

Synthesis, Characterization, Chemical Reactivity Analyses and Biological Investigations of (E)-1-(5-Chloro-2-Hydroxy Benzylidene)Semicarbazide

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ABSTRACT

The synthesis and detailed characterization of Schiff base (E)-1-(5-chloro-2-hydroxybenzylidene)semicarbazide was performed using combination of experimental and theoretical methods. Experimental spectroscopy studies were conducted utilizing FTIR, FT-Raman, NMR and UV–Vis techniques to identify important functional groups and electronic transitions. Density functional theory (DFT) simulations were carried out to optimize the molecule geometry and to examine the structural parameters, vibrational frequencies and electrical characteristics. The experimental findings were found to be in good agreement with the theoretical vibrational spectra, which confirm the proposed chemical framework. Frontier molecular orbital (HOMO-LUMO) analysis, molecular electrostatic potential (MEP) mapping and natural bond orbital (NBO) analysis were used to explain the chemical reactivity, charge distribution and intramolecular interactions of the substance. Molecular docking studies were also carried out to evaluate the biological activity and possible binding interactions of the title chemical with important protein targets. The combined spectroscopic, quantum-chemical and docking studies give a complete insight into the structural, electrical and pharmacological features of this semicarbazide derivative, revealing its potential for additional biological and materials applications.

Keywords: FT-IR; FT-Raman; HOMO-LUMO; MEP; NBO; Molecular Docking;

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1. INTRODUCTION

Schiff bases are an important class of organic compounds containing an azomethine ($-C=N-$) functional group. Schiff bases are usually formed by condensation of a primary amine with an aldehyde or a ketone. These compounds have received considerable attention due to their easy preparation, structural diversity and ability to coordinate metal ions through donor atoms such as nitrogen, oxygen and sulfur. Because of their tunable electronic structures and facile functionalization, they have found applications in coordination chemistry, catalysis, molecular materials, sensors and development of biologically active compounds [1-3].

Semicarbazones are a very important subclass of Schiff bases in which the azomethine function is conjugated with a semicarbazide group containing a carbonyl. The presence of hydrazinic nitrogen atoms, a carbonyl oxygen, and in many cases other phenolic or hetero-atom donor sites offer multiple opportunities for hydrogen bonding, proton transfer, metal coordination and electronic delocalization. These structural features can have a significant impact on the molecular conformation, charge distribution, redox behavior and biological activity. Schiff bases from semicarbazide and hydroxysemicarbazide have been demonstrated to possess significant antitumor activities[4-6]. The activity of these compounds depends on hydrophobicity, molecular polarizability and the presence of oxygen-containing substituents in the aromatic ring.

The addition of a halogen atom into an aromatic Schiff-base framework is a successful approach to change its physicochemical and biological properties. Chlorine substitution can affect the electronic density of the aromatic ring, the acidity of a neighboring phenolic group, enhance intermolecular interactions and influence the lipophilicity and molecular recognition behavior of the compound. In the title molecule, (E)-1-(5-chloro-2-hydroxybenzylidene)semicarbazide, the 5-chloro-2-hydroxybenzylidene fragment is connected to the semicarbazide unit via an azomethine bond. The ortho-hydroxy substituent may allow for intramolecular hydrogen bonding with the imine nitrogen and the chloro substituent may alter the electronic properties of the conjugated system. These properties make the compound a good model for the study of the relation between molecular shape, electronic distribution and reactivity.

Experimental spectra are of course essential for structural identification but theoretical calculations provide a deeper

interpretation of the molecular properties observed. Density functional theory has proved to be a powerful tool for the optimization of molecular geometries, prediction of structural parameters and assignment of vibrational frequencies. The comparison of the calculated and experimental FTIR and FT-Raman spectra allows for the reliable confirmation of the proposed chemical framework and provides detailed information on the influence of conjugation, hydrogen bonding and substituent effects on the molecular framework. The dipole moment, polarizability, hyperpolarizability and other electrical properties of interest to the potential optical and material applications of the compound can also be evaluated using DFT calculations[7-9].

In the present work, (E)-1-(5-chloro-2-hydroxybenzylidene)semicarbazide was synthesized and studied by a combined experimental and theoretical approach. The major functional groups and electronic transitions were characterized by FTIR, FT-Raman and UV-Visible spectroscopic techniques. The Density Functional Theory (DFT) calculations were performed to optimize the molecular geometry, to determine the structural parameters, to assign the vibrational modes and to evaluate the electrical properties of the compound. The frontier orbital behavior, reactive sites, charge distribution and intramolecular donor-acceptor interactions were studied using HOMO-LUMO, MEP, Fukui function and NBO analyzes. Furthermore, molecular docking studies were performed to investigate the possible binding modes of the compound with biologically significant protein targets. The correlation between experimental and theoretical results gives an integrated understanding of the structural, spectroscopic, electronic and potential pharmacological properties of this chlorinated semicarbazide Schiff base.

2. MATERIAL AND METHODS

2.1. Synthesis

A solution of 2-hydroxy-5-chlorobenzaldehyde (2.0 g, 10 mmol) in ethanol (5 mL) was treated with an aqueous solution of semicarbazide hydrochloride (1.11 g, 10 mmol), and 0.5 mL of concentrated HCl was added. The reaction mixture was stirred for 1 h at room temperature on a magnetic stirrer. The precipitated solid was collected by filtration after completion and washed with cold water and air dried. The crude product was recrystallised from ethanol to yield pure 15CHS. The purity of the compound was checked by thin-layer chromatography (TLC). The synthetic route is shown in Fig.1.

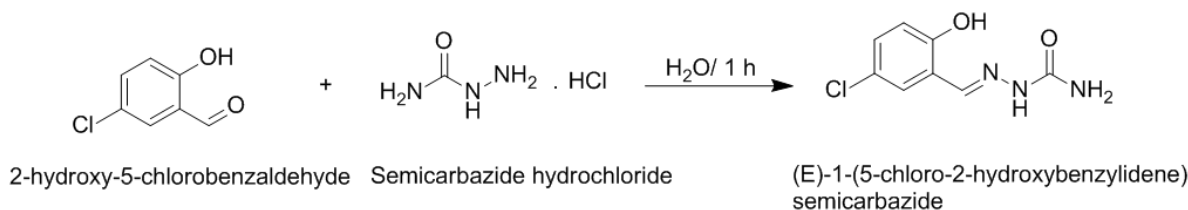


Fig.1. The scheme of the synthesis of 15CHS

2.2 Experimental

The FT-IR and FT-Raman spectrum of 15CHS was recorded at 4000-450 cm⁻¹ and 4000-100 cm⁻¹. The ¹H and ¹³C NMR spectra were recorded in DMSO-d₆ solvent. UV-Vis absorption spectrum was recorded in the range 250-600 nm. All the spectral data was collated at SAIF, IIT Madras, Chennai, India

2.3. Computational information

Initial atomic coordinates for the geometry optimization were obtained from the software package Gaussian 09W [10]. The molecular framework of 15CHS (gas phase) in the ground state was optimised by DFT/B3LYP method with 6-311++G(d,p) basis set and optimised geometry was used for harmonic vibrational calculations. The calculated frequencies were scaled with a factor 0.961. The vibrational frequencies for all the fundamental modes were assigned using the potential energy distribution (PED) analysis with the help of the VEDA 4 program according to the procedures widely adopted in previous investigations [11]. The electronic properties were studied calculating the theoretical UV Vis spectra using time dependent DFT (TD DFT) using the 6 311++G(d,p) basis set in gas phase and DMSO. The ¹H and ¹³C NMR chemical shifts were calculated using the gauge including atomic orbital (GIAO) method at the B3LYP/6311++G(d,p) level and compared with the experimental NMR spectra. Ligand-protein docking simulations were performed using the AutoDock 4.2.6 software package [12].

3. RESULTS AND DISCUSSION

3.1. Molecular geometry

The optimized molecular structure and atom-numbering scheme of 15CHS, obtained using the Gaussian 09W [10] and Chemcraft software packages, are presented in Fig. 2. The calculated geometrical parameters, including bond lengths and bond angles, are listed in Table 1.

To the best of our knowledge, experimentally determined geometrical parameters for 15CHS are not available in the literature. Therefore, the calculated structural parameters of the title compound were compared with the

available crystallographic data for the structurally related molecule

2-[(E)-4-diethylamino-2-hydroxybenzylidene]hydrazinecarboxamide [13]. Such comparisons are useful for assessing the reliability of the optimized geometry. In general, the calculated bond lengths are slightly longer than the corresponding experimental values. This difference is expected because the experimental X-ray diffraction measurements describe the molecule in the crystalline solid state, whereas the DFT calculations generally correspond to an isolated molecule in the gas phase. Similar deviations between theoretical and X-ray structural parameters have been reported for other organic systems.

The 15CHS molecule contains seven C–C bonds, four C–H bonds, three C–N/N–H bonds, two C–O bonds and one each of an N–N, C–Cl and O–H bond. Among the calculated structural parameters, the C6–C7 bond exhibits the greatest length, with experimental and theoretical values of 1.466 and 1.463 Å, respectively. The calculated C–C and C–H bond lengths within the benzene ring fall in the ranges of 1.385–1.446 and 0.960–1.091 Å, respectively, at the B3LYP/6-311++G(d,p) level. These values are in reasonable agreement with the reported experimental data for the related compound, although small deviations are observed for individual bonds.

The calculated bond lengths also reflect the influence of atomic identity and bonding environment. In general, the homonuclear C–C and N–N bonds are longer than the corresponding heteronuclear C–H and N–H bonds. This behavior is mainly associated with differences in atomic size, effective nuclear charge and electronegativity. Furthermore, electrostatic interactions between atoms bearing like charges are repulsive, whereas interactions between oppositely charged atoms are attractive. These effects, together with bond order, resonance and conjugation within the molecule, contribute to the observed variation in the calculated geometrical parameters. The overall agreement between the theoretical and available experimental values supports the reliability of the optimized molecular structure of 15CHS.

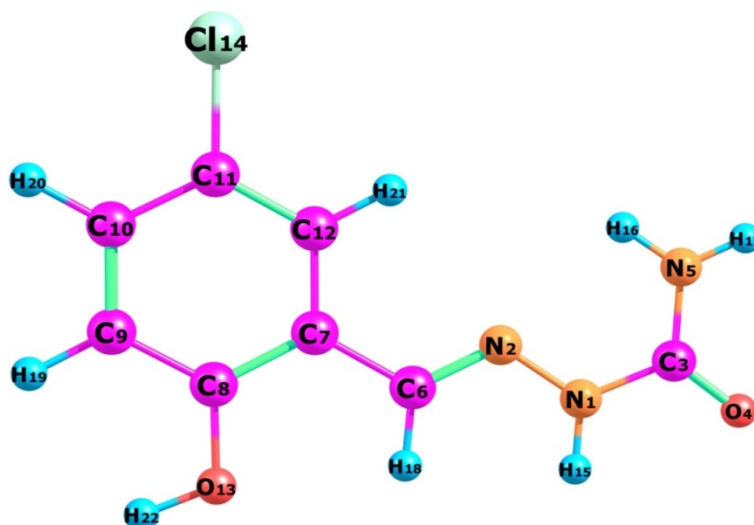


Fig.2. Optimized geometric structure with atoms numbering of 15CHS

Table 1. Geometrical parameters of 15CHS

Parameters	Experimental ^a	B3LYP/ 6-311++G(d,p)	Parameters	Experimental ^a	B3LYP/ 6-311++G(d,p)
Bond length(Å)			Bond angle(°)		
N1-N2	1.378	1.35	N2-N1-C3	120.3	122.7
N1-C3	1.376	1.396	N2-N1-H15	121.8	121.8
N1-H15	0.944	1.016	N1-N2-C6	120.3	118.1
N2-C6	1.287	1.284	C3-N1-H15	117.2	115.5
C3-O4	1.36	1.219	N1-C3-O4	-	120
C3-N5	1.35	1.359	N1-C3-N5	117.4	114.8
N5-H16	0.944	1.006	N2-C6-C7	120.6	121.8
N5-H17	0.944	1.005	N2-C6-H18	118.9	121.3
C6-C7	1.446	1.463	O4-C3-N5	-	125.2
C6-H18	0.96	1.091	C3-N5-H16	124	121
C7-C8	1.406	1.408	C3-H5-H17	117.2	117.8
C7-C12	1.406	1.403	H16-N5-H17	120.5	121.2
C8-C9	1.385	1.394	C7-C6-H18	118.9	117
C8-O13	1.36	1.369	C6-C7-C8	120.8	119.6
C9-C10	1.387	1.39	C6-C7-C12	121.4	122.1
C9-H19	0.96	1.086	C8-C7-C12	117.3	118.3
C10-C11	1.387	1.393	C7-C8-C9	120.8	120.5
C10-H20	0.96	1.082	C7-C8-O13	117.6	117.5
C11-C12	1.387	1.384	C7-C12-C11	120.8	120.6
C11-Cl14	-	1.76	C7-C12-H21	118.3	119
C12-H21	0.96	1.081	C9-C8-O13	120.6	122
O13-H22	0.874	0.963	C8-C9-C10	120.8	120.6
			C8-C9-H19	119.6	119.8
			C8-O13-H22	-	110

			C10-C9-H19	119.6	119.6
			C9-C10-H11	120.6	119
			C9-C10-H20	119.7	120.6
			C11-C10-H20	119.7	120.5
			C10-C11-C12	20.8	121
			C10-C11-C114	-	119.4
			C12-C11-C114	-	119.6
			C11-C12-H21	119.6	120.5

^aTaken from Ref [13].

3.2 Vibrational investigations

Vibrational spectroscopy has a prominent role in the organic chemistry field, in particular in the identification and characterisation of functional groups and in the study of molecular conformations, reaction kinetics and associated chemical processes. The title molecule, 15CHS, contains 22 atoms and hence has 60 normal modes of vibration. A full assignment of these basic vibrational modes with the corresponding values of the potential energy distribution (PED) is given in Table 4.2. the comparative spectra of FTIR and FT-Raman spectra as shown in Fig. 3.

(a) NH₂ vibrations

The NH₂ group has two N-H stretching modes, asymmetric (higher frequency) and symmetric (lower frequency). This pattern is characteristic for primary amines where the asymmetric stretch is expected to be at higher wavenumber than the symmetric stretch[14].

The stretching vibrations of C-H and N-H bonds for aromatic compounds are found above 3000 cm⁻¹, a region that is often used for fast structural identification[15-16]. For the 15CHS having only one NH₂ group, one asymmetric(γ_{as}) and one symmetric(γ_s) N-H stretching vibration are expected. The asymmetric stretch NH₂ for primary aromatic amines usually occurs between about 3500 and 3420 cm⁻¹ [17] and the symmetric stretch between about 3420 and 3340 cm⁻¹.

The calculated NH₂ wavenumbers at 3603(γ_{as}) and 3470 cm⁻¹ (γ_s) with PED contributions of 61% and 100%. Present study the very weak FT-Raman band at 3598 cm⁻¹ is assigned to γ_{as} of NH₂. The NH₂ γ_s stretching shows a very strong band in FT-IR and a very weak band in FT-Raman. The experimental observation is at 3479 cm⁻¹ (strong) in FT-IR and 3470 cm⁻¹ (very weak) in FT-Raman.

(b) C-H vibrations

The C-H stretching vibrations in aromatic systems are usually found in the 3100–3000 cm⁻¹ region, which is a characteristic feature of unsaturation and aromaticity[18]. In this region, band overlap and coupling often complicate matters, making detailed assignment more difficult than for isolated aliphatic C-H stretches.

Furthermore, these aromatic C-H stretching frequencies are relatively insensitive to the nature and position of substituents.

Most of the aromatic compounds show several IR bands in the 3080–3010 cm⁻¹ range due to the stretching of the ring C-H [19]. C-H stretching vibrations in B3LYP/6-311++G(d,p) calculations at 3091, 3080, 3045 and 2975 cm⁻¹ (modes 56-53) are in good agreement with experiment in the present study. The FT-IR spectrum shows bands at 3059cm⁻¹ (very strong) and 2973cm⁻¹ (very strong) while FT-Raman spectrum shows bands at 3071cm⁻¹ (strong) and 2973cm⁻¹ (weak). The corresponding PED values for these essentially pure modes are 100%, 97%, 97% and 100%, respectively (Table 4.2). The band around 2975 cm⁻¹ likely has contributions from aliphatic C-H stretching, which typically lies in the range of about 2970–2850 cm⁻¹.

(c) C–C ring vibrations

C–C stretching vibrations in aromatic rings are expected to occur typically in the range of 1650 to 1100 cm⁻¹ and are not strongly perturbed by substituents[20]. The C–C stretching vibrations were observed in the range 1580–860cm⁻¹ for 15CHS compound.

The C-C stretching bands were observed at 1580 (very strong), 1204 (strong), 1186 (strong), 1180 (very strong), 871 cm⁻¹ (weak) in FT-IR and at 1570 (very strong), 1385 (weak), 1267 (weak), 1207 (medium), 1185 (weak), 1162 (very strong), and 1080 cm⁻¹ (medium) in FT-Raman. Theoretical wavenumbers at 1572, 1391, 1328, 1221, 1189, 1156, 1124 and 862 cm⁻¹ (modes 50, 45, 42, 40, 39, 38, 36, 30) are assigned as C-C stretch vibrations with PED contributions of 42, 60, 48, 15, 18, 25, 21, and 17%, respectively. The intense bands at 1580-1570 cm⁻¹ are consistent with characteristic modes of the aromatic ring normally observed near 1600 and 1500cm⁻¹.

(d) C-N vibrations

The assignment of C–N stretching frequencies is complicated by the congestion of this region and the frequent mixing of several vibrational modes[15]. C–N stretching absorptions are often observed for aromatic amines in the 1350–1250 cm⁻¹ range, but some higher frequency contributions may also be present when coupled to ring modes.

In the present work, the bands at 1603 (very strong), 1342 (very strong) and 871 cm^{-1} (weak) in FT-IR and 1603 (very strong), 1345 (weak), 1303 (very strong) and 934 cm^{-1} (weak) in FT-Raman are assigned to N-C stretching vibrations. The calculated wavenumbers are 1597, 1343,

1322, 946 and 882 cm^{-1} (modes 51, 44, 43, 33, 30) theoretically scaled and assigned to C–N stretching vibrations with PED contributions of 61%, 37%, 25%, 55% and 18% respectively.

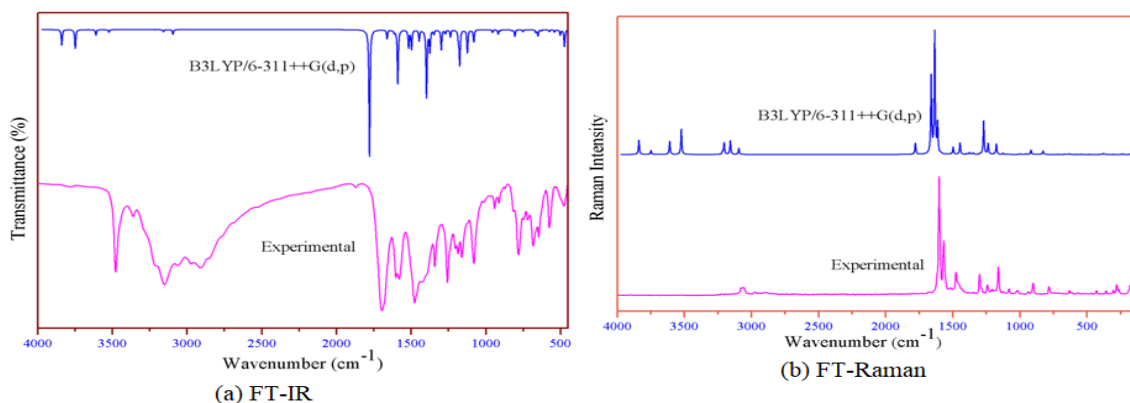


Fig.3. (a) FTIR, (b) FT-Raman spectra of 15CHS (Experimental, B3LYP/6-311++G(d,p))

Table 2. Observed and calculated vibrational frequency of 15CHS molecule

Mode	Experimental wave number (cm^{-1})		Theoretical wave number (cm^{-1}) Scaled ^b	I_{IR}^c	I_{RAMAN}^d	Assignments (PED) ^a
	FTIR	FT-Raman				
60	-	-	3690	12	12	$\gamma_{\text{OH}}(100)$
59	-	3598(vw)	3603	15	3	$\gamma_{\text{asNH}}(61)$
58	3479(vs)	3470(vw)	3470	4	11	$\gamma_{\text{s}} \text{NH}(100)$
57	3361(m)	-	3375	2	21	$\gamma_{\text{NH}}(100)$
56	-	-	3091	0	2	$\gamma_{\text{CH}}(100)$
55	-	3071(s)	3080	0	10	$\gamma_{\text{CH}}(97)$
54	3059(vs)	-	3045	2	11	$\gamma_{\text{CH}}(97)$
53	2973(vs)	2973(w)	2975	4	5	$\gamma_{\text{CH}}(100)$
52	1696(vs)		1710	100	9	$\gamma_{\text{OC}}(69)+\gamma_{\text{NC}}(10)$
51	1603(vs)	1603(vs)	1597	7	64	$\gamma_{\text{NC}}(61)$
50	1580(vs)	1570(vs)	1572	0	100	$\gamma_{\text{CC}}(42)$
49	-	-	1551	2	25	$\gamma_{\text{CC}}(35)+\beta_{\text{CCC}}(14)+\gamma_{\text{CC}}(20)$
48	-	1516(w)	1528	43	1	$\beta_{\text{HNN}}(76)$
47	1477(vs)		1459	14	0	$\beta_{\text{HNN}}(-11)+\beta_{\text{HCC}}(31)$
46	1423(s)	1430(w)	1440	15	6	$\beta_{\text{HNN}}(37)+\beta_{\text{HCN}}(13)$
45	-	1385(w)	1391	9	9	$\gamma_{\text{CC}}(60)+\beta_{\text{HCC}}(15)$
44	1342(vs)	1345(w)	1343	53	1	$\gamma_{\text{NC}}(37)+\beta_{\text{OCN}}(15)+\beta_{\text{HNN}}(13)+\beta_{\text{HNN}}(16)$
43	-	1303(vs)	1322	17	2	$\gamma_{\text{NC}}(25)+\beta_{\text{HCN}}(51)+\beta_{\text{HNN}}(12)$
42	-	1267(w)	1276	3	1	$\gamma_{\text{CC}}(48)+\beta_{\text{HOC}}(22)$
41	1258(vs)	1242(m)	1248	16	0	$\gamma_{\text{OC}}(14)+\beta_{\text{HCC}}(34)$
40	1204(s)	1207(m)	1221	3	28	$\gamma_{\text{OC}}(30)+\gamma_{\text{CC}}(15)+\beta_{\text{HCC}}(21)$
39	1186(s)	1185(w)	1189	6	9	$\gamma_{\text{CC}}(18)+\beta_{\text{HOC}}(16)+\beta_{\text{HCN}}(11)$

38	-	1162(vs)	1156	6	0	γ CC(25)+ β HOC(23)+ β HCC(44)
37	-	1129(w)	1130	28	9	γ NN(51)
36	1080(vs)	1080(m)	1081	18	1	β CCC(10)+ γ CC(21)+ β HOC(13)+ β HCC(12)
35	-		1057	0	0	β HNC(10)+ γ CC(36)+ β HCC(15)
34	1018(w)	1020(m)	1039	10	0	β HNC(51)
33	-	934(w)	946	0	1	β NNC(12)+ γ NC(55)
32	913(w)	912(s)	918	2	1	τ HCNN(82)
31	-	-	898	0	0	τ HCCC(60)
30	871(w)	-	882	4	4	γ ClC(13)+ β CCC(15)+ γ CC(17)+ γ NC(18)
29	-	-	868	1	0	τ CCCC(10)+ τ HCCC(63)
28	-	785(m)	796	1	3	β CCC(26)+ γ OC(17)
27	782(s)		775	5	0	OUT OCCC(14)+ τ HCCC(79)
26	720(m)		724	2	0	OUT ONNC(88)
25	683(s)	674(w)	689	0	0	τ CCCC(32)+OUT CCCC(18)+OUT OCCC(20)
24	646(s)	-	641	2	0	β CCC(30)
23	-	630(w)	627	5	0	β NCC(11)+ γ ClC(18)+ β CCC(12)+ γ OC(12)
22	-	611(w)	604	1	0	β CNN(15)+ β CCC(13)
21	-	565(w)	555	1	0	OUT ClCCC(14)+ τ CCCC(25)+OUT CCCC(14)+ τ HCCC(11)
20	576(s)	-	552	1	0	τ HNCN(73)+ τ HNNC(13)
19	-	523(w)	520	2	0	β OCN(56)
18	478(w)		483	4	0	β NCN(14)+ β CCC(15)+ β OCC(29)
17	-	471(w)	464	0	0	τ HNNC(10)+ τ CCCC(16)+OUT OCCC(26)+OUT CCCC(14)
16	-	430(m)	456	13	0	τ HNNC(68)
15	-	377(m)	383	3	0	β NCN(20)+ β NNC(11)+ β OCC(3)+ β CCC(16)
14	-	361(w)	362	1	1	β CCC(14)+ γ ClC(42)
13	-		344	0	0	OUT ClCCC(22)+ τ CCCC(36)+OUT OCCC(21)
12	-	304(m)	293	1	0	β ClCC(43)+ β NCC(13)
11	-	-	286	2	0	τ NCNN(21)+ τ NNCC(36)+OUT ClCCC(13)+ τ CCCC(11)
10	-	268(m)	256	3	0	τ HOCC(69)
9	-	-	225	0	0	β ClCC(16)+ γ CC(11)+ β OCC(19)
8	-	-	225	20	0	τ CNNC(19)+ τ HOCC(29)+ τ CCCC(11)
7	-	162(m)	171	1	0	β CNN(29)+ β CCC(23)+ β NCN(13)+ β ClCC(15)
6	-	134(w)	123	0	0	τ NCCC(10)+ τ CNNC(13)+ τ CCCC(15)+OUT ClCCC(26)+OUT CCCC(15)
5	-	106(m)	106	0	0	τ CNNC(17)+ τ CCCC(16)+ τ CCCC(35)
4	-	-	57	0	0	β NCC(33)+ β CNN(11)+ β NNC(22)+ β CCC(25)
3	-	-	53	1	0	τ NCNN(40)+ τ NNCC(20)+OUT CCCC(13)+ τ CNNC(15)
2	-	-	24	0	0	τ NCCC(67)+ τ CNNC(26)
1	-	-	16	15	0	τ HNCN(14)+ τ HNCN(35)+ τ NCNN(12)

^a γ -stretching, γ -Symmetrical stretching, γ -as-asymmetrical stretching, β - inplane bending, ω - outplane bending, τ -torsion, vs-very strong, s- strong, m-medium, w-weak, vw-very weak.

^bscaling factor : 0.961 for B3LYP/6-311+G(d,p)

^cRelative absorption intensities normalized with highest peak absorption equal to 100.

^dRelative Raman intensities normalized to 100.

3.3 NMR spectroscopy

NMR spectroscopy is one of the most powerful methods for structural and conformational analysis of organic compounds. The ¹H and ¹³C NMR have been applied in the present work to get detailed information about the electronic environment of the nuclei in the title molecule. The NMR spectra obtained in DMSO-d₆ are given in Fig. 4 and values are displayed in Table 3.

The 15CHS molecule has eight ring carbon atoms. The ¹³C NMR chemical shifts for sp² carbons are typically above 100 ppm[21]. The aromatic carbon resonances of the title compound are found in the range of 120 - 155 ppm. The high electronegativity of the oxygen and

nitrogen atoms polarises the electron distribution in the bonds to the neighbouring carbon atoms, reducing the electron density and adjacent ring positions. The resulting deshielding effect renders the carbons directly attached or much affected by these heteroatoms, relatively downfield. Therefore, in DMSO d₆ the carbon resonances of C3 and C8 are at 154.84 and 135.94 ppm as listed in table 3. These values are consistent with the anticipated effect of electronegative substituents on aromatic ¹³C chemical shifts. The calculated ¹³C NMR chemical shifts are in good agreement with the experimental data.

The molecule also has eight hydrogen atoms linked to the aromatic ring, NH and NH₂ groups. The experimental ¹H NMR chemical shifts are observed at 7.93, 6.85, 6.57, 8.09, 7.15, 7.17, 10.21 and 6.87 ppm and are assigned to H15, H16, H17, H18, H19, H20, H21 and H22, respectively. The corresponding theoretical chemical shifts calculated at the same theoretical level are 7.96, 5.55, 5.11, 8.04, 6.79, 7.09, 8.38 and 6.14 ppm. The calculated ¹H chemical shifts are in overall good agreement with the experimental ones, supporting the proposed structural assignment.

Fig.4. (a) ¹³C NMR, (b) ¹H NMR spectrum of 15CHS (Experimental)

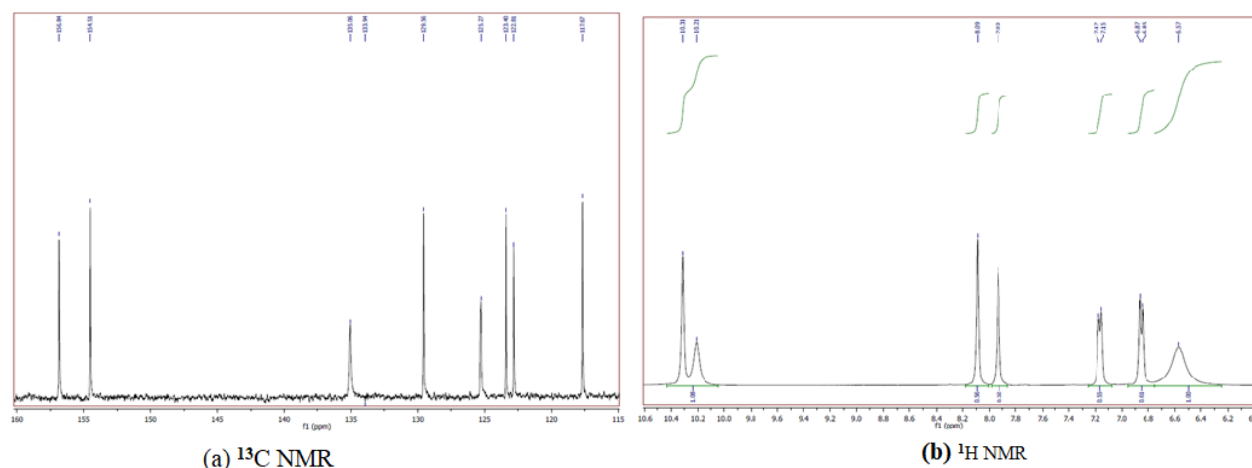


Table 3. Theoretical and experimental ¹³C and ¹H isotropic chemical shifts [with respect to TMS, all values in ppm] for 15CHS molecule

Atom	chemical shifts (ppm)	
	Experimental	B3LYP/6-311++G(d,p)
C3	154.84	153.73
C6	133.94	131.49
C7	117.67	118.19
C8	135.94	136.61
C9	122.81	121.33
C10	125.27	126.17
C11	129.56	129.26

C12	123.40	124.32
H15	7.93	7.96
H16	6.85	5.55
H17	6.57	5.11
H18	8.09	8.04
H19	7.15	6.79
H20	7.17	7.09
H21	10.21	8.38
H22	6.87	6.14

3.4. Electronic properties

The UV–Vis absorption behaviour of 15CHS was examined by experimental observations and TD-DFT computations. TD-DFT has become a commonly utilised approach to probe static and dynamic properties of molecules in their excited states, providing a favourable trade off between accuracy and processing cost.

Fig.5 compares the observed and predicted UV–Vis spectra of the title chemical. The λ maximum was determined. The analysis of the molecular orbitals shows that the principal visible absorption band of 15CHS is due to an electronic transition from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). Experimental UV-Vis spectra were recorded in DMSO, whereas theoretical calculations were performed in DMSO solvent and in gas phase. The comparison of estimated absorption wavelengths, band

gap energies, excitation energies and oscillator strengths with the experimental results are obtained in Table 4.

The observed and computed λ_{max} values are 284 nm (experimental), 290 nm (TD-DFT/DMSO) and 288 nm (TD-DFT/gas phase), as shown in Table 4. The band gap energy (ΔE) is a significant parameter to identify the stability and reactivity of molecule. The HOMO and LUMO energies are closely connected to ionisation potential (I) and electron affinity (A) correspondingly. The optical band gap obtained from the experimental UV–Vis spectrum is 4.3722 eV. The related theoretical band gaps are 4.2866 eV (TD-DFT/DMSO) and 4.3164 eV (TD-DFT/gas phase) while the HOMO-LUMO energy difference from the orbital diagram is 4.2316 eV as shown in Fig. 6. All these values are in good agreement (Tables 4 and 5) indicating the dependability of the computational model and the assigned electronic transitions.

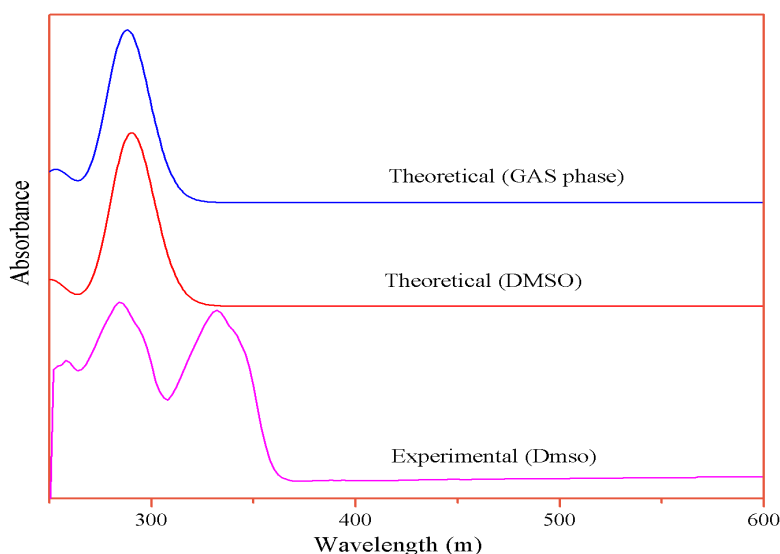


Fig.5. UV–Vis spectra of 15CHS (Experimental, Theoretical (DMSO, GAS phase))

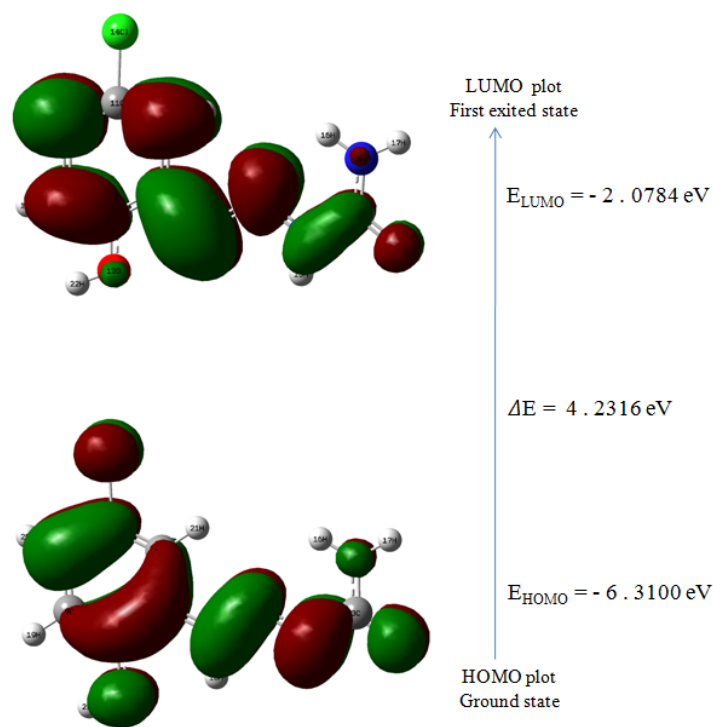


Fig.6. Atomic orbital HOMO – LUMO composition of the frontier molecular orbital for 15CHS

Table 4. The UV–vis wavelength (λ), band gap energy (ΔE), and oscillator strength (f) for 15CHS calculated by TD-DFT/B3LYP method

Experimental		TD-DFT/B3LYP-311++G(d,p)								
$\lambda_{\max}$ (nm)	Band gap (ev)	DMSO				Gas phase				Assignment
		λ_{cal} (nm)	Band gap (eV)	Energy (cm ⁻¹)	f	λ_{cal} (nm)	Band gap (eV)	Energy (cm ⁻¹)	f	
284	4.3722	290	4.2866	34489	0.6228	288	4.3164	34746	0.5074	HOMO->LUMO (96%)
		250	4.9725	39987	0.0954	252	4.9330	39582	0.0975	H-1->LUMO (89%), HOMO->L+4 (7%)
		232	5.3583	43038	0.0005	228	5.4523	43851	0.0006	H-5->LUMO (77%), H-3->LUMO (10%)

Table 5. Calculated energy values of title compound by B3LYP/6-311++G(d,p) method.

Basis set	B3LYP/6-311++G(d, p)
EHOMO (eV)	-6.3100
ELUMO (eV)	-2.0784
Ionization potential	6.3100
Electron affinity	2.0784
Energy gap (eV)	4.2316
Electronegativity	4.1942
Chemical potential	-4.1942

Chemical hardness	2.1158
Chemical softness	0.2363
Electrophilicity index	4.1571

3.5. Molecular Electrostatic Potential (MEP)

Electrostatic potential maps are three-dimensional representations of charge distributions in molecules. These maps are usually employed to identify reactive sites by displaying regions of different electrostatic potential. This facilitates the prediction of how a molecule interacts with electrophiles and nucleophiles. The mapping of MEP is particularly useful in the study of structure-property connections and physicochemical behavior[23]. The electrostatic potential levels are represented by different colours that usually follow the order: red < orange < yellow < green < cyan < blue in the MEP maps.

Regions of high (positive) potential are shown in blue and are indicative of regions of relative lack of electrons, such as in the vicinity of hydrogen atoms attached to electronegative atoms. Red areas, in contrast, indicate high electron density and a strong negative potential. These areas are usually located around electronegative elements (oxygen, nitrogen) as shown in Fig 7.

Oxygen is more electronegative than nitrogen, therefore it usually pulls more electron density, although the actual MEP distribution relies on the molecular environment, bonding, and orientation of the lone pair. In the 15CHS MEP, the electron-rich centers are well localised over both the oxygen and nitrogen atoms, as can be seen from the red and yellow regions. These locations are the most preferred ones for electrophilic contact and hydrogen-bond acceptor.

3.6. NLO activity

Nonlinear optical (NLO) properties are a subject of intense current research as they allow for essential functions such as frequency conversion, optical modulation, switching, logic operations and optical memory for emerging technologies in telecommunications, signal processing and optical interconnects [24]. The total static dipole moment (μ), mean linear polarisability (α) and first-order hyperpolarizability (β) of the new molecular system 15CHS were calculated and the finite-field approach was employed to evaluate the NLO response. The hyperpolarizability values are very sensitive to the basis set and level of theory since electron correlation effects can result in substantial changes in β . The procedures to obtain μ , α and β from the output of Gaussian have been described in detail elsewhere. DFT is widely accepted as an efficient and reliable method to study organic NLO materials.

Urea is the benchmark prototype for the NLO properties and is extensively used as a threshold reference compound. Table 6 shows the values of the dipole moment and hyperpolarizability calculated by B3LYP/6-311++G(d,p). The first order hyperpolarizability of 15CHS is found to be $\beta = 9.4327 \times 10^{-30}$ esu and it is approximately fifteen times larger than urea ($\beta_0 = 0.6230 \times 10^{-30}$ esu). The enhancement in the NLO response is mainly attributed to the intramolecular charge transfer between the benzene rings in the molecular framework which confirms the strong nonlinear optical character of 15CHS.

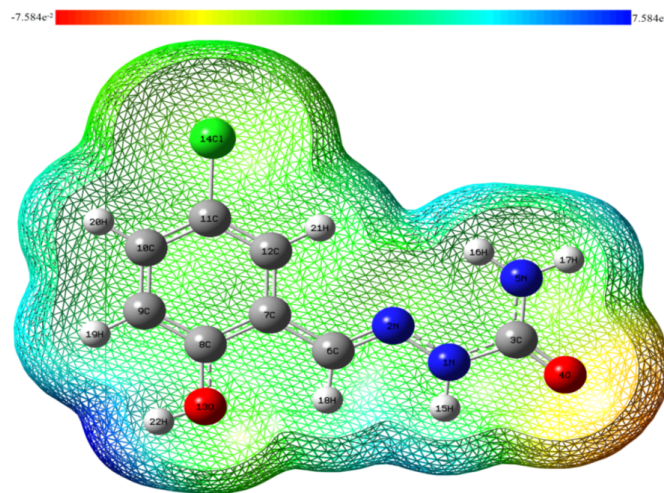


Fig. 7. Total electron density mapped with molecular electrostatic potential surface of 15CHS

Table 6 The values of calculated dipole moment (μ) (D), polarizability (α), first order hyperpolarizability (β_{tot}) components of 15CHS and urea.

Parameters	B3LYP/6-311++G(d,p)	
	15CHS	Urea
$\mu(\text{D})$	2.1516	1.7676
$\alpha_0(\text{e.s.u})$	2.2256×10^{-23}	0.5244×10^{-23}
$\alpha(\text{e.s.u})$	5.0619×10^{-23}	0.6880×10^{-23}
$\beta_{\text{tot}}(\text{e.s.u})$	9.4327×10^{-30}	0.6230×10^{-30}

3.7. NBO Analyses

Natural Bond Orbital (NBO) analysis provides a rigorous framework for identification of donor-acceptor interactions and quantification of their stabilising effect on the molecular electronic structure [25]. The $\sigma(\text{C7-C8})$ bond donates into $\sigma^*(\text{C6-C7})$, $\sigma^*(\text{C7-C12})$ and $\sigma^*(\text{C8-C9})$, and the $\pi(\text{C7-C8})$ conjugates with $\pi^*(\text{N2-C6})$, $\pi^*(\text{C9-C10})$ and $\pi^*(\text{C11-C12})$ with stabilisation energies of 26.58, 19.71 and 25.02 kJ mol⁻¹, respectively.

Important lone pair $\rightarrow$ antibond interactions are: LP(1)N1, LP(2)O4, LP(1)N5 and LP(2)O13 $\rightarrow$ $\sigma^*(\text{N2-C6})$, $\sigma^*(\text{C3-O4})$, $\sigma^*(\text{N1-C3})$, $\sigma^*(\text{C3-N5})$, $\pi^*(\text{C3-O4})$ and $\pi^*(\text{C7-C8})$ with the corresponding E(2) values of 30.72, 52.75, 24.28, 23.55, 54.86 and 25.99 kJ mol⁻¹ (Table 7). These high stabilisation energies, particularly for O and N lone pairs, indicate considerable charge transfer and nucleophilic character, in line with MEP maps and molecular docking results.

Table 7. Second order perturbation theory analysis of Fock matrix in NBO basis for 15CHS

Donor(i)	Type	ED/e	Acceptor(i)	Type	ED/e	^a E(2) (KJ mol ⁻¹)	^b E(J)-E(i) (a.u.)	^c F(I _j) (a.u.)
N ₂ -C ₆	π	1.9221	C ₇ -C ₈	π^*	0.4383	12.89	0.39	0.069
N ₅ -H ₁₆	σ	1.9903	C ₃ -O ₄	σ^*	0.3733	4.12	1.31	0.066
N ₅ -H ₁₇	σ	1.9897	N ₁ -C ₃	σ^*	0.0719	4.33	1.11	0.063
C ₆ -C ₇	σ	1.9615	N ₁ -N ₂	σ^*	0.0244	4.54	1.13	0.064
			N ₂ -C ₆	σ^*	0.0124	4.44	1.39	0.071
			C ₇ -C ₈	σ^*	0.0377	5.69	1.38	0.079
			C ₇ -C ₁₂	σ^*	0.0310	6.78	1.40	0.087
C ₆ -H ₁₈	σ	1.9799	C ₇ -C ₁₂	σ^*	0.0310	4.72	1.19	0.067
C ₇ -C ₈	σ	1.9565	C ₆ -C ₇	σ^*	0.0321	6.19	1.38	0.083
			C ₇ -C ₁₂	σ^*	0.0310	7.06	1.40	0.089
			C ₈ -C ₉	σ^*	0.0273	6.02	1.38	0.082
C ₇ -C ₈	π	1.6056	N ₂ -C ₆	π^*	0.2239	26.58	0.29	0.082
			C ₉ -C ₁₀	π^*	0.3431	19.71	0.31	0.071
			C ₁₁ -C ₁₂	π^*	0.3610	25.02	0.31	0.079
C ₇ -C ₁₂	σ	1.9505	C ₆ -C ₇	σ^*	0.0321	7.23	1.36	0.089
			C ₇ -C ₈	σ^*	0.0377	7.08	1.37	0.088
			C ₈ -O ₁₃	σ^*	0.0273	4.58	1.08	0.063
			C ₁₁ -C ₁₂	σ^*	0.0300	5.37	1.38	0.077
			C ₁₁ -Cl ₁₄	σ^*	0.0350	5.72	0.91	0.065
C ₈ -C ₉	σ	1.9729	C ₇ -C ₈	σ^*	0.0377	6.90	1.39	0.088

			C ₉ -C ₁₀	σ^*	0.0197	4.83	1.41	0.074
O ₁₃ -H ₂₂	σ	1.9900	C ₇ -C ₈	σ^*	0.0377	3.46	1.42	0.063
N ₁	LP(1)	1.6905	N ₂ -C ₆	π^*	0.2239	30.72	0.29	0.086
			C ₃ -O ₄	π^*	0.3733	52.75	0.30	0.114
N ₂	LP (1)	1.9167	N ₁ -H ₁₅	σ^*	0.0334	8.70	0.76	0.074
			C ₆ -H ₁₈	σ^*	0.0371	9.60	0.77	0.078
O ₄	LP (2)	1.8558	N ₁ -C ₃	σ^*	0.0719	24.28	0.68	0.117
			C ₃ -N ₅	σ^*	0.0644	23.55	0.69	0.116
N ₅	LP (1)	1.7898	C ₃ -O ₄	π^*	0.3733	54.86	0.28	0.116
O ₁₃	LP (2)	1.8850	C ₇ -C ₈	π^*	0.4383	25.99	0.38	0.097
Cl ₁₄	LP (2)	1.9721	C ₁₀ -C ₁₁	σ^*	0.0319	4.39	0.97	0.058
			C ₁₁ -C ₁₂	σ^*	0.0300	4.23	0.97	0.057
Cl ₁₄	LP (3)	1.9319	C ₁₁ -C ₁₂	π^*	0.3610	13.10	0.35	0.065

^aE(2) means energy of hyper conjugative interaction (stabilization energy)

^bEnergy difference between donor and acceptor i and j NBO orbitals.

^cF(i,j) is the Fock matrix element between i and j NBO orbitals

3.8. Molecular docking

Molecular docking simulations were carried out using AutoDock 4.2.6 with AutoGrid 4.0 and the AutoDock Tools graphical interface for the preparation, execution and analysis of the docking configurations. The receptor proteins were built by including polar hydrogens, Kollman united atom charges and solvation parameters. Grid maps were generated for each receptor and the Lamarckian Genetic Algorithm (LGA) was used to find the best ligand conformations, with as many as 100 conformers per docking. All calculations were performed on Intel Pentium A1018 (2.10 GHz) with 2 GB RAM under Windows 7.

AutoDock predicts the preferred binding orientation and conformation of small molecules within a receptor of known three-dimensional structure. In an effort to examine the possible antimicrobial activity of 15CHS, docking was carried out at the active sites of three target proteins 2X8R[26], 4HOE[27], and 3EQA[28] obtained from the RCSB Protein Data Bank. The ligand was docked separately into the binding site of each receptor and the lowest binding energy conformation was chosen as the representative binding mode. The binding energies (kcal mol⁻¹) and calculated inhibition constants (μ M) are listed in Table 8.

3EQA showed the most beneficial binding among three receptors with a minimum free energy of -6.50 kcal mol⁻¹. 15CHS forms up to four hydrogen bonds with residues TRP 202 (two interactions) and ARG 329 (two interactions) in this configuration and has an RMSD of 31.25 Å. These results suggest that 15CHS has the most favourable binding conditions for 3EQA among the targets evaluated. Fig. 8 show the expected binding conformations of 15CHS with 2X8R, 4HOE and 3EQA.

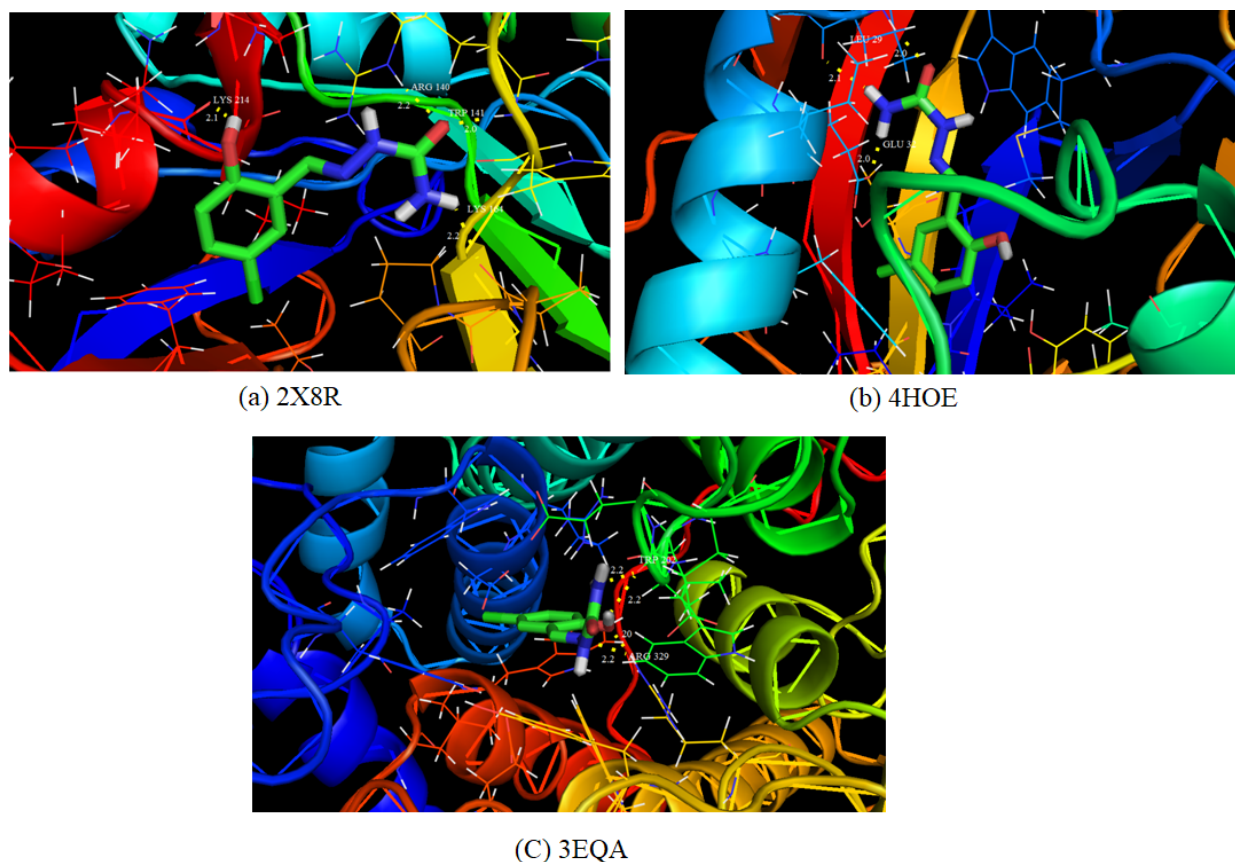


Fig.8. Docking and Hydrogen bond interactions of 15CHS with (a) 2X8R, (b) 4HOE and (c) 3EQA protein structure

Table 8. Hydrogen bonding and molecular docking with antimicrobial protein targets

Protein (PDB ID)	Bonded residues	No. of hydrogen bond	Bond distance (Å)	Estimated Inhibition Constant (μm)	Binding energy (kcal/mol)	Reference RMSD (Å)
2X8R	ARG 140	4	2.2	140.92	-5.25	36.77
	TRP 141		2.0			
	LYS 164		2.2			
	LYS 214		2.1			

4. CONCLUSION

The compound 15CHS was successfully synthesized and extensively characterised by experimental and theoretical methods. The vibrational behaviour was investigated by FT-IR, FT-Raman, UV-Vis and NMR spectroscopies, supported with the density functional theory (DFT) calculations. Generally a good correlation was found between the experimental and the calculated vibrational frequencies. The optimised molecular arrangement, vibrational frequencies, infrared intensities and Raman activities were calculated using the B3LYP/6-311++G(d,p) method and the electronic properties were

compared with the experimental UV-Vis spectrum. The calculated band gap energy (< 4.35 eV) proves that 15CHS is electronically appropriate for biological activity. The first order hyperpolarizability of 15CHS was calculated to be 9.4327×10^{-30} esu, which is comparable to the analogous derivatives and almost 15 times higher than the benchmark NLO reference molecule urea, which highlights the considerable non linear optical capabilities of 15CHS. The Natural Bond Orbital (NBO) analysis revealed that the molecular stability is greatly enhanced by hyperconjugative interactions and large charge delocalisation. Molecular docking studies showed a

minimum binding energy of -6.50 kcal mol $^{-1}$ for 15CHS with the 3EQA protein, indicating strong interactions within the 3EQA binding site, thereby supporting its potential as a biologically active entity.

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