Crystal Growth, Quantum Chemical Calculation and Molecular Docking Studies of 3-(2-(Piperidin-1-yl)Acetamido) Benzofuran-2-Carboxamide.

T.Ramya, S.Geetha, K.P. Nalini

Department of Physics, Chevalier T.Thomas Elizabeth College for Women, Chennai -600011

Abstract

3-(2-(Piperidin-1-yl) Acetamido) Benzofuran-2-Carboxamide (3PAB2C), is a Benzo furan derivative. As Benzo furan has several pharmacological activities, in the present work, 3PAB2C has been synthesized by slow evaporation method. To investigate and confirm the structure of 3PAB2C X- ray crystallography and spectroscopic methods like NMR, FTIR and UV – Vis spectra have been recorded. Finally, molecular docking studies of 3PAB2C was carried out against COVID-19 spike and confirmed with theoretical method using Density functional theory (DFT) calculations.

Key words: 3-(2-(Piperidin-1-yl) Acetamido) Benzofuran-2-Carboxamide, NMR, FTIR, DFT.

Introduction

3-(2-(Piperidin-1-yl) Acetamido) Benzofuran-2-Carboxamide (3PAB2C) is a Benzofuran derivative. 3PAB2C with an aromatic ring structure is a heterocyclic analog of naphthalene. As Benzofuran containing compounds exhibit various medicinal and non-drug applications focus on the synthesis and evaluation of pharmacological activities of various benzofuran coupled with heterocyclic moieties such as pyrazole, thiazole, oxadiazole, thiazolidinone [1] has been carried out. Literature survey reveals that Benzofuran derivatives show pharmacological activities like antimicrobial, anti-inflammatory, antidepressant, antitumor, analgesic and anticonvulsant [2-9]. This interesting information leads to the present investigation. Therefore 3PAB2C is synthesized by slow evaporation method and characterized using various

spectroscopic techniques. Molecular docking study against COVID-19 disease is performed and Density Functional Theory calculations were used to support the molecular docking results.

Experimental

3PAB2C was synthesized at Biochemie Innovations Lab, Tindivanam. The pure sample was used as such for the spectral measurements. FTIR spectrum of 3PAB2C has been recorded in the range 3500 – 350 cm-1 using BRUKER IFS Spectrometer and UV – Vis spectrum in the range of 200-450 nm using UV Visible Spectrophotometer at Biochemie Lab. 1 H NMR and 13 C NMR was recorded using Lab Mann Bruker at School of Chemistry, Madurai Kamaraj University.

Computational details

All the theoretical computations were performed at B3LYP level on a personal computer using Gaussian 09W program package [10]. The geometry optimization was carried out using the initial geometry generated at DFT level methods adopting 6-31 G (d,p) basis set.

Crystal Growth

3PAB2C Crystal was synthesized in four steps. 2-(cyanomethoxy) benzonitrile was obtained from stirred solution of 2-hydroxy benzonitrile (0.083 mol) in DMF (50 mL) to which potassium carbonate (0.166 mol) and 2-chloro acetonitrile (0.101 mol) were added at room temperature. The reaction mixture was refluxed to 80 °C for 3 hours and was poured into ice-cold water and filtered, then washed with hexane and dried.

(2-cyanomethoxy) benzonitrile (0.0632 mol) was mixed with ethanol (100 mL), KOH (0.178 mol) and was refluxed to 75 °C for 3 hours, then poured into the ice-cold water, filtered to obtain 3-amino-1-benzofuran-2-carboxamide.

To 3-amino-1-benzofuran-2-carboxamide (0.0227mol) in dichloromethane (100 mL), triethylamine (0.0646 mol) was added at 0 to 5 °C. To this mixture chloroacetyl chloride (0.0267 mol) was added drop wise over 5 min. After completion of addition, the reaction was stirred at ambient temperature for 4 hours. Then the reaction mixture was diluted, washed to synthesis 3-(2-(substitutedamino) acetamido) benzofuran-2-carboxamide.

Finally, 3-(2-(piperidin-1-yl) acetamido) benzofuran-2-carboxamide was synthesized by adding 3-[(chloroacetyl) amino]-1-benzofuran-2-carboxamide (0.0012 mol) in dichloromethane (10 mL) with triethylamine (0.0024 mol) and piperidine (0.0014 mol). After 6 hours, the reaction mixture was diluted with dichloromethane (50 mL), washed with water and brine (25 mL) then dried over anhydrous sodium sulphate, filtered and evaporated. 3 Dimensional structure of 3PAB2C is given in Fig.1.

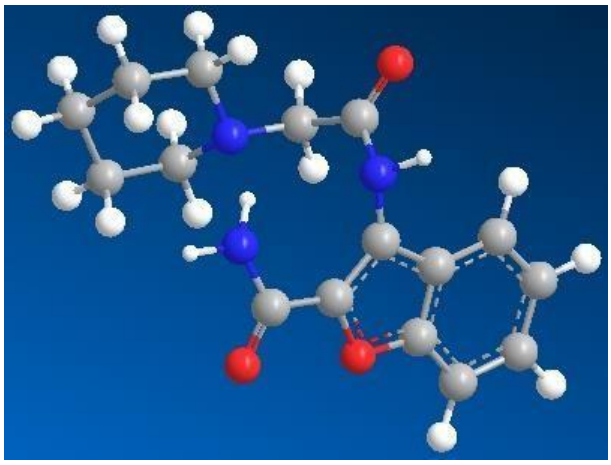


Fig 1. 3D structure of 3PAB2C

Result and discussion

The structure of 3PAB2C was confirmed by X-Ray crystallography method. The ORTEP diagram is given in Fig 2. The geometrical parameters for the crystal like bond length, bond

angle and torsional angle are given in Table 1. The geometrical parameters are in acceptable range [11].

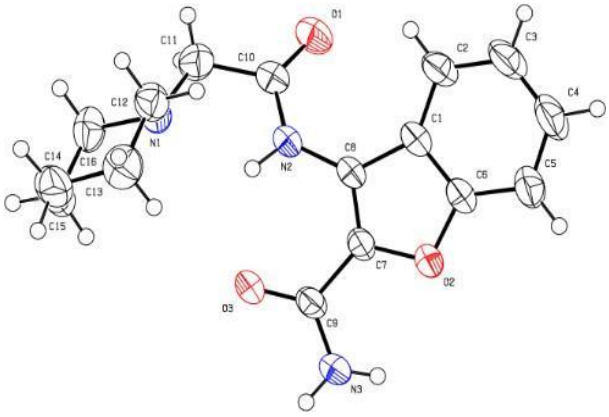


Fig 2. ORTEP diagram of 3PAB2C

Table 1: Geometry of 3PAB2C

S. No.	Bond atoms	Bond length (Å)	Bond angle Atoms	Bond angle	Dihedral angle atoms	Dihedral angles
1	C(22)-H(41)	1.098	H(41)-N(35)- H(40)	119.955	C(12)-C(1)-C(2)-C(4)	177.769
2	C(22)-H(40)	1.097	H(41)-N(35)- C(13)	119.594	C(12)-C(1)-C(2)-H(3)	-2.128
3	C(21)- H (39)	1.096	H(40)-N(35) – (13)	116.675	C(10)-C(1)-C(2)-C(4)	1.370
4	C(21)-H(38)	1.097	C(30)-C(27)-H(29)	109.311	C(10)-C(1)-C(2)-H(3)	-178.528
5	C(20)-H(37)	1.097	C(30)-C(27)-H(28)	109.428	C(12)-C(1)-C(10)-C(8)	-178.785
6	C(20)-H(36)	1.095	C(30)-C(27)-C(24)	111.264	C(12)-C(1)-C(10)-O(37)	-0.626
7	C(19)-H(35)	1.096	H(29)-C(27)-H(28)	107.966	C(2)-C(1)-C(10)-C(8)	-1.366
8	C(19)-H(34)	1.097	H(29)-C(27)-C(24)	109.366	C(2)-C(1)-C(10)-O(37)	176.794
9	C(18)-H(33)	1.098	H(28)-C(27)-C(24)	109.442	C(10)-C(1)-C(12)-C(11)	1.335
10	C(18)-H(32)	1.096	C(27)-C(24)-H(26)	109.547	C(10)-C(1)-C(12)-N(34)	177.209
11	C(16)-H(31)	1.096	C(27)-C(24)-H(25)	109.639	C(2)-C(1)-C(12)-C(11)	-175.408
12	C(16)-H(30)	1.097	C(27)-C(24)-C(21)	110.125	C(2)-C(1)-C(12)-N(34)	0.466

13	N(13)-H(29)	1.018	H(26)-C(24)-H(25)	108.147	C(11)-C(13)-N(35)-H(40)	-173.626
14	N(13)-H(28)	1.010	H(26)-C(24)-C(21)	109.667	C(11)-C(13)-N(35)-H(41)	-15.508
15	N(10)-H(27)	1.019	H(25)-C(24)-C(21)	109.685	O(38)-C(13)-N(35)-H(40)	7.557
16	C(6)-H(26)	1.086	C(24)-C(21)-H(23)	109.558	O(38)-C(13)-N(35)-H(41)	165.675
17	C(3)-H(25)	1.083	C(24)-C(21)-H(22)	109.547	C(21)-C(24)-C(27)-C(30)	-53.648
18	C(2)-H(24)	1.088	C(24)-C(21)-C(18)	110.563	C(21)-C(24)-C(27)-H(28)	-174.704
19	C(1)-H(23)	1.088	H(23)-C(21)-H(22)	108.113	C(21)-C(24)-C(27)-H(29)	67.210
20	N(17)-C(22)	1.475	H(23)-C(21)-C(18)	109.520	H(25)-C(24)-C(27)-C(30)	-174.424
21	C(21)-C(22)	1.528	H(22)-C(21)-C(18)	109.499	H(25)-C(24)-C(27)-H(28)	64.520
22	C(20)-C(21)	1.523	N(33)-C(30)-H(32)	109.727	H(25)-C(24)-C(27)-H(29)	-53.565
23	C(19)-C(20)	1.524	N(33)-C(30)-H(31)	109.682	H(26)-C(24)-C(27)-C(30)	67.046
24	C(18)-C(19)	1.530	N(33)-C(30)-C(27)	109.897	H(26)-C(24)-C(27)-H(28)	-54.010
25	N(17)-C(18)	1.478	H(32)-C(30)-H(31)	108.141	H(26)-C(24)-C(27)-H(29)	-172.096
26	C(8)-C(9)	1.387	H(32)-C(30)-C(27)	109.666	C(24)-C(27)-C(30)-N(33)	57.096
27	C(5)-C(9)	1.425	H(31)-C(30)-C(27)	109.701	C(24)-C(27)-C(30)-H(31)	177.763
28	C(4)-C(5)	1.387	N(33)-C(18)-C(21)	110.127	C(24)-C(27)-C(30)-H(32)	-63.605
29	C(6)-C(5)	1.408	N(33)-C(18)-H(20)	109.660	H(28)-C(27)-C(30)-N(33)	178.160
30	C(1)-C(6)	1.402	N(33)-C(18)-H(19)	109.601	H(28)-C(27)-C(30)-H(31)	-61.172
31	C(2)-C(1)	1.397	C(21)-C(18)-H(20)	109.584	H(28)-C(27)-C(30)-H(32)	57.459
32	C(3)-C(2)	1.398	C(21)-C(18)-H(19)	109.640	H(29)-C(27)-C(30)-N(33)	-63.795
33	C(4)-C(3)	1.392	H(20)-C(18)-H(19)	108.199	H(29)-C(27)-C(30)-H(31)	56.873
34	O(7)-C(4)	1.357	C(30)-N(33)-C(18)	110.675	H(29)-C(27)-C(30)-H(32)	175.504
35	C(8)-O(7)	1.374	C(30)-N(33)-C(15)	114.400	C(18)-C(21)-C(24)-C(27)	53.744
36	C(16)-N(17)	1.484	C(18)-N(33)-C(15)	113.604	C(18)-C(21)-C(24)-H(25)	174.492

37	C(11)-C(16)	1.529	N(33)-C(15)-H(17)	109.154	C(18)-C(21)-C(24)-H(26)	-66.879
38	C(11)-O(15)	1.225	N(33)-C(15)-H(16)	109.135	H(22)-C(21)-C(24)-C(27)	174.507
39	C(12)-O(14)	1.229	N(33)-C(15)-C(14)	112.513	H(22)-C(21)-C(24)-H(25)	-64.745
40	C(12)-N(13)	1.368	H(17)-C(15)-H(16)	107.764	H(22)-C(21)-C(24)-H(26)	53.884
41	C(8)-C(12)	1.446	H(17)-C(15)-C(14)	109.065	H(23)-C(21)-C(24)-C(27)	-67.053
42	N(10)-C(11)	1.384	H(16)-C(15)-C(14)	109.096	H(23)-C(21)-C(24)-H(25)	53.695
43	C(9)-N(10)	1.362	O(36)-C(14)-N(34)	125.749	H(23)-C(21)-C(24)-H(26)	172.324
44			O(36)-C(14)-C(15)	121.426	N(33)-C(18)-C(21)-C(24)	-57.702
45			N(34)-C(14)-C(15)	112.812	N(33)-C(18)-C(21)-H(22)	-178.494
46			H(39)-N(34)-C(14)	114.151	N(33)-C(18)-C(21)-H(23)	63.118
47			H(39)-N(34)-C(12)	116.446	H(19)-C(18)-C(21)-C(24)	62.972
48			C(14)-N(34)-C(12)	128.536	H(19)-C(18)-C(21)-H(22)	-57.820
49			O(38)-C(13)-N(35)	124.136	H(19)-C(18)-C(21)-H(23)	-176.209
50			O(38)-C(13)-C(11)	118.254	H(20)-C(18)-C(21)-C(24)	-178.412
51			N(35)-C(13)-C(11)	117.599	H(20)-C(18)-C(21)-H(22)	60.796
52			O(37)-C(11)-C(13)	119.230	H(20)-C(18)-C(21)-H(23)	-57.593
53			O(37)-C(11)-C(12)	111.622	C(27)-C(30)-N(33)-C(15)	169.241
54			C(13)-C(11)-C(12)	129.109	C(27)-C(30)-N(33)-C(18)	-60.883
55			C(11)-O(37)-C(10)	105.069	H(31)-C(30)-N(33)-C(15)	48.561
56			N(34)-C(12)-C(11)	123.327	H(31)-C(30)-N(33)-C(18)	178.438
57			N(34)-C(12)-C(1)	129.654	H(32)-C(30)-N(33)-C(15)	-70.095
58			C(11)-C(12)-C(1)	106.887	H(32)-C(30)-N(33)-C(18)	59.781
59			O(37)-C(10)-C(8)	124.708	C(21)-C(18)-N(33)-C(15)	-168.173
60			O(37)-C(10)-C(1)	111.597	C(21)-C(18)-N(33)-C(30)	61.529

61			C(8)-C(10)-C(1)	123.667	H(19)-C(18)-N(33)-C(15)	71.130
62			C(10)-C(8)-H(9)	122.165	H(19)-C(18)-N(33)-C(30)	-59.168
63			C(10)-C(8)-C(6)	115.781	H(20)-C(18)-N(33)-C(15)	-47.508
64			H(9)-C(8)-C(6)	122.054	H(20)-C(18)-N(33)-C(30)	-177.806
65			C(8)-C(6)-H(7)	118.991	C(14)-C(15)-N(33)-C(18)	84.663
66			C(8)-C(6)-C(4)	121.959	C(14)-C(15)-N(33)-C(30)	-146.925
67			H(7)-C(6)-C(4)	119.050	H(16)-C(15)-N(33)-C(18)	-36.580
68			C(6)-C(4)-H(5)	119.235	H(16)-C(15)-N(33)-C(30)	91.833
69			C(6)-C(4)-C(2)	121.608	H(17)-C(15)-N(33)-C(18)	-154.121
70			H(5)-C(4)-C(2)	119.158	H(17)-C(15)-N(33)-C(30)	-25.708
71			C(4)-C(2)-H(3)	121.023	N(34)-C(14)-C(15)-N(33)	6.113
72			C(4)-C(2)-C(1)	117.967	N(34)-C(14)-C(15)-H(16)	127.377
73			H(3)-C(2)-C(1)	121.009	N(34)-C(14)-C(15)-H(17)	-115.155
74			C(12)-C(1)-C(10)	104.801	O(36)-C(14)-C(15)-N(33)	-175.122
75			C(12)-C(1)-C(2)	136.125	O(36)-C(14)-C(15)-H(16)	-53.857
76			C(10)-C(1)-C(2)	119.003	O(36)-C(14)-C(15)-H(17)	63.611
77					C(15)-C(14)-N(34)-C(12)	-179.736
78					C(15)-C(14)-N(34)-H(39)	11.428
79					O(36)-C(14)-N(34)-C(12)	1.562
80					O(36)-C(14)-N(34)-H(39)	-167.274
81					C(11)-C(12)-N(34)-C(14)	-151.449
82					C(11)-C(12)-N(34)-H(39)	17.170
83					C(1)-C(12)-N(34)-C(14)	33.277
84					C(1)-C(12)-N(34)-H(39)	-158.103

85					O(37)-C(11)-C(13)-N(35)	-12.001
86					O(37)-C(11)-C(13)-O(38)	166.887
87					C(12)-C(11)-C(13)-N(35)	170.488
88					C(12)-C(11)-C(13)-O(38)	-10.624
89					O(37)-C(11)-C(12)-N(34)	-177.815
90					O(37)-C(11)-C(12)-C(1)	-1.617
91					C(13)-C(11)-C(12)-N(34)	-0.152
92					C(13)-C(11)-C(12)-C(1)	176.047
93					C(12)-C(11)-O(37)-C(10)	1.219
94					C(13)-C(11)-O(37)-C(10)	-176.704
95					C(8)-C(10)-O(37)-C(11)	177.829
96					C(1)-C(10)-O(37)-C(11)	-0.308
97					C(6)-C(8)-C(10)-O(37)	-177.476
98					C(6)-C(8)-C(10)-C(1)	0.442
99					H(9)-C(8)-C(10)-O(37)	2.430
100					H(9)-C(8)-C(10)-C(1)	-179.652
101					C(4)-C(6)-C(8)-C(10)	0.438
102					C(4)-C(6)-C(8)-H(9)	-179.469
103					H(7)-C(6)-C(8)-C(10)	-179.556
104					H(7)-C(6)-C(8)-H(9)	0.537
105					C(2)-C(4)-C(6)-C(8)	-0.378
106					C(2)-C(4)-C(6)-H(7)	179.616
107					H(5)-C(4)-C(6)-C(8)	179.639
108					H(5)-C(4)-C(6)-H(7)	-0.367
109					H(3)-C(2)-C(4)-C(6)	179.346

110					H(3)-C(2)-C(4)-H(5)	-0.671
111					C(1)-C(2)-C(4)-C(6)	-0.552
112					C(1)-C(2)-C(4)-H(5)	179.431

The 3PAB2C was also characterized by 1H NMR, 13C NMR, FTIR and UV-Vis spectral methods Fig 4-7. The appearance of amide NH peak around 10 ppm and the broad singlet around 5.5 ppm & 6.0 ppm indicate the presence of N-substituted amide and carboxamide unit. The two peaks appearing at around 160 ppm in 13C NMR are due to the two carbonyl groups. In FTIR spectrum the N-H stretching vibrations at 3438cm-1 and 3336 cm-1 confirms the existence of amide group [12]. The aromatic C-H stretching vibrations generally appear in the range 3000 – 3250cm-1 in substituted benzenes [13]. In the present case, the vibration at 3223 cm-1 is assigned to CH asymmetric stretching. The vibration at 1664 cm-1 confirms the presence of carbonyl group.

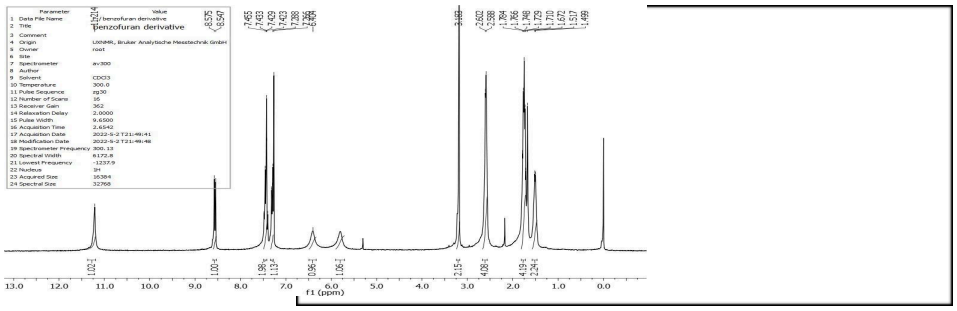


Fig 4. ^{1}H NMR spectrum of 3PAB2C

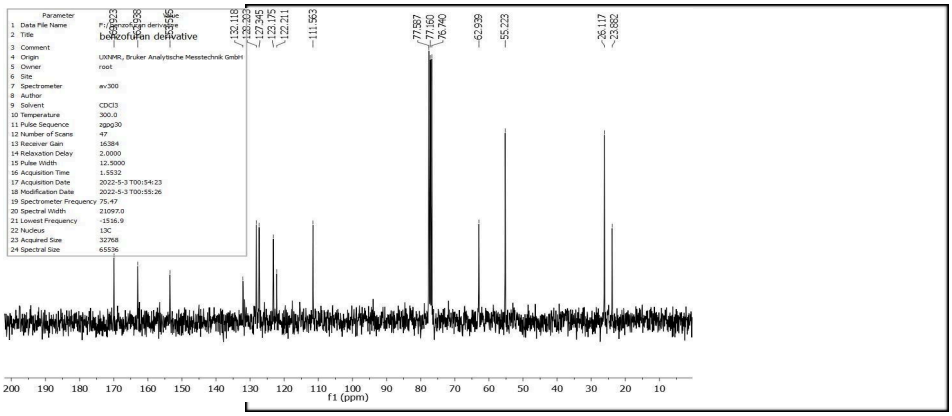


Fig 5. ^{13}C NMR spectrum of 3PAB2C

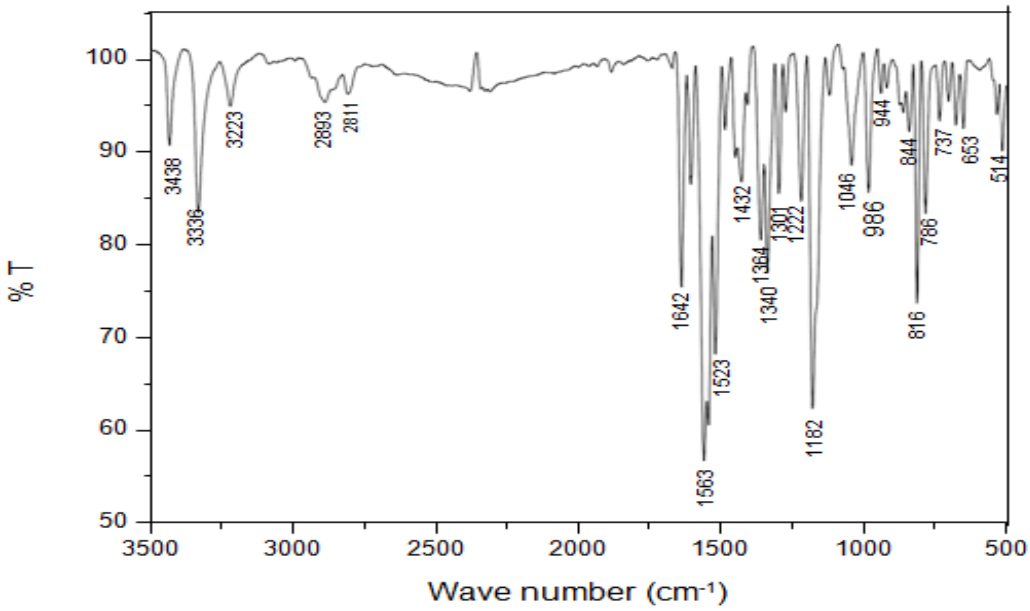


Fig 6: FT-IR spectrum of 3PAB2C

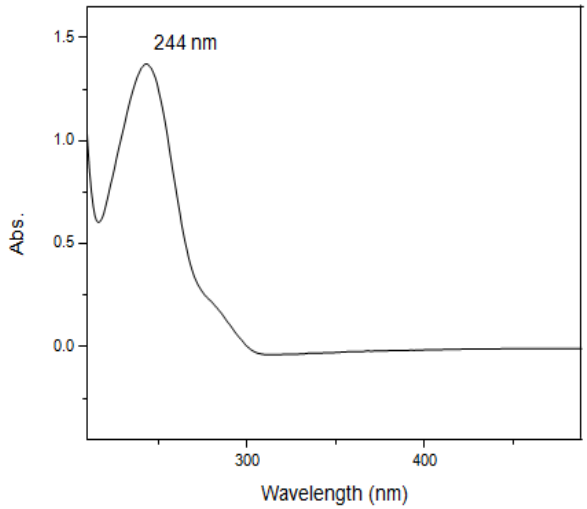


Fig 7. UV-Visible spectrum of 3PAB2C

Molecular Docking Studies

Molecular docking, frequently used in pharmaceutical industries to predict the binding conformation of small molecules to the applicable target binding site to develop new drug. The 3D structure of the Sars-Cov-2 spike protease (6m0j.pdb) was downloaded from the protein data bank. The molecular docking and visualization using discovery studio are performed as followed by Sribalan et al [14]. The 3PAB2C was used to perform for COVID-19 spike protease (6m0j.pdb) Molecular docking was performed using the Auto-Dock Tool (1.5.6). The 3D structure was optimized using Gaussian 09W. All avoidable water and ligand were removed from the enzyme and polar hydrogen was added to the enzyme. The target was generated as a PDBQT format. PDBQT file of the ligands also generated and performed. The grid box size is fixed as 60 x 60 x 60 points in X, Y, and Z directions. Similarly, the grid space is fixed as 0.375 Å and ten runs were created by using Lamarckian genetic algorithm searches. The calculated bonding energy and inhibition constant is found to be -4.47 kcal/mol and 533.42 µM respectively. The compound showed 2 hydrogen bonding interactions which form the conventional hydrogen

bonding interaction with PHE338 and GLY339 respectively. The calculated hydrogen bonding distance is 3.26Å and 2.87Å. The molecular docking result concludes that the 3PAB2C may cure the COVID-19 disease. The docking interaction results are representing in Table 2. The docking interacted image is represented in Fig 8.

Table 2: Docking of 3PAB2C with 6m0j.pdb (Spike protease of Sars-Cov-2)

Sample code	Binding energy kcal/mol	Inhibition constant	No of hydrogen bonding	Hydrogen bonding amino acid residue
1	-4.47 kcal /mol	533.42 µM	2	PHE338(3.26Å) conventional hydrogen bonding interaction GLY339(2.87Å) conventional hydrogen bonding interaction

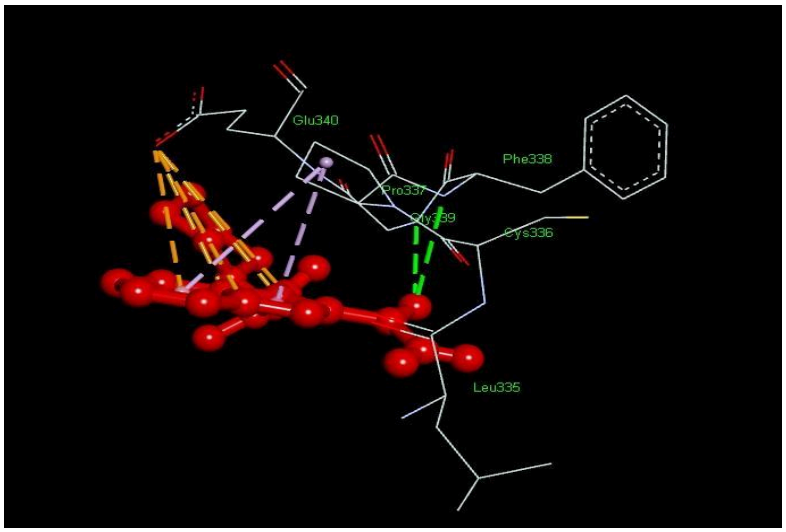
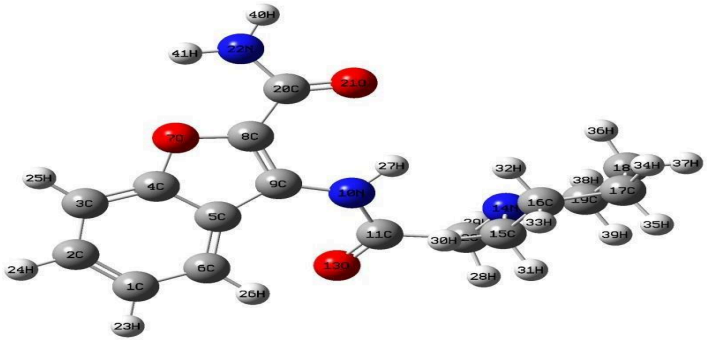


Fig 8: Interaction of 3PAB2C with 6m0j.pdb

DFT Studies

The DFT studies were performed to support the molecular docking studies. The Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) clearly

shows that benzofuran has highest electron cloud. Thus, the benzofuran moiety is responsible for interaction with proteins. The Molecular Electrostatic Potential (MEP) clearly shows that the negative region presents in benzofuran oxygen and carboxamide oxygen is responsible for hydrogen bonding interactions. The mulliken charge analysis shows that the nitrogen and oxygen has highest negative charge thus responsible for hydrogen bonding interactions. The Optimized structure, HOMO, LUMO, MEP and Mulliken charge information are represented the given fig 9-14.



ig 9. Optimized structure of 3PAB2C

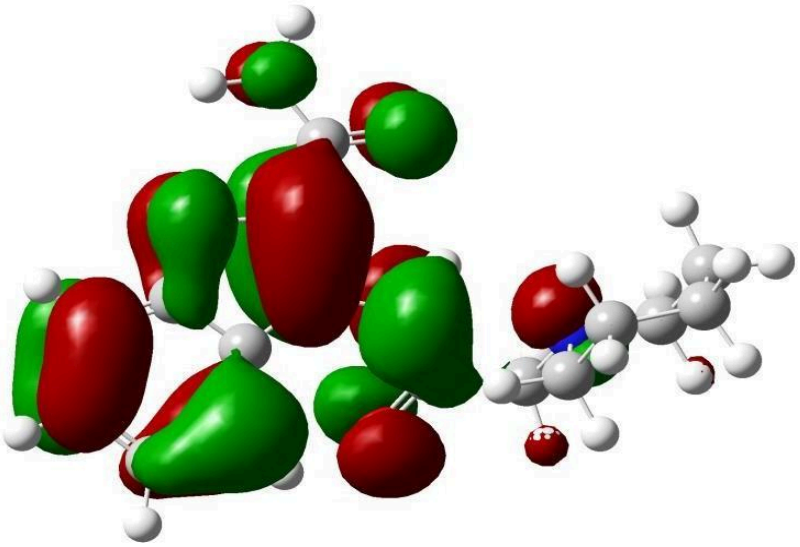


Fig 10. Highest occupied molecular orbital (HOMO) of 3PAB2C

Fig 11. Lowest unoccupied molecular orbital (LUMO) of 3PAB2C

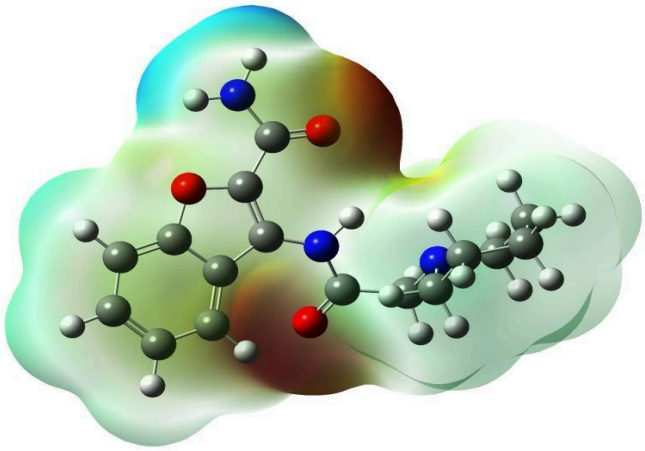


Fig 12. Molecular electrostatic potential of 3PAB2C

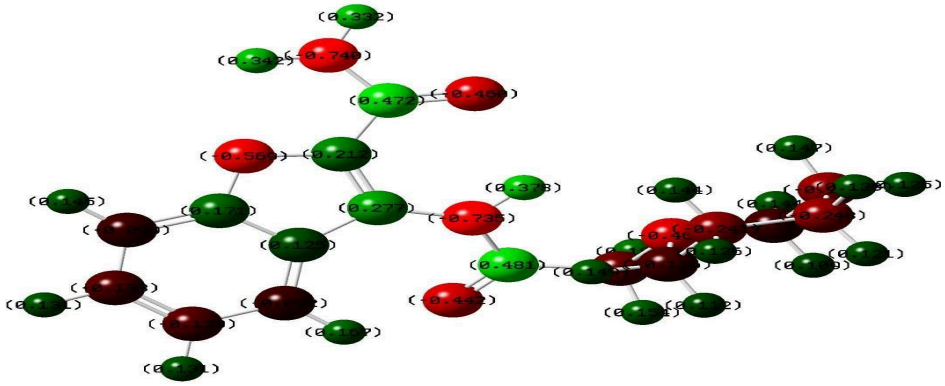


Fig 13. Mulliken charge analysis of 3PAB2C

Conclusion

The 3PAB2C was synthesized and crystalized using ethanol as a solvent by slow evaporation method and the crystal structure was recorded. The geometrical parameter has been studied. Further 3PAB2C has been characterized by 1H NMR, 13C NMR, FTIR and UV-Visible experimental spectrums. The molecular docking studies of 3PAB2C were performed against COVID-19 spike protease and the docking results were appreciable. The density functional theory (DFT) calculations were performed to support the molecular docking studies. The DFT

information was coherent with docking results. Thus the present investigation provides information that 3PAB2C is a bioactive compound for COVID-19 target which may be useful to upgrade further knowledge on 3PAB2C.

References

- H. Khanam, Shamsuzzaman. Eur. J. Med. Chem. 2014, 1-22
- 2.. R. K. Thalji, N. Aiyar, E. A. Davenport, J. A. Erhardt, L. A. Kallal, D. M. Morrow, S. Senadhi, C. L. Burns-Kurtis, J. P. Marino. Bioorg. Med. Chem. Lett., 2010, 20, 4104- 4107.
- X. Bao, S. Lu, J-S. Liow, S. S. Zoghbi, K. J. Jenko, D. T. Clark, R. L. Gladding, R. B. Innis and V. W. Pike. J. Med. Chem., 2012, 55, 2406-2415.
- H. Hao, W. Chen, J. Zhu, C. Lu, Y. Shen. Eur. J. Med. Chem., 2015, 102, 277-287.
- P. Yadav, P. Singh, and A. K. Tewari. Bioorg. Med. Chem. Lett., 2014, 24, 2251-2255.
- X.-Y. Ye, C. L. Morales, Y. Wang, K. A. Rossi, S. E. Malmstrom, M. Abousleiman, L. Sereda, A. Apedo, J. A. Robl, K. J. Miller, J. Krupinski and D. A. Wacker. Bioorg. Med. Chem. Lett., 2014, 24, 2539-2545.
- S.-T. Xue, H.-F. Guo, M.-J. Liu, J. Jin, D.-H. Ju, Z-Y. Liu, Z.-R. Li. Eur. J. Med. Chem.,2015, 96, 151-161.
- R. Romagnoli, P. G. Baraldi, M. K. Salvador, F. Prencipe, L. C. Lopez-Cara, S. S. Ortega, A. Brancale, E. Hamel, I. Castagliuolo, S. Mitola, R. Ronca, R. Bortolozzi, E. Porcù, G. Basso and G. Viola. J. Med. Chem., 2015, 58, 3209–3222.

- E. Baiceanu, K-A. Nguyen, L. Gonzalez-Lobato, R. Nasr, H. Baubichon-Cortay, F. Loghin, M. L. Borgne, L. Chow, A. E. Boumendjel, M. Peuchmaur and P. Falson. Eur. J. Med. Chem., 2016, 122, 408-418.
- R. Nithyabalaji, H. Krishnan, J. Subha, R. Sribalan. J. Mol. Struc. DOI: https://doi.org/10.1016/j.molstruc.2019.127563
- R.Tanka, M.Haramura, A.Tanka, N.hirayama, Anal. Sci.2005.
- S.Gunasekaran, K.Thilak Kumar, S.Ponnuswamy, Indian J. Pure Appl, Phys.79, 2005,171.
- A.Sharms, VP Gupta, A Virdi, Indian Journal of Pure and Applied Physics, 2004, 251-257.
- R. Sribalan, G. Banuppriya, M. Kirubavathi, A. Jayachitra, V. Padmini. Bioorg. Med.
- Chem. Lett. 2016, 26, 5624-5630.