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ARTICLE

An efficient green synthesis and antibacterial activity of 1,3-benzoxazine and 1,3-naphthoxazine using NaCl.SiO_2 as solid catalyst in neat condition

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Abstract

A series of 1,3-oxazine derivatives were synthesized by a one-pot three-component (ie, phenol, formaldehyde, amine) method where SiO_2 bonded with NaCl was used as a reusable, more efficient, easily prepared, and available solid catalyst. The reactions were also carried out at room temperature for greener approach. in vitro studies for the synthesized compounds were also done against two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) to check for their applicability as an antibacterial agent where some of the synthesized compounds gives the best antibacterial activity against selected bacterial strains. Streptomycin was used as a standard control for all the microbial test.

1 | INTRODUCTION

Oxazine, due to their wide and diverse range of biological activities are considered as an important type of heterocycles and are therefore, a special interest for the synthetic areas. The 1, 3-oxazine nucleus features prominently in many biologically important natural products and other bioactive molecules.[1–4] They have gained much importance due to their many biological activities like antitubular,[3] antibacterial,[4] anticancer,[5] anticonversant,[2] and analgesic.[1] Benzo-1,3-oxazines are also generally known to possess biologically properties like cytotoxic,, antianginal, antihypertensive effects, demonstrating antirheumatic, and antiosteoclastic bone resorption activity.[6]

Multicomponent reactions (MCRs) have become highly efficient as they avoid time consumption, has economic purification processes, formation of several bonds in a single step and manufacture of complex molecular architectures.[7] This type of MCRs incorporate synthetic strategy as the products formed are obtained in just a single step and also its variation can be simply developed by changing the reacting components.[8]

Recently, there is a consideration on the use and design of environmentally friendly heterogeneous catalysts to reduce the amount of toxic waste. Among them, nano scale metal oxides[9] have been efficiently used as catalysts for organic transformations.[10–12] Solid-support catalyst has become prominently important in the recent years as they are easily available/easily prepared and are generally less expensive. They can also be easily separated from reaction mixtures and can be reused which make them eco-friendly and also simple to use. This is one of the main reasons why silica-supported catalyst in MCRs has gained much importance and attention.[13] Gabbas et al also synthesized a series of 3,4-dihydro-2*H*-benzo- and naphtho-1,3-oxazine derivatives using methylene bromide for ring-closure reaction where they also confirmed the presence of antibacterial property in almost all of their synthesized compounds via in vitro antimicrobial studies.[14a] As derivatives containing oxazine ring system have shown to

possess great potential as antimicrobial agents,[14] synthesis of some oxazine derivatives as well as their in vitro antibacterial investigation were assessed and presented in this work.

2 | RESULTS AND DISCUSSION

In continuation with our investigation on the methodology of green synthesis of oxazines,[15] this present study, focused on a greener and economical approach, for the synthesis of various substituted [1.3-oxazines] by MCRs which were carried out at room temperature using a solid-support catalyst, SiO_2.NaCl. The catalyst we have chosen proves to be an inexpensive, easily available, nontoxic, environmental friendly, and an efficient catalyst. The catalyst also acts as a solid-support medium and also slightly makes the medium acidic which inhances the reaction rate.[16]

Using the catalyst, the completion of the reactions was achieved within 5 to 10 minutes at room temperature with continuous stirring. The products **4(a-f)** were obtained using substituted phenols (Table 1), while products **6(a-c)** and **8(a, b)** were obtained using 2-naphthol (Table 2), and 1-naphthol (Table 3), respectively. We also observed the production of heat as the reaction progressed which was then monitored and the structure of the purified compounds were deduced by their IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy (Schemes 1-4).

The compounds were also screened for their antibacterial activity which revealed that almost all the compounds which were tested showed moderate to good activity against the bacterial strains. Compounds **4b**, **8a**, and **4f** showed promising results. The results of the antibacterial screening are as shown in Table 4. Further, to quantify its antibacterial activity minimum inhibitory concentration (MIC) of all the compounds were determined. The results are as shown in Table 5.

From Table 5, we can see that compound **8a** showed maximum inhibition against all bacterial strains with MIC of 0.023 for *Bacillus subtilis (B.s.)*, *Escherichia coli (E.c.)*, and *Staphylococcus aureus (S.a.)* and 0.005 for *Klebsiella pneumonia (K.p.)* Whereas, compound **4a**, **4c**, and **4d** showed no activity against *S.a*., *B.s*., and *E.c*., respectively. Overall, compound **8a, 4e**, and **4f** shows promising property as it gave the best antibacterial activity against all bacterial strains.

TABLE 1 Reaction of phenol/substituted phenols with aniline and 4-ipropyl to form compounds **4(a-f)**

Product	R′	R	Yield in %
4a	4-Br	H	78
4b	2-NO_2	H	75
4c	4-NO_2	H	76
4d	2-NH_2	H	70
4e	4-Br	4-ipropyl	73
4f	H	4-ipropyl	76

TABLE 2 Reaction of β-napthanol with aniline and 4-ipropyl to form compounds **6(a-b)**

Product	R	Yield in %
6a	H	80[17]
6b	4-ipropyl	81

TABLE 3 Reaction of α-napthanol with aniline and 4-ipropyl to form compounds **8(a-c)**

Product	R	Yield in %
8a	H	82
8b	4-ipropyl	79
8c	CH_3	79

2.1 | Spectral and analytical data

(4a) 6-bromo-3,4-dihydro-3-phenyl-2*H*-benzo[e][1,3] oxazine- mp: 58°C; IR (KBr, cm^{-1}): 2853.83 and 2924.75 (—CH_2), 504.45 (—C—Br), 1515.44 (C═C), 1081.78 (CH_2—O), 1261.07 (C—N), 1314.83 (C—O), 1612.79 (C—O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.351 (s, 2H, CH_2), 4.150 (s, 2H, CH_2), 6.760-7.060 (m, 7H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 60.45, 79.10, 115.30, 116.21, 117.81, 121.30, 129.63, 131.00, 132.8, 133.4, 147.8, 152.7; MS: m/z = 289.

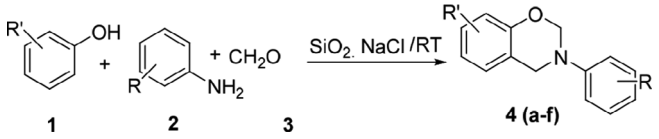
(4b) 3,4-dihydro-8-nitro-3-phenyl-2*H*-benzo[e] [1,3]oxazine- mp: 50°C; IR (KBr, cm^{-1}): 3418.78 (C—H), 2851.76 and 2921.42 (—CH_2), 1538.76 (C—NO_2), 1507.07 (C═C), 1076.22 (CH_2—O), 1263.90 (C—N), 1318.93 (C—O), 1602.77 (C—C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.781(s, 2H, CH_2), 5.610 (s, 2H, CH_2), 6.743-7.912 (m, 8H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 54.79, 87.51, 114.37, 115.59, 118.71, 121.40, 124.12, 123.02, 129.95, 141.05, 149.82, 157.94; MS: m/z = 157 (M^+).

(4c) 3,4-dihydro-6-nitro-3-phenyl-2*H*-benzo[e] [1,3]oxazine- mp: 52°C; IR (KBr, cm^{-1}): 1506.97 (C—NO_2), 3411.85 (—CH), 2926.28-2854.30 (—CH_2), 1074.30 (CH_2—O), 1261.46 (C—N), 1453.69 (C—O), 1602.70 (C—C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.896 (s, 2H, CH_2), 5.298 (s, 2H, CH_2), 6.854-7.200 (m, 8H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 53.57, 68.72, 108.9,

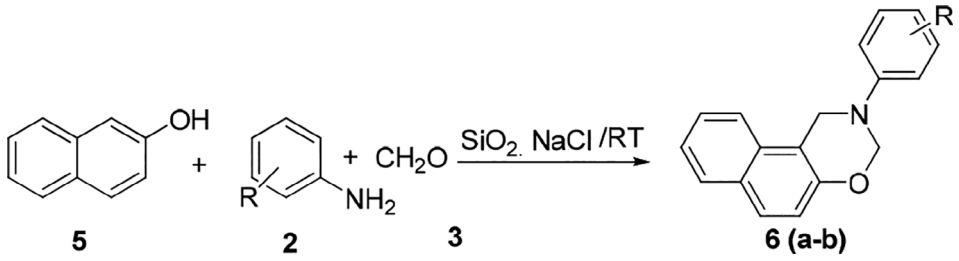
113.75, 115.22, 117.79, 121.04, 129.29, 129.41, 129.56, 148.72, 158.04; MS: m/z = 156.

(4d) 3,4-dihydro-3-phenyl-2*H*-benzo[e][1,3]oxazine-8-amine- mp: 52°C; IR (KBr, cm^{-1}): 3306.98 (C—NH_2), 3376.44 (C—H), 2963.65 and 2850.57 (—CH_2), 1095.68 (CH_2—O), 1261.81 (C—N), 1468.68 (C—O), 1603.11 (C—C), 1509.22 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.174 (s, 2H, CH_2), 3.487 (s, 2H, NH_2), 4.686 (s, 2H, CH_2), 6.704-7.138 (m, 8H, ArH), ^{13}C NMR (400 MHz, $CDCl_3$): δ 57.95, 89.90, 115.27, 116.19, 117.17, 118.74, 119.64, 121.48, 129.43, 155.17, 144.27, 146.42; MS: m/z = 226.

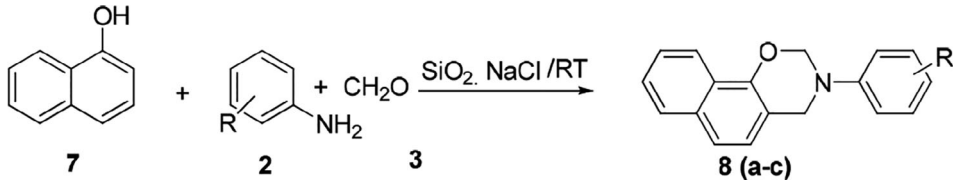
(4e) 6-bromo-3,4-dihydro-3-(4-isopropylphenyl)-2*H*-benzo[e][1,3]oxazine- mp: 99°C; IR (KBr, cm^{-1}): 2861.26 (C—CH_3), 471.98 (C—Br), 3276.63 (C—H), 2960.40 and 2861.26 (—CH_2), 1056.44 (CH_2—O), 1260.80 (C—N), 1489.13 (C—O), 1613.22 (C—C), 1514.17 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.317 (s, 3H, CH_3), 2.991 (s, H, CH) 3.026 (s, 2H, CH_2), 4.511 (s, 2H, CH_2), 6.891-7.452 (m, 7H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.112, 33.313, 49.067, 111.586, 116.298, 118.486, 124.865,



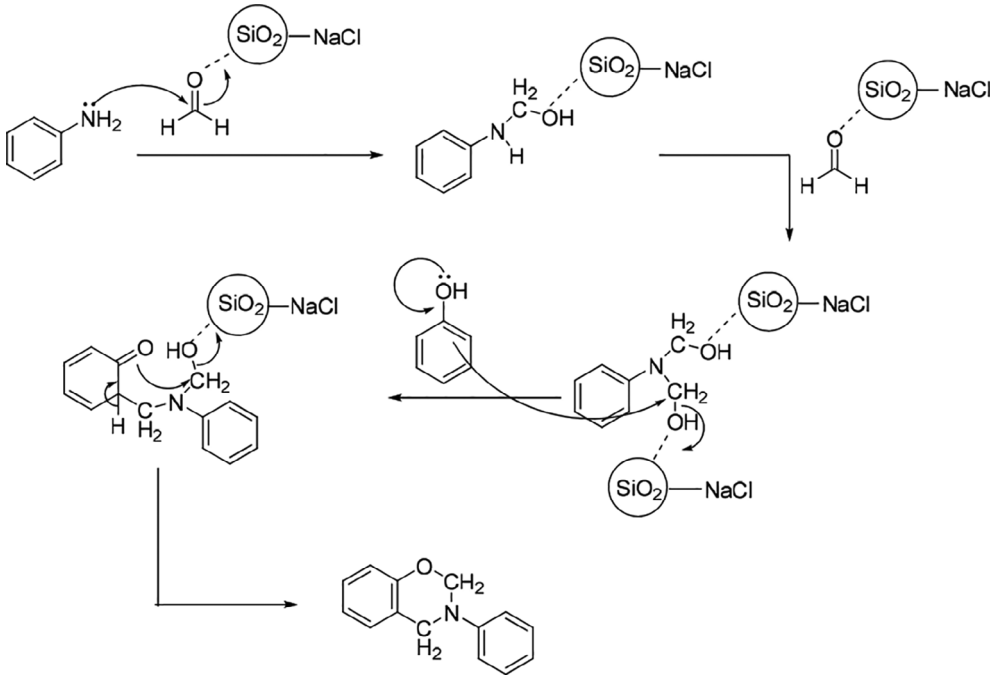
SCHEME 1 Synthesis of 1,3-benzoxazine **4(a-f)**



SCHEME 2 Synthesis of 1,3-napthoxazine **6(a-b)**



SCHEME 3 Synthesis of 1,3-napthoxazine **8(a-c)**



SCHEME 4 Plausible reaction mechanism showing the role of catalyst

127.326, 130.960, 131.776, 142.087, 144.448, 156.256; MS: m/z = 333.

(4f) 3,4-dihydro-3-(4-isopropylphenyl)-2*H*-benzo[e][1,3]oxazine- mp: 70°C; IR (KBr, cm^{-1}): 2855.58 (C—CH_3), 3294.92 (C—H), 2958.34 and 2925.72 (—CH_2), 1059.15 (CH_2—O), 1262.48 (C—N), 1490.97 (C—O), 1611.19 (C—C), 1512.35 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ δ 2.291 (s, 3H, CH_3), 3.026 (s, 2H, CH_2), 3.304 (s, H, CH), 4.651 (s, 2H, CH_2), 6.748-7.954 (m, 8H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.23, 36.37, 60.96, 89.99, 114.01, 114.29, 121.07, 122.33, 127.33, 128.33, 128.15, 129.03, 138.71, 147.33, 158.07; MS: m/z = 253.

(6a) 3,4-dihydro-3-phenyl- 2*H*-naphtho[2,1-e][1.3]oxazine- mp: 160°C;IR (KBr, cm^{-1}): 3298.23(C—H), 2863.55 (—CH_2) 1575.87 (C═C), 1050.75 (—CH_2-O), 1268.70 (C—N), 1243.04 (C—O), 1600.10 (C—C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.454 (s, 2H, CH_2), 6.748-8.147 (m, 11H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 49.60, 115.33, 116.33, 119.43, 121.28, 122.09, 125.23, 125.32, 126.32, 126.37, 127.47, 129.49, 134.16, 147.22, 152.80; MS: m/z = 260 (M^{-1}).

(6b) 3,4-dihydro-3-(4-isopropylphenyl)-2*H*-naphtho[2,1-e][1,3]oxazine- mp: 130°C; IR (KBr, cm^{-1}):2863.90 (C—CH_3), 3275.32 (C—H), 2955.69 and 2863.90 (—CH_2), 1048.01 (CH_2O), 1263.54 (C—N), 1471.07 (C—O), 1614.42(C—C), 1511.56 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.941 (s, 3H, CH_3), 2.975 (s, 3H, CH_3), 3.141 (s, H, CH), 4.948 (s, 2H, CH_2), 6.792-7.929 (m, 10H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.124, 33.202, 112.712, 113.566, 118.263, 121.419, 121.724, 122.748, 126.447, 127.301, 128.834, 129.636, 141.815, 144.857, 145.085, 155.526; MS: m/z = 304 (M^+).

(8a) 2,3-dihydro-2-phenyl-1*H*-naphtho[1,2-e][1,3]oxazine- mp: 98°C; IR (KBr, cm^{-1}): 3294.07 (C—H), 1582.25 (C═C), 1068.10 (CH_2—O), 1271.86 (C—N), 1244.79 (C—O), 1602 (C—C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.50 (s, 2H, CH_2), 5.487 (s, 2H, CH_2), 6.547-7.894 (m, 11H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 51.70, 98.33, 115.49, 116.28, 119.33, 121.83, 124.23, 125.43, 126.72, 127.32, 128.74, 129.50, 135.50, 135.78, 148.47, 155.28; MS: m/z = 262 (M^+).

TABLE 4 Antibacterial activity of the compounds **4(a-f)**, **6(a-c),** and **8(a-b)** (diameter of zone of inhibition in mm)

	Zone of inhibition (mm)			
Compounds (2 mg/mL DMSO)	***B.s.***	***E.c.***	***K.p.***	***S.a.***
4a	10	12	12	NI
4b	11	16	15	10
4c	NI	12	14	16
4d	13	NI	10	12
4e	14	13	14	14
4f	15	11	12	13
6a	13	13	14	11
6b	13	13	12	13
8a	18	18	19	17
8b	13	>10	>10	12
8c	10	11	13	11
Streptomycin	23	22	22	22

Abbreviations: *B.s., Bacillus subtilis*; *E.c., Escherichia coli*; *K.p., Klebsiella pneumonia*; NI, no inhibition; *S.a., Staphylococcus aureus*.

TABLE 5 Determination of MIC of compounds **4(a-f)**, **6(a-c),** and **8(a-b)**

	MIC (mg/ml)			
Compounds (2 mg/mL DMSO)	***B.s***	***E.c.***	***K.p.***	***S.a.***
4a	0.75	0.375	0.375	—
4b	0.75	0.046	0.046	0.75
4c	—	0.375	0.187	0.046
4d	0.187	—	0.75	0.375
4e	0.187	0.187	0.187	0.187
4f	0.187	0.375	0.375	0.187
6a	0.187	0.187	0.187	0.375
6b	0.187	0.187	0.375	0.187
8a	0.023	0.023	0.005	0.023
8b	0.187	0.75	0.75	0.375
8c	0.75	0.375	0.187	0.375
Streptomycin	0.00729	0.00729	0.0029	0.0058

Abbreviations: *B.s.*, *Bacillus subtilis*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumonia*; MIC, minimum inhibitory concentration; *S.a., Staphylococcus aureus*.

(8b) 2,3-dihydro-2-(4-isopropylphenyl)-1*H*-naphtho[1,2-e][1,3]oxazine- mp: 129°C; IR (KBr, cm^{-1}): 2863.90 (C—CH_3), 3275.46 (C—H), 2955.50 and 2890.97 (—CH_2), 1049.92 (CH_2—O), 1263.31 (C—N), 1470.97 (C—O), 1614.38 (C—C), 1510.80 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.299 (s, 3H, CH_3), 2.992 (s, 2H, CH_2), 3.010 (s, H, CH), 4.966 (s, 2H, CH_2), 6.760-7.942 (m, 10H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.216, 33.313, 115.283, 116.123, 119.227, 121.218, 122.746, 122.874, 126.649, 126.744, 127.128, 128.748, 128.920, 129.476, 132.011, 133.124, 146.858, 154.321; MS: m/z = 304 (M^+).

(8c) 3,4-dihyro-3-(4-isopropylphenyl)-6-methyl-2*H*-benzo[e][1,3]oxazine- mp: 100°C; IR (KBr, cm^{-1}): 2857.99 (C—CH_3), 3313.75 (C—H), 2912.26 and 2885.41 (—CH_2), 1040.43 (CH_2—O), 1266.52 (C—N), 1465 (C—O), 1614.62 (C—C), 1512.13 (C═C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.318 (s, H, CH_3), 2.431 (s, 2H, CH_2), 4.716 (s, 2H, CH_2), 6.749-8.377 (m, 10H, ArH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.533, 50.006, 112.712, 115.200, 116.446, 119.170, 121.999, 125.116, 126.155, 126.179, 127.309, 129.866, 130.717, 134.007, 144.576, 152.847; MS: m/z = 276 (M^+).

3 | EXPERIMENTAL SECTION

All the chemicals and reagents were purchased from Merck and HiMedia and were used without further purification. The synthesized compounds were characterized by checking its melting point, IR, H^1 NMR, C^{13} NMR, and Mass spectroscopy. All the evaporation process after reaction completion and Column chromatography completion was done using BUCHI Rotavapor R-300. Melting point was determined in open capillary tubes using IKON melting point apparatus and the results were uncorrected. FT-IR Perkin Elmer instrument was used to record the IR spectra using Kbr and FT-NMR Bruker Avance-II spectrometer (400 MHz) was used to record the ^{1}H NMR and ^{13}C NMR spectra where $CDCl_3$ was used as the solvent. For mass spectroscopy, Compact Mass Spectrophotometer APCI Expression-S instrument was used. Progress of the reaction was monitored by TLC using TLC silica gel 60 Aluminum backed F254 which was developed in UV chamber as well as in iodine chamber. For isolation and separation process Column Chromatography was performed using Silica Gel 100-200 mesh.

3.1 | Preparation of SiO_2.NaCl (Catalyst)

About 4 g of NaCl was dissolved in 20 mL of distilled water and to it 10 g of SiO_2 (100-200 mesh) was added and then continuously stirred for 20 minutes in a magnetic stirrer. The whole mixture was further heated gently on a hot magnetic stirrer plate with occasional stirring until the white solid is free flowing. The resultant catalyst was further activated in a hot air oven maintained at 120°C for at 24 hours prior to use.

3.2 | General procedure for the synthesis of compounds 4(a-f), 6(a-c), and 8 (a-b)

To a mixture of 1 mmol of Amine (**2**), 2 mmol of Formaldehyde (**3**) was added. Subsequently 1 mmol of Substituted-Phenol (**1**)/naphthol (**5 or 7**) and 1 g of the prepared catalyst was added and then stirred at room temperature for 5 to 10 minutes. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was first washed with ethyl acetate and the filtrate was dried using BUCHI Rotavapor R-300. The catalyst after washing was kept to dry in a hot air oven for further use. The synthesized compound was then separated and purified by Column chromatography using hexane: ethylacetate mixture. Further confirmation of the synthesized compound was done by spectral analysis data such as melting point, IR, NMR, and mass spectroscopy.

3.3 | Antibacterial studies

All the synthesized compounds were screened for their antibacterial activity against two gram-positive bacteria viz. *B.s.* and *S.a.*, and two gram-negative bacteria viz. *E.c.*, and *K.p.*. Well diffusion method was used for the in vitro antibacterial studies and the activity was determined by measuring the diameter of inhibition zones (mm), also the MIC were determined by means of standard 2-fold serial broth dilution method.[18] DMSO concentration of 2 mg/mL of was used where DMSO was used as a−ve control and Streptomycin was used as a+ve control.

4 | CONCLUSIONS

In this present study we have successfully synthesized a series of 1,3-oxazines using an efficient and environmental friendly catalyst through a one-pot, three-component condensation reaction. The synthesized compounds were characterized by their melting point, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. They were also screened for their antibacterial activity which included determination of zone of inhibition and MIC. Compounds like **8a**, **4e**, and **4f** showed promising property as it gave the best antibacterial activity against all bacterial strains.

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