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Discovery of Investigational Drug CT1812, an Antagonist of the Sigma-2 Receptor Complex for Alzheimer's Disease

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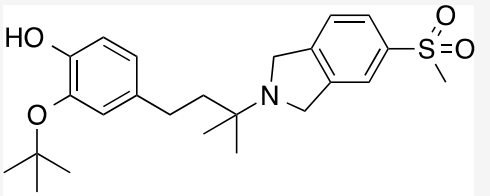
ABSTRACT: An unbiased phenotypic neuronal assay was developed to measure the synaptotoxic effects of soluble Aβ oligomers. A collection of CNS druglike small molecules prepared by conditioned extraction was screened. Compounds that prevented and reversed synaptotoxic effects of Aβ oligomers in neurons were discovered to bind to the sigma-2 receptor complex. Select development compounds displaced receptor-bound Aβ oligomers, rescued synapses, and restored cognitive function in transgenic hAPP Swe/Ldn mice. Our first-in-class orally administered small molecule investigational drug **7** (**CT1812**) has been advanced to Phase II clinical studies for Alzheimer's disease.



Compound **7** (**CT1812**)

KEYWORDS: *CT1812, amyloid oligomer, amyloid oligomer-displacing, AβO, AβO-displacing, Alzheimer's disease drug, sigma-2, transmembrane protein 97, TMEM97, progesterone receptor membrane component 1, PGRMC1, supercritical fluid extraction, SFE, conditioned extraction, CE*

The role of amyloid 1−42 (Aβ) in neurodegeneration and Alzheimer's disease (AD) has been widely studied.[1] Disease-modifying drug development focus has shifted from Aβ fibril and plaque onto soluble Aβ oligomers (AβOs) that are known to be one of the most potently synaptotoxic species of Aβ.[2−12] Particularly synaptotoxic soluble AβOs are freely diffusible within the brain, versus localized fibril- and plaque-associated Aβ.[13] Soluble AβOs exert reversible effects in the human cortex,[7] in neuronal assay systems,[14,15] and in animal models of cognition.[16−18] The pharmacologic blockade of reversible AβO binding to neuronal receptors and its synaptotoxic effects may slow the AβO-induced events associated with synaptic dysfunction and gognitive deficits in MCI and AD. The MTT assay[19] is commonly used as a measure of toxicity in cultured cells and was adapted for use measuring membrane trafficking changes in neurons ("Trafficking Assay", Table 1).[20,21] We used this assay to discover compounds that blocked the synaptotoxic effects of preformed soluble AβOs at sublethal concentrations.[22] Administration of soluble AβOs to this neuronal system demonstrated that both synthetic and human brain-derived soluble AβOs behaved as pharmacologic ligands, bound to and saturated neuronal receptors, and exerted functional synaptotoxic effects related to binding.[22,23] We have utilized this unbiased phenotypic neuronal trafficking assay to screen for CNS druglike small molecules. These molecules also displace bound AβO from neuronal synaptic receptors to prevent and reverse synaptotoxicities.[22−24] The active compounds identified in this assay were able to treat and prevent oligomer-induced deficits in membrane trafficking, reduce the binding of AβOs to neurons, and prevent dendritic spine loss.

The AβO-displacing compounds identified in our unbiased phenotypic neuronal trafficking assay were subsequently screened biochemically against a broad panel of CNS relevant receptors (EuroFins/Cerep panel of 117 trans-membrane proteins and soluble receptors, ion channels, and monoamine transporters). Compound **7** was found to be >100-fold selective for all measured targets save for the sigma-1 receptor for which compound **7** was approximately 10-fold more selective. The effective AβO-displacing compounds reported here were found to be high affinity binders to the sigma-2 receptor.[22,23] We have characterized the binding of our own AβO-displacing compounds in the presence of other known sigma-2 ligands[23] and have found our AβO-displacing compounds to bind potently and selectively to the canonical sigma-2 site.[22−24] A structure−activity relationship emerged between the AβO-displacing activity in our neuronal assay and sigma-2 receptor binding affinity. The sigma-2 receptor has been the subject of ongoing characterization studies and, via affinity purification and micro protein sequencing, identified as

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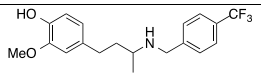
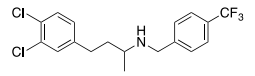
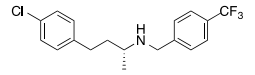
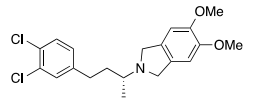
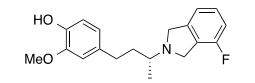
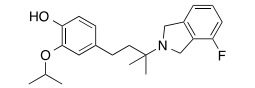
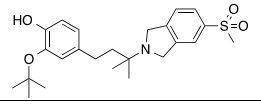
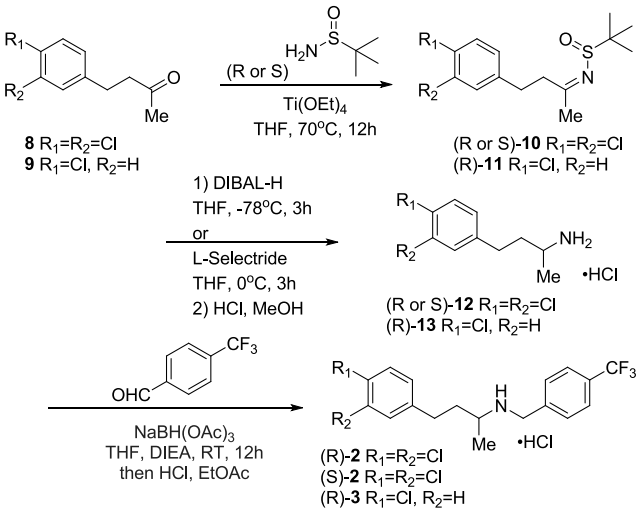
Table 1. Structure–Activity Relationships of the Anti-AβO Compounds 1–7

cmpd	structure	Trafficking assay (uM)	Sigma-2 receptor K_i (nM)	MLM $t_{1/2}$ (min)	hERG EC_{50} (uM)
1		2.2	9	16	nt
(R)-2		2.0	48	33	4.4
(S)-2		11.7	120	53	1.1
3		5.7	50	27	0.78
4		0.7	0.44	61	0.29
5		0.25	79	16	7.0
6		0.25	11	4	9.6
7 (CT1812)		0.31	8.5	45	26

Scheme 1. Synthesis of Anti-AβO Compounds 2 and 3[37–39,45]



transmembrane protein 97 (TMEM97) in tumor cell lines.[25] The TMEM97 protein is likely to form a tight complex with other molecules such as PGRMC1.[26] Sigma-2-selective small molecules have been developed as imaging agents and as potential therapeutic agents for oncologic[27,28] and neurologic[29–32] end points.

AβOs bind saturably and reversibly to a single receptor site at neuronal synapses that mediates synaptotoxic effects and neurodegenerative processes resulting from AβO exposure.[3,33,34] Previous reports indicate this receptor is a multiprotein complex most likely consisting of cellular prion protein (PrP^C),[14] NgR1,[35] and LilRB2.[36]

A CNS druglike chemical library was prepared using our proprietary "conditioned extraction" (CE) platform beginning from supercritical fluid extraction (SFE) of natural materials.[37–39] SFE effectively selects for low molecular weight compounds and simultaneously excludes high molecular weight compounds, polyphenolic compounds, polyionic compounds, and cellulosic materials that would likely interfere with the screening process. The light oil SFE fractions are commonly comprised of relatively volatile low molecular weight components including reactive aldehydes, ketones, lactones, Michael acceptors, epoxides, etc. Extract mixtures containing these reactive natural products are readily "conditioned" by application of one-, two-, or three-step CE procedures including, for example, hydride reduction, organometallic addition, oxidation/reduction, and reductive amina-

Scheme 2. Preparation of Isoindoline Compounds 4–7[45]

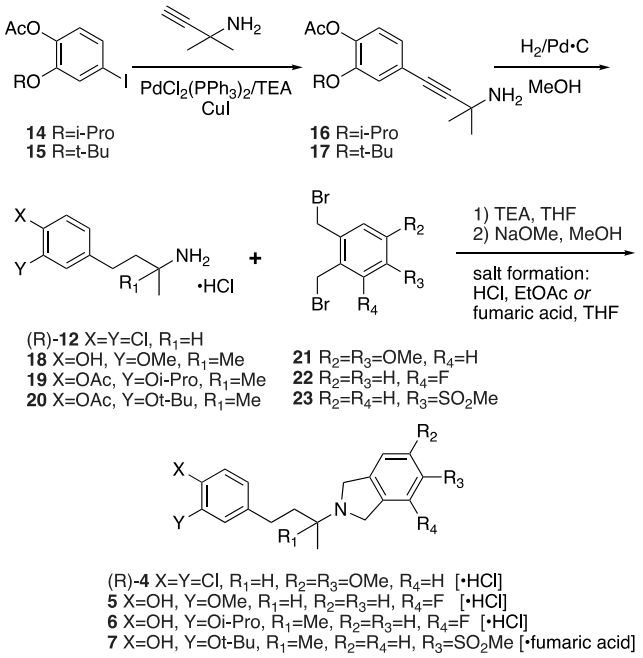


Table 2. Physiochemical Properties and Brain-to-Plasma Ratios (AUC_{brain}/AUC_{plasma}) of Anti-AβO Compounds

cmpd	MW	cLogP	tPSA	Brain/plasma AUC	Brain/plasma 24 h postdose
(R)-2	376.2	6.48	12.3	NT	8.2
3	341.8	5.89	12.0	NT	2.0
6	357.5	4.65	32.7	5.64	5.4
7	431.6	3.26	66.8	2.51	5.7

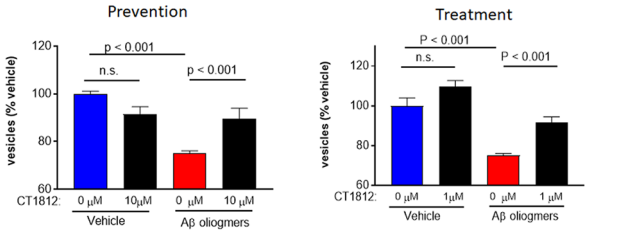


Figure 1. Aβ oligomers (3 μM, red bars) decrease membrane vesicle trafficking rate compared to vehicle (blue bars). Compound 7 (**CT1812**) significantly reduces these deficits when added before Aβ oligomers (Prevention, EC50 = 6.8 uM and 71% recovery) or after Aβ oligomers (Treatment, EC50 = 358 nM and 88% recovery).[24] There is no effect of CT1812 on membrane vesicle trafficking on its own at 30X EC50 for either prevention or treatment bound AβO when added 1 h after addition of AβO.

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Table 3. Compound 7 (CT1812) Reduced Mean Morris Water Maze Swim Length in Transgenic Mice[24]

	nTg + vehicle			Tg + vehicle			Tg + CT1812			nTg + CT1812		
Day	Mean, cm	SEM	N	Mean, cm	SEM	N	Mean, cm	SEM	N	Mean, cm	SEM	N
1	3791	150	12	3518	138	10	3230	115	10	3317	192	12
2	2666	294	12	3252 [a]	329	10	2332	218	10	2739	265	12
3	2173	213	12	2854 [a]	267	10	1987	265	10	2262	259	12
4	1797	219	12	2426	287	10	1611	231	10	2008	160	12

[a] $p < 0.5$ vs nontransgenic + vehicle.

Table 4. Compound 7 (CT1812) Restored Spontaneous Alteration in the Y-Maze Test of Spatial Working Memory

nTg + vehicle			Tg + vehicle			Tg + CT1812			nTg + CT1812		
Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
62.6%	3.7%	11	56.1%	2.8%	11	58.5%	2.8%	11	65.3%	6.0%	12

[a] $p < 0.5$ vs random chance (50%).

Table 5. Compound 7 (CT1812) Restored Freezing Time in the Contextual Fear Conditioning Test

nTg + vehicle			Tg + vehicle			Tg + CT1812			nTg + CT1812		
Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
52%	5	11	32%[a]	6	12	45%	6	12	51%	5	11

[a] $p < 0.5$ vs nontransgenic + vehicle.

tion to provide structurally diverse chemically stable CNS druglike mixtures of alcohols and "alkaloidal" amines and amino alcohols. A two-step reductive amination CE protocol was performed on low molecular weight fractions of SFE ginger oil to produce mixtures of chemically stable low molecular weight natural and unnatural products.[37] The mixtures were fractionated, and the fraction pools were screened in our phenotypic neuronal assay. The racemic secondary amine **1** (Table 1), which was derived from the reductive amination of natural gingerone present in the original SFE ginger oil mixture on reaction with 4-(trifluoromethyl)-benzylamine, protected neurons from AβO-induced synaptotoxicity and displayed anti-AβO activity. The metabolic instability of **1** as measured by mouse liver microsomes (MLM) prompted our search for structurally analogous metabolically stable anti-AβO compounds for oral administration in a transgenic AD mouse model. Substitution for or removal of the HO- and MeO-substituents of compound **1** was investigated to probe the likely possibilities of oxidative and conjugative metabolism at these sites. The chlorinated compounds **2** and **3** exhibited promising anti-AβO activity and significantly improved metabolic stability and so were suitable for advancement to animal behavioral studies. Comparative assay of the enantiomers (R)-**2** and (S)-**2** demonstrated significant differences in neuronal activity and hERG activity (EuroFins, IC_{50} based on hERG-CHO, automated patch-clamp assay). We proceeded to prepare the generally superior (R)-isomers for subsequent analogue work in this series; however, the hERG activity of these relatively lipophilic amine compounds remained a concern. We discovered that reintroduction of phenol and alkoxy aryl substituents resulted in diminished hERG activity likely due to increased polar surface area (PSA) of the oxygenated compounds relative to the chlorinated compounds. However, improvement in hERG activity upon increase in PSA was often accompanied by diminished neuronal activity and metabolic instability. A significant breakthrough came with a series of isoindoline analogues exemplified by compounds **4**–**7**. Introduction of the isoindoline moiety realized submicromolar neuronal activity. Furthermore, it allowed for the reintroduction of oxygenated substituents and seemed to strike a balance between increased PSA (to address hERG activity[40−42]) and improved neuronal activity and metabolic stability. Our advanced candidate isoindolines **6** and **7** benefited from the combination of properties including relatively high PSA, oppositely polarized aromatic rings, a gem-dimethyl substitution alpha to nitrogen, and the favored isoindoline moiety.

Significant increases in PSA were of particular benefit to our advanced candidate isoindolines **6** and **7**. The introduction of the methyl sulfone substituent increased PSA between 2- and 5-fold in our various structural analogue series. However, the dramatic increase in PSA and the accompanying increase in molecular weight often resulted in loss of neuronal activity or metabolic instability. The methyl sulfone substitution was well-tolerated in the isoindoline series and particularly wins the case of the isoindoline compound **7**. Compound **7** exhibited excellent neuronal activity, high affinity binding at sigma-2, and excellent selectivity versus a blockade of hERG ion currents. Notably, the chiral center present in compounds **1**–**4** had been replaced by gem-dimethyl substitution alpha to the isoindoline nitrogen. The gem-dimethyl substitution in compounds **6** and **7** likely blocked oxidative metabolism at the carbon alpha to nitrogen and was also likely responsible for inducing entropically favorable restrictions to bioactive conformations,. The use of the gem-dimethyl substitution is a strategy that has been used by medicinal chemists when designing clinical useful compounds.[43]

The available[37−39] methyl ketones **8** and **9** (Scheme 1) were converted by applying Ellmann's method[44] to the *N*-*tert*-butanesulfinyl ketimines **10** and **11**. Subsequent hydride reduction provided the chiral alpha-methyl amines **12** and **13**. The chiral amines were subsequently converted via reductive amination to the chiral anti-AβO compounds (R)-**2**, (S)-**2**, and **3**. The unoptimized overall yield from ketones **8** or **9** is around 6%.

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A general synthesis of isoindoline anti-AβO compounds was developed (Scheme 2). Ready access to the gem-dimethyl-substituted isoindolines was realized *via* palladium-mediated coupling[46] of aryl iodides **14** and **15**[45] to 2-methyl-3-butyn-2-amine to provide the gem-dimethyl-substituted propargyl-amines **16** and **17** (74% yield for **17**). These were subsequently hydrogenated to the intermediate amines **19** and **20** (100% yield for **20**). The available chiral alpha-methyl amines **12** and **18**[37−39] and the gem-dimethyl-substituted amines **19** and **20** were condensed with the corresponding bis-benzylbromides **21**−**23** to provide the isoindoline anti-AβO compounds **4**−**7** (63% yield for **7**).[45]

Our early lead compounds **2** and **3** had exhibited promising neuronal activity and performed well in animal behavioral models but were exceedingly lipophilic as reflected in their low PSA (∼12). These compounds exhibited problematic off-target activity profiles and hERG activity. Certain trends in physiochemical properties including a substantial increase in total polar surface area (PSA) (Table 2) contributed to progress in the program and enabled the nomination of suitable development candidates. It became clear that increased PSA addressed off-target and hERG activities. The reintroduction of phenol and alkoxy substituents as in compounds **5** and **6** informed the design of next generation compounds. Incorporation of the aryl methyl sulfone increased PSA, minimized off-target activities and hERG activity, and enabled the nomination of our clinical candidate **7**. Compound **7** exhibits good absorption and a particularly high brain-to-plasma ratio of 5.7 at 24 h postdose (Kp,uu = 6.75). The free fraction of compound **7** in the brain is 13% and in the plasma is 5%. Compound **7** is not a Pgp substrate and has a CACO-2 Papp(B-A)/Papp(A-B) = 0.95.

In primary hippocampal and cortical neuronal cultures AβOs were found to bind specifically and to saturate a single receptor site on some but not all neurons.[22,23] The anti-AβO compounds reported here prevented and restored trafficking deficits caused by AβOs in neurons. The offset between sigma-2 binding affinity and potency in the in vitro membrane vesicle trafficking assay likely results from several factors such as (1) lipophilic compound adsorption to microtiter plate plastic that lowers the effective concentration of the compounds, (2) greater than 80% of sigma-2 receptors need to be occupied by compound to achieve an effect on binding and function, or (3) the high concentration of the low potency synthetic Aβ preparations needed to achieve adequate testing windows in the in vitro assays. Clinical candidate **7** prevented and reversed trafficking deficits caused by AβOs but had no effect in the absence of AβOs (Figure 1). Compound **7** also prevented binding AβO to neuronal receptors, displaced prebound AβO, and was determined by a one-site ELISA assay to have no effect on AβO assembly or AβO dissociation.[24,47]

The anti-AβO compounds **2**, **3**, **6**, and **7** restored cognitive function in transgenic hAPP Swe/Ldn mice (Tables 3−5).[22,24,48] The drug-treated transgenic mice performed in the Morris water maze task significantly better than did the transgenic vehicle-treated mice (Table 3). Treatment with compound **7** does not affect nontransgenic animal performance. Transgenic mice treated with compound **7** remembered previous arms entered in the Y-maze task significantly better than chance but vehicle-treated transgenic animals did not (Table 4). Transgenic mice treated with compound **7** demonstrated significant improvements in spatial and cue-dependent learning and memory compared to vehicle-treated animals in the fear conditioning assay (Table 5).

Microelectrodes coated with antibodies for total Aβ or AβOs were inserted into the brains of live transgenic mice and measured soluble Aβ concentration by the minute.[24,49] Intravenous administration of compound **7** increased release of AβO oligomers and increased AβO concentration in the interstitial brain fluid without causing a measurable increase in Aβ monomer concentration. Remarkably, a concomitant increase in the concentration of AβO in the cerebrospinal fluid (CSF) was also observed.[24] This strongly suggests that AβO displacement by compound **7** in the brain facilitates clearance of interstitial AβO to the CSF.

This limited structure−activity relationship represents a medicinal chemistry effort that produced hundreds of structural analogues. Our effort to optimize anti-AβO activity in our neuronal assay was complicated by issues of off-target activities and pharmacokinetics that are beyond the scope of this Letter. However, the structural features required of the anti-AβO pharmacophore are well represented here. In summary, the simple lipophilic benzylic amines **2** and **3** exhibited promising anti-AβO activity and acceptable metabolic stability. They were efficacious in animal behavioral testing[22,24] but suffered from hERG and other off-target activities due, apparently, to their relatively low PSA. The isoindolines **4**−**7** exhibited promising submicromolar neuronal activity. The isoindoline scaffold tolerated polar substitution and allowed dramatic increases in PSA leading to highly active compounds with minimal hERG and off-target activities. Our advanced candidates **6** and **7** benefitted as well from gem-dimethyl substitution alpha to nitrogen that imparts metabolic stability and optimal conformational biases for anti-AβO activity.

The CNS druglike small molecules reported here are potentially first-in-class therapeutics for the treatment and prevention of early cognitive decline and neurodegeneration in MCI and AD patients. Our first-in-class clinical candidate compound **7** (**CT1812**)[45] is an AβO-displacing compound and a potent and highly selective antagonist of the sigma-2 receptor. Compound **7** has been demonstrated to prevent AβO binding to neurons and also to displace bound AβO from neuronal receptors.[22−24] It has been determined to have no effect on AβO assembly or AβO dissociation in a biochemical ELISA assay.[24,47] It is metabolically stable and exhibits good pharmacokinetics and robust brain exposure and restores synapse number and cognitive function in transgenic mouse models.[24,48] Remarkably, compound **7**, upon AβO-displacement in the rodent brain, facilitates the clearance of AβO from brain interstitial fluid to CSF as demonstrated in a mouse microimmunoelectrode study.[24] Unlike other Aβ-targeted therapeutics, by displacing Aβ oligomer binding, CT1812 lowers Aβ oligomer affinity for its receptor, the same phenotype observed with the protective Icelandic mutation that confers 4-fold lower incidence of Alzheimer's disease on carriers.[50]

In phase I clinical studies, compound **7** was determined to be safe and well tolerated at single doses up to 1120 mg and at multiple doses up to 560 mg in healthy elderly volunteers.[51,52] Adverse events, most commonly headache and gastrointestinal symptoms, were mild to moderate in severity. Plasma concentrations of drug were found to be dose proportional, and CSF concentrations upon multiple doses exceeded the expected minimum target concentrations required to improve

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memory in AD patients. Compound **7** (**CT1812**) is an orally administered first-in-class small molecule drug candidate for Alzheimer's disease. When administered to mild to moderate Alzheimer's patients once daily for 28 days, **CT1812** significantly increased CSF concentrations of Aβ oligomers in AD patient CSF, reduced concentrations of synaptic proteins and phosphorylated tau fragments, and reversed expression of many AD-related proteins dysregulated in CSF compared to placebo.[24] Four randomized, double-blind, placebo-controlled phase II clinical studies are currently underway in patients with mild to moderate AD: SNAP (NCT03522129), SPARC (NCT03493282), SHINE (NCT03507790), and SEQUEL (NCT04735536).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00048.

Details of materials and methods used in this work including NMR, MS, and chemical synthesis (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of themanuscript.

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Notes

The authors declare the following competing financial interest(s): Gilbert M. Rishton, Gary C. Look, Nicholas J. Izzo, Kelsie M. LaBarbera, Colleen S. Limegrover, Courtney Rehak, Raymond Yurko, and Susan M. Catalano are current or former employees of Cognition Therapeutics, Inc.

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■ ABBREVIATIONS

AD, Alzheimer's disease; AβO, soluble Aβ oligomer; MCI, mild cognitive impairment; CNS, central nervous system; TMEM97, transmembrane protein 97; SFE, supercritical fluid extraction; CE, conditioned extraction; PSA, polar surface area; CSF, cerebrospinal fluid; MIE, microimmunoelectrode

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