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ARTICLE

Synthesis of Thienyl-Isoxazolines and *in vitro* Screening for their Antimicrobial Activity

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ABSTRACT

A series of thiophene conjugated isoxazoles were efficiently synthesized using a recyclable heterogeneous catalyst Amberlyst-15 in good yields. The synthesized new compounds **5**(**a-j**) were characterized by spectral and elemental analysis and screened *in vitro* for their antimicrobial potencies. Amongst the synthesized series; compounds **5c** and **5j** having chloro substitution on both thiophene and aromatic ring exhibited excellent inhibition against the tested species.

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KEYWORDS

Amberlyst-15, Antibacterial, Antifungal, Chalcone, Cyclocondensation.

INTRODUCTION

An interest in discovery, design and synthesis of novel small-molecules with antimicrobial effects is propelling research across the globe. The compounds with isoxazole core occupy prime position in pharmaceutical chemistry due to their diverse applications. The more commonly applied methods for the synthesis of molecules with isoxazole core being; aluminum chloride catalyzed cyclo-isomerization of α,β-acetylenic oximes [1], 1,3-dipolar cycloaddition reactions involving aldoximes with alkene [2], acetyl acetone [3] and chalcone [4,5], using chloramine-T as catalytic dehydrogenating agent. Synthesis of isoxazoles from 1-copper(I) alkynes and dihaloformaldoximes under base-free conditions circumvents the drawbacks of 1,3-dipolar cycloaddition [6]. Cycloadditions of copper(I) acetylides to nitrile oxides provide access to substituted isoxazoles with high regeoselectivity [7,8]. The *tert*-butyl nitrite found an efficient in one-pot approach for the synthesis of 3,5-disubstituted isoxazoles from substituted aldoximes and alkynes [9]. The reaction of *N*-hydroxyl-4-toluenesulfonamide with α,β-unsaturated carbonyl compounds under mild reaction conditions yields isoxazoles with high regioselectivity [10]. The successive treatment of primary alcohols with $PhI(OAc)_2$ in the presence of TEMPO, NH_2OH and then NCS, followed by reaction with alkynes in the presence of Et_3N forms isoxazoles [11]. The reaction of terminal alkynes with *n*-BuLi and then with aldehydes, followed by the treatment with molecular iodine and subsequently hydroxylamine yields isoxazoles with high regioselectivity [12]. Chemoselective synthesis of disubstituted isoxazoles involves the treatment of α-haloketoximes with phosphine, acyl chloride and a base [13].

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Literature on isoxazoles reveals that, these class of compounds have broad spectrum of medicinal properties like COX-2 inhibitor [14], anti-inflammatory [15], antifungal [16], antioxidant [17], antibacterial [18,19], analgesic [20], antituberculosis [21] and cystic fibrosis transmembrane conductance regulator (CFTR) protein [22], *etc*. The current study presents the synthesis of series of new thiophene conjugated isoxazoles and their *in vitro* antimicrobial evaluation results.

EXPERIMENTAL

Melting points were determined by an open capillary tube method and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Agilent NMR spectrometer using $CDCl_3$ as solvent and the chemical shifts were expressed δ ppm. Mass spectra were obtained on ESI/APCI-Hybrid Quadrupole, Synapt G2 HDMS ACQUITY UPLC model spectrometer. Elemental analysis was obtained on a Thermo-Finnigan Flash EA 1112 CHN analyzer.

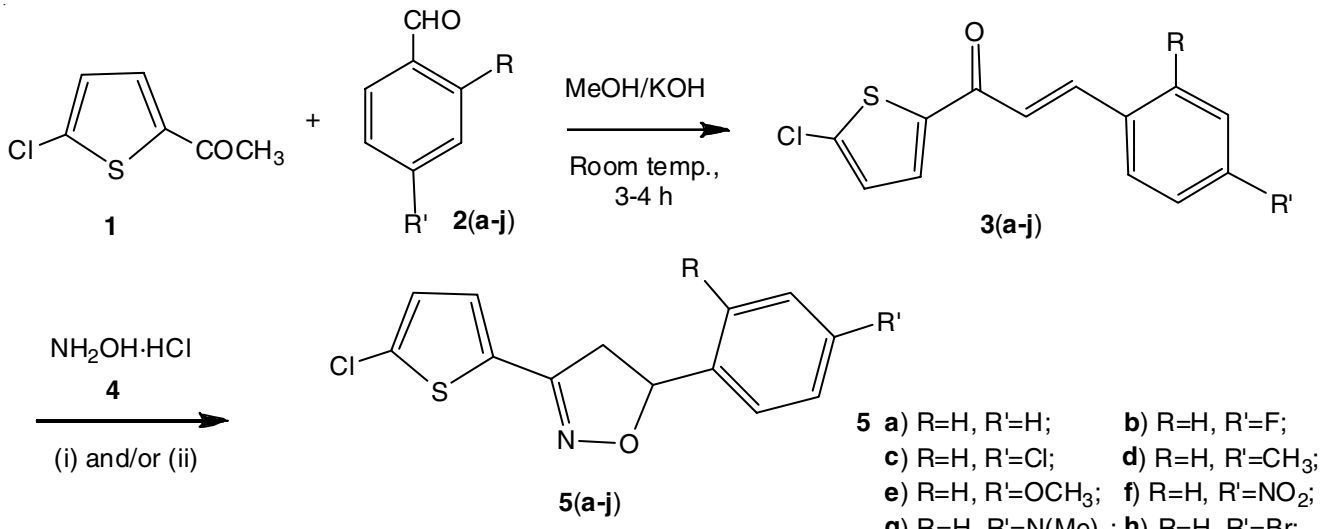
Initially, the intermediate 3-aryl-1-(5-chlorothiophen-2-yl)prop-2-en-1-ones, **3**(**a-j**) prepared *via* Claisen-Schmidt reaction of 5-chloro-2-acetylthiophene (**1**) with aromatic aldehydes, **2**(**a-j**) in methyl alcohol [23]. The reaction of compounds **3**(**a-j**) with hydroxylamine hydrochloride (**4**) in methyl alcohol in the presence of Amberlyst-15 catalyst under reflux conditions yielded thiophene-isoxazole derivatives **5**(**a-j**) in good yields (**Scheme-I**). ^{1}H NMR, ^{13}C NMR, MS and elemental analysis provided the structural proof for the compounds, **5**(**a-j**).

General procedure for synthesis of thienyl-isoxazoles, 5(a-j): To a solution of chalcones **3**(**a-j**) (10 mmol equivalent) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in methyl alcohol (25 mL), Amberlyst-15 (0.1 g) was added. Then the mixture was stirred at room temperature for 30-60 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was extracted into diethyl ether (40 mL), the ethereal layer was washed with water and dried to get the target product **5**(**a-j**). The mother liquor was treated with EtOAc (20 mL), stirred for 10 min and filtered to obtain insoluble catalyst. The recovered catalyst was washed with EtOAc, dried and reused effectively for four times for a reaction.

3-(5-Chlorothiophen-2-yl)-5-phenyl-4,5-dihydroisoxazole (5a): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-phenylprop-2-en-1-one (**3a**) (2.48 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 72% yield, m.p. 101-103 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 3.076 (dd, 1H, *J* = 6.5, 17.2 Hz, C_4-H_a), 3.410 (dd, 1H, *J* = 7.5, 12.9 Hz, C_4-H_b), 5.798 (dd, 1H, *J* = 6.6, 13.0 Hz, C_5-H), 6.854-6.921 (m, 2H, Ar-H), 7.240-7.382 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 43.2 (1C, C-4), 84.1 (1C, C-5), 125.1 (1C), 125.4 (1C), 126.9 (2C), 127.4 (1C), 128.2 (1C), 128.7 (2C), 130.6 (1C), 141.3 (1C), 163.9 (1C, C-3). MS (ES^+) *m/z*: 263.01 (M^+, ^{35}Cl, 100), 265.04 (M+2, ^{37}Cl, 34); Anal. calcd. for $C_{13}H_{10}NOSCl$ (%): C, 59.20 (59.07); H, 3.82 (3.80); N, 5.31 (5.28).

3-(5-Chlorothiophen-2-yl)-5-(4-fluorophenyl)-4,5-dihydroisoxazole (5b): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (**3b**) (2.66 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 75% yield, m.p. 108-110 ºC; ^{1}H NMR ($CDCl_3$, δ ppm): 3.020 (dd, 1H, *J* = 6.8, 16.2 Hz, C_4-H_a), 3.326 (dd, 1H, *J* = 7.1, 12.0 Hz, C_4-H_b), 5.786 (dd, 1H, *J* = 6.1, 12.2 Hz, C_5-H), 6.820-6.871 (m, 2H, Ar-H), 7.116-7.280 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 41.5 (1C, C-4), 81.6 (1C, C-5), 115.8 (2C), 124.5 (1C), 124.9 (1C), 128.0 (1C), 127.5 (2C), 130.6 (1C), 138.1 (1C), 160.1 (1C), 165.6 (1C, C-3). MS (ES^+) *m/z*: 281.06 (M^+, ^{35}Cl, 100), 283.03 (M+2, ^{37}Cl, 33); Anal. calcd. (found) for $C_{13}H_9NOSClF$ (%): C, 55.42 (55.30); H, 3.22 (3.21); N, 4.97 (4.94).

5-(4-Chlorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydroisoxazole (5c): Synthesized by reacting 3-(4-chlorophenyl)-1-(5-chlorothiophen-2-yl)prop-2-en-1-one (**3c**) (2.81 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 67% yield, m.p. 121-122 ºC; ^{1}H NMR ($CDCl_3$, δ ppm): 3.052 (dd, 1H, *J* = 6.5, 16.3 Hz, C_4-H_a), 3.338 (dd, 1H, *J* = 7.4, 12.9 Hz, C_4-H_b), 5.780 (dd, 1H, *J* = 7.0, 13.4 Hz, C_5-H), 6.856-



Scheme-I: Schematic diagram for the synthesis of thienyl-isoxazoles, **5**(**a-j**)

6.918 (m, 2H, Ar-H), 7.262-7.440 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 41.8 (1C, C-4), 85.2 (1C, C-5), 125.0 (1C), 125.4 (1C), 126.8 (2C), 128.6 (1C), 129.5 (2C), 130.0 (1C), 133.8 (1C), 142.1 (1C), 165.1 (1C, C-3). MS (ES^+) *m/z*: 297.01 (M^+, 100), 299.05 (M+2, 63), 301.02 (M+4, 11); Anal. calcd. (found) for $C_{13}H_9NOSCl_2$ (%): C, 52.37 (52.22); H, 3.04 (3.02); N, 4.70 (4.67).

3-(5-Chlorothiophen-2-yl)-5-(*p*-tolyl)-4,5-dihydroisoxazole (5d): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3d**) (2.62 g, 10 mmol) and hydroxyl-amine hydrochloride (**4**) (1.03 g, 15 mmol) in 75% yield, m.p. 126-128 ºC; ^{1}H NMR ($CDCl_3$, δ ppm): 2.112 (s, 3H, CH_3), 3.089 (dd, 1H, *J* = 6.8, 17.0 Hz, C_4-H_a), 3.320 (dd, 1H, *J* = 6.5, 12.2 Hz, C_4-H_b), 5.799 (dd, 1H, *J* = 6.8, 12.6 Hz, C_5-H), 6.832-7.024 (m, 2H, Ar-H), 7.100-7.307 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 20.6 (1C, CH_3), 43.7 (1C, C-4), 82.9 (1C, C-5), 124.4 (2C), 124.8 (1C), 125.0 (1C), 125.7 (2C), 128.8 (1C), 131.5 (1C), 136.2 (1C), 138.0 (1C), 162.1 (1C, C-3). MS (ES^+) *m/z*: 277.08 (M^+, ^{35}Cl, 100), 279.09 (M+2, ^{37}Cl, 31); Anal. calcd. (found) for $C_{14}H_{12}NOSCl$ (%): C, 60.54 (60.41); H, 4.35 (4.37); N, 5.04 (5.01).

3-(5-Chlorothiophen-2-yl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole (5e): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3e**) (2.78 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 66% yield, m.p. 113-114 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 3.108 (dd, 1H, *J* = 6.7, 17.2 Hz, C_4-H_a), 3.410 (dd, 1H, *J* = 7.9, 12.2 Hz, C_4-H_b), 3.824 (s, 3H, OCH_3), 5.801 (dd, 1H, *J* = 6.8, 12.9 Hz, C_5-H), 6.831-6.940 (m, 4H, Ar-H), 7.229-7.354 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 45.0 (1C, C-4), 86.1 (1C, C-5), 55.2 (1C, OCH_3), 113.2 (2C), 124.8 (1C), 125.3 (1C), 126.9 (2C), 128.0 (1C), 130.5 (1C), 133.8 (1C), 158.6 (1C), 162.9 (1C, C-3). MS (ES^+) *m/z*: 293.07 (M^+, ^{35}Cl, 100), 295.09 (M+2, ^{37}Cl, 32); Anal. calcd. (found) for $C_{14}H_{12}NO_2SCl$ (%): C, 57.24 (57.11); H, 4.12 (4.10); N, 4.77 (4.75).

3-(5-Chlorothiophen-2-yl)-5-(4-nitrophenyl)-4,5-dihydroisoxazole (5f): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (**3f**) (2.92 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 78% yield, m.p. 97-98 ºC; ^{1}H NMR ($CDCl_3$, δ ppm): 3.033 (dd, 1H, *J* = 6.7, 16.7 Hz, C_4-H_a), 3.410 (dd, 1H, *J* = 7.7, 12.0 Hz, C_4-H_b), 5.811 (dd, 1H, *J* = 6.4, 12.9 Hz, C_5-H), 6.930-6.955 (m, 2H, Ar-H), 7.666-8.124 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 43.7 (1C, C-4), 86.2 (1C, C-5), 123.6 (2C), 125.1 (1C), 125.5 (1C), 127.7 (1C), 128.0 (2C), 130.6 (1C), 143.2 (1C), 145.1 (1C), 165.8 (1C, C-3). MS (ES^+) *m/z*: 308.01 (M^+, ^{37}Cl, 100), 310.02 (M+2, ^{37}Cl, 34); Anal. calcd. (found) for $C_{13}H_9N_2O_3SCl$ (%): C, 50.57 (50.46); H, 2.94 (2.93); N, 9.07 (9.03).

4-(3-(5-Chlorothiophen-2-yl)-4,5-dihydroisoxazol-5-yl)-N,N-dimethylaniline (5g): Obtained from 1-(5-chlorothiophen-2-yl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (**3g**) (2.91 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 84% yield, m.p. 138-139 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 3.008 (s, 6H, N-CH_3), 3.107 (dd, 1H, *J* = 6.6, 16.5 Hz, C_4-H_a), 3.384 (dd, 1H, *J* = 7.0, 13.4 Hz, C_4-H_b), 5.882 (dd, 1H, *J* = 6.4, 12.6 Hz, C_5-H), 6.890-6.921 (m, 2H, Ar-H), 7.008-7.1826 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 40.4 (2C, N-CH_3), 45.0 (1C, C-4), 85.1 (1C, C-5), 112.8 (2C), 124.7 (1C), 125.2 (1C), 127.4 (2C), 128.4 (1C), 129.9 (1C), 131.6 (1C), 132.7 (1C), 149.8 (1C), 162.7 (1C, C-3). MS (ES^+) *m/z*: 306.04 (M^+, ^{35}Cl, 100), 308.05 (M+2, ^{37}Cl, 34); Anal. calcd. (found) for $C_{15}H_{15}N_2OSCl$ (%): C, 58.72 (58.60); H, 4.93 (4.91); N, 9.13 (9.09).

5-(4-Bromophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydroisoxazole (5h): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(4-bromophenyl)prop-2-en-1-one (**3h**) (3.25 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 61% yield, (gummy mass); ^{1}H NMR ($CDCl_3$, δ ppm): 3.056 (dd, 1H, *J* = 6.8, 16.4 Hz, C_4-H_a), 3.341 (dd, 1H, *J* = 7.4, 12.7 Hz, C_4-H_b), 5.806 (dd, 1H, *J* = 6.5, 13.1 Hz, C_5-H), 6.888-6.921 (m, 2H, Ar-H), 7.267-7.720 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 42.7 (1C, C-4), 85.3 (1C, C-5), 121.7 (1C), 123.0 (1C), 124.9 (1C), 125.4 (1C), 127.5 (2C), 128.3 (1C), 130.5 (1C), 130.9 (2C), 140.9 (1C), 162.5 (1C, C-3). MS (ES^+) *m/z*: 341.01 (M^+, 100), 343.02 (98), 345.03 (32), 347.03 (2); Anal. calcd. (found) for $C_{13}H_9NOSBrCl$ (%): C, 45.57 (45.48); H, 2.65 (2.64); N, 4.09 (4.07).

3-(5-Chlorothiophen-2-yl)-5-(2,4-dimethylphenyl)-4,5-dihydroisoxazole (5i): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(2,4-dimethylphenyl)prop-2-en-1-one, **3i** (2.76 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 81% yield, m.p. 166-168 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 2.240 (s, 6H, CH_3), 3.100 (dd, 1H, *J* = 6.7, 16.7 Hz, C_4-H_a), 3.416 (dd, 1H, *J* = 7.3, 12.7 Hz, C_4-H_b), 5.962 (dd, 1H, *J* = 6.8, 12.2 Hz, C_5-H), 6.944-7.168 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 22.1 (2C, CH_3), 44.4 (1C, C-4), 85.8 (1C, C-5), 125.0 (1C), 125.4 (1C), 126.3 (1C), 128.2 (1C), 128.8 (1C), 130.1 (1C), 130.6 (1C), 133.1 (1C), 136.5 (1C), 137.7 (1C), 163.8 (1C, C-3). MS (ES^+) *m/z*: 291.02 (M^+, ^{35}Cl, 100), 293.04 (M+2, ^{37}Cl, 34); Anal. calcd. (found) for $C_{15}H_{14}NOSCl$ (%):C, 61.74 (61.65); H, 4.84 (4.81); N, 4.80 (4.77).

3-(5-Chlorothiophen-2-yl)-5-(2,4-dichlorophenyl)-4,5-dihydroisoxazole (5j): Synthesized by reacting 1-(5-chlorothiophen-2-yl)-3-(2,4-dichlorophenyl)prop-2-en-1-one, **3j** (3.15 g, 10 mmol) and hydroxylamine hydrochloride (**4**) (1.03 g, 15 mmol) in 61% yield (gummy mass); ^{1}H NMR ($CDCl_3$, δ ppm): 3.066 (dd, 1H, *J* = 6.6, 16.1 Hz, C_4-H_a), 3.409 (dd, 1H, *J* = 7.4, 12.8 Hz, C_4-H_b), 5.790 (dd, 1H, *J* = 6.5, 12.3 Hz, C_5-H), 6.990-7.281 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, δ ppm): 44.7 (1C, C-4), 87.1 (1C, C-5), 124.0 (1C), 124.8 (1C), 126.9 (1C), 128.2 (1C), 128.8 (1C), 130.0 (1C), 130.7 (1C), 132.5 (1C), 133.4 (1C), 136.3 (1C), 163.7 (1C, C-3). MS (ES^+) *m/z*: 331.02 (M^+, 100), 333.00 (95), 335.03 (14), 337.01 (3); Anal. calcd. (found) for $C_{13}H_8NOSCl_3$ (%):C, 46.94 (46.83); H, 2.42 (2.41); N, 4.21 (4.17).

RESULTS AND DISCUSSION

In search of new potent antimicrobial agents, we successfully synthesized a series of new thiophene-isoxazoles derivatives **5**(**a-j**) by an Amberlyst-15 catalyzed reaction of chalcones **3**(**a-j**) with hydroxylamine hydrochloride (**4**) in good yields. The catalyst was washed with EtOAc, dried and reused effectively for four times for a reaction. Compounds **5**(**a-j**) shows a doublet of doublets at δ 3.020-3.114 (dd, 1H, *J* = 6.0-6.9 Hz

and J = 16.0-17.2 Hz) ppm for C_4-H_a; δ 3.326-3.418 (dd, 1H, J = 7.1-7.9 Hz and J = 12.0-12.9 Hz) ppm for C_4-H_b; and δ 5.780-5.812 (dd, 1H, J = 6.1-7.0 Hz and J = 12.2-13.4 Hz) ppm for C_4-H_a protons, respectively. The appearance of three doublets of doublets proves that the methylene protons of newly formed isoxazoles ring are diastereotopic. Other signals appear in the aromatic and substituents proton absorption region in the ^{1}H NMR spectra. The C-4, C-5 and C-3 carbons of newly formed isoxazole ring resonates at δ 41.5-45.0, 82.9-87.1 and 162.1-165.8 ppm, these signals confirms the formation of products and other signals appear in aromatic and substituents carbon region in the ^{13}C NMR spectra. Further, the designed series of compounds shows base peak comparable to their molecular masses and also halogen isotopic mass peaks in the mass spectra and comparable elemental analysis data.

Antimicrobial activity: The antimicrobial activities of compounds **5**(**a-j**) were determined as minimum inhibitory concentrations (MIC) by serial dilution method [24]. Bacterial pathogens *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 737) and fungal strains *Aspergillus niger, Aspergillus flavus* and *Candida albicans* (MTCC 227) were used for the study. Ciprofloxacin and nystatin were used as positive control against bacterial and fungal species, respectively. Dimethyl sulfoxide was used as solvent control. The experiments were carried out in triplicate; the results were taken as a mean of the three determinations.

Table-1 shows that synthesized series of new thienyl-isoxazoles **5**(**a-j**) exhibits wide range of anti-microbial activity against the tested microorganisms. Amongst the series, 5-(4-chlorophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydroisoxazole (**5c**) and 3-(5-chlorothiophen-2-yl)-5-(2,4-dichlorophenyl)-4,5-dihydroisoxazole (**5j**) show excellent inhibition against the tested species; compound **5c** against *S. aureus* (12.5 μg/mL), *E. coli* (12.5 μg/mL), *P. aeruginosa* (6.25 μg/mL), *A. niger* (25.0 μg/mL), *A. flavus* (25.0 μg/mL), *C. albicans* (12.5 μg/mL) and compound **5j** against *S. aureus* (12.5 μg/mL), *E. coli* (12.5 μg/mL), *P. aeruginosa* (12.5 μg/mL), *A. niger* (12.5 μg/mL), *A. flavus* (12.5 μg/mL), *C. albicans* (6.25 μg/mL) species.

Comparable inhibition shown by 3-(5-chlorothiophen-2-yl)-5-phenyl-4,5-dihydroisoxazole (**5a**) against *S. aureus* (25.0 μg/mL), *E. coli* (25.0 μg/mL), *P. aeruginosa* (12.5 μg/mL), *A. niger* (50.0 μg/mL), *A. flavus* (50.0 μg/mL), *C. albicans* (25.0 μg/mL) and 3-(5-chlorothiophen-2-yl)-5-(4-fluorophenyl)-4,5-dihydroisoxazole (**5b**) against *S. aureus* (25.0 μg/mL), *E. coli* (25.0 μg/mL), *P. aeruginosa* (12.5 μg/mL), *A. niger* (25.0 μg/mL), *A. flavus* (25.0 μg/mL), *C. albicans* (25.0 μg/mL) organisms. Compounds 3-(5-chlorothiophen-2-yl)-5-(4-nitrophenyl)-4,5-dihydroisoxazole (**5f**), 4-(3-(5-chlorothiophen-2-yl)-4,5-dihydroisoxazol-5-yl)-N,N-dimethylaniline (**5g**) and 5-(4-bromophenyl)-3-(5-chlorothiophen-2-yl)-4,5-dihydroisoxazole (**5h**) found inactive showing MIC's values of > 200.0 μg/mL against all tested species.

Moderate activities shown by the compounds 3-(5-chlorothiophen-2-yl)-5-(*p*-tolyl)-4,5-dihydroisoxazole (**5d**), 3-(5-chlorothiophen-2-yl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole (**5e**) and 3-(5-chlorothiophen-2-yl)-5-(2,4-dimethylphenyl)-4,5-dihydroisoxazole (**5i**) with the MIC's in the range of against *S. aureus* (75.0-100.0 μg/mL), *E. coli* (75.0-125.0 μg/mL), *P. aeruginosa* (50.0-75.0 μg/mL), *A. niger* (125.0-150 μg/mL), *A. flavus* (125 μg/mL), *C. albicans* (75.0-125.0 μg/mL) organisms.

Conclusion

An efficient synthesis of series of thiophene-isoxazole derivatives using a reusable heterogeneous catalyst Amberlyst-15, were characterized by spectroscopic studies. The newly synthesized target molecules **5**(**a-j**) studied for their antimicrobial potencies. Results of the tests clearly shows that the compounds **5c** and **5j** having chloro substitutions on both thiophene and aromatic ring exhibited excellent inhibition comparable to standards in the range of (6.25-25.0 μg/mL) against the tested organisms and therefore might acts as lead molecules as antimicrobial agents.

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TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED SERIES OF COMPOUNDS **5**(**a-j**)

Entry	Minimum inhibitory concentrations (MIC's) (μg/mL)					
	S. aureus	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. albicans*
5a	25.0	25.0	12.5	50.0	50.0	25.0
5b	25.0	25.0	12.5	25.0	25.0	25.0
5c	12.5	12.5	6.25	25.0	25.0	12.5
5d	75.0	100.0	75.0	150.0	125.0	75.0
5e	100.0	125.0	75.0	150.0	125.0	125.0
5f	>200.0	>200.0	>200.0	>200.0	>200.0	>200.0
5g	>200.0	>200.0	>200.0	>200.0	>200.0	>200.0
5h	>200.0	>200.0	>200.0	>200.0	>200.0	>200.0
5i	75.0	75.0	50.0	125.0	125.0	100.0
5j	12.5	12.5	12.5	12.5	12.5	6.25
Ciprofloxacin	25.0	25.0	12.5	–	–	–
Nystatin	–	–	–	50.0	50.0	25.0

*Values are the mean of three determinations (n = 3).

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