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Research Article

SYNTHESIS AND ANTIMICROBIAL STUDIES OF NEWLY SYNTHESIZED 1-SUBSTITUTED-3-SUBSTITUTED PROPANE-1, 3-DIONES

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ABSTRACT

In this current work, one new series of 1-Substituted-3-substitutedpropane-1, 3-diones or β-diketones 4(a-e) have been synthesized from 4-Hydroxy-3-methoxybenzaldehyde i.e. Vanillin. The structures of titled synthesized compounds of the series have been confirmed by usual chemical characteristics, elemental analysis, IR and NMR spectral studies. They have also been studied for their antimicrobial effects against the growth response of pathogenic microorganisms viz. *E. coli, S. aureus, A. flavus* through agar diffusion method.

Keywords: Propane-1, 3-diones,Vanillin, Spectral studies, Antimicrobial effects.

1. INTRODUCTION

Propane-1, 3-diones which are commonly referred as β-diketones are one of the important classes of organic compounds frequently encountered in synthetic chemistry [1-3]. They are significant intermediates not only as key building blocks for synthesis of core heterocycles such as pyrazole, isoxazole, triazole, flavone, benzodiazepine and pyrimidine [4-9] in medicinal chemistry, but also as an invaluable chelating ligand for various metals of transition and lanthanide series in material chemistry [10].

Aside from their synthetic importance, β-diketones have showed wide assortment of pharmacological activities such as antibacterial [11], antiviral [12], systematic insecticidal [13], antioxidant [14], prophylactic antitumor [15] as well as an anti-sunscreen agent that filters harmful ultra-violet (UV) radiation to protect skin [16]. Furthermore, β-diketones have examined as breast cancer chemo-preventive blocking agent [17], antiestrogenic [18] and anticarcinogenic [19] agent. In addition, β-diketones are well known to have a keto-enol tautomerism [20] and recently it has been reported that β-keto-enols are important pharma-cophore for HIV-I integrase (IN) inhibitor [21].

Presence of such varying pharmacological activities in these molecules developed our interest to synthesize some new β-diketone molecules containing phenolic as well as aldehydic group. With this view here, we have synthesized a new series of 1-Substituted-3-substituted derivatives of propane-1, 3-dione comprising moiety of vanillin, characterized them by usual chemical characteristics, elemental analysis and spectral techniques as well as investigated its antimicrobial activities through method of agar diffusion.

2. EXPERIMENTAL

All chemicals and solvents used during research 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. Elemental analysis was carried out on Thermo Scientific (Flash 2000) CHNS elemental analyzer. IR spectra were recorded over Shimadzu IRAffinity-1 instrument by means of KBr pallet. ^{1}H NMR spectra were scanned by Brucker Avance-II at 400 MHz using DMSO-d_6 as solvent and Trimethylsilane as an internal reference. ^{13}C NMR spectrum of one sample (4a) was recorded on same instrument at 100 MHz.

2.1. Experimental process for synthesis of 1-Substituted-3-substitutedpropane-1,3-diones 4(a-e) [22]:

Synthesis of above titled compounds involves following preparatory steps:

2.1.1. Preparation of 4-Formyl-2-methoxyphenyl acetate (1)

Initially Vanillin (a) was refluxed with acetic anhydride along with sodium acetate for 1 hr. The reaction mixture was cooled and poured over crushed ice by which two distinct layers were formed. The lower organic layer was separated by separating funnel, washed number of times by distilled water and purified by distillation to get 4-Formyl-2-methoxyphenyl acetate (1). M.p. 78-80°C, Yield 86%.

2.1.2. Preparation of 5-Formyl-2-hydroxy-3-methoxyacetophenone (2)

4-Formyl-2-methoxyphenyl acetate (1) and anhydrous $AlCl_3$ (1:3) were heated in an oil bath at 120°C for 1 hr (Fries rearrangement). The cooled reaction mixture was decomposed by 10% ice cold HCl to form crude ketone which was then purified by dissolving it in glacial acetic acid followed by pouring the solution slowly in ice cold distilled water with continuous stirring to get 5-Formyl-2-hydroxy-3-methoxyacetophenone (2). M.p. 102-104°C, Yield 78%.

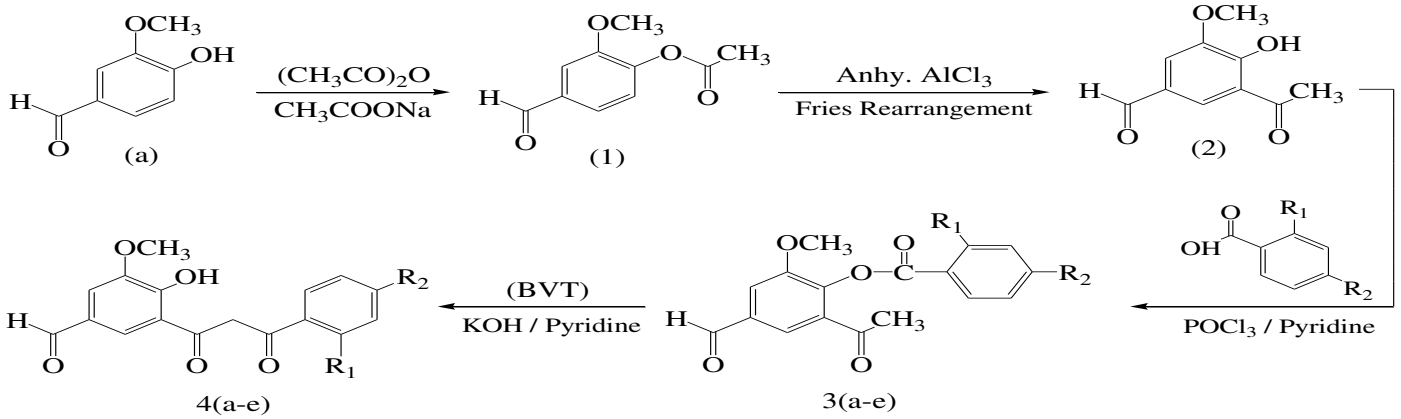
2.1.3. Preparation of 2-(Substitutedbenzoyloxy)-5-formyl-3-methoxyacetophenones 3(a-e)

0.04 mol 5-Formyl-2-hydroxy-3-methoxyacetophenone (2) and 0.05 mol corresponding substituted benzoic acid were dissolved in pyridine and $POCl_3$ was dropwise added with constant stirring below 10°C of temperature. The reaction mixture was kept at room temperature for overnight and then decomposed by ice cold 10% HCl solution. The solid product was separated, filtered, washed with distilled water followed by 10% $NaHCO_3$ washing and again with plenty of distilled water. Finally, they were recrystallized by hot ethanol to obtain 2-(Substitutedbenzoyloxy)-5-formyl-3-methoxyacetophenones 3(a-e) as:

2-(4'-Nitrobenzoyloxy)-5-formyl-3-methoxyacetophenone (3a), M.p.126-130°C, Yield 80%.
2-(4'-Methoxybenzoyloxy)-5-formyl-3-methoxyacetophenone (3b), M.p.116-120°C, Yield 71%.
2-(2'-Chlorobenzoyloxy)-5-formyl-3-methoxyacetophenone (3c), M.p.110-114°C, Yield 75%.
2-(4'-Chlorobenzoyloxy)-5-formyl-3-methoxyacetophenone (3d), M.p.124-128°C, Yield 76%.
2-(2',4'-Dichlorobenzoyloxy)-5-formyl-3-methoxyacetophenone (3e), M.p.134-136°C, Yield 84%.

2.1.4. Preparation of 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl) propane-1, 3-diones or β-diketones 4(a-e) via Baker-Venkataraman Rearrangement

0.05 mol 2-(Substitutedbenzoyloxy)-5-formyl-3-methoxyacetophenone 3(a-e) was dissolved in 40 ml of pyridine. The resulting solution was warmed up to 60°C and pulverized KOH was added gradually with continuous stirring. After 6-8 hrs, the reaction mixture was acidified by ice cold dilute HCl solution (1:1). The solid product was separated, filtered, washed with 10% solution of $NaHCO_3$ and then several times with distilled water. Finally, it was recrystallized from mixture of ethanol and acetic acid to get respective β-diketones namely 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl)propane-1,3-diones 4(a-e). The general experimental scheme for synthesis of above titled compounds is depicted in Fig. 1.



Where, R_1 : H, H, Cl, H, Cl and R_2 : NO_2, OCH_3, H, Cl, Cl

Fig. 1: Experimental scheme for synthesis of 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl)propane-1, 3-diones 4(a-e)

2.2. Antimicrobial study

In this section, all newly synthesized 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(substitutedphenyl) propane-1, 3-diones 4(a-e) were screened for their antimicrobial activities by method of Agar diffusion [23-24] in order to investigate their effects against growth response of pathogenic microorganisms *E. coli*, *S. aureus* and *A. flavus* at six concentrations ranging from 25 μg/ml to 1000 μg/ml. To prepare the solutions of above concentrations DMSO was used as solvent. Nutrient-agar and Czapek-Dox media were utilized respectively for antibacterial and antifungal analysis as well as standard Ciprofloxacin and Amphotericin drugs were utilized for the purpose of comparison.

2.2.1. Antibacterial analysis

First of all, the bacterial stock cultures were revived by inoculation in broth media and allowed to grown at 37°C for about 18 hrs. The plates of agar of above media were prepared and wells or holes were prepared in the plates. Each plate was inoculated with 18 hrs old cultures [100 μl, 10^4 CFU] and spread evenly on the plate. Afterward, the wells or holes were filled with solutions of different concentration of compounds and standard drugs. All the plates were incubated at the temperature of 37°C for at least 24 hrs and zones of inhibition were measured as diameter (in mm).

2.2.2. Antifungal analysis

First of all, the fungal stock culture was revived by inoculation in broth media and allowed to grown at 27°C for about 48 hrs. The plates of agar of above media were prepared and wells or holes were prepared in the plates. Each plate was inoculated with 48 hrs old cultures [100 μl, 10^4 CFU] and spread evenly on plate. Afterward, the wells or holes were filled with solutions of different concentrations of compounds and standard drugs. All the plates were incubated at 27°C temperature for near about 96 hrs and inhibition zones were measured as diameter (in mm).

3. RESULTS AND DISCUSSION

3.1. Spectroscopic data

The spectral data of IR, ^{13}C NMR and ^{1}H NMR showed expected peaks or signals which correspond to various groups present in respective compound. Also, data on elemental analysis was found in full agreement with the proposed structure of compounds. The physical and spectroscopic data of newly synthesized compounds 4(a-e) are summarized below.

3.1.1. 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-nitrophenyl)propane-1, 3-dione (4a):

Brown solid; Yield 76%; M.p. 180-184°C; Elemental Anal. Calcd. for $C_{17}H_{13}NO_7$: C, 59.48; H, 3.82; O, 32.62. Found: C, 59.41; H, 3.75; O, 32.56. IR Spectra (KBr cm^{-1}): 3120 (phenolic OH), 2980 (aromatic C-H), 2850 (aliphatic C-H), 1695 (C=O), 1520 (aromatic C=C), 1285 (C-N). ^{1}H NMR Spectra (400MHz, DMSO-d_6, δ, TMS=0): 2.51 (s, 3H, -OCH_3), 3.43 (s, 2H, -CH_2), 3.80 (s, 1H, -OH), 8.15-8.32 (m, 6H, Ar-H), 13.54 (s, 1H, -CHO). ^{13}C NMR Spectra (100MHz, DMSO-d_6, δ, TMS=0): 40 (-CH_2), 190 (-CHO), 195 (C=O), 123-136 (Ar-C), 149-165 (C=C).

3.1.2. 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-methoxyphenyl)propane-1,3-dione(4b):

Dark brown solid; Yield 68%; M.p. 140-144°C; Elemental Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91; O, 29.24. Found: C, 65.72; H, 4.87; O, 29.03. IR Spectra (KBr cm^{-1}): 3340 (phenolic OH), 2980 (aromatic C-H), 2910 (aliphatic C-H), 1615 (C=O), 1515 (aromatic C=C), 1260 (C-O).^{1}H NMR Spectra (400MHz, DMSO-d_6, δ, TMS=0): 2.51 (s, 3H,-OCH_3), 3.81 (s, 2H, -CH_2), 5.13 (s, 1H, -OH), 6.99-7.90 (m, 6H, Ar-H), 10.10 (s, 1H, -CHO).

3.1.3. 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(2'-chlorophenyl)propane-1,3-dione (4c):

Dark brown solid; Yield 72%; M.p. 133-136°C; Elemental Anal. Calcd. for $C_{17}H_{13}ClO_5$: C, 61.36; H, 3.94; O, 24.04. Found: C, 61.28; H, 3.87; O, 24.0. ^{1}H NMR Spectra (400MHz, DMSO-d_6, δ, TMS=0): 2.50 (s, 3H, -OCH_3), 3.38 (s, 2H, -CH_2), 4.10 (s, 1H, -OH), 6.77-7.65 (m, 6H, Ar-H), 9.88 (s, 1H,-CHO).

3.1.4. 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(4'-chlorophenyl)propane-1,3-dione (4d):

Brown solid; Yield 77%; M.p. 145-148°C; Elemental Anal. Calcd. for $C_{17}H_{13}ClO_5$: C, 61.36; H, 3.94; O, 24.04. Found: C, 61.30; H, 3.83; O, 24.0. IR Spectra (KBr cm^{-1}): 3340 (phenolic OH), 2970 (aromatic C-H), 2885 (aliphatic C-H), 1590 (C=O), 1385 (C-O), 875 (C-Cl). ^{1}H NMR Spectra (400MHz, DMSO-d_6, δ, TMS=0): 2.52 (s, 3H, -OCH_3), 3.36 (s, 2H, -CH_2), 4.30 (s, 1H, -OH), 7.23-7.74 (m, 6H, Ar-H), 10.9 (s, 1H, -CHO).

3.1.5. 1-(5'-Formyl-2'-hydroxy-3'-methoxyphenyl)-3-(2',4'-dichlorophenyl)propane-1,3-dione(4e): Yellow-brown solid; Yield 80%; M.p. 168-170°C; Elemental Anal. Calcd. for $C_{17}H_{12}Cl_2O_5$: C, 55.61; H, 3.29; O, 21.79. Found: C, 55.58; H, 3.21; O, 21.67. ^{1}H NMR Spectra (400MHz, DMSO-d_6, δ, TMS=0): 2.51 (s, 3H, -OCH_3), 3.36 (s, 2H, -CH_2), 4.15 (s, 1H, -OH), 7.48-7.83 (m, 6H, Ar-H), 13.54 (s, 1H, -CHO).

3.2. Antimicrobial activity

In the present work, total five 1-Substituted-3-substituted derivatives of propane-1, 3-dione 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 standard drugs against *E. coli*, *S. aureus* and *A. flavus* with zone of inhibition in mm are tabulated in Table-(1-4) and their photographs are shown under Fig.-(2-4) respectively. From the results on antimicrobial activities, it was observed that, out of all these compounds 4(a-e), compound (4b) and (4c) were not showed any inhibition zones against *E. coli* at all the tested concentrations, while compounds (4a), (4d) and (4e) has showed (3,5,8), (3,3,5) and (3,6,8) mm zones of inhibition at concentrations of 250, 500 and 1000µg/ml respectively. The minimum inhibitory concentration (MICs) at which these compounds (4a), (4d) and (4e) showed inhibition against *E. coli* was at 250 µg/ml but in case of compounds (4b) and (4c) for *E. coli*, MICs was not found. In case of *S. aureus*, compound (4a) showed (3, 5, 10, 11) mm zones of inhibition at 100, 250, 500 and 1000 µg/ml concentrations respectively with a MICs at 100 µg/ml. Compounds (4b) and (4d) showed 4 and 3 mm of inhibition zones at 1000 µg/ml concentration only while compound (4e) showed (3, 5, 7) mm of inhibition zones at 250, 500 and 1000 µg/ml respectively with MICs at 250 µg/ml. The compound (4c) not showed any inhibition zones for *S. aureus* at all the analyzed range of concentrations. The results on antifungal activity were shocked us because all these newly synthesized compounds 4(a-e) were not showed any zones of inhibitions at any concentrations against fungi *A. flavus*.

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

Compd. code	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*

Compd. code	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 Ciprofloxacin drug against *E. coli* and *S. aureus*

Organism	25 µg	50 µg	100 µg	200 µg	400 µg	800 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 Amphotericin drug against fungi *A. flavus*

Organism	25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
A. flavus	NI	NI	NI	NI	7	10	400

NI: No Inhibition; NF: MIC not found; MIC: Minimum Inhibitory Concentration

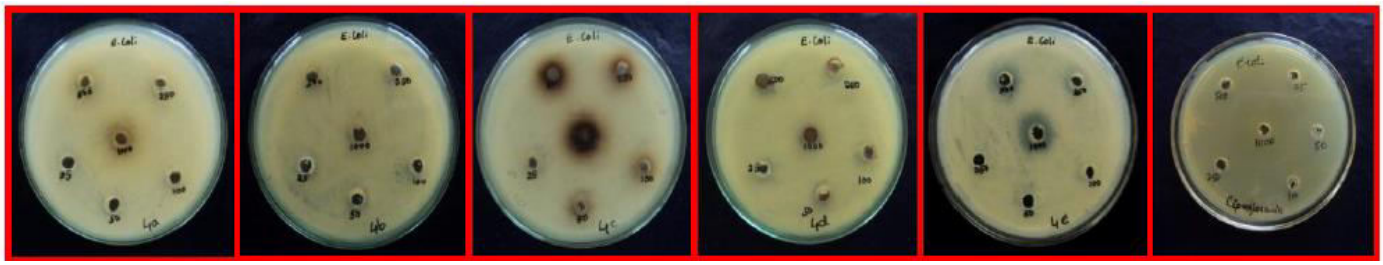


Fig. 2: Effects of synthesized compounds 4(a-e) and std. Ciprofloxacin on the growth response of *E. coli*

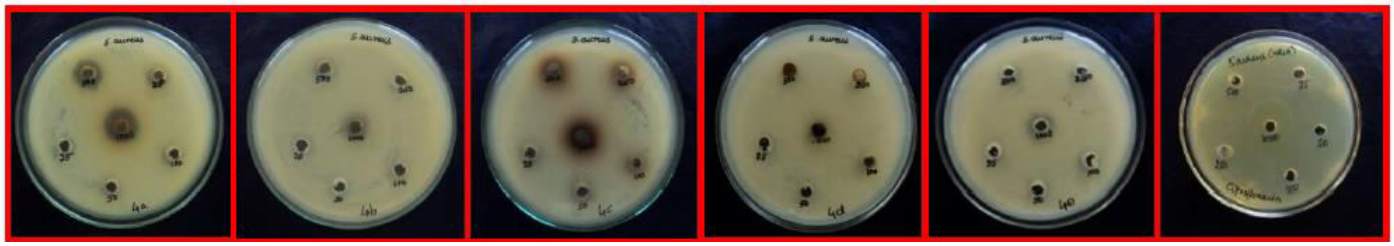


Fig. 3: Effects of synthesized compounds 4(a-e) and std. Ciprofloxacin on the growth response of *S. aureus*

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

4. CONCLUSION

In conclusion, a new series of 1-Substituted-3-substituted derivatives of propane-1, 3-dione 4(a-e) comprising 4-Hydroxy-3-methoxybenzaldehyde i.e. Vanillin moiety were synthesized successfully in satisfactory yield via Baker-Venkataraman Transformation (BVT) of corresponding substituted 2-benzoyloxyacetophenones 3(a-e) and their structures were elucidated or confirmed by chemical characteristics, elemental analysis and IR, ^{13}C NMR and ^{1}H 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 at all analyzed range of concentrations and said to be inactive.

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Conflict of interest

There is no conflict of interest.

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