Supporting Information for

Original article

Discovery of highly selective and orally available benzimidazole-based phosphodiesterase 10 inhibitors with improved solubility and pharmacokinetic properties for treatment of pulmonary arterial hypertension

Yuncong Yang[†], Sirui Zhang[†], Qian Zhou[†], Chen Zhang, Yuqi Gao, Hao Wang, Zhe Li, Deyan Wu, Yinuo Wu, Yi-You Huang[*], Lei Guo[*], Hai-Bin Luo[*]

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China

[†]These authors made equal contributions to this work.

[*]Corresponding authors. Tel./fax: +86 20 39943000.

E-mail addresses: huangyy287@mail.sysu.edu.cn (Yi-You Huang), guolei7@mail.sysu.edu.cn (Lei Guo), luohb77@mail.sysu.edu.cn (Hai-Bin Luo).

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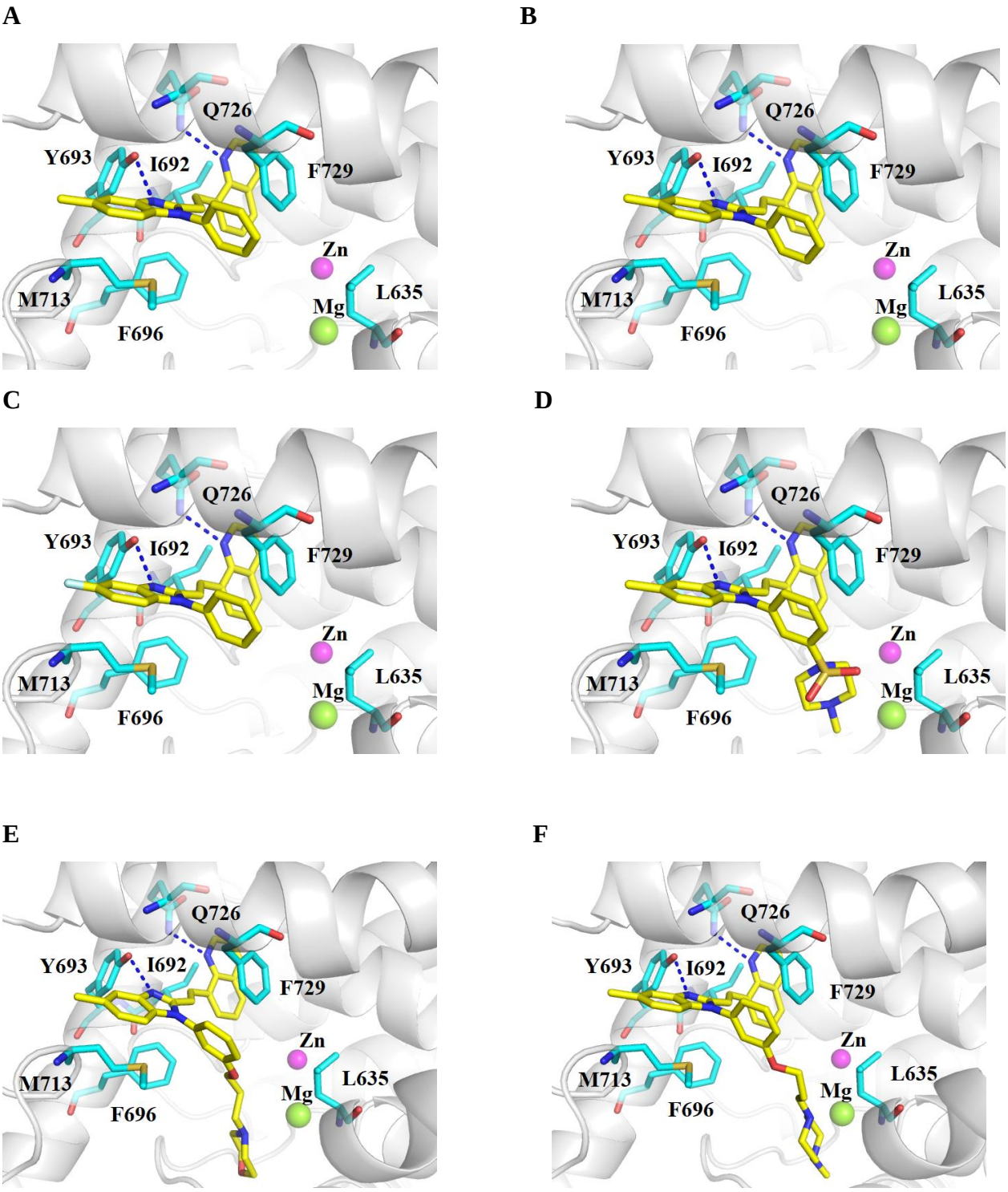


Figure S1 The predicted binding modes of representative compounds **2** (A), **4** (B), **6** (C), **10** (D), **13** (E) and **14** (F) with PDE10.

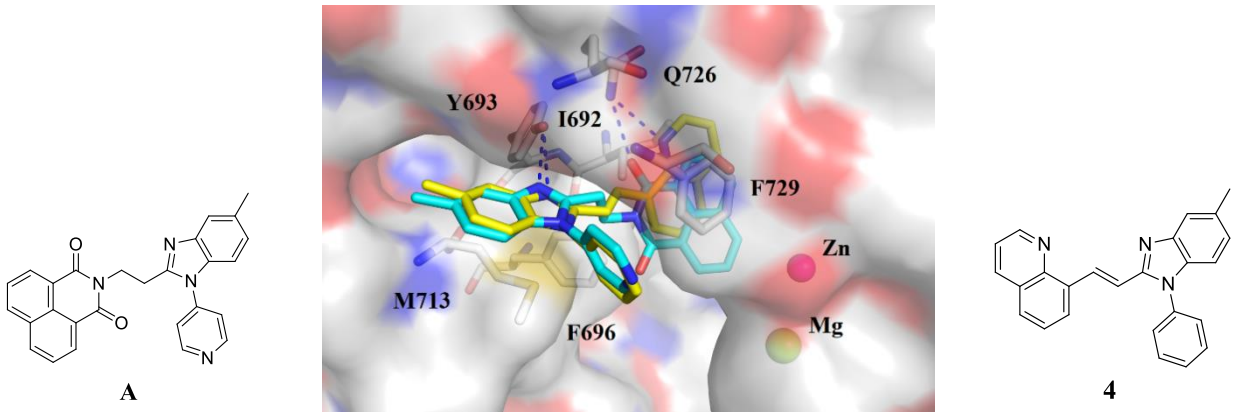


Figure S2 Chemical structures and binding modes of PDE10 inhibitors. Alignment of the crystal structure of PDE10A−**A** with the predicted binding mode of compound **4** (middle). The predicted structure is depicted in cyan while the crystal structure is depicted in silver and yellow.

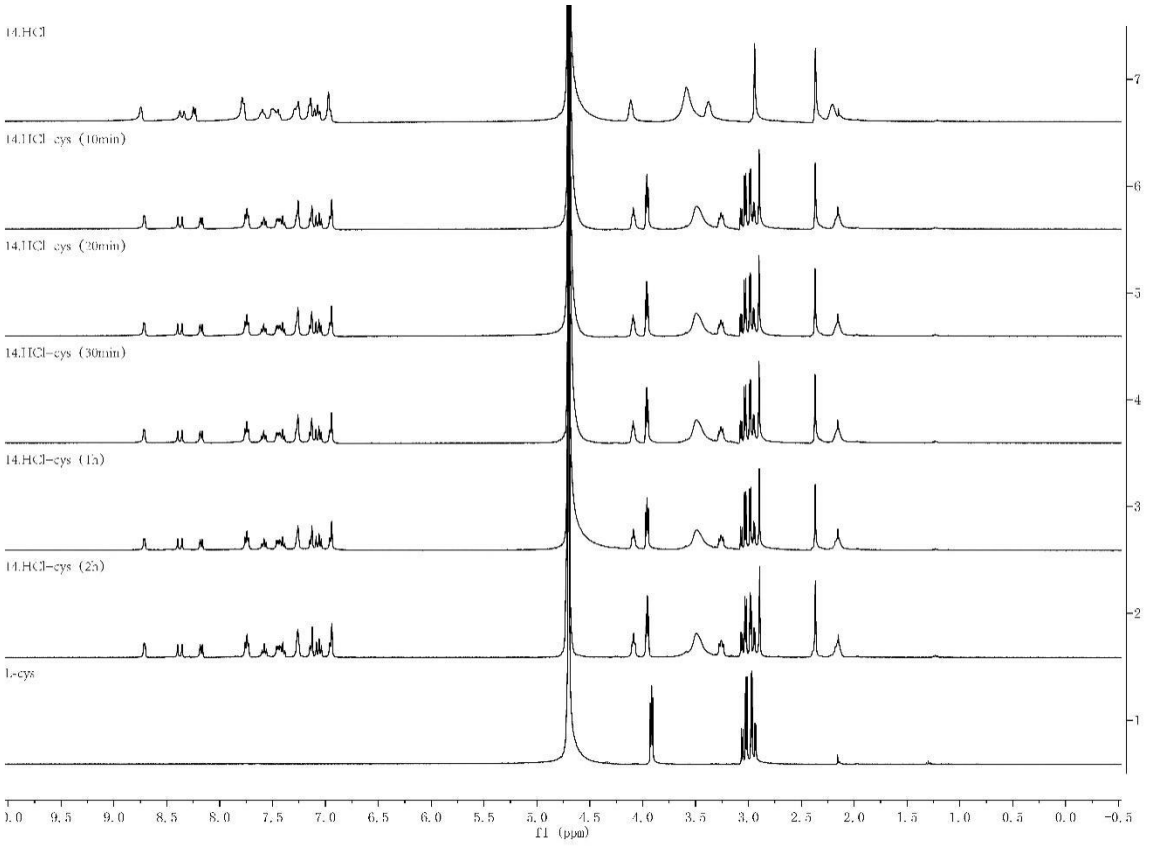
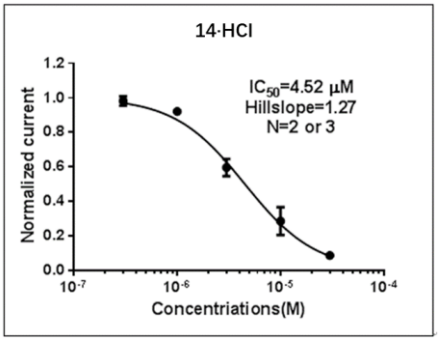


Figure S3 ^{1}H NMR spectra of **14 3HCl** in the presence of Cys (ratio: 1:1) against time measured in D_2O.



Sample	IC50 (μmol/L)	Test cells	Hill slope
Terfenadine	0.057	2	2.4
14 3HCl	4.52	2 or 3	1.27

Figure S4 Concentration−response relationship for peak tail current inhibition by **14 3HCl** with terfenadine as a reference. Solid lines were fitted to the Hill equation. Currents in the presence of **14 3HCl** were normalized to the control amplitudes and plotted as a function of drug concentration. Each drug concentration was applied to 2−3 cells.

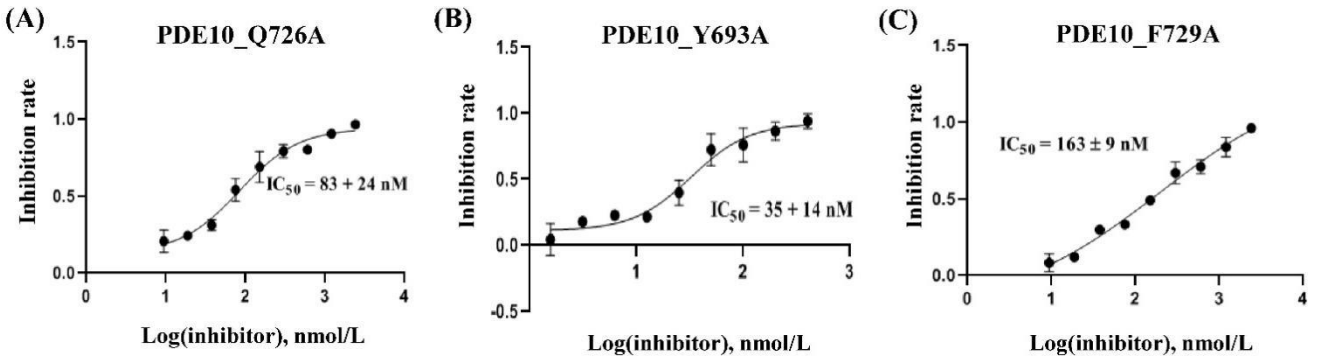


Figure S5 The results of compound **14** inhibiting the catalytic ability of the mutant types of PDE10A including (A) Q726A mutant, (B) Y693A mutant and (C) F729A mutant, respectively.

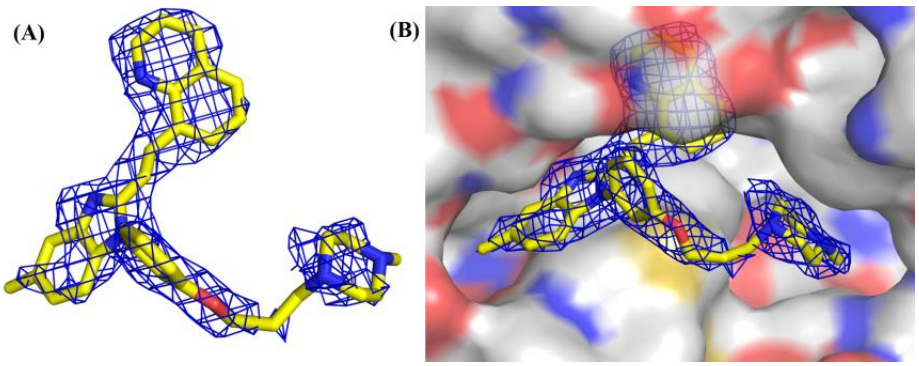


Figure S6 Electron density map (blue mesh) for **14** binding to the PDE10 pocket. (A) Fo-Fc map was calculated from the structure with omission of **14** and contoured at 2.0 σ. (B) The 2Fo-Fc map contoured at 1.0 σ.

Table S1 The predicted binding free energies between compounds and PDE10.

Compd.	$\delta G_{\text{MM-PBSA}}$ (kcal/mol)[a]	$\delta G_{\text{MM-GBSA}}$ (kcal/mol)[b]	Compd.	$\delta G_{\text{MM-PBSA}}$ (kcal/mol)[a]	$\delta G_{\text{MM-GBSA}}$ (kcal/mol)[b]
1	−30.70 ± 3.14	−33.02 ± 2.96	**8**	−33.64 ± 2.79	−35.67 ± 2.40
2	−37.07 ± 3.18	−39.32 ± 2.86	**9**	−34.16 ± 3.12	−35.00 ± 2.48
3	−29.53 ± 2.85	−31.72 ± 2.16	**10**	−37.94 ± 3.38	−40.09 ± 2.88
4	−37.68 ± 3.12	−39.96 ± 2.54	**11**	−42.20 ± 3.23	−42.83 ± 3.28
5	−35.39 ± 3.05	−36.51 ± 2.64	**12**	−39.52 ± 3.42	−38.18 ± 2.69
6	−33.19 ± 3.09	−35.19 ± 2.78	**13**	−39.21 ± 3.85	−41.92 ± 3.61
7	−30.38 ± 3.35	−31.57 ± 3.07	**14**	−41.62 ± 3.46	−42.84 ± 3.19

[a]Calculated binding free energy by MM-PBSA method. [b]Calculated binding free energy by MM-GBSA method.

Table S2 Metabolic stability of **2** and **3** in rat liver microsomes.

Compd.	Species		Percent remaining (%)						$t_{1/2}$ (min)[a]	CL_{int} (ml/min/kg)[b]
			0 min	5 min	10 min	15 min	30 min	45 min		
2	Rat	Mean	100.00	1.89	0.19	0.15	0.13	0.05	0.87	2844.5
		RSD of area ratio	0.02	1.03	0.32	0.20	0.14	0.09		
4	Rat	Mean	100.00	75.40	66.87	55.06	23.91	17.81	17	142.9
		RSD of area ratio	0.15	0.03	0.00	0.12	0.16	0.04		

[a]$t_{1/2}$: elimination half-life; [b]CL_{int} is the *in vitro* intrinsic clearance.

Table S3 Pharmacokinetic profile of compound **A** in Sprague−Dawley rats.

Route	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (h ng/mL)	$AUC_{(0-\infty)}$ (h ng/mL)	$MRT_{(0-t)}$ (h)	CL (mL/h/kg)	F (%)
i.v.[a]	0.58 ± 0.02	0.083 ± 0.001	3501 ± 726	1097 ± 215	1099 ± 215	0.21 ± 0.02	2337 ± 470	/
p.o[b]	1.8 ± 0.7	0.25 ± 0.00	32 ± 10	55 ± 14	70 ± 20	1.5 ± 0.2		2.5 ± 0.6

[a]i.v. = intravenous administration, dose = 2.5 mg/kg. [b]*p.o.* = oral administration, dose = 10 mg/kg.

Table S4 Pharmacokinetic parameters of tadalafil in rats summarized according to the literatures.

Parameters	Results
AUC_{last} (μg·min/mL)	9.50 ± 5.14[a]
$AUC_{0-\infty}$ (μg·min/mL)	10.2 ± 5.1[a]
C_{max} (μg/mL)	0.0821 ± 0.0463[a]
T_{max} (min)	37.5 ± 15.0[a]
Terminal half-life (min)	76.9 ± 27.7[a]
Volume of distribution / V_d (L/kg)	5.2[b]
Clearance / CL (mL/min/kg)	5.1[b]
Elimination half-life / $t_{1/2}$ (h)	2.4[b]
Oral bioavailability / F (%)	63[b]

[a]Oral administration of at a dose of 1 mg/kg in rats (n = 4). Data were shown as mean ± SD according to the reference[1]. AUC_{last}, total area under the plasma concentration–time curve from time zero to time last. $AUC_{0-\infty}$, total area under the plasma concentration–time curve from time zero to time infinity. C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}. [b]Oral administration of at a dose of 5 mg/kg in rats. Data were presented according to the reference[2].

Table S5 Alignment of amino acids at the substrate-binding pocket across PDE families.

	678	685	689	692	693	713	725	726	729	730	762
PDE10A2	V	T	A	I	Y	M	G	Q	F	Y	W
PDE1B	P	H	T	L	M	L	S	Q	F	I	N
PDE2	Q	T	A	I	Y	M	L	Q	F	M	W
PDE3	P	H	T	I	V	F	L	Q	F	I	W
PDE11	V	S	A	V	T	F	L	Q	W	I	W
PDE4	P	Y	T	I	M	M	S	Q	F	I	Y
PDE7	P	S	S	V	T	L	I	Q	F	M	W
PDE8	P	C	A	I	S	V	S	Q	F	I	W
PDE5	I	Q	A	V	A	M	M	Q	F	I	W
PDE6	I	Q	A	V	A	M	L	Q	F	I	W
PDE9	E	A	V	L	L	F	A	Q	F	I	Y

The data shown in the table according to the reference[3].

Table S6 The ion chromatography result of compound **14 3HCl**.

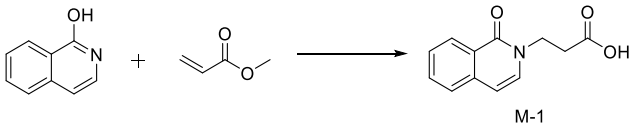
Sample weight (mg)	Detecting Cl^- concentration (mg/L)	Total volume (mL)	Ratio of Cl (w/w)
2.81	45.7028	10	0.1626
7.90	12.7814	100	0.1619

Table S7 The plasma concentration of **14 ·3HCl** (*p.o*) at a dose of 10 mg/kg.

Time (h)	**14 ·3HCl** plasma concentration (ng/mL)			Average ± SD (ng/mL)	Average ± SD （nmol/L)
	Rat 1	Rat 2	Rat 3		
0	BLQ	BLQ	BLQ	NA	
0.25	4.47	4.97	2.40	3.94 ± 1.36	
0.5	20.86	33.71	17.94	24.17 ± 8.39	
1	56.07	82.31	39.59	59.32 ± 21.54	
2	151.55	162.37	100.85	138.26 ± 32.84	266.8 ± 63.4
4	253.92	250.31	184.36	229.53 ± 39.16	442.8 ± 75.5
6	273.61	235.45	265.90	258.32 ± 20.18	498.4 ± 38.9
8	299.45	197.39	205.52	234.12 ± 56.72	451.7 ± 108.0
10	243.68	118.14	161.84	174.56 ± 63.73	336.8 ± 123.0
24	51.63	18.62	18.74	29.67 ± 19.02	

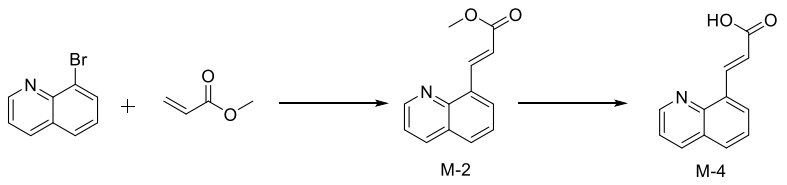
Preparation and characterization of the target compounds

1. Synthesis routine for compounds M-1 to M-35



M-1

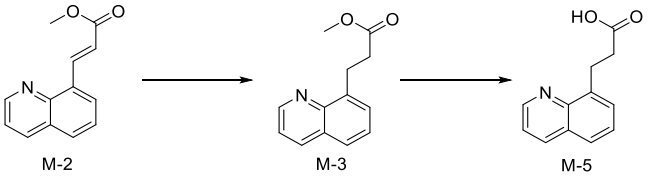
3-(1-Oxoisoquinolin-2(*1H*)-yl)propanoic acid (M-1). To a solution of isocarbostyril (2.374 g, 16.3 mmol) and methyl acrylate (1.689 g, 19.6 mmol) in MeOH (33 mL) was added sodium methanolate (88 mg, 1.63 mmol), and the mixture was refluxed for 2.5 h, then the methyl acrylate (1.689 g, 19.6 mmol) was added again refluxing for another 1.5 h. After completion of the reaction, solvent was removed *in vacuo*, and then the residue was extracted with DCM (100 × 3 mL) and brine (100 mL). The organic phase was collected and dried over anhydrous sodium sulfate. After concentrated by vacuum distillation, the crude was added 1 mol/L HCl (33 mL) and refluxed for 1 h and stirred at room temperature for 16 h. When it finished, white solid was filtered to afford a product (1.36 g). Yield, 38.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 8.1 Hz, 1H), 7.64 (tt, J = 10.1, 2.1 Hz, 1H), 7.53−7.45 (m, 2H), 7.24−7.18 (m, 1H), 6.52 (d, J = 7.4 Hz, 1H), 4.27 (t, J = 6.4 Hz, 2H), 2.95 (t, J = 6.4 Hz, 2H).



M-2 M-4

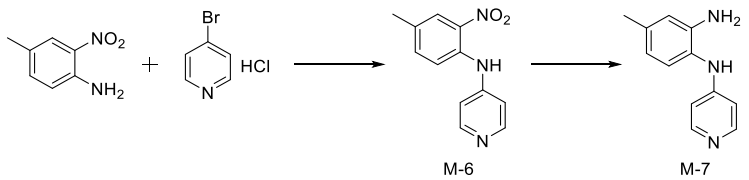
Methyl-(*E*)-3-(quinolin-8-yl)acrylate (M-2). To the solution of 8-bromoquinoline (7.36 g, 35.4 mmol) in dry DMF (27 mL) was added methyl acrylate (3.6 g, 42.5 mmol), TEA (27 mL, 35.4 mmol) and $(PPh_3)_2PdCl_2$ (2.48 g, 3.54 mmol) in sequence, then the mixture was heated to 120 ℃ stirring overnight under argon atmosphere. After cooling to room temperature, the solid was filtered through sufficient Celite, and then poured into water and extracted with EA (200 × 3 mL), then the organic phase was washed with water (50 × 3 mL), brine (50 × 3 mL), and dried over anhydrous sodium sulfate. After concentration *in vacuo* the residue was purified by silica gel column chromatography (DCM/MeOH, 100:1) to afford a product (4.41 g). Yield, 58.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (d, J = 3.9 Hz, 1H), 8.94 (d, J = 16.3 Hz, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 7.2 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.59 (s, 1H), 7.48 (d, J = 12.4 Hz, 1H), 6.85 (d, J = 16.3 Hz, 1H), 3.88 (s, 3H).

(*E*)-3-(Quinolin-8-yl)acrylic acid (M-4). To the solution of **M-2** (202.9 mg, 0.95 mmol) in EtOH (3 mL) was added 6 mol/L NaOH (0.5 mL), and the mixture was stirred at room temperature overnight. After completion of the reaction, solvent was removed *in vacuo*, and then the solution was acidified with 1 mol/L aqueous HCl to pH = 1−2. The white solid was filtered to afford a product (130 mg). Yield, 71.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (d, J = 16.3 Hz, 2H), 8.25 (d, J = 8.2 Hz, 1H), 8.09 (d, J = 7.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 7.55 (dd, J = 8.1, 4.2 Hz, 1H), 6.82 (d, J = 16.2 Hz, 1H).



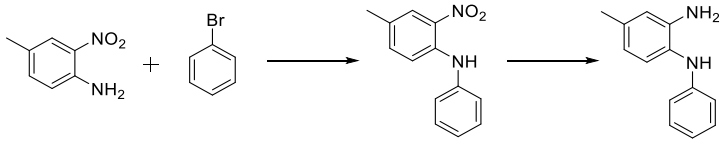
Methyl 3-(quinolin-8-yl)propanoate (M-3). To the solution of **M-2** (220 mg, 1.03 mmol) in EtOH (5 mL) was add Pd/C (22 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 5:1) to afford a product (181 mg). Yield, 81.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98−8.92 (m, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 6.9 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.42 (dd, J = 8.2, 4.2 Hz, 1H), 3.69 (s, 3H), 3.61 (t, J = 7.7 Hz, 2H), 2.90 (t, J = 7.7 Hz, 2H).

3-(Quinolin-8-yl)propanoic acid (M-5). To a solution of **M-3** (181 mg, 0.84 mmol) in EtOH (3 mL) was added 6 mol/L NaOH (0.5 mL), and the mixture was stirred at room temperature overnight. After completion of the reaction, ethanol was removed *in vacuo*, and then the solution was acidified with 1 N aqueous HCl to pH = 1−2. The white solid was filtered to afford a product (150 mg). Yield, 88.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (d, J = 4.0 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 6.9 Hz, 1H), 7.54−7.50 (m, 2H), 3.58 (t, J = 7.2 Hz, 2H), 2.89 (t, J = 7.2 Hz, 2H).



***N*-(4-Methyl-2-nitrophenyl)pyridin-4-amine (M-6).** To the solution of 4-methyl-2-nitroaniline (390 mg, 2.58 mmol) in toluene (9 mL) was added 4-bromopyridine hydrochloride (1.0 g, 5.16 mmol), NaOBu-*t* (743 mg, 7.74 mmol), BINAP (65 mg, 0.1 mmol), $Pd_2(dba)_3$ (50 mg, 0.05 mmol) in sequence, and the mixture was heated to 100 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 2:1) to afford a product (290 mg), Yield, 49.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.50 (d, J = 6.0 Hz, 2H), 8.04 (s, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.42–7.36 (m, 1H), 7.10 (d, J = 6.3 Hz, 2H), 2.40 (s, 3H).

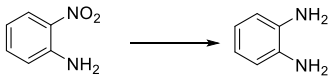
4-Methyl-N^1-(pyridin-4-yl)benzene-1,2-diamine (M-7). To the solution of **M-6** (290 mg, 1.27 mmol) in EtOH (5 mL) was add Pd/C (40 mg, 13%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromate-graphy (DCM/MeOH, 10:1) to afford a product (197 mg). Yield, 77.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.80 (s, 2H), 8.50 (d, J = 6.0 Hz, 2H), 8.04 (s, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.42−7.36 (m, 1H), 7.10 (d, J = 6.3 Hz, 2H), 2.40 (s, 3H).



M-8 M-9

4-Methyl-2-nitro-*N*-phenylaniline (M-8). 4-Methyl-2-nitroaniline (3.08 g 20.2 mmol) was added into bromobenzene (3.17 g, 20.2 mmol) in round bottomed flask and K_2CO_3 (2.8 g, 20.2 mmol), CuI (385 mg, 2.02 mmol) was added into flask successively. The mixture was heated to 200 ℃ stirring for 2 h. After cooling down to room temperature, the mixture was filtered through Celite washed by EA for three times, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 5:1) to afford a product (1.76 g). Yield, 38.2%; ^{1}H NMR (500 MHz, MeOD) δ 7.97 (s, 1H), 7.41 (t, J = 7.9 Hz, 2H), 7.28−7.22 (m, 3H), 7.21−7.19 (m, 1H), 7.18−7.16 (m, 1H), 2.29 (s, 3H).

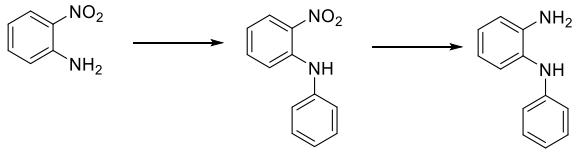
4-Methyl-N^1-phenylbenzene-1,2-diamine (M-9). To the solution of **M-8** (1.76 g, 7.73 mmol) in EtOH (60 mL) was add Pd/C (269 mg, 15%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (1.16 g). Yield, 77.3%; ^{1}H NMR (500 MHz, MeOD) δ 8.27 (s, 2H), 7.87 (s, 1H), 7.43 (t, J = 7.9 Hz, 2H), 7.28−7.22 (m, 3H), 7.21−7.19 (m, 1H), 7.18−7.16 (m, 1H), 2.26 (s, 3H).



M-10

Benzene-1,2-diamine (M-10). To the solution of 2-nitroanline (1.54 g, 11.1 mmol) in EtOH (60 mL) was add Pd/C (174 mg, 11%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography

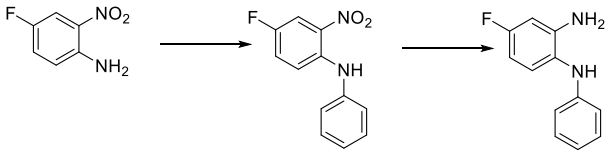
(PE/EA, 10:1) to afford a product (732 mg). Yield, 61.0%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82−6.77 (m, 4H), 3.36 ppm (s, 4H).



M-11 M-12

2-Nitro-*N*-phenylaniline (M-11). 2-Nitroaniline (3.3 g, 23.9 mmol) was added into iodobenzene (7.30 g, 35.8 mmol) in round bottomed flask and K_2CO_3 (6.6 g, 47.8 mmol), CuI (2.1 g, 12.0 mmol) was added into flask successively. The mixture was heated to 200 ℃ stirring for 2 h under Argon atmosphere. After cooling down to room temperature, the mixture was filtered through Celite washed by EA for three times, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromate-graphy (PE/EA, 10:1) to afford a product (2.16 g). Yield, 42.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (br, 1H), 8.19 (dd, J = 8.7, 1.8 Hz, 1H), 7.43 (d, J = 1.8 Hz, 1H), 7.41−7.32 (m, 2H), 7.28−7.23 (m, 4H), 6.76 (1H, dd, J = 1.8, 1.2 Hz).

***N*^{1}-Phenylbenzene-1,2-diamine (M-12).** To the solution of **M-11** (2.16 g, 10.1 mmol) in EtOH (80 mL) was add Pd/C (246 mg, 11%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (1.34 g). Yield, 73.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, J = 15.1, 7.2 Hz, 2H), 7.16 (d, J = 7.8 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 6.86−6.80 (m, 5H), 5.21 (s, 1H), 3.79 (s, 2H).

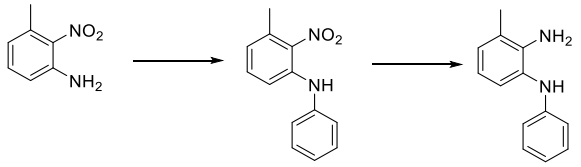


M-13 M-14

4-Fluoro-2-nitro-*N*-phenylaniline (M-13). 4-Fluoro2-nitroaniline (1.18 g 7.6 mmol) was added into iodobenzene (2.31 g, 11.4 mmol) in round bottomed flask and K_2CO_3 (2.12 g, 15.2 mmol), CuI (434 mg, 2.28 mmol) was added into flask successively. The mixture was heated to 200 ℃ stirring for 2 h under argon atmosphere. After cooling down to room temperature, the mixture was filtered through Celite washed by EA for three times, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (1.70 g). Yield, 95.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, J = 7.9 Hz, 2H), 7.04 (dd, J = 8.5, 5.9 Hz, 1H), 6.76 (t, J = 7.3 Hz, 1H), 6.68 (d, J = 7.8 Hz, 2H), 6.47 (dd, J = 10.1, 2.8 Hz, 1H), 6.45 (td, J = 8.4, 2.8 Hz, 1H), 5.00 (s, 1H).

4-Fluoro-*N*^{1}-phenylbenzene-1,2-diamine (M-14). To the solution of **M-13** (1.34 g, 5.78 mmol) in MeOH (30 mL) was added Zinc powder (3.78 g, 57.8 mmol) and 2 mol/L HCl (60 mL) in sequence. The mixture was heated to 80 ℃ and stirred for 2 h. After cooling down to room temperature, the mixture was filtered through Celite washed by MeOH for three times, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (790 mg). Yield, 67.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (t, J = 7.9 Hz, 2H), 7.02 (dd, J =

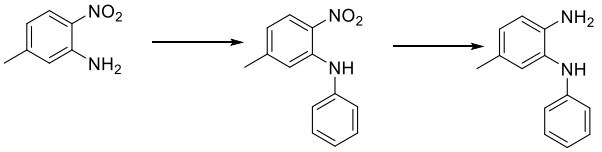
8.5, 5.9 Hz, 1H), 6.79 (t, *J* = 7.3 Hz, 1H), 6.64 (d, *J* = 7.8 Hz, 2H), 6.49 (dd, *J* = 10.1, 2.8 Hz, 1H), 6.42 (td, *J* = 8.4, 2.8 Hz, 1H), 5.00 (s, 1H), 3.93 (s, 2H).



M-15 M-16

3-Methyl-2-nitro-*N*-phenylaniline (M-15). To the solution of 3-methyl-2-nitroaniline (916 mg, 6.0 mmol) in toluene (12 mL) was added bromobenzene (945 mg, 6.0 mmol), Cs_2CO_3 (3.92 g, 12.0 mmol), BINAP (560 mg, 0.9 mmol), $Pd_2(dba)_3$ (550 mg, 0.6 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 5:1) to afford a product (500 mg). Yield, 36.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.40−7.32 (m, 2H), 7.25−7.17 (m, 4H), 7.12 (dd, *J* = 10.6, 4.2 Hz, 1H), 6.74 (dd, *J* = 6.6, 1.3 Hz, 1H), 2.50 (s, 3H).

3-Methyl-N^1-phenylbenzene-1,2-diamine (M-16). To the solution of **M-15** (500 mg, 2.19 mmol) in EtOH (10 mL) was add Pd/C (50 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 5:1) to afford a product (320 mg). Yield, 74.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (dd, *J* = 8.3, 7.5 Hz, 2H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.84 (t, *J* = 7.3 Hz, 1H), 6.77−6.69 (m, 3H), 5.17 (s, 1H), 3.82 (s, 2H), 2.26 (s, 3H).

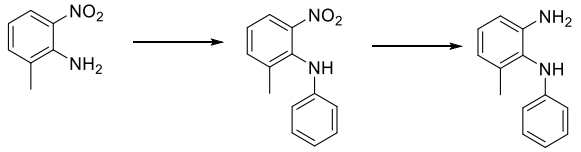


M-17 M-18

5-Methyl-2-nitro-*N*-phenylaniline (M-17). To the solution of 5-methyl-2-nitroaniline (500 mg, 3.29 mmol) in toluene (7 mL) was added bromobenzene (774 mg, 4.94 mmol), Cs_2CO_3 (2.14 g, 6.58 mmol), BINAP (307 mg, 0.49 mmol), $Pd_2(dba)_3$ (300 mg, 0.33 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (710 mg). Yield, 94.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.8 Hz, 1H), 7.43 (dd, *J* = 11.0, 4.7 Hz, 2H), 7.28 (d, *J* = 7.6 Hz, 2H), 7.23 (d, *J* = 7.4 Hz, 1H), 6.99 (s, 1H), 6.58 (dd, *J* = 8.8, 1.7 Hz, 1H), 2.26 (s, 3H).

5-Methyl-N^1-phenylbenzene-1,2-diamine (M-18). To the solution of **M-17** (630 mg, 2.76 mmol) in EtOH (20 mL) was add Pd/C (63 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (400 mg). Yield, 73.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26−7.22 (m, 2H), 6.98 (d, *J* = 1.3 Hz, 1H), 6.86−6.84 (m, 2H), 6.79 (d, *J* = 1.0 Hz, 1H),

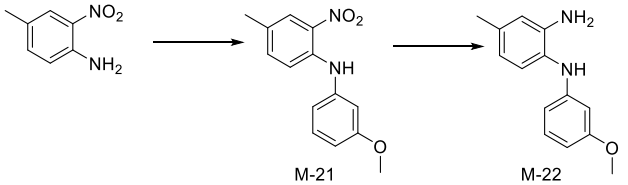
6.78−6.76 (m, 1H), 6.75−6.73 (m, 1H), 5.20 (s, 1H), 3.64 (s, 2H), 2.25 (s, 3H).



M-19 M-20

2-Methyl-6-nitro-*N*-phenylaniline (M-19). To the solution of 6-methyl-2-nitroaniline (500 mg, 3.29 mmol) in toluene (7 mL) was added bromobenzene (774 mg, 4.94 mmol), Cs_2CO_3 (2.14 g, 6.58 mmol), BINAP (307 mg, 0.49 mmol), $Pd_2(dba)_3$ (300 mg, 0.33 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (220 mg). Yield, 29.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.99 (dd, J = 8.4, 1.1 Hz, 1H), 7.45−7.43 (m, 1H), 7.28 (s, 1H), 7.25 (dd, J = 5.6, 3.8 Hz, 1H), 7.10−7.08 (m, 1H), 7.00 (t, J = 7.4 Hz, 1H), 6.81−6.77 (m, 2H), 2.11 (s, 3H).

6-Methyl-N^1-phenylbenzene-1,2-diamine (M-20). To the solution of **M-19** (220 mg, 0.96 mmol) in EtOH (7 mL) was add Pd/C (22 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (79 mg). Yield, 41.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (t, J = 7.8 Hz, 2H), 7.04 (t, J = 7.7 Hz, 1H), 6.79 (t, J = 7.3 Hz, 1H), 6.70 (d, J = 7.7 Hz, 2H), 6.60 (d, J = 7.8 Hz, 2H), 5.00 (s, 1H), 3.89 (s, 2H), 2.20 (s, 3H).

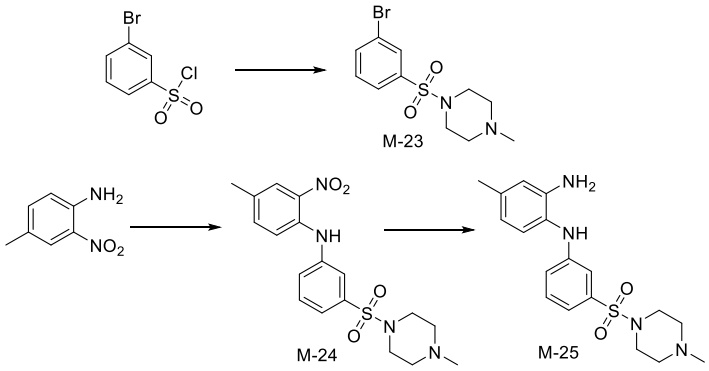


M-21 M-22

***N*-(3-Methoxyphenyl)-4-methyl-2-nitroaniline (M-21).** To the solution of 4-methyl-2-nitro-aniline (500 mg, 3.29 mmol) in toluene (7 mL) was added 3-bromoanisole (1.23 g, 4.94 mmol), Cs_2CO_3 (2.14 g, 6.58 mmol), BINAP (307 mg, 0.49 mmol), $Pd_2(dba)_3$ (300 mg, 0.33 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (450 mg). Yield, 53.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.00 (s, 1H), 7.29 (t, J = 8.1 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.20 (dd, J = 8.8, 1.9 Hz, 1H), 6.85 (dd, J = 7.9, 1.9 Hz, 1H), 6.79 (t, J = 2.2 Hz, 1H), 6.74 (dd, J = 8.1, 2.2 Hz, 1H), 3.82 (s, 3H), 2.30 (s, 3H).

***N*^{1}-(3-Methoxyphenyl)-4-methylbenzene-1,2-diamine (M-22).** To the solution of **M-21** (450 mg, 1.74 mmol) in EtOH (15 mL) was add Pd/C (45 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (285 mg). Yield, 71.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (t, J = 8.1 Hz, 1H), 7.02 (d, J = 7.9 Hz, 1H), 6.65 (s, 1H), 6.59 (d, J = 7.9 Hz,

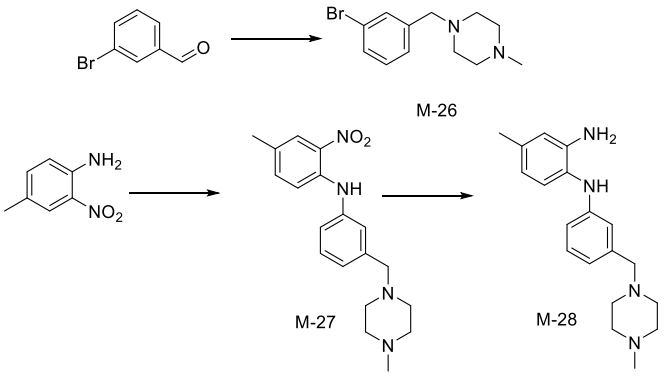
1H), 6.38 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.34–6.32 (m, 1H), 6.27 (t, *J* = 2.2 Hz, 1H), 5.12 (s, 1H), 3.77 (s, 5H), 2.30 (s, 3H).



1-((3-Bromophenyl)sulfonyl)-4-methylpiperazine (M-23). To a solution of 3-bromobenz-enesulfonyl chloride (3.56 g, 13.9 mmol) in DCM (28 mL) was added 1-methylpiperazine (2.79 g, 27.8 mmol) in ice bath, and then stirred for 3 h at room temperature. After completion of the reaction, the mixture was poured in to water and extracted with DCM three times, then the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (DCM/MeOH, 20:1) to afford a product (285 mg). Yield, 90.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.71 (dd, *J* = 15.7, 7.9 Hz, 2H), 7.42 (t, *J* = 7.9 Hz, 1H), 3.06 (s, 4H), 2.47 (dd, *J* = 14.0, 9.1 Hz, 4H), 2.27 (s, 3H).

4-Methyl-*N*-(3-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-2-nitroaniline (M-24). To the solution of 4-methyl-2-nitroaniline (739 mg, 2.32 mmol) in toluene (5 mL) was added **M-23** (363 mg, 2.38 mmol), Cs_2CO_3 (1.58 g, 4.85 mmol), BINAP (217 mg, 0.35 mmol), $Pd_2(dba)_3$ (212 mg, 0.23 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (475 mg). Yield, 52.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.05 (s, 1H), 7.64 (s, 1H), 7.55 (d, *J* = 7.1 Hz, 2H), 7.48 (dd, *J* = 6.7, 2.3 Hz, 1H), 7.30 (d, *J* = 4.7 Hz, 1H), 7.23 (d, *J* = 8.7 Hz, 1H), 3.11 (s, 4H), 2.56−2.48 (m, 4H), 2.36 (s, 3H), 2.31 (s, 3H).

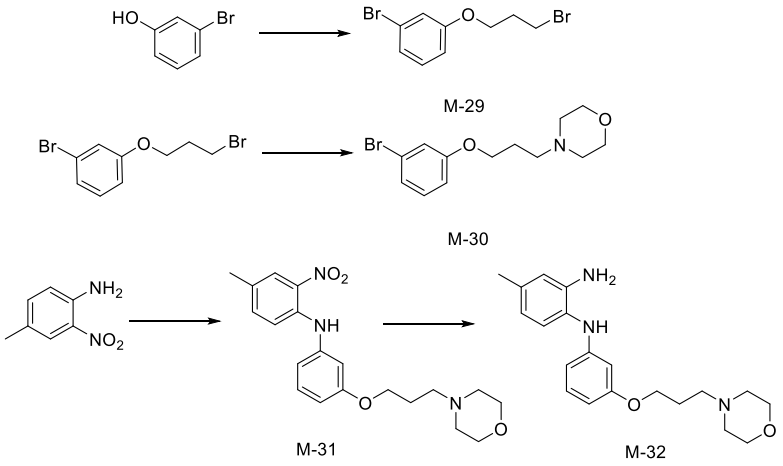
4-Methyl-N^1-(3-((4-methylpiperazin-1-yl)sulfonyl)phenyl)benzene-1,2-diamine (M-25). To the solution of **M-24** (400 mg, 1.02 mmol) in EtOH (10 mL) was add Pd/C (40 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (287 mg). Yield, 71.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, *J* = 5.5 Hz, 1H), 7.13 (d, *J* = 7.7 Hz, 1H), 7.03 (s, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 6.82 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.66 (s, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 5.34 (s, 1H), 3.75 (s, 2H), 3.07 (s, 4H), 2.55–2.45 (m, 4H), 2.31 (d, *J* = 3.0 Hz, 6H).



1-(3-Bromobenzyl)-4-methylpiperazine (M-26). To a solution of 3-bromobenzaldehyde (2.44 g, 13.2 mmol) in DCM (132 mL) was added 1-methylpiperazine (1.98 g, 19.8 mmol) and $Na(OAc)_3BH$ (8.4 g 39.6 mmol) in ice bath, and then stirred overnight at room temperature. After completion of the reaction, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (880 mg). Yield, 25.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.40 (d, J = 7.4 Hz, 1H), 7.22 (t, J = 4.5 Hz, 1H), 7.20−7.18 (m, 1H), 3.54 (s, 2H), 2.93 (s, 4H), 2.74 (d, J = 4.6 Hz, 4H), 2.60 (s, 3H).

4-Methyl-*N*-(3-((4-methylpiperazin-1-yl)methyl)phenyl)-2-nitroaniline (M-27). To the solution of 4-methyl-2-nitroaniline (249 mg, 1.64 mmol) in toluene (3 mL) was added **M-26** (440 mg, 1.64 mmol), Cs_2CO_3 (964 mg, 3.28 mmol), BINAP (138 mg, 0.25 mmol), $Pd_2(dba)_3$ (136 mg, 0.16 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 20:1) to afford a product (300 mg). Yield, 53.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.00 (s, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.26−7.22 (m, 2H), 7.21−7.17 (m, 2H), 7.16 (d, J = 3.0 Hz, 1H), 7.13 (s, 1H), 3.52 (s, 2H), 2.42 (t, J = 28.8 Hz, 8H), 2.30 (s, 3H), 2.29 (s, 3H).

4-Methyl-N^1-(3-((4-methylpiperazin-1-yl)methyl)phenyl)benzene-1,2-diamine (M-28). To the solution of **M-27** (260 mg, 0.76 mmol) in EtOH (5 mL) was add Pd/C (50 mg, 19%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (140 mg). Yield, 53.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (t, J = 7.7 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 6.75−6.71 (m, 2H), 6.63 (s, 1H), 6.57−6.53 (m, 2H), 5.08 (s, 1H), 3.72 (s, 2H), 3.43 (s, 2H), 2.64−2.44 (m, 8H), 2.28 (s, 6H).

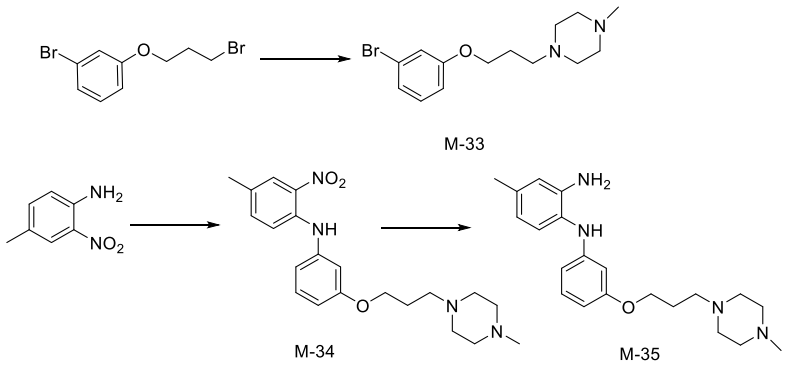


1-Bromo-3-(3-bromopropoxy)benzene (M-29). The solution of 3-bromophenol (4.32 g, 25 mmol) in DMF (10 mL) was dropped in the solution of 1,3-dibromopropane (10.08 g, 50 mmol) and K_2CO_3 (5.18 g, 37.5 mmol) in DMF (25 mL), and the mixture was stirred for 2 h at room temperature, then stirred for another 2 h at 70 ℃. After completion of the reaction, the mixture was poured into water and extracted with EA (100 × 3 mL), then the organic phase was washed with water (25 × 3 mL), brine (25 × 3 mL), and dried over anhydrous sodium sulfate. After concentration *in vacuo* the residue was purified by silica gel column chromatography (PE/EA, 10:1) to afford a product (6.0 g). Yield, 76.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (dd, J = 10.1, 6.4 Hz, 1H), 7.10−7.06 (m, 2H), 6.84 (d, J = 8.2 Hz, 1H), 4.01 (t, J = 6.3 Hz, 2H), 2.53 (t, 2H), 2.00−1.96 (m, 2H).

4-(3-(3-Bromophenoxy)propyl)morpholine (M-30). To the solution of **M-29** (4.82 g, 16.3 mmol) in MeCN (10 mL) was added morpholine (2.84 g, 32.6 mmol) and K_2CO_3 (4.50 g, 32.6 mmol), and the mixture was stirred at 80 ℃ for 3 h. After completion of the reaction, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (PE/EA, 2:1) to afford a product (2.0 g). Yield, 40.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15−7.13 (m, 1H), 7.08 (dd, J = 4.5, 1.9 Hz, 2H), 6.85−6.83 (m, 1H), 4.03 (t, J = 6.3 Hz, 2H), 3.80−3.68 (m, 4H), 2.52 (dd, J = 12.1, 4.8 Hz, 2H), 2.52−2.44 (m, 4H), 2.01−1.93 (m, 2H).

4-Methyl-*N*-(3-(3-morpholinopropoxy)phenyl)-2-nitroaniline (M-31). To the solution of 4-methyl-2-nitroaniline (529 mg, 3.5 mmol) in toluene (14 mL) was added **M-30** (1.05 g, 3.5 mmol), Cs_2CO_3 (2.28 g, 7.0 mmol), BINAP (327 mg, 0.52 mmol), $Pd_2(dba)_3$ (320 mg, 0.35 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 20:1) to afford a product (700 mg). Yield, 53.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.00 (s, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.26 (s, 1H), 7.21 (dd, J = 7.2, 5.4 Hz, 2H), 6.84 (d, J = 8.0 Hz, 1H), 6.79 (d, J = 2.1 Hz, 1H), 6.74−6.72 (m, 1H), 4.02 (t, J = 6.3 Hz, 2H), 3.76−3.68 (m, 4H), 2.56−2.48 (m, 2H), 2.47 (d, J = 4.5 Hz, 4H), 2.30 (s, 3H), 1.97 (dt, J = 13.5, 6.6 Hz, 2H).

4-Methyl-N^1-(3-(3-morpholinopropoxy)phenyl)benzene-1,2-diamine (M-32). To the solution of **M-31** (700 mg, 1.87 mmol) in EtOH (9 mL) was add Pd/C (70 mg, 19%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (410 mg). Yield, 58.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (t, J = 8.1 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 6.62 (s, 1H), 6.56 (d, J = 7.9 Hz, 1H), 6.34 (dd, J = 8.1, 2.2 Hz, 1H), 6.29 (dd, J = 8.0, 1.9 Hz, 1H), 6.23 (t, J = 2.1 Hz, 1H), 5.09 (s, 1H), 3.95 (t, J = 6.3 Hz, 2H), 3.80−3.62 (m, 6H), 2.51−2.47 (m, 2H), 2.49−2.41 (m, 4H), 2.28 (s, 3H), 1.94−1.90 (m, 2H).



1-(3-(3-Bromophenoxy)propyl)-4-methylpiperazine (M-33). To the solution of **M-29** (2.0 g 6.78 mmol) in MeCN (5 mL) was added 1-methylpiperazine (1.36 g 13.5 mmol) and K_2CO_3 (1.87 g 13.5 mmol), and the mixture was stirred at 80 ℃ for 3 h. After completion of the reaction, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (PE/EA, 2:1) to afford a product (597 mg). Yield, 28.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, J = 10.1, 6.4 Hz, 1H), 7.07−7.03 (m, 2H), 6.82 (d, J = 8.2 Hz, 1H), 3.99 (t, J = 6.3 Hz, 2H), 2.60−2.40 (m, 10H), 2.28 (s, 3H), 1.97−1.93 (m, 2H).

4-Methyl-*N*-(3-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)-2-nitroaniline (M-34). To the solution of 4-methyl-2-nitroaniline (1.38 g, 9.07 mmol) in toluene (18 mL) was added **M-33** (2.86 g, 9.07 mmol), Cs_2CO_3 (5.9 g, 18.1 mmol), BINAP (847 mg, 1.36 mmol), $Pd_2(dba)_3$ (830 mg, 0.9 mmol) in sequence, and the mixture was heated to 110 ℃ stirring overnight under argon atmosphere. After completion of the reaction, the mixture was filtered through Celite, and then the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (2.73 g). Yield, 77.7%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.00 (s, 1H), 7.29−7.27 (m, 1H), 7.22 (s, 2H), 6.83 (d, J = 7.7 Hz, 1H), 6.79 (s, 1H), 6.73 (dd, J = 8.3, 2.0 Hz, 1H), 4.01 (t, J = 6.3 Hz, 2H), 2.63−2.43 (m, 10H), 2.30 (d, J = 2.7 Hz, 3H), 2.29 (s, 3H), 1.99−1.95 (m, 2H).

4-Methyl-N^1-(3-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)benzene-1,2-diamine (M-35). To the solution of **M-34** (2.73 g, 7.05 mmol) in EtOH (35 mL) was add Pd/C (270 mg, 10%), and the mixture was stirred overnight at room temperature under hydrogen atmosphere. After completion of the reaction, the mixture was filtered through Celite, and the filtrate was removed *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford a product (2.0 g). Yield, 74.1%; ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.02 (s, 1H), 6.97 (t, J = 8.0 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 6.56 (s, 1H), 6.36 (d, J = 7.4 Hz, 1H), 6.24 (d, J = 7.9 Hz, 1H), 6.19 (d, J = 8.1 Hz, 1H), 6.15 (s, 1H), 4.64 (s, 2H), 3.87 (t, J = 6.3 Hz, 2H), 2.45−2.25 (m, 10H), 2.18 (s, 3H), 2.14 (s, 3H), 1.79 (dd, J = 13.5, 6.7 Hz, 2H).

2. General procedures for synthesis of compounds 1−9

To the solution of appropriate acid (1.0 mmol) in DCM (10 mL) was added HATU (760 mg, 3 mmol), DIPEA (516 mg, 4.0 mmol), and appropriate amine (1.0 mmol), and stirred at room temperature for 12 h. After completion of reaction, the mixture was extracted with EA (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford crude intermediate directly used in the next step without purification. To the residue above was added AcOH (10 mL) and stirred at 90 ℃ overnight. Then the reaction mixture was alkalized with

NaOH to pH 9−10, and extracted with EA (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM/MeOH, 10:1) to afford products **1**−**9** in two steps.

2-(2-(5-Methyl-1-(pyridin-4-yl)-1*H*-benzo[*d*]imidazol-2-yl)ethyl)isoquinolin-1(2*H*)-one (1)
(50 mg, yield 45.3%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (dd, J = 4.6, 1.6 Hz, 2H), 8.30 (d, J = 8.1 Hz, 1H), 7.63−7.59 (m, 2H), 7.47 (t, J = 8.4 Hz, 2H), 7.13 (dd, J = 4.5, 1.6 Hz, 2H), 7.08−7.02 (m, 3H), 6.36 (d, J = 7.4 Hz, 1H), 4.54 (t, J = 6.6 Hz, 2H), 3.38 (t, J = 6.6 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 161.39, 151.95, 151.77, 143.18, 143.01, 137.43, 133.57, 133.34, 132.74, 132.37, 127.32, 127.04, 126.52, 125.75, 124.92, 121.52, 119.32, 110.12, 105.49, 47.42, 27.17, 21.55. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd. for $C_{24}H_{20}N_4O$ 381.1710, Found 381.1700.

8-(2-(5-Methyl-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoline (2)
(51 mg, yield 48.1%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.81 (dd, J = 4.1, 1.8 Hz, 1H), 8.32 (dd, J = 8.3, 1.7 Hz, 1H), 7.80 (dd, J = 6.5, 3.1 Hz, 1H), 7.54−7.40 (m, 7H), 7.26 (dd, J = 7.8, 1.7 Hz, 2H), 7.00 (d, J = 8.2 Hz, 1H), 6.93 (d, J = 8.2 Hz, 1H), 3.62 (t, J = 7.7 Hz, 2H), 3.20 (d, J = 8.0 Hz, 2H), 2.42 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 154.76, 150.03, 146.50, 143.18, 139.37, 136.86, 135.86, 134.58, 131.40, 130.22, 129.41, 128.95, 128.46, 127.36, 127.15, 126.72, 124.12, 121.74, 118.97, 109.83, 30.15, 28.73, 21.62. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd. for $C_{25}H_{21}N_3$ 364.1808, Found 364.1805.

(*E*)-8-(2-(1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (3)
(20 mg, yield 48.8%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.17 (d, J = 16.8 Hz, 1H), 9.09 (dd, J = 4.2, 1.7 Hz, 1H), 8.53 (dd, J = 8.3, 1.7 Hz, 1H), 8.31 (d, J = 7.2 Hz, 1H), 8.18 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 16.8 Hz, 1H), 7.84 (d, J = 3.1 Hz, 1H), 7.82 (t, J = 2.9 Hz, 1H), 7.80−7.78 (m, 1H), 7.71 (dd, J = 8.3, 4.2 Hz, 1H), 7.57 (dd, J = 6.1, 3.1 Hz, 2H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 151.22, 148.63, 145.57, 139.51, 137.73, 131.92, 131.89, 131.77, 129.84, 128.82, 127.29, 126.58, 122.79, 114.24, 112.50. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd. for $C_{18}H_{13}N_3$ 272.1182, Found 272.1180.

(*E*)-8-(2-(5-Methyl-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (4)
(196 mg, yield 46.3%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.18 (d, J = 16.6 Hz, 1H), 8.98 (d, J = 4.1 Hz, 1H), 8.50 (d, J = 8.3 Hz, 1H), 8.15 (dd, J = 7.6, 2.8 Hz, 2H), 7.80 (s, 5H), 7.74 (s, 1H), 7.71−7.67 (m, 2H), 7.55 (d, J = 16.6 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 151.18, 145.64, 137.67, 132.81, 132.11, 132.01, 131.83, 131.33, 130.96, 130.54, 128.77, 128.28, 128.10, 127.22, 122.76, 114.08, 112.62, 111.62, 21.64. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd. for $C_{25}H_{19}N_3$ 362.1652, Found 362.1641.

(*E*)-8-(2-(1-Phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (5)
(46 mg, yield 50.5%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.09 (d, J = 16.2 Hz, 1H), 8.99 (d, J = 2.5 Hz, 1H), 8.42 (d, J = 8.3 Hz, 1H), 8.04 (d, J = 7.2 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.75−7.71 (m, 2H), 7.64 (dt, J = 14.7, 7.3 Hz, 5H), 7.34 (dd, J = 15.5, 6.1 Hz, 2H), 7.27−7.23 (m, 2H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 151.16, 150.74, 145.66, 143.36, 137.11, 136.67, 135.48, 133.65, 133.05, 130.65, 129.68, 129.50, 128.69, 127.94, 127.30, 127.05, 123.60, 123.43, 122.36, 119.52, 116.35, 110.69. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd. for $C_{24}H_{17}N_3$ 348.1495, Found 348.1486.

(*E*)-8-(2-(5-Fluoro-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (6)

(35 mg, yield 59.3%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, J = 16.2 Hz, 1H), 8.93 (dd, J = 4.1, 1.8 Hz, 1H), 8.16 (dd, J = 8.3, 1.7 Hz, 1H), 7.88 (d, J = 6.8 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.69−7.63 (m, 2H), 7.60 (dd, J = 8.0, 2.1 Hz, 1H), 7.57−7.50 (m, 4H), 7.47−7.41 (m, 2H), 7.14 (dd, J = 8.8, 4.7 Hz, 1H), 7.00 (td, J = 9.1, 2.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.92, 159.03, 152.84, 149.94, 146.27, 143.89, 143.78, 136.16, 135.62, 134.67, 134.48, 133.21, 129.96, 128.97, 128.83, 128.50, 127.65, 127.60, 126.26, 121.44, 116.77, 111.33, 111.12, 110.47, 110.39, 105.28, 105.09. HRMS (ESI-TOF) m/z $[M+H]^+$ Calcd. for $C_{24}H_{16}N_3F$ 366.1401, Found 366.1391.

(*E*)-8-(2-(4-Methyl-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (7)

(45 mg, yield 49.3%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.05 (d, J = 16.3 Hz, 1H), 8.99 (dd, J = 4.2, 1.8 Hz, 1H), 8.41 (dd, J = 8.3, 1.7 Hz, 1H), 8.02 (d, J = 6.9 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.72 (dd, J = 10.1, 4.7 Hz, 2H), 7.67-7.63 (m, 1H), 7.63 (d, J = 4.2 Hz, 1H), 7.63−7.59 (m, 2H), 7.59 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 16.2 Hz, 1H), 7.16-7.12 (m, 2H), 7.02−7.00 (m, 1H), 2.68 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 150.69, 150.27, 145.62, 142.66, 137.13, 136.30, 135.69, 133.78, 132.48, 130.62, 129.59, 129.45, 129.19, 128.70, 127.95, 127.07, 123.56, 122.37, 116.54, 108.20, 17.19. HRMS (ESI-TOF) m/z $[M+H]^+$ Calcd. for $C_{25}H_{19}N_3$ 362.1652, Found 362.1642.

(*E*)-8-(2-(6-Methyl-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (8)

(100 mg, yield 36.1%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.02 (d, J = 16.3 Hz, 1H), 8.95 (dd, J = 4.1, 1.8 Hz, 1H), 8.39 (dd, J = 8.3, 1.7 Hz, 1H), 7.96 (d, J = 7.2 Hz, 1H), 7.89 (d, J = 7.3 Hz, 1H), 7.71−7.55 (m, 8H), 7.19−7.17 (m, 1H), 7.05 (d, J = 16.2 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 1.89 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 151.74, 150.68, 137.08, 133.67, 132.55, 130.15, 129.88, 129.85, 129.58, 128.68, 127.04, 126.99, 125.48, 123.06, 122.36, 121.80, 117.57, 116.28, 18.10. HRMS (ESI-TOF) m/z $[M+H]^+$ Calcd. for $C_{25}H_{19}N_3$ 362.1652, Found 362.1652.

(*E*)-8-(2-(7-Methyl-1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)vinyl)quinoline (9)

(15 mg, yield 38.5%) ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.03 (d, J = 16.3 Hz, 1H), 8.96 (dd, J = 4.1, 1.8 Hz, 1H), 8.39 (dd, J = 8.3, 1.7 Hz, 1H), 7.97 (dd, J = 10.7, 7.8 Hz, 2H), 7.71 (t, J = 7.4 Hz, 2H), 7.66-7.63 (m, 2H), 7.62-7.60 (m, 2H), 7.58 (dd, J = 4.4, 3.3 Hz, 2H), 7.29 (d, J = 16.2 Hz, 1H), 7.13 (dd, J = 8.2, 1.1 Hz, 1H), 6.99 (s, 1H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 150.69, 145.64, 141.50, 137.07, 136.89, 135.59, 133.77, 133.18, 132.47, 130.62, 129.52, 129.41, 128.67, 127.97, 127.11, 127.03, 124.98, 122.32, 119.14, 116.45, 110.37, 21.83. HRMS (ESI-TOF) m/z $[M+H]^+$ Calcd. for $C_{25}H_{19}N_3$ 362.1652, Found 362.1657.

Protein expression and purification

Briefly, the plasmid (pET15b-PDE10A) was transferred to *E. coli* strain BL21 (Codonplus, Stratagene). Then, the *E. coli* cells carrying the recombinant plasmid were grown in 2xYT medium (containing 100 μg/mL ampicillin and 30 μg/mL chloramphenicol) at 37 °C until an absorption of OD_{600} = 0.6−0.8. Then, 1 mmol/L isopropyl-*β*-D-thiogalactopyranoside was added to induce the expression of the PDE10A protein and the culture was incubated at 15 ℃ for 48 h. The catalytic domain of PDE10A was purified with three chromatographic columns of nickel-nitrilotriacetic acid (Qiagen), Q-Sepharose (Amersham Biosciences), and Sephacryl S300 (GE Healthcare). A typical batch of purification yielded about 10 mg of PDE10A from 10 liters of cell culture and the purity was verified by SDS-PAGE. The catalytic domains of PDE1B (10-487), PDE2A (580-919), PDE3A (679-1087), PDE4D2 (86-413), PDE5A1(535-860), PDE7A1 (130-482), PDE8A1 (480-820) and PDE9A2 (181-506) were purified by using similar protocols[3-5].

The plasmid for expression of the wide-type PDE10A (449-770) was mutated to Y693A/Q726A/F729A mutant with the Fast Mutagenesis Kit (Vazyme) using the PCR primers as followed:

[Y693A]

5′-TAGCAGCAGAATTCTGGGCTGAGGGTGATGAA-3′/5′-CCAGAATTCTGCTGCTATATCATTTGCCGTCAATTTTGTAA-3′;

[Q726A]

5′-GCCCTTGGGTTCTACAATGCCGTGGCCATTCC-3′/5′-TTGTAGAACCCAAGGGCGCCTTGGGGGACTTCATCC-3′;

[F729A]

5′-AGCTTGGGGCCTACAATGCCGTGGCCATTCCC-3′/5′-ATTGTAGGCCCCAAGCTGGCCTTGGGGGACTT-3′.

All mutations were verified by DNA sequencing. Protein overexpression and purification of mutants were similar to the protocol for the wild type[3].

Statistics on diffraction data and structure refinement of PDE10 with 14

The PDE10A apo crystals were grown by using hanging drop method and protocol was similar to those previously reported[3-5]. Briefly, the unliganded PDE10A2 enzyme (10 mg/mL in a buffer composed of 20 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 1 mmol/L EDTA, and 1 mmol/L *β*-mercaptoethanol) was vapor-diffused against the well buffer of 0.1 mol/L Hepes (pH 7.5), 0.2

mol/L $MgCl_2$, 18% PEG3350, and 50 mmol/L 2-mercaptoethano at 4 ℃. Crystals appeared within a week. Then, the apo crystals were fished and soaked in well buffer containing 20 mmol/L **14** for 24 h. The crystallization buffer containing 20% ethylene glycol was used as the cryosolvent. Diffraction data were collected at 100 K on an in-house Oxford Diffraction Xcalibur Nova diffractometer. The data were processed using the program CrysAlis Pro, and the structures were solved and refined using *CCP4* and *Phenix*[6,7]. The coordinates and structure factors have been deposited in the Protein Data Bank with PDB ID of 7BPI.

The electron density map of 2Fo-Fc and Fo-Fc shown **14** bound to the PDE10 pocket (Fig. S6). Although the conformation of quinoline and benzimidazole portion of **14** was well determined, the density map for the propyl side-chain linker was incomplete and shown low occupancy which might suggest some flexibility.

Data collection	PDE10A−**14**
Wavelength (Å)	1.5418
Temperature (K)	100
Resolution (Å)	19.25−2.40
Space group	$P2_12_12_1$
Unit cell	
a, *b*, *c* (Å)	49.94, 81.68, 162.49
α, *β*, *γ* (°)	90.0, 90.0, 90.0
No. reflections	26047 (2585[a])
Completeness (%)	97.46 (98.85[a])
R_{merge}	0.111 (0.377[a])
$\langle I/\sigma(I) \rangle$	12.3 (2.5[a])
Redundancy	3.4 (3.3[a])
Structure refinement	
R-factor/R-free	0.25/0.32
RMS deviations	
Bond lengths	0.009 Å
Bond angles	1.111
Average B-factor ($Å^2$) (atoms)	
Protein	25.9 (5078)
Inhibitor	29.2 (39)
Zn	35.1 (2)
Mg	23.1 (2)

Waters	22.6 (110)
Ramachandran plot	
Preferred	94.26%
Allowed	5.42%

[a]The numbers in parentheses are for the highest resolution shells of 2.49−2.4 Å.

Molecular docking and dynamics simulations

The X-ray crystal structure of PDE10A−**14** complex was selected for molecular modeling, and Surflex-dock embedded in the software Tripos Sybyl 2.0 was used[7]. Two metal ions crucial for the PDE's catalytic activity in the catalytic domain and water molecules coordinating these two metal ions were retained. hydrogen atoms were added, and the ionizable residues were protonated at the neutral pH. The protomol was generated using the parameters by default. The parameters of proto_thresh and proto_bloat were assigned 0.5 and 0, respectively. After the protomol was prepared, molecular docking was performed for test molecules.

Binding free energy calculations. After 8 ns molecular dynamics simulations with similar parameters to our previous work[5], MM-PBSA binding free energy calculations were performed by extracting 100 snapshots of the last 1 ns trajectories with default parameters assigned. According to the MM-PBSA method, the binding free energies (δG_{bind}) are calculated using Eq. (1), in which $G_{complex}$, G_{rec} and G_{lig} are the representations of the free energies of complex, receptor and ligand, respectively. Each free energy is calculated as the sum of the MM energy E_{MM}, the solvation free energy G_{solv}, and the entropy contribution TS, respectively, leading to Eq. (2). δE_{MM} represents the gas phase interaction energy. It is decomposed into $E_{MM,\ complex}$, $E_{MM,\ rec}$ and $E_{MM,\ lig}$. The solvation free energy represents the sum of the electrostatic solvation free energy and nonpolar solvation free energy. The entropy contribution for each system was omitted since this computational process is extremely time-consuming for large protein−ligand systems.

1. $\delta G_{bind} = G_{complex} - G_{rec} - G_{lig}$ (1)

2. $\delta G_{bind} = \delta E_{MM} + \delta G_{solv} - T\delta S$ (2)

Metabolic stability in the rat liver microsomes

The assays of compounds **2** and **3** were performed at the Medicilon Company, Shanghai, China. The experimental procedures were similar to those in our previous study[8]. Compounds **2** and **3** were dissolved in 100% DMSO to prepare a 0.5 mmol/L stock solution and diluted to a final concentration of 1.5 μmol/L for the experiments.

The assays of compounds **10**−**14** was performed as followed: microsomes in 0.1 mol/L Tris buffer pH 7.4 (final concentration 0.33 mg/mL), co-factor $MgCl_2$ (final concentration 5 mmol/L) and tested compound (final concentration 0.1 μmol/L, co-solvent (0.01% DMSO) and 0.005% Bovin serum albumin (BSA)) were incubated at 37 ℃ for 10 min. The reaction was started by the addition of NADPH (final concentration 1 mmol/L). Aliquots were sampled at 0, 7, 17, 30 and 60 min respectively and methanol (cold in 4 ℃) was added to terminate the reaction. After centrifugation (4000 rpm, 5 min), samples were then analyzed by LC−MS/MS.

***In vivo* pharmacokinetics analysis of 14·3HCl**

The pharmacokinetic properties of **14·3HCl** were analyzed by the Medicilon Company, Shanghai, China. Six male SD rats with a body weight of 230−260 g were purchased from Shanghai SIPPR-BK LAB Animal Ltd., Shanghai, China, and used for the pharmacokinetic analysis of **14·3HCl**. It was dissolved/suspended in 5% DMSO, 10% solutol, and 85% water for intravenous administration (i.v.) and oral administration (*p.o.*). A final dosage of 2.5 and 10 mg/kg rat of the formulated compounds was administered for i.v. and *p.o.* purposes, respectively, and the blood samples were taken at various time points within 24 h. The concentration of the compounds in the blood was analyzed by LC−MS/MS (Shimadzu liquid chromatographic system and API4000 mass spectrometer, Applied Biosystems, Ontario, Canada).

Acute toxicity of compound 14·3HCl

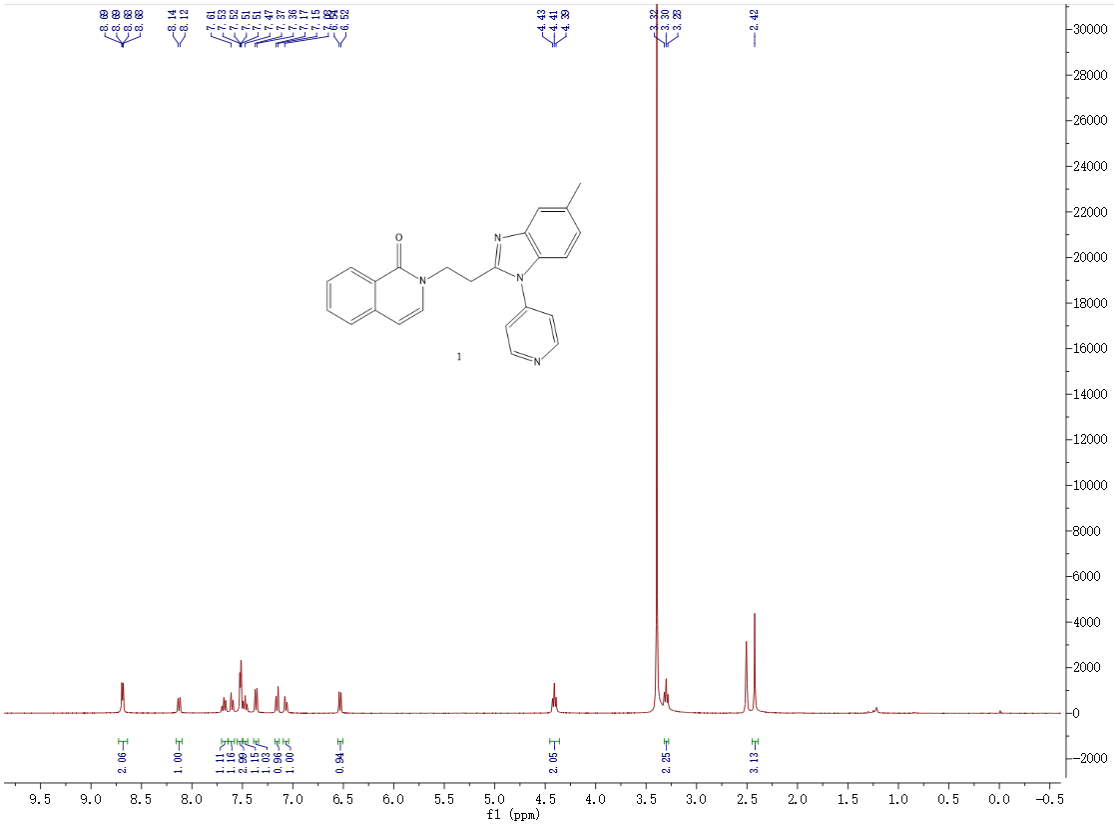
The acute toxicity was tested according to similar protocols that were described in our previous study[2]. Thirty SPF KM mice (22 days, 18−22 g), which were purchased from the Laboratory Animal Center of Southern Medical University (Guangzhou, China), were used to evaluate the acute toxicity of **14·3HCl**. Mice were randomly divided into three groups (half male and female), and each group was given in single oral dose of 0, 0.5, or 1.0 g/kg **14·3HCl** on the first day of the experiment. Mice were maintained on a 12 h light/dark cycle (light from 7:00 to 19:00) at 20−25 ℃ and 45%−70% relative humidity. Sterile food and water were provided according the institutional guidelines. Prior to each experiment, mice were fasted overnight and allowed free access to water. Compound **14·3HCl** was dissolved in 0.5% CMC-Na solution and orally administrated. Mice were observed during initial 12 h for any abnormal behavior and mortality and weighed 4 h after **14·3HCl** administered and then observation once a day for subsequent 14 days. Animals were sacrificed on the 14th day, and tissue samples of the heart, liver, and kidney were macroscopically examined for possible damage.

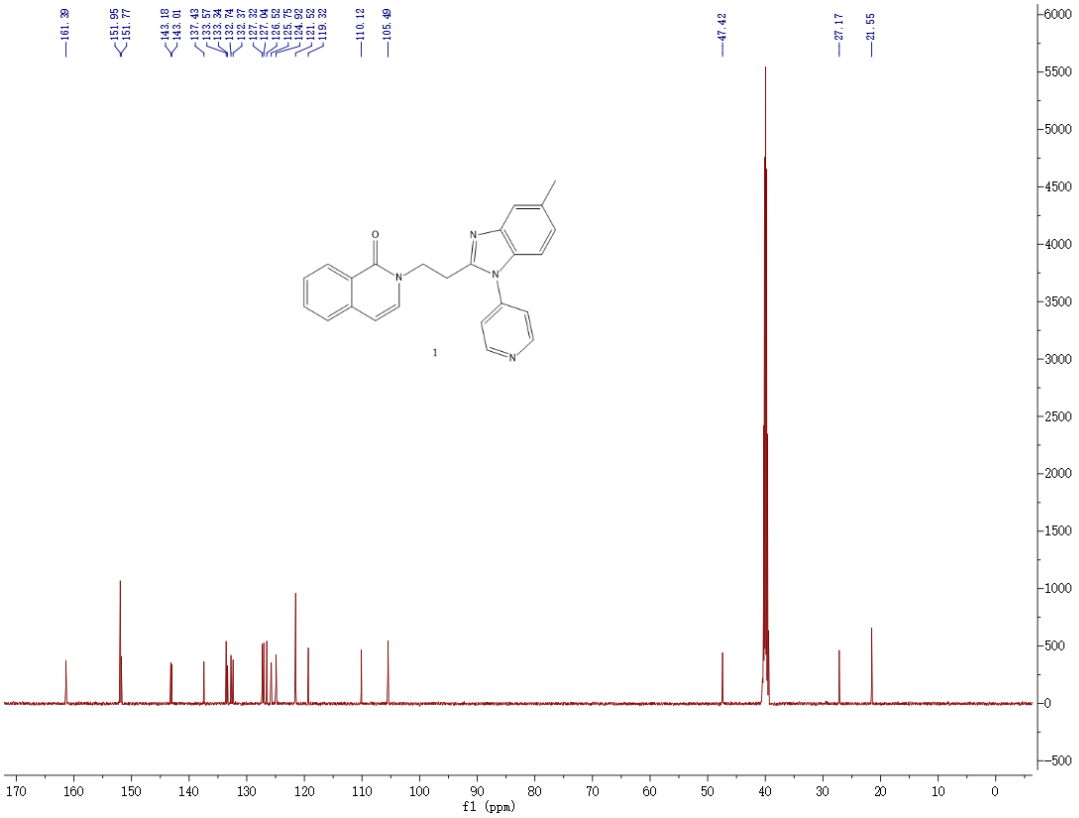
Pharmacodynamics effects of 14·3HCl against PAH in animals

All animal care and experimental protocols were in accordance with “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication, revised 1996, No. 86-23, Bethesda, MD) and were approved by the Institutional Ethical Committee for Animal Research of Sun Yat-sen University. Forty Wister rats (6 weeks, 160−180 g), purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., were used to evaluate the pharmacodynamics effects of **14·3HCl** on PAH. The rats were randomly divided into four groups: control group, model, compound **14·3HCl** (2.5 mg/kg), and positive (tadalafil, 5.0 mg/kg). Rats were maintained on a 12 h light/dark cycle

(light from 7:00 to 19:00) at 24 ± 1 °C and 60%−70% relative humidity. Sterile food and water were given according to the institutional guidelines. Prior to each experiment, the rats were fasted overnight and allowed free access to water. All the rats were administrated i.v. with MCT 60 mg/kg except group control. Then, the rats were orally treated with drug vehicle (control and model groups), compound **14·3HCl** (2.5 mg/kg) and tadalafil (5.0 mg/kg) for 3 weeks, respectively. Compound **14·3HCl** and tadalafil were dissolved in 0.5% CMC-Na solution and orally administrated 0.4 mL per 100 g weight. The method of right cardiac catheter was applied to measure the pulmonary artery pressure and the mean pulmonary artery pressure (mPAP) was used to conduct statistics. Subsequently, the rats were killed and the hearts were dissected into right ventricle (RV) and left S7 ventricle and interventricular septum (LV+S); the 2 parts of the hearts were weighed with electronic scales, the value of RV/(LV+S) was used to conduct statistics. Then a part of lung tissues was taken and fixed with 4% paraformaldehyde. Routine HE staining was performed to observe the pathological changes and calculate the percentage (WT%) of the medial wall thickness of pulmonary arterioles[4,5,8].

^{1}HNMR, ^{13}CNMR and HRMS spectra of the targeted compounds





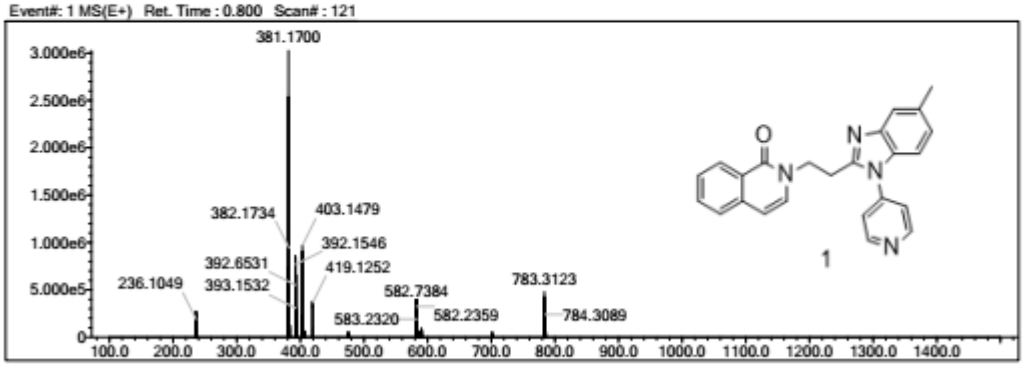
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

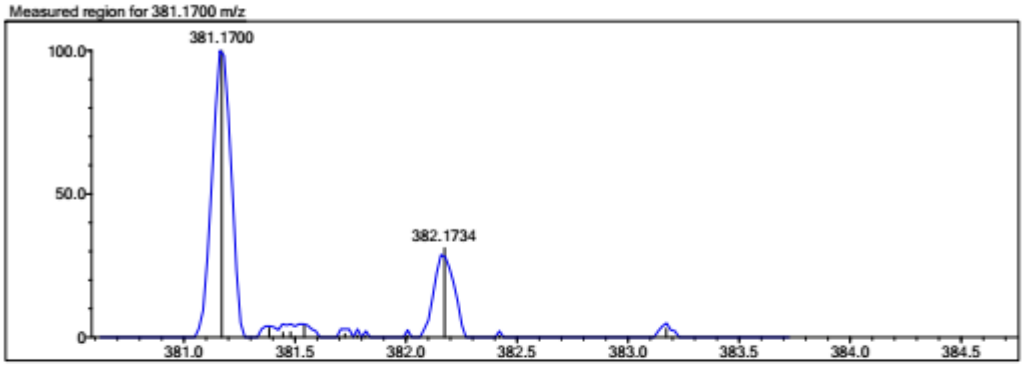
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

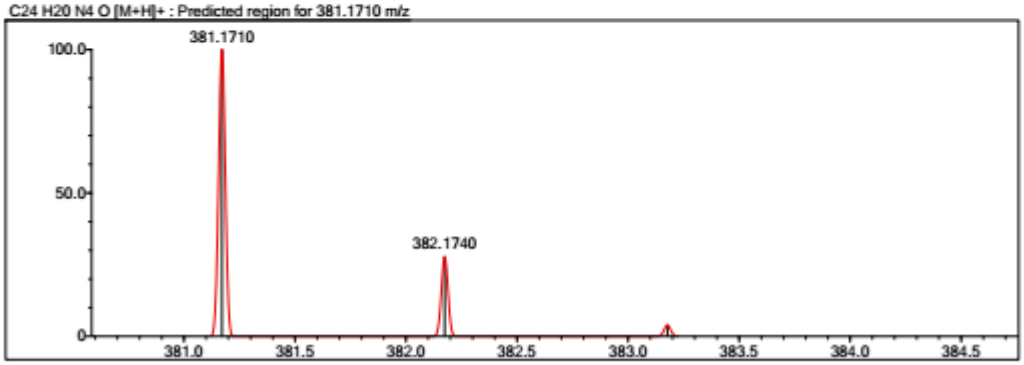
Event#: 1 MS(E+) Ret. Time : 0.800 Scan# : 121



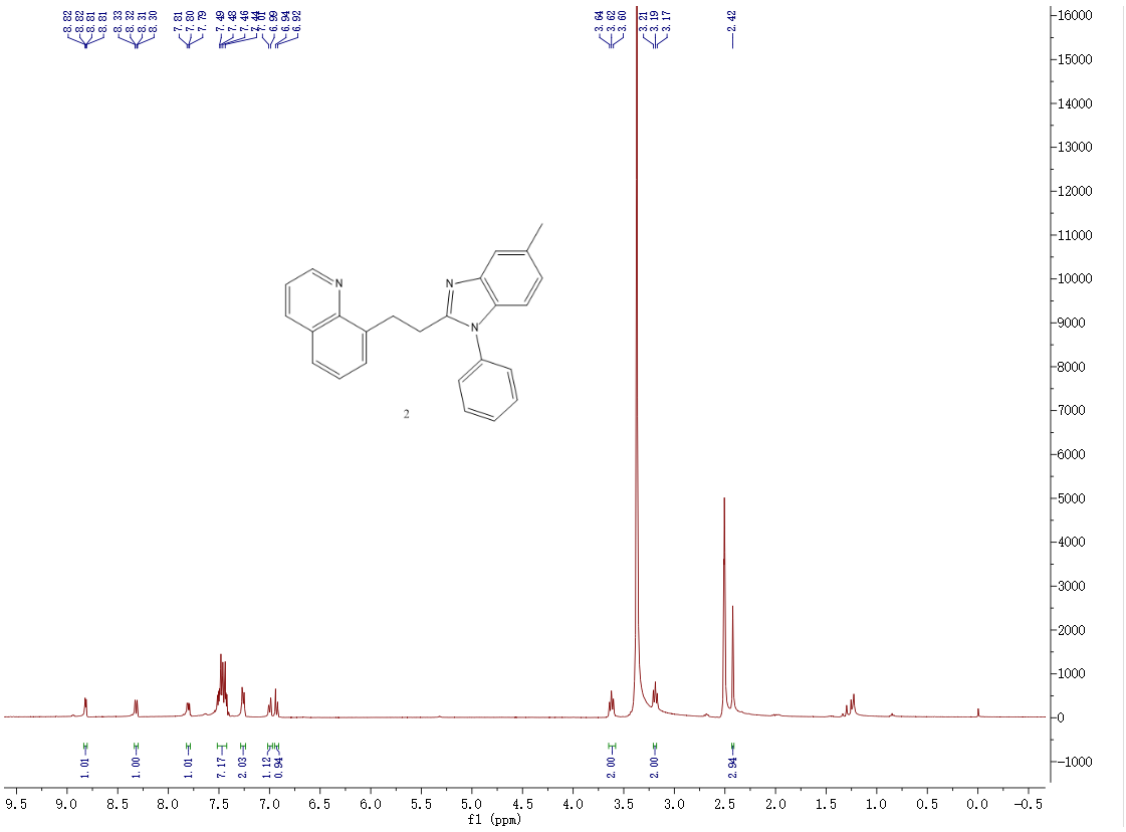
Measured region for 381.1700 m/z

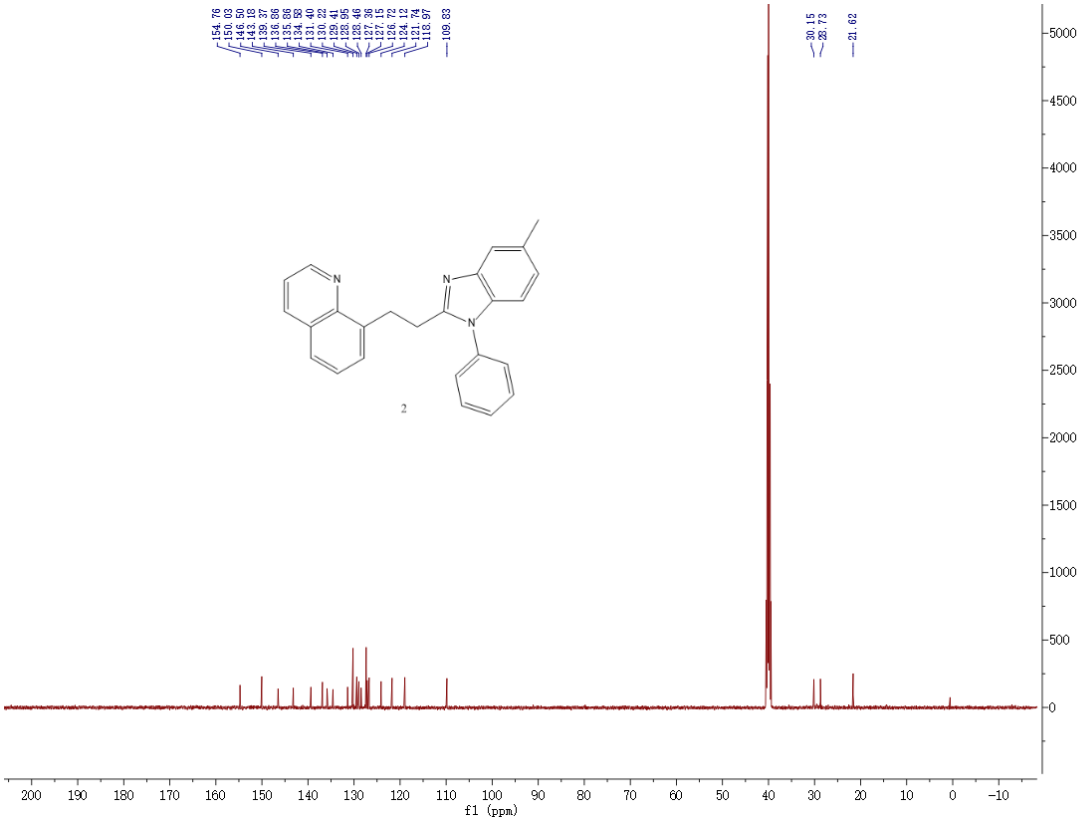


C24 H20 N4 O [M+H]+ : Predicted region for 381.1710 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	74.08	C24 H20 N4 O	[M+H]+	381.1700	381.1710	-1.0	-2.62	77.21	17.0


8.82
8.82
8.81
8.81
8.33
8.32
8.31
8.30
7.81
7.80
7.79
7.49
7.48
7.46
7.44
7.43
6.99
6.94
6.92
3.64
3.62
3.60
3.21
3.19
3.17
2.42
N
N
N
2
1.01
1.00
1.01
7.17
2.03
1.12
0.94
2.00
2.00
2.94
f1 (ppm)


154.76
150.03
146.50
144.19
139.37
136.86
135.86
134.58
131.40
130.22
129.41
128.95
128.46
127.38
127.15
126.72
124.12
121.74
118.97
109.83
30.15
28.73
21.62
N
N
N
2
f1 (ppm)

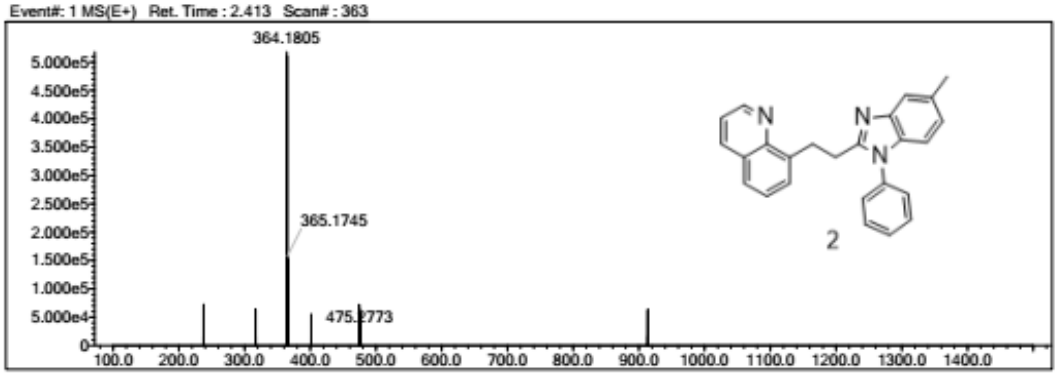
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

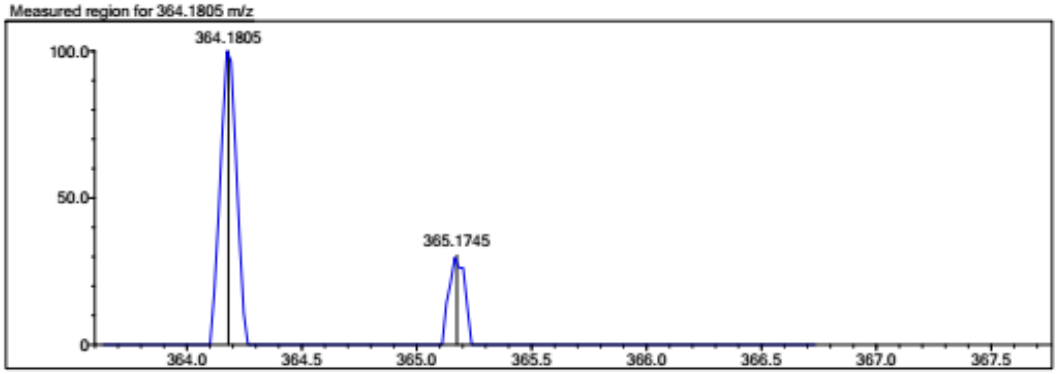
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

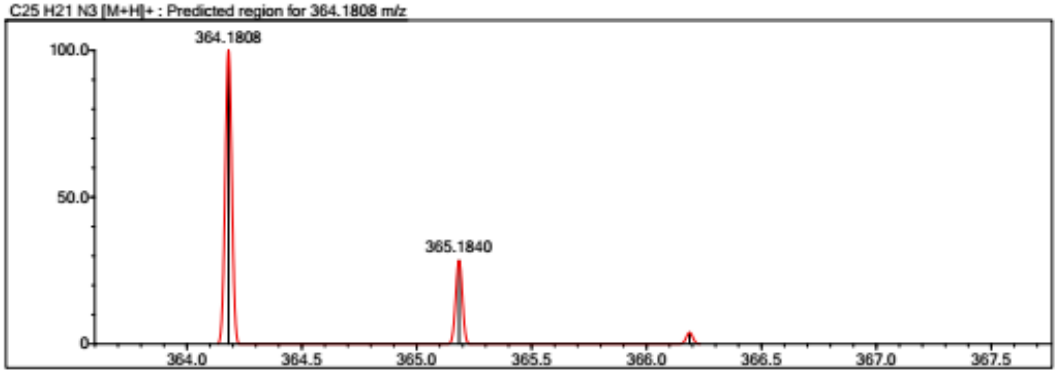
Event#: 1 MS(E+) Ret. Time : 2.413 Scan# : 363



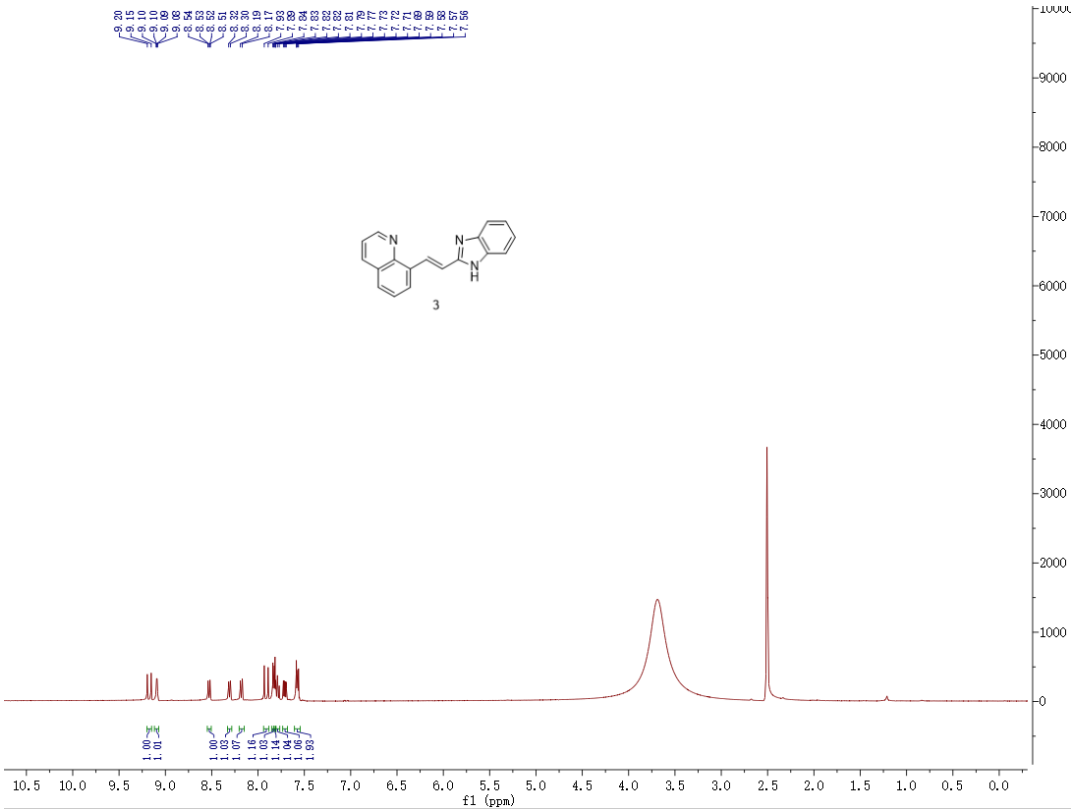
Measured region for 364.1805 m/z

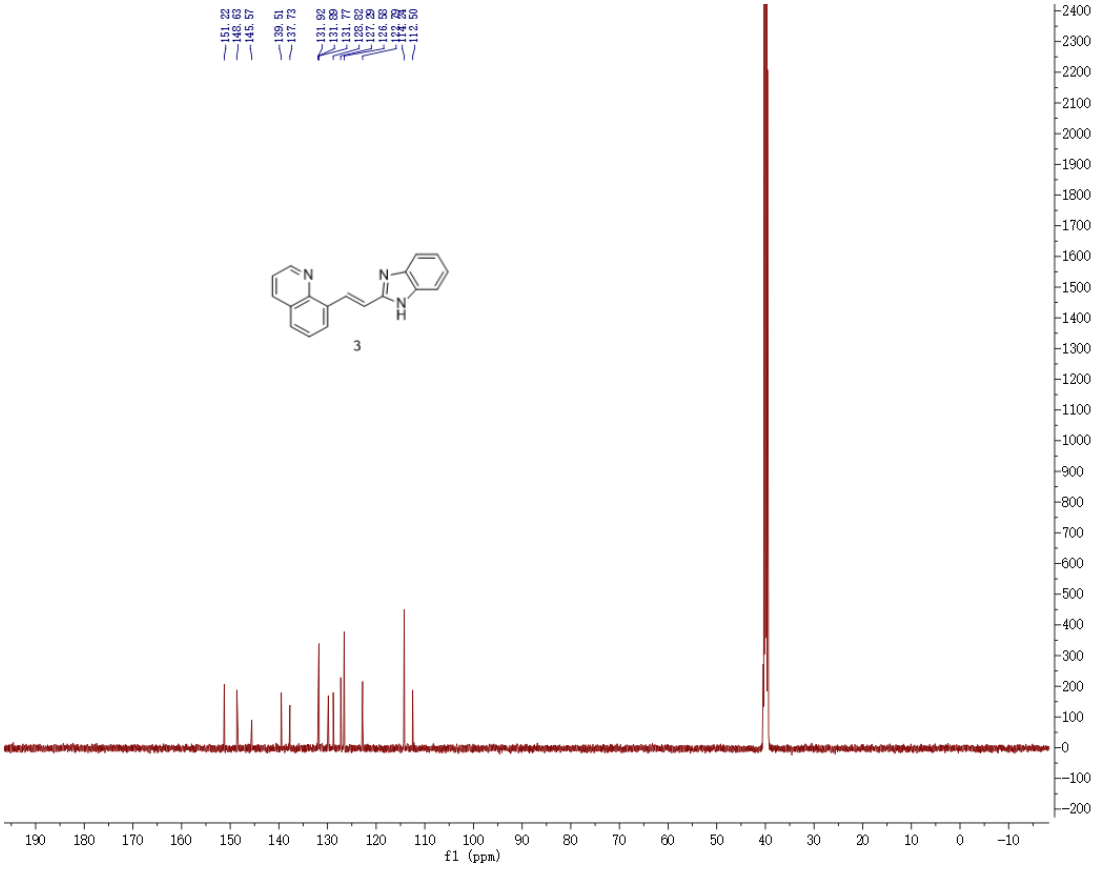


C25 H21 N3 [M+H]+ : Predicted region for 364.1808 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	73.77	C25 H21 N3	[M+H]+	364.1805	364.1808	-0.3	-0.82	73.77	17.0


3
f1 (ppm)


3
f1 (ppm)

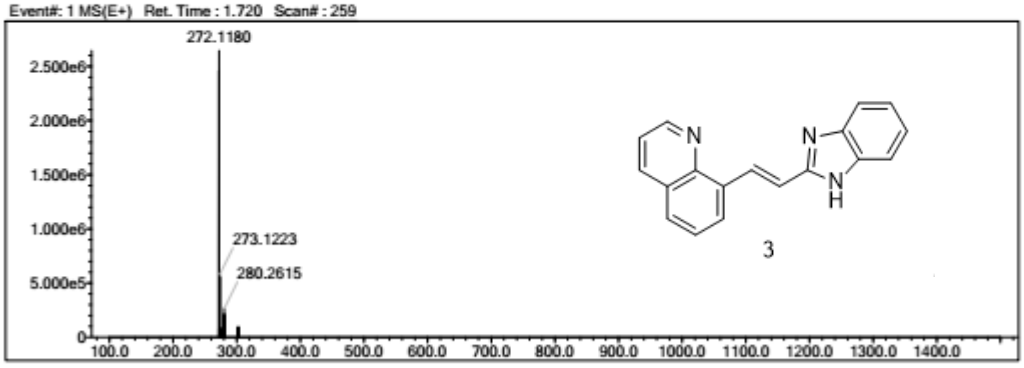
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

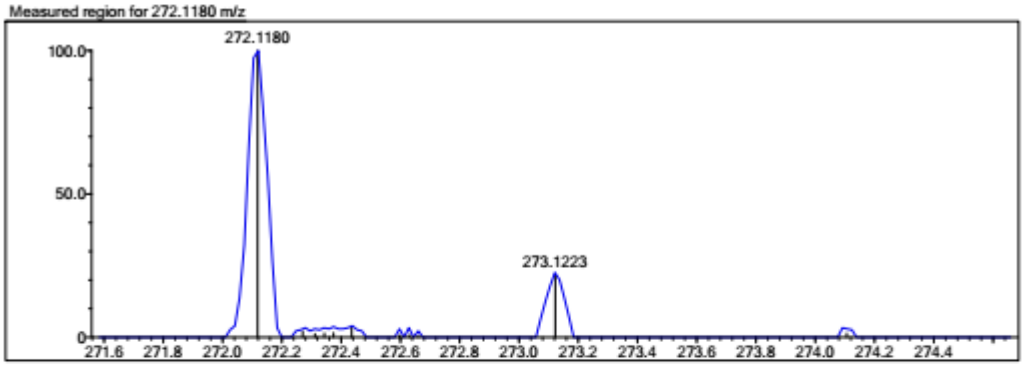
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

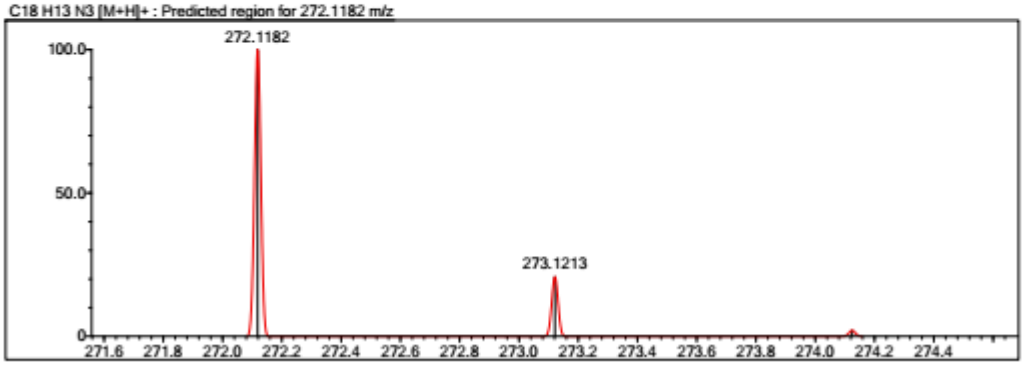
Event#: 1 MS(E+) Ret. Time : 1.720 Scan# : 259



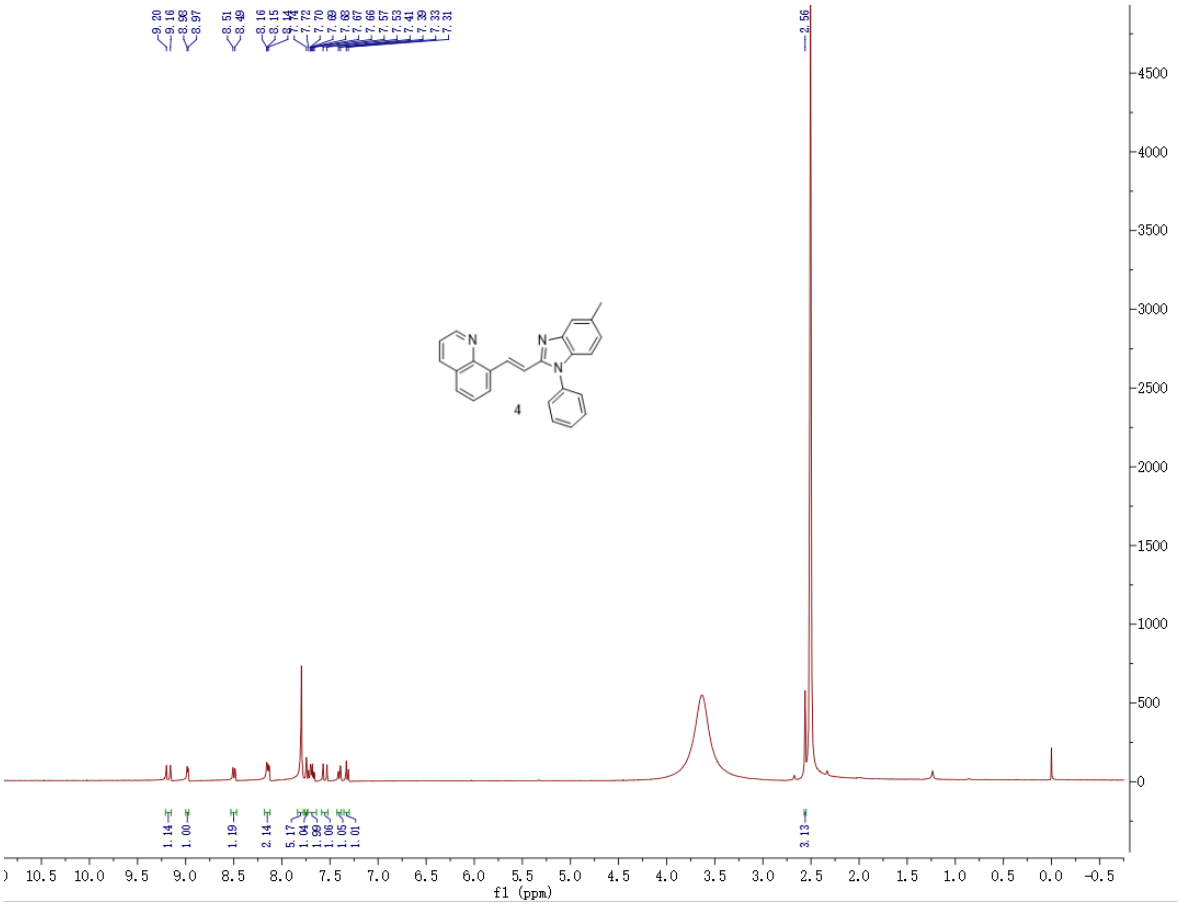
Measured region for 272.1180 m/z

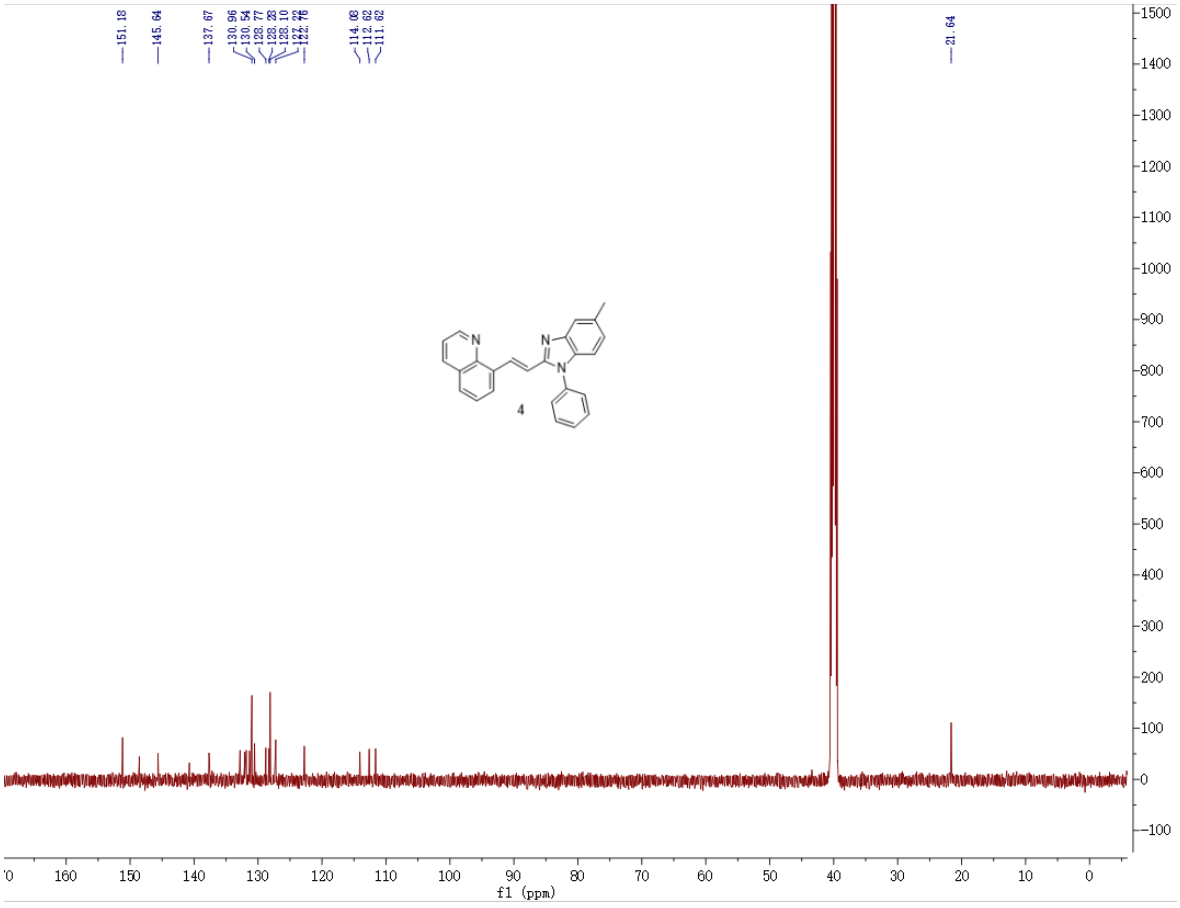


C18 H13 N3 [M+H]+ : Predicted region for 272.1182 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	55.37	C18 H13 N3	[M+H]+	272.1180	272.1182	-0.2	-0.73	55.37	14.0


9.20
9.16
8.98
8.97
8.51
8.49
8.16
8.15
8.14
7.72
7.70
7.69
7.68
7.67
7.66
7.57
7.53
7.41
7.39
7.33
7.31
2.56
4
1.14
1.00
1.19
2.14
5.17
1.04
1.08
1.05
1.01
3.13
f1 (ppm)


151.18
145.64
137.67
130.96
130.54
128.77
128.28
128.10
127.22
122.76
114.08
112.62
111.62
21.64
4
f1 (ppm)

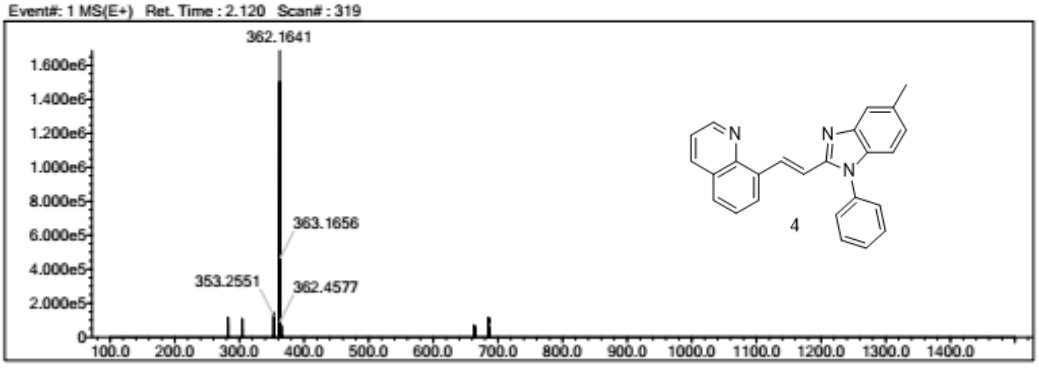
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

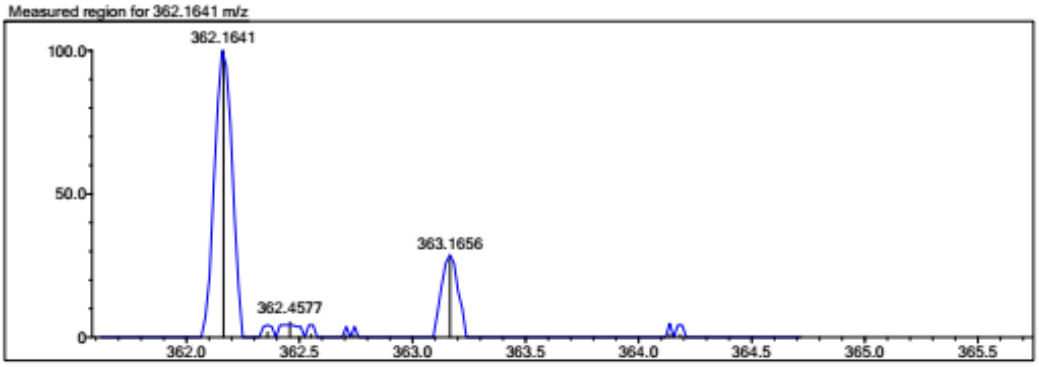
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

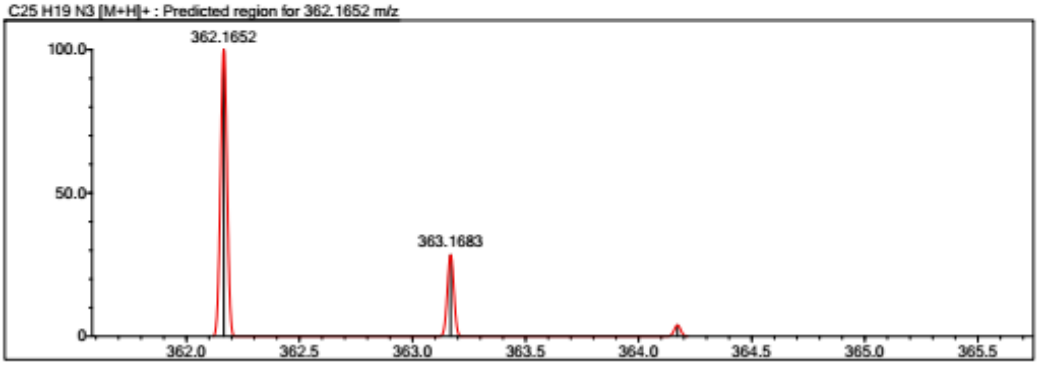
Event#: 1 MS(E+) Ret. Time : 2.120 Scan# : 319



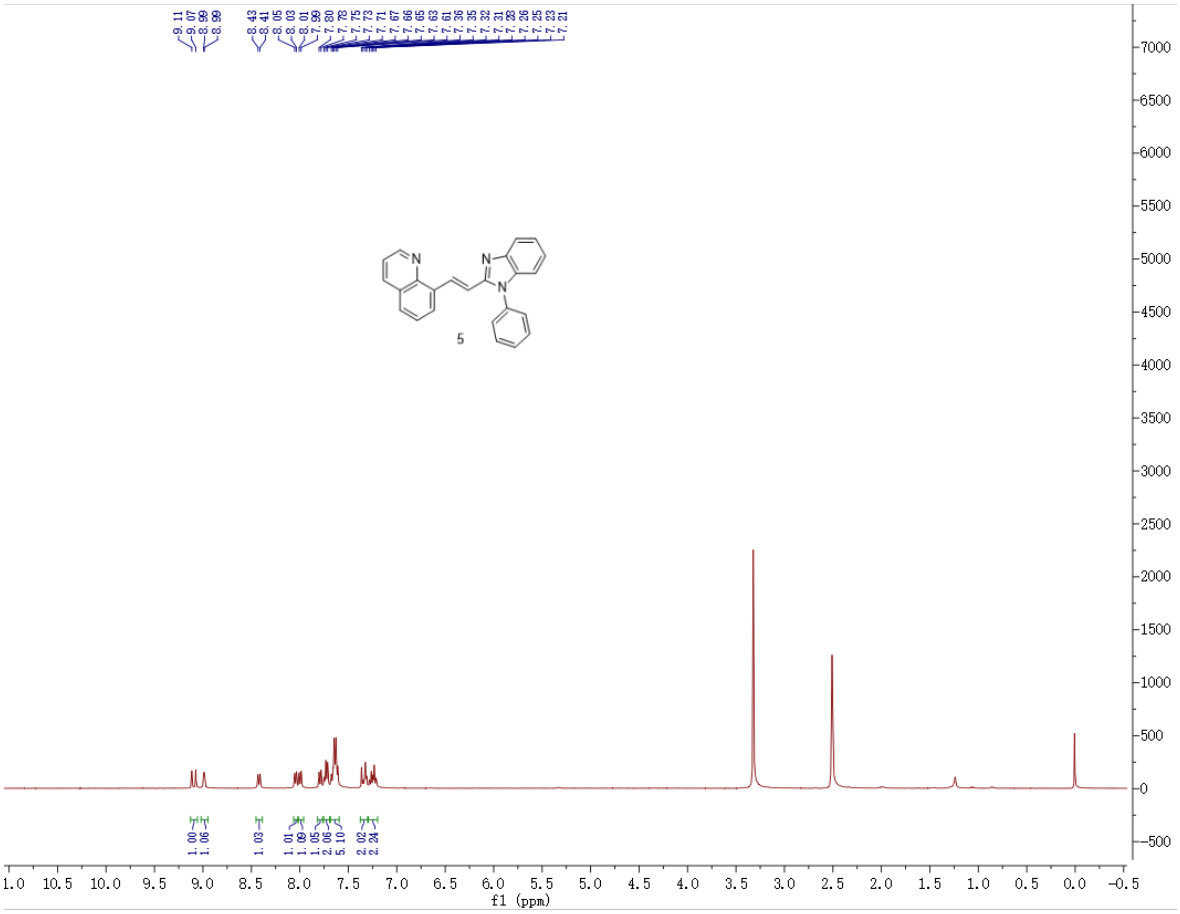
Measured region for 362.1641 m/z

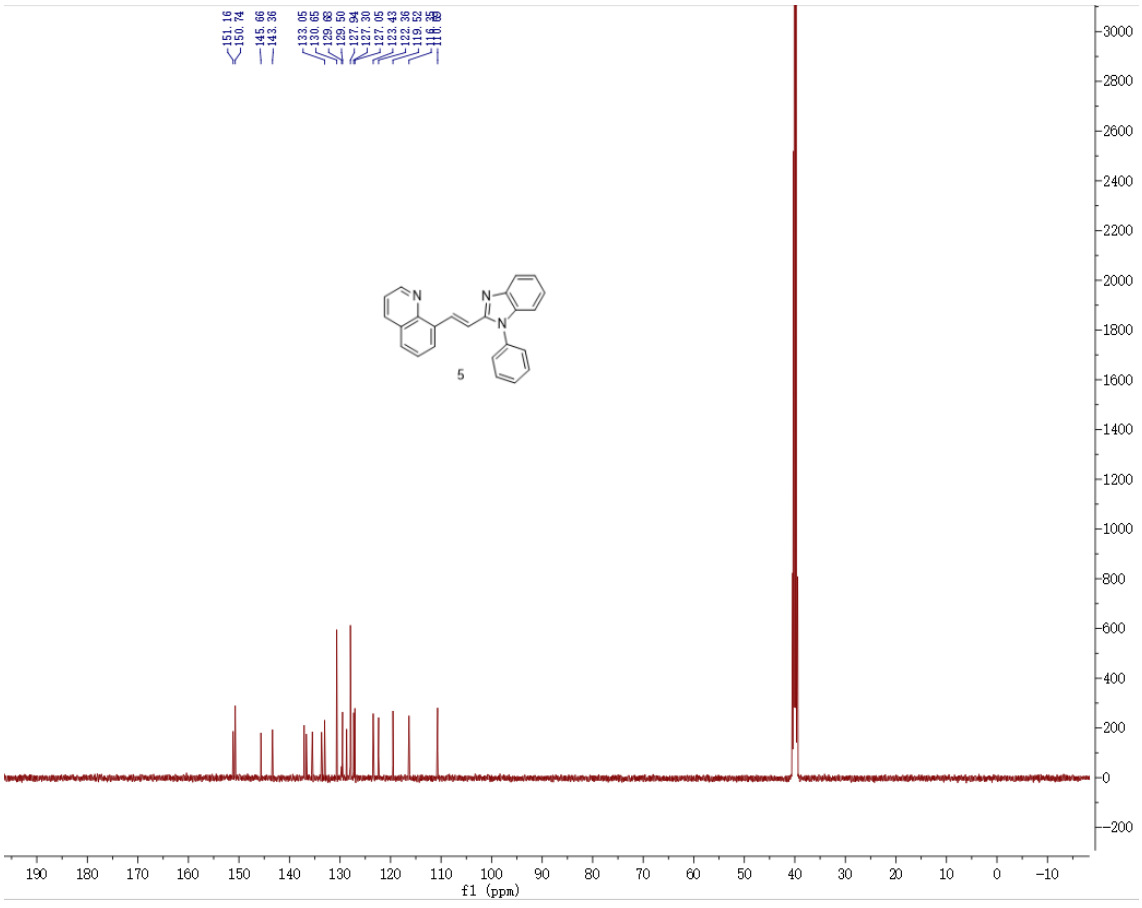


C25 H19 N3 [M+H]+ : Predicted region for 362.1652 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	65.38	C25 H19 N3	[M+H]+	362.1641	362.1652	-1.1	-3.04	68.90	18.0


9.11
9.07
8.90
8.89
8.43
8.41
8.05
8.03
8.01
7.89
7.80
7.79
7.75
7.73
7.71
7.67
7.66
7.65
7.63
7.61
7.36
7.35
7.32
7.31
7.28
7.26
7.25
7.23
7.21
5
1.00
1.06
1.03
1.01
1.09
1.05
2.06
5.10
2.02
2.24
f1 (ppm)


151.16
150.74
145.66
143.36
133.05
130.65
129.88
129.50
127.94
127.30
127.05
123.43
122.36
119.52
5
f1 (ppm)

Elmt	Val.	Min	Max
H	1	10	40
2H	1	0	0
B	3	0	0
C	4	10	35
N	3	0	5

Elmt	Val.	Min	Max
O	2	0	3
18O	2	0	0
F	1	0	1
Al	3	0	0
Si	4	0	0

Elmt	Val.	Min	Max
P	3	0	0
S	2	0	1
Cl	1	0	0
Fe	2	0	0
Se	2	0	0

Elmt	Val.	Min	Max
Br	1	0	0
Te	2	0	0
I	3	0	0

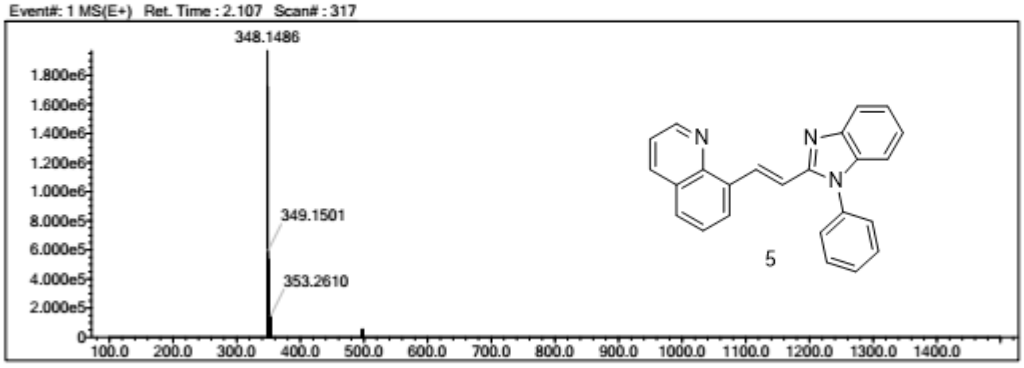
Use Adduct
H
Na

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

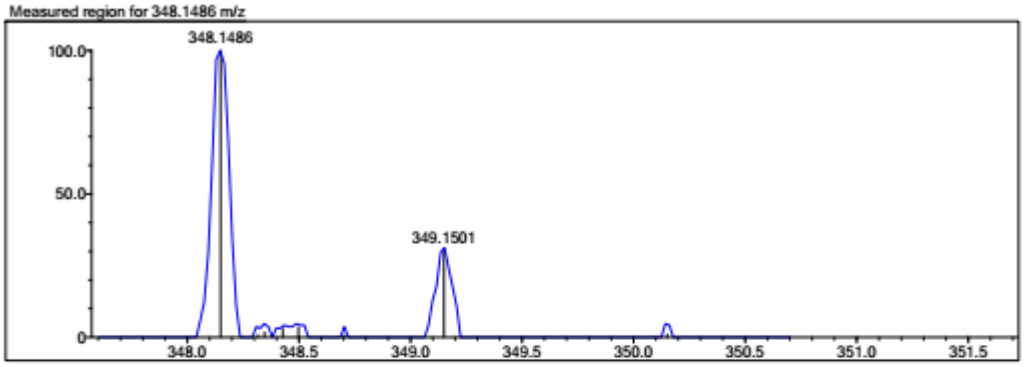
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

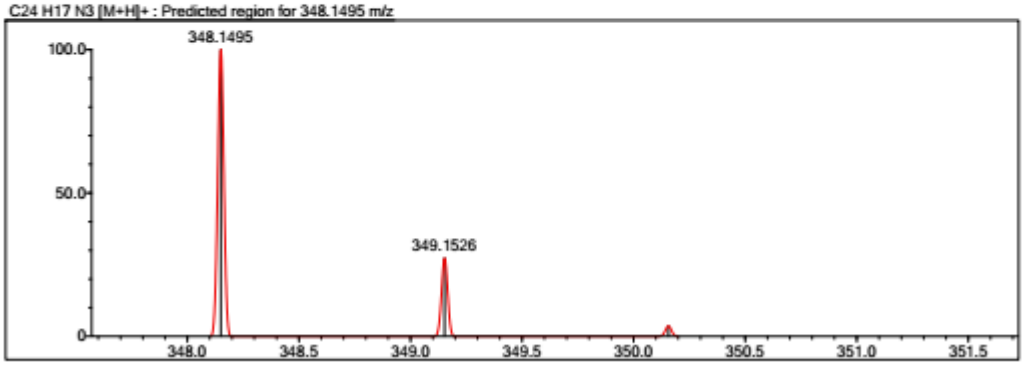
Event#: 1 MS(E+) Ret. Time : 2.107 Scan# : 317



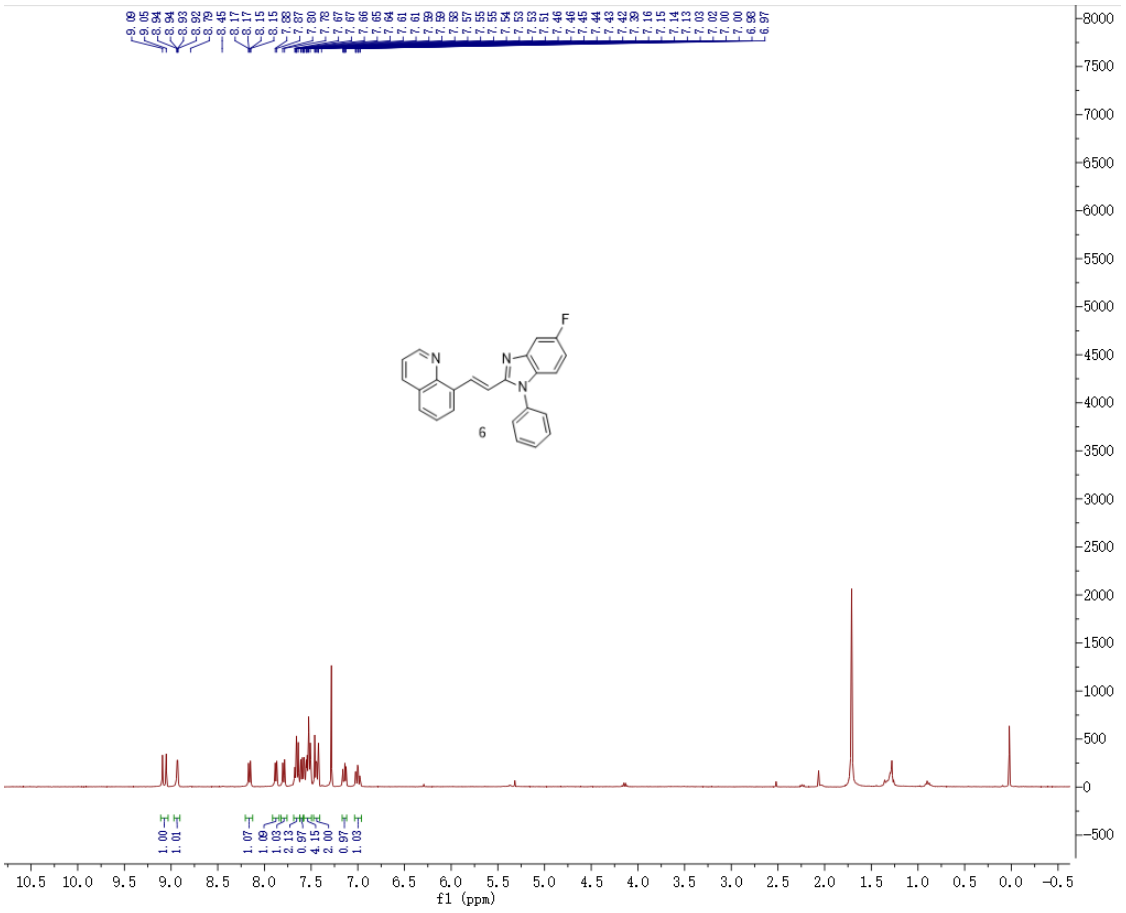
Measured region for 348.1486 m/z

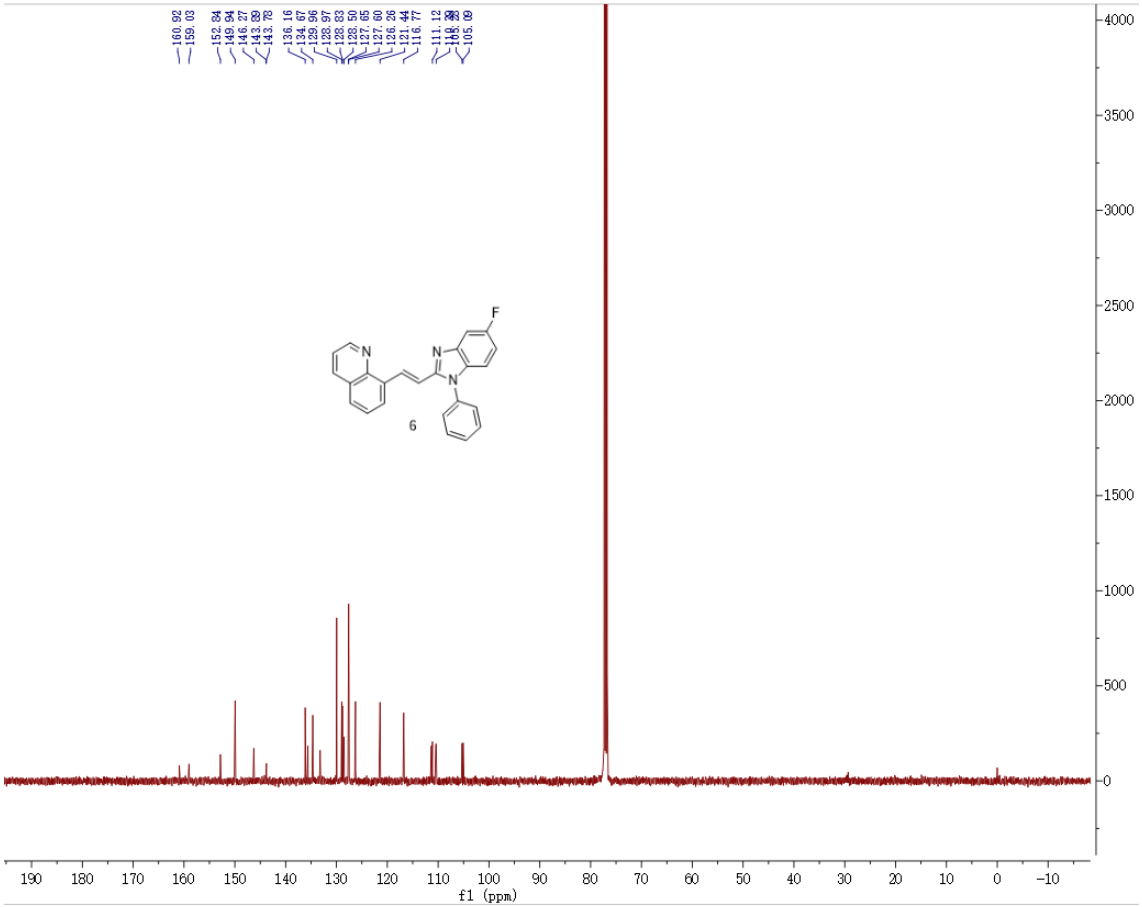


C24 H17 N3 [M+H]+ : Predicted region for 348.1495 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	74.62	C24 H17 N3	[M+H]+	348.1486	348.1495	-0.9	-2.59	77.71	18.0


F
N
N
6
f1 (ppm)


F
N
N
6
f1 (ppm)

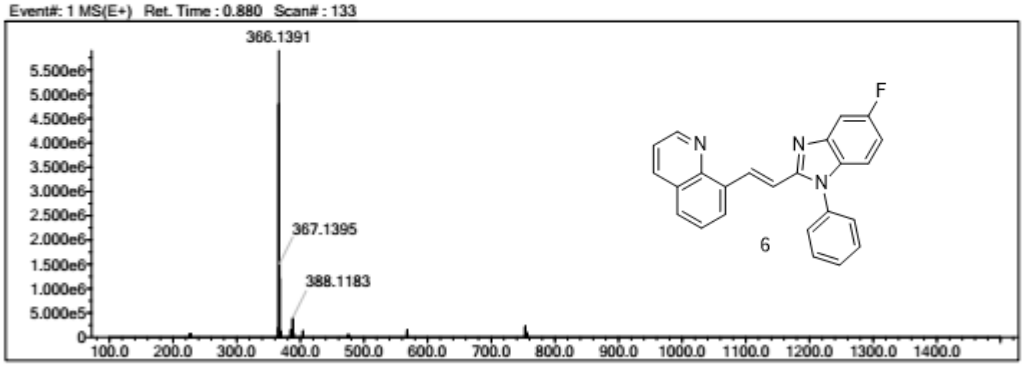
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

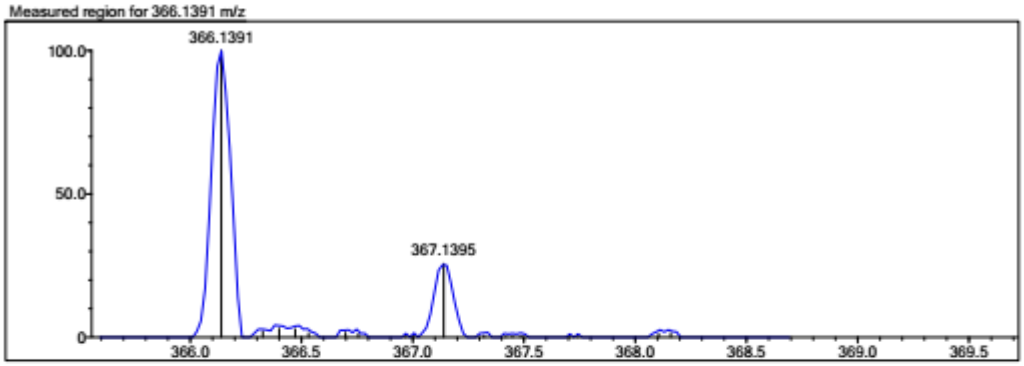
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

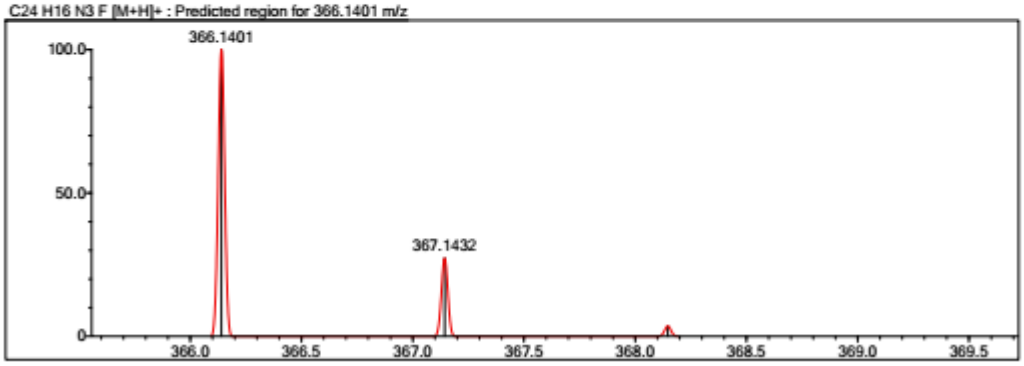
Event#: 1 MS(E+) Ret. Time : 0.880 Scan# : 133



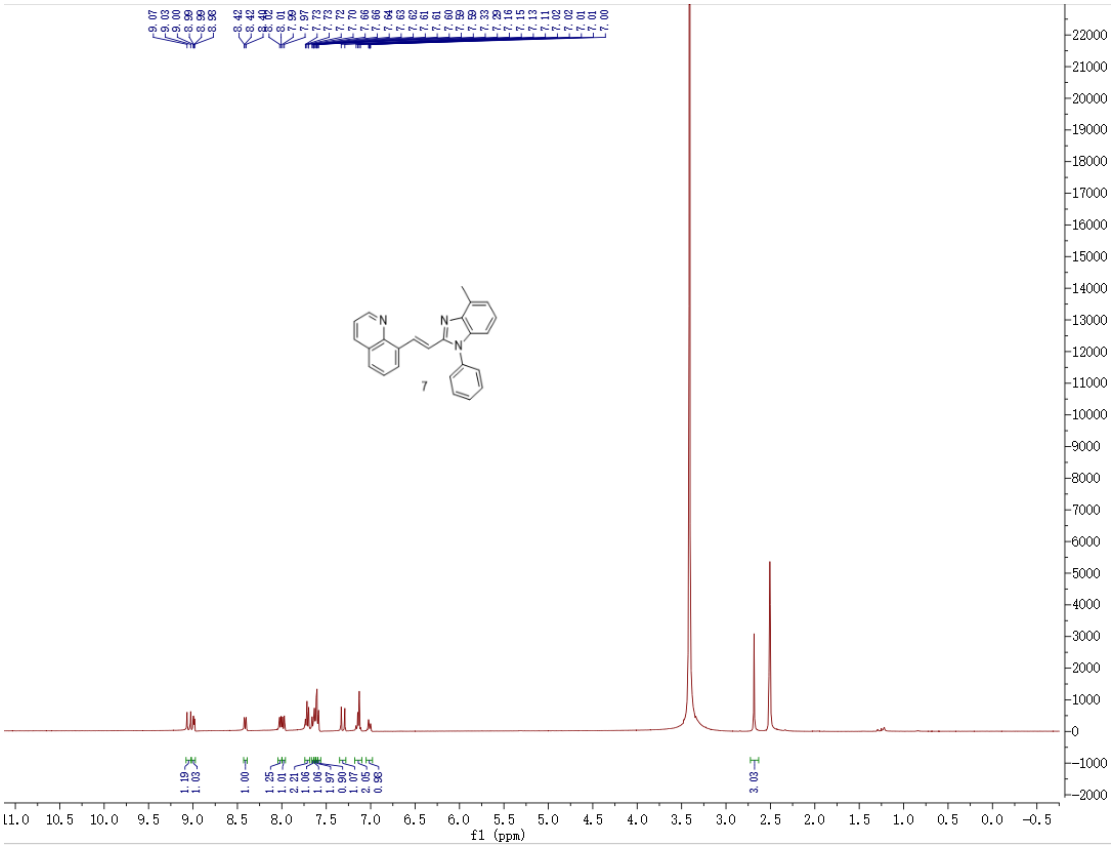
Measured region for 366.1391 m/z

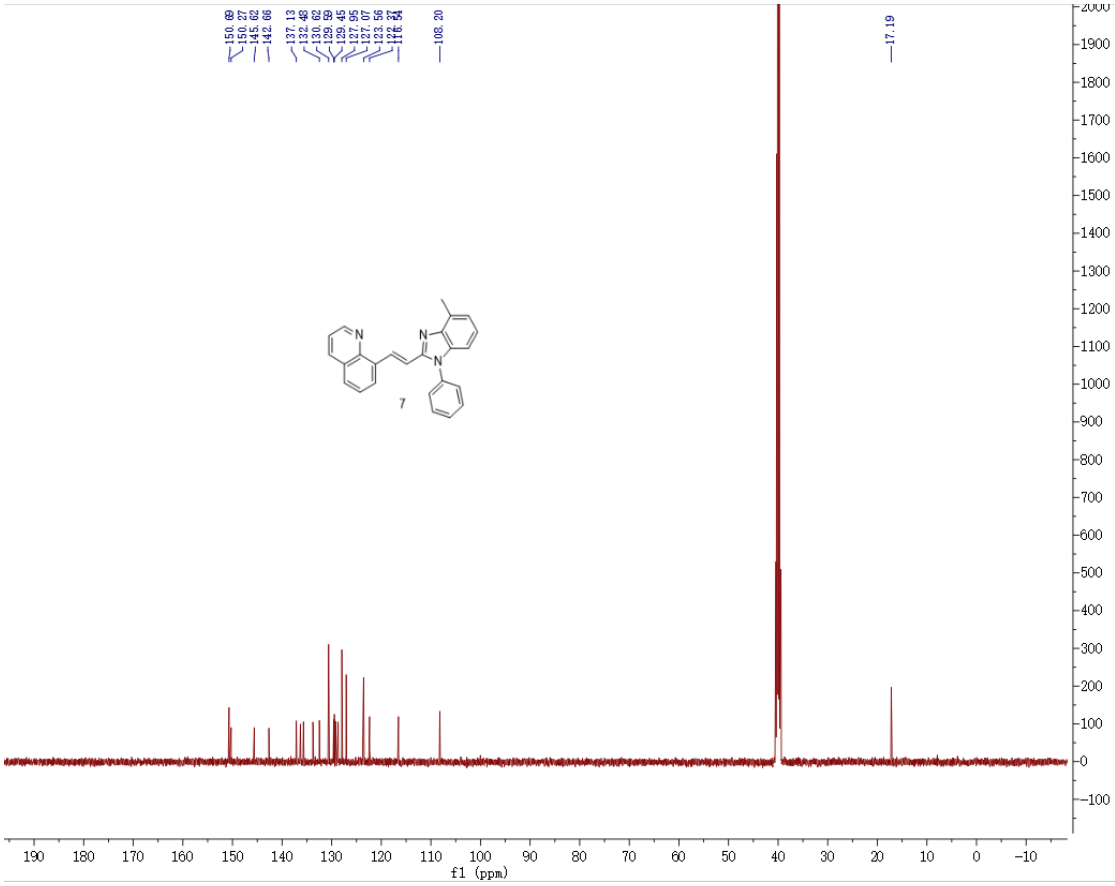


C24 H16 N3 F [M+H]+ : Predicted region for 366.1401 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	79.73	C24 H16 N3 F	[M+H]+	366.1391	366.1401	-1.0	-2.73	83.34	18.0


f1 (ppm)


f1 (ppm)

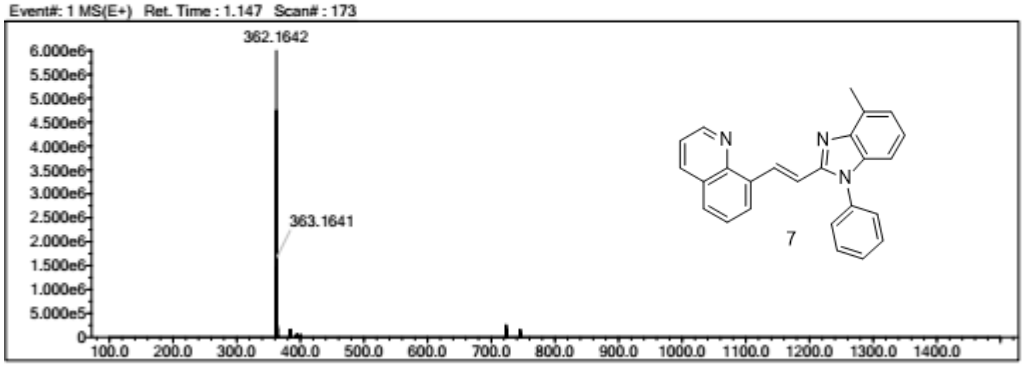
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

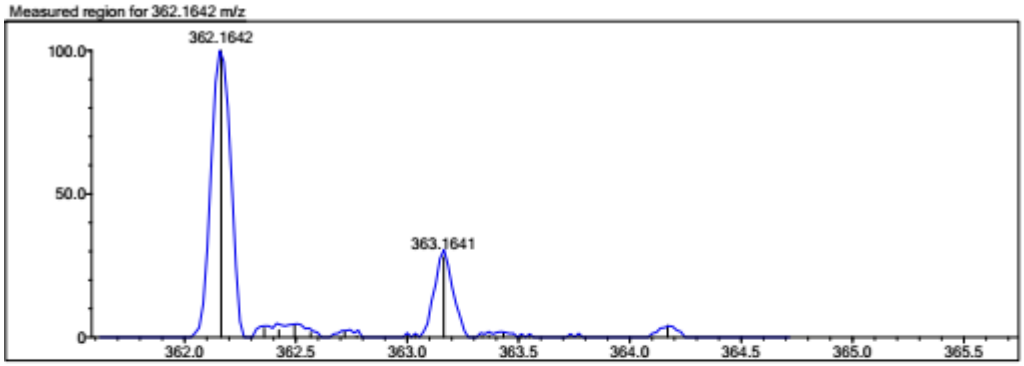
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

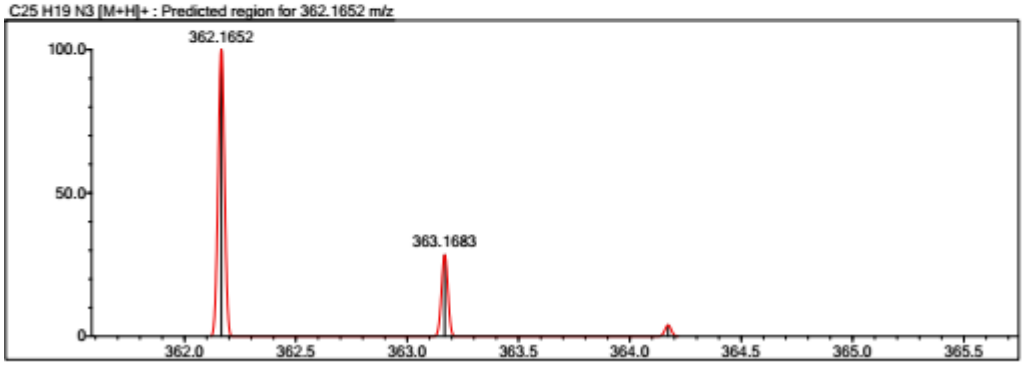
Event#: 1 MS(E+) Ret. Time : 1.147 Scan# : 173



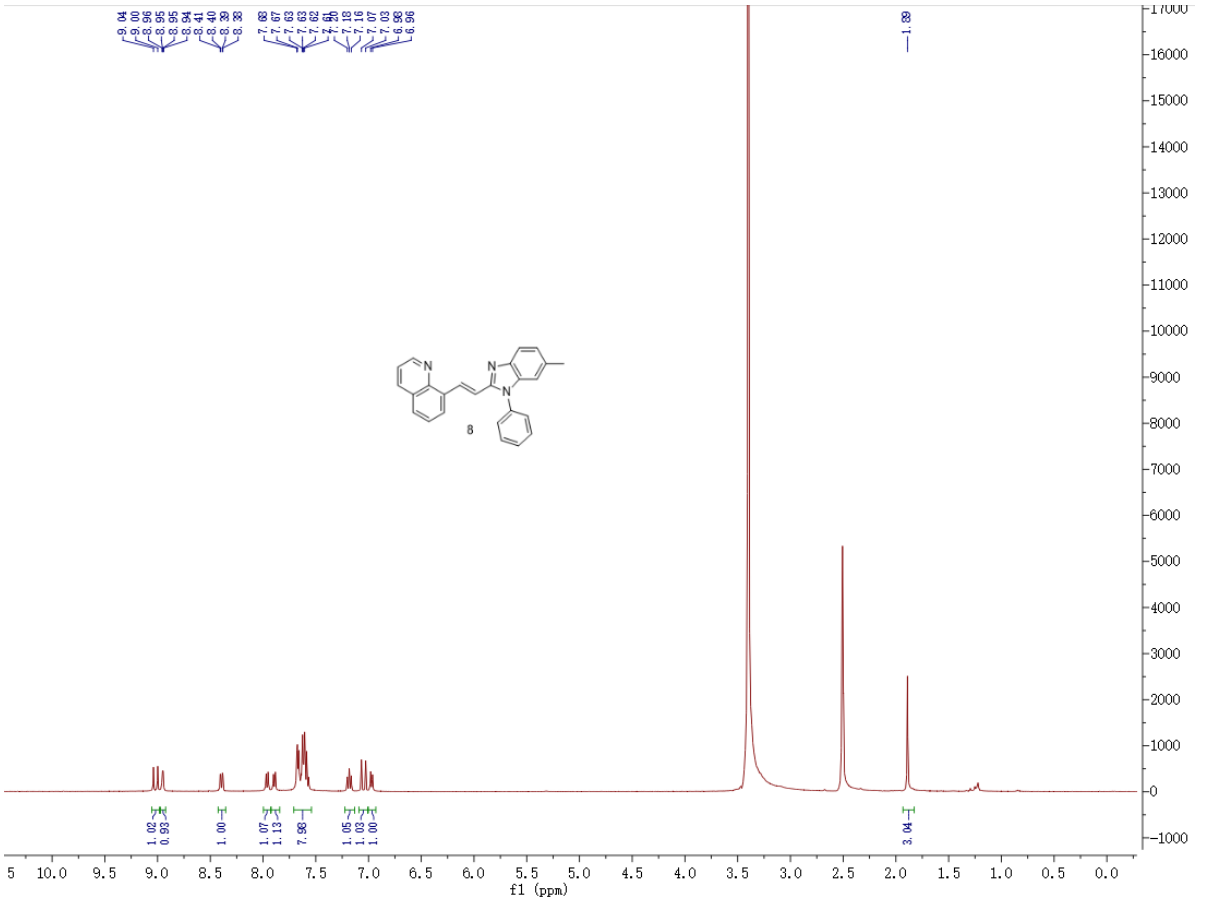
Measured region for 362.1642 m/z

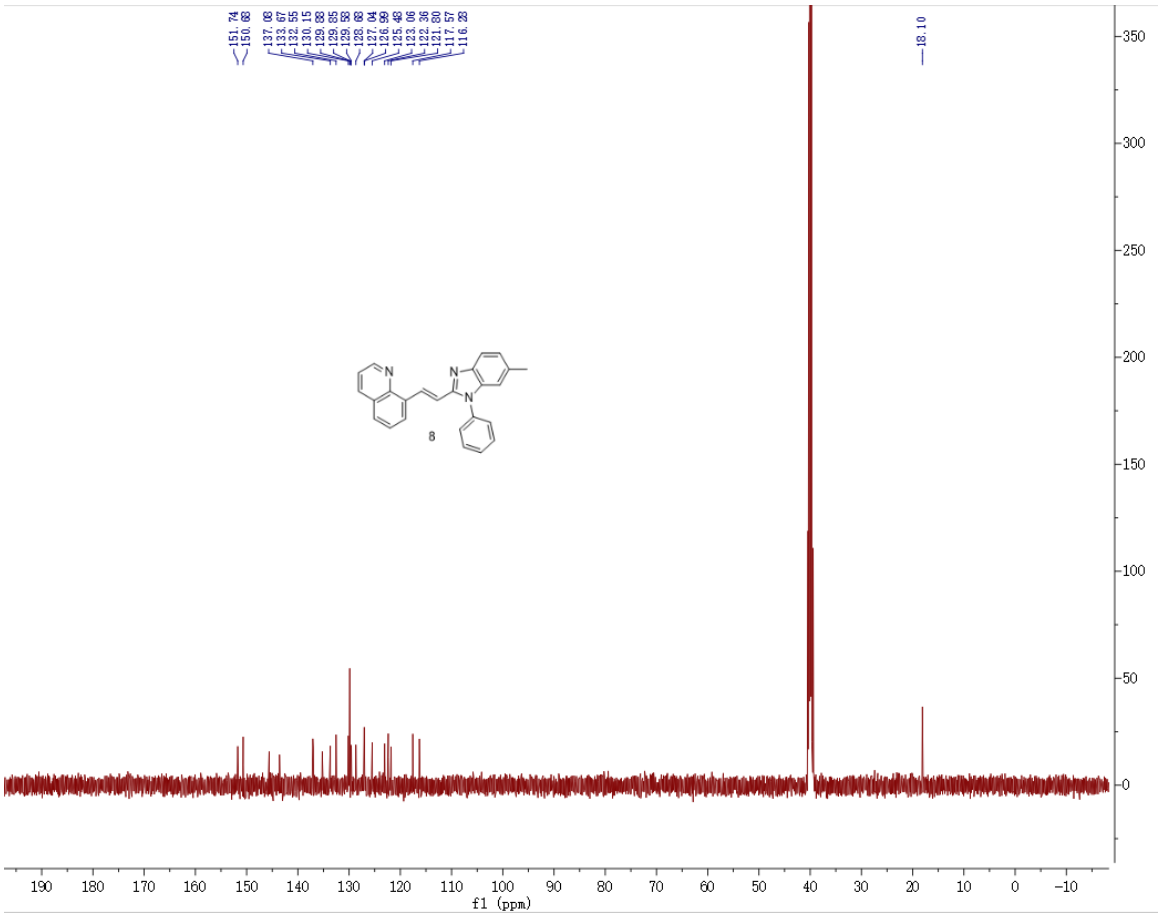


C25 H19 N3 [M+H]+ : Predicted region for 362.1652 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	72.67	C25 H19 N3	[M+H]+	362.1642	362.1652	-1.0	-2.76	76.02	18.0


9.04
9.00
8.96
8.95
8.94
8.41
8.40
8.39
8.38
7.68
7.67
7.63
7.62
7.61
7.18
7.16
7.07
7.03
6.98
6.96
1.89
8
1.02
0.93
1.00
1.07
1.13
7.98
1.05
1.03
1.00
3.04
f1 (ppm)


151.74
150.68
137.08
133.67
132.55
130.15
129.88
129.85
129.83
128.83
127.94
125.48
123.06
122.36
121.80
117.57
116.28
18.10
8
f1 (ppm)

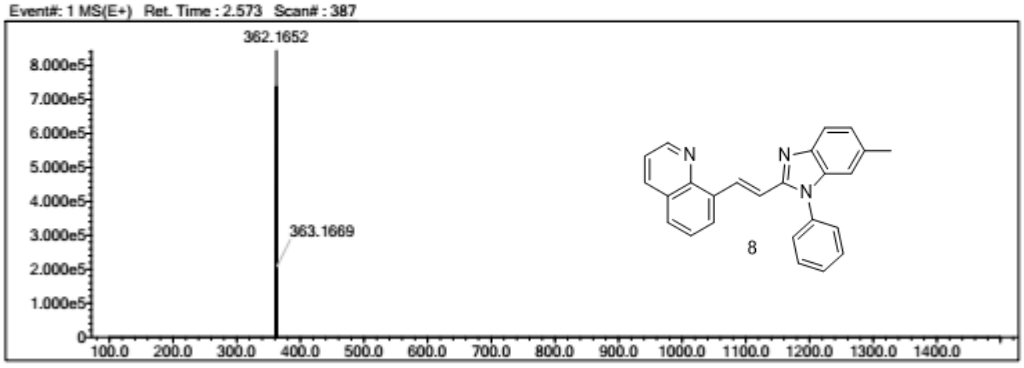
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

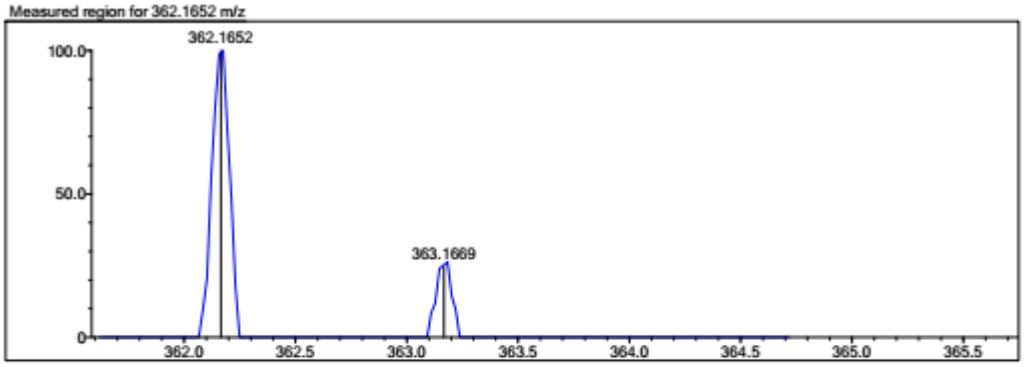
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

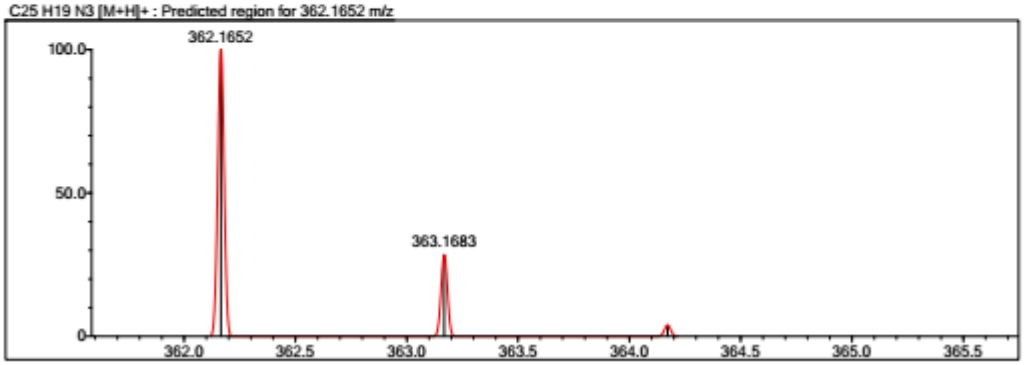
Event#: 1 MS(E+) Ret. Time : 2.573 Scan# : 387



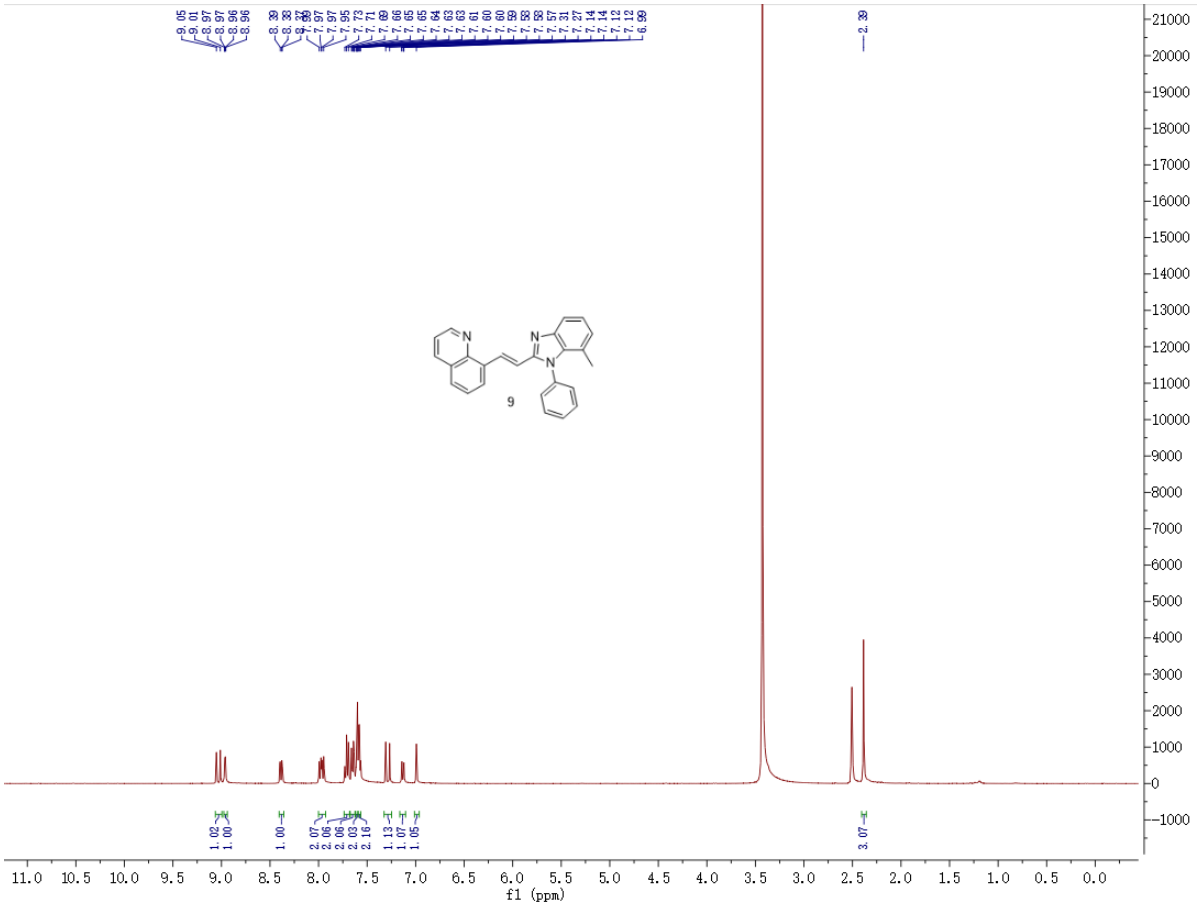
Measured region for 362.1652 m/z

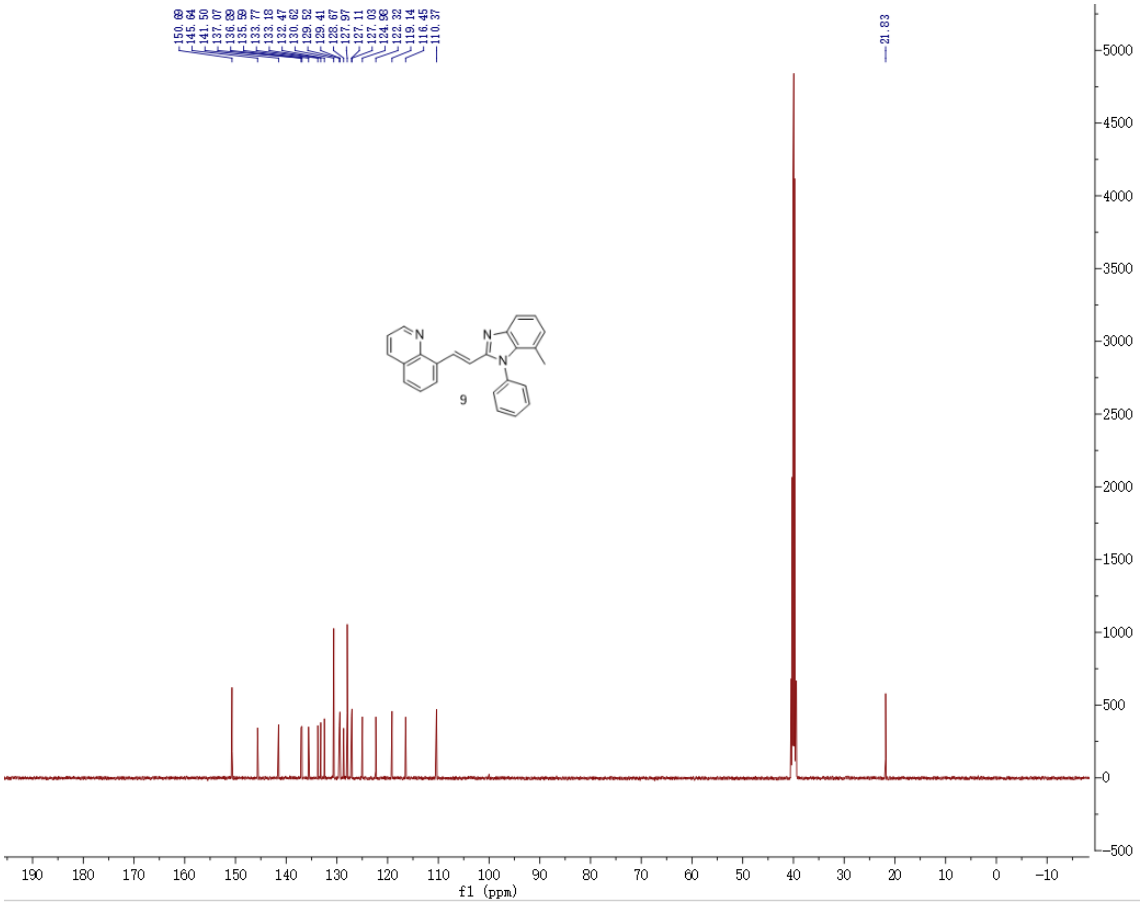


C25 H19 N3 [M+H]+ : Predicted region for 362.1652 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
5	79.67	C25 H19 N3	[M+H]+	362.1652	362.1652	0.0	0.00	79.67	18.0


8.05
8.01
7.97
7.97
7.96
7.96
8.39
8.38
8.37
8.37
7.97
7.97
7.95
7.73
7.71
7.69
7.66
7.65
7.64
7.63
7.63
7.61
7.60
7.60
7.59
7.58
7.58
7.57
7.31
7.27
7.14
7.14
7.12
7.12
6.99
2.39
9
1.02
1.00
1.00
2.07
2.06
2.06
2.03
2.16
1.13
1.07
1.05
3.07
f1 (ppm)


150.89
145.64
141.50
141.07
136.89
135.99
133.77
133.18
132.47
130.62
129.52
129.41
128.67
127.97
127.11
127.03
124.98
122.32
119.14
116.45
110.37
21.83
9
f1 (ppm)

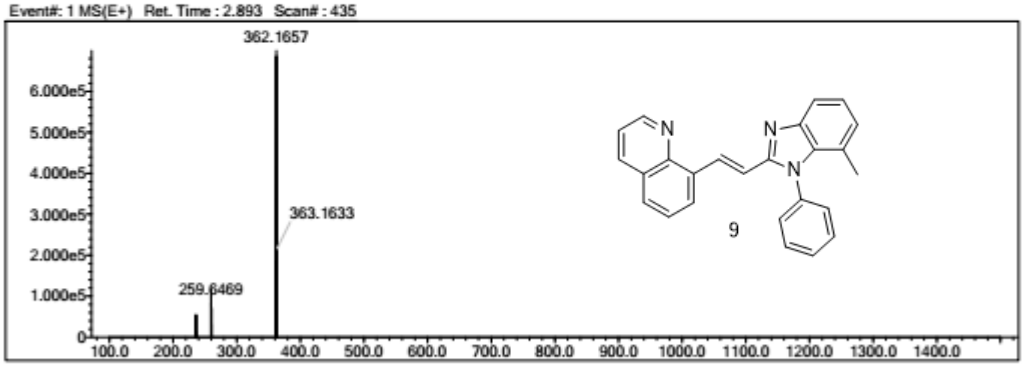
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

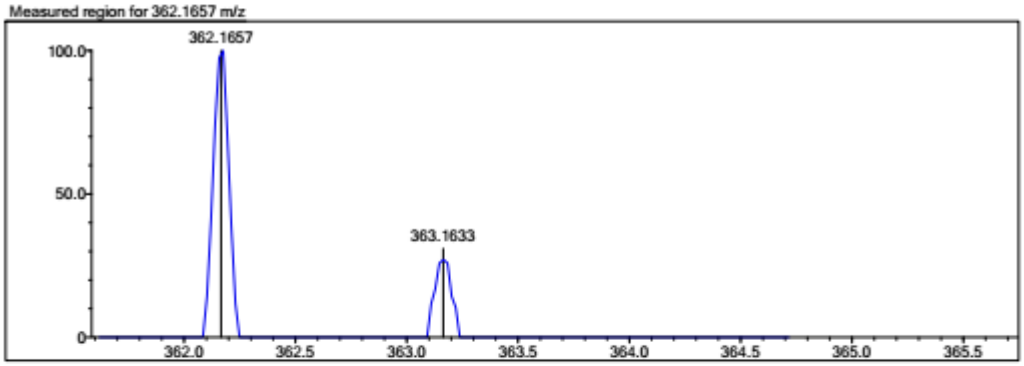
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

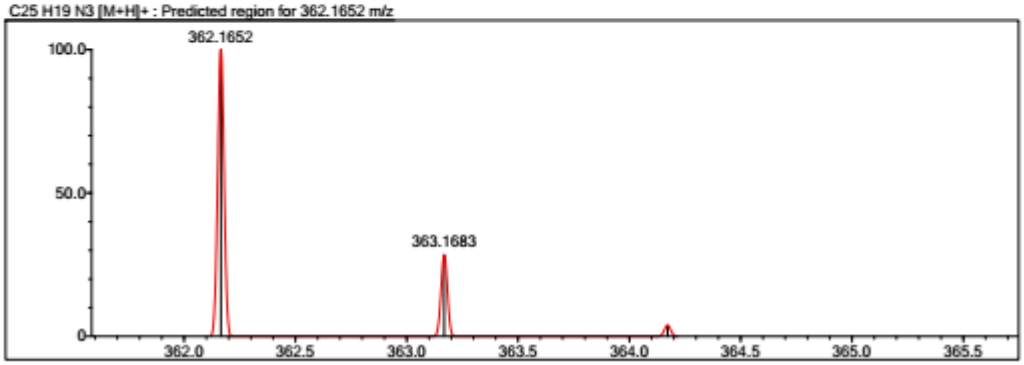
Event#: 1 MS(E+) Ret. Time : 2.893 Scan# : 435



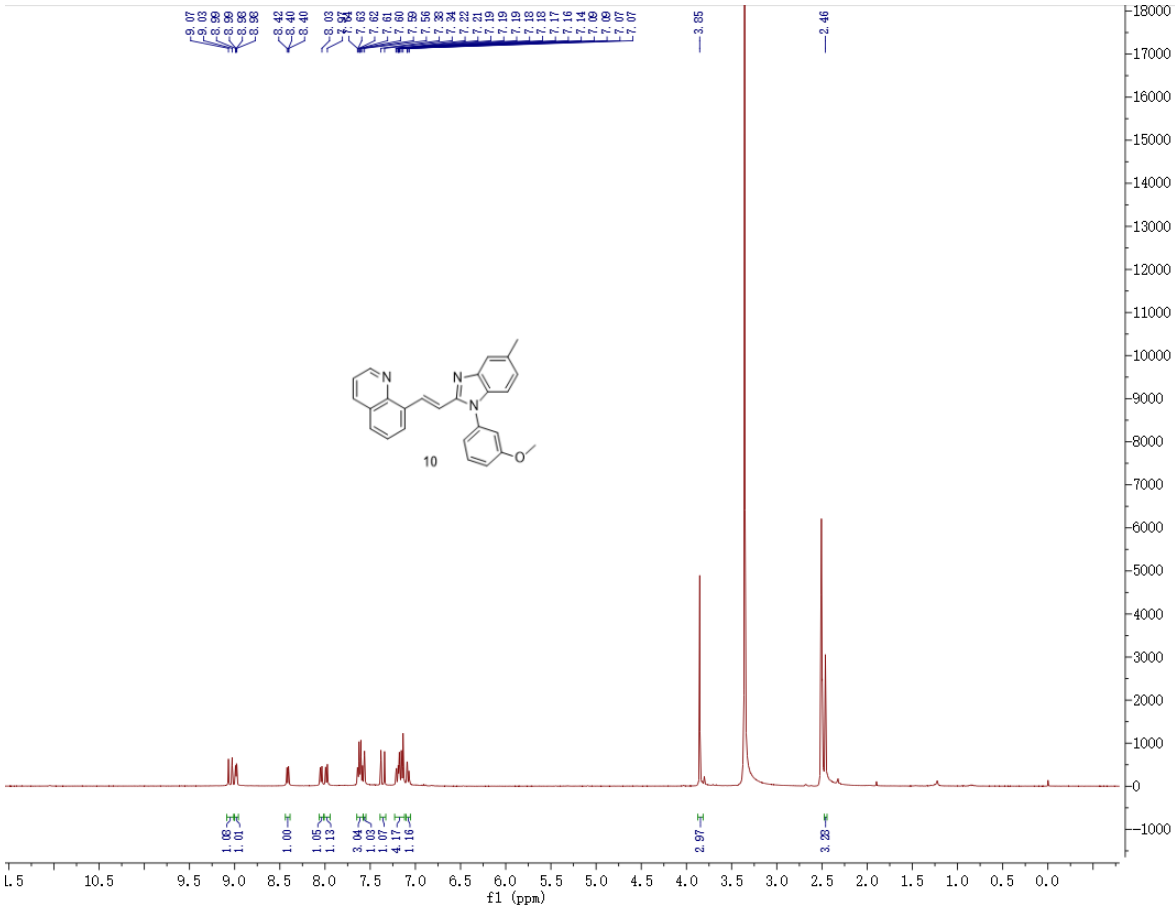
Measured region for 362.1657 m/z

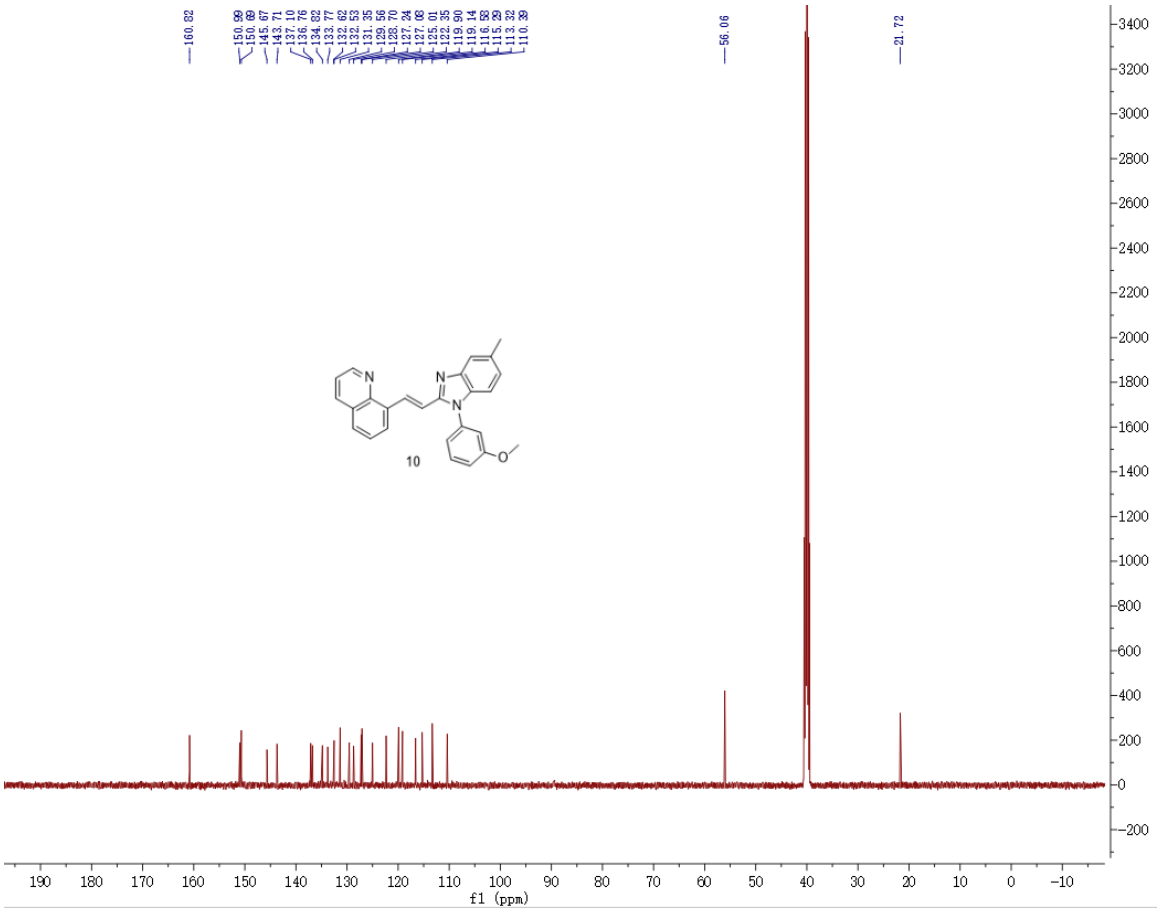


C25 H19 N3 [M+H]+ : Predicted region for 362.1652 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.45	C25 H19 N3	[M+H]+	362.1657	362.1652	0.5	1.38	87.28	18.0


9.07
9.03
8.99
8.98
8.98
8.42
8.40
8.40
8.03
7.63
7.62
7.61
7.60
7.59
7.56
7.38
7.34
7.22
7.21
7.19
7.19
7.19
7.18
7.18
7.17
7.16
7.14
7.09
7.09
7.07
7.07
3.85
2.46
10
1.08
1.01
1.00
1.05
1.13
3.04
1.03
1.07
4.17
1.16
2.97
3.28
f1 (ppm)


160.82
150.99
150.69
145.67
143.71
137.10
136.76
134.82
134.77
132.62
132.53
131.35
129.56
128.70
127.24
127.08
125.01
122.35
119.90
119.14
116.88
115.29
113.32
110.39
56.06
21.72
10
f1 (ppm)

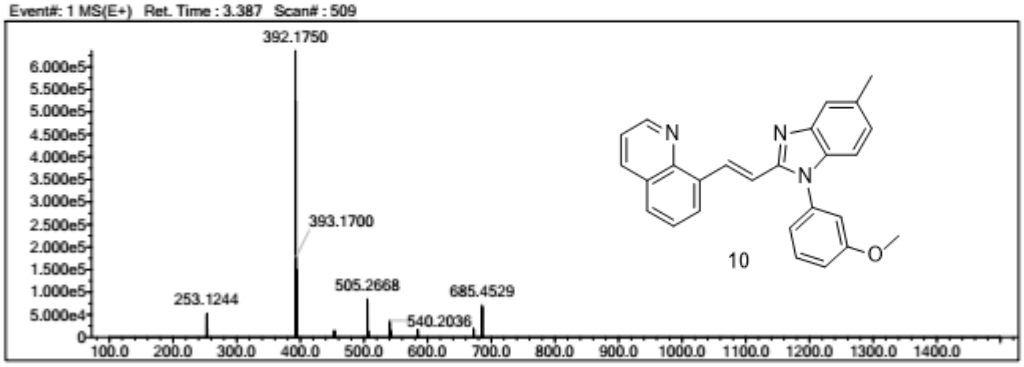
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

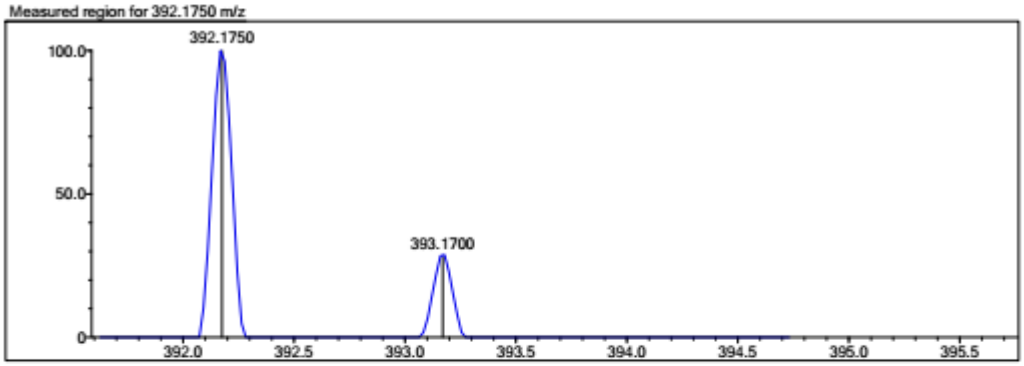
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

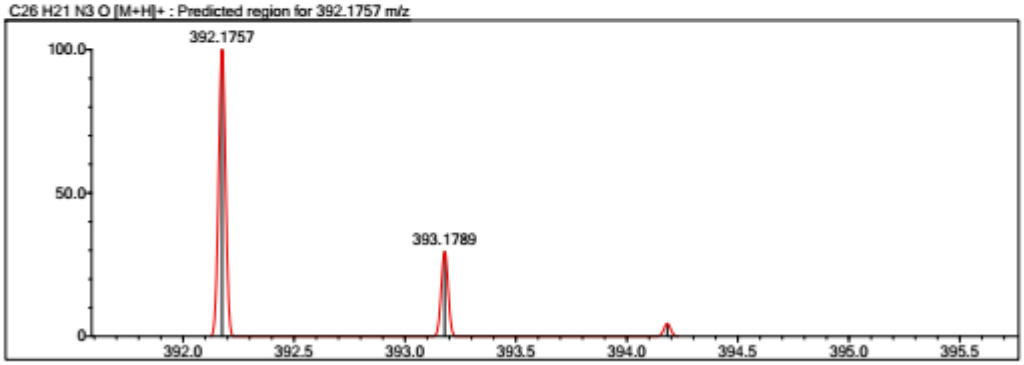
Event#: 1 MS(E+) Ret. Time : 3.387 Scan# : 509



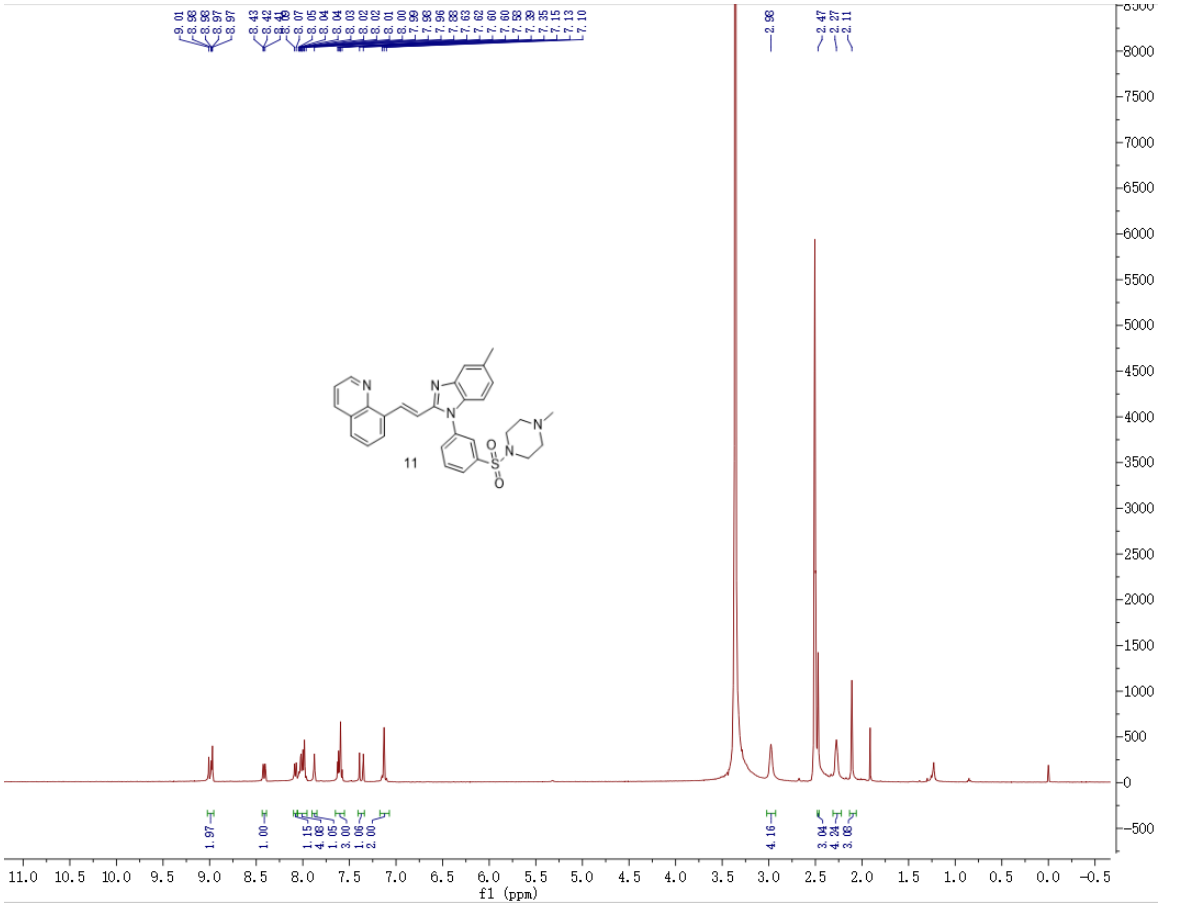
Measured region for 392.1750 m/z

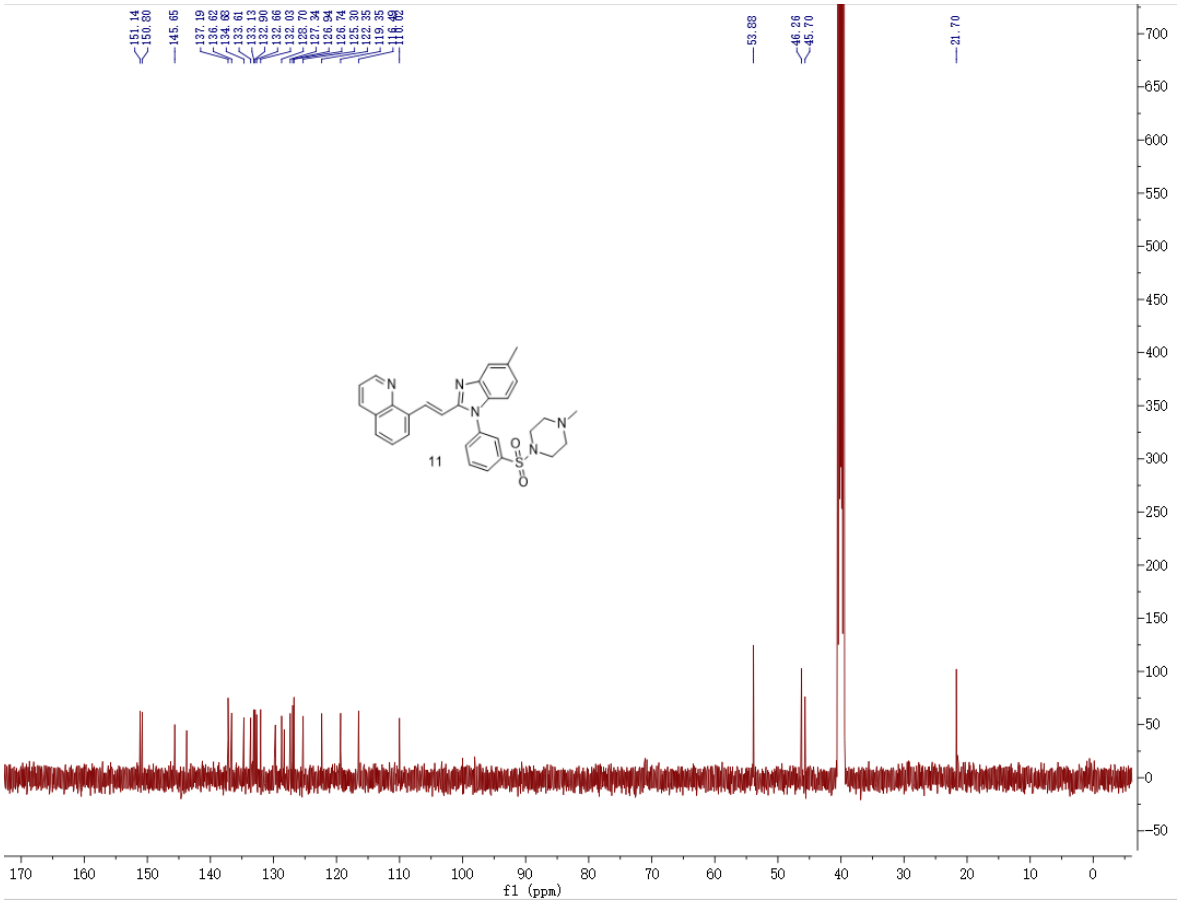


C26 H21 N3 O [M+H]+ : Predicted region for 392.1757 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	79.90	C26 H21 N3 O	[M+H]+	392.1750	392.1757	-0.7	-1.78	81.49	18.0


11
f1 (ppm)


11
f1 (ppm)

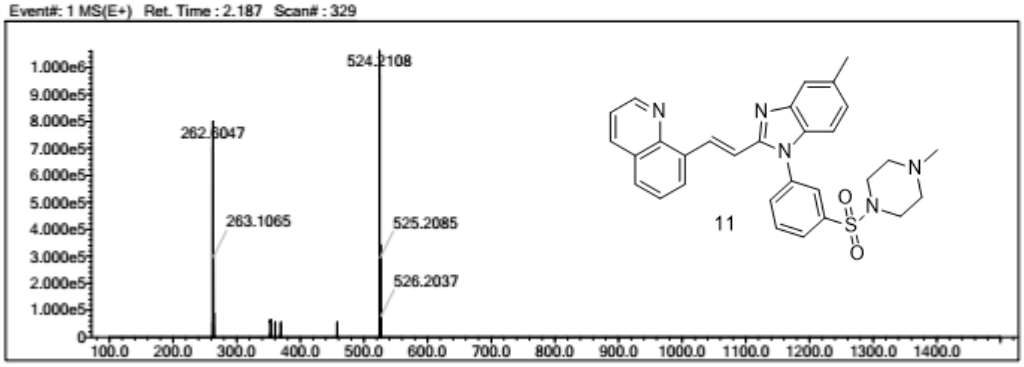
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

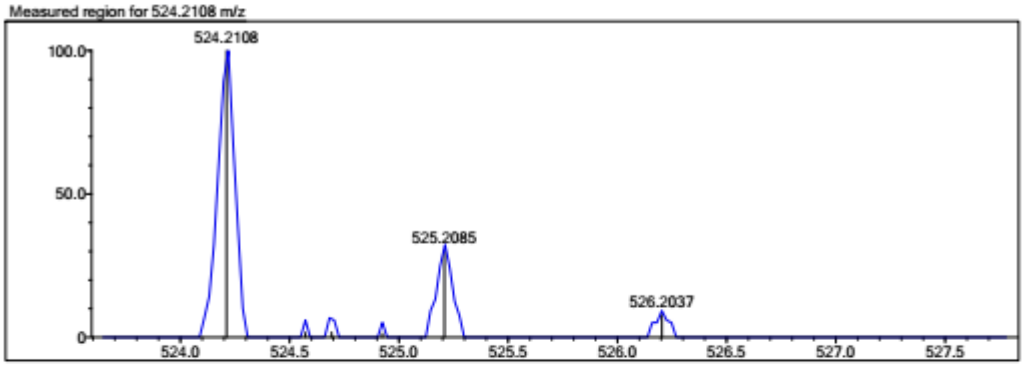
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

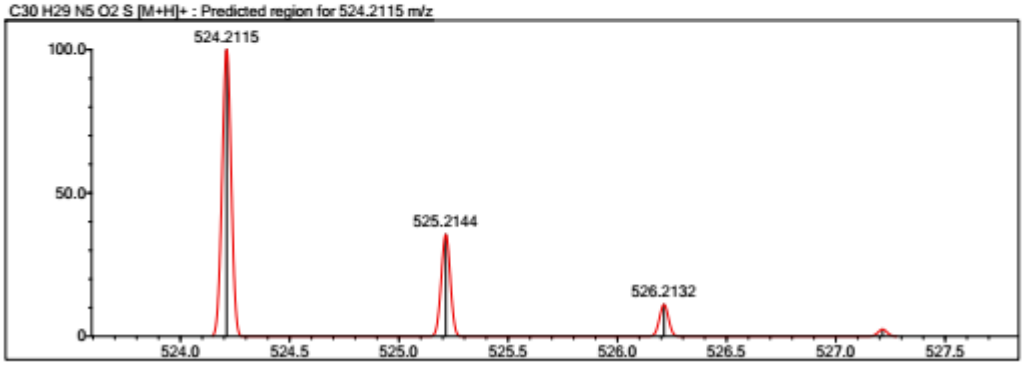
Event#: 1 MS(E+) Ret. Time : 2.187 Scan# : 329



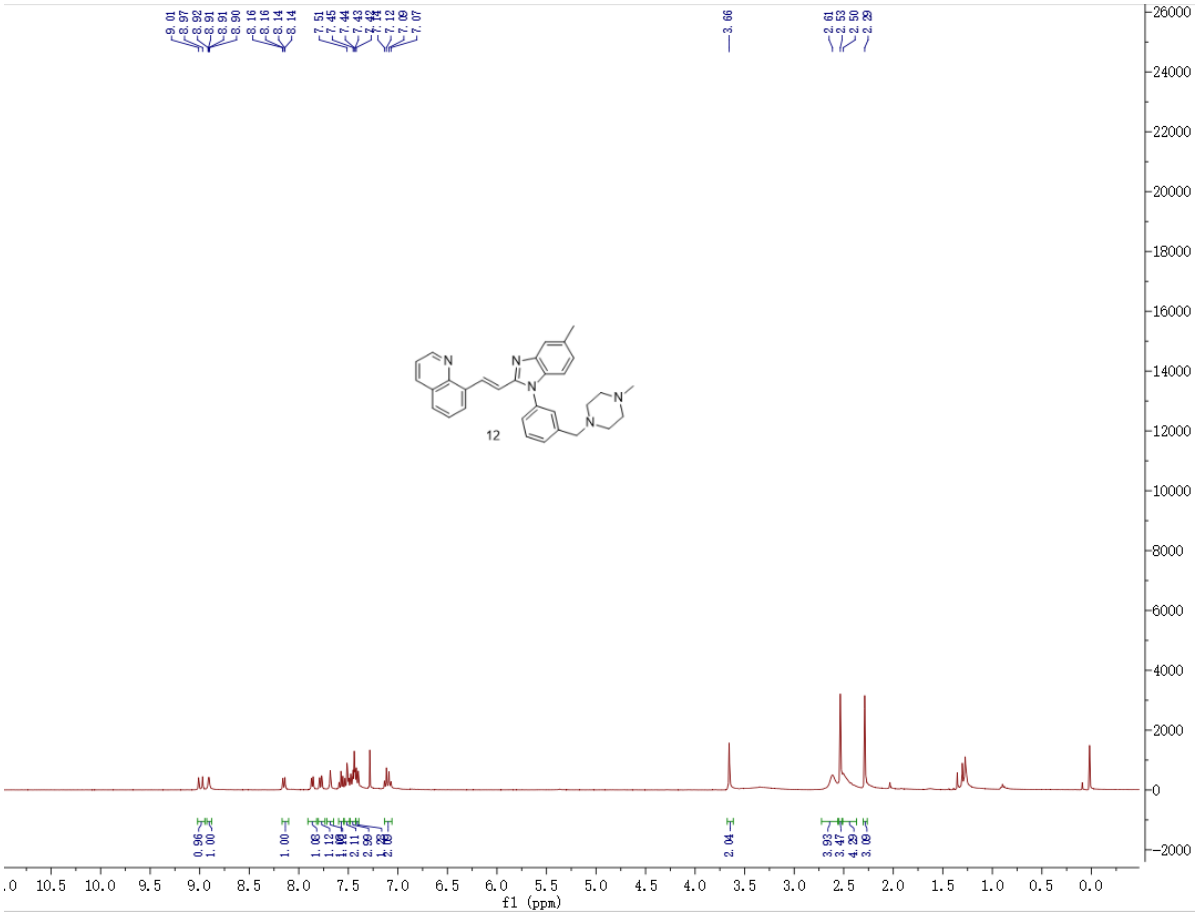
Measured region for 524.2108 m/z

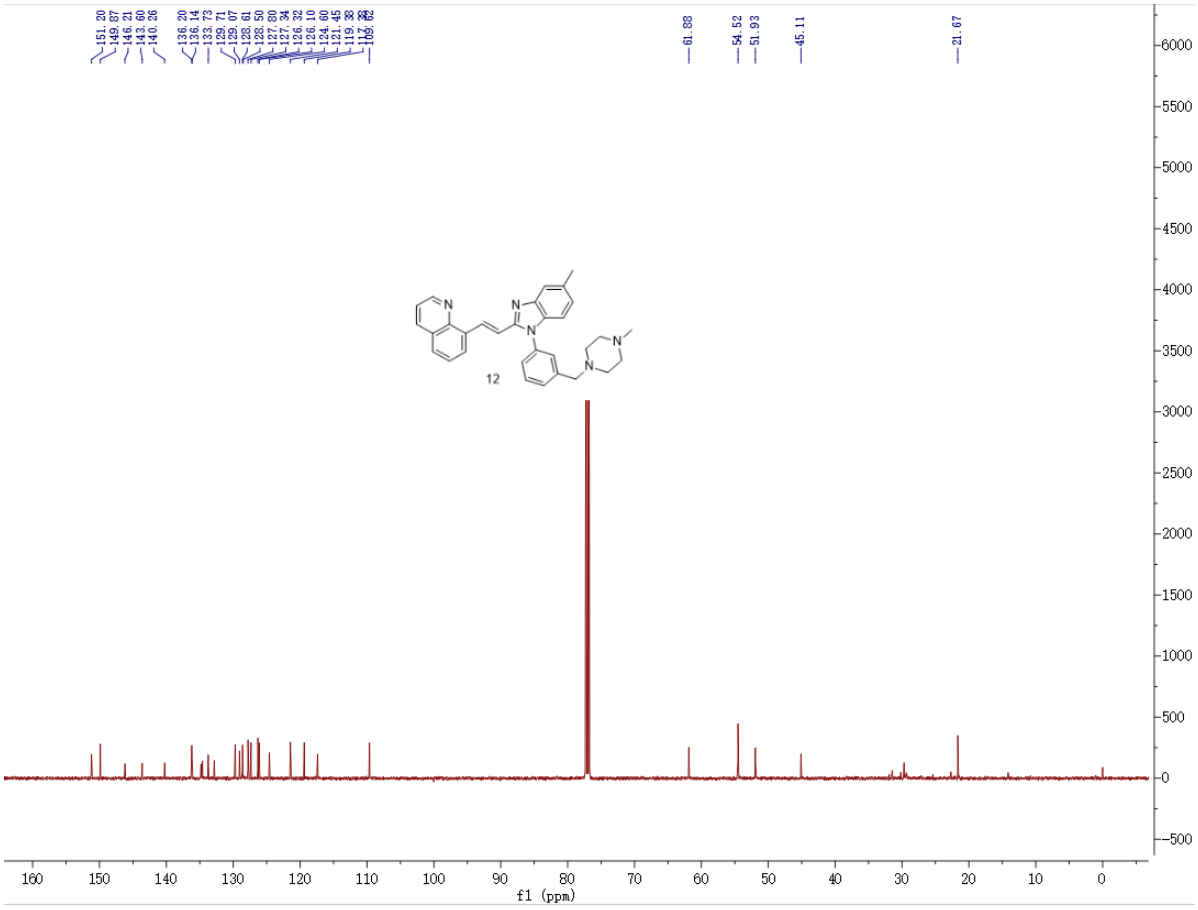


C30 H29 N5 O2 S [M+H]+ : Predicted region for 524.2115 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
7	79.52	C30 H29 N5 O2 S	[M+H]+	524.2108	524.2115	-0.7	-1.34	80.20	19.0


9.01
8.97
8.92
8.91
8.91
8.91
8.90
8.16
8.16
8.14
8.14
7.51
7.45
7.44
7.43
7.14
7.12
7.09
7.07
3.66
2.61
2.53
2.50
2.29
12
f1 (ppm)


151.20
149.87
146.21
143.60
140.26
136.20
136.14
133.73
129.71
129.07
128.61
128.50
127.80
127.34
126.32
126.10
124.60
121.45
119.38
61.88
54.52
51.93
45.11
21.67
12
f1 (ppm)

Elmt	Val.	Min	Max
H	1	10	40
2H	1	0	0
B	3	0	0
C	4	10	35
N	3	0	5

Elmt	Val.	Min	Max
O	2	0	3
18O	2	0	0
F	1	0	1
Al	3	0	0
Si	4	0	0

Elmt	Val.	Min	Max
P	3	0	0
S	2	0	1
Cl	1	0	0
Fe	2	0	0
Se	2	0	0

Elmt	Val.	Min	Max
Br	1	0	0
Te	2	0	0
I	3	0	0

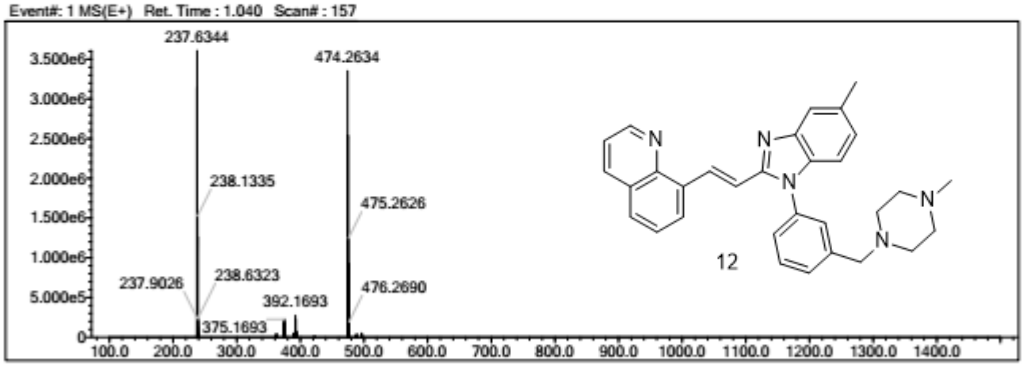
Use Adduct
H
Na

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

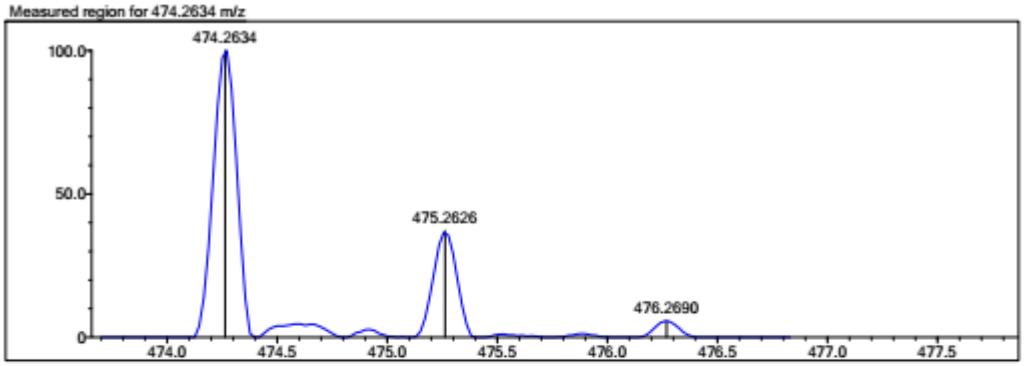
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

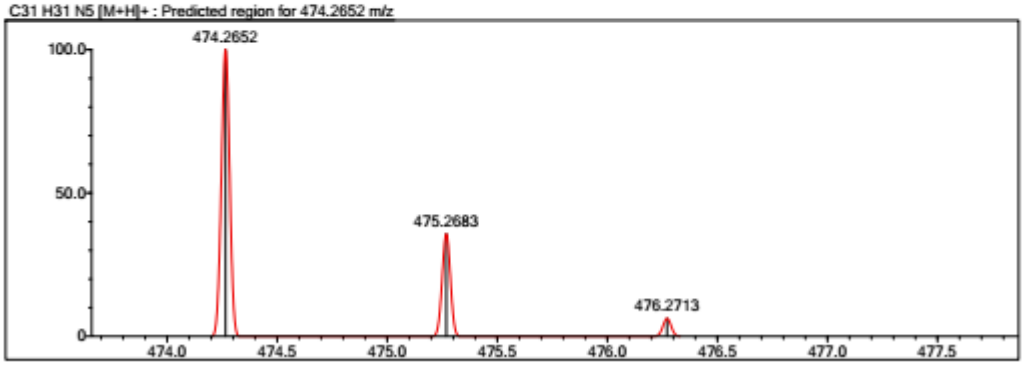
Event#: 1 MS(E+) Ret. Time : 1.040 Scan# : 157



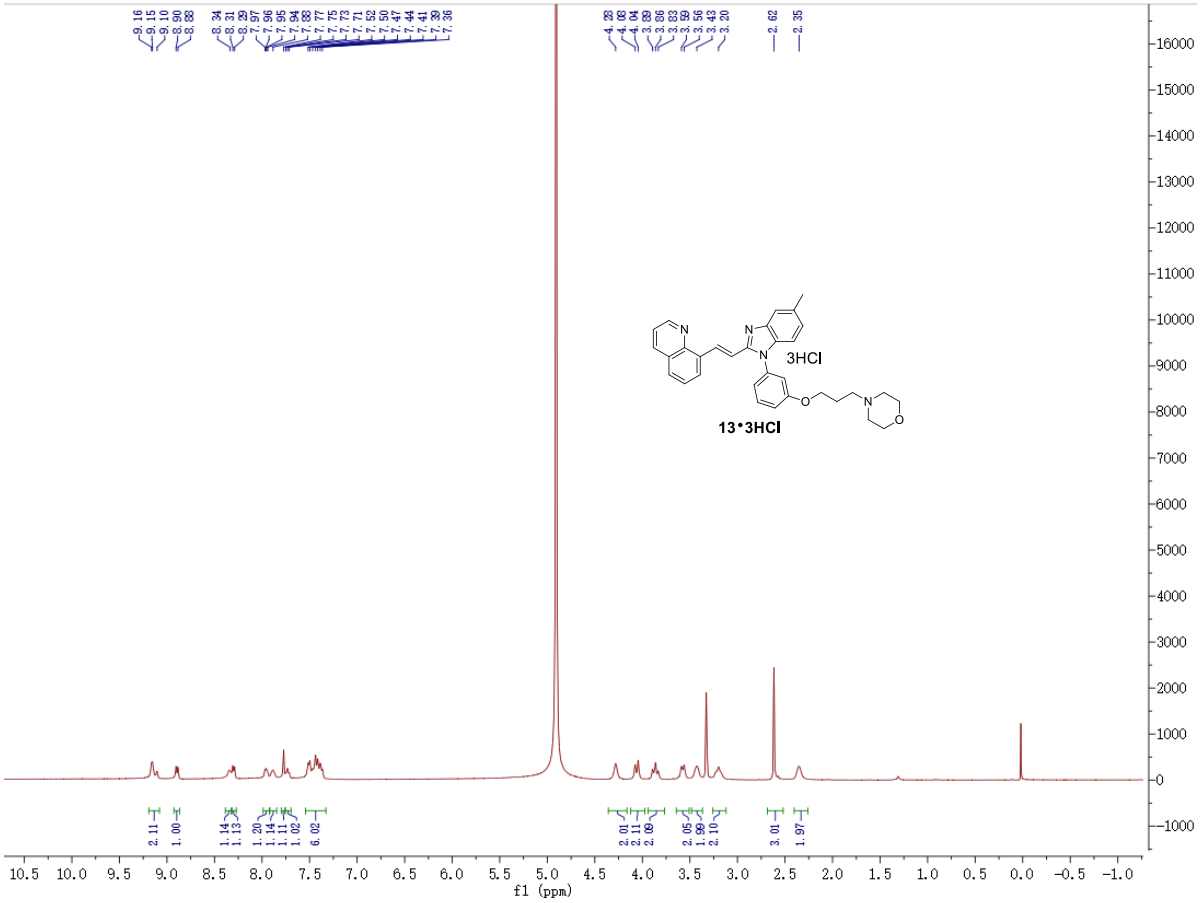
Measured region for 474.2634 m/z

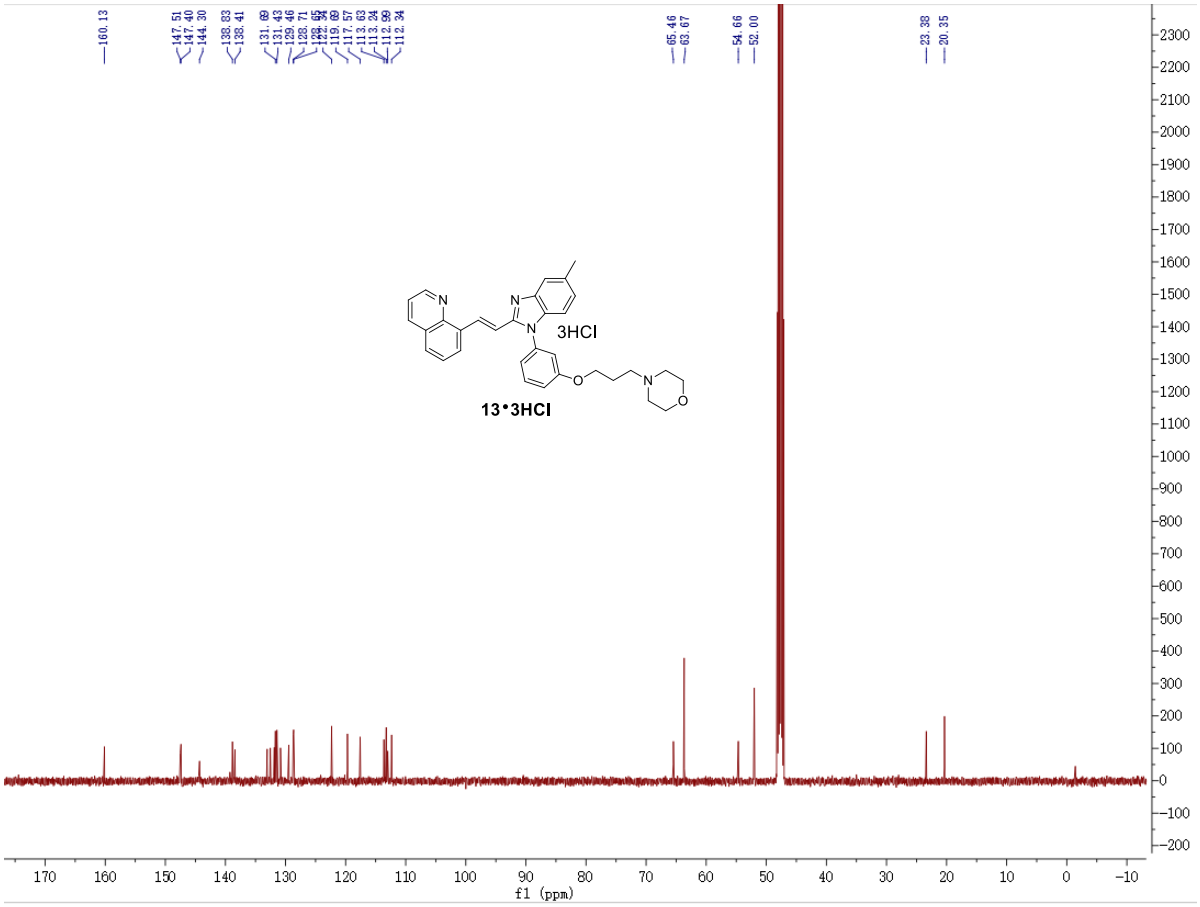


C31 H31 N5 [M+H]+ : Predicted region for 474.2652 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
5	74.89	C31 H31 N5	[M+H]+	474.2634	474.2652	-1.8	-3.80	80.52	19.0


3HCl
13*3HCl
f1 (ppm)


3HCl
13*3HCl
f1 (ppm)

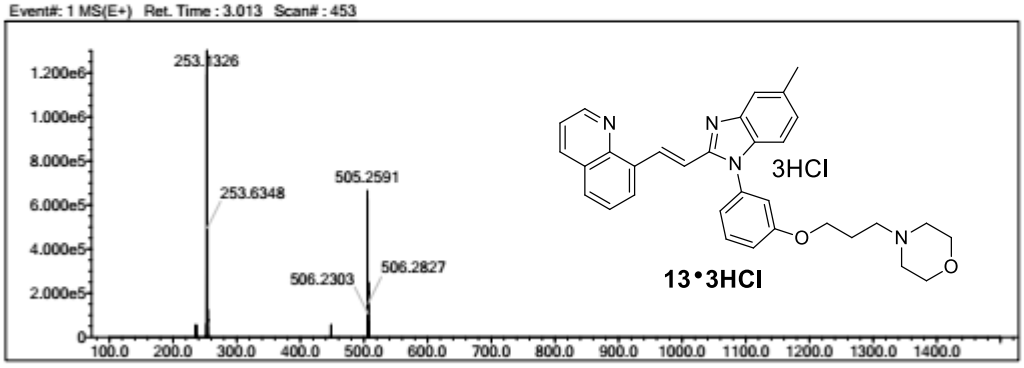
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

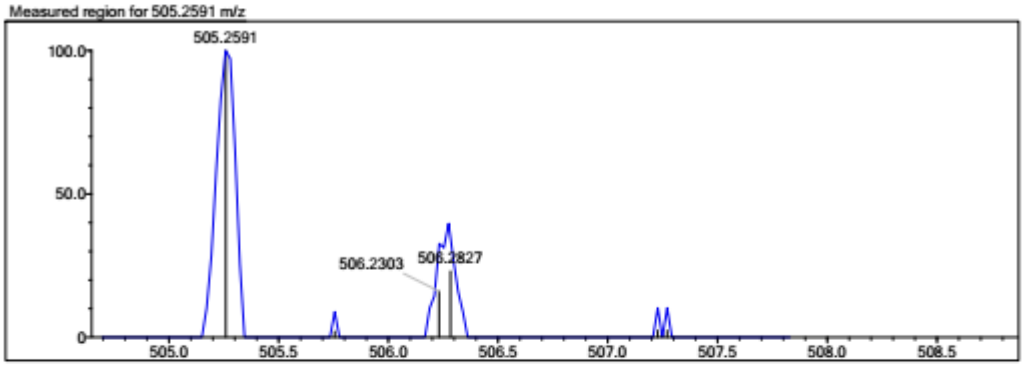
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

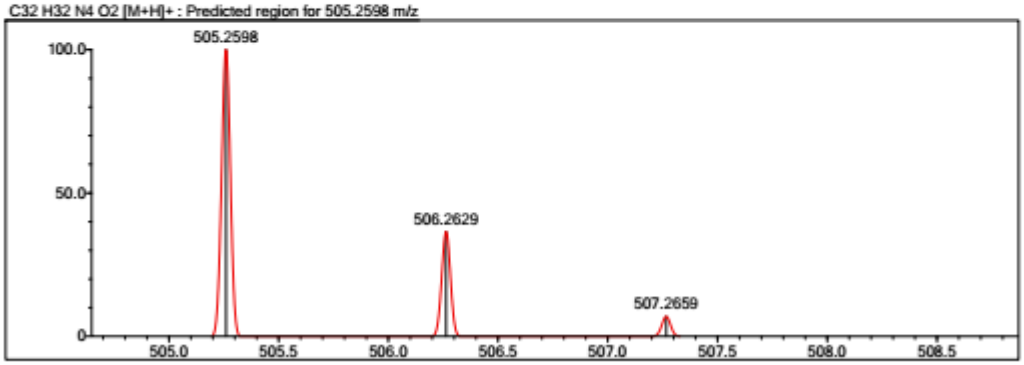
Event#: 1 MS(E+) Ret. Time : 3.013 Scan# : 453



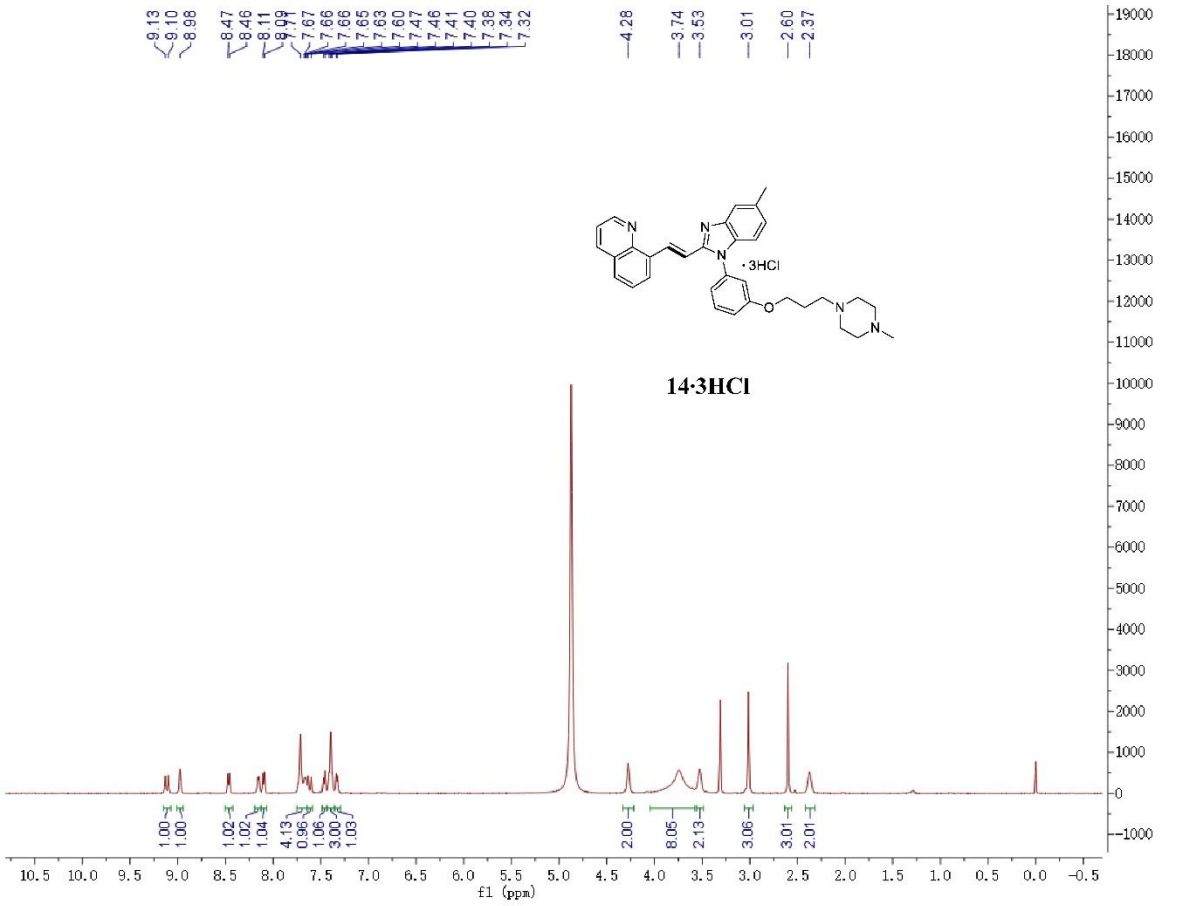
Measured region for 505.2591 m/z



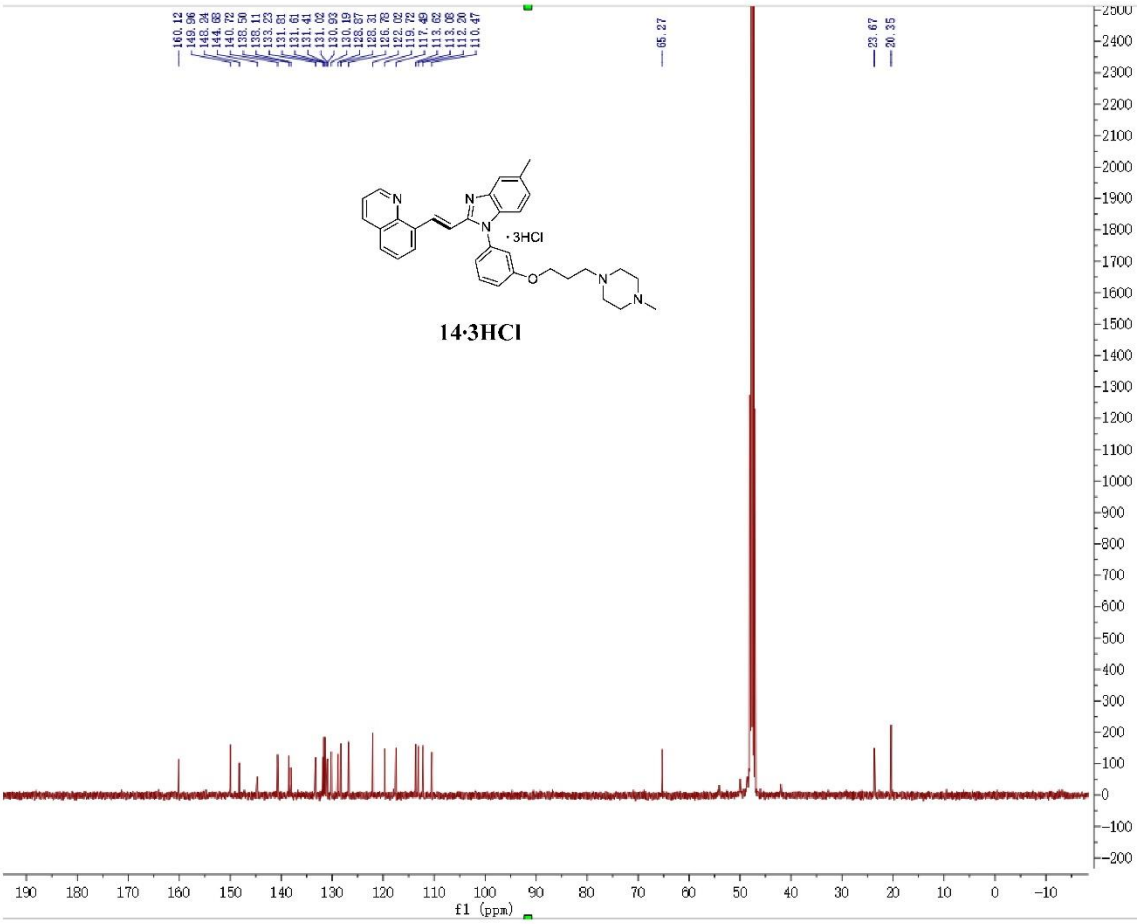
C32 H32 N4 O2 [M+H]+ : Predicted region for 505.2598 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	49.51	C32 H32 N4 O2	[M+H]+	505.2591	505.2598	-0.7	-1.39	50.00	19.0



14·3HCl



14·3HCl

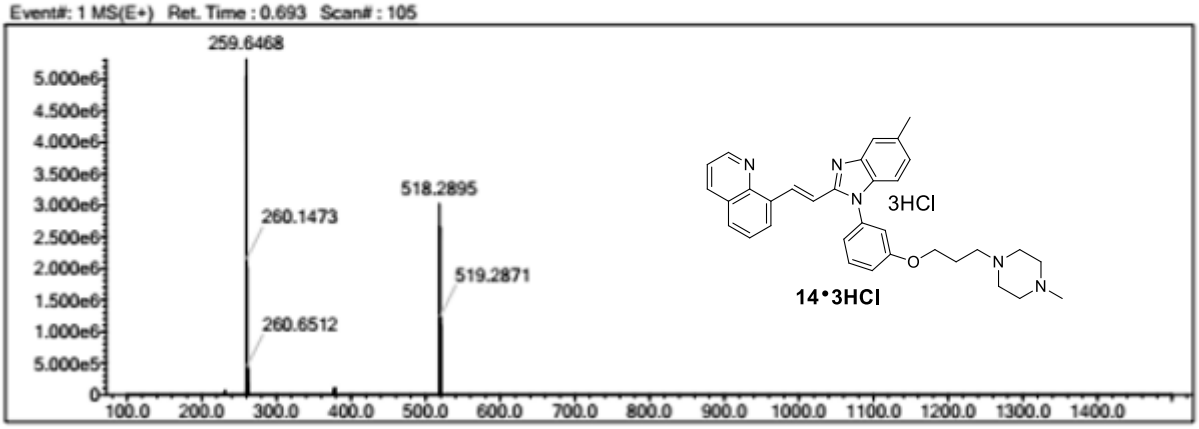
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	40	O	2	0	3	P	3	0	0	Br	1	0	0	H
2H	1	0	0	18O	2	0	0	S	2	0	1	Te	2	0	0	Na
B	3	0	0	F	1	0	1	Cl	1	0	0	I	3	0	0	
C	4	10	35	Al	3	0	0	Fe	2	0	0					
N	3	0	5	Si	4	0	0	Se	2	0	0					

Error Margin (ppm): 100
HC Ratio: 0.0 - 1000.0
Max Isotopes: all
MSn Iso RI (%): 75.00

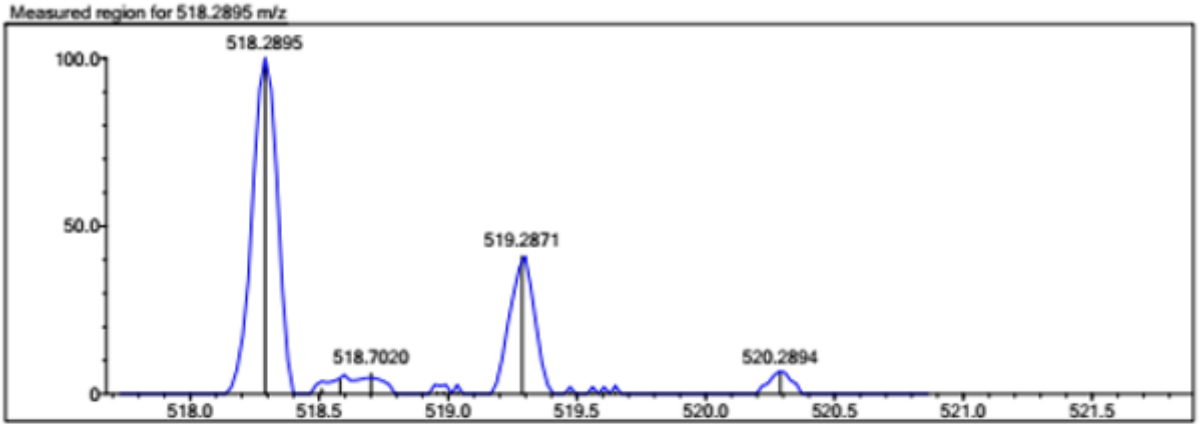
DBE Range: -300.0 - 300.0
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: both
Use MSn Info: no
Isotope Res: 10000
Max Results: 10000

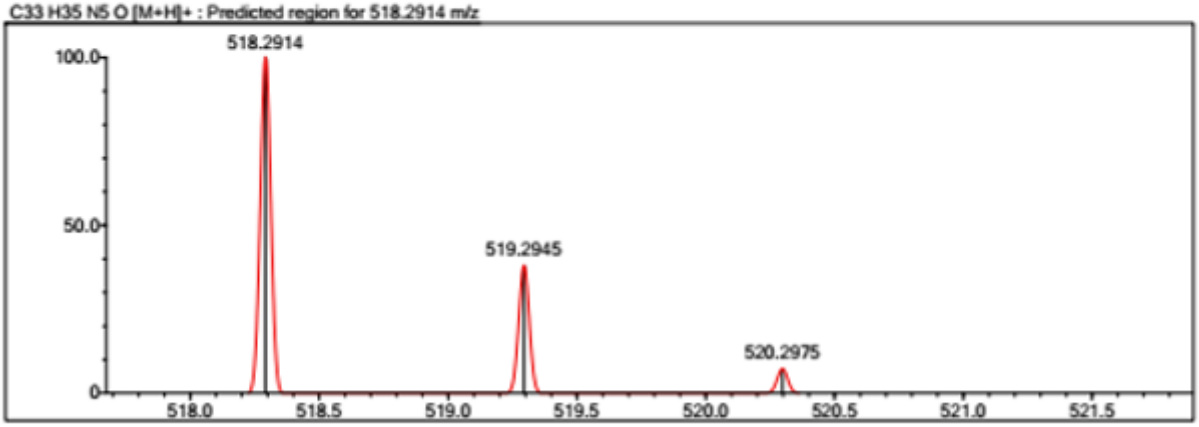
Event#: 1 MS(E+) Ret. Time : 0.693 Scan# : 105



Measured region for 518.2895 m/z



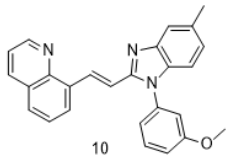
C33 H35 N5 O [M+H]+ : Predicted region for 518.2914 m/z



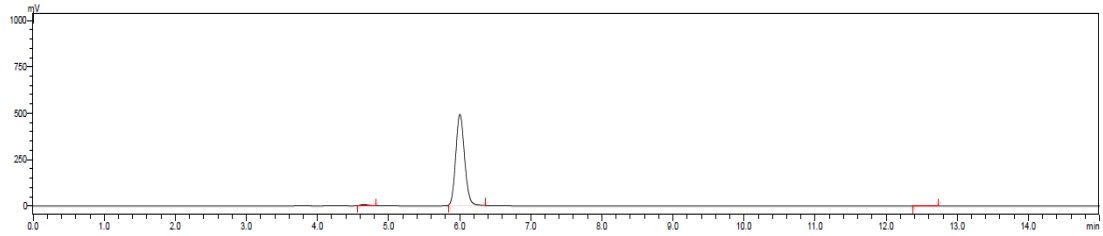
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	77.93	C33 H35 N5 O	[M+H]+	518.2895	518.2914	-1.9	-3.67	83.50	19.0

HPLC spectra data for the purity of tested compounds

Compound	Purity (%)	*t* (min)
10	98.9	6.004
11	98.0	6.853
12	99.8	5.016
13	99.6	9.325
14	99.2	4.972

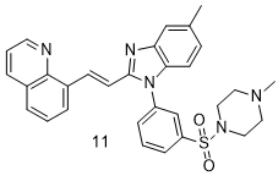


10

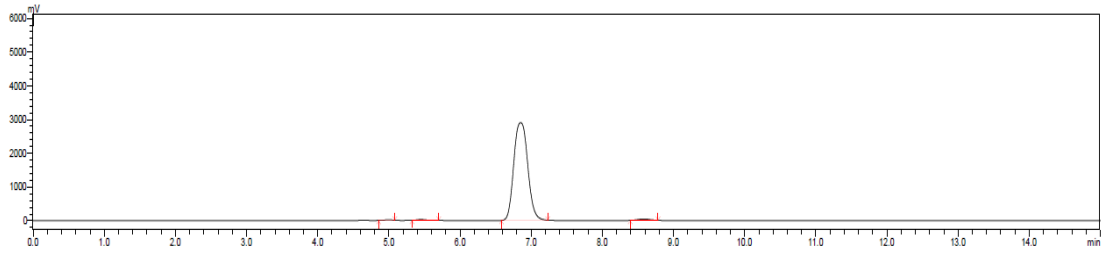


Peak #	Retention time	Peak area	Peak height	Peak area %
1	4.653	44219	6519	1.041
2	6.004	4201657	489675	98.935
3	12.598	1003	68	0.024
In total		4246878	496261	100.00

UV detection at 254 nm; elution, MeOH (100%); T = 25 ℃; flow rate = 0.8 ml/min. Purity：98.9%.

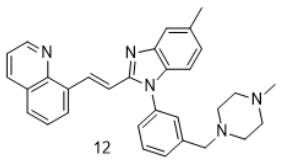


11

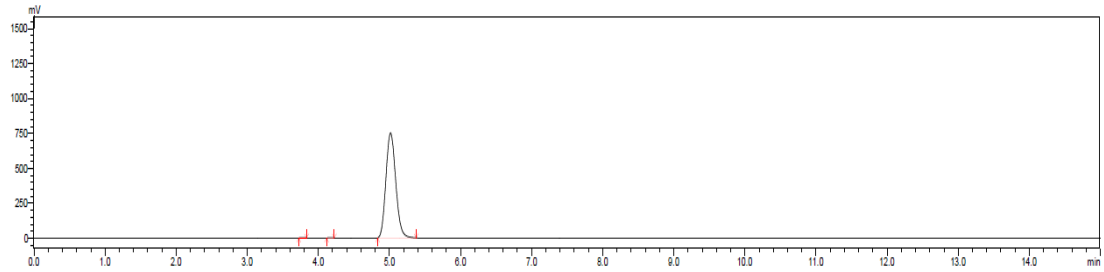


Peak #	Retention time	Peak area	Peak height	Peak area %
1	4.996	128852	17960	0.320
2	5.447	216631	23222	0.537
3	6.853	39518562	2898706	97.998
4	8.583	461861	35320	1.145
In total		40325906	2975208	100.00

UV detection at 254 nm; elution, MeOH in water (90 %, *v*/*v*); T = 25 ℃; flow rate = 0.8 mL/min. Purity: 98.0%

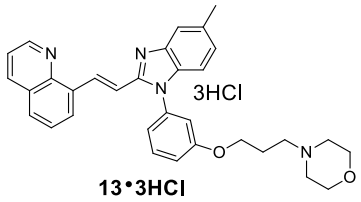


12

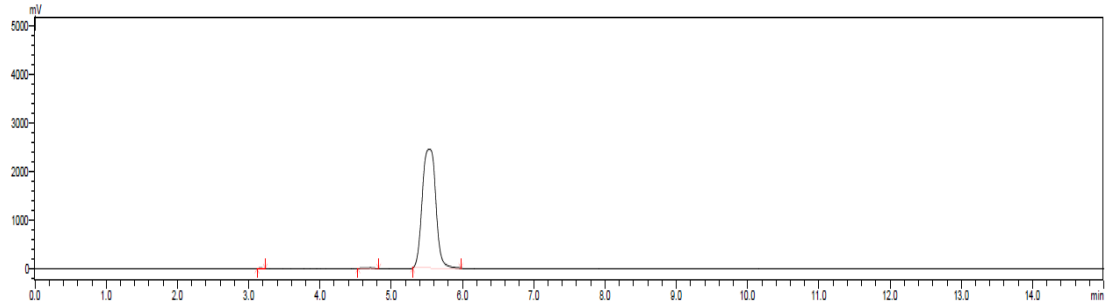


Peak #	Retention time	Peak area	Peak height	Peak area %
1	3.777	8383	2072	0.116
2	4.117	8384	2027	0.116
3	5.016	7214769	750479	99.768
In total		7231536	754578	100.00

UV detection at 254 nm; elution, MeOH in water (97%, *v*/*v*); T = 25 ℃; flow rate = 1.0 mL/min. Purity: 99.8%

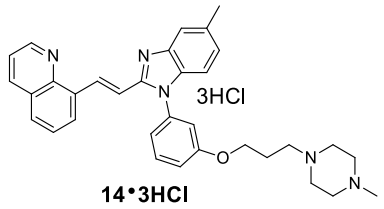


13•3HCl

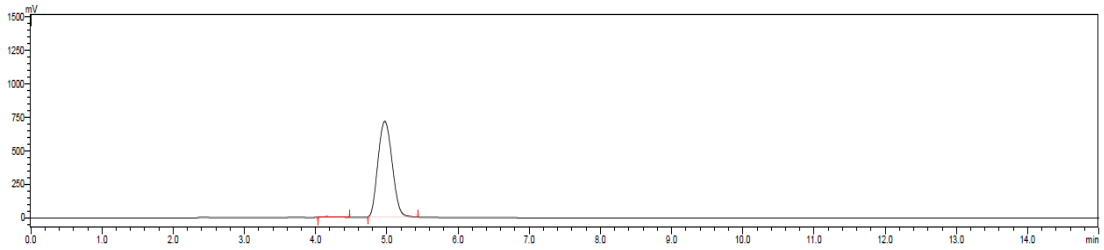


Peak #	Retention time	Peak area	Peak height	Peak area %
1	3.162	35131	8088	0.108
2	4.711	98633	9935	0.303
3	5.535	32437771	2453339	99.589
In total		32571536	2471363	100.00

UV detection at 254 nm; elution, MeOH (100 %); T = 25 ℃; flow rate = 0.8 mL/min. Purity：99.6%



14•3HCl



Peak #	Retention time	Peak area	Peak height	Peak area %
1	4.159	80223	5035	0.806
2	4.972	9873637	713569	99.194
In total		9953861	718605	100.00

UV detection at 254 nm; elution, MeOH in water (97 %, *v*/*v*); T = 25 ℃; flow rate = 1.0 mL/min. Purity：99.2%

References

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(3) Wang H, Liu Y, Hou J, Zheng M, Robinson H, Ke H. Structural insight into substrate specificity of phosphodiesterase 10. *Proc Natl Acad Sci U S A* 2007;**104**:5782–7.

(4) Wu D, Huang Y, Chen Y, Huang YY, Geng H, Zhang T, et al. Optimization of chromeno[2,3-*c*]pyrrol-9(2*H*)-ones as highly potent, selective, and orally bioavailable PDE5 inhibitors: structure–activity relationship, X-ray crystal structure, and pharmacodynamic effect on pulmonary arterial hypertension. J Med Chem 2018;**61**:8468–73.

(5) Huang YY, Yu YF, Zhang C, Chen Y, Zhou Q, Li Z, et al. Validation of phosphodiesterase-10 as a novel target for pulmonary arterial hypertension *via* highly selective and subnanomolar inhibitors. J Med Chem 2019;**62**:3707–21.

(6) Winn MD, Ballard CC, Cowtan KD, et al. Overview of the CCP4 suite and current developments. Acta Crystallogr D Biol Crystallogr 2011;**67**:235–42.

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