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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEW 3, 4-DIHYDRO-2H-BENZO- AND NAPHTHO-1, 3-OXAZINE DERIVATIVES

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ABSTRACT

Five new 3, 4-dihydro-2H-benzo-and naphtho-1, 3-oxazine derivatives were synthesized via a modified step-wise procedure in which formaldehyde was substituted with methylene bromide for ring-closure reaction. The structures of the synthesized compounds were confirmed by FT-IR, ^{1}H and ^{13}C NMR spectral analysis, elemental analysis and mass spectrometry. The *in vitro* antimicrobial activities of the synthesized compounds were assessed against three strains of gram-positive and three strains of gram-negative bacteria as compared to streptomycin standard drug. Some of the synthesized compounds were found to have antibacterial activity ranging from moderate, good, very good and excellent against some of the bacteria strains with only one of the synthesized compound showing no activity against all the bacteria strains used.

Keywords: 1, 3-Benzoxazines, 1, 3-Naphthoxazines, Methylene bromide, Antimicrobial evaluation, Gram-positive bacteria, Gram-negative bacteria.

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INTRODUCTION

A survey of existing literature has revealed that compounds containing 3, 4-dihydro-1, 3-oxazine ring systems show a wide range of pharmacological and antibacterial activities.[1-3]These heterocyclic compounds are therefore studied extensively for the synthesis of biologically active compounds ranging from herbicides and fungicides to therapeutically usable drugs. [4] Biological activities exhibited by these compounds include antimicrobial, antitumor, anthelminthic, antimycobacterial, antituberculosis and Insect Growth Regulatory (IGR) activity among others.[5-7] The fact that some of the synthesized compounds contains 2-aminothiazole in their moiety also suggest these compounds to be potential antimicrobial agents as 2-aminothiazole compounds were reported to show marked biological and pharmacological activities.[8-14] Moreover, these compounds were also reported to be having profound application in the field of polymer science as they are used in the production of polymeric materials through thermally activated ring-opening polymerization.[15-18] More importantly, the polymers obtained from these categories of compounds were reported to possess superior advantages over the known traditional phenolic resins.[19-21] In view of the promising biological activities of these compounds coupled with our quest for the search of potent antibacterial compounds, we report the synthesis of new compounds using a modified step-wise technique in which formaldehyde which is a suspected human carcinogen and a confirmed animal carcinogen [22] was replaced with methylene bromide for the ring-closure reaction. The *in vitro* antimicrobial activities of these compounds were assessed and the result presented in this paper.

EXPERIMENTAL

Materials

All chemicals and reagents used in this work were commercially purchased from Sigma Aldrich, HmbG Chemicals Company, Fisher Chemicals Company, R & M Chemicals and Acros Organics, USA) and are used as received. FT-IR spectra were recorded using Perkin Elmer FTIR model 100 series spectrophotometer (KBr Pellet) in the region 280-4000 cm^{-1}. ^{1}H and ^{13}C NMR spectral analysis were

conducted on JEOL 500 MHz NMR spectrometer using acetone-d_6 as the NMR solvent. Chemical shifts (δ) and coupling constant (*J*) are expressed in ppm. Elemental analysis was performed with a Leco CHNS-932 Elemental Analyzer. GC-MS analysis was conducted using Shimadzu model QP 5050A GC-MS analyzer. Melting points of the synthesized compounds were determined using a Barnstead electrothermal melting point instrument 9100 Model and are uncorrected.

Procedure for the synthesis of Imine compounds 1 (a-e)

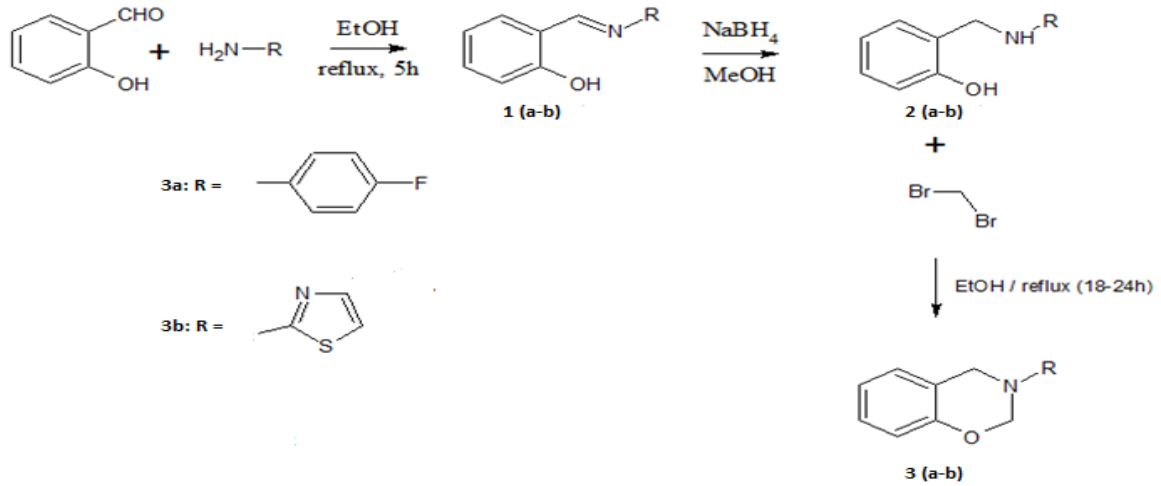
The imine compounds 1 (Schemes-1, 2 and 3) were prepared by refluxing necessary molar proportion of the salicylaldehyde / 2-hydroxy-1-naphthaldehyde and individual amines / diamines in absolute alcohol for 5h under nitrogen atmosphere.

Procedure for the synthesis of 2-hydroxybenzylamines /2-hydroxynaphthylamines 2 (a-e)

In all cases, 200 mmol of the imine compounds were added into a conical flask containing 100 mL of ethanol. To this solution was added 100 mmol of $NaBH_4$ in small portions at ambient temperature while stirring until the reaction is complete. 150 mL of water was then added and the product was extracted with ethyl acetate, washed with water, dried overnight with anhydrous Na_2SO_4 and concentrated to dryness.

Procedure for the synthesis of 1, 3-benzoxazine and naphthoxazine derivatives 3 (a-e)

In all cases, 100 mmol of the 2-hydroxybenzylamines and 200 mmol of methylene bromide were added to 100 mL of absolute ethanol and the mixture refluxed for 18-24h under nitrogen atmosphere. The mixture was allowed to cool to room temperature and the solvent removed by rotary evaporation. 100 mL of water was then added and the compound extracted with ethyl acetate, washed with water, dried overnight with anhydrous Na_2SO_4 and concentrated to dryness. All solid compounds were purified by recrystallization in 50:50 water: ethanol mixture and liquid compounds by column chromatography on silica gel using hexane-ethylacetate (4:1) as eluents to afford the purified compounds.



Scheme-1: Modified step-wise procedure for the synthesis of 3, 4-dihydro-2H-benzo-1, 3- benzoxazine compounds (a-b)

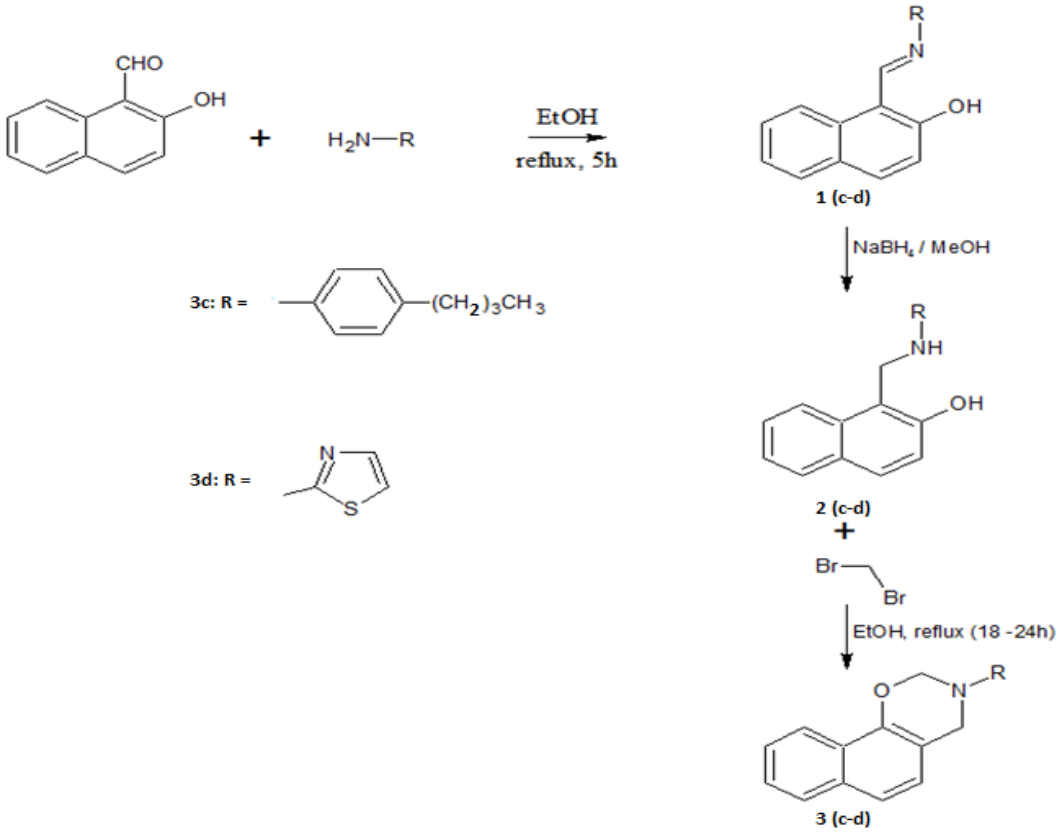
3-(4-fluorophenyl)-3, 4-dihydro-2H-1, 3-benzoxazine **(3a)**

Yield 62%, light brown solid, mp = 101-102°C. IR (KBr): 3038, 2890, 22820, 1866, 1591, 1498, 1375, 1215, 1153, 1032, 935, 823, 748, 662, 583, 520, 442 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6, ppm): δ_H 7.46-6.64 (4H, Ar-H), 5.36 (O-CH_2-N), 4.56 (Ar-CH_2-N), 4.38 (C-F). ^{13}C NMR (500 MHz, Acetone-d_6, ppm): δ_C 156.2 (C-O), 145.4 (C-O), 127.6 (CH), 127.2 (CH), 120.2 (CH), 120.0 (CH), 114.4 (CH), 114.2 (CH), 79.6 (O-CH_2-N), 50.14 (Ar-CH_2-N), 44.8 (C-F). MS: m/z 229 (M^+). Elemental analysis:

$C_{14}H_{12}FNO$ (229.25). Calculated (%): C, 73.28; H, 5.23; N, 6.11. Experimental (%): C, 73.14; H, 5.18; N, 6.08.

3-(1, 3-thiazol-2-yl)-3,4-dihydro-2H-1,3-Benzoxazine **(3b)**

Yield 64%, viscous brown red liquid, bp = 120-123°C. IR (KBr): 3273, 2968, 2754, 1521, 1453, 1368, 1239, 1150, 1042, 962, 855, 749, 692, 612, 509, 433 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6, ppm): δ_H 7.32-6.68 (4H, Ar-H), 7.58 (CH, thiazole), 6.52 (CH, thiazole), 5.46 (O-CH_2-N), 4.42 (Ar-CH_2-N). ^{13}C NMR (500 MHz, Acetone-d_6, ppm): δ_C 158.00 (C, thiazole), 146.20 (C, benzene), 129.20 (CH, thiazole), 128.6 (C, benzene), 122.00-115.20 (4H, Ar-H), 79.00 (O-CH_2-N), 46.20 (Ar-CH_2-N). MS: m/z 218 (M^+). Elemental analysis: $C_{11}H_{10}N_2OS$ (218.27). Calculated (%): C, 60.48; H, 4.58; N, 12.83. Experimental (%): C, 60.34; H, 4.40; N, 12.74.



Scheme-2: Modified step-wise procedure for the synthesis of 3, 4-dihydro-2H-naphtho-1, 3-naphthoxazine compounds (c-d)

3-(4-butylphenyl)-3, 4-dihydro-2H-naphtho [2, 1-e] [1, 3] oxazine **(3c)**

Yield 62%, viscous dark red, bp = 125-127°C. IR (KBr): 3030, 2925, 2861, 1615, 1509, 1463, 1373, 1312, 1226, 1061, 940, 811, 743, 676, 495, 417, 318 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6, ppm): δ_H 8.62-6.94 (6H, Ar-H), 5.46 (2H, O-CH_2-N), 4.92 (2H, Ar-CH_2-N), 2.55 (CH_2 aliphatic), 1.62 (CH_2 aliphatic), 1.34 (CH_2 aliphatic), 0.94 (CH_3 aliphatic). ^{13}C NMR (500 MHz, Acetone-d_6, ppm):δ_C 194.2 (C), 169.2 (C), 164.4 (C), 153.2 (C), 146.6 (C), 134.2 (C), 129.4 (CH), 129.2 (CH), 128.6 (CH), 122.6 (CH), 120.2 (CH), 119.4 (CH), 113.8 (CH), 79.6 (O-CH_2-N), 49.2 (Ar-CH_2-N), 34.6 (CH_2 aliphatic), 34.2 (CH_2 aliphatic), 22.8 (CH_2 aliphatic), 14.2 (CH_3 aliphatic). MS: m/z 317 (M^+). Elemental analysis: C22H23NO (317.42). Calculated (%): C, 83.17; H, 7.25; N, 4.41. Experimental (%): C, 82.96; H, 7.11; N, 4.22.

3-(1, 3-thiazol-2-yl)-3, 4-dihydro-2H-naphtho [2, 1-e] [1, 3] oxazine **(3d)**
Yield 65%, viscous brown red, bp = 106-107.80°C. IR (KBr): 3242, 3061, 2968, 2882, 1612, 1513, 1455, 1366, 1232, 1045, 966, 865, 808, 742, 694, 612, 509, 420 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6, ppm): δ_H 8.18-6.82 (Ar-H), 7.22 (C-4, thiazole), 6.94 (C-5, thiazole), 5.62 (O-CH_2-N), 5.16 (Ar-CH_2-N).^{13}C NMR (500 MHz, Acetone-d_6, ppm):δ_C 170.0 (C-2, thiazole), 151.6 (C, naphthalene), 138.6 (C-4, thiazole), 128.6-118.0 (8H, Ar-H), 114.2 (C, naphthalene), 109.4 (C-5, thiazole). MS: m/z 268 (M^+). Elemental analysis: $C_{15}H_{12}N_2OS$ (268.33). Calculated (%): C, 67.08; H, 4.47; N, 10.43. Experimental (%): C, 66.92; H, 4.33; N, 10.31.

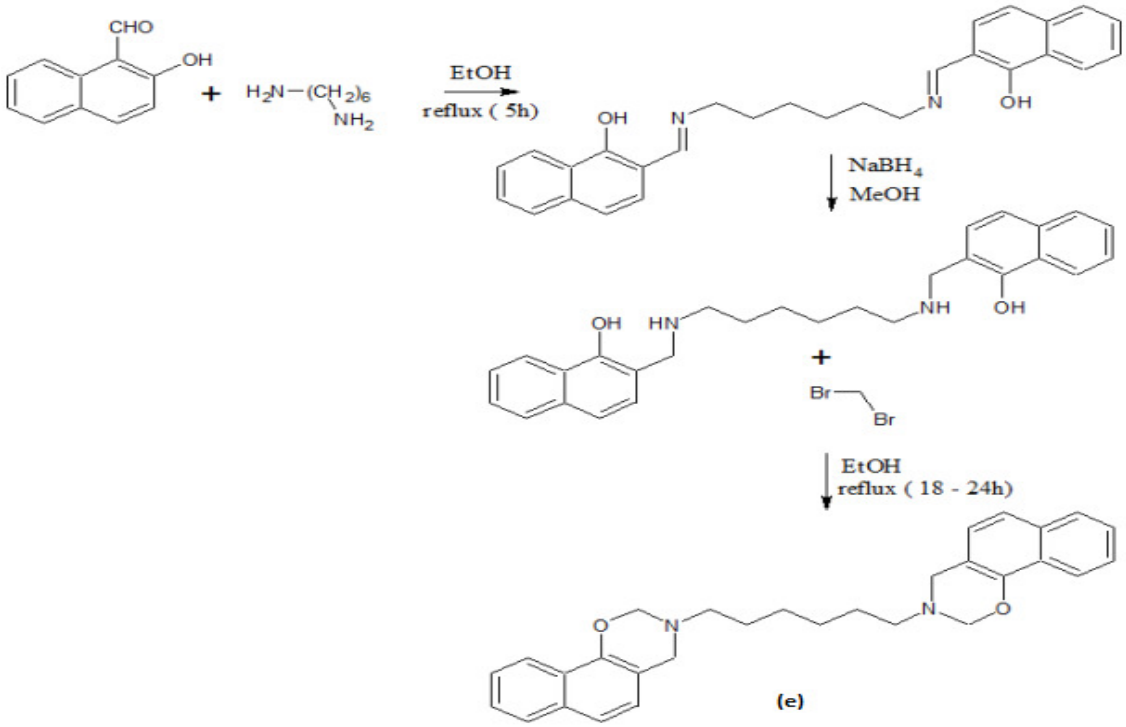
3, 3'-(1, 6-hexamethylene) bis (3, 4-dihydro-2H-1, 3-Naphthoxazine) **(3e)**
Yield 60%, beige brown solid, mp = 108.20-109.42°C. IR (KBr): 3399, 3050, 2925, 2856, 1621, 1513, 1458, 1351, 1269, 1175, 997, 943, 837, 744, 494, 421, 344 cm^{-1}; ^1H NMR (500 MHz, Acetone-d_6, ppm): δ_H 8.32-7.17 (Ar-H), 5.21 (2H, O-CH_2-N), 3.96 (2H, Ar-CH_2-N), 2.42 (CH_2), 1.44 (CH_2), 1.34 (CH_2). ^{13}C NMR (500 MHz, Acetone-d_6, ppm):δ_C 156.2 (C), 134.4 (C), 127.2 (C), 129.6 (CH), 128.2 (CH), 126.2 (CH), 124.6 (CH), 121.6 (CH), 120.4 (CH), 114.1 (CH), 112.8 (C), 78.8 (2H, O-CH_2-N), 52.2 (2H, Ar-CH_2-N), 49.4 (CH_2 aliphatic), 30.6 (CH_2 aliphatic), 28.2 (CH_2 aliphatic). MS: m/z 452 (M^+). Elemental analysis: $C_{30}H_{32}N_2O_2$ (452.59). Calculated (%): C, 79.54; H, 7.07; N, 6.19. Experimental (%): C, 79.44; H, 7.01; N, 6.10.

Biological Evaluation
The synthesized compounds were tested *in-vitro* for their antibacterial activity against three strains of gram positive and three strains of gram negative bacteria using the cup-plate agar diffusion method[23] using streptomycin (100mg / ml) as the reference antibacterial agent. The sterilized mediums (autoclaved at 110°C for 45min.) [1 mg ml^{-1}] were inoculated with suspensions of the micro-organisms (10^5 cfu mL^{-1}) as matched to McFarland barium sulphate solution and poured into petridishes to obtain a depth of 3-4 mm. The mediums were solidified, inverted and incubated for 18-24 hours at 30-37°C until sufficient growth has taken place. After incubation, each plate was examined and the zones of complete inhibition were measured. The zones were measured using sliding calipers to the nearest whole millimeter (mm).

RESULTS AND DISCUSSION

Schemes I-3 shows the synthetic route for the new benzo- and naphtho-1, 3-oxazine compounds. Step 1 and 2 are as reported in the step-wise synthetic process[24,25] and involves condensation of the aromatic aldehydes and the primary amines to give imine compounds 1 (a-e) followed by subsequent reduction with sodium borohydride in methanol to the corresponding 2-hydroxybenzylamines / 2-hydroxynaphthylamines 2 (a-e). Condensation and reduction processes (steps 1 and 2) were ascertained using FT-IR spectroscopy. Appearance of band in of the region 1602-1622 cm^{-1} in the FT-IR spectrum of the imine compounds 1 (a-e) which is due to C=N signifies condensation and the appearance of band near 3300 cm^{-1} in the FT-IR spectrum of the reduced compounds 2 (a-e) which is due to NH and absence of C=N band signifies reduction. Step 3 involves the reflux of the 2-hydroxybenzylamines / 2-hydroxynaphthylamines obtained in excess of methylene bromide which results in ring-closure reaction leading to the target benzo- and naphtho- 1, 3-oxazine compounds 3 (a-e**).**
The structures of the synthesized compounds were established on the basis of their spectroscopic data (FT-IR, ^1H NMR, and ^{13}C NMR) and elemental analysis. All the synthesized compounds showed appropriate characteristic signals necessary to confirm their structures. FT-IR spectrum showed characteristic absorption bands due to trisubstituted benzene rings in the region 935-966 cm^{-1} and 1453-1498 cm^{-1} which are characteristic absorptions of benzoxazine compounds. Other bands observed include that of asymmetric Ar-H stretching vibration in the region of 3012-3016 cm^{-1}, asymmetric stretching for C-O-C in the region 1215-1269 cm^{-1} and aliphatic CH_2 stretching bands between 2820 and 2972 cm^{-1}.
^1H NMR spectrum of all the synthesized compounds showed multiplets due to the aromatic protons and the proton resonance shifts due to the two methylene groups (O-CH_2-N and Ar-CH_2-N) which are necessary to confirm the formation of oxazine ring. ^{13}C NMR spectrums of the synthesized compounds

equally confirm the presence of carbon chemical shifts corresponding to O-CH_2-N and Ar-CH_2-N as contained in the spectral data given.



Scheme-3: Modified step-wise procedure for the synthesis of 3, 4-dihydro-2H-naphtho-1, 3-naphthoxazine compound (e)

Antibacterial activity

Tables 1and 2 gives the result of the antimicrobial activity against three strains of gram positive bacteria and three strains of gram negative bacteria as compared to 100 mg/ml streptomycin which was used as the standard. From the result, compound **3a** was found to exhibit good activity against *Bacillus subtilis* B29 and *Staphylococcus epidermidis* S273. The compound also shows very good activity against *Acinetobacter anitratus* A9 and excellent activity against Escherichia coli E266 and only a moderate activity against *Staphylococcus aureus* S276. Compound **3b** showed moderate activity against *Bacillus subtilis* B29, C *Staphylococcus epidermidis* S273 and *Escherichia coli* E266. Compound **3c** however shows very good activity against all the strains of gram positive bacteria. It also shows very good activity against Acinetobacter anitratus A9 and an excellent activity against *Escherichia coli* E266. Compound **3d** only shows moderate activity against *Bacillus subtilis* B29. Compound **3e** however shows no activity against all the strains of bacteria used.

Table-1: Antimicrobial activity data against gram positive bacteria

Sample(s)	*Bacillus Subtilis B29*			*Staphylococcus aureus S276*			*Staphylococcus epidermidis S273*		
	i	ii	iii	i	ii	iii	i	ii	iii
A	18	20	18	14	14	14	20	18	18
B	12	12	12	13	13	13	10	10	10
C	22	24	22	25	24	25	22	22	24
D	13	13	12	12	11	10	8	8	8
E	8	8	8	7	8	7	10	10	10
Standard	28	28	28	32	33	34	34	34	34

All results are in millimeter (mm)
Standard (Streptomycin 100mg/ ml)

Table-2: Antimicrobial activity data against gram negative bacteria

Sample(s)	Pseudomonas aeruginosa ATCC 15442			*Escherichia coli E266*			*Acinetobacter anitratus A9*		
	i	ii	iii	i	ii	iii	i	ii	iii
A	9	9	8	28	28	28	25	26	26
B	10	10	11	14	16	14	14	16	14
C	15	18	15	38	38	40	24	28	24
D	8	8	9	10	10	10	12	12	12
E	-	-	-	6	8	6	10	10	12
Standard	31	31	31	30	30	30	36	37	36

All results are in millimeter (mm)
Standard (Streptomycin 100mg/ ml)

CONCLUSION

New 3, 4-dihydro-2H-benzo- and naphtho-1, 3-oxazine derivatives were successfully synthesized and characterized using a modified step-wise procedure in which formaldehyde was replaced with methylene bromide for ring-closure reaction. The synthesized compounds were thereafter evaluated for antibacterial activity against some strains of gram positive and gram negative bacteria. Most of the synthesized compounds show very promising antibacterial activity against some of the bacteria strains while some do not show any activity against some of the bacteria strains.

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