Eco Friendly Synthesis and Potent Antimicrobial Activities of Pyrazoles and Isoxazoles

BHOYAR A. D. [1], PARIHAR R.T. [2], RAJPUT P.R. [3]

[1]Department of Chemistry, P.R. Patil College of Enggineering and Technology, Kathora Road, Amravati 444607, Maharasthra, India. email: amolbhoyar@rediffmail.com

[2]Department of Chemistry, Vidnyan Mahavidyalaya, Malakapur, Dist. Buldhana, Maharasthra, India

[3]Department of Chemistry, Vidybharati Mahavidyalaya, Camp, Amravati 444601, Maharasthra, India

Abstract

Condensation of 2-substituted-3,5-dichloroacetophenones 2a-c obtained from the condensation of 2-hydroxy-3,5-dichloroacetophenone 1 and benzoyl chloride were dissolved in NaOH , on treatment under baker venkatraman transformation in presence of KOH with pyridine gives 1-(2-hydroxy-3,5- dichlorophenyl)-3-substituted-1,3-propanedione 3a-c. Then converted into 3–aroyl-6,8-dichloroflavanones 4a-i by using different aromatic aldehyde in the presence of ethanol, Piperidine. The condensation of 4a-i with crystal of iodine in DMF solvent gives 3-aroylflavone 5a-i. The condensation of 5a-i and phenylhydrazinehydrochlorides, piperidine in DMF gives 4-aroyl-3,5-diarylpyrazoles 6a-i and condensation of 5a- i and hydroxylaminehydrochlorides gives 4-aroyl–3,5diarylisoxazoles 7a –i. The above compounds are screened for their antimicrobial activities and have been found to exhibit significant antibacterial and antifungal activities. The zones of inhibition measured in term of mm.

Key words: flavanone, isoxazoline, pyrazoline.

Introduction

In Order to synthesize flavanones, flavones, pyrazoles and isoxazoles the reaction sequence were followed as out line in the scheme I. The required 1-(2-hydroxy –3, 5 – dichlorophenyl)–3-substituted-1,3-propanedio ne was synthesized from 2-hydroxy–3,5-di-chloroacetophenone which on condensation is converted into 2-aroyl–3,5-dichloroaceto phenones 2 were reacted under baker venkatraman transformation in presence of KOH with pyridine gives 1-(2-hydroxy–3,5- dichlorophenyl)-3-substituted-1,3-propanedi-one 3 and then converted into 3-aroyl- flavanones 4 by using different aromatic aldehyde in the presence of ethanol- piperidine. The reaction of 4 and iodine in the presence of DMF yields

flavones 5. The condensation of 5 and phenylhydrazine hydrochloride in presence of DMF and piperidine gives pyrazoles 6 and the Condensation of 5 and hydroxylamine- hydrochloride gives isoxazole 7. The characterization data of compounds (3, 4, 5, 6 and 7) represented in table I . The new compounds prepared were characterized and screened for their antimicrobial activity.

Material and method

All melting points were determined in open capillary tubes and are uncorrected. I.R. spectra were recorded on a Perkin Elmer Infra Red spectrophotometer 1310 using KBr disc. ^{1}H NMR was recorded in $CDCl_3$ on a DRX 300 spectrometer. The reactions were monitored on TLC on silica gel G and the solvent system used was benzene.

2–Aroyloxyacetophenone (2a– c)

2–hydroxy–3,5-dichloroacetophenone (0.04 mol.) and benzoyl chloride (0.05mol.) were dissolved in NaOH (10%) 30 ml (2a), 2–hydroxy-3,5-dichloroacetophenone (0.04 mol) and anisic acid (0.05 mol) were suspended in dry pyridine (30 ml) with $POCl_3$ 3ml, (2b) 2-hydroxy-3,5-dichloroacetophenone (0.04 mol) and salicylic acid (0.05 mol) were suspended in dry pyridine (30ml) with $POCl_3$ 3ml (2c) All the above reaction mixture was kept for overnight and then worked up by dilution and acidification with ice cold HCl (50%) to neutralize pyridine. The solid product was filtered washed with water followed by sodium bicarbonate (10%) washing finally again with water it crystallized from ethanol to obtained 2–Aroyloxyacetophenone (2a- c).

1–(2-hydroxy–3,5-dichlorophenyl)–3-aryl–1 ,3-propanedione (3a – c)

When 2–Aroyloxyacetophenone (2a–c) (0.05 mol) was dissolved in dry pyridine 40ml .The solution was warmed upto 600C and pulverized KOH (15 g) was added slowly with constant stirring. After 4 h the reaction mixture was acidified by adding ice cold dil.HCl (1:1) The product thus separated was filtered washed with sodium bicarbonate solution (10%) and finally again with water. It was then crystallized from ethanol – acetic acid mixture to get 1-(2-hydroxy–3,5–dichlorophenyl)–3-aryl-1,3-propanedione (3a-c) respectively.

3a – IR spectrum recorded in KBr (cm–1) 3030, (v), -OH ; 1600, (s), >C=O ;1170, (s), >C–O; 790,(s), C-Cl. PMR spectrum recorded in δ CDCl3 3.69,(s), 3H, Ar–O-CH3; 4.56,(s), 2H, –CO–CH2–CO– (Keto) ; 6.6, (s), 1H, –C=CH- ; 6.92–8.08, (m), 6H, Ar-H ; 12.75, (s),1H,Ar–OH; 15.71,(s),1H,–CHOH=C(enol) TLC : Solvent (Benzene) height : 2.7 cm, solute height : 2.3 cm; Rf value : 0.85 , m.p.1120C, yield 78%.

3–Aroylflavanone (4a-i)

1–(2–hydroxy–3,5-dichlorophenyl)–3–(4'-methoxyphenyl)-1,3-propanedione 3a (0.01 mol) and anisaldehyde, benzaldehyde, salicylaldehyde(0.012 mol) separately was refluxed in ethanol (25 ml) and piperidine (0.5ml) for 15-20 min. yield 3–arylflavanone (4a-c)resp.1-(2-hydroxy–3,5–dichlorophenyl) -3–phenyl-1,3-propanedione 3b (0.01mol) and anisaldehyde, benzaldehyde, salicyl-aldehyde (0.012 mol) separately was refluxed in ethanol (25 ml) and piperidine (0.5 ml) for 15–20 min. yield 3–aroylflavanone (4d–f) resp. 1-(2-hydroxy–3,5–dichlorophenyl–3-(2'-hydroxyphenyl)–1,3-proponedione 3C (0.01mol) and anisaldehyde, benzaldehyde, salicylaldehyde (0.012 mol) separately was refluxed in ethanol (25 ml) and piperidine (0.5ml) for15–20 min. yield 3–aroylflavanone (4g–i) resp. All above reaction after refluxing, cooling the reaction mixture was acidified with dil. HCl (1:1). The product thus separated was filtered washed with sodium bicarbonate solution (10%) and finally again with water. It was then crystallized from ethanol–acetic acid mixture.

4d IR spectrum recorded in KBr (cm-1)

1637, (s), >C=O ; 1562, (s), >C=O ; 1213,(s), C-O-C ; 825, (s) , C–Cl PMR spectrum recorded in δ CDCl3 3.89, (s), 3H, Ar–OCH3 ; 5.36, (dd) , 1 H, CHA – CH ; 5.76 (dd) , 1H, CH – CHB ; 6.7–8.1, (m), 11H, –Ar–H. TLC: Solvent (Benzene) height: 2.0cm Solute height: 1.7 cm; Rf value: 0.85, m.p.1780C, yield 72%.

3–aroylflavone (5a-i)

3-aroyl-6,8–dichloroflavanone (4a-i) (0.01 mol) was refluxed for 10 minutes with crystals of iodine in DMF (20 ml). After cooling the reaction mixture was dilute with water. The solid product thus separated was filtered, washed with sodium bicarbonate solution and then with water. Finally it was crystallized from ethanol-acetic acid mixture to get the compounds 3-aroylflavone (5a-i)

5d IR spectrum recorded in KBr (cm-1) 1602, (s), >C=O ; 1560,(m), >C=C< ;1257, (s), Ar–O–C ; 879,(s), C-Cl ;PMR spectrum recorded in δ CDCl3 3.9, (s), 3H, Ar–OCH3 ; 7.06–8.12, (m), 11H, Ar–H. TLC : Solvent (Benzene) height : 2.2 cm , Solute height : 1.7 cm Rf Value 0.77 m.p.1770C yield 85%

4-Aroyl-3,5-diaryl-1-Phenylpyrazoles (6a-i)

When 3–aroylflavones (5a–i) (0.01 mol) and phenylhydrazinehydrochloride (0.02 mol) were refluxed in DMF 20 ml containing a few drops of piperidine for 1.5 h. separately, After cooling the mixture was diluted with water HCl (1:1). The product thus separated was filtered and crystallized from ethanol–acetic acid to yield 4–Aroyl-3,5-diaryl–1-Phenyl-pyrazoles (6a–i) respectively.

6d IR spectrum recorded in KBr cm-1

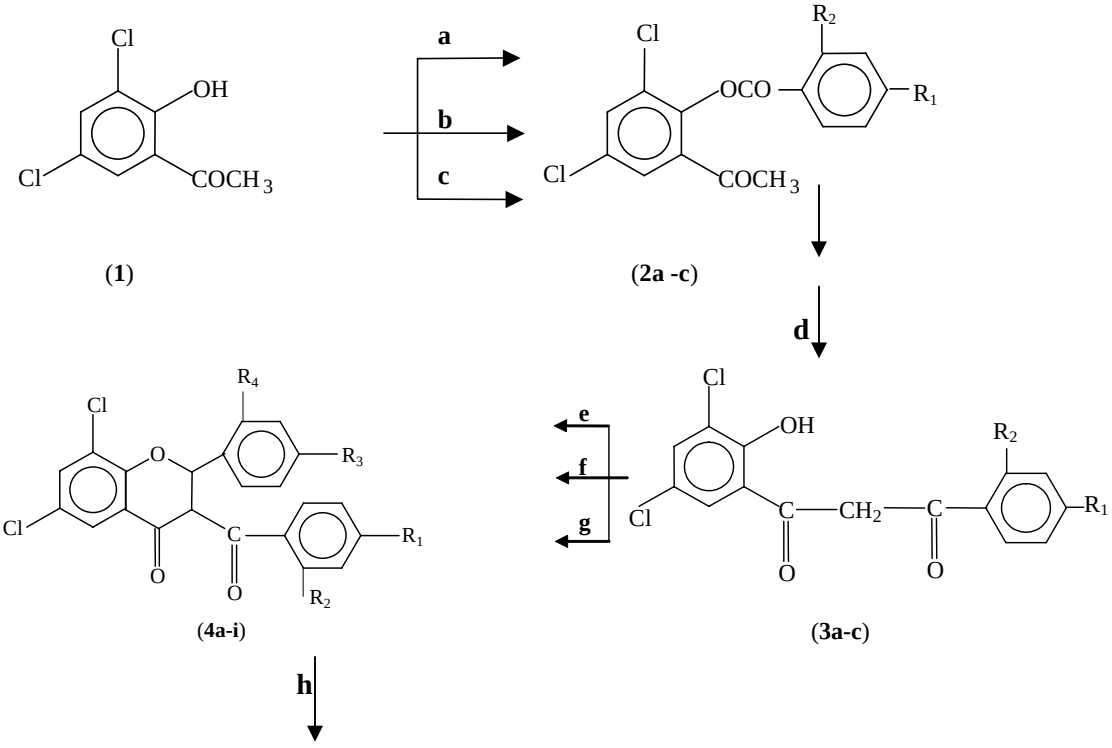
3070,(w,b),–OH; 2831, (s), –C-H ;1645 , (s) >C=O; 1600,(s), >C=N ; 1502, m, >C=C<; 1253, (m), Ar–O–C ; 837, (s), C–Cl; PMR spectrum recorded in δ CDCl3 3.80; (s) , 3H, Ar-OCH3; 6.8 – 7.4 (m) 16 H , Ar – H ;11.48 (s) , 1H, Ar – OH. TLC: Solvent (Benzene) height: 2.0cm Solute height: 1.5 cm; Rf value: 0.75, m.p. 1950C, yield 72%.

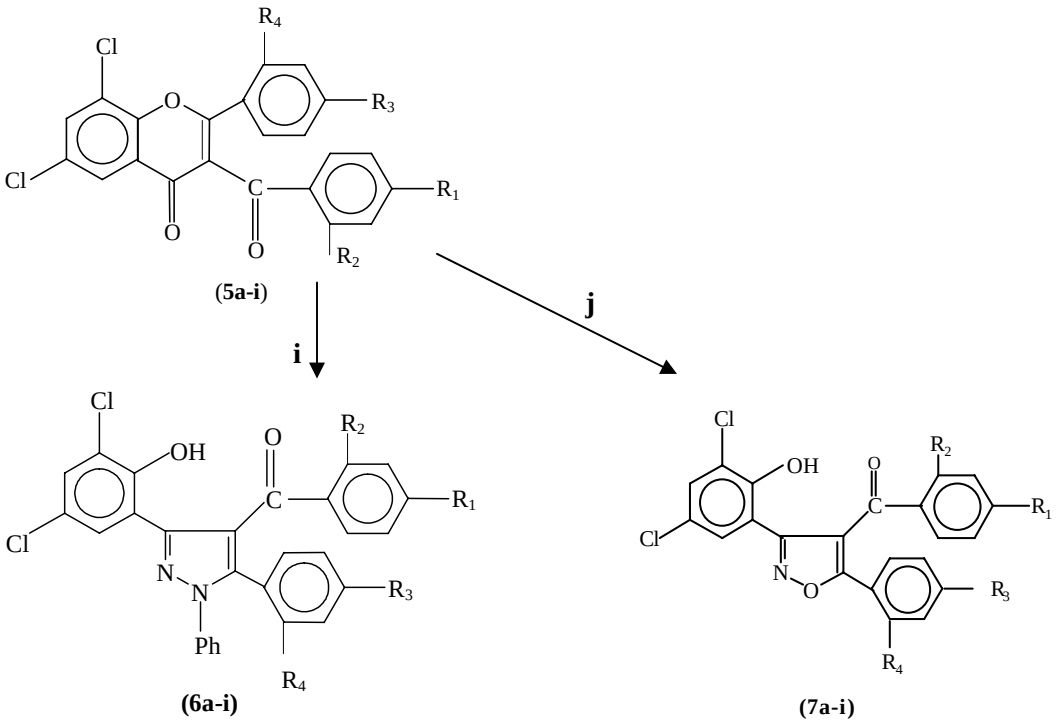
4–aroyl–3,5–diarylisoxzoles (7a– i)

When 3–aroylflavone (6a–i) (0.01 mol) and hydroxylaminehydrochloride (0.02 mol) were refluxed in DMF 20 ml containing few drops of piperidine 0.5 ml for about 1.5 h. separately, After cooling the mixture was acidified with HCl(1:1). The product thus separated, filtered and crystallized from ethanol-acetic acid to yield 4–aroyl–3,5–diarylisoxazoles (7a–i) respectively. 7d IR spectrum recorded in KBr (cm-1) 3367 (wb),–OH; 1608, (s), >C=O; 1515,(s),>C= N ; 1253, (s), Ar–O–C ; 827, (s), C-Cl ; PMR spectrum recorded in δ CDCl3 3.87,(s), 3 H, Ar-O-CH3 ; 6.98– 8.01,(m),11H, Ar–H ; 10.12,(s),1H,Ar–OH; TLC:Solvent (Benzene) height : 2.9 cm , Solute height : 2.3 cm Rf Value 0.79 , m.p.2050C , yield 72%

Results and Discussion

2-hydroxy-3,5-dichloroacetophenone (1) on treating with different aromatic acid in the presence of pyridine or NaOH gives a compounds containing aromatic group,





a: C_6H_5 COCl, NaOH (10 %) **b** : Anisic Acid, $POCl_3$, Pyridine **c** : Salicylic acid, $POCl_3$, Pyridine
d : Pyridine, KOH **e** : Benzaldehyde, Piperidine, Ethanol **f** : Anisaldehyde, Piperidine, ethanol
g : Salicylaldehyde, Piperidine ethanol **h** : I_2 , DMF **i** : $PhNHNH_2$.HCl, Piperidine, DMF
j : NH_2OH.HCl, Piperidine, DMF

These structures are possible for these compounds (2a, 2b, 2c). The IR spectrum of this compound consist of a ester stretching band at 1790 cm^{-1}, thereby suggested that there is reaction between hydroxyl group and benzoyl chloride (2a). However (2b) shows a PMR peak at δ2.60 of Ar-OCH_3 and (2c) shows a IR peak at 3040 cm^{-1}, this confirms there is presence of OH group and peak at 1820 $^{cm-1}$ for ester group.

The acetophenones (2a-c) was formylated by the reaction of pyridine in KOH gives 1-(2-hydroxy-3,5-dichloro- phenyl)-3-aryl-1,3-propanedione (3a-c). This on reaction with different aldehyde gives 3-aroylflavanones (4a-i). When flavanone on treatment with iodine in DMF medium gives flavones (5a-i) whose structure was also corroborated by spectral analysis. These flavones on treatment with phenyl- hydrazinehydrochloride in DMF medium containing small amount of piperidine gives pyrazoles (6a-i) which was confirmed by its spectral analysis

In a similar fashion 3-aroylflavones (5a-i) was treated with hydroxylamine- hydrochloride in DMF medium containing small amount of piperidine gives 3,5-diaryl-4-aroylisoxazoles (7a-i) which was characterized by spectral analysis.

Conclusion

Antimicrobial activity

The compounds 6 and 7 were screened for their antibacterial activity against S. aureus, S. typhi at concentration of 1000 µg using narfloxacin as standard and antifungal activity against A. nigar, A. fumigates at concentration of 1000 µg using griseofulving as standard. Test solution was prepared by dissolving 1mg of (1000 µg) of compound in 1ml of DMF and 0.1 ml (100 µg) of this solution was using for testing. The zones of inhibition were measured in mm (12-16 mm, 17-22 mm, 23-27 mm for weak, moderate and highly active zones respectively).

Table 1 Characterization data of compounds 3, 4, 5 , 6 & 7

Compds	R1	R2	R3	R4	M.P. 0C	Yield
3a	OCH3	H	-	-	112	78
3b	H	H	-	-	122	82
3c	H	OH	-	-	115	75
4a	OCH3	H	OCH3	H	156	62
4b	OCH3	H	H	H	167	75
4c	OCH3	H	H	OH	182	78
4d	H	H	OCH3	H	178	72
4e	H	H	H	H	161	87
4f	H	H	H	OH	156	63
4g	H	OH	OCH3	H	173	82
4h	H	OH	H	H	169	72
4i	H	OH	H	OH	163	83
5a	OCH3	H	OCH3	H	170	70
5b	OCH3	H	H	H	195	72
5c	OCH3	H	H	OH	178	81
5d	H	H	OCH3	H	177	85
5e	H	H	H	H	185	82
5f	H	H	H	OH	196	86
5g	H	OH	OCH3	H	183	76
5h	H	OH	H	H	192	72
5i	H	OH	H	OH	163	79

6a	OCH3	H	OCH3	H	172	78
6b	OCH3	H	H	H	177	85
6c	OCH3	H	H	OH	183	76
6d	H	H	OCH3	H	195	72
6e	H	H	H	H	185	82
6f	H	H	H	OH	192	72
6g	H	OH	OCH3	H	178	81
6h	H	OH	H	H	196	86
6i	H	OH	H	OH	163	79
7a	OCH3	H	OCH3	H	198	79
7b	OCH3	H	H	H	192	82
7c	OCH3	H	H	OH	179	72
7d	H	H	OCH3	H	205	72
7e	H	H	H	H	198	76
7f	H	H	H	OH	188	83
7g	H	OH	OCH3	H	182	85
7h	H	OH	H	H	205	88
7i	H	OH	H	OH	187	79

Narfloxacin showed a zone inhibition of 27 mm for *S. aureus* and 25 mm for *S. typhi.* Griseofulving exhibited a zone inhibition of 28 mm for both *A. nigar* and *A.fumigates* DMF was used as solvent control using agar plate technique[1] and are shown in Table 2.

Table 2 Antimicrobial activity data of compounds 6a- i and 7a- I Antimicrobial activity

	Antibacterial activity		**Antifungal Activity**	
Compd	**S. aureus**	**S. typhi**	**A. nigar**	**A. fumigates**
6a	22	24	21	25
6b	23	19	21	16
6c	20	22	19	20
6d	21	23	19	22
6e	13	18	15	16
6f	19	15	17	12
6g	20	23	25	21

6h	20	15	18	16
6i	12	16	14	19
7a	24	20	22	21
7b	18	22	24	20
7c	20	21	23	24
7d	21	20	19	23
7e	13	16	17	19
7f	14	13	16	19
7g	21	19	23	22
7h	17	16	19	15
7i	15	18	17	20

Presence of methoxy groups invariably increased the antibacterial activity of compound. The isoxazoles and pyrazoles having methoxy group were more active than the other pyrazoles and isoxazoles.

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