

Design, Synthesis and Characterization of Novel Pyridazin-3-one Derivatives: *In Vitro* Vasorelaxant Activity and *In Silico* Insights into eNOS Modulation

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S66	^{13}C NMR of 5g	69
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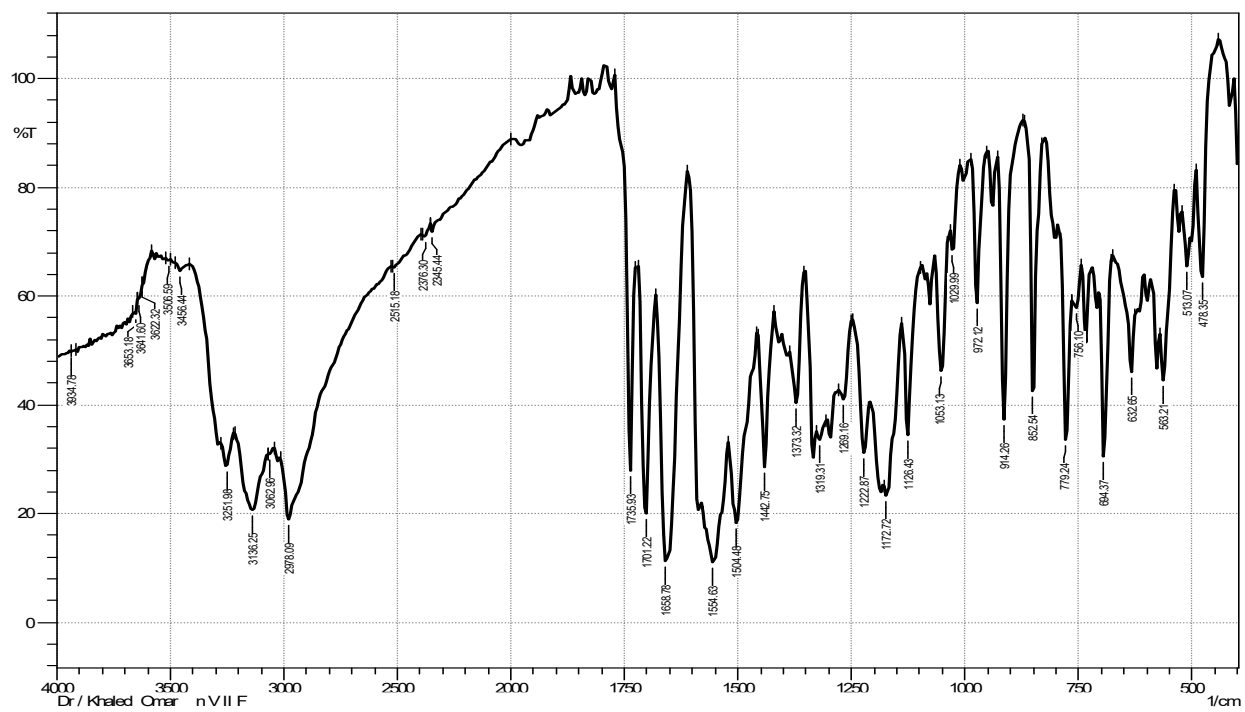


Figure 1. IR chart of 4a

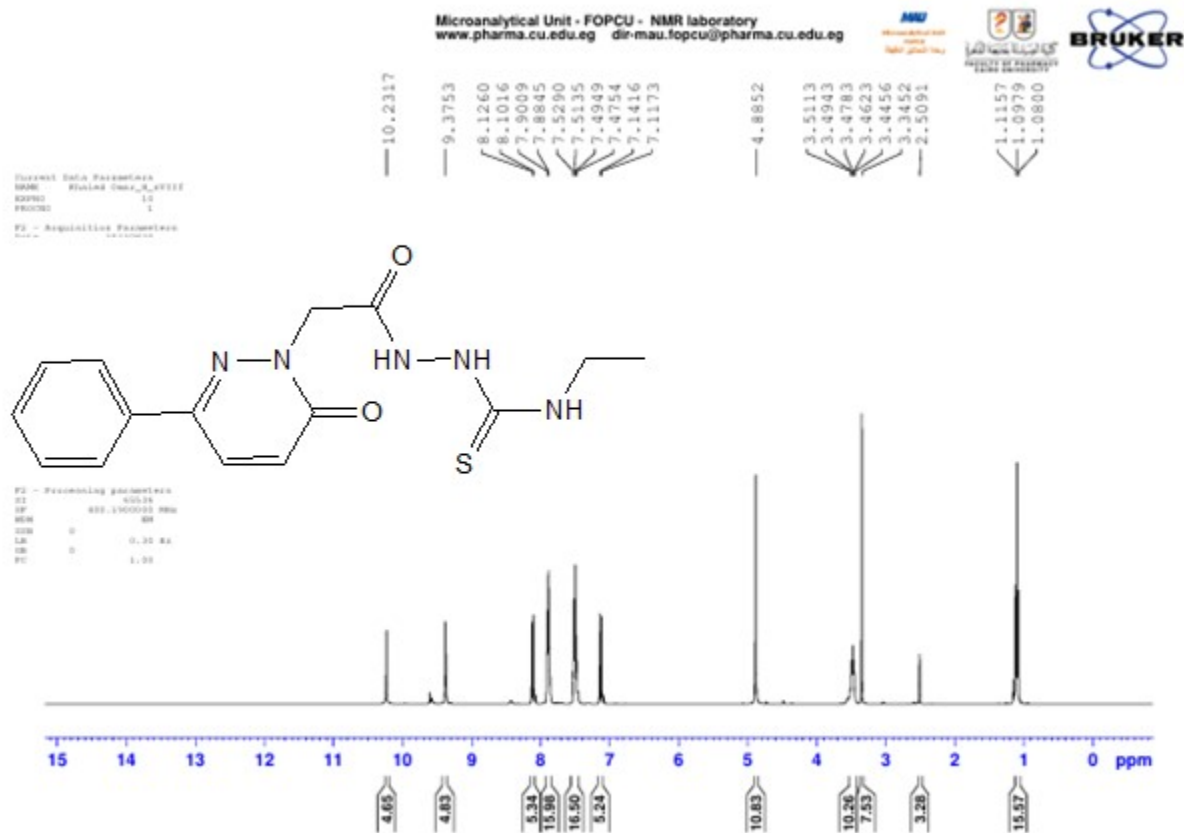


Figure 2. ¹H NMR chart of 4a

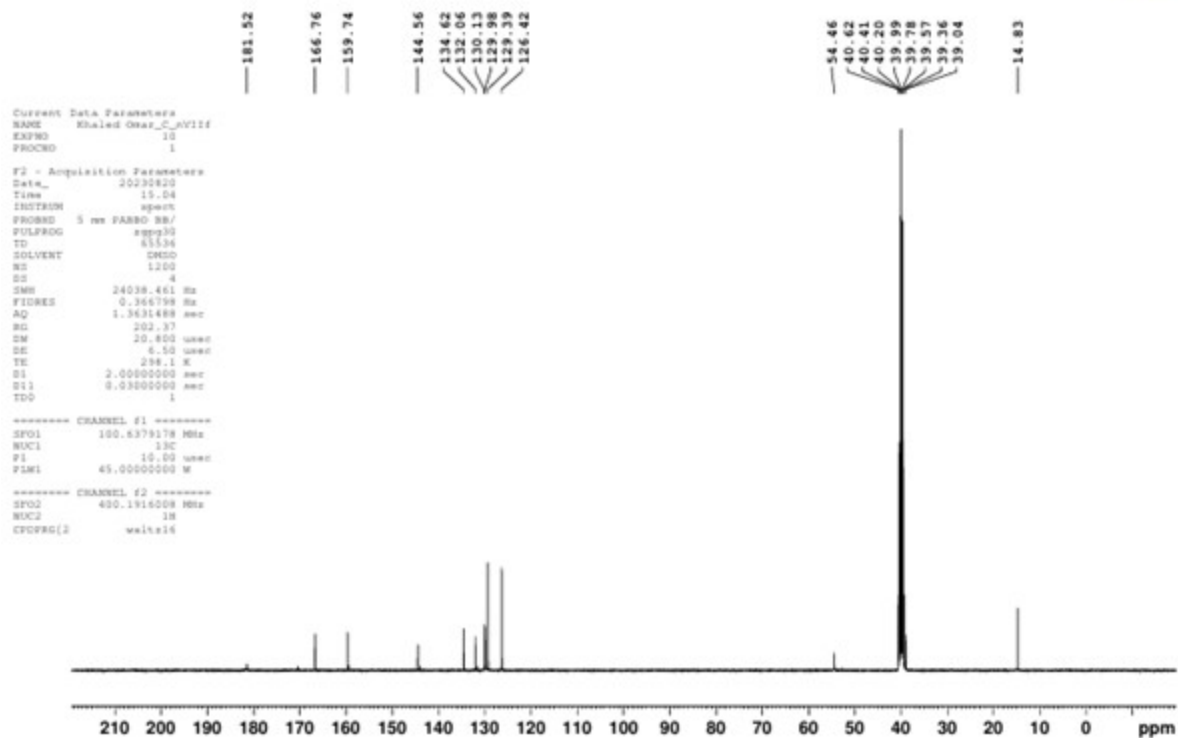


Figure 3. ^{13}C NMR chart of 4a

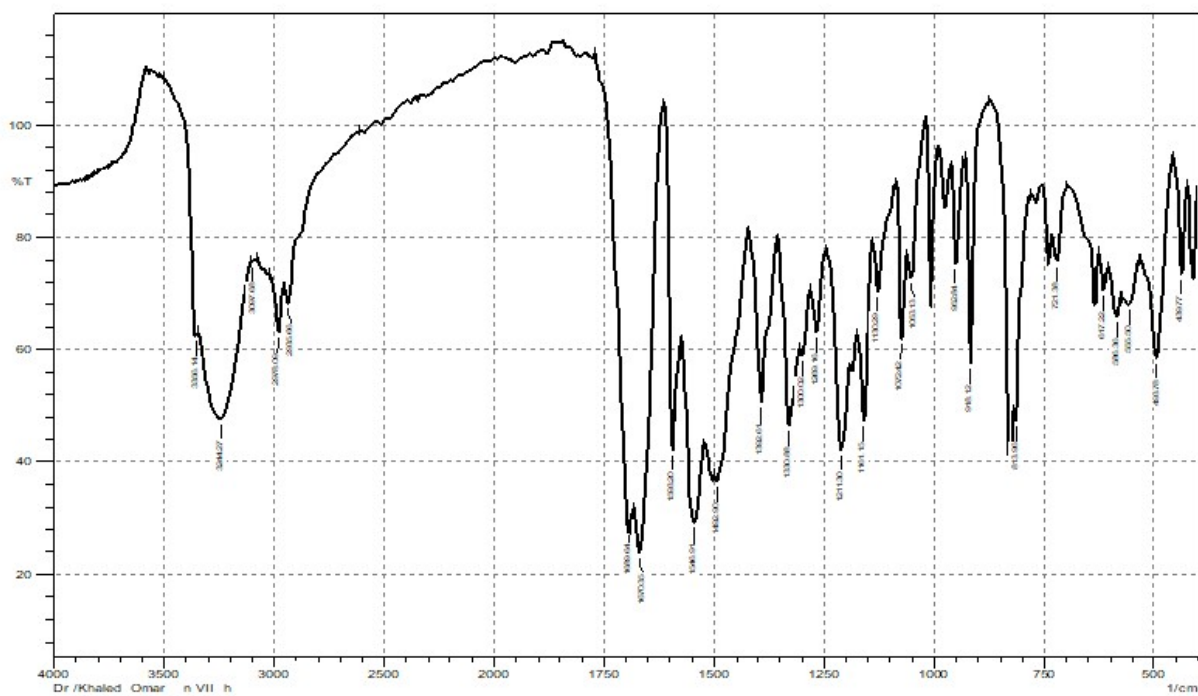


Figure 4. IR chart of 4b

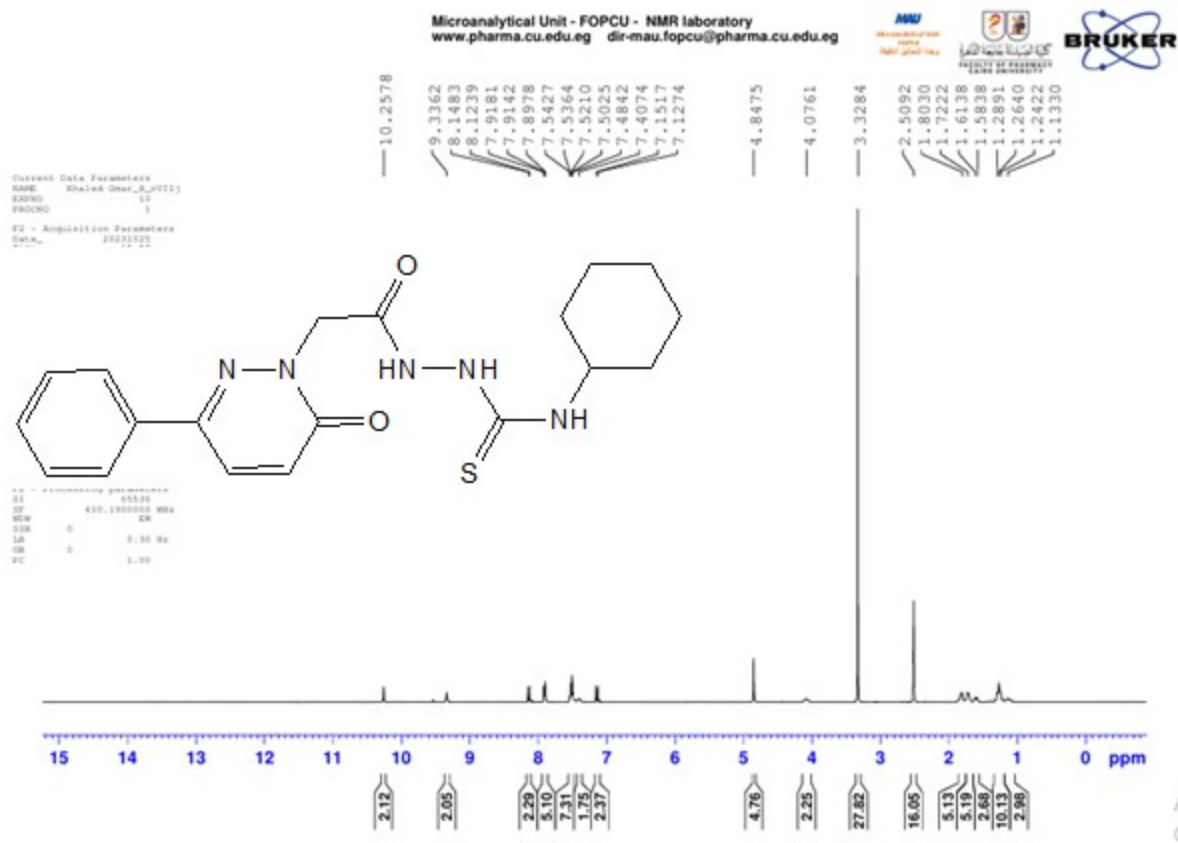


Figure 5. ¹H NMR of 4b

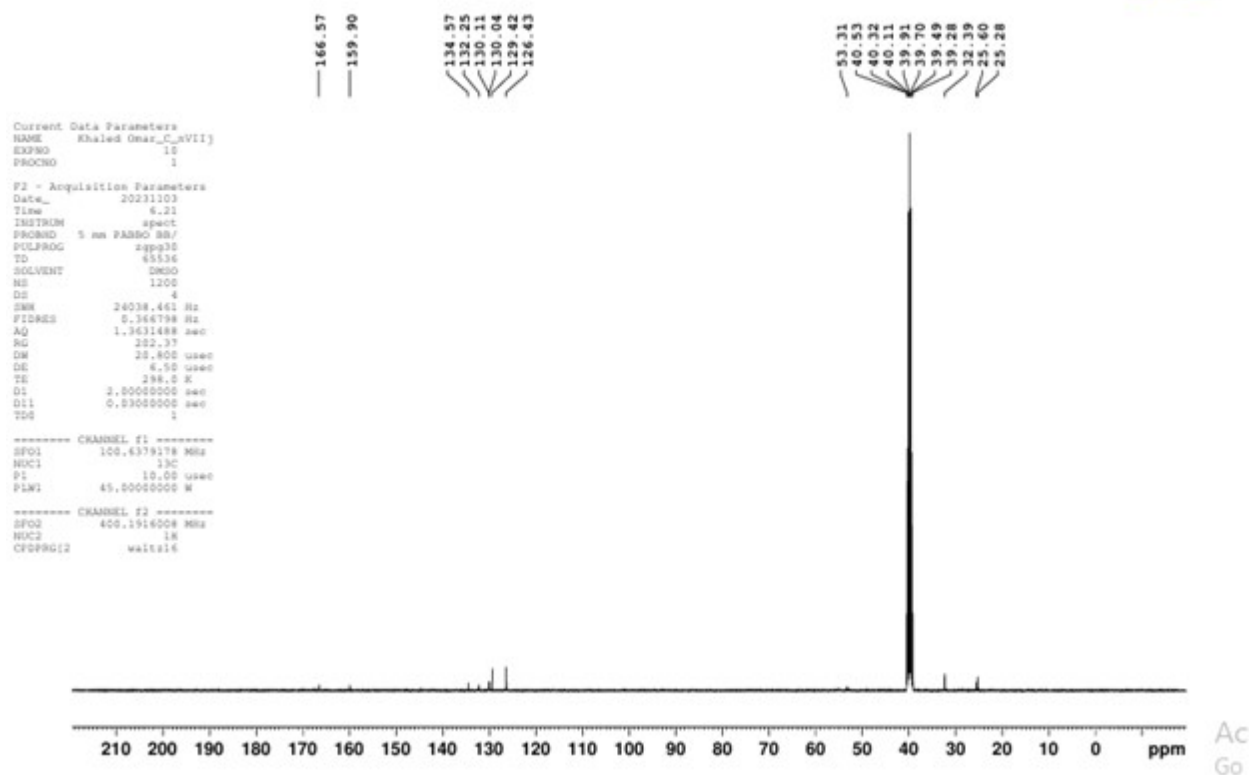


Figure 6. ^{13}C NMR chart of 4b

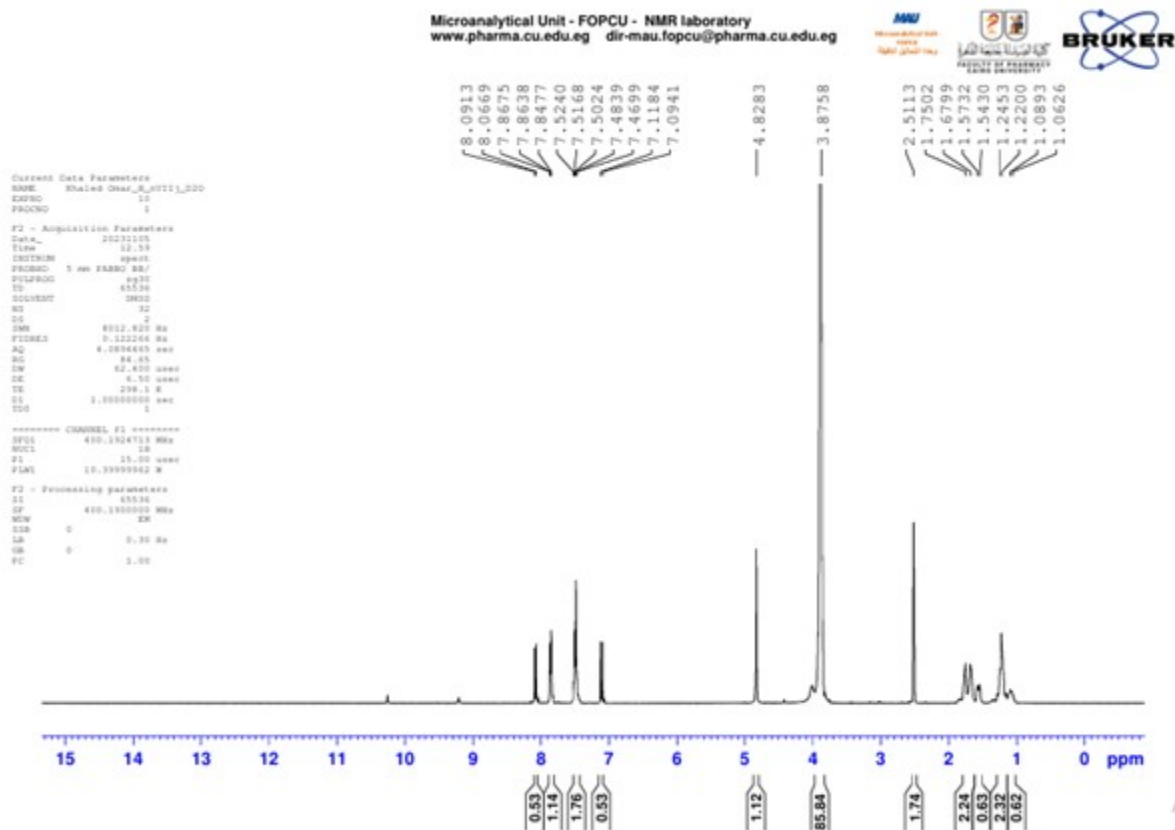


Figure 7. D₂O chart of 4b

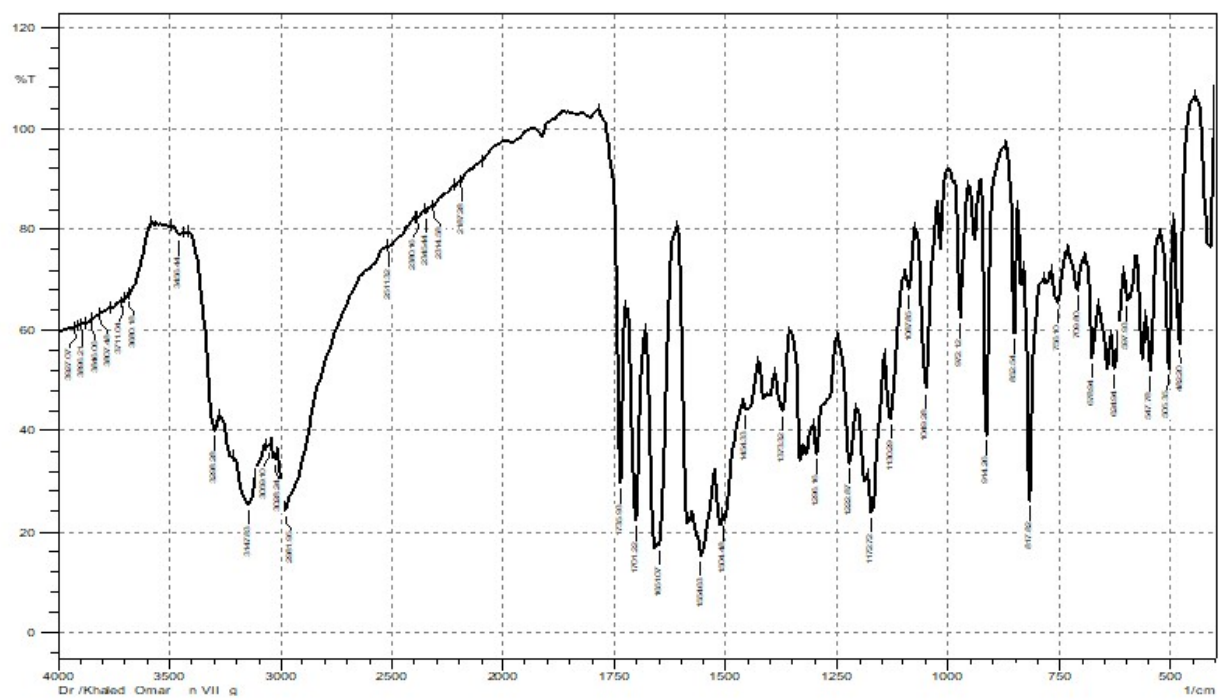


Figure 8. IR chart of 4c

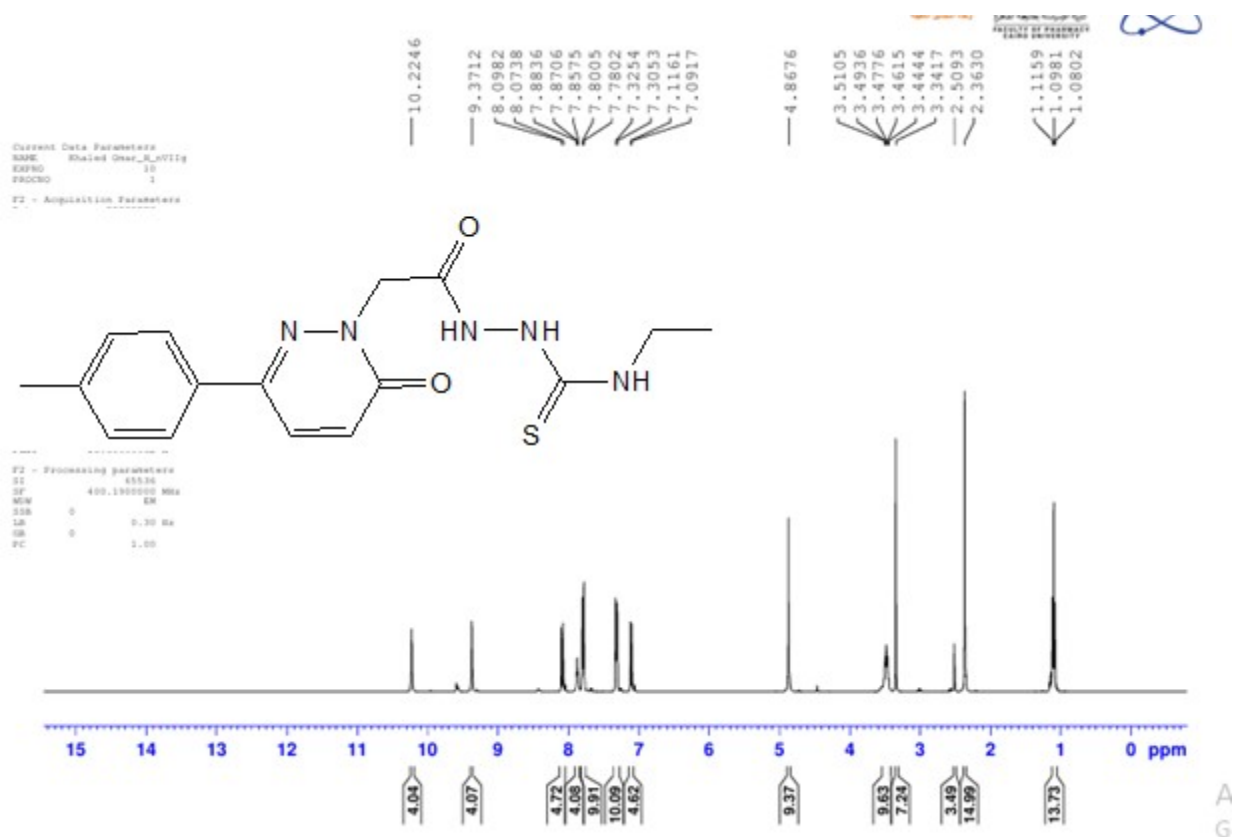


Figure 9. ¹H NMR chart of 4c

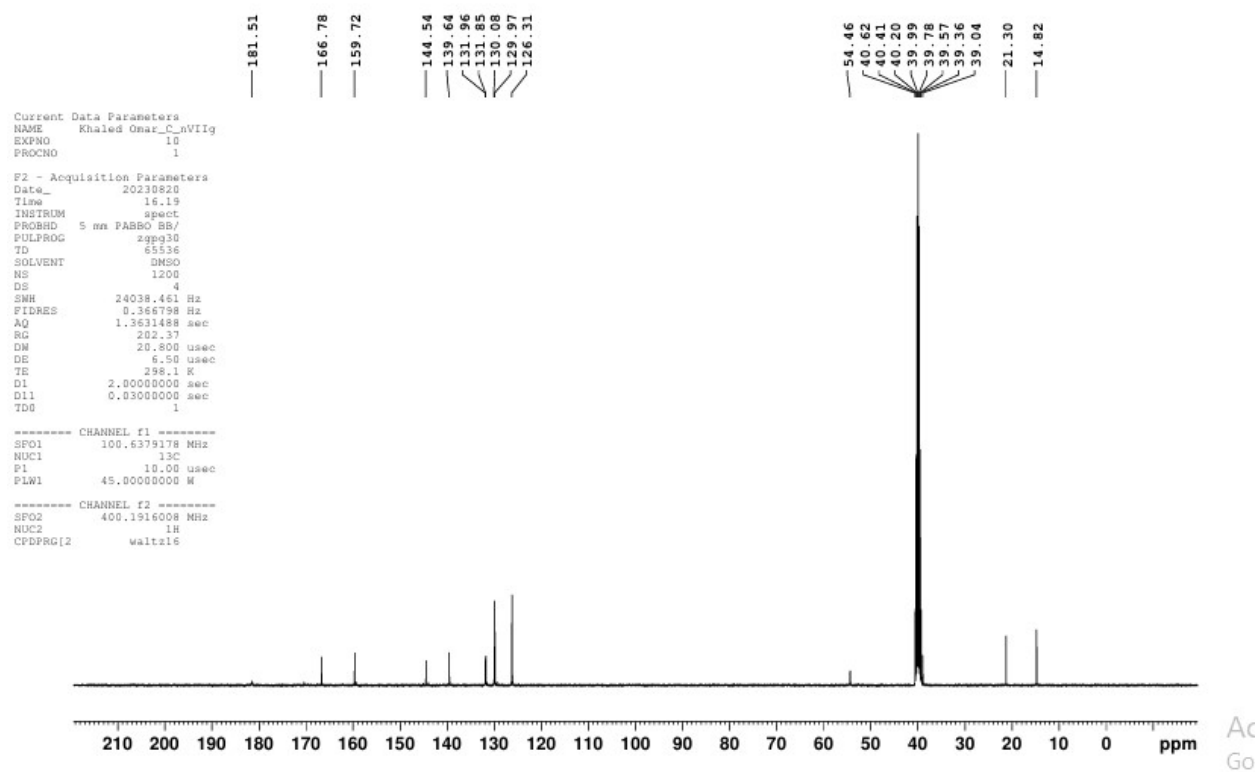


Figure 10. ^{13}C chart of 4c

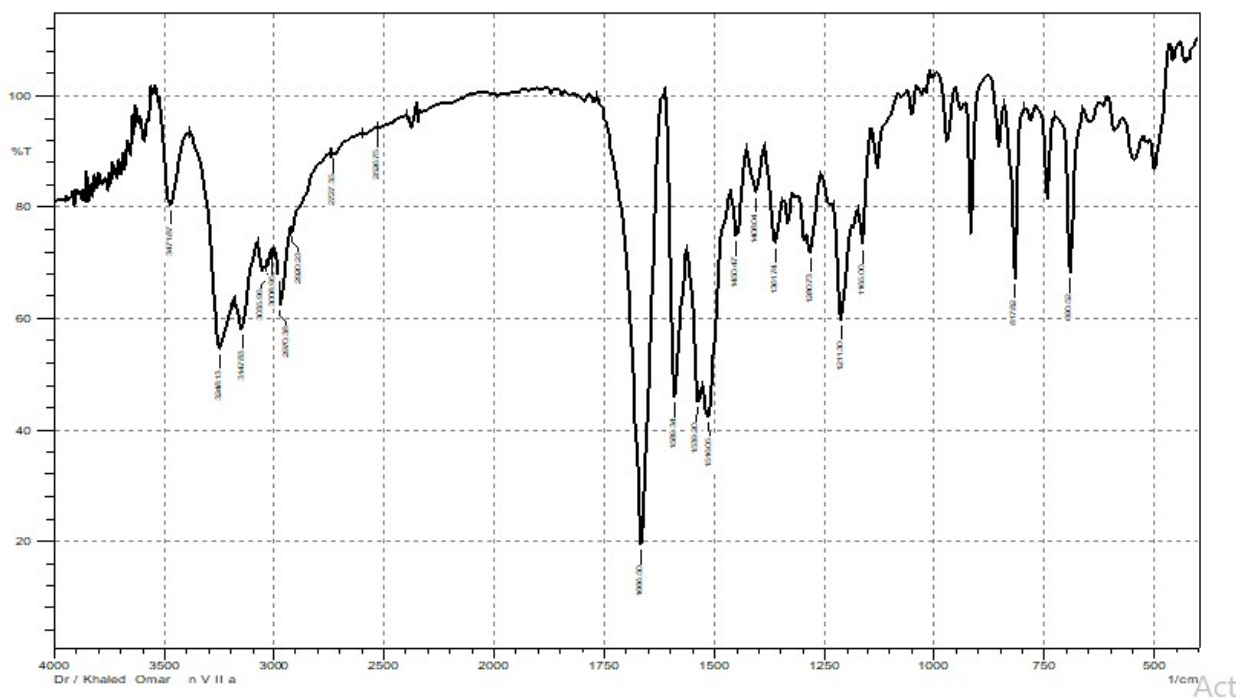


Figure 11. IR chart of 4d

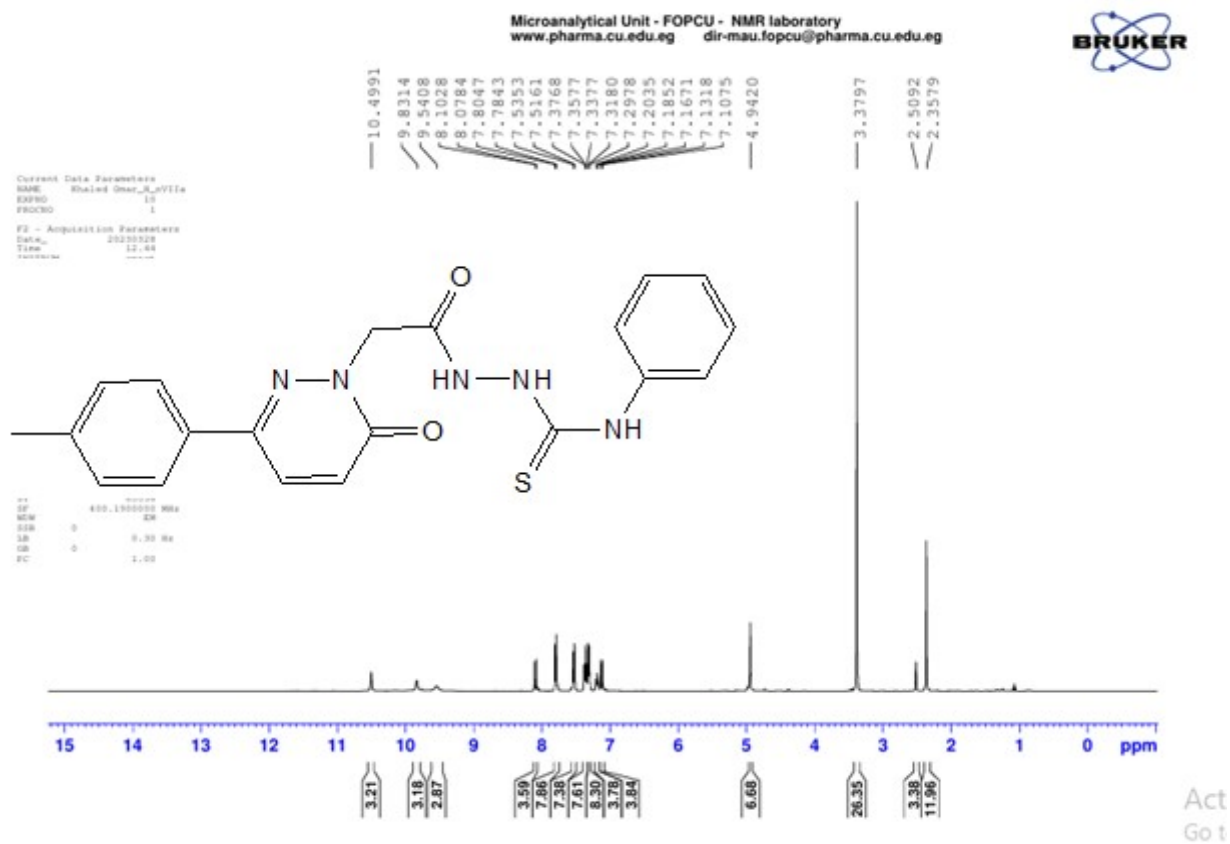


Figure 12. ¹H chart of 4d

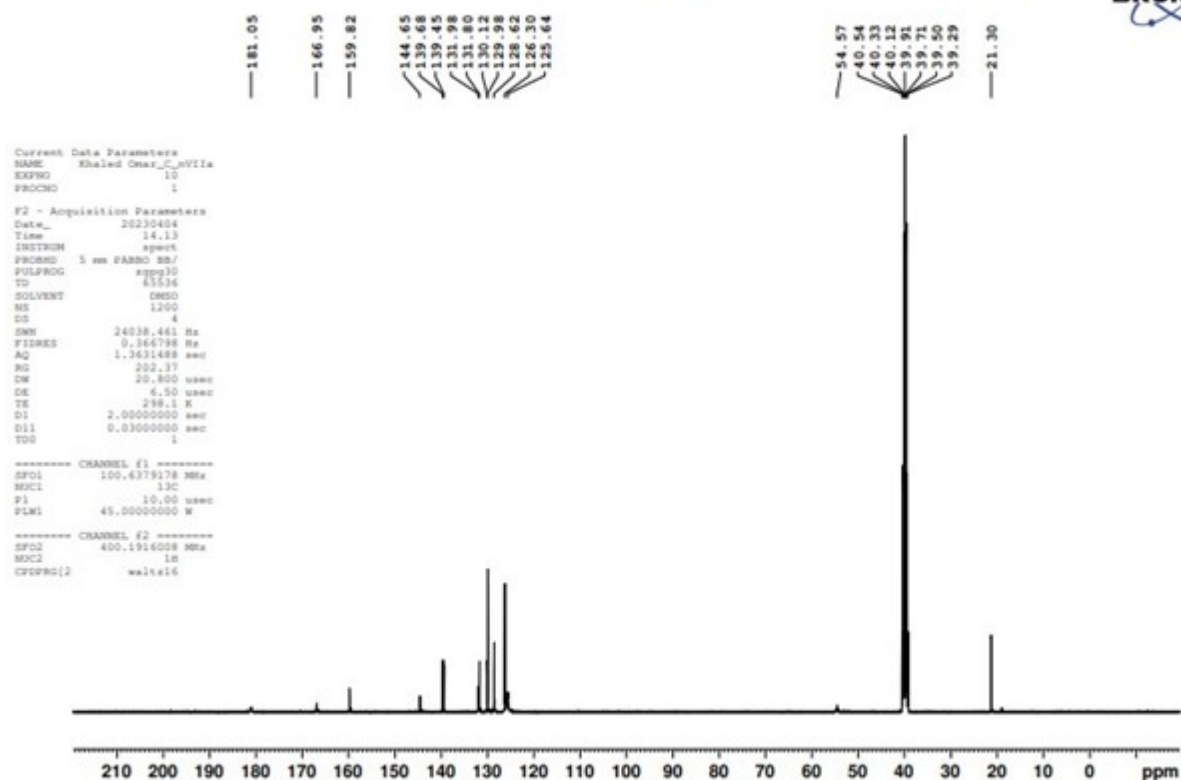


Figure 13. ^{13}C NMR chart of 4d

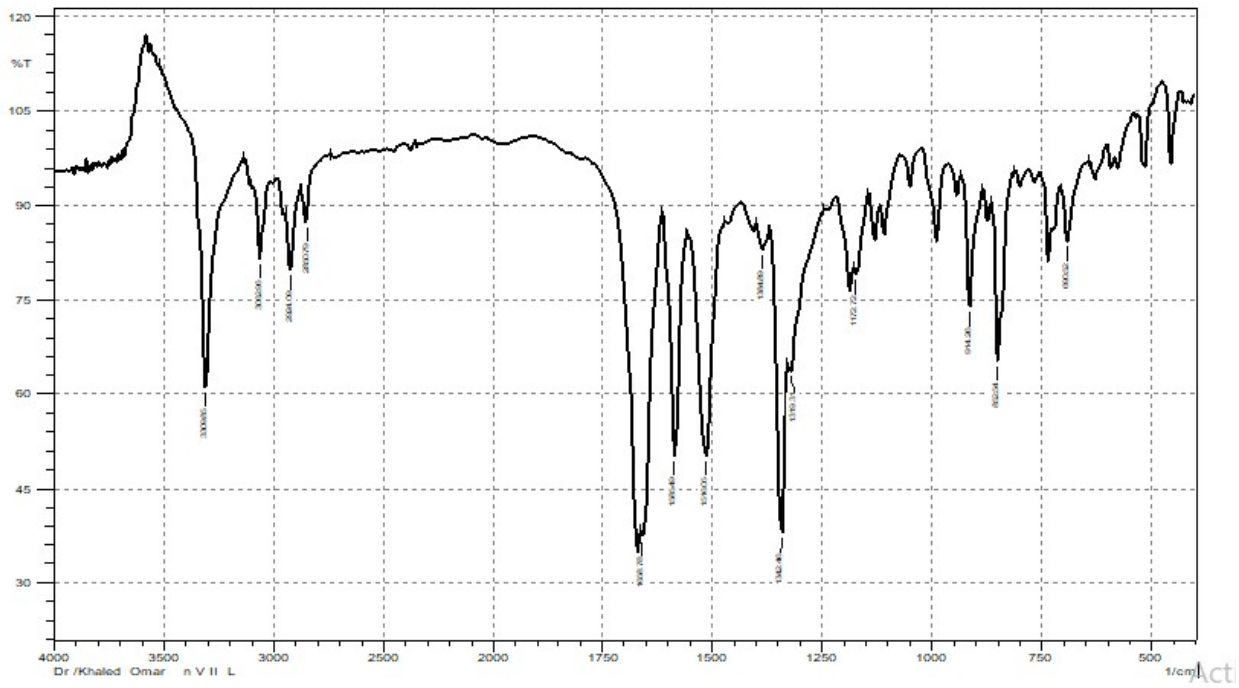


Figure 14. IR chart of 4e

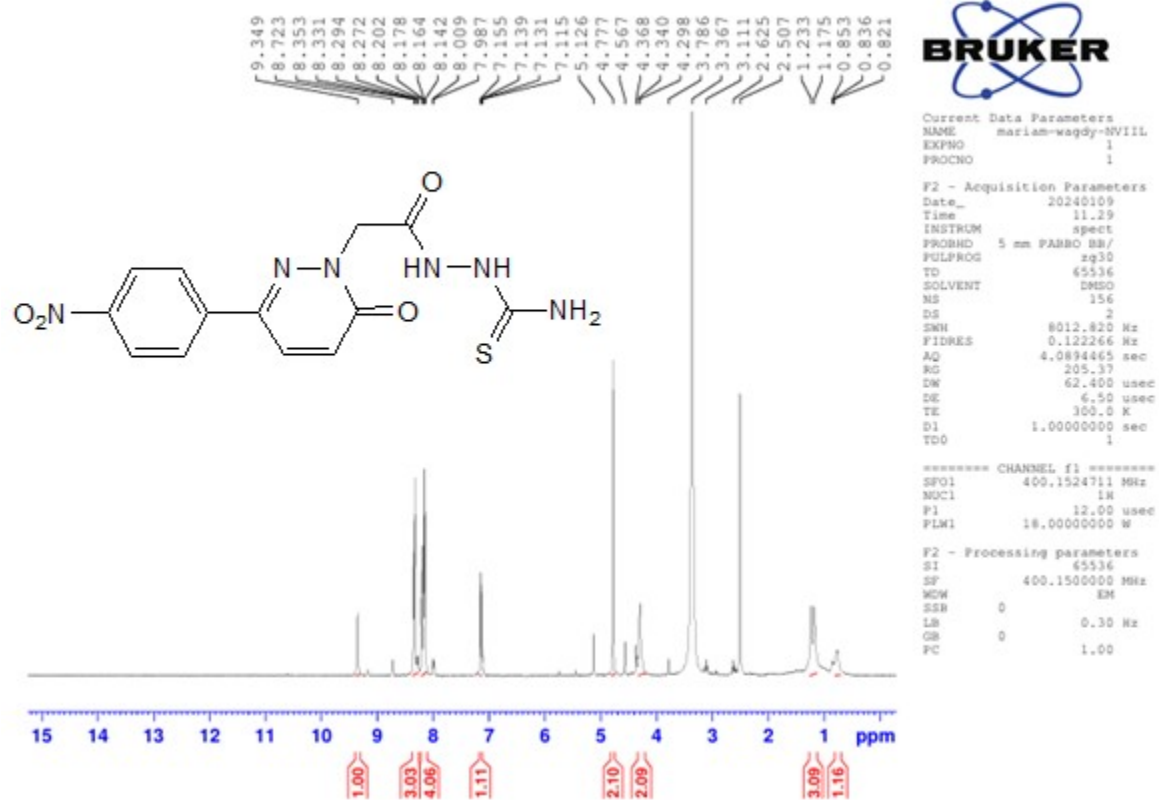


Figure 15. ¹H chart of 4e

A
G

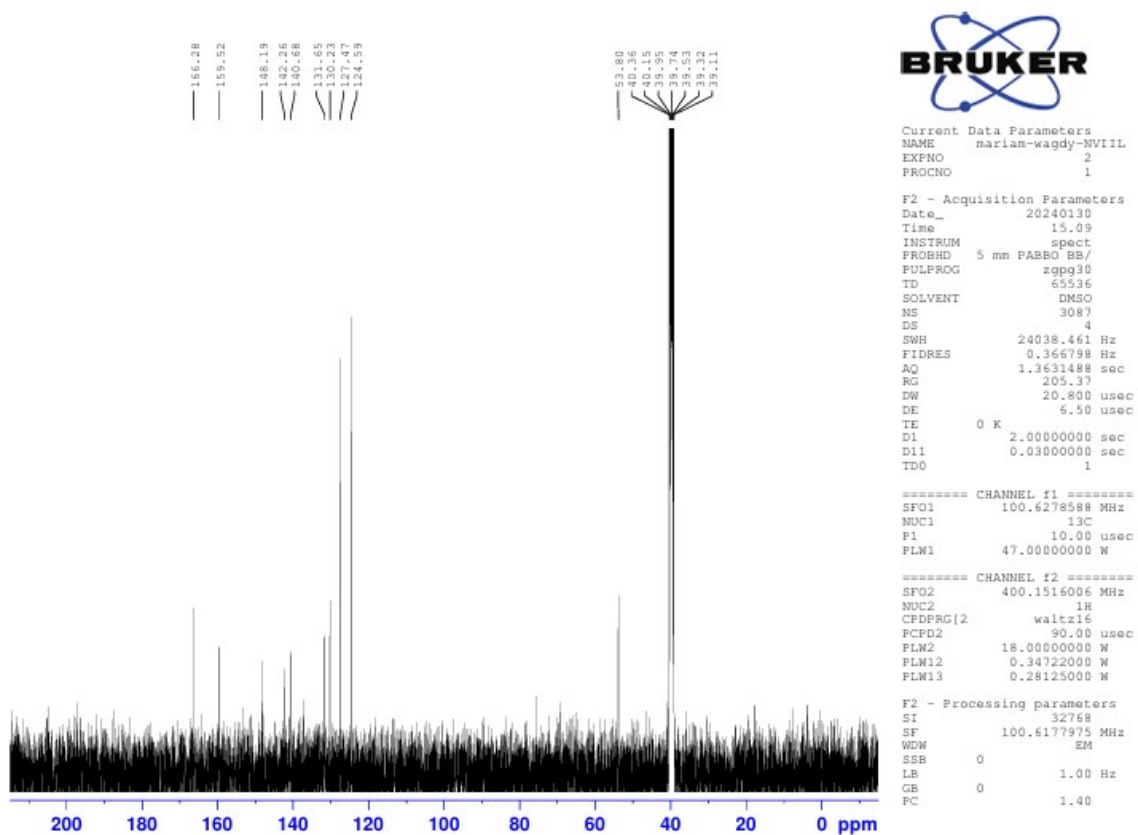


Figure 16. ^{13}C chart of 4e

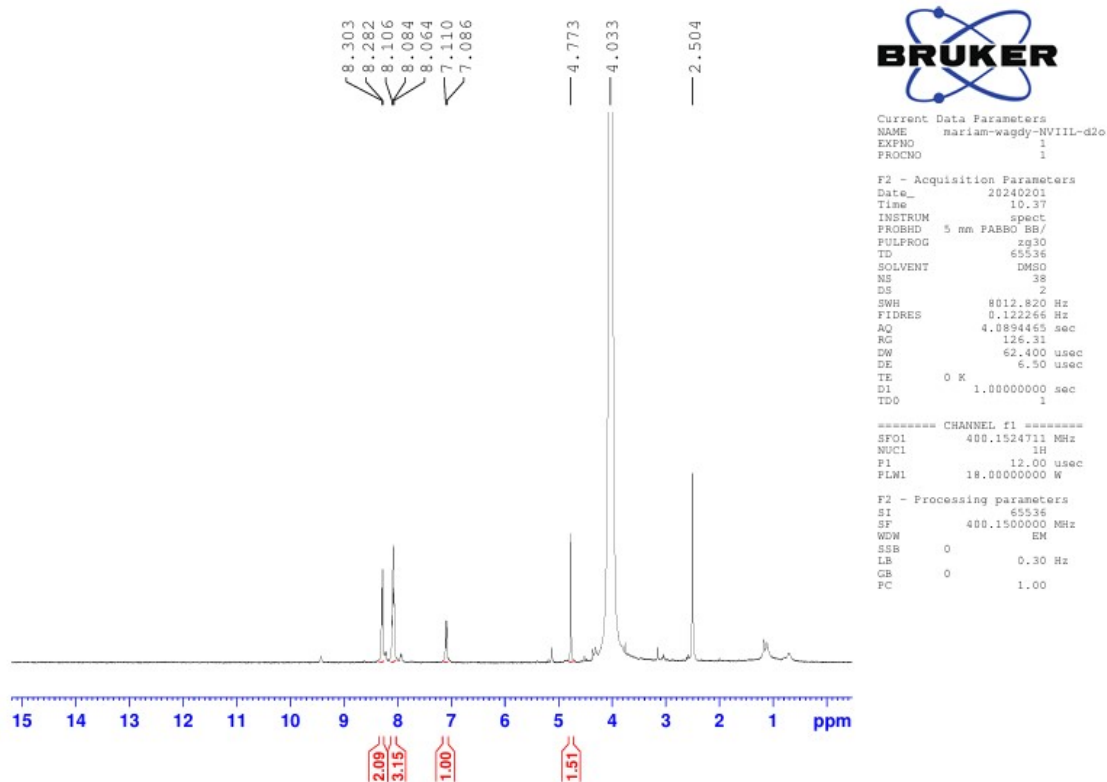


Figure 17. D₂O of 4e

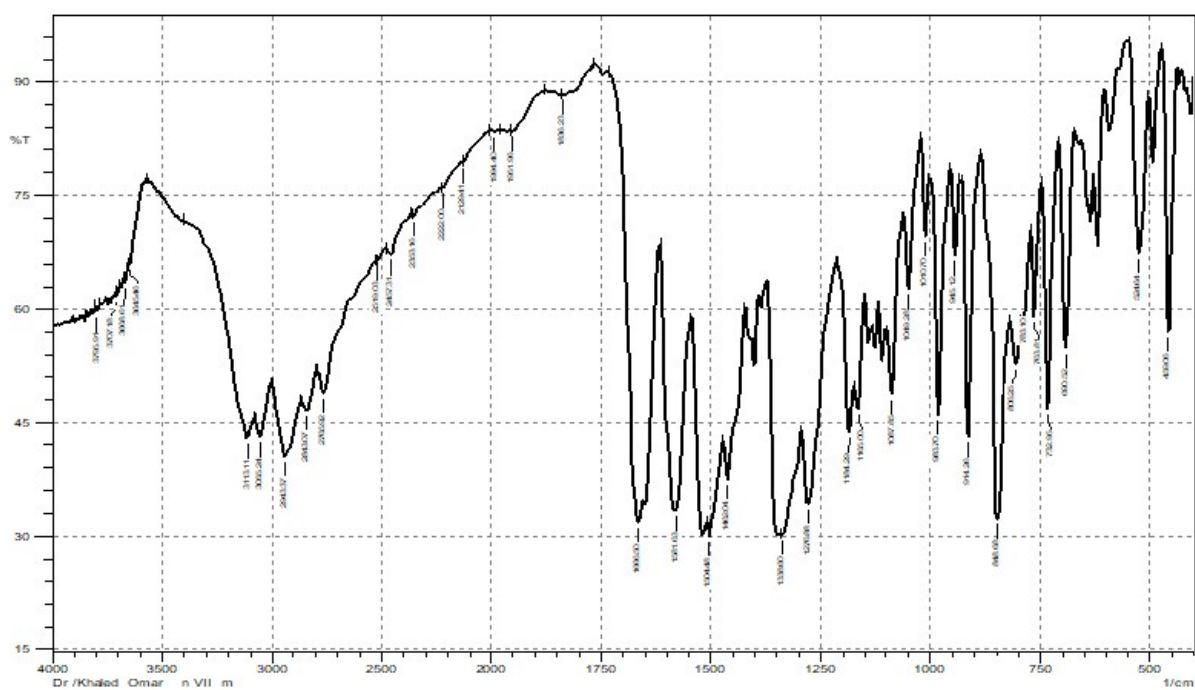


Figure 18. IR chart of 4f

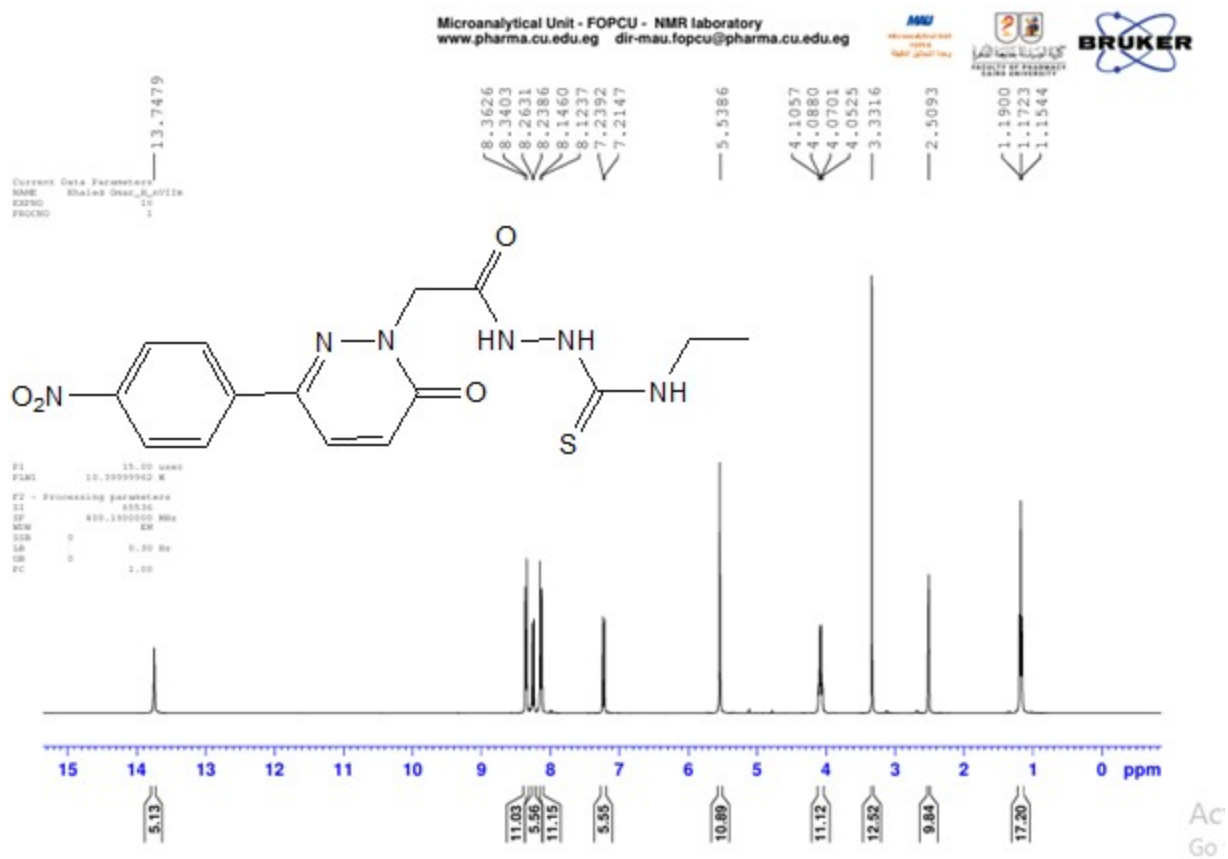


Figure 19. ¹H NMR of 4f

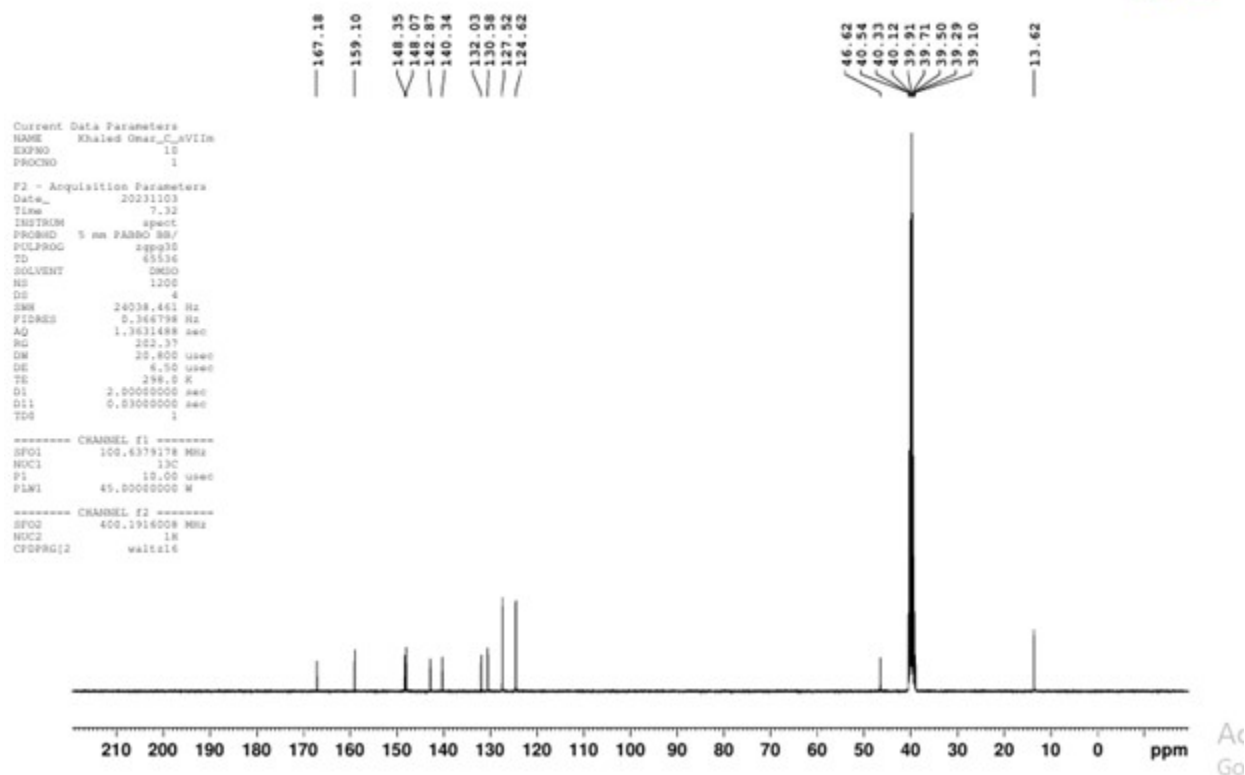


Figure 20. ^{13}C NMR chart of 4f

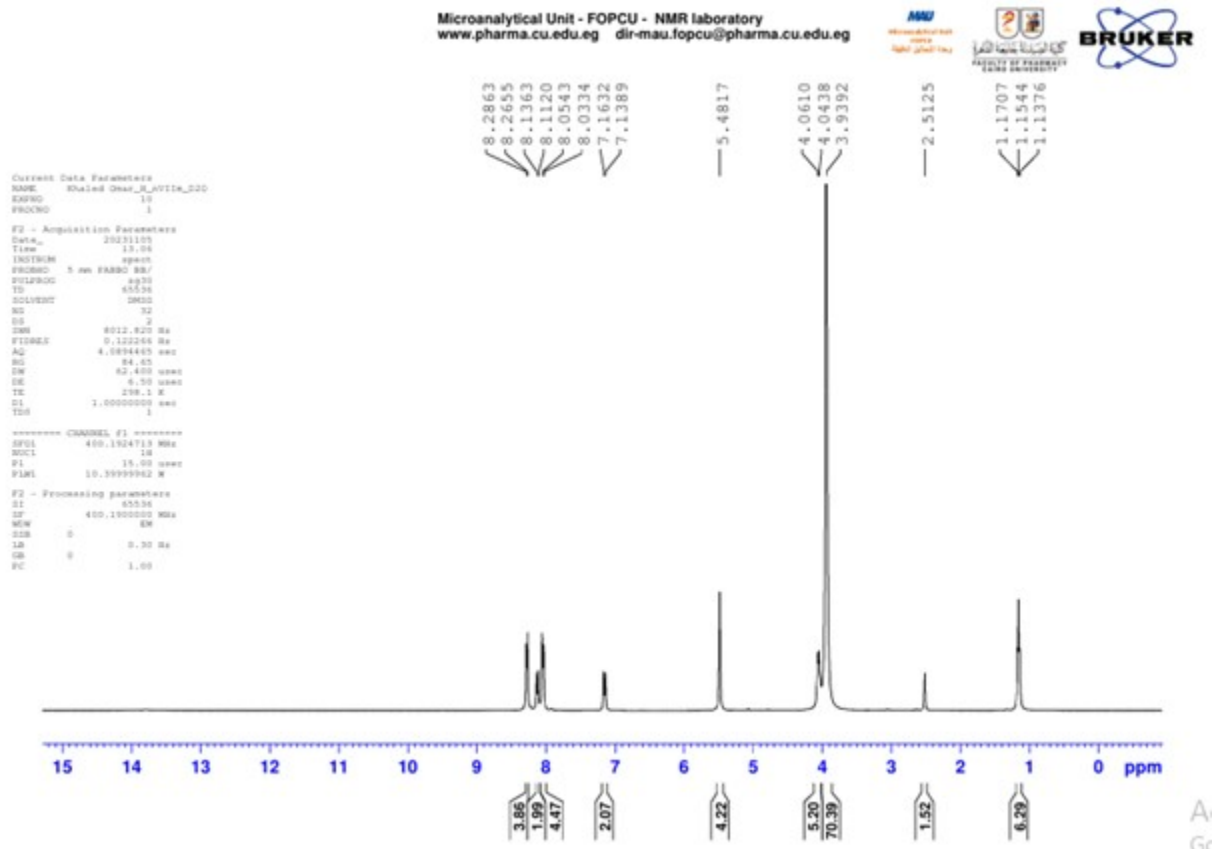


Figure 21. D₂O chart of 4f

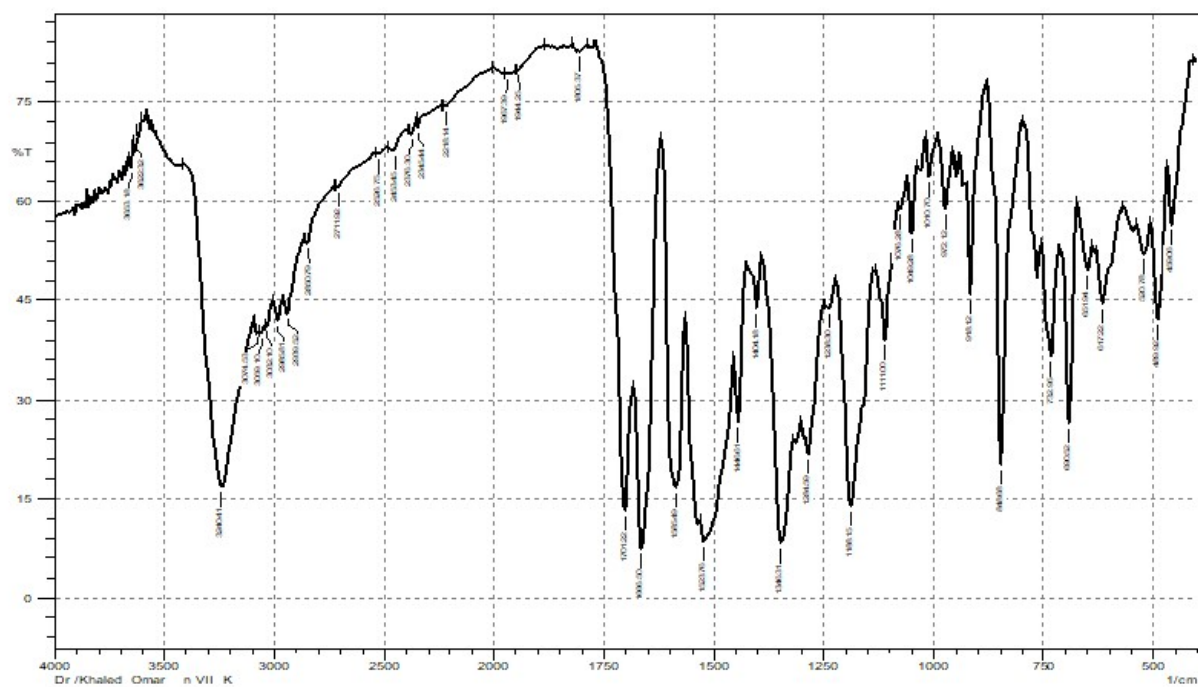


Figure 22. IR chart of 4g

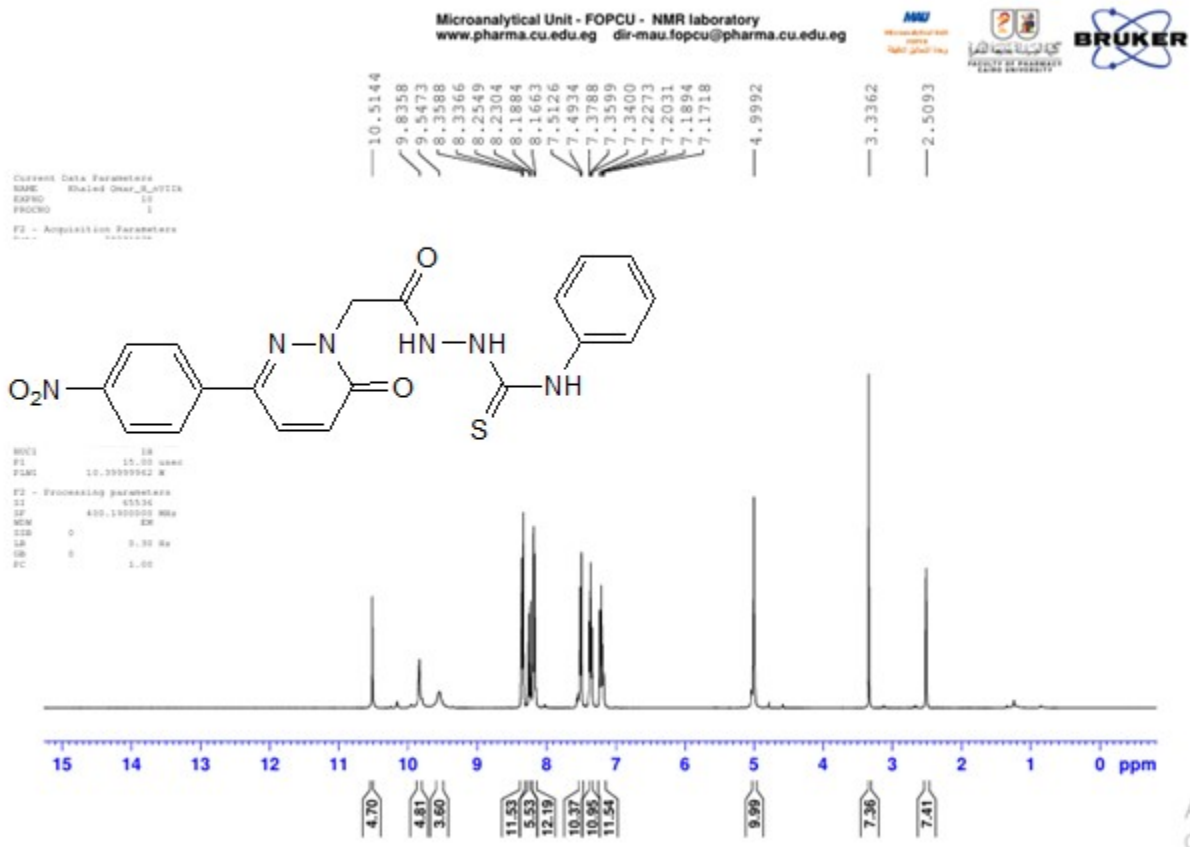


Figure 23. ¹H NMR of 4g

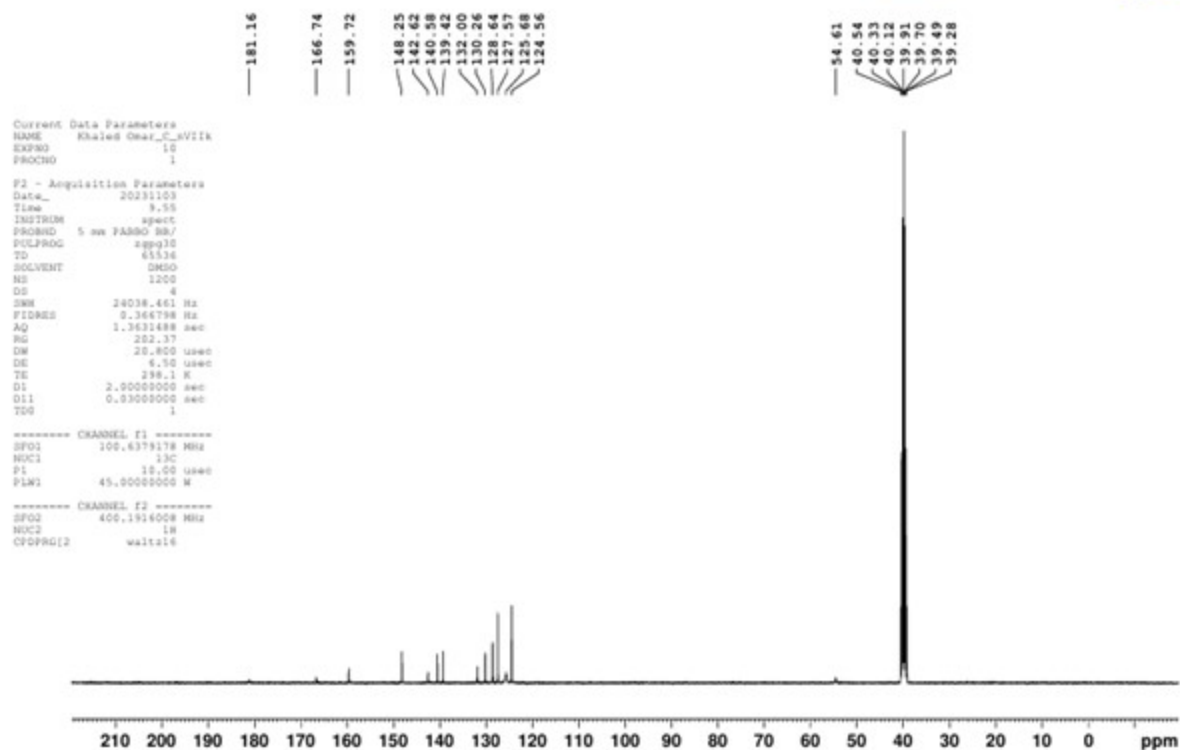


Figure 24. ^{13}C NMR of 4g

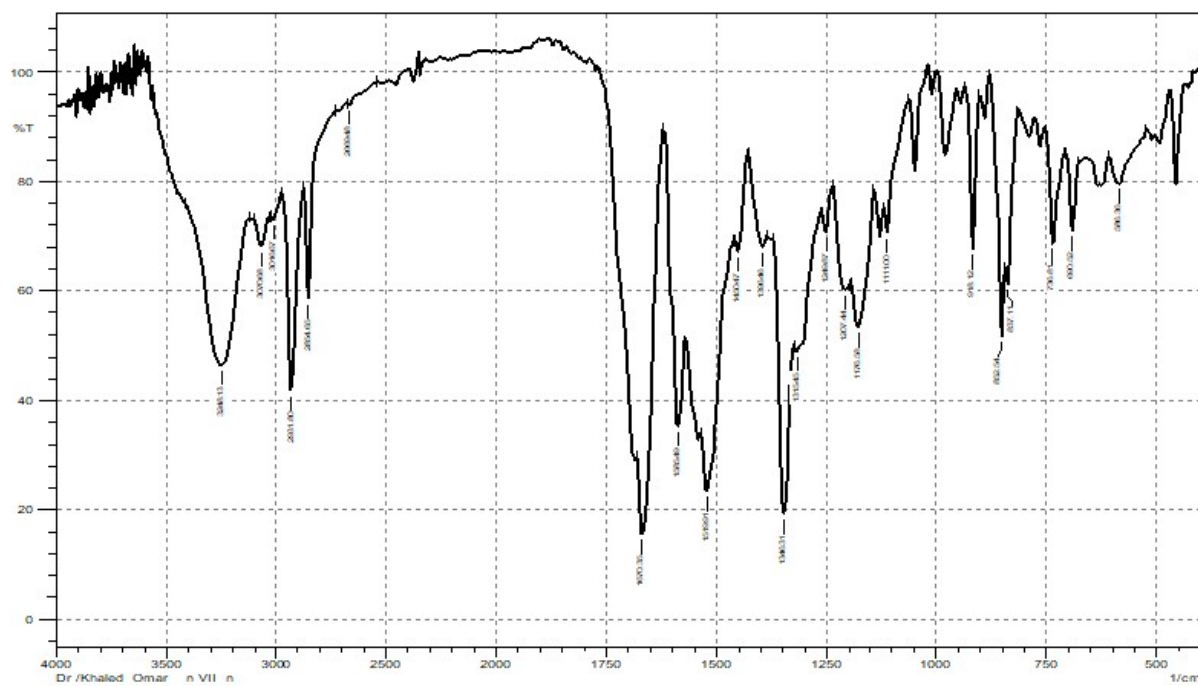


Figure 25. IR chart of 4h

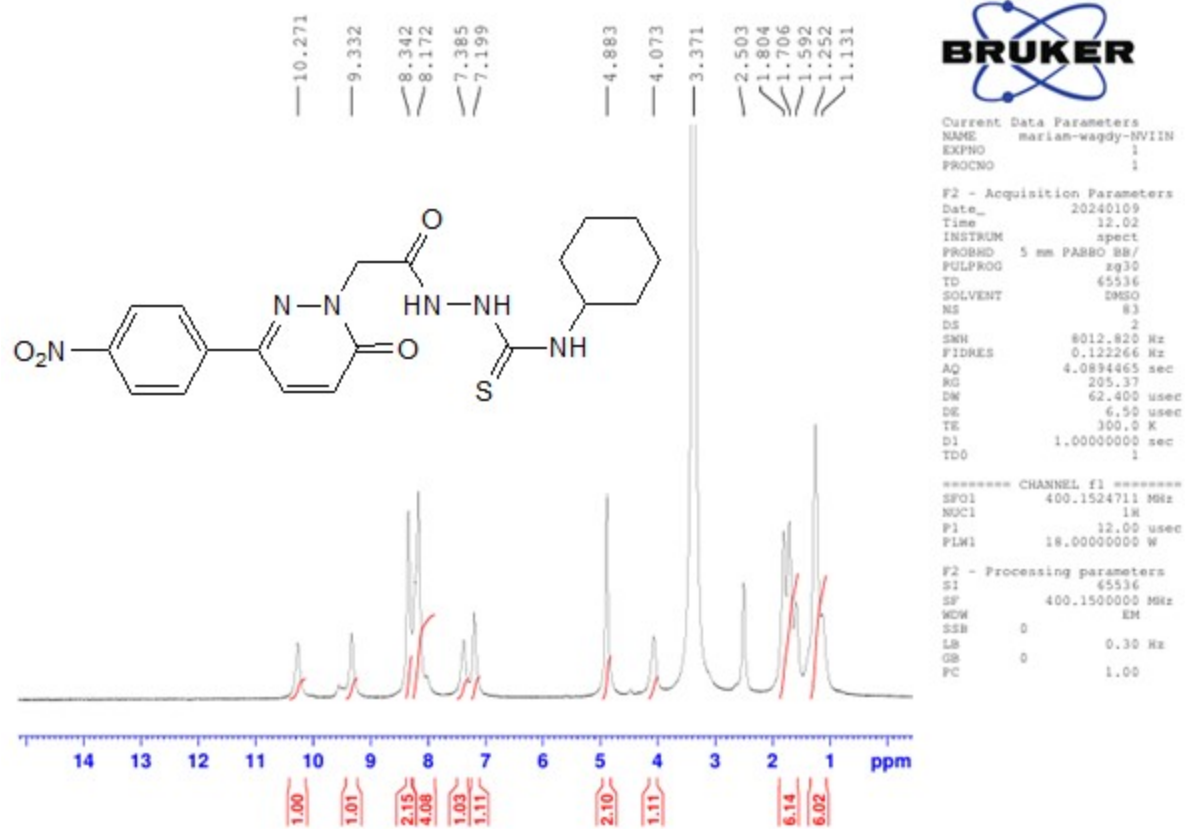


Figure 26. ¹H NMR of 4h

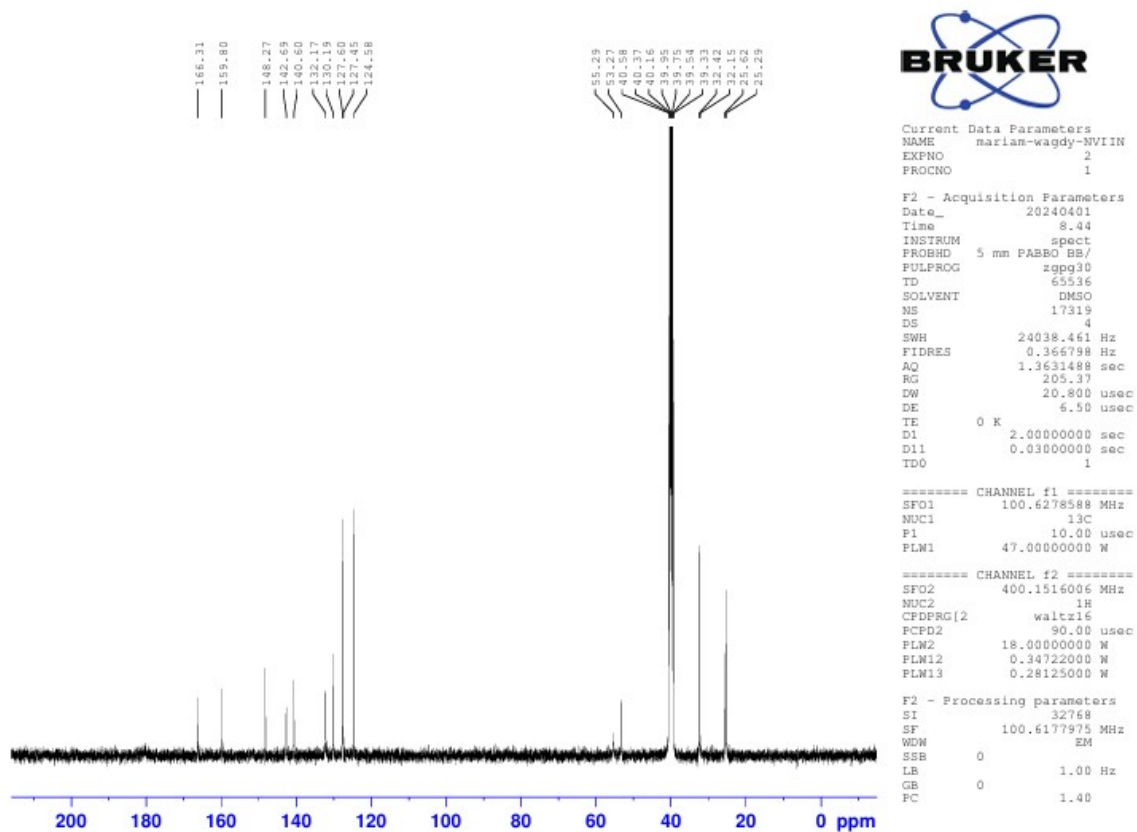


Figure 27. ^{13}C NMR of 4h

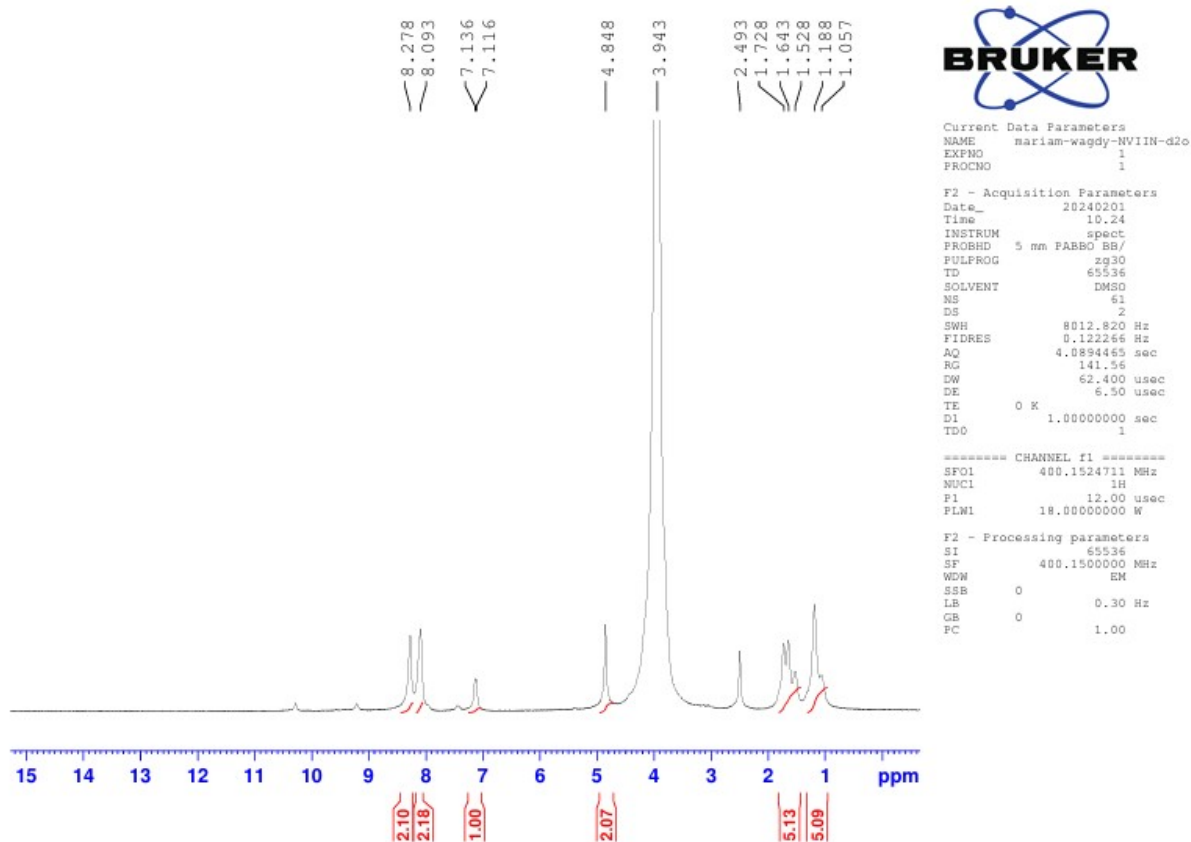


Figure 28. D2O chart of 4h

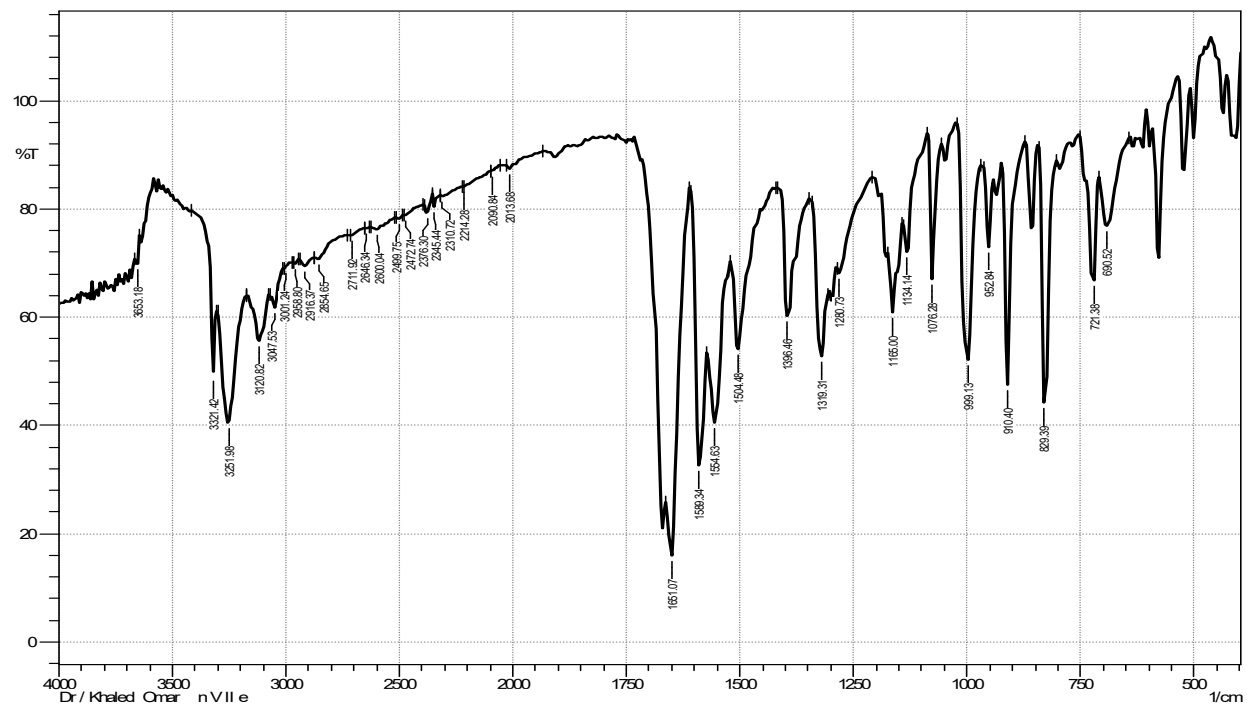


Figure 29. IR chart of 4i

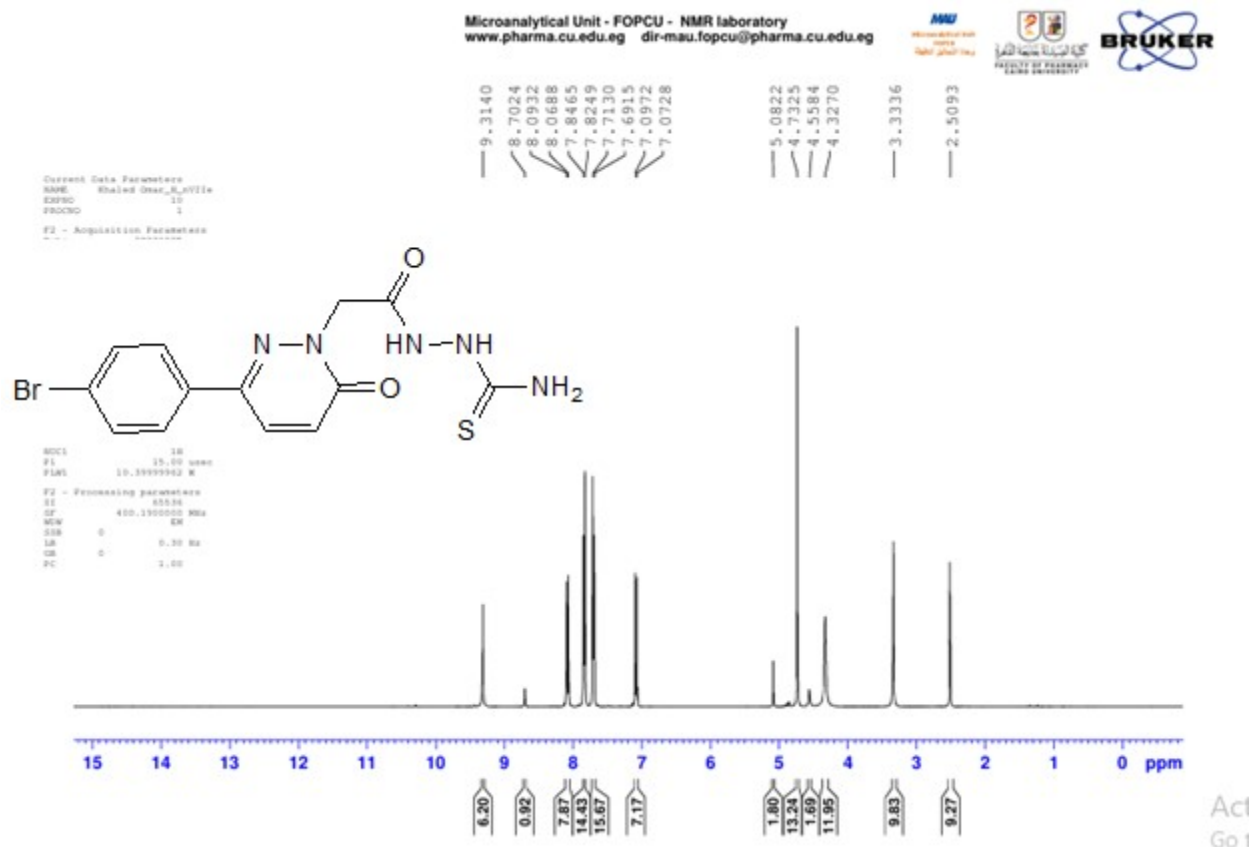


Figure 30. ¹H NMR chart of 4i

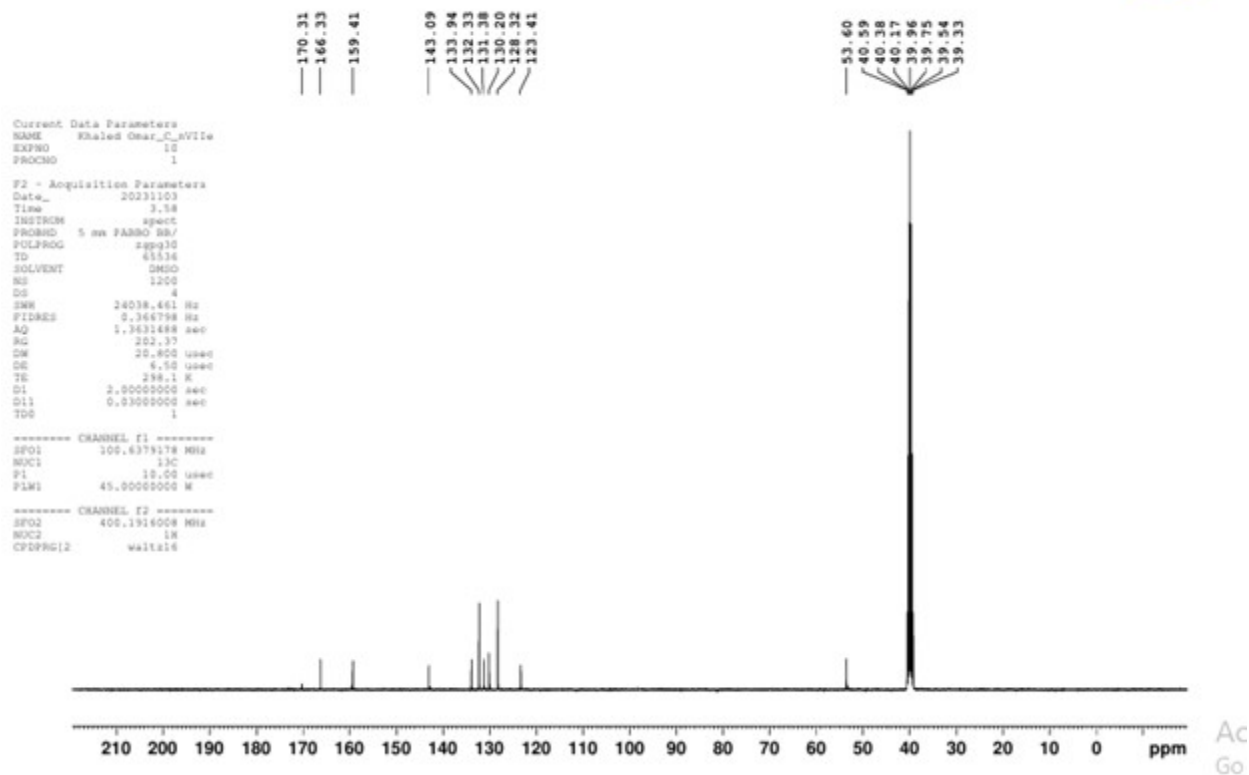


Figure 31. ^{13}C NMR chart of 4i

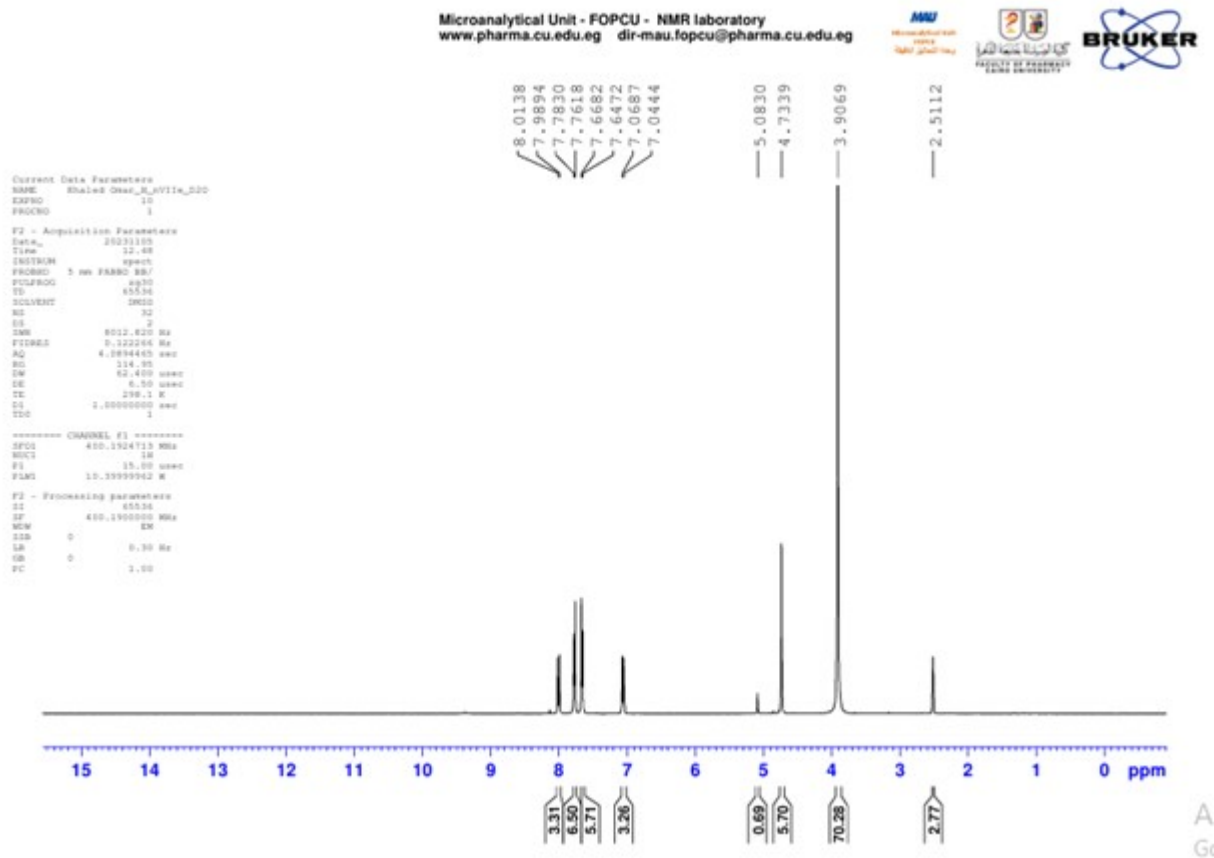


Figure 32. D₂O chart of 4i

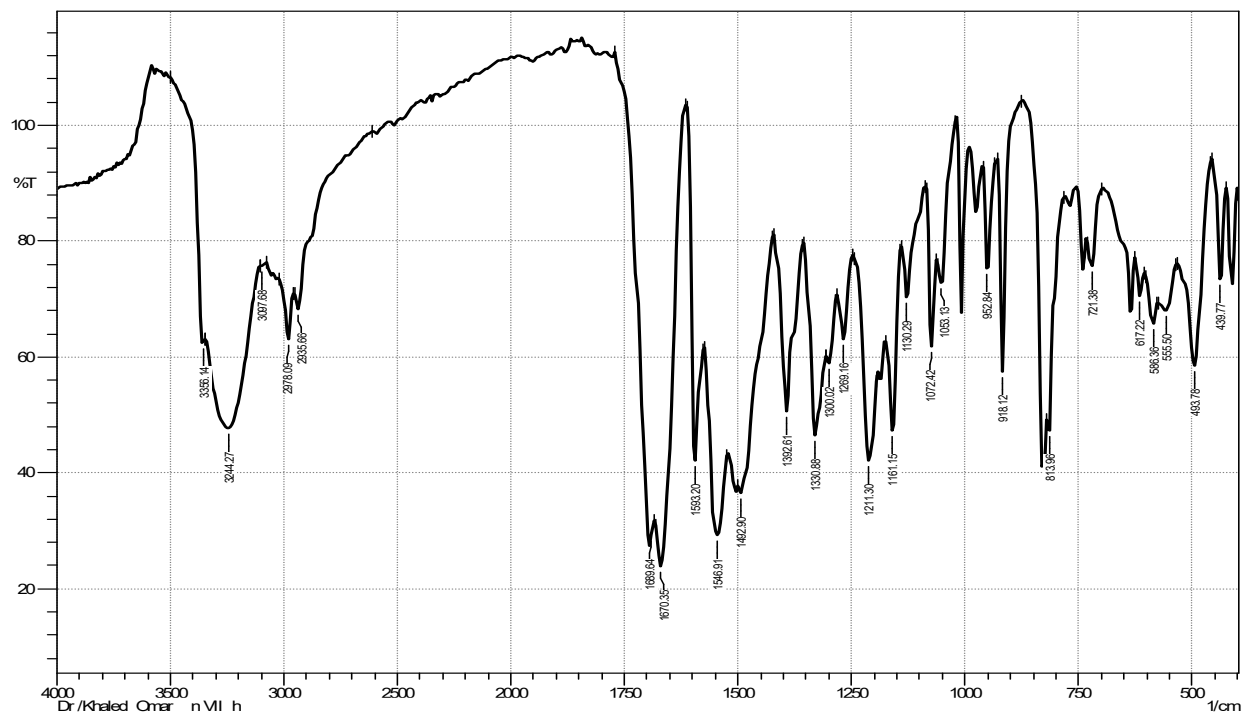


Figure 33. IR chart of 4j

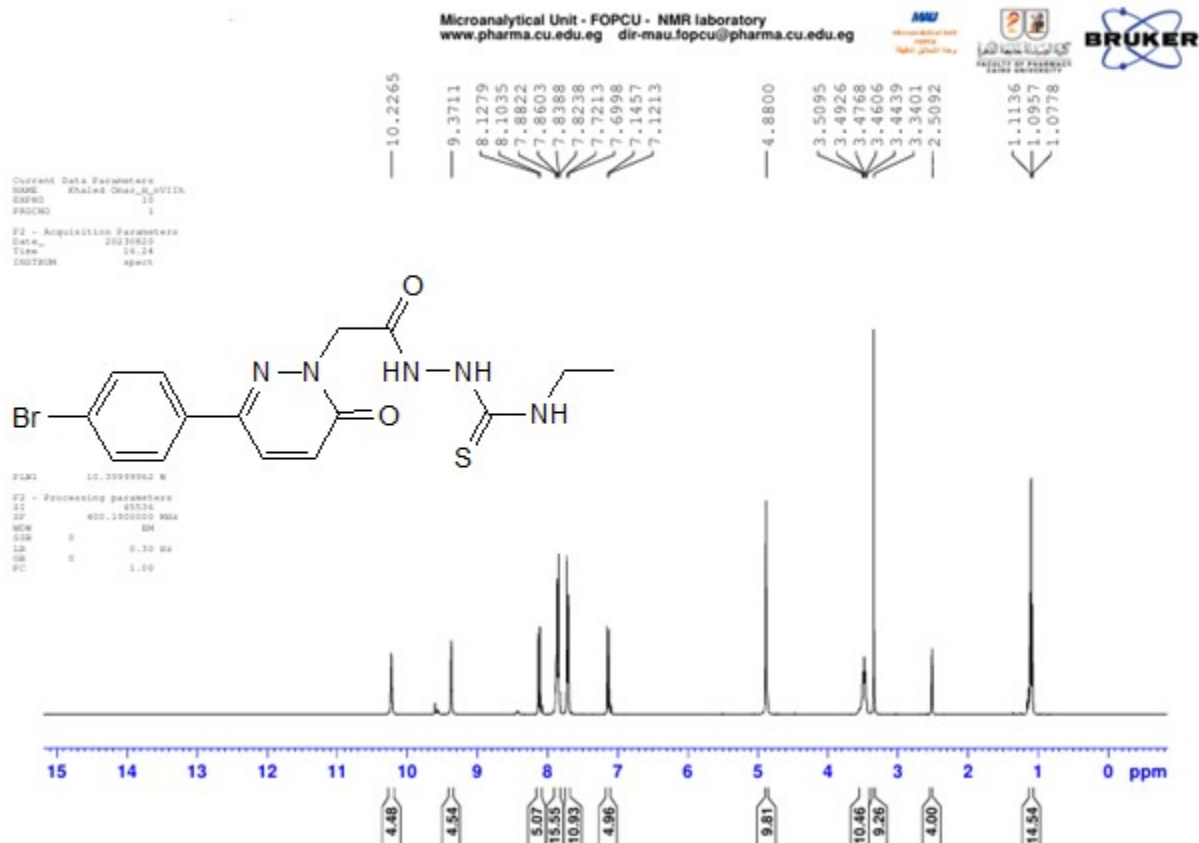


Figure 34. ¹H NMR chart of 4j

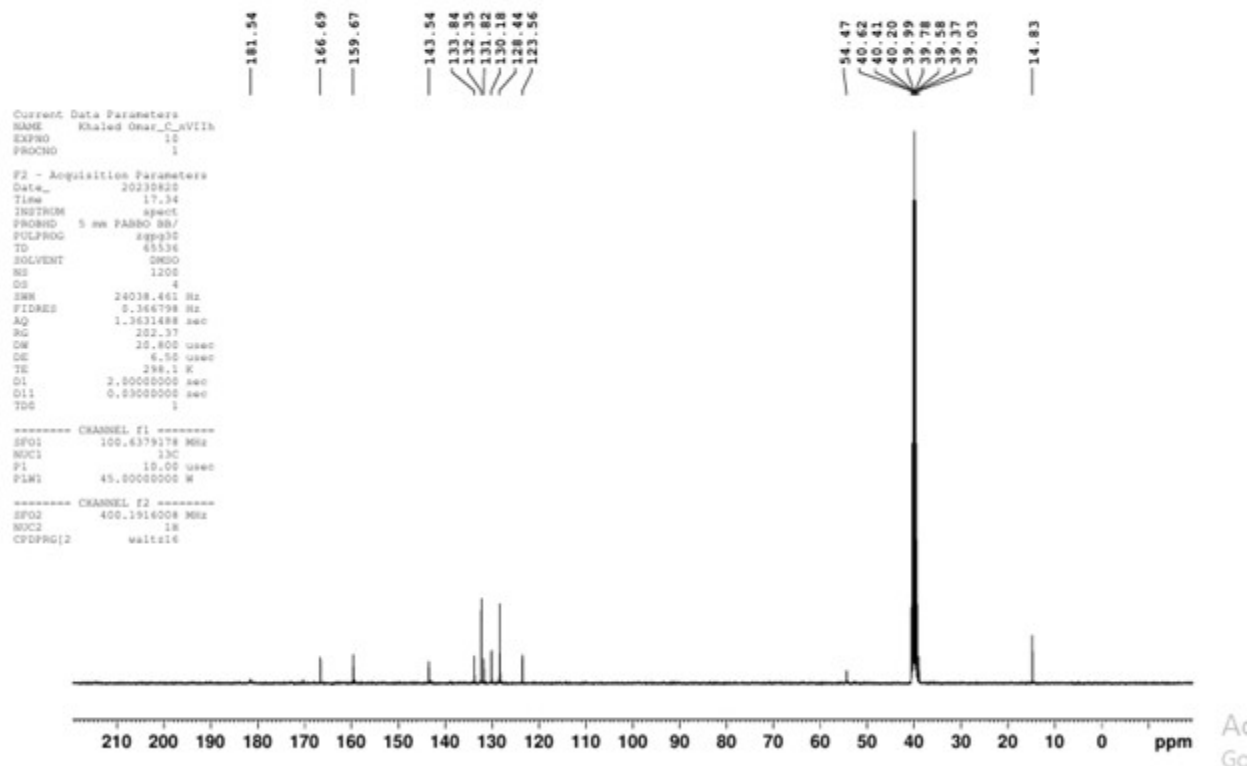


Figure 35. ^{13}C NMR chart of 4j

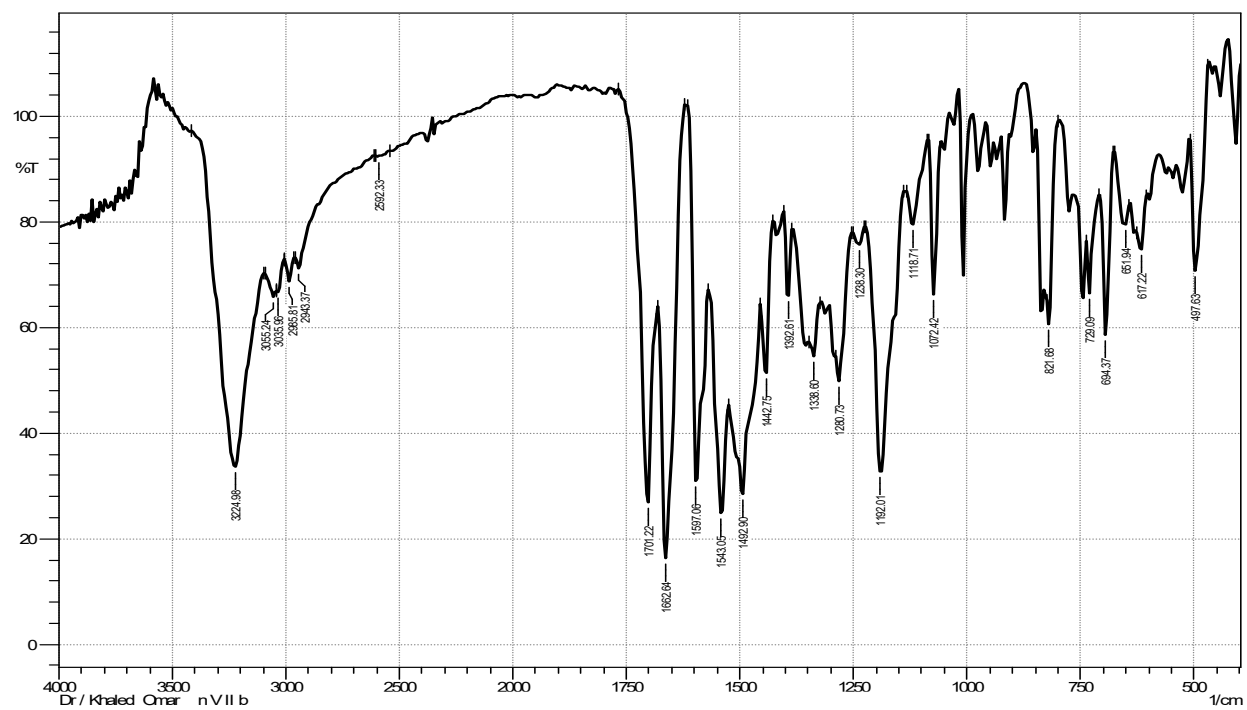


Figure 36. IR chart of 4k

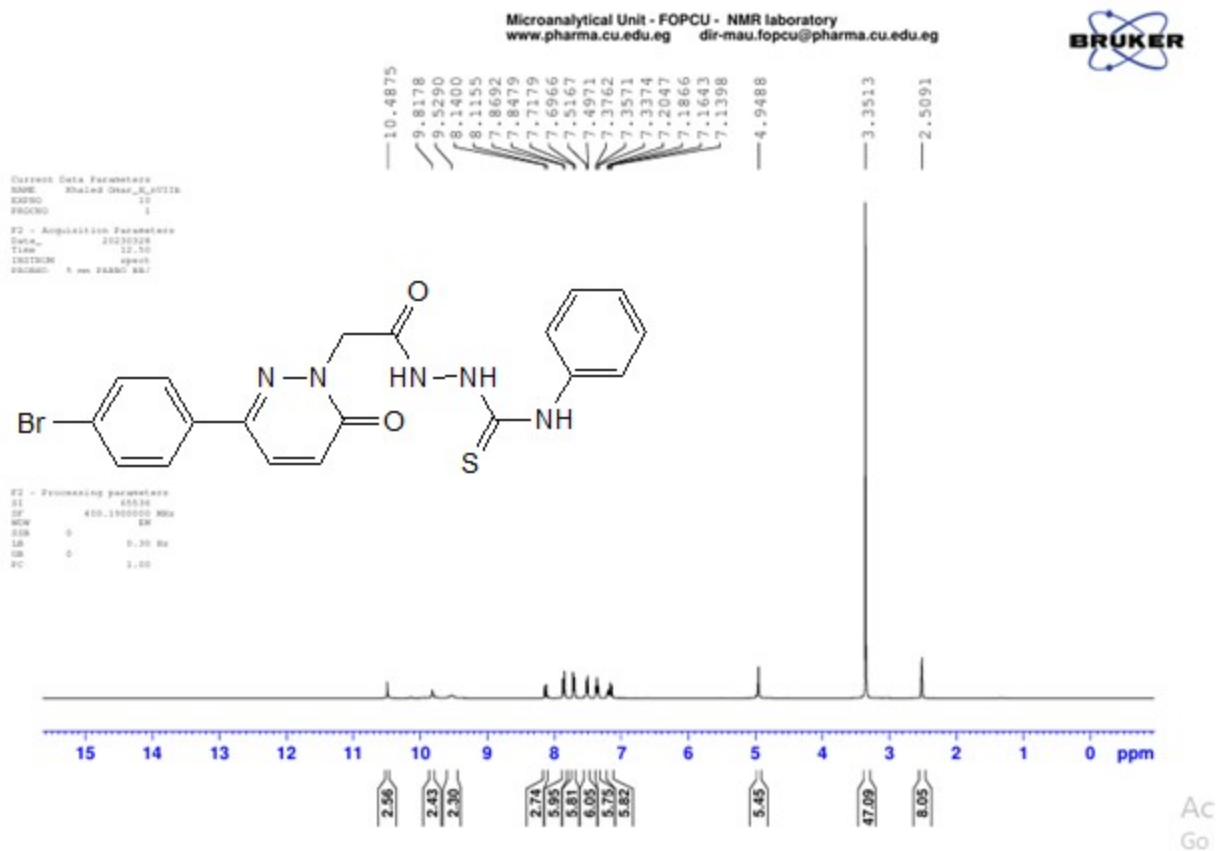


Figure 37. ¹H NMR chart of 4k

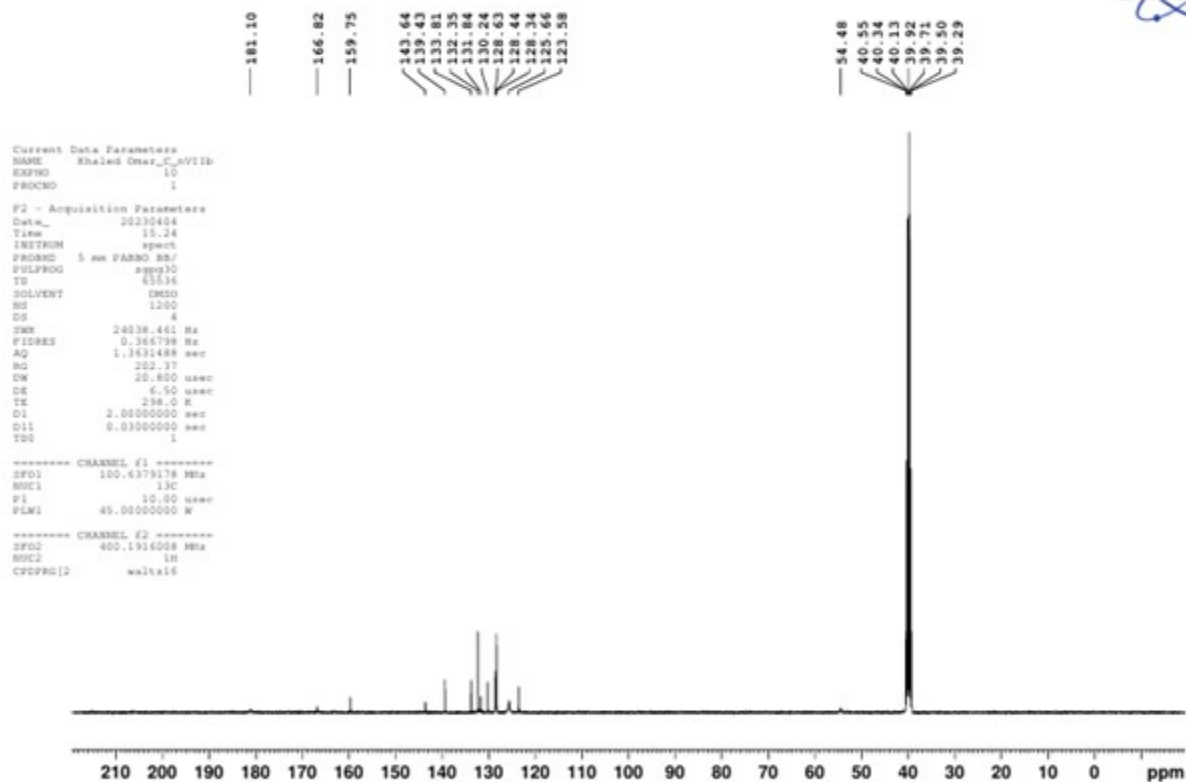


Figure 38. ^{13}C NMR chart of 4k

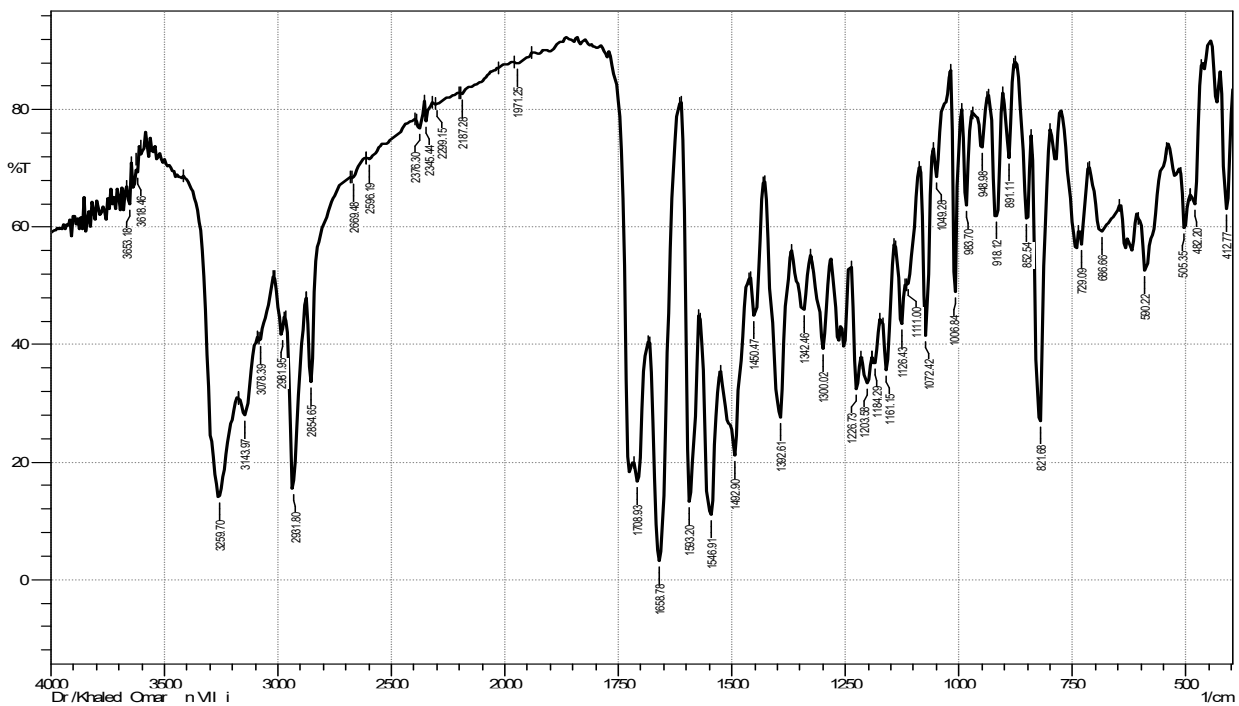


Figure 39. IR chart of 4l

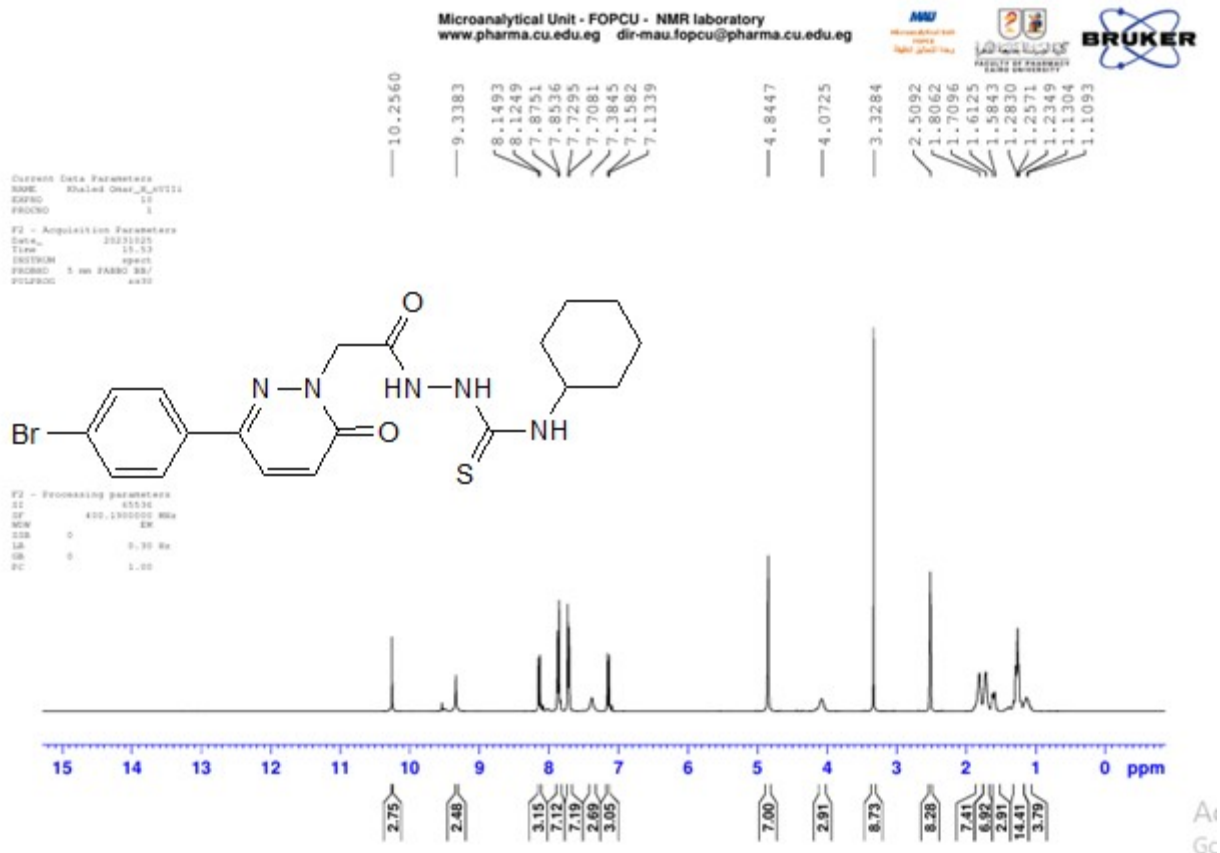


Figure 40. ¹H NMR chart of 41

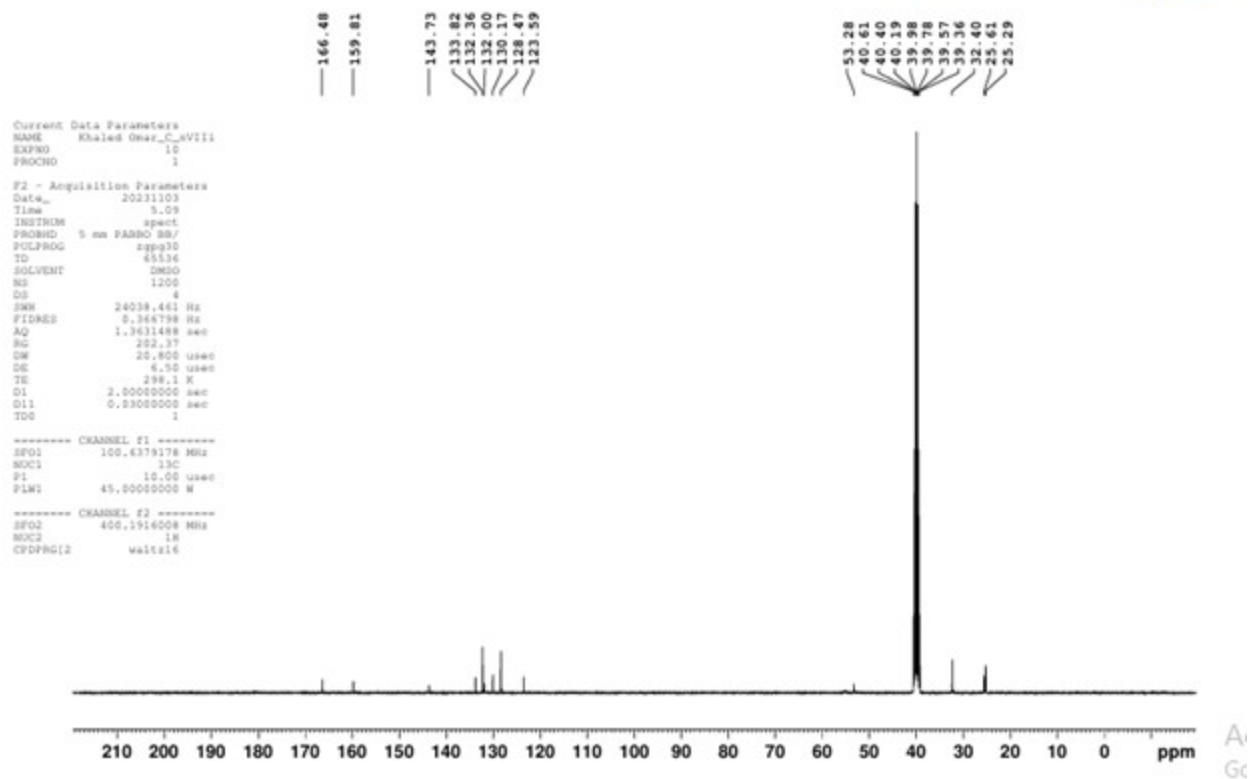


Figure 41. ^{13}C NMR chart of 4l

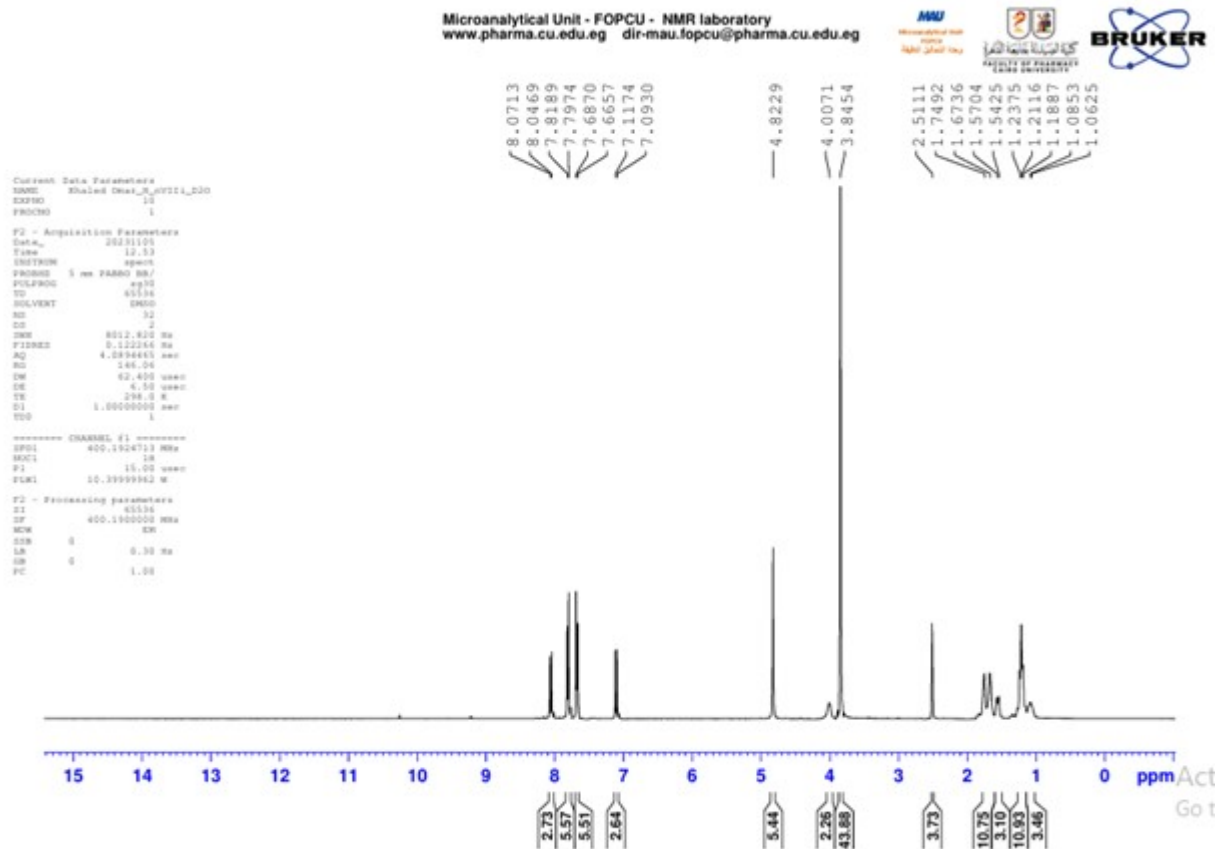


Figure 42. D₂O chart of 4l

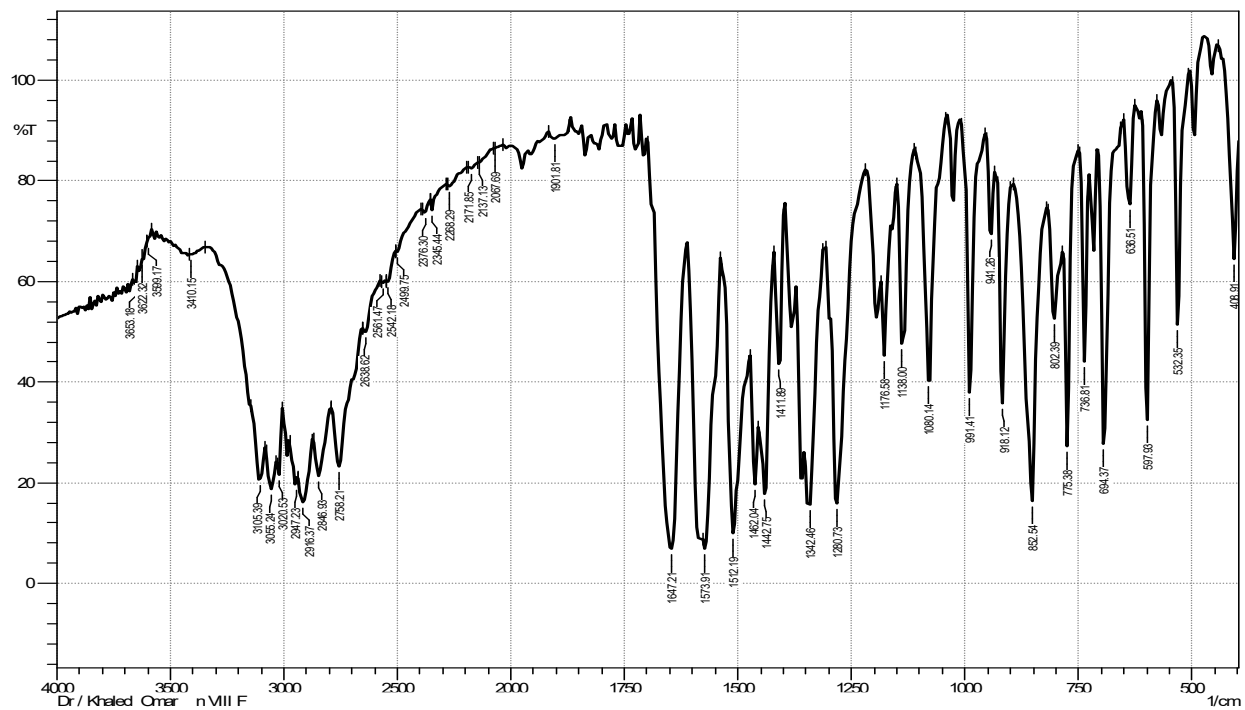


Figure 43. IR chart of 5a

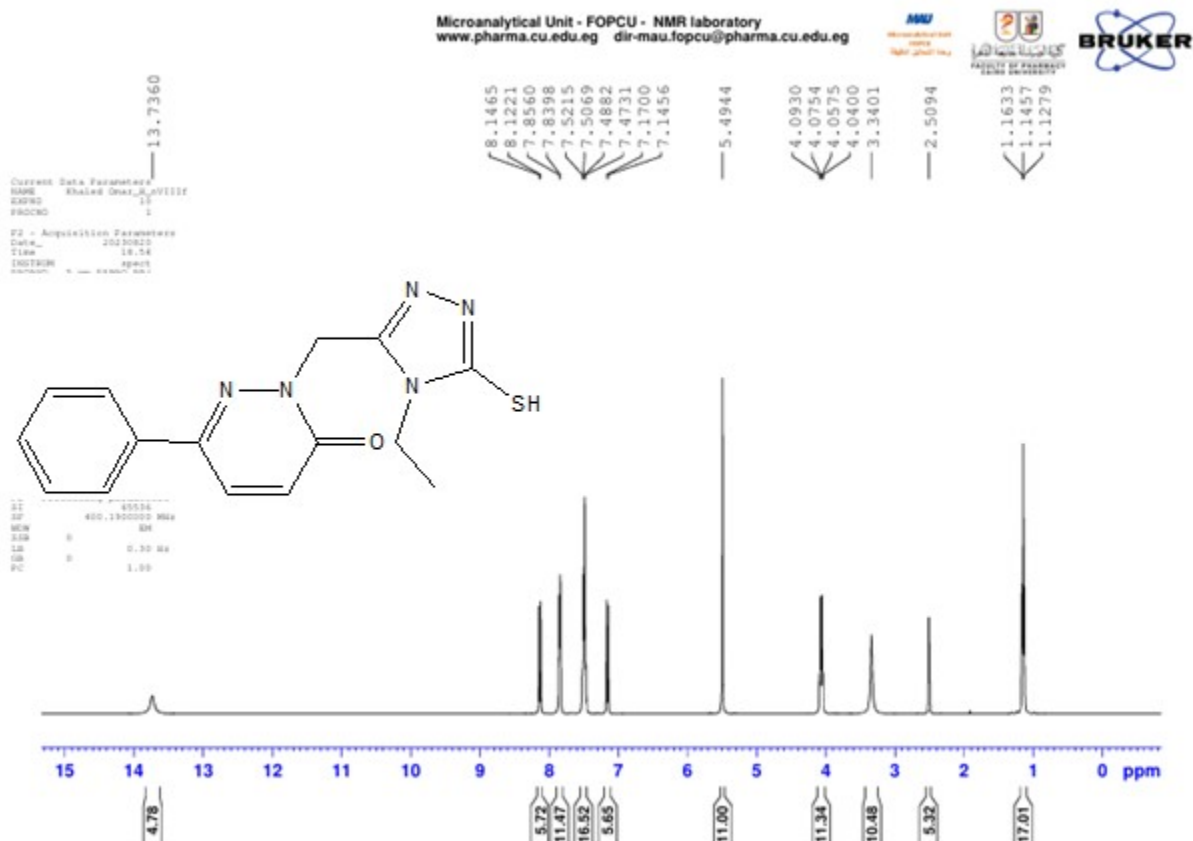


Figure 44. ¹H NMR chart of 5a

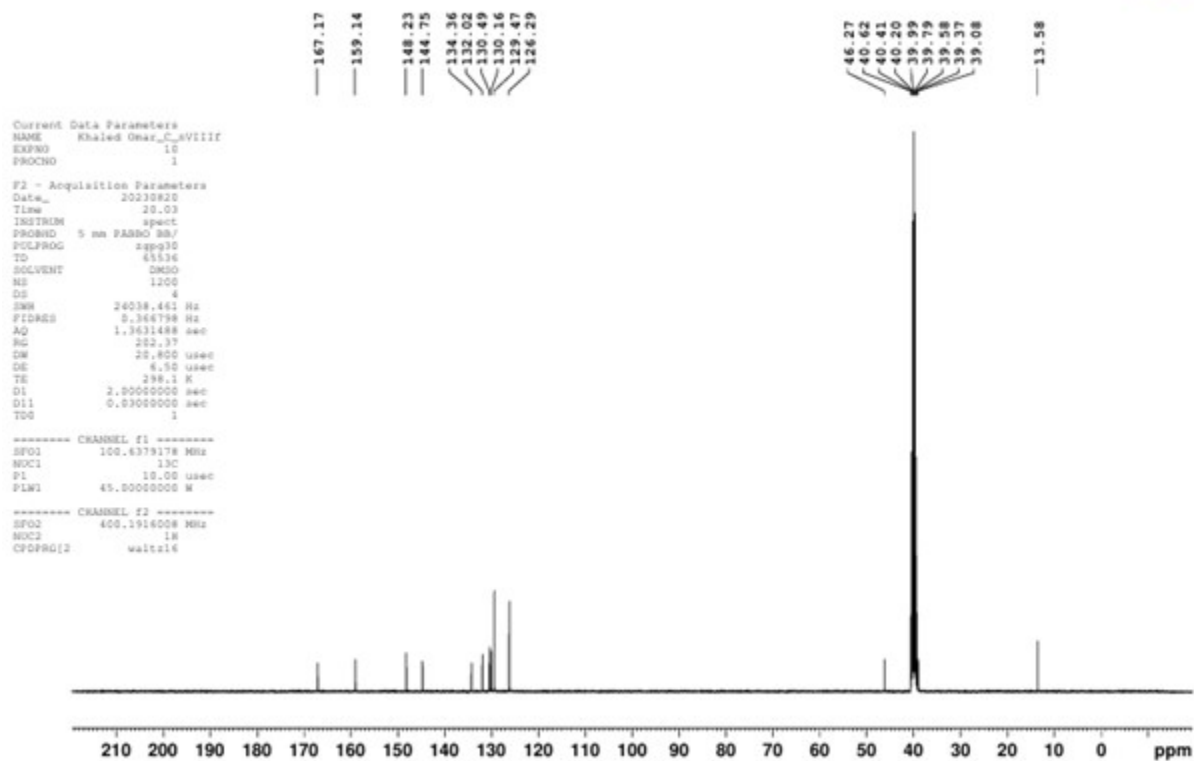


Figure 45. ^{13}C NMR chart of 5a

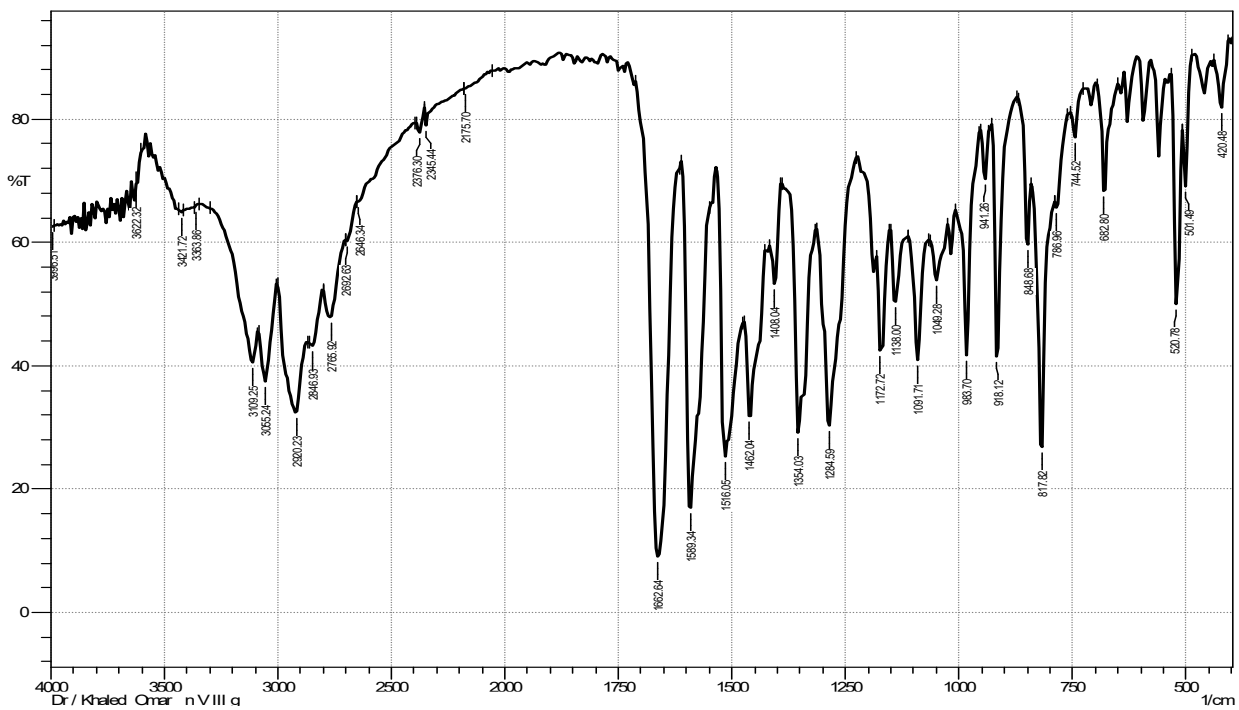


Figure 46. IR chart of 5b

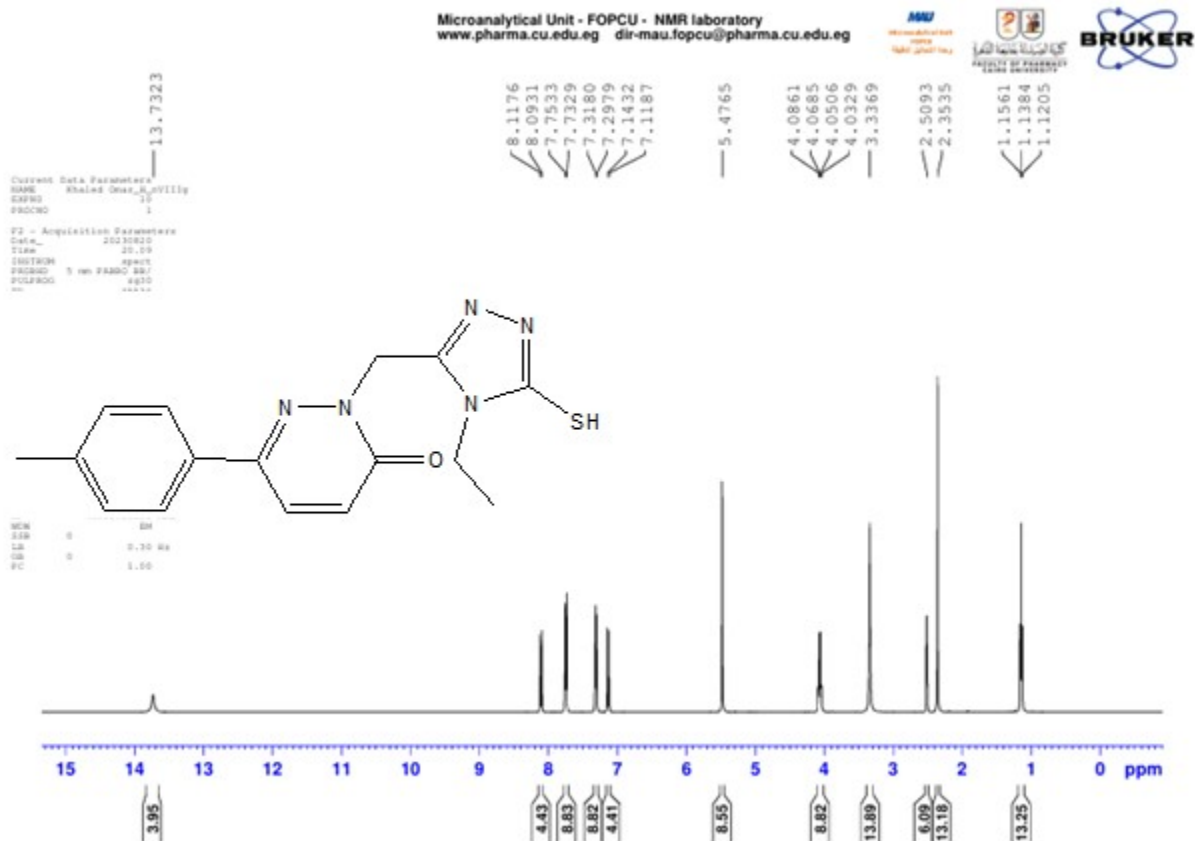


Figure 47. ^1H NMR of 5b

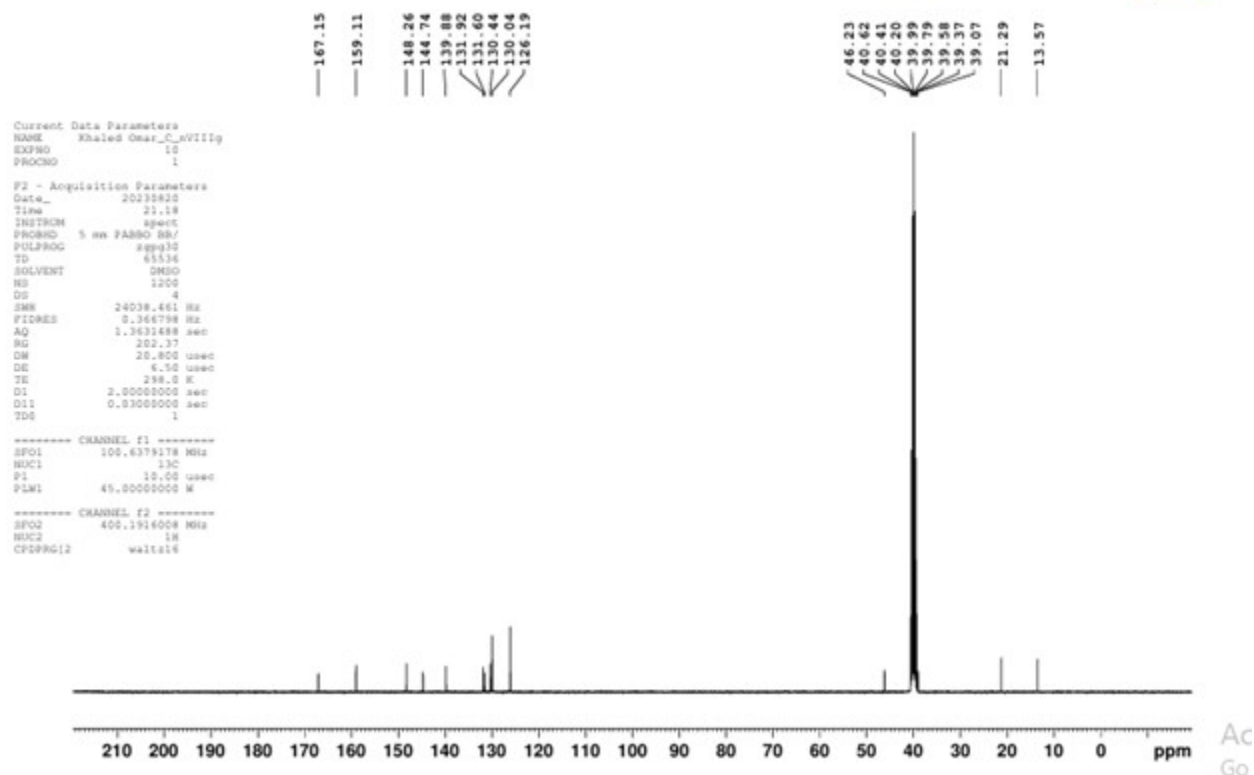


Figure 48. ^{13}C NMR chart of 5b

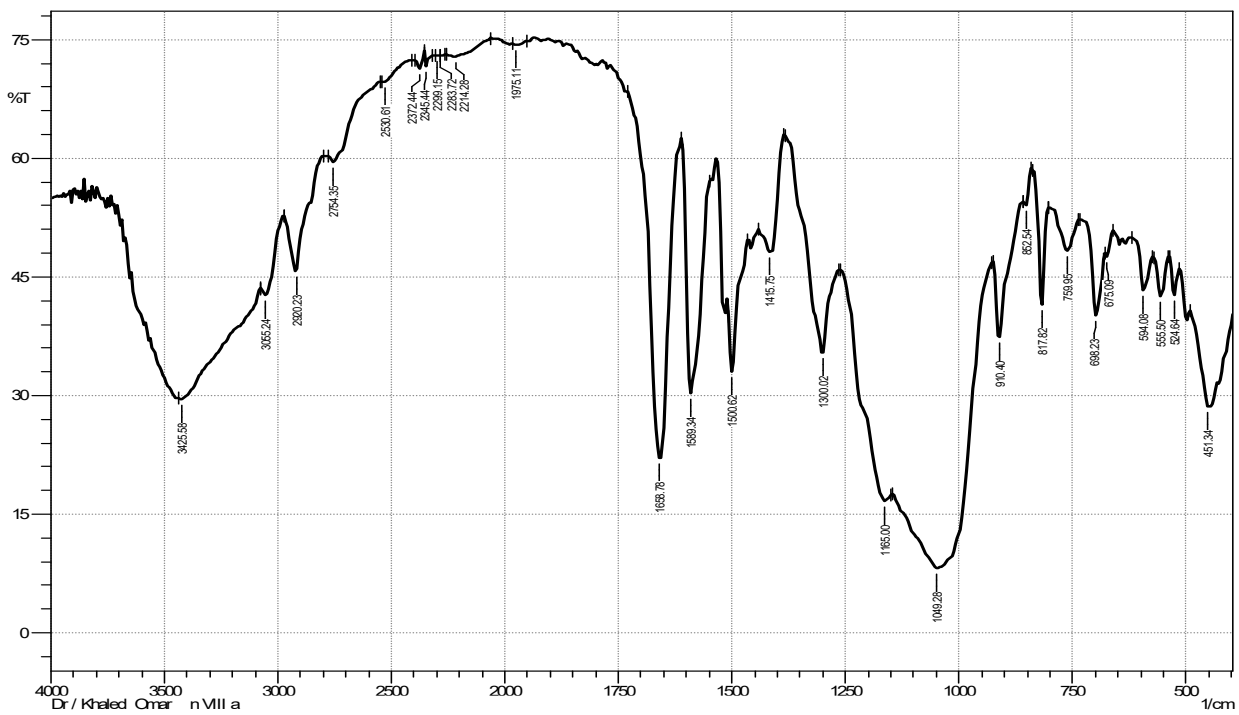


Figure 49. IR chart of 5c

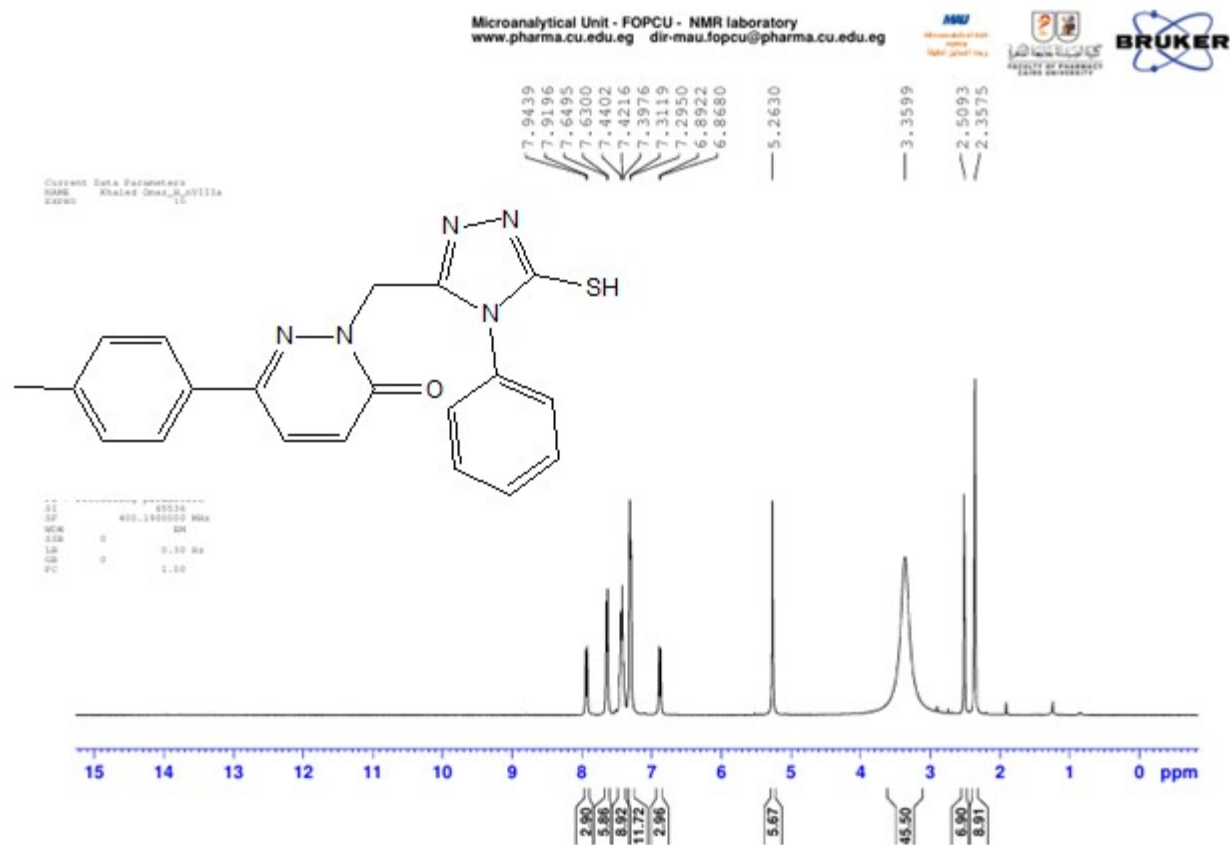


Figure 50. ¹H NMR chart of 5c

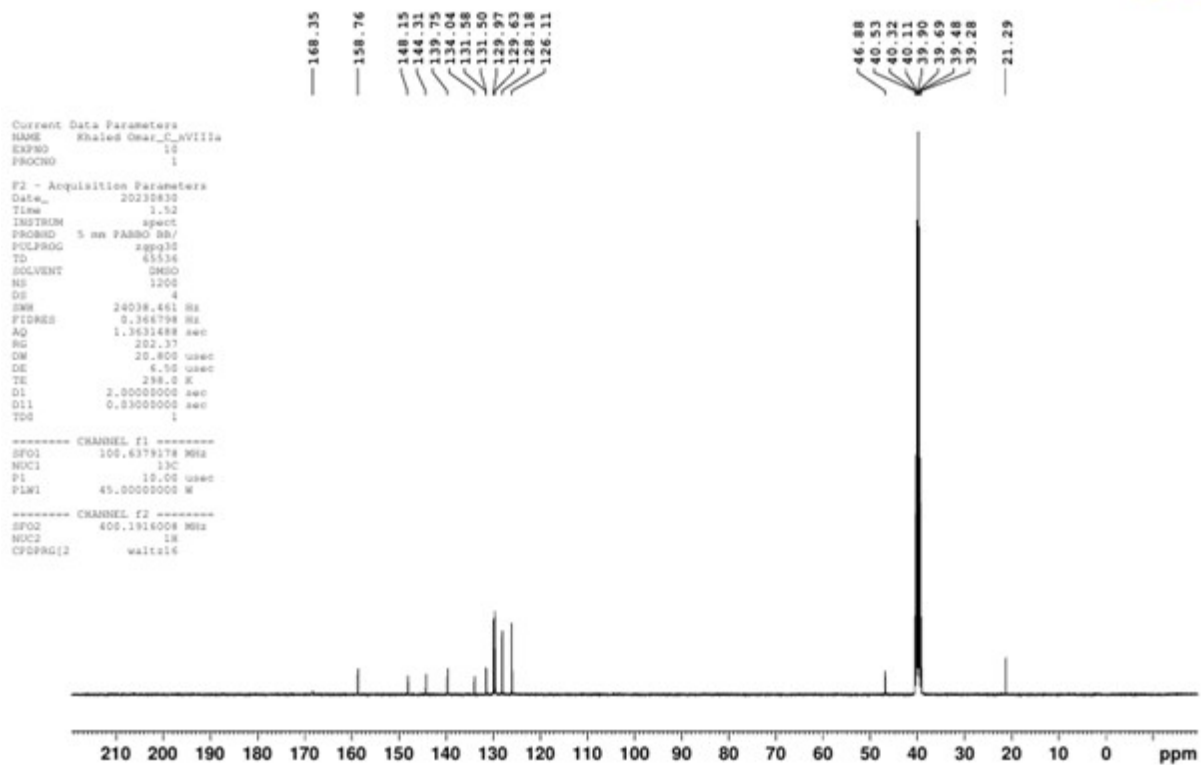


Figure 51. ^{13}C NMR chart of 5c

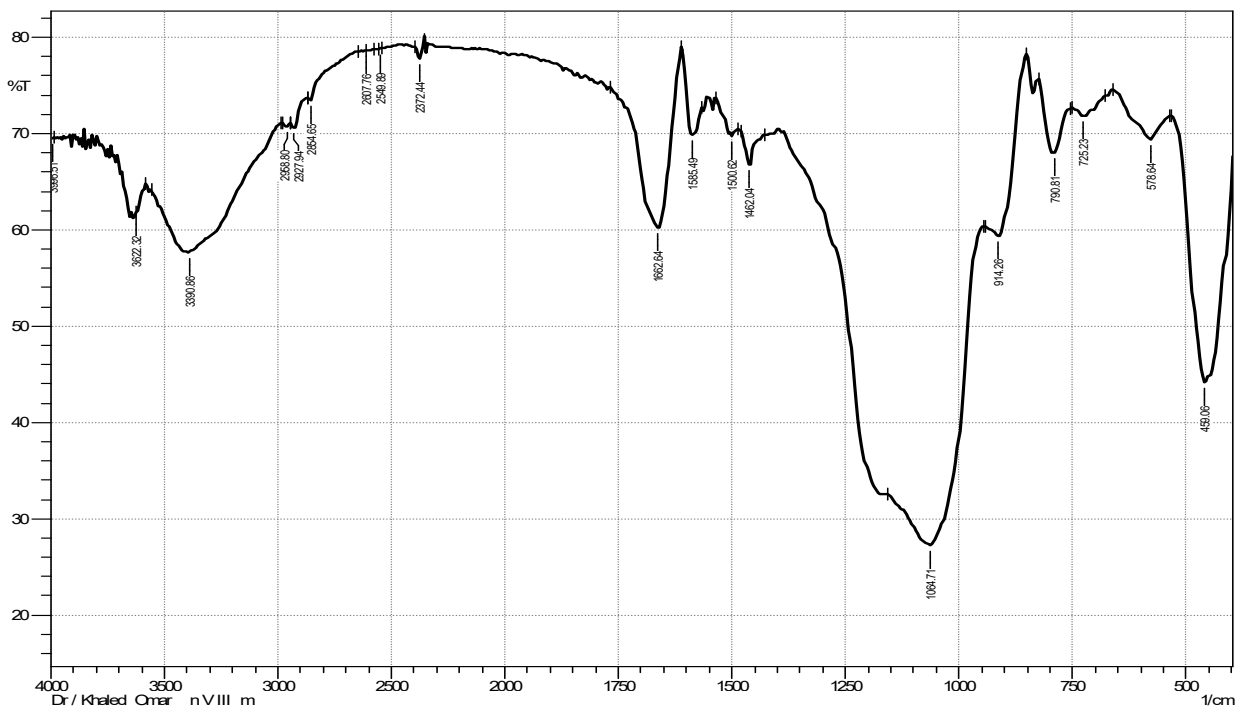


Figure 52. IR chart of 5d

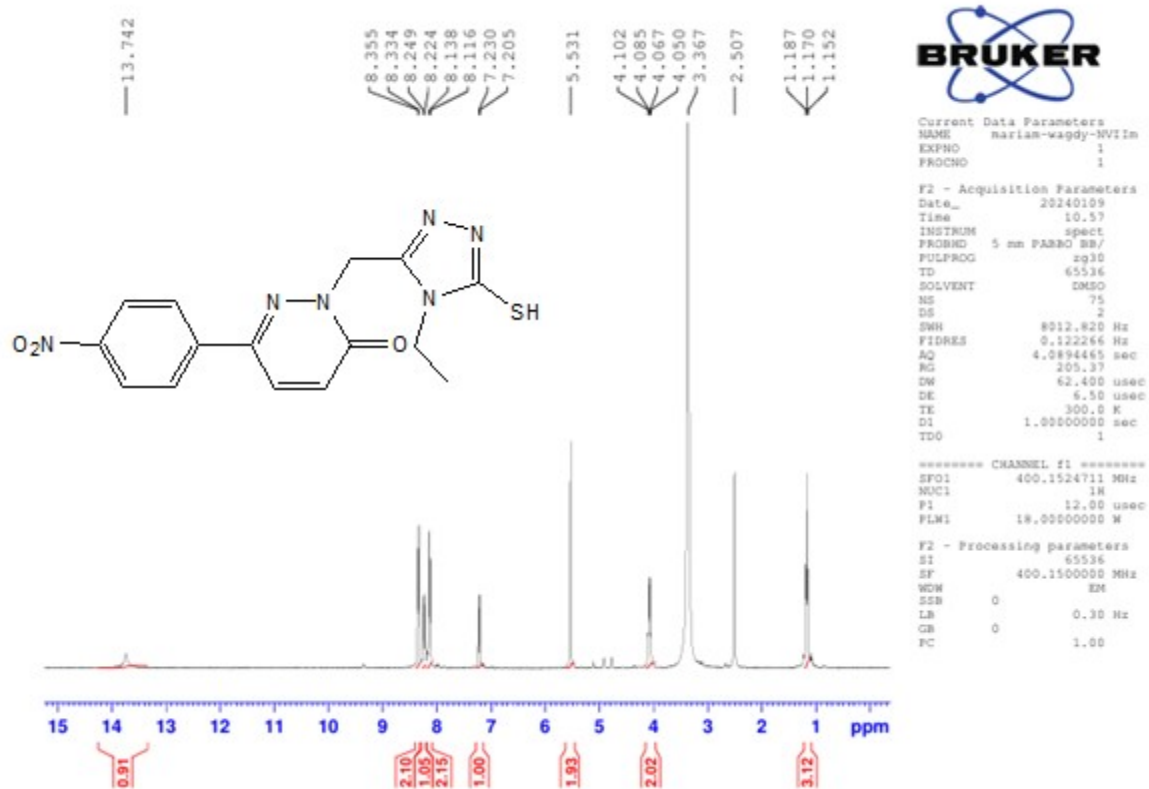


Figure 53. ¹H NMR chart of 5d

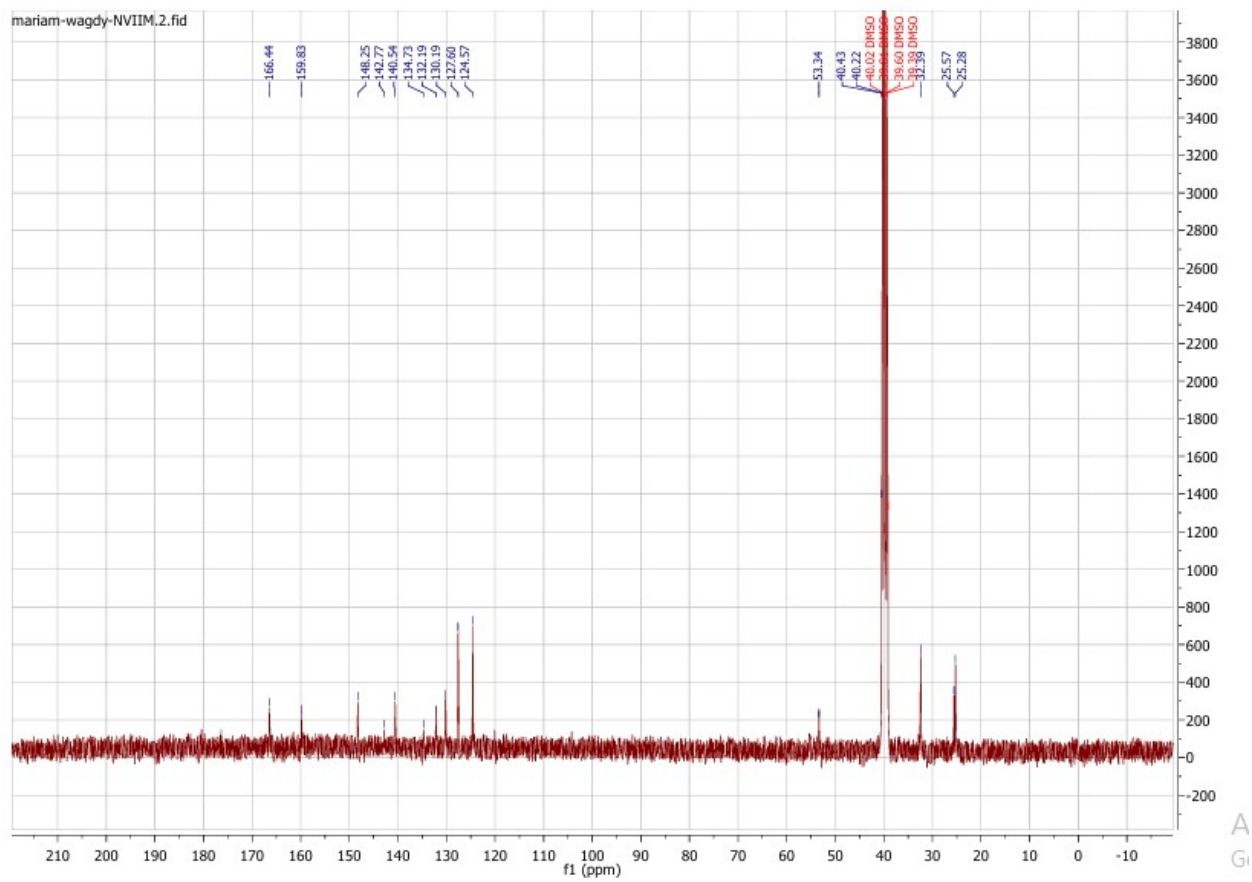


Figure 54. ^{13}C NMR chart of 5d

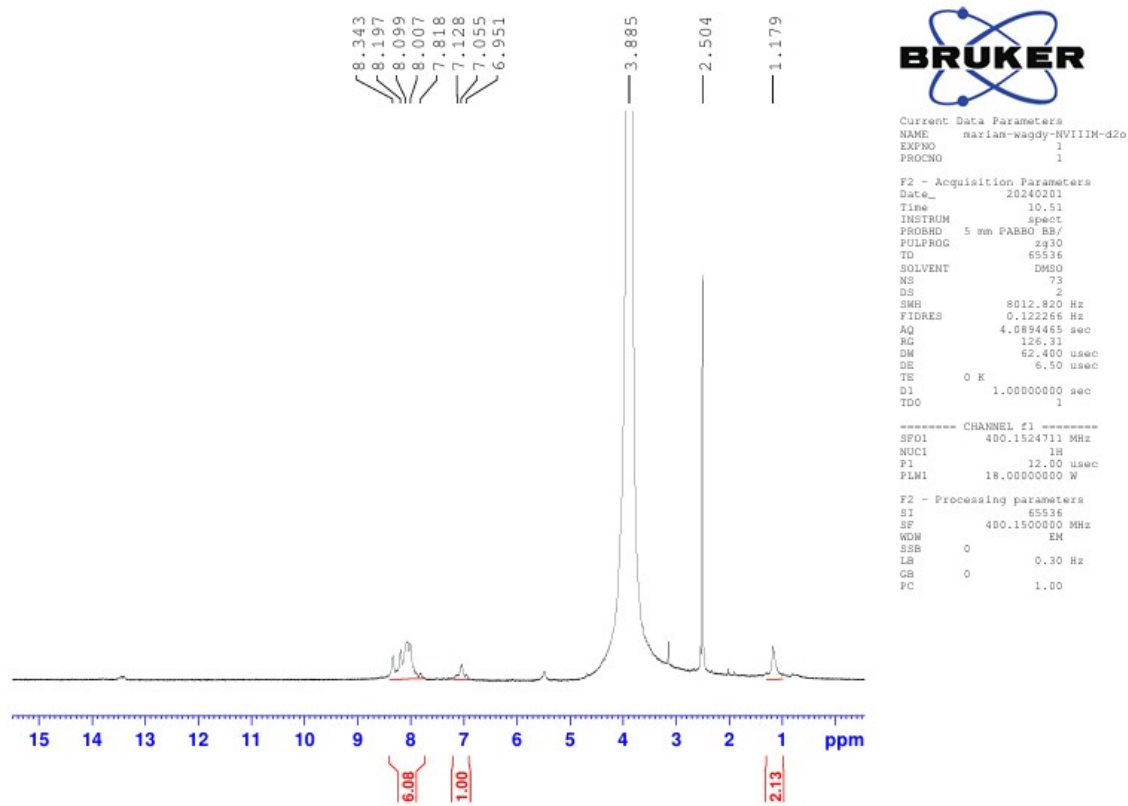


Figure 55. D₂O chart of 5d

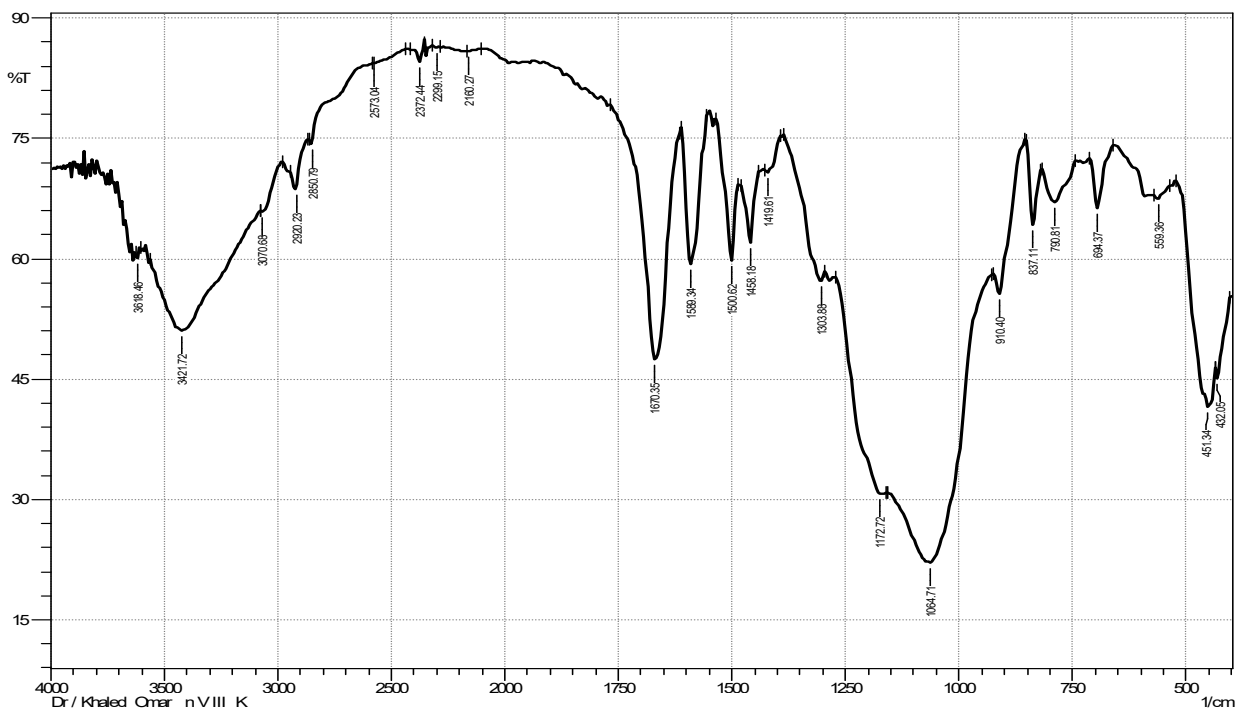


Figure 56. IR chart of 5e

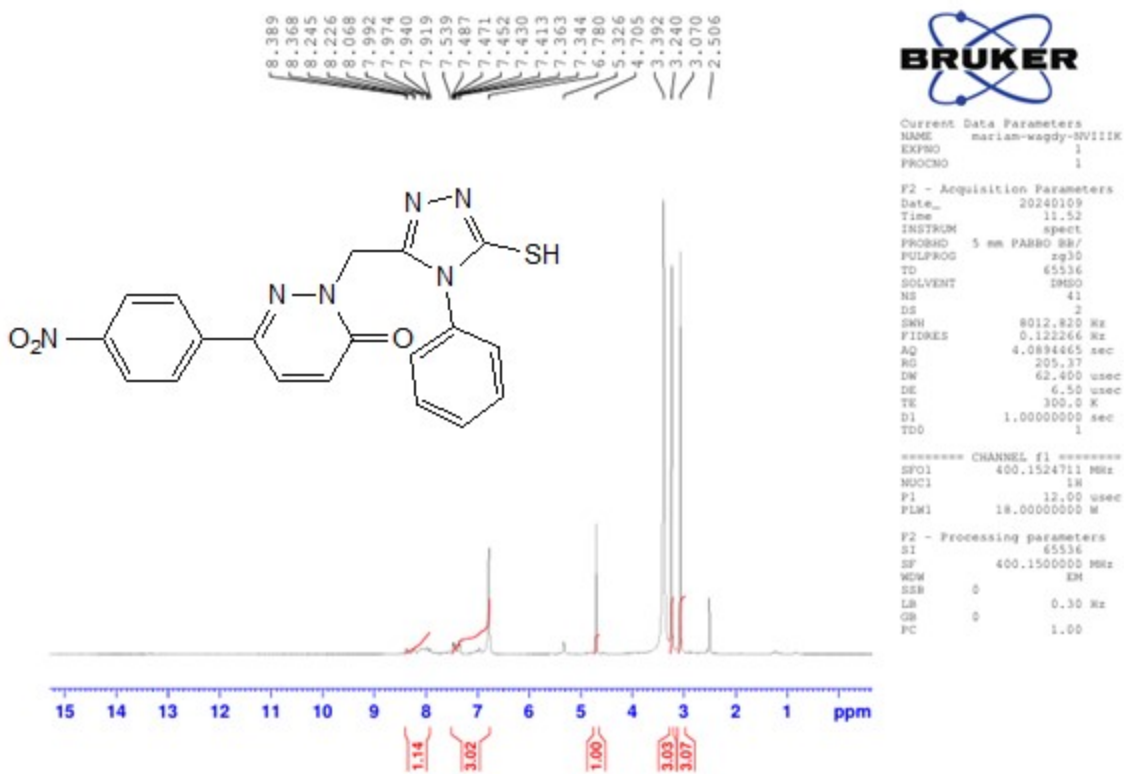


Figure 57. ¹H NMR chart of 5e

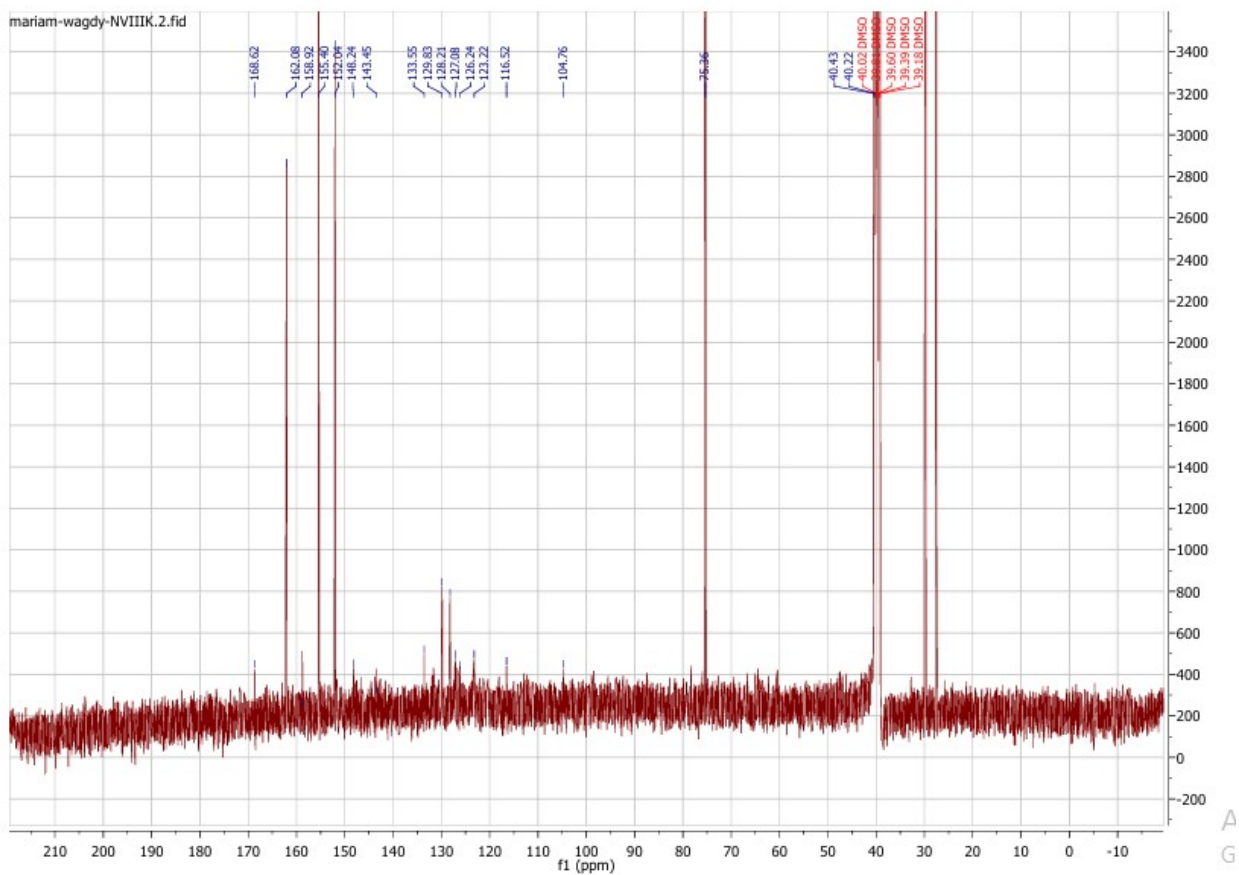


Figure 58. ^{13}C NMR chart of 5e

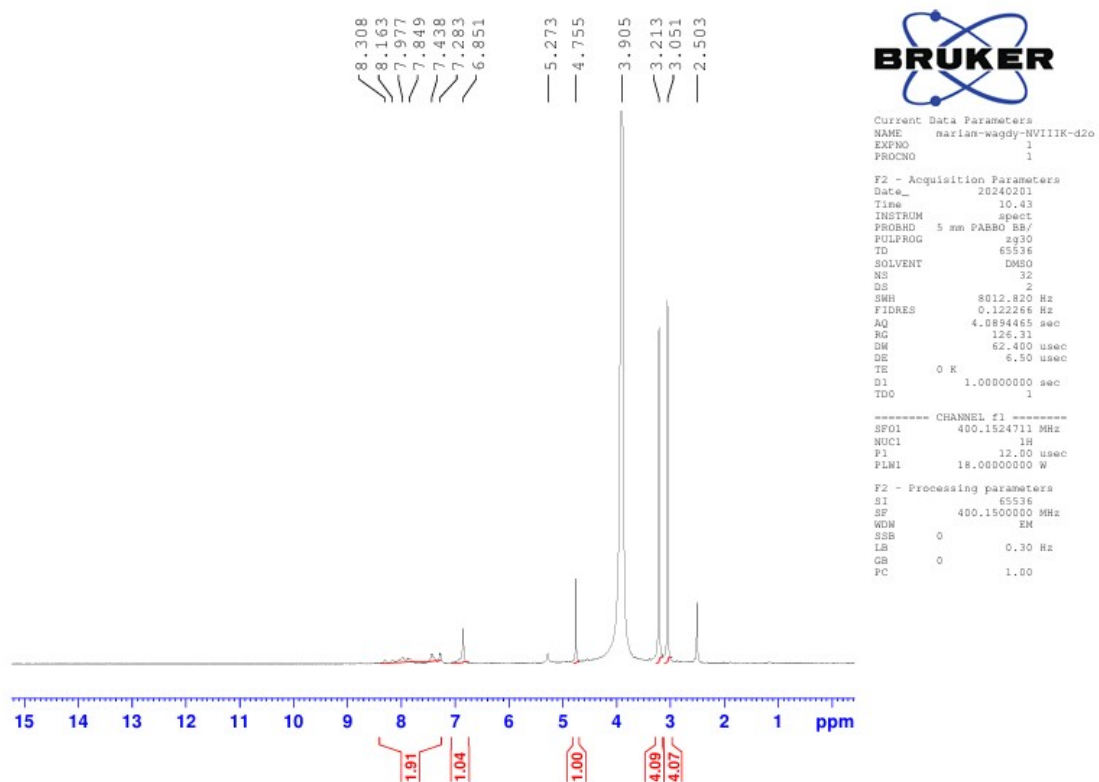


Figure 59. D₂O chart of 5e

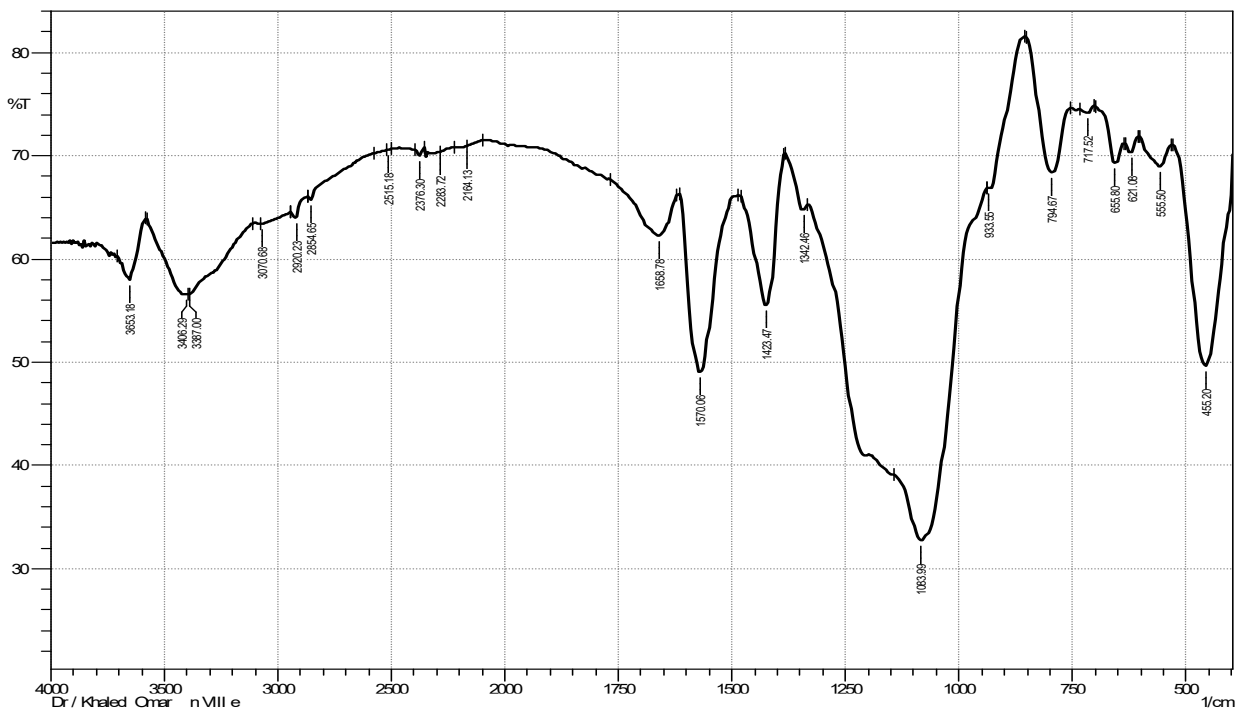
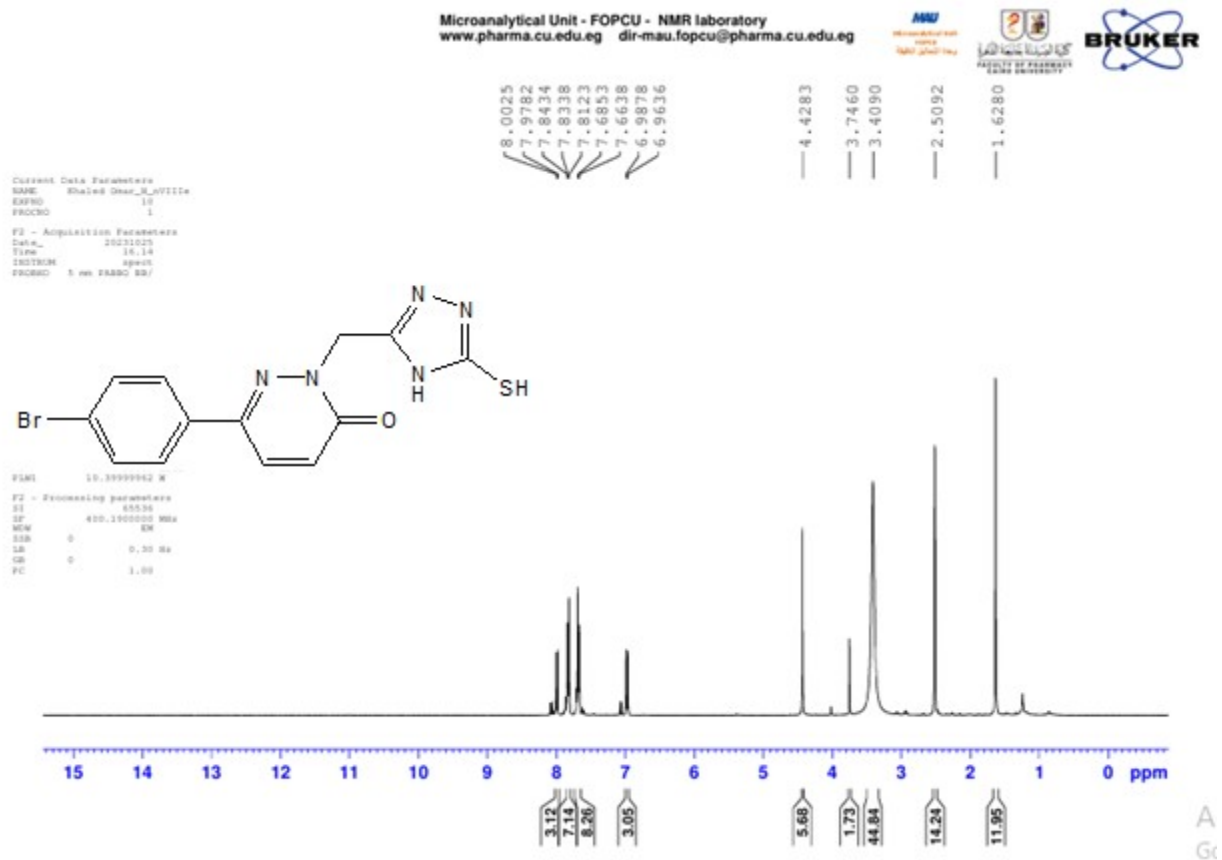


Figure 60. IR chart of 5f



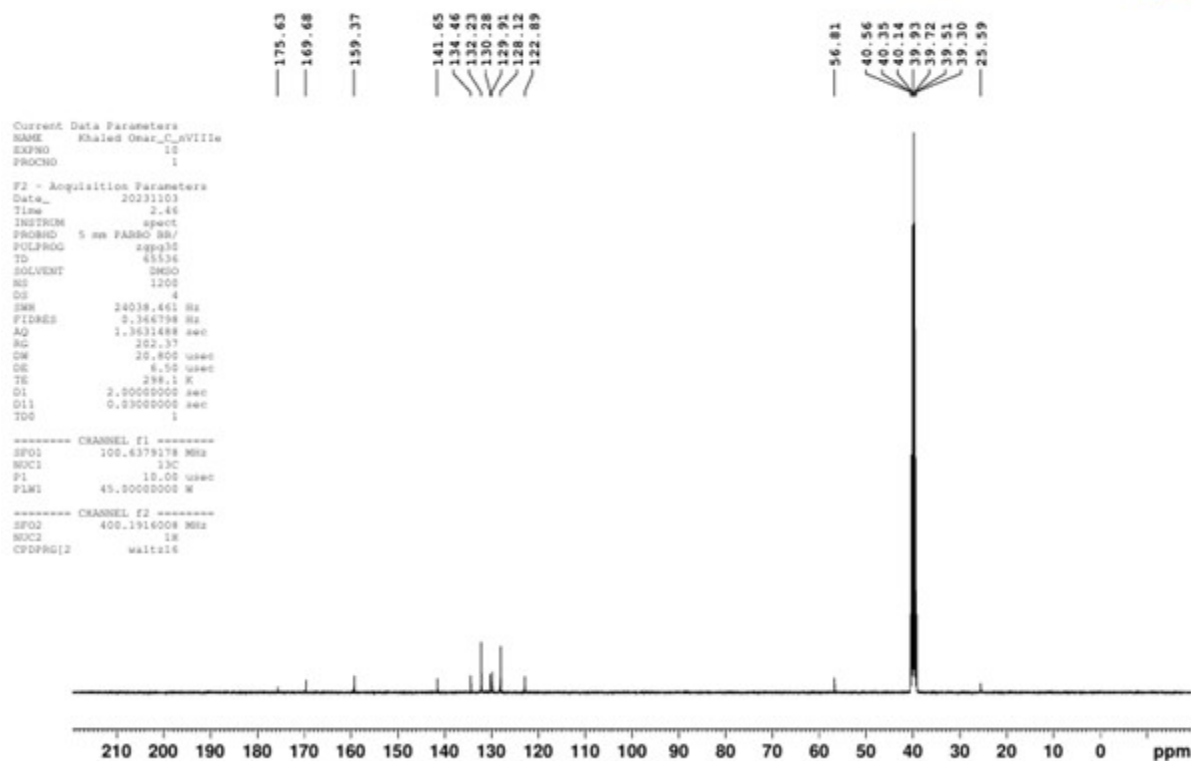


Figure 62. ^{13}C NMR chart of 5f

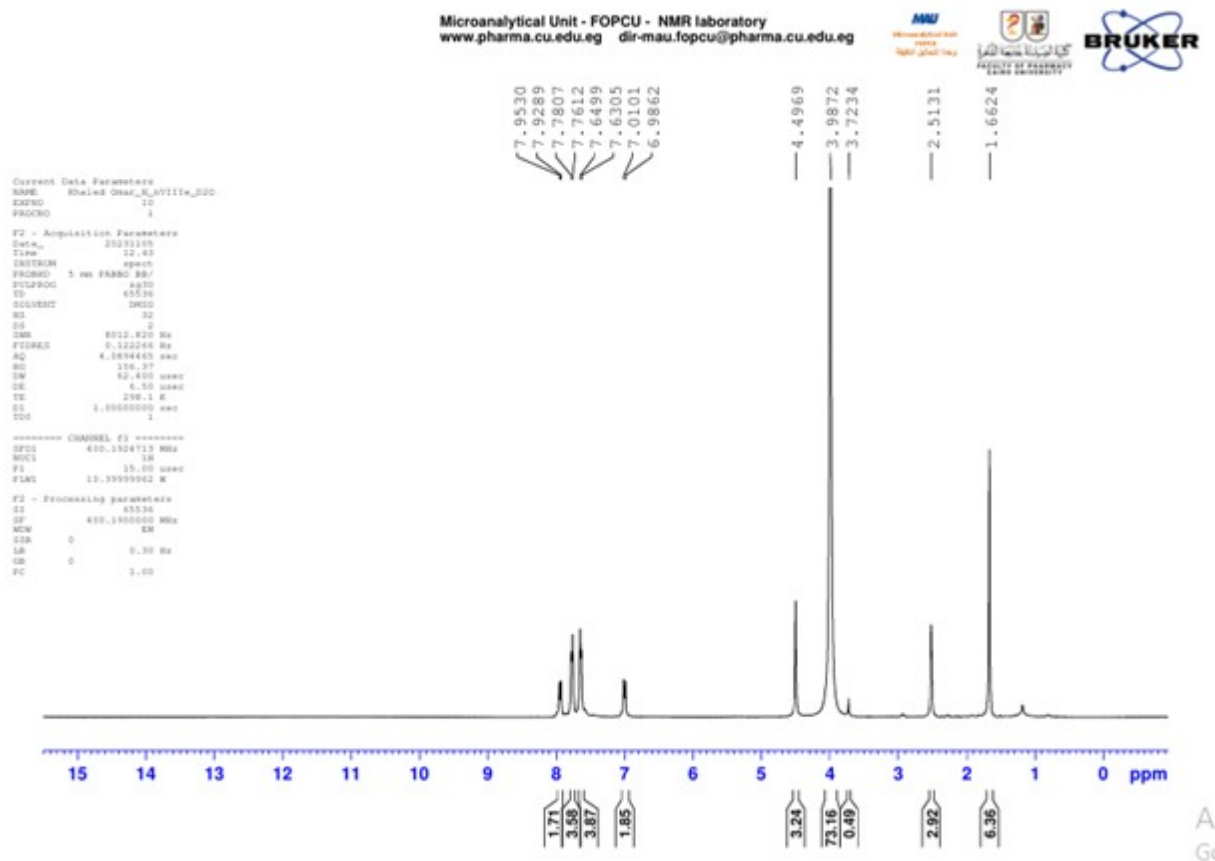


Figure 63. D₂O chart of 5f

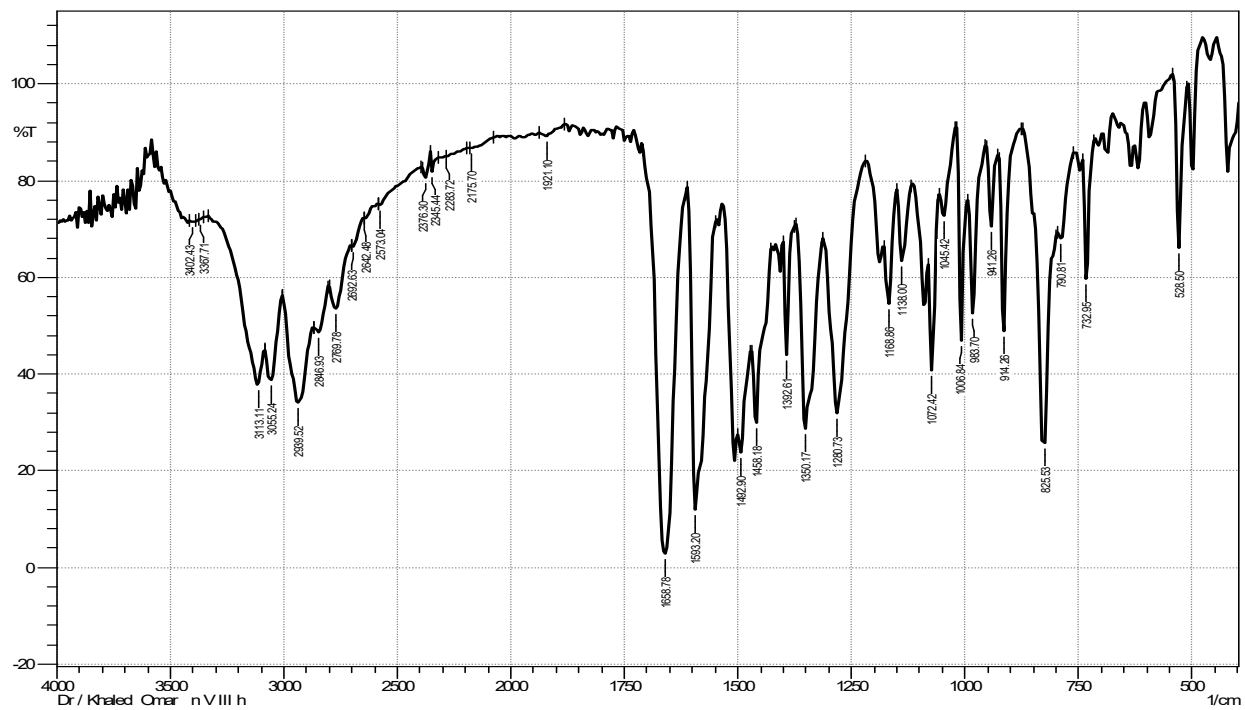


Figure 64. IR chart of 5g

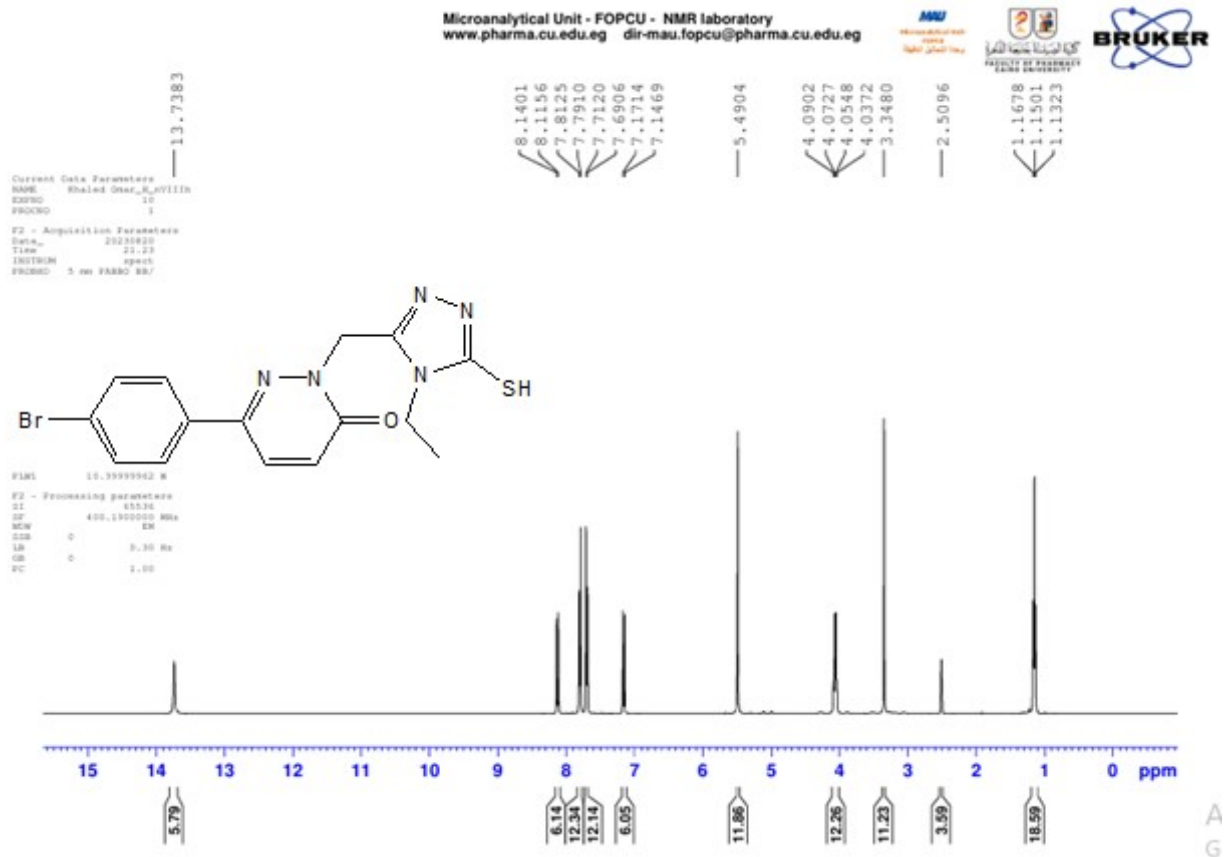


Figure 65. ¹H NMR chart of 5g

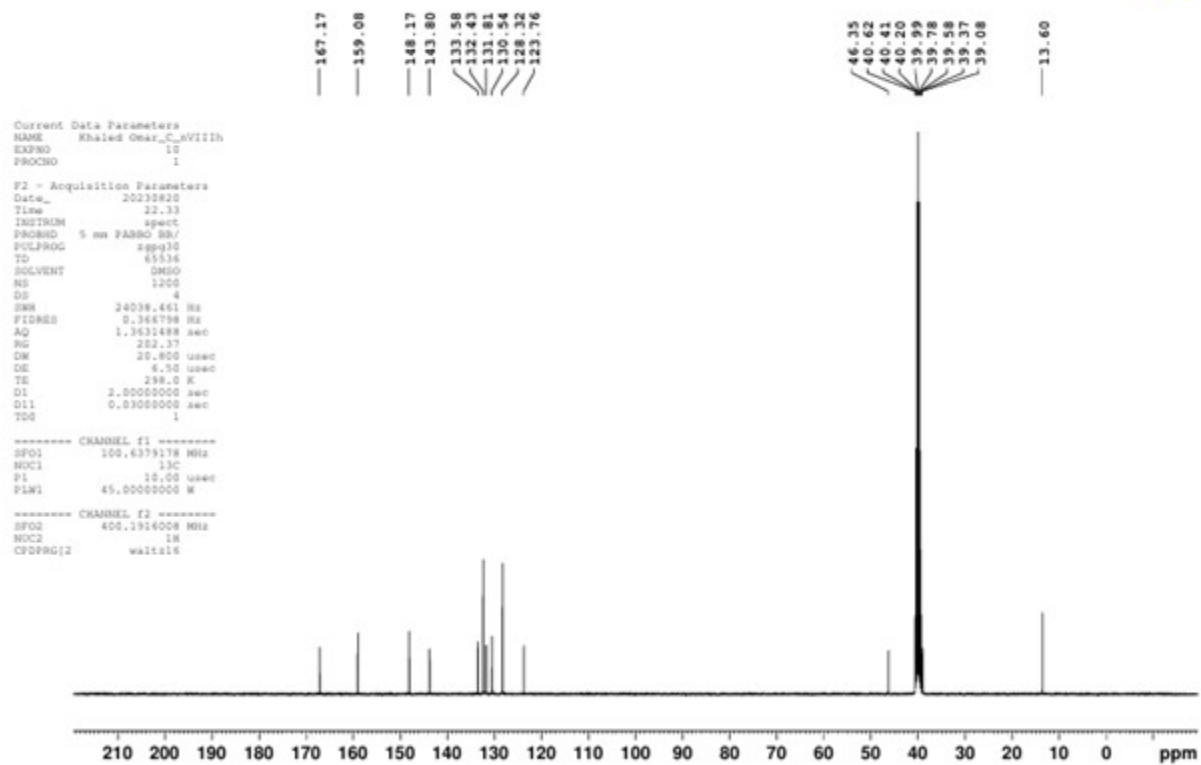


Figure 66. ^{13}C NMR chart of 5g

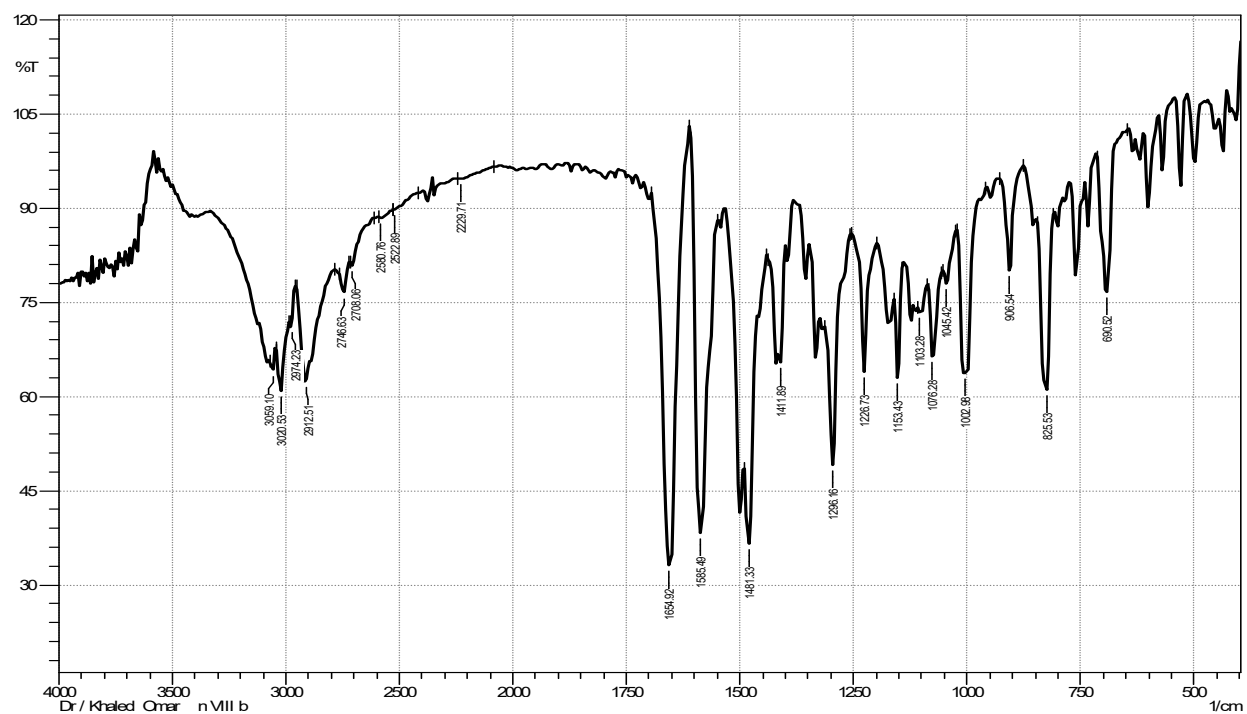


Figure 67. IR chart of 5h

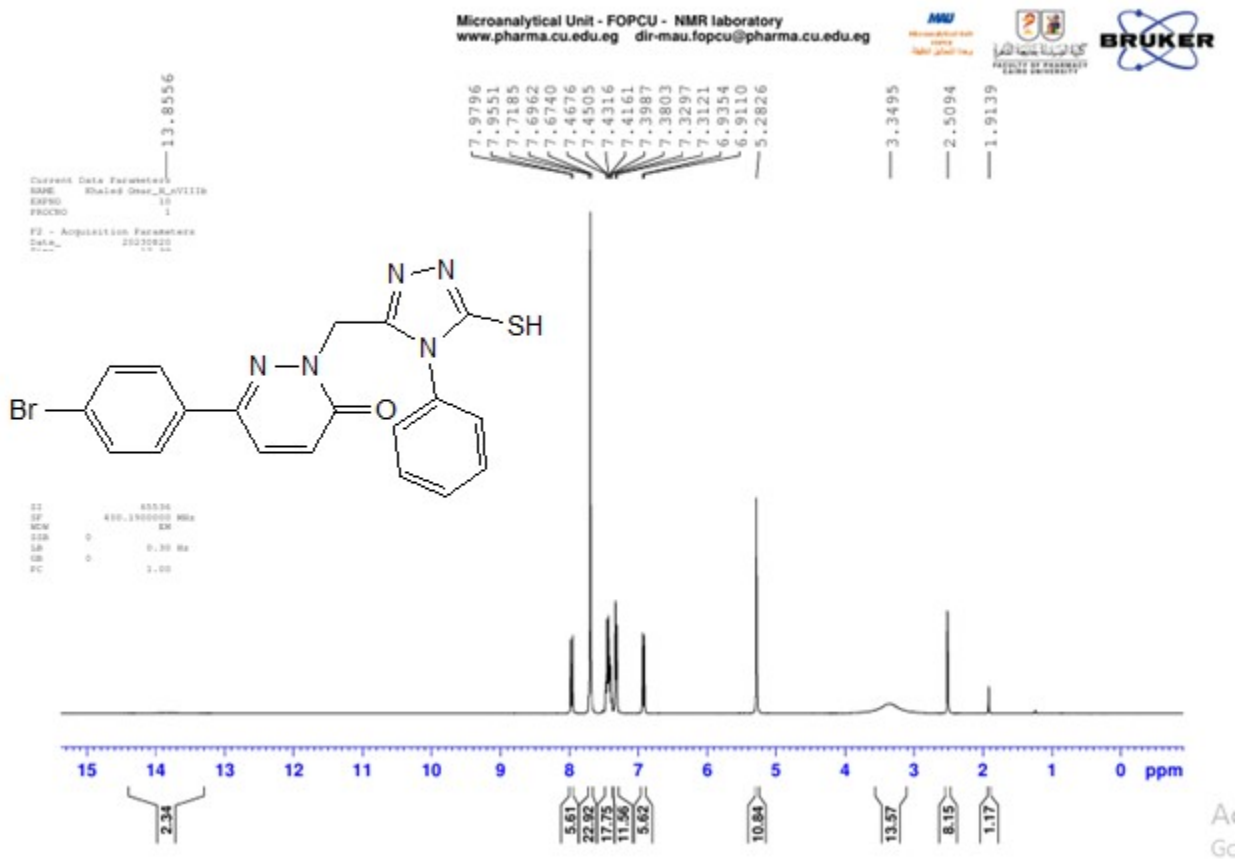


Figure 68. ¹H NMR chart of 5h

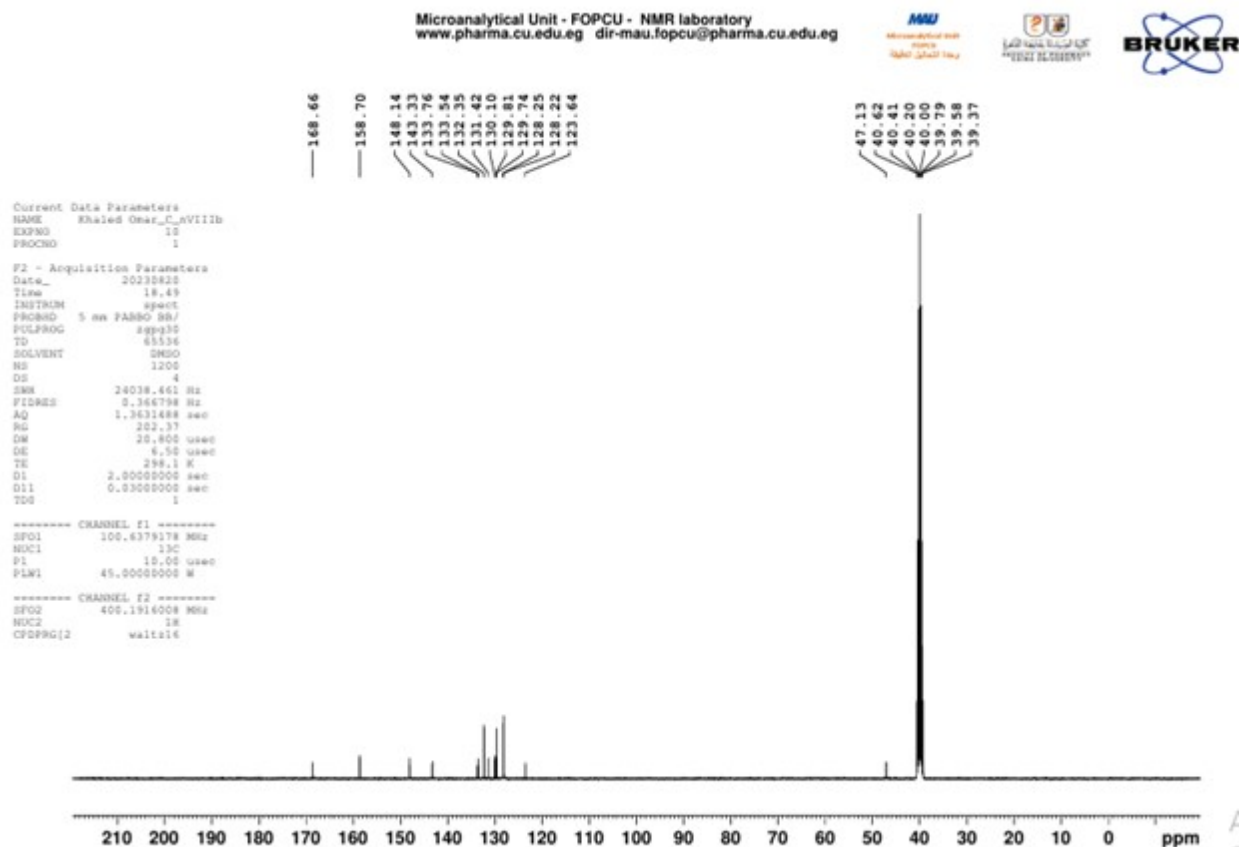


Figure 69. ^{13}C NMR chart of 5h

Additional Data and tables

Data 1	Structure of compounds 4f, 4h, 5d and 5e relative to hydralazine
Data 2	<i>ADMET</i> Description plot illustrating the human intestinal absorption (HIA) and blood brain barrier penetration prediction for the newly synthesized compounds.
Table 1	The ten generated pharmacophore hypotheses with their maximum fit, total cost, cost difference, RMS and correlation coefficient
Table 2	Primer's sequence of all studied genes.

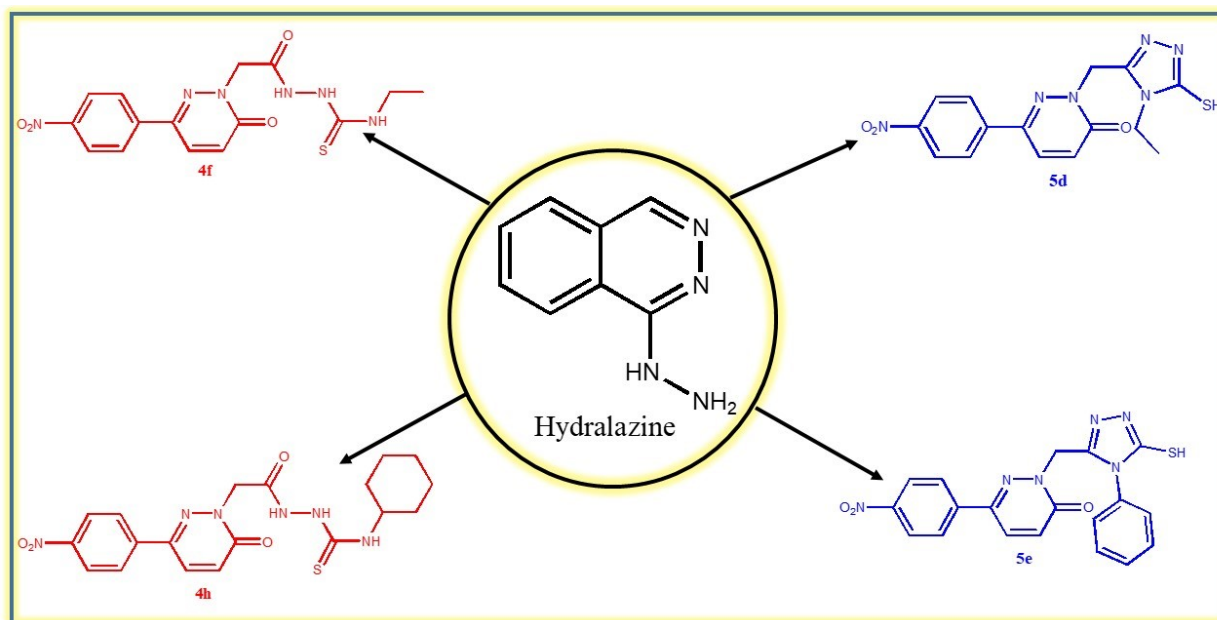


Figure 1. Structure of compounds 4f, 4h, 5d and 5e relative to hydralazine.

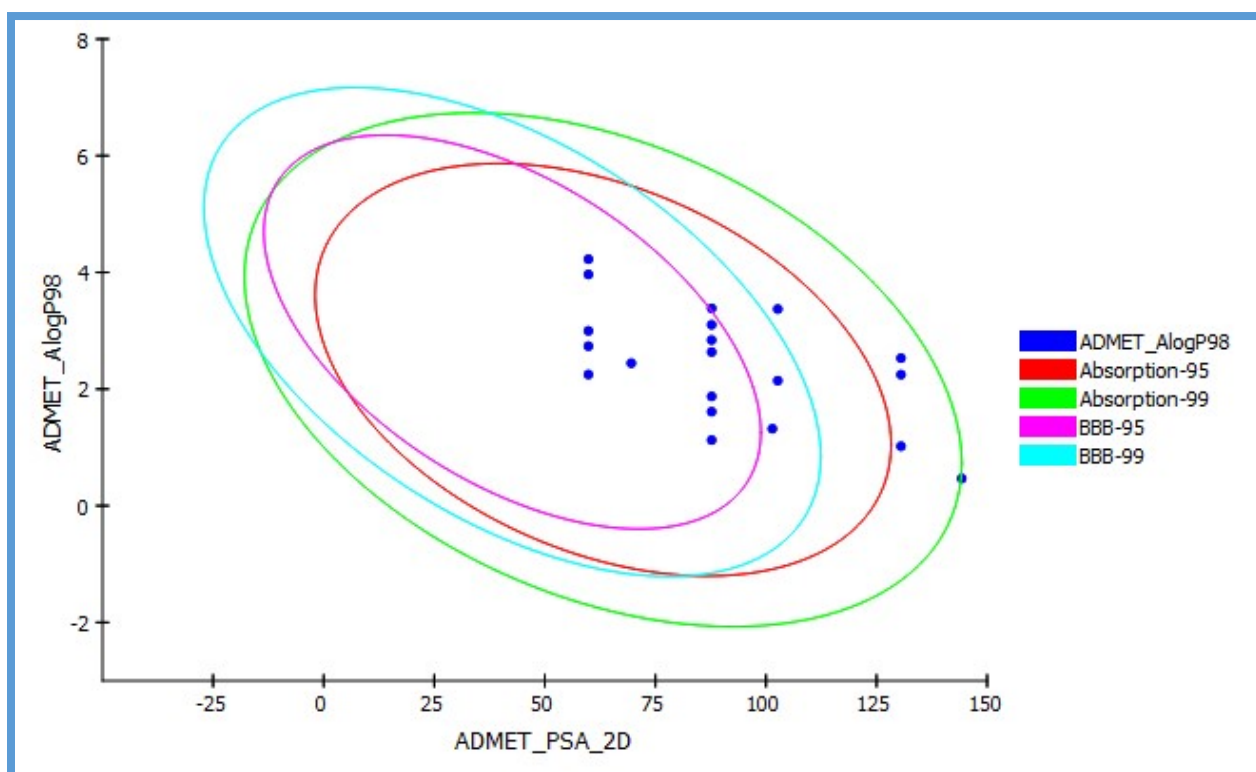


Figure 2. *ADMET* Description plot illustrating the human intestinal absorption (HIA) and blood brain barrier penetration prediction for the newly synthesized compounds.

Table 1. The ten generated pharmacophore hypotheses with their maximum fit, total cost, cost difference, RMS and correlation coefficient.

Hypothesis	Maximum fit	Total cost	Cost difference	RMS	Correlation coefficient
1	6.699	78.203	62.030	2.390	0.813
2	7.330	87.920	52.312	2.662	0.761
3	5.439	91.703	48.530	2.793	0.732
4	4.646	105.191	35.042	3.12	0.649
5	4.916	106.755	33.478	3.168	0.635
6	4.275	110.204	30.029	3.225	0.619
7	5.166	111.228	29.005	3.248	0.614
8	3.937	111.301	28.932	3.232	0.616
9	4.581	112.050	28.182	3.282	0.600
10	4.127	121.294	18.939	3.472	0.534

Table 2. Primer's sequence of all studied genes.

mRNA	Primer sequence 5'-3'
eNOS	F: ATTGCACCCTTCCGGGGATT R: ACGGTTTGCAGGACGCTGGTT
GAPDH Housekeeping gene	F: TGGATTTGGACGCATTGGTC R: TTTGCACTGGTACGTGTTGAT

F: Forward

R: Reverse