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Synthesis, Characterization And Studies Of Antimicrobial Activity Of 2-Pyranone Derivatives

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Abstract

Antimicrobial agents have been in widespread and largely effective therapeutic use since their discovery in the 20th century. However, the emergence of multi-drug resistant pathogens now presents an increasing global challenge to both human and veterinary medicines. It is now widely acknowledged that there is a need to develop novel antimicrobial agents to minimize the threat of further antimicrobial resistance. 2-Pyranones and their derivatives are found to possess pronounced biological activity. Compounds in which the pyranone ring is fused with other aromatic ring have also been found to possess remarkable biological properties. In view of the therapeutcial interest of 2-pyranones, in the present investigation we have synthesized 2-pyranone derivatives by the reaction of ketenes with β-substituted phenyl α,β-unsaturated ketones (chalcones) involving [4+2] cycloadditions products as follows undergo conversion to 2-pyranone.

Keywords: 2-Pyranones, Therapeutic, Antimicrobial Agents, Cycloadditions Products

Introduction

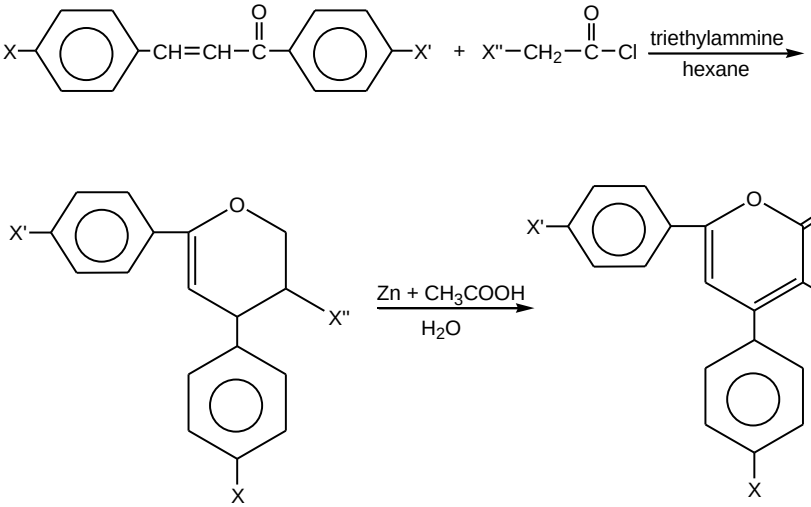
The compounds with pyranone ring system have been known for more than a century, but their versatility in organic synthesis to generate molecular diversity was recognised only after 1960. The presence of this ring system in plants, animals, marine organisms, bacteria, insects and its involvement in different biological processes such as defence against other organisms, biosynthetic intermediates and as metabolites have made this scaffold an important chemical entity. Many of the pyranones have been used as precursors for the synthesis of pharmacologically active compounds such as HIV protease inhibitors[1], antifungals[2], cardiotonics[3], anticonvulsants,[4] antimicrobials,[5] pheromons[6] natural pigments,[7]antitumour agents,[8] and plant growth regulators.[9-15] Microbially derived pyranones, obtained from fungi of various genera, are found to display a wide range of cytotoxic, neurotoxic and phytotoxic properties. Various pyranones have been used for the construction of biological importance natural products such as solanopyrones[16] pheromones,[17] coumarins[18] and inhibitors of α-chymotrypsin,[19] and elastase enzymes.[20] The

aromatic potential of pyranones has been observed through their ease of electrophilic substitution such as nitration,[21] sulphonation and halogenation.[22,23]

Pyrones can exist in one of two major isomeric forms, 2-pyrone and 4-pyrone (also known as α and γ pyrones, respectively) which are assigned on the position of the carbonyl group relative to the oxygen atom within the ring system. The parent compounds for both types of pyrone ring system are susceptible to ring-opening by nucleophilic reagents, particularly hydroxide ions, which will predominantly attack the carbonyl position on the 2-pyrone, or in a Michael fashion in 4-pyrone.[24,25]**.** A vital aspect in drug discovery and design is the metabolism of the test compound, but crucially one needs to know whether by-products affect the host. The formation of this non-toxic chemical, combined with the reactivity of –OH at the C-3, and C-7 positions, make these compounds an excellent starting point for the synthesis of complex natural product targets.[26]

Experimental

In the present investigation we have synthesized 2-pyranone derivatives by the reaction of ketenes with β-substituted phenyl α,β-unsaturated ketones (chalcones) involving [4+2] cycloadditions products as follows undergo conversion to 2-pyranone.



X = H, OCH_3, F, Cl, Br, NO_2

X' = H, OCH_3, F, Cl, Br, NO_2

X'' = H, Cl, C_6H_5

Scheme-1

Methodology

Placed (10m mole) of substituted acetyl chloride and (10m mole) of chalcone in triethylammine and hexane in a round bottom flask equipped with reflux condenser. Refluxed the reaction mixture for till the initial cycloadduct is separated out. The initial cycloadduct was not isolated but treated with excess of Zn in moist acetic acid to afford the 2–pyranone derivative. Check the progress of the reaction by Thin layer Chromatography. The crude product was recrystallized with Pet-Ether.

Result And Discussion

Melting points were determined on electric melting point apparatus and are uncorrected. The purity of all the compounds was checked by thin layer chromatography using various non-aqueous solvents. IR spectra (in KBr pellets) were recorded on Shimadzu-8400S FT-IR spectrophotometer. The ^{1}H NMR spectra were recorded on Jeol AL 300 MHz FT NMR and in $CDCl_3$ using TMS as an internal standerd. The chemical shifts are expressed as δ ppm.

A. 3- Chloro-6-(4-fluorophenyl)4-(4-methoxyphenyl)2-pyranone
B. 4-(4-methoxyphenyl)6-phenyl-2-pyranone
C. 3-Chloro-6-(4-chlorophenyl)4-(4-methoxyphenyl) 2-pyranone
D. 3-chloro 4-(4-chlorophenyl) 6-phenyl 2- pyranone
E. 3,4,6-Triphenyl-2-pyranone
F. 3-chloro-6-(4-fluorophenyl)-4-(4-nitrophenyl)-2-pyranone

Table-I: Physical data of 2-pyranones derivatives

Compds.	**Molecular Formula**	**Molecular weight**	**Yield (%)**	**Melting point (^{0}C)**	**Elemental analysis**			
					C		**H**	
					Found	**Calcd.**	**Found**	**Calcd.**
A	C_{18} $H_{14}O_3$ F C	332.5	55	156-160	64.80	64.96	14.22	14.40
B	C_{18} $H_{15}O_3$	279	52	160-162	77.25	77.4	17.32	17.20
C	C_{18} H_{14} O_3 Cl_2	349	56	165-168	61.45	61.89	13.55	13.75
D	C_{17} H_{12} O_2 Cl_2	319	55	180-184	63.55	63.94	10.35	10.03

E	C_{23} H_{18} O_2	326	48	175-180	85.42	85.18	9.54	9.87
F	C_{17} H_{11} C ClFN	347.5	45	170-175	59.24	59.04	18.24	18.20

Table II: IR and ^{1}H NMR Spectral interpretation of 2-pyranones (A-F)

S.No.	Compounds	IR cm^{-1} (KBr)	^{1}H NMR δ ppm ($CDCl_3$)
1.	A	3040 cm^{-1} (=C-H), 1620 cm^{-1} (C=C), 740 cm^{-1} (C-Cl), 1705 cm^{-1} (C=O) , 1250Cm^{-1} (-C-O), 2920 cm^{-1} (C-H)	3.7 (s, 3H, -OCH_3), 7.10-7.45 (m, 8H,Ar-H),5.8 (d, 1H,=CH-)
2	B	3020 cm^{-1} (=C-H), 1625 cm^{-1} (C=C), 1712 cm^{-1} (C=O), 1210 cm^{-1} (-C-O), 2910 cm^{-1} (C-H)	3.8 (s, 3H,-OCH_3), 7.15-7.35 (m, 9H, Ar-H), 5.5 (s, 1H,=CH-), 5.7(s,1H, =CH)
3.	C	3030 cm^{-1} (=C-H), 1630 cm^{-1} (C=C), 750 cm-^{1} (C-Cl), 2925 cm^{-1} (C-H),1235 cm^{-1} (-C-O), 1710 cm^{-1} (C=O)	7.4-7.9 (m, 8H, Ar-H), 3.5 (s,3H,-OCH_3), 5.4 (s, 1H, =CH-)
4.	D	3020 cm^{-1} (=C-H), 1625 cm^{-1} (C=C), 745 cm^{-1} (C-Cl), 1230 cm^{-1} (C-O), 1702 cm^{-1} (C=O)	7.3-7.8 (m, 9H, Ar-H), 5.8 (s, 1H, =CH-)
5.	E	3015 cm^{-1} (=C-H), 1640 cm^{-1} (C=C), 1705 cm^{-1} (C=O), 1220 cm^{-1} (C-O)	7.3-7.5 (m, 15H, Ar-H), 5.3 (s, 1H,=CH-)
6.	F	3025 cm^{-1} (=C-H), 1620 cm^{-1} (C=C), 1704 cm^{-1} (-C=O), 1250-1350 cm^{-1} (N-O)	7.4-7.6 (m, 8H,Ar-H),5.9(s,1H,=CH-)

Antimicrobial Studies Of Pyranone Derivatives

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. The gram negative bacterium such as Escherichia coli is present in human intestine and causes lower urinary tract infection. The main objective of the present study was to investigate the effects of synthesized compounds on the growth of E. coli.LB media Lysogeny broth (LB), a nutritionally rich medium, is primarily used for the growth of bacteria. T According to its creator Giuseppe Bertani, the abbreviation LB was

actually intended to stand for lysogeny broth.27-35 The general composition of ingredients used to promote growth, including the following:

- Peptides and casein peptones
- Vitamins (including B vitamins) provided by yeast extract.
- Trace elements (e.g. nitrogen, sulfur, magnesium)
- Minerals

AgarMedium

An agar plate is a Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms or small plants like the moss Physcomitrella patens. Selective growth compounds may also be added to the media, such as antibiotics. Individual microorganisms placed on the plate will grow into individual colonies, each a clone genetically identical to the individual ancestor organism (except for the low, unavoidable rate of mutation. The synthesized pyrimidine derivatives were screened for the antibacterial activity against Gram-negative bacteria viz., Escherichia coli by using the cup plate method Benzylpenicillin was used as reference standard for comparing the results.

Nutrient broth was used for the preparation of inoculums of the bacteria and nutrient agar was used for the screening method. The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37℃+-1℃ for 18 hours, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121℃ for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160℃, for an hour. Into each sterilized petriplate, was poured about 125 ml of molten nutrient agar medium which was already inoculated with respective strain of bacteria aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification, the cups of each of 7 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish and labeled accordingly. Each test compound (5 mg) was dissolved in dimethyl sulfoxide to give a concentration of 1000 µg/ml. Benzyl penicillin solution was also prepared to give a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using conc HCl. All the compounds were tested at

dose levels of 50 µg and 100 µg and DMSO used as a control. The solutions of each test compound, control and reference standard were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37+_1 ℃ for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

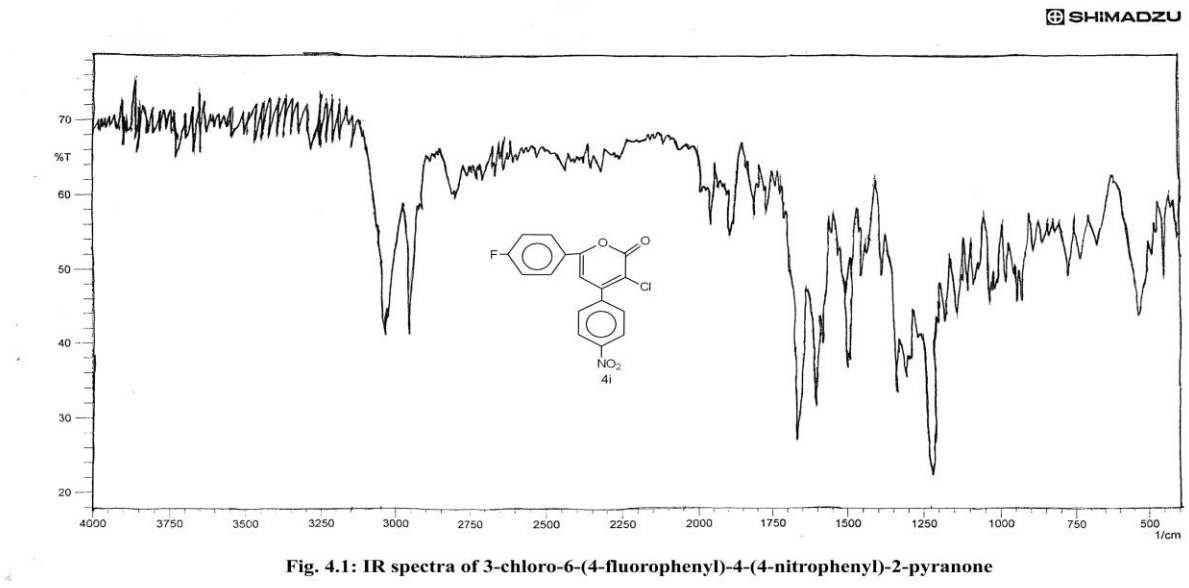


Fig. 4.1: IR spectra of 3-chloro-6-(4-fluorophenyl)-4-(4-nitrophenyl)-2-pyranone

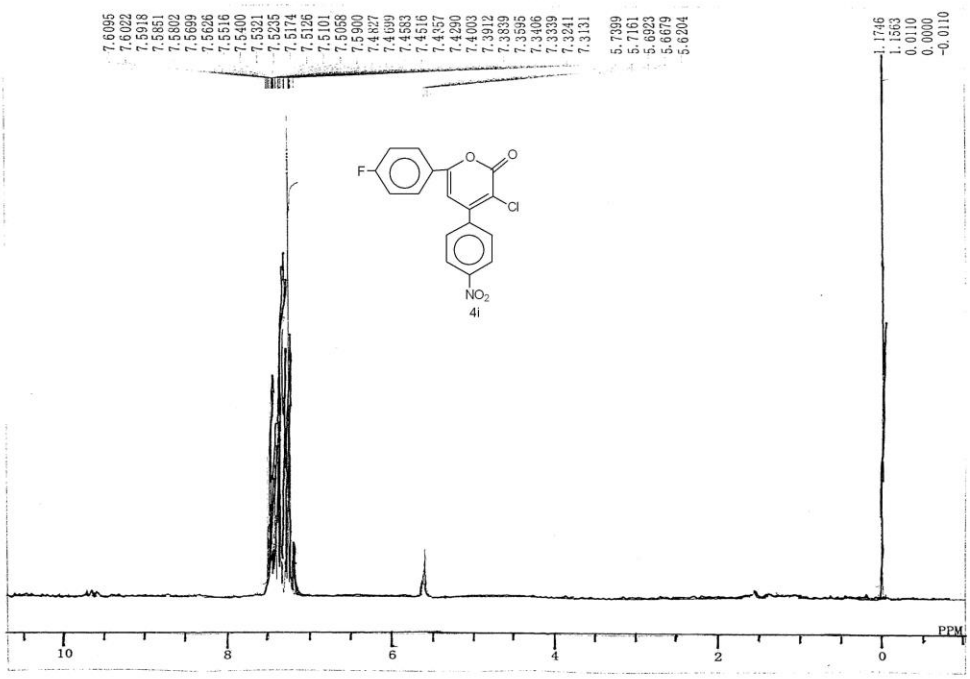


Fig. 4.2: ^{1}H NMR spectra of 3-chloro-6-(4-fluorophenyl)-4-(4-nitrophenyl)-2-pyranone

Table III : Composition of Nutrient agar medium

Peptone	5.0 gm
Sodium chloride	5.0 gm
Beef extract	1.5 gm
Yeast extract	1.5 gm
Agar	15.0 gm
Distilled water	1000 ml
pH	7.4+-0.2

Table IV: Zone of inhibition in centimeters of *E. coli* against the synthesized compounds

Microbial Species		***Escherichia coli***	
Inhibition zone diameter (cm)	Dose	10 µ	25 µ
	A	1.6	1.9
	B	1.7	2.4
	C	1.3	1.7
	D	1.4	1.7
	E	1.7	2.5
	F	1.8	2.5

Table V: Optical density of synthesized compounds

Sr.No	Sample	O.D readings after 5 hours	O.D readings after 24 hours
1	**Control**	1.796	1.709
2	A	10µ=1.784	10 µ =0.640
3	B	10µ=1.820	10µ=0.638
4	C	10µ=1.850,25µ =1.834	10µ=1.680,25µ=1.602
5	D	10µ=1.526, 20µ =1.836	10µ=1.780, 25µ=1.794
6	E	10µ=1.810, 25µ =1.844	10µ=0.620, 25µ=1.755
7	F	10µ=1.822, 25µ =1.870	10µ=1.705, 25µ=1.750

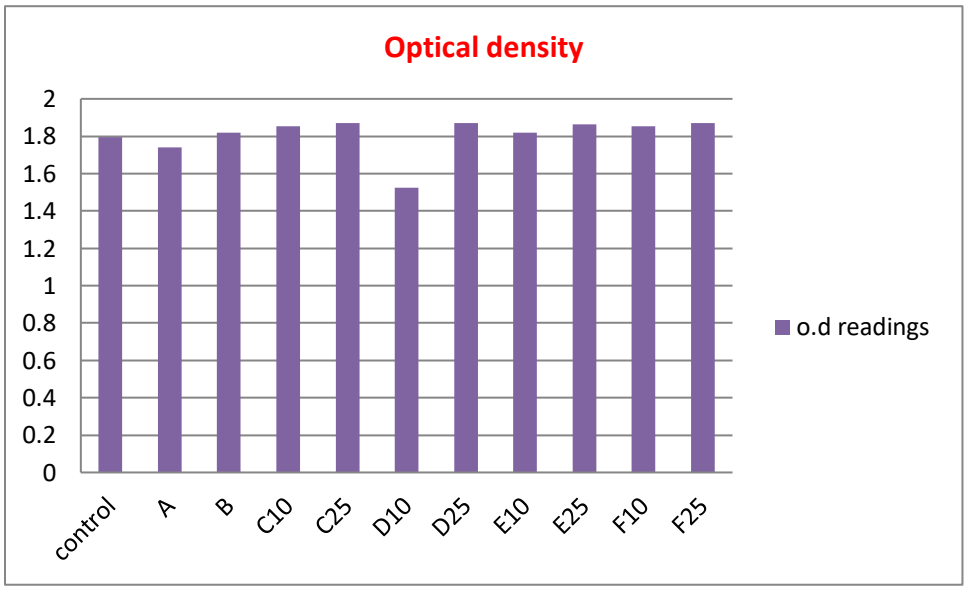


Fig.1: Optical density after 5 hours

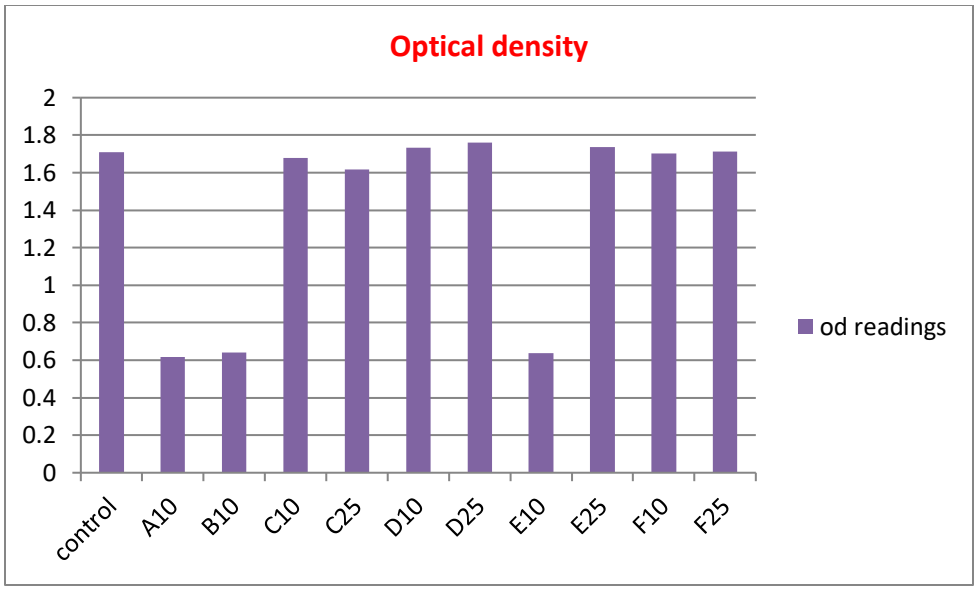


Fig.2: Optical density after 24 hours

Conclusion

Presented in Figure 1 and 2 are the inhibition of the growth of *E. coli* by the action of the 2-pyranone derivatives measured in terms of optical density at different concentration (10µ and25µ) and broth dilution. There has been a remarkable decrease in the bacterial growth rate with increasing concentration and longer period of incubation. It clear from the result that there are higher inhibition of the growth after 24 h than 5 h. It is evident that most of compounds like 3-chloro-6-(4-chlorophenyl)4-(4-methoxyphenyl) 2-pyranone (10µ, 25 µ)[C], 3-chloro 4-(4-chlorophenyl) 6-phenyl 2- pyranone (10µ,25µ)[D], 3, 4, 6 Triphenyl-2-pyranone (25µ)[E] and 3-chloro-6-(4-fluorophenyl)-4-(4-nitrophenyl)-2-pyranone (10µ,25µ)[F] possess very good activity while 3, 4, 6 Triphenyl-2-pyranone (10µ)[E] shows moderate activity against bacterial strains like E.coli. 3- Chloro-6-(4-fluorophenyl)4-(4-methoxyphenyl)2-pyranone (10µ)[A] and 4-(4-methoxyphenyl)6-phenyl-2-pyranone (10µ)[B] possess least activity against selected bacterial strain.

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