Supplementary Data

The synthesis and evaluation of carbocyclic pyrrolo-[2,3-*d*]pyrimidine nucleoside analogs

Jongbok Lee,[a] Hyewon Suh,[b] Sangeun Yoon,[a] Kowoon Choi,[b] Chul-hoon Lee,[b]* and Hakjune Rhee[a,c]*

[a]Department of Bionanotechnology, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea. E-mail: hrhee@hanyang.ac.kr

[b]Department of Pharmacy, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea. E-mail: chhlee@hanyang.ac.kr

[c]Department of Chemistry and Applied Chemistry, Hanyang University, Sa 3-Dong 1271, Ansan, Kyunggi-Do, 426-791, Korea.

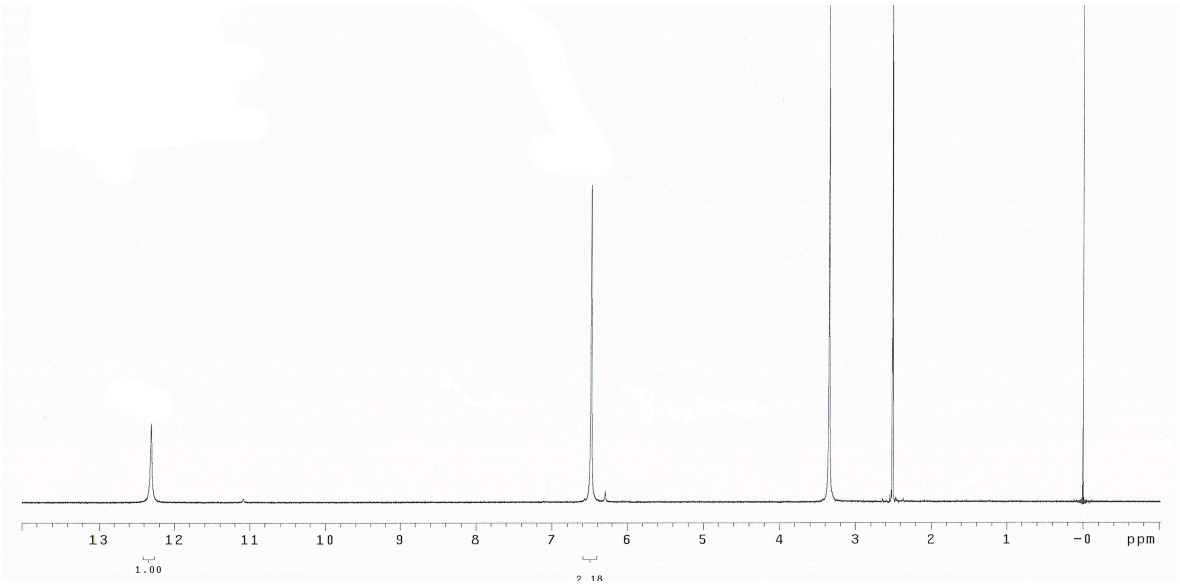
Table of Contents

^{1}H, ^{13}C and ^{1}H-^{1}H NOESY spectra for compounds **S2-S15**

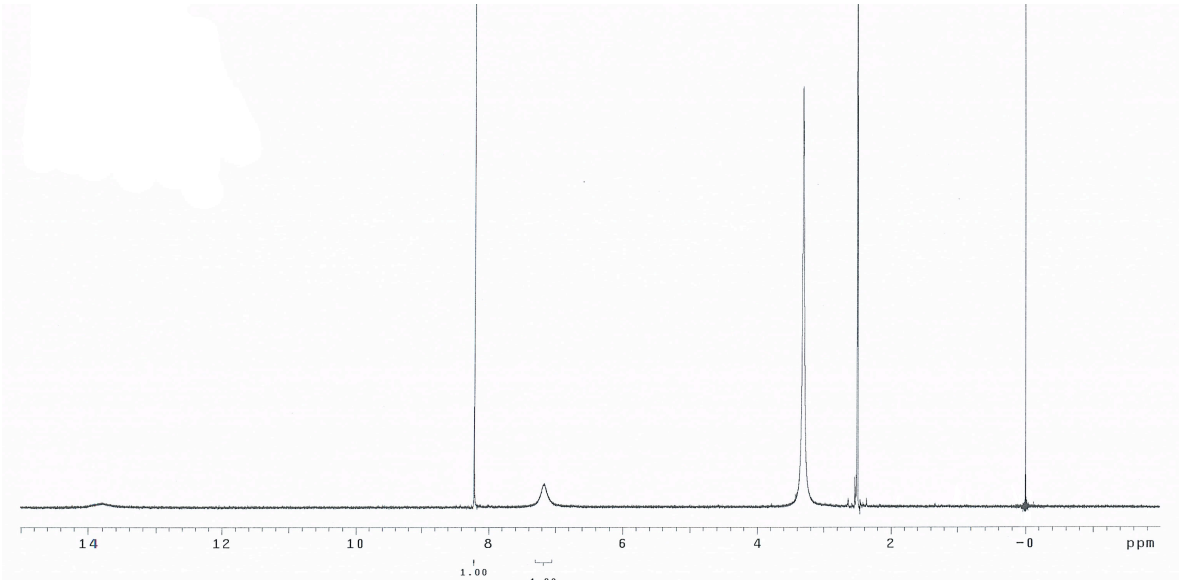
Cell growth inhibition assay (MTT assay) **S16**

X-ray crystallographic data for 3 **S17-S20**

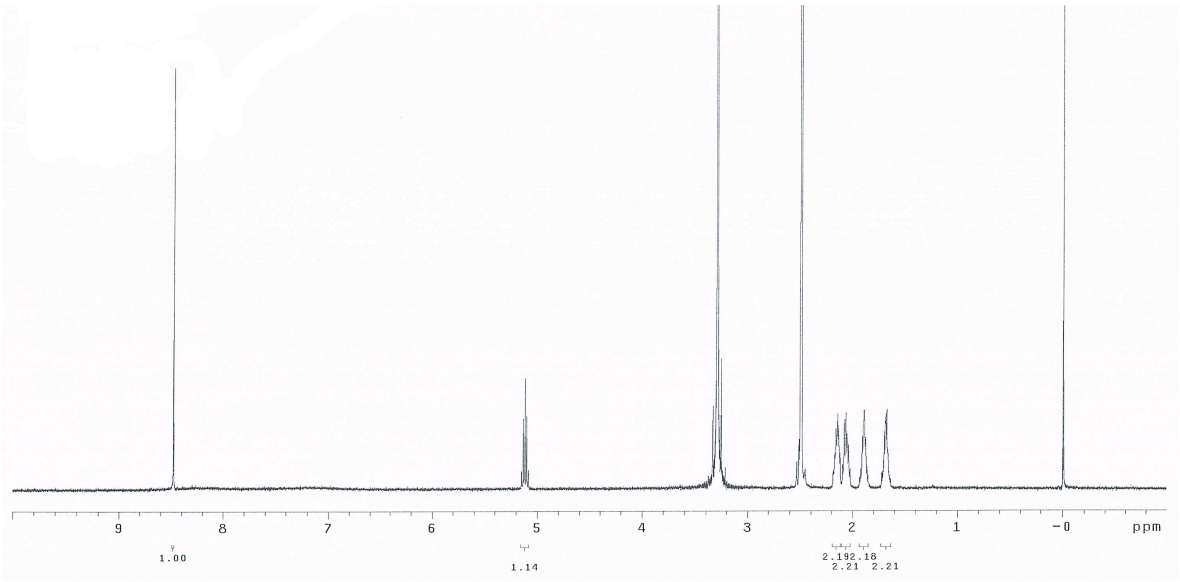
^{1}H NMR of 2-amino-5-bromo-3,4-dicyanopyrrole (1)



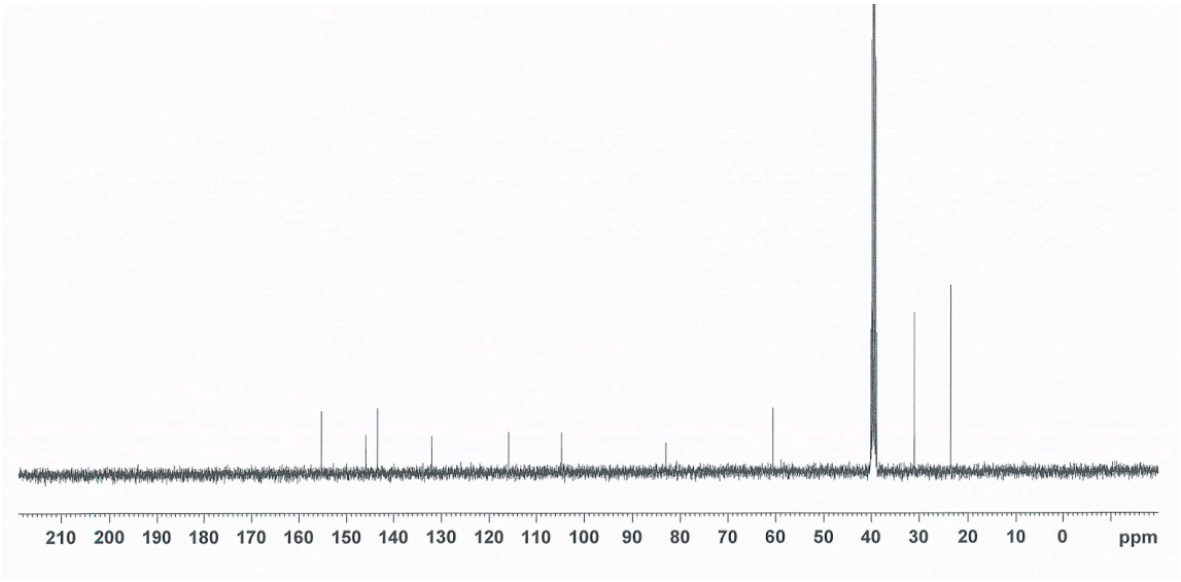
^{1}H NMR of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (2)



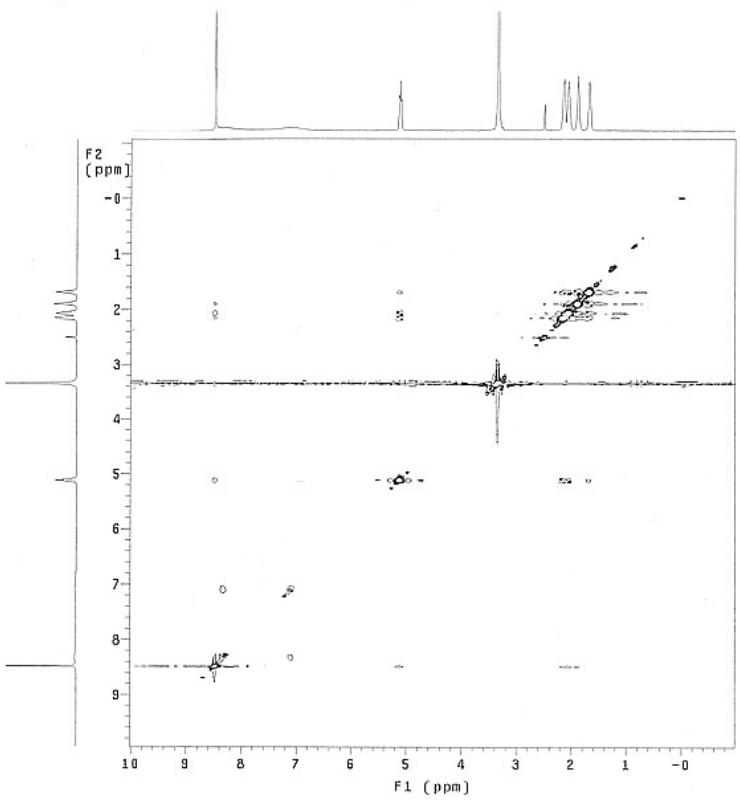
^{1}H NMR of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3)



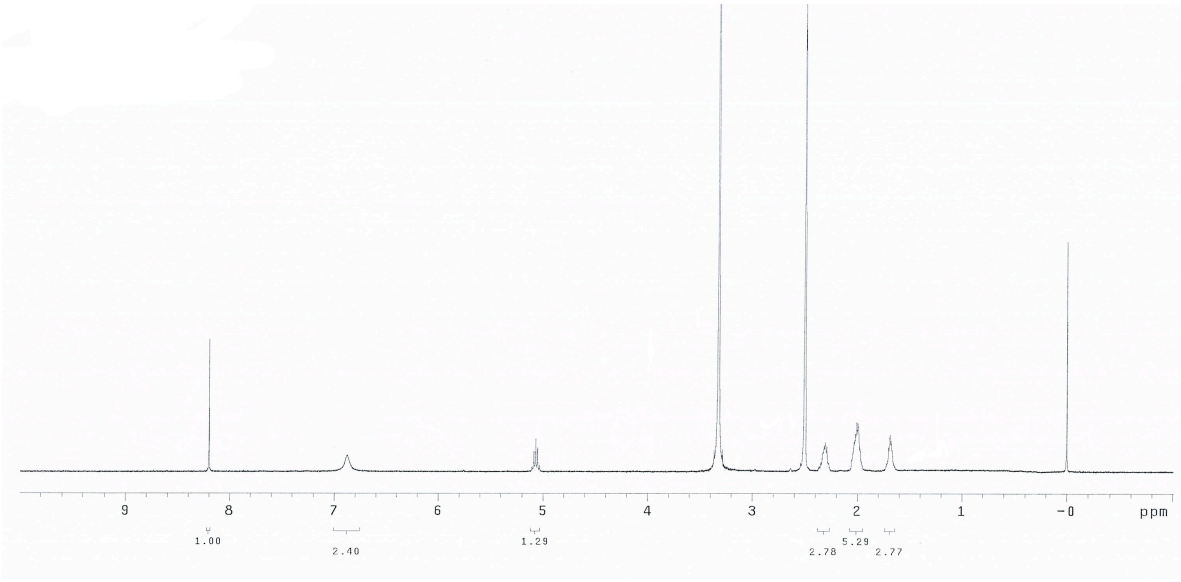
^{13}C NMR of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3)



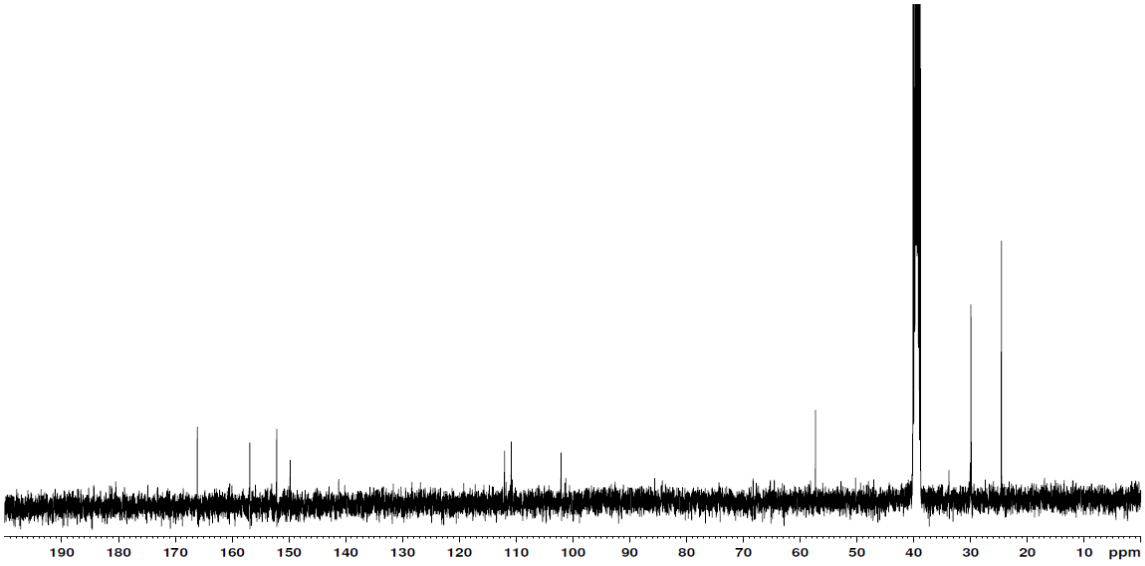
^{1}H-^{1}H NOESY Spectrum of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3)



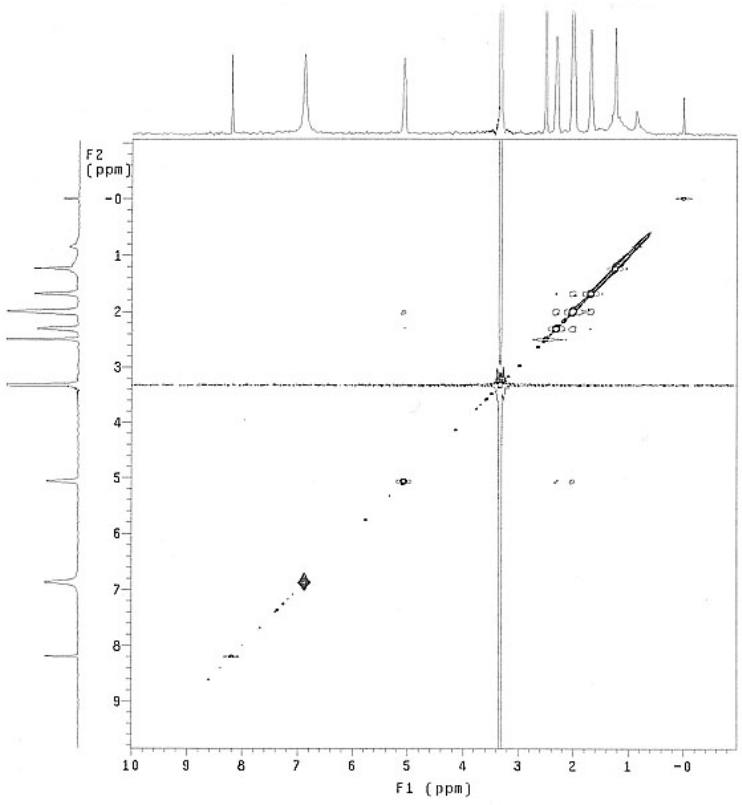
^{1}H NMR of 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4)



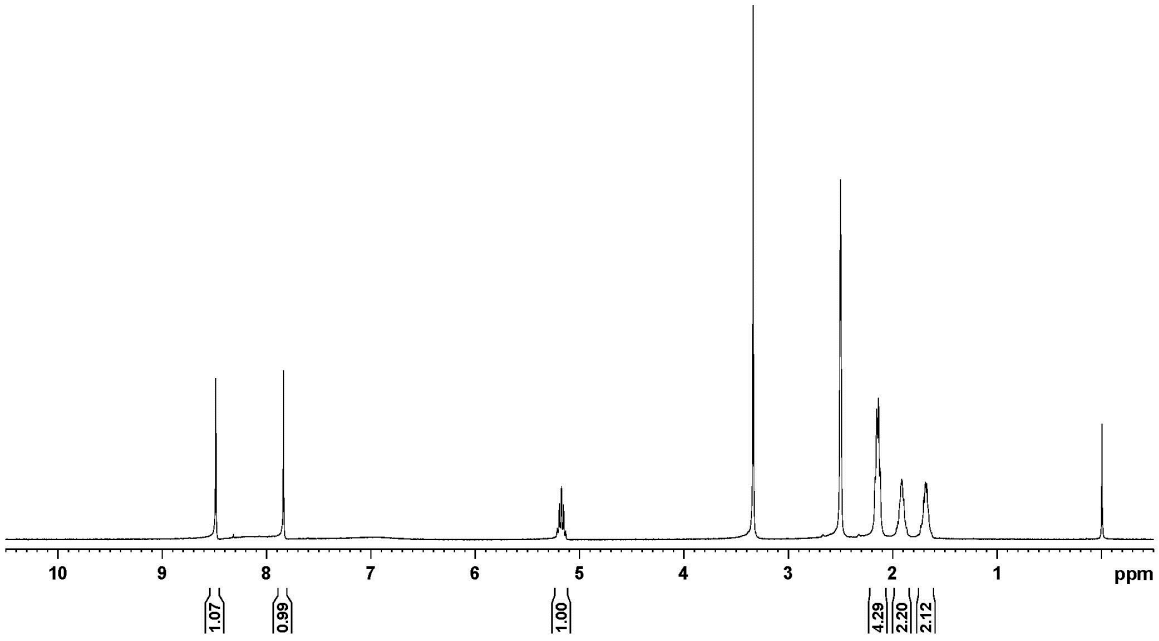
^{13}C NMR of 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4)



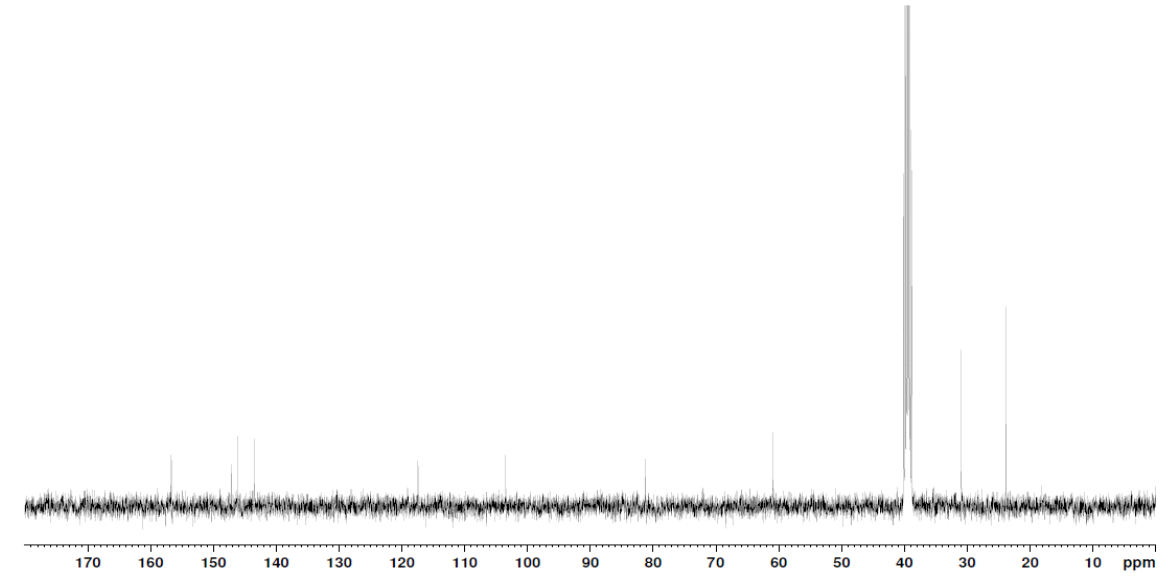
^{1}H-^{1}H NOESY Spectrum of 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4)



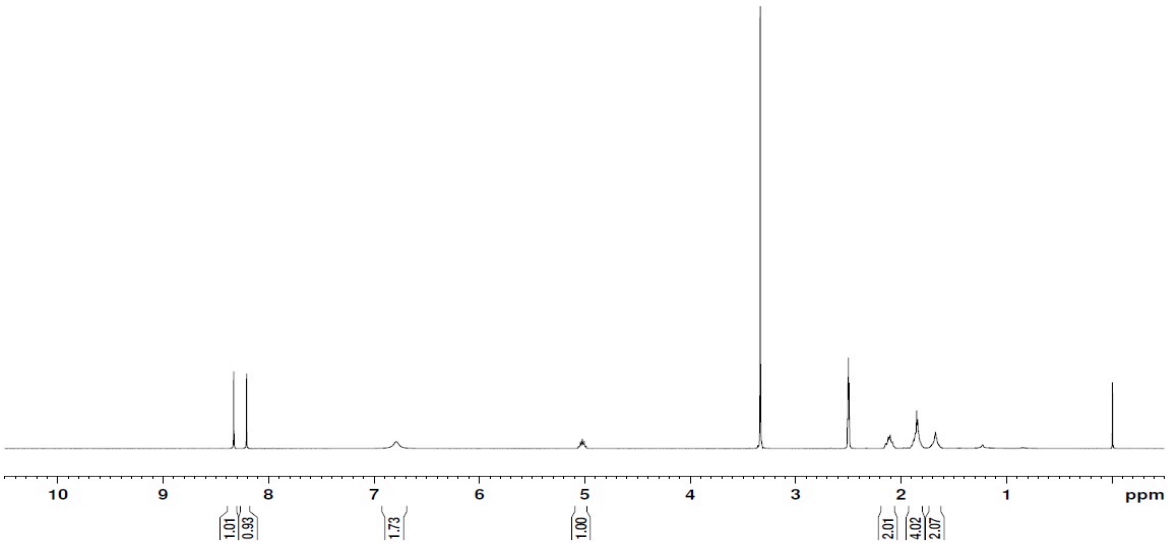
^{1}H NMR of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (5)



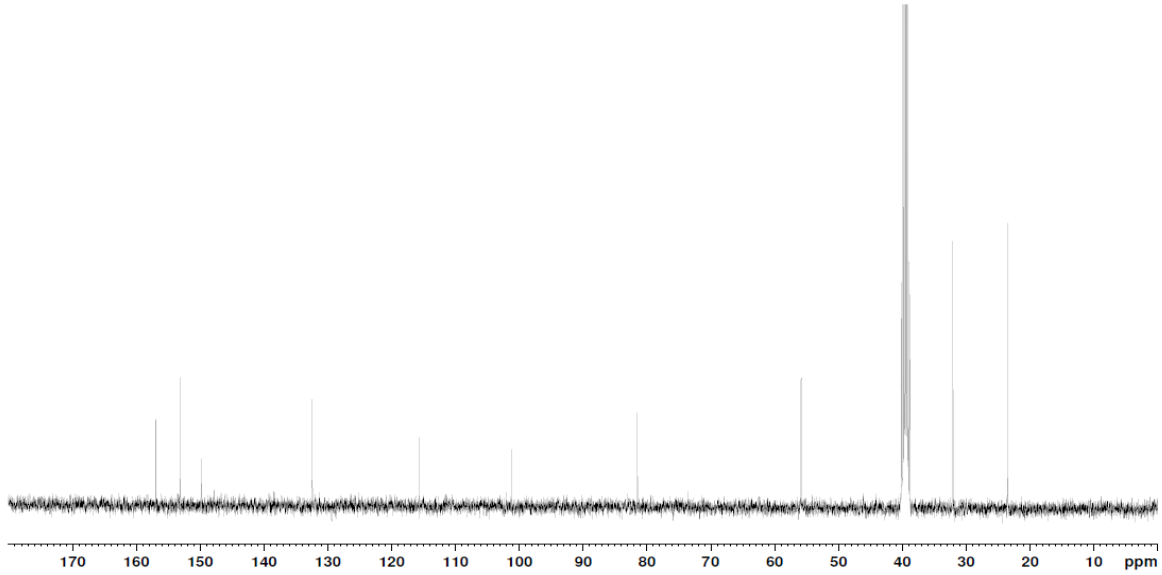
^{13}C NMR of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (5)



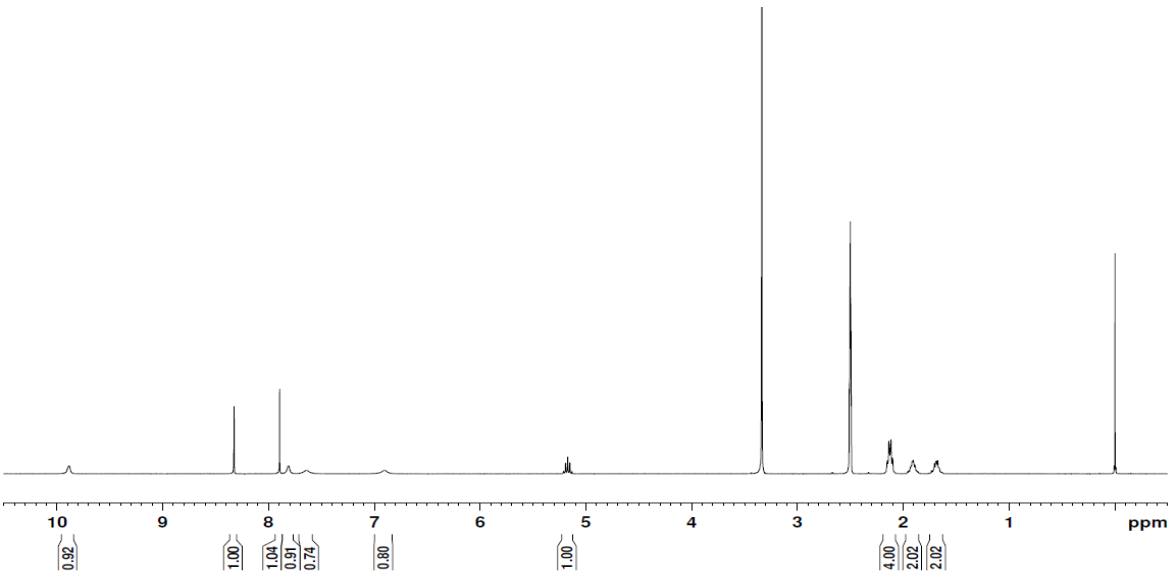
^{1}H NMR of 4-amino-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (6)



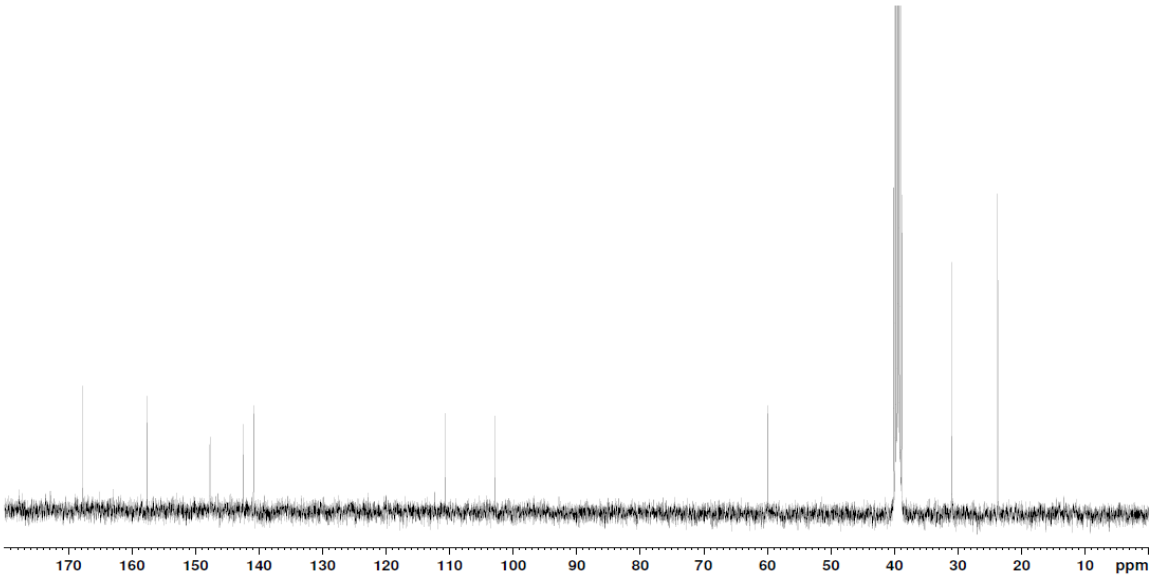
^{13}C NMR of 4-amino-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (6)



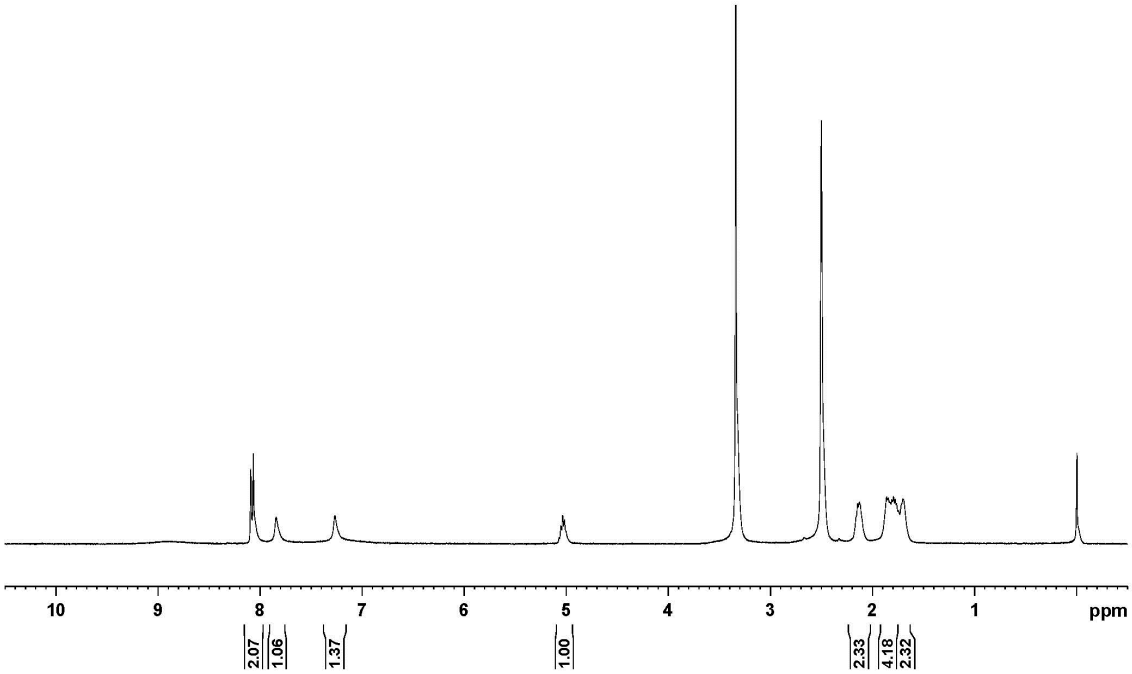
^{1}H NMR of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7)



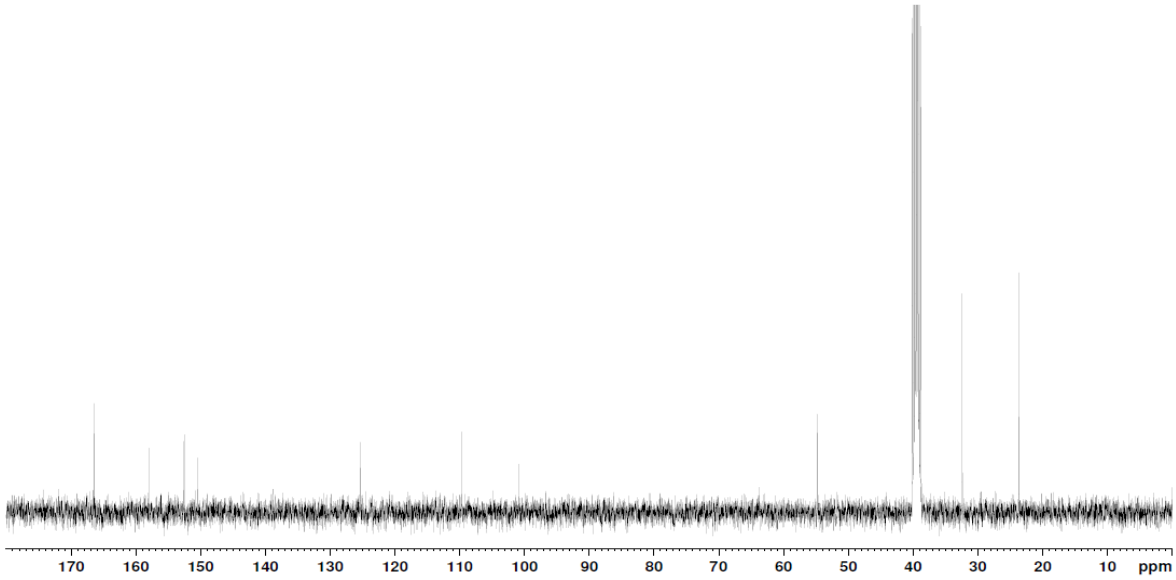
^{13}C NMR of 4-amino-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7)



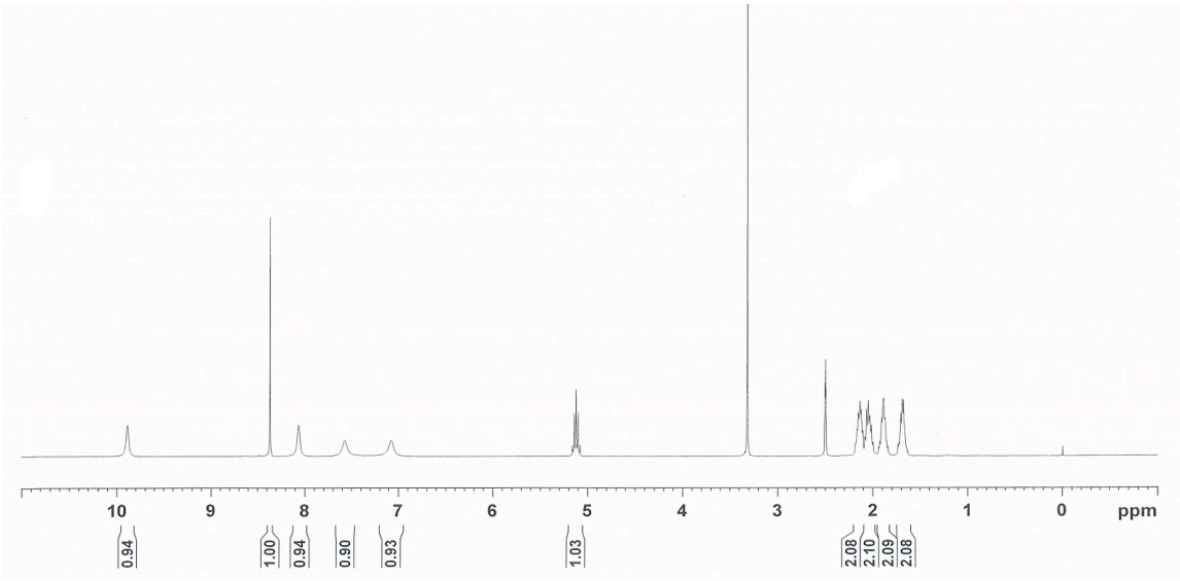
^{1}H NMR of 4-amino-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8)



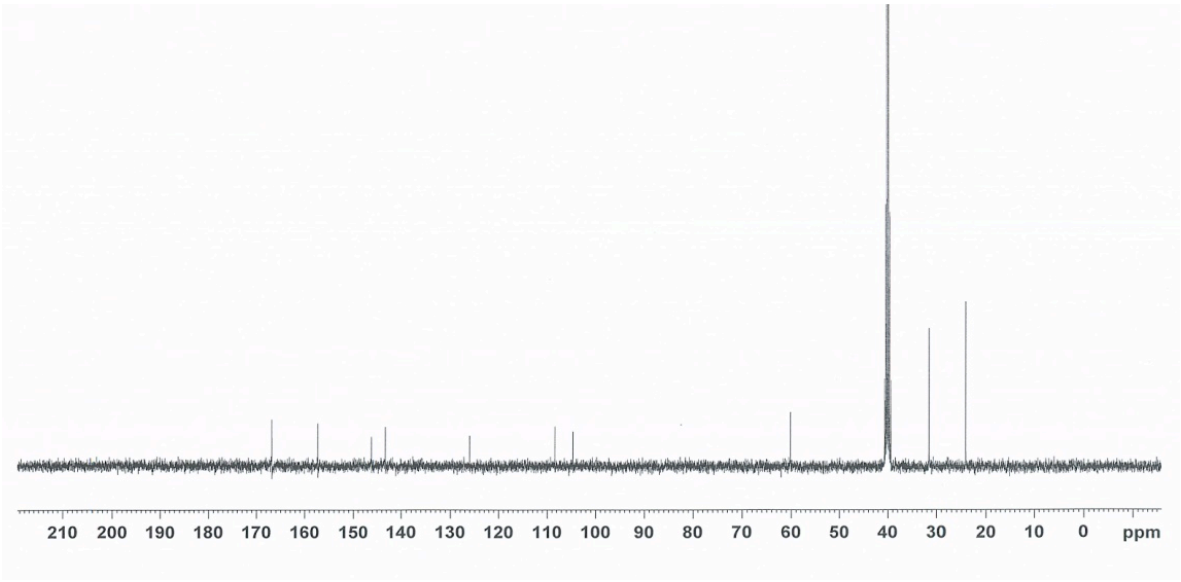
^{13}C NMR of 4-amino-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8)



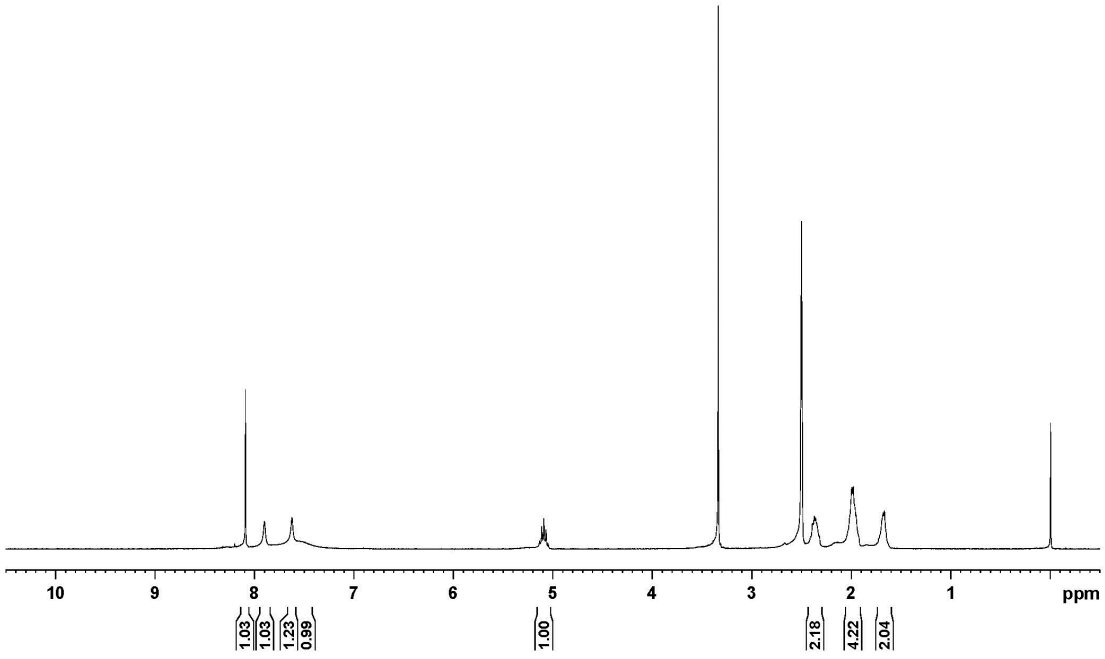
^{1}H NMR of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (9)



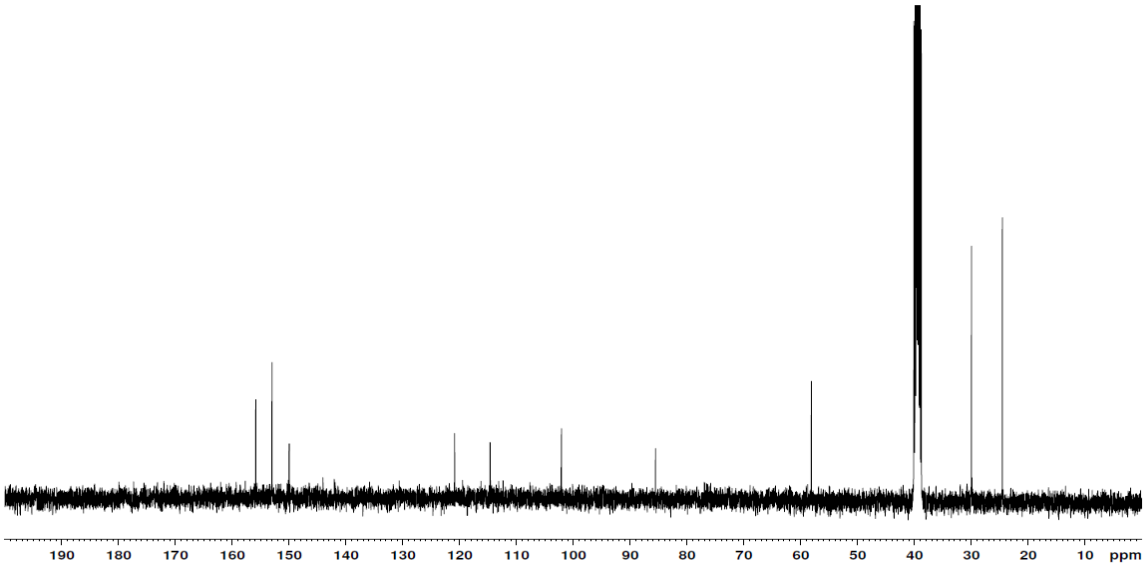
^{13}C NMR of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (9)



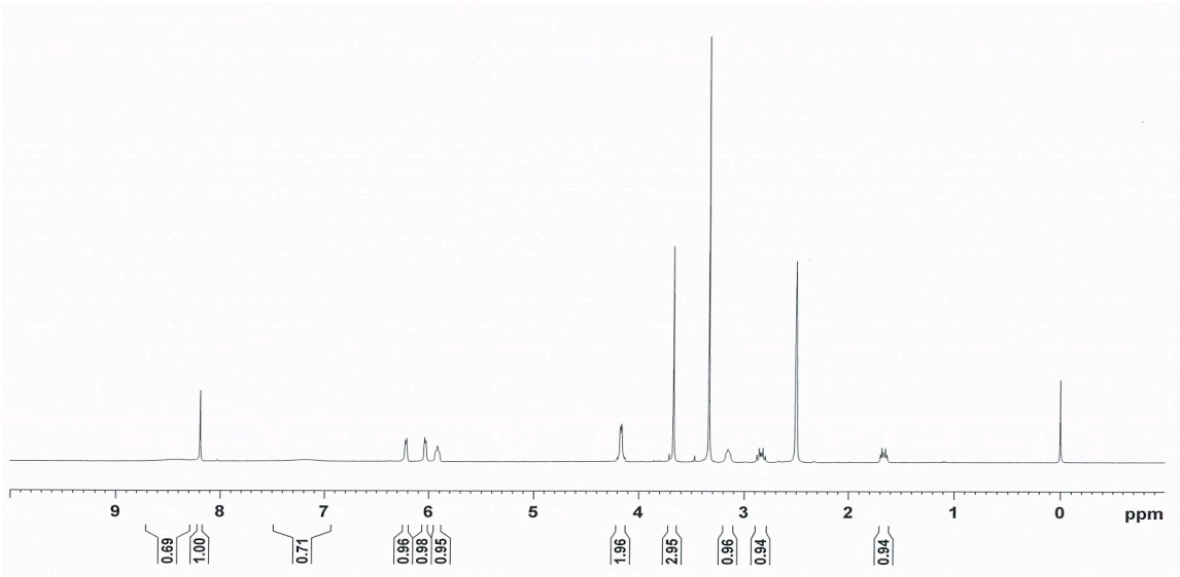
^{1}H NMR of 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (10)



^{13}C NMR of 4-amino-6-bromo-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (10)

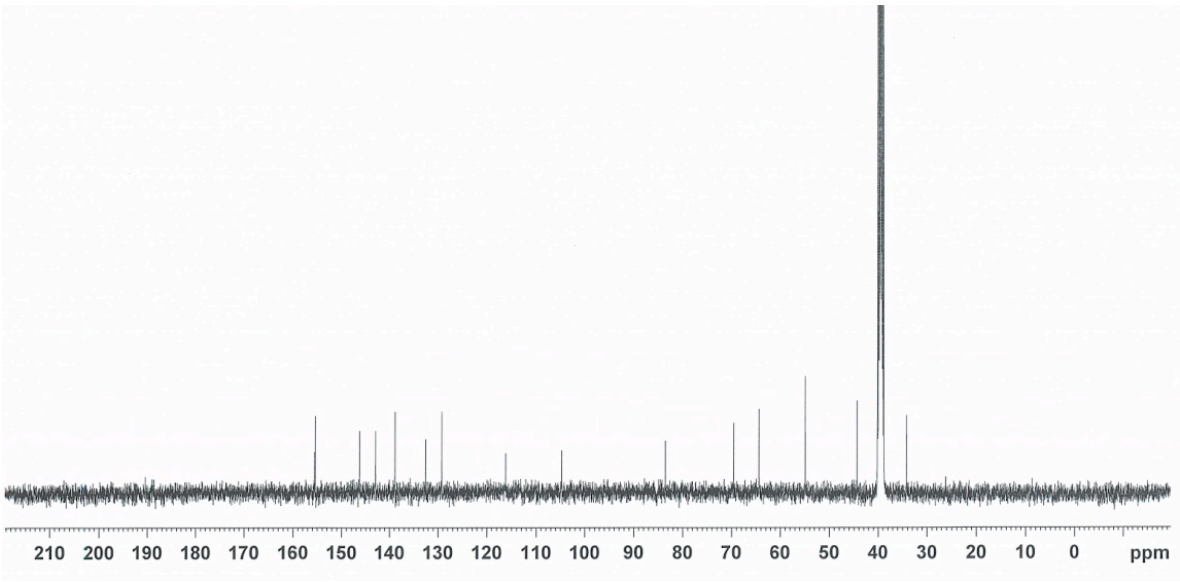


^{1}H NMR of *syn*-[4-(4-amino-6-bromo-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-cyclopenten-1-yl]methyl methyl carbonate (12)

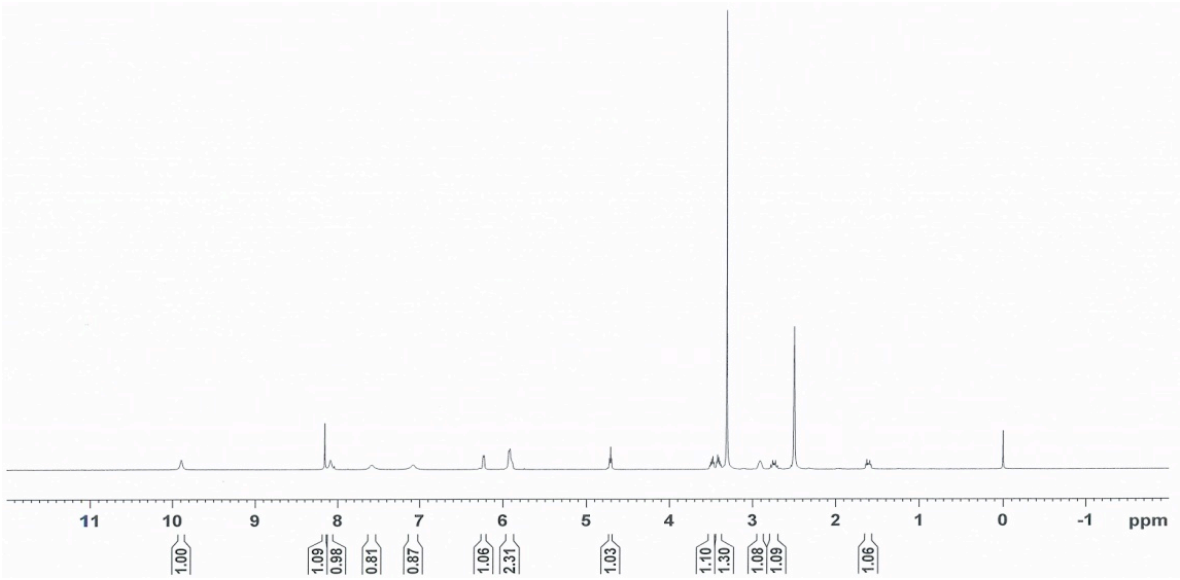


^{13}C NMR of *syn*-[4-(4-amino-6-bromo-5-cyano-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-cyclopenten-

1-yl]methyl methyl carbonate (12)

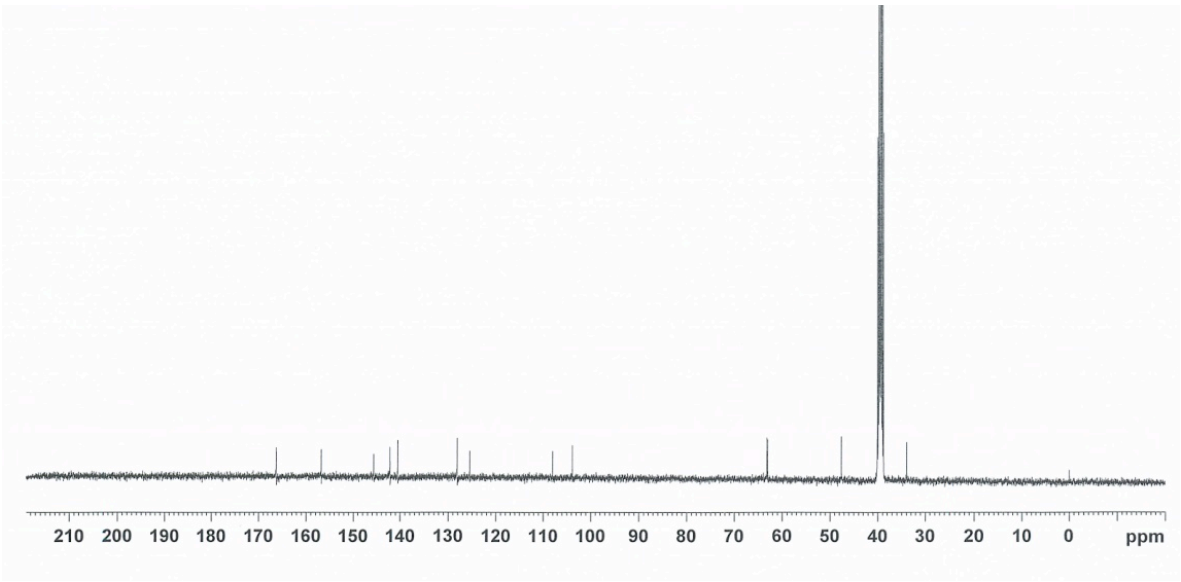


^{1}H NMR of *syn*-4-amino-6-bromo-7-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (13)

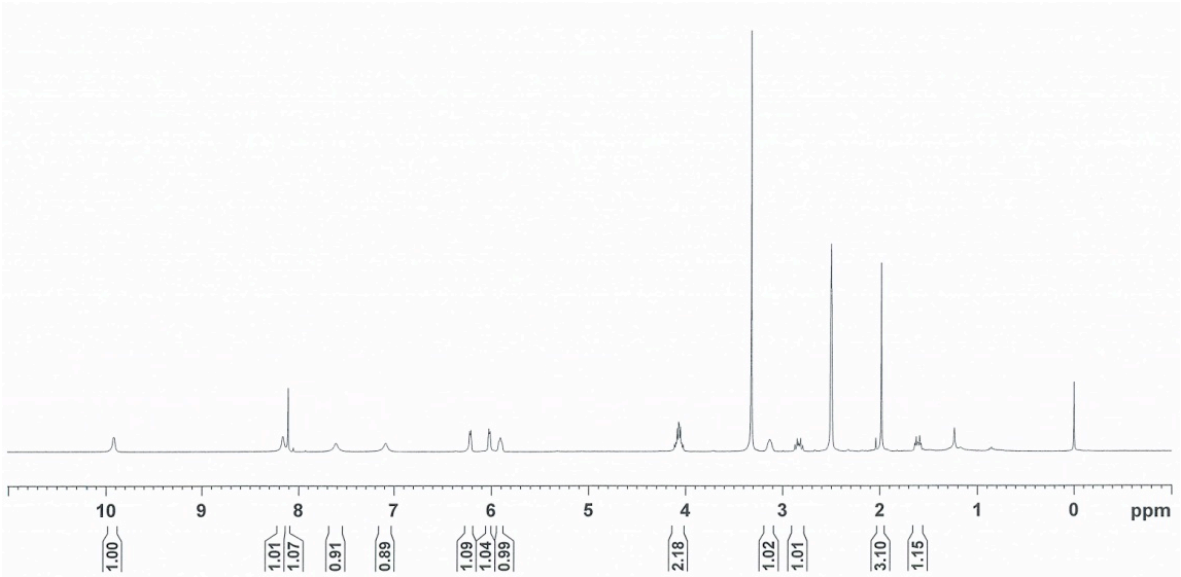


^{13}C NMR of *syn*-4-amino-6-bromo-7-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-

***d*]pyrimidine-5-carboxamide (13)**

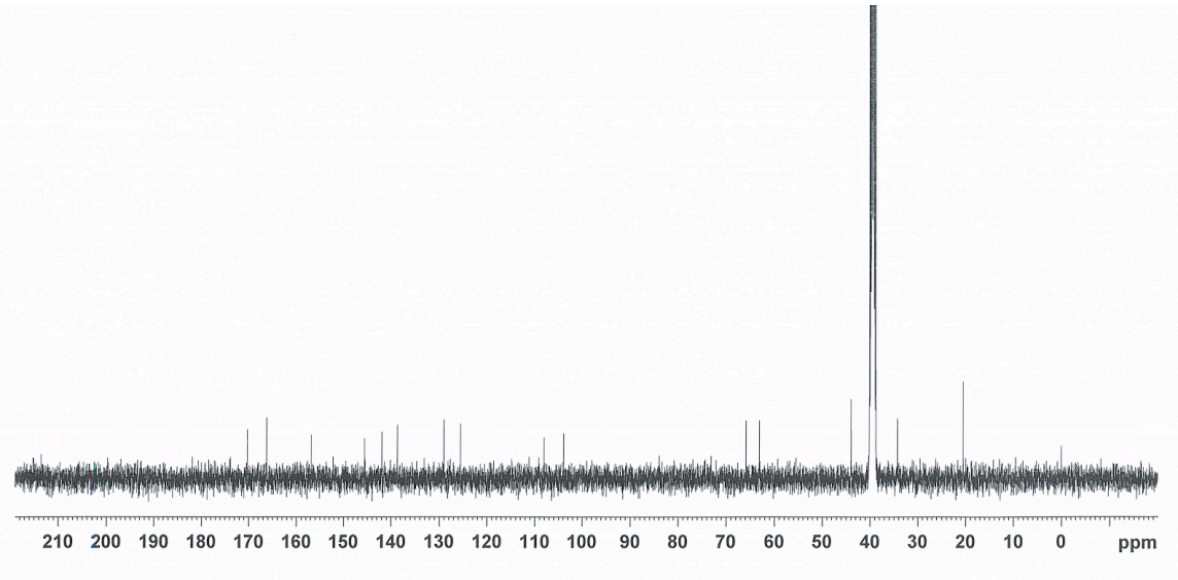


^{1}H NMR of *syn*-[4-(4-amino-6-bromo-5-carbamoyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-cyclopenten-1-yl]methyl acetate (14)

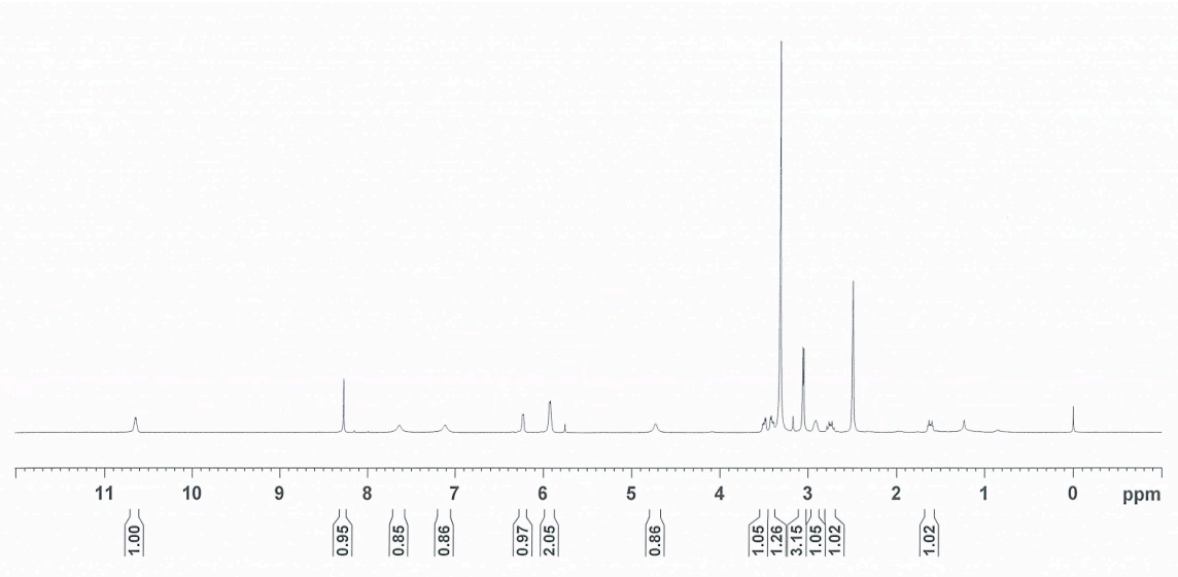


^{13}C NMR of *syn*-[4-(4-amino-6-bromo-5-carbamoyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-

cyclopenten-1-yl]methyl acetate (14)

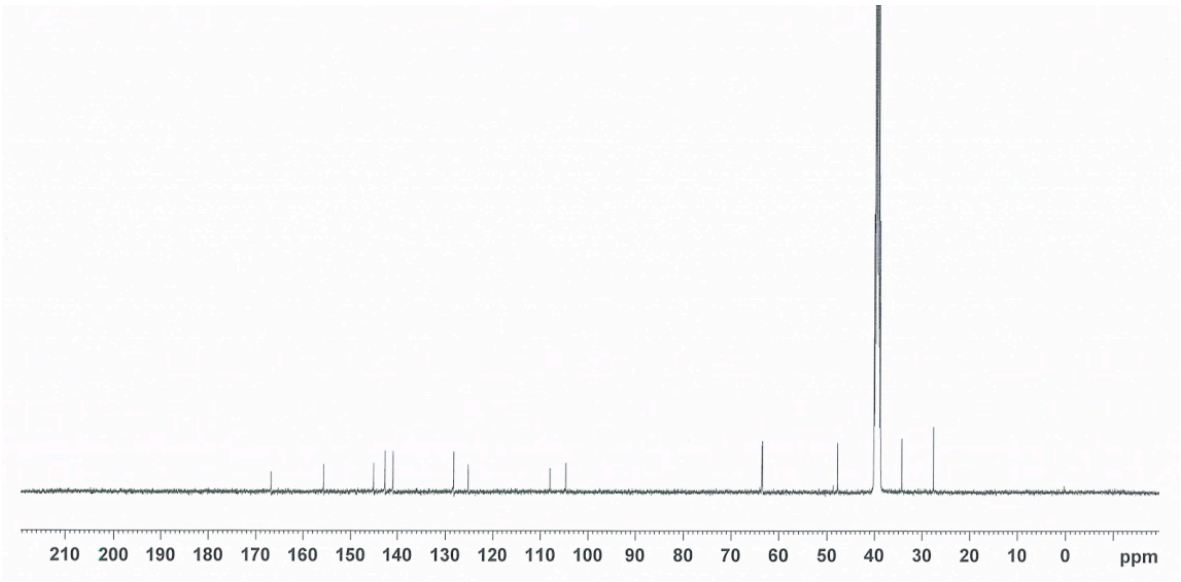


^{1}H NMR of *syn*-4-amino-6-bromo-7-[4-(methoxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (15)



^{13}C NMR of *syn*-4-amino-6-bromo-7-[4-(methoxymethyl)-2-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-

***d*]pyrimidine-5-carboxamide (15).**



Cell growth inhibition assay (MTT assay)

Cell viability was assessed using the conventional colorimetric dye reduction method based on the reduction of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma). The viable cell number was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Device, Sunnyvale, CA, USA).

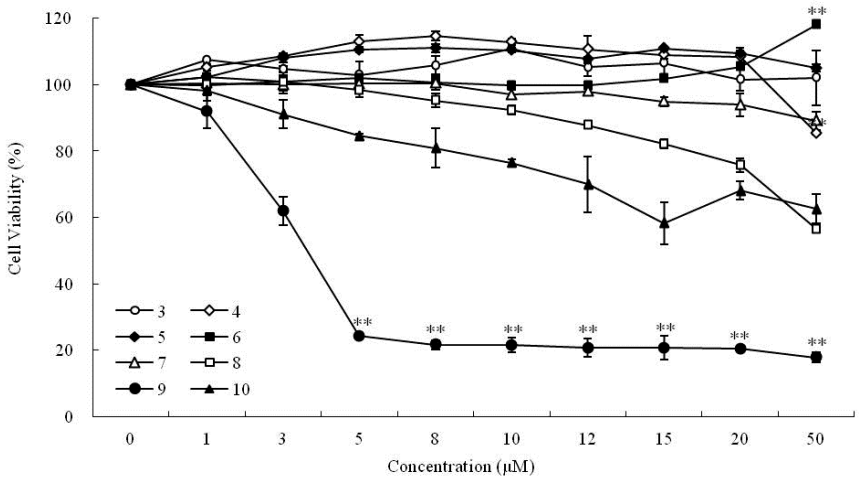


Fig. 1. Anti-proliferative effect of compounds **3**, **7**, **8**, **9**, and **10** in human ovarian cancer PA-1 cells. PA-1 cells were incubated with 1 - 50 μM of each compound for 24 h. The cell viability was determined by MTT assay. The results represent the mean ± SD (standard deviation) of three independent experiments. (**p*<0.05, ***p*<0.01)

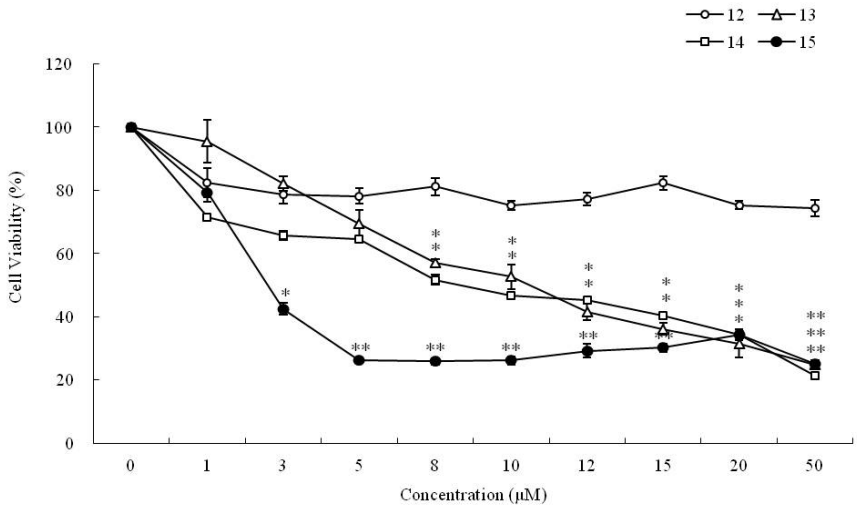


Fig. 2. Anti-proliferative effect of compounds **12**, **13**, **14**, and **15** in human ovarian cancer PA-1 cells. PA-1 cells were incubated with 1 - 50 μM of each compound for 24 h. The cell viability was determined by MTT assay. The results represent the mean ± SD (standard deviation) of three independent experiments. (*p<0.05, **p<0.01)

X-ray crystallographic data for compound 3.

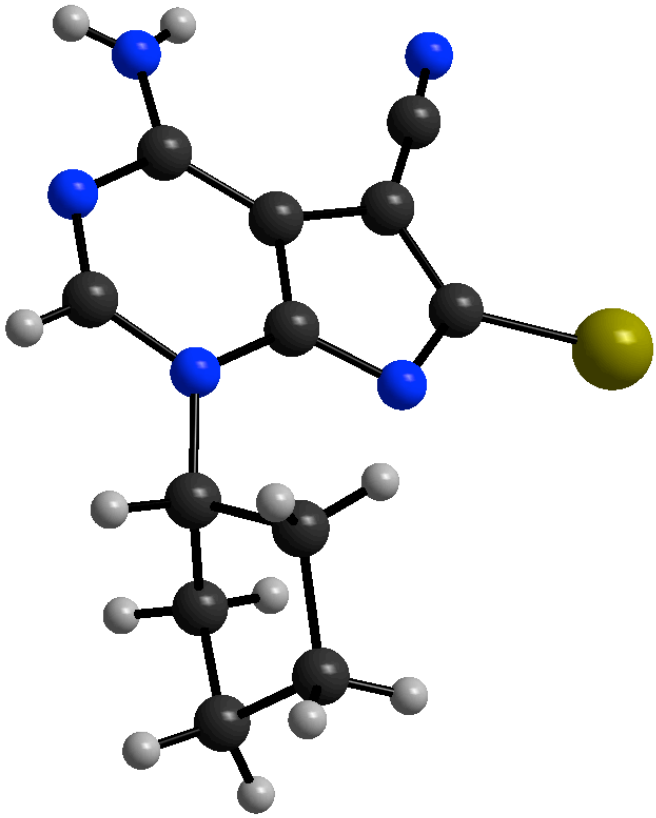


Fig. 3. The molecular structure of 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*] pyrimidine-5-carbonitrile **3**.

For **3**, single crystals were picked up with a cryoloop attached to a goniometer head, and transferred to a cold stream of liquid nitrogen (-183 °C). The data collection was carried out using synchrotron X-ray (λ = 0.82000 Å) equipped with ADSC Quantum 210 CCD detector at Pohang Accelerator Laboratory. After the data integration (HKL2000) and space group determination (XPREP), the structure was solved by direct methods and subsequent difference Fourier techniques (SHEXLTL). All the framework atoms were refined anisotropically. After adding hydrogen atoms to their geometrically ideal positions and the results were attached to the CIF file.

Table 1. Crystal data and structure refinement for 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3**

Identification code	p21c
Empirical formula	C12 H12 Br N5
Formula weight	306.18
Temperature	100(2) K
Wavelength	0.82000 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 7.7690(16) Å α= 90°
	b = 5.1750(10) Å β= 97.21° (3)
	c = 30.128(6) Å γ= 90°
Volume	$1201.7(4)^3$
Z	4
Density (calculated)	1.692 Mg/m^3
Absorption coefficient	3.410 mm^{-1}
F(000)	616
Crystal size	0.32 x 0.10 x 0.02 mm^3
Theta range for data collection	1.57 to 30.00°.
Index ranges	-9<=h<=9, -6<=k<=0, -36<=l<=36
Reflections collected	3495

Independent reflections	2151 [R(int) = 0.1168]
Completeness to theta = 30.00°	93.9 %
Max. and min. transmission	0.9349 and 0.4083
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2151 / 0 / 176
Goodness-of-fit on F^2	1.079
Final R indices [I>2sigma(I)]	R1 = 0.0666, wR2 = 0.1952
R indices (all data)	R1 = 0.0687, wR2 = 0.1982
Extinction coefficient	0.018(4)
Largest diff. peak and hole	1.328 and -1.438 $e.^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (^{2}x 10^3) for 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

	x	y	z	U(eq)
Br(1)	1576(1)	5859(1)	3422(1)	36(1)
N(1)	4235(6)	2184(9)	3569(2)	33(1)
N(2)	6153(6)	-1314(9)	3845(1)	32(1)
N(3)	5582(6)	-3326(10)	4521(2)	34(1)
N(4)	3229(7)	-2351(11)	4878(2)	35(1)
N(5)	-220(7)	2643(11)	4534(2)	43(1)
C(1)	2814(7)	3139(11)	3727(2)	31(1)
C(2)	2432(7)	1998(11)	4121(2)	34(1)
C(3)	3745(7)	89(12)	4218(2)	30(1)
C(4)	4146(7)	-1817(12)	4544(2)	33(1)
C(5)	6470(7)	-3047(12)	4182(2)	33(1)
C(6)	4771(7)	331(12)	3866(2)	32(1)
C(7)	7114(8)	-1528(12)	3452(2)	33(1)
C(8)	8058(8)	940(10)	3337(2)	34(1)
C(9)	8131(10)	744(12)	2827(2)	43(2)
C(10)	7273(8)	-1832(13)	2677(2)	36(1)
C(11)	6012(7)	-2313(11)	3017(2)	33(1)
C(12)	968(7)	2430(10)	4351(2)	33(1)

Table 3. Bond lengths [Å] and angles [^{o}] for 4-amino-6-bromo-1-cyclopentyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3**

Br(1)-C(1)	1.879(6)
N(1)-C(6)	1.341(7)
N(1)-C(1)	1.349(7)
N(2)-C(5)	1.353(7)
N(2)-C(6)	1.378(7)
N(2)-C(7)	1.480(6)
N(3)-C(5)	1.310(7)
N(3)-C(4)	1.371(8)
N(4)-C(4)	1.332(7)
N(5)-C(12)	1.138(8)
C(1)-C(2)	1.390(8)
C(2)-C(12)	1.421(8)
C(2)-C(3)	1.423(8)
C(3)-C(4)	1.401(8)
C(3)-C(6)	1.409(7)
C(7)-C(11)	1.529(7)
C(7)-C(8)	1.534(8)
C(8)-C(9)	1.549(8)
C(9)-C(10)	1.533(9)
C(10)-C(11)	1.523(7)
C(6)-N(1)-C(1)	103.1(5)
C(5)-N(2)-C(6)	116.4(5)
C(5)-N(2)-C(7)	119.2(5)
C(6)-N(2)-C(7)	123.9(5)
C(5)-N(3)-C(4)	119.3(5)

N(1)-C(1)-C(2)	114.7(5)
N(1)-C(1)-Br(1)	119.5(4)
C(2)-C(1)-Br(1)	125.8(4)
C(1)-C(2)-C(12)	128.2(5)
C(1)-C(2)-C(3)	104.2(5)
C(12)-C(2)-C(3)	127.3(5)
C(4)-C(3)-C(6)	119.6(5)
C(4)-C(3)-C(2)	136.3(5)
C(6)-C(3)-C(2)	104.0(5)
N(4)-C(4)-N(3)	115.9(5)
N(4)-C(4)-C(3)	125.7(5)
N(3)-C(4)-C(3)	118.4(5)
N(3)-C(5)-N(2)	126.4(5)
N(1)-C(6)-N(2)	126.1(5)
N(1)-C(6)-C(3)	114.0(5)
N(2)-C(6)-C(3)	119.8(5)
N(2)-C(7)-C(11)	114.9(5)
N(2)-C(7)-C(8)	114.9(5)
C(11)-C(7)-C(8)	105.1(4)
C(7)-C(8)-C(9)	104.3(4)
C(10)-C(9)-C(8)	106.2(5)
C(11)-C(10)-C(9)	103.8(5)
C(10)-C(11)-C(7)	101.2(4)
N(5)-C(12)-C(2)	176.5(6)

Symmetry transformations used to generate equivalent atoms.

Table 4. Anisotropic displacement parameters (2x 10^3) for p21c. The anisotropic displacement factor exponent takes the form: $-2^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	32(1)	40(1)	38(1)	3(1)	14(1)	3(1)
N(1)	31(2)	37(2)	32(2)	1(2)	12(2)	2(2)
N(2)	31(2)	38(2)	29(2)	1(2)	15(2)	2(2)
N(3)	33(2)	39(2)	34(2)	1(2)	14(2)	-2(2)
N(4)	26(3)	47(3)	35(2)	7(2)	18(2)	8(2)
N(5)	37(3)	55(3)	43(3)	4(2)	23(2)	3(2)
C(1)	26(3)	37(3)	32(2)	0(2)	15(2)	2(2)
C(2)	27(3)	42(3)	35(3)	3(2)	12(2)	1(2)
C(3)	23(2)	39(3)	32(2)	0(2)	12(2)	-3(2)
C(4)	27(3)	43(3)	30(2)	3(2)	11(2)	-1(2)
C(5)	23(3)	44(3)	35(3)	3(2)	14(2)	4(2)
C(6)	32(3)	37(3)	30(3)	0(2)	11(2)	-3(2)
C(7)	36(3)	36(3)	29(3)	2(2)	18(2)	2(2)
C(8)	32(3)	35(3)	37(3)	-1(2)	14(2)	-1(2)
C(9)	56(4)	44(3)	35(3)	-1(2)	27(3)	-9(3)
C(10)	32(3)	47(3)	31(2)	1(3)	14(2)	1(2)
C(11)	36(3)	37(3)	30(2)	0(2)	16(2)	-1(2)
C(12)	34(3)	36(3)	32(2)	-3(2)	15(2)	3(2)