Multicomponent synthesis and invitro study of phenyl isoxazole-4-carbonitrile Derivatives Promoted by Transition metal acetates as a catalyst

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ABSTRACT:

A variety of 5-amino-3-phenylisoxazole-4-carbonitrile analogues are produced using a simple new pathway and biological method, followed by a traditional technique. Aromatic aldehyde, malononitrile, and hydroxylamine hydrochloride in the presence of the transition metal acid catalyst Cu $(OAc)_2$ in acetonitrile as a solvent at reflux would yield these derivatives. Advanced spectroscopic analyses including ^{1}HNMR, ^{13}CNMR, and LCMS were used to analyze all the desired analogues, and elemental analysis was used to determine the structural identity of the titled analogues. Additionally, the produced compounds' antimicrobial activity was assessed.

KEYWORDS:

Malanonitrile, hydroxylamine hydrochloride, aromatic aldehyde, 5-amino-3-phenylisoxazole-4-carbonitrile, Cu $(OAc)_2$, antibacterial activity

1. INTRODUCTION:

A wide variety of functionalized heterocyclic scaffolds and their synthesis are significant to medicinal chemists as they provide the ability to expand the available drug-like chemical space. Isoxazole are heterocyclic compounds with five members that contain two heteroatoms, such as nitrogen and oxygen. The isoxazole and its derivatives are an important intermediate for the synthesis of new chemicals in medicinal chemistry, which have been increased in last decades. Isoxazole nucleus had shown a wide spectrum of biological activity. Easy synthesis of isoxazole ring has been as an object of interest for the scientist from research group all over the world. The synthesis of isoxazole compounds have resulted in multiple corresponding antibacterial drugs in the market, Flucloxacillin, Dicloxacillin, Leflunomide, Risperidone, Oxacillin, Sulfamethoxazole, and Cloxacillin) and there are many patents related with isoxazole and Numerous naturally occurring and physiologically active compounds are known to have the isoxazole nucleus system [1]. The isoxazole class includes several antibacterial medications, and they are especially helpful in medicinal chemistry [2]. Two bacteriostatic sulfonamide antibiotics, isoxazole and its derivatives, were used either by themselves or in combination with other antibiotics to treat infections caused by Gram-(+) and Gram-(-) bacteria [3, 4].

Acivicin, an inhibitor of the γ-glut amyl transferee, has antileishmanial, anticancer, and anti-parasitic properties [5]. Numerous pharmacological characteristics, including antifungal, anti-inflammatory, antiplatelet, anti-HIV, anti-Alzheimer, and analgesic, were present in isoxazole derivatives [6–11].

Copper acetate was effectively employed as a catalytic medium in the synthesis of series 5-amino-isoxazole-4-carboni-trile homologous through a multicomponent reaction of malononitrile, hydroxylamine, and other aromatic aldehyde in order to discover applications of other heterocycles. All compounds' in vitro inhibitory efficacy was assessed against a variety of harmful microorganisms, including

2. METHODS AND MATERIALS:

2.1. EXPERIMENTAL:

All starting materials, solvents, and reagents were purchased from commercial sources, including fine chemicals, and were utilized without additional purification. The source of the bacterial culture media was (HI Media). The Agrawal melting point meter was used to assess the newly comparable melting points, which are uncorrected. TLC plates precoated with SiO2 and fluorescent indicator F254 employing EtOAc/n-hexane (3:7) as the mobile phase were used to visualize the reaction process under UV light. A Bruker FT-NMR Ultra Shield-400 spectrometer was used to measure the 1H and 13C NMR spectra at 400 and 100 MHz, respectively. A Thermo Finnigan Flash EA micro analyzer was used to conduct elemental tests (CHNS/O).

2.2. GENERAL PROCEDURE FOR THE SYNTHESIS OF5-AMINO-3-PHENYLISOXAZOLE-4-CARBONITRILE (4a-4i):

As a 50 mL RBF, a combination of malononitrile (1), aromatic aldehydes (2), and hydroxylamine hydrochloride (3) is dissolved in 30 mL ethanol. During the reaction, an acid catalyst, such as Cu $(OAc)_2$ (2 mmol), is gradually added, and the reaction is continued for six hours. TLC was used to track the reaction's development (EtOAc: n-hexane-5:5). After the reaction was finished, cold water was added, neutralized with a saturated NaHCO3 solution, extracted with ethylacetae, and the organic layer was separated. The organic layer was extracted as a byproduct of vacuum distillation.

2.2.1.5-amino-3-phenylisoxazole-4-carbonitrile (4a):

Orange color solid ; Yield: 85 %; M.P : 194–196 °C; ^{1}H NMR (400 MHz, CDCl3) δ ppm: 7.074 (d, J = 7.6 Hz, 2H,Ar-H), 7.758 (d, J = 5.8 Hz, 2H, Ar-H), 8.224 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δppm: 74.85, 114.04 , 117.15, 120.08 , 126.66, 131.35, 161.18, 167.98; Molecular

weight(m/z) : 185.57(M+); Molecular formulae $C_{10}H_7N_3O$: Analysis of elements: Calculated : C-64.85, H- 3.81, N- 22.68. Obtained: C- 64.81, H -3.79, N- 22.72.

2.2.2.5-Amino-3-(4-hydroxyphenyl) isoxazole-4-carbonitrile (4b):

Pale orange color crystals; Yield: - 92%; M.P- 203–205°C;^{1}HNMR (400 MHz, $CDCl_3$) δ ppm :7.174 (d, J = 8.0Hz, 2H,Ar-H), 7.754(d, J = 6.8 Hz, 2H, Ar=H′), 8.156 (s, 2H, NH_2), 9.924 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 78.12, 115.08, 118.08, 119.45, 125.50, 136.21, 159.95, 168.47; Molecular weight(m/z) ; 201.58 (M+); Molecular formulae : $C_{10}H_7N_3O_2$: Analysis of elements: Calculated: C-59.70, H-3.51, N -20.89. Obtained: C -59.63, H-3.50, N-20.96.

2.2.3.5-Amino-3-(2-hydroxy-3-methoxyphenyl) isoxazole-4-carbonitrile (4c):

orange color solid ; Yield- 87%; M.P- 214–216°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.041 (s, 3H, CH_3), 7.271–7.398 (m, 3H, Ar-H), 8.024 (s, 2H, NH_2), 9.354 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δppm: 55.74, 105.58 , 116.47 , 119.59 , 120.71 , 124.05 , 127.28 , 143.07 , 145.65, 156.47 , 159.05 ; Molecular weight(m/z):23295 (M+H); Molecular formulae : $C_{11}H_9N_3O_3$: Analysis of elements: Calculated C- 57.14, H- 3.92, N -18.17. Obtained: C -57.08, H- 3.90, N 18.25.

2.2.4.5-Amino-3-(4-methoxyphenyl) isoxazole-4-carbonitrile (4c):

Brown color solid ; Yield- 89%; M.P- 225–227°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.847 (s, 3H, CH_3), 3.741 (m, 3H, OCH_3), 7.198–7.374 (m, 3H, Ar-H), 8.487 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δppm: 54.74, 106.58 , 118.47 , 120.58 , 121.88 , 125.25 , 128.29 , 142.87 , 146.67, 157.77 , 160.17 ; Molecular weight(m/z):218.25 (M+H); Molecular formulae : $C_{11}H10N_3O2$: Analysis of elements: Calculated C- 58.58, H- 4.08, N -15.28. Obtained: C -58.51, H- 4.06, N-15.35

2.2.5.5-Amino-3-(4-tolyl) isoxazole-4-carbonitrile (4d):

Pale orange color powder; Yield:88%; M.P: 221–223°C; ^{1}HNMR (400 MHz, $CDCl_3$) δ ppm : 2.212 (s, 3H, CH_3), 7.334 (d, J = 7.6 Hz, 2H, Ar-H), 7.617 (d, J = 7.4 Hz, 2H, Ar-H ′), 8.232 (s, 2H, NH_2); ^{13}CNMR (100 MHz, $CDCl_3$) δppm: 22.87 , 82.22 , 115.14, 117.86, 128.41, 131.65, 132.58 ,149.85, 163.77; Molecular weight(m/z) ; 166.58(M+H);Molecular formulae : $C_{11}H_9N_3O$: Analysis of elements: Calculated: C-66.32, H- 4.55, N-21.09. Obtained: C- 66.27, H- 4.53, N 21.16

2.2.6.5-Amino-3-(2, 4-dichlorophenyl) isoxazole-4-carbonitrile (4e):

Orange color solid; Yield-85%; M.P-231–236°C; ^{1}HNMR (400 MHz, $CDCl_3$) δ ppm : 7.680 (s,1H,Ar-H), 7.857 (s, 1H, Ar-H), 8.134 (d, J = 8.8Hz, 1H, Ar-H), 8.275 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 88.54 , 114.33 , 126.57 , 128.70 , 129.41 , 130.04 , 132.66, 138.75 , 148.87 , 157.67 ; Molecular weight(m/z) ; 255.74 (M+2); Molecular formulae ; $C_{10}H_5Cl_2N_3O$:

Analysis of elements: Calculated: C -42.27, H-1.98, N-16. 54. Obtained: C -42.20, H- 1.96, N-16.62.

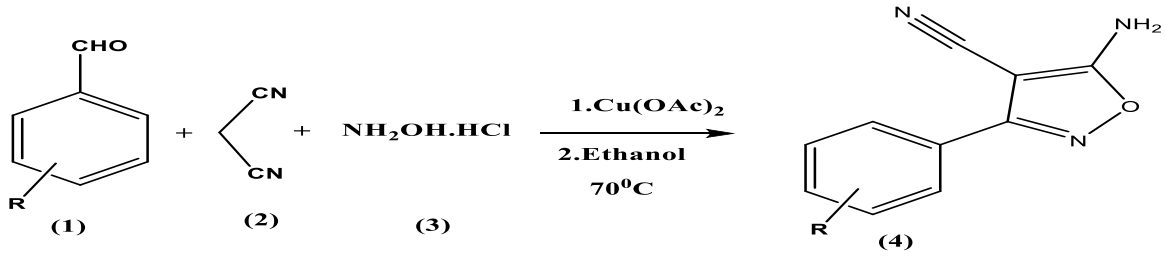
2.2.7. 5-Amino-3-(4-Bromophenyl) isoxazole-4-carbonitrile (4f):

Orange red color compound ; Yield-87%; M.P-239-241°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 1.884(s,3H,CH3), 7.945 (d, J = 7.2 Hz, 2H, Ar-H), 8.025-8.324 (s, 2H, Ar-H), 8.347 (m, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 87.74 , 119.45 , 125.24 , 128.74 ,129.34, 136.78 , 148.65 , 149.44 , 155.65 ; Molecular weight(m/z): 256.77(M+2); Molecular formulae: $C_{10}H_6BrN_4O_3$: Calculated: C-52.18, H- 2.63, N-24.34. Obtained: C- 52.12, H- 2.59, N- 24.37.

2.2.8. 5-Amino-3-(4-nitrophenyl) isoxazole-4-carbonitrile (4f):

Orange color solid ; Yield-82%; M.P-218-220°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.894 (d, J = 9.2 Hz, 2H, Ar-H), 8.214 (s, 2H, Ar-H), 8.447 (m, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δppm : 81.74 , 118.45 , 126.24 , 128.74 , 137.16 , 147.65 , 148.44 , 154.65 ; Molecular weight(m/z): 232.54(M+H); Molecular formulae: $C_{10}H_6N_4O_3$: Calculated: C-52.18, H- 2.63, N-24.34. Obtained: C- 52.12, H- 2.59, N- 24.37.

3. RESULTS AND DISCUSSION:



(4a-4i)

R = H, 4-OH, 4-OCH_3 , 4-CH_3, 4-OH-3-OCH_3, 2,4-$(Cl)_2$, 4-Br, 4-NO_2

(Scheme-1)

First, the reaction mixture of malononitrile (1 mmol), substituted aryl aldehyde (1.2 mmol), and hydroxylamine hydrochloride (1 mmol) was taken in ethanol (30 ml) in 50 mL RBF and fitted on a magnetic stirrer to prepare 5-amino-3-phenylisoxazole-4-carbonitrile derivatives. In an RBF, the catalytic amount of Cu $(OAc)_2$ (3 mmol) was gradually introduced. For six hours at reflux, the reaction mixture was vigorously stirred. The synthetic process's broad substrate range, ease of handling, and readily available, reasonably priced catalyst are its main benefits. As illustrated by scheme I, we synthesized a wide range of 5-amino-3-phenylisoxazole-4-carbonitrile using a considerable number of derivatives and ideal reaction conditions. These analogs' reaction conditions were tuned at multiple catalyst types, catalyst concentrations, and solvent types. Compared to oxidative related catalysts like AgOAc, $Zn(OAc)_2$, and NH4OAc, the highest yield

of compounds was produced in the presence of Cu (OAc)2 catalyst, despite varying amounts of catalyst used throughout the process (Table 1).

Table-1: Effective the various catalysts for the titled derivatives.

Entry	Various catalyst	Time (hrs)	Yield (%)
1	AgOAc	6	64
2	$Zn(OAc)_2$	6	68
3	NH4OAc	6	74
4	Cu(OAc)2	6	92

During the reaction, the different amount of catalyst was applied completion of the reaction, initially 0.1 mmol added in the reaction , traces of product was obtained and gradually increase the amount of catalyst added and slowly increases product obtained. This indicated that 2.0mmol of the $Cu(OAc)_2$ was used in these reaction better results was obtained compared to same amount of other catalyzed as shown table-2.

Table-2: different amounts of catalyst in Ethanol at reflux (4b):

Entry	Amount of catalyst (%)	Time (hrs)	Yield (%)
1	1.0	10	Traces
2	2.0	12	45
3	3.0	05	92
4	4.0	08	92

Usually, the various solvents used in during this reaction, ethyl alcohol is suitable solvent and perfectly maintained reaction compared to the other solvents such as methanol, DMF and Acetonitrile. An ethanol is the best solvent utilizing during the reaction, the advantages of the reaction are no pollution effects, easy to work up and there is no wastage of yield as shown Table-3.

Table-3: The effect of solvents for titled derivatives at reflux (4b) :

Entry	Various Solvent	Time (hrs)	Yield (%)
1	Ethanol	05	92
2	MeOH	05	68
3	Acetonitrile	05	70
4	DMF	05	64

CHARACTERIZATION:

The structure of the titled analogous was performed by the evidence of spectral analysis such as, ^{1}HNMR, ^{13}CNMR, LCMS and elemental analysis. In this study, proton NMR of titled derivatives exhibited by various values of respective groups such as hydroxyl proton is 8.147 δppm, furan is 6.874-8.217δppm, Thiophene is 7.210-7.818δppm, methyl protons 2.258 δppm, , 9.975 δppm of NH_2 protons as well as aromatic protons 7.892-8.254δppm appeared at various range of values.13CNMR of these derivatives appeared at different values.

4. BIOLOGICAL ACTIVITY:

The results of the above table-4 represented that the *anti-bacterial activity* of derivatives 4b, 4c, 4d mostly electron donating group of compounds viz; these derivatives exhibited good active potent while electron withdrawing group of compounds “4f and 4g” displayed an excellent active potent. The compound 4b,4d and 4e exhibited moderate active potential due to Nitro groups present in the compound.

Table-4: Antimicrobial activity screening activity synthesized scaffold (4a-4i):

Entry	Bacteria			
	S.aureus	E.coli	S. typhi	B.substills
4a	08	06	08	07
4b	15	18	20	16
4c	17	19	21	22
4d	20	17	19	18
4e	14	16	18	15
4f	24	23	23	20
4g	23	21	22	22
4h	15	13	10	13
Streptomycin	27	27	25	25
Fluconazole	NA	NA	NA	NA
DMSO	---	----	---	---

5. CONCLUSION:

In conclusion, a novel and practical one-pot synthesis of 5-amino-3-phenylisoxazole-4-carbonitrile analogues via multi-component processes has been revealed by this investigation of named derivatives. Along with a number of additional benefits, such as a fast reaction time, straightforward experimental workup procedures, and the absence of hazardous byproducts, this copper acetate acid catalyst reaction proceeded easily and produced an outstanding yield. The method for generating derivatives systems described here avoids the use of hazardous organic solvents and catalysts. For the synthesis of this family of chemicals, this technique offers a

promising environmentally friendly path. Additionally, the named derivatives' antibacterial activity was investigated. Excellent active potential was shown by derivatives with electron-withdrawing groups

6. AKOWNLDEMENT:

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