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Crystal Structure Study, Hirshfeld Surface Analysis and *in vitro* Antibacterial Activity of *p*-tolyl 4-fluorobenzoate

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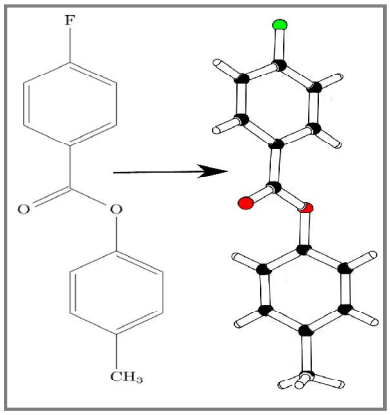
ABSTRACT

The title compound $C_{14}H_{11}FO_2$ crystallizes in orthorhombic crystal system with space group $P2_12_12_1$. The compound exhibits inter-molecular interaction of the types C–H…O, and C–H…π; intra-molecular interaction of the type and C–H…O. The inter-contacts are also studied using Hirshfeld surface analysis. The compound was screened for anti-bacterial activity which revealed medium to low activity against certain bacteria.

Graphical Abstract



Highlights

- This manuscript discusses the procedure to synthesis *p*- tolyl 4- fluorobenzoate.
- Elemental analysis, ^{1}H NMR, FT-IR spectral analysis was studied.
- The X-ray crystal structure study reveals that the compound crystallizes in orthorhombic crystal system with space group $P2_12_12_1$.
- The compound exhibits inter-molecular interaction of the types C–H…O, and C–H…π; intra-molecular interaction of the type and C–H…O.

- The inter-contacts are also studied using Hirshfeld surface analysis.
- The compound was screened for anti-bacterial activity which revealed medium to low activity against certain bacteria.

Keywords: *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Escherichia coli,* fingerprint plots, Streptomycin, Resazurin assay, phenyl benzoate, Hirshfeld surface analysis.

INTRODUCTION

The title compound is a phenyl benzoate derivative. The title compound comprises of halogen fluorine, which is attached at the fourth position to the benzoic acid attached to the tolyl. Phenyl benzoate is a phenyl ester of benzoic acid. Phenyl benzoates are used in the production of polyesters, which find varied applications.

A few of the phenyl benzoate derivatives like liquid crystalline compound possessing a terminal hydroxyl group showed cytotoxic effects on human lung cancer. They also showed potent anti-cancer activity [1]. Some of the phenyl benzoate derivatives show enhanced electro-optical properties; hence they are used in new types of liquid crystal-based display devices [2].

Herein we report the synthesis, characterization, X-ray crystal structure study, Hirshfeld surface analysis and *in vitro* anti-bacterial activity of *p*-tolyl 4-fluorobenzoate.

MATERIALS AND METHODS

Synthesis of *p*-tolyl 4-fluorobenzoate: Chemicals were purchased from Sigma Aldrich Chemical Corporation. *p*-Tolyl 4-fluorobenzoate was synthesized by benzoylation of substituted *p*-cresol (0.1 g, 0.001 mmol) with 4-fluorobenzoyl chloride (0.16 g, 0.001 mmol) using 50 mL, 10% of sodium hydroxide solution. The reaction mass was stirred for 2-3 h at 0°C. The reaction was monitored by thin layer chromatography using n-hexane (4 mL): ethyl acetate (1 mL) solvent mixture. After completion of the reaction the oily product was extracted with 50 mL diethyl ether. Further, diethyl ether layer was washed with 10% of sodium hydroxide solution (3×50 mL) followed by water (3×30 mL) and then dried over anhydrous sodium sulphate and evaporated the solvent under pressure to afford compound p-tolyl 4-fluorobenzoate. Finally, on recrystalization it gives pure white p-tolyl 4-fluorobenzoate crystals. The melting point of the compound is 65-67°C. The schematic diagram is shown in the figure 1.

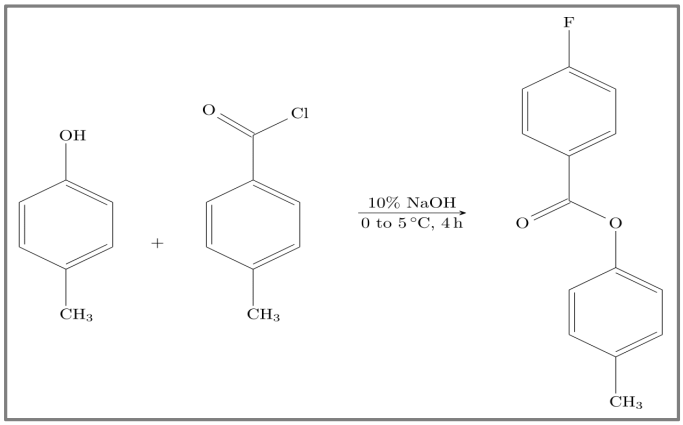


Figure 1. Schematic diagram of the compound p-tolyl 4-fluorobenzoate.

Spectral Data: ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer in DMSO-d_6 solvent and the chemical shifts were recorded in δ (ppm) downfield from tetramethylsilane. Elemental analysis was done using Perkin Elmer 2400 elemental analyzer and results are within 0.4% of the calculated value. Infrared spectra were recorded on a Perkin Elmer spectrophotometer in the range 400-4000 cm^{-1}.

X-ray Data Collection: A suitable single crystal was selected for X-ray diffraction study. Data were collected on a Bruker Kappa Apex II single crystal X-ray diffractometer equipped with Cu K_α radiation and CCD detector [**3**]. Crystal structure was solved by direct methods using *SHELXS-97*. After locating all the non-hydrogen atoms, the structure was refined by full-matrix least-squares method using *SHELXL-97* [**4**]. The model obtained was refined with isotropic thermal parameters, and later with anisotropic thermal parameters. Hydrogens were placed at chemically acceptable positions. A total of 155 parameters were refined with 1943 unique reflections which converged the residual (*R*) value to 0.053.

Biological Activity: Anti-bacterial activity was tested against two gram-positive bacteria, namely *Bacillus subtilis* (MTCC No. 121), *Staphylococcus aureus* (MTCC No. 7443) and two gram-negative bacteria, namely *Proteus vulgaris* (MTCC No. 742) and *Escherichia coli* (MTCC No. 730). The bacterial strains were inoculated in nutrient broth, and kept for overnight culture at 37°C. Minimum inhibitory concentration (MIC) is the lowest concentration at which blue color of the dye (indicator) turns to pink color [**5**]. MIC was determined by microbroth dilution method using resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) as an indicator. This was performed on 96-well microtiter plates [**6**].

For susceptibility testing the plates were prepared in duplicates. Nutrient broth of 50 µL was distributed to all the wells. 50 µL compound was added to third and fourth wells. Serial dilution was performed from the fourth well till the concentration reached 0.39×10^{-2} mg mL^{-1}. Finally, 10 µL of bacterial suspension was added to all the wells.

The concentrations of the prepared solutions were as follows: 0.5 mg mL^{-1}, 0.25 mg mL^{-1}, 0.125 mg mL^{-1}, 0.625×10^{-1} mg mL^{-1}, 0.3125×10^{-1} mg mL^{-1}, 0.156×10^{-1} mg mL^{-1}, 0.78×10^{-2} mg mL^{-1}, 0.39×10^{-2} mg mL^{-1}. Blue color indicates that the compound inhibits the growth of the bacteria, whereas pink color indicates the bacterial growth.

Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 10 µL of resazurin was added to all the wells. The plates were incubated for another hour. The change in color was assessed visually and MIC was recorded.

RESULTS AND DISCUSSION

Elemental Analysis: Carbon (C) and Hydrogen (H) analyses were carried out to confirm the chemical composition of the synthesized compound. The experimental and calculated percentages of C and H are given in table 1. The differences between experimental and calculated percentages of C and H are very small and are within the experimental errors. This confirms the formation of the product in the stoichiometric proportion.

Table 1. Elemental analysis for the title compound

Element	Experimental (%)	Calculated (%)
Carbon	73.05	73.03
Hydrogen	4.86	4.82

^{1}H NMR Spectral Analysis: The ^{1}H NMR spectra of the compound is as shown in the figure 2. The NMR peak at δ 2.45 (s, 3H) clearly indicates that the three hydrogens of methyl group are attached to aromatic ring. The peaks at δ 7.5-8.2 refers to eight aromatic hydrogens of the compound.

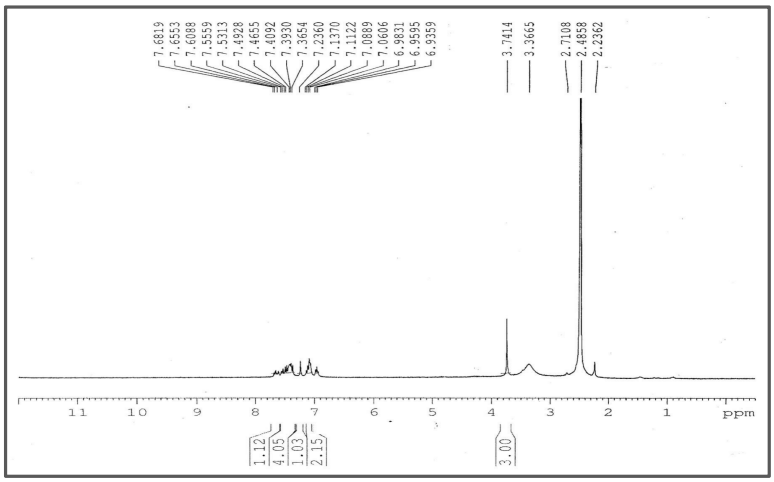


Figure 2. ^{1}H NMR spectra of the title compound.

FT-IR Spectral Analysis: The peak observed at1715 cm^{-1} is assigned to C=O of carbonyl group.

***In vitro* Antimicrobial Activity:** The results of biological activity of the title compound are given in the table 2. The resazurin assay showed that the compound has lower to average activity against the tested bacterial strains. The results of the compound with tested bacterial strains were compared with streptomycin, which is a standard antibiotic drug used in the experiment [7].

The MIC values of the compound and the standard were same when tested against the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. They showed comparatively lesser MIC value against gram-negative bacteria *Proteus vulgaris* and *Escherichia coli*.

Table 2. MIC of the title compound and the standard Streptomycin against various bacterial strains

Bacterial Strains	MIC (mg mL^{-1})	
	Streptomycin (std.)	Compound
Bacillus subtilis	0.3125×10^{-1}	0.3125×10^{-1}
Staphylococcus aureus	0.3125×10^{-1}	0.3125×10^{-1}
Proteus vulgaris	0.156×10^{-1}	0.3125×10^{-1}
Escherichia coli	0.78×10^{-2}	0.156×10^{-1}

X-ray Crystal Structure Determination: The compound $C_{14}H_{14}F_2O$ crystallizes in orthorhombic crystal system, with space group $P2_12_12_1$. The unit cell parameters are *a*=7.2929(14) Å, *b*=11.3195 (19) Å, *c* = 14.395(3) Å. The details of the crystal data and structure refinement are given in table 3. The geometrical calculations were carried out using the program *PLATON* [8]. The molecular and packing diagrams were generated using *Mercury* [9]. Figure 3 shows the *ORTEP* diagram of the molecule with thermal ellipsoids drawn at 50% probability.

The phenyl rings are nearly planar. The r.m.s. deviation for the ring C2–C7 (ring-1) from the mean plane is 0.009(4) Å. The maximum deviation is 0.010(5) Å for the atom C3. The r.m.s. deviation for the ring C10–C15 (ring-2) from the mean plane is 0.006(5) Å. The maximum deviation is 0.006(5) Å for the atom C14. The bond distance between O9 and C8 is 1.203(5) Å; this confirms the double bond between O and C. Selected bond lengths and angles are listed in the table 4.

Table 3. The crystal data and structure refinement details

CCDC Deposit Number	1423293	F_{000}	480
Empirical formula	$C_{14}H_{11}FO_2$	Crystal size	0.210 × 0.210 × 0.210 mm
Formula weight	230.23	θ range for data collection	5° to 64.9°
Temperature	293 K	Index ranges	$-8 \le h \le 8$
Wavelength	1.54178 Å		$-11 \le k \le 12$
Crystal system	Orthorhombic		$-16 \le l \le 16$
Space group	$P2_12_12_1$	Reflections collected	6247
Cell dimensions	a = 7.2929(14) Å	Independent reflections	1943 [R_{int} = 0.044]
	b = 11.3195(19) Å	Refinement method	Full matrix least-squares on F^2
	c = 14.395(3) Å		
Volume	1188.3(4) Å^3	Data / restraints / parameters	1943 / 0 / 155
Z	4	Goodness-of-fit on F^2	1.067
Density(calculated)	1.287 Mg m^{-3}	Final [I> 2σ(I)]	R1 = 0.0531, wR2 = 0.1504
Absorption coefficient	0.798 mm^{-1}	Largest diff. peak and hole	0.148 and −0.151 eÅ^{-3}

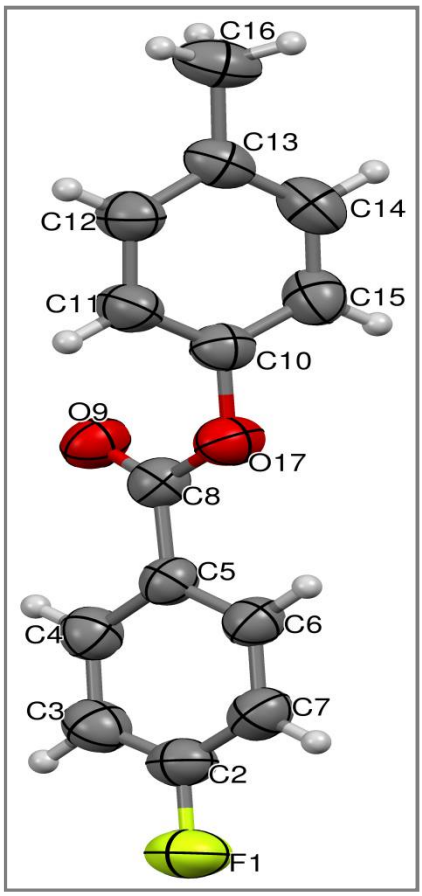


Figure 3. *ORTEP* diagram of the molecule with thermal ellipsoids drawn at 50% probability.

Table 4. Selected bond lengths and bond angles (Å, °)

Atoms	Distances	Atoms	Angles
O(9)-C(8)	1.203(5)	F(1)-C(2)	1.369(5)
C(2)-C(3)	1.357(7)	C(5)-C(6)	1.382(5)
C(10)-C(15)	1.374(6)	O(17)-C(8)	1.354(5)
C(8)-O(17)-C(10)	118.2(3)	O(9)-C(8)-C(5)	125.4(4)
F(1)-C(2)-C(3)	117.9(4)	O(17)-C(8)-C(5)	111.6(3)
C(10)-C(15)-C(14)	118.6(4)	O(17)-C(10)-C(11)	120.3(4)

The rings 1 and 2 are *sp*2 hybridized. They are described by the torsion angles 1.66º and 1.13º respectively which suggest that they adopt *+syn-periplanar* (*+sp*) conformation. The benzoic acid and the *p* tolyl group are also in *+syn-periplanar* (*+sp*) conformation, since the torsion angle between the atoms O9-C8-O17-C10 is 3.4(6)º. Selected torsion angles are listed in the table 5.

Table 5. Selected torsion angles (°).

Atoms	Angle	Atoms	Angle
C(5)-C(8)-O(17)-C(10)	-175.0(3)	C(6)-C(5)-C(8)-O(9)	171.9(4)
O(9)-C(8)-O(17)-C(10)	3.4(6)	F(1)-C(2)-C(3)-C(4)	-179.2(4)
C(16)-C(13)-C(14)-C(15)	-179.3(4)	O(17)-C(10)-C(11)-C(12)	175.2(4)
C(12)-C(13)-C(14)-C(15)	1.4(7)	C(4)-C(5)-C(8)-O(17)	171.0(3)
F(1)-C(2)-C(7)-C(6)	179.7(4)	C(12)-C(13)-C(14)-C(15)	1.4(7)

The bond lengths and angles are in agreement with those of the phenyl benzoate derived molecules reported earlier [**7, 8**]. Ring 2 deviates from ring 1 by a torsion angle of -175.0(3)º (C5-C8-O17-C10) from the mean plane defined for F1/C2/C3/C4/C5/C6/C7/C8/O9. The torsion angle indicates that the section O17-C10 is in an *anti-periplanar* (*-ap*) conformation with respect to the mean plane described above (for ring 1).

The packing diagrams of the molecules when viewed along *a* and *c*-axes are shown in the figures 4 and 5 respectively. The molecular structure exhibits a long centroid *Cg-Cg* interaction between the rings 1 and 2. The weak centroid-centroid distance is 4.850(3) Å with the symmetry code 1/2+*x*, 1/2-*y*, 1-*z*.

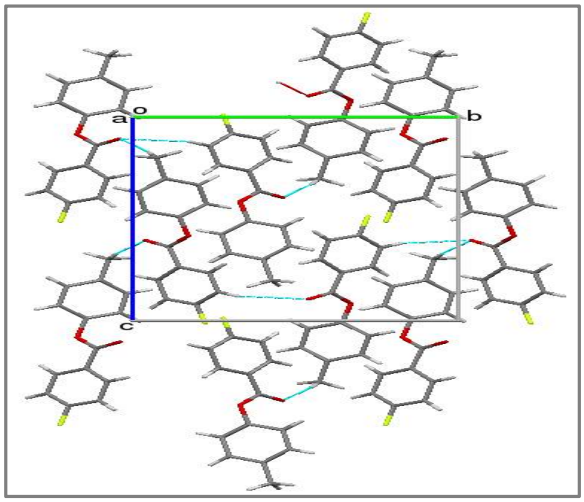


Figure 4. Packing of molecules when viewed along *a*-axis.

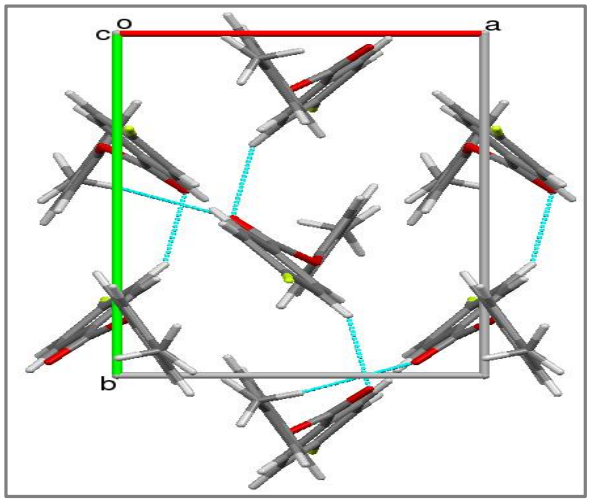


Figure 5. Packing of molecules when viewed along *c*-axis.

The molecular structure is stabilized by C—H…π intermolecular (C12—H12…*Cg*(1) and C16—H16B…*Cg*(1)), with H-centroid distances 2.92 Å and 2.96 Å respectively; C-centroid distances 3.688(6) Å and 3.784(6) Å respectively; the C-H-centroid angles are 141º and 145º respectively. *Cg*(1) is the centroid of the ring C2/C3/C4/C5/C6/C7.

The molecular structure also exhibits inter and intra molecular interactions of the type C—H…O. The hydrogen bond geometries are shown in the table 6.

Table 6. Hydrogen-bond geometry (Å, °)

D—H…A	D—H	H…A	D…A	D—H…A
C(6)—H(6)…O(17)*	0.93	2.4	2.716(5)	100
C(7)—H(7)…O(9)[a]	0.93	2.48	3.315(6)	149
C12—H12…*Cg*(1)[b]	0.93	2.92	3.688(6)	141
C16—H16B…*Cg*(1)[c]	0.96	2.96	3.784(6)	145

*Intramolecular hydrogen bond interactions, [a]Symmetry code: -*x*, -1/2 + *y*, 1/2 – *z*. [b]Symmetry code: -1/2 - *x*, 1 - *y*, 1/2 + *z*., [c]Symmetry code: -1/2 + *x*,1/2 - *y*, 1 – *z*.

Hirshfeld Surface Analysis: *Crystal Explorer 3.1* [9] program was used to understand the interactions and the connectivity among the molecules efficiently**.** The crystallographic information file (.cif) was imported to the *Crystal Explorer* to generate the Hirshfeld surfaces. The Hirshfeld surface is the region around the molecule in the crystal space which can be considered as the boundary separating two regions - the interior (the reference molecule) and the exterior (neighboring molecules) [10].

The Hirshfeld surface of the title compound is as shown in the figure 6. The bright red spot is due to the acceptor O9, which forms C-H. ..O type hydrogen bond with the atom C7 of the neighboring molecule.

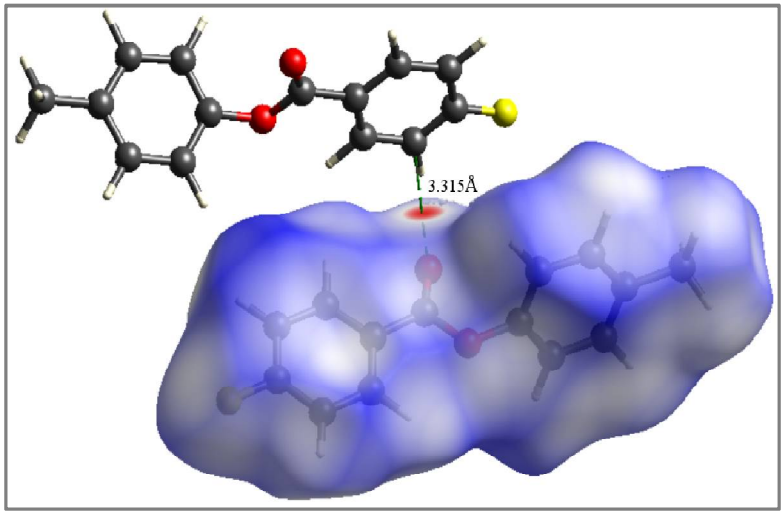


Figure 6. Hirshfeld surface mapped with d_{norm} showing the intermolecular distance of the title compound.

The presence of 'wings' at the top left and bottom right in the fingerprint plot (figure 7(a)) are due to the presence of C-H. ..π interactions. The fingerprint plot shows that percentage contribution to the total Hirshfeld surface area [11]. The fingerprint plot data are shown in table 7. The major contribution is from H…H contacts (35.9%).

Table 7. Percentage of various intermolecular contacts contributing to Hirshfeld surface

Inter-contacts	Contribution (%)	Inter-contacts	Contribution (%)
H…H	35.9	F–H/H…F	14.3
C–H/H…C	29.8	C–C	2.9
O–H/H…O	15.0		

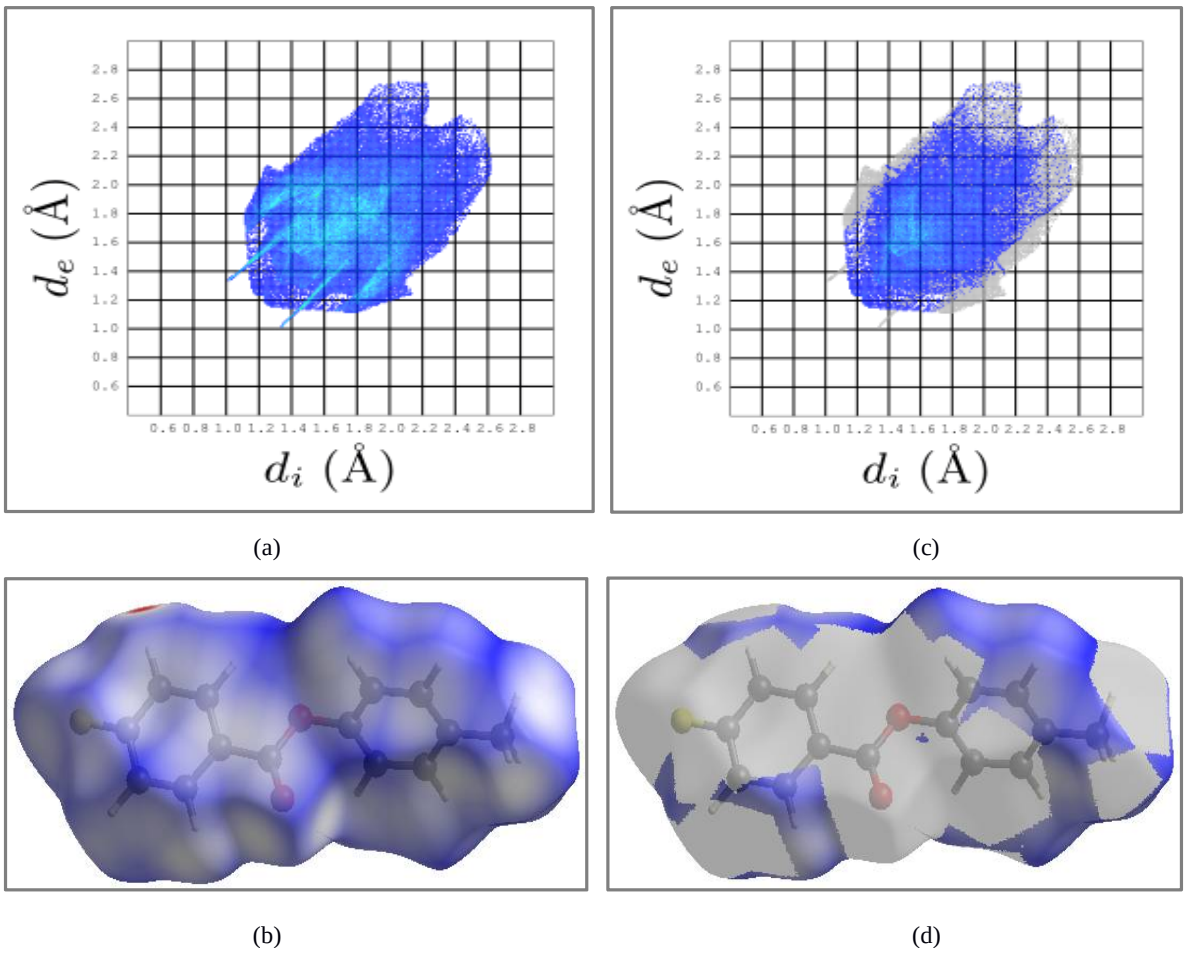


Figure 7. Fingerprint plots and the surface maps of the title compound. (a) Highlights full two dimensional map. (b) All contacts, (c) Two dimensional map resolved into H…H contacts, (d) Surface highlighting H…H contacts.

APPLICATION

This research work is a helpful addition to the library of drug molecules-whenever there is a need for a compound with these properties, one can then lookup for the same in this library.

CONCLUSION

In the present research work we have discussed the synthesis of the compound p-tolyl 4-fluorobenzoate. The compound was characterized by FT-IR, H-NMR. The structure was confirmed by the single crystal X-ray diffraction. Screening for antibacterial activity indicated that the compound has lower MIC value against the bacteria *Escherichia coli*.

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