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Synthesis, Characterisation and Antimicrobial Activity of Benzil and Its Substituted Analogs

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Abstract
Benzils are well known for their bacterial activity, their coordination compounds containing diketone are reported to possess antimicrobial activity. A series of different substituted benzils (benzil, 4,4'-dibromo benzil, 2,2-dichloro benzil, 2'-chloro-4-methoxy-3-nitro benzil) were synthesized and their antimicrobial activities were studied by using disc diffusion method. The compounds were screened for its antibacterial activity against gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and gram negative bacteria such as *E.coli,* and *Klebsiella pneumoniae.* It was observed that 2-chloro-4'-methoxy-3'-nitro benzil and 2,2'-dichloro benzil showed anti bacterial activity in a dose dependent manner. The compound with chloro substituent showed potent antibacterial activity against Staphylococcus aureus bacterial strain in comparison with bromo substituted and unsubstituted benzil. The susceptibility of the microorganisms to the synthesized benzils was compared with each other and with the standard antibiotic streptomycin. The antimicrobial activity of the compound reveals that they act as a source of potent antimicrobial agents.

1. Introduction

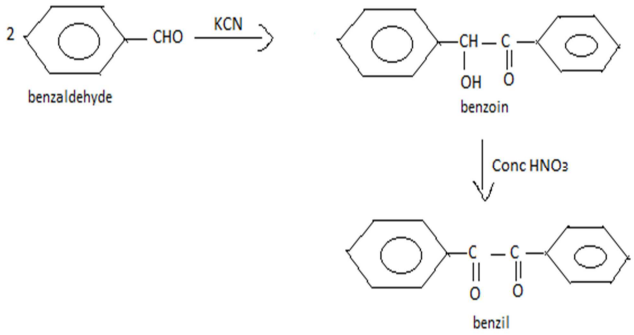
All bacteria are unicellular prokaryotes. They do not have a defined cellular nucleus. Their genetic information is in their nucleoid, a single, circular tightly packed DNA molecule. According to their shape, bacteria are divided into three groups: (1) spirilla (with a spiral body shape); (2) bacillus (with a rod or stick shaped body); (3) cocci (with a spherical body shape) (1,2). Some types of bacteria live on their own and others form colonies. Some bacteria are quite mobile and others stay put for their whole life. Bacteria move using their cytoplasmic tail - flagella, or by secreting slimy substances that allow them to slide along surfaces (3-5). The cell walls of most bacteria contain a polysaccharide called peptidoglycan. A difference in their cell wall structure is a major feature used in classifying these organisms (6) Nowadays research focuses on the synthesis of novel, potent selective antimicrobial drugs with least cell/tissue toxicity and adverse effects. The number of life-threatening infectious diseases caused by multidrug-resistant bacteria have reached an alarming level in many countries around the world. Bacterial resistance to many available antibacterial agents is a growing problem. Accordingly, the development of new antibacterial agents that could overcome the resistance problem has become the subject of an ongoing research (7-12).

Bacteria that cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans, in other animals and also in plants. Some bacteria can only make one particular host ill, others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The disease caused by bacteria are almost as diverse as the bugs themselves and include infectious diseases such as pneumonia, food borne illnesses, tetanus, typhoid fever, diphtheria, syphilis and leprosy and even certain forms of cancer. Bacterial cells grow and divide, replicating repeatedly to form large numbers, present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules. Consequently a number of chemotherapeutic agents are available to combat such organisms. The survived microorganisms have matched the ingenuity in developing their own defenses. As a result such drugs gradually lose their effectiveness in action. Repetition and overdose of such drugs often cause environmental pollution. In order to get rid of this situation, there is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases (Rojas et al., 2003). The present paper is a continuation of such type of investigations. For the purpose, five different substituted benzil compounds have been synthesized, characterized and evaluated their capabilities as antimicrobial agents. From the literature survey, it had been found that benzil has anti tumour activity(13). The variation in the substituent and composition of the benzil reveals that it has been proposed to analyse the anti oxidant and anti proliferative activity with different vitro models. In this work, we report on the synthesis of substituted benzil derivatives and on the biological activities of these compounds against gram-negative and gram positive organisms.

2. Materials and Methods

2.1. Synthesis of Different Substituted Benzils

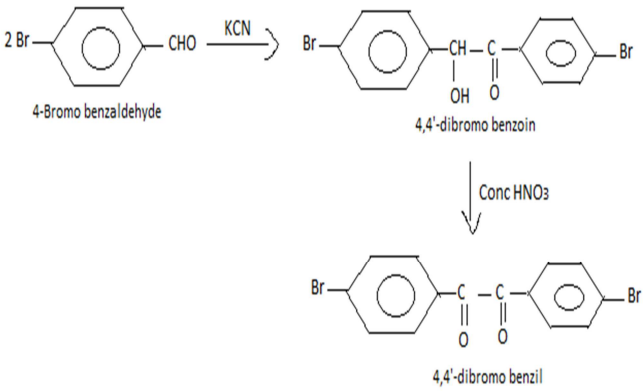
A) BENZIL



The compound benzil is synthesized from two moles of benzaldehyde with ethanol in the presence of KCN as catalyst by steam distillation process. The benzoin obtained after steam distillation process is followed by refluxing for 1 hour with concentrated nitric acid has the melting point of 100.3°c.

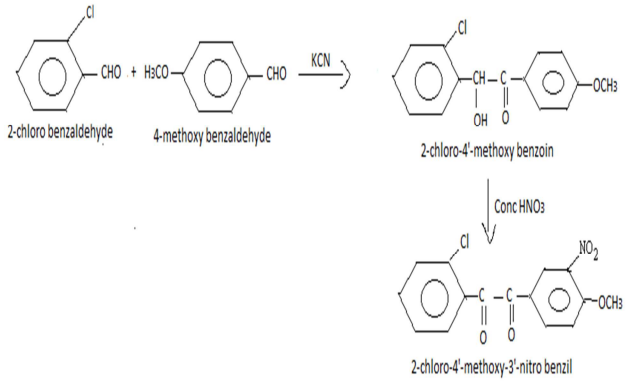
B) 4,4'-DIBROMO BENZIL

The compound 4,4'-dibromo benzil is prepared from two moles of 4-bromo benzaldehyde in ethanol with KCN as catalyst . The 4, 4'-dibromo benzoin is obtained by steam distillation process and the crude 4,4'-dibromo benzoin obtained is then refluxed with con. HNO_3 for 1 hour. It melts at 231.7°c.



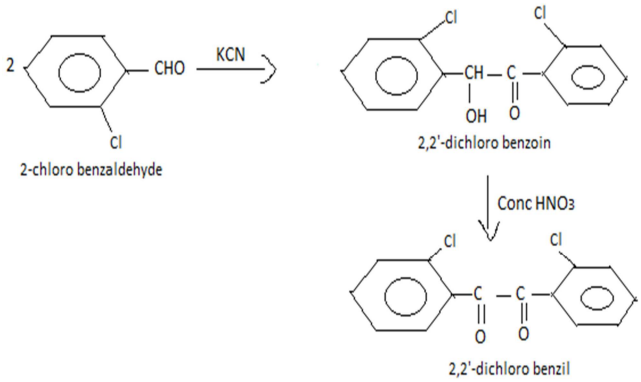
C) 2'-CHLORO-4-METHOXY-3-NITRO BENZIL

2'-chloro-4-methoxy benzoin is prepared by treating 4-methoxy benzaldehyde with 2-chloro benzaldehyde with alcohol in the presence of potassium cyanide on refluxing and steam distillation. The product obtained is refluxed with concentrated nitric acid. The compound 2'-chloro-4-methoxy benzil was found to have melting point at 110°c.



D) 2,2'-DICHLORO BENZIL

The compound 2,2'-dichloro benzil is prepared from two moles of 2-chloro benzaldehyde with ethanol in the presence of a catalyst KCN. The 2,2'-dichloro benzoin is obtained after refluxing and subjected to steam distillation for 1 hour. The crude 2,2'-dichloro benzoin obtained is then refluxed with con. HNO_3 for 1 hour. The product 2,2'-dichloro benzil is obtained with the melting point 80.3°c.



2.2. Experimental

Melting points were measured on an electro thermal 9300 melting point apparatus and are calibrated and is also confirmed by thermal studies. IR spectra were recorded on a Bruker optics (FT-IR) spectrophotometer using KBr-disk. For determination of the preliminary biological activities the disc diffusion method was used.

In vitro Antimicrobial Activity

2.3. Micro Organisms Used

In vitro antimicrobial studies were carried out against human pathogens. The three Gram positive bacteria studied were *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615) and the two Gram negative bacteria studied were *E.coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC15380).

2.4. Disc Diffusion Method

Antibacterial activity of the syntheised benzil, Compound (1) Benzil, Compound (2) 4,4'- Dibromo Benzil, Compound (3) 2'-chloro-4-methoxy-3-nitro benzil and Compound (4) 2,2'-dichloro benzil was investigated by using disc diffusion method (Murray et al., 1995). Petri plates were prepared with 20 ml of sterile MHA (Hi-media, Mumbai). The test culture (100µl of suspension containing 108 CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 minutes (fig 1). Three different concentrations of the compounds (25, 50 and 100 µg/disc) were loaded on a sterile disc and placed on the surface of the medium and left for 30 minutes at room temperature for compound diffusion. Streptomycin (10 µg/disc) was used as a positive control. These plates were incubated for 24 hrs at 37 °C. Zone of inhibition was recorded in millimetres (mm).

3. Result and Discussion

The compound (1) benzil, compound (2) 4,4'-dibromo benzil, compound (3) 2-chloro 4'-methoxy-3'-nitro benzil and compound (4) 2,2'-dichloro benzil were synthesized by the above discussed method. The antimicrobial activity of four different substituted benzils were tested against three gram-positive, two gram-negative bacteria. It was observed that the compound (3) 2'-chloro-4-methoxy-3-nitro benzil and compound (4) 2,2'-dichloro benzil exhibits sufficient antimicrobial activity by showing maximum zone of inhibition (mm) at dose dependent manner. (fig. 1). Compound (3) 2-chloro 4'-methoxy-3'-nitro benzil showed a maximum zone of inhibition of 11(mm) at 100 (µg) and 8 (mm) at 25 and 50 (µg) against *staphylococcus aureus*. In the case of gram negative bacterium, *E. coli* compound 3 showed a zone of inhibition of 10 mm at 100 (µg). Compound (4) 2,2'-dichloro benzil showed a highest zone of inhibition of 12 mm at 100 (µg), 11 mm at 50 (µg) against *Bacillus subtilis* when compared with standard streptomycin which showed a zone inhibition of 14 mm. In the case of gram negative bacterium *Klebsiella pneumonia* compound 4 also showed a zone of inhibition of 10 mm at 100 (µg). The pathogens Bacillus subtilis, Staphylococcus aeureus and E.coli showed higher antimicrobial activity for the compound 3 and the pathogen *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae* showed maximum activity for the compound 2'-chloro-4-methoxy-3-nitro benzil. The pathogens Bacillus subtilis, Staphylococcus epidermidis and Klebsiella pneumoniae were found to exhibit similar anti bacterial activity for the compound 2,2'-dichloro benzil. Thus the activity of compounds against various pathogens is mainly in a dose dependent manner that is by increasing the dose from 25, 50 and 100 (µg) the activity also increases (Table 2).

4. Spectral Analysis

Table 1. *IR spectral data for different benzil compounds*

FTIR Stretching frequency for the group using KBr	Compound 1	Compound 2	Compound 3	Compound 4
-C=O stretching	1688 cm^{-1}, 1612 cm^{-1}	1599 cm^{-1},1688 cm^{-1}	1609 cm^{-1},1673cm^{-1}	1584 cm^{-1},1684 cm^{-1}
-NO_2 stretching	-	-	1536 cm^{-1}	-
Aromatic C-H stretching	2980 cm^{-1}	2982 cm^{-1}	3092 cm^{-1}	3086 cm^{-1}
Aliphatic C-H stretching	2654 cm^{-1}	2838cm^{-1}	2922cm^{-1}	2924cm^{-1}
Aromatic sym C=C stretching	1590 cm^{-1}	1574 cm^{-1}	1589cm^{-1},1609cm^{-1}	1562 cm^{-1}
Presence of Benzene ring	1014 cm^{-1}, 1091 cm^{-1}	1092cm^{-1},1110cm^{-1}	1069cm^{-1},1094cm^{-1}	1068cm^{-1},1085cm^{-1}
substituted benzene ring	972 cm^{-1}	926cm^{-1}	964 cm^{-1}	956cm^{-1}

4.1. Mass Spectral Data for Different Benzils

The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 210 for compound 1, m/e 366 for compound 2, m/e 319 for compound 3 and m/e value 279 for compound 4. From the mass spectral data the existence of the compound could be confirmed.

4.2. NMR Spectral Data for Different Benzils

In the PMR spectrum a singlet at 3.8 ppm indicates the presence of methoxy group in 2-chloro-4'-methoxy-3'-nitro benzil. Signals in the range of 6.8 to 7.48 ppm indicate the presence of aromatic protons for all benzils. In the ^{13}C NMR the aliphatic carbon atoms appear at 58 and 68 ppm for the compound 2-chloro-4'-methoxy-3'-nitro benzil, the aromatic carbon atoms appear in the range of 128 to 132 ppm. The signal at 199 ppm indicating the carbonyl carbon of.aromatic, 1,2-diketone.

Table 2. *Antimicrobial assay of substituted benzils by disc diffusion method.*

Name of the pathogens	Zone of Inhibition (in mm)												Streptomycin
	compound 1(µg)			compound 2(µg)			compound 3(µg)			compound 4(µg)			
	25	50	100	25	50	100	25	50	100	25	50	100	
Bacillus subtilis	_	_	_	_	_	_	_	_	9	7	11	12	14
Staphylococcus aeureus	_	_	_	_	_	_	8	8	11	_	9	10	20
Staphylococcus epidermitis	_	_	_	_	_	_	_	_	9	_	_	_	20
E.coli	_	_	_	_	_	_	_	_	10	_	8	9	19
Klebsiellapneumoniae	_	_	_	_	_	_	_	_	9	_	8	10	20

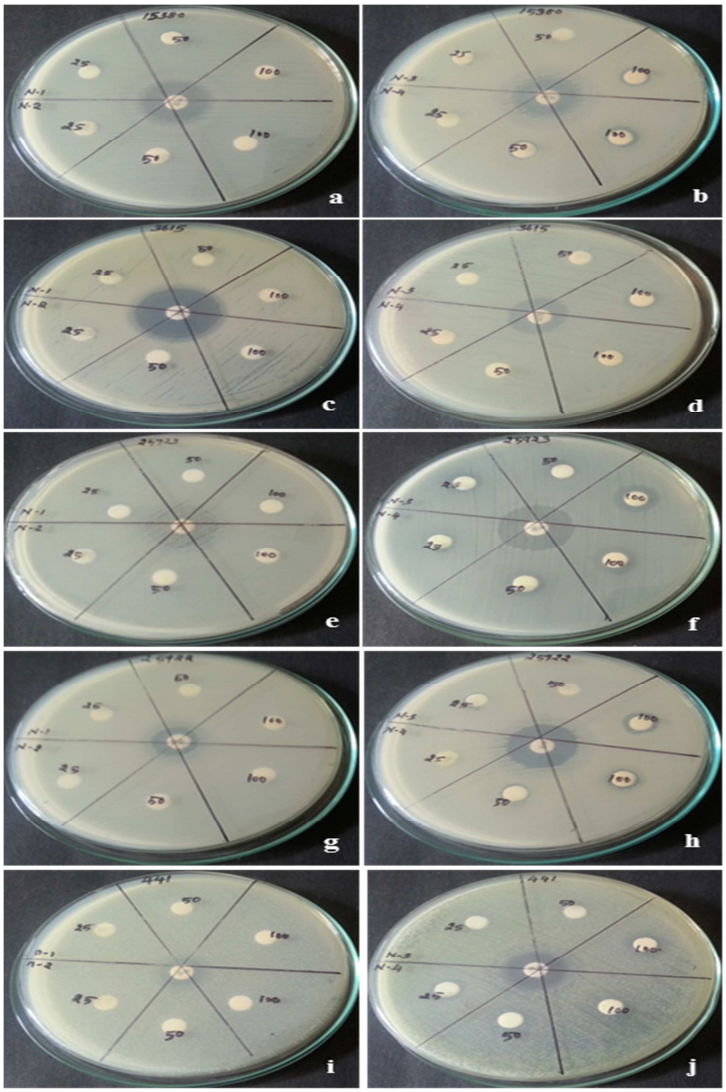


Fig. 1. *Antimicrobial activity dosages for pathogens*

5. Conclusions

Thus it was also concluded that the compound (1) benzil and compound (2) 4,4'-dibromo benzil do not show any anti bacterial activity but the compound with chloro substituent exhibits anti bacterial activity for all pathogens Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, E.coli and Klebsiella pneumoniae. The activity of the compounds were found to be dose dependent i.e., 100 µg/mL showed greater inhibition. The susceptibility of the microbes to the compound was compared with standard antibiotic streptomycin. The thermal stability of the synthesized compounds are comparable to the standard. It can be concluded that this class of compounds certainly holds great promise towards good activity worth to be studied in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

6. Supplementary Materials

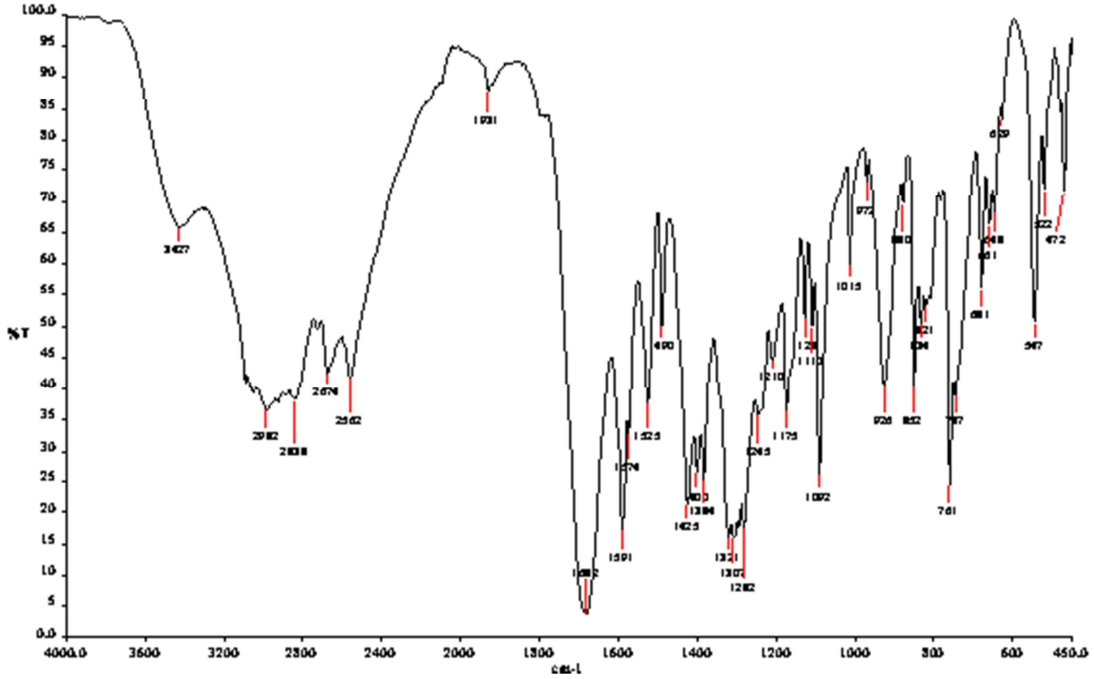


Fig 2(a). *IR spectra for benzil*

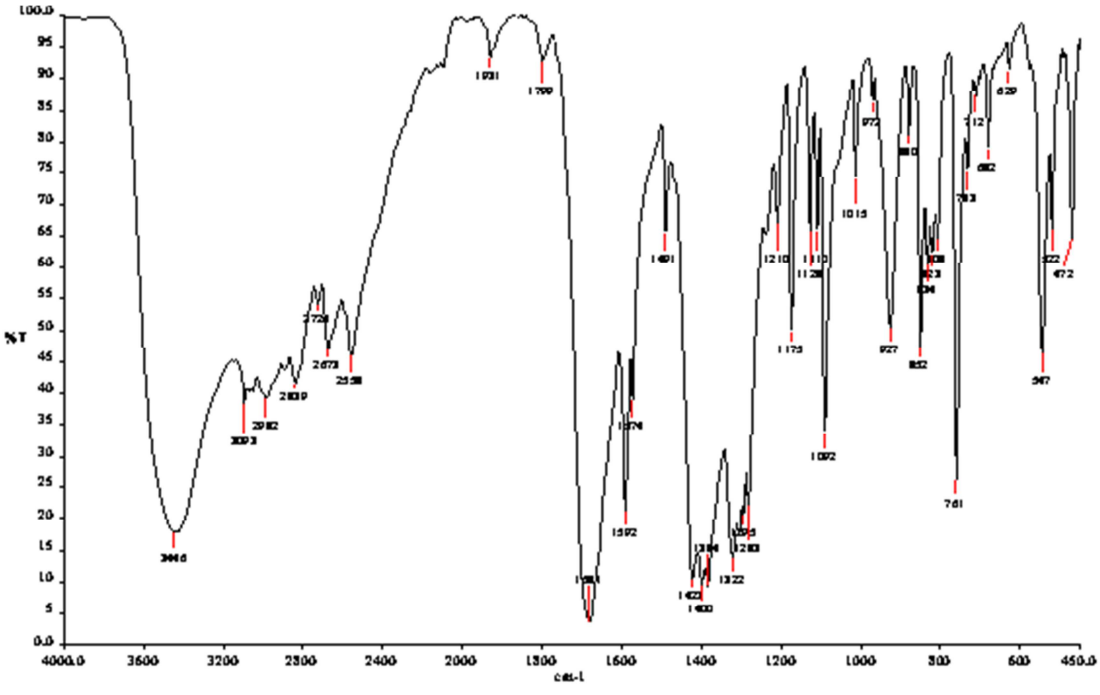


Fig 2(b). *IR spectra for 4,4-dibromo benzil*

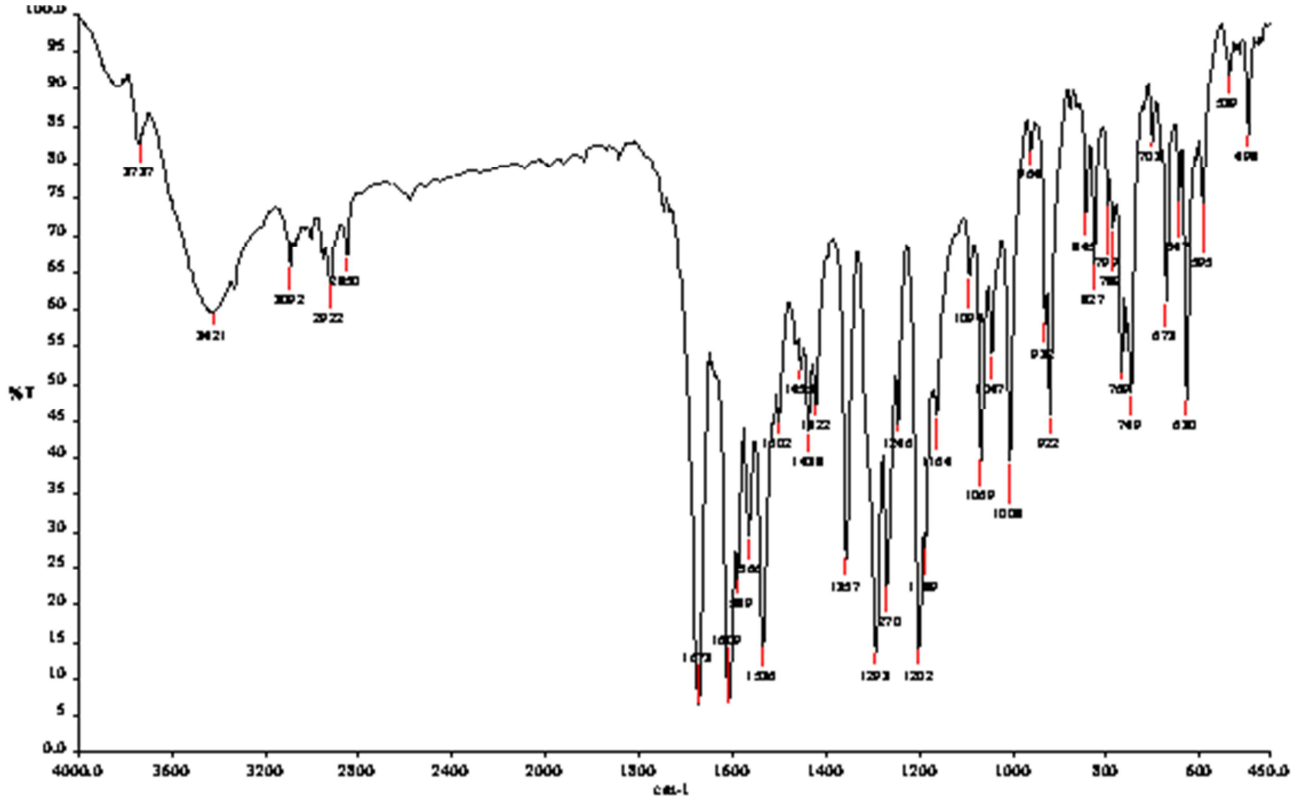


Fig 2(c). *IR spectra for 2'-chloro-4-methoxy-3-nitro benzil*

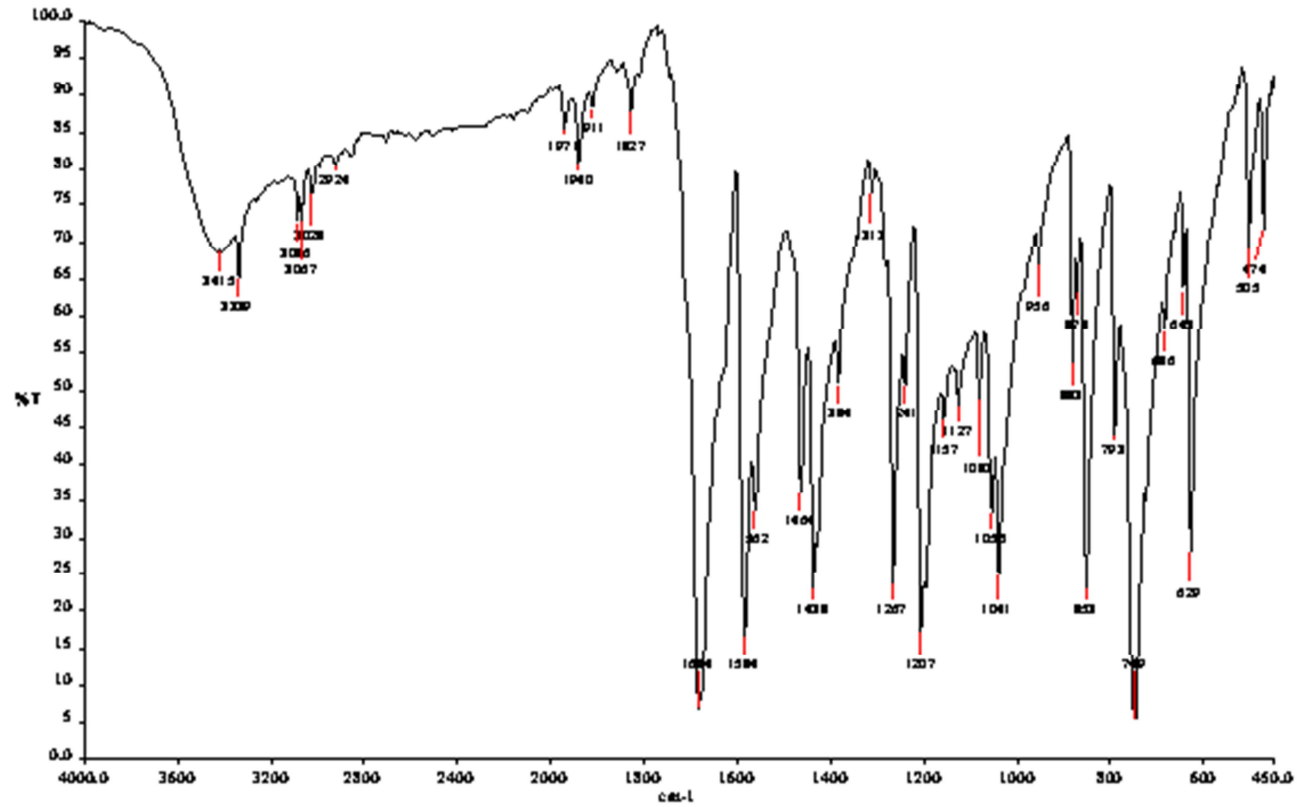


Fig 2(d). *IR Spectra for 2,2'-dichloro benzil*

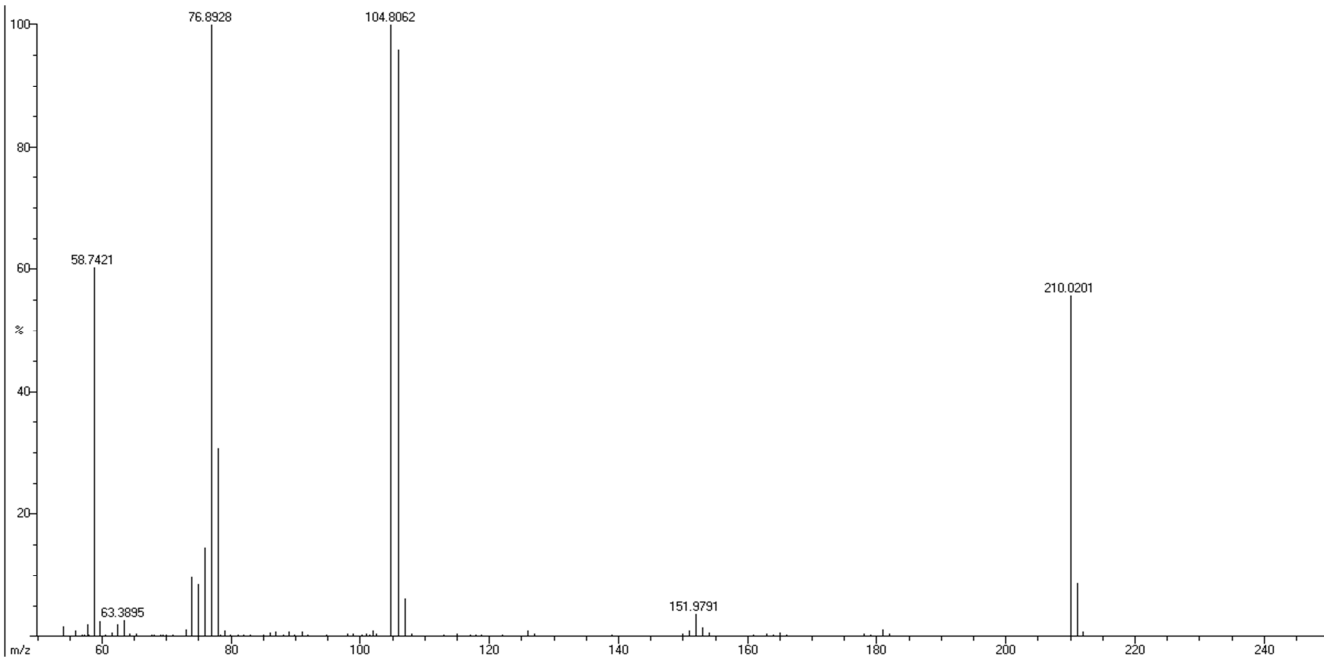


Fig 3(a). *Mass spectra for benzil*

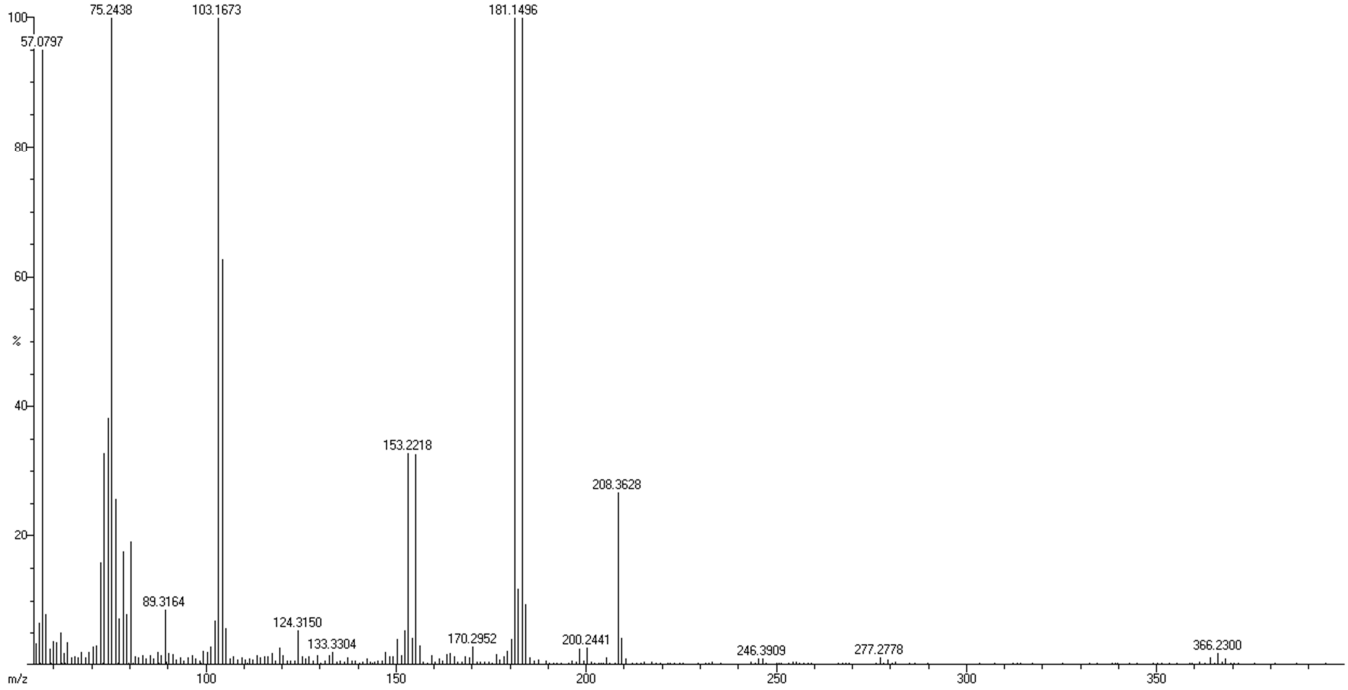


Fig 3(b). *Mass spectra for 4,4'-dibromo benzil*

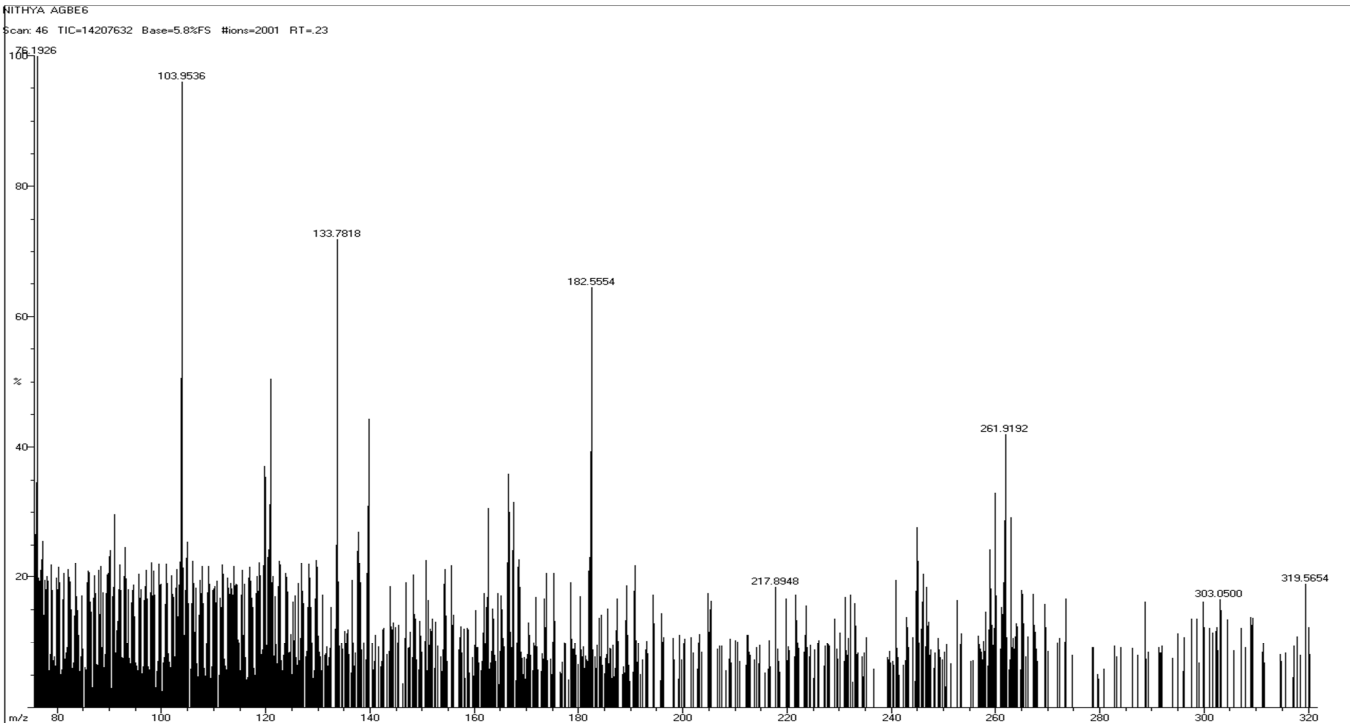


Fig 3(c). *Mass spectra for 2'-chloro-4-methoxy-3-nitro benzil*

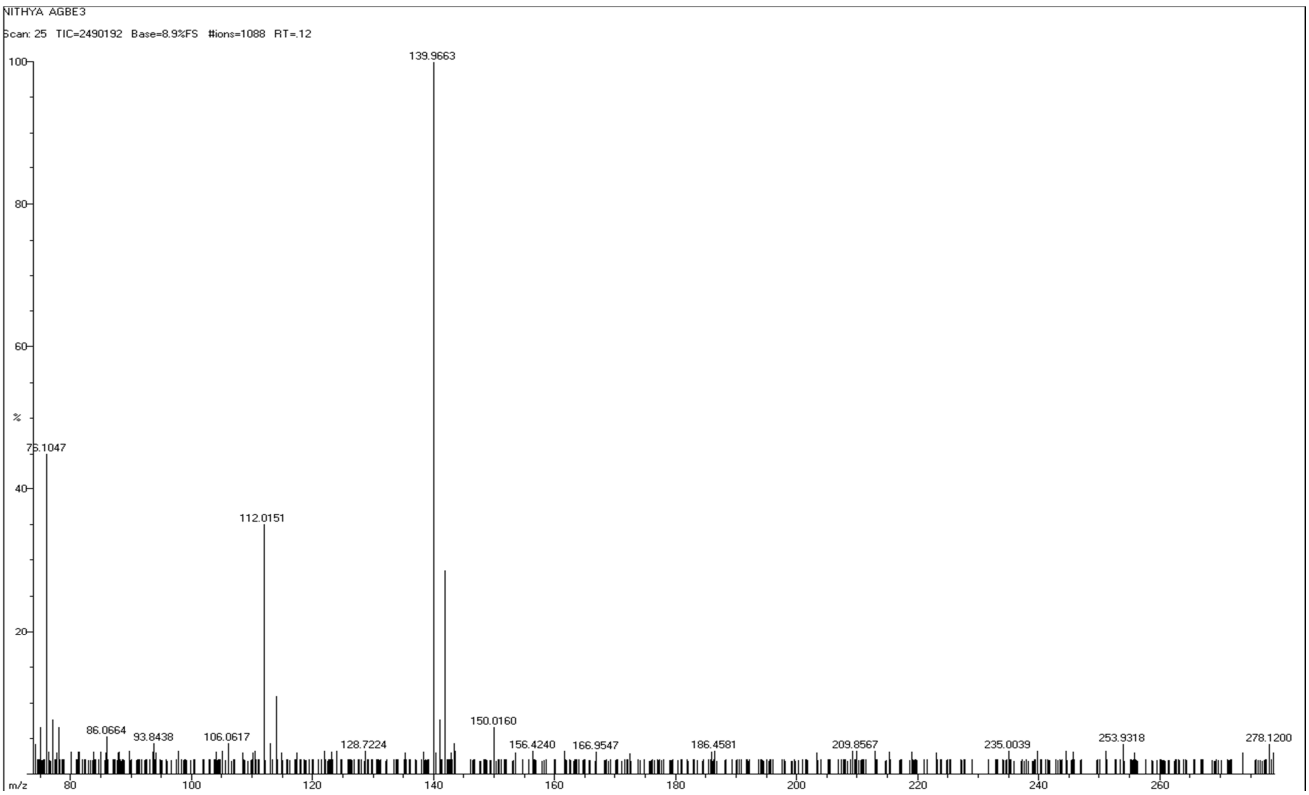


Fig 3(d). *Mass spectra for 2,2'-dichloro benzil*

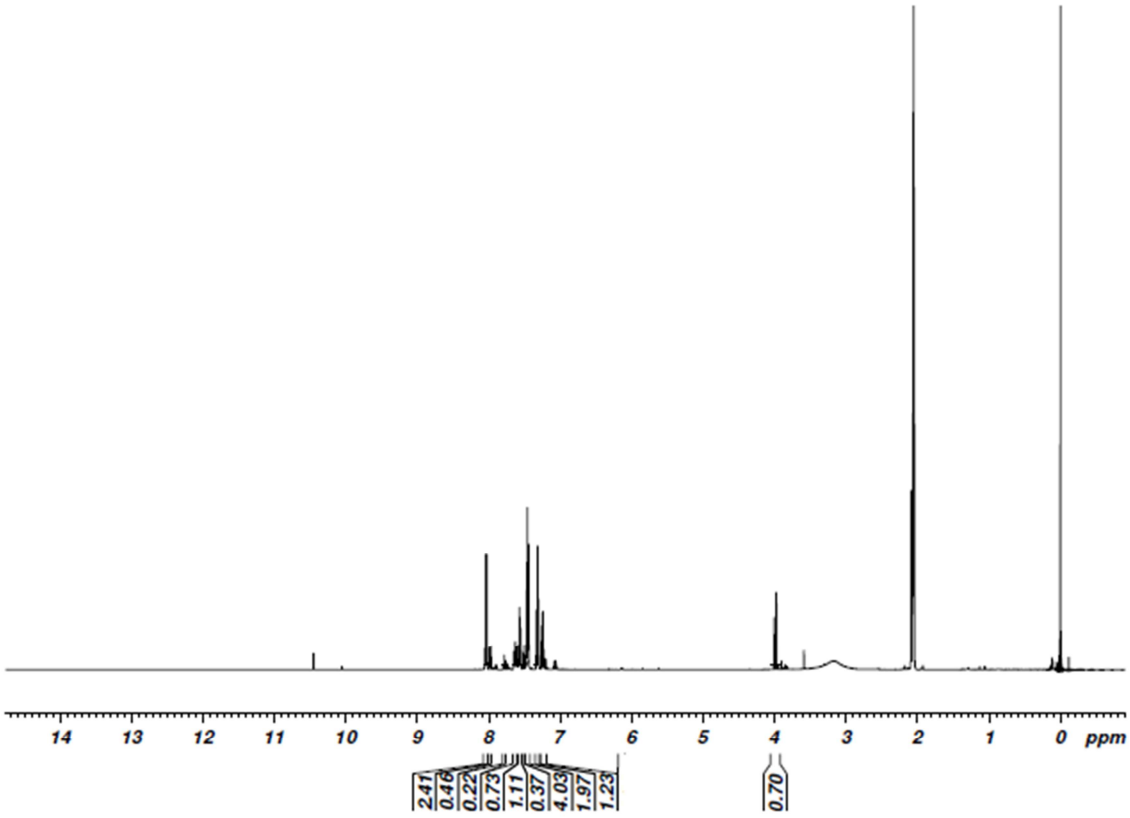


Fig 4(a(i)). *^{1}H NMR spectra for benzil*

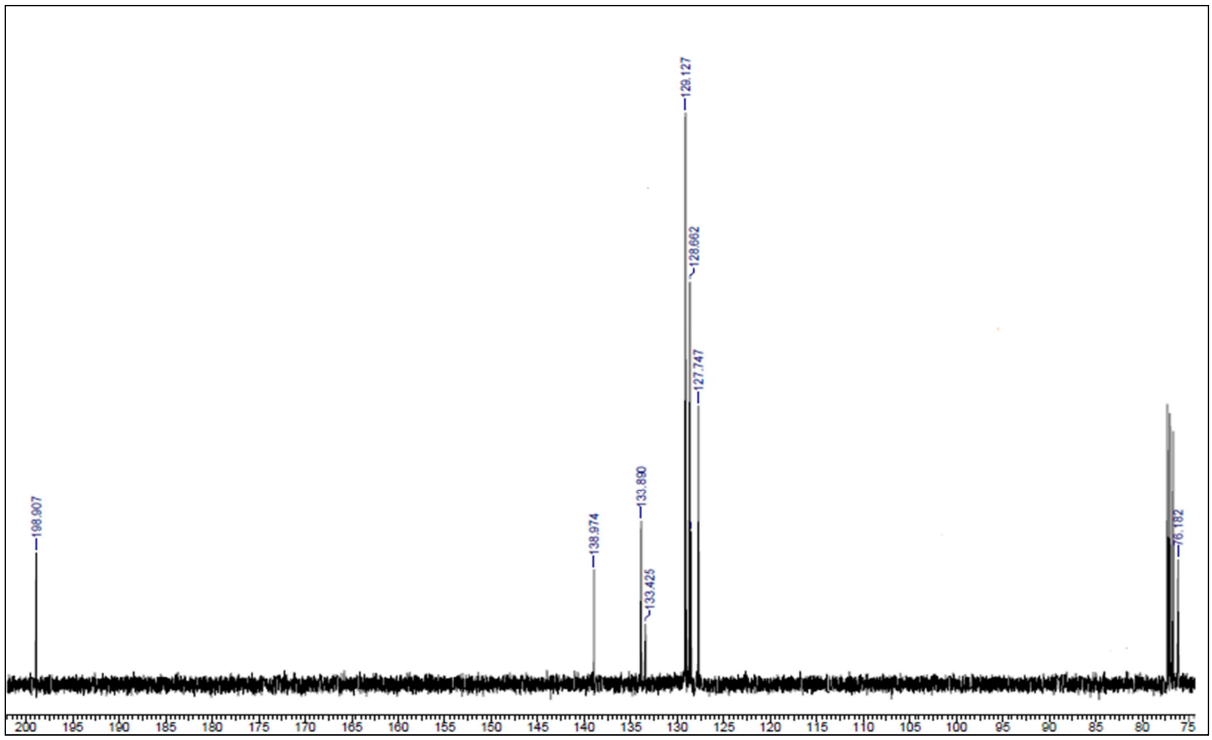


Fig 4(a(ii)). *C^{13} NMR spectra for benzil*

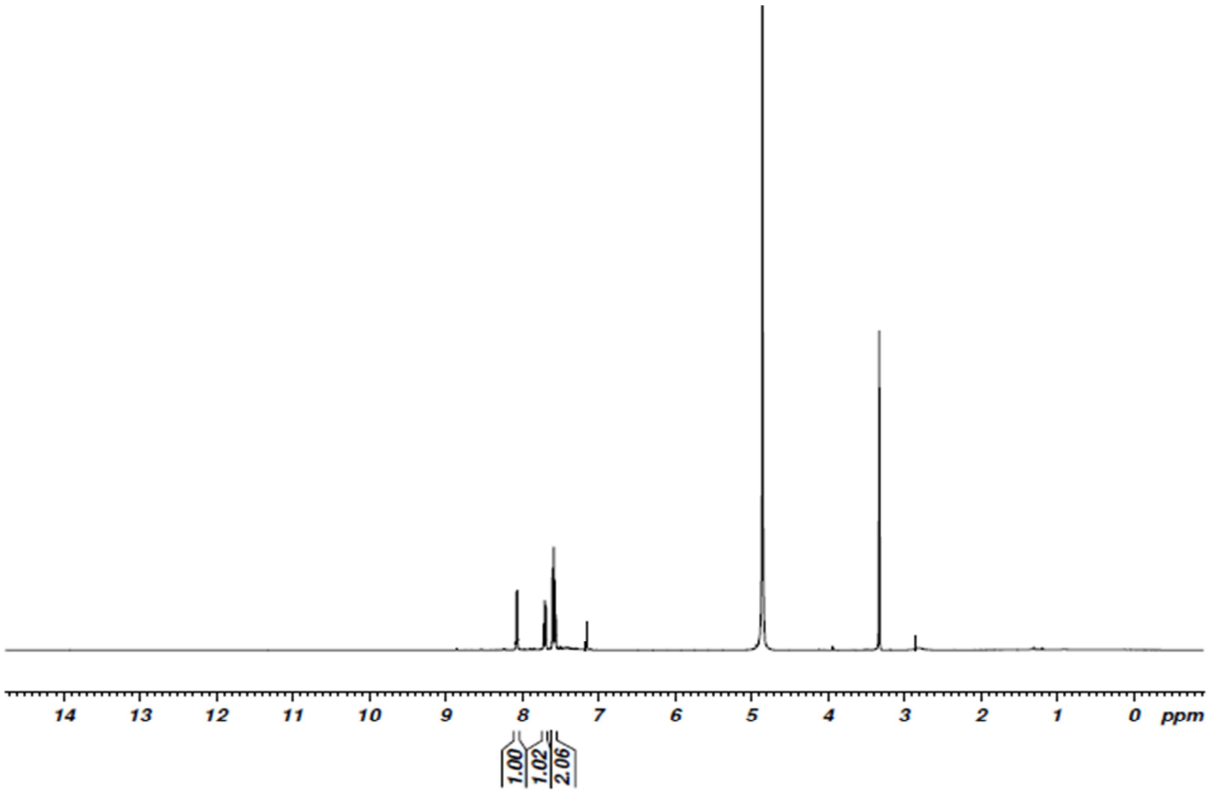


Fig 4(b(i)). *1H NMR spectra for 4,4'-dibromo benzil*

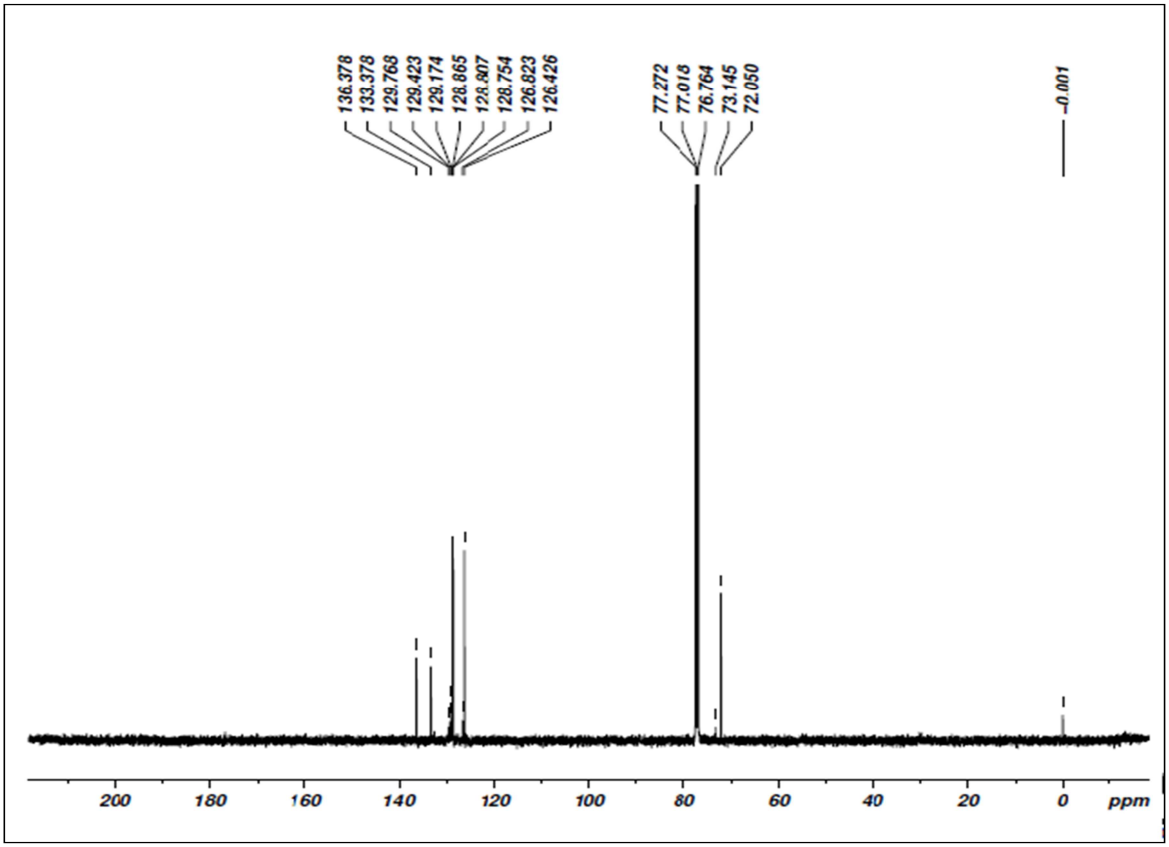


Fig 4(b(ii)). *C^{13} NMR spectra for 4,4'-dibromo benzil*

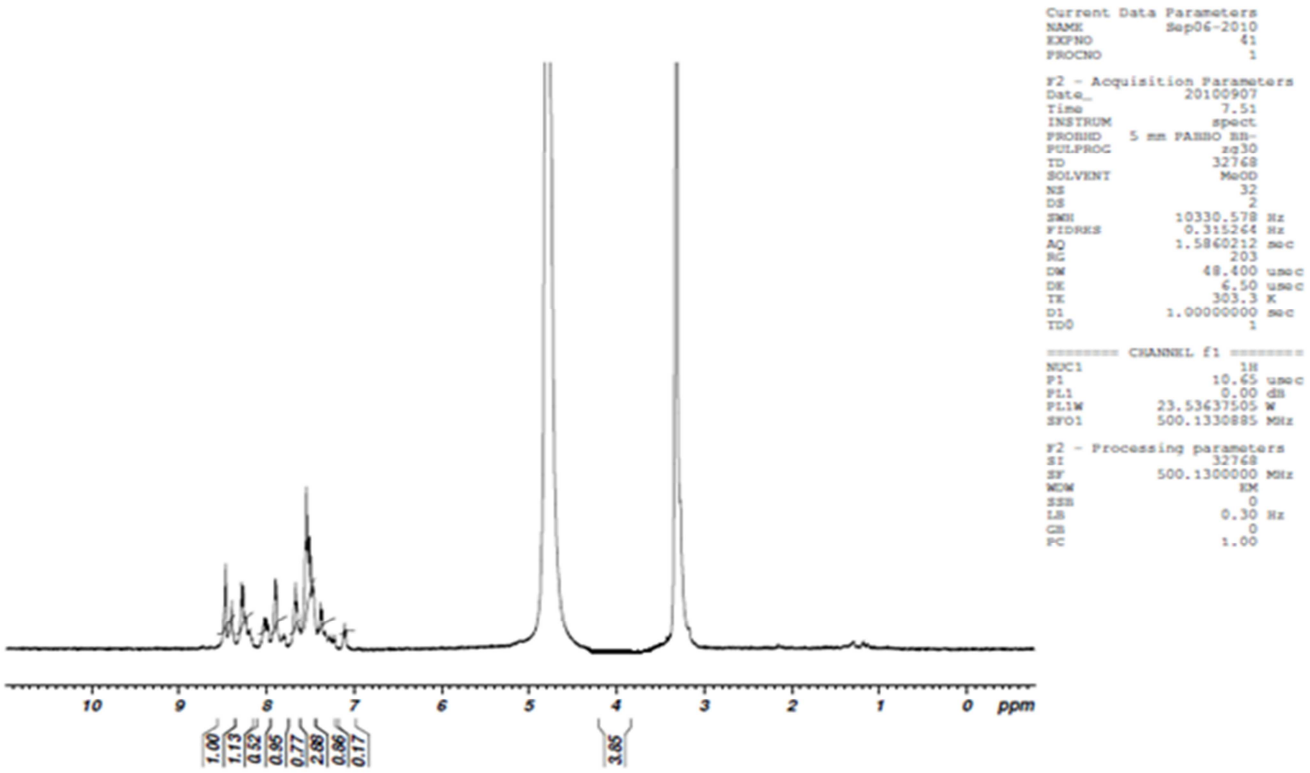


Fig 4(c(i)). *1H NMR spectra for 2'-chloro-4-methoxy-3-nitro benzil*

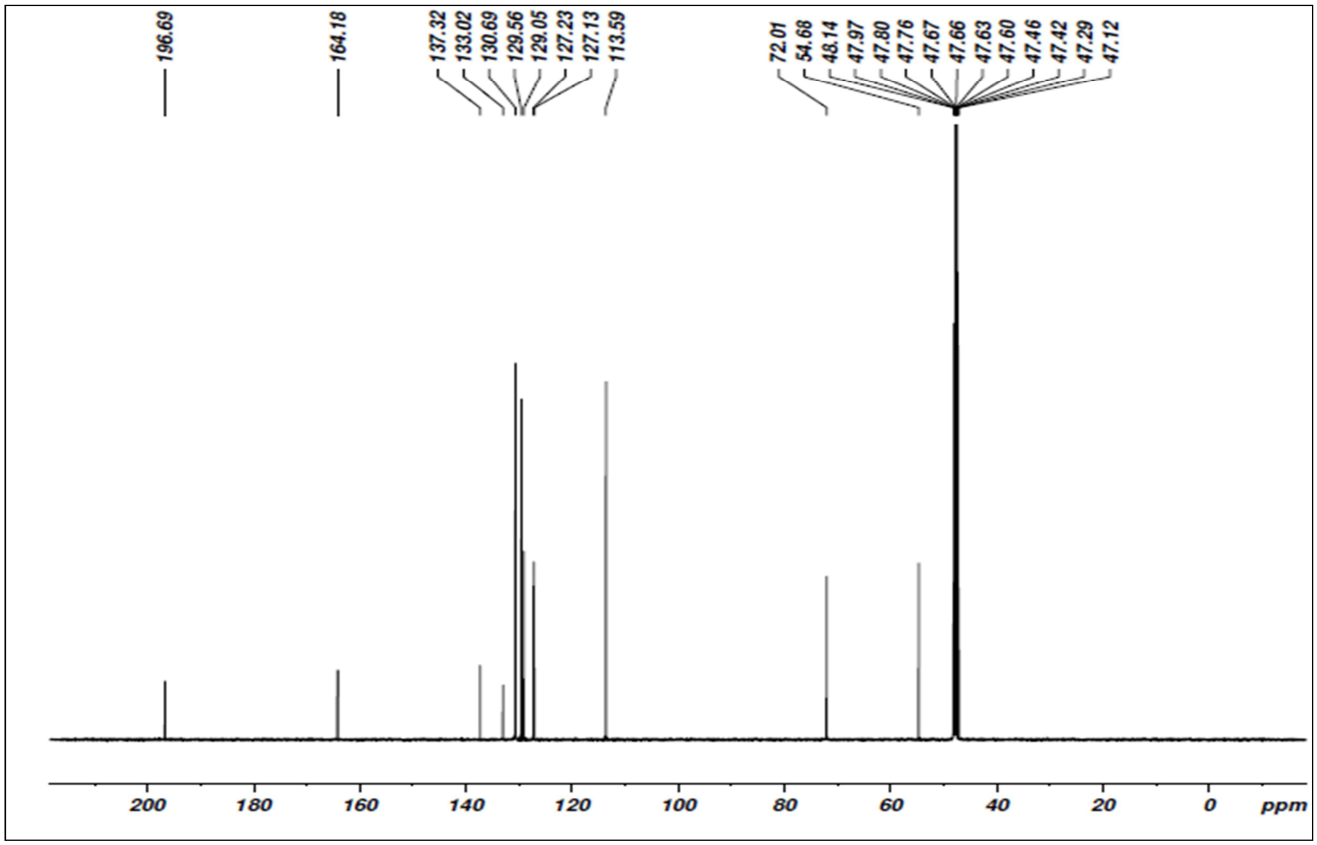


Fig 4(c(ii)). *C^{13} NMR spectra for 2'-chloro-4-methoxy-3-nitro benzil*

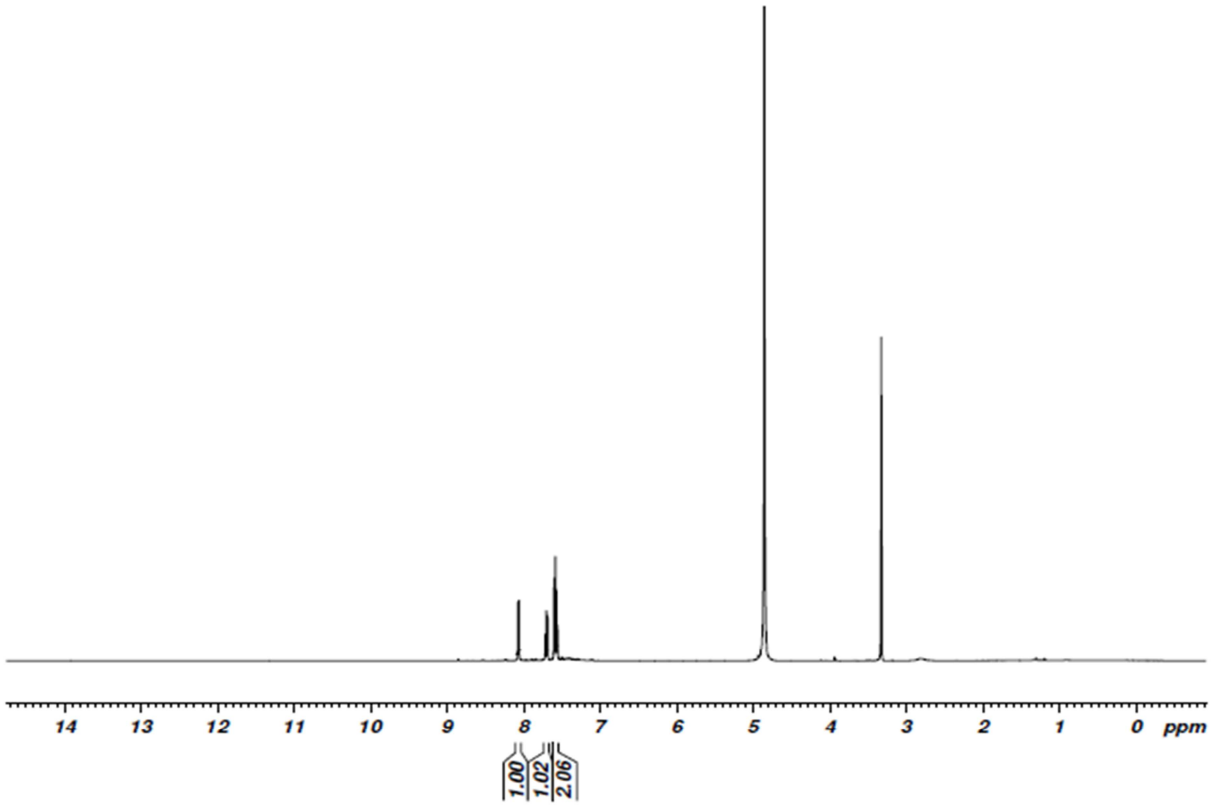


Fig 4(d(i)). *1H NMR spectra for 2,2'-dichloro benzil*

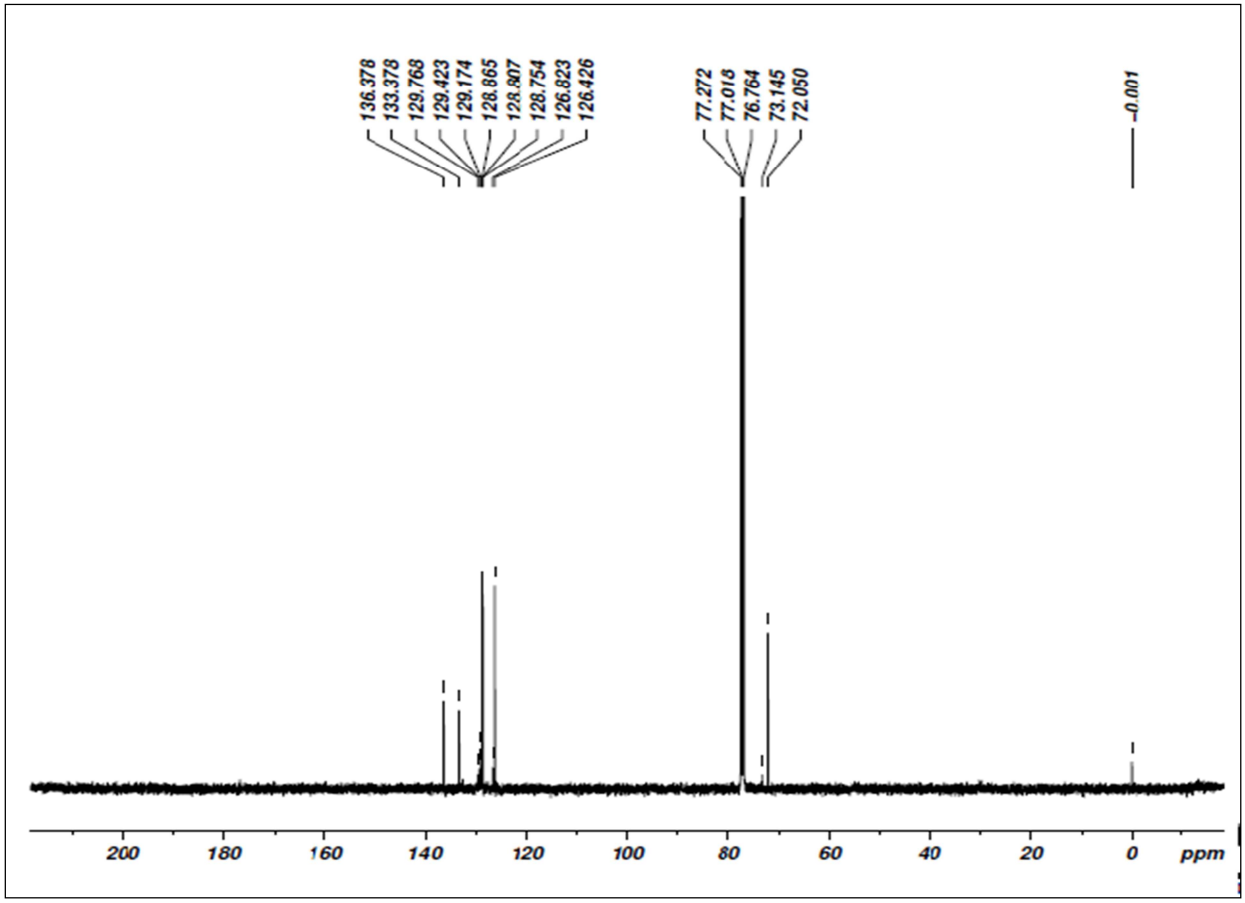


Fig 4(d(ii)). *C^{13} NMR spectra for 2,2'-dichloro benzil*

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