Synthesis And *In-Silico* Anti-Cancer Potential of N-Aryl-Keto-Nitrone As A Spin Adducts

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Abstract

Introduction: This study explores N-Aryl-Keto-Nitrone synthesis and its impact on spin-trapping chemistry, revealing crucial insights into free radicals' roles in diseases. While aldo-nitrones are well-studied, keto-nitrones, especially linear ones, are underexplored as spin traps. Seven novel keto-nitrones are introduced, and their efficacy in capturing carbon-centered radicals is assessed. Methods: Synthesis involves dissolving N-aryl-nitroso compounds in THF, adding NaOH, and introducing dimethyl or diethyl bromo malonate. Thin-layer chromatography monitors the reaction, yielding crude keto-nitrones purified through extraction, drying, and recrystallization. Spin trapping experiments use various radical sources, analyzed by EPR at room temperature. In silico predictions determined ADME properties, P-glycoprotein substrate potential, and molecular docking determined binding orientations with VEGFR1. Results: Diverse N-aryl-keto-nitrones with unique structures and reactivity towards carbon-centered radicals are successfully synthesized. Enhanced interpretability is observed in penta-deuterated compounds N6 and N7. The compounds exhibit varying lipophilicity and resistance to oxidative or reducing agents, broadening their potential applications. *In silico* predictions show favorable properties, and the compounds demonstrate potential as VEGFR1 downregulators, suggesting applications in disrupting angiogenic signals in cancers. Conclusion: This research advances spin-trapping chemistry by introducing linear keto-nitrones as effective agents. The synthesized compounds demonstrate versatility and impact, with ongoing research focusing on additional applications and refinement for practical use.

Keywords. Nitrones, Electron paramagnetic resonance spectroscopy, Spin trap, Free radicals, anti-angiogenesis.

Significance | Advancing spin trapping chemistry through the introduction of effective linear keto-nitrones, expanding applications and refining practical use.

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Introduction

Free radicals have been implicated in playing an important role in the development of many diseases, as well as having a role in many normal physiological processes, ranging from intermediates in enzyme reactions to roles as effectors (especially nitric oxide and, perhaps, superoxide) (Ke Jian Liu et al., 1999, Loredana Maiuolo et al., 2018, Germán et al., 2015). The toxicity of many drugs and chemicals is also known sometimes to be mediated through the generation of free radicals (Ke Jian et al., 1999). While the existence of free radicals in these biological processes can be inferred from end-product analysis and from the effects of antioxidants or enzymes such as superoxide dismutase, the technique of electron paramagnetic resonance (EPR) spectroscopy allows for their direct detection and, potentially, much more extensive characterization of their generation and reactions.

Short-lived free radicals may be detected in many different chemical or biological systems by using the spin trapping approach (E. G. J. A. o. C. R. Janzen, 1971), (Evans, 1979), (Mottley & Mason, 1989),

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(Schneider, Freed, & J. Reuben, 1989). Traditional spectroscopy using electron paramagnetic resonance (E. G. J. A. o. C. R. Janzen, 1971), (Evans, 1979), (Mottley & Mason, 1989), (Schneider, Freed, & J. Reuben, 1989), (Tordo, 1998) may be used to identify an adduct formed when a short-lived free radical reacts with a trapping agent. Common traps include nitrones and nitroso compounds, with the resultant adduct being a nitroxide or aminoxyl radical. We will not go into detail here about the benefits and downsides of these two types of spin traps, since they have been discussed extensively elsewhere (E. G. J. A. o. C. R. Janzen, 1971), (Evans, 1979), (Mottley & Mason, 1989), (Schneider, Freed, & J. Reuben, 1989), (Tordo, 1998), (Buettner & medicine, 1987). In contrast to nitrones, the addend of nitroso compounds is covalently bonded to the nitrogen, resulting in more hyperfine couplings. However, they are harmful and unstable under heat and light, and their oxygen-centered radical adducts typically only last a short period. Because of this, nitrones are often used, especially in biological contexts. Scheme 1 depicted the structure of the frequently used spin trapping DMPO and PBN

For instance, keto-nitrones have been used as synthetic instruments in 1,3-dipolar addition processes (Roubaud et al., 2001), (Holzapfel & Crous, 1998),(Tuffariello, 1984), (Torssell, 1988), (Black & Johnstone, 1984), (Torrente, Noya, Branchadell, & Alonso, 2003), (Fischer, Hyrosova, Fisera, Hametner, & Cyranski, 2005),(Franco, Merchan, Merino, & Tejero, 1995), (Nishi, Hagi, Ide, Murakami, & Makino, 1992). However, a review of the literature reveals that never before has any linear nitrone been utilized as an agent that traps spins, and only a small number of cyclic keto-nitrones have been used in studies (E. G. Janzen & Zhang, 1993),(Rosen et al., 2000),(Bottle, Micallef, & chemistry, 2003),(Boyer, Bernardes-Genisson, Farines, Souchard, & Nepveu, 2004),(Sár, Ősz, Jekő, & Hideg, 2004), (Reybier et al., 2006),(Ionita, 2006), (Rizzi, Lauricella, et al., 1997).

This is especially noteworthy since several aldo-nitrones, which are generated from either (Z)-benzylidene(tert-butyl)azane oxide (PBN) or 5,5-dimethyl-3,4-dihydro-2H-pyrrole N -oxide (DMPO), have been extensively used to identify free radicals in a variety of media. This is partially because the majority of techniques widely employed to generate aldo-nitrones are not transferable to the synthesis of keto-nitrones, contributing to the difficulty experienced in the manufacture of these compounds (Roubaud et al., 2001), (Holzapfel & Crous, 1998),(Tuffariello, 1984), (Torssell, 1988), (Black & Johnstone, 1984), (Torrente, Noya, Branchadell, & Alonso, 2003), (Fischer, Hyrosova, Fisera, Hametner, & Cyranski, 2005),(Franco, Merchan, Merino, & Tejero, 1995), (Domingues et al., 2003). Furthermore, using the EPR spectra of the adduct to determine the identity of the addend is more difficult when using keto-nitrones as spin trapping agents than when using aldo-nitrones because there isn't any hyperfine coupling between the unpaired electron and a hydrogen nucleus in the β-position towards the nitrogen(Buettner & medicine, 1987), (E. G. Janzen & Zhang, 1993),(Rosen et al., 2000),(Bottle, Micallef, & chemistry, 2003),(Boyer, Bernardes-Genisson, Farines, Souchard, & Nepveu, 2004),(Sár, Ősz, Jekő, & Hideg, 2004), (Reybier et al., 2006),(Ionita, 2006), (Rizzi, Lauricella, et al., 1997). But take note that spin trapping in conjunction with other analytical techniques like mass spectrometry and HPLC may yield more details about the structure of the trapped radical (Qian, Yue, Tomer, Mason, & Medicine, 2003),(Kumamoto, Hirai, Kishioka, & Iwahashi, 2004),(Defoin, 2004).

However, the application of the spin trapping technique in biological systems, in vivo in particular, has been limited and sometimes difficult because of the stability, or lack of stability, of the spin adducts in the viable systems where a whole array of reducing systems ready to convert the paramagnetic adducts into EPR silent products. For example, the half-life of DMPO superoxide adduct in neutral media is about 1 min, while under in vivo conditions, the EPR signal has never been observed. Recently, many attempts have been to design and synthesize spin traps that could produce adducts more resistant to bioreduction. One of the newly synthesized traps is DEPMPO, a phosphorylated derivative of the widely used DMPO, which has been reported to produce spin adducts with a longer lifetimes, particularly for the adduct of superoxide (Ke Jian et al., 1999) . The spin adducts of DEPMPO usually have characteristic EPR spectra, which makes it possible to identify the trapped radical unambiguously. Another significant advantage of using DEPMPO in trapping superoxide radicals is that DEPMPO/O2• decomposition does not produce the OHradical dot adduct, which is a significant drawback when using DMPO.

The objective of the study was to investigate the synthesis and biological activity of N-Aryl-Keto-Nitrone in silico. Seven N-aryl-C, C-di-alkoxy carbonyl nitrones (N1–N7) were synthesized, with six being novel, derived from suitable aryl-nitroso compounds. The primary focus was on exploring the efficiency of these keto nitrones in capturing carbon-centered free radicals in aqueous conditions, forming long-lived aminoxyl radicals with persistent EPR spectra. Additionally, the reactivity of specific compounds (N6 & N7) towards methoxyl radicals was examined. The study introduced linear keto nitrones as spin traps for the first time, emphasizing their potential as effective tools for trapping radicals in functioning biological systems, showcasing improvement over the commonly used DMPO trap.

Materials and Methods

The chemicals and solvents were procured exclusively from Sigma-Aldrich, whereas the enzymes were sourced from Boerhinger Mannheim Co. The solvents used were of the utmost purity

attainable in the commercial market and employed without further purification. Aqueous media for spin trapping experiments were prepared using tri-distilled water. The buffer solutions were agitated for 4 hours while a chelating acid resin (4 g dm−3) was present to eliminate small amounts of metallic contaminants. Nitroso benzene and N, N-dimethyl-4-nitrosoaniline were readily obtainable from commercial sources, while the other N-aryl-nitroso compounds were synthesized using previously known techniques (Gowenlock, Maidment, Orrell, Prokes, & Roberts, 2001),(Shine, Zmuda, Kwart, Horgan, & Brechbiel, 1982),(Duling, 1994).

Synthesis

The Following method was utilized to synthesize the spin addcuts (Nishi, Hagi, Ide, Murakami, & Makino, 1992). 1,3-dimethoxy-N-(4-methoxyphenyl)-1,3-dioxopropan-2-imine oxide N1 was prepared. The additional N -aryl-C, C-di-alkoxy-carbonyl-nitrones N1-N7 were synthesized using a comparable procedure, as explained shortly. The proper N-aryl-nitroso compound was dissolved in THF (30 mmol in 30 cm3) and then added to a solution of NaOH thirty millimoles in thirty cubic centimeters of water. After bringing the medium down to 5 degrees Celsius, either 33 mmol of dimethyl bromo malonate or diethyl bromo malonate in twenty cm^3 of THF was gradually over two hours while the mixture was vigorously stirred. After three to five hours, THF was removed under decreased pressure to halt the reaction, which used thin-layer chromatography to monitor. Then, 10 cm^3 of ice water was added. The corresponding crude keto-nitrones N2–N7 was obtained via dichloromethane extraction, drying under $MgSO_4$, and then evaporating off the solvent. Both flash chromatography and recrystallization were used to refine these molecules further. The synthesis produces reported following are individuals determined following the process of purifying. 1H NMR spectra were used to identify the keto-nitrones. The J values are presented in Hz, and the chemical shifts (δ) are recorded and expressed in parts per million (ppm) compared to the Internal Transportation Management System. EPR spectroscopy confirmed that these keto-nitrones were pure of whatever paramagnetic contaminants.

5,5-diethyl-4,6-dioxo-2-(*p*-tolyl)-1,3,2-dioxazinane-2-oxide **N2** was obtained as an oil in 94 % yield after flash- flash-chromatography (silica, 30 % pentane in diethyl-ether). 1H NMR ($CDCl_3$, 300 MHz): δ 2.07 (4H, q, 2 CH_2), 0.74 (6H, t, 2 CH_3), 2.43 (3H, d, CH_3), 7.49 (2H, d, H arom.), 6.94 (2H, d, H arom.).

2-(3-(dimethyl amino) phenyl)-5,5-diethyl-4,6-dioxo-1,3,2-dioxazinane-2-oxide **N3**

a 30% yield was achieved by recrystallization in anhydrous diethyl-ether, resulting in crystals with a melting point of 82°C.1H NMR ($CDCl_3$, 300 MHz): δ 1.91 (4H, t, 2 CH_2), 2.9 (6H, q, 2 CH_3), 3.02 (6H, s, $N(CH_3)_2$), 4.16 (H, q, 2 CH_2), 3.91 (6H, q, 2 CH_3), 7.15 (2H, dd , H arom.), 7.45 (1H, dd, H arom.). 7.65 (1H, dd, H arom.).

2-(3-(methoxy carbonyl) phenyl)-5,5-dimethyl-4,6-dioxo-1,3,2-dioxazinane-2-oxide **N4,** a 70 % yield was achieved by recrystallization in anhydrous diethyl- ether, resulting in crystals with a melting point of 144 ◦C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (3H, s, OCH_3), 1.39 (6 H, s, 2 CH_3), 7.64 (2H, dd, H arom.), 8.16 (2H, d, , H arom) ,8.0(1H ,t, H arom.), 8.2 (1H, dd.H arom.).

2-(3-chlorophenyl)-5,5-diethyl-4,6-dioxo-1,3,2-dioxazinane -2-oxide **N5,** a 37 % yield was achieved by recrystallization in anhydrous diethyl- ether, resulting in crystals with a melting point of 117 ◦C. 1H NMR ($CDCl_3$, 300MHz): δ 1.92 (4H, q, 2 C-CH_2), 0.84 (6H, t, 2 CH_3), 3.94 (3H, s, OCH_3), 7.54 (2H, d, H arom.). 6,94 (2H, d, H arom).

N-(4-chlorophenyl)-2,3-dimethoxy-1,3-dioxopropane-2-imine oxide **N6,** a 54 % yield was achieved by recrystallization in anhydrous diethyl- ether, resulting in crystals with a melting point of 88 ◦C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.87 (6H, s, OCH_3), 7.54 (2H, m, H arom) ,8.44 (2H, m, H arom).

2-(4-chlorophenyl)-5,5-diethyl-4,6-dioxo-1,3,2-dioxazinane -2-oxide **N7** was produced as an oil with a 70% yield using flash chromatography using a mixture of 30% pentane and diethyl ether as the platform. 1H NMR ($CDCl_3$, 300 MHz): δ 2.01 (4H, q, 2 C-CH_2), 0.84 (6H, t, 2 CH_3), 7.55 (2H, d, H arom.). 6,95(2H, d, H arom).

The technique of spin trapping

A hydroxyl radical was produced in an aqueous medium by both the standard Fenton system, which consisted of 0.2% hydrogen peroxide, 2 mmol dm3 of ethylene-diamine-tetra-acetic-acid, and 1 mmol dm3 of ferric sulphate, and UV photolysis of a solution of 3% hydrogen peroxide in water. These two treatments were carried out in the presence of water during the whole process. A Fenton reaction was carried out when there is of either DMSO (10%), MeOH (10%), or EtOH (10%), respectively, in order to form methyl, hydroxymethyl, and hydroxyethyl are free radicals. This was accomplished by combining the three substances. A solution of (0.1) mol dm^3 Potassium persulfate in phosphate buffer-methanol (80:20, volume/volume) was heated for two minutes at a temperature of sixty degrees Celsius. The solution was heated. In order to produce the methoxyl radical $CH_3O•$, this specific action was taken. The xanthine oxidase (X/XO) system is used to create superoxide. The components of this system are as follows: 0.4 mmol dm3 of xanthine, 1 mmol dm3 of diethyl-enamine-pent-acetic acid, and 0.4 units cm^3 of xanthine. During the experiments that included spin trapping, a phosphate buffer with a pH of 7.2 and containing 10-30 mmol dm3 of keto-nitrone

was used. EPR experiments were carried out with the help of a computer-controlled Bruker EMX spectrometer that operated in the X-band with a modulation frequency of 100 kHz. These experiments were carried out in capillary tubes maintained at room temperature. We were able to determine the hyperfine coupling constants for the different spin adducts by simulating EPR signals with the help of a piece of software that was developed by Duling (Duling, 1994).

In silico study of the spin adduct

Prediction (computational study) of physicochemical properties

The PKCSM online program (Douglas et al., 2015) was utilized to assess spin adducts' ADME and physicochemical properties. The oral and CNS activity scores, set as optimal values for new analogues. Predictions of spin adduct absorption, P-glycoprotein (P-gp) substrate characteristics, skin permeability, volume of distribution (VDss), bound/unbound states, renal OCT2 substrate, and total hepatic and renal clearance were made using various models developed by PKCSM. The Caco-2 Permeability model predicted absorption, indicating high permeability if Papp > 8×10-6 cm/s. P-gp substrate predictions were based on inhibitory potential, and skin permeability was assessed using a QSAR model. VDss predictions determined the uniform distribution in plasma and tissue. The bound/unbound state was predicted, indicating the efficacy of new compounds. Renal OCT2 substrate predictions assessed renal clearance, and total hepatic and renal clearance predicted effective doses for steady-state concentration. Toxicity predictions, including LD50, AMES toxicity, T. Pyriformis toxicity, minnow toxicity, maximum tolerated dose, chronic toxicity, hepatotoxicity, skin sensitization, and hERG inhibition, were made using PKCSM. These predictions provide valuable insights into the safety and efficacy of the spin adducts in various aspects of drug development.

Molecular docking

The molecular docking method was used to determine the biological efficacy of the spin adducts with VEGFR1. The Auto Dock Vina program calculated the binding affinity of the spin adducts. The VEGFR1 structure (PDB code 3HNG) was downloaded from a database (www.rcsb.org/pdb), and water molecules and heteroatoms were removed from the protein structure. The charges and hydrogen atoms were added to the compounds before the docking run. We have used flexible docking to allow the compounds to rotate. The docking grid was set to fit the compound with the proper binding site of the protein. A flexible docking algorithm with 50 docking runs, 100,000 energy, and a maximum of 10,000 interactions was set to minimize errors. The binding affinity of compounds with VEGFR1 provides important information about biological activity.

Results and Discussion

We utilized a highly effective method to synthesize seven N-aryl-C and C-di-alkoxy-carbonyl-nitrones, six of which possessed unique structures. The synthetic process demonstrated versatility, allowing for the preparation of different nitrones. Each nitrone showed novel features, such as resistance to oxidative or reducing agents and varying degrees of lipophilicity. However, our study determined the synthesis of N-aryl-keto-nitrones capable of penetrating biological membranes and forming covalent bonds with natural or synthetic macromolecules. We have shown this synthesized chemical reactivity toward free radicals to determine their effective synthesis of more complex compounds. Compounds 1–5 showed potent radical traps in aqueous environments, whch can convert the hydroxymethyl and 1-hydroxyethyl radicals into highly persistent or stable nitroxides. The novel hyperfine couplings with aromatic hydrogen nuclei in these spin adducts showed complex electron paramagnetic resonance (EPR) spectra with many lines.

Penta-deuterated keto-nitrones 6 and 7, lacking aromatic hydrogen, generated less convoluted EPR spectra in their spin adducts. While compounds 6 and 7 were ineffective in capturing hydroxyl and superoxide radicals, compounds 5 and 8 successfully achieved this. The investigation revealed that the methoxyl radical adducts had lower hyperfine coupling constants (hfcc aN) for each nitrone than carbon-centered radical adducts.

The polarity of the medium significantly influenced the sensitivity of aN, indicating the potential of keto-nitrones 6 and 7 as spin-trapping agents in heterogeneous environments. This finding suggests their potential applicability in diverse domains, including chemical synthesis and spin-trapping chemistry. The study presented a groundbreaking use of linear keto-nitrones as spin trapping agents, marking a significant advancement in the field. Ongoing research aims to explore additional applications of N-aryl-keto-nitrones, emphasizing their promising versatility and potential impact across various scientific domains.

Synthesis

By condensing the right carbonyl molecule with a N -substituted hydroxylamine, several different PBN-type nitrones have been made so far with almost quantifiable yields (Hinton & Janzen, 1992), (Dondoni et al., 1994),(Rizzi, Marque, et al., 1997), (E. G. Janzen, Coulter, Oehler, & Bergsma, 1982). It is widely recognized, however, that this approach cannot be directly used to the synthesis of keto-nitrones (Roubaud et al., 2001), (Holzapfel & Crous, 1998), (Tuffariello, 1984), (Torssell, 1988),

(Black & Johnstone, 1984).(Torrente, Noya, Branchadell, & Alonso, 2003), (Fischer, Hyrosova, Fisera, Hametner, & Cyranski, 2005), (Franco, Merchan, Merino, & Tejero, 1995), (Nishi, Hagi, Ide, Murakami, & Makino, 1992). Tomioka et al. (Nishi, Hagi, Ide, Murakami, & Makino, 1992), detailed the prepare of N1, a molecule employed as a starting chemical in the production of indo-lines and indoles of biological interest, by 1,3-dipolar cycloaddition processes of keto-nitrones. We thought modifying their process for making numbers N2-N7 could be cool. These novel keto-nitrones should be viewed as fascinating new synthetic intermediaries based on their possible reactivity towards nucleophiles or alkynes. Potential uses in spin trapping chemistry are also possible. When sodium hydroxide was applied to di-alkyl-malonate, the resulting carbanion was able to react swiftly with the N-aryl nitroso molecule. Bromide anion spontaneously removed, yielding the different N -aryl-C, C-di-alkoxy-carbonyl-nitrones.

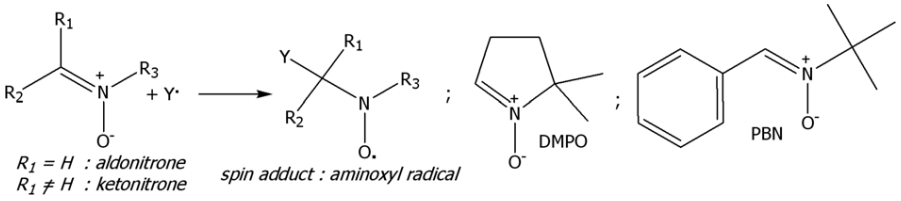
The synthesis efficiency was impacted by exchanging H- atoms for different groups on the initial aryl-nitroso molecule. When N 1and N 2 yields are compared to those of N6 and N7, the detrimental isotope effect becomes clear. Improving the initial aryl-nitroso compound's conjugation considerably reduced its responsiveness when R3 was a group that donated electrons. This was evident in steps 3 and 5's preparation. Interestingly, the presence of an electron-withdrawing group at position R3 did not significantly alter the synthesis yield . The synthesis yield went to zero when R3 had a hydroxyl group.

Experiments with spin trapping

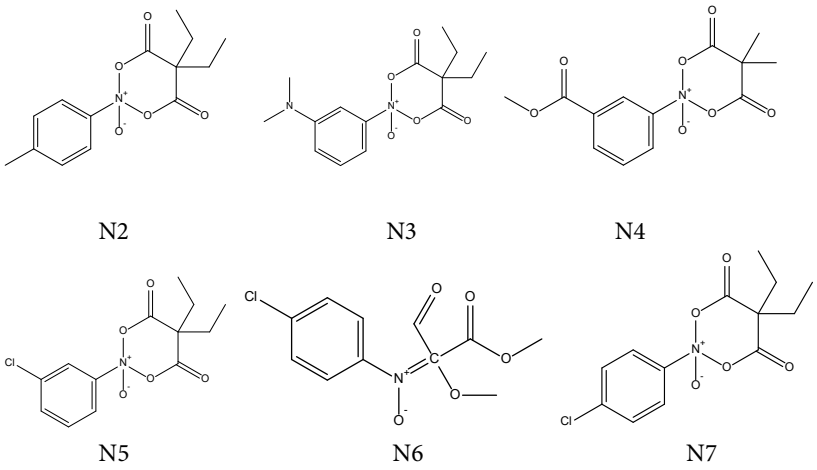
An investigation of the capacity of these compounds to function as spin-trapping agents was carried out by producing free radicals in the presence of N1-N7 in aqueous conditions. To simplify the nomenclature, the aminoxyl produced as a consequence of the nitrone n capturing a free radical X• will be represented by the notation n/•X. This is done in an attempt to simplify the nomenclature. A good illustration of this would be the sign 1/hydroxymethyl radical, which represents the spin adducts of the hydroxymethyl radical, which is 1. We began by concentrating on the reactivity of 1 with carbon-centered radicals in phosphate buffer at a pH of 7.2. This was the first item that we wanted to investigate. Because our principal purpose was to immediately evaluate the potential of this compound to perform the function of a spin trap, only two distinct free radicals, namely hydroxymethyl and hydroxyethyl, have been produced by the presence of 1. Strong EPR signals were detected, and it was discovered that the spin adducts 1/•CH_2OH and 1/•$CH(CH_3)$ OH were associated with those signals. That particular order was discovered for these spinning adducts. An inventory of the hyperfine coupling constants (hfccs) that were created as a result of their simulation may be found in Table 2. No matter which radical was trapped, the EPR spectrum that was recorded was always complicated by the presence of hyperfine couplings between the unpaired electron and the nitrogen nucleus (aN) and the five aromatic hydrogen nuclei (aH-arom; two hydrogen atoms in meta positions, and three hydrogen atoms in ortho and para positions). This was the case regardless of the radical that was trapped. As an example, Figure 1a shows a duplicate of the EPR spectrum of 1/hydroxymethyl radical that has been combined with a simulation overlay. As a consequence of unresolved couplings with aromatic hydrogen nuclei, it is composed of three primary lines that have been greatly extended. These lines are the ones that make up the structure. These lines emerge as a result of the hyperfine interaction with nitrogen, which is responsible for their production. On the other hand, when nitrone N2 was used as the spin trapping agent rather than nitrone 1, the results were equivalent to those obtained with nitrone N1. The findings of the examination into its spin adducts are shown in Table 2, which may be found here. In the process of removing oxygen by using argon bubbling, it is essential to notice that the hyperfine couplings with the aromatic hydrogen nuclei revealed themselves. This can be seen in Figure 1b, which depicts an example of a 2/hydroxyethyl radical. When hydroxy-methyl and 1-hydroxy-ethyl radicals were produced in the presence of the keto-nitrones N3-N5, the same sorts of outcomes were seen (for more information, see Table 2 for more details). It was simple to generate the appropriate spin adduct in every instance, and it had an EPR spectrum at neutral pH that stayed stable for a long time throughout the experiment. The nitroxide disproportionation process is impeded due to the absence of a β-hydrogen, which often interferes with the decay of spin adducts of aldo-nitrones via its interference. The high nitroxide stability may be attributed to this particular reason.

Despite several different substitution groups on the aromatic moiety at the meta and para locations, the trapping reaction was still able to take place. This is a crucial point to keep in mind. The decrease in the number of hyperfine hydrogen nuclei couplings made it feasible to get EPR spectra that were less difficult and more particular. This was accomplished by reducing the number of hyperfine couplings. On the other hand, it is essential to consider that the strength of the recorded EPR spectra reduced when either hydroxymethyl or hydroxy ethyl group was manufactured when three. In light of this, it would seem that in attendance at the para -dimethyl-amino group might the other slow down the process of trapping or speed up the decay of the spin adduct.

These preliminary findings together demonstrated that keto-nitrones N1–N5 effectively scavenged hydroxyl radicals centered on carbon, forming EPR detectable spin adducts. This substantiates the potential usefulness of keto-nitrones with the overall structure shown in scheme 2 for free radical detection. However, the potential uses of these novel spin traps may be



Scheme 1. The structure of the frequently used spin trapping DMPO and PBN



Scheme 2. The structure of the spin adducts (N2 to N7).

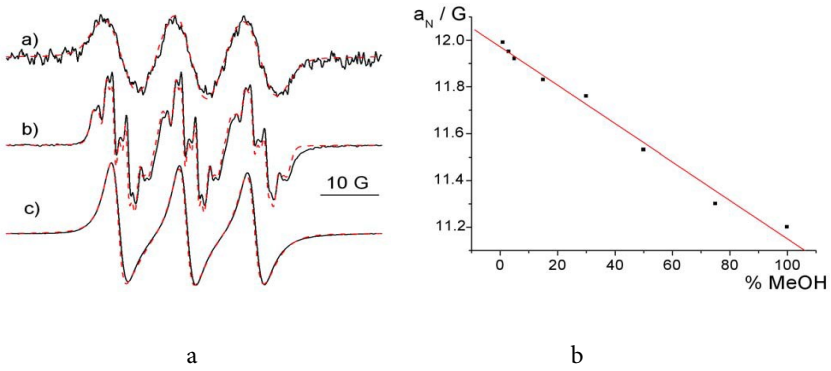


Figure 1. a) The EPR spectrum of 1/hydroxymethyl radical that has been combined with a simulation overlay. b) 2/hydroxyethyl radical. When hydroxymethyl and 1-hydroxy-ethyl radicals were produced in the presence of the keto-nitrones N3-N5.

Table 1. Spin Adduct Examination Results with Keto-Nitrones N3-N5 for Hydroxymethyl and Hydroxyethyl Radicals.

Spin adduct	a*N* / G	a*Harom* / G
1/•CH_2OH	12.00	1.29 (2*H*) ; 2.55 (3*H*)
1/•$CH(CH_3)OH$	11.97	1.41 (2*H*) ; 2.67 (3*H*)
2/•CH_2OH	12.10	1.00 (2*H*) ; 2.36 (3*H*)
2/•$CH(CH_3)OH$	12.29	1.01 (2*H*) ; 2.25 (3*H*)
3/•CH_2OH	13.31	0.63 (2*H*) ; 1.08 (2*H*)
3/•$CH(CH_3)OH$	12.98	0.69 (2*H*) ; 1.20 (2*H*)
4/•CH_2OH	10.97	0.87 (2*H*) ; 2.40 (2*H*)
4/•$CH(CH_3)OH$	11.03	0.96 (2*H*) ; 2.27 (2*H*)
5/•CH_2OH	12.19	2.26 (2*H*)
5/•$CH(CH_3)OH$	12.16	2.25 (2*H*)
6/•CH_2OH	11.95	/
6/•$CH(CH_3)OH$	12.06	/
6/•CH_3	12.55	/
6/•OCH_3	11.67	/
7/•CH_3	12.66	/
7/•CH_2OH	11.97	/
7/•$CH(CH_3)OH$	12.06	/
7/•OCH_3	11.69	/

Table 2. Prediction (computational study) of physicochemical properties of spin adducts.

Spin adducts	SMILES	Molecular Weight	LogP	#Rotatable Bonds	#Acceptors	#Donors	Surface Area
N2	[O-][N+]1(OC(=O)C(CC)(CC)C(=O)O1)C=2C=CC(C)=CC=2	279.292	2.53652	3	5	0	116.521
N3	[O-][N+]1(OC(=O)C(CC)(CC)C(=O)O1)C=2C=C(C=CC=2)N(C)C	308.334	2.2941	4	6	0	128.646
N4	[O-][N+]1(OC(=O)C(C)(C)C(=O)O1)C=2C=C(C=CC=2)C(=O)OC	295.247	1.2345	2	7	0	119.432
N5	[O-][N+]1(OC(=O)C(CC)(CC)C(=O)O1)C=2/C=C(/[Cl])C=CC=2	299.71	2.8815	3	5	0	120.46
N6	[O-][N+]1(OC(=O)C(CC)(CC)C(=O)O1)C=2C=CC([Cl])=CC=2	299.71	2.8815	3	5	0	120.46

Table 3a. Prediction (computational study) of pharmacokinetics properties of spin adducts.

Spin adducts	Water solubility	Caco2 permeability	Intestinal absorption (human)	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitior	CYP2C19 inhibitior	CYP2C9 inhibitior
N2	-3.783	1.293	96.506	-2.803	No	No	No	-0.351	0.244	-0.593	-2.308	No	No	No	No	No
N3	-3.764	1.309	96.702	-2.821	No	No	No	-0.43	0.204	-0.732	-2.471	No	Yes	No	No	No
N4	-3.337	0.574	81.125	-2.826	No	No	No	-0.705	0.26	-0.968	-3.004	No	Yes	No	No	No
N5	-3.943	1.258	95.341	-2.821	No	No	No	-0.415	0.235	-0.773	-2.264	No	No	No	No	No
N6	-4.014	1.283	95.048	-2.821	No	No	No	-0.405	0.232	-0.768	-2.267	No	No	No	No	No

Table 3b. Prediction (computational study) of Toxicity of spin adducts.

CYP2D6 inhibitior	CYP3A4 inhibitior	Total Clearance	Renal OCT2 substrate	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic Toxicity (LOAEL)	Hepatotoxicity	Skin Sensitisation	*T.Pyriformis* toxicity	Minnow toxicity
No	No	0.824	No	Yes	1.28	No	No	2.097	1.299	No	No	0.422	0.231
No	No	0.763	No	Yes	1.067	No	No	2.353	1.273	No	No	0.375	0.606
No	No	0.721	No	No	0.982	No	No	2.693	1.406	No	No	0.345	1.766
No	No	-0.075	No	Yes	1.203	No	No	2.347	1.188	Yes	No	0.394	0.189
No	No	-0.138	No	Yes	1.23	No	No	2.324	1.203	Yes	No	0.423	0.014

Table 4. Binding affinity of spin adducts as anti-angiogenic biological proeprties with VEGR1 cancer biomarker.

Ligand	Target	Binding Energy
N2	VEGFR1	-7.1
N3	VEGFR1	-6.7
N4	VEGFR1	-7.3
N5	VEGFR1	-6.9
N6	VEGFR1	-5.3
N7	VEGFR1	-7

hampered by the intricacy of whether it be the recorded EPR spectra or the width of their lines. One can see this clearly in the spin adducts' spectrum of the N-phenyl modified keto-nitrones 1 and 2, which exhibit several hyperfine couplings. To expand our spin trapping investigations, we thus prepared compounds N6 and N7, their penta-deuterated counterparts of 1 and 2. This finding demonstrates that keto-nitrone N6 is an effective radical trap that produces highly stable spin adducts at the carbon center of radicals. After capturing the methoxyl radical, the spectrum also showed strong spin adduct features. However, 6 was never able to capture superoxide in aqueous environments despite several successful tries. Similarly, a very faint three-line EPR signal (aN = 12.6 G) was seen when $^{\bullet}$OH was generated when a ratio of six was in the buffer. The recorded EPR spectra were quite intense and interpretable for the rest of the samples. Only three thin lines were seen because of the unique hyperfine interaction between the unpaired electron and the nitrogen nucleus. Figure 1a and 1c show the signals of 1 hydroxymethyl radical and 6/ hydroxymethyl radical, respectively, to demonstrate how deuterated spin traps improve the adduct spectra. Because the same outcomes have been seen by substituting 7, rather than 6, for example, we will not discuss them here. The hfcc aN for the methoxyl radical adduct was found to be less than that of the carbon-centered radical adduct for both 6 and 7. The aN value for 6/methyl radical was determined to be 12.55 G, whereas it dropped to 11.67 G for 6/methoxyl radical. It's also worth noting that aN was discovered to change a lot depending on the polarity of the medium. An excellent illustration of this sensitivity to context is the stable spin adducts 6/ hydroxymethyl radical. Pentane (11.06) G, methanol (11.20% G), and dichloromethane (11.40% G) were used to determine aN in this example. As the methanol concentration was raised from 1% to 100%, an EPR spectrum of the adduct was obtained. As can be observed in Figure 2, the hfcc aN was shown to decline straight line between 12.0 G and 11.2 G after signal modelling on a computer. This large amplitude of aN's fluctuation with medium polarity is not unprecedented (see (Tordo, 1998),(Buettner & medicine, 1987),(Roubaud et al., 2001),(Holzapfel & Crous, 1998), (Tuffariello, 1984),(Torssell, 1988), (Black & Johnstone, 1984),(Torrente, Noya, Branchadell, & Alonso, 2003),(Fischer, Hyrosova, Fisera, Hametner, & Cyranski, 2005), (Franco, Merchan, Merino, & Tejero, 1995),(Nishi, Hagi, Ide, Murakami, & Makino, 1992),(E. G. Janzen & Zhang, 1993),(Rosen et al., 2000),(Bottle, Micallef, & chemistry, 2003),(Boyer, Bernardes-Genisson, Farines, Souchard, & Nepveu, 2004),(Sár, Ősz, Jekő, & Hideg, 2004),(Reybier et al., 2006), (Ionita, 2006),(Rizzi, Lauricella, et al., 1997),(Allouch et al., 2003), (Hay, Burkitt, Jones, Hartley, & biophysics, 2005)(Exner, 1951),(Domingues et al., 2003),(Qian, Yue, Tomer, Mason, & Medicine, 2003),(Kumamoto, Hirai, Kishioka, & Iwahashi, 2004), (Defoin, 2004),(Gowenlock, Maidment, Orrell, Prokes, & Roberts, 2001),(Shine, Zmuda, Kwart, Horgan, & Brechbiel, 1982),(Duling, 1994),(E. G. Janzen, Wang, & Shetty, 1978),(Hinton & Janzen, 1992), (Dondoni et al., 1994),(Rizzi, Marque, et al., 1997),(Durand et al., 2003), (E. G. Janzen, Coulter, Oehler, & Bergsma, 1982).The EPR signal of the PBN/ hydroxymethyl radical adduct is weaker in 100% methanol than in water (Saprin, Piette, & biophysics, 1977),(El Hassan, Lauricella, & Tuccio, 2006).

With an N value of 15.6 G compared to 16.0 G. These findings show that the stable adducts of spin generated using a carbon-centered radical that is trapped on a N-aryl-C,C-di-alkoxy-carbonyl-nitrone might be intriguing investigative devices to evaluate the polarity of the environment.

Biological activity potential of spin adducts

Molecular docking simulation with VEGFR1 provides the first measure of a compound's efficacy, which downregulated the VEGFR1 in many cancers, including colon cancer, glioblastoma, prostate cancer, etc. The molecular docking was performed with VEGFR1, possibly due to disruption of upstream protein activity towards the angiogenic signals. We screened the docking binding activity of the designed molecules using Autodock Vina. We kept the receptor and ligand flexible because our objective was to optimize the maximum binding efficiency (Table 4). Specifically, the binding cavity was selected from the docked reference molecule.

The toxicity and the pharmacokinetics study was performed with the PKCSM program to predict compound properties (Table 2, Table 3a, Table 3b). We used an online program called PKCSM to predict how certain spin adduct compounds (N2, N3, N4, N5, N6, N7) would be absorbed, distributed, metabolized, and excreted in the body. The in silico tool showed different properties of a drug, such as oral and CNS activity, permeability in Caco-2 cells, substrate for P-glycoprotein (P-gp), skin permeability, volume of distribution (VDss), serum protein binding, renal clearance, and total clearance. The predicted toxicology of the spin adducts were LD50, AMES mutagenicity, T. Pyriformis toxicity, minnow toxicity, maximum tolerated dose (MRTD), chronic toxicity, hepatotoxicity, skin sensitization, and hERG inhibition. These computational predictions provided the safety, effectiveness, and adverse effects of the new spin derivatives. The results could determine the toxicity of the spin adducts effects on the body. Our study showed the early stages of spin adduct development, helping researchers decide whether these derivatives are suitable for further investigation and possible clinical use.

Conclusions

In conclusion, we represented a synthetic development chemistry for spin-trapping, showing linear keto-nitrones as spin-trapping agents. The method might interest further research in N-aryl-keto-nitrones in diverse scientific fields. The biological efficacy highlights their potency and might be useful for further developing these compounds as cancer therapeutics.

Author contribution

R.S.A. conceptualized, performed the analysis and wrote the Paper.

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Competing financial interests

The authors have no conflict of interest.

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