Synthesis and Antimicrobial Activity of Isoxazole tagged Benzo[*d*]oxazole-2-thiol/Oxazolo[4,5-*b*]pyridine-2-thiol Derivatives

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ABSTRACT *A new series of isoxazole derivatives of mercaptobenzoxazole and oxazolo[4,5-b]pyridine-2-thiol derivatives **7a-7j** were synthesized and evaluated for their antimicrobial activity. The synthesis of isoxazole derivative has been achieved by the sequential reaction of 2-aminophenol/2-aminopyridin-3-ol with CS_2, followed by alkylation and further 1,3-dipolar cycloaddition reaction of nitrile oxide generated from various appropriate aromatic aldoximes with propargylated mercaptobenzoxazole and oxazolo[4,5-b]pyridine-2-thiol. All the synthesized compounds were evaluated for their antimicrobial activity viz. E. coli, P. Aeruginosa, S. Aureus and S. Pyogenus and three pathogenic fungi viz. C. Albicants, A. Aniger and A. Clavatus and promising compounds were identified.*

Keywords: *1,3-dipolar cycloaddition, Isoxazole derivatives, Benzo[d]oxazole-2-thiol; oxazolo[4,5-b]pyridine-2-thiol, antibacterial activity, antifungal activity.*

INTRODUCTION

Recently heterocyclic chemistry is an important tool for the development of new active compound with a number of potential applications. In particular, benzoxazoles and its derivatives[1] exhibit great attention in the field of medicinal chemistry due their important biological properties such as melatonin receptor agonists[2], COX inhibitor[3], anticancer agents[4], 55-HT_3 receptor antagonists[5] and HIV-1 reverse transcriptse inhibitors[6]. Interestingly, 2-mercaptobenzoxazole is a thiol derivative of benzoxazole moiety which exists in tautomeric forms of thiol and thione.[7] The extraordinary therapeutic properties of 2-mercaptobenzoxazole cause them as target compounds in organic synthesis and drug discovery[8].

Oxygen and nitrogen containing isoxazole is most interesting five member heterocyclic rings that present in numerous pharmaceuticals, natural products and chiral ligand.[9] A widely used isoxazole ring generally constructed *via* 1,3-dipolar cycloaddition of alkenes and alkynes with *in-situ* generated nitrile oxides by aldoximes.[10] Isoxazole derivatives are an important class of heterocyclic compounds having remarkable pharmaceutical applications such as antibacterial, antiviral, anticancer, antiplatelet, analgesic, antinociceptive, anticonvulsant, COX-2 inhibitory and immune modulating activities.[11] Naturally occurring Ibotenic acid **1** (Fig. 1) also know ibotenate is psychoactive drug, act as a non-selective glutamate receptor agonist and serve as brain-lesioning agent, Muscimol **2** (Fig. 1) is also another psychoactive drug and known for potent, selective agonist for $GABA_A$ receptor and shows sedative hypnotic, depressant and deliriant psychoactivity. Commercially available drugs like Valdecoxib **3** (Fig. 1) is a nonsteroidal anti-inflammatory drug used for the treatment of rheumatoid, osteoarthritis, arthritis and painful menstruation, Leflunomide **4** (Fig. 1) is trifluoromethyl substituted isoxazole drug used as antirheumatoid and psoriatic arthritis. Pyridine containing isoxazole compound **5** (Fig. 1) known for nicotinic receptor modulator. Cycloserine **6** (Fig. 1) is an antibiotic used to treat tuberculosis bacteria[12] and have been demonstrated to be versatile building blocks in organic synthesis. In belief of medicinal chemistry, the finding focused on synthesis of oxazolopyridine-2-thiol derivatives as this nucleus contain pyridine fragment and might offer some advantages over 2-mercaptobenzoxazole moiety. Water solubility, site for protonation and salt formation are the key benefit of pyridine fragment, which might enhance the interaction with targeted protein *via* hydrogen bonding and may help in modulating the physical and biological property of the molecule.

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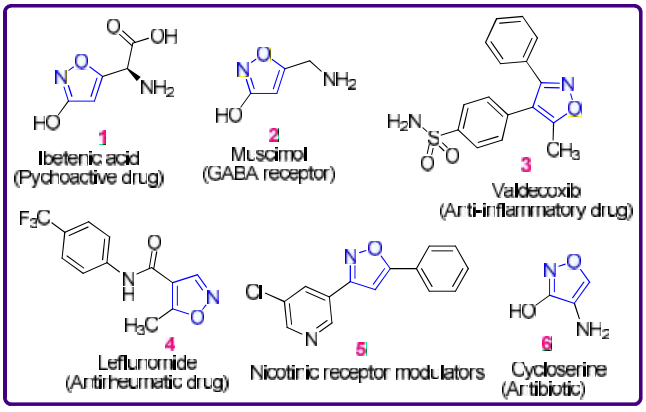


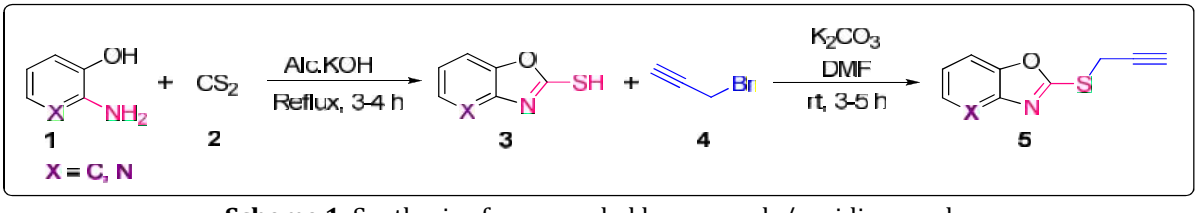
Fig. 1. Bioactive compound containing isoxazole ring.

Therefore, it was anticipated that chemical entities with isoxazole moiety with oxazolopyridine-2-thiol scaffold in single framework would result in improved biological activities. In view of these verdicts, we have attempted to tagged isoxazole moiety with mercaptobenzoxazole and oxazolo[4,5-*b*]pyridine-2-thiol derivatives to evaluate its antimicrobial activity. Hence, here we disclose the synthesis of isoxazole derivative of mercaptobenzoxazole and oxazolo[4,5-*b*]pyridine-2-thiol scaffolds and their anti-microbial activity.

RESULS AND DISCUSSION

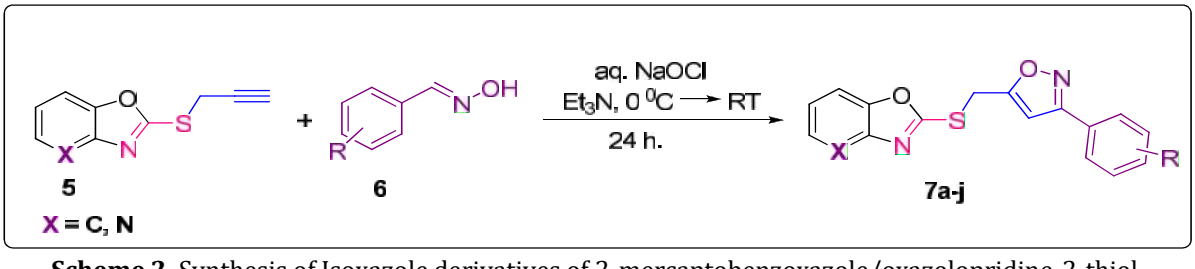
Chemistry

All the present isoxazole were synthesized from appropriate aromatic aldoximes **6** and alkynes **5** as shown in Scheme 2 as per the reported method.[13] First we synthesized the compounds benzo[*d*]oxazole-2-thiol/oxazolo[4,5-*b*]pyridine-2-thiol **3** *via* reactions of 2-aminophenol/2-aminopyridin-3-ol **1** with carbon disulphide **2** using alcoholic potassium hydroxide in water under refluxed condition, affording the respective product in good yield (Scheme 1)[14,15]. The structure of compound **3** was confirmed on the basis of Mass spectrometry. It shows *m/z* value at 152 $[M+H]^+$ for benzoxazole-2-thiol and *m/z* 153 $[M+H]^+$ for oxazolopyridine-2-thiol.



Scheme 1. Synthesis of propargyled benzoxazole/pyridinoxazole.

Compound **3** on reaction with propargyl bromide using two equivalent of inorganic base potassium carbonate in *N*,*N*-dimethyl formamide at ambient temperature resulted in the formation of 2-(prop-2-ynylthio)oxazolo[4,5-*b*]pyridine/ 2-(prop-2-ynylthio)benzo[*d*]oxazole **5** (Scheme 1). The propargylated compound of benzoxazole-2-thiol and oxazolo pyridine-2-thiol were confirmed by ^{1}H NMR spectroscopy which shows signal at 4.09 and 4.17 ppm for two proton of CH_2 which consistent with compound **5**. Further reactions of compound 5 with various aromatic aldoximes dissolved in DCM, Et_3N was added drop wise to a dichloromethane/aqueous sodium hypochlorite biphasic mixture containing excess of the akyne dipolarophile. The in situ generated nitrile oxide from aromatic aldoxime undergo 1,3-dipolar cycloaddition to afford isoxazole tagged 2-mercaptobenzoxazole/oxazole pyridinethiaol derivatives **7a-j** (Scheme 2).The reaction conditions were compatible for various functionalities as shown in Table 1.

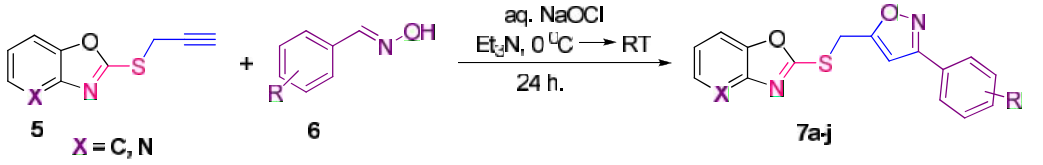


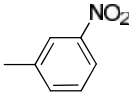
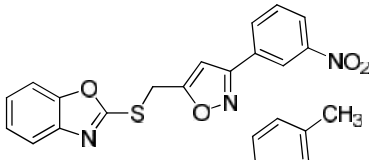
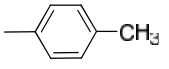
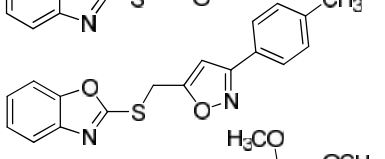
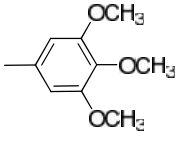
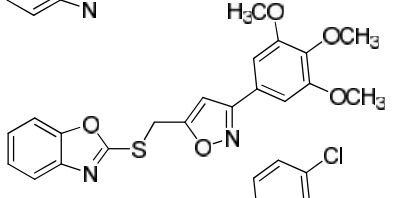
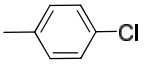
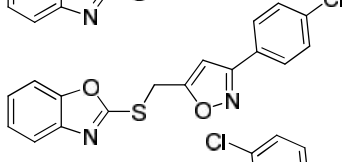
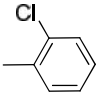
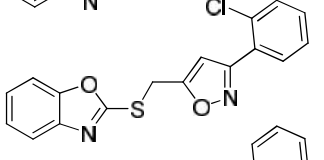
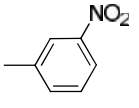
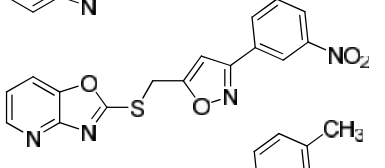
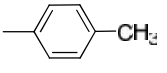
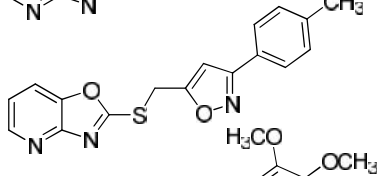
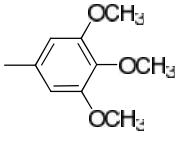
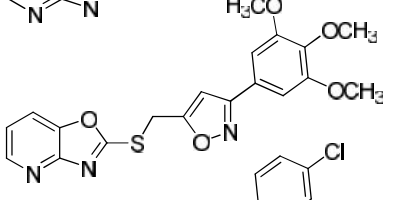
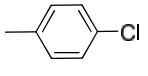
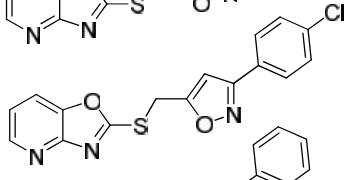
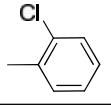
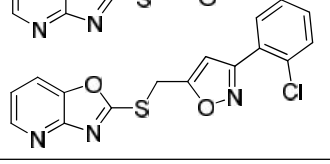
Scheme 2. Synthesis of Isoxazole derivatives of 2-mercaptobenzoxazole/oxazolopridine-2-thiol.

All the isoxazole derivatives were characterized by ^{1}H and ^{13}C NMR spectroscopy which show isoxazole proton in the range 6.28-6.35 ppm for various derivatives and C-atom at range 100.19-103.12 ppm in the ^{13}C NMR.

All the synthesized isoxazole linked 2-mercaptobenzoxazole/oxazole pyridine-2-thiaol derivatives were evaluated for their antimicrobial activities.

Table 1. Isoxazole derivative of 2-mercaptobenzoxazole/oxazole pyridine-2-thiaol.



S.No.	R	X	Product	Yield (%)
7a		C		70
7b		C		74
7c		C		76
7d		C		85
7e		C		78
7f		N		80
7g		N		75
7h		N		78
7i		N		80
7j		N		74

BIOLOGICAL ACTIVITY

Antimicrobial activity

The compounds **7a-j** were screened for their antibacterial activity against *E. coli, P. aeruginosa, S. aureus* and S*. pyogenus and* antifungal activity against *C.albicans, A. niger* and *A. clavatus* by Broth Dilution Method at different concentrations using DMSO as solvent. The serial dilution was prepared in primary and secondary screening. In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized drugs were taken. The synthesized drugs which were found active in this primary screening were further examined in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted for secondary screening to obtain 200 micro/ml 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml concentrations. The control tube was instantly sub cultured [before inoculation] which does not contains antibiotic by spreading a loopful uniformly over a quarter of plate of medium appropriate for the development of the test organism and put for incubation at 37 ^{0}C overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism is recorded as the MIC. The zone of inhibition of the growth was measured. The activity was compared with the standard drugs. A commercial antibacterial Ampiciline, Chloramphenicol, and antifungal Griseofulvin were also tested under similar conditions for comparison. The results are presented in Tables 2 and 3.

Antibacterial activity

The minimum inhibitory concentrations (MIC) of various synthetic compounds were screened against three representative gram-negative organisms, *viz. Escherichia coli* (MTCC443) and *Pseudomonas aeruginosa* (MTCC441) and gram-positive microorganisms, *viz. Staphylococcus aureus* (MTCC96) and *Streptococcus pyogenes* (MTCC442). The assays were performed by broth dilution techniques. Standard antibacterial agents such as ampiciline, chloramphenicol and ciprofloxin were also screened under similar conditions for comparison. The minimum inhibitory concentration (MIC) values are display in Table 2.

All compounds evaluated for their *in vitro* antibacterial activity. These results clearly indicate that compounds **7a–j** displayed significant activity with a high degree of variation against Ampiciline. Based on the results, 80% of the compounds showed considerable high activity against gram-positive *Staphylococcus aureus* (MTCC96) bacterial strains. Compounds **7a**, **7b**, **7h**, **7i** and **7j** shows high activity against Staphylococcus *aureus* (MTCC96) than standard drug Ampiciline. Compound **7e** only display comparatively moderate activity against gram-positive *S. pyogenus* antibacterial strain. In gram-negative bacterial strains, all the compounds show considerable activity against *E. coli* and *P. aeruginosa*. Among the synthesized series, Compounds **7e** comparable activity against *E. coli* with standard drug Ampiciline, while compound **7d** and **7h** demonstrate good activity against *P. aeruginosa*. Comparison with MIC value of tested compound and standard drug, gram-positive bacterial strain show more sensitivity than gram-negative bacterial strains. Among the synthesized series, compounds **7a**, **7e**, **7h** and **7j** are found to be most active candidates as antibacterial agents. However, considering the MIC values of the standard molecule, the order of sensitivity of these bacterial strain toward isoxazole linked 2-mercaptobenzoxazole/oxazole pyridine-2-thiaol derivatives is *S. aureus* > *S. pyogenus* > *P. aeruginosa* > *E. coli*.

Table 2. Antibacterial activity of compounds **7a-7j**

Compound code	MIC (microgram/ml)			
	E. coli MTCC 443	***P. aeruginosa MTCC 441***	***S. aureus MTCC 96***	***S. pyogenus MTCC 442***
7a	200	200	100	200
7b	125	250	200	250
7c	250	250	250	500
7d	200	100	250	250
7e	100	125	250	100
7f	250	250	500	250
7g	250	200	500	250
7h	125	62.5	200	200
7i	125	250	200	200
7j	200	200	125	200
Ampiciline	100	--	250	100
Chloramphenicol	50	50	50	50

MIC values are given in (μg/ml) = Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit bacterial growth

Antifungal activity

In vitro antifungal activity of the newly synthesized compounds were studied against the fungal strains, *Candida albicans* (MTCC 227), *Aspergillus niger (MTCC282)* and *Aspergillus clavatus (MTCC 1323)* using Greseofulvin as a standard antifungal drugs as shown in Table 3. The antifungal screening data of compounds **7a-j** revealed that compound **7a**, **7f**, **7g** and **7j** shows higher antifungal activity against *C. albicans* antifungal strain than Greseofulvin as standard drug, where as compounds **7b, 7c, 7h** and **7i** have equipotent activity with Greseofulvin standard drug aginst *C. albicans* antifungal strain. Compound **7b, 7e** and **7h** show moderate activity against A. niger and *A. clavatus* antifungal strains. Compounds **7d** and **7e** had lower activity in *C. albicans* strain, while compounds **7a**, **7c**, **7d**, **7f**, **7g**, **7i** and **7j** display inferior activity against *A*. niger and *A. clavatus* antifungal strains. Among entire synthesized series compounds **7a**, **7f**, **7g** and **7j** are found to be potent candidate as antifungal agents. Based on the results *C. albicans* strain displayed more sensitivity than other fungal strains with synthesized compounds. Considering the MIC values of the standard drug, the order of sensitivity of these fungal strain toward synthesized compound is *C. albicans* > A. niger > *A. clavatus*.

According to the structure–activity relationship studies, the oxazole pyridine-2-thiaol derivative of isoxazole exhibited comparatively greater antimicrobial activity than isoxazole linked 2-mercaptobenzoxazole derivatives. In particular, compounds with substituent NO_2, and Cl on isoxazole ring displayed enhanced antimicrobial activity.

Table 3. Antifungal activity compound **7a-7j**

Compound code	MIC (microgram/ml)		
	C. albicans MTCC 227	***A. niger MTCC 282***	***A. clavatus MTCC 1323***
7a	250	1000	1000
7b	500	250	500
7c	500	>1000	>1000
7d	>1000	>1000	>1000
7e	1000	500	500
7f	250	1000	1000
7g	250	1000	1000
7h	500	500	500
7i	500	>1000	>1000
7j	250	>1000	>1000
Greseofulvin	500	100	100

MIC values are given in (μg/ml) = Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit fungal growth

Experimental

Materials and methods

All chemicals and reagents were purchased from commercially available source and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C spectra were recorded Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm down field from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI^{+} software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined by an open capillary tube melting point apparatus and were uncorrected.

General procedures

Preparation of benzooxazole/pyridine oxazole (**3**)

2-aminophenol/3-hydroxy-2-aminopyridine **1** (4.5 mmol), carbon disulphide **2** (4 ml) and potassium hydroxide (5.35 mmol) were taken in a 100ml round bottom flask and refluxed in 10ml of 95% ethanol for 7-8 hours. The reaction mixture was cooled to room temperature and concentrated. The 1M aqueous HCl solution was added to this concentrated reaction mixture.

The product was filtered and washed with water (2 x 10 ml) and air dried. the dried product was recrystallized with ethanol.

Preparation of 2-(prop-2-ynylthio)benzo[d]oxazole / 2-(prop-2-ynylthio)oxazolo[4,5-b] pyridine (5)

benzo[*d*]oxazole-2-thiol/oxazolo[4,5-*b*]pyridine-2-thiol **3** (3.0 mmol) was dissolved in dry *N*,*N*-dimethylformamide (10 ml), and K_2CO_3 (6.0 mmol) was added and the reaction mixture was stirred for 25 min at room temperature. The propargyl bromide **4** (3.6 mmol) was added slowly drop wise to the above mixture over a period of 25 min and continued stirring for 4 h. after completion of reaction confirmed by TLC, The reaction was quenched with water and extracted with EtOAc (3 × 20 ml). The combined extracts were washed with water (3 × 25 ml) and brine (20 ml). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Purification by silica gel chromatography (10 % ethyl acetate in hexane) gave the desired product.

Synthesis of Isoxazole linked 2-mercaptobenzoxazole/oxazole pyridine-2-thiaol derivatives (7a-j)

To a mixture of dipolarophile **5** (1mmol) and Et_3N (1mmol) in CH_2Cl_2 were added, a 9-12% aqueous solution of NaOCl (1.6 mmol) dropwise over a period of 1 hr at 0 ^{0}C and the appropriate oxime **6** (1 mmol) in CH_2Cl_2. After being stirred at room temperature for 24 hrs, CH_2Cl_2 was removed under reduced pressure, water was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. This was further purified by column chromatography using ethyl acetate: n-hexane, as eluent to obtain the pure product.

2-((3-(3-nitrophenyl)isoxazol-5-yl)methylthio)benzo[d]oxazole (7a)

Yield 70%; m.p. 109-112^{0}C; IR (KBr): υ_{max} cm^{-1} 2931, 1605, 1538, 1263; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 2H), 6.28 (s, 1H, Isoxazole), 7.12 – 7.22 (m, 2H), 7.36 – 7.40 (m, 1H), 7.65 – 7.71 (m, 1H), 7.78 – 7.85 (m, 1H), 8.20-8.27 (m, 1H), 8.29-8.34 (m, 1H), 8.80(s, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.64, 101.14, 110.53, 121.45, 124.04, 124.54, 129.08, 132.20, 141.72, 150.13, 168.02; MS for $C_{17}H_{11}N_3O_4S$ (ESI): *m/z* 354 $[M+H]^+$.

2-((3-p-tolylisoxazol-5-yl)methylthio)benzo[d]oxazole (7b)

Yield 74%; m.p. 112-115^{0}C; IR (KBr): υ_{max} cm^{-1} 2929, 1538, 1457, 1262, 116; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H), 3.85 (s, 2H), 6.30 (s, 1H, Isoxazole), 6.97 – 7.07 (m, 2H), 7.09 – 7.18 (m, 2H), 7.36-7.50 (m, 2H), 7.72-7.83 (m, 2H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 21.63, 34.50, 100.25, 110.34, 120.55, 123.14, 125.75, 128.11, 129.97, 141.80, 148.82, 156.07, 167.21; MS for $C_{18}H_{14}N_2O_2S$ (ESI): *m/z* 323 $[M+H]^+$.

2-((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methylthio)benzo[d]oxazole (7c)

Yield 76%; m.p. 129-131^{0}C; IR (KBr): υ_{max} cm^{-1} 2921, 1563, 1439, 1280, 1154; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 3H), 3.85 (s, 6H), 4.09 (s, 2H), 6.33 (s, 1H, Isoxazole), 6.91 (s, 2H), 7.10 – 7.28 (m, 2H), 7.41-7.50 (m, 1H), 7.69-7.76 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 31.93, 55.96, 59.94, 101.14, 104.10, 112.04, 120.21, 121.08, 123.40, 132.80, 139.08, 143.20, 153.15, 156.10, 162.02, 167.09; MS for $C_{20}H_{18}N_2O_5S$ (ESI): *m/z* 399 $[M+H]^+$.

2-((3-(4-chlorophenyl)isoxazol-5-yl)methylthio)benzo[d]oxazole (7d)

Yield 85%; m.p. 121-123^{0}C; IR (KBr): υ_{max} cm^{-1} 2989, 1619, 1588, 1484, 1248; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 2H), 6.33 (s, 1H, Isoxazole), 6.99 – 7.09 (m, 2H), 7.13 – 7.19 (m, 2H), 7.37-7.52 (m, 2H), 7.74-7.85 (m, 2H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.08, 101.55, 109.48, 121.76, 123.15, 127.73, 129.67, 136.45, 144.00, 156.75, 161.56, 167.89; MS for $C_{17}H_{11}ClN_2O_2S$ (ESI): *m/z* 343 $[M+H]^+$.

2-((3-(2-chlorophenyl)isoxazol-5-yl)methylthio)benzo[d]oxazole (7e)

Yield 78%; m.p. 112-114^{0}C; IR (KBr): υ_{max} cm^{-1} 2931, 1606, 1538, 1460, 1260; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 2H), 6.32 (s, 1H, Isoxazole), 6.95 – 7.05 (m, 2H), 7.10 – 7.19 (m, 1H), 7.29-7.36 (m, 1H), 7.57-7.64 (m, 2H), 7.68 – 7.78 (m, 2H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.10, 101.51, 109.50, 121.77, 127.74, 129.70, 136.47, 156.76, 161.58, 167.90; MS for $C_{17}H_{11}ClN_2O_2S$ (ESI): *m/z* 343 $[M+H]^+$.

2-((3-(3-nitrophenyl)isoxazol-5-yl)methylthio)oxazolo[4,5-b]pyridine (7f)

Yield 80%; m.p. 111-113^{0}C; IR (KBr): υ_{max} cm^{-1} 2937, 1606, 1537, 1263; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.12 (s, 2H), 6.35 (s, 1H, Isoxazole), 7.10 – 7.14 (m, 1H), 7.32 – 7.40 (m, 1H), 7.73 – 7.81 (m, 1H), 8.19 (d, 1H, *J* = 8.30 Hz), 8.25 (d, 1H, *J* = 8.30 Hz), 8.65 – 8.72 (m, 1H), 8.88 (s, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.91, 101.16, 118.62, 120.70, 121.41, 129.10, 132.21, 141.74, 148.54, 161.54; MS for $C_{16}H_{10}N_4O_4S$ (ESI): *m/z* 355 $[M+H]^+$.

2-((3-p-tolylisoxazol-5-yl)methylthio)oxazolo[4,5-b]pyridine (7g)

Yield 75%; m.p. 150-152^{0}C; IR (KBr): υ_{max} cm^{-1} 2924, 1575, 1437, 1250, 1177; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 3H), 3.90 (s, 2H), 6.31 (s, 1H, Isoxazole), 7.00 – 7.10 (m, 1H), 7.16 (d, 2H, *J* = 8.87 Hz), 7.63 (d, 2H, *J* = 8.87 Hz), 7.73-7.81 (m, 1H), 8.64 - .70 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 21.43, 34.85, 100.22, 120.52,

120.93, 125.72, 128.08, 129.94, 141.76, 148.80, 155.05, 158.13, 169.42; MS for $C_{17}H_{13}N_3O_2S$ (ESI): *m/z* 324 $[M+H]^+$.

2-((3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methylthio)oxazolo[4,5-b]pyridine (7h)

Yield 78%; m.p. 137-139^{0}C; IR (KBr): υ_{max} cm^{-1} 2932, 1585, 1447, 1244, 1127; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H), 3.85 (s, 6H), 4.00 (s, 2H), 6.30 (s, 1H, Isoxazole), 6.89 (s, 2H), 7.19 – 7.27 (m, 1H), 7.72-7.80 (m, 1H), 8.65 - 8.69 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 33.69, 56.20, 60.83, 100.19, 104.05, 120.52, 120.94, 125.75, 139.42, 148.83, 153.39, 157.32, 159.97, 169.89; MS for $C_{19}H_{17}N_3O_5S$ (ESI): *m/z* 400 $[M+H]^+$.

2-((3-(4-chlorophenyl)isoxazol-5-yl)methylthio)oxazolo[4,5-b]pyridine (7i)

Yield 80%; m.p. 130-132^{0}C; IR (KBr): υ_{max} cm^{-1} 2879, 1609, 1586, 1486, 1249, 1163; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.10 (s, 2H), 6.35 (s, 1H, Isoxazole), 7.03 – 7.10 (m, 1H), 7.30 (d, 2H, *J* = 8.91 Hz), 7.61 - 7.67 (m, 1H), 7.77 (d, 2H, *J* = 8.91 Hz), 8.65 – 8.72 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.74, 100.57, 120.47, 120.96, 125.67, 127.68, 128.95, 148.80, 159.95, 170.42; MS for $C_{16}H_{10}ClN_3O_2S$ (ESI): *m/z* 343 $[M+H]^+$.

2-((3-(2-chlorophenyl)isoxazol-5-yl)methylthio)oxazolo[4,5-b]pyridine (7j)

Yield 74%; m.p. 134-136^{0}C; IR (KBr): υ_{max} cm^{-1} 2930, 1608, 1532, 1458, 1252, 1172; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 2H), 6.30 (s, 1H, Isoxazole), 7.13 – 7.22 (m, 1H), 7.29-7.40 (m, 1H), 7.43-7.53 (m, 1H), 7.65 (d, 1H, *J* = 8.68 Hz), 7.83 – 8.00 (m, 2H), 8.64 – 8.73 (m, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): δ 34.78, 103.12, 122.18, 122.97, 129.04, 131.46, 135.80, 147.77, 160.21, 169.17; MS for $C_{16}H_{10}ClN_3O_2S$ (ESI): *m/z* 344 $[M+H]^+$.

CONCLUSION

A series of Isoxazole tagged of 2-mercaptobenzoxazole/oxazole pyridine-2-thiaol derivative have been synthesized through a facile strategy and screened for antimicrobial activity. The results of antibacterial screening reveal that compounds **7a**, **7e, 7h** and **7j** showed good inhibition toward bacteria strain with Ampiciline drug and compounds **7a, 7f, 7g** and **7j** exhibited higher inhibition towards fungal strain with Greseofulvin drug. *S. aureus* antibacterial strain shows more sensitivity toward synthesized compound while in antifungal strain, *C. albicans* is found to be most sensitive. Among the synthesized series, oxazole pyridine-2-thiaol derivative of isoxazole exhibited comparatively greater antimicrobial activity than isoxazole linked 2-mercaptobenzoxazole derivatives.

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Many of life's failures are experienced by people who did not realize how close they were to success when they gave up.

~ Thomas Alva Edison