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5-(Heteroaryl)isoxazole계 화합물의 합성 및 항균 활성

R. Janaki RamaRao, A. K. S. Bhujanga Rao*, N. Sreenivas, B. Suneel kumar, and Y. L. N. Murthy†
Natco Research Centre, Natco Pharma Ltd., Hyderabad, Andhra Pradesh, PIN. 500018, India.
†Department of Organic Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, PIN. 530003, India
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Synthesis and Antibacterial Activity of Novel 5-(heteroaryl)isoxazole Derivatives

R. Janaki RamaRao, A. K. S. Bhujanga Rao*, N. Sreenivas, B. Suneel kumar, and Y. L. N. Murthy†
Natco Research Centre, Natco Pharma Ltd., Hyderabad, Andhra Pradesh, PIN. 500018, India.
**E-mail: nrc@natcopharma.co.in*
†Department of Organic Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, PIN. 530003, India
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요 약. Isoxazole계 화합물을 합성하고 항균활성연구를 수행하였다. 3-Di(alkylamino)acryloalkanones을 hydroxylamine hydrochloride 또는 hydroxylamine-*O*-sulphonic acid와 반응시켜서 target isoxazole계 화합물을 합성하였다.

주제어: Isoxazole계 화합물, 3-di(alkylamino)acryloalkanones, Hydroxylamine hydrochloride, Hydroxylamine-*O*-sulphonic acid, 항균활성

ABSTRACT. The synthesis, characterization and antibacterial activity of novel isoxazole derivatives were reported. 3-Di (alkylamino)acryloalkanones were prepared and used as synthons to get the target isoxazole derivatives via reaction with hydroxylamine hydrochloride or hydroxylamine-*O*-sulphonic acid.

Keywords: Isoxazole derivatives, 3-di(alkylamino)acryloalkanones, Hydroxylamine hydrochloride, Hydroxylamine-*O*-sulphonic acid, Antibacterial activity

INTRODUCTION

Recently the opportunistic infectious diseases have become the most serious problem in chemotherapy. They have been mainly caused by troublesome Gram-negative bacteria, especially Pseudomonas aeruginosa. Furthermore, the infectious diseases caused by other glucose non-fermenting Gram-negative rods and anaerobic bacteria, have gradually increased. The situations have stimulated the search for newer antibacterial agents.

The heterocyclic core in the molecules shows some interesting biological profiles such as antimalarials,[1] and diuretic[2] properties. Compounds containing isoazoline and isoxazole nuclei are known to possess antiinflammatory, antihypertensive, bactericidal, and other types of biological activity. Sulpha drugs such as sulfisoxazole, sulphamethoxazole, and some penicillin derivatives (cloxacillin and oxacillin) having isoxazole moiety were known to exhibit antimicrobial properties.[3-6] The chemical structures of these compounds are mentioned in *Fig.* 1. Some drugs having isoxazole moiety exhibit antipsychotic and neuroleptic activity.[7,8]

The prevalence of isoxazole core in natural and biologically active molecules has stimulated the need for developing novel isoxazole derivatives which exhibit a wide array of biological activities. The preparation of biheterocyclic compounds represents an important strategy in organic synthesis and provides interesting substances for subsequent biological evaluation. Hence in the current work, we aimed to synthesize novel 5-(heteroaryl)isoxazole derivatives and to evaluate antibacterial activity of these compounds. The heteroaryl moiety of these 5-(heteroaryl)isoxazole derivatives has been chosen from 5- and 6-membered heterocyclic compounds, particularly pyridine, pyrazine, thiophene, bromothiophene and furan. A total fourteen 5-(heteroaryl)isoxazole derivatives **1(*a-n*)** were synthesized and the evaluation of antibacterial activity of these compounds was also studied. Among the above isoxazole derivatives compounds **1*b***, **1*d***, **1*f*** and **1*h*** are known in the literature.[9-11]

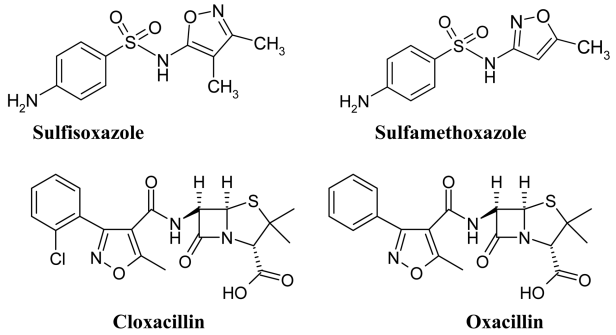


***Fig.* 1.** Chemical structures of sulfisoxazole, sulphamethoxazole, cloxacillin, and oxacillin.

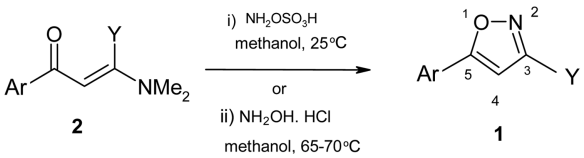
The synthetic process for the preparation of the 5-(heteroaryl)isoxazole derivatives **1(*a-n*)** of the current work involves the familiar [3+2] route of isoxazoles containing **C-C-C** and **N=O** fragments. The **C-C-C** synthon is reacted with **N=O** synthon in a suitable solvent to get the required isoxazole derivative. In the synthesis of novel 5-(heteroaryl)isoxazoles, we employed 3-(dimethylamino)acryloalkanone **(2)** as **C-C-C** synthon and hydroxylamine hydrochloride or hydroxylamine-*O*-sulfonic acid as the **N=O** synthon. 3-(Dimethylamino)acryloalkanone **2(*a-n*)** are reacted with hydroxylamine hydrochloride or hydroxylamine-O-sulfonic acid in methanol to afford the required 5-(heteroaryl)isoxazole derivatives. The synthetic pathway and the structural formulae of 5-(heteroaryl)isoxazoles **1(*a-n*)** of current study are depicted in *Scheme* 1.

The synthesis of the key intermediates, 3-(dimethylamino)acryloalkanone **2(*a-n*)** comprises of, coupling of suitable heteroarylketone (**3**) with *N*,*N*-dimethylformamide dimethyl acetal or with *N*,*N*-dimethylacetamide dimethyl acetal (**4**) in refluxing methanol[12]. The synthetic pathway and the structural formulae of 3-(dimethylamino)acryloalkanone **2(*a-n*)** are given in *Scheme* 2.

Antibacterial activity of the 5-(heteroaryl)isoxazoles **1(*a-n*)** was tested by employing sulfamethoxazole as reference standard.

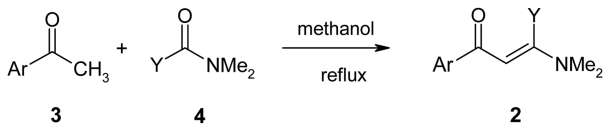
RESULTS AND DISCUSSION

Novel 5-(heteroaryl)isoxazoles **1(*a-n*)** were prepared by reacting 3-dimethylamino acryloalkanone **2(*a-n*)** (**C-C-C synthon**, 1.0 mole), with hydroxylamine-*O*-sulphonic acid or hydroxylamine hydrochloride (**N=O synthon**, 1.1 mole) in methanol. Reactions required reflux temperatures with hydroxylamine hydrochloride whereas room temperature was sufficient with hydroxylamine-*O*-sulphonic acid. Reactions were monitored by TLC and the products were isolated by conventional techniques such as crystallization and column chromatography. Yields of the products were

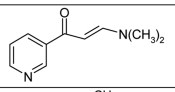
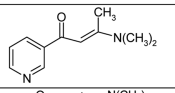
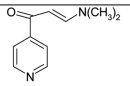
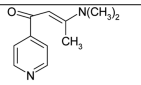
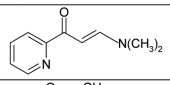
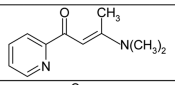
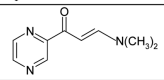
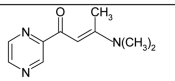
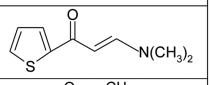
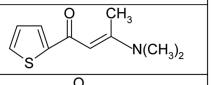
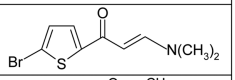
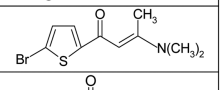
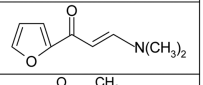
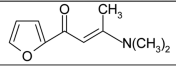


Entry	Ar	Y	Structure
1*a*	3-pyridyl	H	
1*b*	3-pyridyl	CH_3	
1*c*	4-pyridyl	H	
1*d*	4-pyridyl	CH_3	
1*e*	2-pyridyl	H	
1*f*	2-pyridyl	CH_3	
1*g*	pyrazinyl	H	
1*h*	pyrazinyl	CH_3	
1*i*	2-thienyl	H	
1*j*	2-thienyl	CH_3	
1*k*	(5-bromo)-2-thienyl	H	
1*l*	(5-bromo)-2-thienyl	CH_3	
1*m*	2-furyl	H	
1*n*	2-furyl	CH_3	

***Scheme* 1.** Synthesis of novel 5-(heteroaryl)isoxazole compounds **1(*a-n*).**



3 **4** **2**

Entry	Ar	Y	Structure
2a	3-pyridyl	H	
2b	3-pyridyl	CH_3	
2c	4-pyridyl	H	
2d	4-pyridyl	CH_3	
2e	2-pyridyl	H	
2f	2-pyridyl	CH_3	
2g	pyrazinyl	H	
2h	pyrazinyl	CH_3	
2i	2-thienyl	H	
2j	2-thienyl	CH_3	
2k	(5-bromo)-2-thienyl	H	
2l	(5-bromo)-2-thienyl	CH_3	
2m	2-furyl	H	
2n	2-furyl	CH_3	

***Scheme* 2.** Synthesis of 3-(dialkylamino)acryloalkanones **2(*a-n*)**.

found to be moderate to low and purities were greater than 98% (HPLC).

The **C-C-C synthon**, 3-(dimethylamino)acryloalkanones **2(*a-n*)** were synthesized by reacting the suitable heteroarylketone (1 mole) with N,N-dimethylformamide dimethyl acetal or *N*,*N*-dimethylacetamide dimethyl acetal (1.3 mole) in methanol at reflux temperature. The products were isolated by simple crystallization technique with purity (HPLC) above 99%. The yield of the products ranged from moderate to good.

All 5-(heteroaryl)isoxazole compounds (**1*a-n***) were tested for their antibacterial activity against *E. coli* (NCIM 2065), Staphylococcus aureus (NCIM 2079), Pseudomonas aeruginosa (NCIM 2200) by cup plate method. All test strains were maintained on nutrient agar slants and were subcultured.These bacteria also served as test pathogens for the assay.

***Table* 1.** Bacterial activity of isoxazoles at 200 mg/mL-zones of inhibition

Compound	*E. coli*	Pseudomonas areginosa	Staphylococcus aureus
1*j*	15±0.2 mm	18±0.2 mm	30±0.2 mm
1*k*	14±0.2 mm	16±0.2 mm	26±0.2 mm
Sulfamethoxazole (reference)	28±0.2	30±0.2 mm	40±0.2 mm

Solutions of isoxazole molecules were made in DMSO at a concentration of 200 mg/mL under aseptic conditions. The antibacterial activity of these isoxazoles were compared with that of sulfamethaxazole as reference antibacterial agent. Compounds **1*j*** and **1*k*** which contain 2-thienyl and (5-bromo)-2-thienyl moieties in the fifth position of 5-heteroarylisoxazoles displayed good antibacterial activity against *E. coli*, Pseudomonas aeruginosa, Staphylococcus aureus (*Table* 1).

CONCLUSION

In conclusion, fourteen 5-(heteroaryl)isoxazoles compounds **1(*a-n*)** bearing various heteroaryl moieties were synthesized and their antibacterial activity studied against *E. coli* (NCIM 2065), Staphylococcus aureus (NCIM 2079), Pseudomonas aeruginosa (NCIM 2200) employing sulfamethoxazole as reference standard. The potencies of these molecules varied, depending upon the nature of heteroaryl moiety substituted in the fifth position of 5-(heteroaryl)isoxazoles. Significant activity is observed for compounds **1*j*** and **1*k*** which contain 2-thienyl and (5-bromo)-2-thienyl moieties in the fifth position.

EXPERIMENTAL

All reactions were monitored by thin layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. All solvents were obtained from commercial sources and freshly distilled before use. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400-MHz spectrometer. The chemical shifts are reported in d parts per million (ppm) relative to TMS. The IR spectra were recorded in liquid

state in KBr cell and solid state as KBr dispersion using Perkin-Elmer FT-IR spectrometer. The Mass Spectra were recorded on Waters Quattro Micro LC/MS/MS. Analytical HPLC was performed on a Waters system equipped with a UV detector set at 225 nm. Compounds were dissolved in the mobile phase and injected through a 100 μL loop. The following eluent system was used: 0.01 M H_3PO_4 solution and acetonitrile (750:250 ratio). HPLC retention times (t_R) were obtained, at flow rates of 1.0 ml/min, using Kromasil C18 5 (250 × 4.6 mm) column and isocratically run up to 60 min.

Synthesis of 3-Dimethylaminoacryloalkanones (C-C-C synthons), 2(*a-n*)

Synthesis of 3-Dimethylamino-1-(3-pyridyl)-2-propen-1-one (2*a*): 3-Acetylpyridine (20 g, 0.16 moles), *N*,*N*-dimethylformamide dimethyl acetal (25 g, 0.21 moles) and methanol (40 mL) were charged into a 250 mL three-necked round-bottom flask and stirred at reflux temperature for 10 h. Methanol was evaporated under reduced pressure to get a brown crystalline residue (33.9 g). The resulting residue was crystallized from a solvent mixture of EtOAc: isopropyl ether (1:3) (100 mL) at 5-10 ºC to afford a yellow coloured crystalline solid (20 g).

Yield: 60%, mp. 80.2 ºC; purity (HPLC): 99.89%; IR (KBr): cm^{-1} 3423, 2918, 1640.6, 1578.8, 1514.4. ^1H NMR ($CDCl_3$/TMS): δ 2.95 (s, 3H), 3.18 (s, 3H), 5.66 (d,1H, J=12.4 Hz), 7.35 (dd,1H, J=4.8 Hz, 8.0 Hz), 7.85 (d, 1H, J=12.0 Hz), 8.17 (dt, 1H, J=8.0Hz), 8.65 (dd, 1H, J=1.6 Hz, 4.8 Hz), 9.07 (s, 1H). ^{13}C NMR ($CDCl_3$/TMS): δ 37.01, 44.84, 91.38, 122.89, 134.62, 135.25, 148.52, 151.03, 154.33, and 185.84. MS (m/z): 177.4 (M+1) (100%), 132.2 (42%).

Synthesis of 3-Dimethylamino-1-(3-pyridyl)-2-buten-1-one (2*b*): Compound (**2*b***) was prepared in the same manner as described above using *N*,*N*-dimethylacetamide dimethyl acetal instead of *N*,*N*-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 40%, mp. 51.3-56.5 ºC; purity (HPLC): 99.56%; IR (KBr): cm^{-1} 3432, 1610.9, 1568.3, 1539.5, 1405.3. ^1H NMR ($CDCl_3$/TMS): δ 2.68 (s, 3H), 3.11 (s, 6H), 5.61 (s, 1H), 7.33 (m, 1H), 8.15 (dd, 1H, J=2.0 Hz, 8.0 Hz), 8.63 (dd, 1H, J=1.6 Hz, 4.8 Hz), 9.05 (s, 1H). ^{13}C NMR ($CDCl_3$/TMS): δ 16.37, 40.00, 91.77, 122.92, 134.61, 137.87, 148.38, 150.51, 164.62, 185.23. MS (m/z): 191.5 (M+1) (100%), 146.3 (55%).

Synthesis of 3-Dimethylamino-1-(4-pyridyl)-2-propen-1-one (2*c*): Compound (**2*c***) was prepared in the same manner by employing 4-acetylpyridine and *N*,*N*-dimethylformamide dimethyl acetal as substrate and reactant respectively.

Crystallized from EtOAc: isopropyl ether (1:3), yield: 43%, mp. 114 ºC; purity (HPLC): 99.98%; IR (KBr): cm^{-1} 3424.5, 3027.1, 1640.1, 1564.2, 1525.2. ^1H NMR ($CDCl_3$/TMS): δ 2.96 (s, 3H), 3.19 (s, 3H), 5.64 (d, 1H, J=12.4 Hz), 7.68 (dd, 2H, J=1.2 Hz, 4.8 Hz), 7.86 (d, 1H, J=12.4 Hz), 8.70 (dd, 2H, J=1.2 Hz, 4.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.25, 45.13, 91.50, 121.02, 147.03, 150.06, 155.03, 186.36. MS (m/z): 177.4 (M+1) (100%), 159.4 (24%), 134.3 (5%).

Synthesis of 3-Dimethylamino-1-(4 -pyridyl)-2-buten-1-one (2*d*): Compound (**2*d***) was synthesized in the same manner by employing 4-acetylpyridine and *N*,*N*-dimethylacetamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 38%, mp. 91.6 ºC; purity (HPLC): 99.89%; IR (KBr): cm^{-1} 3422.9, 3015.1, 1601.6, 1529.8, 1487.4. ^1H NMR ($CDCl_3$/TMS): δ 2.68 (s, 3H), 3.11 (s, 6H), 5.58 (d, 1H), 7.64 (dd, 2H, J=1.6 Hz, 4.4 Hz), 8.66 (dd, 2H, J=1.6 Hz, 4.8 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.47, 40.09, 91.53, 120.91, 149.59, 149.86, 165.25, 185.31. MS (m/z): 191.5 (M+1) (100%), 174.5 (8%), 173.5 (51%), 146.3 (8%).

Synthesis of 3-Dimethylamino-1-(2-pyridyl)-2-propen-1-one (2*e*): Compound (*2e*) was prepared in the same manner by employing 2-acetylpyridine and N,N-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: isopropyl ether (1:3), yield: 70%, mp. 122.6 ºC; purity (HPLC): 99.85%; IR (KBr): cm^{-1} 3431, 3015.9, 1638.5, 1565.6, 1534.9. ^1H NMR ($CDCl_3$/TMS): δ 2.99 (s, 3H), 3.18 (s, 3H), 6.46 (d, 1H, J=12.4Hz), 7.35 (m, 1H), 7.80 (m, 1H), 7.90 (d, 1H, J=12.4 Hz), 8.15 (d, 1H, J=8.0 Hz), 8.63 (1H, m). ^{13}C NMR ($CDCl_3$/TMS): δ 37.27, 44.95, 90.94, 121.81, 125.21, 136.53, 148.07, 154.56, 156.03, 186.65. MS (m/z): 177.5 (M+1) (100%), 159.4 (16%), 134.3 (23%).

Synthesis of 3-Dimethylamino-1-(2-pyridyl)-2-buten-1-one (2*f*): Compound (**2*f***) was prepared in the same manner by employing 2-acetylpyridine and *N*,*N*-dimethylacetamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 44%, mp. 69 ºC; purity (HPLC): 98.72%; IR (KBr): cm^{-1} 3432.8, 2913.3, 1604.6, 1541.1, 1465.9. ^1H NMR ($CDCl_3$/TMS): δ 2.71 (s, 3H), 3.14 (s, 6H), 6.55 (s, 1H), 7.31 (m, 1H), 7.78 (m, 1H), 8.14 (dt, 1H, J=8 Hz), 8.59 (ddd, 1H, J=0.8 Hz, 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.40, 40.06, 90.74, 121.57, 124.64, 136.47, 147.79, 157.88, 164.91, 185.46. MS (m/z): 191.5 (M+1) (100%), 173.4 (33%), 146.3(24%).

Synthesis of 3-Dimethylamino-1-(2-pyrazinyl)-2-propen-1-one (2*g*): Compound (**2*g***) was prepared by employing

acetylpyrazine and *N*,*N*-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 38%, mp. 131.0 °C; purity (HPLC): 99.74%; IR (KBr): cm^{-1} 3433.2, 2946.9, 1637.3, 1582.1, 1544.4. ^{1}H NMR ($CDCl_3$/TMS): δ 3.01 (s, 3H), 3.21 (s, 3H), 6.34 (d, 1H, J=12.4 Hz), 7.93 (d, 1H, J=12.8 Hz), 8.56 (dd, 1H, J=1.6 Hz, 2.8 Hz), 8.65 (d,1H, J=2.4 Hz), 9.34 (d, 1H, J=1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.50, 45.25, 90.92, 142.76, 144.29, 146.02, 150.45, 154.99, 185.35. MS (m/z): 178.5 (M+1) (100%), 160.4 (16%).

Synthesis of 3-Dimethylamino-1-(2-pyrazinyl)-2-buten-1-one (2*h*): The above compound (**2*h***) was prepared using acetylpyrazine and N,N-dimethylacetamide dimethyl acetal**.**

Crystallized from EtOAc: *n*-hexane (1:1), yield: 53%, mp. 131.8 °C; purity (HPLC): 99.14%; IR (KBr) cm^{-1}: 13422.9, 2922.4, 1617.3, 1537.1, 1444. ^{1}H NMR ($CDCl_3$/TMS): δ 2.73 (s, 3H), 3.15 (s, 6H), 6.44 (s, 1H), 8.52 (dd, 1H, J=1.2 Hz, 2.4 Hz), 8.60 (d, 1H, J=2.8 Hz), 9.32 (d, 1H, J=1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.48, 40.08, 90.37, 142.28, 143.99, 145.19, 152.06, 165.55, 183.57. MS (m/z): 192.5 (M+1) (100%), 174.4 (24%).

Synthesis of 3-Dimethylamino-1-(2-thienyl)-2-propen-1-one (2*i*): The above compound (**2*i***) was synthesized using 2-acetylthiophene and *N*,*N*-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: isopropyl ether (1:3), yield: 59%, mp. 110.9°C; purity (HPLC): 99.85%; IR (KBr): cm^{-1} 3422.8, 3070.9, 1636.0, 1544.1, 1513.0. ^{1}H NMR ($CDCl_3$/TMS): δ 2.92 (s, 3H), 3.14 (s, 3H), 5.61 (d, 1H, J=12.0 Hz), 7.07 (dd, 1H, J=3.6 Hz, 4.8 Hz), 7.47 (dd, 1H, J=1.2 Hz, 5.2 Hz), 7.63 (dd, 1H, J=0.8 Hz, 3.6 Hz), 7.79 (d, 1H, J=12.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.10, 44.86, 91.22, 127.94, 143.74, 147.18, 150.17, 154.60, 184.80. MS (m/z): 182.4 (M+1) (100%), 111.1 (60%).

Synthesis of 3-Dimethylamino-1-(2-thienyl)-2-buten-1-one (2*j*): Compound (**2*j***) was synthesized by employing 2-acetylthiophene and *N*,*N*-dimethylacetamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 70%, mp. 94.5 °C; purity (HPLC): 99.93%; IR (KBr): cm^{-1} 3417.7, 2952.5, 1588.9, 1544.0, 1515.0. ^{1}H NMR ($CDCl_3$/TMS): δ 2.64 (s, 3H), 3.07 (s, 6H), 5.64 (s, 1H), 7.04 (dd, 1H, J=3.6 Hz, 4.8 Hz), 7.41 (dd, 1H, J=1.2 Hz, 4.8 Hz), 7.55 (dd, 1H, J=1.2 Hz, 4.0 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.13, 39.73, 91.23, 126.86, 127.18, 129.12, 149.85, 163.44, 179.72. MS (m/z): 196.5 (M+1) (100%), 112.2 (21%), 111.1 (76%).

Synthesis of 3-Dimethylamino-1-(5-bromo-2-thienyl-)-2-propen-1-one (2*k*): Preparation of compound (**2*k***) was prepared from 5-bromo-2-acetylthiophene and *N*,*N*-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: isopropyl ether (1:3), yield: 70%, mp. 114.1 °C; purity (HPLC): 100%; IR (KBr): cm^{-1} 3415.4, 2900.9, 1631.6, 1548.3, 1521.8. ^{1}H NMR ($CDCl_3$/TMS): δ 2.91 (s, 3H), 3.15 (s, 3H), 5.5 (d, 1H, J=12.4 Hz), 7.03 (d, 1H, J=4.0 Hz), 7.34 (d, 1H, J=4.0 Hz), 7.78 (d, 1H, J=12.0 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.21, 45.01, 90.57, 118.12, 128.09, 130.57, 148.96, 153.73, 179.39. MS (m/z): 262.4 (M+2) (97%), 260.4 (M^+) (100%), 189.3 (18%).

Synthesis of 3-Dimethylamino-1-(5-bromo-2-thienyl)-2-buten-1-one (2*l*): Compound (**2*l***) was prepared by reacting 5-bromo-2-acetylthiophene with *N*,*N*-dimethylacetamide dimethyl acetal.

Crystallized from EtOAc: *n*-hexane (1:1), yield: 79%, mp. 126.7 °C; purity (HPLC): 99.89%; IR (KBr): cm^{-1} 2915.9, 1580.7, 1540.2, 1491.3 ^{1}HNMR ($CDCl_3$/TMS): δ 2.61 (s, 3H), 3.07 (s, 6H), 5.49 (s, 1H), 7.00 (d, 1H, J=4.0 Hz), 7.28 (d, 1H, J=4.0 Hz). ^{13}CNMR ($CDCl_3$/TMS): d 16.32, 39.94, 90.34, 116.91, 126.76, 130.36, 151.53, 164.01, 178.44. MS (m/z): 276.4 (M+2) (98%), 274.4 (M^+) (100%), 191.3 (29%).

Synthesis of 3-Dimethylamino-1-(2-furyl)-2-propen-1-one (2*m*): Compound (**2*m***) was synthesized by using 2-acetylfuran and *N*,*N*-dimethylformamide dimethyl acetal.

Crystallized from EtOAc: isopropyl ether (1:3), yield: 68%, mp. 82.1 °C; purity (HPLC): 99.94%; IR (KBr): cm^{-1} 3421.4, 2918.6, 1641.8, 1576.9, 1541.1. ^{1}H NMR ($CDCl_3$/TMS): δ 2.92 (s, 3H), 3.14 (s, 3H), 5.66 (d, 1H, J=12.4 Hz), 6.48 (dd, 1H, J=1.6 Hz, 3.6 Hz), 7.06 (dd, 1H, J=0.8 Hz, 3.6 Hz), 7.49 (dd, 1H, J=0.4 Hz, 1.2 Hz), 7.79 (d, 1H, J=12.4 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 37.10, 44.82, 91.24, 111.62, 113.12, 143.98, 153.3, 154.63, 177.24. MS (m/z): 166.4 (M+1) (100%), 98.0 (30%), 95.0 (49%).

Synthesis of 3-Dimethylamino-1-(2-furyl)-2-buten-1-one (2*n*): The above compound (**2*n***) was synthesized by using 2-acetylfuran and *N*,*N*-dimethylacetamide dimethyl acetal.

Crystallized from EtOAc: hexane (1:1), yield: 66%, mp. 76.7 °C; purity (HPLC): 99.41%; IR (KBr): cm^{-1} 3403.9, 3112.8, 1603.9, 1571.0, 1557.6. ^{1}H NMR ($CDCl_3$/TMS): δ 2.65 (s, 3H), 3.07 (s, 6H), 5.71 (s, 1H), 6.44 (dd, 1H, J=1.6 Hz, 3.2 Hz), 7.00 (d, 1H, J=3.6 Hz), 7.43 (dd, 1H, J=0.8 Hz, 1.6 Hz). ^{13}C NMR ($CDCl_3$/TMS): δ 16.39, 39.89, 90.95, 111.54, 112.07, 143.16, 156.27, 163.84, 176.58. MS (m/z): 180.5 (M+1) (100%), 112.2 (25%), 95.0 (26%).

Synthesis of novel 5-(Heteroaryl)isoxazoles 1(*a-n*): General procedure

Method-A: 3-Dimethylaminoacryloalkanone (**2**), (1.0 mole equivalents), and methanol were charged into a three necked round bottomed flask and the reaction mass was cooled to 0-5 °C. A solution of hydroxylamine-*O*-sulphonic acid (1.1 mole equivalents) in methanol was added drop wise to the above reaction mass. Then the reaction mass was allowed to warm to room temperature, and stirred for 1-2 h. TLC was checked for completion of reaction. Methanol was evaporated under reduced pressure to yield a brown coloured residue. The organic residues were quenched into aq. saturated sodium bicarbonate solution and extracted into methylene chloride. The layers were separated, organic layer was dried over sodium sulphate, and solvent was distilled off under vacuum to get brown coloured products. The crude products were purified by column chromatography to get pure 5-heteroarylisoxazoles.

Method-B: 3-Dimethylaminoacryloalkanone (**2**), (1.0 mole equivalents), methanol, and hydroxyl- amine hydrochloride (1.1 mole equivalents) were charged into a three necked round bottomed flask at room temperature. Then the reaction mass was stirred at 65-70°C for 3-4h for completion reaction by TLC. The products were isolated as per the process mentioned above.

Synthesis of 5-(3-pyridyl)isoxazole (1*a*): (Method A)

3-Dimethylamino-1-(3-pyridyl)-2-propen-1-one (**2*a***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 30%, mp. 76-77.8 °C; purity (HPLC): 98.75%; IR (KBr): cm^{-1} 3432.4, 3063.3, 1620.5, 1603.4, 1583.3, 1459.1, 1423.0 ^{1}H NMR (DMSO-D6/TMS): δ 7.22 (d, 1H, J=1.6 Hz), 7.59 (dd, 1H, J=4.8 Hz, 7.6 Hz), 8.3 (dt, 1H, J=8.0 Hz), 8.70 (dd, 1H, J=1.6 Hz, 4.8 Hz), 8.75 (d, 1H, J=1.6 Hz), 9.13 (d, 1H, J=2 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 101.60, 123.57, 124.96, 133.78, 146.81, 151.35, 152.34, 166.30, MS (m/z): 147.3 (M+1) (100%), 129.2 (10%), 119.2 (29%).

Synthesis of 3-Methyl-5-(3-pyridyl)isoxazole (1*b*): (Method B)

3-Dimethylamino-1-(3-pyridyl)-2-buten-1-one (**2*b***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 30%, mp. 70.4-70.9 °C; purity (HPLC): 99.85%; IR (KBr): cm^{-1} 3415.4, 3035.4, 1615.5, 1562.5, 1485.7, 1406.3 ^{1}H NMR (DMSO-D6/TMS): δ 2.31(s, 3H), 7.05 (s, 1H), 7.55-7.59 (dd, 1H, J=4.8 Hz, 7.6 Hz), 8.21-8.24 (dt, 1H, J=1.6 Hz, 2.0 Hz), 8.68 (dd, 1H, J=1.2 Hz, 4.8 Hz), 9.06 (d, 1H, J=1.6 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.29, 102.60, 123.55, 124.64, 133.29, 146.61, 151.10, 160.99, 166.43, MS (m/z): 161.4 (M+1) (100%), 134.3 (21%), 133.3 (13%).

Synthesis of 5-(4-pyridyl)isoxazole (1*c*): (Method A)

3-Dimethylamino-1-(4-pyridyl)-2-propen-1-one (**2*c***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 27%, mp. 93.5-97.6 °C; purity (HPLC): 98.77%; IR (KBr): cm^{-1} 3446.1, 2964.1, 1637.5, 1597, 1412.3, 1262.2 ^{1}H NMR (DMSO-D6/TMS): δ 7.35 (d, 1H, J=1.6 Hz), 7.87 (dd, 2H, J=1.6 Hz, 4.4 Hz), 8.78 (m, 3H). ^{13}C NMR (DMSO-D6/TMS): δ 103.31, 120.00, 133.81, 151.04, 152.44, 166.29, MS (m/z): 147.0 (M+1) (100%), 120.0 (13%), 119.0 (38%).

Synthesis of 3-Methyl-5 -(4-pyridyl)isoxazole (1*d*): (Method A)

3-Dimethylamino-1-(4-pyridyl)-2-buten-1-one (**2*d***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 31%, mp. 58-65.3 °C; purity (HPLC): 99.25%; IR (KBr): cm^{-1} 3447.7, 3125.6, 1587.1, 1550.6, 1414.5 ^{1}H NMR (DMSO-D6/TMS): δ 2.33 (s, 3H), 7.18 (s, 1H), 7.79-7.81 (dd, 2H, J=1.6 Hz, 4.4 Hz), 8.75 (dd, 2H, J=1.6 Hz, 4.4 Hz). ^{13}C NMR (DMSO-D6): δ 11.21, 104.26, 119.58, 133.76, 150.88, 161.05, 166.30, MS (m/z): 161.0 (M+1) (100%), 146.0 (8%), 134.0 (8%), 133.0 (13%), 120.0 (12%).

Synthesis of 5-(2-pyridyl)isoxazole (1*e*): (Method B)

3-Dimethylamino-1-(2-pyridyl)-2-propen-1-one (**2*e***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 21%, oily liquid; purity (HPLC): 89.51%; IR (KBr) cm^{-1}: cm-13424.0, 3060.1, 1576.6, 1562.0, 1456.7, 1427.0 ^{1}H NMR (DMSO-D6/TMS): δ 7.11 (d, 1H, J=2 Hz), 7.51 (m, 1H), 7.98 (m, 2H), 8.73 (m, 2H). ^{13}C NMR (DMSO-D6/TMS): δ 102.30, 121.22, 125.20, 137.92, 145.74, 150.32, 152.01, 168.14, MS (m/z): 147.3 (M+1) (100%), 138.3 (24%), 120.2 (28%).

Synthesis of 3-Methyl-5 -(2-pyridyl)isoxazole (1*f*): (Method A)

3-Dimethyl amino-1-(2-pyridyl)-2-buten-1-one (**2*f***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 20%, crude; purity (HPLC): 99.85%; IR (KBr): cm^{-1} 3424.8, 1580.0, 1564.1, 1422.4 ^{1}H NMR (DMSO-D6/TMS) δ 2.33 (s, 3H), 6.98 (s, 1H), 7.49-7.52 (m, 1H), 7.92-7.99 (m, 2H), 8.72 (dd, 1H, J=1.6 Hz, 4.8 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.28, 103.63, 121.12, 125.19, 137.97, 146.04, 150.37, 160.99, 168.56, MS (m/z): 161.4 (M+1) (100%), 138.3 (36%), 120.2 (40%).

Synthesis of 5-(2-pyrazinyl)isoxazole (1*g*): (Method A)

3-Dimethylamino-1-(2-pyrazinyl)-2-propen-1-one (**2*g***) was used as substrate. Product was isolated by column chromatography using ethyl acetate: *n*-hexane (1:1) as

eluent. Yield: 21%, mp. 120-130.4 °C; purity (HPLC): 100%; IR (KBr): cm^{-1} 3423.8, 3144.9, 1608.4, 1562.2, 1400.7. ^1H NMR (DMSO-D6/TMS): δ 7.30 (d, 1H, J=1.6 Hz), 8.76-8.81 (m, 3H), 9.24 (d, 1H, J=1.2 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 104.26, 141.94, 142.53, 145.53, 146.23, 152.44, 166.00, MS (m/z): 148.3 (M+1) (100%), 138.3 (11%).

Synthesis of 3-Methyl-5-(2-pyrazinyl)isoxazole (1*h*): (Method A)

3-Dimethylamino-1-(2-pyrazinyl)-2-buten-1-one (**2*h***) was used as substrate. Product was isolated by column chromatography using ethyl acetate as eluent. Yield: 22%, mp. 113.2-114.9 °C; purity (HPLC): 99.20%; IR (KBr): cm^{-1} 3431.2, 3145.8, 1616.0, 1562.8, 1443.2, 1411.6. ^1H NMR (DMSO-D6/TMS): δ 2.34 (s, 3H), 7.16 (s, 1H), 8.76 (d, 1H, J=2.4 Hz), 8.79 (m, 1H), 9.19 (d, 1H, J=1.2 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.44, 105.48, 142.09, 142.37, 145.51, 146.10, 161.48, 166.28, MS (m/z): 162.4 (M+1) (100%), 139.3 (14%), 121.2 (32%).

Synthesis of 5-(2-thienyl)isoxazole (1*i*): (Method B)

3-Dimethylamino-1-(2-thiophenyl)-2-propen-1-one (**2*i***) was used as substrate. Yield: 93%, oily crude; purity (HPLC): 98.37%; IR (KBr): cm^{-1} 3137.0, 3108.1, 1611.5, 1591.9, 1467.1. ^1H NMR (DMSO-D6/TMS): δ 6.89 (d, 1H, J=2.0 Hz), 7.26 (dd, 1H, J=3.6 Hz, 4.8 Hz), 7.74 (dd, 1H, J=1.2 Hz, 3.6 Hz), 7.84 (dd, 1H, J=0.8 Hz, 4.8 Hz), 8.67 (d, 1H, J=2.0 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 98.21, 126.82, 127.84, 128.76, 150.53, 164.08. MS (m/z): 152.3 (M+1) (100%), 124.2 (19%).

Synthesis of 3-Methyl-5-(2-thienyl)isoxazole (1*j*): (Method B)

3-Dimethylamino-1-(2-thiophenyl)-2-buten-1-one (**2*j***) was used as substrate. Yield: 89.09%, oily crude; purity (HPLC): 99.77%; IR (KBr): cm^{-1} 3109.4, 2930.8, 1614.4, 1603.4, 1477.3, 1419.3. ^1H NMR (DMSO-D6/TMS): δ 2.27 (s, 3H), 6.71 (s, 1H), 7.24 (dd, 1H, J=4.0 Hz, 5.2 Hz), 7.66 (d, 1H, J=4.0Hz), 7.80 (dd, 1H, J=0.8 Hz, 4.8 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.26, 100.79, 127.82, 128.96, 129.28, 161.05, 164.41. MS (m/z): 166.3 (M+1) (100%), 165.4 (24%), 138.3(16%), 126.3 (12%).

Synthesis of 5-(5-bromo-2-thienyl)isoxazole (1*k*): (Method B)

3-Dimethylamino-1-(2-thophenyl-5-bromo)-2-propen-1-one (**2*k***) was used as substrate. Product was isolated by crystallization from *n*-hexane. Yield: 72%, mp. 64.6-65.8 °C; purity (HPLC): 98.67%; IR (KBr): cm^{-1} 3425.4, 3132.0, 1603.8, 1463.2, 1416.5. ^1H NMR (DMSO-D6/TMS): δ 6.92 (d, 1H, J=1.6 Hz), 7.40 (d, 1H, J=3.6 Hz), 7.56 (d, 1H, J=3.6 Hz), 8.67 (d, 1H, J=1.6 Hz). ^{13}C NMR (DMSO-D6): δ 99.97, 115.23, 128.68, 130.05, 132.21, 152.08, 162.56. MS (m/z): 232.4 (M+2) (95%), 230.4 (M^+) (100%), 177.3 (36%).

Synthesis of 3-Methyl-5-(5-bromo-2-thienyl)isoxazole (1*l*): (Method B)

3-Dimethylamino-1-(2-thophenyl-5-bromo)-2-buten-1-one (**2*l***) was used as substrate. Product was isolated by crystallization from *n*-hexane. Yield: 72%, mp. 79.8-80.2 °C; purity (HPLC): 99.43%; IR (KBr): cm^{-1} 3416.0, 3095.1, 2976.0, 1611.8, 1476.0, 1443.0, 1413.8. ^1H NMR (DMSO-D6/TMS): δ 2.26 (s, 3H), 6.76 (s, 1H), 7.38 (d, 1H, J=3.6 Hz), 7.51 (d, 1H, J=3.6 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.45, 101.54, 115.37, 128.77, 130.53, 132.53, 161.50, 163.17. MS (m/z): 246.4 (M+2) (94%), 244.4 (M^+) (100%), 185.4 (6%), 184.6 (17.5%), 165.4 (24%).

Synthesis of 5-(2-furyl)isoxazole (1*m*): (Method B)

3-Dimethylamino-1-(2-furanyl)-2-propen-1-one (**2*m***) was used as substrate. Product was isolated by column chromatography using ethyl acetate: *n*-hexane (1:10) as eluent. Yield: 57%, oily crude; purity (HPLC): 99.37%; IR (KBr): cm^{-1} 3133.2, 1641.1, 1622.1, 1492.5, 1447.5. ^1H NMR (DMSO-D6/TMS): δ 6.74 (dd, 1H, J=1.6 Hz, 3.2 Hz), 6.81 (d, 1H, J=1.6 Hz), 7.16 (d, 1H, J=3.2 Hz), 7.97 (d, 1H, J=1.6 Hz), 8.70 (d, 1H, J=2.0 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 99.34, 111.33, 112.65, 142.78, 145.40, 151.59, 160.52. MS (m/z): 136.3 (M+1) (100%), 102.1 (20%).

Synthesis of 3-Methyl-5-(2-furyl)isoxazole (1*n*): (Method B)

3-Dimethyl amino-1-(2-furanyl)-2-buten-1-one (**2*n***) was used as substrate. Product was isolated by column chromatography using ethyl acetate: *n*-hexane (1:1) as eluent. Yield: 72%, oily crude; purity (HPLC): 99.20%; IR (KBr): cm^{-1} 3135.7, 2933.6, 1644.2, 1626.3, 1546.5, 1503.1, 1455.4, 1416.9. ^1H NMR (DMSO-D6/TMS): δ 2.28 (s, 3H), 6.64-6.72 (m, 2H), 7.09 (dd, 1H, J=1.2Hz, 2.8 Hz), 7.93 (d, 1H, J=1.6 Hz). ^{13}C NMR (DMSO-D6/TMS): δ 11.02, 100.55, 111.00, 112.55, 142.80, 145.28, 160.54, 160.76. MS (m/z): 150.3 (M+1) (100%), 149.3 (8%), 123.2 (7%), 122.2 (40%).

Antibacterial activity studies-Cup Plate method

Media

All experiments with E. Coli, Pseudomonas aeruginosa, Staphylococcus aureus, were carried out in media as follows: Bactopeptone (6.0 g), pancreatic digest of casein (4.0 g) yeast extract (3.0 g), beef extract (1.5 g), dextrose anhydrous (1.0 g), agar (15 g) are dissolved in 1000 mL of distilled water and pH adjusted to 6.6[13].

Evaluation of antibacterial activity

A solution of the experimental isoxazole was prepared

in DMSO at a concentration of 200 mg/mL under aseptic conditions. All the solutions were sterilized by membrane filtration. Solid agar media were used for all the test organisms. Antimicrobial activity of the isoxazoles against different strains of bacteria was determined by cup plate method and the activity was expressed in terms of zone of inhibition in mms. Inoculum was prepared by washing a medium slant of test organisms with 5 ml of fresh aq. NaCl and further diluting 1 mL to 10 mL to make inoculum containing 10^6 CFU/mL. This suspension was added to 25 ml melted medium at temp 45-50 °C and plates were prepared. Holes of diameter 6 mm were made into the agar plates with sterile borer and filled with the drug. The plates were incubated at 35 °C for 24 hrs. The results were compared by using sulfamethoxazole as reference standard.

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REFERENCES

1. Abdel Rehman, M. M.; Mangouma, S. A.; EL-Bitar, H. L. *Bull. Pharm. Sci.* **1990**, *13*, 137.
2. He, F.; Snider, B. B. *J. Org. Chem.* **1999**, *64*, 1397.
3. Whest, H. M.; Hoffer, M. *US 2430094*, 1947.
4. Wornser, G. P.; Keusch, G. T.; Hell, R. C. *Drugs* **1982**, *24*, 459.
5. Doyle, F. P.; Long, A. A. W.; Nayler, J. H. C.; Stove, E. R. *J. Chem. Soc*. **1963**, 5838.
6. Doyle, F. P.; Long, A. A. W.; Nayler, J. H. C.; Stove, E. R. *Nature* **1961**, *192*, 1183.
7. Gelders, Y. G.; Heylen, S. L. E.; Bussche, G. V.; Reyntjens, A. J. M.; Janssen, P. A. J. *Pharmacopsychiatry* **1990**, *23*, 206.
8. Strupczehki, J. T.; Allen, R. C.; Gardner, B. A.; Schmid, B. L.; Stache, U.; Glamkowski, E. J.; Jones, M. C.; Ellis, D. B.; Huger, F. P.; Dunn, R. W. *J. Med. Chem*. **1985**, 761.
9. Elena, B.; Ricardo, B.; Francesco, D. S.; Stefano, M.; Roberto, P. *Heterocycles* **1983**, *20*, 501.
10. Takao, S.; Yoshinori, K.; Daishi, U.; Hiroshi, Y. *Tetrahedron* **1991**, *47*, 5111.
11. Nilda, M. S.; Jorge, L. M T.; Ana, L. P. M.; Eliezer, J. B.; Carlos, A. M. F. *Eur. J. Med. Chem.* **2002**, *37*, 163.
12. Georgy, B. B.; Robert, B. M.; Lee, J. A.; James, B. R. Jr. *J. Med. Chem.* **1978**, *21*, 623.
13. Takeoueda, Kiyoshi, T.; Shigeshi, T.; Seizaburo, K. *The Pharmaceutical Society of Japan*. **1952**, *1*, 16.