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SYNTHESIS OF SOME NOVEL ISOXAZOLES CARRYING 2-(5-PHENYLISOXAZOL-3-YL) PHENOL MOIETY AS POTENTIAL ANTI-INFLAMMATORY AND ANTI-MICROBIAL AGENTS

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ABSTRACT

A series of novel isoxazoles (III a-j) have been synthesized from 2-hydroxy acetophenone (I) with aryl aldehydes to get chalcones i.e. α, β- unsaturated ketones (II). Then cyclization of chalcones with hydroxyl amine hydrochloride in ethanol leads to synthesis of title compounds. The structures of newly synthesized derivatives have been confirmed by different spectral analysis like IR, ^{1}H NMR, Mass spectral analysis. The novel compounds have been tested for their anti-microbial activity and invitro anti-inflammatory activity. Some of the compounds showed encouraging results.

Key words: Isoxazoles, chalcones, anti-inflammatory, anti-microbial activity.

INTRODUCTION

Five-member heterocyclic compounds isoxazoles are important for pharmaceutical industry and material science due to their various applications. Isoxazoles are present in the structures of many natural products and pharmaceutical actives. In fact, isoxazoles have long been targeted in organic synthesis due to the broad spectrum of their biological and pharmacological activities, which include anticonvulsant ([1]), apoptotic ([2]), hypolipidemic

[3], anthelmintic [4], anti-inflammatory [5], anti-microbial [6], antioxidant [7] and anti-tubercular activities [8].

Isoxazoles have illustrious history; their chemistry is associated with Ludwig Claisen, who first recognized the cyclic structure of 3- methyl-5-phenylisoxazole in 1888 and was shown to possess typical properties of an aromatic system under certain reaction conditions; particularly in basic media, it is very highly labile. Dunstan and Dymond were the first to synthesize the isoxazole ring. They isolated a liquid base by heating nitro ethane with aqueous alkalis to obtain 3, 4, 5- trimethylisoxazole. A very significant contribution to the development of isoxazole chemistry came between 1930–1946 from Quilico's studies on the synthesis of ring system from nitrile oxides and unsaturated compounds.

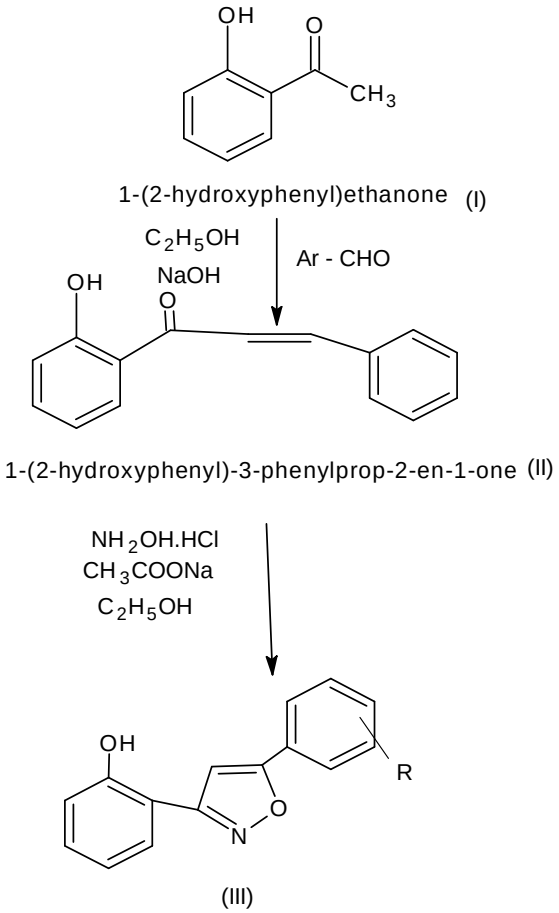
Although there are many methods to prepare such compounds, new variants continue to appear since they exhibit a wide range of biological and medicinal activities. In this article, reaction with acetophenone with various aromatic aldehydes to get α, β- unsaturated ketones and then cyclization with hydroxyl amine hydrochloride in ethanol for the synthesis of title compounds.

Bacteria are becoming resistant to ever more antimicrobial agents. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus, future research in antimicrobial therapy may focus on finding ways to overcome resistance to antimicrobials, or methods to treat infections with alternative means.

Similarly isoxazole derivatives have been known to exhibit the anti-inflammatory activity and in this respect form interesting targets in synthesis since their structures have potential for the development of anti-inflammatory drugs. This has precisely been the reason for selecting these molecules for the present study.

MATERIALS AND METHODS

Melting points were determined with open capillary and are uncorrected. IR spectra were recorded on a Shimadzu FTIR model 8010 spectrophotometer, ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker supercon FT-NMR instrument using TMS as internal standard. Mass spectra were recorded on GCMS in dimethyl sulphoxide.



H, 2-OH, 4-N $(CH_3)_2$, 2-OH 4-OCH_3, 2, 4, 6-$(OCH_3)_3$, 4-Cl, 4-NO_2, 2- OCH_3, 3-NO_2, 3-OCH_3 Aromatic aldehyde derivatives and acetophenone were dissolved in ethanol to react in the presence of aqueous NaOH solution, heated and refluxed for 12 hr. After cooling poured in water and kept in refrigerator. The resulted precipitate was filtered and purified by recrystallization. Thus the compound II obtained was made to react with NH_2OH. HCl [9] in sodium acetate refluxed for 6 hr. The mixture was concentrated by distilling out the solvent under reduced pressure. Finally the compound III obtained was filtered, washed and recrystallized from ethanol.

The in vitro antimicrobial screenings of synthesized compounds (III a-j) were performed by using Cup-plate method[10]. The anti-bacterial activity of final compounds were studied at 50 µg/ml concentration against the following standard bacterial strains: *B. subtilis, S. aureus, E. coli, S. typhi* in Muller-Hinton agar medium and the strains were incubated for 37°C for 24 hr. The standard antibiotic Ampicillin was used as reference drug at a concentration of 50 µg/ml.

Anti fungal activity of newly synthesized compounds (III a-j) were studied at 50 µg/ml concentration against *C. albicans*, and *A. niger* in Sabouraud's agar and fungal strains were incubated under aerobic conditions at 25°C for 48 hours. Clotrimazole was used as standard drug at 50 µg/ml. concentration. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated and compared with that produced by standard.

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification [[11]]. The standard drug Ibuprofen and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different conc. of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken.

RESULTS AND DISCUSSION

In this study new isoxazole derivatives have been synthesized by Claisen condensation and later on cyclization with hydroxylamine hydrochloride. All the compounds were characterized by IR, ^{1}H NMR, Mass and Elemental analysis. The final structure of various isoxazoles IIIa were confirmed by IR spectrum of compound showed peak at 1601.5 due to stretching of C=N, singlet peak at δ 5.28 ppm by CH and molecular peak at 237 by Mass spectrum analysis.

The antimicrobial activity of these synthesized compounds (III a-j) was determined in-vitro by using Cup-plate method. The results of which are summarized in table- 2. The compounds IIIc and IIIj showed potent activity against S. aureus, IIIc and IIIF showed good activity against B.subtilis and E. Coli, IIIe, IIIh showed potent activity against S.typhi. The compounds IIIc ,IIIe and IIIh exhibit promising activity against C. albicans and A. niger. The anti-inflammatory activity was determined by Muzushima and Kabayashi method. The

results were reported in table-3, in which the compound IIId and IIIf showed good activity. However, the tested compounds were less active in comparison to the standard drugs.

Table-1: Physico-Chemical data of final compounds

Sample code	R	Mol. Formula	% yield	R_f	m.p. ˚C
IIIa	H	$C_{15}H_{11}N\,O_2$	47	0.31	124-26
IIIb	2-OH	$C_{15}H_{11}\,N\,O_3$	61	0. 53	113-15
IIIc	4-N$(CH_3)_2$	$C_{17}H_{16}N_2\,O_2$	52	0.42	127-29
IIId	2-OH, 4-OCH_3	$C_{16}H_{13}\,N\,O_4$	44	0.68	123-25
IIIe	2,4,6-$(OCH_3)_3$	$C_{18}H_{17}\,NO_5$	36	0. 51	106-08
IIIf	4-Cl	$C_{15}H_{10}Cl\,NO_2$	58	0.62	112-14
IIIg	4-NO_2	$C_{15}H_{10}N_2\,O_4$	64	0. 79	156-58
IIIh	2- OCH_3	$C_{16}H_{13}NO_3$	76	0.37	141-43
IIIi	3-NO_2	$C_{15}H_{10}N_2\,O_4$	37	0.55	119-21
IIIj	3-OCH_3	$C_{16}H_{13}\,NO_3$	69	0.46	135-37

2-(5-phenylisoxazol-3-yl) phenol (IIIa)

$C_{15}H_{11}\,NO_2$: Calculated: C 75.94%, H 4.67%, and N 5.90%; Found: C 75.98%, H 4.30%, N 5.84%; m.p. = 124-26^0 C; yield 47.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 7.78- 6.12 (m, 9H, Ar-H), 5.28 (s, 1H, CH), 4.18 (s, 1H, OH). IR (KBR) γ max: 3431.2(OH) 3054.1(CH-Ar), 1601.5 (C=N), 1015 (C-O). MS: m/z (%) 237[M+].

2, 2'-isoxazole-3, 5-diyldiphenol (IIIb)

$C_{15}H_{11}N\,O_3$: Calculated: C 71.14%, H 4.38%, and N 5.53%; Found: C 71.25%, H 4.32%, N 5.47%; m.p. = 113-15^0 C; yield 61.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 7.90- 6.51 (m, 8H, Ar-H), 5.33 (s, 1H, CH), 4.13 (s, 1H, OH). IR (KBR) γ max: 3344.7(OH) 3078.5(CH-Ar), 1620.1 (C=N), 1102.3 (C-O). MS: m/z 253[M+].

2-{5-[4-(dimethylamino) phenyl] isoxazol-3-yl} phenol ((IIIc)

$C_{17}H_{16}N_2O_2$: Calculated: C 72.84%, H 5.75%, and N 9.99%; Found: C 72.78%, H 5.81%, N 9.96%; m.p. = 127-29^0 C; yield 52.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.10- 6.73

(m, 8H, Ar-H), 5.13 (s, 1H, CH), 4.21 (s, 1H, OH), 3.12-2.35(s, 6H, -N(CH_3)$_2$). IR (KBR) γ max: 3451.4(OH) 3025.6(CH-Ar), 1640.6 (C=N), 1015.2 (C-O). MS: m/z 280[M+].

2-[3-(2-hydroxyphenyl) isoxazol-5-yl]-5-methoxyphenol (IIId)

$C_{16}H_{13}N\,O_4$: Calculated: C 67.84%, H 4.63%, and N 4.94%; Found: C 67.87%, H 4.72%, N 4.89%; m.p. = 123-25^0 C; yield 44.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.21- 6.58 (m, 7H, Ar-H), 5.23 (s, 1H, CH), 4.30 (s, 1H, OH), 3. 23(s,3H, -O CH_3). IR (KBR) γ max: 3445.8(OH) 3057.2(CH-Ar), 1652.4 (C=N), 1021.3 (C-O). MS: m/z 283[M+].

2-[5-(2,4,6-trimethoxyphenyl)isoxazol-3-yl]phenol (IIIe)

$C_{18}H_{17}N\,O_5$: Calculated: C 66.05%, H 5.23%, and N 4.28%; Found: C 66.08%, H 5. 18%, N 4.29%; m.p. = 106-08^0 C; yield 36.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 7.86- 6.52 (m, 6H, Ar-H), 5.11 (s, 1H, CH), 4.11 (s, 1H, OH), 3. 53 (s, 9H, -(OCH_3)$_3$). IR (KBR) γ max: 3428.8(OH) 2982.5(CH-Ar), 1623.6 (C=N), 1018.4 (C-O). MS: m/z 327[M+].

2-[5-(4-chlorophenyl) isoxazol-3-yl] phenol (IIIf)

$C_{15}H_{10}\,ClN\,O_2$: Calculated: C 66.31%, H 3.71%, and N 5.16%; Found: C 66.38%, H 3. 81%, N 5.22%; m.p. = 112-14^0 C; yield 58.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.12-7.60 (m, 8H, Ar-H), 4.98 (s, 1H, CH), 4.05 (s, 1H, OH). IR (KBR) γ max: 3432.2(OH) 3022.5(CH-Ar), 1631.2 (C=N), 1012.8 (C-O), 675 (C-Cl). MS: m/z 271[M+].

2-[5-(4-nitrophenyl) isoxazol-3-yl] phenol (IIIg)

$C_{15}H_{10}\,N_2\,O_4$: Calculated: C 63.83%, H 3.57%, and N 9.92%; Found: C 63.79%, H 3. 61%, N 9.88%; m.p. = 156-58^0 C; yield 64.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.22-6.75 (m, 8H, Ar-H), 5.14 (s, 1H, CH), 4.23 (s, 1H, OH). IR (KBR) γ max: 3428.2(OH) 3021.1(CH-Ar), 1613.2 (C=N), 1027.1 (C-O). MS: m/z 282[M+].

2-[5-(2-methoxyphenyl) isoxazol-3-yl] phenol (IIIh)

$C_{16}H_{13}\,N\,O_3$: Calculated: C 71.90%, H 4.90%, and N 5.24%; Found: C 71. 97 %, H 4. 82%, N 5.18%; m.p. = 141-43^0 C; yield 76.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.08-6.34 (m, 8H, Ar-H), 5.26 (s, 1H, CH), 4.50 (s, 1H, OH)3.22 (s,3H, OCH_3). IR (KBR) γ max: 3431.5(OH) 3011.4(CH-Ar), 1640.2 (C=N), 1012.7 (C-O). MS: m/z 267[M+].

2-[5-(3-nitrophenyl) isoxazol-3-yl] phenol (IIIi)

$C_{15}H_{10}\,N_2\,O_4$: Calculated: C 63.83%, H 3.57%, and N 9.92%; Found: C 63.89%, H 3. 55%, N 9.91%; m.p. = 119-21^0 C; yield 37.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 8.16-6.55 (m,

8H, Ar-H), 5.08 (s, 1H, CH), 4.15 (s, 1H, OH). IR (KBR) γ max: 3428.2(OH) 3038.3(CH-Ar), 1652.3 (C=N), 1061.7 (C-O). MS: m/z 282[M+].

2-[5-(3-methoxyphenyl) isoxazol-3-yl] phenol (IIIj)

$C_{16}H_{13}NO_3$: Calculated: C 71.90%, H 4.90%, and N 5.24%; Found: C 71. 89%, H 4. 87%, N 5.28%; m.p. = 135-37^0 C; yield 69.0%; ^{1}H NMR (DMSO D_6, 400 MHz) δ ppm. 7.98-6.78 (m, 8H, Ar-H), 5.19 (s, 1H, CH), 4.95 (s, 1H, OH) 3.14 (s,3H, OCH_3). IR (KBR) γ max: 3473.6(OH) 3017.2(CH-Ar), 1657.2 (C=N), 1019.3 (C-O). MS: m/z 267[M+].

Table-2: Data of Antimicrobial activity shown by final compounds

Diameter of zone of inhibition							
Compounds	**R**	***S. aureus***	***B. subtilis***	***E. coli***	***S . typhi***	***C. albicans***	***A. niger***
III a	H	11	11	16	12	13	10
III b	2-OH	15	13	17	12	15	13
III c	4-$N(CH_3)_2$	19	19	20	11	16	17
III d	2-OH, 4-OCH_3	14	14	18	15	10	09
III e	2,4,6-$(OCH_3)_3$	15	17	14	18	18	14
III f	4-Cl	14	19	20	10	05	12
III g	4-NO_2	05	12	16	05	15	05
III h	2- OCH_3	08	15	11	17	16	15
III i	3-NO_2	12	10	16	13	13	10
III j	3-OCH_3	18	13	15	10	12	11
	Ampicillin	24	22	25	20	-----	-----
	Clotrimazole	----	----	----	-----	22	18

Table-3: Data of Anti-inflammatory activity shown by final compounds

Sl. No.	**Compound code**	**Absorbance value (Mean ± SE)**	**Inhibition of denaturation (in %)**
1	Control	0.0165±0.000245	--
2	Standard (Ibuprofen)	0.0306±0.000294	85.45%
3	III a	0.0284±0.000216	17.57 %
4	III b	0.0191±0.000327	25.65 %
5	III c	0.0187±0.000245	13.34 %
6	III d	0.0194±0.000356	**72.12%**
7	III e	0.0223±0.000408	35.15 %

8	III f	0.0276±0.000356	**67.27%**
9	III g	0.0256±0.00051	55.15 %
10	III h	0.0184±0.000294	11.51 %
11	III i	0.0266±0.000572	21 .15 %
12	III j	0.0209±0.0001633	26.67 %

CONCLUSION

In conclusion, 10 new isoxazole derivatives were prepared and their structures were confirmed by spectral analysis. The physico-chemical data of the final compounds are describe in table 1. These compounds were screened for their antimicrobial activity and anti-inflammatory activity. The data reported in table 2 and 3 showed that effect of variation in chemical structure on activity was rather unpredictable. However a particular structural modification leads to generalize in terms activity. The results of this evaluation revealed that substitution of 4-$N(CH_3)_2$, 4-Cl, and OCH_3 groups at 2 and 3 positions are comparatively active when compared to other substitutions. In summary the present study may be helpful for the medicinal chemist who are working on isoxazole nucleus.

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