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Research Article

ONE-POT MULTI-COMPONENT SYNTHESIS OF [1,4]BENZOXAZINE-ISOXAZOLE HYBRIDS AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

A one-flask strategy for the synthesis of novel 4-((3-(aryl) isoxazol-5-yl) methyl)-2H-benzo[b][1,4] oxazin-3(4H)-one derivatives (**4a-j**) were synthesized by the Cu(I)-catalyzed reaction of *in situ* generated nitrile oxides with *in situ* generated N-propargyl 1,4-benzoxazine in good yields and their antibacterial activity was investigated. Among all the synthesized compound **4j** have shown very good inhibition against all the tested Gram-positive and Gram-negative bacterial strains with MIC values ranging from 3.12 to 12.5 μg mL^{-1}. Compound **4f** against *B. subtilis* and *K. pneumoniae* with MIC value 6.25 µg mL^{-1} and Compound **4g** have shown potent activity against *B. subtilis* and *E. coli* with MIC value 12.5 μg mL^{-1}. Remaining compounds are shown moderate to poor activity as compared to the standard drug Streptomycin.

Keywords: 1,4-benzoxazine; Isoxazole; Chloramine-*T*; Antibacterial activity.

INTRODUCTION

The chemistry of 1,4-benzoxazinones and their derivatives have received considerable attention due to their importance in organic, medicinal, and material sciences (**Fig.1**). For example, a large number of 1,4-benzoxazinones have been incorporated into a wide variety of therapeutically interesting drug candidates possessing antibacterial,[1] anticancer,[2-5] anticonvulsant[6] and antithromobotic activities.[7]

The literature survey reveals that benzoxazinone derivatives also known as 5-HT6 receptor antagonists,[8] bladder-selective potassium channel openers,[9] dual selective serotonin reuptake inhibitors and 5HT1A receptor,[10] dopamine agonists[11] and inhibitors of PI3Kinase.[12] Some substituted [1,4]-oxazines are also related to blocking the TXA2 receptor and activate the PGI2 receptor.[13]

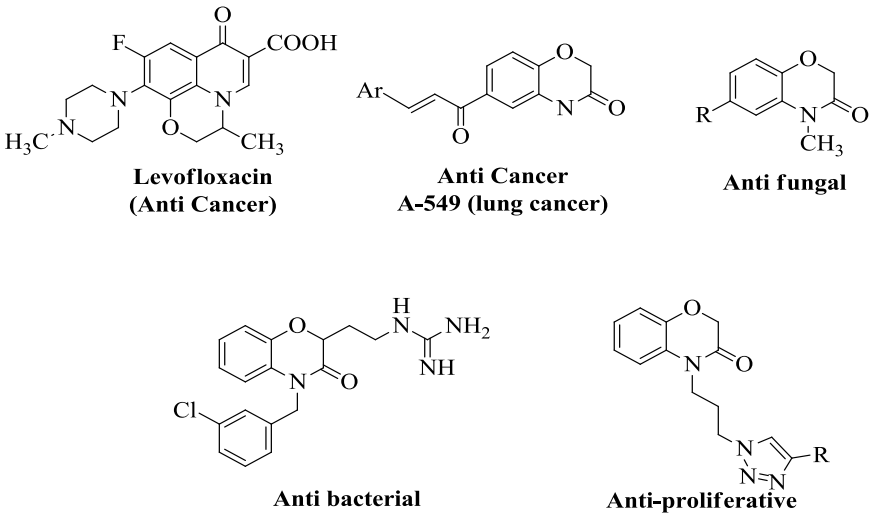


Fig. 1: Some biologically active 1,4-benzoxazine derivatives

Isoxazole and its derivatives have attracted much awareness because of their unique structure and applications.[14] The isoxazole ring system is a five-membered heterocyclic ring structure composed of nitrogen and oxygen atoms at the 1,2 positions and is used in the synthesis of pharmaceuticals.[15,16] The isoxazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the past few years, the therapeutic interest of isoxazole derivatives in the pharmaceutical and medicinal fields has been given great attention by medicinal chemist.[17,18] The synthesis of isoxazole derivatives is obviously an important assignment in modern medicinal chemistry research. Isoxazole is the basic moiety for several drugs, such as zonisamide (an anti-convulsant), leflunomide (a disease-modifying anti rheumatic drug, DMARD) and valdecoxib (a COX-2 inhibitor), **Fig. 2**.

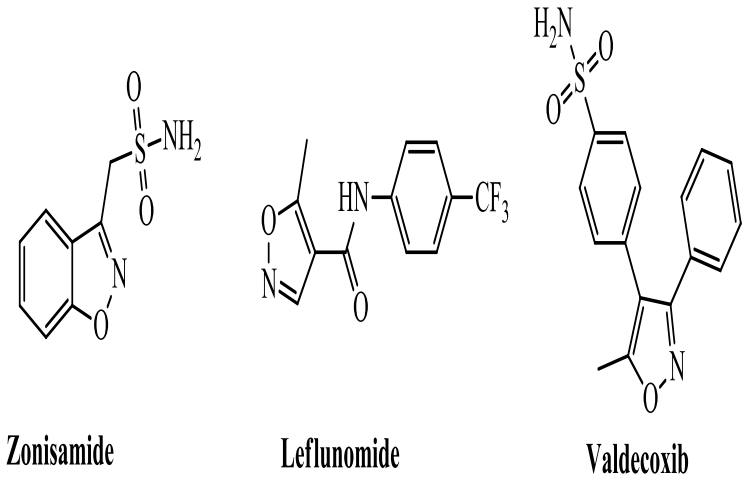
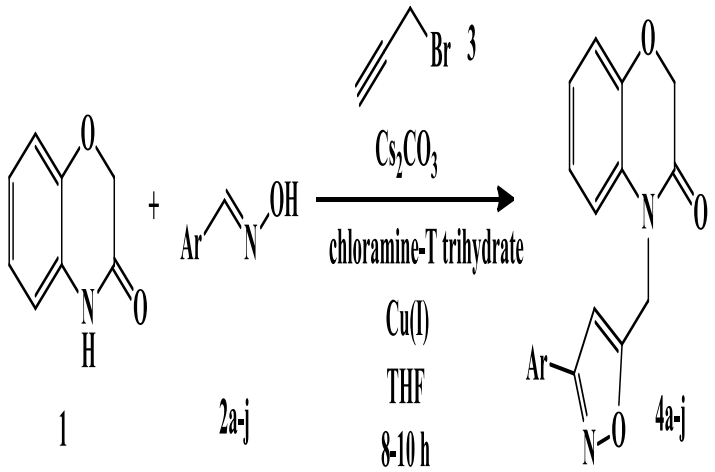


Fig. 2: Structures of isoxazole-congaing drugs

RESULTS AND DISCUSSION

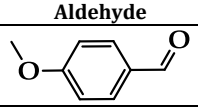
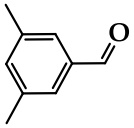
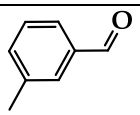
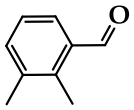
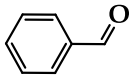
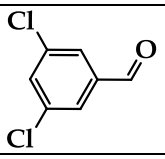
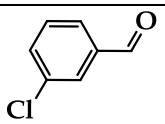
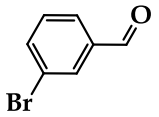
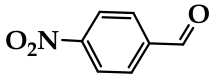
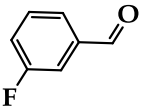
We described to synthesize 4-((3-(aryl) isoxazol-5-yl) methyl)-2H-benzo[b][1,4] oxazin-3(4H)-one scaffold as outlined in **Scheme I**. A series of 3,5-disubstituted isoxazoles (**4a-j**) were synthesized by employing Cu(I)-catalyzed cyclization between in situ generated nitrile oxide and the terminal alkyne as shown in Scheme 1. The aldehydes were converted to the corresponding aldoximes using hydroxylammonium chloride and 1M NaOH in t-BuOH:H_2O at room temperature. These aldoximes were converted to the corresponding nitrile oxide using chloramine-T trihydrate.[19] The *in situ* generated nitrile oxide, commercially available 2H-benzo[b][1,4]oxazin-3(4H)-one (**1**) and propargyl bromide in the presence of copper(I) catalyst and Cs_2CO_3 at room temperature yielded 3,5-disubstitued isoxazoles (**4a-j**) in good to excellent yields, **Table 1**.[20]



Scheme I: Synthesis of 1,4-benzoxazine containing isoxazole derivatives

The structures of the newly synthesized compounds **4a-j** were confirmed by analytical and spectral data (^{1}H-NMR, ^{13}C-NMR, IR, ESI-MS) and elemental (CHN) analysis. All the spectral and analytical data of the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound **4a**. From the IR spectrum, the appearance of a broad absorption band at 3098 cm^{-1}, sharp bands at 1680 and 1588 cm^{-1} are described with respect to –Ar-H, -C=O, and–C=N stretching frequencies, respectively. From the ^{1}H-NMR spectrum, the signals appearance at δ 8.24 – 7.00 (m, 8H, Ar-H), δ 6.79 (s, 1H, Isoxazole-H), δ 5.35 (s, 2H, N-C***H₂***), δ 4.23 (s, 2H, O-C***H₂***), δ 3.80 (s, 3H, O-C***H₃***), and from the ^{13}C NMR, the presence of signals at 162.54 ppm (O–*C*=C), 160.65 ppm (C=O), 158.15 ppm (***C***–OCH$_3$), 57.21 ppm (–O***C***H$_2$), 55.50 ppm (–O***C***H$_3$), and 40.09 ppm (N-*C*H$_2$), and the ESI-mass spectra of **4a** showed[M+1] ion peak at m/z 337, which confirmed the structure of compound **4a**. The elemental analyses (CHN) data (C, 67.81; H, 4.72; N, 8.39) confirmed the purity of compound **4a**.

Table 1: Synthesized 3,5-disubstituted isoxazoles 4a-j

S.No	Aldehyde	Product	Time(h)	Yield(%)
1		4a	10	71
2		4b	12	69
3		4c	12	72
4		4d	12	66
5		4e	9	78
6		4f	8	82
7		4g	9	80
8		4h	10	75
9		4i	8	88
10		4j	8	81

Antibacterial activity

The antibacterial screening results (Table II) revealed that some of the synthesized compounds exhibited good to moderate inhibition against the tested bacterial strains. Compounds bearing 3-flourophenyl group (**4j**) on the isoxazole core registered prominent inhibition against all the tested Gram-positive and Gram-negative microorganisms with MIC values ranging from 3.12 to 12.5 µg mL^{-1}, as compared with the standard drug streptomycin. Compounds possessing 3,5-dichlorophenyl (**4f**) and 3-chlorophenyl (**4g**) groups on the isoxazole core registered good inhibition against tested bacterial strains, with MIC values ranging from 6.25 to 50 μg mL^{-1}. Remaining compounds have shown moderate to poor activity as compared with standard drug.

Table II: *In vitro* antibacterial activity data of the synthesized compounds as *MIC* / μg mL^{-1}

Compound	*B.subtilis*	*S.aureus*	*E.coli*	*K.pneumoniae*
4a	>100	>100	>100	>100
4b	**12.5**	>100	>100	>100
4c	>100	>100	>100	>100
4d	>100	>100	50	>100
4e	>100	>100	50	>100
4f	**6.25**	50	50	**6.25**
4g	**12.5**	50	**12.5**	50
4h	>100	>100	>100	>100
4i	50	>100	>100	>100
4j	**3.12**	**12.5**	**12.5**	**6.25**
Streptomycin	**6.25**	**6.25**	**6.25**	**3.12**

In vitro antibacterial activity

All the synthesized compounds (**4a–4j**) were examined for their *in vitro* antibacterial activity against Gram-positive organisms, *i.e.*, *Bacillus subtilis*, *Staphylococcus aureus,* Gram-negative organisms, *i.e.*, *Escherichia coli*, and *Klebsiella pneumoniae*, using the broth dilution method.[21,22] The antimicrobial activity was evaluated in terms of the minimum inhibitory concentration (MIC) value (which corresponds to the lowest concentration that inhibits visible microbial growth) by the broth dilution method recommended by the National Committee for Clinical Laboratory (NCCL), standard protocol in liquid medium (nutrient agar) distributed in 96-well plates. The test compounds were dissolved in dimethylformamide (DMF) and further dilutions were made at the required concentrations of 300, 150, 75, 37.5, 18.75, 9.75, 6.25, 3.125 and 1.56 μg mL^{-1}. Streptomycin used as reference standards for the antibacterial activity.

Experimental Section

Melting points were determined in open capillaries using Stuart SMP30 apparatus and are uncorrected. NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d_6 (^{1}H-NMR &^{13}C-NMR) as solvent and TMS as internal standard. Elemental analyses were performed on a Perkin-Elmer 240CHN analyser. FTIR spectra were recorded on a Bruker spectrometer, Mass spectra (ESI-MS spectrum) were recorded. Coupling constants (*J*) values are presented in Hertz and spin multiples are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublet) and m (multiplet).

Experimental procedure for synthesis of 3,5-disubstituted isoxazoles (16a-j)

Substituted benzaldehyde (500 mg, 3.67 mmol) was added to a solution of hydroxylamine hydrochloride (4.5 mmol) in 10 mL of 1:1 *t*-BuOH:H_2O. To this was added NaOH (4.00 mmol), and after stirring for 30 min at ambient temperature, TLC analysis indicated that the oxime formation was complete. Chloramine-T trihydrate (5.00 mmol) was added in small portions over 10 min, followed by CuI (0.233 mmol). 1,4-benzoxazine (4.00 mmol), propargyl bromide (5.00 mmol) and Cs_2CO_3 was added, and stirring was continued for a further 10 h. The reaction mixture was poured into cold water (50 mL), and 5 mL of dilute NH_4OH was added to remove all copper salts. Isoxazole **4a-j** wes collected by filtration, re dissolved, and passed through a short plug of silica gel (ethyl acetate: hexanes 1:6) affording of 4-((3-(aryl)isoxazol-5-yl)methyl)-2H-benzo[*b*][1,4] oxazin-3(4H)-ones as good yields.

4-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4a)

Pale-yellow solid; M.p.: 211-213 ^oC; IR (KBr) ν_{max} (cm^{-1}): 3098 (Ar-H), 1680 (C=O), 1588 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J*=8 Hz, 1H), 7.99 (d, *J*=8 Hz, 1H), 7.75 (t, *J*=4 Hz, 1H),

7.64 (d, *J*=8 Hz, 2H), 7.38 (t, *J*=4 Hz, 1H), 7.00 (d, *J*=8 Hz, 2H), 6.76 (s, isoxazole-CH, 1H), 5.35 (s, 2H, N-C***H₂***), 4.23 (s, 2H, O-C***H₂***), 3.80 (s, 3H, O-C***H₃***); ^{13}C-NMR(100 MHz, $CDCl_3$): δ 162.54, 160.65, 158.15, 144.71, 142.25, 134.72, 132.28, 128.68, 126.46, 124.21, 120.50, 118.31, 116.37, 113.81, 98.33, 57.21, 55.50, 40.09; MS (ESI) *m/z*: 337 [M+H]. Anal. Cal for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.79; N, 8.33; found: C, 67.81; H, 4.72; N, 8.39.

4-((3-(3,5-dimethylphenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4b)

Pale-yellow solid; M.p.: 194-196 °C; IR (KBr) ν_{max} (cm^{-1}): 3102(Ar-H), 1675(C=O), 1576(C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (t, *J* =8.0 Hz, 1H), 7.96 (dd, *J* =4.0, 4.0 Hz, 1H), 7.74 (t, *J* =8.0 Hz, 1H), 7.36 - 7.25 (m, 3H), 7.06 (s, 1H), 6.70 (s, isoxazole-CH, 1H), 5.35 (s, 2H, N-C***H₂***), 4.26 (s, 2H ,SO_2-C***H₂***), 2.38 (s, 6H, Ar-C***H₃***); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 162.32, 160.34, 143.18, 140.64, 137.73, 135.70, 128.72, 125.79, 121.66, 119.05, 117.73, 117.66, 98.67, 56.97, 40.58, 21.13; MS (ESI) *m/z*: 334 [M+H]; Anal. Cal for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38; found: C, 71.81; H, 5.48; N, 8.35.

4-((3-(m-tolyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4c)

Pale-yellow solid; M.p.: 177-179 °C; IR (KBr) ν_{max} (cm^{-1}): 3087 (Ar-H), 1673 (C=O), 1567 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18-8.13 (m, 1H), 8.00 -7.92 (m, 1H), 7.75 (t, *J* =8.0 Hz, 1H), 7.43- 7.27 (m, 5H), 6.71 (s, isoxazole-CH, 1H), 5.39 (s, 2H, N-C***H₂***), 4.25 (s, 2H, SO_2-C***H₂***), 2.17 (s, 3H, Ar-C***H₃***); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 161.98, 160.33, 143.53, 140.38, 137.38, 135.26, 133.45, 130.55, 129.09, 127.37, 125.87, 124.84, 123.33, 118.98, 98.32, 57.36, 40.38, 18.46; ESI-MS m/z: 321 [M+H]; Anal. Cal for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74; found: C, 71.20; H, 5.07; N, 8.79.

4-((3-(2,3-dimethylphenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4d)

Yellow solid; M.p.: 188-190 °C; IR (KBr) ν_{max} (cm^{-1}): 3027 (Ar-H), 1670 (C=O), 1575 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* =8.0 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.75 (t, *J* = 8 Hz, 1H), 7.37 (t, *J* =8 Hz, 1H), 7.31 (d, *J* =4.0 Hz, 1H), 7.20 (t, *J*=8 Hz, 1H), 7.11 (d, *J* =8.0 Hz, 1H), 6.77 (s, isoxazole-CH, 1H), 5.39 (s, 2H, N-C***H₂***), 4.25 (s, 2H, SO_2-C***H₂***), 2.35 (s, 3H, Ar-C***H₃***), 1.96 (s, 3H, Ar-C***H₃***); ^{13}C-NMR(100 MHz, $CDCl_3$): δ 161.96, 160.98, 139.88, 137.38, 134.39, 131.51, 127.25, 125.15, 123.88, 123.37, 122.81, 119.01, 117.80, 98.60, 57.27, 40.83, 20.39, 15.26; MS (ESI) *m/z*: 335 [M+H]; Anal. Cal for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38; found: C, 71.78; H, 5.47; N, 8.32.

4-((3-phenylisoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4e)

White solid; M.p.: 154-156 °C; IR (KBr) ν_{max} (cm^{-1}): 3091 (Ar-H), 1676 (C=O), 1578 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.00-7.92 (m, 1H), 7.78-7.67 (m, 3H), 7.57-7.49 (m, 2H), 7.47-7.40 (m, 1H), 7.38-7.30 (m, 1H), 6.71(s, isoxazole-CH, 1H), 5.37 (s, 2H, N-C***H₂***), 4.26 (s, 2H, SO_2-C***H₂***); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 162.33, 160.34, 143.08, 139.45, 137.57, 136.49, 130.12, 126.72, 124.88, 123.02, 120.29, 117.80, 114.26, 98.65, 57.16, 40.12; MS (ESI) *m/z*: 307 [M+H]; Anal. Cal for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15; found: C, 70.52; H, 4.67; N, 9.11.

4-((3-(3,5-dichlorophenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4f)

Yellow solid; M.p.: 219-221 °C; IR (KBr) ν_{max} (cm^{-1}): 3109 (Ar-H), 1679 (C=O), 1574 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, *J* =8 Hz, 1H), 7.98 (d, *J* =8 Hz, 1H), 7.74 (t, *J*=8 Hz, 1H), 7.67 (s, 2H), 7.43 (s, 1H), 7.39 (m, 1H), 6.82 (s, isoxazole-CH, 1H), 5.36 (s, 2H, N-C***H₂***), 4.26 (s, 2H ,SO_2-C***H₂***); ^{13}C-NMR(100 MHz, $CDCl_3$): δ 162.88, 160.87, 144.17, 137.70, 136.05, 135.25, 127.77, 127.22, 126.54, 123.36, 121.11, 117.63, 117.57, 57.21, 40.71; MS (ESI) *m/z*: 376 [M+H]. Anal. Cal for $C_{18}H_{12}Cl_2N_2O_3$: C, 57.62; H, 3.22; N, 7.47; found: C, 57.57; H, 3.20; N, 7.53.

4-((3-(3-chlorophenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4g)

Yellow solid; M.p.: 183-185 °C; IR (KBr) ν_{max} (cm^{-1}): 3092 (Ar-H), 1683 (C=O), 1581 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12-8.17 (m, 1H), 7.98 (s, 1H), 7.92-7.84 (m, 1H), 7.78-7.72 (m, 1H), 7.44-7.28 (m, 4H), 6.80 (s, isoxazole-CH, 1H), 5.33 (s, 2H, N-C***H₂***), 4.25 (s, 2H, SO_2-C***H₂***); ^{13}C-NMR (100 MHz, $CDCl_3$):δ 162.32, 160.69, 144.82, 140.55, 137.35, 135.44, 134.15, 131.60, 128.47, 127.89, 125.27, 121.90, 119.30, 118.58, 117.35, 98.77, 57.12, 40.33; MS (ESI) *m/z*: 341 [M+H]; Anal. Cal for $C_{18}H_{13}ClN_2O_3$: C, 63.44; H, 3.85; N, 8.22; found C, 63.39; H, 3.80; N, 8.18.

4-((3-(3-bromophenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one(4h)

Yellow solid; M.p.: 192-194 °C; IR (KBr) ν_{max} (cm^{-1}): 3081 (Ar-H), 1677 (C=O), 1573 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (brs, 1H), 8.00-7.92 (m, 1H), 7.78-7.70 (m, 1H), 7.76-7.56 (m, 4H), 7.40-7.30 (m, 1H), 6.76 (s, isoxazole-CH, 1H), 5.37 (s, 2H, N-C***H₂***), 4.25 (s, 2H, SO_2-C***H₂***);

^{13}C-NMR (100 MHz, $CDCl_3$): δ 161.97, 160.70, 144.75, 142.75, 137.10, 132.47, 127.88, 125.93, 123.11, 122.23, 119.31, 117.37, 114.63, 98.66, 57.07, 40.22; MS (ESI) *m/z*: 385 [M+2H]; Anal. Cal for $C_{18}H_{13}BrN_2O_3$: C, 56.12; H, 3.40; N, 7.27; found: C, 56.12; H, 3.40; N, 7.27.

4-((3-(4-nitrophenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4i)

Pale-red yellow solid; M.p.: 178-180 ^oC; IR (KBr) ν_{max} (cm^{-1}): 3104(Ar-H), 1682(C=O), 1559 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 - 7.90 (m, 3H), 7.83 (brs, 2H), 7.77 - 7.68 (m, 2H), 7.42-7.30 (m, 1H), 6.83 (s, isoxazole-CH, 1H), 5.35 (s, 2H, N-C***H₂***), 4.31 (s, 2H ,SO_2-C***H₂***); MS (ESI) *m/z*: 352 [M+H]; Anal. Cal for $C_{18}H_{13}N_3O_5$: C, 51.18; H, 3.10; N, 13.26; found: C, 51.11; H, 3.17; N, 13.33.

4-((3-(3-fluorophenyl)isoxazol-5-yl)methyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (4j)

Red solid; M.p.: 168-170 ^oC; IR (KBr) ν_{max} (cm^{-1}): 3108(Ar-H), 1691 (C=O), 1584 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17-8.11 (m, 1H), 7.96-7.88 (m, 2H), 7.78-7.72 (m, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.32-7.27 (m, 1H), 7.25-7.20 (m, 1H), 7.18-7.10 (m, 1H), 6.81 (s, isoxazole-CH, 1H), 5.38 (s, 2H, N-C***H₂***), 4.26 (s, 2H, SO_2-C***H₂***); MS (ESI) *m/z*: 325 [M+H]; Anal. Cal for $C_{18}H_{13}FN_2O_3$: C, 66.66; H, 4.04; N, 8.64; found: C, 66.61; H, 4.07; N, 8.58.

CONCLUSION

In conclusion, a new series of novel 4-((3-(aryl) isoxazol-5-yl) methyl)-2H-benzo[b][1,4] oxazin-3(4H)-one derivatives were synthesized by the Cu(I)-catalyzed reaction of *in situ* generated nitrile oxides with *in situ* generated N-propargyl 1,4-benzoxazine in good yields. The final 3,5-disubstituted isoxazoles were characterized by using NMR, IR, Mass and CHN-analysis. Compounds **4j**, **4f** and **4g** have shown good antibacterial activity against tested bacterial strains. The biological activity of these compounds suggests that the synthesized compounds could be good candidates for future investigations.

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