International Conference on Emerging Trends in Chemical Sciences, Department of Chemistry, Manipal Institute of Technology, Manipal University, Manipal (14-16 September 2017)
Journal of Chemical and Pharmaceutical Sciences ISSN (Print 0974-2115) (Online 2349-8552)

Novel Isoxazoline-1, 2, 4-oxadiazoles: Synthesis, Characterization and Antimicrobial screening

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ABSTRACT

We have synthesized 5-substituted-3-(3-substituted-4, 5-dihydroisoxazol-5-yl)-1, 2, 4-oxadiazole 4(a-f) and studied antimicrobial activities. The *5*-substituted-1, 2, 4-oxadiazole were obtained by treating benzaldehyde oxime 1(a-c) with acrylonitrile in presence of oxidizing agent chloramine-T (CAT) to give isoxazoline 2(a-c), the isoxazoline 2(a-c) is react with NH_2OH HCl in presence of Na_2CO_3 to give the corresponding isoxazoline-amidoxime 3(a-c). Finally, the amidoxime 3(a-c) coupled with aromatic acid in presence of coupling agent EDC. HCl to give isoxazoline-1, 2, 4-oxadiazole 4(a-f). The newly synthesized 1, 2, 4-oxadiazole 4(a-f) have been characterized by elemental analysis and spectral studies.

KEY WORDS: Isoxazoline, Amidoxime, 1, 2, 4-Oxadiazole, Antimicrobial Activity.

1. INTRODUCTION

Isoxazoles are five membered heterocycles which contain nitrogen and oxygen in the ring. Due to their outstanding biological portrait, A- and D-ring coalesce heterosteroids with isoxazole moieties were prepared and many of which are emerged as potent antimicrobial agents (Shyamalee, 2012). Apart from this, isoxazoles show various biological activities such as antiviral, anticancer (Yong, 2015), antituberculosis (Jialin, 2010), anti-HIV (Srirastara, 1999), anticonvulsant and immune modulating activities (Giovannoni, 2003).

1, 2, 4-oxadiazole is an important motif show antitrypanosomal activity (Filho, 2009), peptide inhibitory activity (Borg, 1993), and also acts as anti-inflammatory agents (Nicolaides, 1998). Furthermore, in 2017 several new class of 1, 2, 4-oxadiazole based antibiotics and anticancer agents were described (Sergey, 2017).

Heterocyclic moieties with biological interest (Umesha, 2017), we synthesized the isoxazoline based 1, 2, 4-oxadiazole and their antimicrobial studies. Potent 1, 2, 4-oxadiazole with C-5 position of isoxazoline compounds to show the good result of antimicrobial activities.

2. MATERIALS AND METHODS

All chemicals were used without further purification. Aluminum sheets with pre-coated silica gel 60 F_{254} (0.2 mm, Merck) was used for Thin-layer chromatography (TLC) and their spots were observed by using iodine chamber and UV light. FT-IR Shimadzu 8300 spectrometer, Hewlett-Packard HP GS/MS 5890/5972 and Bruker Avance (400 MHz ^{1}H NMR) instrument were used for spectral studies.

Antimicrobial activity: *S*ynthesized compounds isoxazoline-amidoxime 3(a-c) and *bis*-heterocycles 4(a-f) were tested (dose of 100µg) for *in vitro* anti-bacterial activity against *Bacillus cereus, Staphylococcus aureus (gram positive), Escherichia coli, Klebsiella pneumonia and Shigella flexneri* (gram negative) by disc diffusion method (Simmons, 1996). Organism was inoculated into nutrient agar for 24 h in an incubator at 37ºC and Chloramphenicol was used as a positive control. Also compounds 3(a-c) and 4(a-f) were screened (dose of 100µg) for antifungal activity against *Aspergillus flavus* and *Aspergillus niger* using Fluconazole as a standard drug. After the period of incubation the inhibition zones were measured in mm (Table.1).

Synthesis of 3-phenyl-4,5-dihydroisoxazole-5-carbonitriles (2a-c): (Rai, 1997): Isoxazole-5-carbonitrile 2a was achieved by refluxing aldoxime (1a, 1.0g, 0.0082 mol) and acrylonitrile (1.3g, 0.0246 mol) with oxidizing agent chloramine-T (2.77 g, 0.0098 mol) in alcohol for about 3h to give a white solid isoxazole-5-carbonitrile (2a, 1.10g).

Synthesis of (Z)-N'-hydroxy-3-phenyl-4, 5-dihydroisoxazole-5-carboximidamide (3a-c): (Srikantamurthy, 2013): A mixture of isoxazole-5-carbonitrile (2a, 1.0g, 0.0058 mol), $NH_2OH.HCl$ (2g) and Na_2CO_3 (2g) in dilute ethanol (50%) were refluxed on water bath for about 4 to 5 hours to give light yellow solid isoxazoline-amidoxime (3a. 1.07g).

Synthesis of 5-phenyl-3-(3-phenyl-4, 5-dihydroisoxazol-5-yl)-1, 2, 4-oxadiazole (4a-f): (Kayukova, 2010): A mixture of benzoic acid (0.5g, 0.004mol) in dry CH_2Cl_2 (5ml) and ethyl-(*N', N'*-dimethylamino) propyl-carbodiimide hydrochloride (EDC. HCl) (0.94g, 0.004mol) was cooled to 10-15°C and stirred for 30 min. Then isoxazoline-amidoxime (3a, 0.84g, 0.004mol) was added to the reaction mixture for another 30min and then it was boiled on water bath about 110°C for 8 h. After the reaction the mixture was cooled to 25ºC and extracted with ethyl acetate (2 x 25 ml). Ethyl acetate was distilled off and the obtained product was purified by column chromatography

(*n*-hexane/EtOAc 95/05) to give 4, 5-dihydroisoxazol-1,2,4-oxadiazole with 78% yield (4a, 0.93g). The same procedure was used in all cases.

Spectral data of compound 4(a-f): 5-phenyl-3-(3-phenyl-4,5-dihydroisoxazol-5-yl)-1,2,4-oxadiazole 4a.

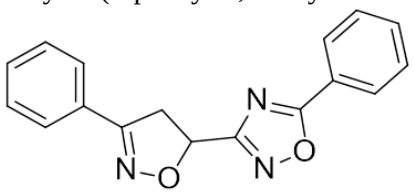


Figure.1. Structure of the compound 4a

IR: 1620 cm^{-1} (C=N), 1270 cm^{-1} (C-N), 1236 cm^{-1} (C-O); ^1H NMR (DMSO-d_6): δ 3.10-3.20 (dd, 1H), 3.64-3.67 (dd, 1H), 4.60-4.64 (dd, 1H), 7.22-7.55 (m, 10H); MS: *m/z* 292.1 $(M+1)^+$. Anal. % Calc: C, 70.10; H, 4.50; N, 14.43; Found; C, 70.09; H, 4.30; N, 14.62.

3-(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)-5-phenyl-1,2,4-oxadiazole 4b.

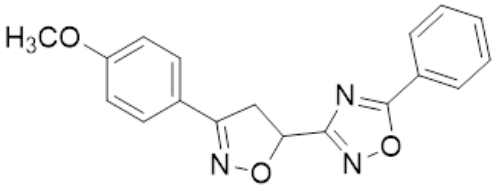


Figure.2. Structure of the compound 4b

IR: 1625 cm^{-1} (C=N), 1275 cm^{-1} (C-N), 1244 cm^{-1} (C-O), 2830 cm^{-1} (O-CH_3); ^1H NMR (DMSO-d_6): δ 3.11-3.20 (dd, 1H), 3.61-3.66 (dd, 1H), 3.88 (s, 3H, -OCH_3), 4.61-4.65 (dd, 1H), 7.25-7.59 (m, 9H, Ar-H); MS: *m/z*322.2 $(M+1)^+$. Anal. % Calc: C, 67.30; H, 4.71; N, 13.08; Found; C, 67.30; H, 4.69; N, 13.10.

3-(3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-5-phenyl-1,2,4-oxadiazole 4c.

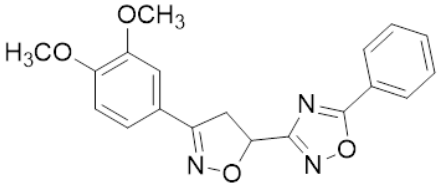


Figure.3. Structure of the compound 4c

IR: 1628 cm^{-1} (C=N), 1272 cm^{-1} (C-N), 1238 cm^{-1} (C-O), 2832 cm^{-1} (O-CH_3); ^1H NMR (DMSO-d_6): δ 3.15-3.27 (dd, 1H), 3.63-3.66 (dd, 1H), 3.85 (s, 6H, -OCH_3), 4.60-4.65 (dd, 1H), 7.23-7.66 (m, 8H, Ar-H); MS: *m/z* 352.4 $(M+1)^+$. Anal. % Calc: C, 64.95; H, 4.90; N, 11.96; Found; C, 64.95; H, 4.88; N, 11.90.

5-(2-methoxyphenyl)-3-(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1,2,4-oxadiazole 4d.

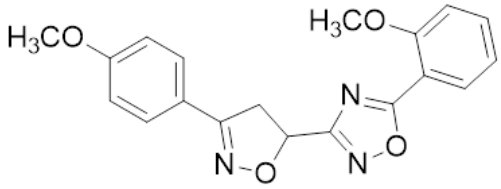


Figure.4. Structure of the compound 4d

IR: 1620 cm^{-1} (C=N), 1268 cm^{-1} (C-N), 1242 cm^{-1} (C-O), 2836 cm^{-1} (O-CH_3); ^1H NMR (DMSO-d_6): δ 3.10-3.20 (dd, 1H), 3.63-3.66 (dd, 1H), 3.84 (s, 6H, -OCH_3), 4.59-4.64 (dd, 1H), 7.24-7.68 (m, 8H, Ar-H); MS: *m/z* 352.3 $(M+1)^+$. Anal. % Calc: C, 64.95; H, 4.88; N, 11.97; Found; C, 64.90; H, 4.88; N, 11.95.

5-(3,4-dimethoxyphenyl)-3-(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1,2,4-oxadiazole 4e.

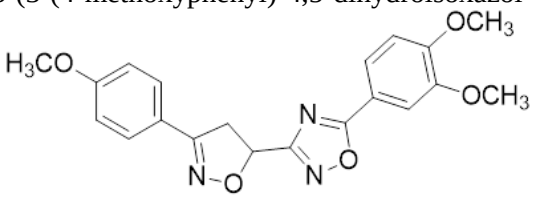


Figure.5. Structure of the compound 4e

IR: 1621cm^{-1} (C=N), 1249 cm^{-1} (C-N), 1240 cm^{-1} (C-O), 2828 cm^{-1} (O-CH_3); ^1H NMR (DMSO-d_6): δ 3.13-3.25 (dd, 1H), 3.65-3.69 (dd, 1H), 3.81 (s, 9H, -OCH_3), 4.59-4.64 (dd, 1H), 7.23-7.78 (m, 7H, Ar-H); MS: *m/z* 382.3 $(M+1)^+$. Anal. % Calc: C, 63.00; H, 5.02; N, 11.02; Found; C, 62.98; H, 5.04; N, 11.00.

5-(3,4-dimethoxyphenyl)-3-(3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl)-1,2,4-oxadiazole 4f.

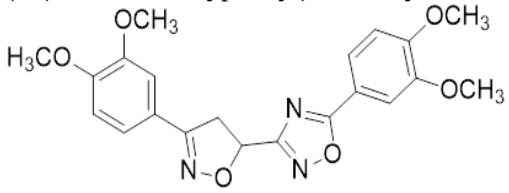
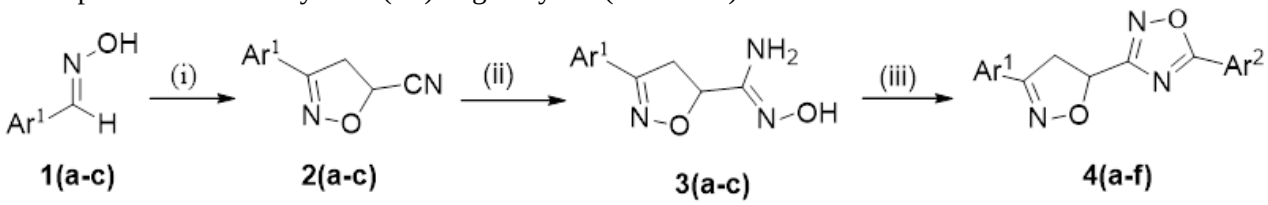


Figure.6. Structure of the compound 4f

IR: 1626cm^{-1} (C=N), 1244 cm^{-1} (C-N), 1244 cm^{-1} (C-O), 2830 cm^{-1} (O-CH_3); ^{1}H NMR (DMSO-d_6): δ 3.13-3.20 (dd, 1H), 3.65-3.70 (dd, 1H), 3.80 (s, 12H, -OCH_3), 4.60-4.66 (dd, 1H), 7.28-7.82 (m, 6H, Ar-H); MS: *m/z*412.5 $(M+1)^+$. Anal. % Calc: C, 61.31; H, 5.14; N, 10.21; Found; C, 61.30; H, 5.15; N, 10.18.

3. RESULTS AND DISCUSSION

The synthesized amidoximes 3(a-c) were coupled with aromatic acids using EDC.HCl in dichloromethane to give respective *bis*-heterocycles 4(a-f) in good yield (Scheme.1).



Scheme.1. Reagents and conditions: (i) acrylonitrile, Chloramine-T, EtOH, reflux 3h; (ii) $NH_2OH.HCL$, Na_2CO_3, aq. EtOH, reflux, 5-6h; (iii) Ar^2-COOH, EDC, rt for 6h then reflux for 8h.

For instance, in IR spectra the appearance signals shows at 1628-1620 cm^{-1} for (C=N), 1275-1244 cm^{-1} for (C-N), and 1244-1236 cm^{-1} for (C-O) and absence of NH_2 stretch at 3248 cm^{-1}. Similarly the ^{1}H NMR spectra of the isoxazoline ring exhibited a typical ABX spin system with H-atom attached to 5-carbon atom of the ring as a doublet of doublets in the range of δ 3.1 to 3.6 and also the absence of NH_2 and OH peak at 5.49 and 9.30 ppm respectively. Finally, it confirms the formation of isoxazoline-1, 2, 4-oxadiazoles (4a-f).

Antimicrobial activity: The synthesized compounds showed good antibacterial activity against the tested strains of bacteria except *S. flexneri* and moderate activity against *S. aureus* and *E.coli*. The 3, 4-dimethoxy substituted derivatives 3c, 4c, 4e and 4f exhibited an excellent antibacterial activity compare to standard chloramphinicol. This revealed that 3,4-dimethoxy substituent on benzene ring of both heterocycles increase the antibacterial activity and all compounds 3a-c and 4a-f exhibit good antifungal activity against *A.niger* but were weakly active against *A. flavus.*

Table.1. Antibacterial activity of synthesized isoxazolyl-1, 2, 4-oxadiazole derivatives 3(a-c) and 4(a-f)

Compounds	*Antibacterial activity*					*Antifungal activity*	
	Gram positive		*Gram negative*				
	B. cereus	***S. aureus***	***E. coli***	***K. pneumonia***	***S. flexneri***	***A. flavus***	***A. niger***
3a	08 ± 0.27	06 ± 0.11	10 ± 0.10	09 ± 0.08	08 ± 0.10	12 ± 0.12	14 ± 0.11
3b	08 ± 0.25	06 ± 0.24	10 ± 0.22	09 ± 0.19	08 ± 0.11	16 ± 0.23	12 ± 0.20
3c	10 ± 0.11	08 ± 0.17	14 ± 0.21	10 ± 0.13	10 ± 0.22	14 ± 0.22	30 ± 0.18
4a	08 ± 0.21	10 ± 0.16	08 ± 0.09	10 ± 0.18	10 ± 0.18	14 ± 0.09	16 ± 0.14
4b	09 ± 0.18	09 ± 0.11	10 ± 0.11	08 ± 0.11	08 ± 0.16	18 ± 0.11	20 ± 0.08
4c	12 ± 0.16	10 ± 0.19	16 ± 0.18	14 ± 0.12	14 ± 0.12	12 ± 0.19	26 ± 0.10
4d	08 ± 0.13	08 ± 0.10	10 ± 0.14	08 ± 0.21	08 ± 0.20	10 ± 0.20	22 ± 0.11
4e	12 ± 0.19	12 ± 0.20	14 ± 0.13	10 ± 0.26	10 ± 0.19	10 ± 0.18	28 ± 0.16
4f	09 ± 0.14	12 ± 0.16	14 ± 0.17	16 ± 0.16	18 ± 0.14	12 ± 0.16	30 ± 0.23
Chloramphenicol	14 ± 0.18	12 ± 0.14	18 ± 0.06	20 ± 0.09	24 ± 0.21	-----	-----
Fluconazole	-----	-----	-----	-----	-----	28 ± 0.08	30 ± 0.11

[a]Zone of inhibition (Mean six replicate ± standard deviation).

4. CONCLUSIONS

We synthesized the simple and novel isoxazolyl-amidoxime 3(a-c) and its *bis*-heterocycles 4**(**a-f) have been investigated for their *in-vitro* antibacterial and antifungal activity. In our newly synthesized compounds, it is cleared that the highest antimicrobial activity for compound 4c 4e and 4f were observed. Compare to antibacterial and antifungal activity, especially the compound 3c showed better antifungal activity against *A.niger*. The results suggest that these isoxazolyl-amidoxime 3(a-c) and its *bis*-heterocycles 4a-f as a valuable and useful antibacterial and antifungal agents.

5. ACKNOWLEDGEMENT

One of the authors Dr. K. B. Umesha, thanks to the University Grant Commission for Sanctioned the Minor Research Project (Science).

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