

Supplementary information for

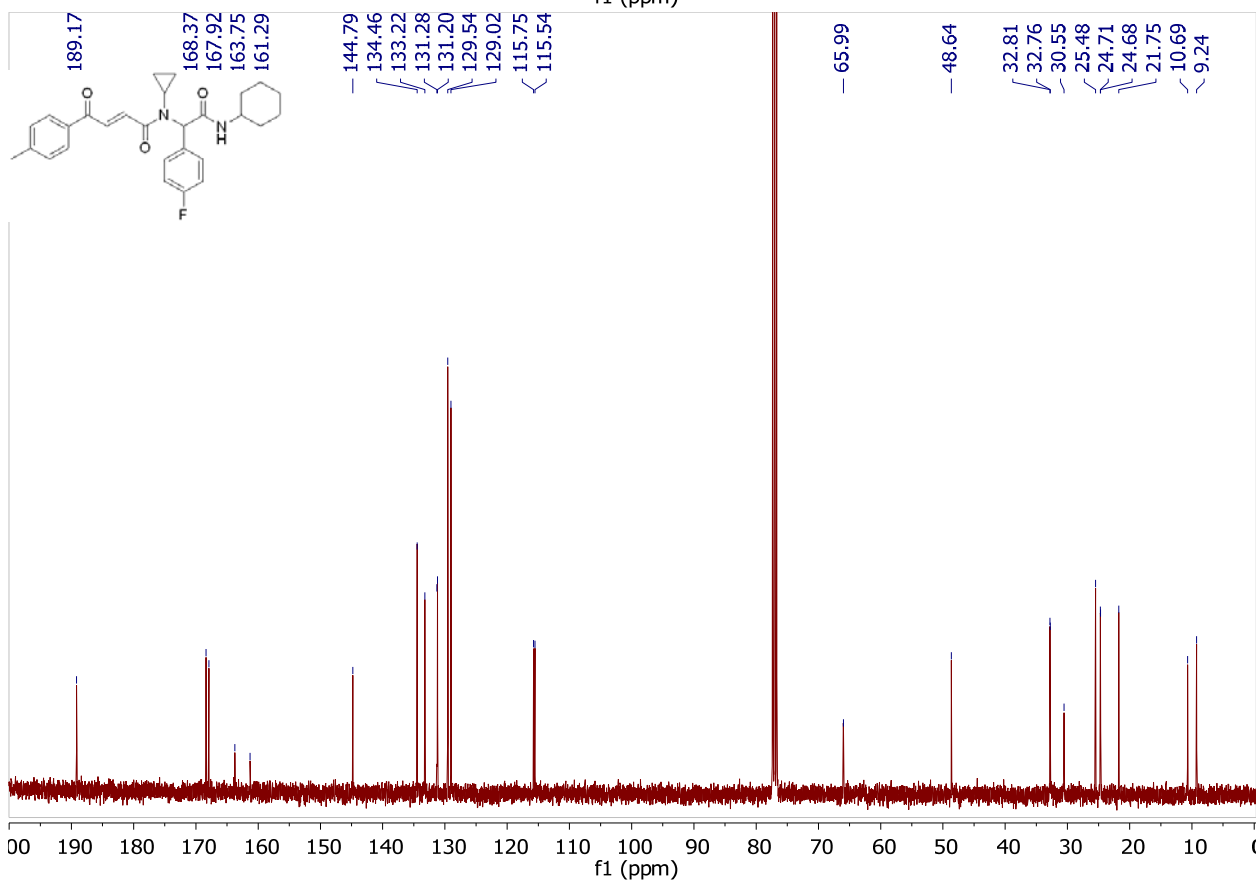
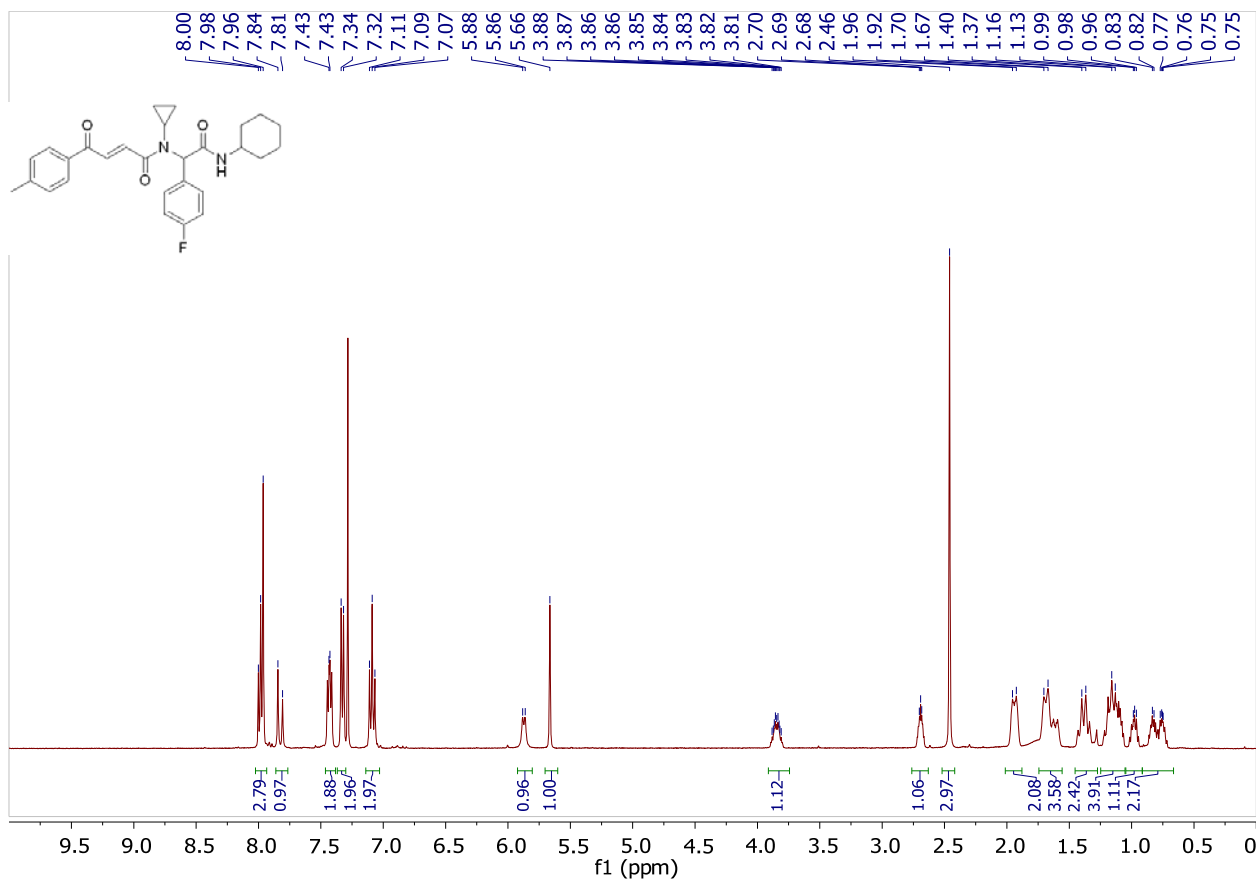
Novel electrophilic amides amenable by the Ugi reaction perturb thioredoxin system via thioredoxin reductase 1 (TrxR1) inhibition: identification of DVD-445 as a new lead compound for anticancer therapy

Mirna Jovanović, Daniil Zhukovsky, Ana Podolski-Renić, Ilona Domračeva, Raivis Žalubovskis, Milan Senčanski, Sanja Glišić, Vladimir Sharoyko, Tatiana Tennikova, Dmitry Dar'in, Milica Pešić* and Mikhail Krasavin*

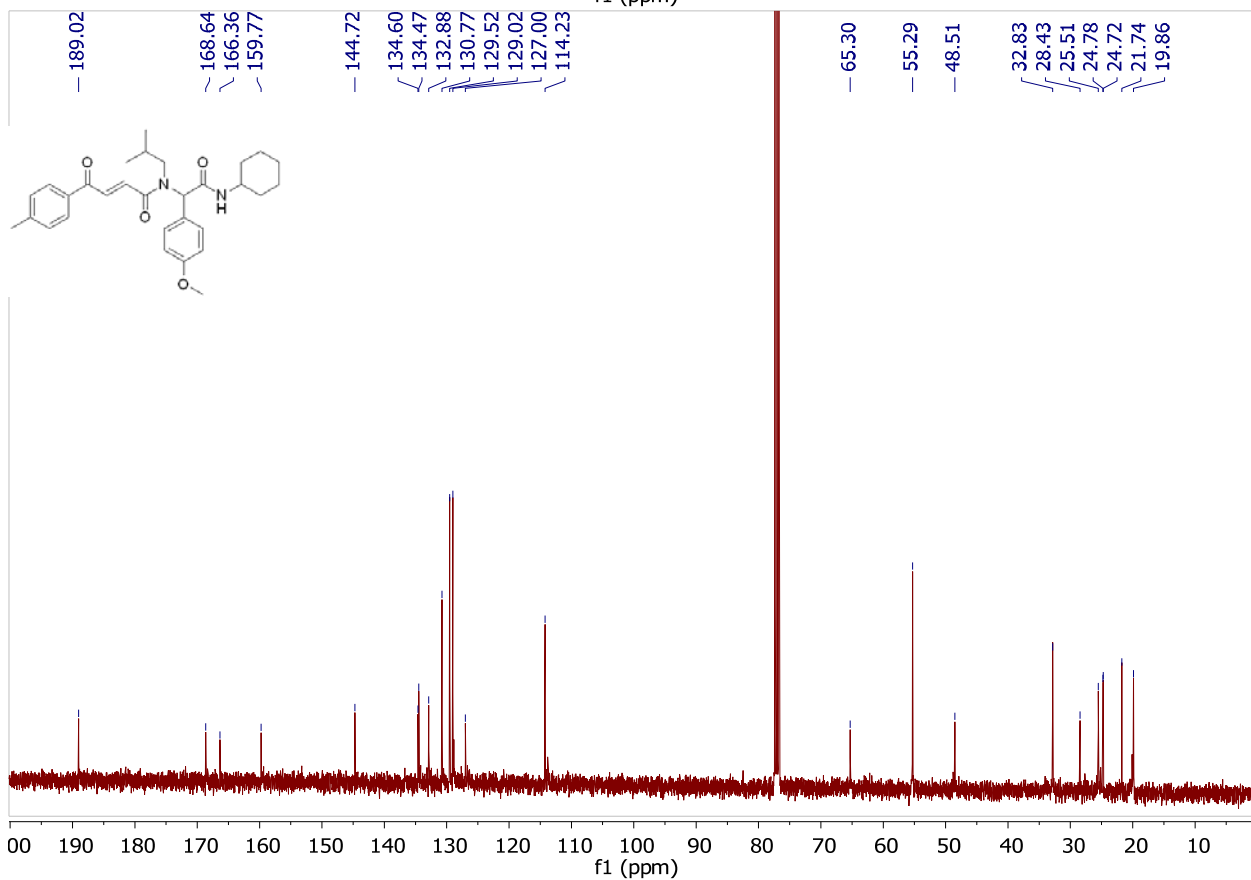
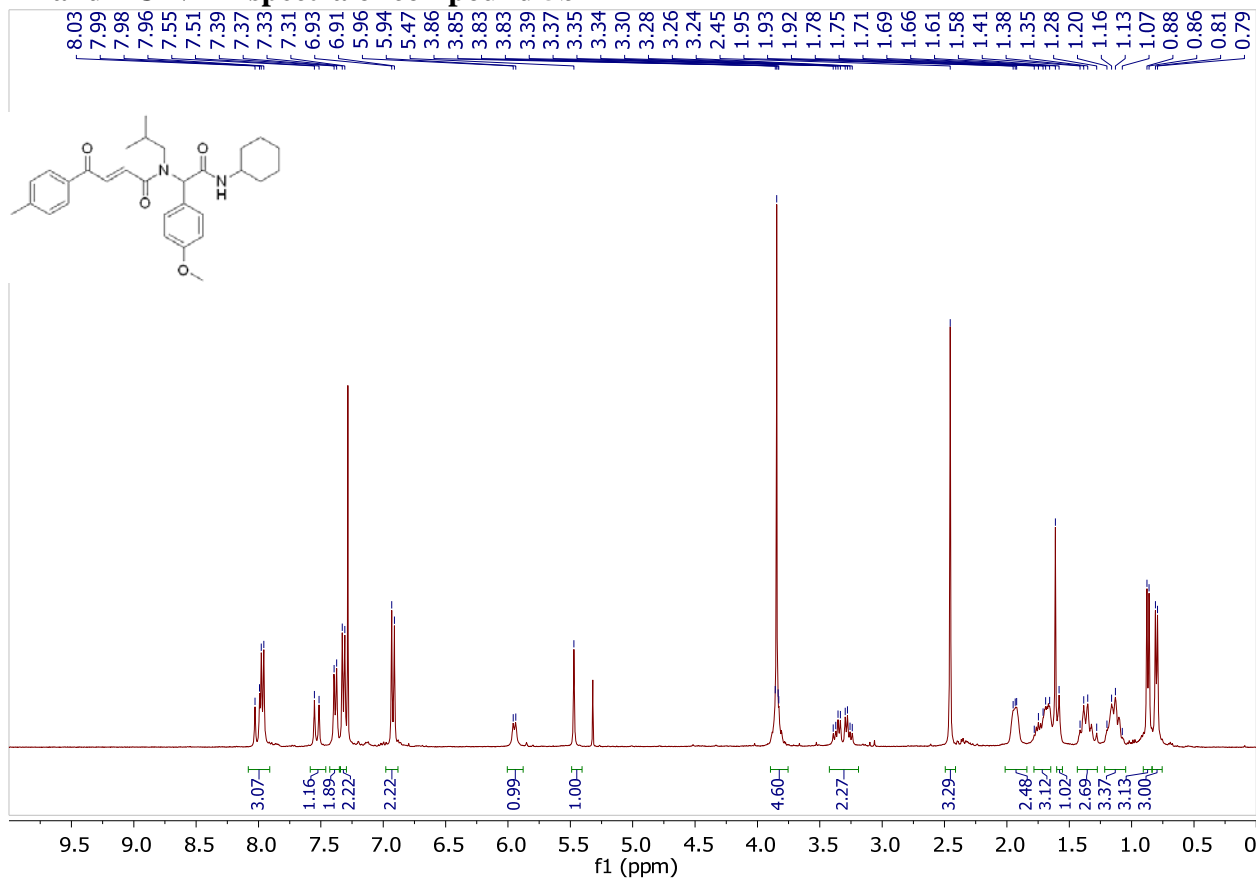
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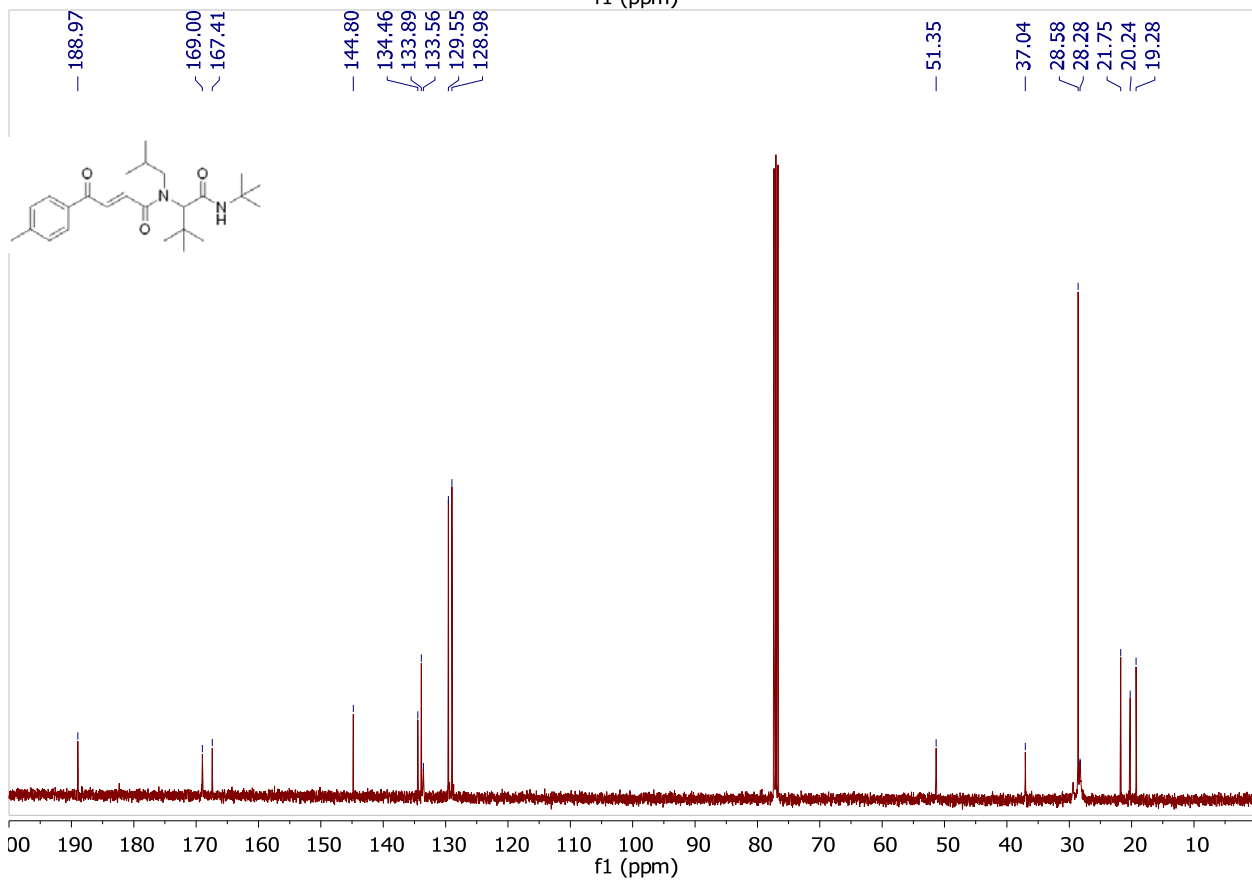
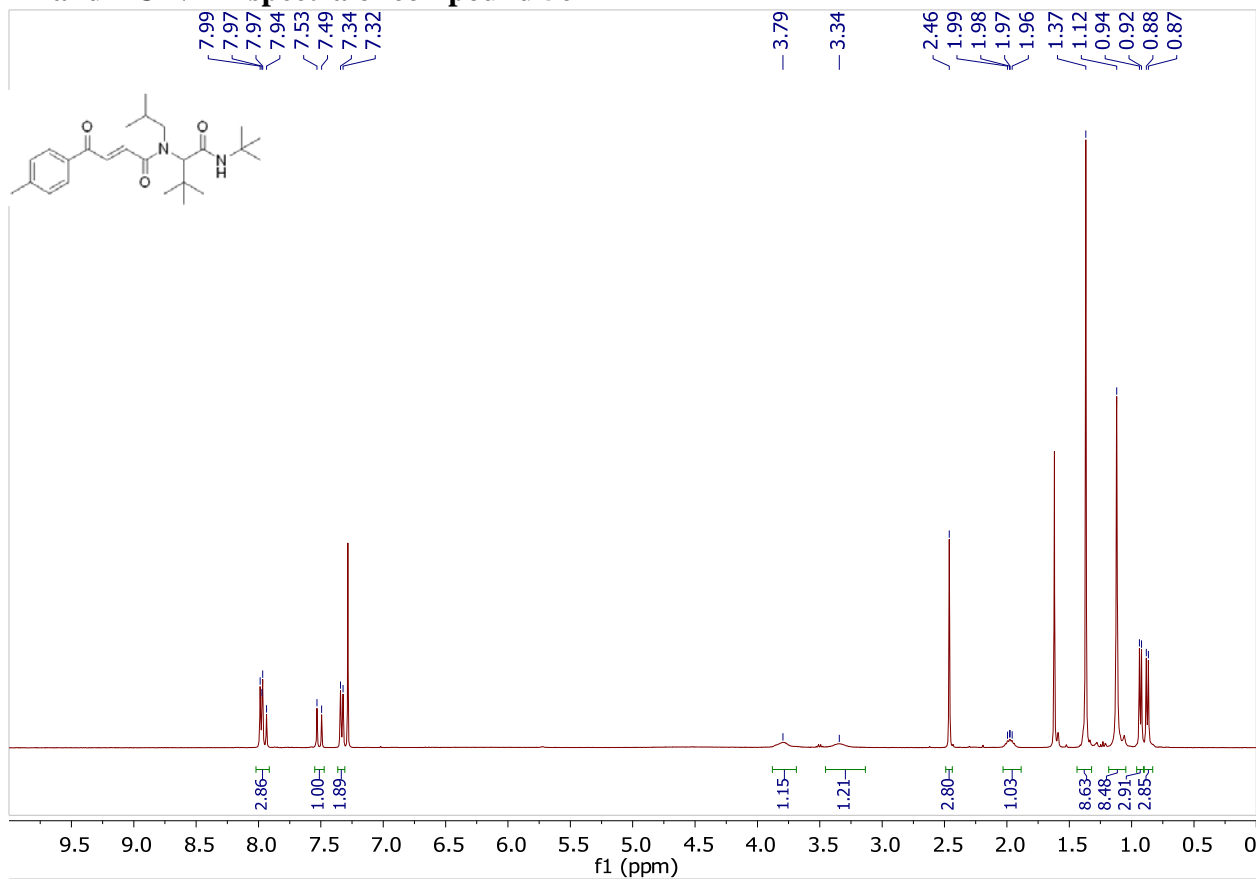
¹H and ¹³C NMR spectra of compound 5a



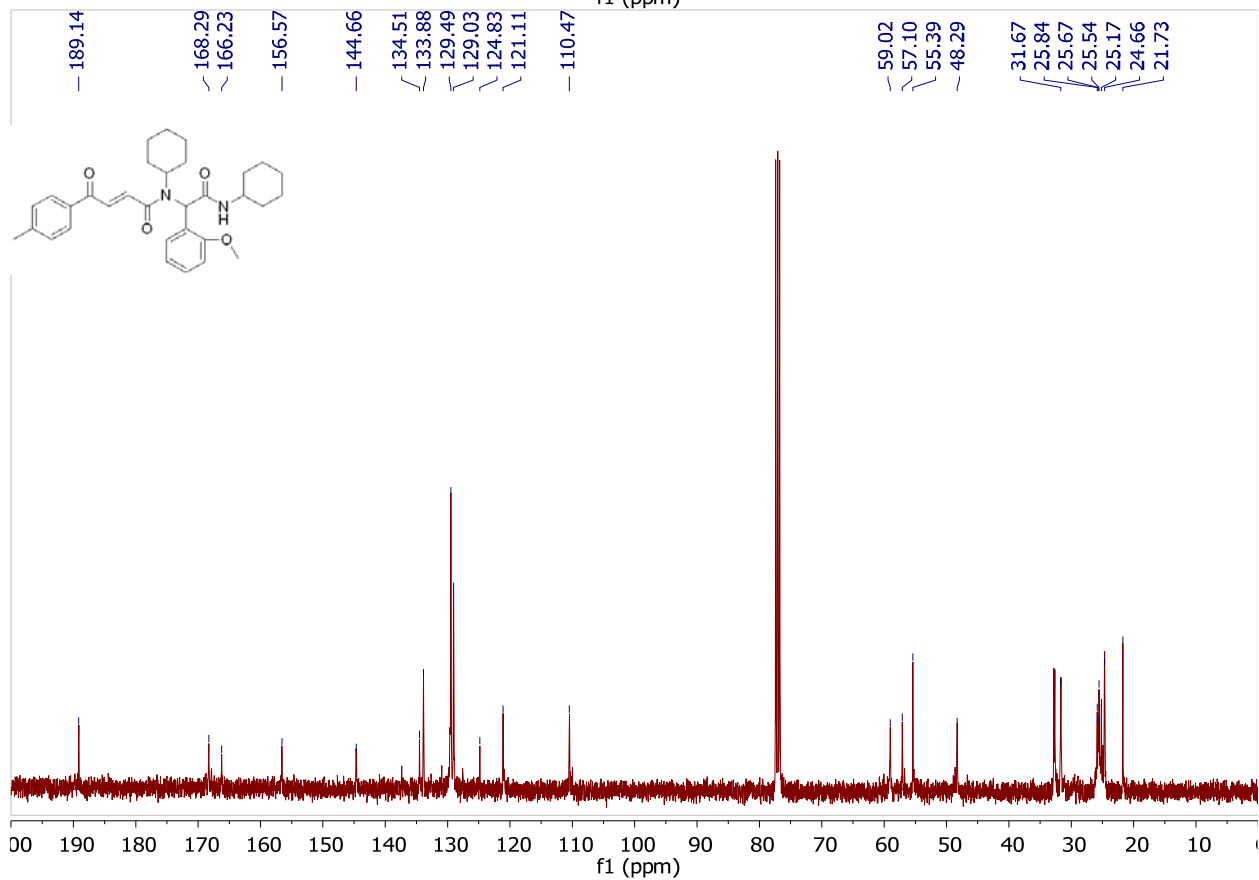
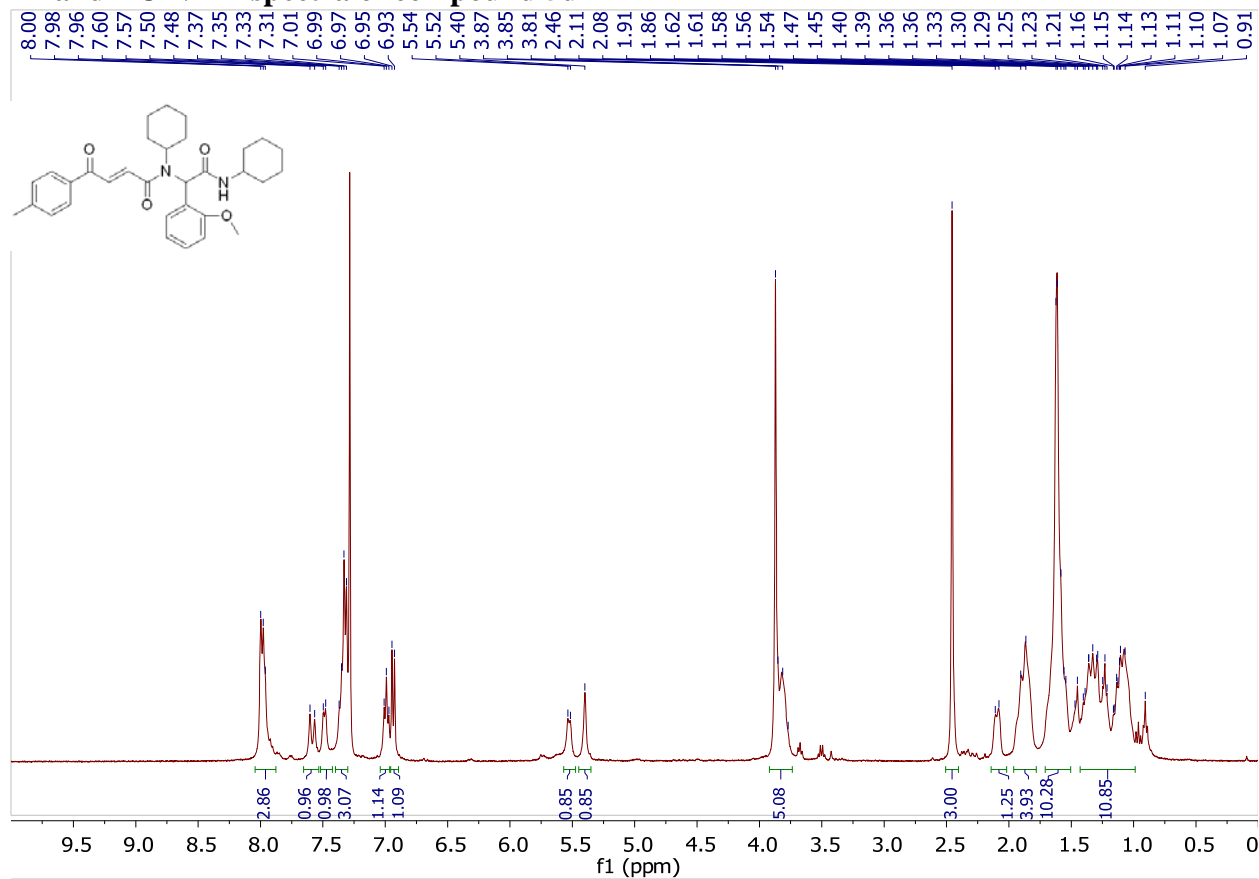
¹H and ¹³C NMR spectra of compound 5b



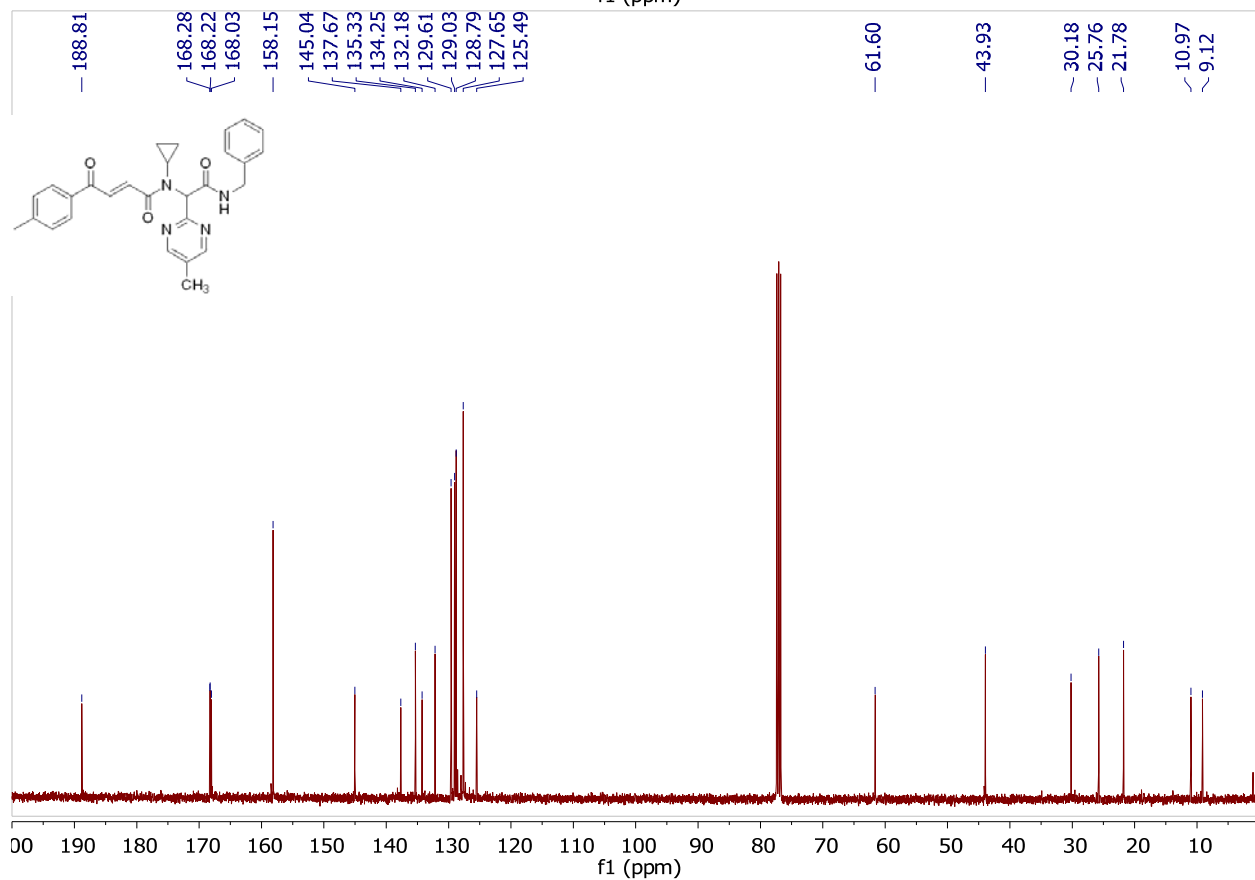
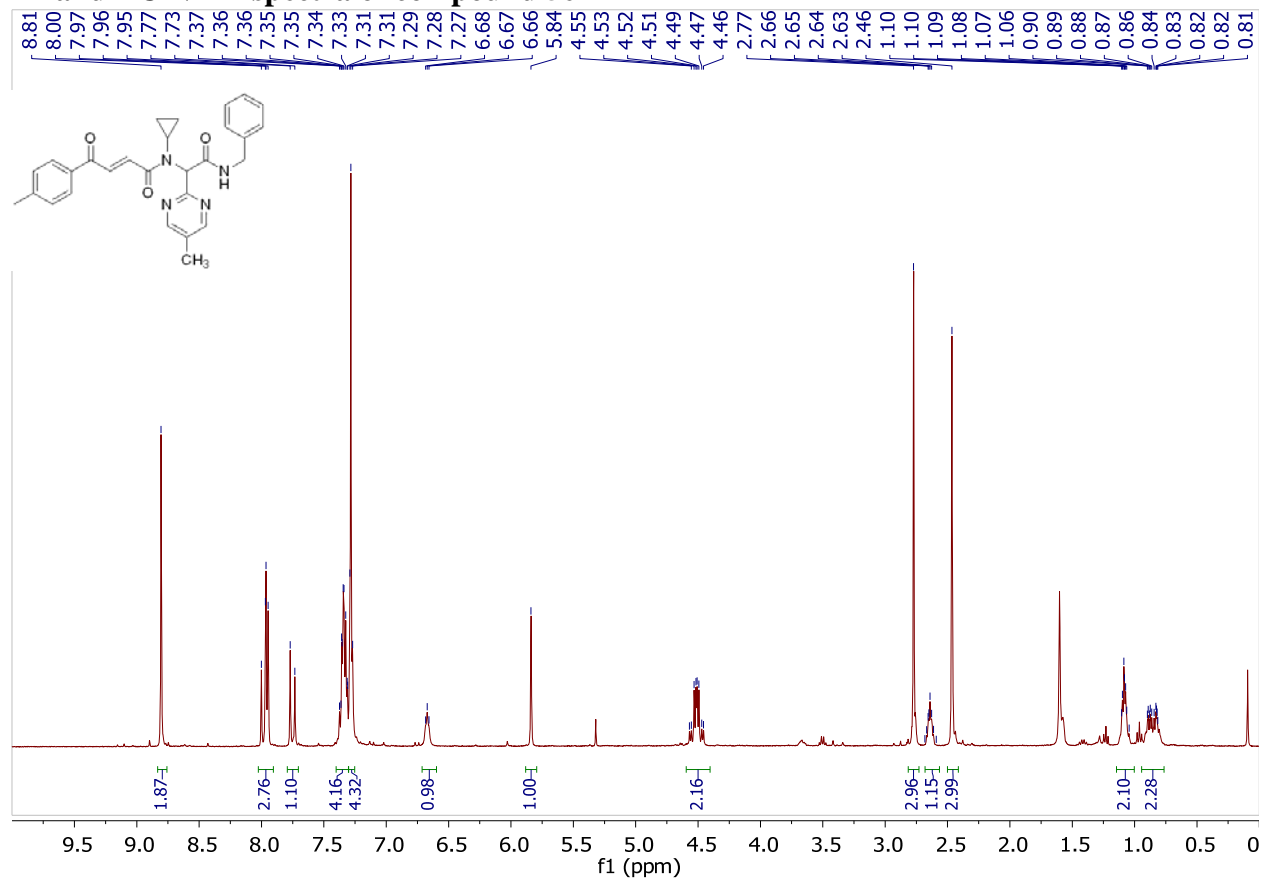
¹H and ¹³C NMR spectra of compound 5c



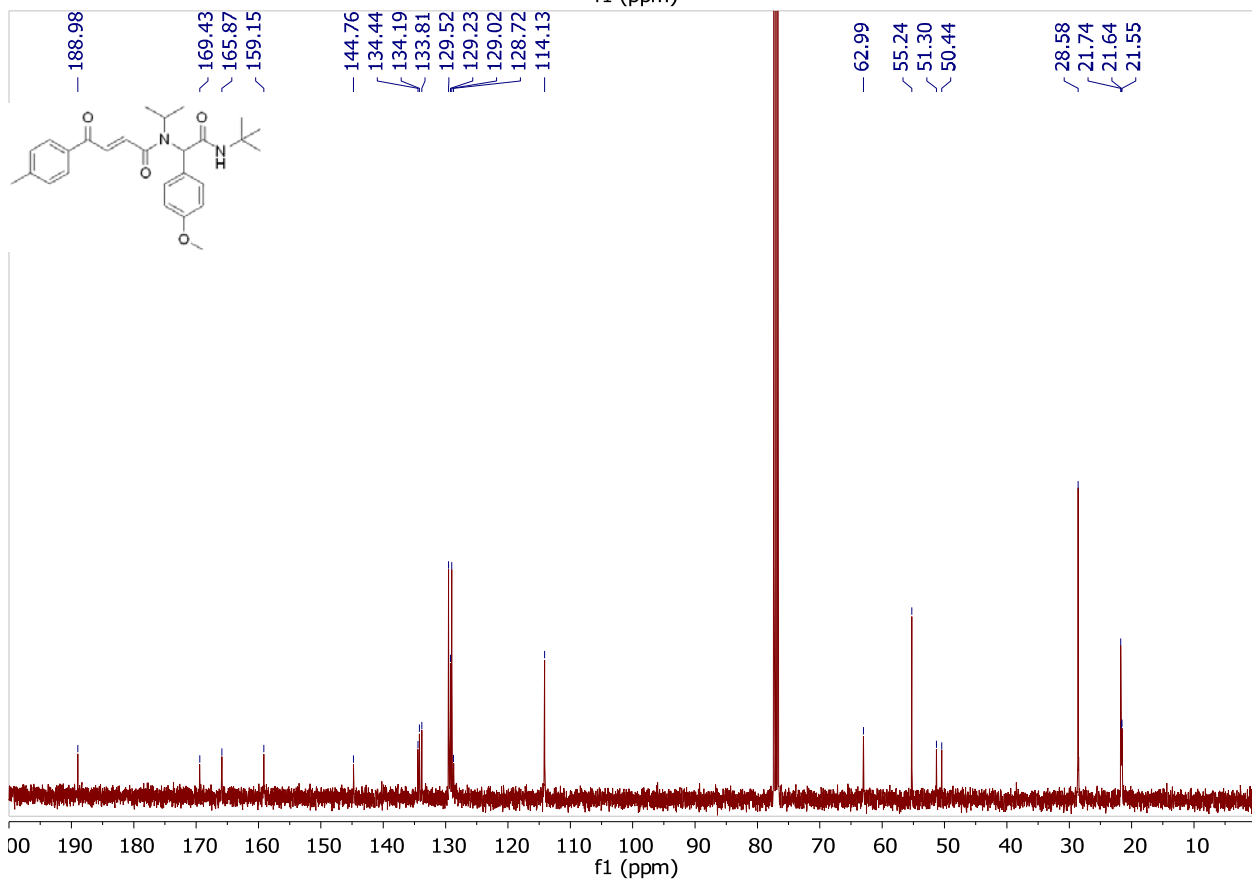
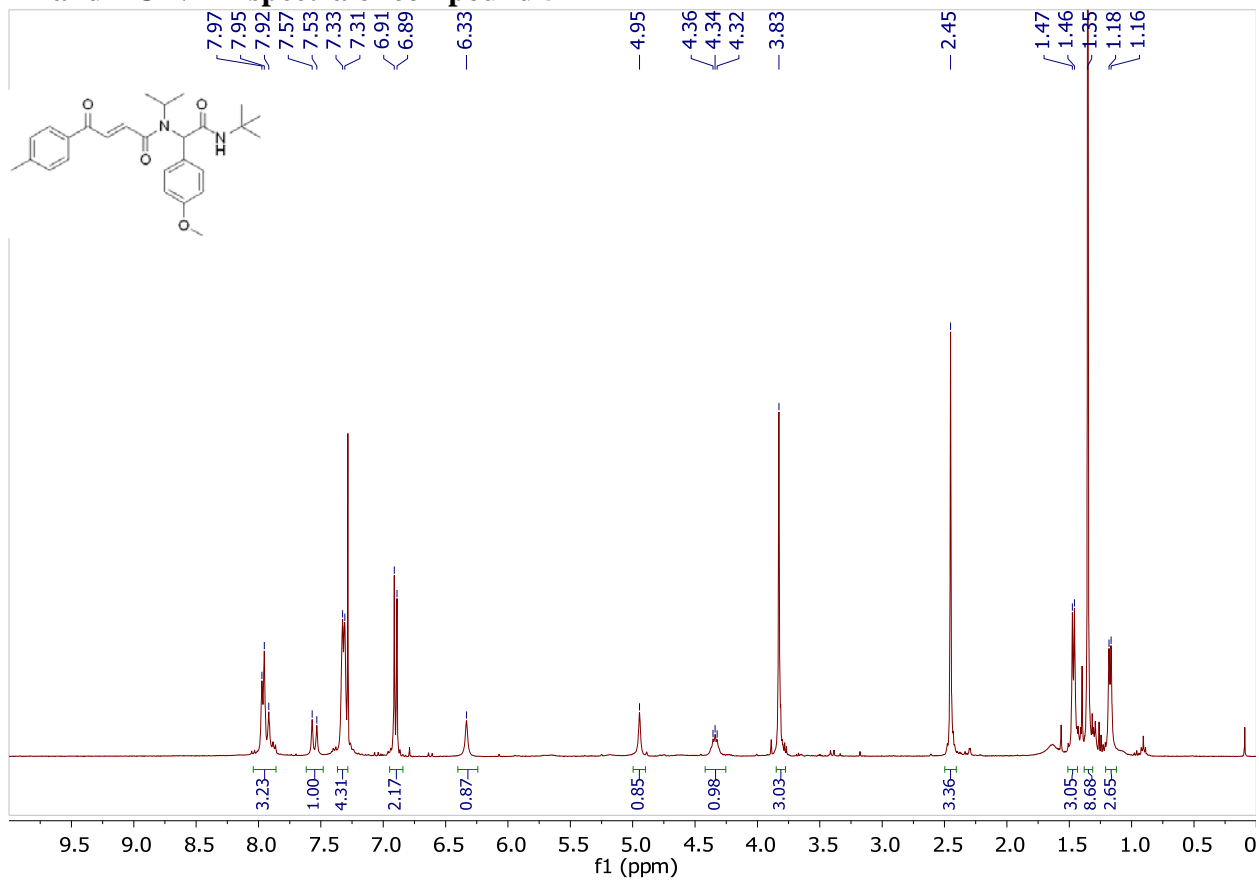
¹H and ¹³C NMR spectra of compound 5d



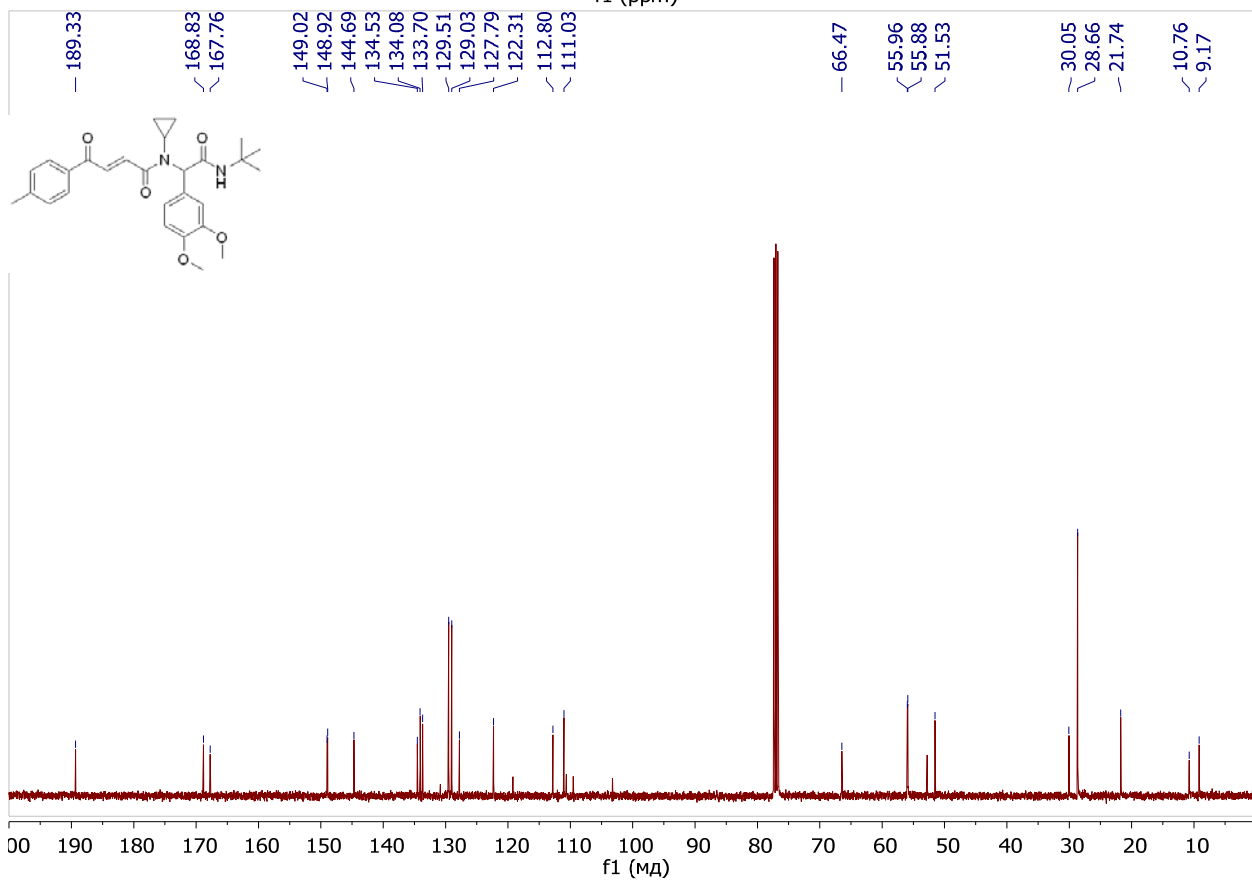
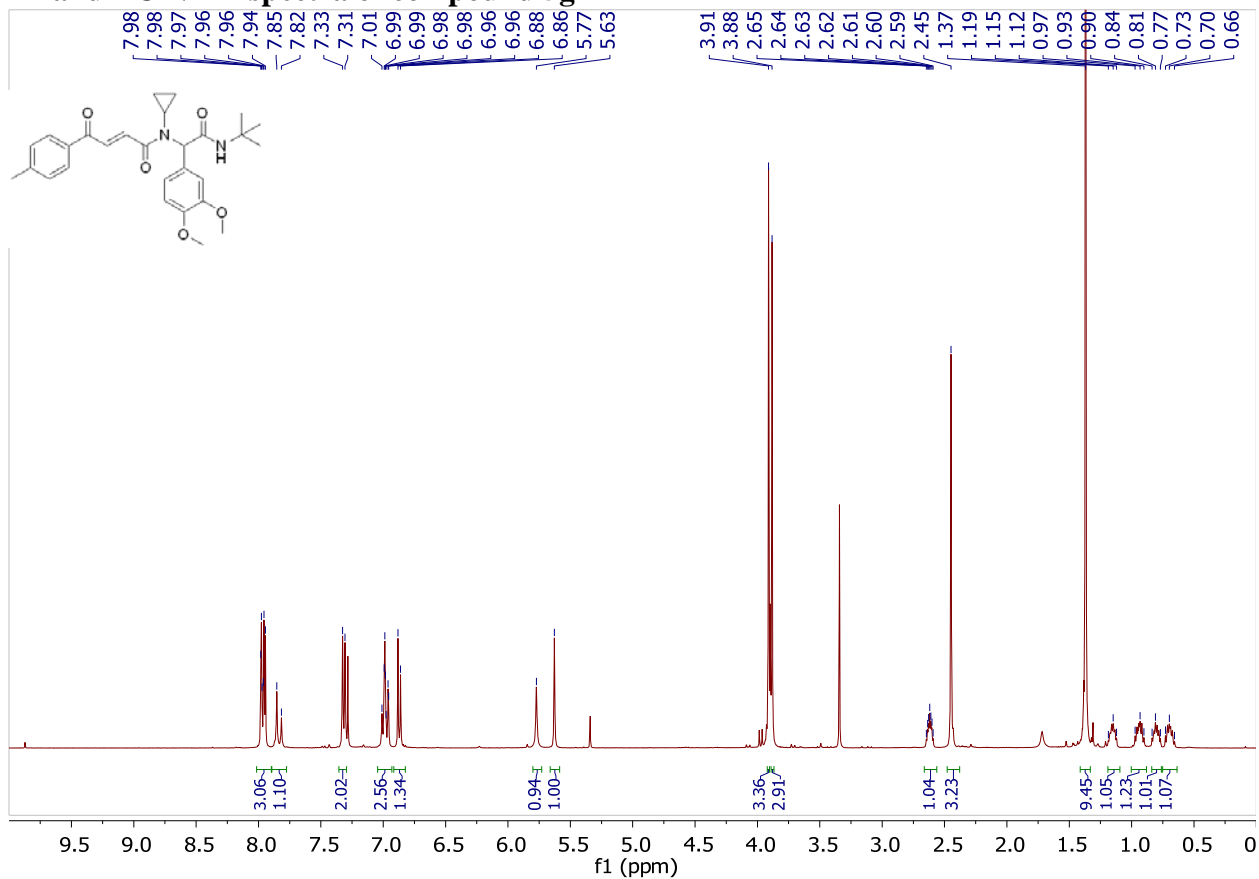
¹H and ¹³C NMR spectra of compound 5e



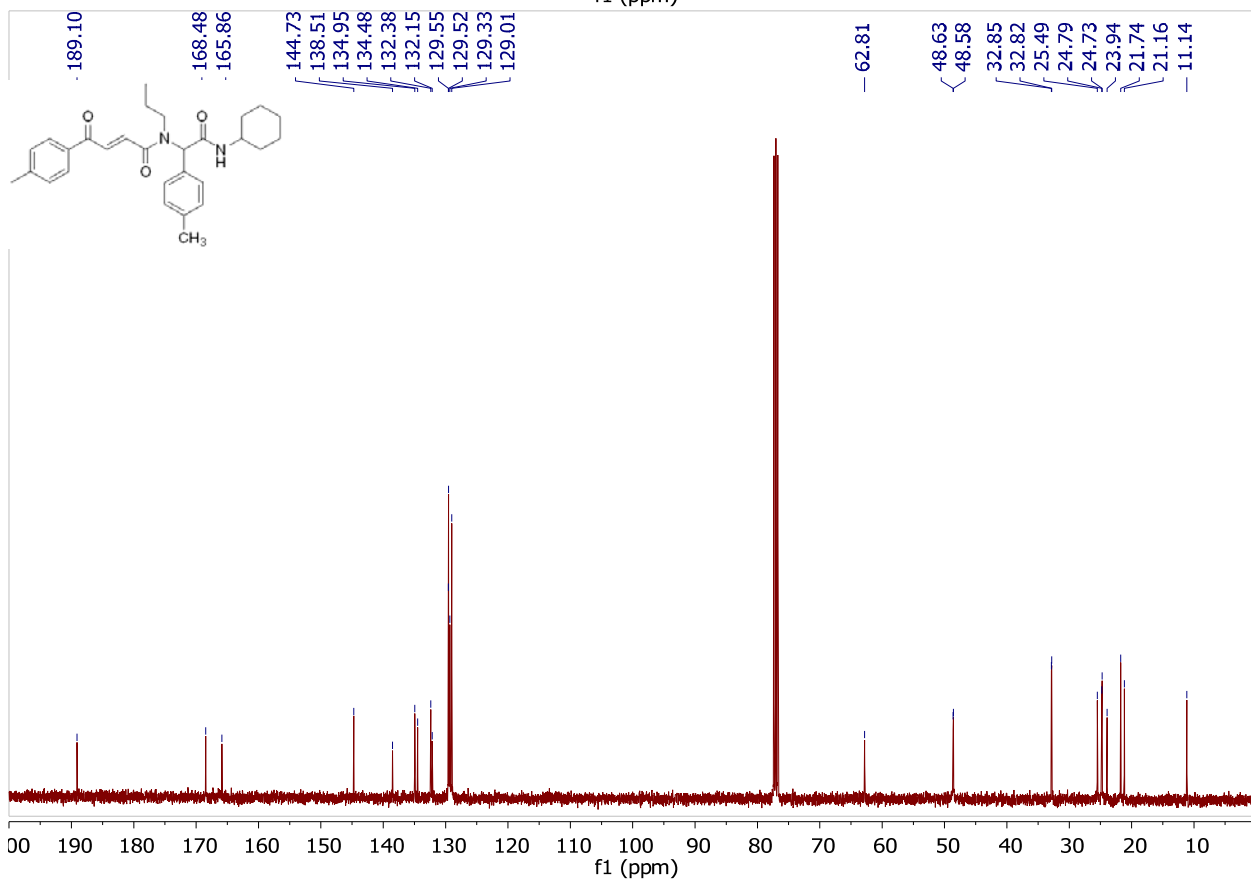
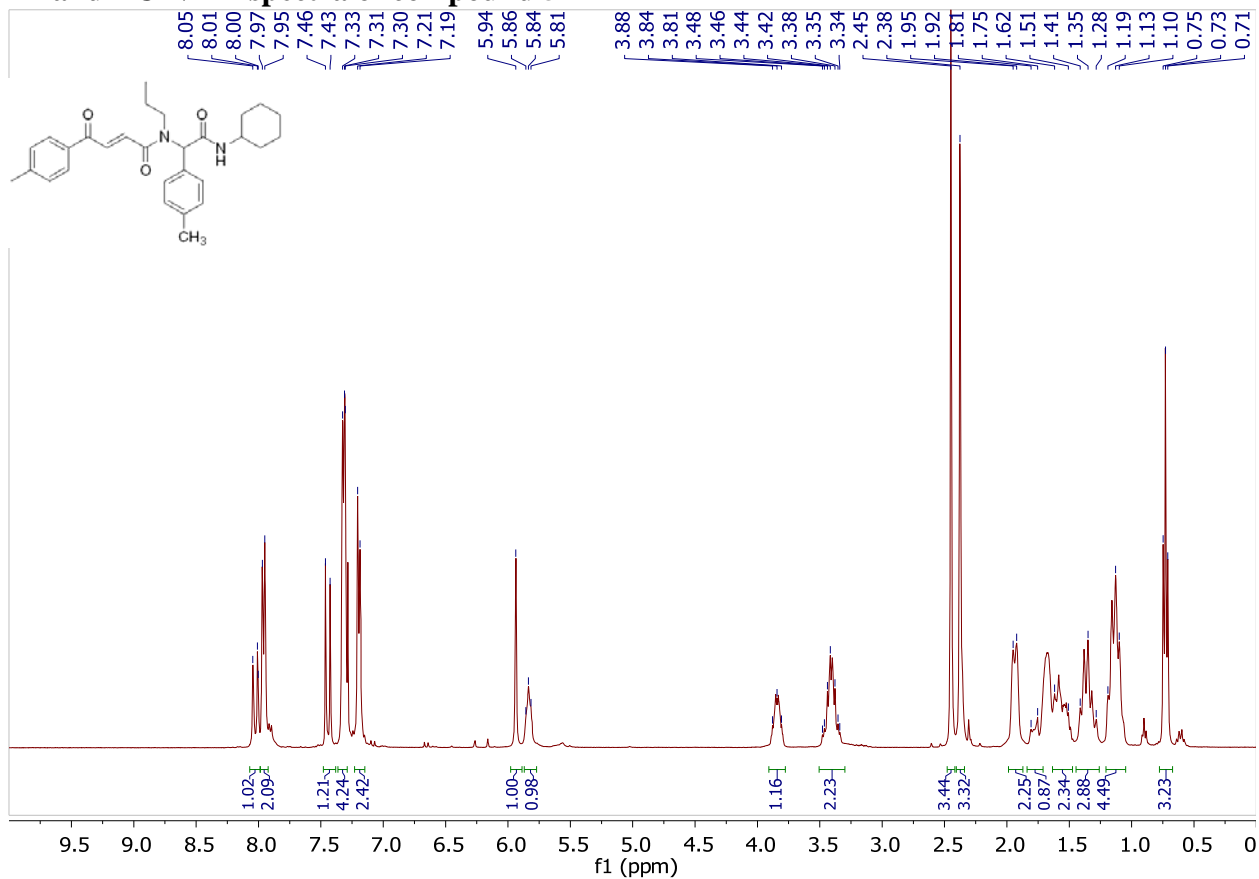
^1H and ^{13}C NMR spectra of compound 5f



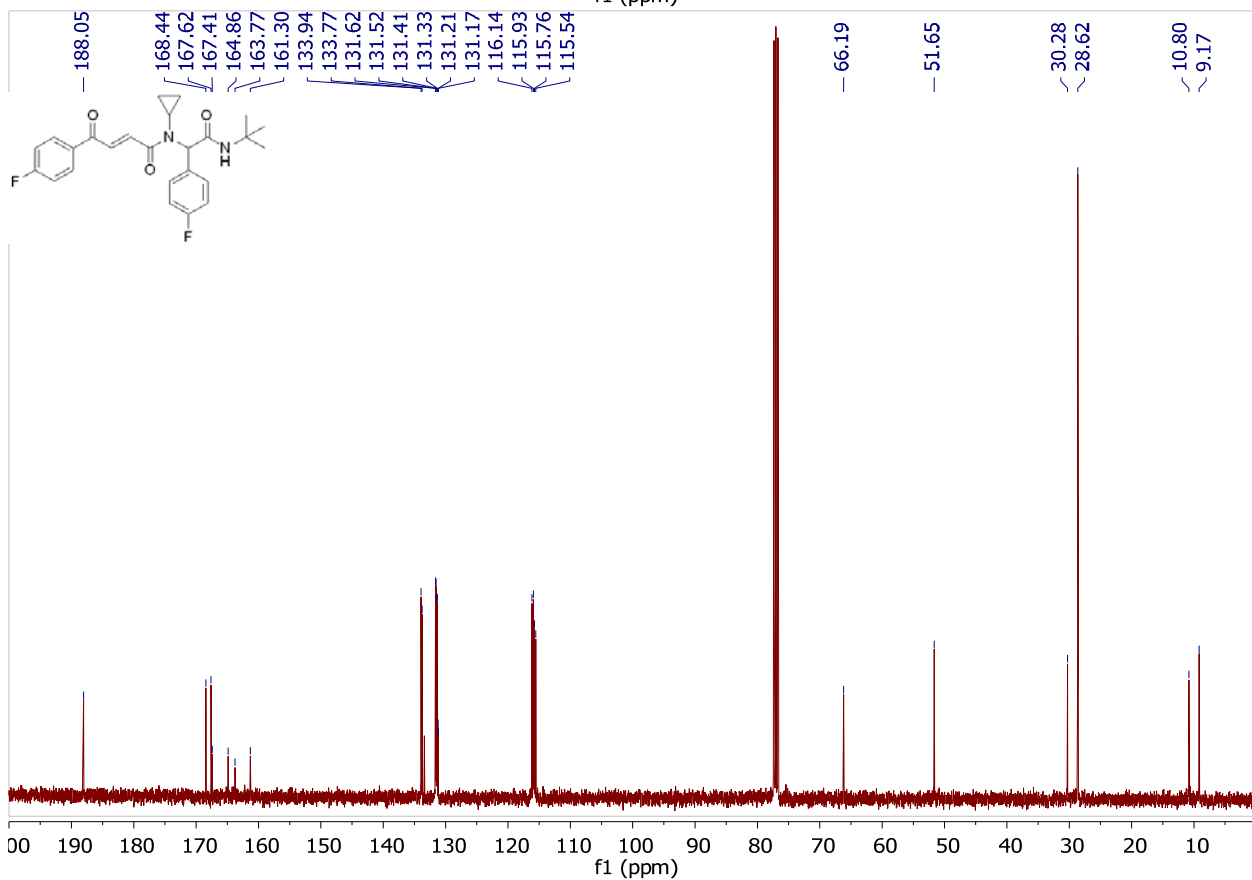
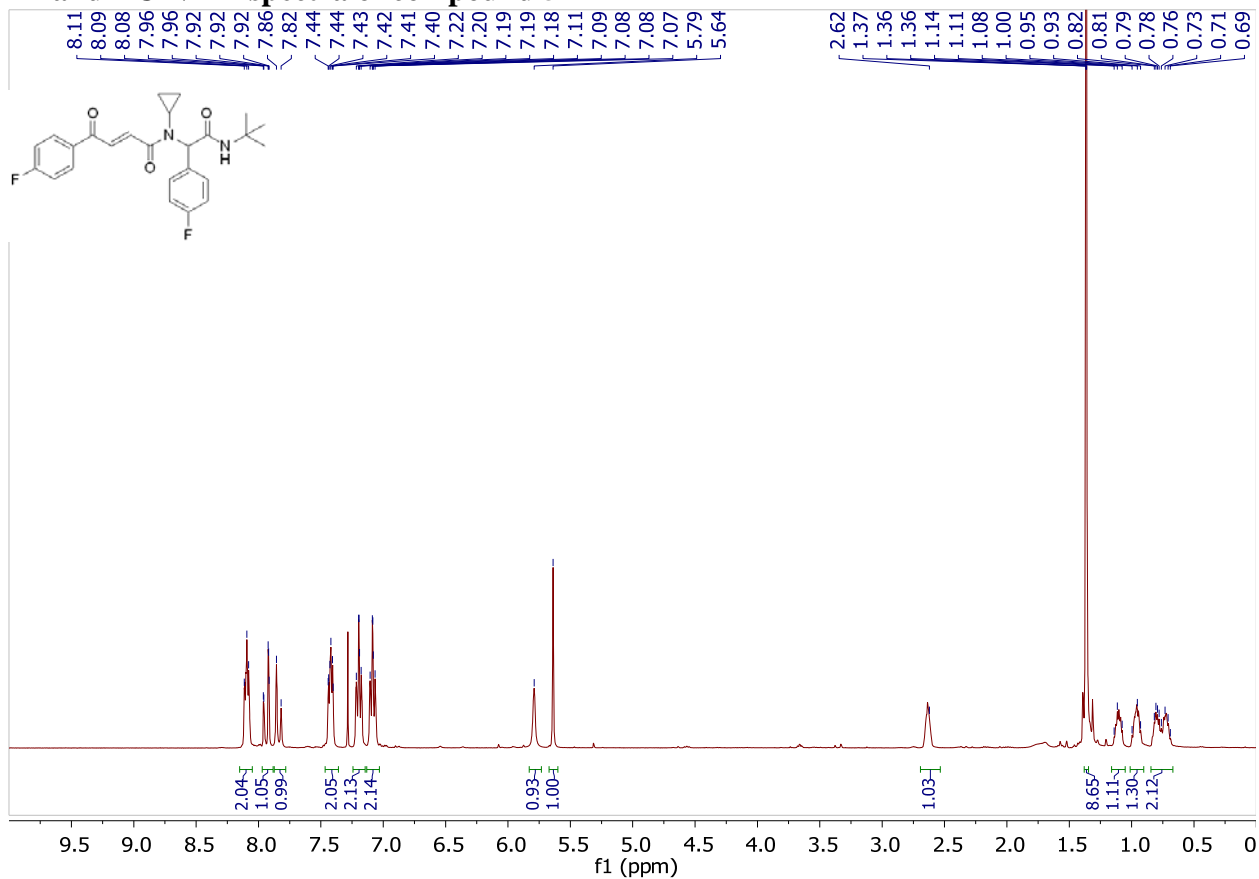
¹H and ¹³C NMR spectra of compound 5g



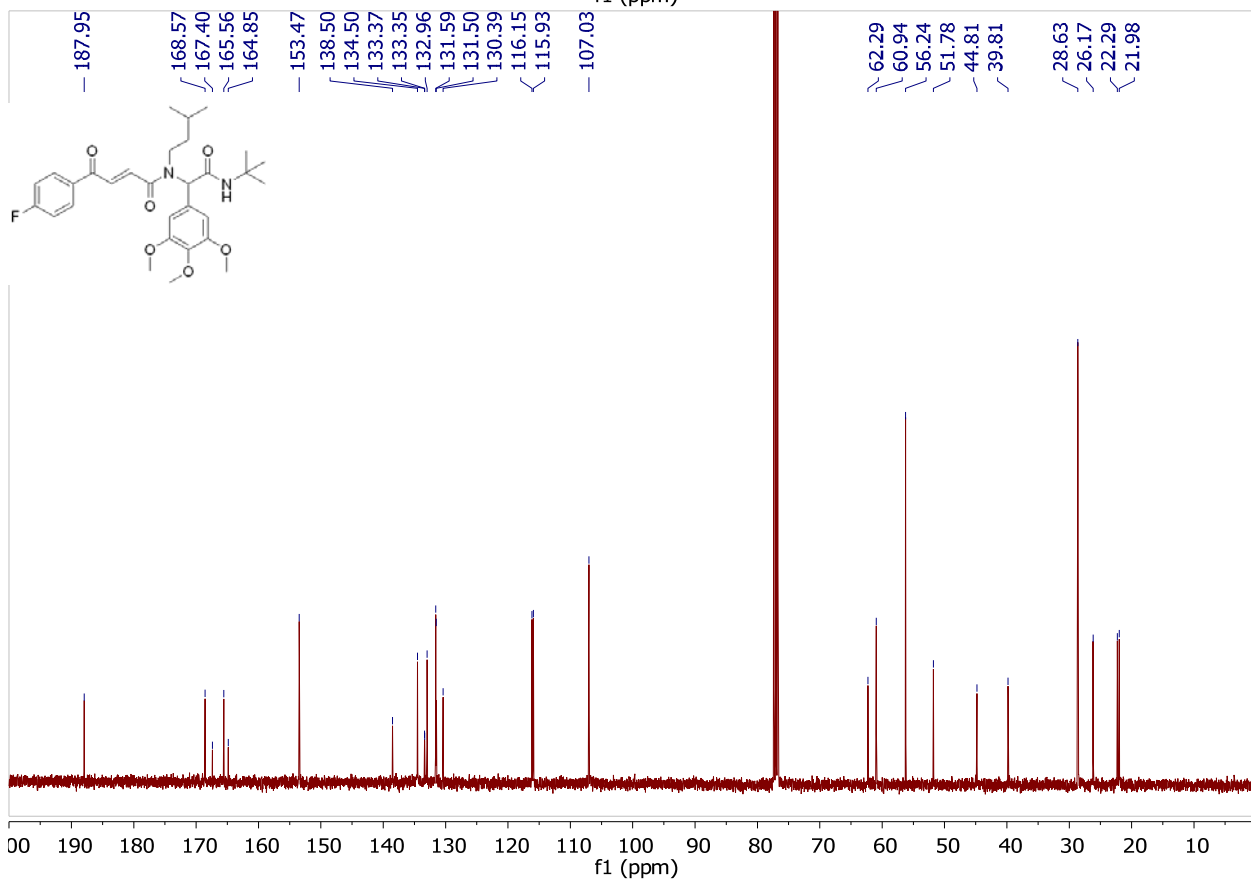
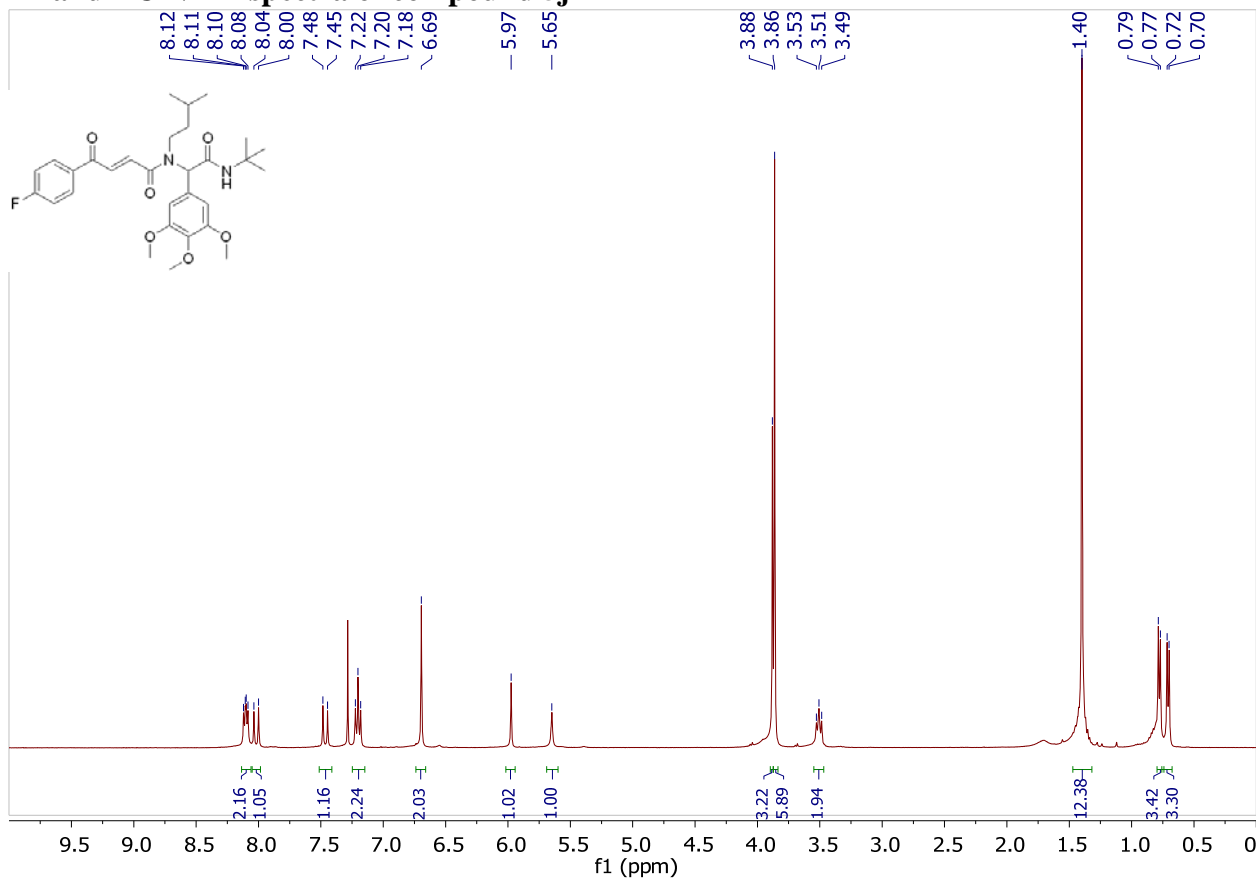
¹H and ¹³C NMR spectra of compound 5h



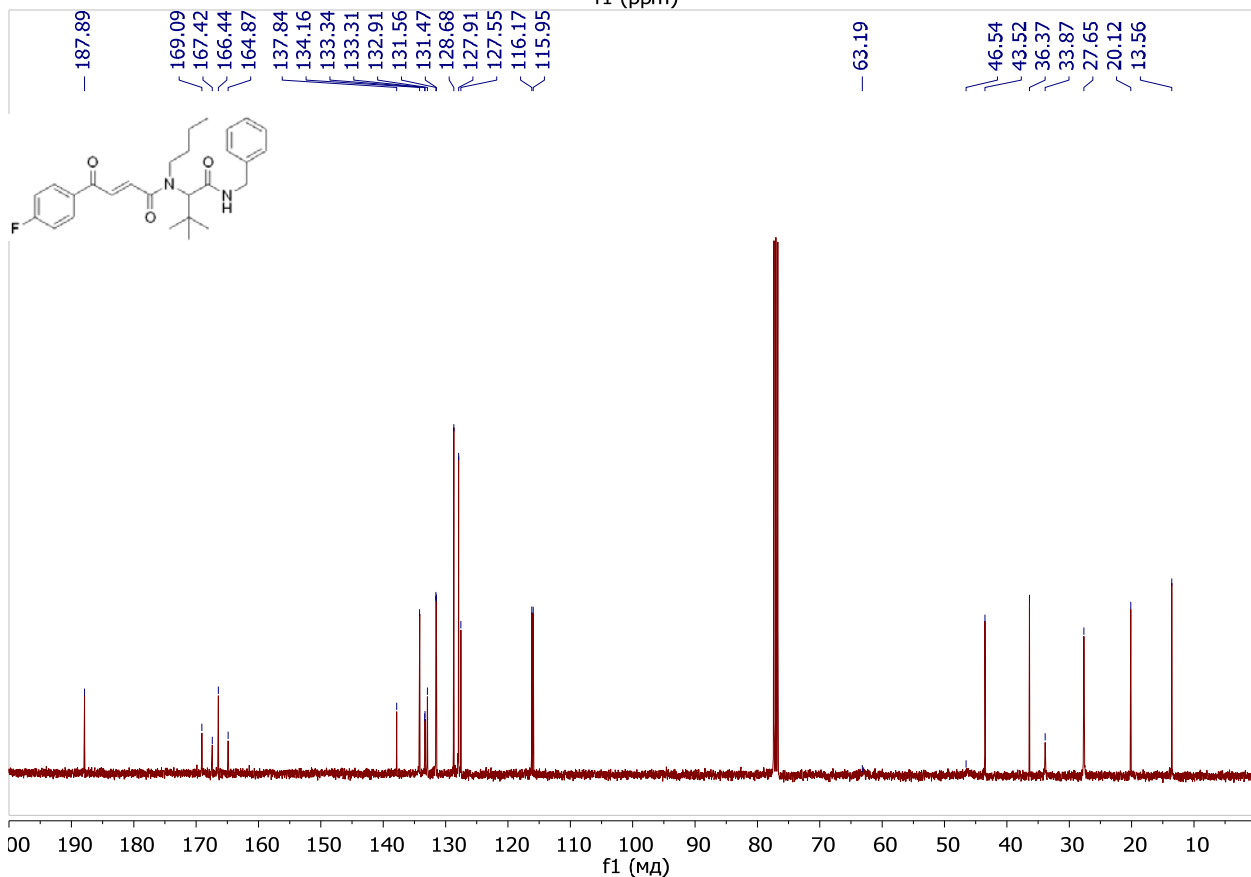
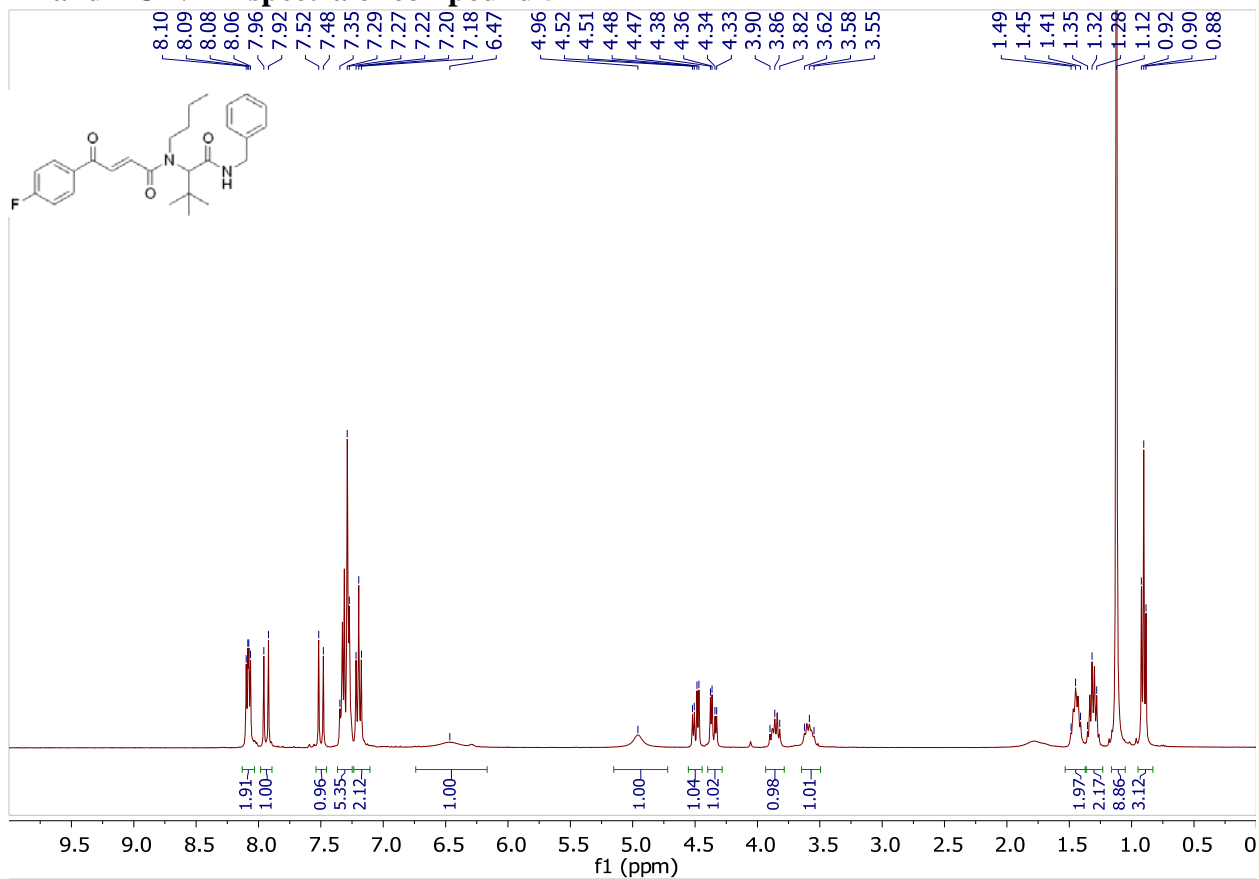
¹H and ¹³C NMR spectra of compound 5i



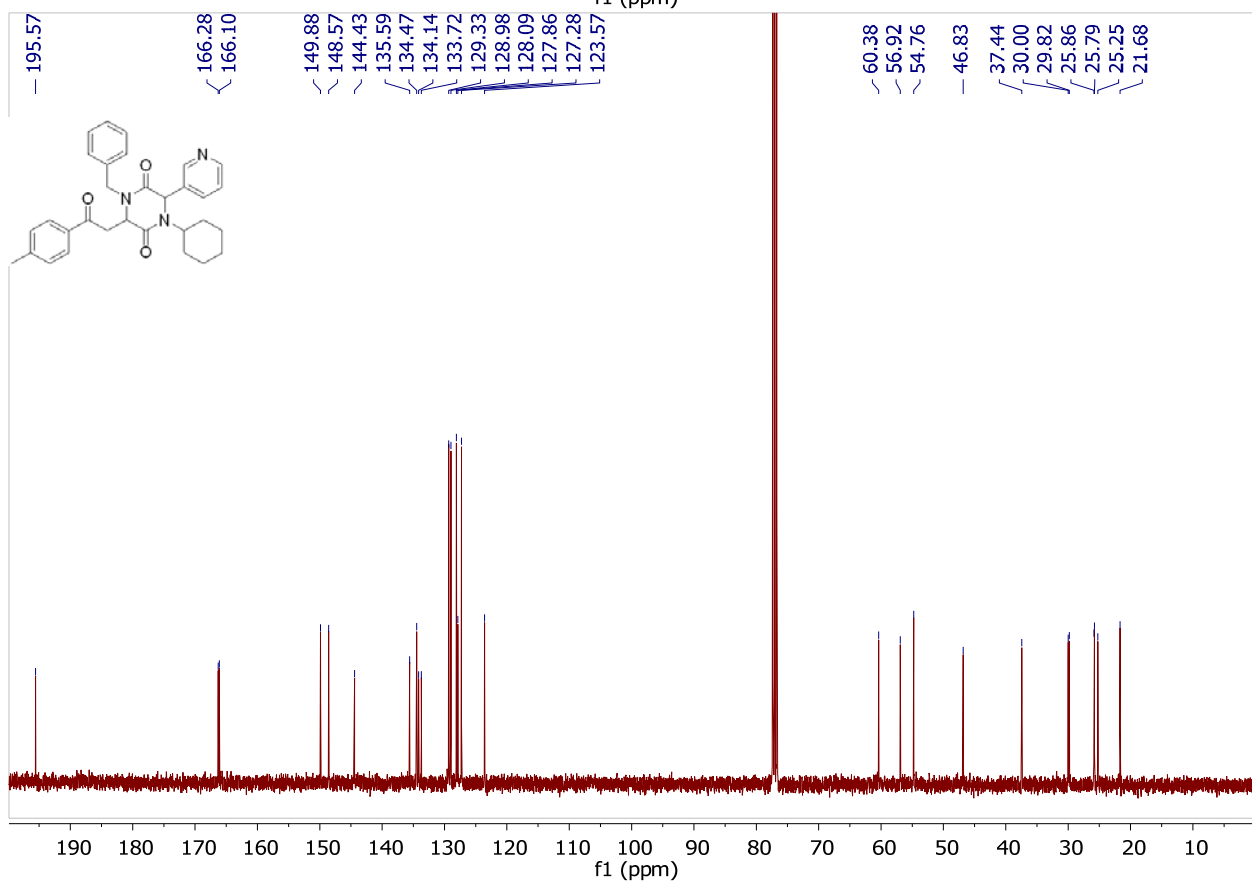
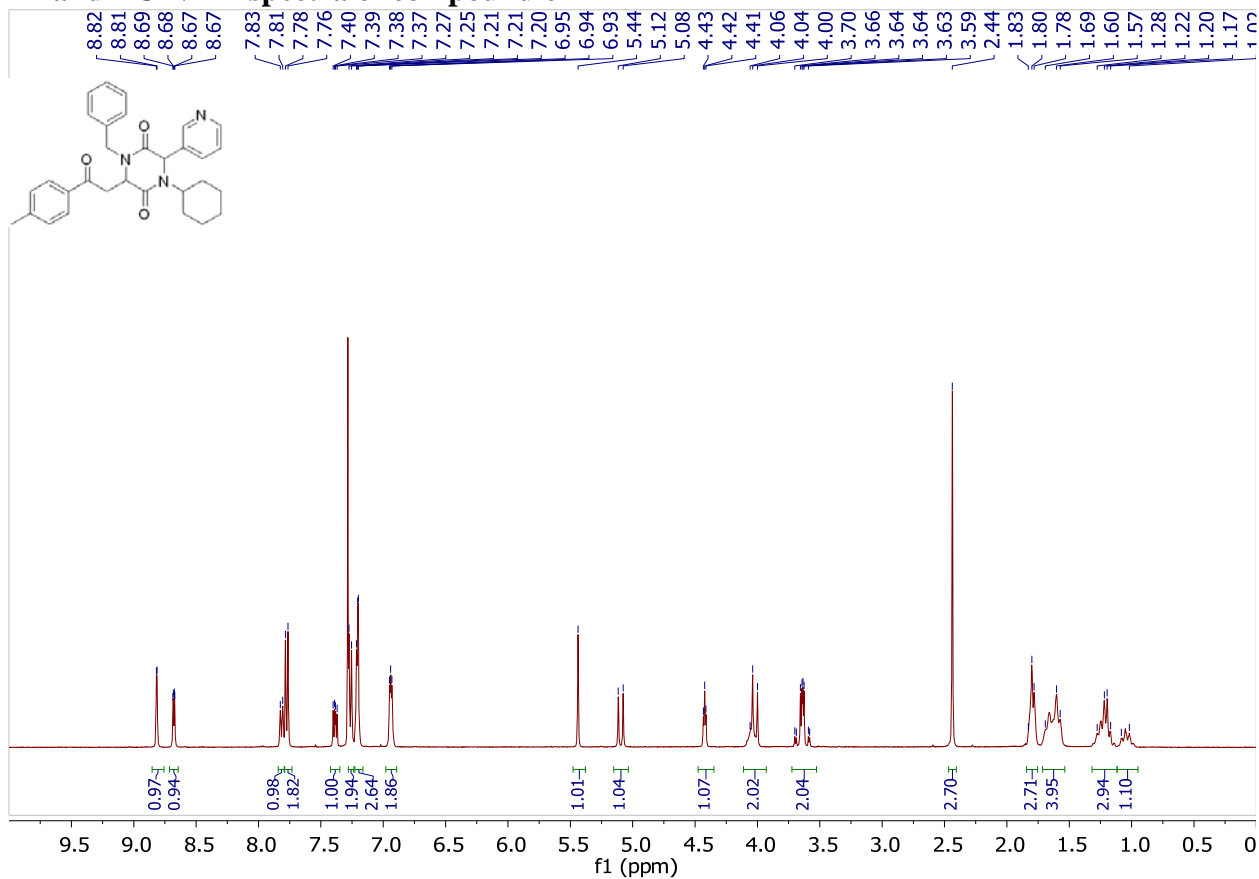
¹H and ¹³C NMR spectra of compound 5j



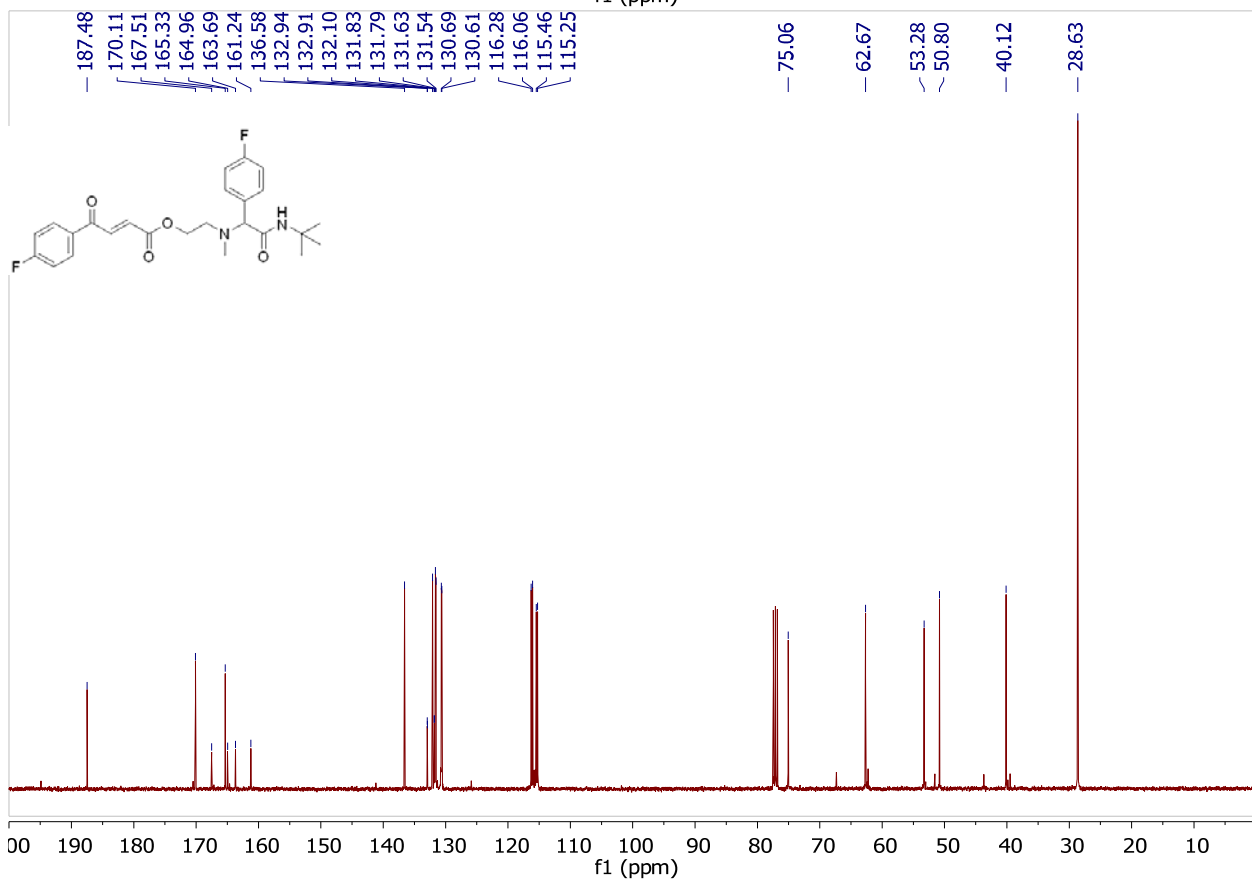
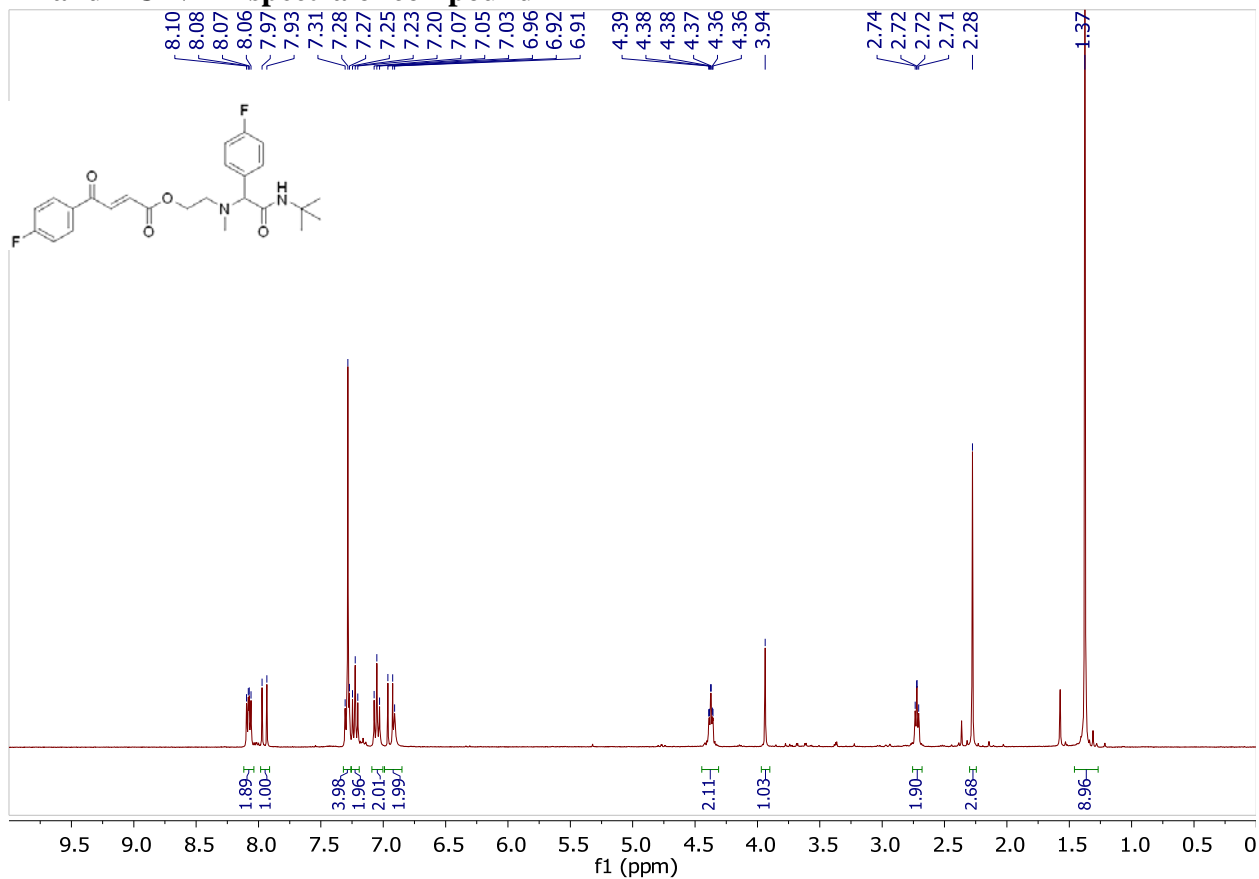
¹H and ¹³C NMR spectra of compound 5k



¹H and ¹³C NMR spectra of compound 6

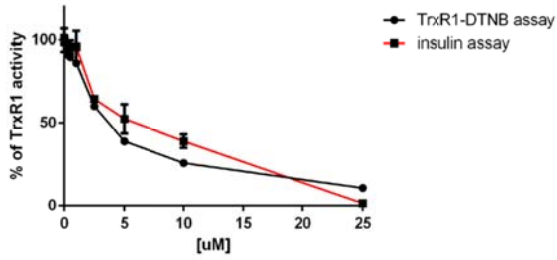


¹H and ¹³C NMR spectra of compound 7

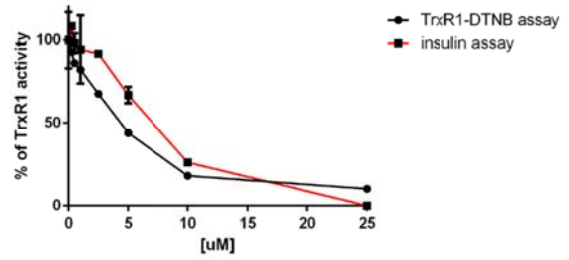


Supplement 1. Correlation between inhibition of TrxR1 on DTNB and insulin assay.

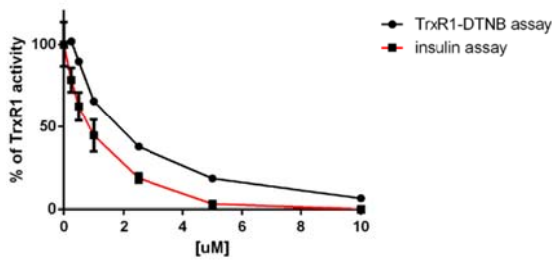
5d



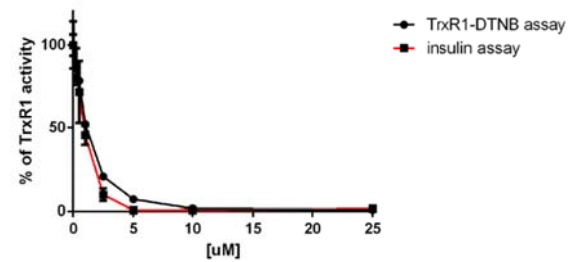
5e



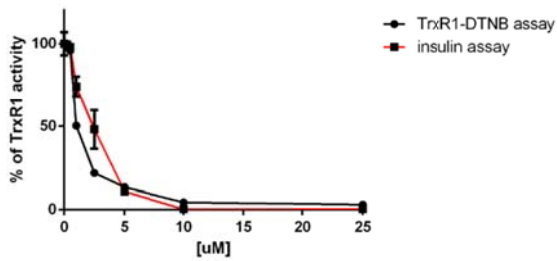
5f



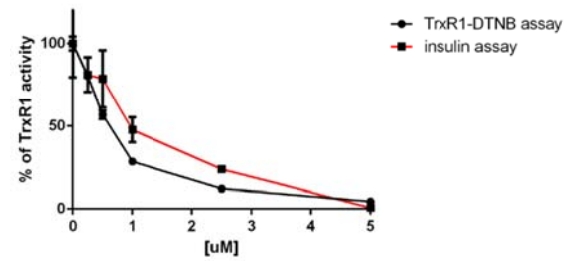
5i



5j

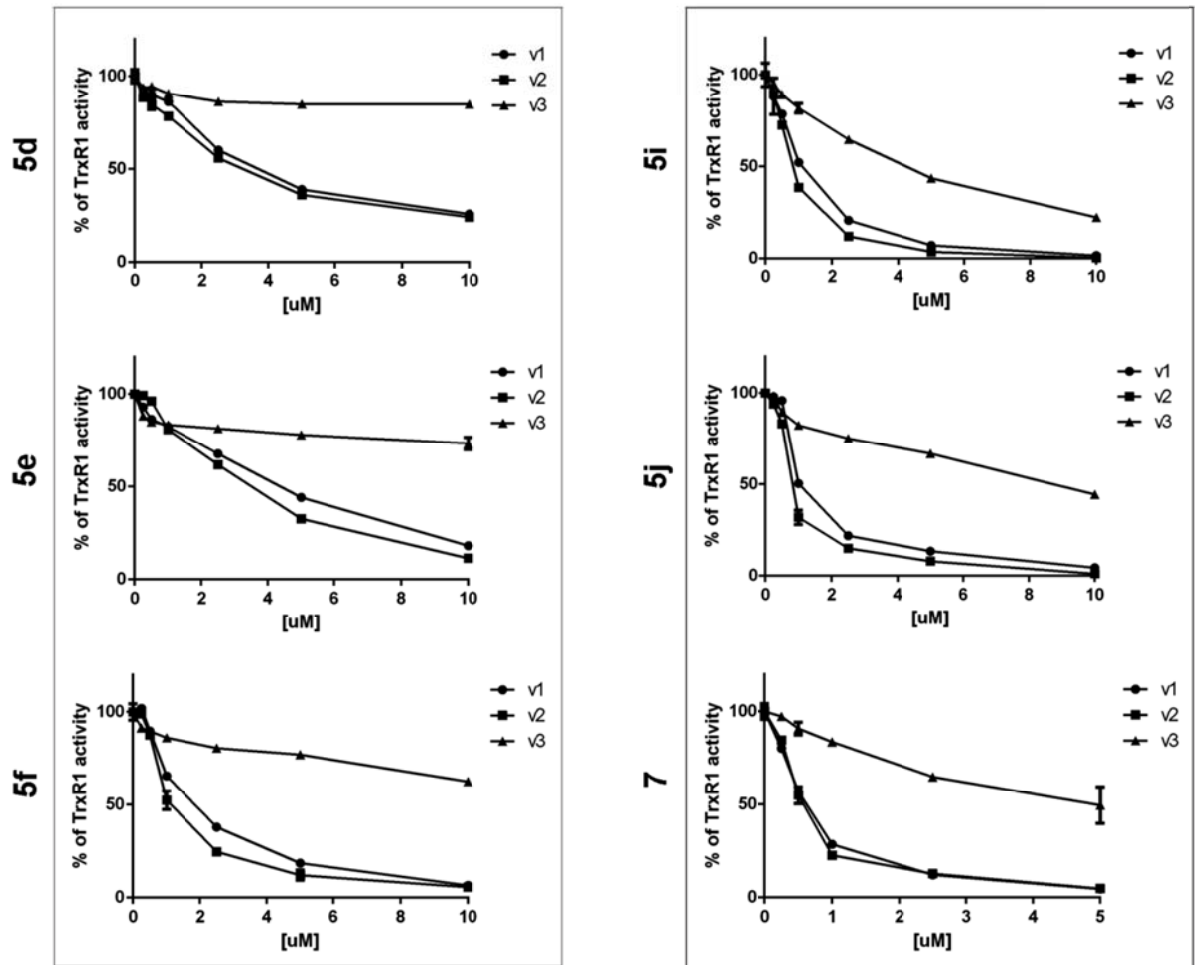


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| The correlation (r) between TrxR1 inhibition in DTNB reduction and insulin assay | | | |
|--|------|----------|------|
| | r | P | |
| 5d | 0.98 | < 0.0001 | **** |
| 5e | 0.96 | 0.0002 | *** |
| 5f | 0.97 | 0.0001 | *** |
| 5i | 0.98 | < 0.0001 | **** |
| 5j | 0.98 | < 0.0001 | **** |
| 7 | 0.98 | < 0.0001 | **** |

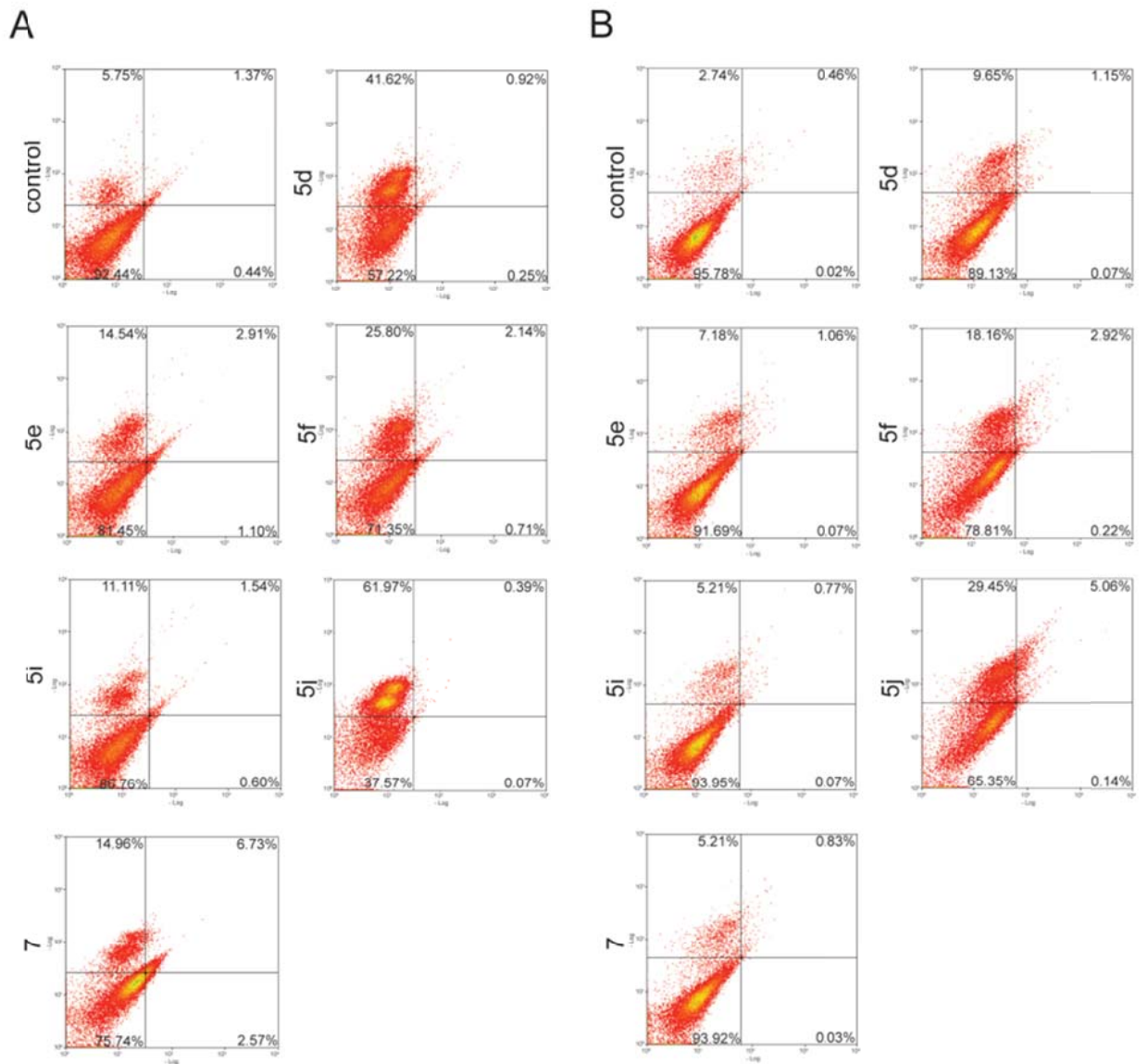
Supplement 2. Variations in incubation volume with and without NADPH.



Rat TrxR1, was incubated for 15 min with same amount of inhibitors, with or without NADPH.

v1 represents incubation of TrxR1 with NADPH in 90 μl volume; v2 represents incubation of TrxR1 with NADPH in 50 μl volume v3 represents incubation of TrxR1 without NADPH in 50 μl volume. Incubation period was followed by adding the substrate, up to a final volume of 100 μl in all reaction variations. Concentration of all components of reaction mixtures after adding the substrate were the same.

Supplement 3. Representative experimental data for cell death induction by UMAs.



Flow cytometric assay distinguishes viable (low left quadrant: AV- PI-), early apoptotic (low right quadrant: AV+ PI-), late apoptotic (up right quadrant: AV+ PI+) and necrotic (up left quadrant: AV- PI+) cells