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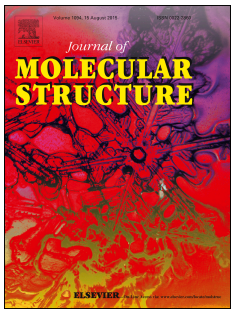

Structural characterization and Hirshfeld surface analysis of racemic baclofen

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Structural characterization and Hirshfeld surface analysis of racemic Baclofen

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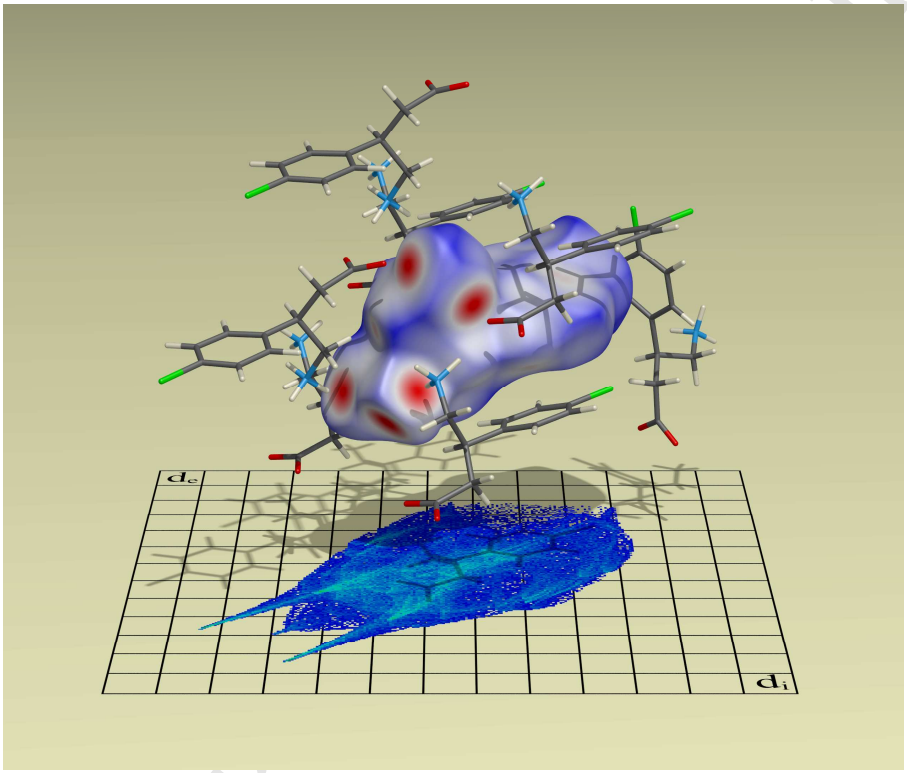

H I G H L I G H T S

* The first single crystal structure of baclofen.
* The molecules exist as zwitterions, adopting a *gauche* conformation with respect to the C_α–C_β bond.
* The crystal packing is governed mainly by N–H···O and C–Cl···π interactions.

Key words : Baclofen - Structure characterization- Hirshfeld surfaces - Hydrogen bonding - GABA derivative

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Abstract

The crystal structure of baclofen, (R,S) [4-amino-3-(4-chlorophenyl)butanoic acid], ($C_{10}H_{12}ClNO_2$, M_r = 213.66) has been determined by single crystal X-ray diffraction analysis. The title compound crystallizes in the orthorhombic space group *Pbca* (No. 61) with *a* = 9.2704(5), *b* = 7.0397(4), *c* = 30.4015(15) Å, *V* = 1984.0(2) Å^3 and *Z* = 8. The molecules exist as zwitterions, adopting a *gauche* conformation with respect to the C_α–C_β bond, and held in a cross-linked chain arrangement by strong N–H···O hydrogen bonds and C–Cl···π interactions. The electrostatic molecular potential as well as the intermolecular interactions of the title compound were analyzed by the Hirshfeld surfaces. The FT-IR spectrum is also reported. The DTA, TG and DTG results indicate that baclofen is stable up to 205°C.

1. Introduction

The spasticity is very common potentially disabling and bothersome complications affecting individuals with spinal cord lesion [1]. It may be defined as a motor disorder characterized by a overactivity of stretch reflexes [2]. Clinically, this can cause for instance weakness of voluntary movement, increased muscle tone, and increased tendon reflexes. Curtis (et al.) has postulated that γ-aminobutyric acid (GABA), which is the hyperpolarizing inhibitory transmitter responsible for the prolonged postsynaptic inhibition of spinal motoneurones can be used in the treatment of spasticity [3]. However, attempts to use GABA in such a therapy appeared ineffective. For this reason a number of GABA derivatives were synthesized with lipophilic substituents and subsequently tested; of these, baclofen, γ-aminobutyric acid ($GABA_B$) receptor agonist, was found to be the most active one and now is regarded as a drug of choice in the treatment of spasticity and trigeminal neuralgia [4-7]. It is thus somewhat surprising that, despite the fact of using the title compound as a drug by more than 30 years no crystal structure of pure baclofen or any its solvate has yet been reported. On the basis of a literature review we have only found reports on crystal structures of (R,S)- or R-baclofen hydrochloride salts [8], baclofen cocrystal with ferulic acid [9] and very recently multicomponent co-crystals formed between baclofen and selected monocarboxylic and dicarboxylic acids [10]. It was also confirmed by various analytical techniques, that baclofen can exist in two crystalline forms, the anhydrate and monohydrate. Since, there are distinct differences in the powder diffraction patterns of the anhydrate and monohydrate, indicating that these two solid forms have unique crystal structures [11]. We report

here the crystal and molecular structure of racemic baclofen as part of our ongoing study of the structural characterization and properties of drug molecules.

2. Experimental

The baclofen was obtained by neutralization of baclofen hydrochloride using $NH_3 \cdot H_2O$. For this purpose, methanol solution of the baclofen hydrochloride was heated to 60°C and 12% $NH_3 \cdot H_2O$ was dropped carefully to the reaction mixture to pH 6.9 – 7.0 and then the reaction mixture was intensively stirred for 2 hours. Crystallization was conducted from methanol. Very thin needle crystals were filtered and washed with cold water. The product was dried at 60°C. Yield of synthesis was 41%. Despite repeated attempts it appeared very difficult to obtain really good quality crystals for X-ray diffraction analysis.

2.1. Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectrum was obtained by using a Thermo Nicolet 6700 spectrometer (Thermo Scientific, USA) with MCT detector (photoconductive detector HgCdTe). Sample was scanned by transmission through KBr pellet. FT-IR spectrum was recorded at room temperature in the range of 4000-500 cm^{-1}. Infrared spectrum exhibited characteristic bands at 3050-2950 cm^{-1} (C–H stretch), 1680 cm^{-1} (C=O, stretch), 1585 cm^{-1} (N–H, bending), 860cm^{-1} (C–Cl, stretch). The FT-IR spectrum is illustrated in Fig. 1S enclosed in Supporting Information.

2.2 Thermal Analysis

The thermal properties of baclofen were studied by DTA-TG-DTG techniques in the range of temperature from 25 up to 700°C and at a heating rate of 10°Cmin^{-1}. The SETSYS-16/18 (Setaram) apparatus was used. The sample (9.91 mg) was studied in flow of air atmosphere using aluminum crucibles. The DTA-TG-DTG methods were used to describe of thermal properties of baclofen in air atmosphere. The DTA-TG-DTG curves are illustrated in Fig. 2S enclosed in Supporting Information. At about 205°C, a sharp mass loss starts due to the degradation of the compound and there is no mass loss observed up to this temperature, hence the crystal might not have any solvent in it.

2.3 X-Ray Crystallography

X-ray diffraction data were collected at 100K by the ω-scan technique using a Bruker AXS Smart APEX-II CCD diffractometer with MonoCap capillary and 30W Incoatec Microfocus Source IμS with Montel optics and Cu-Kα radiation (λ = 1.54178Å). Data collection, cell refinement, data reduction, analysis and absorption correction were carried out with the SMART and SAINTPLUS [12]. The structure was solved by direct methods with SHELXS [13] and refined by a full-matrix least-squares technique on F^2 using SHELXL-2014 [14] with anisotropic thermal parameters for the non-H atoms. All H-atoms were located using difference Fourier techniques and refined with isotropic temperature factors. Molecular graphics used: program Mercury [15]. Additional details of the data collection and refinement are listed in Table 1. Selected bond lengths and angles are given in Table 2.

Table 1. Crystal and structure refinement data.

Formula	$C_{10}H_{12}ClNO_2$
CCDC no.	1455243
Formula weigh	213.66
Temperature, K	100
Wavelength, Å	CuK_α
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions, Å	9.2704(5) 7.0397(4) 30.4015(15)
Volume, $Å^3$	1984.0(2)
Z	8
Calculated density, g cm^3	1.431
Absorption coefficient mm^{-1}	3.197
θ range for data collection	5.6 - 66.0
Limiting indices (h, k, l)	-10/10, -7/7, -35/36
Reflections collected/unique	13172/1558
Data/restraints/parameters	1558/0/139
R_{int}	0.053
Goodness-of-fit on F^2	1.146
$R[F^2 > 2 \sigma(F^2)]$	0.0858
R (all data)	0.0888
$wR[F^2 > 2 \sigma(F^2)]$	0.240
$wR(F^2)$	0.241
Largest diff. peak and hole, e $Å^{-3}$	1.03, -0.45

$w = 1/[\sigma^2(Fo^2) + (0.0803P)^2 + 14.7802P]$ where $P = (Fo^2 + 2Fc^2)/3$

Table 2. Selected bond lengths (Å) and angles (°)

Cl1–C8	1.759(7)	O2–C1–C2	118.1(4)
O1–C1	1.273(6)	O1–C1–O2	123.3(5)
O2–C1	1.250(6)	O1–C1–C2	118.7(4)
N1–C4	1.485(8)	C1–C2–C3	115.5(4)
C1–C2	1.523(8)	C2–C3–C4	108.4(4)
C2–C3	1.538(8)	C2–C3–C5	111.1(4)
C3–C4	1.527(8)	C4–C3–C5	111.3(5)
C3–C5	1.528(8)	C3–C4–N1	112.9(4)
C5–C10	1.391(8)	C6–C5–C10	118.6(5)
C5–C6	1.388(8)	C3–C5–C6	120.8(5)
C6–C7	1.381(8)	C3–C5–C10	120.5(5)
C7–C8	1.370(9)	C5–C6–C7	121.2(5)
C8–C9	1.376(9)	C6–C7–C8	118.3(5)
C9–C10	1.386(9)	C7–C8–Cl1	119.0(5)
		C9–C8–Cl1	118.1(5)
		C7–C8–C9	122.9(6)
		C8–C9–C10	117.9(5)
		C5–C10–C9	121.1(5)

2.4. Hirshfeld Surface Analysis

The electrostatic molecular potential for baclofen was mapped on Hirshfeld surfaces (HS) over the range -0.05 au (red), through 0 (white), to 0.05 au (blue). Ab initio wavefunctions were obtained using Tonto program [16]. A 6-311++G (3df, 2pd) basis set at the Hartree–Fock level and molecular geometries directly from the relevant crystal structure was employed. The HS divide the crystal space into smooth non-overlapping molecular volumes and give a unique information about each molecule in a crystal. This enables a convenient analysis of intermolecular interactions in a crystal. The Hirshfeld surfaces [17] and the related 2D-fingerprint plots [18] were calculated using CrystalExplorer (Ver. 3.1) [19]. Before starting the calculations the bond lengths to hydrogen atoms were set to standardized neutron values (O–H 0.983, N–H 1.009 and C–H 1.083 Å).

3. Results and Discussion

3.1. Crystal structure

The structure of the title compound is shown in Fig. 1. The C–C bond lengths appear consistent with the general pattern of amino acids. As expected the molecules exist as zwitterions.

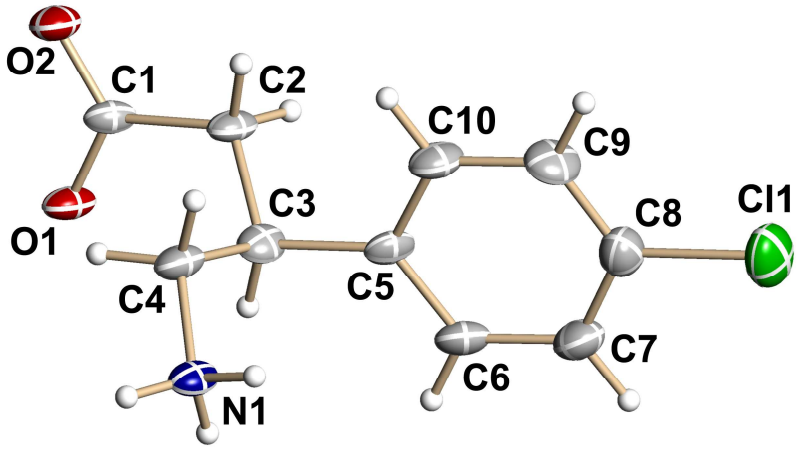
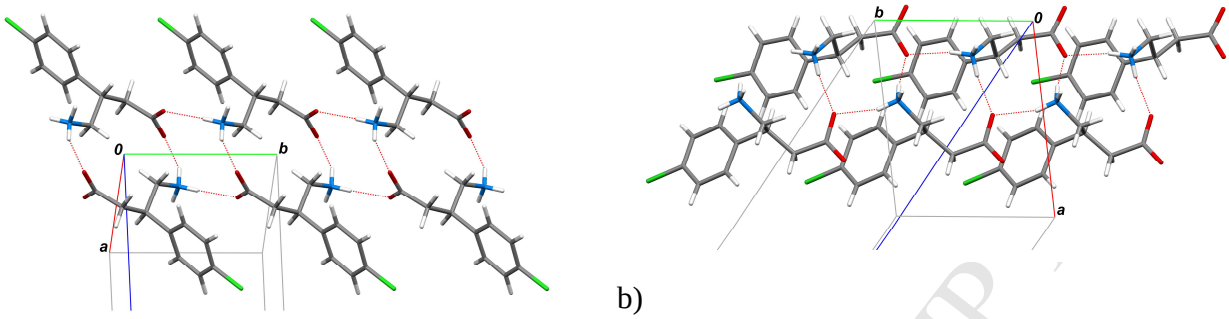


Fig. 1. *A perspective view of the baclofen with atom labeling scheme. Thermal ellipsoids are drawn at the 50% probability level.*

The N(1)–C(4) bond length of 1.485(8) Å is longer than the standard value (1.47 Å) while the two almost equal C–O bond lengths (1.273(6) and 1.250(6) Å) are consistent with the deprotoneted carboxyl group. Three tetrahedrally bonded hydrogen atoms to the nitrogen atom in the ammonium group, form almost linear intermolecular hydrogen bonds with oxygen atoms of neighboring molecules. One of the hydrogen bonds links the molecules in infinite chains in the **b** direction (Table 3, Fig 2a). These chains are cross-linked to form a three-dimensional structure by the remaining two hydrogen bonds (Table 3, Fig. 2b). The latter, create heterointermolecular synthons [20] described in Etter's graph-set notation [21] as a $R^2_2 8$ ring. The strong N–H···O hydrogen bonds, are likely responsible for the extension of the N(1)–C(4) bond length. In addition, the crystal structure of baclofen is stabilized by the intermolecular C–Cl···π interactions, in which a Cl1 atom approaches a benzene ring atom C8 in a "edge-on" manner [C8–Cl1····C8$^{(i)}$ 3.221(7)Å; 174.8(3)°; $^{(i)}$1-x,1/2+y,1/2-z].



a) b)

Fig. 2*. A packing diagram depicting the three dimensional hydrogen bonding network. Hydrogen bonds are indicated by dotted lines.*

Table 3. Hydrogen bonding (Å, °)

D–H···A	**D–H**	**H···A**	**D···A**	**D–H···A**
N1–H11···O1^{i}	0.94(8)	1.85(8)	2.777(6)	171(7)
N1–H12···O2^{ii}	0.97(5)	1.83(5)	2.742(6)	156(5)
N1–H13···O1^{iii}	0.97(8)	1.84(8)	2.801(6)	170(7)

Symmetry code : (i)1/2-x, ½+y, z; (ii) 1-x,1-y,-z; (iii) x,1+y,z.

It is generally known that due to reinforcement by charge assistance in the presence of anion-cation interactions [22] hydrogen bonds between ammonium and carboxylate groups are stronger than those between the corresponding neutral groups. This can explain the significant torsion-angle distortion around the C_{α}–C_{β} bond. In the title compound the ammonium and carboxylic groups are twisted by an angle (C1–C2–C3–C4) of -63.3(6)°. Since the *gauche* conformation about the C_{α}–C_{β} bond, is not the lowest energy state for the conservative molecule [23]. This suggests the possibility that more than one conformer of baclofen may exist in solution, which may be of importance in relation to the physiological activity of the molecule. The conformational freedom in the title molecule can also be described using the torsion angles presented in Fig. 3. These angles describe rotation of the $–CH_2–COO^-$ and $–COO^-$ fragments, the movement of the $–NH_3^+$ group and rotation of the aromatic ring relative to the amino acid skeleton. The torsion angles τ_1 (O2-C1-C2-C3), τ_2 (C1-C2-C3-C5), τ_3 (C5-C3-C4-N1) and τ_4 (C4-C3-C5-C10) are 140,2(5)°, 174,2(4)° , -62,8(6)° and -68,2(7)°, respectively.

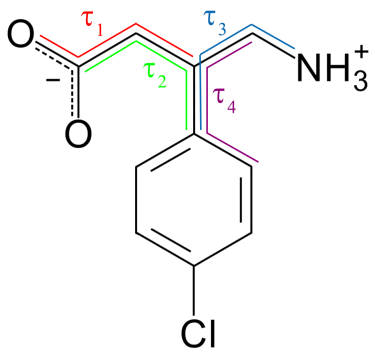
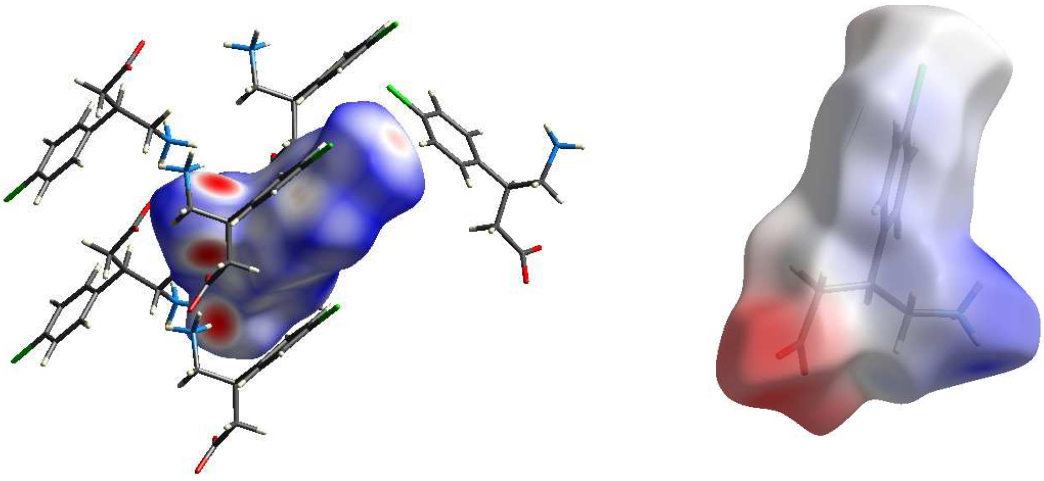


Fig. 3. *The conformational freedom of baclofen defined by* $\tau_{1\text{-}4}$ *torsion angles.*

3.2 Hirshfeld surfaces

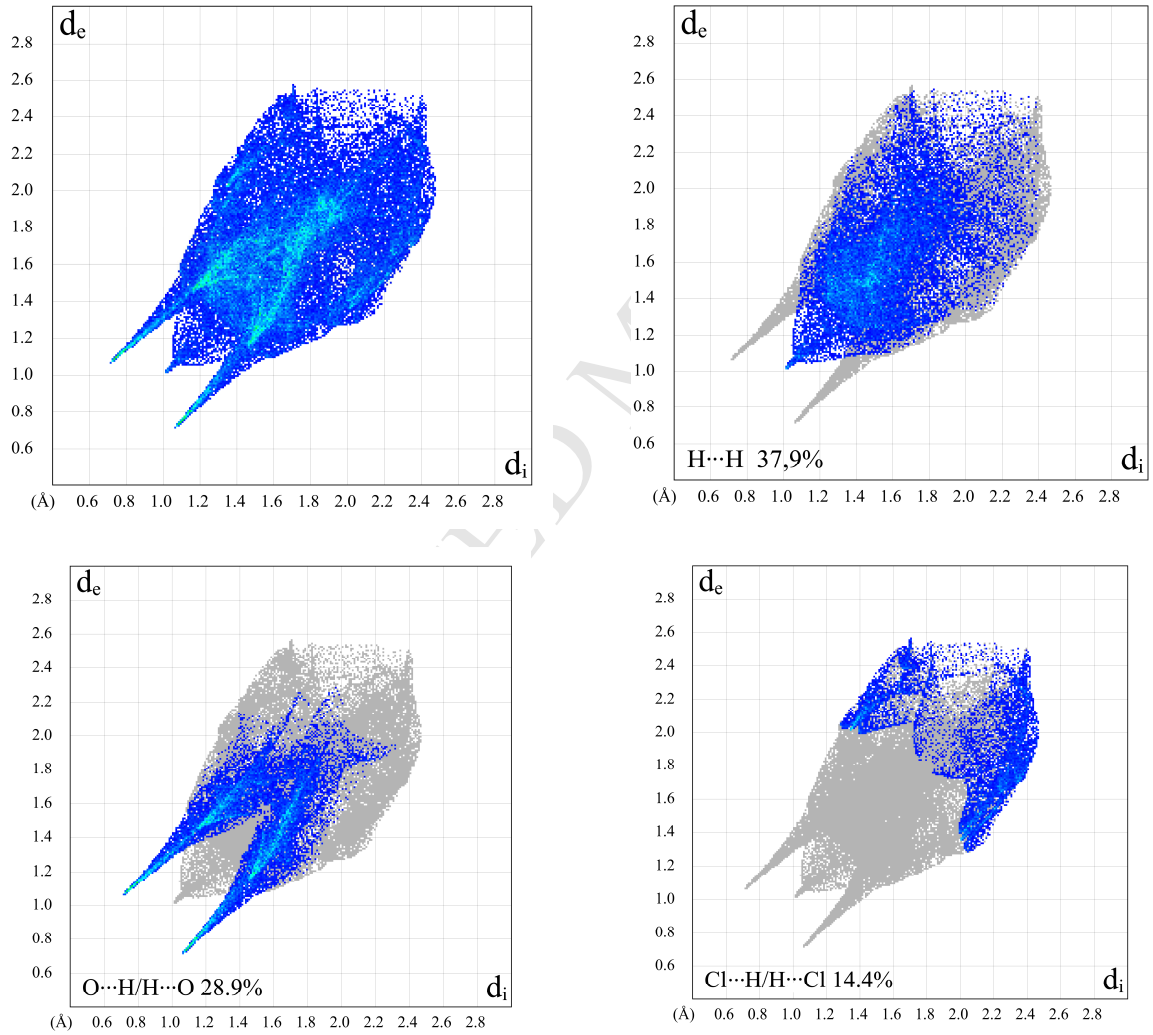
The analysis of the Hirshfeld surfaces extends the information about intermolecular contacts in the analyzed structure. These surfaces show the susceptible areas to strong and weak contacts. It can be identify in the color pattern on the surface as concave red curvature for the acceptor region around to N and O atoms with free electron pairs (Fig. 4a). The convex blue curvature for the donor groups is observed mainly in the N–H, O–H and C–H regions [24,25]. The electrostatic potential formed by baclofen molecule is separated into positive and negative parts (Fig. 4b). These results show that baclofen can approach either positively or negatively charged molecules in biological systems by adjusting its own orientation.



a) b)

Fig 4. *HS of Baclofen mapped with a)* d_{norm} *and b) electrostatic potential, respectively.*

The Fig. 5 illustrates the full fingerprint plot and decompose fingerprint plots (FP), which highlight separately the most important intermolecular contacts for baclofen (to provide context, the outline of the full fingerprint is shown in grey in Fig. 5). The FP present a symmetric behavior where the two sharp peaks projecting towards the bottom of the fingerprint plot are due to strong N–H···O hydrogen bonds [26]. This analysis shows that the H···H intercontacts contribute in largest value to the Hirshfeld surface with a 37.9%. While the O···H interactions contribute with a 28.9%, followed by Cl···H, C···H and Cl···C intercontacts with 14.4, 9.3 and 6.0%, respectively.



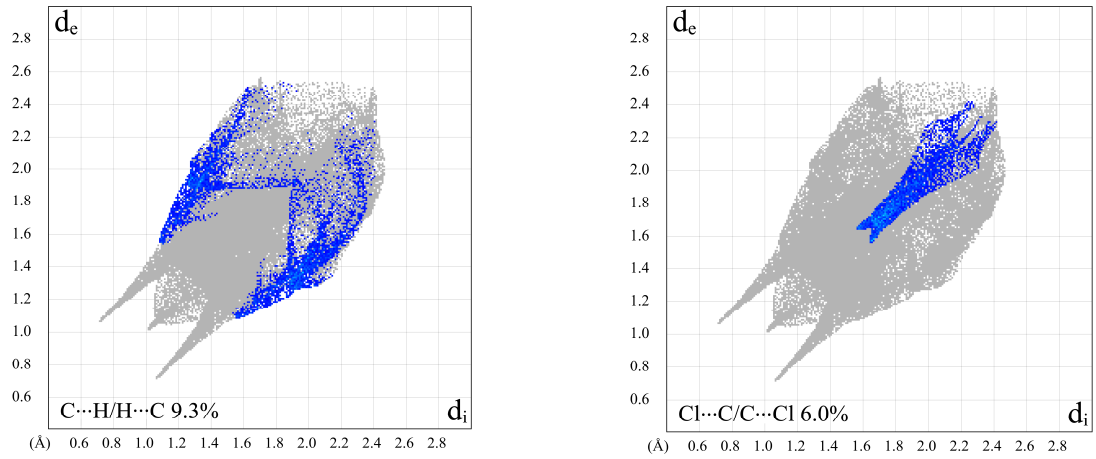


Fig. 5. *Full fingerprint plot and plots resolved (above 3%) into interactions contributed to the total HS area for baclofen molecule, where d_i is the closest internal distance from a given point on the HS, while d_e is the closest external contacts.*

4. Conclusion

In conclusion, we have structurally characterized the racemic baclofen. The molecules of baclofen in the crystal exist as zwitterions, adopting a *gauche* conformation with respect to the C_α–C_β bond. This suggests the possibility that more than one conformer of baclofen may exist in solution. The crystal structure presents the strong N–H···O hydrogen bonds, as well as weak C–Cl···π intermolecular interactions, that build the 3D arrangement. This was also verified by the Hirshfeld surface analysis, which identified in a quantitative way, all relevant interaction in the crystal of baclofen.

Supplementary Data

Supplementary crystallographic data are contained in CCDC-1455243. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: 144-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk) or from the authors.

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Supplementary Information

Structural characterization and Hirshfeld surface analysis of racemic Baclofen.

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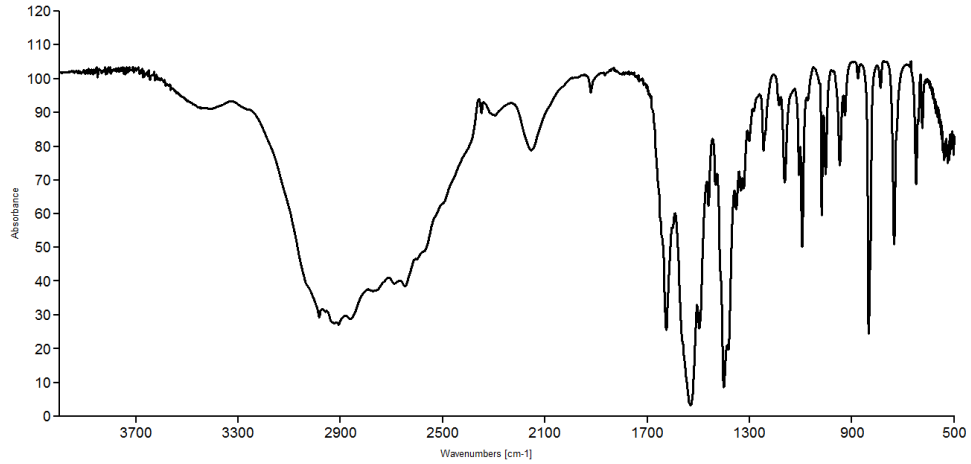

Fig. 1S. *The FT-IR spectrum of the title compound.*

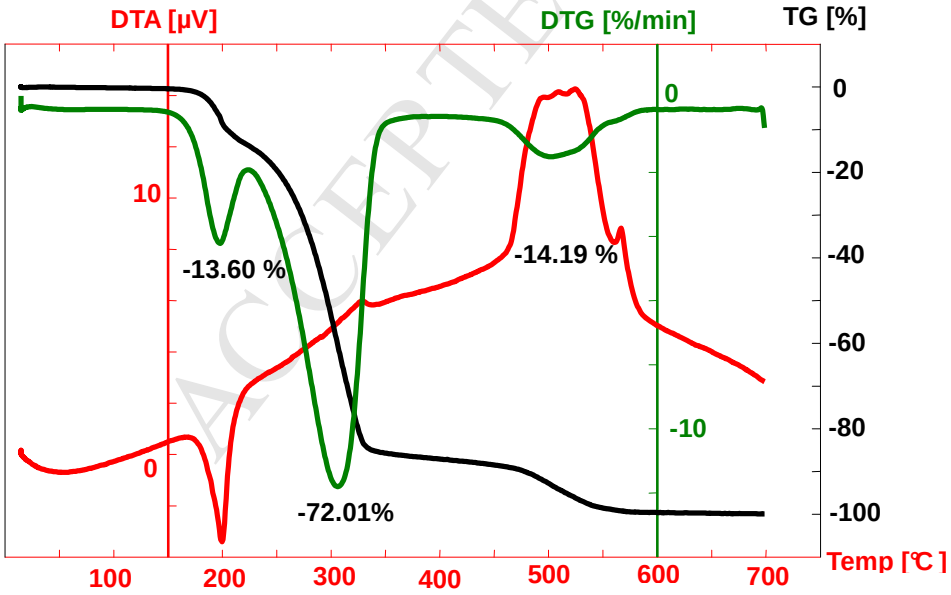

Fig. 2S. *DTA, DTG and TG curves of thermal decomposition of Baclofen recorded in air atmosphere.*