Synthesis, Biological Activity, Molecular Docking and Structural Studies of N-(O-Fluor phenyl)-4,6-Dimethyl-2-Pyridone-3-Nitrile

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Abstract: The title compound crystallizes in space group, P21/c with unit cell parameters, a=7.926(5), b=11.156(3), c=14.261(3) Å, β = 102.01(1)°,V = 1233.4(9) Å3 containing four asymmetric molecules. The molecules are packed in the crystal lattice with bifurcated hydrogen bonding of the type C-H---O. Oxygen atom O(1) is involved in the bifurcated hydrogen bonding with bridge distances 3.511(2) Å and 3.466(2) Å, respectively. Fluorine F(1) of the phenyl ring does not involve in either intra- or inter-hydrogen bonding. The dihedral angle between the phenyl group and pyridone ring is 87.7(1)° indicating that pyridonyl group is perpendicular to the phenyl group making the way for its maximum interaction through weak hydrogen bondings. Molecular docking study done on this compound with Phospholipase (PLA2) indicates that there are hydrogen bond and hydrophobic interactions at the active sites on the PLA2. The molecule is stabilized through these interactions with distances varying from 2.5 to 4 Å and hence it is a good candidate for the study of inhibition activity of the enzyme based on its experimental evidences. Also a study of in-vitro biological activity carried out indicates that the compound has antioxidant and hydrogen-peroxide-radical scavenging properties and more potent than ascorbic acid taken as standard.

Index Terms- Anti-inflammatory, Active site, Crystal, Molecular Docking, Structure.

1. Introduction

The title compound is a derivative of the compound, 3-cyano-4,6-dimethyl-2-pyridone (4,6-dimethyl-2-pyridone-3-nitrile), known as 'Guareschi pyridone', has been known for long time perhaps more than a century [1]. It is known that organic compounds containing fluorine atoms exhibit biological and pharmacological significance [2]. The fluorine atoms in such compounds play a very significant role in biomolecular interactions [2, 3]. The study of such interactions involving fluorine atoms in crystal structures has been considered to be of great importance in crystal engineering [4]. It is well known that a number of Schiff's bases and thiophene derivatives exhibit different kinds of biological activities [5,6,7,8]. In order to study the biological and pharmacological properties different types of thiophene derivatives which have been prepared by us, the synthesized compound is used as a starting material for the synthesis of such derivatives. The synthesized compound has been screened for its anti-bacterial and anti-oxidant properties. The conducted *in-vitro* study indicates that the compound exhibits significant bacterial inhibition at low-to-moderate concentrations. Also it is nice to know its three dimensional structure as it exhibits a pronounced biological activity.

The molecular docking study of the compound with the *Phospholipase* A_2 (PLA_2) enzyme (E.C. 3.1.1.4). shows that the it has anti-inflammatory activity due to atoms involvement in interactions less than 4.0 Å with the atoms of amino acid residues of Phospholipase (PLA_2) enzyme. The molecule is stabilized through hydrogen bonding and hydrophobic interactions with distances varying from 2.5 to 4Å, respectively. Also a study of *in-vitro* biological activity carried out indicates that the compound has anti-oxidant and hydrogen-peroxide-radical-scavenging properties and it can be considered more potent than ascorbic acid taken as standard.

The crystal structure of the title compound determined by X-ray method shows that its staking pattern in the crystalline state exhibits along its crystallographic b-axis. The dihedral angle between the phenyl group and pyridone ring is found to be 87.7(1)° indicating that pyridonyl group is perpendicular to the phenyl group and probably making the way for its interaction through hydrogen bonds.

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2. Materials and methods

Title compound was synthesized using reactants O-fluoroaniline and ethylcyanoacetate in acetic anhydride medium by heating the mixture taken in a conical flask for about seven hours at 160-165ºC on an oil bath. The reaction was the initial step for the expected final synthesis of pharmaceutically important thiophenyl derivatives based on the Gewald's reaction mechanism [9]. The resultant compound obtained from the reaction was found to be the title compound. Its molecular constituents were found to be $C_{14}H_{11}FN_2O$. Its experimentally determined melting point was found to be 156°C. The yield of the product was found to be more than 90%. The probable reaction scheme could be represented as:

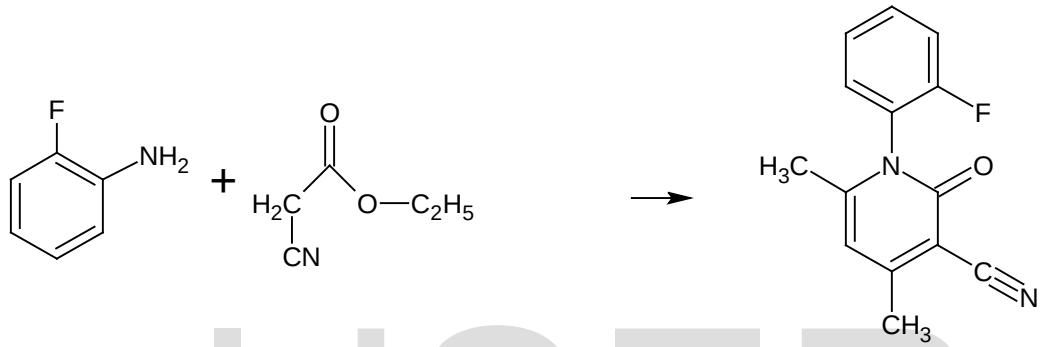


Fig. 1. Schematic representation of the reaction.

3. X-Ray Crystal Structure Determination

3.1. Crystallization of the Compound

Single crystals of the compound were obtained from a solution in isopropanol by slow evaporation technique [10] at room temperature. Several pale yellow parallelepiped crystals were obtained.

3.2. X-ray Data Collection, Reduction and Structure Solution

A suitable crystal was selected after examining it under an analytical polarizing microscope for its uniform extinction for data collection. Unit cell parameters were obtained from a set of different weighted intensity reciprocal lattice points. These parameters were refined to obtain unit cell parameters and based on these parameters a set of weighted intensity reflections data were collected after correcting for Lorentz and polarization effects [10]. These intensities were then put on an absolute scale and used in the structure determination of the molecule by direct method of phase determination [10]. The structure of the molecule thus obtained was refined by the several cycles of the least-squares method by incorporating positional parameters and isothermal parameters followed by anisothermal parameters for non-hydrogen atoms. All the positions of hydrogen atoms were geometrically fixed and their parameters were not refined. The final values of R and Rw as 0.0407 and 0.1040 were obtained, respectively. The refinement of the structure was considered to be satisfactory as parameters obtained were within the allowed range [11,12]. SHELXL97 [13] was used in the structure determination and refinement of the structure. The data collection parameters and other associated parameters are given in Table 1.

4. Results and Discussion

The final fractional coordinates and the equivalent isothermal parameters of the non-hydrogen-atoms along with the hydrogen atoms are given in Table 2. The bond distances and angles are given in Table 3. A molecular ORTEP [14] diagram representing the thermal ellipsoids plot is depicted in Fig. 2, and the crystal packing of the asymmetric molecules is depicted in Fig 3. The six-membered heterocyclic pyridonyl group has a well-defined diene-like structure with the bond distances C(8) - C(9) and C(10) - C(11) are shorter than the bonds C(7) – C(8) and C(9) – C(10) by more than 3 e.s.d's and these parameters agree with the reported values [1] . It may be noted that all the bond distances are found to be normal within 3 times of their e.s.d's. However, the distance between C(5) – F(1) is found to be 1.346 (3) Å slightly less than the expected value of 1.362(3) Å. But, this value agrees with the reported values [11.12]. This may be probably due to the partial negative charge present on the fluorine atom on account of resonance nature of the phenyl group. The molecules are packed nicely in

the crystal lattice with weak bifurcated hydrogen bonding involving C(4)-H(4) and C(8)-H(8) atoms of the pyridone ring with O(1) of carbonyl group of the phenyl with bridge distances 3.511(2) Å and 3.466(2) Å, respectively. The display of the hydrogen bonding connecting molecules occurs in layers along the longest crystallographic c-axis (Fig.3). [15,16].

Table 1. Shows the crystal unit cell parameters and other associated data collection Parameters.

Crystal Data	
Identification code	Shelexl
Empirical formula	C14 H11 F N2 O
Formula weight	242.25
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, P21/c
Unit cell dimensions	a = 7.926(5) A alpha = 90.000(2) deg. b = 11.156(3) A beta = 102.007(2) deg c = 14.261(3) A gamma = 90.000(4) deg.
Volume	1233.4(9) $Å^3$
Z, Calculated density	4, 1.305 Mg/m^3
Absorption coefficient	0.094 mm^{-1}
F(000)	504
Crystal size	0.30 x 0.25 x 0.20 mm
Theta range for data collection	2.34 to 26.83 deg.
Limiting indices	-10<=h<=9, -14<=k<=14, -18<=l<=12
Reflections collected / unique	11917 / 2630 [R(int) = 0.0288]
Completeness to theta = 26.83	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9814 and 0.9222
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2630 / 0 / 165
Goodness-of-fit on F^2	1.048
Final R indices [I ≥2σ (I)]	R1 = 0.0407, wR2 = 0.1040
R indices (all data)	R1 = 0.0628, wR2 = 0.1186
Largest diff. peak and hole	0.200 and -0.188 e. $Å^{-3}$

Table 2. The final fractional coordinate (X10^4 Å) and the equivalent isothermal parameters (X10^2 Å^2) of the non-hydrogen atoms and hydrogen atoms.

Atom	X	Y	Z	U (eq)
C (1)	8739(2)	2486(2)	-882(1)	57(1)
C (2)	9695(3)	2002(2)	-1495(1)	67(1)
C (3)	9161(3)	974(2)	-1991(1)	65(1)
C (4)	7682(3)	410(2)	-1885(1)	65(1)
C (5)	6760(2)	895(2)	-1263(1)	53(1)
C (6)	7260(2)	1924(1)	-764(1)	43(1)
C (7)	4865(2)	3169(1)	-599(1)	41(1)
C (8)	3832(2)	3648(1)	27(1)	39(1)
C (9)	4087(2)	3351(1)	981(1)	44(1)
C (10)	5441(2)	2575(2)	1358(1)	47(1)
C (11)	6492(2)	2124(1)	808(1)	44(1)
C (12)	2504(2)	4463(2)	-402(1)	44(1)
C (13)	2980(3)	3858(2)	1615(1)	67(1)
C (14)	7960(2)	1316(2)	1191(1)	64(1)
N (1)	6201(2)	2415(1)	-150(1)	40(1)
N (2)	1450(2)	5124(2)	-735(1)	61(1)
O (1)	4645(2)	3372(1)	-1460(1)	60(1)
F (1)	5291(2)	372(1)	-1141(1)	83(1)
H (1)	9097	3191	-552	69
H (2)	10707	2376	-1571	80
H (3)	9810	657	-2405	78
H (4)	7310	-286	-2225	78
H (10)	5625	2362	2001	57
H (13A)	3303	4675	1768	101
H(13B)	3134	3397	2195	101
H(13C)	1792	3826	1289	101
H(14A)	7961	1128	1848	97
H(14B)	9023	1707	1152	97
H(14C)	7847	591	821	97

Table 3: Bond lengths (Aº) and Angles (º)

Atoms	Bond length
C(1)-C(6)	1.371(2)
C(1)-C(2)	1.380(2)
C(1)-H(1)	0.9300
C(2)-C(3)	1.367(3)
C(2)-H(2)	0.9300
C(3)-C(4)	1.367(3)
C(3)-H(3)	0.9300
C(4)-C(5)	1.373(3)
C(4)-H(4)	0.9300
C(5)-F(1)	1.346(2)
C(5)-C(6)	1.365(2)
C(6)-N(1)	1.4410(19)
C(7)-O(1)	1.2256(18)
C(7)-N(1)	1.398(2)
C(7)-C(8)	1.434(2)
C(8)-C(9)	1.373(2)
C(8)-C(12)	1.429(2)
C(9)-C(10)	1.397(2)
C(9)-C(13)	1.496(2)
C(10)-C(11)	1.354(2)
C(10)-H(10)	0.9300
C(11)-N(1)	1.3762(19)
C(11)-C(14)	1.483(2)
C(12)-N(2)	1.141(2)
C(13)-H(13A)	0.96
C(13)-H(13B)	0.96
C(13)-H(13C)	0.96
C(14)-H(14A)	0.96
C(14)-H(14B)	0.96
C(14)-H(14C)	0.96

Atoms	Angles
C(6)-C(1)-C(2)	119.69(18)
C(6)-C(1)-H(1)	120.2
C(2)-C(1)-H(1)	120.2
C(3)-C(2)-C(1)	120.26(19)
C(3)-C(2)-H(2)	119.9
C(1)-C(2)-H(2)	119.9
C(4)-C(3)-C(2)	120.65(17)
C(4)-C(3)-H(3)	119.7
C(2)-C(3)-H(3)	119.7
C(3)-C(4)-C(5)	118.27(18)
C(3)-C(4)-H(4)	120.9
C(5)-C(4)-H(4)	120.9
F(1)-C(5)-C(6)	117.56(15)
F(1)-C(5)-C(4)	120.21(17)
C(6)-C(5)-C(4)	122.22(18)
C(5)-C(6)-C(1)	118.90(15)
C(5)-C(6)-N(1)	119.56(15)
C(1)-C(6)-N(1)	121.51(14)
O(1)-C(7)-N(1)	120.38(14)
O(1)-C(7)-C(8)	125.08(15)
N(1)-C(7)-C(8)	114.55(13)
C(9)-C(8)-C(12)	121.38(14)
C(9)-C(8)-C(7)	122.76(14)
C(12)-C(8)-C(7)	115.86(13)
C(8)-C(9)-C(10)	118.26(14)
C(8)-C(9)-C(13)	121.59(15)
C(10)-C(9)-C(13)	120.14(14)
C(11)-C(10)-C(9)	121.58(14)
C(11)-C(10)-H(10)	119.2
C(9)-C(10)-H(10)	119.2
N(2)-C(12)-C(8)	179.15(18)
C(9)-C(13)-H(13A)	109.5
C(9)-C(13)-H(13B	109.5
C(9)-C(13)-H(13C)	109.5
C(9)-C(13)-H(13C)	109.5
C(11)-C(14)-H(14A)	109.5
C(11)-C(14)-H(14B)	109.5
C(11)-C(14)-H(14C)	109.5
C(11)-N(1)-C(7)	123.43(13)
C(10)-C(11)-C(14)	122.86(15)
N(1)-C(11)-C(14)	117.78(14)

Table 4. Hydrogen bonds (Å) and Angles (°)

D-H. . .A	d(D-H)	D(H. . .A)	D(D. . . A)	<(DHA)
C(4)-H(4). . .O1#1	0.93	2.64	3.511(2)	157.1
C(8)-H(8). . .O1#2	0.93	2.61	3.466(2)	153.9

Symmetry transformations used to generate equivalent atoms:

O1#1: -x+1, y-1/2, -z-1/2 ; O1 #2: x, -y+1/2, z+1/2

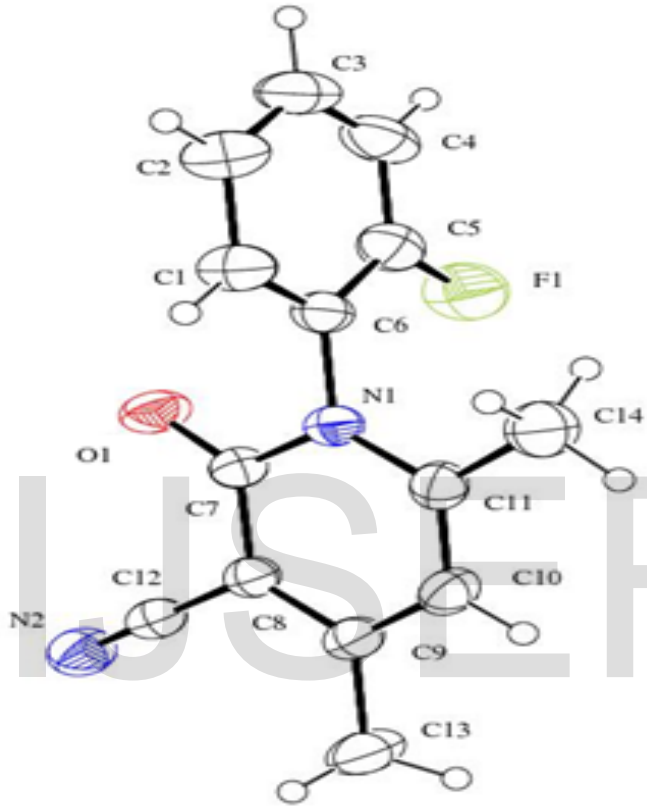


Fig. 2. An ORTEP diagram showing name and numbering scheme of atoms in the synthesized compound.

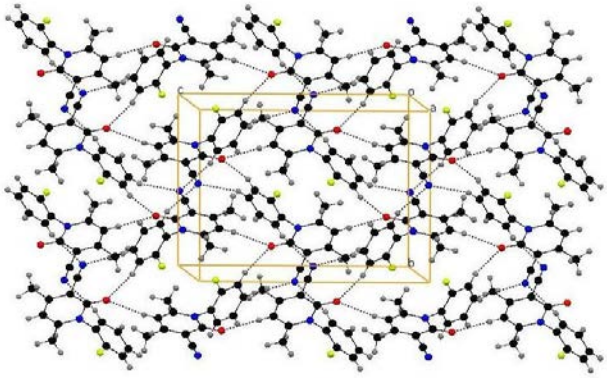


Fig. 3. Shows packing of molecules of the compound in the unit cell as viewed along the b-axis.

The asymmetric molecules are arranged compactly in layers parallel to the c-axis. The dihedral angle between the phenyl group and pyridine ring is 87.7(1)° indicating that pyridyl group is almost

perpendicular to the phenyl group making the way for its maximum interaction through weak hydrogen bonds. The dihedral angle could be a possible indication for the non-occurrence of either intra- or inter-hydrogen bonding between F(1) and O(1) atoms.

3.3. Antioxidant Property of the Compound

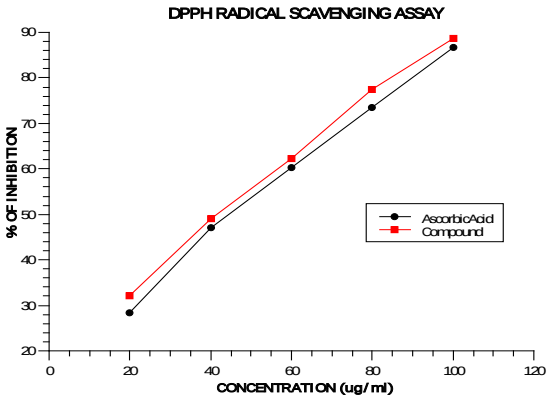
The anti-oxidant activity of the compound was estimated using thermostable radical scavenging assay method (Cottel et.al, 1996). In this assay, a mixture containing 200ml of DPPH (100µM in methanol) and the compound (20-100µg/ml) was incubated in dark for 20 minutes and observance of the mixture was measured at 517nm using spectrophotometer with suitable control according to the procedure. From the results obtained the percentage inhibition was calculated. The compound concentration required to reduce the initial DPPH radical by 50% was calculated using the following equation.

$$\% \text{ of Inhibition} = \frac{(AC - AT)*100}{AC} \quad (1)$$

where AC and AT stand for Absorbance of the Control (Ascorbic acid) and Absorbance of the test solutions (compound), respectively
The antioxidant property of the compound is found to be very close to ascorbic acid taken as standard. The values are given in Tables (5 and 6).

Table 5. **IC_{50} Concentration of compounds taken in for DPPH assay**

Sample	Absorption At (100 µg/ml)	IC_{50} (µg/ml)
Ascorbic Acid	**0.007**	**49.689(1)**
Compound	**0.006**	**48.180(1)**



3.4. Hydrogen Peroxide Radical Scavenging Assay

The hydrogen peroxide scavenging activity of the compound was determined by the modified method of (Dehpour et.al, 2009). Hydrogen peroxide solution (1%) was prepared in phosphate buffer (pH 7.4). The concentration with 0.1mg/ml was added toH_2O_2 solution and absorbance was measured at 230nm using UV-Spectrophotometer (Pc based UV-VIS Spectrophotometer UV-5704 SS) against the blank solution containing phosphate buffer without H_2O_2.

The percentage of H_2O_2 scavenging activity of the compound and the standard was calculated using equation (1).

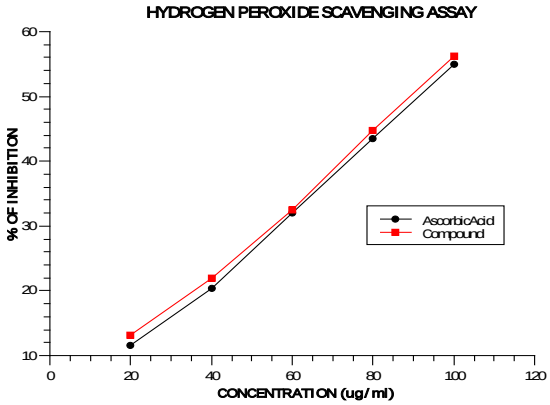


Table 6. IC_{50} Concentration of compound. (Hydrogen Peroxide Scavenging Assay)

Sample	Absorption at(100 µg/ml)	IC_{50} (µg/ml)
Ascorbic Acid	**0.737**	**90.992(1)**
Compound	**0.716**	**88.920(1)**

Docking Analysis

In the present work, *in silico* docking of the synthesized compound with snake venom enzyme namely, Phospholipase (PLA_2: PDB id:1FV0) (Fig.4) was performed using the molecular modeling software Maestro Schrodinger Software [17]. Induced Fit Docking (IFD) was carried out using Grid-based Ligand Docking with Energetics (GLIDE). IFD identifies the possible binding modes of ligand and associated conformational changes in the active site of the receptor molecule. In IFD, the first step is the protein and ligand preparation followed by energy minimization. Minimized conformation of protein and ligand was subjected to docking and the best Docked Poses were considered based on the Glide Energy (GE), Docking Score (DS), Hydrogen Bonds (HB) and Hydrophobic Interactions (HI). The present docking study of the compound has been taken up in order to know its inhibitory effect with the enzyme PLA_2.
The co-crystal ligand molecule aristolochic acid was re-docked using the method as described by Chandra et al [18] with the target and the best conformation was chosen as a template for comparison with the docked conformation of the synthesized compound.
It is known that Phospholipase A_2 (PLA_2) hydrolyzes the fatty acid at the sn-2 position of membrane phospholipids resulting in the production of eicosanoids and related bioactive lipid mediators of inflammation. PLA_2 enzyme was found to be the major component of the snake venom and chosen as the target for molecular docking studies to check the

anti-inflammatory potential of the synthesized compound. Co-crystal ligand aristolochic acid [18] was re-docked at the active site of PLA_2 enzyme resulting in docking energy and glide score of -49.45and -7.14, respectively. Re-docked aristolochic acid formed hydrogen bonds and hydrophobic interactions with the amino acid residues present at the active site on the enzyme (PLA_2). The active site is formed by the amino acid residues such as Serine (S), Isoleucine(I), Phenylalanine(F), Lysine(K), Cystine(C), and Tyrosine(T) as has been found similar to the co-crystal complex. The ligand was docked with PLA_2 to check the anti-inflammatory potential. The best conformation of the docked complex was identified based on the DS, GE and also based on the Mode of Ligand Binding (MLB). The ligand makes hydrogen bonds and several hydrophobic interactions at the active site on the enzyme. The Table 7 lists the active site interactions and Fig. 5 shows the ligplot representation of the active site interactions with the synthesized compound. A schematic representation of the electrostatic surface view model of the enzyme (PLA_2) with the bound ligand is shown in Fig.7.

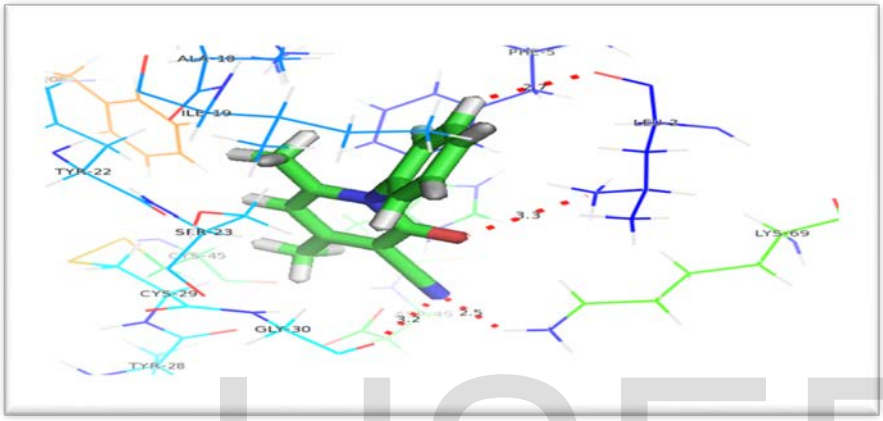


Fig 5. Diagram showing the molecular interactions of the synthesized compound with the enzyme (PLA_2).

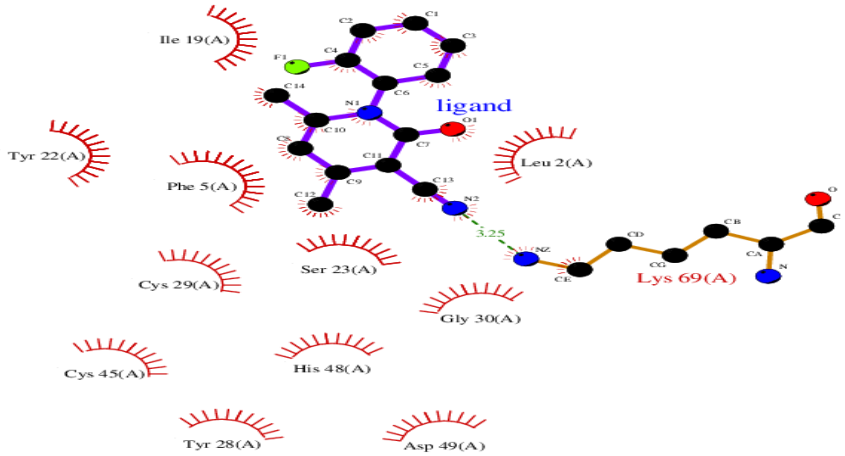


Fig 6. Ligplot representation showing the active site interactions. Hydrogen bond interaction between [N(2) of the ligand and Lys NZ (69) of PLA2] is shown in dotted lines and hydrophobic interactions are shown in red curves

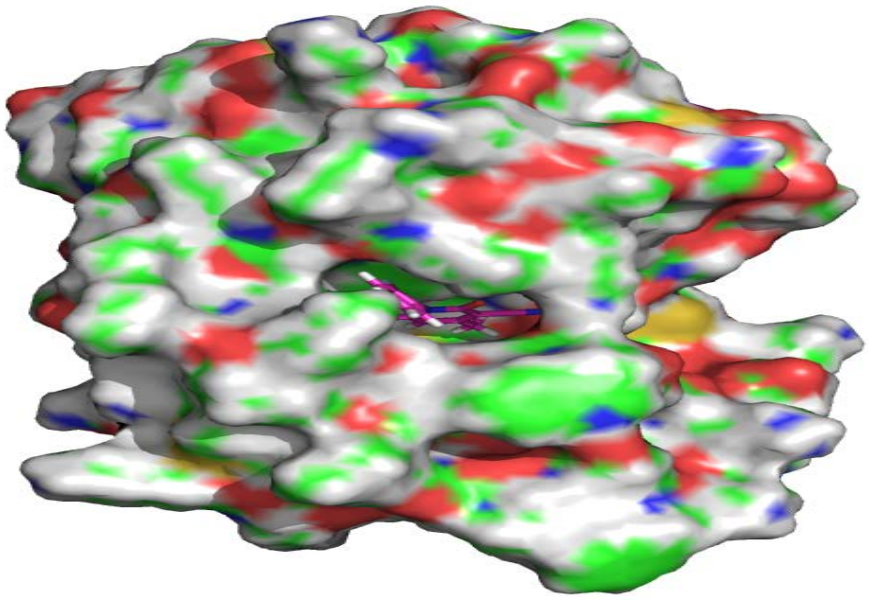


Fig.7. Surface view model showing the bound ligand (shown as stick model) at the active site of the snake venom enzyme (PLA_2).

The Molecular Docking Analysis of the title compound as ligand at the active of the enzyme PLA_2. (Fig.6) shows a preferred orientation of the compound with the enzyme and its stability through hydrogen bonding interaction at 3.25 Å along with hydrophobic interactions (Table 9) indicating that the compound may be considered as a good candidate for anti-inflammation activity. It is well known that the hydrogen bonding interaction [19] is one of the important parameter that is associated with biological activity such as the anti-inflammatory activity [20]. The Table (7) also contains the docking score and glide energy score. The compound may be considered as a potential candidate for use as an anti-oxidant and anti-inflammatory agent with glide energy of -36.7134 [20, 21] with the hydrogen bond distance involving atoms (Lys A 69 NZ) and N(2) of the compound(Table 8).This has been supported by the data of anti-oxidant and DPPH assay. The interactions of the molecule with PLA_2 enzyme are shown in Figs.4 and 6.

Table 7. Results of Molecular Docking Interactions of the compound

Entry ID	Docking score	Glide gscore	Glide Energy	XP GScore	XP HBond	XP Pose Rank	IFD Score
1	-6.26902	-6.269	-30.6092	-6.26902	-0.3500	5	-230.16
2	-5.72011	-5.7201	-32.0903	-5.72011	0.000	4	-229.98
3	-5.60604	-5.606	-33.0515	*-5.60604*	*-0.3500*	4	-229.83
4	-5.63882	-5.6388	-36.7134	-5.63882	**-0.1134**	3	-229.52
5	-5.10277	-5.1028	-32.0863	-5.10277	-0.5095	6	-229.09
6	-4.94928	-4.9493	-32.3803	-4.94928	-0.5426	4	-228.91
7	-4.78398	-4.784	-27.9839	-4.78398	-0.7000	4	-228.57

Table 8. Hydrogen bond interactionfrom Docking Study.

Donor (PLA2)	Acceptor (compound)	Distance (Å)
LYS A 69 NZ	N(2)	3.25

Table 9. Hydrophobic interactions due to Molecular Docking.

Atom 1	Atom 2	Distance (Å)
C(13)	LYS A 69 CE	3.85
C(12)	CYS A 45 CB	3.75
C(12)	CYS A 29 CA	3.73
C(5)	SER A 23 CB	3.50
C(3)	SER A 23 CB	3.76
C(14)	TYR A 22 CB	3.54
C(14)	TYR A 22 C	3.51
C(3)	ILE A 19 CD1	3.77
C(1)	ILE A 19 CD1	3.62
C(3)	ILE A 19 CG1	3.48
C(1)	ILE A 19 CG1	3.51
C(10)	PHE A 5 CE2	3.62
C(9)	PHE A 5 CE2	3.89
C(8)	PHE A 5 CE2	3.67
C(4)	PHE A 5 CD2	3.86

Conclusion and Future Work

The molecule of the synthesized compound exhibits profound anti-oxidant property as has been observed from both DPPH and Scavenger Assay experiments. The molecular docking score study on the compound with PLA_2 enzyme indicates that the compound is a good candidate as an anti-inflammatory agent based on the results of glide energy of the molecule which is -36.714 with glide score of -5.63. The molecules are staked along the longest c-axis held by both intra- and inter-hydrogen bonding interactions and thereby rendering stability to the crystalline lattice. The fluorine atom of the compound is not involved in hydrogen bonding. In future we are interested in studying the inhibitory action of several different thiophene derivatives with the enzyme and also compare with respect to those used to neutralize the PLA_2 induced actions through synthetic herbal compounds and anti PLA_2 rabbit antiserum in *in vivo* and *in vitro* models [21].

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