SYNTHESIS, SPECTRAL CHARACTERIZATION AND INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF SOME NOVEL SUBSTITUTED PROPANE-1, 3-DIONES DERIVED FROM P-CHLORO-M-CRESOL

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A B S T R A C T

In this study, a series of novel substituted propane-1,3-diones i.e. β-diketones (4a-d) were synthesized in high yields *via* Baker-Venkataraman rearrangement of different substituted 2-benzoyloxy acetophenones (3a-d) with potassium hydroxide in pyridine medium. The structures of titled compounds were established on the basis of spectral data studies of IR and ^{1}H NMR. Furthermore, these compounds were tested *in-vitro* against human pathogens in order to assess their antibacterial and antifungal activity using agar diffusion method.

1. Introduction

In recent years, β-diketones or 1,3-diketones have attracted considerable attention of chemists, biochemists and pharmacologists because of their ready access, predictable reactivity, non-toxicity and serve as precursors for the synthesis of various biologically active heterocyclic compounds[1-2] such as pyrazoles, isoxazoles, imidazoles, benzimidazoles, diazepines and benzodiazepines. They have been used as chelating ligand for various lanthanides and transition metals in material chemistry[3]. Aside from their synthetic importance, they have been found to exhibit significant pharmacological activities like antibacterial[4], antioxidant[5], antiviral[6], systematic insecticidal[7], prophylactic antitumour[8] and breast cancer chemo-preventive blocking agent[9]. In addition, substituted β-diketones like (4-*tert*-butyl-4'-methoxydibenzoylmethane), 1-(4-*tert*-butylphenyl)propane-1,3-dione and 1-p-cumenyl-3-phenylpropane-1,3-dione have been used in UV sunscreen cosmetics that filters ultraviolet rays to protect skin[10] and recently it is reported that β-diketones in its keto-enol form are also the important pharmacophores for the HIV-1 integrase (1N) inhibitors[11]. As β-diketones are having such diverse biological and pharmacological applications, we decided to prepare a new series of substituted propane-1, 3-diones. Hence, in the present work, we have synthesised some novel substituted propane-1,3-diones i.e. β-diketones (4a-d) containing p-chloro-m-cresol moiety by Baker-Venkataraman rearrangement of corresponding substituted 2-benzoyloxyacetophenones (3a-d), characterized them by IR and ^{1}H NMR spectral data and investigate their antimicrobial activity.

2. Materials and Methods

All chemicals and solvents used in this study were of analytical grade obtained from S.D.Fine Chemicals, Merck and Alfa Aesar Company Ltd. The melting points were determined by open tube capillary method and were found uncorrected. The structures of newly synthesized products were characterized by spectral data studies of IR and ^{1}H NMR. The IR spectra were recorded in KBr on Shimadzu (IRAffinity-1) FTIR spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz NMR spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. All the products were purified by recrystalization and their purity was checked by thin layer chromatography (TLC) on silica gel-G plates.

3. Synthesis of substituted propane-1,3-diones i.e. β-diketones (4a-d)

The synthesis of β-diketones involves following steps:

3.1 General procedure for synthesis of p-chloro-m-cresyl acetate (1)

Initially p-chloro-m-cresol (a) was refluxed with acetic anhydride in presence of anhydrous sodium acetate for 1.5 hour. The reaction mixture was allowed to cool followed by decomposition in cold water. The two layers are formed, out of which lower organic layer was separated by means of separating funnel and purified by distillation to get p-chloro-m-cresyl acetate (1).

3.2 General procedure for synthesis of 5-chloro-2-hydroxy-4-methylacetophenone (2)

The p-chloro-m-cresyl acetate (1) and anhydrous $AlCl_3$ were heated at 120^oC for 1 hour undergone Fries rearrangement followed by decomposition in 10% ice cold HCl to form crude ketone. It was purified by dissolving it in acetic acid and pouring the solution drop wise into cold water with stirring to get 5-chloro-2-hydroxy-4-methylacetophenone (2).

3.3 General procedure for synthesis of substituted 2-benzoyloxy acetophenones (3a-d)

The 5-chloro-2-hydroxy-4-methylacetophenone (2) (0.04 mol) and appropriate substituted benzoic acids (0.05mol) were dissolved in dry pyridine and $POCl_3$ was added drop by drop with stirring till the viscous mass is obtained. Maintain the temperature below 10^oC during the addition of $POCl_3$. The reaction mixture was kept overnight at room temperature and then decomposed by 10% HCl. The solid product thus separated was filtered, washed with water followed by 10% $NaHCO_3$ solution and then again with water. Finally it was crystallized from hot ethanol to get corresponding substituted 2-benzoyloxy acetophenones (3a-d).

3.4 General procedure for synthesis of substituted propane-1,3-diones (4a-d) through Baker-Venkataraman rearrangement

Substituted 2-benzoyloxy acetophenones (3a-d) (0.05mol) was dissolved in dry pyridine (40 ml). The solution was warmed up to 60^oC and pulverized KOH was added slowly with constant stirring. After 6-8 hours the reaction mixture was acidified by ice cold dil.HCl (1:1). The coloured solid product thus separated was filtered, washed with 10% $NaHCO_3$ and finally several times by water. It was then recrystalized from ethanol-acetic acid mixture to get corresponding substituted propane-1, 3-diones i.e. β-diketones (4a-d) as shown in Figure 1. The physical characterization data are given in Table 1.

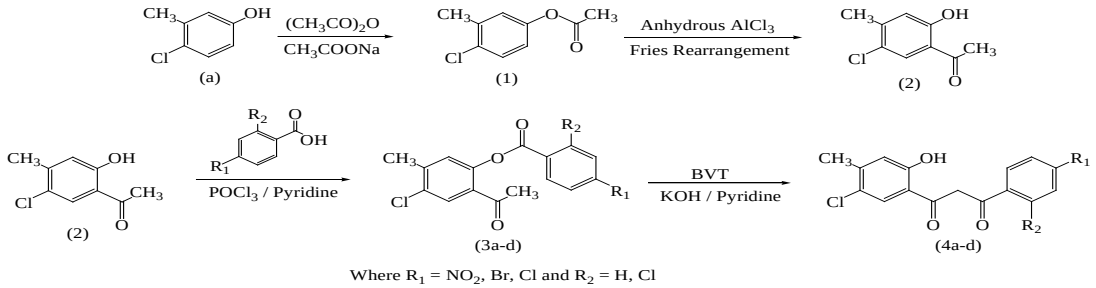

Fig.1: Reaction scheme for synthesis of substituted propane-1,3-diones (4a-d)

4. Spectral data of substituted propane-1, 3-diones (4a-d)

1-(5'-chloro-2'-hydroxy-4'-methylphenyl)-3-(4'-nitrophenyl)propane-1,3-dione (4a)

IR (KBr): 3388 cm^{-1} (Phenolic -OH stretch), 2918 cm^{-1} (Aromatic C-H stretch), 2848 cm^{-1} (Aliphatic C-H stretch), 1695 cm^{-1} (C=O stretch), 1590 cm^{-1} (Aromatic C=C stretch), 1338 cm^{-1} (C-N stretch), 715 cm^{-1} (C-Cl stretch). ^1H NMR (DMSO-d_6): δ 4.2 (S, 1H of OH), δ 2.5 (S, 3H of CH_3), δ 3.34 (S, 2H of CH_2), δ 6.78-8.33 (m, 6H of Ar-H).

1-(5'-chloro-2'-hydroxy-4'-methylphenyl)-3-(4'-bromophenyl)propane-1,3-dione (4b)

IR (KBr): 3202 cm^{-1} (Phenolic -OH stretch), 3073 cm^{-1} (Aromatic C-H stretch), 2929 cm^{-1} (Aliphatic C-H stretch), 1709 cm^{-1} (C=O stretch), 1564 cm^{-1} (Aromatic C=C stretch), 707 cm^{-1} (C-Cl stretch), 545 cm^{-1} (C-Br stretch). ^1H NMR (DMSO-d_6): δ 4.75 (S, 1H of OH), δ 2.3 (S, 3H of CH_3), δ 2.5 (S, 2H of CH_2), δ 6.91-8.04 (m, 6H of Ar-H).

1-(5'-chloro-2'-hydroxy-4'-methylphenyl)-3-(4'-chlorophenyl)propane-1,3-dione(4c)

IR (KBr): 3413 cm^{-1} (Phenolic -OH stretch), 3136 cm^{-1} (Aromatic C-H stretch), 2950 cm^{-1} (Aliphatic C-H stretch), 1720 cm^{-1} (C=O stretch), 1540 cm^{-1} (Aromatic C=C stretch), 710 cm^{-1} (C-Cl stretch). ^{1}H NMR (DMSO-d_6): δ 4.54 (S, 1H of OH), δ 1.69 (S, 3H of CH_3), δ 2.28 (S, 2H of CH_2), δ 6.69-7.82 (m, 6H of Ar-H).

1-(5'-chloro-2'-hydroxy-4'-methylphenyl)-3-(2',4'-dichlorophenyl)propane-1,3-dione (4d)

IR (KBr): 3734 cm^{-1} (Phenolic -OH stretch), 3096 cm^{-1} (Aromatic C-H stretch), 2919 cm^{-1} (Aliphatic C-H stretch), 1653 cm^{-1} (C=O stretch), 1538 cm^{-1} (Aromatic C=C stretch), 731 cm^{-1} (C-Cl stretch). ^{1}H NMR (DMSO-d_6): δ 4.75 (S, 1H of OH), δ 2.3 (S, 3H of CH_3), δ 2.5 (S, 2H of CH_2), δ 6.95-8.02 (m, 5H of Ar-H).

Table 1: Physical characterization data of substituted propane-1,3-diones (4a-d)

Sr.No.	Compound	R_1	R_2	Mol. Formula	Mol.Wt.	M.P. (^{o}C)	Colour	% Yield
1	4a	NO_2	H	$C_{16}H_{12}ClNO_5$	333.72	218-220	Yellow	70
2	4b	Br	H	$C_{16}H_{12}BrClO_3$	367.62	112-114	Yellow	68
3	4c	Cl	H	$C_{16}H_{12}Cl_2O_3$	323.17	140-142	Yellow	72
4	4d	Cl	Cl	$C_{16}H_{11}Cl_3O_3$	357.62	142-144	Yellow	75

5. Antimicrobial Activity

5.1 Antibacterial activity

The newly synthesized compounds (4a-d) were investigated for their *in-vitro* antibacterial activity against *Escherichia coli* (Gram -ve), *Pseudomonas aeruginosa* (Gram -ve) and *Bacillus subtilis* (Gram +ve) at different concentrations ranging from 25-1000 µg/ml by agar diffusion method[12-13]. DMSO was used as solvent to prepare the solutions of compounds and nutrient agar was used as media. Ciprofloxacin was used as standard antibiotic for reference. Initially the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hrs old cultures (100 µl, 10^4 cfu) and spread evenly on the plate. After 20 minutes, the wells were filled with of different concentrations of compounds and antibiotic. All the plates were incubated at 37ºC for 24 hrs and diameter of inhibition zone were noted. The antibacterial activity along with values of MICs of compounds and antibiotic are given in Table 2-5

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Table 2: Antibacterial activity of substituted propane-1,3-diones (4a-d) against *Escherichia coli*

Compound	25 µg	50 µg	100 µg	250 µg	500µg	1000 µg	MIC µg
4a	0	0	0	0	0	0	NF
4b	0	0	0	0	0	0	NF
4c	0	0	0	0	0	0	NF
4d	0	0	0	0	0	0	NF

Table 3: Antibacterial activity of substituted propane-1,3-diones (4a-d) against *Pseudomonas aeruginosa*

Compound	25 µg	50 µg	100 µg	250 µg	500µg	1000 µg	MIC µg
4a	0	0	0	0	0	5	1000
4b	0	0	0	0	0	0	NF
4c	0	0	0	0	0	0	NF
4d	0	0	0	0	0	5	1000

Table 4: Antibacterial activity of substituted propane-1,3-diones (4a-d) against *Bacillus subtilis*

Compound	25 µg	50 µg	100 µg	250 µg	500µg	1000 µg	MIC µg
4a	0	0	0	0	4	7	500
4b	0	0	0	0	0	0	NF
4c	0	0	0	0	0	0	NF
4d	0	0	0	0	0	4	1000

Table 5: Antibacterial activity of standard Ciprofloxacin against human pathogens

Organism	25 µg	50 µg	100 µg	250 µg	500µg	1000 µg	MIC µg
E.coli	26	29	32	34	38	*	25
P.aeruginosa	30	32	34	35	38	*	25
B.subtilis	20	24	27	30	36	*	25

NF-MIC not found and *Zones could not be measured due to merging.

5.2 Antifungal activity

The antifungal activity of newly synthesized compounds (4a-d) was also investigated in-*vitro* against *Aspergillus flavus*, *Candida albicans* and *Neurospora crassa* at different concentrations ranging from 25-800 µg/ml by agar diffusion method. DMSO was used as solvent to prepare the solutions of compounds and Czapek-Dox agar was used as media. Amphotericin was used as standard antibiotic for reference. Initially the stock cultures of fungi were revived by inoculating in broth media and grown at 27ºC for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 hrs old cultures (100 µl, 10^4 cfu) and spread evenly on the plate. After 20 minutes, the wells were filled with of different concentrations of compounds and antibiotic. All the plates were incubated at 27ºC for 96 hrs and diameter of inhibition zone were noted. It is observed that, the compounds have not shown any inhibition zone. The antifungal activity of standard Ampotericin along with their MICs is given in Table 6.

Table 6: Antifungal activity of standard Amphotericin against human pathogens

Organism	25 µg	50 µg	100 µg	200 µg	400µg	800 µg	MIC µg
A.flavus	0	0	0	0	7	10	400
C.albicans	0	2	7	9	13	15	50
N.crassa	0	0	0	0	7	9	400

Result and Discussion

In the present work, a series of novel substituted propane-1,3-diones (4a-d) were synthesized in high yields from corresponding substituted 2-benzoyloxy acetophenones (3a-d) through Baker-Venkataraman rearrangement. The structures of these compounds were established from their physical and spectral data. The IR and ^{1}H NMR spectra showed all the expected signals /peaks which correspond to various groups present in each compound. All the newly synthesized substituted propane-1,3-diones were investigated for their *in vitro* antibacterial and antifungal activities. It was observed that all the compounds 4a, 4b, 4c, and 4d has not showed any zones of inhibition against *E.coli* at all the concentrations tested. The compounds 4a and 4d both showed 5mm of inhibition zones at 1000 µg/ml concentration whereas compounds 4b and 4c has not showed any zones of inhibition at all concentrations against *P.aeurigonosa.* The compound 4a showed 4 and 7mm of zones at 500 and 1000 µg/ml concentrations respectively and also the compound 4d showed 4mm of zone at 1000 µg/ml concentrations against *B.subtilis* whereas for the compound 4b and 4c inhibition zones were not observed against *B.subtilis*. From the results of antifungal activity, it was observed that, all the synthesized compounds 4a-d have not showed any inhibition against *Aspergillus flavus*, *Candida albicans* and *Neurospora crassa.*

Conclusion

In this study, a new series of substituted propane-1,3-diones (4a-d) were successfully derived from p-chloro-m-cresol, analysed for their structures and investigated for their antimicrobial activity. The results of antimicrobial activity reveals that, all the compounds 4a-d showed negative antifungal activity against all the fungi tested and found to be inactive at all the analysed range of concentrations. But in case of antibacterial activity, we concluded that these compounds are poorly active against tested bacteria as compared to standard ciprofloxacin. If the concentration of these synthesized compounds increases, its activity may also increases.

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