Supporting Information

6H-Indolo-[2,3-b]-Quinoxaline derivatives as promising bifunctional SHP1 inhibitors

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Contents

Methods

1. Reagents and instruments

All chemicals were reagent grade and utilized as purchased. The spectra were recorded using a Bruker AVIII 600 MHz spectrometer (Bruker, Billerica, MA, USA) with ^{1}HNMR and ^{13}CNMR techniques. The chemical shifts are reported in parts per million (ppm) using dimethyl sulfoxide-d6 as internal standards. ESI mass spectra (MS) were obtained using a SHIMADZU 2020 and an Agilent 6110 MSD liquid chromatograph mass spectrometer. Thin-layer chromatography (TLC) was performed on glass plates coated with silica gel F254, and the resulting chromatograms were visualized using ultra-violet irradiation. The measurements were conducted using the following instruments: a Shimadzu spectrophotometer (UV-2550) and a Shimadzu spectrophotofluorometer (RF-6000).

2. General procedure for the synthesis of compounds 5a-5e

2.1 General procedure for the synthesis of indoline-2,3-dione derivatives 2a-2e

A glass vial was charged with 6-bromoindoline-2,3-dione (1 g, 4.44 mmol, 1.0 equiv), triethylamine (1 mL), ethynylbenzene derivatives (5.34 mmol, 1.2 equiv), CuI (0.178 mmol, 0.04 equiv), $Pd(pph3)_2Cl_2$ (0.09 mmol, 0.02 equiv), and THF (3 mL). The resulting mixture was placed in a monowave 200 microwave synthesis reactor and stirred at 50 °C for 15 minutes. After completion of the reaction detected by TLC, the resulting solid was washed three times with methanol and dried at room temperature, resulting in the compounds **2a-2e**, which were yellow solids in yields of 76-87% respectively.

2.2 General procedure for synthesis of 6H-indolo[2,3-b]quinoxaline derivatives 3a-3e

To a solution of indoline-2,3-dione derivatives **2a-2e** (400 mg, 1.6 mmol, 1.0 equiv) in a solvent of glacial acetic acid (6 mL) was added benzene 1,2-diamine (1.6 mmol, 1.0 equiv), and then the mixture was stirred at 80 ℃ for 10 min. After completion of the reaction detected by TLC. The precipitated solid was washed three times with methanol, filtered, and dried at room temperature, resulting in the compounds **3a-3e**, which were yellow solids in yields of 52%-56% respectively.

2.3 General procedure for synthesis of ethyl 2-(6H-indolo[2,3-b]quinoxalin-6-yl)acetate 4a-4e

To a solution of compounds **3a-3e** (150 mg, 0.63 mmol, 1.0 equiv) and K_2CO_3 (1.89 mmol, 3 equiv) in DMF (3mL) was added ethyl 2-bromoacetate (0.94 mmol, 1.2 equiv), and then stirred for 1 h at room temperature. After completion of the reaction detected by TLC, the reaction mixture was slowly added to an ice water and stirred for

30 min. Then the resulting solid was filtered, washed with water, and dried at room temperature, resulting in the compounds **4a-4e**, which were yellow solids in yields of 83%-87% respectively.

2.4 General procedure for synthesis of compounds 5a-5e

To a solution of compounds **4a-4e** (50 mg, 0.24 mmol, 1.0 equiv) in a mixed solvent of THF (1 mL) and H_2O (0.5 mL) was added LiOH (1.2 mmol, 5 equiv), and then stirred for 6 h at room temperature. After completion of the reaction detected by TLC, Then the THF was removed under reduced pressure and the residual water layer washed with EtOAc, and acidified to pH = 2 by HCl solution (2 M). Then the resulting solid was filtered, washed with water, and dried at room temperature, resulting in the compounds **5a-5e**, which were yellow solids in yields of 88%-96% respectively.

3. Evaluation of the fluorescence properties of the compounds

3.1 UV-vis and emission spectral experiments

The compounds were analyzed qualitatively and quantitatively using a UV spectrophotometer (UV-2550) and a fluorescence spectrophotometer (RF-6000). The compound was first diluted with DMSO to a solution with a concentration of 1 mM, and then 20 μL of the above solution and 180 μL of DMSO solution were placed in a cuvette, and the sample was placed in a UV spectrophotometer instrument to measure the excitation wavelength. The tested sample was placed in a fluorescence spectrophotometer instrument and the emission wavelength of the sample was measured using the measured excitation wavelength. The fluorescence quantum yield was measured by using a UV spectrophotometer to measure the excitation wavelength when the absorbance of the compound was less than 0.05, and then the fluorescence integral area was obtained by measuring the fluorescence spectrum.

3.2 Theoretical calculations of compounds

SwissADME website was used to predict and obtain the corresponding values of physicochemical properties to explore the influence of different structures on the physicochemical properties of compounds.

3.3 Fluorescence response of compounds to $SHP1^{PTP}$

The assay was performed in 384-well plates at a reaction volume of 15 μL in combination with different concentrations of $SHP1^{PTP}$ protease. The compounds were prepared at a concentration of 20 μM. Recombinant SHP1PTP was included in a typical 15 μL assay mixture, the $SHP1^{PTP}$ were dissolved in HEPES buffer and diluted by a factor of five, down from a starting concentration of 4000 nM. This was in a total of five gradients. Fluorescence signals were quantified using an Envision microplate

reader at an excitation wavelength of 360 nm to assess the impact of varying concentrations of $SHP1^{PTP}$ on the fluorescence intensity of the compounds.

3.4 Confocal cell imaging

A 20 μM solution of the compound in DMSO was prepared and incubated with the MDA-MB-231 cells for 16 hours at 37°C under a 5% CO_2 atmosphere. Following the incubation period, the cells were washed with phosphate-buffered saline (PBS) and fixed in a 4% paraformaldehyde solution for 15 minutes at room temperature. Subsequently, the cells were washed three times with phosphate-buffered saline (PBS). Subsequently, the excitation wavelength was set to 405 nm and 488 nm. The cells were imaged by confocal laser scanning fluorescence microscopy (CLSM, Ti2-E+A1) in order to analyze the imaging data.

4. Evaluation of biological activities of compounds

4.1 Molecular-level activity evaluation based on SHP1 protease

A colorimetric assay was conducted to assess the inhibitory effect of the test compounds against SHP1 in 384-well plates. In brief, the test compounds were solubilized in dimethyl sulfoxide (DMSO) and serially diluted to create a concentration gradient for the subsequent inhibitory assay. Following the solubilization and dilution of the test compounds in room temperature, the enzymatic activities of SHP1 were then determined by the dephosphorylation of the substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), as monitored by a plate reader. Subsequently, quantification of DiFMUP products was performed using the EnVision multilabel plate reader (Perkin-Elmer Life Sciences, Boston, MA, USA). The excitation and emission wavelengths were 355 nm and 460 nm, respectively. The assays were conducted in a final volume of 50 μL, which consisted of 60 mM HEPES, pH 7.2, 75 mM NaCl, 75 mM KCl, 1 mM EDTA, 0.05% Tween-20, 5 mM DTT, the specified concentration of enzyme, and the concentration of the inhibitor under investigation. Subsequently, the substrate was introduced in order to determine the respective Michaelis constants (Km). The linear region of the enzyme response curve represents the initial rate of dephosphorylation, and the inhibitory activity of the compounds was continuously monitored in order to ascertain their efficacy. The dose–response curves for the inhibitors were analysed using control-based, normalised IC_{50} regression curve fitting.

4.2 Reversibility studies of active molecules

A 100-fold concentration of SHP1 protease and a 10-fold IC_{50} concentration of the compound were incubated together for 30 minutes to allow for optimal interaction between the compound and the enzyme. Solvent mixtures of the same concentration of SHP1 protein and the same volume of the compound were employed as controls. Subsequently, the aforementioned mixture was diluted 100-fold and utilized as the reaction solution. The reaction was initiated by the addition of the substrate, and the enzyme-time curve was subsequently examined. The diluted enzyme promotion curves were then compared with the experimental samples.

4.3 Molecular docking studies of active molecules

To elucidate the potential mechanism between ligand and SHP1 protein in molecular level, molecular docking was conducted using Schrodinger software (https://www.schrodinger.com/). Initially, the crystal structure of SHP1 was extracted from the RCSB protein database (PDB ID: 4GS0). Thereafter, 4GS0 was preprocessed using Schrodinger software, with the B chain and ions of 4GS0 being removed, and the structure was optimized. A docking box was generated with residues near the catalytic domain of the protein as the center, with a box size of 20 Å*20 Å*20 Å. Additionally, the molecular structure of ligand was constructed using Chemdraw software, and then the ligand was introduced into Schrodinger software for molecular docking with 4GS0. The docking was performed in standard precision mode using default parameters. Finally, the binding state of the ligand and SHP1 protein was analyzed by the Pymol software. The optimal conformation with the lowest docking score was selected for the result analysis.

4.4 Evaluation of cell proliferation inhibition activity

MV-4-11 was selected to test the effect of compounds on cell proliferation and was cultured in IMDM medium (10% fetal bovine serum). MV-4-11 cells in good growth condition were harvested and, after counting, 1×10^4 cells/well were seeded in 96-well plates, and 20 μM compound was added to the cell suspension at a 5-fold concentration dilution. After 72 hours of incubation, 20 μL MTS solution was added to each well. After incubation for 4 hours, the fluorescence signals of the samples in each well were measured at the wavelengths of 490 nm and 690 nm using the Envision multifunctional enzyme reader.

4.5 Cytotoxicity assays of active molecules

Human peripheral blood mononuclear cells (PBMC) and MDA-MB-231 cells were used to evaluate the cytotoxicity. PBMC were cultured with 1640 medium (1%

penicillin/streptomycin, 20% fetal bovine serum (NBCS)) and maintained at 37 °C in a 5% CO_2 incubator. For the cell viability assay, 96-well microplates were seeded with 1×10^4 cells per well and the compounds were subsequently added at different concentrations. At 72 hours, 30 μL of CTG reagent was added, and after 10 minutes of incubation, cell viability was calculated by recording absorbance using a 96-well plate reader. The cytotoxicity of MDA-MB-231 was evaluated in accordance with the aforementioned methodology.

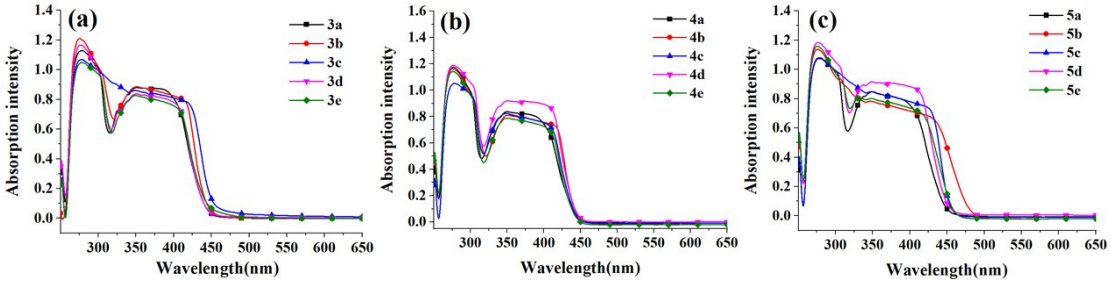


Figure S1. The ultraviolet excitation spectrum of all the studied compounds **3a**-**3e**, **4a**-**4e**, and **5a**-**5e** in DMSO.

As shown in Figure S2, it could be seen that the H in methylene of compound **4a** interacted with the O of the D419 residue through a C-H···O interaction (2.6 Å). The benzene ring in compound **4a** formed a π-alkyl interaction with the alkyl center of residue I279 (5.1 Å), while its quinoline ring also engaged π-cation interactions with the N of residue R358 at distances of 3.5 Å and 4.3 Å.

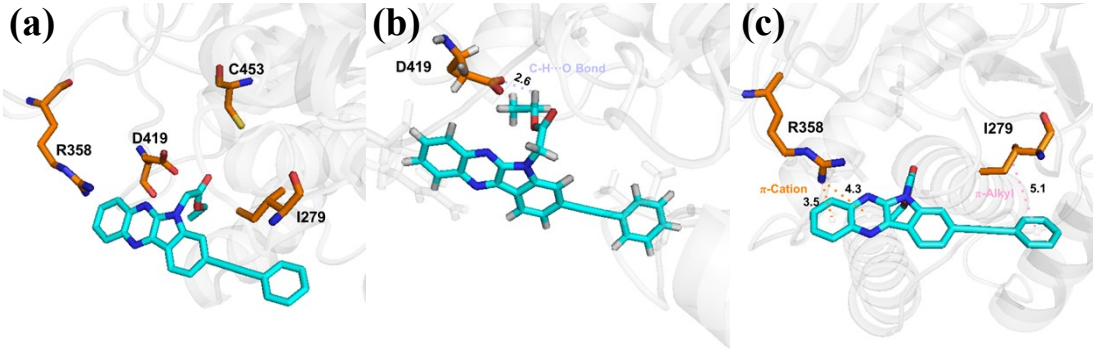


Figure S2. Four hot residues in the vicinity of ligand compound **4a** (a); Interactions between ligand compound **4a** and D419 (b); Interactions between ligand compound **4a** and R358 and I279 (c).

The raw bio-activity data and dose-response curves for compounds **5a-5e** with more than 50% inhibition rate were shown in Table S1, Table S2 and Figure S3, suggesting the dephosphorylation process and the accurate determination of IC_{50} values. During the bio-activity experiments, the signal data was collected 10 times within 5 minutes after the initiation of enzyme reaction. And the amount of product produced by enzyme hydrolysis substrate was increased with the increased time. Finally, we divided the increase of product fluorescence signal by the time to obtain the initial reaction speed. Thus, for avoiding the potential fluorescence interference of fluorescent substance, the spontaneous fluorescence of the initial compound had been effectively eliminated in the bio-activity experiment.

Table S1. The raw bio-activity data of **5a-5e** (with more than 50% inhibition rate) against $SHP1^{PTP}$.

Concentration (μM)	**5a**		**5b**		**5c**		**5d**		**5e**	
2%DMSO	1022317	996393	1082664	1082136	1069515	1044293	1015805	985588	955975	947505
0.07	1001895	1016403	1032551	1055415	1006971	1000845	984461	972170	937560	950286
0.21	929537	907088	1011993	1077889	958986	1034588	905399	925683	956747	904982
0.62	1034693	958574	1043906	996663	1049791	975784	863279	782438	957452	899076
1.85	436168	414244	1018859	989161	897701	851005	680511	606978	917047	906810
5.56	388525	391272	996308	979621	194438	142685	424581	357464	859867	815366
16.67	10145	10857	880484	863530	8132	6177	118125	103240	782616	772738
50.00	-6534	-9534	266174	323806	-5505	-8948	1888	4467	374291	312582

Table S2. The data of **5a-5e** in dose-response curves.

LOG	**5a**		**5b**		**5c**		**5d**		**5e**	
-1.16	98	102	95	98	94	96	97	99	98	100
-0.69	91	91	93	100	90	99	89	94	100	96
-0.21	101	96	96	92	98	93	85	79	100	95
0.27	43	42	94	91	84	81	67	62	96	96
0.74	38	39	92	91	18	14	42	36	90	86
1.22	1	1	81	80	1	1	12	10	82	82
1.70	-1	-1	25	30	-1	-1	0	0	39	33

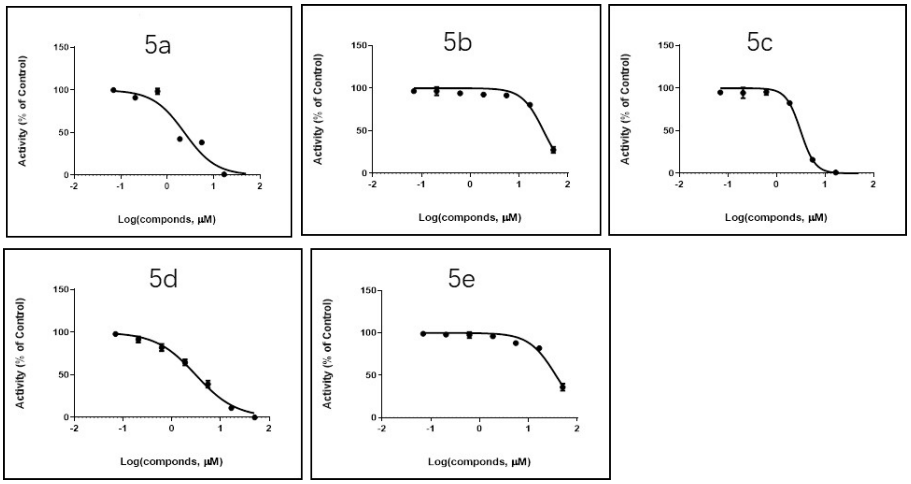


Figure S3. The dose-response curves for the studied compounds **5a-5e**.

Experimental studies

S1. Chemical characterization data of intermediary (3a-3e and 4a-4e) and final compounds (5a-5e).

8-(phenylethynyl)-6H-indolo[2,3-b]quinoxaline

(**3a**): Yellow solid. Yield:54%. m.p.: > 330 °C. IR: 1611, 1592, 1483, 1469, 1400, 1338, 1324, 1246, 1202, 1123, 1106, 1024, 824, 756, 684 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 12.20 (s, 1H), 8.39 (d, J = 8.1 Hz, 1H), 8.26 (dd, J = 8.4, 1.2 Hz, 1H), 8.09 (dd, J = 8.4, 1.2 Hz, 1H), 7.85-7.81 (m, 1H), 7.75 (td, J = 7.5, 6.9, 1.2 Hz, 1H), 7.72 (s, 1H), 7.67-7.63 (m, 2H), 7.53 (dd, J = 8.1, 1.2 Hz, 1H), 7.48 (dd, J = 5.1, 1.8 Hz, 3H); MS (ESI): m/z calcd for $C_{22}H_{14}N_3$ $[M+H]^+$ 320.1, found 320.1.

8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline

(**3b**): Yellow solid. Yield:54%. m.p.: > 330 °C. IR: 1600, 1507, 1398, 1324, 1244, 1202, 1166, 1103, 1023, 822, 756 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 12.17 (s, 1H), 8.36 (d, J = 7.8 Hz, 1H), 8.28-8.24 (m, 1H), 8.10-8.06 (m, 1H), 7.84-7.81 (m, 1H), 7.74 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.68 (s, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.49 (dd, J = 8.1, 1.5 Hz, 1H), 7.05-7.01 (m, 2H), 3.82 (s, 3H); MS (ESI): m/z calcd for $C_{23}H_{16}N_3O$ $[M+H]^+$ 350.1, found 350.1.

8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxaline

(**3c**): Yellow solid. Yield:52%. m.p.: > 330 °C. IR: 1613, 1593, 1469, 1400, 1340, 1325, 1246, 1127, 1106, 1026, 1013, 822, 808, 753, 676 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 12.19 (s, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.83 (t, J = 7.5 Hz, 1H), 7.74 (t, J = 7.5 Hz, 1H), 7.70 (s, 1H), 7.54 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 7.8 Hz, 2H), 2.36 (s, 3H); MS (ESI): m/z calcd for $C_{23}H_{16}N_3$ $[M+H]^+$ 334.1, found 334.0.

8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline

(**3**d): Yellow solid. Yield: 54%. m.p.: > 330 °C. IR: 1612, 1592, 1566, 1467, 1442, 1398, 1324, 1244, 1202, 1126, 1105, 1024, 1011, 822, 752, 686 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 12.18 (s, 1H), 8.37 (d, J = 8.1 Hz, 1H), 8.26 (dd, J = 8.4, 1.2 Hz, 1H), 8.08 (dd, J = 8.4, 1.2 Hz, 1H), 7.83 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.74 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.71-7.67 (m, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.51 (dd, J = 8.1, 1.4 Hz, 1H), 7.31 (d, J = 8.1 Hz, 2H), 2.66 (q, J = 7.5 Hz, 2H), 1.21 (t, J = 7.5 Hz, 3H); MS (ESI): m/z calcd for $C_{24}H_{18}N_3$ $[M+H]^+$ 348.1, found 348.1.

8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline

(**3e**): Yellow solid. Yield: 56%. m.p.: > 330 °C. IR: 1612, 1591, 1399, 1339, 1324, 1246, 1202, 1122, 1106, 1026, 1011, 821, 789, 762 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 12.19 (s, 1H), 8.38 (d, J = 7.8 Hz, 1H), 8.26 (dd, J = 8.4, 1.5 Hz, 1H), 8.09 (dd, J = 8.4, 1.5 Hz, 1H), 7.83 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.75 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.70 (s, 1H), 7.59-7.53 (m, 2H), 7.51 (dd, J = 7.8, 1.2 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 2.63-2.59 (t, J = 7.2 Hz, 2H), 1.64-1.59 (q, J = 7.5 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H); MS (ESI): m/z calcd for $C_{24}H_{20}N_3$ $[M+H]^+$ 362.1, found 362.0.

ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate

(**4a**): Yellow solid. Yield: 87%. m.p.: > 330 °C. IR: 1739, 1611, 1583, 1426, 1372, 1326, 1213,

1194, 1122, 1049, 1020, 855, 750, 685 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.45 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.11 (s, 1H), 7.88 (t, J = 8.1 Hz, 1H), 7.80(t, J = 8.1 Hz, 1H), 7.65 (s, 2H), 7.61 (d, J = 8.1 Hz, 1H), 7.49 (s, 3H), 5.45 (s, 2H), 4.20 (q, J = 6.6 Hz, 2H), 1.24 (t, J = 6.6 Hz, 3H); MS (ESI): m/z calcd for $C_{19}H_{20}N_3O_2$ $[M+H]^+$ 406.1, found 406.0.

ethyl 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate
(**4b**): Yellow solid. Yield: 86%. m.p.: > 330 °C. IR: 1739, 1603, 1582, 1428, 1410, 1372, 1327, 1196, 1169, 1123, 1073, 1032, 857, 833, 792, 755, 692 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.41 (d, J = 8.1 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.04 (s, 1H), 7.85 (t, J = 7.2 Hz, 1H), 7.78 (t, J = 7.2 Hz, 1H), 7.57 (t, J = 7.2 Hz, 3H), 7.04 (d, J = 8.7 Hz, 2H), 5.43 (s, 2H), 4.19 (q, J = 7.2 Hz, 2H), 3.82 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H); MS (ESI): m/z calcd for $C_{27}H_{22}N_3O_3$ $[M+H]^+$ 436.1, found 436.0.

ethyl 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate
(**4c**): Yellow solid. Yield: 84%. m.p.: > 330 °C. IR: 1736, 1611, 1589, 1427, 1369, 1326, 1245, 1196, 1120, 1048, 1019, 853, 814, 756 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.43 (d, J = 7.8 Hz, 1H), 8.30 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 8.1 Hz, 1H), 8.07 (s, 1H), 7.86 (t, J = 7.5 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 7.8 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 5.44 (s, 2H), 4.19 (q, J = 7.2 Hz, 2H), 2.37 (s, 3H), 1.23 (t, J = 7.2 Hz, 3H); MS (ESI): m/z calcd for $C_{27}H_{22}N_3O_2$ $[M+H]^+$ 420.1, found 420.1.

ethyl 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate
(**4d**): Yellow solid. Yield: 86%. m.p.: > 330 °C. IR: 1736, 1612, 1584, 1426, 1407, 1325, 1245, 1213, 1194, 1125, 1023, 830, 753 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.42 (d, J = 8.1 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 7.86 (t, J = 7.5 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 5.44 (s, 2H), 4.19 (q, J = 7.2 Hz, 2H), 2.67 (q, J = 7.8 Hz, 2H), 1.23 (t, J = 6.0 Hz, 3H), 1.21 (t, J = 6.6 Hz, 3H); MS (ESI): m/z calcd for $C_{28}H_{24}N_3O_2$ $[M+H]^+$ 434.1, found 434.1.

ethyl 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate
(**4e**): Yellow solid. Yield: 83%. m.p.: > 330 °C. IR: 1736, 1611, 1586, 1426, 1409, 1371, 1322, 1198, 1123, 1048, 1019, 862, 819, 766, 754 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.42 (d, J = 8.1 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 7.86 (t, J = 7.5 Hz, 1H), 7.80-7.77 (m, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 7.8 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 5.44 (s, 2H), 4.19 (q, J = 6.9 Hz, 2H), 2.61 (t, J = 7.5 Hz, 2H), 1.62 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 6.9 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H); MS (ESI): m/z calcd for $C_{29}H_{26}N_3O_2$ $[M+H]^+$ 448.1, found 448.1.

2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid
(**5a**): Yellow solid. Yield: 96%. m.p.: 317-319 °C. IR: 1611, 1584, 1428, 1326, 1197, 1127, 821, 753, 687 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.42 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 9.3 Hz, 1H), 8.13 (d, J = 9.3 Hz, 1H), 8.08 (s, 1H), 7.87-7.84 (m, 1H), 7.78 (td, J = 7.8, 7.0, 1.3 Hz, 1H), 7.65 (d, J = 4.2 Hz, 1H), 7.64 (d, J = 1.8 Hz, 1H), 7.59 (dd, J = 7.8, 1.2 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H), 7.48 (d, J = 1.8 Hz, 2H), 5.34 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 169.56, 145.55, 144.35, 139.87, 139.20, 138.95, 131.52 , 129.53, 129.19, 128.88, 128.72, 127.54, 126.68, 124.76, 124.54,

122.40, 121.95, 118.69, 113.54, 91.45, 89.87, 42.59; MS (ESI): m/z calcd for $C_{24}H_{14}N_3O_2$ $[M-H]^-$ 376.1, found 376.0.

2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid
(**5b**): Yellow solid. Yield: 90%. m.p.: 325-326 °C. IR: 1602, 1429, 1398, 1321, 1244, 1198, 1128, 1026, 825, 757, 697 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.36 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.82-7.79 (m, 1H), 7.74-7.70 (m, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.50-7.47 (m, 1H), 7.01 (d, J = 8.7 Hz, 2H), 4.90 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 168.59, 159.78, 145.72, 145.10, 140.03, 139.16, 138.81, 133.16, 129.04, 127.47, 126.00, 124.85, 123.48, 122.28, 122.05, 118.09, 114.46, 113.95, 113.51, 91.50, 88.82, 67.00, 55.30; MS (ESI): m/z calcd for $C_{25}H_{18}N_3O_3$ $[M+H]^+$ 408.1, found 408.0.

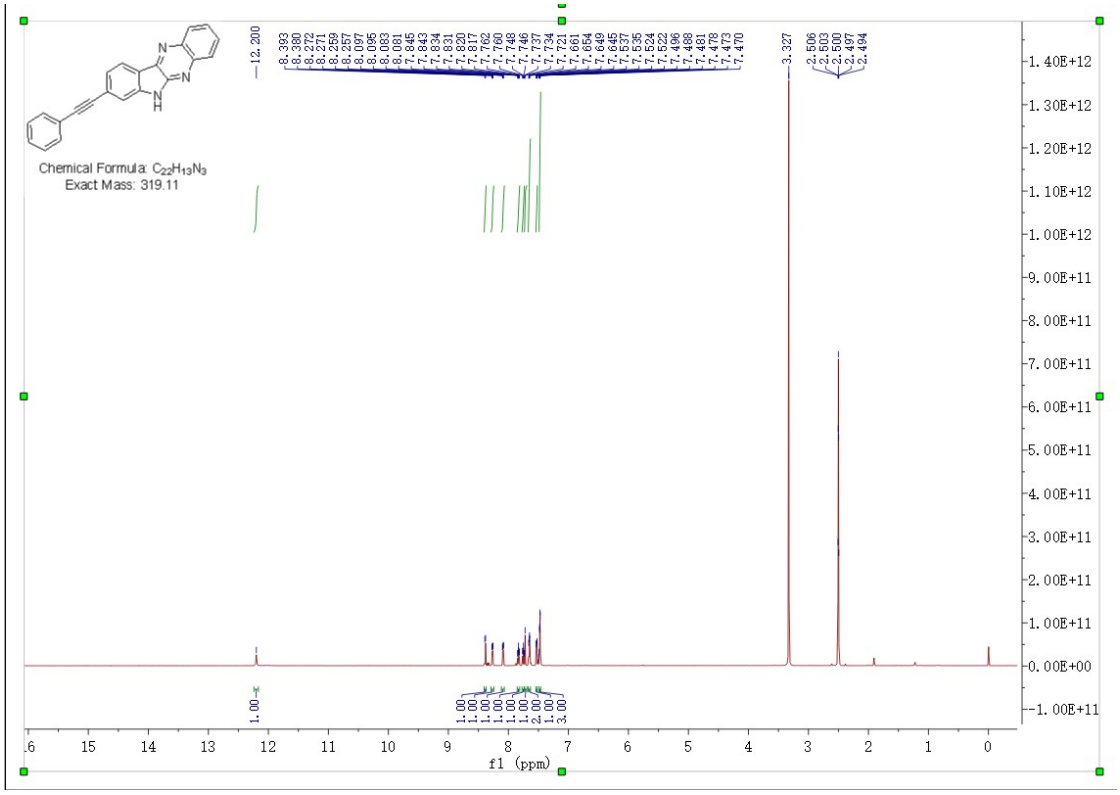
2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid
(**5c**): Yellow solid. Yield: 96%. m.p.: 315-317 °C. R: 1610, 1583, 1428, 1326, 1193, 1126, 867, 812, 751 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.41 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 9.3 Hz, 1H), 8.13 (d, J = 9.3 Hz, 1H), 8.05 (s, 1H), 7.87-7.84 (m, 1H), 7.79-7.76 (m, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.53 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 5.33 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 169.56, 145.55, 144.37, 139.85, 139.20, 139.08, 138.98, 131.44, 129.50, 129.17, 127.54, 126.86, 126.66, 125.00, 124.47, 122.38, 118.93, 118.54, 113.44, 91.75, 89.35, 42.57, 21.09; MS (ESI): m/z calcd for $C_{25}H_{16}N_3O_2$ $[M-H]^-$ 390.1, found 390.0.

2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid
(**5d**): Yellow solid. Yield: 96%. m.p.: 309-311 °C. IR: 1603, 1408, 1325, 1286, 1243, 1191, 1123, 1023, 817, 751cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.41 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 8.03 (s, 1H), 7.85 (t, J = 7.5 Hz, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.56 (t, J = 8.1 Hz, 3H), 7.31 (d, J = 7.8 Hz, 2H), 5.31 (s, 2H), 2.66 (q, J = 7.5 Hz, 2H), 1.21 (t, J = 7.5 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 169.58, 145.57, 145.21, 144.43, 139.86, 139.17, 138.99, 131.54, 129.47, 129.17, 128.31, 127.54, 126.61, 124.97, 124.42, 122.35, 119.19, 118.52, 113.45, 91.73, 89.36, 42.76, 28.10, 15.22; MS (ESI): m/z calcd for $C_{26}H_{20}N_3O_2$ $[M+H]^+$ 406.1, found 406.0.

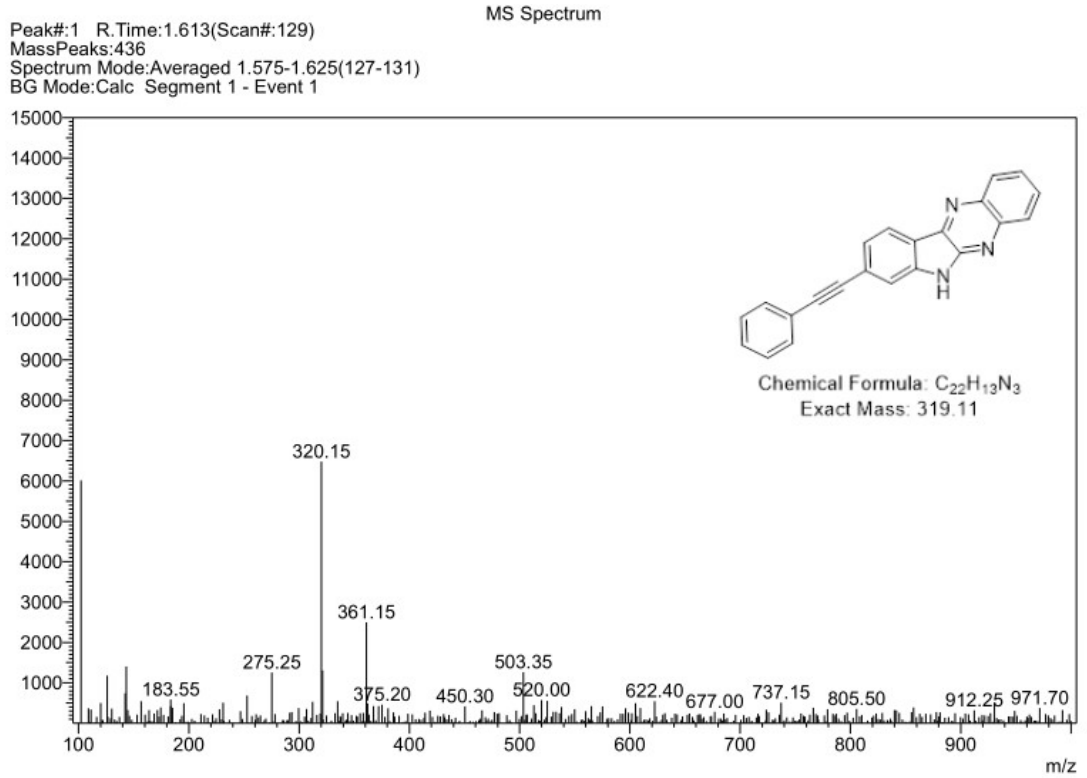
2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid
(**5e**): Yellow solid. Yield: 88%. m.p.: 320-322 °C. IR: 1610, 1582, 1427, 1325, 1243, 1195, 1126, 1049, 826, 752cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ: 8.38 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 8.10 (d, J = 9.0 Hz, 1H), 7.92 (s, 1H), 7.82 (t, J = 8.1 Hz, 1H), 7.74 (t, J = 8.1 Hz, 1H), 7.55-7.50 (m, 3H), 7.26 (d, J = 8.1 Hz, 2H), 5.20 (s, 2H), 2.59 (t, J = 7.5 Hz, 2H), 1.61 (q, J = 7.5 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.89, 145.60, 144.56, 143.55, 139.90, 139.07, 139.01, 131.43, 129.31, 129.12, 128.82, 127.51, 126.42, 124.86, 124.19, 122.26, 119.24, 118.46, 113.45, 91.63, 89.41, 40.04, 37.09, 23.76, 13.58; MS (ESI): m/z calcd for $C_{27}H_{22}N_3O_2$ $[M+H]^+$ 420.1, found 420.1.

S2. ^{1}H NMR spectra、^{13}C NMR spectra and MS spectra of intermediary and final compounds.

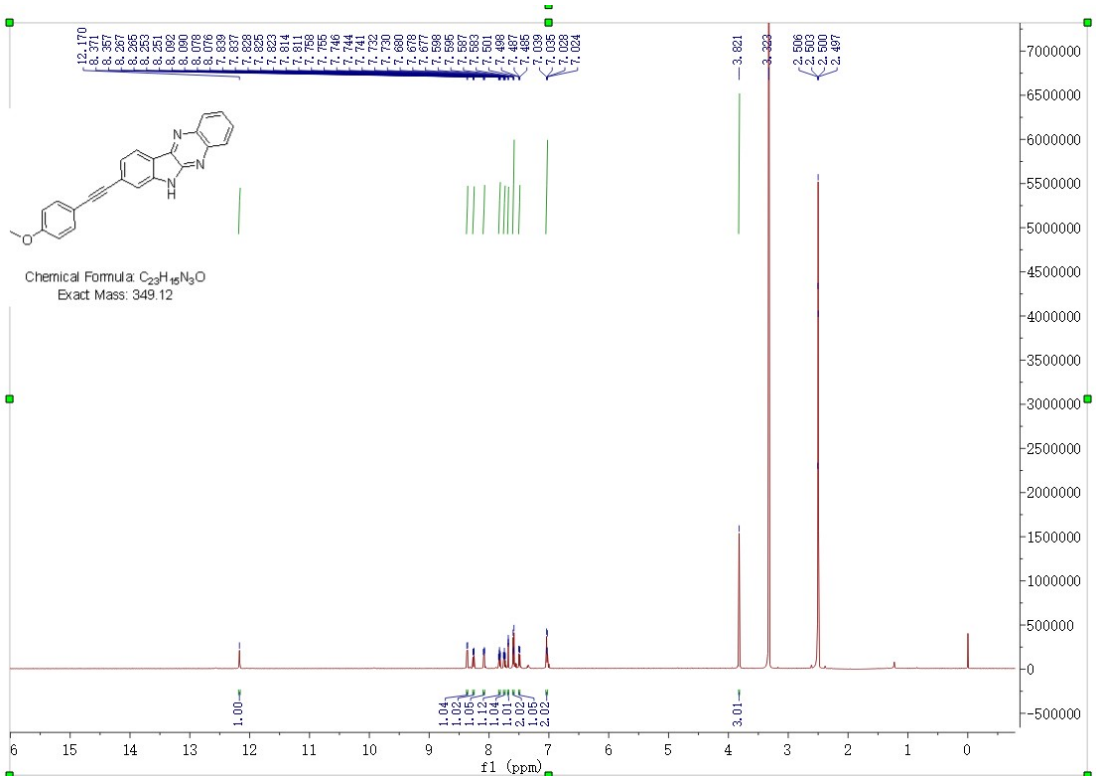
^{1}H NMR spectrum of 8-(phenylethynyl)-6H-indolo[2,3-b]quinoxaline (**3a**)



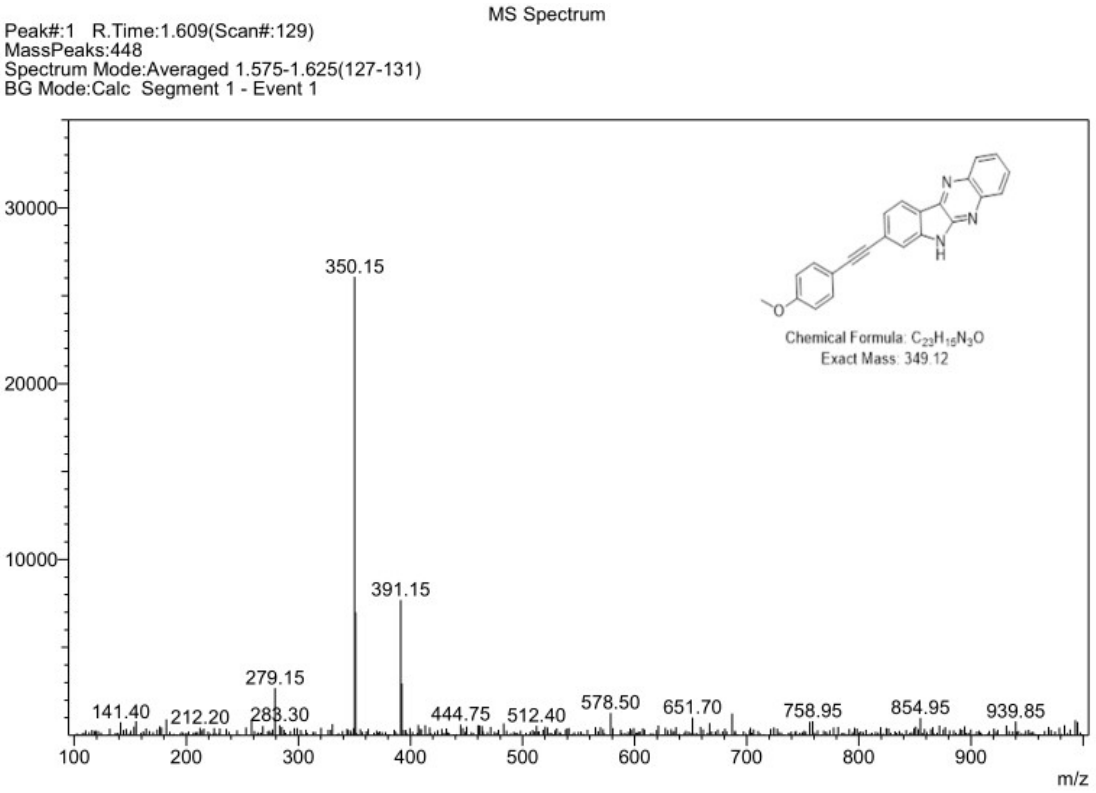
MS spectrum of 8-(phenylethynyl)-6H-indolo[2,3-b]quinoxaline (**3a**)



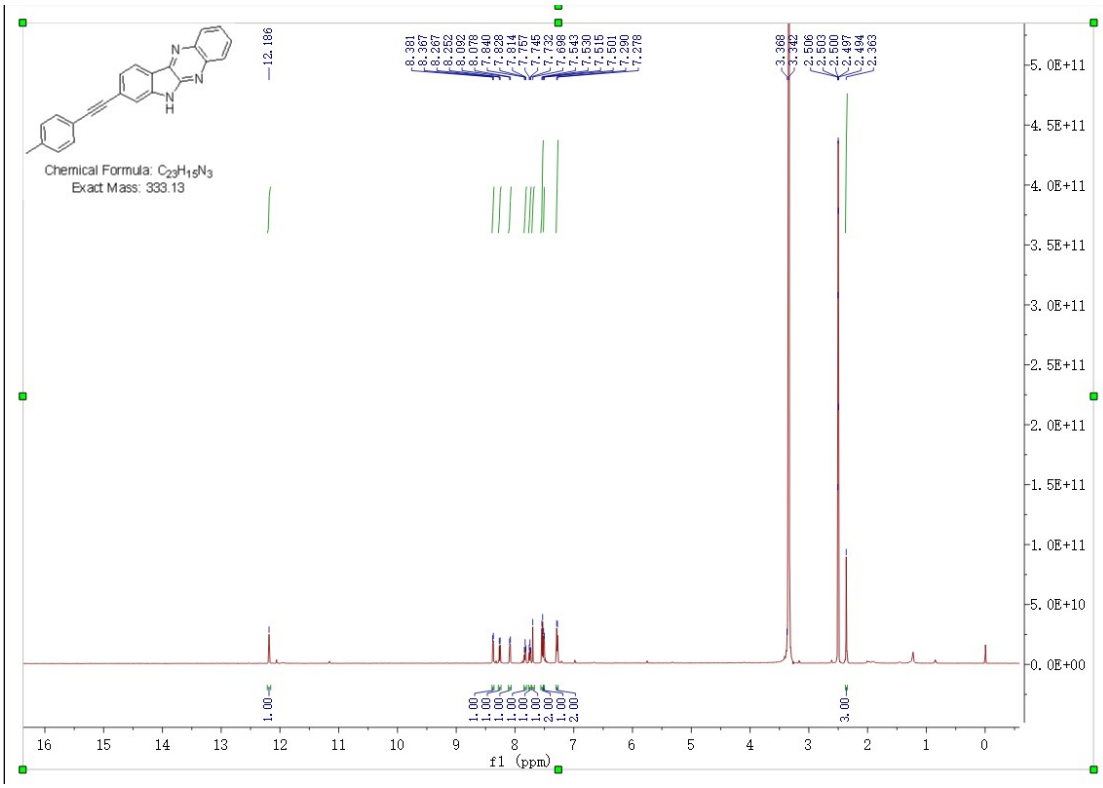
^{1}H NMR spectrum of 8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3b**)



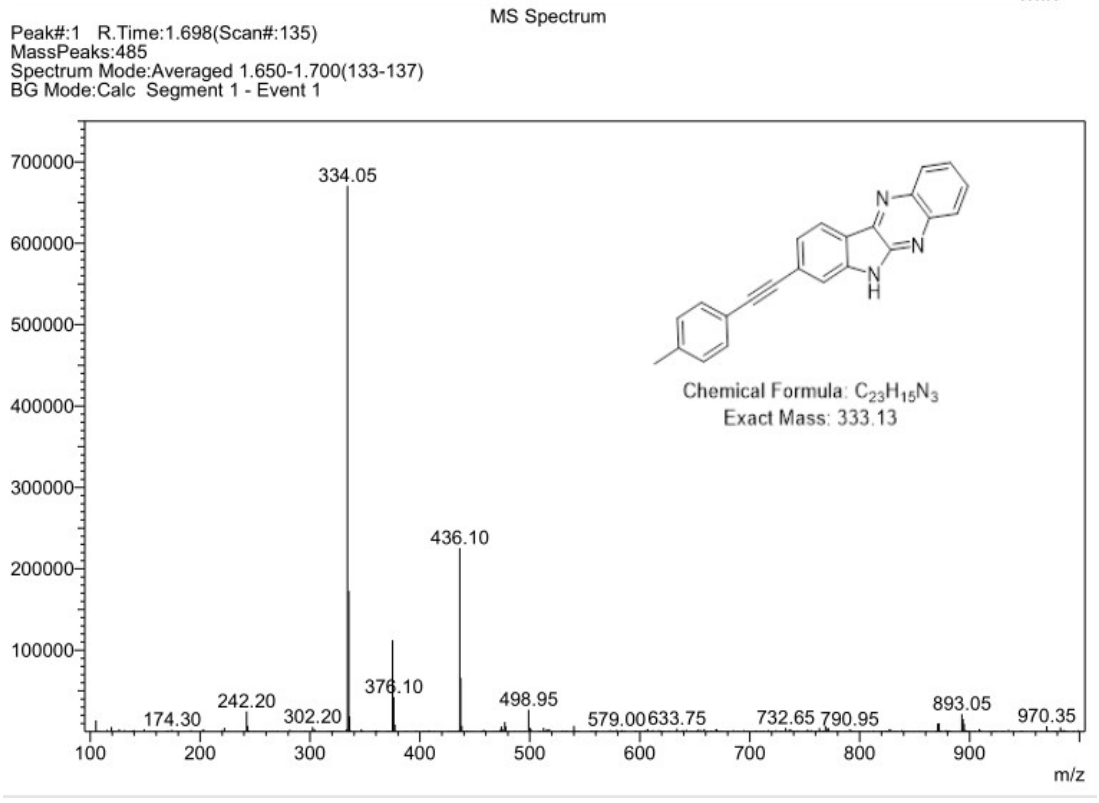
MS spectrum of 8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3b**)



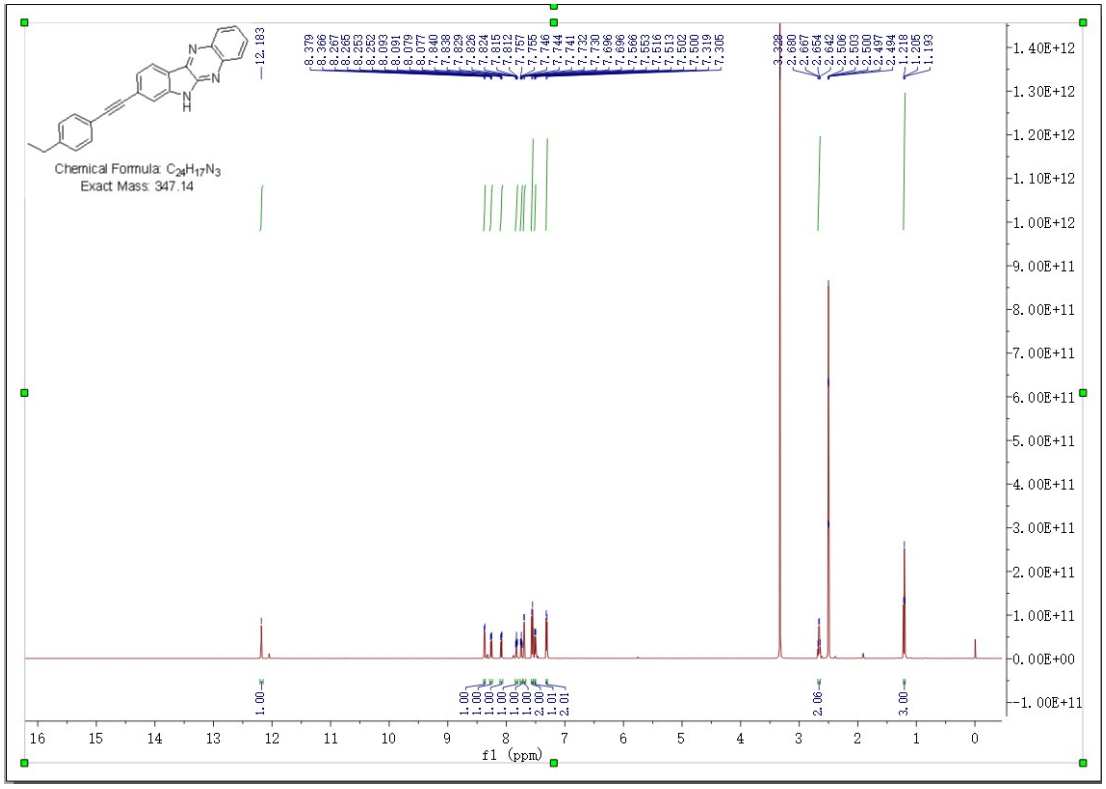
^{1}H NMR spectrum of 8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxaline (**3c**)



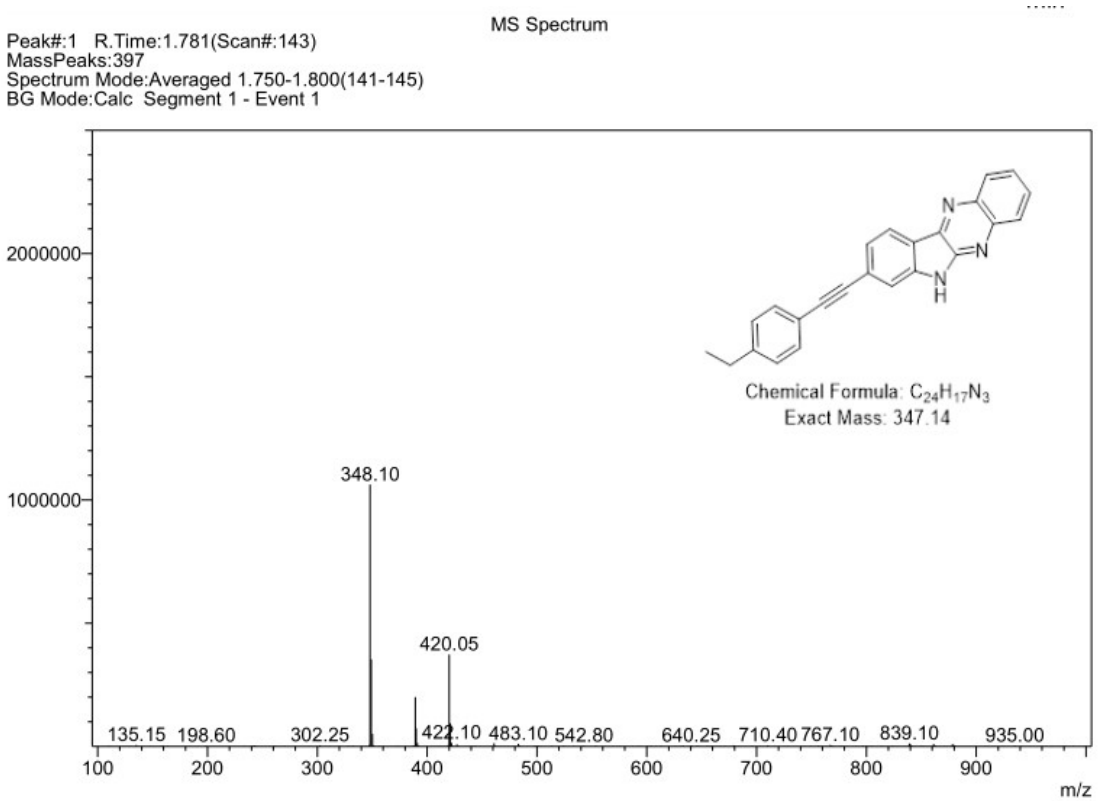
MS spectrum of 8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxaline (**3c**)



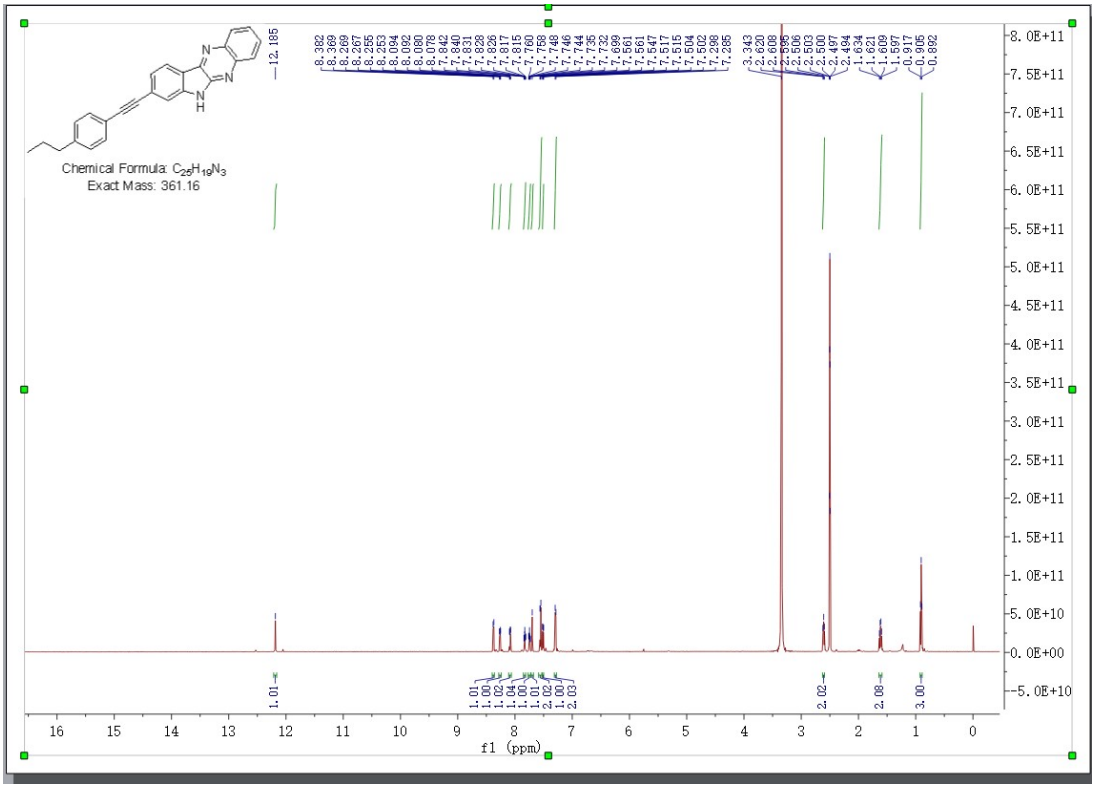
^{1}H NMR spectrum of 8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3d**)



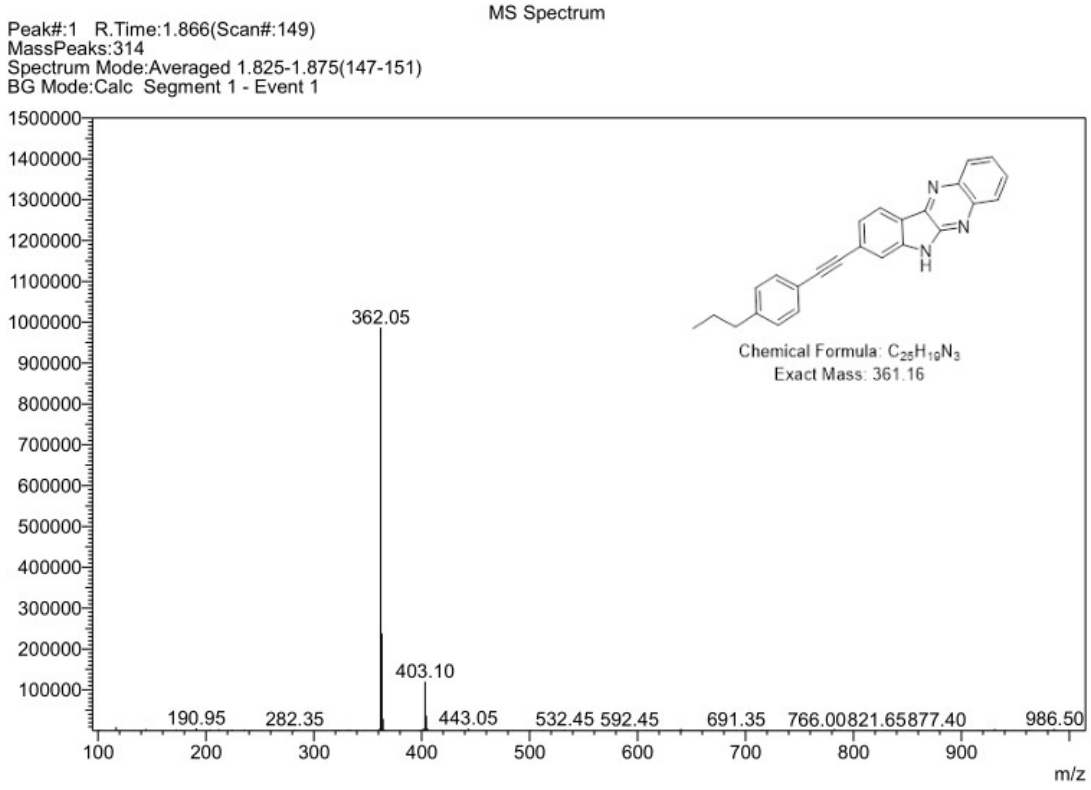
MS spectrum of 8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3d**)



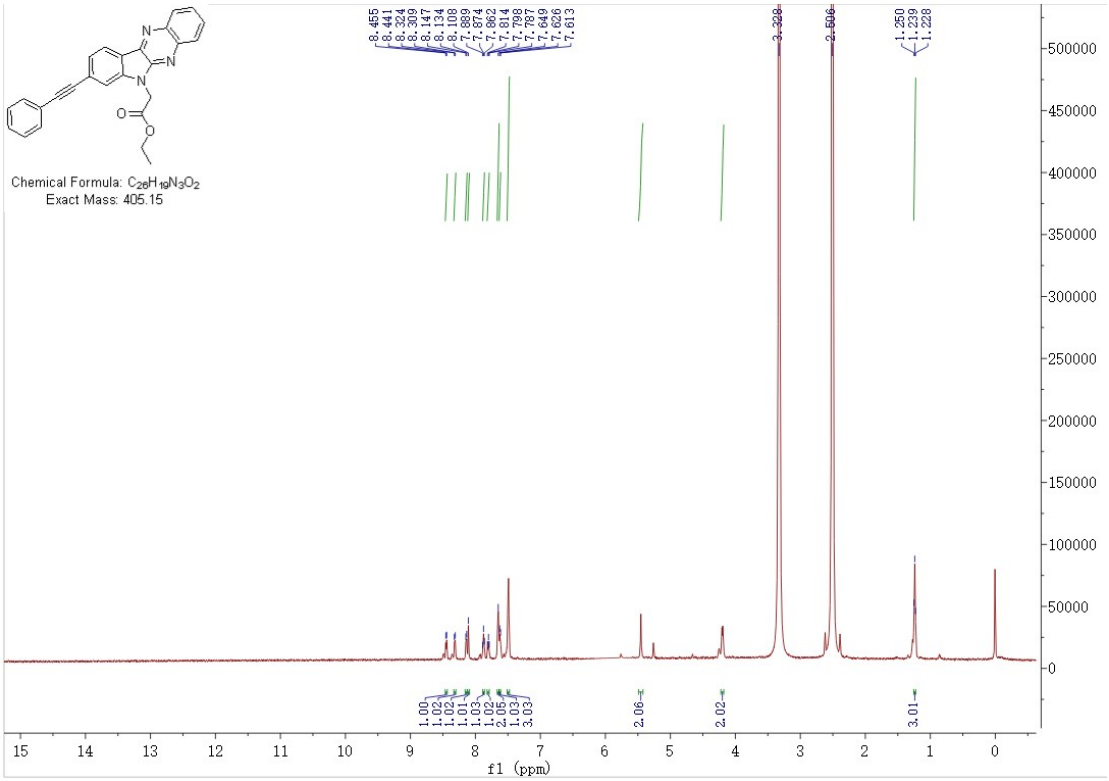
^{1}H NMR spectrum of 8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3e**)



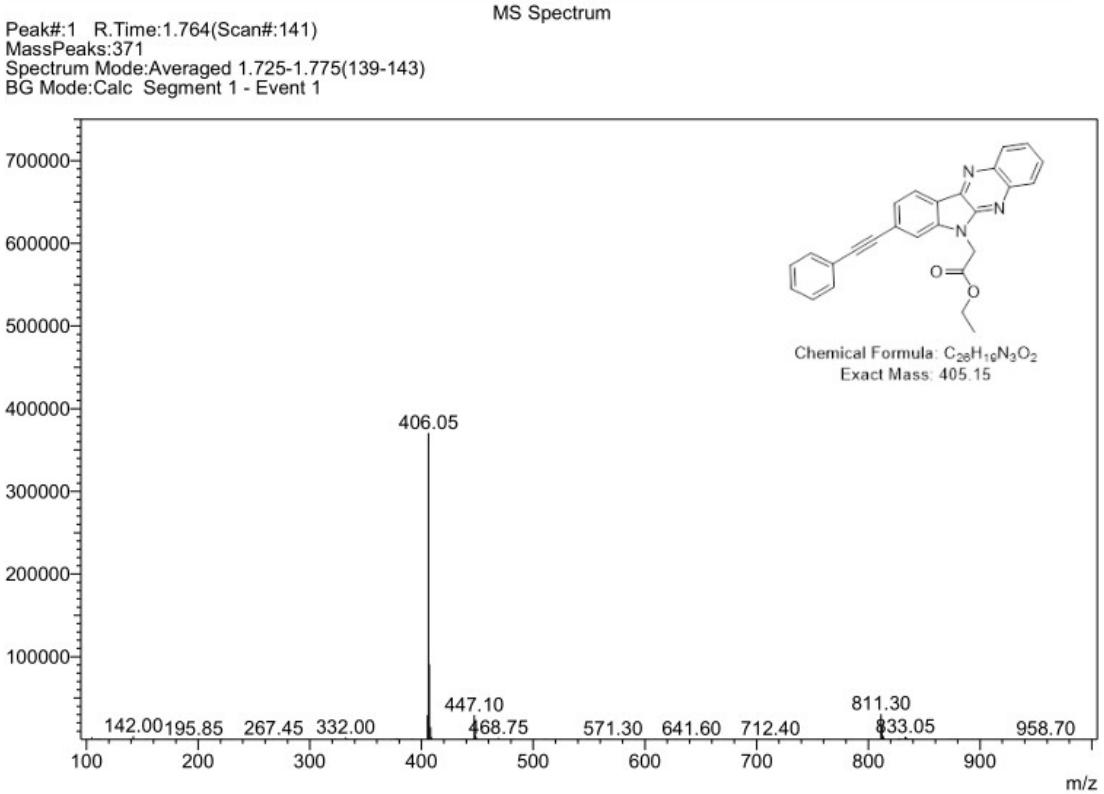
MS spectrum of 8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxaline (**3e**)



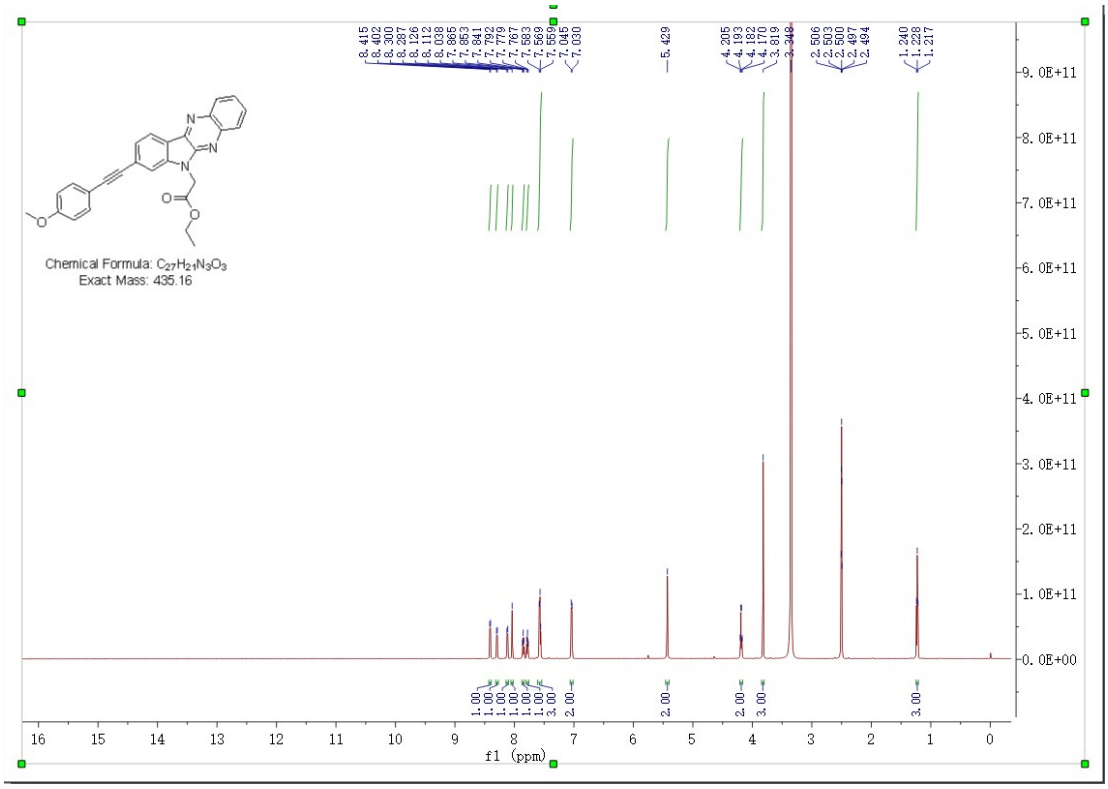
^{1}H NMR spectrum of ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4a**)



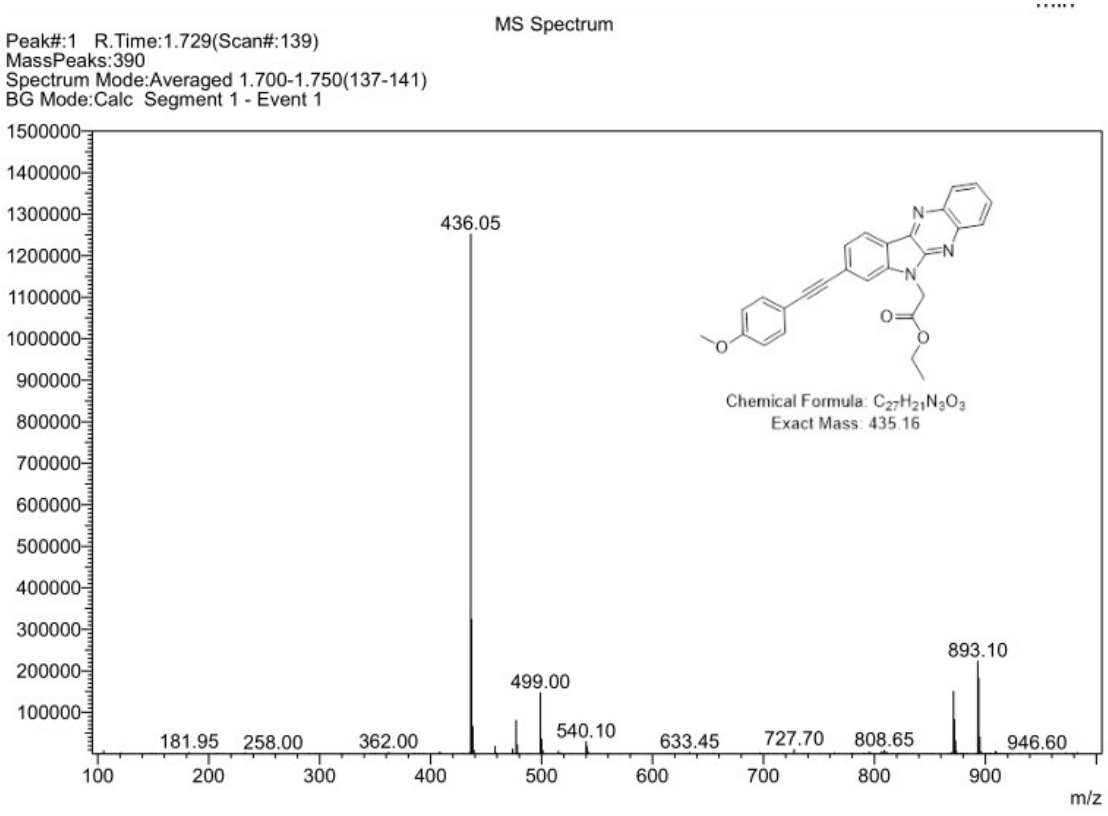
MS spectrum of ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4a**)



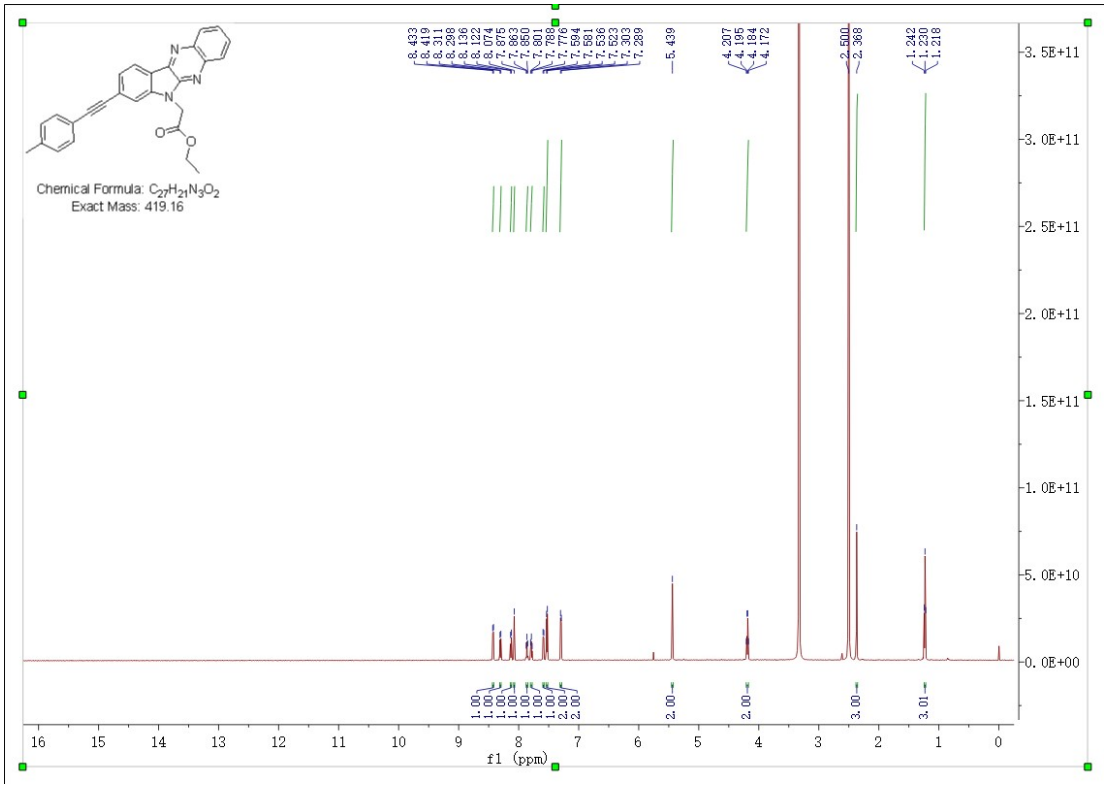
^{1}H NMR spectrum of ethyl 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl) acetate (**4b**)



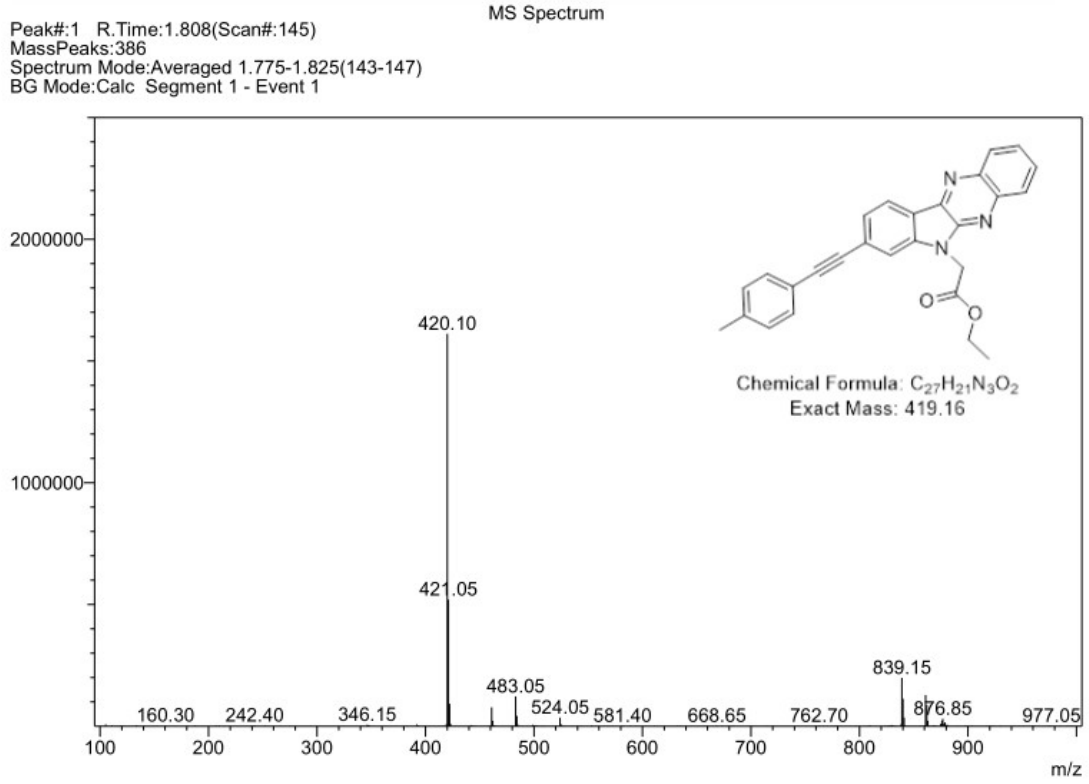
MS spectrum of ethyl 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4b**)



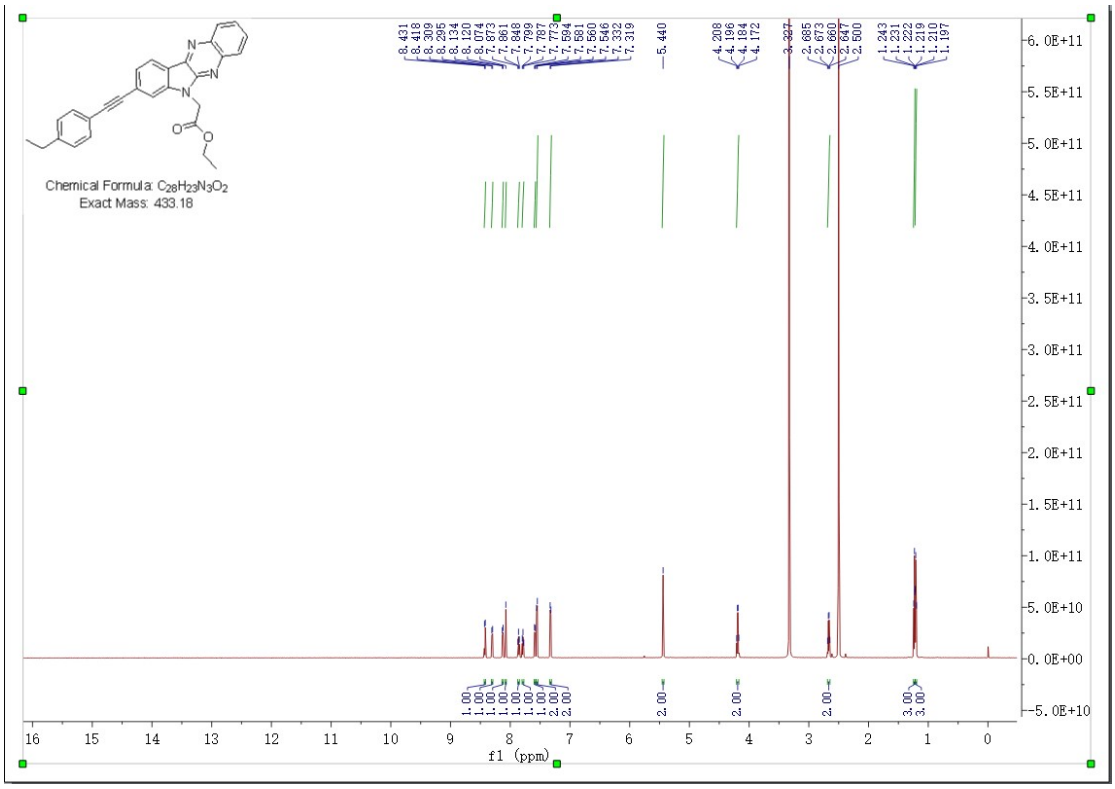
^{1}H NMR spectrum of ethyl 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4c**)



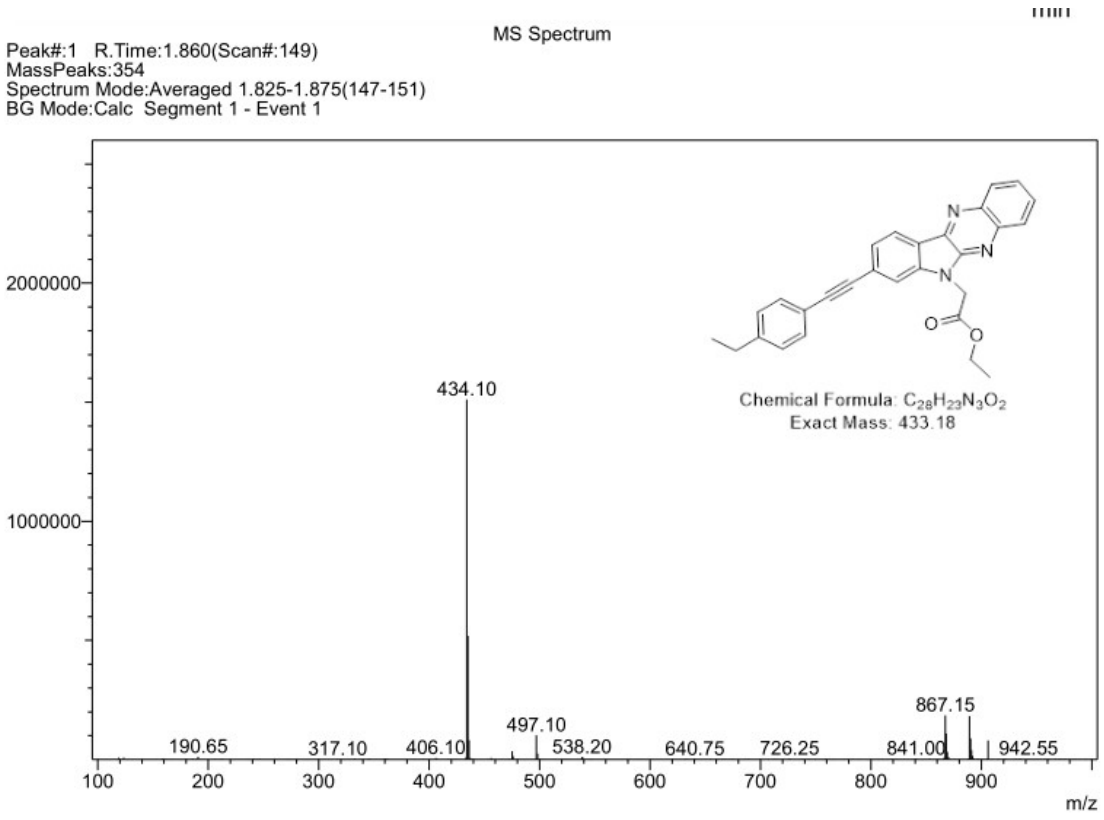
MS spectrum of ethyl 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4c**)



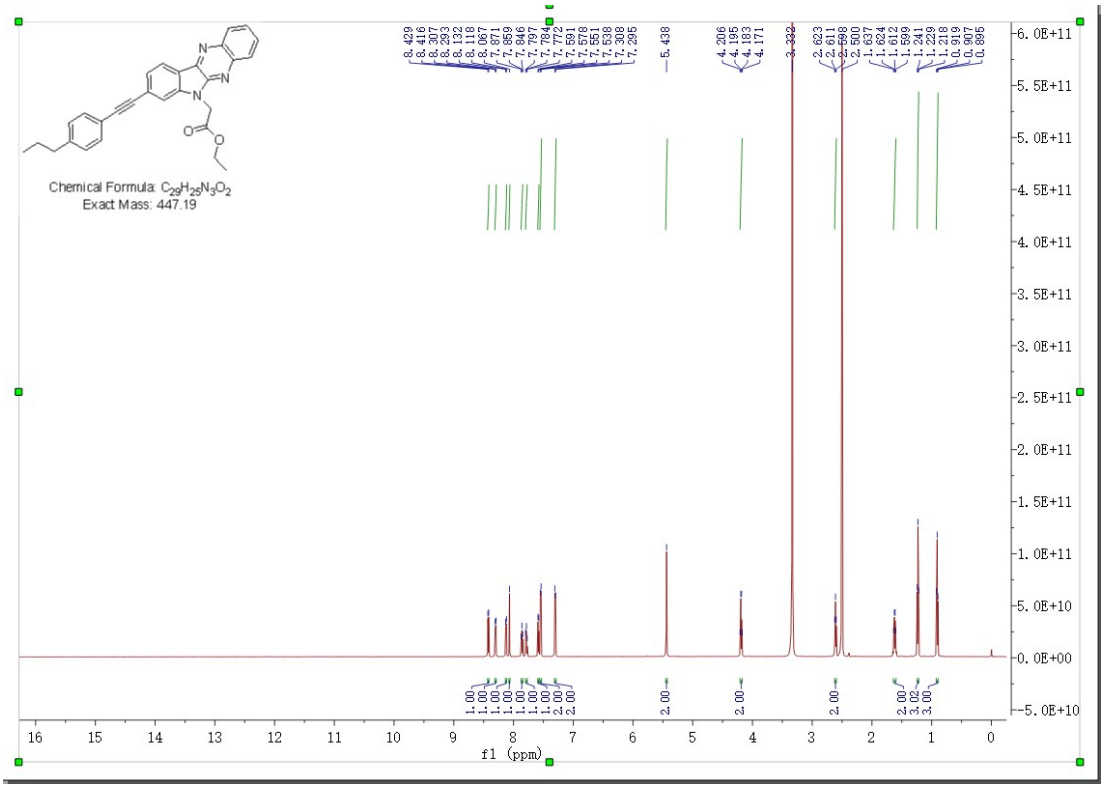
^{1}H NMR spectrum of ethyl 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4d**)



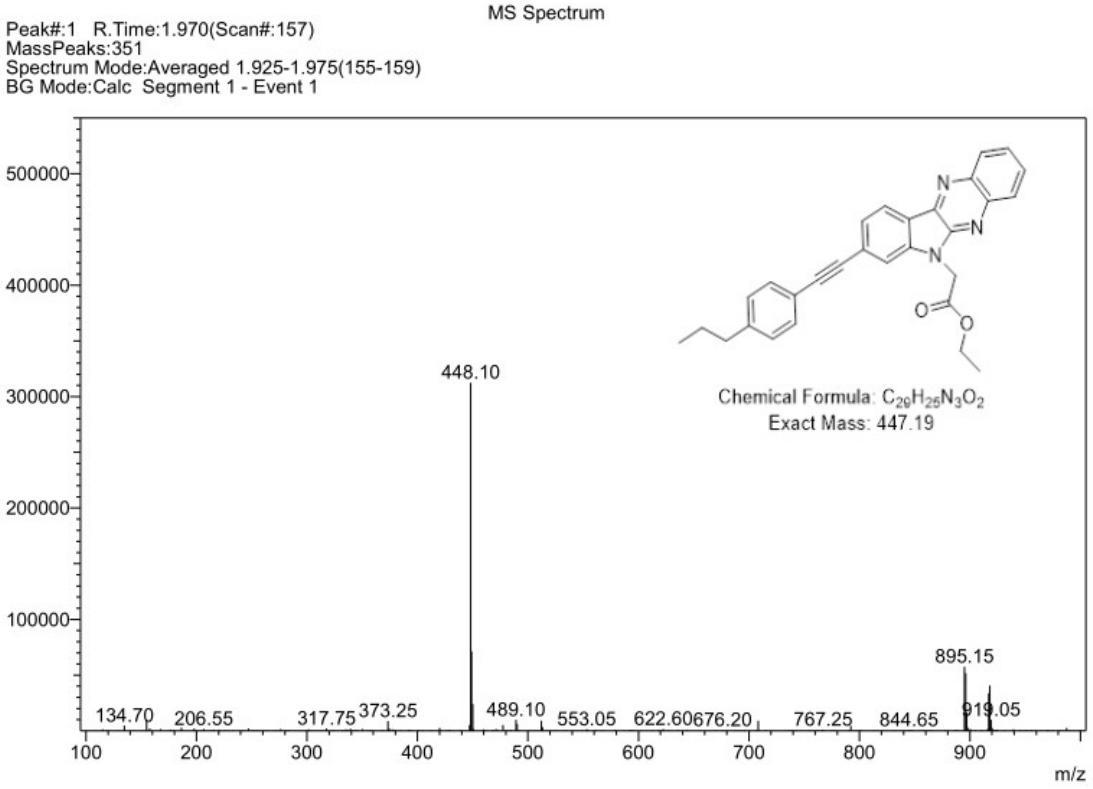
MS spectrum of ethyl 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4d**)



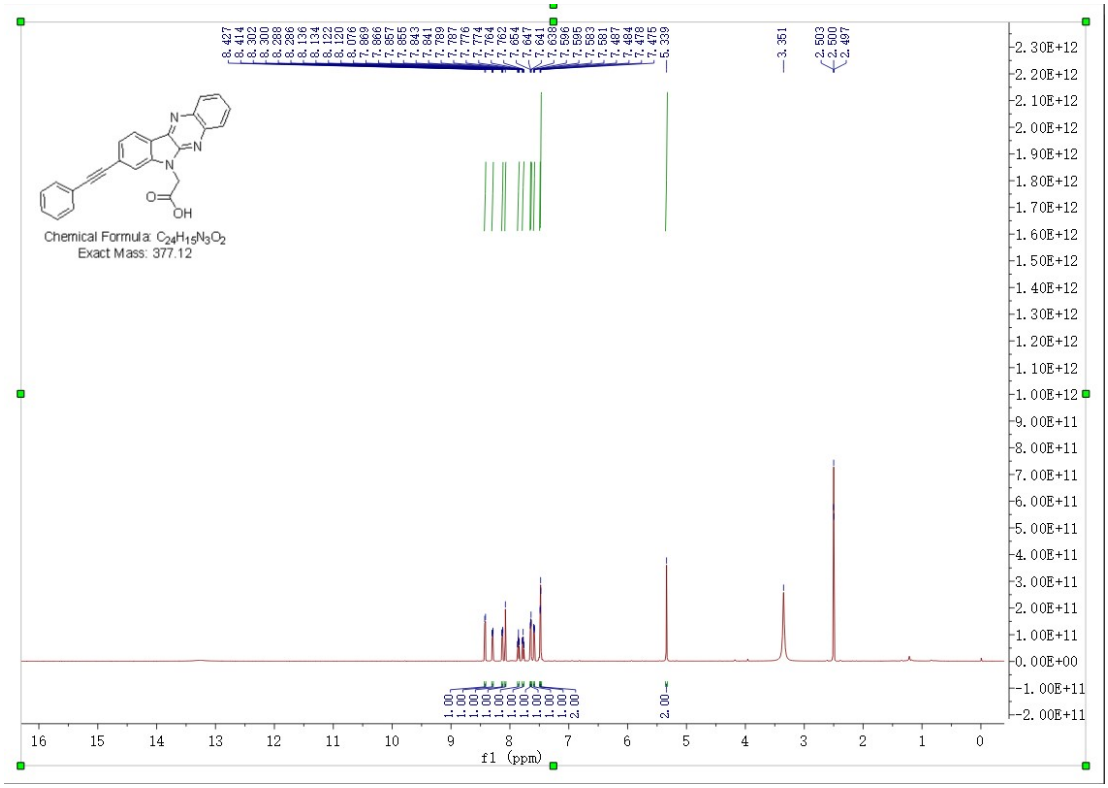
^{1}H NMR spectrum of ethyl 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl) acetate (**4e**)



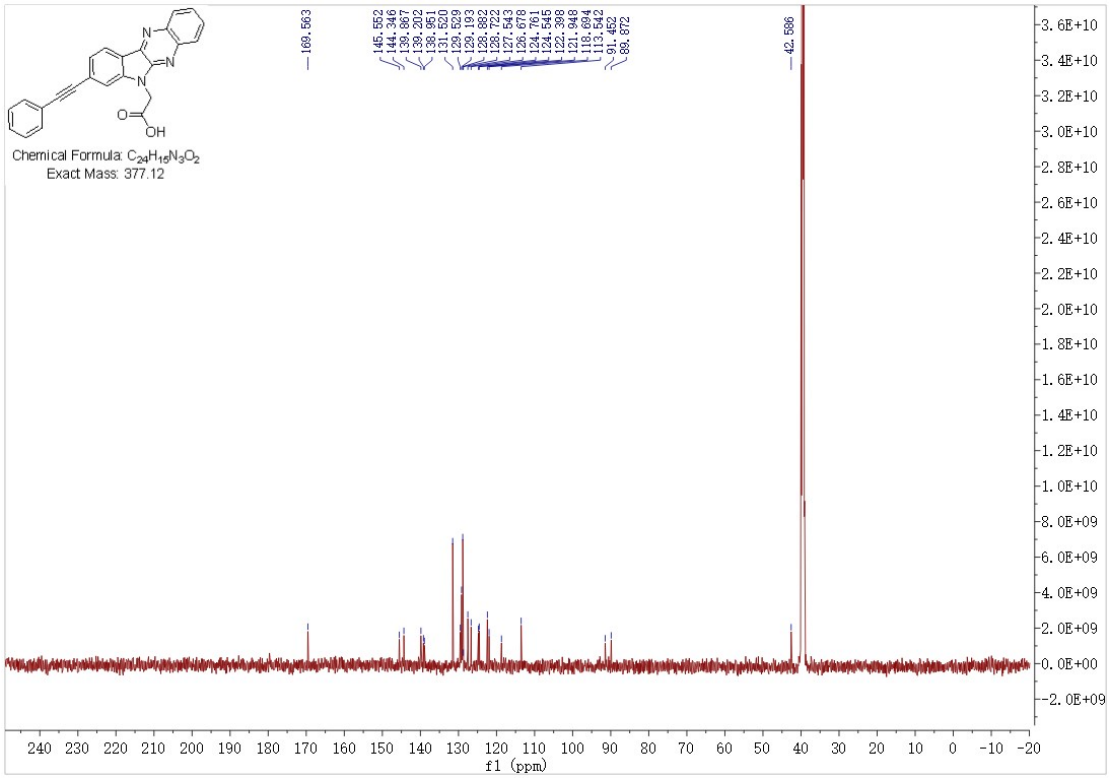
MS spectrum of ethyl 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetate (**4e**)



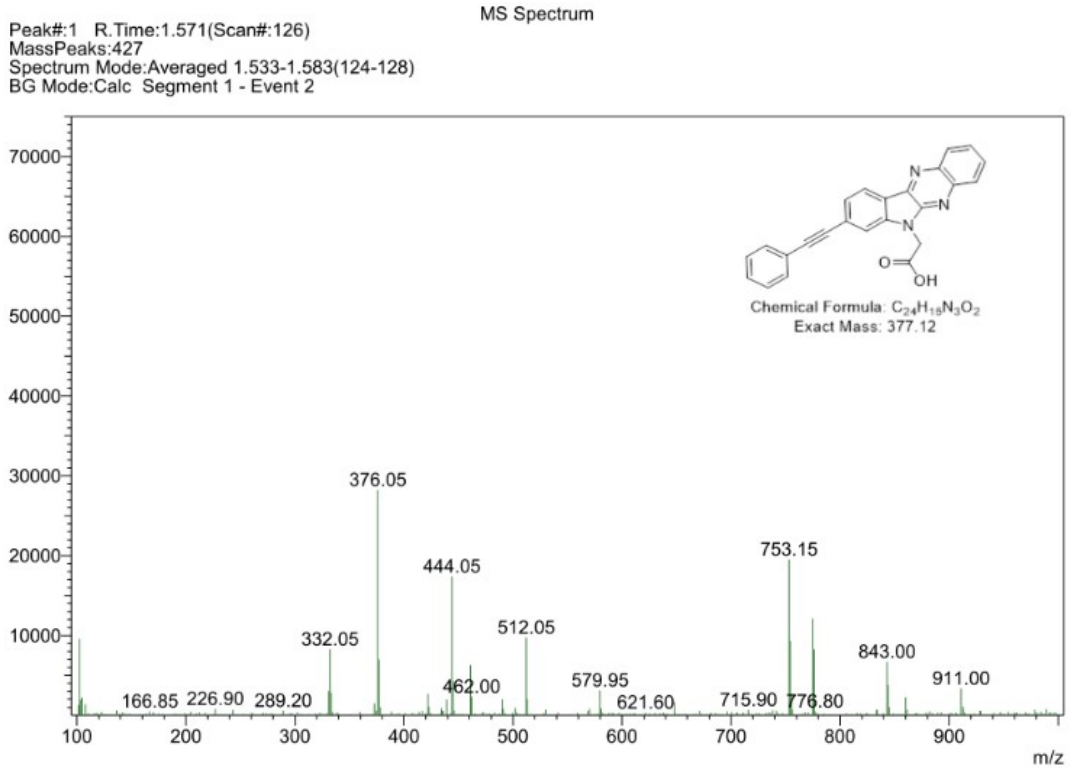
^{1}H NMR spectrum of ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5a**)



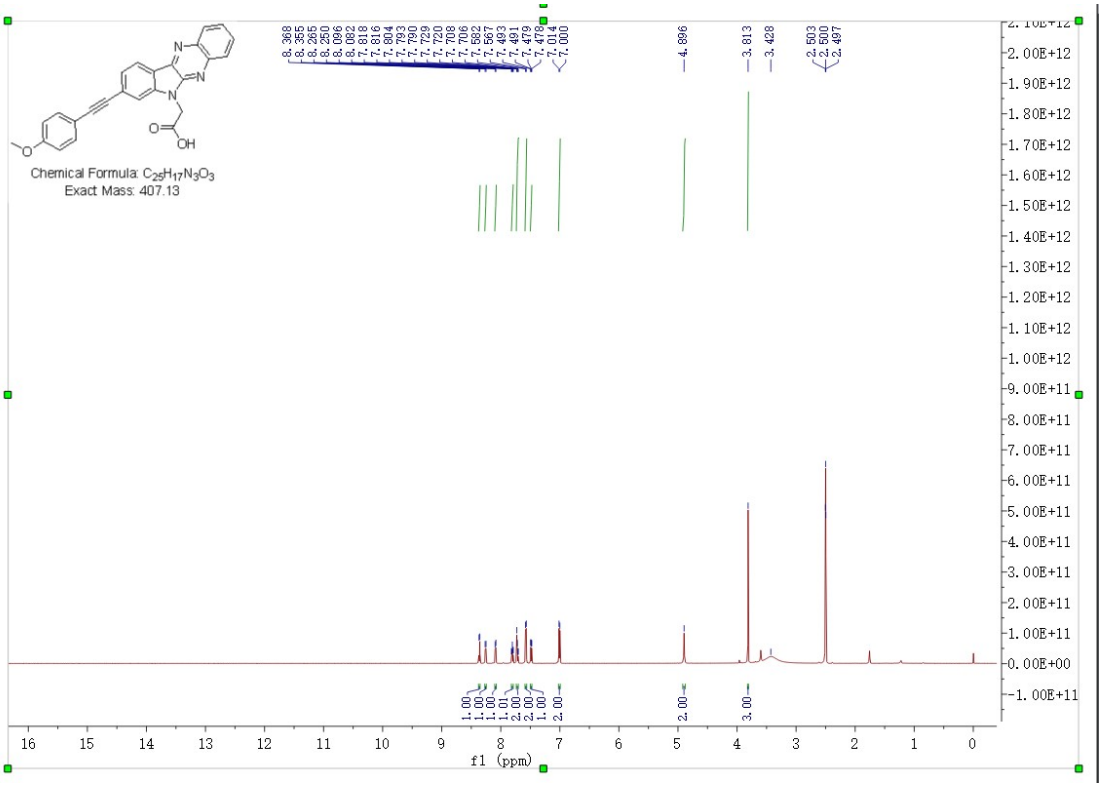
^{13}C NMR spectrum of ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5a**)



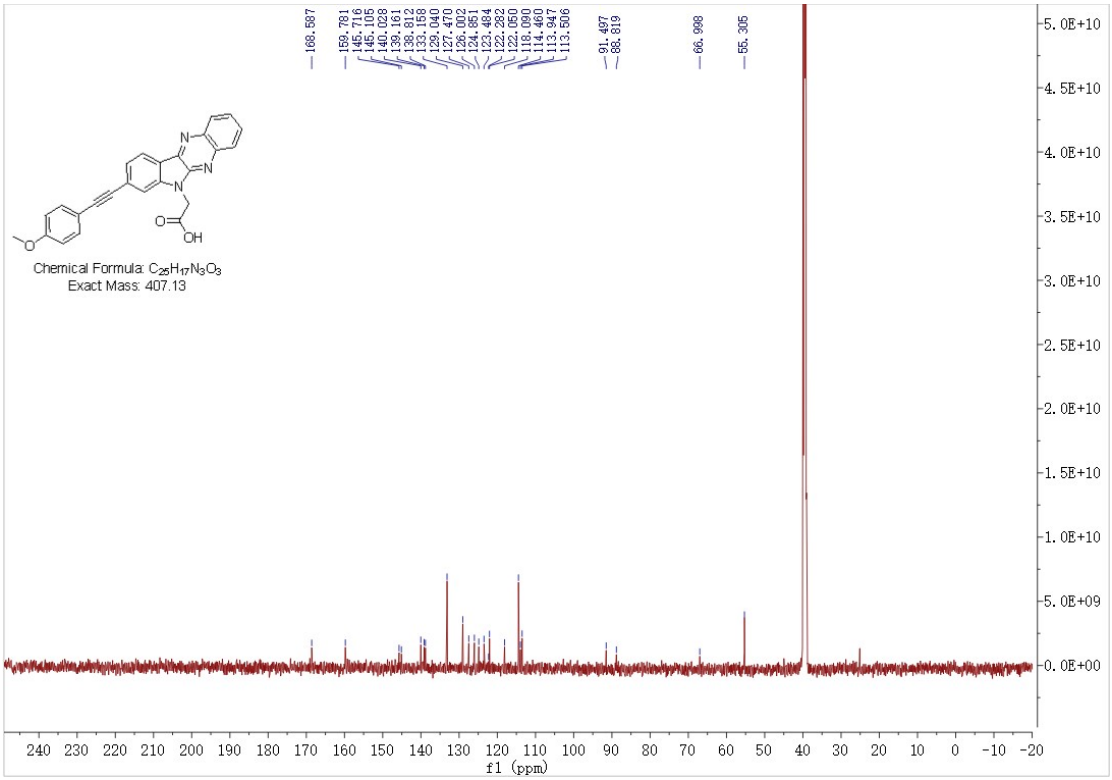
MS spectrum of ethyl 2-(8-(phenylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5a**)



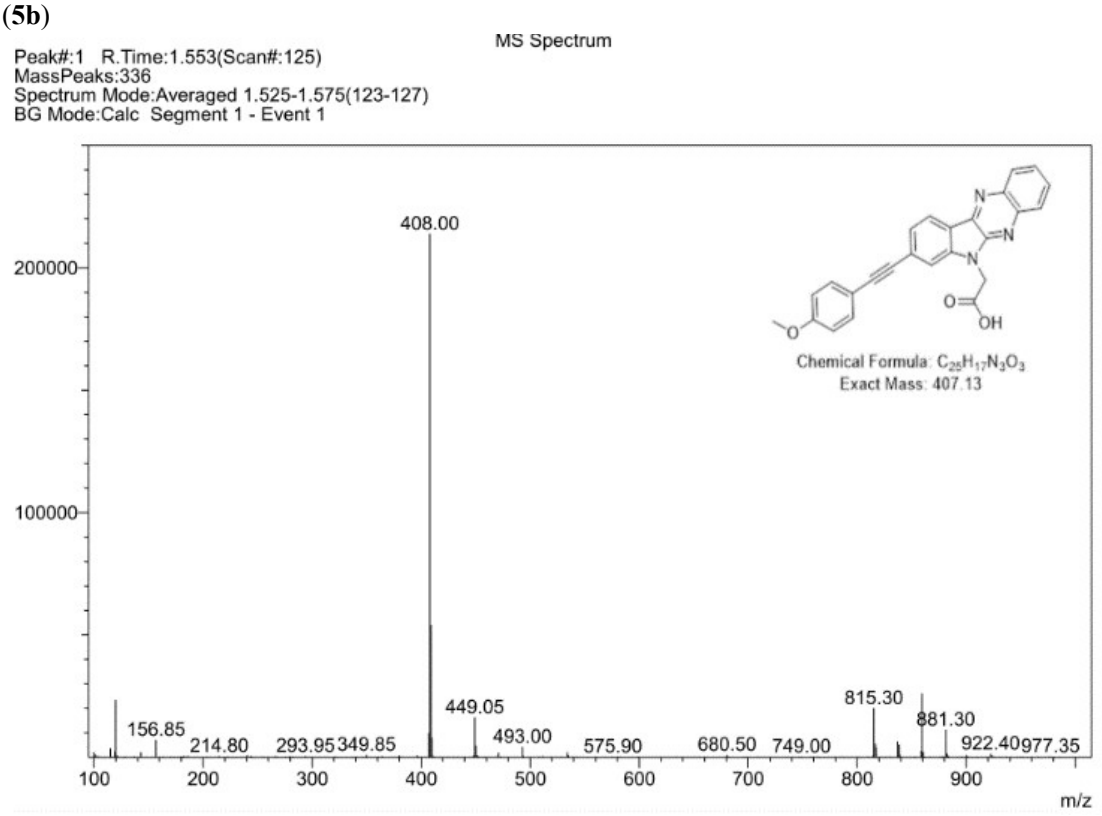
^{1}H NMR spectrum of 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5b**)



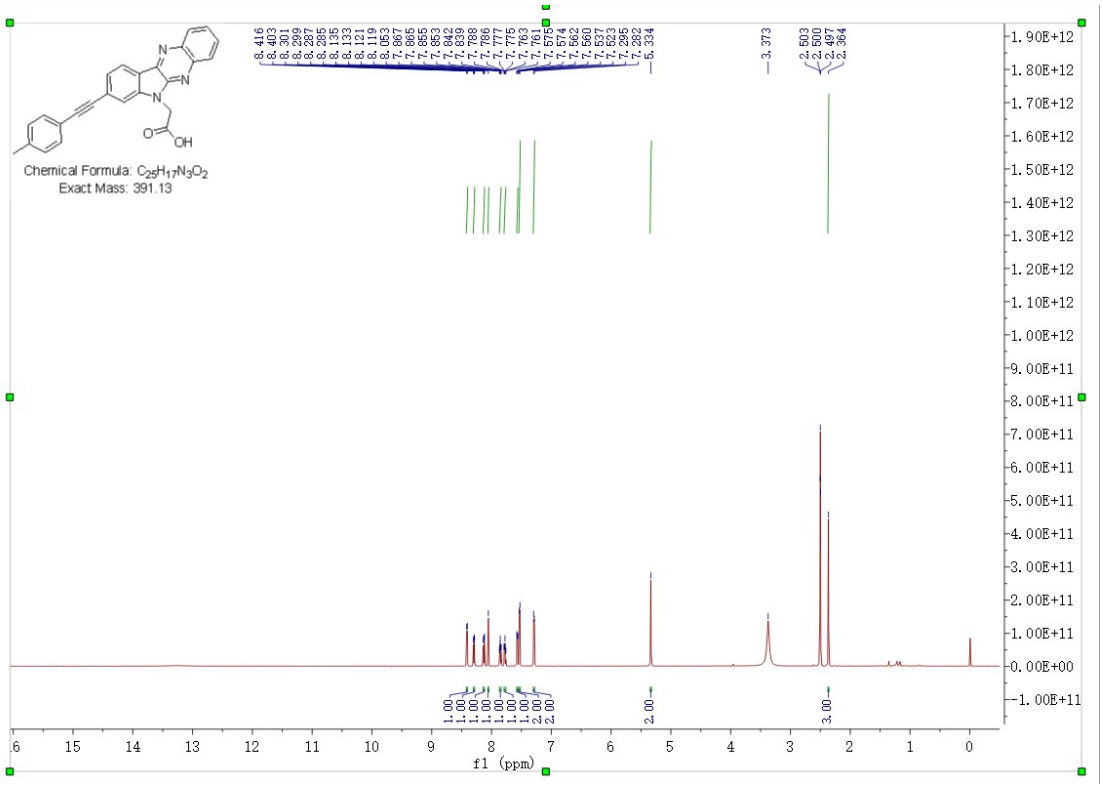
^{13}C NMR spectrum of 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5b**)



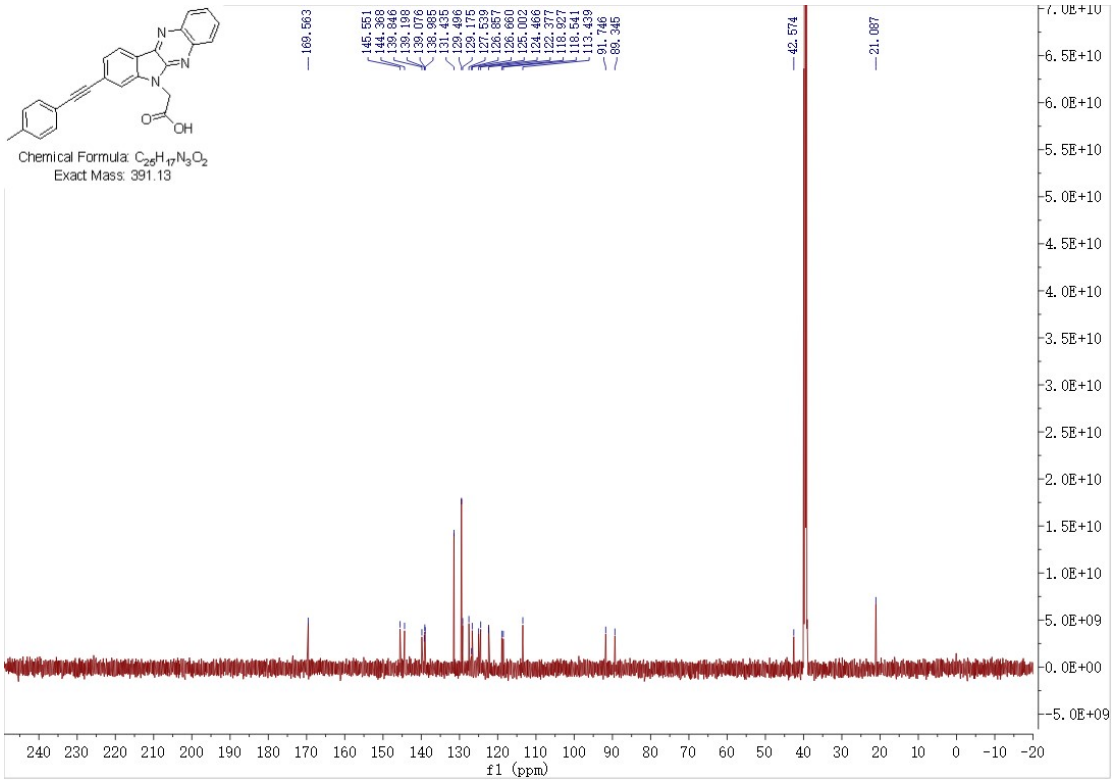
MS spectrum of 2-(8-((4-methoxyphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5b**)



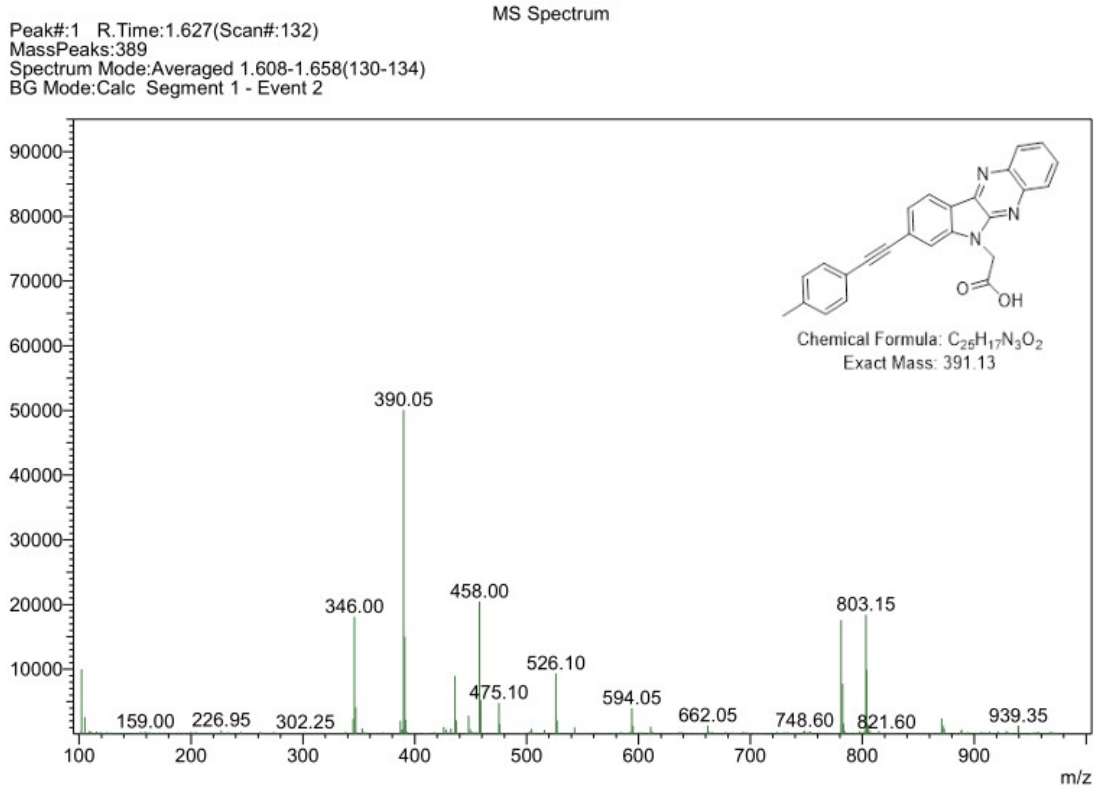
^{1}H NMR spectrum of 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5c**)



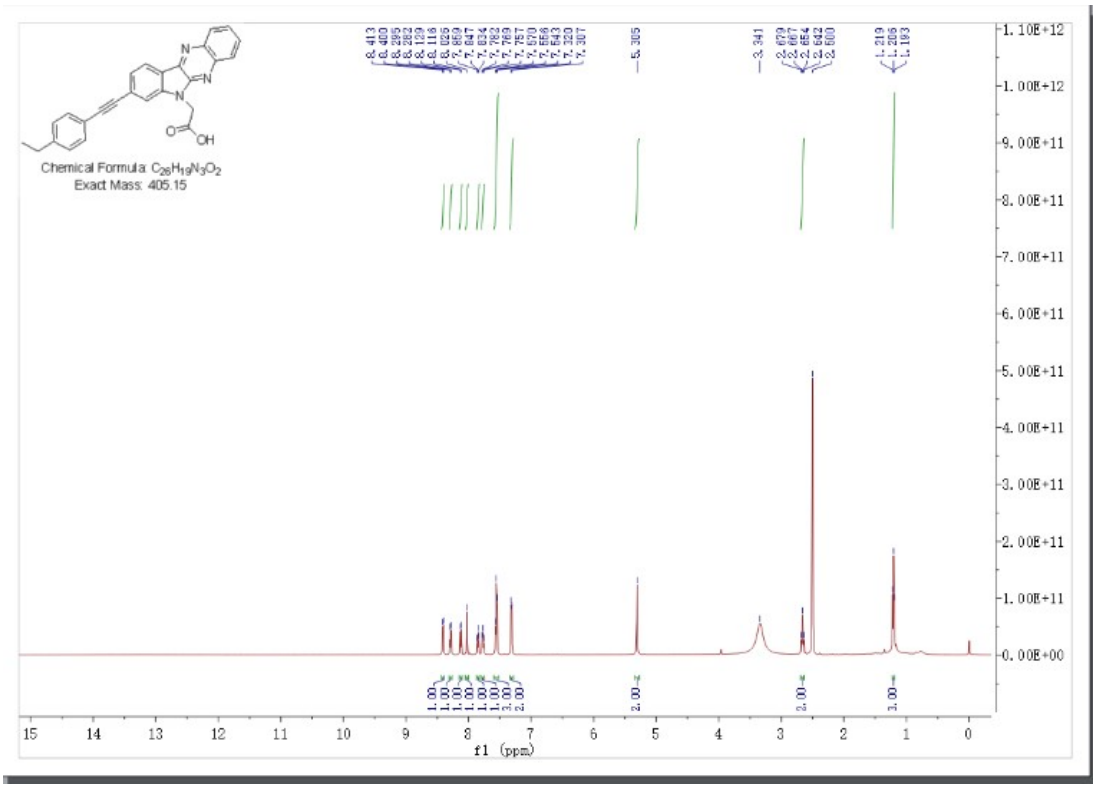
^{13}C NMR spectrum of 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5c**)



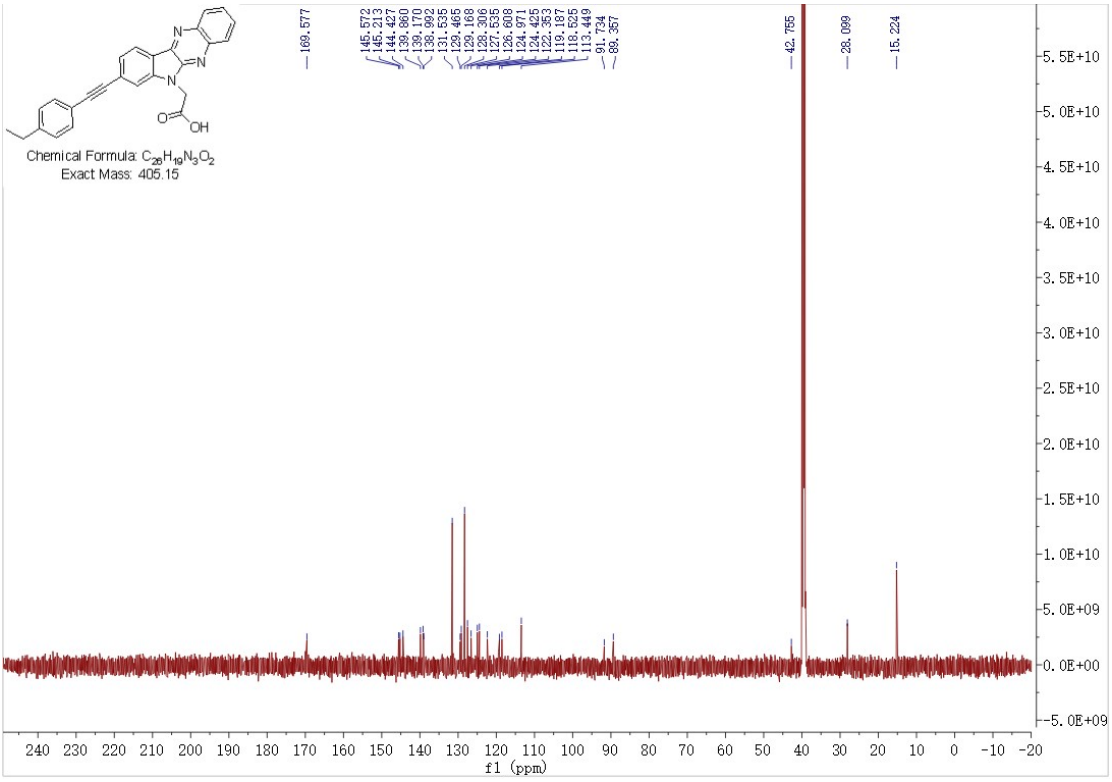
MS spectrum of 2-(8-(p-tolylethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5c**)



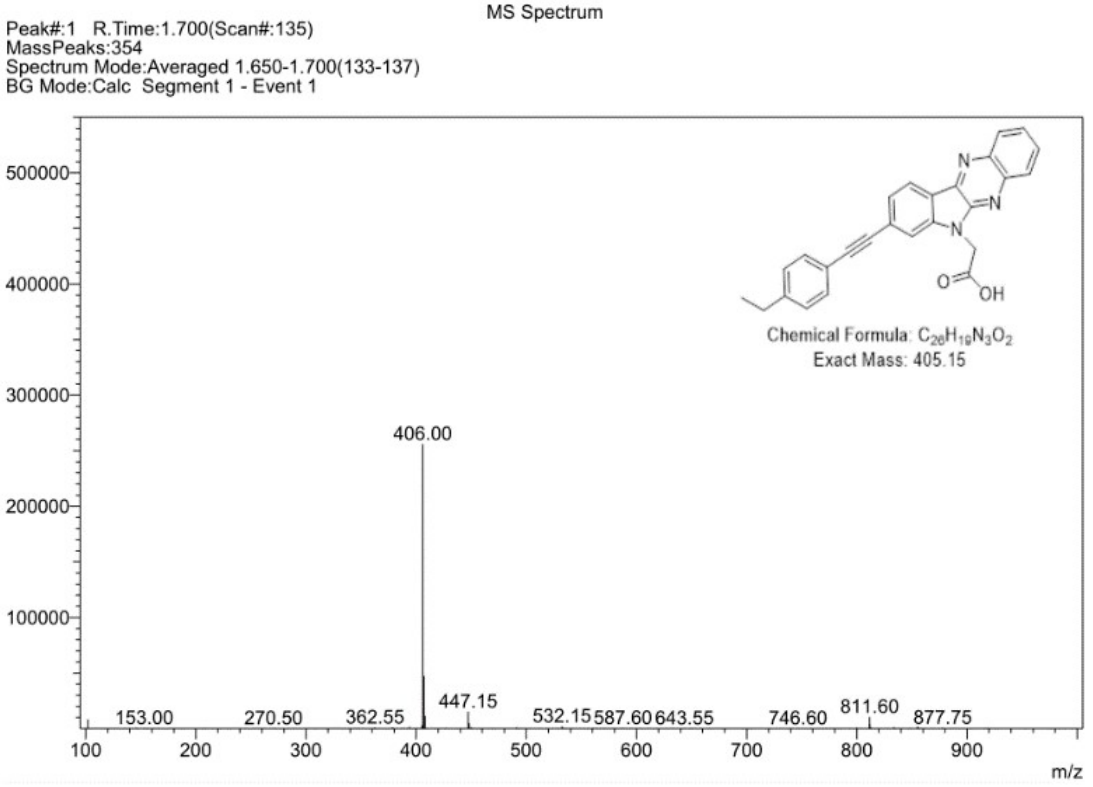
^{1}H NMR spectrum of 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5d**)



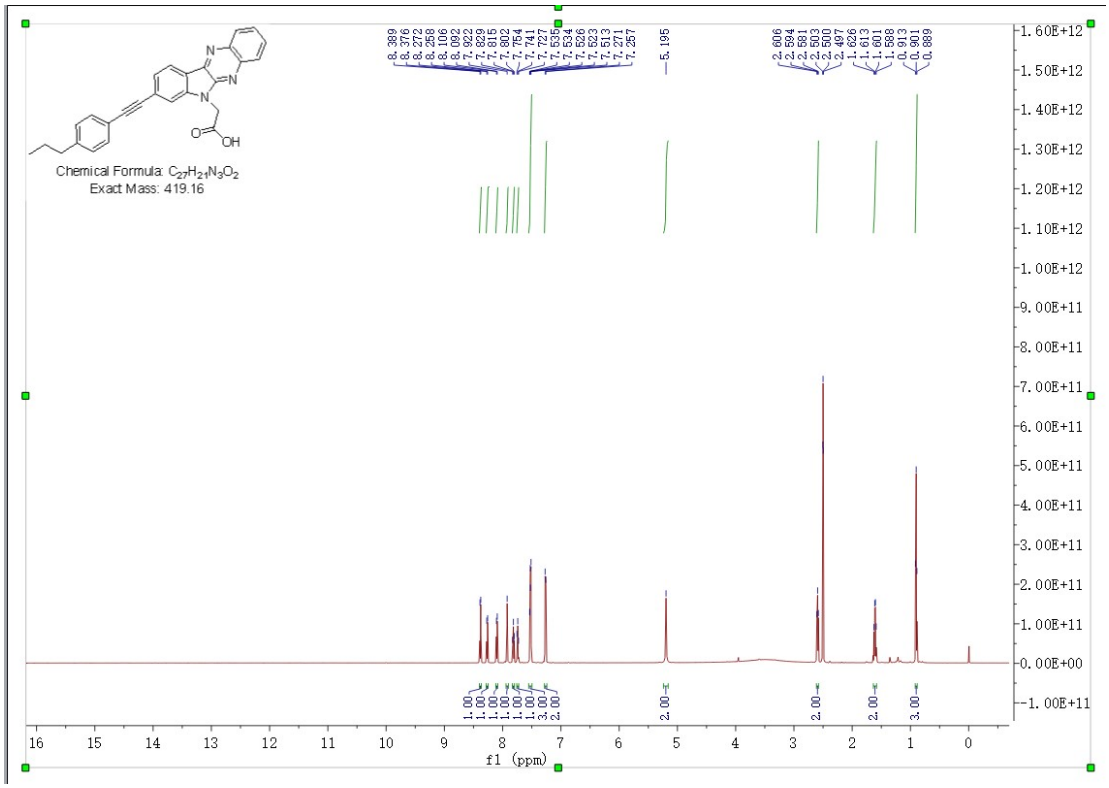
^{13}C NMR spectrum of 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5d**)



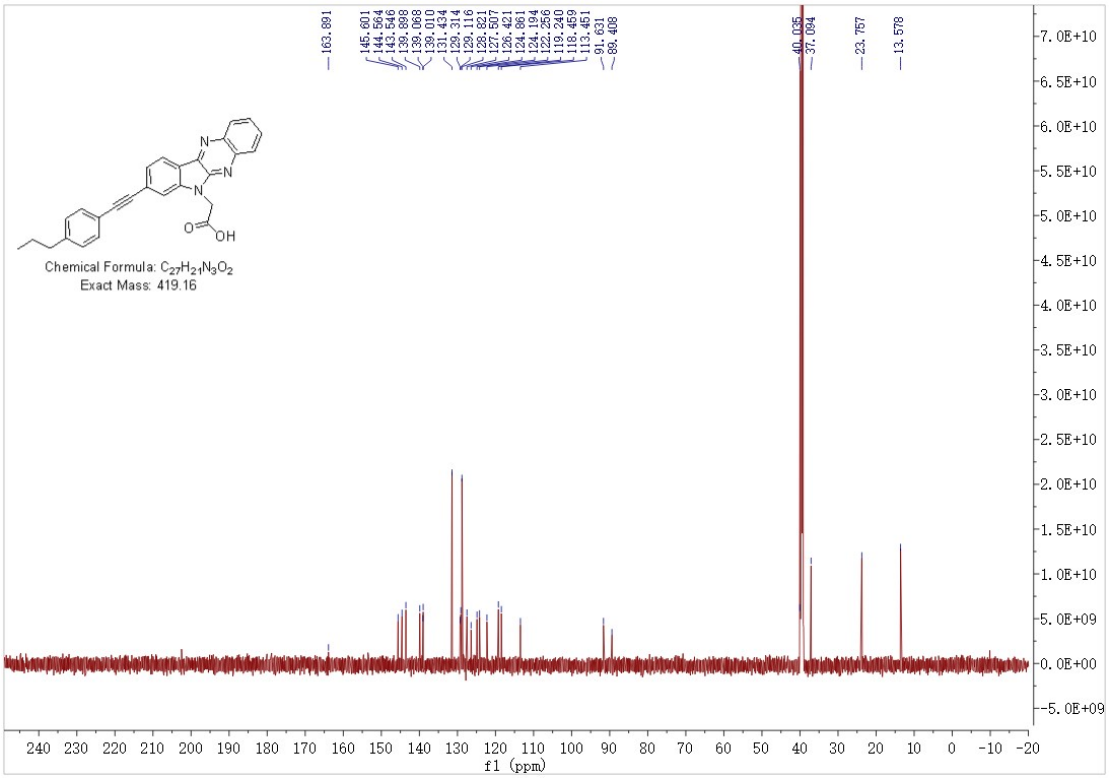
MS spectrum of 2-(8-((4-ethylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5d**)



^{1}H NMR spectrum of 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5e**)



^{13}C NMR spectrum of 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5e**)



MS spectrum of 2-(8-((4-propylphenyl)ethynyl)-6H-indolo[2,3-b]quinoxalin-6-yl)acetic acid (**5e**)