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RESEARCH ARTICLE

Synthesis, Characterization and Study of its *In Vitro* Biological Activity of the compound 4,4'-Dimethoxy benzil and its Iron Oxide Nano Composite

Nithya G*, Sudha R
Department of Chemistry, School of Basic Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai.
*Corresponding Author E-mail: **nithyaranju@gmail.com**

ABSTRACT:
To synthesize 4,4'-dimethoxy benzil iron oxide nano composite. The presence of compound is identified by using FTIR, NMR, MASS and SEM analysis. The biological activity of the compound like antioxidant, anticancer and antibacterial activity of the synthesized compound has been carried out. To know about the molecular docking activity of the compound 4,4'-dimethoxy benzil. The variation in the substituent and composition of the benzil reveals that it has been proposed to analyse the antioxidant and antimicrobial activity with different vitro models. In this work, we report on the synthesis of polysubstituted benzils and on the biological activities of these compounds.

KEYWORDS: Synthesis, SEM analysis, microbial activity and docking study.

INTRODUCTION:
In our approach, we have selected benzil, a organic compound which is used in the treatment of cancer. Benzils and its derivatives have attracted significant attention in the past decades. Benzil compounds having aromatic α-diketone as a functional group are used as an antidepressant, astringent, anti-inflammatory, carminative, deodorant, diuretic, expectorant, sedatives and antibacterial agents. Benzil derivatives exhibit radical scavenging, antibacterial and hypertensive (Mahabusarakam et al., 2004), antiprotozoal (Ganapaty et al., 2009), antiproliferative and antimitotic (Mousset et al., 2008) activities.

The emergence and spread of antimicrobial resistance have become one of the most serious public health concerns across the world. The search for new antimicrobial compounds is a challenging task as bacteria are continuously developing resistance to antimicrobial compounds; however, infections due to such bacterial strains are infrequent although potentially fatal[1-3]. Accordingly, the development of new antibacterial agents that could overcome the resistance problem has become the subject of an ongoing research[4-9]. The ever growing resistance to antibiotics leads to continuous screening for new biologically effective compounds of either natural or synthetic origin.

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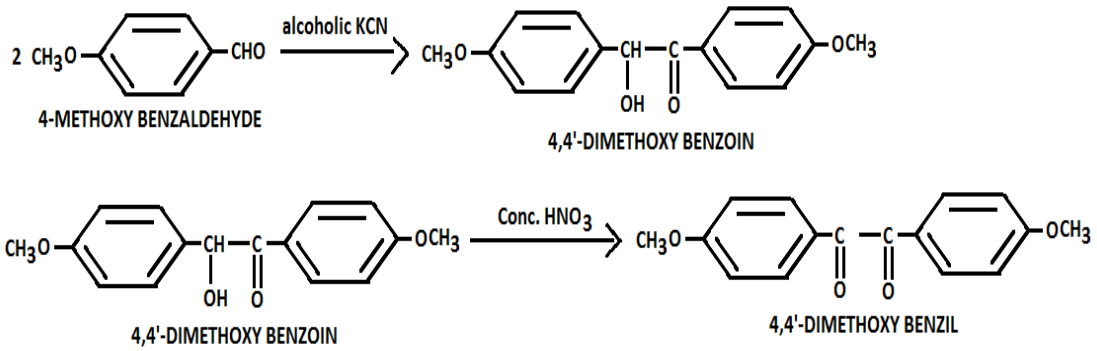
Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with overall minimum energy. With this information we can find out new drugs and also make new synthetic compounds and lead molecules with different mechanism of actions and thereby different target organisms especially against drug resistant bacteria and emerging microbes.

For the purpose, three different substituted benzil compounds have been synthesized, characterized and evaluated their capabilities in biological activity[10-19]. From the literature survey, it had been found that benzil has anti tumour activity[20].

SYNTHESIS OF THE COMPOUND 4,4'-DIMETHOXY BENZIL AND ITS IRON OXIDE NANO COMPOSITE:
The title compound was synthesized by benzoin condensation using 4g of KCN dissolved in 75cc of water in a one litre flask. To this was added 12g (0.05mole) of 4-methoxy benzaldeyde and 75cc of 95% ethanol. The mixture formed a solution at the boiling temperature and was refluxed for one and half hours. Steam was then passed through the solution until all the alcohol and nearly all the unchanged aldehyde were removed. The condensed water was decanted from the product and then the crude 4,4'-dimethoxy benzoin obtained is treated with 10ml concentrated nitric acid. It is then heated over in a water bath for one hour and latter

set aside for crystallization. The product was then pressed as free as possible from oily material on a suction funnel and washed with cold alcohol. In this way about 9.8g (yield: 70%) of crude product was obtained (scheme 1). Melting point of the compound was found to be 115.3ºC. The yield of pure 4,4'-dimethoxy benzil amounted to 50% of the expected product.



Scheme 1. Schematic representation of synthesis of 4,4'-dimethoxy benzil

The synthesized compound was purified and the melting point of the compound was found to be 100±1ºC. In order to improve the purity of the synthesized compound, the basic material was purified by recrystallisation from ethanol. This process was carried out repeatedly and the purity of the material was monitored by TLC and measuring its melting point each time.

The compound 4,4'-dimethoxy benzil is dissolved in the solvent ethanol and the iron oxide nano material is also dissolved with the solvent ethanol. It is the mixed together to get a homogenous solution and is then stirred well for 1 hour at 50^0c in a magnetic stirrer. It is then calcined at about 400^0c over night. The product obtained is 4,4'-dimethoxy benzil iron oxide composite.

MASS SPECTRAL ANALYSIS:

The results of mass spectral analysis (Table 1) confirm the molecular weight (Fig.1) and molecular formula of the compound.

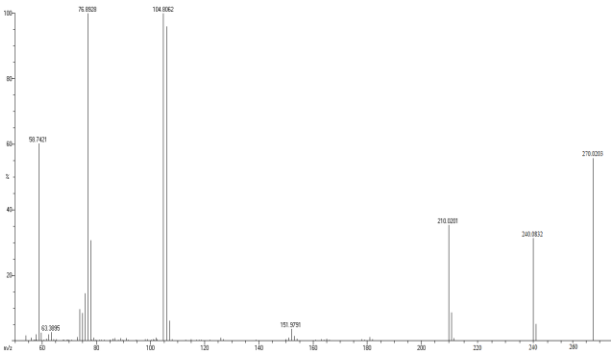


Figure.1 Mass spectra of 4,4'-dimethoxy benzil

Table 1 Mass spectral fragmentation peaks for 4,4'-dimethoxy benzil

Peaks	Fragmentation
270.0203	M^+
240.0532	$[M^+-OCH_3]$
210.0201	$[M^+-O_2C_2H_6]$
104.8062	$[M^+-CO-C_6H_5]$
76.8928	$[C_6 H_5]^+$
58.7421	$[CO-CO]^+$

FTIR SPECTRAL ANALYSIS:

The FTIR spectrum of the compound 4,4'-dimethoxy benzil was recorded in the frequency region 4000 cm^{-1} - 400 cm^{-1}. The FTIR spectrum of the title compound shows the presence of keto group with very strong intensity at 1642 cm^{-1} is attributed to the C=O stretching modes. The bands around 2918 cm^{-1} in FTIR are assigned to the $–OCH_3$ stretching modes. The aromatic C=C symmetric stretching vibrations appear at 1590 cm^{-1}.The in-plane deformation of C=O appears around 1321-1282cm^{-1} as a medium band in the FTIR. The C=O out of plane deformation is observed as strong bands around 1072cm^{-1} in FTIR. The band at 1014cm^{-1} and 1091 cm^{-1} in FTIR is assigned to the benzene ring deformation. The band at 761 cm^{-1} in FTIR established the presence of monosubstituted benzene ring (Fig.2a). The IR Spectral band at 606 cm^{-1} corresponds to the presence of iron oxide composite in fig. 2b.

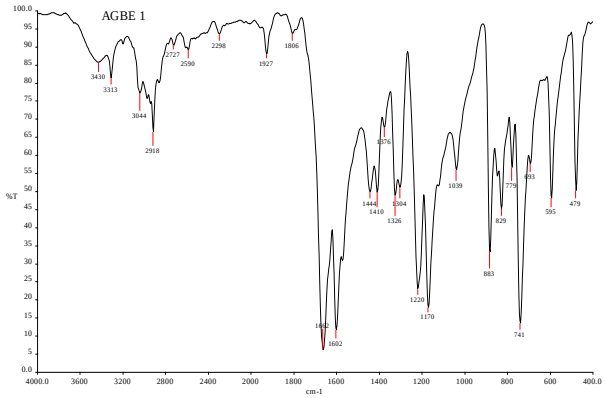


Figure 2a IR spectra of 4,4'-dimethoxy benzil in solid KBr

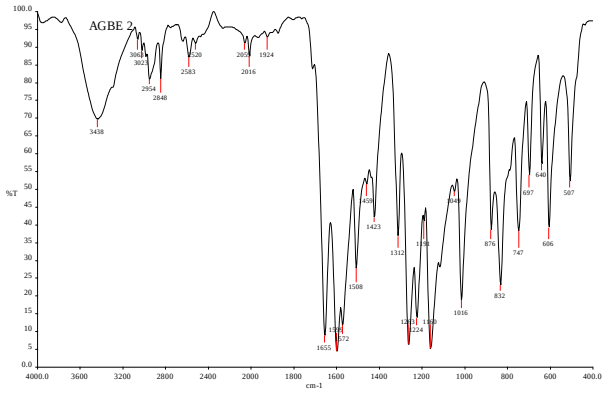


Figure 2b IR spectra of 4,4'-dimethoxy benzil iron oxide composite in solid KBr

HR SEM ANALYSIS:

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. Fig. 3a illustrates the SEM image for 4,4'-dimethoxy benzil and the fig.3b corresponds to SEM image of iron oxide doped with 4,4'-dimethoxy benzil.

Figure 3a SEM image of 4,4'-dimethoxy benzil

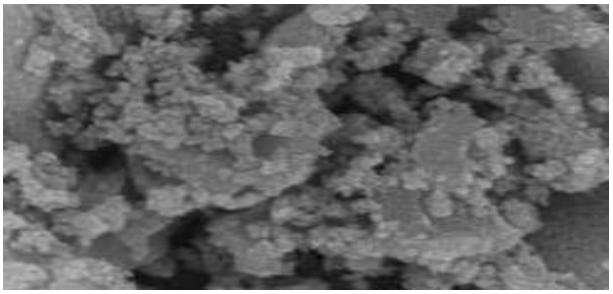


Figure 3b SEM image of 4,4'-dimethoxy benzil doped with iron oxide nano composite

IN VITRO BIOLOGICAL ACTIVITY OF 4,4'-DIMETHOXY BENZIL AND ITS IRON OXIDE NANO COMPOSITE

DISC DIFFUSION METHOD:

Antibacterial activity of the synthesized compounds were 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. 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).

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).

ANTIMICROBIAL STUDY:

The antimicrobial activity of 4,4'-dimethoxy benzil and its iron oxide composite were tested against three gram-positive, two gram-negative bacteria. It was observed that the compound 4,4'-dimethoxy benzil exhibits sufficient antimicrobial activity by showing maximum zone of inhibition (mm) at dose dependent manner. Compound 4,4'-dimethoxy benzil showed a maximum zone of inhibition of 12(mm) at 100 (µg) and 11 (mm) and 50 (µg) against staphylococcus aureus. In the case of gram negative bacterium, E. coli 4,4'-dimethoxy benzil showed a zone of inhibition of 10 mm at 100 (µg).

Compound 4,4'-dimethoxy benzil showed a highest zone of inhibition of 11 mm at 100 (µg), 9 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 also showed a zone of inhibition of 10 mm at 100 (µg). The pathogens Bacillus subtilis, Staphylococcus aeureus and E.coli (fig. 5a) showed higher antimicrobial activity for the compound 4,4'-dimethoxy benzil and the pathogen Bacillus subtilis, Staphylococcus aureus and Klebsiella pneumonia (fig. 5b) showed maximum activity for the compound 4,4'-dimethoxy benzil. The pathogens Bacillus subtilis, Staphylococcus epidermidis and Klebsiella pneumoniae were found to exhibit similar anti bacterial activity for the compound 4,4'-dimethoxy 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).

Table 2 Antimicrobial assay of substituted benzils by disc diffusion method.
ZONE OF INHIBITION (in mm)

Name of the pathogens	4,4'-dimethoxy benzil (µg)			4,4'-dimethoxy benzil iron oxide composite (µg)			Streptomycin
	25	50	100	25	50	100	
	ZONE OF INHIBITION (in mm)						
Bacillus subtilis	7	11	12	-	-	9	14
Staphylococcus aeureus	-	9	11	8	8	10	20
Staphylococcus epidermitis	_	_	-	_	_	9	20
E.coli	_	_	10	_	7	8	19
Klebsiellapneumoniae	_	8	10	_	-	9	20

DOCKING STUDIES:

Docking studies of synthesized compounds were performed using GLIDE 4.0 and IFD script from Schrödinger, LLC (New York) as our primary docking engine. The docking algorithm in GLIDE utilises a hierarchical search protocol. The scoring function, called GLIDE score, for computing binding affinity is an extension of an empirically based Chem-Score function of Eldridge *et al*. We employed OPLS-AA molecular mechanics potential energy function throughout our calculations. The extra precision mode of GLIDE, which has higher penalties for unfavourable and unphysical interactions, was used for docking. Computations were carried out on a Linux with Red hat 9.0 computer platform. The protein used for docking study is alpha tubulin. The pictures were taken using Pymol

Molecular docking revealed all the synthesized molecules showed good binding energy toward the target protein. The dock score for the compound 4,4'-dimethoxy benzil is high which is attributed to the dipole-dipole and hydrogen bond interaction with amino acids of targeted protein. It was observed that the most active compound having significant antibacterial activity are also found to have good docking activity. The acting force of this binding mode mainly depends on hydrogen bonding, vander Waals forces and hydrophobic interaction due to non-polar residue interaction. The docked molecule with protein structure is given in Fig 4.

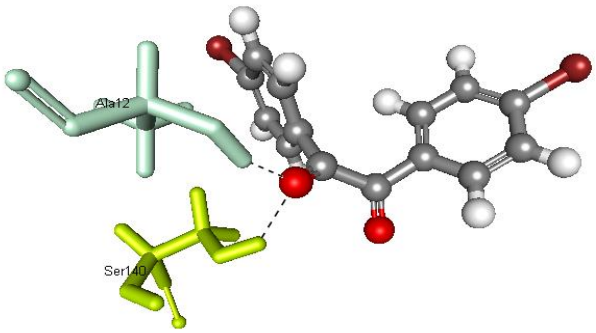


Figure 4 Docking activity of 4,4'-dimethoxy benzil

It was interesting to observe that even though the core structure of the compound 4,4'-dimethoxy benzil was same, the degree of interaction and binding site were found to be different. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

CONCLUSION:

It is observed that the methoxy substituted benzil are more potent anti-tumor agents compared with the iron oxide substituted compound. The antioxidant studies showed that the presence of electron donor substituents such as methoxy group at para position enhances the DPPH scavenging ability. The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial activities. These observations may promote a further development of our research in this field. Thus it was also concluded that the compound 4,4'-dibromo benzil iron oxide nano composite shows lower anti bacterial activity but the

compound with methoxy substituent exhibits anti bacterial activity for all pathogens. 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.

REFERENCES:

1. Foucault. C, Brouqui. P, FEMS Immunology and Medical Microbiology, 2007, 49, 173.
2. Neu. H.C, Science, 1992, 257, 1064.
3. Wise. R, Hart. T, Carsetal. O, British Medical Journal., 1998, 317, 609.
4. Travis. J, Reviving the antibiotics miracle?, Science, 1994, 264, 360–362.
5. Hata. K, Hata. J, Miki. HT, Toyosawa. T, Nakamura. T, Katsu. K, In-vitro and in-vivo antifungal activities of ER-30346, a novel oral triazole with a broad antifungal spectrum, Antimicrob. Agents Chemother., 1996, 40, 2237–2242.
6. Sugawara.T, Shibazaki.M, Nakahara.H, Suzuki.K, YM-47522, a novel antifungal antibiotic produced by Bacillus sp. II, Structure and relative stereochemistry. J. Antibiot., (Tokyo), 1996, 49, 345–348.
7. Moellering. R C, Vancomycin-Resistant Enterococci. Clin. Infect. Dis., 1998, 26, 1196–1199.
8. Davies. J, Inactivation of antibiotics and the dissemination of resistance genes. Science, 1994, 264, 375–382.
9. Thomson.J.M, Bonomo.R.A., The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril. Curr Opin Microbiol., 2005, 8, 518–524.
10. G Nithya, B Thanuja, G Chakkaravarthi, Charles C Kanagam, 2'-chloro-4-methoxy-3-nitro benzil, Acta crystallographica section E, 2011, E67, o1535.
11. G. Nithya, Charles C.Kanakam, R.Sudha, Synthesis, characterization, study of biological activity and molecular docking of benzil and its substituted analogs, International Journal of Chem.Tech Research, 2015, 8(6), 241-249.
12. R Sudha, Charles C Kanakam, G Nithya, Synthesis Characteraisation and Antimicrobial Activity of Substituted Benzilic Acids, International Journal of ChemTech Research, 2015, 8 (10), 383-387.
13. R. Sudha, Charles C Kanakam, G. Nithya, In vitro antioxidant activity of different substituted benzilic acid using 2,2-Diphenyl-1-Picryl Hydrazyl radical, ABTS assay method, Asian Journal of Pharmaceutical and Clinical Research, 2016, 9(3), 127-130.
14. R. Sudha, Brindha devi, Charles C Kanakam and G.Nithya, Docking studies for various anti bacterial benzilate derivatives, Asian Journal of Pharmaceutical and Clinical Research, 2017, 10 (4), 268-271.
15. R. Sudha, Charles C Kanakam, G. Nithya and Brindha devi, Docking anti oxidant activity on hydroxyl (diphenyl) acetic acid and its derivatives, Asian Journal of Pharmaceutical and clinical Research, 2017, 10 (7), 263-265.
16. G. Nithya, Charles C Kanakam and R.Sudha, Polymorphic behaviour of an organic compound using a dynamic thermal and x-ray diffraction technique 2'Chloro-4-methoxy-3-nitro benzyl, Asian Journal of Pharmaceutical and Clinical Research, 2017, 10 (4), 259-262.
17. G. Nithya, R.Sudha, Charles C Kanakam, Study of polymorphic behavior of 2,2'-dichloro benzil using dynamic thermal and X-ray diffraction technique, Asian Journal of Chemistry, 2017, 29, 2335-2339.
18. G Nithya, R Sudha, P Brindha Devi, Charles C Kanakam, In silico studies for various antibacterial benzil and its substituted analogs, Asian Journal of Pharmaceutical and Clinical Research, 2017, 10 (12), 1-4.
19. G Nithya, Charles C Kanakam, R Sudha, V Vanitha, Synthesis, Characterization and Antimicrobial Activity of Benzil and its Substituted Analogs, International Journal of Chemical and Biomedical Science, 2015, 1(2), 25-37.
20. Schulz. H.; Jorgensen. B., Annu Rev Microbiol, 2001, 55, 105.