

# Supporting Information

## GFP- related Chromophores: Photoisomerization, Thermal Reversion, and DNA Labelling

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United States.

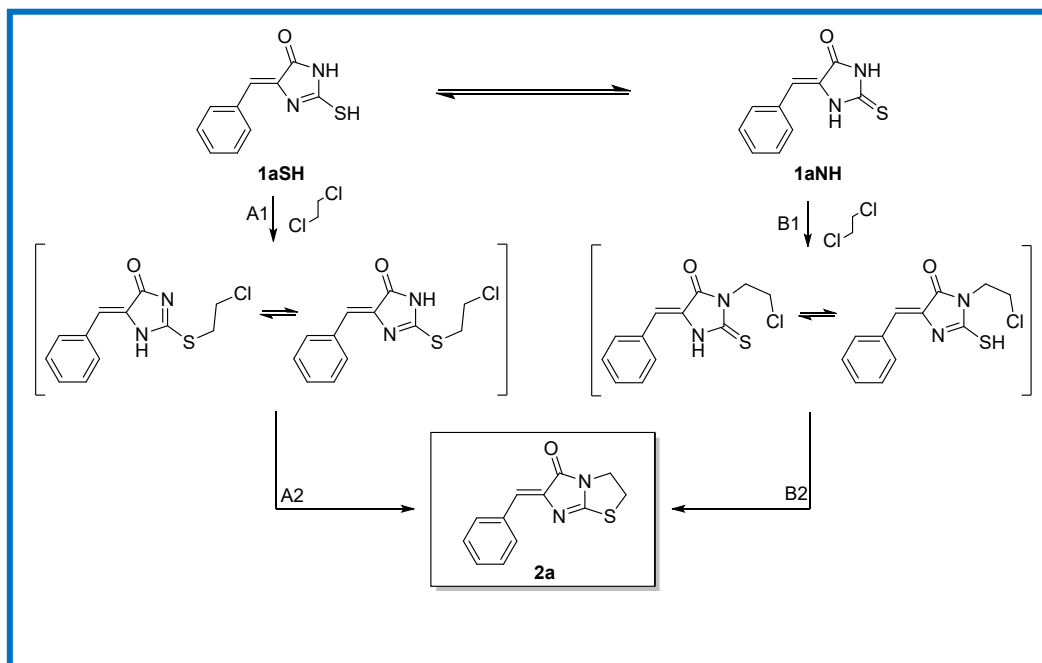
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# 1. Mechanistic study

The formation of *Z*-6-benzylidene-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one **2a** can be postulated considering two different paths, Scheme S1. The nucleophilic attack to the alkyl group can be initiated either by the thiol group or the nitrogen lone pair, path A and B, respectively. To address this question, a reaction with 1-bromoethane was performed with **1a**. If the reaction occurs via the nitrogen, the compound **1aNethyl** shown in Scheme S2 should be detected. Whilst if the nucleophilic attack occurs via the sulfur atom, the compound **1aSethyl** would be detected.



After completing the reaction with 1-bromoethane (Scheme S2) the compound **4** resulted as the main product of this synthesis. Additionally, a sub-product **5** was detected, which incorporated two ethyl groups. These compounds were elucidated by <sup>1</sup>H-NMR and GC-MS analyses, as described in Figures S1 and S2, respectively.

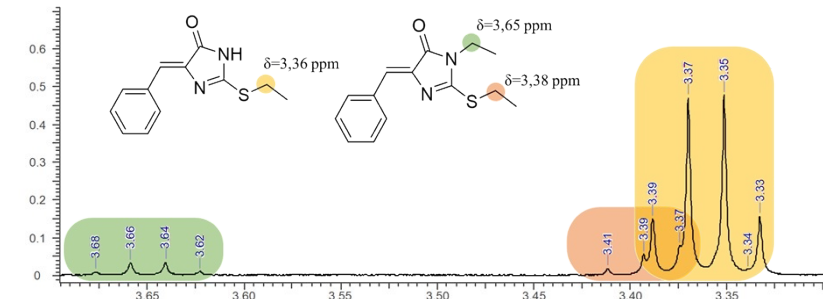
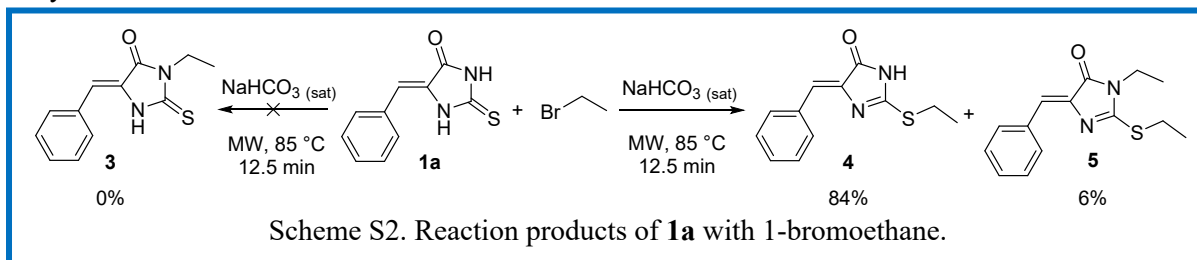
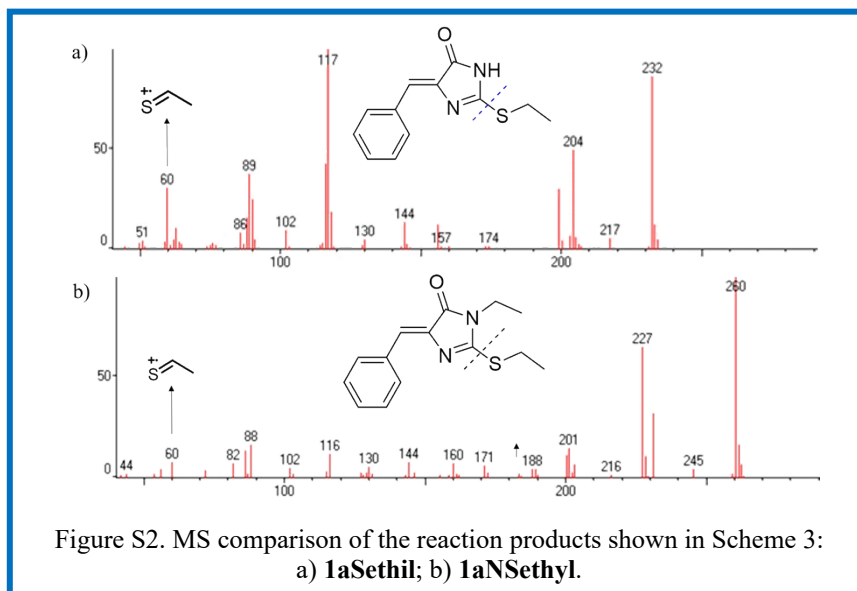


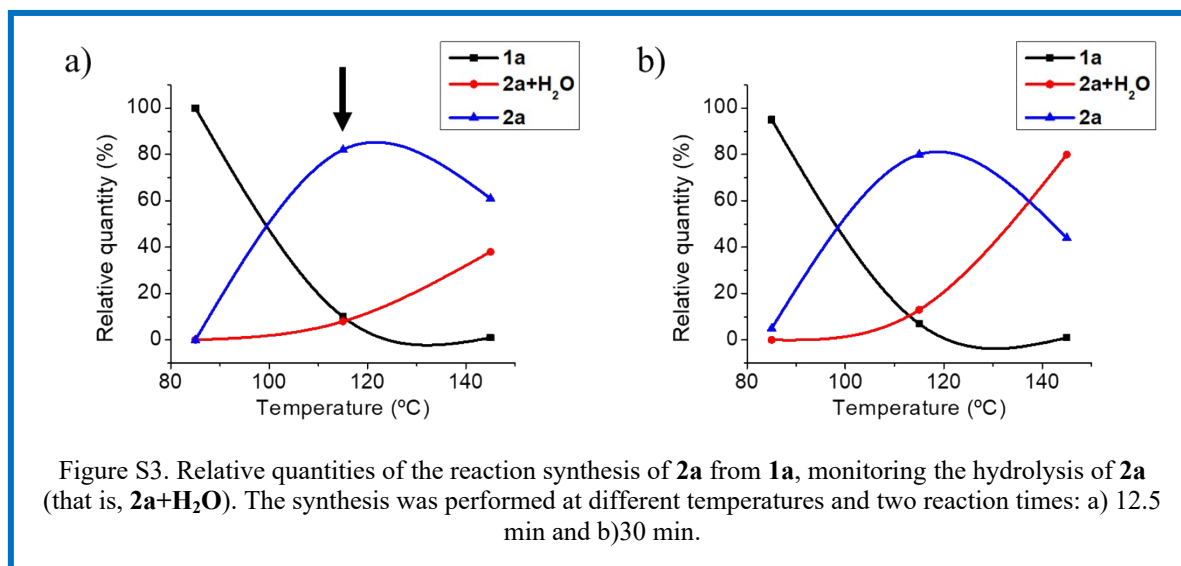
Figure S1. <sup>1</sup>H-NMR of the reaction product of illustrated in Scheme 3.



## 2. Hydrolysis of 2a

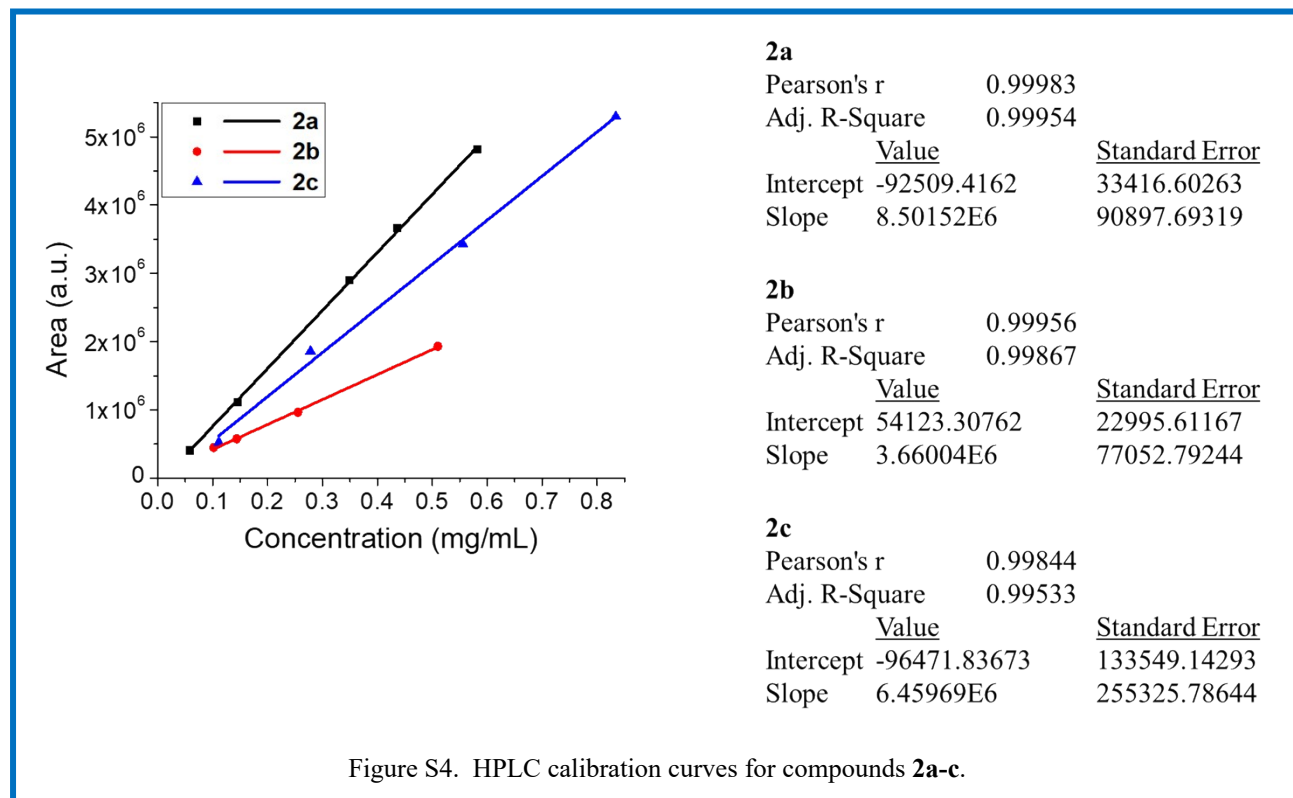
The synthesis of compound **2a** can be carried out by two different pathways. One of them consisted of a two-reaction step, where the second step starts from compound **1a**. In this synthesis, we detected the hydrolysis of the desired compound, **2a**, and some attempts varying the microwave conditions were done to diminish the formation of this sub-product (**2a+H<sub>2</sub>O**).

The relative quantities of the reaction products varying the microwave conditions are summarized in Figure S3. We explored the synthesis at 85, 115 and 150 °C and two reaction times, 12.5 and 30 min, Figure S3a and S3b, respectively. The quantification was performed by GC. From the results, we could conclude that the hydrolysis is rather ruled by changes in the temperature than the reaction time. The best condition, where the amount of **2a+H<sub>2</sub>O** was the least, was at 115 °C and 12.5 min, as denoted with an arrow in Figure S3a.



### 3. Yield determinations of 2a-c by HPLC

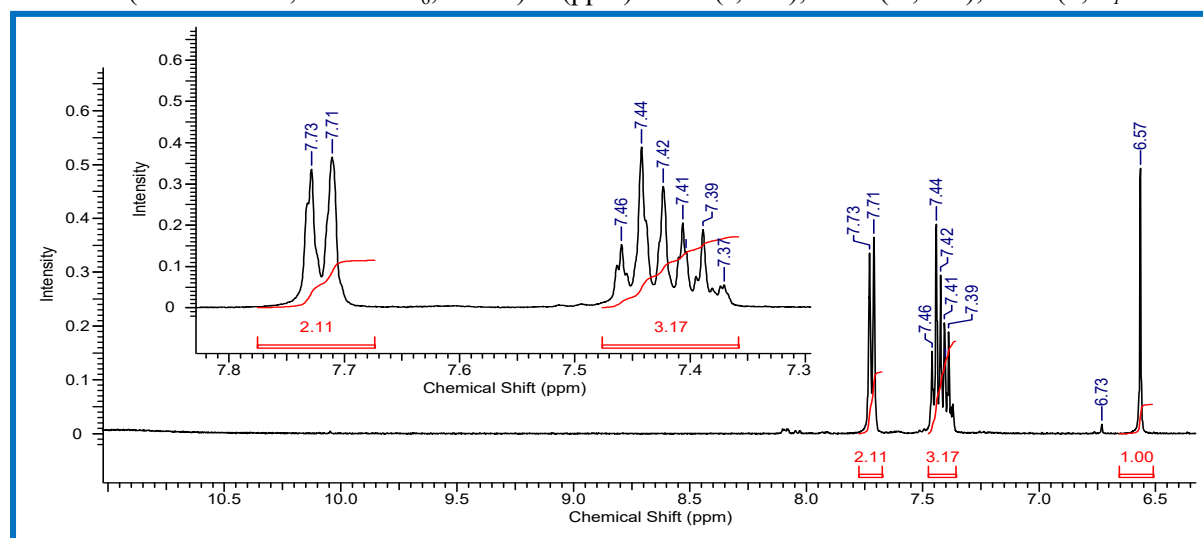
Elution was performed with a mixture of MeOH:H<sub>2</sub>O (90:10) at a flow rate of 0.8 mL min<sup>-1</sup>. The retention times under these conditions were: **2a** (5.4 min); **2b** (6.5 min); **2c** (4.8 min).



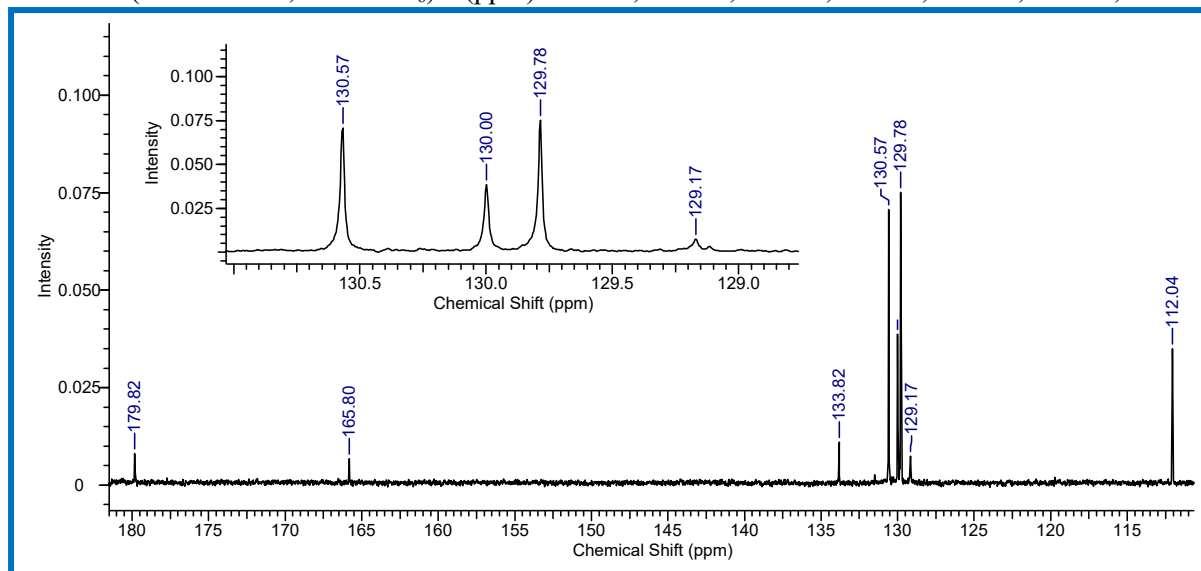
### 4. Chemical characterization of synthesized compounds

#### Compound 1a. 5-benzylidene-2-thioxoimidazolidin-4-one

<sup>1</sup>H-NMR (400.16 MHz, acetone-d<sub>6</sub>, 22 °C) δ (ppm): 6.57 (s, 1H); 7.42 (m, 3H); 7.72 (d; *J*<sub>H</sub>=7.15 Hz; 2H).

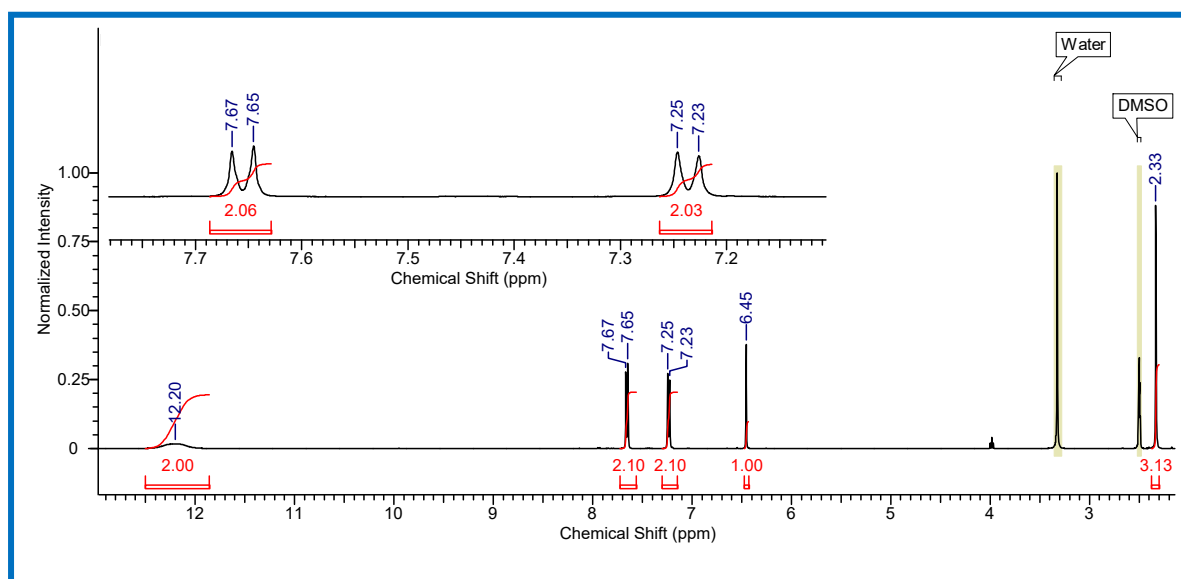


$^{13}\text{C}$ -NMR (100.56 MHz, acetone- $d_6$ )  $\delta$  (ppm): 112.04; 129.17; 129.78; 130.00; 130.57; 165.80; 179.82.

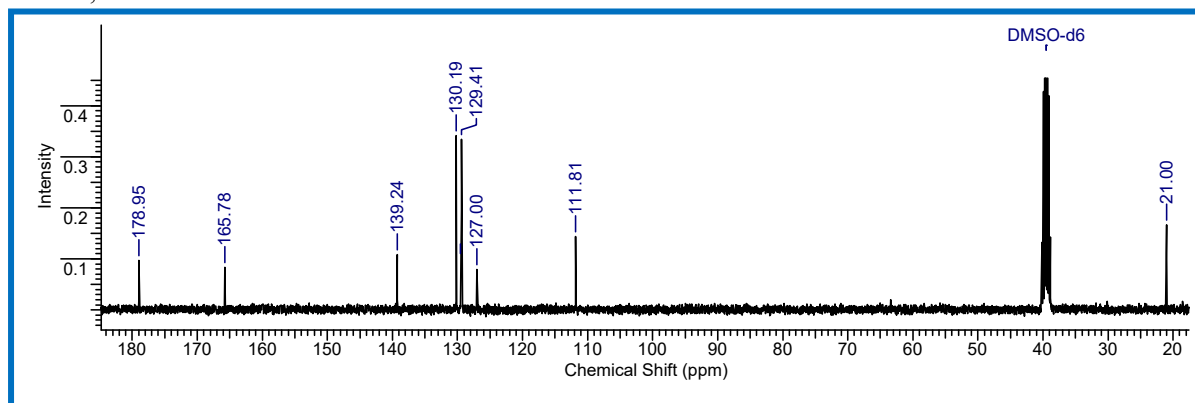


### Compound 1b. 5-(4-methylbenzylidene)-2-thioxoimidazolidin-4-one

$^1\text{H}$ -NMR (400.16 MHz, DMSO- $d_6$ , 22 °C)  $\delta$  (ppm): 2.33 (s, 3H); 6.45 (s, 1H); 7.24 (d;  $J_f=8.10$  Hz; 2H); 7.66 (d;  $J_f=8.10$  Hz; 2H), 12.20 ( $s_{\text{exchanges}}$ , 2H).

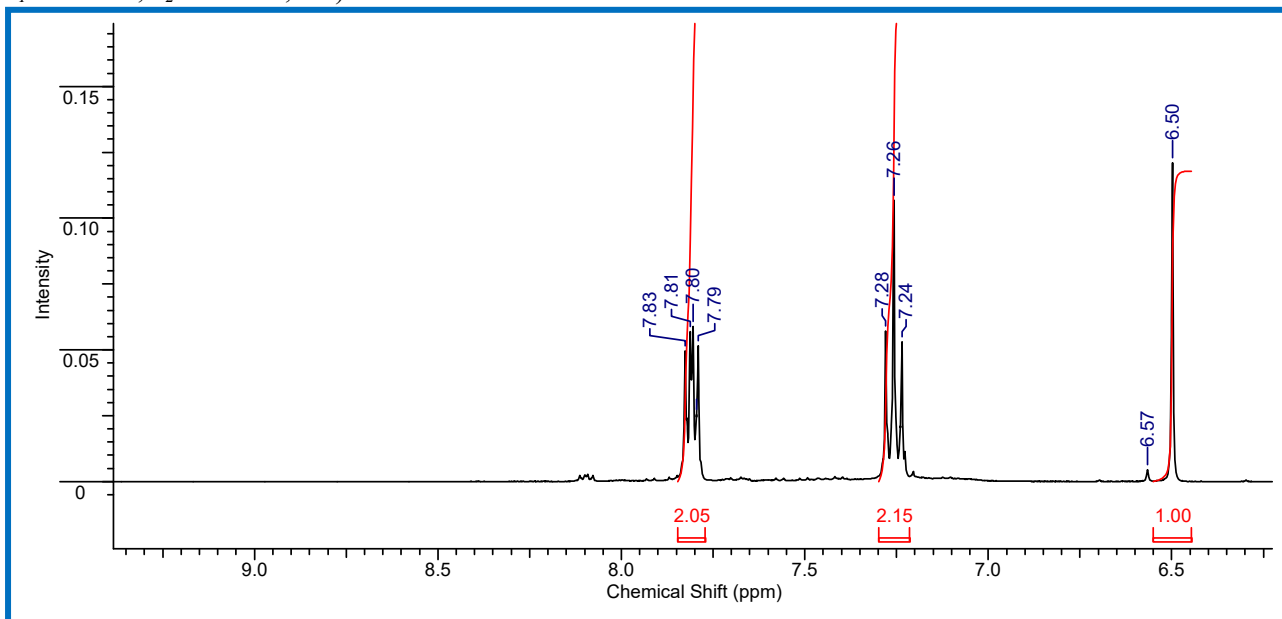


$^{13}\text{C}$ -NMR (100.56 MHz, acetone- $d_6$ )  $\delta$  (ppm): 21.00; 111.81 (2C); 127.00; 129.41 (2C); 130.19 (2C); 139.24; 165.95; 178.95.

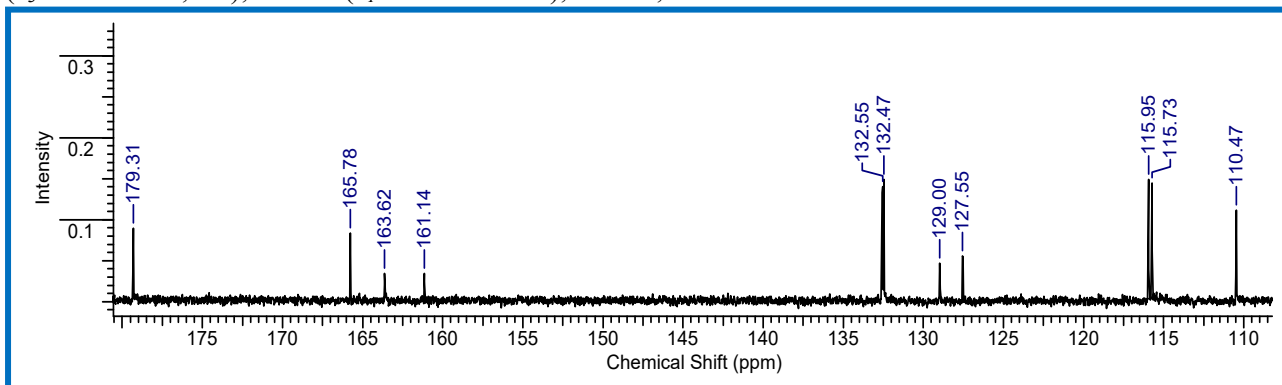


**Compound 1c.** 5-(4-fluorobenzylidene)-2-thioxoimidazolidin-4-one

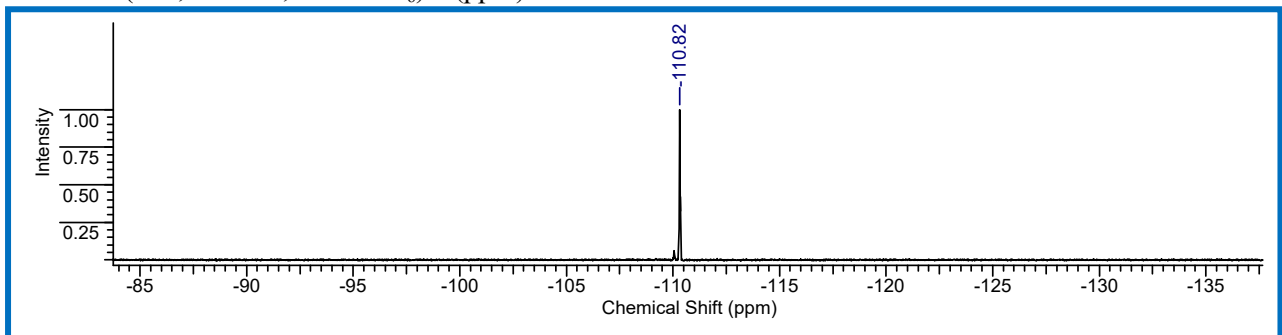
$^1\text{H-NMR}$  (400.16 MHz,  $\text{DMSO-}d_6$ , 22 °C)  $\delta$  (ppm): 6.50 (s, 1H); 7.26 (t;  $J_1=8.92$  Hz; 2H); 7.81 (dd;  $J_1=8.92$  Hz;  $J_2=5.58$  Hz; 2H).



$^{13}\text{C-NMR}$  (100.56 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm): 110.47; 115.84 ( $J_2^{C-F}=21.61$  Hz; 2C); 127.75; 129.00; 132.51 ( $J_3^{C-F}=8.18$  Hz; 2C); 162.38 ( $J_1^{C-F}=248.74$  Hz); 165.78; 179.31.

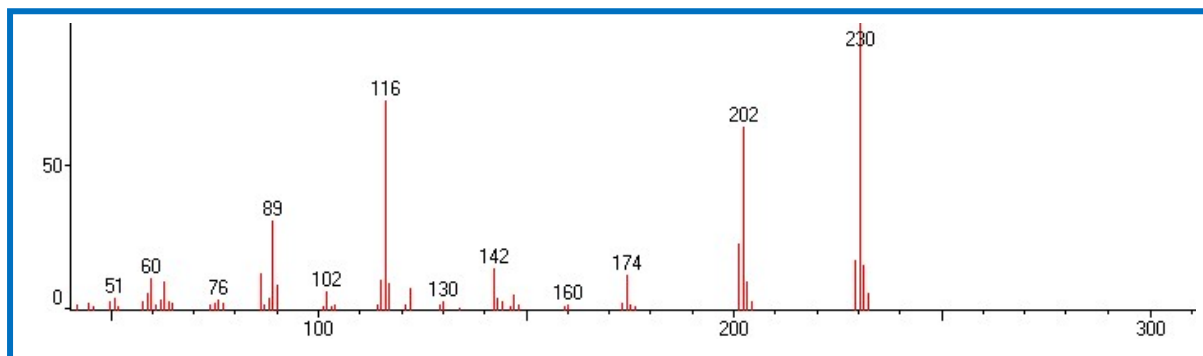


$^{19}\text{F-NMR}$  (376.53 MHz;  $\text{DMSO-}d_6$ )  $\delta$  (ppm): -110.82.

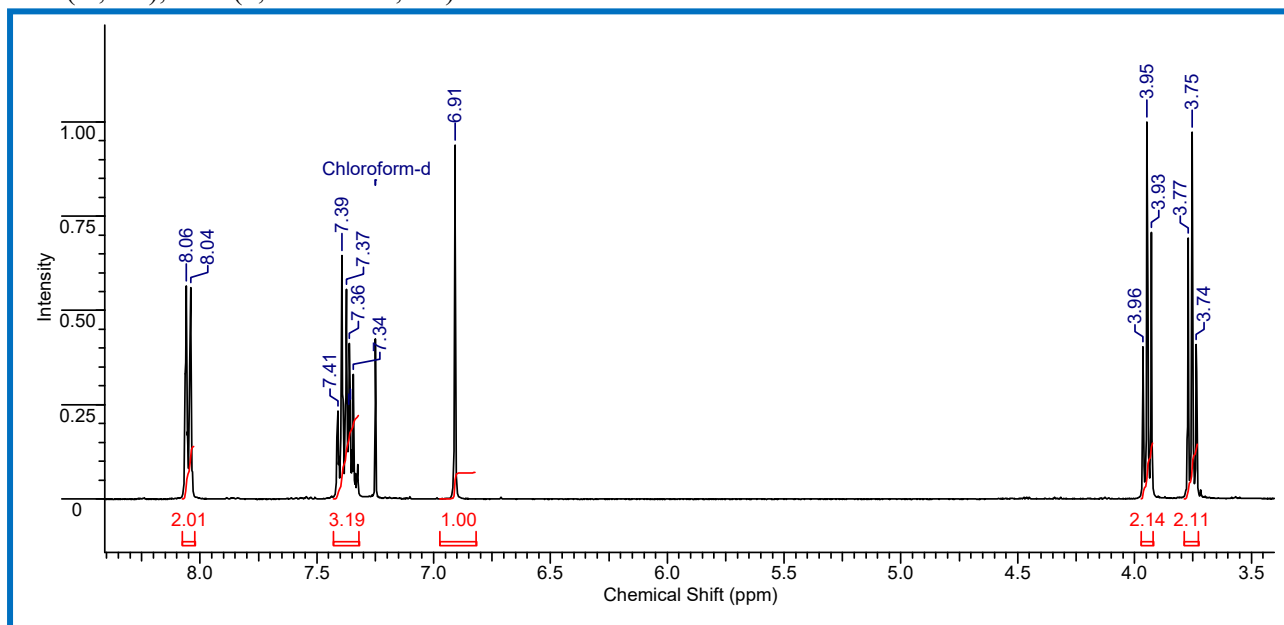


**Compound 2a.** 6-benzylidene-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one

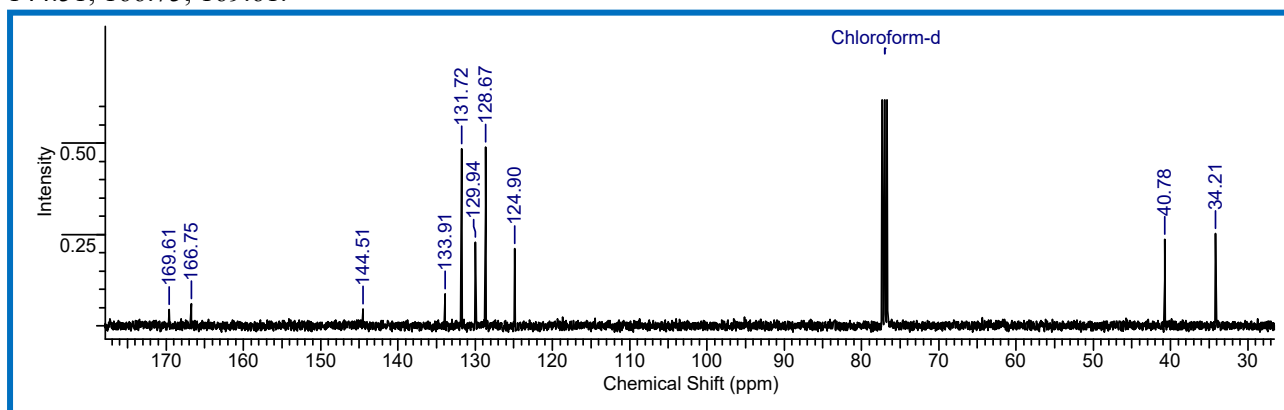
GC-MS (200 to 250 °C, 10 °C/min; Retention time: 11.278 min):  $m/z$  (%) = 231 (M+1, 15), 230 (M<sup>+</sup>, 100), 229 (18), 203 (10), 202 (63), 201 (23), 174 (12), 142 (15), 116 (72), 115 (11), 90 (9), 89 (30), 86 (13), 63 (10), 60 (11).



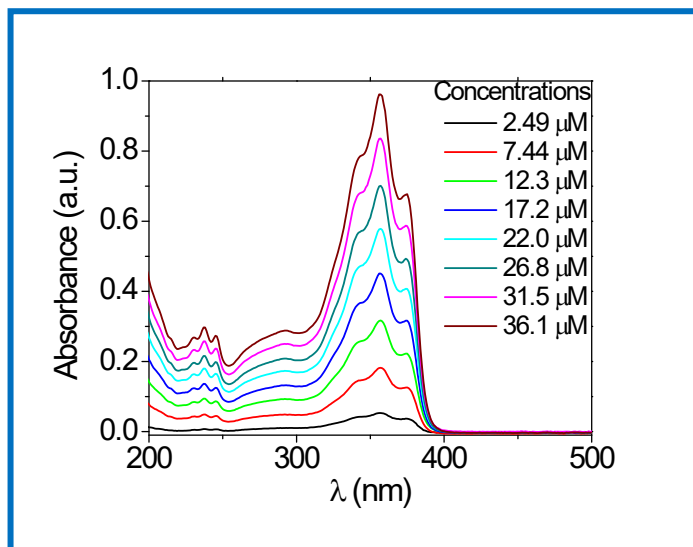
<sup>1</sup>H-NMR (400.16 MHz, CDCl<sub>3</sub>, 22°C)  $\delta$  (ppm): 3.95 (t,  $J=7.07$  Hz, 2H); 3.75 (t,  $J=7.07$  Hz, 2H); 6.91 (s, 1H); 7.37 (m, 3H); 8.05 (d;  $J=8.50$  Hz, 2H).



<sup>13</sup>C-NMR (100.56 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 34.21; 40.78; 124.90; 128.67 (2C); 129.94; 131.72 (2C); 133.51; 144.51; 166.75; 169.61.

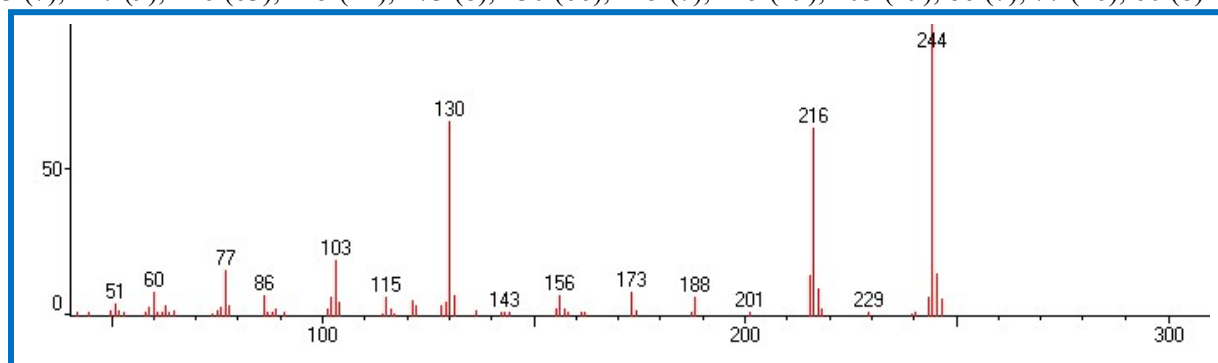


UV-Vis (ACN):  $\epsilon_{356} = 2.70 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .

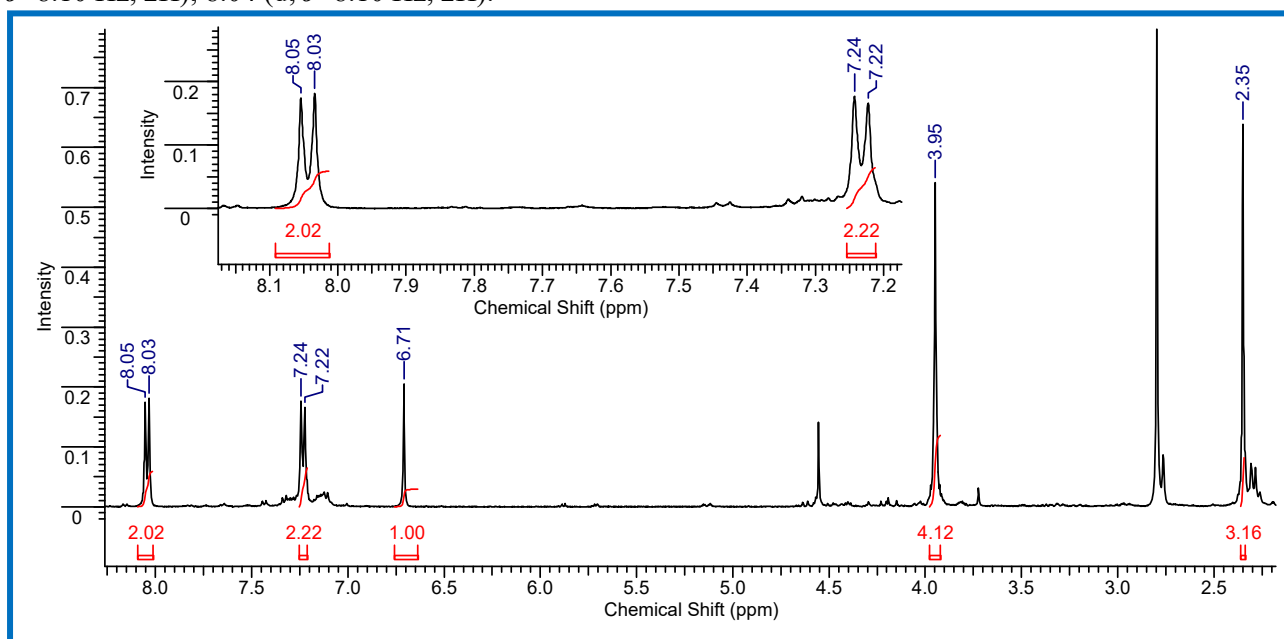


**Compound 2b.** 6-(4-methylbenzylidene)-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one

GC-MS (100 to 250  $^{\circ}\text{C}$ , 15  $^{\circ}\text{C}/\text{min}$ ; Retention time: 19,800 min):  $m/z$  (%) = 245 ( $\text{M}+1$ , 15), 244 ( $\text{M}^+$ , 100), 243 (7), 217 (9), 216 (63), 215 (14), 173 (8), 130 (66), 115 (7), 115 (19), 103 (19), 86 (7), 77 (16), 60 (8).

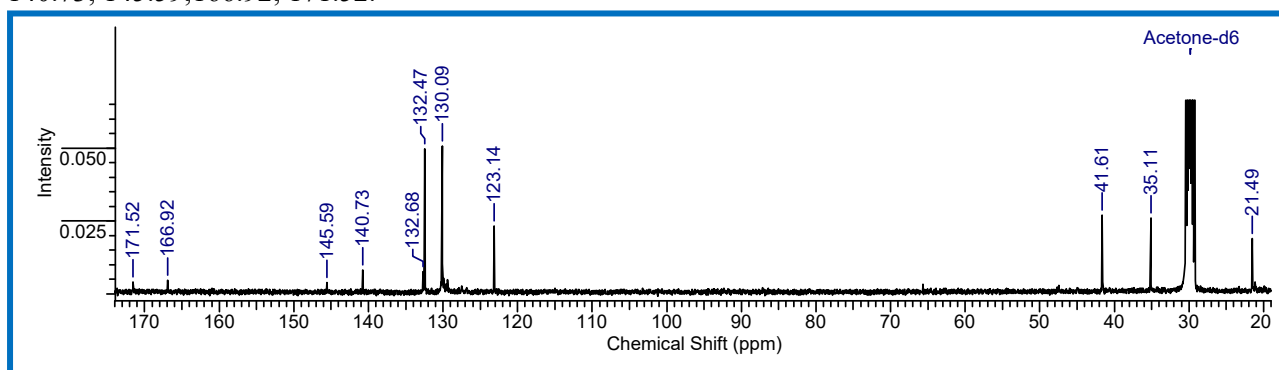


$^1\text{H-NMR}$  (400.16 Mhz, acetone- $d_6$ , 22 $^{\circ}\text{C}$ )  $\delta$  (ppm): 2.35 (s, 3H); 3.95 (s, 4H); 6.71 (s, 1H); 7.23 (d,  $J=8.10$  Hz, 2H); 8.04 (d;  $J=8.10$  Hz, 2H).

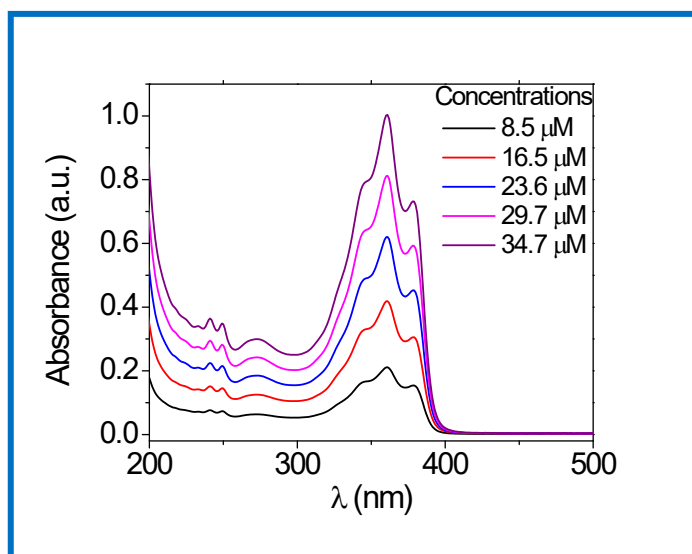




$^{13}\text{C}$ -NMR (100.56 MHz, acetone- $d_6$ )  $\delta$  (ppm): 21.48; 35.11; 41.61; 123.14; 130.09 (2C); 132.47 (2C); 132.68; 140.73; 145.59; 166.92; 171.52.

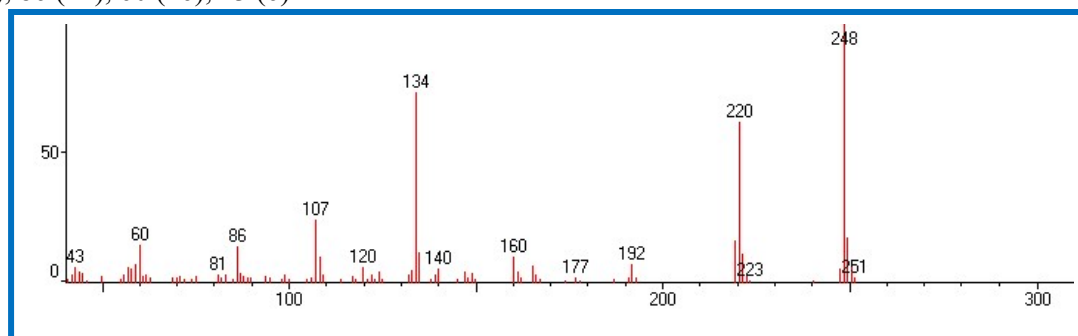


UV-Vis (ACN):  $\epsilon_{361} = 2.99 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .

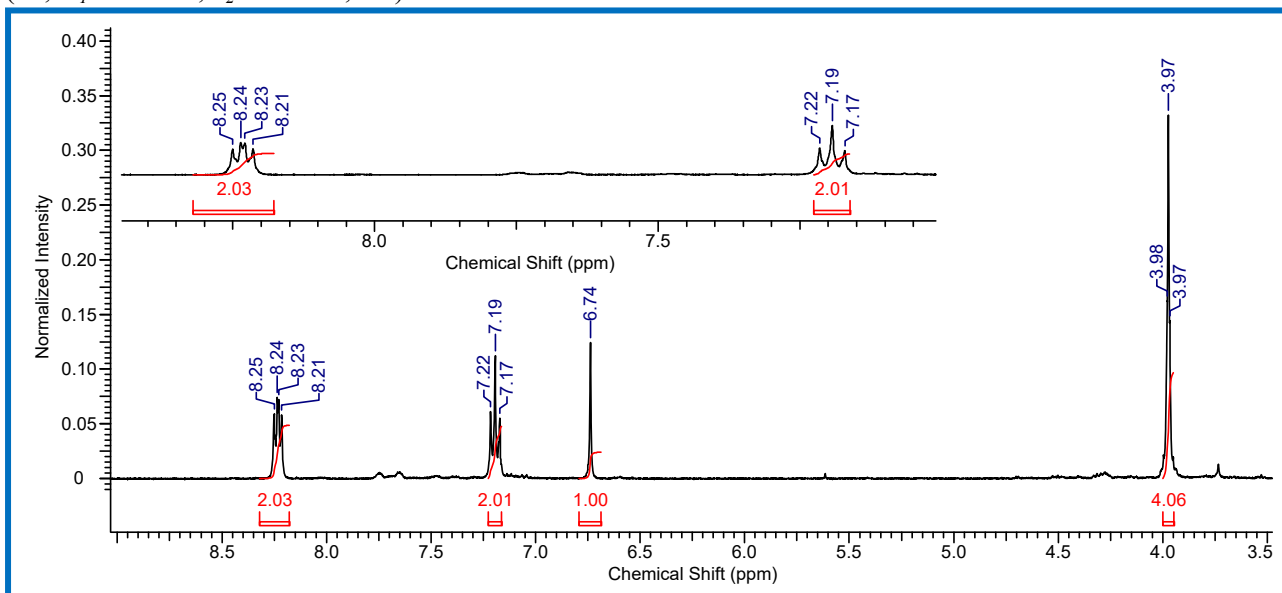


**Compound 2c.** 6-(4-fluorobenzylidene)-2,3-dihydroimidazo[2,1-*b*]thiazol-5(6*H*)-one.

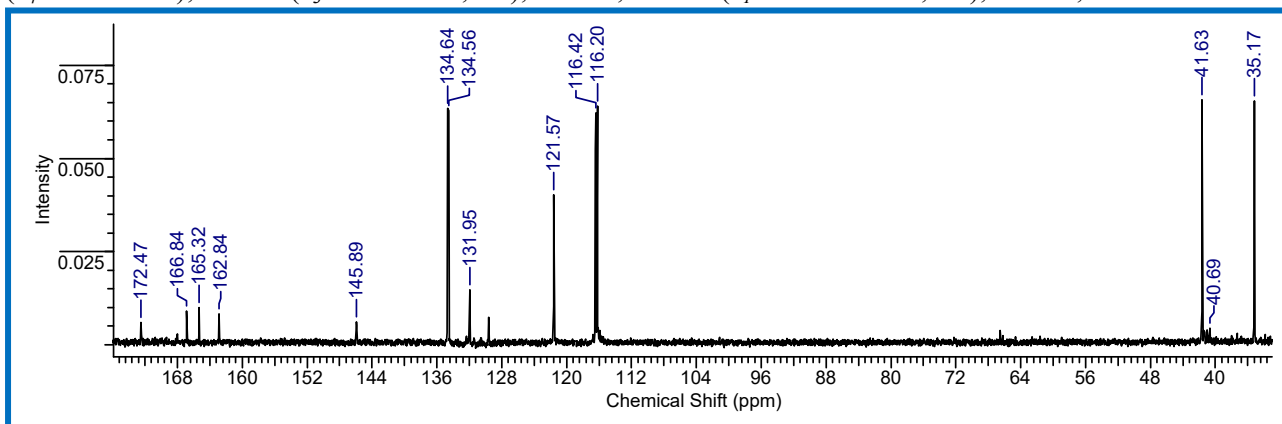
GC-MS (150 to 250 °C, 15 °C/min; Retention time: 14.315 min):  $m/z$  (%) = 250 ( $M+2$ , 7), 249 ( $M+1$ , 17), 248 ( $M^+$ , 100), 221 (11), 220 (62), 219 (16), 165 (6), 160 (10), 140 (6), 135 (11), 134 (73), 108 (10), 86 (7), 107 (24), 86 (14), 60 (16), 43 (6).



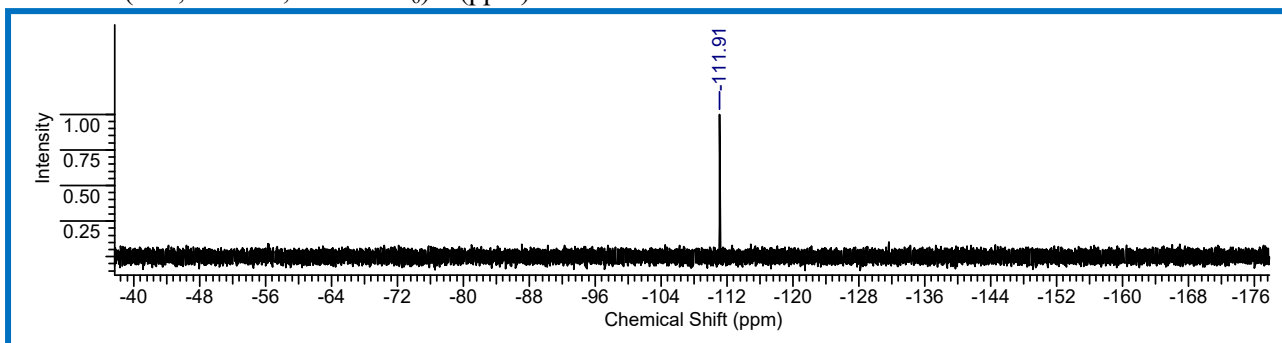
$^1\text{H-NMR}$  (400.16 MHz, acetone- $d_6$ , 22°C)  $\delta$  (ppm): 3.97 (s, 4H); 6.74 (s, 1H); 7.19 (t,  $J_1=8.86$  Hz, 2H); 8.24 (dd,  $J_1=8.86$  Hz,  $J_2=5.77$  Hz, 2H).



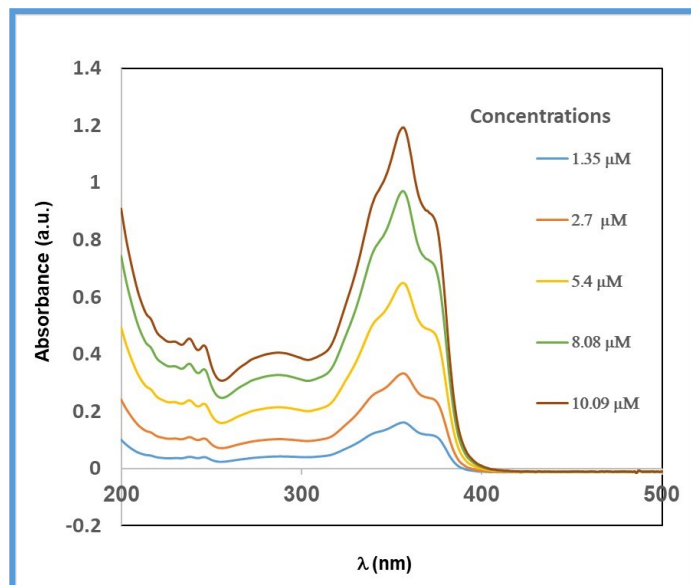
$^{13}\text{C-NMR}$  (100.56 MHz, acetone- $d_6$ )  $\delta$  (ppm): 35.17; 41.63; 164.08 ( $J_2^{-F}=21.88$  Hz, 2C); 121.57; 131.99 ( $J_4^{C-F}=3.70$  Hz); 134.47 ( $J_3^{C-F}=8.42$  Hz, 2C); 145.89; 164.08 ( $J_1^{C-F}=249.76$  Hz, 2C); 166.84; 172.47.



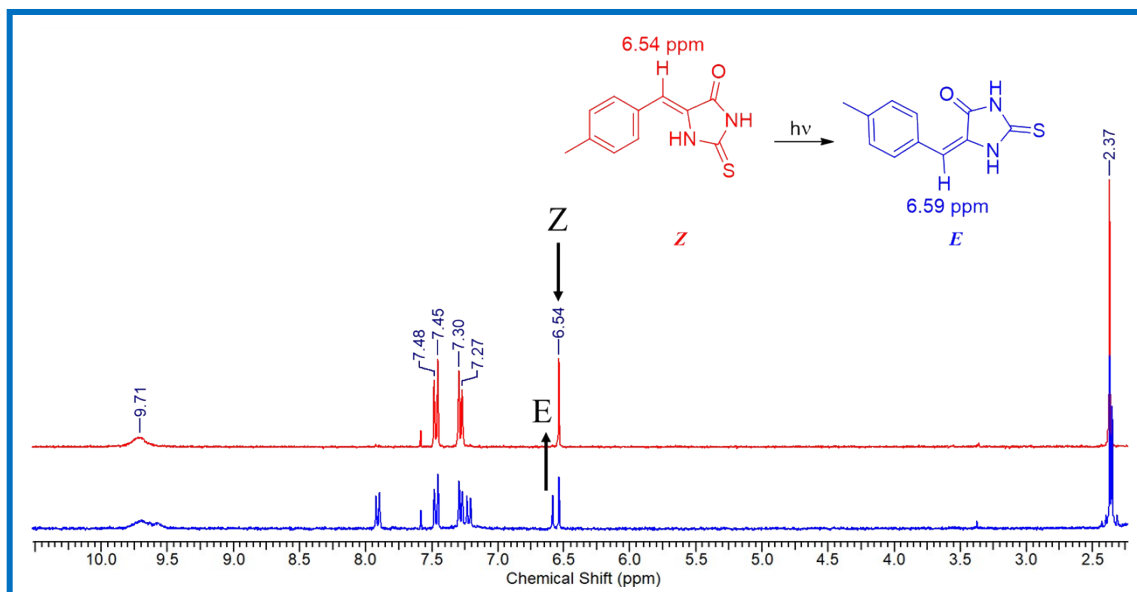
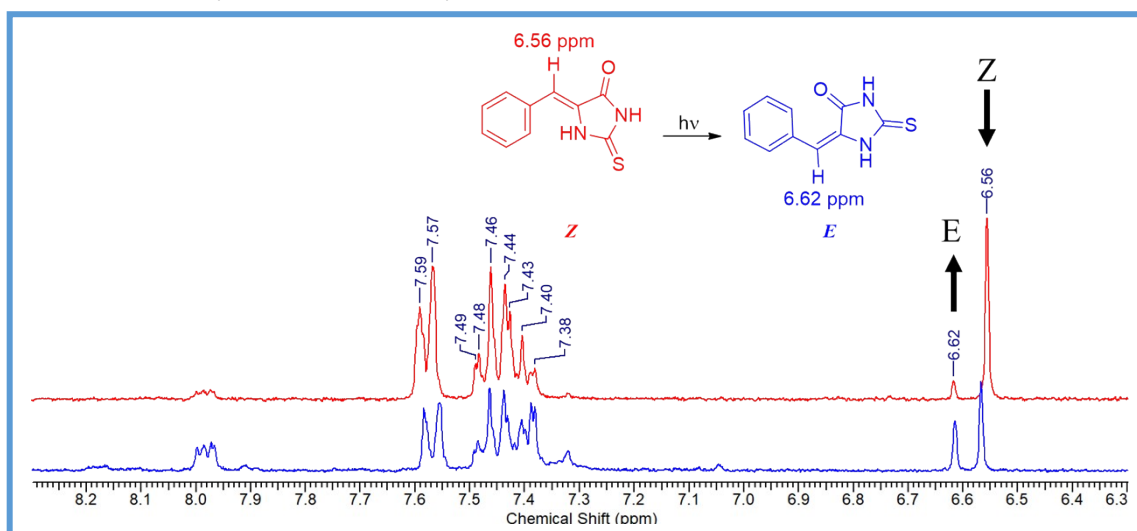
$^{19}\text{F-NMR}$  (376.53 MHz; acetone- $d_6$ )  $\delta$  (ppm): -111.91.

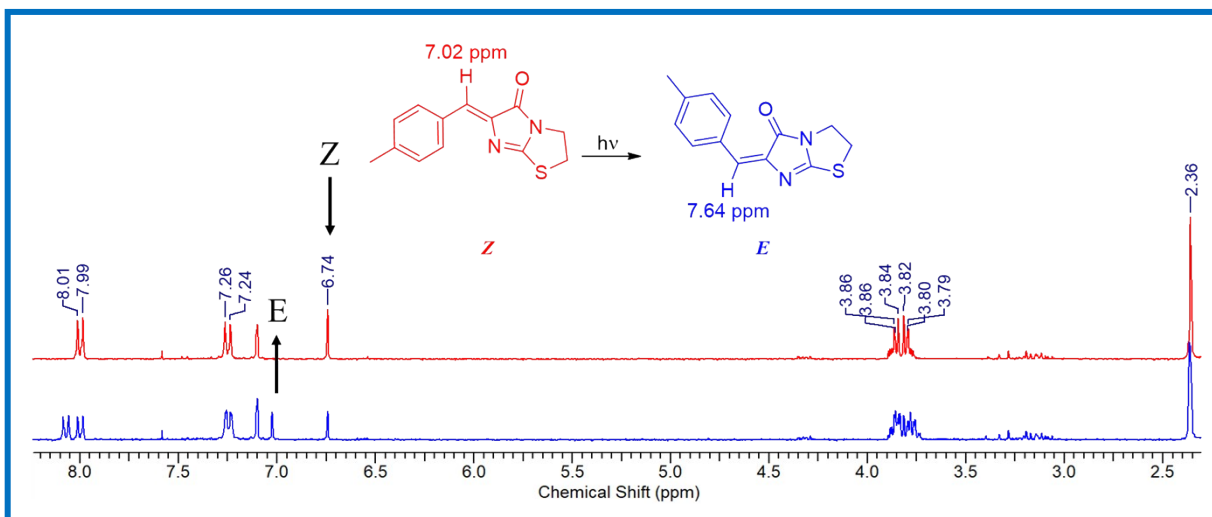
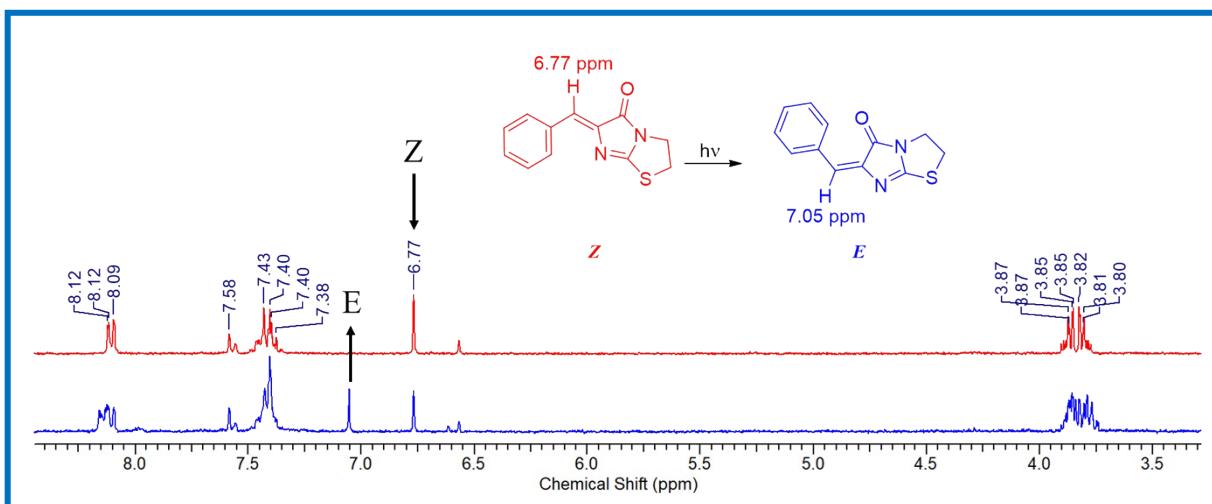
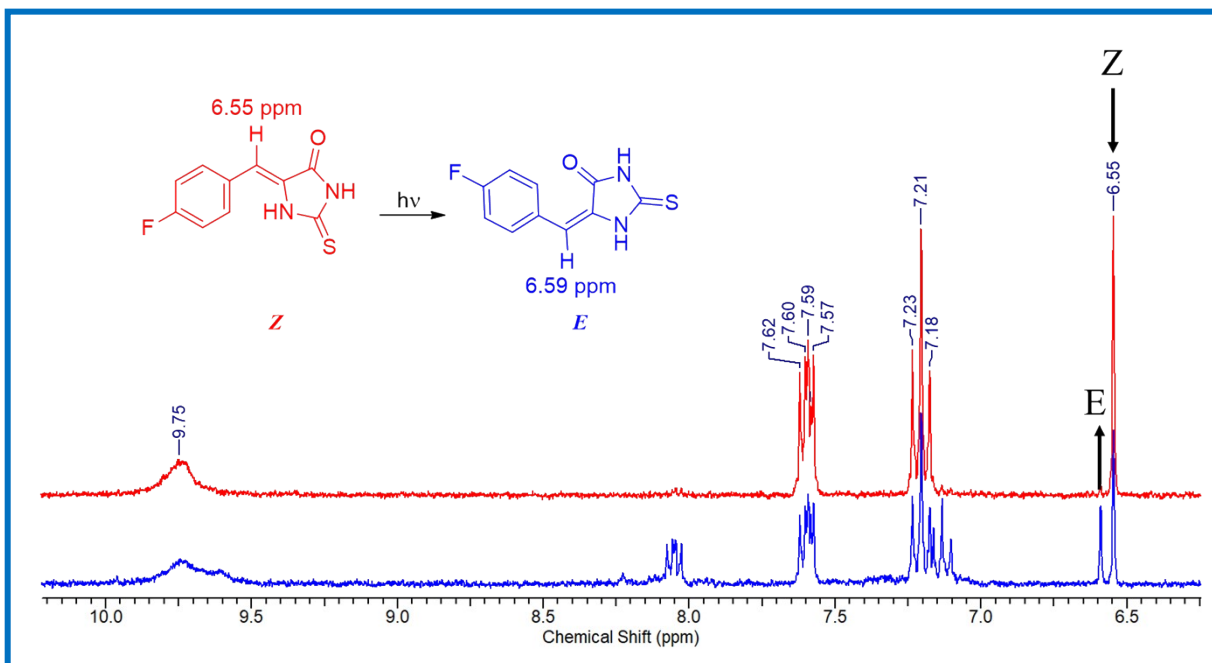


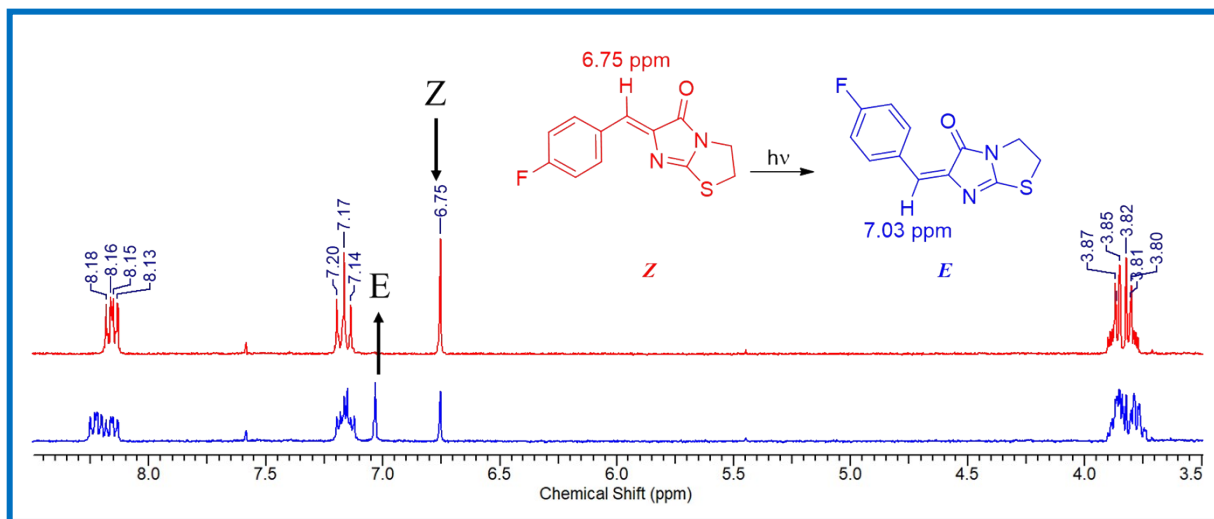
UV-Vis (ACN):  $\epsilon_{357} = 11.76 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .



## 5. $^1\text{H-NMR}$ spectra in the photoisomerization







## 6. Thermal reversion

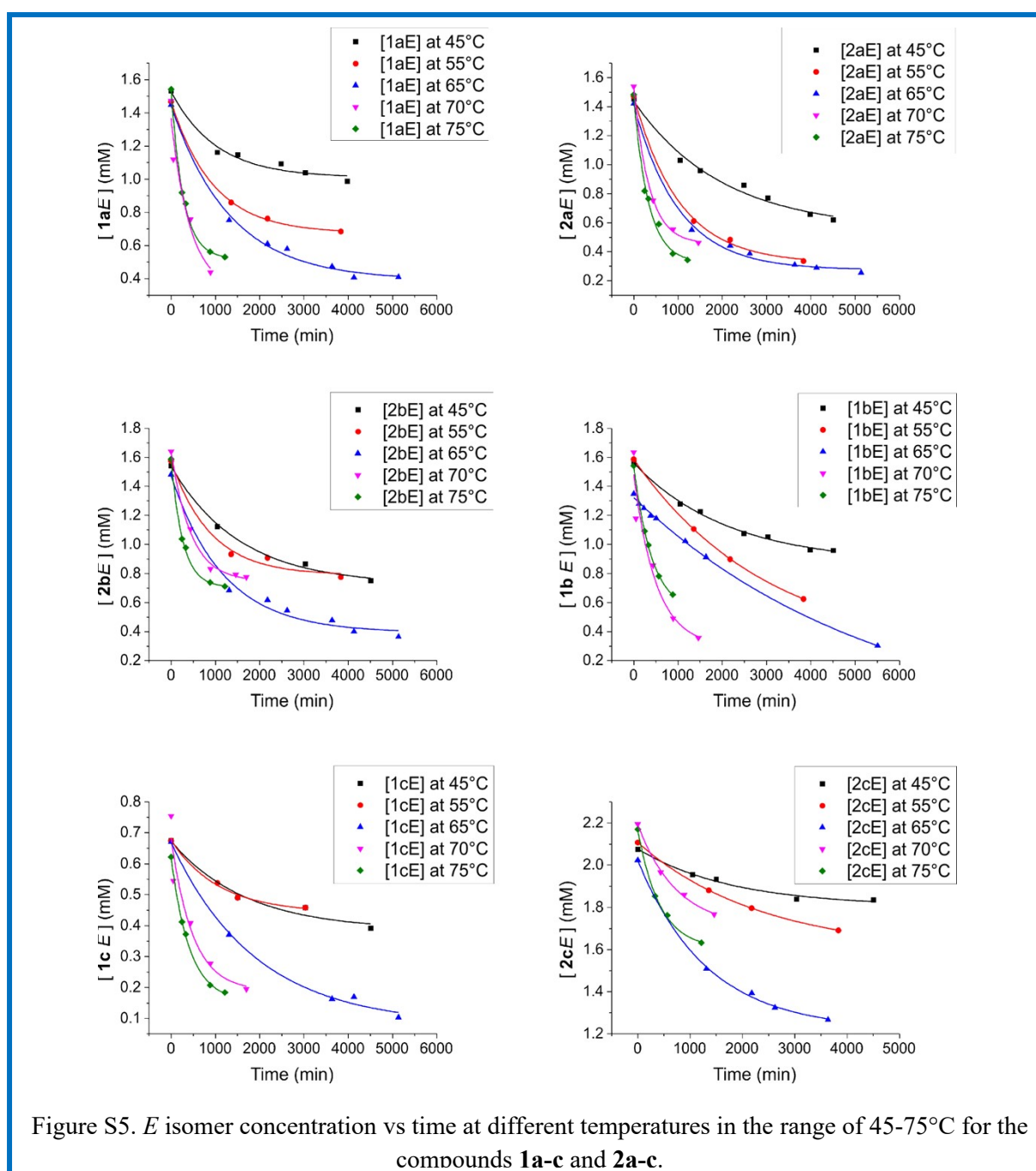


Figure S5. *E* isomer concentration vs time at different temperatures in the range of 45-75°C for the compounds **1a-c** and **2a-c**.

## 7. Equations employed in the data analysis

Eq. 1 Exponential decay  $[E] = [E]_0 e^{-k t}$

Eq. 2 Arrhenius equation  $\ln(k) = \ln(A) - \frac{Ea}{RT}$

Eq. 3 Activation entropy  $\Delta S^\ddagger = 4.575 \log A - \dots$

Eq. 4 Activation enthalpy  $\Delta H^\ddagger = Ea - RT$

Eq. 5 Free energy of activation  $\Delta G^\ddagger = \Delta H^\ddagger - T \Delta S^\ddagger$

## 8. Energy from B3LYP/6-311+G(d,p) and CAS(6/5)/6-31g(d)

DFT (6,311+G(d,p)) IN ACETONITRILE

Compound	E isomer hartree	Z isomer hartree	energia-TS-S0	T1 minimum	S0-ISC_I-T1	S0-ISC_II-T1	Z isomer kcalmol <sup>-1</sup>
1a T1-H	- 969.015177100	- 969.018776800	- 968.916338900	- 968.956858000	- 968.956561200	- 968.954272300	- 2.258845555
1b T3-CH3	- 1008.343887000	- 1008.347325000	- 1008.254871000	- 1008.285502000	- 1008.285356000	-1008.283697000	- 2.157377286
1c T4-F	- 1068.284472000	- 1068.287491000	- 1068.185541000	- 1068.225705000	- 1068.225508000	-1068.223061000	- 1.894450851
2a T1c-H	- 1046.421851000	- 1046.427757000	- 1046.344551000	- 1046.364513000	- 1046.364482000	-1046.364261000	- 3.706070463
2b T3c-CH3	- 1085.750419000	- 1085.756151000	- 1085.672138000	- 1085.692886000	- 1085.692769000	-1085.692876000	- 3.596883829
2c T4c-F	- 1145.691324000	- 1145.697150000	- 1145.585871000	- 1145.633557000	- 1145.633470000	-1145.633469000	- 3.655869712

DFT (6,311+G(d,p)) IN ACETONITRILE

Compound	Ea-ST-S0 kcalmol <sup>-1</sup>	DH <sup>‡</sup> -ST-S0	DG <sup>‡</sup> -ST-S0	DS <sup>‡</sup> -ST-S0	T1 minimum kcalmol <sup>-1</sup>	S0 to ISC_I-T1 kcalmol <sup>-1</sup>	S0 to ISC_II-T1 kcalmol <sup>-1</sup>	DH <sup>‡</sup> to ISC_II-T1 kcalmol <sup>-1</sup>	DG <sup>‡</sup> to ISC_II-T1 kcalmol <sup>-1</sup>	DS <sup>‡</sup> to ISC_II-T1 ue
1a T1-H	62.021898689	60.188818257	60.258471799	-0.234000000	36.595782925	36.782027712	38.218333957	36.92077004	35.7429349	3.951
1b T3-CH3	55.858375949	54.568216641	54.934054616	-3.215000000	36.637135794	36.728752165	37.769790244	37.0556846	35.4348278	3.451
1c T4-F	63.974582412	60.275414552	60.914219113	-2.145000000	36.876844381	37.000463731	38.535979211	37.27280281	36.1332458	3.821
2a T1c-H	48.506475924	48611.202849092	48613.210251634	-0.234000000	35.980133461	35.999586252	36.138265828	34.57953250	32.8225062	5.892
2b T3c-CH3	49.122062637	48604.917715032	48607.943565315	-3.215000000	36.102497792	36.175916391	36.108772886	34.5676098	33.0961003	4.935
2c T4c-F	66.172747809	48611.390474400	48613.877921626	-2.145000000	36.249334990	36.303928307	36.304555816	34.80418086	33.0684899	5.819

DFT (6,311+G(d,p)) IN GAS PHASE

Compound	E isomer hartree	Z isomer hartree	energia-TS-S0	Z isomer kcalmol <sup>-1</sup>	Ea-ST-S0 kcalmol <sup>-1</sup>
1a T1-H	- 969.000539700	- 969.004516100	- 968.880713200	- 2.495228342	75.192254041
1b T3-CH3	- 1008.329042000	- 1008.332843000	- 1.008.227882000	- 2.385163195	63.478849994
1c T4-F	- 1068.269247000	- 1068.272245000	- 1.068.149144000	- 1.881273154	75.365760387
2a T1c-H	-1046.408577	-1046.414033	-1046.331316	-3.423691237	48.48200306
2b T3c-CH3	-1085.736932	-1085.742203	-1085.651986	-3.307602	53.30441273
2c T4c-F	-1145.67788	-1145.683226	-1145.565228	-3.354665204	70.69018791

CAS(6/5)/6-31g(d) IN GAS PHASE

Compound	E isomer hartree	Z isomer hartree	energia-TS-S0	Z isomer kcalmol	Ea-ST-S0 kcalmol <sup>-1</sup>
1a T1-H	-964.672365	-964.6633498	-964.5320232	5.657122662	88.06579745
1b T3-CH3	-1003.709886	-1003.700617	-1003.57365	5.816384545	85.48936939
1c T4-F	-1063.508503	-1063.540787	-1063.400176	-20.25851318	67.9762098
2a T1c-H	-1041.54021	-1041.542734	-1041.446759	-1.583833703	58.6413801
2b T3c-CH3	-1080.587877	-1080.580028	-1080.473498	-4.92532121	66.84857542
2c T4c-F	-1140.401756	-1140.4082	- 1140.267043000	-4.043670516	84.53367259

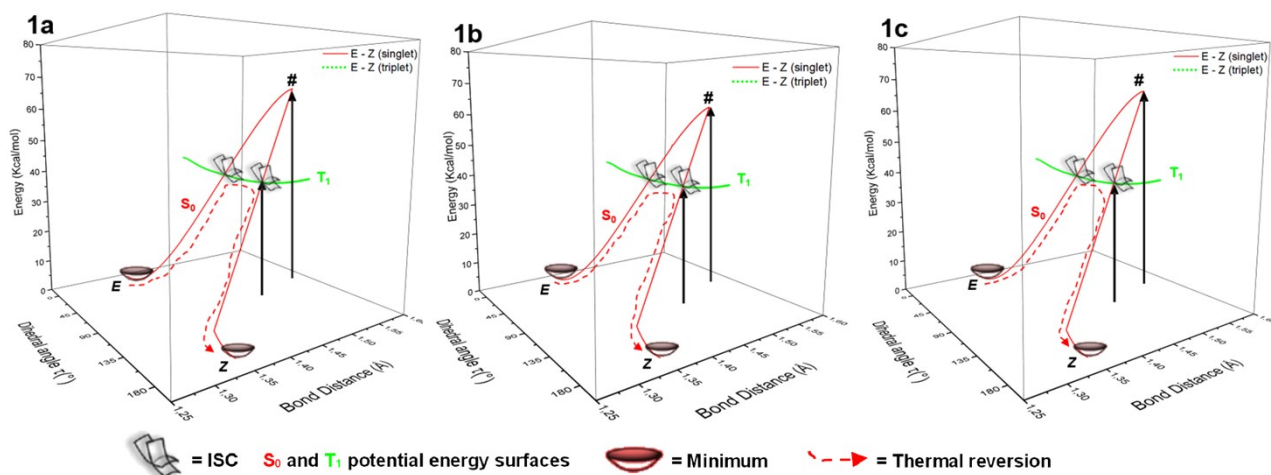


Figure S6. S<sub>0</sub> and T<sub>1</sub> potential energy surfaces (PES) obtained for compounds **1a-c**. B3LYP/6-311+g(d,p) in acetonitrile.

**1a**

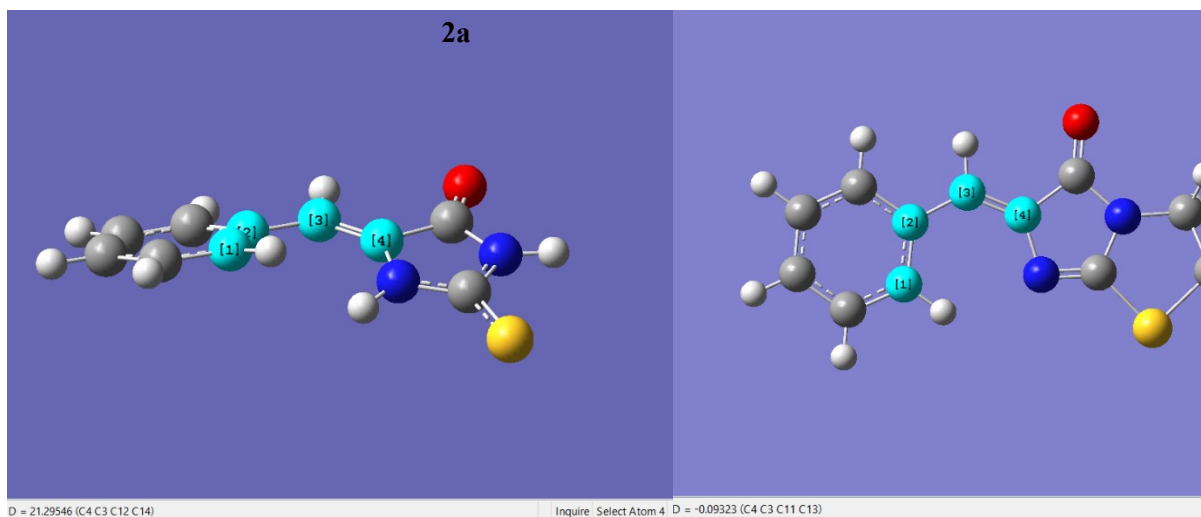


Figure S7. Dihedral angles of the styryl moiety for compounds **1a** and **2a** respectively (B3LYP/6-311+g(d,p)).