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Research Article

MOLECULAR DOCKING STUDIES AND BIOACTIVITY OF VARIOUS BENZILIC ACIDS AND ITS ANALOGS

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ABSTRACT

Objective: Various benzilic acids and its analogs have been synthesized using the protocol, obtain good to exceptional yield and their biological activity, and its docking studies have been discussed.

Methods: Molecular docking studies were performed by discovery studio - LibDock docking program. 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*.

Conclusion: From the antibacterial results prove that the synthesized compounds showed the potential activity. These remarks may give the encouragement of further development of our research in this field. The antioxidant activity was also performed for the compound benzilic acid and its substituted analogs.

Keywords: Synthesis, Molecular docking, Antimicrobial, Antioxidant, Anticancer.

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INTRODUCTION

The benzilic acids and its analog have been found to exhibit a wide variety of biological activity. There is a serious of difficulties obtained in the treatment of cancer and infectious diseases due to the development of resistance to current anticancer/antibiotic drugs. There should be a good revolution in the biomedical research to discover and development of new anticancer/antibiotic agents with prior intense. In the human body due to the activation of oncogenes [1] or inactivation of tumor suppressor genes involving in multistep process. The numerous molecular studies show that functional relationship between polyunsaturated fatty acid metabolism, inflammation, and carcinogenesis has revealing potential on novel targets like arachidonic acid metabolizing enzymes such as cyclooxygenases and lipoxygenases [2,3].

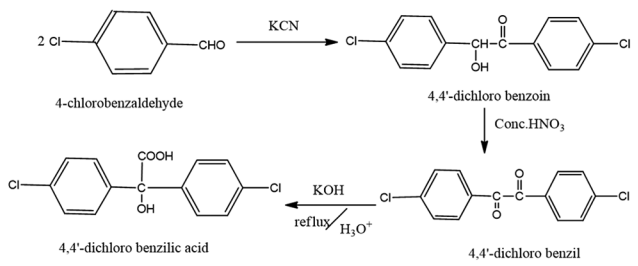
There will be an adverse gene mutation and post-translational modifications of key cancer-related proteins concerned in the regulation of cellular proliferation, apoptosis, differentiation, and senescence [4-6] due to the formation by these enzymes of various lipid peroxides and bioactive lipids, free radicals, and aldehydes. Across the world, the antimicrobial resistance becomes most serious public health concern. Hence, the search and developing new antibacterial drug becomes the challenging task; however, such bacterial strains cause infections are infrequent and serious [7-9]. Ongoing research [10-15] shows that the development of new antibacterial agents that could overcome the resistance problem. The interaction of two molecules could be determined by a well-established technique like molecular docking [16]. Using this study, the best orientation of ligand forms a complex with overall minimum energy would be found out. The new drugs can be synthesized and find out molecules with different mechanism of actions, with this information and thereby different target organisms, especially against drug-resistant bacteria and emerging microbes developed. Different substituted benzilic acids and its analogs have been synthesized, characterized, and evaluated their capabilities in biological activity [17-20]. The different derivatives of benzilic acid prove that it has been anticipated and to analyze the antioxidant and antimicrobial activity with different vitro models [21].

METHODS

Synthesis of different substituted benzilic acids and its analogs

4,4'-dichlorobenzilic acid

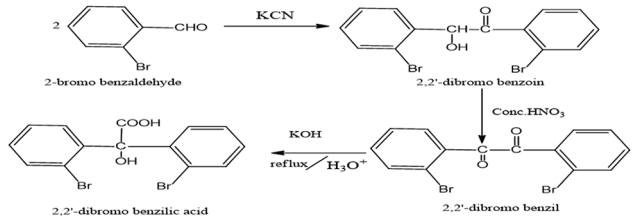
To 1.0 g of 4,4'-dichlorobenzil, 3 mL of 95% ethanol was added, and the mixture was heated with constant stirring until the benzil was completely dissolved. 3 mL of an aqueous potassium hydroxide (1 N) was added dropwise to this mixture. The reaction mixture was stirred and refluxed well for 25 min. After completion of reaction, mixture was cooled in an ice-water bath for an additional 20 min when potassium benzilate was formed. This solution was cooled and poured into crushed ice containing 10 mL of 1 M HCl. The precipitate was filtered, washed with water, and dried to afford solid benzilic acid of about 50% yield. The pH of the reaction mixture was maintained at 2.



2,2'-dibromobenzilic acid

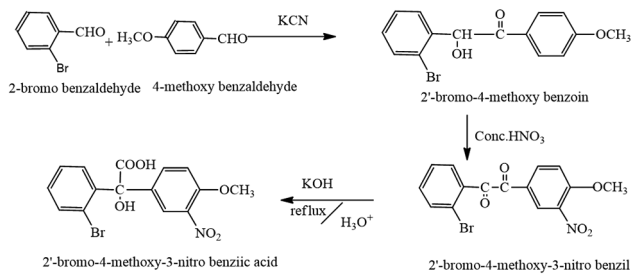
To 1.0 g of 2,2'-dibromobenzil, 3 mL of 95% ethanol was added, and the mixture was heated with constant stirring until the benzil was

completely dissolved. 3 mL of an aqueous potassium hydroxide (1 N) was added dropwise to this mixture. The reaction mixture was stirred and refluxed well for 25 min. After completion of reaction, mixture was cooled in an ice-water bath for an additional 20 min when potassium benzilate was formed. This solution was cooled and poured into crushed ice containing 10 mL of 1 M HCl. The precipitate was filtered, washed with water, and dried to afford solid benzilic acid of about 50% yield. The pH of the reaction mixture was maintained at 2.



2'-bromo-4-methoxy-3-nitrobenzilic acid

To 2'-bromo-4-methoxy-3-nitrobenzil, 3 mL of 95% ethanol was added, and the mixture was heated with constant stirring until the benzil was completely dissolved. 3 mL of an aqueous potassium hydroxide (1 N) was added dropwise to this mixture. The reaction mixture was stirred and refluxed well for 25 min. After completion of reaction, mixture was cooled in an ice-water bath for an additional 20 min when potassium benzilate was formed. This solution was cooled and poured into crushed ice containing 10 mL of 1 M HCl. The precipitate was filtered, washed with water, and dried to afford solid benzilic acid of about 50% yield. The pH of the reaction mixture was maintained at 2.



EXPERIMENTAL

The melting point of synthesized compounds was measured on an electrothermal 9300 melting point apparatus and is calibrated. The thermal study used to confirm the melting point. Infrared (IR) spectra were recorded on Bruker optics (Fourier transform IR) spectrophotometer using KBr pellet. Disk diffusion method was used for the determination of the preliminary biological activities.

Molecular docking studies

The molecular interaction can be determined by the entrenched technique like molecular docking study and find the best orientation of ligand would form a complex with overall minimum energy. The pharmacological data obtained were confirmed by the molecular docking studies. All the synthesized compounds were docked. The ligand molecules were drawn and analyzed using Chem Draw Ultra 8.0. Three-dimensional coordinates were prepared using dock server.

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[25]. 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, 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 570 nm in a microtiter plate reader. DMSO and cyclophosphamide used as a negative and positive control (PC).

Cell survival was calculated by the following formula:

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

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

The ability of the extracts to annihilate the 1,1-diphenyl-2-picrylhydrazyl (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 extract was added, at an equal volume, to methanolic solution of DPPH (0.1 mM). 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:

% of inhibition = (A of control–A of test)/A of control*100

Cytotoxicity % = 100–Viability%

Disc diffusion method

Antibacterial activity of the synthesized compounds was investigated using disc diffusion method (Murray *et al*., 1995). Petri plates were prepared with 20 ml of sterile Mueller-Hinton agar (Hi-Media, Mumbai). The test culture (100 µl of suspension containing 108 colony-forming unit /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 µg/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 µg/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* (ATCC 15380).

RESULTS AND DISCUSSION

Molecular docking activity

The results were obtained for molecular docking study shows that the good binding energy toward the target protein and the synthesized compounds. The compound 2'-bromo-4-methoxy-3-nitrobenzilic acid shows high dock score which is due to the hydrogen bond interaction and dipole-dipole with amino acids of targeted protein. Compound (3) having significant antibacterial activity compared with other two. 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 other compounds such as (1) and (2) having moderate antibacterial activity are also found to have good docking activity. The docked molecule with protein structure is given in Fig. 1, and the hydrogen bonding is also presented in Table 1.

Cytotoxic activity

The cytotoxic activity results show that all synthesized compounds have medium-to-high activity. Percentage of viability and cytotoxic activity in MCF-7 cancer cells had been performed. The synthesized compound had shown the appreciably highest activity, compared to the standard

cyclophosphamide taken as 100% (Table 2). The chlorosubstituted moiety is more potent toward the antitumor agents compared with other alkyl substituted compounds, but the presence of an alkyl group on the phenyl ring enhances the activity (Fig. 2).

Antioxidant activity

The synthesized compounds were investigated by scavenging of DPPH is shown in Fig. 3 and their values were given in Table 3. From the results, it signifies that synthesized compound is time dependent and a relatively slow process. The result confirms there is a moderate-to-high change in antioxidant activity due to the presence of different substituents on phenyl ring. 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. The scavenging capacity is extremely enhanced in the presence of the substituted benzilic acid and electron donor group on the phenyl ring.

Antimicrobial activity

The substituted benzilic acids were tested against three Gram-positive and two Gram-negative bacteria. Compound (2) and compound (3) were observed that exhibit sufficient antimicrobial activity by showing maximum zone of inhibition (mm) at dose-dependent manner. Compound (3) showed a maximum zone of inhibition of 11 mm at 100 µg and against *S. aureus*. In the case of Gram-negative bacterium, *E. coli* compound (2) showed a zone of inhibition of 11 mm at 100 µg. Compound (1) showed a highest zone of inhibition of 12 mm at 100 µg, 11 mm at 50 µg against *B. subtilis* when compared with standard streptomycin which showed a zone of inhibition of 14 mm. In the case of Gram-negative bacterium, *K. pneumonia* compound (1) also showed a zone of inhibition of 9 mm at 100 µg. The pathogens *B. subtilis*, *S. epidermidis,* and *K. pneumoniae* were found to exhibit similar antibacterial activity for the compound 2'-bromo-4-methoxy-3-nitrobenzilic acid. 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 4).

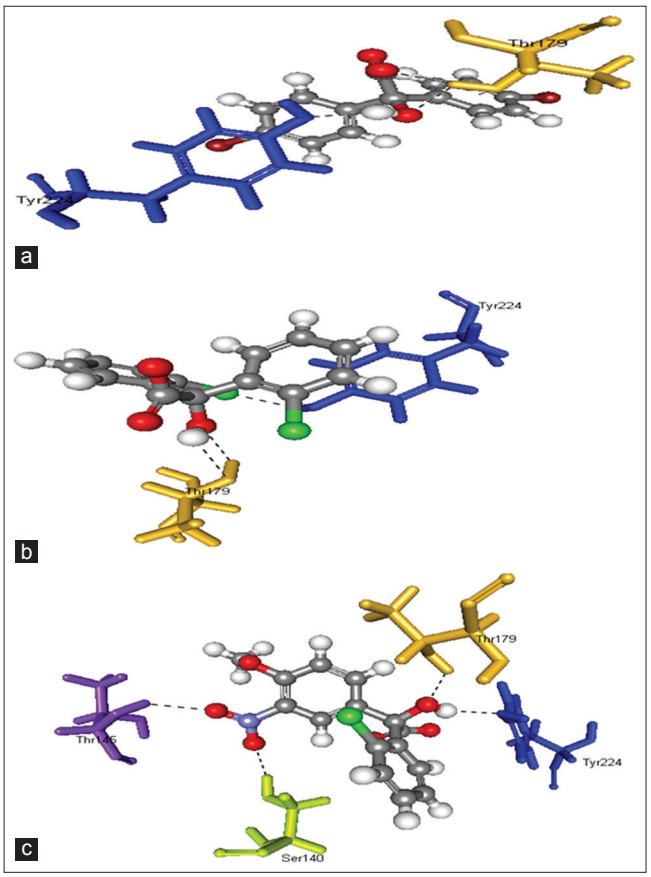


Fig. 1: (a-c) Molecular docking of 4,4'-dichlorobenzilic acid, 2,2'-dibromobenzilic acid, and 2'-bromo-4-methoxy-3-nitrobenzilic acid

CONCLUSION

The bromosubstituted benzilic acid is more potent toward antitumor agents, antioxidant studies, and antimicrobial activity compared with the alkyl-substituted compounds. Due to the presence of electron donating substituent like alkyl group enhances the antioxidant activity by DPPH scavenging method. There will be most promising antibacterial activities results were obtained by the synthesized compounds. The activity of the compounds was found to be dose dependent, i.e., 100 µg/mL showed greater inhibition. These results perhaps support a future research in this field. The susceptibility of the microbes to the compound was compared with standard antibiotic streptomycin. It can be concluded that the synthesized compounds positively holds assure toward studies in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress. The docking studies show that the necessity of hydrogen bond formation for enhancing the activity of synthesized compounds.

ACKNOWLEDGMENT

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AUTHOR'S CONTRIBUTION

R. Sudha - has synthesized the compounds in the laboratory. Charles C Kanakam - has gave the protocol to prepare the compounds. G. Nithya - has minorly helped to synthesis compound.

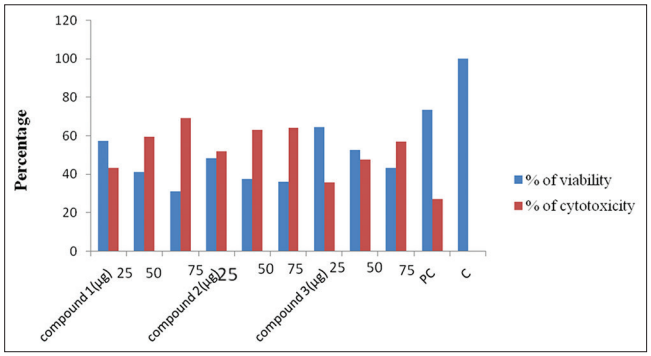

Fig. 2: Effect of cell viability and cytotoxicity of compound in MCF-7 cancer cells; C - control; PC - positive control (cyclophosphamide)

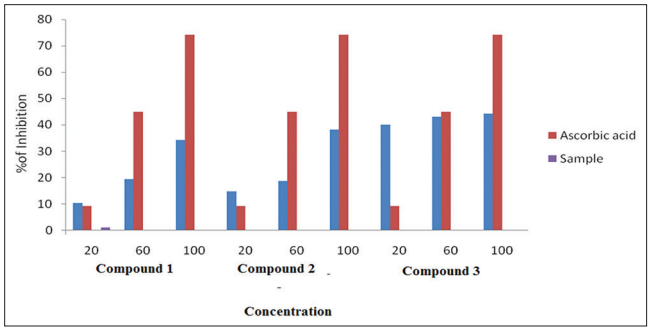

Fig. 3: Antioxidant activity for the compound

Table 1: Hydrogen bonding for molecular docking

Compound	LibDock score	Number of H bonds	H bonds	H bond distance
1	93.4823	2	THR179 (2), TYR224	2.30, 1.88, 1.74
2	92.1212	2	THR145, THR179 (2)	2.41, 2.48, 1.87
3	106.763	4	THR179, TYR224 (2)	1.83, 2.41, 1.78

Table 2: % 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		
% of viability	57.03	40.81	31.04	48.28	37.24	36.08	64.34	52.45	43.05	73.22	100
% of cytotoxicity	42.96	59.18	68.95	51.71	62.75	63.91	35.65	47.54	56.94	26.77	0

PC: Positive control (cyclophosphamide), C: Control

Table 3: 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	10.18	19.33	34.23	14.74	18.64	38.12	39.98	43.06	44.17
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 4: Antimicrobial assay of substituted benzilic acids by disc diffusion method

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

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

CONFLICTS OF INTEREST

The author declared that there are no conflicts of interest regarding the publication of this article.

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