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REVIEW

Sigma 1 Receptors *In vitro* and *In vivo* Imaging Studies in Different Disease States

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The sigma receptor system has been classified into two distinct subtypes, Sigma 1 (σ1R) and Sigma 2 (σ2R). Sigma 1 receptors (σ1Rs) are involved in many neurodegenerative diseases and different central nervous system disorders such as Alzheimer's disease, Parkinson's, schizophrenia, drug addiction, and pain. This makes them attractive targets to develop radioligands as tools to gain better understandings of disease pathophysiology and clinical diagnosis. Over the years, several σ1R radioligands have been developed to image the changes in σ1R distribution and density providing insights into their role in disease development. Moreover, the involvement of both σ1R and σ2R with cancer make these ligands, especially those that are σ2R selective, great tools for imaging different types of tumors. This review will discuss the principals of molecular imaging using PET and SPECT, known σ1R radioligands and their applications for labelling σ1Rs in different disease conditions that have shown considerable potential as biomarkers and an opportunity to fulfill the ultimate goal of better health care outcomes and improving human health.

Introduction

The concept of what sigma 1 receptors (σ1Rs) are has evolved significantly over the past 40 years. Currently, σ1Rs are known to be a unique class of chaperone proteins that regulate protein folding, oxidative stress, cell homeostasis, and are involved in many pharmacological events that make them an attractive, validated therapeutic target. σ1Rs gained a lot of interest in the past 25 years with total of 1,102 articles published from 1992-2017 demonstrating intensive efforts employed in the area of medicinal chemistry to develop selective ligands to probe the associated, putative pharmacologies.[1] σRs are classified into 2 subtypes: sigma 1 (σ1Rs) and sigma 2 (σ2Rs). They differ in protein size, tissue expression, pharmacological, and drug selectivity profiles.[2-4] σRs are widely distributed in the central nervous system (CNS) in areas involved in pain modulation, memory, emotions, motor functions; and the periphery where they are expressed mainly in the heart, liver, spleen, lung, kidney, adrenal gland, and gastrointestinal tract.[2, 5-11] Previously, they were misclassified as opioid receptors due to their high affinity to (+)-benzomorphans.[2-4] Subsequently, they were further incorrectly thought to be the phencyclidine (PCP) binding site at the glutamate NMDA receptors because SKF-10,047 can bind to the PCP site and PCP can bind to σRs.[12] Later, it was confirmed that σRs are orphan receptors and are now recognized as unique class of chaperon proteins.[5, 13-15]

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The σ1R is comprised of 223 amino acids. The amino acid sequence shares more than 90% identity across species with no similarity to any other mammalian protein and less than 30% homology with fungal enzyme C8-C7 sterol isomerase, although it lacks C8-C7 isomerase activity.[5, 16] The sequence of the ligand-binding domain of σ1Rs is highly conserved across species, while the transmembrane helices are poorly conserved.[17] The σ2R has a molecular weight between 18–22 kDa. Previously, it has been claimed that σ2R binding sites are located in the progesterone receptor membrane component 1 (PGRMC1) protein complex,[18] but recent studies emphasized that both are different proteins.[19] In 2017, the σ2R was cloned by Alon *et al*.[20] who identified the σ2R as the endoplasmic reticulum (ER)-resistant TMEM97 transmembrane protein, which is involved in cholesterol trafficking, homeostasis, and cell growth regulation. The crystal structure of the σ2R has not been resolved due to the lack of selective ligands.

σ1Rs are chaperone proteins located at the mitochondria associated membrane (MAM) of the endoplasmic reticulum (ER). At the MAM, the ER supplies Ca^{2+} directly to the mitochondria through inositol 1,4,5-trisphosphate receptors (IP3Rs). σ1Rs are a Ca^{2+} sensitive chaperone that form a complex with another chaperone protein, immunoglobulin heavy chain-binding protein (BiP). Upon ER stress and depletion of Ca^{2+}, σ1Rs dissociate from BiP, sustain the proper conformation of the IP3Rs, and regulate Ca^{2+} signalling into the mitochondria. The location of σ1Rs at the MAM and the fine-tuning mechanism they exert on mitochondrial Ca^{2+} signaling supports many of their reported functions such as; regulation of protein folding/degradation, involvement in cell survival and cellular stress responses.[13, 21, 22] Also, several studies have

reported σ1Rs translocate to the plasma membrane and the nuclear membrane where they can associate with different protein targets to regulate their action through protein-protein interactions, such as ion channels (potassium, calcium, sodium) and G protein-coupled receptors mainly glutamate, NMDA, and μ opioid.[21, 23-25]

σ1Rs were first cloned in 1996 from guinea pig, followed with subsequent cloning from human placental choriocarcinoma, mouse, and rat tissues.[5, 16, 26, 27] Since the cloning of σ1Rs and the generation of σ1R knockout mice in 2003,[28] the research on σ1Rs has made enormous progress and has provided a greater understanding of the σ1Rs physiological and pathological roles.[1] The human σ1Rs were crystalized with two ligands in 2016. The crystal structure of σ1Rs showed a triangular trimer with a single transmembrane domain in each protomer.[17] Interestingly, σ1Rs exist in different dynamic oligomerization states that change based on the bound ligands. Fluorescence Resonance Energy Transfer (FRET) studies revealed that antagonists stabilize higher oligomeric states, while agonists favour dissociation of these complexes.[29, 30] Although many efforts have been employed to find the endogenous ligand, no small molecule endogenous ligand has been concretely identified yet for σ1Rs. Interestingly, some endogenous molecules show high/moderate binding affinities to σ1Rs, nevertheless no consensus has been reached on a single ligand. Some of the proposed σ1Rs endogenous ligands are neurosteroids; such as progesterone (K_i σ1 = 270 nM). Also, *N*-alkyl amines, sphingosine and their derivatives such as L-threo-sphingosine (K_i σ1= 20 nM), D- erythro-sphingosine (K_i σ1= 140 nM) were presented as endogenous ligands. Most recently, *N,N*-dimethyltryptamine (DMT) (K_i σ1= 14750 nM) was suggested as the endogenous ligand for σ1Rs.[4, 31-35] Over the past five decades, ligands with diverse structures and flexibility that bind to σ1Rs with high to moderate affinity and low selectivity were reported. Some of these ligands are marketed prescription drugs such as; haloperidol (antipsychotic, dopamine antagonist), fluoxetine (antidepressant, selective serotonin reuptake inhibitor), donepezil (Alzheimer's disease, cholinesterase inhibitor), pentazocine (analgesic, opioid agonist). Although these compounds were very useful in aiding to identify the role of σ1Rs in different diseases, some of these compounds are not selective enough for σ1Rs to draw definitive conclusions. Some of these drugs displayed higher or equal affinity at sigma receptors compared to their approved therapeutic target. For example, haloperidol was reported to bound with equal affinity to both sigma receptors and D2 receptors in rat brain (Ki = 2.8 nm).[36] However, another study reported haloperidol to have lower affinity at D2R in rat (total) striatum (Ki = 10 nM).[37]

Many inconsistent results or off target activities have been reported, which complicate the interpretation of the actual contribution of σ1Rs. Recently, with the help of ligand design strategies and imaging techniques, high affinity, selective ligands have been discovered to probe the receptor and explore its diverse biological contributions. However, no feasible *in vitro* functional assays for σ1Rs have been accepted to determine downstream signalling pathways that discriminate between agonist and antagonists. Functional activity of σ1R ligands remain challenging and really needs further investigation.[22, 38]

Identification of σ1R ligand functional activity (how to differentiate/discriminate between agonist and antagonist in absence of functional assays)?

Generally, σ1R ligands are characterized by radioligand binding assays and some predictive approaches have been used to identify the agonist/antagonist profile which include:

Behavioral pharmacologic assays. Pain related behaviors (allodynia and hyperalgesia) were evaluated against sigma ligands. It has been well established that σ1R agonists (e.g.: pentazocine) diminish opioid analgesic activity, while σ1R antagonists (e.g.: haloperidol) have been demonstrated to potentiate opioid analgesia in both CD-1 mice and Sprague--Dawley rats, as well as being endowed with antiallodynic effects in different pain models.[39-41] Ligands that induce the same phenotype as pentazocine are commonly accepted as σ1R agonists, whereas compounds that show same effect as haloperidol are considered as antagonists. Responses have been measured by using different animal models of pain such as; formalin or capsaicin induced pain models, chronic constriction injury (CCI) assay, tail flick assay, and Von Frey assay.[42]

On the other hand, animal behavioral studies using a cocaine-induced convulsion model have been helpful to discriminate between σR agonists and antagonists. Pretreatment of mice with σR antagonists before the administration of a convulsive dose of cocaine were reported to have protective effects and attenuate cocaine induced behavioral toxicity, lethality, and locomotor stimulatory effects. While σR agonists worsened the behavioral toxicity of cocaine and exacerbated the convulsive effects of cocaine.[43-45]

Furthermore, agonist or antagonist profiles of novel compounds could also be determined by their effects on 1,3-di(2-tolyl)guanidine (DTG)-induced acute dystonic reactions in rats, an established functional assay for σR activity. Compounds are microinjected into the rat red nucleus where they are considered to be agonists if they elicit dystonia. Antagonists were reported to attenuate σR agonist-induced dystonic head postures.[46, 47]

Genetic (knockout mice). Knockout mice help to understand the role and the function of genes that have been inactivated. Whereas, the difference in the normal behavior or physiology of knockout mice compared to wild type infer the possible gene function. For example, in σ1Rs knockout mice, attenuation of pain behaviors in different pain models and enhancement of morphine mechanical antinociception was observed, which is consistent with the observation that σ1R antagonists showed antinociception in pain models.[28, 48-50] Knockout mice are a helpful tool that may predict the functional activity of σ1R ligands. There is always the caveat of compensatory mechanisms, however.

Molecular biology (antisense oligonucleotide). Antisense oligonucleotides are utilized as a knockdown expression method in which downregulation of the targeted receptor

occurs to study gene functions.[51] In σ1Rs antisense oligodeoxynucleotides, enhanced analgesia to morphine as well as blockade of cocaine acquisition, attenuation of cocaine induced convulsions, and reduction in cocaine induced locomotor stimulatory effects.[43, 52] These effects are all consistent with σ1Rs antagonist activity.

Competition binding assay with phenytoin (DPH). DPH was proposed as an allosteric modulator of σ1Rs that modify the binding affinity of σ1R ligands.[53] DPH increases the affinity of σ1R agonists to the active state and did not increase the affinity of the antagonists. Thus, these results suggest that DPH can be used as a predictive tool to differentiate between σ1R agonists and antagonists.

Fluorescence Resonance Energy Transfer (FRET) biosensor assay. Biosensor assays have the ability to detect ligand-mediated conformational changes of σ1Rs induced by agonist or antagonist binding. The technique is based on the use of cyan and yellow fluorescent proteins (CFP and YFP, respectively), which upon ligand binding based on their agonist or antagonist profile will lead to real-time fluorescence resonance energy transfer (FRET) changes in living cells. The agonist binding will lead to a decrease in FRET signal, while the antagonist will increase the FRET signal. Thus, σ1R ligand agonist/antagonist profiles can be predicted.[54]

The alteration of σ1Rs oligomerization state upon binding of agonists and antagonists. Agonist binding resulted in dissociation of the multimers to monomers and dimers and induce an outward facing conformation of dopamine transporter (DAT), thus enhancing cocaine binding and behavioral responses. Whereas the antagonist stabilized the higher order of oligomerization without changing the DAT conformation.[29, 55]

σ1R ligands in clinical trials

Both agonists and antagonists are of great interest as potential therapeutic candidates against σ1Rs related diseases. Selective and high affinity σ1R ligands (10 compounds) have been developed previously and advanced to clinical trials for Alzheimer's disease, depression, neuropsychiatric disorders, schizophrenia, major depressive disorder, and anxiety. Unfortunately, these compounds failed and were discontinued in clinical development.[56]

To date, three σ1R agonists are in clinical trials (Fig 1); the first one is ANAVEX®2-73 (Blarcamesine), a mixed muscarinic receptor/σ1R ligand (Ki σ1 = 850 nM; Ki σ2= inactive).[57] ANAVEX®2-73 is currently in a phase III clinical trials for Alzheimer's disease (ClinicalTrials.gov identifier: NCT03790709), as well as a phase II clinical trial for treatment of cognitive impairment in Parkinson's disease patients with dementia (ClinicalTrials.gov Identifier: NCT03774459), an observational study for event-related potential (ERP) biomarkers in subjects with schizophrenia and healthy volunteer subjects (ClinicalTrials.gov identifier: NCT04025502), and a phase II clinical trial in Rett syndrome patients. Recently, Anavex Life Sciences announced that FDA granted Fast Track designation for clinical development program for the treatment of Rett syndrome (ClinicalTrials.gov Identifier: NCT03758924).[58] The second ligand is SA4503 (Cutamesine), a selective σ1R agonist (Ki σ1= 4.6 nM; Ki σ2= 63 nM), which has completed a phase II clinical trial for acute ischemic stroke (ClinicalTrials.gov identifier: NCT00639249), and a phase II clinical trial for major depressive disorder (ClinicalTrials.gov identifier: NCT00551109).[59] The third agonist is pridopidine (ACR16 or Huntexil). Initially, it was classified as dopamine stabilizer, however recently it was found to be a selective σ1R agonist (Ki σ1 = 70 nM) at the lower end of the active dose known to produce neurochemical and behavioral effects in rats. At this dose, it displayed 100 fold selectivity over dopamine D2 receptor (Ki = 7520nM).[60, 61] Pridopidine is currently in a phase II clinical trial to evaluate its safety and efficacy for treating levodopa induced dyskinesia in patients with Parkinson's disease (ClinicalaTrial.gov Identifier: NT03922711).[62] It also completed a phase III clinical trial for the treatment of motor symptoms in Huntington's disease (ClinicalTrial.gov Identifier: NCT00665223). Recently, it was selected for inclusion in a novel platform trial for Amyotrophic Lateral Sclerosis (ALS) by the Sean M. Healey & AMG Center for ALS at Massachusetts General Hospital.

On the other hand, σ1R antagonists in clinical trials (Fig 1) are led by E-52862 (S1RA), the first-in-class potential σ1R antagonist, (K_i σ1= 17 nM; K_i σ2= 6300 nM), currently in a phase II clinical trial in Europe (EudraCT Number: 2012-000398-21) for pain management as monotherapy for neuropathic pain of different etiology and as an adjuvant therapy to opioids.[63] In addition, [^{18}F]FTC-146 is the most highly selective σ1R antagonist reported to date, (K_i σ1= 0.0025 nM; K_i σ2= 364 nM), and is currently in a phase I clinical trial as PET/MRI diagnostic agent to pinpoint sites of nerve damage, identify the source of pain generation and monitor treatment response in complex regional pain syndrome (CRPS), sciatica patients, chronic neuropathic and/or nociceptive pain to investigate changes in σ1Rs expression in chronic pain. [^{18}F]FTC-146 is tool to help identify the correlation between nerve injury, σ1Rs expression, and pain generation. (ClinicalTrials.gov identifier: NCT02753101).[64-66]

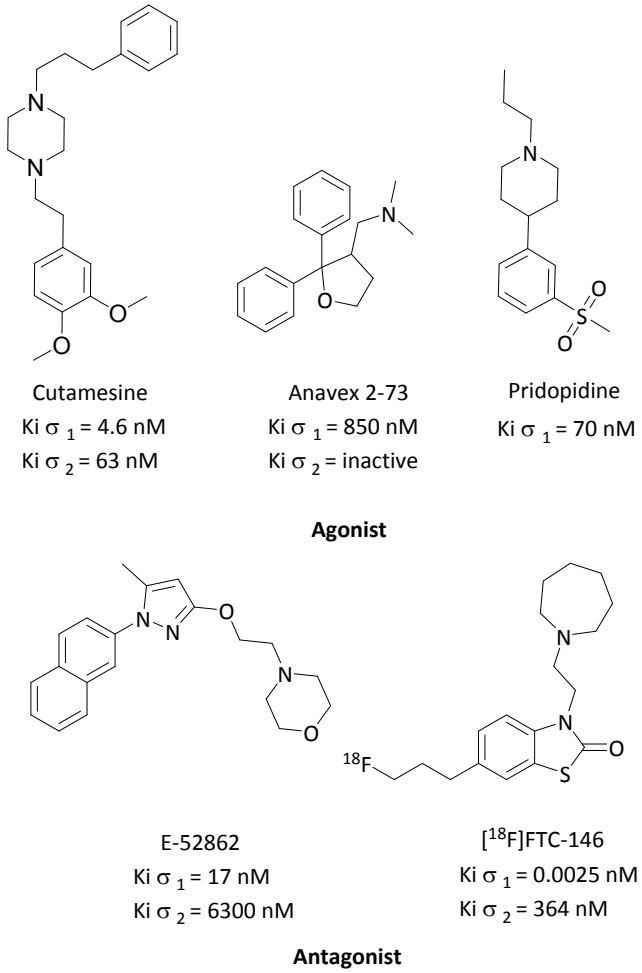


Fig 1: Successful σ1Rs ligands in clinical trials.

Role of σ1R activation or inhibition in chronic neurological diseases

σ1Rs are involved in many pharmacological events and functions throughout the CNS, such as signal transduction,[67] memory, recognition, emotion, and modulation of the neurotransmitters; dopamine,[68] acetylcholine,[69] serotonin,[70] and glutamate.[71]

Neurodegenerative diseases

The pathophysiology of neurodegenerative diseases is complex; however, there is a common factor that involves dysfunction at the mitochondrial, endoplasmic reticulum, and synapse axis.[72] Therefore, the location of σ1Rs at the MAM makes them attractive targets for studying neurodegenerative diseases and develop diagnostic biomarkers to monitor disease progression and develop potential therapeutics. Several studies have shown the involvement of σ1Rs in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's Disease (HD), and juvenile amyotrophic lateral sclerosis (ALS).[73, 74] Remarkably, the expression levels of σ1Rs were found to be altered in brain of patients who suffer from different neurodegenerative diseases.[75, 76]

Furthermore, the activation of σ1Rs attenuates reactive oxygen species (ROS) at the ER, suppresses oxidative stress, and are involved in cellular defense against neurodegenerative disorders.[73, 76-79] Of additional interest is the resultant increase in the expression of protective genes such as the antiapoptotic protein bcl-2, after activation of σ1Rs. Thus, σ1Rs may also contribute to neuroprotection.[57, 80, 81] Therefore, determination of the expression level of σ1Rs in the CNS could be useful in the diagnosis of AD, PD, and ALS.

Alzheimer's disease (AD). AD is a progressive brain disorder characterized by slow destruction of memory and thinking skills and considered as the most common cause of dementia. Both σRs are involved in many cellular pathways that affect brain plasticity, learning and memory processes, and AD progression. For recent reviews on the role of sigma receptors in AD, see these selected references.[78, 82-84] Additionally, postmortem and PET neuroimaging studies revealed that AD patients had experienced an idiopathically low density of σ1Rs in the hippocampus comparted to healthy individuals.[84-86] So, activation of σ1Rs were examined for the treatment of AD, whereas σ1Rs agonist were reported to attenuate memory deficit and showed neuroprotective and anti-amnesic properties. This is thought to be due to the σ1Rs modulatory role on Ca^{2+} mobilization, regulation of oxidative stress, antiapoptotic effect, regulation of glutamate release and increases in acetylcholine secretion.[85-89] The σ1R agonist, ANAVEX®2-73, is in a phase III clinical trial for AD, which activates σ1Rs and has demonstrated the ability to reduce crucial pathophysiological signs of AD such as beta amyloid, hyperphosphorylated tau, and increased inflammation. ANAVEX®2-73 has also shown dose dependent improvement in cognitive functions. Moreover, the σ2R allosteric antagonist Elayta (CT1812), is currently in phase I/II clinical trial for mild to moderate AD treatment (ClinicalTrials.gov identifier: NCT02907567). Elayta displaces the toxic beta amyloid oligomers and prevents their binding to neurons, which in turn prevent downstream synaptotoxicity and protect against memory loss.[90]

Both σ1R agonists and σ2R antagonists showed a neuroprotective effect, anti-amnesic activity, and improvement in patient's cognitive functions. Brain imaging of σRs could be used as potential diagnostic biomarkers to afford insights about AD pathophysiology and monitor therapeutic efficacy.

Amyotrophic Lateral Sclerosis (ALS). ALS is a neurodegenerative disease that is characterized by loss of motor neurons in the brain and spinal cord leading to paralysis and early death. Complex pathophysiological mechanisms have contributed to ALS such as; neuronal injury from excitotoxicity, mitochondrial dysfunction, increased reactive oxygen species, and endoplasmic reticulum stress responses (which initiate protein degradation). Also, motor neuron damage can lead to activation of microglia and astrocytes, which further contributes to neurodegeneration.[91] Since, σ1Rs are located at the MAM, regulate Ca^{2+} homeostasis, modulate neuronal

excitability, and are highly expressed on the motor neurons in the spinal cord, it is believed that σ1Rs could be involved with ALS progression and serve as a potential target for ALS pharmacotherapy. Moreover, several lines of evidence suggest that, σ1R alterations or mutation lead to motor neuron degeneration and progression of ALS. It is of potential importance that low level of σ1Rs were observed in ALS patients.[92-95] σ1R activation by agonists, such as PRE-084 , pridopidine, and SA4503, showed neuroprotection, and reduced microglial and astroglial reactivity in the transgenic superoxide dismutase 1 (SOD1) mouse model.[96, 97] It also prevented the loss of neuromuscular connections, motor axons, and motor neuron cell bodies in the spinal cord and increased animal survival.[96-99]

Parkinson's disease (PD). PD is a neurodegenerative disease, which affects motor function, characterized by the gradual loss of dopaminergic neurons in the substantia nigra. Several studies have suggested that σ1Rs are linked to PD because they are expressed in the substantia nigra and are known to modulate dopamine release via different mechanisms.[100, 101] Moreover, in two different human clinical trials, low σ1R density has been observed in early PD patients compared to healthy volunteers when the PET radioligand [^{11}C]SA4503 was utilized.[102, 103] In patients with PD, the binding potential of [^{11}C]SA4503 to σ1Rs in the anterior putamen (10.8 ±4.2) was lower compared to normal individuals (12.2 ±5.0). In addition, the binding potential of [^{11}C]SA4503 was significantly lower on the more affected (9.1 ± 4.4) than the less affected (12.4 ± 4.3) side of the anterior putamen of PD patients. While, σ1R knockout mice showed increases in α-synuclein aggregation and phosphorylation, a major constituent of Lewy bodies that are believed to play a critical role in the pathogenesis of PD. Also, loss of dopaminergic neurons in substantia nigra was observed in σ1Rs knockout mice.[101] Consequently, σ1R activation has been reported to restore synaptic connectivity and protect nigrostriatal dopamine neurons against degeneration.[104, 105] In a unilateral 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in mice, the σ1R agonist, pridopidine demonstrated a neuroprotective effect, and showed an increase in dopaminergic fiber density in the striatum, restorative plasticity, and upregulation of neurotrophic factor (BDNF). While, 6-OHDA-lesioned mice with σ1R knockout did not show the beneficial effects of pridopidine. [104] It was reported that daily administration of PRE-084, σ1R agonist, utilizing a murine model with induced nigrostriatal degeneration has shown significant motor recovery and an increase in striatal dopaminergic fiber density suggesting the therapeutic potential of σ1R agonists in PD.[105]

Huntington's Disease (HD). HD is a hereditary neurodegenerative disease associated with the production of mutant huntington protein (mHtt) and characterized by gradual, progressive loss of neurons in the brain, which leads to motor and cognitive impairments.[106] The loss in function of normal proteins and the production of mutant proteins results in the disruption of multiple intracellular pathways, apoptosis, mitochondrial dysfunction, oxidative stress, ER stress, and autophagy.[107] Since σ1Rs are activated in ER-stress, they may be implicated in the ER-related degradation of the mHtt. Several studies have shown that activation of σ1Rs provides a neuroprotective role in HD, for example; the σ1R agonist PRE084 demonstrated a neuroprotective effect by decreasing ROS levels, exerting antioxidant effects as well as increasing antiapoptotic effects by affecting NF-kB signaling.[108] Administration of the σ1R agonist, (+)-3-PPP resulted in a neuroprotective effect and increase in the density of the neuronal cultures in mice.[109] While pridopidine improved motor performance and survival in the R6/2 and Yac128 HD mouse models.[110] It was also suggest that the agonistic activity of pridopidine at σ1Rs resulted in modulation of ER stress, especially the PKR-like ER-localized eIF2a kinase (PERK) pathway.[111]

Neuropsychiatric disorders

The first pharmacological activity reported for σRs upon binding of the prototypic ligand, (±)-SKF10,047 (Ki σ1 = 44.8 nM, σ2/σ1 = 95.1), was psychotomimetic effects.[34] Later, σ1Rs were reported to be involved in neuronal plasticity. Neuronal plasticity is the ability of the nervous system to form new neuronal connections and compensate for injury. Basically, changes in the structure, function and organization of neurons, occur in response to new experiences and injuries. Disruption in neuronal plasticity and reduction in dendric spine density have been reported to be implicated in the pathophysiology of neuropsychiatric disorders such as depression, and schizophrenia.[112] These findings suggested the contribution of σRs to neuropsychiatric disorders. Moreover, σ1Rs provide a defense mechanism against oxidative and ER stress that might be triggered under psychological stress or neuropathological conditions.[113] Thus, the antipsychotic potential of σ1R ligands has been explored with great interest.[114-116]

Depression. σ1Rs are involved in the pathophysiology of depression because of depressive-like behaviors that develop in σ1R knock-out mice.[117] Additionally, σ1R agonists have been reported to have antidepressant activity.[118, 119] It is suggested that σ1Rs antidepressant activity is due to modulation of serotonin, noradrenaline, and glutamate neurotransmission.[70, 71, 120, 121] Remarkably, clinically used antidepressants (e.g.: imipramine) bind with high to moderate affinity to σ1Rs. It was suggested that antidepressants enhance the brain-derived neurotrophic factor (BDNF) signaling, which induce glutamate release through activation of PLC-γ/IP3/Ca^{2+} pathway due to their binding to σ1Rs.[71]

Schizophrenia. σ1Rs are also involved in the pathophysiology of schizophrenia as a result of their modulation of dopaminergic neurotransmitters. The symptoms of schizophrenia include positive and negative symptoms, cognitive impairment, and social isolation. E-5842, σ1Rs ligand, was reported to increase dopamine release in striatum and its neurochemical profile is similar to atypical antipsychotics.[122-124] It was reported that σ1R agonists (e.g.: pregnenolone and dehydroepiandrosterone) were effective against negative and cognitive symptoms of schizophrenia and demonstrated antipsychotic activity without producing extrapyramidal side effects.[116, 125-129] The

antidepressant drug, fluvoxamine is a selective serotonin reuptake inhibitor (SSRI) as well as a σ1R agonist with high affinity (Ki = 36 nM). Fluvoxamine at therapeutic doses binds to σ1Rs, which was confirmed using PET imaging studies in human brain.[130] Its efficacy in treating cognitive impairments and negative symptoms in some schizophrenic patients is suggested to be through σ1Rs activation.[131-133] Furthermore, σ1R density was reported to be low in the postmortem brain, predominantly in temporal cerebral cortex, of schizophrenic patients compared to age-matched, normal postmortem controls.[134, 135] Previously, five σ1R antagonists [panamesine (EMD57445), eliprodil (SL82.0715), rimcazole (BW234U), BMY14802 (BMS181100), and DuP734] were progressed to clinical trials for the treatment of positive symptoms of schizophrenia. However, they were not effective against positive symptoms. Interestingly, eliprodil and rimcazole were effective against negative symptoms of schizophrenia.[77, 116, 136]

Pain and analgesia

Under normal physiological (non-sensitizing) conditions, σ1Rs do not modify normal sensory mechanical or thermal perception. However, they are activated and effective under pathological or sensitizing conditions such as nerve injury, chronic pain, inflammation, allodynia, hyperalgesia, and they are associated with neurophysiopathological changes.[56] σ1Rs are considered an endogenous anti-opioid system. Since, σ1R agonists reduce opioid analgesia, while σ1R antagonists potentiate opioid analgesia and restore normal nociceptive thresholds. It is well established that σ1Rs have a significant role in pain modulation and have been associated with nerve injury and neuroinflammation.[137, 138] Moreover, σ1R antagonists demonstrated antiallodynic effects in neuropathic and neurogenic pain.[38, 49, 138-140] σ1R antagonists are considered as potential biomarkers to locate nerve injury and neuroinflammation.[64] CM304, a σ1R antagonist, has shown promising anti-allodynic activity in different animal models of neuropathic pain and nociceptive pain.[141] Also, σ1Rs are known to be upregulated at the site of partial sciatic nerve ligation.[64, 142] Interestingly, two σ1R antagonists are now in clinical trials; E5286 (phase II) for pain management /neuropathic pain, and [^{18}F]FTC-146 (phase I) as a diagnostic agent to pinpoint nerve damage in sciatica and complex regional pain syndrome (CRPS). These findings together with the large body of literature about the role of σ1Rs in pain modulation suggest that σ1Rs are promising class of pharmacotherapeutics for pain in the future. The proposed mechanism of antinociception of σ1R antagonists involves inhibition of glutamate release, regulation of the activity of different targets involved in pain pathways such as ion channels (Na^+, K^+, Ca^+), and G-protein coupled receptors (cannabinoid CB_1 receptors, serotonin 5-HT_{1A} and 5-HT_{2A} receptors, glutamate NMDA and mu opioid receptor), and activation of descending inhibitory systems.[56]

Addiction

σ1R activation is associated with the addictive, neurotoxic, and reinforcing effects of many drug of abuse (cocaine, methamphetamine, and alcohol). Preclinical studies in male rodents, suggested that σ1R antagonists inhibit behaviors related to alcohol use disorders (AUDs); reduce alcohol consumption, and alcohol-seeking behavior.[143] So, σ1Rs might be a promising target for treating AUDs and superior to the current Food and Drug Administration (FDA)-approved drugs for AUDs that have limited efficacy such as disulfiram, naltrexone, and acamprosate.[143] Further human studies are needed to confirm the efficacy in human.[143] Additionally, σ1Rs upregulation was found after chronic self-administration of methamphetamine to rats. σ1Rs are a promising therapeutic target as well for the treatment of methamphetamine addiction.[124, 144] Moreover, σ1Rs antagonism involved in drug abuse treatment and attenuation of psychostimulant-induced effect such as blocking cocaine induced seizures, hyper-locomotion, sensitization, and change the gene and protein expression that was upregulated by cocaine administration.[145, 146]

Role of σ1Rs in cancer

σ1Rs are highly expressed in different types of cancer, such as brain, chronic myeloid leukemia, breast, prostate, and colorectal cancer cell lines. Their upregulation in several cancers, has attracted much research focused around tumor imaging. Hence, imaging of σ1Rs with radioligands might contribute to a better understanding of the tumor physiology, the pathophysiological function of σ1Rs, and aid in the development of novel antineoplastic drugs. For a comprehensive review about σ1R radioligands developed for cancer imaging readers are referred to the following reference.[147] Besides, σ1R antagonists show a strong ability to inhibit cancer cell proliferation *in vitro* and *in vivo*.[148-151] For example, the σ1R antagonist, 1-(4-Iodophenyl)-3-(2-adamantyl) guanidine (IPAG), resulted in an unfolded protein response (UPR) followed by autophagy and apoptosis in cancer cells.[151] Additionally, σ1R ligands were reported to regulate cancer cell electrical plasticity.[149, 152] Although the exact mechanism is still inconclusive, σ1Rs are reported to inhibit cancer cell proliferation via upregulation of anti-apoptotic pathways, involvement in protein homeostasis, a pathway known to be involved in cell death and cancers, and regulate membrane electrical activities. It is noteworthy that, both σRs are overexpressed in different tumors,[153] but σ2Rs show higher expressions and overwhelming evidence to be useful biomarkers for tumor proliferation. σ2R ligands have been demonstrated to be useful tools in imaging solid tumors, and as potential therapeutics for cancer treatment.[154-156]

Role of σ1Rs in cardiac dysfunction

Although both σRs are found to be expressed in the heart by ligand binding studies in early 1990s,[157] the physiological function of cardiac σRs remains unknown and limited studies have been done to explore their role in the heart. Recently, Chowdhury *et.al*. reported that σ1Rs regulate normal

mitochondrial organization and size in the heart of mice. Additionally, σ1Rs knockout mice demonstrated cardiac dysfunction associated with accumulations of irregularly shaped mitochondria and defects in its respiratory function.[158] These finding suggested that σ1Rs exert a cytoprotective effect, regulate cardiac hemodynamics and are needed to maintain normal cardiac contractility. More clinical research is required to define the physiological function of σ1Rs in the heart and evaluate the potential therapeutic role of σ1Rs in cardiovascular disease.

Shedding light on σ1Rs and molecular imaging

σ1Rs are now recognized as potential therapeutic targets that have a putative role in many diseases. It has been reported that σ1Rs can be involved in reducing the symptoms of some neurodegenerative disorders, but can lead to the establishment of other diseases.[73, 159] Consequently, σ1Rs have been considered as an attractive target and have gained more attention in drug discovery field for their potential therapeutic value. These observations confirmed the importance of studying σ1Rs in many neurodegenerative diseases, CNS disorders, tumor progression, pain, addiction, and cardiac dysfunction to assess their possibility as a promising target for therapeutic development.[77, 160, 161]

Over the past decades, σ1Rs have been studied using different imaging techniques. These techniques made and continue to have a significant impact in the recognition of σ1Rs as "a Pluripotent Modulator in Living Systems".[21] Atomic force microscopy imaging and confocal imaging techniques have been used to identify the interaction between σ1Rs and other proteins (receptors and ion channels), which is not the scope of this review. This in turn helped with understanding the pathophysiology and the functional crosstalk between σ1Rs and other proteins.[21] Moreover, developing radioligands for imaging σ1Rs *in vitro* and *In vivo* helped to confirm that the σ1R is a unique protein and does not belong to opioid or NMDA receptor families.[162] It also provided insights about the available σRs subtypes, their anatomical distribution,[9] and identified high affinity σ1Rs ligands.

In addition, σ1R radioligands had a critical role in understanding the receptor pharmacology and its contributions in many diseases. Bibliometric analysis of the scientific publications focused on σ1Rs in the last 25 years indicated that research efforts were previously focused more on neuroimaging, addiction, and psychiatric disorders; however, neurodegenerative diseases, neuroprotection, and pain are currently attracting the most attention.[1] Interestingly, the top two keywords were "Positron Emission Tomography (PET)," and "Neuroprotection," respectively.[1] This indicates the importance of imaging in the discovery of σ1Rs roles in preclinical and clinical studies in neurodegenerative and neuropsychiatric diseases. The following sections discuss the role of imaging in drug discovery, frequently used techniques and radioligands, and the application of σ1Rs radioligands in molecular imaging.

Imaging in drug discovery

Nuclear medicine functional imaging (molecular imaging) is a type of medical imaging that noninvasively creates a visual representation of the internal aspects of the body and determines the biological/molecular processes in normal and diseased states to identify abnormalities. The principles of *in vivo* molecular imaging depend on detecting the energetic particles (radiation) emitted from a radioactive material (radioisotope) upon decaying by gamma scintigraphy, single photon emission computed tomography (SPECT), or positron emission tomography (PET). Imaging data obtained are processed by computers to produce 2D and 3D images. These images can be used for diagnosis and detection of functional processes in living systems quantitatively.

Functional imaging nowadays has a great impact on drug discovery and development and valuable contributions in pharmaceutical industry. It is considered as a major tool in preclinical development, translational research, clinical diagnosis, clinical trials, and life sciences. It facilitates the visualization of the biological activities in animals, without the need to use invasive techniques, which require sacrifice of the animals. For a review on the advantages and limitations of *ex vivo* autoradiography versus molecular imaging the reader is directed to this reference.[163] Molecular Imaging has been utilized by different fields such as oncology, cardiology and mostly neuroscience due to the inaccessibility of human brain.

The advancement in nuclear medicine technology and the use of powerful non-invasive instrumentation allow for the use of radioligands as diagnostic biomarkers. Radioligands have become important tools in improving the drug discovery process through the quantitative assessment of radioligand distribution, determination of target expression levels in different tissues, characterization and validation of many targets, and confirmation of target engagement in many pathological conditions in living system.[164] The assessment of target distribution and expression levels became easier, which in turn helped with the design of safer and more efficacious treatments. Moreover, the use of these imaging techniques in animals and humans has helped to delineate normal physiological and pathological conditions resulting in improvements in understanding disease pathophysiology, monitoring disease progression, and earlier diagnosis as well as follow-up treatments.

Radioligands used in clinical diagnosis

Classification of radioligands. Radioligands used for PET or SPECT can be classified depending on how the radiolabel is introduced into the imaging agent.[165] In the first class, the imaging agent is the radionuclide itself (e.g.: [^{18}F]sodium fluoride is a PET imaging agent for osteosarcoma). The second class have the radiolabels attached (atomic substitute) to or pendant from the target molecule (e.g.: [^{18}F]fluorodeoxyglucose (^{18}F-FDG) is a marker for tissue glucose uptake and monitoring tumor metabolism). While in class three, the radionuclides are incorporated within a molecule as an isotopic modification. The selected molecule usually is a ligand

that binds specifically to the target of interest, for detailed information the reader is encouraged to read this reference.[165]

Criteria of ideal radioligand. The ideal imaging agent should demonstrate high affinity (at nanomolar or picomolar range), with a high selectivity profile over other targets, high *in vivo* stability, and high uptake at the target tissue. Also, high specific binding with minimal nonspecific binding is important to ensure more detailed results and avoiding incorrect interpretation of the imaging data. Mintun *et.al*. reported a mathematical model that can provide a quantitative characterization of drug binding sites for *in vivo* PET imaging.[166] This can be achieved by calculating the binding potential (BP), which is equivalent to the product of the maximum drug specific binding concentration (B_{max}) and the reciprocal of the radioligand binding affinity (K_D), BP = $B_{max}K_D^{-1}$. Thus, BP reflects the potential of a given tissue for ligand-binding site interaction and provide accurate characterization of drug-receptor kinetics in living subjects. A suitable tissue kinetic profile is desirable as well; good radioligands should demonstrate fast and reversible binding kinetics, a considerable washout period and adequate clearance because slow pharmacokinetics will limit the clinical utility of the radioligand. Moreover, the radiation risk should be within an acceptable range with low potential toxicity and relatively low total radiation dose to the patient per unit of initial activity after administration.[167] In case of brain imaging, high brain to blood ratios and good blood-brain barrier (BBB) permeabilities are essential. Moreover, for radioligands with short half-lives, a rapid uptake into the brain is essential to ensure a pseudo-equilibrium has been reached before the decay of the radionuclide. In addition to the aforementioned, radiometabolites generated via peripheral metabolism should not be able to cross the BBB.

Challenges of imaging agents in human. One of the challenges of imaging agents is their stability in human body, for example a [^{18}F] PET radioligand can be metabolized by defluorination and result in non-specific accumulation of [^{18}F]fluoride radioactivity in bone, which will affect the quality of imaging, the quantitation of PET signals and the utility of the radioligand. So, with fluorine radioligands little to no accumulation in the bone is desirable during the scan time to ensure reliable results and quantification of the PET signals.[168] Also, the same concept is applied to [^{123}I] as it can undergo deiodination.[169] Moreover, the presence of radiometabolites would limit the usefulness of radioligand and affect the kinetic analysis. Especially, if the metabolite is active and binds to the target with different affinity, this will complicate the quantification of the signals. While if the radiometabolite is inactive, this may increase nonspecific binding that will affect the signal to noise ratio.[163] Therefore, radioligands are preferred to have good *in vivo* metabolic stability.

Another challenge is the incorporation of the radioisotope into a molecule may change its chemical and physical properties that may affect its binding affinity, pharmacokinetic properties or biological activity.[163, 169] One of the factors to be considered when choosing the type of the radiolabelled nuclei is the radiosynthesis step, the time of the introduction of the radiolabelled atom. Among the challenges face the [^{18}F]fluoride PET tracers. For example, If the radiosynthesis of the [^{18}F]fluoride is the last step to get the final PET tracer, the radiochemical yield will be high. But if more steps are required after the introduction of the radiolabelled [^{18}F], reduction of the radiochemical yield will occur due to the longer production time and their relatively short half-life (109.8 min).[170]

Non-invasive imaging techniques (PET and SPECT) in drug discovery

The use of SPECT and PET imaging in drug discovery is common.[164, 171]. Different factors should be considered before the choice of the imaging technique such as the resolution, sensitivity, cost, availability of scanners and equipment, the availability of radiolabelled tracer and the ease of synthesis, and the clinical use (e.g.: repeated dosing).[165]

Single-photon emission computed tomography (SPECT) imaging

Principle: A nuclear medicine 3D tomographic imaging technique that directly detects the gamma rays emitted from a radioactive isotope upon decaying, using gamma cameras that surround the body. The cameras acquire many 2-D images from multiple angles, then a tomographic reconstruction algorithm is applied to generate a 2D or 3D data set. The total time of a scan is around 15-20 minutes.

Generally, the patient is injected with a diagnostic radiolabelled probe that has affinity for a specific target, where it will accumulate. When the radioisotope decays, gamma radiation is emitted, and captured. The resultant computationally generated images show the distribution of the radiolabelled probe within the patients' body that can be interpreted and used for diagnosis. The isotopes suitable for SPECT are Thallium ^{201}TI, Technetium ^{99m}TC, Gallium ^{67}Ga, Iodine ^{123}I, Iodine ^{125}I, and Iodine ^{131}I. The half-lives for their gamma emission are 73 h,6 h, 78.26 h, 13.2 h, 59.49 days, and 8 days, respectively. The most widely used SPECT radiolabels for biomolecules labelling are the radiometal ^{99m}Tc and radioiodine ^{123}I. [^{99m}Tc] Tc- isotope has the advantages of moderate half-life (6 h), which is suitable for clinical use. Besides, their convenient production and the availability of in-house generator (Molbedynum99). While, ^{123}I has been used clinically as a radionuclide for SPECT because its longer half-life (13.2 h), easy synthesis, and its gamma emissions are ideal for sodium-iodide-based SPECT detectors.[165] The radioisotope ^{131}I is used for therapeutic applications. While, the radioisotope ^{125}I has been used in nuclear medicine imaging mainly for *in vitro* or *ex vivo* assays due to its radioactive emission of a total of 21 low-energy (~2O-500 eV) Auger electrons compared to 11 Auger electrons emitted from ^{123}I. These Auger electrons have been found to do little cellular damage and their radiotoxicity depends strongly on their distribution within the cell. Thus, in order to reduce the exposure risk of Auger electrons, the subcellular distribution should be considered. For example, the diagnostic use of these radiopharmaceuticals should localize the Auger electrons in the cytoplasm of cells. While, therapeutic use in cancer should

direct the radiochemical to the tumor cell nucleus.[172] The radioactive emission has limited the utility of ^{125}I as an *in vivo* diagnostic agent. However, it has been reported to be used for *in vivo* SPECT, or SPECT/CT studies mainly for tumor imaging of small animals.[173-177] The long half-life of ^{125}I isotope has enabled *ex vivo* biodistribution studies to verify the *in vivo* data. Moreover, ^{125}I is the radionuclide of choice for radioimmunoassays. SPECT imaging is most common in clinical imaging because of its advantages over PET that make it widely available. Some of its advantages are the radioisotopes are more easily obtained, less expensive and have long half-lives that allow for the observation of biological processes up to several hours after the administration of the radioisotope.[164] The gamma scanning equipment is less expensive and no need to use a cyclotron for preparation of the radioisotopes on site, which add to the reduced cost.

Positron emission tomography (PET) imaging

Principle: A radioactive nuclide (PET tracers) emits positron and neutrino upon conversion of a proton to a neutron using a cyclotron. When the positron collides with an electron (antiparticle), an annihilation process occurs; two gamma photons are generated in opposite directions. The resulting signals are recorded when a PET scanner detect these emissions concurrently. Thus, the origin of the irradiation can be identified. In this case, PET scanners detect gamma rays emitted indirectly from a positron-emitting radioligand.[170, 178]

PET scans produce higher spatial resolution images and exhibit higher sensitivity compared to SPECT and other imaging methods such as computed tomography (CT) or standard magnetic resonance imaging (MRI). The improved resolution and sensitivity permit better detection of detailed brain areas and early dementia where there is no clinical signs or little structural changes have occurred that are hard to detect to identify pathological conditions by CT or MRI. Together with the quantitative nature of PET scans, PET imaging is a useful tool in diagnosis of brain diseases, neuroimaging, cancer biology, and neurodegenerative diseases.[171, 179] The main drawback of PET scanning is the short half-lives of the radionuclides. The PET radioligands decay rapidly, so they have to be synthesized prior to imaging studies. This requires the synthesis and the use of the tracers to be within the half-lives of the radiolabelled molecule. Consequently, a limited time is allowed for clinical use and detection in the body is dedicated for short tasks. This is in addition to considering the availability of onsite cyclotron to prepare the radioligands and the high cost.

The most commonly used non-metallic positron-emitting radionuclides are ^{11}C, ^{13}N, ^{15}O, ^{18}F and less commonly ^{76}Br, and ^{124}I, while their half-lives are 20.4 min, 9.96 min, 2.03 min, 109.8 min, 16.1 h, and 4.18 days, respectively. Early PET tracers utilized ^{11}C isotopes due to their synthetic feasibility. However, if a potent ligand containing a fluorine atom is available, ^{18}F isotope is a superior PET tracer due to the longest decay half-life that enables enough time for radiosynthesis and detection. Because of this, ^{18}F ligands do not require a cyclotron close to the bedside and they can be synthesized offsite and shipped to imaging clinics.[165] Also, the lower positron energy (0.64 MeV) of ^{18}F isotope compared to ^{11}C isotope (0.96 MeV) results in the production of images with higher resolution.[180]

Dual modalities

Hybrid biomedical imaging modalities combine CT or MRI with SPECT or PET such as SPECT/CT or PET/CT and more recently, PET/MRI scanners. They allow the correlation of the functional imaging information to the anatomic information, which resulted in tremendous advancements in the imaging field that produce more precise 3D localization of the tissues that expressed high radioactivity. These multimodality diagnostic imaging techniques have become important tools in clinical diagnosis, treatment planning, and therapy monitoring. Remarkably, PET/MRI has a great advantage of combining the high sensitivity and molecular imaging properties of PET with the ability of MRI to penetrate the tissues and provide superior soft tissue contrast, and detect anatomical details with high spatial resolution and low noise.[181, 182] The complementary role of PET/MRI has opened new opportunities in non-invasive imaging to visualize both biochemical and anatomical changes and provide more accurate measurements of radioligand uptake.[64, 183] Clinical use of PET/MRI and [^{18}F]FTC146 PET tracer was reported for imaging peripheral nerve injury and the origin of chronic pain in human successfully, which was not accessible using only CT or MRI.[64, 65]

Radiotheranostics

Radiotheranostics is a term used in nuclear medicine that describes the use of radiolabelled probes that have both diagnostic imaging and targeted therapeutic components. Currently, this is a highly active area of research mainly, in the field of oncology. Radiotheranostics are contributing to the concept of personalized precision medicine, and represent a tool for improving patient outcomes, enhancement of therapy efficacy, and predicting adverse effects.[184-186]

σ1Rs are highly expressed in different types of tumors and several peer-reviewed studies show the therapeutic and diagnostic potential of σ1R ligands in cancer.[154, 187-189] Therefore, σ1R targeted radionuclide therapies are considered to be radiotheranostics.[186] The imaging, or diagnostic component, identifies the extent of sigma receptor expression in the tumor. This information is used as a diagnostic biomarker that can determine the efficacy of the σ1R probe as a therapy and measure tumor shrinking.[186] Ogawa, K. introduced some σ1R radiolabelled probes as "a companion diagnostic test of therapeutic agents,"[184] and reported the use of a radiolabelled σR ligand for receptor radionuclide therapy for the first time.[190] The iodinated vesamicol derivative (+)-2-[4-(4-iodophenyl) piperidino] cyclohexanol [(+)-pIV, is a σ1R ligand that showed high affinity (Ki = 1.30 nM) at σ1Rs over VAChT (Ki = 1260 nM).[191] The analogous radioiodine labelled derivative (+)-[^{125}I]pIV showed high accumulation in DU-145 tumor-bearing mice, where DU-145 is a human prostate cancer cell line overexpressing the σ1Rs. Accordingly, Ogawa, K et.al. supposed that the use of the therapeutic radioiodine ^{131}I, that emits beta

particles, instead of ^{125}I to label the sigma ligand (+)-pIV would create a radiotheranostic agent.[190] (+)-[^{131}I]pIV was prepared and showed a significant tumor growth inhibition in DU-145-bearing cancer mice compared to control group upon single administration, Fig 2.[190] The finding suggested that (+)-[^{131}I]pIV could be a potential radionuclide therapy and further studies are required to reduce the nonspecific radioactivity reported at liver and kidney due to the high lipophilicity.

Moreover, further studies for development of radiohalogen labelled σ1R ligands were reported. Different (+)-pIV analogs having the α-particle emitting radionuclide halogen, astatine-211 (^{211}At), were also reported as a radionuclide therapy, that gained much consideration as a candidate for clinical use in the future. However, its properties have not yet been fully characterized.[192]

In addition, σ1R radiobrominated analogs of pIV were synthesised. (+)-pBrV (Ki σ1 = 2.4 nM) exhibited high tumor uptake in mice. However, the radioactivity was retained in the liver and kidney after blocking studies expected due to its high lipophilicity. Modified analogs with an extra hydroxyl group were developed that exhibited lower lipophilicity. (+)-4-[1-(2-hydroxycyclohexyl)piperidine-4-yl]-2-bromophenol, (+)-BrV-OH,(Ki σ1 = 60.3 nM) was selected for distribution and blocking studies. Initially, the ^{77}Br isotope was developed because of its long half-life of 57.0 h, which is an Auger electron emitter and can be used for radiotherapy. It displayed lower lipophilicity than the parent compound and high tumor uptake at early time points but faster clearance even from the brain and the tumor which may be due to its lower affinity. Moreover, the PET tracer, (+)-[^{76}Br]BrV-OH, showed high uptake in tumor via σ1Rs. This PET tracer might be a promising imaging agent, but its affinity is not sufficient and further modification is warranted to increase the affinity without increasing the lipophilicity to improve its biodistribution.[193]

Recently, a series of aza-vesamicol derivatives, with varying alkyl chain lengths between a piperazine ring and a benzene ring was developed to improve the radioiodine labeled probes for σ1R imaging. The binding affinity at σ1Rs increased depending on the length of the alkyl chain and the highest affinity derivative 2-(4-(3-phenylpropyl)piperazin-1-yl)cyclohexan-1-ol (Ki=5.8 nM) is compound 1, Fig 2. Its radioiodine labeled probe [^{125}I] showed high accumulation in σ1R expressing DU-145 cells both *in vitro* and *in vivo*, which was confirmed by blocking studies using haloperidol. Compared to the parent compound, [^{125}I]1 showed better biodistribution as a σ1R imaging probe 24 h post-injection.[184]

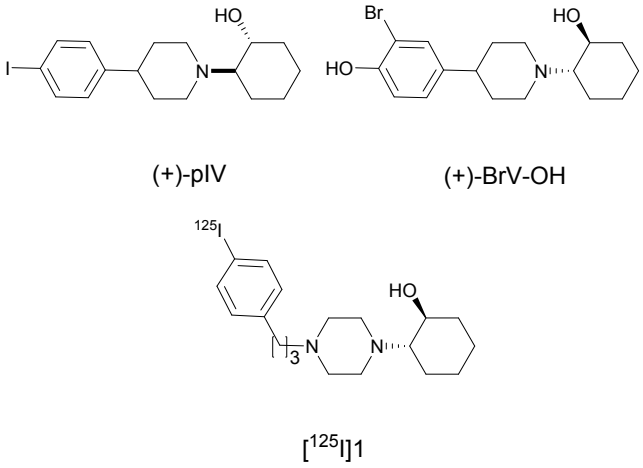


Fig 2: Structure of (+)-pIV. And (+)-BrV-OH, modified aza-vesamicol derivative 1.

A radiotheranostic could be also applied in pain management and [^{18}F]FTC-146 could be considered as a potential agent. The diagnostic agent [^{18}F]FTC-146 was able to accumulate at injured sciatic nerves created in a rat model and accurately detected the peripheral nerve injury and neuroinflammatory areas, which correlated to pain sensitivity, using PET/MR imaging and ex vivo autoradiography. Also, this study indicated that σ1Rs are upregulated in areas of nerve damage at the site of partial sciatic nerve ligation in the spared nerve injury (SNI) rat model.[64] In a human clinical trial using [^{18}F]FTC-146, a successful treatment course was realized after the source of chronic knee pain was localized. This led to the surgical removal of an intraarticular synovial lipoma that showed high [^{18}F]FTC-146 uptake using PET/MRI, which resulted in complete reversal of the chronic knee pain.[183] Interestingly, the analogous cold ligand, CM304, showed antiallodynic activity in mouse neuropathic pain models; chronic constriction injury assay, and cisplatin-Induced neuropathy assay.[141] Moreover, CM304 displayed antinociceptive activity in induced chemical and inflammatory pain.[141] In addition, ultrasound-guided direct injection of CM304 in the neuroma of the SNI rat resulted in reduction of the mechanical allodynia in animals experienced neuropathic pain.[64] Thus, [^{18}F]FTC-146 could be considered as a radiotheranostic agent that has the potential to precisely identify location of σ1Rs and its expression level to diagnose peripheral nerve injury, and enable image-guided treatment and at the same time provide pain relief. However, the short plasma half-life of CM304 ($t_{1/2}$ = 2.3h) in Sprague Dawley rats has hindered development as a therapeutic/analgesic.[194]

Applications of σ1R radioligands in molecular imaging

The development of σ1R radioligands have been under investigation for a number of years. These radioligands helped with understanding σ1Rs pathophysiology and linking the apparent pharmacological events to σ1R binding. Therefore, radioligand imaging probes provide a powerful tool in studying the complex role of σ1Rs in physiological and pathological

conditions, quantifying the down- or upregulation, and monitoring disease progression and therapeutic outcomes. In theory, imaging studies could also allow for improved diagnosis and the development of new therapeutic approaches.

σR Radioligands tools for preclinical imaging studies.

In vitro radioligand binding studies are important for probing new receptors and confirming their existence in certain tissues as well as identifying high affinity and selective ligands that can be selected for further evaluation. These assays continue to play a central role in drug discovery, and preclinical studies.

However, most of the earlier studies used to visualize both σRs were carried out with nonselective compounds that did not completely discriminate between both subtypes. In addition, some previous compounds that are reported to bind σRs were not highly selective over other drug targets or proteins. To add further ambiguity, as reported by Leitner ML *et al*., usually both sigma receptor subtypes are co-localized, but exist in different ratios.[195]

Some of the pharmacological tools used as σ1R agonists are PRE-084, (+)-pentazocine, DTG, and (+)-SKF-10,047, which could induce some action or change in receptor function or location. Antagonists that have been studied such as; BD-1047, BD-1063, and NE-100 maybe more suited to understand localization of receptors. These agonists and antagonists were the most used blocking agents in radiolabelled binding studies. Some of them ([^{3}H] NE-100, [^{3}H] pentazocine, [^{3}H] DTG, [^{3}H] SKF-10,047) were used as the radioligand in binding assays. Even with their shortcomings, these hallmark ligands played a critical role in assessing the involvement of σ1Rs in different pharmacological activities.

[^{3}H](+)-pentazocine, a benzomorphan derivative, is the prototype σ1R agonist, (Ki σ1= 3.1 nM; Ki σ2= 1542 nM; σ1/σ2= 500), and is used as the gold standard radioligand in binding assays. It was developed into an enantiomerically pure radioligand by De Costa *et.al*., Fig 3.[196] However, pentazocine has significant limitations; it is difficult to synthesize, degrades over time, resulting in increased background levels. Several ligands were synthesized to label σ1Rs and develop better and selective radioprobes and proposed as a replacement of [^{3}H](+)-pentazocine but few candidates displayed a real selectivity at σ1Rs over other targets and none have been widely accepted as a replacement. Two of the best candidates, [^{3}H]-BHDP and [^{3}H]-SN56, have not seemed to gain traction as replacements, although they are much more selective. Table 1 summarizes the radioligand affinity (K_d) and the density of available receptors (B_{max}) of the proposed replacement compared to pentazocine.

[^{3}H]-BHDP is a potent and selective σ1Rs ligand that displayed high affinity in rat liver mitochondria and rat brain membranes with similar Kd values (Kd = 2-3 nM), Fig 3. It demonstrated 100 fold selectivity over σ2 and low affinity (μM range) for most of the 32 receptors examined.[197] The receptor profile of [^{3}H]-BHDP suggests that it could be a potent and selective σ1R ligand in binding experiments.

It is noteworthy that, SN56 is reported as a highly selective σ1R ligand (Ki σ1= 0.56 nM; Ki σ2= nM; σ1/ σ2 > 1000) and demonstrated a high selectivity profile over 16 targets, Fig 3.[198] its tritiated derivative [^{3}H]-SN56 was examined for its application as a tritium radioligand in competition binding assays. [^{3}H]-SN56 displayed several advantages over pentazocine; high affinity (70-fold higher than pentazocine) and selectivity for σ1Rs with specific, saturable, and reversible binding to the σ1Rs, facile synthesis in high yields, and chemical stability. These results suggested [^{3}H]-SN56 to be a favorable alternative for [^{3}H](+)-pentazocine in radioligand binding assays to study σ1Rs.[199]

Table 1: Equilibrium dissociation constant (Kd) and maximal density of binding sites (Bmax) values of the most selective σ1Rs radioligands in rat brain.

Compound	K_d	B_{max}
[^{3}H]-(+)-Pentazocine [a]	4.8 ± 0.4 nM	1,419 ± 11 fmol/mg
[^{3}H]-BHDP [b]	2.08 ± 0.28 nM	0.42 ± 0.11 pmol/mg)
[^{3}H]-SN56 [c]	0.069 ± 0.0074 nM	340 ± 10 fmol/mg

a: data from refence [200], b: data from reference[197], c: data from reference[199]

[^{3}H]DTG, a tritiated radiolabelled analog of DTG, is a non-selective sigma receptor agonist that has high affinity for both σRs, Fig 3. However, it is still used for *in vitro* binding assays to determine the binding affinities of new compounds at σ2Rs in the presence of (+)-pentazocine (to block binding to σ1Rs sites). This is because no selective σ2Rs ligand have been accepted and used in binding assays up to this time.[6, 201] Therefore, further investigations to develop selective σ2Rs probes are warranted to explore the pharmacological/physiological role in different diseases.

Some of the compounds used previously for studying σRs have affinity to other therapeutic targets such as, haloperidol. Haloperidol is a dopamine D2 antagonist and marketed as antipsychotic drug. It has high affinity at both σRs (K_i σ1 = 3.0 nM, K_i σ2 = 54.0 nM) and demonstrates a nonselective σRs antagonist activity, Fig 3.[202] Haloperidol is the most frequently used σRs antagonist for *in vitro* and *in vivo* biodistribution blocking studies as a blocking agent to confirm the uptake, distribution, blood brain barrier penetration, specific binding and selective labelling of σRs by the tested radioligands.

REVIEW

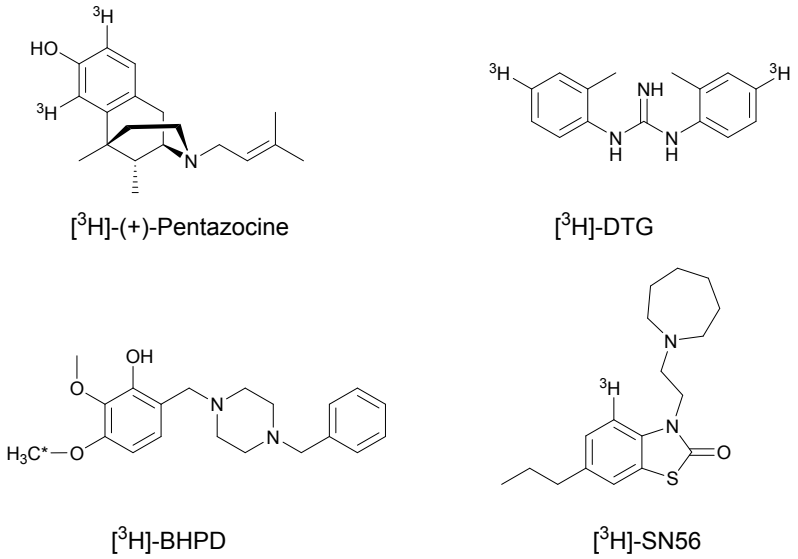


Fig 3: Radiolabelled σRs selective ligand for preclinical studies. *: indicate the radiolabelled proton site

σ1R Radioligands investigated in human clinical trials.

Selective σ1Rs radiolabelled compounds have been developed for studying *in vitro* and *in vivo* biological activities to elucidate their role in different diseases. Accordingly, the development of imaging probes for σ1Rs in human body, especially the brain, has become of great interest to many research groups. Despite many PET and SPECT radioligands being developed, few compounds have been evaluated in human to visualize σ1Rs and investigate their density in human brain; [^{11}C]SA4503, [^{18}F]FPS, [^{11}C]nemonapride, [^{123}I]TPCNE, (S)-[^{18}F]fluspidine, and [^{18}F]FTC-146, Fig 4. Interestingly, [^{18}F]haloperidol PET tracer has been used to study brain uptake and distribution in healthy volunteers and schizophrenic patients, but it could not be used for selective labelling of σRs due to its high affinity at D2 receptor and low σRs selectivity profile.[203]

[^{11}C]SA4503. In 2000, the first selective σ1Rs PET radioligand, [^{11}C]SA4503, was developed by Kawamura *et.al*.[204] and evaluated in human brain in 2001.[205] SA4503 showed high σ1R affinity (Ki σ1= 4.4 nM, Ki σ2 = 242), and moderate affinity for the vesicular acetylcholine transporters (VAChT, Ki=50.2 nM), emopamil binding protein (EBP), and low affinity over other 29 targets.[206-208] However, [^{11}C]SA4503 did not bind to VAChT in rat brain.[207] Preclinical evaluation of [^{11}C]SA4503 using PET studies suggested that it is a potential radioligand for mapping σ1Rs in human brain.[209-211] PET imaging studies in Alzheimer's and Parkinson's patients showed successful visualization of σ1Rs where reduced σ1R density in their brains were reported.[86, 102] Tumor uptake studies have been conducted using [^{11}C]SA4503 that support the role of σ1Rs in cancer.[212-214]

Currently, SA4503 (Cutamesine), in clinical trials for the treatment of many σ1R involved diseases. However, there are some limitations of [^{11}C]SA4503 such as the short half-life of ^{11}C isotope, which limits its use as a diagnostic agent, the requirement of onsite cyclotron, and the relatively slow kinetics due to its high affinity and low rate of dissociation.[215]

[^{18}F]FPS and derivatives. The second PET radioligand evaluated in healthy human volunteers for brain imaging was [^{18}F]FPS (Ki σ1 = 4.3 nM). However, it displayed high affinity at σ1Rs (*K* d = 0.5 nM), which resulted in slow clearance with no significant washout from the brain and did not reach transient equilibrium by 4 h after administration.[216] [^{18}F]FPS is not suitable candidate for neuroimaging, so different analogs were developed to improve its pharmacokinetic parameters and synthesize tracers with lower affinity such as [^{18}F]SFE, the fluoroethyl derivative of [^{18}F]FPS. It exhibited lower affinity for σ1Rs (Kd = 5 nM) and faster clearance,[217, 218] but no human clinical data has been reported for this compound. Recently, the synthesis of fluorinated ligands related to [^{18}F]FPS has been reported. The authors claimed these compounds might have potential as σR ligands (binding data not available).[219]

[^{11}C]Nemonapride. [^{11}C]Nemonapride binds with high affinity to dopamine D2 receptor in the striatum and sigma receptor in cerebral cortex and cerebellum where there are no D2 receptors. It was used in PET imaging studies to image σRs in the cerebellum of PD patients who are suffering from levodopa-

induced dyskinesia (LID). PET studies indicated increase in the σRs cerebellar binding in dyskinetic patients with PD, which was reduced after pallidal surgery. This reduction in σRs binding and the improvement of dyskinesia, suggested the association of σRs in the pathogenesis of PD.[220]

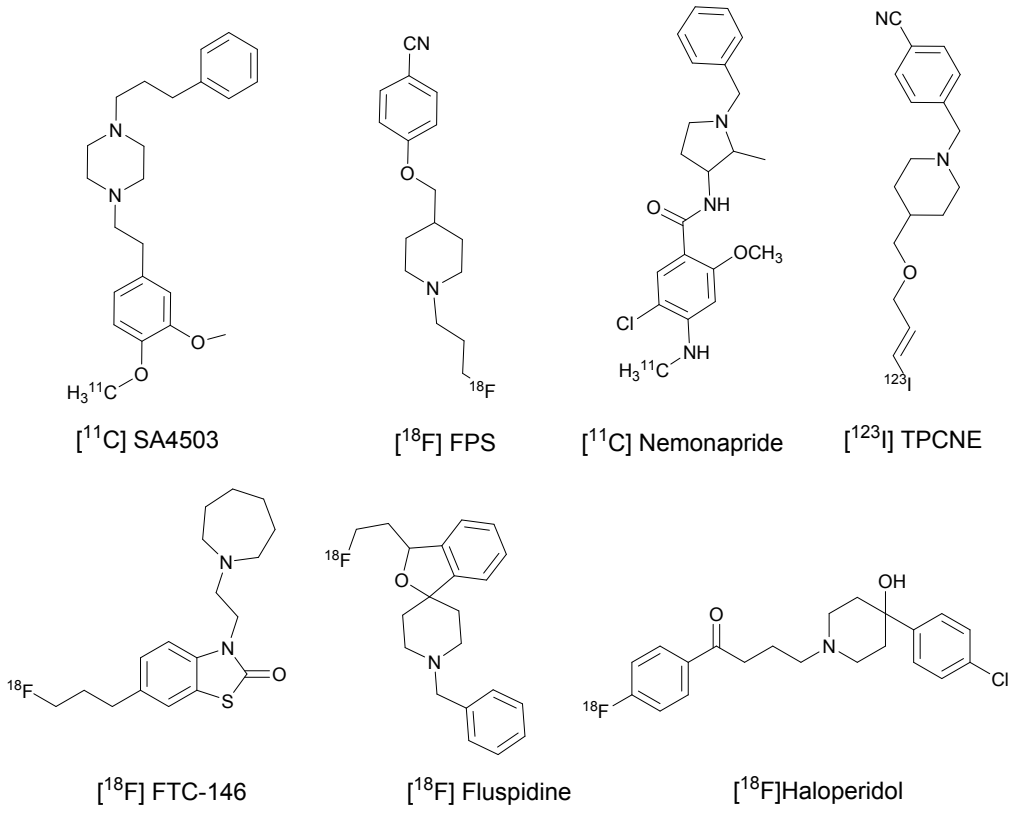


Fig 4: Radiolabelled σ1Rs ligands tested in human.

[123I]TPCNE. [123I]TPCNE (1(trans-iodopropen-2-yl)-4-[(4-cyanophenoxy)methyl]piperidine) is a σ1Rs ligand (*K*i σ1 = 0.67 nM, Ki σ2 = 38.8 nM) that showed a low selectivity profile over σ2Rs, (σ1/ σ2 = 50). It was employed in human trials utilizing SPECT imaging and demonstrated high brain uptake. A blocking study using haloperidol suggested binding was specific to σ1Rs. However, binding in the posterior cingulate area was not affected by haloperidol pretreatment, which could not be accounted for.

The high affinity resulted in irreversible binding profile and the radioligand did not clear over 30 h. Thus, no further studies have been reported for [123I]TPCNE.[221]

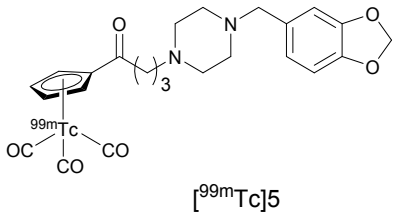
[18F]Fluspidine. [18F]Fluspidine is a spirocyclic piperidine derivative that exhibited high affinity and selectivity toward σ1Rs, and high metabolic stability *in vitro* and *in vivo*.[222] It has two enantiomers that show different affinities toward σ1Rs, R isomer (Ki σ1 = 0.57 nM, σ2/ σ1 = 1,330) and S isomer (Ki σ1 = 2.3 nM).[222, 223] Both isomers have been used to image σ1Rs in mice and piglets to investigate their respective *in vivo* kinetics and suitability for σ1R imaging in humans.[224, 225] Both enantiomers were also investigated in several tumor cell lines, and PET/CT imaging of brain tumors in mice were conducted. High tumor uptake supports the use of both tracers as potential PET imaging agents for brain tumor.[188] Also, (S)-(-)-[18F]Fluspidine exhibited fast and reversible kinetics in brain and was selected for a first-in-human PET/CT study to investigate σ1Rs in brain, (German clinical trial register ID: DRKS00008321).[226] The results indicate that (S)-(-)-[18F]Fluspidine is a potential PET imaging agent for clinical investigation of σ1Rs. Hence, the utility of (S)-(-)-[18F]Fluspidine for quantifying pathological changes (via determining σ1Rs expression) in major depressive disorder was evaluated.[226] Recently, metabolic stability studies have been conducted *in vitro* and in human for (S)-(-)-[18F]Fluspidine. Human plasma metabolic stability studies for (S)-(-)-[18F]Fluspidine showed 91% of the drug remained unchanged 30 min post injection. This data indicates that (S)-(-)-[18F]Fluspidine is a suitable candidate for PET imaging of σ1Rs.[227] However, no more information is currently available about its imaging performance in humans.

[18F]FTC-146. [18F]FTC-146 is a selective σ1Rs antagonist (>1000 fold over σ2Rs) that showed a picomolar affinity at σ1Rs (Ki σ1 = 0.00025 nM) that might be responsible for its slow pharmacokinetics in human. Preclinical studies showed high brain uptake and favourable pharmacokinetics in rodents (mouse, rat) and non-human primates (monkey).[168, 228] It is used as a PET/MRI diagnostic agent currently in Phase 1 clinical trials for identifying the source of pain generation in complex regional pain syndrome (CRPS), sciatica patients, chronic neuropathic and/or nociceptive pain.[65]

Successful σ1Rs radioligands in animal studies

In the past two decades, different classes of compounds have been evaluated for imaging both σRs by PET and SPECT. Previous comprehensive overviews discuss the development of PET and/or SPECT radioligands for both σRs.[77, 170, 229-232] Therefore, we will introduce the recent successful radioligands used for imaging σ1Rs in animals.

To date, there is no potential ^{99m}Tc-labeled σ1Rs SPECT imaging agent that has advanced to human clinical trials. Previous agents developed for preclinical tumor imaging either did not report *in vitro* affinity or have micromolar affinities. In addition, there are several challenges that hinder the development of ^{99m}Tc-based CNS receptor imaging agents. First, there is a need for a chelating agent to form a complex with the transition metal (^{99m}Tc). Then, there must be integration of the metal complex into the σ1R ligand. This change to the parent molecule might affect the size and configuration of the final tracer, ultimately effecting brain uptake and target engagement. In 2014, Wang, X. *et.al*., reported a series of cyclopentadienyl tricarbonyl ^{99m}Tc complexes as potent σ1R SPECT radioligands.[233] This study used ^{99m}Tc-labeled σ1R-targeting radioligands which contained a [(Cp-R)99mTc(CO)3] core that allowed for integration of the σ1R ligand to the metal complex via linkers. Initially, rhenium (Re) analogs were synthesized to determine if these complexes could retain binding affinity at σ1Rs, then ^{99m}Tc labelled radioligands were synthesized. [^{99m}Tc]5 radioligand, Fig 5, has advantages of σ1R nanomolar affinity (Ki σ1 =2.11, σ2/σ1 = 14.5), high initial brain uptake (2 min post-injection), and specific binding to σ1Rs in the normal brain confirmed by the reduction in radioligand uptake upon pre-treatment with haloperidol.

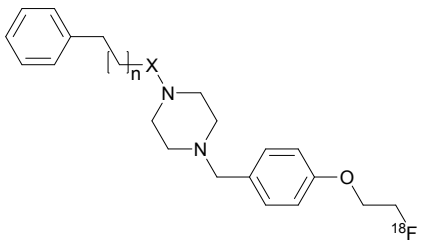


[^{99m}Tc]5

Fig 5: Structure of the σ1R SPECT imaging agent [^{99m}Tc]5.

Compound [^{99m}Tc]5 demonstrated high metabolic stability in mouse brain, where 94% of radioactive species present in the mouse brain corresponded to the parent compound 15 min post-injection and radiometabolites detected in the plasma did not enter the brain. The radioligand uptake in C6 glioma and DU145 cell lines was significantly reduced in a time and dose dependent manner when haloperidol, DTG, and SA4503 were utilized as pre-blocking agents. Further evaluation of [^{99m}Tc]5 as a potential *in vivo* SPECT radioligand for imaging σ1Rs in solid tumors was conducted in C6 glioma-bearing mice, high specific binding of [^{99m}Tc]5 to σ1Rs was observed in the tumor. These results represent a nice advancement in the development of ^{99m}Tc- labelled radioligands and further investigations are warranted.[233]

Efforts are ongoing in the search for an optimal ^{18}F-labeled benzylpiperazine derivative for PET imaging. Among them are a new series of benzylpiperazine derivatives, which were reported as selective σ1R ligands with high affinity (Ki σ1 = 0.31-4.19 nM), and high subtype selectivity (Ki σ2/σ1 = 50-2448).[234] Three of the fluoroethoxy analogs also exhibited high selectivity toward the vesicular acetylcholine transporter, VAChT, (Ki = 99-18252) and were chosen for radiolabeling. Radioligands [^{18}F]2, [^{18}F]3, and [^{18}F]4 displayed high initial brain uptake in mouse (8.37-11.48% ID/g at 2 min), Fig 6. In addition to the high selectivity for σ1Rs, these ligands are not substrates for permeability-glycoprotein (P-gp) and had limited defluorination in vivo. [^{18}F]2 and [^{18}F]3 display fast kinetics in the mouse brain and low brain-to-blood ratios. While [^{18}F]4 displayed high brain-to-blood ratios and high *in vivo* metabolic stability. However, [^{18}F]4 displayed slow kinetics in the mouse brain that limit its application for human neuroimaging. [^{18}F]4 can serve as a lead compound for further structural modifications to explore new potential radioligands for σ1Rs with suitable kinetics for imaging σ1Rs in the brain.



[^{18}F] 2; n =3, X=CO
[^{18}F] 3: n=1, X=CO
[^{18}F] 4: n=1, X=CH_2

Fig 6: Structure of ^{18}F-labeled benzylpiperazine derivatives [^{18}F]2, [^{18}F]3, [^{18}F]4.

Generally, studies that monitor long-term brain recovery post-stroke are limited; however, it is important to study the changes that occur in brain after stroke to allow for better treatment. Hence, Henderson et. al.[235] used a multi-modal imaging approach in rats to image the biological recovery process after stroke, neuroinflammation, and neurodegeneration. This approach combines MRI, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and PET imaging. MRI was used for visualizing the infarct 48 h after stroke, while PET and MALDI-MS were used for depiction of biological mechanisms occurring in the long-term recovery (3 months post-stroke).

MALDI-MS imaging has the advantage of providing high spatial resolution imaging for more than one compound, either exogenous or endogenous, in the same experiment. Accordingly, translocator protein 18kDa (TSPO) tracer [^{18}F]DPA-714 was used as a biomarker of brain injury and inflammation, whereas σ1R radioligand *N*-(2-Benzofuranylmethyl)-*N*'-[4-(2-fluoroethoxy) benzyl]piperazine [^{18}F]IAM6067 (Ki σ1 = 2.6 nM, σ2/σ1= 187),[236] was used as a biomarker for neurodegeneration, Fig 7. Since stroke can cause a disruption of calcium signaling that may lead

to neuronal cell death,[237] σ1Rs can serve as a potential neuronal biomarker. [^{18}F]IAM6067 PET scans showed no decrease in σ1Rs tracer in the infarct area compared with the rest of the brain, and [^{18}F]DPA-714 PET scans showed no inflammation or TSPO over-expression. These results suggested that the brain has stabilized post-stroke and remodeling of the brain structure had occurred. However, *ex vivo* MALDI-MS imaging was carried out to investigate lipid biomarkers changes that cannot be detected by PET scans in stroke recovery. MALDI-MS imaging showed differences in the lipid profile (e.g. phosphatidylcholine and sphingomyelin) between the scar region and the rest of the brain. This finding indicates that lipid metabolism remains altered in the brain 3 months after the ischemic attack, suggesting that recovery processes are still in play. Clearly, further investigations into the exact role of the lipid biomarkers are needed.[235]

Most PET radioligands used for σ1R imaging are labeled either with carbon-11 or fluorine-18, which have short half-lives (20.4 min and 109.8 min, respectively). Gangangari. *et.al*. claimed that tumor imaging requires a radioligand with a long half-life. [238] The long half-life radioligand will compensate for the slow possible binding kinetics of σ1R ligands. It will also help to achieve equilibrium, allow for monitoring the kinetics and receptor occupancy not only for hours, but also for days post administration. This in turn, will permit the visualization of drug induced apoptosis in cancer cells and assess the efficacy of σ1R antagonists in animal models and clinical trials. The iodine radioisotopes, iodine-123 ($t_{1/2}$ = 13.22 h), iodine-124 ($t_{1/2}$ = 4.2 days), iodine-125 ($t_{1/2}$ = 59.49 days) offer this advantage of long half-lives. These radioiodinated ligands will provide the longer time necessary for assessment of drug efficacy in cancer treatment and have a potential application in tumor imaging.

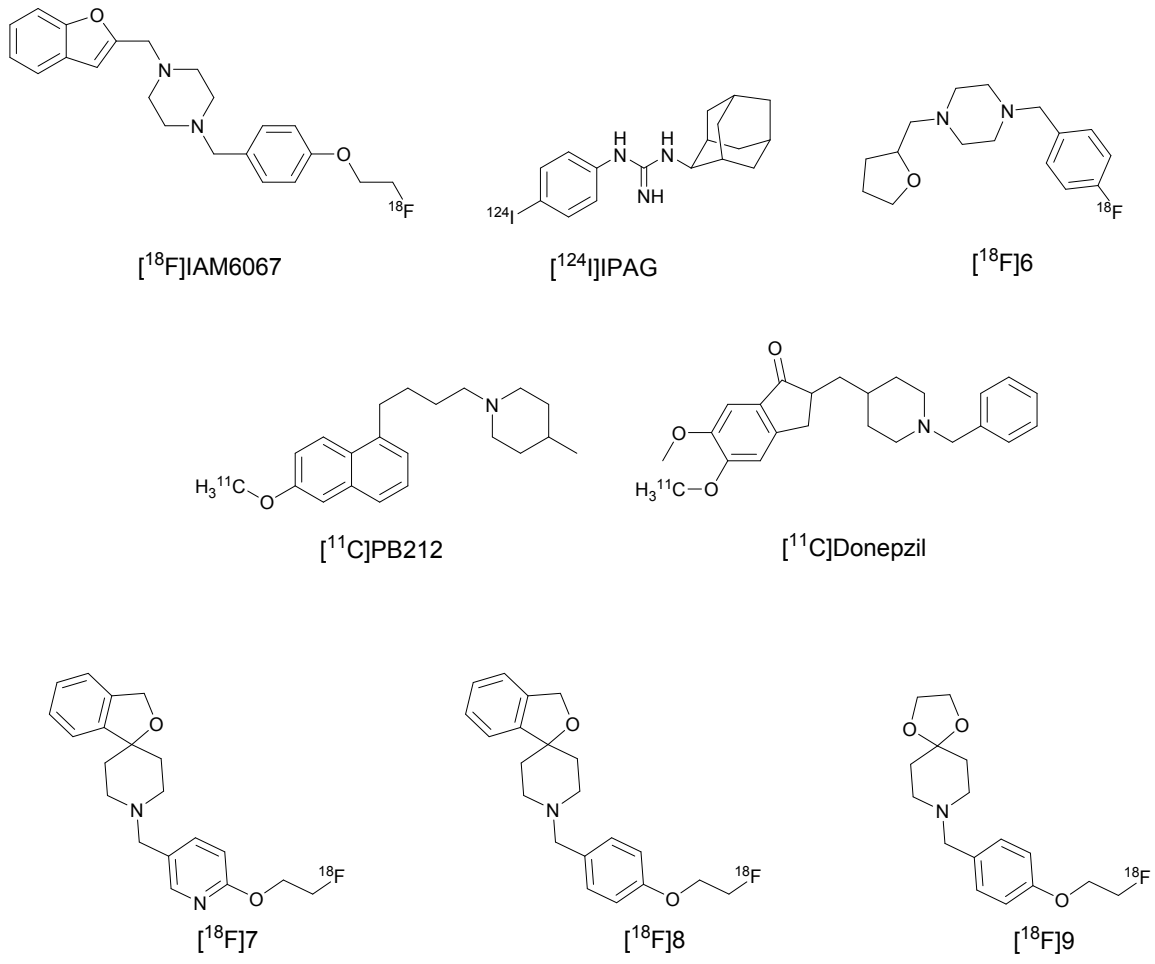


Fig 7: Selected successful σ1Rs radioligands tested in animal studies

For example, σ1R antagonists bearing a benzamide scaffolded labelled with [^{123}I] and [^{125}I] were reported for SPECT imaging of σ1Rs in melanoma.[239, 240] These compounds were able to image the tumor and the results were confirmed by the *ex vivo* biodistribution studies. Unfortunately, the non-selectivity of the benzamide derivatives makes it difficult to assess σ1R expression due to simultaneous binding to melanin receptors. Another example is the iodine radiolabeled analog, [^{124}I]IPAG, Fig 7. IPAG [1-(4-Iodophenyl)-3-(2-adamantyl) guanidine] is a high affinity selective σ1R antagonist (K_i = 2.8 nM) with a slow

clearance from background tissues (around 24 h).[241] [^{124}I]IPAG, was used to image the upregulation of σ1Rs *in vitro* in the MCF-7 breast cancer cell line and *in vivo* in two separate PET imaging studies of MCF-7 tumor–bearing mice and mice bearing LNCaP (prostate) tumors, where tumors were established in athymic nude mice by injecting 107 cells subcutaneously. PET images 24 h post administration in MCF7 tumor–bearing mice depicted that [^{124}I]IPAG accumulated in tumor and was clearly visualized. Interestingly, the tumor could not be delineated after 4 h because of the slow clearance from tissues. The high background activity associated with non-target binding clears over time and allow for better detection at 24 h.[238] The exponential one phase decay curve of [^{124}I]IPAG showed preferential clearance of the radioligand from blood, liver, spleen, and muscles while being retained in the tumor for 72 h. The long half-life of ^{124}I isotope allows PET imaging for extended time post [^{124}I]IPAG administration (144 h, ~ 0.17 % ID/ g) that facilitates preferential retention of the radioligand activity only in σ1R expressing tissues (tumor, liver, and salivary glands) that retain the radioactivity and improve tumor delineation.[238] PET studies using LNCaP tumors bearing mice revealed comparable results with studies done using MCF7 tumor–bearing mice. Biodistribution of [^{124}I]IPAG in mouse bearing MCF-7 tumors 4 h post injection was 0.28 ±0.01 %ID/g in brain.[238] Previously, in different study the distribution of [^{125}I]IPAG in the mouse brain were reported (specific binding in cerebellum, 0.64% injected dose; striatum, 0.58%; thalamus, 0.54%; cortex, 0.53%; and hippocampus, 0.46%).[242] About 0.6% of the injected dose/g of [^{125}I]IPAG penetrated the blood brain barrier in mouse brain. Thereby, [^{124}I]IPAG could be potentially used as an imaging agent for brain tumors as hypnotized by the authors, which will require further investigations. The availability of high affinity σ1R PET or SPECT radioligands with long half-lives could be a useful tool to image σ1Rs upregulation in cancer. This in turn, helps with monitoring the efficacy of cancer therapy in a noninvasive manner.

Compound [^{18}F]6, is a promising PET tracer as a biomarker for early diagnosis in AD animal model, Fig 7. [^{18}F]6 , 1-(4-Fluorobenzyl)-4-[(tetrahydrofuran-2-yl)methyl]piperazine, has nanomolar affinity at σ1Rs and moderate selectivity against σ2Rs (Ki σ1 = 3.2, Ki σ2 =168 , σ2/σ1= 52) with more than 2000-fold selectivity over VAChT and negligible affinity at other 10 CNS targets. PET/MRI studies demonstrated high specific binding at σ1Rs in rat brain, high brain uptake with high brain-to-blood ratios. In addition, [^{18}F]6 was highly stable *in vivo* as it represented 95% of the total radioactivity in the mouse brain at 60 min post-injection. No signs of peripheral radiometabolites were observed as being able to enter the brain. The compound also has low lipophilicity (log D = 0.76) with suitable kinetics, as the maximum concentration in the brain was reached within 2 min, and then washed out steadily with time. Moreover, [^{18}F]6 was used to investigate the changes in σ1Rs expression in pre-AD stage using SAMP8 mice, a model of AD that display age-related cognitive decline close to that in human. In *ex vivo* autoradiography, a significant reduction in [^{18}F]6 uptake was found in the cortex, striatum, hippocampus, and cerebellum of SAMP8 compared to control mice. Indicating the ability of [^{18}F]6 to predict changes in σ1Rs in AD animal model before the emergence of Aβ deposition. Thus, σ1Rs might be a useful biomarker for early diagnosis of AD in preclinical stages and more investigations are warranted for greater understanding of the role of σ1Rs in AD progression.[167]

PB212 is a selective σ1R antagonist that exhibits high affinity (*K*i = 0.030 nM) and 596 fold selectivity over σ2Rs (*K*i = 17.9 nM), Fig 7.[243] *In vitro* autoradiography experiments using [^{11}C]PB212 revealed high binding in the brain of both the wild type and σ1Rs knockout mice. This was indicative of nonspecific binding and unsuitability for brain imaging of sigma receptors. While high and specific binding of [^{11}C]PB212 was observed in the spleen tissues of both CD1 mice and Wistar rats. This result was confirmed by *in vivo* PET imaging studies in Wistar rats and blocking studies using haloperidol and fluspidine. Therefore, [^{11}C]PB212 could be used to image σ1Rs expression in the periphery and further studies are required.[244]

Recently, σ1Rs were reported to be involved in maintaining normal cardiac contractility. σ1R knockout mice showed cardiac contractile dysfunction, while σ1R inhibition (in wildtype mice) resulted in atrial fibrosis and atrial electrical remodelling.[158, 245] Moreover, Cutamesine (SA4503) is a σ1R agonist in phase II clinical trial for ischemic stroke.[246] However, the physiological role of cardiac σ1Rs remains unknown and further investigations (clinical research is encouraged) targeting cardiac diseases should be done to evaluate the therapeutic potential in cardiovascular disease. Utilization of [^{11}C]donepezil for PET imaging opens the gates for revisiting or reassessing an overlooked σ1R ligand as a target for cardiac pharmacological intervention, Fig 7. Donepezil is a reversible acetylcholinesterase (AChE) inhibitor, which also has high affinity at σ1Rs and is used for the treatment of Alzheimer's patients. Donepezil displayed a cardioprotective effect in Alzheimer's patients who seem to have low cardiovascular mortality risk. However, the mechanism of cardioprotection is unknown and could be caused by AChE inhibition, σ1R binding, or both. However, Horsager *et.al*. speculated that the increased binding and uptake of [^{11}C]donepezil in human heart with age and it is cardioprotective effect may be primarily due to the upregulation of σ1Rs.[247] Future studies to compare the cardiac uptake of the highly selective σ1R PET ligand, [^{18}F]FTC- 146, and blocking studies using the σ1Rs selective ligand SA4503 and donepezil in different age groups were suggested to confirm the results on elucidating which of the target proteins is upregulated.

A series of radiolabelled spirocyclic piperidine derivatives were reported and some of them are promising PET radioligands for σ1R imaging. Among them are fluspidine and its derivative [^{18}F]7, where its cold ligand demonstrates high σ1Rs affinity (Ki σ1= 2.3, σ2/σ1 = 142), Fig 7. PET imaging evaluation in rhesus monkeys of [^{18}F]7 showed high brain uptake, high specific binding, fast reversible kinetics, and 3 times higher binding potential than (S)-fluspidine. Hence, these results suggested viability of [^{18}F]7 as a σ1Rs PET radioligand in human brain.[248] In contrast, [^{18}F]8 unlabelled ligand displayed subnanomolar affinity (Ki σ1= 0.79, σ2/σ1 = 351). Consequently, [^{18}F]8 exhibited slow, irreversible kinetics in monkey brain with no

significant washout during the 4-h scan session. This finding made [^{18}F]8 unsuitable for human neuroimaging.[248]

In order to develop σ1R tumor radioligands and ensure high tumor uptake and low background accumulation, less lipophilic tracers are desired. So, the authors proposed the replacement of the spirocyclic piperidine moiety in [^{18}F]8 (log D7.4 = 2.41) with a more hydrophilic group 1,4-dioxa-8-azaspiro[4.5]decane to reduce the lipophilicity. Accordingly, compound [^{18}F]9 showed low lipophilicity (log D7.4 = 0.81) and was selected as a PET radioligand from the series to image tumors *in vitro* and *in vivo*, Fig 7. [^{18}F] 9 displayed high *in vitro* stability in human plasma at room temperature for 2 h and specific binding to σ1Rs in four different cell lines that were confirmed by blocking studies using SA4503, haloperidol or fluspidine. Furthermore, high accumulation of the radioligand was observed in dynamic PET studies utilizing A431 tumor bearing NMRI *nu/nu* mice. This accumulation was significantly reduced upon pre-treatment with haloperidol. The results indicated the specific binding of [^{18}F]9 to σ1Rs in the tumors *in vivo* and that [^{18}F]5 is a promising tumor imaging agent.[249]

Comp54, the first σ1Rs PET radioligand with a 6-hydroxypyridazinone core structure, Fig 8. It was reported to be a promising σ1Rs antagonist with high binding affinity (Ki σ1=1.4 nmol/L) and apparent good selectivity (σ2/σ1 = 1365.7).[250] Further modification to incorporate [^{11}C] isotope without significantly changing the main scaffold, and preserving high affinity and selectivity were described. Lan, Y *et.al*. reported the radiosynthesis and evaluation of two novel [^{11}C] radiolabelled PET tracers as derivatives of comp54; [^{11}C]HCC0923 and [^{11}C]HCC0929. Both unlabelled compounds showed decreased affinity and selectivity at σ1Rs (HCC0923, Ki σ1 =10.3 nmol/L, σ2/σ1 =111.3; while HCC0929, Ki σ1 =5.6 nmol/L, σ2/σ1 = 272.8).[251]

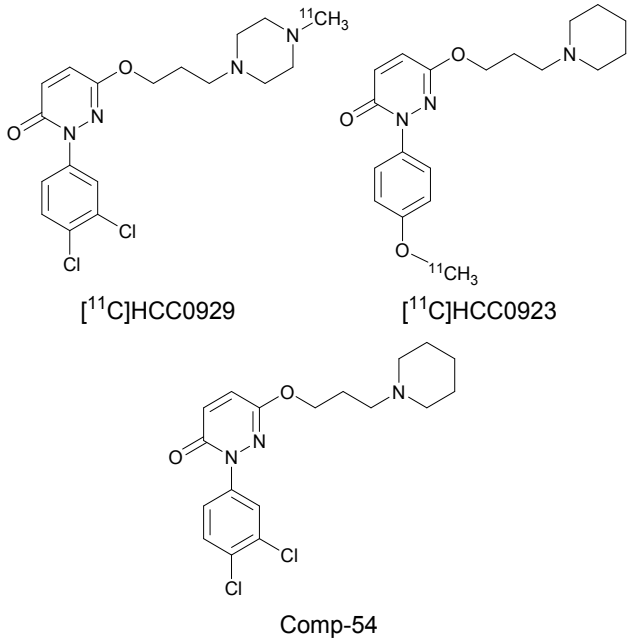


Fig 8: Structure of comp-54 and its derivatives.

However, in PET/CT studies, both radioligands bind to σ1Rs in the mouse brain. They demonstrated good selectivity and specificity toward σ1Rs in self-blockade studies using the unlabelled ligands. [^{11}C]HCC0923 exerted high BBB penetration and fast uptake after intravenous bolus injection that reached a maximum uptake within few minutes, and sustained binding over the scanning time (60 min). While, [^{11}C]HCC0929 showed better affinity, specificity, higher BBB penetration and faster brain clearance kinetic properties.

[^{11}C]HCC0929 was further investigated in PET/CT brain imaging with positive blocking studies to confirm the specificity to σ1R binding using SA4503 (σ1R agonist) and PD144418 (σ1R antagonist).

The radiolabelled uptake of [^{11}C]HCC0929 was extensively decreased in mice brain, with different kinetic uptake and washout properties. In addition, the biodistribution studies indicated that the major brain functional regions (cortex, cerebellum, brain stem, thalamus, hypothalamus, striatum, hippocampus, and amygdala) were labelled by [^{11}C]HCC0929 and a moderate wash-out rate during the scanning period (60 min) was observed. Also, other organs such as heart, lung, and kidney showed high uptake at 5 min that washed out gradually. While the maximum uptake in liver and spleen was behind and peaked at 30 min, and 15 min respectively and slightly washed out. So, [^{11}C]HCC0929 could be a promising PET imaging agent for σ1Rs visualization in neurological disorders especially when introducing a new scaffold that might expand the chemical diversity of σ1Rs PET radioligands that warrants further investigation.[251]

Conclusion

σ1Rs are attractive targets for the development of pharmacotherapeutic agents for different diseases due to their involvement in many physiological and pathological events. The use of imaging studies has indicated alteration of σ1Rs expression levels, which have a correlation with the age, disease states, and type of tissue affected. Reduced σ1Rs densities were noted in certain brain areas of Alzheimer's and Parkinson's patients, while increases in expression were seen in areas of nerve damage in chronic pain and different types of tumors. Imaging studies have significantly contributed to the successful identification and classification of σ1Rs as a unique class of chaperone proteins. They also aided in the identification of σ1R expression levels in mammalian brain, assessment of receptor engagement, and quantifying receptor occupancy. This is in addition to confirming the correlation of σ1R expression with disease progression. Nevertheless, imaging studies continue to give insights about σ1Rs role and value its importance in normal and diseased states, which opens new perspectives in pharmacological intervention for many disease state diagnoses and treatments. These studies might also uncover new roles of this receptor in other diseases. Evidently, imaging studies in mice depicted high expression of σ1Rs in salivary glands,[238] where the literature revealed no previous reports on σ1Rs expression in the salivary glands. The only reported information are the pharmacological effect of σ1Rs agonists that stimulate

salivary gland secretions,[252] and the physiologic uptake of σ1Rs radioligands [^{11}C]SA4503 in submandibular gland.[253] Now, after confirming their expression level in mice, it will be interesting to investigate the role of σ1Rs in salivary gland diseases. As it might have a role with the abnormal Ca^{2+} metabolism or formation of Ca^{2+} stones. Also, PET imaging studies in human using [^{18}F]FTC-146 showed significant uptake of [^{18}F]FTC-146 in the human thyroid that confirm high expression of σ1Rs in thyroid for the first time.[65] These findings create a research curiosity to investigate why there is high uptake of σ1Rs antagonist in the thyroid? what is its role? since it remains unclear, more studies are warranted . This knowledge could be used to develop future treatments for salivary gland or thyroid gland. However; more studies are required to assess the role of σ1Rs in these tissues that may envision σ1Rs as a new therapeutic target for new pharmacological intervention and future drug development.

Since, σ1Rs research efforts are driven by the desire to explore the vast/huge involvement of σ1Rs in many pharmacological activities and diseases with the hope to translate the basic research into therapeutic drugs in the future. Therefore, molecular imaging using radiolabelled probes to determine the σ1Rs expression could be used as a diagnostic tool that can guide surgery or treatment, monitor disease progression or exert its therapeutic effect at the targeted tissue. The developed PET and SPECT tracers has a great value especially in the study of CNS diseases due to the inaccessibility of the human brain and the fact that σ1Rs are widely distributed throughout the CNS. In this review, we introduced the recent radiolabelled σ1Rs targeting probes. The use of imaging studies in drug discovery is inevitable; however, limited number of successful radioligands are available. Moreover, until now, there are no successful σ1Rs tumor imaging agents in the clinical trial. Thus, more investigations to synthesize an ideal σ1R radioligands with appropriate kinetics and selectivity is still needed for practical clinical translation.

Conflicts of interest

The authors declare no conflicts of interest.

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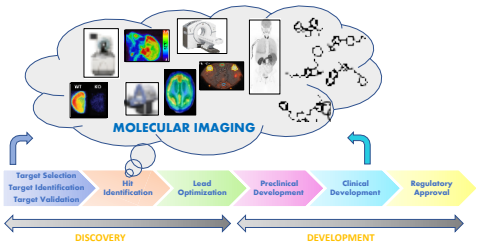

Molecular imaging studies have paved the road for the development of successful σ1R ligands currently in clinical trials