Supporting Information

Design and Development of an Isatin-1,2,3-Triazole Hybrid Analogueg as a Potent Anti-Inflammatory Agent with Enhanced Efficacy and Gene Expression Modulation

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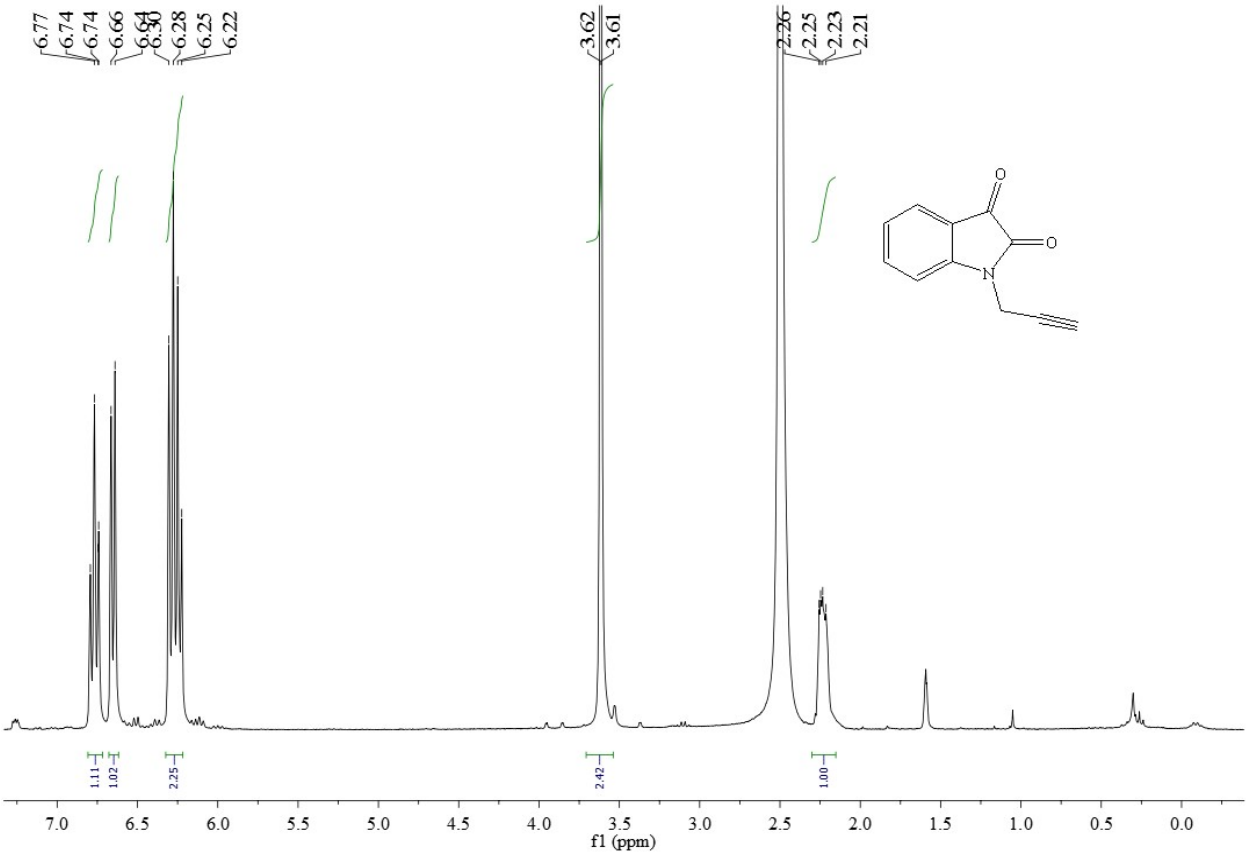
ABSTRACT: Isatin (1H-indole-2,3-dione) and its derivatives have been found to exhibit various biological activities, including anticancer and antidiabetic properties. In this study, a series of nine isatin-1,2,3-triazole conjugates were synthesized and evaluated for their anti-inflammatory potential via in vitro experiments. The synthesis involved the propargylation of isatin **1** with propargyl bromide to obtain N-propargyl isatin **2**, which was then subjected to click reactions with different aromatic azides to yield isatin-N-1,2,3-triazoles (**3a-i**). The structures of all the compounds were confirmed via NMR and HR-MS. The final isatin analogues were tested for their ability to attenuate the production of proinflammatory cytokines in Lipopolysaccaride (LPS)-induced human leukemia monocytic cell line THP-1 cells. Importantly, none of the compounds had any negative effect on THP-1 cell viability at the tested concentrations of 4 mM and 8 mM respectively. LPS induced the production of the cytokines: Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) and Monocyte chemoattractant protein-1 (MCP-1) by 351.4, 7.9 and 14.3 fold respectively, in THP-1 cells. However, treatment with compound **3e** markedly attenuated the levels of TNF-α (by 6.6 fold and 1.5 fold), IL-6 (by 1.03 fold and 1.41 fold) and MCP-1 (by 3.3 fold and 1.7 fold) by several fold at 4 mM and 8 mM concentrations respectively. Furthermore, in the gene expression modulation studies, **3e** was found to downregulate the genes responsible for the production of TNF-α (24 and 25 fold), IL-6 (148 and 502 fold) and MCP-1 (50 and 25 fold) at the two tested concentrations compared with their expression in LPS induced THP-1 cells (135 fold, 6612 fold, and 68.8 fold respectively). Thus, **3e** markedly attenuated the secretion of TNF-α, IL-6 and MCP-1 from LPS treated THP-1 cells, and also the expression of concerned genes. At the lowest dose tested, i.e., 4mM, **3e** had the greatest effect on both gene expression and marker secretion.

Keywords: Isatin; 1,2,3-Triazole, Anti-inflammatory, Cytokines, Nuclear factor

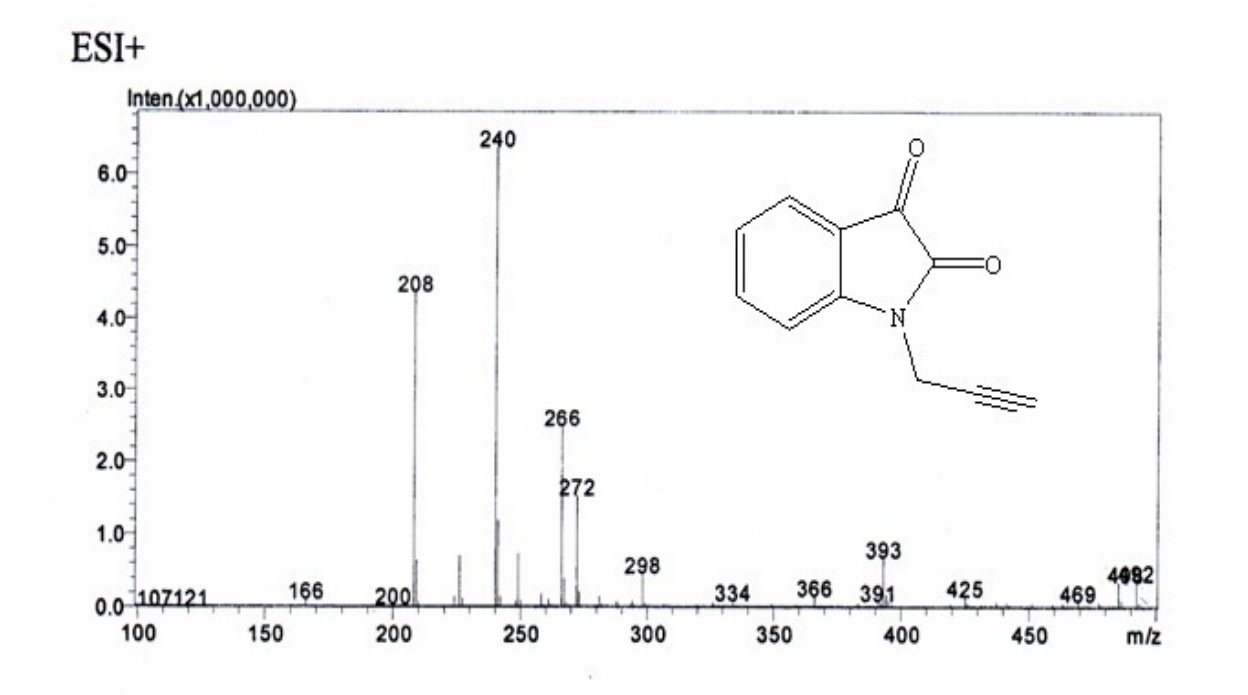
Instrumentation and chemicals

^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature using, 400/500 MHz spectrometers DMSO-*d6* using TMS as internal standard. IR data are given only for compounds with significant functions (OH, C=O) and were recorded as neat or KBr plate and are reported in wave number (cm-1). The data are reported as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz) and integration. High resolution mass spectra were obtained by peak matching. Analytical thin layer chromatography was performed using Kieselgel 60 F254 (E. Merck) on silica gel plates. All reactions were carried out under an atmosphere of nitrogen in glassware, which had been oven-dried. All chemicals were commercially obtained from AVRA Synthesis Pvt. Ltd and used directly without further purification.

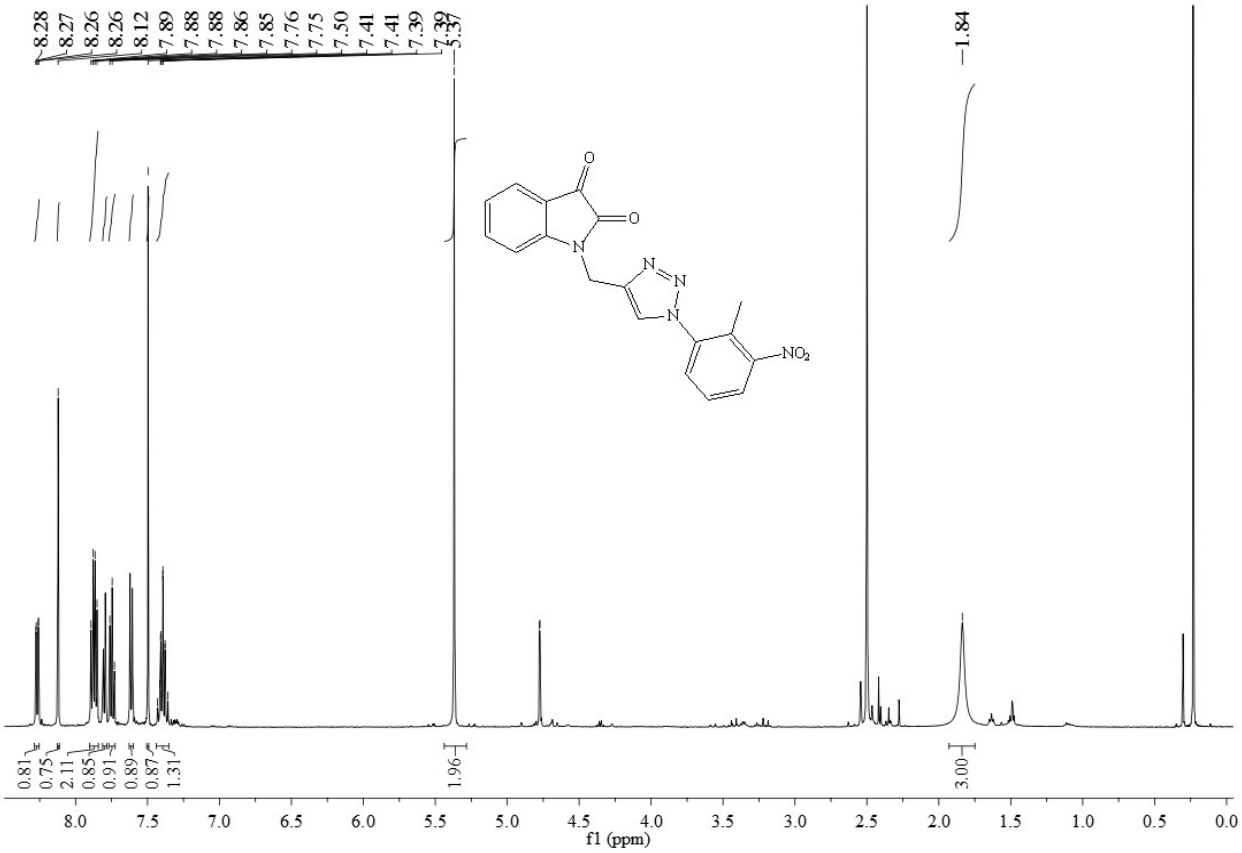
^{1}H NMR SPECTRA of 2 (500 MHz, DMSO-*d6*):



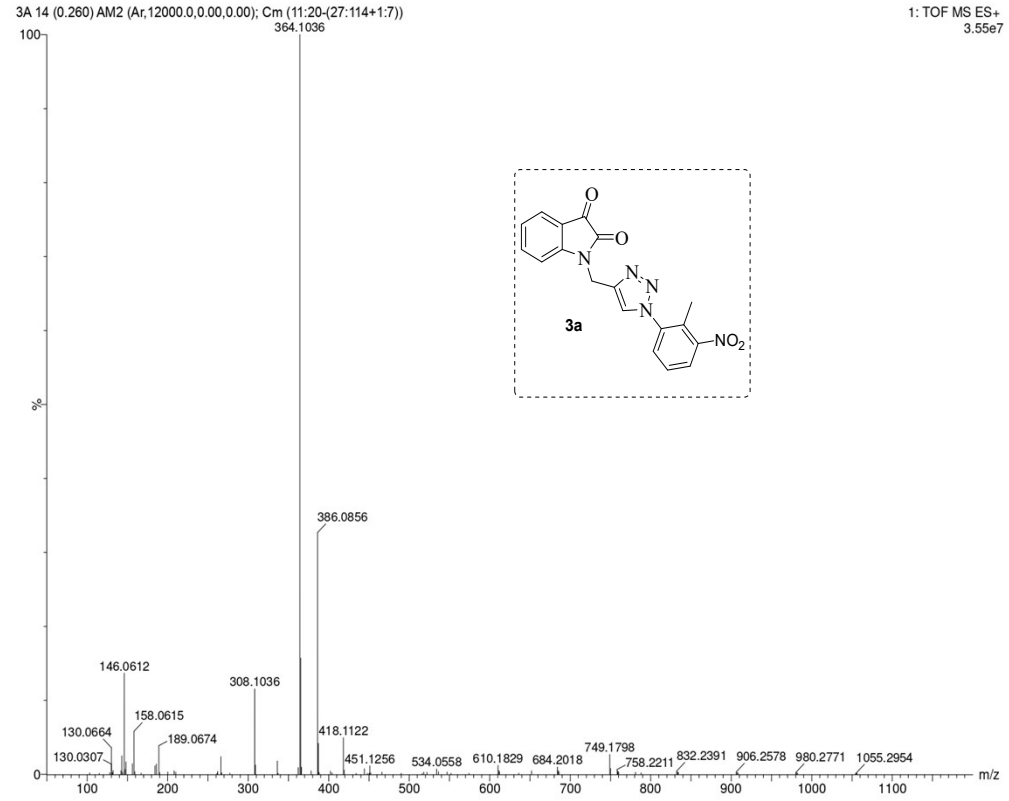
ESI-MASS SPECTRA of 2:



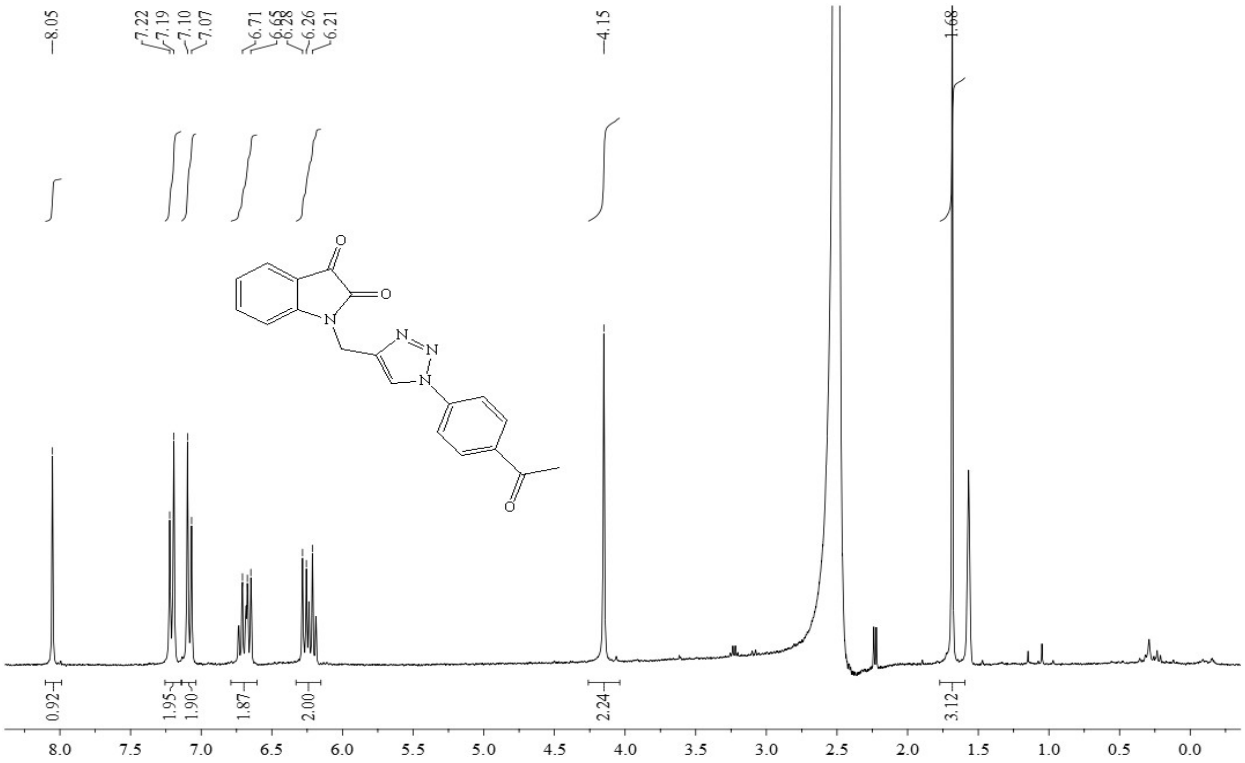
^{1}H NMR SPECTRA of 3a (500 MHz, DMSO-*d6*):



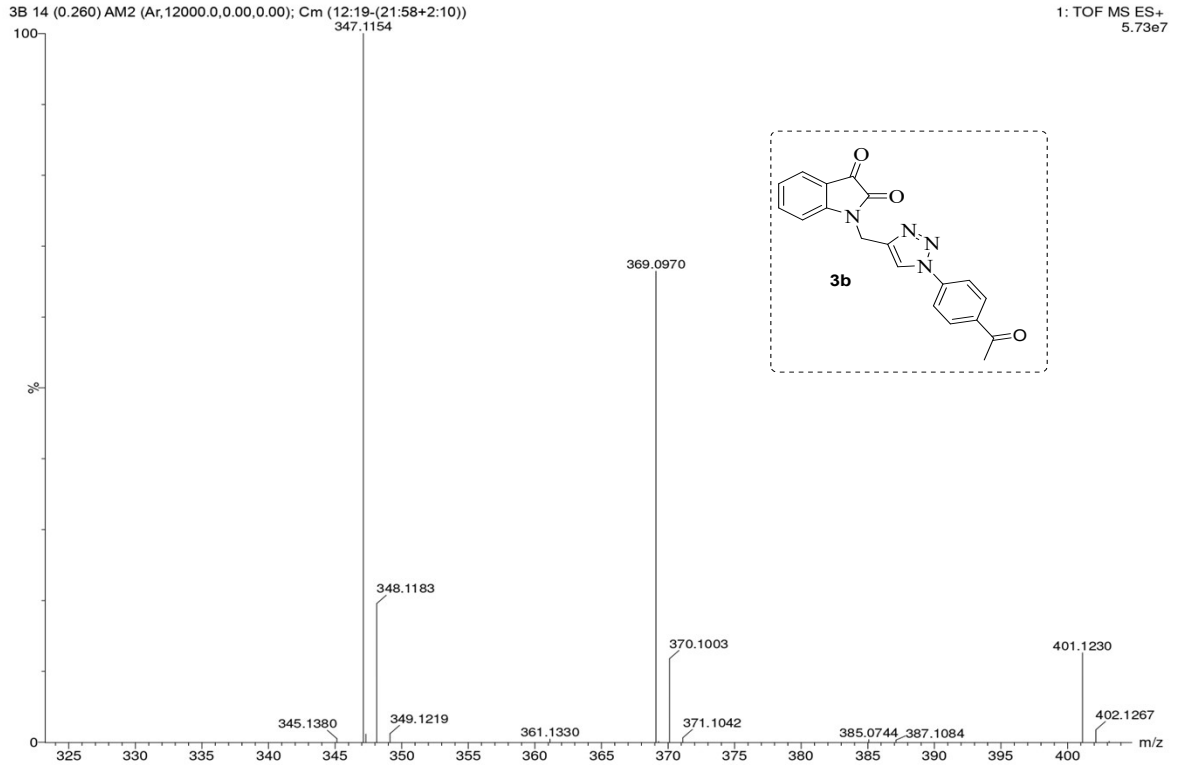
HR-MS SPECTRA of 3a:



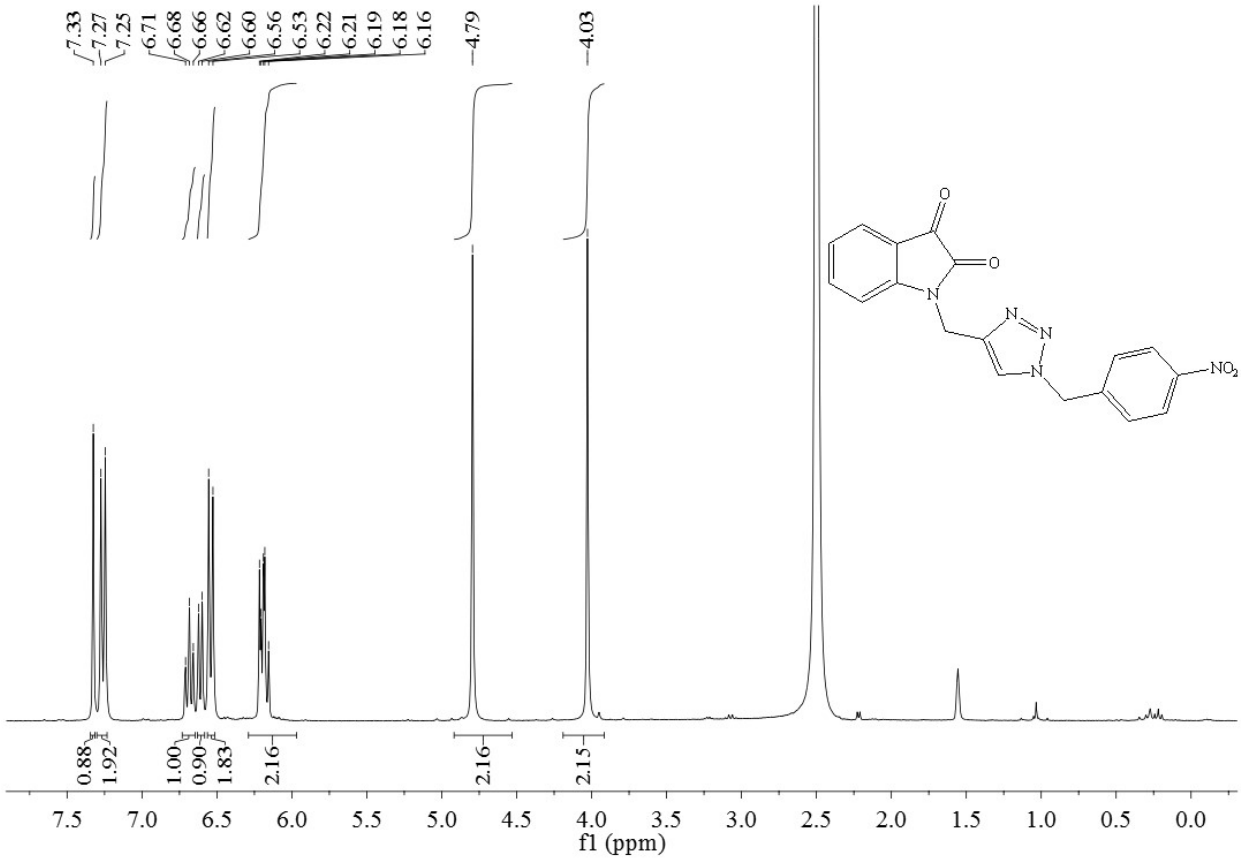
^{1}H NMR SPECTRA of 3b (500 MHz, DMSO-*d6*):



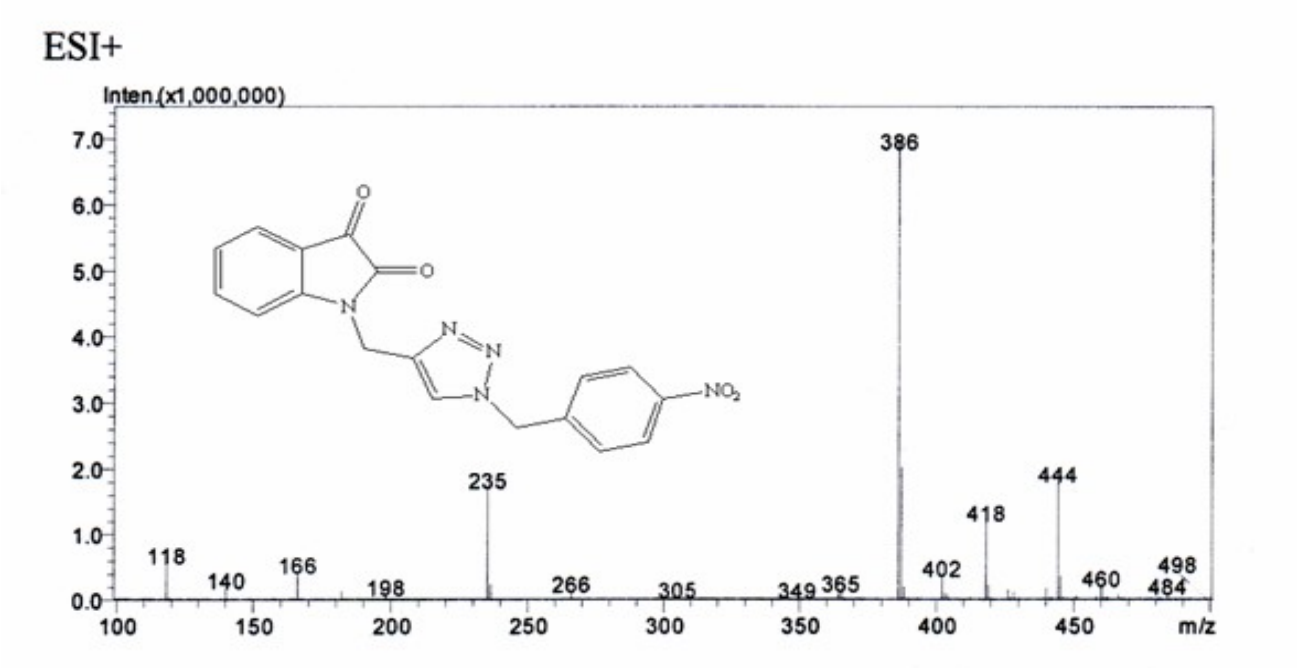
HR-MS SPECTRA of 3b:



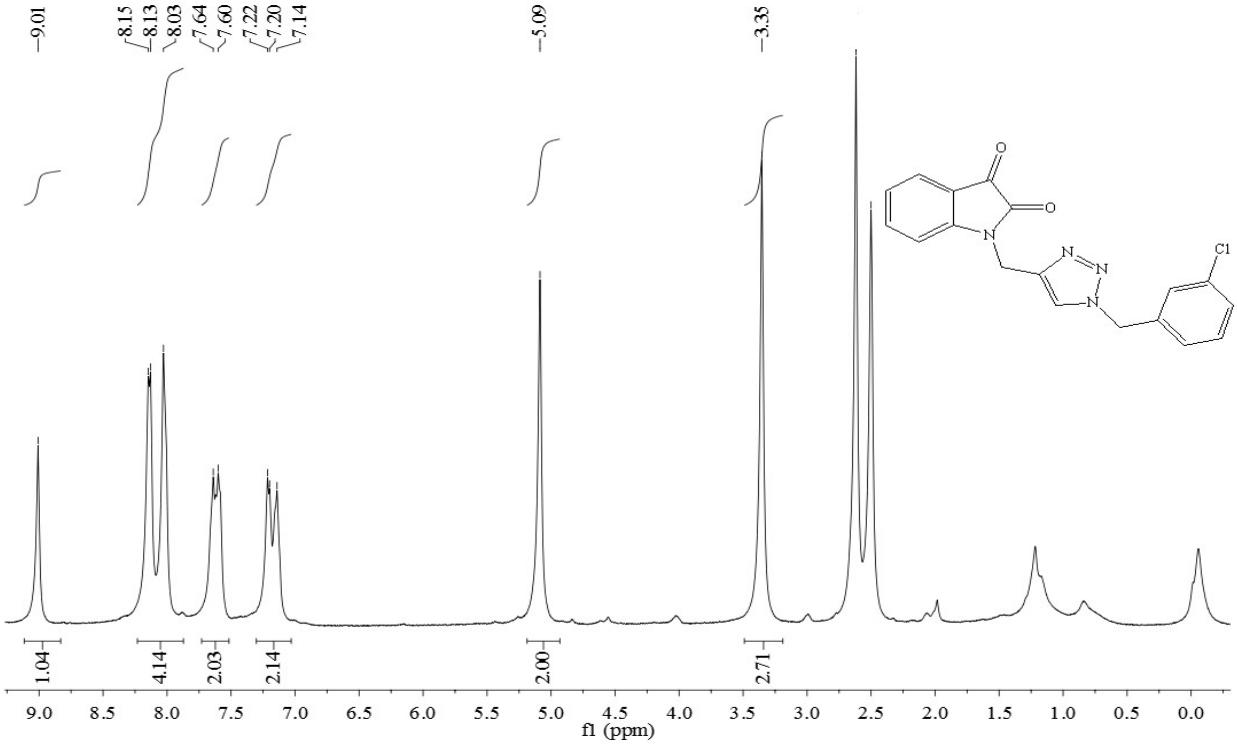
^{1}H NMR SPECTRA of 3c (500 MHz, DMSO-*d6*):



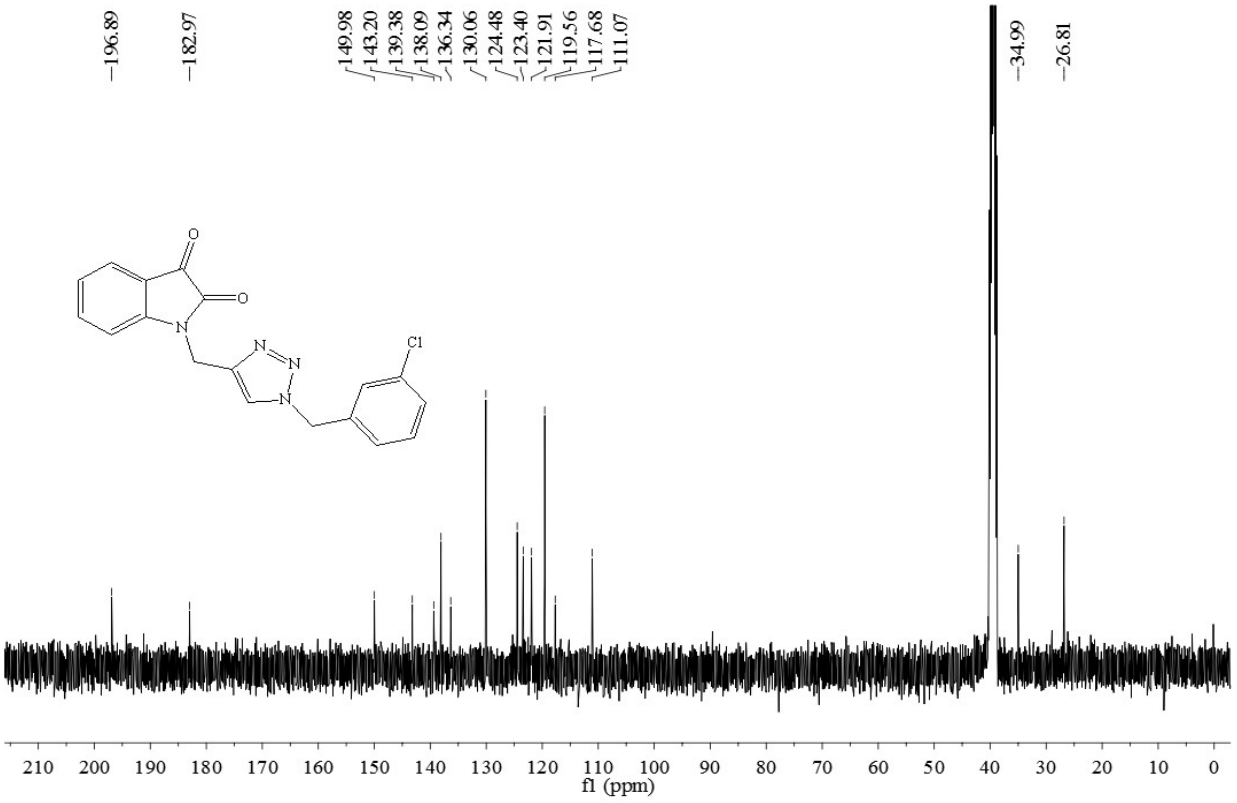
ESI-MASS SPECTRA of 3c:



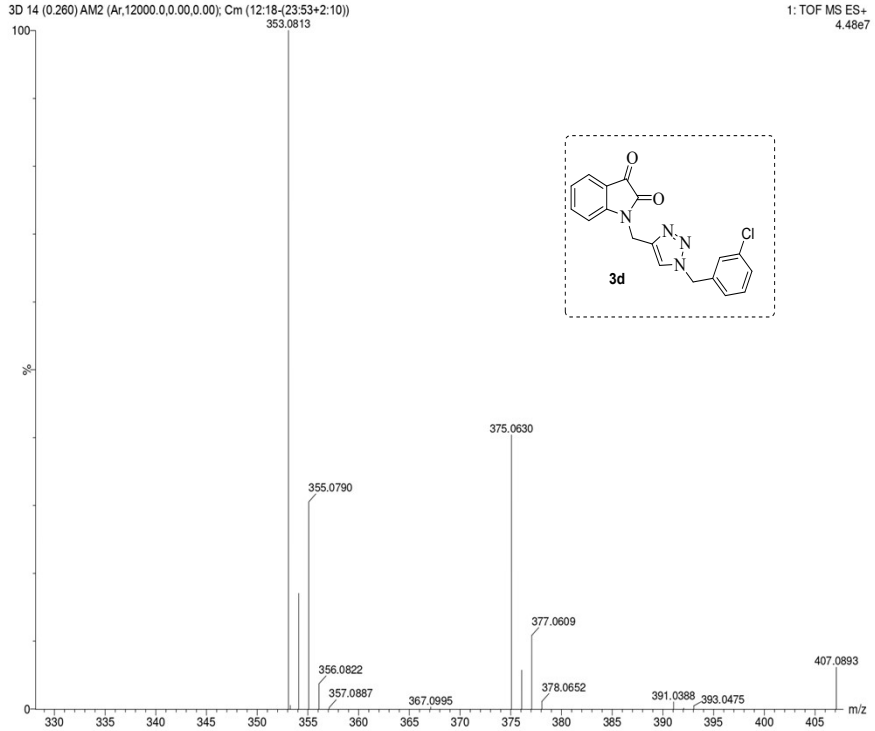
^{1}H NMR SPECTRA of 3d (500 MHz, DMSO-*d6*):



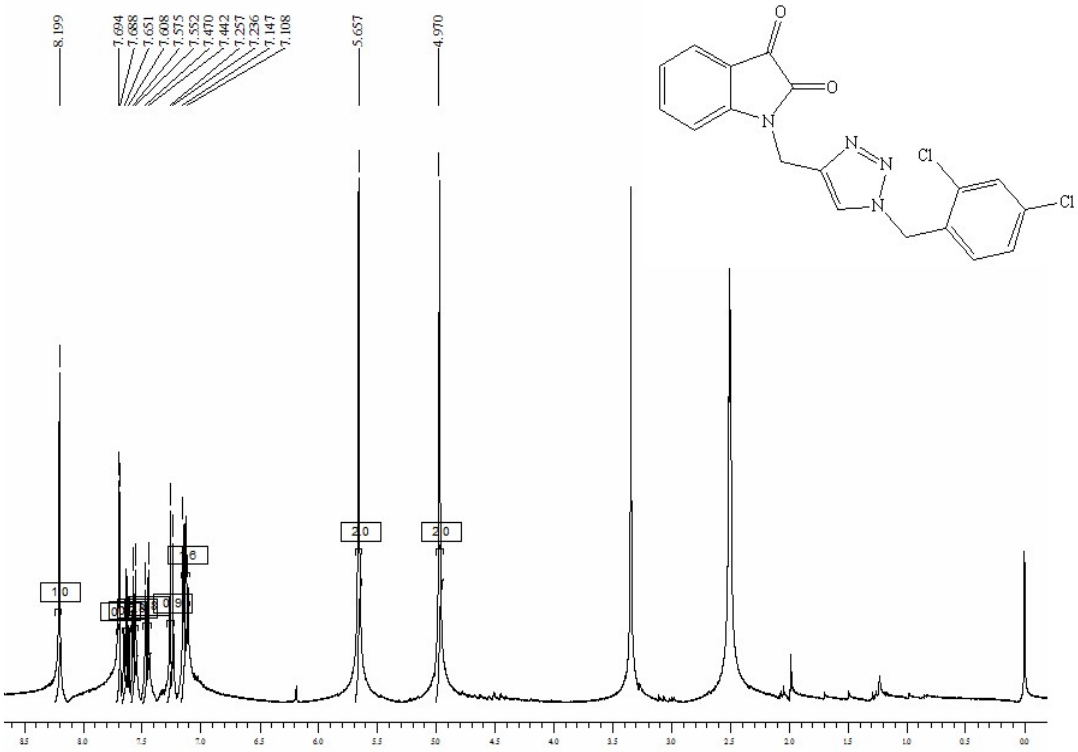
^{13}C NMR SPECTRA of 3d (75 MHz, DMSO-*d6*):



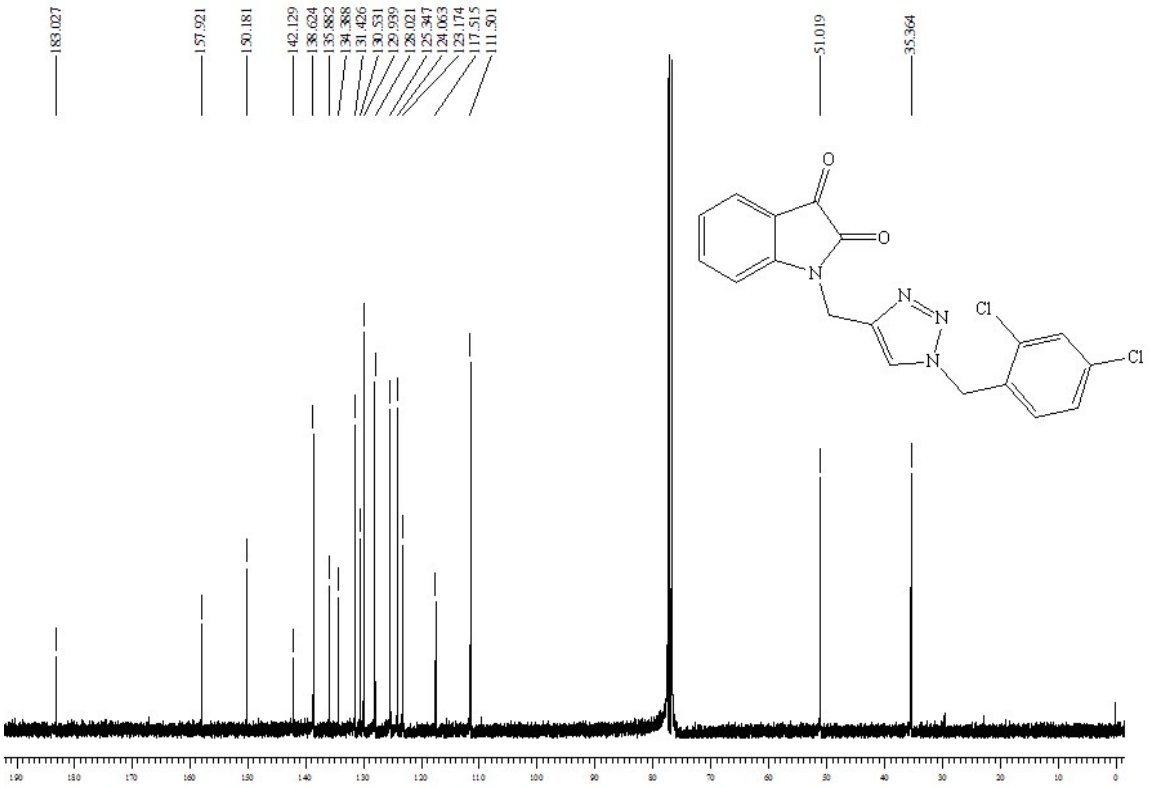
HR-MS SPECTRA of 3d:



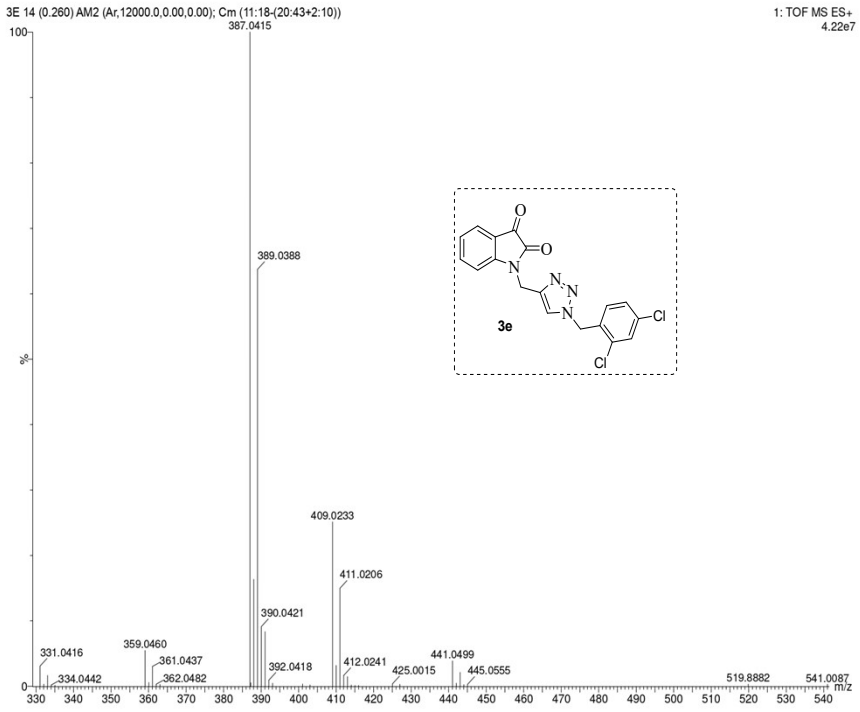
^{1}H NMR SPECTRA of 3e (400 MHz, DMSO-d_6):



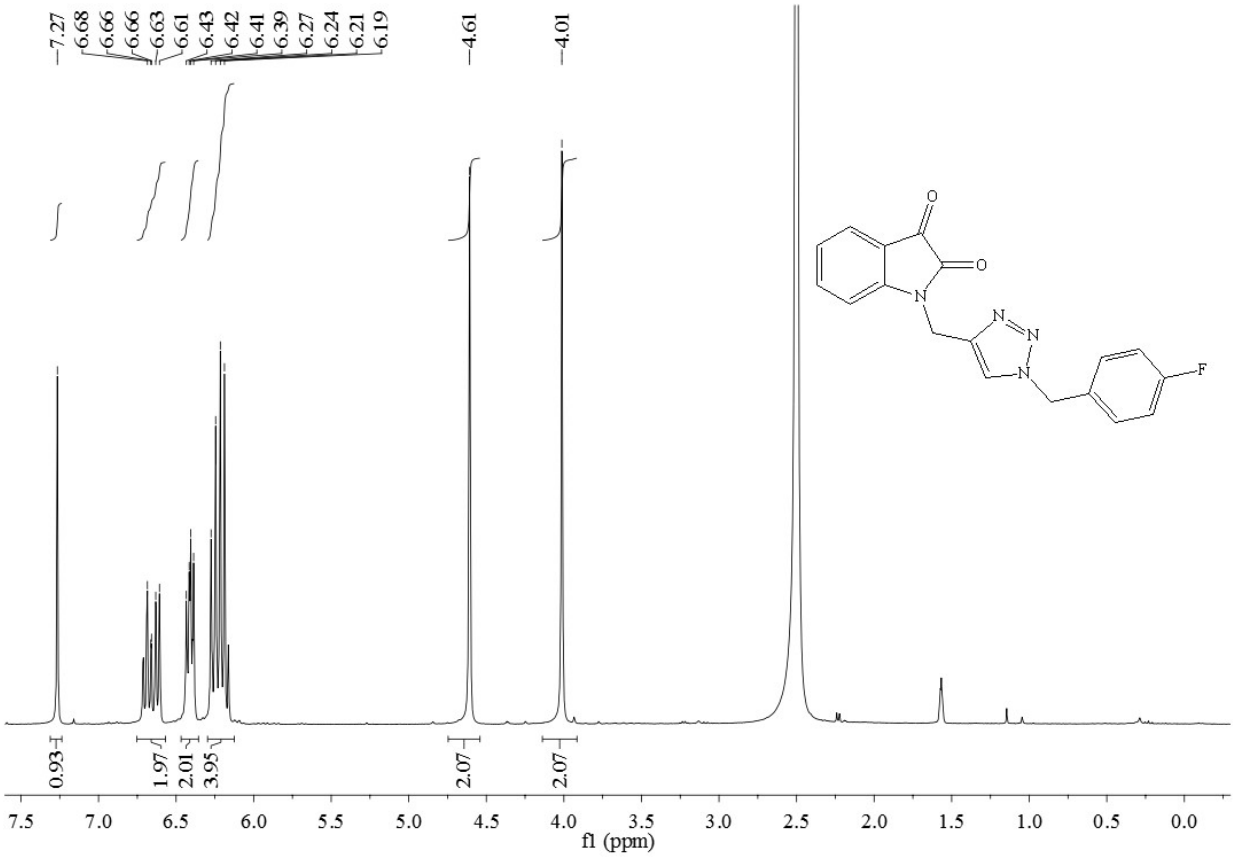
^{13}C NMR SPECTRA of 3e (75 MHz, $CDCl_3$):



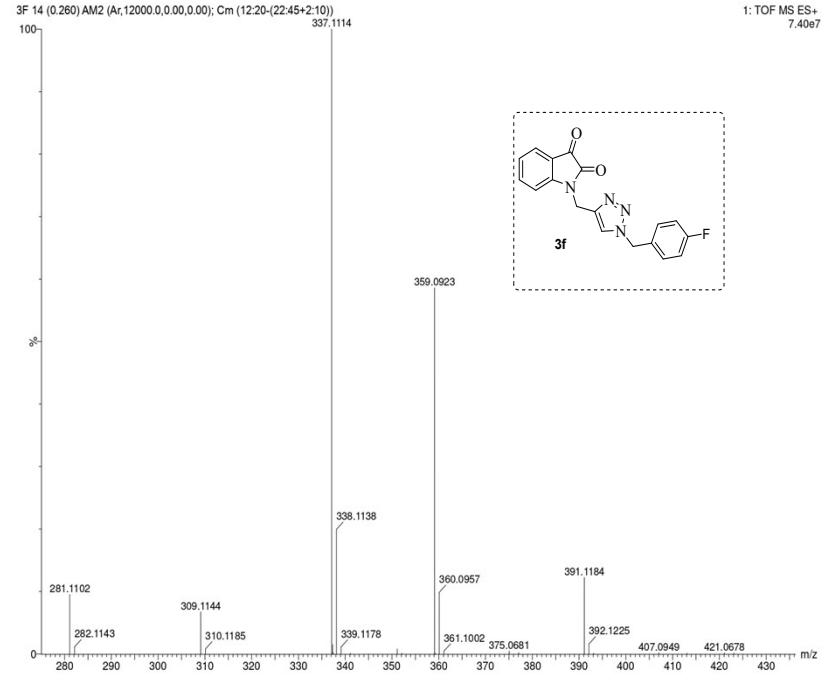
HR-MS SPECTRA of 3e:



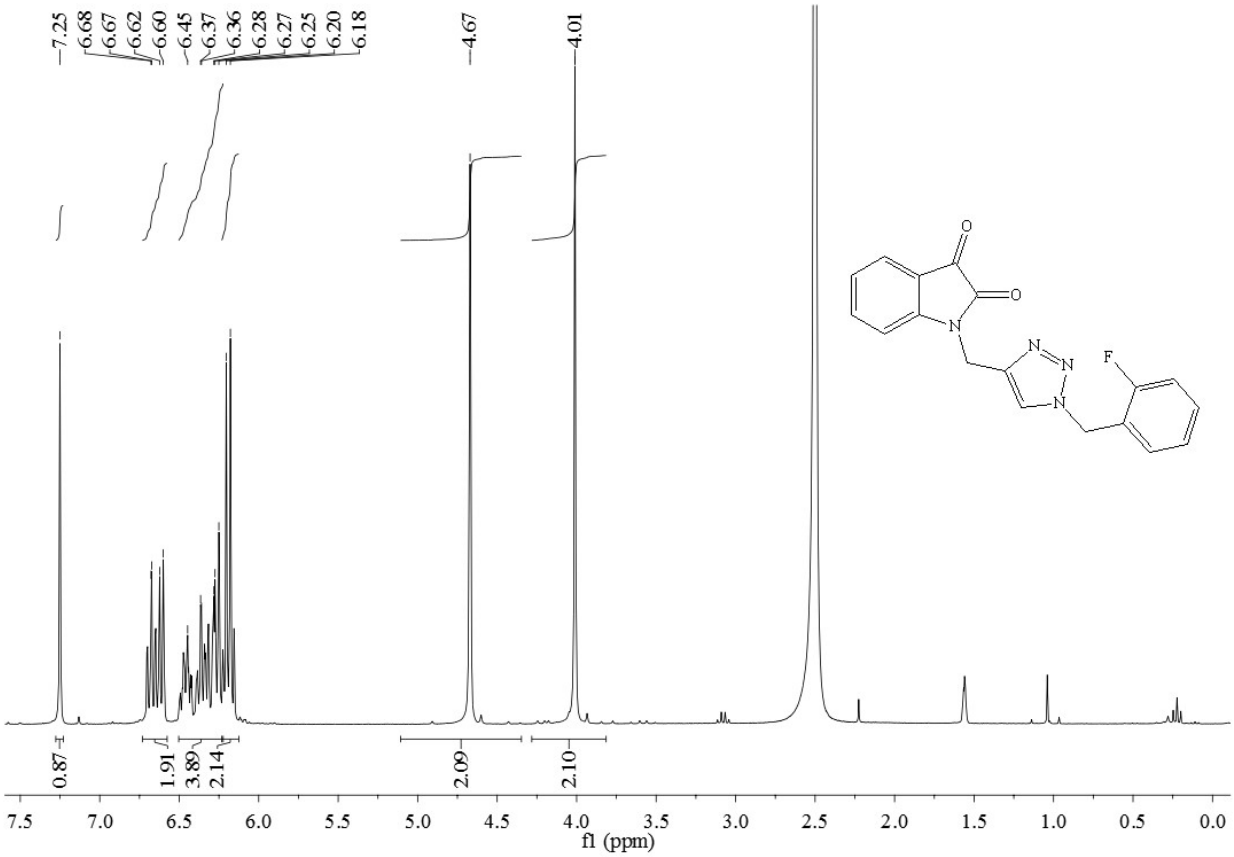
^{1}H NMR SPECTRA of 3f (500 MHz, DMSO-*d6*):



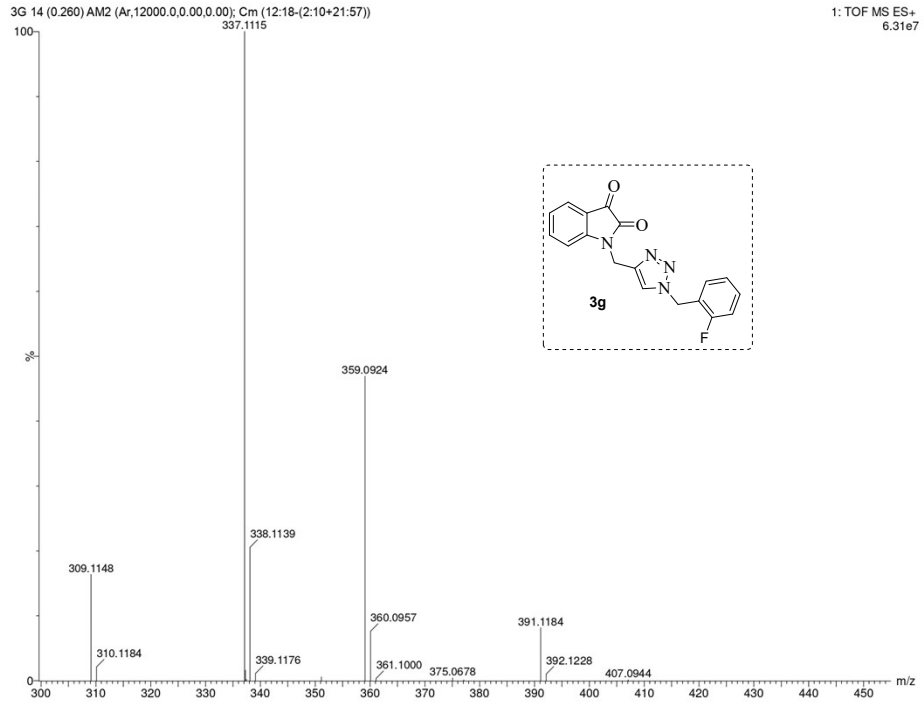
HR-MS SPECTRA of 3f:



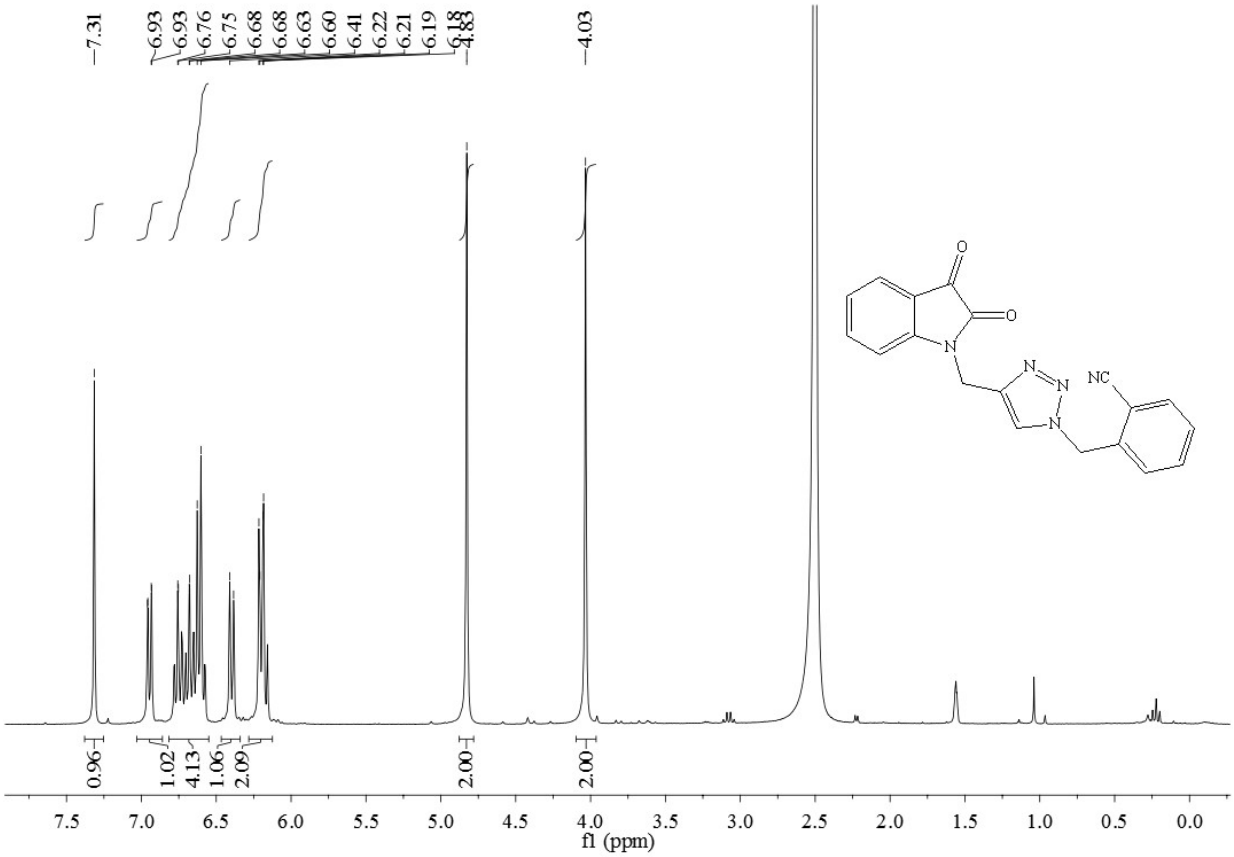
^{1}H NMR SPECTRA of 3g (500 MHz, DMSO-*d6*):



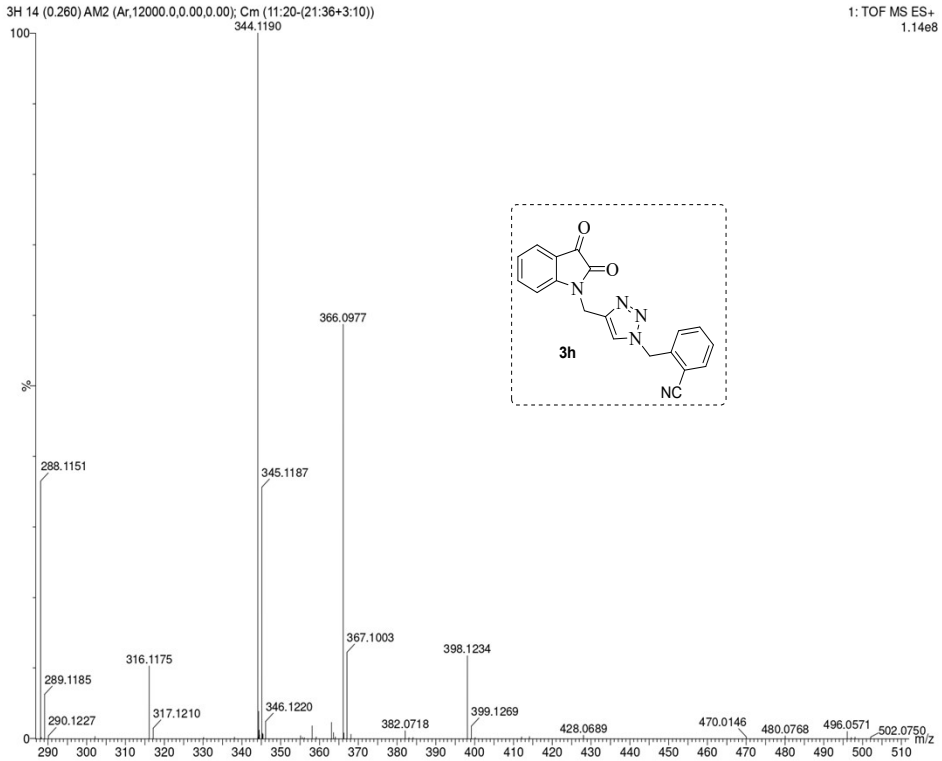
HR-MS SPECTRA of 3g:



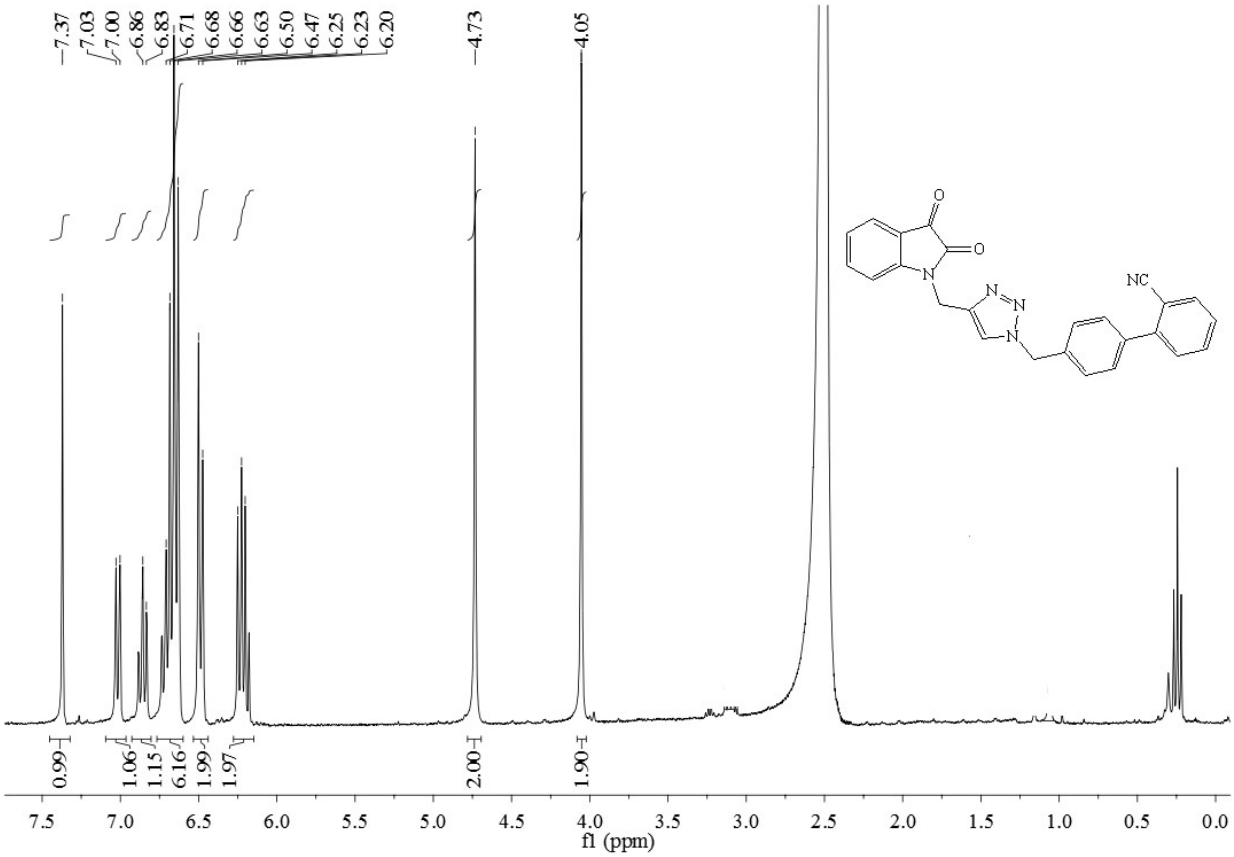
^{1}H NMR SPECTRA of 3h (500 MHz, DMSO-*d6*):



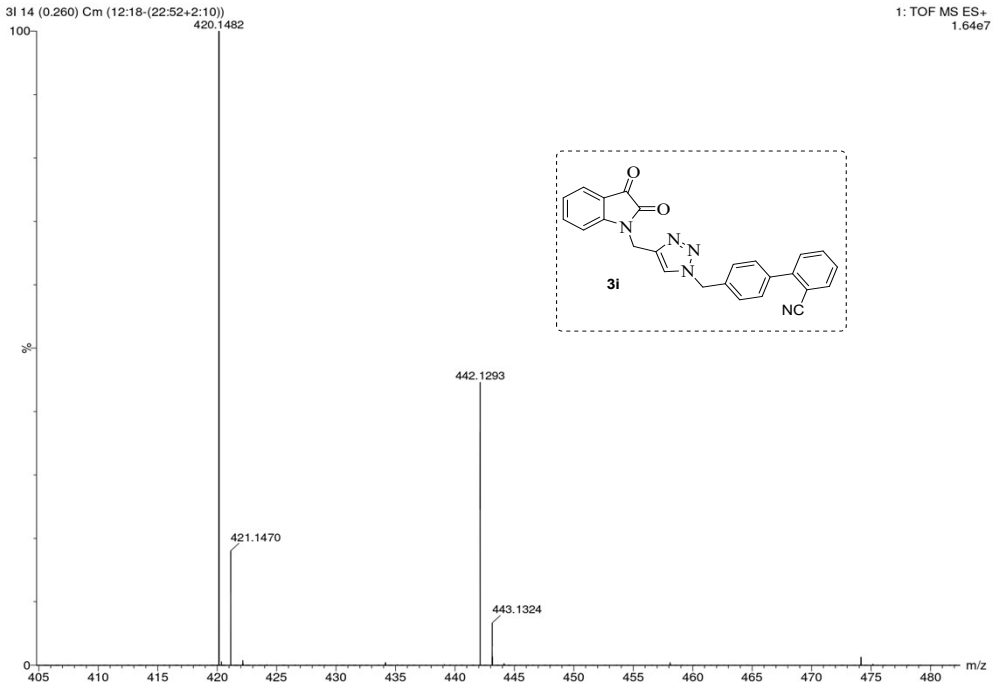
HR-MS SPECTRA of 3h:



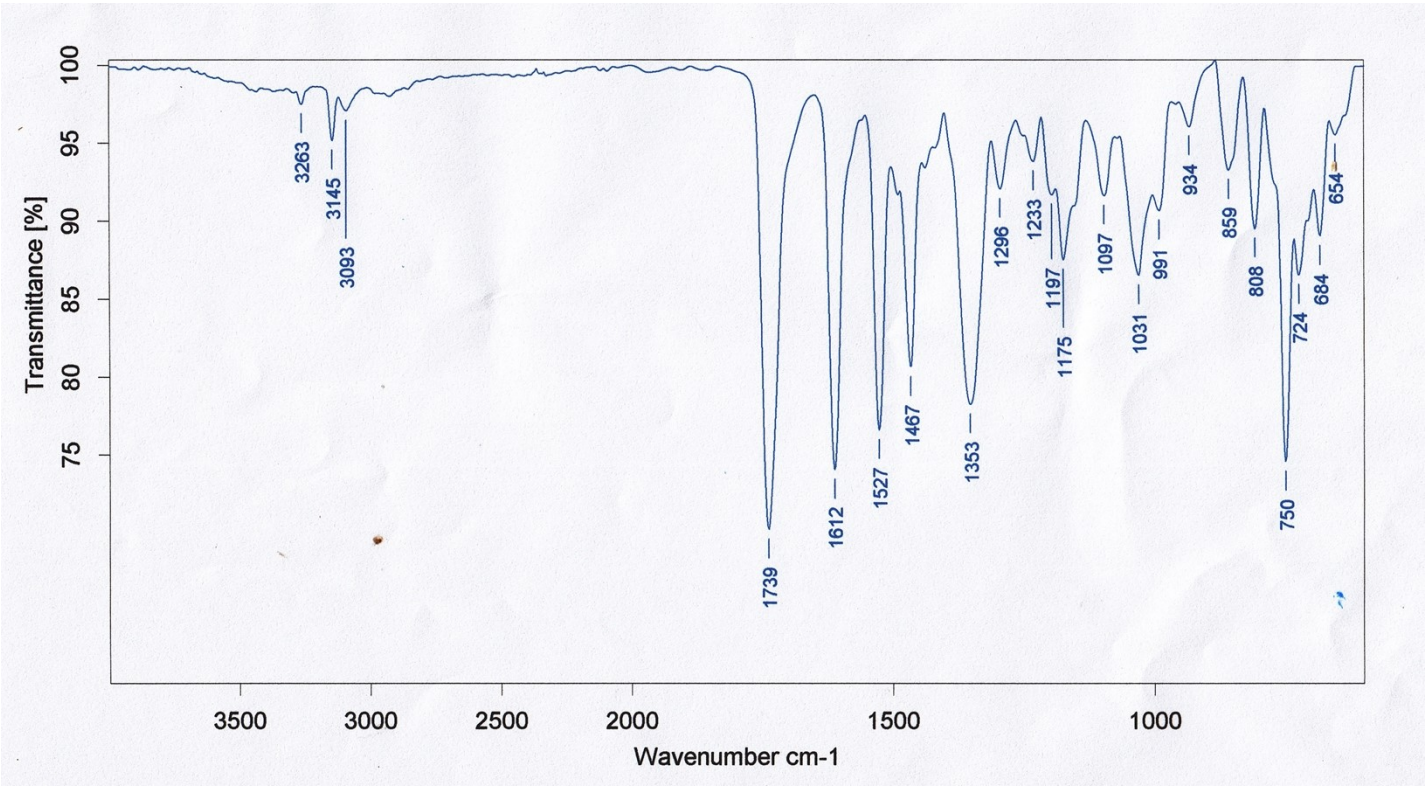
^{1}H NMR SPECTRA of 3i (500 MHz, DMSO-*d6*):



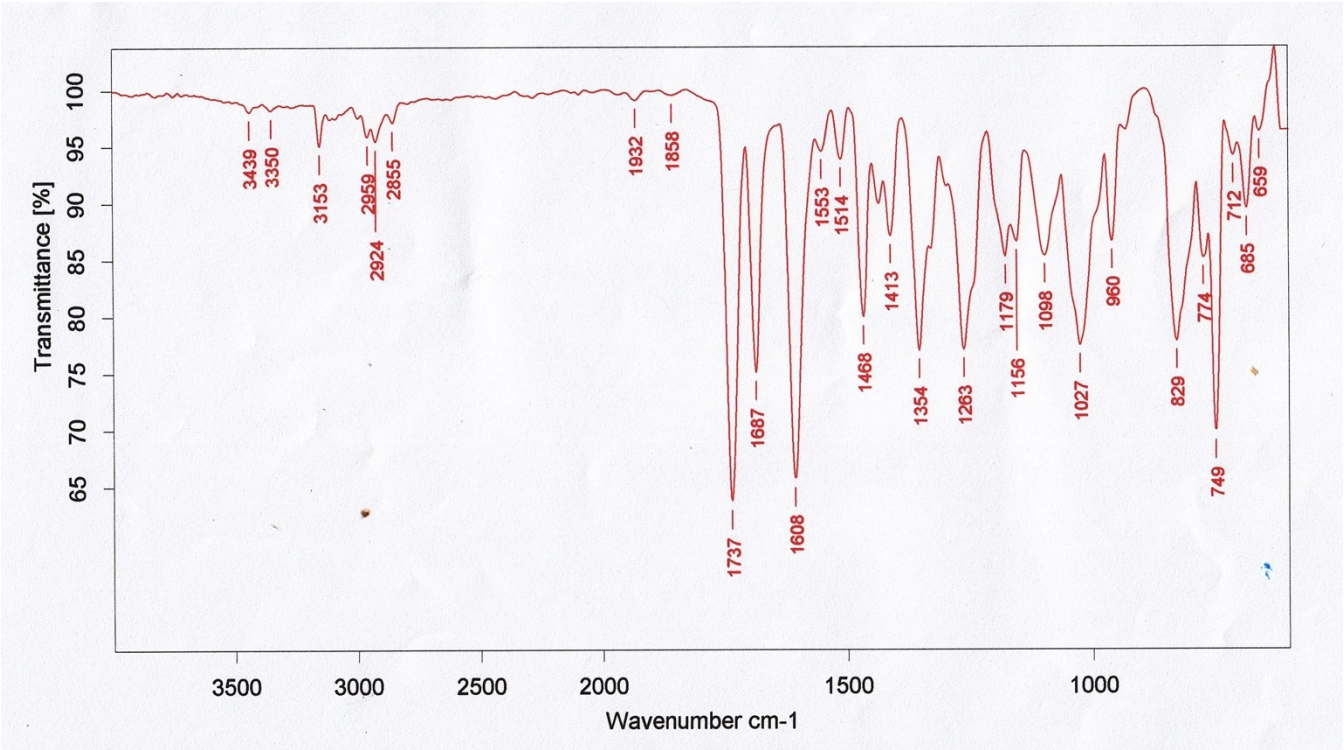
HR-MS SPECTRA of 3i:



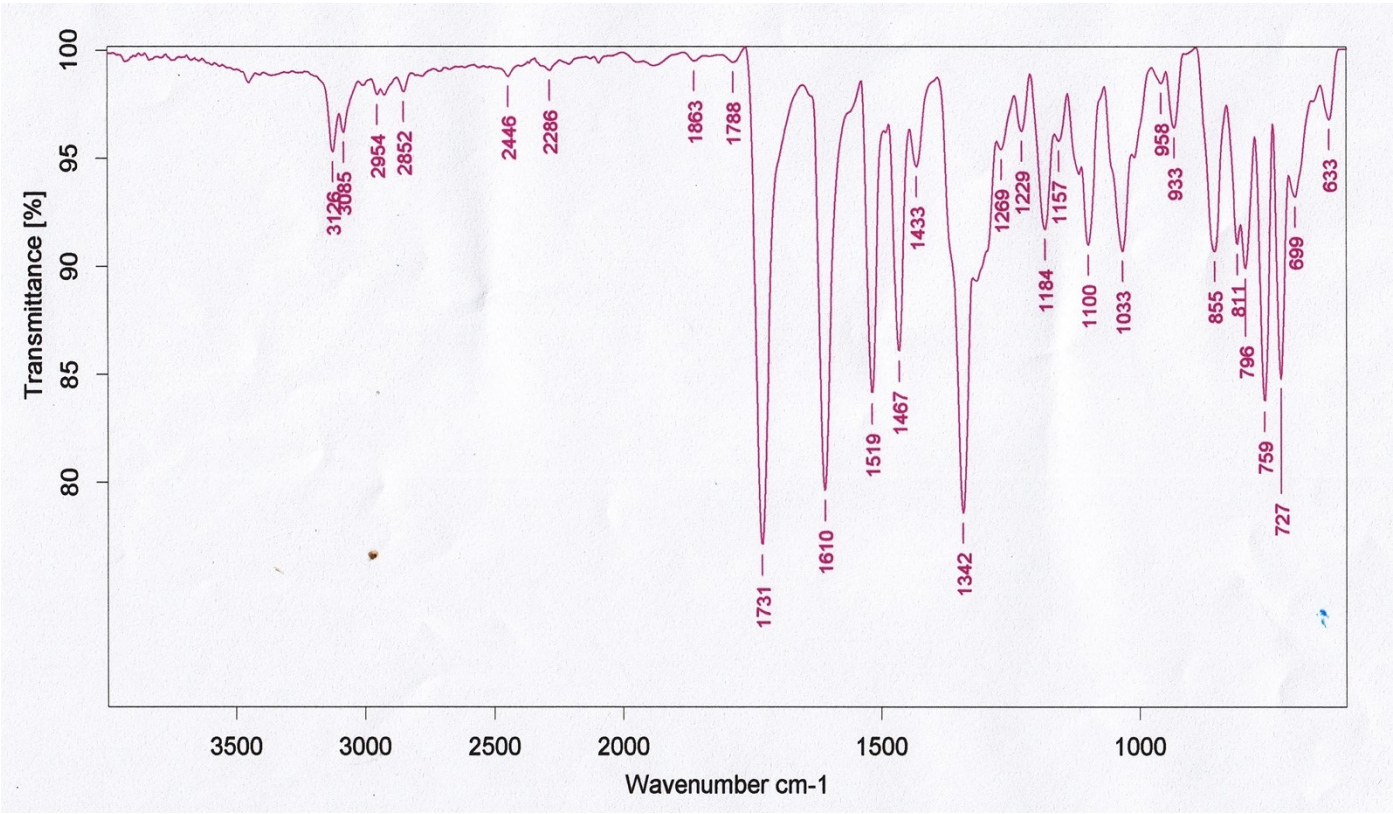
IR SPECTRA of 3a:



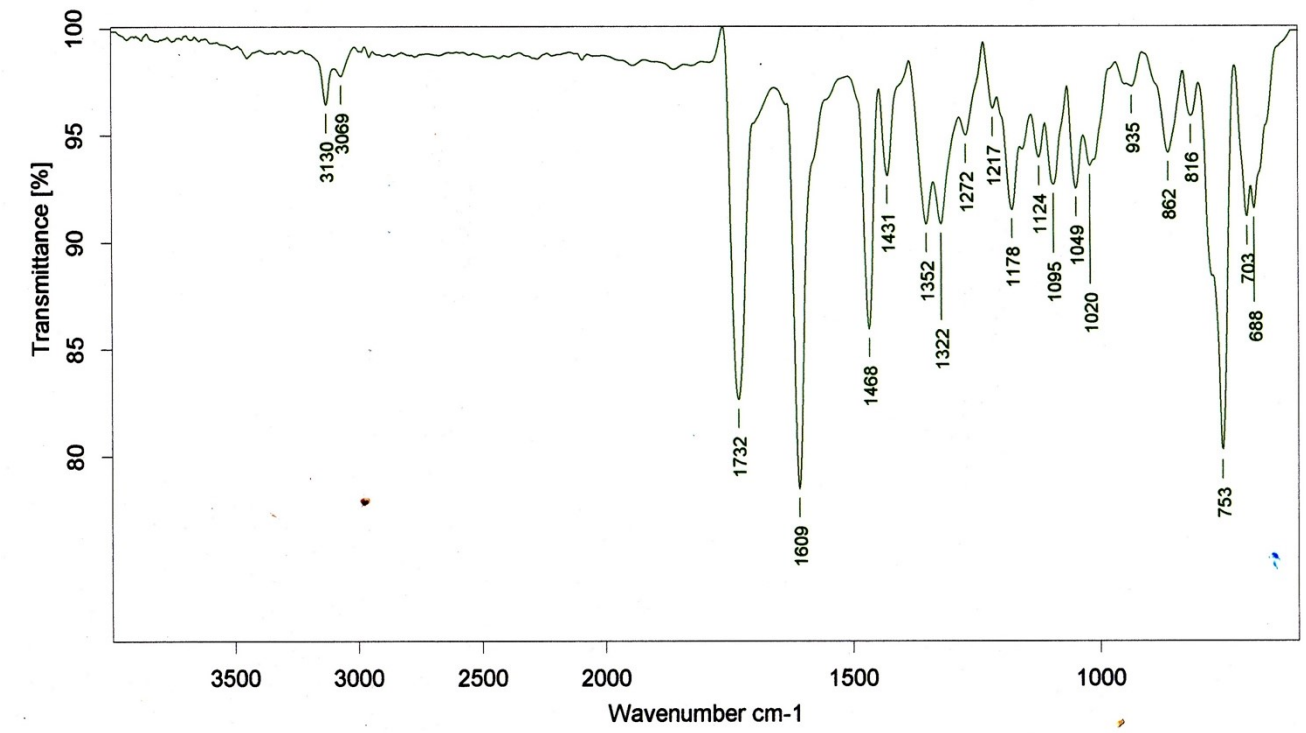
IR SPECTRA of 3b:



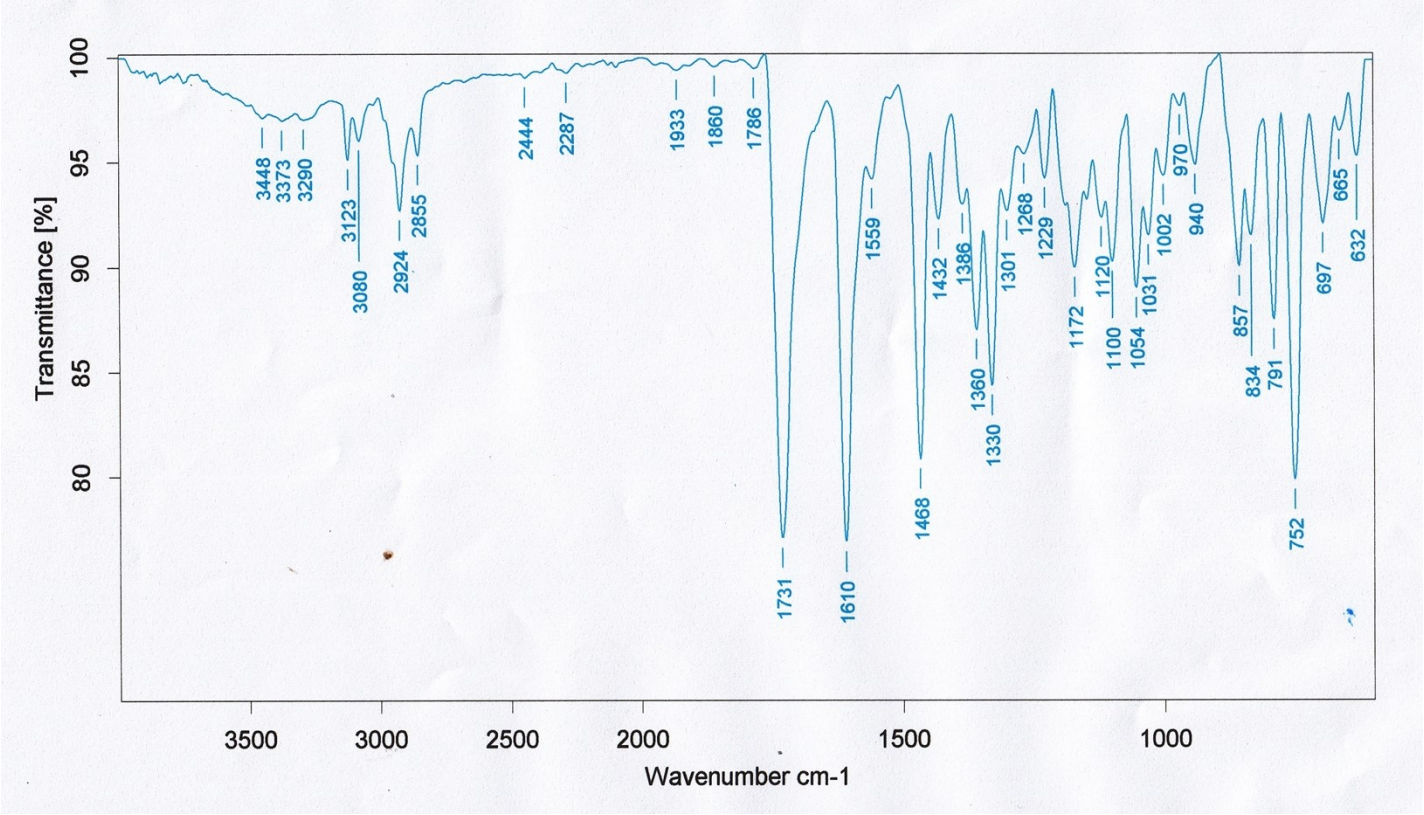
IR SPECTRA of 3c:



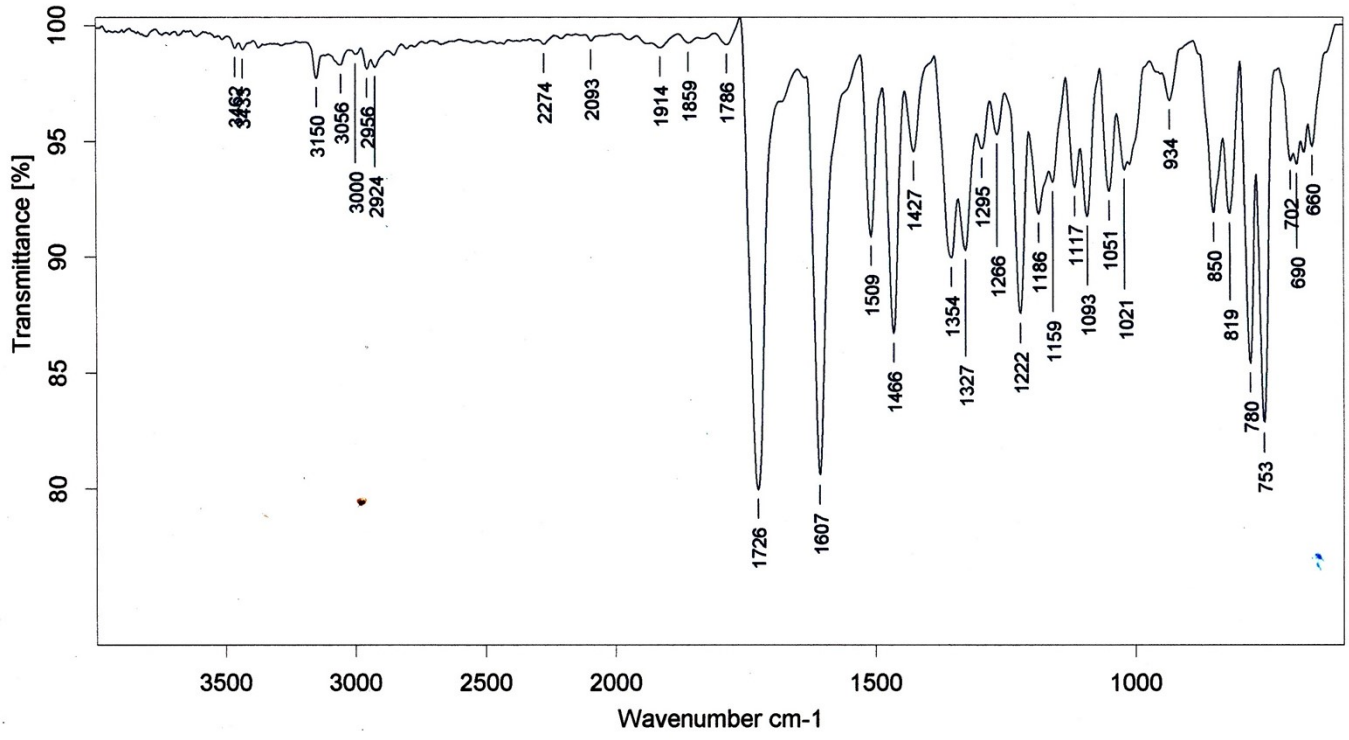
IR SPECTRA of 3d:



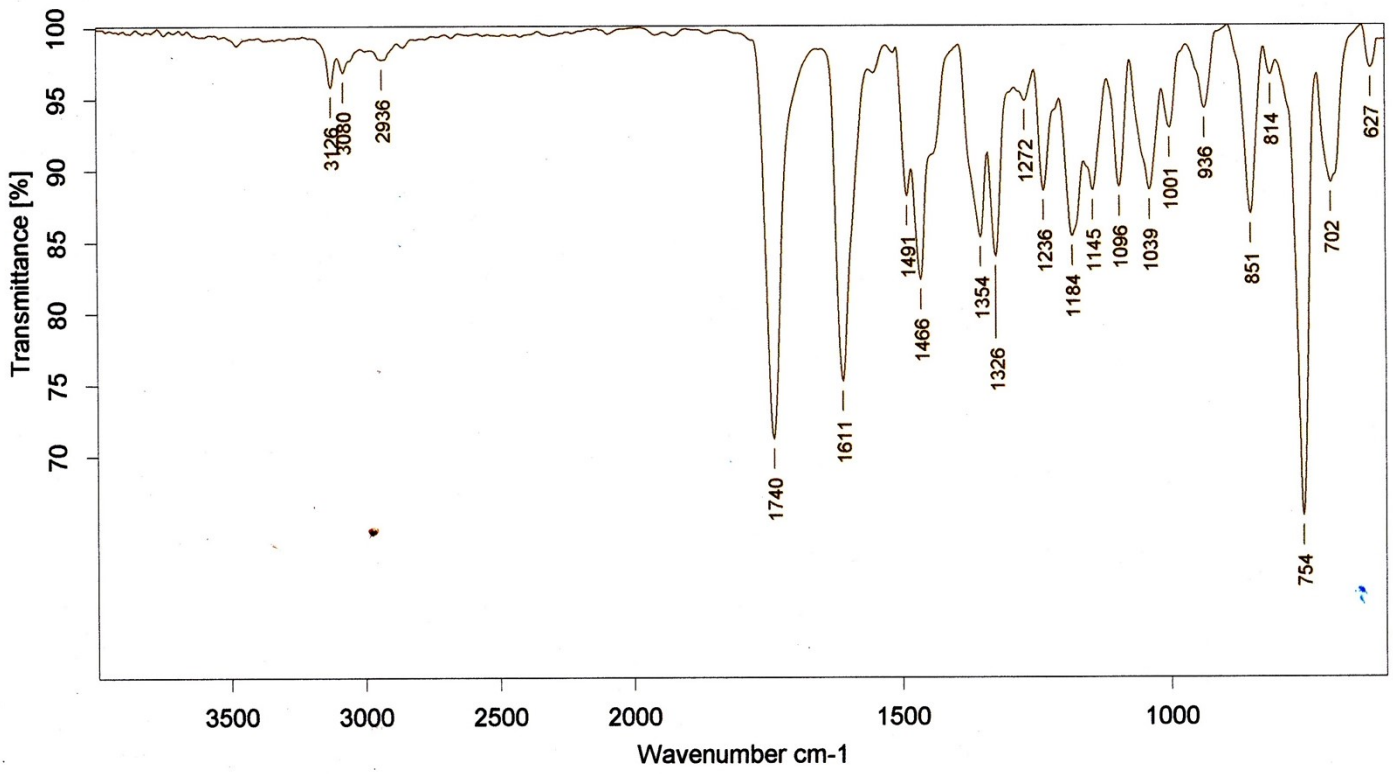
IR SPECTRA of 3e:



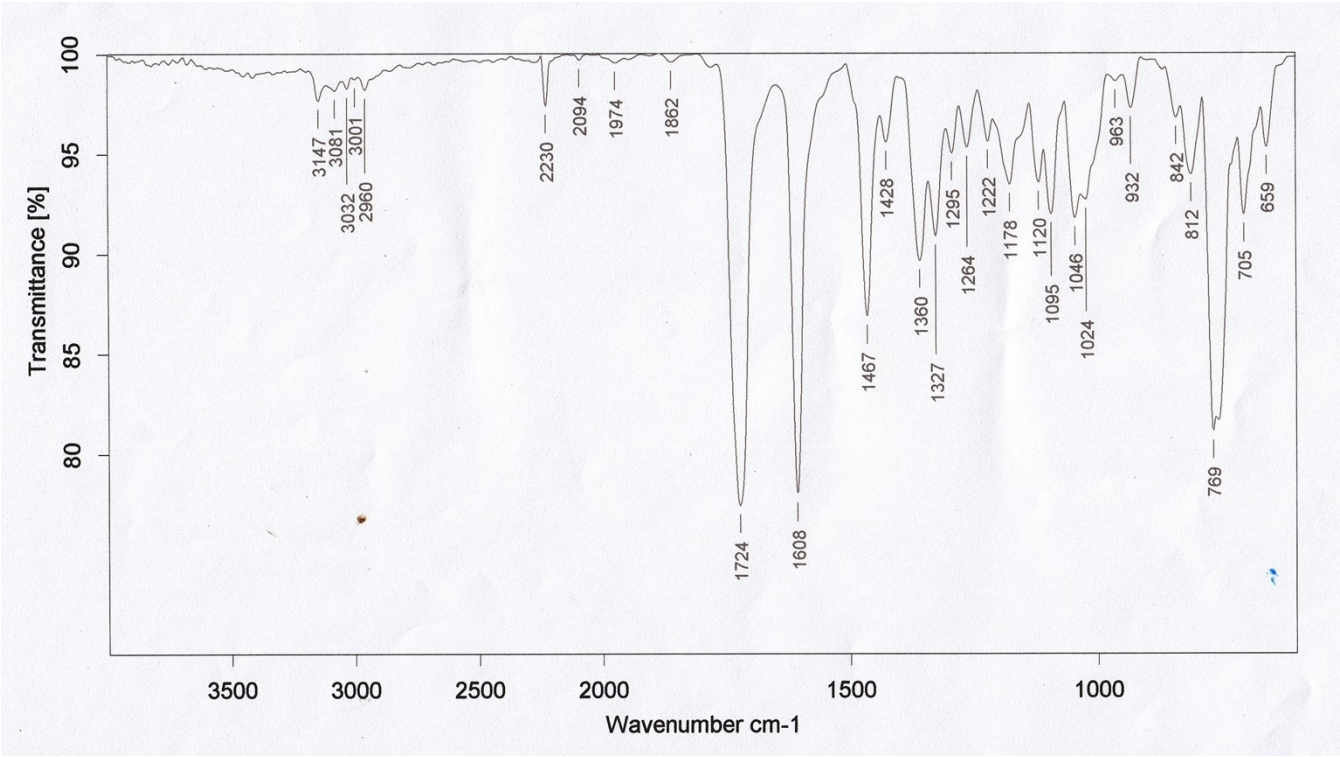
IR SPECTRA of 3f:



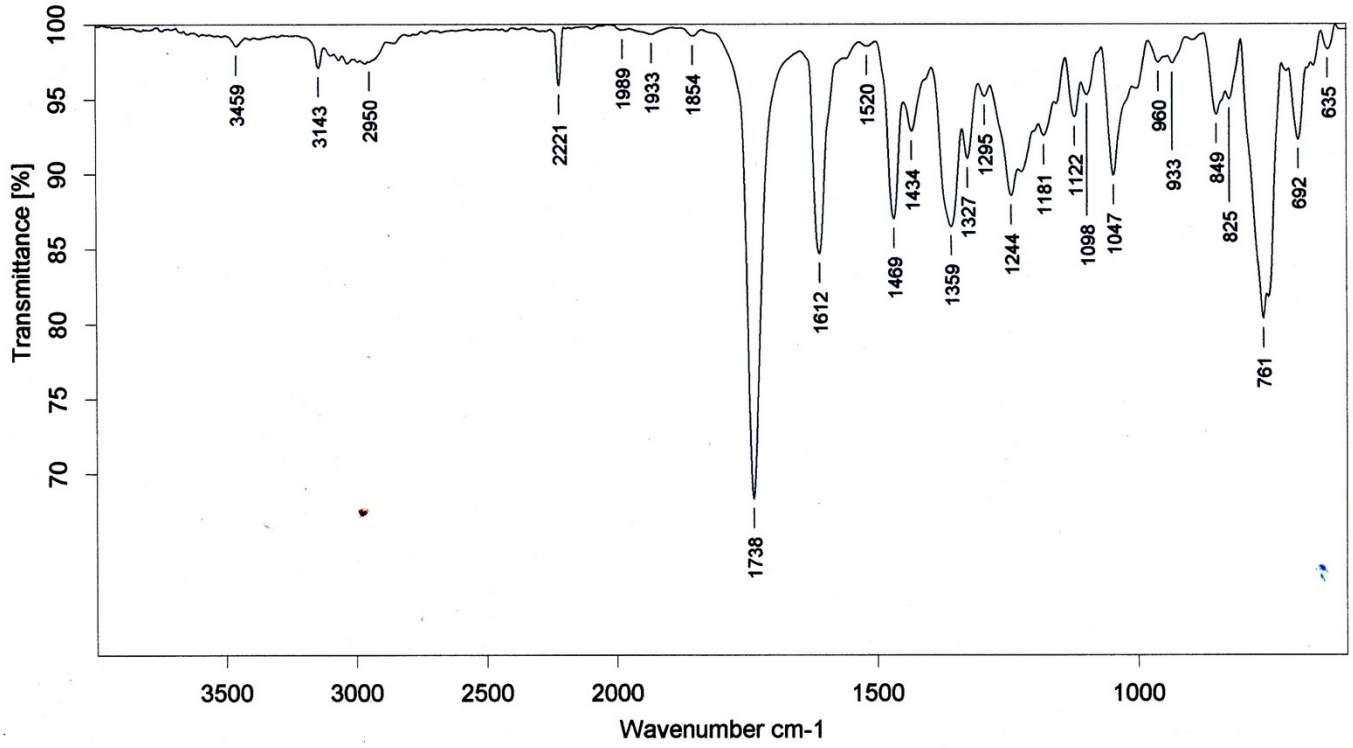
IR SPECTRA of 3g:



IR SPECTRA of 3h:



IR SPECTRA of 3i:



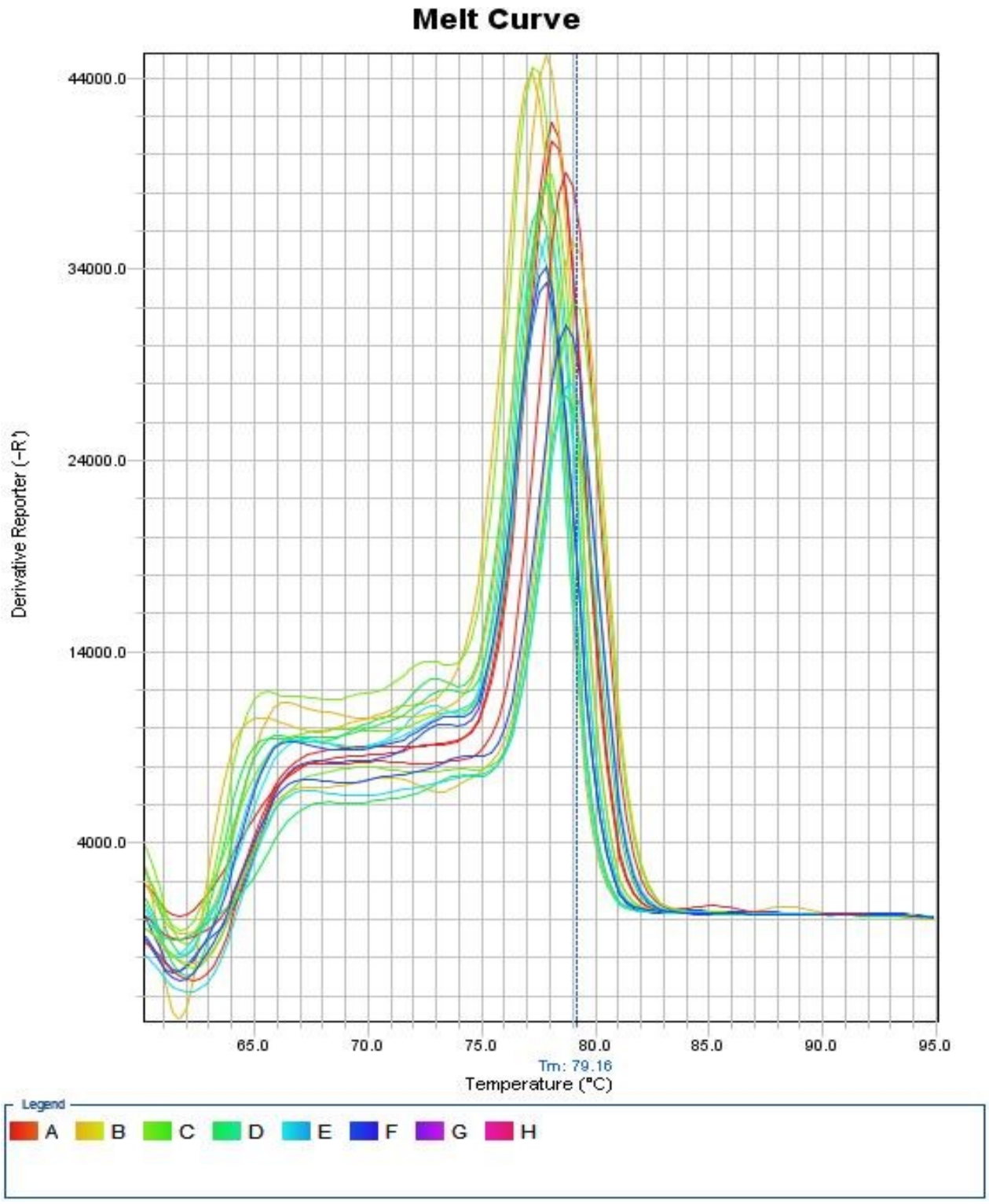


Figure 1: Melt curve analysis of RT-PCR product of GAPDH gene with respect to parent compound Isatin.

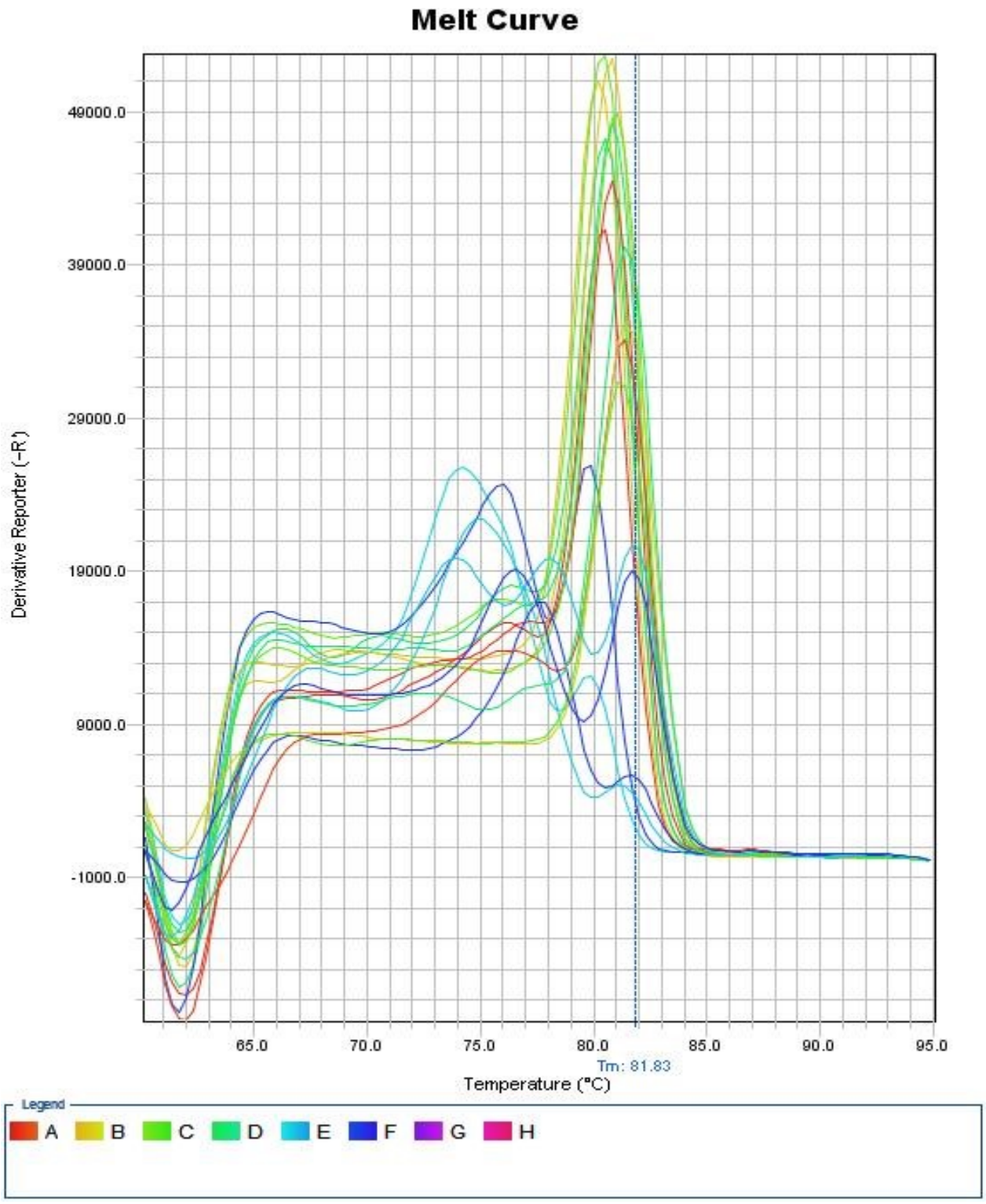


Figure 2: Melt curve analysis of RT-PCR product of TNF-α gene with respect to parent compound Isatin.

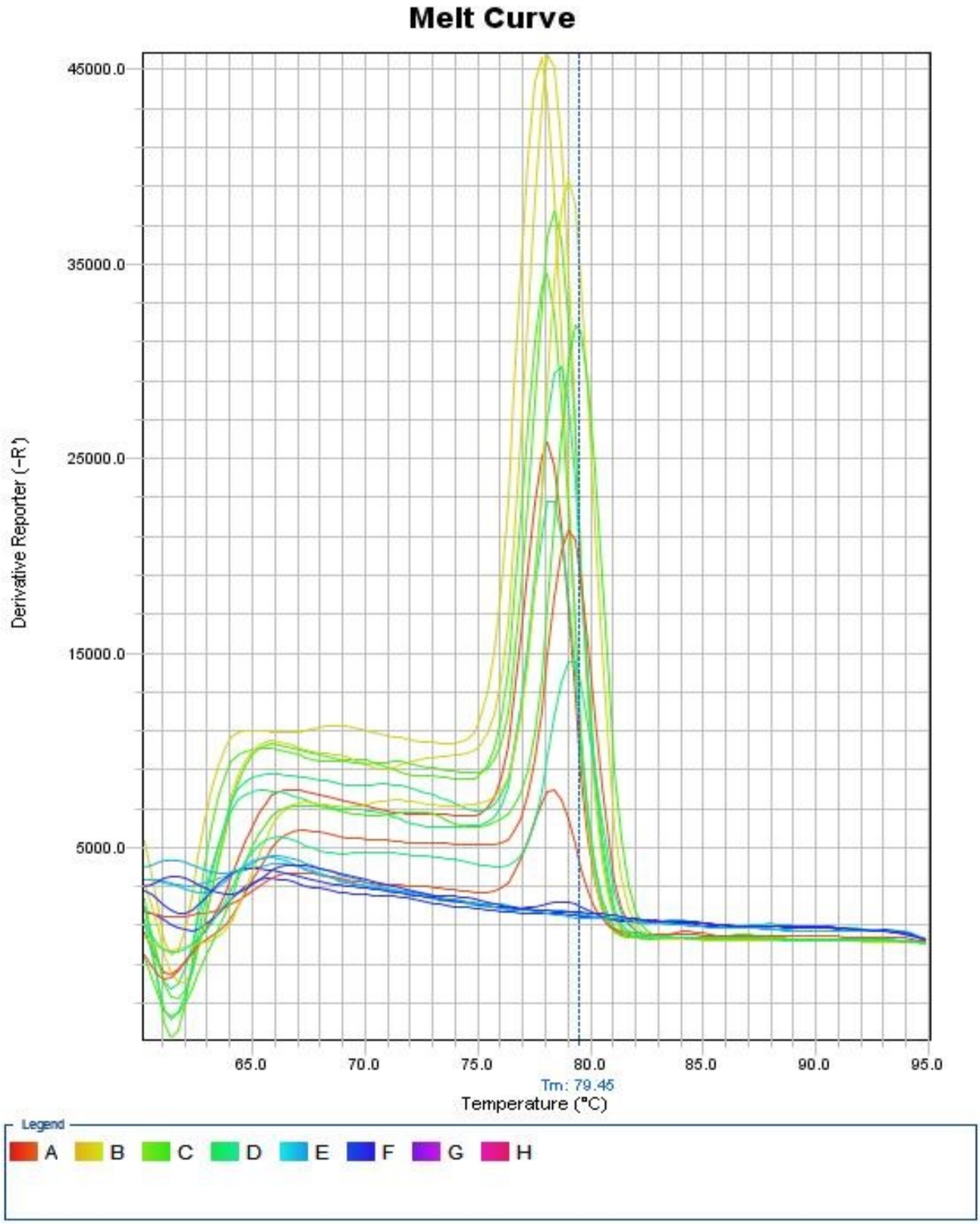


Figure 3: Melt curve analysis of RT-PCR product of IL-6 gene with respect to parent compound Isatin.

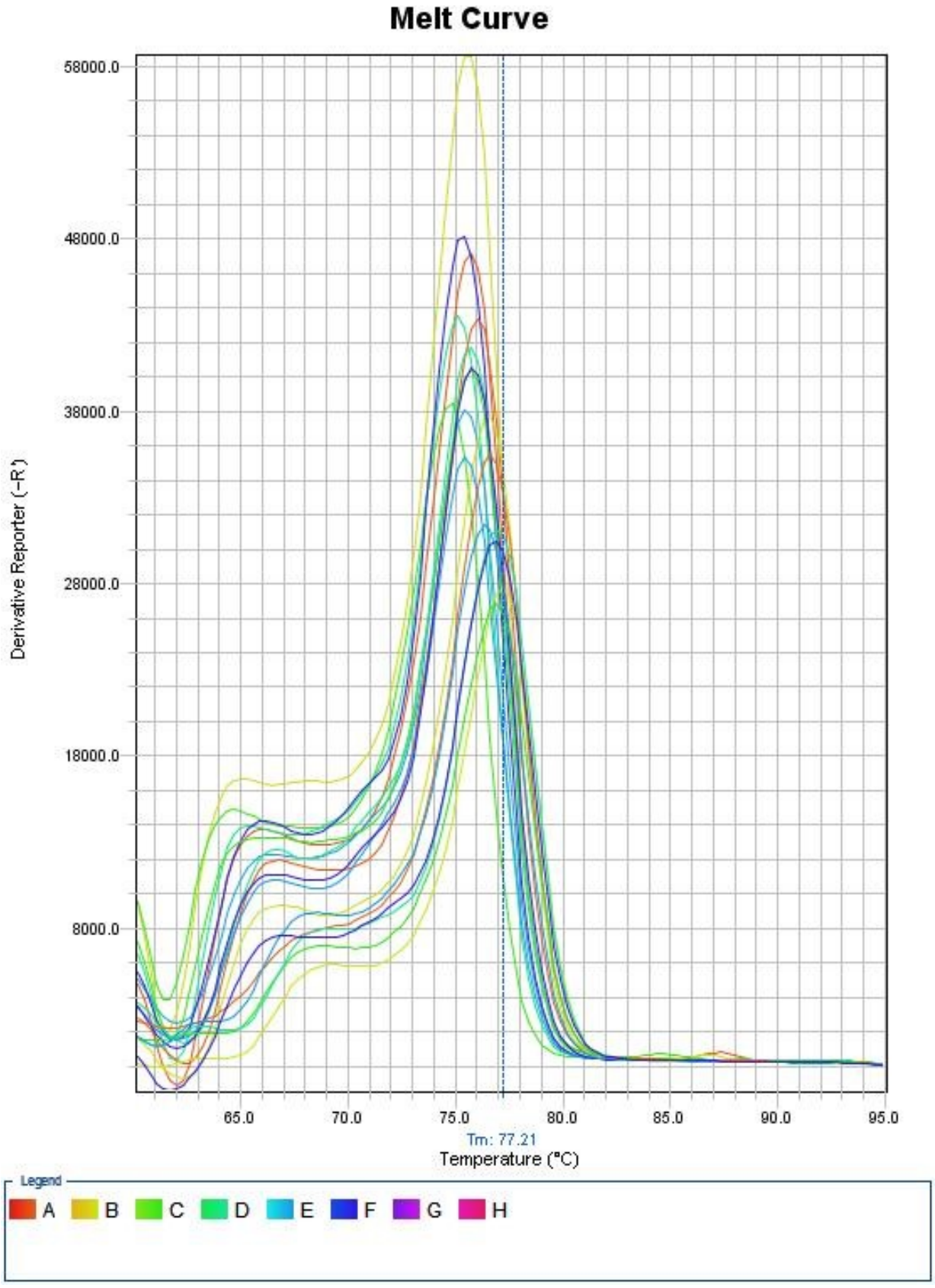


Figure 4: Melt curve analysis of RT-PCR product of MCP-1 gene with respect to parent compound Isatin.

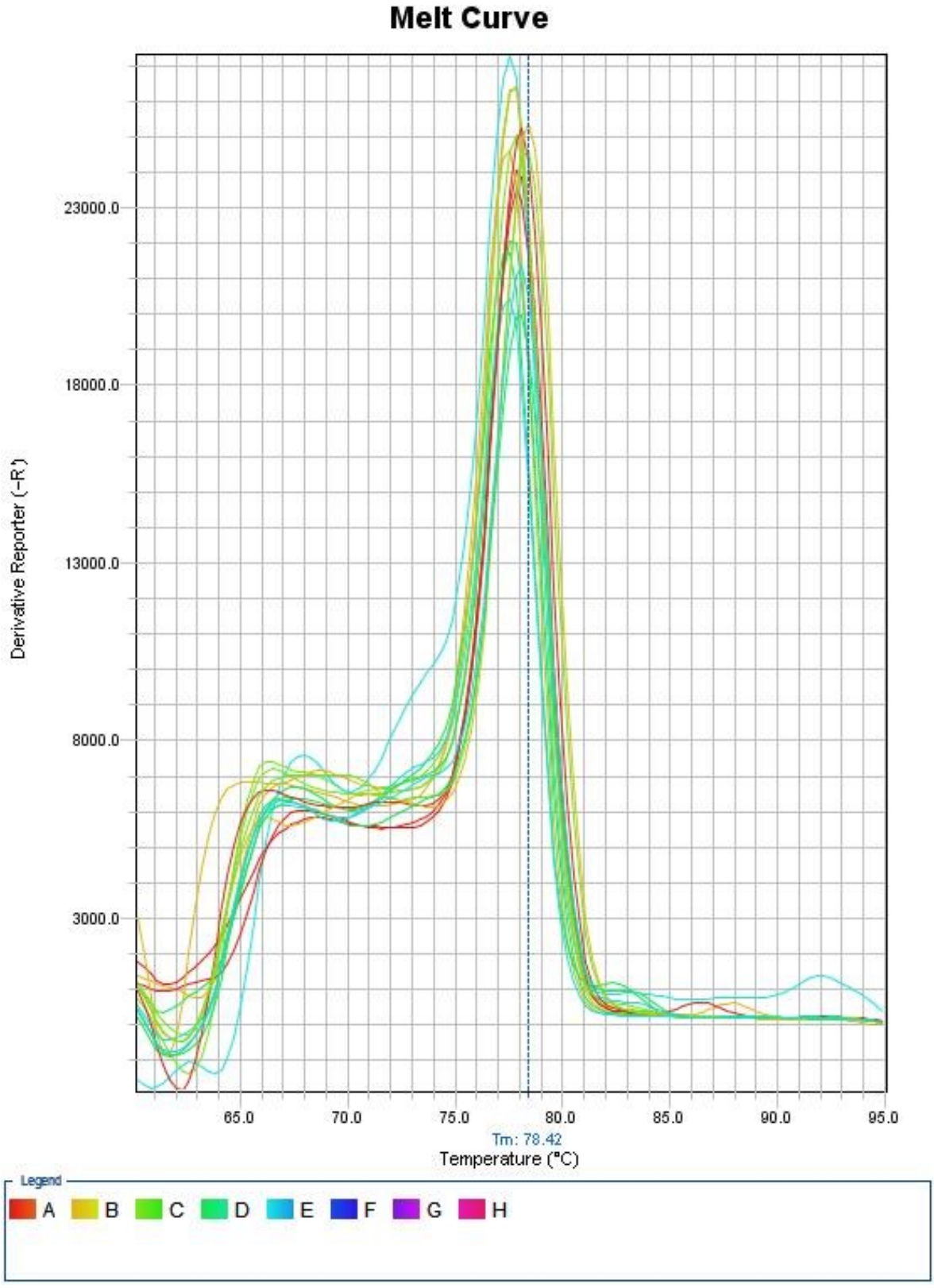


Figure 5: Melt curve analysis of RT-PCR product of GAPDH gene with respect to Isatin derivative compound 3e.

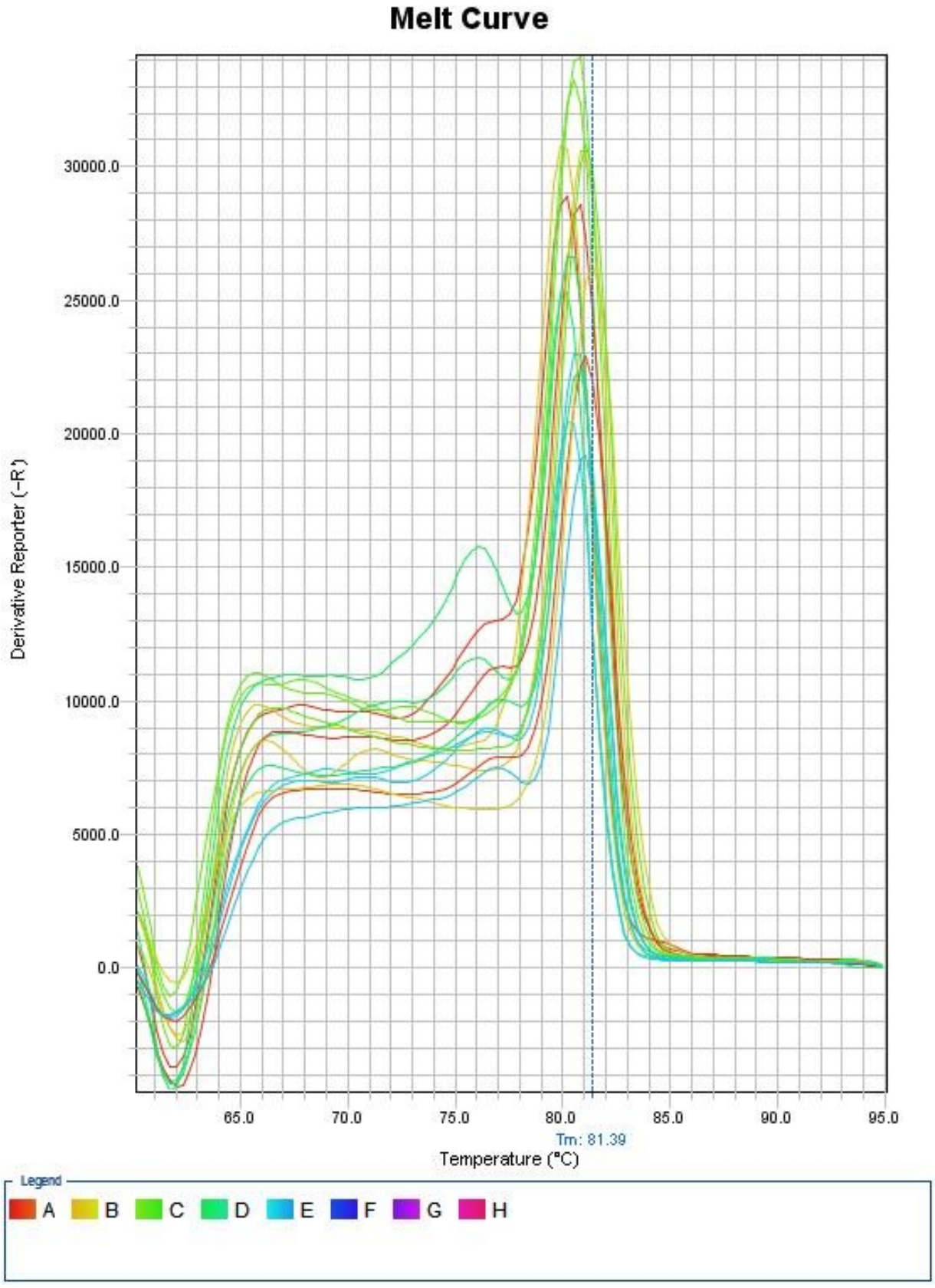


Figure 6: Melt curve analysis of RT-PCR product of TNF-α gene with respect to Isatin derivative compound 3e.

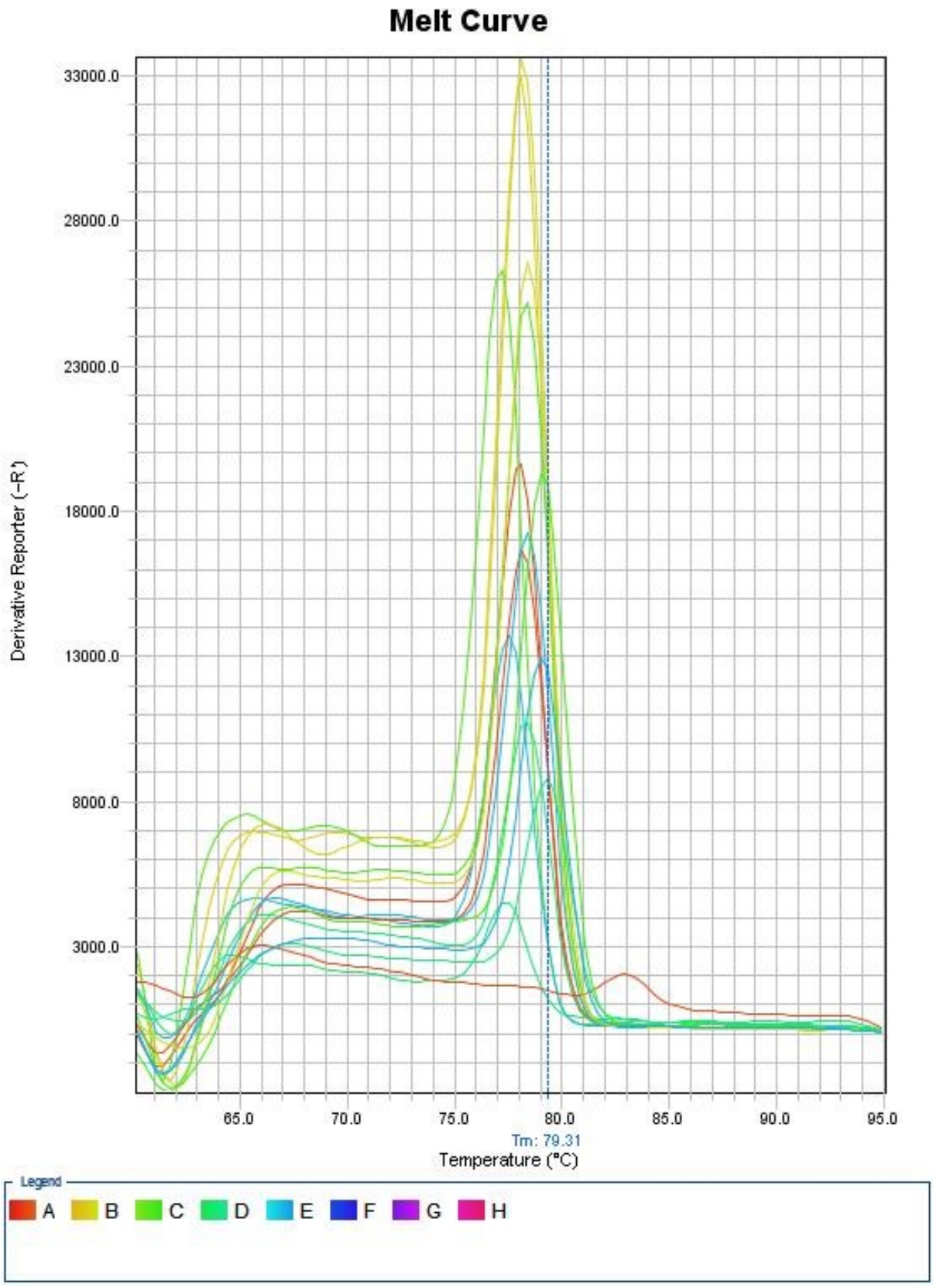


Figure 7: Melt curve analysis of RT-PCR product of IL-6 gene with respect to Isatin derivative compound 3e.

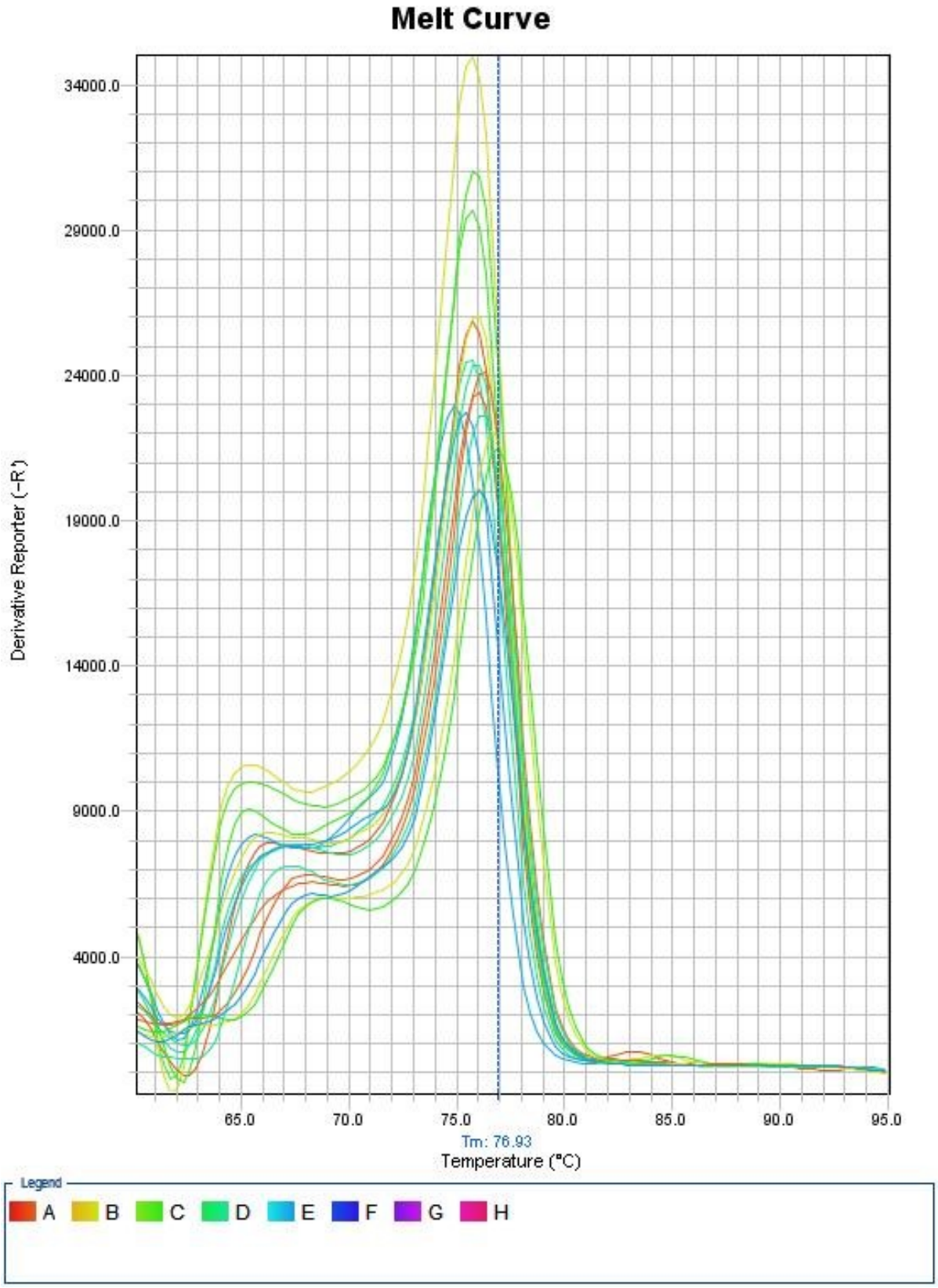


Figure 8: Melt curve analysis of RT-PCR product of MCP-1 gene with respect to Isatin derivative compound 3e.