ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

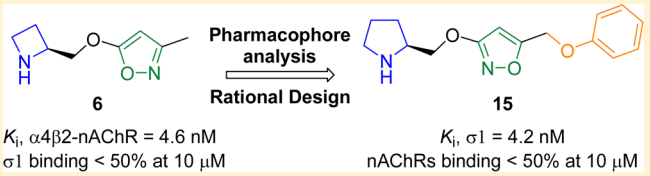
From α4β2 Nicotinic Ligands to the Discovery of σ1 Receptor Ligands: Pharmacophore Analysis and Rational Design

Li-Fang Yu, Han-Kun Zhang, Hendra Gunosewoyo, and Alan P. Kozikowski*

Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

Supporting Information

ABSTRACT: Comparative analyses of the pharmacophoric elements required for σ1 and nicotinic ligands led to the identification of a potent and selective σ1 ligand (**15**). Compound **15** displayed high selectivity for the σ1 receptor (K_i, σ1 = 4.1 nM; K_i, σ2 = 1312 nM) with moderate binding affinity for the DAT (K_i = 373 nM) and NET (K_i = 203 nM) in the PDSP broad screening panel of common CNS neurotransmitter transporters and receptors. The key finding in this present work is that a subtle structural modification could be used as a tool to switch a ligand's selectivity between nAChRs and sigma receptors.



KEYWORDS: *Nicotinic acetylcholine receptor, sigma-1 receptor, alkoxyisoxazole, pharmacophore, broad screening*

Originally described as an opioid receptor subtype and subsequently as a member of the *N*-methyl-D-aspartate/phencyclidine (NMDA/PCP) receptor family, the sigma receptors are now known to modulate diverse physiological functions relevant to not only various neurological disorders but also certain forms of cancer. There are two types of sigma receptors: σ1 and σ2, which possess distinct pharmacological profiles in the central and peripheral nervous systems. The σ1 receptor is a 223 amino acid protein with two transmembrane domains located at the mitochondrial-associated endoplasmic reticulum membrane.[1] On the other hand, the σ2 receptor is overexpressed in aggressive tumor cells and as such has been proposed as a biomarker for tumor cell proliferation.[2] Various reports have demonstrated the involvement of σ receptors in the modulation of potassium and calcium channels as well as the release of important central nervous system (CNS) neurotransmitters such as dopamine, acetylcholine, 5-hydroxytryptamine (5-HT), and glutamate.[3] A number of synthetic compounds targeting sigma receptors have been reported by both academia and pharmaceutical companies.[4] In 2009, it was reported that *N*,*N*-dimethyltryptamine (DMT) could be a low potency endogenous ligand for the σ1 and σ2 receptors.[5] Other endogenous σ1 ligands such as D-*erythro*-sphingosine and sphinganine have also been studied.[6] A diverse class of nonendogenous compounds is also known to bind to the σ2 receptor, which include the psychoactive natural product ibogaine and various synthetic arylalkylamines. However, the lack of both structural information on this sigma receptor subtype and availability of highly selective ligands has hampered studies of the biomolecular mechanisms of σ2 receptor action.

Sigma receptors (Figure 1) are also known to regulate neurotrophic factor signaling and exhibit promising neuroprotective properties. Moreover, assessment of the currently approved SSRIs has demonstrated that the σ1 receptor is often

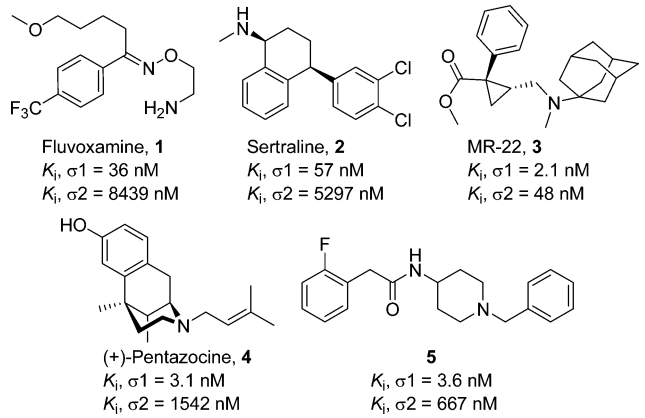


Figure 1. Structures of selected σ1 receptor ligands.

targeted by these agents, and specifically in the cases of fluvoxamine (**1**) and sertraline (**2**), the binding affinities for σ1 are found to be in the range of 36−57 nM.[7] Taken together, these studies suggest that the σ1 receptor could potentially be a novel target for the development of antidepressants with a novel mechanism of action. In addition, the σ ligand (−)-MR-22 (**3**) (K_i, σ1 = 2.1 nM; K_i, σ2 = 48 nM) has also been reported to confer procognitive and neuroprotective effects in rats with selective cholinergic lesion and amyloid infusion.[8] Other notable σ1-selective synthetic ligands include the benzomorphan(+)-pentazocine (**4**), which is 500-fold more selective at the σ1 (K_i = 3.1 nM) over the σ2 receptor and is commonly used as the radioligand of choice to study specific σ1

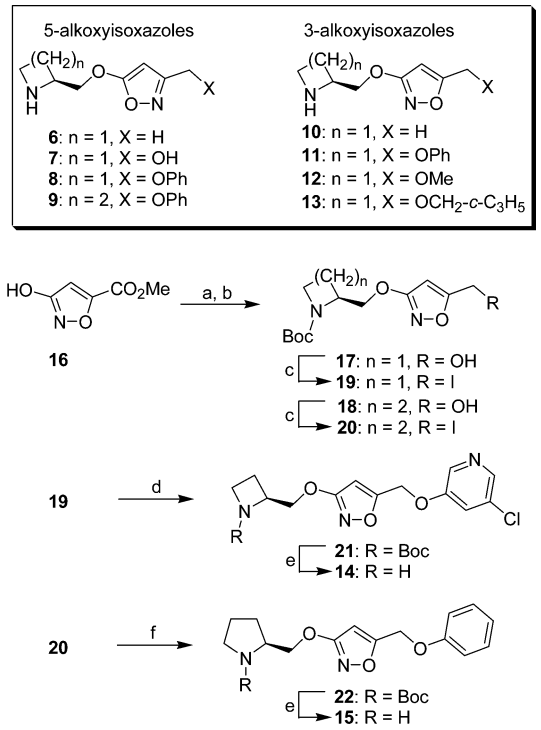
Received: September 4, 2012
Accepted: October 17, 2012
Published: October 17, 2012

receptor binding.[9] The piperidyl amide **5** has also been reported as a synthetic derivative of haloperidol and displayed a binding affinity of 3.6 nM at the σ1 receptor with 185-fold selectivity over the σ2 receptor.[10]

During our ongoing campaign for the discovery of potent and selective $\alpha4\beta2$ nicotinic acetylcholine receptor (nAChR) partial agonists as novel antidepressant agents, structure–activity relationship (SAR) studies have been undertaken on the alkoxyisoxazoles, exemplified by compounds **6**–**13**, as an alternative scaffold to the reported pyridyl ethers.[11] In this ongoing project, a broad screening of selected promising compounds in a panel of approximately 50 common neurotransmitter receptors and transporters was being conducted by the Psychoactive Drug Screening Program (PDSP) at UNC, Chapel Hill, to confirm the global CNS selectivity of these ligands. In this manuscript, a serendipitous discovery of novel potent and selective σ1 receptor ligands emanating from our efforts in the nicotinic ligands program is reported. Herein we show how a subtle structural change can serve as a tool to fine-tune ligand selectivity away from nAChRs to sigma receptors.

The synthesis of compounds **6**–**13** has been reported previously.[11] The 3-alkoxyisoxazoles **14** and **15** were synthesized utilizing the routes shown in Scheme 1. 3-

Scheme 1[a]



[a]Reagents and conditions: (a) 1-(*tert*-butoxycarbonyl)-2(*S*)-azetidinylmethanol or 1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolindylmethanol, diisopropylazodicarboxylate, PPh_3, THF; (b) $LiBH_4$, THF; (c) I_2, PPh_3, imidazole, CH_2Cl_2; (d) 5-chloropyridin-3-ol, K_2CO_3, DMF; (e) TFA, CH_2Cl_2; (f) phenol, K_2CO_3, DMF.

Hydroxyisoxazole-5-carboxylic acid methyl ester (**16**) and Boc-protected 2(*S*)-azetidinylmethanol or 2(*S*)-pyrrolidinylmethanol underwent Mitsunobu reaction to form 3-alkoxyisoxazoles, which were subsequently reduced with $LiBH_4$ to form the alcohols **17** and **18**. These alcohols were transformed to the corresponding iodides **19** and **20**. The pyridinyl ether **21** and the phenyl ether **22** were obtained through nucleophilic substitution following standard methods. After acidic deprotection and subsequent purification by HPLC, compounds **14** and **15** were obtained as trifluoroacetates. The number of equivalents of trifluoroacetic acid (TFA) in these non-stoichiometric compounds was determined by elemental analysis.

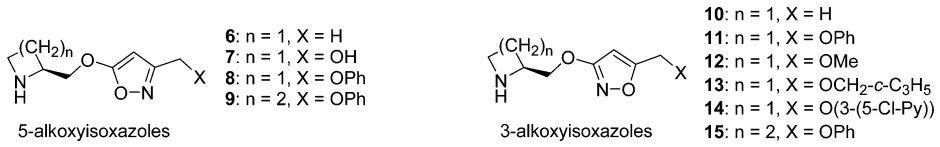
In our previous studies, the 5-alkoxyisoxazoles were identified as novel nicotinic ligands, exemplified by the lead compound **6**, which displayed high selectivity for the β2-containing nAChRs ($\alpha2\beta2$-, $\alpha3\beta2$-, $\alpha4\beta2$-, and $\alpha4\beta2$*-nAChRs) over the β4-containing nAChRs ($\alpha3\beta4$-, $\alpha2\beta4$-, and $\alpha4\beta4$-nAChRs) compared to nicotine.[11] Compound **6** displayed favorable druglike properties in the preliminary absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) studies and demonstrated potent antidepressant-like activity in the classical mouse forced swim test. The selectivity profile of compound **6** was determined by the PDSP broad screening, where it was found to show a high selectivity for the nAChRs among the panel's 50 CNS-related neurotransmitter receptors and transporters, including σ1 and σ2 receptors. During the SAR investigation of this series, a 3-(phenoxymethyl)-substituted isoxazole (compound **8**) was found to bind to the σ1 receptor with moderate affinity (K_i = 328 nM) but not to the σ2 receptor (<50% binding in the primary assay at 10 μM concentration), while maintaining affinity to the nAChRs (see Table 1). Deletion of the benzene ring of **8** gives the corresponding hydroxymethyl analog **7**, which did not show any significant binding affinity to either σ1 or σ2 receptors and also exhibited reduced affinities to the nAChRs. When the azetidine ring of compound **8** was replaced by the larger pyrrolidine ring to give compound **9**, binding to the σ1 receptor was found to be increased 80-fold (K_i = 4.2 nM). At the same time, the binding affinities to the nAChRs were reduced by about 2- to 12-fold compared to the case of compound **7** (see Table 1).

As evident in the preliminary SAR above (Table 1), the phenoxymethyl substituent at the 3-position of the isoxazole was found to be crucial for optimal binding at the σ1 receptor. Intrigued by this phenomenon, we sought more information in the literature on the pharmacophoric elements required for σ1 receptor binding,[12] which include (1) a substituted nitrogen-containing heterocycle (exemplified by piperidine, piperazine, or pyrrolidine)[4] and (2) an aromatic ring, typically a phenyl ring, at a distance of approximately 6–10 Å. Applying this pharmacophore model of the σ1 receptor to compounds **8** and **9**, we found a good match, as shown in the top panel of Figure 2. A side-by-side comparison of the pharmacophores for the σ1 receptors and the nicotinic receptors is provided in Figure 2. For the nicotinic receptors, the hydrogen bond acceptor element is required to be positioned approximately 5–9 Å away from the cationic nitrogen.[14] Once the phenoxymethyl substituent is attached to the isoxazole, the binding affinity to the σ1 receptor begins to manifest itself (cf. compound **7** vs **8** and **9**), in addition to nAChR binding. This is consistent with the known pharmacophore models for the σ1 and nicotinic receptors.

In our previous study with nAChRs, it was observed that the N–O flip in the 5-alkoxyisoxazoles to afford the 3-alkoxyisoxazoles, exemplified by compounds **10**–**14**, diminished affinity to all seven investigated nAChR subtypes, which is possibly due to the misalignment of the key pharmacophoric elements (see Table 2).[11] Inspired by the σ1 binding affinities

dx.doi.org/10.1021/ml3002715 | ACS Med. Chem. Lett. 2012, 3, 1054–1058

Table 1. *In Vitro* Binding Affinities of the 5-Alkoxyisoxazoles 6−15 at Seven Rat nAChRs, σ1 Receptors, and σ2 Receptors[13]



	K_i (nM)[a]								
	nAChRs[b]								
ID	α2β2	α2β4	α3β2	α3β4	α4β2	α4β2*[c]	α4β4	σ1	σ2
6	4.3	311	8.7	692	4.6	12.0	86.0	NA[d]	NA
7	197	>10^4	521	>10^4	137	637	4900	NA	NA
8	47.9	58.0	362	186	23.8	172	27.5	328	NA
9	176	320	2040	809	160	2120	55.1	4.2	NA
10	3340	>10^4	6670	>10^4	1950	7380	>10^4	NA	NA
11	NA	NA	NA	NA	NA	NA	NA	33	1472
12	NA	NA	4100	NA	NA	NA	NA	NA	NA
13	NA	NA	NA	NA	NA	NA	NA	NA	369
14	>10^4	1820	>10^4	6100	2220	>10^4	5580	103	NA
15	NA	NA	NA	NA	NA	NA	NA	4.1	1312
haloperidol[e]								7.0	74
nicotine[e]	5.5	70.0	29.0	260	4.9	9.8	23.0		

[a]See Supporting Information. Radioligands: nAChRs: [^{3}H]epibatidine; σ1: [^{3}H]pentazocine; σ2: [^{3}H]DTG (ditolylguanidine). [b]The K_i values of nAChRs for **6**−**13** are cited from the literature.[11] [c]α4β2*, endogenous receptors prepared from rat forebrain. Besides α4 and β2, other unidentified subunits may also be present. [d]Not active, defined as <50% binding in the primary assay at 10 μM. [e]The K_i values for nicotine and haloperidol are taken from the PDSP Assay Protocol Book.

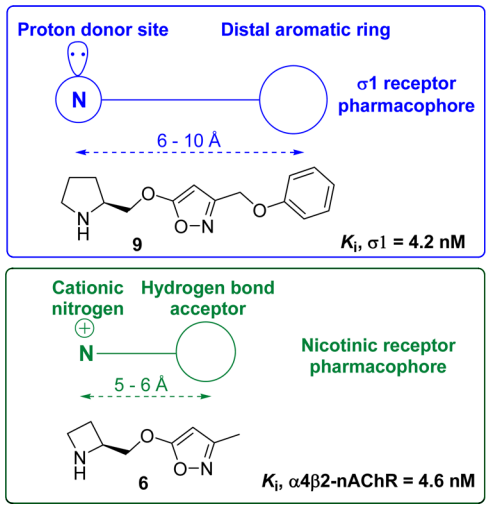


Figure 2. σ1 receptor pharmacophore model (top) and nicotinic receptor pharmacophore model (bottom) and proposed match of substructures with pharmacophore elements for compounds **9** and **6**.

of some of the 5-alkoxyisoxazoles, the 3-alkoxyisoxazoles **10**−**14** were subsequently evaluated for their binding affinity to σ receptors. Encouragingly, the aromatic ethers **11** and **14** were found to be relatively potent binders to the σ1 receptor (K_i = 33 and 103 nM). The phenoxymethyl compound **11**, in particular, is found to be a more potent σ1 binder than the corresponding 5-alkoxyisoxazole **8** (K_i = 328 nM), suggesting that the N−O reversion (isoxazole flip) is highly important for nAChRs binding, but less so for σ1 receptor binding. Compound **10** and the aliphatic ethers **12** and **13** were found to be inactive at the σ1 receptor, as the distal aromatic ring was absent in all these three compounds, consistent with the proposed σ1 pharmacophore (Figure 2). Interestingly, the cyclopropyl analog **13** is found to be a moderately potent σ2 binder (K_i = 369 nM). Given that the replacement of the azetidine ring with a pyrrolidine ring resulted in increased σ1 activity (as seen in **8** and **9**) in the 5-alkoxyisoxazoles, the pyrrolidine analog **15** was then designed and evaluated by PDSP. As expected, the binding affinity of **15** to the σ1 receptor increased by about 8-fold (K_i, σ1 = 4.1 nM, K_i, σ2 = 1312 nM) compared to that of **11**.

A broad-range screening study was carried out for compounds **11** and **15** to further determine their global selectivity profiles among 42 other CNS neurotransmitter receptors and transporters, including serotonin receptors, dopamine receptors, GABA receptors, biogenic amine transporters, adrenergic receptors, muscarinic receptors, opioid receptors, and histamine receptors (tested at 10 μM concentration, NIMH-PDSP, University of North Carolina, Chapel Hill). As shown in Table 3, the two compounds demonstrated good selectivity profiles vs the listed biological targets during this primary screening. A secondary binding study of compounds **11** and **15** was next carried out at those receptors and transporters where the binding efficacy at 10 μM was greater than 50%, which include the serotonergic receptors (5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_6$), the β_2 adrenergic receptor, histaminergic receptors (H$_1$ and H$_2$), the DAT, and the NET. Their K_i values were determined and are shown in Table 4. There were no significant binding activities observed at the serotonergic 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, and 5-HT$_6$ receptors, the β_2-adrenergic receptor, and the H$_1$- and H$_2$-histaminergic receptors (K_i > 4000 nM). Compound **15** was found to be the more selective of the two σ1 ligands (K_i = 4.1 nM) with approximately 90-fold and 50-fold selectivity vs the DAT and NET, respectively. Compound **11** has similar binding affinities at both the σ1 receptor (K_i = 33 nM) and the DAT (K_i = 24 nM) together with moderate NET affinity (K_i = 191 nM).

In the context of the possible use of σ1 ligands as novel antidepressants, compounds **11** and **15** could be considered as triple inhibitors of the σ1 receptor, DAT, and NET. Many

dx.doi.org/10.1021/ml3002715 | ACS Med. Chem. Lett. 2012, 3, 1054−1058

Table 2. Primary Binding Competition Efficacies (%) of Compounds 11 and 15 at Various Neurotransmitter Receptors and Transporters[a]

		serotonergic receptors								
target	ID	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_3	5-HT_{5A}
inhibition	**15**	34.4	−1.2[b]	15.7	−4.7	68	54.5	76.9	7.0	32.4
	11	26.3	2.3	14	5.9	77.9	43.3	68.8	−0.1	15.2

		serotonergic receptors		GABA	dopaminergic receptors					
target	ID	5-HT_6	5-HT_7	$GABA_A$	D_1	D_2	D_3	D_4	D_5	BZPR[c]
inhibition	**15**	28.3	16.9	5.7	−5.7	23.9	35.9	32	36.5	27.1
	11	51.6	42.4	2.5	6.1	22.9	12.9	30	4.9	23.5

		adrenergic receptors								
target	ID	α_{1A}	α_{1B}	α_{1D}	α_{2A}	α_{2B}	α_{2C}	β_1	β_2	β_3
inhibition	**15**	21	27.8	25.2	36.1	44.4	39.3	−1.2	55.6	7.2
	11	17.5	18.1	22.4	17.4	34.8	43.8	22.1	29.4	−1.5

		histaminergic receptors				muscarinic receptors				
target	ID	H_1	H_2	H_3	H_4	M_1	M_2	M_3	M_4	M_5
inhibition	**15**	55	57.2	−4.9	26.5	4.4	11.8	12.2	33.6	36.6
	11	49.2	40.9	4.7	42.6	5.7	12.6	47.6	35.1	44.5

		opioid receptors[d]			transporters[e]		
target	ID	DOR	KOR	MOR	DAT	NET	SERT
inhibition	**15**	10.3	9.4	29.8	59.4	95.9	3.4
	11	4.9	6.7	38.1	91.8	97.2	8.8

[a]The default concentration for primary binding experiments is 10 μM (n = 4). For details see the Supporting Information. [b]Negative inhibition represents a stimulation of binding. [c]BZPR: benzodiazepine receptors (rat brain site). [d]DOR: δ opioid receptor; KOR: κ opioid receptor; MOR: μ opioid receptor. [e]DAT: dopamine transporter; NET: norepinephrine transporter; SERT: serotonin transporter.

Table 3. Secondary Binding Affinities of Compounds 11 and 15 at Selected Neurotransmitter Receptors and Transporters[a]

	K_i (nM)[a]									
ID	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_6	β_2	H_1	H_2	DAT	NET	σ1
11	6405	NA	$>10^4$	$>10^4$	NA	NA	NA	24	191	33
15	4406	5097	$>10^4$	NA[b]	$>10^4$	$>10^4$	5157	373	203	4.1

[a]See Supporting Information. K_i values were determined for those targets where the binding efficacy at 10 μM was greater than 50%. [b]NA: not active, defined as <50% binding in the primary assay at 10 μM.

SSRIs currently in the market inhibit the reuptake of monoamines in the brain. For example, the tricyclic antidepressant desipramine inhibits norepinephrine reuptake, and bupropion is a dual inhibitor of both DAT and NET. A triple reuptake inhibitor of serotonin, dopamine, and norepinephrine, JZAD-IV-22, has also been reported to be an effective antidepressant in a clinical trial.[15] Further work is needed on the functional properties of compounds **11** and **15** to determine whether they are agonists or antagonists at these receptors and transporters.

In conclusion, we report the identification of compound **15** as a selective σ1 ligand resulting from a comparative analysis of the pharmacophoric elements required for σ1 and nicotinic ligands. Attachment of the phenoxymethyl substituent to previously reported $\alpha4\beta2$-nAChR-selective 5-alkoxyisoxazoles provides the required pharmacophoric element to fill in the distal hydrophobic region. A further improvement of selectivity for the σ1 receptor away from the nicotinic receptors was achieved via an N−O flip in the isoxazole ring. Tested at the 50 common CNS neurotransmitter transporters and receptors of the PDSP panel, compound **15** displayed high selectivity for the σ1 vs the σ2 receptor (K_i, σ1 = 4.1 nM, K_i, σ2 = 1312 nM) with moderate binding affinity for the DAT (K_i = 373 nM) and NET (K_i = 203 nM). The main SAR information from this work is that a subtle structural modification in our alkoxyisoxazole nicotinic ligands could be used as a tool to fine-tune their selectivity between the nAChRs and the sigma receptors.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1-312-996-7577. E-mail: kozikowa@uic.edu.

Funding

This research was supported by Award Number U19MH085193 from the National Institute of Mental Health.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health. We thank the PDSP for performing binding affinity assays. We thank Dr. Werner Tückmantel for his assistance in proofreading the manuscript.

■ ABBREVIATIONS

NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine [1-(1-phenylcyclohexyl)piperidine]; CNS, central nervous system; 5-HT, 5-hydroxytryptamine; DMT, *N*,*N*-dimethyltryptamine; nAChR, nicotinic acetylcholine receptor; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; SAR, structure−activity relationship; ADME-Tox, absorption, distribution, metabolism, excretion, and toxicity; TFA, trifluoroacetic acid

■ REFERENCES

(1) Hayashi, T.; Su, T. P. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* **2007**, *131* (3), 596−610.

(2) Wheeler, K. T.; Wang, L. M.; Wallen, C. A.; Childers, S. R.; Cline, J. M.; Keng, P. C.; Mach, R. H. Sigma-2 receptors as a biomarker of proliferation in solid tumours. *Br. J. Cancer* **2000**, *82* (6), 1223−32.

(3) Bowen, W. D. Sigma receptors: recent advances and new clinical potentials. *Pharm. Acta Helv.* **2000**, *74* (2−3), 211−8.

(4) Narayanan, S.; Bhat, R.; Mesangeau, C.; Poupaert, J. H.; McCurdy, C. R. Early development of sigma-receptor ligands. *Fut. Med. Chem.* **2011**, *3* (1), 79−94.

(5) Fontanilla, D.; Johannessen, M.; Hajipour, A. R.; Cozzi, N. V.; Jackson, M. B.; Ruoho, A. E. The hallucinogen N,N-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science (New York, N.Y.)* **2009**, *323* (5916), 934−7.

(6) Ramachandran, S.; Chu, U. B.; Mavlyutov, T. A.; Pal, A.; Pyne, S.; Ruoho, A. E. The sigma1 receptor interacts with N-alkyl amines and endogenous sphingolipids. *Eur. J. Pharmacol.* **2009**, *609* (1−3), 19−26.

(7) Stahl, S. M. The sigma enigma: can sigma receptors provide a novel target for disorders of mood and cognition? *J. Clin. Psychiatry* **2008**, *69* (11), 1673−4.

(8) Antonini, V.; Marrazzo, A.; Kleiner, G.; Coradazzi, M.; Ronsisvalle, S.; Prezzavento, O.; Ronsisvalle, G.; Leanza, G. Anti-amnesic and neuroprotective actions of the sigma-1 receptor agonist (−)-MR22 in rats with selective cholinergic lesion and amyloid infusion. *J. Alzheimer's Dis.* **2011**, *24* (3), 569−86.

(9) Maeda, D. Y.; Williams, W.; Bowen, W. D.; Coop, A. A sigma-1 receptor selective analogue of BD1008. A potential substitute for (+)-opioids in sigma receptor binding assays. *Bioorg. Med. Chem. Lett.* **2000**, *10* (1), 17−8.

(10) Huang, Y.; Hammond, P. S.; Whirrett, B. R.; Kuhner, R. J.; Wu, L.; Childers, S. R.; Mach, R. H. Synthesis and quantitative structure-activity relationships of N-(1-benzylpiperidin-4-yl)phenylacetamides and related analogues as potent and selective sigma1 receptor ligands. *J. Med. Chem.* **1998**, *41* (13), 2361−70.

(11) Yu, L. F.; Tückmantel, W.; Eaton, J. B.; Caldarone, B.; Fedolak, A.; Hanania, T.; Brunner, D.; Lukas, R. J.; Kozikowski, A. P. Identification of novel alpha4beta2-nicotinic acetylcholine receptor (nAChR) agonists based on an isoxazole ether scaffold that demonstrate antidepressant-like activity. *J. Med. Chem.* **2012**, *55* (2), 812−23.

(12) Glennon, R. A.; Ablordeppey, S. Y.; Ismaiel, A. M.; el-Ashmawy, M. B.; Fischer, J. B.; Howie, K. B. Structural features important for sigma 1 receptor binding. *J. Med. Chem.* **1994**, *37* (8), 1214−9.

(13) K_i determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, Ph.D., at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda, MD, USA.

(14) Beers, W. H.; Reich, E. Structure and activity of acetylcholine. *Nature* **1970**, *228* (5275), 917−22.

(15) Caldarone, B. J.; Paterson, N. E.; Zhou, J.; Brunner, D.; Kozikowski, A. P.; Westphal, K. G.; Korte-Bouws, G. A.; Prins, J.; Korte, S. M.; Olivier, B.; Ghavami, A. The novel triple reuptake inhibitor JZAD-IV-22 exhibits an antidepressant pharmacological profile without locomotor stimulant or sensitization properties. *J. Pharmacol. Exp. Ther.* **2010**, *335* (3), 762−70.