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Synthesis and spectral analysis

PREPARATION, CHARACTERISATION BY SPECTRAL ANALYSIS, BIOLOGICAL ACTIVITY AND DOCKING STUDIES OF AROMATIC 2,2′-DIFLUORO BENZILIC ACID

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ABSTRACT

2,2′-difluoro benzilic acid is prepared by benzoin-benzilic acid rearrangement method. The starting material benzoin was prepared by the reaction with simple benzaldehyde followed by the oxidation to get benzil. The target compound was synthesised and then purified. The compounds were characterised using IR, UV and NMR spectral studies which are used to determine the presence of functional groups and types of transitions. *In vitro* antibacterial activity was studied and docking studies were also carried out. Biochemical investigations revealed significant biological activity compared to those of respective controls.

Keywords: preparation, docking studies, antibacterial activity, characterisation.

AIMS AND BACKGROUND

Benzilic acid and its derivatives are of wide interest because of their diverse biological activity and clinical applications. They are remarkably effective compounds both with respect to their inhibitory activity and their favourable selectivity ratio. Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of a fate of mankind. The resistance of bacteria against antimicrobial agents has become a wide spread medical problem[1]. The current interest in the development of new antimicrobial agents can be partially ascribed both to the increasing emergence of bacterial resistance to antibiotic therapy and to newly emerging pathogens. The antimicrobial activity of chemical compound is influenced by its physical and biological characteristics[2]. It has been well established that physiological activity is a function of the chemical structure of compound.

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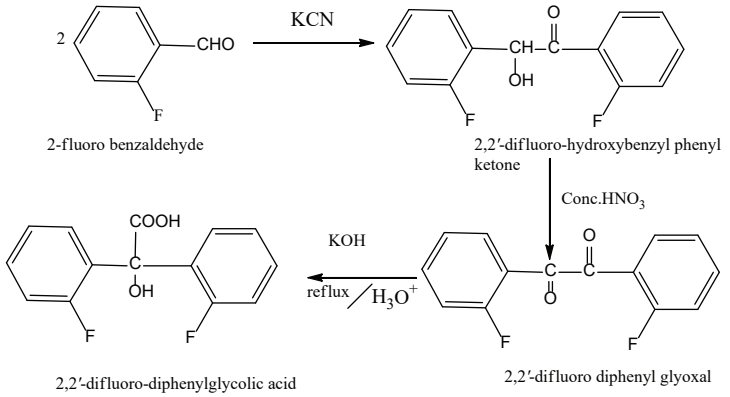
Ongoing research shows the development of new antibacterial agents that could overcome the resistance problem. The interaction of two molecules could be determined by a well established technique like molecular docking[3–5]. Using this study, the best orientation of ligand forms a complex with overall minimum energy would be found out. The new drugs can be synthesised 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 have been developed. Different substituted benzilic acids and analogs have been synthesised, characterised, and evaluated their capabilities in biological activity. The different derivatives of benzilic acid prove that it can be anticipated and analyse the antioxidant and antimicrobial activity with different *in vitro* models[6–9].

Bactericidal drugs kill bacteria and bacteriostatic drugs slow or stop the bacterial growth. These definitions are not absolute; bacteriostatic drugs may kill some bacteria, and bactericidal drugs may not kill all of the bacteria *in vitro*. More precise quantitative methods identify the minimum *in vitro* concentration at which an antibiotic can inhibit growth (minimum inhibitory concentration, or MIC) or kill (minimum bactericidal concentration, or MBC). Molecular docking study is a well-established technique to determine the interaction of two molecules and also to know the best orientation of ligand that forms a complex with overall minimum energy. Using this study, new synthetic compounds which lead to different mechanisms and thereby different target organisms against drug resistant bacteria and emerging microbes[10–15] could be developed. Based on this, new substituted benzilic acid was synthesised, characterised and evaluated.

EXPERIMENTAL

Method of preparation of 2,2′-difluorobenzilic acid. 2,2′-difluoro hydroxybenzyl phenylketone was prepared from 2.0 g of 2-fluorobenzaldehyde in 40 ml of ethanol in the presence of 0.25 g KCN catalyst. To 1.0 g of 2,2′-difluoro hydroxybenzyl phenylketone 25 ml of concentrated nitric acid were added slowly and allowed to reflux for 30 min until no more NO_2 gas was apparent. To the reaction mixture 30 ml of water were added, cooled to room temperature and repeatedly washed with water to get the yellow coloured substance with yield of about 50%. About 2.0 g of 2,2′-difluoro-diphenyl glyoxal, 5 ml of 95% ethanol were added and the mixture was heated with constant stirring until the 2,2′-difluoro-diphenyl glyoxal was completely dissolved. This was followed by the dropwise addition of 3.5 ml of aqueous (1N) potassium hydroxide. The reaction mixture was stirred and allowed to reflux well for 30 min. After completion of reaction, the mixture was cooled in an ice-water bath, potassium diphenyl glycolate was formed. This solution was cooled and poured into crushed ice containing 10 ml of 1M HCl. The precipitate was filtered, washed with water and dried to afford 2,2′-diflourobenzilic acid with yield of about 65%. The pH of the reaction mixture was maintained at 2 (Scheme 1).

Scheme 1
Schematic representation of 2,2′-difluorobenzilic acid



Preparation of antibacterial solution. 1,2-bis (4-amino phenyl) ethane-1,2-dione was dissolved in dimethyl sulphoxide (DMSO). Proper drug controls were used. The zone of inhibition of the control and the compound was taken at concentration of 25, 50, 75 and 100 µg/ml for testing the antibacterial activity. The compound diffused into the medium producing a concentration gradient 5–12. After the incubation period, the zones of inhibition were measured in mm.

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 synthesised 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 synthesised compounds were docked. The ligand molecules were drawn and analysed using Chem Draw Ultra 8.0. 3D, coordinates were prepared using dock server.

Antibacterial activity of the syntheised compound 2,2′-difluorobenzilic acid was carried by using disc diffusion method[16]. Petri plates were prepared with 20 ml of sterile MHA (Hi-media, Mumbai). The test culture (100 µl of suspension containing 108 CFU/ml bacteria) were 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[17]. Streptomycin (10 µg/disc) was used as a positive control[18]. These plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in mm.

Micro organisms used. *In vitro* antimicrobial studies were carried out against different 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) and *Klebsiella pneumonia*.

Mass spectral analysis of 2,2′-difluorobenzilic acid. The mass spectral analysis results (Fig. 1, Table 1) confirm the molecular weight of the compound.

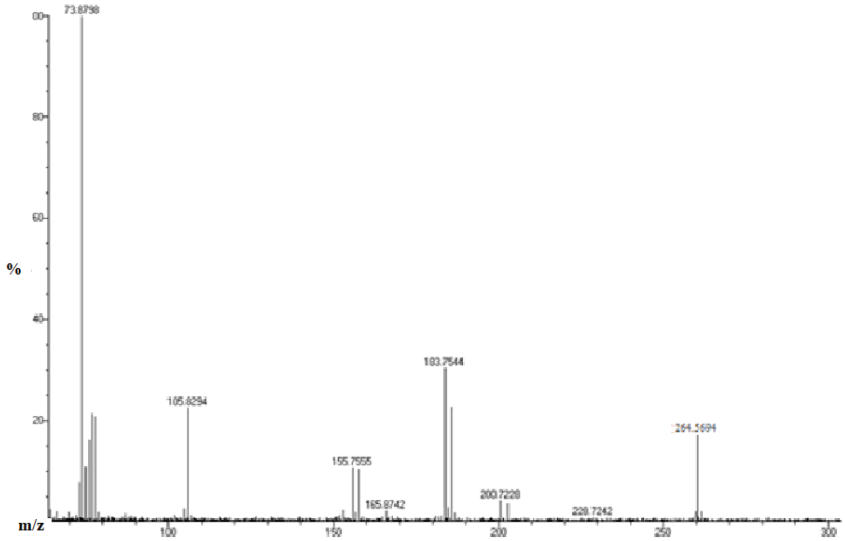


Fig. 1. Mass spectra of 2,2′-difluorobenzilic acid in solid KBr

Table 1. Mass spectral fragmentation peaks for 2,2′-difluorobenzilic acid

Peaks	Fragmentaion
264.5694	M^+
228.7242	$[M^+\text{-}F2]$
155.9787	$2[C_6H_5]^+$
105.8187	$[C_6H_5\text{-}C\text{-}OH]^+$

FT-IR spectral analysis. The FT-IR spectrum of the compound 2,2′-difluorobenzilic acid was recorded in the frequency region 4000–450 cm^{-1} (Fig. 2 and Table 2). 2,2′-difluorobenzilic acid molecules in the crystal lattice are greatly evident by the broadened envelope due to –OH stretch around 3437 cm^{-1}. The FT-IR spectrum of the title compound shows the presence of a strong intensity absorption at 1724 cm^{-1} attributed to the C=O stretching of the carboxylic acid group. The bands around 3066 cm^{-1} in the FT-IR spectrum are assigned to the C–H stretching. The C=C symmetric stretching vibrations of the aromatic ring are observed by the band at 1567 cm^{-1}. The C–O stretching of the alcohol and carboxylic acid group are observed by the bands at 1157 and 1065 cm^{-1}.

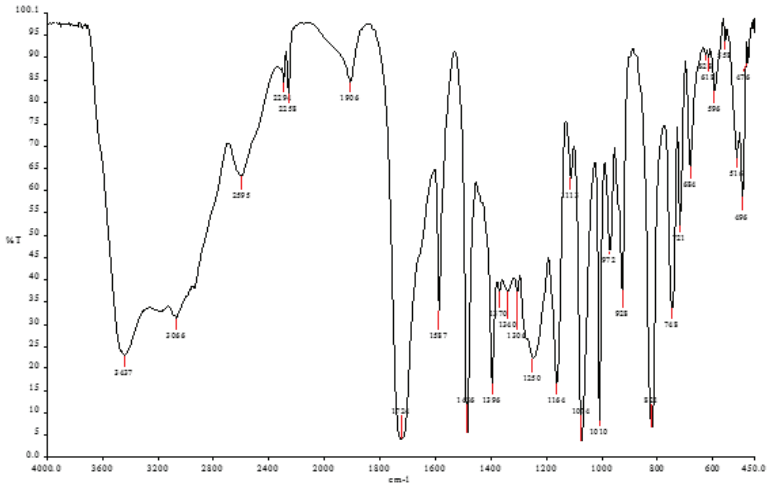


Fig. 2. IR spectrum of 2,2′-difluorobenzilic acid in solid KBr

Table 2. Vibrational assignments of the 2,2′-difluorobenzilic acid

FT-IR for MB (wave number) cm^{-1})	Band assignments
3437 cm^{-1}	–O–H stretching
1724 cm^{-1}	–C=O stretching
3066 cm^{-1}	Aliphatic C–H stretching
1567 cm^{-1}	Aromatic sym C=C stretching
1157cm^{-1}, 1065 cm^{-1}	C–O stretching

FT-NMR spectral analysis. The ^{1}H-NMR and ^{13}C-NMR spectra of the title compound are presented in Figs 3 and 4, respectively, and the chemical shifts are presented as δ value. The chemical shifts are tabulated with the assignments in Table 3. In the ^{1}H-NMR spectrum, the signals in the range of 7.1 to 7.5 ppm indicate the presence of aromatic protons. In the ^{13}C-NMR the aromatic carbon atoms appear in the range of from 126 to 159 ppm. The signal in the range of 70 ppm indicates the presence of aliphatic carbon in C–O–H. The signal at 176 ppm corresponds to the carbon of the –COOH group.

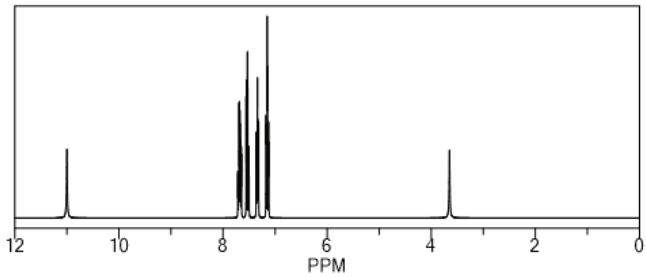


Fig. 3. ^{1}H -NMR of 2,2′-difluorobenzilic acid in DMSO-d_6

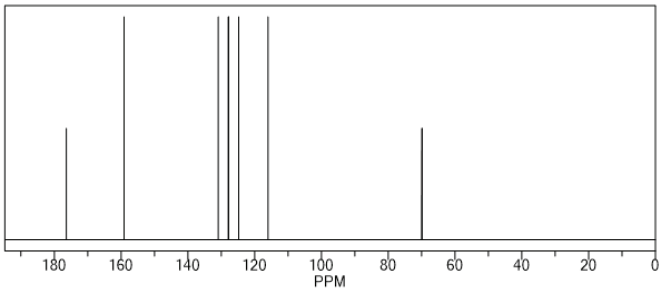


Fig. 4. ^{13}C-NMR of 2,2′-difluorobenzilic acid in DMSO-d_6

Table 3. Chemical shifts in ^{1}H-NMR and ^{13}C-NMR spectra of 2,2′-difluorobenzilic acid

Spectrum	Signal at δ (ppm)	Group identification
^{1}H-NMR	7.1 to 7.5 (multiplet)	Aromatic protons
	11.0 ppm	Carboxylic acid group
	3.65 ppm	Hydroxy group
^{13}C-NMR	176 ppm	Carbon of carbonyl group
	126 to 159 ppm	Aromatic carbon atoms
	70 ppm	C–O–H
		Aliphatic carbon

Table 4. Antibacterial activity for 2,2′-difluorobenzilic acid using disc diffusion method

Name of the pathogens	Antibacterial activity – Disc diffusion method (μg)			Streptomycin (S10)
	Zone of inhibition (mm)			
	25	50	100	
Bacillus subtilis	–	–	–	9
Staphylococcus aeureus	–	–	11	20
Staphylococcus epidermitis	8	–	8	24
E. coli	–	–	10	20
Klebsiella pneumoniae	–	9	10	21

Antimicrobial activity. The results revealed that the activity was considerably affected by various substitutions on the aromatic ring of benzilic acid. For antibacterial activity it was observed that introduction of electron withdrawing group on benzene ring of benzilic acid showed considerable increase in antibacterial potency of the compound. It was also observed that the synthesised compound containing difuoro substituent showed significant potency against Gram-positive *Staphylococcus aeureus* bacteria (MIC = 11 Mm). The same substitution showed maximum inhibition against Gram–negative bacteria like *Klebsiella pneumoniae* and *E. coli*. This is further enhanced by increasing the concentration of the compound dosage for all pathogens (Table 4). The activity of the compounds was 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.

Docking score and ligand interaction results. The compound 2,2′-difluorobenzilic acid was found to inhibit the bacteria at a distance of 6 mm. This is quite lesser than the other molecules. This makes this molecule more effective binding and the docking score was found to be –6.121 kcal mol^{-1}. The binding analysis of this compound reveals that the compound well fitted into the active site pocket and the fluorophenyl group was found to interact with non-polar amino acids Tyr25 and Trp64 which reveals that there are two stacking interactions making this compound more stable for further processing as better drug compound. 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. 5 and the hydrogen bonding is also presented in Table 6. The binding analysis and ligand interaction for the compound 2,2′-difluorobenzilic acid was depicted in Fig. 6.

Table 5. Docking score and ligand interaction results for the synthesised compound

S.No	Compound name	Docking score (kcal/mol)	Ligand interaction
1	2,2′-difluorobenzilic acid	–6.121	Tyr157

Table 6. Hydrogen bonding for molecular docking

Lib dock score	No of H bonds	H bonds	H bond distance
89.97	2	ALA226, SER140	2.13, 1.98

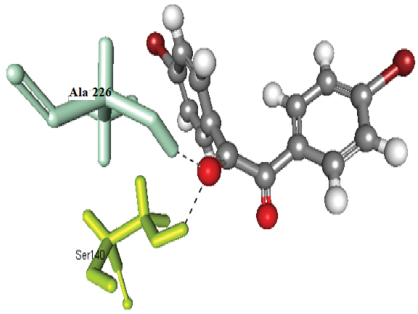


Fig. 5. Docked molecule with protein structure

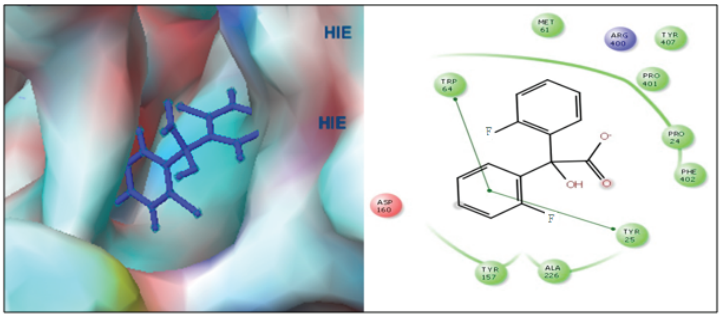


Fig. 6. Binding analysis and ligand interaction diagram of 2,2′-difluorobenzilic acid

CONCLUSIONS

The mass and the presence of functional groups of the synthesised compound 2,2′-difluorobenzilic acid and its derivative were analysed and confirmed by FT-IR, Mass, ^{1}H-NMR, ^{13}C-NMR and UV analyses. The ability of characterising physicochemical behaviour of the medium and its information about molecular behaviour of liquids and solutions was studied by ultrasonic technique. To conclude, we have synthesised the 2,2′-difluorobenzilic acid and its derivatives were docked into the protein which provides good binding energy and the interaction pattern. Compound 2,2′-difluorobenzilic acid possessed good docking score with related to the reference ligand and it also possessed cancer cell toxic nature with the inhibition. Additional studies will be carried out with respect to the protein analysis and the binding affinity of the ligand through differential scanning fluorimetry experiments.

A further study to acquire more information concerning pharmacological activity is in progress. More 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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