International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.6, pp 241-249, 2015

Synthesis, Characterization, Study of Biological Activity and Molecular Docking of Benzil and its Substituted Analogs.

Nithya.G[1]*, Charles C.Kanakam[2], Sudha.R[1].

[1]Department of Chemistry, School of Engineering, Vels University, Pallavaram, Chennai-600117, Tamil Nadu, India.
[2]Depatment of Chemistry, Formerly Presidency College, University of Madras, Tamil Nadu, India.

Abstract: A series of benzil compounds have been synthesized by oxidation of corresponding benzoins which in turn was prepared from respective aldehydes. Using this protocol, four new benzils were prepared in good to excellent yields and their biological activity has been delineated. Molecular docking studies were conducted in order to validate the obtained pharmacological data and to provide understandable evidence for the observed antimicrobial activity of all synthesized compounds. Several benzils exhibited excellent antimicrobial and cytotoxic activity. In order to determine the cytotoxic effects we used an MTT viability assay. The results showed that cell growth is significantly lower in extract treated cells compared to untreated control. The effect of inhibition of cell growth was shown in different concentration dosages for cytotoxic, anti bacterial and anti oxidant activity *in vitro*. The anti oxidant activity was also performed for the compound benzil and its substituted analogs.
Key Words : Synthesis, Molecular docking, antimicrobial, antioxidant, anticancer.

1. Introduction

Treatment of cancer and infectious diseases faces serious difficulties due to the development of resistance to current anticancer/antibiotic drugs. Therefore the discovery and development of new anticancer/antibiotic agents is a high priority in biomedical research. In the human body cancer is a multi-step process that often involves the inactivation of tumor suppressor genes or activation of oncogenes[1], caused by many factors. Other than genetic mutation, different chemical species which interfere with the enzyme's structure or activity are also responsible for cancer. Reactive oxygen species (ROS), produced as by-products of metabolic reactions in living organisms which initiate toxic oxidative reactions in biomolecules, causes oxidative stress. A state of oxidative stress has deleterious effects on almost all tissues and can initiate or enhance the rate of pathological conditions such as neurodegeneration, inflammation, aging process, cancer and cardiovascular diseases[2–4].

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[5-7]. Accordingly, the development of new antibacterial agents that could overcome the resistance problem has become the subject of an ongoing research[8-13]. The ever growing resistance to antibiotics leads to continuous screening for new biologically effective compounds of either natural or synthetic origin.

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.

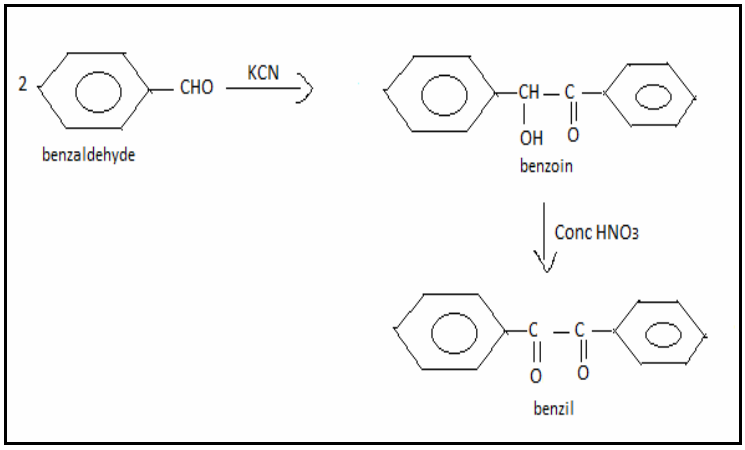
For the purpose, four different substituted benzil compounds have been synthesized, characterized and evaluated their capabilities in biological activity. From the literature survey, it had been found that benzil has anti tumour activity[14]. The variation in the substituent and composition of the benzil reveals that it has been proposed to analyse the anti oxidant and anti microbial activity with different vitro models. In this work, we report on the synthesis of polysubstituted benzils and on the biological activities of these compounds.

2. Materials and Methods

Synthesis of Different Substituted Benzils

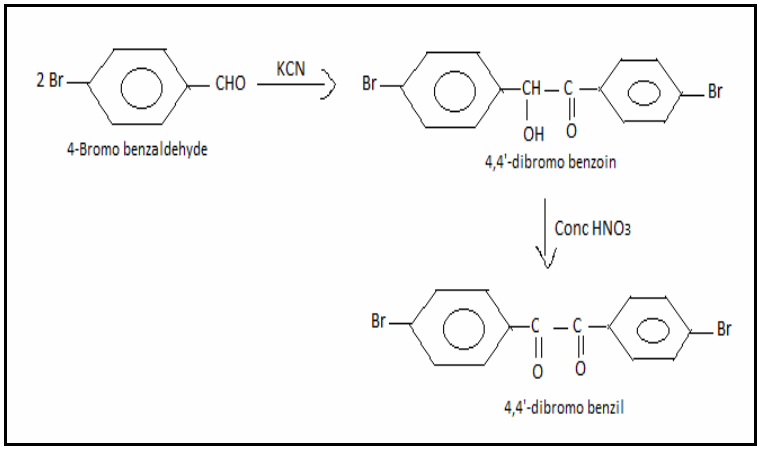
A) Benzil

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.



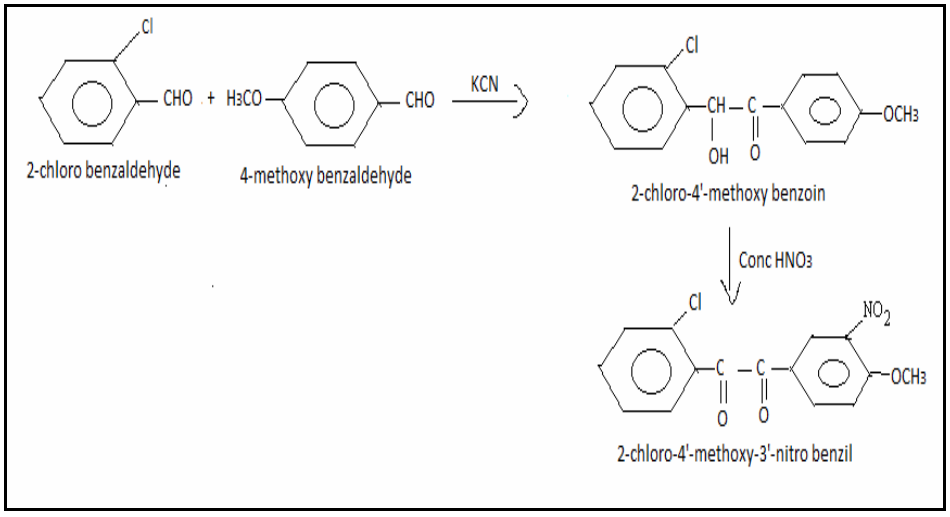
B) 4,4'-Dibromo Benzil

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.



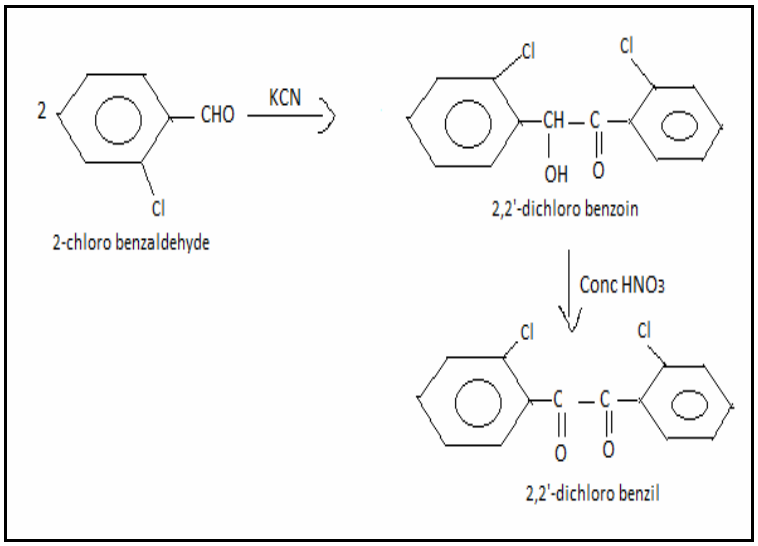
C) 2'-Chloro-4-Methoxy-3-Nitro Benzil

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 distiilation for 1 hour. The crude 2,2'-dichloro benzoin obtained is then refluxed with con. HNO_3 for 1 hour. The produt 2,2'-dichloro benzil is obtained with the melting point 80.3°c.



3. 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.

3.1 Molecular Docking Studies

Molecular docking studies were conducted in order to validate the obtained pharmacological data and to provide understandable evidence for the observed antimicrobial activity of all synthesized compounds. 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. All the synthesized compounds were docked. The ligand molecules were drawn and analysed using Chem Draw Ultra 8.0. 3D, coordinates were prepared using dock server.

***IN VITRO* Cytotoxic Activity, Antioxidant Activity and Antimicrobial Activity**

3.2 Mtt Assay for Cell Viability

The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO_2[15]. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the compound (25, 50, 75 µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a micro titre plate reader. DMSO and Cyclophosphamide used as a negative and positive control.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

3.3 DPPH Free Radical Scavenging Assay

The ability of the extracts to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Blois (1958). Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM)[16]. The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic Acid was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula

% of Inhibition = (A of control – A of Test)/A of control * 100

Cytotoxicity % = 100 – Viability%

3.4 Disc Diffusion Method

Antibacterial activity of the syntheised benzil, Compound 1 (N1) Benzil, Compound 2 (N2) 4,4'-Dibromo Benzil, Compound 3 (N3) 2,2'-dichloro benzil and Compound 4 (N4) 2'-chloro-4-methoxy-3-nitro 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. 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).

4. Result and Discussion

4.1. Synthesis and Characterization

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 method discussed. The identities of the products were established by mass spectral analysis, IR spectroscopy (Table 1). The agreement between experimental and calculated values for elemental analysis confirmed the successful synthesis and purity of desired compounds. Compounds (3) 2'chloro-4-methoxy-3-nitro benzil and (4) 2,2'-dichloro benzyl

were found to exhibit polymorphism by XRD and thermal studies. CCDC 817058 and 879618 contain the supplementary crystallographic data for compound (3) 2'-chloro-4-methoxy-3-nitro benzil and CCDC 879851 and 915833 contain the supplementary crystallographic data for polymorphic behavior of compound (4) 2,2'-dichloro-benzil respectively. These data can be obtained via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033.

Spectral Analysis

Table 1: IR Spectral Data for Compounds

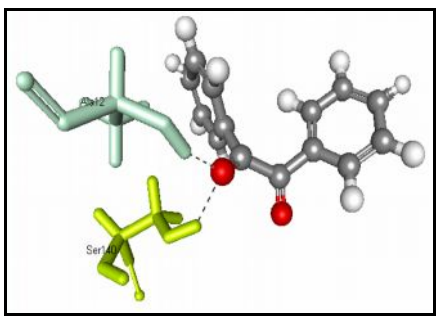
FTIR Stretching frequency for the group using KBr	Compound 1	Compound 2	Compound 3	Compound 4
-C=O stretching	1688 cm^{-1}, 1612	1599 cm^{-1},1688	1609 cm^{-1}	1584 cm^{-1},1684 cm^{-1}
$-NO_2$ stretching	-	-	1536 cm^{-1}	-
Aromatic C-H	2980 cm^{-1}	2982 cm^{-1}	3092 cm^{-1}	3086 cm^{-1}
Aliphatic C-H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

Mass Spectral Data for Different Benzils

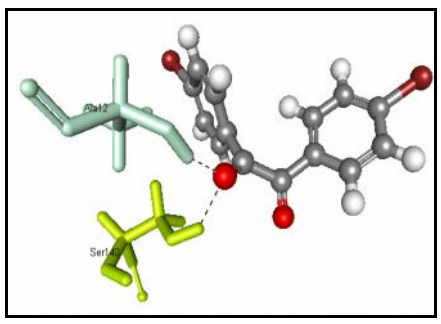
The molecular mass of the compound is identified by mass spectral analysis and it was found to be m/e value 210 for compound 1, m/e 366 for compound 2, m/e 319 for compound 3 and m/e value 279 for compound 4. From the mass spectral data the existence of the compound could be confirmed.

4.2 Molecular Docking Activity

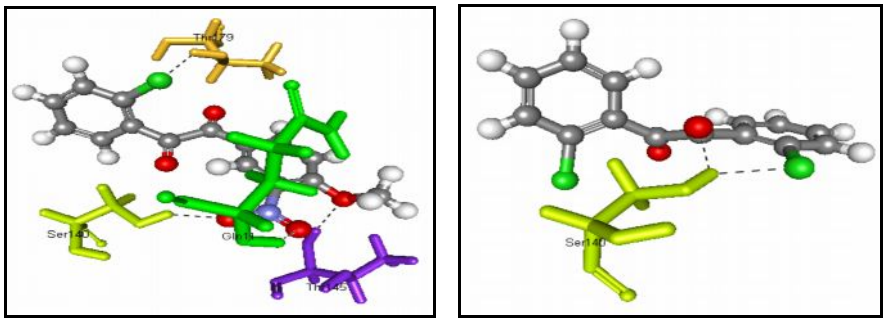
Molecular docking revealed all the synthesized molecules showed good binding energy toward the target protein. The dock score for the compound 2'-chloro-4-methoxy-3-nitro benzil is high which is attributed to the dipole-dipole and hydrogen bond interaction with amino acids of targeted protein. It was observed that the most active compound of the series, i.e., compound (3) was predicted to be most active too. The other compounds like (1), (2) and (4) 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 1 and the hydrogen bonding is also presented in table 2.



(1)



(2)



(3) **(4)**

Fig. 1 Moleclar Docking For Benzil, 4,4'-Dibromo Benzil, 2'-Chloro-4-Methoxy-3-Nitro Benzil And 2,2'-Dichloro Benzil

Table 2. Hydrogen Bonding for Molecular Docking

Compound	Lib Dock Score	No. of H bonds	H bonds	H bond distance
1	81.3232	2	ALA 12, SER 140	2.05, 1.75
2	80.2121	2	ALA 12, SER 140	2.08, 1.78
3	103.463	4	GLN 11, SER 140, THR 145, THR 179	2.27, 2.00, 2.22, 2.49
4	82.3993	1	SER 140(2)	1.93,2.43

4.3 Cytotoxic Activity

The results indicated that all of the compounds have significant cytotoxic activity. Percentage of viability and cytotoxic activity in MCF – 7 cancer cells had been performed. All the compounds had shown the appreciably highest activity, 80% compared to the standard cyclophosphamide taken as 100% (Table 3). It is observed that the chloro substituted are more potent anti-tumor agents compared with alkyl substituted compounds but the presence of an alkyl group on the phenyl ring enhances the activity (Fig. 2).

Table 3 % of Viability and Cytotoxic Activity

Test	compound 1(µg)			compound 2(µg)			compound 3(µg)			compound 4(µg)			PC	C
	25	50	75	25	50	75	25	50	75	25	50	75		
% of viability	56.31	48.68	41.48	64.34	52.45	43.05	48.28	37.24	36.08	60.09	47.81	38.40	73.22	100
% of cytotoxicity	43.68	51.31	58.51	35.65	47.54	56.94	51.71	62.75	63.91	39.90	52.18	61.59	26.77	0

PC- Positive control (Cyclophosphamide); C- Control

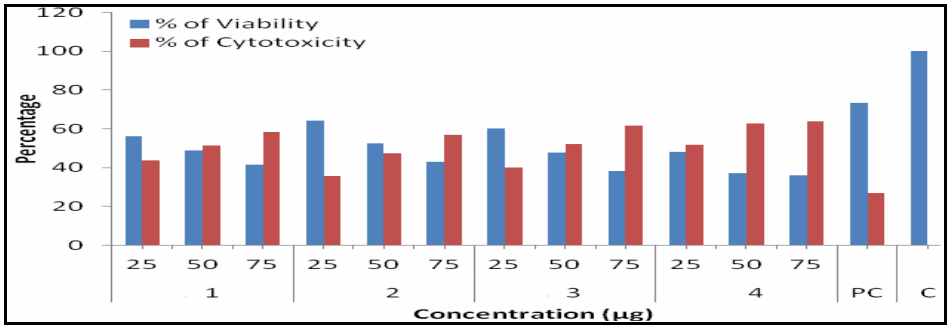


Figure 2 Effect of Cell Viability and Cytotoxicity of Compound in Mcf-7 Cancer Cells; C- Control; Pc- Positive Control (Cyclophosphamide)

4.4 Anti Oxidant Activity

The anti-oxidant behavior of the synthesized compounds was investigated and the percent scavenging of DPPH is shown in Figure 3. The results indicate that the scavenging of DPPH by the tested compounds is time dependent and a relatively slow process. It is also observed that scavenging of DPPH by different benzils can be correlated to the substituent attached at N′ position. Generally the presence of electron donor substituent such as alkyl group enhances the antioxidant property while electron withdrawing group suppresses the DPPH scavenging ability. Among the different benzil substituents, the scavenging ability is remarkably improved in the presence of the unsubstituted benzil and electron donor group on the phenyl ring.

Table 4 Anti Oxidant Behavior by Dpph Assay

Test	compound 1(µg)			compound 2(µg)			compound 3(µg)			compound 4(µg)		
	20	60	100	20	60	100	20	60	100	20	60	100
Sample	17.33	59.72	82.58	13.47	16.97	37.36	12.81	17.83	33.94	38.04	41.03	42.12
Ascorbic acid	23.70	55.37	81.28	9.06	44.78	74.16	9.06	44.78	74.16	9.06	44.78	74.16

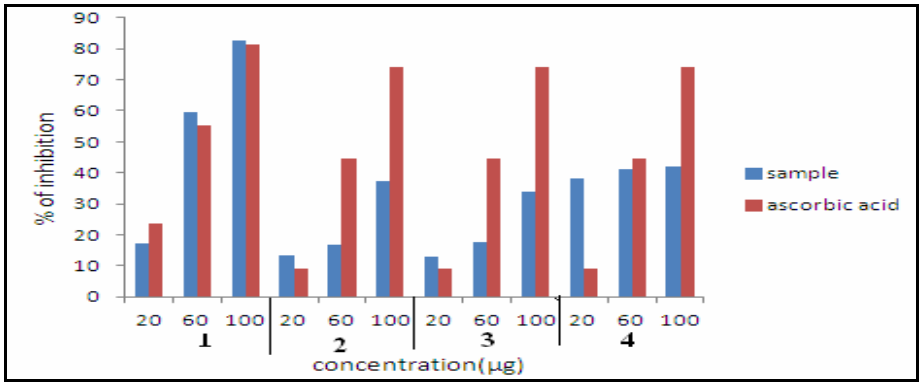


Fig 3 Antioxdant Activitity for the Compound

4.5 Anti Microbial Activity

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. Compound (3) 2,2'-dichloro 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) 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 2,2'-dichloro benzil. The pathogens Bacillus subtilis, Staphylococcus epidermidis and Klebsiella pneumoniae were found to exhibit similar anti bacterial activity for the compound 2'-chloro-4-methoxy-3-nitro 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 5).

Table 5 Antimicrobial Assay of Substituted Benzils by Disc Diffusion Method. Zone of Inhibition (in mm)

Name of the pathogens	compound 1(µg)			compound 2(µg)			compound 3(µg)			compound 4(µg)			Streptomycin
	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

Conclusion

The chloro substituted benzils are more potent anti-tumor agents compared with the alkyl substituted compounds. The antioxidant studies showed that the presence of electron donor substituents such as alkyl group at N 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 (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. 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. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial infection. 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.

Acknowledgement

The author acknowledges Vels University for providing the lab facilities and for giving the opportunity to do the research work. The author also acknowledges the SAIF, IIT Madras for doing characterization studies.

References

1. Spannhoff. A, Sippl. W. and Jung, M., Cancer treatment of the future: Inhibitors of histone methyltransferases., Int. J. Biochem. Cell Biol., 2009, 41, 4–11.
2. Finkel. T, Holbrook. N.J, Oxidants, oxidative stress and the biology of ageing. Nature, 2000, 408, 239–247.
3. Kang. T.S, Jo. H.O, Park. W.K, Kim.J.P, Konishi. Y, Kong. J.Y, Park. N.S, Jung.Y.S,Synthesis and antioxidant activities of 3,5-dialkoxy-4-hydroxycinnamamides, Bioorg. Med.Chem. Lett., 2008, 18, 1663–1667.
4. Noguchi. N, Novel insights into the molecular mechanisms of the antiatherosclerotic properties of antioxidants: the alternatives to radical scavenging, Free Radical Biol. Med., 2002, 33, 1480–1489.
5. Foucault.C, Brouqui.P, FEMS Immunology and Medical Microbiology, 2007, 49, 173.
6. Neu. H.C, Science, 1992, 257, 1064.
7. Wise.R, Hart.T, Carsetal.O, British Medical Journal., 1998, 317, 609.
8. Travis. J, Reviving the antibiotics miracle?, Science, 1994, 264, 360–362.
9. 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.
10. 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.

11. Moellering.R C, Vancomycin-Resistant Enterococci. Clin. Infect. Dis., 1998, 26, 1196–1199.
12. Davies. J, Inactivation of antibiotics and the dissemination of resistance genes.Science, 1994, 264, 375–382.
13. 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.
14. Schulz.H.; Jorgensen.B., Annu Rev Microbiol, 2001, 55, 105.
15. Mossman.T., Rapid colorimetric assay for cellular growth and survival – application to proliferation and cytotoxicity assays., J.Immunol.Methods, 1983, 65, 55-63.
16. Blois.MS, Antioxidant determinations by the use of a stable free radical, Nature, 1958, 29, 1199-1200.

*****