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Research Article

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A highly efficient, simple and ecofriendly microwave-induced synthesis of indolyl chalcones and isoxazoles

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ABSTRACT

Solvent free synthesis of 3-Aryl-1-(2-aryl-1H-indol-3-yl)prop-2-en-1-one has been successfully accomplished in high yields and lesser time by using green chemistry techniques(Grindstone chemistry and microwave irradiation). 3-Acetyl-2-phenyl indoles on treatment with either acidic or basic catalyst generate the corresponding enolates which reacts with aryl aldehydes affording the desired title products. All the synthesized compounds have been characterized by elemental analyses and spectral data (IR, ^{1}*HNMR and Mass). They have also been screened for their antibacterial and anti spermicidal activities and some of them have shown promising results against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.*

Keywords: Indolyl-isoxazole, indolyl chalcones, antibacterial activity, grindstone chemistry, microwave irradiation

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INTRODUCTION

Isoxazoles were reported for their various biological activities such as antimicrobial, anti-inflammatory.[1-3]. The reactive intermediate chalcones involved in their synthesis also exhibit wide range of these activities [4-6]. The ability of indole to exhibit anti-inflammatory, antimicrobial, antifungal activities [7-9] prompted the selection of indole as starting compound. In the light of these properties, it is interesting to synthesize some new indole-isoxazole derivatives and to evaluate their antibacterial and antispermicidal activities. 2-Aryl-1H-indoles were subjected to acetylation with acetyl chloride in glacial acetic acid to give 3-Acetyl-2-aryl-1H-indoles which on claisen schimdt reaction with substituted benzaldehydes gave desired 3- Aryl-1-(2-aryl-1H-indol-3-yl)-prop-2-ene -1-ones 4(a-j). Chalcones were condensed with hydroxyl amine hydrochloride in presence of sodium acetate and glacial acetic acid to obtain isoxazoles 5(a-j). The synthetic sequence leading to the formation of targeted compounds is depicted in scheme-1.

Fluorinated and poly fluoro substituted chalcones are known to act as antineoplastic agents[10]. Using aldol condensation reactions of chalcones many important compounds have been synthesized in synthetic organic chemistry[11-13]. These reactions can be carried out using many elegant synthetic procedures. Conventional methods are usually noxious and results in the generation of significant quantities of waste containing metal salts such as Li salts [14]. So we have designed various strategies for the synthesis of 3-aryl-1-(2-aryl-1H-indol-3-yl)-prop2-ene-1-one and assessed the efficiency of these synthetic routes. In order to make a comparative study we have used conventional and solvent free synthesis methods (Grindstone chemistry and Microwave irradiation) and inexpensive catalyst like KSF and $Mg(HSO_4)_2$ are used to obtain target molecules. All the synthesized compounds were screened for their antibacterial and antispermicidal activities.

In the present work grindstone technique was used first time for the synthesis of titled compounds. This method is superior since it is ecofriendly, high yielding, requires no special apparatus, non hazardous, simple and convenient. The significance of this approach is that chalcone can be obtained by grinding with dramatic improvement in yield [15]].further the work up process is also simplified since there is no involvement of any volatile organic solvent to be removed but only requires pouring and washing in water.

Another solvent free popular technique is microwave chemistry [16] since microwave irradiation leads to rate enhancement, product selectivity and less by product contamination. Supported reagents such as clay efficiently induce reactions under safe and simple conditions with domestic microwave oven instead of specialized expensive commercial microwave systems. In grinding method we have used $Mg(HSO_4)_2$ as catalyst. Grinding together of 3-Acetyl-2-aryl-1H-indole, arylaldehyde and catalytic amount of montmorilonite KSF clay yielded no desired product. Therefore the mixture was irradiated in a microwave oven which resulted in the formation of chalcones in good yield.

Montmorillonite KSF showed catalytic activity for chalcone synthesis and has many advantages over catalysts like NaOH, KOH such as ease of handling, low cost and elimination of metal wastes. In addition the catalyst can be utilized upto three cycles.

EXPERIMENTAL SECTION

General information-
Melting points were determined in open capillaries and are uncorrected. All solvents were then purified according to standard procedures. The ^{1}HNMR spectra were recorded at JEOL AL 300MHz FTNMR instrument, ν in cm^{-1}. Chemical shifts were calibrated to tetramethylsilane as an external reference. Coupling constants are given in hertz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet.IR spectra (cm^{-1}) were recorded on FTIR Nicolet Magna 550 and Shimadzu 8400s spectrophotometer in KBr disc and noteworthy absorptions levels (cm^{-1}) were listed. Mass spectra were determined by JEOL SX-102 spectrophotometer. TLC using silica gel G as adsorbent checked the purity of the compounds, UV light or iodine accomplished visualization. The reactions were carried out in a domestic microwave oven (Samsung M 1630 N/M 1610 W with maximum 600W Power).

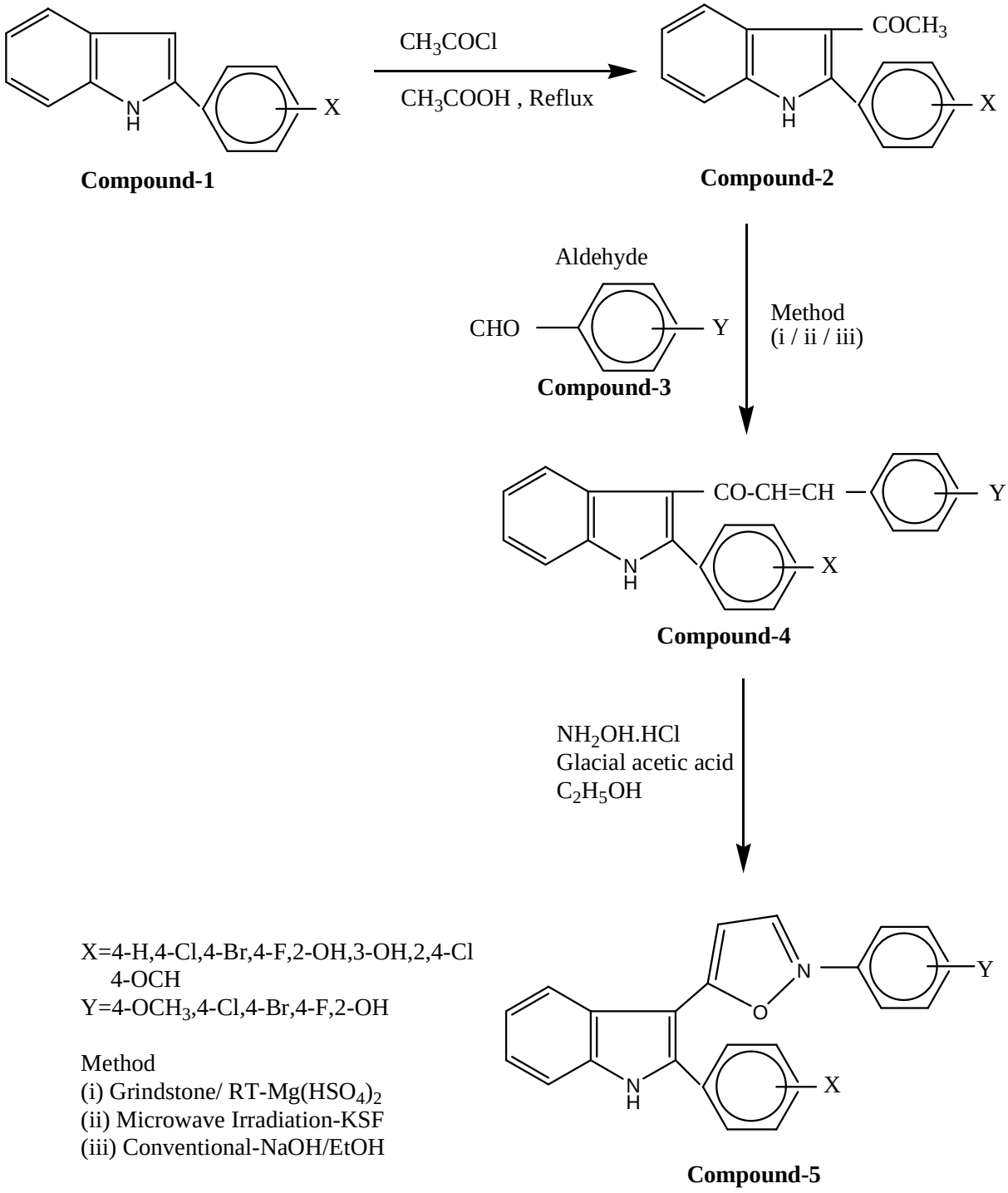
Synthesis of 2-aryl indole:-
The mixture of substituted acetophenones (10mmol) and phenyl hydrazine of 1.08 gm (10mmol) heated about 60-90 min. over water bath. Then we cooled it at room temperature and filtered. The cold mixture was washed with dil. HCl followed by about 12 ml of cold rectified spirit. A small portion of mixture was recrystallised and pure 4-fluoro aceto phenyl hydrazone as dark yellow crystal were obtained. Thus obtained hydrazone was cyclised with PPA (polyphosphoric acid, 10mmol) on an oil bath at 140-150^{o}C temperature maintained about 1-10 min. Then it poured in the ice water (crushed ice), filtered and washed with water for five times (in Bruckner funnel), recrystallised from ethanol and pet ether.

Synthesis of 3-Acetyl-2-aryl-1H-indole:-
A mixture of 2-fluoro aryl indole (5gm), glacial acetic acid (5ml) and acetyl chloride (150ml) was refluxed for 24 hr. Excess acetyl chloride and acetic acid were removed in vacco, the resultant product was poured into water and filtered. The solid mass was dissolved in ethanol (25ml) and sodium hydroxide solution (2N,10 ml) with stirring and heating on a water bath. The resultant solution was diluted with water and filtered off. The residual solid was recrystallised from ethanol and gave a single spot on TLC. The 3-acetyl-2-(fluoroaryl) indoles have been characterized by 2,4-dinitro phenyl hydrazones.

Synthesis of 3-Aryl-1-(2-aryl-1H-indol-3-yl)prop-2-en-1-ones 4(a-j):-
Method (i)- Solvent free Grindstone method-
A mixture of 3-acetyl-2-aryl-1H-indoles (3mmol) and $Mg(HSO_4)_2$ (0.04gm) was grinded together in a mortar using a pestle to generate yellow colored enolate. Then aryl aldehydes (3mmol) was added to it and grinding continued further to give orange red colored tacky solid within 10-20 minutes. The reaction proceeds exothermically indicated by rise in temperature (5-10^{o}C). After the reaction was complete (when no starting material was detectable by TLC analysis) CH_2Cl_2 was added, the mixture was filtered over sintered glass funnel and residue washed several times

with CH_2Cl_2. The filtrate was concentrated and the solid thus obtained was purified by recrystallisation from ethyl alcohol to afford pure desired compound 4(a-j) as yellow needles.

Scheme-1



X=4-H,4-Cl,4-Br,4-F,2-OH,3-OH,2,4-Cl
4-OCH
Y=4-OCH_3,4-Cl,4-Br,4-F,2-OH

Method
(i) Grindstone/ RT-$Mg(HSO_4)_2$
(ii) Microwave Irradiation-KSF
(iii) Conventional-NaOH/EtOH

Method (ii)-Microwave-assisted solvent free method-
3-acetyl-2-aryl-1H-indoles (3mmol), Aryl aldehyde (3mmol) and montmorillonite KSF (0.4gm) were thoroughly mixed in a pestle mortar. This mixture was then transferred into a conical flask ($100cm^3$) and irradiated with microwave for 60-190 seconds at 800 watts. After the reaction was complete (indicated by absence of starting material in TLC;(C_6H_6:EtOAc:: 95:5). 95% ethanol was added and the mixture was filtered. The filtrate was concentrated in vacco and then the crude product so obtained was recrystallised from ethanol to afford pure needles of desired compound. The catalyst left as residue during filtration 2-3 times with hot ethanol and dried in vaccum for reuse.

Method(iii)-Conventional method-
3-Acetyl-2-aryl-1H-indoles (3mmol) and substituted aryl aldehydes (3mmol) were dissolved in minimum amount of ethanol(25ml) and then stirred at room temperature for about 5 minutes. Sufficient 2N NaOH solution (40ml) was added to it, to render the stirred solution alkaline and the whole mixture was further stirred for about half an hour. This was then neutralized with 2N HCl (3ml) diluted with water and left overnight. The precipitated chalcone was filtered, washed well with water and recrystallised from ethanol.

3-(2-Aryl indole)-5-(substituted phenyl)-isoxazoles 5(a-j)-
Anhydrous sodium acetate (0.73gm, 0.01mol)was dissolved in a little amount of hot glacial acetic acid, to that ethanolic (10ml) solution of hydroxylamine hydrochloride (0.7gm,0.01mol) was added. The mixture was cooled and added to the solution of chalcone and then refluxed for 8-10 hrs. The mixture was concentrated and neutralized with NaOH solution. The product thus isolated was recrystallised from ethanol.

Table-1: Physical and analytical data's of 3-Aryl-1-(2-aryl-1H-indole-3yl) prop-2-en-1-ones 4(a-j)

Compounds	X	Y	Molecular Formula	Molecular Weight	Melting Point (°C)	Elemental analysis Found (calcd) %		
						C	H	N
4a	4-H	4-OCH_3	$C_{24}H_{19}NO_2$	353	90	81.5	5.38	3.96
4b	4-Cl	4-Cl	$C_{23}H_{15}NOCl_2$	396	80	69.6	3.78	3.53
4c	4-Br	4-Br	$C_{23}H_{15}NOBr_2$	485	115	56.9	3.09	2.88
4d	4-F	4-F	$C_{23}H_{15}NOF_2$	363	100	76.03	4.13	3.85
4e	2-OH	2-OH	$C_{23}H_{17}NO_3$	355	70	77.74	4.78	3.94
4f	3-OH	2-OH	$C_{23}H_{17}NO_3$	355	80	77.74	4.78	3.94
4g	2,4-Cl	4-Cl	$C_{23}H_{14}NOCl_3$	426.5	95	64.7	3.28	3.28
4h	2,4-Cl	4-OCH_3	$C_{24}H_{17}NO_2Cl_2$	422	100	68.24	4.02	3.31
4i	4-OCH_3	4-OCH_3	$C_{25}H_{21}NO_3$	371	120	77.62	5.66	3.77
4j	4-OCH_3	4-F	$C_{24}H_{18}NO_2F$	359	110	76.88	5.01	3.89

Table-2: Physical and analytical data's of 3-(2-aryl indole)-5-(substituted phenyl) isoxazoles 5(a-j)

Compounds	X	Y	Molecular Formula	Molecular Weight	Melting point (°C)	Elemental analysis Found (calcd) %		
						C	H	N
5a	4-H	4-OCH_3	$C_{24}H_{18}N_2O_2$	366	153	78.68	4.91	7.65
5b	4-Cl	4-Cl	$C_{23}H_{14}N_2OCl_2$	405	120	68.14	3.45	6.91
5c	4-Br	4-Br	$C_{23}H_{14}N_2OBr_2$	494	135	55.87	2.83	5.66
5d	4-F	4-F	$C_{23}H_{14}N_2OF_2$	372	115	74.19	3.67	7.52
5e	2-OH	2-OH	$C_{23}H_{16}N_2O_3$	368	110	75	4.34	7.6
5f	3-OH	2-OH	$C_{23}H_{16}N_2O_3$	368	115	75	4.34	7.6
5g	2,4-Cl	4-Cl	$C_{23}H_{13}N_2OCl_3$	439.5	125	62.79	2.95	6.37
5h	2,4-Cl	4-OCH_3	$C_{24}H_{16}N_2O_2Cl_2$	435	135	66.2	3.67	6.43
5i	4-OCH_3	4-OCH_3	$C_{25}H_{20}N_2O_3$	396	150	75.75	5.05	7.07
5j	4-OCH_3	4-F	$C_{24}H_{17}N_2O_2F$	384	145	75	4.42	7.29

Biological Activities-
Spermicidal activity-
Sperm motility scheme was followed to see the effect of chemicals. Compounds 5(a-j) were evaluated for spermicidal activities, briefly the motility of sperms was observed under 40x magnification of motic digital microscope with image processing software and CCD colored camera attached to a computer assembly in a cell counting chamber. While observing the motility ; 25µl solution (compound + alcohol) was added to the 25µl semen and it was observed that addition of compounds to semen produces a reduction of sperm motility in HF cattle spermatozoa; which was dose and time dependent. At 100ppm concentration of compound 5h, HF cattle sperm

motility was reduced to 25% in 2.5 min. Compound 5g at 100ppm inhibited motility to 30% in 3 min. After 3 and 2.5 min. sperm motility was reduced to 35% by compound 5d,5i and 5j respectively. Whereas compound 5a, 5b, 5c,5e,5f compounds reduces the motility to 50% in 3.5-5 min. at 100ppm concentration only. The results have been summarized in table and shown by graph. (figrure-1).

Table-3: Spectral data's of 3-(2-aryl indole)-5-(substituted phenyl) isoxazoles 5(a-j)

Compounds	IR νmax (cm^{-1}) (KBr)	1HNMR δ (ppm) ($CDCl_3$)	Mass (m/z) (M^+)
5a	3220 (-N-H), 1600 (>C=N),1240 (C-O-N), 3030 (=CH str.)	6.00 (s,1H,Isoxazole ring proton) 7.41-7.68 (m,8H,Aro.), 11.06 (s,1H,N-H)	365
5b	3225 (-N-H), 1615 (>C=N),1245 (C-O-N), 3020 (=CH str.)	6.18 (s,1H,Isoxazole ring proton) 7.31-7.80 (m,8H,Aro.), 11.02 (s,1H,N-H)	404
5c	3218 (-N-H), 1605 (>C=N),1230 (C-O-N), 3035 (=CH str.)	6.05 (s,1H,Isoxazole ring proton) 7.40-7.98 (m,8H,Aro.), 11.10 (s,1H,N-H)	493
5d	3230 (-N-H), 1610 (>C=N),1220 (C-O-N), 3025 (=CH str.)	6.12 (s,1H,Isoxazole ring proton) 7.32-7.78 (m,8H,Aro.), 11.16 (s,1H,N-H)	371
5e	3228 (-N-H), 1610 (>C=N),1236 (C-O-N), 3028 (=CH str.)	6.10 (s,1H,Isoxazole ring proton) 7.21-7.87 (m,8H,Aro.), 11.08 (s,1H,N-H)	367
5f	3210 (-N-H), 1620 (>C=N),1235 (C-O-N), 3015 (=CH str.)	6.06 (s,1H,Isoxazole ring proton) 7.34-7.98 (m,8H,Aro.), 11.03 (s,1H,N-H)	367
5g	3224 (-N-H), 1608 (>C=N),1245 (C-O-N), 3010 (=CH str.)	6.08 (s,1H,Isoxazole ring proton) 7.21-7.70 (m,8H,Aro.), 11.16 (s,1H,N-H)	438.5
5h	3226 (-N-H), 1620 (>C=N),1220 (C-O-N), 3025 (=CH str.)	6.10 (s,1H,Isoxazole ring proton) 7.31-7.90 (m,8H,Aro.), 11.09 (s,1H,N-H)	434
5i	3236 (-N-H), 1615 (>C=N),1238 (C-O-N), 3038 (=CH str.)	6.02 (s,1H,Isoxazole ring proton) 7.45-8.08 (m,8H,Aro.), 11.05 (s,1H,N-H)	395
5j	3215 (-N-H), 1606 (>C=N),1232 (C-O-N), 3020 (=CH str.)	6.00 (s,1H,Isoxazole ring proton) 7.11-7.79 (m,8H,Aro.), 11.09 (s,1H,N-H)	383

Table-4

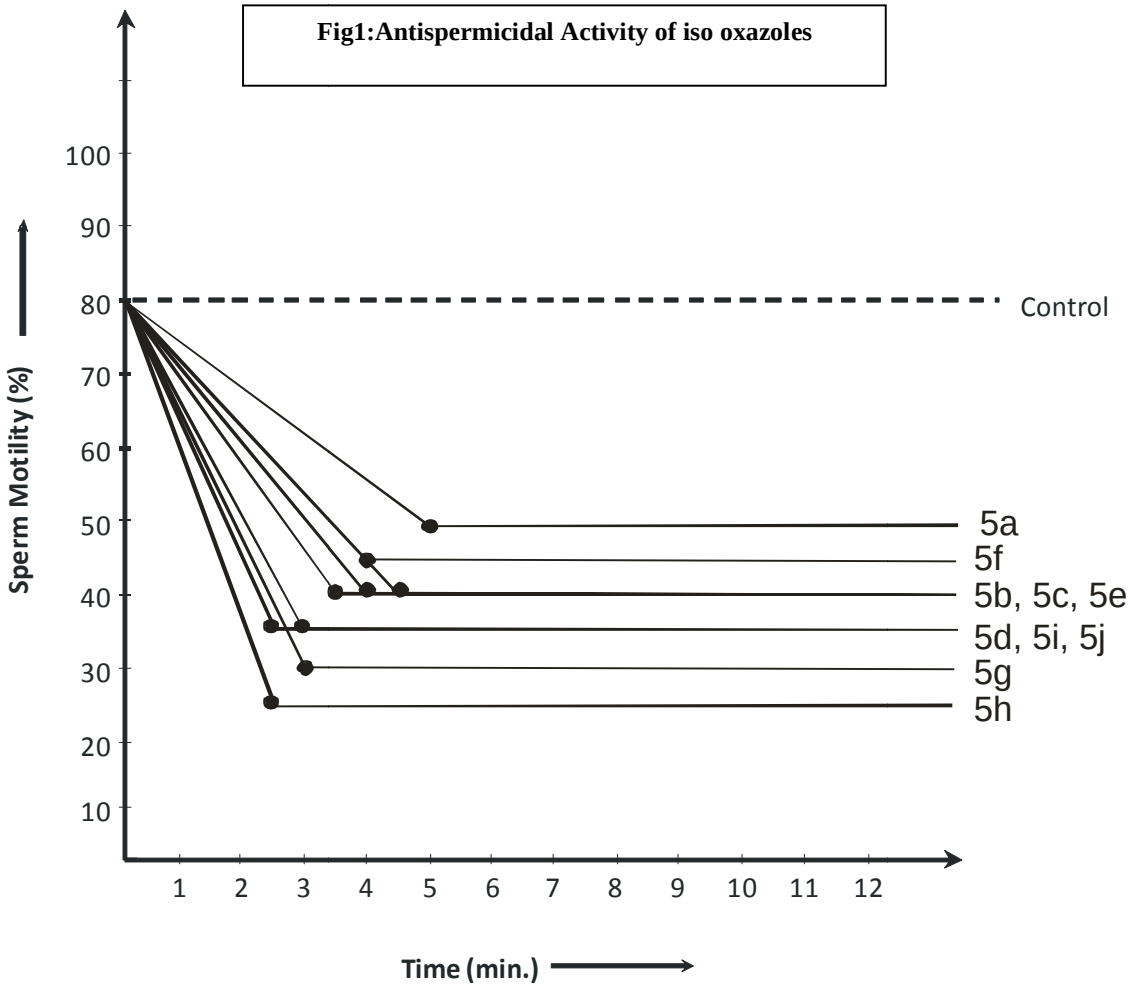
Compounds	Dose (ppm)	Motility reduced (%)	Time	Base
5a	100	50	5.0	Alcohol
5b	100	40	4.5	Alcohol
5c	100	40	3.5	Alcohol
5d	100	35	3.0	Alcohol
5e	100	40	4.0	Alcohol
5f	100	45	4.0	Alcohol
5g	100	30	3.0	Alcohol
5h	100	25	2.5	Alcohol
5i	100	35	3.0	Alcohol
5j	100	35	2.5	Alcohol

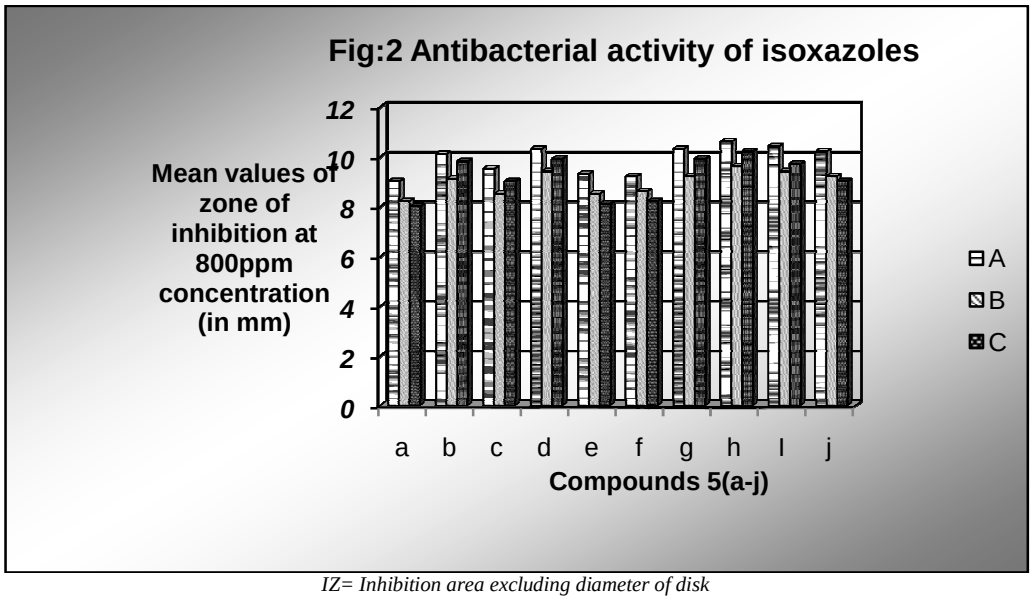
Antibacterial Activity-

All the synthesized compounds were evaluated in vitro for antibacterial activity by using filter paper disc method [19-26] against different strains of bacteria viz. Staphylococcus *aureus*, Escherichia *coli*, Pseudomonas *aeruginosa*. All the compounds test along with standard antibacterial Ibuprofen were used at 200,400,600,800ppm concentrations.

Procedure: Solution of known concentrations (200,400,600,800ppm) of the test samples were made by dissolving in DMF. Dried and sterilized filter paper discs (6mm in diameter) soaked with known amount of test agents were placed on the nutrient agar media solidified in petridishes (120mm in diameter) and incubated with the test organisms. These plates were then kept at temperature (37^oC) for 18 hours to allow maximum growth of the organisms. The antibacterial activity was determined by measuring the average diameter of zone of inhibition in mm. (figure-2)

Fig1:Antispermicidal Activity of iso oxazoles





IZ= Inhibition area excluding diameter of disk
AI= Activity index = inhibition area of sample/inhibition area of standard
A= Staphylococcus aureus , B= Escherichia coli , C= Pseudomonas aeruginosa

RESULTS AND DISCUSSION

The synthesis of the target compounds was accomplished according to the reaction sequences illustrated in scheme - 1. As visualized, the reaction proceeded smoothly through solvent free methods and the chalcones were obtained excellent yields (82-92%) within a few minutes (6-14 min.)

Grinding together the solid aldehydes and ketones without addition of catalyst reveals an interesting phenomenon, in some cases a liquid melt is observed while in others the solid reagents remained as discrete crystalline phases [17]. More important, upon addition of the solid catalyst a rise in temperature (5-10^{o}C) is observed and the reaction is only observed in those systems that exhibit a phase change to a melt. Thus, the existence of a liquid phase is a prerequisite for reaction in these systems. In all of the cases where reaction is observed, the mixture solidifies as the solid dehydration product separates from the melt. In case of KSF, the reaction mixture was irradiated for 60-190 seconds in a domestic microwave oven. The assigned molecular structure of all indolyl chalcone 4(a-j) were based on spectroscopic analysis (IR,^{1}HNMR and MS) and elemental analysis data.

In the IR spectra of 2-aryl-1H-indoles, N-H stretching absorption appears as a strong absorption band from 3470-3420 cm^{-1}. The strong absorption band in the range 1275-1200 cm^{-1} has been attributed to Ar-F stretching modes. The C-N frequencies have been assigned to the 1200-975 cm^{-1} region. In 3-Acetyl indole derivatives the N-H stretching band is shifted towards lower wave numbers (3375-3125 cm^{-1}) due to the presence of >C=O group at position 3 of the indole ring. >C=O absorption band is observed downfield between 1620-1605 cm^{-1} due to conjugation of >C=O with the indole ring. One of the special features of the aceylated indoles is the occurrence of two broad strong peaks in the N-H stretching region. This may be due to the intermolecular hydrogen bonding between the N-H group of one molecule and the >C=O group of second molecule [18].

In the IR spectra of 4(a-j) >N-H absorption appears as a broad band from 3375-3200 cm-^{1}. Characteristic absorption due to >C=O group appears in the range of 1625-1605 cm^{-1}. This downfield shift is due to conjugation of the carbonyl group with the olefinic double bond and aryl groups, which results in delocalization of the electrons of carbonyl group giving ionic resonance structures. The olefinic double bond (>C=C<) appears between 1600-1594 cm^{-1} .

In ^{1}NHMR spectra of 2-aryl-1H-indole, methine proton at C-3 position of indole moiety shows a resonance signal at δ 6.4 ppm and N-H resonance signal is observed in the region δ 7.8-8.2 ppm as a broad singlet. Aromatic protons are observed as multiplet from δ 6.8-7.7 ppm. In 3-acetyl-2-aryl-1H-indole resonance signal due to methine proton at C-3 disappears and a singlet due to CH_3 proton appears in the region δ 2.1-2.3 ppm. Aromatic and N-H resonance signals remains unaltered. The ^{1}NHMR spectra of 4(a-j) exhibit a complex splitting pattern. The two olefinic protons constitute an AB system and appear as a pair of doublet, the downfield doublet in the region δ 7.30-7.63ppm with coupling constant J=8.1-8.4 Hz due to –CH=CH-proton and the doublet in the region δ 6.97-7.1ppm with coupling constant J=8..1-8.4 Hz due to –CO-CH= proton. This strong downfield shift of these two protons is due to extended conjugation with aromatic protons. High value of coupling constant suggests trans geometry across the double bond. Aromatic protons appear as a complex multiplet in the region δ 6.7-7.6 ppm. N-H resonance signal appears as a broad singlet from δ 8.0-8.4 ppm. The IR and ^{1}HNMR data of 3-Aryl-1-(2-aryl-1H-indole-3yl) prop-2-en-1-ones are summarized in table-1.

It has been reported that α,β-unsaturated ketone which are attractive due to their electrophillic properties can react with nitrogenous bases to give the corresponding isoxazoles. Thus a series of isoxazoline derivatives 5(a-j) were obtained by treating chalcones with hydroxylamine hydrochloride in ethanol in the presence of catalytic amount of glacial acetic acid. To accelerate the reaction and to improve the yield of products, the microwave irradiation technique was applied. The microwave-assisted reaction in solvent and catalyst-free environment proceeded efficiently and completed within 6-14 min. with excellent yield of products (76-89%).

In their IR (cm-^{1}) spectral studies characteristic absorptions are observed in region 3210-3236 due to(-NH-) group, 1600-1620 due to (>C=N) group, 1220-1245 (C-O-N) and 3015-3035 cm-^{1} due to =CH.

In ^{1}HNMR spectra of 5(a-j), δ 6.00(s,1H, isoxazole ring proton), 7.41-7.68(m,8H,aromatic protons), 11.06 (s, 1H,N-H).

CONCLUSION

We developed an easier, facile and practically convenient green methodology for the synthesis of indolyl chalcones and isoxazoles. The notable merits offered by this methodology are mild reaction conditions, simple procedure, short reaction time and excellent yield of the products.

Compounds with electron releasing groups such as methoxy, hydroxyl exhibit better antibacterial activity than others not having such groups. Compounds having pharmacophores such as chloro, dichloro and fluoro groups have exhibited more activity on all the three bacteria than the others. The results suggests that the chalcones derivatives have excellent scope for further development as commercial antimicrobial agents. Further experiments were needed to elucidate their mechanism of action.

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