REGIO-AND STEREOSELECTIVITY OF 1,3-DIPOLAR CYCLOADDITION REACTION OF CINNARIZINE DRUG WITH CHIRAL NITRONES, AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

2-Methyl-3-(Aryl)-4(phenyl)-5-(1-methylene-4-diphenylmethyl)piperazine isoxazolidine (**3a-j**) have been prepared by 1,3-dipolar cycloaddition reaction of the synthesized C-aryl-N-methylnitrones (**1a-j**) with Cinnarizine and some physical properties (melting points (m.p), Retardation Factor (Rf), etc) that were taken of these novel isoxazolidines. The structural properties of isoxazolidine and nitrones were analyzed by Fourier-transform infrared FT-IR spectroscopy, ¹H nuclear magnetic resonance NMR spectroscopy, ¹³C-NMR spectroscopy. The relative product formulations were determined from ¹H-¹H NOESY, ¹H-¹³C-HMQC NMR spectrum for some of the compounds and biological evaluation for synthesized derivatives were studied using the disc diffusion process against selected *Escherichia coli*, and *Staphylococcus aureus* bacteria also against pathogenic fungi *Candida Albicans* and *Microsporum gypsum* compared with the standard drugs isoxazolidine (**3d,3f**) revealed high activity against *staphylococcus aureus*, and *Escherichia coli* as well as complete inhibition of growth against pathogenic fungi *Candida Albicans* and *Microsporum gypsum*.

Keywords: Nitrones, 1,3-Dipolar Cycloaddition Reaction, Isoxazolidines, Cinnarizine.

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INTRODUCTION

One of the most significant ways of synthesizing five-membered heterocycles is the 1,3-dipolar cycloaddition reactions, in which we can use simple starting materials through it. Nitrones are known to be highly desirable among different 1,3-dipoles due to the large applications of cycloadducts, the cycloaddition reactions of nitrones with olefines are one of the more effective methods to get isoxazolidines.¹⁻³ The regio, stereo, and chemoselectivity of nitron cycloaddition reactions are extraordinary, as well as the formation of multiple chiral stereocenters, have made it an appealing and efficient approach in the synthesis of a vast scope of biologically important natural compounds as isoxazolidines.⁴ The undoubted benefit of this strategy is the potential to produce a molecule containing many stereocenters⁵ also, provide a significant degree of stereochemical control.⁶ Alternatively, in drug production, isoxazolidines are important scaffolds that imitate groups of natural building blocks largely and have fascinating, diverse biological activities. The isoxazolidine ring structure has the biological uses of including cytotoxic activity through DNA intercalation^{7,8} and transcriptional activators⁹, antiviral activity¹⁰, antifungal and antimicrobial activities^{11,12}, anti-Inflammatory activity¹³, advanced glycation end inhibitor activity¹⁴, etc. We used cinnarizine drug (1-cinnamyl-4-(diphenylmethyl) piperazine) as dipolarophile in this search. Cinnarizine is a piperazine derivative often used to treat cerebral arteriosclerosis and cerebral apoplexy and post-trauma cerebral Symptoms and it is mainly used as an antihistamine, antiserotonergic.¹⁵ In 1955, Janssen Pharmaceutica synthesized the cinnarizine for the first time¹⁶. Stereospecificity is an important aspect of this reaction which Huisgen has discovered the effect of alkene geometry which is held completely in the cycloadduct. However, it is still important to solve the problems of regio and stereoselectivity of this kind of reaction. According to the electronic character of the substituent, which is on alkene, two regioisomeric

types can be produced in the reaction between nitrones and monosubstituted olefins 5-isoxazolidine where a substituent group is on the carbon atom alpha to the oxygen atom, and 4-isoxazolidines a substituent group is on the carbon atom beta to the oxygen atom, reactions of the alkenes that carry electron-donating substituents are controlled by interactions of lowest unoccupied molecular orbital (LUMO)_{dipole} and highest occupied molecular orbital (HOMO)_{dipolarophile} orbitals which give of 5-isoxazolidines. On the other side regioselectivity to reactions of the alkenes that carry strongly electron-accepting substituents are controlled by interactions of HOMO_{dipole} and LUMO_{dipolarophile} orbitals leading solely to 4-isoxazolidines according to the theory of Frontier Molecular Orbitals (FMO), whereas in alkenes that contain moderately electron-accepting substituent groups, such as with 1,2-disubstituted olefins, the generation of a mixture of regioisomers is predicted.¹⁷⁻¹⁹ This problem is approached by substrates control or by add suitable catalysts. Another problem is the stereochemistry of the reaction as products can be produced on Endo and Exo intermediate states paths, which can also not be readily predicted.²⁰⁻²³ The topic of diastereoselectivity and enantioselectivity was solved by the application of chiral catalysts or by the application of chiral nitrones or alkenes in cycloaddition reaction.^{1, 24} In this study, we synthesized ten derivatives of nitrones by reacting to the substituted benzaldehyde with N-methylhydroxylamine hydrochloride and its reaction with the cinnarizine drug. This reaction exhibited high selectivity, which was studied, in addition to the biological activity of isoxazolidine derivatives. The product compounds were identified by using FTIR spectroscopy, ¹H-NMR spectroscopy, ¹³C-NMR spectroscopy ¹H-¹H -NOESY spectra ¹H-¹³C- HMQC NMR spectrum.

EXPERIMENTAL

Material and Methods

Measurement of melting points was done on the SMP1/4 device. Reactions were followed by TLC on silica gel F254, with detection by the exposure to iodine vapor. IR spectra were recorded on Avatar 320 FT-I (Shimadzu Company /Sana'a), ¹H-NMR spectra were carried out using a Bruker AC400 spectrometer at 400 MHz for solutions in CDCl₃ and ¹³C-NMR Spectra were measured using a Bruker spectrometer at 300MHz (AL-Baath University/Faculty of Science/Chemistry Department /Homs/Syria). Chemical shifts were denoted in the δ units (ppm) (parts per million), with the internal reference tetramethylsilane(TMS) at δ =0.00 ppm, and J in Hz. Starting materials and reagents are commercially available Sigma-Aldrich).

General Procedure for the Synthesis of Some Selected C-aryl-N-methyl nitrones(1a-j)

The first nitrones were synthesized as per ref. (1a-j)²⁵ with the amendment. In a 250 ml round flask bottle with magnetic stirring the N-methyl hydroxylamine (250mg, 3mmol) was solved in (25ml) dichloromethane, sodium bicarbonate (252mg, 3mmol) was added to this stirred solution besides substituted benzaldehyde(3mmol). The resulting mixture was subjected to reflux at 40°C for(3-6hrs). When TLC(Benzene: MeOH(8:2) showed consummation of the reaction, water (20ml) was added and the organic layer was isolated, dried over anhydrous magnesium sulfate, sifted, and dissolvable was dispersed and recrystallized by hot ethanol, then purity of compounds was monitored using column chromatography on silica (84 g, 9835; 5cm x 45cm) and eluted using Benzene: MeOH (8:2) to give C-aryl-N-methyl nitrones(1a-j).

Synthesized of C-(4-methylphenyl)-N-methylnitrone(1b)

The mixture was stirred for (5hrs); yield 72%; m.p. 116-118; Rf 0.38, IR(KBr): ν =3087(=C-H),3027,2943(-CH₃),1587(C=N),1505,1414(-C=C-aromatic), 1272,1163(N⁺-O⁻),944,838 cm^{-1} (Para-disubstituted benzene). ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 7.90-7.95(m,2H, aromat. H,a,e),7.10-7.15(m, 2H, aromat.H, b,d) 7.02 (CH=N), 3.60 (s,3H, N-CH₃), 2.03-2.06 (t, 3H, Ar-CH₃). ¹³C-NMR (300 MHz, CDCl₃): δ (ppm) = 21.00 (Ar-CH₃), 54.00(N-CH₃), 126.50,128.00(Aromat. C), 135.00 (C=N).

Synthesized of C- (4-florophenyl)-N-methylnitrone(1d)

The mixture was stirred for(4hrs); yield 66%; m.p. 98-100; Rf 0.50, IR(KBr): ν =3145(=C-H),3087,2943 (CH₃)1596(C=N),1505,1489(-C=C- aromatic) ,1166(N⁺-O⁻) ,838 cm^{-1} (Para-disubstituted benzene). ¹H-NMR (400 MHz, CDCl₃): δ (ppm)=7.30- 7.32(m,2H, aromat. H, b, d) 7.25-7.28(m, 2H, aromat.H a,e),7.18

(CH=N), 3.78(s, 3H, N-CH₃). ¹³C-NMR(300MHz, CDCl₃): δ (ppm) = 58.00 (N-CH₃), 124.00-162.00, (Aromat. C), 134.00 (CH=N).

Synthesized of C-(4-chlorophenyl)-N-methylnitrone(1f)

The mixture was stirred for (5hrs) ; yield 70%; m.p. 117-119; Rf 0.45 IR(KBr): ν=3102(=C-H), 3087, 2983(-CH₃), 1560(C=N), 1505, 1455(-C=C- aromatic), 1196 (N⁺-O⁻), 838 Cm⁻¹ (Para-disubstituted benzene). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) = 8.15 - 8.20 (m, 2H, aromat.H.b,d), 7.35-7.40 (m, 2H, aromat.H.a,e), 7.30 (CH=N), 3.85 (s, 3H, N-CH₃). ¹³C-NMR(300 MHz, CDCl₃): δ (ppm) = 54.00 (N-CH₃), 127.00-128.00, (Aromat. C), 134.00 (CH=N).

Synthesized of C-(2,6-Dichlorophenyl)-N-methylnitrone(1g)

The mixture was stirred for (4hrs); yield 65%; m.p. 63-65; Rf 0.48 IR(KBr): ν=3210(=C-H), 308(-CH₃), 1596(C=N), 1535, 1447(-C=C-ar-matic), 1187(N⁺-O⁻), 834, 734, 663 Cm⁻¹ (ortho- disubstituted benzene). ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 7.60-7.64 (m, 2H, aromat.H.b,d), 7.54 (m, 2H, aromat.H.c), 7.55 (CH=N), 3.73(s, 3H, N-CH₃). ¹³C-NMR (300 MHz, CDCl₃): δ (ppm) = 59.02 (N-CH₃), 126.80-135.80, (Aromat.C), 135.06(CH=N).

Synthesized of C-(4-methoxyphenyl)-N-methylnitrone(1h)

The mixture was stirred for (4hrs); yield 64%; m.p. 37-39; Rf 0.34 IR(KBr): ν=3083(=C-H), 3044, 2946(CH₃), 1680(C=N), 1599, 1461(-C=C-aromatic), 1251(C-O), 1155(N⁺-O⁻), 1091(C-N), 832, 774, 733 Cm⁻¹ (Para-disubstituted benzene). ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 7.33-7.36 (m, 2H, aromat.H.a, e), 7.05-7.08 (m, 2H, aromat.H.b,d), 7.44 (CH=N), 3.91-3.94 (s, 3H, CH₃), 3.83 (s, 3H, N-CH₃). ¹³C-NMR (300 MHz, CDCl₃): δ (ppm) = 54.7(-CH₃), 59.08 (N-CH₃), 129.00-132.00, (Aromat. C), 134.00 (CH=N).

Synthesized of C-(5-hydroxymethyl furfural)-N-methylnitrone(1j)

The mixture was stirred for (6hr) ; yield 52%; m.p. 118-120 ; Rf 0.53, IR(KBr): ν=3375(OH), 2929(-CH₃), 1523(C=N), 1196(N⁺-O⁻), 1072, (C-O-C) Cm⁻¹. ¹H-NMR(400 MHz, CDCl₃): δ(ppm)= 10.05(s, 1H, -OH), 6.56-6.48 (m, 2H, aromat.H.b,c), 7.55 (CH=N), 3.82 (s, 3H, N-CH₃). ¹³C-NMR(300 MHz, CDCl₃): δ(ppm) = 59.00 (N-CH₃), 110.04, 112.06, 143.04, 145.23, (Furfural C), 166.40 (CH=N).

General Procedure for Isoxazolidine Derivatives Formation (3a-j)

According to ref²⁵, with slight modification, the isoxazolidine derivatives were prepared in (100ml) a round bottom flask. The nitron (1.44mmol) was dissolved and stirred in toluene, and (1.44mmol) from cinnarizine was added to the solution. The reactants solution was heated for (21-48h) by reflux at 110 °C. When TLC indicates that the reaction finished, the resulting mix was cooled and the reaction solvent was evaporated, then handled with water (20ml) and chloroform (30ml) was added, then the organic layer separated and dried over anhydrous sodium sulfate, after that filtered, and the solvent was evaporated in vacuo. Crystallization of the residue was done by hot ethanol, for purification, column chromatography was used, eluted Benzene: MeOH (6: 4).

Preparation of (2-Methyl-3-(4-methylphenyl)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3b)

The mixture was stirred for (22hr); yield 91%; m.p. 104 -106; Rf 0.44, IR(KBr): ν= 3284(=C-H), 3065, 2996(-CH₃), 1552, 1492(C=C aromatic), 1168(N-O), 1056, 1059(C-N), 865, 794 Cm⁻¹ (Para-substituted benzene). ¹H-NMR (CDCl₃, 400MHz): δ=1.23(m, 4H, 2CH₂), 2.08(s, 3H, CH₃), 2.28 (m, 2H, CH₂), 2.57 (d, 4H, 2CH₂), 3.05(s, 3H, N-CH₃), 3.51-3.55(d, J_{H4-H3} = 16.5 Hz 1H, C4-H), 3.86-3.94 (m, 1H, C5-H), 4.05-4.09(d, J_{H3-H4} = 16.5 Hz 1H, C3H), 4.50 (s, 1H, C-H), 7.24-8.10 (m, 19H, Ar-protons). ¹³C-NMR(CDCl₃, 300MHz): δ=27.14(-CH₃), 50.44(C6), 52.43 (2C7,7'), 53.00 (C4-H), 54.10(N-CH₃), 56.89 (2C8,8'), 78.87(C3), 80.20(C9), 85.55(C5), 126.35-142.45(Ar- carbones).

Preparation of (2-Methyl-3-(4-Fluorophenyl)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3d)

The mix was stirred for (18hr); yield 82%; m.p. 105-107; Rf 0.63, IR(KBr): $\nu=3067(=C-H)$, 295, (-CH₃), 1500, 1491 (C=C aromatic), 1141(N-O), 1072, 1089(C-N), 963, 741 cm^{-1} (Para-di-substituted benzene). ¹H-NMR(CDCl₃, 400MHZ): =1.24 (m, 4H, 2CH₂), 2.21(m, 2H, CH₂), 2.61 (d, 4H, 2CH₂), 2.98(s, 3H, N-CH₃), 3.51-3.55(d, $J_{H4-H3} = 16.3$ Hz 1H, C4-H), 3.89-3.98(m, 1H, C5-H), 4.05-4.10 (d, $J_{H3-H4} = 16.5$ Hz 1H, C3H), 4.45(s, 1H, C-H), 7.26-8.22(m, 19H, Ar-protons). ¹³C-NMR(CDCl₃, 300MHZ): =50.78(2CH₂), 51.05(-C6), 52.01 (2C7,7'), 53.63(C4-H), 55.43(N-CH₃), 56.49(2C8,8'), 78.11(C3), 81.45(C9), 85.88 (C5) 156.00(C-F) 126.12-156.45(Ar-carbones).

Preparation of (2-Methyl-3-(4-Chlorophenyl)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3f)

The mixture was stirred for (22hr); yield 88%; m.p. 97-99; Rf 0.45, IR(KBr): $\nu=3065(=C-H)$, 2923, 2851(-CH₃), 1620, 1495(C=C aromatic), 1165(N-O), 1076(C-N), 963, 841 cm^{-1} (Para-disubstituted benzene). ¹H-NMR(CDCl₃, 400MHZ): =1.22 (m, 4H, 2CH₂), 2.20 (m, 2H, CH₂), 2.60 (d, 4H, 2CH₂), 2.95(s, 3H, N-CH₃), 3.50-3.54(d, $J_{H4-H3} = 16$ Hz, 1H, C4-H), 3.88-3.98(m, 1H, C5-H), 4.03-4.09(d, d, $J_{H3-H4} = 16$ Hz 1H, C3-H), 4.43(s, 1H, C-H), 7.28-8.21(m, 19H, Ar-protons). ¹³C-NMR (CDCl₃, 300MHZ): =49.43(2C7,7'), 50.44(C6), 53.00(C4-H), 54.10(N-CH₃), 56.89(2C8,8') 77.87(C3), 80.20(C9), 85.55(C5), 133.45(CCl), 126.23-141.45(Ar-carbones).

Preparation of (2-Methyl-3-(2,6-dichlorophenyl)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3g)

The mixture was stirred for (17hr); yield 84%; m.p. 110-112; Rf 0.48, IR(KBr): $\nu=3068(=C-H)$, 2958(-CH₃), 1552, 1491(C=C aromatic), 1163(N-O), 1034, 1076 (C-N), 795, 707 cm^{-1} (Ortho-disubstituted benzene). ¹H-NMR(CDCl₃, 400MHZ): =1.25 (m, 4H, 2CH₂), 2.26(m, 2H, CH₂), 2.62 (d, 4H, 2CH₂), 2.89(s, 3H, N-CH₃), 3.53-3.57(d, $J_{H4-H3} = 16.1$ Hz 1H, C4-H), 3.92-4.02 (m, 1H, C5-H), 4.06-4.10(d, $J_{H4-H3} = 16.1$ Hz 1H, C3-H), 4.51 (s, 1H, C-H), 7.25, 8.23 (m, 18H, Ar-protons). ¹³CNMR(CDCl₃, 300MHZ): =49.50 (2C7,7'), 51.38(C6), 54.05(C4-H), 54.67(N-CH₃), 57.28(2C8,8'), 78.27(C3H), 82.04(C9), 86.43(C5), 126.45-130.18(Ar-carbones).

Preparation of (2-Methyl-3-(4-methoxyphenyl)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3h)

The mixture was stirred for (23hr); yield 55%; m.p. 84-86; Rf 0.44, IR(KBr): $\nu=3277(O-CH_3)$, 3022(=CH), 2934(-CH₃), 1509, 1448(C=C aromatic), 1174(N-O), 1151(C-O-C), 1060, 1078(C-N), 860, 706 cm^{-1} (Para-disubstituted benzene). ¹H-NMR(CDCl₃, 400MHZ): =1.26(m, 4H, 2CH₂), 2.27(m, 2H, CH₂), 2.64(d, 4H, 2CH₂), 2.89(s, 3H, N-CH₃), 3.52-3.56(d, $J_{H4-H3} = 16.4$ Hz 1H, C4-H), 3.73 (s, 3H, O-CH₃), 3.91-4.02(m, 1H, C5-H), 4.08-4.12(d, $J_{H4-H3} = 16.5$ Hz 1H, C3H), 4.53(s, 1H, C9-H), 6.98-7.60(m, 19H, Ar-protons). ¹³C-NMR(CDCl₃, 300M HZ): = 50.03(2C 7,7'), 51.78(C6), 53.10 (C4-H), 54.10(N-CH₃), 56.89 (2C8,8'), 55.13(O-CH₃), 78.35 (C3), 82.21(C9), 86.98 (C5), 120.12-150.29 (Ar-carbons).

Preparation of (2-methyl-3-(5-hydroxymethylfuran)-4-phenyl-5-(1-methylene-4-diphenylmethyl)piperazine)-2-isoxazolidine(3j)

The mixture was stirred for (26hr); yield 95%; m.p. 130-132; Rf 0.50, IR(KBr): $\nu=3202 (-OH)$, 3003(=C-H), 2935(-CH₃), 1624, 1555, 1446(aromatic), 1181(N-O), 1147, 1178(C-O-C), 1074, 1088(C-N), 937, 802 cm^{-1} (benzene). ¹H-NMR(CDCl₃, 400MHZ): =1.30(m, 4H, 2CH₂), 2.32 (m, 2H, CH₂), 2.67(d, 4H, 2CH₂), 2.98(s, 3H, N-CH₃), 3.49-3.53(d, $J_{H4-H3} = 16$ Hz 1H, C4-H), 3.98-4.03(m, 1H, C5-H), 4.09-4.13(d, $J_{H4-H3} = 16$ Hz 1H, C3H), 4.50(s, 1H, C-H), 6.59(d, 1H, C4-furfural), 6.98(d, 1H, C3-furfural), 7.26-7.86(m, 15H, Ar-protons), 9.89(s, 1H, OH). ¹³C-NMR(CDCl₃, 300MHZ): =50.43 (2C7,7'), 50.68(C6), 53.18(C4), 55.22(N-CH₃), 57.19 (2C8,8'), 77.87(C3H), 80.20(C-H), 86.01 (C5), 118.47 (C4-furfural), 120.00(C3-furfural), 144.08(C2-furfural), 160.08(C5-furfural), 125.23-138.45(Ar-carbones).

Antimicrobial Assay

Selected Microbes for antimicrobial activity studies were *Escherichia coli* (ATCC 25922) and *staphylococcus aureus* (ATCC 25923) bacteria against pathogenic fungi *Candida Albicans* and *Microsporum gypsum*. The assay of antibacterial was through disc diffusion on agar media method.²⁶ A sterile solution of Nutrient agar medium was added into glass Petri dishes. 20 mL was spilled into a glass petri dish with 100 × 15 mm and allowed to harden. In the next step, the medium bore three bores (0.5ml of all one) by cork, both fresh bacteria (*E. coli* and *S. aureus*) respectively, were spread on agar media in a glass petri dish. To bores were added drops of saturated tested compounds of various concentrations at 1.00, 0.250, and 0.100 (mg/mL); negative control discs have content of DMSO solvent, and positive control discs (nitrofurazone and Fluconazole) were arranged for media overgrown with bacteria. The culture was incubated at 37°C has taken 24 hours. Antibacterial test results are Average diameter of inhibition zone was measured in mm to all compound using a rule.

RESULTS AND DISCUSSION

Chemistry

Within the framework of our study aimed at the utilization of 1,3-dipolar cycloaddition reactions to heterocycles system and also to bioactive molecules program synthesis. In this research, the 1,3-dipolar cycloaddition of prepared nitrones to the cinnarizine drug has been documented. Although the drug is a commercial product and some aspects of its chemistry have been verified, its potential as a reactive chiral olefinic dipolarophil has been careless so far. The new isoxazolidines(3a-j), 2-Methyl-3-(Aryl)-4(phenyl)-5-(1-methylene-4-diphenylmethylpiperazine) isoxazolidines were prepared and identified based on IR and NMR data. The biological activity of some novel isoxazolidines was studied. Consequently, this alkene is most employed in asymmetric 1,3-dipolar cycloaddition with nitrones.²⁷ The mechanism for the addition of the nitrones to the asymmetric olefin was addressed briefly. Nitrones (**1a-j**) which were synthesised according to the published process as far as possible²⁸ via condensation reaction between the appropriate aldehydes and N-methylhydroxylamine offered the desired nitrones in good yields as a (Scheme-1). The nitrones(**1a-j**) are separated in a pure form and then treated with cinnarizine.

Scheme-1: Synthesis of Nitrones

1,3-Dipolar cycloaddition reaction of nitrones to olefin E-1-cinnamyl-4-(diphenylmethyl) piperazine (cinnarizine) (**2**) which was accomplished in toluene at 110°C under reflux conditions afforded isoxazolidines(**3a-j**) in a high yield and selectivity.

Scheme-2: 1,3-Dipolar Cycloaddition Reaction of Nitrones to Cinnarizine

Spectral Analysis

FTIR Analysis

In the ranges ($1146\text{--}1196\text{ cm}^{-1}$) and ($1500\text{--}1600\text{ cm}^{-1}$), the IR spectra of nitrones showed two absorption bands which assigned to stretching vibrations of $\text{N}^+\text{--O}^-$ and C=N groups, respectively²⁹ bands in the IR spectra confirmed the formation of nitrones via condensation reaction of substituted benzaldehyde with N-methylhydroxylamine. The IR spectra of the isoxazolidines indicated the disappearance of the band of C=C in the olefine, which appears at (1597 cm^{-1}), also the disappearance of the C=N band at (1552 cm^{-1}) in nitron is evidence of the formation of isoxazolidine as shown in (Fig.-1), and new absorption bands at ($1076\text{--}1183\text{ cm}^{-1}$) were assigned to C-N , N-O groups of isoxazolidine ring respectively.

Fig.-1: FTIR Spectra for Nitron(1f) and Isoxazolidine (3f)

^1H -NMR Analysis

In each case, a mixture of Z, E-isomers was detected using NMR analysis. All nitrones **1a-j** were diastereomerically pure. When preparing the nitrones, when non-polar solvent(dichloromethane) was used for the preparation of nitrones, the E isomers ratio was very high compared to the Z isomer ratio (E: Z) (20: 1). We used (**1f**) as a model compound, the composition and configuration of nitron(**1f**) were specified by the analysis of its spectral data. It has been stated that nitrones have a pure product in the E-configuration. The structure of nitron (**1f**) has been proved by ^1H -NMR spectroscopy. The phenyl ring protons were noticed as multiple and (N=CH) protons as singlet with chemical shifts of 7.35-8.20 and 7.02-7.55ppm, respectively as in (Figs.-2 to 5). Cinnarizine is 1,2-disubstituted alkene and the product of the 1,3-dipolar cycloaddition reaction have to be a mixture of regioisomers but due to steric hindrance, just one stereoisomer (anti-anti) can be differentiated from the individual cycloaddition products obtained in a pure state, allowing us to assign the stereochemistry of the recently created isoxazolidine using ^1H -NMR where the oxygen atom in a nitron attacked the carbon atom that is the most electrophilic and is associated with the methylene group and the positively charged carbon atom in the nitron was bound to the carbon atom that carries the phenyl group because that alkene(cinnarizine) has an electron-withdrawing group (phenyl group). The $\text{HOMO}_{\text{dipole}}$ $\text{LUMO}_{\text{dipolarophile}}$ interaction mainly regulates the 1,3-dipolar cycloaddition reaction of the different N-methyl-C-phenylnitrones(**1a-j**). In this case, at the oxygen atom, the HOMO dipole has the biggest coefficient, while at the terminal carbon atom, the LUMO dipolarophile has the biggest coefficient. There are three additional chiral centers C-3, C-4, and C-5 were produced in the cycloaddition. Therefore, four diastereomeric products, anti-syn 4, and syn-syn 4 anti-anti 3 syn-anti 3 could be formed (Scheme -3).

Scheme-3: Four possible Diastereomeric Cycloadducts of Isoxazolidines

Fig.-2: ^1H - NMR Spectrum for C-(4-Chlorophenyl)-N-methylnitron(1f) Fig.-3: ^{13}C - NMR Spectrum for C-(4-Chlorophenyl)-N- methylnitron(1f)

The regio and stereo-selectivity of most nitrones cycloaddition were due to steric hindrance and the trans configuration of the olefine. Asymmetric introduction in nitron-olefin cycloaddition has been accomplished by a combination of chirality in both the dipole and dipolarophile. The anti-isoxazolidines (**3**) arise in an Exo-mode by cycloaddition of the E-nitron.

Fig.-4: ^1H - ^{13}C -HMQC NMR spectrum for C-(4-Chlorophenyl)-N-methylnitron(1f)

Fig.-5: ^1H - ^1H -COSY NMR spectrum for C-(4-Chlorophenyl)-N-methylnitron(1f)

The E-isomer of the nitrones yields the anti-adduct (**3**), Exo transition states would be preferred for steric reasons. The ^1H -NMR spectrum of the cycloadducts provided us information of their structures be anti-anti isomer (**3**). We used (**3f**) as a model compound. The spectra of (**3f**) contained signals of ten methine protons of the compound which appeared in regions (1.22-2.60ppm). In this case, they appeared to overlap with each other as aliphatic protons. The signals of H3 in the isoxazolidine ring have shown in the regions (4.05-4.09ppm) as a doublet with coupling constant $J_{\text{H3,H4}} = 16$ Hz. This large value of coupling constant indicates the trans-relationship between them, and H5 at (3.87-3.95ppm), which is (m) but there overlap together to look multiple while H4 proton appears at (3.50-3.54ppm) as a doublet with coupling constant $J_{\text{H4,H3}} = 16$ Hz. The protons H-4 and H-5 fail to offer coupling since $(\Phi)90^\circ$. This aspect of the H-NMR spectrum is uniquely diagnostic for the protons H-4, H-5 anti-relationship. The relationship between the protons H3, H4, and H4, H5 always trans which was indicated to coupling constant values and examination of molecular models such in (Figs.-6 and 7). Thus, after analyzing the ^1H -NMR spectrum, anti-anti (**3**) isomer was obtained.

Fig.-6: ^1H -NMR Spectrum for Isoxazolidine (**3f**)

Antimicrobial Activity

The elucidated results in a (Table-1), show the biological activity of the isoxazolidines (**3b**, **3d**, **3h**, **3g**, **3f**, **3j**). Antibacterial activity of the compounds (**3d**, **3f**) was observed very high activity against microorganisms compared with standard drugs, the compound (**3b**) showing moderated activity against *E. coli* and high activity against *S. aureus*, *Candida*, the compound (**3g**) showing moderated activity against all microorganisms used. The compound (**3j**) was appeared to have weak activity against all microorganisms, cinnarizine was found to be inactive against all microorganisms used.

Fig.-7: ^{13}C -NMR Spectrum for Isoxazolidine (3f)

As it is clear, Cinnarizine does not have biological activity, but after the cycloaddition of nitrones and cinnarizine, isoxazolidines have high activity against microorganisms. In addition to *S. aureus*, *E. coli*, and *candida*. We used another fungus in our search, the fungus was *M. gypsum*. In tropical countries, *M. gypseum* was recorded as the causal agent of dermatomycoses in man and cattle.³⁰ It also causes infection of monkeys, dogs, mice, and rats. This fungus is occasionally causing tinea corporis and tinea capitis in man, its infection in AIDS patients expand widely. The results revealed that the highest activity is clear for examined compounds (3d, 3f), this means that the evaluation of the antifungal activity of the compounds (3d, 3f) recorded the highest activity against fungus of *Microsporum gypsum* and high inhibition of these compounds. Finally, the remainder examined compounds (3b, 3h, 3g, 3j) revealed moderated activity against fungus, while (cinnarizine drug) was found to be inactive against microorganisms used.

Table -1: Antimicrobial Screening Results of the Some Selected Compounds

Inhibition Zone Diameter (mm)											
Entry	Concentration (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>	<i>M. gypsum</i>	Entry	Concentration (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>Candida albicans</i>	<i>M. gypsum</i>
3b	1	18	15	18	17	3j	1	13	15	12	14
	0.250	14	11	13	11		0.250	9	10	8	9
	0.100	9	-	7	-		0.100	-	-	-	-
3d	1	26	28	22	24	Cinnarizine	1	10	5	10	5
	0.250	21	20	17	21		0.250	5	-	5	-
	0.100	18	17	10	15		0.100	4	-	-	-
3h	1	19	20	18	16	Nitrofurazone	1	25	30	-	-
	0.250	14	14	12	10		0.250	19	26	-	-
	0.100	-	8	-	-		0.100	15	20	-	-
3g	1	16	13	14	15	Fluconazole	1	-	-	20	18
	0.250	8	-	10	7		0.250	-	-	17	15
	0.100	-	-	-	-		0.100	-	-	15	12
3f	1	25	29	28	21	DMSO		-	-	-	-
	0.250	20	23	23	17						
	0.100	17	18	20	14						

CONCLUSION

From through study of regio and stereoselectivity of 1,3-dipolar cycloaddition reactions of nitrones to alkenes, the following conclusions can be gotten from this research work that 1,3-Dipolar cycloaddition of C-aryl-N-methyl nitrones and cinnarizine affords anti- anti-isoxazolidines as one isomer, and the formation of anti-adduct has been rationalized by Z/E isomerization of nitrones and by NMR spectroscopic study. Highly regio and stereoselectivity synthesis of novel isoxazolidines by 1,3-dipolar cycloaddition of nitrones with cinnarizine was assigned. This search identified the synthesis of ten novel isoxazolidines(3a-j) derivatives that were not yet prepared in any previous research. Studied and examined the antimicrobial evaluation of isoxazolidines (3b, 3d, 3h, 3g, 3f, 3j, and cinnarizine). These new compounds that were not synthesized in

any previous study may be useful in the potential development of cinnarizine drug as a novel antimicrobial medication.

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REFERENCES

1. J. Malinina, T.Q. Tran, A.V. Stepanov, V.V. Gurzhiy, G.L. Starova, R.R. Kostikov, and A.P. Molchanov, *Tetrahedron Letters*, **55** (27) 3663(2014), <https://doi.org/10.1016/j.tetlet.2014.04.107>
2. H. Pellissier, *Tetrahedron*, **68**(10), 2189 (2012), <https://doi.org/10.1016/j.tet.2011.10.103>
3. S. Karlsson, and H. Högborg, *Tetrahedron Asymmetry*, **12**(14), 1975(2001), [https://doi.org/10.1016/S0957-4166\(01\)00366-4](https://doi.org/10.1016/S0957-4166(01)00366-4)
4. B. Chakraborty, and P.K. Sharma, *Rasayan Journal of Chemistry*, **3**(3), 454(2010).
5. A. Viktor, V. Dmitrieva, M. Mariia, M.M. Efremovaa, S. Alexander, and A. Novikov, *Tetrahedron Letters*, **59**(24), 2327(2018), <https://doi.org/10.1016/j.tetlet.2018.04.066>
6. M. Djahieche, H. Boufenaya, and A. Boukhari, *Revue Roumaine de Chimie*, **64**(11), 973(2019), <https://doi.org/10.33224/rch.2019.64.11.05>
7. A. Rescifina, U. Chiacchio, A. Corsaro, A. Piperno, and R. Romeo, *European Journal of Medicinal Chemistry*, **46**(1), 129 (2011), <https://doi.org/10.1016/j.ejmech.2010.10.023>
8. R. Mandal, M. Singh, A. Krishnan, Y. H. Dahat, P. Bharitkar, V. Ravichandiran, and A. Hazra. *Natural Product Research*, **34**(15), 1(2019), <https://doi.org/10.1080/14786419.2019.1582045>
9. A. R. Minter, B. B. Brennan, and A. K. Mapp, *Journal of the American Chemical Society*, **126**(34), 10504 (2004), <https://doi.org/10.1021/ja0473889>
10. B. Loh, L. Vozzolo, B. J. Mok, C. C. Lee, R. J. Fitzmaurice, S. Caddick, and A. Fassati, *Chemical Biology & Drug Design*, **75**(5), 461(2010), <https://doi.org/10.1111/j.1747-0285.2010.00956.x>
11. F. Chen, P. Han, P. Liu, N. SiJ. Liu, and X. Liu, *Scientific Reports*, **4**, 1(2014).
12. P. Chen, J. R. Fan, T. Zhou, X. L. Liu, J. L. Liu, and G. Schnabel, *Plant Disease*, **96**(3), 416,(2012), <https://doi.org/10.1094/PDIS-06-11-0495>
13. M. Setoguchi, S. Iimura, Y. Sugimoto, Y. Yoneda, J. Chiba, T. Watanabe, F. Muro, Y. Iigo, G. Takayama, and M. Yokoyama, *Medicinal Chemistry*, **21**, 42(2013), <https://doi.org/10.1016/j.bmc.2012.11.003>
14. A. Kaur, B. Singh, and A. S. Jaggi, *Bioorganic & Medicinal Chemistry Letters*, **23**(3), 797 (2013), <https://doi.org/10.1016/j.bmcl.2012.11.080>
15. A. Shi, H. Chen, Y. Cui, and X. Tang, *International Journal of Pharmaceutics*, **373**(1-2), 147 (2009), <https://doi.org/10.1016/j.ijpharm.2009.02.006>
16. S. A. Allen, A. Tomilov, and G. A. Cortopassi, *Biochemical Pharmacology*, **155**, 298 (2018), <https://doi.org/10.1016/j.bcp.2018.07.013>
17. M. Kissane, and A. R. Maguire, *Chemical Society Reviews*, **39**, 845 (2010), <https://doi.org/10.1039/B909358N>
18. K. Torssell, Nitrile Oxides, Nitrones, and Nitronates in Organic Synthesis, *Verlagsgesellschaft, Weinheim publishers*, **22**, 828 (1988)
19. K. Żelechowski, W.M. Gołębiewski, and M. Krawczyk, *Monatshefte für Chemie*, **146**, 1895 (2015), doi.org/10.1007/s00706-015-1568-7
20. N. Nishiwaki, H. Asahara, *Methods Applications Cycloaddition Reactions in Organic Synthesis*, *John Wiley*, p223,240 (2014).
21. F.M. Cordero, S. Bonollo, F. Machetti, and A. Brandi, *European Journal of Organic Chemistry*, **2006**(14), 3235 (2006), <https://doi.org/10.1002/ejoc.200600104>
22. M. Burdisso, R. Gandolfi, P. Grunanger, and A. Rastelli, *The Journal of Organic Chemistry*, **55**(11), 3427, (1990), <https://doi.org/10.1021/jo00298a001>
23. E. Tyrrell, J. Allen, K. Jones, and R. Beauchet, *Synthesis*, **14**, 2393 (2005), <https://doi.org/10.1055/s-2005-869992>.

24. H. Pellissier, *Tetrahedron*, **63**(16) 3235(2007), <https://doi.org/10.1016/j.tet.2007.01.009>
25. A.Vasella, *Helvetica Chimica Acta*, **60**(426),1273(1977), <https://doi.org/10.1002/hlca.19770600417>
26. HP. Cane, N. Saidi, M. Mustanir, D. Darusman, R. Idroes, and M. Musman, *Rasayan Journal of Chemistry*, **13**(4), 2215(2020), <http://dx.doi.org/10.31788/RJC.2020.1345818>
27. H. Zhang, W.H. Chan, A.W Lee, P.F. Xia, and W.Y.Wong, *Letters in Organic Chemistry*, **1**(1), 63, (2004), <http://doi.org/10.2174/1570178043488644>
28. S.R. Sandler, and W. Daro, In *Organic Functional Group Preparations*, Second Ed., Academic Press, San Diego, p.3, 351 (1989).
29. M. A. Hamer, G. Voinov, T. V. Tikhon, Y. V. Shevelev, N.V. Rybalova, A.B. Gatilov, Pervukhina, Burdukov and A. Grigor'ev, *Organometallics*, **26**(7),1607 (2007), <https://doi.org/10.1021/om060883o>
30. K. Domsch, W. Gams, and T.H Anderson, *Compendium of Soil Fungi*, Academic Press (London) Ltd, p.1 860(1980).

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