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In silico docking analysis of piperine with cyclooxygenases

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Abstract

The structure of 1-[5-(1,3-benzodioxol- 5-yl)-1-oxo-2,4-pentadienyl]piperidine (Piperine), $C_{17}H_{19}O_3N$, a versatile bioactive molecule has been redetermined at 100(2) K by X-ray crystallography to explore their potential utilization in inhibition of prostaglandin release. The crystal structure is stabilized by weak nonclassical intermolecular C-H…O hydrogen bonds and also intermolecular C-H…π interactions. The crystallographic coordinates of the compound were extrapolated to docking studies to elucidate the action of piperine against the enzymes, cyclooxygenases (COX-1 and COX-2) involved in biosynthesis of prostaglandin release. Using AutoDock suite, piperine was docked at the binding site of COX-1 and COX-2 enzyme and a strong affinity (-9.06kcal/mol, Ki =227.73nM and -8.77kcal/mol, Ki = 375.62nM, respectively) was formed by Hydrogen bonds and hydrophobic interactions. These results suggest that piperine can be a promising lead for the development of COX family inhibitors.

Key words: Prostaglandin, Molecular Docking, X-ray crystallography, COX-1, COX-2.

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Introduction

Piperine is the principal alkaloid present in the fruits of black pepper (Piper nigrum Linn) and long pepper (Piper longum Linn.) belongs to the plant family Piperaceae. Administration of piperine has proven significant increase in the bioavailability of plasma concentrations of rifampin, phenytoin, propranolol and theophylline in humans(Bano et al. 1991).

Compound possesses a diverse biological activities including anti-oxidant, anti-platelet, (Park et al. 2007)anti-inflammatory, analgesic (Agus 2005), anti-hyperlipidemic (Jin et al. 2009), antidepressant and bioavailability-enhancing activity (Srinivasan 2007). The underlying molecular mechanism of such broad range activities of piperine is rather poorly understood and previous molecular interaction studies have shown only with β-lactoglobulin (Zsila et al. 2005), human serum albumin(Suresh et al. 2007) and human monoamine oxidase (Rahman and Rahmatullah). The piperine (figure 1) consists of methylenedioxyphenyl ring, basic piperidine moiety attached through a carbonyl amide linkage and side chain with conjugated double bond. The crystal structure of piperine was redetermined at 100(2) K to accurately determine the position of hydrogen atoms along with the associated supramolecular features.

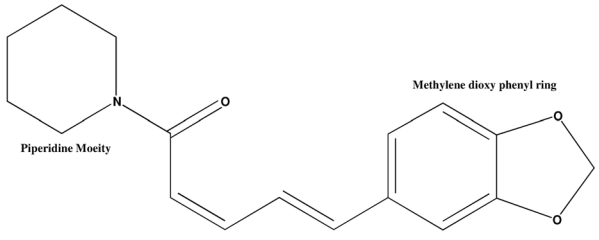

Figure 1: Structure of Piperine

From the experimental evidence, it was shown that piperine has anti-inflammatory properties attributed to inhibition of

prostaglandin release(Agus 2005).Under the influence of COX-1, prostaglandins maintain the integrity of the gastric mucosa, support normal platelet function and control renal blood flow. COX-2 produces prostaglandins through stimulation and its expression is highly restricted; During inflammation COX-2 is dramatically upregulated causing pain and fever.(Crofford 1997) Both COX-1 and COX-2 are the pharmacological targets of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), which are utilized for the treatment of pain and inflammation(Blobaum and Marnett 2007). There are clear differences in DNA, mRNA structure and function between COX-1 and COX-2, less difference between the protein structure and function of these enzymes. The core sequences of both enzymes as well as their crystal structures are 60% identical but their catalytic regions are widely conserved (Picot 1994). The flexibility associated with the side chain of Leu-531 is responsible for increasing the volume at the opening of the cyclooxygenase channel subsequently allowing COX-2 to oxygenate a wide-ranging array of substrates compared to COX-1(Blobaum and Marnett 2007).

These two COX isoforms differ in many respects. COX-1 is constitutively expressed in most tissues and plays an important role in homeostasis. In particular, it is implicated in maintaining the protective lining of the stomach mucosa, regulating the renal blood flow and mediating platelet aggregation at sites of vascular injury (Dubois et al. 1998). By contrast, COX-2 is absent from most normal tissues, except for some specific regions in the brain, kidney and uterus. COX-2 expression is rapidly induced by inflammatory cytokines such as Tumor Necrosis Factor (TNF) and Interleukin (IL) -1 or -6 in leukocytes. Numerous oncogenic mutations in many genes, including c-src, Ras, APC, p53 and STK11/Lkb1, also stimulate COX-2 transcription. Increased amounts of COX-2 are found commonly in both precancerous and cancerous tissues(Christopher S Williams et al. 1999; Tsuji et al. 2001). Furthermore, COX-2 has been shown to be induced in response to chemotherapy and radiotherapy(Steinauer et al. 2000; Subbaramaiah et al. 2000). This increase in COX-2 expression results in enhanced synthesis of PGs in neoplasic and inflamed tissues (Dubois et al. 1998; Subbaramaiah and Dannenberg 2003). COX-2 expression has been documented in most human cancers (Koki et al. 2002). Tumor cells, activated stromal fibroblasts, tumor infiltrating inflammatory cells and angiogenic endothelial cells can also express COX-2 (Masferrer et al. 2000).

Encouraged by these wide varieties of piperine applications and as part of their continuing program to discover COX inhibitory compounds from Indian medicinal plants as reported by the authors previously using curcumin (Girija et al. 2010) against COX-2 In the present study, intermolecular interactions of piperine and its possible mode of biological interaction with COX enzymes were attempted. Docking methodologies were used to test the bioactivity of piperine with COX enzymes. In addition, ligands known to bind COX enzymes were also used to establish relationships between their biological activity and predicted binding affinity.

Materials and Methods

Single Crystal X-ray Diffraction Studies of Piperine

Single crystal with dimensions of (0.38 × 0.30 × 0.15) mm3 was chosen for X-ray diffraction studies. The X-ray diffraction data were collected on a Bruker Smart CCD Area Detector System, at IISc, Bangalore, using MoKa (0.71073 Å) radiation. The structure was solved by direct methods and difference Fourier synthesis using SHELXS97(Sheldrick 1997). The positions of all non-hydrogen atoms were included in the full-matrix least-square refinement using SHELXL97(Sheldrick 1997). The hydrogen atoms were fixed geometrically and allowed to ride on their parent C atoms and refined isotropically. Molecular diagrams were generated using ORTEP(Farrugia 1997). The C-H bond lengths are in the range of 0.94(2)-1.04(3) Å. The experimental conditions, crystal data and structure refinement results are summarized in (Table 1); the atomic coordinates and equivalent isotropic displacement parameters are listed in (Supplementary 1); selected bond lengths and angles are given in (Supplementary 2). The intermolecular hydrogen bonds including the weak interactions are listed in (Supplementary 3).

Table1. Crystallographic details

Empirical Formula	$C_{17}H_{19}NO_3$
Formula weight	285.33
Temperature	273(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	a = 8.722(2)Å b = 13.397(3)Å c = 12.905(3) Å β = 107.449(4)°.
Volume	1438.7(6) $Å^3$
Z, Calculated density	4,1.317 Mg/m^3
Absorption coefficient	0.090 mm^{-1}
F(000)	608
Crystal size	0.3 x 0.2 x 0.1 mm
Theta range for data collection	2.25 to 24.71°.
Limiting indices	-10<=h<=10, -15<=k<=14, -15<=l<=11
Reflections collected / unique	7143 / 2447 [R(int) = 0.0432]
Completeness to theta = 24.71	99.9 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2447 / 0 / 267
Goodness-of-fit on F^2	1.108
Final R indices [I>2sigma(I)]	R1 = 0.0522, wR2 = 0.1035
R indices (all data)	R1 = 0.0740, wR2 = 0.1108
Extinction coefficient	0.0013(12)
Largest diff. peak and hole	0.210 and -0.183 $e.Å^{-3}$

Molecular Docking Studies

Molecular docking simulation of Piperine to COX-1 and COX-2 were performed in order to gain functional and structural insight into the mechanism of inhibition. AutoDock 4.0(Morris et al. 1998) suite was used as molecular-docking tool.

Protein and ligand structure preparation

X-Ray Crystallographic structures of the 2.6Å model of ovine COX-1 complexed with Ibuprofen (PDB: 1EQG) and Crystal structure of arachidonic acid (ACD) bound to the cyclooxygenase active site of COX-2 (PDB: 1CVU) were obtained from the protein databank (www.pdb.org). COX-2 structures (PDB: 1CVU, 1CX2 and 1PXX) have minor differences in their structures (sequence identity >99.5 and RMSD<0.507 Å) (Maldonado-Rojas and Olivero-Verbel). In the present study 1CVU was used as the structure was solved at high resolution of 2.4 Å as compared to other two with 3 Å and 2.90Å, respectively. The structures were edited by deleting all the HETATOMS, water molecules and co-crystallized compounds. Topology file and other force field parameters were generated for piperine using the PRODRG program (Aalten et al. 1996). Flexible torsions for piperine, Ibuprofen and ACD were defined using AUTOTORS. In the present study, the co-crystallized ligands (Ibuprofen and ACD for 1EQG and 1CVU, respectively) were redocked within the inhibitor binding site for the evaluation of the accuracy of AutoDock4.0. As shown in figure 4, the RMSD of

0.131 and 1.705 were observed between the best docked confirmation and crystal structures of Ibuprofen and ACD respectively. This convinced to perform further docking studies of piperine with COX family using AutoDock 4.0.The docking site for piperine on 1EQG and 1CVU was defined at the position of the co-crystallized ligands by using PyRX 0.8 interface (Wolf 2009) with grid box size of 61x50x55, spacing of 0.375, grid centre 25.290, 32.999 and 201.736 for 1EQG and 27.588, 23.209 and 46.329 for 1CVU and assigning 3 Degrees of Freedom. The Lamarckian Genetic Algorithm (LGA) (Morris et al. 1998) was employed with the population size of 150 individuals, maximum number of generations and energy evaluations of 27,000 and 2.5 million respectively. From the estimated free energy of ligand binding (ΔG), the inhibition constant (Ki) for each ligand was calculated. Only the best pose (the one with the lowest binding energy) was considered for each ligand. The best confirmation was analyzed for proteins-piperine interaction using Ligplot+ (Wallace et al. 1995). PyMOL (DeLano 2002) was used for docking confirmation representation.

Results and Discussions

Crystal Structure Analysis

The determination of the crystal and molecular structure of (E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadieny piperidine [C17H19O3N] has been performed at 100(2) K to accurately determine the position of hydrogen atoms along with the associated supramolecular features. The precision of the structure is significantly improved in this new study. It belongs to the space group P21/n with cell parameters a = 8.7223(2), b = 13.3974(2), c = 12.9054(3) Å and β = 107.45(3)°. The piperine molecule is entirely planar and the piperidine ring assumes a chair conformation. This allows the bond to rotate and have 3 degrees of freedom between C1-C2, C4-C3, C5-C51 which in turn has a flexible interaction with COX enzymes. The packing of the molecule is stabilized by synergistic interplay of C-H...O and C-H...π intermolecular interactions in the crystal lattice. These noncovalent interactions not only structurally stabilize the compound but also allow predicting the probable Hydrogen bond formation between the piperine and the active site residues of protein (K. Ramanathan et al. 2011).

Previous report on the determination of the crystal structure (Nethaji and Vasantha 1989) of piperine has highlighted only the significant conformational features of this molecule but not packing features. All the crystallographic studies on piperine have been performed at room temperature. We have performed a determination of the molecular and crystal structure at 100(2)K in order to gain better insights into the structure and especially weak interactions. Subsequent analysis of the crystal packing reveals the importance of intermolecular interactions, which play a pivotal role in the crystal engineering of these molecules. A view of (1) with the atom labeling scheme is given in (Fig.2)

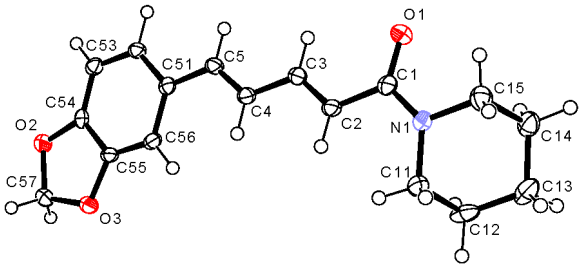

Figure 2: ORTEP diagram of the title compound with 50% ellipsoidal probability

All the bond lengths and bond angles are normal (Table 3) and the molecule is essentially planar. N-Substituted piperidines have been the subject of several X-ray crystallographic investigations and most of them were found to adopt a nearly planar conformation (that is with the N- C = O group lying in the mean plane of the piperidyl ring). Such a conformation would not be preferred in terms of nonbonded interactions alone and is to be ascribed to restricted rotation of freedom with the COX enzymes around the N-C partial bond, as can be easily proved by comparing the observed N-C distances with those expected for a pure single bond.

The piperidyl ring tends to be coplanar with the amide group owing to the partial double bond character of the C(7)-N(1) bond. This is evident from the comprehensive discussion of the C(sp2)-N(piperidyl) bond by Gilli and Bertolasi(Gilli.G and Bertolasi.V 1979) The piperidine ring exhibits a perfect chair conformation(Cremer and Pople 1975) with puckering parameters QT= 0.5620(2) Å , θ = 175.47 (2)° and ψ2= 98.97(2)°.The asymmetry parameter for the piperidine ring ΔCs is 0.345. There is a fairly large spread in the endocyclic torsion angles at N in the piperidyl moiety (N- C15-C14-C13=-56.5° (3), N-C11-C12-C13= 54.0°(3), C15-C14-C13-C12=+52.2° (3), C14-C13-C12-C11 =54.0° (3), C12-C11-N-C15=-59.5° (2), C14-C15-N-C11=61.2° (2). This variation in the values of torsion angles are attributed to the packing effects. Packing effects will influence torsion angles more than bond angles or bond lengths.

Intermolecular Interactions

The packing of molecules is essentially via the involvement of weak intermolecular contacts. C-H...O hydrogen bonds, involving H57A form dimers which are linked to the other molecule by C-H...π dimers, involving H11B and these dimeric motifs are held by additional C-H...O hydrogen bond, involving H57B to generate tetrameric motifs in the crystal lattice (Fig. 3). (Table-4) C57 acts as bifurcated donor. The C-H…O and C-H… π interactions generate structural motifs, which form cavities, and the potential of these cavities to serve as hosts in forming complexes is currently being explored.

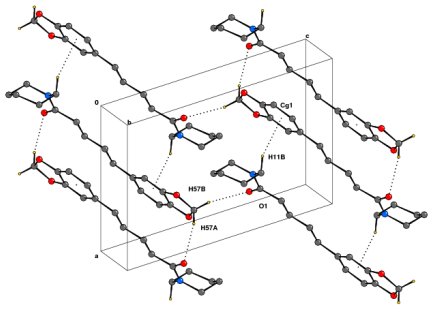

Figure 3: Packing of the molecule showing the tetramer formed by C-H…O and C-H…π Intermolecular interactions

Docking Analysis

Docking affinities of piperine and cocrystallized ligands with COX-1 and COX-2 are depicted in Table 5. Piperine was docked into the active site of COX-1 surrounding by Val116, Arg120, Leu352, Val349, Tyr355, Phe381, Leu384, Tyr385, Trp387, Phe518, Ile523, Gly526, Ala527, Ser530, Leu531 (figure 5a) and with COX-2 residues Phe205, Val344,Tyr348, Val349, Leu352, Ser353, Phe381,Tyr385, Trp387, Phe518, Met522, Val523, Ala527, Ser530, Leu534 as illustrated in (figure 6a).

The least energy confirmation from the docking of piperine into the active site of COX-1 is similar to that of crystallographic ligand Ibuprofen. The conformation with the least binding energy, having maximum cluster size at the active site of the respective protein was considered for interaction studies. Oxygen at the second position of methylene dioxy phenyl ring hydrogen bonded to Tyr355 (A) with bond distance of 2.74Å (O-H...O) and 3.05 Å (N-H…O) with Arg120 (A) as evident by the ligplot diagram 5b. Similar hydrogen bond formation can also be observed with Ibuprofen (not shown in figure). Formation of such hydrogen bonds contributes significantly to interact piperine with COX-1 at the active site. The inhibition constant value (K_i) of piperine is much smaller of 227.73nM than the Ibuprofen 855.46nM.

Table 5: Molecular interaction of piperine and co-crystallized compounds with COX-1 and COX-2.

Target proteins	Compound	IC	BLP	HBL	Interacting residues
COX-1 (1EQG)	Ibuprofen	855.4	-8.28	2.96 2.71 2.76	Arg120(A) Arg120(A) Tyr355(A)
	Piperine	227.7	-9.06	3.05 2.74	Arg120(A) Tyr355(A)
COX-2 (1CVU)	arachidonic acid (ACD)	82760	-5.57	-	-
	Piperine	375.06	-8.77	-	-

IC- Inhibition Constant(Ki)(nM); BLP: Best ligand pose energy (Kcal/mol) HBL: Hydrogen bond length (A˚)

Interaction of piperine with the active site of COX-2 was hydrophobic, which is similar to crystallographic bound substrate Arachidonic Acid as evident by the ligplot diagram 6b. Binding energy of -8.77 Kcal/mol and very low inhibition constant (K_i) of 375.06nM was observed for piperine as compared to ACD of -5.57Kcal/Mol and 82760nM.

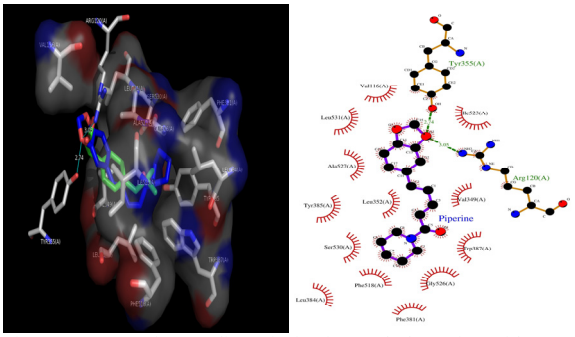

Figure 5: Interaction studies of piperine and Ibuprofen with COX-1. a) Interaction of piperine (blue) and ibuprofen (green) at binding site of COX-1 as shown using PyMOL. b) Ligplot+ representation of interaction of piperine with COX-1. Residues represented in Ligplot+ are labeled and shown in PyMOL as sticks and molecular surface. Hydrogen bonds are represented by lines in PyMOL (a) and dashed lines in Ligplot+ (b)

Conclusion

Although piperine is used worldwide, in the present study its topological analysis of weak and strong noncovalent interactions using crystallographic method is performed and further extrapolated to molecular docking analysis to know the structure-activity relationship. From the analysis it was elucidated that piperine can inhibit the activity of both COX-1 and COX-2 enzymes, hence co-crystallisation of piperine with COX family can be taken up for further design of novel class of compounds in treatment of biosynthesis of prostaglandin release.

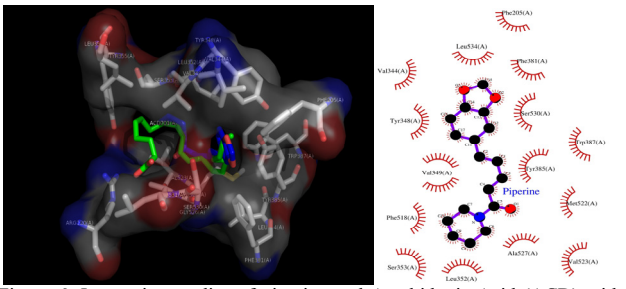

Figure 6: Interaction studies of piperine and Arachidonic Acid (ACD) with COX-2. a) Interaction of piperine (blue) and ACD (green) at binding site of COX-2 as shown using PyMOL.b) Ligplot+ representation of interaction of piperine with COX-2. Residues represented in Ligplot+ are labeled and shown in PyMOL as sticks and molecular surface

Supplementary data are available from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK on request, by quoting the deposition number (288124) for (E, E)-1-[5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl] Piperidine (Piperine)

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Supplementary 1.

Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) of NDA.

$U_{eq} = (1/3)\Sigma_i\Sigma_j U_{ij}(a_i^* a_j^*)(a_i.a_j)$

Atom	x	y	z	U(eq)
O(2)	1609(2)	3668(1)	-3480(1)	26(1)
O(1)	11832(2)	4523(1)	3465(1)	28(1)
C(55)	3770(2)	4410(1)	-2307(2)	19(1)
C(56)	5086(3)	4498(2)	-1423(2)	21(1)
O(3)	3100(2)	5132(1)	-3068(1)	27(1)
C(5)	6970(3)	3677(2)	220(2)	21(1)
C(54)	2887(2)	3541(2)	-2552(2)	20(1)
C(2)	10332(3)	5242(2)	1801(2)	22(1)
N(1)	12634(2)	6052(1)	3082(1)	23(1)
C(51)	5559(2)	3651(1)	-746(2)	19(1)
C(1)	11644(2)	5250(2)	2841(2)	21(1)
C(4)	8011(3)	4432(2)	521(2)	21(1)
C(53)	3290(3)	2705(2)	-1914(2)	22(1)
C(3)	9329(2)	4473(2)	1509(2)	21(1)
C(52)	4648(2)	2777(2)	-1010(2)	21(1)
C(15)	13899(3)	6073(2)	4126(2)	29(1)
C(11)	12358(3)	7037(2)	2566(2)	26(1)
C(12)	11868(3)	7779(2)	3298(2)	32(1)
C(13)	13074(3)	7805(2)	4423(2)	36(1)
C(57)	1769(3)	4665(2)	-3854(2)	25(1)
C(14)	13430(3)	6763(2)	4908(2)	32(1)

Supplementary 2.

Bond lengths [Å] and angles [°]

O(2)-C(54)	1.381(2)
O(2)-C(57)	1.441(2)
O(1)-C(1)	1.242(2)
C(55)-C(56)	1.359(3)
C(55)-O(3)	1.377(2)
C(55)-C(54)	1.380(3)
C(56)-C(51)	1.416(3)
O(3)-C(57)	1.436(3)
C(5)-C(4)	1.337(3)
C(5)-C(51)	1.466(3)
C(54)-C(53)	1.372(3)
C(2)-C(3)	1.331(3)
C(2)-C(1)	1.480(3)
N(1)-C(1)	1.354(3)
N(1)-C(15)	1.464(3)
N(1)-C(11)	1.466(3)
C(51)-C(52)	1.398(3)
C(4)-C(3)	1.439(3)
C(53)-C(52)	1.394(3)
C(15)-C(14)	1.511(3)
C(11)-C(12)	1.518(3)
C(12)-C(13)	1.517(4)
C(13)-C(14)	1.523(3)

Angles	
C(54)-O(2)-C(57)	105.73(15)
C(56)-C(55)-O(3)	127.52(18)
C(56)-C(55)-C(54)	122.57(19)
O(3)-C(55)-C(54)	109.91(18)
C(55)-C(56)-C(51)	117.69(19)
C(55)-O(3)-C(57)	106.18(15)
C(4)-C(5)-C(51)	126.1(2)
C(53)-C(54)-C(55)	121.8(2)
C(53)-C(54)-O(2)	128.07(19)
C(55)-C(54)-O(2)	110.15(17)
C(3)-C(2)-C(1)	121.7(2)
C(1)-N(1)-C(15)	118.63(18)
C(1)-N(1)-C(11)	127.15(19)
C(15)-N(1)-C(11)	112.10(18)
C(52)-C(51)-C(56)	118.8(2)
C(52)-C(51)-C(5)	119.85(18)
C(56)-C(51)-C(5)	121.35(19)
O(1)-C(1)-N(1)	121.5(2)
O(1)-C(1)-C(2)	119.85(19)
N(1)-C(1)-C(2)	118.66(19)
C(5)-C(4)-C(3)	125.4(2)
C(54)-C(53)-C(52)	116.5(2)
C(2)-C(3)-C(4)	124.2(2)
C(53)-C(52)-C(51)	122.69(19)
N(1)-C(15)-C(14)	110.27(19)
N(1)-C(11)-C(12)	110.21(18)
C(13)-C(12)-C(11)	111.5(2)
C(12)-C(13)-C(14)	111.7(2)
O(3)-C(57)-O(2)	107.82(16)
C(15)-C(14)-C(13)	110.4(2)

Supplementary 3

Nonbonded interactions and possible hydrogen bonds (Å).

(D-donor; A-acceptor; H-hydrogen)

D—H· · ·A	**D—H**	**H· · ·A**	**D· · ·A**	**D—H· · ·A**
C57–H57A· · ·O1[i]	1.00(2) 0.99 (2)	2.55(3) 2.51(2)	3.499(3) 3.482(3)	158.1(2) 167.6(2)
C57-H57B· · ·O2[ii]		2.7919(2) 2.8395 (2)	3.629(2) 3.721(2)	137.92(3) 141.69)3)
C13-H13B· · ·Cg3[iii]				
C17-H17A· · ·Cg1[iii]				

Symmetry code: (i) – x+1,-y+1, -z (ii) x-1, +y, +z-1 (iii) 1-x , -y, 1-z