< Supporting Information >

Phenotype-based Discovery of a HeLa-Specific Cytotoxic Molecule that Downregulates HPV-mediated Signaling Pathways via Oxidative Damage

Sanghee Lee[1),†], Wansang Cho[2),†], Sungyoul Hong[3)], Sihyeong Yi[2)], Heejun Kim[2)], Soo Yeon Baek[1),4)], Hankum Park[2)], Jinjoo Jung[5)], Young Kee Shin[3)], Seung Bum Park*[,2),5)]

1) Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Seoul 02792, Korea
2) CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University, Seoul 08826, Korea
3) Department of Pharmacy, Seoul National University, Seoul 08826, Korea
4) Department of Chemistry, Sogang University, Seoul 04107, Korea
5) Department of Biophysics and Chemical Biology, Seoul National University, Seoul 08826, Korea

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I. Supplementary Figures and Table

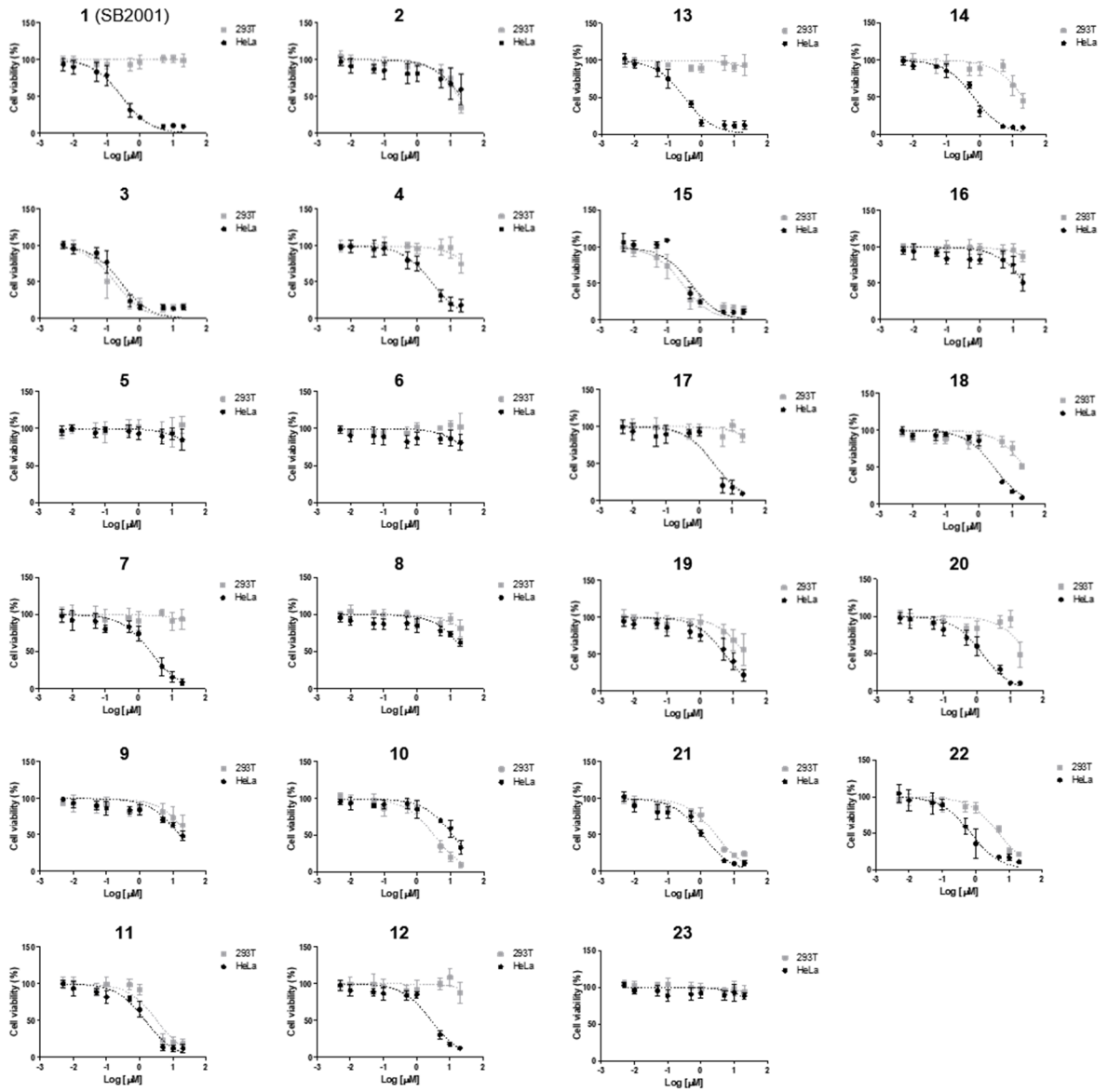


Fig. S1. Investigation of dose-responses for pyrazolopyridine analogs in this focused library. Cell viability was measured after 72-h incubation of indicated compounds in HeLa (black) or 293T cells (gray). IC_{50} values were listed in Figure 2c. Graphs were fitted by the mean and S.D. and data represented at least two independent experiments.

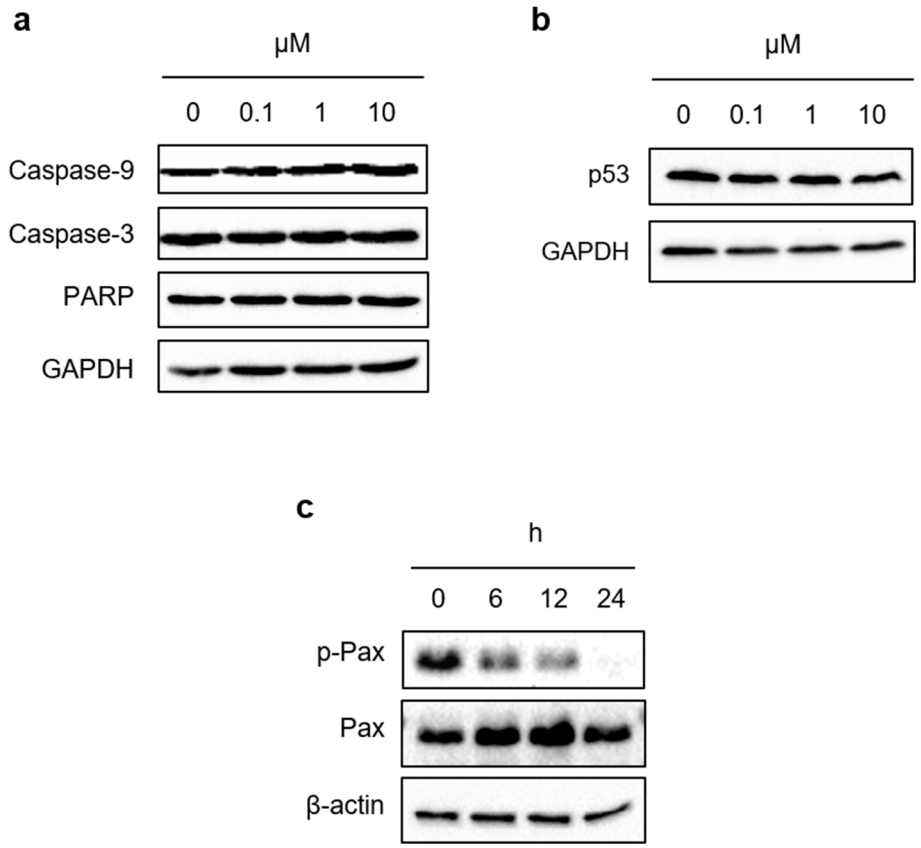


Fig. S2. Mechanistic elucidation of SB2001-induced HeLa cell death. (a) SB2001 showed no effect on apoptosis and p53 in 293T cells. Western blot analysis for caspase-3, caspase-9, and PARP. Cells were treated with SB2001 for 72 h. (b) Cells were incubated with SB2001 for 24 h, then subjected to western blot analysis for p53. Data represented in two independent experiments. (c) SB2001 suppressed E6 downstream signaling in HeLa cells. Cells were treated with SB2001 (10 μM). Western blot analysis for phospho-paxillin (p-Pax) and paxillin (Pax). Data represented in two independent experiments.

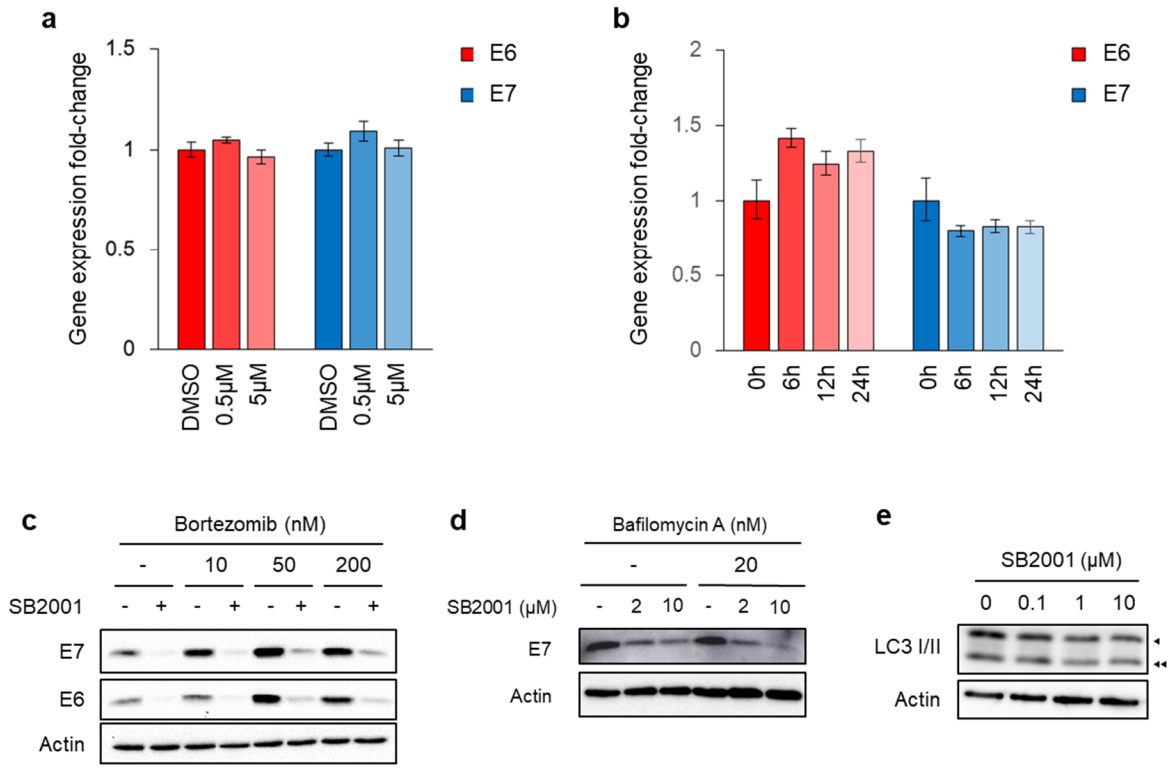


Fig. S3. **SB2001-mediated suppression of HPV oncoproteins were independent by gene expression and protein degradation in HeLa cells.** (a, b) The fold-changes in gene expression were analyzed by qPCR upon SB2001 treatment for 24 h or with 10 μM, respectively. qPCR data were normalized to GAPDH expression for each sample. Graphs depicted with RQ (relative quantification) and RQ_{min}/RQ_{max}. (c) Co-treatment test of SB2001 with bortezomib, a proteasome inhibitor, in HeLa cells. Cells were incubated with SB2001 (10 μM) for 24 h in the presence or absence of bortezomib, then subjected to western blotting for E6 and E7. (d) Co-treatment test of SB2001 with bafilomycin A, a late-stage autophagy inhibitor. Cells were incubated with SB2001 for 24 h in the presence or absence of bafilomycin A (nM), then subjected to western blotting for E7. Data represented in two independent experiments. (e) Western blot analysis for LC3. Cells were incubated with SB2001 for 24 h for analysis. Single- and double-arrow heads indicated LC3 I and LC3 II, respectively.

No.	Kinase	No.	Kinase	No.	Kinase	No.	Kinase	No.	Kinase
1	ABL1	61	Ck1g 3	121	GSK3b	181	MST3/STK24	241	RAF1
2	ABL2/ARG	62	CK1g1	122	Haspin	182	MST4	242	RET
3	ACK1/TNK2	63	CK2a	123	HCK	183	MUSK	243	RIPK2
4	AKT1	64	CK2a2	124	HGK/MAP4K4	184	NEK1	244	RIPK5
5	AKT2	65	CLK1	125	HIPK2	185	NEK11	245	ROCK1
6	AKT3	66	CLK3	126	HIPK3	186	NEK2	246	ROCK2
7	ALK	67	CLK4	127	HIPK4	187	NEK3	247	RON/MST1R
8	ALK1/ACVRL1	68	CLK2	128	HIPK1	188	NEK4	248	ROS/ROS1
9	ALK2/ACVR1	69	COT1/MAP3K8	129	IGF1R	189	NEK6	249	RSK1
10	ALK4/ACVR1B	70	CSK	130	IKKa/CHUK	190	NEK7	250	RSK2
11	ALK5/TGFBR1	71	CTK MATK	131	IKKb/IKBKB	191	NEK9	251	RSK3
12	ARAF	72	DAPK1	132	IKKe/IKBKE	192	NIK/MAP3K14	252	RSK4
13	ARK5/NUAK1	73	DAPK2	133	IR	193	NLK	253	SGK1
14	ASK1/MAP3K5	74	DCAMKL2	134	IRAK4	194	OSR1/OXSR1	254	SGK2
15	Aurora A	75	DDR2	135	IRAK1	195	P38a/MAPK14	255	SGK3/SGKL
16	Aurora B	76	DMPK	136	IRR/INSRR	196	P38b/MAPK11	256	SIK2
17	Aurora C	77	DRAK1/STK17A	137	ITK	197	P38d/MAPK13	257	SLK/STK2
18	AXL	78	DYRK1/DYRK1A	138	JAK1	198	P38g	258	SNARK/NUAK2
19	BLK	79	DYRK1B	139	JAK2	199	p70S6K/RPS6KB1	259	SRMS
20	BMX/ETK	80	DYRK2	140	JAK3	200	p70S6Kb/RPS6KB2	260	SRPK1
21	BRAF	81	DYRK3	141	JNK1	201	PAK1	261	SRPK2
22	BRK	82	DYRK4	142	JNK2	202	PAK2	262	STK16
23	BRSK1	83	EGFR	143	JNK3	203	PAK3	263	STK22D/TSSK1
24	BRSK2	84	EPHA1	144	KDR/VEGFR2	204	PAK4	264	STK25/YSK1
25	BTK	85	EPHA2	145	KHS/MAP4K5	205	PAK5	265	STK33
26	c-Kit	86	EPHA3	146	LCK	206	PAK6	266	STK39/STLK3
27	c-MER	87	EPHA4	147	LIMK1	207	PASK	267	SYK
28	c-MET	88	EPHA5	148	LKB1	208	PBK/TOPK	268	TAK1
29	c-Src	89	EPHA7	149	LOK/STK10	209	PDGFRa	269	TAOK1
30	CAMK1a	90	EPHA8	150	LRRK2	210	PDGFRb	270	TAOK2/TAO1
31	CAMK1b	91	EPHB1	151	LYN	211	PDK1/PDPK1	271	TAOK3/JIK
32	CAMK1d	92	EPHB2	152	LYN B	212	PHKg1	272	TBK1
33	CAMK1g	93	EPHB3	153	MAPKAPK2	213	PHKg2	273	TEC
34	CAMK2a	94	EPHB4	154	MAPKAPK3	214	PIM1	274	TGFBR2
35	CAMK2b	95	ERBB2 HER2	155	MAPKAPK5/PRAK	215	PIM2	275	TIE2/TEK
36	CAMK2d	96	ERBB4/HER4	156	MARK1	216	PIM3	276	TRKA
37	CAMK2g	97	ERK1	157	MARK2/PAR-1Ba	217	PKA	277	TRKB
38	CAMK4	98	ERK2/MAPK1	158	MARK3	218	PKCa	278	TRKC
39	CAMKK1	99	FAK/PTK2	159	MARK4	219	PKCb1	279	TSSK2
40	CAMKK2	100	FER	160	MEK1	220	PKCb2	280	TTK
41	CDK1/cyclin A	101	FES/FPS	161	MEK2	221	PKCd	281	TXK
42	CDK1/cyclin B	102	FGFR1	162	MEKK2	222	PKCepsilon	282	TYK/LTK
43	CDK2/cyclin A	103	FGFR2	163	MEKK3	223	PKCeta	283	TYK2
44	CDK2/cyclin E	104	FGFR3	164	MELK	224	PKCg	284	TYRO3/SKY
45	CDK3/cyclin E	105	FGFR4	165	MINK/MINK1	225	PKCiota	285	ULK1
46	CDK4/ cyclin D1	106	FGR	166	MKK6	226	PKCmu/PRKD1	286	ULK2
47	CDK4/cyclin D3	107	FLT1/VEGFR1	167	MLCK/MYLK	227	PKCnu/PRKD3	287	VRK1
48	CDK5/p25	108	FLT3	168	MLCK2/MYLK2	228	PKCtheta	288	WEE1
49	CDK5/p35	109	FLT4/VEGFR3	169	MLK1/MAP3K9	229	PKCzeta	289	WNK2
50	CDK6/cyclin D1	110	FMS	170	MLK2/MAP3K10	230	PKD2/PRKD2	290	WNK3
51	CDK6/cyclin D3	111	FRK/PTK5	171	MLK3/MAP3K11	231	PKG1a	291	YES/YES1
52	CDK7/cyclin H	112	FYN	172	MNK1	232	PKG1b	292	ZAK/MLTK
53	CDK9 cyclin K	113	GCK/MAP4K2	173	MNK2	233	PKG2/PRKG2	293	ZAP70
54	CDK9 cyclin T1	114	GRK2	174	MRCKa/CDC42BPA	234	PKN1/PRK1	294	ZIPK/DAPK3
55	CHK1	115	GRK3	175	MRCKb/CDC42BPB	235	PKN2/PRK2		
56	CHK2	116	GRK4	176	MSK1/RPS6KA5	236	PLK1		
57	CK1a1	117	GRK5	177	MSK2/RPS6KA4	237	PLK2		
58	CK1d	118	GRK6	178	MSSK1/STK23	238	PLK3		
59	CK1epsilon	119	GRK7	179	MST1/STK4	239	PRKX		
60	Ck1g 2	120	GSK3a	180	MST2/STK3	240	PYK2		

Table S1. **Kinase activity profiling of SB2001.** Inhibitory effect of SB2001 was examined against 294 kinases with 10 μM of SB2001 in duplicate. Kinases were listed in table.

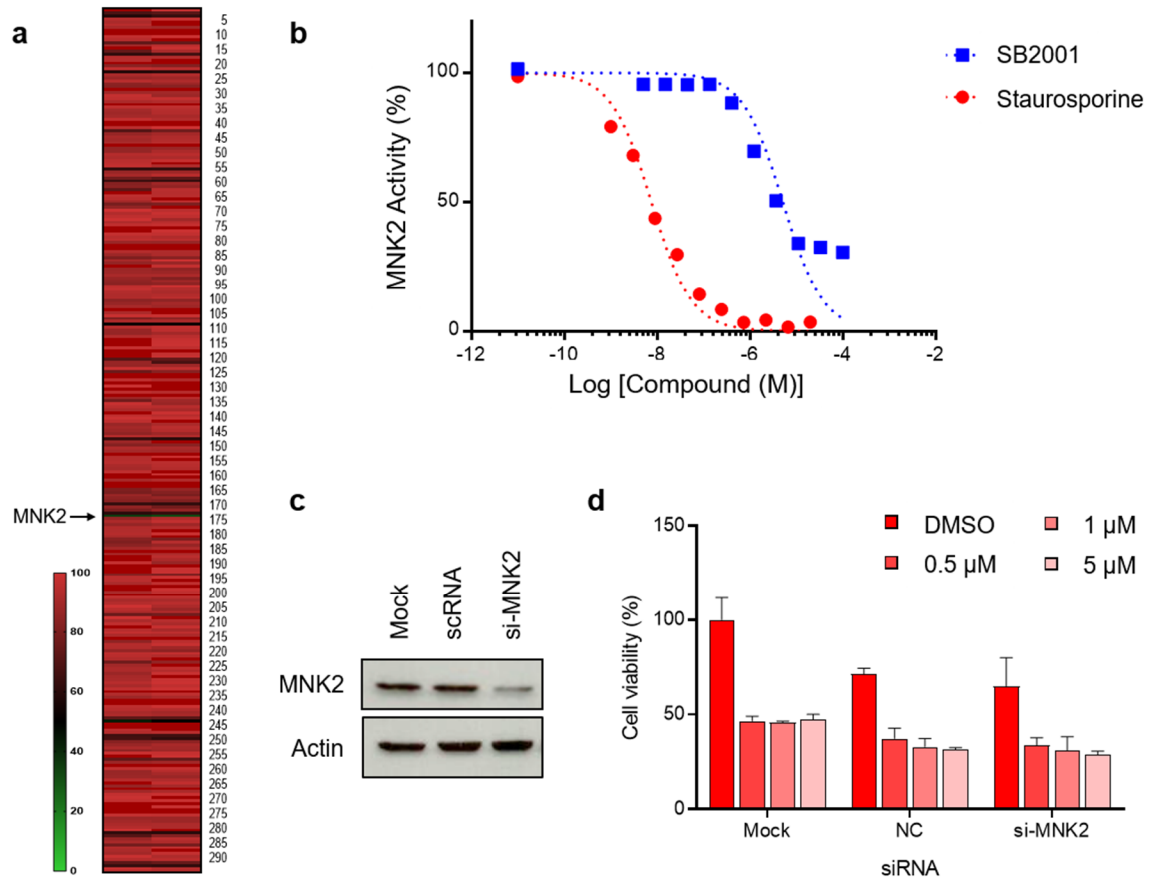


Fig. S4. Invalidation for MNK2 as a direct target for SB2001. (a) Heat map of kinase activity profiling about SB2001. Heat map indicates enzyme activity (%) compared to DMSO control. Rows indicate different kinases aligned in an alphabetic order listed in the Table S1 and columns show in a replicate result. Activity in out of range due to over 100 % was displayed in dark red. (b) Dose-response of SB2001 for MNK2 activity in *in vitro* kinase assay. Staurosporine was used as a positive control. (c) Knockdown of MNK2. Western blotting of cell lysate after vehicle, scramble siRNA (NC), or si-MNK2 transfection for 48 h in HeLa cells. (d) Evaluation of SB2001-induced cytotoxicity against HeLa cells under MNK2 knockdown. Cells were transfected with indicated siRNA for 48 h and incubated with SB2001 in last 24 h, then viability was measured. Bar graph depicts the mean and S.D. Data represented in two independent experiments.

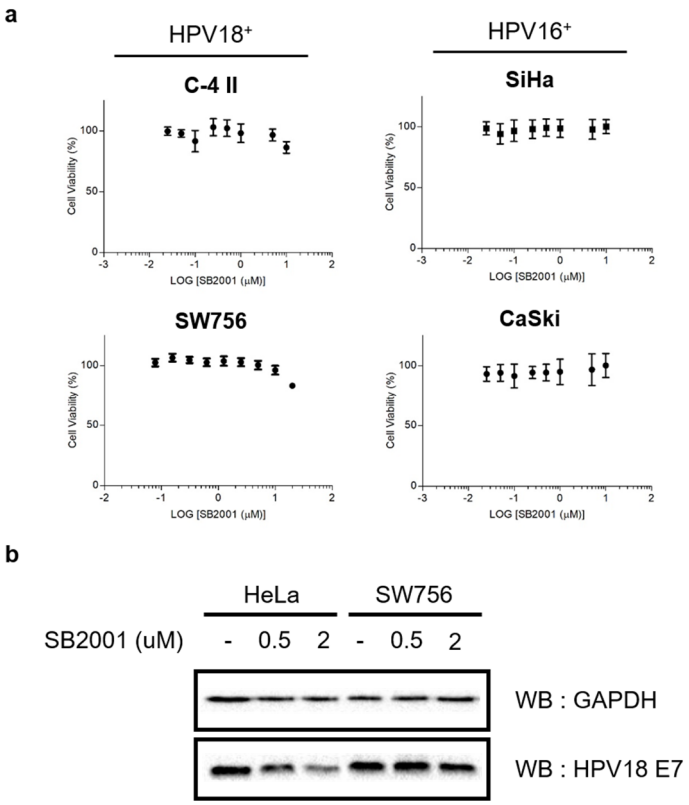
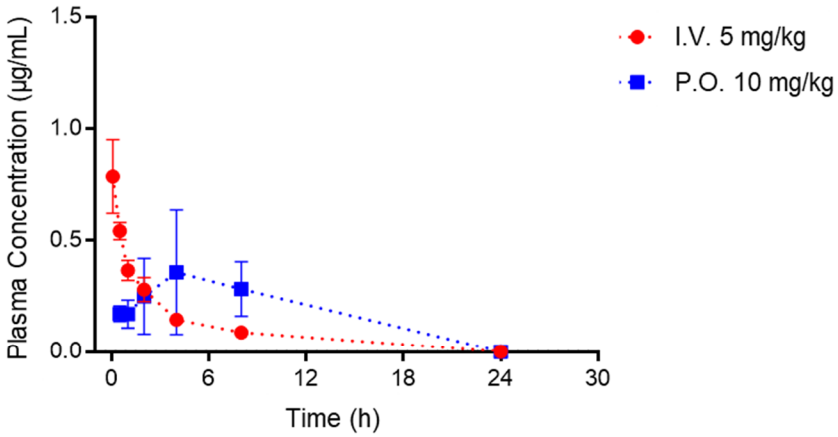


Fig. S5. SB2001-induced cytotoxicity and perturbation of HPV oncoprotein signaling are specific in HeLa cells. (a) Dose-response for SB2001 against various HPV-positive cancer cell lines. Graph depicts the mean and S.D. (b) Western blot analysis for HPV18 E7 in HeLa and SW756 cell line. Cells were incubated with SB2001 for 48 h, then subjected to western blotting. Data represented in two independent experiments.



Parameters	I.V. 5 mg/kg	P.O. 10 mg/kg
T_{max} (h)	NA	6.40 ± 2.19
C_{max} (μg/mL)	NA	0.45 ± 0.23
$T_{1/2}$ (h)	1.47 ± 0.08	1.82 ± 0.35
AUC_t (μg·h/mL)	2.47 ± 0.30	4.38 ± 0.66
AUC_∞ (μg·h/mL)	2.47 ± 0.30	4.51 ± 1.18
CL (L/h/kg)	2.05 ± 0.27	NA
V_{ss} (L/kg)	8.30 ± 1.10	NA
F_t (%)	NA	91.3

Fig. S6. Plasma concentration and pharmacokinetic parameters of SB2001 for pharmacokinetic study. Graph depicts the mean and S.D.

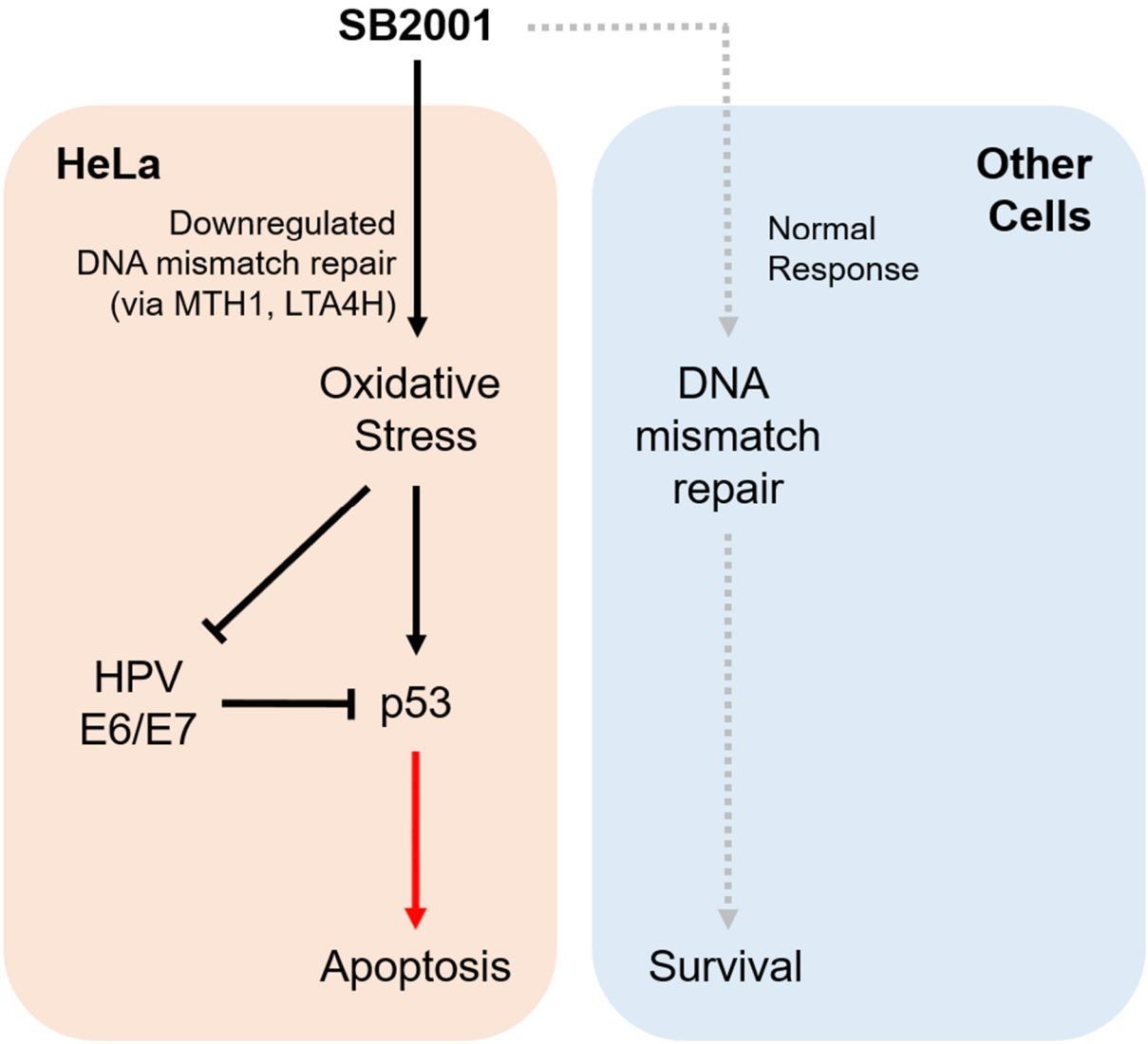


Fig. S7. Mechanistic figure of SB2001-induced cytotoxicity in HeLa cell.

II. General Information for Biological Experiments

Mice and cells. Hsd:ICR (CD-1®) mice and Koat:Athymic NCr-nu/nu mice were from KOATECH [Pyeongtaek, Korea]. Human HeLa (CCL-2), human CaSki (CRL-1550), human ME-180 (HTB-33), human C-4 II (CRL-1595), human SW756 (CRL-10302), human SiHa (HTB-35), human C-33A (HTB-31), human HCT116 (CCL-247), human SW480 (CCL-228), human MCF7 (HTB-22), human DU145 (HTB-81), human PC3 (CRL-1435), human U266B1 [u266] (TIB-196), human 293T (CRL-3216), rat L6 (CRL-1456), mouse NIH/3T3 (CRL-1658), and mouse Raw264.7 (TIB-71) cell lines were from ATCC [Manassas, VA].

Kits, reagents, and antibodies. Dulbecco's modified eagle medium (DMEM), RPMI 1640 medium, heat-inactivated fetal bovine serum (FBS), and antibiotic-antimycotic solution were purchased from Gibco, Invitrogen [Karlsbad, CA]. Phosphate-buffered saline (PBS) was purchased from WELGENE [Gyeongsan, Korea]. 100-mm cell culture dish, T-75 flask, transparent 96-well plate, and transparent 6-well plate were purchased from CORNING [Corning, NY]. Confocal dish was purchased from SPL Life Science [Pocheon, Korea]. DMSO was purchased from Acros Organics [Waltham, MA]. Triton-X-100 and staurosporine were purchased from Sigma-Aldrich [St. Louis, MO]. Propidium iodide was purchased from Calbiochem [Burlington, MA]. Ez-Cytox reagent for WST assay was purchased from Daeil Bio Co. Ltd. [Seoul, Korea] SDS-PAGE equipments and polyvinylidene difluoride (PVDF) membrane were purchased from Bio-Rad [Hercules, CA]. Amersham ECL prime western blotting detection system (Amersham ECL prime solution) was purchased from GE Healthcare Life Science [Chicago, IL]. To measure the protein concentration of cell lysate, Micro BCA™ protein assay kit was purchased from PIERCE [Waltham, MA]. Hoechst 33342 reagent for nucleus staining was purchased from Life Technologies [Karlsbad, CA]. Kapa SYBR Green 2× ABI Prism reagent was purchased from Kapa Biosystems [Wilmington, MA]. RNase A for cell cycle analysis and RNeasy mini kit for RNA extraction were purchased from QIAGEN [Hilden, Germany]. Accupower cyclescript RT premix (dT20) and all the qPCR primers, scramble siRNA (negative control) and targeted siRNAs were purchased from Bioneer Inc [Daejeon, Korea]. Lipofectamine RNAiMAX was purchased from ThermoFisher Scientific [Waltham, MA]. Anti-GAPDH (#2118), anti-β-Actin (#4970), anti-caspase-9 (human specific) (#9502), anti-caspase-3 (#9662), anti-cleaved caspase-3 (#9664), anti-PARP (#9532), anti-p53 (#2527), anti-paxillin (#2542), and anti-phospho-paxillin (Tyr118) (#2541) antibodies were from Cell Signaling Technology [Danvers, MA]. Anti-p21 (ab18209), anti-HPV16 E6+HPV18 E6 (ab70), anti-HPV18 E7 (ab100953), anti-LC3B (ab51520), anti-MNK2 (ab84345), and TRITC goat anti-rabbit secondary antibody (ab6718) were from Abcam [Cambridge, UK].

siRNA and qPCR primer sequences. siRNA and qPCR primers were designed from references;

siRNA[1]	Sense	Antisense
HPV18 E7 #1	5′-CAACCGAGCACGACAGGAA-3′	5′-UUCCUGUCGUGCUCGGUUG-3′
HPV18 E7 #2	5′-CCAACGACGCAGAGAAACA-3′	5′-UGUUUCUCUGCGUCGUUGG-3′

qPCR primer[2]	Forward	Reverse
HPV18 E6	5′-GCGACCCTACAAGCTACCTG-3′	5′-GTTGGAGTCGTTCCTGTCGT-3′
HPV18 E7	5′-GCATGGACCTAAGGCAACAT-3′	5′-TGTTGCTTACTGCTGGGATG-3′

Instruments and programs. Measurement of absorbance in 96-well plates for WST assay was performed with Synergy HT microplate reader from BioTek [Winooski, VT]. Transfer of protein from SDS-PAGE gel to PVDF membrane was performed with Transblot Turbo from Bio-Rad [Hercules, CA]. Chemiluminescence detection for western blot was performed with ChemiDoc™ MP from Bio-Rad. Quantification of chemiluminescence was done with ImageLab 4.0 software from Bio-Rad. Flow cytometry for cell cycle analysis was performed with BD FACSAria II from BD Bioscience [San Jose, CA], equipped with 488-nm coherent sapphire laser. Immunofluorescence imaging was carried out with Olympus Inverted Microscope Model IX71, equipped for epi-illumination using a halogen bulb (Philips No. 7724). Emission signal of each experiment was observed at two spectral settings: blue channel, using a 330–385 nm band pass exciter filter, a 400 nm center wavelength chromatic beam splitter, a 420-nm long pass barrier filter (Olympus filter set U-MWU2); and red channel using a 510–550 nm band pass exciter filter, a 570 nm center wavelength chromatic beam splitter, a 590-nm long pass barrier filter (Olympus filter set U-MWG2). Emission signal of each experiment was detected with 12.5M pixel recording digital color camera (DP71) from Olympus [Tokyo, Japan]. Brightfield imaging was performed with Zoe™ Fluorescent Cell Imager from Bio-Rad [Hercules, CA]. RNA quantification before qPCR was conducted with NanoView from GE Healthcare [Chicago, IL]. Quantitative PCR was performed with StepOne Plus Real-Time PCR System from Applied Biosystems [Foster City, CA]. Pharmacokinetic parameters were obtained after the analysis of plasma concentration−time plot with WinNonlin software from Pharsight [St. Louis, MO]. Provided graphs were analyzed and displayed using GraphPad Prism 5 from GraphPad Software [La Jolla, CA].

III. Synthetic Procedures and Characterization of New Compounds

Compound 1 (**SB2001**), 5-chloro-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 6-chloroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product was precipitated out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was further purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. NMR spectra of this compound was previously reported.[10] R_f=0.3 (EA:Hexane:MeOH=20:20:1), HRMS (ESI+) *m/z* cald for $C_{13}H_{12}ClN_4^+$ $[M+H]^+$: 259.0745; Found: 259.0744.

Compound 6, 2-(1-butyl-3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of *N*-Boc-indole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction, the solution was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtered solid was dissolved into DMF (100 mM), and to the solution was added NaH (1.2 equiv.). After 10 min of stirring, the reaction mixture was treated with *n*-BuBr (1.2 equiv.) and stirred at the ambient temperature for 2 h. After the reaction completion monitored by TLC the reaction mixture was quenched with ddH_2O and the organic phase was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 (s) and the filtrate was concentrated under reduced pressure. Without purification, the crude mixture was dissolved into 20 % trifluoroacetic acid (TFA) in acetone (100 mM), and stirred at the ambient temperature until the full conversion of the starting material. The remaining TFA was removed by azeotropic evaporation with toluene, and the crude mixture was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.2 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, J = 1.2 Hz, 1H), 8.13 (d, J = 1.2Hz, 1H), 7.12 (m, 2H), 6.86 (d, J = 4.8 Hz, 1H), 6.74 (s, 1H), 4.46 (br s, 1H),

4.43 (d, J = 4.2 Hz, 2H), 2.05 (s, 3H), 1.91 (t, 2H), 1.35 (dd, 2H), 0.95 (t, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.5, 149.4, 140.1, 131.0, 129.6, 129.3, 128.8, 128.2, 124.1, 117.7, 115.7, 114.9, 46.1, 31.9, 22.3, 13.4, 11.7; LRMS (ESI+) *m/z* cald for $C_{17}H_{21}N_4^+$ $[M+H]^+$: 281.18; Found: 281.10.

Compound 7, 3-bromo-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 4-bromoindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product was precipitated out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.28 (s, 1H), 8.21 (d, J = 1.2 Hz, 1H), 8.04 (d, J = 1.2 Hz, 1H), 7.01 (t, 1H), 6.88 (dd, 1H), 6.77 (t, 1H), 4.96 (br s, 2H), 2.51 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.4, 150.8, 149.4, 141.8, 131.5, 130.7, 126.7, 125.6, 123.8, 120.0, 114.9, 114.4, 12.9; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}BrN_4^+$ $[M+H]^+$: 303.02; Found: 302.95.

Compound 8, methyl 3-amino-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)benzoate

To a solution of 3-formylindole-4-carboxylic acid methyl ester and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.20 (br s, 1H), 8.17 (d, J = 0.6 Hz, 1H), 7.96 (s, 1H), 7.20 (t, 1H), 7.04 (d, J = 4.8 Hz, 1H), 6.97 (d, J = 4.8 Hz, 1H), 4.88 (br s, 2H), 3.39 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.4, 152.1, 150.0, 147.9, 141.4, 132.5, 130.0, 128.8, 125.8, 122.8, 118.3, 117.5, 114.4, 52.0, 12.7; LRMS (ESI+) *m/z* cald for $C_{15}H_{15}N_4O_2^+$ $[M+H]^+$: 283.12; Found: 283.05.

Compound 9, 2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)-3-nitroaniline

To a solution of 4-nitroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a yellow solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using MeOH in dichloromethane (DCM) with 1 % triethylamine (TEA) as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.33 (br s, 1H), 8.25 (d, *J* = 3.3 Hz, 1H), 8.08 (d, *J* = 3.3 Hz, 1H), 7.31 (t, 1H), 7.11 (dd, 1H), 7.05 (s, 1H), 5.34 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 152.4, 151.7, 149.7, 130.6, 129.6, 122.1, 118.8, 115.7, 114.7, 110.9, 12.7; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}N_5O_2^+$ $[M+H]^+$: 270.10; Found: 270.05.

Compound 10, 4-chloro-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 5-chloroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.24 (br s, 1H), 8.45 (s, 1H), 8.18 (s, 1H), 7.09 (d, *J* = 4.8 Hz, 1H), 7.06 (s, 1H), 6.77 (d, *J* = 4.8 Hz, 1H), 5.08 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 151.7, 149.1, 145.1, 141.3, 129.9, 129.4, 128.0, 126.3, 124.7, 119.7, 116.6, 114.0, 12.2; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}ClN_4^+$ $[M+H]^+$: 259.07; Found: 259.05.

Compound 11, 4-bromo-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 5-bromoindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction

monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.24 (br s, 1H, 8.45 (s, 1H), 8.18 (s, 1H), 7.21 (d, *J* = 1.2 Hz, 1H), 7.20 (s, 1H), 6.73 (d, *J* = 5.1 Hz, 1H), 5.12 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 159.5, 145.9, 133.1, 131.3, 129.8, 126.7, 125.7, 117.5, 107.5, 12.7; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}BrN_4^+$ $[M+H]^+$:303.02 Found: 303.00.

Compound 12, 5-fluoro-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 6-fluoroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.21 (br s, 1H), 8.41 (d, *J* = 0.9 Hz, 1H), 8.12 (d, *J* = 1.2 Hz, 1H), 7.03 (t, 1H), 6.54 (d, *J* = 6.9 Hz, 1H), 6.42 (t, 1H), 5.22 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 164.8, 161.6, 152.1, 149.8, 148.5, 148.4, 141.5, 132.8, 132.6, 129.7, 127.1, 120.1, 120.0, 114.4, 103.4, 103.1, 101.5, 101.2, 12.7; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}FN_4^+$ $[M+H]^+$: 243.10; Found: 243.00.

Compound 13, 5-bromo-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 6-bromoindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.25 (br s, 1H), 8.43 (d, *J* = 1.2 Hz, 1H), 8.16 (d, *J* = 0.9 Hz, 1H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.96 (s, 1H), 6.78 (dd, 1H), 5.25 (br s, 1H), 2.51 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ

152.3, 149.8, 148.6, 141.8, 133.3, 129.9, 127.1, 123.0, 122.1, 119.42, 117.6, 114.7, 12.9; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}BrN_4^+$ $[M+H]^+$: 303.02; Found: 302.95.

Compound 14, 5-methyl-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 6-methylindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.19 (br s, 1H), 8.43 (d, *J* = 1.2 Hz, 1H), 8.12 (d, *J* = 0.9 Hz, 1H), 6.92 (d, *J* = 4.5 Hz), 6.60 (s, 1H), 6.48 (d, *J* = 4.8 Hz, 1H), 4.82 (br s, 2H), 2.51 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.2, 150.1, 146.3, 141.7, 138.2, 131.4, 129.6, 128.3, 121.2, 118.4, 116.42, 116.39, 114.6, 21.7, 12.9; LRMS (ESI+) *m/z* cald for $C_{14}H_{15}N_4^+$ $[M+H]^+$: 239.13; Found: 239.15.

Compound 15, 5-azido-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 6-azidoindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using MeOH in DCM with 1 % TEA as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.21 (br s, 1H) 8.43 (s, 1H), 8.14 (s, 1H), 7.07 (d, *J* = 5.1 Hz, 1H), 6.54 (s, 1H), 6.38 (d, *J* = 1.2 Hz, 1H), 5.21 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.6, 149.3, 147.4, 141.1, 139.3, 132.3, 129.1, 126.7, 120.6, 113.9, 107.2, 104.7, 12.2. LRMS (ESI+) *m/z* cald for $C_{13}H_{12}N_7^+$ $[M+H]^+$: 266.11; Found: 266.00.

Compound 16, methyl 3-amino-4-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)benzoate

To a solution of 3-formylindole-6-carboxylic acid methyl ester and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%),

and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.2 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.27 (br s, 1H), 8.50 (d, J=1.9Hz, 1H), 8.23 (s, 1H), 7.45 (d, J=1.5Hz, 1H), 7.24 (dd, 1H), 7.18 (d, J=7.9Hz, 1H), 5.26 (br s, 2H), 3.85 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (75MHz, DMSO-*d*$_6$) δ 166.2, 151.7, 148.9, 141.4, 131.5, 130.5, 129.72, 129.65, 125.8, 120.3, 118.2, 114.0, 52.2, 12.2; LRMS (ESI+) *m/z* cald for $C_{15}H_{15}N_4O_2^+$ $[M+H]^+$: 283.12; Found: 283.00.

Compound 17, 2-bromo-6-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline]

To a solution of 7-bromoindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.29 (br s, 1H), 8.44 (dd, 1H), 8.20 (d, *J* = 0.6 Hz, 1H), 7.44 (m, 1H), 7.07 (m, 1H), 6.64 (m, 1H), 4.93 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 152.4, 149.8, 143.7, 141.9, 132.6, 131.2, 130.2, 127.6, 126.0, 114.6, 109.5, 12.9; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}BrN_4^+$ $[M+H]^+$: 303.02; Found: 302.95.

Compound 18, 2-fluoro-6-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of 7-fluoroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a white solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. R_f=0.5 (EA:DCM:MeOH=60:30:1), ^{1}H NMR (300 MHz, acetone-*d*$_6$) δ 12.39

(br s, 1H), 8.52 (d, J = 0.9 Hz, 1H), 8.19 (d, J = 1.2 Hz, 1H), 7.05 (t, 1H), 6.97 (d, J = 4.5 Hz, 1H), 6.74 (m, 1H), 4.58 (br s 2H), 2.56 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 152.7, 150.9, 149.6, 134.3, 134.2, 129.2, 127.1, 127.0, 126.71, 126.68, 126.59, 126.57, 117.07, 117.05, 117.01, 116.98, 114.5, 114.4, 114.2, 11.8; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}FN_4^+$ $[M+H]^+$: 243.10; Found: 243.05.

Compound 19, 2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)-6-nitroaniline

To a solution of 7-nitroindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. As a reaction proceeded, a desired product came out as a yellow solid. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature, and the resulting solid was slowly filtered and washed with cold MeOH and diethyl ether. The filtrate was purified by silica-gel flash column chromatography using DCM with 1 % TEA as eluent system. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.34 (br s, 1H), 8.43 (d, J = 1.2 Hz, 1H), 8.24 (d, J = 0.9 Hz, 1H), 8.09 (d, J = 6.3 Hz, 1H), 7.41 (d, J = 5.4 Hz, 1H), 6.97 (br s 2H), 6.78 (t, 1H), 2.51 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.0, 149.2, 144.1, 141.3, 137.7, 131.5, 130.2, 128.5, 125.5, 125.0, 115.6, 114.1, 12.2; LRMS (ESI+) *m/z* cald for $C_{13}H_{12}N_5O_2^+$ $[M+H]^+$: 270.10; Found: 270.00.

Compound 20, *N*-methyl-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of *N*-methylindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was concentrated under reduced pressure, and then purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. The desired product was obtained as a white solid. R_f=0.2 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.22 (br s, 1H), 8.39 (s, 1H), 8.12 (s, 1H), 7.22 (m, 1H), 7.01 (d, J = 4.2 Hz, 1H), 6.65 (m, 1H), 6.61 (m, 1H), 4.95 (br s, 1H), 2.64 (d, J = 1.8 Hz, 3H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.1, 150.0, 147.6, 131.1, 130.0, 129.4, 127.7, 124.49, 116.3, 114.5, 110.0, 30.7, 12.7; LRMS (ESI+) *m/z* cald for $C_{14}H_{15}N_4^+$ $[M+H]^+$: 239.13; Found: 239.10.

Compound 21, *N*-butyl-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

To a solution of *N*-butylindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was concentrated under reduced pressure, and then purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. The desired product was obtained as a white solid. R_f=0.3 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, acetone-*d*$_6$) δ 12.47 (br s, 1H), 8.46 (s, 1H), 8.10 (s, 1H), 7.23 (t, 1H), 7.06 (d, *J* = 4.2 Hz, 1H), 6.76 (m, 1H), 6.71 (m, 1H), 4.45 (br s, 1H), 3.13 (m, 2H), 2.54 (s, 3H), 1.55 (m, 2H), 1.36 (m, 2H), 0.89 (m, 3H); ^{13}C NMR (75 MHz, acetone-*d*$_6$) δ 150.7, 147.2, 131.7, 130.2, 129.8, 128.8, 125.4, 117.2, 115.3, 111.2, 44.1, 31.9, 21.0, 14.2, 12.4; LRMS (ESI+) *m/z* cald for $C_{17}H_{21}N_4^+$ $[M+H]^+$: 281.18; Found: 281.10.

Compound 22, *N*-benzyl-2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)aniline

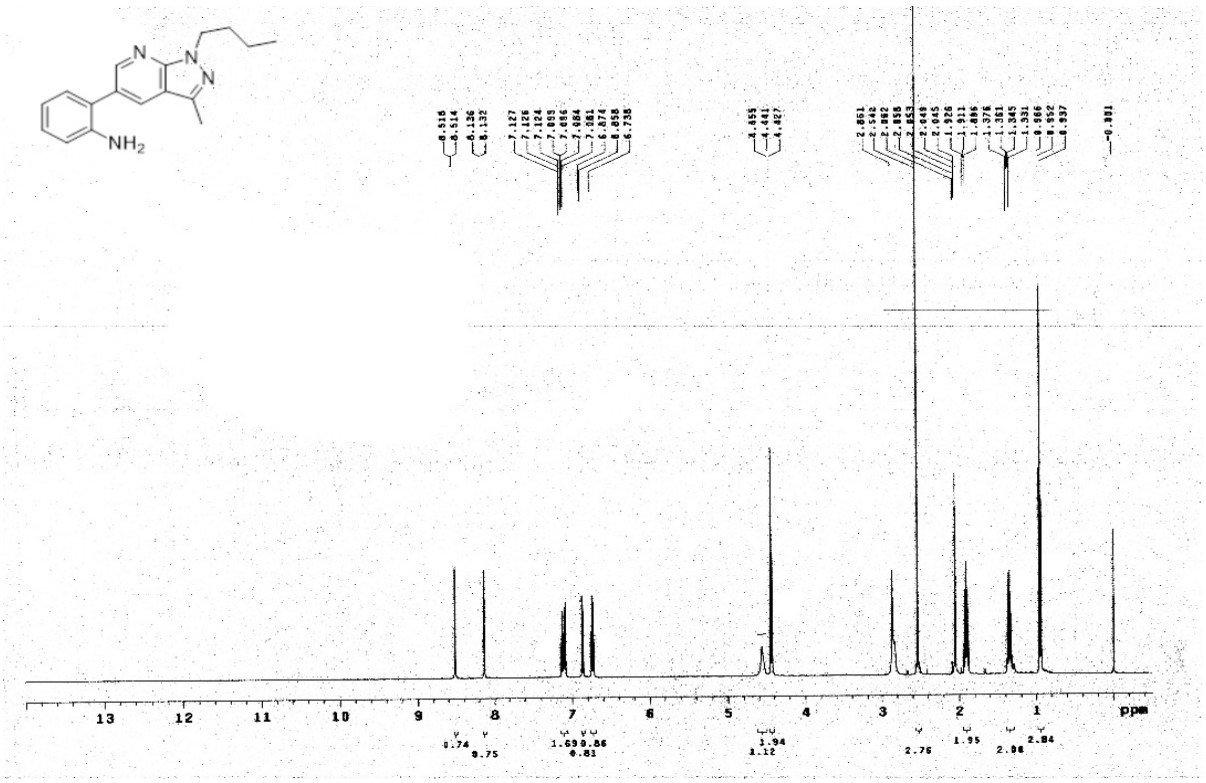
To a solution of *N*-benzylindole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was concentrated under reduced pressure, and then purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. The desired product was gained as a white solid. R_f=0.6 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.24 (br s, 1H), 8.47 (s, 1H), 8.17 (s, 1H), 7.31 (m, 2H), 7.28 (m, 2H), 7.18 (s, 1H), 7.06 (t, 1H), 7.01 (d, *J* = 4.2 Hz, 1H), 6.63 (t, 1H), 6.48 (d, *J* = 4.8 Hz, 1H), 5.58 (br s, 1H), 4.26 (d, *J* = 2.1 Hz, 2H), 2.51 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 152.4, 150.2, 146.3, 141.0, 131.5, 130.3, 129.3, 129.0, 128.0, 127.5, 127.2, 125.0, 116.9, 114.8, 111.4, 47.1, 12.9; LRMS (ESI+) *m/z* cald for $C_{20}H_{19}N_4^+$ $[M+H]^+$: 315.16; Found: 315.05.

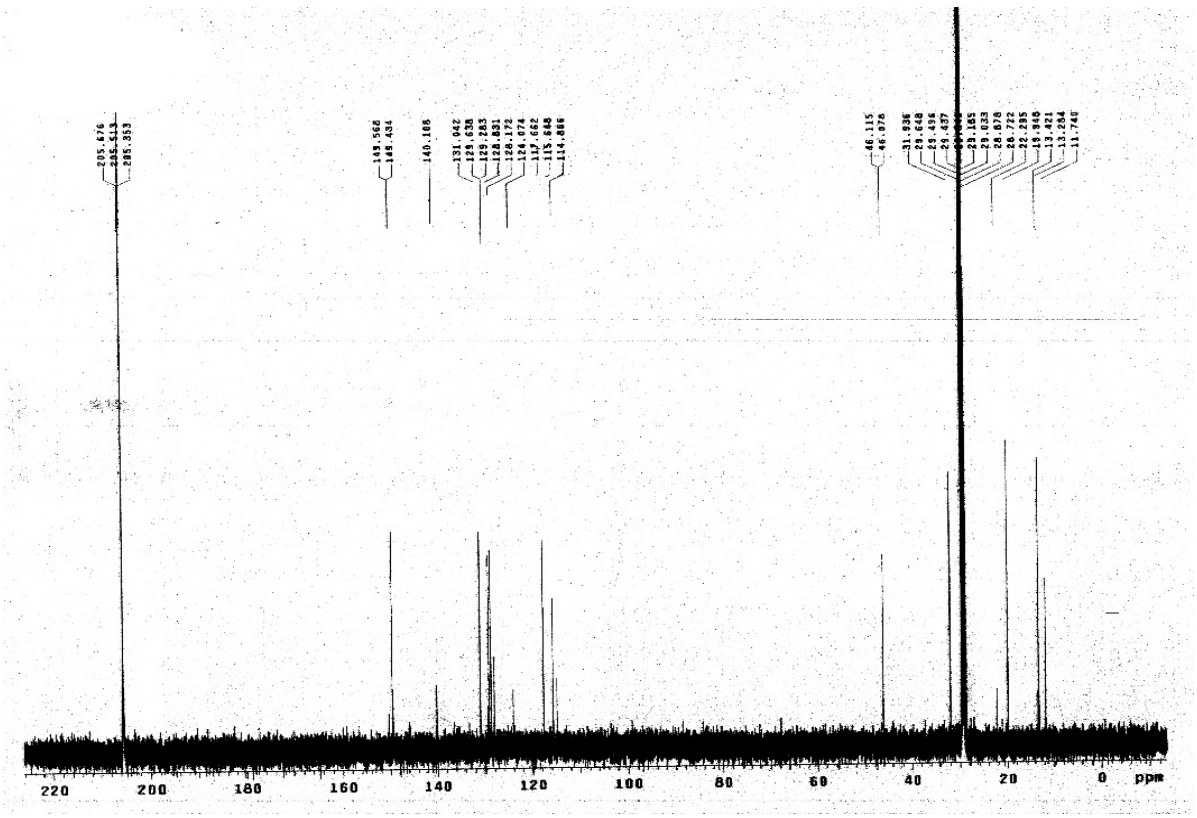
Compound 23, benzyl-(2-(3-methyl-1*H*-pyrazolo[3,4-b]pyridin-5-yl)phenyl)carbamate

To a solution of *N*-Cbz-indole-3-carboxaldehyde and 3-amino-5-methylpyrazole (1.0 equiv.) in MeOH (50 mM) was added aluminum trichloride (10 mol%), and the reaction mixture was stirred under reflux condition of 70 ºC in 20 mL-vial for 3~5 h. After the completion of the reaction monitored by TLC, the reaction mixture was cooled to the ambient temperature. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The combined organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The filtrate was concentrated under reduced pressure, and then purified by silica-gel flash column chromatography using EtOAc in hexane as eluent system. The desired product was obtained as a white solid. R_f=0.5 (EA:Hexane:MeOH=20:20:1), ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 13.30 (br s, 1H), 8.41 (s, 1H), 8.16 (s, 1H), 7.98 (d, *J* = 4.8 Hz, 1H), 7.57 (br s, 1H), 7.34–7.18 (m, 6H), 7.11 (t,1H), 6.89 (br s, 1H), 4.22 (d, *J* = 3.3 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 156.1, 152.4, 150.1, 149.9, 141.9, 140.8, 138.1, 130.4, 129.1, 128.9, 128.8, 127.9, 127.6, 127.4, 127.2, 122.6, 114.7, 43.4, 12.9; LRMS (ESI+) *m/z* cald for $C_{21}H_{19}N_4O_2^+$ $[M+H]^+$: 358.17; Found: 358.05.

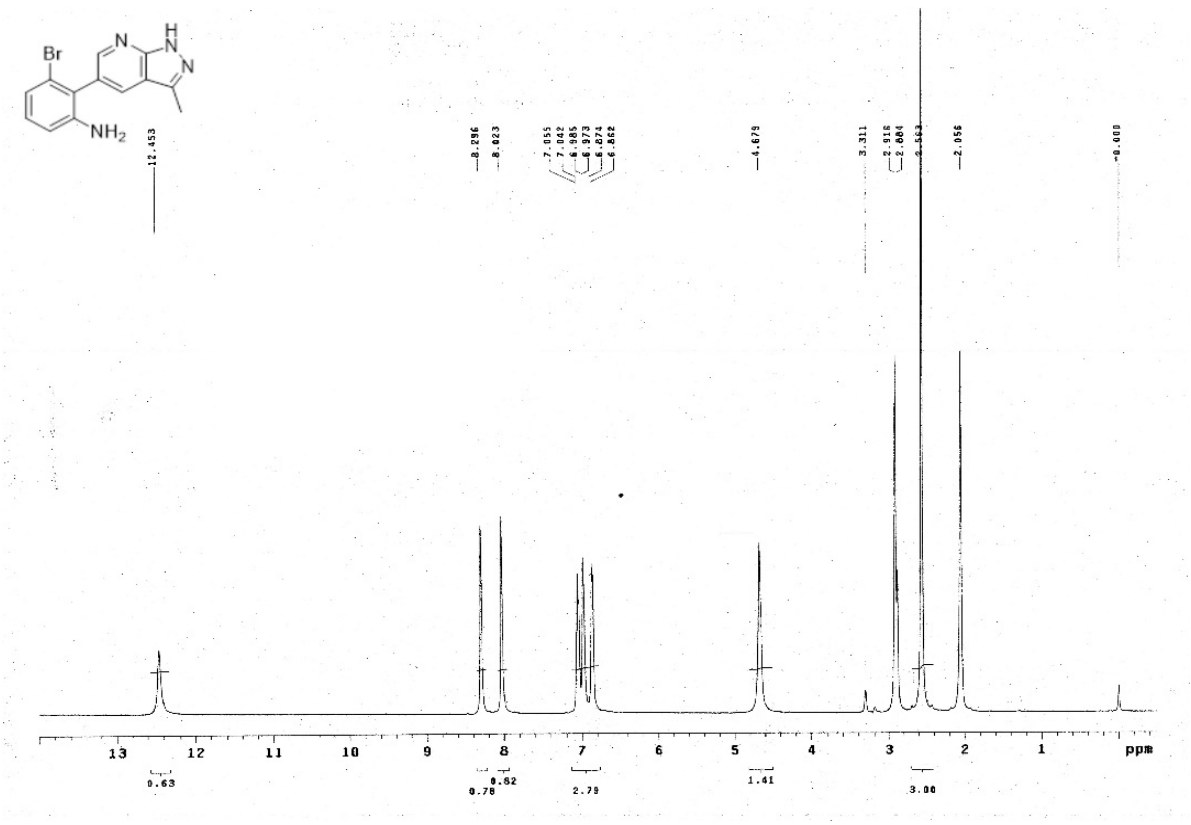
IV. ^{1}H and ^{13}C NMR Spectra

Compound 6





Compound 7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2.563
2.056
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13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
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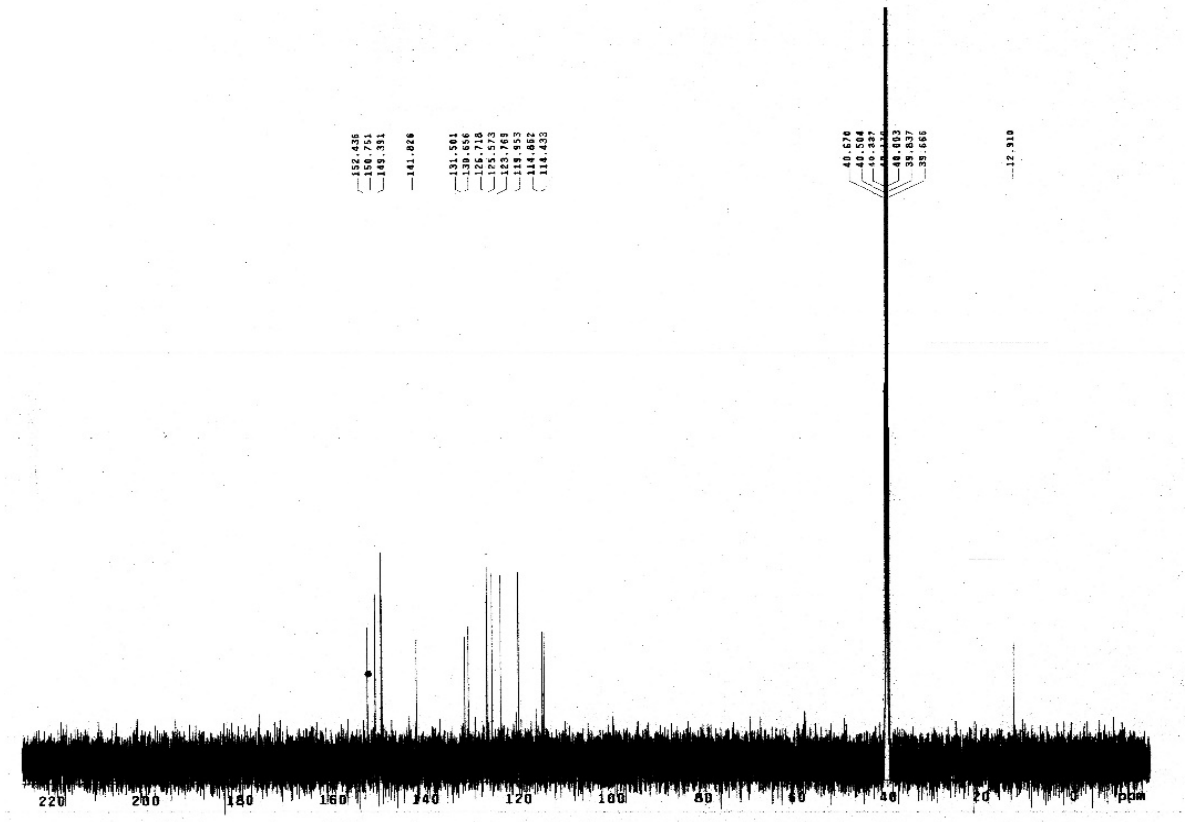
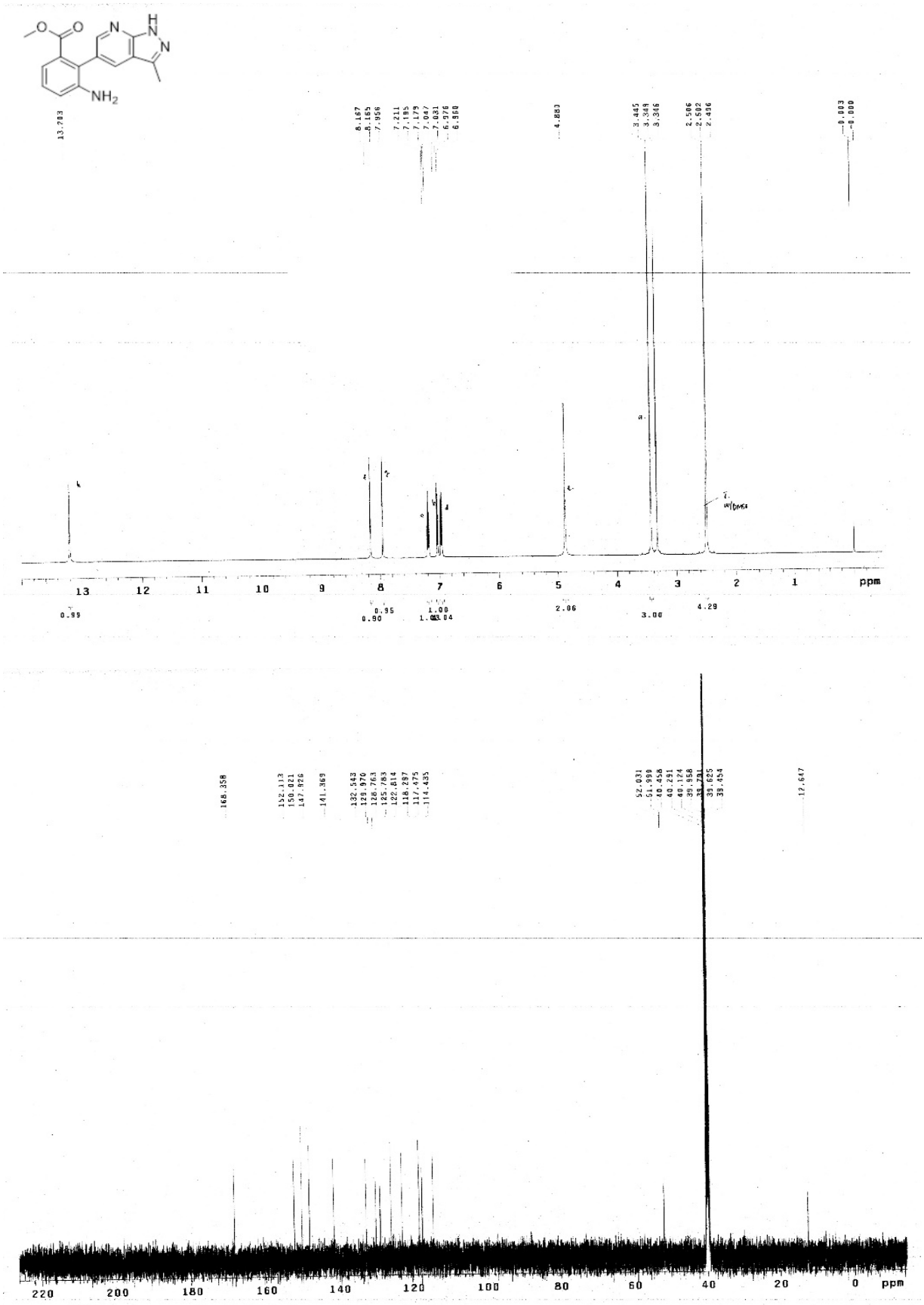
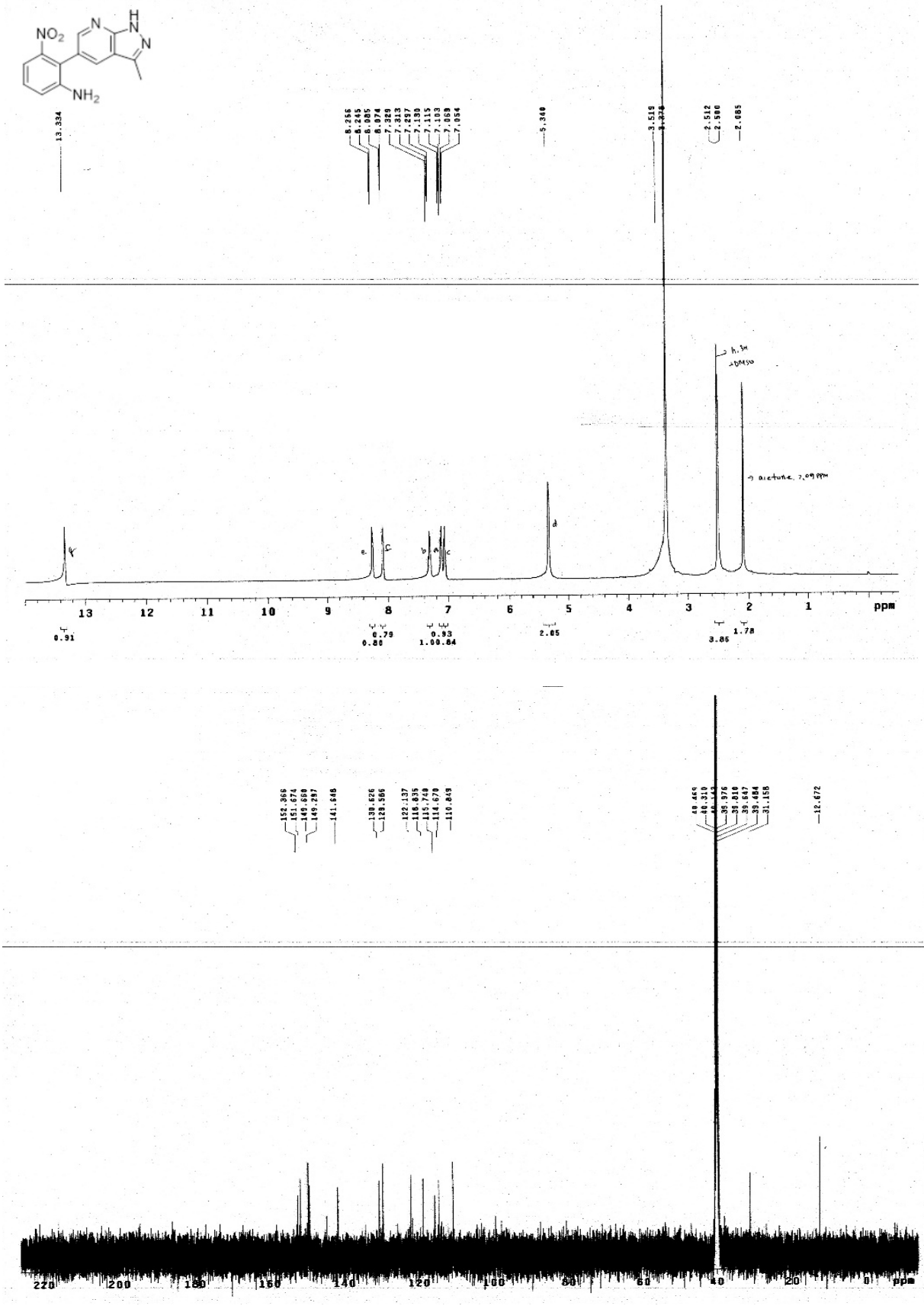
0.78
0.82
2.79
1.41
3.00


152.436
150.751
149.391
141.026
131.501
130.656
126.718
125.573
123.769
119.953
114.662
114.433
40.670
40.504
40.337
40.170
40.003
39.837
39.666
12.910
220
200
180
160
140
120
100
80
60
40
20
0
ppm

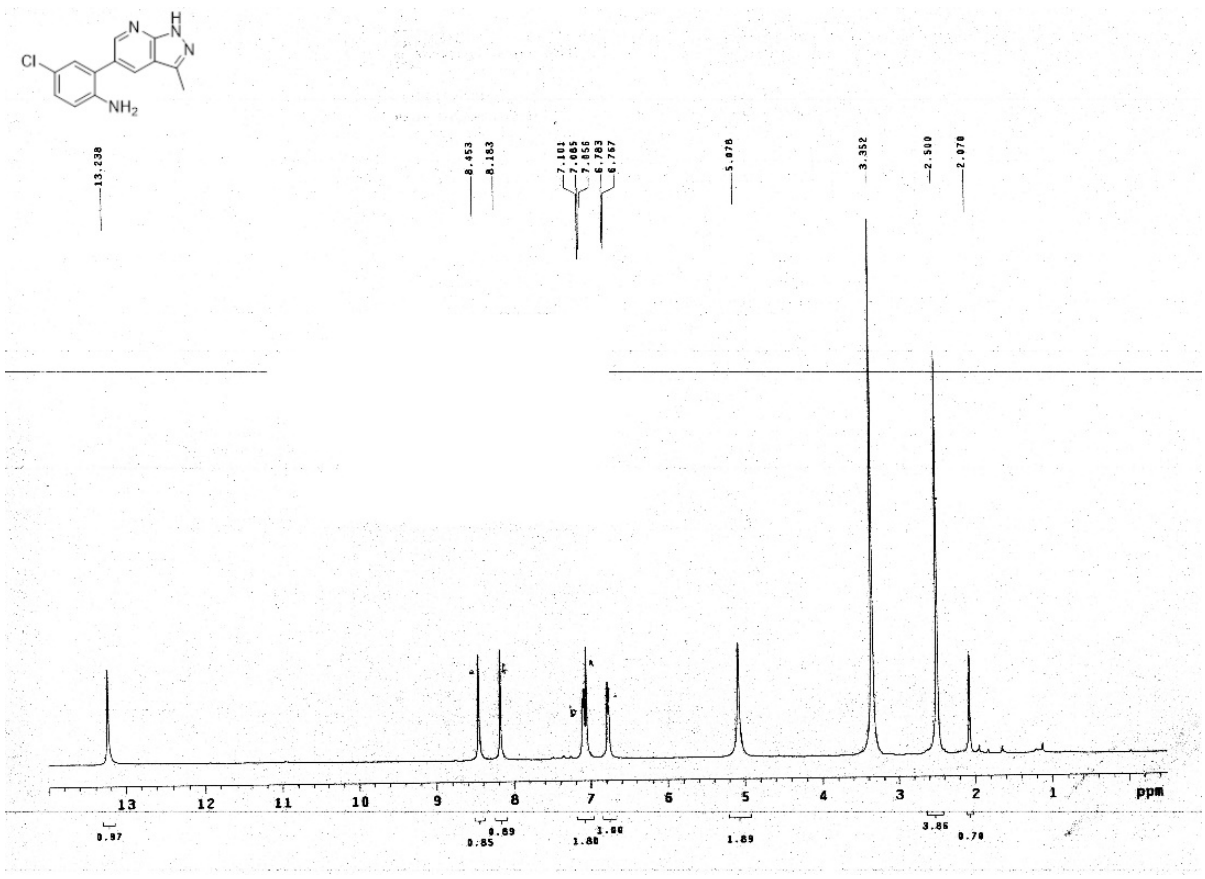
Compound 8

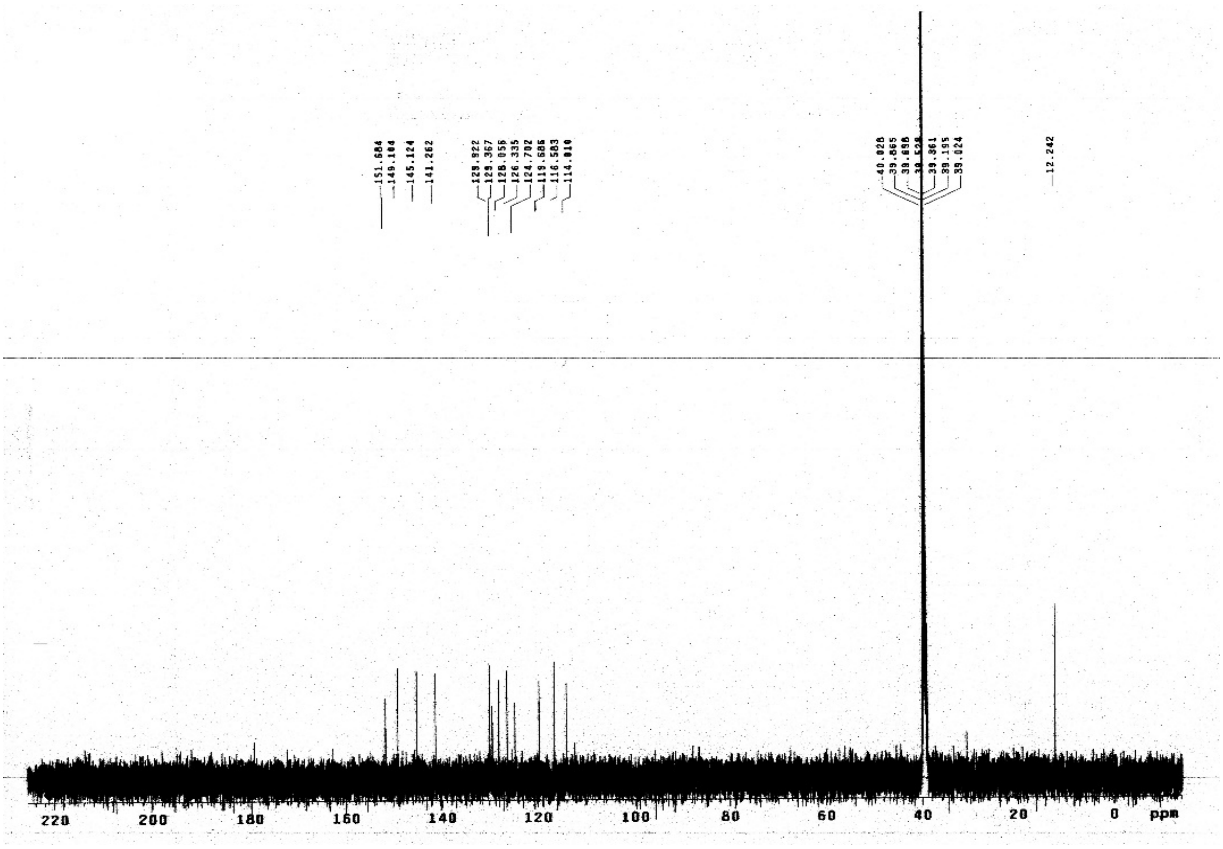

13.703
8.167
8.165
7.956
7.211
7.195
7.179
7.047
7.031
6.376
6.360
4.880
3.445
3.349
3.346
2.506
2.502
2.496
0.003
0.000
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.99
0.95
0.90
1.00
1.04
2.06
3.00
4.29
168.358
152.113
150.021
147.926
141.369
132.543
129.976
126.763
125.783
122.814
118.287
117.475
114.435
52.031
51.990
40.458
40.291
40.124
39.958
39.791
39.625
39.454
12.647
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 9

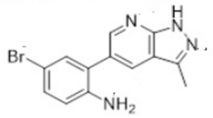

NO2
NH2
N
H
N
13.334
8.256
8.245
8.085
8.074
7.329
7.313
7.297
7.130
7.115
7.103
7.069
7.054
5.340
3.519
3.376
2.512
2.500
2.085
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.91
0.79
0.80
0.93
1.00.84
2.85
3.86
1.78
153.366
151.674
149.668
149.297
141.646
130.626
128.566
122.137
116.835
115.749
114.670
110.849
39.976
39.810
39.647
39.484
31.158
12.672
220
200
180
160
140
120
100
80
60
40
20
0
ppm

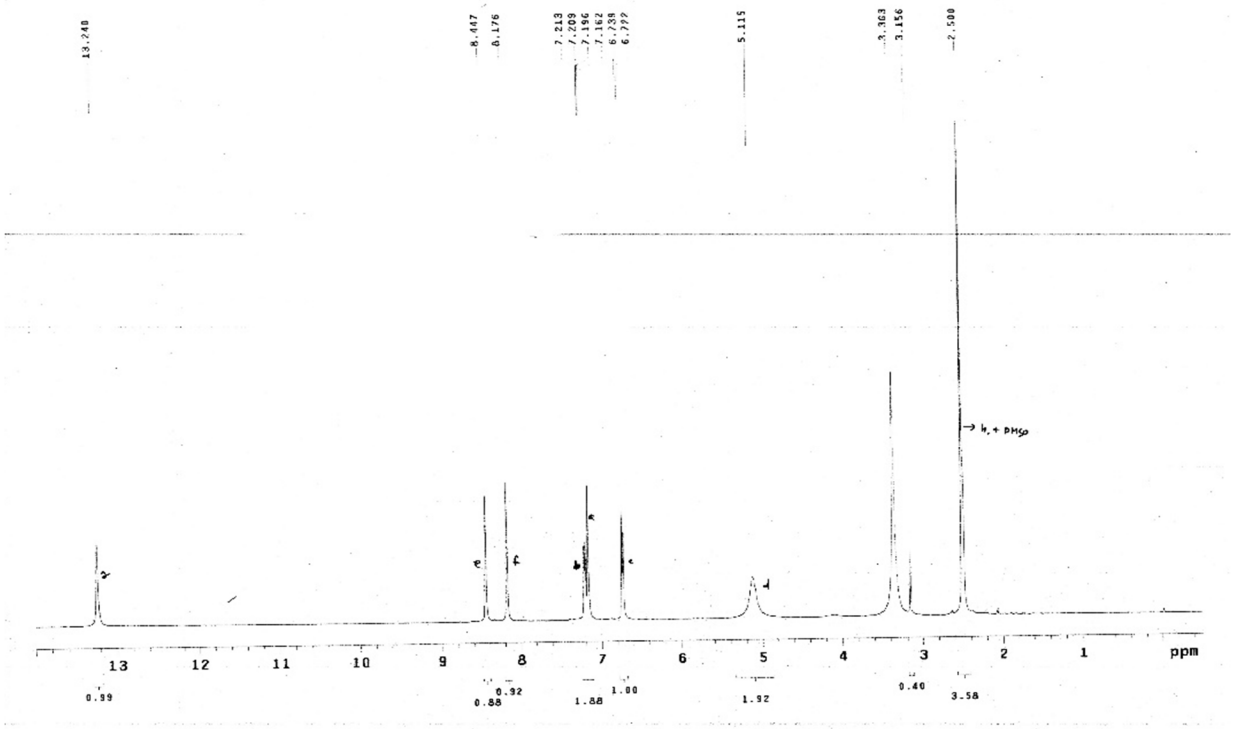
Compound 10

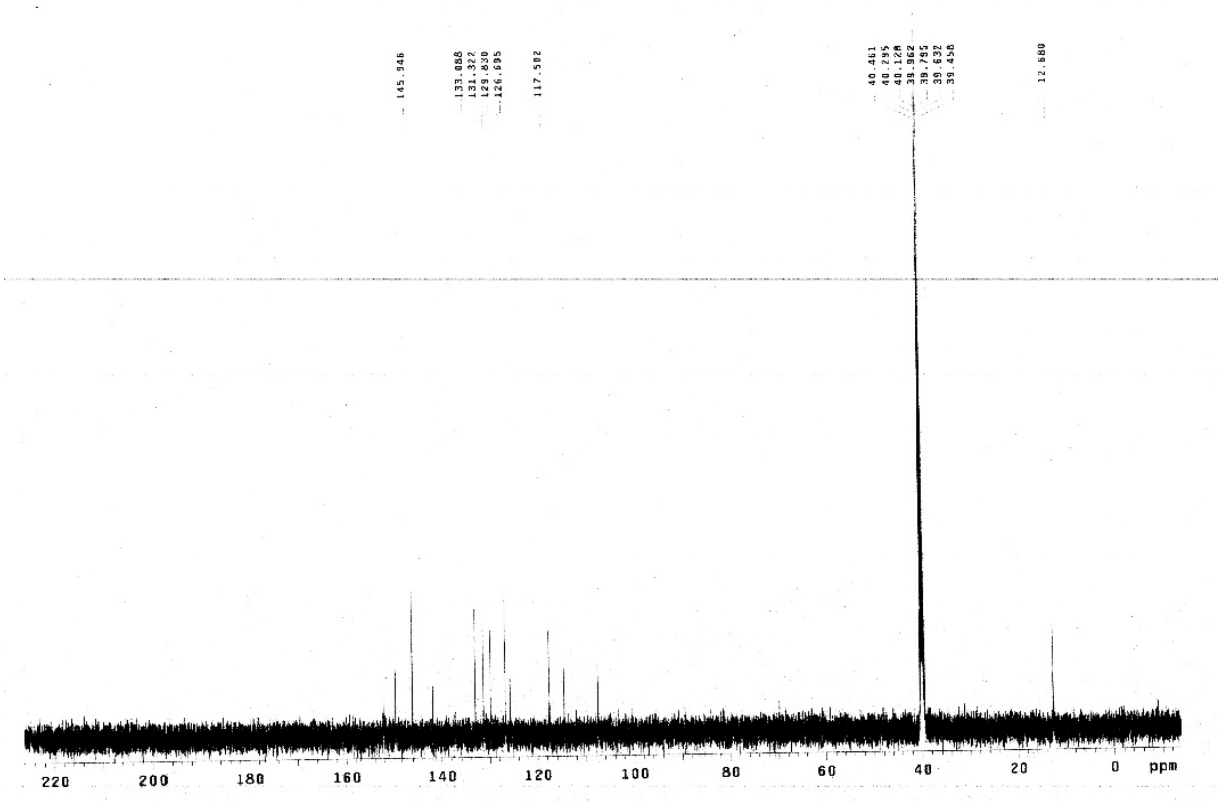

Cl
NH2
N
H
N
13.238
8.453
8.183
7.101
7.085
7.056
6.783
6.767
5.078
3.352
2.510
2.078
ppm


151.684
149.104
145.124
141.262
129.922
129.367
128.056
126.335
124.782
119.686
116.583
114.810
40.828
39.665
39.456
39.247
39.038
38.829
38.620
12.242
ppm

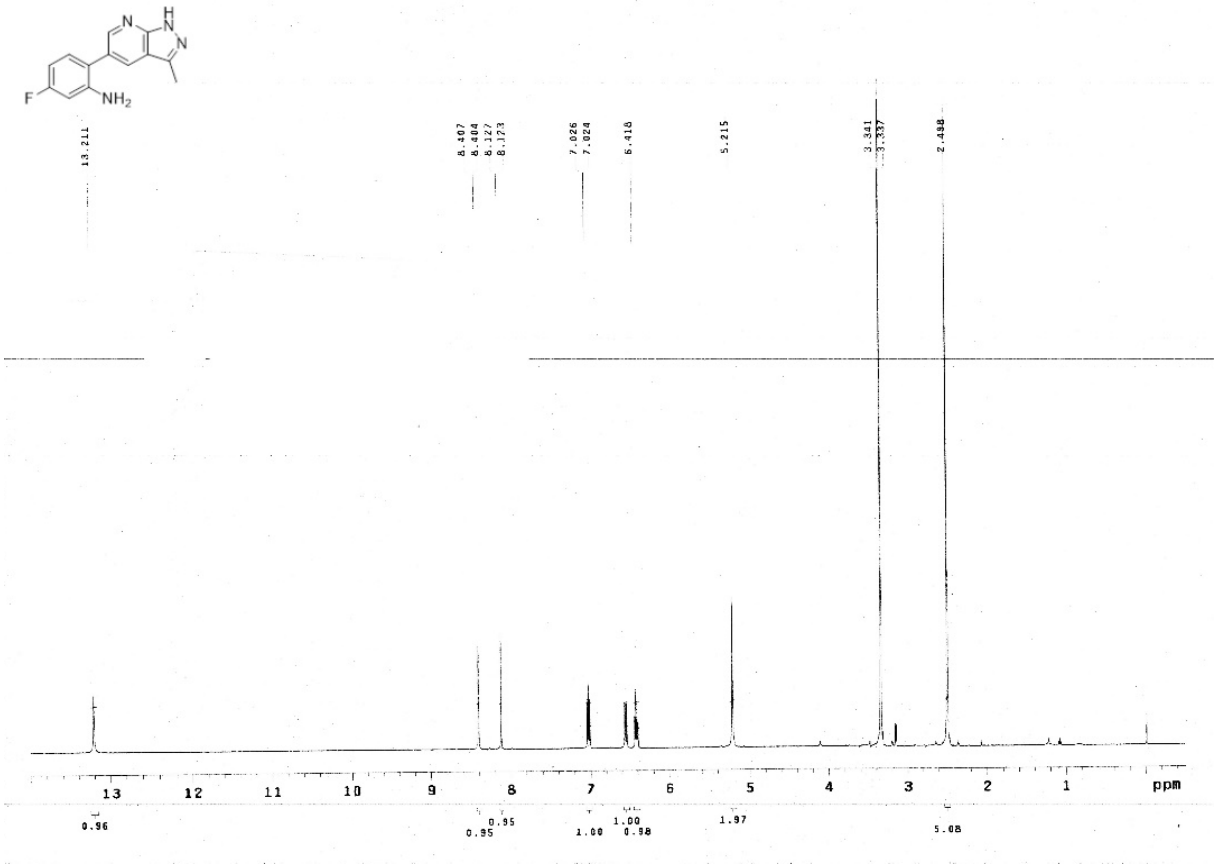
Compound 11

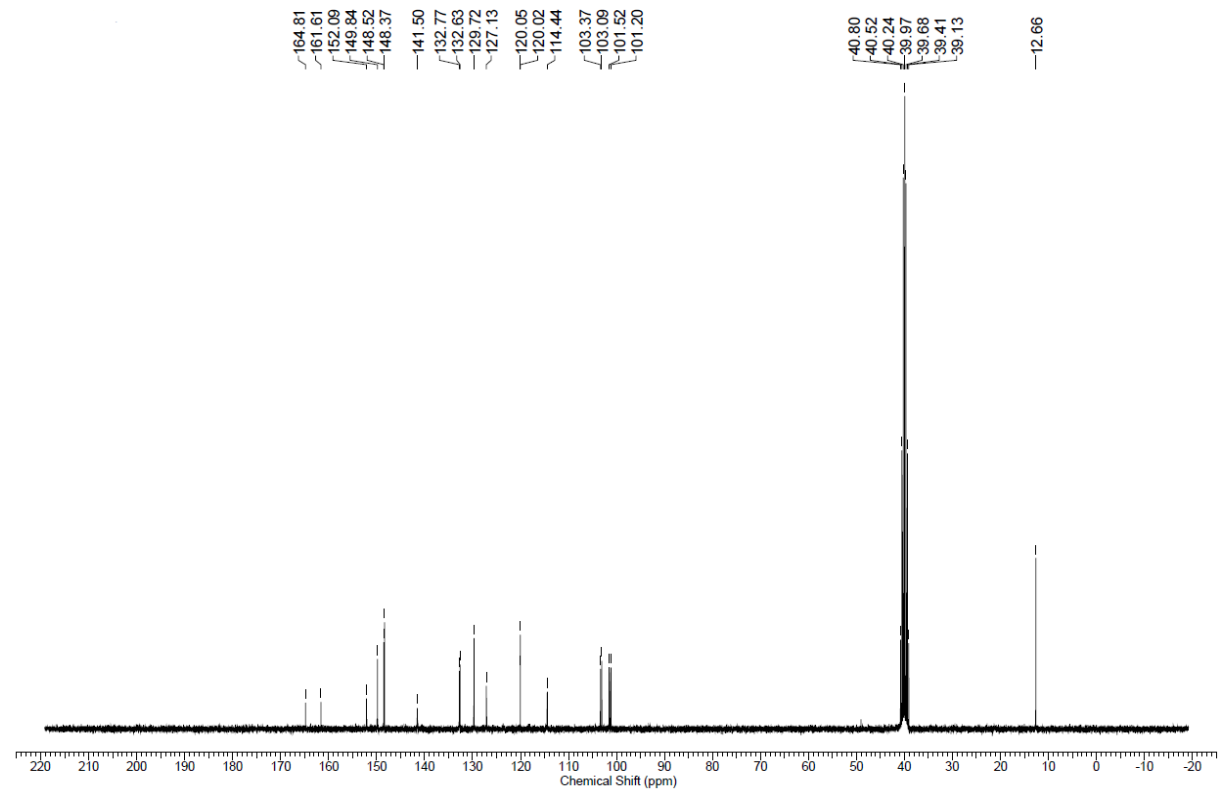

Br
N
H
N
N
NH2


13.240
8.447
8.176
7.213
7.209
7.196
7.162
6.788
6.779
5.115
3.363
3.156
2.500
ppm
0.99
0.88
0.92
1.88
1.00
1.92
0.40
3.58

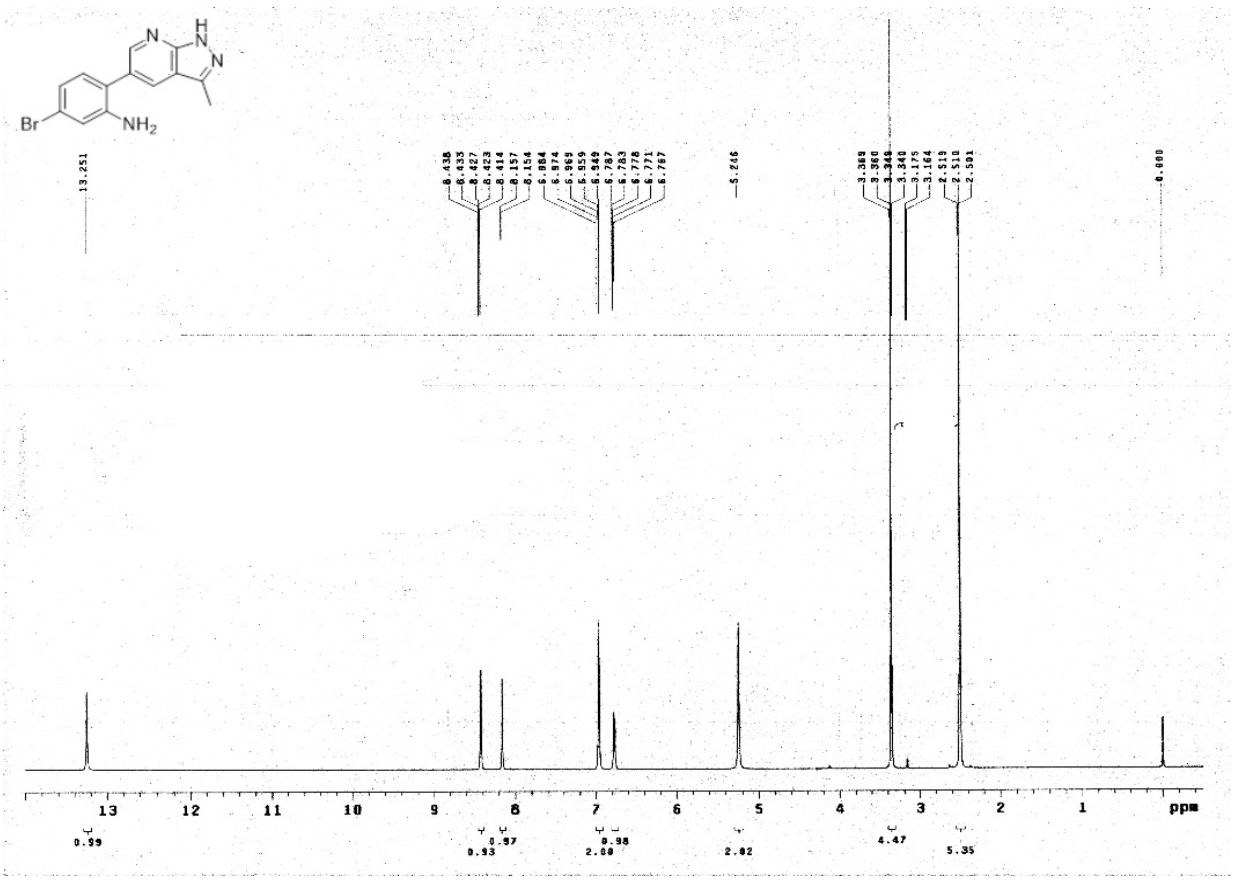

145.946
133.868
131.327
129.348
126.695
117.582
40.461
40.295
40.128
39.962
39.795
39.632
39.458
12.680
ppm

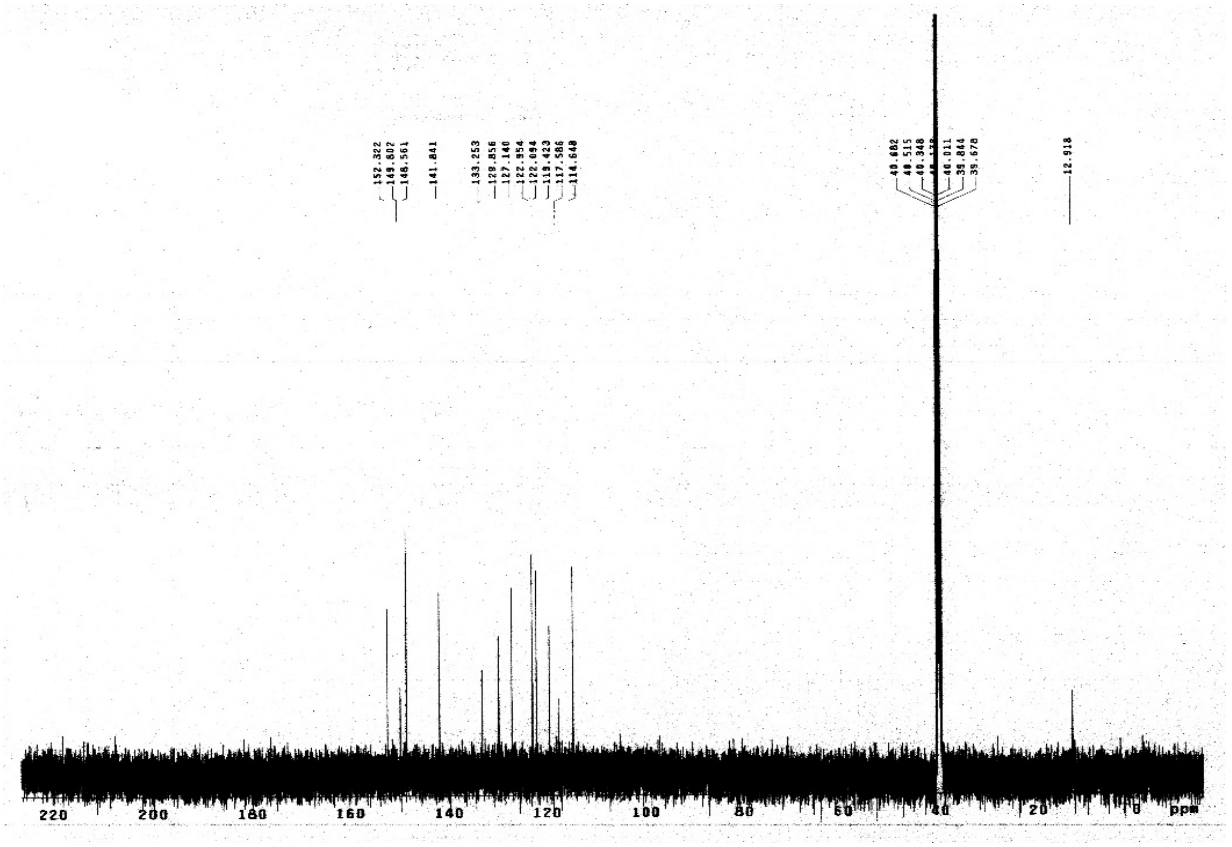
Compound 12


13.211
8.407
8.404
8.127
8.123
7.026
7.024
6.418
5.215
3.341
3.337
2.498
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.96
0.95
0.95
1.00
1.00
0.98
1.97
5.08

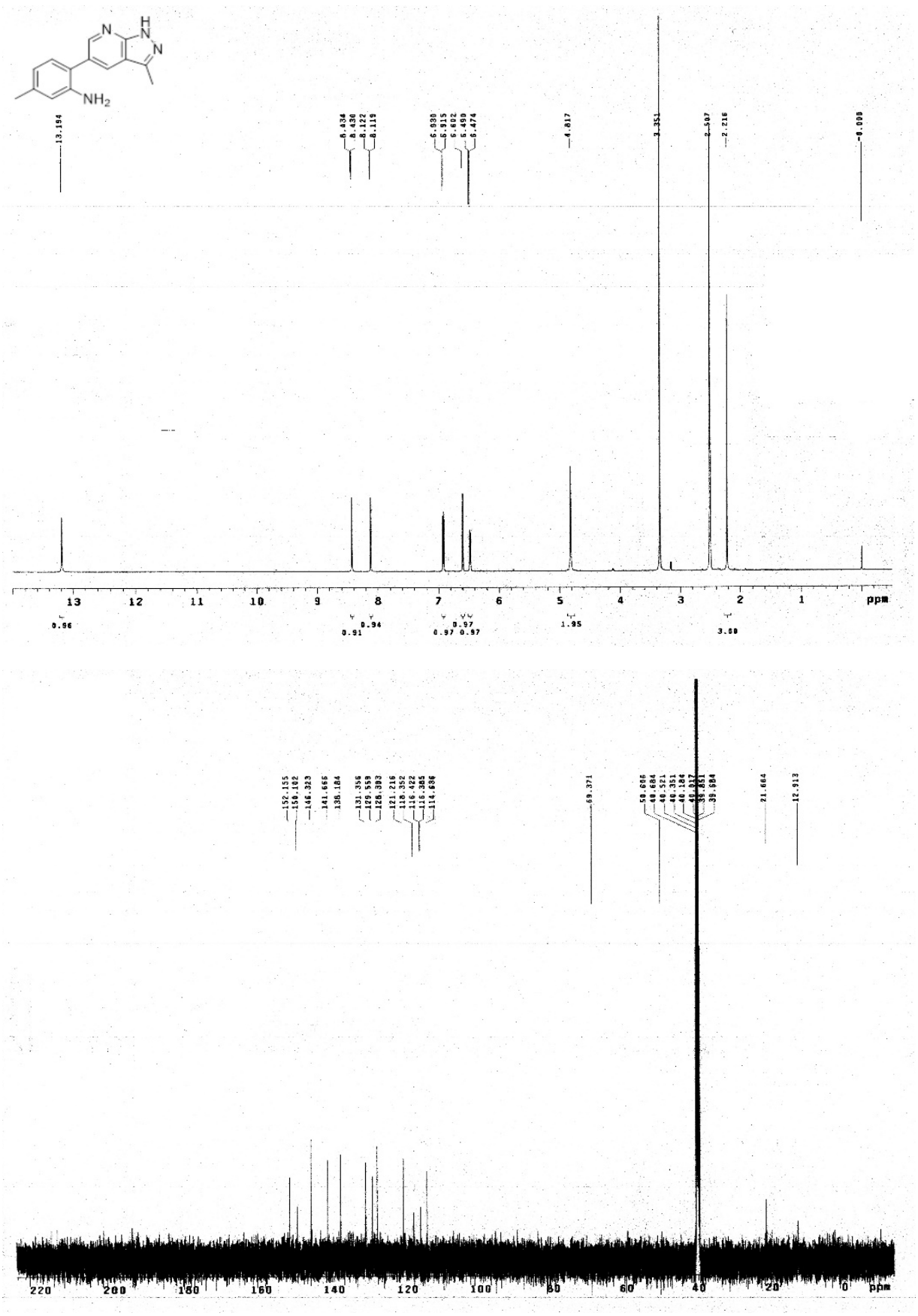

164.81
161.61
152.09
149.84
148.52
148.37
141.50
132.77
132.63
129.72
127.13
120.05
120.02
114.44
103.37
103.09
101.52
101.20
40.80
40.52
40.24
39.97
39.68
39.41
39.13
12.66
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
Chemical Shift (ppm)

Compound 13

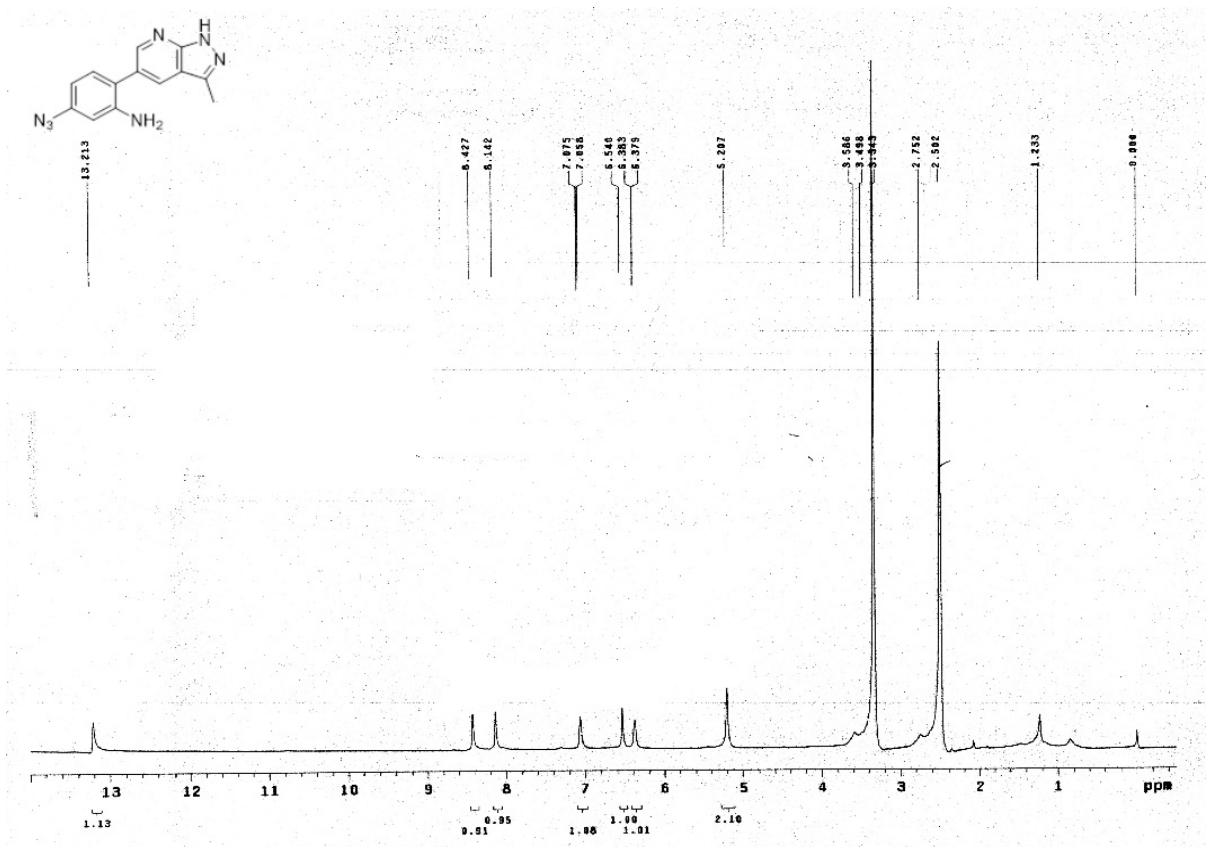

Br
NH2
N
H
N
13.251
8.438
8.433
8.427
8.423
8.414
8.157
8.154
6.984
6.974
5.969
5.959
5.949
5.940
5.787
6.783
6.778
6.771
6.767
5.246
3.368
3.360
3.346
3.340
3.175
3.164
2.519
2.510
2.501
0.000
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.99
0.93
0.97
2.00
0.98
2.02
4.47
5.35

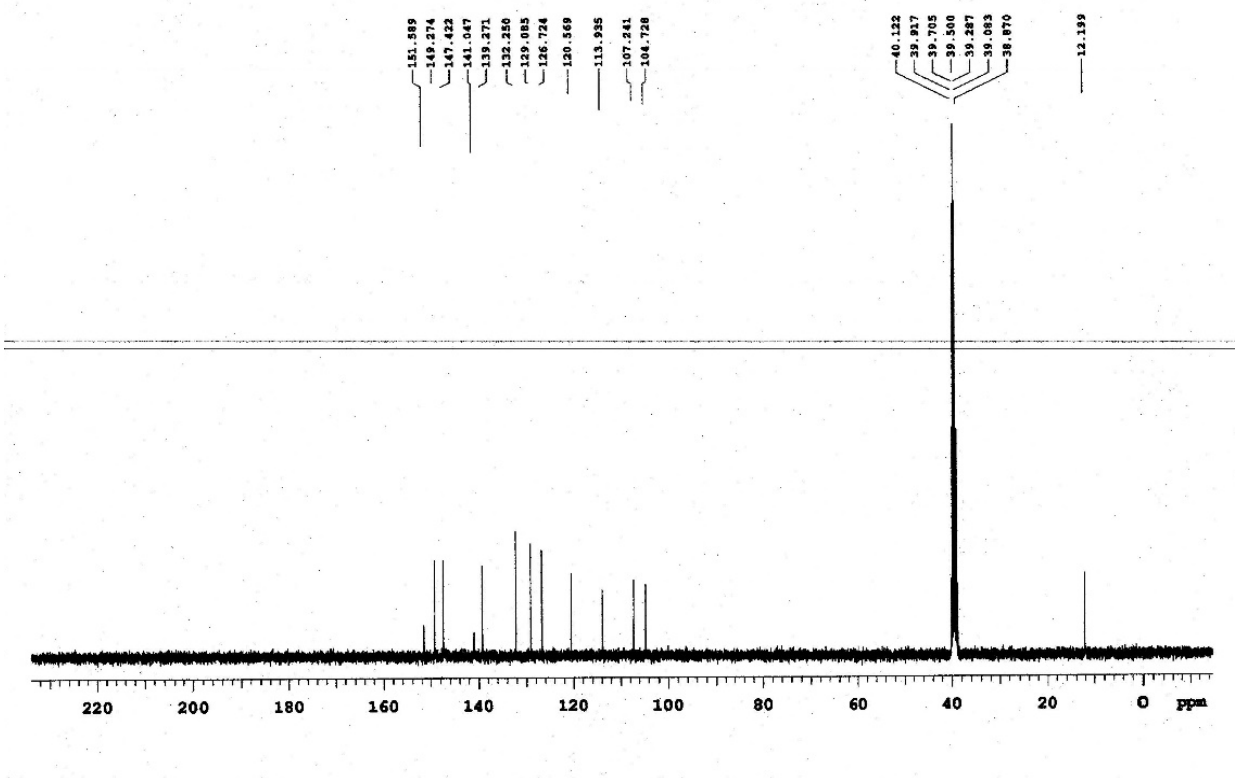

152.322
149.807
148.561
141.841
133.253
129.856
127.140
122.954
122.094
119.423
117.586
114.640
49.682
49.515
49.348
49.181
48.844
48.677
48.511
39.044
39.678
12.918
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 14

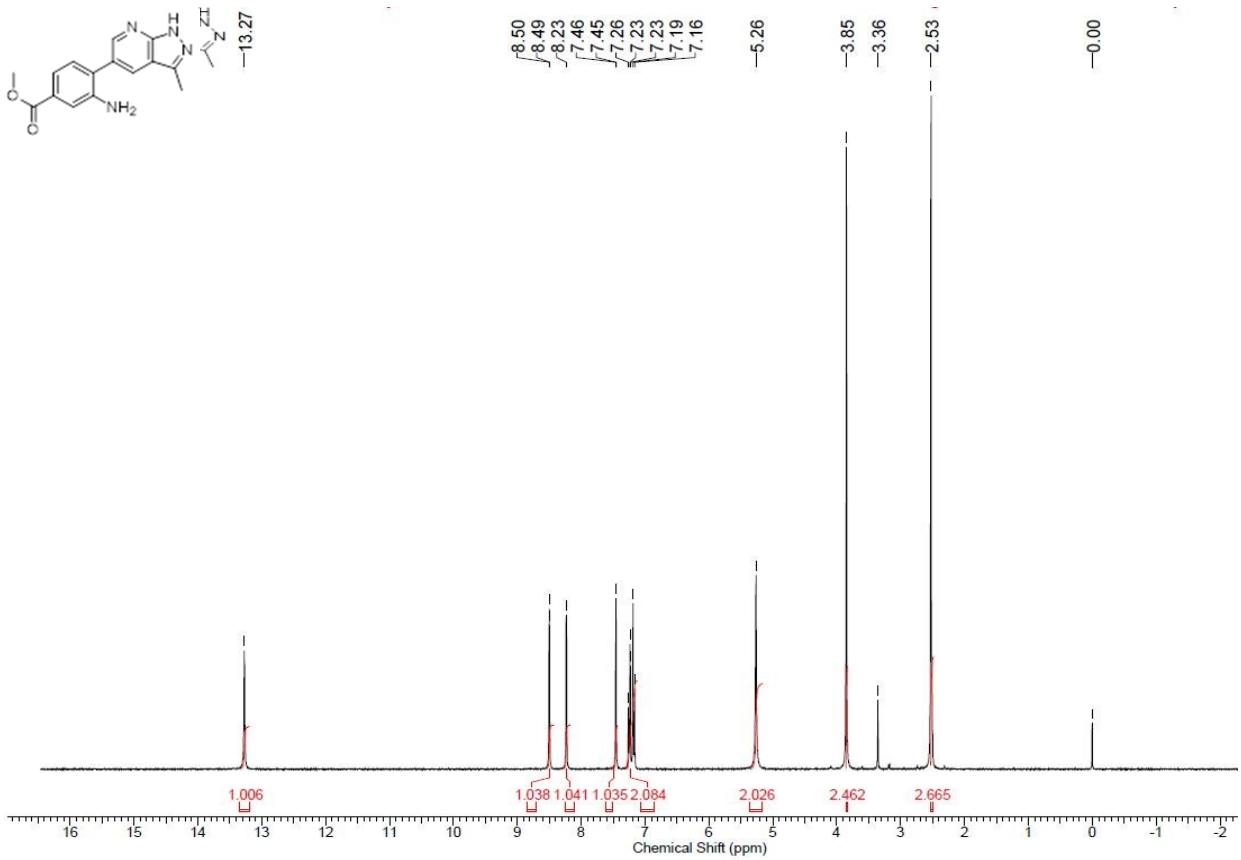

N
H
N
N
NH2
13.194
8.434
8.430
8.122
8.118
6.938
6.915
6.602
6.490
6.474
4.817
3.351
2.507
2.216
0.000
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.96
0.91
0.94
0.97
0.97
0.97
1.95
3.00
152.155
150.102
146.323
141.665
136.184
131.356
129.559
126.303
121.216
118.352
116.422
116.385
114.696
69.371
50.686
40.684
40.521
40.351
40.184
39.684
21.664
12.913
220
200
180
160
140
120
100
80
60
40
20
0
ppm

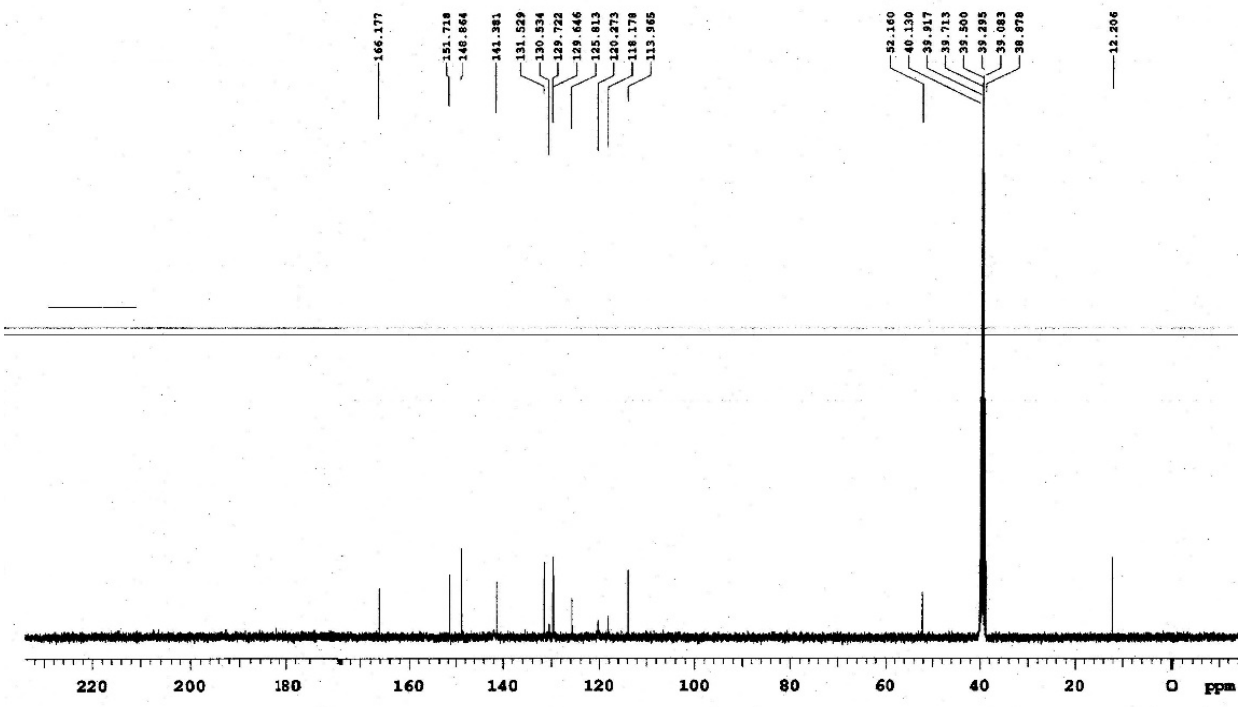
Compound 15


13.213
8.427
8.142
7.875
7.858
6.546
6.383
6.379
5.207
3.586
3.498
3.343
2.752
2.502
1.233
0.884
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.13
0.91
0.95
1.88
1.00
1.01
2.10

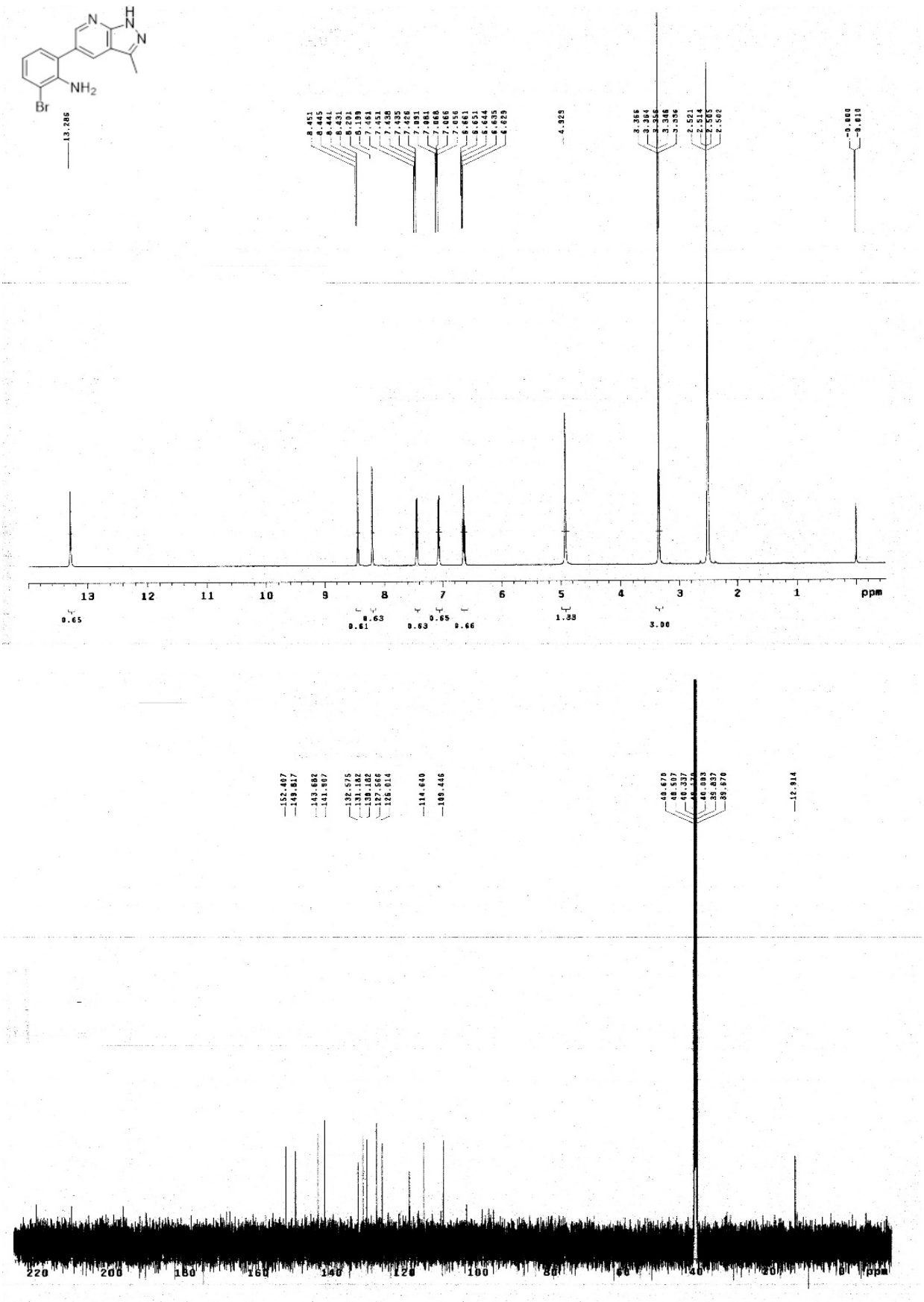

151.589
149.274
147.422
141.047
139.271
132.250
129.085
126.724
120.569
113.995
107.241
104.720
40.122
39.917
39.705
39.500
39.287
39.083
38.870
12.139
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 16

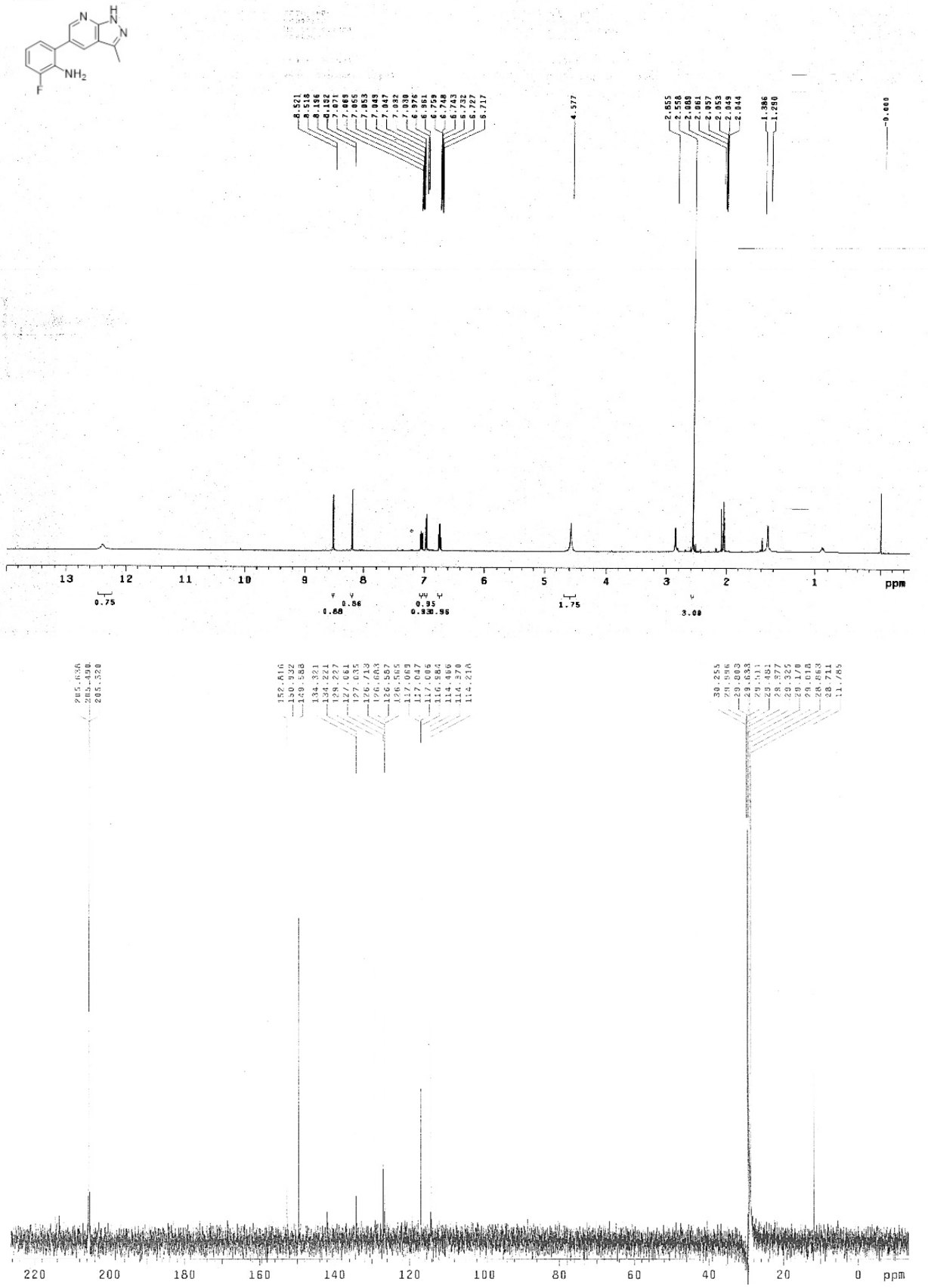

13.27
8.50
8.49
8.23
7.46
7.45
7.26
7.23
7.23
7.19
7.16
5.26
3.85
3.36
2.53
0.00
1.006
1.038
1.041
1.035
2.084
2.026
2.462
2.665
Chemical Shift (ppm)


166.177
151.718
148.864
141.381
131.529
130.534
129.722
129.646
125.613
120.273
118.178
113.965
52.160
40.130
39.917
39.713
39.500
39.295
39.083
38.870
12.206
ppm

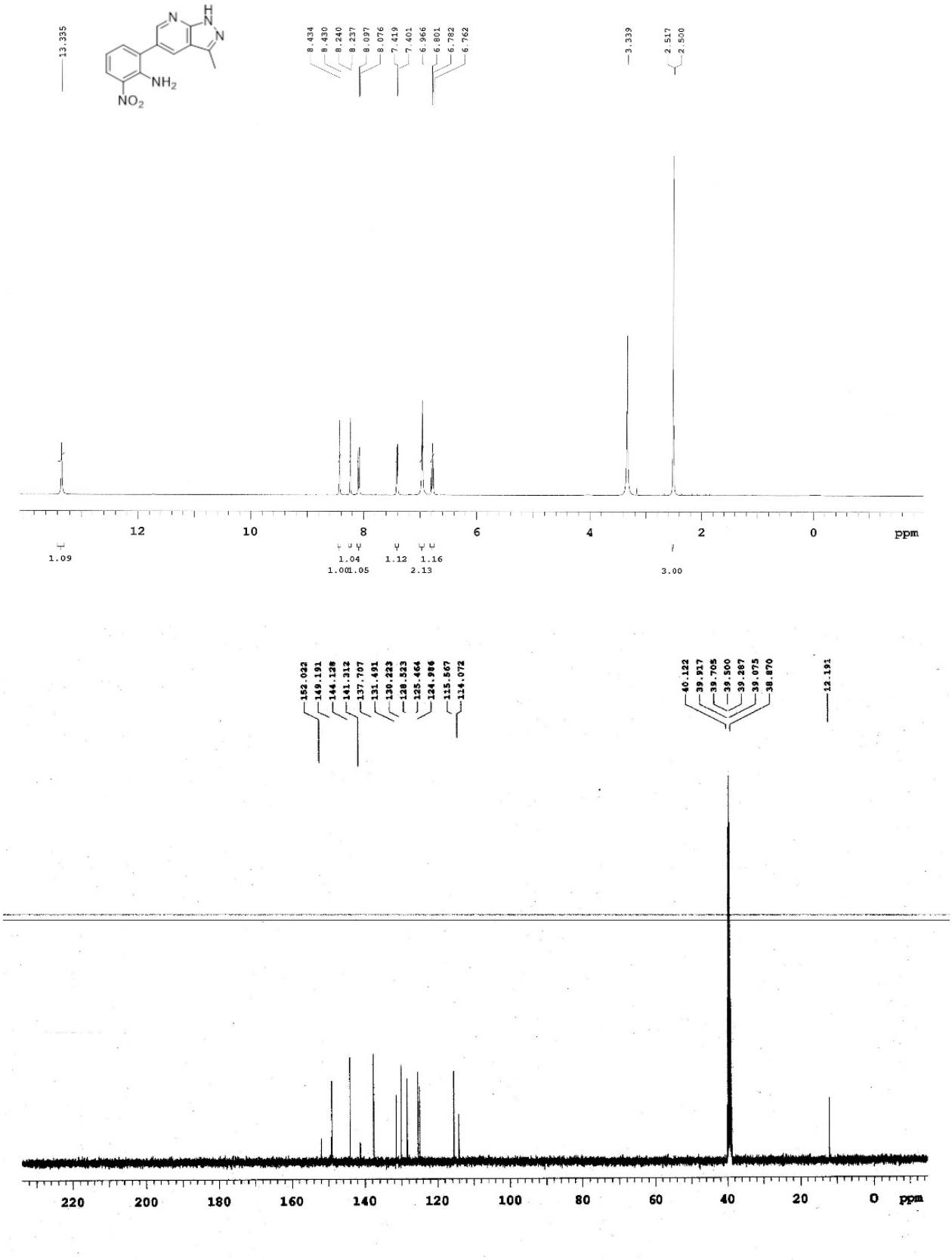
Compound 17


NH2
Br
13.286
8.451
8.445
8.441
8.431
8.201
8.199
7.461
7.451
7.438
7.435
7.426
7.091
7.081
7.068
7.066
7.056
6.651
6.644
6.635
6.629
4.929
3.366
3.364
3.356
3.346
3.336
2.521
2.514
2.505
2.502
-0.000
-0.010
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.65
0.61
0.63
0.63
0.65
0.66
1.33
3.00
152.407
149.817
143.682
141.907
132.575
131.182
130.182
127.566
126.014
114.640
109.446
40.670
40.587
40.337
40.083
39.837
39.670
12.914
220
200
180
160
140
120
100
80
60
40
20
0
ppm

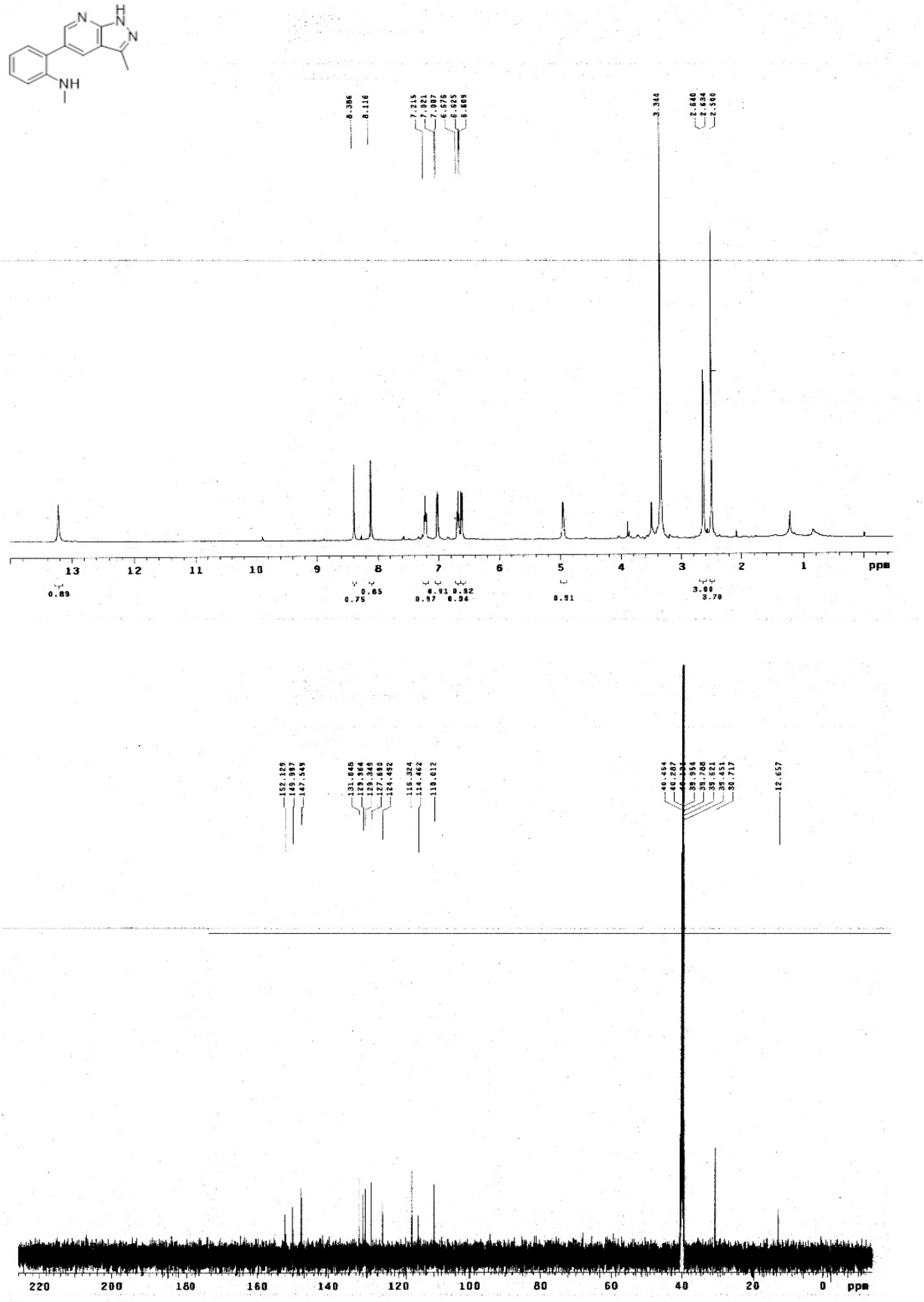
Compound 18



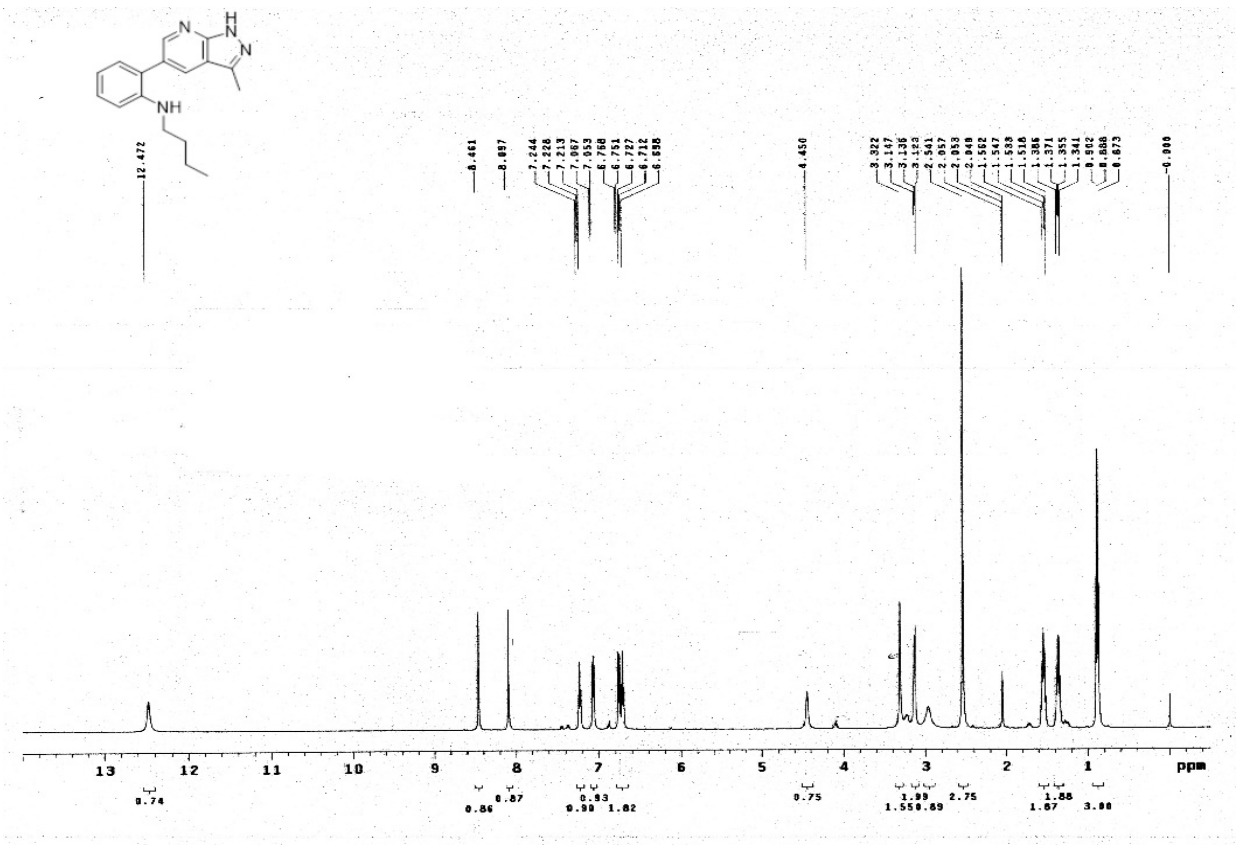
Compound 19

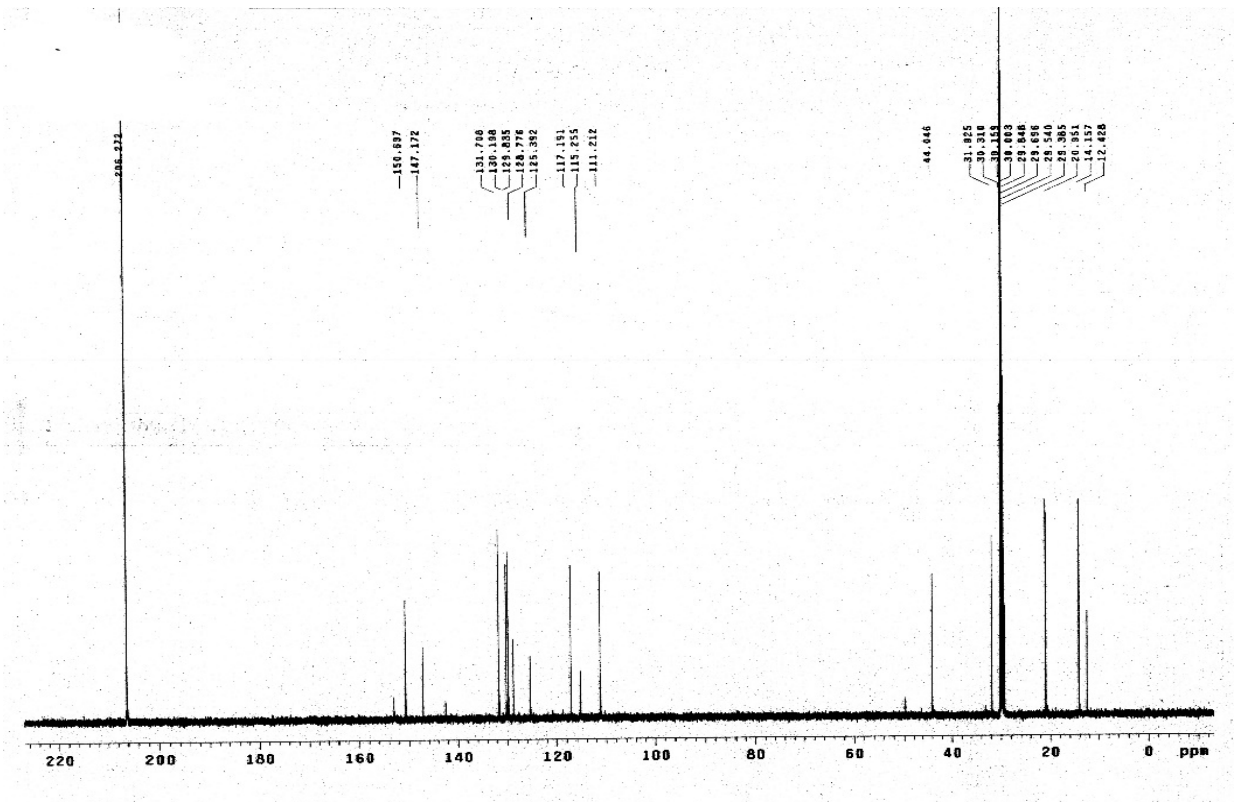

13.335
8.434
8.430
8.240
8.237
8.097
8.076
7.419
7.401
6.966
6.801
6.782
6.762
3.339
2.517
2.500
NH2
NO2
12
10
8
6
4
2
0
ppm
1.09
1.04
1.00
1.05
1.12
1.16
2.13
3.00
152.022
149.191
144.128
141.312
137.707
131.491
130.223
128.523
125.464
124.986
115.567
114.072
40.122
39.917
39.705
39.500
39.287
39.075
38.870
12.191
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 20

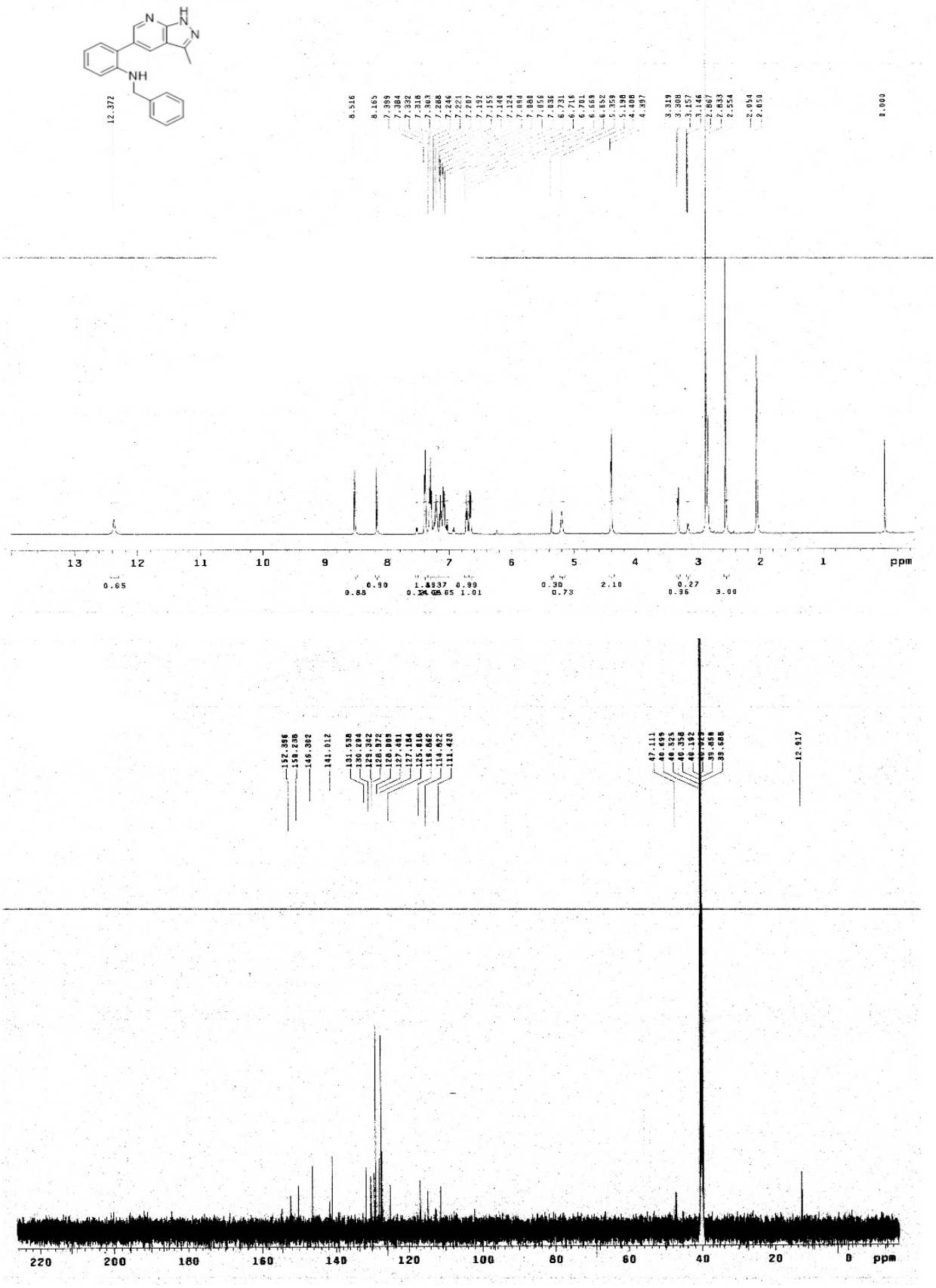

8.386
8.116
7.215
7.021
7.007
6.676
6.825
6.809
3.344
2.640
2.634
2.500
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.89
0.85
0.75
0.91
0.92
0.97
0.94
0.91
3.00
3.70
152.129
149.987
147.549
131.845
129.364
129.346
127.690
124.492
115.324
114.462
110.612
40.464
40.267
40.071
39.954
39.788
39.621
39.451
30.717
12.657
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 21

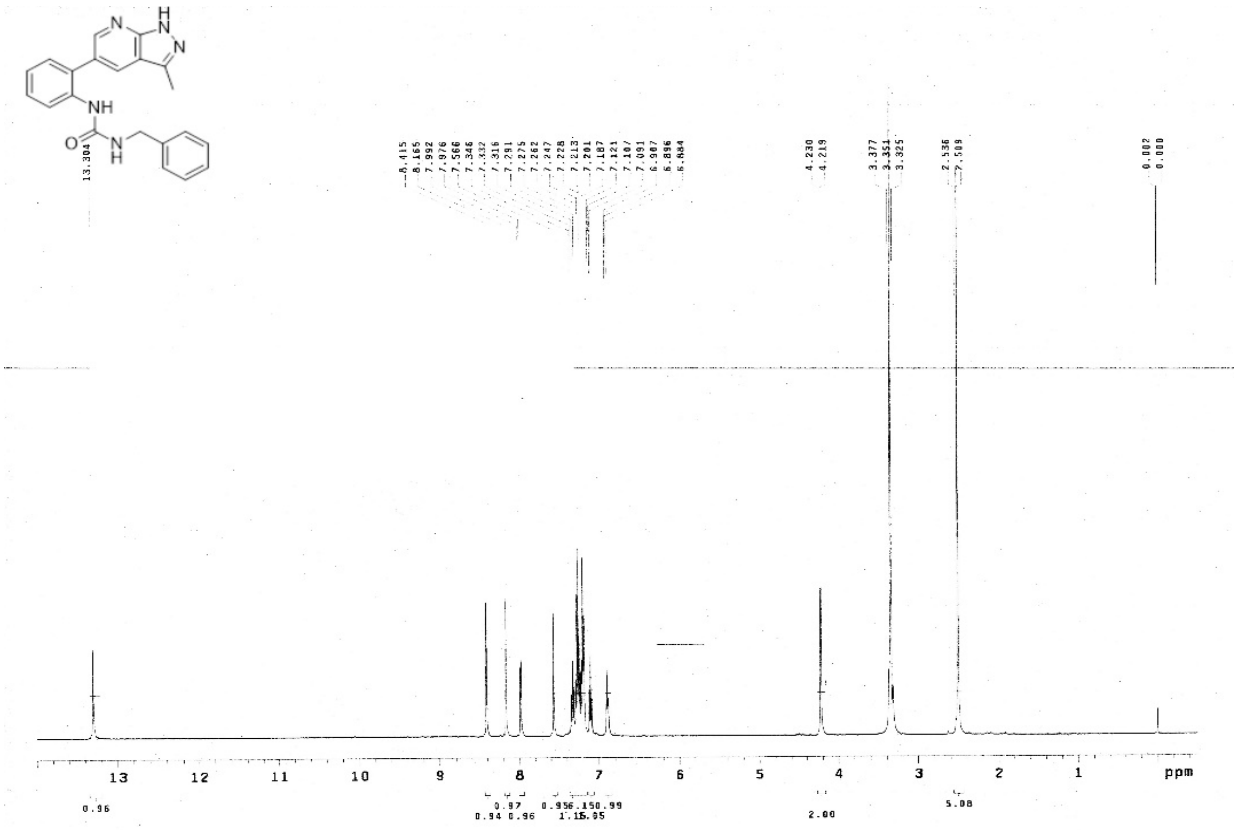


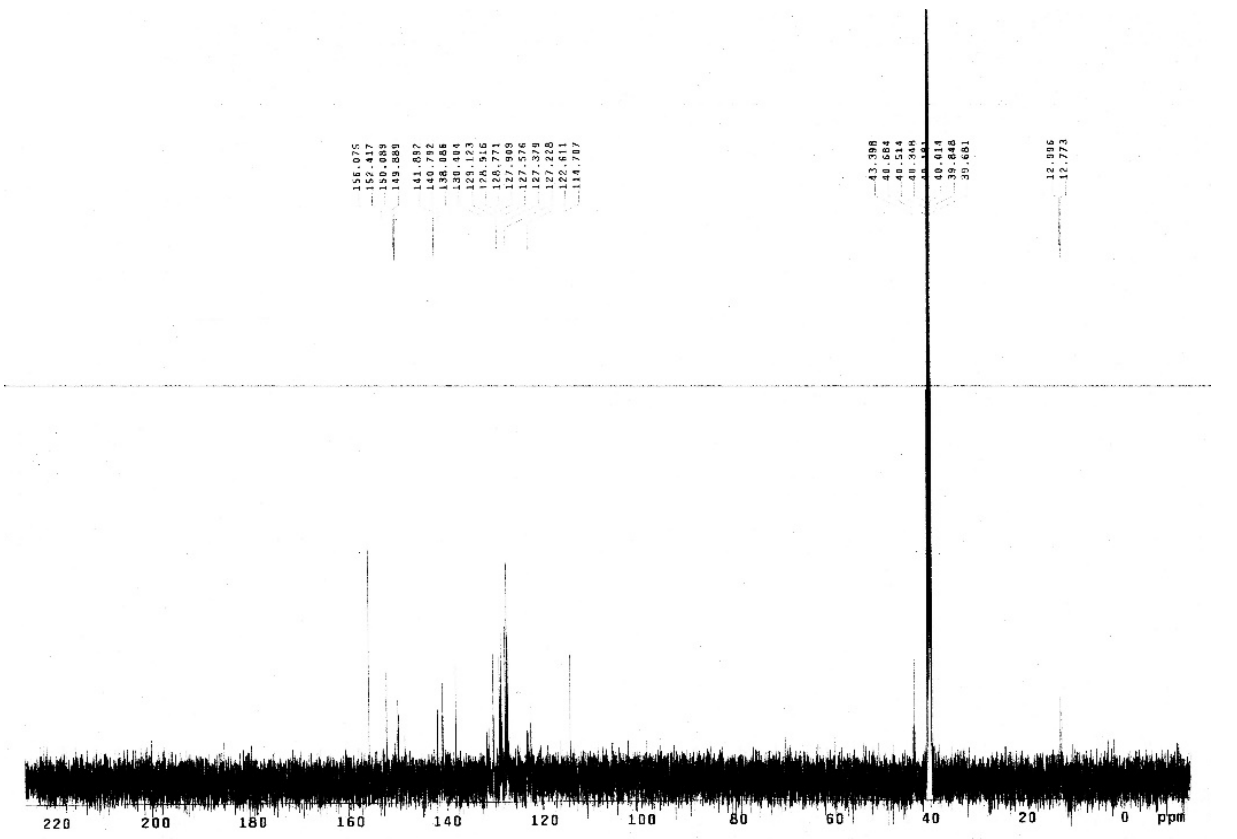


Compound 22



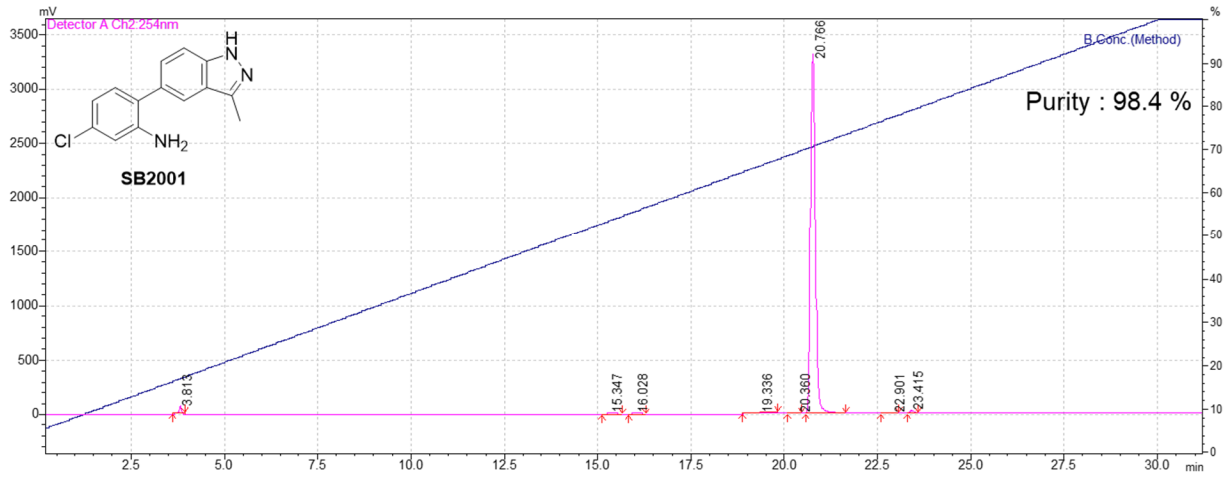
Compound 23





V. HPLC Spectra for Compound Purity

Reverse phase HPLC analysis was performed on a YMC Pack ODS-A C-18 column (250 × 4.6 mm) at a flow rate of 1.0 mL/min, using Shimadzu LC-6 AD pump and SPD-10A detector (Japan). HPLC solvents consist of water containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). Absorbance was detected by 254 nm. Samples were analyzed starting from 5% B in A to 100% B for 30 min.



VI. References

(1) Jung, H. S.; Rajasekaran, N.; Song, S. Y.; Kim, Y. D. S.; Hong, S.; Choi, H. J.; Kim, Y. D. S.; Choi, J. S.; Choi, Y. La; Shin, Y. K. Human Papillomavirus E6/E7-Specific SiRNA Potentiates the Effect of Radiotherapy for Cervical Cancer in Vitro and in Vivo. *Int. J. Mol. Sci.* **2015**, *16* (6), 1–12.

(2) Chang, J. T. C.; Kuo, T. F.; Chen, Y. J.; Chiu, C. C.; Lu, Y. C.; Li, H. F.; Shen, C. R.; Cheng, A. J. Highly Potent and Specific SiRNAs against E6 or E7 Genes of HPV16-or HPV18-Infected Cervical Cancers. *Cancer Gene Ther.* **2010**, *17* (12), 827–836.