

SUPPLEMENTARY INFORMATION

Acridine Based *N*-Acylhydrazone Derivatives as Potential Anticancer Agents: Synthesis, Characterization and ctDNA/HSA Spectroscopic Binding Properties

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1 General information

Table S1. Physico-chemical data for compounds **3a–e** and **7b–d**.

	Name	Melting point [°C]	Color	Formula	Mass (calcd./found)	Yield [mg]	Yield [%]
3a	<i>N'</i> -[(<i>E</i>)-(Acridin-4-yl)methylidene]benzohydrazide	235–236	bright yellow	C ₂₁ H ₁₆ N ₃ O	326.1293/326.1291	141	90
3b	<i>N'</i> -[(<i>E</i>)-(Acridin-4-yl)methylidene]-4-fluorobenzohydrazide	229–230	bright yellow	C ₂₁ H ₁₅ N ₃ OF	344.1199/326.1201	135	82
3c	<i>N'</i> -[(<i>E</i>)-(Acridin-4-yl)methylidene]-4-chlorobenzohydrazide	266–267	bright yellow	C ₂₁ H ₁₅ N ₃ OCl	360.0904/360.0905	158	91
3d	<i>N'</i> -[(<i>E</i>)-(Acridin-4-yl)methylidene]-4-bromobenzohydrazide	269–270	bright yellow	C ₂₁ H ₁₅ N ₃ OBr	404.0399/404.0396	143	73
3e	<i>N'</i> -[(<i>E</i>)-(Acridin-4-yl)methylidene]-4-methoxybenzohydrazide	249–250	bright yellow	C ₂₂ H ₁₈ N ₃ O ₂	356.1399/356.1398	172	82
7b	<i>N'</i> -[(<i>E</i>)-(4-Fluorophenyl)methylidene]acridine-4-carbohydrazide	255–256	bright yellow	C ₂₁ H ₁₅ N ₃ OF	344.1199/344.1196	130	90
7c	<i>N'</i> -[(<i>E</i>)-(4-Chlorophenyl)methylidene]acridine-4-carbohydrazide	243–244	bright yellow	C ₂₁ H ₁₅ N ₃ OCl	360.0904/360.0901	121	80
7d	<i>N'</i> -[(<i>E</i>)-(4-Bromophenyl)methylidene]acridine-4-carbohydrazide	240–241	bright yellow	C ₂₁ H ₁₅ N ₃ OBr	404.0399/404.0395	140	82

Table S2. ^1H NMR (DMSO- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-3a-e}$. Chemical shifts are reported in δ (ppm) relative to DMSO- d_5 signal (2.50 ppm), multiplicities, and coupling constants (Hz) are given in parentheses.

	3a	3b	3c	3d	3e
H-2	12.25 (s)	12.26 (s)	12.30 (s)	12.30 (s)	12.12 (s)
H-4	9.93 (s)	9.91 (s)	9.92 (s)	9.92 (s)	9.90 (s)
H-1'	8.30 (d, 8.4)	8.31 (d, 8.6)	8.31 (d, 7.6)	8.30 (d, 9.2)	8.29 (d, 8.3)
H-2'	7.76 (t, 7.2)	7.76 (t, 7.7)	7.76 (t, 7.7)	7.75 (t, 7.2)	7.75 (t, 7.7)
H-3'	8.51 (d, 7.3)	8.51 (d, 6.7)	8.51 (d, 5.9)	8.50 (d, 6.3)	8.49 (d, 6.5)
H-5'	8.19 (d, 8.7)	8.19 (d, 8.5)	8.19 (d, 9.3)	8.19 (d, 8.8)	8.20 (d, 8.7)
H-6'	7.93 (ddd, 8.8, 6.5, 1.4)	7.93 (ddd, 8.5, 6.6, 1.4)	7.93 (ddd, 8.5, 6.5, 1.4)	7.93 (ddd, 8.5, 6.6, 1.4)	7.93 (ddd, 8.4, 6.5, 1.4)
H-7'	7.68 (ddd, J 8.0, 6.6, 1.1)	7.68 (ddd, 8.1, 6.5, 1.0)	7.68 (ddd, 8.1, 6.6, 1.1)	7.67 (t, 8.0)	7.68 (t, 7.5)
H-8'	8.23 (d, 8.3)	8.23 (d, 8.7)	8.23 (d, 8.6)	8.23 (d, 8.5)	8.23 (d, 8.3)
H-9'	9.22 (s)	9.23 (s)	9.23 (s)	9.22 (s)	9.22 (s)
H-2'',6''	8.04 (d, 7.1)	8.11 (d, 8.4)	8.06 (d, 8.5)	7.99 (d, 8.5)	8.03 (d, 8.4)
H-3'',5''	7.56 (t, 7.5)	7.40 (d, 8.7)	7.64 (d, 8.5)	7.78 (d, 8.5)	7.09 (d, 8.8)
H-4''	7.63 (t, 7.3)	-	-	-	
CH₃	-	-	-	-	3.86 (s)

Table S3. ^{13}C NMR (DMSO- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-3a-e}$. Chemical shifts are reported in δ (ppm) relative to DMSO- d_6 signal (39.52 ppm).

	3a	3b	3c	3d	3e
C-1	163.1	162.1	162.1	162.2	162.5
C-4	144.7	144.8	145.0	145.1	144.0
C-1'	130.5	130.6	130.6	130.6	130.4
C-2'	125.7	125.7	125.7	125.7	125.7
C-3'	126.8	126.8	126.8	126.8	126.6
C-4'	131.0	130.9	130.9	130.9	131.1
C-5'	128.8	128.8	128.8	128.8	128.8
C-6'	131.2	131.3	131.3	131.3	131.2
C-7'	126.2	126.2	126.2	126.2	126.2
C-8'	128.7	128.7	128.7	128.7	128.7
C-9'	137.1	137.1	137.1	137.1	137.1
C-8'a	126.2	126.2	126.2	126.2	126.2
C-9'a	126.2	126.2	126.2	126.2	126.2
C-4'a	146.2	146.2	146.2	146.2	146.2
C-10'a	147.9	147.9	147.9	147.9	147.9
C-1''	133.4	129.8	132.1	132.4	125.4
C-2'',6''	127.8	130.5	129.7	129.9	129.7
C-3'',5''	128.4	128.6	128.6	131.5	113.7
C-4''	131.8	164.2	136.6	125.6	162.0
CH₃	-	-	-	-	55.4

Table S4. ^{15}N NMR (DMSO- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-3a-e}$. Chemical shifts are reported in δ (ppm) relative to external CH_3NO_2 (0.00 ppm).

	3a	3b	3c	3d	3e
N-2	-206.0	-206.3	-206.0	-206.0	-207.3
N-3	-57.3	-57.6	-57.9	-58.0	-56.6
N-10'	-83.3	nd	nd	nd	-83.3

Table S5. ^1H NMR (acetone- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-7b-d}$. Chemical shifts are reported in δ (ppm) relative to acetone- d_5 signal (2.50 ppm), multiplicities, and coupling constants (Hz) are given in parentheses.

	Z_{N-N-7b}	E_{N-N-7b}	Z_{N-N-7c}	E_{N-N-7c}	Z_{N-N-7d}	E_{N-N-7d}
H-2	14.98 (s)	14.98 (s)	15.00 (s)	15.00 (s)	15.05 (s)	15.05 (s)
H-4	8.88 (s)	8.86 (s)	8.85 (s)	8.83 (s)	8.85 (s)	8.84 (s)
H-1'	8.44 (dd, 8.3, 1.6)	8.44 (dd, 8.3, 1.6)	8.41 (dd, 8.3, 1.6)	8.41 (dd, 8.3, 1.6)	8.44 (dd, 8.3, 1.6)	8.44 (dd, 8.3, 1.6)
H-2'	7.82 (dd, 8.3, 7.1)	7.82 (dd, 8.3, 7.1)	7.80 (dd, 8.3, 7.1)	7.80 (dd, 8.3, 7.1)	7.82 (dd, 8.3, 7.1)	7.82 (dd, 8.3, 7.1)
H-3'	9.00 (d, 7.0)	9.00 (d, 7.0)	8.98 (d, 7.0)	8.98 (d, 7.0)	9.00 (d, 7.0)	9.00 (d, 7.0)
H-5'	8.49 (d, 8.8)	8.49 (d, 8.8)	8.46 (d, 8.8)	8.46 (d, 8.8)	8.49 (d, 8.5)	8.49 (d, 8.5)
H-6'	8.02 (m)	8.02 (m)	7.99 (ddd, 8.4, 6.6, 1.2)	7.99 (ddd, 8.4, 6.6, 1.2)	8.01 (ddd, 8.4, 6.6, 1.2)	8.01 (ddd, 8.4, 6.6, 1.2)
H-7'	7.75 (ddd, 8.0, 6.6, 1.0)	7.75 (ddd, 8.0, 6.6, 1.0)	7.73 (ddd, 8.4, 6.6, 1.2)	7.73 (ddd, 8.4, 6.6, 1.2)	7.74 (ddd, 8.4, 6.6, 1.2)	7.74 (ddd, 8.4, 6.6, 1.2)
H-8'	8.27 (d, 8.5)	8.27 (d, 8.5)	8.24 (d, 8.5)	8.24 (d, 8.5)	8.26 (d, 8.5)	8.26 (d, 8.5)
H-9'	9.32 (s)	9.32 (s)	9.28 (s)	9.28 (s)	9.32 (s)	9.32 (s)
H-2'',6''	7.99 (dd, 8.8, 5.6)	7.99 (dd, 8.8, 5.6)	7.94 (d, 8.5)	7.94 (d, 8.5)	7.88 (d, 8.5)	7.88 (d, 8.5)
H-3'',5''	7.27 (t, 8.7)	7.27 (t, 8.7)	7.52 (d, 8.5)	7.52 (d, 8.5)	7.69 (d, 8.5)	7.69 (d, 8.5)

Table S6. ^{13}C NMR (acetone- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-7b-d}$. Chemical shifts are reported in δ (ppm) relative to acetone- d_6 signal (39.52 ppm).

	Z_{N-N-7b}	E_{N-N-7b}	Z_{N-N-7c}	E_{N-N-7c}	Z_{N-N-7d}	E_{N-N-7d}
C-1	162.6	162.5	162.6	162.7	162.5	162.6
C-4	147.7	147.7	147.6	147.5	147.6	147.6
C-1'	134.4	134.4	134.5	134.4	134.5	134.5
C-2'	126.4	126.4	126.3	126.3	126.3	126.4
C-3'	136.8	136.8	136.8	136.8	136.9	136.9
C-4'	128.9	128.9	128.8	128.8	128.8	128.8
C-5'	129.6	129.6	129.6	129.6	129.6	129.6
C-6'	132.9	133.0	132.9	133.0	132.9	133.0
C-7'	127.6	127.6	127.6	127.6	127.6	127.6
C-8'	129.5	129.5	129.4	129.4	129.4	129.4
C-9'	140.0	140.0	140.0	140.0	140.0	140.0
C-8'a	127.2	127.2	127.2	127.2	127.2	127.2
C-9'a	128.0	128.0	127.9	127.9	127.9	127.9
C-4'a	146.7	146.7	146.6	146.6	146.6	146.6
C-10'a	148.5	148.5	148.5	148.4	148.5	148.4
C-1''	132.7	132.7	135.1	135.1	135.5	135.5
C-2'',6''	130.3	130.3	129.8	129.8	130.0	130.0
C-3'',5''	116.5	116.5	129.8	129.8	132.7	132.7
C-4''	164.7	164.7	136.0	136.0	124.3	124.3

Table S7. ^{15}N NMR (acetone- d_6) data for compounds $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-7b-d}$. Chemical shifts are reported in δ (ppm) relative to external CH_3NO_2 (0.00 ppm).

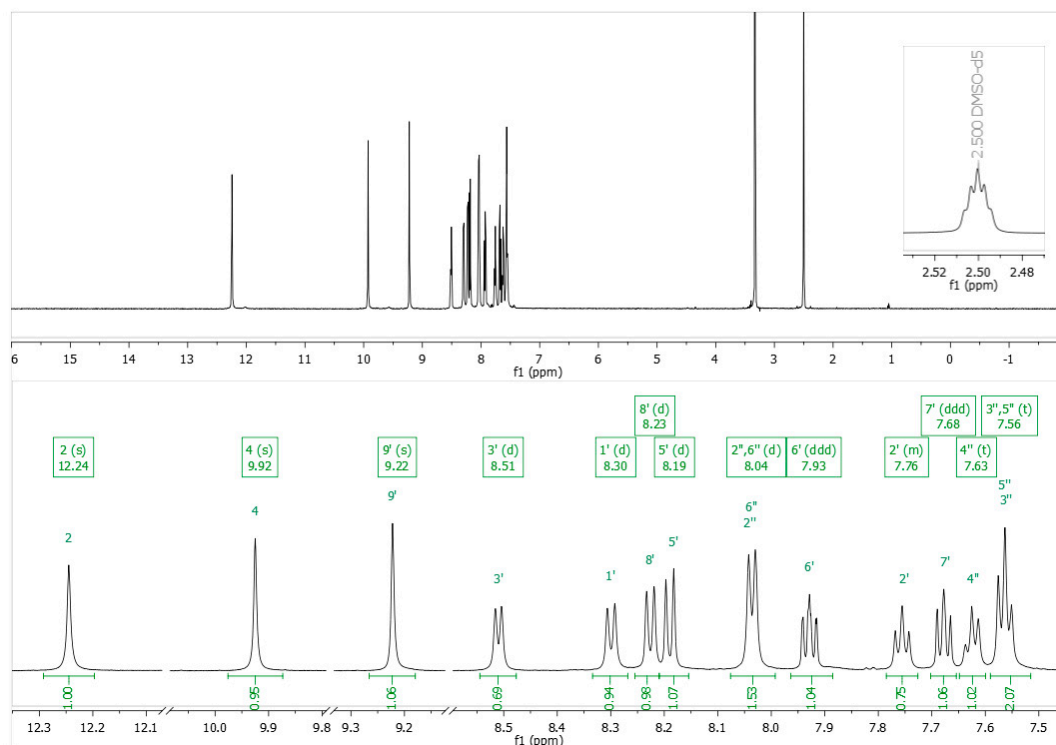
	$Z_{\text{N-N-7b}}$	$E_{\text{N-N-7b}}$	$Z_{\text{N-N-7c}}$	$E_{\text{N-N-7c}}$	$Z_{\text{N-N-7d}}$	$E_{\text{N-N-7d}}$
N-2	-200.6	-199.5	-200.0	-199.0	-199.6	-198.9
N-3	-60.2	-59.7	-57.9	-57.3	-57.5	-57.2
N-10'	-95.9	-95.9	-96.3	-96.3	-96.3	-96.3

Table S8. Selected heteronuclear spin-spin coupling constants for derivatives $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-3a-e}$ (DMSO- d_6) and $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}\text{-7b-d}$ (acetone- d_6).

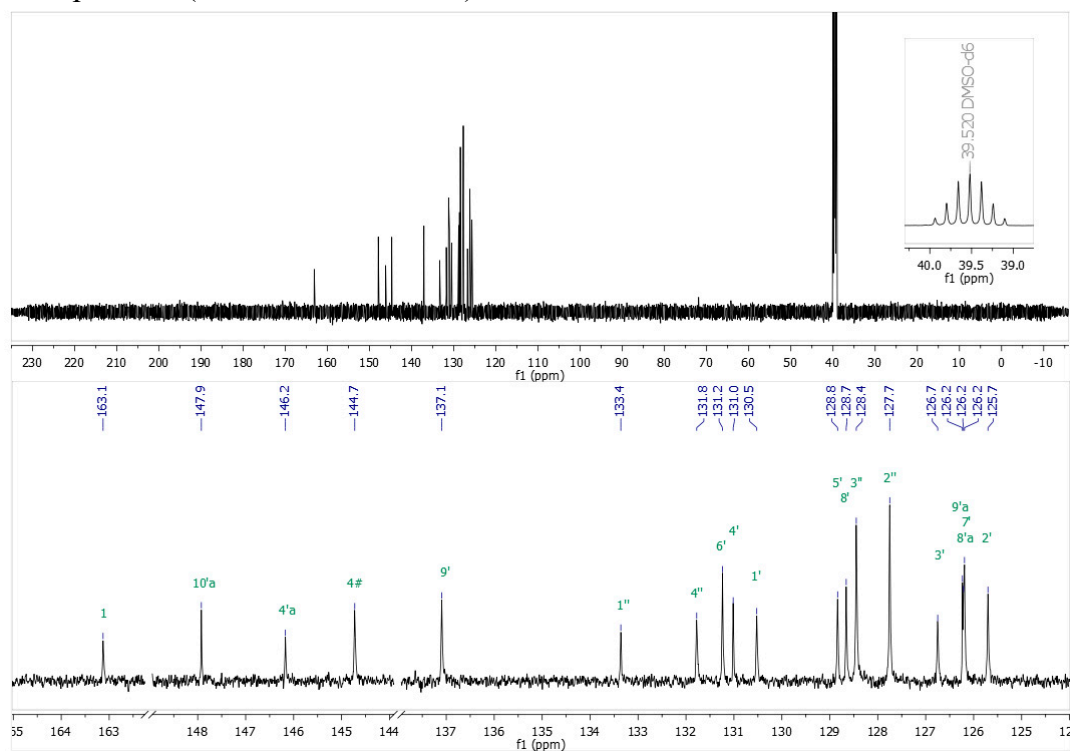
J [Hz]	3a	3b	3c	3d	3e	7b	7c	7d
$^1J_{\text{C4H4}}$	168.0	168.0	168.0	168.6	167.4	162.6	162.6	-
$^2J_{\text{C1H2}}$	10.8	-	11.4	-	-	-	-	-
$^1J_{\text{N2H2}}$	93.0	93.6	93.0	94.8	93.6	91.8	91.8	91.8

2 NMR spectra of derivatives 3a–e and 7b–d.

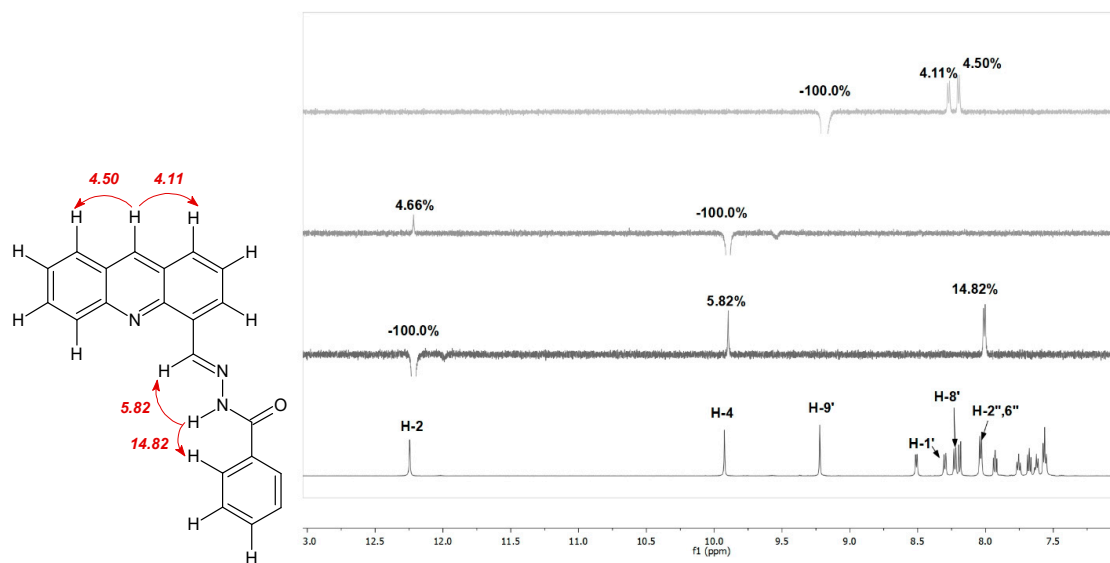
1.1 *N'*-[(*E*)-Acridin-4-yl)methylidene]benzohydrazide (3a)



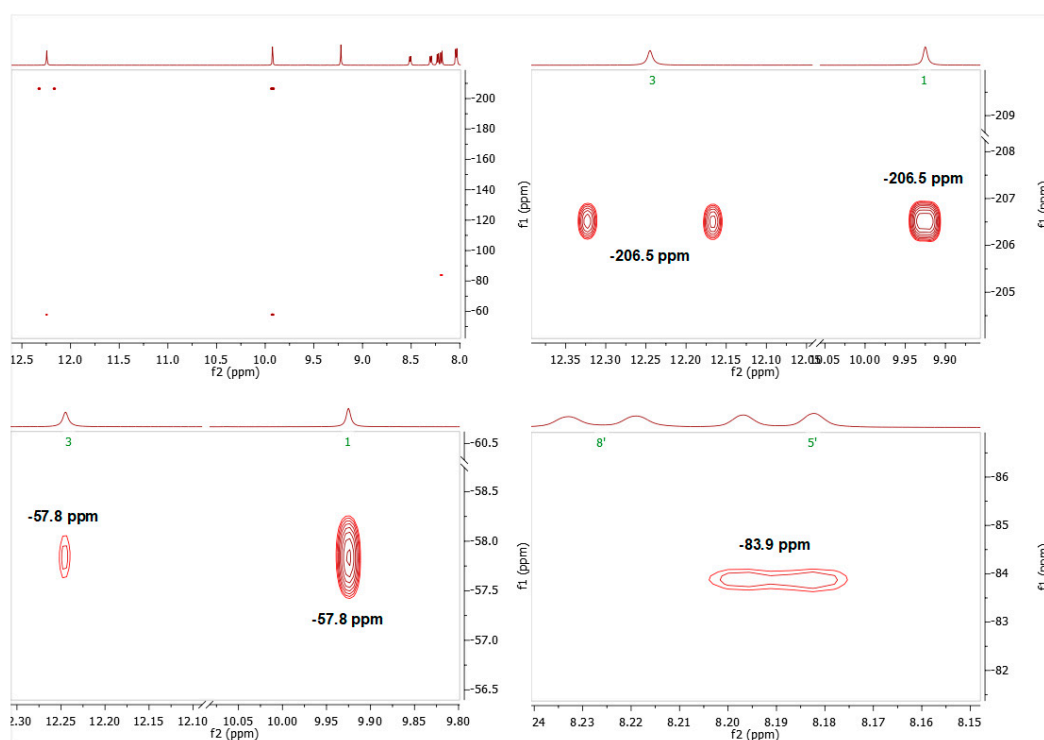
¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **3a**.



¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **3a**.

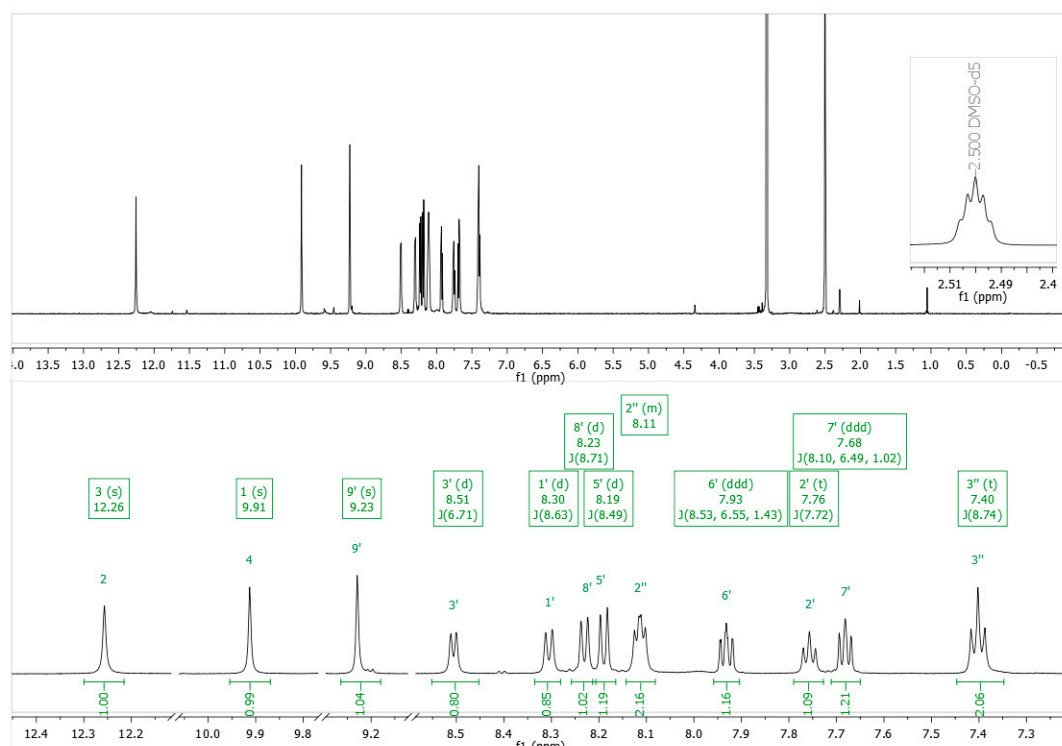


1D NOESY spectra of derivative $E_{C=N}Z_{C(O)-N}$ -**3a**.

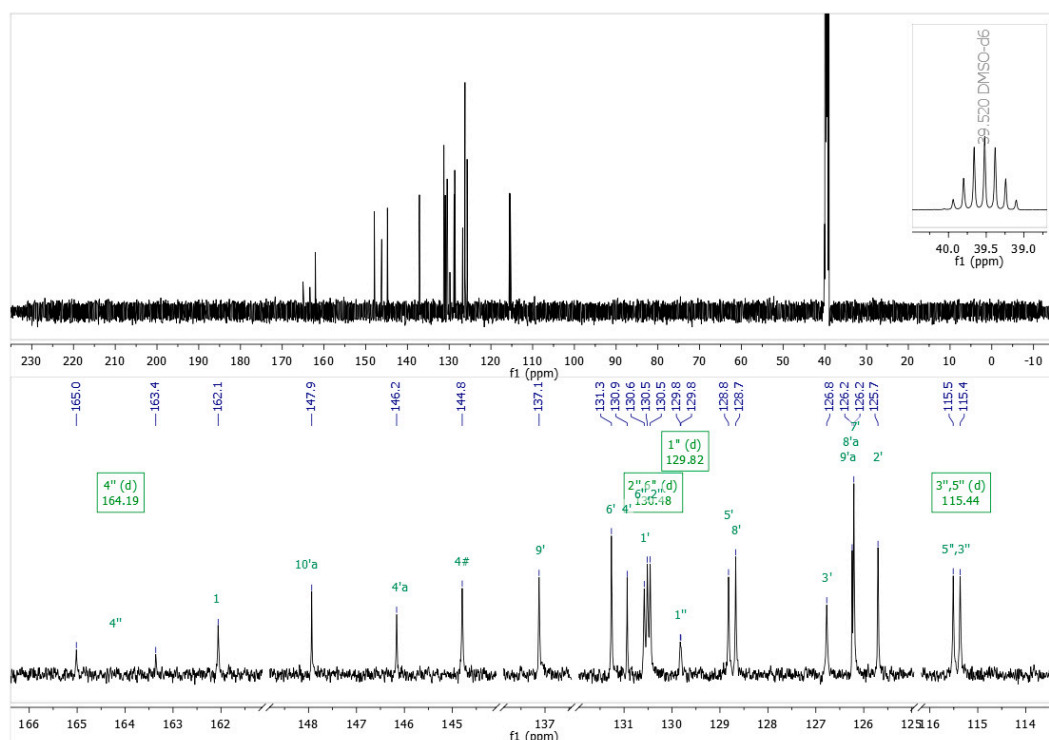


1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **3a**.

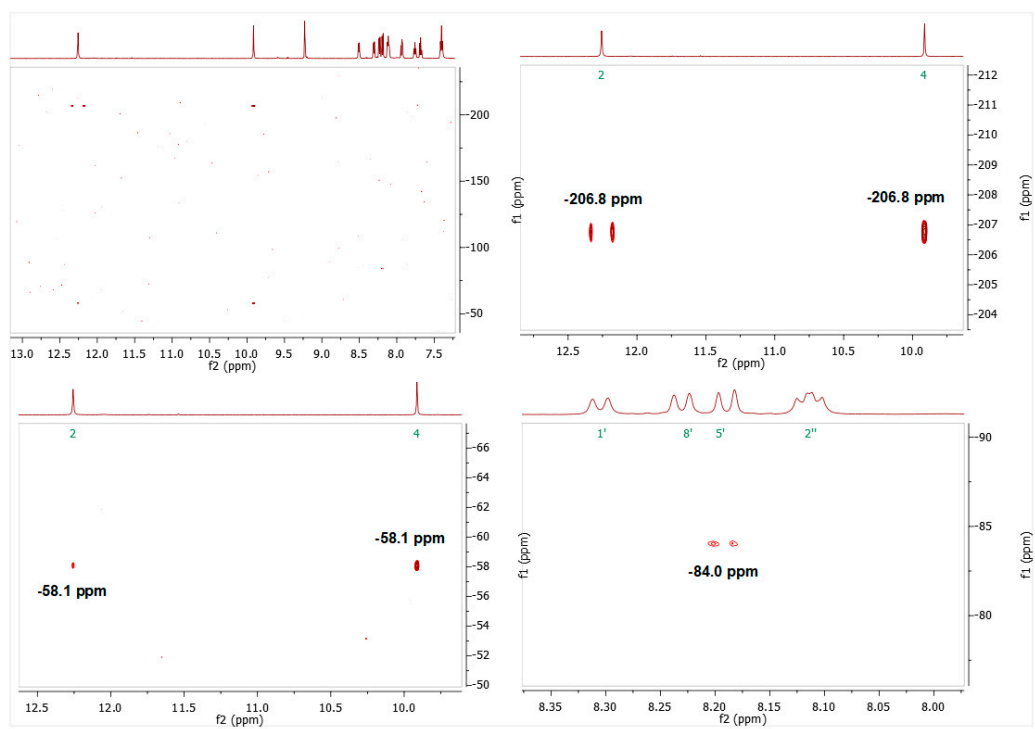
1.2 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-fluorobenzohydrazide (**3b**)



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **3b**.

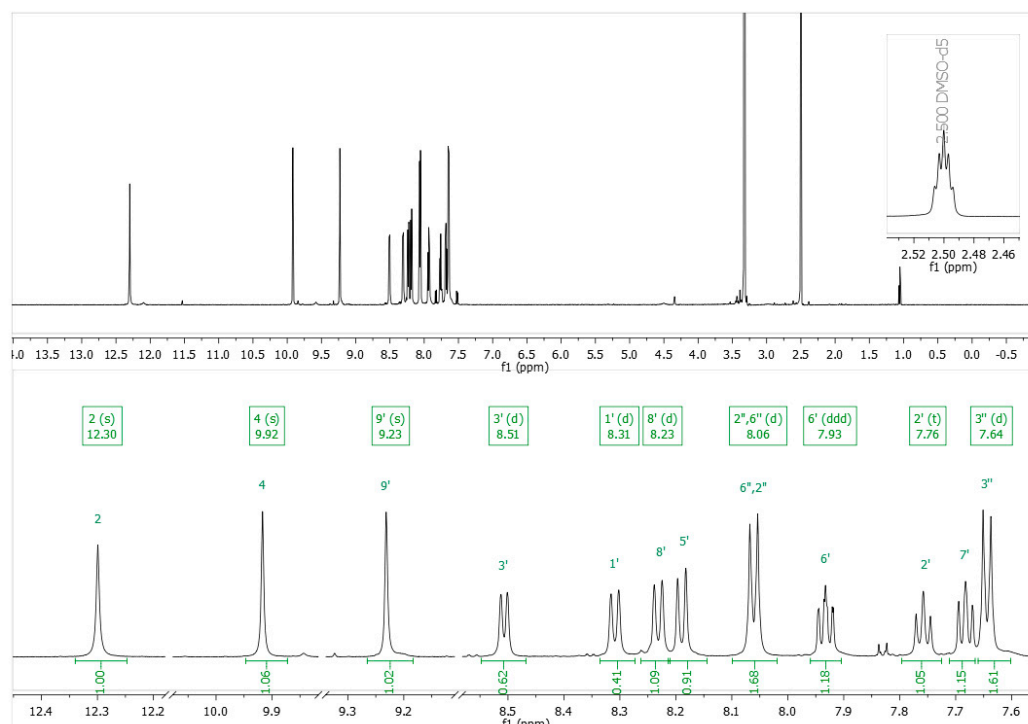


¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **3b**.

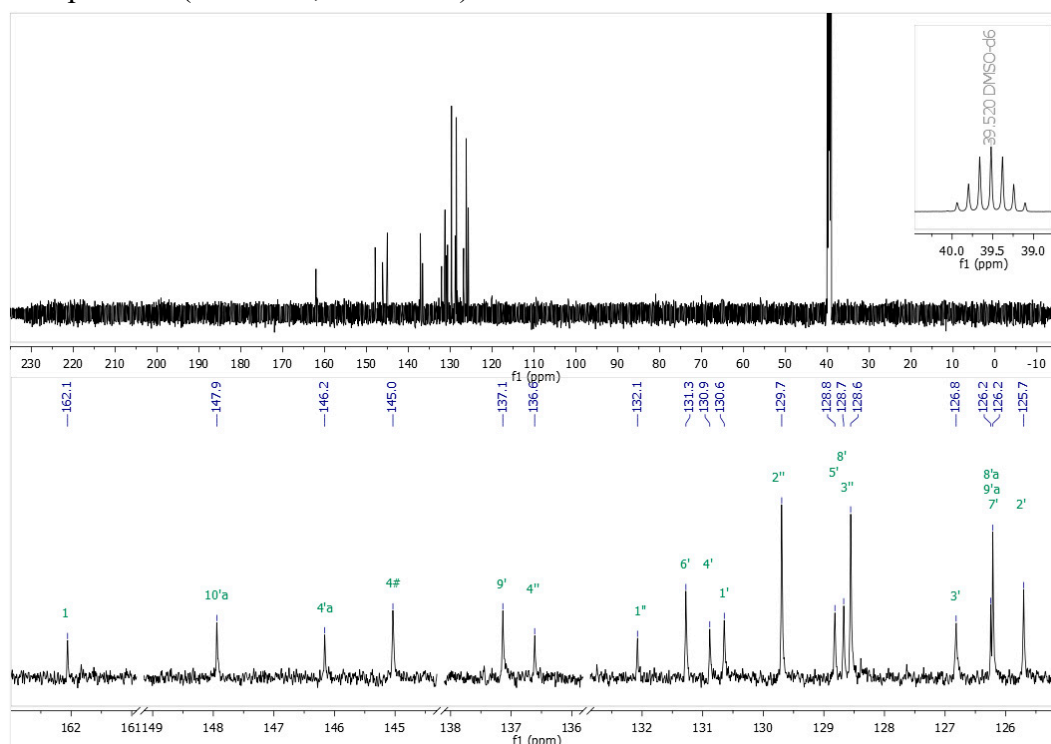


^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **3b**.

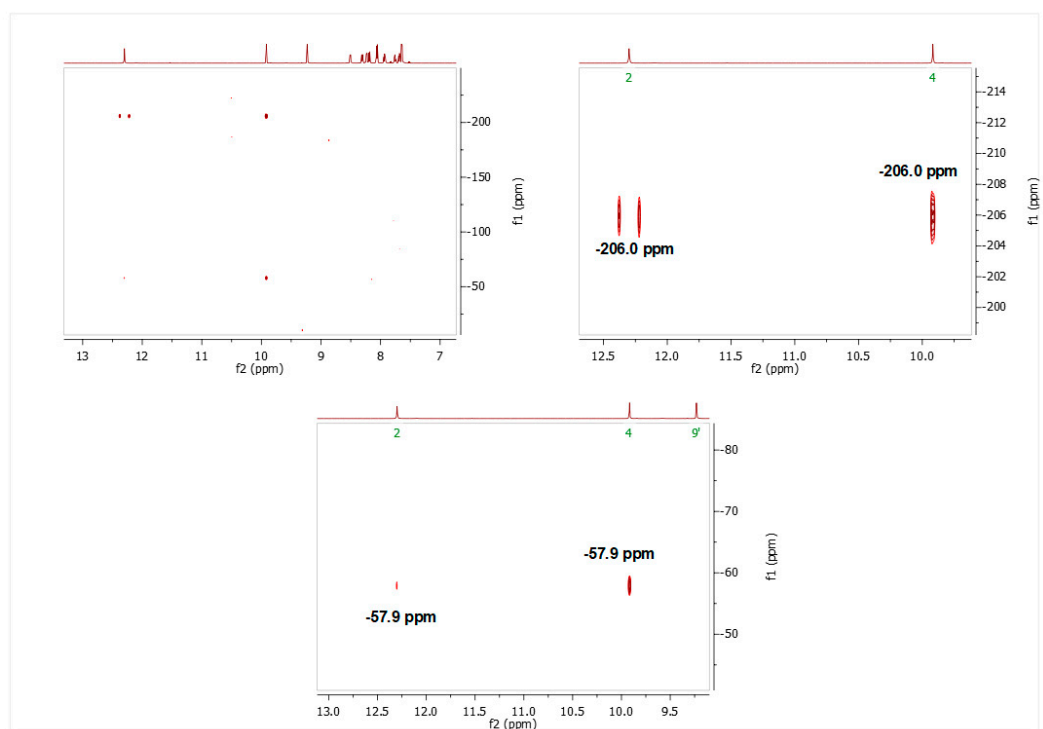
1.3 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-chlorobenzohydrazide (**3c**)



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **3c**.

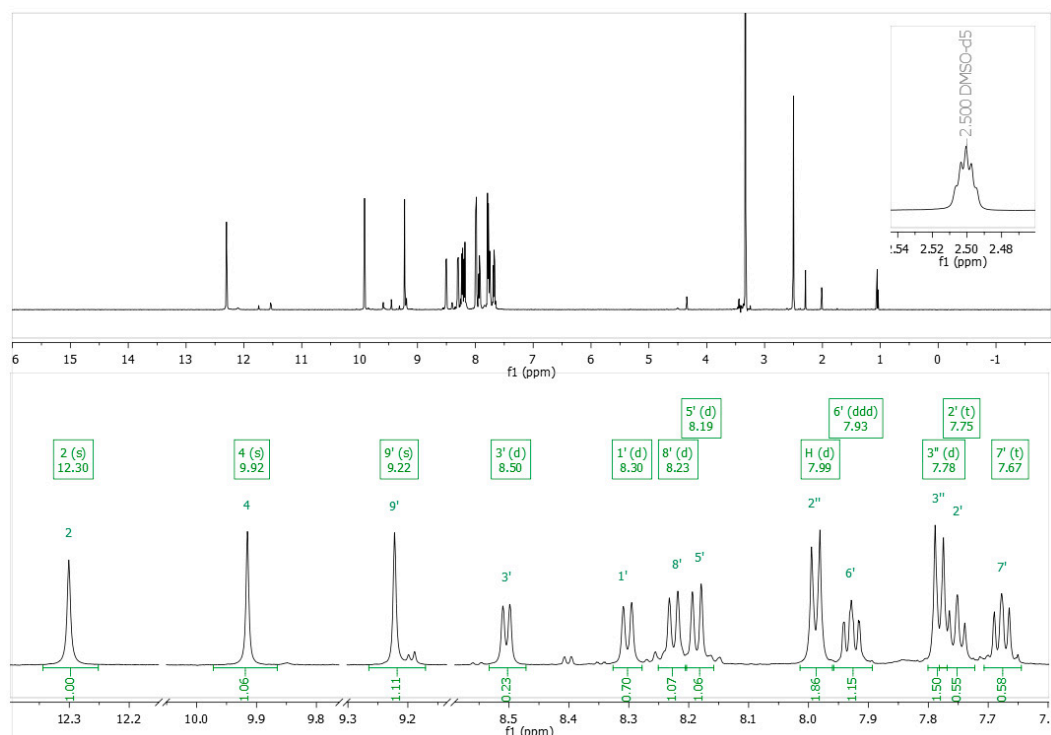


¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **3c**.

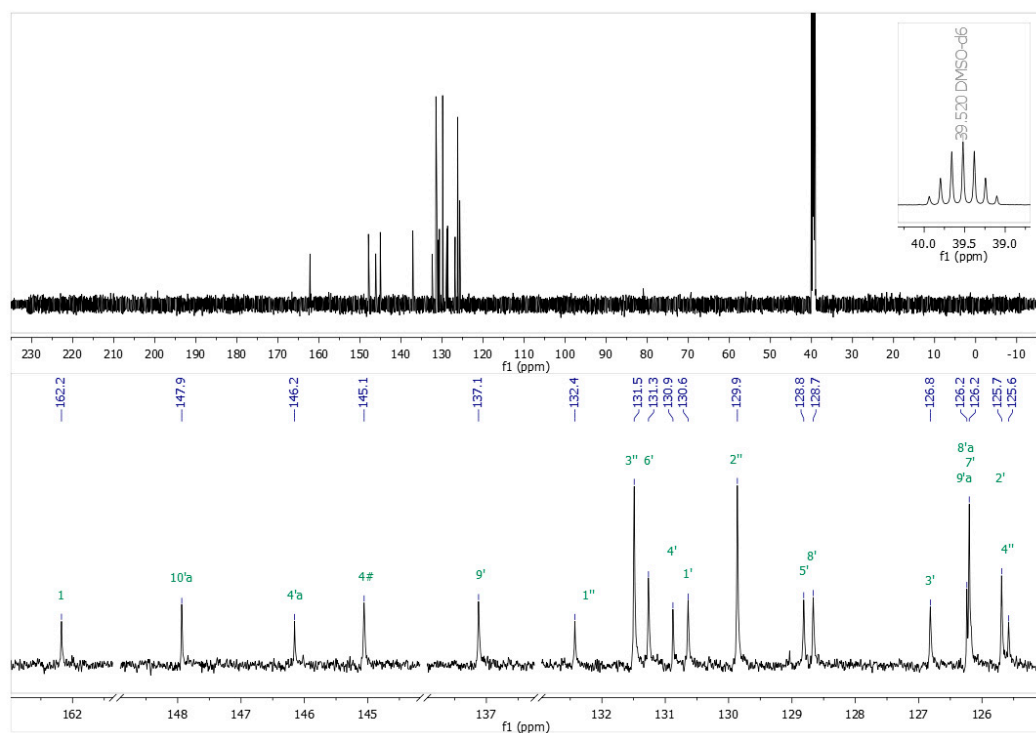


^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO-d_6) of the derivative **3c**.

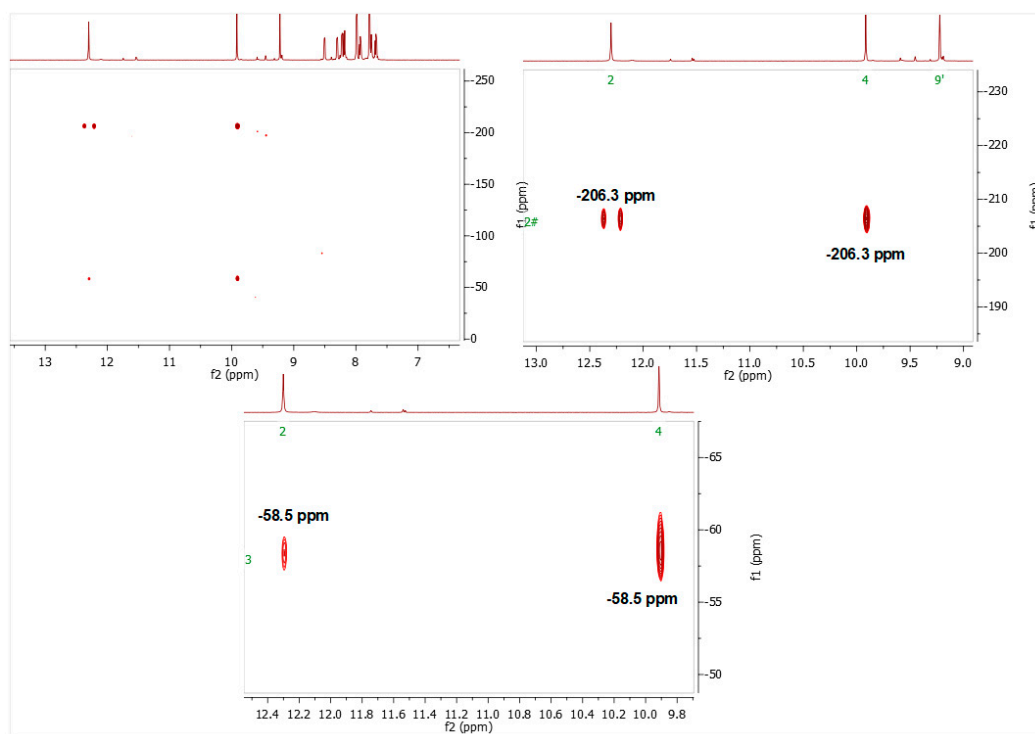
1.4 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-bromobenzohydrazide (**3d**)



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **3d**.

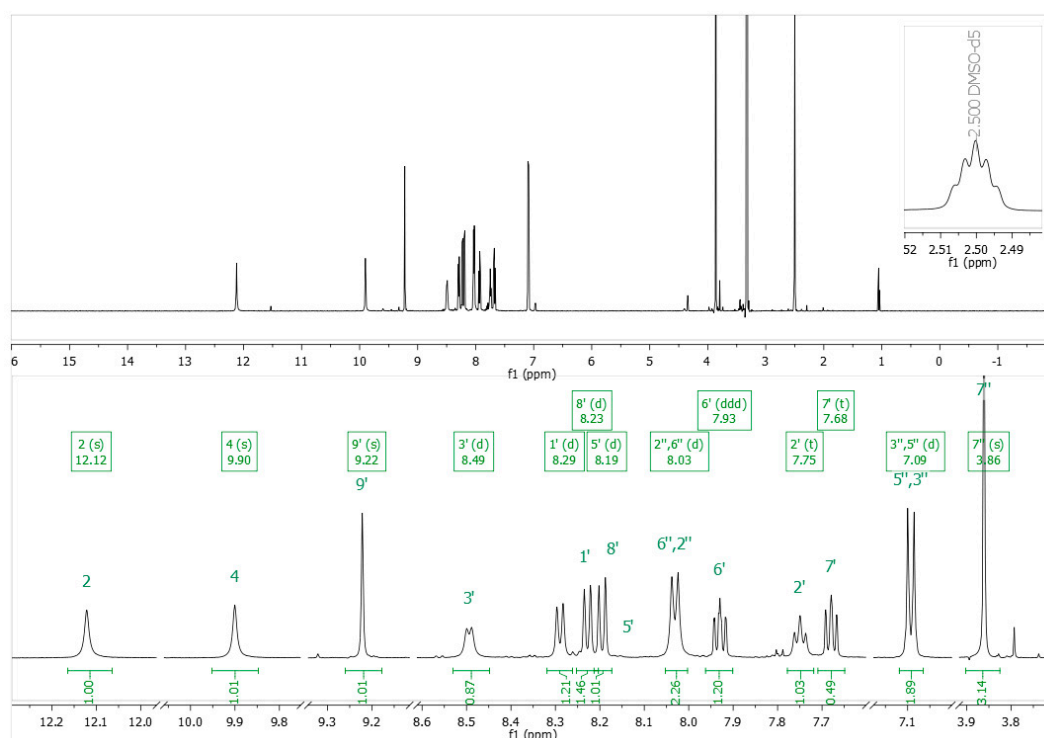


¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **3d**.

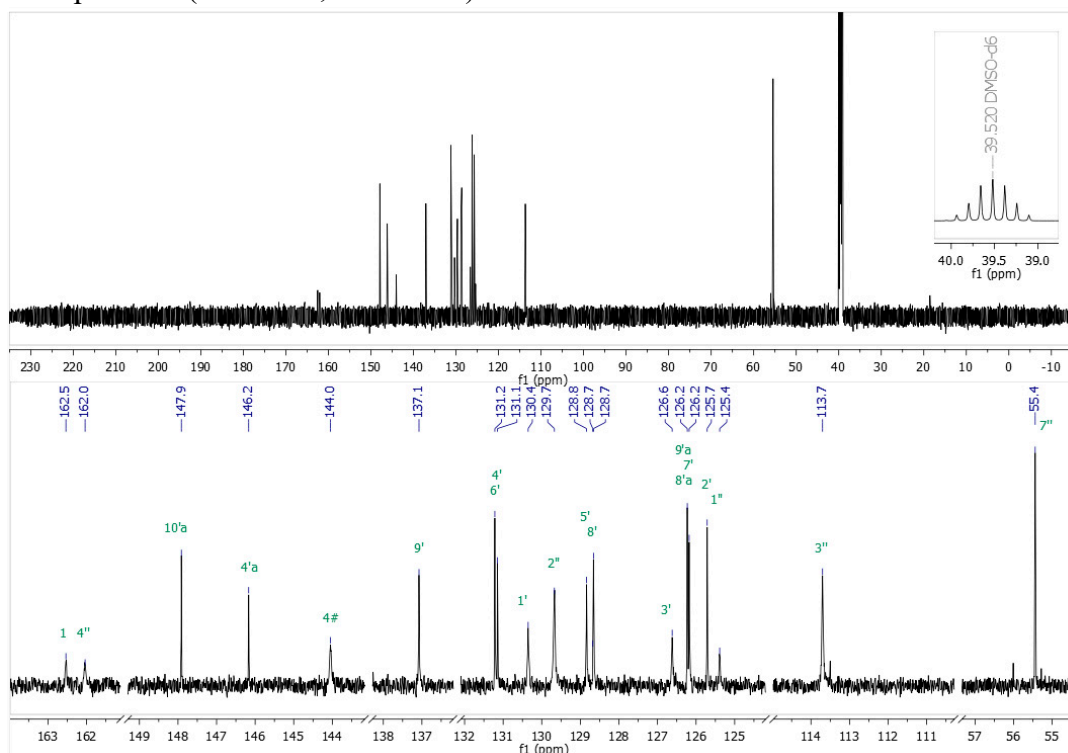


^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO-d_6) of the derivative **3d**.

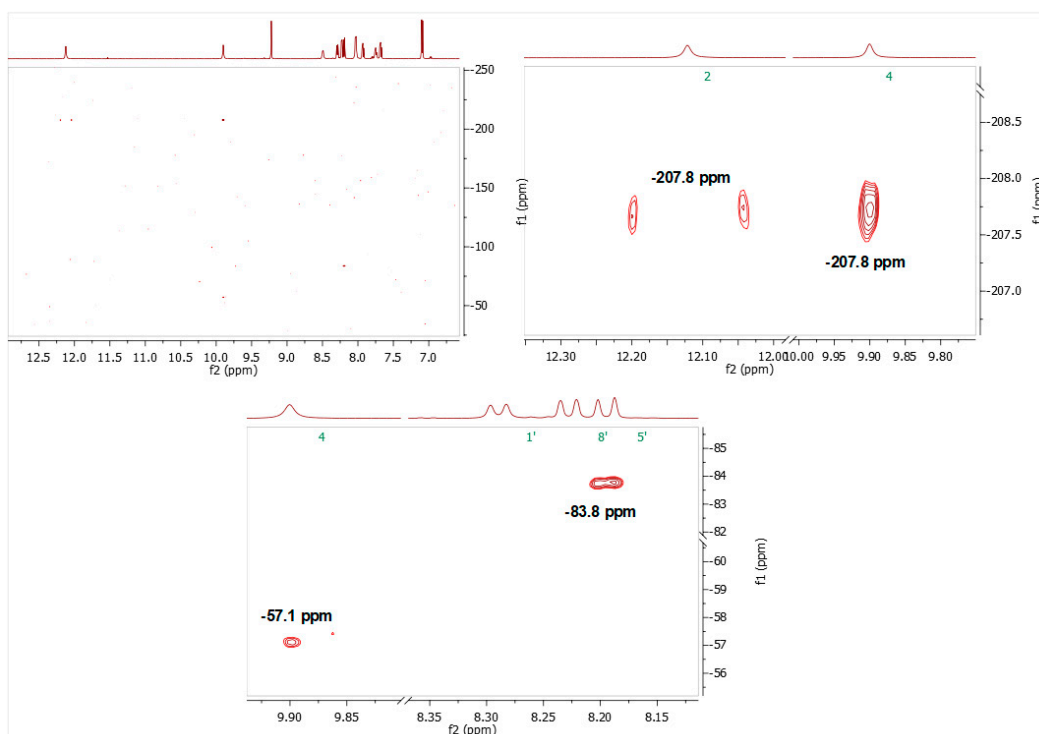
1.5 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-methoxybenzohydrazide (**3e**)



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **3e**.

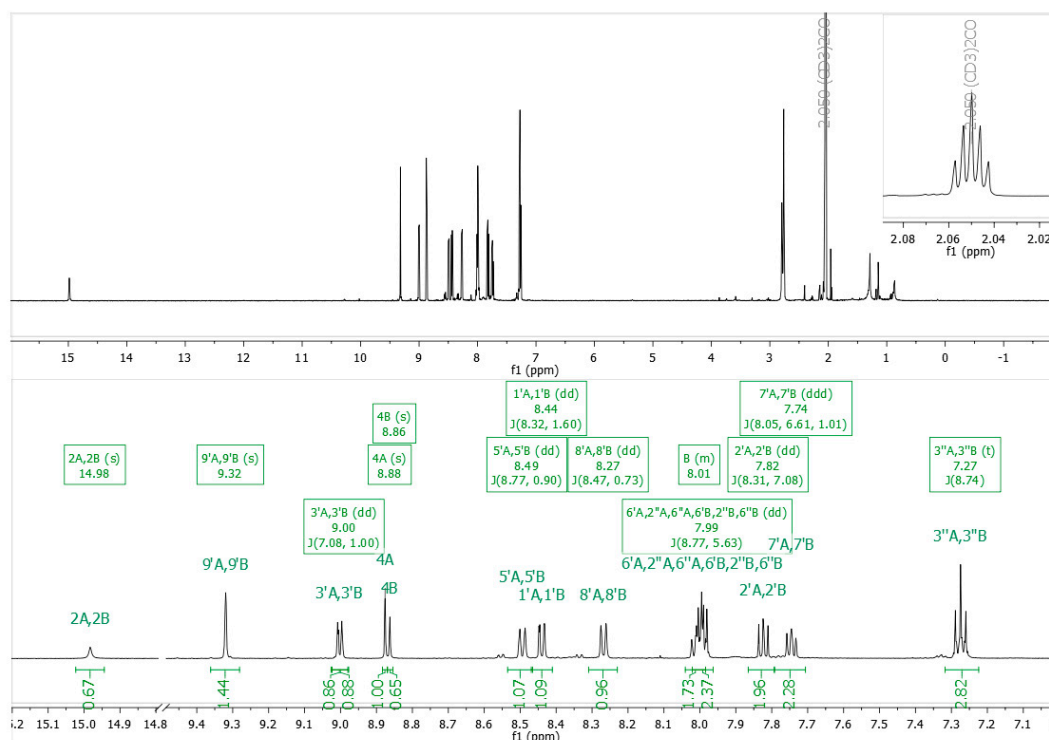


¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **3e**.

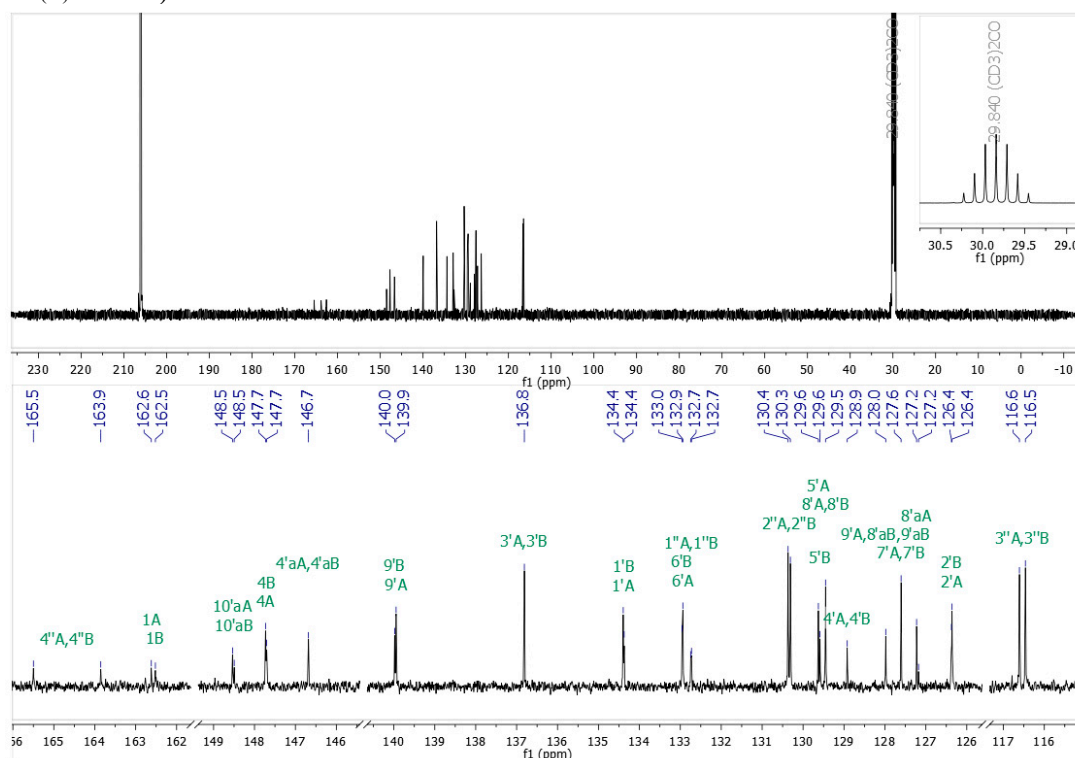


^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **3e**.

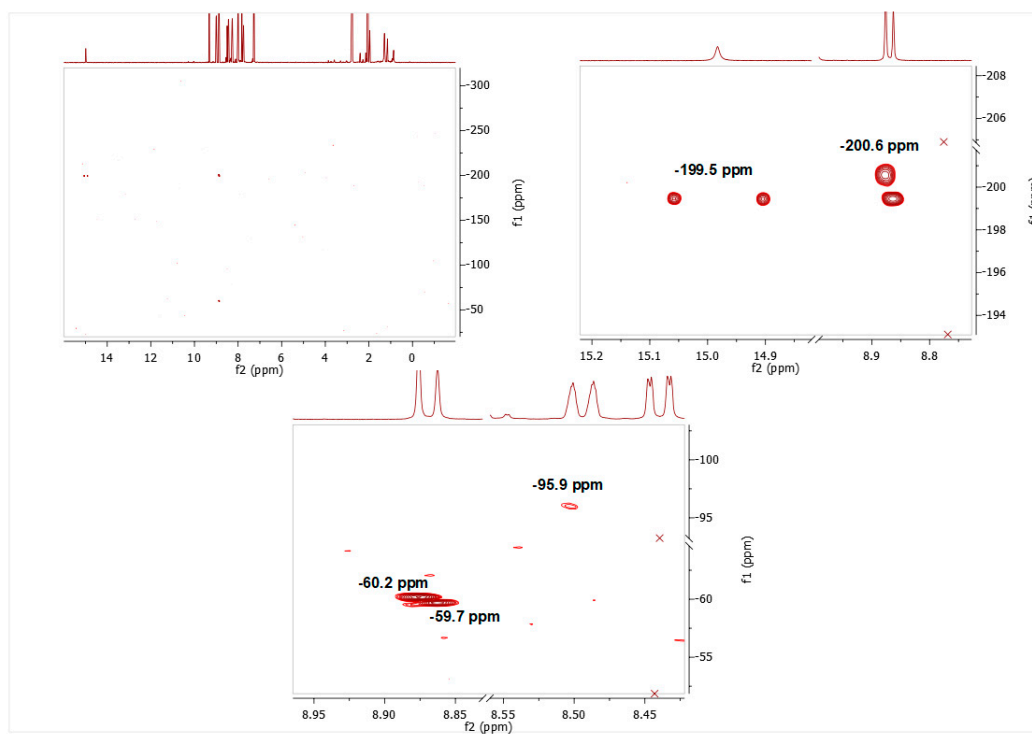
1.6 *N'*-[(*E*)-4-fluorophenylmethylidene]acridine-4-carbohydrazide **7b**



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **7b** (A: $E_C=NZC(O)-NZN-N$, B: $E_C=NZC(O)-NE_N-N$).

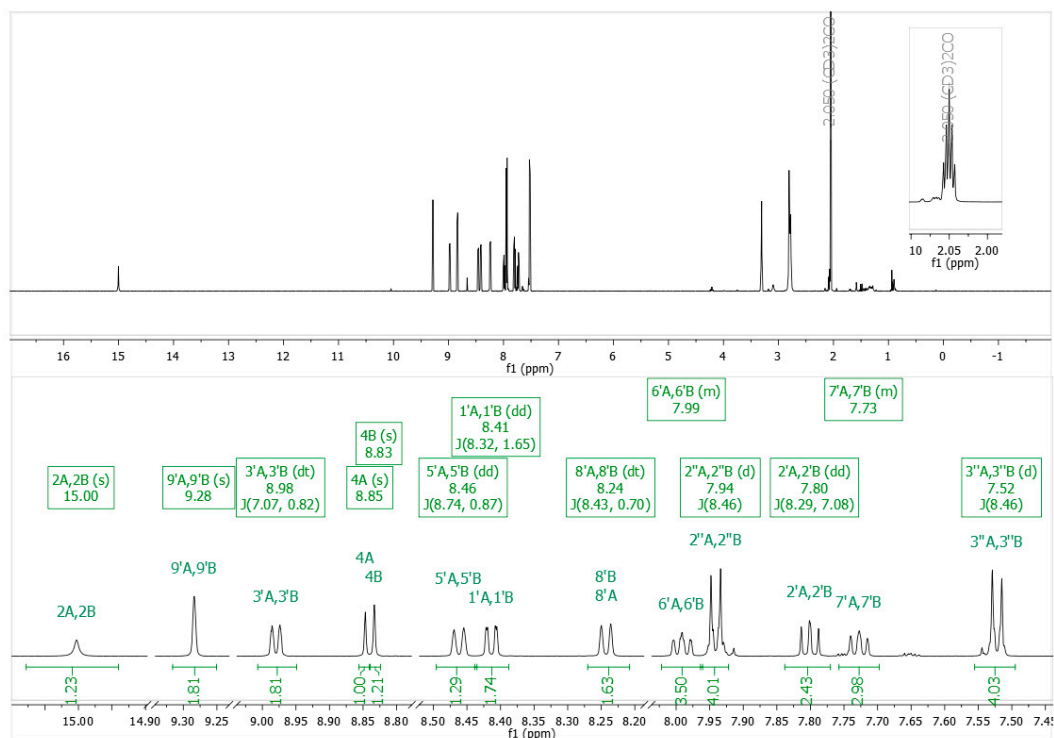


¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **7b** (A: $E_C=NZC(O)-NZN-N$, B: $E_C=NZC(O)-NE_N-N$).

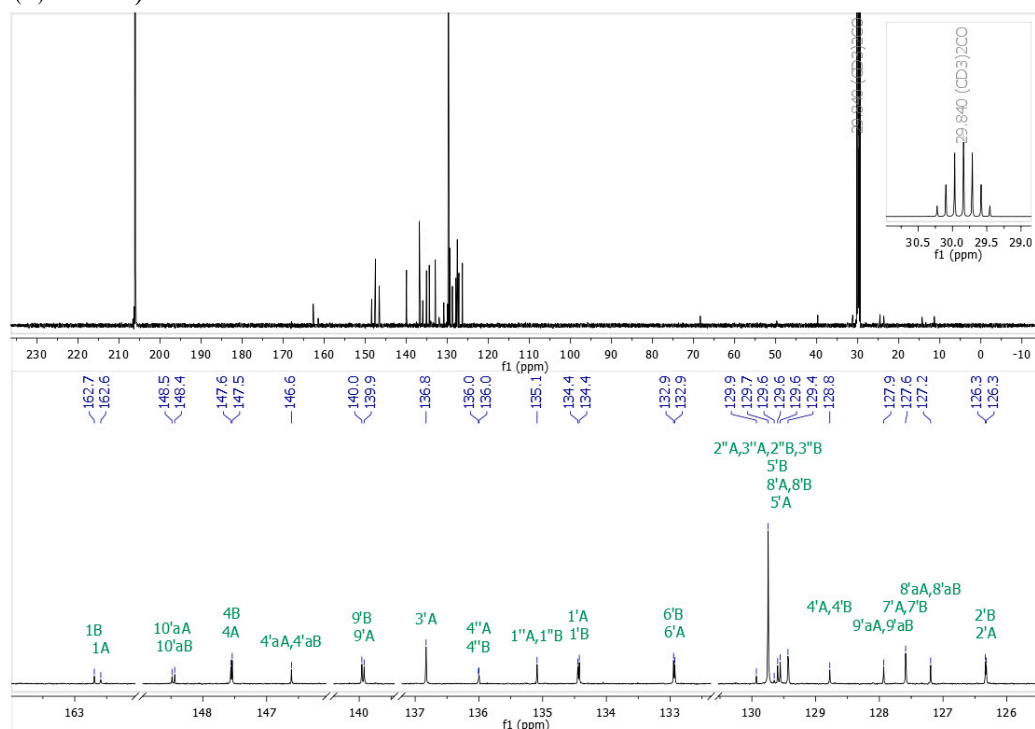


^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **7b** (A: $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}Z_{\text{N}-\text{N}}$, B: $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}E_{\text{N}-\text{N}}$).

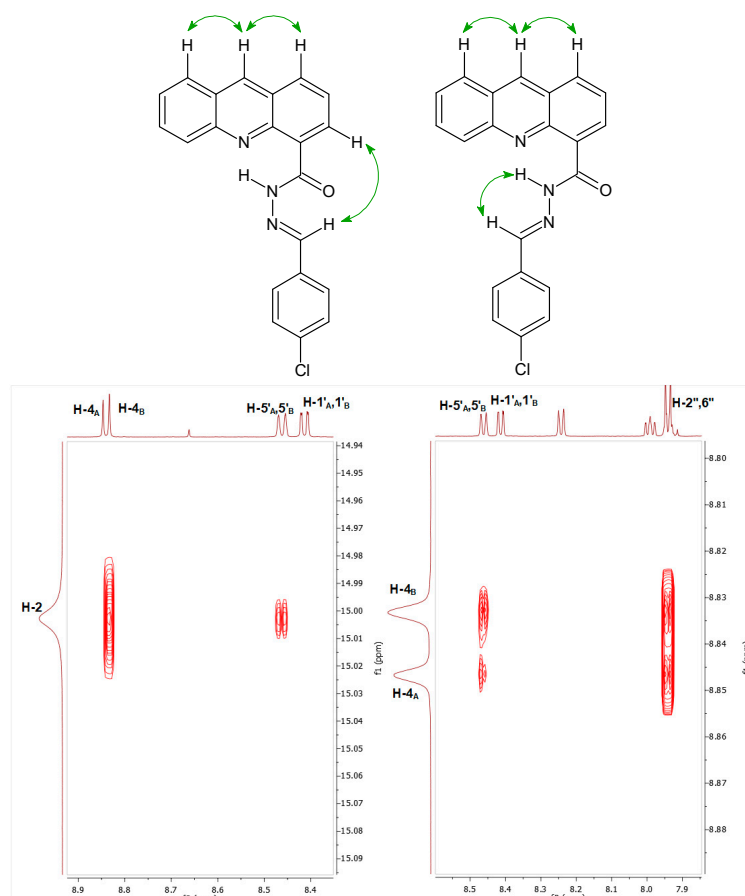
1.7 *N'*-[*(E)*-4-chlorophenylmethylidene]acridine-4-carbohydrazide **7c**



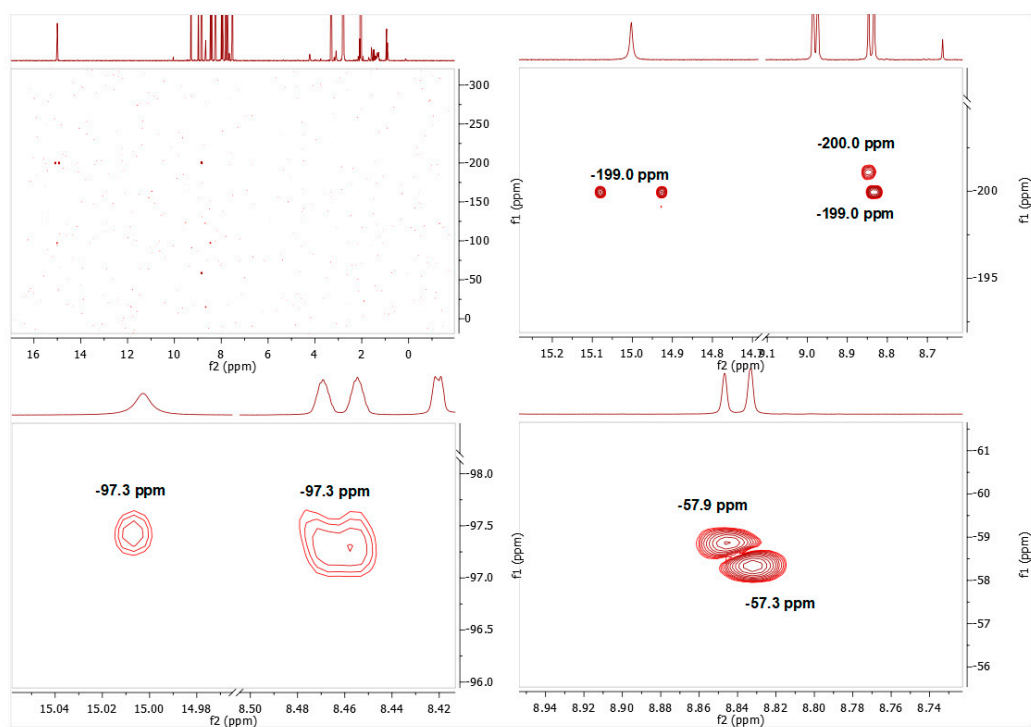
¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **7c** (A: *E*_{C=N}Z_{C(O)}-N_{ZN}-N, B: *E*_{C=N}Z_{C(O)}-N_{EN}-N).



¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **7c** (A: *E*_{C=N}Z_{C(O)}-N_{ZN}-N, B: *E*_{C=N}Z_{C(O)}-N_{EN}-N).

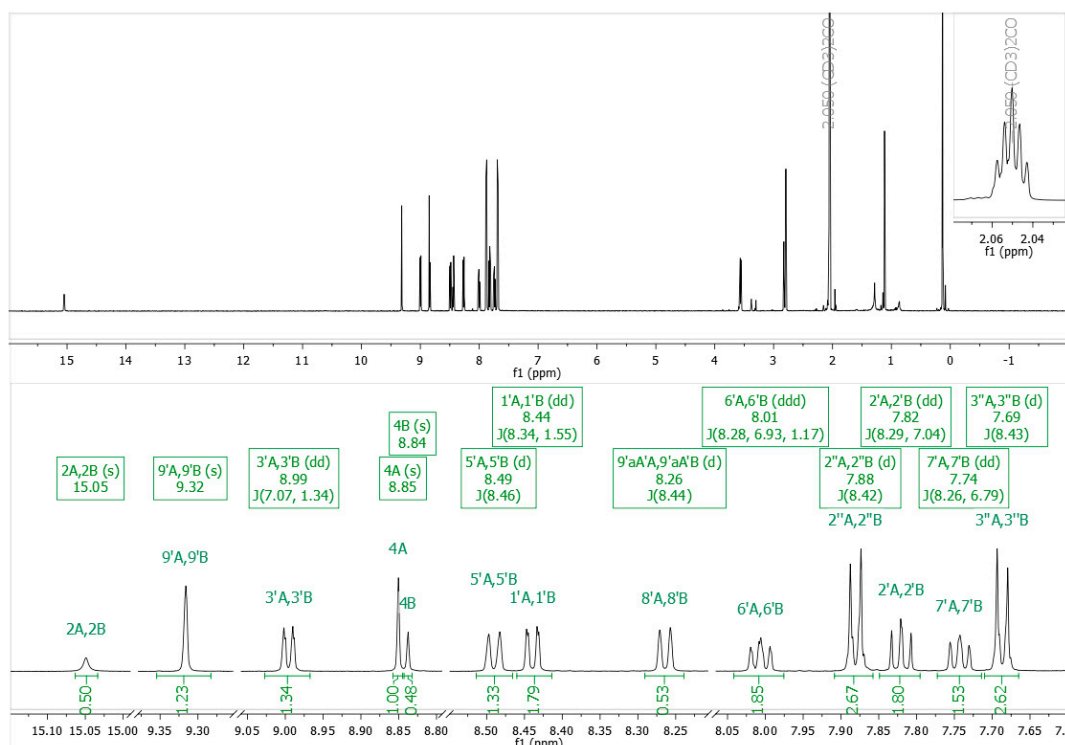


The selected areas of 2D NOESY spectra of derivative $EC=NZC(O)-N-7c$ showing key NOESY cross peaks.

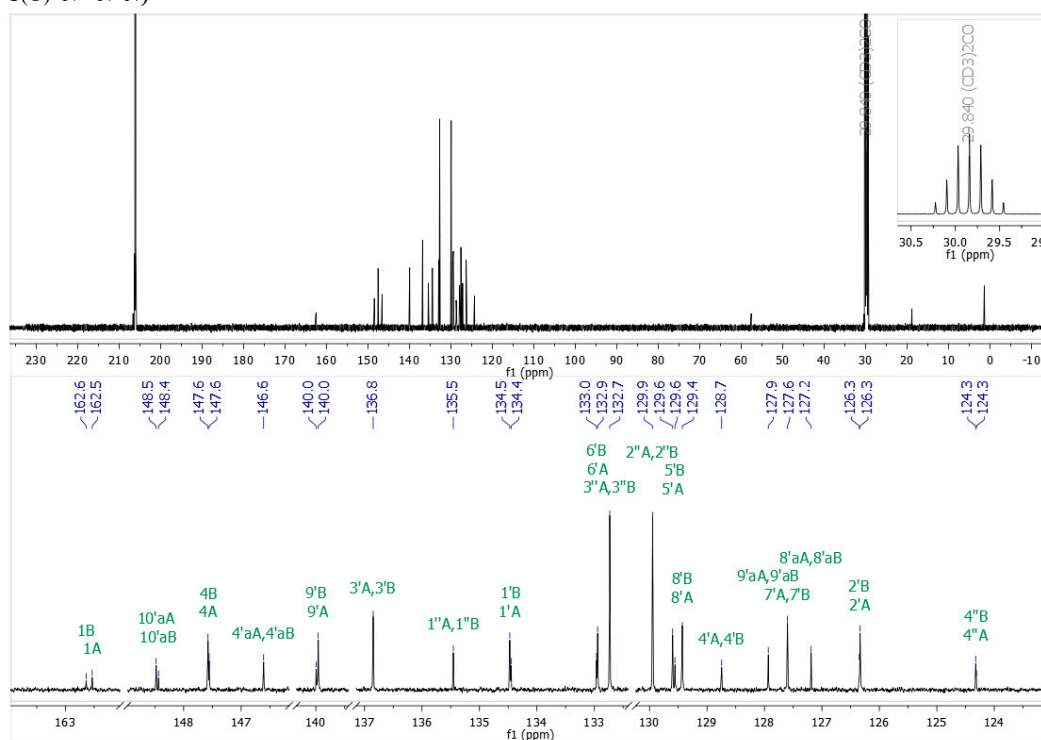


$^1H, ^{15}N$ -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **7c** (A: $EC=NZC(O)-NZN-N$, B: $EC=NZC(O)-NEN-N$).

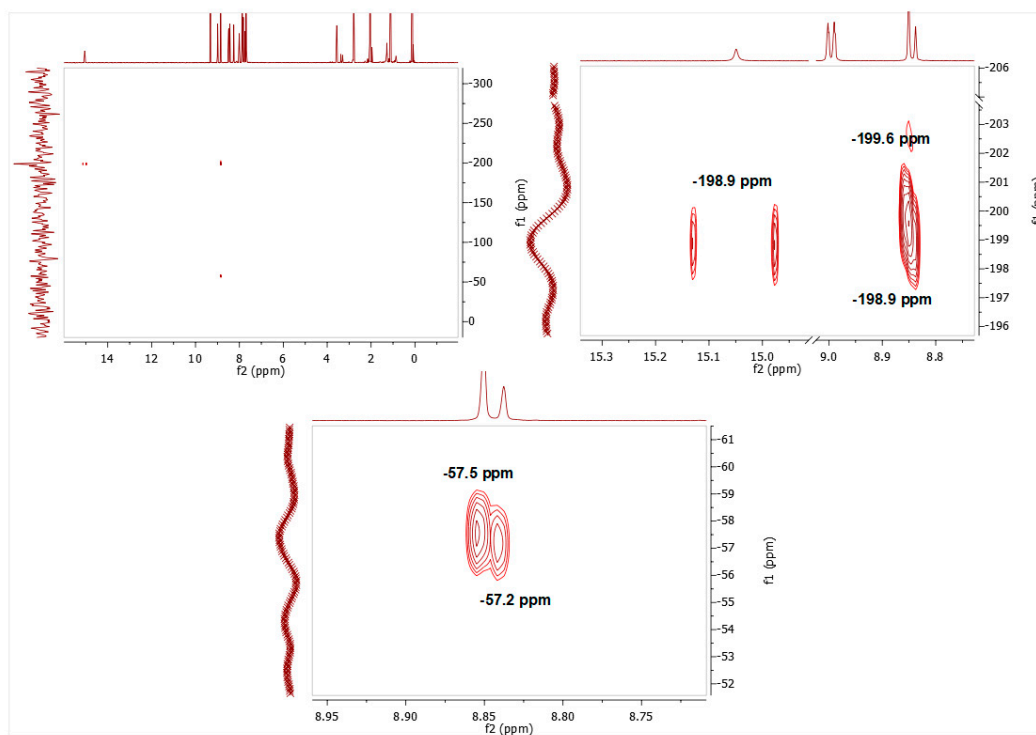
1.8 *N'*-[(*E*)-4-bromophenylmethylidene]acridine-4-carbohydrazide **7d**



¹H NMR spectrum (600 MHz, DMSO-d₆) of the derivative **7d** (A: $E_C=NZ_{C(O)-N}Z_{N-N}$, B: $E_C=NZ_{C(O)-N}E_{N-N}$).



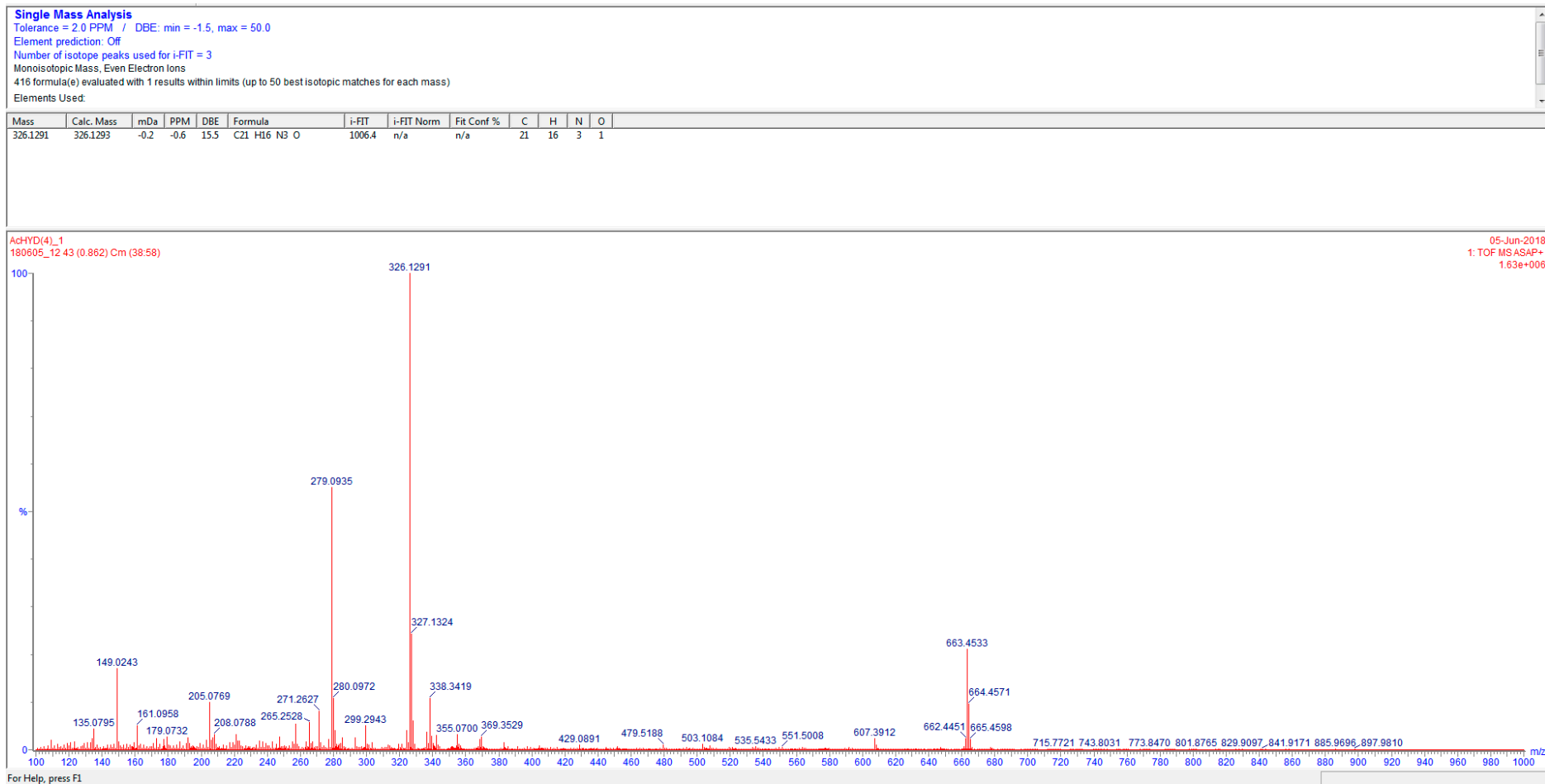
¹³C NMR spectrum (151 MHz, DMSO-d₆) of the derivative **7d** (A: $E_C=NZ_{C(O)-N}Z_{N-N}$, B: $E_C=NZ_{C(O)-N}E_{N-N}$).



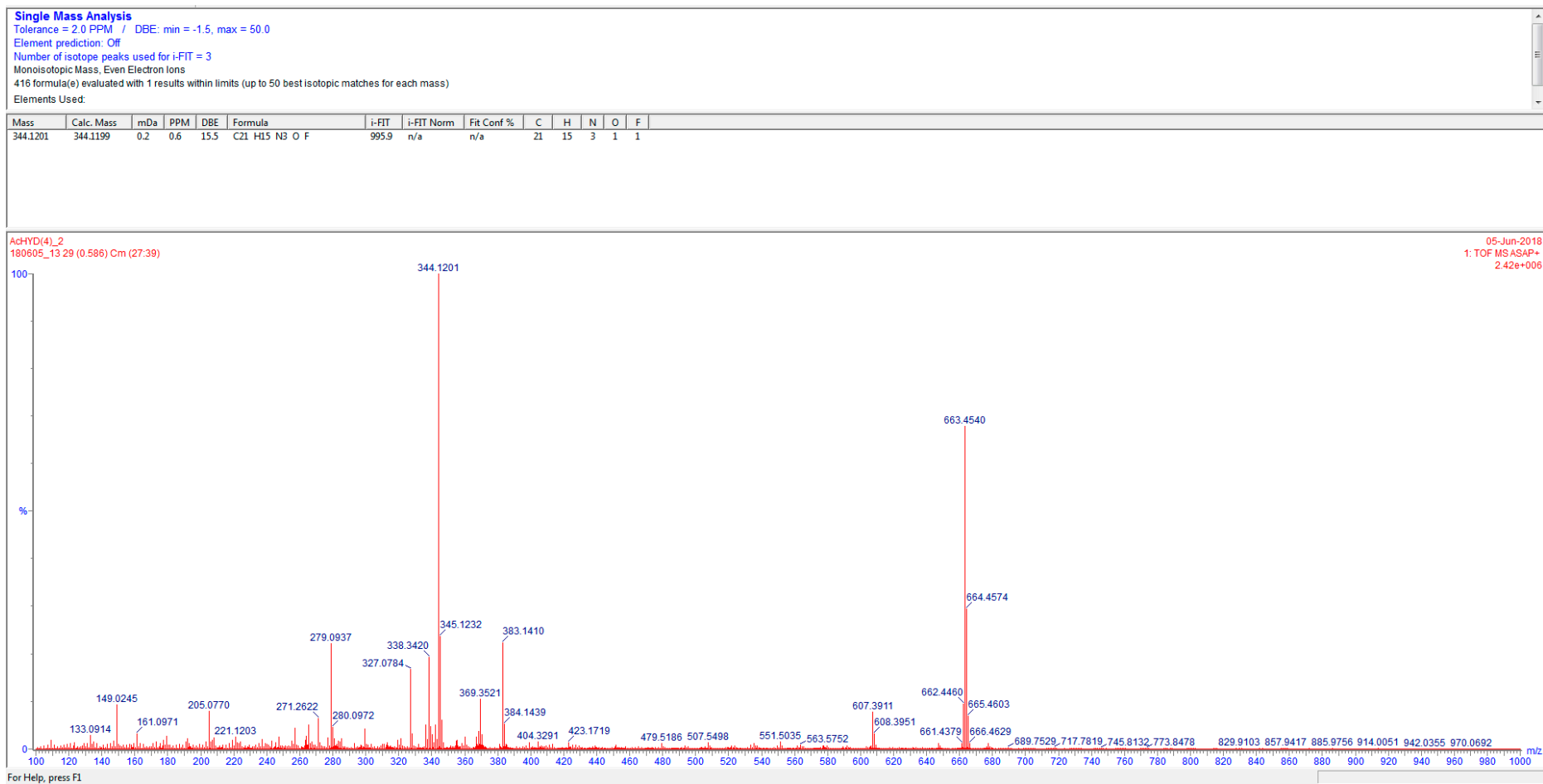
^1H , ^{15}N -HMBC spectrum (600/61 MHz, DMSO- d_6) of the derivative **7d** (A: $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}Z_{\text{N}-\text{N}}$, B: $E_{\text{C}=\text{N}}Z_{\text{C}(\text{O})-\text{N}}E_{\text{N}-\text{N}}$).

3 HR MS spectra of derivatives 3a–e and 7b–d

1.9 *N'*-[(*E*)-Acridin-4-yl)methylidene]benzohydrazide (3a)



1.10 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-fluorobenzohydrazide (3b)



1.11 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-chlorobenzohydrazide (3c)

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

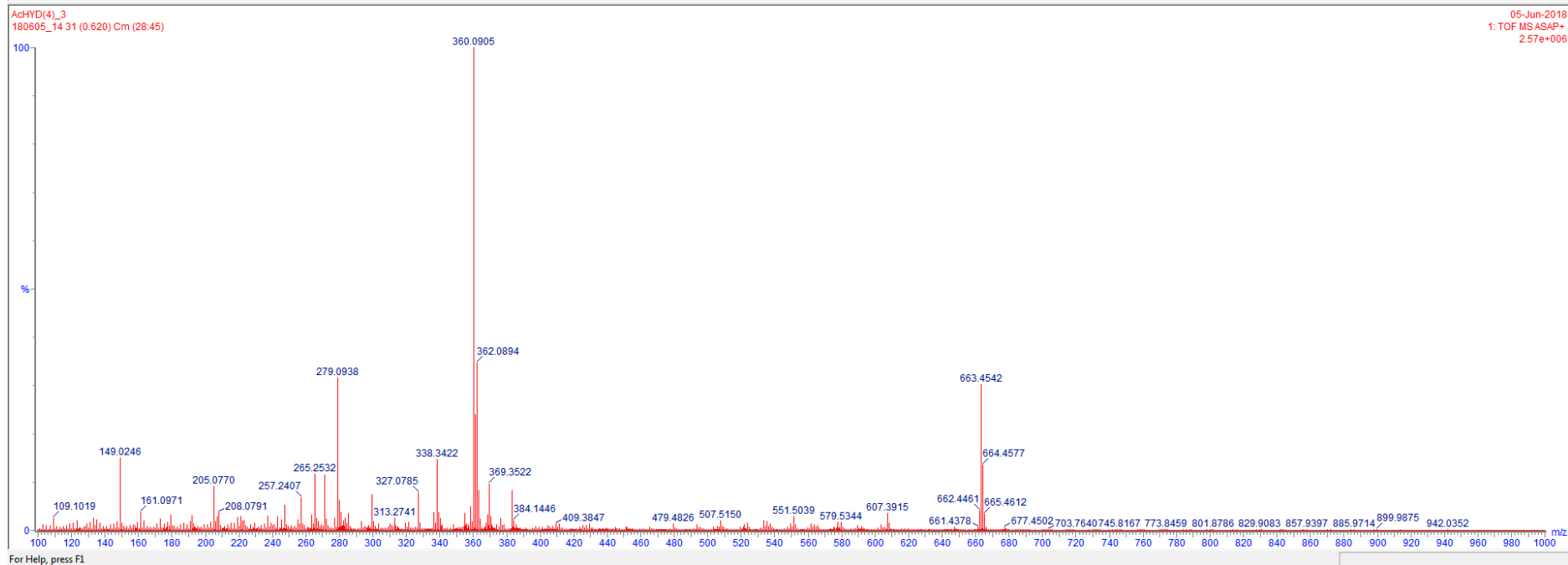
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

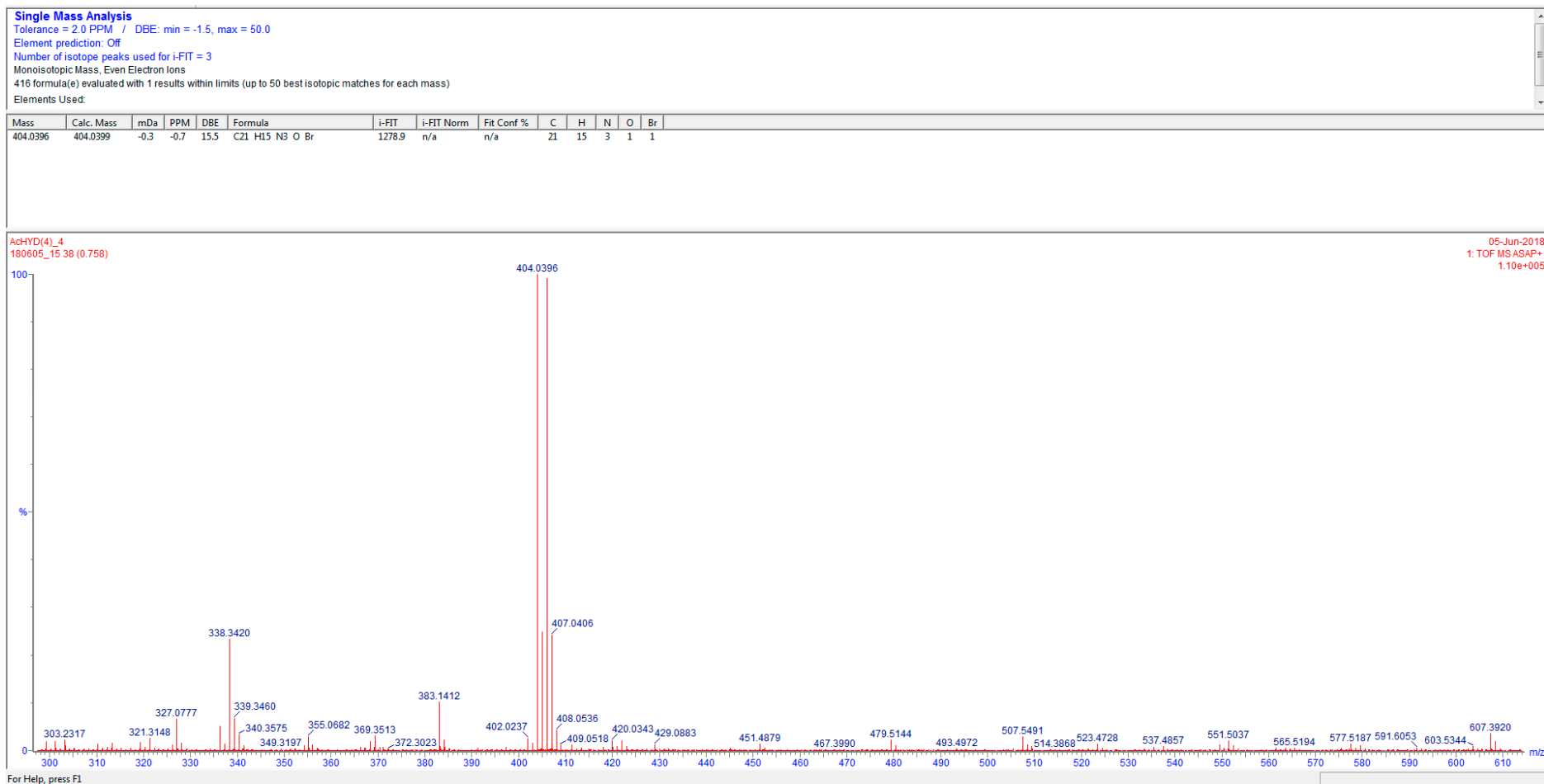
416 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

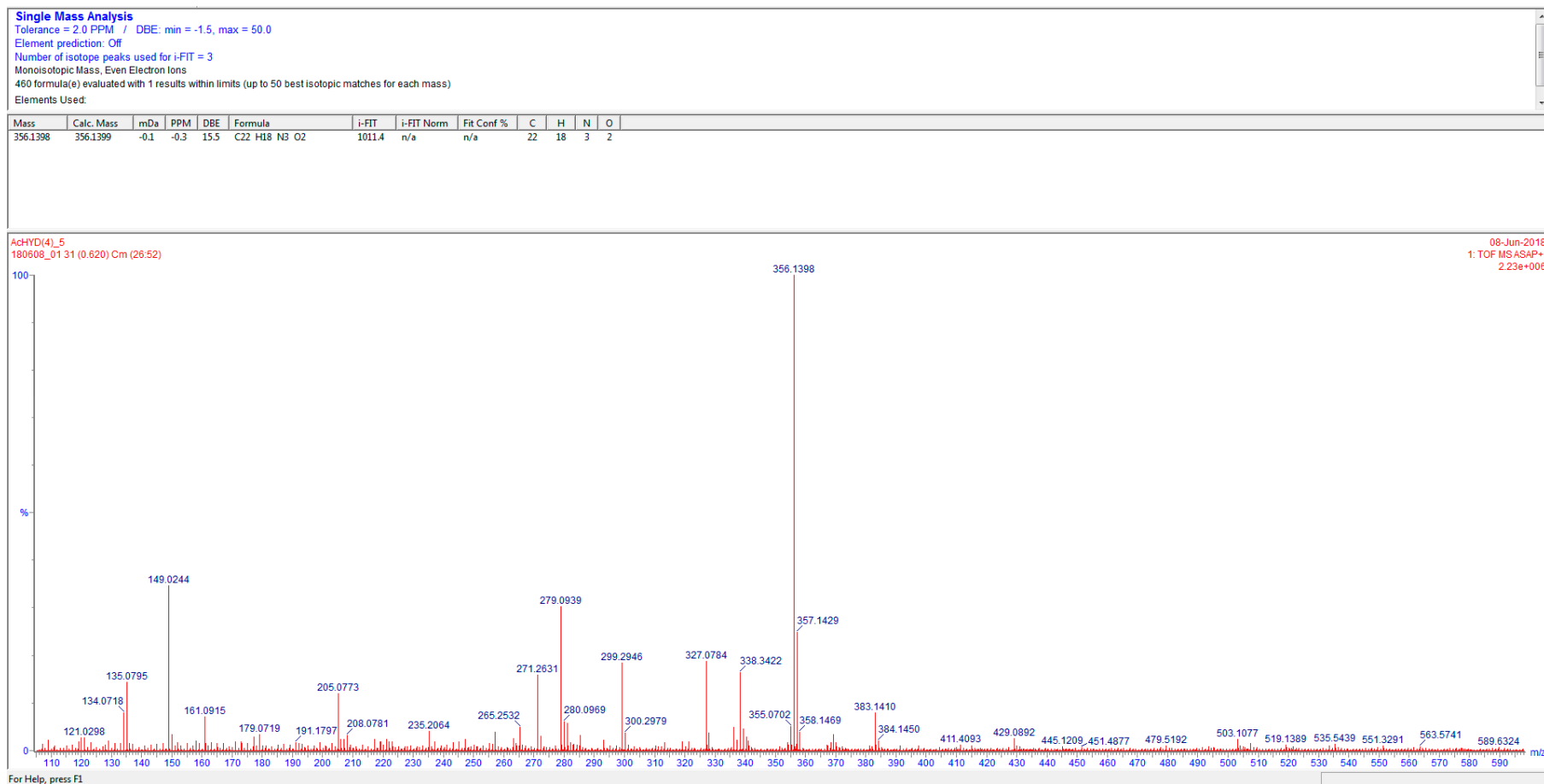
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Cl
360.0905	360.0904	0.1	0.3	15.5	C ₂₁ H ₁₅ N ₃ OCl	1033.1	n/a	n/a	21	15	3	1	1



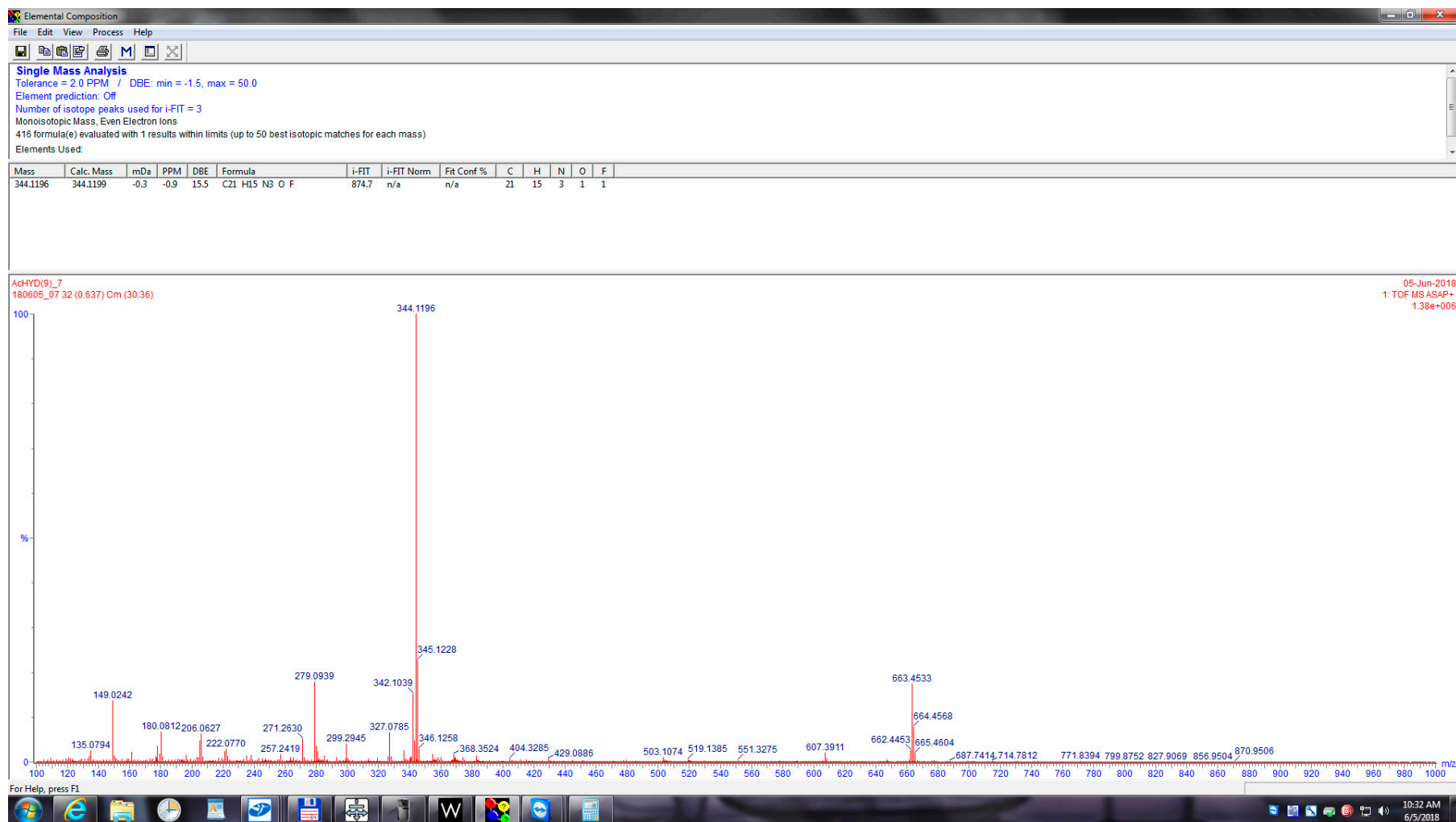
1.12 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-bromobenzohydrazide (3d)



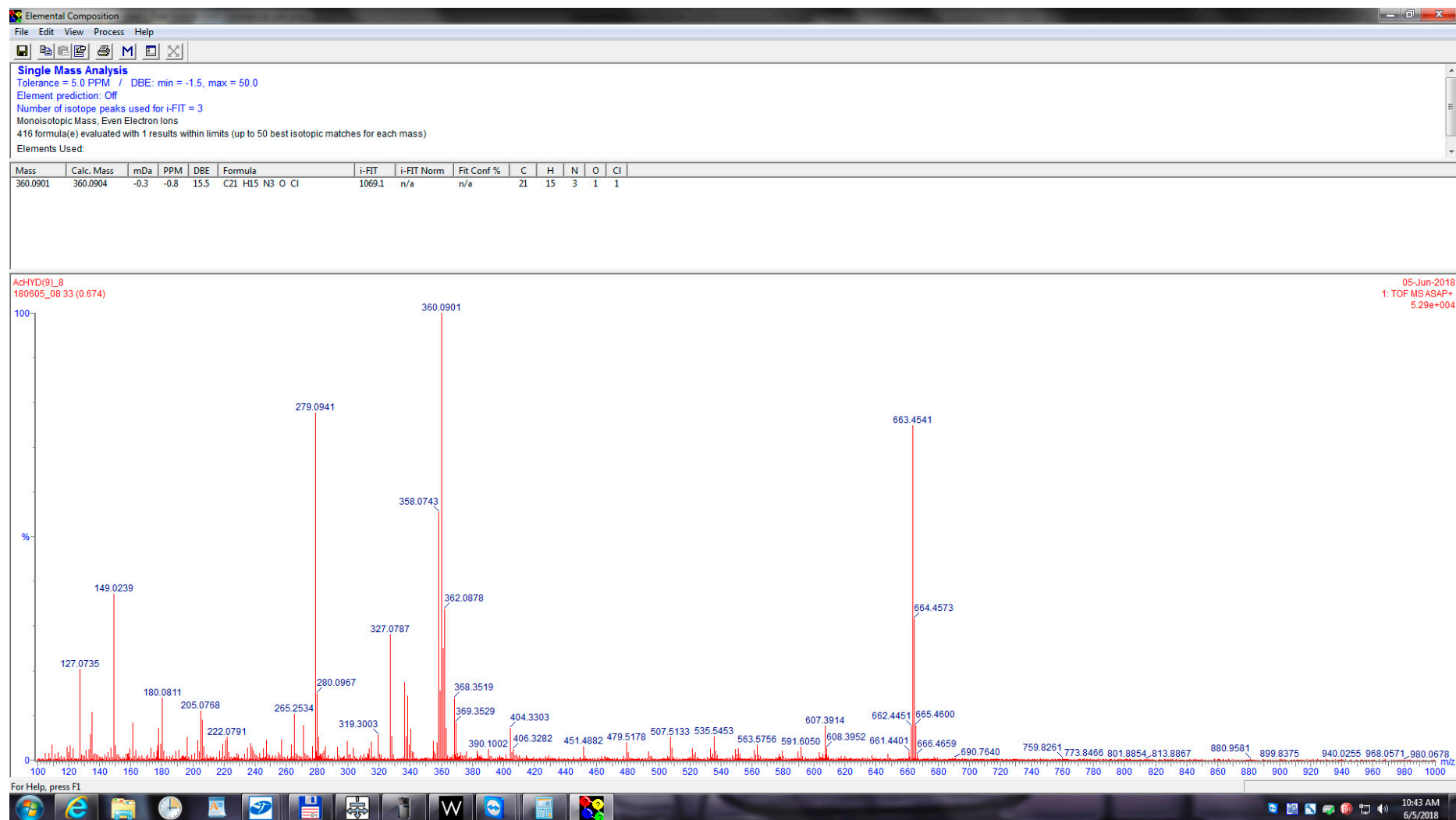
1.13 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-methoxybenzohydrazide (3e)



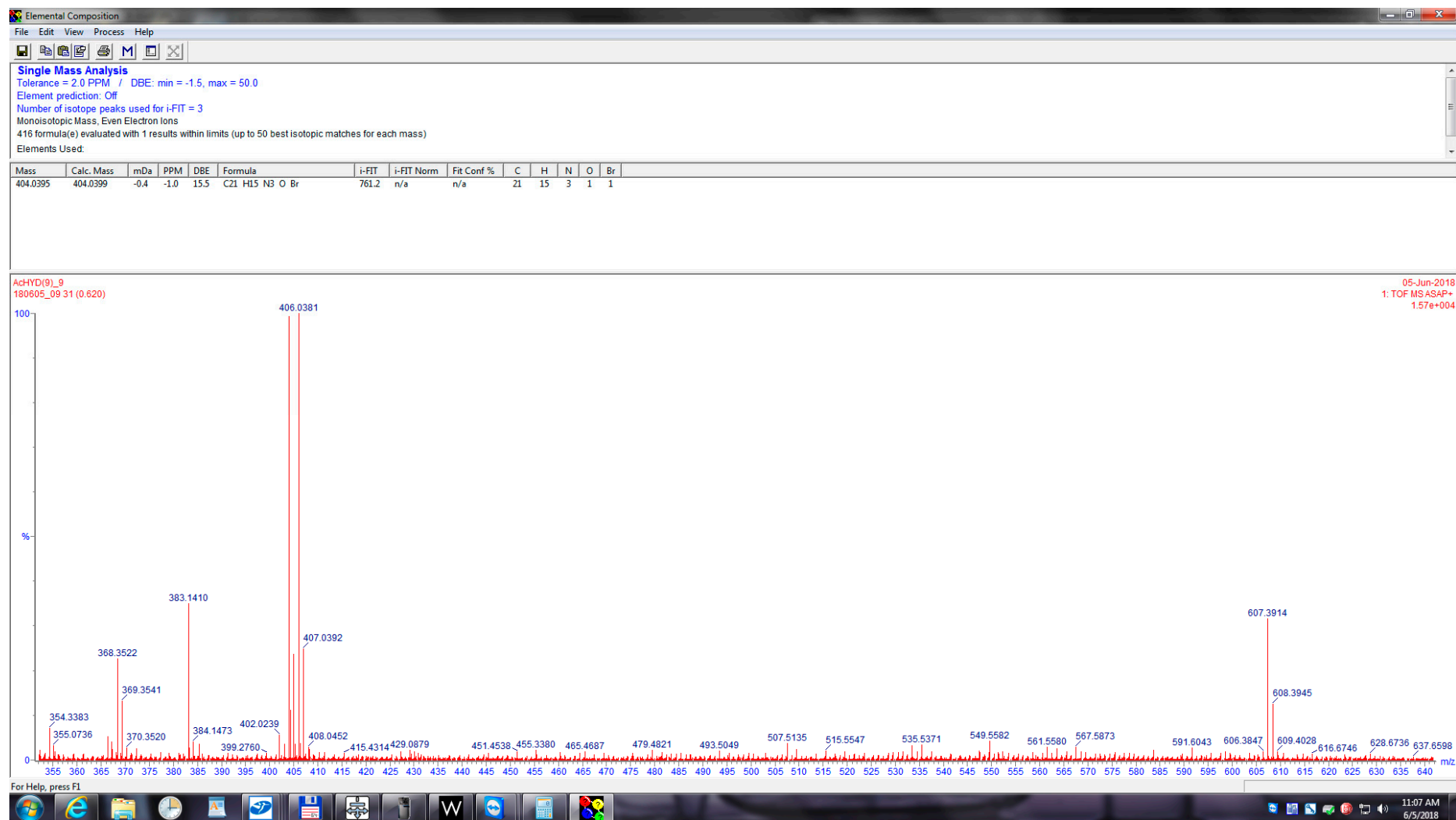
1.14 *N'*-[(*E*)-4-Fluorophenylmethylidene]acridine-4-carbohydrazide (7b)



1.15 *N'*-[(*E*)-4-Chlorophenylmethylidene]acridine-4-carbohydrazide (7c)



1.16 *N'*-[(*E*)-4-Bromophenylmethylidene]acridine-4-carbohydrazide (7d)



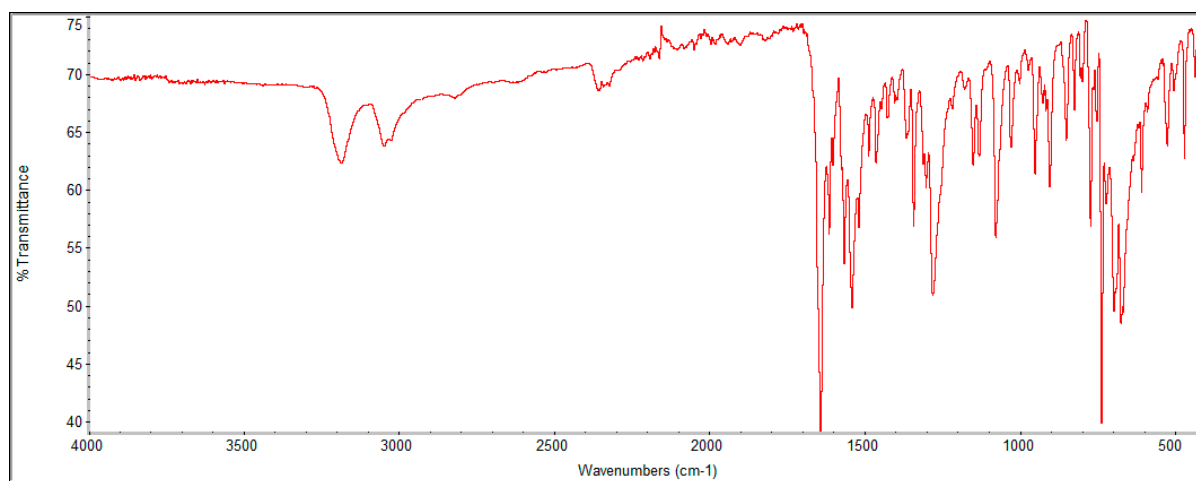
4 IR spectra of compounds 3a–d and 7b, 7d

Table S9. Assignment of characteristic IR absorption bands with corresponding wavenumbers (in cm^{-1}) and intensity.

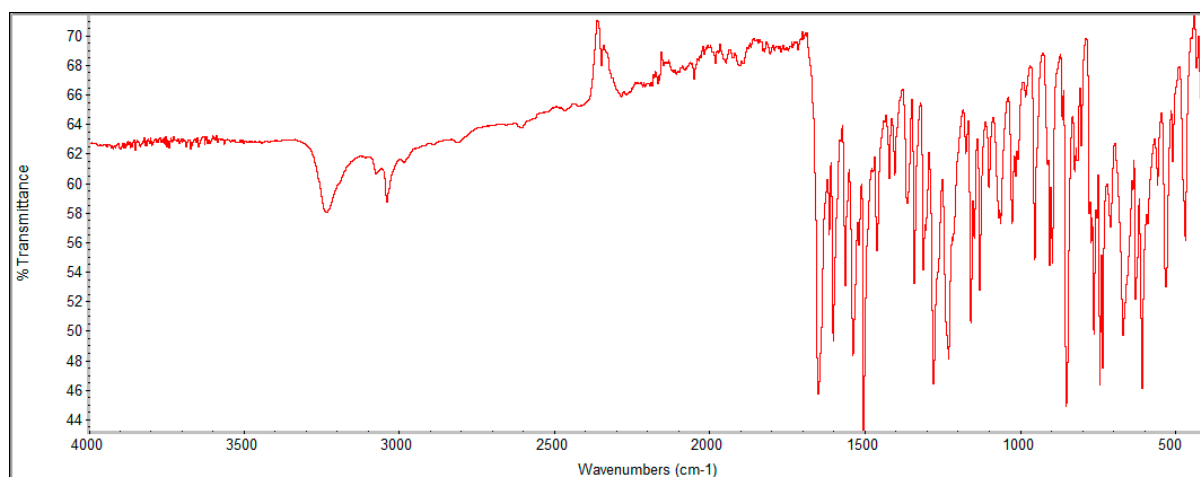
	$\nu(\text{NH})$	$\nu(\text{CH})_{\text{ar}}$	$\nu(\text{CH})_{\text{aliph}}$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{CC})_{\text{ar}}$	$\delta(\text{CCH})_{\text{ar}}$	$\gamma(\text{CCH})_{\text{ar}}$
3a	3196 (w, br)	3058(w) 3026(w)	X	1651(s)	1623(w)	1589(w) 1568(w) 1515(w)	1013(s)	760(s)
3b	3190 (w, br)	3064(w) 3041(w)	X	1655(s)	1626(w)	1602(s) 1563(w) 1506(s)	1012(s)	757(s)
3c	3194 (w, br)	3084(w) 3060(w) 3037(w)	X	1652(s)	1625(w)	1595(m) 1570(w) 1516(s)	1014(s)	754(s)
3d	3195 (w, br)	3082(w) 3058(w) 3038(w)	X	1653(s)	1626(w)	1591(m) 1568(w) 1517(s)	1011(s)	754(s)
3e	3209 (w, br)	3062(w) 3040(w) 3010(w)	2939(w) 2841(w)	1645(s)	1627(w)	1603(s) 1575(w) 1509(s)	1017(s)	756(s)
7b	3369 (m, br)	3077(w) 3062(w) 3050(w) 3013(w)	X	1645(s)	N	1603(s) 1507(m)	1024(m)	749(s)
7d	3201 (w, br)	3089(w) 3052(w)	X	1655(s)	1621(m)	1587(m) 1561(m) 1518(s)	1011(m)	737(s)

ar - aromatic, aliph - aliphatic, w - weak, m - medium, s - strong, br - broad, N - not observed, X - the bond is not present in the compound

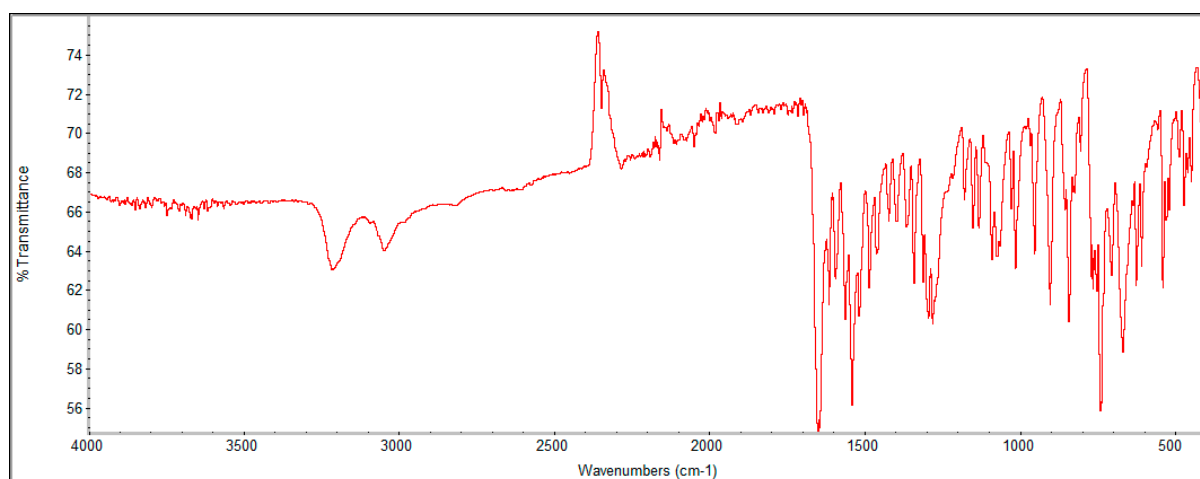
1.17 *N'*-[(*E*)-Acridin-4-yl)methylidene]benzohydrazide (3a)



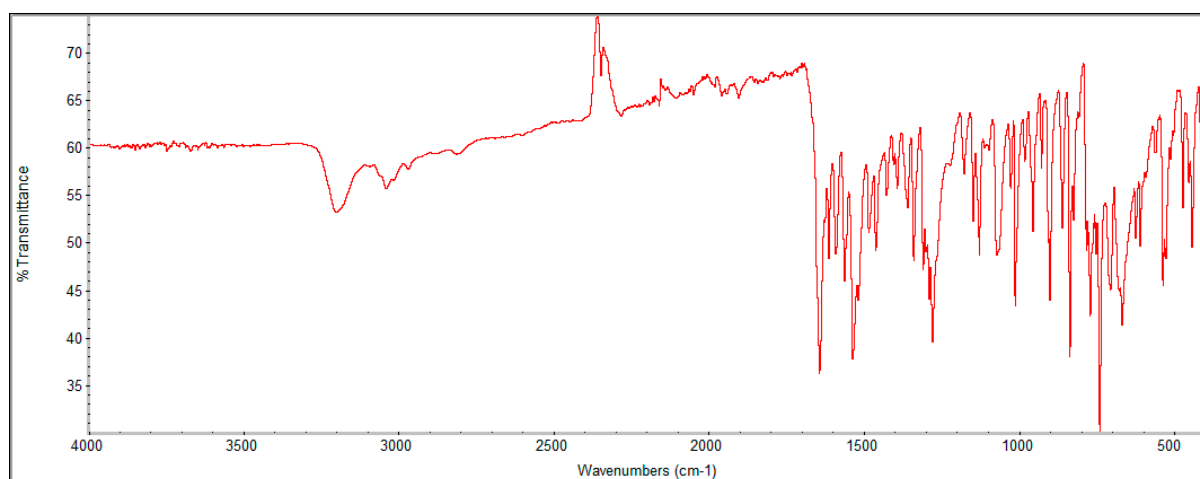
1.18 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-fluorobenzohydrazide (3b)



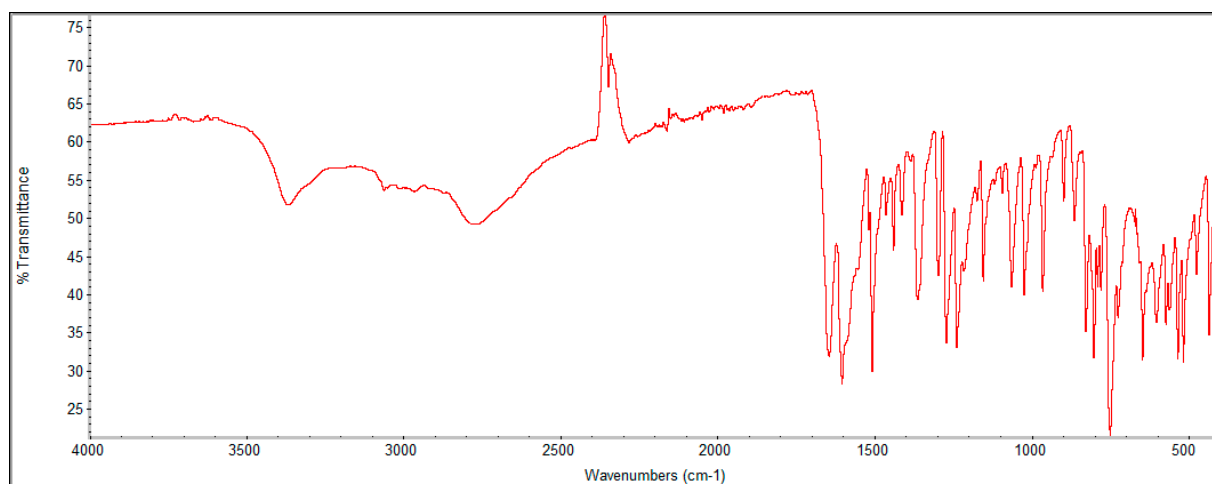
1.19 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-chlorobenzohydrazide (3c)



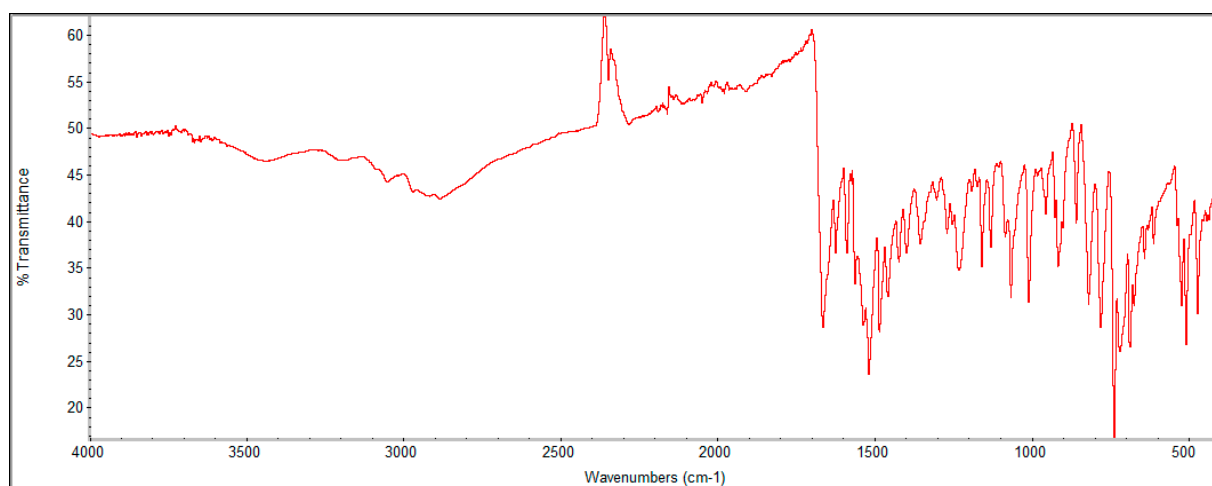
1.20 *N'*-[(*E*)-Acridin-4-yl)methylidene]-4-bromobenzohydrazide (3d)



1.21 *N'*-[(*E*)-4-Fluorophenylmethylidene]acridine-4-carbohydrazide (7b)



1.22 *N'*-[(*E*)-4-Bromophenylmethylidene]acridine-4-carbohydrazide (7d)



5 HSA binding experiments

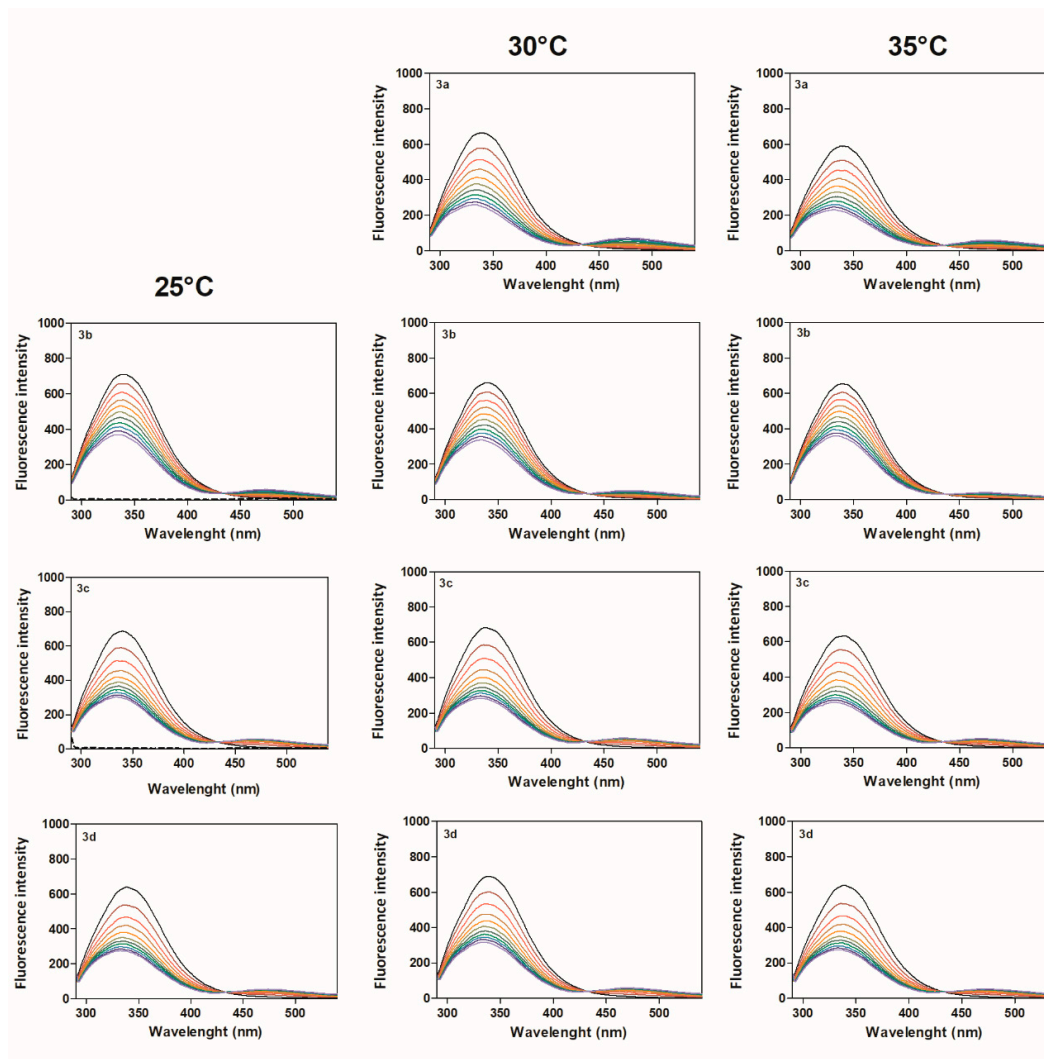


Figure S1. Fluorescence quenching spectra of HSA by different concentration of compound **3a–3d** with $\lambda_{\text{ex}} = 280$ nm in Tris-HCl/NaCl buffer, pH = 7.4, at three temperatures 25, 30 and 37 °C. (The plain black line [HSA] = 4 μM ; plain color line [3a–3d] = 0–6.2 μM).

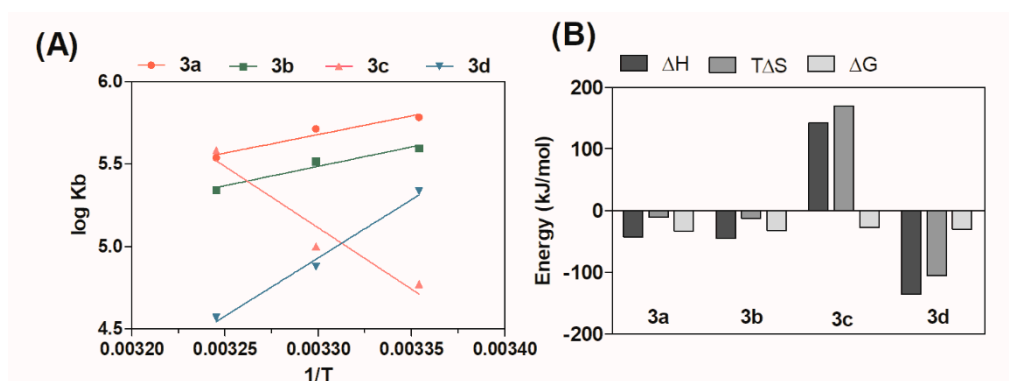


Figure S2. Van't Hoff plot for the interaction of **3a–3d** (A) and thermodynamic profil (B) with HSA at three temperatures, [HSA] = 4 μM ; [3a–3d] = 0–6,2 μM .

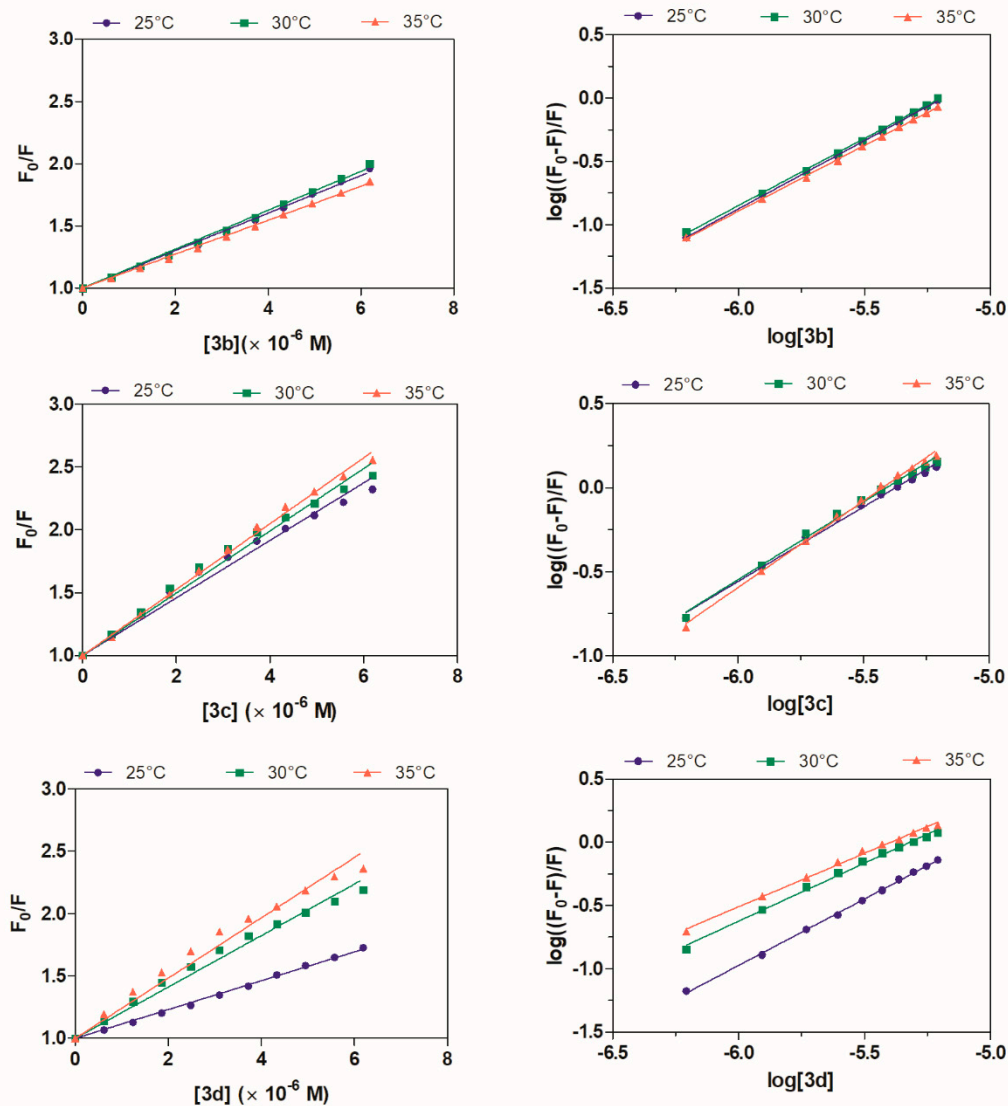


Figure S3. Stern-Volmer plots of HSA + **3b–3d** and double logarithm plot of HSA + **3b–3d** at three different temperature. ([HSA] = 4 μ M; [**3a–3d**] = 0–6.2 μ M).

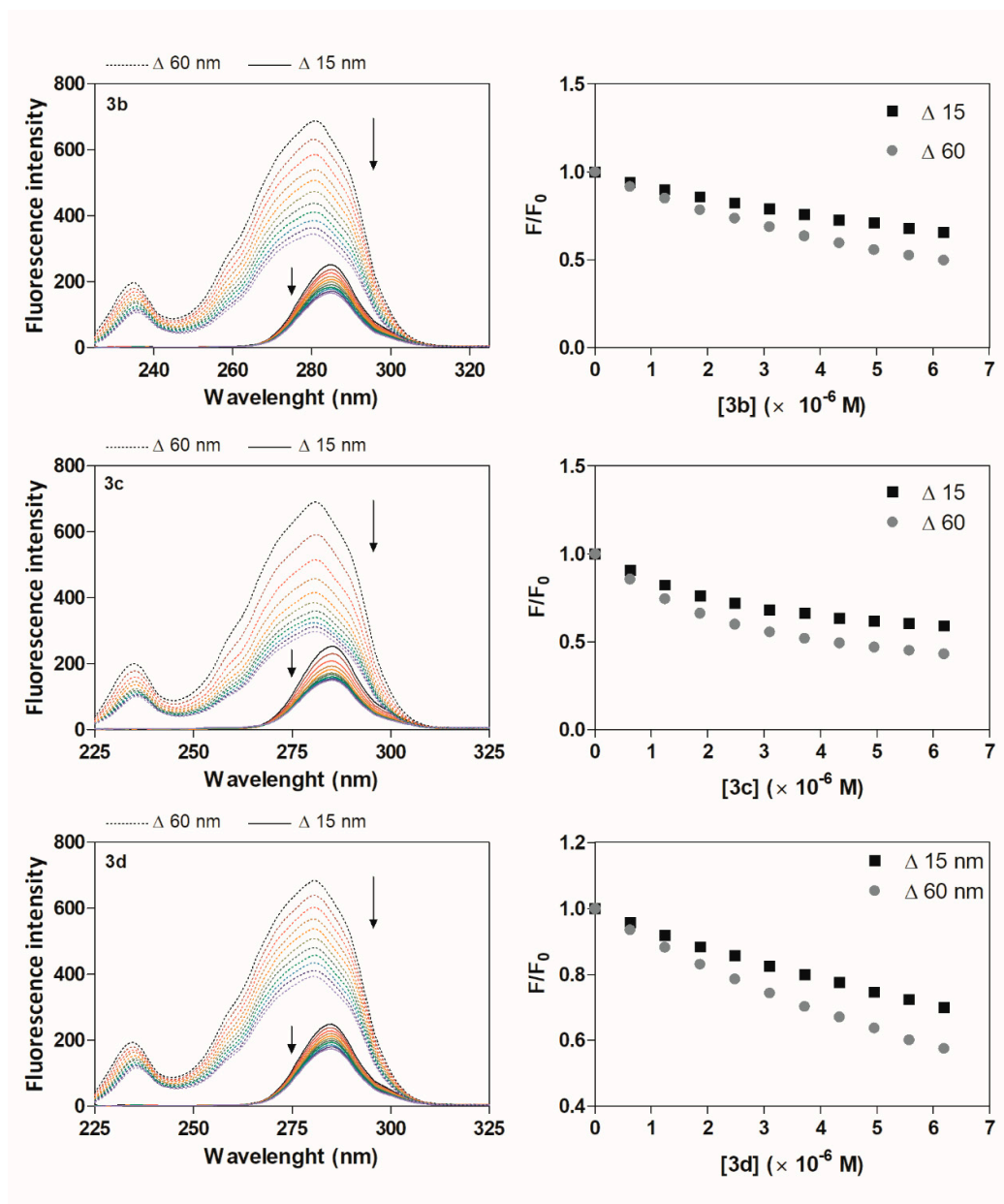


Figure S4. Synchronous fluorescence spectra of HSA + **3b–3d** and corresponding F/F_0 plots at room temperature. ($[HSA] = 4 \mu M$; $[3b-3d] = 0 - 6.2 \mu M$; dashed line for $\Delta\lambda = 60$ nm and plain line for $\Delta\lambda = 15$ nm). Arrow shows the intensity change upon increasing concentration of the quencher.

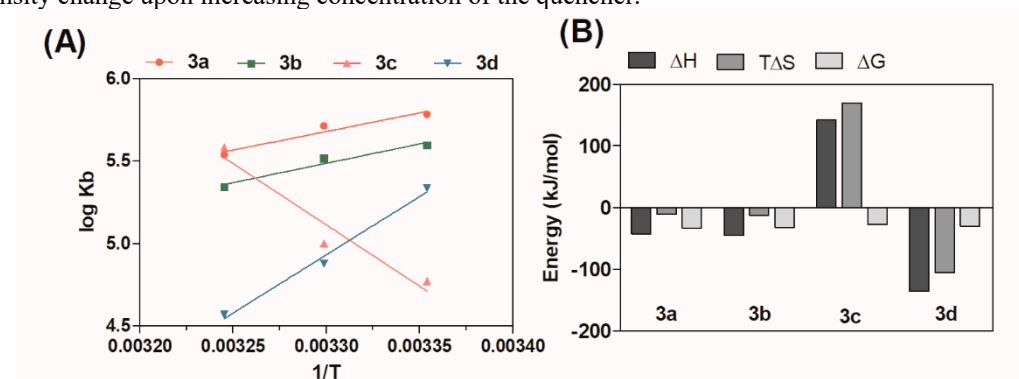
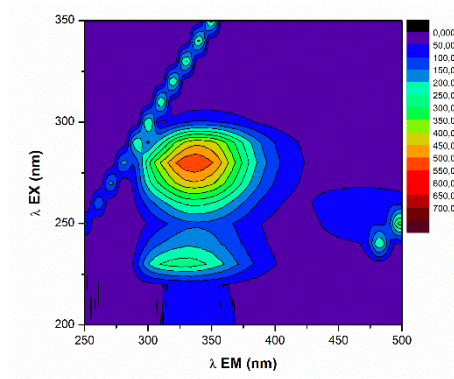
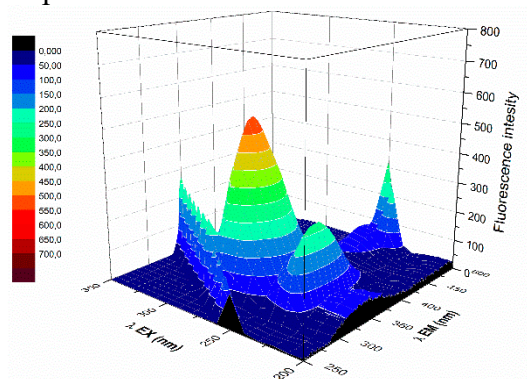
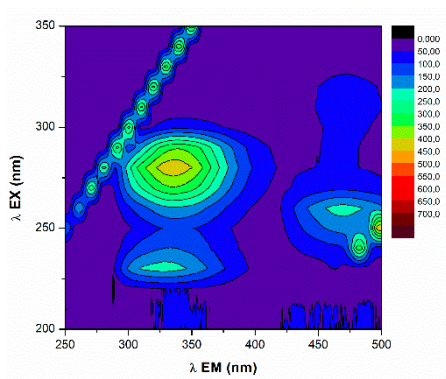
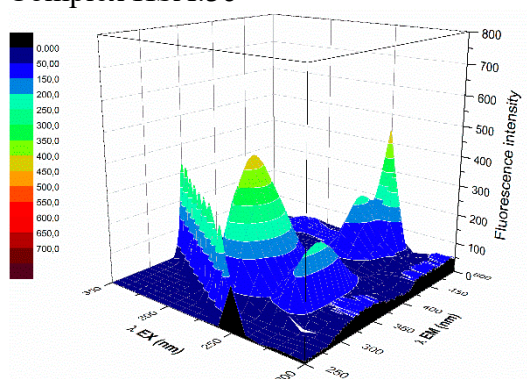


Figure S5. Van't Hoff plot for the interaction of **3a–3d** (A) and thermodynamic profile (B) with HSA at three temperatures. ($[HSA] = 4 \mu M$; $[3a-3d] = 0 - 6.2 \mu M$).

Complex HSA:3b



Complex HSA:3c



Complex HSA:3d

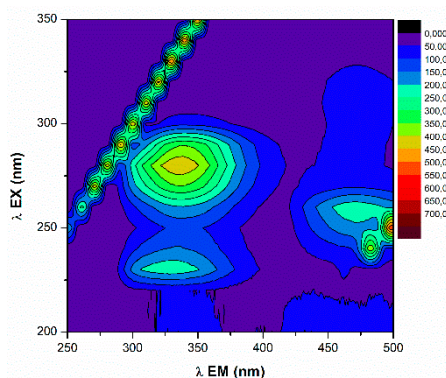
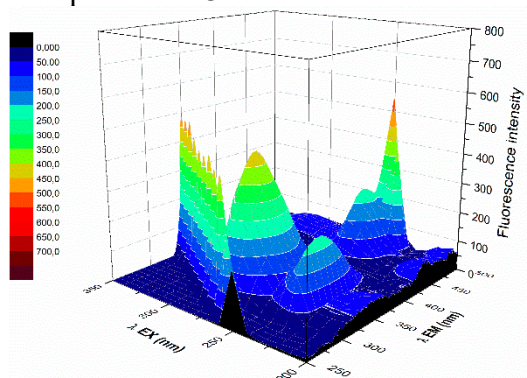


Figure S6. Three-dimensional fluorescence spectra and corresponding contour diagrams of free HSA, HSA:3b, HSA:3c, HSA:3d systems in Tris-HCl/NaCl, pH = 7.4 at 25°C. ([HSA], [3b–3d] = 4 μM).

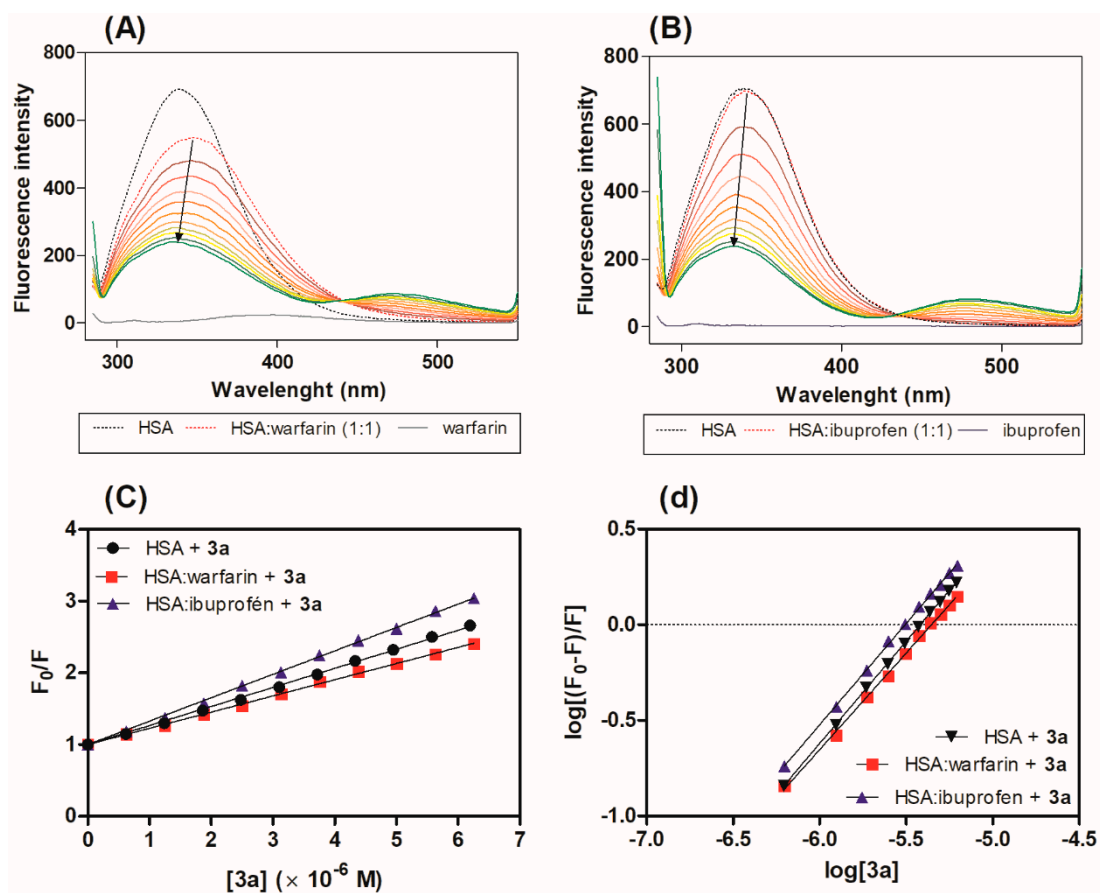


Figure S7. HSA competitive experiments - reverse titration. Emission spectrum (A) HSA:warfarin (1:1), (B) HSA:ibuprofen in presence increasing concentration of **3a** (0.6–6 μM), (C) Stern-Volmer plots and (D) double logarithm plot. ($[\text{HSA}] = 4 \mu\text{M}$).

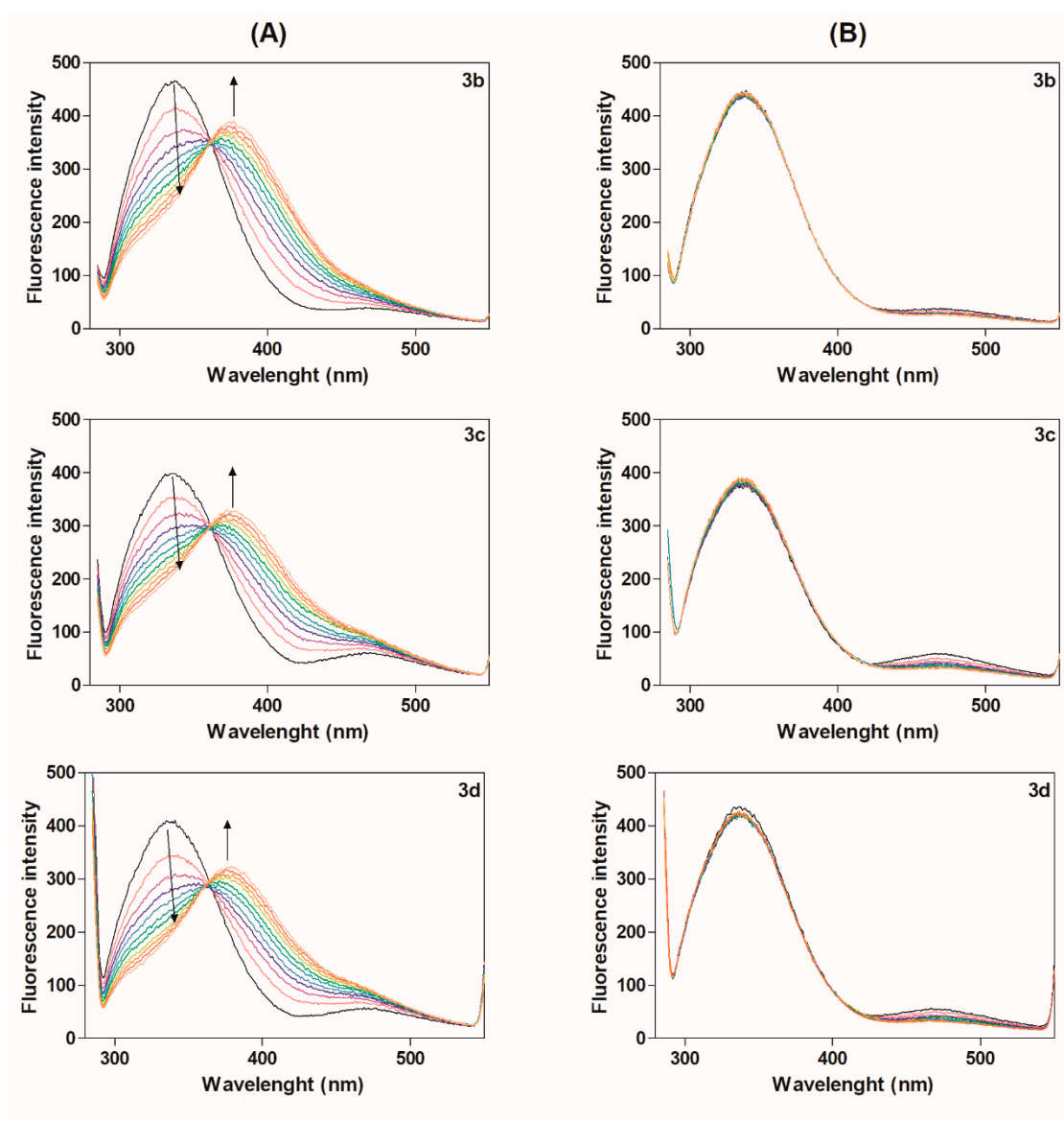


Figure S8. Emission spectrum HSA:acridine derivatives **3b–3d** (1:1, black line) in presence increasing concentration site marker (A) warfarin and (B) ibuprofen (colored line). Molar ratio HAS:marker is: 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5 and 1:5, [warfarin/ibuprofen] = 0–20 μM .