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Synthesis and Microbial Studies of Some New Oxygen Heterocycles

Poonam Yadav[a] and Nalini V Purohit[b]*

[a]*School of Science and Education, Navrachana University, Vadodara*
[b]*Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara*

ABSTRACT

In an attempt to find a new class of antimicrobial agents, a series of oxygen heterocyclic isocoumarins, dihydroisocoumarins, aromatic / aliphatic amino methyl isocoumarin derivatives having benzoyl group at 3rd position in isocoumarin moiety was synthesized using simple appropriate starting material o-acyl benzoic acid and bromoacetophenone derivatives in presence of anhy. K_2CO_3 in ethyl methyl ketone. These compounds were screened for their antibacterial activity against gram (+ve) S. Aureus and gram (-ve) E. Coli bacteria. Most of the isocoumarin derivatives were active against gram (+ve) bacteria and the best result was with dihydroisocoumarin.

Key words: isocoumarin derivatives, dihydroisocoumarins, amino methyl isocoumarins, antibacterial activity, antifungal activity.

INTRODUCTION

Oxygen heterocycles especially coumarin isocoumarins and dihydroisocoumarins are reported to posses various physiological activities [1-6]. The biological activities of these heterocycles have been known for many years. Two types of oxygen heterocycles, isocoumarins and dihydroisocoumarins are found in nature. Substituted isocoumarins are obtained from *Coriandrum sativum L*, an umbelliferous plant [7-8], *C. glabrum* [9], *P. scoparius*, a toxic and endemic plant from North Africa [10] and 3,4 dihydroisocoumarins from Periconia macrospinosa and Sapornia affinits [11]. In our earlier studies we found an increase in the microbial / biological properties of the isocoumarins by introducing various substituted alkyl / aroyl / aryl linkage at various positions, especially at 3rd & 4th position of isocoumarin nucleus [12-14]. We have also used isocoumarin / coumarin moieties together for its varied biological activities. But 4-alkyl-3-aroyl substituted dihydroisocoumarins are still not reported in the literature, using the same starting material as isocoumarin does. Isocoumarins have important role in medicinal filed with varied biological activities like Antibacterial, antifungal, anti cancer [15], enzyme inhibiting [16], cytotoxic [17], hepatoprotective [18] and anti influenza [19].

On the other hand careful literature survey have also revealed that isocoumarin ring system have occupied a unique position in the design and synthesis of novel biological agents with remarkable analgesic and anti-inflammatory activities [20] in addition to their well documented potential antimicrobial activities. Literature survey shows amino methyl coumarins as novel coumarins [21] for their antibacterial activity which also propelled us to synthesize some new amino methyl isocoumarins by extending the series.

MATERIALS AND METHODS

Chemistry

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254. IR were recorded on FTIR Perkin Elmer spectrophotometer and ^{1}H NMR spectra on a Bruker spectrometer (400 MHz) using TMS as internal standard. Mass spectrums were recorded on Thermo Scientific Corporation, DSQ II Mass Spectrometer. All the compounds gave satisfactory elemental analysis. O-acyl benzoic acid **1** [23-24], 4-alkyl-3-aroyl isocoumarin **6** [13] and bromo compounds **2** and **4** [25] were prepared by literature method.

General procedure for synthesis of 4-alkyl-3- aroyl isocoumarin 3a-3b

A mixture of o-acetyl benzoic acid **1a** (0.41 g, 0.0025 moles), 5-methyl-2-hydroxy bromoacetophenone **2** (0.6 g, 0.0025 moles) anhy.K_2CO_3 (1.0 g, 0.0052 moles) in ethyl methyl ketone was refluxed for 10- 12 hrs, solvent was removed completely; water added and reaction mixture was extracted with ethyl acetate. Solvent layer was first washed with sat. sodium bicarbonate and then with water and finally dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture.

General procedure for synthesis of Dihydroisocoumarin 5a-5b

A mixture of o-acetyl benzoic acid **1a** (0.41 g, 0.0025 moles), α – bromo-4-hydroxy propiophenone **4** (0.6 g, 0.0025 moles) anhy.K_2CO_3 (1.0 g, 0.0052 moles) in ethyl methyl ketone was refluxed for 10- 12 hrs, solvent was removed completely; water added and reaction mixture was extracted with ethyl acetate. Solvent layer was first washed with sat. sodium bicarbonate and then with water and finally dried over anhy. Na_2SO_4. After removal of solvent the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture.

General procedure for synthesis of 4-alkylbromide – 3- aroyl isocoumarin 7

A mixture of 4-methyl - 3-(4’ – hydroxybenzoyl) isocoumarin **7** (0.42g, .0025 mole), N – bromo succinamide (0.882g, .0052 moles), benzoyl peroxide 25-30 mg in carbon tetrachloride was refluxed for 6 hrs under light, solvent was removed completely and the crude product was purified by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate mixture.

General procedure for synthesis of 4-aminomethyl – 3- aroyl isocoumarin 9

3-(4’-hydroxy-benzoyl) - 4-methylbromide – isocoumarin **7** (0.5 g, 0.0013 mole), piperidine **8a** (0.3 ml, 0.0026 mole) and DMF were taken in a round bottom flask and refluxed for 8 hrs. After the reaction was over the reaction mixture was poured into ice and the solid obtained was filtered. The crude product was purified by column chromatography using petroleum ether (60-80^{0}C)-ethyl acetate (60:40).

Antibacterial Activity

Antibacterial activity of the target compounds were tested in vitro against bacterial strains *E. Coli* (gram negative) and *S. Aureus* (gram positive) using serial agar dilution (cup plate method) [26].

The two microorganisms were cultured in dishes containing agar medium. Four cups (8 mm) were put onto the dishes and each tested compound (0.1ml of 2mg/ml) was then added into the cups under aseptic condition. Then the dishes were incubated at 37^{0}C for 24h. The zone of inhibition of the growth of the bacteria, which was produced by diffusion of the compounds from the cup into the surrounding medium, was measured in milli meters to evaluate the antibacterial activity. Each experiment was repeated twice. DMSO was used as a positive control for all the experiments.

Antifungal activity

The standard fungal culture *T. Paradoxa & P. Mangiferae* were grown on PDA slants at room temperature. Mycelial growth inhibition of *T. Paradoxa & P.Mangiferae* was evaluated by the poisoned food technique [27], where the inhibition in growth of the fungal strain was observed on PDA. The stock solutions (1000ppm) were made from each of the test compounds. The required % concentrations of the compounds (mg/ml) were obtained by mixing the appropriate amount of the stock solution with 20 ml of molten PDA. The amended PDA was poured into Petri dishes and allowed to set.

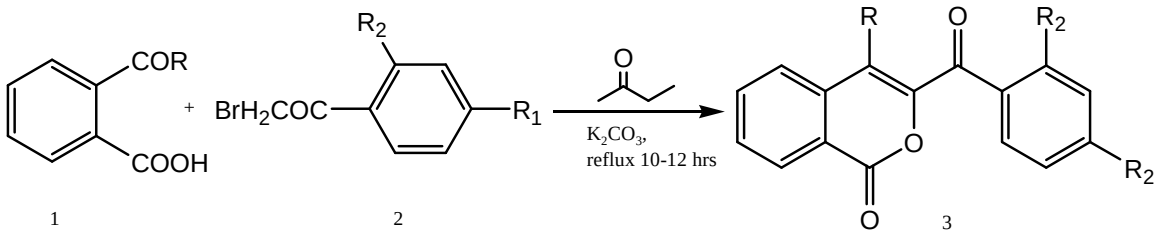
An inoculum of the fungus obtained from 7 days old axenic culture, grown as above, was placed at the centre of the amended agar medium. Each experiment was performed in triplicate. The diameter of the fungal colony was measured after 4 days, then after 7 days at 26+1^{0}C and the % inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Growth area in reference - growth area in sample} \times 100}{\text{Growth area in reference}}$$

RESULTS AND DISCUSSION

In the present paper attempts have been made to synthesize some new 3-aroyl-4-alkyl isocoumarins by refluxing o-acyl benzoic acid **1** with bromoacetophenone **2** in presence of K_2CO_3 in ethyl methyl ketone (Scheme I). No side product was found from the reaction mixture (TLC). On the routine work in the extension of our previous work using bromopropiophenone **4** instead of bromoacetophenone, surprisingly we got dihydroisocoumarin **5** (Scheme II). Isocoumarin ring was dehydrogenated due to bromine present in α-carbon and cyclisation is basic need followed by hydrolysis and 3,4 –dialkyl – 4 hydroxy dihydroisocoumarin **5** was formed. Methyl group at the 4th position of isocoumarin moiety was used in allylic bromination with NBS (Scheme III), which afforded amino methyl isocoumarins **9** on reaction with different amines **8**. In all series physical data was in complete agreement with all the compounds synthesized. In all isocoumarins IR peak for lactone carbonyl was at 1738 cm^{-1}and aroyl carbonyl at 1680cm^{-1}. The elemental analysis confirms its formation. All isocoumarins in ^{1}H NMR spectrum showed characteristic signal at δ 8.4 for C_8 proton and ^{1}H singlet for methyl groups in **5a** (Scheme II). Due to poor yield of dihydroisocoumarins, their stereochemistry was not studied. In compound **9**$_{a-e}$, the IR spectra showed four peaks, each for 1669cm^{-1} for aroyl carbonyl, 1738 cm^{-1} for lactonic carbonyl, 1176 – 1275 cm^{-1} (C-N) and 3300-3450 cm^{-1} (N-H), in compounds where primary amines have been used. In compounds **9**, where secondary amines were used, all the peaks except for NH at 3300 cm^{-1} was found. The ^{1}H NMR showed two proton peaks at δ 4.45 – 4.48 corresponding to the methylene group of amino function and other peaks were observed at 6.8 – 8.3 for aromatic protons. All compounds were screened for antimicrobial activity.

Scheme I



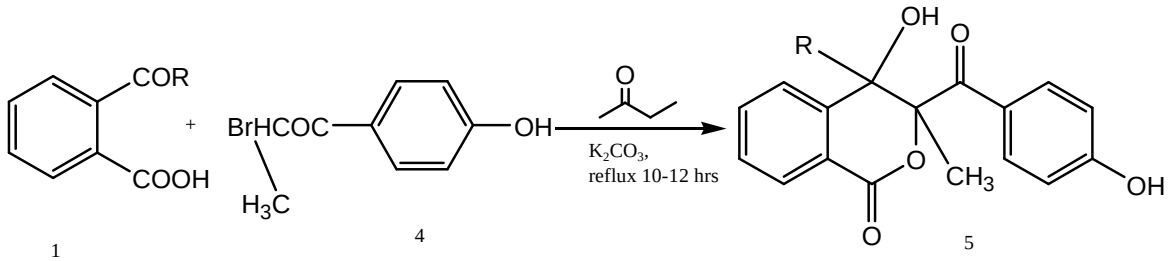
1 2 3

R = -CH_3 **(1a)**
-C_2H_5 **(1b)**

R^1 = OH
R^2 = CH_3

R = CH_3, R^1 = OH, R^2 = CH_3 **(3a)**
R = C_2H_5, R^1 = OH, R^2 = CH_3 **(3b)**

Scheme II

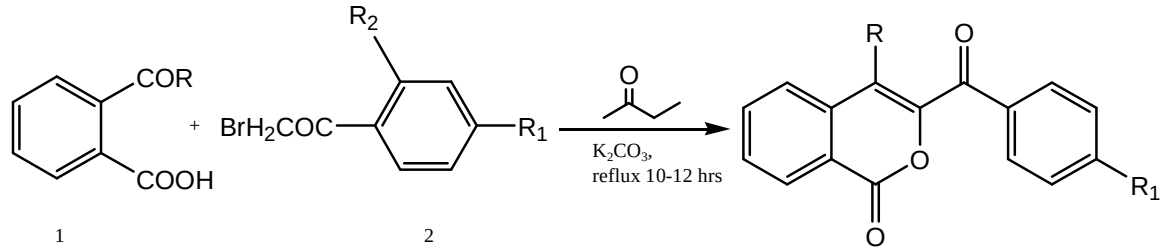


1 4 5

R = -CH_3 **(1a)**
-C_2H_5 **(1b)**

R = CH_3, **(5a)**
R = C_2H_5**(5b)**

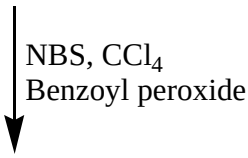
Scheme III

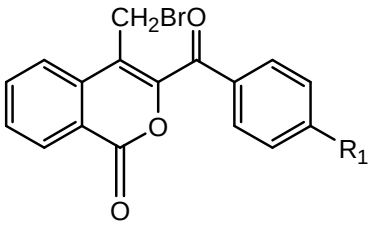


1 2 6

R = -CH_3 **(1a)**

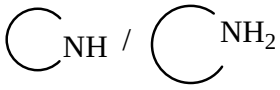
R^1 = OH
R^2 = H



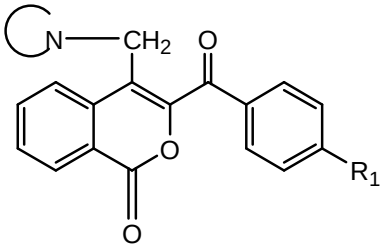


7

+



8 a,b 8 c,d



9

R = CH_3, amines = piperidine (**9a**)
morpholine (**9b**)
aniline (**9c**)
p-toludine (**9d**)

Structure Activity Relationship

A comparison of the compounds activity with that of standard antibiotic ampicillin is effectively presented in Table III. All the compounds showed moderate to good activity against gram positive *S. Aureus* except **9b** & **9c**, where zone of inhibition is less as compared to the standard drug. Increase in the length of alkyl chain does not have much effect on the activity. The same series of compounds were screened against gram negative *E. Coli* bacteria where except **3a** & **3b** all compounds were inactive against *E. Coli.*

The antimicrobial screening was further extended to antifungal activity against *T. Paradoxa & P. Mangiferae.* In **9b**, where both O & N heteroatom are present as morpholine substituent group at 4th position of isocoumarin, % growth inhibition was less as compared to other compounds. Best result was obtained with dihydoisocoumarins (**5a**, **5b**). Literature survey also supports the same [22]. **9a** was found to active against both fungi as well as bacteria's.

Table I: - Physical Data of synthesized compounds

Compound	Mol. Formula	Mol. Weight (g)	Yield	M.P (^{0}C)	% C (Cal.)	% H (Cal.)	% N (Cal.)
3a	$C_{18}H_{14}O_4$	294	70.37	60	72.30 (73.46)	4.73 (4.76)	-
3b	$C_{19}H_{16}O_4$	308	65.02	56	74.00 (74.02)	5.22 (5.19)	-
5a	$C_{18}H_{16}O_5$	312	32.00	82	69.07 (69.23)	5.44(5.12)	-
5b	$C_{19}H_{18}O_5$	326	27.91	77	70.04 (69.93)	5.57 (5.52)	-
8	$C_{17}H_{11}BrO_4$	358.9	62.00	48	57.00(56.84)	3.15 (3.06)	-
9a	$C_{22}H_{21}NO_4$	363	45.00	105	72.99 (72.72)	5.49 (5.78)	3.88 (3.85)
9b	$C_{21}H_{19}NO_5$	365	48.05	87	69.00 (69.04)	5.41 (5.20)	3.91 (3.83)
9c	$C_{23}H_{17}NO_4$	371	53.21	102	74.43 (74.39)	4.67 (4.58)	3.95 (3.77)
9d	$C_{24}H_{19}NO_4$	385	62.03	84	74.65 (74.80)	4.89 (4.93)	3.74 (3.63)

Table II: - Characterization data of synthesized compounds

Compound	NMR	m/z (ESI$^+$)
3a	2.1 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 5.5 (s, 1H, OH), 6.8-8.4 (m, 7H, Ar. Protons)	295 (M^+ + H)
3b	1.9 (t, 3H, CH_2), 1.0 (q, 2H, CH_2), 2.4 (s, 3H, CH_3), 5.5 (s, 1H, OH), 6.8-8.4 (m, 7H, Ar. Protons)	309 (M^+)
5a	1.7 (s, 3H, CH_3), 2.0 (s, 3H, CH_3), 3.8 (s, 1H, OH), 5.0 (s, 1H, OH), 6.8-8.0 (m, 8H, Ar. Protons)	314 (M^+ +2H)
5b	1.7 (s, 3H, CH_2), 1.0 (t, 3H, CH_3), 1.9 (q, 2H, CH_2), 3.4 (s, 1H, OH), 6.8 (s, 1H, OH), 7.0-8.4 (m, 8H, Ar. Protons)	325 (M^+ -H)
8	4.4 (s, 2H, CH_2), 9.0 (s, 1H, OH), 6.9-8.4 (m, 8H, Ar. Protons)	360 (M^+ + H)
9a	3.1 (s, 2H, CH_2), 1.6 (s, 6H, CH_2-CH_2-CH_2), 3.4 (s, 4H, CH_2-N-CH_2), 6.6 (s, 1H, OH), 6.9-8.25 (m, 8H, Ar. Protons)	361 (M^+ - 2H)
9b	3.2 (s, 2H, CH_2), 3.4 (t, 4H, CH_2-N-CH_2), 3.7 (t, 4H, CH_2-O-CH_2), 5.3 (s, 1H, OH), 7.1-8.4 (m, 8H, Ar. Protons)	363 (M^+ - 2H)
9c	3.0 (s, 2H, CH_2), 5.8 (s, 1H, OH), 8.9 (s, 1H, NH), 6.3-8.1 (m, 13H, Ar. Protons)	371 (M^+)
9d	2.7 (s, 3H, CH_3), 3.3 (s, 2H, CH_2), 5.0 (s, 1H, OH), 12.2 (s, 1H, NH), 6.1-8.1 (m, 12H, Ar. Protons)	386 (M^+ + H)

Table III: - Antimicrobial Activity

Compound	Zone of Inhibition (mm)		% Growth inhibition	
	S. Aureus	*E. Coli*	*T. Paradoxa*	*P. Mangiferae*
3a	15	11	23.68	66.66
3b	13	10	21.99	40.03
5a	14	-	55.65	54.00
5b	13	-	50.29	50.41
9a	12	-	63.21	65.53
9b	7	-	11.94	13.55
9c	8	-	48.62	44.52
9d	12	-	51.27	33.27
Control	-	-	-	-
Ampicillin	15	5	-	-

CONCLUSION

In conclusion, we have synthesized a series of 3,4-disubstituted isocoumarins **3**, 3,4-disubstituted-3,4 dihydroisocoumarins **5** and amino methyl isocoumarins **9** using simple starting materials. Amongst the potent compounds, **9a** & **5a** have shown significant antifungal activity against *T. Paradoxa* and *P. Mangiferae,* while 10d was effective against *T. Paradoxa*. All the compounds exhibited marked antibacterial activity against *S. Aureus* and only **3a** & **3b** were active against both. Antifungal activity was better with –NH linkage with any substituent group. Presence of electron releasing methyl group as p-toludine could not show remarkable effect on the activity, much contrary to the earlier known studies.

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