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Research Article

SYNTHESIS, CHARACTERIZATION AND MICROBIAL SCREENING OF ISOXAZOLE DERIVATIVES OF 2, 6-DICHLORO-1-(*N*-SUBSTITUTED PHENYL)-1, 4-DIHYDROPYRIDINE-3, 5-DICARBALDEHYDE

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ABSTRACT

A series of new isoxazoles **5a-f** were prepared by reaction of propenones **4** with hydroxylamine hydrochloride while the propenones **4a-f** were prepared by the condensation of 2, 6 – dichloro -1- (*N*-substituted phenyl)-1, 4 –dihydropyridine -3, 5 – dicarbaldehydes **3** with different aromatic ketones. The structures of the newly synthesized compounds have been confirmed on the basis of elemental analysis and spectral studies. The newly synthesized title compounds have been screened for their in *vitro* antimicrobial activities. Some of the compounds exhibited encouraging results.

Keywords: Dihdropyridines, Propenones, Isoxazoles, Antimicrobial activity.

INTRODUCTION

Dihydropyridines and their derivatives are an important class of bioactive molecules in the pharmaceutical field[1,2]. The development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities. Isoxazole is a five membered heterocyclic ring system containing oxygen and nitrogen atoms. In recent years, the synthesis of novel isoxazole derivatives remains a main focus of medicinal research[3]. Isoxazole have been reported to posses anthelmantic[4], antibacterial[5,6], antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antiviral, antitumor, antifungal and antidepressant activities[7-12]. Among aromatic heterocycles, the isoxazole unit constitutes an easily accessible nucleus that is present in a number of natural pharmacological compounds[13] and displays a wide range of organic reactivities and could be used as an effective means of preparing new molecular scafolds. Also isoxazoles have been repeatedly shown as useful synthons in organic synthesis[14].

In view of the above and in continuation of our work in the synthesis of fused heterocyclic compounds[15-21], we herein report a new series of propenones 4a-f and isoxazoles 5a-f. (Scheme-I).

MATERIALS AND METHODS

All melting points were determined in open capillary and are uncorrected. The IR spectra were recorded on FT-IR spectrophotometer. ^{1}HNMR spectra were recorded on varian USA Mercury plus 300 MHz NMR spectrometer with DMSO-d_6 as a solvent using TMS as internal reference (chemical shift in δ ppm). The starting compounds were synthesized according to **scheme-I.** Glutaric acid **1** was converted into *N*-substituted phenyl glutarimides 2a-f which were then diformylated using Vilsmeier-Haack reaction to form **3a-f.** Compounds **4a-f (i),(ii)** were prepared by using following general method [21].

General procedure for preparation of propenones 4a-f(i),(ii):- Different aromatic methyl ketones (2.0 mmole) in ethanol (95%, 20 ml) were added to the mixture of **3** (1.0 mmole), ethanol (95%, 30ml) and aq. Sodium hydroxide (40% just to alkaline) and stirred for 24 hr. The contents were poured on to crushed ice and isolated by acidification and recrystalised from ethanol to give **4.**

General procedure for preparation of isoxazoles 5a-f (i),(ii):- To a mixture of hydroxylamine hydrochloride (2 mmole) in ethanol and anhydrous sodium acetate (2 mmole) dissolved in minimum amount of hot acetic acid was added a solution of propenone (1 mmole) in ethanol (25ml). The contents were refluxed for 4-5 hr, concentrated and neutralized with NaOH. The product was isolated and recrystalised from ethanol to give **5a-f (i),(ii).** Physical and elemental analysis data of **5a-f (i),(ii)** are listed in **Table-1**

2,6-dichloro-3,5-bis [3-(phenyl)-isoxazole]-1-(phenyl)-1,4-dihydropyridine 5a (i):- IR (KBr): 1566 (C=N), 1411 (ArC=C), 1250 (C-N),1342 (N-O str.), 1020 (C-O str.), 758 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.49 (s, 2H, CH_2), 6.80 (s, 2H, 2CH, isoxaz.), 7.02-7.51(m, ArH).

2,6-dichloro-3,5-bis [3-(phenyl)-isoxazole]-1-(4-methyl phenyl)-1,4-dihydropyridine 5b (i):- IR (KBr): 1570 (C=N), 1411 (ArC=C), 1244 (C-N), 1383 (N-O str.),1021 (C-O str.), 800 (C-Cl), 2923 (CH_3) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.30 (s, 3H, CH_3), 2.50 (s, 2H, CH_2), 6.79 (s, 2H, 2CH, isoxaz.), 7.02-7.50 (m, ArH).

2,6-dichloro-3,5-bis[3-(phenyl)-isoxazole]-1-(2-chloro phenyl)-1,4-dihydropyridine 5c (i):- IR (KBr): 1588 (C=N), 1440 (ArC=C), 1247 (C-N), 1367 (N-O str.), 1022 (C-O str.), 756 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.48 (s, 2H, CH_2), 6.75 (s, 2H, 2CH, isoxaz.), 7.05-7.63 (m, ArH).

2,6-dichloro-3,5-bis[3-(phenyl)-isoxazole]-1-(4-chloro phenyl)-1,4-dihydropyridine 5d (i):- IR (KBr): 1566 (C=N), 1413 (ArC=C), 1243 (C-N), 1350 (N-O str.), 1021 (C-O str.), 802 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.52 (s, 2H, CH_2), 6.76 (s, 2H, 2CH, isoxaz.), 7.06-7.70 (m, ArH).

2,6-dichloro-3,5-bis[3-(phenyl)-isoxazole]-1-(3-chloro phenyl)-1,4-dihydropyridine 5e (i):- IR (KBr): 1578 (C=N), 1410 (ArC=C), 1243 (C-N), 1370 (N-O str.), 1020 (C-O str.), 796 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.38 (s, 2H, CH_2), 6.82 (s, 2H, 2CH, isoxaz.), 7.04-7.67 (m, ArH).

2,6-dichloro-3,5-bis[3-(phenyl)-isoxazole]-1-(4-methoxy phenyl)-1,4-dihydropyridine 5f (i):- IR (KBr): 1578 (C=N), 1411 (ArC=C), 1245 (C-N), 1370 (N-O str.), 1022 (C-O str.), 799 (C-Cl), 1296 (OCH_3) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.50 (s, 2H, CH_2), 3.67 (s, 3H, OCH_3), 6.79 (s, 2H, 2CH, isoxaz.), 7.03-7.62 (m, ArH).

2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(phenyl)-1,4-dihydropyridine 5a (ii):- IR (KBr): 3453 (OH), 1570 (C=N), 1411 (ArC=C), 1247 (C-N),1350 (N-O str.),1021 (C-O str.), 751 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.49 (s, 2H, CH_2), 6.78 (s, 2H, 2CH, isoxaz.), 7.20-7.77 (m, ArH), 10.90 (br s, 2H, 2OH).

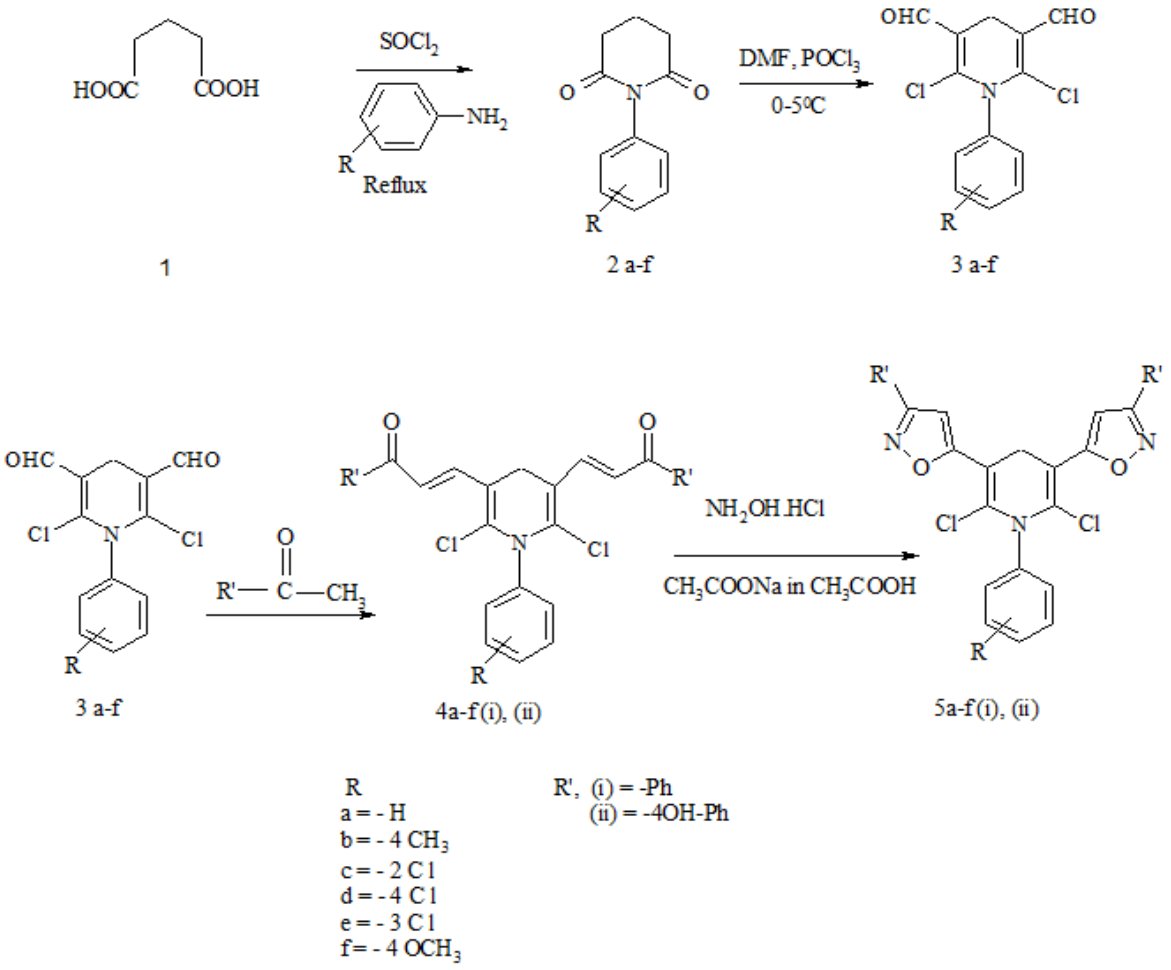
2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(4-methyl phenyl)-1,4-dihydropyridine 5b (ii):- IR (KBr): 3446 (OH), 1562 (C=N), 1409 (ArC=C), 1260 (C-N),1336(N-O str.),1021 (C-O str.), 802 (C-Cl), 2928 (CH_3) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.07 (s, 3H, CH3), 2.49 (s, 2H, CH_2), 6.50 (s, 2H, 2CH, isoxaz.), 7.09-7.31 (m, ArH), 10.88 (br s, 2H, 2OH).

2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(2-chloro phenyl)-1,4-dihydropyridine 5c (ii):- IR (KBr): 3470 (OH), 1572 (C=N), 1421 (ArC=C), 1242 (C-N), 1330 (N-O str.),1019 (C-O str.), 758 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.47 (s, 2H, CH_2), 6.70 (s, 2H, 2CH, isoxaz.), 7.02-7.54 (m, ArH), 10.97 (br s, 2H, 2OH).

2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(4-chloro phenyl)-1,4-dihydropyridine 5d (ii):- IR (KBr): 3481 (OH), 1590 (C=N), 1409 (ArC=C), 1246 (C-N), 1325 (N-O str.),1019 (C-O str.), 825 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.49 (s, 2H, CH_2), 6.77 (s, 2H, 2CH, isoxaz.), 7.21-7.62 (m, ArH), 10.92 (br s, 2H, 2OH).

2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(3-chloro phenyl)-1,4-dihydropyridine 5e (ii):- IR (KBr): 3385 (OH), 1580 (C=N), 1411 (ArC=C), 1245 (C-N), 1335 (N-O str.),1021 (C-O str.), 785 (C-Cl) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.57 (s, 2H, CH_2), 6.80 (s, 2H, 2CH, isoxaz.), 7.09-7.59 (m, ArH), 10.86 (br s, 2H, 2OH).

2,6-dichloro-3,5-bis[3-(4-hydroxy phenyl)-isoxazole]-1-(4-methoxy phenyl)-1,4-dihydropyridine 5f (ii):- IR (KBr): 3467 (OH), 1577 (C=N), 1450 (ArC=C), 1247 (C-N), 1383 (N-O str.),1022 (C-O str.), 828 (C-Cl),1295(OCH_3) cm^{-1}., ^{1}HNMR(DMSO-d_6): δ 2.50 (s, 2H, CH_2), 3.39 (s, 3H, OCH_3), 6.79 (s, 2H, 2CH, isoxaz.), 7.02-7.51 (m, ArH), 10.98 (br s, 2H, 2OH).



R
a = - H
b = - 4 CH_3
c = - 2 Cl
d = - 4 Cl
e = - 3 Cl
f = - 4 OCH_3

R', (i) = -Ph
(ii) = -4OH-Ph

Scheme I

Antimicrobial Activity

The compounds **5a-f (i),(ii)** were screened for their in vitro antimicrobial activities against *B. subtilis, E. coli, S. aureus, P.aeroginosa, A. niger* and *C. albicans*. The agar diffusion assay (Well method, Disc size 6mm, Hi media) was used. The compounds were tested at the concentration of 100µg/ml in DMF. The results were compared with respective standards Chloramphenicol and Nystatin. All the compounds showed moderate to good antimicrobial activity. All the compounds found less active against *A. niger*, but the compounds **5a(i), 5a(ii), 5e(i)** and **5e(ii)** are found more potent than standard against *C. albicans.* **(Table 2)**

Cultures used

Culture code	Culture Name	Name of Culture collection centre.
Bacteria	*Bacillus subtilis 2250*	NCIM, Pune
	Staphylococcus aureus 2079	NCIM, Pune
	Escherichia coli 2109	NCIM, Pune
	Pseudomonas aeruginosa 2036	NCIM, Pune
Yeast	*Candida albicans 3471*	NCIM, Pune
Fungi	*Aspergillus niger* 545	NCIM, Pune

- **Media used**
  - For Bacteria : Muller Hinton agar (Hi-media)
  - For Yeast : MGYP
  - For Fungi : Potato dextrose agar (Hi-media)
- **Inoculum Size**
  - Bacteria : 1 X 10^8 bacteria per ml
  - Fungi : 1 X 10^6 spore per ml
- **Concentration of compound** : 100µg/ml (Prepared in DMF)
- **Method used** : Agar diffusion assay (Well method, Disc size 6 mm)
- **Dilution of Drug** : Stock prepared 1000 µg per ml prepared in DMF

[100µg per well]

RESULTS AND DISCUSSION

The reaction sequences for the synthesis of title compounds are shown in Scheme-I. The key intermediate propenones 4a-f were prepared by treating 2, 6 – dichloro -1- (*N*-substituted phenyl)-1, 4 – dihydropyridine -3, 5 – dicarbaldehyde 3 with substituted aromatic methyl ketones in presence of sodium hydroxide. These propenones 4a-f are used as suitable precursors for the synthesis of isoxazoles 5a-f . The intermediates 4a-f, when treated with hydroxylamine hydrochloride in the presence of sodium acetate in glacial acetic acid yielded isoxazoles 5a-f. All the newly synthesized compounds were characterized by analytical, FTIR, ^{1}HNMR spectral data.

The conversion of propenones 4a-f to isoxazoles 5a-f was confirmed by FTIR, ^{1}HNMR spectral studies in addition to elemental analysis. IR spectrum of 5b(i) showed absorption bands at 2923, 1570, 1411, 1383, 1021 and 800 cm^{-1} indicating the presence of CH_3, C=N, C=C, N-O, C-O and C-Cl groups respectively. Its ^{1}HNMR spectrum displayed three singlets at δ 2.30, 2.50 and 6.79 due to CH_3, CH_2 and CH of isoxazole ring respectively. Further multiplet appeared at δ 7.02-7.50 was due to aromatic protons.

Table 1: Shows Physical data of compounds 5a-f (i),(ii)

Compound No.	R	R^1	M.F.	M.P.(°C)	Yield (%)	% Found (Calcd.)		
						C	H	N
5a(i)	-H	-Ph	$C_{29}H_{19}O_2N_3Cl_2$	104	98.03	67.80 (67.97)	3.61 (3.73)	8.13 (8.20)
5b(i)	-4CH_3	-Ph	$C_{30}H_{21}O_2N_3Cl_2$	120	63.80	68.37 (68.44)	3.92 (4.02)	7.91 (7.98)
5c(i)	-2Cl	-Ph	$C_{29}H_{18}O_2N_3Cl_3$	150	66.17	63.62 (63.69)	3.25 (3.31)	7.60 (7.68)
5d(i)	-4Cl	-Ph	$C_{29}H_{18}O_2N_3Cl_3$	128	70.64	63.61 (63.69)	3.27 (3.31)	7.63 (7.68)
5e(i)	-3Cl	-Ph	$C_{29}H_{18}O_2N_3Cl_3$	170	68.67	63.59 (63.69)	3.25 (3.31)	7.61 (7.68)
5f(i)	-4OCH_3	-Ph	$C_{30}H_{21}O_3N_3Cl_2$	154	55.95	66.40 (66.43)	3.82 (3.90)	7.68 (7.74)
5a(ii)	-H	-4OH-Ph	$C_{29}H_{19}O_4N_3Cl_2$	160	65.71	63.90 (63.98)	3.40 (3.51)	7.67 (7.71)
5b(ii)	-4CH_3	-4OH-Ph	$C_{30}H_{21}O_4N_3Cl_2$	126	98.56	64.48 (64.52)	3.69 (3.79)	7.47 (7.52)
5c(ii)	-2Cl	-4OH-Ph	$C_{29}H_{18}O_4N_3Cl_3$	140	68.75	60.09 (60.17)	3.03 (3.13)	7.21 (7.25)
5d(ii)	-4Cl	-4OH-Ph	$C_{29}H_{18}O_4N_3Cl_3$	80	98.26	60.11 (60.17)	3.10 (3.13)	7.18 (7.25)
5e(ii)	-3Cl	-4OH-Ph	$C_{29}H_{18}O_4N_3Cl_3$	172	96.52	60.07 (60.17)	3.05 (3.13)	7.20 (7.25)
5f(ii)	-4OCH_3	-4OH-Ph	$C_{30}H_{21}O_5N_3Cl_2$	190	94.73	62.63 (62.72)	3.57 (3.68)	7.28 (7.31)

Table 2: Shows Results of antimicrobial activity of the compounds 5a-f (i), (ii)

Compound	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeroginosa*	*A. niger*	*C. albicans*
5ai	9.47	8.14	8.47	7.10	-	9.57
5aii	10.52	8.18	9.52	9.20	-	9.67
5bi	9.89	9.43	8.88	8.43	-	-
5bii	8.70	8.23	7.70	7.10	-	-
5di	10.99	13.51	8.99	14.50	6.45	7.30
5dii	9.00	8.81	7.58	7.56	-	9.47
5ei	8.05	12.68	7.05	13.68	-	13.48
5eii	11.14	10.95	11.20	11.90	-	12.99
5fi	8.44	12.02	8.50	10.02	-	-
5fii	9.90	12.83	7.90	13.23	-	-
Chloramphenicol (10 mcg/disc)	30.94	20.52	30.94	20.52	NA	NA
Nyastatin (100 U/ml)	NA	NA	NA	NA	9.53	9.53

Diameter in mm calculated by digital Vernier Caliper.

"-" means no zone of inhibition,NA "Not Applicable"

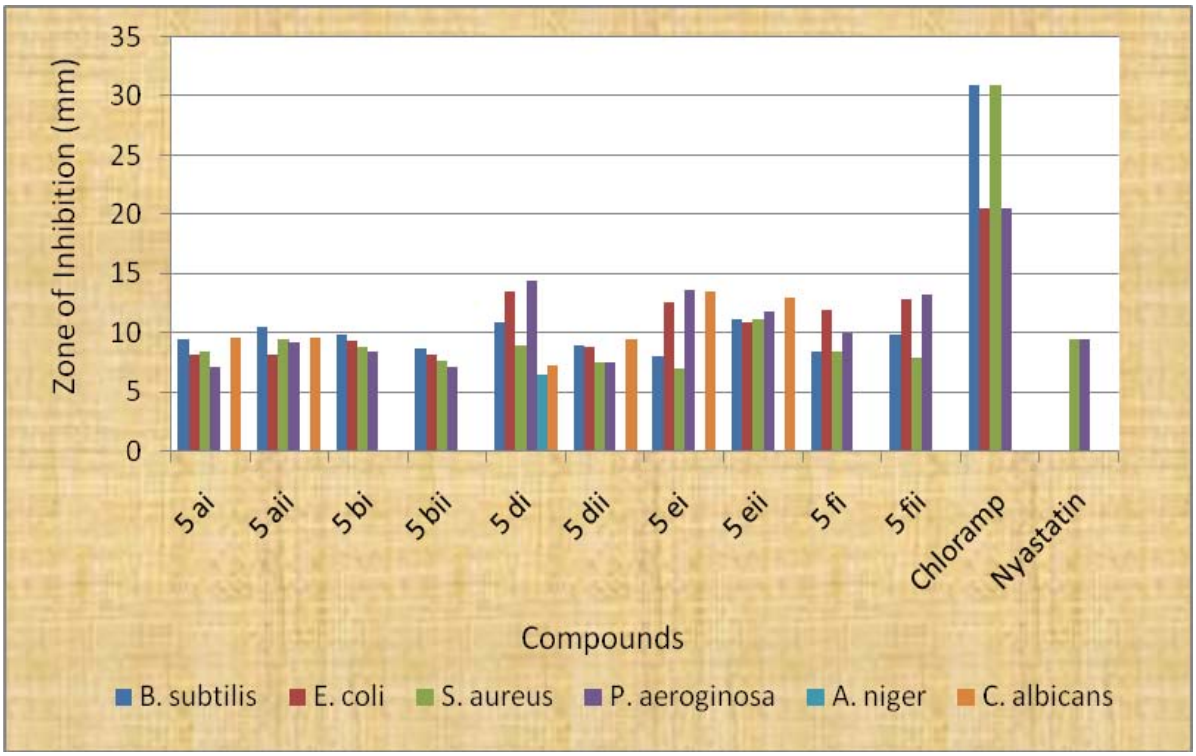


Fig. 1: Shows antimicrobial activity of compounds 5a-f (i), (ii)

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