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SYNTHESIS OF NOVEL α,β-UNSATURATED *N*-METHYL ALDONITRONES USED ASANTIOXIDANT AGENTS

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ABSTRACT

Novel stilbene based nitrones were synthesized and their structures were determined using high-resolution mass spectrometry, ^{1}H-NMR, IR and single crystal X- ray studies. The antioxidant activities of stilbenenitrones were evaluated with the free radical scavenging model 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). It was observed that a series of novel nitrones ***C_1-C_{12}*** *were synthesized in good yield. Compound* ***C_3*** *and* ***C_{10}*** *showed maximum antioxidant activity.*

Key words: *Antioxidant activity, DPPH, ABTS, stilbene and nitrones*

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INTRODUCTION

Oxidative metabolism is an essential phenomenon for the survival of cells. Out of the total oxygen intake, about 2-3% oxygen is converted to harmful intermediates that are termed as reactive oxygen species (ROS) which leads to cumulative damage to cellular proteins, DNA, enzymes and membrane lipids thus shutting down cellular respiration.[1][2] Free radicals that lead to cancer[3]respiratory tract disorders,[4]heart diseases,[5]stroke, diabetics,[6] atherosclerosis[7]and intestinal diseases.[8]Moreover free radicals directly promote various neurodegenerative diseases[9][10]such as Parkinson disease[11]and Alzheimer disease.[12]

Resveratrol, 3,4',5-trihydroxy-*trans*-stilbene found in grapes and a variety of medicinal plants,[13] is a naturally occurring phytoalexin that protects against fungal infections. Its biological properties[14] include antifungal,[15]antibacterial,[16]anticancer,[17]antiviral

,[18] estrogenic,[19]platelet antiaggregating[20]and heart protecting activities.[21]Free radicals, particularly reactive oxygen species (ROS) can accumulate to such a degree as to cause a biochemical chain reaction that leads to cellular injury, death and behavioural deficits.[22][23] Accordingly, considerable attention has been given the possibility of successfully preventing the pathophysiological consequences associated with free radical-induced oxidative stress following ischemic stroke,[24-28] with the expectation that this might provide neuroprotection and reduce secondary reperfusion-induced damage after the onset of ischemia.

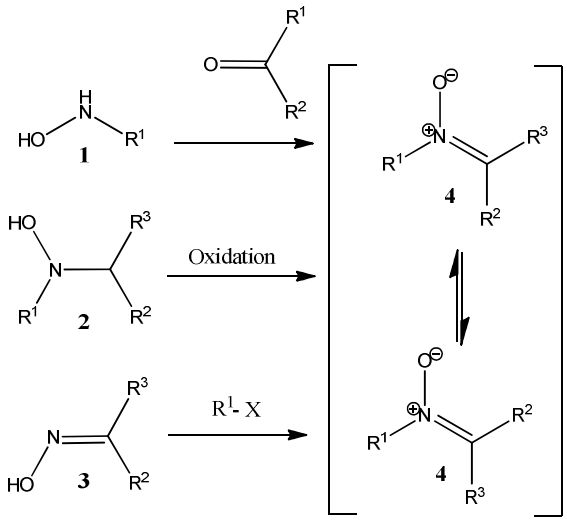
The most widely studied spin trap agents are members of the nitrone class of free radical spin trap agents in which a nitrone moiety traps ROS in addition to other free radical species.[29-31]Nitrone spin traps have become increasingly attractive prospects for the treatment of a variety of pathological conditions in which free radical oxidative stress is suspected to be the major culprit, particularly because of the stable nitroxides that are formed after ROS trapping.[32]The parent compound of the nitrone spin trap family is α-phenyl-*N*-tert-butyl nitrone (PBN), which traps short-lived free radicals such as alkoxyl,[33] superoxide,[34]and hydroxyl,[35]radicals and forms a more stable nitroxide, thereby removing harmful free radicals from circulation. PBN also has the ability to prevent oxidation of lipids like low-density lipoprotein.[36]While many PBN-type spin traps have been synthesized, the most extensively studied in vivo have been the analogs of PBN, sodium 2-sulfophenyl- *N*-tert- butyl nitrone (S-PBN). NXY-059 is also structurally related to the parent compound PBN, but unlike PBN, NXY-059 contains two sulfonyl groups, which reduces the lipid solubility of the compound and appears to alter several aspects of the 192 drug's biochemical properties. A simple and efficient multi-step scheme for the synthesis of NXY-059 from the precursors 2-methyl-2-nitropropane and t-butylhydroxylamine has been published.[37]

OBJECTIVE

To the best of our knowledge stilbene based nitrones were not known in the literature, we focused to synthesize novel nitrones and evaluate for its antioxidant activity. We have recently undertaken the design, synthesis, and pharmacological evaluation of a nitrone series, possessing a substituted stilbene moiety as the salient feature of their chemical structure. It was anticipated that the presence of a extended conjugation would improve the bioavailability and reduce the toxicity characteristics of these nitrones in comparison to compounds described in the literature.[38]

EXPERIMENTAL WORK

The most convenient approach for the generation of these is condensation between secondary hydroxylamines**1** and an aldehyde or a ketone, but other methods also exist such as oxidation of tertiary hydroxylamines**2** or alkylation of oximes**3** with alkyl halides to give nitrones**4** (Scheme 1). The stilbenenitrone was synthesized from stilbenecarbaldehyde which is not commercially available. The aldehyde choice was based on the previous literature.[39]

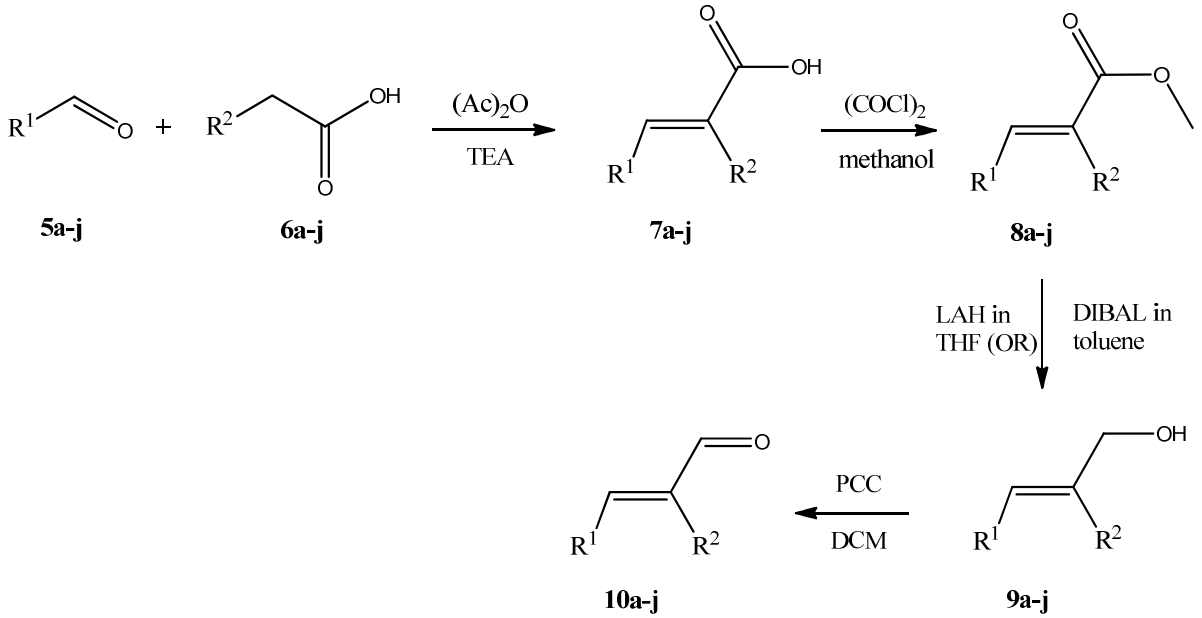


Scheme 1

Synthesis of substituted phenyl acrylic carbaldehydes10a-k:

Substituted phenyl acrylic carbaldehyde was not commercially available. Which is prepared by using substituted phenyl acrylic acids **7a-k**and this carboxylic acid was prepared by the condensation

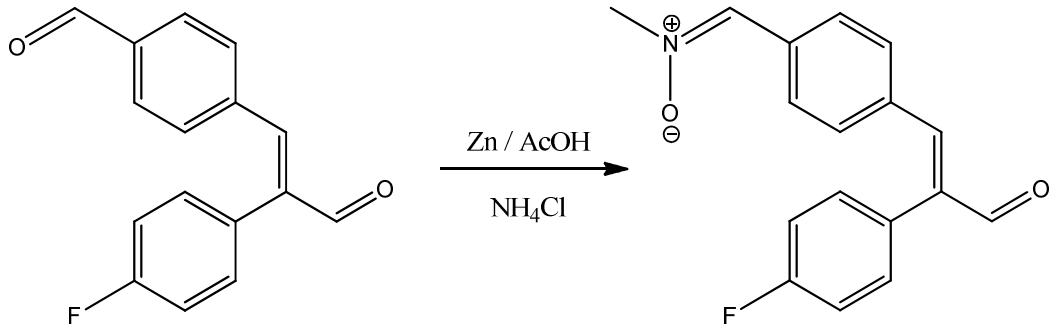
reaction between the corresponding aldehyde **5a-k**and corresponding phenyl acetic acid **6a-k**as shown in scheme 2.



5a-j **6a-j** **7a-j** **8a-j** **9a-j** **10a-j**

Scheme 2

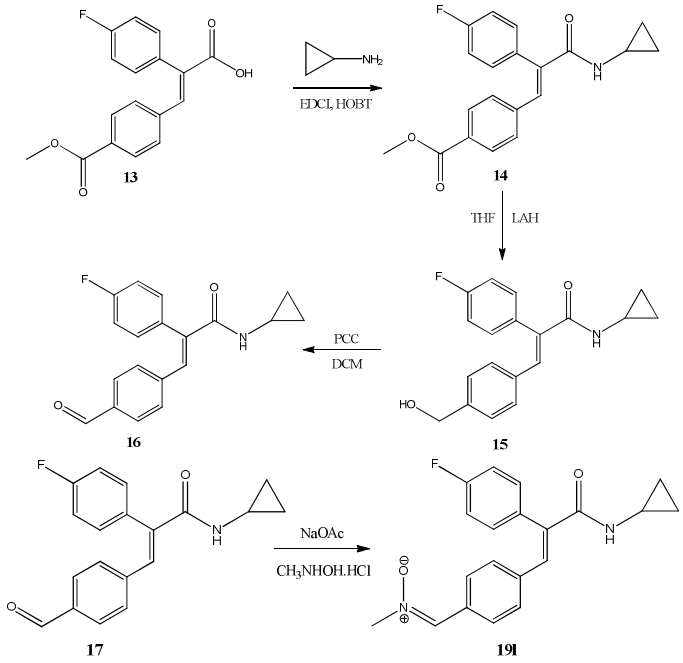
In our laboratory we tried to synthesized dinitrone derivatives but unfortunately mono nitrone product **19k** only formed (scheme 3). This product was confirmed by mass and NMR.



11 **19k**

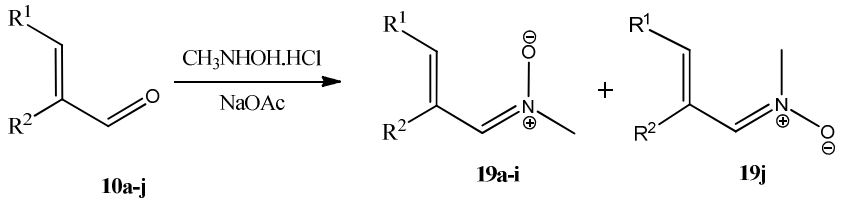
Scheme 3

For cross checking of this compound **19k**, we synthesized another compound **19l**.Compound **13** taken in DMF followed by EDCI(1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and HOBt(1-Hydroxybenzotriazole hydrate) in the presence of base DIPEA (N,N-Diisopropylethylamine) and cyclopropylamine resulting product was treated with LAH (Lithium aluminium hydride) followed by oxidation using PCC (pyridiniumchloro chromate) to get compound **16**.Compound**16** was treated with *N*-methyl hydroxylamine hydrochloride to get nitrone**19l** and this compound showed similar peaks in NMR as in the compound **19k**. This results are showed aromatic aldehydes highly reactive than the α,β-unsaturated carbaldehyde.



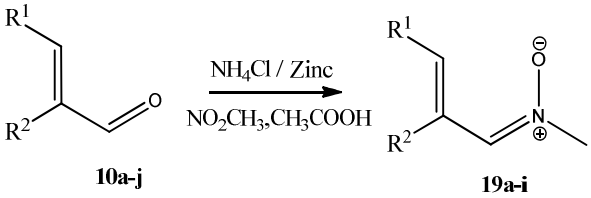
Scheme 4

α,β-unsaturated *N*-methyl aldonitrones were also synthesized from the corresponding carboxaldehyde with *N*-methyl hydroxylamine hydrochloride in the presence of sodium acetate in ethanol under cooling condition as shown in scheme 5.



Scheme 5

To selective synthesis of *Z*-nitrones**19a-i** were prepared by the reduction of nitro compound and carboxaldehyde with zinc dust, ammonium chloride and acetic acid as catalyst in ethanol as shown in scheme 6.



Scheme 6

Synthesized nitrones**19a-i**are highly stable and dimerization could also be controlled under this condition. Nitrone exists exclusively in the *Z* configuration.The structure of the *Z*nitrone was confirmed by single crystal X-ray studies (CCDC – 979568) as shown in fig. 1. and close packing diagram of the *Z*-nitrone as shown in fig. 2.

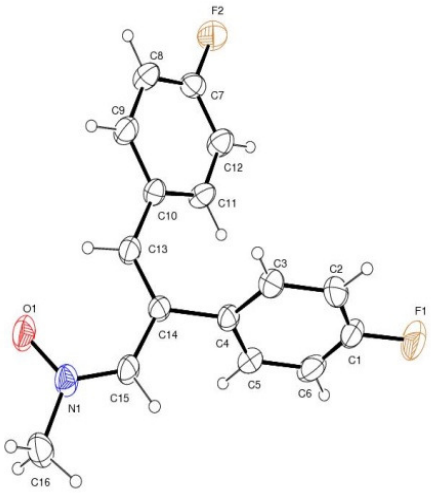


Fig 1. Compound 19i - X-ray structure of the *Z*-nitrone

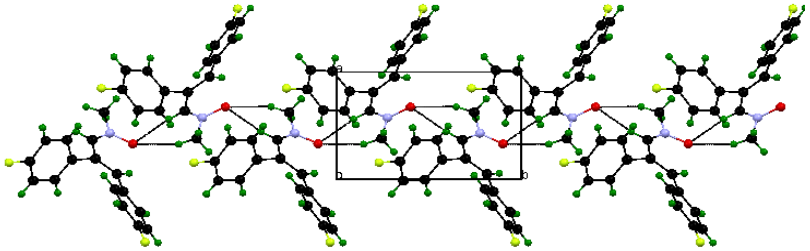


Fig 2. Compound 19i - Close packing diagram of the *Z*-nitrone

Crystal data

Empirical formula	C_{16} H_{13} F_2 N O
Formula weight	273.27
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system, space group	Orthorhombic, P212121
Unit cell dimensions	a = 5.7072(4) A alpha = 90 deg.
	b = 9.2885(7) A beta = 90 deg.
	c = 26.3540(18) A gamma = 90 deg.
Volume	1397.06(17) A^3
Z, Calculated density	4, 1.299 Mg/m^3
Absorption coefficient	0.099 mm^-1

General Methods:

Reactions were monitored by TLC using precoated silica gel aluminium plates containing a fluorescent indicator (Merck, 5539). Detection was done by UV (254 nm) followed by charring with sulfuric- acetic acid spray, 1% aqueous potassium permanganate solution or 0.5% phosphomolybdic acid in 95% EtOH. Anhydrous Na_2SO_4 was used to dry organic solutions during work-ups and the removal of solvents was carried out under vacuum with a rotary evaporator. Flash column chromatography was performed using Silica Gel 60 (230–400 mesh, Merck). Melting points were determined on a Kofler block and are uncorrected.

IR spectra were obtained on a Perkin–Elmer Spectrum One spectrophotometer. ^{1}H NMR spectra were recorded with a Varian VXR-200S spectrometer, using tetramethylsilane as internal standard and ^{13}C NMR spectra were recorded with a Bruker WP-200-SY.

Synthesis of substituted phenyl acrylic acids 7a-j:

To a solution of substituted phenyl acetic acids (1 equiv) and acetic anhydride (2 equiv) followed by triethylamine (3 equiv) added drop by drop to get clear solution and stirring was continued for 15 min. To this substituted aromatic aldehydes (2 equiv) added drop by drop and stirring was continued for 2 hours at RT. Reaction progress was monitored by TLC. After completion, the reaction mixture poured into the ice water and acidified by 1:1 HCl to get solid. Crude solid was dissolved by ethyl acetate and this layer was basified by using 2N NaOH solution. Aqueous layer was washed by ethyl acetate two times to remove excess aldehydes. Aqueous layer was acidified by using 1:1 HCl to get pure solid and it was filtered, dried at the vacuum pump.

Preparation of 2,3-Bis-(4-fluoro-phenyl)-acrylic acid 7i:

To a solution of 4-fluoro phenyl acetic acid (5g, 32.44 mmol, 1 equiv), 4-fluoro benzaldehyde (4 mL, 32.44 mmol, 1 equiv) and triethylamine (13.6 mL, 97.31 mmol, 3 equiv) in acetic anhydride (50 mL). After 2 hours, purification, compound **7i**(4.2 g, 50%): IR ν_{max}(KBr) 1682, 3064 cm^{-1}; Mass = 261.1 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.06-7.14 (4H, m, Ar-H); 7.20-7.29 (4H, m, Ar-H); 7.78 (1H, s, =CH); 12.77 (1H, s, -COOH).

Synthesis of substituted phenyl acrylic esters 8a-j:

A double-necked, round bottomed flask equipped with magnetic stirring bar is charged substituted phenyl acrylic acid (1 equiv) and methanol (10 v). The flask is closed with a rubber septum and placed under N_2 by piercing the septum with an needle. Oxalyl chloride (1.5 equiv) was added via dropping funnel with gaud tube followed by the addition of 2 drops of DMF. Reaction was carried out under cooling condition (0̊ C) and bring to RT for another two hours. Reaction progress was monitored by TLC. After completion of the reaction, solvent was evaporated and added crushed ice into the reaction mixture, solid was precipitateout, filtered and dried at the vacuum pump.

Preparation of 2,3-Bis-(4-fluoro-phenyl)-acrylic acid methyl ester 8i:

To a solution of 2,3-Bis-(4-fluoro-phenyl)-acrylic acid 7**i** (4.2 g, 16.15 mmol, 1 equiv) and oxalyl chloride (2.77 mL, 32.3 mmol, 2 equiv) in methanol (40 mL) and two drops of DMF. After 2 hours, purification, compound **8i** (4 g, 91%); IR ν_{max}(KBr) 1711 cm^{-1}; Mass = 275.0 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.79 (3H, s, -OCH_3); 6.85-6.89 (2H, m, -ArH); 7.00-7.09 (2H, m, -ArH); 7.16-7.19 (2H, m, -ArH); 7.27 (2H, m, -ArH); 7.82 (1H, s, =CH).

Synthesis of substituted phenyl acrylic alcohols 9a-j:

Dissolved substituted phenyl acrylic ester in toluene, stirred, cooled to -78̊ C under N_2 inlet. Added DIBAL (1M solution in toluene). After half an hour, the reaction was quenched with dil. HCl under -78̊ C, stirred for 30 min and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation.The product was isolated by column chromatography on silica gel using EtOAc-Hexane as eluant. The product was a clearcolorless oil.

Preparation of 2,3-Bis-(4-fluoro-phenyl)-prop-2-en-1-ol 9i:

Reaction of 2,3-Bis-(4-fluoro-phenyl)-acrylic acid methyl ester (4g, 14.58 mmol, 1 equiv), and DIBAL (14 mL, 14.58 mmol, 1 equiv) in toluene 40 mL under -78̊ C. After completion of the reaction, purification, column chromatography (Hex:EtOAc 30:70), compound **9i** (2.2 g, 61%); IR ν_{max}(KBr) 3257 cm^{-1}; Mass = 229.0 (M^+-17); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.59-1.63 (1H, t, -OH); 4.43 (2H, d, -CH_2); 6.66 (1H, s, =CH); 6.80-6.85 (2H, m, -ArH); 6.93-6.97 (2H, m, -ArH); 7.01-7.05 (2H, m, -ArH); 7.17-7.20 (2H, m, -ArH).

Synthesis of substituted phenyl acrylic carbaldehydes10a-j:

Dissolve substituted phenyl acrylic alcohol in methylene chloride and place in an addition funnel. Place pyridiniumchloro chromate (1.5 equiv) in a

RB flask and add methylene chloride and add a magnetic stir bar to the flask. Add the alcohol solution dropwise to the PCC suspension with gentle stirring using the magnetic stir bar. Once the addition is complete, stirring was continued for 30 min. Decant the solvent layer from the tarry black residue, and rinse the residue twice with methylene chloride. Evaporate the combined methylene chloride solution on a rotary evaporator. Dilute the residual material with diethyl ether and filter through cotton plug to remove the insoluble chromic salts. Wash the ether layer with 1M NaOH, followed by saturated salt solution, then dry the product solution with anhydrous Na_2SO_4. The organic layer was evaporated to get the product.

Preparation of 2,3-Bis-(4-fluoro-phenyl)-propenal10i:

To a solution of 2,3-Bis-(4-fluoro-phenyl)-prop-2-en-1-ol (2.2 g, 8.94 mmol, 1 equiv) and pyridiniumchloro chromate (1.93 g, 8.94 mmol, 1 equiv) in DCM (40 mL). After 30 min, product purified by column chromatography (Hexane:EtOAc 80:20) to get the compound **10i** (1 g, 46%); IR ν_{max}(KBr) 3223, 1599 cm^{-1}; Mass = 245.1 (M^++1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H6.93-6.97 (2H, m, -ArH); 7.12-7.22 (6H, m, -ArH); 7.36 (1H, s,=CH); 9.74 (1H, s, -CHO).

General procedure for the synthesis of nitrones19a-j:

Method A:

Nitromethane (2 equiv) and 25 mL of 95% ethanol was mixed with ammonium chloride (2 equiv) in 25 mL of water. The mixture was cooled in an ice bath and stirred vigorously while adding zinc dust (4 equiv) in portion wise every 2 to 3 minutes. After stirring for 30 minutes at 0̊ C to 10̊ C, the resulting white suspension was treated with aldehyde (1 equiv) in 10 mL of acetic acid. A clear solution was immediately obtained. After 15 minutes, the nitrone began to precipitate. The reaction mixture was stirred for 30 minutes at room temperature, then poured into 100 mL of water. The crude*Z*-nitrone was collected by filtration and purified by silica gel (60-120 mesh) column chromatography using hexane and ethyl acetate (70:30) to give pure compounds.

Method B:

In a 100 mL RB flask, the aldehyde (1 equiv) in ethanol was added into the stirring solution of N-methyl hydroxylamine hydrochloride (2 equiv) in sodium acetate (3 equiv) under cooling condition (10̊ C). The mixture was stirred for 15 minutes and the progress of the reaction was monitored by TLC. Ice water (20 mL) was added into the reaction mixture,*E*- and *Z*-nitrone was precipitate out and the resulting solid was purified by silica gel (60-120 mesh) column chromatography using hexane and ethyl acetate as a eluent to give pure compounds.

Preparation of *Z*-[2-(2-Chloro-phenyl)-3-phenyl-allylidene]-*N*-methylnitrone 19a:

Following the general procedure (method A), reaction of (*2E*)-2-(2-chlorophenyl)-3-phenylprop-2-enal (2) (1g, 4.13 mmol, 1 equiv), nitromethane (0.5 mL, 8.26 mmol, 2 equiv), zinc (0.81 g, 12.39 mmol, 3 equiv), acetic acid (1.5 mL, 24.79 mmol, 6 equiv), ammonium chloride (0.22 g, 4.13 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour, and column chromatography (hexane/EtOAc, 1:1), gave compound **19a** (0.79g, 79%): IR ν_{max}(KBr) 3430, 2931, 1598, 1179 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H3.74 (3H, s, -NCH_3); 6.95-6.98 (4H, m, ArH); 7.18-7.21 (2H, m, ArH); 7.32 (1H, s, =CH); C^{13}NMR (400MHz, DMSO) δc 38.91, 54.14, 115.79, 116.01, 118.24, 120.43, 125.24, 126.52, 126.64, 127.21, 128.48, 128.81, 129.14, 131.56, 134.98, 135.43

Preparation of *Z*-[2-(4-Fluoro-phenyl)-3-phenyl-allylidene]-*N*-methyl nitrone 19b: Following the general procedure (method A), reaction of (2*E*)-2-(4-fluorophenyl)-3-phenylprop-2-enal (1g, 4.4 mmol, 1 equiv), nitromethane (0.54 mL, 8.85 mmol, 2 equiv), zinc (0.86 g, 13.27 mmol, 3 equiv), acetic acid (1.5 mL, 26.55 mmol, 6 equiv), ammonium chloride (0.23 g, 4.43 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour, and column chromatography (hexane/EtOAc, 1:1), gave compound **19b** (0.74g, 74%): IR ν_{max} (KBr) 3429, 2929, 1601, 1508, 1225, 1179 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H3.74 (3H, s, -NCH_3); 6.92-6.94 (2H, m, ArH); 7.14-7.23 (6H, s, ArH); 7.33 (1H,

s, =CH); 9.09 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 38.78, 54.28, 115.43, 116.43, 118.41, 121.23, 122.32, 124.63 125.65, 126.32, 127.48, 128.46, 129.64, 130.13, 134.92, 161.36

Preparation of *Z*-[2-(4-Methoxy-phenyl)-3-phenyl-allylidene]-*N*-methyl nitrone 19c: Following the general procedure (method A), reaction of (2*E*)-2-(4-methoxyphenyl)-3-phenylprop-2-enal (1g, 4.2 mmol, 1 equiv), nitromethane (0.50 mL, 8.40 mmol, 2 equiv), zinc (0.8 g, 12.60 mmol, 3 equiv), acetic acid (1.5 mL, 25.21 mmol, 6 equiv), ammonium chloride (0.22 g, 4.20 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour, and column chromatography (hexane/EtOAc, 1:1) gave compound **19c** (0.62g, 62%): IR ν_{max}(KBr) 3430, 2934, 1606, 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.74 (3H, s, -NCH_3); 3.76 (3H, s, -OCH_3); 6.92-6.97 (4H, m, ArH); 7.09-7.15 (5H, m, ArH); 7.28 (1H, s, =CH); 9.06 (1H, s, N=CH); ^{13}CNMR (400MHz, DMSO) δc 40.12, 54.21, 59.24, 114.86, 115.47, 116.41, 118.61, 121.21, 122.42, 124.13 125.65, 126.23, 127.86, 127.91, 128.43, 134.96, 161.27

Preparation of *Z*-[3-(4-Fluoro-phenyl)-2-(4-methoxy-phenyl)-allylidene]-*N*-methyl nitrone19d:Following the general procedure (method A), reaction of (2*E*)-3-(4-fluorophenyl)-2-(4-methoxyphenyl)prop-2-enal(1g, 3.9 mmol, 1 equiv), nitromethane (0.48 mL, 7.8 mmol, 2 equiv), zinc (0.76 g, 11.72 mmol, 3 equiv), acetic acid (1.4 mL, 23.43 mmol, 6 equiv), ammonium chloride (0.21 g, 3.91 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour, and column chromatography (hexane/EtOAc, 1:1) gave compound **19d** (0.76g, 76%) IR ν_{max}(KBr) 3429, 2933, 1608, 1175 cm^{-1}1H NMR (400 MHz, $CDCl_3$) δ_H 3.73 (3H, s, NCH_3); 3.76 (3H, s, -OCH_3); 6.94 (2H, d, J=8Hz, Ar-H); 6.99 (4H, d, J=8Hz, Ar-H); 7.09-7.12 (2H, d, Ar-H); 7.28 (1H, s, =CH); 9.05 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 40.12, 54.21, 59.24, 114.86, 115.47, 116.41, 118.61, 121.21, 122.42, 124.13 125.65, 126.23, 127.86, 127.91, 128.43, 134.96, 161.27,161.38.

Preparation of*Z*-(2,3-Diphenyl-allylidene)-*N*-methylnitrone 19e:

Following the general procedure (method A) reaction of (2*E*)-2,3-diphenylprop-2-enal **(1)** (1g, 4.81 mmol, 1 equiv), nitromethane (0.62 mL, 9.62 mmol, 2 equiv), zinc (0.94 g, 14.42 mmol, 3 equiv), acetic acid (1.7 mL, 28.8 mmol,6 equiv), ammonium chloride (0.25 g, 4.81 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour, and column chromatography (hexane/EtOAc, 1:1), gave compound **19e** (0.63g, 60%): IR ν_{max}(KBr) 3434, 2934, 1591, 1179 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H3.75 (3H, s, -NCH_3); 6.91-6.93 (2H, m, Ar-H); 7.12-7.13 (3H, m, Ar-H); 7.19-7.21 (2H, m, Ar-H); 7.32 (1H, s, =CH); 7.35-7.41 (3H, m, Ar-H); 9.12(1H, s, N=CH); ^{13}C NMR (400MHz, DMSO) δc38.91, 54.14, 115.79, 116.01, 118.24, 120.43, 125.24, 126.52, 126.64, 127.21, 128.48, 128.81, 129.14, 134.98, 134.93

Preparation of *Z*-[2-(4-Methyl-phenyl)-3-phenyl-allylidene]-*N*-methyl nitrone 19f:Following the general procedure (method A), reaction of (2*E*)-2-(4-methylphenyl)-3-phenylprop-2-enal(1g, 4.5 mmol, 1 equiv), nitromethane (0.55 mL, 9 mmol, 2 equiv), zinc (0.88 g, 13.5 mmol, 3 equiv), acetic acid (1.6 mL, 27.03 mmol, 6 equiv), ammonium chloride (0.24 g, 4.51 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19f** (0.78g, 78%): IR ν_{max}(KBr) 3434, 2923, 1597, 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.32 (3H, s, -CH_3); 3.74 (1H, s, -OCH_3); 6.94-6.96 (2H, m, ArH); 7.07-7.08 (2H, m, Ar-H); 7.12-7.14 (3H, m, Ar-H); 7.17-7.19 (2H, m, Ar-H); 7.28 (1H, s, =CH); 9.09 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 38.91, 40.46, 54.34, 115.23, 116.51, 118.48, 120.54, 125.26, 126.53, 126.24, 127.29, 128.61, 128.81, 129.18, 134.16, 134.52

Preparation of *Z*-[2-(4-Methoxy-phenyl)-3-thiophen-2-yl-allylidene]-*N*-methyl nitrone 19g:Following the general procedure (method A), reaction of (2*E*)-2-(4-methoxyphenyl)-3-(thiophen-2-yl)prop-2-enal (1g, 4.1 mmol, 1 equiv), nitromethane (0.50 mL, 8.19 mmol, 2 equiv), zinc (0.79 g, 12.29 mmol, 3 equiv), acetic acid (1.48 mL, 24.58 mmol, 6 equiv), ammonium chloride (0.22 g, 4.1 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19g** (0.65g, 65%): IR ν_{max}(KBr) 3435,

2924, 1606, 1581, 1166cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.69 (3H, s, -NCH_3); 3.80 (3H, s, -OCH_3); 6.92-6.95 (1H, m, Ar-H); 7.02-7.08 (3H, m, Ar-H); 7.16-7.21(3H, m, Ar-H); 7.38-7.39 (1H, m, Ar-H); 9.37 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc38.56, 56.81, 59.68, 114,75, 126.81, 127.13, 127.83, 130.32, 136.65, 161.23

Preparation of *Z*-[3-(4-Fluoro-phenyl)-2-p-tolyl-allylidene]-*N*-methyl nitrone 19h:Following the general procedure (method A), reaction of (2*E*)-3-(4-fluorophenyl)-2-(4-methylphenyl)prop-2-enal (1g, 4.17 mmol, 1 equiv), nitromethane (0.50 mL, 8.33 mmol, 2 equiv), zinc (0.8 g, 12.49 mmol, 3 equiv), acetic acid (1.5 mL, 24.99 mmol, 6 equiv), ammonium chloride (0.22 g, 4.17 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19h** (0.63g, 63%): IR ν_{max}(KBr) 3435, 2948, 1656, 1512, 1425, 1243 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.33 (3H, s, -CH_3); 3.81 (3H,s, -NCH_3); 6.77-6.82 (2H, m, Ar-H); 6.94-7.01(5H, m, Ar-H); 7.14-7.16 (1H, m, Ar-H) 7.26 (1H, s, =CH); 9.19 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 38.78, 40.52, 54.28, 115.43, 116.43, 118.41, 121.23, 122.32, 124.63 125.65, 126.32, 127.48, 128.46, 129.64, 130.13, 134.92, 161.36

Preparation of *Z*-[2,3-Bis-(4-fluoro-phenyl)-allylidene]-*N*-methyl nitrone 19i (*Z*-nitrone):Following the general procedure (method A), reaction of (2*E*)-2,3-bis(4-fluoro phenyl)prop-2-enal (1g, 4.09 mmol, 1 equiv), nitromethane (0.50 mL, 8.19 mmol, 2 equiv), zinc (0.8 g, 12.29 mmol, 3 equiv), acetic acid (1.5 mL, 24.58 mmol, 6 equiv), ammonium chloride (0.22 g, 4.10 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19i** (0.76g, 76%): IR ν_{max}(KBr) ; 3434, 2994, 1906, 1601, 1506, 1231, 1178 cm^{-11}H NMR (400 MHz, $CDCl_3$) δ_H 3.82 (3H, s, -NCH_3); 6.79-6.84 (2H, m, Ar-H); 6.93-6.99 (1H,m, Ar-H); 7.03-7.07 (2H, m, Ar-H); 7.14-7.18 (3H, m, Ar-H); 7.26 (1H, s, Ar-H); 9.18 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 38.89, 39.93, 40.14, 54.24, 115.79, 116.01, 129.67, 131.64, 132.86, 134.32, 134.99, 135.02, 160.43, 162.86

Preparation of *E*-[2,3-Bis-(4-fluoro-phenyl)-allylidene]-*N*-methyl nitrone 19j (*E*-nitrone):Following the general procedure (method B), reaction of (2*E*)-2,3-bis(4-fluoro phenyl)prop-2-enal(1g, 4.09 mmol, 1 equiv), N-methylhydroxylaminehydrochloride (0.68 g, 8.19 mmol, 2 equiv), sodium acetate (1 g, 12.27 mmol, 3 equiv) in ethanol, after 15 min, and column chromatography (hexane/EtOAc, 1:1), gave compound **19j**(0.76g, 76%): IR ν_{max}(KBr) 3430, 2934, 1603, 1509, 1228, 1182 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.76 (1H, s, NCH_3); 7.00 (1H, s, Ar-H); 7.15-7.19 (2H, m, Ar-H); 7.22-7.26 (2H, m, Ar-H); 7.36-7.39 (2H, m, Ar-H); 7.43-7.47 (2H, m, Ar-H); 7.71 (1H, s, =CH); C^{13}NMR (400MHz, DMSO) δc 38.89, 54.24, 115.79, 116.01, 129.67, 131.64, 132.86, 134.32, 134.99, 135.02, 160.43, 162.86

Preparation of*Z*-[2-(4-Fluoro-phenyl)-3-(4-*N*-methyl nitrone-phenyl)-propenal19k:Following the general procedure (method A), reaction of 4-[(1*E*)-2-(4-fluorophenyl)-3-oxoprop-1-en-1-yl]benzaldehyde (1g, 3.94 mmol, 1 equiv), nitromethane (0.48 mL, 7.87 mmol, 2 equiv), zinc (0.78 g, 11.81 mmol, 3 equiv), acetic acid (1.4 mL, 23.62 mmol, 6 equiv), ammonium chloride (0.21 g, 3.94 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1 hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19k**(0.76g, 76%): IR ν_{max}(KBr) 3430, 2920, 1672, 1512, 1171cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.88 (3H, s, NCH_3); 7.08-7.13 (2H, m, Ar-H); 7.16-7.19 (2H, m, Ar-H); 7.24-7.26 (1H, m, Ar-H); 7.34-7.39 (2H, m, Ar-H); 8.08 (2H, d, Ar-H, J = 8 Hz); 9.77 (1H, s, -CHO); C^{13}NMR (400MHz, DMSO) δc38.96, 54.43, 115.43, 127.81, 128.69, 130.52, 135.23, 139.32, 142.13, 161.64, 190.16

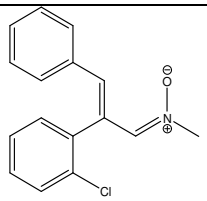
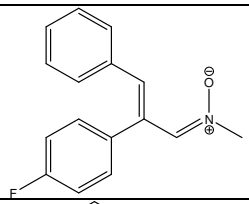
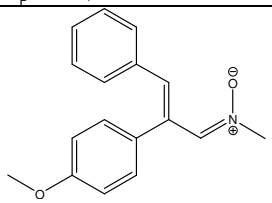
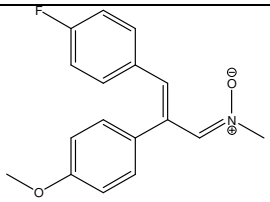
Preparation of*Z*-[*N*-cyclopropyl-2-(4-fluoro-phenyl)-3-(4-*N*-methyl nitrone -phenyl)-acrylamide 19l:Following the general procedure (method A), reaction of (2*E*)-*N*-cyclopropyl-2-(4-fluorophenyl)-3-(4-formylphenyl)prop-2-enamide (1g, 3.24 mmol, 1 equiv), nitromethane (0.39 mL, 6.47 mmol, 2 equiv), zinc (0.63 g, 9.7 mmol, 3 equiv), acetic acid (1.16 mL, 19.42 mmol, 6 equiv), ammonium chloride (0.17 g, 3.24 mmol, 1 equiv) in equimolar mixture of ethanol and water, after 1

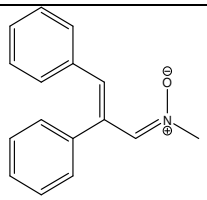
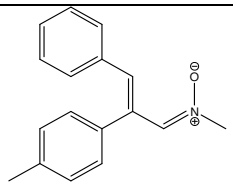
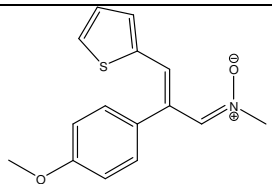
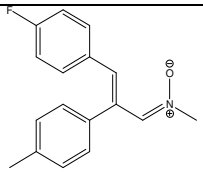
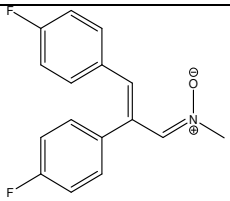
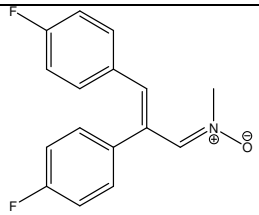
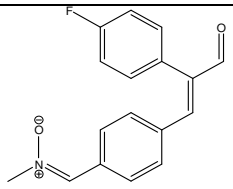
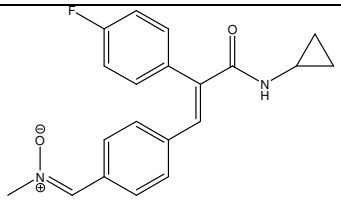
hour and column chromatography (hexane/EtOAc, 1:1), gave compound **19l** (0.76g, 76%):IR ν_{max}(KBr) 3434, 3227, 3039, 1643, 1509, 1168 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H 0.36-0.39 (2H, m, $-CH_2$); 0.62-0.66 (2H, m, Ar-H); 2.79-2.81 (1H, t, -CH); 3.78 (3H, s, $-NCH_3$); 7.04 (1H, s, Ar-H); 7.23-7.27 (2H, m, Ar-H); 7.51-7.58 (4H, m, Ar-H); 7.85 (1H, s, =CH); 8.19-8.21 (2H, m, Ar-H); 8.53-8.54 (1H, s, N=CH); C^{13}NMR (400MHz, DMSO) δc 15.83, 20.61, 38.12, 40.18, 54,81, 66.23, 115.42, 115.89, 118.43, 127.84, 128.04, 130.54, 132.43, 135.76, 161.36

RESULTS AND DISCUSSION

In the antioxidant study, DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay[40] and ABTS assay were chosen to evaluate antioxidant potential of the newly synthesized compounds **(19a-l)**.The percentage of inhibition (IC_{50}) was graphically estimated using a linear regression algorithm and the results were depicted in Table I and compared with that of standard L-ascorbic acid. The antioxidant activity of the synthesized compounds are associated with their electron donating capability to DPPH radical and convert into stable diamagnetic molecules. Compounds **19b**and**19f** showed good radical scavenging activity, this may be due to the presence of electron withdrawing fluoro groups and electron donating methyl group at phenyl ring. Remaining compounds **(19c, 19e, 19h, 19i, 19j** and**19l)** showed moderate activity. Among the synthesized compound **19b**showed good DPPH radical scavenging activity (IC_{50} 20.28 µM/mL).It was observed that most of the tested compounds showed good to moderate antioxidant activity.

Table I50% Inhibition of DPPH radical and ABTS assay by compounds (19a-l). Each value represents mean ± SD (n=3)

Compound	Structures	DPPH Assay(IC_{50} µm)	ABTS Assay(IC_{50} µm)	Time (h)	Yield (%)
19a		30.94	53.41	1.2	70
19b		**20.28**	**35.92**	1.2	72
19c		**23.93**	**39.69**	1.5	67
19d		28.02	46.83	2	58

19e		**23.86**	**42.87**	1.2	75
19f		**20.45**	**32.67**	1.2	71
19g		32.64	52.89	1	55
19h		**22.76**	**36.81**	1.2	76
19i		**21.27**	**33.71**	1.2	80
19j		**21.14**	**35.59**	1.2	76
19k		26.58	41.18	1.2	70
19l		**24.85**	**45.16**	1.2	72
Standard	**L-Ascorbic acid**	**12.35**	**24.23**		

In ABTS assay, the synthesized compounds **(19a-l)**having different concentrations (5, 20, 50 and 100 µm/mL) were tested in $ABTS^{\cdot+}$ scavenging activity,[41][42] ABTS radical scavenging method is a rapid and easy method to test the antioxidant activity of the synthesized compounds. In this assay the reaction between ABTS and potassium persulfate directly produced the green or blue colour of $ABTS^{\cdot+}$ radical and interaction of this radical with synthesized compounds leads to less coloured product. Compounds**19b**and **19f** showed good ABTSradical scavengingactivity, where as the compounds (**19c, 19e, 19h, 19i, 19j**and**19l**) showed moderate activity. Compound **19f** showed good ABTS radical scavenging activity (IC_{50} 32.67 µM/mL). Remaining compounds possessing much lower activity than that of other tested compounds. The results were depicted in Table I.

In conclusion, we have synthesized a series of novel nitrones**(19a-l)** in short reaction time with good yield. The newly synthesized analogues were evaluated for their *in vitro* antioxidant activity. Among the tested compounds **19b** and**19f**showed maximum antioxidant activity in comparison with all other tested compounds.The activity of those compounds was comparable with that of standard drugs. These results suggested that the further structural modifications on these molecules might provide lead compounds with potent antioxidant agents.

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