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Synthesis, antimicrobial and antioxidant activities of 2-isoxazoline derivatives

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Abstract

A series of derivatives of *trans*-3-(2,4,6-trimethoxy phenyl)-4,5-dihydro isoxazolo-4,5-bis(aroylcarbohydrazide) and of trans-3-(2,4,6-trimethoxyphenyl)4,5-dihydroisoxazolo-4,5-bis[carbonyl-(4'phenyl)thiosemi- carbazide (**9**) were synthesized from *trans*-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-4,5-bis(hydrazenocarbonyl) Isoxazole (**8**). The structures of the Compounds were elucidated by elemental and spectral (IR, NMR, and MS) analysis. The compound 9 show activity against some bacterial species. Whereas, no activity was observed for compounds **10a, 10b** and **10c** against all bacterial species. The antioxidant activity of new compounds has been screened. Compound 9 showed higher antioxidant activity using the DPPH and ATBS method.

Keywords: Isoxazolines; nitrile oxide; 1,3-dipolar cycloaddition; antibacterial activity; antioxidant activity.

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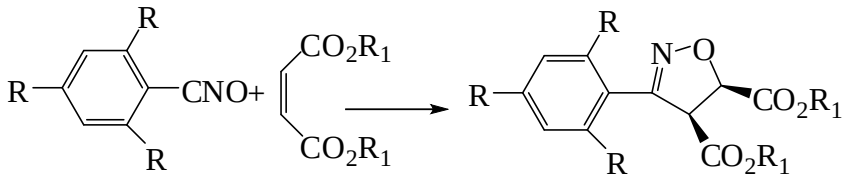
1. Introduction

Isoxazolines are an important class of nitrogen and oxygen containing heterocycles that belong to the azoles family which have gained much importance in the field of medicinal chemistry as the anticancer agents [1-8]. Isoxazolines are also reported to possess good antimicrobial, analgesic, anti-inflammatory activity [4]. Several isoxazolines were generated by 1,3-dipolar cycloaddition of nitrile oxides. Abu-Orabi and Al-Ghezawi reported that 2,4,6-trimethoxybenzonitrile oxide (**1a)** or 2,4,6-trimethylbenzonitrile oxide (**1b)** reacted with dimethyl maleate (**2a)** or diethyl maleate **2b**, to afford only *cis*-cycloadduct **3a-d**, equation 1, [9].



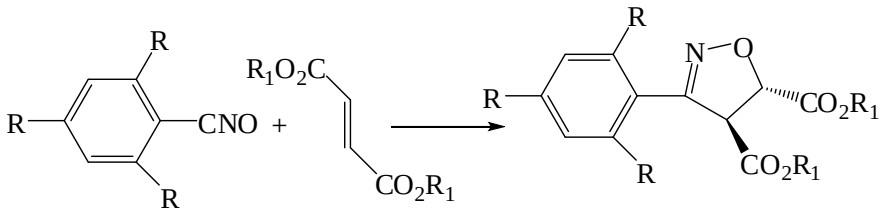
..equ(1)

1a: R= OCH_3
1b: R=CH_3

2a: R_1= CH_3
2b: R_1= C_2H_5

3a: R=OCH_3, R_1=CH_3
3b: R=OCH_3, R_1=C_2H_5
3c: R=CH_3, R_1=CH_3
3d: R=CH_3, R_1=C_2H_5

While the reaction of nitrile oxides **1a** and **1b** with dimethyl fumarate (**4a)** and diethyl fumarate (**4b)** gave the *trans*-isomers **5a-d**, equation 2, [9, 10].



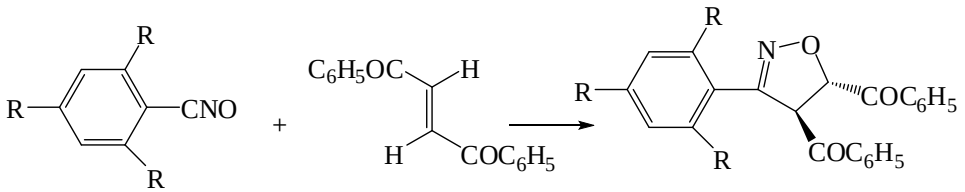
...equ(2)

1a: R=OCH_3
1b: R=CH_3

4a: R_1= CH_3
4b: R_1=C_2H_5

5a: R=OCH_3, R_1=CH_3
5b: R=OCH_3, R_1=C_2H_5
5c: R=CH_3, R_1=CH_3
5d: R=CH_3, R_1=C_2H_5

Similarly, the reaction of compounds **1a** and **1b** with *trans*-dibenzoylethylene (**6)** afforded the corresponding 2-isoxazoline derivatives **7a** and **7b** were formed in good yields as shown in equation 3, [10].



..equ(3)

1a: R= OCH_3
1b: R=CH_3

6

7a: R=OCH_3
7b: R=CH_3

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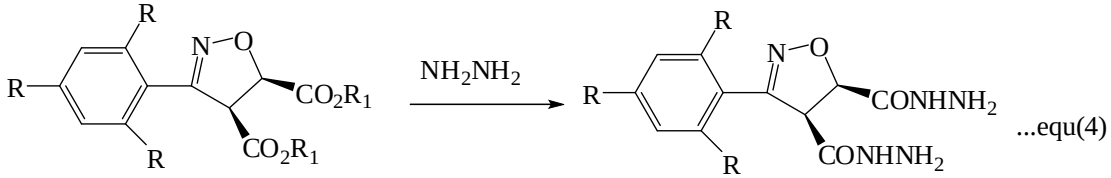
The isoxazoline derivatives **5a-d** bearing an ester group react with excess hydrazine hydrate in ethanol under reflux to give high yield of bis(hydrazinocarbonyl) compounds derivatives **8a-b,** equation 4,[11].



...equ(4)

5a: R = OCH_3, R_1 = CH_3
5b: R = OCH_3, R_1 = C_2H_5
5c: R = CH_3, R_1 = CH_3
5d: R = CH_3, R_1 = C_2H_5

8a: R = OCH_3
8b: R = CH_3

Isoxazolines derivatives exhibit biological, and pharmaceutical activities. In addition, they have wide industrial and analytical applications. These observations promoted us to study the synthesis of some compounds that possess isoxazoline moiety (scheme 1) in order to study the antibacterial activity of these compounds at different bacterial strains.

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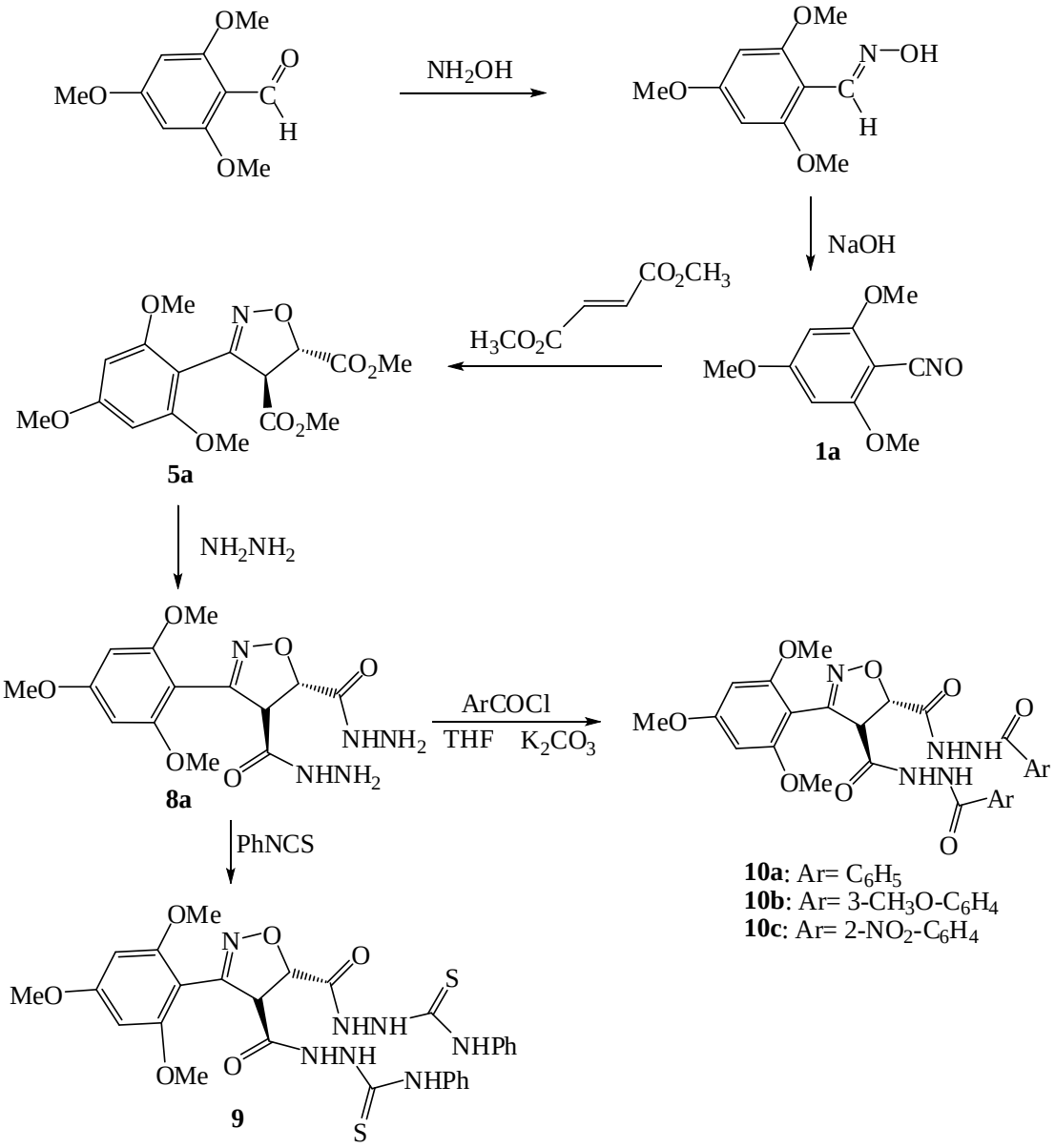

Scheme 1

2. Results and Discussion

Compound 9 was prepared from the reaction of *trans*-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-4,5-bis(hydrazinocarbonyl) isoxazole (8a), with phenylisothiocyanate in absolute ethanol at room temperature, scheme 1.

The HR-MS for compound 9 displays a molecular ion peak at m/z = 622.16207 corresponding to the ion $[C_{28}H_{29}N_7O_6S_2\text{-}H]^-$ as expected from its calculated m/z = 622.15425. The IR spectra of the thiosemicarbazides derivative 9 show absorption bands in the range 3028-3363 cm^{-1}, which are assigned for the N-H stretching frequency. The band at 1703 cm^{-1} is assigned for carbonyl group, while the band in the range of 1200-1256 cm^{-1} is corresponding to the thiocarbonyl group. The ^{1}H-NMR spectrum for compound 9 shows peaks in the range 9.75 - 10.52 ppm, which belong to

the NH protons, these protons are deuterium exchangeable, the ten aromatic protons are detected as a multiplet in the range 7.15 - 7.44 ppm, the two phenyl protons appear as a singlet at 6.20 ppm. The protons on carbon atoms 4 and 5 of the isoxazoline ring appear as two doublets at 4.85 and 5.40 ppm with a coupling constant of 7.8 Hz. the NH peaks was confirmed by addition of deuterium oxide to the NMR tubes of compound 9.

The ^{13}C-NMR spectrum of compound 9 shows the carbon of C=O groups at 159 and 162 ppm. The signal at 180 ppm is assigned to the carbon in C=S bond, ten aromatic carbons were observed in the range of 124-128 ppm. The quaternary carbons in the aromatic rings appear at 138 ppm.

2.1. Preparation of *trans*-3-(2,4,6-trimethoxyphenyl)-4,5-dihydroisoxazolo-4,5-bis(phenylcarbohydrazide) 10a-c:

Compounds 10a-c were prepared from the reaction of *trans*-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-4,5-bis(hydrazinocarbonyl) Isoxazole 8a with two equivalents of aroyl chloride derivatives as shown in scheme 1. The dicarbohydrazides 10a-c were characterized by the IR, ^{1}H-NMR, ^{13}C-NMR and HR-MS.

The HR-MS for compound 10a displays a molecular ion peak at m/z = 560.17793 corresponding to the ion $[C_{28}H_{26}N_5O_8\text{-}H]^-$ as expected from its calculated m/z = 560.17814. IR spectra show broad band in the range 3008-3306 cm^{-1}, which is assigned for NH stretching frequency. The band at 1650 cm^{-1} indicates the amide carbonyl group. In compound 10c, the band at 1535 cm^{-1} corresponds to the Nitro (NO_2) group. Figures 10 and 11 show IR spectra for compounds 10b and 10c, respectively.

The ^{1}H-NMR spectra of compound 10a-c show that the three methoxy groups appear as two singlets, one at 3.76 ppm corresponds to six protons, it belongs to the methoxy at the ortho positions of the phenyl group, while the singlet at 3.79 ppm corresponds to the protons of the methoxy group in para position. The two protons on carbon atoms 4 and 5 of the isoxazoline ring appear as two doublets at 5.02 and 5.31 ppm with a coupling constant of 7.8 Hz. The two protons of aryl group appear at 6.32 ppm as a singlet. Compound 10a show ten aromatic protons as a multiplet in the range 6.93- 7.97 ppm. The broad peak in the range 10.27 - 10.59 ppm is assigned for the four NH amide protons.

The ^{1}H-NMR spectra of compound 10b shows singlet peak at 3.83 ppm corresponds to the protons of the two methoxy group in meta position on the benzoyl rings, and eight aromatic protons appear as a multiplet in the range of 7.11 – 7.50 ppm, The broad peak

in the range 10.22 - 10.53 ppm is assigned for the four NH amide protons. While compound 84c shows eight aromatic protons as a multiplet in the range 7.60 – 8.12 ppm. Three singlets in the range 10.57-10.81 ppm are assigned to the four NH amide protons. The ^{13}C-NMR spectra of the compounds 10a-c show signal at 55.3 and 55.5 ppm that assigned to the three methoxy carbon on the phenyl group, the signal at 90 ppm assigned to the two CH aromatic carbon, The C=O carbons appear at 164-167 ppm. In the compound 10a the aromatic carbons in the two benzoyl rings appear in range of 124 – 139 ppm, while in compound 10b, the aromatic carbons appear in the range 112-133 ppm. The two methoxy groups attached to the benzoyl ring appear at 55.2 ppm. In compound 10c, signal at 146 and 147 ppm are assigned for the carbons attached to Nitro group, other aromatic carbons appear in the range 124-133 ppm. The full assignments of ^{13}C–NMR and ^{1}H–NMR chemical shifts of the compound 10b were confirmed by HMQC and HMBC.

2.2. Antimicrobial activity:

The antibacterial activities of the newly synthesized compounds were evaluated *in vitro* against three Gram-positive and three Gram-negative bacterial strains by the agar well diffusion. The results of the *in vitro* antibacterial screen of new compounds are shown in Table 1. Compound 9 shows activity against four bacterial species Micrococcus *Luteus, Staphylococcus Aureus, Serratia Marcescens* and *Bacillus Cereus* (Table 1) whereas, compounds 10a, 10b and 10c no activity was observed for these compounds against all bacterial species.

Table 1: Mean antibacterial activities (mm inhibition zone diameter ± SE) and MIC values of compounds, and positive control ampicillin (500 µg/ml) against selected bacterial species. Widest diameters of inhibition zones by the oils are indicated in boldface (n=3). The negative control (25% methanol) did not show any antibacterial activity against all tested bacterial species.

Bacterial species	9	10a	10b	10c
Gram – positive bacteria				
*Micrococcus Luteus (*ATCC 9341))	+	-	-	-
Staphylococcus Aureus (ATCC 29213)	+	-	-	-
Bacillus Cereus (ATCC 11778)	+	-	-	-
Gram – negative bacteria				
Serratia Marcescens (ATCC 27117)	+	-	-	-
Pseudomonas Aeruginosa (ATCC 27853)	-	-	-	-

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Salmonella Typhi (ATCC 6539)	-	-	-	-

2.3. Antioxidant activity

The antioxidants activity from organic compounds play important role through their scavenging activity that are a valuable for treatment of many diseases. In this study, the free radical scavenging activity of all compounds was carried out in the presence of the (1,1-diphenyl-2-picrylhydrazyl) DPPH and (2'-Azino–bis(3-ethylbenzoline-6-sulfonic acid) diammonium salt) ABTS using ascorbic acid and α-Tocopherol antioxidant agents as positive control. Although a number of methods are available for determination of the antioxidant activity, the DPPH and ABTS methods are very common, rapid and have been shown to be two of the most appropriate methods [12,13]. The IC50 (effective concentration for scavenging 50% of the inhibition) of synthesized compounds on DPPH and ABTS radical are presented in Tables 2. Based on the experimental results, among all the compounds synthesized, showed higher scavenging activity towards DPPH and ABTS. The higher antioxidant activity of compound 9 can be explained by the existence of the thiourea fragment [14]. However, the order of the radical scavenging power found in both models was 10c > 10b > 10a due to the presence of the nitro on phenyl in compound 10c determines a slight increase of antioxidant activity of compound 10c compared with 10b and 10a.

Table 2**.** DPPH and ABTS antioxidant activities of compounds **9**, **10a, 10b, 10c** and positive controls (ascorbic acid and α-tocopherol). Values expressed are means ± S.D. of three parallel measurements.

Compound	**DPPH IC50 (mg/mL)**	**ABTS IC50 (mg/mL)**
9	$0.07 \pm 4.7\times10^{-3}$	$0.06 \pm 5.7\times10^{-3}$
10a	$0.20 \pm 3.0\times10^{-3}$	$0.12 \pm 7.1\times10^{-3}$
10b	$0.17 \pm 8.3\times10^{-2}$	$0.10 \pm 1.0\times10^{-2}$
10c	$0.09 \pm 1.9\times10^{-3}$	$0.08 \pm 5.0\times10^{-3}$
α-Tocopherol	$2.3\times10^{-3} \pm 1.7\times10^{-5}$	$1.8\times10^{-3} \pm 4.7\times10^{-6}$
Ascorbic acid	$1.7\times10^{-3} \pm 2.3\times10^{-6}$	$1.6\times10^{-3} \pm 4.7\times10^{-6}$

Mean values are significantly different (p<0.05), B: Butanol fraction; A: aqueous methanol fraction; W: water fraction

3. Materials and Methods

3.1. Materials:

2,4,6-trimethoxybenzaldehyde, hydroxylammonium chloride, Dimethyl fumarate, hydrazine hydrate, substituted benzoyl chloride, phenyl isothiocyanate, 1,1-Diphenyl-1-picrylhydrazyl (DPPH, purity N 99%), ascorbic acid (purity = 99%), α-tocopherol

(purity = 99%), 2,2'-Azino–bis(3-ethylbenzoline-6-sulfonic acid) diammonium salt (ABTS, purity N 99%), organic solvents and reagents were purchased from Aldrich, Fluka, Across and Janssen chemicals companies and used without any purification. Melting points were measured on electrothermal digital melting point apparatus and were uncorrected. Infrared spectra (IR) were recorded over the range (400-4000 cm^{-1}) on FT-IR Spectrometer, Bruker spectrum 2000. Potassium bromide pellets were used. High Resolution Mass spectra (HR-MS) were measured in positive ion mode using electrospray ionization (ESI) technique on Bruker APEX-2 instrument.

3.2. Preparation of 2,4,6-Trimethoxybenzaldoxime:

2,4,6-trimethoxybenzaldehyde (11.7g, 60 mmol) was dissolved in 100 mL ethanol / NaOH (10%) mixture (1:1ratio), Excess hydroxylammonium chloride was dissolved in water (75 mL). Then the two solutions were mixed, and the mixture was heated at 60 $^{\circ}C$ for 30 min. Then the mixture was allowed to cool to room temperature. White crystals were collected by suction filtration and recrystallized from ethanol [15].

3.3. Preparation of 2,4,6-Trimethoxybenzonitrile Oxide 1a:

2,4,6-trimethoxybenzaldoxime (4.8 g, 24 mmol) was dissolved in NaOH solution (1 *N*, 50ml) and pyridine (20ml). The clear solution was added dropwise with stirring over a period of 1 hr to a previously prepared solution of Br_2 (3.5 g) in of ice-cooled 1 *N* NaOH (80 mL). The temperature was kept during the addition at 0 $^{\circ}C$. After the addition was completed, the resulting solution was stirred at 0 $^{\circ}C$ for further 30 min. The resulted white precipitate was filtrated as quickly as possible through a large Büchner funnel, washed several times with ice-H_2O and dried under vacuum [16].

3.4. Preparation of *trans*-Dimethyl 3-(2,4,6-trimethoxy phenyl)-4,5-dihydro-4,5-isoxazole Dicarboxylate 5a:

Dimethyl fumarate (2.2 g, 15 mmol) was added to a solution of 2,4,6-trimethoxybenzonitrile oxide (3.15 g, 15 mmol) in dry THF (80 mL). The mixture was heated under reflux for 6 hr. The solvent was removed using rotatory evaporator and the residue was recrystallized from methanol-petroleum ether (60-80 °C) [16].

^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm = 3.54 (s, 6H), 3.70 (s, 6H), 3.76 (s, 3H), 4.73 (d, 1H, *J* = 6.8 Hz), 5.45 (d, 1H, *J* = 6.8 Hz), 6.05 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm = 52.7, 52.9, 55.4, 56.0, 59.1, 80.5, 90.6, 98.0, 149.6, 159.7, 162.9, 167.8, 169.8.

3.5. Preparation of *trans*-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-4,5 -bis(hydrazenocarbonyl) Isoxazole 8a:

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An excess hydrazine hydrate was added to a solution of trans-dimethyl-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-4,5- isoxazoledicarboxylate (1.5 g, 4.3 mmol) in ethanol (80 mL). The mixture was heated under reflux for 50 hr. The solvent was removed and the residue was recrystallized from methanol-petrolueum ether (60-80 °C) to give product (1g, 67% yield); mp: 178-180 °C. the HR-MS displayed a molecular ion peak at m/z 376.12275 corresponding to the ion $[C_{14}H_{19}N_5O_6+Na]^+$ as expected from its calculated m/z = 376.12330. IR (KBr; cm^{-1}): 3300 (N-H), 3050 (C-H arom), 2940 (C-H aliph), 1650 (C=O), 1600 (C=C), 1450 (C=N), 1100 (C-O). ^{1}H-NMR (400MHz, DMSO-d6): δ ppm = 3.71 (s, 6H), 3.80 (s, 3H), 4.29 (bs, 4H, NH_2), 4.50 (d, 1H, *J* = 6.8 Hz), 5.05 (d, 1H, *J* = 6.8 Hz), 6.23 (s, 2H), 9.14 (bs, 1H, NH), 9.50 (bs, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d6): δ ppm = 55.4, 55.9, 58.7, 81.3, 90.9, 97.7, 150.5, 159.4, 162.2, 165.8, 166.9.

3.6. Preparation of trans-3-(2,4,6-trimethoxyphenyl)4,5-dihydroisoxazolo-4,5-bis[carbonyl-(4'phenyl) thiosemicarbazide] 9:

To a solution of hydrazinocarbonyl compound (3.1 g, 5 mmol) in absolute ethanol (30 mL), phenyl isothiocyanate (0.82 g, 6 mmol) was added. The reaction mixture was stirred at room temp for about 20 hr. The mixture was poured into cold water. The resulted precipitate was filtred and recrystallized from ethanol giving 2.5 g (80%) of product **9**; mp: 192-194 °C, IR (KBr; cm^{-1}): 3028-3363 (N-H), 1703 (C=O), 1200-1256 (C=S). ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm = 3.72 (s, 6H), 3.74 (s, 2H), 4.85 (d, 1H, J=7.8 Hz), 5.40 (d, 1H, *J* = 7.8 Hz), 6.20 (s, 2H), 7.15-7.44 (m, 10H), 9.75 (bs, 4H, NH), 10.34 (bs, 1H, NH), 10.52 (bs, 1H, NH). ^{13}C NMR (100MHz, DMSO-d6): δ ppm = 55.3, 56.0, 58.1, 81.6, 91.0, 97.3, 124.1, 125.2, 125.8, 128.0, 128.2, 138.6, 138.8, 139.0, 150.5, 159.4, 162.4, 180.3.

3.7. Preparation of *trans*-3-(2,4,6-trimethoxy phenyl)-4,5-dihydro isoxazolo-4,5-bis(aroylcarbohydrazide) 10a-c:

To a solution of acid dihydrazide (1.12g, 2 mmol) and potassium carbonate (4 mmol) in aq. tetrahydrofuran (160 mL 1:3), a solution of benzoyl chloride (4mmol) in tetrahydrofuran (20 mL) was added dropwise with stirring at 25 °C. The reaction mixture was stirred for 3-6 hr, during which time a precipitate was formed. The precipitate was collected by filtration and washed with water then recrystallized from dimethylsolufoxide (DMSO) / water (1:1)

For compound trans-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro isoxazolo-4,5-bis(phenylcarbohydrazide) **10a:**

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Yield: 1.48g (83%); mp: 202-204 °C, the HR-MS displayed a molecular ion peak at m/z 560.17869 corresponding to the ion $[C_{28}\ H_{27}N_5O_8\text{-}H]^-$ as expected from its calculated m/z = 560.17814. IR (KBr; cm^{-1}): 3145 (N-H), 3026 (C-H aromatic), 1675 (C=O), 1471 (C=N). ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm = 3.41 (s, 6H), 3.83 (s, 3H), 5.02 (d, 1H, *J* = 7.8 Hz), 5.31 (d, 1H, *J* = 7.8 Hz), 6.32 (s, 2H), 6.93-7.97 (m, 10H), 10.27 (bs, 1H, NH),10.44 (bs, 1H, NH), 10.59 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d6): δ ppm = 55.4, 55.9, 58.2, 81.4, 91.0, 97.5, 124.9, 127.4, 127.5, 128.3, 128.5, 131.7, 131.9, 132.3, 132.4, 139.2, 150.2, 159.6, 162.3, 165.0, 165.2, 166.3, 167.4.

For compound trans-3-(2,4,6-trimethoxyphenyl)-4,5-dihydroisoxazolo-4,5-bis(aroylcarbohydrazide) **10b:**

Yield: 0.86g (73%); mp: 225-227 °C.

IR (KBr; cm^{-1}): 3145 (N-H), 3026 (C-H aromatic), 1675 (C=O), 1471 (C=N).

^{1}H-NMR (400 MHz, DMSO-d6): δ ppm = 3.76 (s, 6H), 3.79 (s, 3H), 3.83 (s, 6H), 4.97 (d, 1H, *J* = 7.8 Hz), 5.24 (d, 1H, *J* = 7.8 Hz), 6.27 (s, 2H), 7.11-7.50 (m, 8H), 10.22 (bs, 1H, NH),10.37 (bs, 1H, NH), 10.53 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d6): δ ppm = 55.2, 55.3, 55.4, 55.9, 58.2, 81.5, 91.0, 97.5, 112.4, 112.4, 117.8, 119.7, 119.7, 129.5, 129.7, 133.7, 133.7, 150.2, 159.0, 159.2, 159.6, 162.3, 164.7, 163.9, 166.4, 167.3.

For compound trans-3-(2,4,6-trimethoxyphenyl)-4,5-dihydroisoxazolo-4,5bis(aroylcarbohydrazide) **10c:**

Yield: 0.63g (52%) of product; mp: 255-257 °C,

IR (KBr; cm^{-1}): 3145 (N-H), 3026 (C-H aromatic), 1608 (C=O), 1471 (C=N), 1535 (NO_2). ^{1}H-NMR (400 MHz, DMSO-d6): δ ppm = 3.74 (s, 6H), 3.80 (s, 3H), 4.93 (d, 1H, *J* = 7.8 Hz), 5.28 (d, 1H, *J* = 7.8 Hz), 6.24 (s, 2H), 7.60-8.12 (m, 8H), 10.57 (bs, 1H, NH), 10.61 (bs, 1H, NH), 10.81 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d6): δ = 55.3, 55.9, 58.2, 81.2, 90.8, 97.4, 124.2, 124.3, 129.5, 129.5, 130.1, 130.3, 131.3, 131.7, 146.9, 147.2, 150.2, 159.5, 162.3, 163.7, 164.0, 165.7, 166.8.

3.8. Antimicrobial Activity:

In vitro antimicrobial activity of new compounds was screened against six different bacterial isolates (obtained from the Department of Biological Sciences, Yarmouk University, Jordan) using the agar well diffusion methods. The six bacterial isolates investigated included three *Gram*-positive bacteria *Micrococcus Luteus, Bacillus Cereus* and *Salmonella Typhi* and three *Gram*-negative bacteria *Staphylococcus*

Aureus, *Serratia Marcescens* and *Pseudomonas Aeruginosa*. Bacterial strains were cultured overnight at 37ºC in Trypton Soy broth (TSA).

3.9. Antioxidant activity

The antioxidant activity of compound was determined using the DPPH and ABTS according to the procedures described in the literature [17-20]. Positive controls used included α-tocopherol and ascorbic acid while methanol was the negative control. All determinations of the IC_{50} by the three assay methods were conducted in triplicates. The IC50 of the extracts and the positive controls, expressed as mean ± SD, are shown in Table 3. All determinations of the IC50 by the three assay methods were conducted in triplicates.

4. Conclusions

New 2-isoxazoline derivatives were successfully synthesized and characterized using spectroscopic techniques (IR and NMR) and elemental analysis. All the synthesized compounds have been investigated for their antioxidant activity by DPPH and ABTS assays, and the results indicated that these compounds have good scavenging activities. They were evaluated for their antimicrobial activities against some gram positive and gram-negative bacteria. The results showed that compound 9 show activity against some bacterial species, whereas the rest of the compounds have no considerable effects on microbial growth.

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References

1. Jung, H. K.; Doddareddya MR, Cha JH, Rhim H, Cho YS, Koh HY, et al. Synthesis and biological evaluation of novel T-type Ca^{2+} channel blockers. Bioorg Med Chem. 2004;12:3965–70.

2. Popat KH, Nirmavat KS, Kachhadia VV, Joshi HS. Synthesis and biological activity of 3-aryl-5-(3'-bromo/chlorophenyl) isoxazoles. J Indian Chem Soc. 2003;80:707–8.

3. Norman BH, Lander PA, Gruber JM, Kroin JS. Cyclohexyl-linked tricyclic isoxazoles are potent and selective modulators of the multidrug resistance protein (MRP1) Bioorg Med Chem Lett. 2005;15:5526–30.

4. Nyati M, Rao SN, Srivastav KY, Verma LB. Microwave induced synthesis and antimicrobial activity of some 3-benzimidazolyl-5 aryl-2-isoxazolines. Indian J Hetro Chem. 2006;15:295–6.

5. Diana, G. D., McKinlay, M. A., Brisson, C. J., Zalay, E. S., Miralles, J. V., & Salvador, U. J. (1985). Isoxazoles with antipicornavirus activity. *Journal of medicinal chemistry*, *28*(6), 748-752.

6. Kiran Kumar, A. B. V., Uma Ravi Sankar, A., & Kim, S. H. (2014). A Simple, Efficient One-Pot Synthesis of 2-Isoxazoline Derivatives and their Antimicrobial Activity. *Journal of Heterocyclic Chemistry*, *51*(S1), E146-E151.

7. Prajapti, S. K., Shrivastava, S., Bihade, U., Gupta, A. K., Naidu, V. G. M., Banerjee, U. C., & Babu, B. N. (2015). Synthesis and biological evaluation of novel Δ 2-isoxazoline fused cyclopentane derivatives as potential antimicrobial and anticancer agents. *MedChemComm*, *6*(5), 839-845.

8. Antczak, C., Bauvois, B., Monneret, C., & Florent, J. C. (2001). A new acivicin prodrug designed for tumor-targeted delivery. *Bioorganic & medicinal chemistry*, *9*(11), 2843-2848.

9. Abu-Orabi, S. T., & Al-Ghezawi, N. M. (1987). 1, 3-Dipolar cycloaddition of nitrile oxides with cis-and trans-ethylene-substituted. DELTA. 2-isoxazoline derivatives. *Journal of Chemical and Engineering Data*, *32*(3), 383-384.

10. Abu-Orabi, S. T. (1995). 1, 3 Dipolar Cycloaddition Reactions of Aromatic Nitrile Oxides with Disubstituted Acetylenes and Disubstituted Ethylenes. Part IV. *Mutah Journal of Research And Studies Natural And Applied Sciences Series*, *10*, 79-88.

11. Abu-Orabi, S. T., Al-Hamdany, R., Al-Momany, L. A., & Ta'an, E. A. (1999). Reactions of Isoxazoline and Isoxazole Derivatives with Hydraaaazine Hydrate. *Asian Journal of Chemistry*, *11*(4), 1276.

12. Koparir, M.; Orek, C.; Parlak, A.E.; Söylemez, A.; Koparir, P.; Karatepe, M.; Dastan, S.D. Synthesis and biological activities of some novel aminomethyl derivatives of 4-substituted-5-(2- thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones. Eur. J. Med. Chem. 2013, 63, 340–346.

13. Kumar, A.; Sharma, P.; Kumari, P.; Kalal, B.L. Exploration of antimicrobial and antioxidant potential of newly synthesized 2,3-disubstituted quinazoline-4(3H)-ones. Bioorg. Med. Chem. Lett. 2011, 21, 4353–4357

14. Yehye, W.A.; Rahman, N.A.; Alhadi, A.A.; Khaledi, H.; Ng, S.W.; Ariffin, A. Butylated hydroxytoluene analogs: Synthesis and evaluation of their multipotent antioxidant activities. Molecules 2012, 17, 7645–7665

15. Brewster, R. Q., VanderWerf, C. A., & McEwen, W. E. (1977). *Unitized experiments in organic chemistry* (Vol. 577). Van Nostrand.

16. Grundmann, C., & Dean, J. M. (1965). Nitrile Oxides. V. Stable Aromatic Nitrile Oxides1, 2. *The Journal of Organic Chemistry*, *30*(8), 2809-2812.

17. Al-Qudah M.A., Allahham, F. E., Obeidat, S. M., Al-Jaber, H. I. Lahham, J. N., Abu Orabi, S. T., 2020a. In vitro Antioxidant Activities, Total Phenolics and Total Flavonoids of the Different Extracts of Capparis spinosa L. and Capparis decidua Edgew (Forssk.) from Jordan, International Journal of Pharmaceutical Research, 12(3), 1226-1236.

18. Al-Qudah, M. (2016). Antioxidant Acitvity and Chemical Composition of Essential Oils of Fresh and Air-dried Jordanian Nepeta curviflora Boiss. Journal of Biologically Active Products from Nature, 6(2), 101-111.

19. Al-Qudah, M. A.; Saleh, A. M.; Alhawsawi, N. L.; Al-Jaber, H. I.; Rizvi, S. A.; Afifi, F. U. 2017. Composition, Antioxidant and Anticancer activities of the Essential Oil from Fresh and Air-Dried Aerial Parts of *Pallenis spinosa*. Chem. Biodiversity, 14, e1700146.

20. Abu-Orabi, S. T., Al-Qudah, M. A., Saleh, N. R., Bataineh, T. T., Obeidat, S. M., Al-Sheraideh, M. S., Al-Jaber, H.I., Tashtoush, H.I., Lahham, J. N. (2020). Antioxidant Activity of Crude Extracts and Essential Oils from Flower Buds and Leaves of *Cistus creticus* and *Cistus salviifolius*. *Arabian Journal of Chemistry*, 13(7), 6256-6266.

Sample Availability: Samples of the compounds are available from the authors.