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Synthesis and Evaluation of Antimicrobial Activities of Some Novel Isoxazole Derivatives

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Abstract: *4-Methylthiobenzaldehyde **(1)** was condensed with various arylketones **(2)** in the presence of potassium hydroxide to obtain a series of 1-aryl-3-(4-methylthiophenyl)-2-propene-1-ones **(3)**. The compound **(3)** were brominated with bromine in chloroform to get dibromopropanones **(4)** later they were treated with hydroxylamine hydrochloride in presence of aqueous alkali to get 3,5-diaryllisoxazoles **(5a-k)**. The structures of newly synthesized compounds are characterized by elemental analysis, FT-IR, ^{1}H-NMR and mass spectroscopic studies and screened for their antimicrobial activities. The preliminary results revealed that some of the compounds exhibited promising antimicrobial activities.*

Keywords: *Antibacterial, Antifungal, isoxazoles and dibromopropanones*

1. INTRODUCTION

Isoxazoles are a class of heterocyclic compounds having numerous applications and have been proved to be important building blocks in organic synthesis. The wide range of biological activities displayed by isoxazole compounds include cytotoxic[1], antimicrobial[2,3], anthelmintic[4], antirhinovirus[5], centrally acting muscle relaxants[6,7], analgesic[8], anti-inflammatory[9]. Some isoxazole derivatives displayed agrochemical properties namely herbicidal[10-14], soil fungicidal[15,16] activity and have applications as pesticides and insecticides[17]. Isoxazoles have also been used as dyes, electric insulating oils, high temperature lubricants and polyisoxazoles have applications as semiconductors. The literature survey on substituted isoxazole derivatives indicated that they posses significant biological activities. Prompted by the various biological activities of these compounds, we decided to synthesize some novel isoxazole derivatives and were screened for their *in vitro* antibacterial and antifungal.

2. MATERIALS AND METHODS

The newly synthesized compounds were confirmed by their spectral analysis. The chemicals used for the synthesis of novel isoxazoles were of standard quality. 4-Methylthiobenzaldehyde, different arylketones and bromine were procured from Sigma-Aldrich, Bengaluru, India. Potassium hydroxide, Hydroxylamine hydrochloride from Spectrochem, Mumbai-India. Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded on a SHIMADZU-FTIR Infrared Spectrometer in KBr (ν_{max} in cm^{-1}). ^{1}H-NMR spectra were recorded in $CDCl_3$ on amx (400 MHz) spectrometer using TMS as internal standard. Elemental analysis was carried out on a Euro-E-300, FABMS spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer using argon/xenon (6kv, 10mA). Completion of the reaction was monitored by thin layer chromatography (TLC) using Merck silica gel 60 F_{254} coated alumina plates. The synthetic pathway is presented in **Scheme**. The data of the novel isoxazole derivatives are presented in **Tables 1** and **2** and the biological activity data are tabulated in **Tables 3** and **4**.

3. REACTION SCHEME

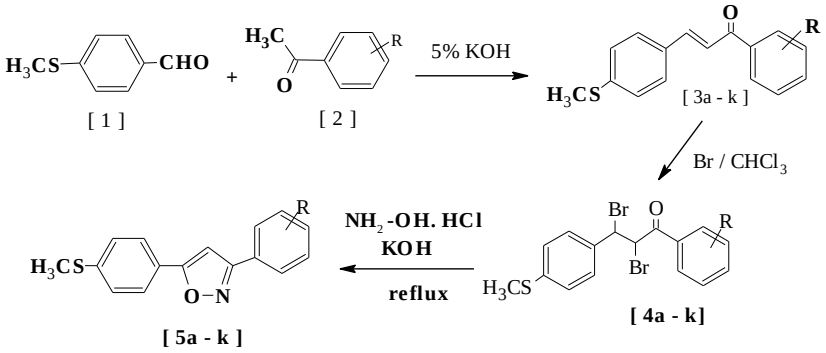

Table1. *Characterization Data Of Dibromopropanones (4a-K)*

Compd	R_1	MF	M.W	Yield (%)	M.P(^{0}C)
4a	4-CH_3	$C_{17}H_{16}OSBr_2$	428.00	97	163-167
4b	4-OCH_3	$C_{17}H_{16}O_2SBr_2$	443.99	94	160-164
4c	4-Cl	$C_{16}H_{13}OSClBr_2$	448.44	98	165-168
4d	4-Br	$C_{16}H_{13}OSBr_3$	492.97	96	158-162
4e	4-NO_2	$C_{16}H_{13}O_3SNBr_2$	459.00	96	156-158
4f	4-F	$C_{16}H_{13}OSFBr_2$	432.00	95	144-148
4g	4-SCH_3	$C_{17}H_{16}OS_2Br_2$	460.00	86	158-162
4h	2,4-Cl_2	$C_{16}H_{12}Cl_2OSBr_2$	482.90	90	157-160
4i	2,3,4-Cl_3	$C_{16}H_{11}Cl_3OSBr_2$	517.35	90	187-191
4j	2,4-Cl_2-5-F	$C_{16}H_{11}Cl_2OSFBr_2$	500.90	86	198-202
4k	4-C_6H_5	$C_{22}H_{18}OSBr_2$	490.00	89	183-187

Table2. *Characterization Data of Isoxazoles (5a-K)*

Compd.	R_1	MF	M.W	M.P(^{0}C)	Analysis (%)Found (Calculated)		
					C	H	N
5a	4-CH_3	$C_{17}H_{15}NOS$	281.00	135-139	72.57 72.49	5.47 5.33	5.25 4.98
5b	4-OCH_3	$C_{17}H_{15}NO_2S$	297.00	137-140	68.76 68.68	5.28 5.00	4.88 4.71
5c	4-Cl	$C_{16}H_{12}NOSCl$	301.79	137-140	63.82 63.69	4.01 3.98	4.75 4.64
5d	4-Br	$C_{16}H_{12}NOSBr$	346	198-202	55.60 55.46	3.59 3.46	4.25 4.04
5e	4-NO_2	$C_{16}H_{12}N_2O_3S$	312.34	157-161	61.66 61.53	3.98 3.84	9.22 8.97
5f	4-F	$C_{16}H_{12}NOSF$	285.33	123-127	67.51 67.36	4.39 4.21	5.30 4.91
5g	4-SCH_3	$C_{17}H_{15}NOS_2$	313.43	145-148	65.36 65.17	4.91 4.79	4.62 4.47
5h	2,4-Cl_2	$C_{16}H_{11}NOSCl_2$	336.23	154-157	57.29 57.15	3.38 3.27	4.31 4.16
5i	2,3,4-Cl_3	$C_{16}H_{10}NOSCl_3$	370.68	133-136	51.99 51.84	2.91 2.70	3.88 3.72
5j	2,4-Cl_2-5-F	$C_{16}H_{10}NOSCl_2F$	354.22	109-112	54.44 54.25	2.97 2.82	4.23 3.91
5k	4-C_6H_5	$C_{22}H_{17}NOS$	343.44	166-171	77.99 76.96	5.27 4.95	4.37 4.08

3.1. 3-(aryl)-1-[4-(methylthio)phenyl]prop-2-en-1-ones were prepared as per the procedure reported in the literature[18] (3).

3.2. Preparation of 2,3-Dibromo-1-(aryl)-3-[4-(methylthio)phenyl]prop-2-en-1-ones (4).

Bromine (0.1 mol) in chloroform (25 ml) was added slowly to a solution of 1,3-diaryl-2-propen-1-ones (0.1 mol) in chloroform (50 ml) with continuous stirring. The stirring was continued for 24h.

Excess of chloroform was distilled off under reduced pressure. The solid formed was collected by filtration, dried and recrystalized from chloroform. The characterization data of dibromo propenones are given in **Table 1.**

4a: **IR(KBr, cm^{-1}):** 3031(Ar-H), 2863(C-H), 1666(C=O), 1602 and 1448(C=C), 772 and 693(C-Br), **^{1}H-NMR(CDCl$_3$):** δ 2.16(s, 3H, CH_3), 2.49(s, 3H, SCH_3), 7.22(d, 2H, *J*=8.8Hz, 4-methylphenyl), 7.29(d, 2H, *J*=8.8Hz, 4-methylphenyl), 7.31(d, 2H, *J*=8.2Hz, 4-methylthiophenyl), 7.41(d, 2H, *J*=8.2Hz, 4-methylthiophenyl), 5.54(d, 1H, *J*=11.7Hz, α-proton of propanone), 5.31(d, 1H, *J*=11.7Hz, β -proton of propanone); **FAB MS (*m/z*, %):** 426(M$^+$, 100), 428(M+2, 82), 430(M+4, 47), 391(61), 327(46), 223(22).

4b: **IR(KBr, cm^{-1}):** 3068(Ar-H), 2883(C-H), 1679(C=O), 1607 and 1456(C=C), 773 and 679(C-Br), **^{1}H-NMR(CDCl$_3$):** δ 2.44(s, 3H, SCH_3), 3.91(s, 3H, OCH_3), 7.74(d, 2H, *J*=8.6Hz, 4-methoxyphenyl), 7.87(d, 2H, *J*=8.6Hz, 4-methoxyphenyl), 7.36(d, 2H, *J*=8.1Hz, 4-methylthiophenyl), 7.47(d, 2H, *J*=8.1Hz, 4-methylthiophenyl), 5.65(d, 1H, *J*=12.1Hz, α-proton of propanone), 5.36(d, 1H, *J*=12.7Hz, β -proton of propanone); **FAB MS (*m/z*, %):** 442(M$^+$, 100), 444(M+2, 87), 446(M+4, 41), 385(56), 328(38), 283(15).

4c: **IR(KBr, cm^{-1}):** 3046(Ar-H), 2873(C-H), 1678(C=O), 1610 and 1470(C=C), 927, 889 and 679(C-Cl and C-Br), **^{1}H-NMR(CDCl$_3$):** δ 2.62(s, 3H, SCH_3), 7.83(d, 2H, *J*=8.8Hz, 4-chlorophenyl), 7.92(d, 2H, *J*=8.8Hz, 4-chlorophenyl), 7.28(d, 2H, *J*=8.7Hz, 4-methylthiophenyl), 7.34(d, 2H, *J*=8.7Hz, 4-methylthiophenyl), 5.65(d, 1H, *J*=11.9Hz, α-proton of propanone), 5.82(d, 1H, *J*=11.9Hz, β-proton of propanone); **FAB MS (*m/z*, %):** 446(M$^+$, 100), 448(M+2, 87), 450(M+4, 64), 452(M+6, 23), 385(56), 328(38), 283(15).

4d: **IR(KBr, cm^{-1}):** 3077(Ar-H), 2879(C-H), 1680(C=O), 1606 and 1487(C=C), 695 and 648(C-Br), **^{1}H-NMR(CDCl$_3$):** δ 2.51(s, 3H, SCH_3), 7.69(d, 2H, *J*=8Hz, 4-bromophenyl), 7.95(d, 2H, *J*=8Hz, 4-bromophenyl), 7.08(d, 2H, *J*=8.2Hz, 4-methylthiophenyl), 7.23(d, 2H, *J*=8.2Hz, 4-methylthiophenyl), 5.93(d, 1H, *J*=11.2Hz, α-proton of propanone), 5.73(d, 1H, *J*=11.2Hz, β-proton of propanone); **FAB MS (*m/z*, %):** 491(M$^+$, 100), 493(M+2, 89), 495(M+4, 56), 497(M+6, 18), 459(28), 438(38), 412(42), 307(57).

3.3. Preparation of 5-[4-(methylthio)phenyl]-3-phenylisoxazole (5a-k).

An equimolar mixture of dibromo propanones **(5)** (0.01 mol) and hydroxyl amine hydrochloride in ethanol (25 ml) was taken in a round bottom flask. The contents were refluxed on a water bath for 30 minutes. A solution of potassium hydroxide (30%, 5 ml) was added slowly and the mixture was refluxed until the colour of the reaction mixture turned red with simultaneous deposition of potassium bromide. It was allowed to stand for 5 hours at room temperature. The contents were then poured on to crushed ice with vigorous stirring and neutralized with dil. HCl. The resulting solid was collected by filtration, washed with water and recrystalized from ethanol. The characterization data of isoxazole, synthesized are given in **Table 2.**

5a: **IR (KBr, cm^{-1}):** 3022(Ar-H), 2972 and 2854(C-H, SCH_3), 1614, and 1492(C=N, C=C); **^{1}H-NMR (CDCl$_3$):** δ 2.36(s, 3H, CH_3), 2.45(s, 3H, SCH_3), 7.52(s, 1H, isoxazole), 7.35(d, 2H, *J*=8 Hz, 4-methylphenyl), 7.41(d, 2H, *J*=8 Hz, 4-methylphenyl), 7.82(d, 2H, *J*=8.4 Hz, 4-methylyhiophenyl), 7.79 (d, 2H, *J*=8.4 Hz, 4-methylyhiophenyl).

5b: IR (KBr, cm^{-1}): 3048(Ar-H), 2877(C-H, SCH_3), 1603 and 1573(C=N, C=C,); **^{1}H-NMR (CDCl$_3$):** δ 2.53(s, 3H, SCH_3), δ 3.82(s, 3H, OCH_3), 7.49(s, 1H, isoxazole), 7.08(d, 2H, *J*=8.4Hz, 4-methylthiophenyl), 7.41(d, 2H, *J*=8.4Hz, 4-methylthiophenyl), 7.8(d, 2H, *J*=8.8Hz, 4-methoxyphenyl), 7.83(d, 2H, *J*=8.8 Hz, 4-methoxyphenyl).

5d: IR (KBr, cm^{-1}): 3033(Ar-H), 2916(C-H, SCH_3), 1666, 1661 and 1550(C=N, C=C), 742 and 721(C-Br); **^{1}H-NMR (CDCl$_3$):** δ 2.45(s, 3H, SCH_3), 7.59(s, 1H, isoxazole), 7.42(d, 2H, *J*=8.4 Hz, 4-methylthiophenyl), 7.75(d, 2H, *J*=8.4Hz, 4-methylthiophenyl), 7.82(d, 2H, *J*=8Hz, 4-bromphenyl), 7.85(2d, 4H, *J*=8 Hz, 4-bromophenyl), **FAB MS (*m/z*, %):** 266(M$^+$, 36), 348(M+2, 53), 289(44), 232(49).

5k: **IR (KBr, cm^{-1}):** 3063(Ar-H), 2859 (C-H, SCH_3), 1612, 1567 and 1473(C=N, C=C,); **^{1}H-NMR (CDCl$_3$):** δ 2.52(s, 3H, SCH_3), 7.76(s, 1H, isoxazole), 7.32(d, 2H, *J*=8.28Hz, 4-methylthio phenyl), 8.24(d, 2H, *J*=8.28Hz, 4-methylthiophenyl), 7.41-7.98(m, 9H, biphenyl), **FAB MS (*m/z*, %):** 343(M$^+$, 38), 344965), 307(63), 289(76), 221(31).

4. BIOLOGICAL ACTIVITY

4.1. Antibacterial Activity

The newly synthesized compounds **(5a-k)** were screened for antibacterial activities against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25922) *Pseudomonas aeruginosa (ATTC-27853)* and *Klebsiella pneumoniae* bacterial strains by the disc diffusion method[18, 19]. Ciprofloxacin was used as standard drug. The results of such studies are given in **Table 3.**

Table3. A*ntibacterial activity data of compounds (5a-l)*

Compd.	Diameter of the inhibition zone (in mm)			
	S. aureus	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
5a	11	13	17	11
5b	18	10	15	13
5c	16	11	18	15
5d	10	16	10	12
5e	12	15	11	12
5f	**19**	**16**	**24**	**17**
5g	**17**	**18**	**23**	**20**
5h	13	11	21	16
5i	**19**	**18**	**25**	**19**
5j	15	12	22	13
5k	17	15	21	17
Ciprofloxacin	19	18	25	20

Diameter of the inhibition zone (in mm) at 10 µg/ml concentration.

4.2. Antifungal Activity

All the newly synthesized compounds **(5a-k)** were screened for their antifungal activity against *Candida Albicans* (NICM No.300*), Aspergillus Fumigatus* (NICM No.902), *Aspergillus Flavus* (NICM No.524) *and Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[20, 21,]. Amphotericin B was used as the standard drug. The results of antifungal studies are given in **Table 4.**

Table4. *Antifungal*

Compound	Diameter of the inhibition zone (in mm)			
	A. fumigatus	***A. flavus***	***C. albicans***	***T. mentagraphytes***
5a	**20**	**17**	**19**	**20**
5b	**22**	**18**	**18**	**19**
5c	15	17	12	12
5d	20	16	15	19
5e	12	12	17	15
5f	**20**	**16**	**17**	**17**
5g	17	13	14	13
5h	16	18	13	11
5i	**21**	**18**	**20**	**19**
5j	15	10	10	17
5k	14	17	11	15
Amphotericin-B	22	18	20	20

activity data of compounds (5a-k) Diameter of the inhibition zone (in mm) at 10 µg/ml concentration.

5. RESULTS AND DISCUSSION

The investigation of the antibacterial and antifungal screening studies revealed that all the tested compounds **(5a-k)** showed moderate to good inhibition in respective solvents used for testing. The compounds **5f, 5g & 5i** showed comparatively good activity against all the bacterial strains. The good activity can be attributed to the presence of pharmacologically active groups 4-fluoro, 4-methylthio, 2,3,4-trichloro which are directly attached to the phenyl ring of the isoxazole system. The compounds**, 5a, 5b, 5f & 5i** showed comparatively good activity against all the tested fungal strains. The groups 4-methyl, 4-methoxy, 4-Fluoro and 2,3,4-trichloro which are directly attached to the phenyl ring of the isoxazole system were responsible for the good antifungal activity.

6. CONCLUSION

This article reports the successful synthesis and antimicrobial activity of new isoxazole derivatives carrying 4-thiomethyl moiety. The antimicrobial activity results indicated that **5f, 5g & 5i** showed the most promising antibacterial and **5a, 5b, 5f & 5i** antifungal activity. Thus these compounds can be recommended antimicrobial agents.

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