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Synthesis of isoxazolidine and isoxazoline rings via 1,3-dipolar cycloaddition reaction tethered to 2-benzothiazolethiol and study their antibacterial activity

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Abstract: New 2-benzothiazolethiol derivatives containing isoxazolidine and isoxazoline rings were made throughout 1,3-dipolar cycloaddition reactions. 4-methyl-*o*-phenylenediamine was treated with carbon disulfide to form benzo[*d*]thiazole-2-thiol 1. The *S*-alkylation reaction of thiol group using allyl bromide and propargyl bromide gave compounds 2 and 3 in 83% and 90% yields respectively. Then, freshly prepared nitrones which are obtained *in stiu* by the reaction of *N*-methylhydroxylamine with deferent substituted aromatic aldehydes; 4-hydroxybenzaldehyde, 4-hydroxy-1-naphthaldehyde, 5-Methyl-2-thiophenecarboxaldehyde, 3-nitrobenzadehyde and 3-bromorobenzaldehyde, then, were reacted with compounds 2 and 3 which gave compounds 4a-e and 5a-e containing isoxazolidine and isoxazoline rings respectively. All the obtained compounds were characterized using infrared (IR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectrophotometer and all were very matched the data of the structure. Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria were used to evaluate the anti-bacterial activities for the obtained compounds, and the results displayed significant activity towards the selected bacteria by comparison to the standard drug amoxicillin.

Keywords: *Cycloaddition, Isoxazolidine, Isoxazoline, Nitrone, Ntibacterial activity.*

1. Introduction

Nitrones is an important reactive intermediate which is useful in organic synthetic reaction [1, 2]. Multifaceted applications of nitrones including 1,3-dipolar cycloaddition to construct the cyclic ring and complex natural products structures [3-5]. Heterocyclic chemistry has attracted interest of many researchers to syntheses and develop new compounds related to the heterocyclic family, which have used extensively in different sectors; medicine, agricultural, pharmaceutical, as well as material sciences [6, 7]. Azoles are crucial family of heterocyclic compounds and it is core structure of enzymes and many receptors that found in various organisms which interact through noncovalent bond and exhibit many biological activities [8] like anti-biotic [9], antimycotic agents [10], anti-viral [11], anti-inflammatory [12], anti-cancer [13], and anti-oxidant agents[14].

2-Aminobenzenethiol compound containing nitrogen and sulfur atoms is an important fused rings heterocycle. Another types of heterocyclic ring are isoxazolidine and isoxazoline, this structural nature an important intermediate in the synthesis [15, 16] and its derivatives exhibit various bioactivities for their special properties, resulted in wide potential utilization in medicine field [13, 15, 17]. Since there are many strategies that could be used to form isoxazolidine and isoxazoline rings using classical method [18, 19]. The cycloaddition chemistry is a crucial in the synthesis of chemical compounds which can provide quick and control access to a number of natural products [19-21].

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2. Material and Methods

2.1. Experimental Section

All reagents were brought from the market and were put to use with no more effort to purify; the melting points were detected using stuart smp3 electronic devise. Shimadzu FT-IR 8400S, spectrophotometer was used to obtain the IR spectra. The ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra were obtained using D6-DMSO on Bruker 400 MHz spectrometer and the TMS as reference. The reactions were followed using TLC; the spots were detected using $KMnO_4$ dipping and UV cabinet.

2.2 Synthesis of 2-Benzothiazolethiol **1**

Carbon disulfide (1.73 mL, 0.0289 mol) was added to a mixture of 2-aminobenzenethiol (2.5 mL, 0.024 mol) and (1.9 g, 0.048 mol) NaOH in distilled H_2O (20 mL) and heated at 80 °C under stirr. After 4 h, neutralization with 3 N HCl solution, followed by extraction with EtOAc, then the organic layers were dried using $MgSO_4$. Solvent evaporation under vacuum gave compound **1** in (85%) yield; IR υ_{max}(film)/cm^{-1} 3124, 3091, 2567, 1618, 1521, 1492. Data consistence with literature [22].

2.2. The Method for the Synthesis of Compounds 2 and 3

To 2-Benzothiazolethiol **1** (2.0 g, 0.012 mol) in *N*,*N*-Dimethylformamide (20 mL) and K_2CO_3 (0.0015 mol), 3-Bromo-1-propene (1.2 mL, 0.013 mol) or propargyl bromide (1.0 mL, 0.013 mol) was added dropwise at 25 °C, the completion of reaction was monitored using TLC silica plate. After 3 h, water was added and the mixture was extracted with EtOAc (3 x 20 mL). The layers of organic solvent were collected, washed with water, and the obtained organic layer was dried over $MgSO_4$, filtration and solvent evaporation under reduced pressure gave; products **2** (83%) yield in solid phase: m.p 75- 76 °C, IR υ_{max}/cm^{-1} 3421, 3059, 1618, 1525, 1498. Data consistent with literature [23]. Product **3** (90%) yield in solid phase: m.p 197-199 °C, IR υ_{max}/cm^{-1} 3429, 3211, 3059, 2939, 2839, 1622, 1517, 1452, 2117.

2.3. Synthesis of Compounds 4a-e and 5a-e

The aldehydes (0.001 mol), *N*-methylhydroxylamine hydrochloride (0.0011 mol) and triethylamine (0.0012 mol) in PhMe (5.0 mL) were refluxed for 30 min. Compounds **2** or **3** (0.001 mol) was added and the reaction mixture was heated under reflux for 10 h, the completion of the reaction was followed by TLC silica plate. Then, the reaction mixture temperature was reduced to 25 °C and followed by solvent evaporation under vacuum pressure gave;

2.4. Data for Compounds 4a-e

Compound 4a (67%) yield as a black in solid phase: m.p 195-197 °C, IR υ_{max}/cm^{-1} 3336, 3280, 3003, 2922, 2810, 1618, 1450, 1583: ^{1}H-NMR (400 MHz, *d6*-DMSO) δ 8.27-8.24 (8 H, m, Ar CH), 5.82-5.55 (1H, m, O-CH), 4.6 (1H, s, OH), 3.10 (2H, d, SCH_2), 2.95 (2H, t, CH_2), 2.29 (3H, s, N-CH_3), 2.12 (1H, d, N-CH).

Compound 4b (70%) yield as a black in solid phase, m.p 257-259 °C, IR υ_{max}/cm^{-1} 3450, 3037, 2980, 2939, 1637, 1473, 1519; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 7.03-6.57 (10 H, m, Ar CH), 5.81-5.55 (1H, m, CH), 2.87 (1H, t, CH), 2.81 (3H, s, CH_3), 2.75 (2H, d, CH_2), 1.49 (2H, t, CH_2).

Compound 4c (77%) yield as a black in solid phase, m.p 215-218 °C, IR υ_{max}/cm^{-1} 3423, 3025, 2937, 2974, 1624, 1469, 1527. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 8.73-8.31 (6H, m, Ar CH), 5.45-3.39 (1H, m, CH), 2.97 (1H, t, CH), 2.82 (3H, s, CH_3), 2.70 (2H, d, CH_2), 1.58 (s, 3H, CH_3), 1.46 (2H, t, CH_2).

Compound 4d (70%) yield as a black in solid phase, m.p: 230-228 °C, IR υ_{max}/cm^{-1} 3331, 3013, 2939, 2980, 1625, 1402, 1502, 1348, 1535. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 7.03-6.18 (8 H, m, CH), 5.67-5.61 (1H, m, CH), 3.86 (2H, d, CH_2), 2.86 (3H, s, CH_3), 2.76 (1H, d, CH), 1.74 (2H, t, CH_2).

Compound 4e (75%) yield in solid phase, m.p: 210-212 °C, IR υ_{max}/cm^{-1} 3446, 3035, 2974, 2939, 1638, 1475, 1598, 680; ^{1}H NMR (400 MHz, *d6*-DMSO) δ 7.03-6.29 (8 H, m, CH), 4.45-4.24 (1H, m, CH), 3.12 (2H, d, CH_2), 3.21 (3H, s, NCH_3), 2.72 (1H, t, CH), 1.42 (2H, t, CH_2).

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2.5. Data for Compounds 5a-e

Compound 5a (85%) yield as a black in solid phase, m.p: 225-227 °C, IR υ_{max}/cm^{-1} 3450, 3215, 3022, 2941, 2976, 1627, 1475, 1581, ^{1}H NMR (400 MHz, *d6*-DMSO) δ 8.78-7.84 (10 H, m, CH), 4.47 (1H, s, OH). 3.13 (2H, d, CH_2), 2.93 (1H, d, CH), 2.29 (3H, s, CH_3), 2.19 (1H, d, CH).

Compound 5b (87%) yield as a solid, m.p: 170-172 °C, IR υ_{max}/cm^{-1} 3448, 3030, 2974, 2939, 1635, 1475, 1510. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 7.03-6.20 (10 H, m, CH), 2.89 (3H, s, CH_3), 2.89 (1H, d, CH), 2.79 (2H, d, SCH_2), 1.49 (1H, d, CH).

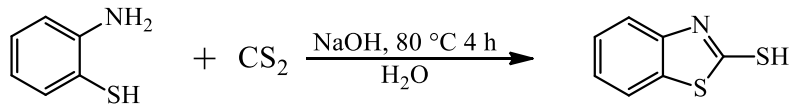
Compound 5c (84%) yield as a brown in solid phase, m. p: 200-202 °C, IR υ_{max}/cm^{-1} 3446, 3015, 2976, 2939, 1625, 1475, 1530, ^{1}H NMR (400 MHz, *d6*-DMSO) δ 8.73-8.31 (6H, m, CH), 2.96 (1H, d, NCH), 2.82 (3H, s, CH_3), 2.70 (2H, d, CH_2), 1.58 (3H, s, CH_3), 1.41 (1H, d, CH_2).

Compound 5d (75%) yield as a brown in solid phase, m.p: 198-200 °C, IR υ_{max}/cm^{-1}, 3269, 3032, 2976, 2939,1627, 1475, 1514, 1367, 1575. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 8.79-7.47 (10 H, m, Ar CH), 3.89 (2H, d, S-CH_2), 2.86 (3H, s, N-CH_3), 2.76 (1H, d, N-CH), 1.72 (1H, d, CH).

Compound 5e (90%) yield as a grey in solid phase, m.p: 230-232 °C, IR υ_{max}/cm^{-1} 3454, 3022, 2976, 2871, 1635, 1475, 1515, 750. ^{1}H NMR (400 MHz, *d6*-DMSO) δ 8.67-6.70 (8H, m, CH), 3.57 (2H, d, CH_2), 3.17 (3H, s, CH_3), 2.89 (1H, d, CH), 1.5 (1H, d, CH).

3. Result and Discussion

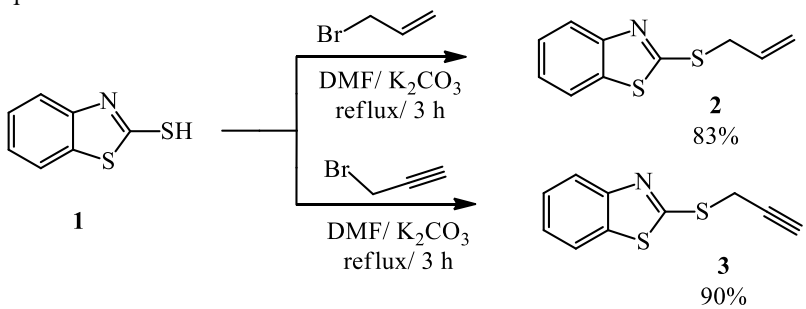
The aim is to synthesize new compounds contain five membered rings throughout the cycloaddition reaction. The synthetic pathways involve the formation of 2-benzothiazolethiol followed by *S*-alkylation, then cyclization step to form the isoxazoline and isoxazolidine rings. However, to explore the ability to try the cycloaddition that could lead to five membered rings, compound **1** was needed to obtain compounds 2 and 3. To do so, 2-Mercaptoaniline and CS_2 was heated in the presence of NaOH which gave 2-benzothiazolethiol **1** in good yield [22] see (scheme 1).



1
85%

Scheme 1. Synthesis of 2-benzothiazolethiol.

The *S*-alkylation was carried out using allyl bromide which gave compound **2** in good yield and using propargyl bromide gave compounds **3** in excellent yield see (scheme 2) [23]. The completion of reaction was controlled using TLC silica plate. The FT-IR spectra confirmed the presence of the double and triple bonds in products.



Scheme 2. *S*-alkylation step using allyl bromide and propargyl bromide.

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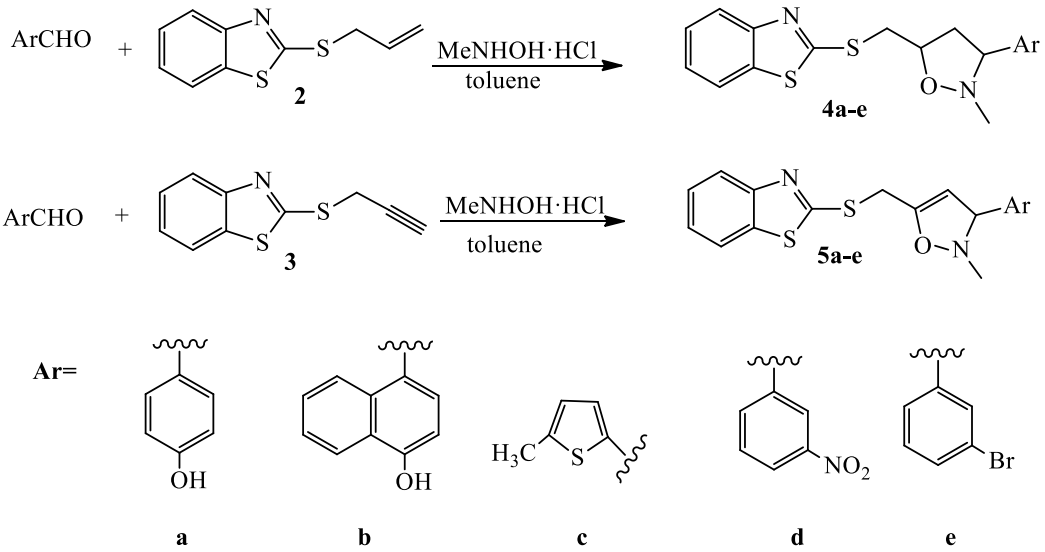
The two compounds 2 and 3 in-hand would allow testing the cycloaddition reactions using oximes in which can be prepared freshly by treating different substituted aldehydes with *N*-methylhydroxylamine hydrochloride. However, aldehyde 1.0 equivalent was reacted with *N*-methylhydroxylamine hydrochloride 1.2 equivalents and triethylamine in methyl benzene which was refluxed for 30 min to give nitrones *in situ*. This was followed by the addition of compound **2** and heating under reflux for 15 hours gave the desired products 4a-e in good yields see (Scheme 3). Based on the FT-IR spectra the double and triple bonds were disappeared.

The protons of the isoxazoline ring was assigned on its ^{1}H NMR spectra in which the proton adjacent to nitrogen appeared as triplet at 2.97-2.12 and the proton adjacent oxygen appeared as quintet at 5.81-4.24 ppm.

For the formation of isoxazolidine ring, the same nitrones and method with compound **3** were used and gave the desired products 5a-e in good yields see (Scheme 3). ^{1}H NMR spectra for the obtained compounds shows peak at 2.96-219 ppm as doublet belong to proton adjacent to nitrogen.



Scheme 3. Cycloaddition reaction to form isoxazoline and isoxazolidine ring.

3. Antibacterial Activity

The antibacterial activities for the synthesized compounds 4a-e and 5a-e were investigated against two types of bacteria; gram negative; *Esherichia coli* and *Pseudomonas aeruginosa*, and gram positive; *Staphylococcus aureus* and *bacillus subtilis*. Uutilizing the disc diffusion method, agar-agar gel [24, 25], two (50 and 100) mg/mL concentrations were used. The inhibition zones were measured in millimetre and were balanced to amoxicillin as a reference. The results clearly indicate that some compounds have good effect against the selected bacteria.

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Table 1.
Antibacterial effect of compounds 4a-e and 5a-e.

Comp. No.	Conc. (mg/mL)	Zone of inhibition (in mm)			
		Gram negative		Gram positive	
		E. Coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*
4a	50	10	5	10	11
	100	14	10	15	16
4b	50	14	7	14	10
	100	15	9	12	14
4c	50	-	13	-	-
	100	20	16	15	14
4d	50	9	5	15	-
	100	11	11	27	13
4e	50	10	-	10	9
	100	15	11	15	14
5a	50	12	10	10	10
	100	12	13	17	15
5b	50	11	10	13	12
	100	14	12	20	15
5c	50	-	8	9	10
	100	14	14	16	15
5d	50	11	8	-	11
	100	20	14	15	17
5e	50	-	11	14	12
	100	-	17	20	16
Amoxicillin	25	-	-	11	8
DMSO	-	-	-	-	-

4. Conclusion

1,3-dipolar cycloaddition reaction was successfully utilized for the formation of five membered ring isoxazolidine and isoxazoline in good yield which led to obtain new heterocyclic compounds. I have demonstrated the formation of oximes by the condensation of an aldehyde and *N*-methylhydroxylamine, then cyclization on to prepared compounds having terminal double and triple bonds. The antibacterial study for the synthesized compounds was done against the selected bacteria, the derivatives shows moderate to good activity.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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