

Supporting Information:

Orally Bioavailable Isothioureas Block Function of the Chemokine Receptor CXCR4 In Vitro and In Vivo

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¹H-NMR data for compounds 1

1a: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.78 (s, 1 H), 4.53 (s, 2 H), 4.48 (m, 2 H), 4.35 (m, 2 H), 3.82 (m, 2 H), 1.87-1.05 (m, 20 H); HR-MS [M+H]⁺ observed = 379.1992, estimated = 379.1990.

1b: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.78 (s, 1 H), 4.43 (m, 2 H), 4.32 (m, 2 H), 4.23 (s, 2 H), 4.08 (m, 1 H), 3.71 (m, 1 H), 3.09 (s, 3 H), 1.77-1.05 (m, 20 H); HR-MS [M+H]⁺ observed = 393.2141, estimated = 383.2141.

1c: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.68 (s, 1 H), 4.52 (s, 2 H), 4.34 (m, 2 H), 4.22 (m, 2 H), 4.17 (m, 1 H), 4.02 (m, 1 H), 1.90-1.40 (m, 16 H); HR-MS [M+H]⁺ observed = 351.1677, estimated = 351.1677.

1d: ¹H-NMR CH₃OD δ= 6.50 (s, 1 H), 4.29 (m, 2 H), 4.18 (m, 2 H), 4.10 (s, 2 H), 4.05 (m, 1 H), 3.69 (m, 1 H), 0.98 (d, 12 H, J=7.5 Hz); HR-MS [M+H]⁺ observed = 299.1359, estimated = 299.1359.

1e: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.80 (s, 1 H), 4.81 (s, 2 H), 4.48 (m, 2 H), 4.30 (m, 2 H), 4.10 (m, 1 H), 3.85 (m, 1 H), 1.85-1.32 (m, 24 H); HR-MS [M+H]⁺ observed = 407.2304, estimated = 407.2303.

1f: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.68 (s, 1 H), 4.45 (s, 2 H), 4.40 (m, 2 H), 4.25 (m, 2 H), 4.85 (m, 2 H), 1.80-1.32 (m, 28 H); HR-MS [M+H]⁺ observed = 435.2612, estimated = 435.2616.

1g: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.72 (s, 1 H), 4.50 (s, 2 H), 4.41 (m, 2 H), 4.28 (m, 2 H), 3.88 (m, 1 H), 3.65 (m, 1 H), 1.85-1.00 (m, 22 H); HR-MS [M+H]⁺ observed = 393.2141, estimated = 393.2141.

1h: ¹H-NMR DMSO[d6]/D₂O (10:1) δ= 6.69 (s, 1 H), 4.47 (s, 2 H), 4.40 (m, 2 H), 4.28 (m, 2 H), 3.90 (m, 1 H), 3.67 (m, 1 H), 1.80-1.00 (m, 24 H); HR-MS [M+H]⁺ observed = 407.2308, estimated = 407.2303.

1i: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1), tautomers observed δ = 6.78 (m, 1 H), 4.55 (m, 2 H), 4.50 (m, 2 H), 4.33 (m, 2 H), 4.10 (m, 1 H), 3.75 (m, 1 H), 2.03-1.05 (m, 18 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 365.1828, estimated = 365.1828.

1j: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1), tautomers observed δ = 6.75 (m, 1 H), 4.53 (s, 2 H), 4.42 (m, 2 H), 4.28 (m, 2 H), 3.70-3.26 (m, 3 H), 3.67 (m, 1 H), 1.80-1.00 (m, 18 H), 0.82 (m, 3 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 381.2146, estimated = 381.2147.

1k: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1), tautomers observed δ = 6.73 (s, 1 H), 4.56-4.40 (m, 2 H), 4.42 (m, 2 H), 4.28 (m, 2 H), 3.75 (m, 1 H), 2.08-1.00 (m, 25 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 431.2299, estimated = 431.2301.

1l: $^1\text{H-NMR}$ D_2O δ = 6.70 (s, 1 H), 4.52 (m, 2 H), 4.42 (m, 2 H), 4.35 (s, 2 H), 4.00 (m, 1 H), 3.80 (m, 1 H), 2.05-1.00 (m, 25 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 431.2301, estimated = 431.2301.

1m: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1) δ = 6.75 (s, 1 H), 4.52 (s, 2 H), 4.43 (m, 2 H), 4.28 (m, 2 H), 4.00 (m, 1 H), 3.65 (m, 2 H), 1.85-0.90 (m, 19 H), 0.81 (s, 9 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 435.2612, estimated = 435.2616.

1n: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1) δ = 7.48 (t, 2 H, J =8.5 Hz), 7.41 (t, 1 H, J =8.5 Hz), 7.17 (d, 2 H, J =8.5 Hz), 6.72 (s, 1 H), 4.27 (m, 2 H), 4.16 (m, 2 H), 4.05 (m, 2 H), 3.74 (m, 1 H), 1.95-1.05 (m, 10 H), 0.81 (s, 9 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 373.1516, estimated = 373.1515.

1o: $^1\text{H-NMR}$ D_2O (50°) δ = 7.36 (m, 2 H), 7.28 (m, 1 H), 7.05 (m, 2 H), 6.50 (s, 1 H), 4.70 (s, 2 H), 4.18 (s, 2 H), 4.95 (m, 2 H), 3.86 (m, 2 H), 3.70 (m, 1 H), 2.00-1.10 (m, 10 H), 0.81 (s, 9 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 387.1673, estimated = 387.1672.

1p: $^1\text{H-NMR}$ DMSO[d_6]/ D_2O (10:1) δ = 7.38 (m, 4 H), 7.32 (m, 1 H), 4.46 (s, 2 H), 3.60 (m, 2 H), 1.74-0.95 (m, 20 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 331.2207, estimated = 331.2208.

1q: ^1H -NMR DMSO[d_6]/ D_2O (10:1) δ = 6.92 (s, 1 H), 4.50 (s, 2 H), 3.62 (m, 2 H), 3.52 (s, 3 H), 2.96 (s, 3 H), 1.70-0.97 (m, 20 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 381.2141, estimated = 381.2141.

1r: ^1H -NMR DMSO[d_6]/ D_2O (10:1) δ = 8.10 (d, 1 H, J =8.0 Hz), 7.72 (d, 1 H, J =8.0 Hz), 7.41 (t, 1 H, J =8.0 Hz), 7.38 (t, 1 H, J =8.0 Hz), 7.12 (s, 1 H), 4.93 (s, 2 H), 3.58 (m, 1 H), 3.12 (m, 1 H), 1.80-0.10 (m, 20 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 427.1989, estimated = 427.1990.

1s: ^1H -NMR DMSO[d_6]/ D_2O (10:1) δ = 6.80 (s, 1 H), 4.50 (s, 2 H), 4.18 (m, 2 H), 3.67 (m, 2 H), 4.48 (m, 2 H), 2.08 (m, 2 H), 1.82-1.05 (m, 20 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 393.2141, estimated = 383.2141.

1t: ^1H -NMR DMSO[d_6]/ D_2O (10:1) δ = 6.78 (s, 1 H), 4.42 (m, 2 H), 4.20 (s, 2 H), 3.67 (m, 2 H), 1.80-1.00 (m, 20 H), 1.48 (s, 6 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 407.2303, estimated = 407.2303.

1u: ^1H -NMR DMSO[d_6]/ D_2O (10:1) δ = 6.78 (s, 1 H), 4.52 (m, 2 H), 4.20 (s, 2 H), 3.88 (m, 2 H), 1.85-1.30 (m, 20 H), 1.48 (s, 6 H); HR-MS $[\text{M}+\text{H}]^+$ observed = 435.2613, estimated = 435.2616.

The purity of the compounds was assessed by ¹H-NMR spectroscopy as well as by LC/MS applying two methods. The compounds showed purities > 95%.

HPLC Methods

Method 1: Synergy Fusion RP, 1 x 50 mm, 4µm; mobile phase: (A) water + 3.2 mM ammonium acetate + 0.03% formic acid, (B) CH₃CN + 0.03% formic acid;

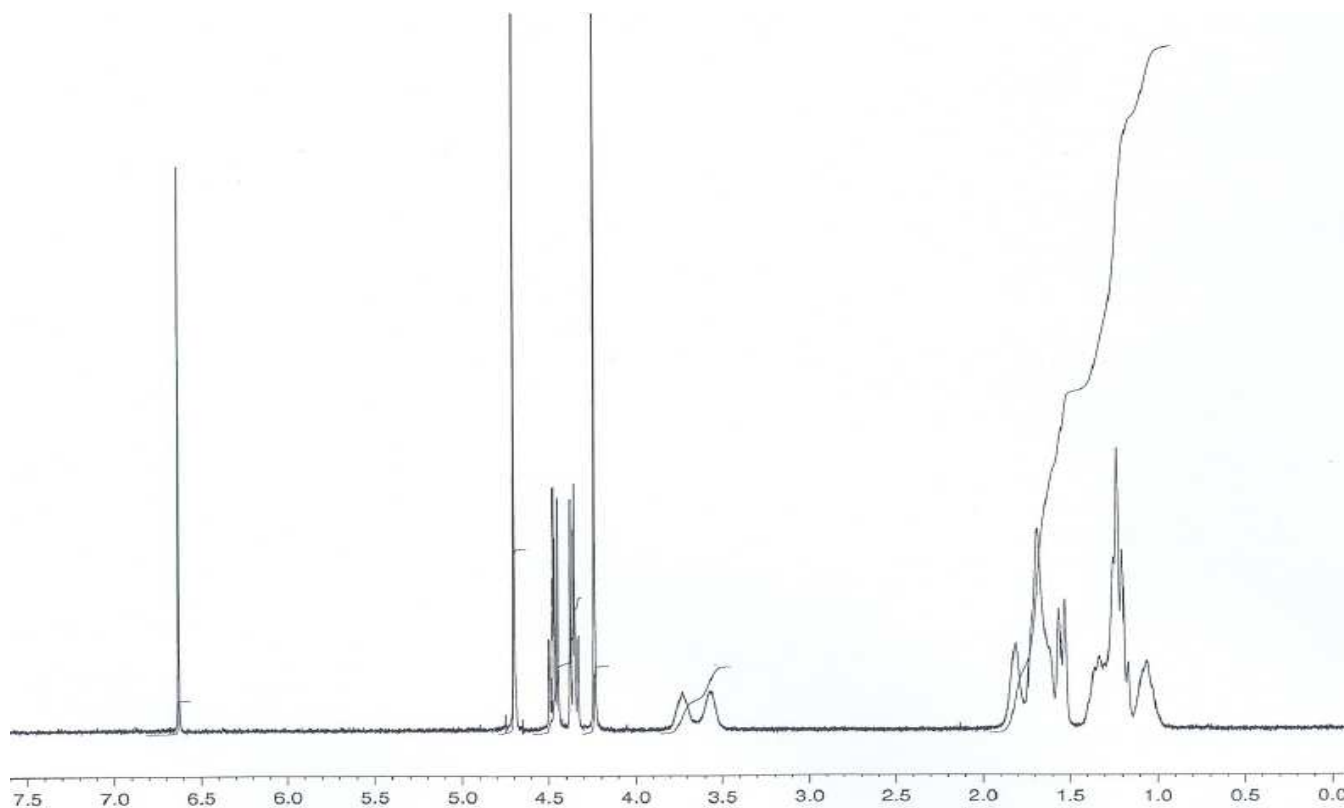
Time [min]	% A	% B	Flow [µL/min]
0	98	2	90
15	2	98	90
20	2	98	90
21	98	2	90
35	2	2	90

Method 2: Xterra-C18 MS, 1 x 150 mm, 3.5 µm; mobile phase: (A) water + 3.2 mM ammonium acetate + 0.03% formic acid, (B) CH₃CN + 0.03% formic acid

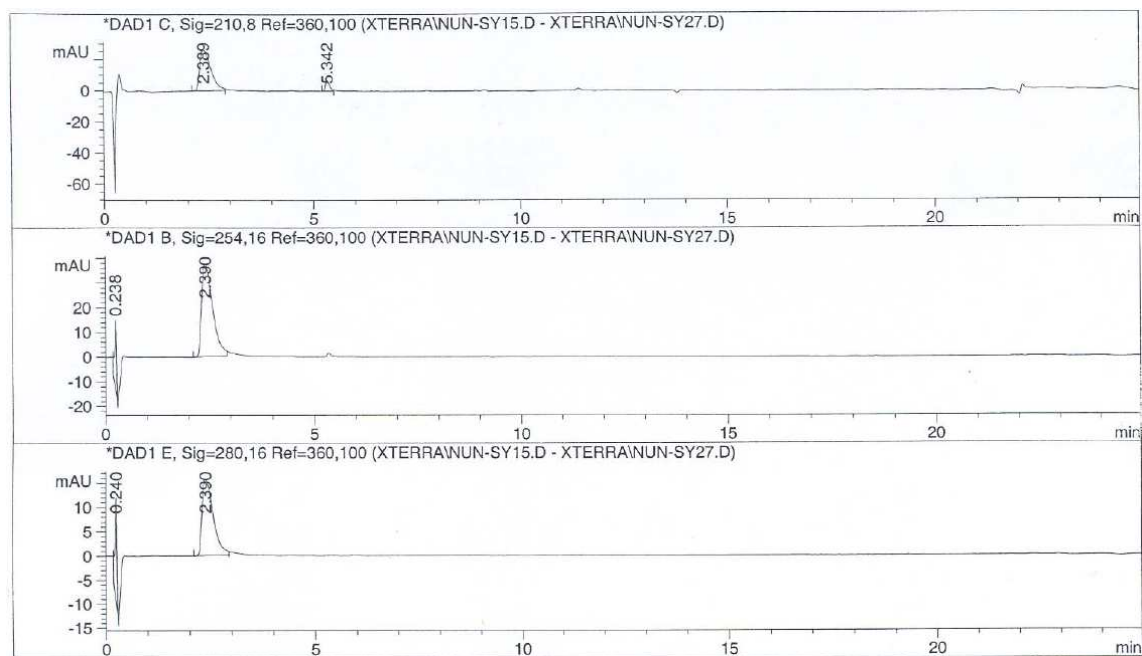
Time [min]	% A	% B	Flow [µL/min]
0	98	2	300
18	2	98	300
20	2	98	300
21	98	2	300
25	2	2	300

Compound 1a

^1H -NMR (D_2O)

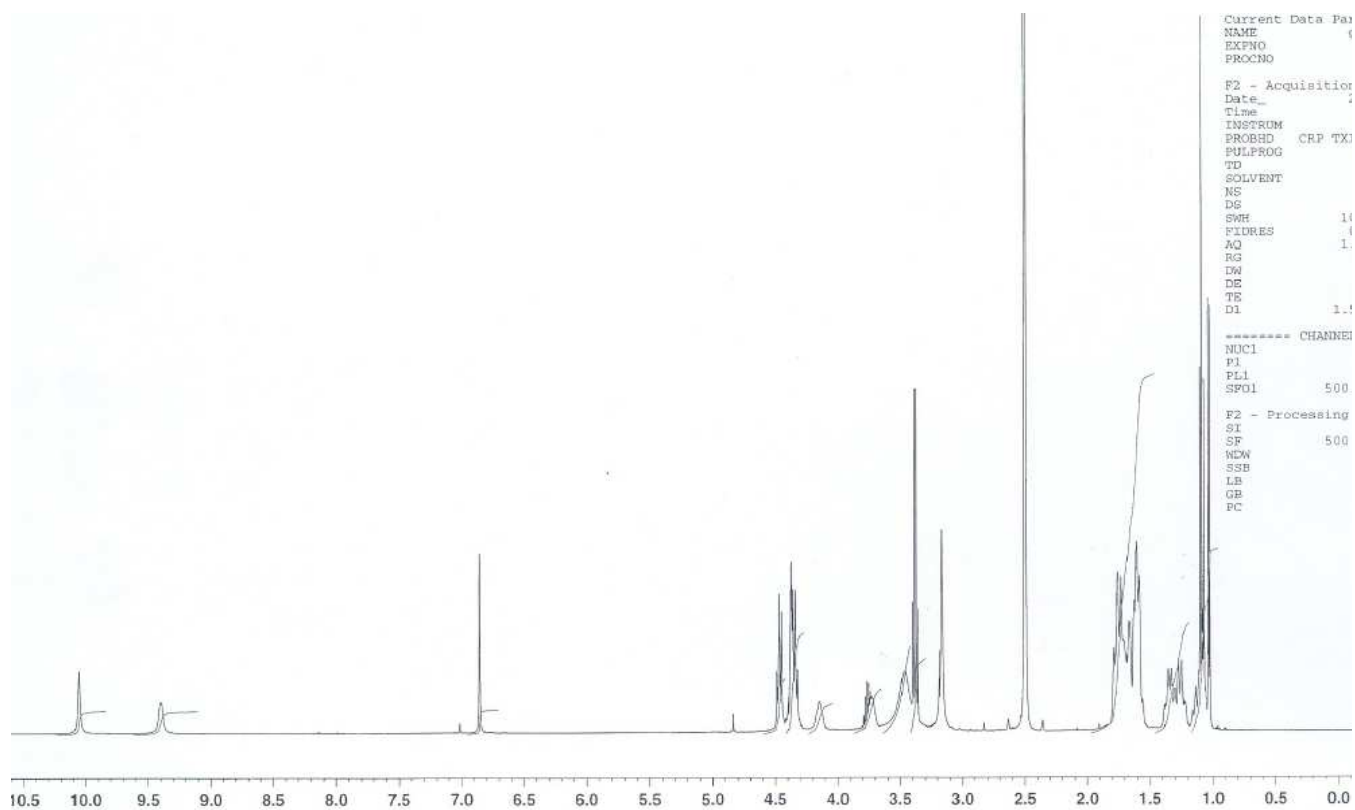


HPLC (Method 1)

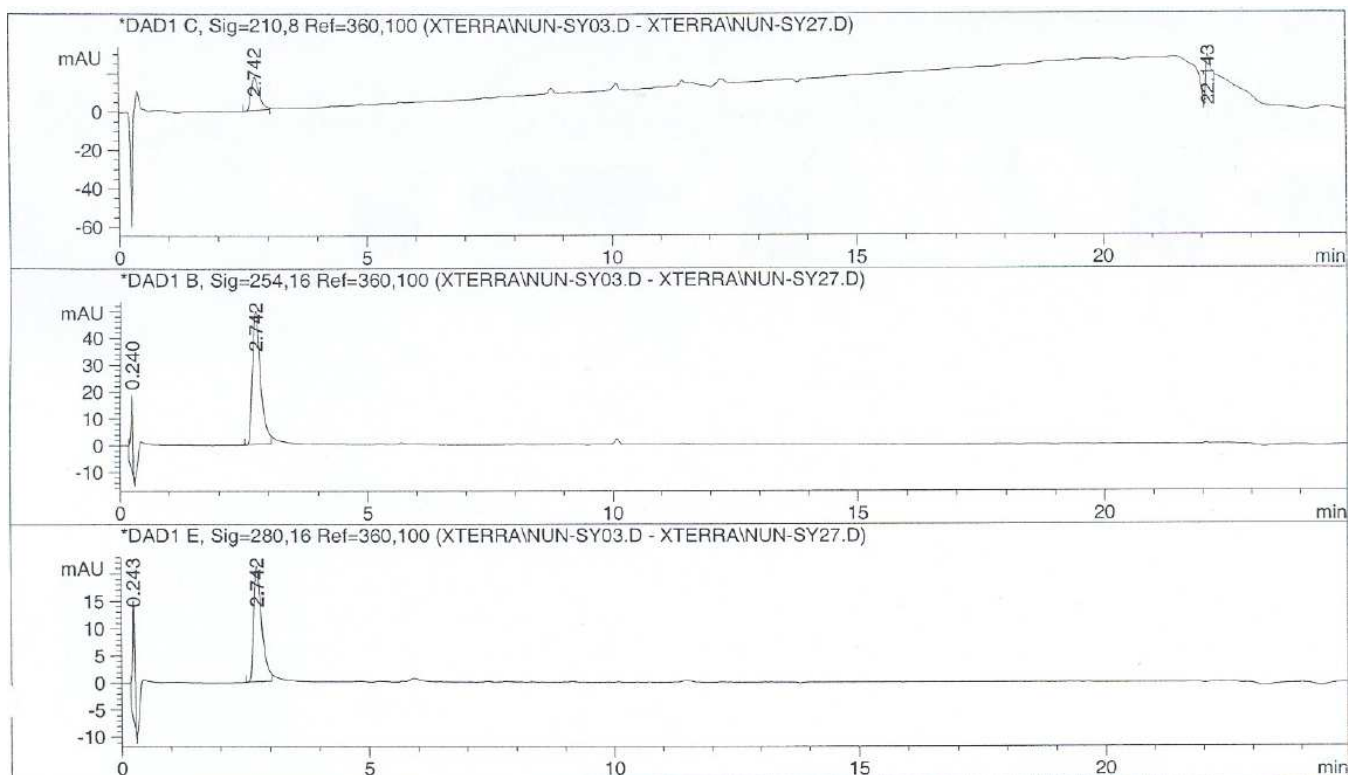


Compound 1b:

$^1\text{H-NMR}$ (DMSO-d_6)

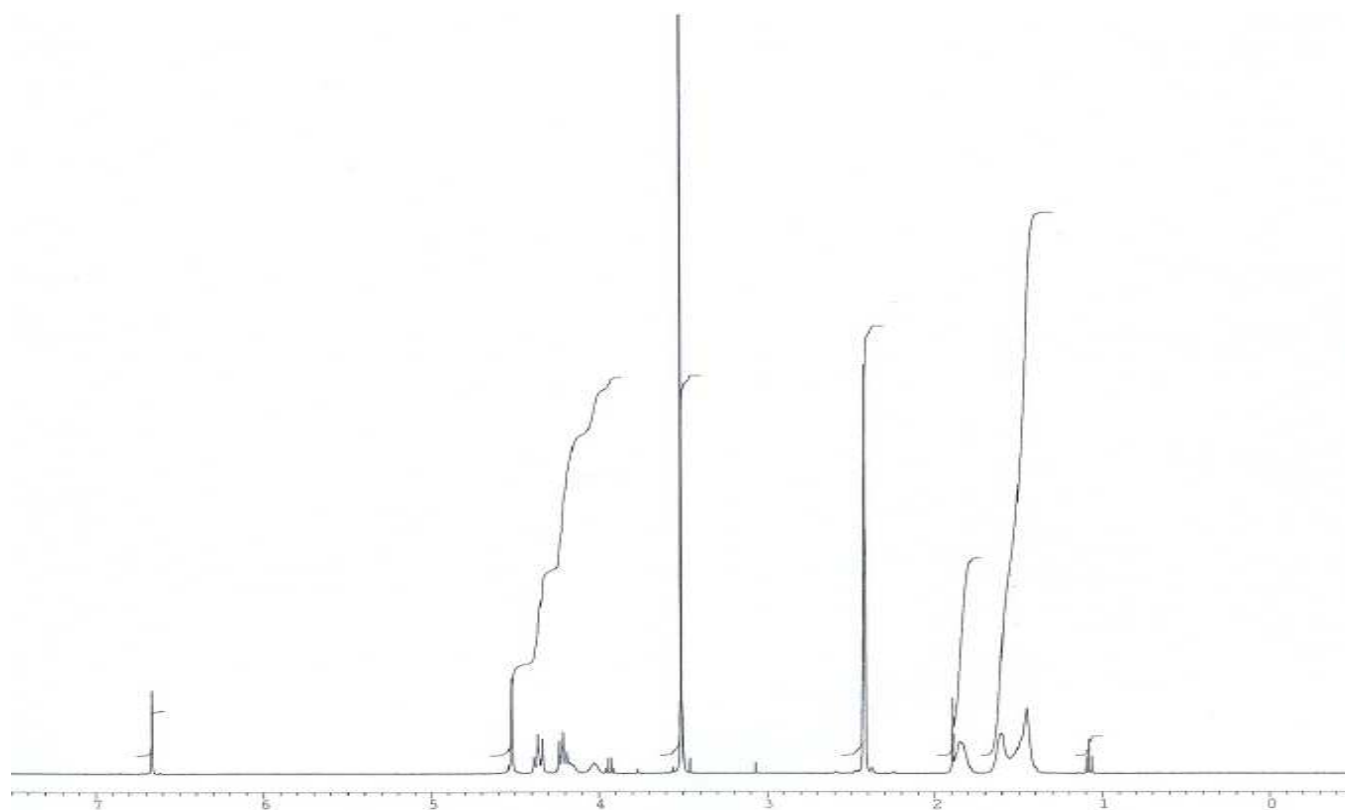


HPLC (Method 1)



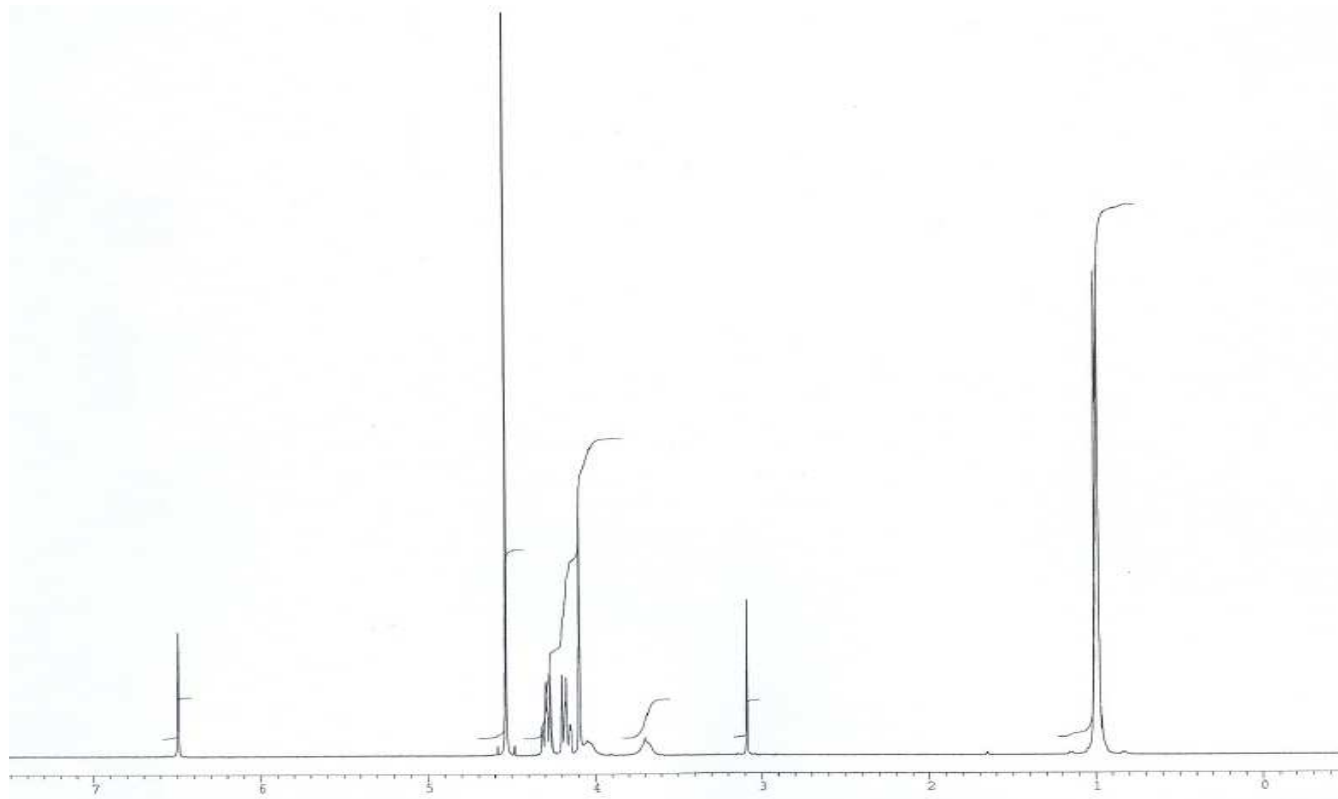
Compound 1c

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

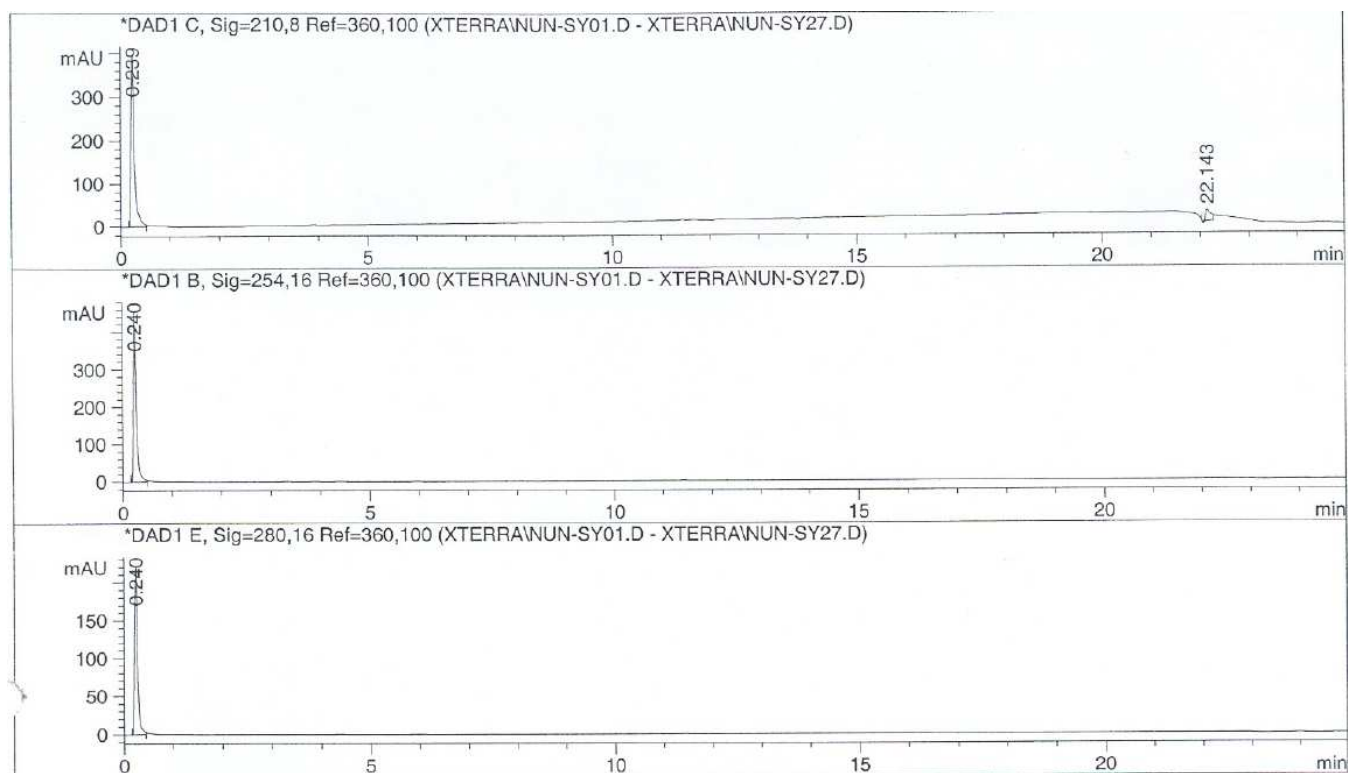


Compound 1d

^1H -NMR (CD_3OD)

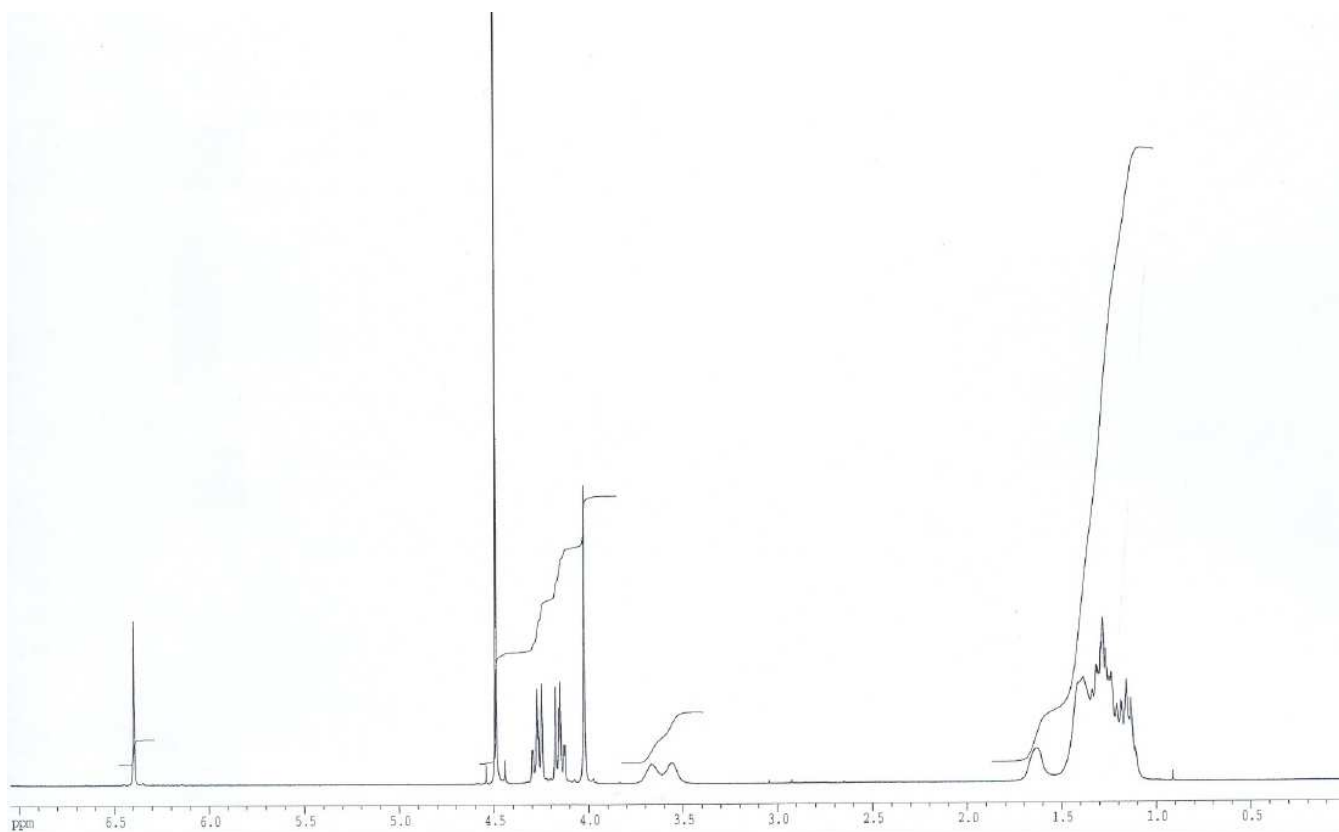


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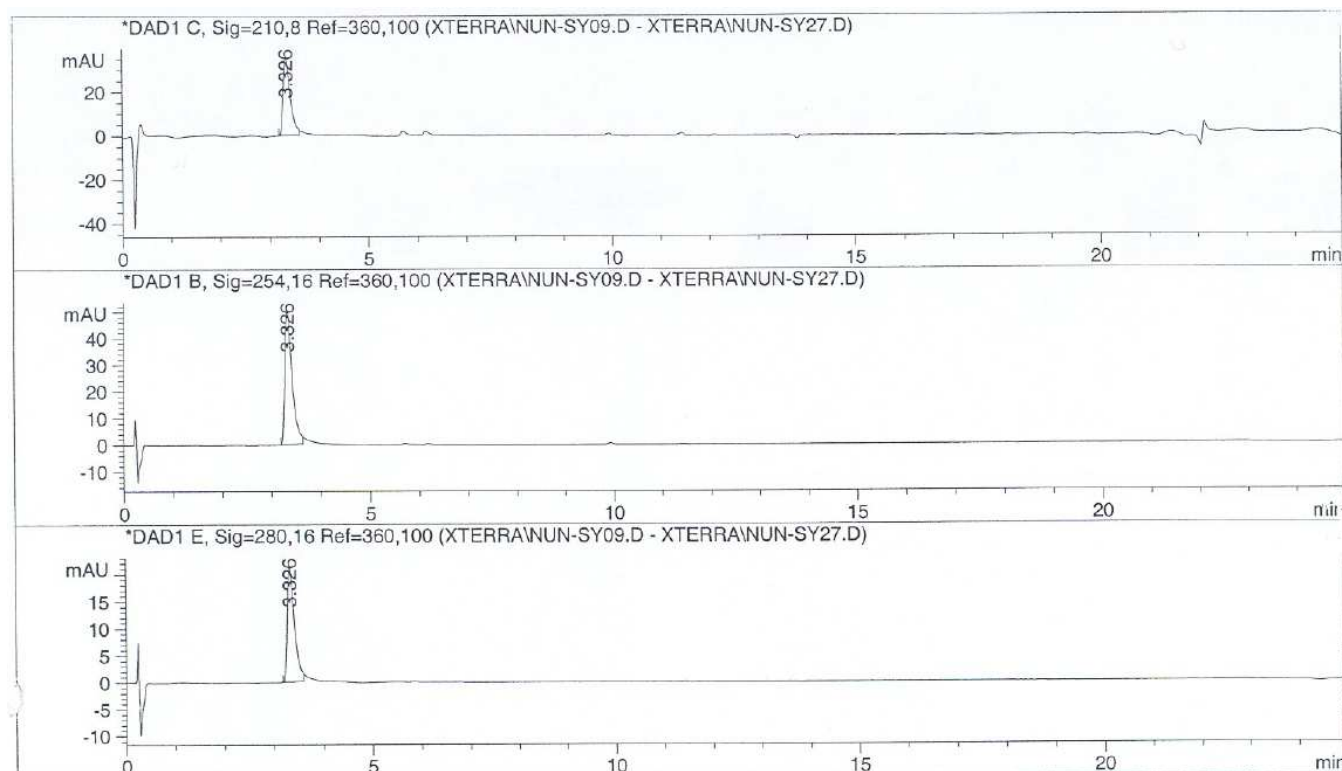


Compound 1e

^1H -NMR (D_2O)

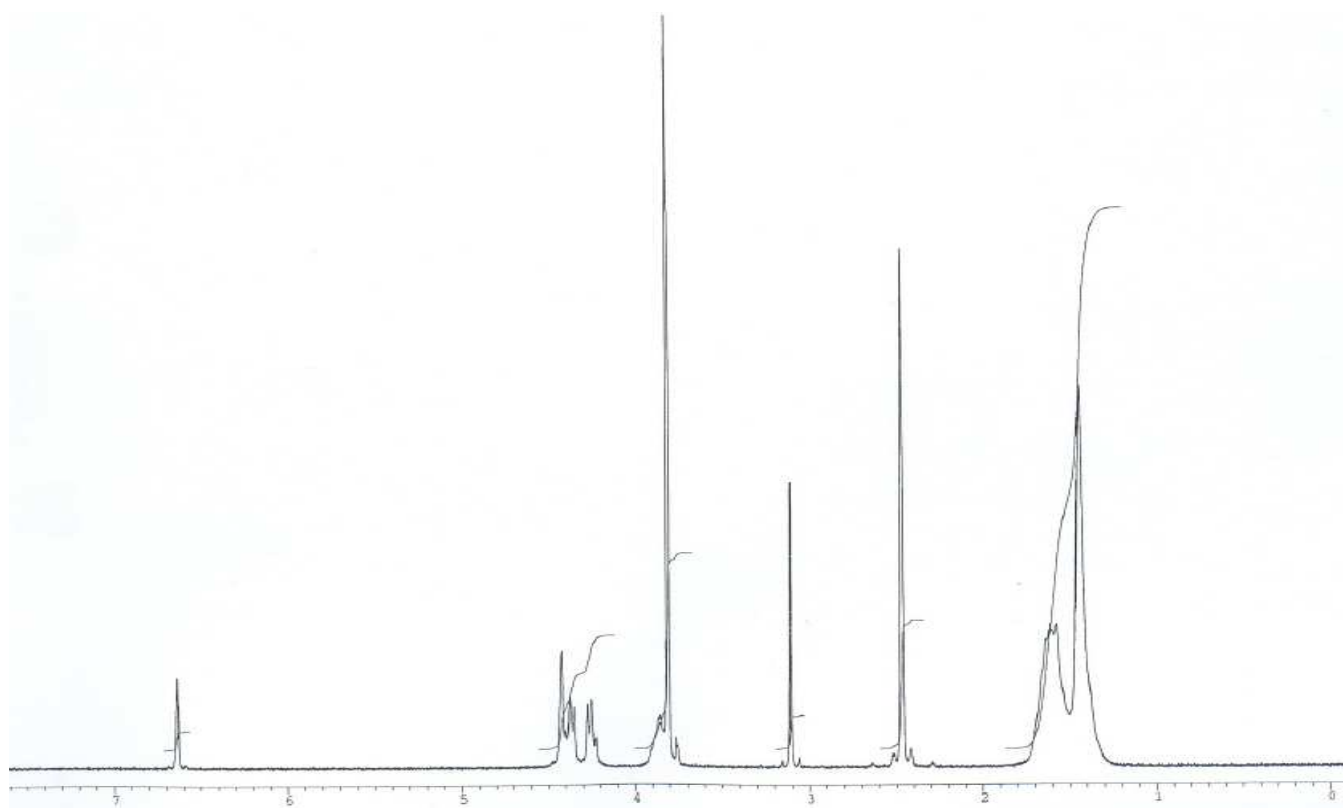


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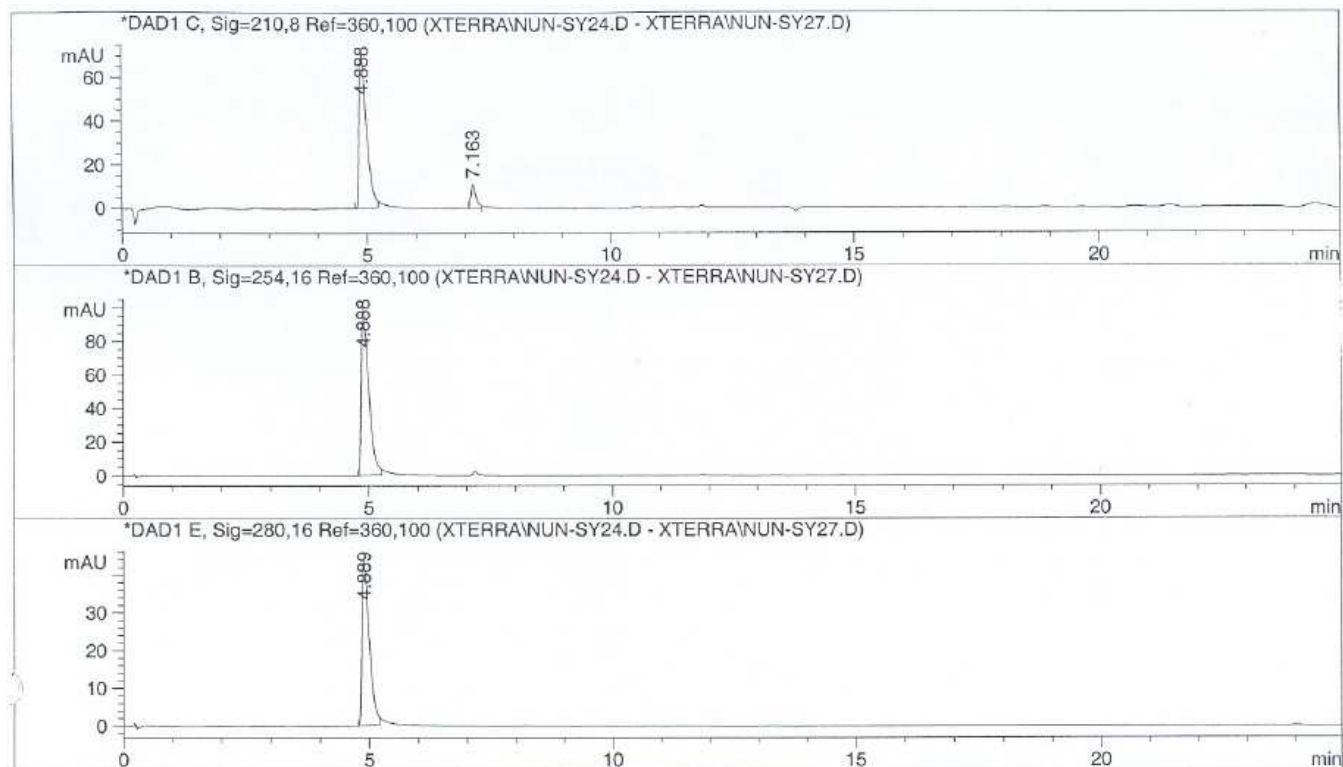


Compound 1f

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

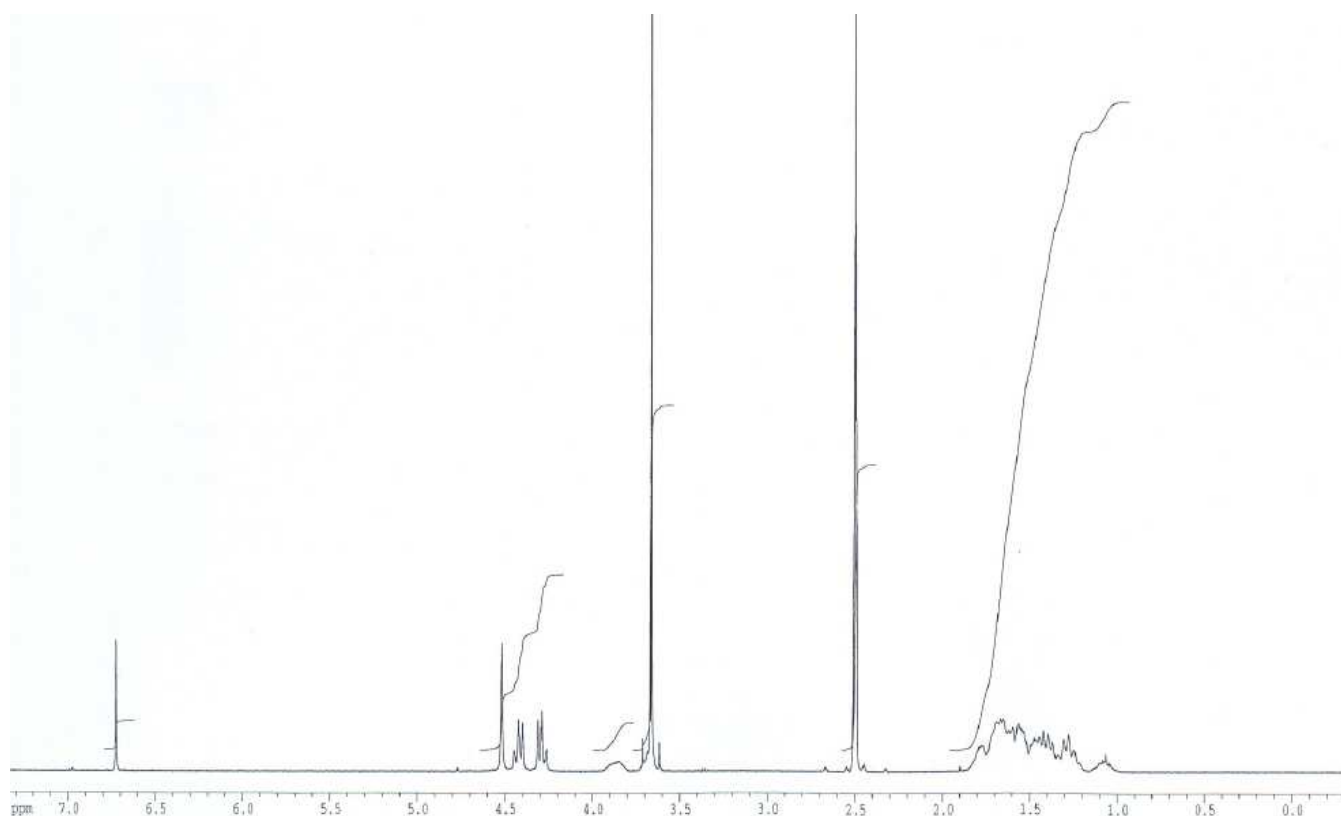


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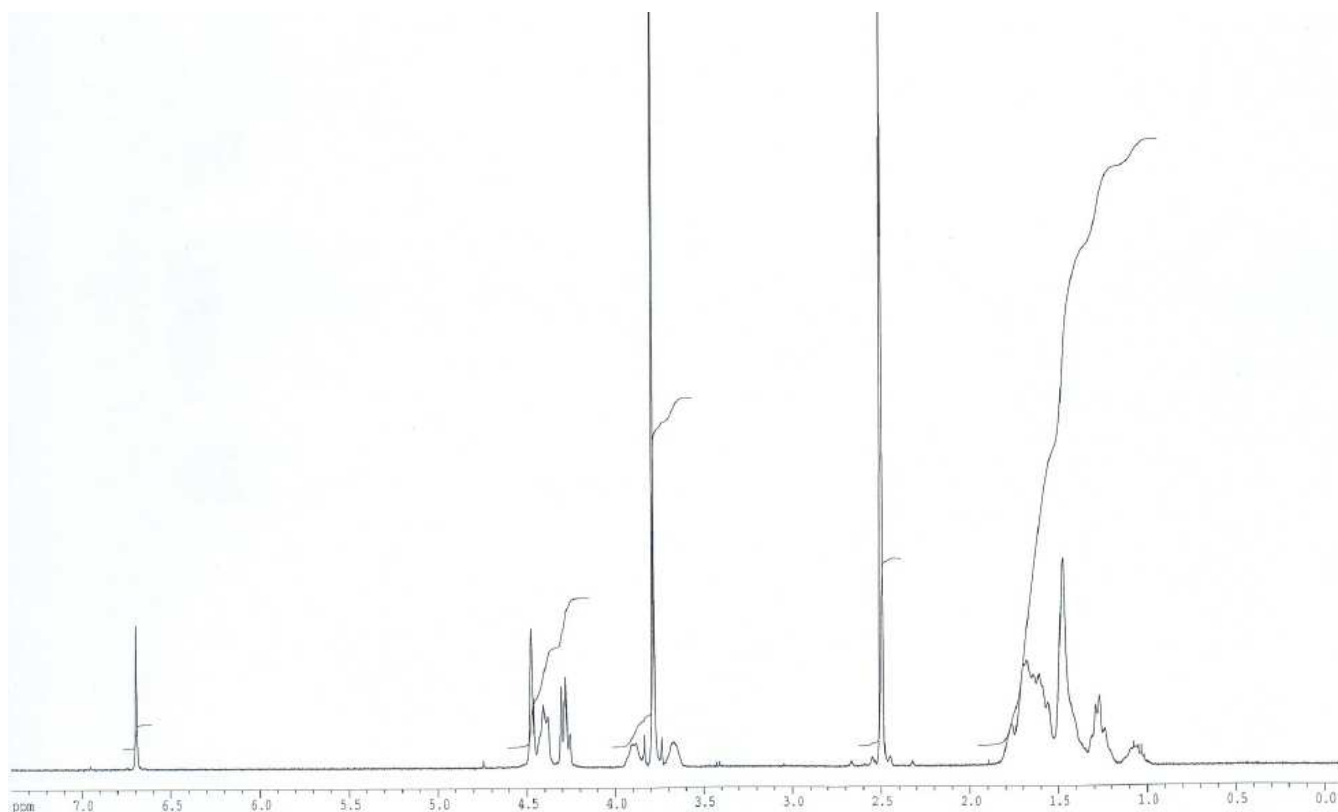
Compound 1g

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

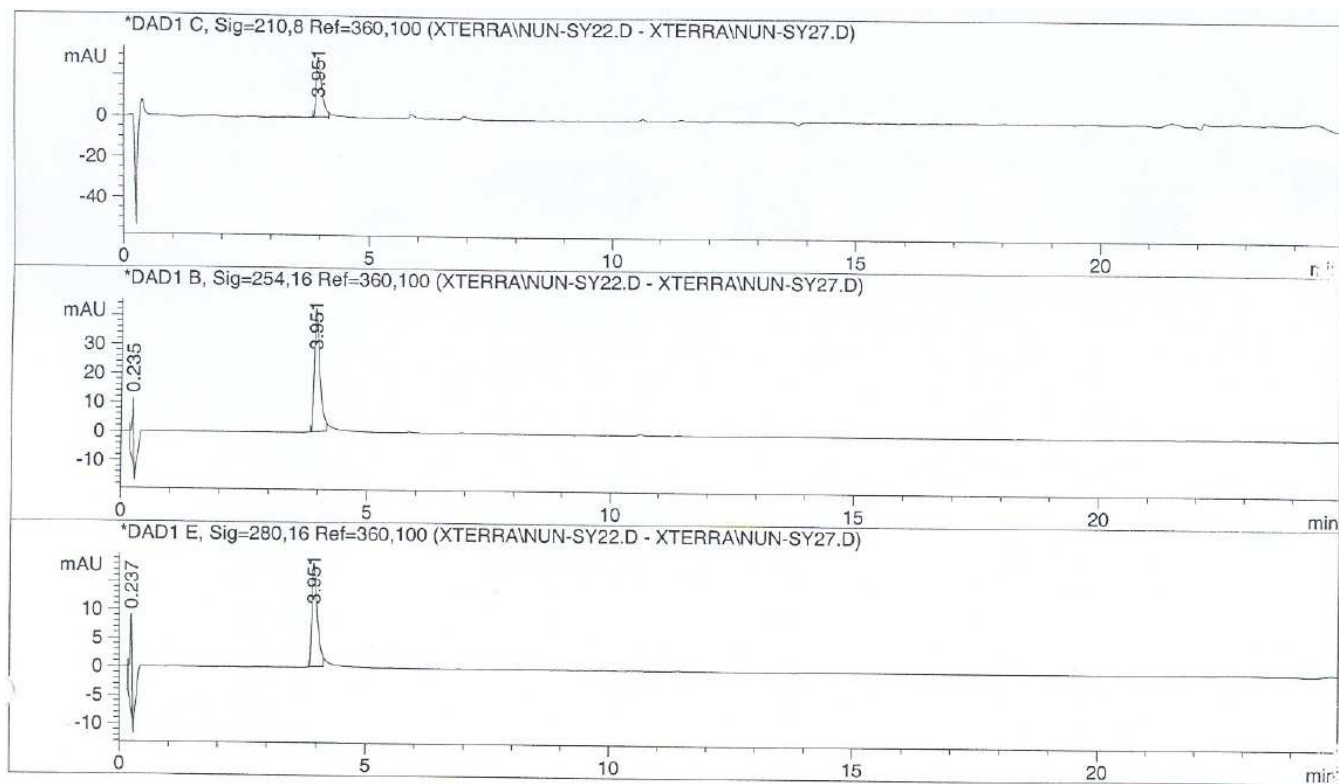


Compound 1h

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

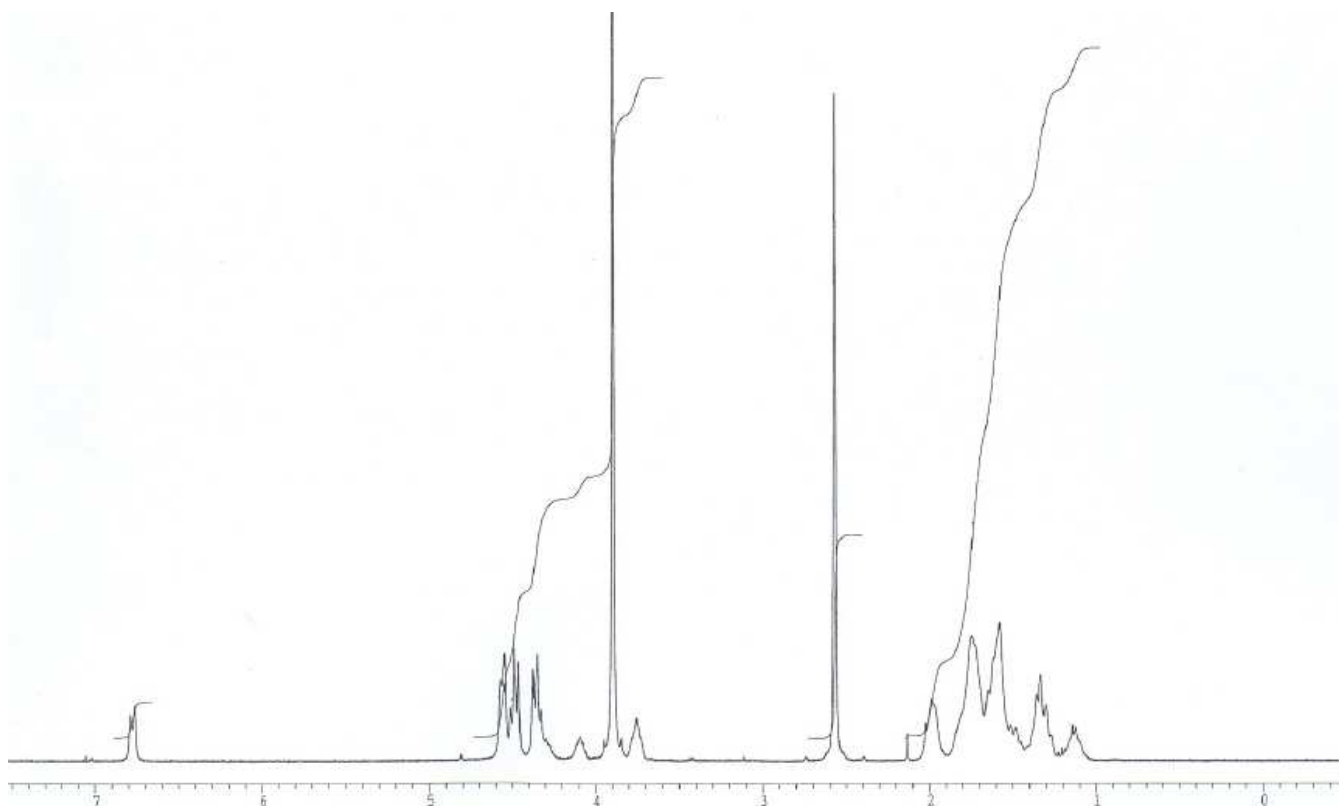


HPLC (Method 1)

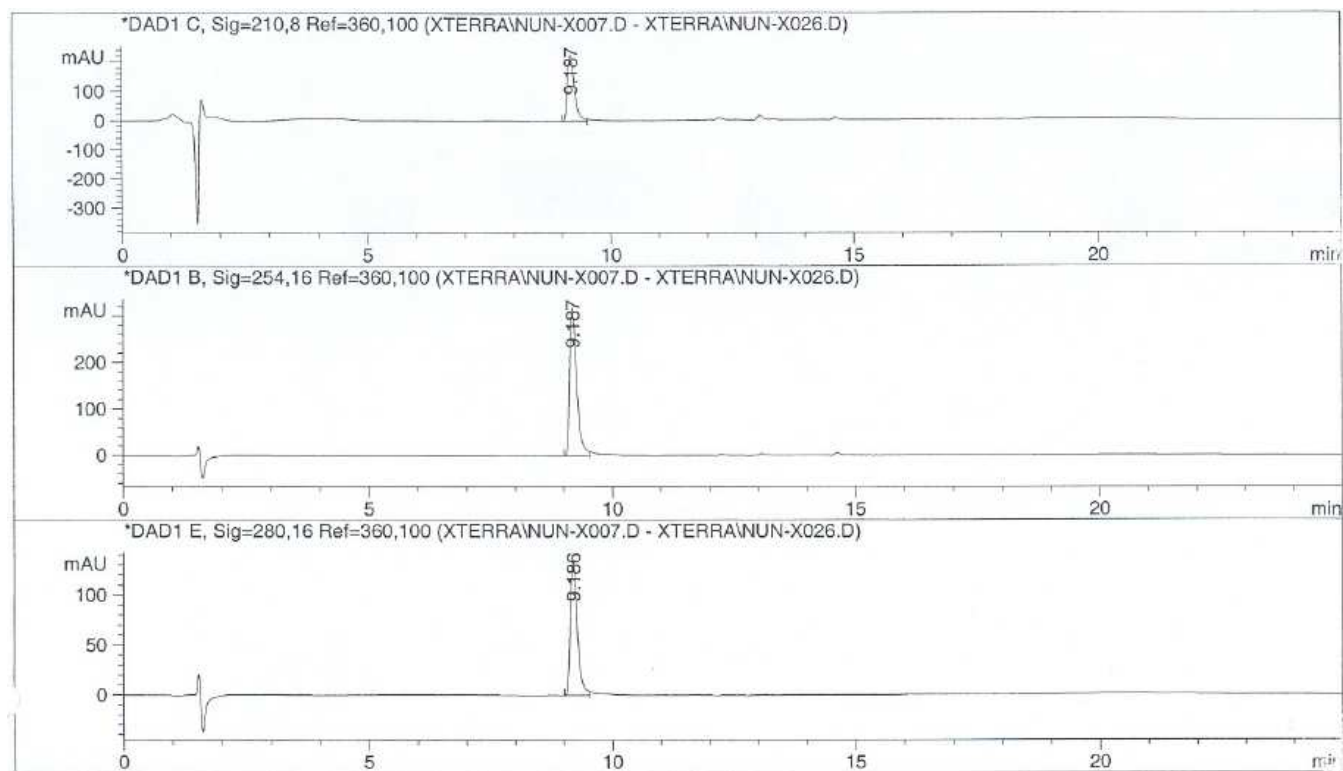


Compound 1i

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

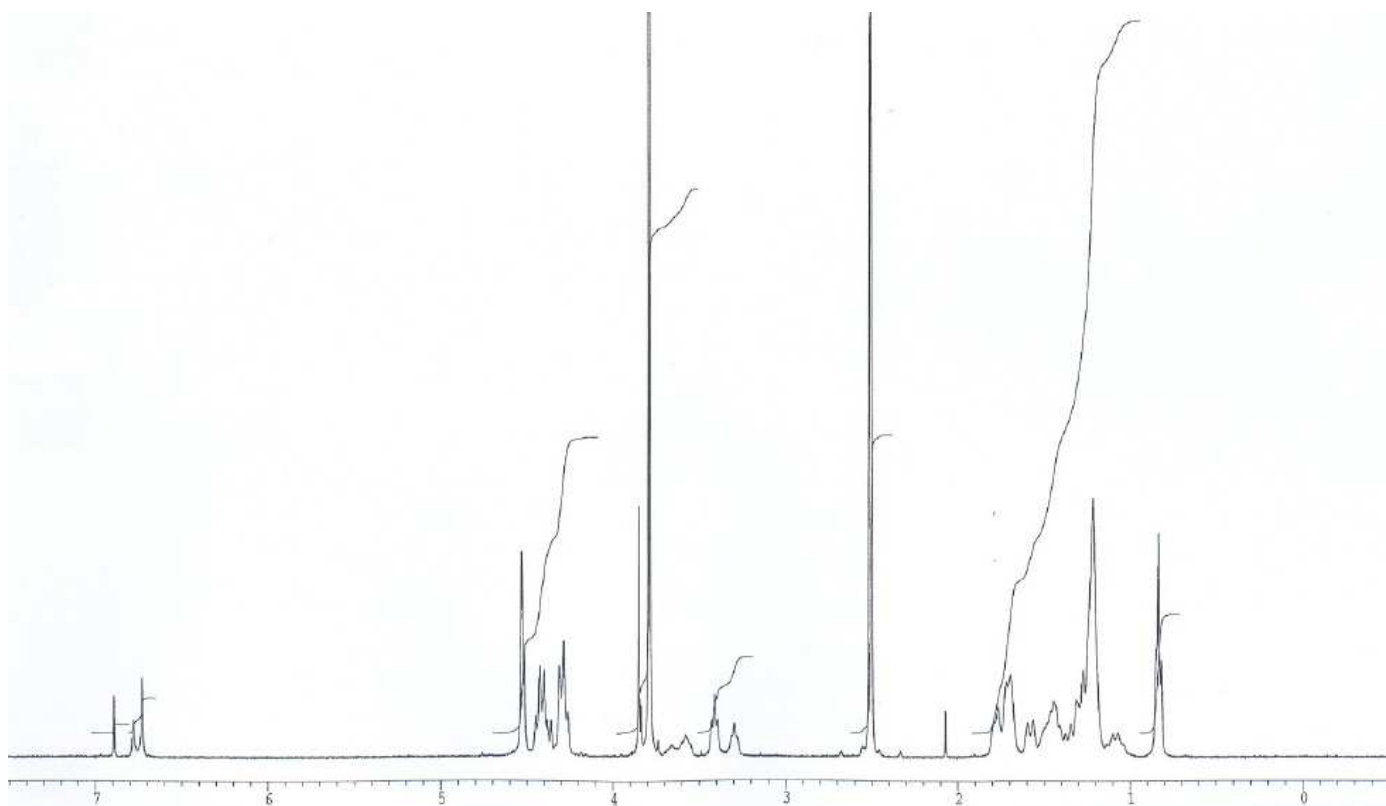


HPLC (Method 2)

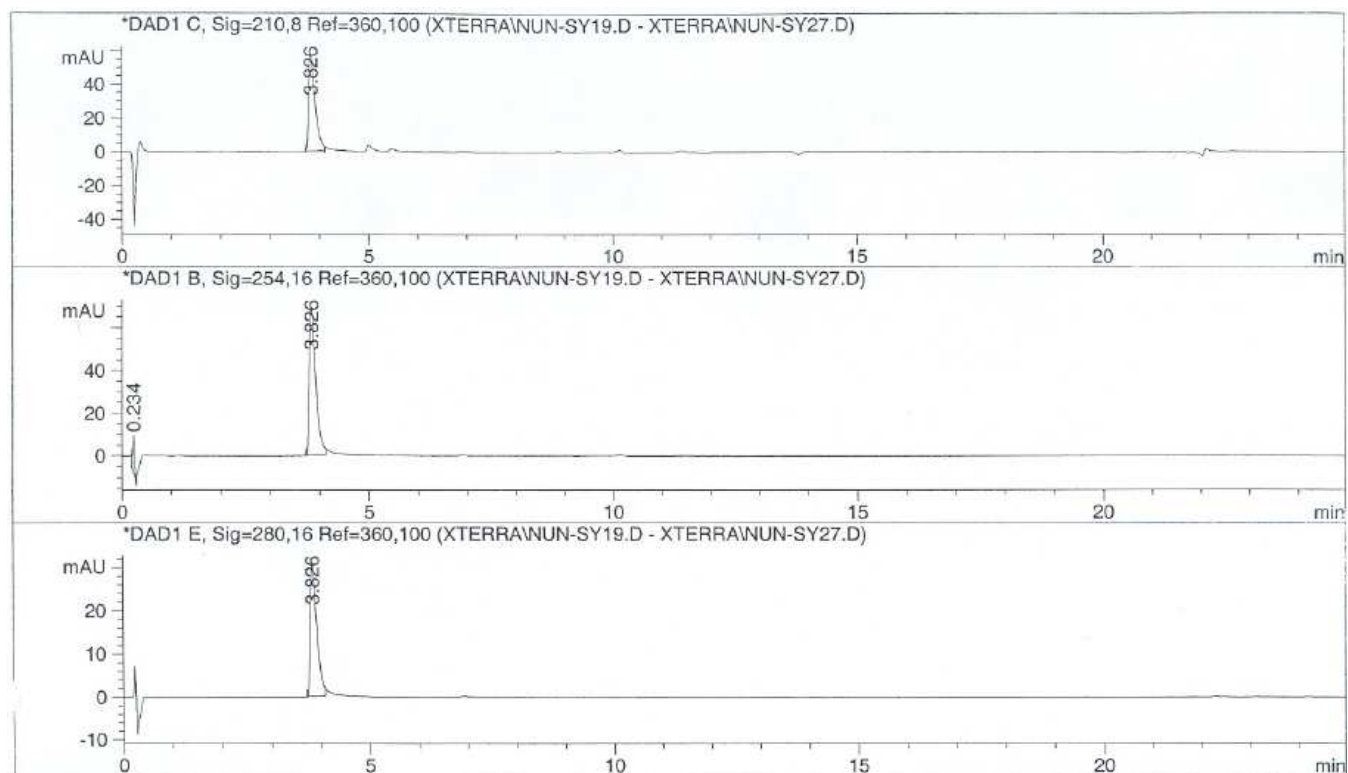


Compound 1j

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

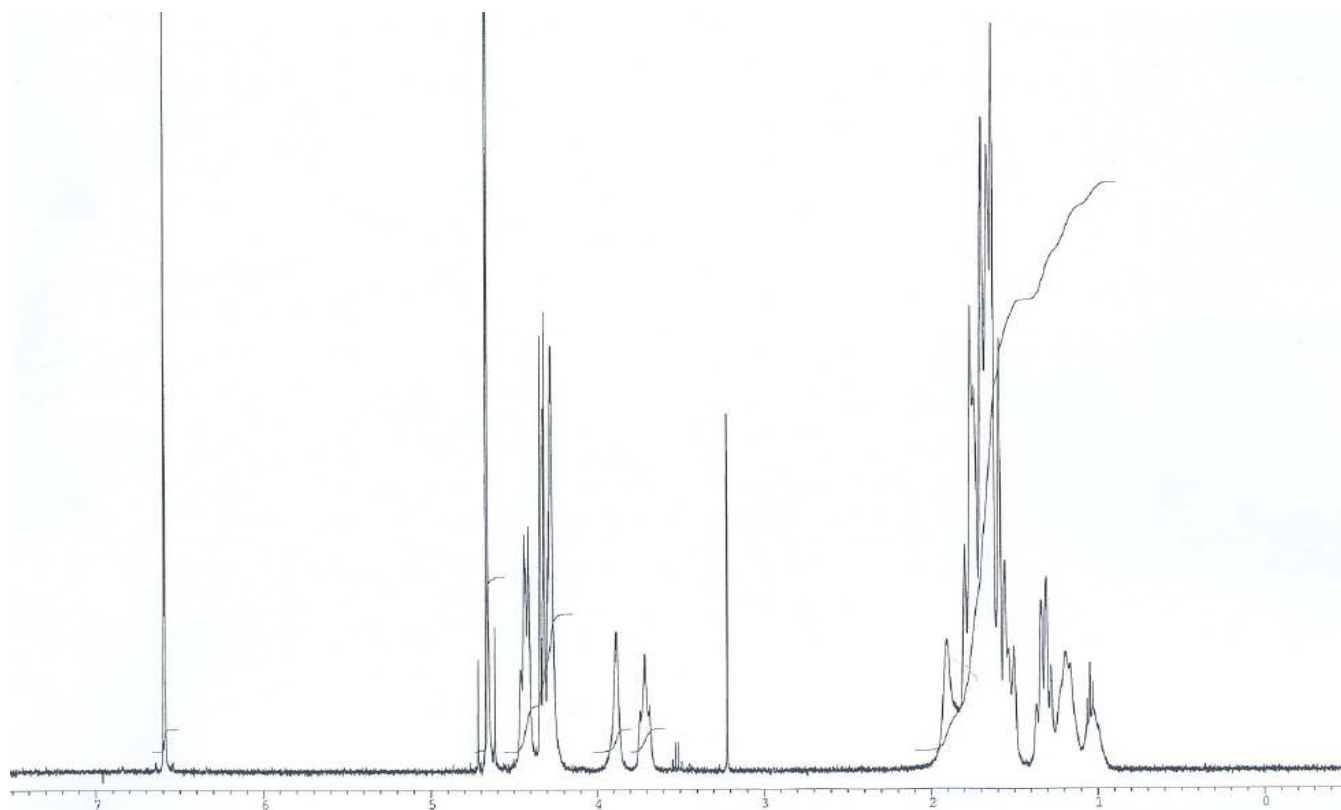


HPLC (Method 1)



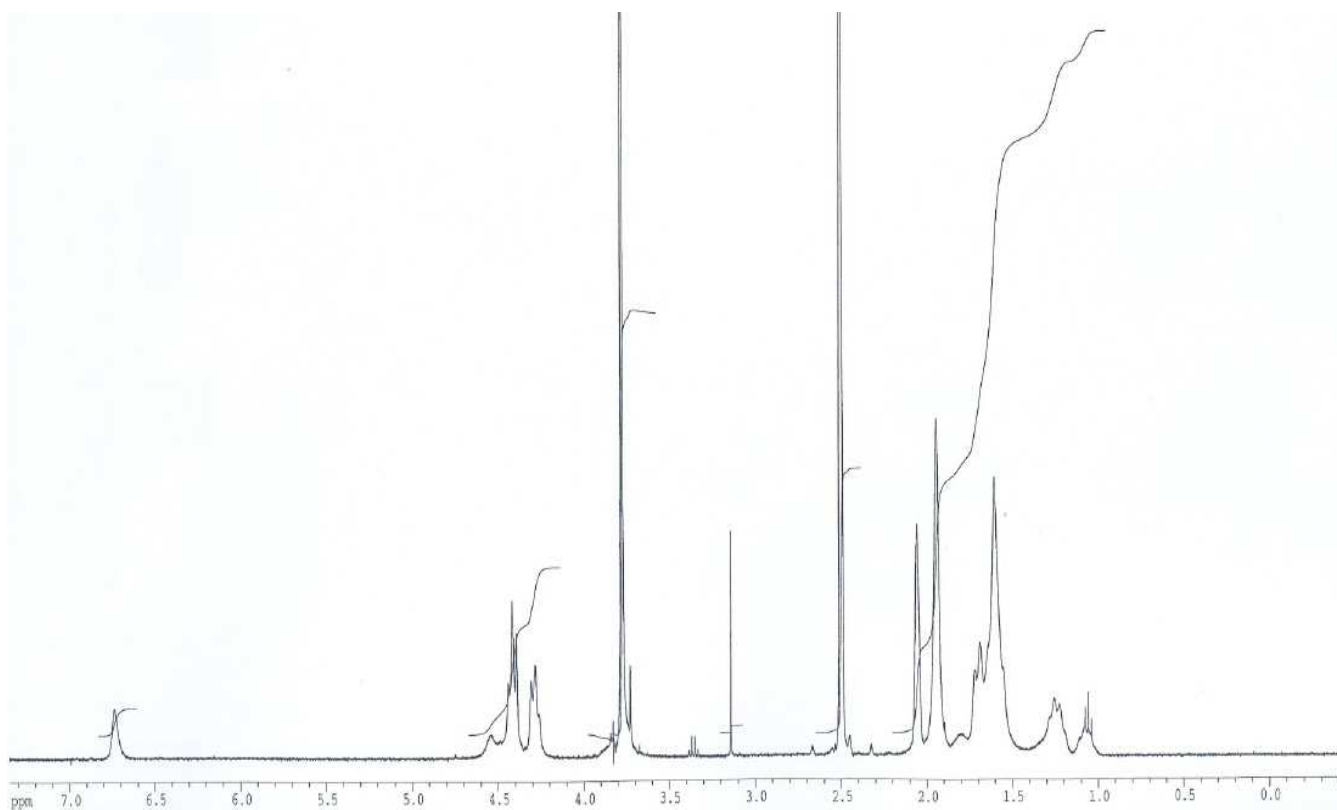
Compound 1k

^1H -NMR (D_2O)

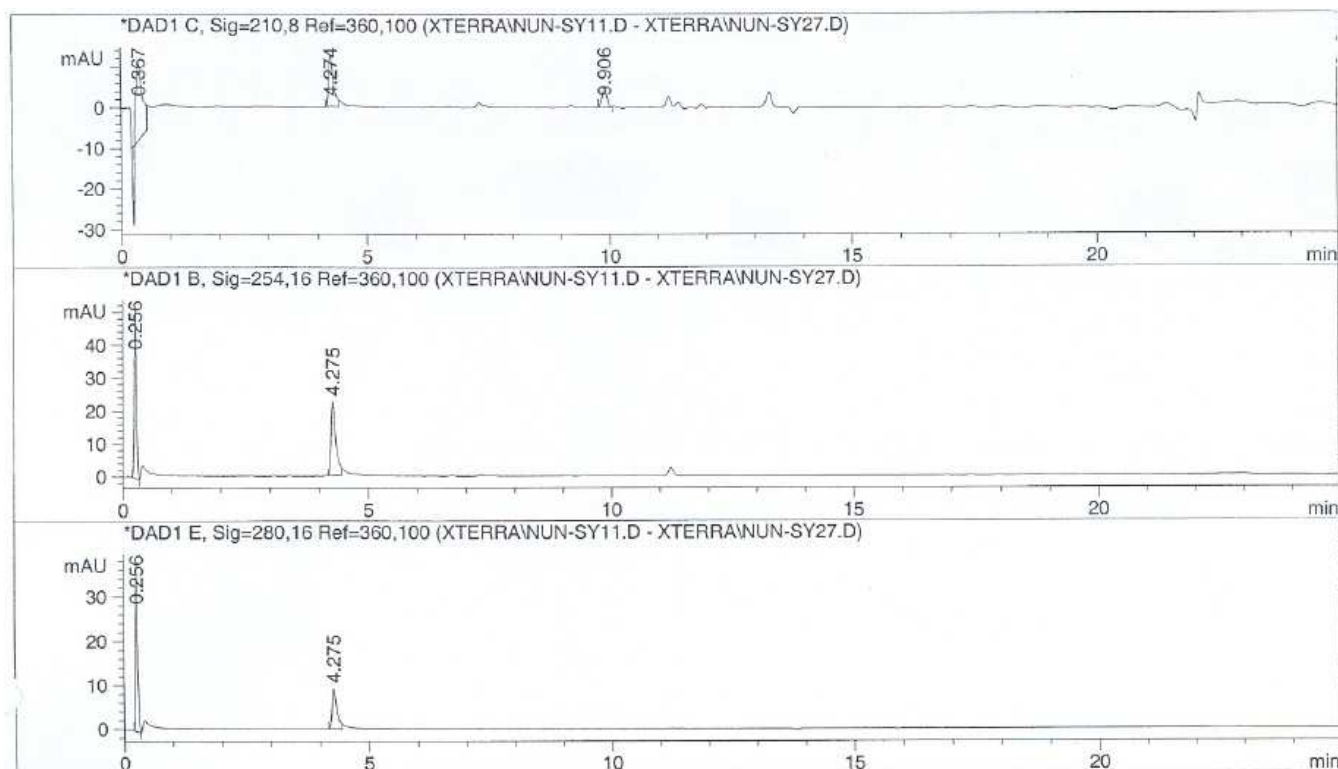


Compound 11

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

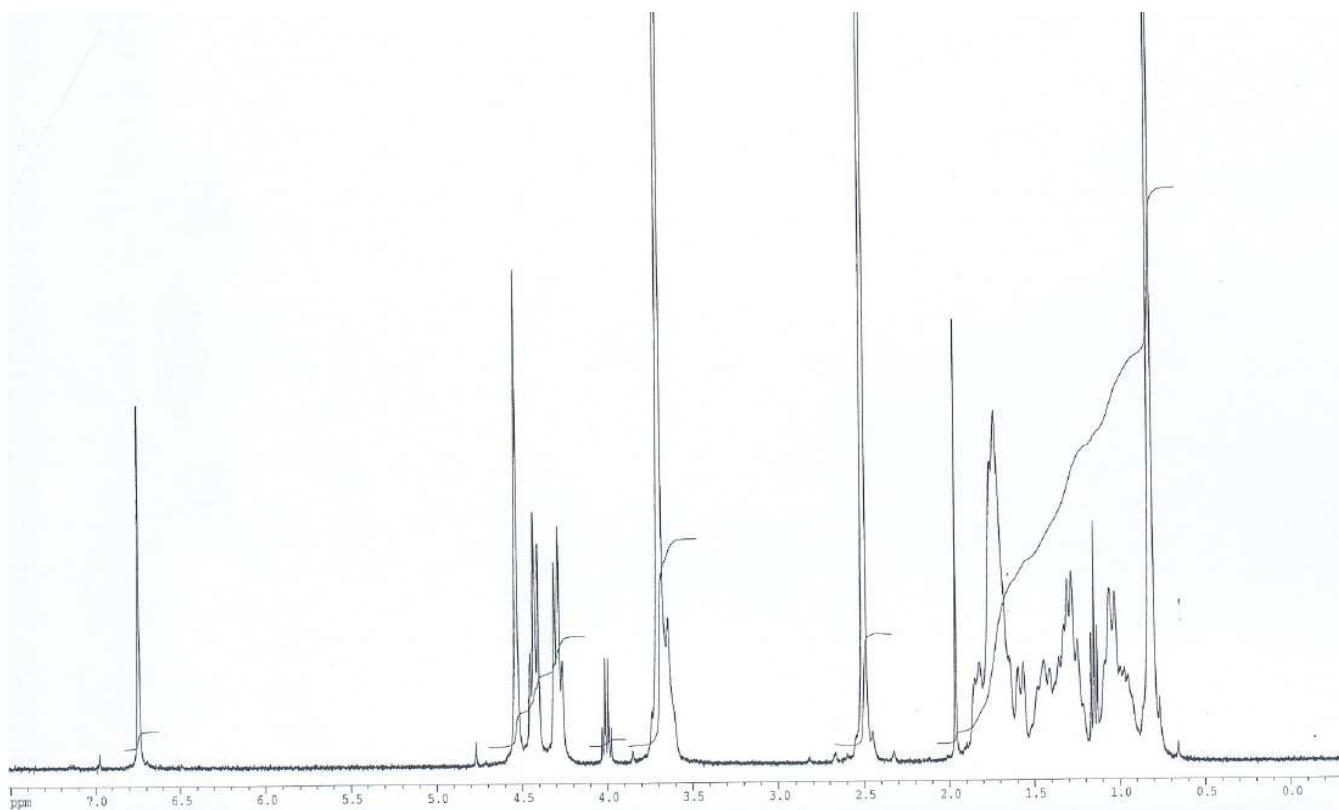


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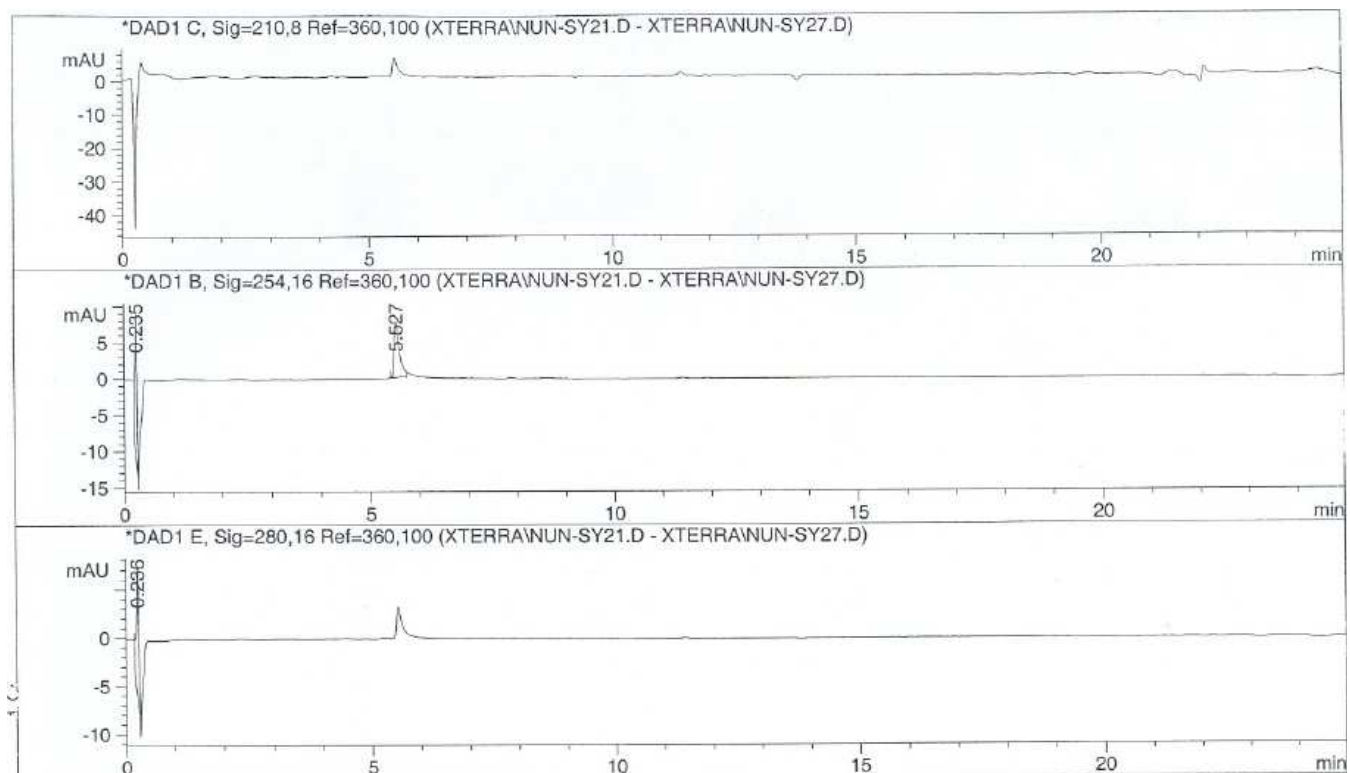


Compound 1m

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

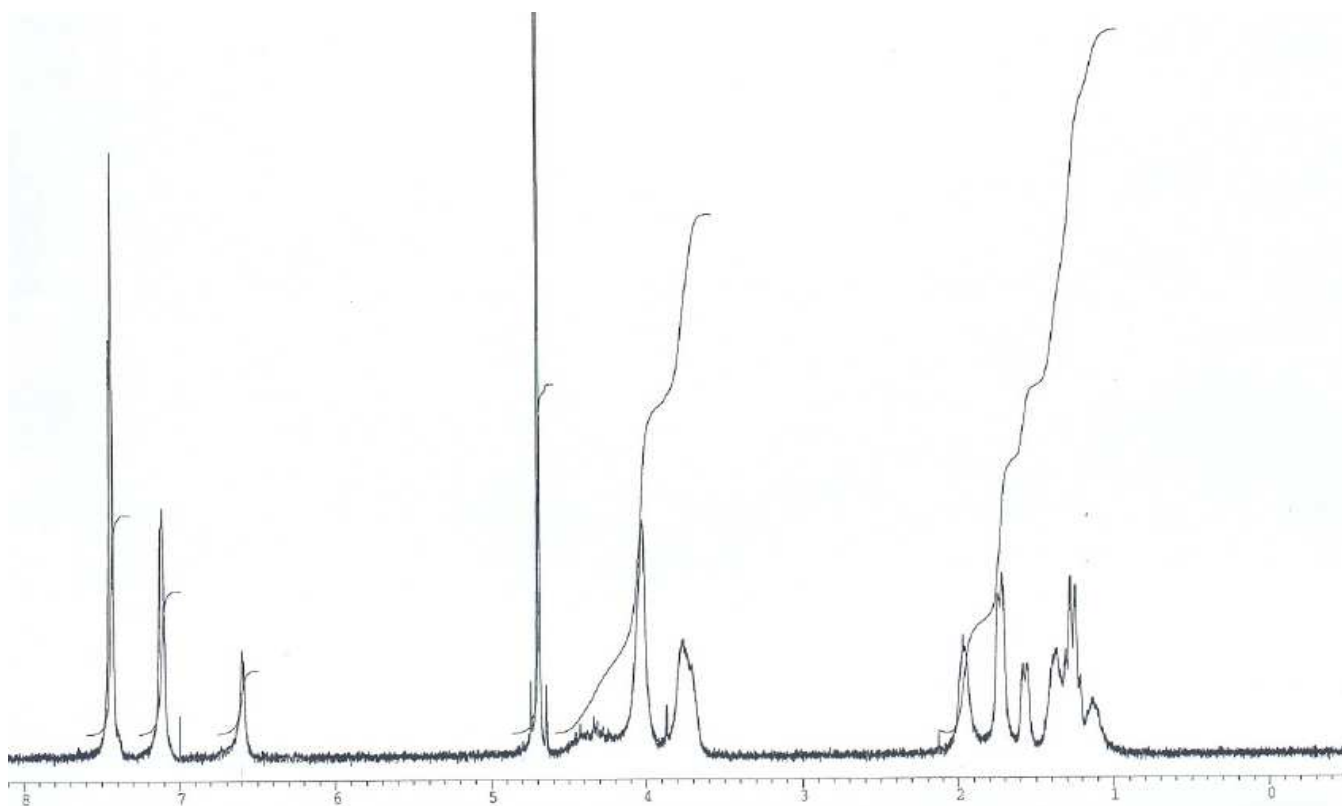


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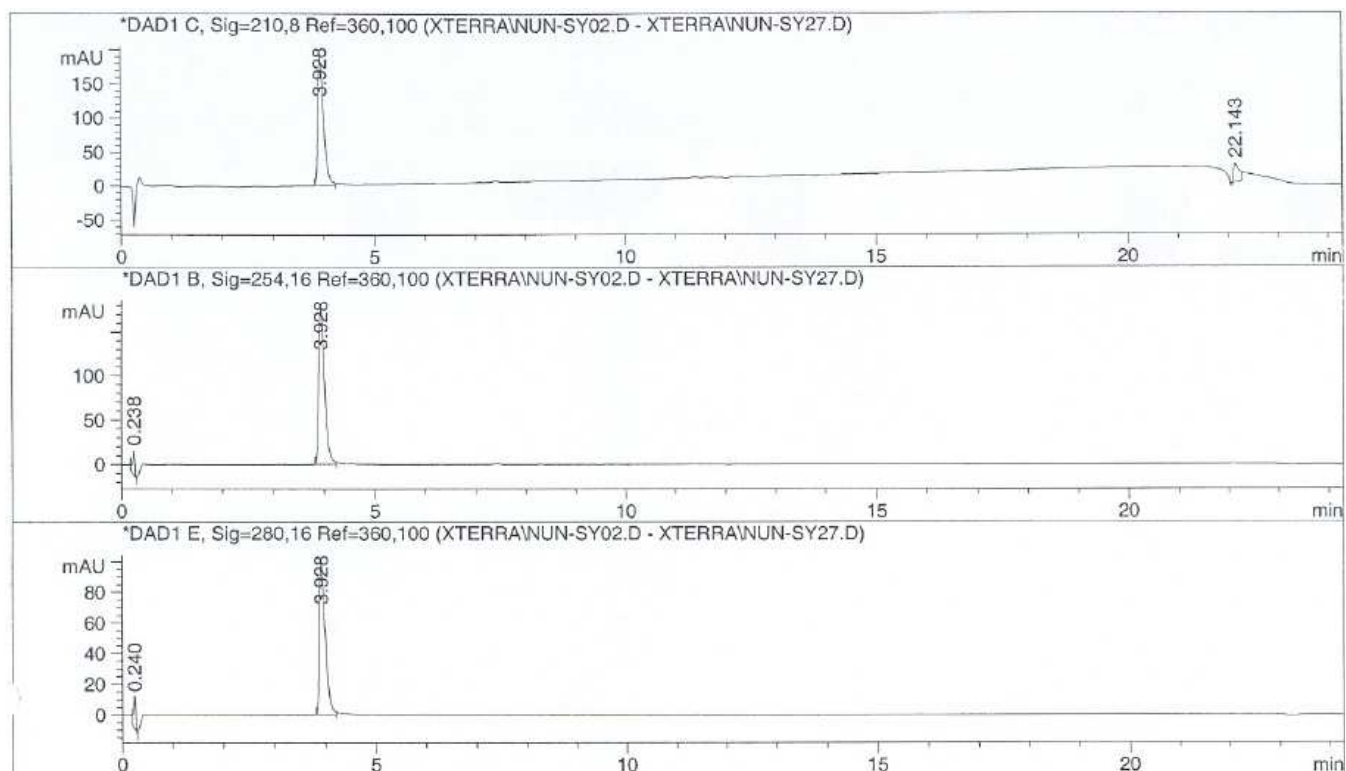


Compound 1n

^1H -NMR (D_2O)

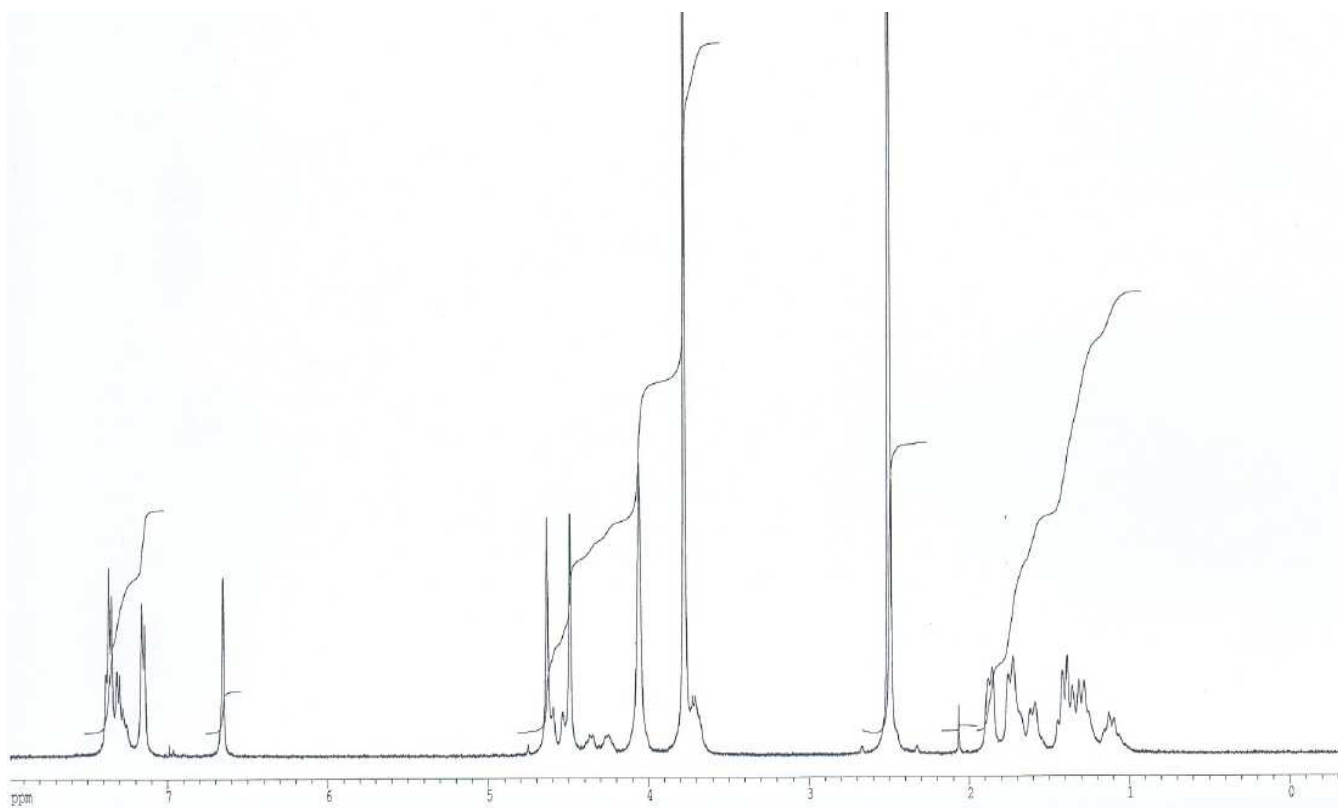


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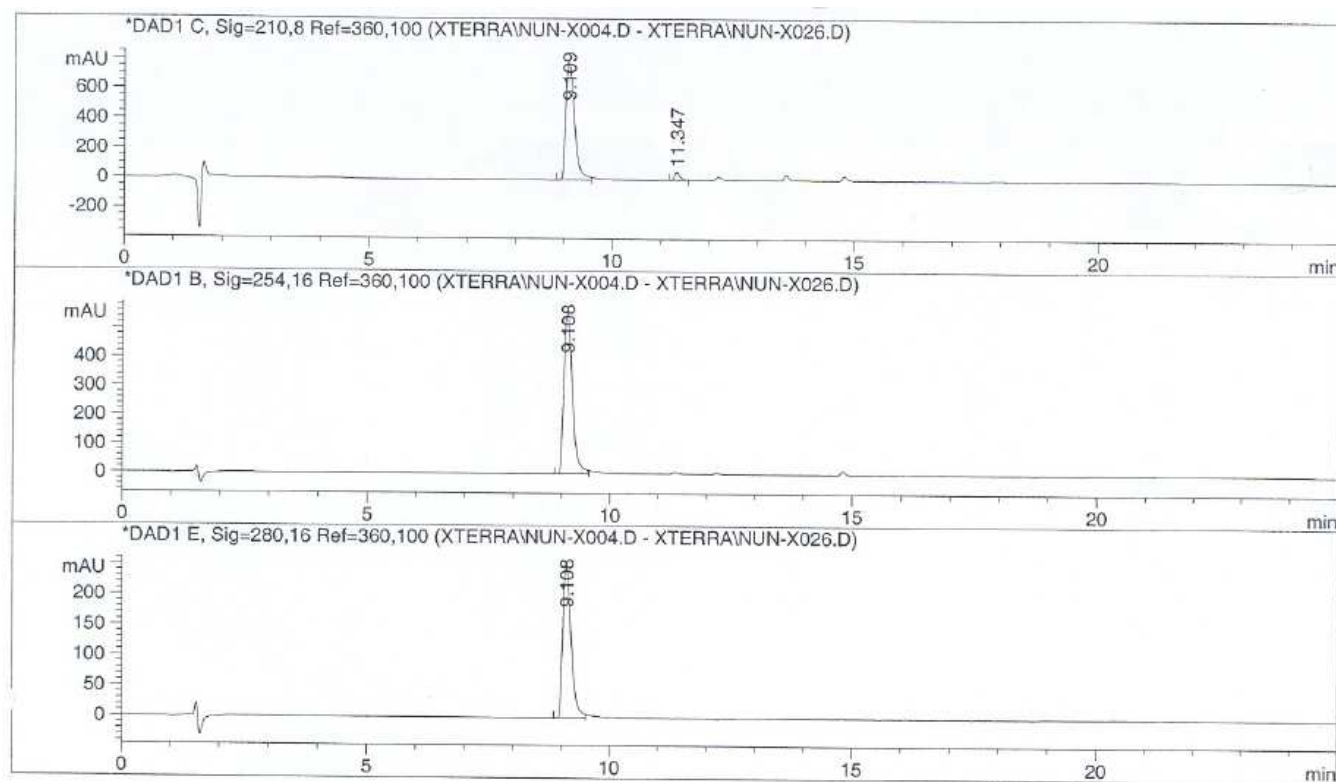


Compound 1o

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

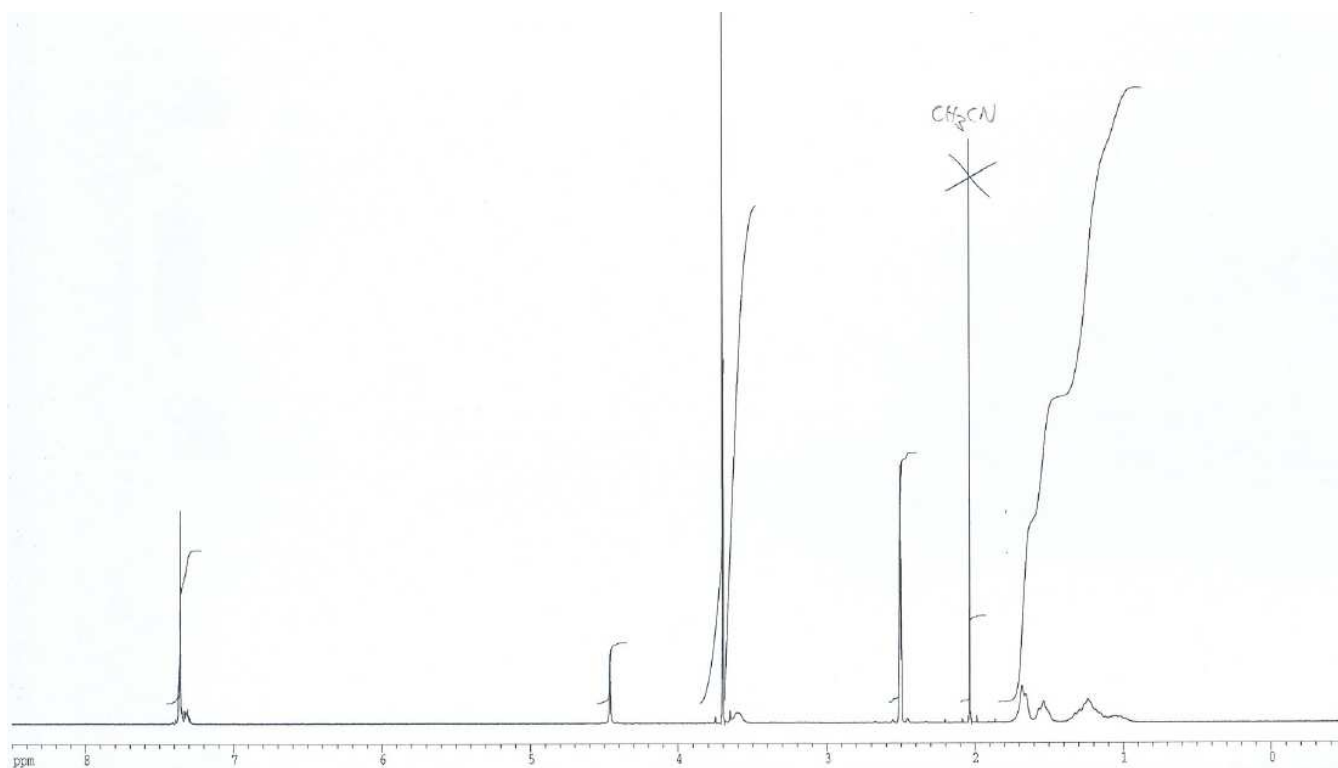


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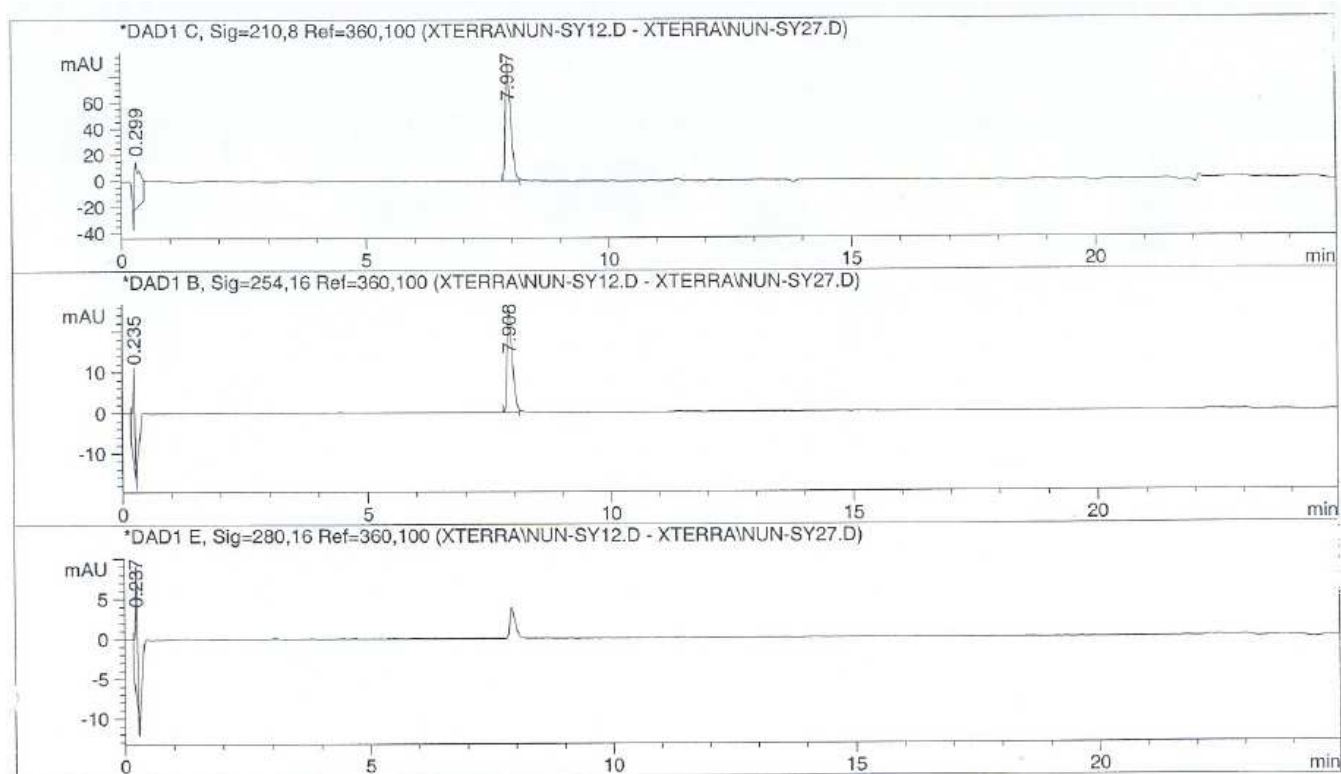


Compound 1p

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

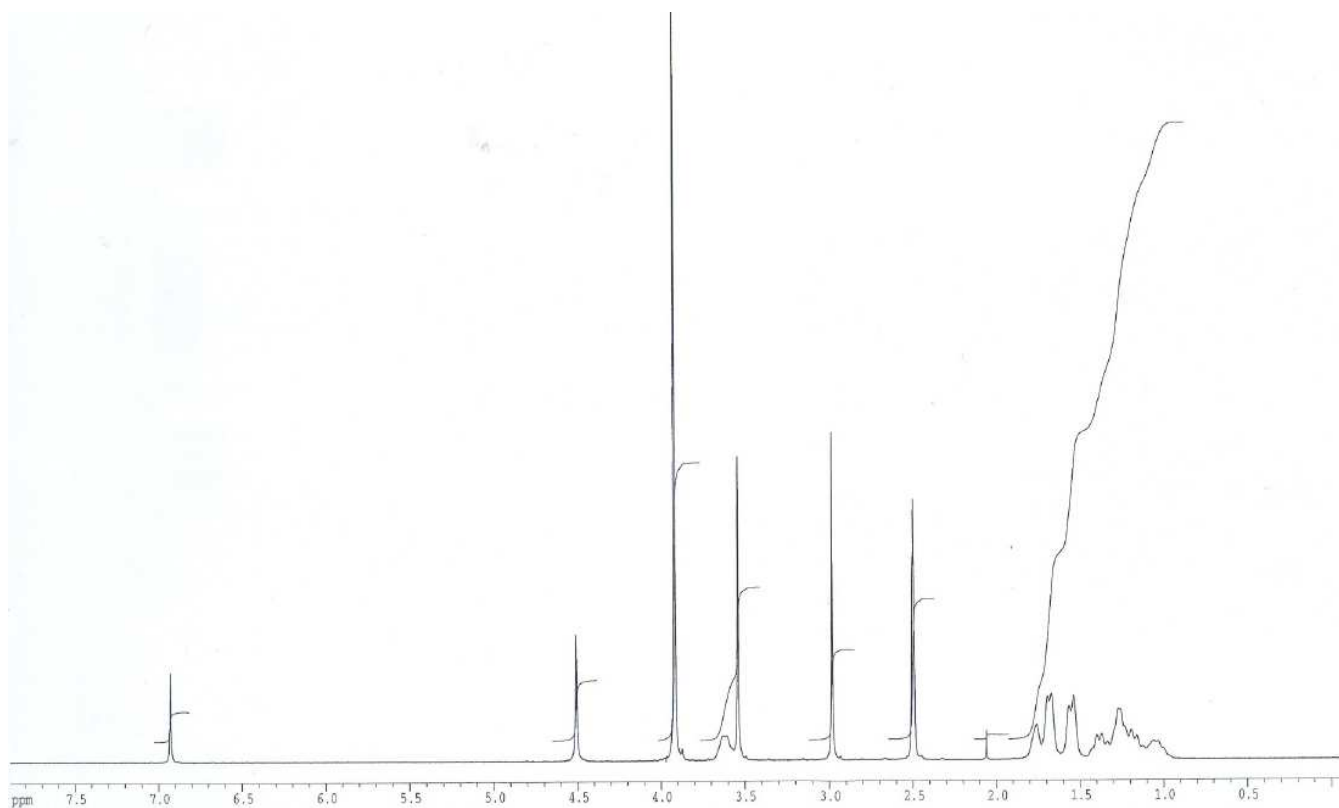


HPLC (Method 1)

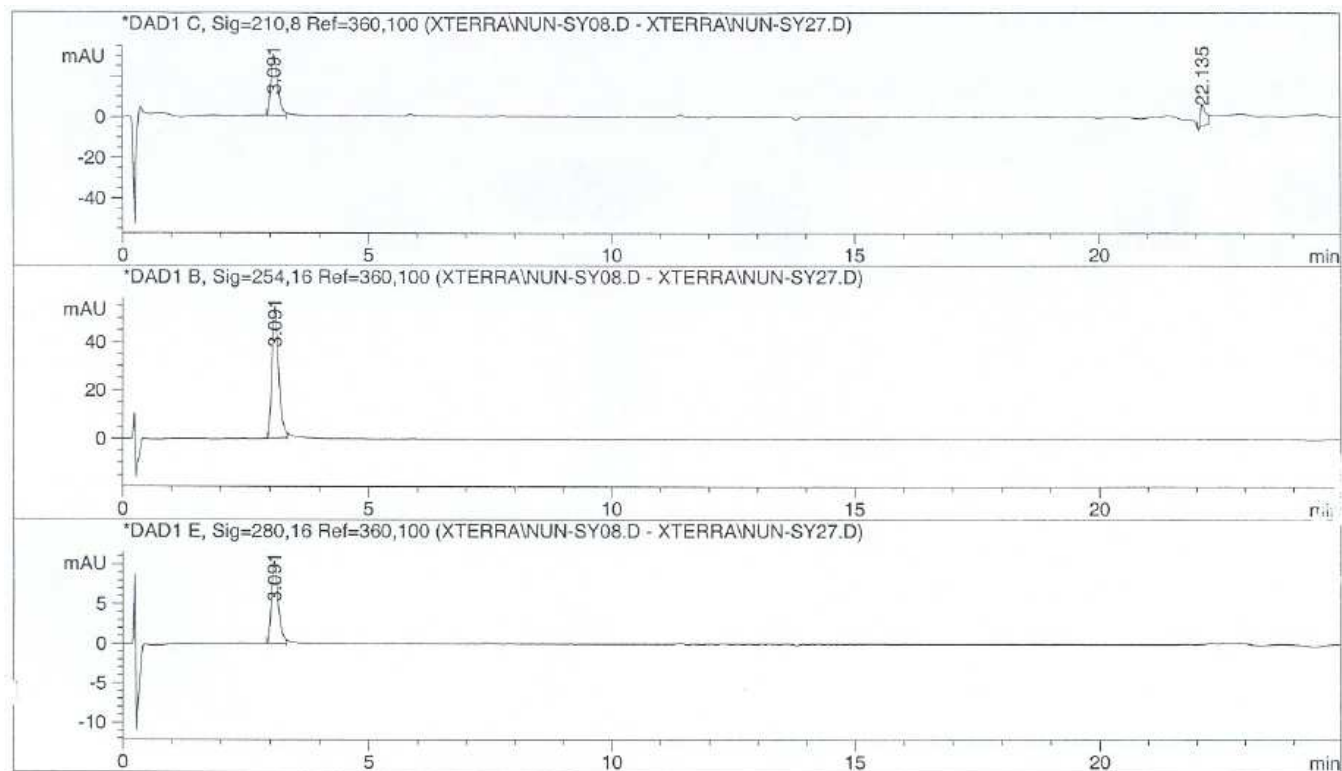


Compound 1q

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

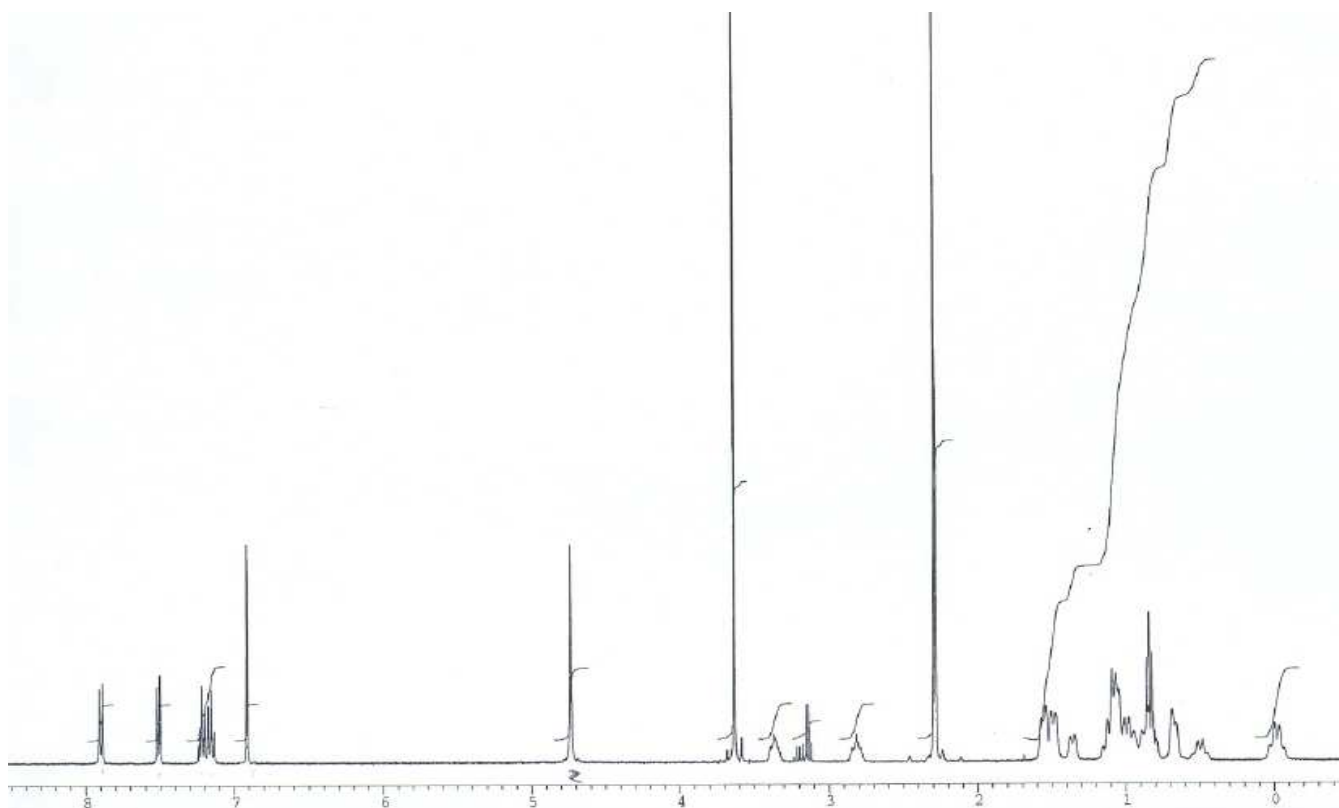


HPLC (Method 1)

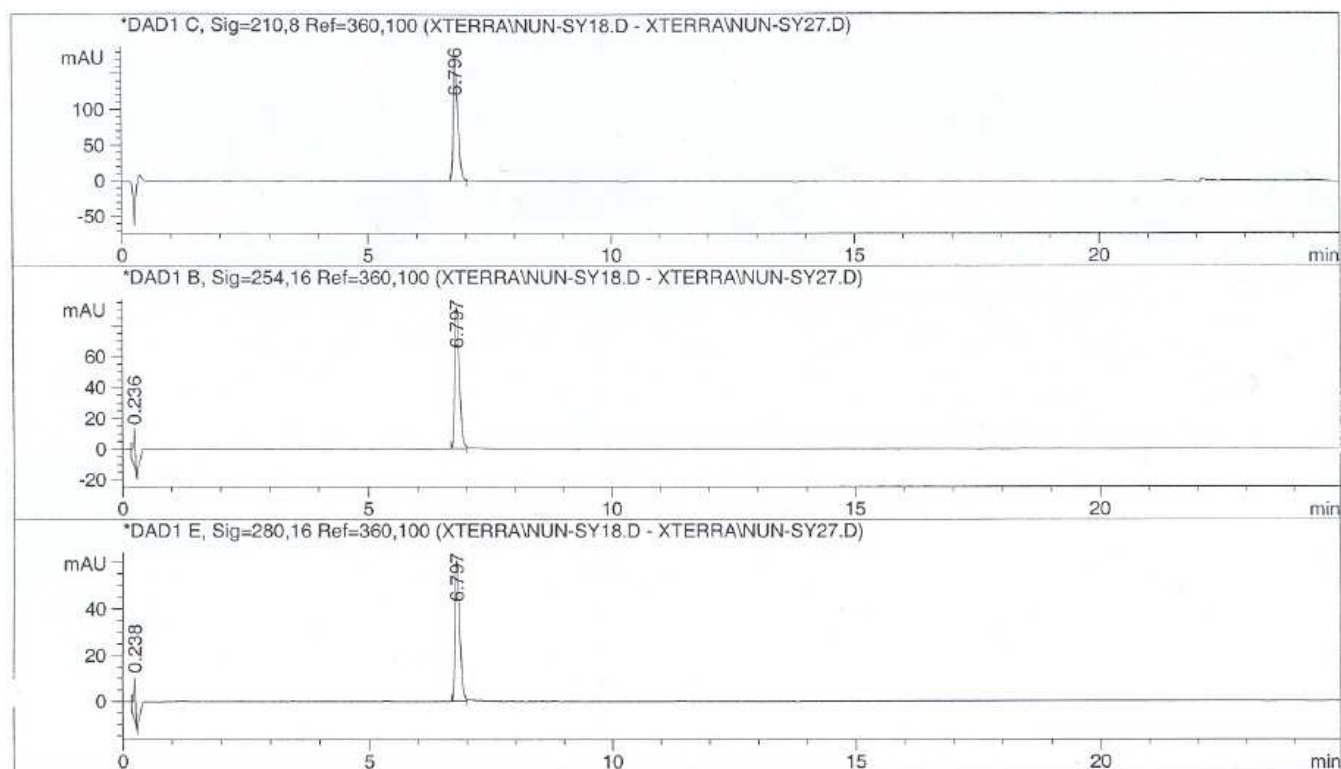


Compound 1r

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

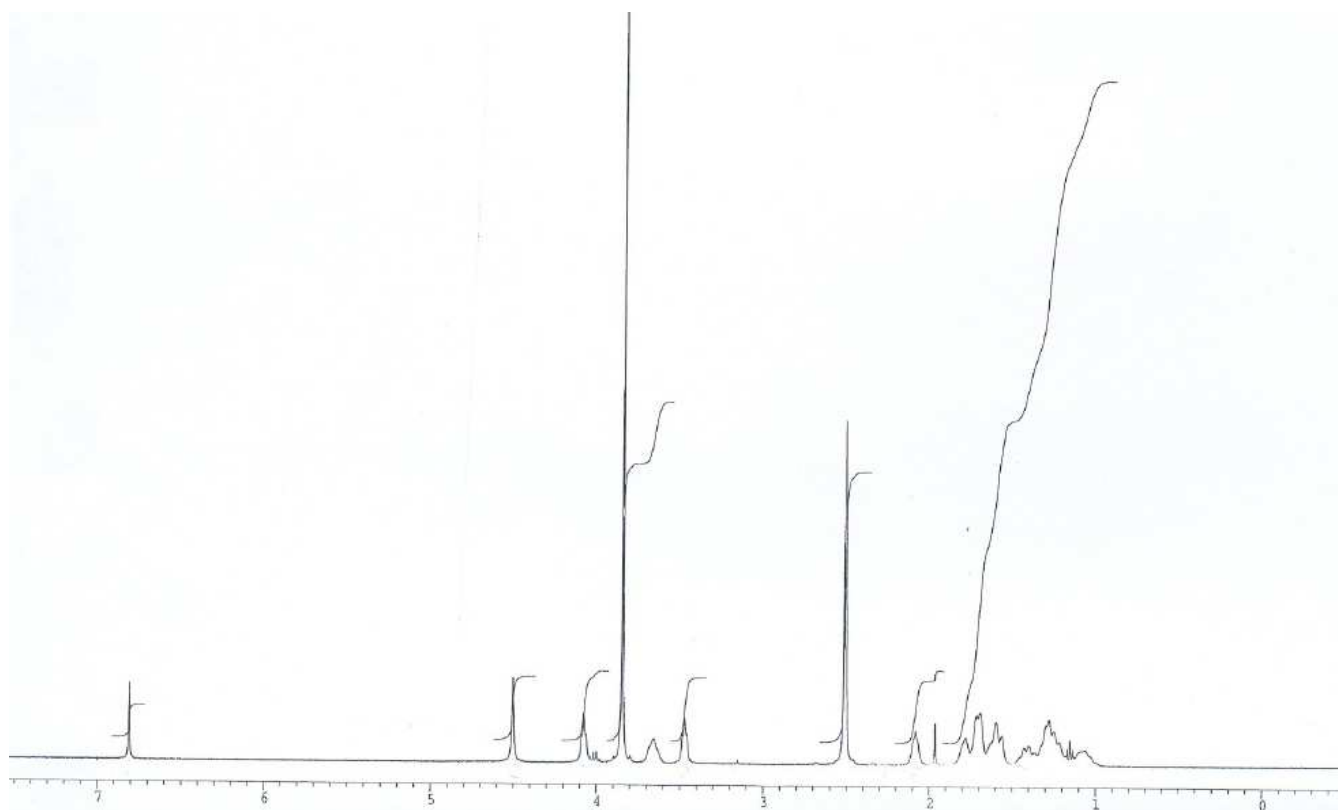


HPLC (Method 1)

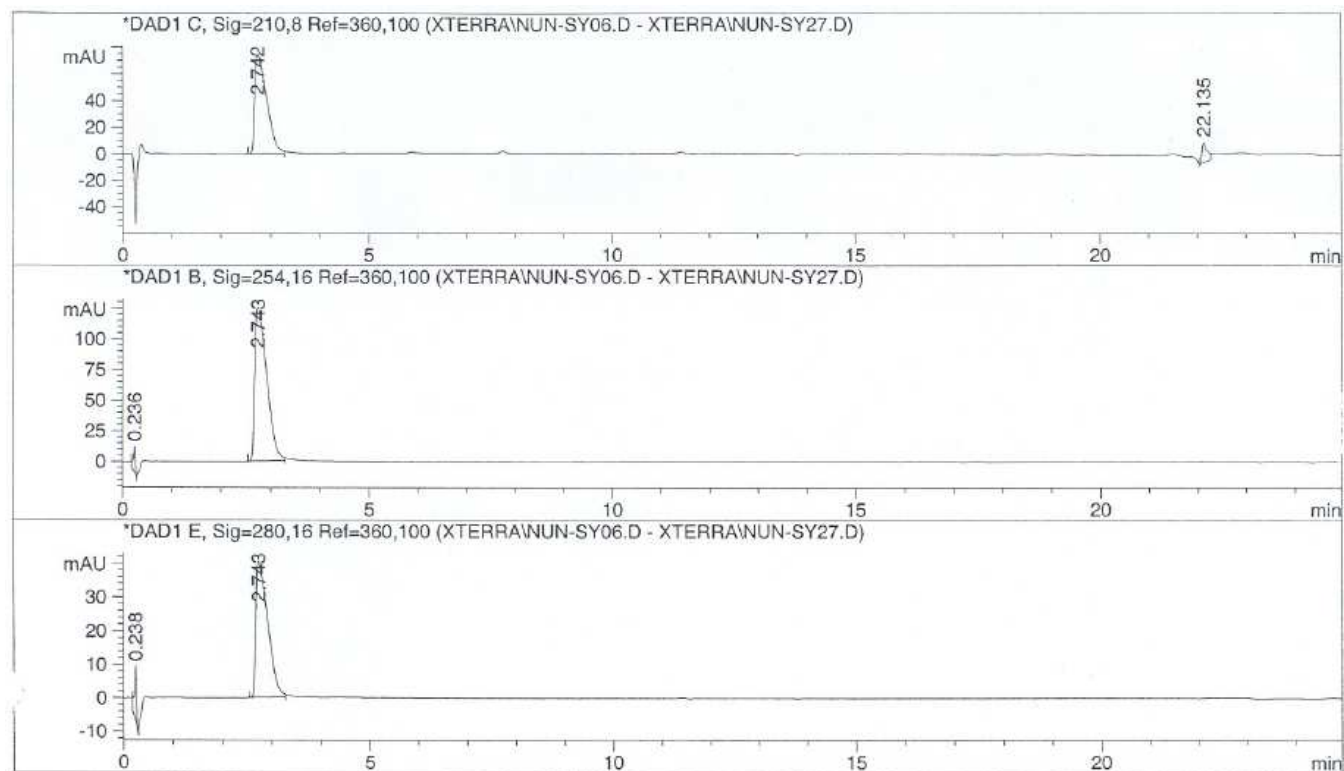


Compound 1s

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

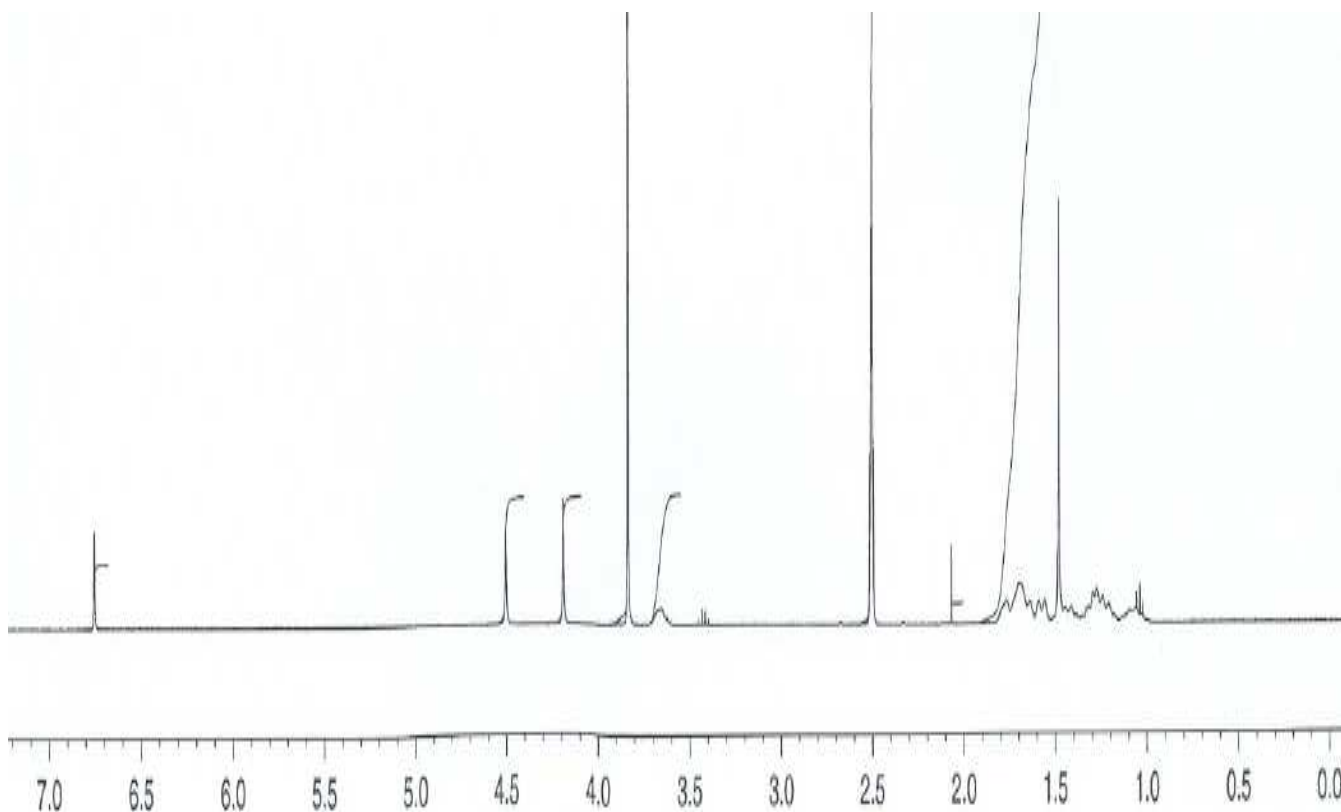


HPLC (Method 1)

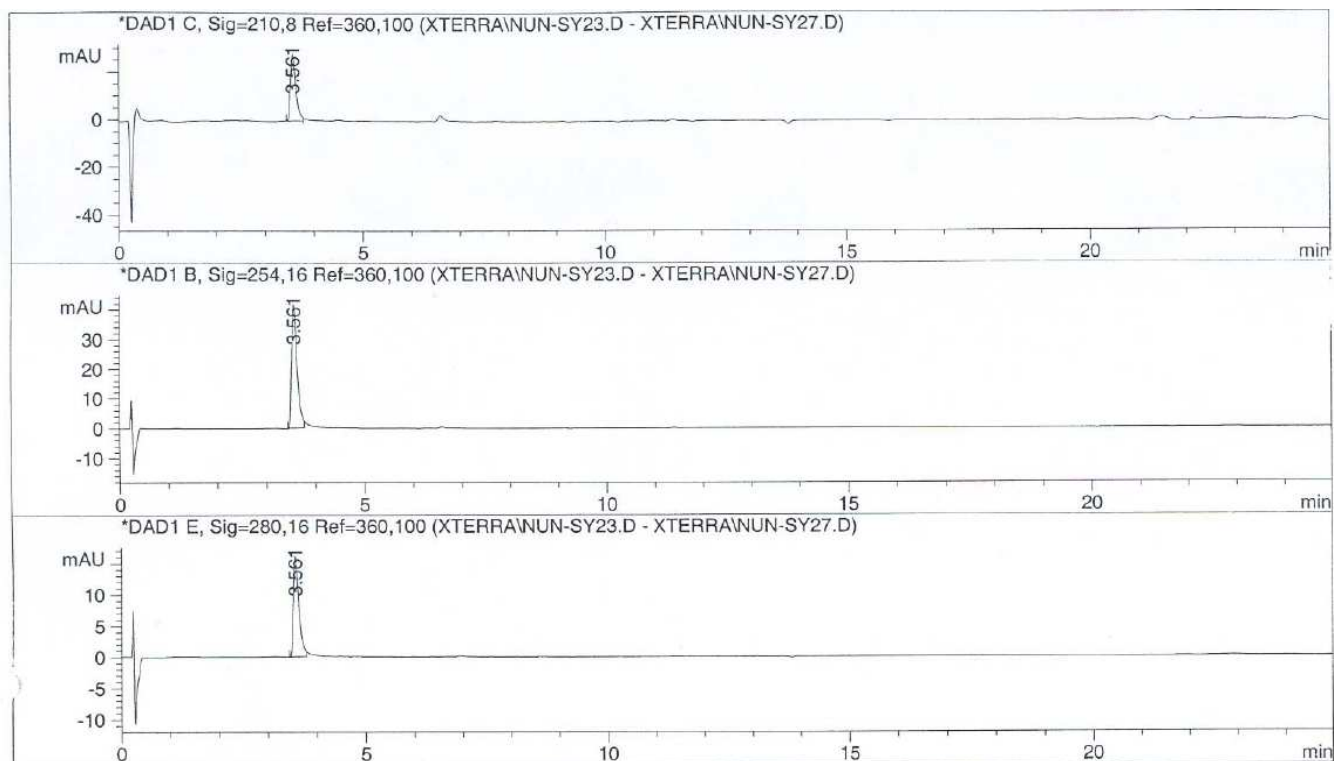


Compound 1t

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)

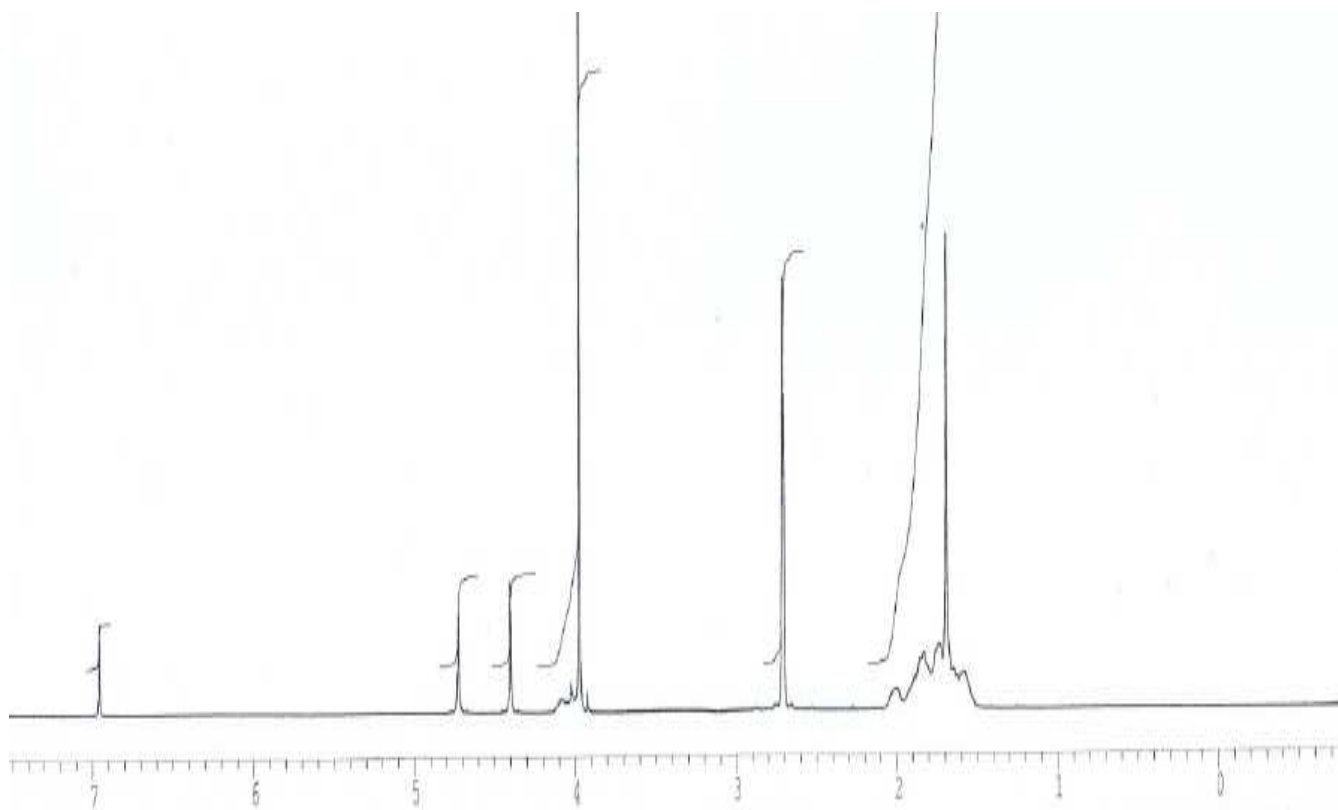


HPLC (Method 1)

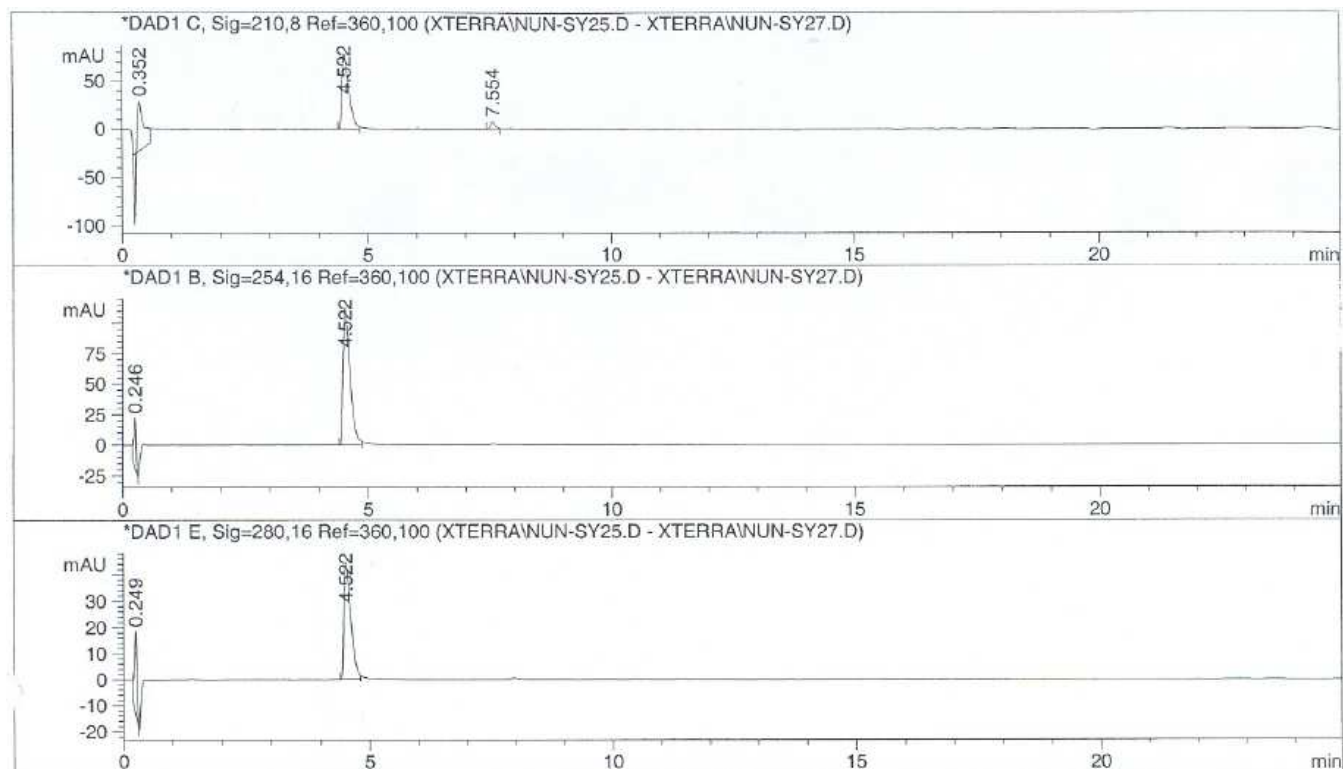


Compound 1u

^1H -NMR ($\text{DMSO-d}_6/\text{D}_2\text{O}$)



HPLC (Method 1)



Purity of compounds 1a-u assessed by HPLC or ¹H-NMR

compound	HPLC Method 1 Purity (254 nM)	HPLC Method 2 Purity (254nM)	¹ H- NMR Purity
1a	100 %	---	
1b	100 %	100 %	
1c	---	---	> 95 %
1d	100 %	---	
1e	100 %	100 %	
1f	100 %	90 %	
1g	---	---	> 95 %
1h	100 %	100 %	
1i	---	100 %	
1j	100 %	100 %	
1k	---	---	> 95 %
1l	---	---	> 95 %
1m	100 %	100 %	
1n	100 %	100 %	
1o	---	100 %	
1p	100 %	100 %	
1q	100 %	100 %	
1r	100 %	100 %	
1s	100 %	100 %	
1t	100 %	100 %	
1u	100 %	100 %	