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Synthesis and antimicrobial activity of some ester functionalized isoxazoles incorporating anthracene moieties via nucleophilic substitution reaction

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Abstract

Background and objective: Five-membered heterocycle compounds having single oxygen and nitrogen atom at adjacent positions are known as isoxazoles. Isoxazole compounds have a broad range of biological activities and therapeutic value. In view of a strategic design of antimicrobial compounds, several new ester-functionalized isoxazoles were synthesized and characterized.

Methods: A regioselective isoxazole incorporating an anthracene moiety was adducted via an effective 1,3-dipolar cycloaddition between anthracene nitrile oxide and propargyl bromide as a dipolarophile.

Results: Synthesized isoxazole 4 underwent nucleophilic substitution reaction to produce unprecedented ester-functionalized isoxazoles 6a-j, by condensation with equimolar amounts of different generated *in situ* sodium carboxylate upon dissolving in acetonitrile with refluxing. The chemical structure of all target compounds was proved by (FT-IR, ^{1}H-NMR, and APT^{13}C-NMR) techniques and their antibacterial and antifungal activities was evaluated.

Conclusion: Allnewly synthesizedcompounds6 a-j have been obtained in good yields after purification by column chromatography. They showed significant antibacterial and antifungal activity after screening against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus* and a fungi strain, *Candida albicans*, using disc diffusion method.

Keywords: Nitrile oxide; 1,3-dipolar cycloaddition; Ester-functionalized isoxazole; Antimicrobial activity.

Introduction

Heterocyclic compounds, which contain one or more heteroatoms, have garnered a great deal of attention as they act as a bridge between chemical and life sciences, including medicinal drugs, herbicides, pesticides and veterinary products.[1,2] Isoxazoles are a member of five-membered heterocycle compounds, these compounds are composed of two adjacent heteroatoms, oxygen, and a nitrogen atom,[3, 4] because of their two adjacent electronegative heteroatoms contribute to hydrogen donor-acceptor interactions with certain target enzymes and receptors that are unavailable with other ring structures, they have desirable pharmacological activity.[5] Carbon-carbon and carbon-nitrogen double bonds are responsible for displaying biological properties which include anticancer,[6] antifungal,[7] antimicrobial,[3] antiviral,[8] anti-TB and anti-inflammatory.[9, 10]

The inclusion of isoxazole may contribute to enhanced pharmacokinetic profiles, higher effectiveness, and lower toxicity,[11] as a consequence of their pivotal role in drug formulation, functionalized isoxazole scaffolds are considered necessary to be an essential part of new drug design, attracting the significant interest of many research groups to develop this

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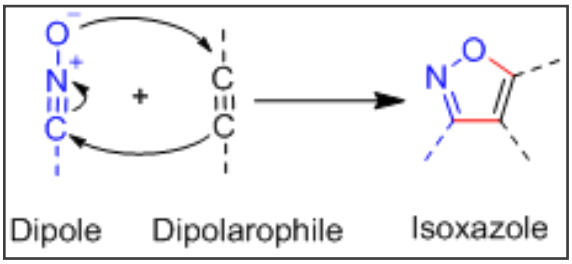
important component of heterocyclics.[12, 13] A variety of synthetic protocols for producing the isoxazole core have been explored.[14] The condensation reaction of hydroxyl amine with each 1,3-dicarbonyl compound, α,β-unsaturated carbonyl compounds and nitriles are used in the classical approach for the synthesis of these remarkable compounds. Furthermore, another elegant and straight forward protocol to synthesize isoxazoles and their derivatives is conducting 1,3-dipolar cycloaddition reaction on alkynes as a dipolarophile with nitrile oxides as the dipole.[15, 16]

In light of the interesting chemistry of isoxazoles, anthracene and their derivatives and their wide applications in pharmaceutical and biological fields.[17] This investigation delves into an efficient and sustainable synthetic protocol to synthesize and characterize distinct ester-functionalized isoxazoles 6a-j containing anthracene moiety and it is thought to be an advancement to biologically active isoxazoles.



Scheme 1 General synthesis of isoxazoles by 1,3-dipolar cycloaddition reaction.

Methods

The present study was performed at Hawler Medical University/college of pharmacy in the postgraduate research lab. All melting points for the prepared compounds in this study were recorded using Electro thermal melting point apparatus model 9100 (capillary method), and JASCO 4600 scientific instrument was utilized to record FT-IR spectra in the range 4000-600 cm^{-1}. ^{1}H and ^{13}C-NMR spectra were measured on a Bruker600 MHz in the deuterated dimethyl sulfoxide (DMSO-d_6)(Day PetronicCompany,Iran).

All chemical shifts were recorded in parts per million (ppm) relative to internal TMS. Coupling constants (J) were expressed in hertz (Hz), broad signals were abbreviated to b, singlet to s, broad singlet to bs, doublet to d, triplet to t, quartet to q, multiplet to m. Solvents and reagents that are commercially accessible were employed without any further purification. The reactions were monitored by TLC and run-on silica gel plates and visualized with UV light. Column chromatography and TLC: silica gel H60 and GF_{254}, respectively; eluants: cyclohexane/ethyl acetate 9:1 to pure ethyl acetate

Synthesis of 9-anthracenenitrile oxide 2

A mixture of 9-anthraldehyde oxime 1 (3.56 g, 16.09 mmol) and (1.33 mL, 16.41 mmol) of pyridine was dissolved in 100 mL of chloroform, the mixture was cooled to 0°C and (2.02g, 15.12mmol) of N-Chlorosuccinimide (NSC) was added portion-wise.[18] The solution was further stirred for three additional hours at room temperature, afterwards, the organic layer was washed with distilled water (2X100 ml) and then dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the obtained nitrile oxide 2 was recrystallized from diethyl ether.

Synthesis of starting material 4

In an Erlenmeyer flask enfolded with aluminum foil, (1.28mL, 14.32mmol) of propargyl bromide 3 was dissolved in 50 ml of anhydrous dichloromethane, then (3.14g, 14.32mmol) of the afforded nitrile oxide 2 was added slowly.[19] after continues stirring the mixture at room temperature for 2 days, the organic phase was washed with brine (3X100 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum and the crude product 4 was purified by column chromatography.

General procedure for the synthesis of ester-functionalized isoxazoles 6a-j

Equimolar amounts of carboxylic acids

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5a-j and sodium bicarbonate (0.01mol) were dissolved in (15 mL) of acetonitrile and refluxed with stirring for 3 hours,[20] followed by the addition of (0.01mol) of cyclo adducted isoxazole4. The reflux was continued and the progression of the reaction was monitored by TLC. After 24 hours, cooled, and poured into crushed ice, the organic phase was extracted with DCM (3X30mL).The solvent was removed under vacuum and the crude products 6 a-j were obtained, which were purified by column chromatography.

Antibacterial Activity

Antibacterial activity was achieved against two distinct microorganisms *E. coli* and *S. aureus* by utilizing the agar well diffusion technique.[21] Newly prepared molten MHA (Muller Hinton Agar) medium was poured at a uniform depth of 5 mm into 9-cm petri plates and then left to cool to room temperature. On the other hand, various quantities (5, 15, 30 µg) of the synthesized isoxazole derivatives 6a-j were mixed with KBr powder, the mixtures were converted to disc form under high pressure, applied on the discs and KBr was used as a control disc. Subsequently, the plates were left in an incubator at 37°C for twenty-four hours to culture organisms. After incubating, a clear ruler was utilized to determine the size of the well's surrounding inhibitory zone in millimeters (mm). the activity results are shown in Table 3.

Antifungal Activity

The sabouraud dextrose agar medium plate disc diffusion method was employed to assess the antifungal activity at a concentration of 50 µg per disc.[22] The antifungal activity of each synthesized isoxazole derivative was examined in vitro against *Candida albicans*. To get the ideal concentration each synthesized compound was dissolved in DMSO. The discs (6 mm in diameter) were impregnated; air dried and placed on the sabouraud dextrose agar media, previously seeded with 0.2 mL of a broth culture of each organism for 18 hours. Nearly 250 µL of samples from the different concentrations were then filled in wells. The inhibitory zones were measured in millimeters after the plates had been incubated at 37 °C for 24 hours, the activity results are shown in Table 3.

Results

The conversion of 9-anthraldehyde oxime to its nitrile oxide form was the initial step of this study, which is reacted with propargyl bromide to obtain compound 4 as a yellow powder after purification by column chromatography. In the quest to synthesize some novelester-functionalized isoxazole, anucleophilic substitution reaction was conducted on the prepared isoxazole4 with different carboxylic acid derivatives, as a consequence all synthesized compounds6 a-j were collected as a powder with a good to high percentage of the yield as shown in Table 1

Table 1: Some physical constants of the synthesized compounds

Compounds	Chemical formula	Yield %	M.P °C	Color
4	$C_{18}H_{12}BrNO$	78	81-85	Yellow
6a	$C_{25}H_{17}NO_3$	92	142-144	White
6b	$C_{25}H_{18}N_2O_3$	94	168-170	White
6c	$C_{25}H_{16}N_2O_5$	91	186-188	Yellow
6d	$C_{25}H_{16}FNO_3$	75	138-140	Pale yellow
6e	$C_{25}H_{16}BrNO_3$	79	121-123	Orange
6f	$C_{25}H_{17}NO_4$	89	139-141	White
6g	$C_{25}H_{15}N_3O_7$	92	221-223	Yellow
6h	$C_{27}H_{18}N_2O_5$	88	173-175	Yellow
6i	$C_{25}H_{16}ClNO_3$	75	137-139	White
6j	$C_{20}H_{15}NO_3$	54	141-143	White

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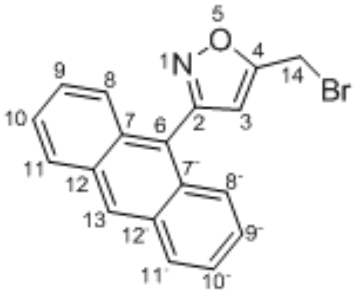
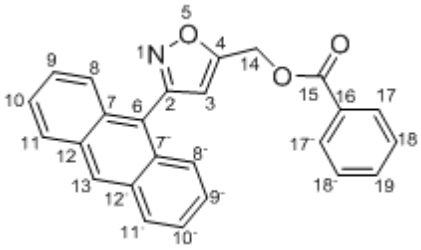
Different spectroscopic techniques have been utilized for the structure elucidation of the synthesized compounds; FT-IR spectroscopy is regarded as an indispensable characterization option, for the identification of functional groups, which is given strong evidence to obtain some primary information about the progress of the study. All synthesized isoxazole derivatives 6a-j showed some characteristic peaks, for instance, a strong absorption band between 1662-1743 cm^{-1} corresponding to C=O stretching of the ester groups, in addition to vibration frequencies at 1203-1268 cm^{-1} due to C-O stretching. Compounds 6c,g and h are further characterized by presenting two prominent absorption bands around 1500 and 1330 cm^{-1} referring to asymmetrical and symmetrical stretching of the NO_2 groups respectively. In the ^{1}H-NMR spectra, all methylene and aromatic protons were observed at the expected region, as presented in Table 2. Attached Proton Test NMR (APT NMR) are ^{13}C-NMR experiments, pointed up the carbons bonded to an odd number of protons (CH and CH_3)while quaternary and even proton bonded carbons are pointed down, for example, signals for all carbonyl of the ester groups C=O at d 163.9-167.9 ppm and methylene groups at d56-68 ppm are pointed down. All of these data suggested that the nucleophilic substitution reaction was effectively completed and the target molecules was achieved.

Table 2 FT-IR, ^{1}H-NMR and APT ^{13}C-NMR chemical shift assignments in ppm for synthesized compounds

Compounds	FT-IR in cm^{-1}, ^{1}H-NMR and ^{13}C-NMR chemical shifts in ppm
 **4**	**FT-IR in cm^{-1}**: 3178 (C-H str. isoxazole),1585 (C=N str.), 1245 (C-O str.). **^{1}H-NMR** (300 MHz, DMSO-d_6, 25°C): d = 4.68 (s, 2H, C_{14}-**H_2**), 5.59 (s, 1H, C_3-**H**), 7.47-7.54 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.81 (d, H, $C_{11,11'}$-**H**), 8.06 (d, 2H, $C_{8,8'}$-**H**), 8.59 (s, 1H, C_{13}-**H**). **^{13}C-NMR** (75 MHz, DMSO-d_6, 25°C): δ C: (18.6: **C_{14}**, 107.2: **C_3**, 122.4: **$C_{9,9'}$**, 125.0: **$C_{10,10'}$**, 125.3:**$C_{7,7'}$**, 125.4: **$C_{11,11'}$**, 126.6: **$C_{8,8'}$**, 128.5: **C_{13}**,129.1: **$C_{12,12'}$**, 131.0: **C_6**,161.2: **C_2**, 167.8: **C_4**.
 **6a**	**FT-IR in cm^{-1}**: 3143 (C-H str. isoxazole), 1708 (C=O str.),1604 (C=N str.), 1265 (C-O str.). **^{1}H-NMR** (600 MHz, DMSO-d_6, 25°C): d = 5.74 (s, 2H, C_{14}-**H_2**), 7.09 (s, 1H, C_3-**H**), 7.54-7.57 (m, 6H, $C_{9,9'}$,$_{10,10'}$,$_{18,18'}$-**H**), 7.69 (t, H, *J*= 6.8 Hz, C_{19}-**H**), 7.75 (d, 2H, *J*= 8 Hz, $C_{17,17'}$-**H**), 8.09 (d, 2H, *J*= 8 Hz, $C_{11,11'}$-**H**), 8.18 (d, 2H, *J*= 8 Hz,$C_{8,8'}$-**H**), 8.81 (s, 1H, C_{13}-**H**). **APT ^{13}C-NMR** (150 MHz, DMSO-d_6, 25°C): δ C: **Upside**:107.93: **C_3**, 125.47: **$C_{9,9'}$**, 126.13: **$C_{10,10'}$**, 127.47: **$C_{11,11'}$**, 129.10: **$C_{18,18'}$**, 129.50: **$C_{8,8'}$**, 130.37: **C_{13}**, 131.10: **$C_{17,17'}$**,134.25: **C_{19}** **Downside**: 57.70: **C_{14}**, 125.43:**$C_{7,7'}$**, 129.07: **C_{16}**, 131.13: **$C_{12,12'}$**, 143.28: **C_6**, 160.92: **C_2**, 165.68: **C_{15}**, 168.24: **C_4**.

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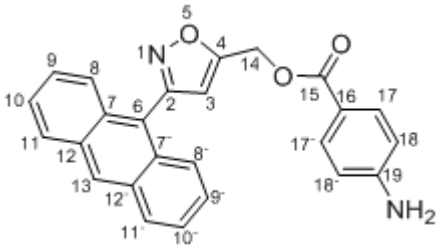


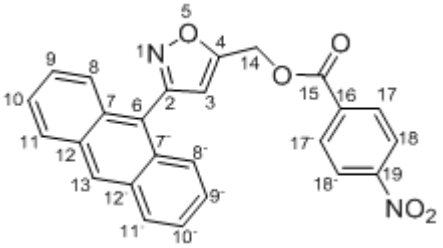
6b

FT-IR in cm^{-1}: 3471, 3363(NH_2str.asym. and sym.), 3131 (C-H str. isoxazole), 1700 (C=O str.),1519 (C=N str.), 1261 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 5.62 (s, 2H, C_{14}-**H_2**), 6.14 (s, 2H, **NH_2**), 6.63 (d, 2H, J= 8.6 Hz, $C_{18,18'}$-**H**) 7.02 (s, 1H, C_3-**H**), 7.52-7.57 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.75 (d, 2H, J= 8.9 Hz, $C_{17,17'}$-**H**), 7.79 (d, 2H, J= 8.7 Hz, $C_{11,11'}$-**H**), 8.18 (d, 2H, J= 7.7 Hz, $C_{8,8'}$-**H**), 8.80 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C: **Upside**: (107.55: **C_3**, 113.23:**$C_{18,18'}$**, 126.11:**$C_{9,9'}$**, 127.44: **$C_{10,10'}$**, 129.04: **$C_{11,11'}$**, 130.36: **$C_{8,8'}$**, 131.11: **C_{13}**, 132.05: **$C_{17,17'}$**.

Downside: 56.74: **C_{14}**, 125.48: **C_{16}**, 127.41: **$C_{7,7'}$**,129.08: **$C_{12,12'}$**, 132.07: **C_6**, 154.51: **C_{19}**, 160.86: **C_2**, 165.72: **C_{15}**, 168.93: **C_4**.



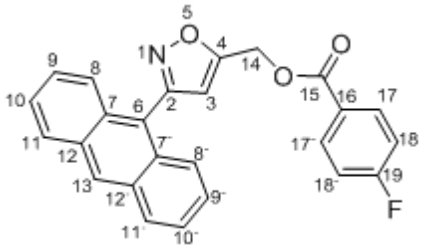
6c

FT-IR in cm^{-1}: 3139 (C-H str. isoxazole), 1716 (C=O str.),1604 (C=N str.), 1515, 1338(NO_2str. asym. and sym.),1265 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 5.78 (s, 2H, C_{14}-**H_2**), 7.13 (s, 1H, C_3-**H**), 7.54-7.60 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.75 (d,2H, J= 8.3 Hz, $C_{11,11'}$-**H**), 8.20 (d, 2H, J= 7.5 Hz, $C_{8,8'}$-**H**), 8.30 (d, 2H, J= 8.9 Hz, $C_{17,17'}$-**H**), 8.35 (d, 2H, J= 8.9 Hz, $C_{18,18'}$-**H**), 8.82 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C:**Upside**: (108.17: **C_3**, 124.40: **$C_{18,18'}$**, 125.44:**$C_{9,9'}$**, 127.48: **$C_{10,10'}$**, 129.11: **$C_{11,11'}$**, 129.52: **$C_{8,8'}$**:130.35: **C_{13}**, 131.12: **$C_{17,17'}$**.

Downside: 58.37: **C_{14}**, 126.11: **$C_{7,7'}$**, 127.40: **$C_{12,12'}$**,129.06: **C_{16}**, 130.27: **C_6**, 150.60: **C_2**, 160.93: **C_{19}**, 164.30: **C_{15}**, 167.78: **C_4**.



6d

FT-IR in cm^{-1}: 3135 (C-H str. isoxazole), 1708 (C=O str.),1504 (C=N str.),1268 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 5.73 (s, 2H, C_{14}-**H_2**), 7.10 (s, 1H, C_3-**H**), 7.33 (d, 2H, J= 8.9 Hz, $C_{18,18'}$-**H**), 7.53-7.57 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.75 (d, 2H, J= 8.9 Hz, $C_{17,17'}$-**H**), 8.13-8.16 (d, 2H, J= 11.2 Hz, $C_{11,11'}$-**H**), 8.18 (d, 2H, J= 8.6 Hz, $C_{8,8'}$-**H**), 8.80 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C:**Upside**: (107.94: **C_3**, 116.57: **$C_{18,18'}$**, 125.46:**$C_{9,9'}$**, 126.11: **$C_{10,10'}$**, 127.45: **$C_{11,11'}$**, 129.06: **$C_{8,8'}$**:130.36: **C_{13}**, 131.12: **$C_{17,17'}$**.

Downside: 57.82: **C_{14}**, 126.08: **C_{16}**,127.41: **$C_{7,7'}$**, 129.07: **$C_{12,12'}$**, 132.90: **C_6**, 160.92: **C_2**, 164.74: **C_{19}**, 166.86: **C_{15}**, 168.15: **C_4**.

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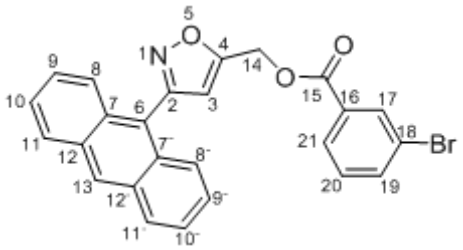


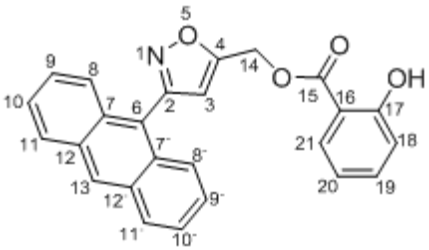
6e

FT-IR in cm^{-1}: 3127 (C-H str. isoxazole), 1712 (C=O str.),1565 (C=N str.),1249 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d$_6$, 25°C): d = 5.76 (s, 2H, C_{14}-**H_2**), 7.13 (s, 1H, C_3-**H**), 7.51 - 5.58 (m, 6H, $C_{9,9}$-,$_{10,10}$-,$_{19,20}$-**H**), 7.76 (d, 2H, *J*= 7.7 Hz, $C_{11,11}$-**H**), 7.91 (d, 1H, *J*= 7.5 Hz, C_{21}-**H**), 8.08 (s, 1H, C_{17}-**H**), 8.19 (d, 2H, *J*= 6.6 Hz, $C_{8,8}$-**H**), 8.81 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d$_6$, 25°C): δ C: **Upside**:(108.08: **C_3**,125.46: **$C_{9,9}$**-126.15: **C_{21}**, 127.43: **$C_{10,10}$**-, 129.12: **$C_{11,11}$**-, 129.53: **$C_{8,8}$**-, 130.38: **C_{13}**, 131.62: **C_{20}**, 132.32: **C_{17}**, 137.02: **C_{19}**.

Downside: 58.08: **C_{14}**,125.46: **C_{18}**, 126.09: **$C_{7,7}$**-, 127.48: **$C_{12,12}$**-, 129.00: **C_{16}**, 131.15: **C_6**, 160.94: **C_2**, 164.44: **C_{15}**, 167.98: **C_4**.



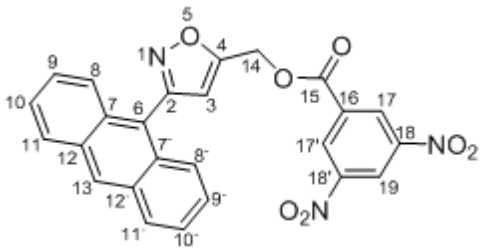
6f

FT-IR in cm^{-1}: 3458 (OH str.), 3189 (C-H str. isoxazole), 1662 (C=O str.),1608 (C=N str.),1203 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d$_6$, 25°C): d = 5.77 (s, 2H, C_{14}-**H_2**), 6.96 (t, 2H, *J*= 7.6 Hz,C_{20}-**H**),7.03 (d, 1H, *J*= 8.3 Hz, C_{18}-**H),** 7.11 (s, 1H, C_3-**H**), 7.53 - 8.59 (m, 6H, $C_{9,9}$-,$_{10,10}$-, $_{19}$-**H**), 7.76 (d, 2H, *J*= 8.2 Hz, $C_{11,11}$-**H**), 7.92 (d, 1H, *J*= 8.0 Hz, C_{21}-**H**), 8.19 (d, 2H, *J*= 7.8 Hz, $C_{8,8}$-**H**), 8.81 (s, 1H, C_{13}-**H**), 10.41 (s, 1H, **OH**).

APT ^{13}C-NMR (150 MHz, DMSO-d$_6$, 25°C): δ C: **Upside**:(109.75: **C_3**, 113.52: **C_{18}**, 117.99: **C_{20}**, 125.41: **$C_{9,9}$**-, 126.10: **$C_{10,10}$**-, 127.44: **$C_{11,11}$**-, 129.11: **$C_{8,8}$**-, 130.32: **C_{13}**, 131.13: **C_{21}**, 136.32: **C_{19}**.

Downside: 57.78: **C_{14}**,125.47: **C_{16}**, 127.48: **$C_{7,7}$**-, 129.07: **$C_{12,12}$**-, 130.94: **C_6**, 160.38: **C_{17}**, 160.93: **C_2**, 167.97: **C_{15}**, 168.05: **C_4**.



6g

FT-IR in cm^{-1}: 3097 (C-H str. isoxazole), 1735 (C=O str.),1619 (C=N str.), 1538, 1338 (NO_2str. asym. and sym.),1265 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d$_6$, 25°C): d = 5.85 (s, 2H, C_{14}-**H_2**), 7.17 (s, 1H, C_3-**H**), 7.56-7.60(m, 6H, $C_{9,9}$-,$_{10,10}$-**H**), 7.74 (d, H, *J*= 8.4 Hz, $C_{11,11}$-**H**), 8.20 (d, 2H, *J*= 7.8 Hz, $C_{8,8}$-**H**), 8.83 (s, 1H, C_{13}-**H**), 8.99 (s, 2H, $C_{17,17}$-**H**), 9.01 (s, 2H, C_{19}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d$_6$, 25°C): δ C: **Upside**:(108.29: **C_3**, 125.40: **C_{19}**, 126.13: **$C_{9,9}$**-, 127.53: **$C_{10,10}$**-, 129.64: **$C_{11,11}$**-, 130.35: **$C_{8,8}$**-, 131.09: **$C_{17,\ 17}$**-, 132.33: **C_{13}**.

Downside: 58.81: **C_{14}**,126.08: **$C_{7,7}$**-, 127.48: **$C_{12,12}$**-,129.36: **C_6**, 130.28: **C_{16}**, 148.63: **$C_{18,18}$**-, 160.92: **C_2**, 167.62: **C_{15}**, 179.19: **C_4**.

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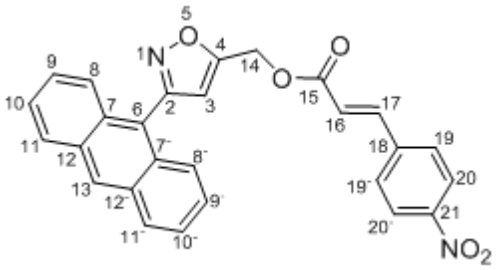


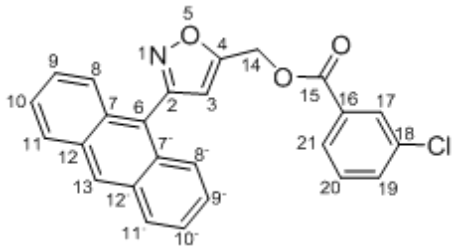
6h

FT-IR in cm^{-1}: 3120 (C-H str. isoxazole), 1716 (C=O str.),1596 (C=N str.), 1508, 1334 (NO_2str. asym. and sym.),1253 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 5.73 (s, 2H, C_{14}-**H_2**), 7.02 (d, 1H, *J*= 16.1 Hz, C_{16}-**H**), 7.07 (s, 1H, C_3-**H**), 7.50-7.60 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.74 (d, 1H, *J*= 8.3 Hz, $C_{19,19'}$-**H**), 7.91 (d, 1H, *J*= 16.1 Hz, C_{17}-**H**), 8.05 (d, 2H, *J*= 8.5 Hz, $C_{11,11'}$-**H**), 8.20 (d, 2H, *J*= 7.6 Hz, $C_{8,8'}$-**H**), 8.24 (d, 2H, *J*= 8.5 Hz, $C_{20,20'}$-**H**), 8.83 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C: **Upside**:(108.12: **C_3**, 121.87: **C_{16}**, 124.37: **$C_{20,20'}$**, 125.40: **$C_{9,9'}$**, 126.14: **$C_{10,10'}$**, 127.49: **$C_{11,11'}$**, 129.13: **$C_{8,8'}$**, 130.07: **$C_{19,19'}$**, 131.13: **C_{13}**, 143.60: **C_{17}**.

Downside: 57.22: **C_{14}**, 124.34: **$C_{7,7'}$**, 126.11: **$C_{12,12'}$**, 130.09: **C_6**, 143.62: **C_{18}**,148.65: **C_{21}**,160.90: **C_2**, 165.60: **C_{15}**, 168.15: **C_4**.



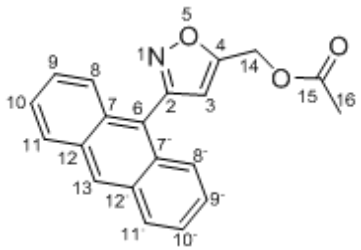
6i

FT-IR in cm^{-1}: 3127 (C-H str. isoxazole), 1724 (C=O str.),1527 (C=N str.), 1253 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 5.81 (s, 2H, C_{14}-**H_2**), 7.16 (s, 1H, C_3-**H**), 7.55-7.61 (m, 4H, $C_{9,9'}$,$_{10,10'}$-**H**), 7.75 (d, 2H, *J*= 5.9 Hz, $C_{11,11'}$-**H**), 7.88 (t, 1H, *J*= 10 Hz,C_{20}-**H**), 8.21 (d, 2H, $C_{8,8'}$-**H**), 8.50 (d, 1H, *J*= 7 Hz, C_{19}-**H**), 8.55 (d, 1H, *J*= 8.2 Hz, C_{21}-**H**), 8.73 (s, 1H, C_{17}-**H**), 8.84 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C: **Upside**:(108.19: **C_3**,124.42: **$C_{9,9'}$**, 125.46:**$C_{10,10'}$**, 127.50: **$C_{11,11'}$**, 128.61: **C_{21}**, 129.13: **$C_{8,8'}$**, 129.54: **C_{17}**, 130.33: **C_{20}**, 131.35: **C_{13}**, 136.04: **C_{19}**.

Downside: 58.34: **C_{14}**, 126.11: **$C_{7,7'}$**,128.67: **$C_{12,12'}$**, 129.13: **C_{16}**, 130.33: **C_{18}**, 136.08: **C_6**, 160.68: **C_2**, 163.91: **C_{15}**, 167.82: **C_4**.



6j

FT-IR in cm^{-1}: 3127 (C-H str. isoxazole), 1743 (C=O str.),1600 (C=N str.), 1218 (C-O str.).

^{1}H-NMR (600 MHz, DMSO-d_6, 25°C): d = 3.32 (s, 3H, C_{16}-**H_3**), 5.25 (s, 2H, C_{14}-**H_2**), 7.07 (s, 1H, C_3-**H**), 7.53-7.58 (m, 6H, $C_{9,9'}$,$_{10,10'}$-**H**), 8.08 (d, 2H, *J*= 8 Hz, $C_{11,11'}$-**H**), 8.19 (d, 2H,*J*= 8 Hz, $C_{8,8'}$-**H**), 8.82 (s, 1H, C_{13}-**H**).

APT ^{13}C-NMR (150 MHz, DMSO-d_6, 25°C): δ C: **Upside**:33.91: **C_{16}**107.83: **C_3**, 124.47: **$C_{9,9'}$**, 126.13: **$C_{10,10'}$**, 128.47: **$C_{11,11'}$**,129.70: **$C_{8,8'}$**, 130.37: **C_{13}**.

Downside: 57.82: **C_{14}**, 125.53:**$C_{7,7'}$**,131.23: **$C_{12,12'}$**, 143.38: **C_6**, 160.93: **C_2**, 165.78: **C_{15}**, 168.33: **C_4**.

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The synthesized isoxazole derivatives 6a-jevaluated for their antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans.* The diameter of the inhibition zone was measured to determine microbial growth inhibition (using disc agar diffusion method) and the in vitro results of the achieved compounds revealed that they exhibited sensitivity against *E. Coli* and resistance against *S. aureus* except compounds 6e and 6g. Regarding antifungal activity, most of them are active at a specific concentration.

Discussion

The described synthetic approach toward novel esters-functionalized isoxazole was confirmed to be strong and dependable. Concerning yields, all of the synthetic stages produced extremely good results, and the product quality was determined to be acceptable. Because of the quick access to the compounds with large quantities appropriate for characterization and biological assays. In the first step, commercially available 9-anthraldehyde oxime was converted to nitrile oxide form in a very good yield (81%), up on typical N-Chlorosuccinimide treatment in chloroform solution at 0°C for 3 hours, which is stable for along time at low temperature.[23] A potent synthesis route for the creation of a wide range of heterocycles, which serve as crucial scaffolds in many biologically active molecules, is 1,3-dipolar cycloaddition. Here in, 3-(anthracen-9-yl)-5 -(bromomethyl) isoxazole4 was adducted as a single regioisomerina very good yield after chromatographic purification; from conducting 1,3-dipolar cycloaddition on the obtained 9-anthraldehyde nitrile oxide as an active dipolar with propargyl bromide as dipolarophile, the reaction was performed in a dark place to protect the dipolarophile from sunlight, anhydrous dichloromethane was used as a reaction medium and the reaction was monitored by TLC, after 48 hours all starting materials vanished, the synthetic route illustrated in Scheme 2.

The structure of the obtained cycloadduct4 was verified relying upon analytical and spectroscopic data; in the FT-IR spectrum, disappearance of the absorption bands at 2292 &3200 cm^{-1} attributed to N-O stretching of the nitrile oxide and C-H stretching of the propargyl bromide respectively, exhibiting two new distinctive absorption bands at 1585 and 3178cm^{-1} due to C=N and C-H stretching of the

Table 3 Antibacterial and antifungal activity of the synthesized compounds

Compds	Escherichia coli			Staphylococcus aureus			Candida albicans			
	5µg	15µg	30µg	5µg	15µg	30µg	400ppm	600ppm	800ppm	1000ppm
6a	8	12	17	--	--	--	8	12	14	17
6b	22	30	36	--	--	--	6	8	--	--
6c	20	21	27	--	--	--	14	--	--	--
6d	21	24	28	--	--	--	11	--	--	--
6e	14	18	20	16	18	23	--	--	--	--
6f	13	14	26	--	--	--	14	--	--	--
6g	20	21	27	15	18	22	13	--	--	--
6h	20	22	25	--	--	--	13	--	--	--
6i	30	32	37	--	--	--	12	--	--	--
6j	16	27	31	--	--	--	--	--	--	--

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isoxazole ring respectively, it's adequate evidence to prove achievement of the desired isoxazole4. The ^{1}H-NMR spectrum in deuterated DMSO is unambiguously consistent for the assigned structure by showing a singlet signal at d 4.68 ppm related to the protons of the methylene group, additional singlet signal at d 6.59 ppm referred to the single proton attached to the isoxazole ring (C_3-H).All aromatic protons are presented at the predicted region at d 7.47 to 8.59 ppm as shown in Table 2. Further verification for the formation of compound 4 was attained from the ^{13}C-NMR spectrum with the signals at d18.6 and 107.2ppm due to C_{14} andC_3 respectively. The depicted signals in the aromatic region at different chemical shifts were inconvenient with different types of carbons as follows 122.4: $C_{9,9}$-, 125.0: $C_{10,10}$-, 125.3:$C_{7,7}$-, 125.4: $C_{11,11}$-, 126.6: $C_{8,8}$-, 128.5: $C_{13,}$129.1: $C_{12,12}$-, 131.0: C_6,161.2: C_2, 167.8: C_4.

From a synthetic chemistry perspective, the ester group was introduced to the skeleton. The synthesized isoxazole 4 was used as a central core to prepare target compounds 6a-j, Scheme 3. It was designed through a sustainable nucleophilic substitution reaction, an equimolar of sodium bicarbonate with different derivatives of carboxylic acid was heated under reflux in acetonitrile for 3 hours. Afterwards, the synthesized isoxazole 4 was added portion-wise and the reactions were left under reflux overnight, nucleophilic substitution reactions were completed successfully. Quenching was performed by pouring water, extraction with DCM allowed for the obtaining of organic phases that were dried over anhydrous sodium sulphate, Scheme 3. Residues were submitted to chromatographic separation to isolate the products and purification. The products were collected as a powder in very good yields, and high purity. Compounds 6 c, g and h obtained higher yields compared to the other products, this referred to the presence of a nitro group attached to carboxylic acids that enhance their acidity and easily convert into sodium carboxylate. FT-IR spectra for all compounds given some primitive information about the function groups, the newly linked C=O group as the most characteristic band in infrared spectra appeared as strong and intense bands between 1662 to 1743 cm^{-1}, all C-O stretching bands located between 1203-1268cm^{-1}as a strong broadband, in addition, short weak vibration frequency at 3120-3178 cm^{-1} corresponding to C-H stretching for isoxazole rings. Moreover, an inspection and comparison of the ^{1}H-NMR spectra for compounds 6a-j with starting material revealed marked differences between them, signals that referred to the methylene group shifted to the more downfield, for all compounds located between d 5.58 to 5.85 ppm and the aromatic region become more complicated due to introducing newly phenyl ring. The APT ^{13}C-NMR spectra revealed the most important feature for all compounds, by presenting a more de shielded signal downside between d 56.7-58.8ppm due to $C_{14,}$ owing to the influence of electron-withdrawing group (OC=O), and the last more downfield signal between d 163.9-167.9 ppm due to quaternary carbon of the newly linked carbonyl groups.

In this study for example, the structure of compound 6a is chosen as a representative of their series, it was assigned through a diligent examination of all the collected analytical and spectroscopic data. The FT-IR spectrum presented two new elegant strong absorption bands at 1708 and 1265 cm^{-1} due to C=O and C-O stretching of the ester group respectively, the presence of the isoxazole ring is confirmed by the existence of vibration frequencies centred at 3143 and 1143cm^{-1}due to C-H and C=N respectively, Fig.1A and Table 2.In the ^{1}H-NMR spectrum, the two protons of the methylene (CH_2O) and isoxazole ring (C_3-H) occur red as sharp singlets at d 5.74 and 7.09 ppm respectively, while

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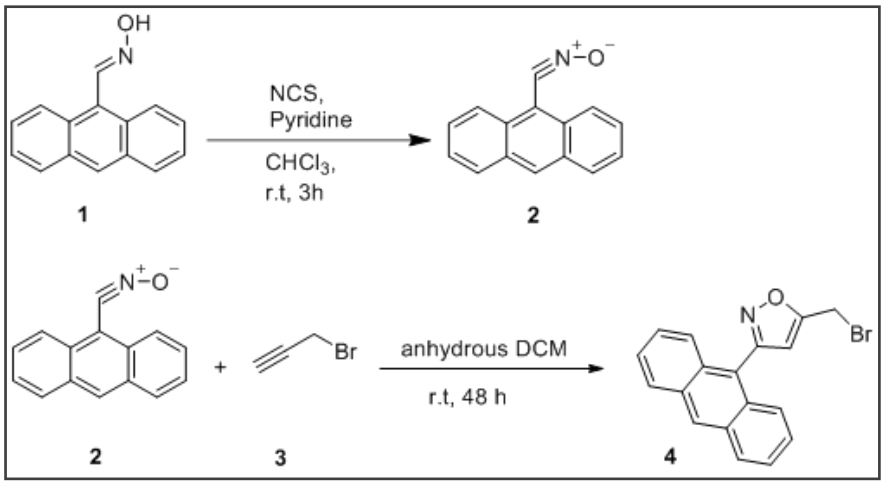
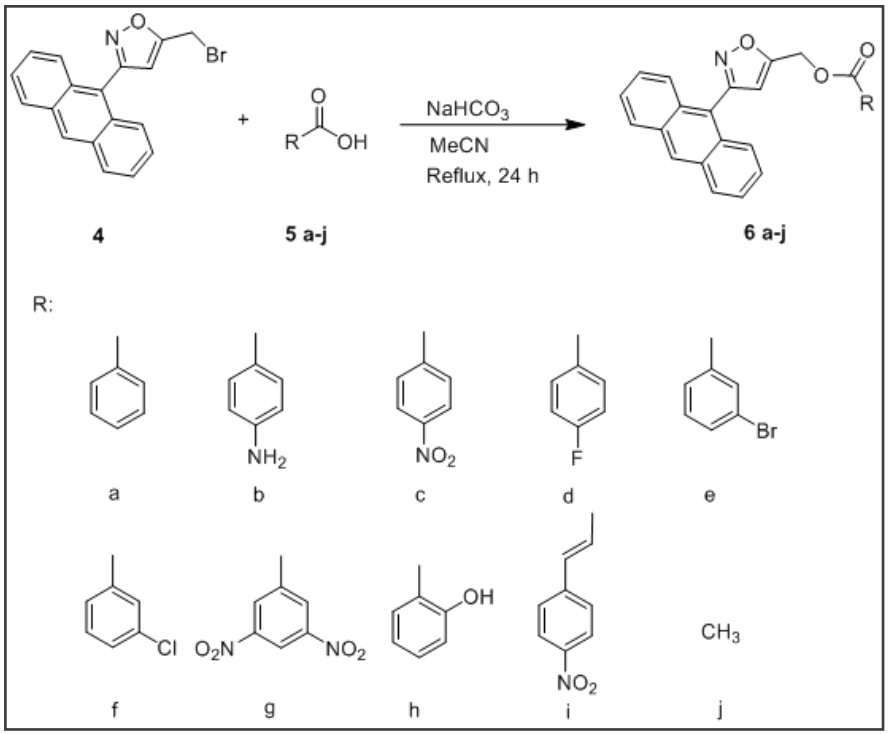
the phenyl and anthryl protons resonated between d7.54-8.81 ppm and integrated to fourteen (5 phenyl, 9 anthracene) Figure 1B and Table 2. The ^{13}C-NMR presented nine pointed-up signals for nine types of carbons bonded to odd protons (107.93: C_3, 125.47: $C_{9,9}$, 126.13: $C_{10,10}$, 127.47: $C_{11,11}$, 129.10: $C_{18,18}$, 129.50: $C_{8,8}$, 130.37: C_{13}, 131.10: $C_{17,17}$,134.25: C_{19}), quaternary and even proton bonded carbons were depicted in the downside (57.70: C_{14}, 125.43:$C_{7,7}$, 129.07: C_{16}, 131.13: $C_{12,12}$, 143.28: C_6, 160.92: C_2, 165.68: C_{15}, 168.24: C_4), Likewise, all spectroscopic analyses attested the formation of the other desired products, Figure 1C and Table 2.



Scheme 2 Reaction scheme for the synthesis of 3-(anthracen-9-yl)-5(bromomethyl) isoxazole 4.



Scheme 3 Ester-functionalized isoxazole6a-jsynthetic pathway.

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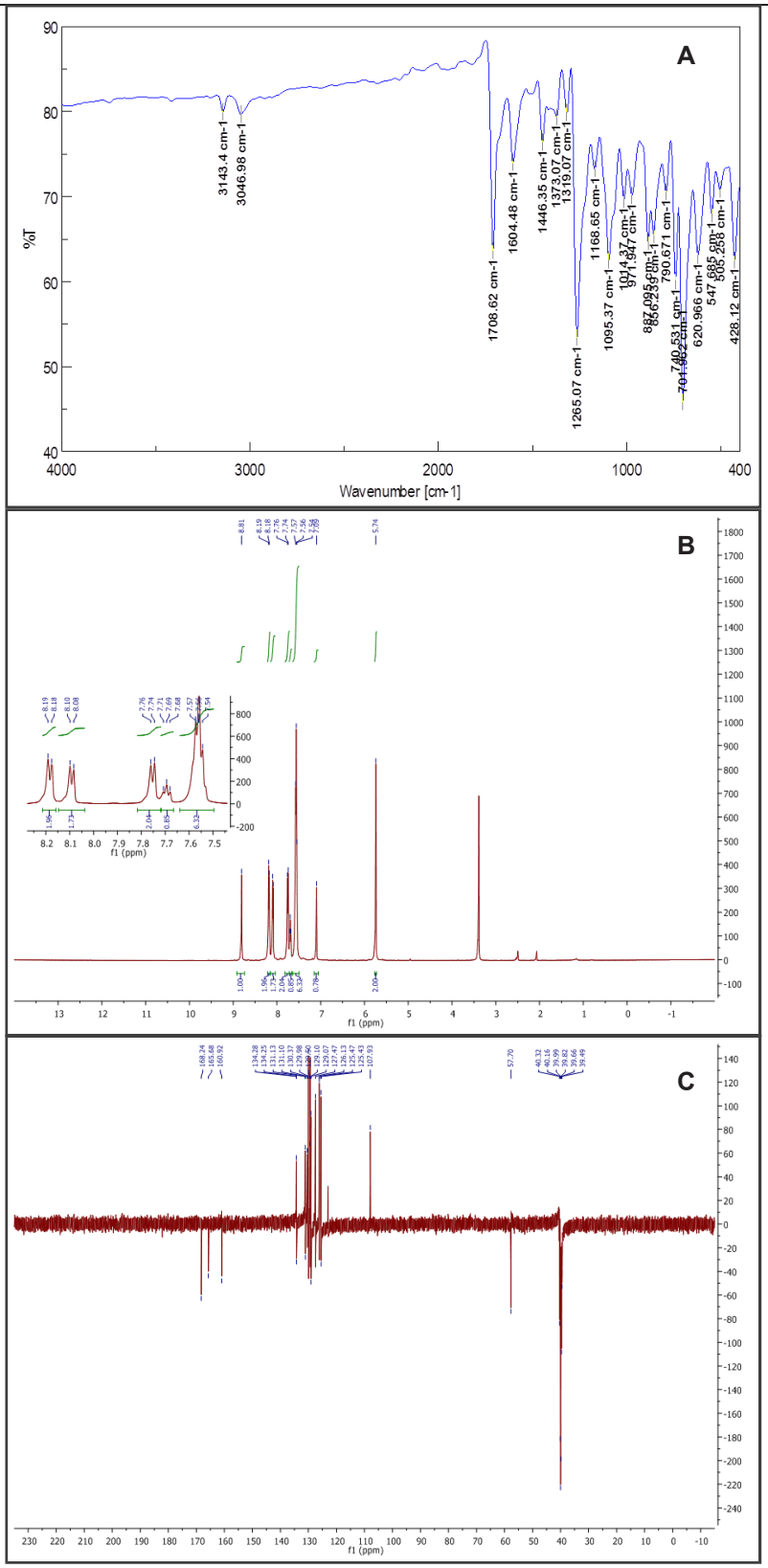

Figure 1 Spectroscopic data for compound 6a, (A) FT-IR, (B) ^{1}H-NMR, and (C) APT ^{13}C-NMR spectra.

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Antibacterial activity

The antibacterial potency was assessed against *Escherichia coli* and *Staphylococcus aureus*, it was checked by measuring the minimum inhibitory zone (using disk agar diffusion method), and the results were shown in Table 3.

The results of *in vitro* antibacterial activity of the synthesized compounds showed almost good antibacterial activity against the gram-negative bacterial strain, with a growth inhibition zone of (17 – 37 mm) against *E. coli*. The most active compound against *E. coli* was compound 6i with a growth inhibition zone of 37 mm. On the other hand, the synthesized compounds showed nearly no antibacterial activity against the gram-positive bacterial strain, except for compounds 6e and 6g, which exhibit an accepted inhibition range for both bacterial strains, *E. coli* and *S. aureus* with inhibition zones of 20, 27, and 23, 22 mm, respectively, Table 3.

By talking about compound 6e, experiments to quantify the effect of bromine on a chemical basis, the presence of bromine in such compounds, revealed to be more reactive than their chlorine analogues.[24]

Regarding compound 6g with nitro groups, the different chemical and physical properties of the nitro group ($-NO_2$) including its electron-withdrawing ability, polarity, size, capability to form hydrogen bonds and redox properties provide a central role in the action of many drugs, especially antimicrobial agents.[25] Despite the antimicrobial properties, especially, the antibacterial and antiparasitic properties of nitroaromatic antibiotics, drugs having nitro groups frequently exhibit mutagenicity and inappropriate toxicity profiles, which have retarded further progress of this drug class. The strong biological activity of these compounds guides the search for non-mutagenic and selectively toxic nitroaromatic antibiotics that target the infectious agent without harming host cells. In addition, reprocessing existing nitroaromatic antibiotics that have been proven safe represents a less risky and cost-efficient strategy in the search for novel and effective drugs to treat mistreated tropical diseases.[26]

By comparing the obtained results with standard antibiotics used in this field we can conclude the effectiveness of the synthesized compound for their antibacterial activity, for example, the Doxycycline antibiotic at 30 µg disk concentration, shows an inhibition zone range from 18 to 24 mm for *E. coli*, which is even less than what had seen with most of our compounds. The same condition could be applied to Kanamycin, which shows an inhibition zone of 17 – 25 mm.

Regarding the antibacterial effectiveness of the two active compounds against *S. aureus*, 6e and 6g, their inhibition zones lie in the range of Kanamycin inhibition zone at 30 µg disk concentration, which is between 19 – 26 mm, when 6e and 6g have inhibition zones of 23 mm and 22 mm, respectively. Vancomycin shows a similar pattern to what just have been mentioned regarding Kanamycin, Vancomycin shows an inhibition zone of 17 – 21 mm at 30µg disk concentration.[27]

Antifungal activity

The antifungal activity of the synthesized isoxazole derivatives was tested against *Candida albicans*, the antifungal activity was checked by measuring the minimum inhibitory zone (using the reliable sabouraud dextrose agar medium plate disc diffusion method), and the results were shown in Table 3.

The results of *in vitro* antifungal activity of the synthesized compounds showed variable results, with a growth inhibition zone ranging between 6 to17 mm against *C. albicans*. The most active compound against the tested fungi was compound 6a with a maximum growth inhibition zone of 17 mm.while two of the synthesized compounds showed no antifungal activity, namely compounds 6e and 6j, Table 3.

Phenolic compounds, especially those isolated from natural sources, possess antifungal properties of interest.

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Particularly, phenolic acids have shown promising *in vitro* and *in vivo* activity against *Candida* species.[28]
Compound 6a, the simplest compound among this series of synthesized compounds, showed the perfect sequence of antifungal activity, in such a way that increment in the concentration shows a consistent increase in the activity. This supports the idea that phenolic compounds that occur naturally have antifungal activity of interest especially because they are simple phenolic compounds such as Stilbenes. As seen with Cryptolepine, in general, the analogues with electron-donating groups are more active than those with electron-withdrawing groups, at least against *C. albicans*.[29]

Conclusion

In conclusion, we have reported a regioselective approach to synthesize 3-(anthracen-9-yl)-5-(bromomethyl) isoxazole 4 through a valuable 1,3- dipolar cycloaddition protocol between 9-anthraldehyde nitrile oxide and propargyl bromide. Different derivatives of ester-functionalized isoxazole were synthesized and characterized from their spectroscopic data, by conducting nucleophilic substitution reaction on the synthesized compound 4. All products were purified by column chromatography. A notable advantage of this protocol is that the reaction condition was mild and the products were obtained in good yields at a suitable time. Unpresented synthesized isoxazole derivatives 6a-j were evaluated for their inhibitory activity by screening against *Escherichia coli* as G(-ve) and *Staphylococcus aureus* as G(+ve). They possessed a significant growth inhibition against *E-coli* and exhibit resistance against *S. aureus* except for compounds 6 e and 6 g. In terms of their antifungal activity, most of them have significant activity at a definite concentration, Table 3.

Funding

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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