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SYNTHESIS, STRUCTURAL ELUCIDATION AND ANTIMICROBIAL ACTIVITY EVALUATION OF NEW DERIVATIVES OF 2-MERCAPTOAMINOACETYLENICBENZOXAZOLE

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ABSTRACT

The objectives are to synthesize of 2-mercaptobenzoxazole derivatives with amino acetylenic side chain as new and possibly with excellent antimicrobial agents, to reach structural selectivity of higher antifungal activity without or low antibacterial interference. A new series of 2-{[4-(t-amino-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole derivatives (**HZ1-HZ8**) were synthesized, and structural elucidation was confirmed through UV-HPLC, FT-IR,^{1}H-NMR,^{13}C-NMR by the aid of Bruker FT-IRand elemental analyses, by the aid of the Varian 300MHZ spectrometer. An antimicrobial activity evaluation was done by agar diffusion methods, broth dilution test, and kinetic ofthe killing against *Staphylococcus aureus* (ATCC 6538p), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Candida. albicans* (ATCC10231) and *Aspergillus. Brasiliensis* (ATCC16404). The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentration (MBC) were determined and compared to two positive control drugs (ciprofloxacin 85.51% and fluconazole 99.9%). The synthesized compounds were consistent with the assigned structures as illustrated by UV-HPLC, FT-IR, DSC, ^{1}H-NMR,^{13}C-NMR and elemental analysis results. Compounds 2-{[4-(pyrrolidin-1-yl)but-2-yn-1-yl]sulfanyl}-

1,3-benzoxazole**HZ2**, 2-{[4-(2-methyllindolin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole **HZ3**, 2{[4-(4-methylpiperazine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole**HZ4**, 2-{[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole **HZ5**, 2-{[4-(2-morphline-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole **HZ6**, 2-{[4-(piperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole **HZ7** and 2-{[4-(azepane-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole**HZ8** demonstrated the highest antifungal activity against *C. albicans*with MIC value of 125 µg/ml. The most active compound against *A. brasiliensis* was 2-{[4-(2-morphline-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole **HZ6** with zone of inhibition of35 mm. In conclusion these results verified the selectivity of these synthesized compounds to antifungal activity compared to their antibacterial activity. These data promote our interest for further structural modification to reach structural selectivity for higher antifungal activity.

Keywords: 2-mercaptobenzoxazole, Aminoacetylenic, antibacterial, antifungal, ciprofloxacin, fluconazole

INTRODUCTION

The discovery of antibiotics is rightly considered one of the most significant health-related events of modern times [1], but unfortunately the development of antibiotic resistance was followed [2, 3]. The antibiotic resistance is now emerging and becoming recent phenomenon [4]. To overcome this problem we need to synthesize new synthetic antimicrobials. The complexity of the natural product-based drugs make use of these compounds challenging [5]. To minimize resistant development some researchers suggested that compounds containing benzoxazole moiety, have remarkable biological activities, for example, anti-viral [6], anti-parasitic [7]and analgesic [8].Benzoxazole is an organic compound, which has benzene fused with an oxazole ring. Oxazole is 1, 3 azole having oxygen atom and a nitrogen atom at the 3-position, a slight change in the substitution pattern of benzoxazole nucleus causes a distinguishable difference in their pharmacological activities [9]. Kaur *et al*., in 2014 synthesized a new series of 5 (or 6)-nitro/amino-2-(substituted phenyl) benzoxazole derivatives (Figure1) and tested them for their antibacterial against *S. aureus, B. subtilis, P. aeruginosa, E. coli,* and antifungal activities against *C. albicans*and drug-resistant isolates, the synthesized compounds were found to exhibit noticeable antibacterial activity [9]. A series of biologically active phenoxy derivatives of 2-substituted benzoxazole organophosphates have been synthesized (Figure 2), and their antibacterial and

antifungal activity had been estimated against pathogenic bacteria as S. aureus (Gram+ve) and *E. coli* (Gram–ve) and against pathogenic fungi (*A.niger* and *A. Fusarium oxysporium),* all the compounds were found to have moderate antibacterial and antifungal activities [10].

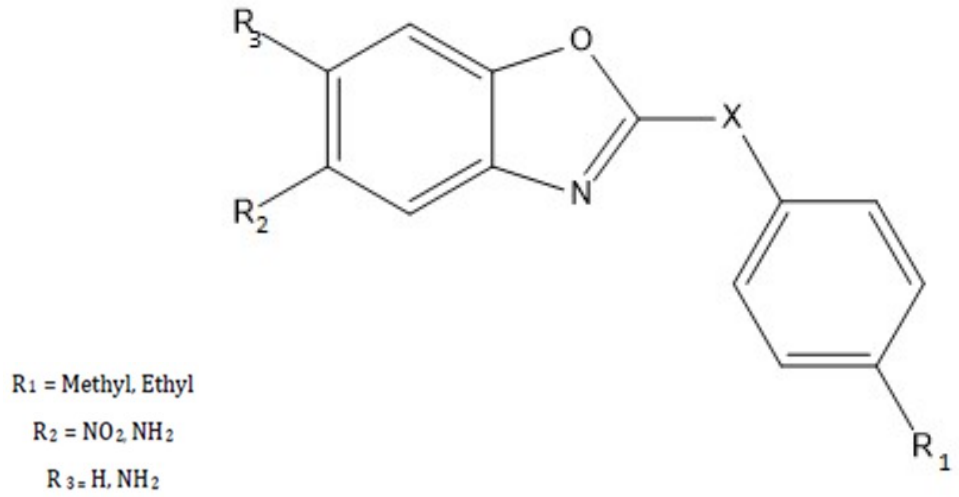


Figure 1: The structure of 5 (or 6)-nitro/amino-2-(substituted phenyl) benzoxazole derivative [9].

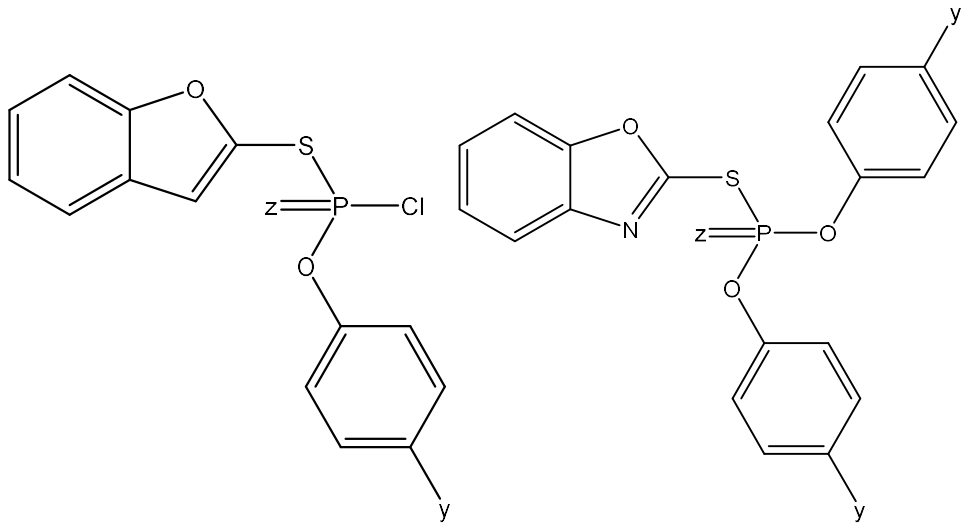


Figure 2: The structure of 2-substitutedbenzoxazolephenoxy derivatives [10].

As a result of reviewing the previous studies, we envision a unique series of 2-mercaptobenzoxazole namely 2-{[4-(amino – 1-yl)but-2-yn-1-yl] sulfanyl}-1,3-benzoxazole derivatives (**HZ1-HZ8**). Based on the concept of fractional base analysis with the following unique substitutes, 2-mercacptobenzoxazole as a directing moiety towards different sites in bacteria and fungi with structural selectivity for pure antifungal activity for the following reasons; amino acetylenic chain intensify this effect and provide the following binding interaction: the cyclic amine group provide ionic or hydrogen bonding with their receptor in fungi or bacteria, electrostatic interaction, the 2-butynyl group provide the appropriate distance between 2-mercaptobenzoxazole and cyclic amine**,** all these unique groups are expected to generate effective antifungal and antibacterial agents with selectivity of these structures to antifungal activity in compares to their antibacterial activity.

MATERIALS AND METHODS:

Experimental:

Chemistry

Chemicals

2-mercaptobenzoxazole 95%, 2-MBO (0.01 mole, 1.51 g), propargyl bromide solution (80wt. %toluene), 2-methylpiperidine 98% , 1-methylpiperazine 99%, cis2,6-dimethyl-piperidine 98%, pyrolidine99%, 2-methyl-lindoline, hexamethylenediamine 99% , morpholine, piperidine99%, all of these chemical materials were from(Sigma Aldrich, USA), potassium carbonate anhydrous (Gialand chemical company GCC, UK), paraformaldehyde polymer (BDH chemical ltd poole, England), cuprous chloride (East Anglia chemicals Hadleigh Ipswich), magnesium sulphate anhydrous (Lonover, UK), potasium bromide (Scharlau, Spain), acetonitrile99.7% (PanRe AC Quimca SA, EU), 1,4-dioxane (Full time, China), chloroform extra pure (TEDIA, USA), diethyle-ether (Lonover, England), dimethyl sulfoxide (DMSO) (BBCchemicals for lab, EU), acetone 99% (Scharlau, Spain), absolute ethanol 99.9% (Super Chem), distilled water.

Instrumentation:

Hot plate with magnetic stirrer (Dragon MS7-H55-S, China), analytical balance with aprecision of 0.01mg (Phoenix instrument, USA), rotary evaporator0-100Kpa/0-700mmHg (Rocker 600, Germany) with digital water bath RF 300DB (Stuart, Germany), Gallenkamp melting point apparatus (U.S.A), HPLC-UV (FinniGan Surveyor, USA), UV-VIS (evolution 160, USA), DSC (Mettler Toledo, Int Co), Varian 300MHZ spectrometer, Varian VX 500

NMR, Bruker FT-IR spectrophotometer 7800 to 400cm^{-1} (Evisa, Poland), elemental analyzer with variation range (±0.4) (Euro Vector, Italy).

Synthesis:

Synthesis of 2-(prop 2-yn-1-ylsulfanyl)-1,3-benzoxazole (HZ0):

A mixture of 2-MBO (0.01 mole, 1.51g), potassium carbonate anhydrous (2g, >0.01mole) and 35ml acetonitrile was heated and stirred under reflux for 30min until the temperature of the mixture became 70^{o}C then the propargyl bromide (2ml, >0.01mole) was added to the mixture drop wise then the reaction was heated to 80^{o}C and stirred for 2 h, the resulted brown mixture was filtrated and concentrated under reduced pressure, the product was extracted by chloroform and water for three times then the chloroform layer was collected, and dried using magnesium sulphate, the chloroform layer concentrated by reduced pressure, the result brownish liquid take 24 h to become solid, the crystals **HZ0**($C_{10}H_7NOS$), 70% yield, melting point: (58.06-60) retention time HPLC-UV(8.517 min), FT- IR (KBr cm-1): Acetylenic C-H stretching (3270.68 cm^{-1}), C-H stretching Ar (3062.406cm^{-1}), CΞC stretching-acetylinic (2121.91, 2244.74 cm^{-1}), C=C stretching aromatic (1506.20 cm^{-1}), C=N stretching thiazole (1596.70 cm^{-1}) , C-N 3^{o}aromatic (1359.76 cm^{-1}), C-H out of plane bending aromatic (750.17 cm^{-1}), S-C stretching (636.89 cm^{-1}),C-O stretching cyclic(941.09cm^{-1}), ^{1}H-NMR (DMSO-d_6): δ; 7.63ppm(doublet, aromatic protons, 1H), 7.47 ppm(doublet, aromatic protons, 1H), 7.32ppm, *J* = 1.4 Hz (doublet, aromatic proton, 2H), 4.09ppm, *J* = 2.6 Hz(doublet, S-CH_2-C, 2H), 2.30 ppm (singlet, acetylenic proton C-H, 1H).

^{13}C-NMR (CDCL3): δ; (162.95ppm), (141.63ppm), (151.91ppm), (109.96ppm), (124.12ppm), (118.6ppm), (20.6ppm), (76.99ppm), (73.51ppm).

Synthesis of 2-{[4-(t-amino-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole derivatives by Mannich reaction(HZ1-HZ8):

A mixture of 2-(prop-2yn-1-yl sulfanyl)-1,3-benzoxazole (**HZ0**), (cyclic amine (2-methylpiperidine, 1-methylpiperazine, cis2,6-dimethyl-piperidine, pyrolidine, 2-methyl-lindoline, hexamethylinimine, morpholine, piperidine), cuprous chloride in excess amount as a catalyst, and paraformaldehyde (0.01mol, 0.46g) in 25ml 1,4 dioxane was stirred for 15min before it was heated and stirred under reflux at 70-75 ^{o}C for 3h, then the resulted brown liquid was filtrated and concentrated under reduced pressure, then it was dissolved in diethyl ether and concentrated under reduced pressure, the

ending products were **HZ1, HZ2, HZ3, HZ4, HZ5, HZ6, HZ7** and **HZ8.**

2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole(HZ1)

The title compound **HZ1** was synthesized using the same procedure mentioned above (Mannich reaction) in 65.27% yield, retention time HPLC-UV =3.73 min, FT-IR (NaCl): C-H stretching aromatic (3052.76 cm^{-1}), C-H stretching with tertiary amine (2707.57 cm^{-1}), C=C stretching aromatic (1604.48 cm^{-1}), C=N stretching (1477.21 cm^{-1}), C-N stretching 3° aromatic(1365.36 cm^{-1}), C-N stretching in cyclic amine (1229.72 cm^{-1}), C-H out of plane bending aromatic(742.46cm^{-1}, 804.17 cm^{-1}), C-O stretching cyclic (9292.52 cm^{-1}), ^{1}H-NMR (DMSO d6): δ; 7.65 ppm (multiplet, aromatic protons, 1H), 7.45 ppm (multiplet, aromatic protons, 1H), 7.35 ppm (triplet, aromatic protons, 2H), 3.61 ppm (singlet, S-CH_2-C, 2H), 3.6 ppm (singlet, C-CH_2-N, 2H), 2.32 ppm (multiplet, cyclic amine proton, 2H), 1.5ppm(triplet, cyclic amine proton, 4H), 1.47ppm(multiplet, cyclic amine proton, 2H), 1.05ppm(singlet, CH_3 on cyclic amine, 6H), ^{13}C-NMR ($CDCL_3$): δ; (163.07ppm), (151.82 ppm), (141.73ppm), (124.33 ppm), (118.53 ppm), (109.87ppm), (78.74 ppm), (76.73ppm), (54.55 ppm), (37.41ppm), (34.95ppm), (24.32ppm), (21.37 ppm), (20.64ppm), elemental analysis: calculated for$C_{18}H_{22}N_2OS$: C 68.75 %, H 7.05 %, N 8.91 %, found: C 68.40 %, H 6.98 %, N 8.72 %.

2-{[4-(Pyrrolidin – 1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole (HZ2)

The title compound **HZ2** was synthesized using the same procedure mentioned above (Mannich reaction) in 76.10% yield, melting point: (39-41) ◦C, FT-IR (KBr cm^{-1}): C-H stretching aromatic (2975.63 cm^{-1}), C-H stretching tertiary amine (2796.21 cm^{-1}), C=C stretching aromatic (1569.06 cm^{-1}), C=N stretching (1496.49 cm^{-1}), C-N stretching 3°aromatic (1374.99cm^{-1}), C-N stretching tertiary cyclic amine (new) (1224.58 cm^{-1}), C-H out of plane bending aromatic (752.10 cm^{-1}), C-O stretching cyclic (912.17 cm^{-1}), ^{1}H-NMR (DMSO d6): δ; 7.61 ppm (doublet, aromatic protons, 1H), 7.45 ppm (doublet, aromatic protons, 1H), 7.35 ppm (multiplet, aromatic protons, 2H), 3.43 ppm (singlet, S-CH_2-N, 2H), 3.4ppm(singlet, C-CH_2-N, 2H), 2.54 ppm (triplet, cyclic amine protons, 4H) 1.71 ppm (multiplet, cyclic amine protons, 4H), ^{13}C-NMR ($CDCL_3$): δ; (163.25ppm), (151.83 ppm), (141.70ppm), (124.41ppm), (118.49ppm), (109.98 ppm), (80.22 ppm), (76.72ppm), (53.97 ppm), (43.09ppm), (23.55ppm), (21.25 ppm), elemental analysis: calculated

for $C_{15}H_{16}N_2OS$: C 66.15%, H 5.92%, N 10.29%, found: C 66.63%, H 5.81%, N 10.62%.

2-{[4-(2-methyllindolin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole (HZ3)

The title compound **HZ3** was synthesized using the same procedure mentioned above (Mannich reaction) in 61.73% yield, FT-IR (NaCL):C-H stretching aromatic (3010.99 cm^{-1}), C-H stretching with tertiary amine (2489.51cm^{-1}), C=C stretching aromatic (1571.70 cm^{-1}), C=N stretching thiazole (1571.70 cm^{-1}), C-N stretching 3^o aromatic(1365.36 cm^{-1}), C-N stretching in cyclic amine (1234.22 cm^{-1}), C-H out of plane bending aromatic(761.74 cm^{-1}), ^{1}H-NMR(DMSO d6):δ;7.65 ppm (doublet, aromatic protons, 1H), 7.45 ppm (multiplet, aromatic protons, 2H), 7.35 ppm (multiplet, aromatic protons, 2H), 7.09ppm(triplet, cyclic amine protons, 1H), 6.92ppm(doublet, cyclic amine protons, 4H), 6.44 ppm (multiplet, cyclic amine protons, 1H), 4.10 ppm (singlet, C-CH_2-N, 2H) , 3.62 ppm (singlet, S-CH_2-C, 2H), 3.10ppm(multiplet, cyclic amine protons, 1H), 2.85 ppm (doublet, cyclic amine protons, 2H), 1.28ppm(singlet, CH_3 on cyclic amine, 3H).Elemental analysis: calculated for $C_{20}H_{18}N_2OS$: C 71.83 %, H 5.43 %, N 8.38 %, found: C 71.51 %, H 5.22 %, N 8.11 %.

2-{[4-(4-methylpiperazine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole (HZ4)

The title compound **HZ4** was synthesized using the same procedure mentioned above (Mannich reaction) in 73.72% yield, FT-IR (NaCL): C-H stretching aromatic (3056.62 cm^{-1}), C-H stretching with tertiary amine (2825.20 cm^{-1}), C=C stretching aromatic (1604.49 cm^{-1}), C=N stretching thiazole (1496.42 cm^{-1}), C-N stretching 3^o aromatic(1355.71 cm^{-1}), C-N stretching in cyclic amine (1290.14 cm^{-1}), C-H out of plane bending aromatic (809.96 cm^{-1}, 746.52 cm^{-1}), C-S stretching (659.59 cm^{-1}), ^{1}H-NMR (DMSO d6):δ;7.59 ppm (doublet of triplet, aromatic protons, 1H, J = 6.3, 1.3 Hz), 7.45 ppm (doublet of triplet, aromatic protons, 2H, J = 8.6, 1.2 Hz), 7.35 ppm (multiplet, aromatic protons, 2H), 4.08 ppm (2H , singlet, S-CH_2-C, 2H),3.28 ppm (singlet, C-CH_2-N, 2H), 3.28 ppm (singlet, cyclic amine protons, 2H), 2.40ppm(multiplet, cyclic amine protons, 4H), 2.25ppm(doublet of doublet, CH_3 on cyclic amine protons, 3H, J = 3.1, 1.3 Hz), elemental analysis: calculated for $C_{15}H_{17}N_3OS$: C 62.69 %, H 5.96 %, N 14.62 %, found: C 62.24 %, H 6.80 %, N 14.31 %.

2–{[4-(2-methylpiperidine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole(HZ5)

The title compound **HZ5** was synthesized using the same procedure mentioned above (Mannich reaction) in 54.37% yield, melting point: 50°C, FT-IR (KBr cm^{-1}):C-H stretching aromatic (2954.41 cm^{-1}), C-H stretching tertiary amine (2954.41 cm^{-1}), C=C stretching aromatic (1190.092 cm^{-1}), C=N stretching thiazole (1496.42 cm^{-1}), C-N stretching 3° aromatic (1361.50 cm^{-1}), C-N stretching tertiary cyclic amine (1224.58 cm^{-1}), C-H out of plane bending aromatic (765.60 cm^{-1}), ^{1}H-NMR (DMSO d6):δ;7.65 ppm (doublet, aromatic protons, 2H), 7.31 ppm (doublet of doublet, aromatic protons, 2H, *J* = 7.9, 3.1, 1.6 Hz), 3.62 ppm (singlet, S-CH_2-C, 2H), 3.28 ppm (singlet, C-CH_2-N, 2H), 2.36 ppm (multiplet, cyclic amine protons, 2H), 2.36ppm(triplet of doublet, cyclic amine protons, 2H, *J* = 11.6, 3.1 Hz), 1.57 ppm (multiplet, cyclic amine protons, 2H), 1.55 ppm (multiplet, cyclic amine protons, 2H), 1.48ppm(multiplet, cyclic amine protons, 2H),1.09ppm(singlet, CH_3 on cyclic amine, 3H), ^{13}C–NMR(CDCL3):δ;(165.16ppm), (151.84 ppm), (141.73ppm), (124.34ppm), (118.53ppm), (109.89 ppm), (78.94 ppm), (76.71ppm), (54.52ppm),(43.43 ppm), (34.47 ppm), (26.07ppm), (24.29ppm), (21.3 ppm), (19.83ppm), elemental analysis: calculated for $C_{17}H_{20}N_2OS$: C 67.97%, H 6.71%, N 9.32%, found: C 67.62%, H 6.41%, N 9.64%.

2-{[4-(2-morpholine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole(HZ6)

The title compound **HZ6** was synthesized using the same procedure mentioned above (Mannich reaction) in 64.81% yield, melting point: (80-82) ◦C, HPLC-UV: retention time =3.592min, FT-IR (KBr cm-1):C-H stretching aromatic (3000.70 C), C-H stretching tertiary amine (2676.71 cm^{-1}), C=C stretching aromatic (1602.56cm^{-1}), C=N stretching thiazole (1602.56 cm^{-1}), C-N stretching 3° aromatic (1355.71 cm^{-1}), C-N stretching tertiary cyclic amine (1220.72 cm^{-1}), C-H out of plane bending aromatic (752.10, 891.91cm^{-1}). ^{1}H-NMR (DMSO d6): δ; 7.62 ppm (triplet, aromatic protons, 2H), 7.32 ppm (doublet of doublet, aromatic protons: *J* = 8.1, 1.2 Hz), 3.68 ppm (triplet, S-CH_2-C *J* = 4.7 Hz), 3.67ppm(multiplet, cyclic amine protons, 4H), 3.29 ppm (singlet, C-CH_2-N), 2.50ppm(doublet, cyclic amine protons, 4H*J* = 4.7 Hz), ^{13}C–NMR(CDCL3):δ; (163.14ppm), (151.85 ppm), (141.66ppm), (124.9 ppm), (118.52 ppm), (110.42ppm), (79.28 ppm), (76.97ppm), (66.65 ppm**),** (52.77 ppm), (47.43ppm), (22.63ppm), elemental analysis: calculated for $C_{15}H_{16}N_2O_2S$: C 62.48%, H

5.59 %, N 9.71 %, found: C 62.22%, H 5.19 %, N 9.43 %.

2-{[4-(piperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole (HZ7)

The title compound **HZ7** was synthesized using the same procedure mentioned above (Mannich reaction) in 71.66% yield, melting point: 60.09◦C, FT-IR (KBr cm-1): C-H stretching aromatic (3000.70 cm^{-1}) C-H stretching tertiary amine (2762.78 cm^{-1}), C=C stretching aromatic (1569.06 cm^{-1}), C=N stretching (1492.63 cm^{-1}), C-N stretching 3° aromatic (1352.71 cm^{-1}), C-N stretching new tertiary cyclic amine bond (1224.58 cm^{-1}), C-H out of plane bending aromatic (755.96 cm^{-1}), ^{1}H-NMR (DMSO d6): δ; 7.61 ppm (multiplet, aromatic proton, 1H), 7.31 ppm (multiplet, aromatic proton, 2H), 4.11 ppm (singlet, S-CH_2 – C, 2H), 3.26 ppm (doublet, C-CH_2-N, 2H J = 2.2 Hz), 2.42 ppm (multiplet, cyclic amines proton, 4H), 1.52 ppm (multiplet, cyclic amines proton, 4H), 1.33ppm (multiplet, cyclic amines proton, 2H),^{13}C-NMR (CDCL3):δ; (163.14ppm), (151.83 ppm), (141.71ppm), (124.41ppm), (118.50ppm), (109.88 ppm),(79.88 ppm), (76.72ppm), (54.39 ppm), (47.78ppm), (25.71ppm),(23.63 ppm), elemental analysis: elemental analysis: calculated for $C_{16}H_{18}N_2OS$: C 67.10 %, H 6.34 %, N 9.78 %, found: C 66.90 %, H 6.01 %, N 9.75 %.

2-{[4-(azepane-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole (HZ8)

The title compound **HZ8** was synthesized using the same procedure mentioned above (Mannich reaction) in 99.10% yield, melting point: 62.67 ◦C, FT-IR (KBr cm^{-1}): C-H stretching aromatic (3000.70 cm^{-1}), C-H stretching tertiary amine (2979.91 cm^{-1}), C=C stretching aromatic (1602.56 cm^{-1}), C=N stretching thiazole (1602.56 cm^{-1}), C-N stretching 3° aromatic (1365.86 cm^{-1}), C-N stretching(new bond) tertiary cyclic amine (1224.58 cm^{-1}), C-H out of plane bending aromatic (759.62 cm^{-1}), ^{1}H-NMR (DMSO d6): δ; 7.61 ppm (multiplet, aromatic proton, 2H), 7.30 ppm (multiplet, aromatic proton, 2H), 4.10 ppm (singlet, S-CH2 – C, 2H), 3.36 ppm (triplet, cyclic amine proton, 4H, J = 2.2 Hz), 3.35 ppm (singlet, C-CH2-N, 2H), 2.16 ppm (doublet, cyclic amine proton, 4H J = 0.9 Hz), 1.61ppm(multiplet, cyclic amine proton, 4H),**^{13}C**-NMR($CDCL_3$):δ; (163.31ppm), (151.89 ppm), (141.78ppm), (124.46 ppm), (123.9 ppm), (118.57 ppm) (109.94ppm), (80.11 ppm), (76.81ppm), (50.47 ppm), (48.37ppm), (28.42ppm), (26.95ppm), (21.39 ppm), elemental analysis: calculated for $C_{17}H_{20}N_2OS$: C 67.97%, H

6.71%, N 9.32 %, found: C 67.65 %, H 6.98%, N 8.77%.

Culture media Muller Hinton broth (MHB) (Mastgrp Ltd, UK), Mueller Hinton agar (MHA) (Mastgrp Ltd, UK)/ (Himedia, India), Sabourauds dextrose broth (SDB) (Himedia, India), Sabourauds dextrose agar (SDA) (Mastgrp Ltd, UK).

Microorganisms

Staphylococcus aureus (S. aureus ATCC 6538), *Bacillus subtilis (B. Subtilis* ATCC 6633), *Pseudomonas aeruginosa (P. aeruginosa* ATCC 9027), *Escherichia coli* (*E. coli* ATCC 8739), *Candida albicans (C. albicans*ATCC 10231), *Aspergillus. Brasiliensis* (A.*brasiliensis* ATCC 16404), all these pure cultures of bacterial and fungal strains were obtained from Dar Al Dawa (Na'ur , Jordan).

Evaluation of antimicrobial activity:

All of the synthesized compounds (2-mercaptoaminoacetylenicbenzoxazole derivatives**HZ1-HZ8**) were tested for their antimicrobial activity in vitro against*S. Aureus* (ATCC 6538p), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *B. subtilis* (ATCC 6633), *C. albicans* (ATCC10231) and *A. brasiliensis* (ATCC16404), by agar diffusion method[11], in this method a petridish containing MHA or SDA was inoculated over its surface with 0.1 ml (1×10^6 CFU/ml) of overnight bacterial culture in MHB broth, or 2 d culture of *C. albicans* (ATCC10231) in the SDB broth or a spore suspension of *A. brasiliensis* (ATCC16404), then wells were cut and filled with 0.2ml of the synthesized compounds in different concentrations (10000 µg/ml,5000 µg/ml,2000 µg/ml,1000 µg/ml,500 µg/ml,250 µg/ml,125 µg/ml), then the plates were left at room temperature for 30 min, then the plates were incubated at 37°C for 24 h for bacteria, and at 25°C for 48 h for fungi. The zone of inhibition was determined by measuring the diameter of the zone formed around each wells and the results were compared with a positive control (5 µg/ml ciprofloxacin dissolved in distilled water for bacteria, 500 µg/ml fluconazole dissolved in DMSO for fungi), and negative control (DMSO alone) was included also. The agar diffusion method was designed to allow for statistical analysis and was performed in triplicate. Experimental data presented in this study represent the mean±SD of that triplicate data set. The second method used to be broth dilution test to determine the minimum inhibitory concentration [12,13].In this method a stock solution of 2-{[4-(t-amino-1-yl)but-2-yn-1-yl] sulfanyl}-1,3-benzoxazole derivatives (**HZ1-HZ8**) were prepared at concentrations

at least 2000 µg/ml, in which 20000µg was weighed and dissolved in 1ml DMSO. This stock solution of the synthesized compounds was diluted with MHB for bacteria or with SDB for *C. albicans* to give concentrations ranging from 2000 µg/ml to 31.25 µg/ml, 0.1ml of overnight bacterial culture or 3d fungal culture (with count=1×10^6)were added to each tube. Positive control tube containing 5ml of sterile MHB/SDB and 0.1ml of bacterial or fungal culture. Negative control tube containing 5ml of the tested compound diluted in sterile MHB/SDB was added. The tubes were incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for fungi. The MIC was determined by comparison the turbidity of each tube with the positive and the negative control tube, the MIC tube was the lowest concentration of the synthesized compound in which no turbidity was observed, to determine the minimum bactericidal/fungicidal concentration (MBC/MFC) the MIC tube and the tubes with concentration higher than the MIC tube were sub-cultured onto MHA/SDA and the plates were incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for fungi. The tube which contains the lowest concentration and gave no growth was the MBC/MFC, the Broth dilution test was designed to allow for statistical analysis and were performed in triplicates, the MIC and the MBC/MFC for the synthesized compounds were compared to the positive control (ciprofloxacin 85.51 % dissolved in distilled water for bacteria and fluconazole 99.90 % dissolved in DMSO for fungi), in the same concentrations and the procedure used for the synthesized compounds (**HZ1-HZ8**). The third method used was the kinetic of killing test, in this method the time exposure viability for the 2-{[4-(hexamethylamine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole and 2-{[4-pyrolidine-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole derivatives (**HZ2 and HZ8**) was determined, to evaluate the killing rate of **HZ2** and **HZ8** within a given exposure time, the MBC/MFC concentrations were prepared for **HZ2** and **HZ8** compounds, each compound was tested against certain microorganism (**HZ2**was tested against *B. subtilis* and *P. aeruginosa*, and **HZ8**was tested against *S. aureus, E.coli*, and *C. albicans)*, broth cultures were prepared using MHB for bacteria and incubated at 37°C for 24 h, or using SDB for *C.albicans* (ATCC10231) and incubated at 37°C for 2 d, the control was prepared by adding 0.1 ml of bacterial overnight culture or 48 h fungal culture to 10 ml sterile distilled water, then serial dilution was done using sterile distilled water and the viable count was determined

from the appropriate dilutions (control 1). Control 2 was prepared using the MBC/MFC concentration of each compound dissolved in 1ml DMSO, then serial dilutions were done using sterile distilled water (1/10, 1/100, 1/1000, 1/10000, 1/100000), 0.1 ml of bacterial overnight culture or 48 h fungal culture was added to each concentration tube, then serial dilutions were done for each concentration and viable count was determined from the appropriate dilution. The first dilution of the synthesized compound which showed no effect on the microorganism was chosen to be used in the next test, dilution 1/10 was enough to neutralize the synthesized compounds activity.0.1 ml of each broth culture with inoculum size as follow (*S. aureus*=9×10^5, *E. coli*=1.5×10^6, *P. aeruginosa*=9.6×10^4, *B. subtilis*=3.8×10^4 and *C. albicans*=4.4×10^3 CFU/ml) were challenged with sterile distilled water contain the MBC/MFC concentrations for **HZ2** or **HZ8** compounds. Then 1 ml was taken from each sample at different time intervals (0.15, 0.5, 0.45, 1, 2, 3, 4, 5 24 h) and added to 9ml of sterile distilled water. Then serial dilutions were done for each time interval and viable count was determined from the appropriate dilution. The kinetic of killing for compounds **HZ2** and **HZ8** was compared to the kinetic of killing of the positive control (ciprofloxacin 85.51% dissolved in distilled water at MBC concentration for bacteria and fluconazole 99.9% dissolved in DMSO at MFC concentration for fungi).

Statistical analysis Statistical analysis was carried using statistical packages for social science software (SPSS) for student's t-test. Values are expressed as mean ±SD.

RESULTS

Antimicrobial activity

According to the zone of inhibition results, all the synthesized derivatives (**HZ1-HZ8**) had antifungal activity against *A. brasilince* with a zone of inhibition diameter ranging from 14-17 mm, and against *C. albicans* with a zone of inhibition diameter ranging from 11-17 mm, for the concentrations used, in comparison to the positive control (fluconazole 500 µg/ml) which gave zone of inhibition diameter of 15 mm, the same synthesized compounds (**HZ1-HZ8**) gave antibacterial activity against *B. subtilis* with a zone of inhibition diameter ranging from 5-12mm for all concentrations used, in comparison to the positive control (ciprofloxacin5µg/ml) which gave 24 mm zone of inhibition diameter, and the antibacterial activity against *S. aureus* with a zone of inhibition diameter ranging from 22-10mm for all concentrations, in comparison

to the positive control (ciprofloxacin5µg/ml) which gave 22 mm zone of inhibition diameter. The synthesized compounds didn't show any activity against *P.aeruginosa* and *E. coli.* According to the minimum inhibitory concentration (MIC) results compounds (**HZ2-HZ8**) gave the highest antifungal activity against *C. albicans* with the lowest MIC value of 125µg/ml, compounds (**HZ1-HZ3**) gave the highest antibacterial activity against *B. subtilis* with the MIC value of 125µg/ml, most of the synthesized compounds showed good antimicrobial activity against *S. aureus with a* MIC value of 250 µg/ml, compounds **HZ2, HZ6, HZ8**showed antimicrobial activity against *E. coli* with a MIC value of 250 µg/ml, compounds **HZ2, HZ8**showed antimicrobial activity against *P. aeruginosa* with MIC value of 250 µg/ml. For the MBC and MFC values all compounds showed the same MFC values against *C. albicans* with value of 500µg/ml, and MBC value of 2000µg/ml against *P. aeruginosa, E. coli, S. aureus and B. subtilis* except **HZ2** which gave MBC value of 1000µg/ml against *P. aeruginosa*. All the MBC values against bacteria were higher than the MBC given by the positive control ciprofloxacin. According to the kinetic of killing test results of the **HZ2** and **HZ8** against bacteria and fungiare shown in table3 and figure3, **HZ2** against *B. subtilis*, showed that the count of bacteria decreased slowly within the first 3 h and the viability of the microorganism was abolished after 4 h, the LT 90 % value (time at which 90% of the original population was killed by the antimicrobial agent) for this experiment was in the period between 3 and 4 h. The kinetic of killing of the positive control(Ciprofloxacin) against *B. subtilis* showed that the count of bacteria decreased slowly within the first 2 h, The viability of the microorganism was abolished after 3 h, the LT 90 % value for this experiment was in the period between 2 and 3 h. The kinetic of killing of **HZ2** against *P. aeruginosa*, showed that the count of bacteria decreased rapidly within the first 0.5 h, and then was decreased slowly within the next 4 h, the viability of the microorganism was abolished after 5 h, the LT 90 % value for this experiment was in the period between 4 and 5 h. The kinetic of killing curve of the positive control ciprofloxacin against *P. aeruginosa* showed that the count of bacteria decreased rapidly within the first 0.5 h, and then was decreased slowly within the next 4 h, the viability of the microorganism was abolished after 5 h, the LT 90 % value for this experiment was in the period between 4 and 5 h. The kinetic of killing of **HZ8** against

S. aureus showed that the count of bacteria decreased slowly within the first 2 h, then it decreased between 2 to 3 h, the viability of the microorganism was abolished after 5 h and the LT 90 % value for this experiment was in the period between 4 and 5 h. The kinetic of killing curve of the positive control ciprofloxacin against *S. aureus* showed that the count of bacteria decreased slowly within the first 2 h, then it decreased rapidly between 2 to 3 h, the viability of the microorganism was abolished after 5 h, the LT 90 % value for this experiment was in the period between 4 and 5 h. The kinetic of killing of **HZ8** against *E. coli* showed that the count of bacteria decreased within the first 0.5 h, then the count decreased slowly within the next 2 h, the viability of the microorganism was abolished after 3 h, the LT 90 % value for this experiment was in the period between 2 and 3 h. The kinetics of the killing of the positive control ciprofloxacin against *E. coli* showed that the count of bacteria decreased within the first 0.5 h, then the count decreased slowly within the next 4 h, the viability of the microorganism was abolished after 5 h, the LT 90 % value for this experiment was in the period between 4 and 5 h. The kinetic of killing of **HZ8** against *C. albicans* showed that the count decreased slowly within the first 45min, then it decreased between 45 min and 1h, the viability of the microorganism was abolished after 3 h, the LT 90 % value for this experiment was in the period between 2 and 3 h. The kinetic of the killing of the positive control, fluconazole against *C. Albicans* showed that the count of bacteria decreased slowly within the first 45min, then it decreased rapidly between 45 min and 1h, the viability of the microorganism was abolished after 3 h, the LT 90 % value for this experiment was in the period between 2 and 3 h.

Table1: The zone of inhibition diameter (in mm) of the synthesized compounds (HZ1-8) at 500µg/ml concentration

Microorganism	*B. Subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A.brasiliensis*
Concentration / Compound	500	500	500	500	500	500
HZ1	12±2	-	-	4±0.5	17±2	17±1
HZ2	-	-	-	-	14±3	15±2.5
HZ3	-	10±1	-	-	11±2	16±2
HZ4	10±2	10±2	-	-	16±6	16±4
HZ5	11±1	-	-	-	12±2.4	15±2
HZ6	7±1	-	-	-	16±4	15±1
HZ7	5±1	-	7±1	-	14±3	16±0
HZ8	10±3	10±2	-	8±1.3	12±2	14±0
Ciprofloxacin(5µg/ml)	24±2	22±1.5	16±1.5	20±2		
Fluconazole (500µg/ml)					15±1	15±2
Negative control	-	-	-	-	-	-

Values are the mean±SD, (n=3), (-): no zone of inhibition, HZ1: 4-{|(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole, HZ2: 2-{|4-(Pyrrolidin – 1-yl)but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole, HZ3: 4-{|(2-methyllindolin-1-yl) but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole, HZ4: 4-{|(4-methylpiperazine-1-yl) but-2-yn-1-yl| sulfanyl}-1,3-benzoxazole, HZ5: 4-{|(2-methylpiperidine-1-yl) but-2-yn-1-yl| sulfanyl}-1,3-benzoxazole, HZ6: 4-{|(2-morphline-1-yl)but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole, HZ7: 2-{|4-(piperidin-1-yl)but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole, HZ8: 2-{|4-(azepane-1-yl)but-2-yn-1-yl|sulfanyl}-1,3-benzoxazole

Table 2: The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) of the synthesized compounds (HZ1-HZ8) in µg/ml against *B. Subtilis, S. aureus, E. coli, P. aeruginosa*and *C. albicans*

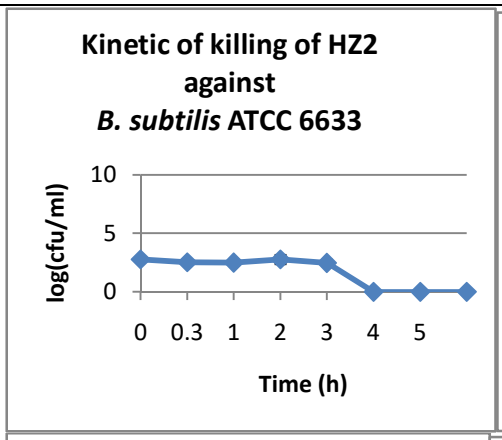
Microrganism	*B. Subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
Concentration / Compound	MIC/MBC(µg/ml)	MIC/MBC (µg/ml)	MIC/MBC (µg/ml)	MIC/MBC (µg/ml)	MIC/MFC (µg/ml)
HZ1	125/2000	250/2000	1000/2000	500/2000	250/500
HZ2	125/2000	250/2000	250/2000	250/1000	125/500
HZ3	125/2000	250/2000	1000/2000	500/2000	125/500
HZ4	250/2000	250/2000	500/2000	500/2000	125/500
HZ5	250/2000	250/2000	500/2000	500/2000	125/500
HZ6	250/2000	500/2000	250/2000	500/2000	125/500
HZ7	250/2000	250/2000	500/2000	500/2000	125/500
HZ8	250/2000	250/2000	250/2000	250/2000	125/500
Ciprofloxacin (5µg/ml)	62.5/250	250/125	62.5/125	125/250	
Fluconazole(500µg/ml)					125/500
Negative control	-	-	-	-	-

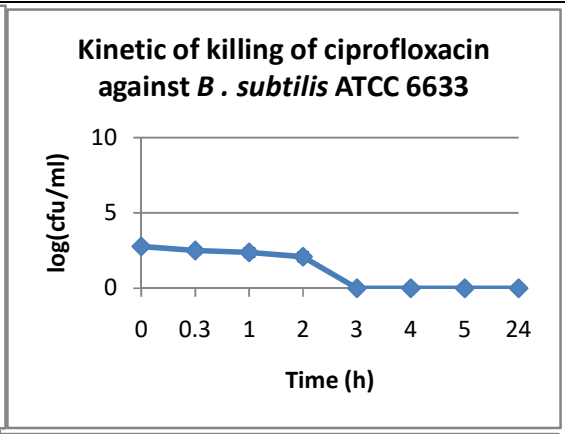
HZ1: 4-{[(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ2: 2-{[4-(Pyrrolidin – 1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ3: 4-{[(2-methyllindolin-1-yl) but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ4: 4-{[(4-methylpiperazine-1-yl) but-2-yn-1-yl] sulfanyl}-1,3-benzoxazole, HZ5: 4-{[(2-methylpiperidine-1-yl) but-2-yn-1-yl] sulfanyl}-1,3-benzoxazole, HZ6: 4-{[(2-morphline-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ7: 2-{[4-(piperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ8: 2-{[4-(azepane-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration.The results were the mean of that triplicate data set (n=3).

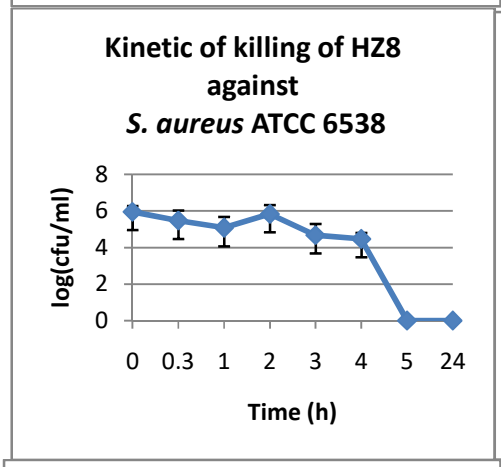
Table 3: Kinetic of killing results for HZ2&HZ8 against *B. Subtilis, S. aureus, E. coli, P. aeruginosa* and *C. albicans,* showing CFU/ml.

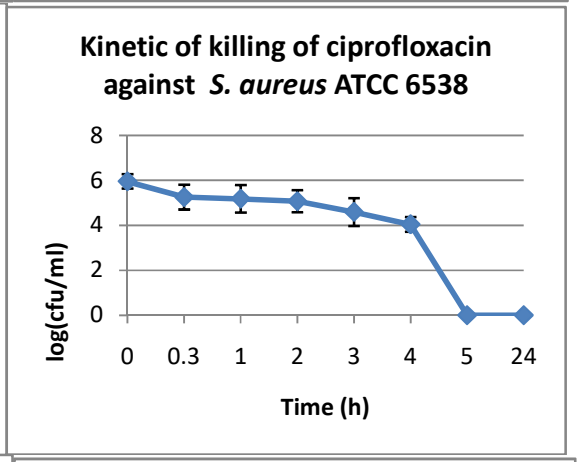
Microrganism	*B. Subtilis*		*S. aureus*		*E. coli*		*P. aeruginosa*		*C. albicans*	
Compound / Time (h)	HZ2	Cipro.	HZ8	Cipro.	HZ8	Cipro.	HZ2	Cipro.	HZ8	Fluc.
0	6.1×10^{2}	6.1×10^{2}	9×10^{5}	9×10^{5}	1.5×10^{6}	1.5×10^{6}	3×10^{5}	3×10^{5}	4.4×10^{3}	4.4×10^{3}
0.15	ND	ND	ND	ND	ND	ND	ND	ND	3×10^{3}	2×10^{3}
0.3	3.5×10^{2}	3.2×10^{2}	3×10^{5}	1.78×10^{5}	1.48×10^{5}	6.1×10^{5}	2.6×10^{4}	5.2×10^{3}	2.1×10^{3}	1.44×10^{3}
0.45	ND	ND	ND	ND	ND	ND	ND	ND	1.17×10^{3}	1.02×10^{3}
1	3×10^{2}	2.5×10^{2}	1.2×10^{5}	1.53×10^{5}	1.48×10^{5}	5.3×10^{5}	1.46×10^{4}	3.3×10^{3}	6×10^{1}	3.8×10^{2}
2	*6×10^{2}*	*1.25×10^{2}*	6.8×10^{5}	1.2×10^{5}	1.08×10^{5}	3.3×10^{5}	1.2×10^{4}	2×10^{3}	4×10^{1}	2.3×10^{2}
3	3×10^{2}	0	4.7×10^{4}	3.9×10^{4}	0	2.2×10^{4}	1.05×10^{4}	1.2×10^{3}	0	0
4	0	0	3×10^{4}	1.1×10^{4}	0	8×10^{3}	8×10^{3}	6.3×10^{2}	0	0
5	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0

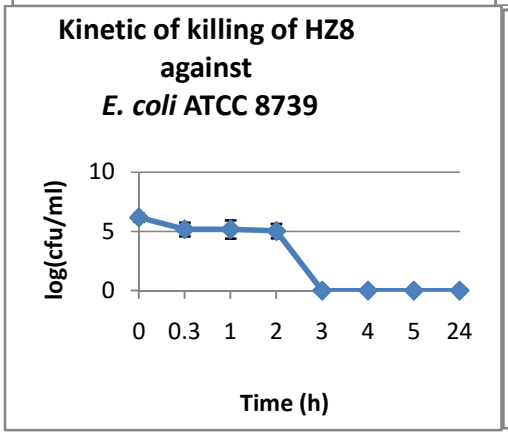
HZ2: 2-{[4-(Pyrrolidin – 1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ8: 2-{[4-(azepane-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, cfu/ml: colonies forming unit per 1ml, Cipro: ciprofloxacin, Fluc: fluconazole 99.9%,(ND): Not detected, 0: no growth.

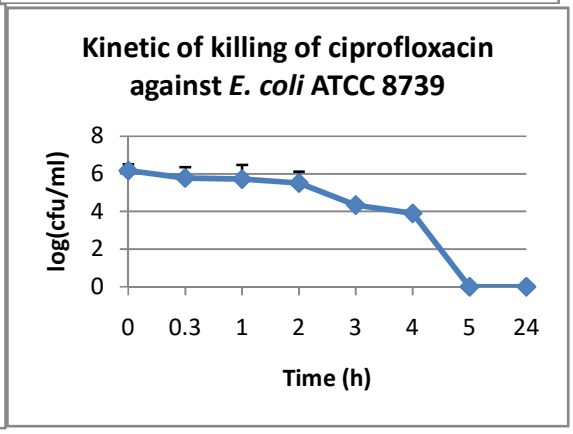

Kinetic of killing of HZ2 against B. subtilis ATCC 6633
log(cfu/ml)
10
5
0
0 0.3 1 2 3 4 5
Time (h)


Kinetic of killing of ciprofloxacin against B . subtilis ATCC 6633
log(cfu/ml)
10
5
0
0 0.3 1 2 3 4 5 24
Time (h)


Kinetic of killing of HZ8 against S. aureus ATCC 6538
log(cfu/ml)
8
6
4
2
0
0 0.3 1 2 3 4 5 24
Time (h)


Kinetic of killing of ciprofloxacin against S. aureus ATCC 6538
log(cfu/ml)
8
6
4
2
0
0 0.3 1 2 3 4 5 24
Time (h)


Kinetic of killing of HZ8 against E. coli ATCC 8739
log(cfu/ml)
10
5
0
0 0.3 1 2 3 4 5 24
Time (h)


Kinetic of killing of ciprofloxacin against E. coli ATCC 8739
log(cfu/ml)
8
6
4
2
0
0 0.3 1 2 3 4 5 24
Time (h)

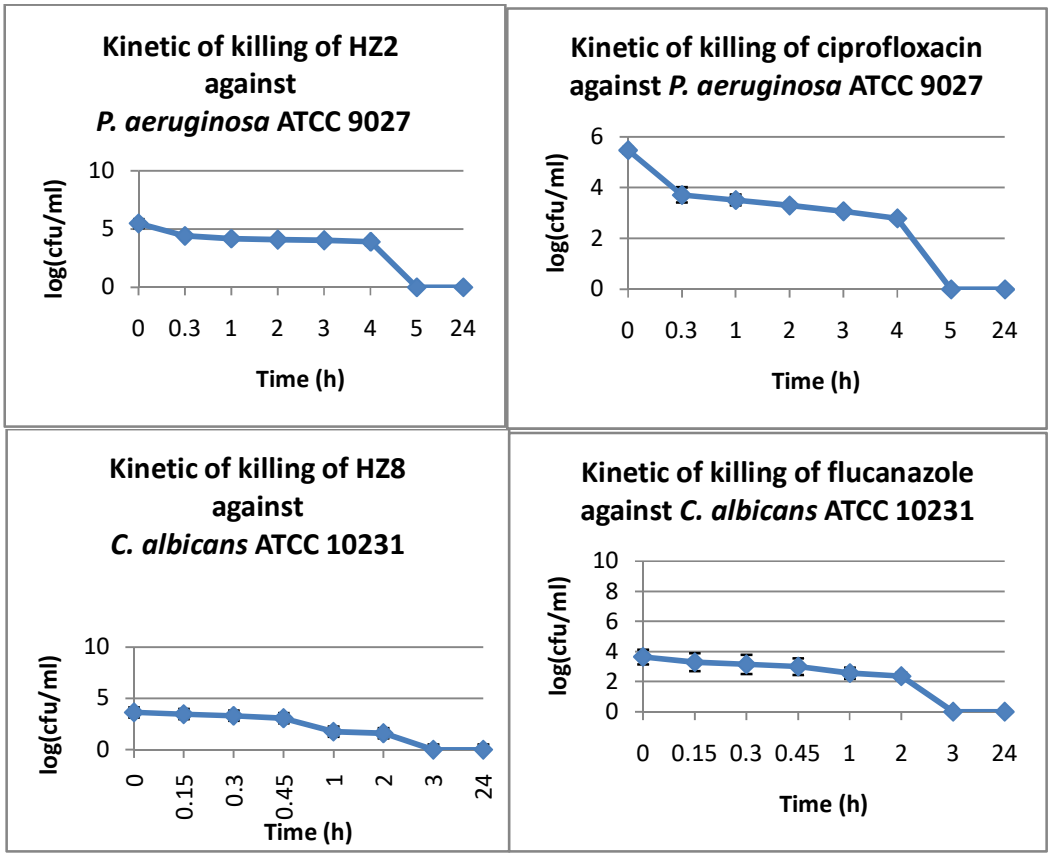


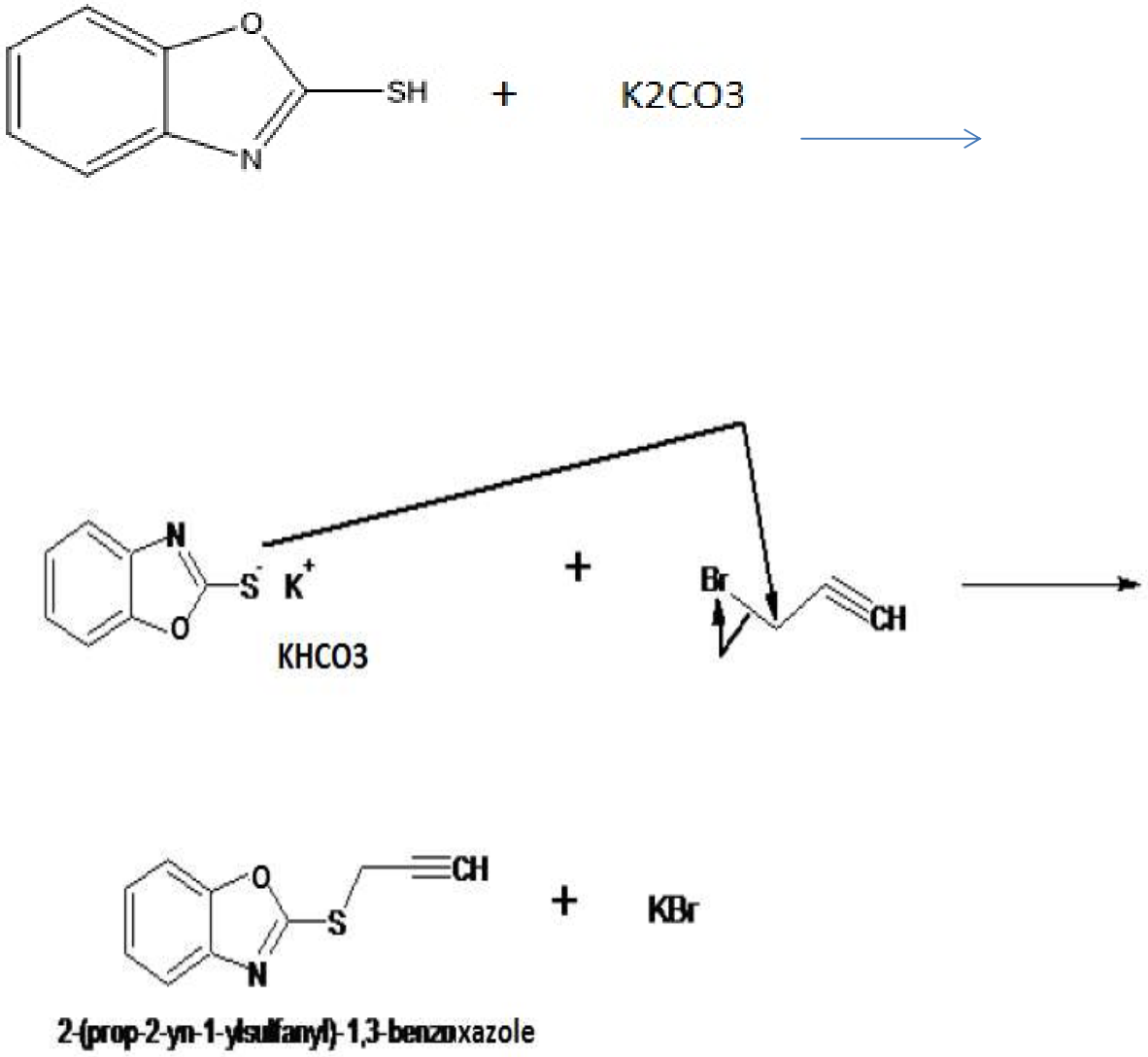
Figure 3: Time exposure viability curve for addition of HZ2: 2-{[4-(Pyrrolidin – 1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, HZ8: 2-{[4-(azepane-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole, ciprofloxacin and fluconazole at MBC values to culture of *B. subtilis, S. aureus, E. coli, P. aeruginosa*and *C. albicans*

DISSCUSION

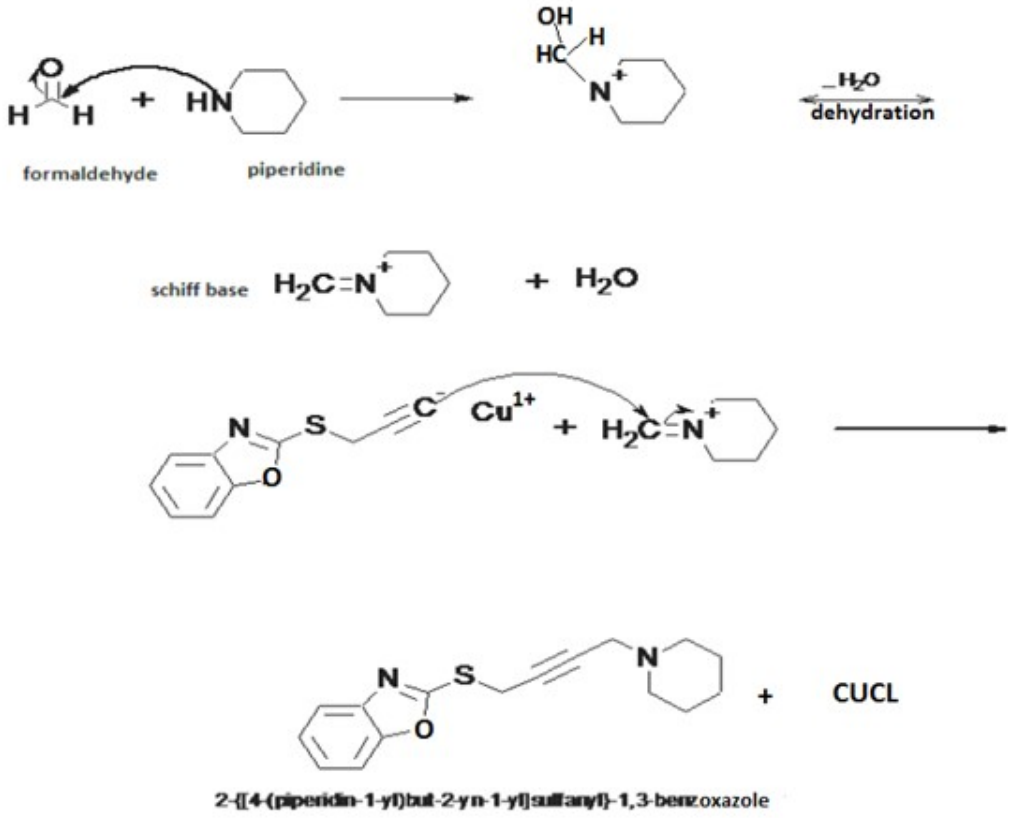
Chemistry

The designed compounds were synthesized as shown in (scheme 1) which involved the alkylation of 2-mercaptobenzoxazole with 3-bromoprop-1-yne (propargyl bromide) in the presence of acetonitrile as a solvent with K_2CO_3 as a base. The reaction ninvolve direct displacement of the anionic sulfur in the mercaptobenzoxazole ring tobromide of the β-carbon of the propargyl bromide to generate 2-(prop-2-yn-1-ylsulfunyl)-1, 3-benzoxazole **HZ0**. The Mannich reaction of 2-(prop-2-yn-1-ylsulfanyl)-1, 3- benzoxazole **HZ0**, paraformaldehyde, cyclic amines, and cuprous chloride as catalyst in peroxide-free 1, 4-dioxane was heated up to 70◦C to generate compounds (**HZ1-HZ8**). The yield obtained was ranged from 54.37% to 99% as solid and semisolid products. The suggested mechanism for Mannich reaction is shown in (scheme 2), the mechanism showed that the reactive immoniumion intermediates should be formed from the condensation of the formaldehyde with the appropriate cyclic amines (Schiff base formation). The attack of the carbanion in 2-(prop-2-yn-1-ylsulfanyl)-1,3-benzoxazole on the Schiff base generate

the appropriate Mannich product(**HZ1-HZ8**). The synthesized compounds were consistent with UV-HPLC, FT-IR, DSC, ^{1}H-NMR, ^{13}C-NMR, and the elemental analysis results.



Scheme 1: Alkylation reaction of 2-MBO



Scheme 2: Mannich reaction of HZ0

The microbiological evaluation

To determine the in-vitro the antimicrobial activity of newly synthesized compounds, broth dilution method is used to determine the MIC and MBC [12], agar diffusion method and kinetic of the killing test were used. The variability of the results using the agar diffusion method and the MIC method may be due to the lipophilicity of the of 2-{[4-(t-amino-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzoxazole derivatives (**HZ1-HZ8**), making their diffusion in agar medium slow and giving small zone of inhibition diameter, the synthesized compounds showed good antifungal activity against *C. albicans,* according to the results of the three methods used, and very good activity against *A. brasiliensis* according to the agar diffusion results. The activity may be attributed to the effects of the lipophilicity, electronegativity, ionic nature of cyclic amine and the appropriate distance of the (benzoxazole) and cyclic amine in these compounds. The most active compounds against *C. albicans* were **HZ2-HZ8**, and the most active compound act against *A. brasiliensis* was **HZ5**. The synthesized compounds were active against Gram positive bacteria more than Gram negative bacteria, this may result from the peptidoglycan layers of the Gram positive

cell wall were more receptive to antimicrobial agents due to the absence of the outer membrane, while Gram negative bacteria possess cell wall, which is not readily penetrated by hydrophobic compounds. The synthesized compounds have higher antimicrobial activity against *B. subtilis* than against *S. aureus*. This may be related to the high resistance and the virulence nature of *S. aureus.* The most active compounds against *B. subtilis* were **HZ1, HZ2, HZ3**, with MIC value of 125 µg/ml, this may assume to the lipophilicity and the conformational effect that facilitate the passage through the cell wall of Gram positive bacteria, all the synthesized compounds gave activity against *S. aureus*with a MIC value of 250 µg/ml except **HZ6** which gave a MIC value of 500 µg/ml, which may be due to the presence of morpholine ring which differentiate this compound from other cyclic amines. The activity of the synthesized compounds against *P. aeruginosa* are much lower than the activity against *E. coli,* which may be due to the resistance nature of *P. aeruginosa* by releasing metalo beta lactamase enzyme[13], the most active compounds against *P. aeruginosa* were **HZ2** and **HZ8** with MIC value of 250 µg/ml, the activity may be due to the lipophilicity and the steric effect of the compounds. The most active compounds against *E.coli* were **HZ2, HZ6** and **HZ8**, with a MIC of 250 µg/ml, *E.coli* strains have a lacking in the pore forming proteins, especially OmpF[3], so the only way to enter bacteria cell is the permeation lipid bilayer by high lipophilic compounds like **HZ8** due to the presence of azepane. Most of the synthesized compounds (**HZ1-HZ8**) showed the high MBC value of 2000µg/ml against all tested bacteria (*B. subtilis, S. aureus, P. aeruginosa* and *E. coli)* in comparison to the positive control (ciprofloxacin), but they showed good MFC results against *C. albicans (*500 µg/ml) which was the same as the positive control (fluconazole).

CONCLUSION

In conclusion, we have described the synthesis of a series of amino acetylenic 2-mercaptobenzoxazole derivatives. A unique aminoacetylenic side chain provides additional forces of interaction with the microorganism. The data generated from this investigation clearly indicate the selectivity of these structures to antifungal activity compared to their antibacterial activity. These results promote our interests to investigate structural variation and selectivity in finding a pure antifungal activity, also for greater potency as antifungal agents relative to antibacterial agents.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ACKNOWLEDGMENT

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