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Structural Study and Antibacterial Activity of a Benzophenone Derivative: [2-Bromo-4-(2-chloro-benzoyl)-phenoxy]-acetic acid ethyl ester

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ABSTRACT

The title compound $C_{17}H_{14}BrClO_4$ was synthesized by refluxing a mixture of (3-Bromo-4-hydroxy-phenyl)-(2-chloro-phenyl)-methanone and ethyl chloroacetate in dry acetone and anhydrous potassium carbonate. Elemental analysis confirms the formation of the compound in the stoichiometric proportion. The compound was also characterized by FT-IR spectral analysis, ^1H NMR spectral analysis. Single crystal X-ray diffraction reveals that the compound crystallizes in monoclinic crystal system with space group $P\,2_1/c$. The unit cell parameters are a=7.4733(6) Å, b=32.205(3) Å, c=7.5203(6) Å, β= 110.110(3)°. In addition to this, the compound was screened for its anti-bacterial activity against two gram-positive and two gram-negative bacteria.

Keywords: Benzophenone, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*.

INTRODUCTION

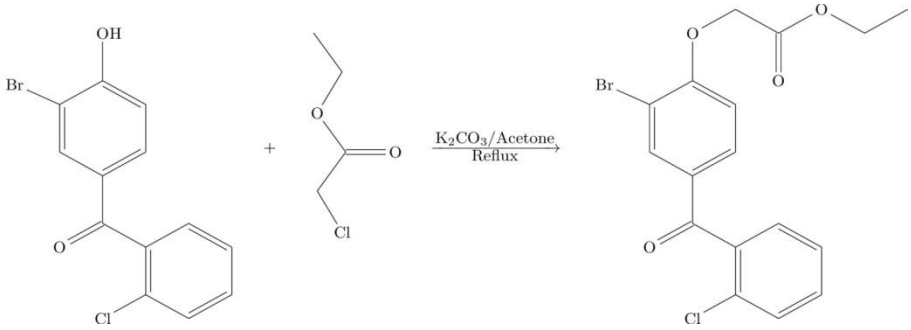
Literature shows that the benzophenone and its derivatives are pharmacologically active. The presence of halogen group compounds shows potent anti-inflammatory activity [1]. It is reported that the phenoxy acetic acid shows very good anti tumor activity on Ehrlich ascites tumor cells. It also shows anti-ulcerogenic activity, cyclooxygenase activity, anti-convulsant activity and anti-microbial activities [2]. The title compound consists of: two halogen compounds, bromine and chlorine are attached to each phenyl rings at meta and ortho positions respectively of a benzophenone moiety. The phenoxy acetic acid ethyl ester is also attached to the benzophenone.

In connection with this, it was found worthwhile to synthesis the title compound. Herein, we report the synthesis, preliminary characterizations, crystal structure and anti-bacterial activity of [2-Bromo-4-(2-chloro-benzoyl)-phenoxy]-acetic acid ethyl ester.

MATERIALS AND METHODS

Chemicals were purchased from Sigma Aldrich Chemical Corporation. Thin Layer Chromatography was performed on aluminum-backed silica plates from Merck & Co., and visualized under UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. IR spectra were recorded on Perkin Elmer spectrum version 10.03.09. ^{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.

Synthesis of [2-Bromo-4-(2-chloro-benzoyl)-phenoxy]-acetic acid ethyl ester: Compound [2-Bromo-4-(2-chloro-benzoyl)-phenoxy]-acetic acid ethyl ester was obtained by refluxing a mixture of compound (3-Bromo-4-hydroxy-phenyl)-(2-chloro-phenyl)-methanone (0.00645 mol) and ethyl chloroacetate (0.0129 mol) in dry acetone (50 mL) and anhydrous potassium carbonate (0.0129 mol) for 12 hours. The reaction mixture was cooled and the solvent was removed by distillation; the reaction was monitored by TLC (thin layer chromatography) using benzene : ethyl acetate (10:1) as an eluent. The residual mass was triturated with cold water to remove potassium carbonate and extracted with ether (3×50 mL). The ether layer was washed with 10% sodium hydroxide solution (3×50 mL) followed by water (3×30 mL). It was dried over anhydrous sodium sulphate and evaporated to dryness to obtain a crude solid, which on recrystalization with ethanol afforded desired compound in good yield 92%, m.p. 65-67°C.



Scheme : Schematic diagram of the title compound

Single Crystal X-ray Diffraction: A colorless, rectangular shaped single crystal of dimension 0.23 × 0.22 × 0.21 mm was used to study X-ray diffraction. The experimental analysis reveals that the synthesized compound $C_{17}H_{14}BrClO_4$ crystallizes in monoclinic crystal system, with space group $P\,2_1/c$. The cell parameters and other details of refinement are shown in table 2. The data were collected on a Bruker CCD diffractometer equipped with CuK_α radiation. Data reduction and applying of absorption corrections were carried out using the *APEX 2* package [3]. Crystal structure was solved by direct methods using *SHELXS-97* and was refined by full-matrix least squares against F^2 using *SHELXL-97* [4]. All the non-hydrogen atoms were refined anisotropically and the hydrogen atoms were placed at chemically acceptable positions. A total of 209 parameters were refined from 2009 unique reflections. Initially, the residual factor was high which was finally reduced to R = 5.41%. The bond lengths and angles are within the expected range.

***In vitro* antibacterial activity:** The compounds were screened for antibacterial activity 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.

Antibacterial activity was determined by resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) assay using broth microdilution method performed in 96 well microtiter plate [5], by dissolving 1 mg of sample in 1 mL of ethanol.

For susceptibility testing the plates were prepared in duplicates. Nutrient broth of 50 µL was distributed to all the wells. 50 µL compounds were added to third and fourth wells. Serial dilution was performed from the fourth well till the concentration reached $0.39x10^{-2}$ 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}. MIC (minimum inhibitory concentration) is defined as the lowest concentration at which the color changed [6]. 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 the wells. The plates were incubated for another hour. The change in color was assessed visually. Any change in color from blue to pink is recorded. The lowest concentration at which the change occurred was taken as MIC.

RESULTS AND DISCUSSION

Elemental Analysis: In order to confirm the chemical composition of the synthesized compound, Carbon (C), Hydrogen (H) analysis was carried out. 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 title compound

Element	Experimental (%)	Calculated (%)
Carbon	51.32	51.35
Hydrogen	3.53	3.55

FT-IR Spectral Analysis: The peaks observed at 1760 cm^{-1} are assigned to the C=O stretching of the ethyl ester. The peak at 1674 cm^{-1} corresponds to the C=O stretching of the benzophenone which gives the evidence for the structure conformation.

^{1}H NMR Spectral Analysis: The NMR peak at δ 1.23 triplet is for three hydrogens in CH_3 of ester, the quartet peak at δ 4.12 is for the CH_2 ester group, the singlet peak at δ 4.88 is for two hydrogens of -OCH_2, the peaks at δ 6.56 – 7.81 clearly indicate the seven aromatic hydrogens of the compound, thus confirming the structure.

Crystallography: The *ORTEP* and the packing diagrams are shown in the figures 1, 2, and 3.Bond lengths and angles, torsion angles, hydrogen bond geometry are shown in tables 3, 4, and 5 respectively. The geometrical calculations were carried out using the program *PLATON* [7]. The molecular and packing diagrams were generated using *Mercury* [8].

Table 2. Crystal data and structure refinement details.

CCDC Deposit Number	1476403
Empirical formula	$C_{17}H_{14}BrClO_4$
Formula weight	397.63
Temperature	296 K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Cell dimensions	a = 7.4733(6) Å, b = 32.205(3) Å, c = 7.5203(6) Å, β = 110.110(3)°
Volume	1699.6(3) $Å^3$
Z	4
Density(calculated)	1.554 Mg m^{-3}
Absorption coefficient	4.894 mm^{-1}
F_{000}	800
Crystal size	0.23 × 0.22 × 0.21 mm
θ range for data collection	2.74° to 64.39°
Index ranges	$-8 \le h \le 8$ $-36 \le k \le 36$ $-8 \le l \le 8$
Reflections collected	7291
Independent reflections	2009 [R_{int} = 0.0569]
Refinement method	Full matrix least-squares on F^2
Data / restraints / parameters	2009 / 0 / 209
Goodness-of-fit on F^2	1.050
Final [I > 2σ(I)]	$R1$ = 0.0541, $w R2$ = 0.1503
Largest diff. peak and hole	0.526 and −0.391 e $Å^{-3}$

The phenyl rings (C2–C7) and (C10–C15) are bridged by carbonyl group C8=O9. These rings are *sp2* hybridized. They are well described by the torsion angles 0.09° and 1.23° respectively; which suggest that they adopt +*syn-periplanar* (+*sp*). The phenyl rings are highly planar. The r.m.s. deviation for the rings (C2–C7) and (C10–C15) are 0.016(8) Å and 0.012(5) Å respectively. The dihedral angle between the least-square planes of the two phenyl rings (C5–C8–C10–C11) is 5.1(8)°.

Table 3. Selected bond lengths and bond angles (Å, deg.)

Atoms	**Bond Lengths/Angles**	**Atoms**	**Bond Lengths/Angles**
O(9)–C(8)	1.216(9)	O(20)–C(19)	1.189(6)
Br(16)–C(12)	1.892(5)	Cl(1)–C(6)	1.726(5)

O(17)–C(13)	1.360(6)	O(21)–C(22)	1.448(9)
C(10)–C(11)	1.386(7)	C(14)–C(15)	1.379(7)
C(5)–C(6)	1.390(8)	C(6)–C(7)	1.363(10)
C(13)–O(17)–C(18)	118.7(4)	Br(16)–C(12)–C(11)	119.6(4)
Br(16)–C(12)–C(13)	119.6(4)	C(19)–O(21)–C(22)	116.9(4)
Cl(1)–C(6)–C(7)	119.3(5)	Cl(1)–C(6)–C(5)	118.9(4)
C(19)–O(21)–C(22)	116.9(4)	C(3)–C(2)–C(7)	118.8(10)
C(10)–C(11)–C(12)	120.9(5)	O(20)–C(19)–O(21)	126.0(6)

The bond lengths and angles agree well with those of previously reported benzophenone derivatives. The double bond between the carbonyl groups are confirmed by the bond lengths 1.216(9) Å for O9 – C8 and 1.189(6) Å for O(20) - C(19).

Table 4. Selected torsion angles (deg.)

Atoms	**Torsion Angles**	**Atoms**	**Torsion Angles**
C(5)–C(8)–C(10)–C(15)	5.1(8)	C(6)–C(5)–C(8)–O(9)	97.7(8)
O(9)–C(8)–C(10)–C(15)	-172.8(7)	Cl(1)–C(6)–C(7)–C(2)	-177.4(6)
Br(16)–C(12)–C(13)–O(17)	-3.2(6)	Br(16)–C(12)–C(13)–C(14)	177.9(3)
C(4)–C(5)–C(6)–Cl(1)	177.3(4)	Cl(1)–C(6)–C(7)–C(2)	-177.4(6)
O(9)–C(8)–C(10)–C(11)	5.9(9)	C(10)–C(11)–C(12)–C(13)	-0.1(7)
C(23)–C(22)–O(21)–C(19)	-179.9(5)	O(20)–C(19)–O(21)–C(22)	1.8(7)

Table.5. Hydrogen-bond geometry (Å, deg.)

D—H...A	**D—H**	**H...A**	**D...A**	**D—H...A**	**Symmetry code**
C7 – H7 … O9	0.93	2.40	3.214(11)	146	*1+x, y, z*

The torsion angle between the phenyl ring and the carbonyl group (C6 – C5 – C8 – O9) is found to be 97.7(8)°. This is lesser when compared with the torsion angle 135.94(15)° for 3,3'-dinitrobenzophenone at the same position [9]. The molecules are connected by the intermolecular interaction of the type C—H…O.

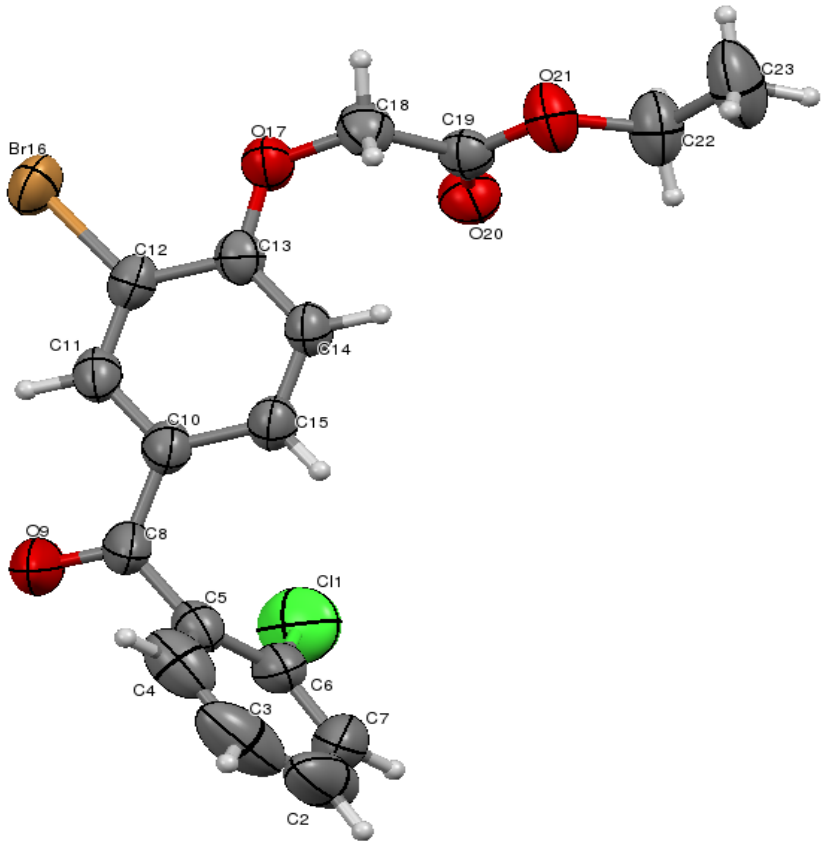


Figure.1. The *ORTEP* diagram of the title compound

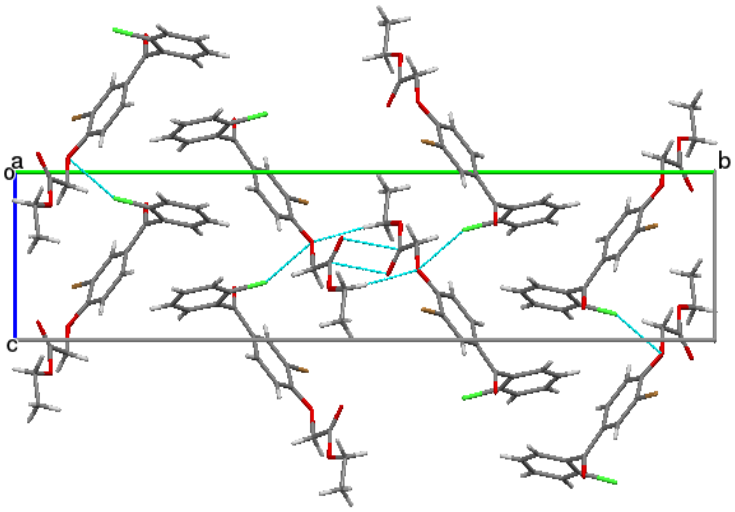


Figure 2. The molecular packing view of the title compound down the *a* axis

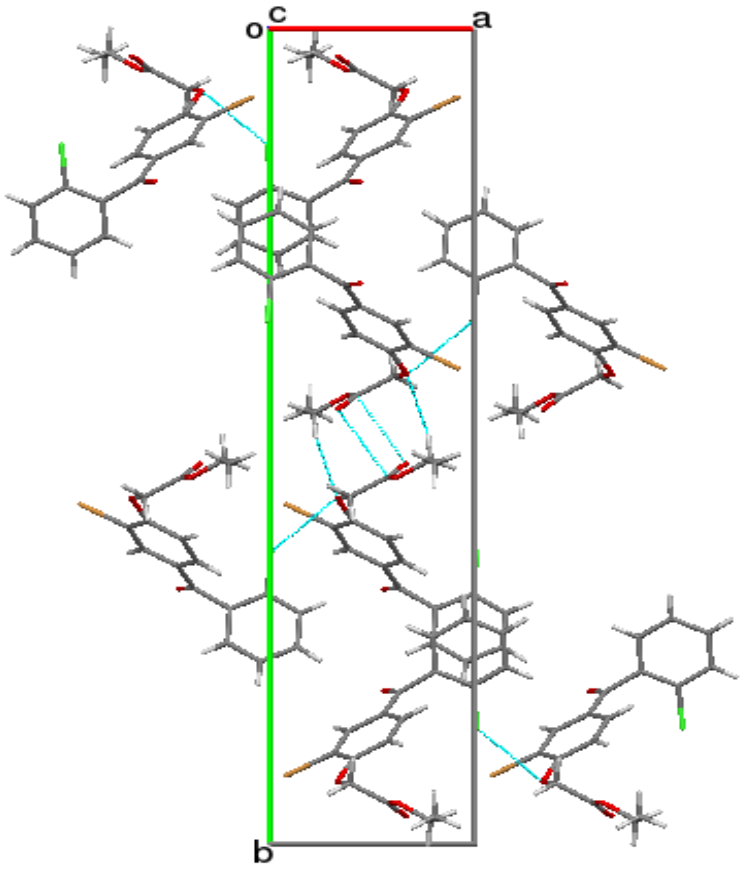


Figure 3. The molecular packing view of the title compound down the *c* axis

Biological Activity: The synthesized compound was screened for antibacterial activity. The result is reported in table 6. The MIC recorded for the gram-negative bacteria *Escherichia coli* is less. This indicates that the compound is more active against *Escherichia coli* compared to other bacteria. The compound showed less to average activity against different bacterial strains.

Table 6. MIC of the title compound against different bacterial strains

Bacterial Strains	**MIC x 10^{-1} (mg/mL)**
Bacillus subtilis	0.1560
Staphylococcus aureus	0.3125
Proteus vulgaris	0.3125
Escherichia coli	0.078

APPLICATIONS

Literature shows that halogen containing compounds show better biological activity. The presence of halogen might have enhanced the activity of the compound. This research work is useful for the creation of

a library. Whenever there is a need for a molecule with these properties, one can make use of the title compound.

CONCLUSIONS

In the present research work we have discussed the synthesis of the 2-Bromo-4-(2-chloro-benzoyl)-henoxy]-acetic acid ethyl ester. The compound was characterized by FT-IR, H NMR. The structure was confirmed by 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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REFERENCES

[1] Shaukath A. Khanum, Bushra A. Begum, V. Girish, Noor Fatima Khanum. Synthesis and Evaluation of Benzophenone-N-ethylMorpholine Ethers as Anti-inflammatory Agents, *International J. Biomedical Science,* **2010,** 6(1), 60-65.
[2] T. Prashanth, V. Lakshmi Ranganatha, M.K. Usha, Shaukath Ara Khanum, D. Revannasiddaiah, Sumati Anthal, Rajni Kant, Vivek K. Gupta. Ethyl 2-[4-(2-chlorobenzoyl)-2,6-dimethyl phenoxy] ethanoate, *Acta Cryst*, **2012,** E68, o2435.
[3] Bruker, *APEX2, SAINT, SADABS*, **2009**, Bruker AXS Inc, Madison, Wisconsin, USA.
[4] G.M. Sheldrick, *SHELXS 97, A program for crystal structure determination,* Univ. of Göttingen, **2015**, Germany.
[5] Deborah A. Hudman and Neil Sargentini, Resazurin-based assay for screening bacteria for radiation sensitivity, *Methodology,* **2013,** 2:55, 1-6.
[6] Satyajit D. Sarker, Lutfun Nahar, Yashodharan Kumarasamy. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, *Methods*, **2007,** 42, 321-324.
[7] A.L. Spek, *Acta Cryst,* **2009,** D65, 148-155.
[8] C.F. Macrae, I.J. Bruno, J.A. Chisholm, P.R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P.A. Wood, J. *Appl. Cryst*, **2008,** 41, 466-470.
[9] N. Latha Rani, C.S. Karthik, P. Mallu, L. Mallesha, M.A. Sridhar. Synthesis, Crystal Structure and Hirshfeld Study of 3,3'-dinitrobenzophenone, *J. Applicable Chem*, **2016,** 5(1), 231-241.

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