

Supporting information

Excavating precursors from pair herbs *Polygala tenuifolia* and *Acori tatarinowii*: Synthesis, anticonvulsant activity evaluation of 3,4,5-trimethoxycinnamic acid (TMCA) piperazine amide derivatives

Zefeng Zhao ^{a, *}, Mengchen Lei ^a, Yongqi Wang ^a, Yujun Bai ^b and Haifa Qiao ^{a, **}

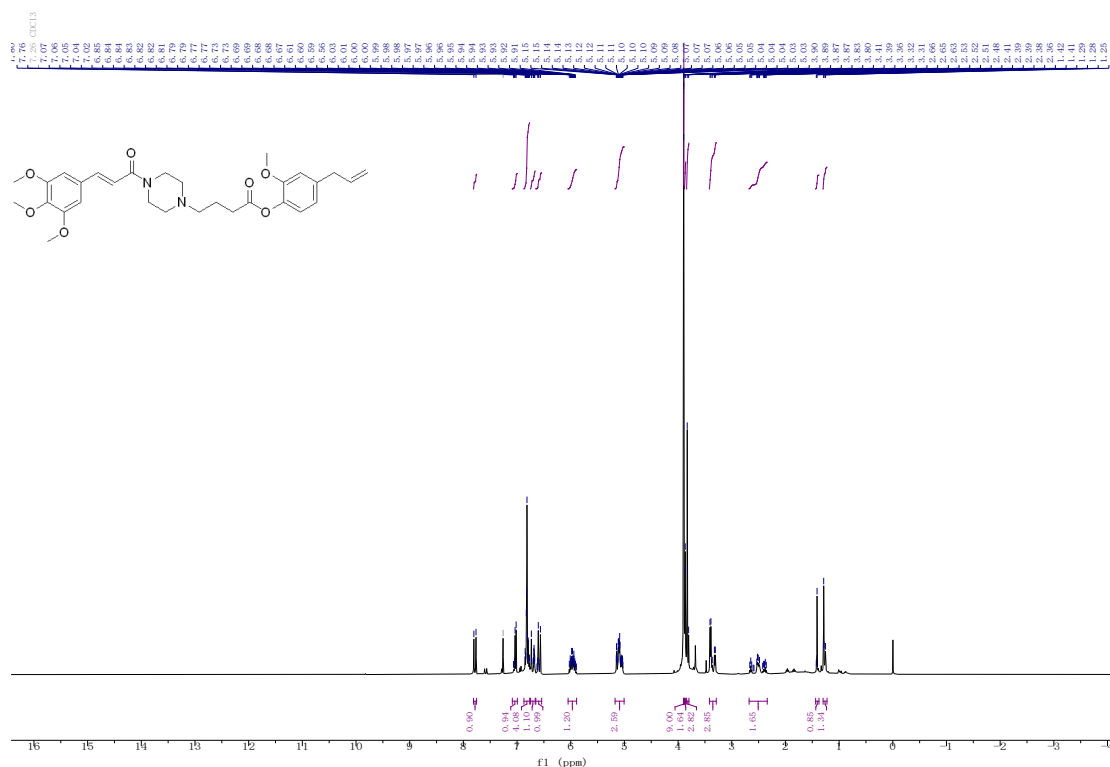
^a Shaanxi University of Chinese Medicine, Xianyang, Shaanxi Province 712046, P. R. China;

^b Northwest University, 229 Taibai Road, Xi'an, 710069, P. R. China;

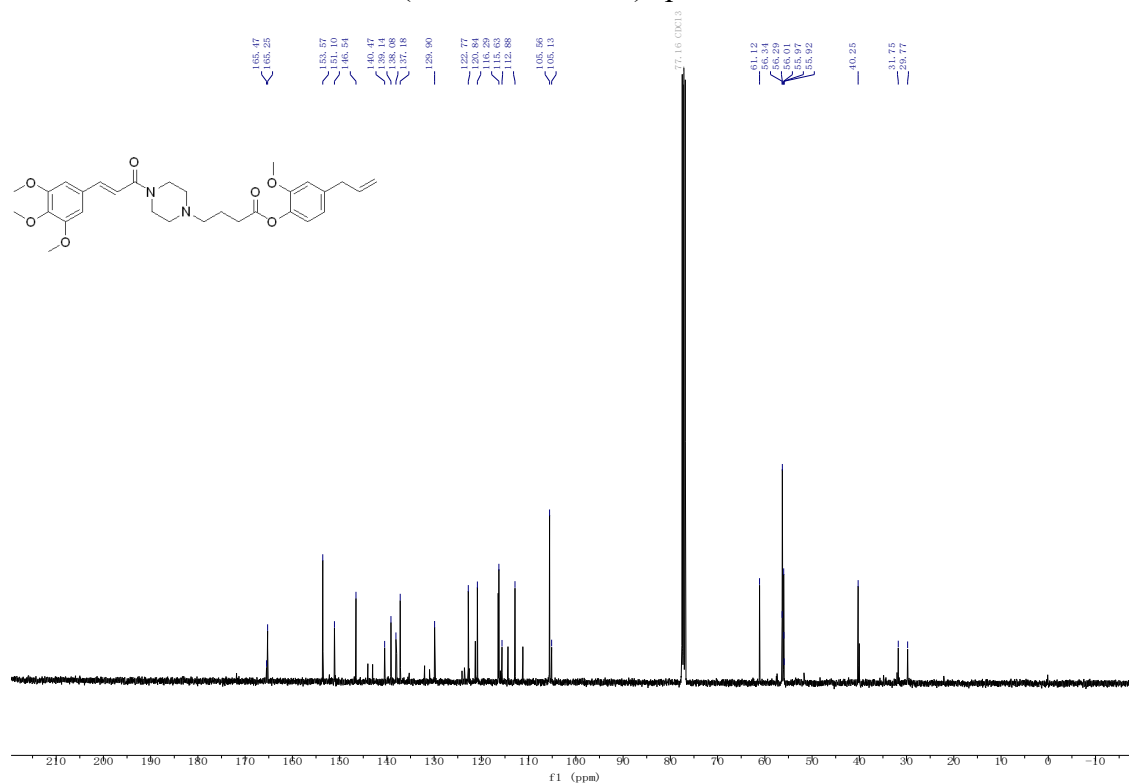
* Correspondence: 2061046@sntcm.edu.cn and 1511006@sntcm.edu.cn.

NMR spectrum of synthesized derivatives

4-allyl-2-methoxyphenyl (*E*)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**A2**):



¹H NMR (600 MHz, CDCl₃) spectrum of **A2**



¹³C NMR (600 MHz, CDCl₃) spectrum of **A2**

Chemical structure of compound 10: COc1cc(OC)cc(C=Cc2cc(OC)cc(OC)c2)cc1C(=O)N3CCN(CC3)C(=O)CC(=O)Oc1ccc(C=C)cc1

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents the chemical shift in ppm, ranging from 0 to 16. The spectrum shows several peaks, with integration values provided below the baseline. The chemical shifts (δ) are listed at the top of the spectrum.

Chemical shifts (δ) in ppm: 7.64, 7.59, 7.58, 7.57, 7.56, 7.55, 7.54, 7.53, 7.52, 7.51, 7.50, 7.49, 7.48, 7.47, 7.46, 7.45, 7.44, 7.43, 7.42, 7.41, 7.40, 7.39, 7.38, 7.37, 7.36, 7.35, 7.34, 7.33, 7.32, 7.31, 7.30, 7.29, 7.28, 7.27, 7.26, 7.25.

Integration values: 0.93, 0.99, 5.09, 1.11, 2.34, 9.00, 2.89, 1.91, 2.23, 1.85, 1.85.

Chemical structure of the compound is shown above the spectrum. The spectrum displays peaks corresponding to the chemical structure, with the following chemical shifts (ppm) labeled on the right side:

- 171.57
- 165.78
- 153.56
- 150.90
- 143.94
- 139.89
- 138.16
- 137.16
- 130.66
- 122.71
- 120.40
- 118.57
- 112.82
- 105.16
- 61.13
- 56.35
- 55.99
- 40.22
- 36.02
- 31.76
- 29.24
- 28.05

 ^{13}C NMR (600 MHz, CDCl_3) spectrum of **B2**

Chemical structure of compound 10: COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CC2)CCCC(=O)Oc1ccc(C=C)cc1OC

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents the chemical shift in ppm, ranging from 0 to 16. The spectrum shows several peaks with corresponding integrations and a list of chemical shifts on the right.

Chemical shifts (ppm): 7.286, 6.96, 6.95, 6.94, 6.74, 6.72, 6.71, 6.69, 6.59, 6.58, 6.57, 6.54, 5.90, 5.89, 5.88, 5.86, 5.85, 5.84, 5.04, 5.03, 5.02, 5.02, 5.00, 4.98, 4.98, 4.97, 4.96, 4.95, 4.94, 4.93, 3.65, 3.34.

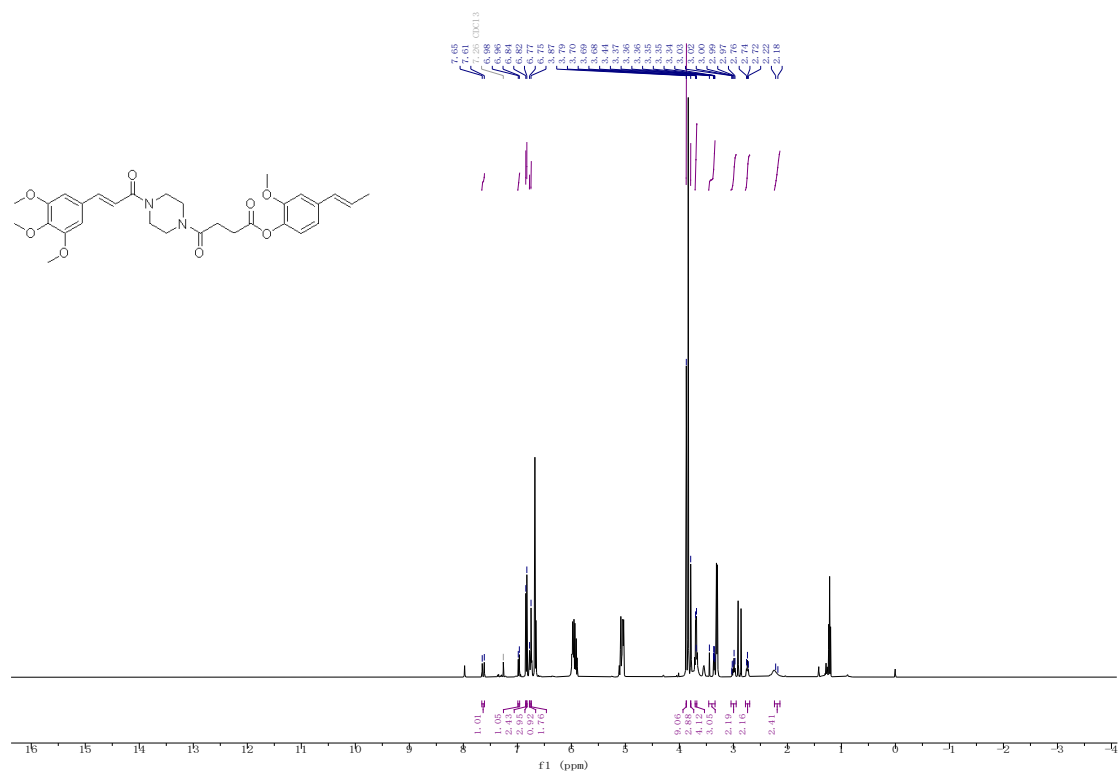
Integrations: 0.97, 1.96, 1.96, 2.16, 2.88, 2.88.

Chemical structure of compound 10 is shown above the spectrum. The spectrum displays peaks corresponding to the chemical shifts listed in the table below.

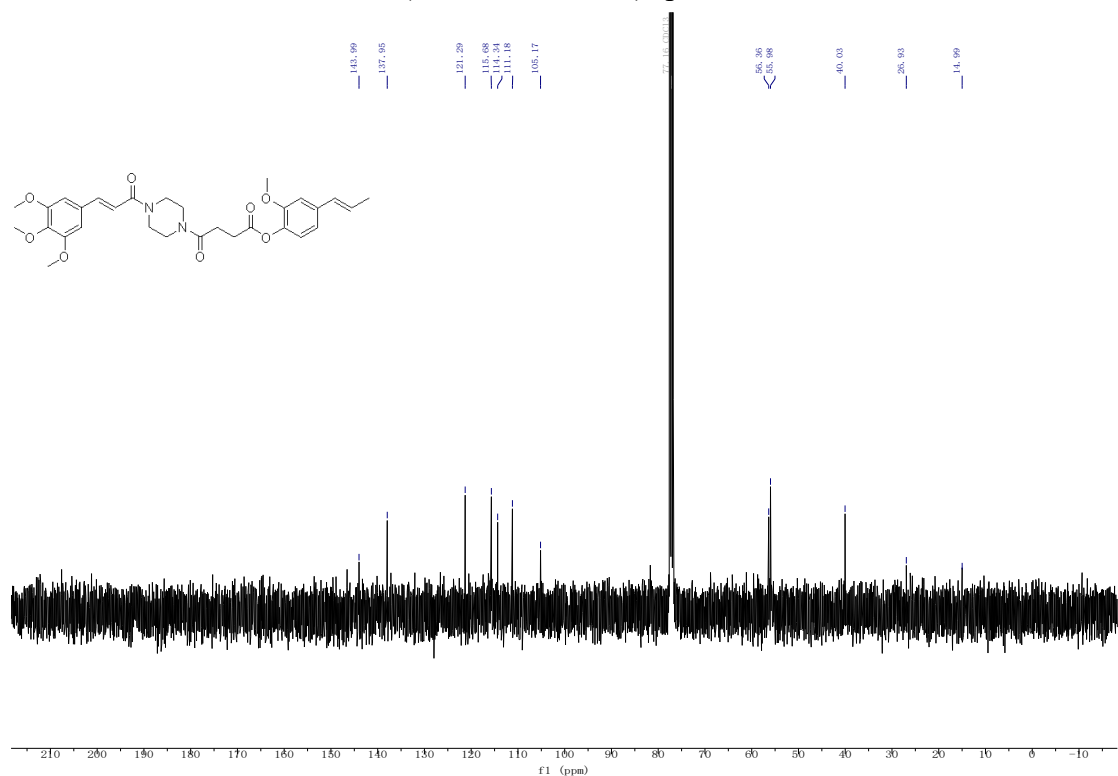
Chemical Shift (ppm)
171.59
165.26
153.39
153.35
150.95
149.45
143.87
139.90
137.85
137.08
131.64
122.63
121.12
120.98
115.46
111.13
105.39
105.04
105.04
60.92
60.89
60.03
55.77
55.75
40.08
39.85
39.62

 ^{13}C NMR (600 MHz, CDCl_3) spectrum of **A3**

2-methoxy-4-((*E*)-prop-1-en-1-yl)phenyl 4-oxo-4-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**B3**):

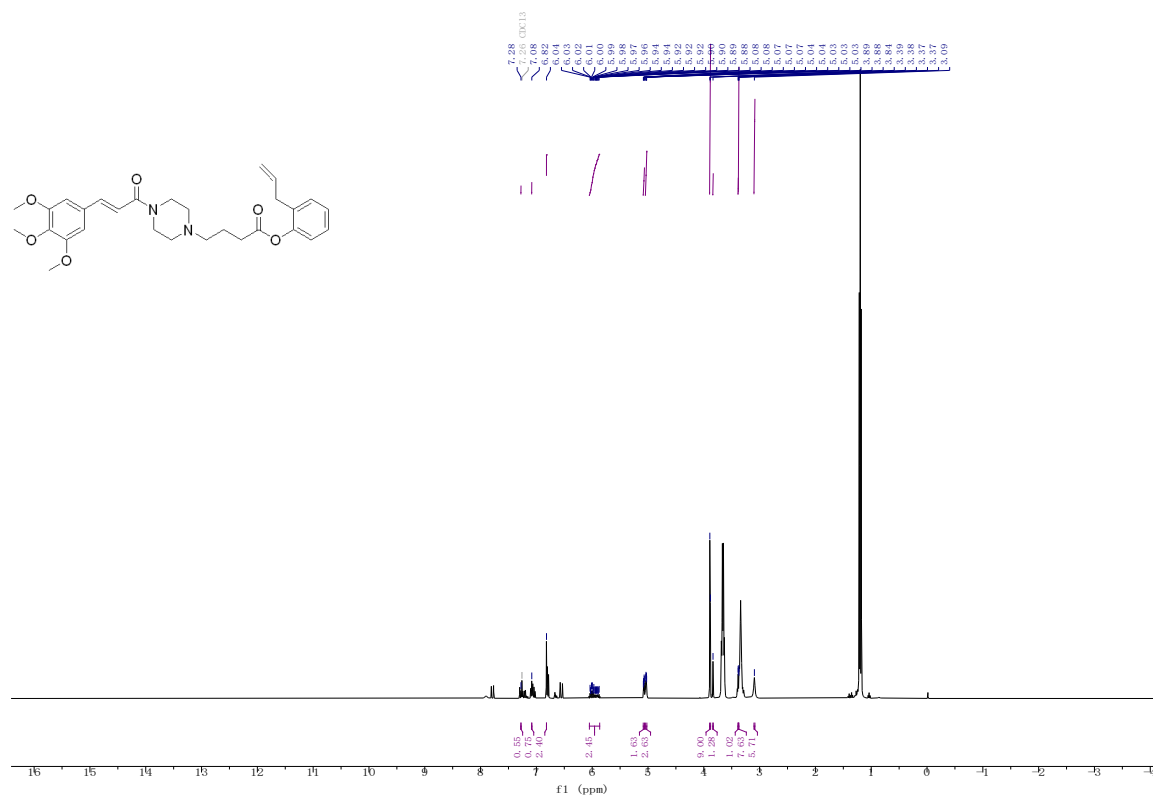


¹H NMR (600 MHz, CDCl₃) spectrum of **B3**



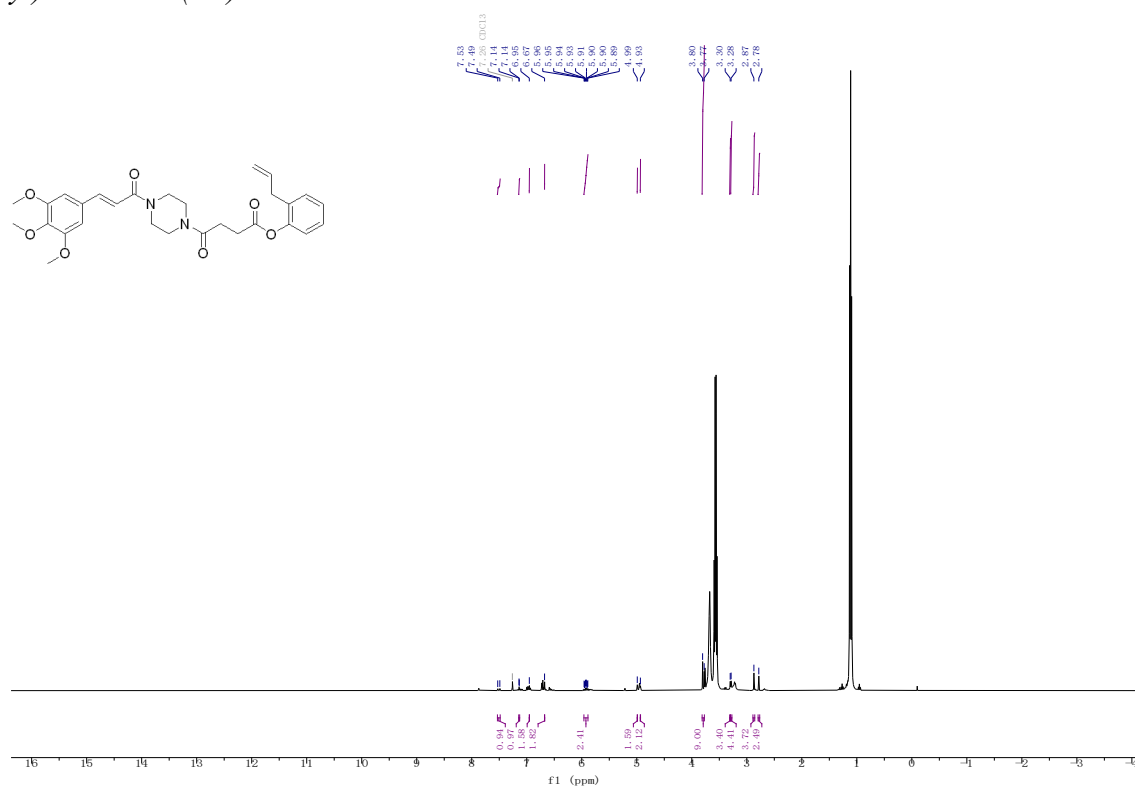
¹³C NMR (600 MHz, CDCl₃) spectrum of **B3**

2-allylphenyl (E)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (A4):

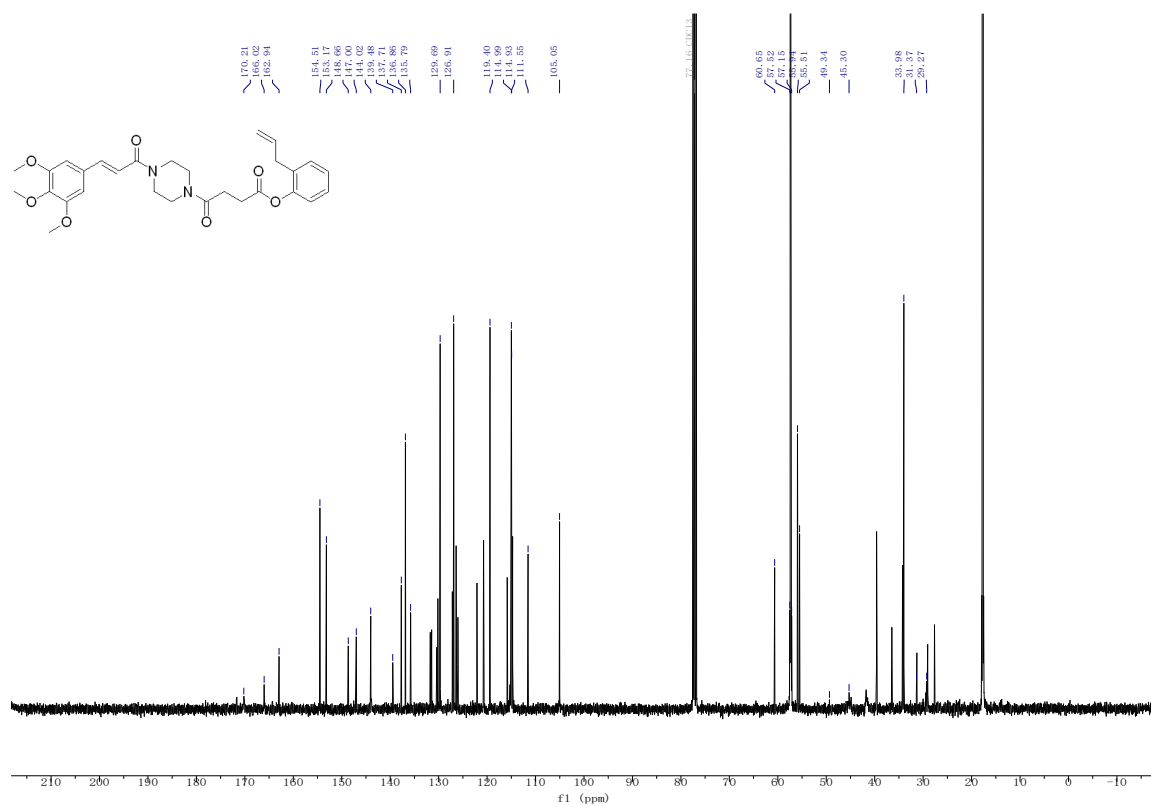


¹H NMR (600 MHz,

2-allylphenyl (*E*)-4-oxo-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**B4**):

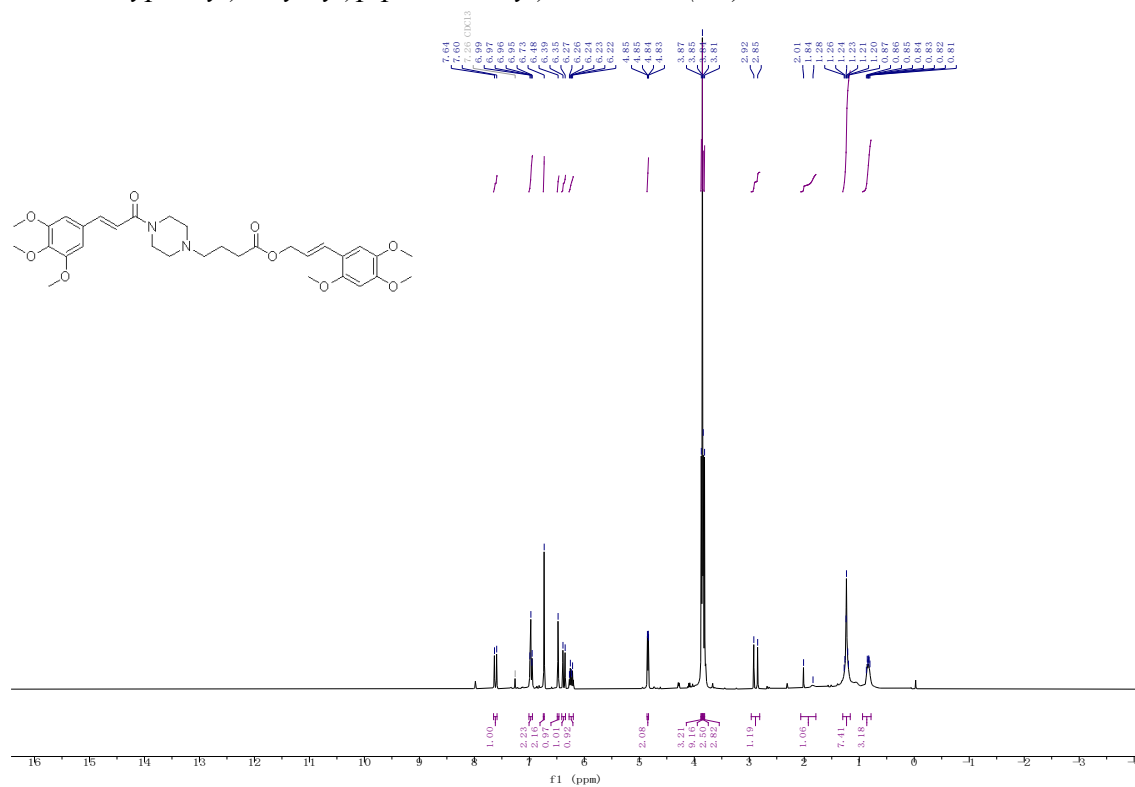


¹H NMR (600 MHz, CDCl₃) spectrum of **B4**

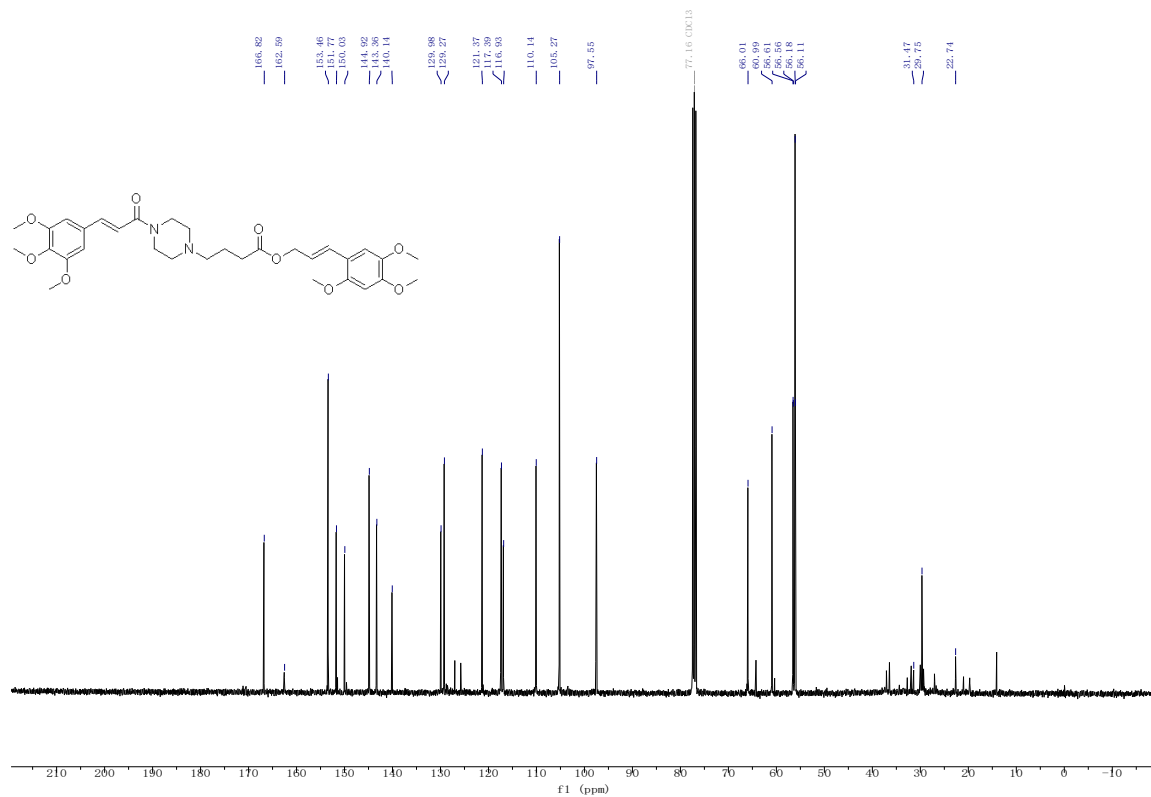


¹³C NMR (600 MHz, CDCl₃) spectrum of **B4**

(E)-3-(2,4,5-trimethoxyphenyl)allyl 4-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**A5**):

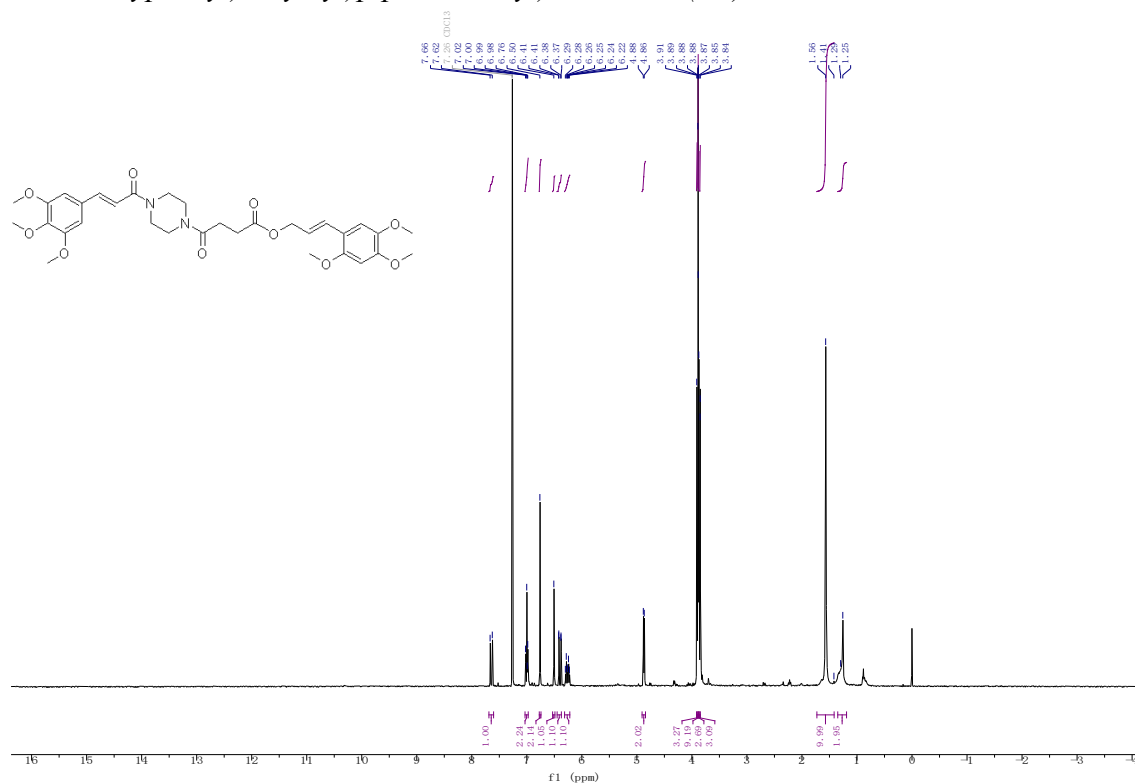


¹H NMR (600 MHz, CDCl₃) spectrum of **A5**

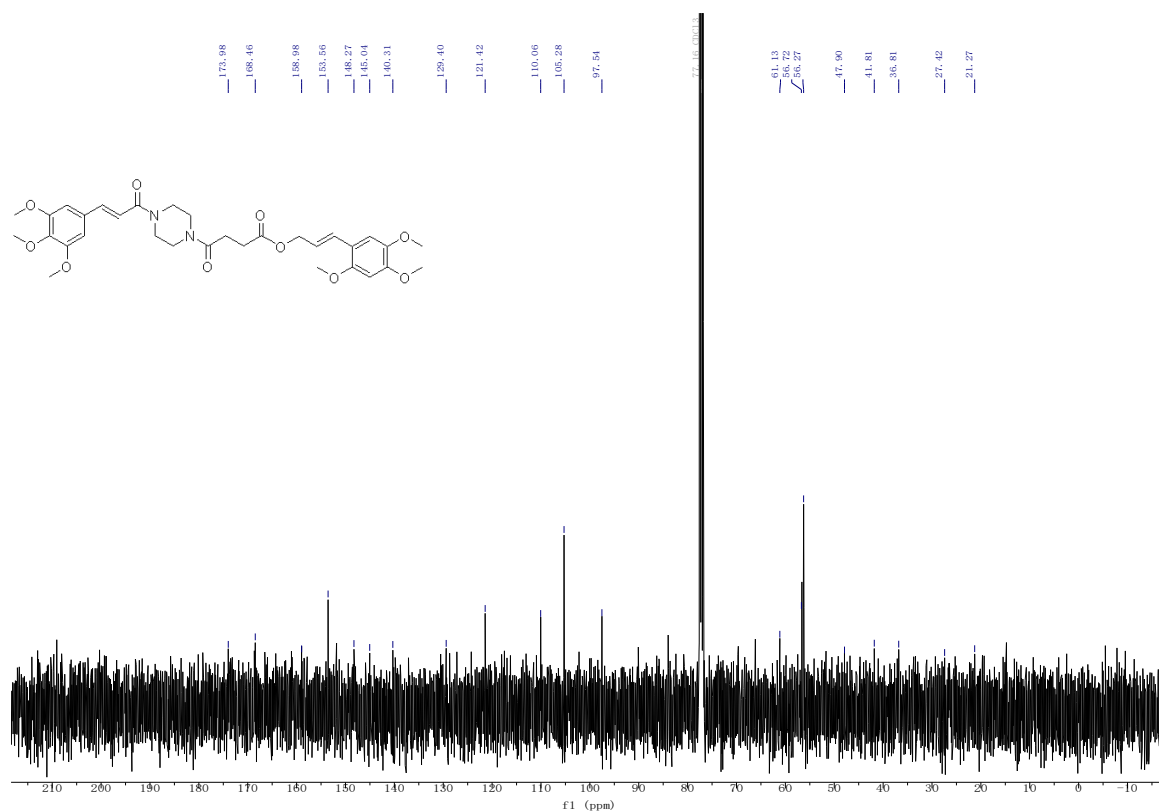


¹³C NMR (600 MHz, CDCl₃) spectrum of **A5**

(E)-3-(2,4,5-trimethoxyphenyl)allyl 4-oxo-4-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**B5**):

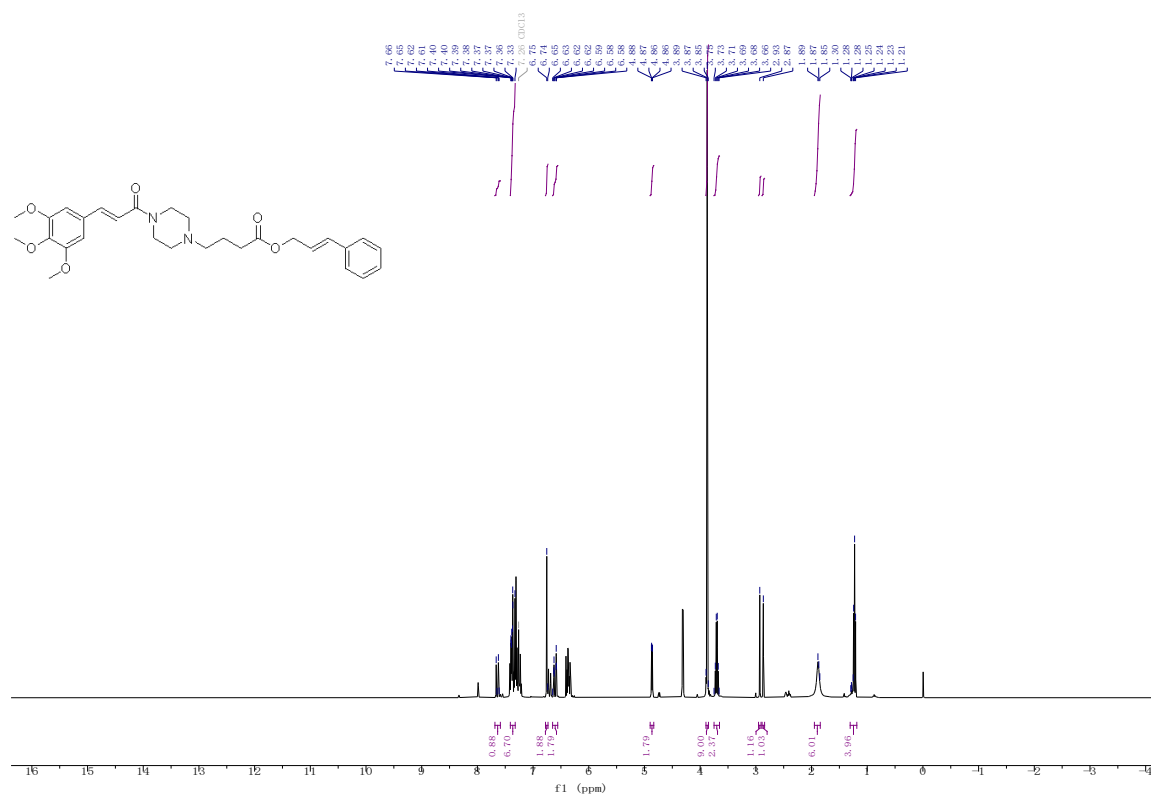


¹H NMR (600 MHz, CDCl₃) spectrum of **B5**

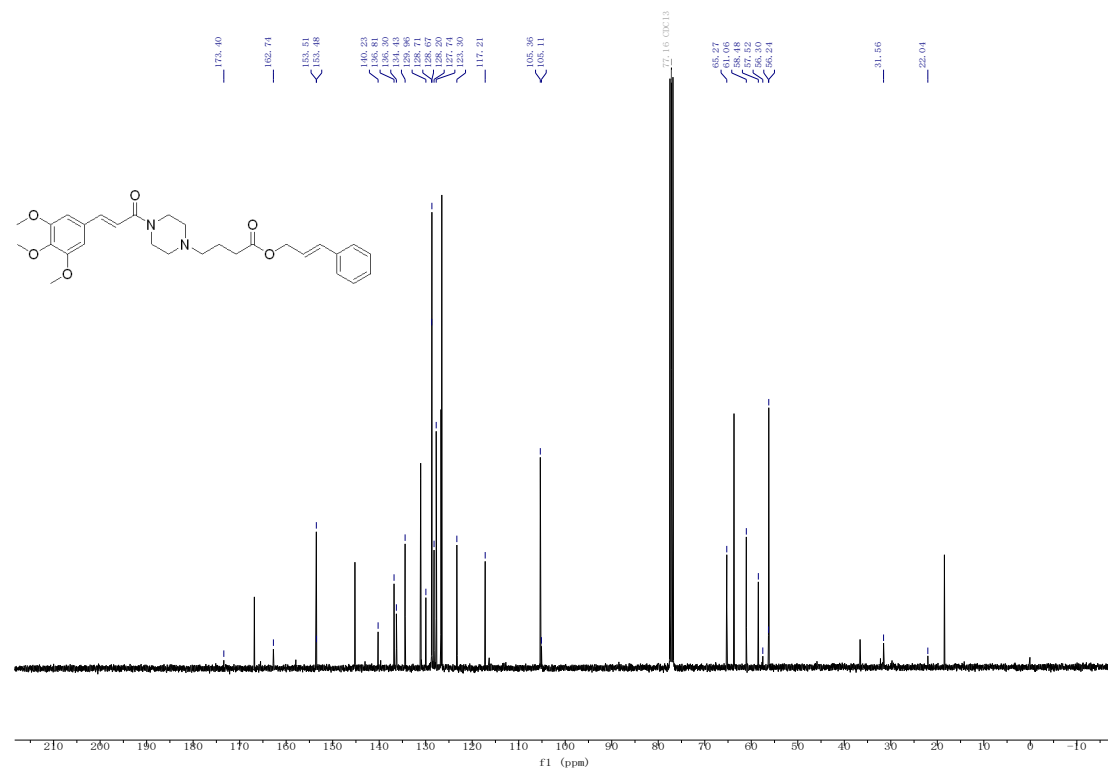


¹³C NMR (600 MHz, CDCl₃) spectrum of **B5**

cinnamyl 4-(4-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (A6):

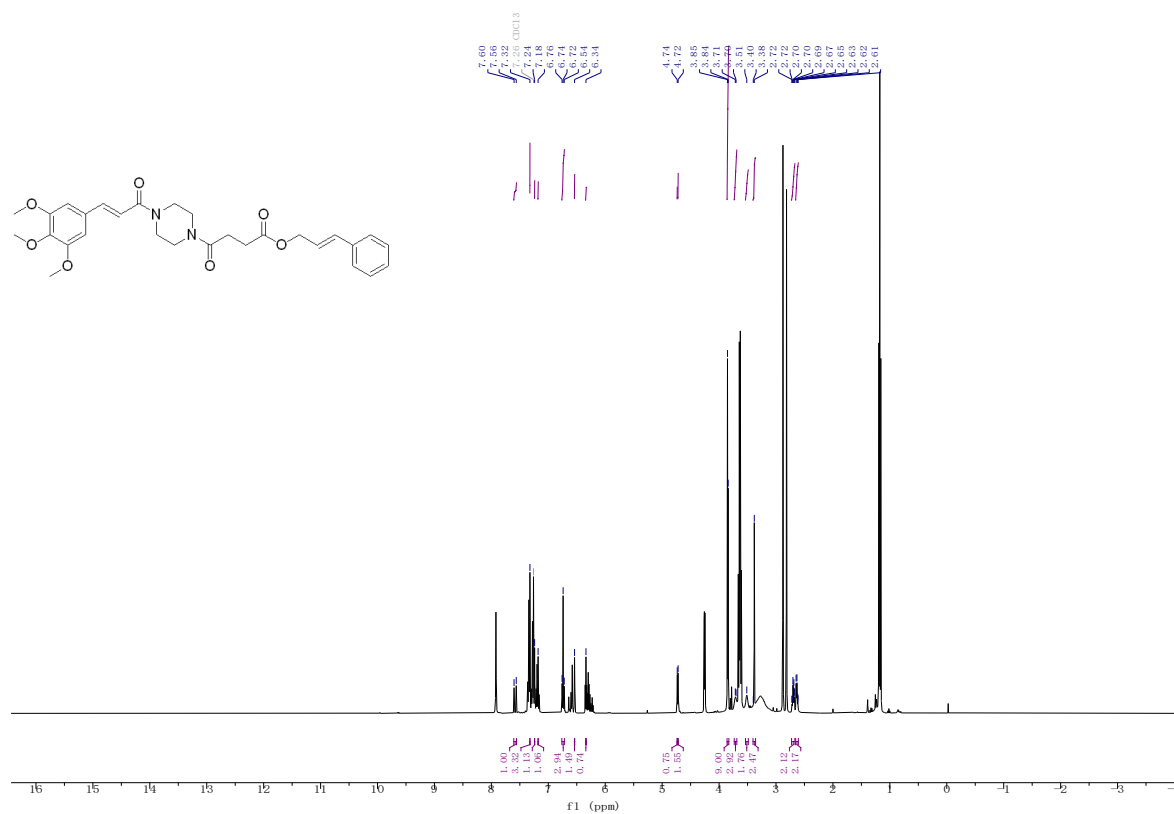


¹H NMR (600 MHz, CDCl₃) spectrum of A6

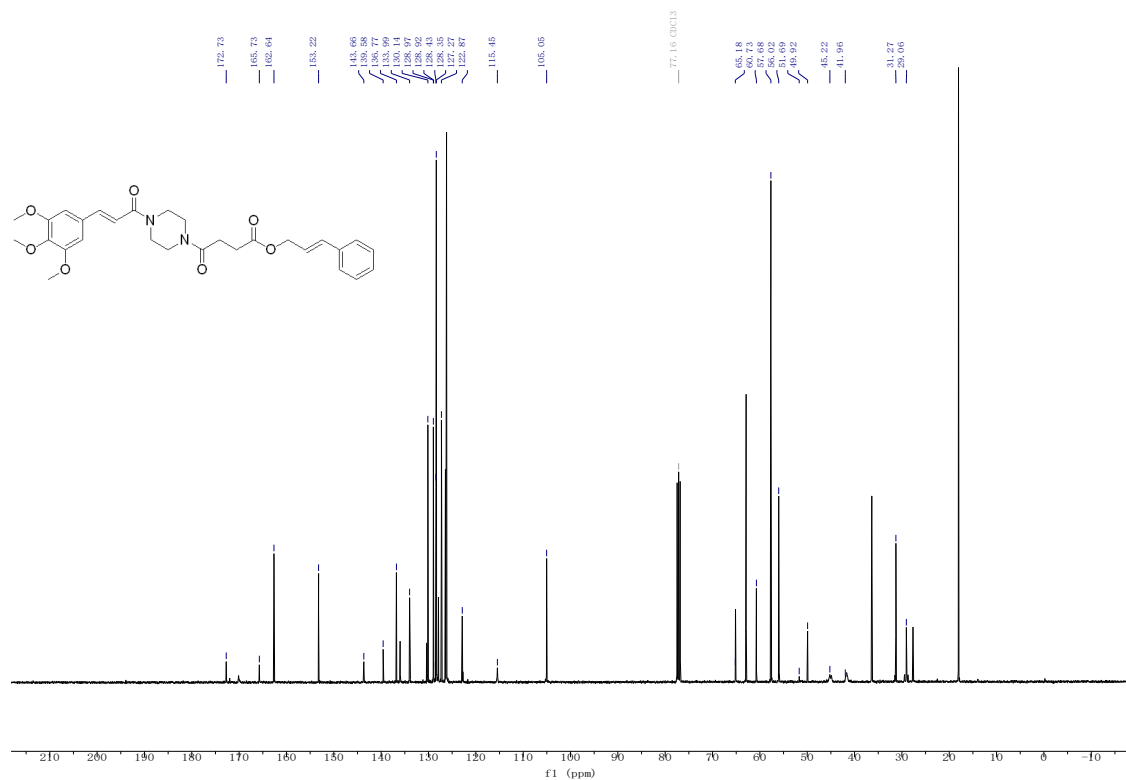


¹³C NMR (600 MHz, CDCl₃) spectrum of A6

cinnamyl 4-oxo-4-(4-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (B6):

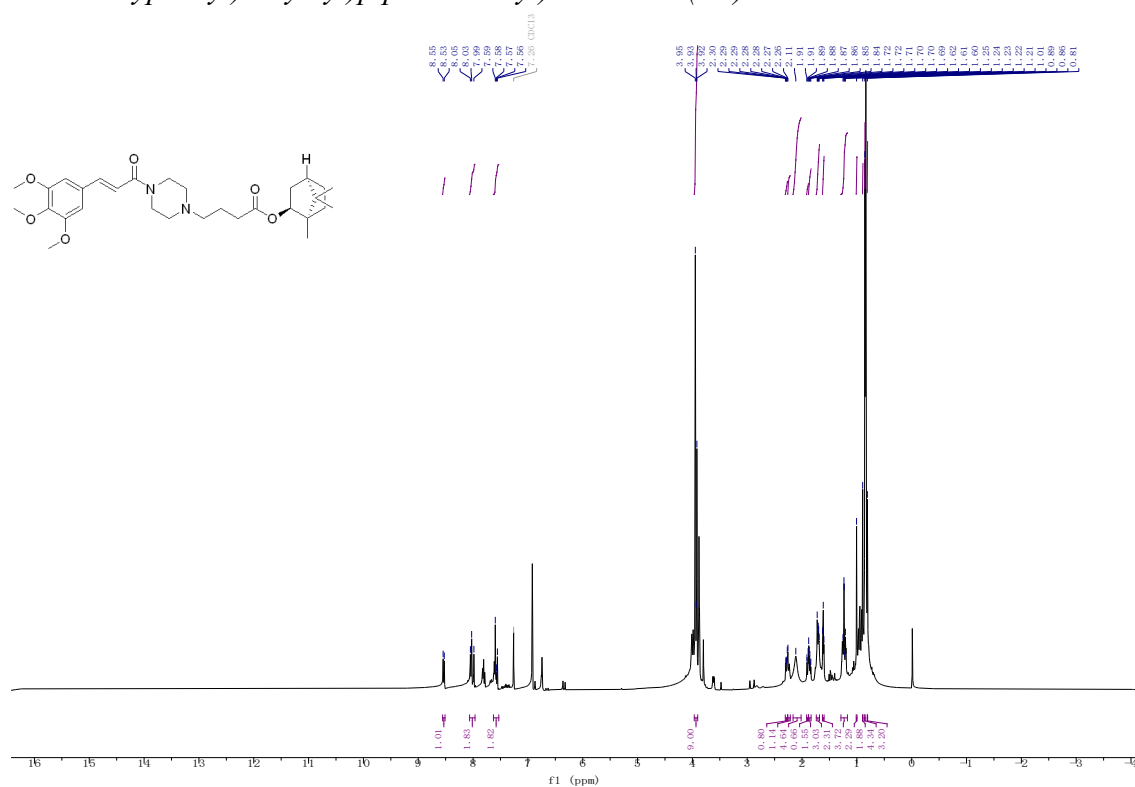


¹H NMR (400 MHz, CDCl₃) spectrum of B6

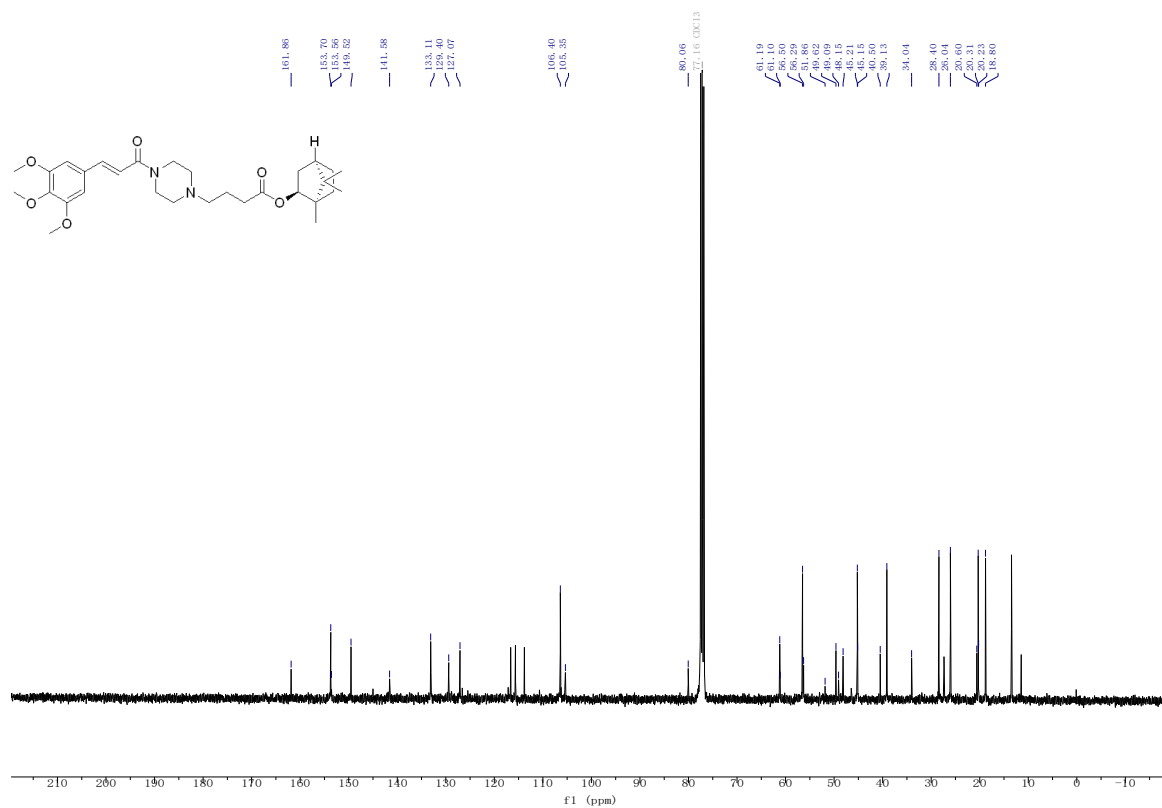


¹³C NMR (101 MHz, CDCl₃) spectrum of B6

rel-(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 4-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**A7**):

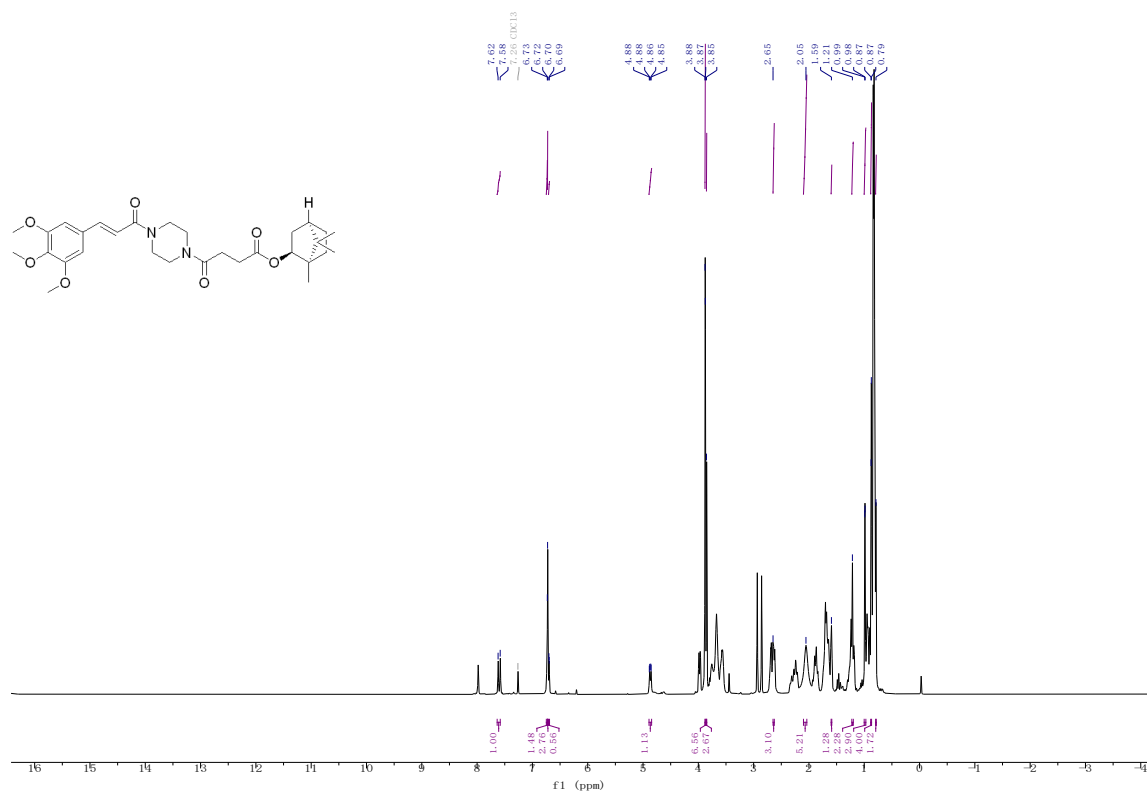


¹H NMR (400 MHz, CDCl₃) spectrum of **A7**

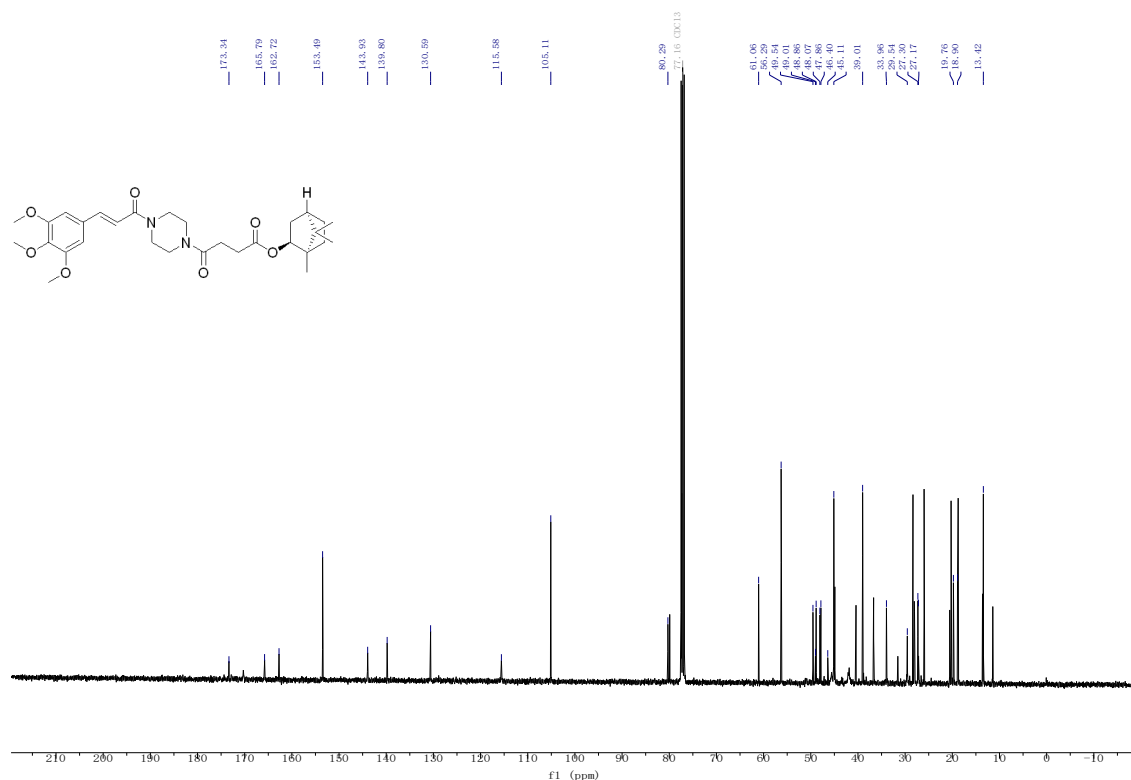


¹³C NMR (101 MHz, CDCl₃) spectrum of **A7**

(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 4-oxo-4-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butanoate (**B7**):



¹H NMR (400 MHz, CDCl₃) spectrum of **B7**



¹³C NMR (101 MHz, CDCl₃) spectrum of **B7**

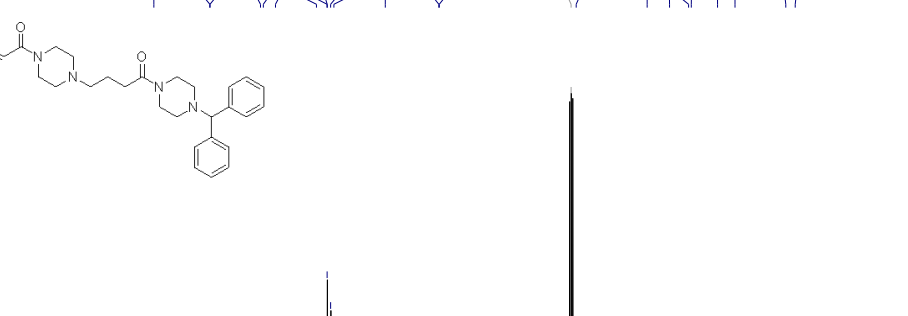
COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CCCN(C2)C(=O)N3CCN(C4C5=CC=CC=C5)C5=CC=CC=C4)CC3

1H NMR spectrum (CDCl₃) of compound 10. The spectrum shows peaks from 0 to 8 ppm. Aromatic and vinylic protons appear between 6.5 and 7.6 ppm. The piperazine ring protons are in the 3.5-4.1 ppm range. The dimethoxyphenyl group shows a singlet at 3.8 ppm and a doublet at 3.9 ppm. Integration values are provided below the baseline.

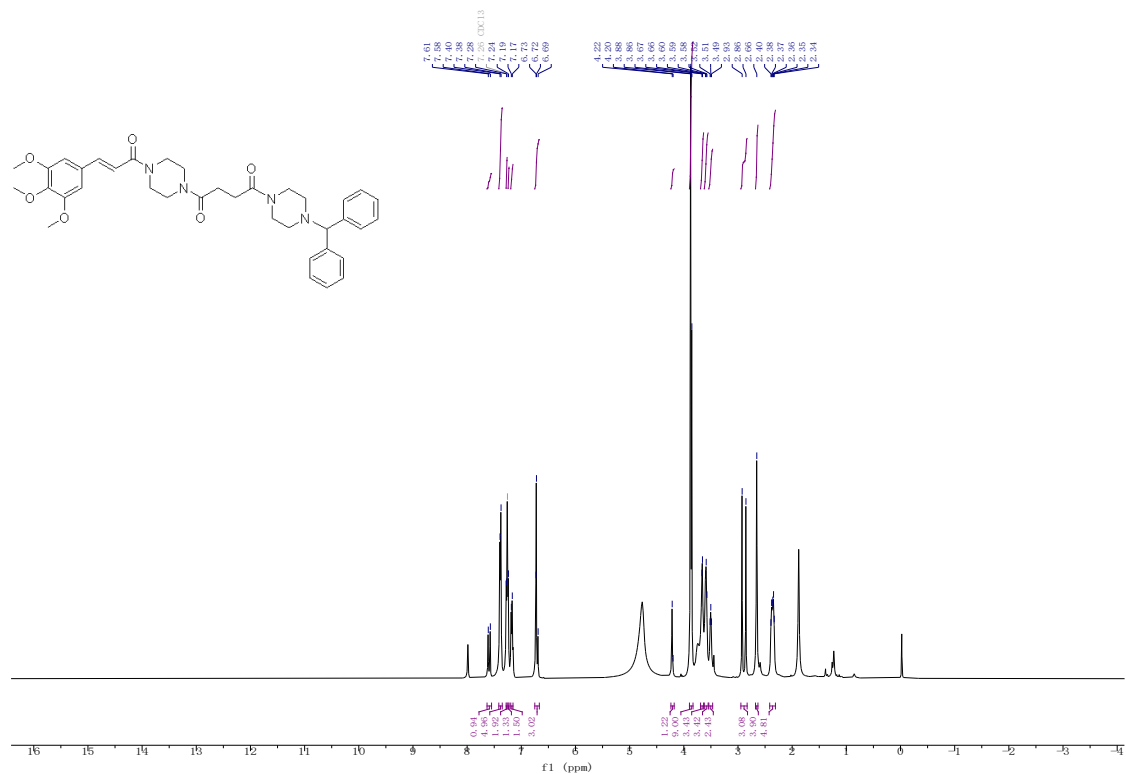
Chemical Shift (ppm)	Integration
7.59	1.00
7.57	4.16
7.55	2.20
7.53	2.20
7.42	2.21
7.41	
7.39	
7.30	
7.30	
7.29	
7.28	
7.26	
7.21	
7.19	
7.17	
6.72	
6.69	
4.26	
4.23	
3.89	
3.86	
3.86	
3.85	
3.83	
3.83	
3.73	
3.68	
3.67	
3.67	
3.60	
3.44	
3.44	
2.42	
2.37	
2.37	
2.36	
2.33	
2.33	
1.84	
1.83	
1.75	
1.44	
1.41	
1.41	
1.38	
1.33	
1.31	
1.30	
1.25	
1.21	

Chemical structure of the compound is shown above the spectrum. The spectrum displays peaks corresponding to the chemical structure, with the following chemical shifts (ppm) labeled above the peaks:

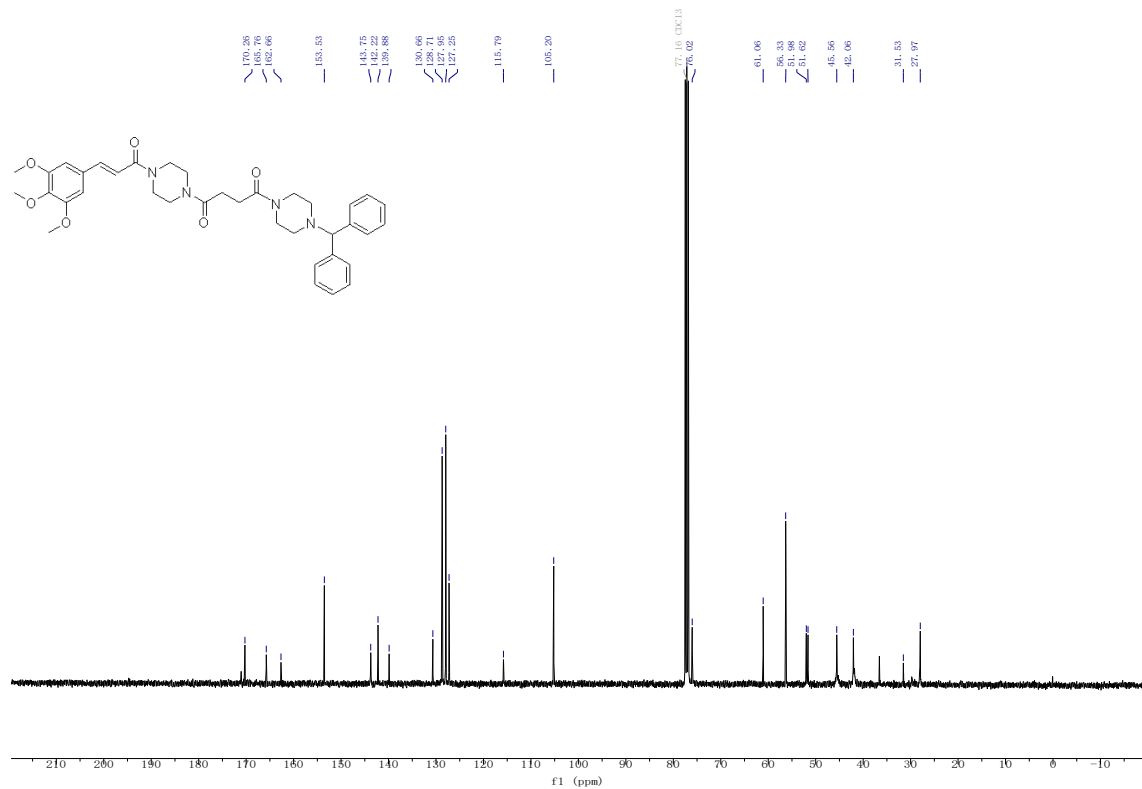
- 165.38
- 153.54
- 153.51
- 145.63
- 142.23
- 139.66
- 131.00
- 128.75
- 128.73
- 128.71
- 127.31
- 116.45
- 105.12
- 105.03
- 77.16 (CDCl₃)
- 76.98
- 61.08
- 56.54
- 56.52
- 51.74
- 46.19
- 42.50
- 31.77
- 29.77

COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CC2)CCCN(=O)N3CCN(CC3C4=CC=CC=C4)C5=CC=CC=C5¹³C NMR (101 MHz, CDCl₃) spectrum of **A8**

(E)-1-(4-benzhydrylpiperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B8**):



¹H NMR (400 MHz, CDCl₃) spectrum of **B8**



¹³C NMR (101 MHz, CDCl₃) spectrum of **B8**

Chemical structure of compound 10: COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CC2)CCCN3CCN(C(C3)c4ccc(F)cc4)C(=O)N5CCN(C(C5)c6ccc(F)cc6)C(=O)N7CCN(C(C7)c8ccc(F)cc8)C(=O)N9CCN(C(C9)c10ccc(F)cc10)C(=O)N11CCN(C(C11)c12ccc(F)cc12)C(=O)N13CCN(C(C13)c14ccc(F)cc14)C(=O)N15CCN(C(C15)c16ccc(F)cc16)C(=O)N17CCN(C(C17)c18ccc(F)cc18)C(=O)N19CCN(C(C19)c20ccc(F)cc20)C(=O)N21CCN(C(C21)c22ccc(F)cc22)C(=O)N23CCN(C(C23)c24ccc(F)cc24)C(=O)N25CCN(C(C25)c26ccc(F)cc26)C(=O)N27CCN(C(C27)c28ccc(F)cc28)C(=O)N29CCN(C(C29)c30ccc(F)cc30)C(=O)N31CCN(C(C31)c32ccc(F)cc32)C(=O)N33CCN(C(C33)c34ccc(F)cc34)C(=O)N35CCN(C(C35)c36ccc(F)cc36)C(=O)N37CCN(C(C37)c38ccc(F)cc38)C(=O)N39CCN(C(C39)c40ccc(F)cc40)C(=O)N41CCN(C(C41)c42ccc(F)cc42)C(=O)N43CCN(C(C43)c44ccc(F)cc44)C(=O)N45CCN(C(C45)c46ccc(F)cc46)C(=O)N47CCN(C(C47)c48ccc(F)cc48)C(=O)N49CCN(C(C49)c50ccc(F)cc50)C(=O)N51CCN(C(C51)c52ccc(F)cc52)C(=O)N53CCN(C(C53)c54ccc(F)cc54)C(=O)N55CCN(C(C55)c56ccc(F)cc56)C(=O)N57CCN(C(C57)c58ccc(F)cc58)C(=O)N59CCN(C(C59)c60ccc(F)cc60)C(=O)N61CCN(C(C61)c62ccc(F)cc62)C(=O)N63CCN(C(C63)c64ccc(F)cc64)C(=O)N65CCN(C(C65)c66ccc(F)cc66)C(=O)N67CCN(C(C67)c68ccc(F)cc68)C(=O)N69CCN(C(C69)c70ccc(F)cc70)C(=O)N71CCN(C(C71)c72ccc(F)cc72)C(=O)N73CCN(C(C73)c74ccc(F)cc74)C(=O)N75CCN(C(C75)c76ccc(F)cc76)C(=O)N77CCN(C(C77)c78ccc(F)cc78)C(=O)N79CCN(C(C79)c80ccc(F)cc80)C(=O)N81CCN(C(C81)c82ccc(F)cc82)C(=O)N83CCN(C(C83)c84ccc(F)cc84)C(=O)N85CCN(C(C85)c86ccc(F)cc86)C(=O)N87CCN(C(C87)c88ccc(F)cc88)C(=O)N89CCN(C(C89)c90ccc(F)cc90)C(=O)N91CCN(C(C91)c92ccc(F)cc92)C(=O)N93CCN(C(C93)c94ccc(F)cc94)C(=O)N95CCN(C(C95)c96ccc(F)cc96)C(=O)N97CCN(C(C97)c98ccc(F)cc98)C(=O)N99CCN(C(C99)c100ccc(F)cc100)C(=O)N101CCN(C(C101)c102ccc(F)cc102)C(=O)N103CCN(C(C103)c104ccc(F)cc104)C(=O)N105CCN(C(C105)c106ccc(F)cc106)C(=O)N107CCN(C(C107)c108ccc(F)cc108)C(=O)N109CCN(C(C109)c110ccc(F)cc110)C(=O)N111CCN(C(C111)c112ccc(F)cc112)C(=O)N113CCN(C(C113)c114ccc(F)cc114)C(=O)N115CCN(C(C115)c116ccc(F)cc116)C(=O)N117CCN(C(C117)c118ccc(F)cc118)C(=O)N119CCN(C(C119)c120ccc(F)cc120)C(=O)N121CCN(C(C121)c122ccc(F)cc122)C(=O)N123CCN(C(C123)c124ccc(F)cc124)C(=O)N125CCN(C(C125)c126ccc(F)cc126)C(=O)N127CCN(C(C127)c128ccc(F)cc128)C(=O)N129CCN(C(C129)c130ccc(F)cc130)C(=O)N131CCN(C(C131)c132ccc(F)cc132)C(=O)N133CCN(C(C133)c134ccc(F)cc134)C(=O)N135CCN(C(C135)c136ccc(F)cc136)C(=O)N137CCN(C(C137)c138ccc(F)cc138)C(=O)N139CCN(C(C139)c140ccc(F)cc140)C(=O)N141CCN(C(C141)c142ccc(F)cc142)C(=O)N143CCN(C(C143)c144ccc(F)cc144)C(=O)N145CCN(C(C145)c146ccc(F)cc146)C(=O)N147CCN(C(C147)c148ccc(F)cc148)C(=O)N149CCN(C(C149)c150ccc(F)cc150)C(=O)N151CCN(C(C151)c152ccc(F)cc152)C(=O)N153CCN(C(C153)c154ccc(F)cc154)C(=O)N155CCN(C(C155)c156ccc(F)cc156)C(=O)N157CCN(C(C157)c158ccc(F)cc158)C(=O)N159CCN(C(C159)c160ccc(F)cc160)C(=O)N161CCN(C(C161)c162ccc(F)cc162)C(=O)N163CCN(C(C163)c164ccc(F)cc164)C(=O)N165CCN(C(C165)c166ccc(F)cc166)C(=O)N167CCN(C(C167)c168ccc(F)cc168)C(=O)N169CCN(C(C169)c170ccc(F)cc170)C(=O)N171CCN(C(C171)c172ccc(F)cc172)C(=O)N173CCN(C(C173)c174ccc(F)cc174)C(=O)N175CCN(C(C175)c176ccc(F)cc176)C(=O)N177CCN(C(C177)c178ccc(F)cc178)C(=O)N179CCN(C(C179)c180ccc(F)cc180)C(=O)N181CCN(C(C181)c182ccc(F)cc182)C(=O)N183CCN(C(C183)c184ccc(F)cc184)C(=O)N185CCN(C(C185)c186ccc(F)cc186)C(=O)N187CCN(C(C187)c188ccc(F)cc188)C(=O)N189CCN(C(C189)c190ccc(F)cc190)C(=O)N191CCN(C(C191)c192ccc(F)cc192)C(=O)N193CCN(C(C193)c194ccc(F)cc194)C(=O)N195CCN(C(C195)c196ccc(F)cc196)C(=O)N197CCN(C(C197)c198ccc(F)cc198)C(=O)N199CCN(C(C199)c200ccc(F)cc200)C(=O)N201CCN(C(C201)c202ccc(F)cc202)C(=O)N203CCN(C(C203)c204ccc(F)cc204)C(=O)N205CCN(C(C205)c206ccc(F)cc206)C(=O)N207CCN(C(C207)c208ccc(F)cc208)C(=O)N209CCN(C(C209)c210ccc(F)cc210)C(=O)N211CCN(C(C211)c212ccc(F)cc212)C(=O)N213CCN(C(C213)c214ccc(F)cc214)C(=O)N215CCN(C(C215)c216ccc(F)cc216)C(=O)N217CCN(C(C217)c218ccc(F)cc218)C(=O)N219CCN(C(C219)c220ccc(F)cc220)C(=O)N221CCN(C(C221)c222ccc(F)cc222)C(=O)N223CCN(C(C223)c224ccc(F)cc224)C(=O)N225CCN(C(C225)c226ccc(F)cc226)C(=O)N227CCN(C(C227)c228ccc(F)cc228)C(=O)N229CCN(C(C229)c230ccc(F)cc230)C(=O)N231CCN(C(C231)c232ccc(F)cc232)C(=O)N233CCN(C(C233)c234ccc(F)cc234)C(=O)N235CCN(C(C235)c236ccc(F)cc236)C(=O)N237CCN(C(C237)c238ccc(F)cc238)C(=O)N239CCN(C(C239)c240ccc(F)cc240)C(=O)N241CCN(C(C241)c242ccc(F)cc242)C(=O)N243CCN(C(C243)c244ccc(F)cc244)C(=O)N245CCN(C(C245)c246ccc(F)cc246)C(=O)N247CCN(C(C247)c248ccc(F)cc248)C(=O)N249CCN(C(C249)c250ccc(F)cc250)C(=O)N251CCN(C(C251)c252ccc(F)cc252)C(=O)N253CCN(C(C253)c254ccc(F)cc254)C(=O)N255CCN(C(C255)c256ccc(F)cc256)C(=O)N257CCN(C(C257)c258ccc(F)cc258)C(=O)N259CCN(C(C259)c260ccc(F)cc260)C(=O)N261CCN(C(C261)c262ccc(F)cc262)C(=O)N263CCN(C(C263)c264ccc(F)cc264)C(=O)N265CCN(C(C265)c266ccc(F)cc266)C(=O)N267CCN(C(C267)c268ccc(F)cc268)C(=O)N269CCN(C(C269)c270ccc(F)cc270)C(=O)N271CCN(C(C271)c272ccc(F)cc272)C(=O)N273CCN(C(C273)c274ccc(F)cc274)C(=O)N275CCN(C(C275)c276ccc(F)cc276)C(=O)N277CCN(C(C277)c278ccc(F)cc278)C(=O)N279CCN(C(C279)c280ccc(F)cc280)C(=O)N281CCN(C(C281)c282ccc(F)cc282)C(=O)N283CCN(C(C283)c284ccc(F)cc284)C(=O)N285CCN(C(C285)c286ccc(F)cc286)C(=O)N287CCN(C(C287)c288ccc(F)cc288)C(=O)N289CCN(C(C289)c290ccc(F)cc290)C(=O)N291CCN(C(C291)c292ccc(F)cc292)C(=O)N293CCN(C(C293)c294ccc(F)cc294)C(=O)N295CCN(C(C295)c

Chemical structure of the compound is shown above the spectrum. The structure is a symmetrical molecule consisting of two 4-fluorophenyl rings connected by a central chain. The chain includes a carbonyl group, a piperazine ring, and a methylene group. The spectrum shows peaks corresponding to the chemical shifts of the various atoms in the molecule.

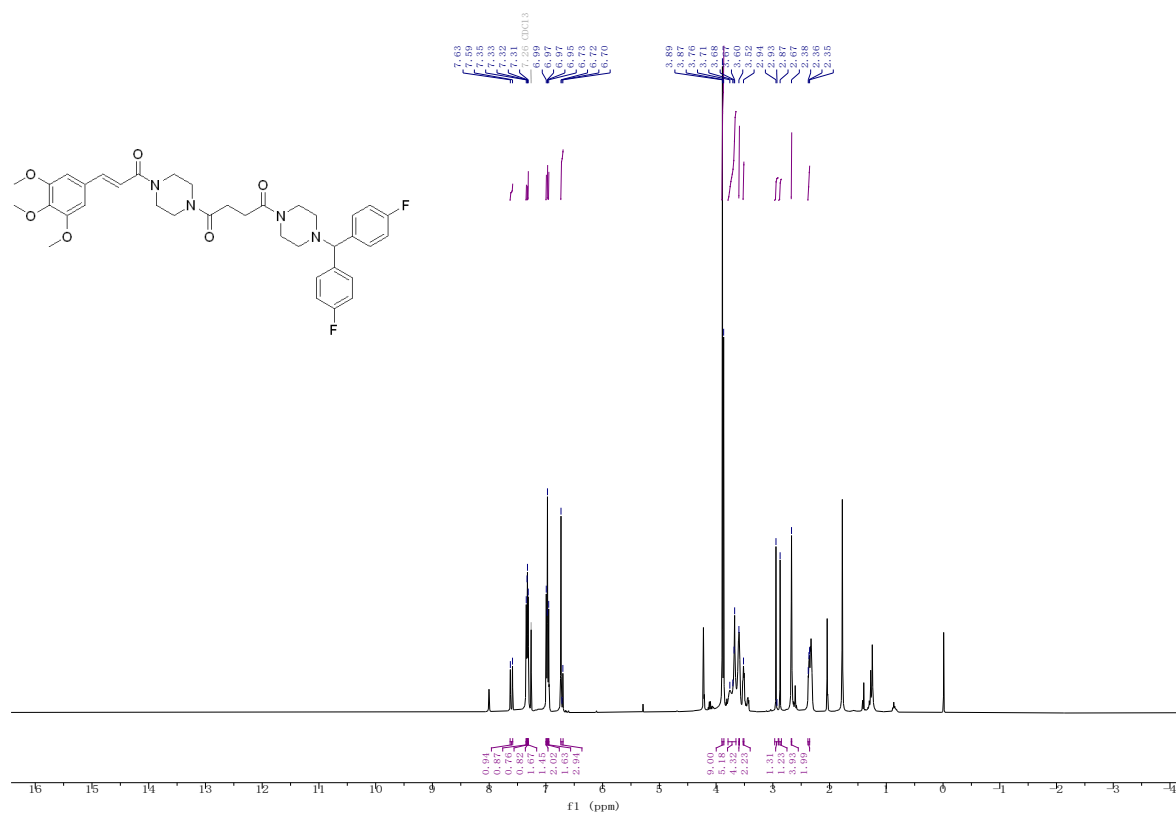
Chemical structure: COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CCC(=O)N3CCN(C4=CC=C(F)C=C4)CC3)CC2

Peak list (ppm):

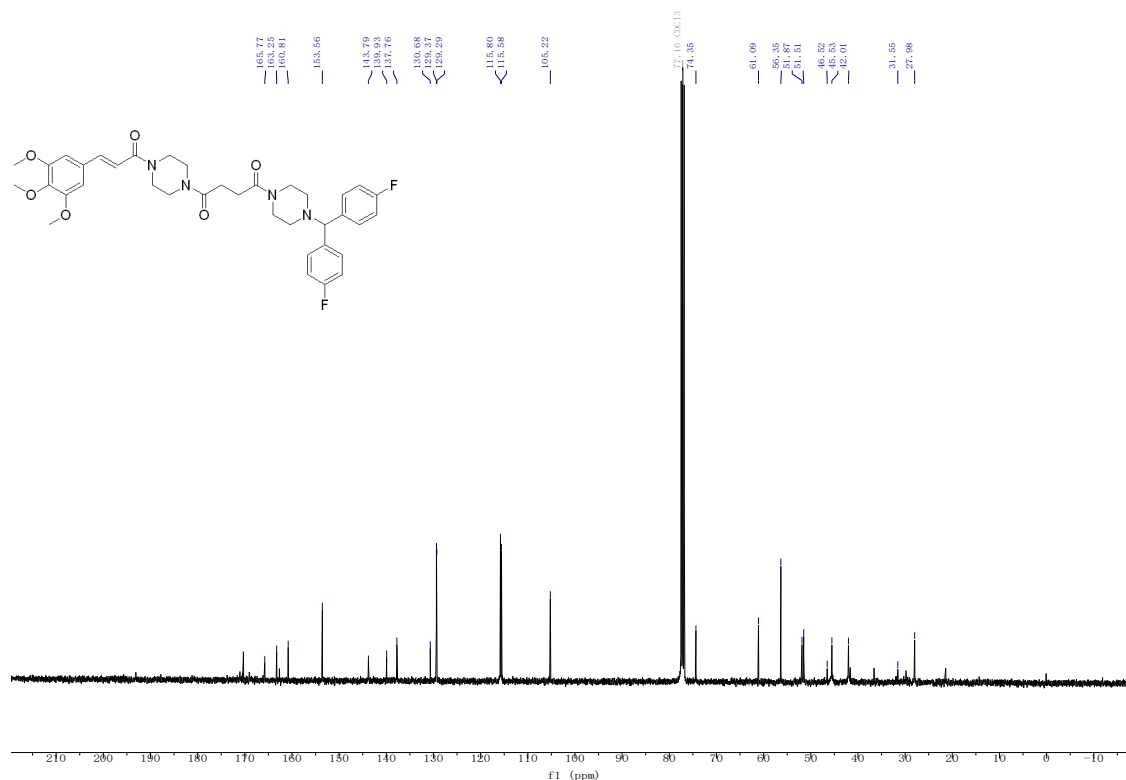
Peak (ppm)
165.39
153.32
143.04
139.64
137.74
130.93
129.39
129.31
122.41
116.54
115.65
115.64
104.97
92.30
77.43
77.43
61.12
56.29
51.65
34.48
28.81

 ^{13}C NMR (101 MHz, CDCl_3) spectrum of **A9**

(E)-1-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B9**):

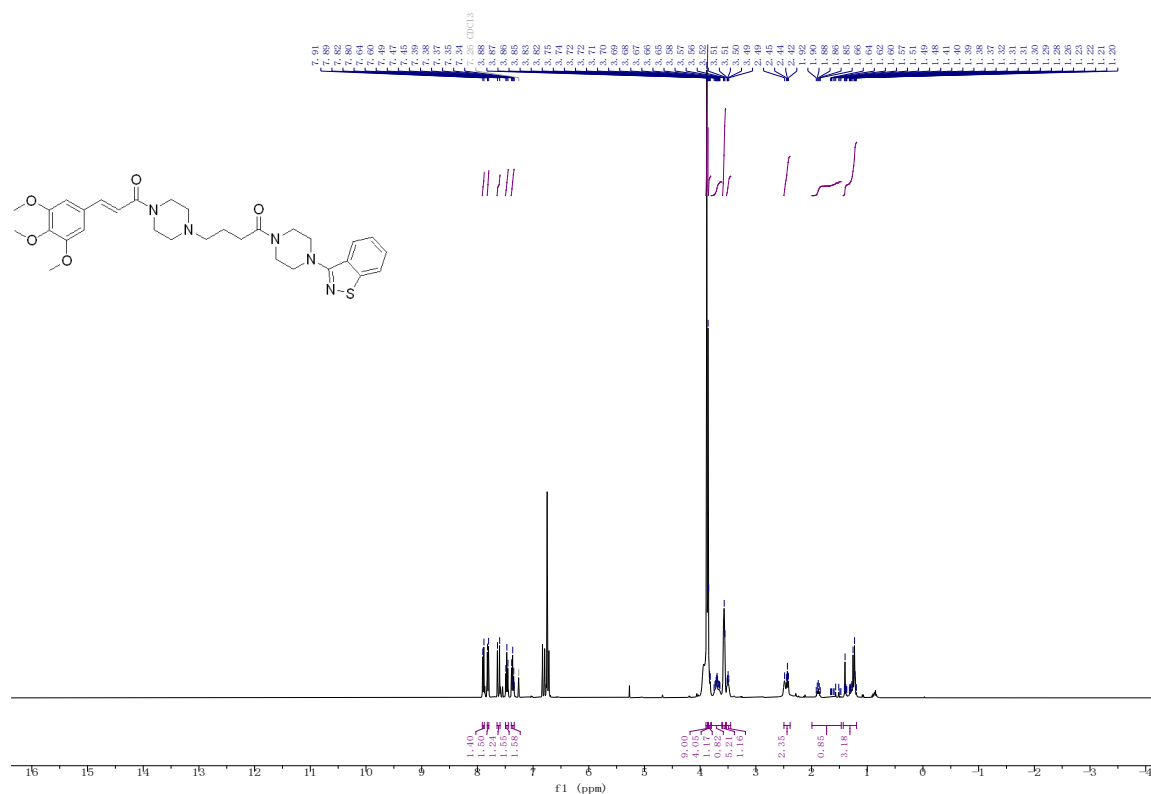


¹H NMR (400 MHz, CDCl₃) spectrum of **B9**

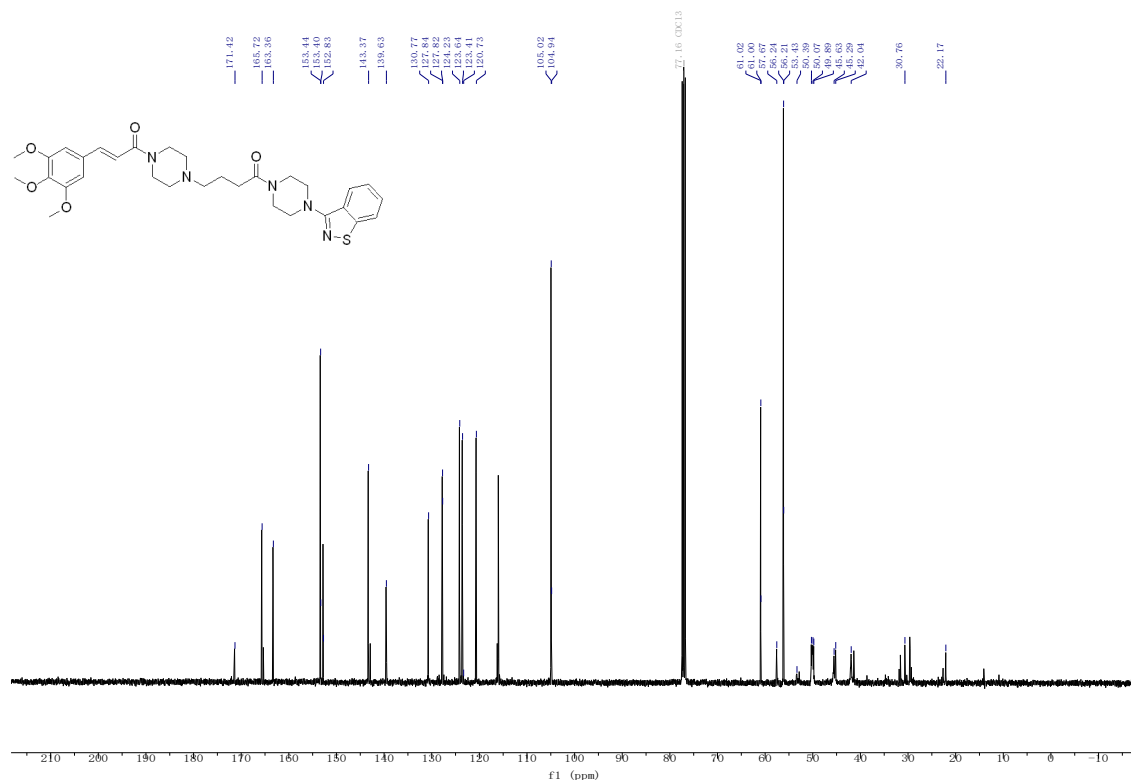


¹³C NMR (101 MHz, CDCl₃) spectrum of **B9**

(E)-1-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A10**):

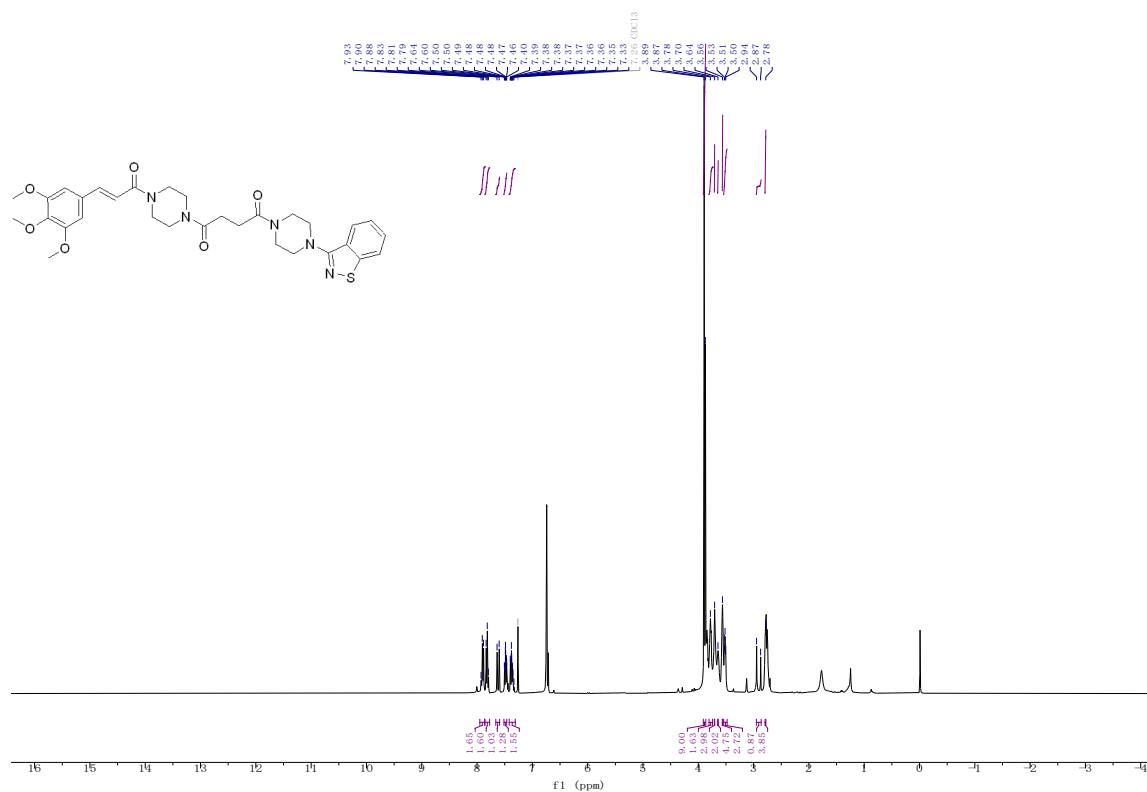


¹H NMR (400 MHz, CDCl₃) spectrum of **A10**

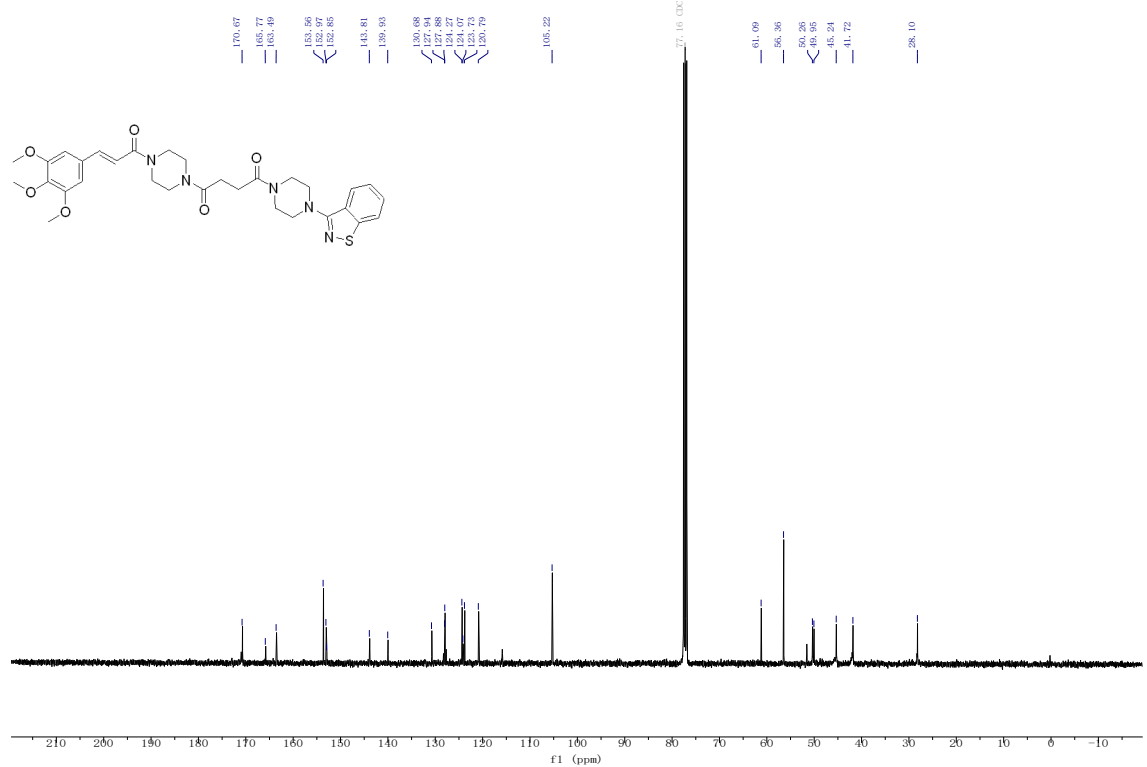


¹³C NMR (101 MHz, CDCl₃) spectrum of **A10**

(E)-1-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B10**):

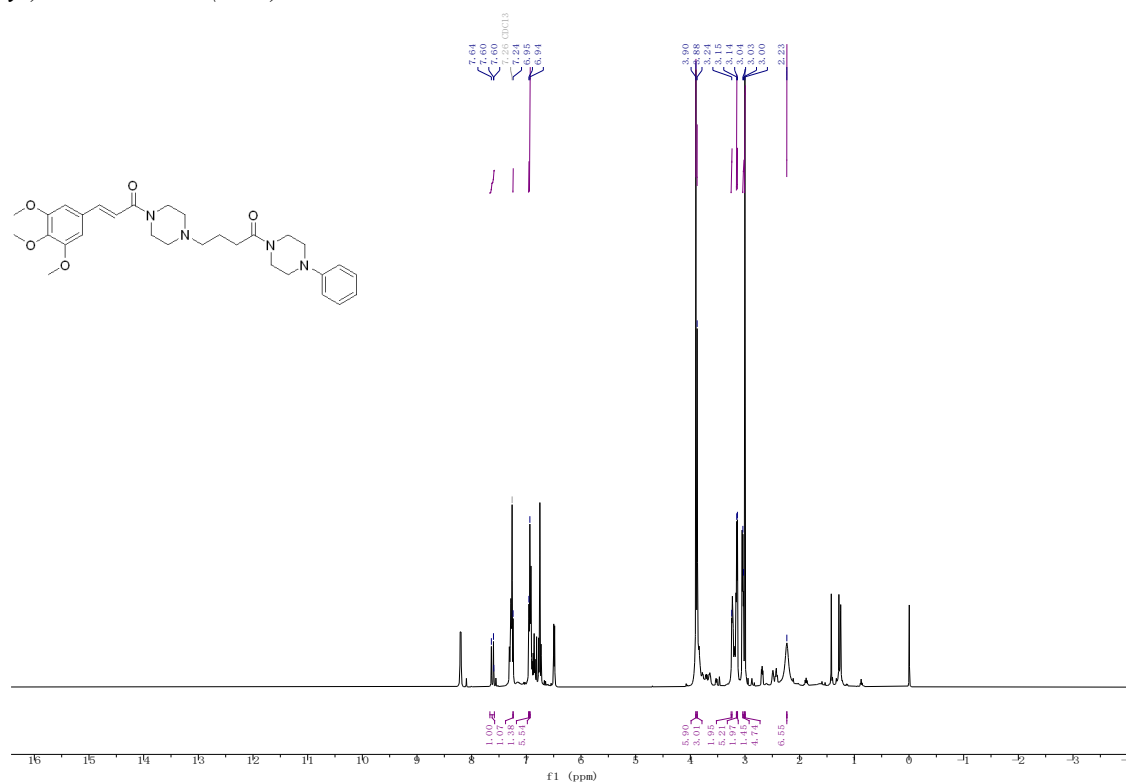


¹H NMR (400 MHz, CDCl₃) spectrum of **B10**

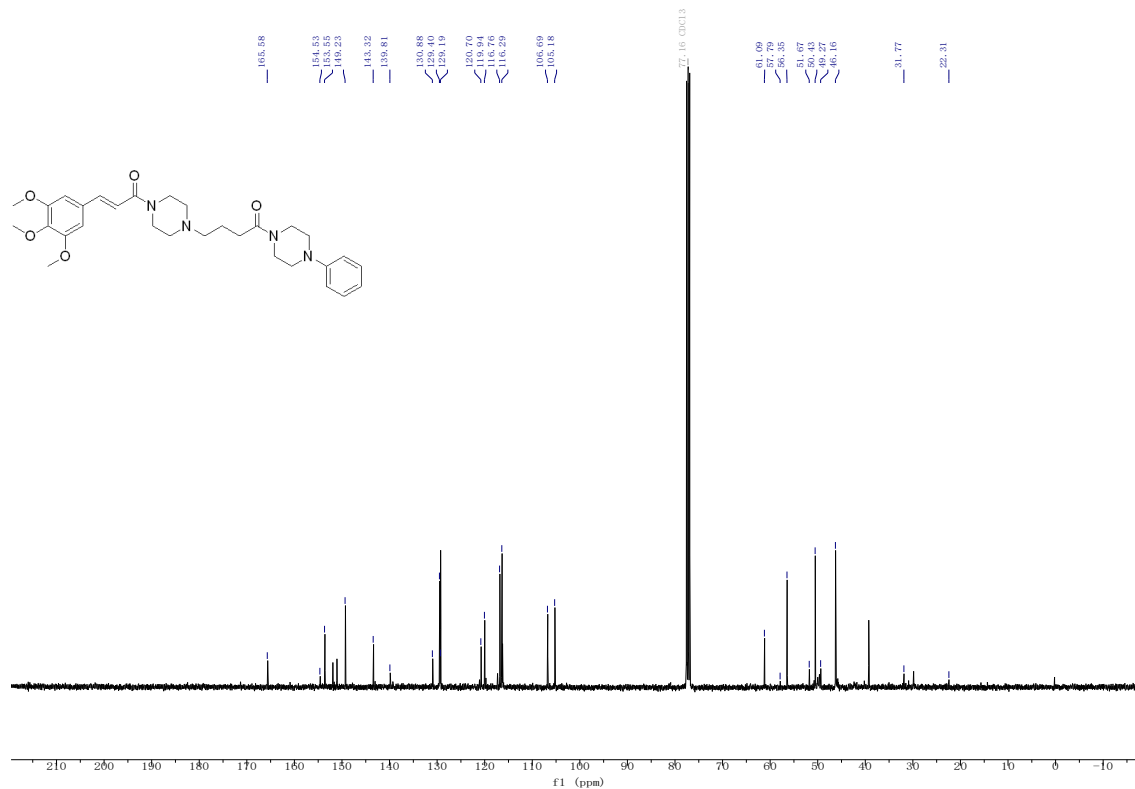


¹³C NMR (101 MHz, CDCl₃) spectrum of **B10**

(E)-1-(4-phenylpiperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A11**):

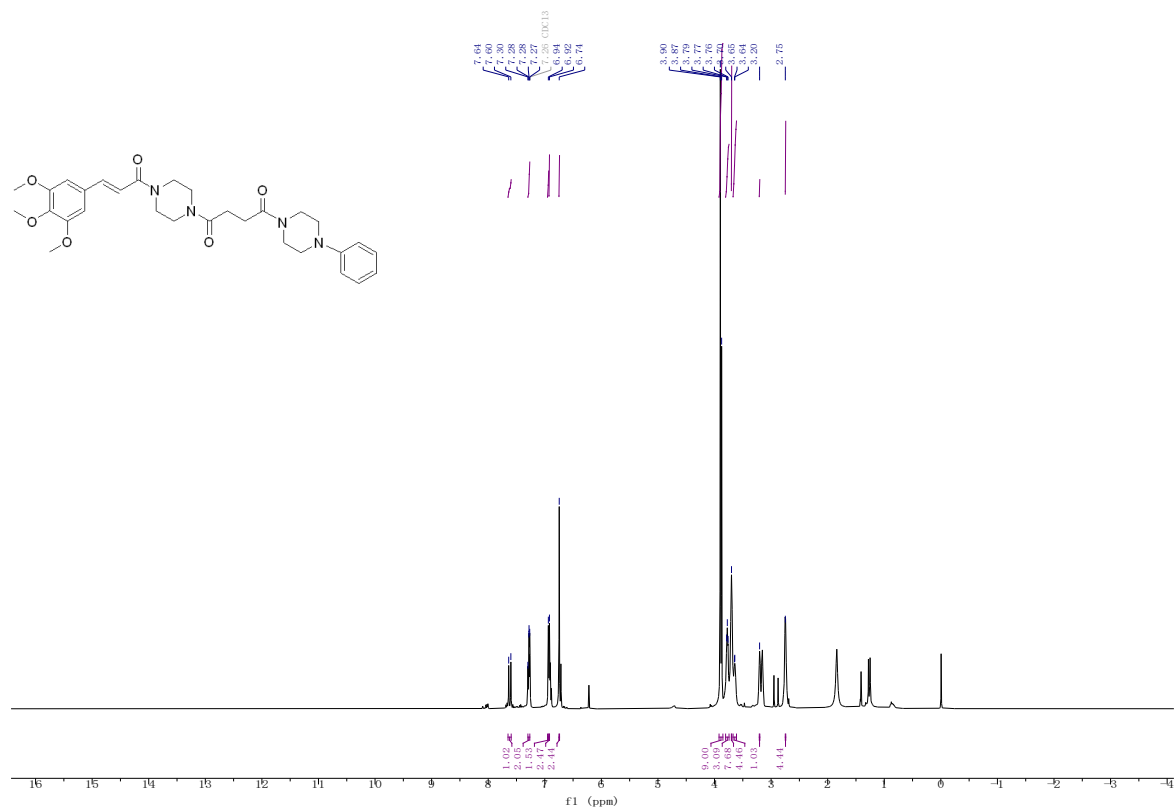


¹H NMR (400 MHz, CDCl₃) spectrum of **A11**

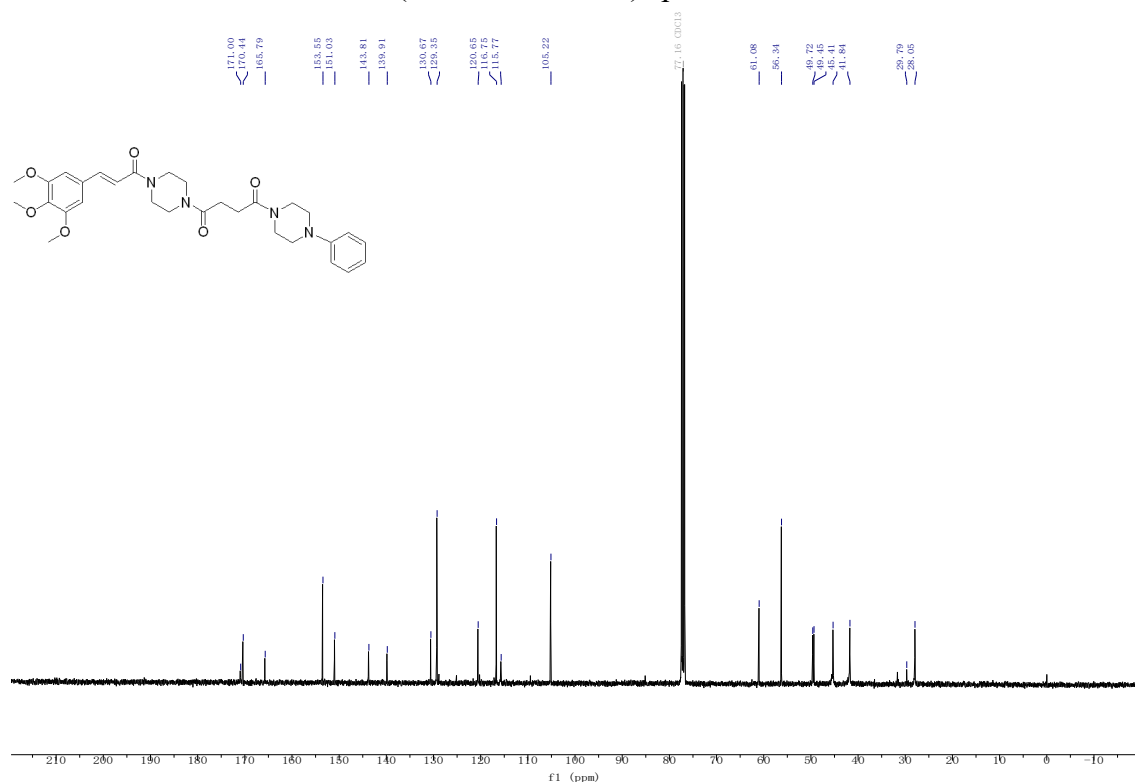


¹³C NMR (101 MHz, CDCl₃) spectrum of **A11**

(E)-1-(4-phenylpiperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B11**):



¹H NMR (400 MHz, CDCl₃) spectrum of **B11**



¹³C NMR (101 MHz, CDCl₃) spectrum of **B11**

Chemical structure of compound 10: COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CCC(=O)N3CCN(c4cc(Cl)c(Cl)cc4)CC3)CC2

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents the chemical shift in ppm, ranging from 0 to 8. The spectrum shows several peaks with corresponding integration values:

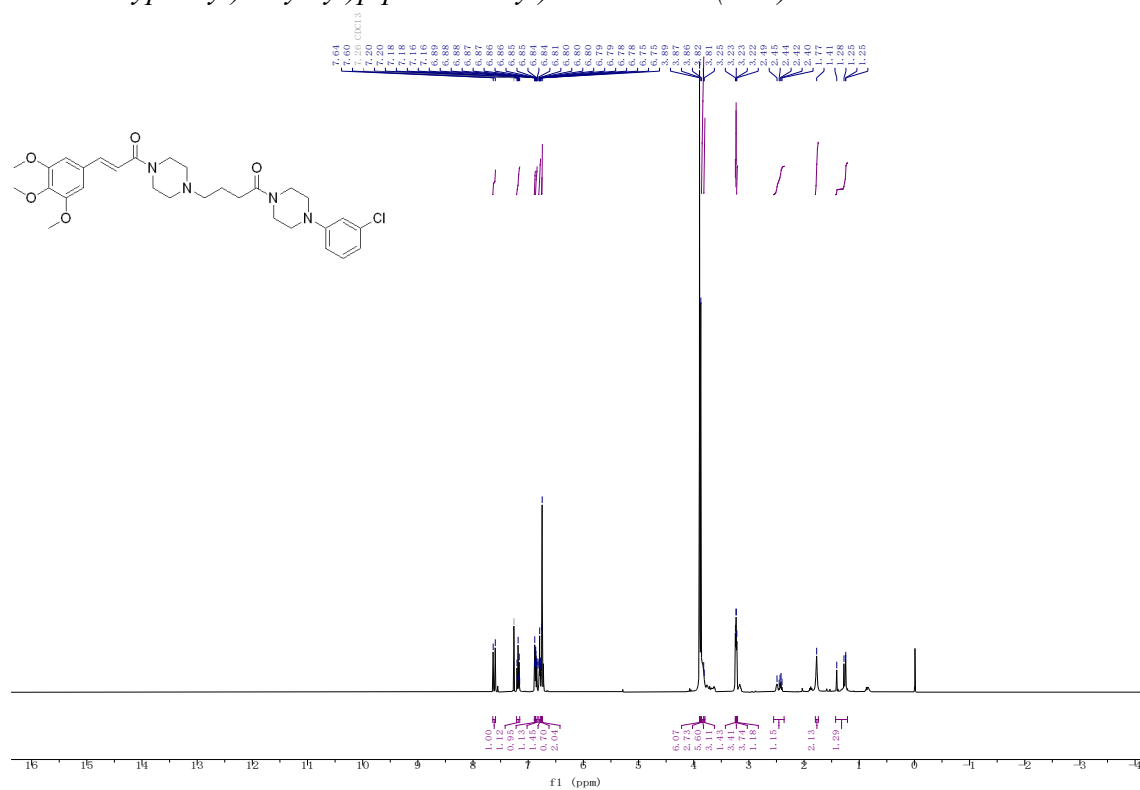
- 7.55, 7.61, 7.26, 7.20, 7.18, 6.94, 6.92, 6.92, 6.92, 6.92, 6.78, 6.75 (Aromatic protons, integration: 1.38, 1.42, 1.43, 1.29, 2.52)
- 6.92 (CH=, integration: 1.00)
- 3.94, 3.94, 3.94, 3.89, 3.87, 3.87, 3.87, 3.85, 3.85, 3.07, 3.06, 2.50 (N-CH₂ and CH₂ protons, integration: 9.00, 5.34, 1.33)
- 1.28, 1.24, 1.24 (CH₃ protons, integration: 4.66)

COc1cc(OC)c(OC)cc1/C=C/C(=O)N2CCN(CCCN3CCN(C3C4=CC=CC=C4Cl)C4=O)CC2

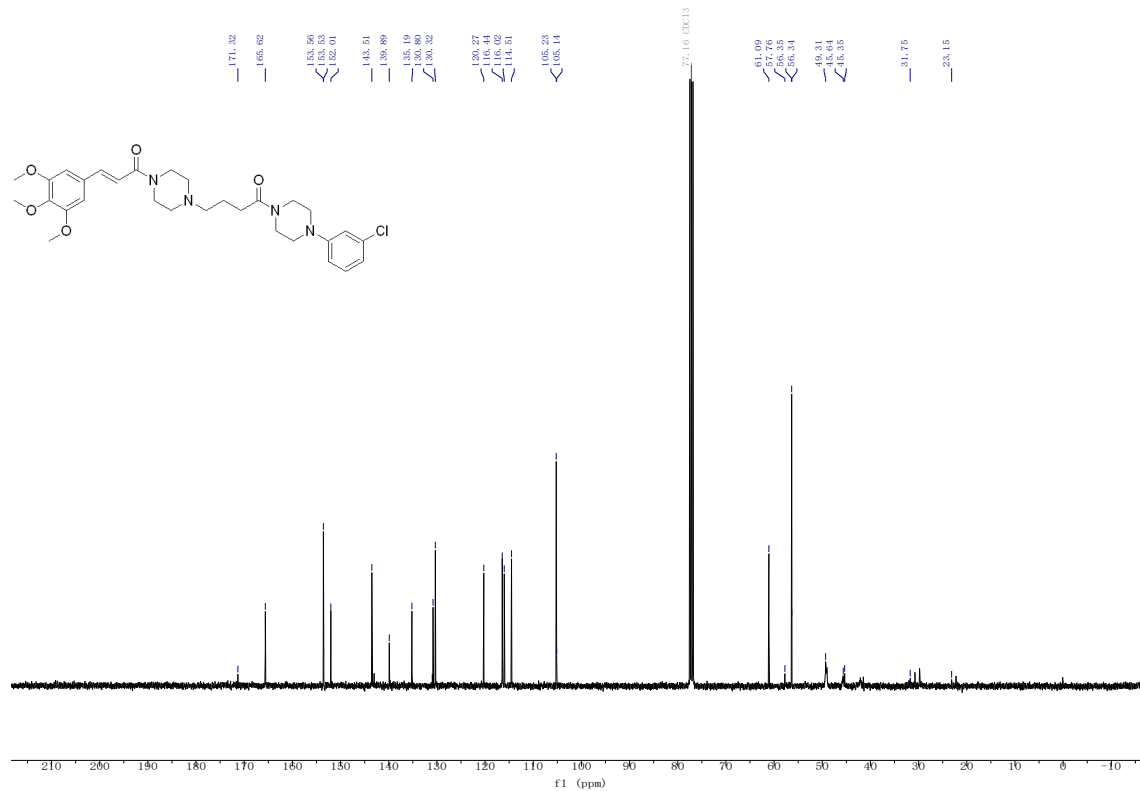
7.11, 41
 6.11, 11
 5.81, 11
 4.61, 11
 4.21, 11
 3.21, 11
 2.91, 11

 ^{13}C NMR (101 MHz, CDCl_3) spectrum of **A12**

(E)-1-(4-(3-chlorophenyl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A13**):

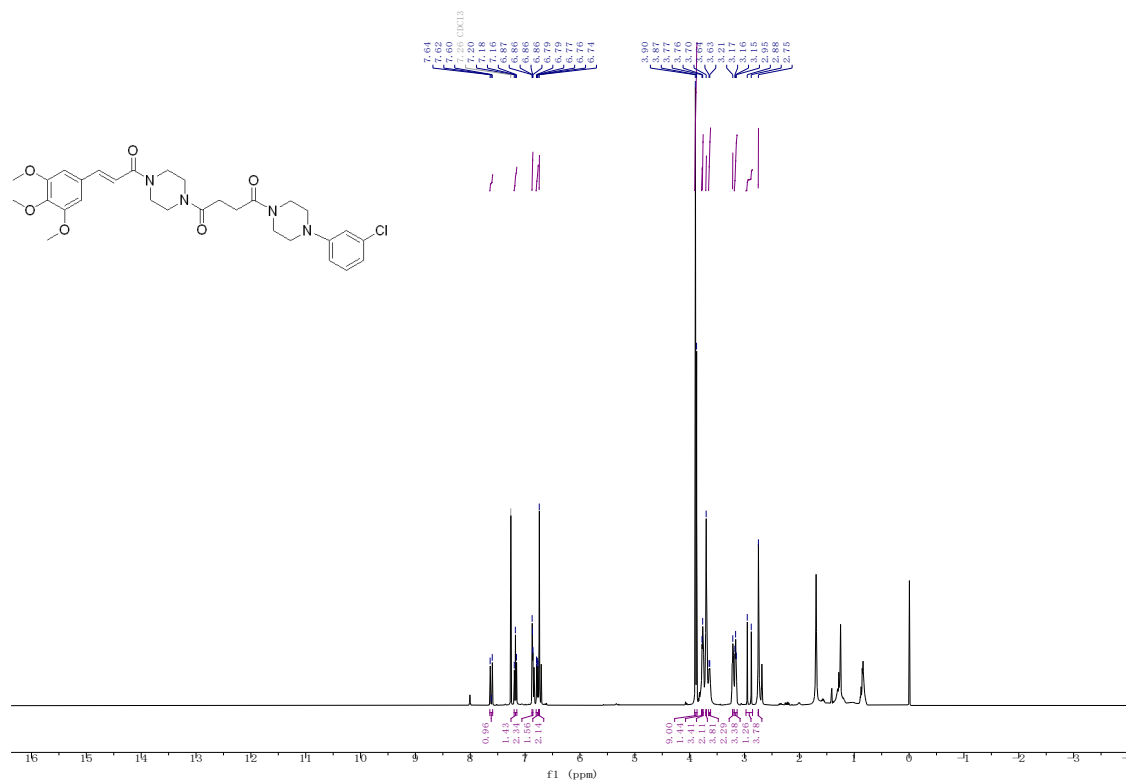


¹H NMR (400 MHz, CDCl₃) spectrum of **A13**

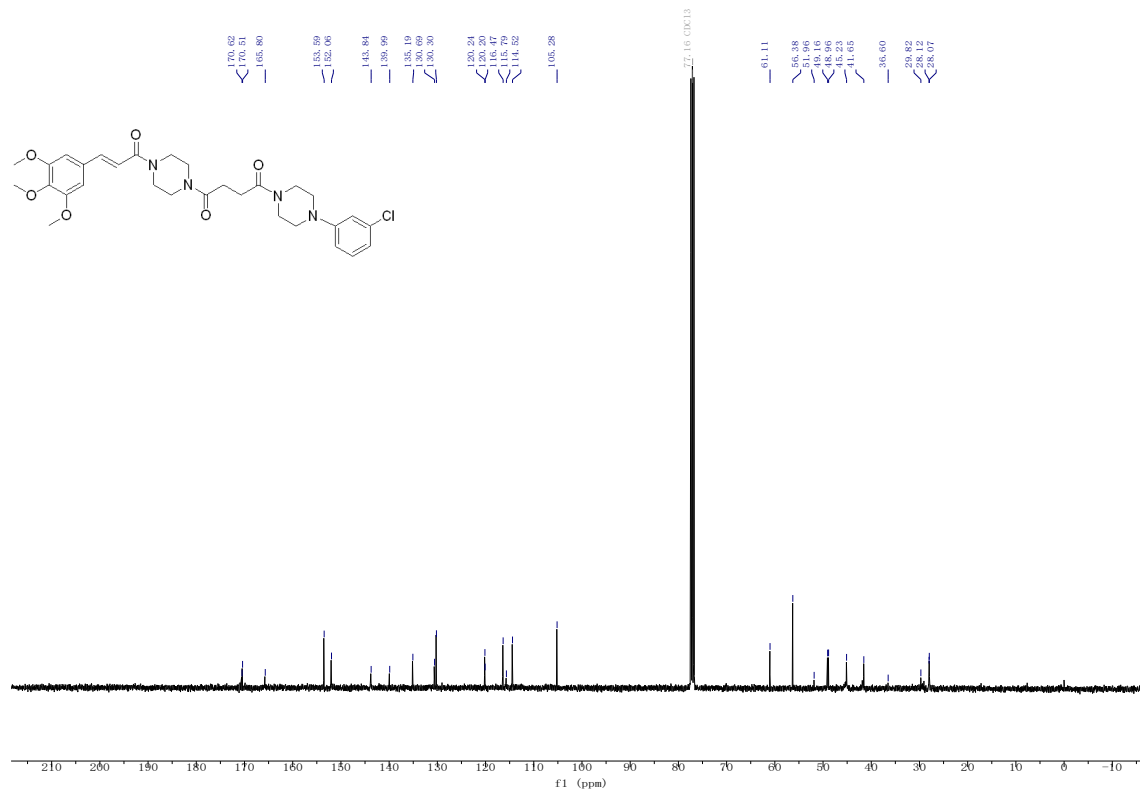


¹³C NMR (101 MHz, CDCl₃) spectrum of **A13**

(E)-1-(4-(3-chlorophenyl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-ylbutane-1,4-dione (**B13**):

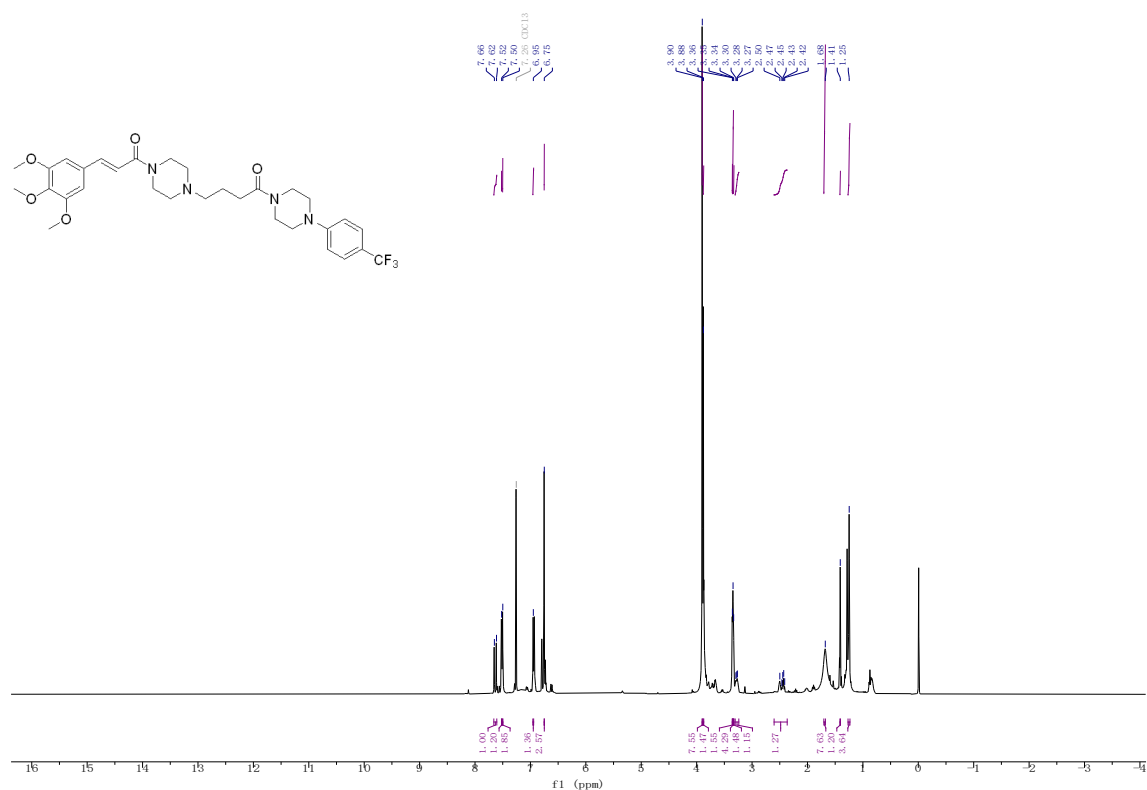


¹H NMR (400 MHz, CDCl₃) spectrum of **B13**

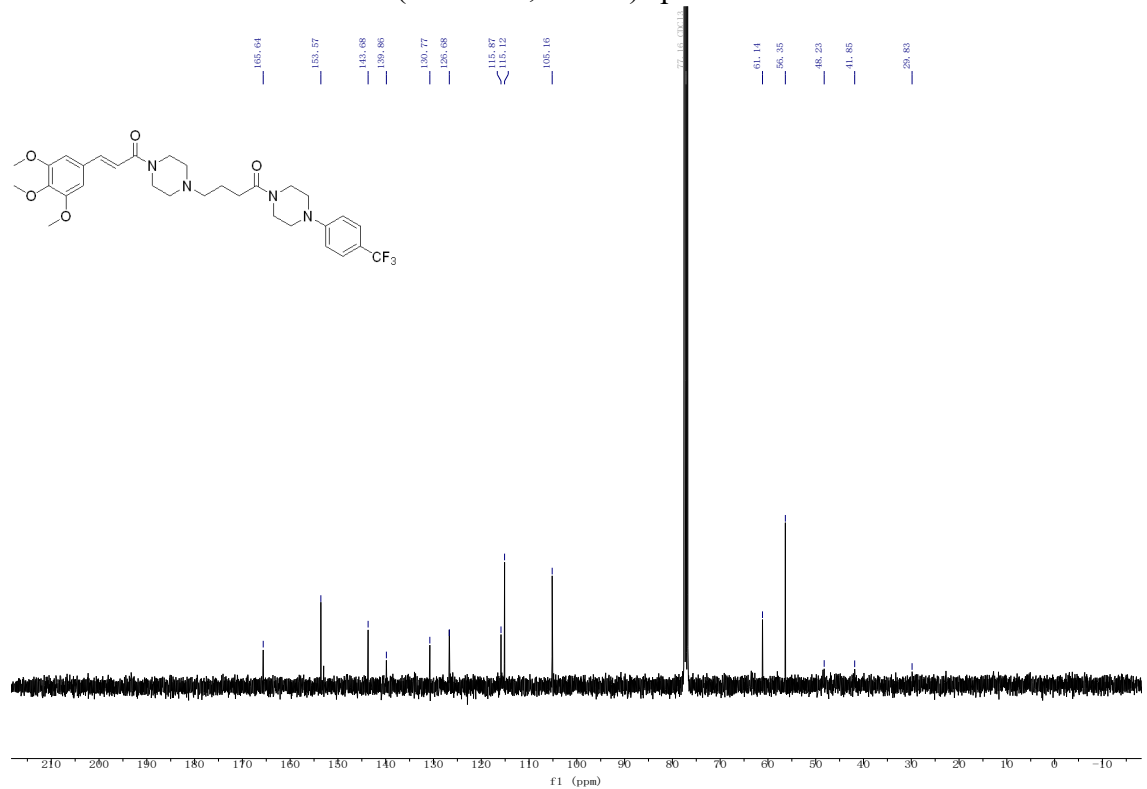


¹³C NMR (101 MHz, CDCl₃) spectrum of **B13**

(E)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A14**):

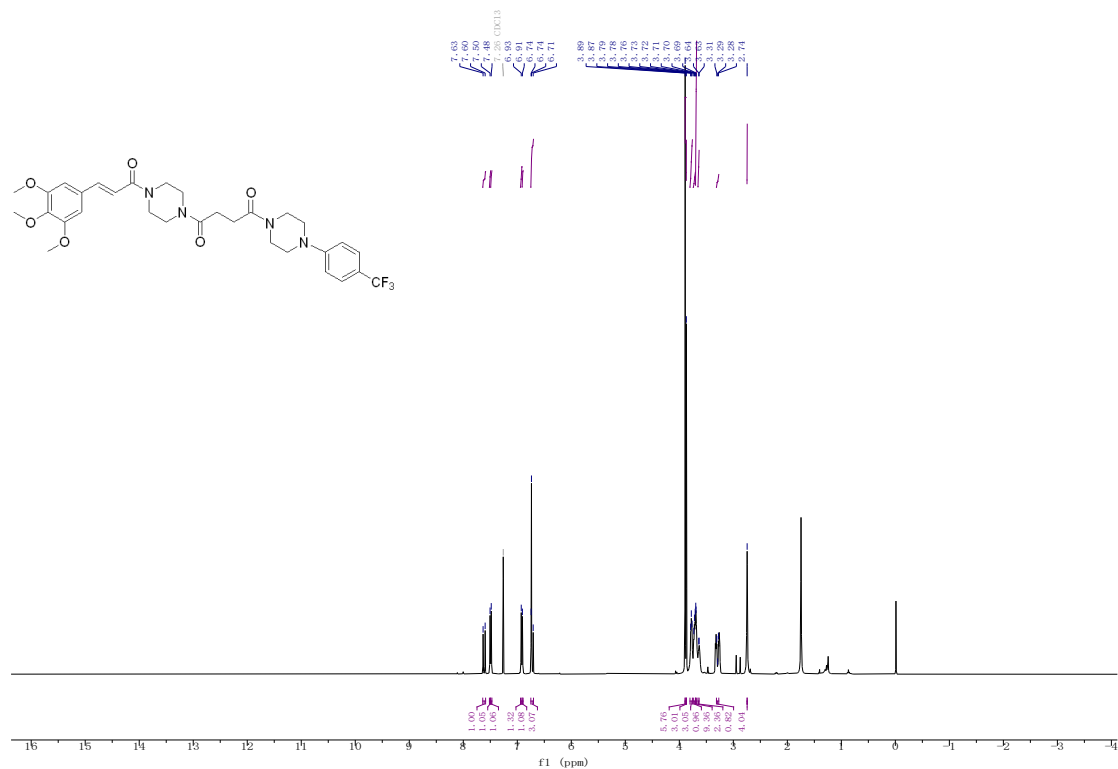


¹H NMR (400 MHz, CDCl₃) spectrum of **A14**

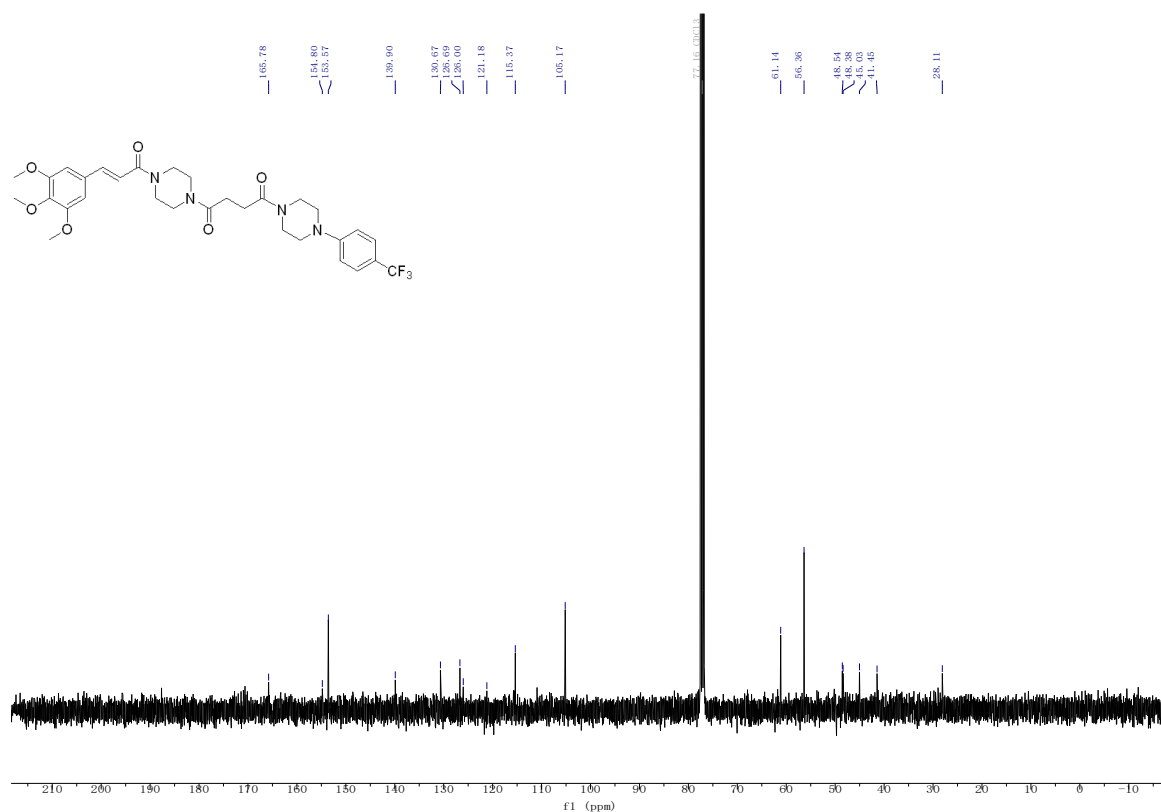


¹³C NMR (101 MHz, CDCl₃) spectrum of **A14**

(E)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B14**):

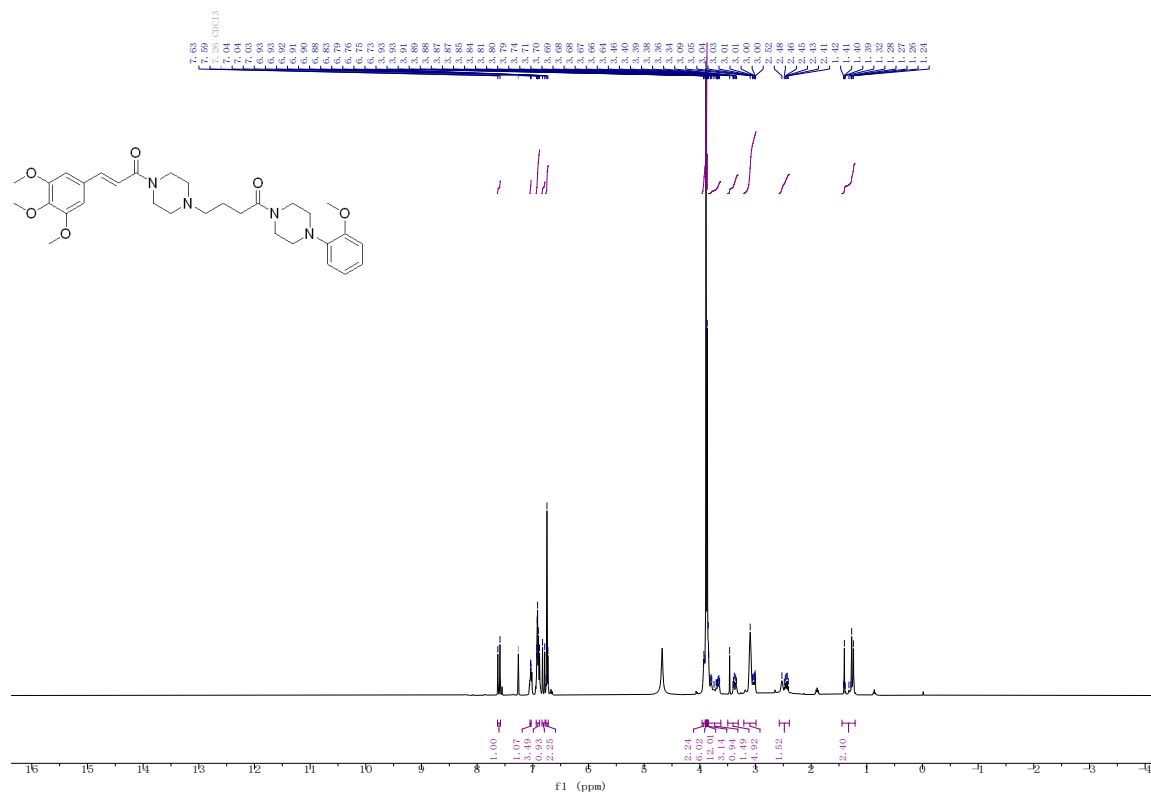


¹H NMR (400 MHz, CDCl₃) spectrum of **B14**

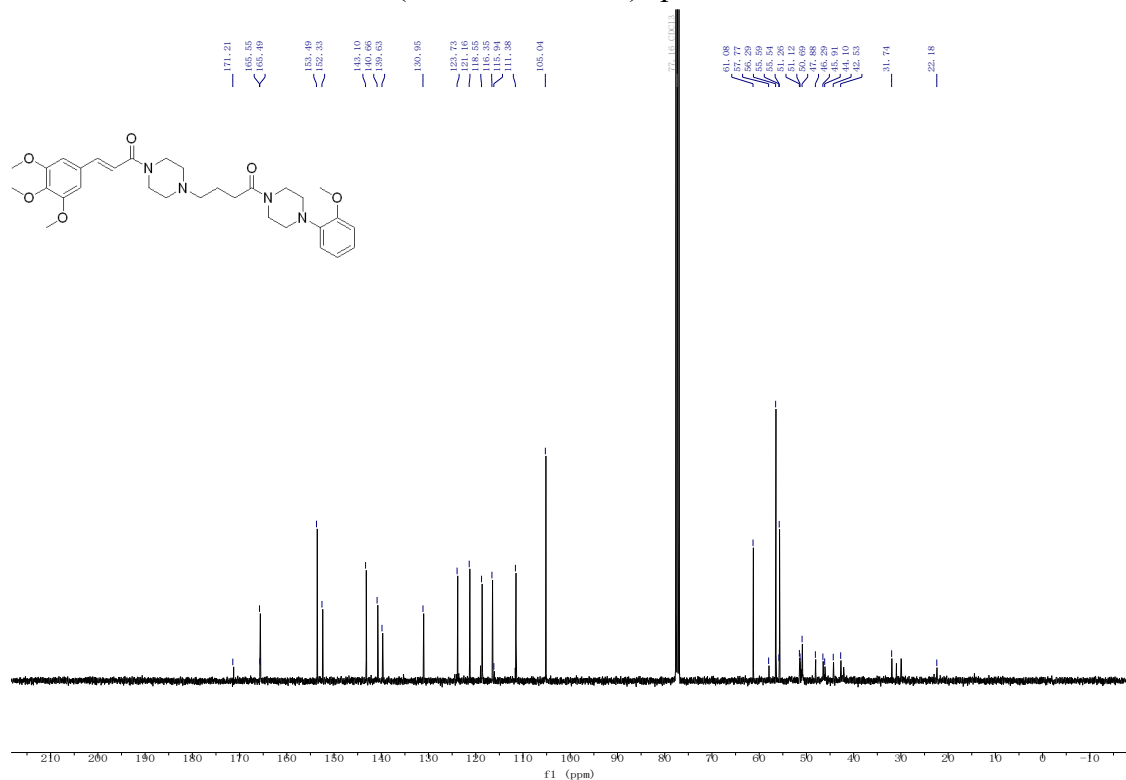


¹³C NMR (101 MHz, CDCl₃) spectrum of **B14**

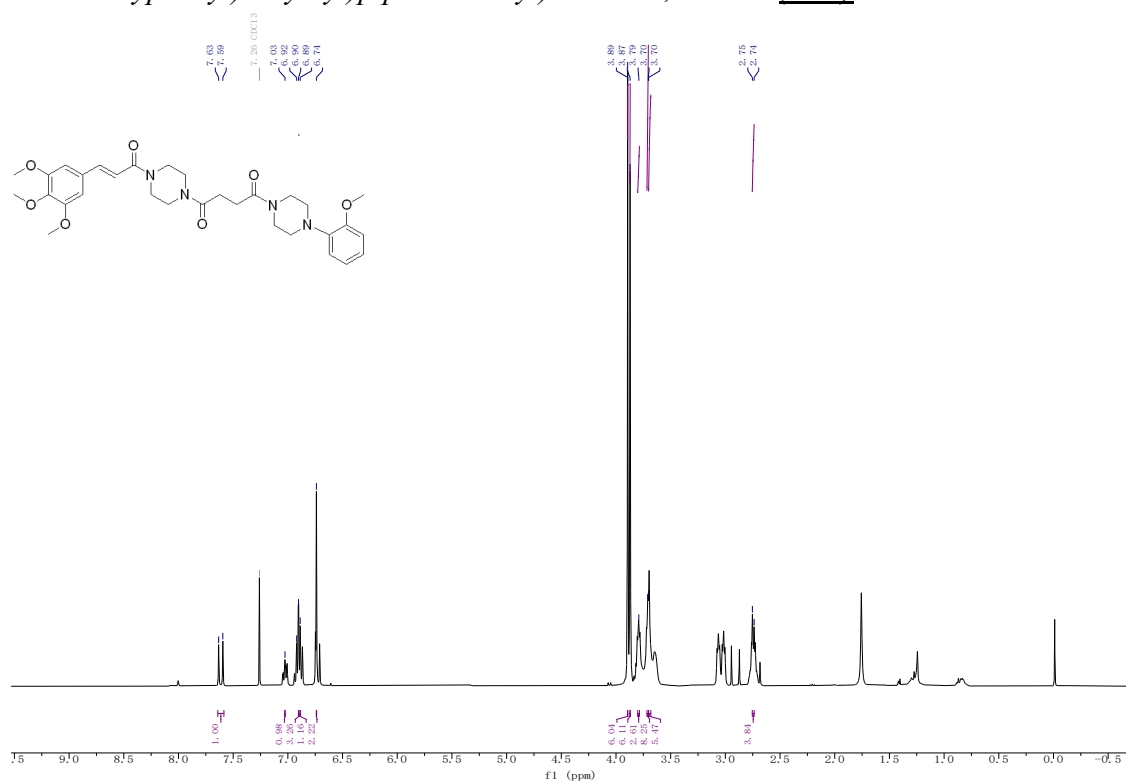
(E)-1-(4-(2-methoxyphenyl)piperazin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (A15):



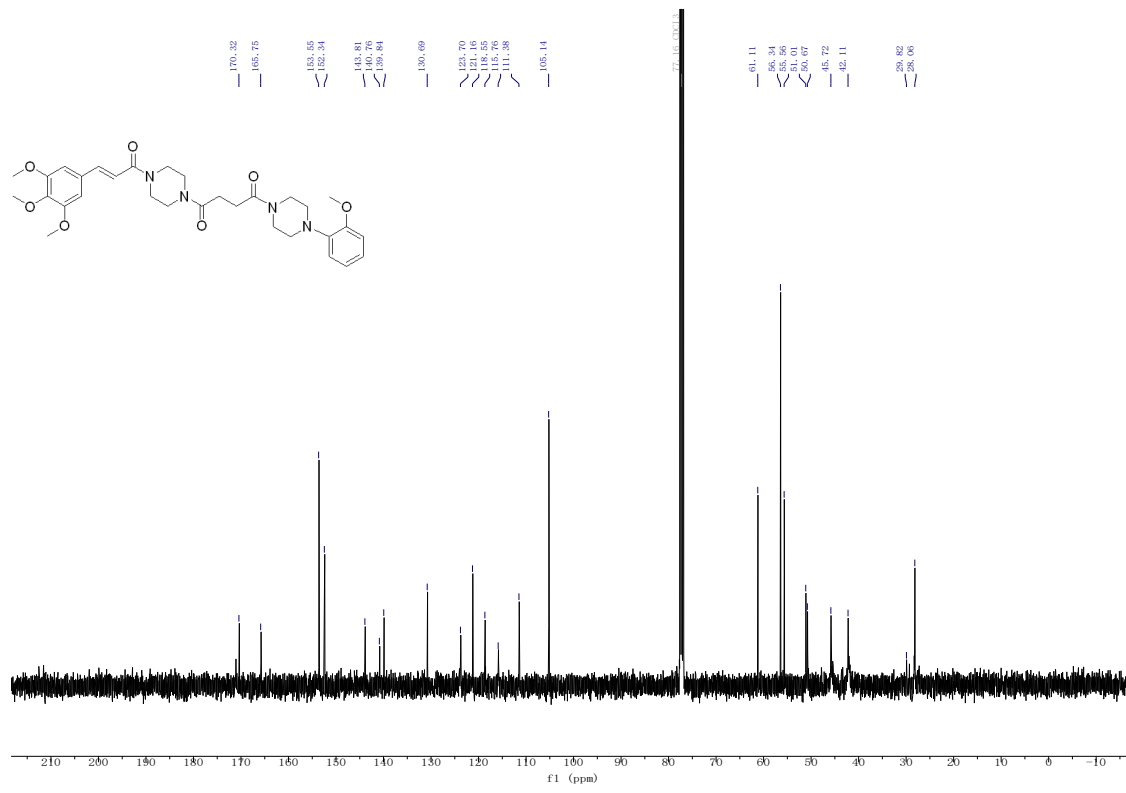
¹H NMR (400 MHz, CDCl₃) spectrum of A15



(E)-1-(4-(2-methoxyphenyl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B15**):

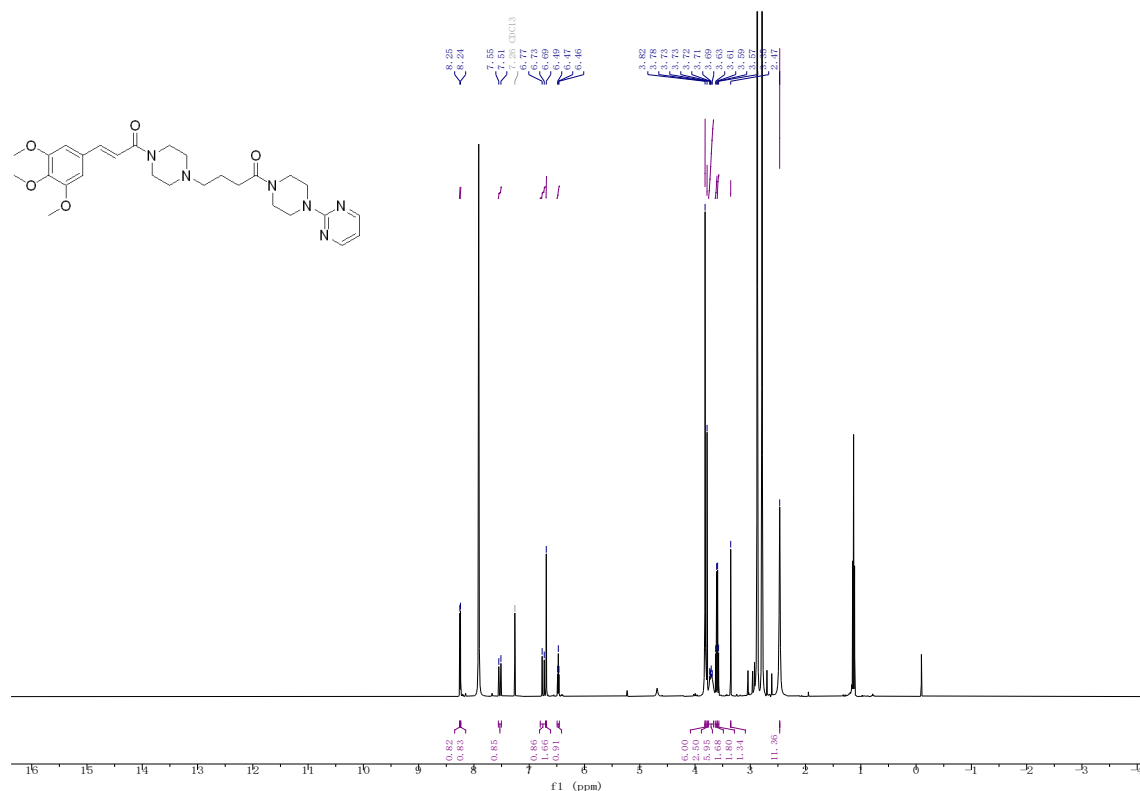


¹H NMR (400 MHz, CDCl₃) spectrum of **B15**

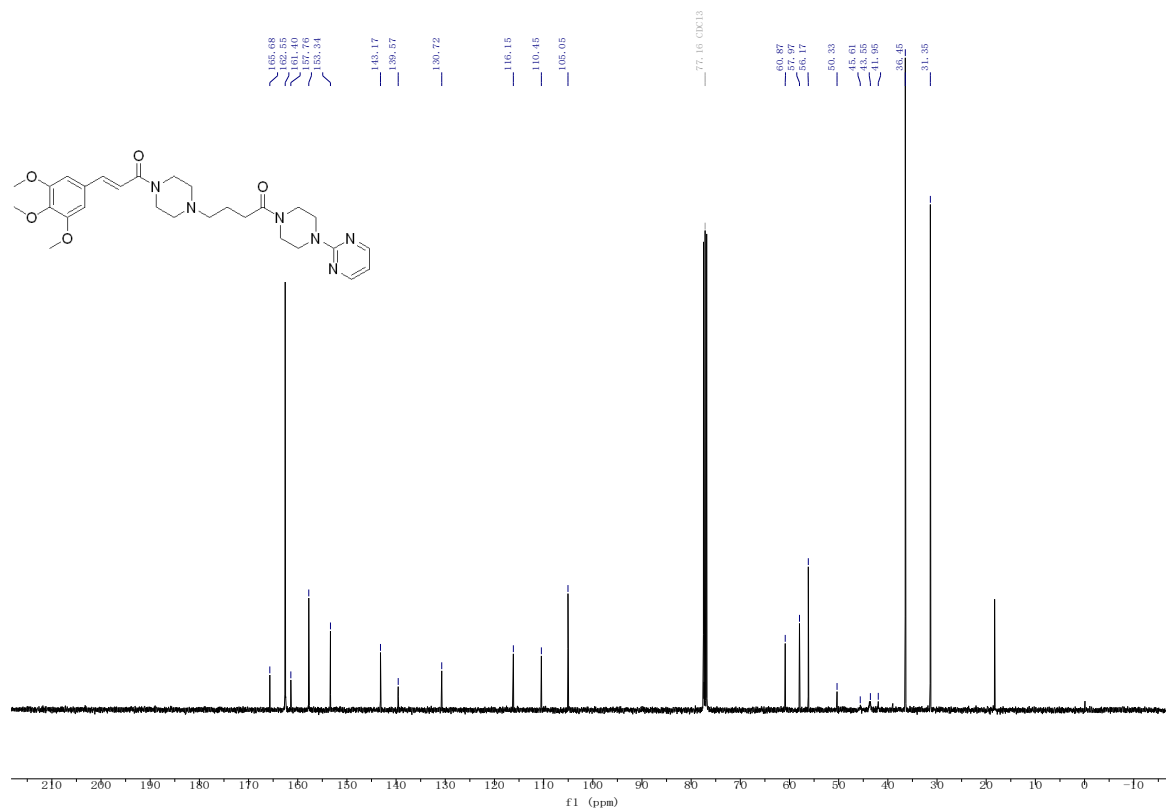


¹³C NMR (101 MHz, CDCl₃) spectrum of **B15**

(E)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A16**):

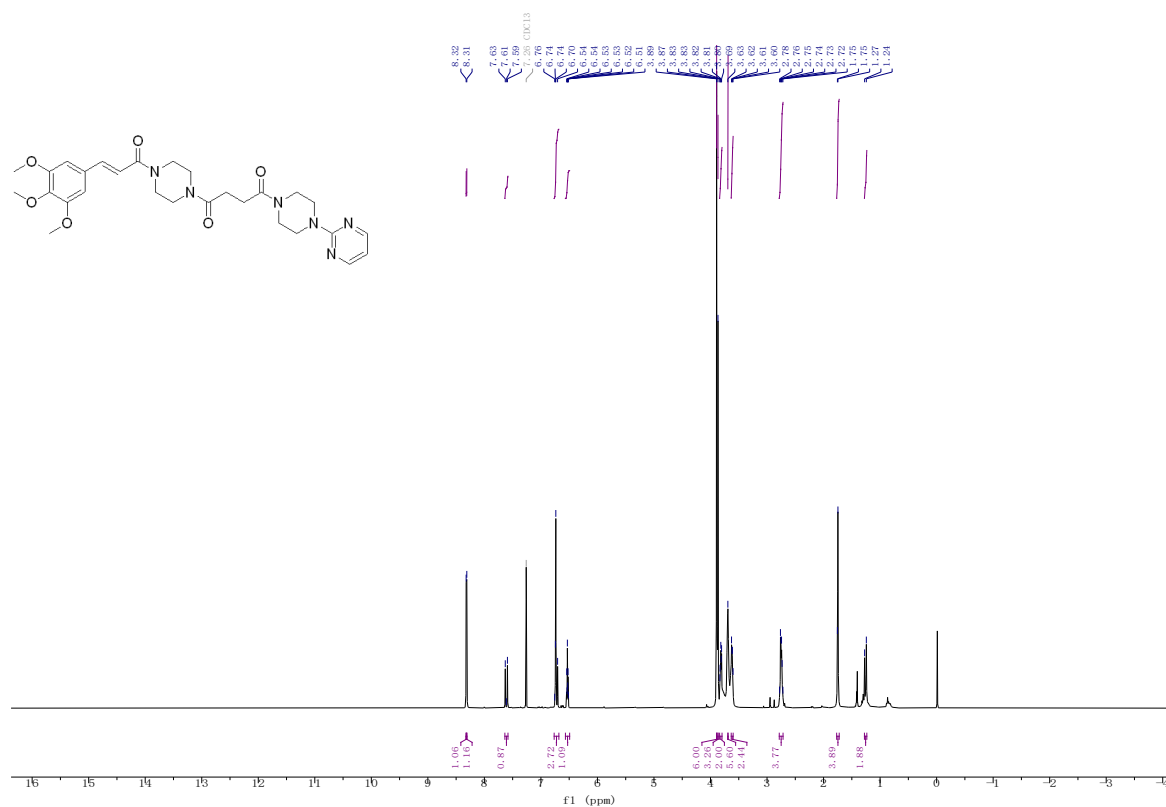


¹H NMR (400 MHz, CDCl₃) spectrum of **A16**

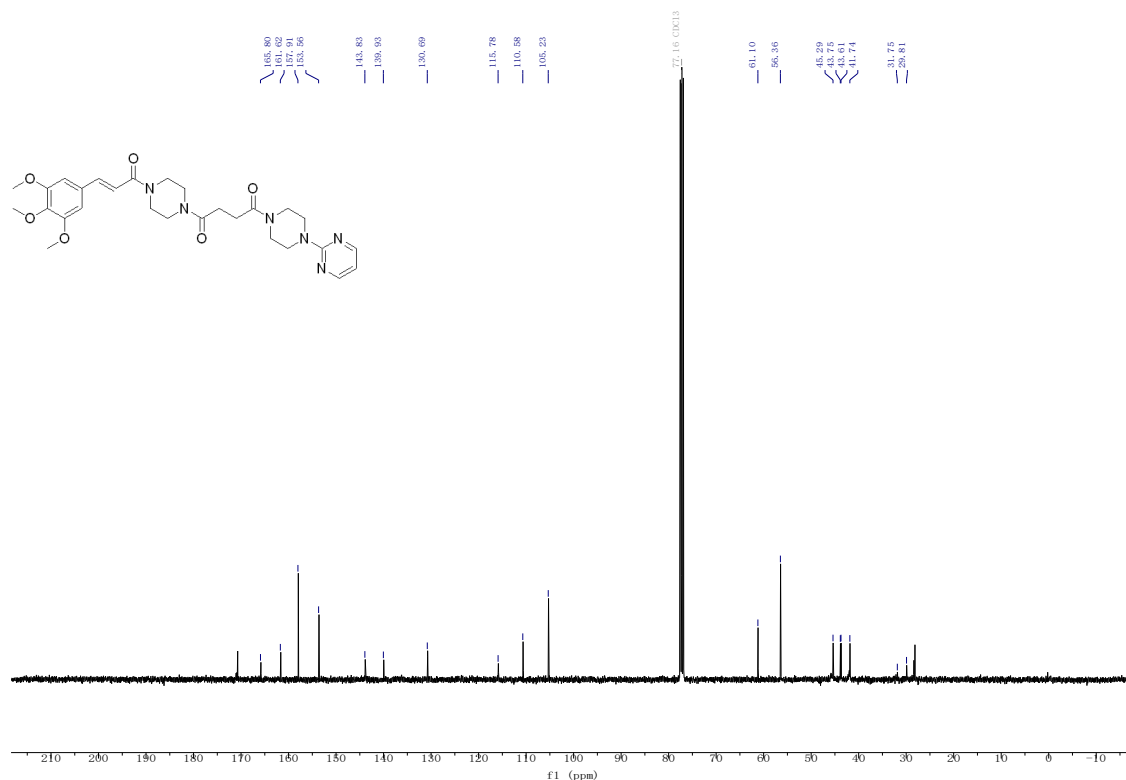


¹³C NMR (101 MHz, CDCl₃) spectrum of **A16**

(E)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)-4-(4-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B16**):

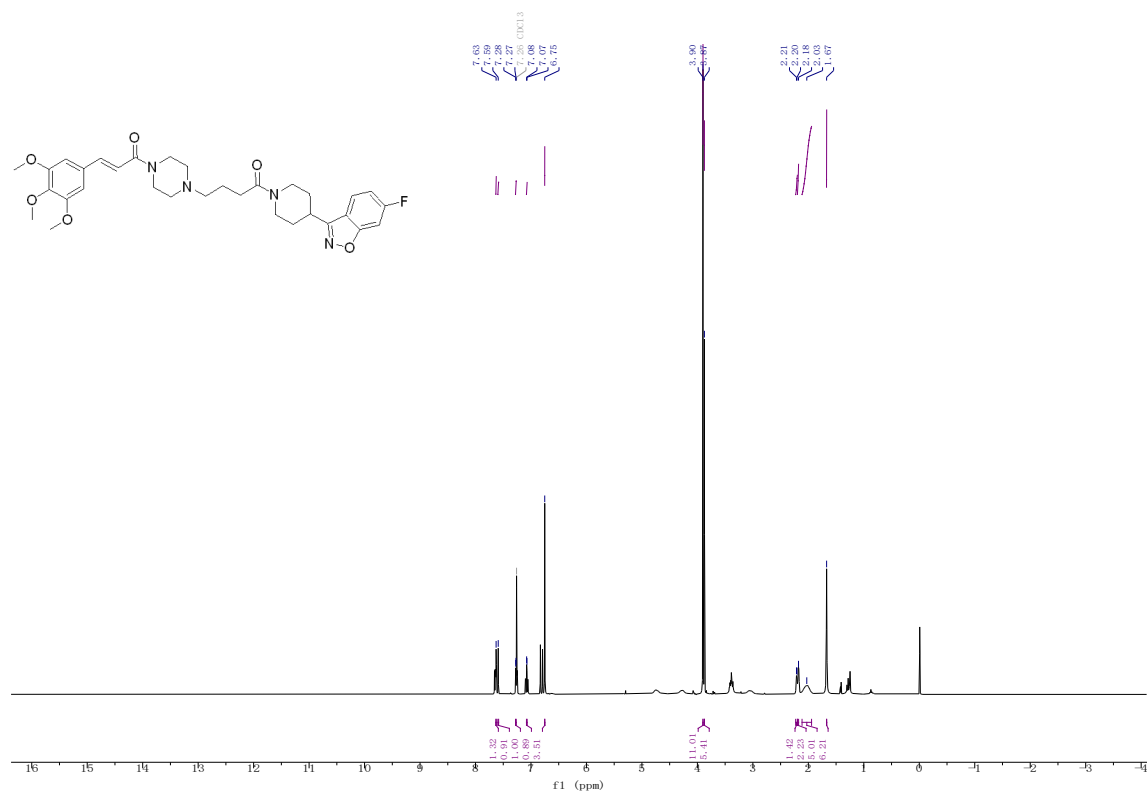


¹H NMR (400 MHz, CDCl₃) spectrum of **B16**

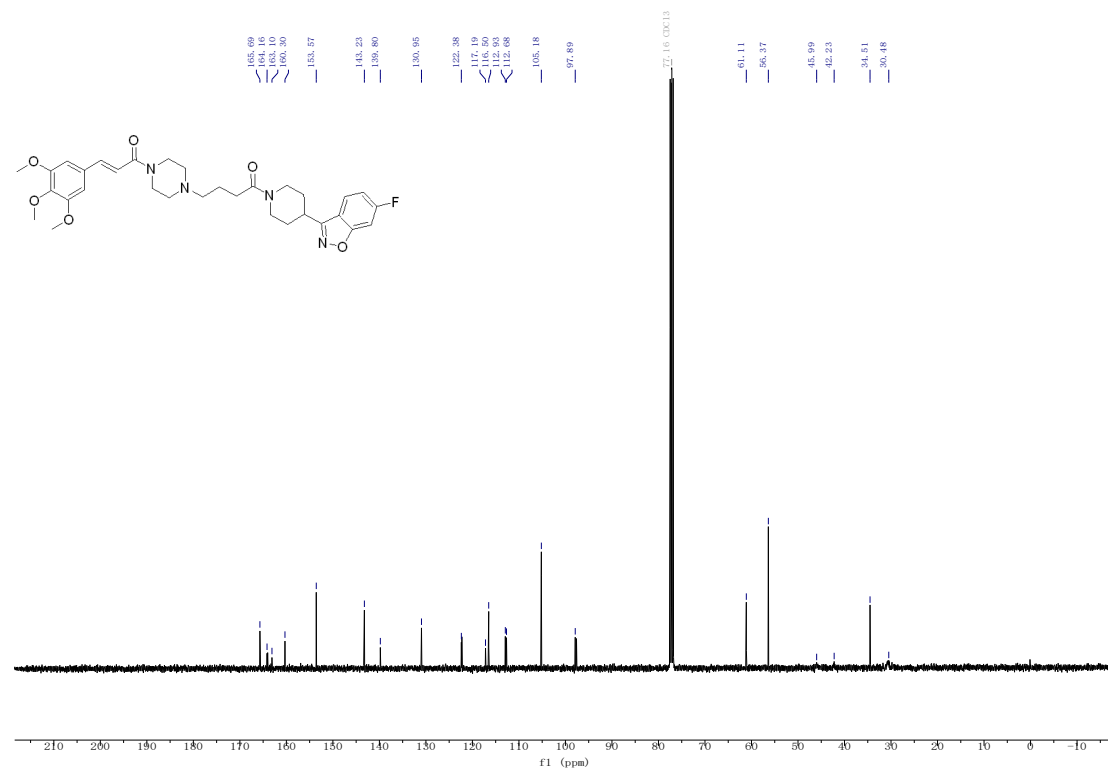


¹³C NMR (101 MHz, CDCl₃) spectrum of **B16**

(E)-1-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butan-1-one (**A17**):

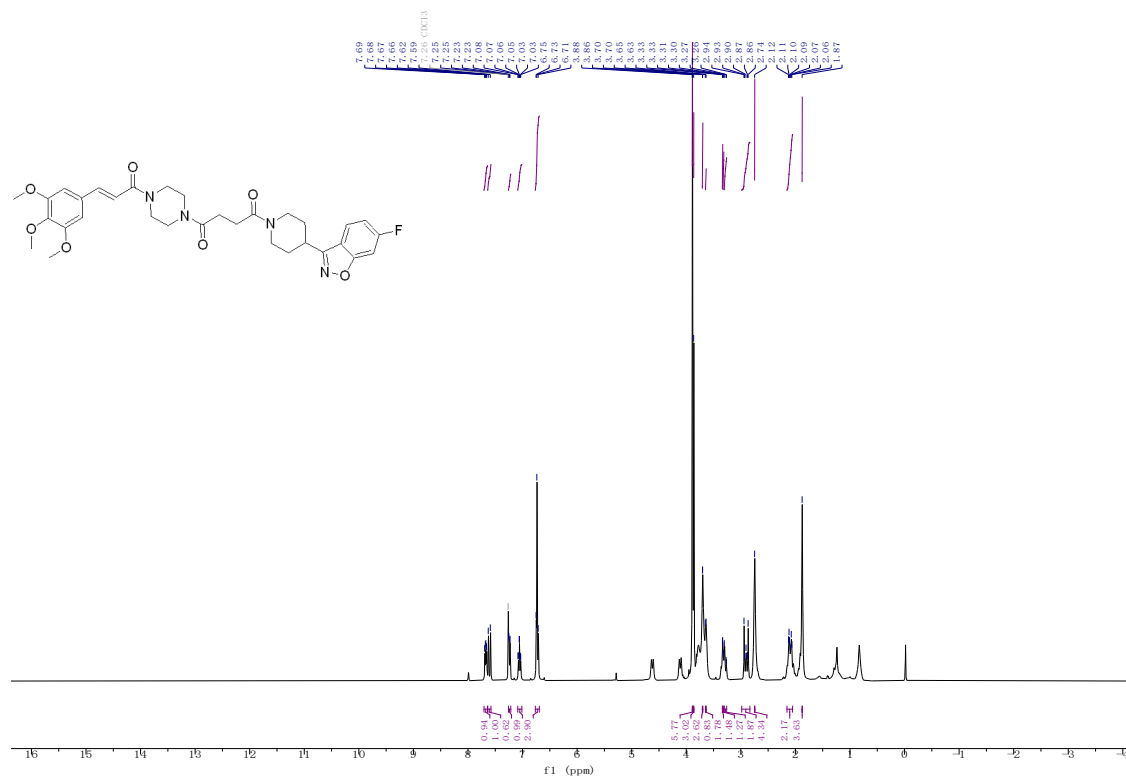


¹H NMR (400 MHz, CDCl₃) spectrum of **A17**

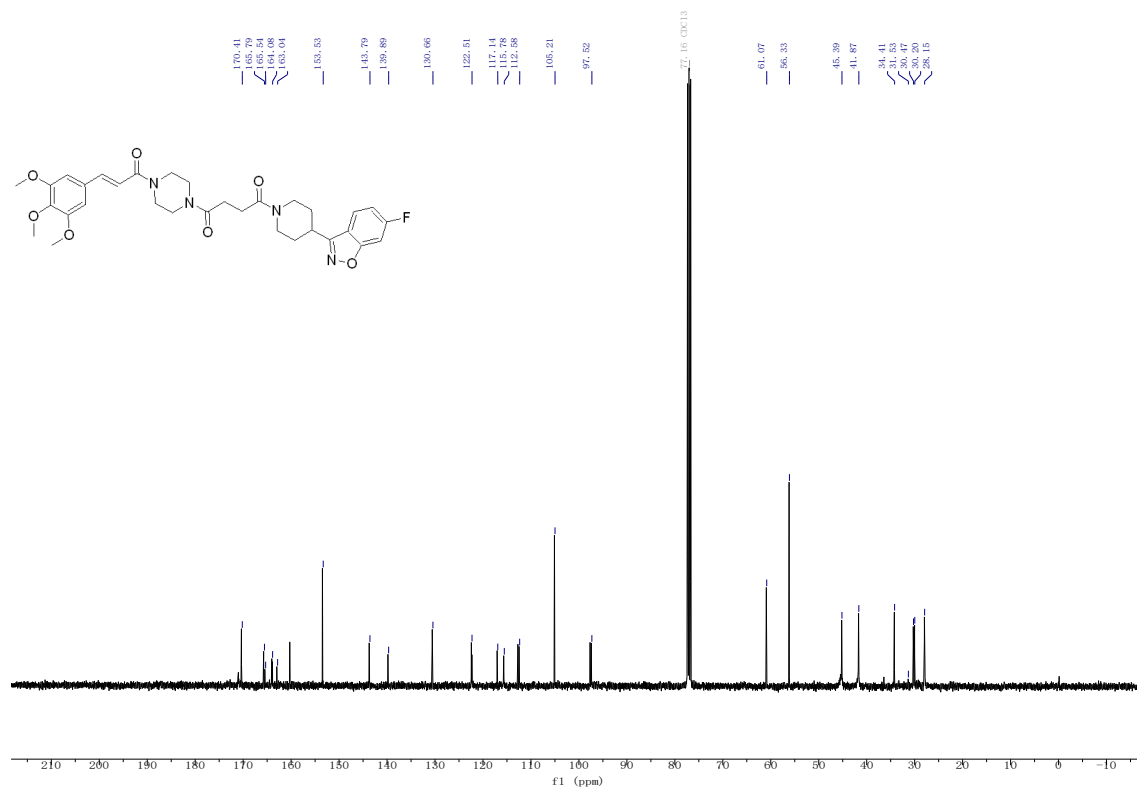


¹³C NMR (101 MHz, CDCl₃) spectrum of **A17**

(E)-1-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)-4-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)piperazin-1-yl)butane-1,4-dione (**B17**):



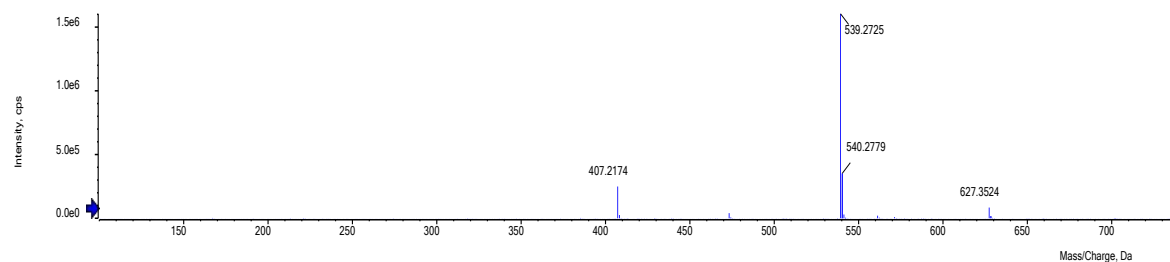
¹H NMR (400 MHz, CDCl₃) spectrum of **B17**



¹³C NMR (101 MHz, CDCl₃) spectrum of **B17**

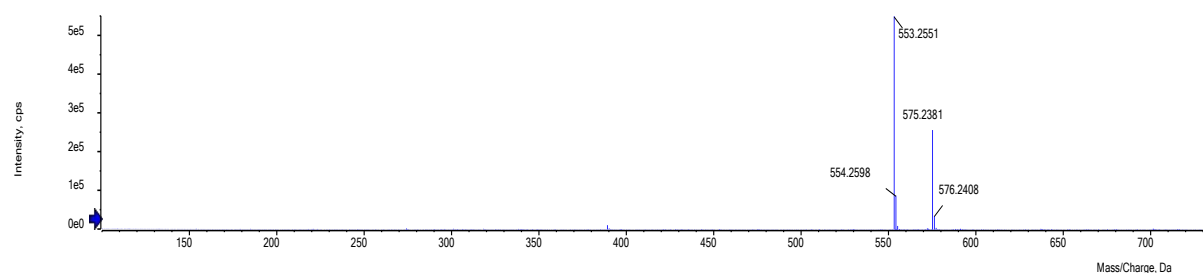
LC-MS spectrum of synthesized derivatives

Spectrum from DataSET13.wiff (sample 42) - 230505 synthetic 017, +TOF MS (100 - 1300) from 0.074 min



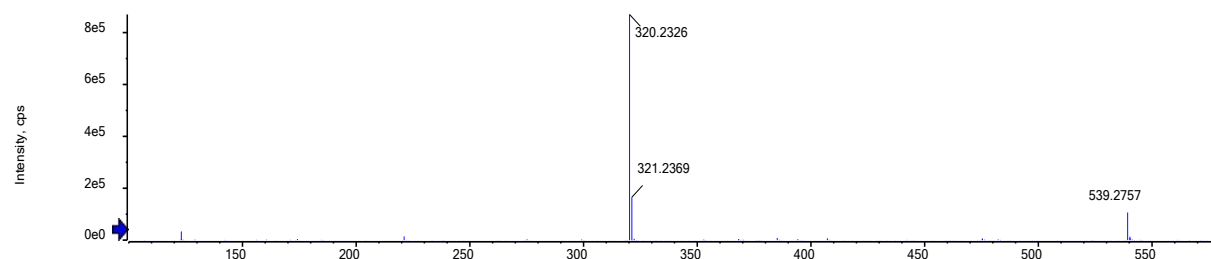
LC-MS spectrum of A2

Spectrum from DataSET14.wiff (sample 38) - 230529 syn030, +TOF MS (100 - 1300) from 0.107 min



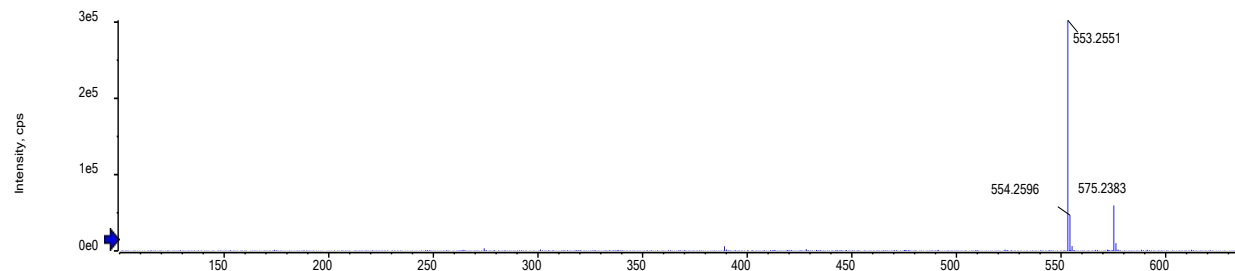
LC-MS spectrum of B2

Spectrum from DataSET14.wiff (sample 24) - 230529 syn016, +TOF MS (100 - 1300) from 0.051 min



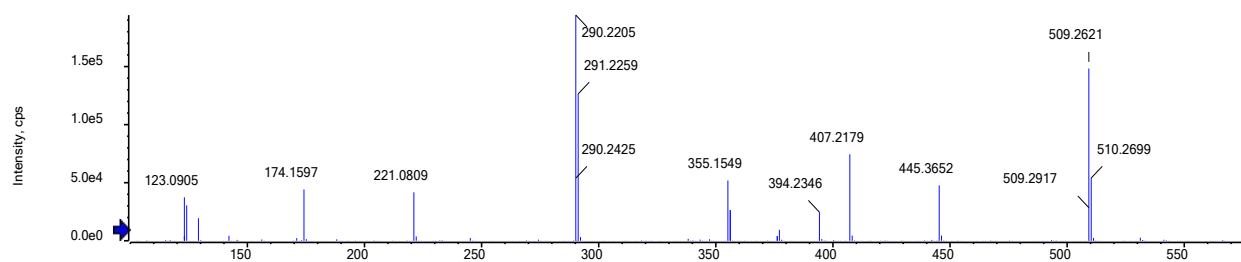
LC-MS spectrum of A3

Spectrum from DataSET14.wiff (sample 28) - 230529 syn020, +TOF MS (100 - 1300) from 0.088 min



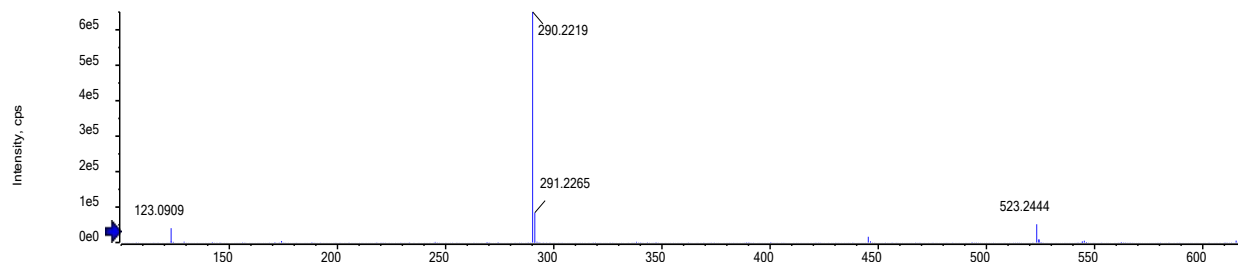
LC-MS spectrum of B3

Spectrum from DataSET14.wiff (sample 25) - 230529 syn017, +TOF MS (100 - 1300) from 0.046 min



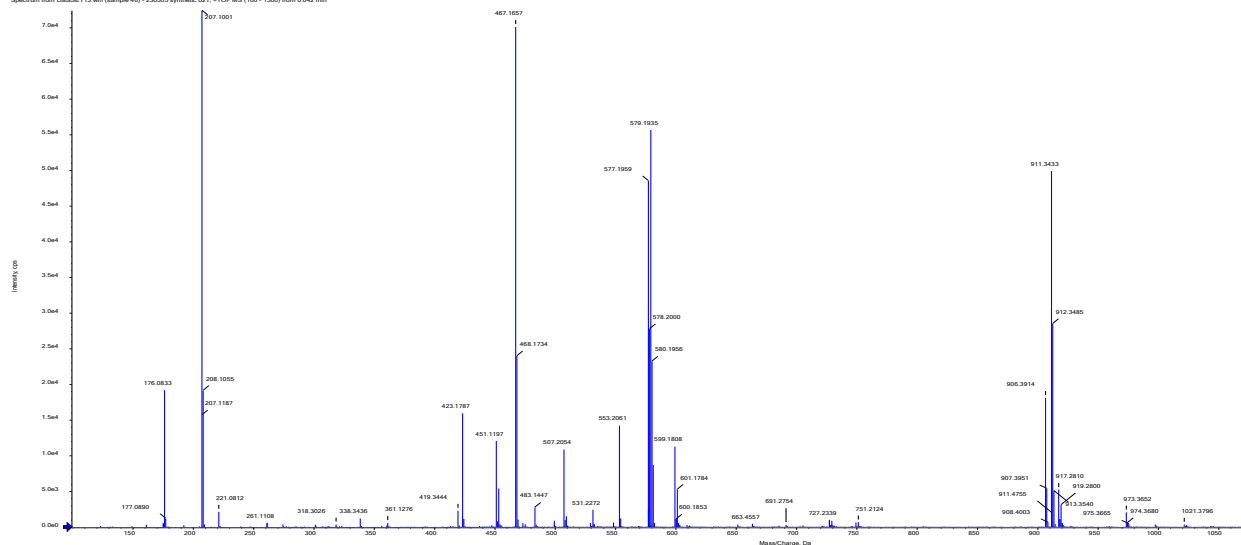
LC-MS spectrum of A4

Spectrum from DataSET14.wiff (sample 48) - 230529 syn040, +TOF MS (100 - 1300) from 0.051 min



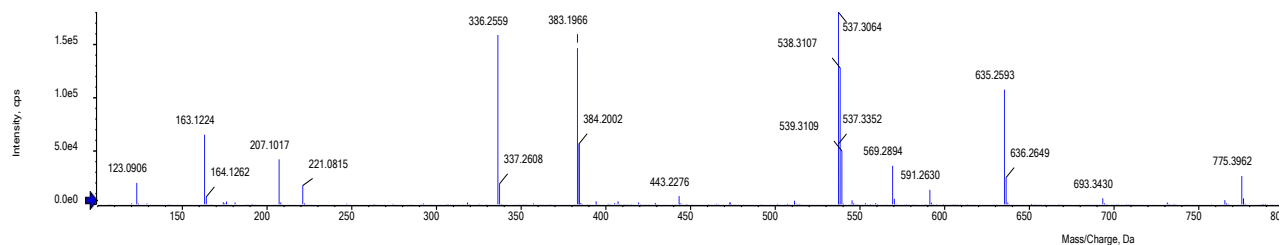
LC-MS spectrum of B4

Spectrum from DataSET13.wiff (sample 46) - 230505 synthetic 021, +TOF MS (100 - 1300) from 0.042 min



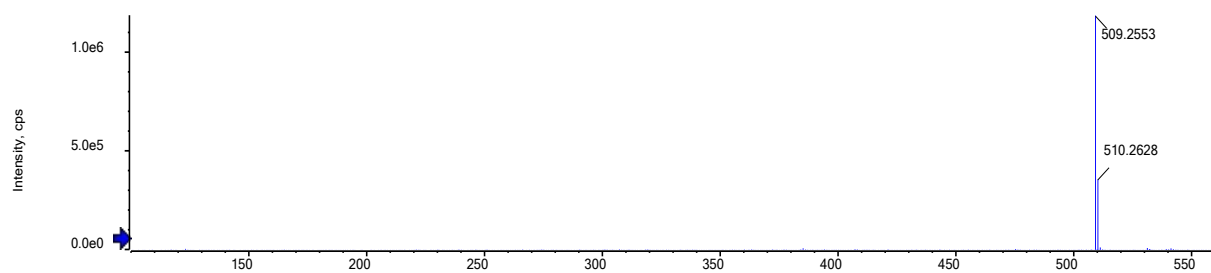
LC-MS spectrum of A5

Spectrum from DataSET13.wiff (sample 47) - 230505 synthetic 022, +TOF MS (100 - 1300) from 0.046 min



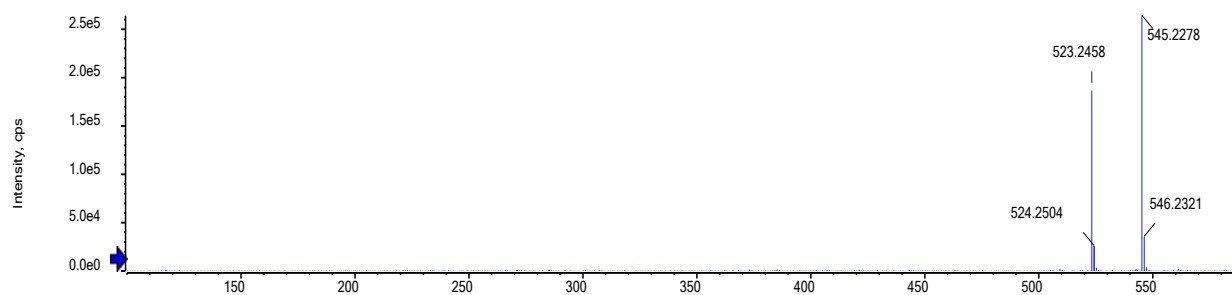
LC-MS spectrum of B5

Spectrum from DataSET14.wiff (sample 11) - 230529 syn003, +TOF MS (100 - 1300) from 0.088 min



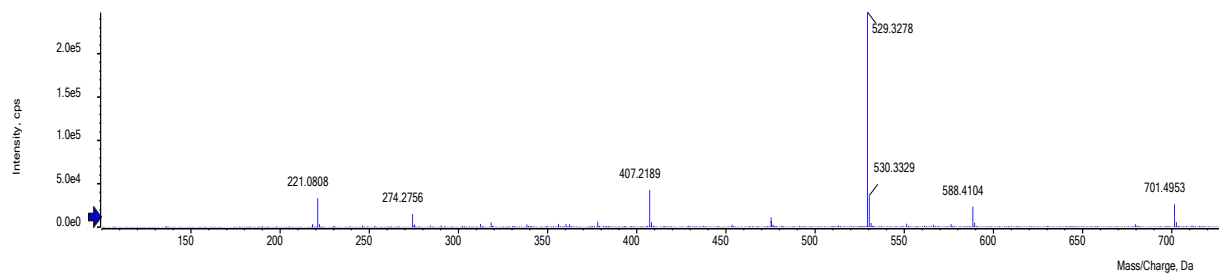
LC-MS spectrum of A6

Spectrum from DataSET14.wiff (sample 30) - 230529 syn022, +TOF MS (100 - 1300) from 0.139 min



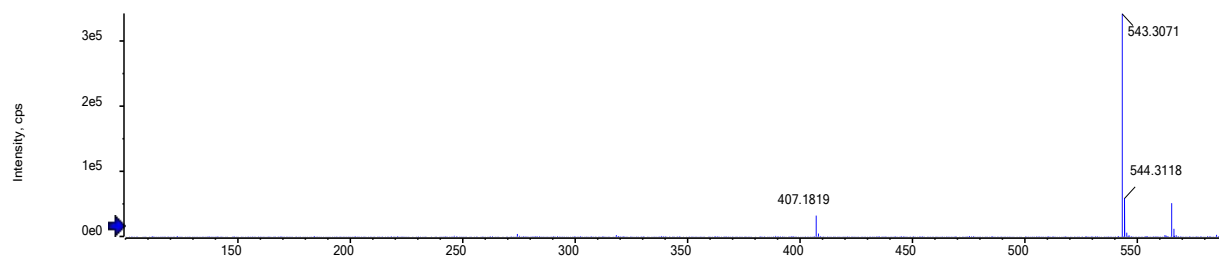
LC-MS spectrum of B6

Spectrum from DataSET14.wiff (sample 20) - 230529 syn012, +TOF MS (100 - 1300) from 0.060 min



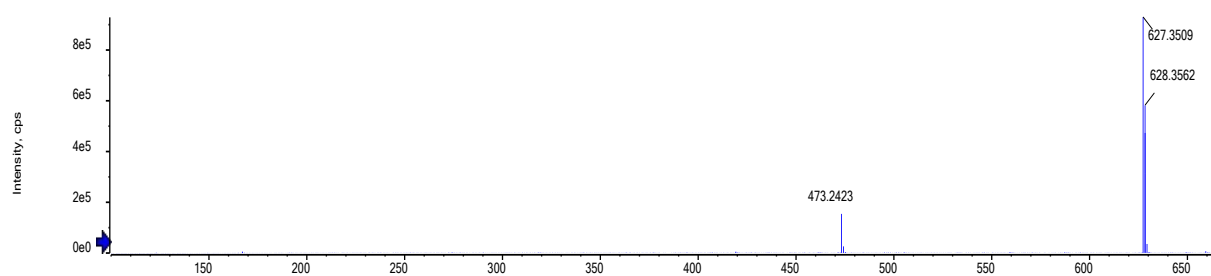
LC-MS spectrum of A7

Spectrum from DataSET14.wiff (sample 34) - 230529 syn026, +TOF MS (100 - 1300) from 0.074 min



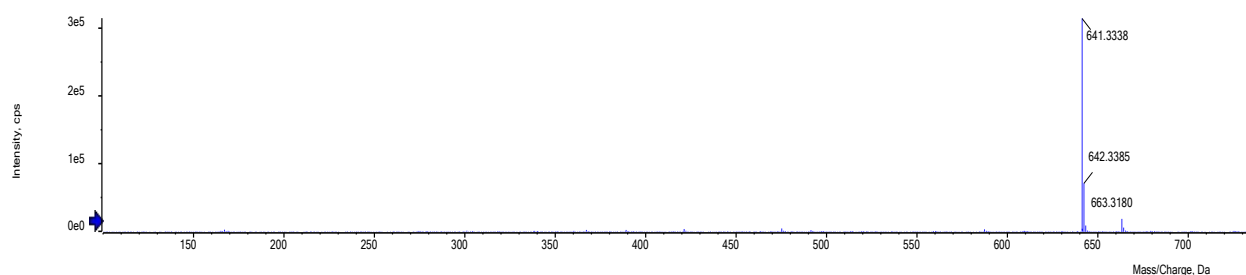
LC-MS spectrum of B7

Spectrum from DataSET13.wiff (sample 27) - 230505 synthetic 002, +TOF MS (100 - 1300) from 0.070 min



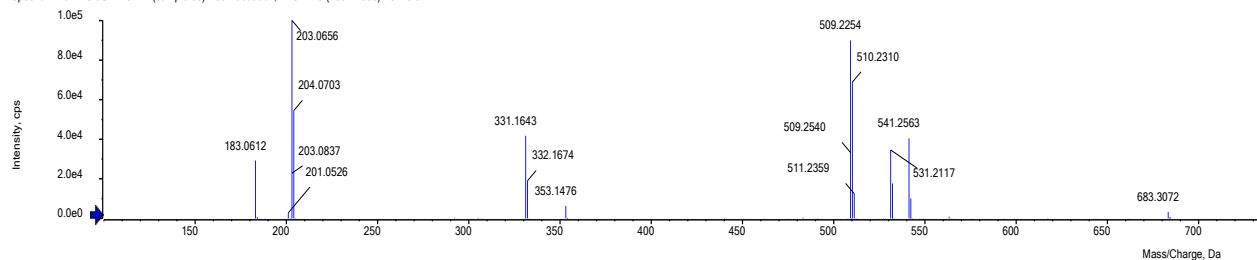
LC-MS spectrum of A8

Spectrum from DataSET14.wiff (sample 37) - 230529 syn029, +TOF MS (100 - 1300) from 0.139 min



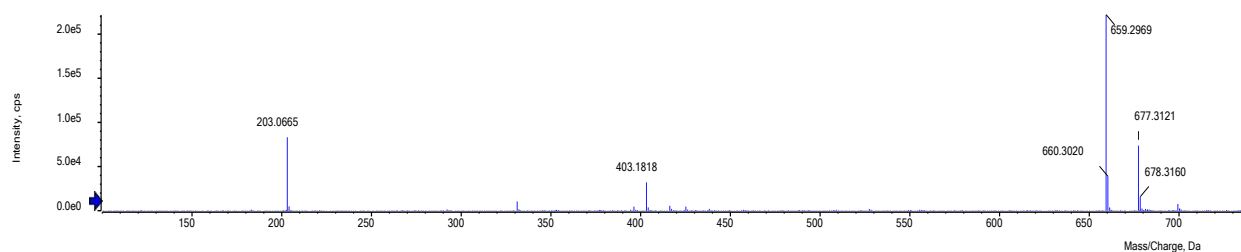
LC-MS spectrum of B8

Spectrum from DataSET13.wiff (sample 58) - Sam0609004, +TOF MS (100 - 1300) from 0.042 min



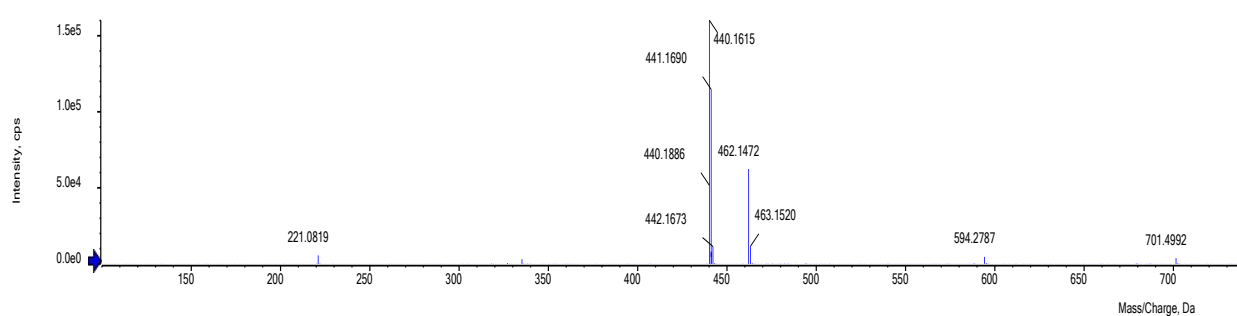
LC-MS spectrum of A9

Spectrum from DataSET13.wiff (sample 66) - Sam0609012, +TOF MS (100 - 1300) from 0.116 min



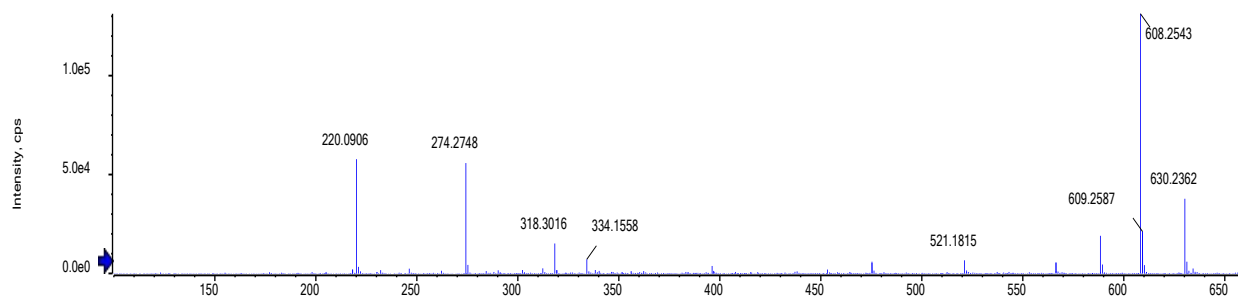
LC-MS spectrum of B9

Spectrum from DataSET13.wiff (sample 55) - Sam0609001, +TOF MS (100 - 1300) from 0.046 min



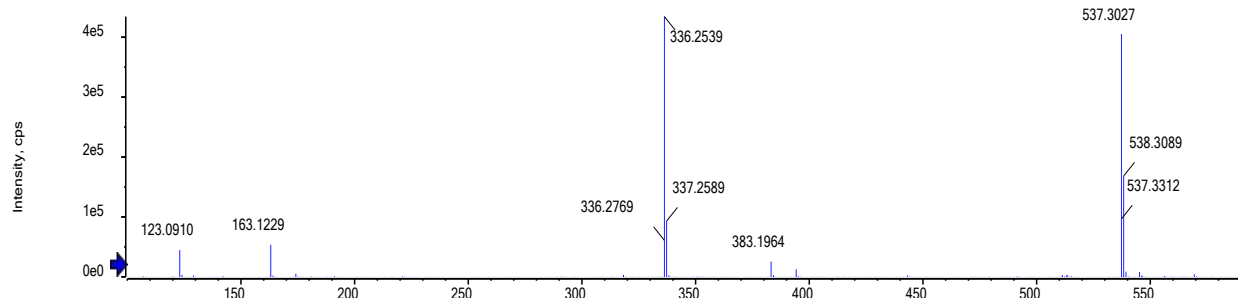
LC-MS spectrum of A10

Spectrum from DataSET14.wiff (sample 43) - 230529 syn035, +TOF MS (100 - 1300) from 0.065 min



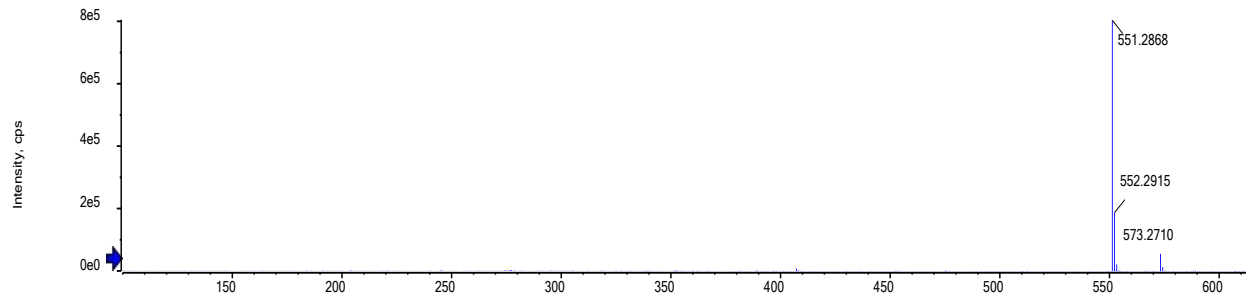
LC-MS spectrum of B10

Spectrum from DataSET13.wiff (sample 31) - 230505 synthetic 006, +TOF MS (100 - 1300) from 0.051 min



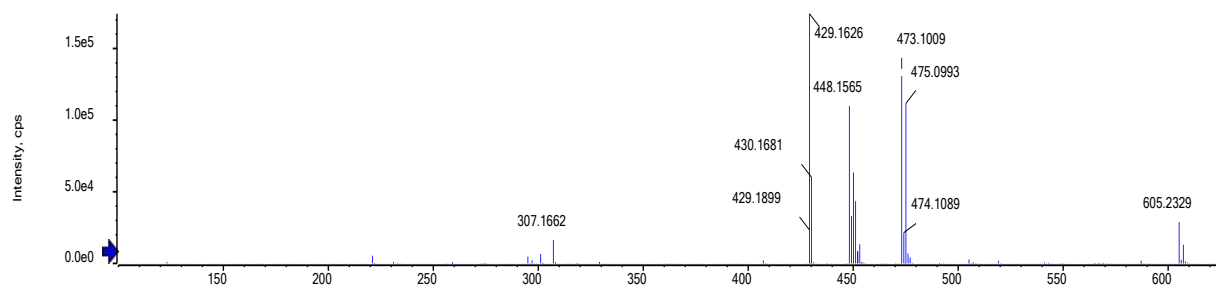
LC-MS spectrum of A11

Spectrum from DataSET14.wiff (sample 36) - 230529 syn028, +TOF MS (100 - 1300) from 0.107 min



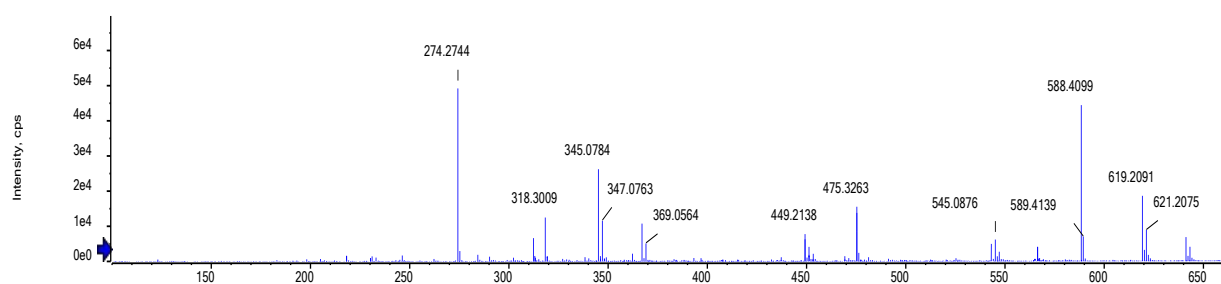
LC-MS spectrum of B11

Spectrum from DataSET13.wiff (sample 29) - 230505 synthetic 004, +TOF MS (100 - 1300) from 0.046 min



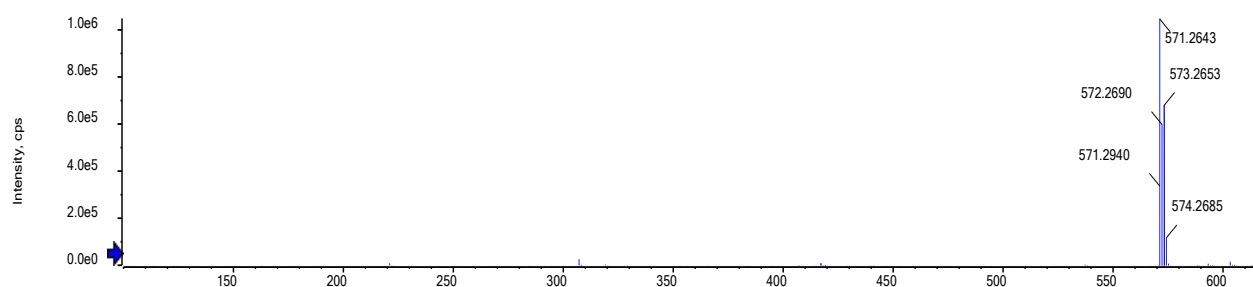
LC-MS spectrum of A12

Spectrum from DataSET14.wiff (sample 31) - 230529 syn023, +TOF MS (100 - 1300) from 0.070 min



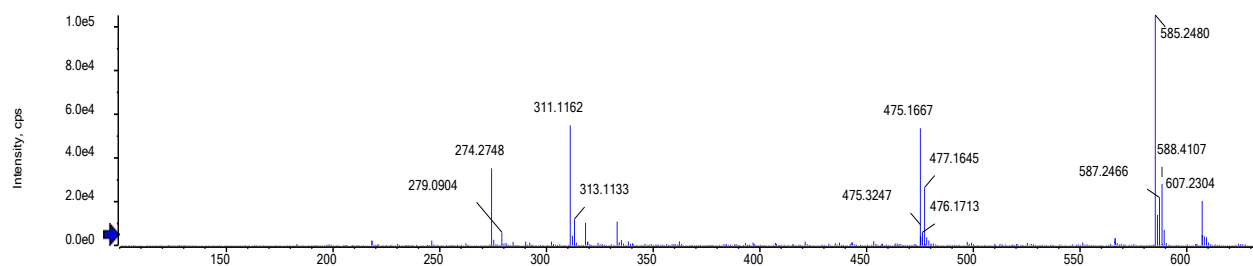
LC-MS spectrum of B12

Spectrum from DataSET13.wiff (sample 32) - 230505 synthetic 007, +TOF MS (100 - 1300) from 0.056 min



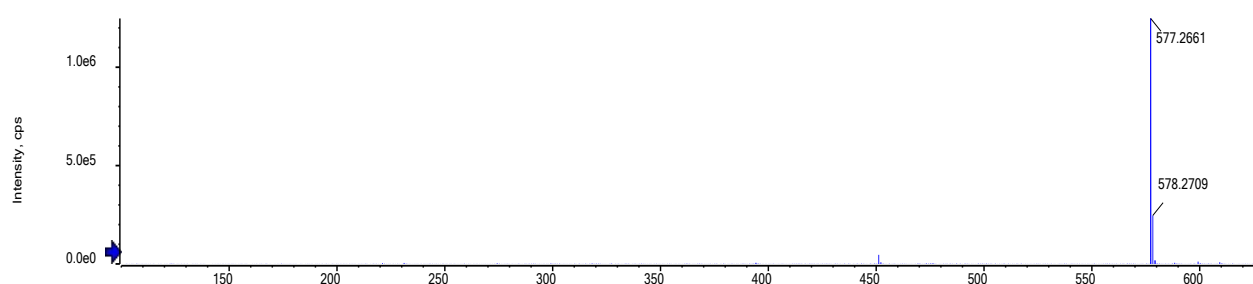
LC-MS spectrum of A13

Spectrum from DataSET14.wiff (sample 39) - 230529 syn031, +TOF MS (100 - 1300) from 0.056 min



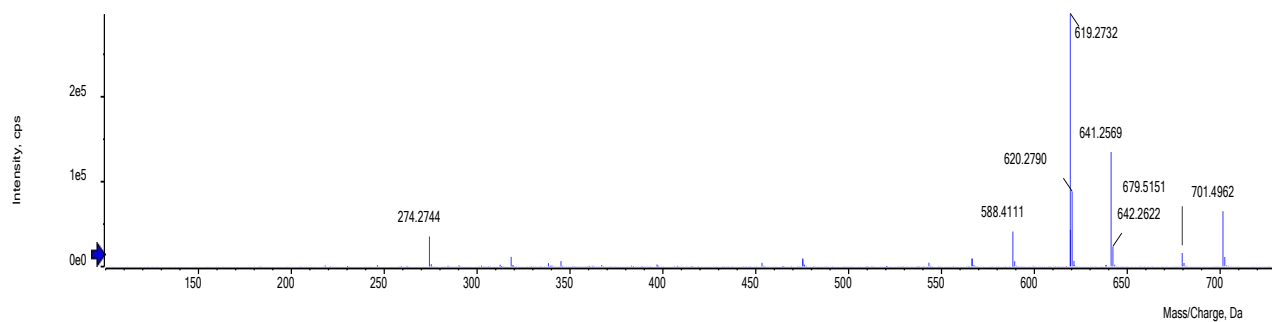
LC-MS spectrum of B13

Spectrum from DataSET13.wiff (sample 38) - 230505 synthetic 013, +TOF MS (100 - 1300) from 0.088 min



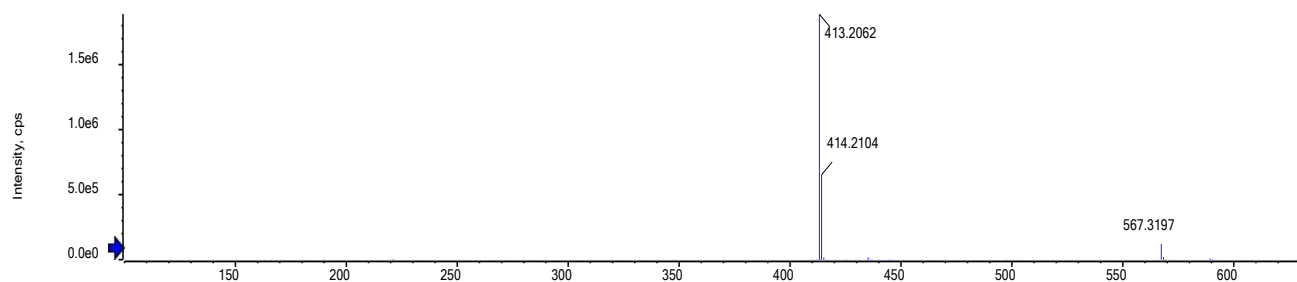
LC-MS spectrum of A14

Spectrum from DataSET14.wiff (sample 32) - 230529 syn024, +TOF MS (100 - 1300) from 0.051 min



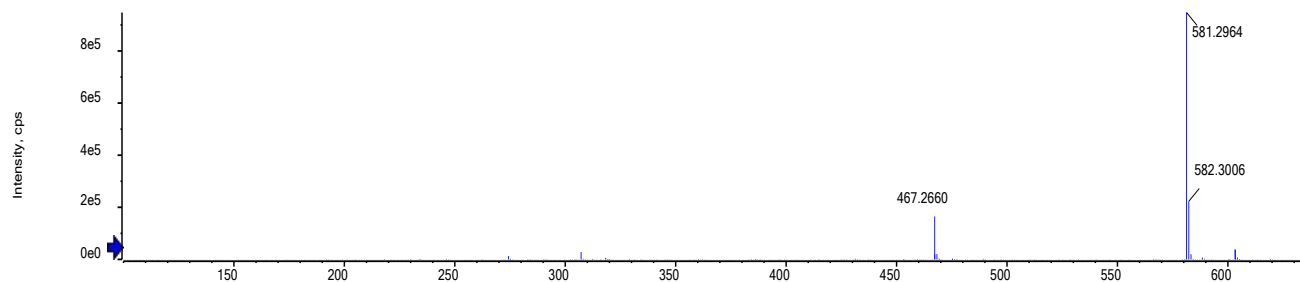
LC-MS spectrum of B14

Spectrum from DataSET13.wiff (sample 33) - 230505 synthetic 008, +TOF MS (100 - 1300) from 0.121 min



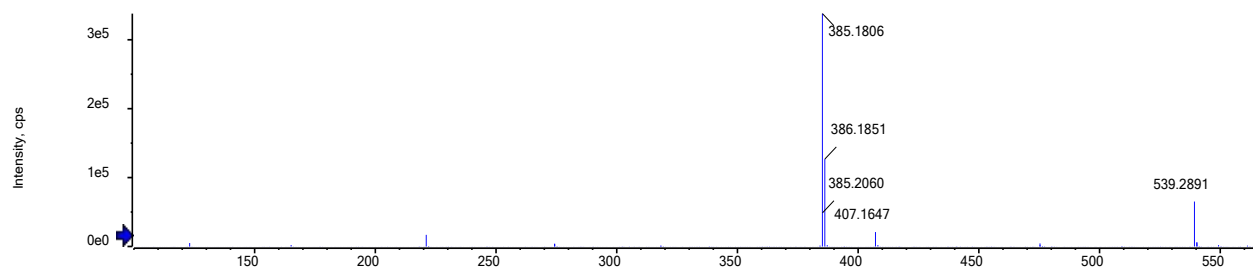
LC-MS spectrum of A15

Spectrum from DataSET14.wiff (sample 40) - 230529 syn032, +TOF MS (100 - 1300) from 0.065 min

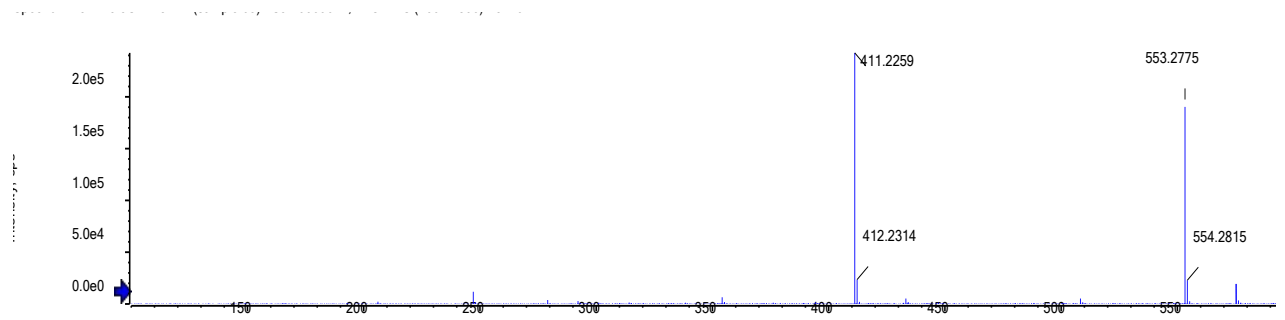


LC-MS spectrum of B15

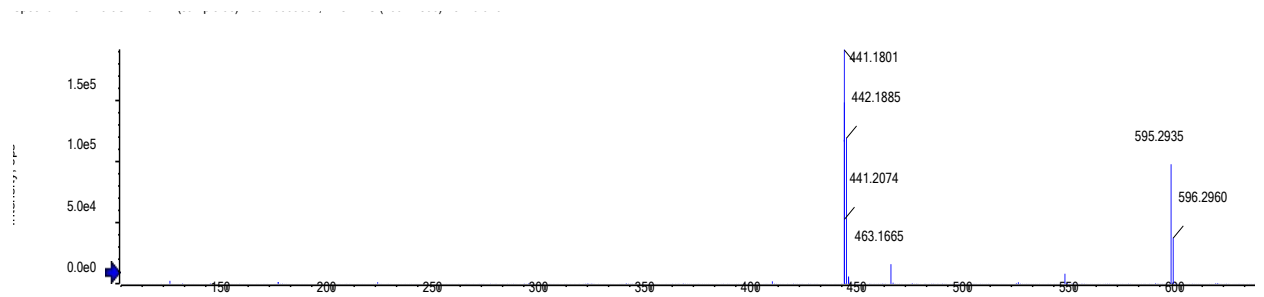
Spectrum from DataSET14.wiff (sample 12) - 230529 syn004, +TOF MS (100 - 1300) from 0.051 min



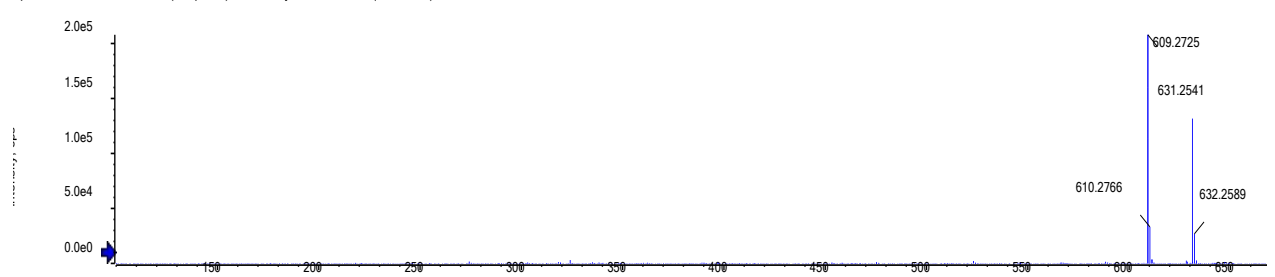
LC-MS spectrum of A16



LC-MS spectrum of **B16**



LC-MS spectrum of **A17**



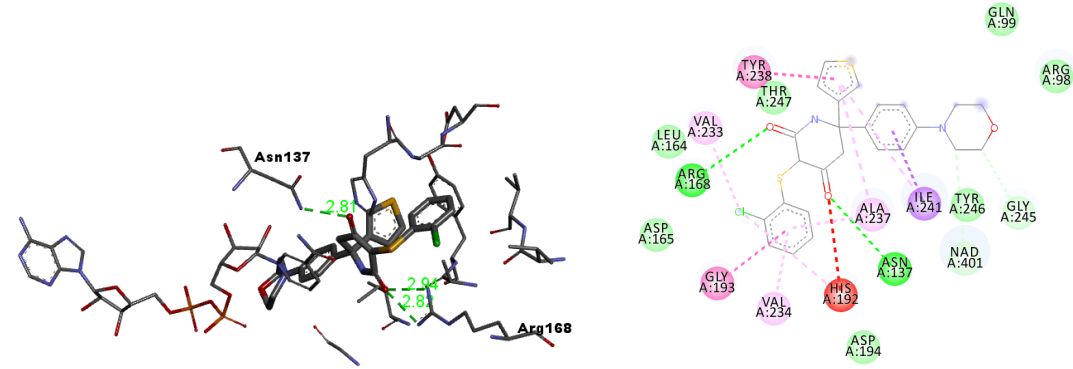
LC-MS spectrum of **B17**

Table S1. ADMET properties of compounds **A14** and **STP**.

Category	Property	ADMET lab 3	VNN-ADMET	PreADMET	PkCSM	Consensus
A14						
Absorption	Water solubility	-	-	14.512 mg/L	-5.446 log mol/L	Low
	Caco-2 permeability	-4.898 log unit	-	51.220 nm/sec	1.199 log Papp in 10 ⁻⁶ cm/s	Moderate
	Human intestinal absorption (%)	-	-	97.562	93.621	High
Distribution	Skin permeability (log Kp)	-	-	-2.215	-2.756	Low
	P-gp inhibitor	Yes	No	Yes	Yes	Yes
	VDss (human, log L/kg)	-	-	-	0.729	Low
Metabolism	BBB permeability (log BB)	No	Yes	Yes	-1.435	Yes
	CYP1A2 inhibitor	No	No	-	No	No
	CYP2C9 inhibitor	No	No	No	No	No
Excretion	CYP2C19 inhibitor	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No
	CYP3A4 inhibitor	Yes	No	Yes	Yes	Yes
Toxicity	Total clearance (log ml/min/kg)	-	-	-	0.509	Low
	AMES toxicity	No	No	No	No	No
	hERG blocker	Yes	Yes	Medium risk	No	Yes
STP						
Absorption	Water solubility	-	-	373.226 mg/L	-3.098 log mol/L	Moderate
	Caco-2 permeability	-4.492 log unit	-	44.979 nm/sec	1.945 log Papp in 10 ⁻⁶ cm/s	Moderate
	Human intestinal absorption (%)	-	-	93.304	93.322	High
Distribution	Skin permeability (log Kp)	-	-	-2.161	-2.444	Low
	P-gp inhibitor	Yes	Yes	No	Yes	Yes
	VDss (human, log L/kg)	-	-	-	0.371	Low
Metabolism	BBB permeability (log BB)	No	No	Yes	0.079	Yes
	CYP1A2 inhibitor	Yes	No	-	Yes	Yes
	CYP2C9 inhibitor	No	No	Yes	No	No
Excretion	CYP2C19 inhibitor	Yes	No	Yes	Yes	Yes
	CYP2D6 inhibitor	Yes	No	No	No	No
	CYP3A4 inhibitor	Yes	No	Yes	No	Yes
Toxicity	Total clearance (log ml/min/kg)	-	-	-	-0.02	Low
	AMES toxicity	No	No	No	No	No
	hERG blocker	No	No	No	No	No

Docking results of original ligand GEN-140 interacting with LDH (PDB:4ZVV).

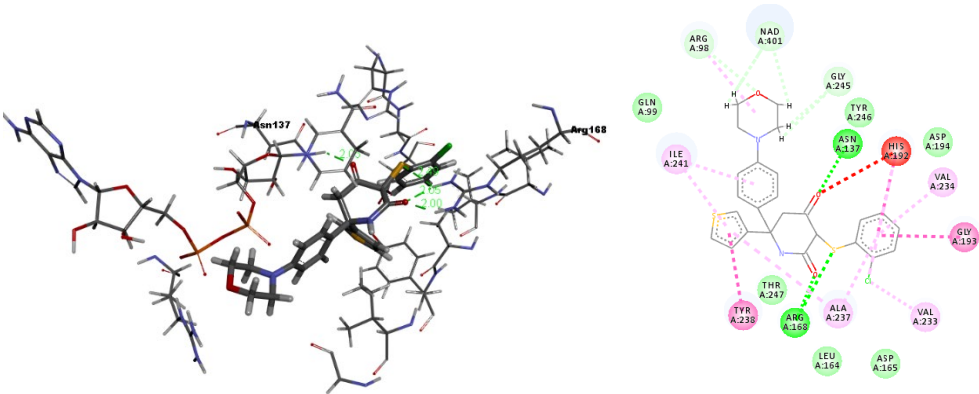
Original co-crystallised interaction



Data result

mode	affinity	dist from best mode	
	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-7.8	0.000	0.000
2	-7.8	1.105	1.518
3	-7.2	4.455	8.159
4	-7.2	5.898	7.647
5	-7.1	4.473	6.889
6	-7.1	5.019	7.268
7	-7.1	2.652	3.234
8	-7.0	4.453	8.035
9	-7.0	3.073	4.708
10	-6.9	1.748	3.177

Typical interaction



Conformational superposition results and RMSD values

