Synthesis and evaluation of 6-substituted 3-arylcoumarin derivatives as multifunctional acetylcholinesterase/monoamine oxidase B dual inhibitors for the treatment of Alzheimer's disease

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Table S1. HPLC analysis data of compounds **5a-t**. The purities of compounds were determined by the methods as shown given in the following table. The peak purity was checked with UV spectra.

Equipment		Agilent 1200 with binary pump, photodiode array detector (DAD)	
Column		Agilent Zorbax Exlipse SB-C18 (250×4.6 mm, 5 μm particle size)	
Method		CH_3OH/H_2O, 65% (v/v) of CH_3OH gradient, flow rate: 1.0 mL/min, calculated the relative purity of each compound at 254 nm	
Results	Compds	Retention time (min)	Relative purity (%)
	5a	7.54	99.16
	5b	6.88	98.25
	5c	6.68	98.77
	5d	7.96	98.11
	5e	5.91	96.67
	5f	7.31	96.44
	5g	8.65	98.25
	5h	6.73	99.23
	5i	7.78	98.59
	5j	6.94	97.36
	5k	7.68	98.52
	5l	7.93	96.32
	5m	8.21	95.45
	5n	8.47	96.73
	5o	6.41	95.88
	5p	5.42	96.84
	5q	6.64	97.24
	5r	6.78	97.31
	5s	8.17	96.46
	5t	7.34	98.77

Table S2. Permeability (Pe × 10^{-6} cm s^{-1}) in the PAMPA-BBB assay for 11 commercial drugs, used in the experiment validation.

Commercial drugs	Bibliography [a)]	Experiment [b)]
Testosterone	17	22.06 ± 3.21
Verapamil	16	16.71 ± 1.45
β-Estradiol	12	13.96 ± 2.17
Progesterone	9.3	13.15 ± 1.52
Clonidine	5.3	8.10 ± 1.26
corticosterone	5.1	3.91 ± 0.78
Piroxicam	2.5	3.27 ± 0.94
Hydrocortisone	1.9	2.78 ± 0.63
Lomefloxacin	1.1	3.72 ± 1.75
Ofloxacin	0.8	3.22 ± 0.48
Dopamine	0.2	1.34 ± 0.25

[a)] Taken from Ref. [1]

[b)] Data are the mean ± SD of three independent experiments.

Ref.[1] L. Di, E. H. Kerns, K. Fan, O. J. McConnell and G. T. Carter, *Eur J Med Chem*., 2003, 38, 223-232.

Table S3. Effects of compounds **5o** and **5p** on cell viability. [a)]

Compounds	**Viability(%) PC12 cells**				
	50 μM	25 μM	12.5 μM	6.25 μM	3.125 μM
5o	91.0 ± 5.0	95.3 ± 3.5	99.0 ± 1.5	101.3 ± 3.2	101.7 ± 3.1
5p	87.3 ± 4.7	94.1 ± 4.9	97.3 ± 3.3	102.0 ± 3.6	101.3 ± 3.2

[a)] Cell viability was measured as MTT reduction and data were normalized as % control. Data are expressed as the means ± SEM of triplicate of four different cultures. All compounds were assayed at increasing concentrations (3.125-50 μM).

Figure S1. Molecular modeling studies of **5o** with hAChE and hMAO-B.

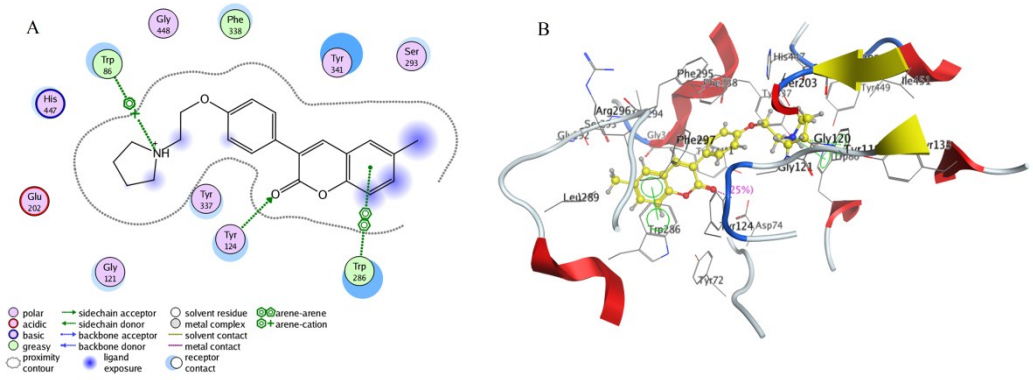


Figure S1.1. 5o with hAChE

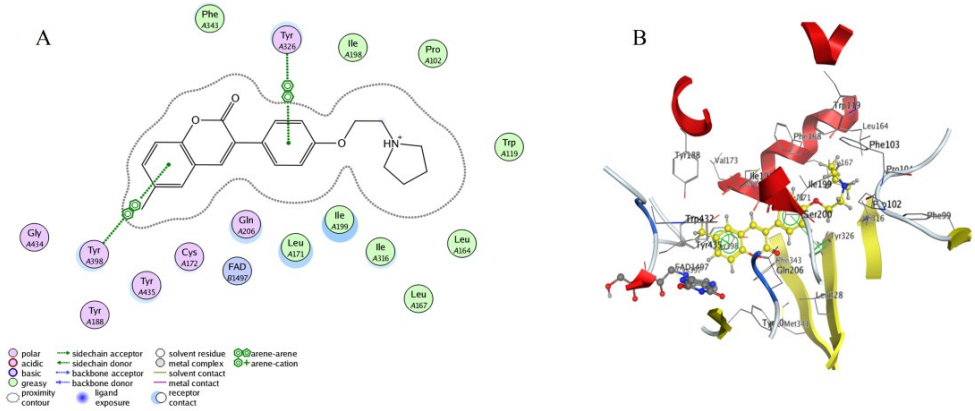


Figure S1.2. 5o with hMAO-B

Figure S2. Lineal correlation between experimental and reported permeability of 11 commercial drugs using the PAMPA-BBB assay. Pe (exp.) = 1.0999Pe (bibl.) + 1.2648 (R^2= 0.9475)

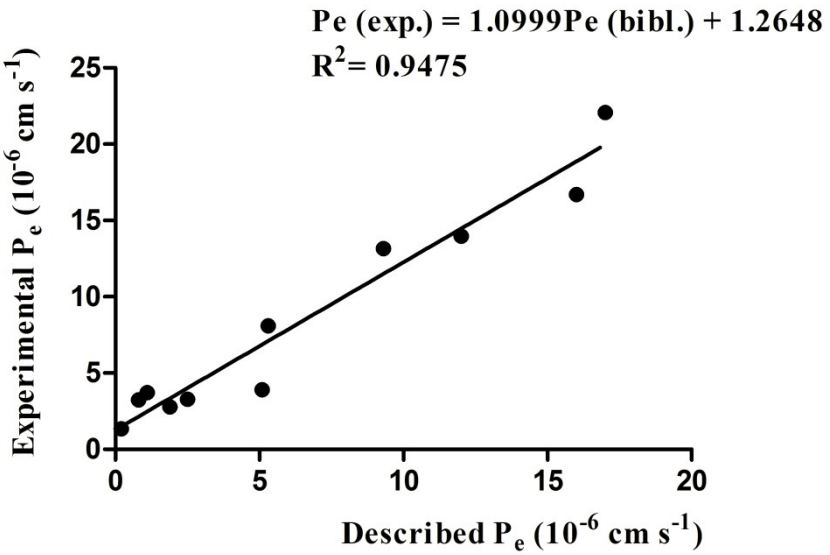
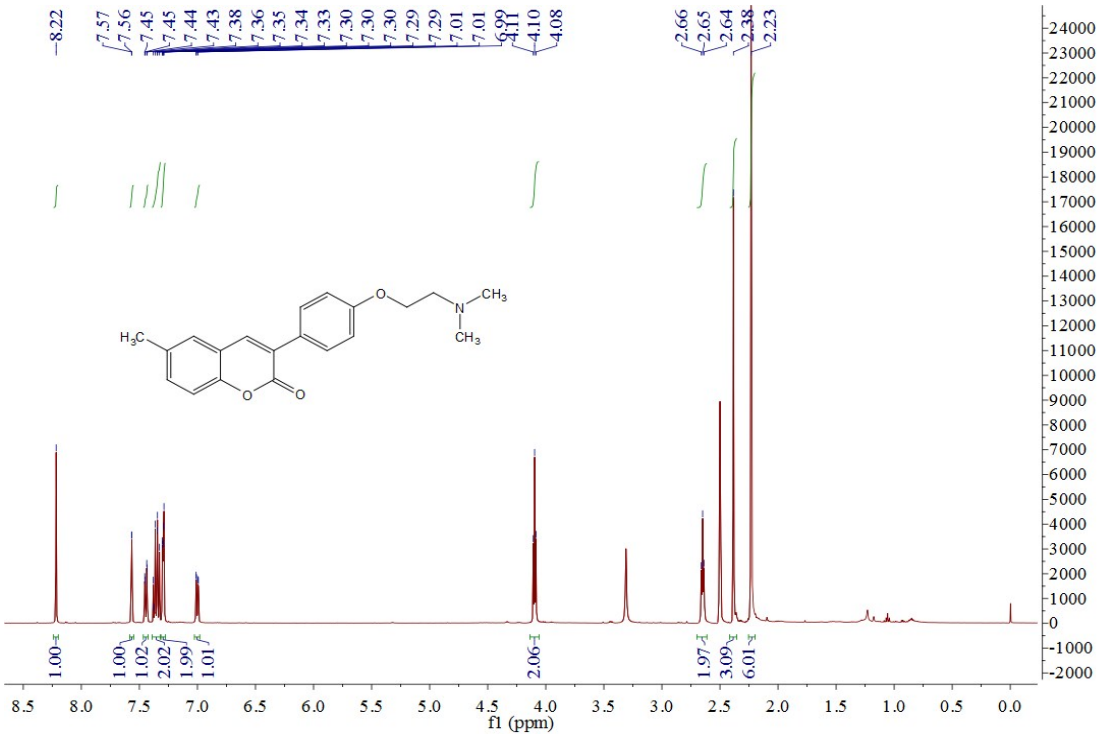
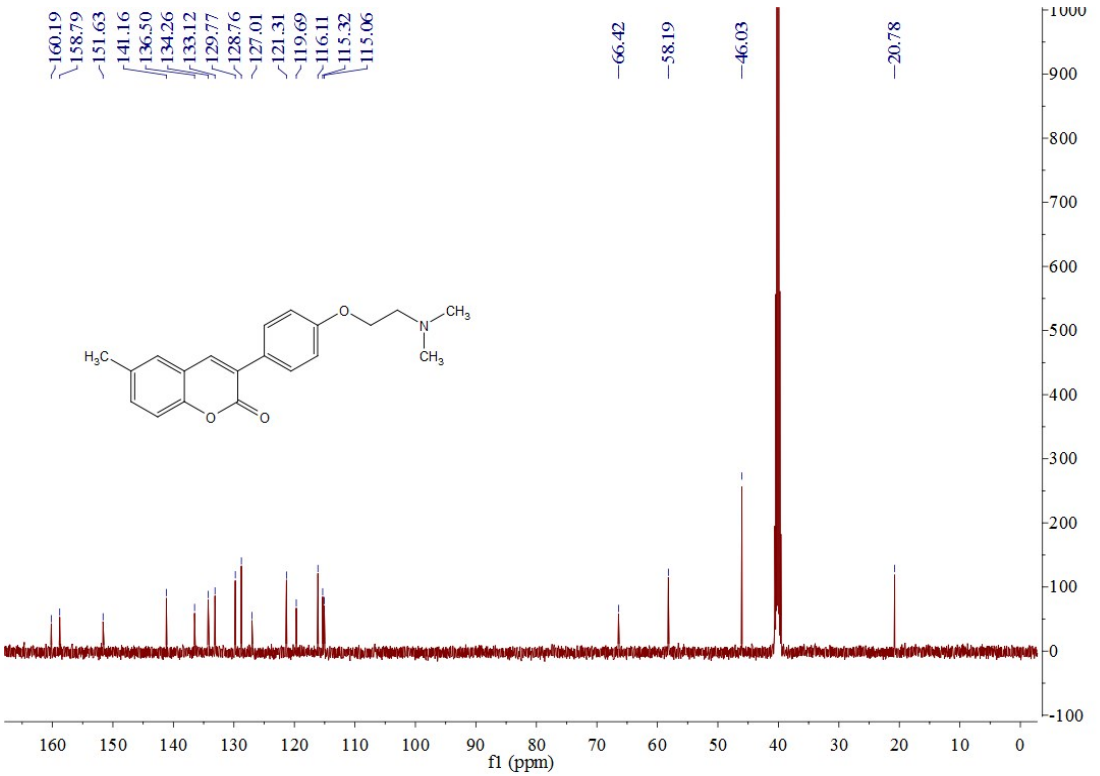


Figure S3. The representative ^{1}H NMR , ^{13}C NMR, IR and HRMS (ESI) spectrums of **5a**, **5b**, **5e**, **5h**, **5l**, **5o** and **5p**.

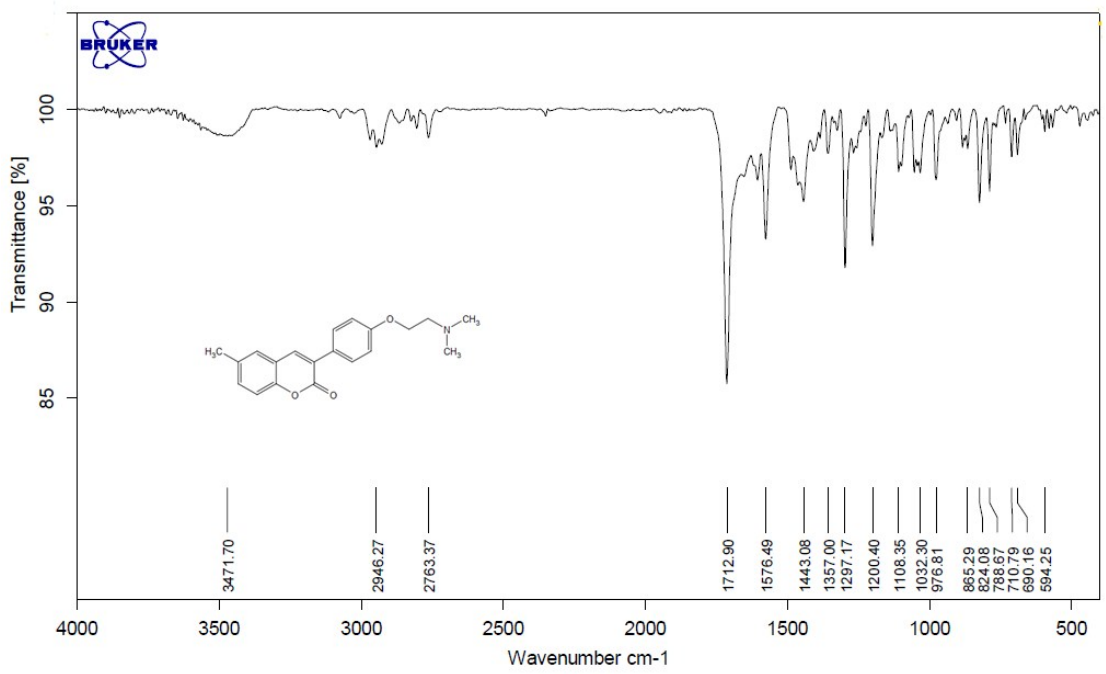
^{1}H NMR spectrum of compound 5a in DMSO



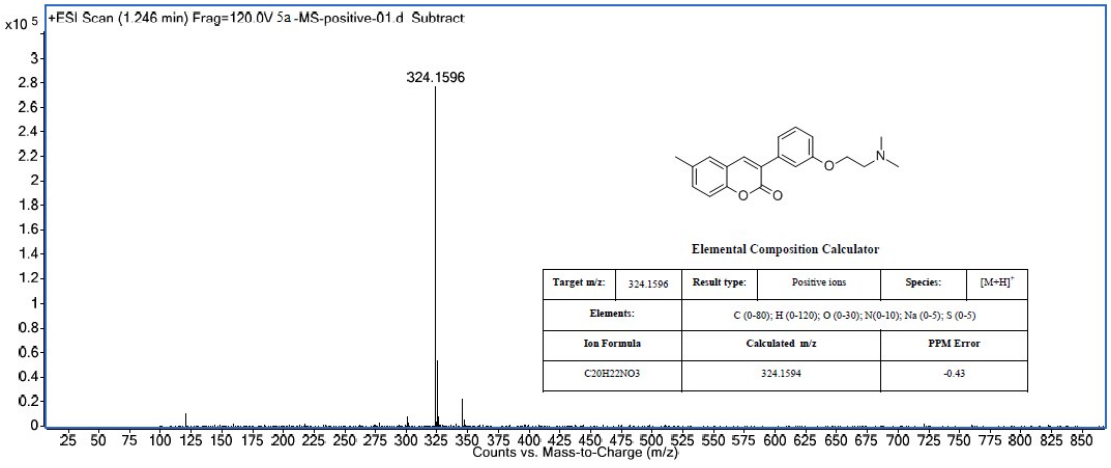
^{13}C NMR spectrum of compound 5a in DMSO



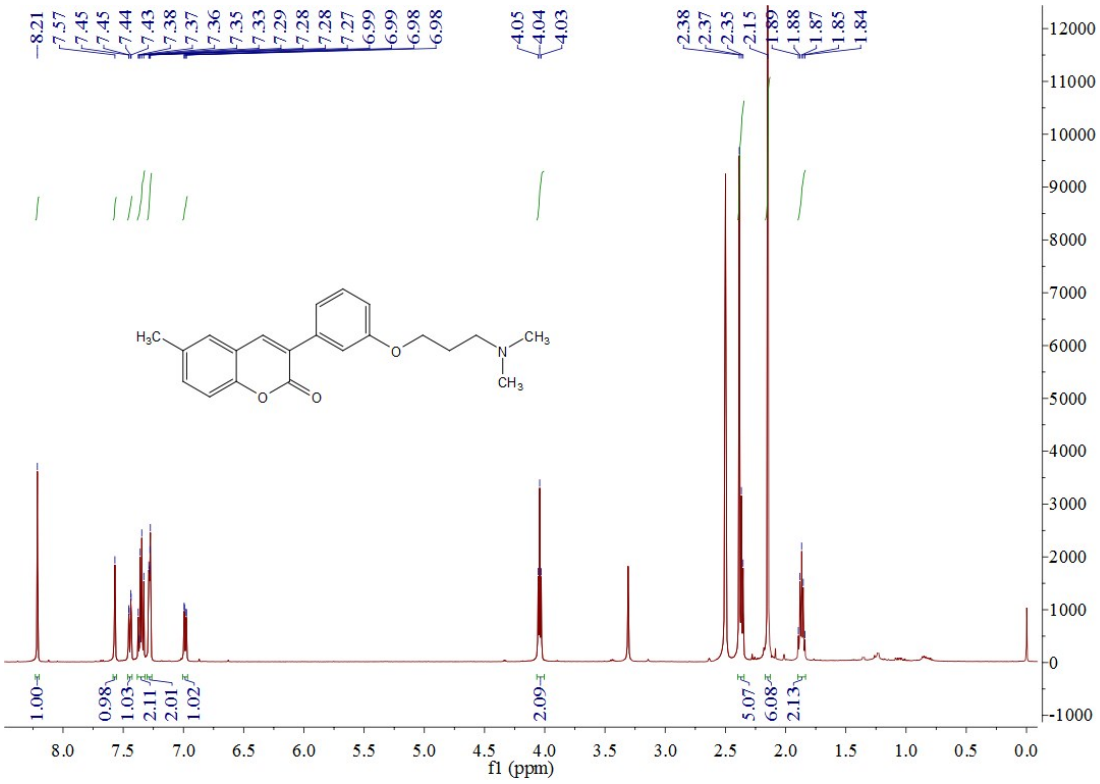
IR (KBr) spectrum of compound 5a



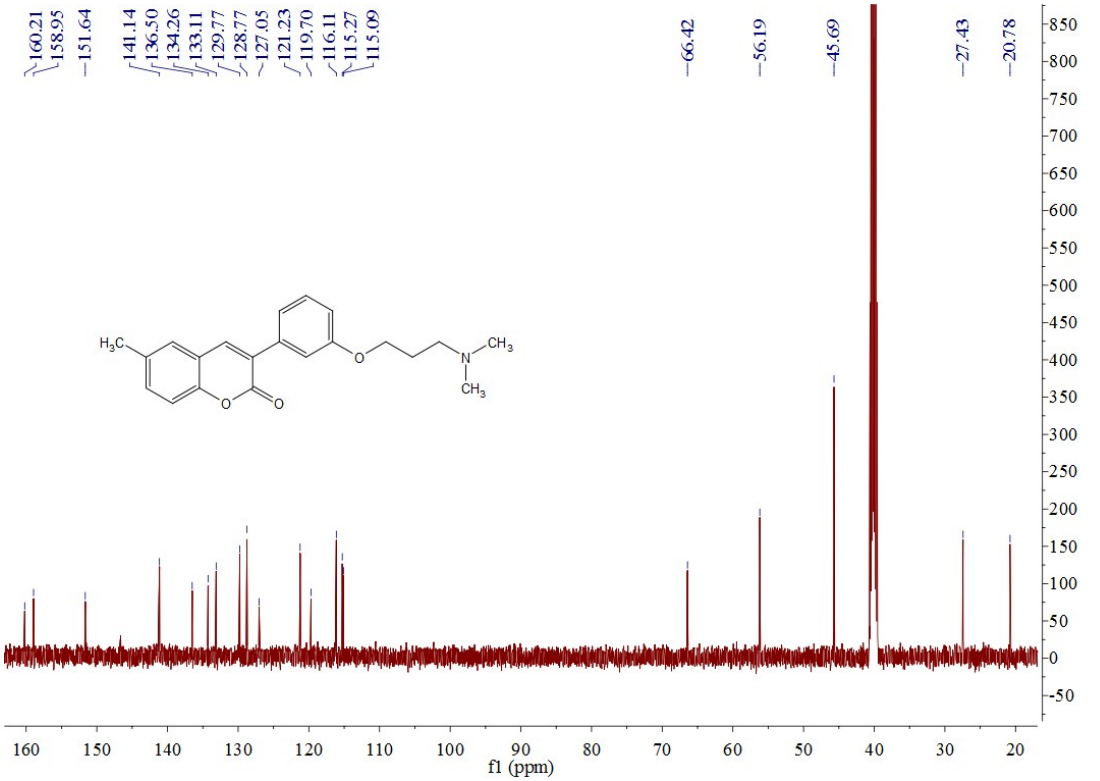
HRMS (ESI) spectrum of compound 5a



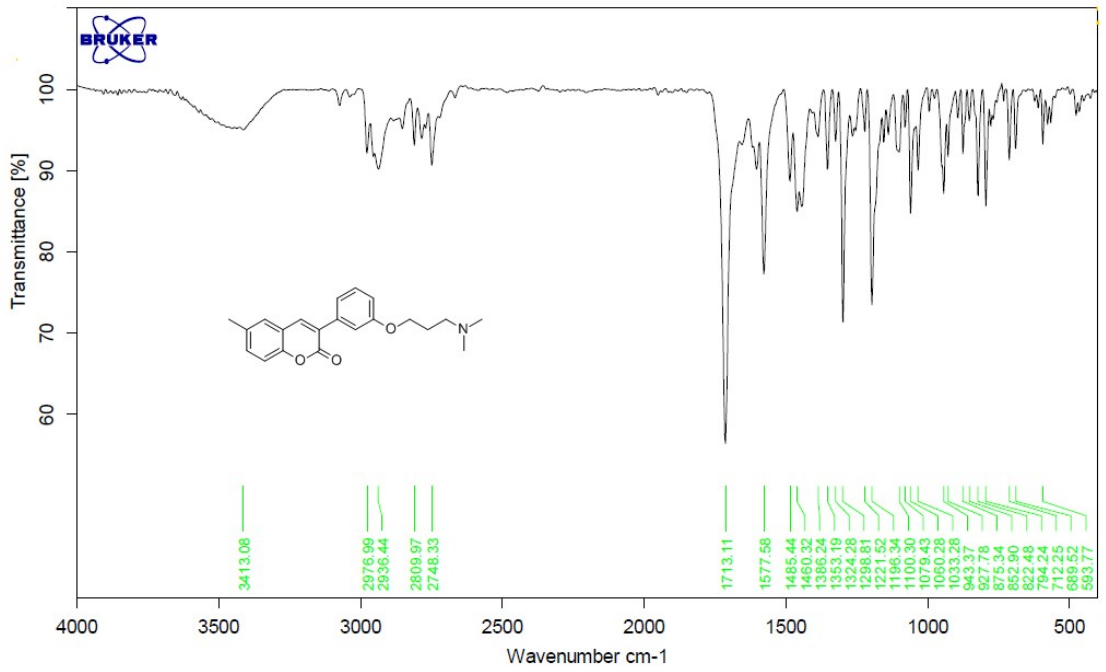
^{1}H NMR spectrum of compound 5b in DMSO



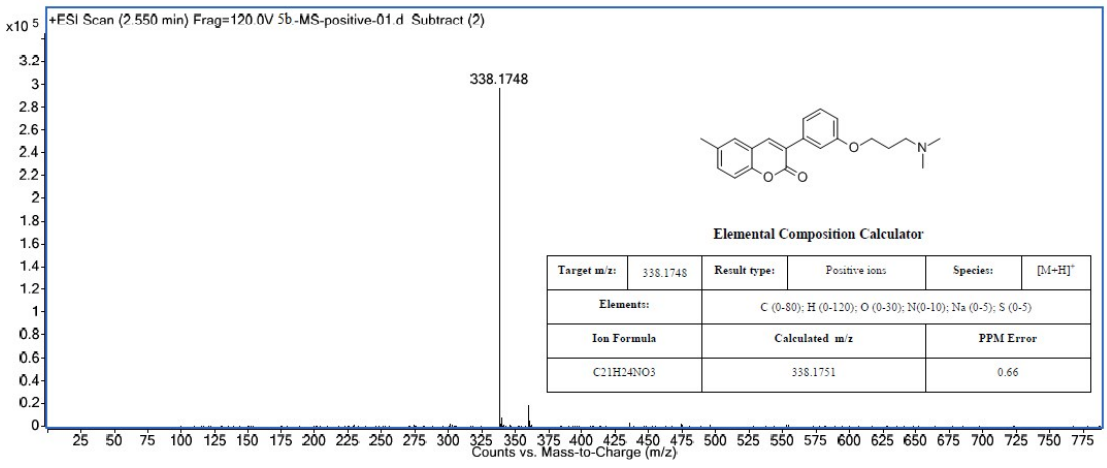
^{13}C NMR spectrum of compound 5b in DMSO



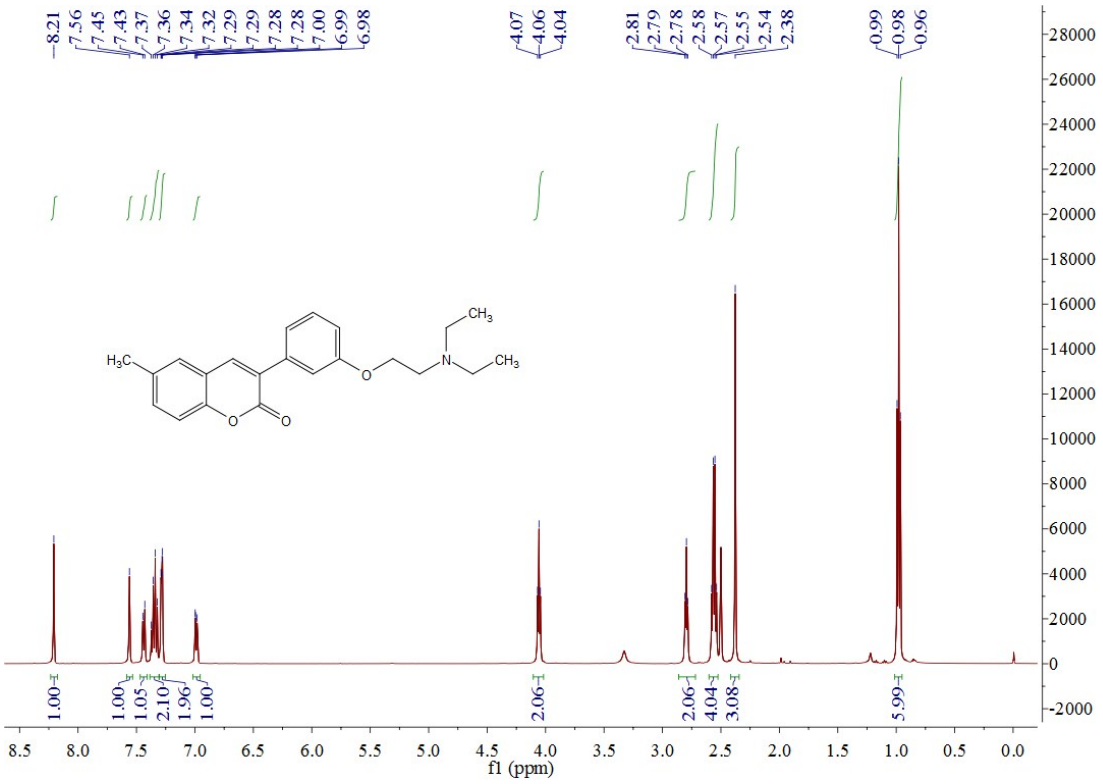
IR (KBr) spectrum of compound 5b



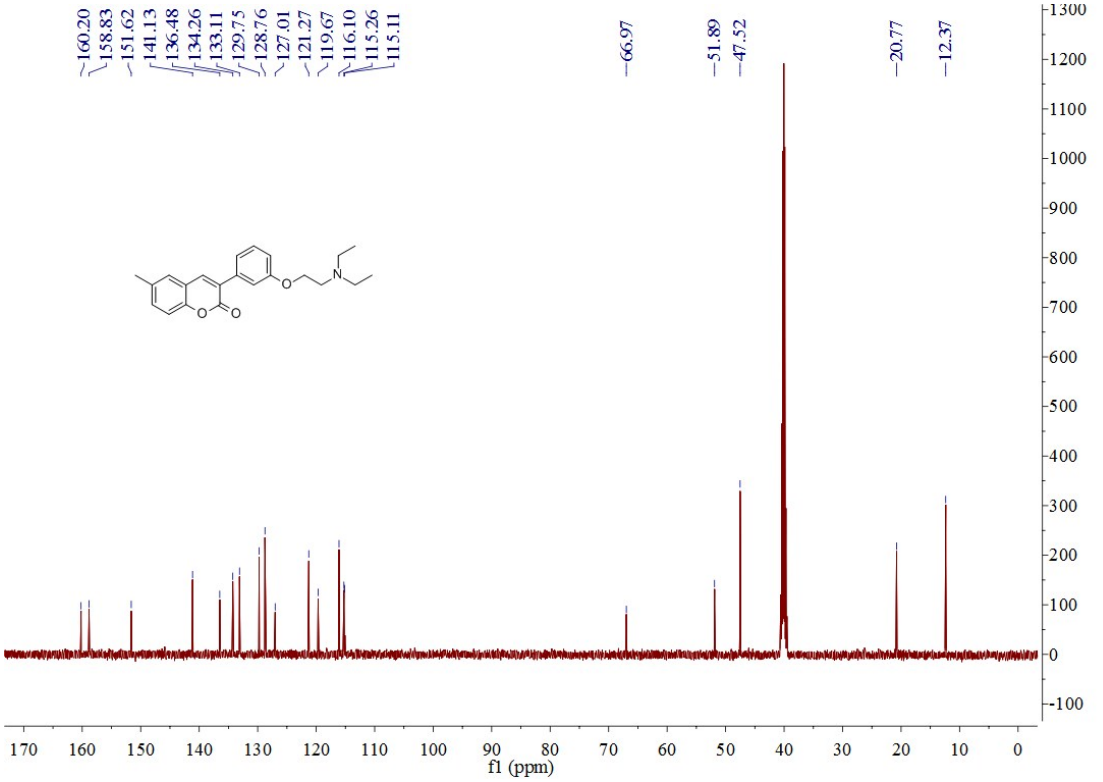
HRMS (ESI) spectrum of compound 5b



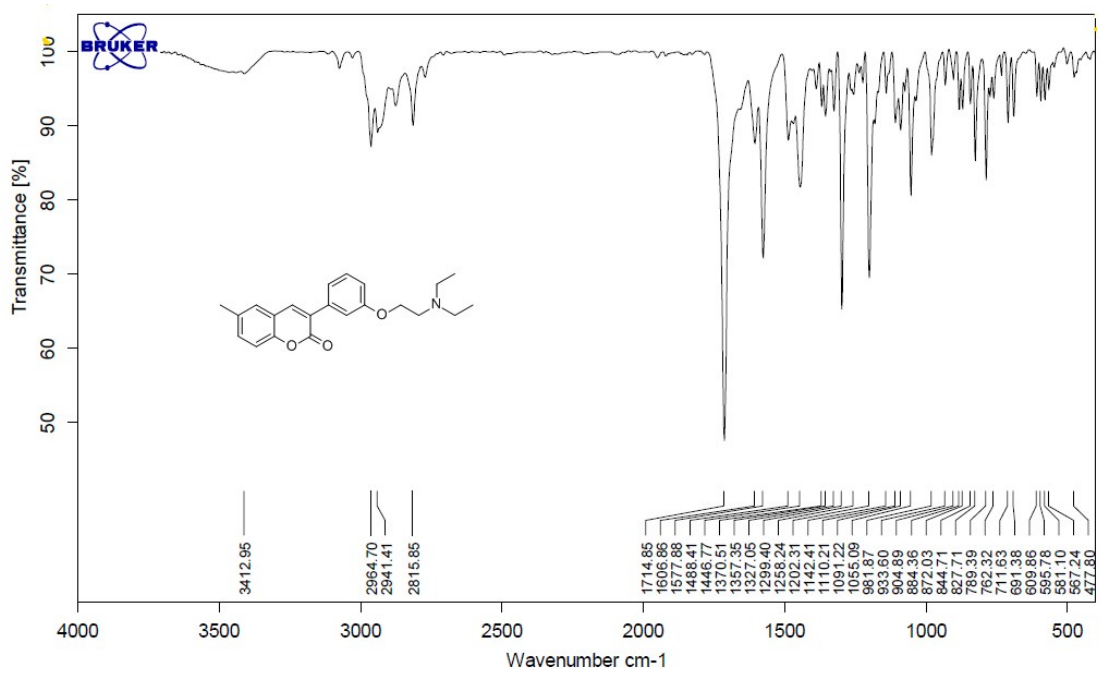
^{1}H NMR of compound 5e in DMSO



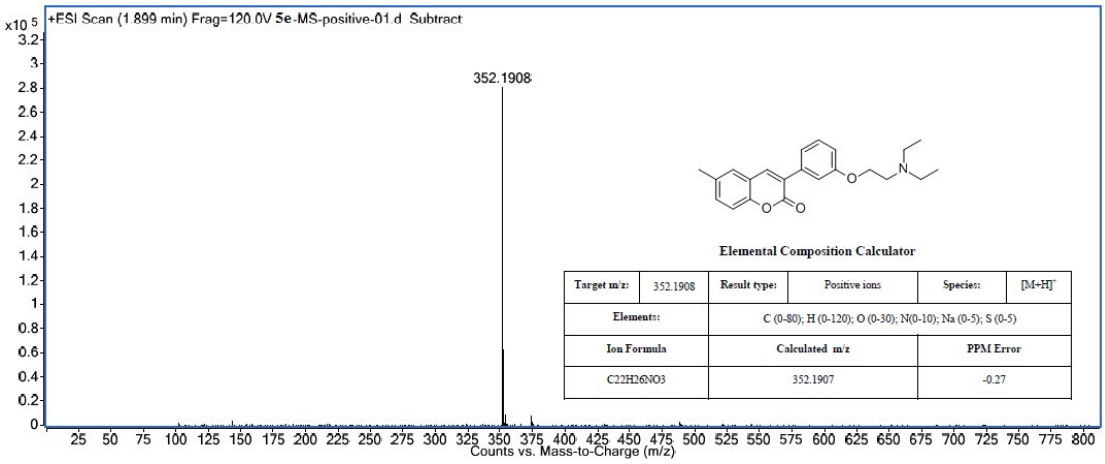
^{13}C NMR of compound 5e in DMSO



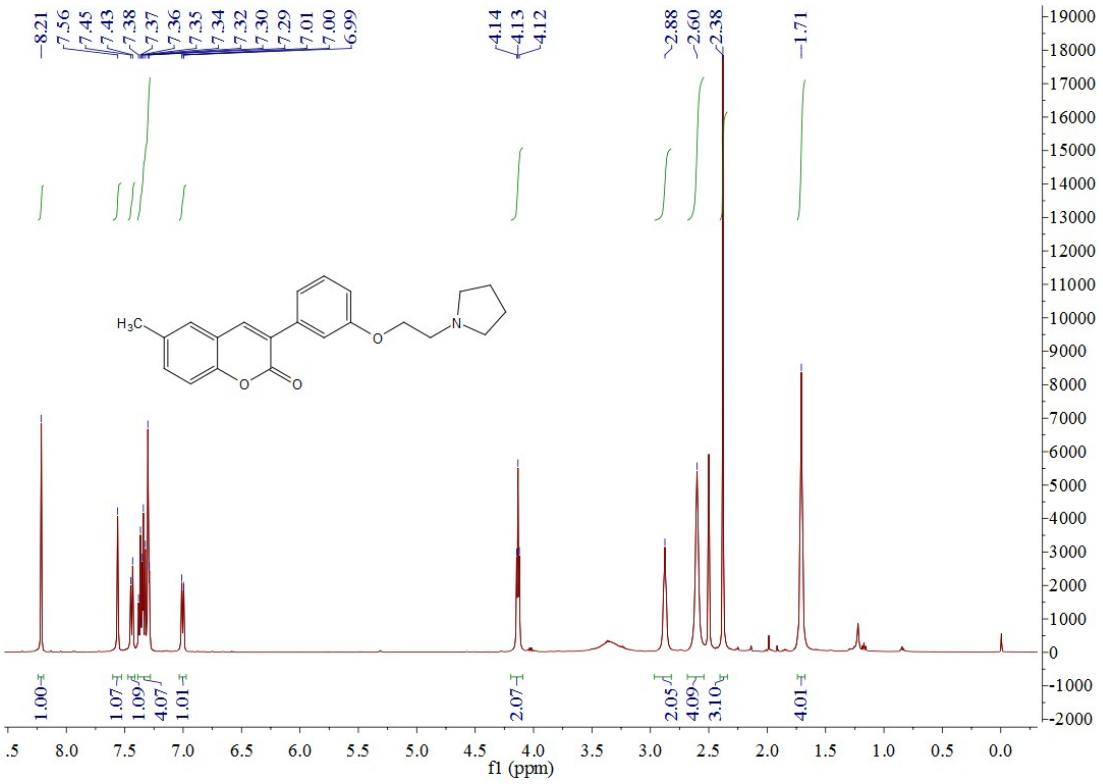
IR (KBr) spectrum of compound 5e



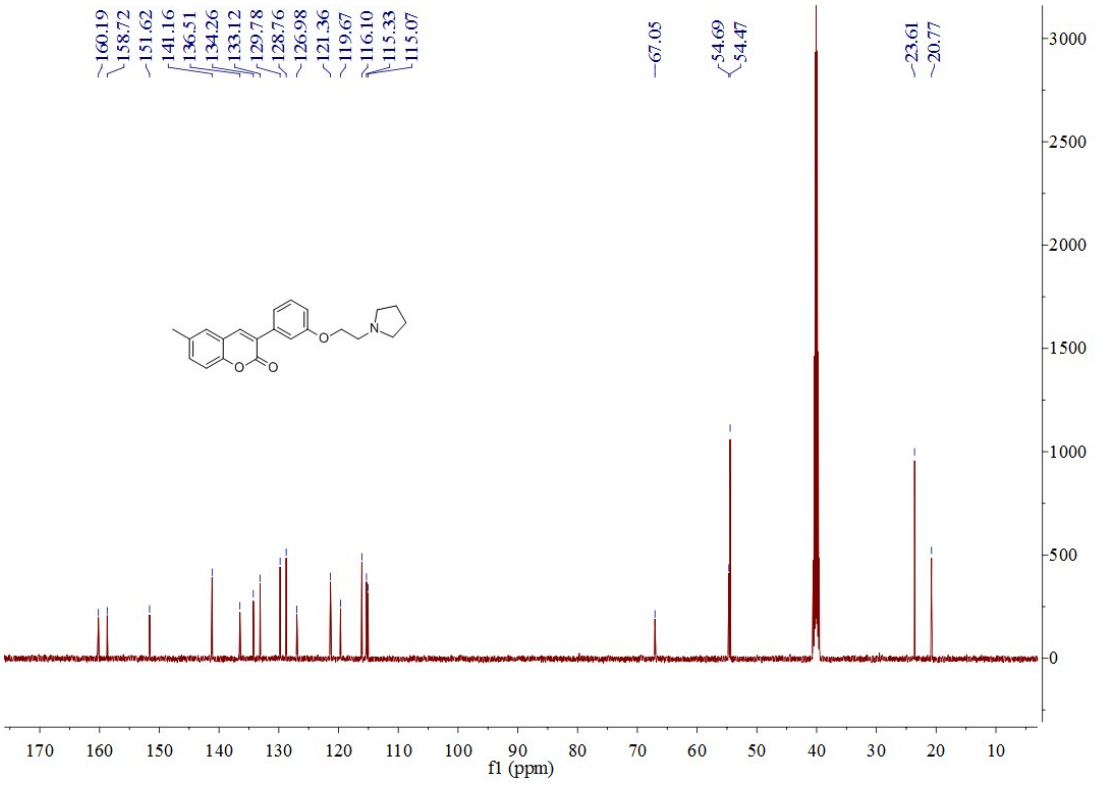
HRMS (ESI) spectrum of compound 5e



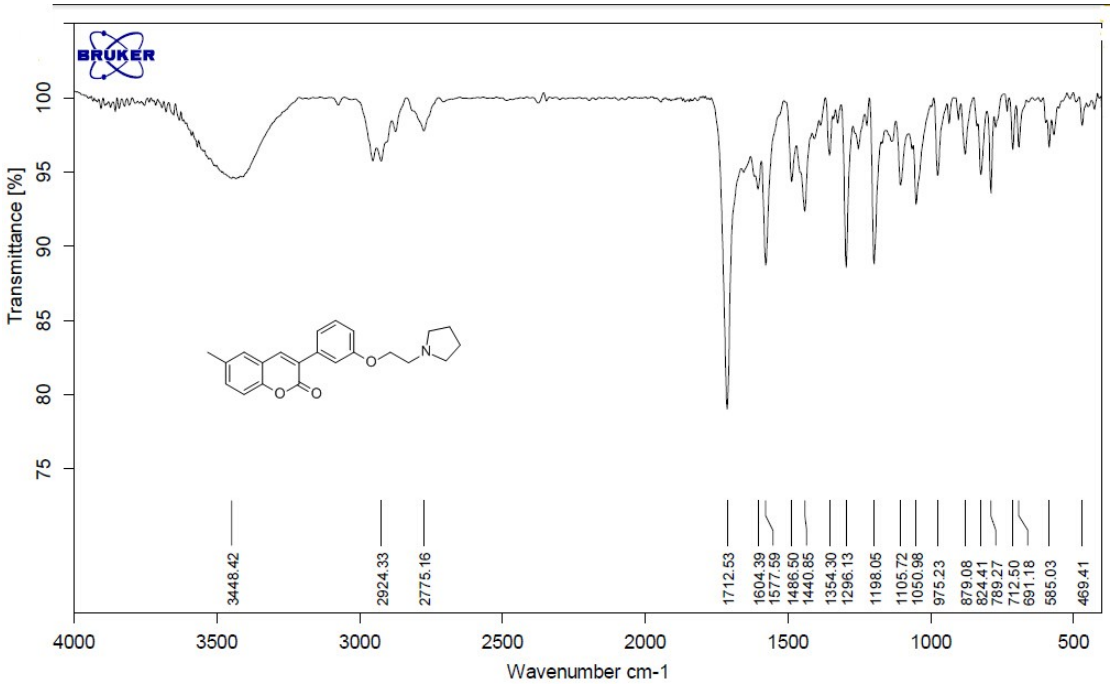
^{1}H NMR of compound 5h in DMSO



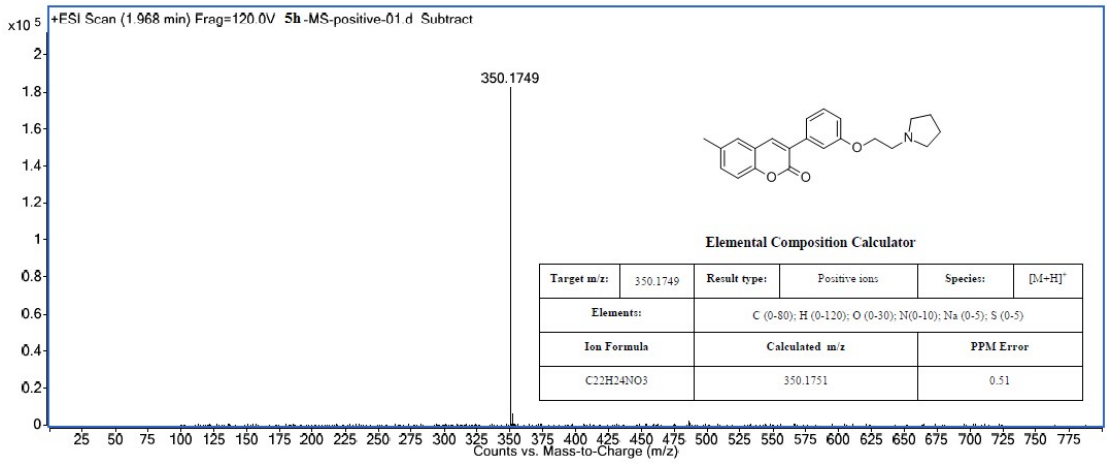
^{13}C NMR of compound 5h in DMSO



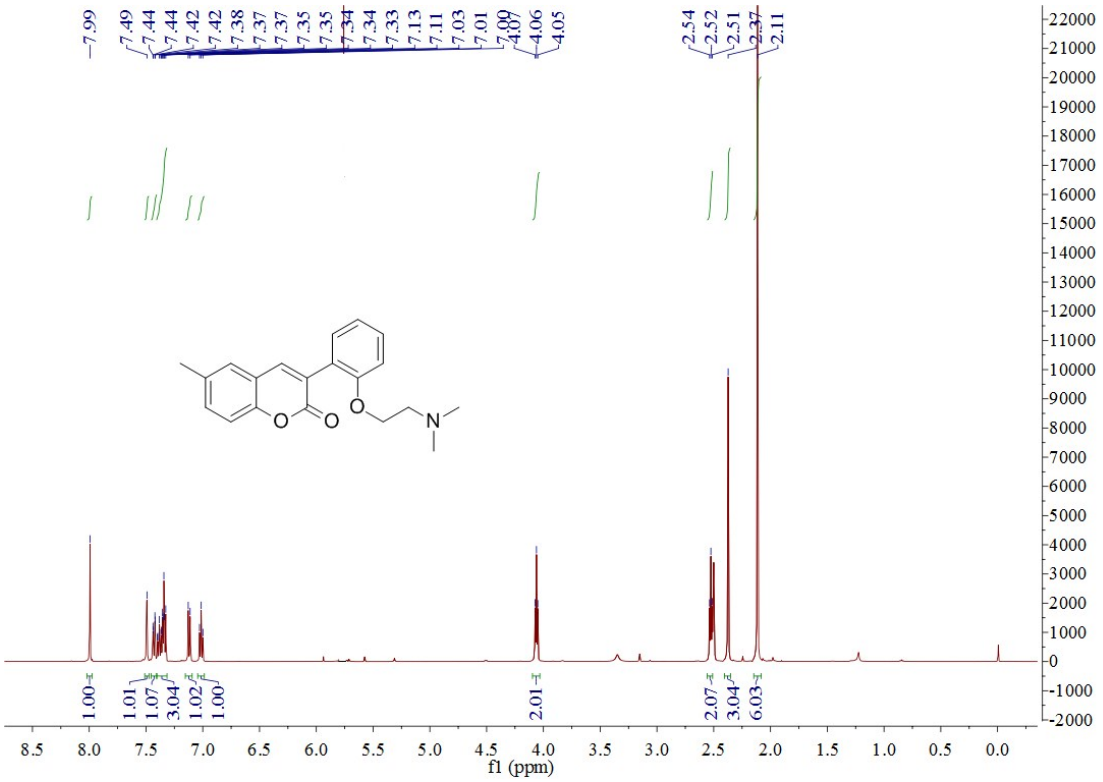
IR (KBr) spectrum of compound 5h



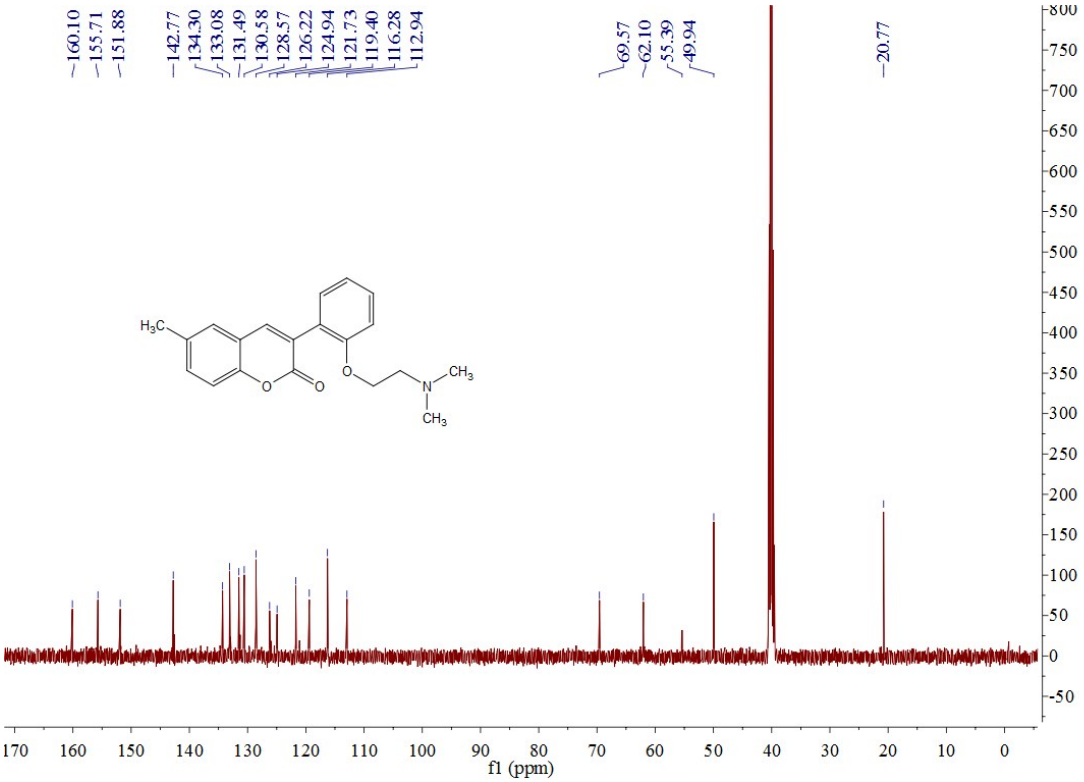
HRMS (ESI) spectrum of compound 5h



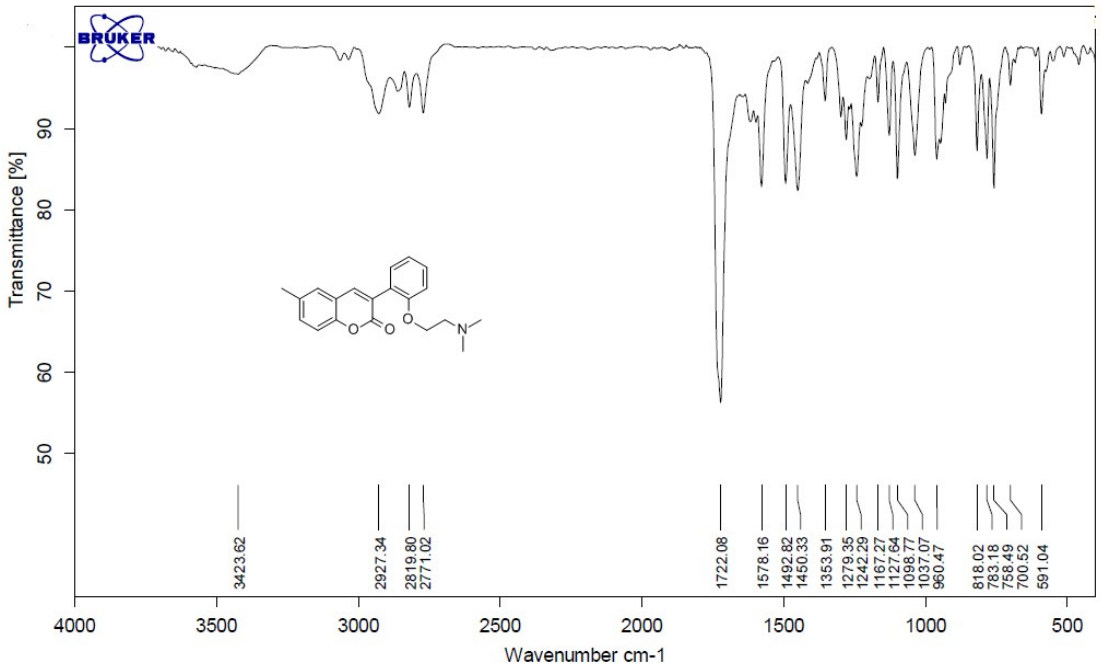
^{1}H NMR spectrum of compound 5l in DMSO



^{13}C NMR spectrum of compound 5l in DMSO



IR (KBr) spectrum of compound 5l



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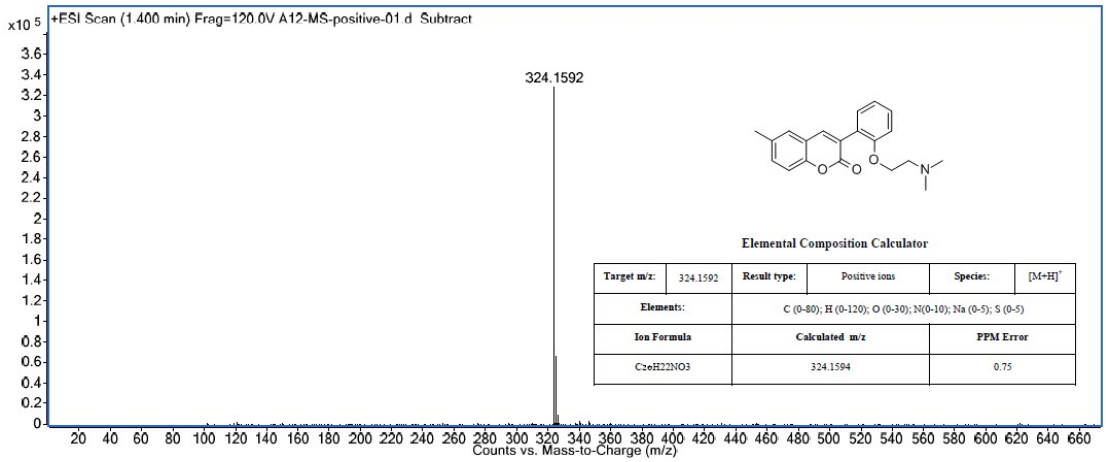
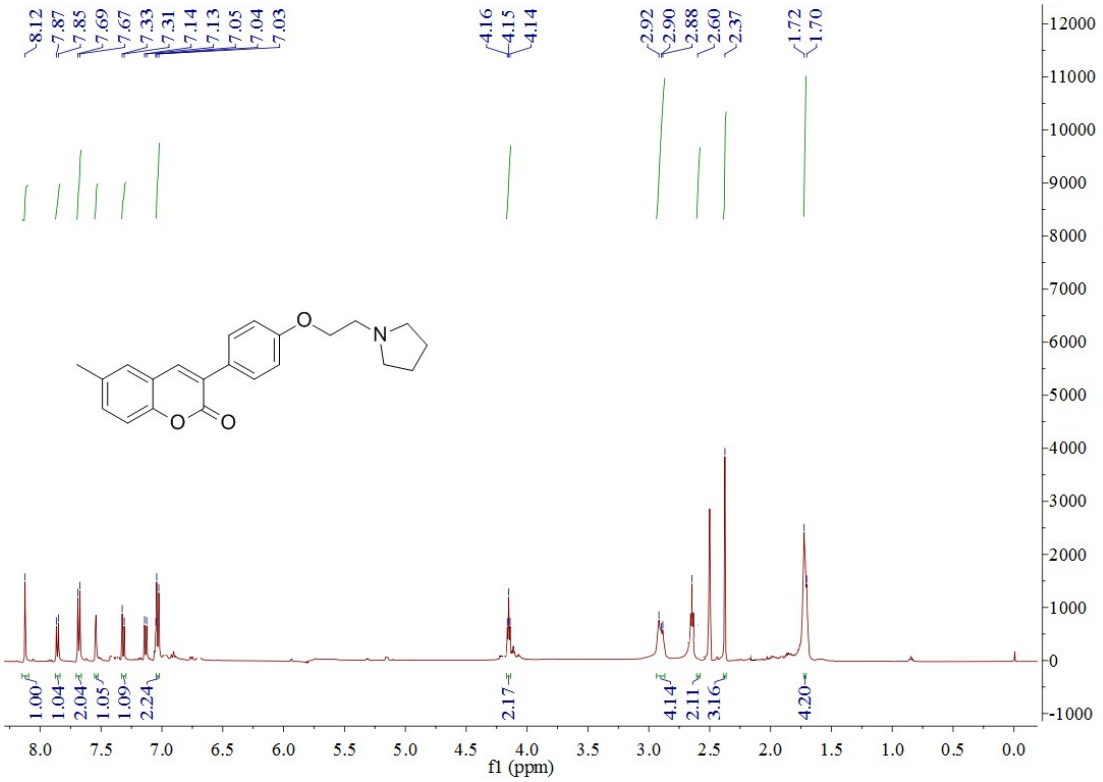
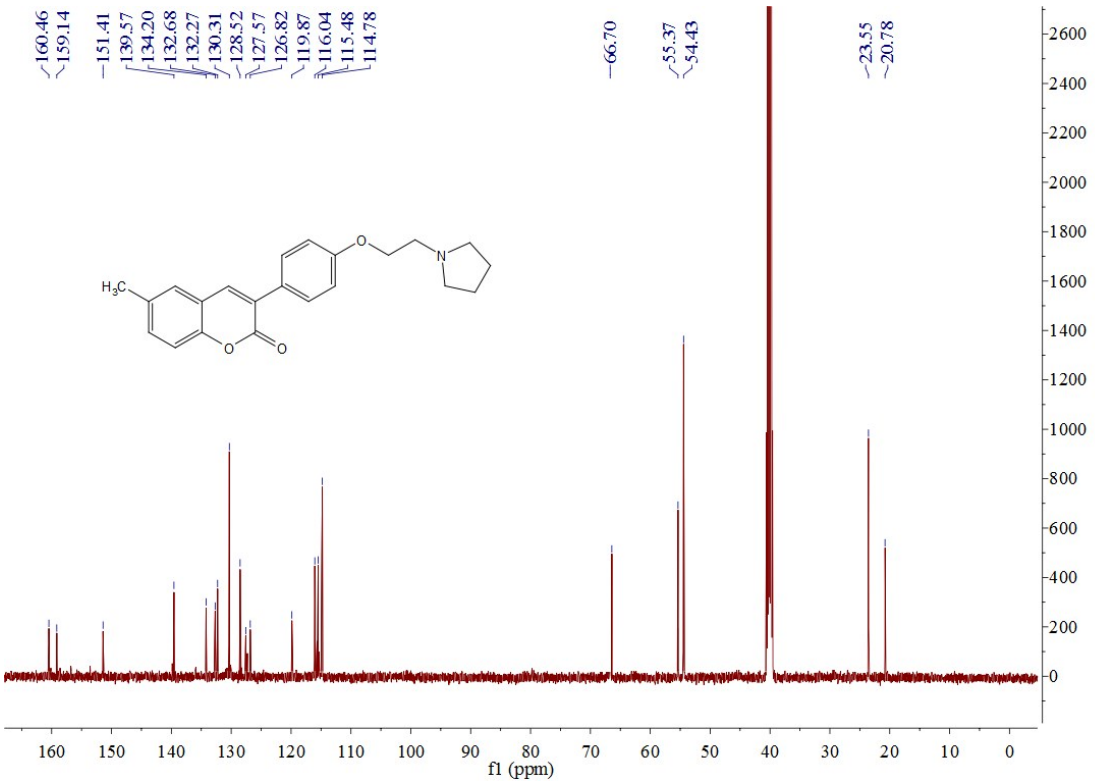


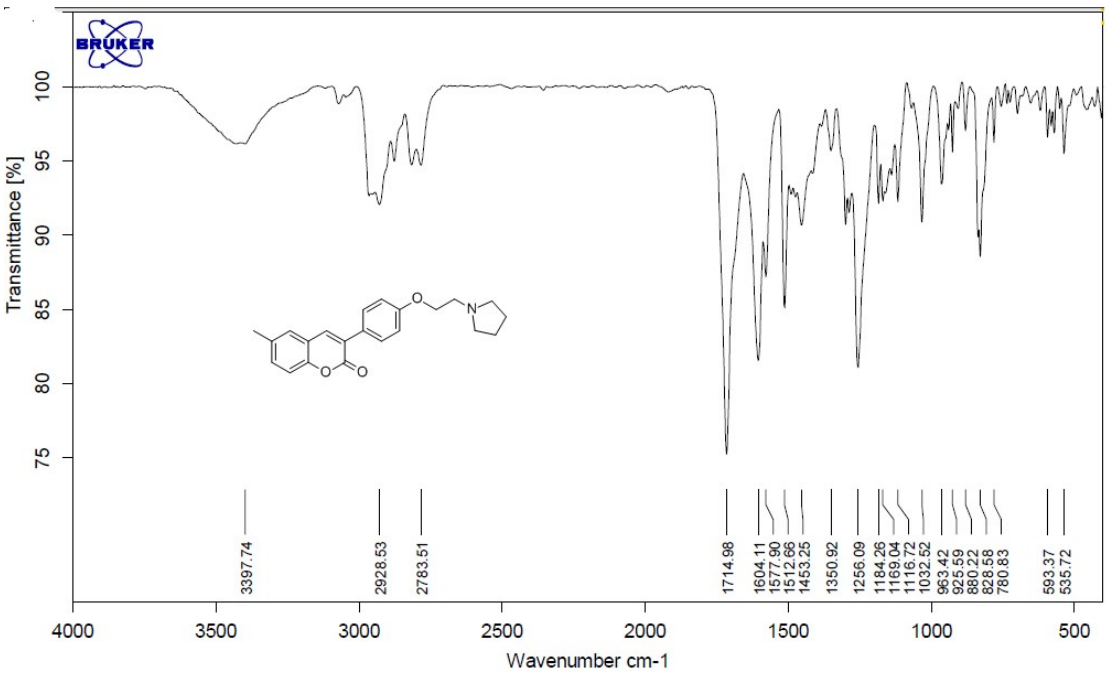
^{1}H NMR spectrum of compound 5o in DMSO



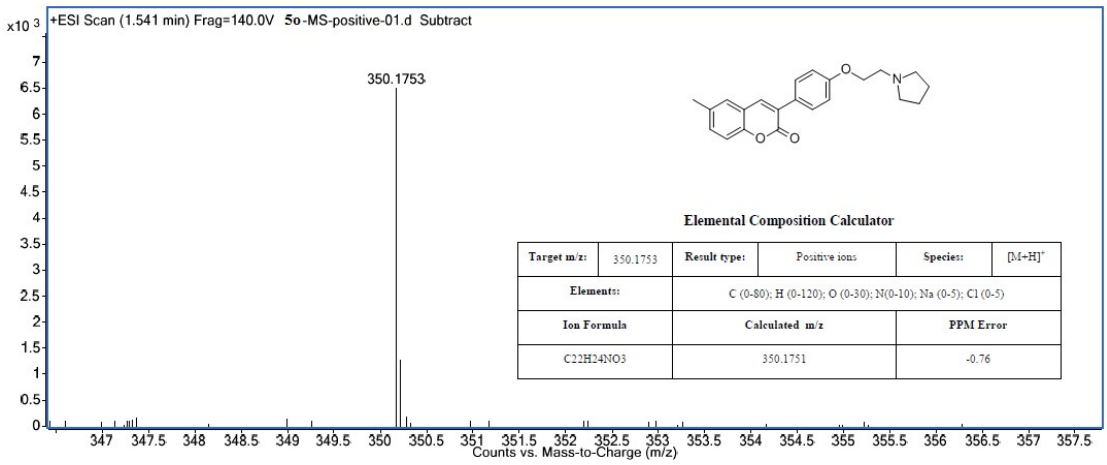
^{13}C NMR spectrum of compound 5o in DMSO



IR (KBr) spectrum of compound 5o



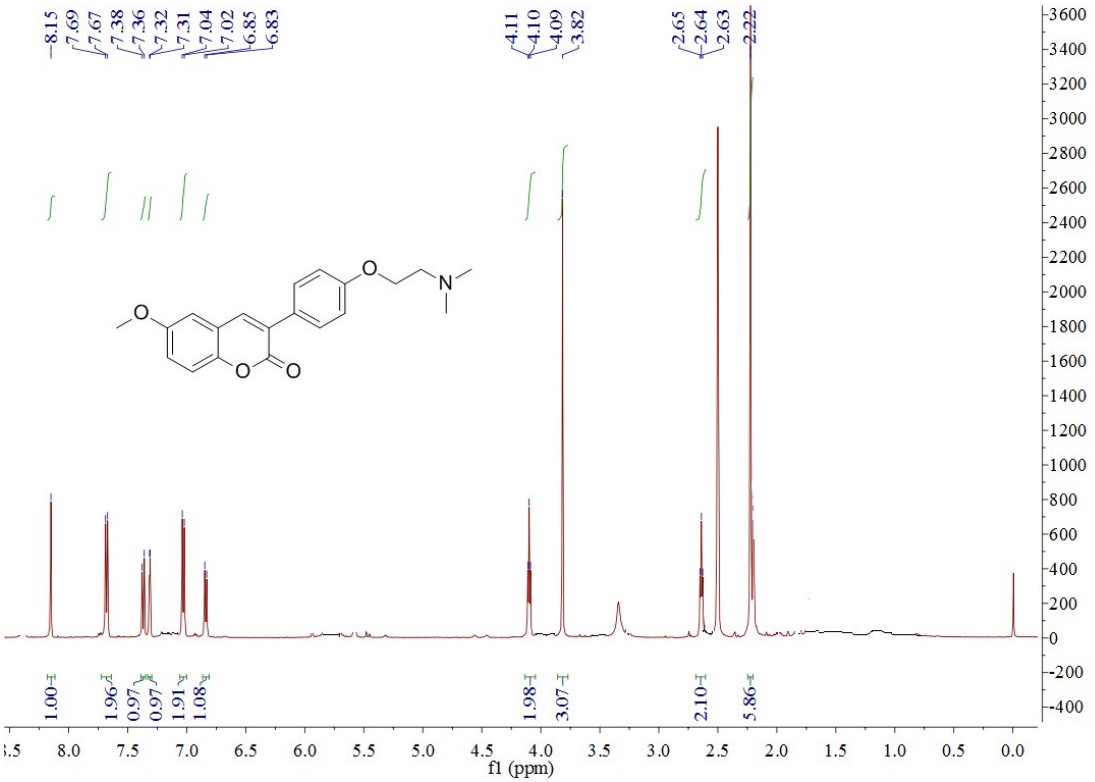
HRMS (ESI) spectrum of compound 5o



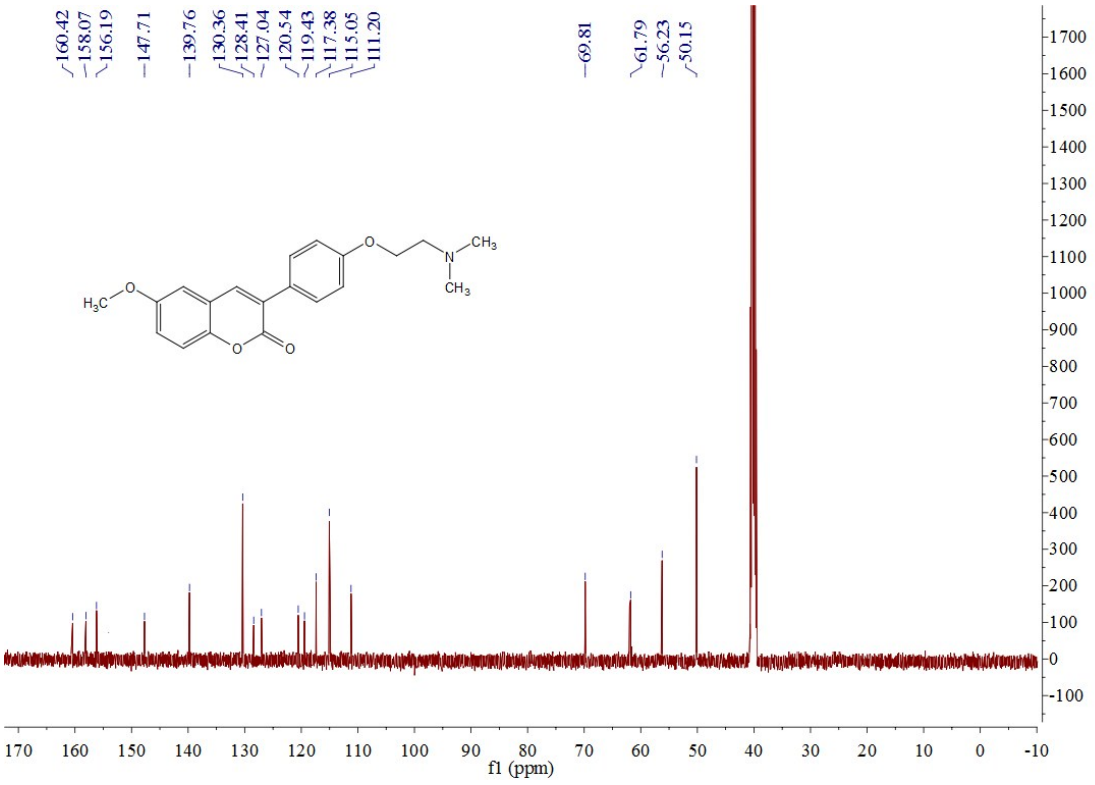
Elemental Composition Calculator

Target m/z:	350.1753	Result type:	Positive ions	Species:	$[M+H]^+$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Na (0-5); Cl (0-5)			
Ion Formula		Calculated m/z		PPM Error	
$C_{22}H_{24}NO_3$		350.1751		-0.76	

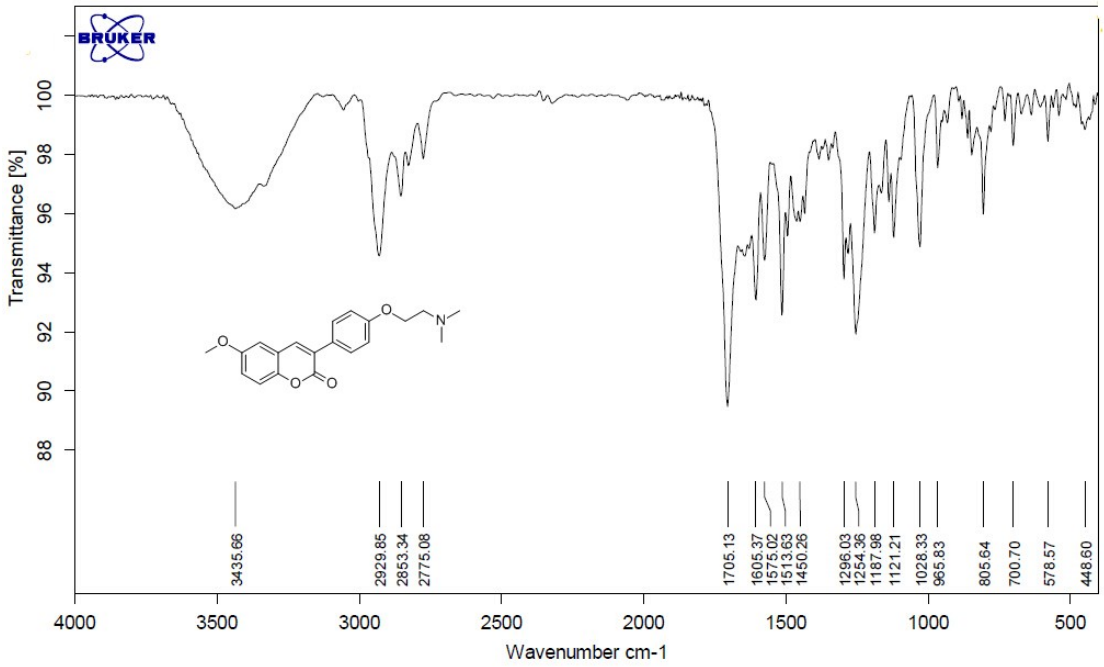
^{1}H NMR of compound 5p in DMSO



^{13}C NMR of compound 5p in DMSO



IR (KBr) spectrum of compound 5p



HRMS (ESI) spectrum of compound 5p