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Synthesis And Antimicrobial Studies of Newly Synthesized 3-(Substituted)-1-(2-Hydroxy-5-Methyl-3-Nitro Phenyl) Prop-2-En-1-Ones

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ABSTRACT

In this study, a new series of 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones i.e., chalcones 4(a-e) have been synthesized from 4-methyl phenol i.e. p-cresol. The structures of newly synthesized compounds of the series have been established on the basis of usual chemical characteristics, elemental analysis and spectral studies of IR and NMR. They have also been studied for their antimicrobial effects against growth response of bacterial and fungal strains through agar diffusion method.

Keywords: p-cresol; Spectral studies; Antimicrobial effects.

INTRODUCTION

Alpha-beta unsaturated ketones or commonly referred as chalcones are one of the important classes of organic compounds frequently encountered in synthetic chemistry. They are important intermediates not only as key building blocks for the synthesis of core heterocycles such as pyrazole, isoxazole, triazole, flavone, benzodiazepine and pyrimidine in medicinal chemistry, but also as an invaluable chelating ligand for various lanthanide and transition metals in material chemistry. Aside from their synthetic importance, chalcones have showed wide assortment of pharmacological activities like antibacterial, antiviral, systematic insecticidal, antioxidant, prophylactic antitumor as well as an anti-sunscreen agent that filters harmful U.V. radiation to protect skin. In addition, chalcones have examined as breast cancer chemo-preventive blocking agent, antiestrogenic and anticarcinogenic agent[1]. Presence of such varying pharmacological activities in chalcones developed our interest to synthesize some new chalcones molecules containing phenolic. With this view here, we have synthesized a new series of 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones i.e. chalcones containing moiety of p-cresol, characterized them by usual chemical characteristics, elemental analysis and spectral techniques as well as investigated its antimicrobial activities through method of agar diffusion.

The chemicals and solvents used were of highest purity purchased commercially from Merck, S.D. Fine and Alfa Aesar Company Ltd. The melting points of all the synthesized compounds were recorded by Thiele's melting point apparatus as uncorrected values. The elemental analysis was carried out on Thermo Scientific CHNS elemental analyser. IR spectra were recorded on a Shimadzu instrument using KBr pallet. 1H NMR spectra were scanned by Brucker at 400 MHz using DMSO-d6 as solvent and TMS as an internal reference. 13C NMR spectrum of one sample (4a) was recorded on same instrument at 100 MHz. Experimental procedure for synthesis of 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones 5(a-e).

Preparation of p-methylphenyl acetate (1)

The p-cresol was refluxed along with acetic anhydride and anhydrous sodium acetate for an hour. The reaction mixture was cooled and poured into the ice-cold water containing crushed ice. Acetate layer was separated by means of separating funnel and several times washed with water. It was finally purified by distillation and the distillate fraction was collected at about 236^0C, to get the compound (1) b.p. 236^0C yield: 84.74%.

Preparation of 2-hydroxy-5-methyl acetophenone (2)

p-methyl phenyl acetate (1) was mixed with anhydrous $AlCl_3$ (1) and heated at 120^0C for 45minutes on an oil bath. The reaction mixture was decomposed in ice cold water containing 10% hydrochloric acid and allowing the solution to fall drop by drop into ice cold water with constant stirring. Green solid compound i. e crude ketone (2) was obtained, m.p. 47^0C, yield: 89%.

Preparation of 2-hydroxy-3-nitro-5-methyl acetophenone (3):

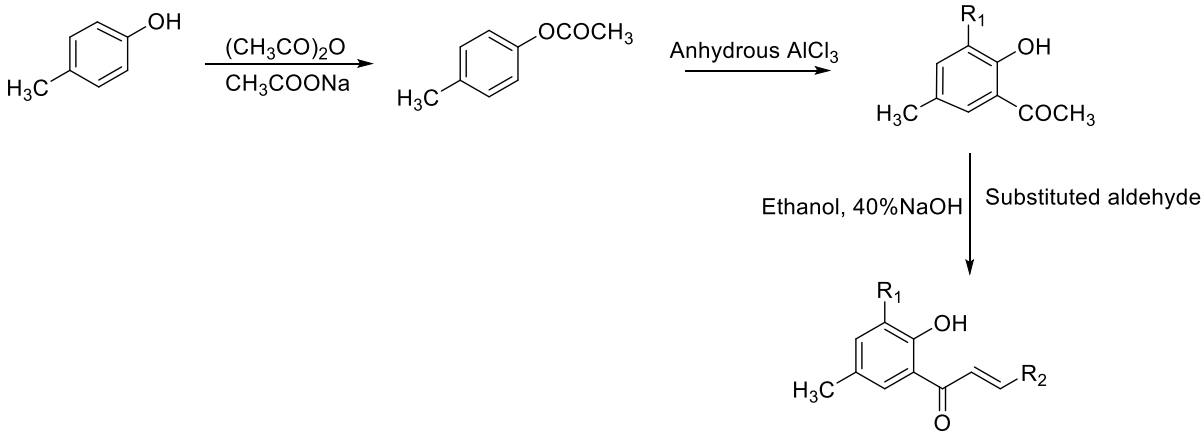
2-hydroxy-5-methyl acetophenone (2) was dissolved in acetic anhydride in a beaker and reaction mixture was kept in ice bath by maintain temperature below 5^0C. To this reaction mixture conc. HNO_3 was added dropwise with constant stirring till the solution becomes orange coloured and kept for 4-5 hrs. it was then decomposed with ice cold water. Yellow granules obtained were filtered and washed with water and then crystallized from ethanol, m.p. yield: 72%.

Preparation of β-unsaturated chalcones (4a–4e)

In this study, α, β-unsaturated chalcones were synthesized by using a Claisen–Schmidt reaction To a solution of substituted acetophenone (0.01mol) and substituted aldehyde , indole 3-carboxyaldehyde, piconaldehyde and furfuraldehyde(0.01mol) in 15ml of ethanol and 40% sodium hydroxide solution added drop by drop. The reaction mixture was continuously stirred on magnetic stirrer at room temperature, up to cake formation followed by decomposition with ice cold HCl (1:1). The crude Chalcones precipitate out ware filtered, washed with 10% NaHCO3 solution and then recrystallized from hot ethanol to obtained 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones 4(a-e)compounds (4a-e) as.

- 3-(2-chlorophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4a) M.P. 112^0C, yield: 74%.
- 3-(3-chlorophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4b) M.P 118^0C, yield: 84%.
- 1-(2-hydroxy-5-methyl-3-nitrophenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4c) M.P 154^0C, yield: 76%.
- 1-(2-hydroxy-5-methyl-3-nitrophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (4d) M.P 126^0C, yield: 64%.
- 3-(furan-2-yl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4e) M.P 78^0C, yield: 54%.

The complete experimental scheme for synthesis of above titled compounds is given below.



Scheme of 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones 4(a-e).

R_1= NO_2; R_2=2-Chloro benzaldehyde, 3- Chloro benzaldehyde, Indol-3-carboxyaldehyde, piconaldehyde and furfural.

Antimicrobial Study

In this section, all newly synthesized 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones 4(a-e) were screened for their antimicrobial activities by Agar diffusion method[2,3]in order to investigate their effects against growth response of two strains of bacteria viz. E. coli (Gram -ve), and S. aureus (Gram +ve) and one strain of fungi viz. A. flavus at six different concentrations ranging from 25

µg/mL to 1000 µg/mL. DMSO was used to prepare the solutions of above concentrations. The Nutrient-agar and Czapek-Dox media were used respectively for antibacterial and antifungal analysis as well as reference drugs Ciprofloxacin and Amphotericin were utilized for the purpose of comparison [4].

Antibacterial Analysis

First of all, the stock cultures of bacteria were revived by inoculating in broth media and grown at the temperature 37ºC for 18 hrs. The agar plates of above media were prepared and wells were made in the plates. Each plate was inoculated with 18 hrs old cultures (100 µL, 104 CFU) and spread evenly on the plate. After 20 minutes, the wells were filled with different concentration of compounds and antibiotic. All the plates were incubated at temperature 37ºC for 24 hrs and diameter of inhibition zones were measured in mm[5].

Antifungal Analysis

First of all, the stock culture of fungus was revived by inoculating in broth media and grown at temperature 27ºC for 48 hrs. The agar plates of the above media were prepared and wells were made in the plates. Each plate was inoculated with 48 hrs old cultures (100 µL, 104 CFU) and spread evenly on the plate. After 20 minutes, the wells were filled with different concentrations of compounds and antibiotic. All the plates were incubated at temperature 27ºC for 96 hrs and diameter of inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Spectroscopic Data The IR, 1H NMR and 13C NMR spectral data showed expected signals or peaks which correspond to various groups present in each compound. Also, elemental analysis was found in full agreement with the proposed structures. The elemental analysis, IR, 1H NMR and 13C NMR spectral data of compounds 4(a-e) are shown below

3-(2-chlorophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4a)

Brown solid; M.P. 112^{0}C, yield: 74%. ; Elemental Anal. Calcd. for $C_{16}H_{12}ClNO_4$.: C: 60.49 , H: 3.81, O:4.41,Cl: 11.16 Found: C: 53.10 , H: 2.40, O:3.98,Cl: 21.00. IR (KBr) cm-1: 3120 (Phenolic OH stretch), 2980 (Aromatic C-H stretch), 2850 (Aliphatic C-H stretch), 1695 (C=O stretch), 1520 (Aromatic C=C stretch),).1H NMR (400 MHz, DMSO-d6) δ (ppm): 1.51 (s, 3H, -CH3), 3.43 (s, 2H, -CH2), 3.80 (s, 1H, -OH), 8.15-8.32 (m, 6H, Ar-H).13C NMR (100MHz, DMSO-d6) δ (ppm): 40 (-CH2), , 195 (C=O), 123-136 (Ar-C), 149- 165 (C=C).

3-(3-chlorophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4b)

1. Dark brown solid; M.P 118^{0}C, yield: 84%. ; Elemental Anal. Calcd. for $C_{16}H_{12}ClNO_4$.
: C:53.10; H:2.40; N:3.98, Cl: 21.00. Found: C: 60.49; H: 3.81; N: 4.41, Cl:20.91. IR (KBr) cm-1: 3340 (Phenolic OH stretch), 2980 (Aromatic C-H stretch), 2910 (Aliphatic C-H stretch), 1615 (C=O stretch), 1515 (Aromatic C=C stretch), 1260 (C-O stretch).1H NMR (400MHz, DMSO-d6) δ (ppm): 1.51 (s, 3H, -CH3), 3.81 (s, 2H, -CH2), 5.13 (s, 1H, -OH), 6.99-7.90 (m, 6H, Ar-H),.

1-(2-hydroxy-5-methyl-3-nitrophenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4c)

Peach color solid; M.P 154^{0}C, yield: 76%; Elemental Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.08; H: 4.38; N: 3.98, found C: 53.10; H: 3.40; N: 7.90. 1H NMR (400MHz, DMSO-d6) δ (ppm): 1.50 (s, 3H, -CH3), 3.38 (s, 2H, -CH), 4.10 (s, 1H, -OH), 6.77-7.65 (m, 6H, Ar-H).

1-(2-hydroxy-5-methyl-3-nitrophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (4d)

Red color solid; M.P 126^{0}C, yield: 64%; Elemental Anal. Calcd. For $C_{16}H_{12}O_4N_2$: C: 53.25; H:2.66; N: 24.04. Found: C: 53.10; H: 2.40; N :3.98,. IR (KBr) cm-1: 3340 (Phenolic OH stretch), 2970 (Aromatic C-H stretch), 2885 (Aliphatic C-H stretch), 1285 (C-N stretch),1590 (C=O stretch), 1385 (C-O stretch), 875 (C-Cl stretch).1H NMR (400MHz, DMSO-d6) δ (ppm): 1.52 (s, 3H, -CH3), 3.36 (s, 2H, -CH), 4.30 (s, 1H, -OH), 7.23-7.74 (m, 6H, Ar-H),.

3-(furan-2-yl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one (4e)

Black color solid; M.P 78^{0}C, yield: 54%; Elemental Anal. Calcd. for $C_{14}H_{13}O_5N$: C, 55.61; H, 3.29; O, 21.79. Found: C, 55.58; H, 3.21; O, 21.67. 1H NMR (400MHz, DMSO-d6) δ (ppm): 1.51 (s, 3H, -CH3), 3.36 (s, 2H, -CH2), 4.15 (s, 1H, -OH), 7.48-7.83 (m, 6H, Ar-H),.

Antimicrobial Activity

In the present work, total five 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones i.e chalcones 4(a-e) were synthesized, purified by recrystallization and used individually to investigate their antimicrobial effects against pathogenic microorganisms viz. E. coli, S. aureus and A flavus. The resulting data on antimicrobial activity of newly synthesized compounds 4(a-e) and antibiotics against E. coli, S. aureus and A. flavus with zone of inhibition in mm are tabulated in (Table1 and 4) and their photographs are shown under respectively[6.7]. From the results on antimicrobial activities, it was observed that, out of all these compounds 4(a-e), compound (4b) and (4c) has not showed any inhibition zone against E. coli at all the concentrations tested, while the compounds (4a), (4d) and (4e) showed (3,5,8), (3,3,5) and (3,6,8) mm of inhibition zones at 250, 500 and 1000 µg/mL concentrations respectively. The minimum inhibitory concentration at which these compounds (4a), (4d) and (4e) showed inhibition against the growth of E. coli was at 250 µg/mL but in case of compounds (4b) and (4c) for E. coli, MICs was not found [5]. In case of S. aureus, compound (4a) showed (3, 5, 10, 11) mm of zones at 100, 250, 500 and 1000 µg/mL concentrations respectively with a MICs at 100 µg/mL. Compound (4b) and (4d) showed 4 and 3 mm of inhibition zones at 1000 µg/mL concentrations only while compound (4e) showed (3, 5, 7) mm of zones at 250, 500 and 1000 µg/mL respectively with MICs at 250 µg. The compound (4c) not showed any inhibition zones for S. aureus at all the tested concentrations. The results on antifungal activity was shocked us because all these newly synthesized compounds 4(a-e) not gave any zones of inhibitions and showed negligible activity against fungi Aflavus.

Table 1: Antibacterial activity of synthesized compounds 4(a-e) against E. coli

Compound	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
4a	NI	NI	NI	3	5	8	250
4b	NI	NI	NI	NI	NI	NI	NF
4c	NI	NI	NI	NI	NI	NI	NF
4d	NI	NI	NI	3	3	5	250
4e	NI	NI	NI	3	6	8	250

Table 2: Antibacterial activity of synthesized compounds 4(a-e) against S. aureus

Compound	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
4a	NI	NI	3	5	10	11	100
4b	NI	NI	NI	NI	NI	4	1000
4c	NI	NI	NI	NI	NI	NI	NF
4d	NI	NI	NI	NI	NI	3	1000
4e	NI	NI	NI	3	5	7	250

Table 3: Antibacterial activity of std. Ciprofloxacin against human pathogens

Organism	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
E. coli	18	20	23	26	28	31	25
S. aureus	13	18	21	25	27	34	25

Table 4: Antifungal activity of std. Amphotericin against fungi A. flavus

Organism	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
A. flavus	NI	NI	NI	NI	7	10	400

Figure 1: Effects of synthesized compounds 4(a-e) and std. Ciprofloxacin on the growth response of E. coli

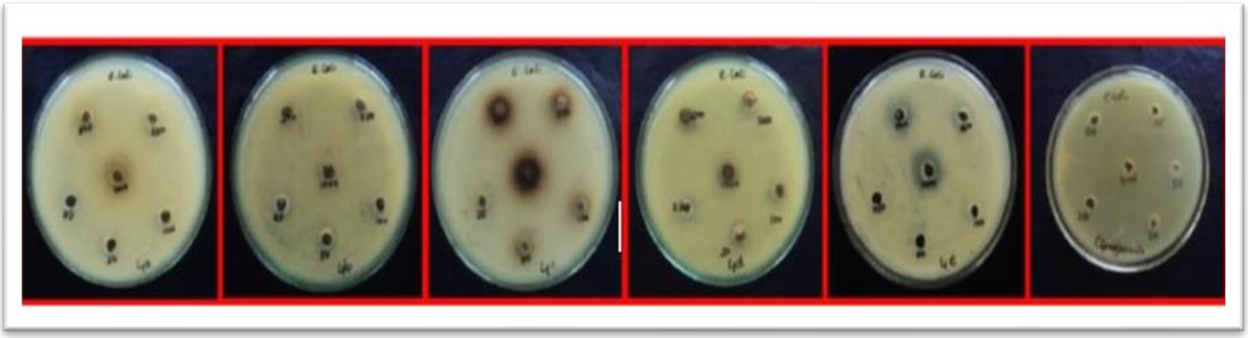


Figure 2: Effects of synthesized compounds 4(a-e) and std. Ciprofloxacin on the growth response of S. aureus

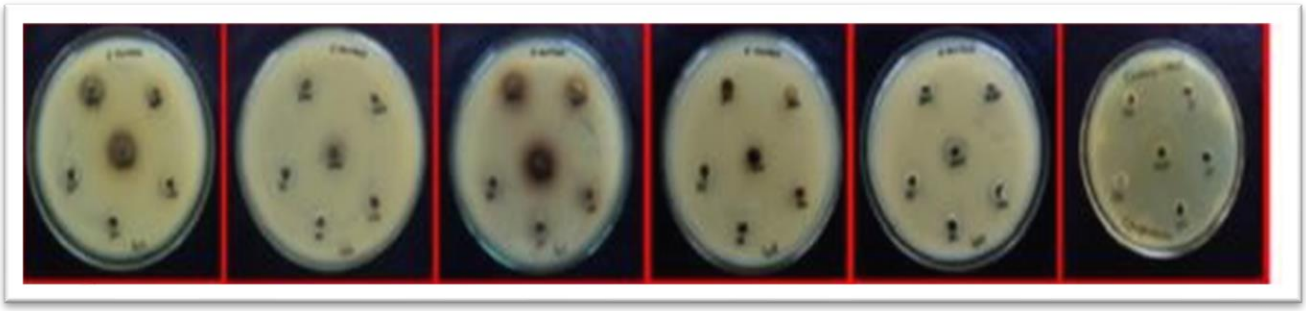
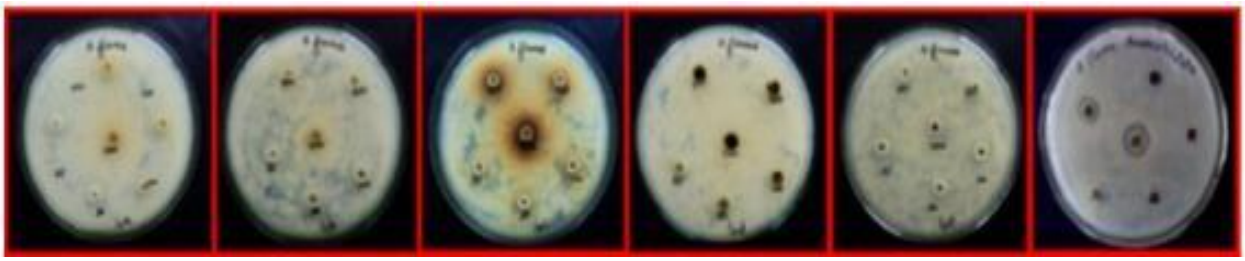


Figure 3: Effects of synthesized compounds 4(a-e) and std. Amphotericin on the growth response of A. flavus



CONCLUSION

In conclusion, a new series of 3-(substituted)-1-(2-hydroxy-5-methyl-3-Nitro phenyl) prop-2-en-1-ones i.e chalcones 4(a-e) bearing 4-methyl phenol i.e. p-cresol moiety were successfully synthesized in satisfactory yield by employing Claisen-Schmidt condensation of corresponding substituted 2-hydroxy-3-nitro-5-methyl acetophenone (3) and their structures were elucidated by chemical characteristics, elemental analysis and IR, 1H NMR and 13C NMR spectroscopic techniques. The results on antimicrobial studies reveals that all the five compounds 4(a-e) were found to have low to moderate antibacterial effects against the growth response of pathogens E. coli and S. aureus as compared to std. Ciprofloxacin drug but in case of antifungal activity against a pathogen A. flavus, they were found to have negligible effects or said to be inactive at all the analyzed range of concentrations.

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REFERENCES

1. Negi A, Gill BS. Pharma Tutor. 2013; 1(2), 45-53.
2. Deshmukh S.Y. Padole N.S. , Wadekar M.P. , Chaudhari M.A.; Journal of Chemical and Pharmaceutical Research, 2021, 13(7):01-06
3. Shubhangi Y. Deshmukh1*, Nilesh S. Padole2; JETIR,2022,9(2): 794-801.
4. Sofowra A. Scientific Res. 1993; 57, 191-289.
5. R.Y.Deshmukh, S.Y. Deshmukh, K.P. Kakde and P. A. fandi; International Journal od advance innovative research;2017, 4(10)
6. Trease GE, Evans WC. Pharmacognosy. 1989; 79, 45-50.
7. Vishwanathan A, Basavaraju R. Eur J Biol Sci. 2010; 3(1), 30-42. [5] Vitex negundo L. India Biodiversity Portal. 2020