^{15}N-Labeled 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl

[^{15}N]TEMPONE

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Created: April 30, 2008; Updated: June 9, 2008.

Chemical name:	^{15}N-Labeled 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl	
Abbreviated name:	[^{15}N]TEMPONE	
Synonym:	^{15}N-Labeled 2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyl, ^{15}N-labeled 2,2,6,6-tetramethyl-4-oxo-piperidine-N-oxy free radical, ^{15}N-labeled triacetoneamine-N-oxyl, ^{15}N-labeled-4-oxo-TEMPONE	
Agent catagory:	Small molecule	
Target:	Other	
Target category:	Other –reactive oxygen species (ROS)	
Method of detection:	Electron paramagnetic resonance imaging (EPRI), magnetic resonance imaging (MRI), proton electron double resonance imaging (PEDRI), Overhauser-enhanced MRI (OMRI)	
Source of signal/ contrast:	Nitroxide	
Activation:	No	
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals	No structure is currently available in PubChem.

Background

[PubMed]

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NLM Citation: Zhang H. ^{15}N-Labeled 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl . 2008 Apr 30 [Updated 2008 Jun 9]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Reactive oxygen species (ROS) are various free radicals generated in a biological milieu (1, 2). They are propagated through a cascade of reactions in the pathogenesis in many diseases, including cancer, stroke, atherosclerosis, ischemia-reperfusion injury, Alzheimer's disease, diabetic vascular diseases, and inflammatory diseases (2). In particular, ROS interact with glutathione (GSH), NADPH, and ascorbates to maintain cellular redox status (3). Therefore, the distribution of ROS in tissue can be used as a surrogate marker to characterize the redox status/environment in disease-related physiological and pathological conditions (1). Because all free radicals contain unpaired electrons, the electron paramagnetic resonance (EPR) technique, also called electron spin resonance (ESR), is specific for detecting and quantifying ROS (2). EPR spectra can provide a wealth of information for unequivocal identification of free radicals, such as fine, hyperfine, and superhyperfine structures, g-factor, and lineshape (2). EPR imaging (EPRI) technique allows for non-invasive mapping of free radicals in animals/organs (4).

EPR is fundamentally similar to nuclear magnetic resonance (NMR) (5). However, the differences in the physical and chemical properties of the resonance species (unpaired electrons *versus* nuclear spin) lead to three major differences in acquiring the spectra/images: gyromagnetic ratio, relaxation time, and concentration (5). The gyromagnetic ratio of an electron spin is 658 times larger than that of a proton nuclear spin, resulting in a 658-fold increase in its magnetic moment and resonant frequency. For instance, with a magnet of 0.34 T, the EPR frequency of X-band is 9.5 GHz, and the NMR frequency of proton nuclei is 14.4 MHz. As a result of the presence of strong non-resonant water absorption, a high radiofrequency such as 9.5 GHz is not suitable for examining tissue samples. Thus, much lower EPR frequencies in the range of 1.2 GHz (L-band) to 300 MHz are used instead, corresponding to a penetration depth of a few cm. The increase in the magnetic moment of electron spin provides ~700 times greater intrinsic sensitivity with EPR on a molar basis than with NMR. Because the excited electron spins relax on a nanosecond time scale, which is several orders of magnitude shorter than the nuclear spin (measured in ms), pulsed EPR (Fourier transformation EPR (FT EPR) or time-domain EPR) is only applicable to those free radicals with an extremely narrow line, whereas most ERP spectrometers use the continuous wave technique (CW EPR). The lack of high concentrations of naturally occurring paramagnetic species such as free radicals often requires the addition of paramagnetic species. This in turn allows for the quantification of exogenous paramagnetic species but also requires the acquisition of the anatomic information with different imaging modalities such as magnetic resonance imaging (MRI). Proton electron double resonance imaging (PEDRI), also called Overhauser-enhanced magnetic resonance imaging (OMRI), is a double resonance technique that encodes characteristic EPR spectral information on a high-resolution MRI (6). This method uses EPR irradiation to saturate paramagnetic species and leads to polarization of water protons through the dynamic nuclear polarization (DNP) effect. The polarized protons produce enhanced signal intensity in MRI. PEDRI offers good sensitivity, high spatial resolution, and signal enhancement of approximately two orders of magnitude (7).

Nitroxides are stable organic free radicals that have a single unpaired electron delocalized between the nitrogen and the oxygen (8). The steric hindrance around the nitroxide group

makes these compounds very stable. They can be obtained in pure form, and they can be stored and handled in the laboratory with no more precautions than most organic substances (9). Nitroxides used as the contrast agent in EPRI can detect the redox status on the basis of their reduction to EPR-silent hydroxylamine (10), and nitroxides have been extensively used in cells, tissues, and living animals (11). Inside cells, nitroxides are reduced to hydroxylamine by cellular antioxidants such as ascorbate, thioredoxin, reductase, ubiquinol, NADPH and GSH . Nitroxides also can function as superoxide dismutase mimics and repair DNA damage caused by ultraviolet irradiation. In addition to the use as an EPRI contrast agent, nitroxides are T_1 relaxation agents in MRI for having an unpaired electron (12). Because their reduced form, hydroxylamine, is diamagnetic, the reduction process is accompanied by a decrease in T_1 relaxivity. This decrease reflects the alterations in the redox status and can be used to map the redox status. Although the T_1 relaxivity of nitroxides is much lower than that of gadolinium chelates (one unpaired electron *versus* seven unpaired electrons), their high cellular permeability leads to a significantly greater volume distribution in tissues and compensates for their lower relaxivity (12). Various nitroxides are designed to target different cellular compartments (8). For example, a neutral nitroxide can be distributed throughout the intracellular and extracellular environments, whereas a charged nitroxide is unable to cross the plasma membrane and can be used to measure oxygen levels in extracellular compartments.

^{15}N-Labeled 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl ([^{15}N]TEMPONE) is a ^{15}N-labeled (spin = 1/2) neutral nitroxide (13). Commercially available TEMPONE exhibits low toxicity with a maximum tolerated dose (MTD) of ~1 mmol/kg (14). Compared to the commercial TEMPONE, which has a natural isotope of nitrogen (^{14}N, spin = 1) with a triplet EPR hyperfine structure, [^{15}N]TEMPONE has a doublet EPR hyperfine structure (13). The decrease in the number of hyperfine structures from three to two leads to a 1.5-fold increase in EPR signal intensity and DNP enhancement, assuming that the motional correlation time of hydroxides is faster than the inverse spin precession (Larmor) frequency of the electron spin (15). The characteristic doublet of ^{15}N-labeled nitroxides is easily distinguished from the triplet structure in the natural isotope of ^{14}N in other nitroxides. Thus, multiple nitroxides labeled with different isotopes (^{14}N and ^{15}N) can be used to separate different reduction or oxidation processes *in vivo* (13).

Synthesis

[PubMed]

[^{15}N]TEMPONE was synthesized by reaction of commercial phorone ($(CH_3)_2CCHCOCHC((CH_3)_2)$) with ^{15}N-labeled ammonium chloride (16).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publication is currently available.

Animal Studies

Rodents

[PubMed]

Utsumi et al. examined the distribution of [^{15}N]TEMPONE in mice (5 weeks old) in the presence of commercial ^{14}N-nitroxides by OMRI (13). After intragastric administration of 200 µl of 10 mM [^{15}N]TEMPONE followed by intravenous injection of 200 µl of 300 mM 3-carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidinyl-N-oxyl (3CP) solution, OMRI was conducted on a custom-built MRI spectrometer. The EPR excitation frequency values (B_0^{EPR}) for ^{14}N-nitroxide and ^{15}N-nitroxide were 6.103 mT and 6.563 mT, respectively, and the NMR frequency for proton nuclei was 617 kHz. Interleaved ESR excitation was used in the OMRI sequence to alternately excite the ^{15}N-radicals ([^{15}N]TEMPONE) and the ^{14}N-enriched radicals (3CP). The images were collected at 1.5, 4.5, 7.5, and 11.5 min after the injection of 3CP. The signal of ^{15}N-radicals appeared to be confined to the deposited site (i.e., the stomach), whereas the signal of ^{14}N-radicals exhibited a global distribution over the whole mouse and their signal intensities were dependent on organ/region. This study demonstrated the possibility of tracking ^{14}N- and ^{15}N-labeled nitroxides simultaneously as well as the co-registration of their distribution in the same anatomic image.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

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