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Synthesis and Antimicrobial Evaluation of Bis-3,5-disubstituted Isoxazoles Based Chalcones[1]

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Abstract—A series of bis-isoxazoles blended chalcones **7a–7j** are synthesized in high yields. The combination of three pharmacologically active moieties in a single scaffold results in the synergistic effect in their bioactivity. All the newly synthesized compounds are characterized by IR, NMR and Mass spectroscopy. The target compounds **7a–7j** are assessed for their antimicrobial activity and these demonstrate high to excellent activity against tested bacterial and fungal strains. The products **7f**, **7h**, **7j,** and **7i** demonstrate potent antimicrobial activity at concentrations 75 and 100 μg/mL.

Keywords: chalcones, 1,5-disubstituted isoxazoles, antimicrobial activity

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INTRODUCTION

Isoxazoles, chalcones and their derivatives demonstrate remarkable biological activity and are used as versatile building blocks in organic and natural compounds chemistry. Among interesting biological activities of isoxazoles is potent and selective antagonism of the *N*-methyl-*D*-aspartate receptor (NMDA). These are structural blocks of well known drugs such as valdecoxib (non-steroidal anti-inflammatory), leflunomide (antirheumatic), isocarboxazid (antidepressant), sulfisoxazole (antibacterial), risperidone (antipsychotic), sulfamethoxazole (antibacterial), and zonisamide (anticonvulsant). Some biologically active chalcone and isoxazole based heterocyclic molecules (**1** [1], **2** [2], and **3** [3]) are presented in the figure.

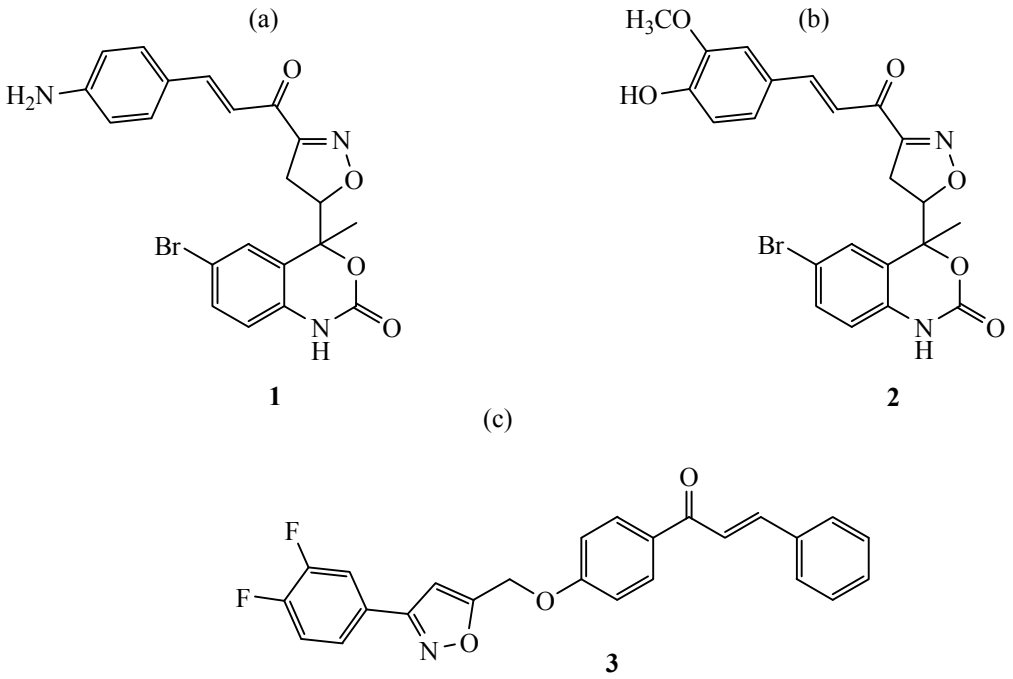
Chalcones (1,3-diarylprop-2-en-1-one) also demonstrate such biological activities as antileishmanial [4], antibacterial [5], antifungal [6], antitumour [7], antimalarial [8, 9], antiviral [10], antitubercular [11], anti-invasive [12], anticancer [13], antioxidant [14], antihyperglycemic [15], anti-inflammatory [16], analgesic [17], antiplatelet [18], and many more.

The above mentioned biological activities of isoxazoles and chalcones encouraged us to make an attempt to conjugate these two important pharmacophores in one molecular structure. So, bis-isoxazoles based chalcones **7a–7j** were synthesized.

RESULTS AND DISCUSSION

2,5-Disubstituted isoxazoles based chalcones **7a–7j** were synthesized by a 4-step protocol (Scheme 1). Initially, the aromatic aldehydes **1a–1e** were converted to oximes **2a–2e** using hydroxylamine hydrochloride. Synthesis of compound **4** was readily achieved by refluxing 1-(2,4-dihydroxyphenyl)ethanone **3** with propargyl bromide and potassium carbonate in dry acetone for 12 h. Structure of the product was confirmed by ^{1}H NMR spectrum, which demonstrated two doublets at 4.79 and 4.74 ppm assigned to two OCH_2 protons and characteristic triplet at 2.57 ppm attributed to two acetylenic (≡CH) protons of dipropagylated compound **4**. Dioxazolic aceto-

[1] The text was submitted by the authors in English.



Representative bioactive chalcone based isoxazoles: (a) (*E*)-4-{3-[3-(4-aminophenyl)acryloyl]-4,5-dihydroisoxazol-5-yl}-6-bromo-4-methyl-1*H*-benzo[*d*][1,3]oxazin-2(4*H*)-one (**1**), antibacterial, (b) (*E*)-6-bromo-4-{3-[3-(4-hydroxy-3-methoxyphenyl)acry-loyl]-4,5-dihydroisoxazol-5-yl}-4-methyl-1*H*-benzo[*d*][1,3]oxazin-2(4*H*)-one (**2**), antibacterial, and (c) 1-{4-[(3-{3,4-difluorophenyl}-isoxazol-5-yl)methoxy]phenyl}-3-phenylprop-2-en-1-one (**3**), mushroom tyrosinase and melanin synthesis in murine B16 cells.

phenones **5a–5e** were synthesized with high yields by reacting dipropargylated compound **4a** with the nitrile oxides. The letter were generated *in situ* by treatment of oximes **2a–2e** with the electrophilic reagent *N*-chlorosuccinimide (NCS).

Bis-isoxazoles based chalcones **7a–7j** were synthesized by treatment of dioxazolic acetophenones **5a–5e** with benzaldehydes **6a–6b** in the presence of ethanolic KOH. The structures of all the newly synthesized compounds were elucidated from IR, NMR and MASS spectra.

Antibacterial activity. The newly synthesized bis-isoxazoles based chalcones **7a–7j** were screened for their antibacterial activity. These compounds demonstrated significant inhibition of the tested four gram positive and four gram negative bacterial strains compared to the standard drug Gentamicin sulphate (Table 1). Among the tested compounds the products **7f**, **7h**, **7j**, and **7i** were highly active at concentrations 75 and 100 μg/mL against *Micrococcus luteus*, Methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris* (Table 1). These results indicated that presence of the electron withdrawing atoms (Br or Cl) or group (NO_2) on isoxazole molecules could stimulate the antibacterial activity more significantly than the electron donating group (CH_3) on isoxazole. Among all products **7a–7j**, *para* methoxy substituted chalcones with the electron withdrawing atoms or group on isoxazole (**7f** and **7h**) demonstrated higher activity than the other compounds.

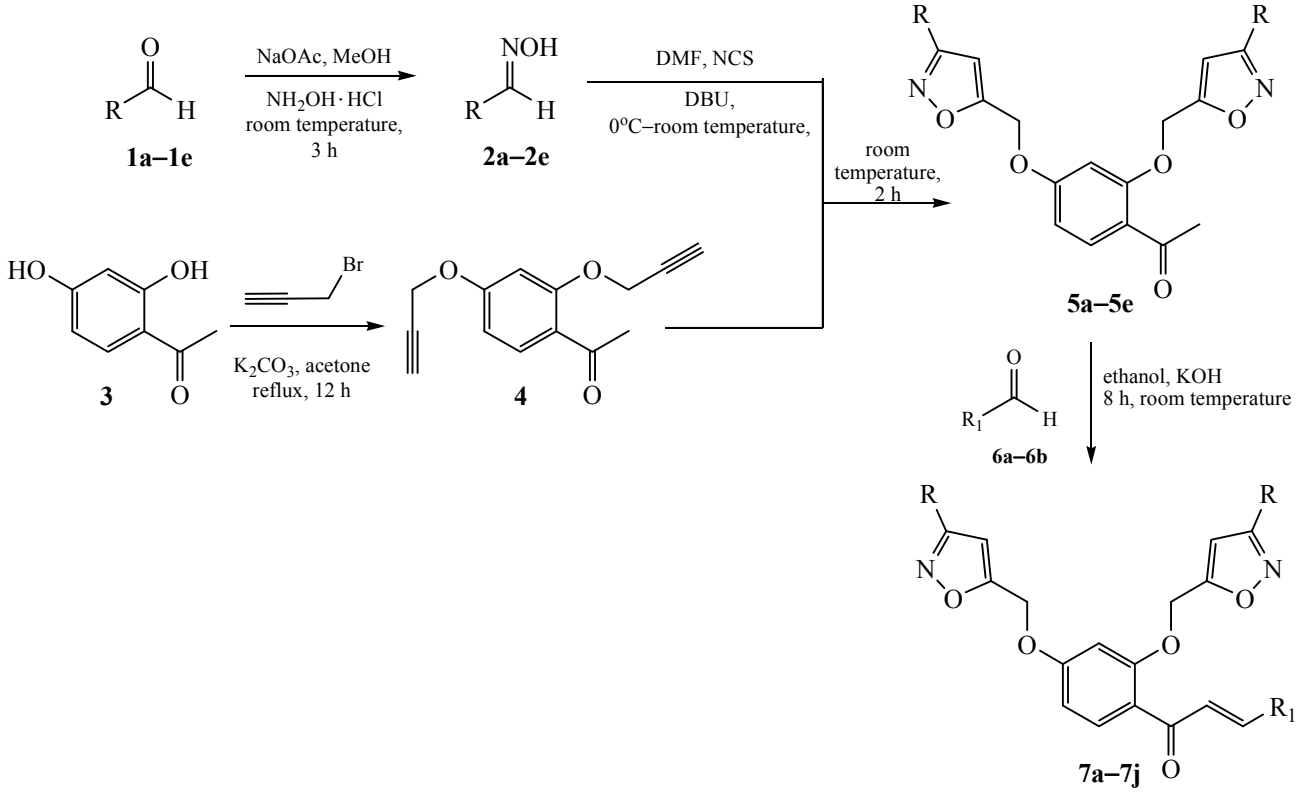
Antifungal activity. Antifungal activity studies were conducted for the compounds **7a–7j** in concentrations 75 and 100 μg/mL against three dermatophytes vize, *Microsporum canis*, *Microsporum gypseum*, *Epidermophyton floccosum*. Nystatin was used as the standard drug. According to the accumulated data (Table 2) antifungal activity was determined to be the highest for the compounds **7f**, **7h**, **7j**, and **7i**.

EXPERIMENTAL

Commercially available reagents were used as supplied, and all solvents were distilled before use. All reactions were performed in an oven-dried glassware.

Melting points were measured in open capillaries and are uncorrected. IR spectra were recorded on a Perkin−Elmer 337 IR spectrophotometer for solid samples pelleted in KBr. NMR spectra were measured on a Bruker AV-400 and AV-300 NMR spectrometers

Scheme 1. Synthesis of bis-3,5-disubstituted isoxazoles based chalcones **7a–7j**.



R = Ph, 4-(Me)Ph, 4-(Cl)Ph, 4-(Br)Ph, 4-(NO_2)Ph; R_1 = Ph, 4-(OMe)Ph.

for $CDCl_3$ solutions with TMS used as an internal standard. CHN analysis was carried out on a Perkin Elmer Model 2400 CHNS elemental analyzer. Electron Spray Ionization (ESI) mass spectra were measured on a QSTARXL hybrid MS system (Applied Bio Systems). TLC was carried out on Merck TLC silica gel 60 F254 plates. The spots were visualized in UV light at 254 nm or by staining with aqueous basic potassium permanganate. Column chromatography was performed on a Merck silica gel 60A (100–200 mesh).

Synthesis of 1-(2,4-dihydroxyphenyl)ethanone (3). A mixture of acetic acid (3 g, 0.05 mol) with freshly fused zinc chloride (6.8 g, 0.05 mol) was heated to 120°C for 30 min, then, upon addition of resorcinol (5.5 g, 0.05 mol), the reaction mixture was heated to 140°C for 30 min while monitoring it by TLC. Upon completion of the process, the reaction mixture was cooled down to room temperature and poured into ice cold water (100 mL), then extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with 20% HCl (50 mL), saturated $NaHCO_3$ (25 mL) and brine (2×25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography using 100–200 mesh silica gel, eluted by 10% ethyl acetate/pet ether to afford 1-(2,4-dihydroxyphenyl)-ethanone as reddish-brown needles. Yield 76%, mp 142–144°C [19].

Synthesis of 1-(2,4-bis-(prop-2-yn-1-yloxy) phenyl)ethanone (4). To a stirred mixture of 1-(2,4-dihydroxyphenyl)ethanone (**3**) (3 g, 0.019 mol) with K_2CO_3 (7.86 g, 0.057 mol) in dry acetone propargyl bromide 80% in toluene (6.78 g, 0.057 mol) was added dropwise, and the reaction mixture was refluxed for ca 12 h. Upon completion of the process (TLC), the reaction mixture was cooled down to room temperature and the excess of acetone was evaporated under reduced pressure. Then the mixture was diluted by water (50 mL) and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with brine (2×25 mL), dried over anhydrous sodium sulphate, filtered, and concentrated in vacuum. The crude product was purified by column chromatography using 100–200 mesh silica gel, eluted by 10% ethyl

Table 1. Antibacterial activity of the compounds **7a–7j**

Compound	Concentration, μg/mL	Zone of inhibition, mm							
		gram positive bacteria				gram negative bacteria			
		Micrococcus luteus	Methicillin-resistant *Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*	*Proteus vulgaris*
7a	75	06	No activity	No activity	05	08	06	07	No activity
	100	09	No activity	No activity	08	11	08	09	No activity
7b	75	19	22	20	23	18	19	23	20
	100	22	25	23	26	21	23	26	22
7c	75	13	14	12	13	11	12	15	14
	100	16	16	15	16	14	15	17	16
7d	75	22	26	24	27	23	20	26	23
	100	25	29	27	30	26	23	29	26
7e	75	15	14	12	13	12	12	15	14
	100	18	17	15	16	14	15	18	17
7f	75	36	35	36	31	31	33	30	28
	100	40	49	49	34	33	36	33	31
7g	75	08	No activity	No activity	07	07	06	No activity	07
	100	11	No activity	No activity	09	10	09	No activity	09
7h	75	33	30	28	29	29	30	31	30
	100	36	33	31	32	31	33	34	33
7i	75	25	27	27	28	26	24	26	25
	100	28	31	30	31	29	26	30	28
7j	75	32	28	26	27	26	26	28	27
	100	35	32	28	30	29	29	31	30
Gentamicin	75	27	31	30	31	28	27	31	29
	100	30	33	33	34	31	30	33	31

acetate in pet ether to afford 1-(2,4-bis(prop-2-yn-1-yloxy)phenyl)ethanone (**4**) as a white solid. ^{1}H NMR spectrum, δ, ppm: 7.85 d (J = 8.97 Hz, 1H), 6.68–6.63 m (2H), 4.79 d (J = 2.38 Hz, 2H), 4.75 d (J = 2.39 Hz, 2H), 2.61 s (3H), 2.57 t (J = 2.29 Hz, 2H).

Synthesis of aldoximes 2a–2e. To a solution of an aldehyde (1 equiv.) in methanol was added hydroxylamine hydrochloride (1 equiv.) followed by sodium acetate (1.5 equiv.). The resulting reaction mixture was stirred at room temperature for 3 h. After completion of the process as monitored by TLC, the reaction mixture was quenched by adding crushed ice, and the precipitate formed was filtered off, washed with hexane and dried to afford substituted aldoximes **2a–2e**.

Synthesis of isoxazoles 5a–5e. For chlorination of aldoximes **2a–2e**, the solutions of aldoximes (1 equiv.) in DMF at room temperature were mixed with NCS

Table 2. Antifungal activity of the compounds **7a–7j**

Compound	Concentration, μg/mL	Zone of inhibition, mm		
		Microsporum canis	*Microsporum gypseum*	*Epidermophyton floccosum*
7a	75	No activity	08	No activity
	100	No activity	10	No activity
7b	75	12	10	09
	100	15	13	11
7c	75	No activity	12	No activity
	100	No activity	15	No activity
7d	75	No activity	No activity	No activity
	100	No activity	No activity	No activity
7e	75	18	16	15
	100	21	18	17
7f	75	25	22	22
	100	28	26	24
7g	75	05	03	05
	100	08	06	07
7h	75	24	19	18
	100	27	22	21
7i	75	19	17	16
	100	22	20	19
7j	75	21	17	16
	100	24	20	18
Nystatin	75	25	20	20
	100	28	24	23

(1 equiv.) and stirred for 30 min. The reaction mixture was cooled down to 0°C and the catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.2 equiv.) was slowly added. This was followed by addition of compound **4**. After consumption of the starting materials, as indicated by TLC, ice-cold water was added to the reaction mixture, precipitate so obtained was filtered off, washed with water and cold MeOH to afford the corresponding pure product.

Synthesis of 3,5-disubstituted isoxazoles based chalcones 7a–7j. To a solution of isoxazoles **5a–5e** (100 mg, 1 equiv.) in EtOH was added KOH (1.5 equiv.), and the mixture was stirred at room temperature for 15 min. An aldehyde **6a** or **6b** (1 equiv.) was added and stirring was continued for 8 h at the same temperature. After consumption of the starting material, as indicated by TLC, ice-cold water was added to the reaction mixture and it was neutralized by 0.1–0.2 N HCl, at which point precipitation occurred. The precipitate was filtered off, washed with water and cold MeOH to afford the corresponding pure 3,5-disubstituted isoxazoles based chalcones **7a–7j**.

(*E*)-1-{2,4-Bis[(3-phenylisoxazol-5-yl)methoxy]-phenyl}-3-phenylprop-2-en-1-one (7a). White solid, yield 90%, mp 171–173°C. IR spectrum, ν, cm^{-1}: 1648 (C=O). ^{1}H NMR spectrum, δ, ppm: 7.92–7.38 m (18H), 7.08 d (*J* = 7.57 Hz, 1H), 6.83 s (1H), 6.67 s

(1H), 6.64 s (1H), 5.30 s (2H), 5.24 s (2H). ^{13}C NMR spectrum, δ_C, ppm: 187.8, 166.1, 161.6, 161.0, 160.9, 158.1, 158.1, 142.2, 130.4, 130.0, 129.5, 129.3, 129.0, 129.0, 128.8 (2C), 128.2 (2C), 127.5 (2C), 127.4 (2C), 127.2 (2C), 126.9 (2C), 124.6, 124.4, 121.9, 118.2, 118.0, 107.9, 101.0, 62.1, 61.6. ESI+ MS: *m/z:* 555 [M + H]$^+$. Found, %: C 75.54; H 4.48; N 4.74. $C_{34}H_{25}ClN_6O_4$. Calculated, %: C 75.80; H 4.73; N 5.05.

(*E*)-1-{2,4-Bis[(3-phenylisoxazol-5-yl)methoxy]-phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one (7b). White solid, yield 88%, mp 157–159°C. IR spectrum, ν, cm^{-1}: 1658 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.00–7.38 m (15H), 7.02 d (J = 7.53 Hz, 1H), 7.00–6.86 m (2H), 6.80 s (1H), 6.67–6.59 m (2H), 5.38–5.21 m (4H), 3.81 s (3H). ^{13}C NMR spectrum, δ_C, ppm: 187.8, 166.1, 161.6, 161.0, 160.9, 158.1, 158.1, 142.2, 130.4, 130.0, 129.5, 129.3, 129.0, 129.0, 128.8 (2C), 128.2 (2C), 127.5 (2C), 127.4 (2C), 127.2 (2C), 126.9 (2C), 124.6, 124.4, 121.9, 118.2, 118.0, 107.9, 101.0, 62.1, 61.6. ESI+ MS: *m/z:* 585 [M + H]$^+$. Found, %: C 73.44; H 4.47; N 4.33. $C_{36}H_{28}N_2O_6$. Calculated, %: C 73.96; H 4.83; N 4.79.

(*E*)-1-{2,4-Bis[(3-{*p*-tolyl}isoxazol-5-yl)methoxy]-phenyl}-3-phenylprop-2-en-1-one (7c). White solid, yield 85%, mp 203–205°C. IR spectrum, ν, cm^{-1}: 1659 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.00–7.22 m (16H), 7.04 d (J = 7.32 Hz, 1H), 6.80 s (1H), 6.71 s (1H), 6.59 s (1H), 5.39–5.19 m (4H), 2.41–2.19 m (6H). ^{13}C NMR spectrum, δ_C, ppm: 187.6, 166.2, 161.7, 161.0, 160.9, 158.2, 158.0, 142.3, 131.7, 131.6, 129.4, 129.3, 129.1 (2C), 129.0, 128.7, 128.4 (2C), 127.9 (2C), 127.5 (2C), 127.3 (2C), 127.0 (2C), 124.5, 124.2, 121.8, 118.2, 118.0, 107.9, 101.1, 62.2, 61.1, 21.5, 21.3. ESI+ MS: *m/z:* 583 [M + H]$^+$. Found, %: C 76.01; H 4.89; N 4.62. $C_{37}H_{30}N_2O_5$. Calculated, %: C 76.27; H 5.19; N 4.81.

(*E*)-1-{2,4-Bis[(3-{*p*-tolyl}isoxazol-5-yl)methoxy]-phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one (7d). White solid, yield 90%, mp 183–185°C. IR spectrum, ν, cm^{-1}: 1657 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.00–7.21 m (15H), 7.07–7.01 m (2H), 6.98 s (1H), 6.78 d (J = 7.29 Hz, 1H), 6.77–6.62 m (2H), 5.39–5.20 m (4H), 3.82 s (3H), 2.41-2.20 m (6H). ^{13}C NMR spectrum, δ_C, ppm: 189.1, 166.1, 161.6, 161.0, 160.8, 159.2, 158.2, 158.0, 143.3, 131.8, 130.3, 130.1, 129.3 (2C), 129.1, 128.8, 128.5 (2C), 127.5 (2C), 127.4 (2C), 127.3 (2C), 127.1 (2C), 124.6, 124.3, 121.9, 118.1, 118.0, 108.0, 101.2, 62.4, 61.2, 56.1, 21.4, 21.2. ESI+ MS: *m/z:* 613 [M + H]$^+$. Found, %: C 74.08; H 5.01; N 4.19.$C_{38}H_{32}N_2O_6$. Calculated, %: C 74.49; H 5.26; N 4.57.

(*E*)-1-{2,4-Bis[(3-{4-chlorophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-phenylprop-2-en-1-one (7e). White solid, yield 78%, mp 179–181°C. IR spectrum, ν, cm^{-1}: 1655 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.00–7.38 m (16H), 7.14–7.03 m (1H), 6.82 s (1H), 6.69 s (1H), 6.60 s (1H), 5.43–5.18 m (4H). ^{13}C NMR spectrum, δ_C, ppm: 187.3, 166.3, 161.6, 161.1, 160.7, 158.1, 158.0, 142.1, 132.4, 132.3, 129.5, 129.4, 129.2 (2C), 129.1, 129.0, 128.8 (2C), 128.7 (2C), 127.9 (2C), 127.7 (2C), 126.3 (2C), 124.4, 124.1, 121.5, 118.1, 118.0, 107.8, 101.3, 62.0, 61.3. ESI+ MS: *m/z:* 623 [M + H]$^+$. Found, %: C 67.14; H 3.56; N 4.17. $C_{35}H_{24}Cl_2N_2O_5$. Calculated, %: C 67.42; H 3.88; N 4.49.

(*E*)-1-{2,4-Bis[(3-{4-chlorophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one (7f). White solid, yield 75%, mp 156–158°C. IR spectrum, ν, cm^{-1}: 1656 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.11–7.25 m (13H), 7.07–7.02 m (1H), 7.01–6.93 s (1H), 6.83 d (J = 7.26 Hz, 1H), 6.78–6.59 m (2H), 5.38–5.21 m (4H), 3.81 s (3H). ^{13}C NMR spectrum, δ_C, ppm: 189.1, 166.2, 161.7, 161.0, 160.7, 159.3, 158.1, 158.0, 143.4, 131.9, 130.4, 130.0, 129.2 (2C), 129.0, 128.7, 128.1 (2C), 127.5 (2C), 127.3 (2C), 127.2 (2C), 127.0 (2C), 124.9, 124.3, 121.0, 118.0, 117.7, 108.3, 101.2, 62.5, 61.3, 56.3. ESI+ MS: *m/z:* 653 [M + H]$^+$. Found, %: C 65.89; H 3.88; N 4.07.$C_{36}H_{26}Cl_2N_2O_6$. Calculated, %: C 66.16; H 4.01; N 4.29.

(*E*)-1-{2,4-Bis[(3-{4-bromophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-phenylprop-2-en-1-one (7g). White solid, yield 75%, mp 207–209°C. IR spectrum, ν, cm^{-1}: 1656 (C=O). ^{1}H NMR spectrum, δ, ppm: 7.99–7.28 m (16H), 7.15–7.05 m (1H), 6.83 s (1H), 6.68 s (1H), 6.59 s (1H), 5.44–5.17 m (4H). ^{13}C NMR spectrum, δ_C, ppm: 187.2, 166.2, 161.7, 161.3, 160.4, 158.3, 158.1, 142.2, 132.7, 132.2, 129.9, 129.7, 129.6 (2C), 129.3, 129.1, 128.9 (2C), 128.6 (2C), 127.8 (2C), 127.6 (2C), 126.4 (2C), 124.0, 123.2, 121.7, 118.3, 118.1, 107.9, 101.5, 62.1, 61.6. ESI+ MS: *m/z:* 711 [M + H]$^+$. Found, %: C 58.81; H 3.18; N 3.71. $C_{35}H_{24}Br_2N_2O_5$. Calculated, %: C 59.01; H 3.40; N 3.93.

(*E*)-1-{2,4-Bis[(3-{4-bromophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one (7h). White solid, yield 80%, mp 186–188°C. IR spectrum, ν, cm^{-1}: 1657 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.08–7.21 m (13H), 7.08–7.01 m (1H), 6.95 s (1H), 6.89 d (J = 7.34 Hz, 1H), 6.76–6.58 m (H), 5.37–

5.21 m (4H), 3.80 s (3H). ^{13}C NMR spectrum, δ_C, ppm: 189.4, 166.4, 161.4, 161.2, 160.5, 159.4, 158.2, 158.1, 142.6, 132.8, 130.4, 130.1, 129.4 (2C), 128.9, 128.4, 128.3 (2C), 127.8 (2C), 127.6 (2C), 127.4 (2C), 127.2 (2C), 124.7, 124.3, 121.3, 118.1, 117.9, 108.4, 101.0, 62.1, 61.6, 56.4. ESI+ MS: *m/z:* 741 $[M + H]^+$. Found, %: C 58.01; H 3.30; N 3.48. $C_{36}H_{26}Br_2N_2O_6$. Calculated, %: C 58.24; H 3.53; N 3.77.

(*E*)-1-{2,4-Bis[(3-{4-nitrophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-phenylprop-2-en-1-one (7i). Yellow solid, yield 88%, mp >300°C. IR spectrum, ν, cm^{-1}: 1646 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.40–8.22 m (4H), 7.95–7.35 m (12H), 7.06 d (*J* = 7.33 Hz, 1H), 6.82 s (1H), 6.70 s (1H), 6.61 s (1H), 5.34 s (2H), 5.25 s (2H). ^{13}C NMR spectrum, δ_C, ppm: 187.0, 166.4, 162.0, 161.6, 160.6, 158.5, 158.4, 148.2, 147.9, 133.3 (2C), 133.2 (2C), 130.1, 129.9, 129.7, 129.6 (2C), 129.3, 129.1, 127.9 (2C), 127.8 (2C), 126.6 (2C), 124.2, 123.3, 121.8, 118.2, 118.0, 108.1, 101.5, 62.0, 61.2. ESI+ MS: *m/z:* 645 $[M + H]^+$. Found, %: C 64.95; H 3.48; N 8.40. $C_{35}H_{24}N_4O_9$. Calculated, %: C 65.22; H 3.75; N 8.69.

(*E*)-1-{2,4-Bis[(3-{4-nitrophenyl}isoxazol-5-yl)-methoxy]phenyl}-3-(4-methoxyphenyl)prop-2-en-1-one (7j). Yellow solid, yield 90%, mp >300°C. IR spectrum, ν, cm^{-1}: 1649 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.38–8.20 m (4H), 7.93–7.36 m (9H), 7.08–6.92 s (3H), 6.78 d (*J* = 7.30 Hz, 1H), 6.72–6.59 m (2H), 5.32 s (2H), 5.24 s (2H), 3.81 s (3H). ^{13}C NMR spectrum, δ_C, ppm: 189.3, 166.2, 161.3, 161.1, 160.6, 159.3, 158.3, 158.1, 148.2, 147.9, 143.2, 133.2 (2C), 133.0 (2C), 131.8, 129.7 (2C), 129.6 (2C), 127.9 (2C), 127.8, 124.2, 123.3, 121.8, 118.2, 118.0, 116.3 (2C), 108.1, 101.5, 62.0, 61.7, 56.2. ESI+ MS: *m/z:* 675 $[M + H]^+$. Found, %: C 63.82; H 3.61; N 8.08. $C_{36}H_{26}N_4O_{10}$. Calculated, %: C 64.09; H 3.88; N 8.31.

Antimicrobial activity. *Bacterial and fungal strains.* The gram-positive strains *Micrococcus luteus* (ATCC 10240), methicillin-resistant *Staphylococcus aureus* (MRSA, NCTC 13616), *Bacillus subtilis* (ATCC 6633), and *Bacillus cereus* (ATCC 14579), and the gram-negative strains *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 43816), *Escherichia coli* (ATCC 8739), and *Proteus vulgaris* (ATCC 13315) were purchased from the American Type Culture Collection. Methicillin-resistant *Staphylococcus aureus* was purchased from the Public Health England Culture Collections. Fungal strains *Microsporum canis* (ATCC-36299), *Microsporum gypseum* (ATCC-24102), *Epidermophyton floccosum* (ATCC-15694) were collected from department of biotechnology, Chaitanya postgraduate college (autonomous), Kishanpura, Hanamkonda, Warangal-506001, Telangana, India. All microbial strains were stored at –80°C, streaked on Luria–Bertani (LB) agar plates (Hi-media Laboratories, Mumbai, India) and incubated at 37°C for 20−24 h. A few isolated colonies were selected from each plate and suspended in 5 mL of LB broth in a sterile culture vessel. The vessel was plugged with cotton and incubated with gentle shaking (140 rpm) at 37°C for 20 h.

Preparation of inoculums. Following the protocol of the Kirby–Bauer disk diffusion assay [20], four or five well-isolated colonies of the same morphological type were picked with an inoculating loop, transferred into 5 mL of nutrient broth, and incubated at 37°C for 24 h until a slightly visible turbidity appeared. The turbidity of the actively growing broth cultures was then adjusted with broth to a density equivalent to that of a 0.5 McFarland standard, and the resulting suspensions were used as the initial inocula in the assay.

Antimicrobial assay. The initial inocula of the test organisms, 100 μL, were swabbed over the surface of the agar media (20 mL) in Petri dishes and let to be absorbed for 15 min. Wells, 8 mm in diameter, were made with a sterile cork borer in the seeded agar plates. Solutions of the test compounds in DMSO (100 μL; *c* 75 and 100 mg/mL) were then loaded into the wells and incubated in the air at 37°C for 24 h. Inhibition zone diameters were measured with a zone reader (HiAntibiotic Zone Scale). The standard drug Gentamicin was used as the positive control.

CONCLUSIONS

Bis-isoxazoles based chalcones **7a–7j** are synthesized with high yields and characterized by different spectroscopic methods. The synthesized compounds are tested for their antimicrobial activity. The highest activities are determined for compounds (**7f**, **7h**, **7j**, and **7i**).

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CONFLICT OF INTERESTS

No conflict of interests was declared by the authors.

REFERENCES

1. Baraldi, P., Barco, A., Benetti, S., Pollini, G., and Simoni, D., *Synthesis*, 1987, vol. 1987, no. 10, p. 857. doi 10.1093/jac/20.6.857
2. Verma, N., Kumar, S., and Ahmed, N., *RSC Advances*, 2016, vol. 6, no. 56, p. 51183. doi 10.1039/C6RA08710H
3. Niu, C., Yin, L., Nie, L.F., Dou, J., Zhao, J.Y., Li, G., and Aisa, H.A., *Bioorganic Med. Chem.*, 2016, vol. 24, no. 21, p. 544. doi 10.1016/j.bmc.2016.08.066
4. da Silva, G.D., da Silva, M.G., Souza, E.M., Barison, A., Simões, S.C., Varotti, F.P., Barbosa, L.A., Viana, G.H., and Villar, J.A., *Molecules*, 2012, vol. 17, no. 9, p. 10331. doi 10.3390/molecules170910331
5. de Mello, T.F., Bitencourt, H.R., Pedroso, R.B., Aristides, S.M., Lonardoni, M.V., and Silveira, T.G., *Exp. Parasitol.*, 2014, vol. 136, p. 27. doi 10.1016/j.exppara.2013.11.003
6. Liu, X.L., Xu, Y.J., and Go, M.L., *Eur. J. Med. Chem.*, 2008, vol. 43, no. 8, p. 1681. doi 10.1016/j.ejmech.2007.10.007
7. Tsuchiya, H., Sato, M., Akagiri, M., Takagi, N., Tanaka, T., and Iinuma, M., *Die Pharmazie*, 1994, vol. 49, no. 10, p. 756.
8. Go, M., Wu, X., and Liu, X., *Curr. Med. Chem.*, 2005, vol. 12, no. 4, p. 483. doi 10.2174/0929867053363153
9. Hans, R.H., Guantai, E.M., Lategan, C., Smith, P.J., Wan, B., Franzblau, S.G., Gut, J., Rosenthal, P.J., and Chibale, K., *Bioorganic Med. Chem. Lett.*, 2010, vol. 20, no. 3, p. 94. doi 10.1016/j.bmcl.2009.12.062
10. Mishra, N., Arora, P., Kumar, B., Mishra, L.C., Bhattacharya, A., Awasthi, S.K., and Bhasin, V.K., *Eur. J. Med. Chem.*, 2008, vol. 43, no. 7, p. 1530. doi 10.1016/j.ejmech.2007.09.014
11. Cheenpracha, S., Karalai, C., Ponglimanont, C., Subhadhirasakul, S., and Tewtrakul, S., *Bioorganic Med. Chem.*, 2006, vol. 14, no. 6, p. 1710. doi 10.1016/j.bmc.2005.10.019
12. Chiaradia, L.D., Mascarello, A., Purificação, M., Vernal, J., Cordeiro, M.N.S., Zenteno, M.E., Villarino, A., Nunes, R.J., Yunes, R.A., and Terenzi, H., *Bioorganic Med. Chem. Lett.*, 2008, vol. 18, no. 23, p. 622. doi 10.1016/j.bmcl.2008.09.105
13. Mukherjee, S., Kumar, V., Prasad, A.K., Raj, H.G., Bracke, M.E., Olsen, C E., Jain, S.C., and Parmar, V.S., *Bioorganic Med. Chem.*, 2001, vol. 9, no. 2, p. 337. doi 10.1016/S0968-0896(00)00249-2
14. Padhye, S., Ahmad, A., Oswal, N., Dandawate, P., Rub, R.A., Deshpande, J., Swamy, K.V., and Sarkar, F.H., *Bioorganic Med. Chem. Lett.*, 2010, vol. 20, no. 19, p. 5818. doi 10.1016/j.bmcl.2010.07.128
15. Vogel, S., Barbic, M., Juergenliemk, G., and Heilmann, J., *Eur. J. Med. Chem.*, 2010, vol. 45, no. 6, p. 2206-2213. doi 10.1016/j.ejmech.2010.01.060
16. Satyanarayana, M., Tiwari, P., Tripathi, B.K., Srivastava, A., and Pratap, R., *Bioorganic Med. Chem.*, 2004, vol. 12, no. 5, p. 883. doi 10.1016/j.bmc.2003.12.026
17. Bandgar, B.P., Gawande, S.S., Bodade, R.G., Totre, J.V., and Khobragade, C.N., *Bioorganic Med. Chem.*, 2010, vol. 18, no. 3, p. 1364. doi 10.1016/j.bmc.2009.11.066
18. De Leon, E., Alcaraz, M., Dominguez, J., Charris, J., and Terencio, M., *Inflamm. Res.*, 2003, vol. 52, no. 6, p. 246. doi 10.1007/s00011-003-1164-x
19. Zhao, L.-M., Jin, H.-S., Sun, L.-P., Piao, H.-R., and Quan, Z.-S., *Bioorganic Med. Chem. Lett.*, 2005, vol. 15, no. 22, p. 5027. doi 10.1016/j.bmcl.2005.08.039
20. Sunitha, V., Kumar, A.K., Shankar, B., Kumar, A.A., Krishna, T., Lincoln, C.A., and Pochampalli, J., *Russ. J. Gen. Chem.*, 2017, vol. 87, no. 2, p. 322. doi 10.1134/S1070363217020281
21. Bauer, A., *Am. J. Clin. Pathol.*, 1966, vol. 45, p. 493. doi 10.1093/ajcp/45.4_ts.493