Electronic supplementary file for manuscript

# Crystal engineering with 1,3,4-oxadiazoles derivatives: On the importance of CH···N and CH···π interactions

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## Contents:

**In vitro Lipoxygenase inhibition studies (1-3).**

Anti-inflammatory potential can be determined by using soybean lipoxygenase enzyme as an *in-vitro* biochemical model.[1.] Similarly, indomethacin was used as reference drug. Spectrophotometric method with slight modification[1,2] was used to check the lipoxygenase activity of synthesized compounds (**1-3**). Inhibition was determined by measuring the loss of soybean 15-LOX activity (5 µg) with 0.2 $\mu$M linoleic acid as the substrate prepared in borate buffer (0.2 M, pH 9.0). The inhibition in triplicate at various concentrations of synthetic compounds were recorded at 234 nm using UV-Vis spectrophotometer. Indomethacin was used as positive control, while methanol was used as negative control. $IC_{50}$ indicating the concentration of 50% inhibition was calculated. Soybean lipoxygenase inhibition data is reported in $IC_{50}$ values.

**Molecular modeling study.**

All docking studies were performed using Autodock Vina (ver. 1.5.6).[3] For this purpose, the crystal structure of soybean lipoxygenase complexed with 13(S)-hydroproxy-9(Z)-2,11(E)-octadecadienoic acid (code ID: 1IK3) were retrieved from protein data bank. The co-crystallized ligand and water molecules were removed, and the protein was converted to pdbqt format using Autodock Tools.[4] The 2D structures of ligands were sketched using Chemdraw 12.0. the 2D structures were converted to 3D format by Openbabel (ver. 2.3.1).[5] PDBQT files were prepared in MGL Tools.[4] The docking parameters were set as follow: size_x = 40, size_y = 40 size_z = 40 center_x = 27.458; center_y = 4.218; center_z = 15.623. The other parameters were left as default. Finally, the conformations with the most favorable free energy of binding were selected for analyzing the interactions between the target enzyme and inhibitors. PyMOL version 1.8.8.2[6] and Chimera 1.6 software[7] was used for 3D molecular graphics, structural alignments and visualizations. Physicochemical properties were calculated using SwissADME.[8]

**Molecular docking Studies**

In order to investigate the binding mode of the inhibitors and their interaction with amino acid residues of lipoxygenase (PDB ID: 1IK3), molecular docking study of synthesized compounds was performed. Docking study further assisted in the identification of the relative location of the co-crystallized inhibitors and reference molecule in the protein architecture of lipoxygenase. Indomethacin was used as the reference drug in biological

screening; therefore, it was also docked with the enzyme to know its binding interaction.

**Lipoxygenase Structure**

The first crystal structure of a LOX[9,10] from Soybean, described by Boyington *et al.,* established the molecular framework common to both plant and animal enzyme. Crystal structure of lipoxygenase bears two major domains: an amino terminal β-barrel, now known as a PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain and a much larger α-helical domain that houses the catalytic iron.[9,11-14] The plant enzymes are significantly larger than the animal enzymes (~900 vs. ~650 amino acids, respectively), and the smaller animal enzymes are simply trimmed down by the omission of several plant-specific loop regions. Despite the differences, a large helical core, along with the relative placements of most of the ~17 helices that comprise it, is conserved. At the heart of the core is the catalytic iron, positioned by invariant histidine side chains contributed by the two longest helices in the common core as well as the main chain carboxyl at the C-terminus provided by an invariant Ile. An unusual structural feature of helix α8, a unique insertion which gives it a distinct curvature[15] has been observed in all LOX structures to date. Some inhibitors have been reported to bind either directly or indirectly to the adjacent amino acid residues of cofactor.[16]

**Molecular docking and Binding Analysis**

For elucidation of the molecular basis of the mechanism of inhibition for synthesized 2-aryl-5-substituted benzyl thio-1,3,4-oxadiazoles (**1-3**), the compounds were docked computationally to the active site of soybean LOX. In this study, first the protein structure (1IK3: 2.0 Å) was retrieved from Protein Data Bank (PDB).

To investigate the orientation of synthesized compounds, 2-((4-(*tert*-butyl)benzyl)thio)-5-phenyl-1,3,4-oxadiazol **(1)**, 2-((4-(*tert*-butyl)benzyl)thio)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole **(2)**, 2-(2,4-dichlorophenyl)-5-((3-fluorobenzyl)thio)-1,3,4-oxadiazole **(3)** and the reference compound indomethacin in the active pocket, all compounds were docked into the active site of LOX (PDB code: 1IK3) using Autodock Vina.

**Table S1**. Inhibitory activity against LOX

| Compound | $IC_{50}$ (μM), LOX |
|---|---|
| **1** | 0.626 |
| **2** | 0.223 |
| **3** | 0.248 |
| Indomethacin | 0.116 |

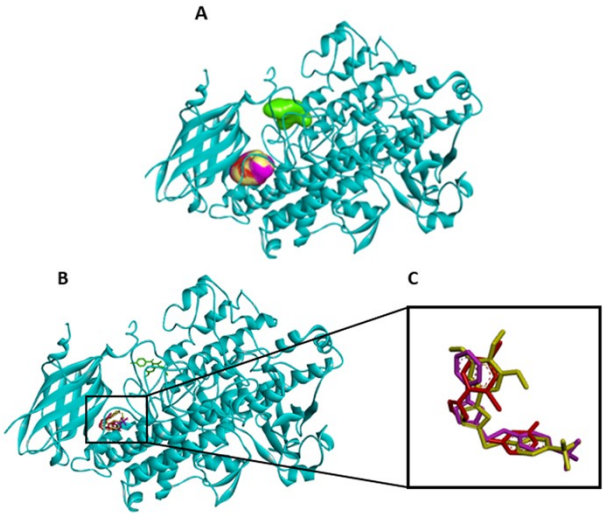


**Fig. 12** A; An overlay of the docked orientations of the most preferred conformations of compounds **1** (magenta), **2** (yellow), **3** (red) and indomethacin (green) (surface view) in the active pocket of lipoxygenase (cyan) shown in ribbons. B; Relative positioning of all 3 ligands in ball and stick model. C; Minimal energy conformations of oxadiazole **1** (magenta), **2** (yellow) and **3** (red).

In Fig. 12 is illustrated the relative positioning of 2-aryl-5-substituted benzyl thio-1,3,4-oxadiazoles (**1-3**) and indomethacin in their minimal energy conformation (out of 20 different conformations for each compound) in the active site of Lipoxygenase. The corresponding binding energies of ligands with most preferred conformation are shown in Table S1. As can be seen, the oxadiazoles 1, 2, 3 preferred to bind the enzyme at a place different in position to that for 2-aryl-5-substituted benzyl thio-1,3,4-oxadiazoles owing to structural differences in indomethacin and 2-aryl-5-substituted benzyl thio-1,3,4-oxadiazoles (**1-3**). The compounds 2-aryl-5-substituted benzyl thio-1,3,4-oxadiazoles (**1-3**) have potential to block the entry of substrate by binding to amino acid

residues lying near the pocket opening of α-helical domain. The enzyme/inhibitor complexes are stabilized by hydrogen bonds in the hydrophilic region and by π–π and van der Waals interactions in the hydrophobic region.

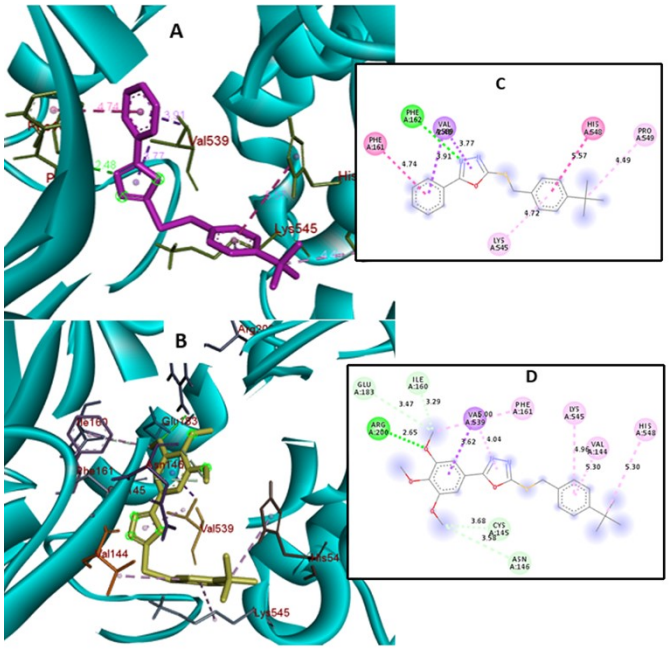


**Fig. S1** Docking pose of ligands in 3D and 2D display; A) oxadiazole **1** (magenta), B) oxadiazole **2** (yellow). Ligands are shown in stick mode while receptor is shown in cyan colored ribbons and key residues are shown in stick mode. 2D-Interaction diagram of compound **1** and **2** are represented in C and D respectively.

Compounds **1** and **2** showed additional hydrogen bond interaction (Fig. S1). In 2-((4-(*tert*-butyl)benzyl)thio)-5-phenyl-1,3,4-oxadiazol **(1)**, the nitrogen of oxadiazole form hydrogen bonding with main chain amide of Phe162 as hydrogen bond acceptor at a distance 3.91Å, while no such interaction was observed in 2-((4-(*tert*-butyl)benzyl)thio)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole **(2)** (Fig. S1). However, the compound 2 showed hydrogen bond with arginine Arg200 forming H-bond with oxygen of methoxy in compound **2** at a distance 4.74 Å (Fig. S1).

A prominent π–π interaction was observed with both the phenyl rings of 2-((4-(*tert*-butyl)benzyl)thio)-5-phenyl-1,3,4-oxadiazol **(1)** i.e, Phe161 forms π–π stacking interaction with the phenyl at 2 position of oxadiazole at a distance 4.74 Å while the phenyl of the thiobenzyl moiety forms π–π interaction with His545 at a distance 5.57 Å. In addition to hydrogen bonding and π–π stacking interactions, the molecule interacted with the binding site via π-alkyl, alkyl alkyl interactions (Fig. S1).

Docking analysis of 2-(2,4-dichlorophenyl)-5-((3-fluorobenzyl)thio)-1,3,4-oxadiazole **(3)** is represented in Fig. S2. the oxadiazole 3 adopts a more bent conformation. Most of the interactions between compound 3 and LOX enzyme were pi-alkyl with Val539 at a distance 3.45 and 3.80 Å, and with Lys545 and Val144 at a distance 4.68 and 5.11 Å respectively. Binding analysis showed that the oxadiazole 3 interacted more with non-polar amino acid residues (Fig. S2) however oxadiazole **2** and **1** showed more interaction with slightly polar amino acid residues (Fig. S2). This Molecular docking showed that the oxadiazoles **1**-**3** showed different mode of binding than that of indomethacin that exhibited higher potency than other 3 ligands.

Molecular docking results indicate that certain structural modifications may be necessary to enhance the effectiveness of the candidates for LOX activity.

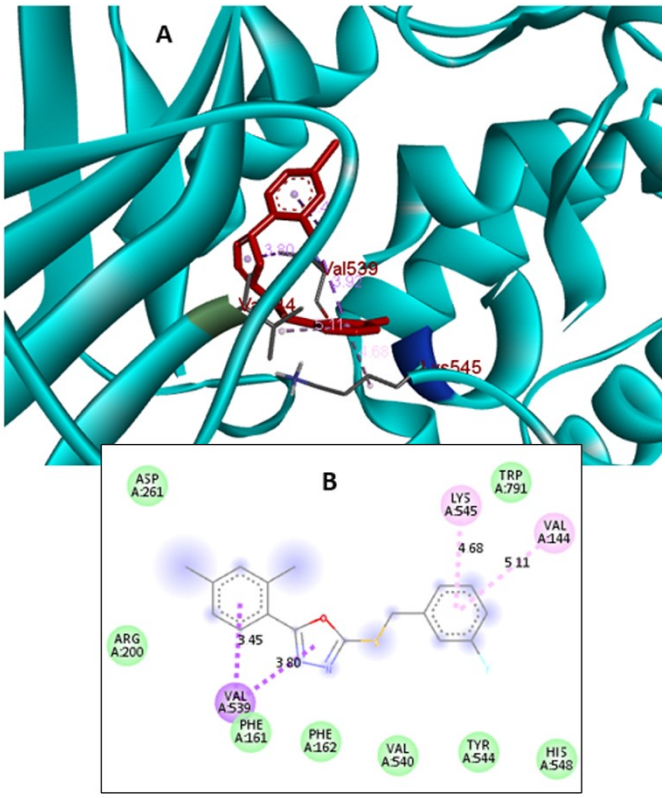


**Fig. S2** Docking pose of ligand 3 in 3D and 2D display; (Left) Compound **3** (red) shown in stick mode while receptor is shown in cyan colored ribbons and key residues are shown in stick mode. (Right) 2D-Interaction diagram of compound **3**.

## 2. NMR spectra

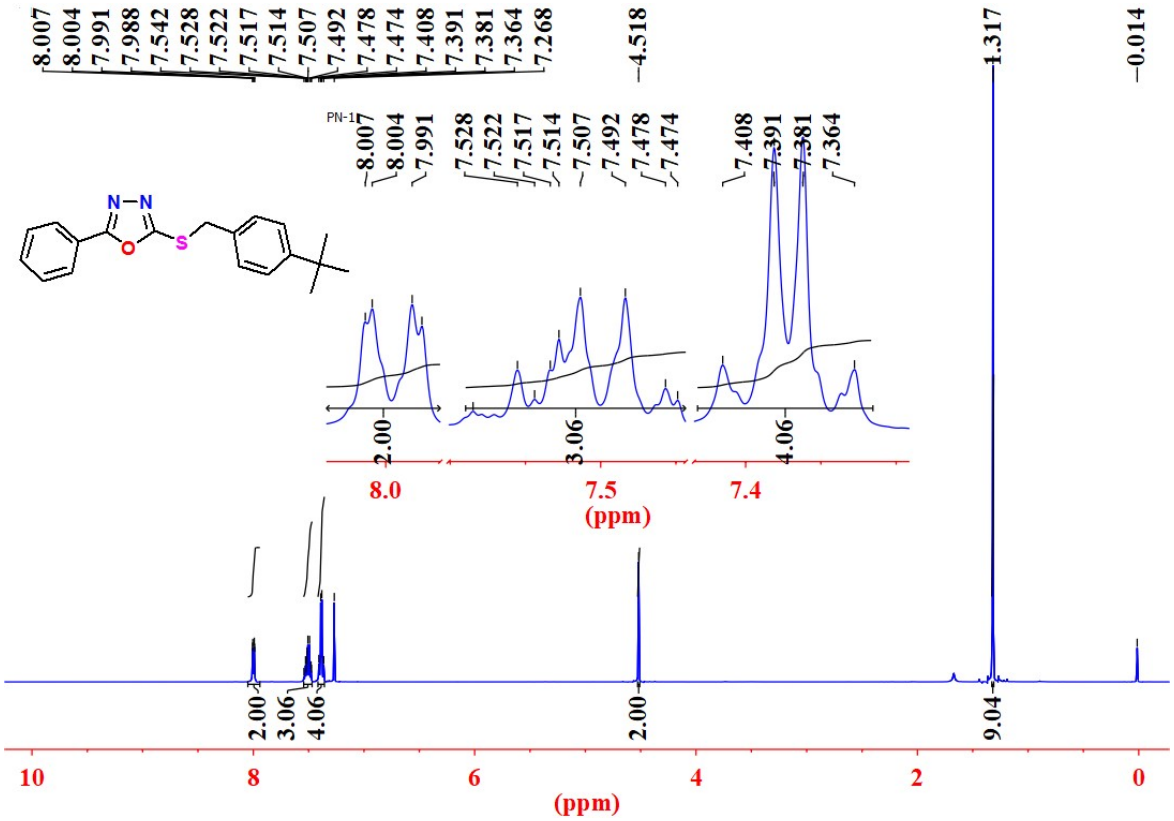


**Fig. S3** $^{1}$H NMR spectrum of compound **1**

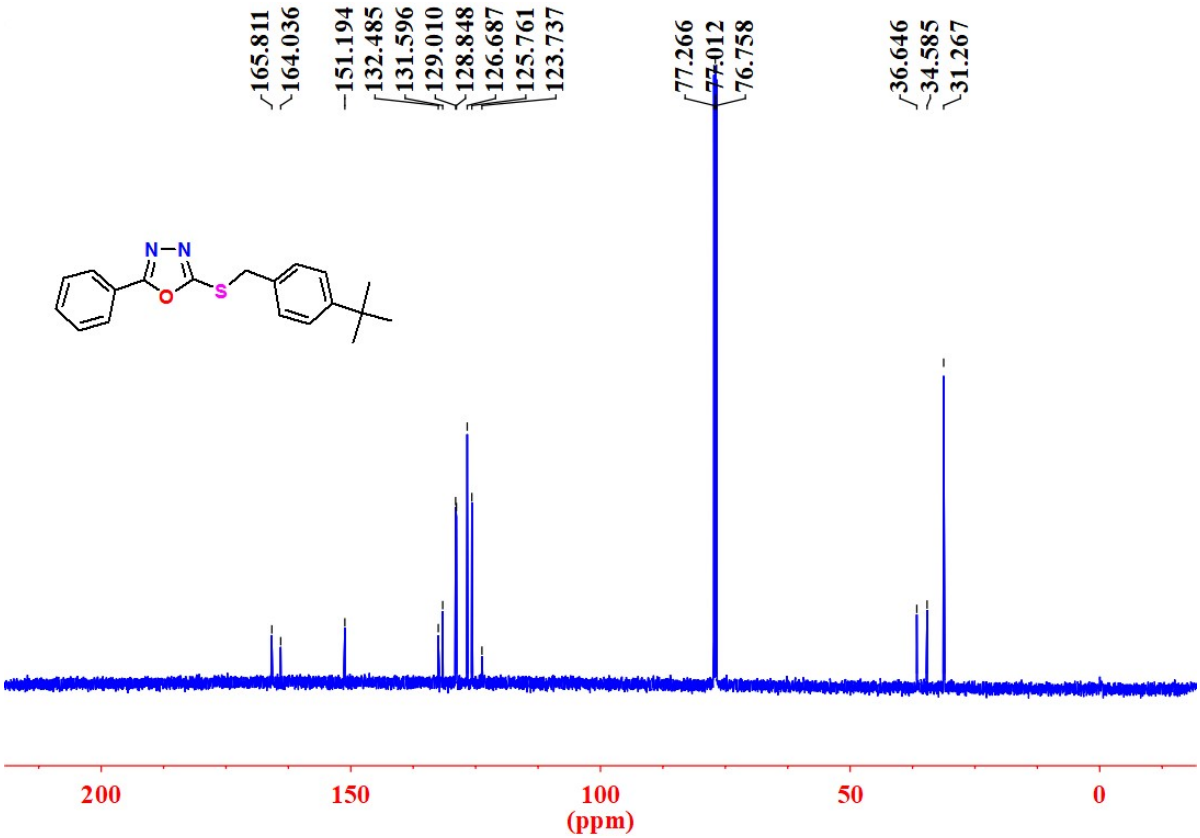


**Fig. S4** $^{13}$C NMR spectrum of compound **1**

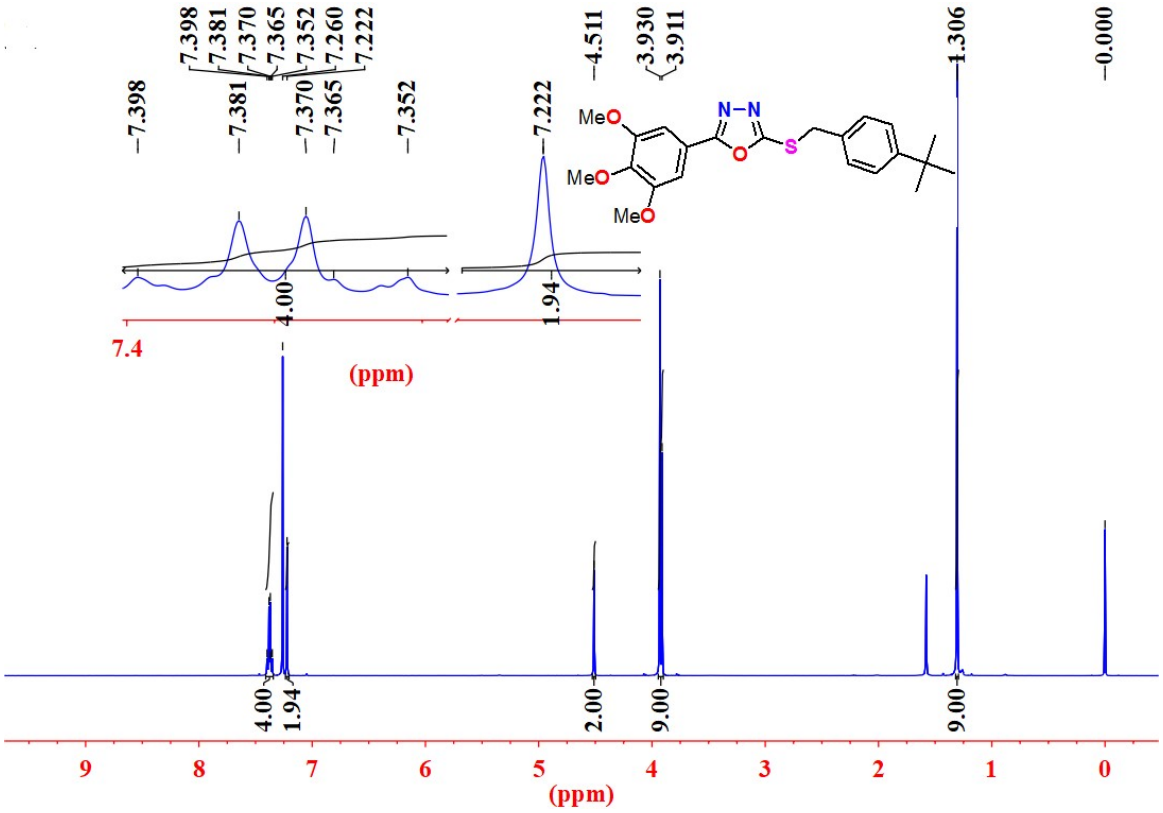


**Fig. S5** $^{1}$H NMR spectrum of compound **2**

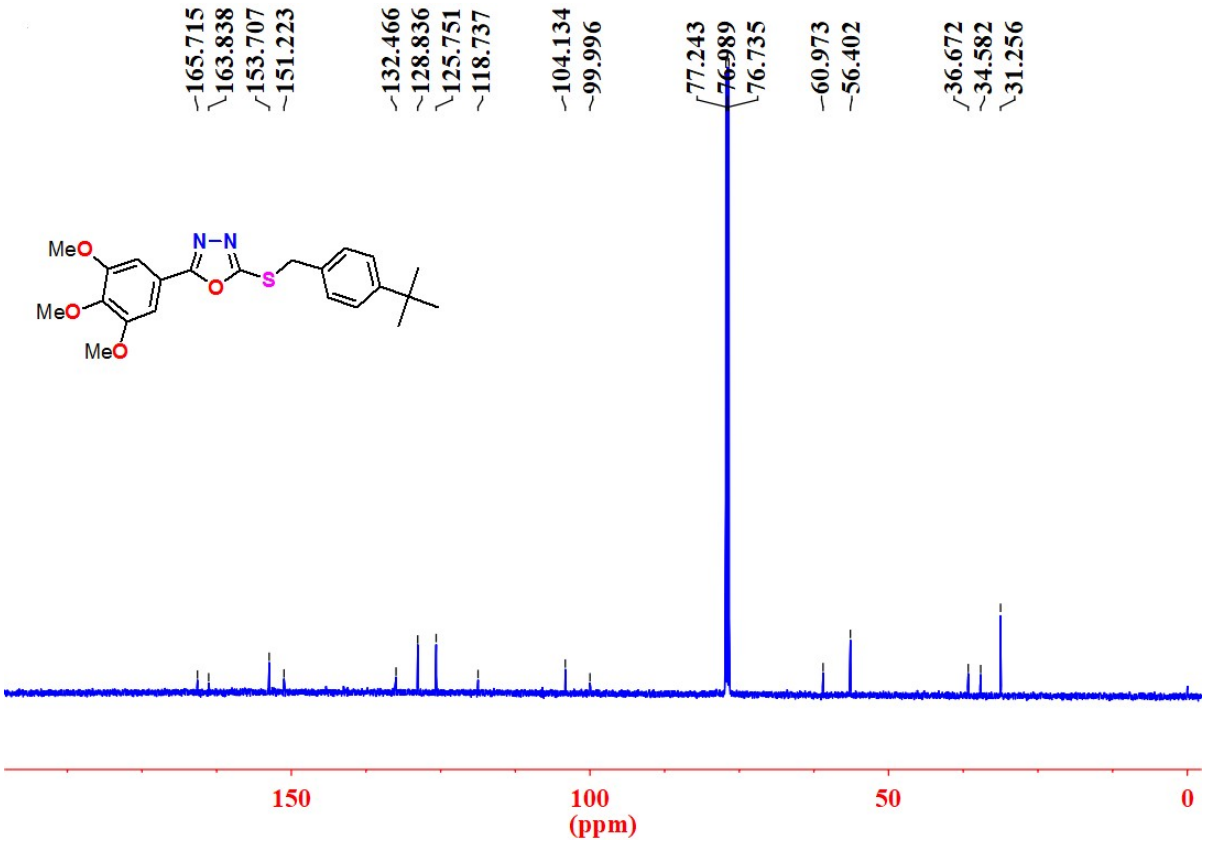


**Fig. S6** $^{13}$C NMR spectrum of compound **2**

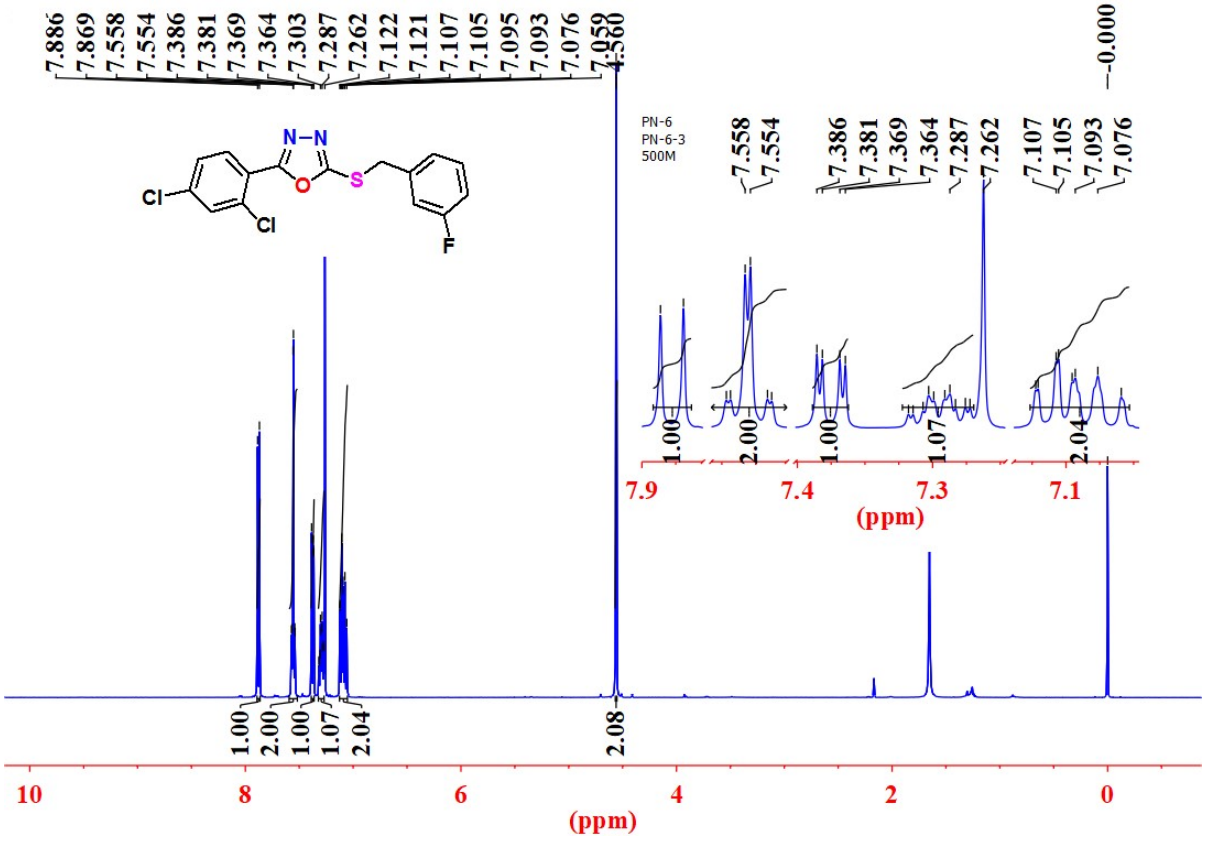


**Fig. S7** $^{1}$H-NMR spectrum of compound **3**

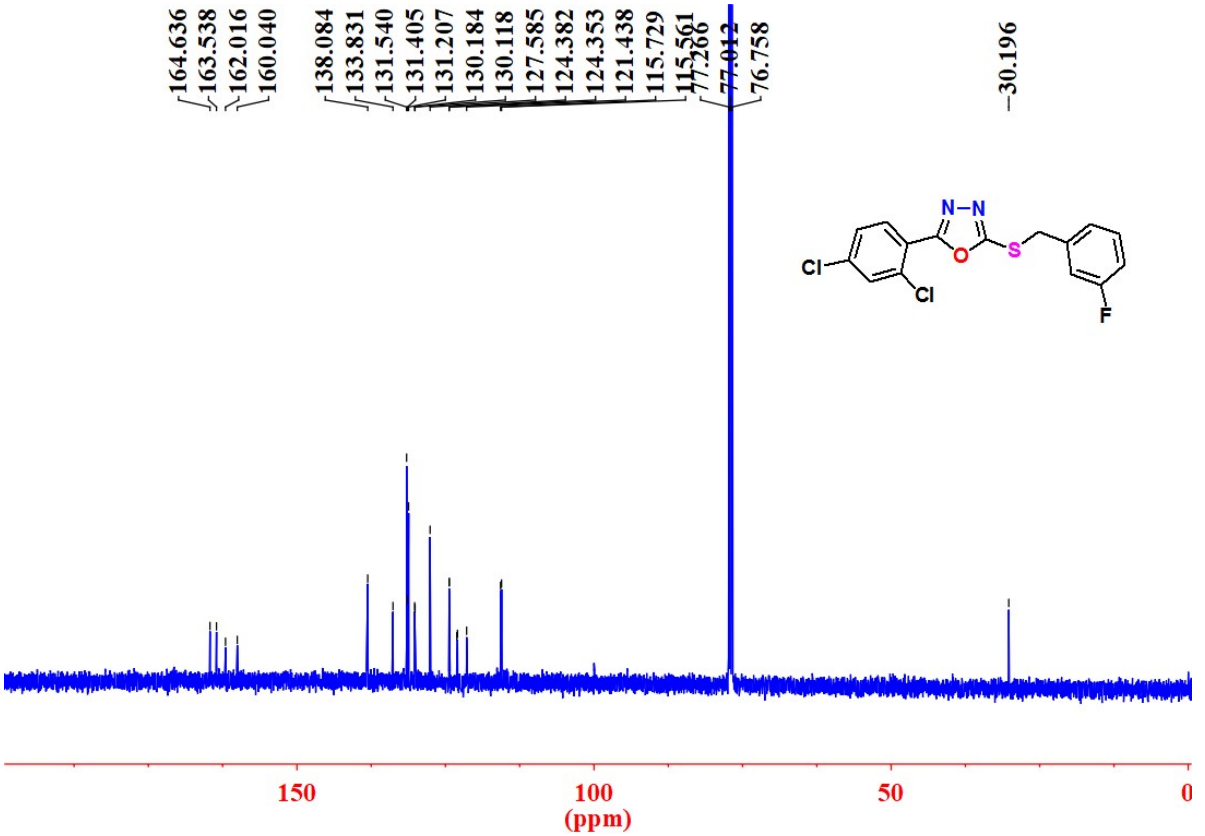


**Fig. S8** $^{13}$C-NMR spectrum of compound **3**

**3. References**


1. U. S. Akula and B. Odhav, J. Med. Plants Res., 2008, 2, 207-212.

2. M. N. Ahmed, M. Arif, H. Andleeb, S. W. A. Shah, I. Arshad, M. N. Tahir, M. Rocha and D. M. Gil, CrystEngComm, 2021, 23, 955-971.

3. O. Trott and A. J. Olson, J. Comput. Chem., 2010, 31, 455-461.

4. M. F. Sanner, J Mol Graph Model, 1999, 17, 57-61.

5. N. O'Boyle, J. Cheminformatics, 2011, 3, 33.

6. The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrodinger, Schrodinger LLC, New York, NY, 2008.

7. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, J. Comput. Chem.,2004, 25, 1605-1612.

8. A. Daina, O. Michielin and V. Zoete, Sci. Rep., 2017, 7, 42717.

9. D. B. Neau, N. C. Gilbert, S. G. Bartlett, W. Boeglin, A. R. Brash and M. E. Newcomer, Biochem., 2009, 48, 7906-7915.

10. H. Sadeghian and A. Jabbari, Expert opinion on therapeutic patents, 2016, 26, 65-88.

11. Y. Xu, A. J. Streets, A. M. Hounslow, U. Tran, F. Jean-Alphonse, A. J. Needham, J.-P. Vilardaga, O. Wessely, M. P. Williamson and A. C. Ong, J. Am. Soc. Nephr: JASN, 2016, 27, 1159.

12. M. E. Newcomer and A. R. Brash, Protein Science, 2015, 24, 298-309.

13. E. Skrzypczak-Jankun, J. Chorostowska-Wynimko, S. H. Selman and J. Jankun, Curr. Enz. Inhibit., 2007, 3, 119-132.

14. J. C. Boyington, B. J. Gaffney and L. M. Amzel, Science, 1993, 260, 1482-1486.

15. A. Parkin, G. Barr, W. Dong, C. J. Gilmore, D. Jayatilaka, J. J. McKinnon, M. A. Spackman and C. C. Wilson, CrystEngComm, 2007, 9, 648-652.

16. Y. Wang, X. Lu, J. Shi, J. Xu, F. Wang, X. Yang, G. Yu, Z. Liu, C. Li and A. Dai, , Monatsh. Chem., 2018, 149, 611-623.