[^{125}I](*E*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3'',4''-dimethoxyphenethyl)-piperazine

[^{125}I]*E*-1

Liang Shan, PhD[1]

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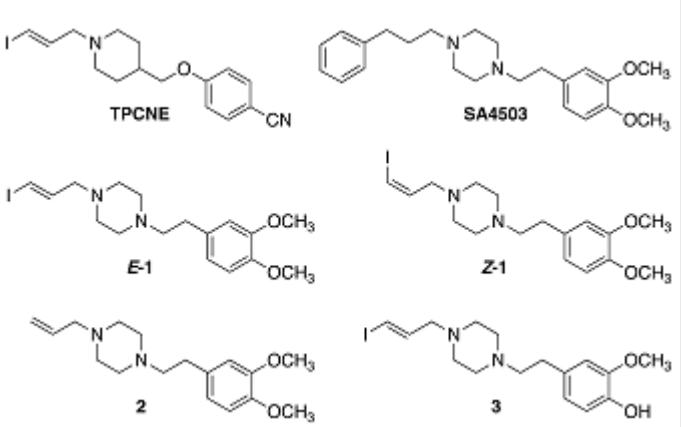
Chemical name:	[^{125}I](*E*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3'',4''-dimethoxyphenethyl)-piperazine
Abbreviated name:	[^{125}I]***E*-1**
Synonym:	
Agent Category:	Compounds
Target:	Sigma-1 (σ1) receptor
Target Category:	Receptors
Method of detection:	Single-photon emission computed tomography (SPECT)
Source of signal / contrast:	^{125}I
Activation:	No
Studies:	• *In vitro* • Rodents



Structures of **E**-1, **Z**-1, 2, and 3 (1).

[1] National Center for Biotechnology Information, NLM, NIH; Email: micad@ncbi.nlm.nih.gov.

Corresponding author.

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Background

[PubMed]

[^{125}I](*E*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3'',4''-dimethoxyphenethyl)-piperazine, abbreviated as [^{125}I]***E*-1**, is a hybrid structure of two known ligands: TPCNE (1(*trans*-iodopropen-2-yl)-4-[(4-cyanophenoxy)methyl]piperidine) and SA4503 (1-[2-(3,4-dimethoxyphenethyl)]-4-(3-phenylpropyl)piperazine). [^{125}I]***E*-1** was synthesized for use with single-photon emission computed tomography (SPECT) of sigma-1 (σ1) receptors (1).

σ1 receptor is a protein that is widely distributed in both the central nervous system (CNS) and peripheral organs. There are at least two subtypes of σ receptors, σ1 receptor and σ2 receptor. Although the functions of σ2 receptor are poorly understood, σ1 receptor is believed to act as a modulator of the signal transduction in neurotransmitter systems (2, 3). σ1 receptor primarily resides at the interface between the endoplasmic reticulum and mitochondria, where it modulates Ca^{2+} flux by acting as a molecular chaperone for type 3 inositol-1,4,5-triphosphate receptors. σ1 receptor can also translocate to the plasma membrane, where it regulates the voltage-dependent Ca^{2+} channels, K^+ channels, and other membrane-bound proteins (2, 4).

Increasing evidence suggests that σ1 receptor is involved in a range of CNS diseases such as affective disorders, psychosis, schizophrenia, substance abuse, Parkinson's disease, and Alzheimer's disease (4, 5). Studies on postmortem human brains have shown that the density of σ1 receptor decreased in patients with schizophrenia and Alzheimer's disease (6). Discovery of specific ligands for σ1 receptors has further prompted development of efficient imaging probes for neuropsychiatric diseases by targeting σ1 receptor (3, 5).

TPCNE and SA4503 are among the radiotracers investigated for σ receptor imaging. TPCNE has an affinity of K_i = 0.67 nM (log *P* = 3.36) for σ1 receptors, and SA4503 has an affinity of K_i = ~5 nM (log *P* = 2.52) for σ1 receptors (7-9). Both exhibit good specific binding to σ1 receptors in rat brains and in human brains. Lever et al. synthesized four hybrid structure–related *N*-1-allyl-*N*'-4-phenethylpiperazines (***E*-1**, ***Z*-1**, **2**, and **3**) from TPCNE and SA4503 on the basis of the hypothesis that hybrid structures might be suitable scaffolds for developing radioiodinated ligands that have high σ1 receptor affinities and appropriate lipophilicities for *in vivo* studies (1). Lever et al. selected ***E*-1** and ***Z*-1** to probe the effect of geometric isomerism upon σ receptor binding and to discriminate the contribution of the iodine atom through comparison with the allyl analog **2** (1). Compound **3**, which is a monomethyl analog and is likely to be a metabolite of ***E*-1**, was used in the studies to determine the radiometabolites of [^{125}I]***E*-1**. The results obtained by Lever et al. showed that [^{125}I]***E*-1** was a selective σ1 receptor antagonist that exhibited properties amenable to *in vitro* and *in vivo* studies, with possible extension to SPECT using iodine-123 (1).

Related Resource Links:

The nucleotide and protein sequences of sigma-1 (σ1) receptors

Sigma-1 (σ1) receptor-related compounds in PubChem

Synthesis

[PubMed]

Lever et al. described the synthesis of **E**-1, **Z**-1, 2, and 3 in detail (1). **E**-1 and **Z**-1 were prepared with comparable overall yields (**E**-1, 31%; **Z**-1, 36%) over four-step synthesis. Synthesis of the allyl analog **2** was accomplished by alkylation of commercially available N-allylpiperazine in one step, with an unoptimized yield of 29%. The synthesis of compound 3 was achieved through multiple steps, with a yield of 70% for the final step. The full chemical names of **E**-1, **Z**-1, 2, and 3 are (*E*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3'',4''-dimethoxyphenethyl)-piperazine, (*Z*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3'',4''-dimethoxyphenethyl)-piperazine, *N*-1-allyl-*N*'-4-(3',4'-dimethoxyphenethyl)-piperazine, and (*E*)-*N*-1-(3'-iodoallyl)-*N*'-4-(3''-methoxy-4''-hydroxyphenethyl)-piperazine, respectively.

[^{125}I]**E**-1 and [^{125}I]**Z**-1 were prepared from their corresponding stannyl precursors, with radiochemical yields of 74%–82%, radiochemical purities of >98%, and specific radioactivities of 71.6–77.3 GBq/μmol (1.94–2.09 Ci/μmol) (1). Incorporation of radioactivity was ~90%. Both [^{125}I]**E**-1 and [^{125}I]**Z**-1 showed >95% radiochemical purity after 2 weeks of storage at −20°C in the dark in EtOH, and the agents maintained 95% purity after 30 days.

The lipophilicity (log $D_{7.4}$) of [^{125}I]**E**-1 and [^{125}I]**Z**-1 was determined by partitioning 74–148 kBq (2–4 μCi) of each agent between equal volumes of n-octanol and Dulbecco's phosphate-buffered saline (0.1 M, pH 7.4) (n = 5) (1). The results showed that the log $D_{7.4}$ values (means ± SD) of [^{125}I]***E*-1** (2.25 ± 0.008) and [^{125}I]**Z**-1 (2.27 ± 0.006) were in the optimal range for *in vivo* utility and were similar to the log *P* value of SA4503 (2.52), but lower than the log *P* value of TPCNE (3.36) (7, 9). The calculated lipophilicities of compounds **2** (1.77) and **3** (2.06) were lower than those of [^{125}I]**E**-1 and [^{125}I]**Z**-1.

The protein binding of [^{125}I]**E**-1 was assessed with the trichloroacetic acid method after incubation with mouse plasma for 30 min at 37°C (1). [^{125}I]**E**-1 showed a modest binding with plasma proteins (48.2 ± 1.0%, n = 4). The protein binding was not assessed for other compounds.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding and selectivity of **E**-1, **Z**-1, 2, and 3 for σ receptors in guinea pig brain were measured with competition binding assays using [^{3}H](+)-pentazocine (σ_1) and [^{3}H]ditolylguanidine ([^{3}H]DTG) (σ_2) as radioligands (1).

Binding assays with guinea pig brain homogenates revealed significant differences in binding affinity and σ_1/σ_2 selectivity for the four compounds (Table 1). **E**-1, **Z**-1, and 3, which contained the N-iodoallyl moiety, displayed similar, moderately high σ_1 receptor affinities. By contrast, compound 2, which contained the N-allyl group, showed a σ_1 receptor affinity one order of magnitude lower. These results indicated that the σ_1 receptor affinity was not sensitive to the **E** and **Z** geometric isomerism, but iodine contributed to the binding at σ_1 sites. For σ_2 receptor binding, the sensitivity was high to the structure, where a 300-fold range was observed for the four analogs. Compound 3 displayed the highest affinity; **E**-1 and **Z**-1 exhibited relatively poor affinity, and compound 2 exhibited the poorest affinity with a $K_i > 10$ μM. These results indicated that a free phenol led to a much stronger σ_2 binding interaction than a methoxy group. Overall, **E**-1 had the highest affinity and best selectivity for σ_1 receptor binding. Compared with SA4503 and TPCNE, **E**-1 exhibited greater selectivity for binding to σ_1 sites over σ_2 sites, accompanied by a 3- to 20-fold weaker apparent σ_1 binding affinity.

Table 1. *In vitro* binding properties for σ receptors.

Compound	σ1 (K_i = nM)	σ2 (K_i = nM)	σ1/σ2 ratio
***E*-1**	15.1 ± 1.3	1,263 ± 166	84
***Z*-1**	19.9 ± 1.3	455 ± 66	23
2	132 ± 12	10,950 ± 1,295	83
3	13.0 ± 0.5	36.6 ± 1.0	3
SA4503	4.6 ± 0.2[a]	63.1 ± 4.3[a]	14
TPCNE	0.67[b]	38.8[b]	58

Values are means ± SEM (*n* = 3–4) from competition assays against [^{3}H](+)-pentazocine (σ1) and [^{3}H]DTG (σ2) in membrane homogenates from male guinea pig brains.
[a]Data from Lever et al. (8).
[b]Data from Waterhouse et al. (10).

Compound **E**-1 was further checked for its binding to other receptors against a panel of six well-characterized radioligands in brain membrane homogenates (data not shown). Low displacements were noted for κ (6%), δ (6%), and μ (34%) opioid receptors, as well as dopamine (8%), norepinephrine (2%), and serotonin transporters (35%). Thus, K_i values for **E**-1 against opioid receptors and monoamine transporters were likely >10 μM.

A σ1 competition assay was also conducted in the presence of phenytoin. Phenytoin is an allosteric modulator of the σ_1 receptor binding that induces a shift to higher affinity for a number of known agonists, but a slight shift to lower affinity for many antagonists (11). The competition assay showed that the half maximal inhibitory concentration for **E**-1 exhibited a modest but significantly different shift to lower affinity (30.4 ± 2.6 nM; Hill slope 1.03 ± 0.06) in the presence of phenytoin as compared to the NaOH vehicle control

(21.1 ± 0.06 nM; Hill slope 1.03 ± 0.04) (P = 0.02). These findings are consistent with designation of **E**-1 as a putative antagonist.

To define the nonspecific binding of [^{125}I]**E**-1 *in vitro*, Lever et al. conducted a series of assays using membrane homogenates from whole CD1 mouse brains (1). Association studies indicated that the pseudoequilibrium was reached within 60 min, and ≥95% specific binding was observed over the plateau from 60 min to 240 min. Specific binding of [^{125}I]**E**-1 increased linearly (r^2 = 1.0) over a protein range of 100–400 mg (data not shown). Homologous competition studies with a 60-min incubation time and 0.2 mg protein showed saturable binding for [^{125}I]**E**-1 that fitted a one-site model (r^2 = 1.0). The K_d was 3.79 ± 0.21 nM, and the B_{max} was 599 ± 28 fmol/mg protein.

Inhibitory potency studies for several σ receptor ligands in competition against [^{125}I]**E**-1 showed a rank order of potency: haloperidol > (+)-pentazocine > BD1063 >> DTG (1). These data supported the selective, saturable binding of [^{125}I]**E**-1 to σ_1 receptor sites in mouse brain membrane homogenates, with highly specific binding and moderately high binding affinity.

Animal Studies

Rodents

[PubMed]

The pharmacokinetics of [^{125}I]**E**-1 and [^{125}I]**Z**-1 were determined after intravenous administration of each radioligand (92.5 kBq; 2.5 μCi) to male CD1 mice (*n* = 4 mice/time point) (1). Within 15 min, there was a trend for [^{125}I]**E**-1 to show higher organ uptake than [^{125}I]**Z**-1. The blood level of [^{125}I]**E**-1 was significantly lower than that of [^{125}I]**Z**-1 (P = 0.01). The brain/blood ratio for [^{125}I]**E**-1 at 15 min was 13.4, while that for [^{125}I]**Z**-1 was 5.8. By 60 min, the levels of [^{125}I]**E**-1 were significantly higher than those of [^{125}I]**Z**-1 in all examined organs (P ≤ 0.006) except for pancreas, which maintained through 120 min.

[^{125}I]**E**-1 and [^{125}I]**Z**-1 readily crossed the blood–brain barrier. [^{125}I]**E**-1 exhibited higher brain uptake and longer retention than [^{125}I]**Z**-1. [^{125}I]**E**-1 clearance from brain was monoexponential (r^2 = 0.99; $t_{½}$ = 36 min), with 5.8% injected dose per gram tissue (ID/g) peak uptake at 15 min, which decreased to 1.5% ID/g by 120 min. [^{125}I]**Z**-1 was also cleared from brain as a first-order decline (r^2 = 0.99; $t_{½}$ = 16 min), from 4.8% ID/g at 15 min to 0.6% ID/g at 120 min. Washouts of both radioligands from heart, lung, and muscle also fit monoexponential curves (r^2 ≥ 0.98) over 15–120 min. In these organs, the half-life values for [^{125}I]**E**-1 were 1.5–2.5 times longer than those for [^{125}I]**Z**-1 (data not shown).

Specific binding of [^{125}I]**E**-1 and [^{125}I]**Z**-1 was evaluated with various σ receptor ligands including haloperidol, BD1063 (σ1), Br-Mach (σ2), and nonradioactive **E**-1 and **Z**-1 compounds (1). Uptake of [^{125}I]***E*-1** in most organs was saturable by pretreatment with

nonradioactive ***E*-1** and blocked by pretreatment with haloperidol (σ1/σ2) and the σ1 receptor-selective BD1063, but not by pretreatment with σ2 receptor-selective Br-Mach. As defined by BD1063, the specific binding of [^{125}I]***E*-1** to σ1 receptors was 80% in brain, 44% in heart, 61% in lung, 68% in spleen, 65% in pancreas, and 57% in liver. Uptake of the [^{125}I]***E*-1** in muscle (0.70% ID/g) was reduced significantly by BD1063 (48%) and ***E*-1** (58%), but any changes with haloperidol or Br-Mach were not significant. Kidney uptake (3.71% ID/g) was not affected by pretreatment with any of these ligands (data not shown). Uptake of the [^{125}I]**Z**-1 at 60 min was blocked significantly by haloperidol in all organs ($P < 0.01$) with the exception of heart ($P = 0.08$). Muscle uptake (0.46%ID/g) was also inhibited (19%; $P = 0.02$), but kidney uptake (3.42% ID/g) was not ($P = 0.71$) (data not shown). As defined by haloperidol, the specific binding of [^{125}I]**Z**-1 was 74% in brain, 40% in lung, and 57%–65% across spleen, pancreas, and liver.

Radiometabolite studies were performed with brain and liver extracts, as well as serum and urine samples at 60 min after intravenous administration of [^{125}I]***E*-1** (3.7 MBq; 100 μCi) to a male CD1 mouse (1). Radioactivity was extracted efficiently (98%) from brain tissue and consisted primarily of the unmetabolized [^{125}I]***E*-1** (92%, t_R = 8.75 min). One minor (8%), less lipophilic metabolite was observed at 3.75 min with high-performance liquid chromatography (HPLC). By contrast, only 69% of the total radioactivity could be extracted from liver. Two minor metabolites, one at 3.75 min (3.5%) and the other at 1.75 min (6.5%), were noted along with unmetabolized [^{125}I]***E*-1** (90%). Recovery of radioactivity from heart, lung, spleen, and pancreas was nearly complete (99%), and the proportion of unmetabolized [^{125}I]***E*-1** ranged from 88% to 96% (data not shown). Low levels of the two minor metabolites observed in the liver extract were also observed to varying degrees in the other peripheral organs. Serum showed 12% unmetabolized [^{125}I]***E*-1** along with 88% polar radiometabolites. Urine consisted almost exclusively of the polar radiometabolites. The data indicated that the polar radiometabolites in serum did not cross the blood–brain barrier.

Aryl methoxy groups are prone to *O*-demethylation *in vivo* by cytochrome P450 enzymes in liver and, to a lesser extent, brain. Therefore, the radiometabolite of [^{125}I]***E*-1** having intermediate lipophilicity (t_R = 3.75 min) could be a simple phenol. Another possibility is that it was a des-methyl congener of [^{125}I]***E*-1**. The monomethyl analog **3** had the same retention time as this radiometabolite under the HPLC conditions (data not shown). Thus, the radiometabolite with t_R = 3.75 min observed in brain and peripheral organs was likely to be [^{125}I]**3** or the des-methyl congener of [^{125}I]***E*-1**.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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