

## Electronic Supplementary Information

### Synthesis and biological evaluation of FJ-809, a compound originally described as MIM-1 and inhibitor of the anti-apoptotic protein Mcl-1.

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<sup>5</sup>Normandie Université, UNICAEN, Structure Fédérative 4206 ICORE, Centre de Microscopie appliqué à la Biologie (CMABIO<sup>3</sup>).

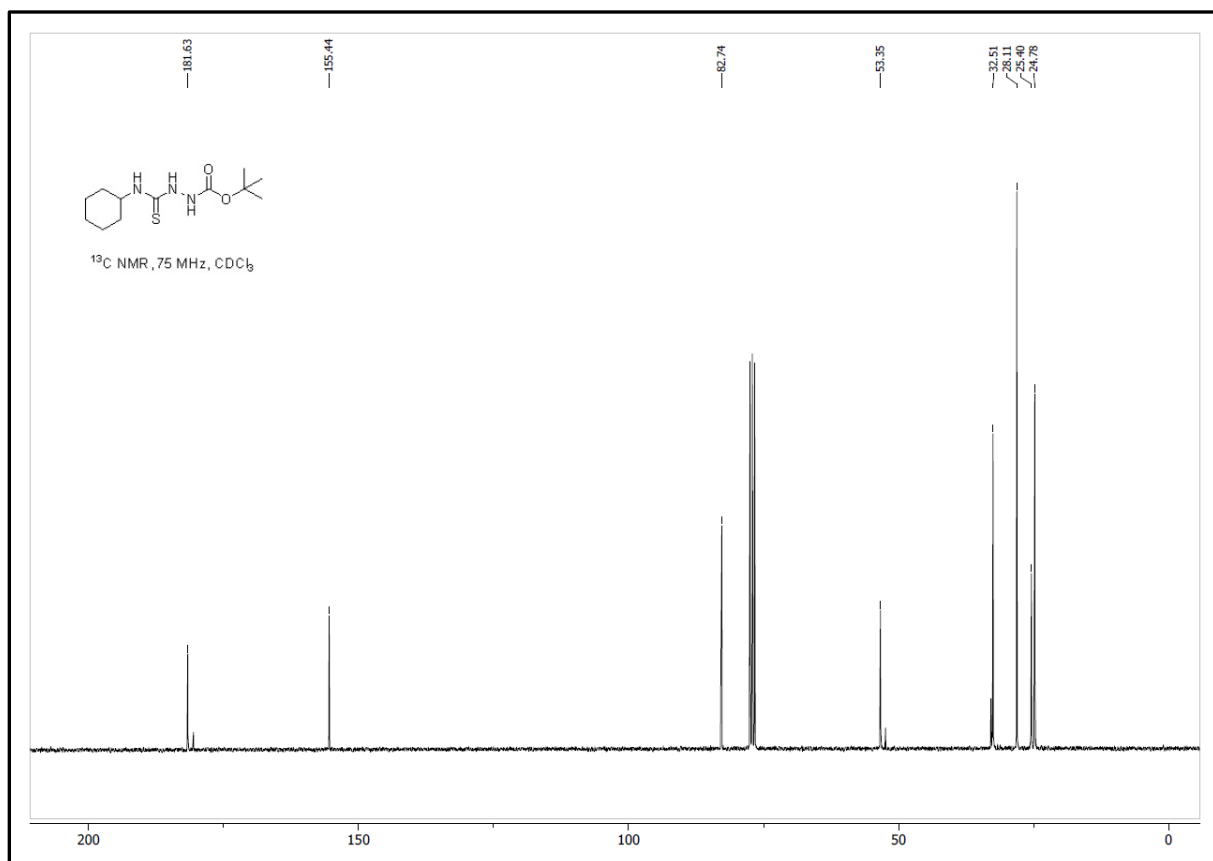
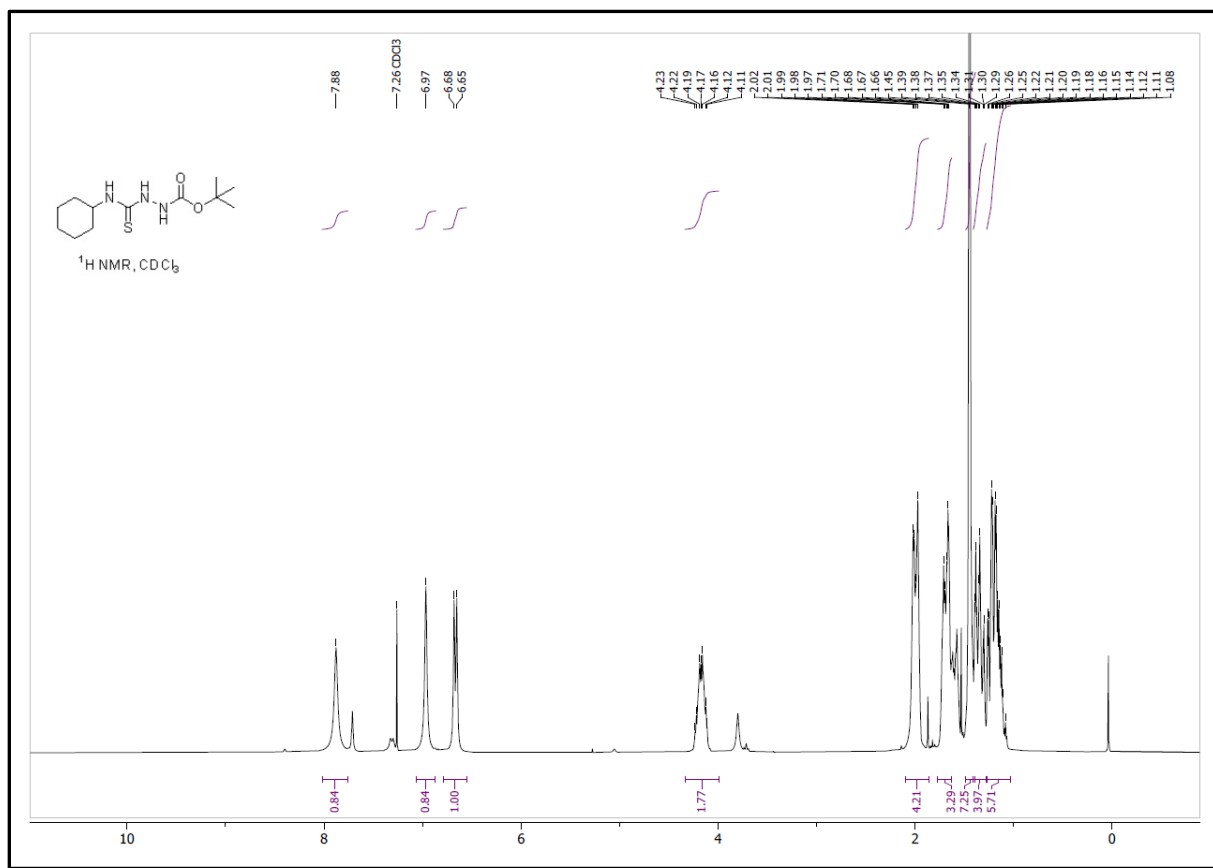
<sup>6</sup>Univ Rennes, CRMPO (Centre Régional de Mesures Pysiques de l'Ouest), Avenue du Général Leclerc, 35042 Rennes Cedex, France.

<sup>7</sup>Bioprojet-Biotech, 4 rue du Chesnay Beaugard, BP 96205, 35762, Saint Grégoire, France.

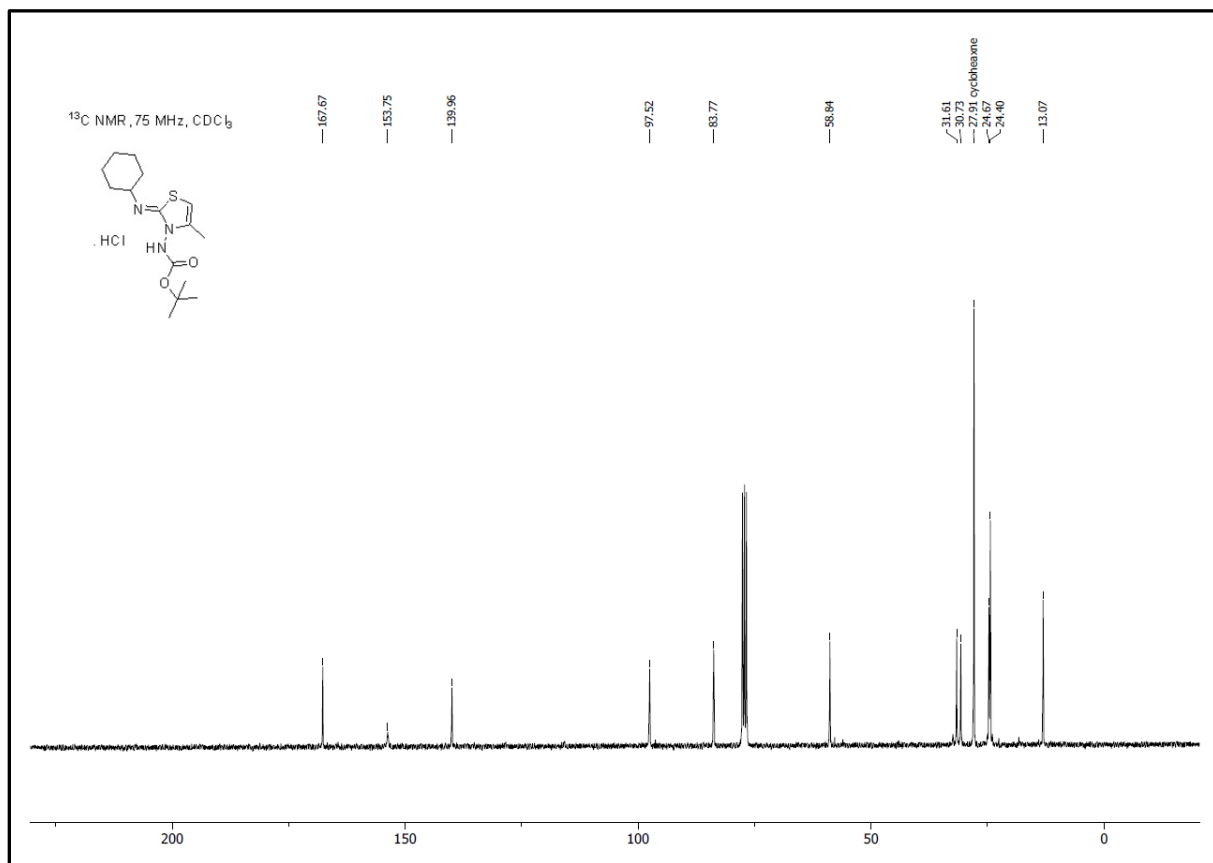
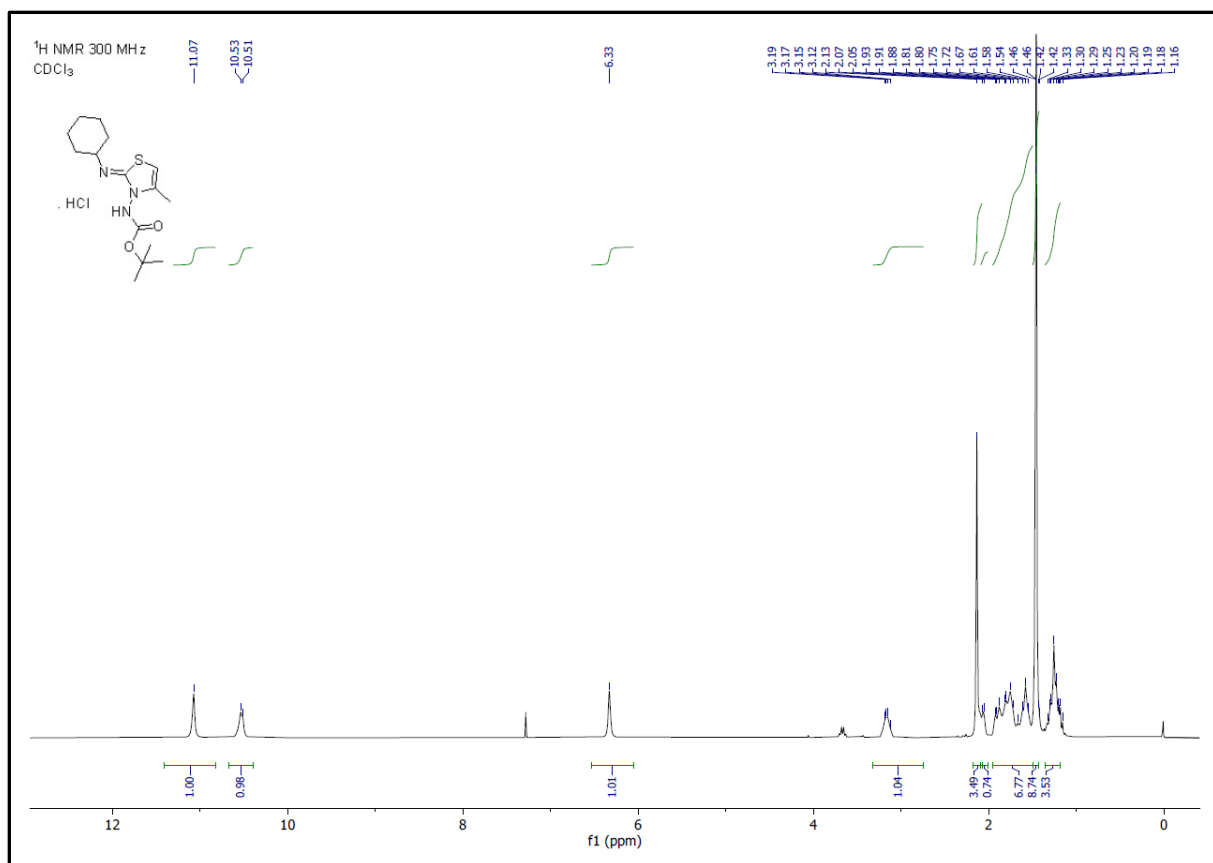
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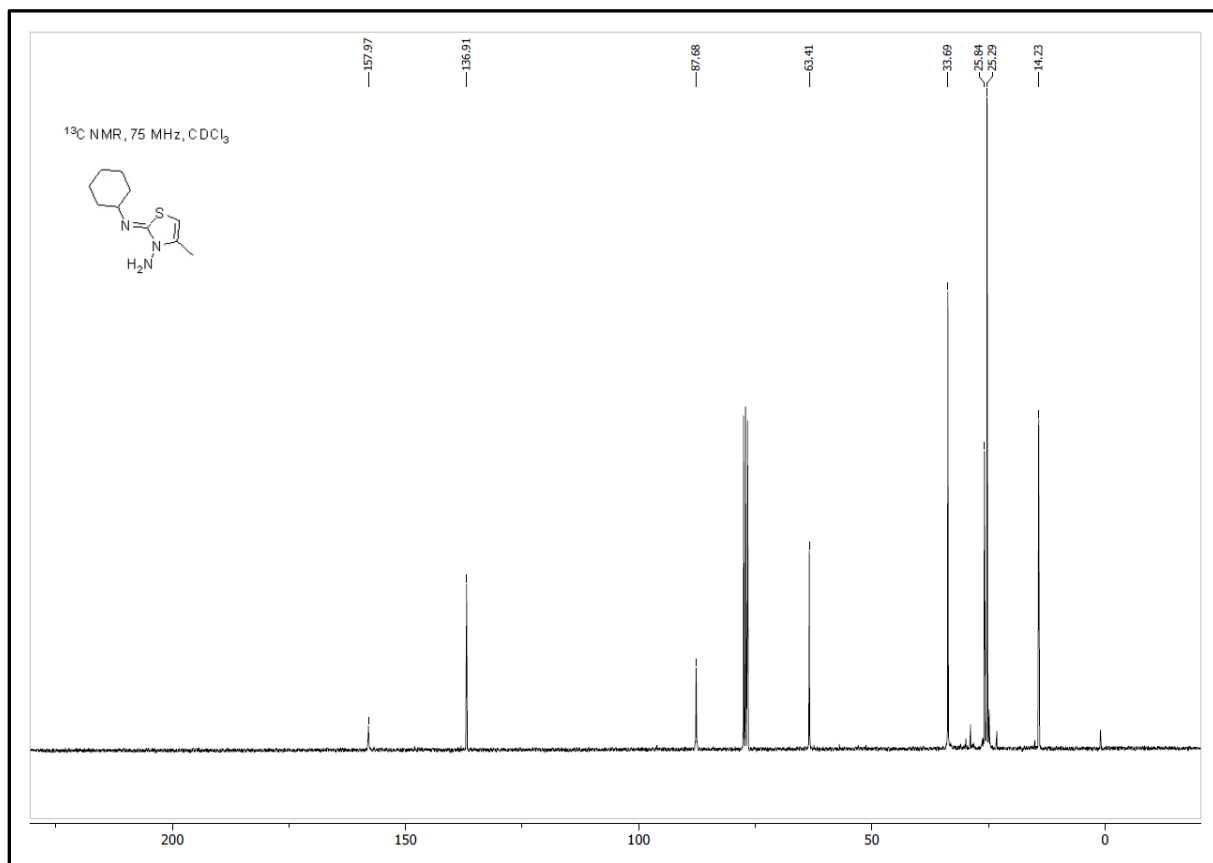
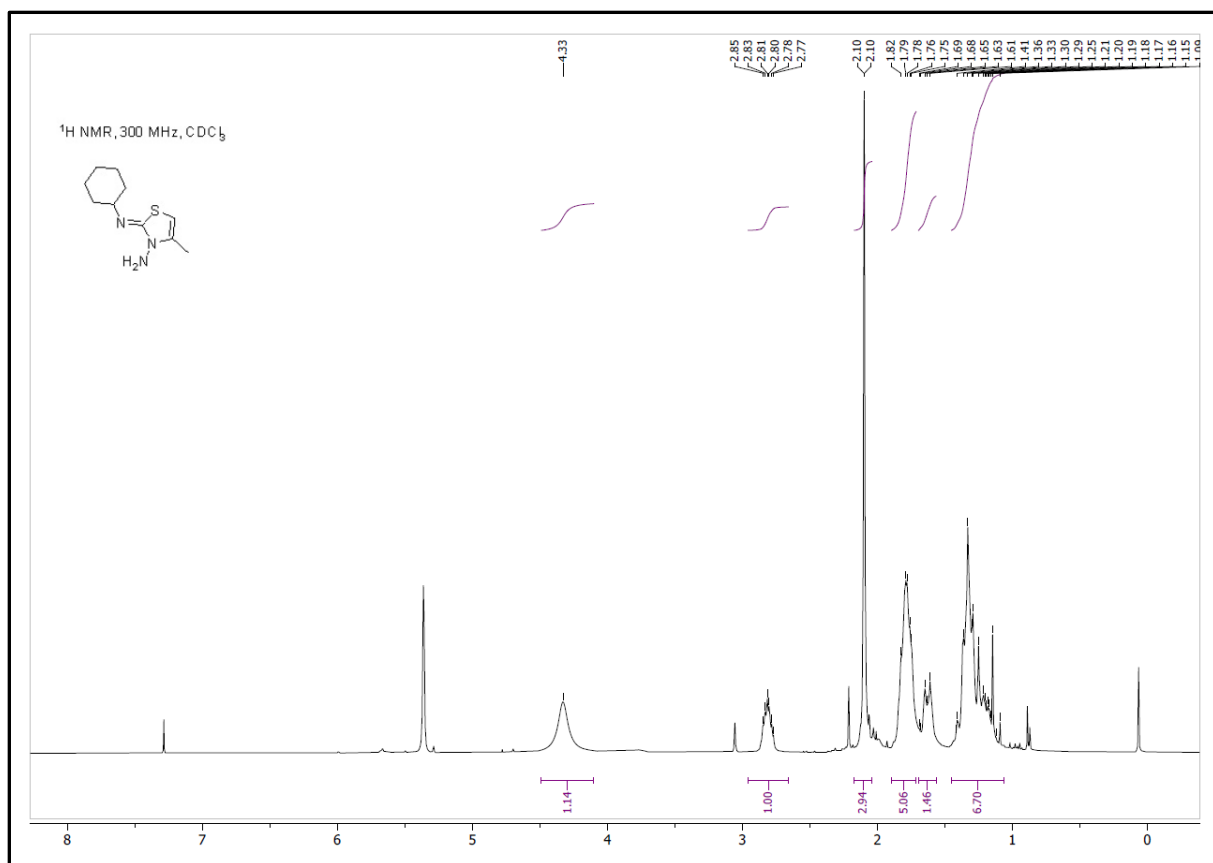
tert-butyl 2-(cyclohexylcarbamothioyl)hydrazinecarboxylate (10)



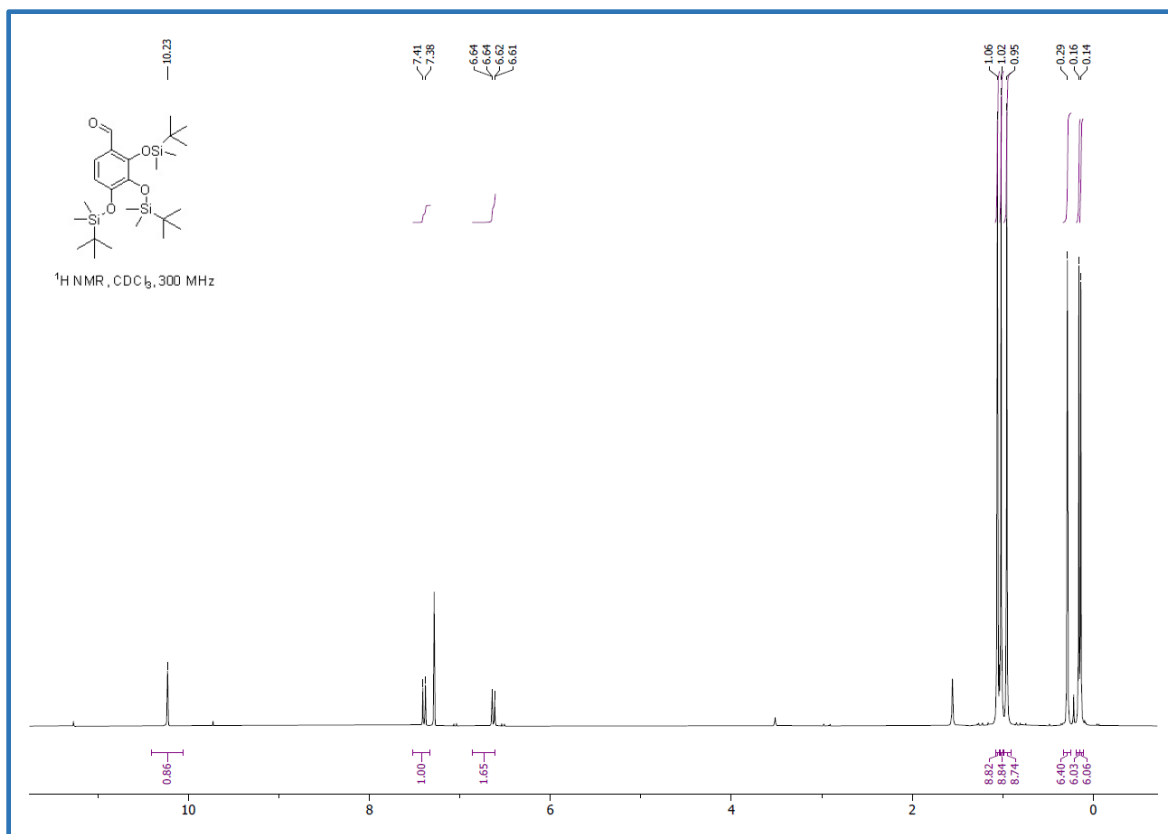
**(Z)-tert-butyl (2-(cyclohexylimino)-4-methylthiazol-3(2H)-yl)carbamate hydrochloride (11)**



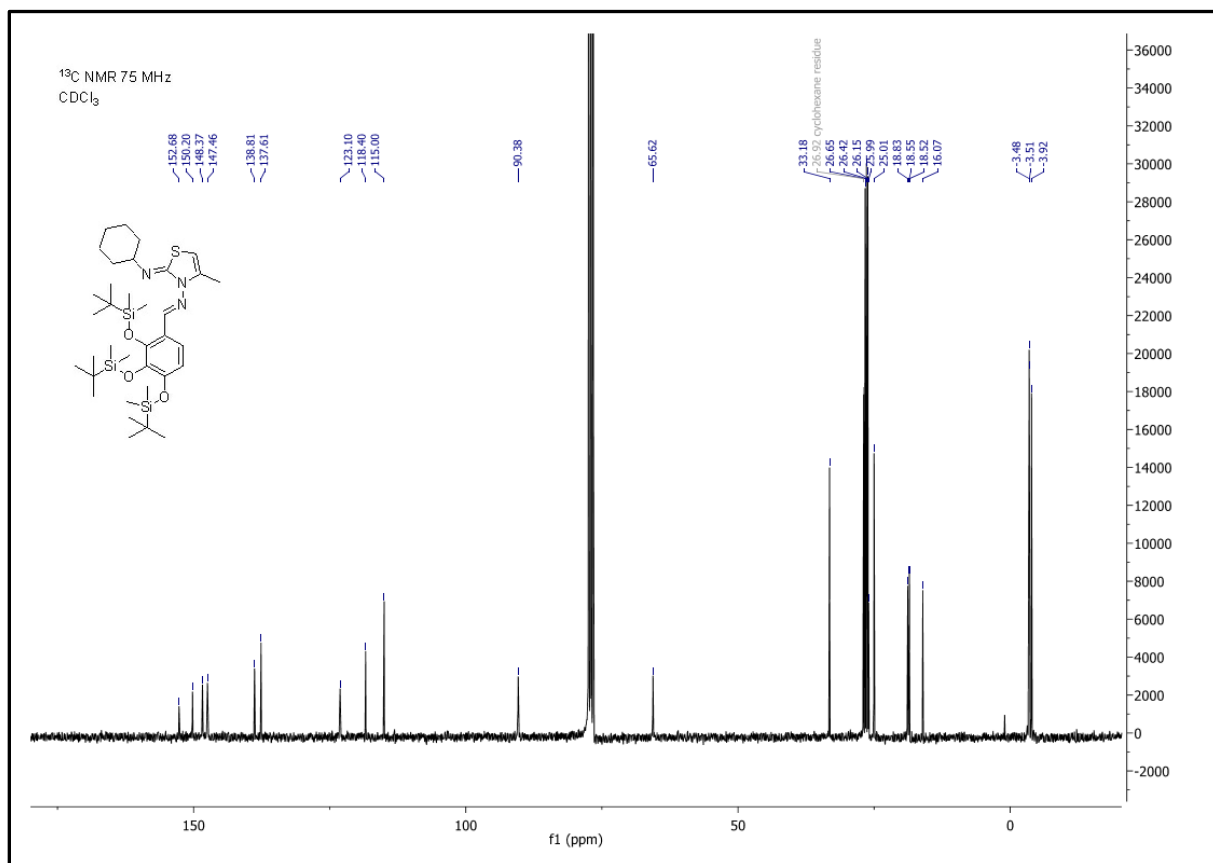
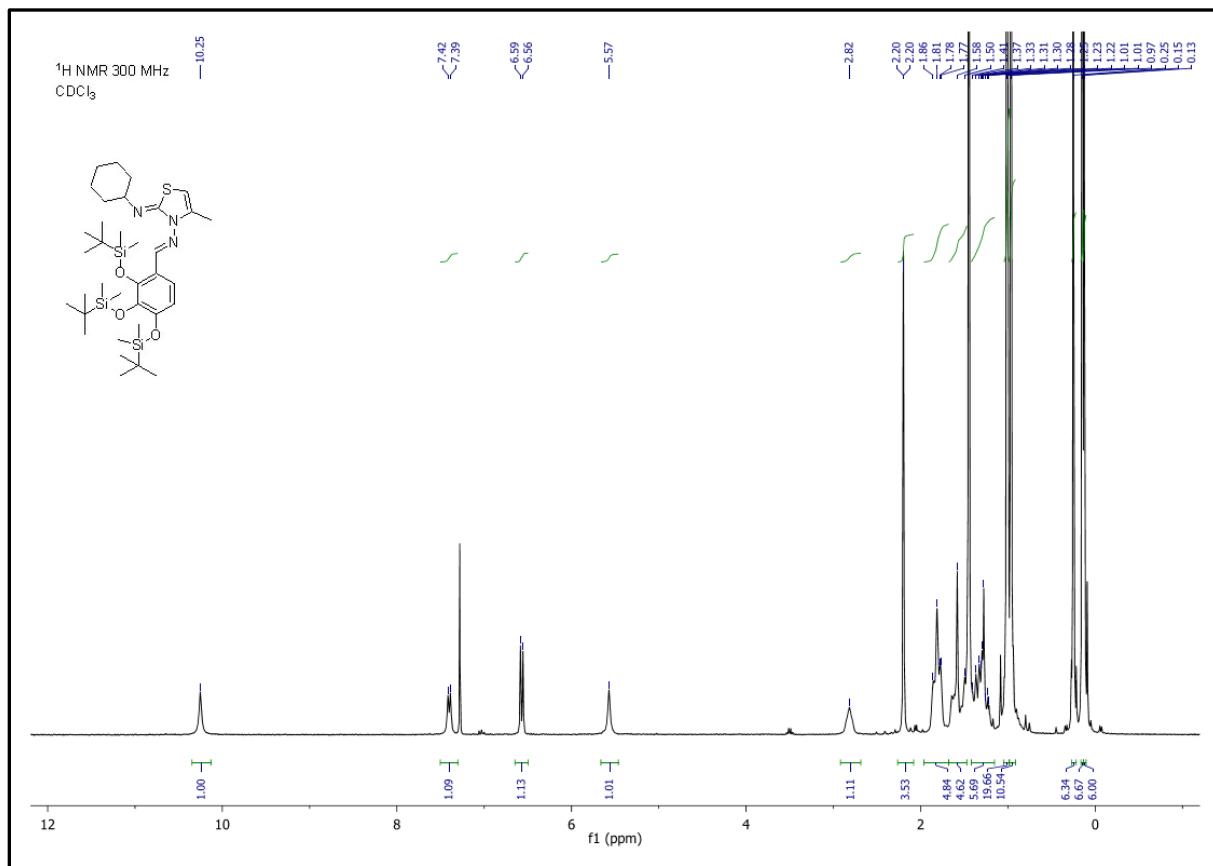
## 2-cyclohexylimino-4-methyl-2,3-dihydro-1,3-thiazol-3-amine (12)



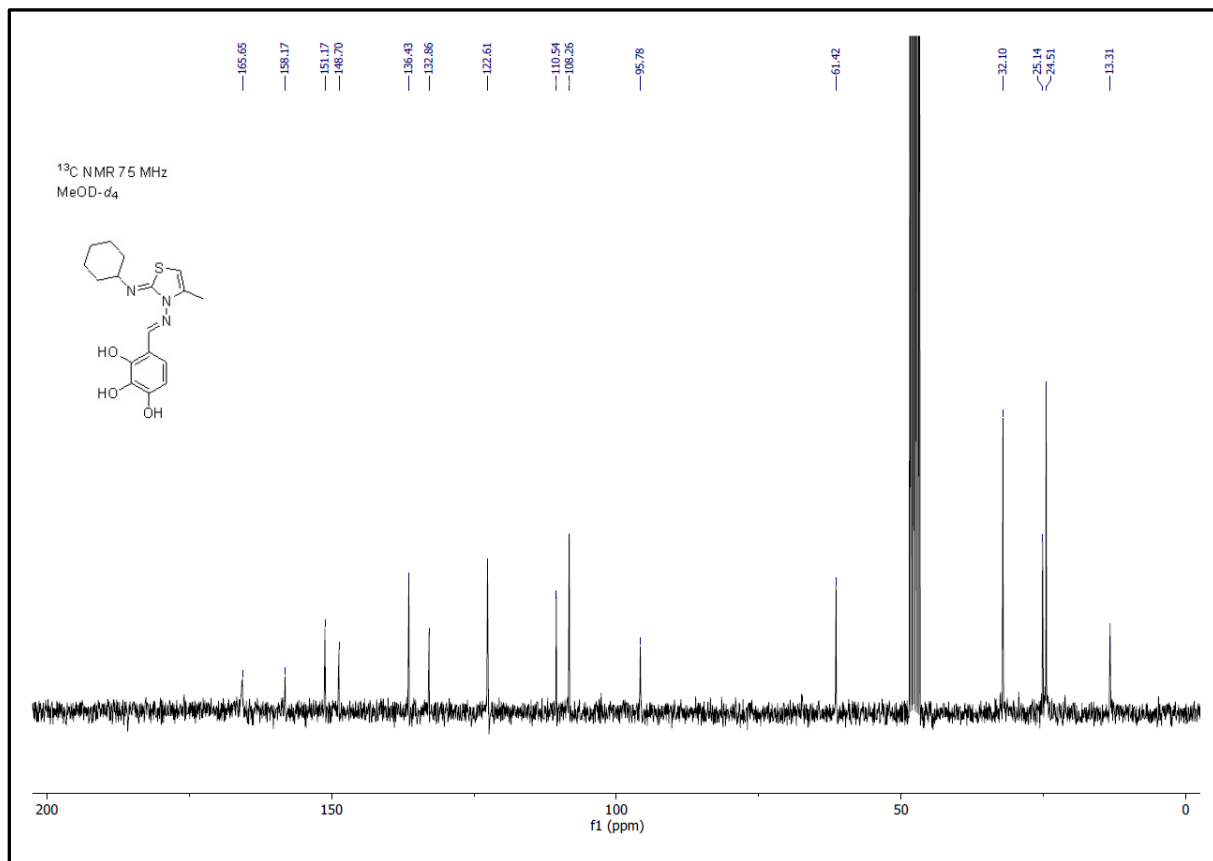
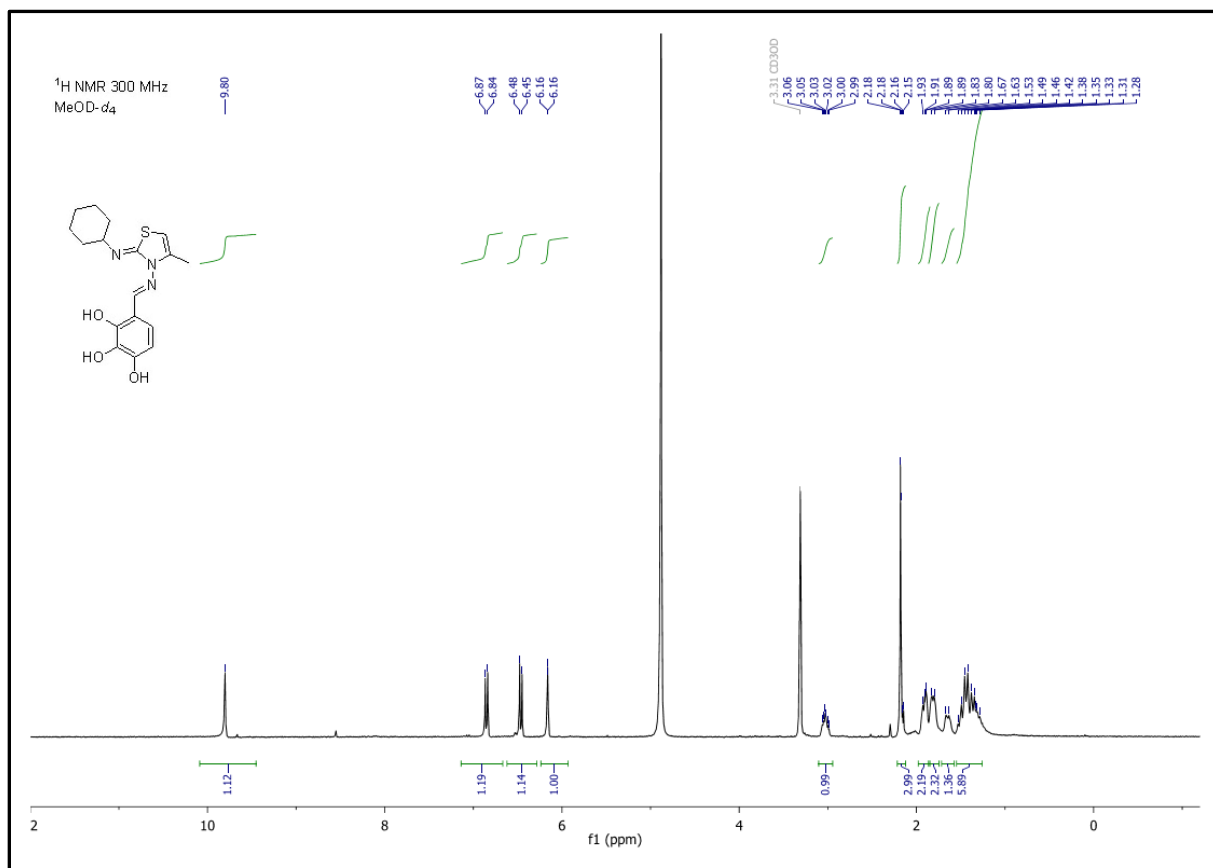
2,3,4-tris((tert-butyl dimethylsilyl)oxy)benzaldehyde (13)



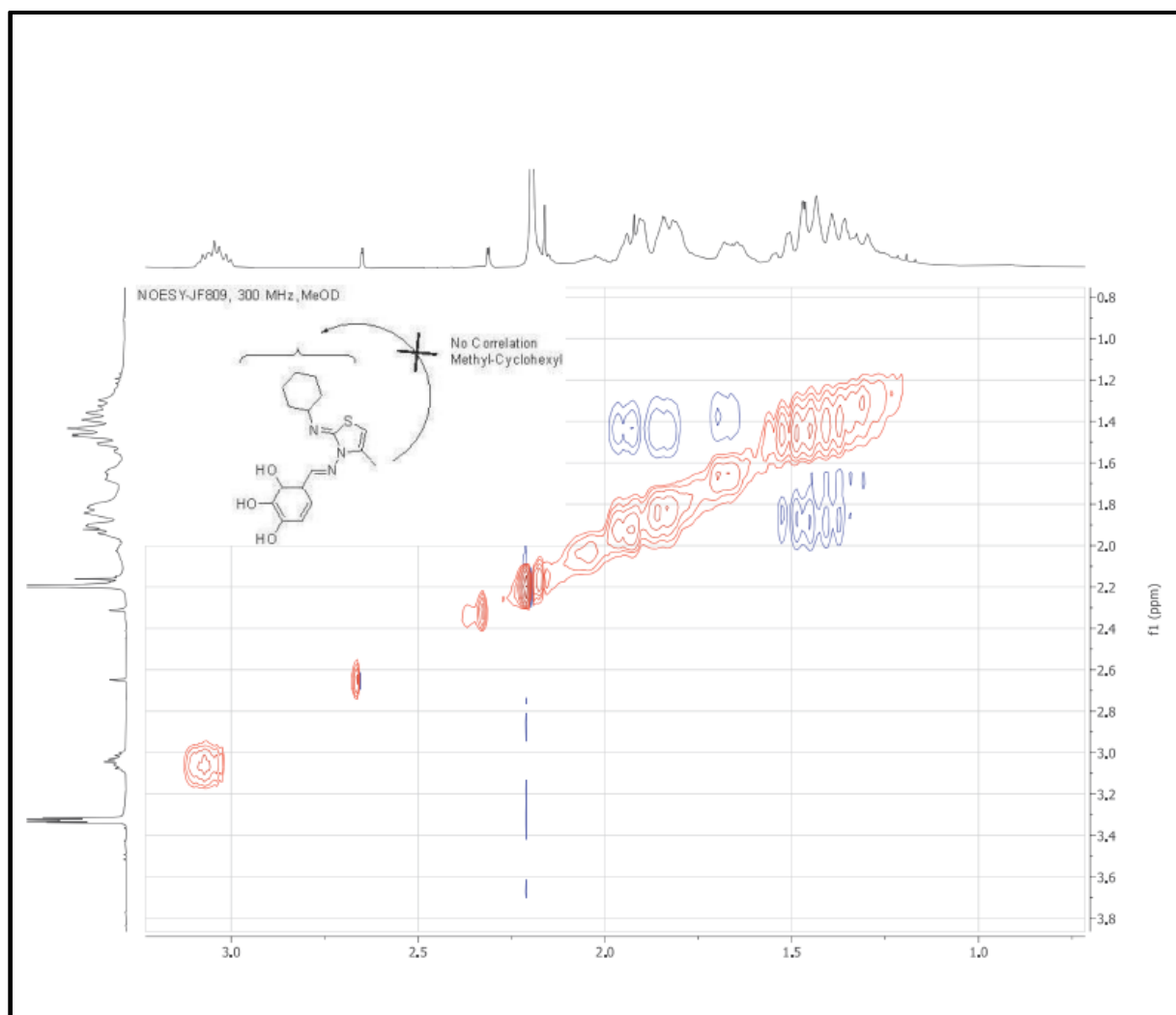
**(2Z,NE)-2-(cyclohexylimino)-4-methyl-N-(2,3,4-tris((tert-butyl)dimethylsilyloxy)benzylidene)thiazol-3(2H)-amine (14)**



4-((E)-(((Z)-2-(cyclohexylimino)-4-methylthiazol-3(2H)-yl)imino)methyl)benzene-1,2,3-triol (FJ-809)

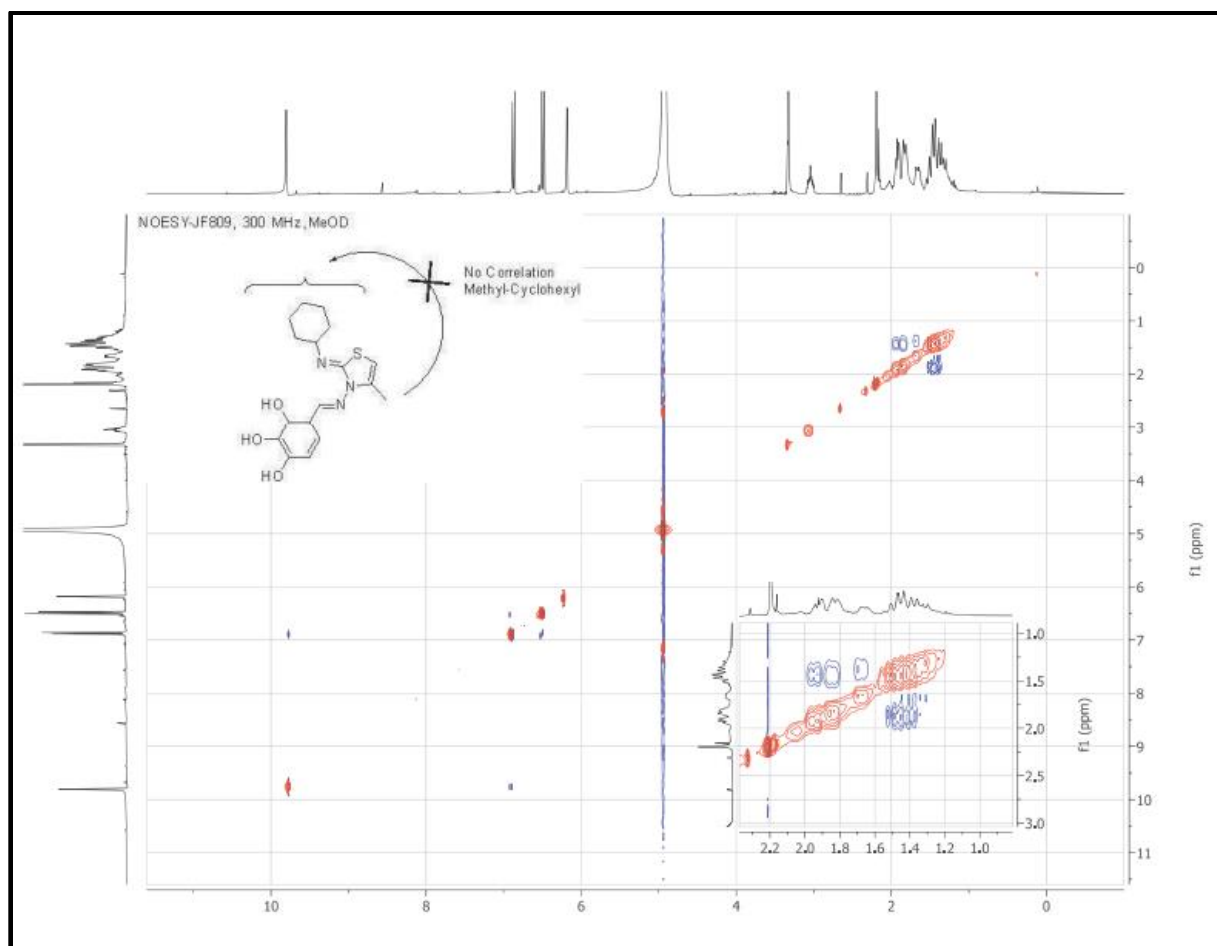


# NOESY spectrum (1) of compound FJ-809





NOESY spectrum (2) of compound FJ-809



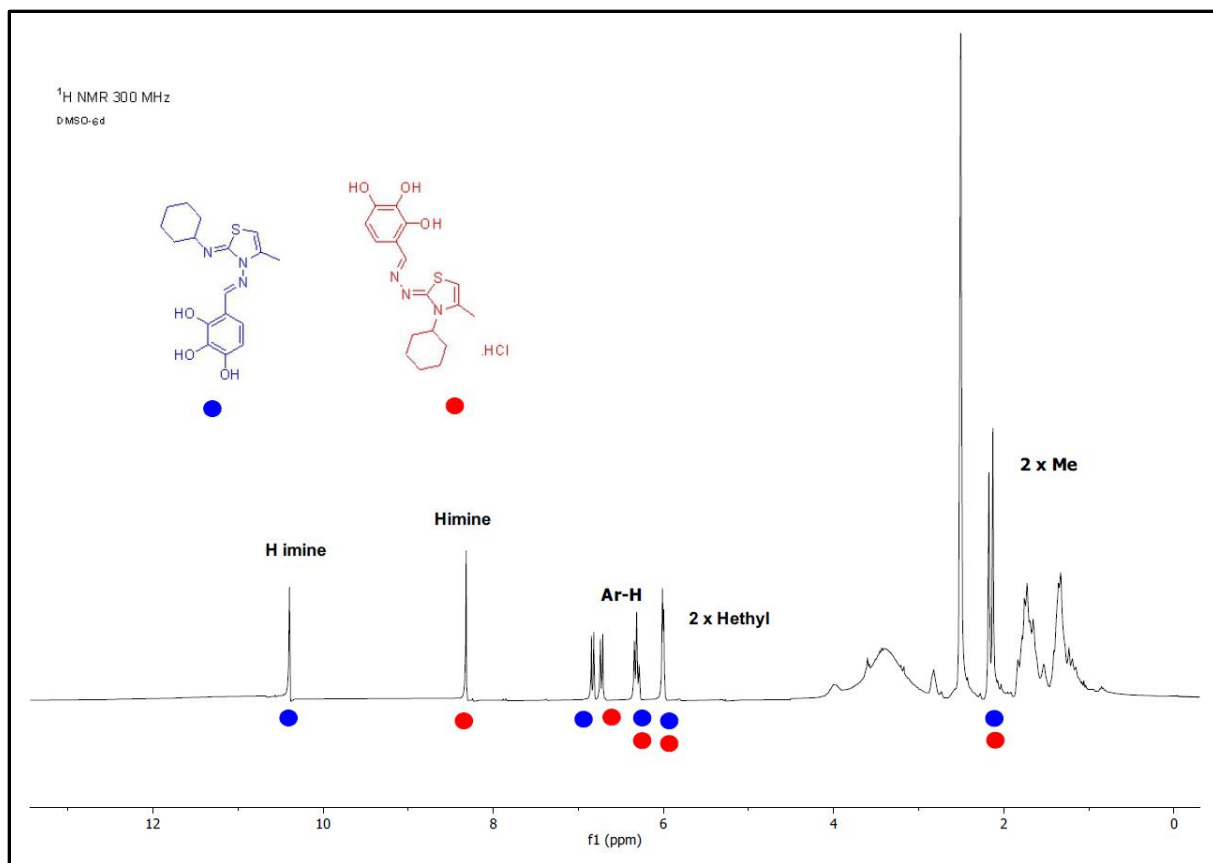
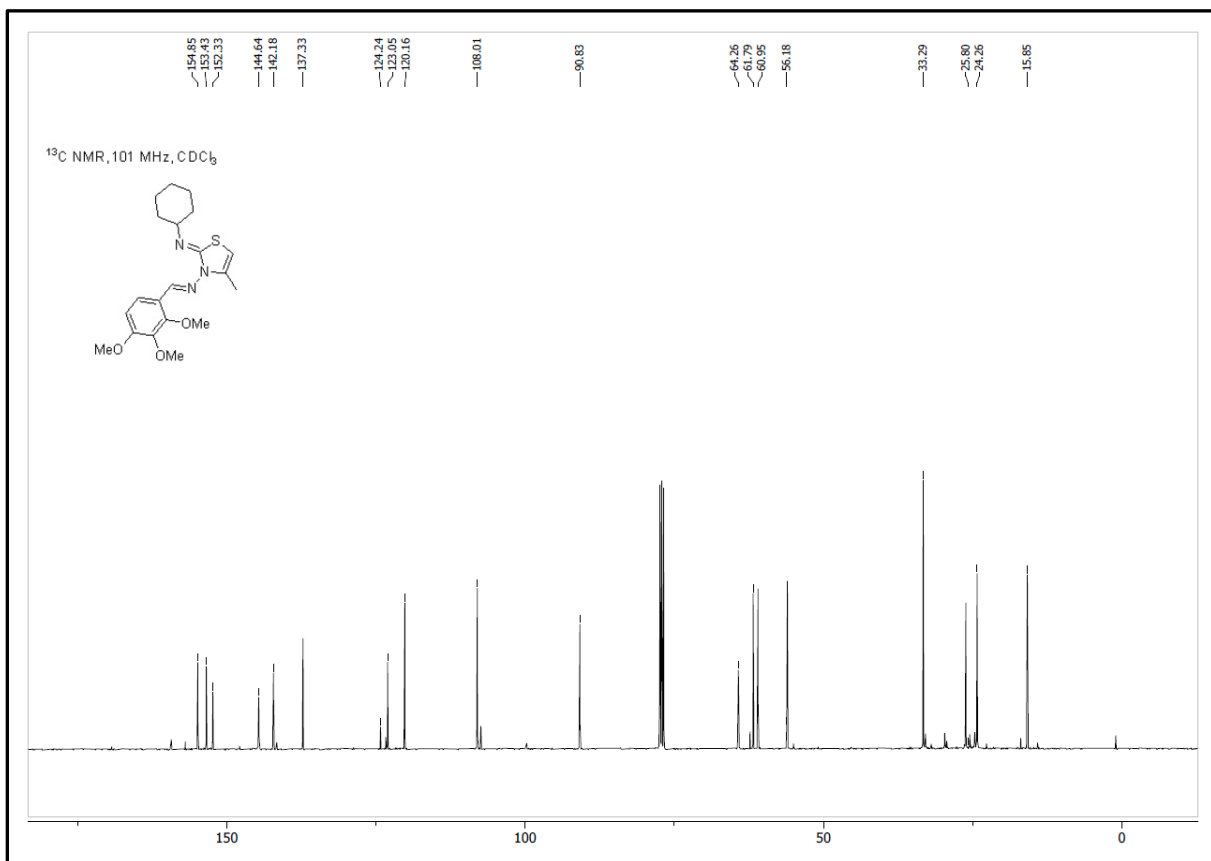
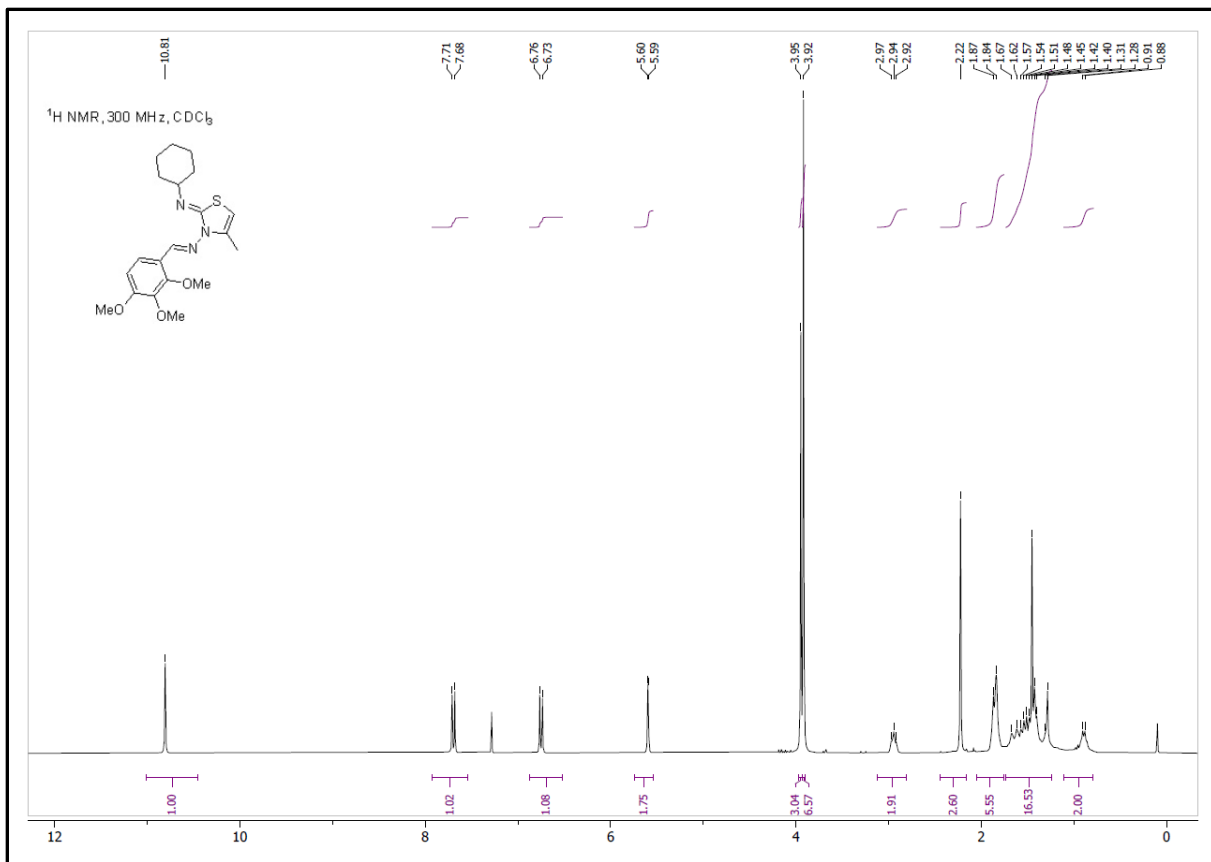


Figure S1: <sup>1</sup>H NMR spectrum of a 1:1 mixture of **FJ-809 (1)** and **MIM1 (2)**

**(2Z,NE)-2-(cyclohexylimino)-4-methyl-N-(2,3,4-trimethoxybenzylidene)thiazol-3(2H)-amine (15)**



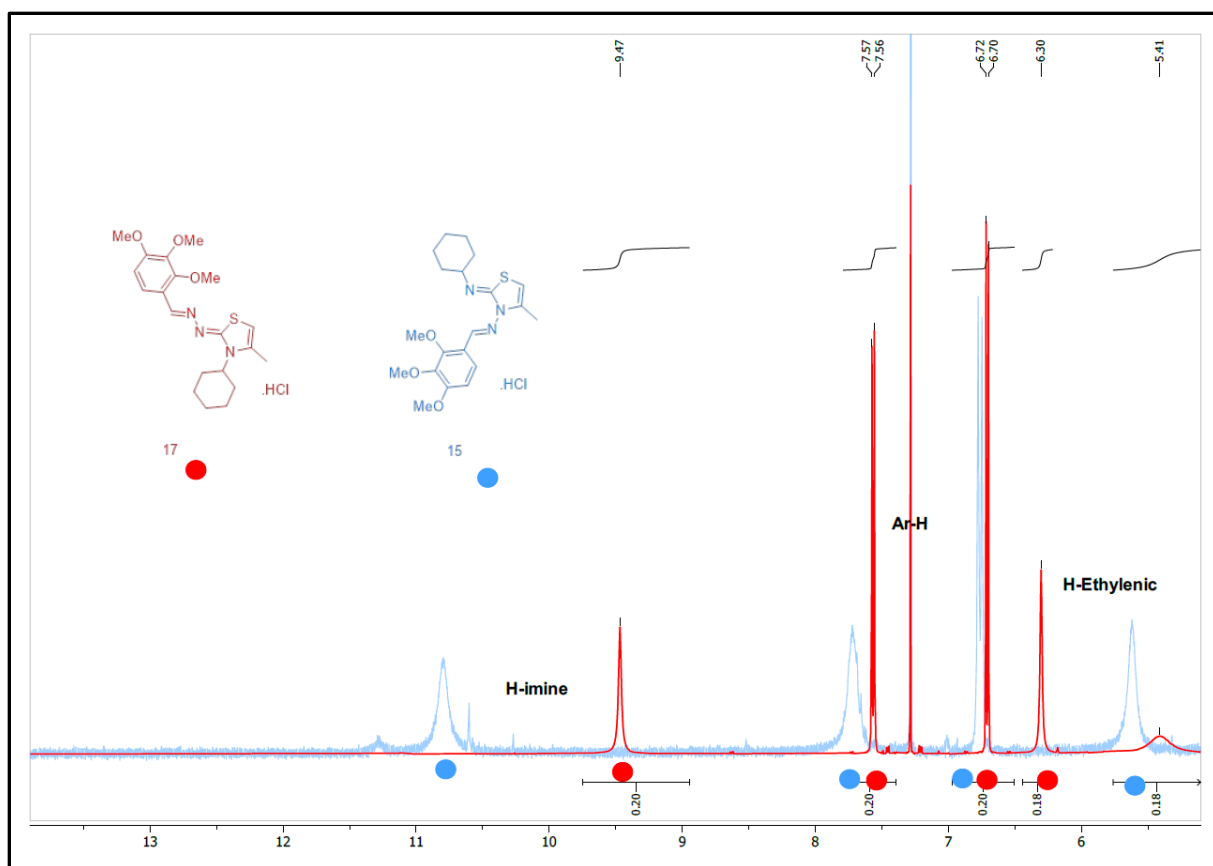
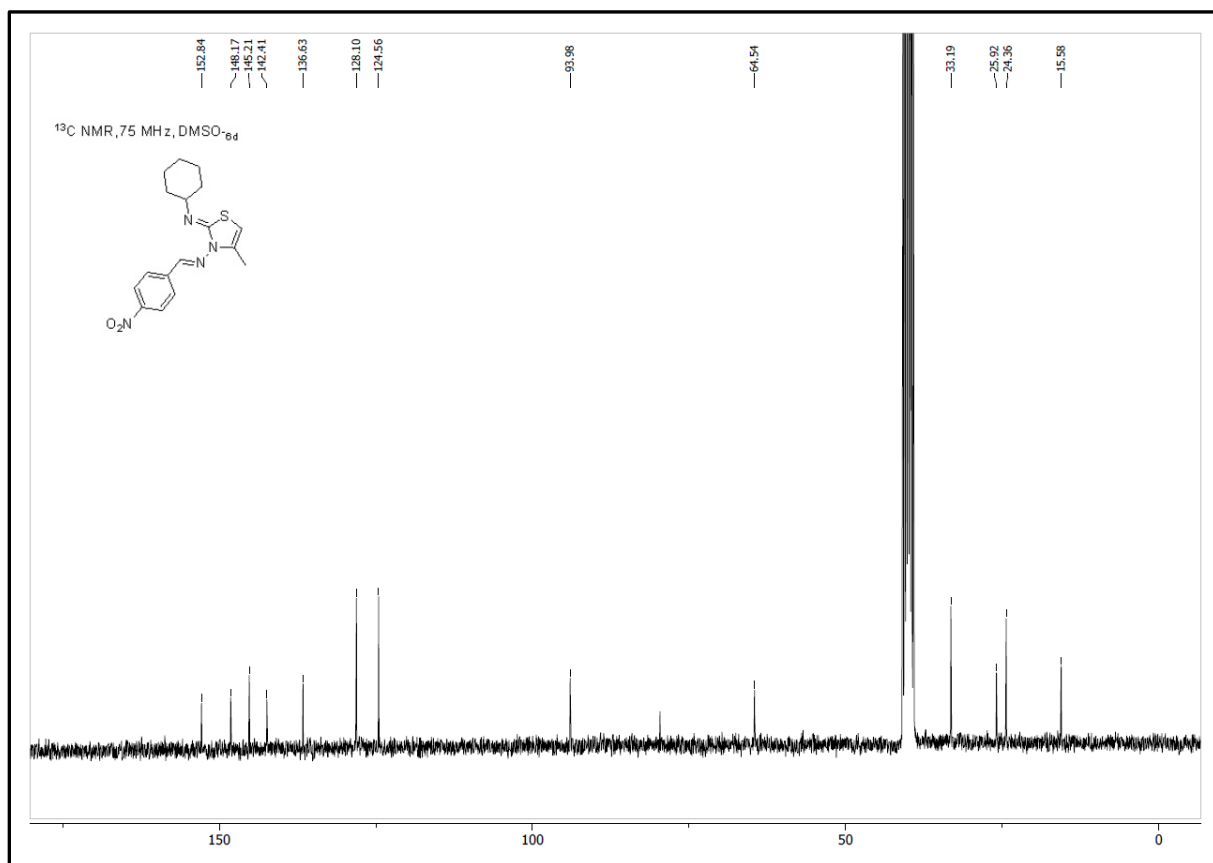
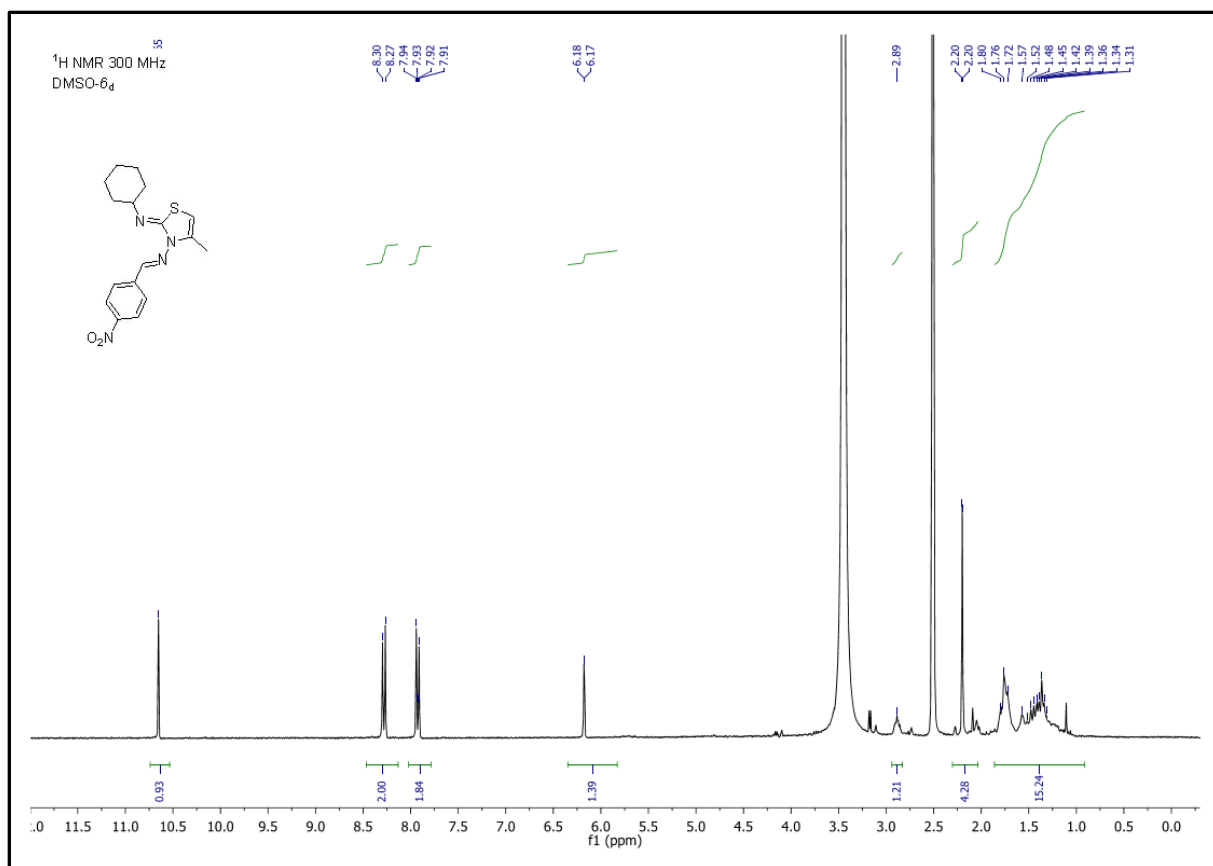


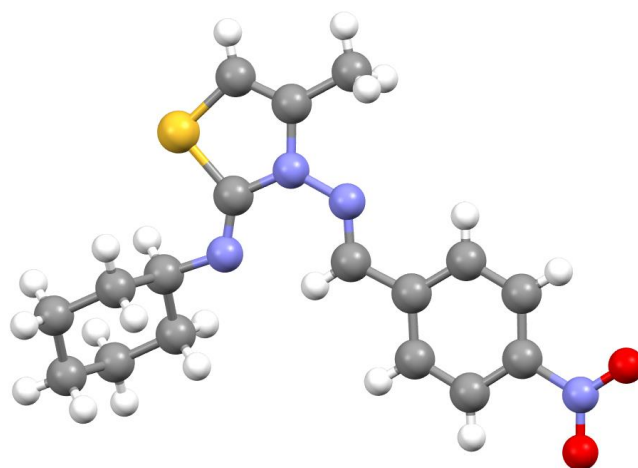
Figure S2:  $^1\text{H}$  NMR spectrum of a 1:1 mixture of **15** and **17**

**(2Z,NE)-2-(cyclohexylimino)-4-methyl-N-(4-nitrobenzylidene)thiazol-3(2H)-amine (16)**



X-Ray data for **16**

CCDC 2123510 (for compound **16**), contain the supplementary crystallographical data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax, +44(0) 1223 336 033; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)



## checkCIF/PLATON report

Structure factors have been supplied for datablock(s) I

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No syntax errors found.      CIF dictionary      Interpreting this report

### Datablock: I

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                                    alpha=90                      beta=96.538 (3)              gamma=90

Temperature:              150 K

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Space group	P 21/n	P 21/n
Hall group	-P 2yn	-P 2yn
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Sum formula	C17 H20 N4 O2 S	C17 H20 N4 O2 S
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Dx, g cm <sup>-3</sup>	1.343	1.343
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Mu (mm <sup>-1</sup> )	0.207	0.207
F000	728.0	728.0
F000'	728.76	
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Nref	3901	3887
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Tmin'	0.887	

Correction method= # Reported T Limits: Tmin=0.422 Tmax=0.986

AbsCorr = MULTI-SCAN

Data completeness= 0.996                      Theta (max)= 27.515

R(reflections)= 0.0829 ( 3311)

wR2(reflections)=  
0.2163 ( 3887)

S = 1.156

Npar= 218

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Click on the hyperlinks for more details of the test.

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PLAT941_ALERT_3_G	Average HKL Measurement Multiplicity .....	4.5	Low
PLAT978_ALERT_2_G	Number C-C Bonds with Positive Residual Density.	11	Info

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- 0 **ALERT level A** = Most likely a serious problem - resolve or explain  
0 **ALERT level B** = A potentially serious problem, consider carefully  
7 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight  
6 **ALERT level G** = General information/check it is not something unexpected
- 0 ALERT type 1 CIF construction/syntax error, inconsistent or missing data  
7 ALERT type 2 Indicator that the structure model may be wrong or deficient  
4 ALERT type 3 Indicator that the structure quality may be low  
2 ALERT type 4 Improvement, methodology, query or suggestion  
0 ALERT type 5 Informative message, check
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## checkCIF publication errors

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- 0 **ALERT level A** = Data missing that is essential or data in wrong format  
1 **ALERT level G** = General alerts. Data that may be required is missing
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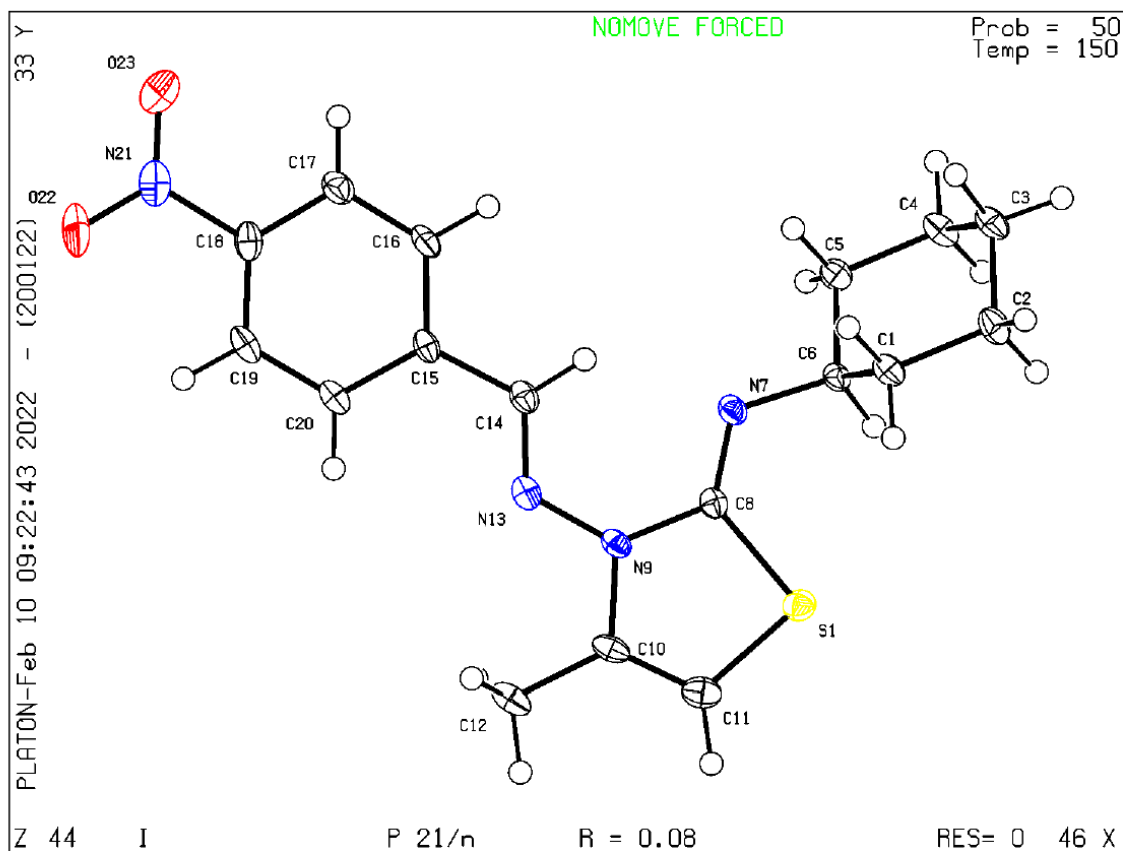
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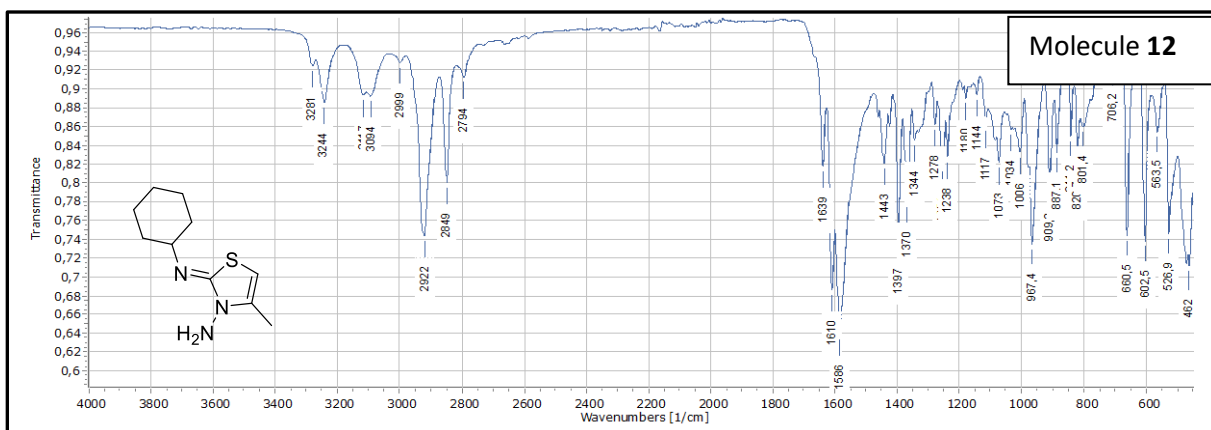
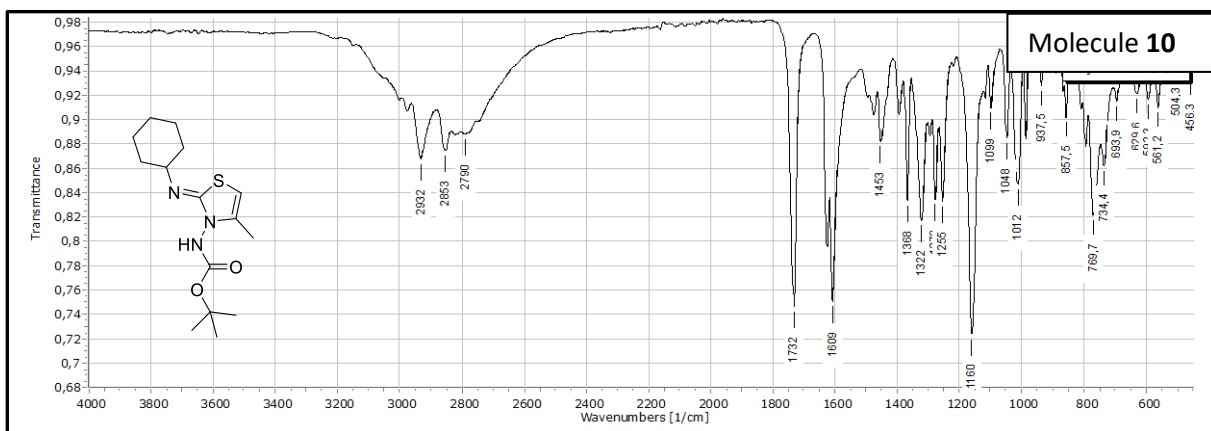
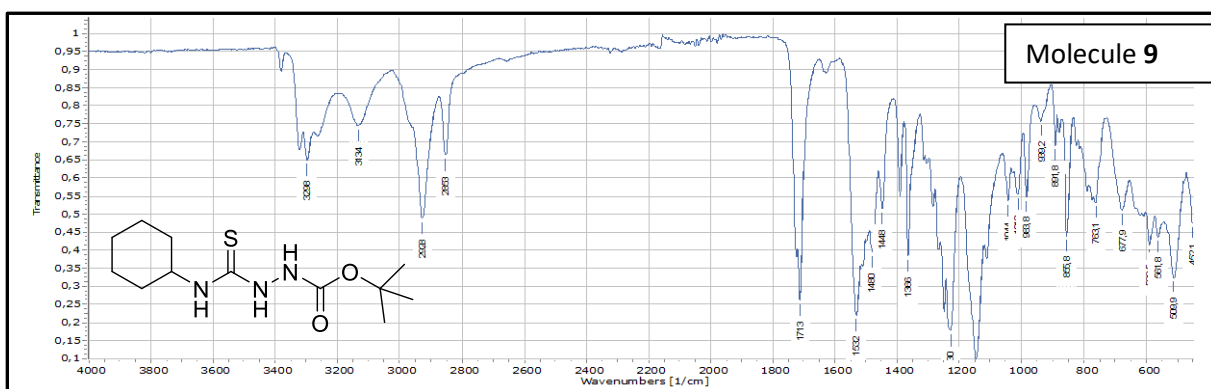
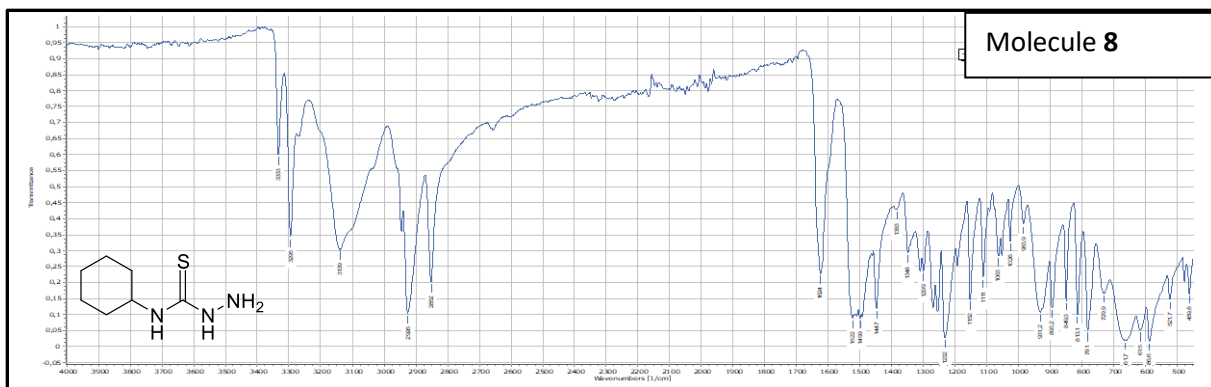
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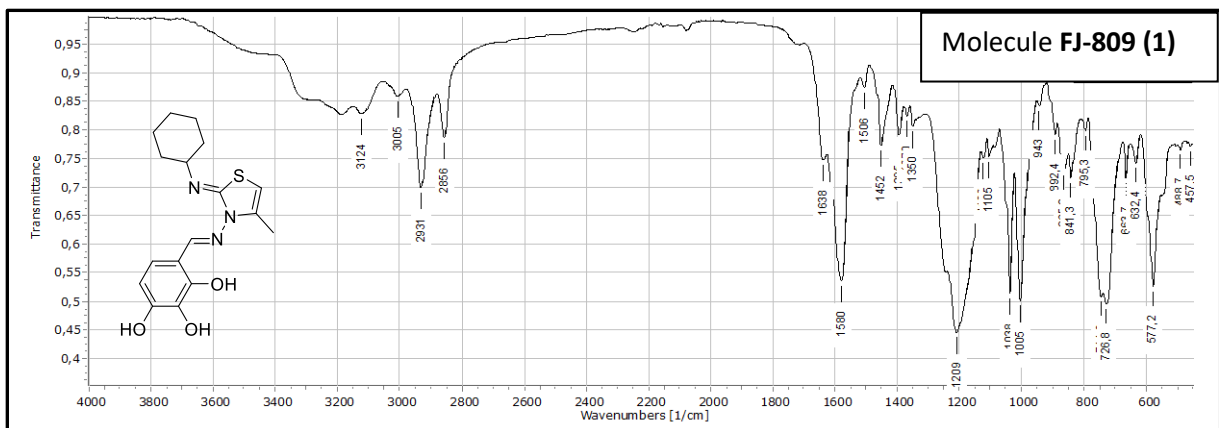
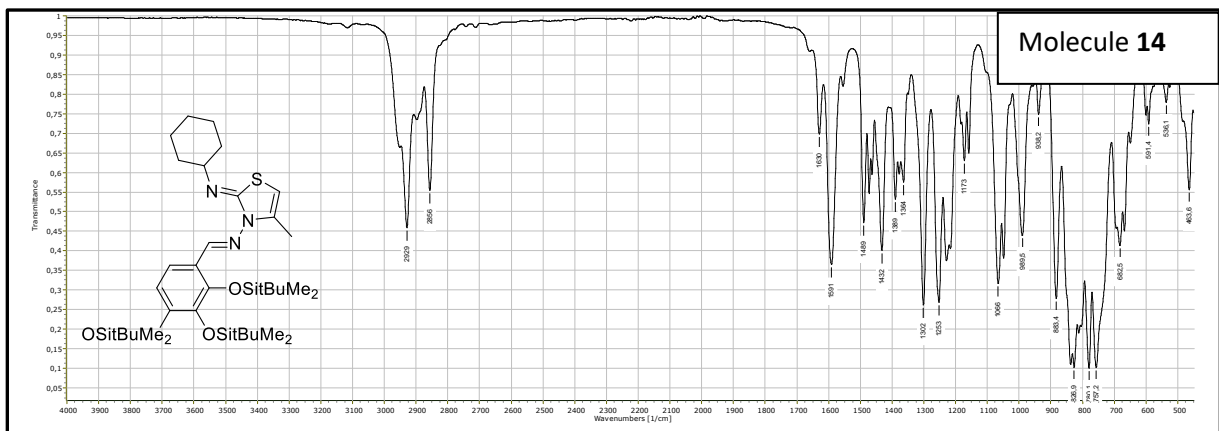
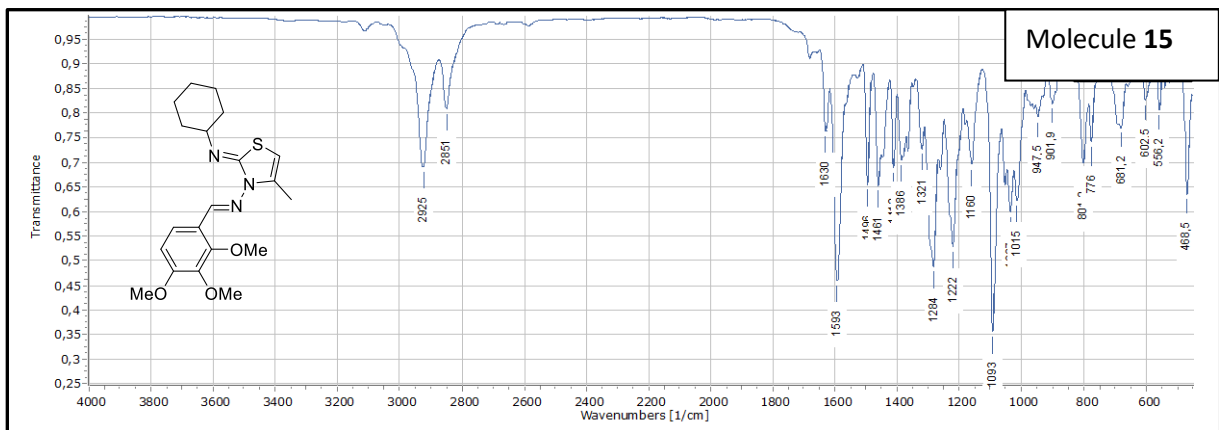
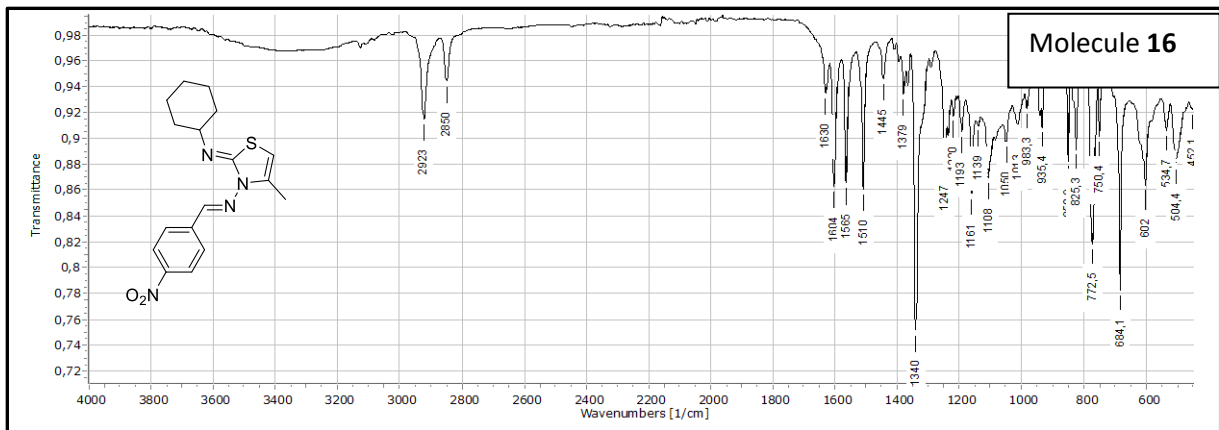
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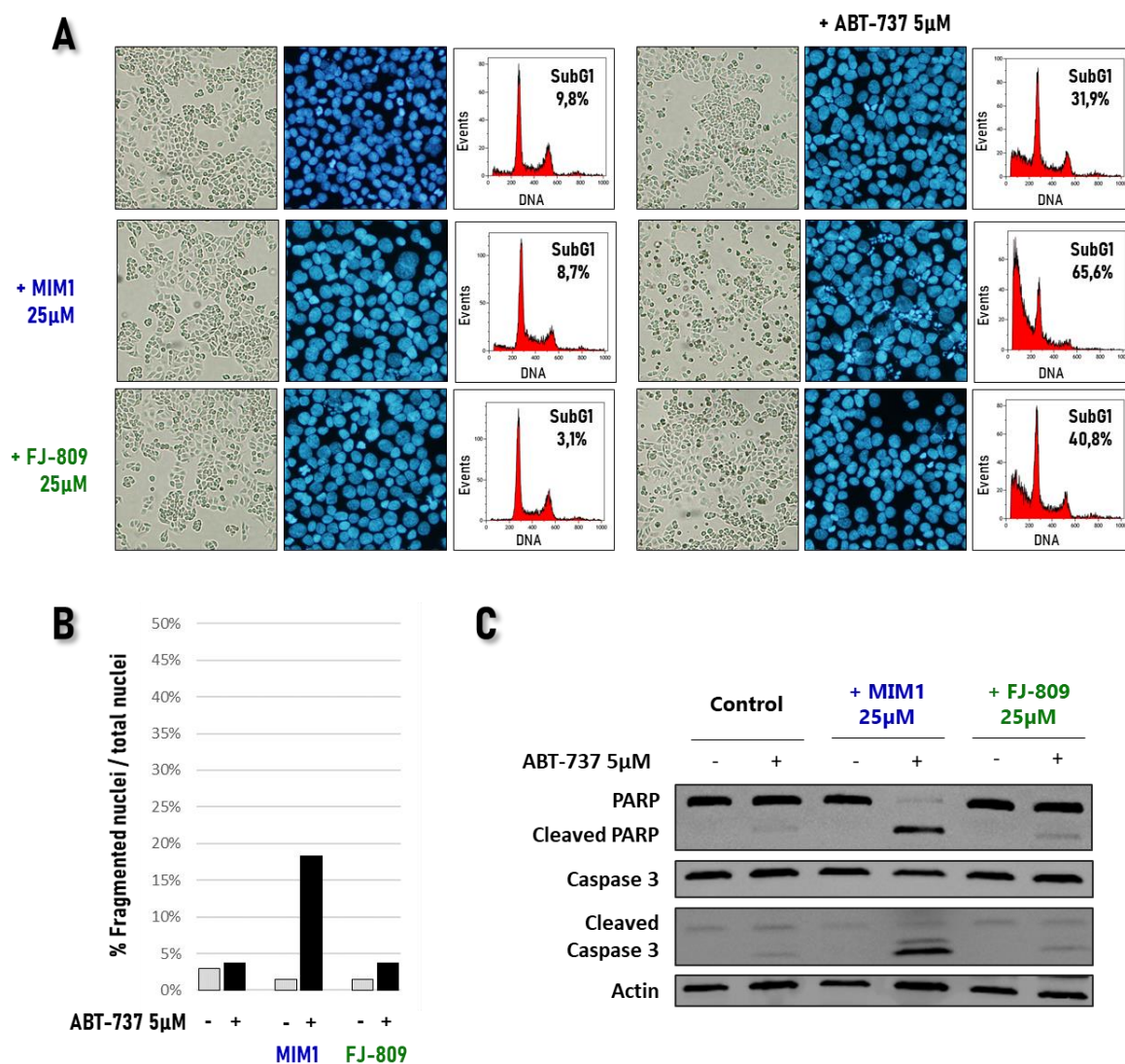
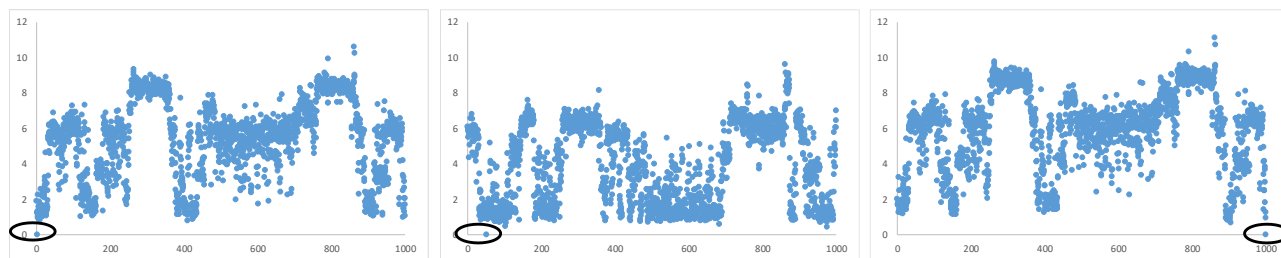


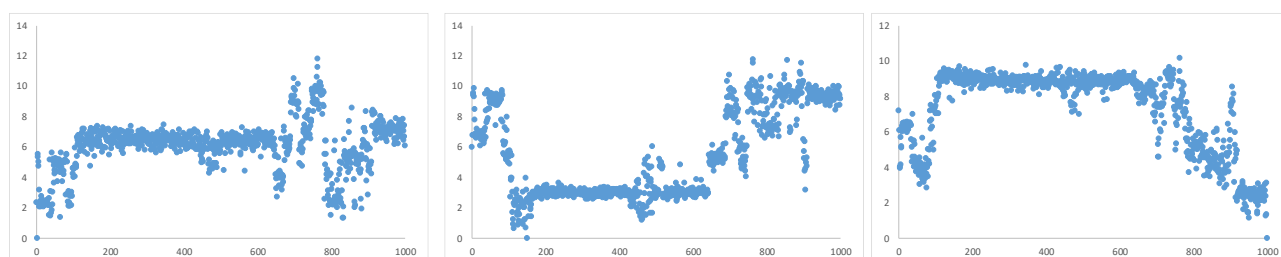
Figure S3: Evaluation of the biological effect of **MIM1** (2) and **FJ-809** (1) on IGROV1-R10 cell line. IGROV1-R10 chemoresistant ovarian cancer cell line was exposed either to **MIM1** or to **FJ809** (25 $\mu$ M) combined or not with ABT-737 (5 $\mu$ M). Effect on cellular and nuclear morphologies as well as cell cycle repartition is represented (A) and nuclei fragmentation is quantified as described in the experimental section (B). PARP and Caspase 3 cleavage is evaluated by western blot (C).

## SUPPLEMENTARY DATA Molecular dynamics experiments

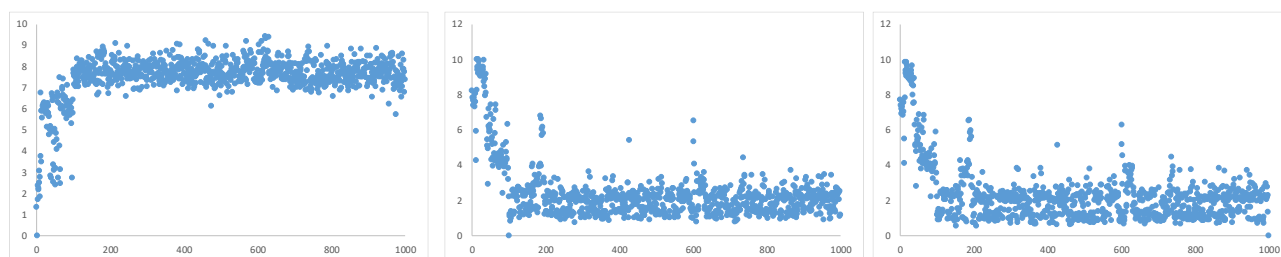
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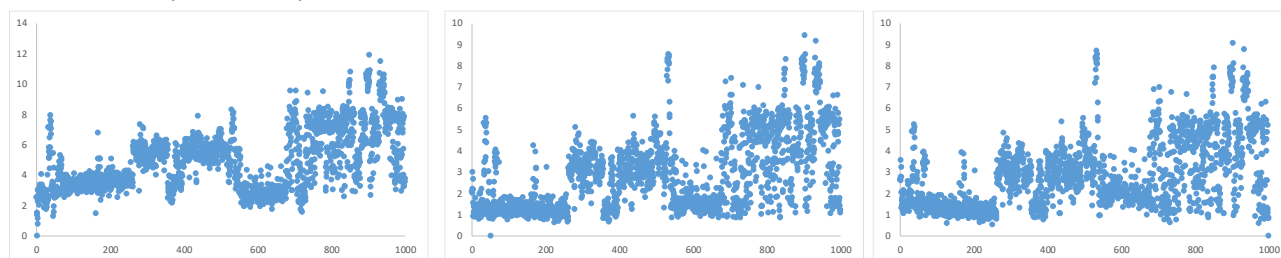
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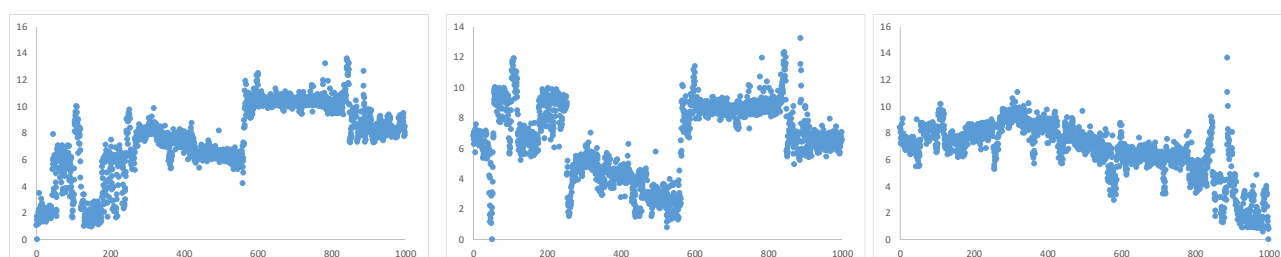
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### Molecular dynamics experiment n° 4



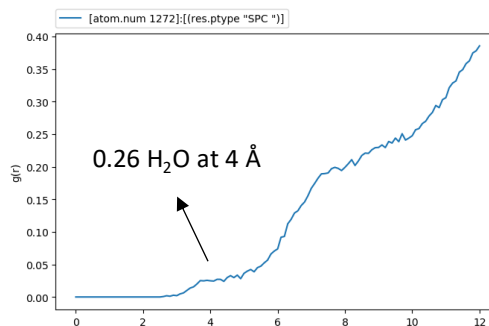
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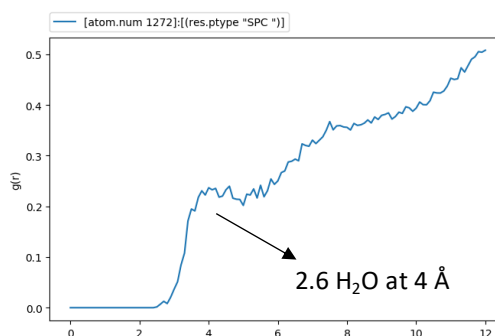
**Figure S4**

RMSD of **MIM1 (2)** in Mcl-1, as calculated from the initial state (left, see the ellipse), a early pseudo steady-state (middle), and final state (right). X-axis: time (ns), y-axis: RMSD (Å).

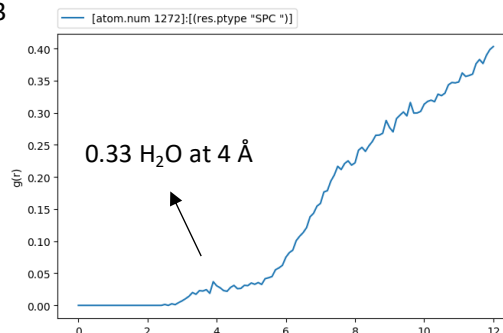
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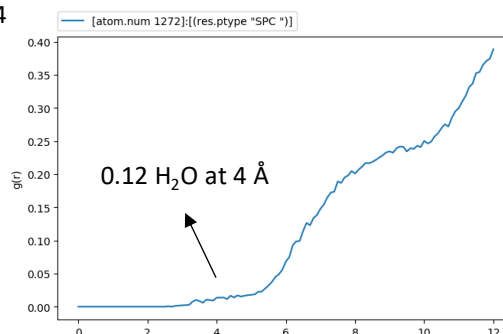
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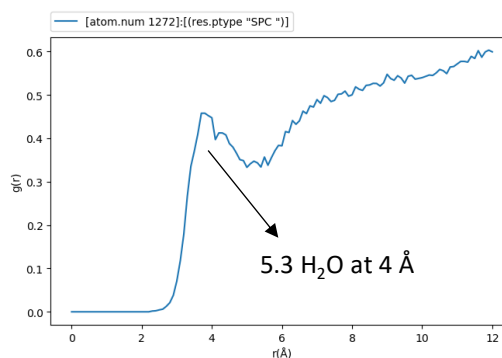
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Molecular dynamics experiment n° 4



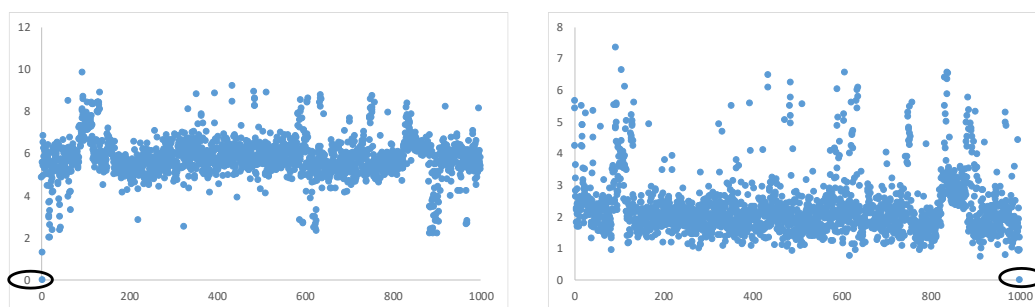
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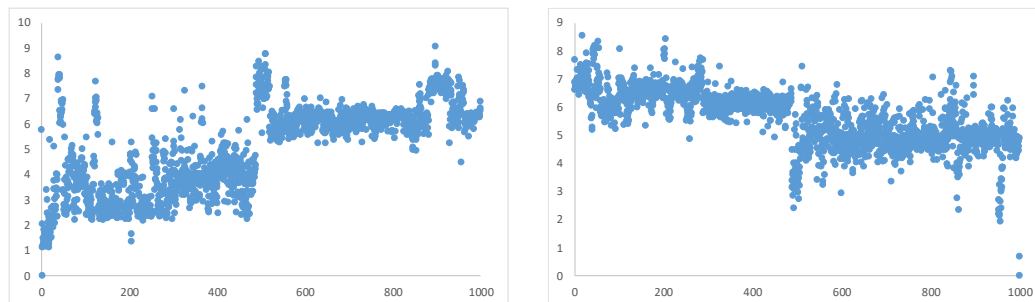
**Figure S5**

RDF of water molecules around the methyl group of **MIM1 (2)**. Integration of the RDF give an approximation of the average coordination number, here given at 4 Å.

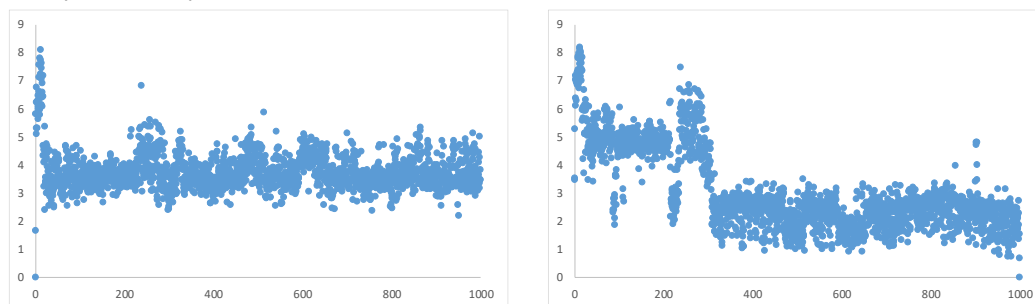
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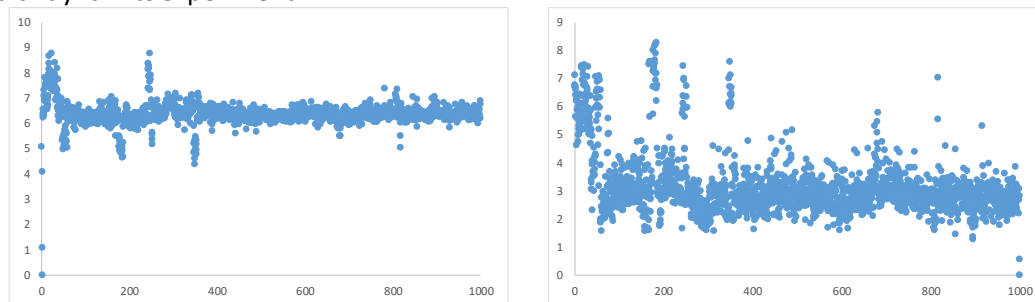
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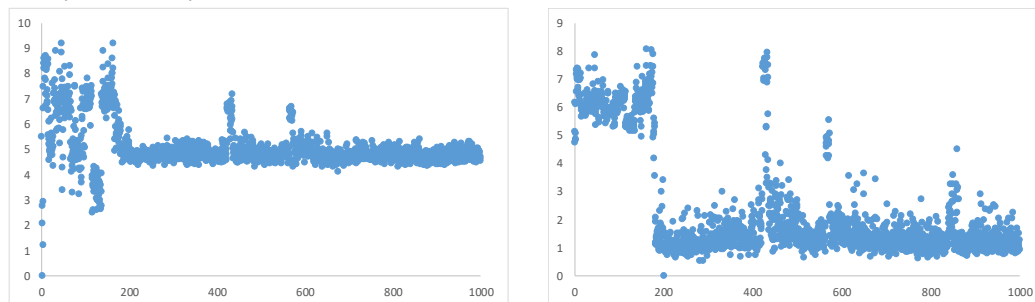
Molecular dynamics experiment n° 3



Molecular dynamics experiment n° 4



Molecular dynamics experiment n° 5

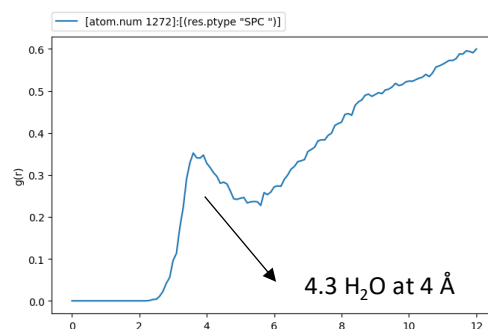


**Figure S6**  
RMSD of **FJ-809 (1)** in Mcl-1, as calculated from the initial state (left, see the ellipse) and final state (right).

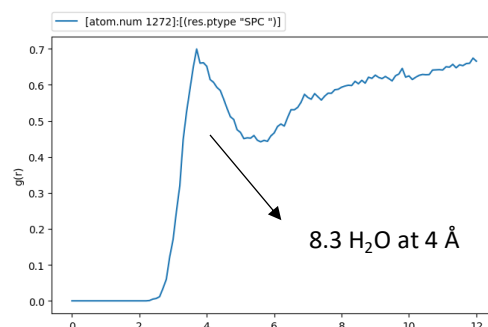


X-axis: time (ns), y-axis: RMSD (Å).

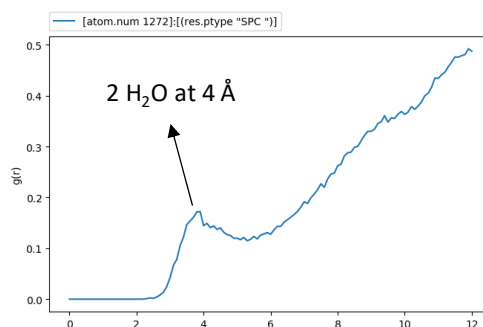
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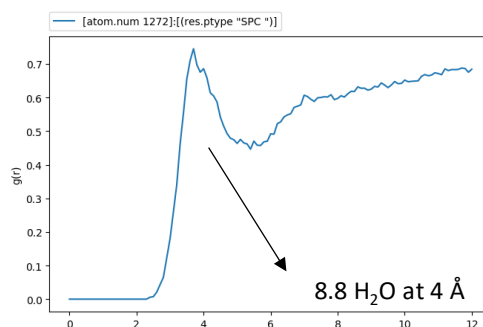
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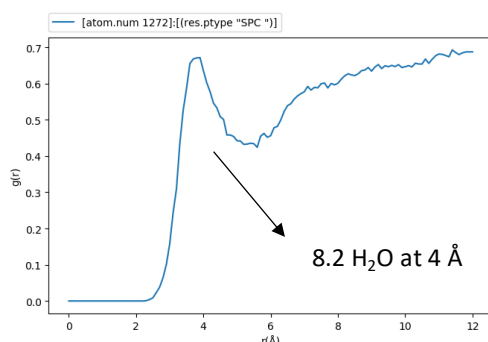
Molecular dynamics experiment n° 3



Molecular dynamics experiment n° 4



Molecular dynamics experiment n° 5



**Figure S7**

RDF of water molecules around the methyl group of **FJ-809 (1)**. Integration of the RDF give an approximation of the average coordination number, here given at 4 Å.