Supplementary Information

Design and synthesis of phosphonate functionalized naphthalenediimide: Application to induce mitochondria mediated apoptosis in the human skin melanoma cells (SKMEL2)

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Experimental Section

HPLC analysis:

The HPLC analysis of the NDI 1 and NDI 2 was performed by Agilent ProStar C-18 HPLC (equipped with Agilent and UV detector) using Agilent HPLC C18 column (250x 4.6mm, 5µm). The mobile phase, Acetonitrile 40% (v/v), 60% (v/v) and 80 % (v/v) in water was used separately as mobile phase for NDI 1 and NDI 2. Samples were analysed by Agilent software, and UV absorbance scans of the resultant peaks were measured on220 nm (Fig. S1)

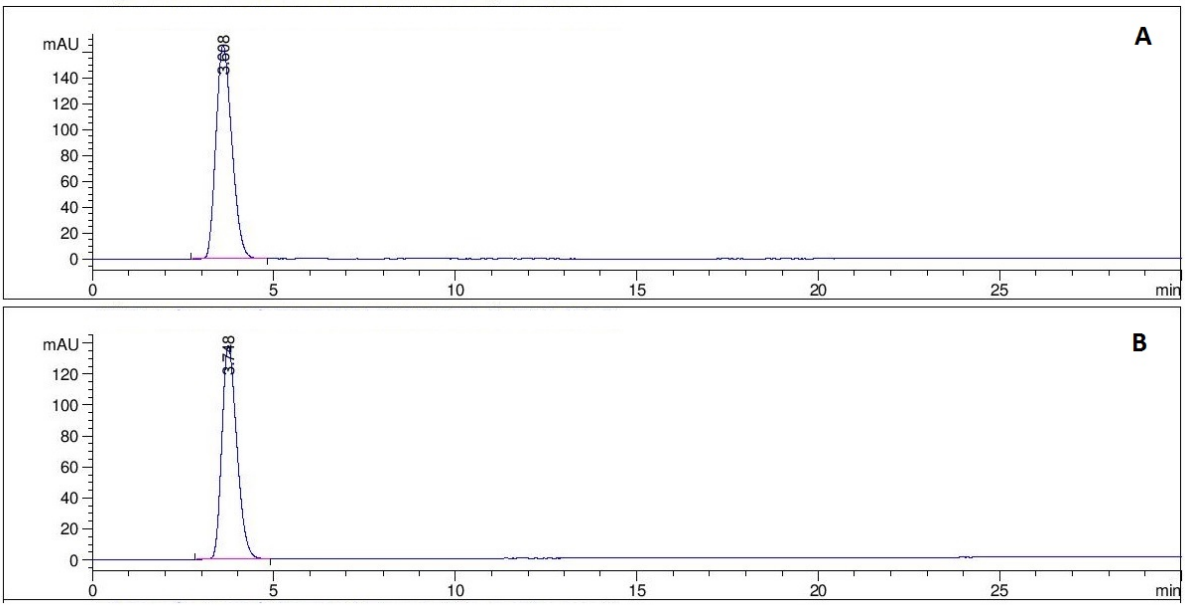


Fig. S1 — HPLC analysis of **NDI-1** and **NDI-2**

Where, **A** is the **NDI-1** and **B** is **NDI-2** compounds. The mobile phase, Acetonitrile 40% (v/v), 60% (v/v) and 80 % (v/v) in water was used separately as mobile phase for **NDI-1**and **NDI-2**. Samples were analysed by Agilent software, and UV absorbance scans of the resultant peaks were measured on 220 nm.

Detection of Phosphatidylserine on the membranes of the apoptotic cells

In order to confirm the presence of apoptotic cells in the population, flow cytometric analysis of the phosphotidylserine on the membranes of the apoptotic cells was undertaken. The SKMEL2 cells were used for the study. The cells incubated with various concentrations

of 4 mM **NDI-1**and **NDI-2** compounds without phosphonates. We found that only 11% apoptotic cells

were present in **NDI-1**treated cells, whereas 6.5% cells were found apoptotic in **NDI-2** treated cells.

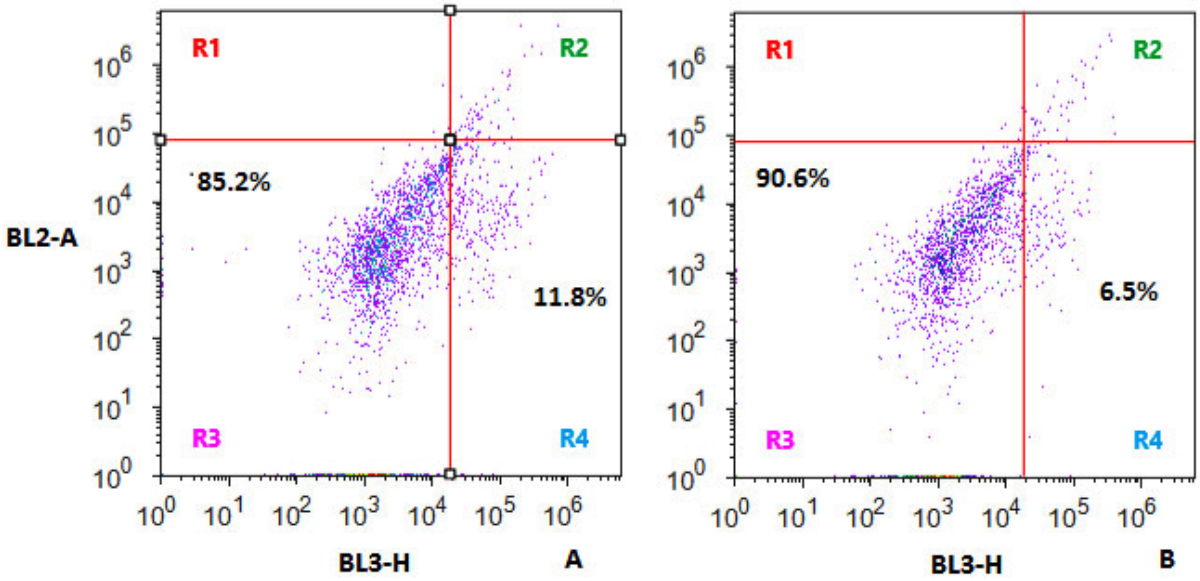


Fig. S2 — Analysis of exposure of Phosphatidylserine on SKMEL2 cells by flow cytometry. **(A)NDI-1** without phosphonate in SKMEL2 cells **(B) NDI-2** without phosphonatein SKMEL2 cells. Flow cytometry analysis of apoptosis induced by the NDI derivatives using the Annexin V-FITC/PI double staining method

Table S1— Primers used for the gene expression by Real Time PCR

Name of the primer	**Forward**	**Reverse**
Bcl2	ATGTGTGTGGAGAGCGTCAA	ACAGTTCCACAAAGGCATCC
Bax	TTTTGCTTCAGGGTTTCATC	CAGTTGAAGTTGCCGTCAGA
Bak1	GCCTTTGCAGTTGGACTCTC	GGGTTGGGAGCAAGTGTCTA
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

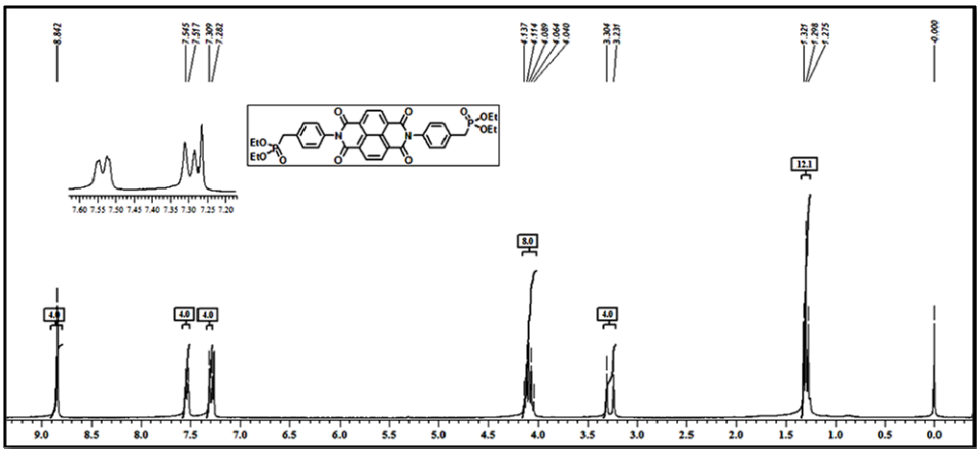


Fig. S3 — ^{1}H NMR spectrum of **NDI-1** in $CDCl_3$

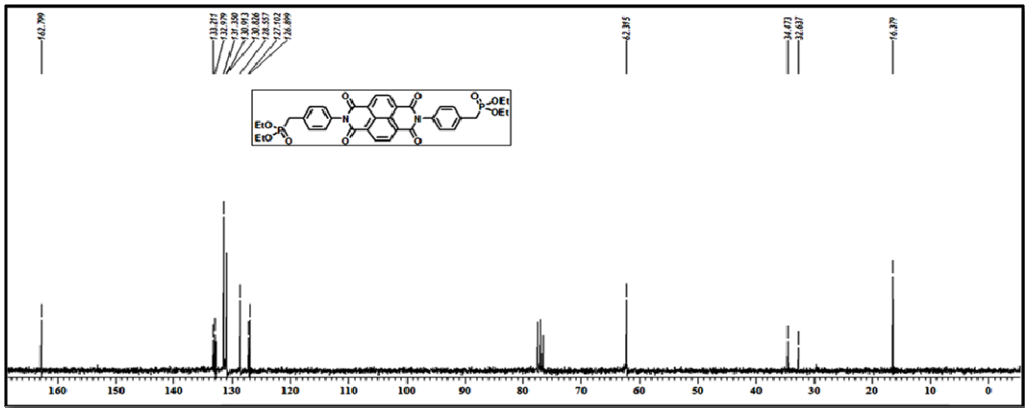


Fig. S4 — ^{13}C NMR spectrum of **NDI-1** in $CDCl_3$

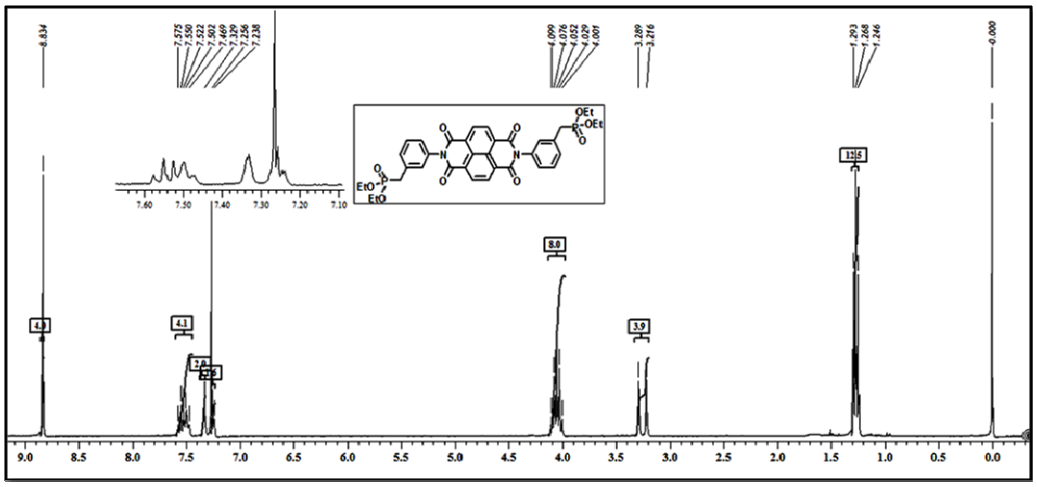


Fig. S5 — ^{1}H NMR spectrum of **NDI-2** in $CDCl_3$

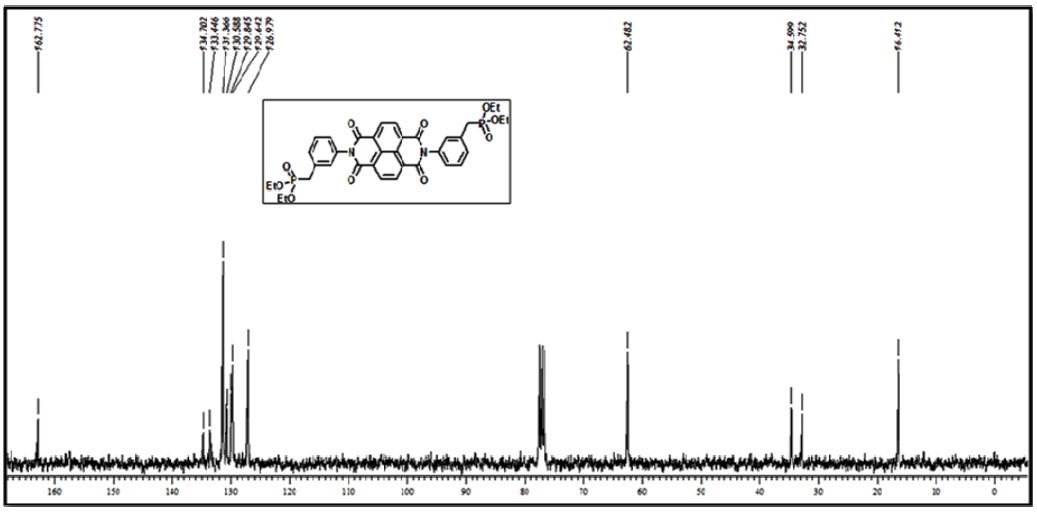


Fig. S6 — ^{13}C NMR spectrum of **NDI-2** in $CDCl_3$

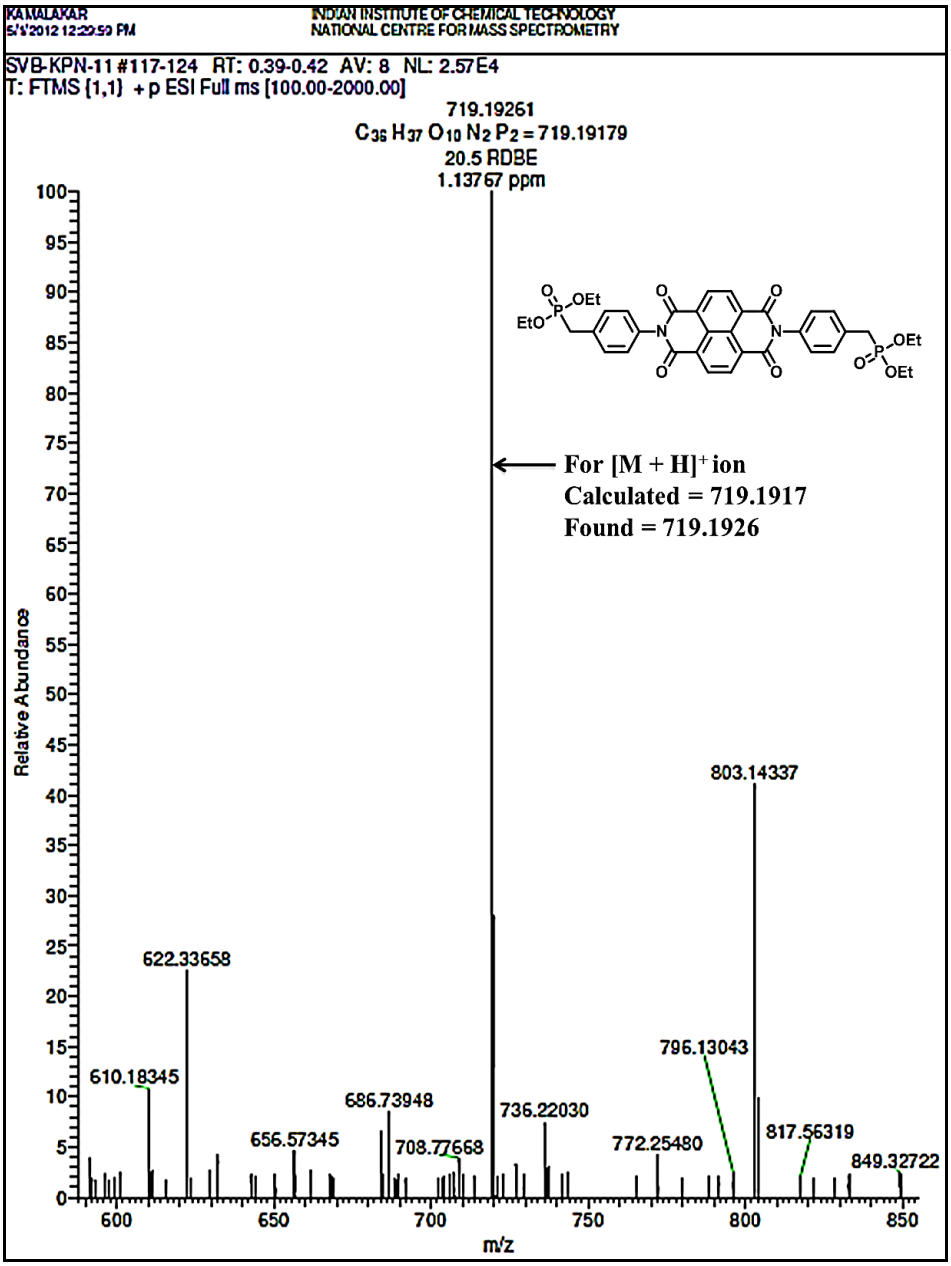


Fig. S7— HRMS spectrum of **NDI-1**

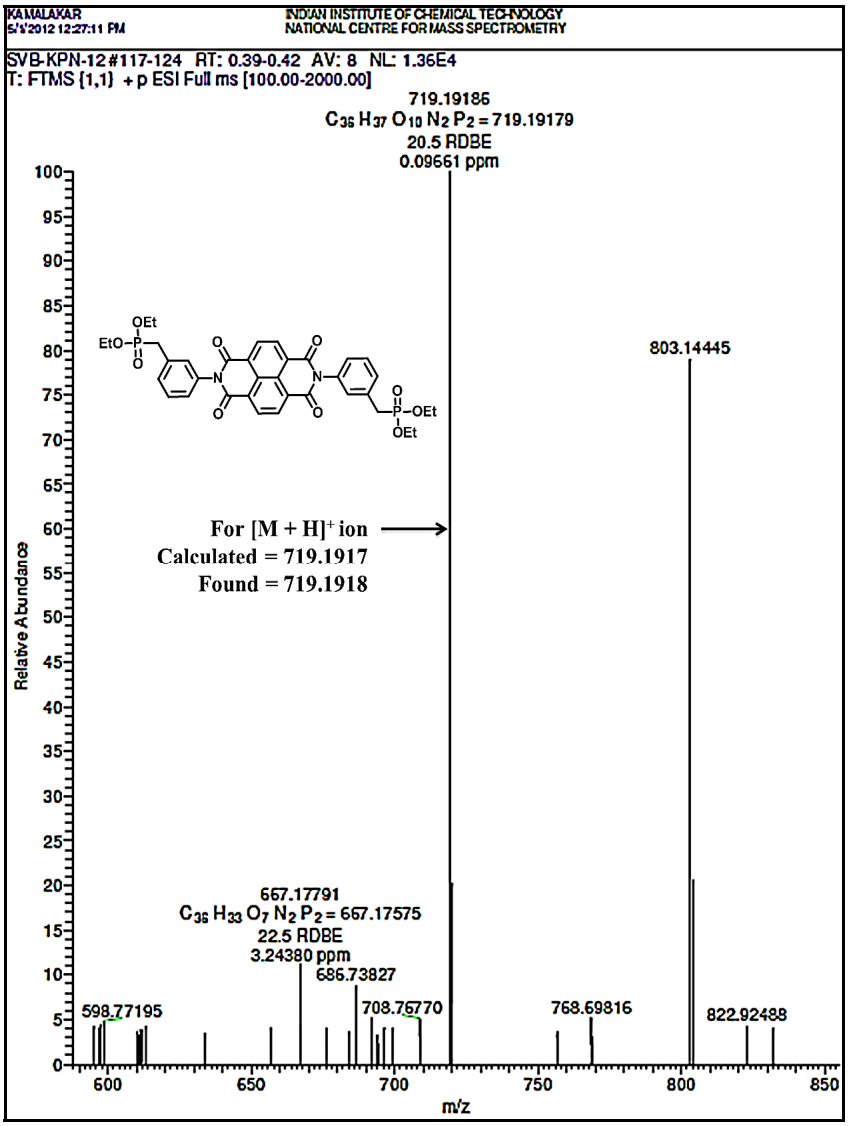


Fig. S8— HRMS spectrum of **NDI-2**

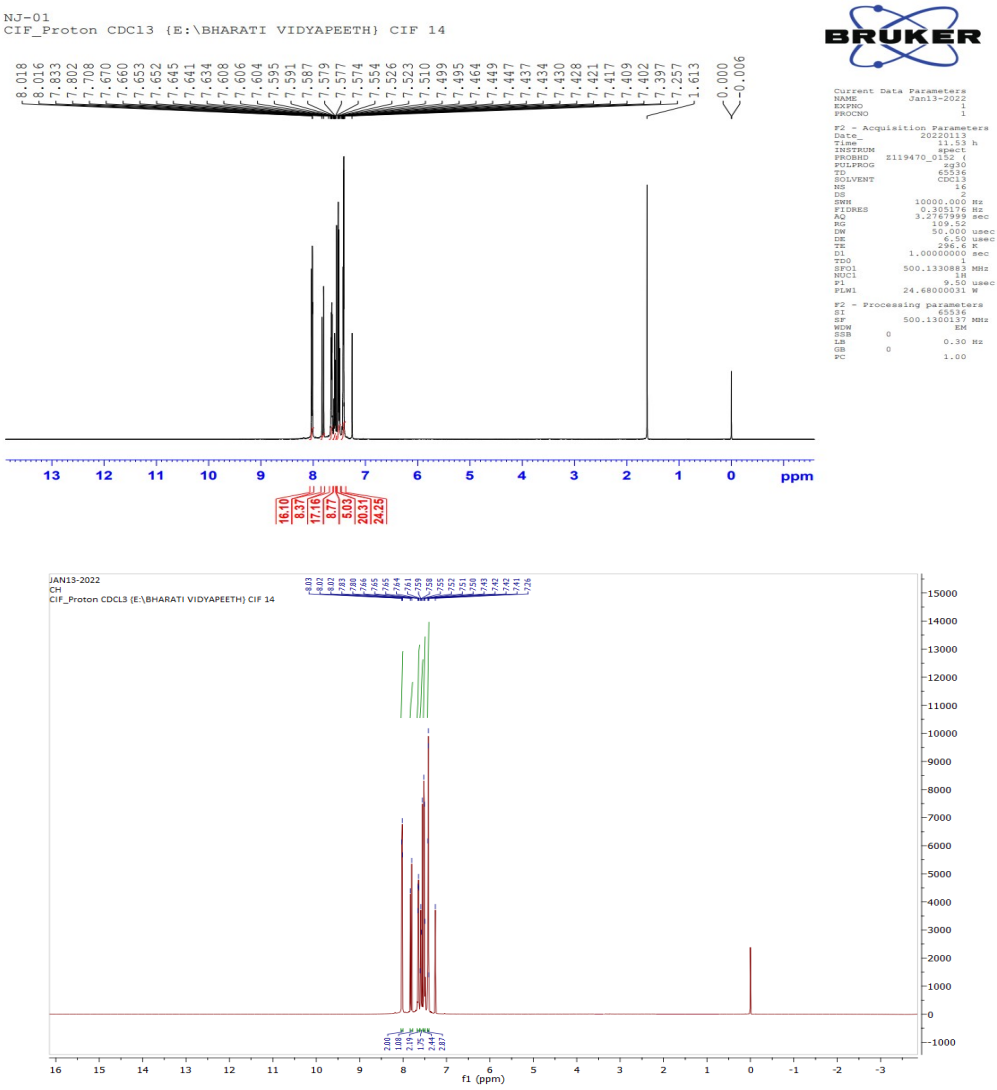


Fig. S4 — ^{1}H NMR spectra of **CH**

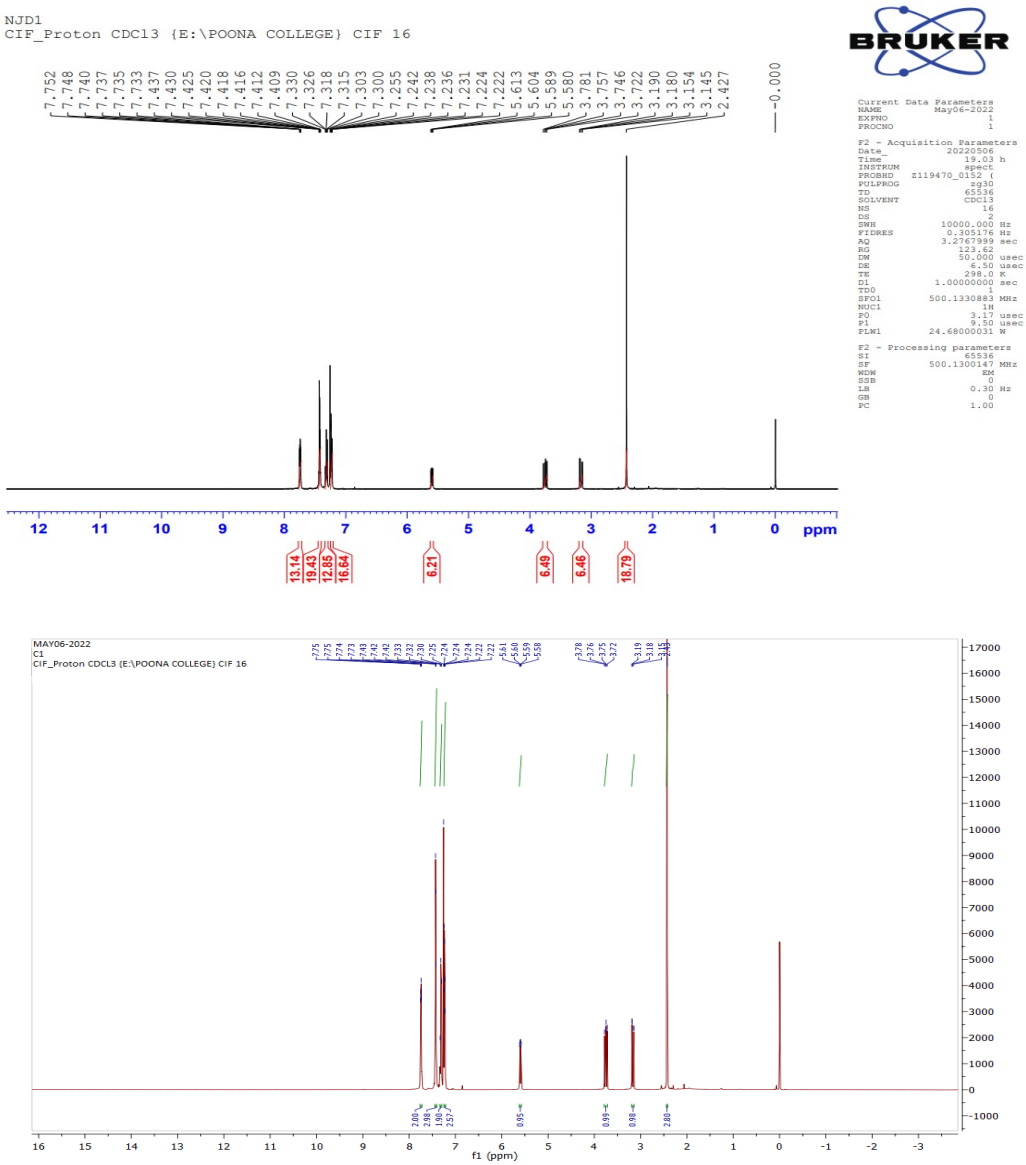


Fig. S5 — ^{1}H NMR spectra of **NDJ1**

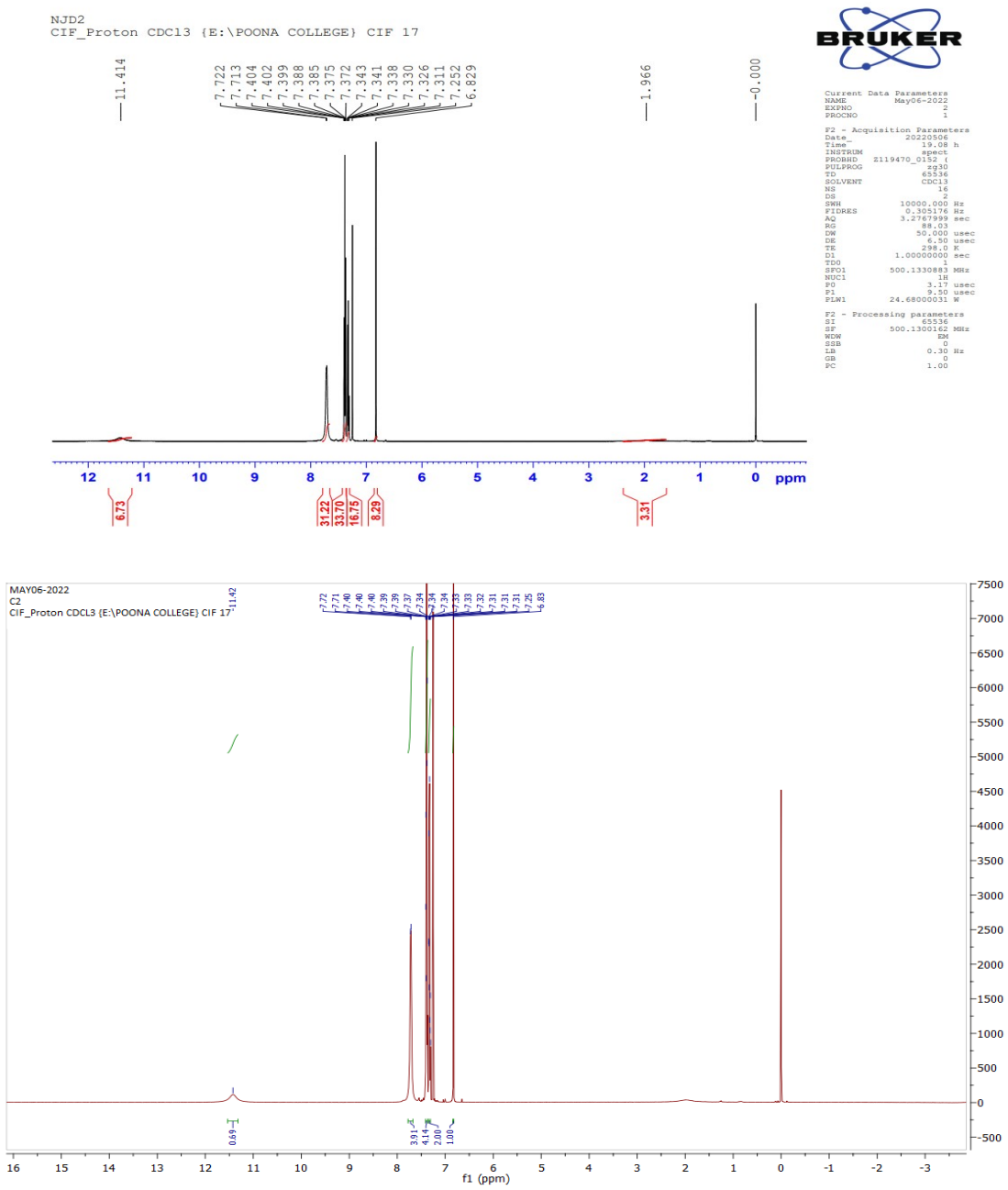


Fig. S6 — ^{1}H NMR spectra of **NDJ2**

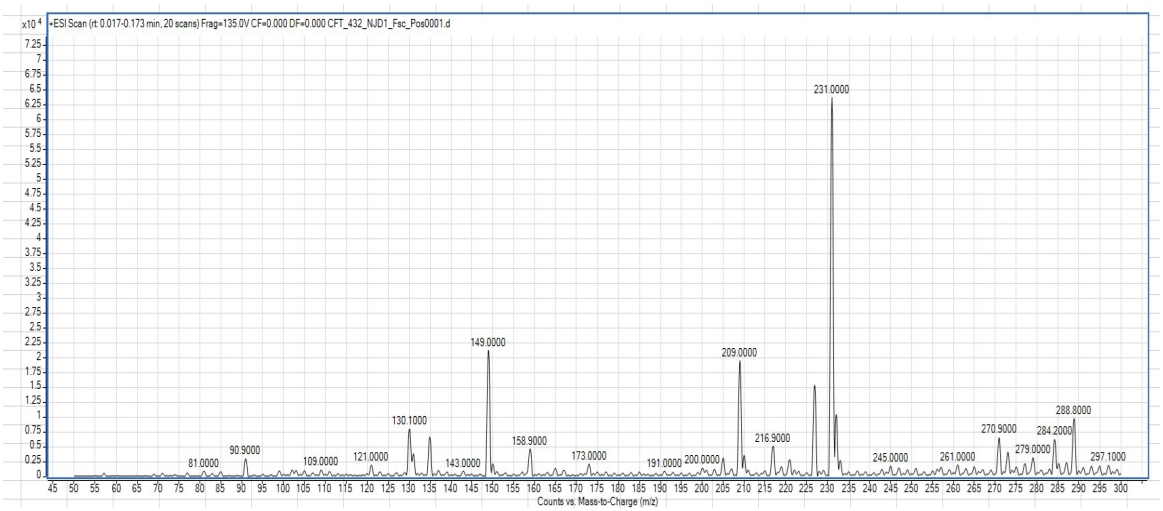


Fig. S7 — Mass spectrum of **CH**

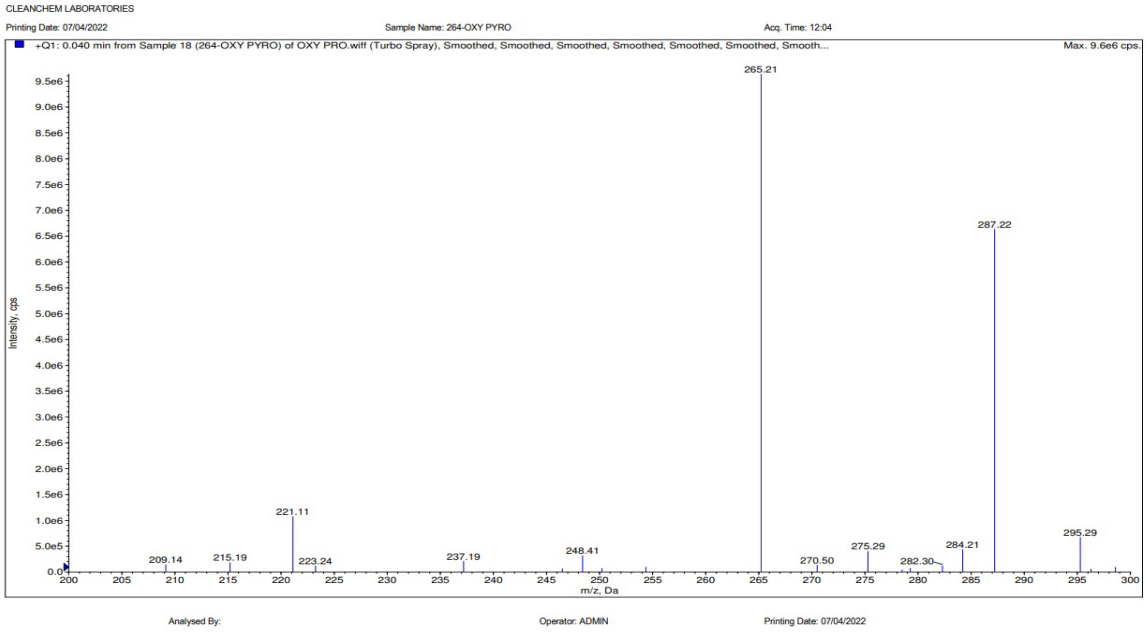


Fig. S8 — Mass spectrum of **NDJ1**

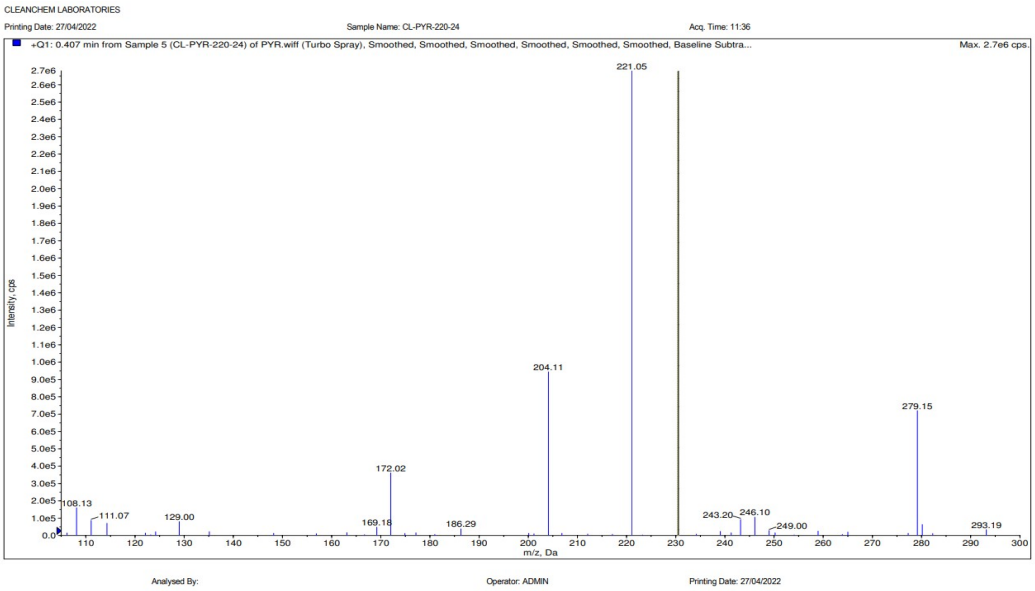
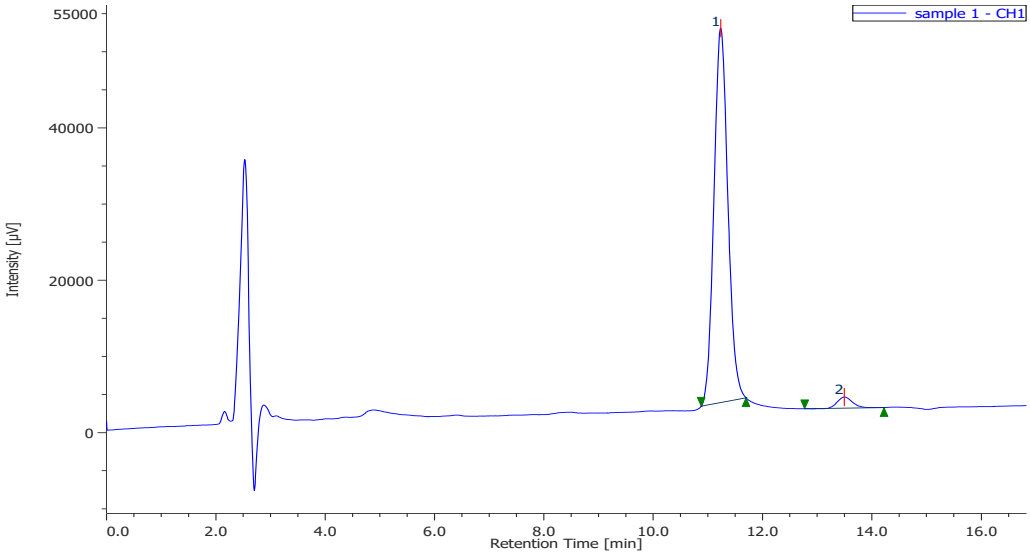
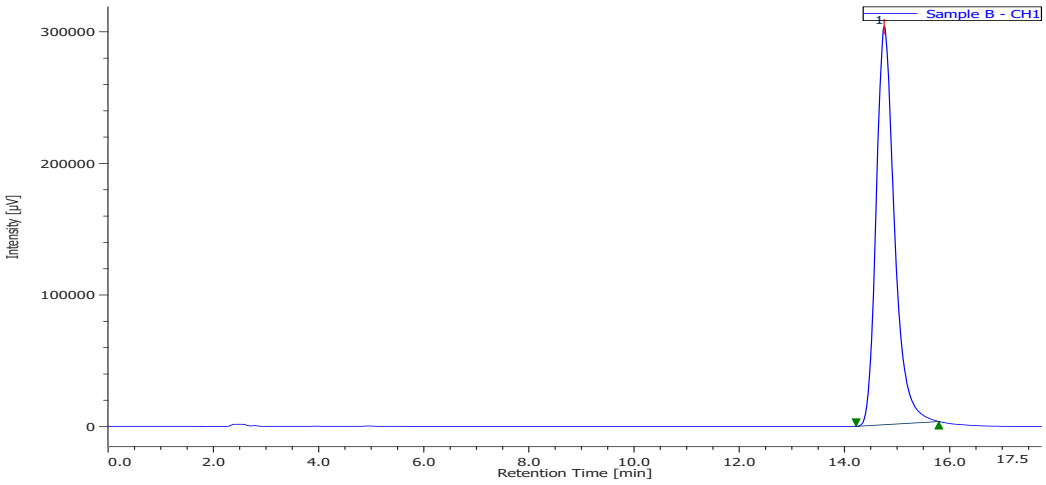


Fig. S9 — Mass spectrum of **NDJ2**



RT	Area	Height	Area%	Height% (Purity)	Quantity
11.233	868454	49108	96.816	97.104	N/A
13.492	28562	1465	3.184	2.896	N/A

Fig. S10 — HPLC percent purity graph of **NDJ1**



RT	Area	Height	Area%	Height% (Purity)	Quantity
14.7	868454	28108	99.121	99	N/A

Fig. S11 — HPLC percent purity graph of **NDJ2**