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Research Article

Synthesis and antimicrobial evaluation of substituted benzoic acid-(5-oxo-pentylidene)-hydrazides

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Abstract

A series of substituted benzoic acid-(5-oxo-pentylidene)-hydrazides were synthesized and studied for their antimicrobial activity. The synthesized compounds were characterized by physicochemical and spectral (IR and NMR) means. All the compounds were evaluated for their in vitro antimicrobial activity against two Gram positive strains (Bacillus subtilis and Staphylococcus aureus) and one Gram negative strains (Escherichia coli) and fungal strain Candida albicans and Aspergillus niger. Among the synthesized hydrazides 3,5-dinitro-bezoic acid-(5-oxo-pentylidene)-hydrazide (10) was found to be most effective antimicrobial agent. In general the synthesized compounds were found to be bacteriostatic and fungistatic in nature.

Keywords: - :Substituted benzoic acid derivatives; antimicrobial evaluation; Minimum inhibitory concentration

INTRODUCTION

The emergence of antibiotic-resistant pathogens is a serious health problem worldwide. Certain infections those were previously treatable with standard antibiotic therapies can now being caused by multi-drug-resistant organisms that are no longer susceptible to conventional treatment. In order to overcome this problem there is a need of development of new chemical entities. Literature survey demonstrated that hydrazides moiety is having a diverse spectrum of biological activities viz. Anticancer (Moorthy et al. 2010, Iradyan et al. 2008, Morgan et al. 2002), antimicrobial (Li. 2009, Rasras et al. 2009, Abdel-Aziz et al. 2009, Ajani et al. 2010), anti-inflammatory (Moldovan et al. 2010, Tributino et al. 2009, Silva et al. 2004), antimalarial (Gemma et al. 2009, Walcourt et al. 2003), AChE inhibitors (Alptuzun et al. 2010), anti-HIV (Jin et al. 2009, Zhan et al. 2009, Vicini et al. 2009) activities.

Material and methods

Starting material was obtained from commercial sources and was used without further purification. Reaction progress was observed by thin layer chromatography making use of commercial silica gel plates (Merck). Melting points were determined in open capillary tubes on a sonar melting point apparatus and are uncorrected. 1H nuclear magnetic resonance (1H NMR) spectra were determined by Bruker Avance II 400 NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethyl silane (internal standard) NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on a Shimadzu FTIR spectrometer.

Synthesis of substituted benzoic acid-(5-oxo-pentylidene)-hydrazide (1-11)

Different aroyl chlorides were synthesized by refluxing corresponding benzoic acid (0.25 mol) with excess of thionyl chloride (0.3 mol). The excess of thionyl chloride was removed by distillation. Substituted benzoyl chloride (0.1 mol) prepared above in petroleum ether was added to hydrazine hydrate (0.1 mol) in petroleum ether and the mixture was stirred for 30 min. The resultant crude product was filtered off, dried and recrystallized from methanol. To the 50 ml methanolic solution of 0.05 mol of substituted benzoic acid hydrazide prepared above added a 50 ml methanolic solution of 0.05 mol of glutaraldehyde and the mixture was refluxed for 90 min. The reaction mixture was allowed to cool at room temperature and the precipitate thus obtained was filtered, dried and then recrystallized from methanol to yield the title compounds.

Benzoic acid (5-oxo-pentylidene)-hydrazide (1): Mp(°C) 95-98; Yield-88%; 1H NMR (CDCl3): δ 7.26-8.01 (m, 8H of ArH), δ 9.16 (s, H of CHO), δ 0.14 (m, 2H, CH2 adjacent to N=CH), δ 1.62-2.1 (m, 2H, H of CH2 adjacent to CHO); IR (KBr pellets) cm-1:3053.32 (C-H stretching of aromatic ring), 1666.50 (C=O stretching of secondary amide), 1575.84 (C=C

stretching of aromatic ring), 1631.78 (C=N stretching of Schiff base), 1764.87 (C=O stretching of aldehyde).

2-Chloro-benzoic acid (5-oxo-pentylidene)-hydrazide (2): Mp(°C) 234-237; Yield-83%; 1H NMR (CDCl3): δ 7.26-8.01 (m, 5H, H of ArH), δ 9.18 (s, H of CHO); IR (KBr pellets) cm-1: 3093.82 (C-H stretching of aromatic ring), 1573.91(C=C stretching of aromatic ring), 725.23 (C-Cl stretching of monosubstituted aromatic ring).

4-Chloro-benzoic acid (5-oxo-pentylidene)-hydrazide (3): Mp(°C) 196-199; Yield 81%; 1H NMR (CDCl3): δ 2.48-2.50 (m, 2H, H of CH2 adjacent to CHO), δ 7.59-7.93 (m, 4H, H of aromatic ring), δ 10.63 (s, H of CHO); IR (KBr pellets) cm-1: 3072.60 (C-H stretching of aromatic ring), 1670.35 (C=O stretching of secondary amide), 1562.34 (C=C stretching of aromatic ring), 1786.08 (C=O stretching of aldehyde).

Evaluation of antimicrobial activity

Determination of MIC

The antimicrobial activity was performed against Gram-positive bacteria: Staphylcococcus aureus, Bacillus sublitis, Gram-negative bacterium: Escherichia coli and fungal strains: Candida albicans and Aspergillus niger using tube dilution method (Cappucino et. al. 1999). Dilutions of test and standard compounds were prepared in double strength nutrient broth-I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) (Pharmacopoeia of India, 2007). The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (A. niger) and at 37 °C for 48 h (C. albicans) and the results were recorded in terms of Minimum Inhibitory Concentration (MIC).

Determination of Minimum Bactericidal /Fungicidal Concentration

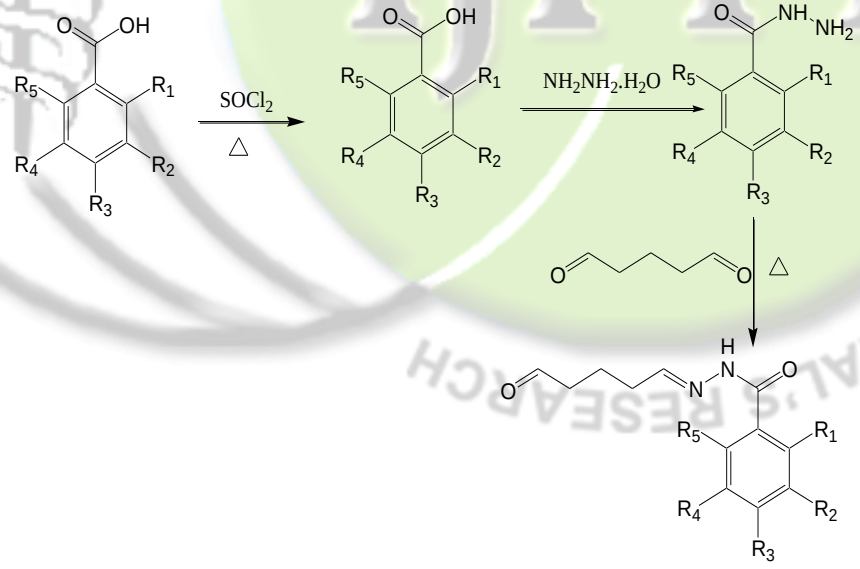
The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by subculturing 100 µL of culture from each tube (which remained clear in the MIC determination) on fresh medium. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction (Rodriguez-Arguelles et al. 2005).

preparation of gels weighed quantity of Lutrol F-127 was placed in the beaker. To that weighed quantity of propylene glycol and Suc-chi was added. Then mixture was kept in an ice bath having temperature ranging from 2-4 oC on magnetic stirrer. Then accurate quantity of pre-cooled distilled water was added and stirred for 30 mins. Then this dispersion was kept in a freezer overnight to remove the air bubble.

Results and Discussion

A series of substituted benzoic acid-(5-oxo-pentylidene)-hydrazides were synthesized using Scheme 1 and their physicochemical properties are presented in Table 1.

Scheme 1. Scheme for the synthesis of substituted benzoic acid-(5-oxo-pentylidene)-hydrazides **(1-11)**



(1-11)

Comp.	R_1	R_2	R_3	R_4	R_5
1.	H	H	H	H	H
2.	Cl	H	H	H	H
3.	H	H	Cl	H	H
4.	H	H	OMe	H	H
5.	H	NO_2	H	H	H
6.	Br	H	H	H	H

7.	F	H	H	H	H
8.	H	H	F	H	H
9.	H	H	CHO	H	H
10.	H	NO_2	H	NO_2	H
11.	H	H	OH	H	H

Table 1. Physicochemical properties of synthesis of substituted benzoic acid-(5-oxo-pentylidene)-hydrazides.

Comp.	Mol. Formula	M. Wt.	M.p (^oC)	R*f* Value*	% Yield
1.	$C_{12}N_2O_2H_{14}$	218.25	95-98	0.56	88
2.	$C_{12}N_2O_2H_{13}Cl$	252.70	234-237	0.79	83
3.	$C_{12}H_{13}ClN_2O$	236.70	196-199	0. 76	81
4.	$C_{13}H_{16}N_2O_3$	248.28	217-220	0.47	79
5.	$C_{12}H_{13}N_3O_4$	263.70	233-236	0.53	84
6.	$C_{12}H_{13}BrN_2O_2$	297.15	237-240	0.76	82
7.	$C_{12}H_{13}FN_2O_2$	236.24	181-184	0.54	79
8.	$C_{12}H_{13}FN_2O_2$	236.24	186-189	0.42	80
9.	$C_{13}H_{14}N_2O_3$	246.26	138-141	0.69	85
10.	$C_{12}H_{12}N_4O_2$	308.25	198-201	0.40	87
11.	$C_{12}H_{14}N_2O_3$	234.25	178-181	0.67	76

*TLC mobile phase: Toluene: Chloroform (7:3)

In general, IR spectra of synthesized compounds showed absorption band at around 1666.50-1670.35 cm^{-1}, 1764.87-1786.08 cm^{-1} and 1631.78 cm^{-1} regions, conforming the presence of C=O of secondary amide, C=O of aldehyde and C=N respectively. In ^1H NMR, characteristic signal were observed at δ 7.25- 8.01(m, 4H of ArH), δ 1.05-1.56 (m, 2H, CH_2 adj. to N=CH) confirmed the formation of hydrazide derivatives.

The synthesized substituted benzoic acid-(5-oxo-pentylidene)-hydrazides derivatives (**1-11**) were screened *in vitro* for their antimicrobial potential by tube dilution method using norfloxacin and fluconazole as reference drugs for antibacterial and antifungal activities, respectively. The results of antimicrobial activity are presented in Table 2.

Table 2. Antimicrobial activity of synthesized substituted benzoic acid-(5-oxo-pentylidene)-hydrazides.

Minimum Inhibitory Concentration (µM/mL)					
Comp.	***S. aureus***	***B. subtilis***	***E. coli***	***C. albicans***	***A. niger***
1	0.23	0.23	0.06	0.11	0.06
2	0.10	0.10	0.05	0.05	0.10
3	0.21	0.05	0.05	0.11	0.05
4	0.10	0.10	0.10	0.10	0.05

5	0.04	0.04	0.09	0.09	0.04
6	0.17	0.08	0.04	0.04	0.08
7	0.21	0.05	0.11	0.05	0.05
8	0.05	0.05	0.11	0.05	0.05
9	0.20	0.05	0.05	0.10	0.05
10	0.04	0.04	0.04	0.04	0.04
11	0.05	0.05	0.05	0.05	0.05
Std.	0.0002*	0.0002*	0.0002*	0.0001**	0.0001**

*Norfloxacin, **Fluconazole

Antimicrobial screening results indicated that 3,5-dinitro-bezoic acid-(5-oxo-pentylidene)-hydrazide (**10**) demonstrated potent antimicrobial activity against the tested microbial strains (MIC=0.04µM/mL). The presence of electron withdrawing group improved the antimicrobial activity of synthesized hydrazides. The role of electron withdrawing group in improving antimicrobial activity as supported by studies of (Abdel-Aziz *et al*. 2009). In general according to the result of MBC/MFC studied (Table 3) the synthesized compounds were bacteriostatic and fungistatic in their action as their MBC/MFC values were more than 3 fold higher than their MIC values (Emami *et al.* 2004).

Table 3. Minimum Bactericidal/Fungicidal Concentration of synthesized substituted benzoic acid-(5-oxo-pentylidene)-hydrazides.

Minimum Bactericidal/Fungicidal Concentration (µM/ml)					
Comp.	***S. aureus***	***B. subtilis***	***E. coli***	***C. albicans***	***A. niger***
1	>0.22	>0.22	>0.22	>0.22	0.05
2	>0.19	>0.19	>0.19	>0.09	0.04
3	>0.21	>0.21	>0.21	>0.21	0.05
4	>0.20	>0.20	>0.20	0.20	0.05
5	>0.18	>0.18	>0.18	>0.18	0.04
6	>0.16	>0.16	>0.16	0.16	0.04
7	>0.21	>0.21	>0.21	0.21	0.05
8	>0.21	>0.21	>0.21	>0.21	0.05
9	>0.20	>0.20	>0.20	0.20	0.05
10	>0.16	>0.16	>0.16	>0.16	0.04
11	>0.21	>0.21	>0.21	0.21	0.05
Std.	0.0019*	0.0019*	0.0019*	0.004**	0.004**

Conclusion

8. Li, W.H. (2009). Synthesis, crystal structure and

A series of substituted benzoic acid-(5-oxo-pentylidene)-hydrazide derivatives (**1-11**) were synthesized in appreciable yield. The synthesized compounds were characterized by physicochemical and spectral (IR and NMR) means. The spectral data was found in agreement with the assigned molecular structures. The antimicrobial activity was evaluated against five representative microorganisms. Among the synthesized hydrazides, 3, 5-dinitro-bezoic acid-(5-oxo-pentylidene)-hydrazide (**10**) was found to be the most effective antimicrobial agent. In general the synthesized compounds were found to be bacteriostatic and fungistatic in nature.

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