Supplementary Information (SI) for Molecular Systems Design & Engineering.

Design and Evaluation of Novel Hydrazide Derivatives as NS1 Inhibitors for Dengue Virus: Synthesis, Experimental, and Computational Studies

Swathi Venkatesan,[a] Prathima T S,[a] Badruzzaman Choudhury,[b] Kaushik Chanda,[c]* Balamurali MM[a]*

[a]Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Chennai Campus, Chennai, Tamil Nadu, India, 600027

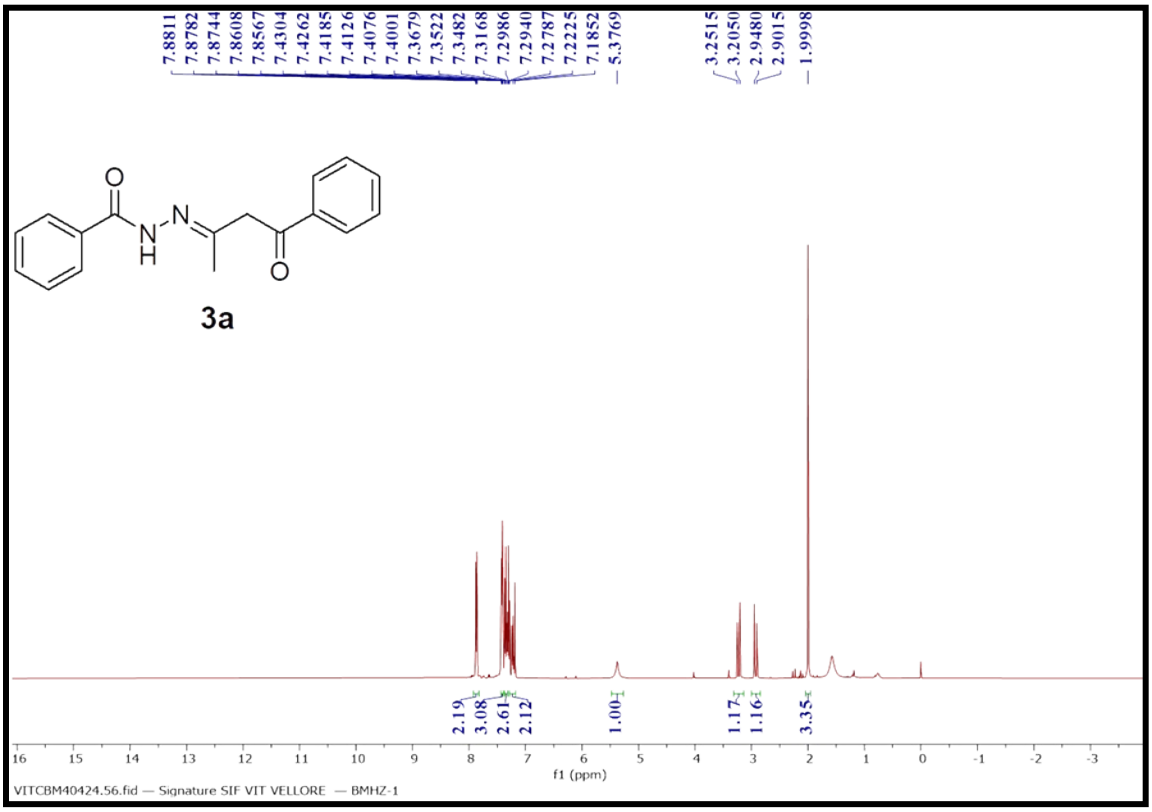
[b]Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, Tamil Nadu, India, 632014.

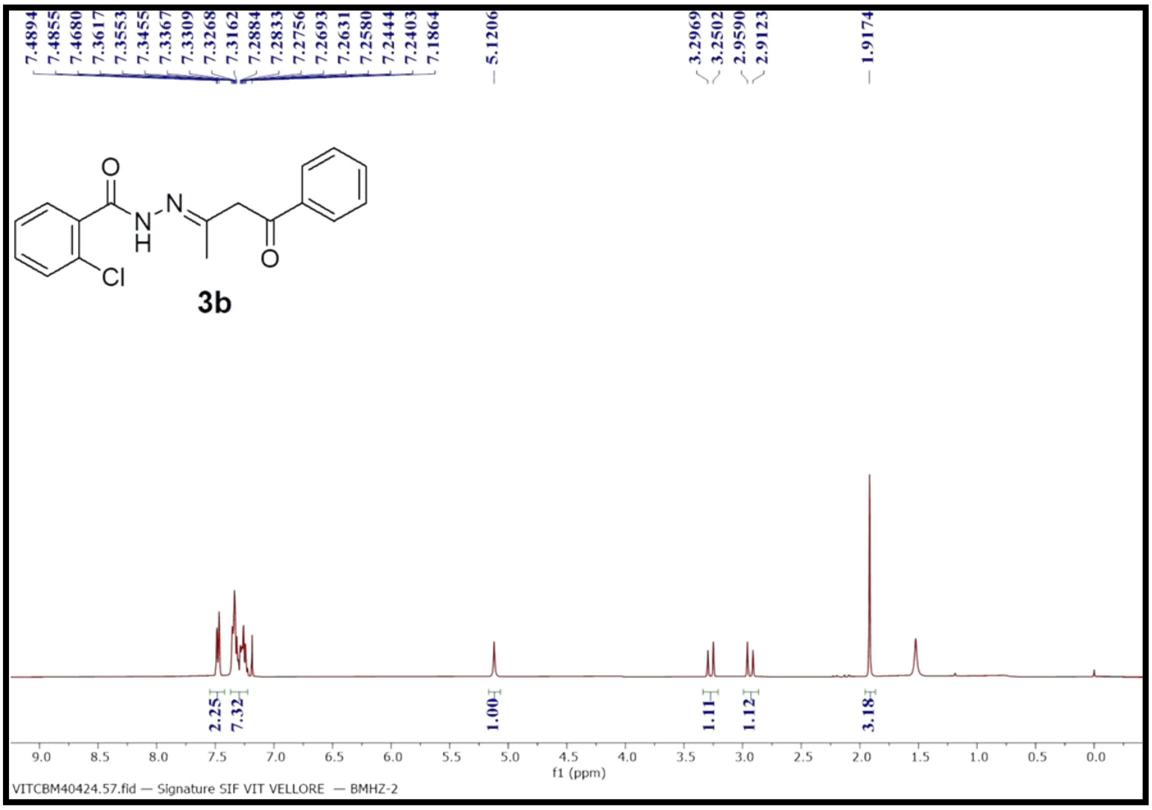
[c]Department of Chemistry, Rabindranath Tagore University, Hojai 782435, Assam, India

Email: chandakaushik1@gmail.com

mmbala@gmail.com

^{1}H NMR spectra of compounds 3a-j

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5.1206
3.2969 3.2502 2.9590 2.9123
1.9174
Cl
3b
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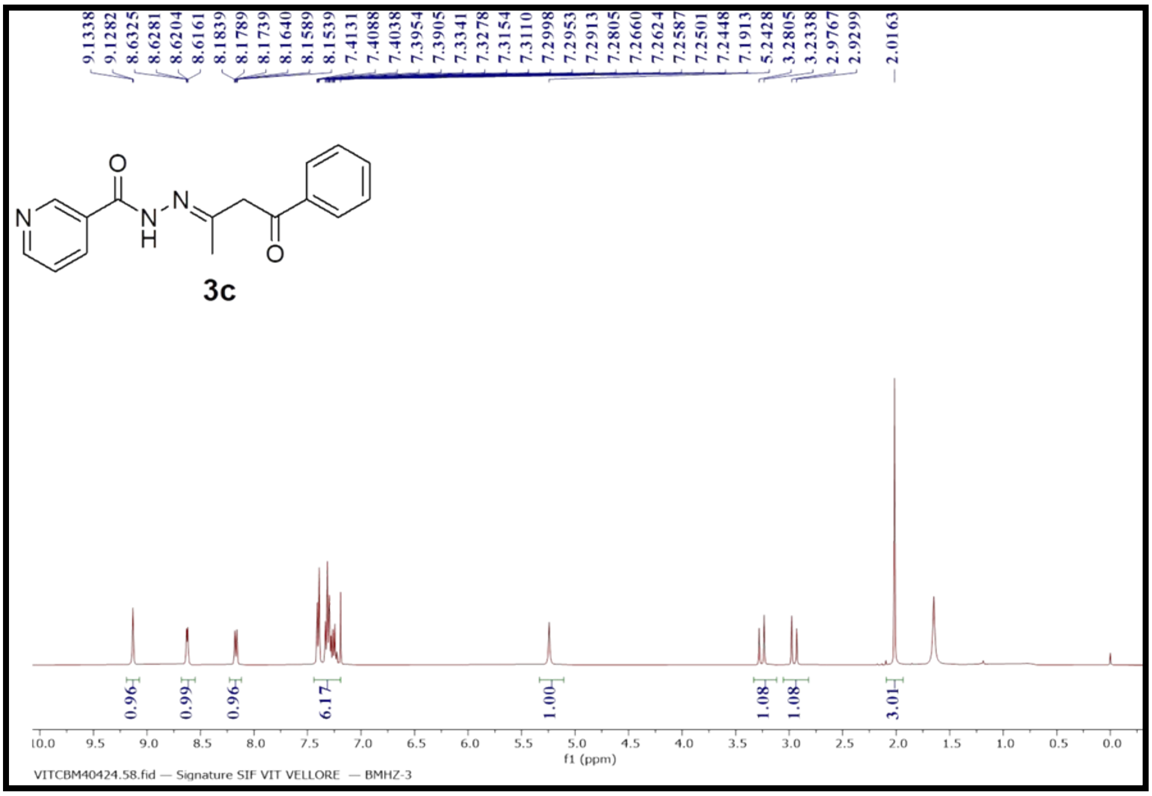
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5.2428
3.2805 3.2338 2.9767 2.9299
2.0163
3c
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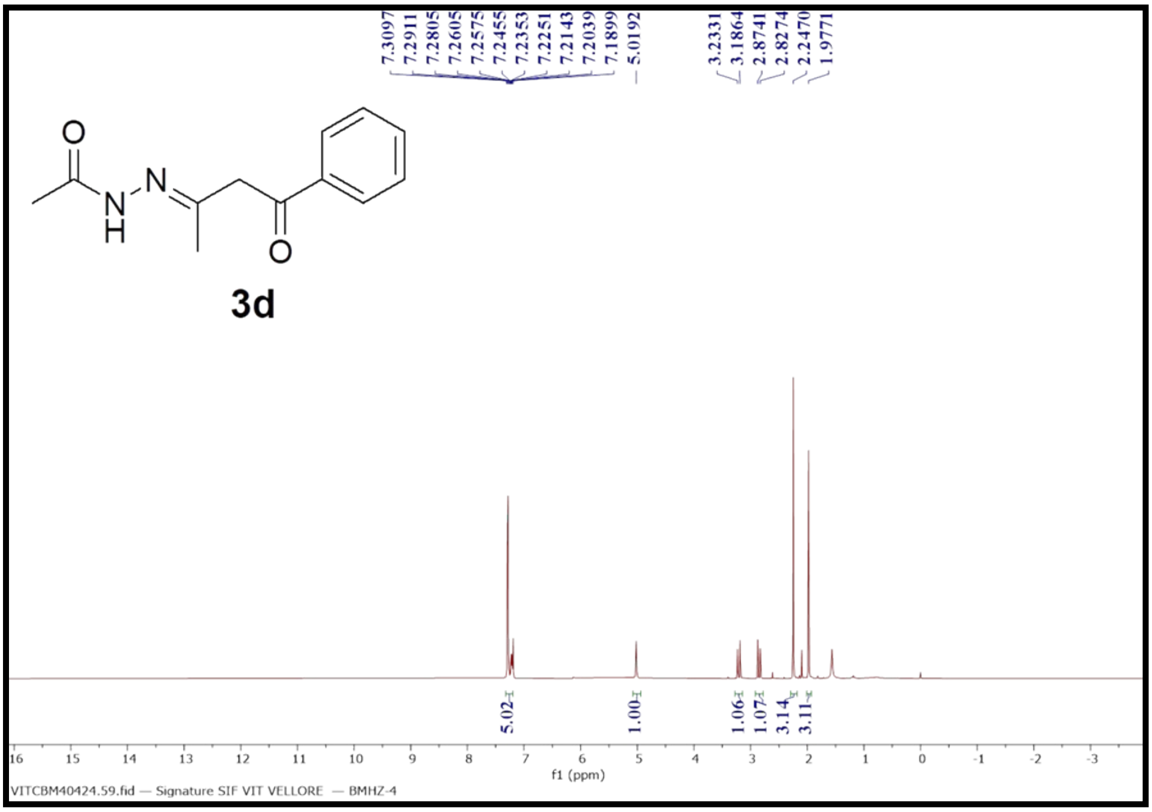
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5.0192
3.2331
3.1864
2.8741
2.8274
2.2470
1.9771
O
N
N
H
O
3d
5.02
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1.07
3.14
3.11
f1 (ppm)
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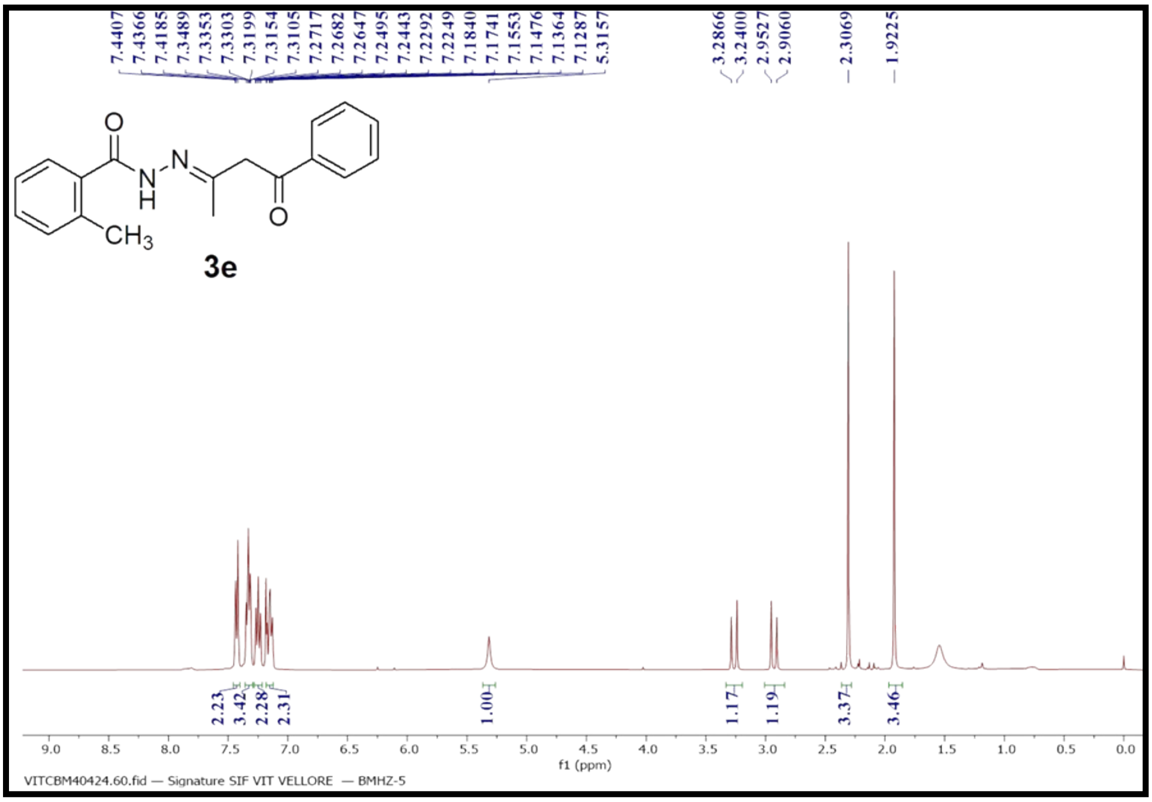
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7.1287
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3.2866
3.2400
2.9527
2.9060
2.3069
1.9225
O
N
N
H
O
CH3
3e
2.23
3.42
2.28
2.31
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f1 (ppm)
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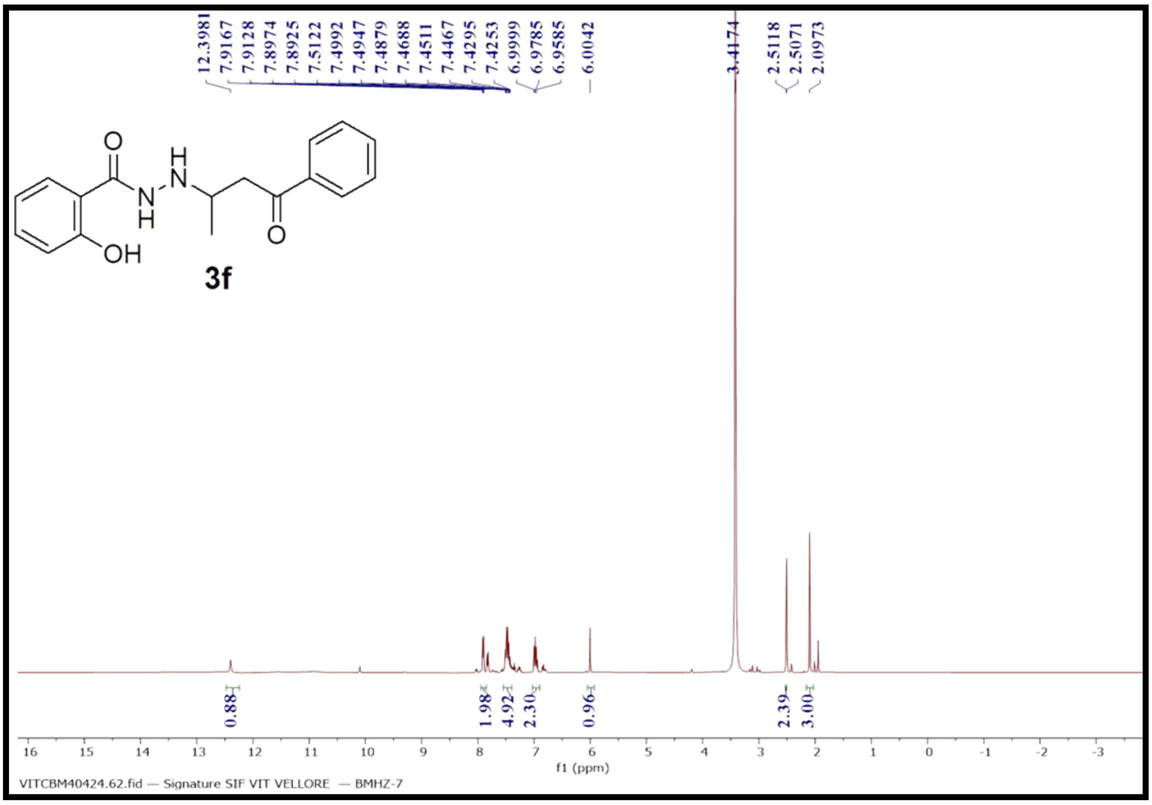
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7.4467
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7.4253
6.9999
6.9785
6.9585
6.0042
3.4174
2.5118
2.5071
2.0973
OH
3f
0.88
1.98
4.92
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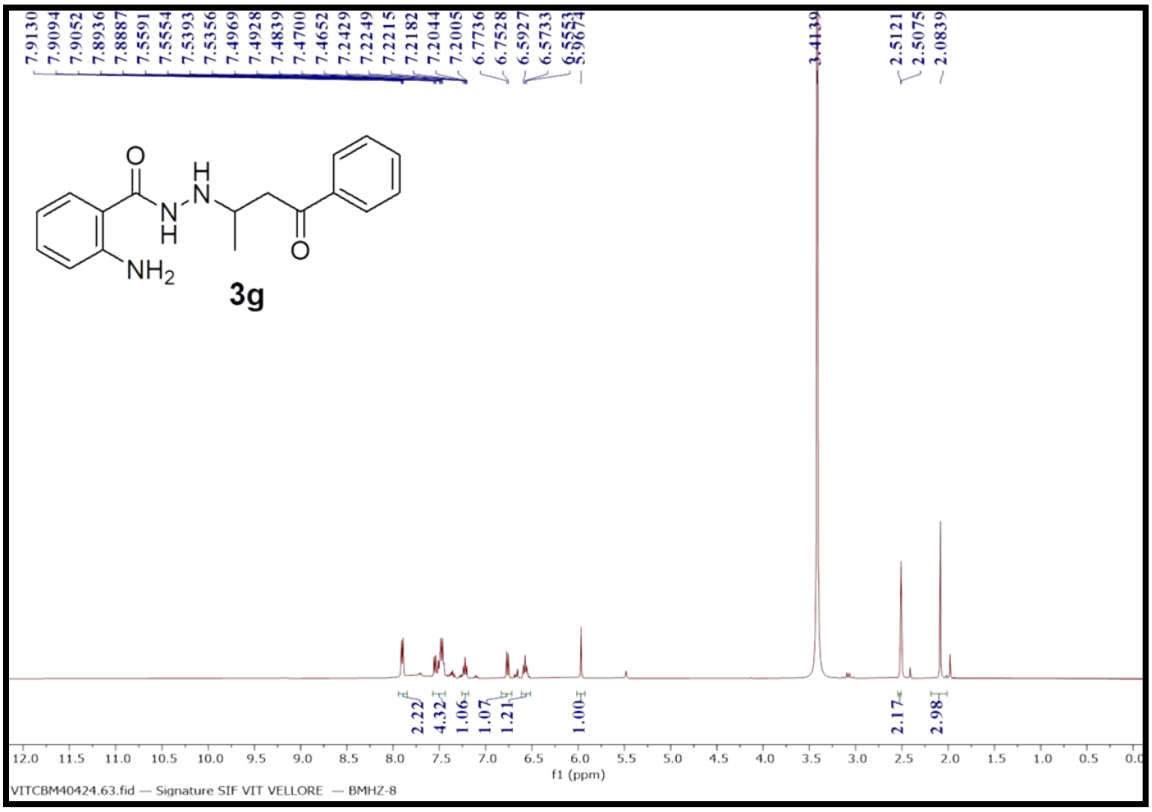
0.96
2.39
3.00
f1 (ppm)
VITCBM40424.62.fid — Signature SIF VIT VELLORE — BMHZ-7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7.2429
7.2249
7.2215
7.2182
7.2044
7.2005
6.7736
6.7528
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6.5733
6.5553
5.9674
3.4139
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NH2
3g
2.22
4.32
1.06
1.07
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1.00
2.17
2.98
f1 (ppm)
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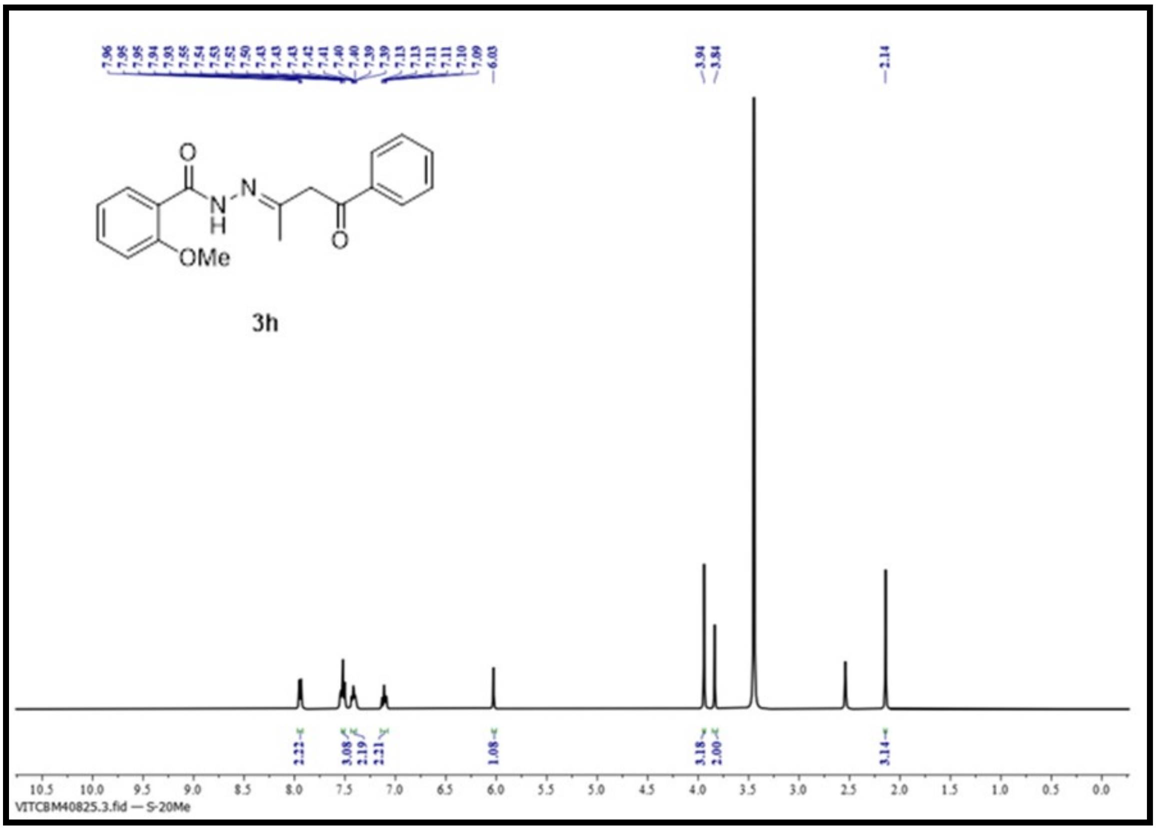
3h
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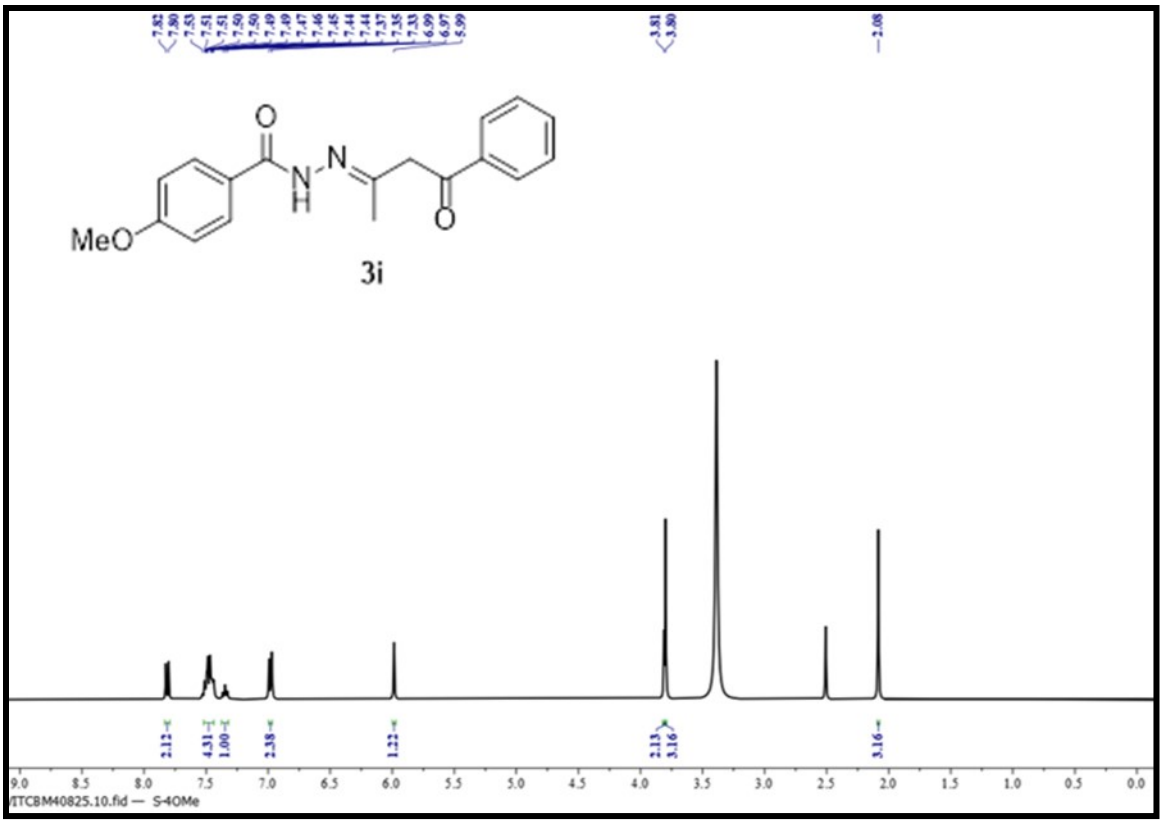
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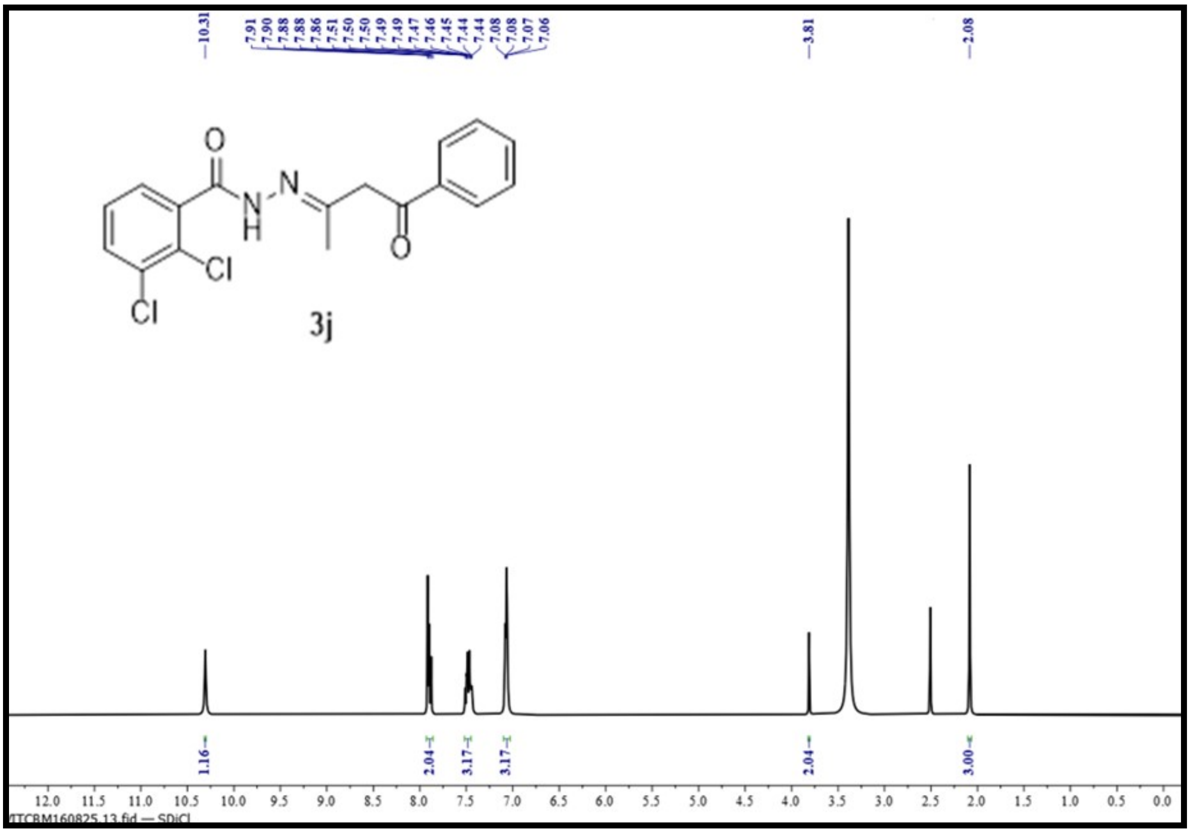
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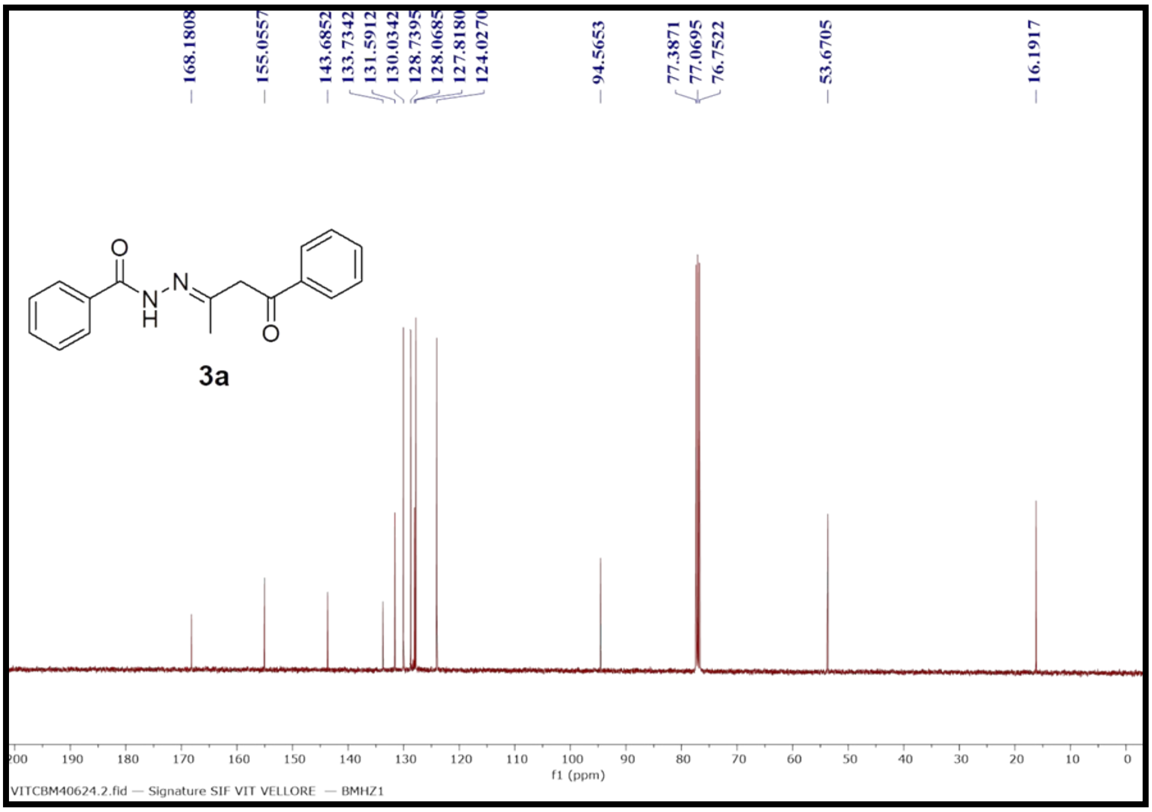
7.45
7.44
7.44
7.08
7.08
7.07
7.06
3.81
2.08
1.16
2.04
3.17
3.17
2.04
3.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

^{13}C NMR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3a
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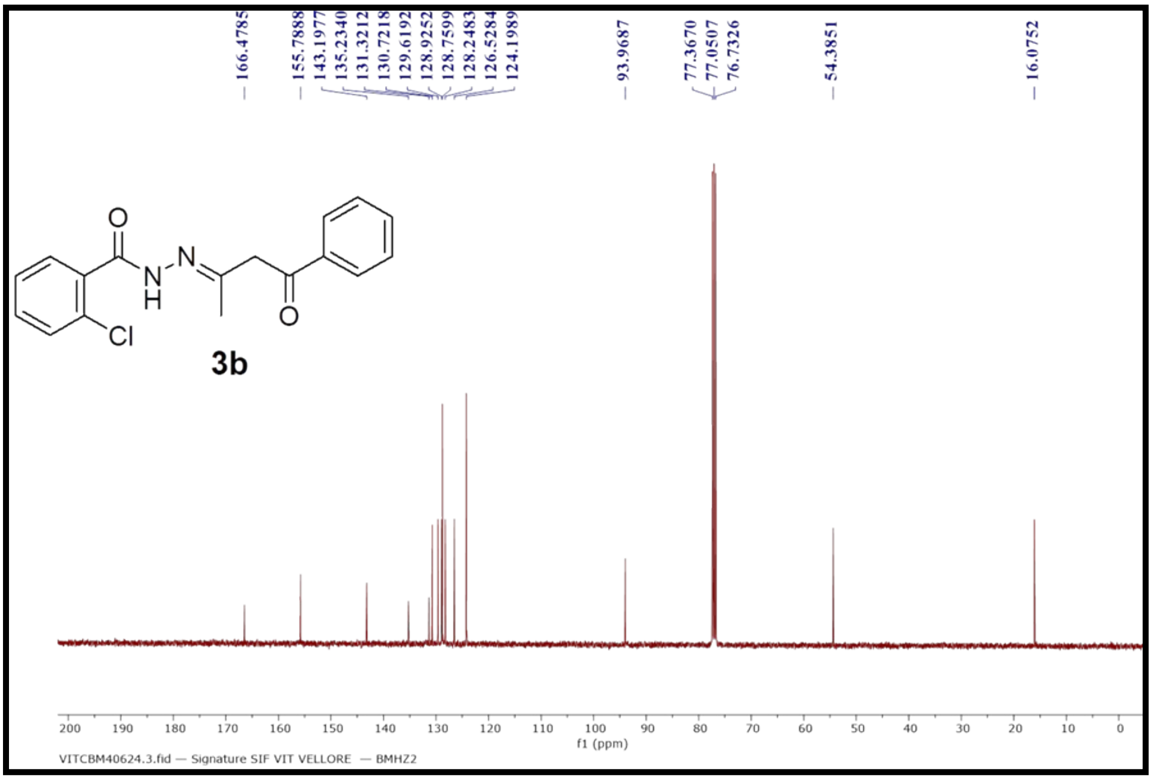
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126.5284
124.1989
93.9687
77.3670
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3b
f1 (ppm)
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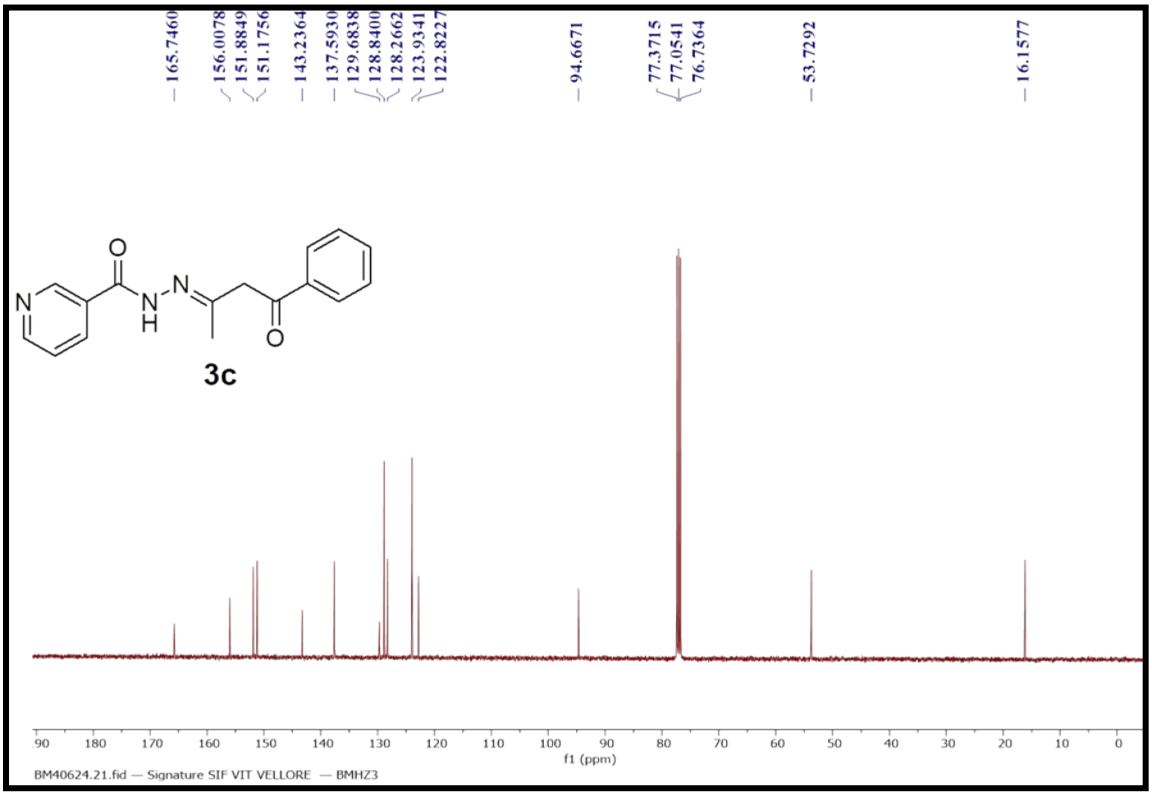
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143.2364
137.5930
129.6838
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128.2662
123.9341
122.8227
94.6671
77.3715
77.0541
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53.7292
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3c
f1 (ppm)
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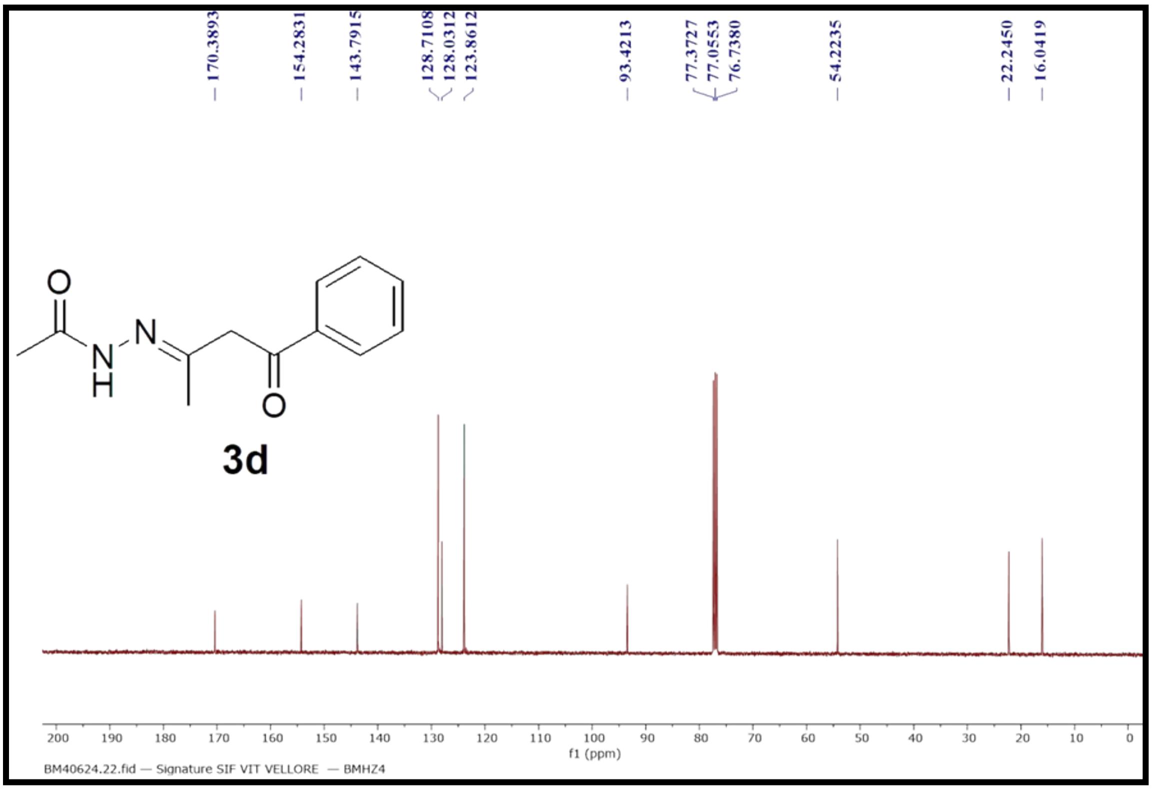
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123.8612
93.4213
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77.0553
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22.2450
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3d
f1 (ppm)
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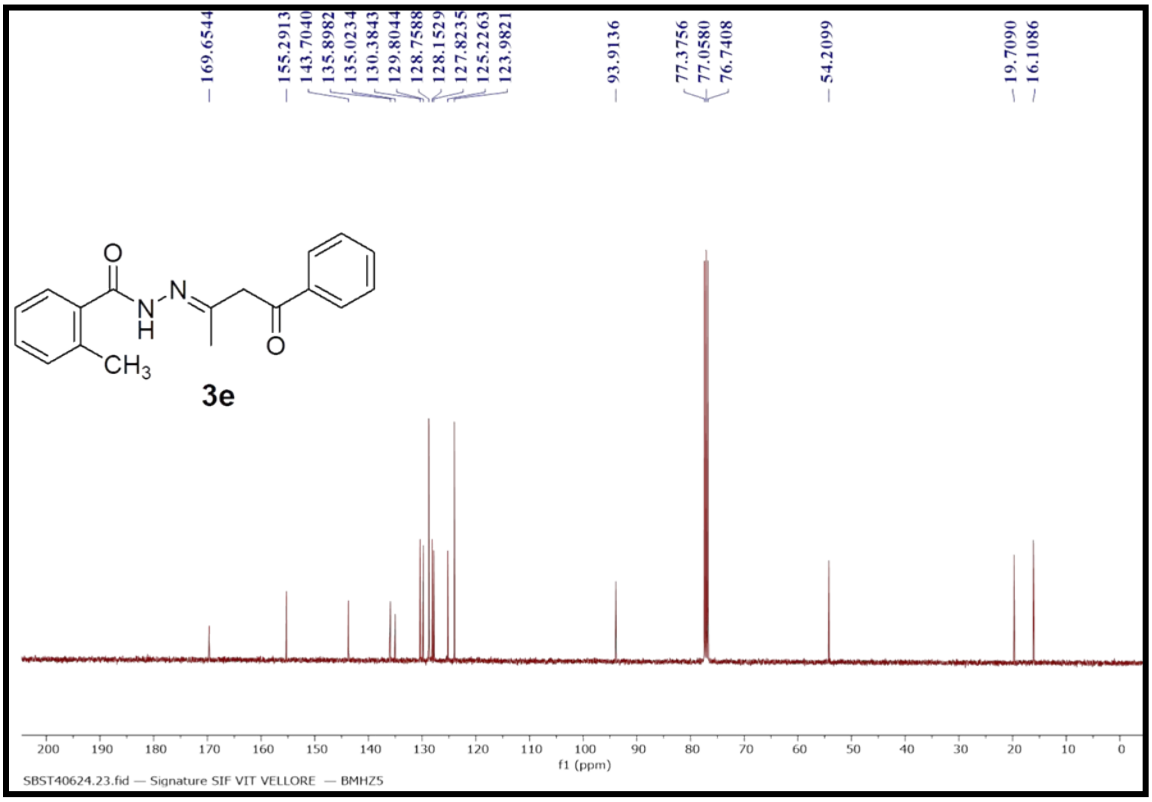
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128.1529
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125.2263
123.9821
93.9136
77.3756
77.0580
76.7408
54.2099
19.7090
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O
N
N
H
O
CH_3
3e
f1 (ppm)
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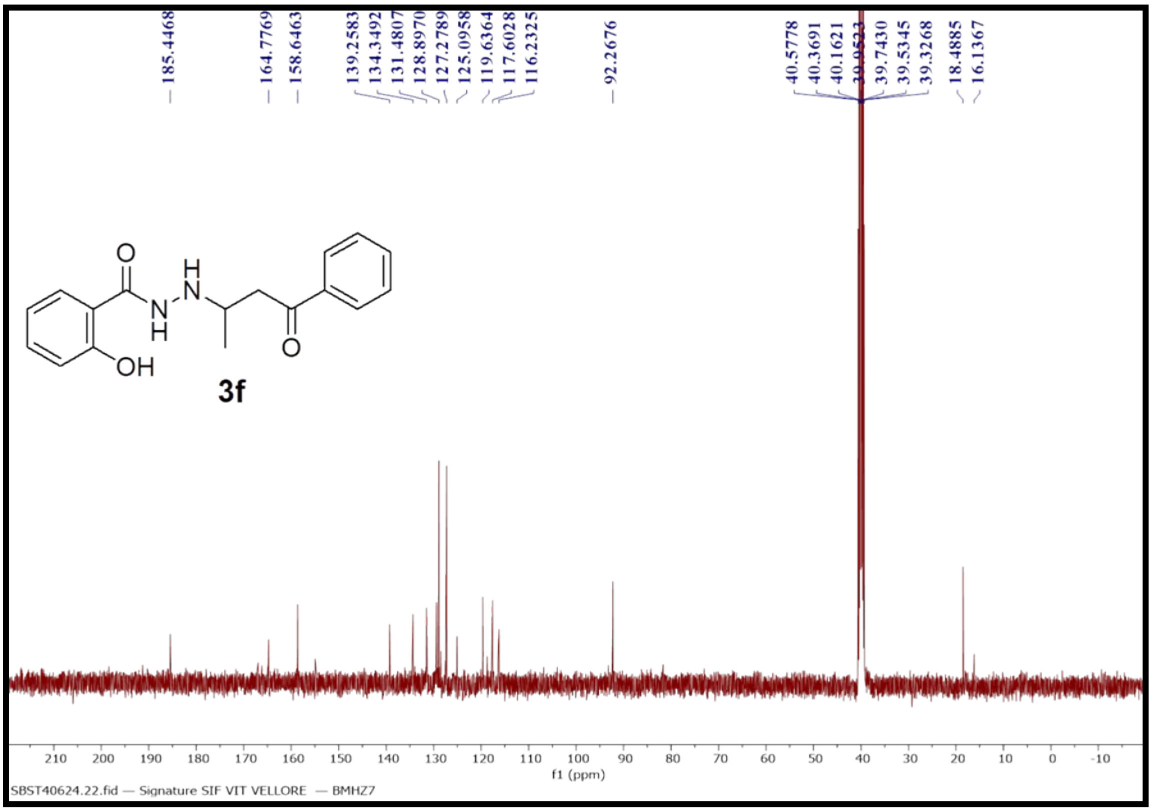
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92.2676
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H
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OH
3f
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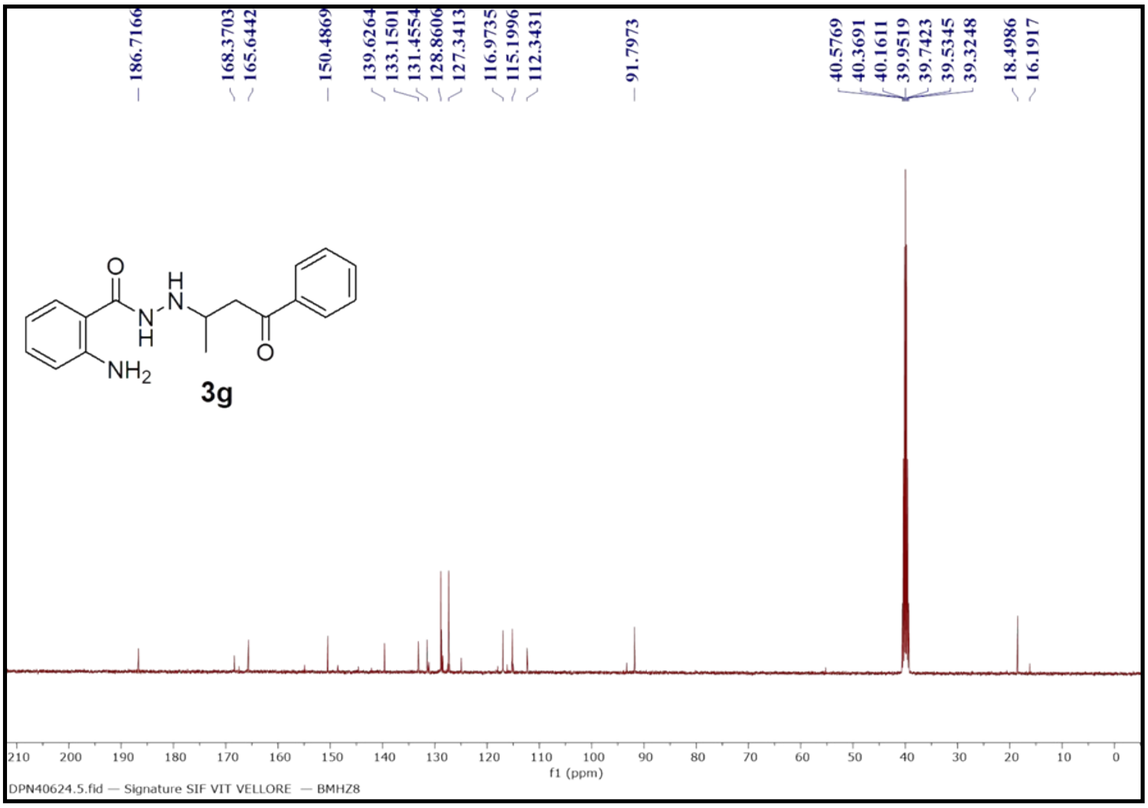
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133.1501
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112.3431
91.7973
40.5769
40.3691
40.1611
39.9519
39.7423
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18.4986
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3g
f1 (ppm)
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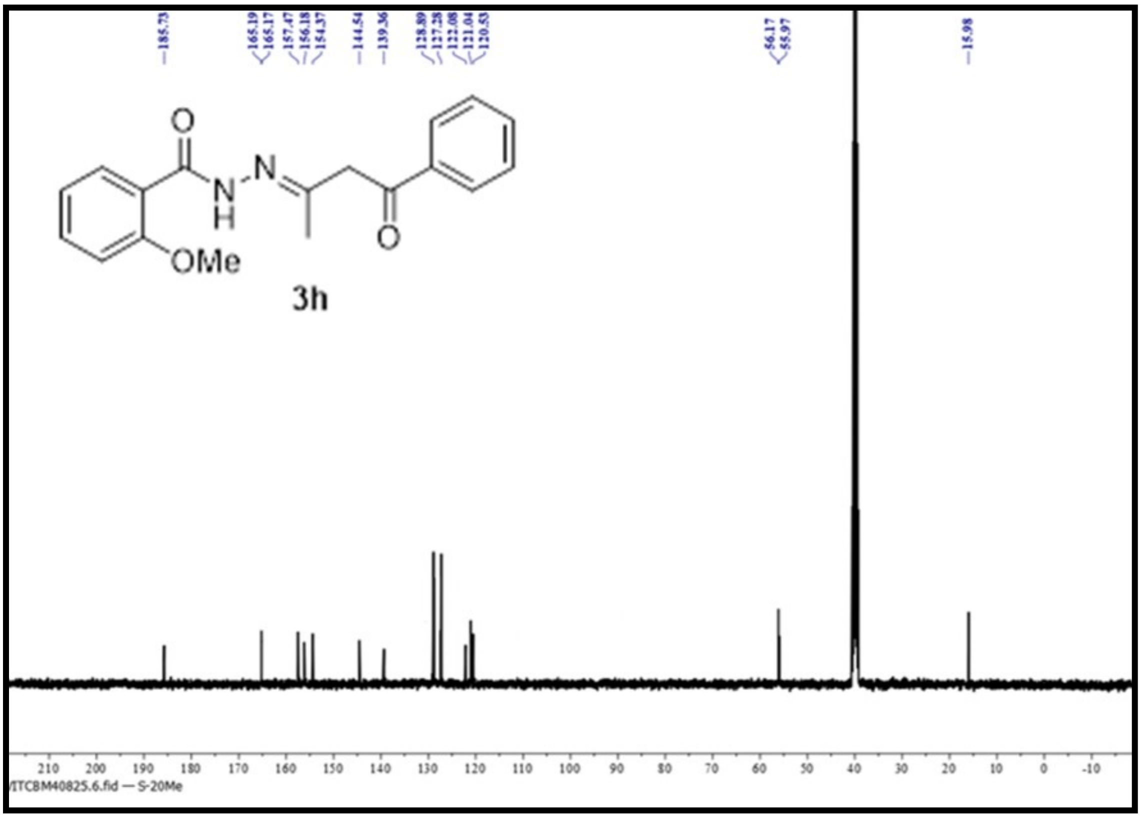
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3h
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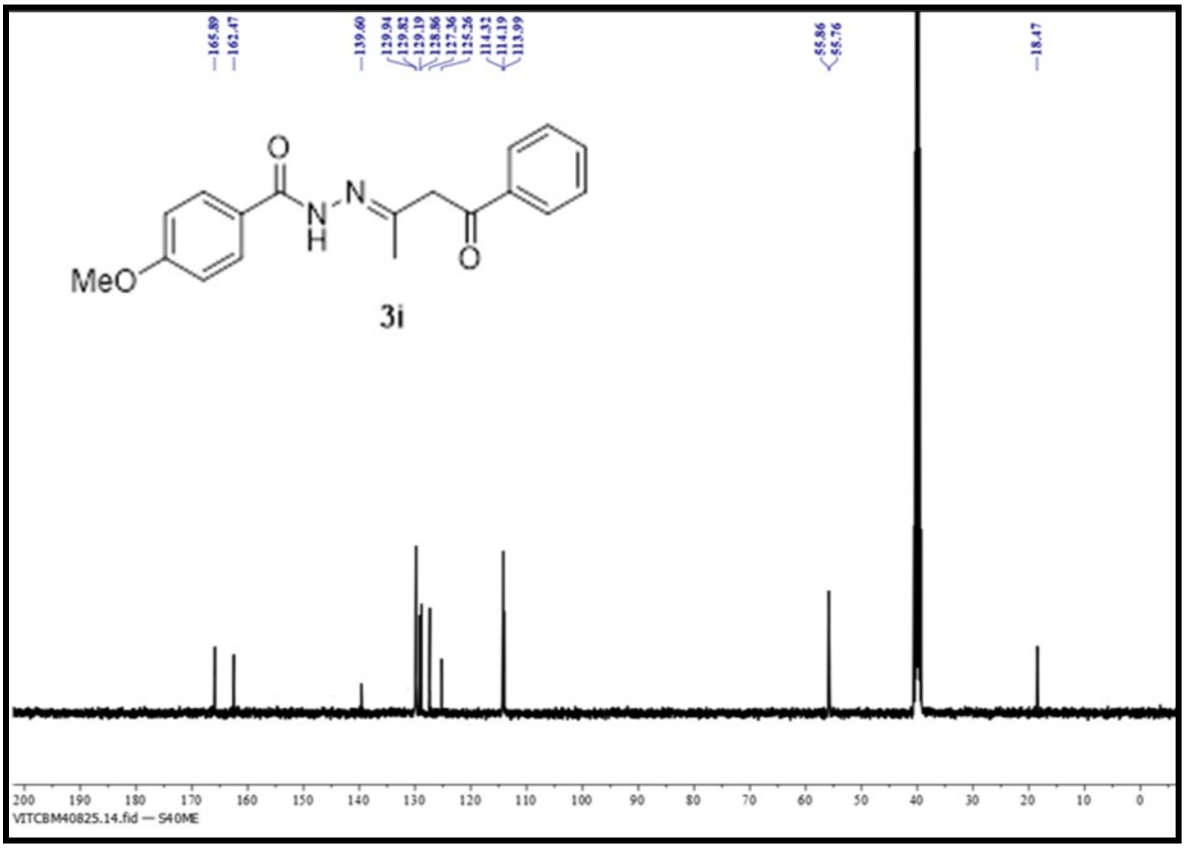
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127.36
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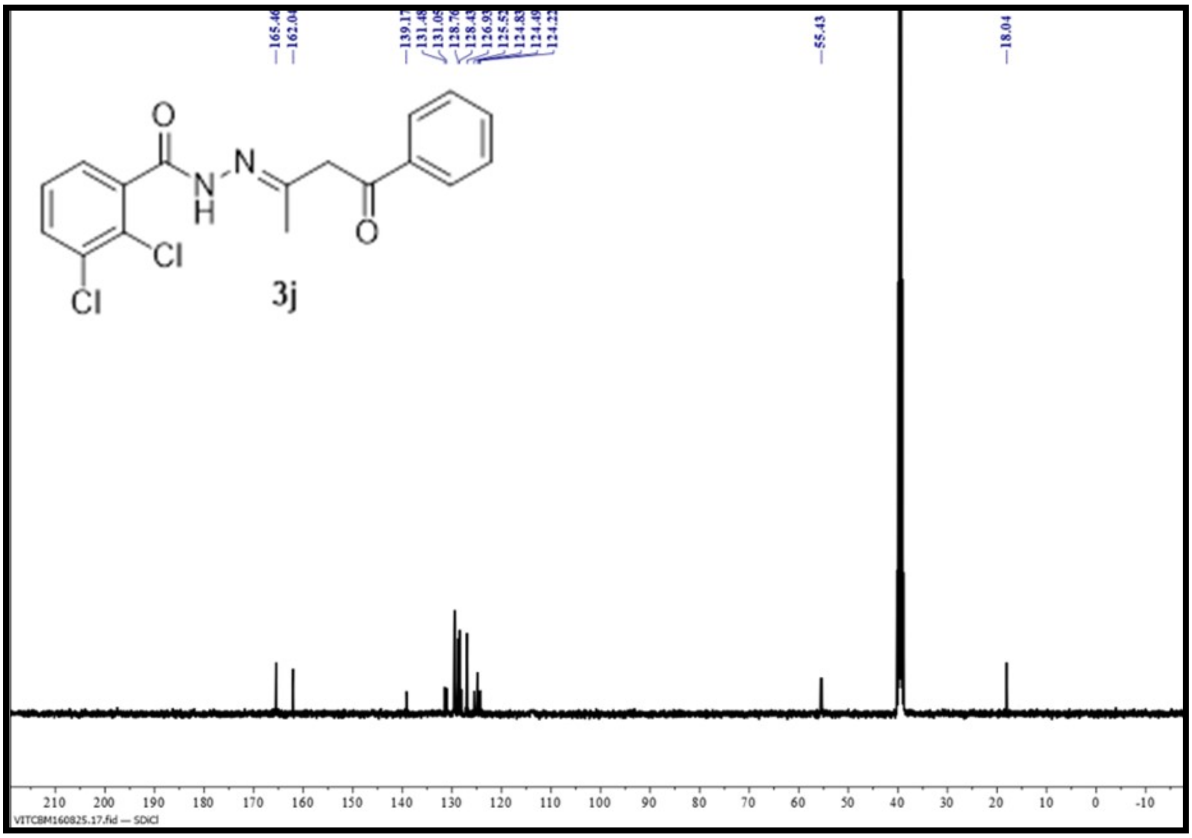
55.86
55.76
18.47
MeO
3i
VITCBM40825.14.fid — S4OME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Cl
Cl
3j
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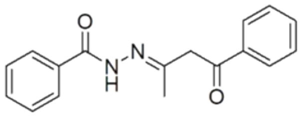
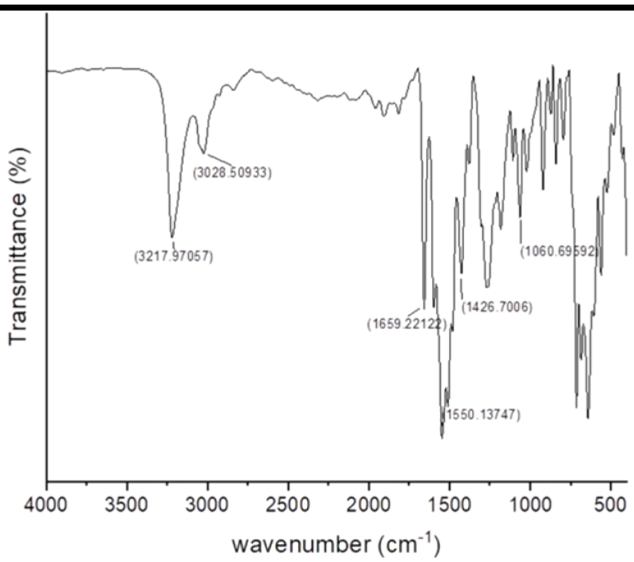
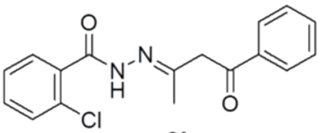
IR Spectra



3a

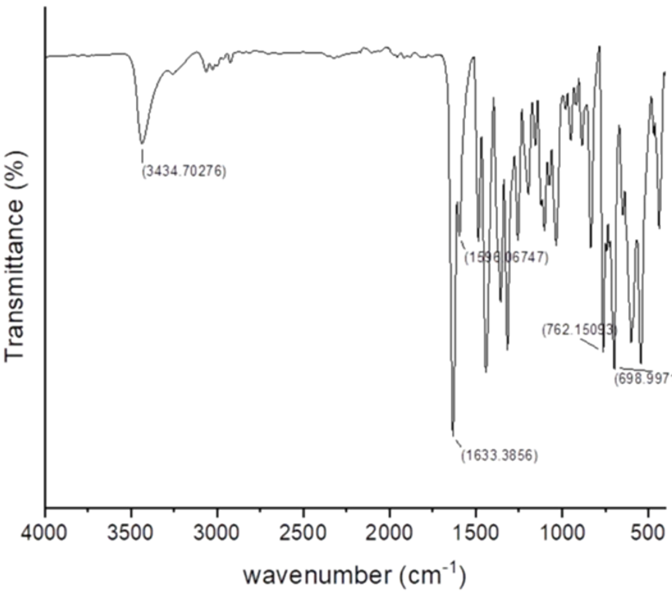
- N-H ~ 3217 cm^{-1}
- Aromatic & Aliphatic H ~ 3028 cm^{-1}
- C=O ~ 1659 cm^{-1}
- C=N ~ 1550 cm^{-1}
- Benzyl H ~ 1500 – 1430 cm^{-1}
- N-H in plane deformation vibration ~ 1426 cm^{-1}
- Bending deformation Of (CH_3)C-H ~ 1060 cm^{-1}

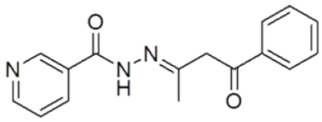




3b

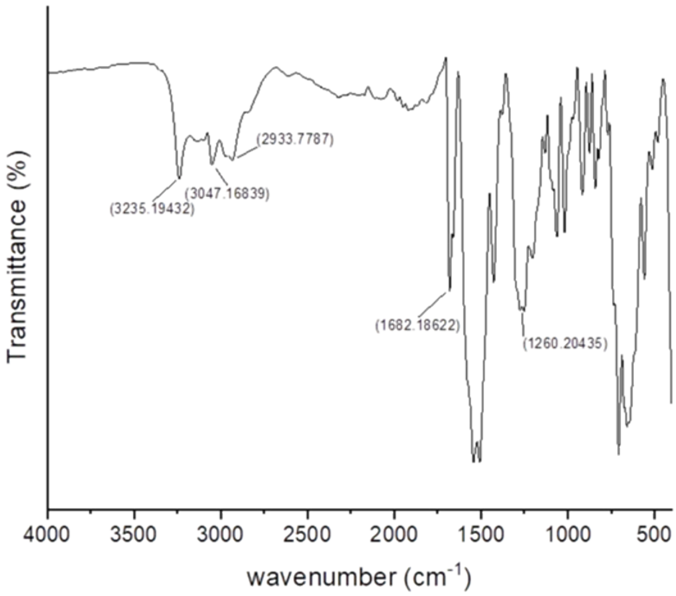
- N-H ~ 3434 cm^{-1}
- Aromatic & aliphatic H ~ 3065 – 2919 cm^{-1}
- C=O ~ 1633 cm^{-1}
- C=N ~ 1596 cm^{-1}
- C-Cl ~ 762 – 698 cm^{-1}

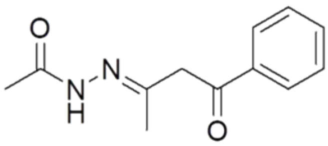




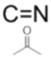
3c

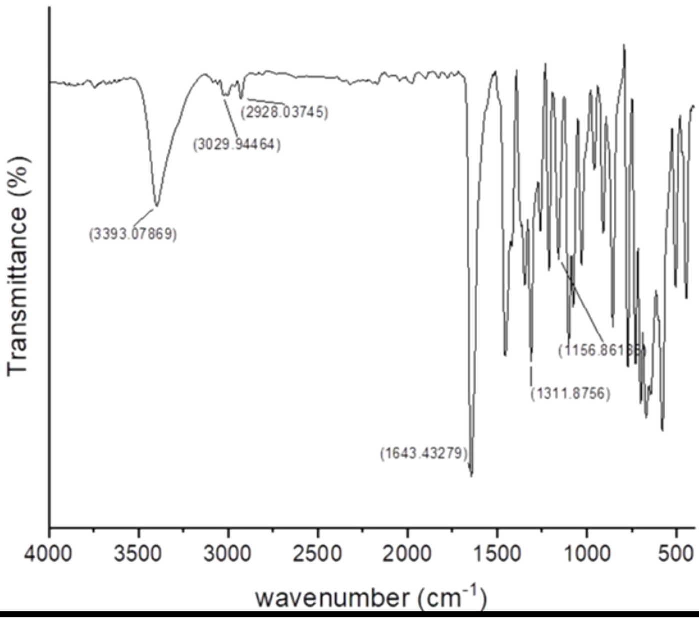
- N-H ~ 3235 cm^{-1}
- Aromatic & aliphatic H ~ 3056 - 2933 cm^{-1}
- C=O ~ 1682 cm^{-1}
- Aromatic C=C ~ 1500 – 1430 cm^{-1}
- N-H in plane deformation vibration ~ 1428 cm^{-1}
- C=O vibration Wrt. Pyridine ~ 1260 cm^{-1}

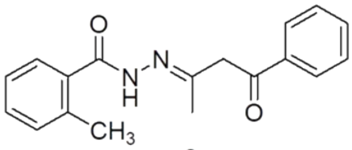




3d

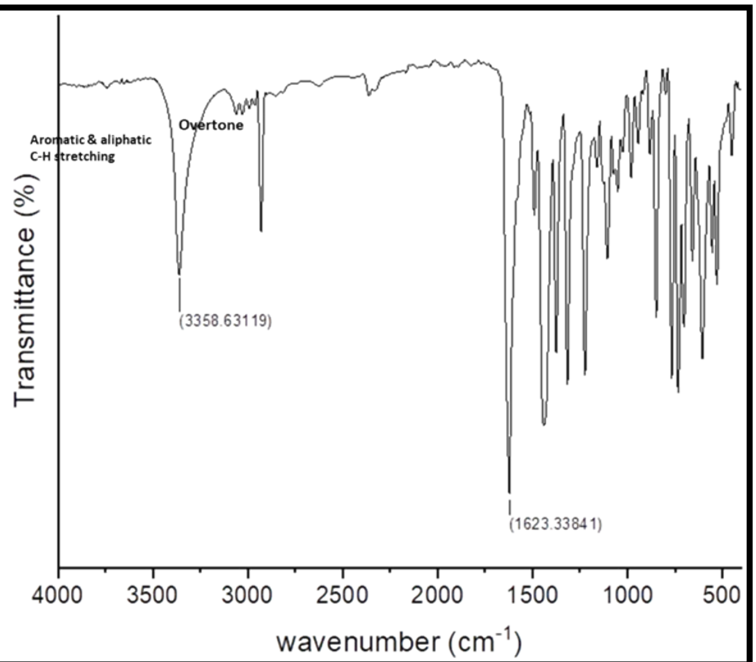
- N-H ~ 3393 cm^{-1}
- Aromatic & aliphatic H ~ 3029 - 2928 cm^{-1}
- C=N ~ 1643 cm^{-1}
-  ~ 1156 cm^{-1}
- CH_3C=O bend ~ 1311 cm^{-1}

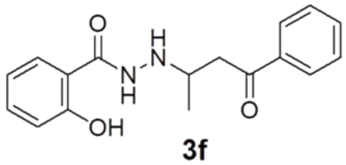




3e

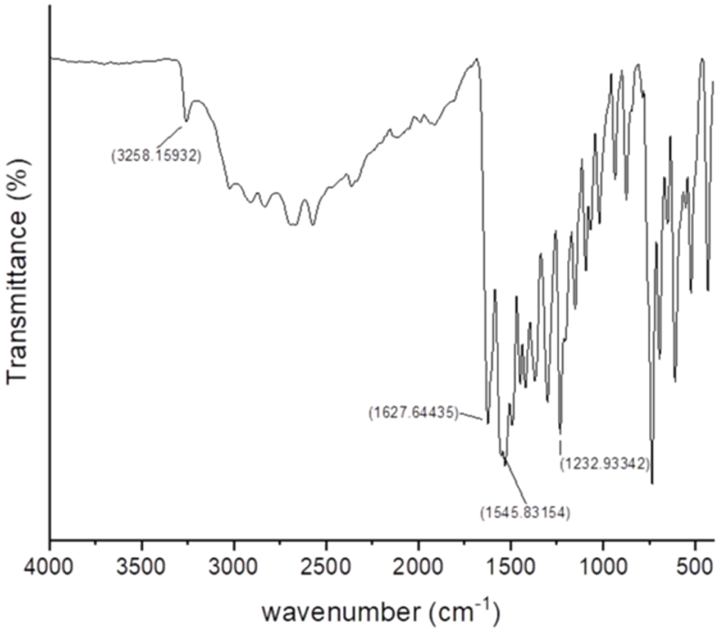
- N-H ~ 3358 cm^{-1}
- Combined C=O strectching and C=N vibration ~ 1623 cm^{-1}

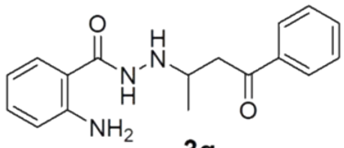




3f

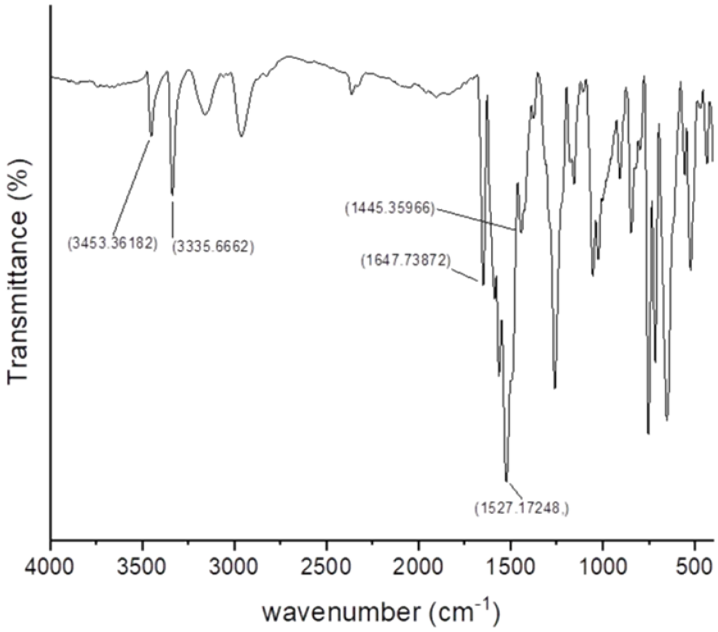
- N-H ~ 3258 cm^{-1}
- C=O ~ 1627 cm^{-1}
- Phenolic O-H ~ 1545 cm^{-1}
- C-O stretching ~ 1232 cm^{-1}

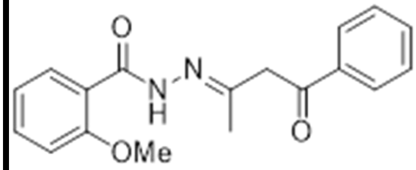




3g

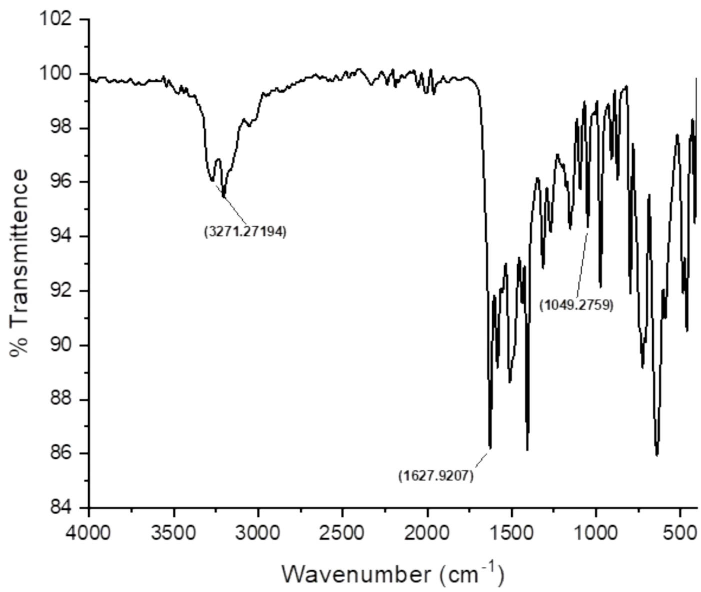
- N-H ~ 3453cm^{-1} and 3335 cm^{-1}
- C=N ~ 1527 cm^{-1}
- C=O ~ 1647 CM^{-1}
- C=C ~ 1445 cm^{-1}

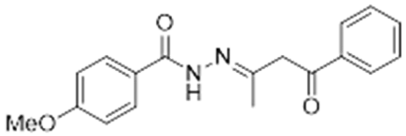




3h

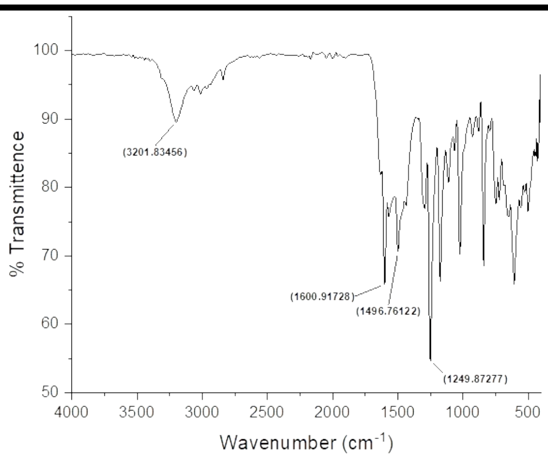
- N-H stretching ~ 3260.921 cm^{-1}
- C-N stretching ~ 1627.67 cm^{-1}
- C-O stretching of OCH_3 ~ 1049.276 cm^{-1}

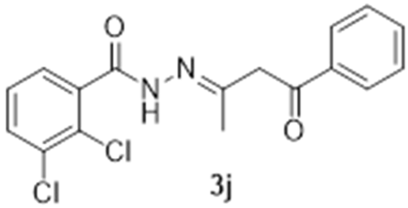




3i

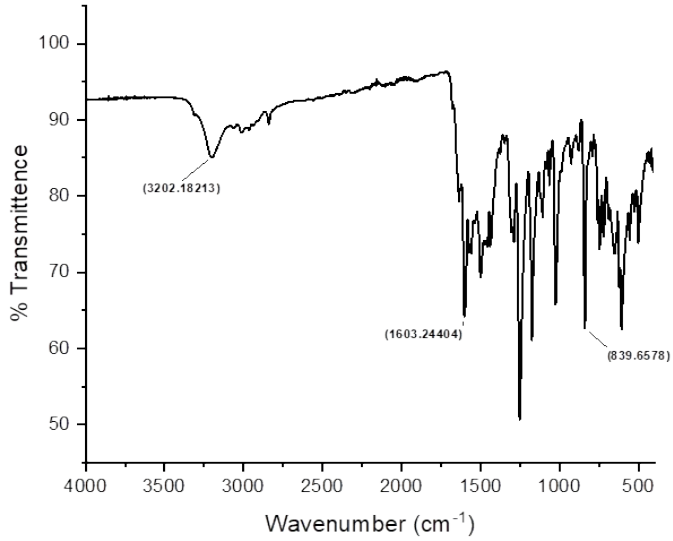
- N-H stretching ~ 3201.835 cm^{-1}
- C=O stretching ~ 1600.917 cm^{-1}
- C=C aromatic stretching ~ 1445 cm^{-1}
- C-O stretching of OCH3 ~ 1249.872 cm^{-1}





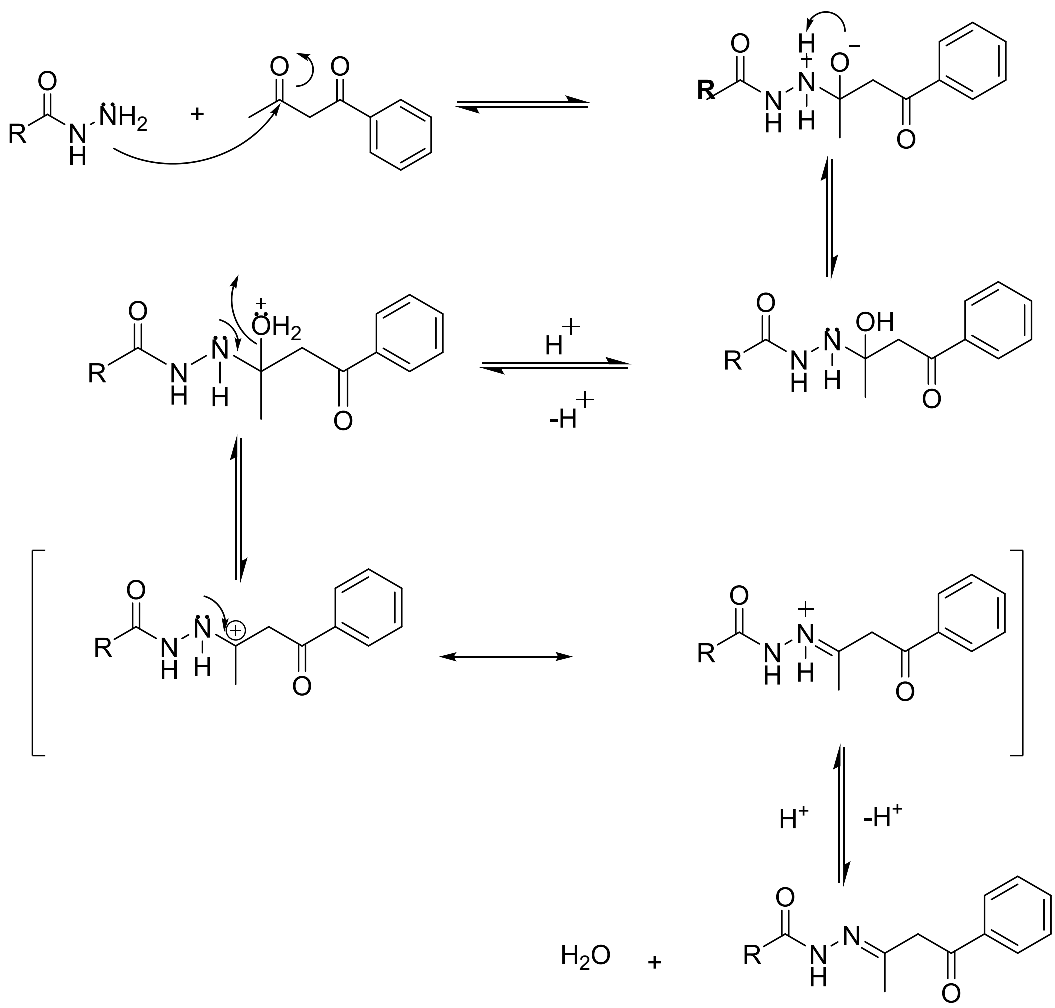
3j

- N-H strectching ~ 3202.182 cm^{-1}
- C=N stretching ~ 1603.244 cm^{-1}
- C-Cl stretching ~ 839.658 cm^{-1}



General mechanism for the synthesis of various hydrazide derivatives

Reaction between hydrazine and 1-phenyl-1,3-butanedione produces hydrazone. Initially, the amine containing a lone pair undergoes electrophilic addition with the carbonyl compound forming semicarbazone intermediate. Then the hydrogen atom in nitrogen will be deprotonated creating carbinolamine intermediate. Secondly, the intermediate hydroxyl group undergoes protonation and introducing a good leaving group – water molecule. Thus the seconds step involves dehydration of the molecule. Finally, after rearrangement, hydrazone inhibitor is crystallized. The mechanistic scheme for the synthesis is shown in the below scheme.



Evaluation of interaction potential of various hydrazide derivatives to the binding pocket of dNS1 protein.

The ability of various hydrazide derivatives (**3a-j**) to interact with the binding pocket of dNS1 protein was evaluated by following the changes observed in the intrinsic fluorescence of the protein with increasing concentrations of various hydrazides (0 – 500 μM). The reaction was performed in a volume of 3 ml at room temperature and at a fixed protein concentration of ~10-5 M in 1x PBS, pH 7.2. The reaction mixture was excited at 280 nm and the emission was monitored in the range of 290 to 550 nm. The quenching of fluorescence was observed for every incremental addition of hydrazide derivative.

Molecular docking

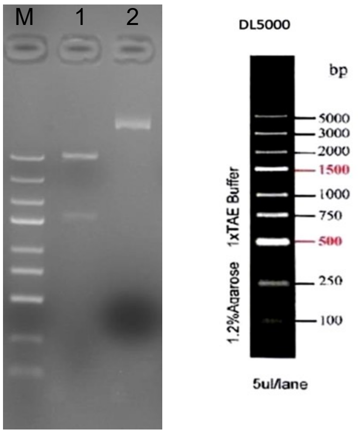
Structure retrieval and preparation: Three dimensional protein structure of Dengue type 4 was retrieved from RSCB protein databank (PDB ID: 8wbb) with a structural resolution of 2.9Å. Using the same as template file, the target protein sequence (dNS1) model was built from Swiss-model. Further the prepared protein macromolecule was optimized by protein preparation wizard of Schrodinger Suite before docking. The 2D ligand structures were drawn using ChemDraw Ultra 8.0. Optimization of the ligand was carried out using Gaussian 09 program by means of density functional theory – B3LYP involving 6-31 + + G(d,p) basis set to get 3D confrontation . Which was further optimised using LigPrep Module of Schrodinger suite. Here, the ligands were retained in their original state, hydrogen bonds, bond angles, with single conformations after which it was utilized in docking. The protein was pre-processed, followed by minimization and then optimisation in order to retain the protein's functionality. This may prevent any erroneous output and further used for docking. The binding interactions and binding energy were calculated with Glide Module of Schrodinger suite. Appropriate grid was generated around the target protein's binding site for the prepared ligand to precisely interact with the target site. Further, MM-GBSA procedure was followed to ascertain the binding interactions as evaluated by glide docking.

MD-Simulation

DESMOND module of Schrodinger Suite was used for Simulation. The protein-ligand complex was built through system builder panel and localized in an orthorhombic box of TIP3P solvent module. Further physiological salt (NaCl) of 0.15M was added to neutralise the environment. The complex was simulated using OPLS4 force field under 100ns period using NPT ensemble class at 300k and 1.013 bar atmospheric pressure.

Molecular cloning, Protein expression and purification

The codon optimized (for E.coli) gene sequence of dNS1 was commercially procured and cloned into pET28A vector between NdeI and XhoI restriction sites. The clone was constructed in such a way to possess N-terminal 6X His tag. The confirmation of constructed clone was done by sequencing. Further confirmation was performed by digestion with restriction endonucleases NdeI and XhoI to release a pop out of ~1200 bp.



Lane M: DNA Ladder
Lane 1: DNS1_pET28a plasmid digested by NdeI and XhoI
Lane 2: Uncut DNS1_pET28a plasmid

Figure S1. Agarose gel depicting the release of NdeI and XhoI double digested fragment (~1150 bp) and the undigested plasmid (~6 kb)

Protein sequence

MGSSHHHHHHSSGLVPRGSHMDMGCVVSWNGKELKCGSGIFVVDNVHTWTEQYK
FQPESPARLASAILNAHKDGVCGIRSTTRLENIMWKQITNELNYVLWEGGHDLTVVA
GDVKGVLTKGKRALTPPVNDLKYSWKTWGKAKIFTPEARNSTFLIDGPDTSECPNER
RAWNFFEVEDYGFGMFTTNIWMKFREGSSEVCDHRLMSAAIKDQKAVHADMGYWI
ESSKNQTWQIEKASLIEVKTCLWPKTHTLWSNGVLESQMLIPRSYAGPFSQHNYRQG
YATQTAGPWHLGKLEIDFGECPGTTVTIQEDCDHRGPSLRTTTASGKLVTQWCCRSC
TMPPLRFLGEDGCWYGMEIRPLSEKEENMVKSQVTA

Gene sequence

atgggcagcagccatcatcatcatcatcacagcagcggcctggtgccgcgcggcagccatatggatatgggttgcgttgttagctgga
acggtaaagagctgaaatgcggttcgggcatcttcgttgtggataacgttcacacctggaccgaacagtacaaatttcagccggaaagt

ccggcacgtctggcatctgcaattctgaacgcgcataaagacggcgtttgcggtattcgtagtaccacccgcctggaaaacatcatgtg
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gaaattcgtccgctgagcgaaaaagaagaaaacatggtcaaaagccaggtcaccgcataactcgag

Further the expression of the above construct was done by transforming the above plasmid into BL21D3 competent cells followed by 0.5 mM IPTG induction at OD600 ~0.5 for 4 hours. Subcellular fractionation was performed by lysing the cells in the presence of 0.5 M PMSF followed by centrifugation at 10000 xg for 30 minutes at 4 oC. The fractionated cell lysates were subjected to SDS-PAGE. The expressed protein was found to be localized as inclusion bodies. In order to solubilize the inclusion bodies protein, the pellet was treated with 6 M guanidinium hydrochloride for 6 hours followed by centrifugation at 10000 xg for 45 minutes. The above supernatant was subject to Ni-NTA affinity purification using 30 mM imidazole in 1x PBS, pH 7.4 as wash buffer and 300 mM imidazole in 1XPBS as elution buffer. Thus eluted protein was extensively dialyzed against phosphate-buffered saline (PBS) to remove imidazole.

Optimization of protein expression

The expression of dNS1 protein in BL21DE3 was optimized under various conditions of temperature (30 and 37 oC), IPTG concentration (0.5 mM and 1 mM) and post induction time (4 hours and overnight). SDS-PAGE results have revealed that best expression was observed at 37 oC with 0.5 mM IPTG for 4 hours of induction. The optimized expression of dNS1 is depicted in figure S1.

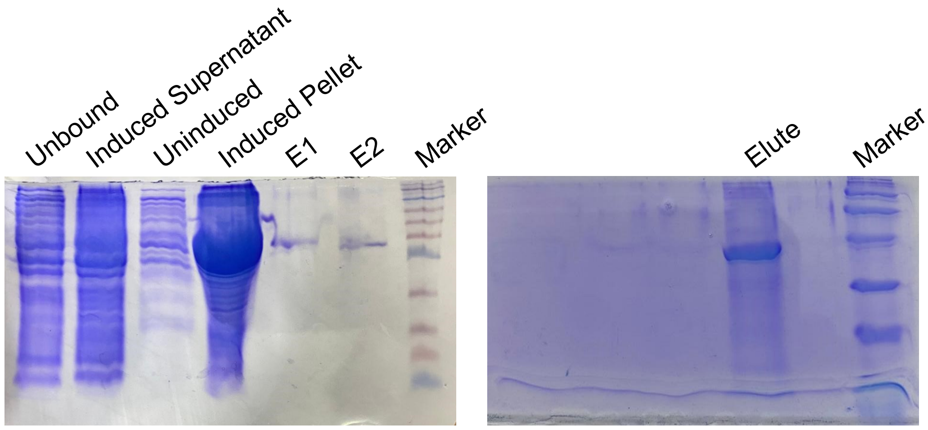


Figure S2. SDS-PAGE depicting the expression, sub-cellular localization and purification of dNS1 protein from pET28a- DNS1 construct.

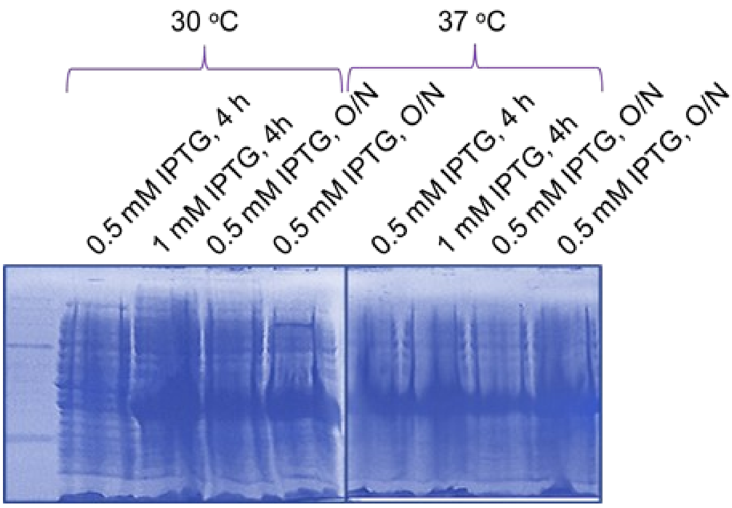


Figure S3. SDS-PAGE depicting the optimized expression of dNS1 in BL21-DE3 cells.

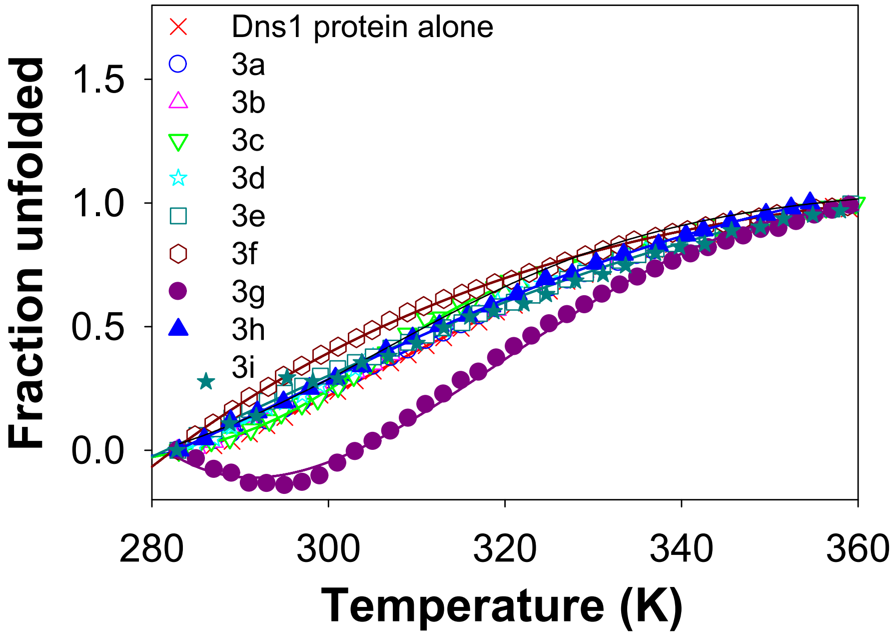
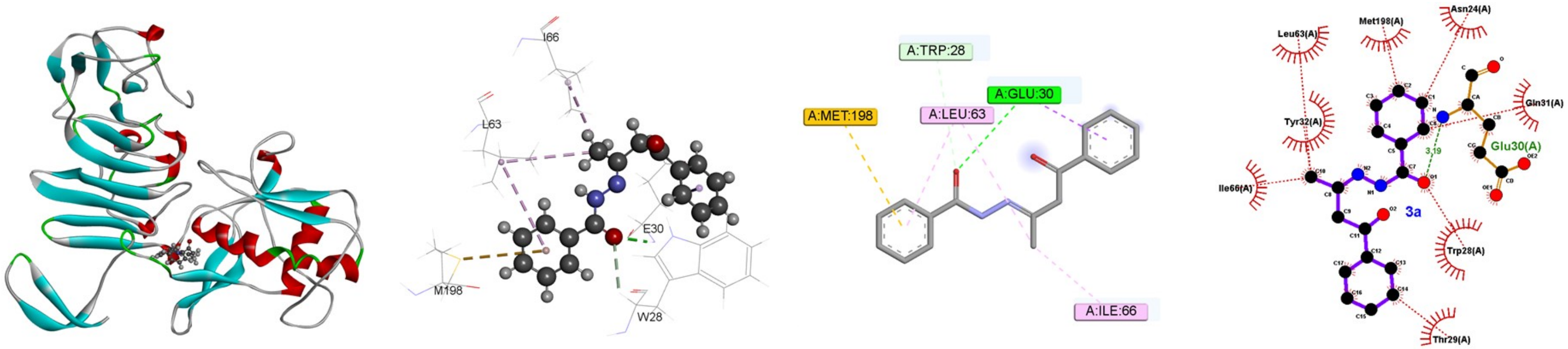


Figure S4. Plot depicting the melting temperature of dNS1 protein in the presence and absence of various hydrazides.

3a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A:LEU:63
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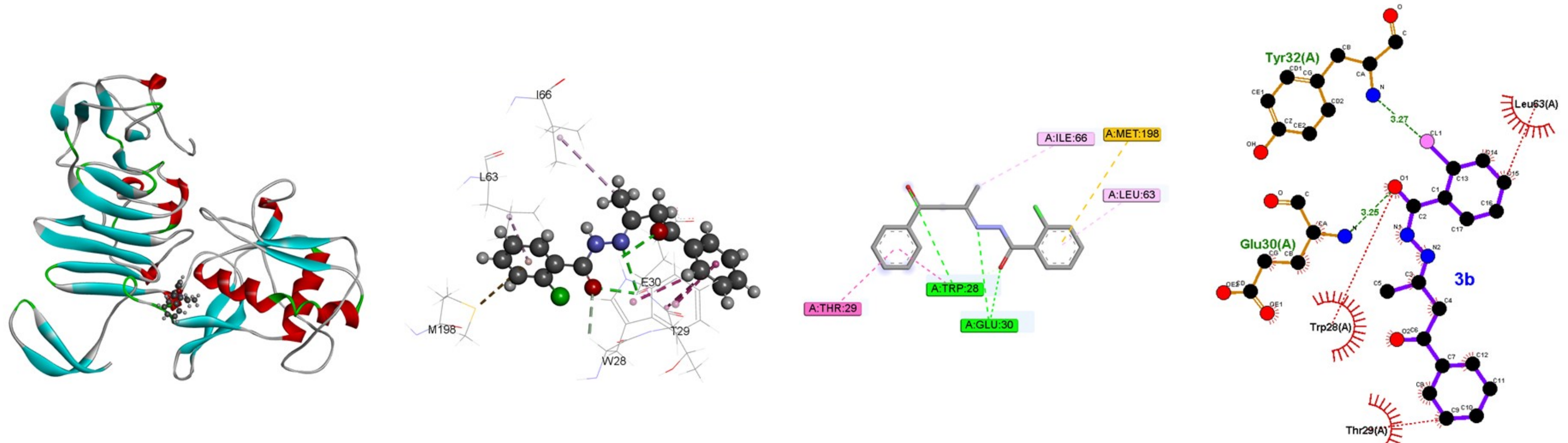
Met198(A)
Asn24(A)
Gln31(A)
Tyr32(A)
Glu30(A)
3.19
Ile66(A)
3a
Trp28(A)
Thr29(A)

3b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3.25
Glu30(A)
3b
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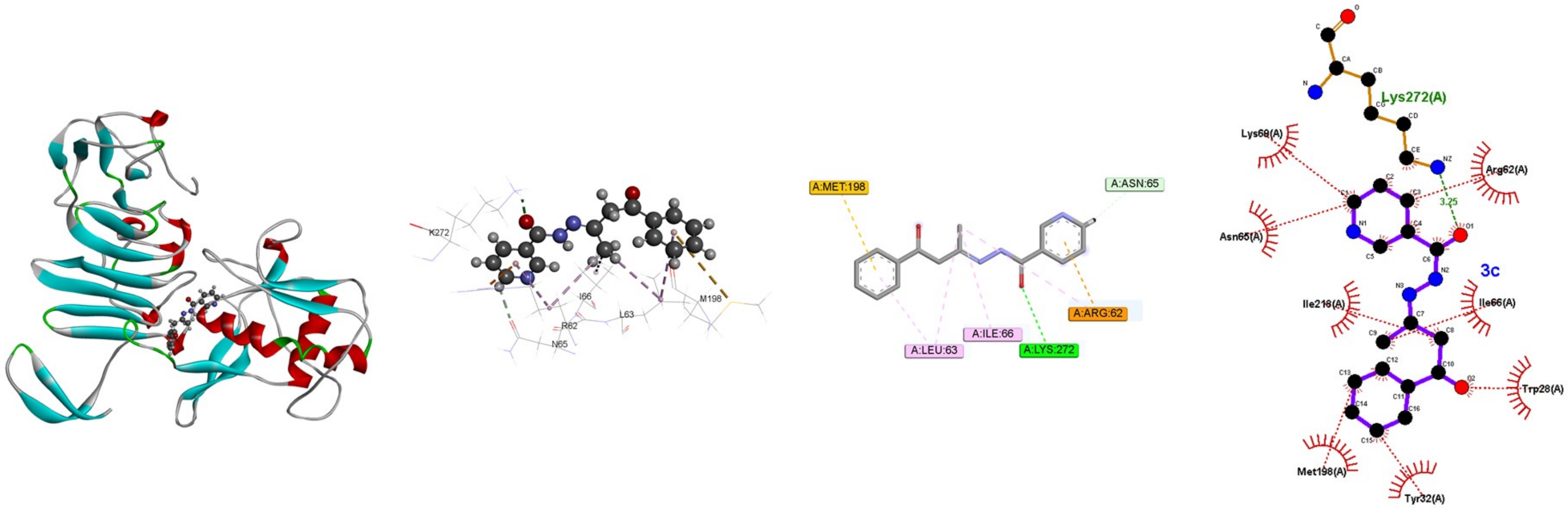
Thr29(A)

3c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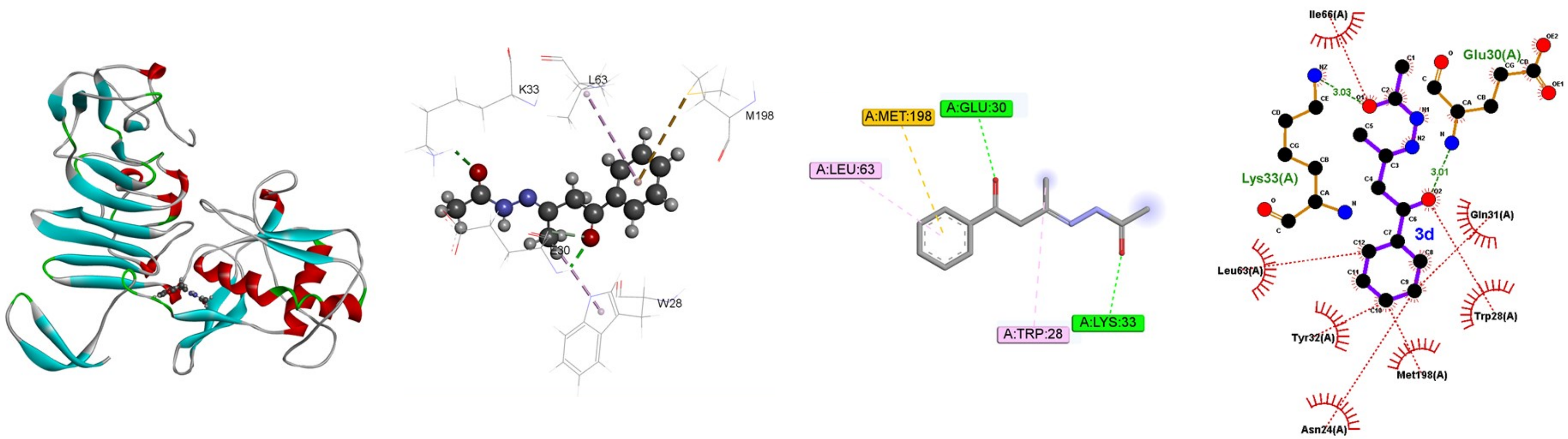
3.25
3c

3d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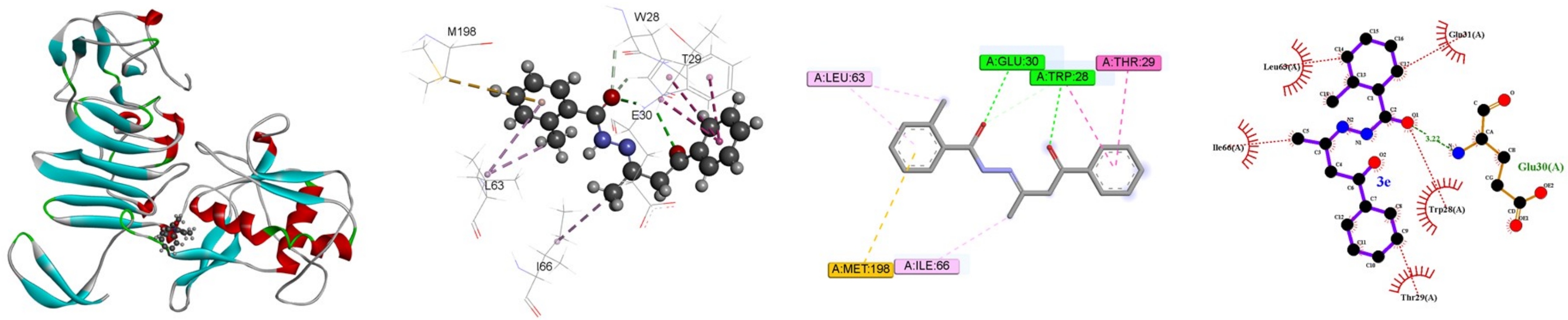
3.01
3d

3e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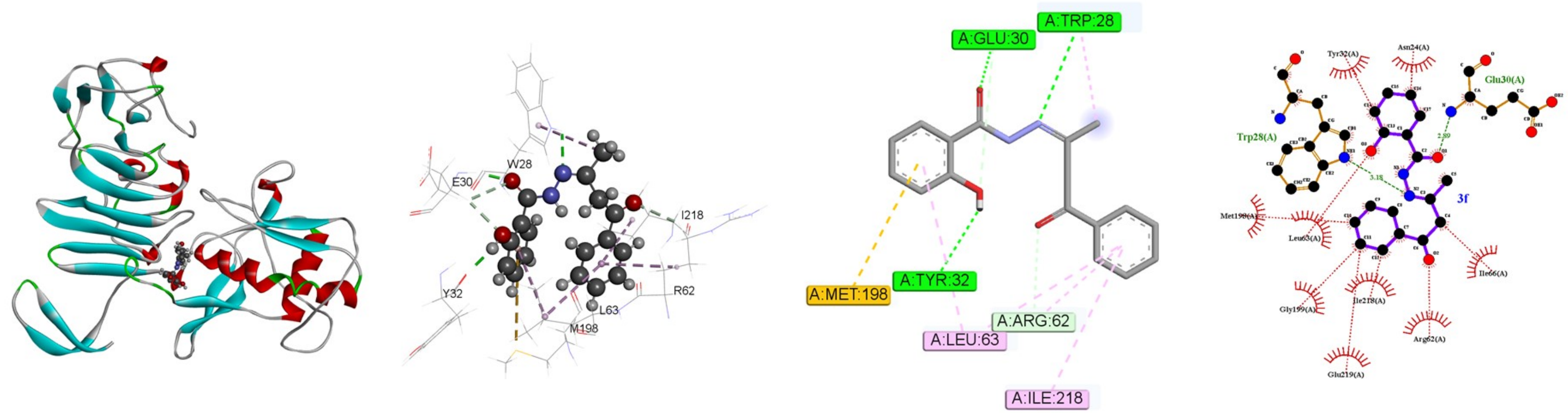
3f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Asn24(A)
Glu30(A)
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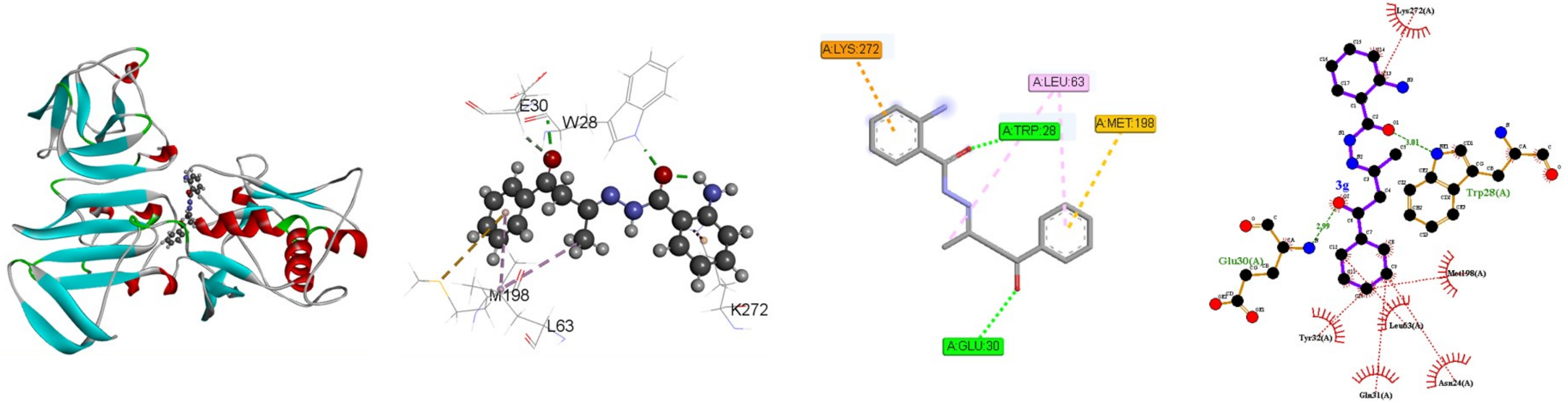
3g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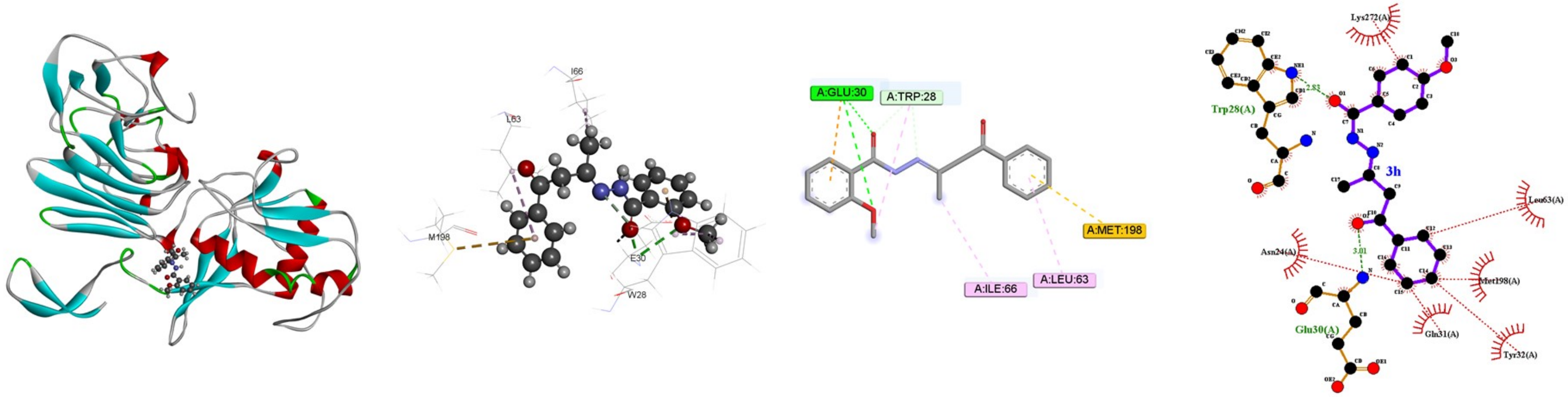
3h



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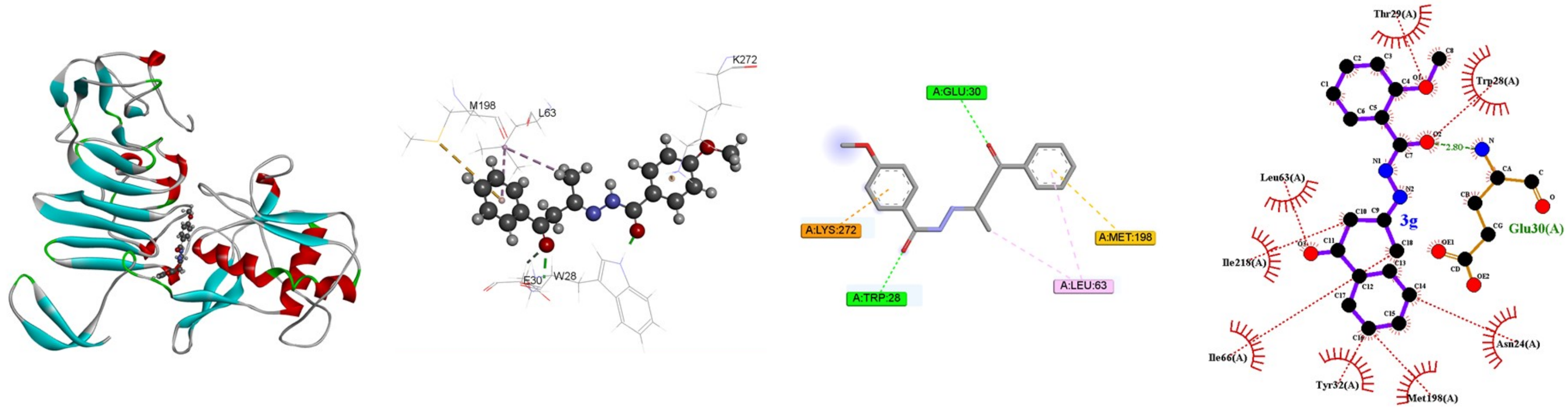
3i



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3j

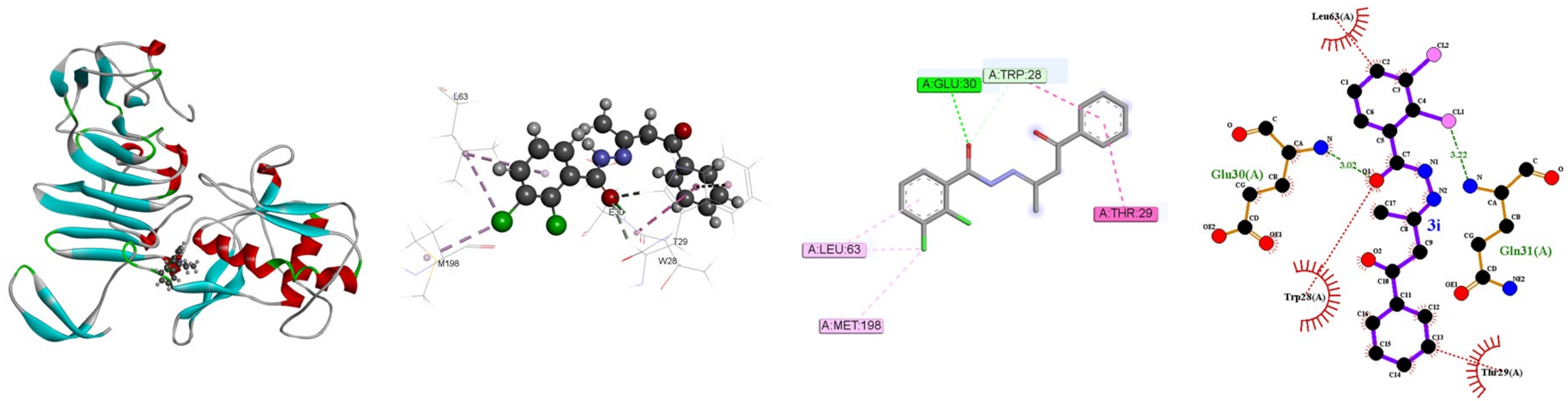

Figure S5. Docked poses of various hydrazide derivatives with dNS1.

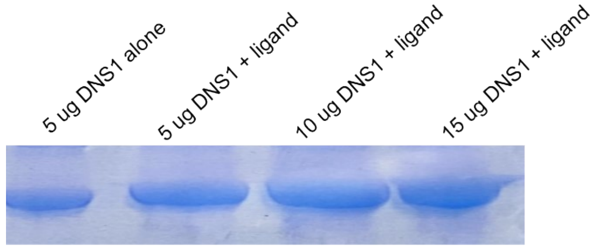

Figure S6. Native gel electrophoresis depicting the migration of DNS1 protein in the presence and absence of ligand **3b**.

Pharmacokinetic properties

Analyzing the Pharmacokinetic parameters of a drug exhibiting their potential treatment towards the target has an important role before synthesis. To understand the target quality such as Absorption, Distribution, Metabolism, Excretion, Toxicity study was performed by QikProp module of Schrödinger suite. So, the descriptors like HOA (Human Oral Absorption), stars, Lipinski rule of five were under study. Human Oral Absorption predicted on 1,2 or 3 scale for low, medium and high, indicates the values greater than 1 can become a potent drug candidate. Star is a number of property or descriptor that dis-obey when compared to 95% of known drugs. Descriptors such as molecular weight, dipole, Ionization Potential, Electron Affinity, Solvent Accessible Surface Area, hydrophobic component of SASA between saturated carbon and hydrogen(FOSA), hydrophilic component of SASA on N,O, and H on heteroatom (FISA), π – component of SASA (PISA), weakly polar component of the SASA of halogens, phosphorous, and sulfur atoms (WPSA), van der waals surface area of polar nitrogen and oxygen atoms (PSA), Globularity descriptor, volume, #rotor, donor hydrogen bond, acceptor hydrogen bond, polarizability in cubic angstroms (QPpolrZ), hexadecane/gas partition coefficient (QPlogPC16), octanol/gas partition coefficient ($QPlogP_{oct}$), water/gas partition coefficient (qplogPw), QPlogPo/w, aqueous solubility (QlogS), binding to human serum albumin (QPlogKhsa), Blood-Brain partition coefficient (QPlogBB), number of likely metabolic reactions (#metabol) were involved in the determination of star. A large number of stars tell us the molecule is unlikely for a drug. Besides, Lipinski rule of five provides suggestions for a compound's druggability: molecular weight (<500), Donor hydrogen bond (≤5), octonal-water coefficient (<5), acceptor hydrogen bond (≤10), aqueous solubility (-6.5 to 0.5). Table 3 shows the results of ADME analysis. Except 2-aminohydrazone (**3g**) rest of the compounds **3a** – **j** are satisfactory and therefore further analysis was preceded. QPlogPW should be from4 to 45. QPlogPo/W should be between -2 to 6.5. QPPCaCo is CaCo-2 cell permeability which has to greater than 25 and not beyond 500. From the discussion, except 2-aminohydrazone (**3g**), every other molecule tends to satisfy the parameters and could be considered potential against dNS1.