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A facile and environmentally benign synthesis of 2*H*-benzo[*b*] [1,4] oxazines of potential biological importance

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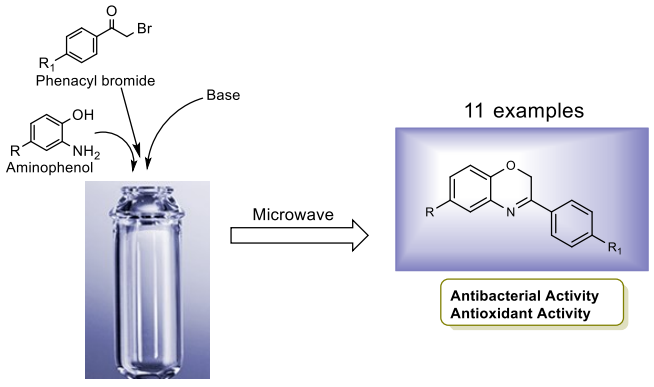
Abstract

A simple and efficient microwave-assisted protocol is reported for the synthesis of fifteen 2*H*-benzo[b] [1,4] oxazine from phenacylbromides and aminophenols using Cs_2CO_3 as a base catalyst, out of which four compounds were found to be novel. The method gave 70 to 86% yields in 3-5 minutes. Microwave assistance proved to have reduced the reaction time, improved the yield, purity, and made the work-up easier. Characterization was done by FT-IR, ^{1}HNMR, ^{13}CNMR, and mass spectrometric analysis. The in vitro anti-bacterial and in vitro antioxidant properties of the synthesized oxazines were assessed. The results suggested that they possess good antibacterial and antioxidant activity. The compound 3h having chlorine and methyl as substituents exhibits higher antibacterial activity with inhibition zones of 14-19mm. The compound 3d having methoxy and methyl as substituents showed high antioxidant activity with IC_{50} value of 53.33(µg/ml).



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Introduction

The importance of 1,4 oxazines has grown significantly over the decade by virtue of its wide biological activities[1–10] and their natural occurrence.[11,12] The interest in these molecules is also attributed to the fact that these heterocycles have a structural scaffold that can serve as building blocks for syntheses of more useful heterocycles.[13,14] A substantial number of 1,4 oxazine derivatives are found in a wide range of therapeutically promising drug candidates possessing biological activities such as being anti-cancer,[4,5,15] anti-microbial,[10,16–19] antimycobacterial tuberculosis,[2,20] anti-oxidant, [10] platelet aggregation inhibition,[21] anticonvulsant,[6] HGF-mimetic agents,[7] transthyretin amyloid fibril inhibition[22] and anti-stress oxidative.[3] The most important 1,4-benzoxazine derivatives that are currently in clinical are Ofloxacin and Levofloxacin.[23,24] They are also reported to possess herbicidal properties.[12]

The large number of papers on the synthesis of oxazines that have appeared in recent years attests to the huge contemporary interest owing to its usefulness.[13,25–27] However, most of the earlier published techniques have at least one of the following drawbacks: low yields resulting from the fairly lengthy, laborious workup, hazardous catalysts used and difficult reaction assemblage etc.

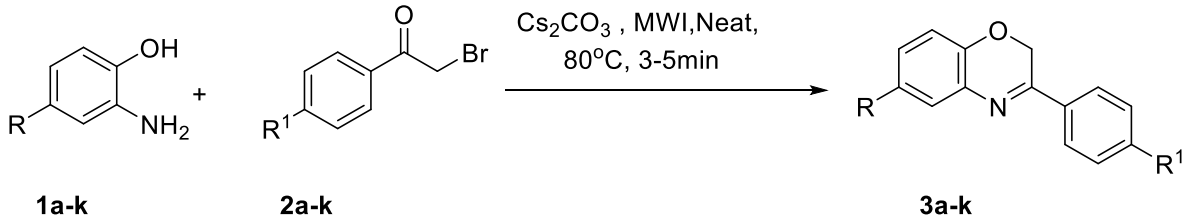
Drug resistance in the modern era possess a challenge in tackling existing illnesses and emerging strands of new micro-organism leading to humanitarian crisis which necessitates the importance of renewed focus on scientific research to synthesize new classes of chemicals that will help in developing new medications. Also, taking into consideration the rising importance placed on sustainability of research and emerging environmental challenges has resulted in the surge of research dedicated to the design and development of green and sustainable methods of organic syntheses of chemicals. The range of starting materials for 1,4 Oxazine synthesis has also evolved in response to the necessity to create and develop novel medications and materials to tackle the ever-increasing challenges associated with anti-microbial drug resistance.[13,28] Despite the growing need and challenges, the literature on the syntheses and biological evaluation of 1,4 Oxazines are limited in number as compared to 1,3-benzoxazines, research on 1,4 Oxazines should be scaled up to achieve desired outcome.

Based on all the above consideration and adding to our research endeavor, to design and develop novel methods for the synthesis of oxazines,[29–31] we have devised an efficient, mild, and convenient method for the synthesis of 2*H*-Benzo[*b*][1,4] oxazines (Scheme 1). Microwaves interact directly with the molecules of the starting reagents in the reaction mixture, generating a rapid temperature rise. On comparative analysis, it was observed that reactions which took hours under reflux conditions were completed in a matter of minutes under microwave irradiation, it proved not only to be time saving but also resulted in achieving desired results such as increase in yields and lesser by-products. An important aspect of this technique is that the requirement for toxic solvents is eliminated, also this method aligns with fulfilling the principles of green chemistry and has proved to be more efficient and advantageous over the existing methodologies [32,33]

Results and Discussion

The reaction conditions were optimized on the reaction of 4-bromo phenacylbromide (1a, 1.0 mmol) and 2-aminophenol (2a, 1.0 mmol) in the different solvents and bases like Na_2CO_3, K_2CO_3, and Cs_2CO_3. The best results were obtained by employing Cs_2CO_3 in ethanol. With Cs_2CO_3 as a base catalyst and ethanol as solvent, the conversion was observed even at room temperature when stirred for 5.0 h. After an extractive workup, the separation was carried out via silica gel chromatography to afford a pure product (yield 40%), which was identified as 3-(4-bromophenyl)-2*H*-benzo[b] [1,4] oxazine (**3a**). In general observance, the use of Cs_2CO_3 as the

base was easier to workup, and yields were higher which can be attributed to its high solubility in solvents. The reaction couldn't proceed without the base. The same reaction was performed under microwave irradiation at 150 W power output and 80°C temperature and high purity of product was obtained in a matter of minutes. With microwave assistance, the reaction time was reduced and the need for solvent was also avoided. The by-products were also eliminated. The reaction proceeds efficiently with both electron-donating and electron-withdrawing substituents on the aromatic aldehydes. (Table 1)



1a-k **2a-k** **3a-k**

Scheme 1. Synthesis of 2*H*-Benzo[*b*] [1,4] Oxazines.

Table 1. List of *synthesized* compounds

SL/No.	Product	R	R^1	Time taken (minutes)	Yield (%)	M.P (°C)	Reported Mp (°C)
1	**3a**	H	Br	3	70	160	162[34]
2	**3b**	H	CH_3	3	78	88	-
3	**3c**	CH_3	Br	2	70	142	143[35]
4	**3d**	CH_3	OCH_3	3	86	120	-
5	**3e**	Cl	CH_3	2	72	119	120[36]
6	**3f**	H	H	2	74	87	88[37]
7	**3g**	H	Cl	3	80	158	159[36]
8	**3h**	CH_3	Cl	3	85	108	109[36]
9	**3i**	Cl	H	2	75	93	95[34]
10	**3j**	CH_3	H	3	76	129	131[34]
11	**3k**	H	O CH_3	3	71	130	131[34]
12	**3l**	CH_3	CH_3	4	70	126	-
13	**3m**	Cl	O CH_3	5	76	118	-
14	**3n**	Cl	Cl	5	75	128	132[34]
15	**3o**	Cl	Br	4	77	124	126[34]

Reaction Conditions: Cs_2CO_3, MWI, neat, 80°C

Antibacterial activity

Despite the fact that oxazines have been extensively utilized as antimicrobial agents, no publications on antibacterial studies of the presently synthesized 1,4 Oxazine compounds have been found in literature.[34-37] This brought us to evaluate the antibacterial activities of the synthesized compounds against selected human pathogens. Table 2 gives the result of the antimicrobial activity against two strains of gram-negative bacteria and two trains of gram-positive bacteria. Streptomycin which was used as the standard. Compound **3h** showed higher inhibition against two out of four bacteria strains with inhibition zone of 19mm for *Escherichia coli* (EC) and 17mm for *Bacillus subtilis* (BS). Compound **3j** also showed good inhibition against all the bacteria strains with inhibition zone of 18mm for *Klebsiella pneumonia* (KP), and 15mm for the other three strains. Compound **3b** showed maximum inhibition zone of 18mm for *Staphylococcus aureus* (SA). Compound **3k** showed maximum inhibition zone of 19mm for *Bacillus subtilis* (BS), compound **3i** showed the most negligible inhibition to almost no inhibition.

Table 2. Minimum Inhibition Zone (mm) of synthesized compounds

Compound (2mg/L)	Minimum Inhibition Zone (mm)			
	Gram-Negative Bacteria		Gram-Positive Bacteria	
	KP	*EC*	*BS*	*SA*
3a	14	14	16	16
3b	10	11	15	18
3c	13	11	14	13
3d	15	10	16	16
3e	14	12	14	14
3f	12	11	12	13
3g	14	12	15	15
3h	19	14	17	17
3i	13	11	11	12
3j	18	15	15	15
3k	16	19	13	14
3l	17	17	15	15
3m	16	13	16	14
3n	16	16	15	15
3o	13	13	13	12
Streptomycin	23	22	22	22

Further, Minimum inhibitory concentration (MIC) of all the compounds was determined. Minimum Inhibitory Concentration (MIC) is a test that determines the lowest concentration of an antimicrobial agent needed to inhibit the visible in-vitro growth of a challenge microorganism. The results are shown in Table 3.

Overall, compounds with methyl **(3b, 3h**) and methoxy group (**3d, 3j, 3k**) exhibited the most inhibition as compared to the others and are observed to be promising antibacterial agents.

Table 3. Minimum Inhibition Concentration of synthesized compounds

Compound	Minimum Inhibition Concentration (mM)			
	Gram Negative Bacteria		Gram-Positive Bacteria	
	KP	*EC*	*BS*	*SA*
3a	$0.67x10^{-3}$	$0.67x10^{-3}$	$1.60\ x10^{-3}$	$1.60\ x10^{-3}$
3b	$3.36\ x10^{-3}$	$3.36\ x10^{-3}$	$0.20\ x10^{-3}$	$0.10\ x10^{-3}$
3c	$0.62\ x10^{-3}$	$2.49\ x10^{-3}$	$0.62\ x10^{-3}$	$0.76\ x10^{-3}$
3d	$7.39\ x10^{-3}$	$7.39\ x10^{-3}$	$1.81\ x10^{-3}$	$0.74x10^{-3}$
3e	$0.73\ x10^{-3}$	$1.46\ x10^{-3}$	$0.73\ x10^{-3}$	$0.73\ x10^{-3}$
3f	$0.89\ x10^{-3}$	$1.79\ x10^{-3}$	$1.79\ x10^{-3}$	$0.89\ x10^{-3}$
3g	$0.77\ x10^{-3}$	$1.54\ x10^{-3}$	$0.19x10^{-3}$	$0.19\ x10^{-3}$
3h	$0.02\ x10^{-3}$	$1.46\ x10^{-3}$	$0.09\ x10^{-3}$	$0.09x10^{-3}$
3i	$0.77x10^{-3}$	$3.09\ x10^{-3}$	$3.09\ x10^{-3}$	$1.54\ x10^{-3}$
3j	$0.10\ x10^{-3}$	$0.84\ x10^{-3}$	$0.21\ x10^{-3}$	$0.84\ x10^{-3}$
3k	$0.20\ x10^{-3}$	$0.02\ x10^{-3}$	$1.57\ x10^{-3}$	$1.57\ x10^{-3}$
3l	$0.10\ x10^{-3}$	$0.10\ x10^{-3}$	$0.20\ x10^{-3}$	$0.20\ x10^{-3}$
3m	$0.17\ x10^{-3}$	$0.68\ x10^{-3}$	$0.17\ x10^{-3}$	$0.68\ x10^{-3}$
3n	$0.17\ x10^{-3}$	$0.17\ x10^{-3}$	$0.17\ x10^{-3}$	$0.17\ x10^{-3}$
3o	$0.58\ x10^{-3}$	$0.58\ x10^{-3}$	$0.58\ x10^{-3}$	$1.16\ x10^{-3}$
Streptomycin	$0.01\ x10^{-3}$	$0.01\ x10^{-3}$	$0.01\ x10^{-3}$	$0.01\ x10^{-3}$

Antioxidant Activities

Oxidative stress, which is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, has been linked to the development of diseases like diabetes, cancer, and cardiovascular diseases.[38,39]

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: The radical scavenging activities of the synthesized oxazines at different concentrations (20, 40, 60, 80 and 100 µg/ml) were studied with 2,2-diphenyl-1-picrylhydrazyl (DPPH) using Trolox as the positive control**.** The findings are shown in Table 4 and Figure 1. The concentration of each compound required to scavenge 50% of the DPPH radical present in the assay medium was established and referred to as IC_{50}. IC_{50} is thus defined as the amount of the antioxidant needed to halve the concentration of DPPH present in the test solution. The Lower the value of IC_{50} value the better the DPPH radical scavenging activity. The reduction in absorbance of DPPH radicals at 517nm caused by antioxidants was used to assess their capacity for reduction. The compounds **3d, 3a, 3f and 3e** presented the lowest values of IC_{50} which showed higher antioxidant activity than Trolox whose IC_{50} was $34.2x10^{-5}$ mM.

Table 4. IC_{50} values and standard deviations of the samples and Trolox

Compound	IC_{50} (mM) $\pm$ SD (n=3)
3a	$22.0 \times 10^{-5} \pm 0.000018$
3b	$26.8 \times 10^{-5} \pm 0.000026$
3c	$24.3 \times 10^{-5} \pm 0.000034$
3d	$21.1 \times 10^{-5} \pm 0.000021$
3e	$22.6 \times 10^{-5} \pm 0.000024$
3f	$27.1 \times 10^{-5} \pm 0.000031$
3g	$24.9 \times 10^{-5} \pm 0.000012$
3h	$23.8 \times 10^{-5} \pm 0.000010$
3i	$25.6 \times 10^{-5} \pm 0.000013$
3j	$29.5 \times 10^{-5} \pm 0.000019$
3k	$26.9 \times 10^{-5} \pm 0.000023$
3l	$37.1 \times 10^{-5} \pm 0.000031$
3m	$29.1 \times 10^{-5} \pm 0.000014$
3n	$31.1 \times 10^{-5} \pm 0.000010$
3o	$25.9 \times 10^{-5} \pm 0.000028$
Trolox	$34.2 \times 10^{-5} \pm 0.000020$

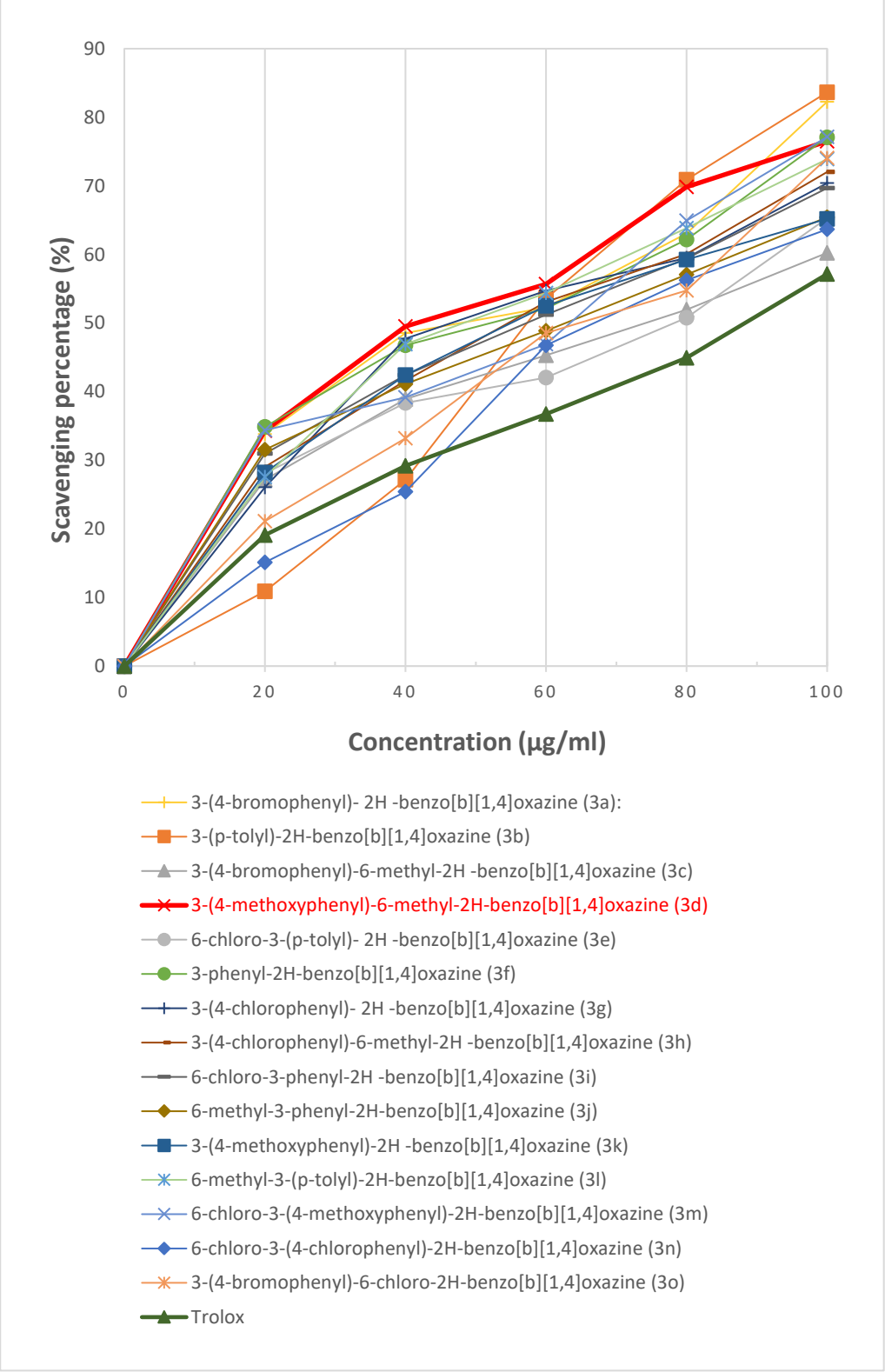


Figure 1. DPPH free-radical scavenging activity in the presence of different concentrations of Trolox and the *synthesized* oxazines.

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay depends on reducing ferric ion ($Fe3^{+}$) into ferrous ion (Fe^{2+}). This assay primarily assesses the reducing capacity of an antioxidant when it reacts with Fe^{3+} ($K_3Fe\ (CN)_6$) to produce a colored Fe^{2+} ($K_3Fe\ (CN)_6$) complex. Potassium ferricyanide is employed as a ferric-

binding reagent, resulting in a blue-colored ferrous complex that was spectrophotometrically evaluated, demonstrating the antioxidant's reducing power. Increased absorbance at 700 nm suggests antioxidant activity. The ability of any compound to donate an electron or hydrogen atom to a metal atom is accountable for its reducing power. The synthesized compounds were assayed over a range of dilutions (20, 40, 60, 80 and 100 µg/ml) and the results in the form of absorbance obtained have been presented in Figure 2.

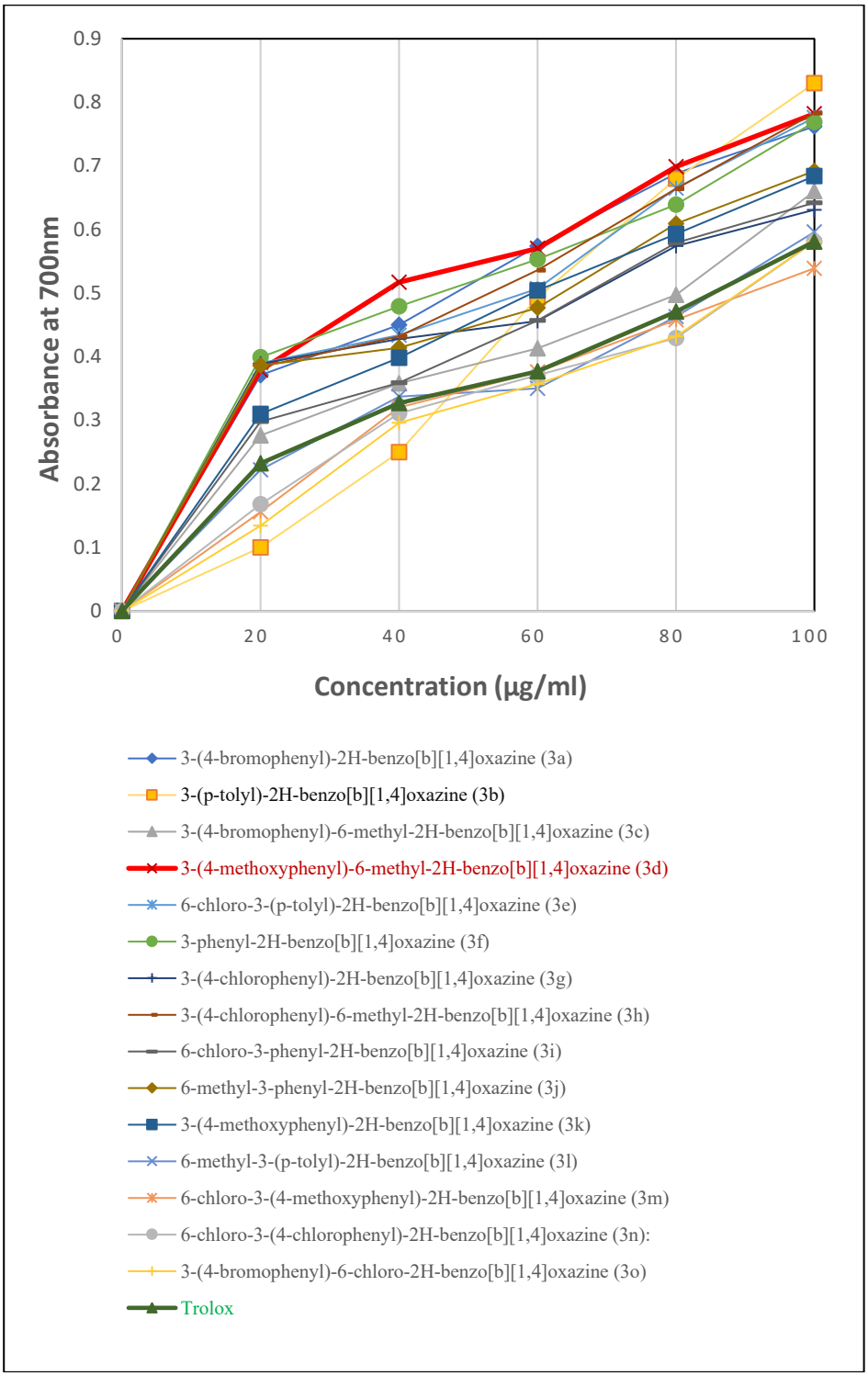


Figure 2. Antioxidant activity of standard (Trolox) and the *synthesized* oxazines using FRAP assay.

Structure activity relationship (SAR) studies

Substitutions (R/R') on the aromatic ring of compounds (**3a-o**) significantly impact their antimicrobial activity. The introduction of electron withdrawing and donating groups at the para positions of aromatic rings increased the compounds' antibacterial potency. In general, the compounds showing the highest inhibitions all had methyl substituents on either R or R' position of the benzene rings. Compound **3h** with methyl at R showed maximum effect against KP, i.e., inhibition at MIC 0.77 x 10^{-3} mM. The good inhibition activity against KP was maintained even with different substitutions on R' as shown by compounds **3j**, **3l** and **3c** with H, methyl, and bromo substitution on R' respectively. Installation of methoxy group at R' showed very good activity against EC as shown by compounds 3k and 3l with MIC 0.02 x 10^{-3} mM and 0.10 x 10^{-3} mM respectively. In case of antibacterial activity against BS, compound 3h having electron withdrawing chloro group on R' position was found to be the most active. The inhibition activity was maintained even with the chloro group at R position as shown by Compounds **3m** and **3n.** Electron donating groups methyl and methoxy at R and R' on compounds **3b**, **3h**, and **3d** added to the results with good activity shown against SA bacteria. Data of antioxidant activity revealed that methyl and methoxy group on R and R' positions of benzene ring showed excellent activity. Compound **3d** exhibited maximum potency with IC_{50} value of 21.1x10^{-5}mM.

Conclusions

In summary, we report a technique that is more simplified, following the principles of green chemistry for the synthesis of 1,4-oxazines, resulting in excellent yields. The increase in the number of publications on the applications of the microwave assisted reactions indicates its increasing utility, at the same time emphasising the importance on such research techniques in promoting green chemistry and must be further studied. The present study provides evidence that the synthesized 1, 4 Oxazine compounds can be good nominees for future investigations to synthesise new anti-bacterial and anti-oxidants. Further studies on the different application of the synthesised compounds are currently at different stages and emphasis on continued research is going on to design more efficient methods for organic syntheses of oxazines.

Experimental Section

General. The starting reagents and catalyst were purchased from Merck and used without further purification. Microwave reactions were carried out in Microwave Digester (Anton Paar Monowave 400). Solvent was extracted using a rotary evaporator (BUCHI Rotavapor 3-300). Melting points were measured on the Ikon melting point apparatus and compared with reported values of known compounds. IR spectra were recorded on an FTIR spectrometer (Perkin Elmer 1725X, Model: Spectrum Two FT-IR). Mass spectra were recorded on mass spectrophotometer (Advion expressions). NMR spectra were recorded with a Bruker spectrometer at 400 MHz (^{1}H NMR) and at 100 MHz (^{13}C NMR) in $CDCl_3$ as solvent and with TMS as internal standard, and chemical shifts are expressed as d/ppm.

General procedure for preparation of 2*H*-benzo[*b*][1,4] oxazines. Aminophenol **1** (1 mmol), phenacylbromide **2** (1mmol) along with base Cs_2CO_3(0.05mmol) were taken in a Pyrex test-tube (Glass vial G10ml) and irradiated for 2-3minutes at 80^{o}C. The reaction progress was monitored by TLC using Silica coated aluminium TLC using 20% ethyl acetate in hexane as the solvent system. After the completion of the reaction, as indicated by TLC,

the resultant mixture was extracted from the vial using ethyl acetate. The solvent was removed under reduced pressure using a rotary evaporator. The crude product was purified by column chromatography (Merck silica gel 100-200 mesh) using n-hexane/EtOAc 3:1 as eluent or by repeated recrystallization in hot ethanol.

The structures of all the synthesized molecules were established by spectral analyses (IR, ^{1}H NMR, ^{13}CNMR, and mass) data. All the synthesized compounds showed appropriate characteristic signals, which confirm their structures. The representative spectral data is given below.

3-(4-Bromophenyl)- 2*H* -benzo[*b*][1,4]oxazine (3a). Mp 162 °C. IR (KBr) cm^{-1}: 3031 (Ar-H str.), 2915 (Al-H str.), 1668 (C=N str.), 1237 (C-N str.), 1081 (C-O-C str.), 586 (C-Br str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.9(s, 2H, CH_2); 6.84(dd,1H);7.1-7.8 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 60.7, 115.2, 115.9, 120.2, 124.1, 129.2, 129.9, 130.9, 134.3, 135.2, 152.4, 155.6 ; MS: *m/z* 288 $(M)^+$.

3-(*p*-Tolyl)-2*H*-benzo[*b*][1,4]oxazine (3b). Mp 88 °C. IR (KBr) cm^{-1}: 3063 (Ar-H str.), 2924 (Al-H str.), 1582 (C=N str.), 1274(C-N str.), 1089 (C-O-C str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38(s, 3H); 5.81(s, 2H, CH_2); 6.96-7.65 (m, 8H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ:21.1, 60.58, 109.59, 118.59, 119.35,123.37, 126.88, 128.00, 130.62, 131.44, 132.33, 140.09, 152.27; MS: *m/z* 224 $(M)^+$.

3-(4-Bromophenyl)-6-methyl-2*H* -benzo[*b*][1,4]oxazine (3c). Mp 142 °C. IR (KBr) cm^{-1}: 3031 (Ar-H str.), (Al-H str.), (C=N str.), (C-N str.), 1071 (C-O-C str.), 643 (C-Br str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ:2.33(s, 3H, Al); 4.87(s, 2H, CH_2); 6.61-7.61(m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ:20.81, 60.81, 115.30, 116.24, 124.16, 125.14, 129.24, 129.67, 130.52, 131.14, 141.27, 150.70, 153.40; MS: *m/z* 302 $(M)^+$.

3-(4-Methoxyphenyl)-6-methyl-2*H*-benzo[*b*][1,4]oxazine (3d). Mp 120 °C. IR (KBr) cm^{-1}: 3033 (Ar-H str.), 2899(Al-H str.), 1576(C=N str.), 1265(C-N str.), 1087(C-O-C str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36(s,3H); 4.11(s, 3H, Al); 4.96(s, 2H, CH_2); 6.95-7.94 (m, 7H, Ar) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.08, 57.67, 71.89, 118.86, 123.91, 124.57, 126.96, 127.42, 129.97, 132.54, 136.47, 137.34, 140.43, 154.05; MS: *m/z* 254 $(M)^+$.

6-Chloro-3-(*p*-tolyl)- 2*H* -benzo[*b*][1,4]oxazine (3e). Mp 121 °C. IR (KBr) cm^{-1}: 2954 (Ar-H str.), 2925 (Al-H str.), 1608 (C=N str.), 1377 (C-N str.), 1090 (C-O-C str.), 726 (C-Cl str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.16(s,3H, Al); 4.82(s, 2H, CH_2); 6.52-7.24 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.72, 61.81, 116.28, 116.74, 124.23, 124.34, 129.56, 129.99, 135.14, 140.65, 149.58, 151.43, 155.20; MS: *m/z* 258 (M^+).

3-Phenyl-2*H*-benzo[*b*][1,4]oxazine (3f). Mp 107 °C. IR (KBr) cm^{-1}: 2973 (Ar-H str.), 2915 (Al-H str.), 1669 (C=N str.), 1258 (C-N str.), 1081 (C-O-C str.), 797 (C-Cl str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.86(s, 2H, CH_2); 6.52(d, 1H, Ar); 6.70-7.78(m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 61.33, 115.16, 116.13, 120.25, 128.91, 128.93, 129.02, 130.20, 135.60, 135.82, 152.33, 157.36; MS: *m/z* 209(M^+).

3-(4-Chlorophenyl)- 2*H* -benzo[*b*][1,4]oxazine (3g). Mp 158 °C. IR (KBr) cm^{-1}: 2925 (Ar-H str.), 1605 (C=N str.), 1461 (C=C str.), 1256 (C-N str.), 1025 (C-O-C str.), 746 (C-Cl str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.81(s, 2H, CH_2); 6.67(d, 1H, Ar); 7.10-7.96 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 56.21, 116.41, 117.97, 124.88, 125.09, 127.01, 127.76, 128.67, 130.06, 136.85, 154.47, 158.38 ; MS: *m/z* 245 (M^+).

3-(4-Chlorophenyl)-6-methyl-2*H* -benzo[*b*][1,4]oxazine (3h). Mp 108 °C. IR (KBr) cm^{-1}: 3031 (Ar-H str.), 2932(Al-H str.), 1453(C=N str.), 1275(C-N str.), 1020(C-O-C str.), (788C-Cl str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.30(s,3H, Al); 4.85(s, 2H, CH_2); 6.89 (d, 1H, Ar); 6.96-7.53 (m, 6H, Ar) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.07, 60.58, 117.90, 123.31, 127.58, 128.95, 130.95, 130.97, 131.90, 134.64, 139.98, 152.45, 152.65; MS: *m/z* 257 (M^+).

6-Chloro-3-phenyl-2*H* -benzo[*b*][1,4]oxazine (3i). Mp 96 °C. IR (KBr) cm^{-1}: 2953 (Ar-H str.), 2924 (Al-H str.), 1590 (C=N str.), 1250 (C-N str.), 1091 (C-O-C str.), 736 (C-Cl str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.30 (s, 3H, Al), 4.88(s, 2H, CH_2); 6.89(d, 1H, Ar); 6.94-7.36(m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 60.57, 115.62, 115.91, 120.36, 124.13, 128.52, 129.29, 130.43, 134.48, 141.14, 152.87, 153.84; MS: *m/z* 244 (M^+).

6-Methyl-3-phenyl-2*H*-benzo[*b*][1,4]oxazine (3j). Mp 112 °C. IR (KBr) cm^{-1}: 3095 (Ar-H str.), 2922 (Al-H str.), 1610 (C=N str.), 1256 (C-N str.), 1090 (C-O-C str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.56(s,3H, Al); 4.79(s, 2H, CH_2);

6.83-7.54 (m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.31, 60.85, 115.57, 157.99, 120.35, 123.26, 125.79, 130.00, 130.12, 134.12, 137.42, 152.64, 153.97; MS: *m/z* 241 (M^+).

3-(4-Methoxyphenyl)-2*H* -benzo[*b*][1,4]oxazine (3k). Mp 131 °C. IR (KBr) cm^{-1}: 3093 (Ar-H str.), 2935 (Al-H str.), 1597 (C=N str.), 1494 (C=C str.) 1269 (C-N str.), 1091 (C-O-C str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.95(s,3H, Al), 4.83(s, 2H, CH_2), 6.54-7.58 (m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 56.04, 62.20, 108.84, 110.54, 114.62, 116.67, 124.56, 128.86, 129.34, 135.68, 152.21, 156.98, 168.05 ; MS: *m/z* 240 (M^+).

6-Methyl-3-(p-tolyl)-2*H*-benzo[*b*][1,4]oxazine (3l). Mp 131 °C. IR (KBr) cm^{-1}: 3099 (Ar-H str.), 2924 (Al-H str.), 1597 (C=N str.), 1494 (C=C str.) 1269 (C-N str.), 1091 (C-O-C str.); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32(s,6H, Al); 4.81(s, 2H, CH_2); 6.72-7.64 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ:29.64, 30.97, 61.18, 114.43, 115.63, 119.94, 124.01, 127.40, 130.59, 136.61, 141.18, 151.88, 152.63, 157.14; MS: *m/z* 238 (M^+).

6-Chloro-3-(4-methoxyphenyl)-2*H*-benzo[*b*][1,4]oxazine (3m). Mp 131 °C. IR (KBr) cm^{-1}: 3090(Ar-H str.), 2958 (Al-H str.), 1586 (C=N str.), 1488 (C=C str.) 1273 (C-N str.), 1088 (C-O-C str.), 767 (C-Cl); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.92(s,3H, Al); 4.81(s, 2H, CH_2); 6.72-7.42 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ:51.11, 61.05, 115.93, 117.28, 118.41, 119.60, 120.48, 128.27, 131.36, 132.74, 157.95, 160.55, 163.96; MS: *m/z* 275 (M^+).

6-Chloro-3-(4-chlorophenyl)-2*H*-benzo[*b*][1,4]oxazine (3n). Mp 131 °C. IR (KBr) cm^{-1}: 3066 (Ar-H str.), 1566 (C=N str.), 1501 (C=C str.) 1272 (C-N str.), 1083 (C-O-C str.), 723(C-Cl); (^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.06(s, 2H, CH_2); 6.91-7.65 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 61.69, 108.84, 110.54, 114.62, 116.67, 124.56, 128.86, 129.15, 129.34, 135.68, 152.21, 156.98; MS: *m/z* 277 (M^+).

3-(4-Bromophenyl)-6-chloro-2*H*-benzo[*b*][1,4]oxazine (3o). Mp 131 °C. IR (KBr) cm^{-1}: 2997 (Ar-H str.), 1579 (C=N str.), 1490 (C=C str.) 1275 (C-N str.), 1083 (C-O-C str.), 763(C-Cl), 603(C-Br) ; (^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.90(s, 2H, CH_2); 6.86-7.54 (m, 7H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ:61.81, 115.42, 116.45, 120.45, 128.91, 129.02, 130.20, 131.77, 135.60, 135.82, 151.99, 157.11 ; MS: *m/z* 322 (M^+).

***In vitro* antibacterial studies.** The synthesized compounds were screened for their anti-bacterial activity against two Gram-positive bacteria viz. *Bacillus subtilis* (BS) and *Staphylococcus aureus* (SA), and two Gram-negative bacteria viz. *Escherichia coli* (EC) and *Klebsiella pneumonia* (KP). Well diffusion method was used for the *in-vitro* anti-bacterial studies and the activity was determined by measuring the diameter of inhibition zones (mm); also, the Minimum inhibitory concentrations [MIC] were determined employing standard two-fold serial broth dilution method. 2mg/ml of DMSO concentration was used where DMSO was used as a negative control and Streptomycin was used as a positive control.

***In vitro* antioxidant assays.** The antioxidant activity of the sample was evaluated utilizing two separate assays: DPPH and FRAP. The antioxidant activity of the sample was tested using the two assays and compared with the standard Trolox. The experiments were carried out in triplicate and the results were averaged. The IC_{50} values for the standard and the sample were derived for the DPPH assay. The DPPH free-radical scavenging percentage was calculated using the measured absorbance as follows:

DPPH scavenging activity (%) = $(A_{control} - A_{sample}) / A_{control} * 100$

where $A_{control}$ is the absorbance of the control (DPPH +methanol) and A_{sample} is the absorbance of the sample compound. From the obtained DPPH scavenging activity (%) values, the IC_{50} value, which represents the concentrations of compounds that caused 50% neutralization, was determined by linear regression analysis. For the FRAP assay, the absorbance of the reaction mixture was measured at 700 nm using a UV/Vis spectrophotometer. Greater absorbance indicated greater reducing power.

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Supplementary Material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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