much to be done to define the mechanisms of action of Sigma1 ligands. Although their classification as agonists and antagonists is still commonly used in the literature (including this review), these putative pharmacological activities have remained undefined at the molecular level and may be inaccurate designations. Insights into the specific pharmacological and biochemical mechanisms by which Sigma1 ligands suppress cancer cell growth and survival are just beginning to emerge. As Sigma1 has no clearly defined enzymatic or signaling activity, most cellular responses to Sigma1 ligands are defined by the proteins and/or cellular systems engaged by Sigma1 (illustrated in Figs. 2 and 3). Thus, it may be more accurate to describe compounds with activity at Sigma1 as allosteric modulators of Sigma1 associated proteins (as illustrated in Fig. 2).

The concept of Sigma1 is rapidly evolving. A growing body of evidence supports the notion that Sigma1 is a novel chaperone or scaffolding protein that engages in diverse but selective protein interactions (see Sects. 4 and 5). Given the number of proteins with which it interacts, it is likely that Sigma1 has multiple "innate" functions. However, although Sigma1 modulators alter multiple processes and systems in cancer cells, the effects of Sigma1 ligands are not pleiotropic (see Sect. 4). Thus, Sigma1 is a selectively multifunctional drug target (concept illustrated in Fig. 3).

Multifunctional drug targets such as Sigma1 can have a number of advantages over single activity targeted therapies, which are prone to adaptive drug resistance (Komarova and Wodarz 2005; Bozic et al. 2012; Pao et al. 2005; Schwartz et al. 2015). In contrast to specific target-based therapies such as tyrosine kinase inhibitors, selective receptor antagonists, and monoclonal antibodies, Sigma1 modulators used alone or in combination with these agents may prolong or even prevent drug resistance. Most complex pathologies and disorders, including cancer, are not usually driven by a single cellular factor. Indeed, cancer is a heterogeneous, highly adaptive, and constantly evolving disease. Consequently, drug resistance in cancer is often accelerated by the targeted agents designed specifically to suppress individual oncogenic driver proteins. Therefore, a major challenge is to address not only the primary, existing target, but also latent targets that emerge as a result of mutations or other adaptive, compensatory mechanisms. This, of course, is the

rationale behind drug combinations. However, the potential efficacy of combining multiple targeted drugs must be balanced against potential adverse drug–drug interactions and differences in drug metabolism and pharmacokinetic (DMPK) properties that can add to the complexity of designing combinations. The development of a single drug addressing an array of targets (i.e., polypharmacology) also poses several challenges as well as advantages (Antolin et al. 2016; Azmi 2013). Modulation of Sigma1 would enable the selective inhibition of multiple nodes through one drug target (Fig. 3). Harnessing the strengths of these approaches would offer promising new possibilities to enhance therapeutic efficacy and bypass or prevent drug resistance.

Additionally, a number of studies demonstrate that Sigma1 modulators are not necessarily cytotoxic agents, and that they may be considerably more versatile (see Sects. 4 and 6). It is tempting to speculate that certain Sigma1 modulator compounds may be used not only as antineoplastic agents, but also to improve the quality of life of cancer patients, with decreased side effects and even benefits such as attenuation of cancer-associated pain (see Sect. 6).

Despite the number of studies suggesting that it is a valid drug target, there still are no Sigma1 drugs in the clinic to treat cancer. This is in large part because fundamental questions regarding the mechanism of action of Sigma1 modulators in the context of cancer remain unanswered or only partially answered. To understand how to use Sigma1 modulators for therapeutic benefit in cancer, there is a need for more detailed and definitive studies leading to a deeper understanding of Sigma1's role in tumor biology.

Acknowledgements We thank Drs. Paul McGonigle and James Barrett and members of the Kim Lab for critical reading of this manuscript. We thank John Vaillancourt for technical support in performing the *SIGMAR1* gene expression analysis in Fig. 1.

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