

BIOLOGICAL ACTIVITY AND MOLECULAR DOCKING OF 2'-BROMO-4-METHOXY-3-NITRO BENZIL, 2,2'-DIBROMO BENZIL, AND 4,4'-DICHLORO BENZIL

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ABSTRACT

Objective: A series of benzil compounds have been synthesized by oxidation of corresponding benzoin which in turn were prepared from respective aldehydes. Using this protocol, three new benzils were prepared in good-to-excellent yields and their biological activity has been delineated.

Methods: Molecular docking studies were conducted 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. To determine the cytotoxic effects, we used an MTT viability assay.

Results: 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, antibacterial, and antioxidant activity *in vitro*.

Discussion: 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. The antioxidant activity was also performed for the compound benzil and its substituted analogs.

Keywords: Molecular docking, Antimicrobial, Antioxidant, Anticancer.

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INTRODUCTION

Treatment of cancer and infectious diseases faces serious difficulties due to the development of resistance to current anticancer/antibiotic drugs. In biomedical field, the invention and development of new anticancer/antibiotic agents are of great demand. The inactivation of tumor suppressor genes or activation of oncogenes [1] in the human body cancer is a multistep process which was caused due to a large number of factors. More than genetic mutation, the other factors responsible for cancer are the different chemical species which interfere with the enzyme's structure or activity. The oxidative stress is caused by reactive oxygen species, which acts as a by-product of metabolic reactions in living organisms and also initiates toxic oxidative biomolecular reactions. 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].

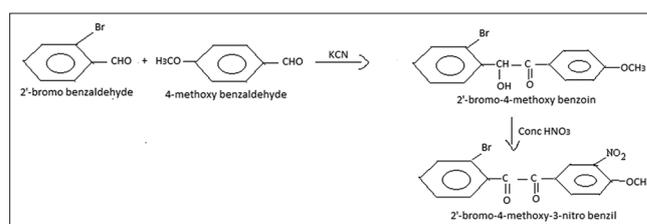
The majority of public health issues across the world is mainly due to the emergence and spread of antimicrobial resistance. As bacteria develop resistance to antimicrobial agents, the search for new antimicrobial compounds has become a challenging task, even though infections due to such bacterial strains are infrequent although potentially fatal [5-7]. The ongoing research has also focused on the development of new antibacterial agents that could overcome the resistance problem [8-13]. The new biologically active compounds of natural or synthetic origin lead to continuous screening for new biologically effective compounds.

To determine the interaction of two molecules and also to know the best orientation of ligand that forms a complex with overall minimum energy, molecular docking study is a well-established technique. By this technique, it is also possible to find a new drug and also prepare new synthetic compounds and lead molecules with different mechanisms 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 [14-23]. From the literature survey, it had been found that benzil has antitumor activity [24]. The variation in the substituent and composition of the benzil reveals that it has been proposed to analyze the antioxidant and antimicrobial activity with different *in vitro* models. In this work, we report on the synthesis of polysubstituted benzils and on the biological activities of these compounds.

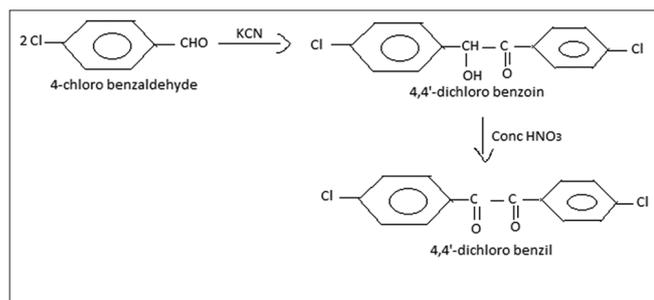
METHODS**Synthesis of different substituted benzils***2'-bromo-4-methoxy-3-nitro benzil*

2'-bromo-4-methoxy benzoin is prepared by treating 4-methoxy benzaldehyde with 2-bromo 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'-bromo-4-methoxy-3-nitro benzil was found to have a melting point at 140°C.

*4,4'-dichloro benzil*

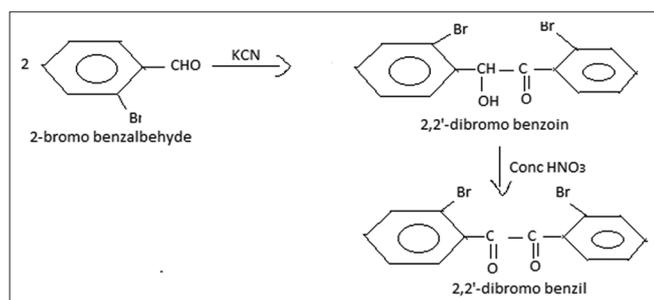
The compound 4,4'-dichloro benzil is prepared from two moles of 4-chloro benzaldehyde in ethanol with KCN as catalyst. The 4,4'-dichloro benzoin is obtained by steam distillation process and the

crude 4,4'-dichloro benzoin obtained is then refluxed with concentrated HNO_3 for 1 h. It melts at 192°C .



2,2'-dibromo benzil

The compound 2,2'-dibromo benzil is prepared from two moles of 2-bromo benzaldehyde with ethanol in the presence of a catalyst KCN. The 2,2'-dibromo benzoin is obtained after refluxing and subjected to steam distillation for 1 h. The crude 2,2'-dibromo benzoin obtained is then refluxed with concentrated HNO_3 for 1 h. The product 2,2'-dibromo benzil is obtained with the melting point of 153°C .



EXPERIMENTAL

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

Molecular docking studies

Molecular docking studies were conducted 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 orientation of ligands forming a complex with minimum energy. All the synthesized compounds were docked. The ligand molecules were drawn and analyzed using Chem Draw Ultra 8.0. 3D, and coordinates were prepared using dock server.

In vitro cytotoxic activity, antioxidant activity, and antimicrobial activity

MTT assay for cell viability

The MTT assay (Mossmann, 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 and supplemented with 10% fetal bovine serum, at 37°C in humidified atmosphere with 5% CO_2 [25]. The cells were plated in 96-well flat bottom tissue culture plates at a density of approximately 1.2×10^4 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, and 75 μg) for 24 h. After the incubation, medium was discarded and 100 μl fresh medium was added with 10 μl of MTT (5 mg/ml). After 4 h, the medium was discarded and 100 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtiter plate reader. DMSO and cyclophosphamide were used as a negative and positive control (PC).

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD}/\text{Control OD}) \times 100$$

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

The ability of the extracts to annihilate the DPPH radical was investigated by the method described by Blois (1958). Stock solution of leaf extracts was prepared to the concentration of 1 mg/ml. 100 μg of each extracts was added, at an equal volume, to methanolic solution of DPPH (0.1 mM) [26]. The reaction mixture is incubated for 30 min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for 3 times. Ascorbic acid was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula:

$$\% \text{ of inhibition} = (\text{A of control} - \text{A of Test})/\text{A of control} * 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability \%}$$

Disc diffusion method

Antibacterial activity of the synthesised benzil, compound 1 (N1) 2'-bromo-4-methoxy-3-nitro benzil compound 2 (N2) 4,4'-dichloro benzil, and compound 3 (N3) 2,2'-dibromo benzil was investigated using disc diffusion method (Murray et al., 1995). Petri plates were prepared with 20 ml of sterile MHA (HiMedia, Mumbai). The test culture (100 μl of suspension containing 108 CFU/ml bacteria) was swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations of the compounds (25, 50, and 100 $\mu\text{g}/\text{disc}$) were loaded on a sterile disc and placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Streptomycin (10 $\mu\text{g}/\text{disc}$) was used as a PC. These plates were incubated for 24 h at 37°C . Zone of inhibition was recorded in millimeters (mm).

Microorganisms 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), and *Staphylococcus epidermidis* (MTCC 3615) and the two Gram-negative bacteria studied were *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC15380).

RESULT AND DISCUSSION

Synthesis and characterization

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

Spectral analysis

The presence of compound has been confirmed by spectral analysis like IR, UV, NMR and mass spectral datas.

Molecular docking activity

Molecular docking revealed that all the synthesized molecules showed good binding energy toward the target protein. The dock score for the compound 2'-bromo-4-methoxy-3-nitro benzil is high which is attributed to the dipole-dipole and hydrogen bond interaction with amino acids of targeted protein [27,28]. It was observed that the most active compound of the series, i.e., compound (1) was predicted to be most active too. The other compounds such as (2) and (3) having significant antibacterial activity are also found to have good docking activity. The acting force of this binding mode mainly depends on hydrogen bonding, Van der 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.

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 benzils exhibit more potent antitumour activity. but the presence of an alkyl group on the phenyl ring enhances the activity (Fig. 2).

Antioxidant activity

The antioxidant behavior of the synthesized compounds was investigated and the percentage scavenging of DPPH is shown in Fig. 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. In general, 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 substituted benzil and electron donor group on the phenyl ring (Table 4).

Antimicrobial activity

The antimicrobial activity of four different substituted benzils was tested against three Gram-positive and two Gram-negative bacteria. It was observed that the compound (1) 2'-bromo-4-methoxy-3-

nitro benzil and compound (3) 2,2'-dibromo benzil exhibit sufficient antimicrobial activity by showing a maximum zone of inhibition (mm) at dose-dependent manner. Compound (3) 2,2'-dibromo benzil showed a maximum zone of inhibition of 11 mm at 100 µg and 8 mm at 25 and 50 µg against *S. aureus*. In the case of Gram-negative bacterium, *E. coli* compound 3 showed a zone of inhibition of 10 mm at 100 µg. Compound (1) showed a highest zone of inhibition of 12 mm at 100 µg and 11 mm at 50 µg against *B. subtilis* when compared with standard streptomycin which showed a zone inhibition of 14 mm. In the case of Gram-negative bacterium, *K. pneumoniae* compound 1 also showed a zone of inhibition of 10 mm at 100 µg. The pathogens *B. subtilis*, *S. aureus*, and *E. coli* showed higher antimicrobial activity for the compound 3, and the pathogen *B. subtilis*, *S. aureus*, and *K. pneumoniae* showed the maximum activity for the compound. The pathogens *B. subtilis*, *S. epidermidis*, and *K. pneumoniae* were found to exhibit similar antibacterial activity for the compound 2'-bromo-4-methoxy-3-nitro benzil. The activity of benzyl substituted compounds against various pathogens is mainly in a dose-dependent manner by increasing the dose from 25, 50, and 100 µg the activity also increases (Table 5).

CONCLUSION

The bromo substituted benzils are more potent antitumor 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

Table 1: IR spectral data for compounds

FTIR stretching frequency for the group using KBr	Compound 1	Compound 2	Compound 3
-C=O stretching	1609/cm, 1673/cm	1599/cm, 1688/cm	1584/cm, 1684/cm
-NO ₂ stretching	1536/cm	-	-
Aromatic C-H stretching	3092/cm	2982/cm	3086/cm
Aliphatic C-H stretching	2922/cm	2838/cm	2924/cm
Aromatic sym C=C stretching	1589/cm, 1609/cm	1574/cm	1562/cm
Presence of benzene ring deformation	1069/cm, 1094/cm	1092/cm, 1110/cm	1068/cm, 1085/cm
Substituted benzene ring deformation	964/cm	926/cm	956/cm

FTIR: Fourier-transform infrared

Table 2: Hydrogen bonding for molecular docking

Compound	Lib Dock score	Number 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

Table 3: Percentage of viability and cytotoxic activity

Test	Compound 1 (µg)			Compound 2 (µg)			Compound 3 (µg)			PC	C
	25	50	75	25	50	75	25	50	75		
Percentage of viability	60.09	47.81	38.40	64.34	52.45	43.05	48.28	37.24	36.08	73.22	100
Percentage of cytotoxicity	39.90	52.18	61.59	35.65	47.54	56.94	51.71	62.75	63.91	26.77	0

PC: Positive control (cyclophosphamide); C: Control

Table 4: Antioxidant behavior by DPPH assay

Test	Compound 1 (µg)			Compound 2 (µg)			Compound 3 (µg)		
	20	60	100	20	60	100	20	60	100
Sample	38.04	41.03	42.12	13.47	16.97	37.36	12.81	17.83	33.94
Ascorbic acid	9.06	44.78	74.16	9.06	44.78	74.16	9.06	44.78	74.16

DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 5: Antimicrobial assay of substituted benzils by disc diffusion method

Name of the pathogens	Zone of inhibition (in mm)									
	Compound 1 (μg)			Compound 2 (μg)			Compound 3 (μg)			Streptomycin
	25	50	100	25	50	100	25	50	100	
<i>B. subtilis</i>	25	50	100	-	-	-	-	-	9	14
<i>S. aureus</i>	7	11	12	-	-	-	8	8	11	20
<i>Staphylococcus epidermidis</i>	-	9	10	-	-	-	-	-	9	20
<i>E. coli</i>	-	-	-	-	-	-	-	-	10	19
<i>K. pneumoniae</i>	-	8	9	-	-	-	-	-	9	20

B. subtilis: Bacillus subtilis, *S. aureus*: Staphylococcus aureus, *E. coli*: Escherichia coli, *K. pneumoniae*: Klebsiella pneumoniae

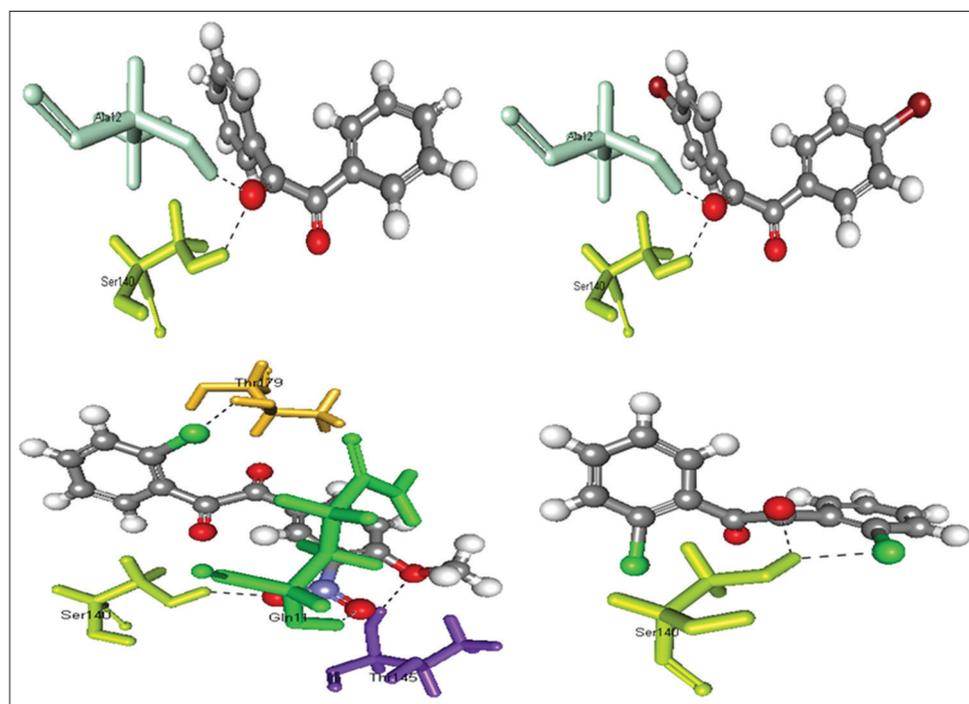


Fig. 1: Molecular docking for 2'-bromo-4-methoxy-3-nitro benzil, 4,4'-dichloro benzil, and 2,2'-dibromo benzil

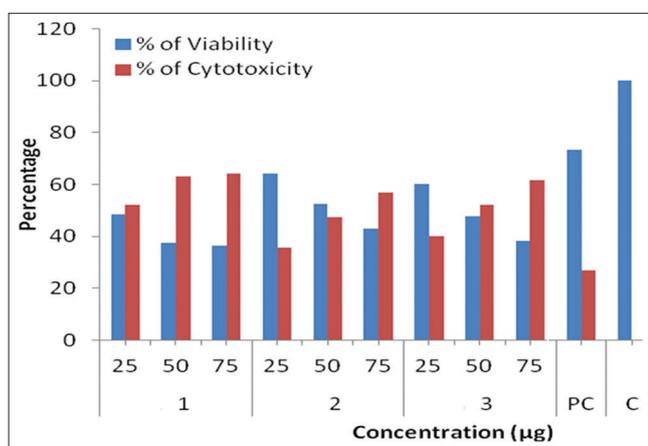


Fig. 2: Effect of cell viability and cytotoxicity of compound in MCF-7 cancer cells; C- control; PC- positive control (cyclophosphamide)

the compound (1) and compound (2) with chloro substituent exhibit antibacterial activity for all pathogens. The activity of the compounds was found to be dose dependent, i.e., 100 $\mu\text{g}/\text{mL}$ which showed greater inhibition. The susceptibility of the microbes to the compound was compared with standard antibiotic streptomycin. The thermal stability of the synthesized compounds is comparable

to the standard. It can be concluded that this class of compounds certainly holds great promise toward 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.

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AUTHORS CONTRIBUTION

Our research concept was thought of all authors, first two authors were conducted the research, whereas the third author has guided. The first author has drafted the manuscript, whereas the other authors have reviewed. Hence, all authors are equally contributed for this research.

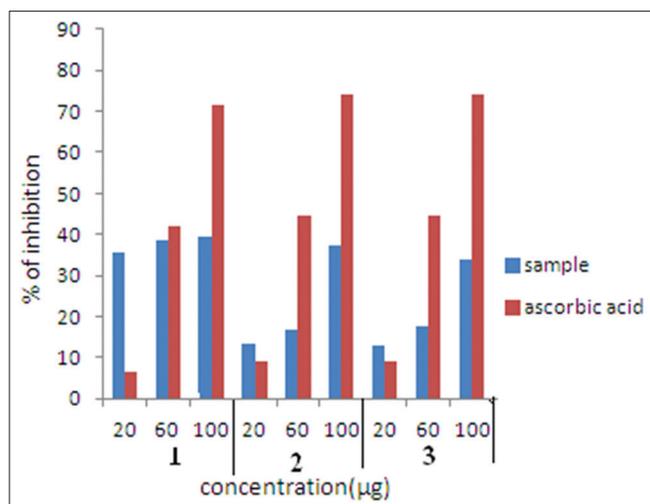


Fig. 3: Antioxidant activity for the compound

CONFLICTS OF INTEREST

There is no conflict of interest in the publication of this research paper.

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