SUPPORTING INFORMATION

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-amine (PQR309): a Potent, Brain-Penetrant, Orally Bioavailable, pan-Class I PI3K/mTOR Inhibitor as Clinical Candidate in Oncology

Florent Beaufils[1,†,$], Natasa Cmiljanovic[1,†,$], Vladimir Cmiljanovic[1,†,$], Thomas Bohnacker[1], Anna Melone[1], Romina Marone[1], Eileen Jackson[1], Xuxiao Zhang[2], Alexander Sele[1], Chiara Borsari[1], Jürgen Mestan[3], Paul Hebeisen[3], Petra Hillmann[3], Bernd Giese[4,††], Marketa Zvelebil[1], Doriano Fabbro[3], Roger L. Williams[2], Denise Rageot[1,*], Matthias P. Wymann[1,*]

[1]Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland;

[2]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK;

[3]PIQUR Therapeutics AG, Hochbergerstrasse 60C, 4057 Basel, Switzerland;

[4]Department of Chemistry, University of Basel, St. Johanns-Ring 19, 4056 Basel, Switzerland.

Current address:

[†]PIQUR Therapeutics AG, Basel, Switzerland;

[††]Department of Chemistry, University of Fribourg, Fribourg, Switzerland.

[$]These authors contributed equally to this work.

*Correspondence to:

Matthias.Wymann@UniBas.CH and Denise.Rageot@UniBas.CH

Dept. Biomedicine, University of Basel

Mattenstrasse 28

4058 Basel, Switzerland

Tel. +41 61 207 5046; Fax. +41 61 207 3566

Overview, Summary

Table S1: Data collection and refinement statistics of the PQR309 - PI3Kγ complex

Space group	
Cell dimensions	
a, *b*, *c* (Å)	43.1, 86.7, 86.7
α, β, γ (°)	118.1, 99.6, 95.4
Resolution (Å)	61.0-2.70 (2.85-2.70)
R_{sym}	0.062 (0.49)
$I/\sigma I$	12.6 (2.4)
Completeness (%)	97.0 (92.9)
Redundancy	3.4 (3.3)
Refinement	
Resolution (Å)	
No. reflections (free)	24405 (1269)
R_{work}/ R_{free}	0.19/0.26
No. atoms	
Protein	6791
Ligand/ion	39
Water	36
B-factors	
Protein	64
Ligand/ion	69
Water	53
R.m.s deviations	
Bond lengths (Å)	0.02
Bond angles (º)	2.0

The coordinates of the PQR309-PI3Kγ complex are deposited under the PDB ID 5OQ4

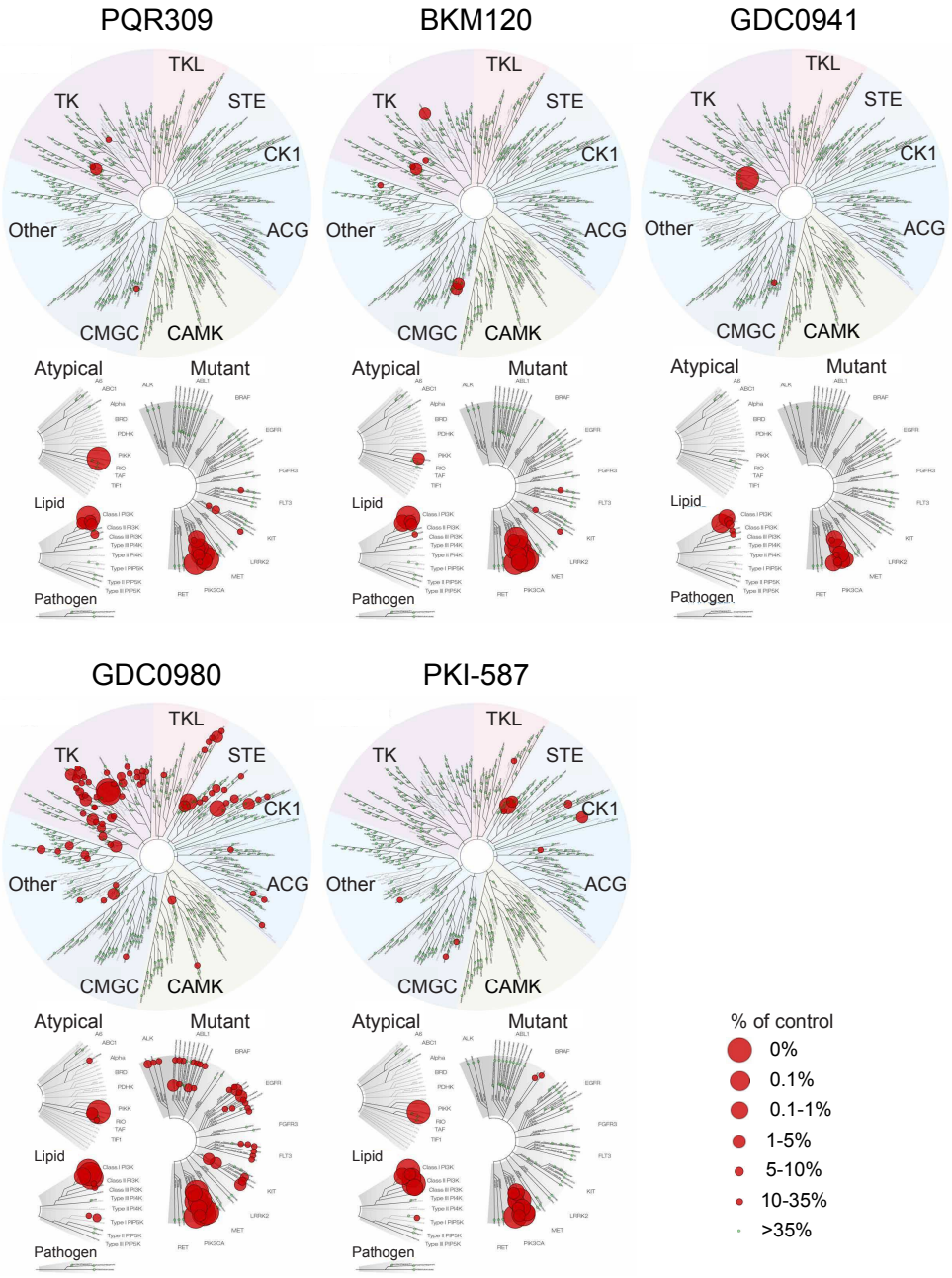


Figure S1. TREEspot™ data visualization of KINOMEScan interactions of indicated PI3K inhibitors. Kinases are organized according to phylogeny and into kinase families, including atypical kinases, lipid kinases, mutated kinases and some pathogen-derived kinases. Kinases targeted by indicated compounds are marked with red circles, where increasing circle size indicates a higher-affinity. AGC: PKA, PKG and PKC kinases; CAMK: Calcium- Calmodulin-dependent protein kinases; CK1: Casein kinase 1; CMGC: CDK, MAPK, GSK3, CLK family kinases; STE: Homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases; TK: Tyrosine kinases; TKL: Tyrosine kinase-like. The raw data used for the TREEspot™ representation is shown in Table S2.

Table S2: Kinase Interactions of PQR309 and Reference Compounds at 10 µM (KINOMEscan™)

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
AAK1	83.0	84.0	100.0	50.0	99.0
ABL1(E255K)-phosphorylated	83.0	62.0	100.0	24.0	100.0
ABL1(F317I)-nonphosphorylated	100.0	97.0		100.0	100.0
ABL1(F317I)-phosphorylated	100.0	94.0	100.0	80.0	100.0
ABL1(F317L)-nonphosphorylated	100.0	97.0		88.0	79.0
ABL1(F317L)-phosphorylated	66.0	100.0	100.0	47.0	100.0
ABL1(H396P)-nonphosphorylated	92.0	82.0		2.0	100.0
ABL1(H396P)-phosphorylated	96.0	100.0	100.0	18.0	99.0
ABL1(M351T)-phosphorylated	100.0	100.0	100.0	20.0	100.0
ABL1(Q252H)-nonphosphorylated	89.0	61.0		7.5	100.0
ABL1(Q252H)-phosphorylated	88.0	100.0	100.0	13.0	94.0
ABL1(T315I)-nonphosphorylated	100.0	92.0		34.0	100.0
ABL1(T315I)-phosphorylated	80.0	100.0	100.0	16.0	100.0
ABL1(Y253F)-phosphorylated	100.0	100.0	100.0	21.0	97.0
ABL1-nonphosphorylated	62.0	69.0		25.0	100.0
ABL1-phosphorylated	86.0	76.0	100.0	23.0	99.0
ABL2	95.0	82.0	100.0	61.0	92.0
ACVR1	77.0	63.0	100.0	89.0	75.0
ACVR1B	99.0	94.0	100.0	100.0	100.0
ACVR2A	89.0	99.0	100.0	100.0	94.0
ACVR2B	76.0	63.0	100.0	86.0	83.0
ACVRL1	89.0	98.0	100.0	91.0	100.0
ADCK3	80.0	70.0	100.0	78.0	60.0
ADCK4	99.0	80.0	100.0	38.0	93.0
AKT1	75.0	72.0	100.0	96.0	93.0
AKT2	92.0	100.0	100.0	96.0	100.0
AKT3	97.0	72.0	100.0	100.0	95.0
ALK	94.0	72.0	100.0	17.0	80.0
ALK(C1156Y)	95.0	76.0		9.5	95.0
ALK(L1196M)	95.0	74.0		28.0	100.0
AMPK-alpha1	78.0	92.0	100.0	74.0	76.0
AMPK-alpha2	100.0	82.0	100.0	87.0	100.0
ANKK1	79.0	96.0	100.0	23.0	86.0
ARK5	97.0	80.0	100.0	72.0	82.0
ASK1	55.0	80.0	100.0	83.0	95.0
ASK2	100.0	96.0	100.0	72.0	100.0
AURKA	37.0	29.0	100.0	77.0	95.0
AURKB	81.0	94.0	100.0	100.0	100.0
AURKC	80.0	85.0	100.0	100.0	95.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
AXL	86.0	60.0	100.0	11.0	81.0
BIKE	87.0	88.0	100.0	78.0	80.0
BLK	61.0	76.0	100.0	11.0	100.0
BMPR1A	100.0	96.0	100.0	73.0	72.0
BMPR1B	67.0	100.0	100.0	72.0	99.0
BMPR2	82.0	100.0	100.0	93.0	97.0
BMX	100.0	78.0	100.0	34.0	93.0
BRAF	75.0	63.0	100.0	78.0	20.0
BRAF(V600E)	65.0	46.0	100.0	77.0	14.0
BRK	100.0	96.0	100.0	57.0	84.0
BRSK1	98.0	83.0	100.0	80.0	78.0
BRSK2	61.0	92.0	100.0	95.0	97.0
BTK	83.0	94.0	100.0	4.5	93.0
BUB1	100.0	71.0		99.0	100.0
CAMK1	76.0	80.0	100.0	35.0	77.0
CAMK1D	83.0	88.0	100.0	78.0	91.0
CAMK1G	93.0	88.0	100.0	67.0	99.0
CAMK2A	88.0	78.0	100.0	80.0	70.0
CAMK2B	92.0	89.0	100.0	88.0	92.0
CAMK2D	100.0	84.0	100.0	93.0	94.0
CAMK2G	84.0	81.0	100.0	77.0	91.0
CAMK4	84.0	100.0	100.0	76.0	100.0
CAMKK1	100.0	85.0	100.0	73.0	93.0
CAMKK2	100.0	81.0	100.0	74.0	98.0
CASK	100.0	89.0		86.0	100.0
CDC2L1	100.0	77.0	100.0	100.0	91.0
CDC2L2	100.0	99.0	100.0	92.0	100.0
CDC2L5	80.0	74.0		100.0	96.0
CDK11	70.0	86.0	100.0	100.0	92.0
CDK2	93.0	86.0	100.0	93.0	88.0
CDK3	99.0	91.0	100.0	96.0	84.0
CDK4-cyclinD1	91.0	84.0		94.0	97.0
CDK4-cyclinD3	97.0	100.0		100.0	100.0
CDK5	85.0	69.0	100.0	73.0	98.0
CDK7	100.0	100.0	100.0	45.0	86.0
CDK8	100.0	81.0	100.0	87.0	84.0
CDK9	70.0	65.0	100.0	99.0	96.0
CDKL1	93.0	86.0		100.0	100.0
CDKL2	100.0	97.0	100.0	95.0	85.0
CDKL3	85.0	98.0	100.0	100.0	99.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
CDKL5	83.0	100.0	100.0	100.0	100.0
CHEK1	84.0	75.0	100.0	98.0	98.0
CHEK2	100.0	92.0	100.0	9.1	93.0
CIT	100.0	72.0	100.0	37.0	65.0
CLK1	55.0	2.4	100.0	95.0	50.0
CLK2	84.0	47.0	25.0	60.0	90.0
CLK3	100.0	57.0	100.0	96.0	100.0
CLK4	32.0	2.2	100.0	87.0	29.0
CSF1R	7.4	15.0	100.0	12.0	98.0
CSF1R-autoinhibited	2.8	3.6		19.0	100.0
CSK	76.0	88.0	100.0	25.0	81.0
CSNK1A1	91.0	70.0		97.0	100.0
CSNK1A1L	95.0	90.0	100.0	100.0	99.0
CSNK1D	76.0	74.0	100.0	90.0	83.0
CSNK1E	78.0	43.0	100.0	83.0	3.2
CSNK1G1	100.0	77.0	100.0	80.0	75.0
CSNK1G2	82.0	100.0	100.0	73.0	56.0
CSNK1G3	100.0	90.0	100.0	85.0	81.0
CSNK2A1	59.0	57.0	100.0	97.0	91.0
CSNK2A2	100.0	69.0	100.0	89.0	95.0
CTK	91.0	100.0	100.0	57.0	97.0
DAPK1	86.0	77.0	100.0	82.0	89.0
DAPK2	94.0	79.0	100.0	75.0	81.0
DAPK3	94.0	68.0	100.0	78.0	78.0
DCAMKL1	84.0	50.0	100.0	70.0	73.0
DCAMKL2	100.0	95.0	100.0	100.0	100.0
DCAMKL3	85.0	100.0	100.0	100.0	100.0
DDR1	65.0	53.0	100.0	90.0	69.0
DDR2	100.0	100.0	100.0	66.0	73.0
DLK	81.0	98.0	100.0	15.0	87.0
DMPK	100.0	84.0	100.0	40.0	97.0
DMPK2	81.0	81.0	100.0	100.0	100.0
DRAK1	97.0	96.0	100.0	67.0	91.0
DRAK2	79.0	100.0	100.0	63.0	63.0
DYRK1A	100.0	40.0	100.0	96.0	90.0
DYRK1B	61.0	68.0	100.0	100.0	97.0
DYRK2	100.0	76.0	100.0	76.0	94.0
EGFR	73.0	91.0	100.0	11.0	78.0
EGFR(E746-A750del)	95.0	90.0	100.0	14.0	79.0
EGFR(G719C)	54.0	62.0	100.0	30.0	98.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
EGFR(G719S)	64.0	100.0	100.0	40.0	100.0
EGFR(L747-E749del, A750P)	85.0	86.0	100.0	17.0	98.0
EGFR(L747-S752del, P753S)	90.0	88.0	100.0	31.0	77.0
EGFR(L747-T751del,Sins)	84.0	72.0	100.0	25.0	100.0
EGFR(L858R)	90.0	90.0	100.0	18.0	100.0
EGFR(L858R,T790M)	78.0	79.0	100.0	3.0	85.0
EGFR(L861Q)	63.0	88.0	100.0	15.0	100.0
EGFR(S752-I759del)	73.0	68.0	100.0	22.0	100.0
EGFR(T790M)	92.0	84.0		4.3	100.0
EIF2AK1	100.0	97.0		100.0	100.0
EPHA1	76.0	85.0	100.0	72.0	100.0
EPHA2	100.0	79.0	100.0	100.0	100.0
EPHA3	79.0	88.0	100.0	50.0	99.0
EPHA4	83.0	92.0	100.0	98.0	92.0
EPHA5	98.0	73.0	100.0	78.0	96.0
EPHA6	89.0	40.0	100.0	100.0	90.0
EPHA7	93.0	92.0	100.0	88.0	89.0
EPHA8	80.0	97.0	100.0	80.0	78.0
EPHB1	70.0	87.0	100.0	92.0	92.0
EPHB2	95.0	100.0	100.0	78.0	92.0
EPHB3	84.0	44.0	100.0	100.0	79.0
EPHB4	84.0	48.0	100.0	77.0	91.0
EPHB6	54.0	98.0	100.0	8.6	97.0
ERBB2	65.0	3.2	100.0	72.0	97.0
ERBB3	61.0	96.0	100.0	29.0	92.0
ERBB4	79.0	92.0	100.0	24.0	96.0
ERK1	77.0	100.0	100.0	98.0	100.0
ERK2	89.0	71.0	100.0	79.0	94.0
ERK3	78.0	100.0	100.0	100.0	100.0
ERK4	100.0	100.0	100.0	100.0	100.0
ERK5	86.0	61.0	100.0	99.0	82.0
ERK8	100.0	79.0	100.0	88.0	82.0
ERN1	73.0	76.0	100.0	73.0	100.0
FAK	79.0	78.0	100.0	83.0	99.0
FER	96.0	95.0	100.0	9.1	100.0
FES	76.0	28.0	100.0	94.0	84.0
FGFR1	100.0	85.0	100.0	100.0	94.0
FGFR2	93.0	64.0	100.0	100.0	100.0
FGFR3	100.0	81.0	100.0	91.0	98.0
FGFR3(G697C)	65.0	100.0	100.0	87.0	99.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
FGFR4	84.0	99.0	100.0	89.0	75.0
FGR	86.0	57.0	100.0	14.0	83.0
FLT1	98.0	93.0	100.0	62.0	82.0
FLT3	52.0	54.0	100.0	30.0	78.0
FLT3(D835H)	57.0	69.0	100.0	16.0	100.0
FLT3(D835Y)	54.0	55.0	100.0	11.0	96.0
FLT3(ITD)	90.0	93.0	100.0	19.0	97.0
FLT3(K663Q)	69.0	76.0	100.0	18.0	89.0
FLT3(N841I)	35.0	18.0	100.0	34.0	100.0
FLT3(R834Q)	87.0	75.0		60.0	100.0
FLT3-autoinhibited	78.0	100.0		58.0	100.0
FLT4	83.0	91.0	100.0	86.0	82.0
FRK	100.0	98.0	100.0	74.0	88.0
FYN	100.0	92.0	100.0	23.0	73.0
GAK	97.0	92.0	100.0	70.0	98.0
GCN2(Kin.Dom.2,S808G)	100.0	89.0	100.0	3.4	100.0
GRK1	100.0	75.0	100.0	96.0	100.0
GRK4	91.0	82.0	100.0	68.0	60.0
GRK7	100.0	100.0	100.0	89.0	100.0
GSK3A	61.0	63.0	100.0	98.0	88.0
GSK3B	92.0	98.0	100.0	100.0	100.0
HASPIN	70.0	75.0		98.0	44.0
HCK	95.0	63.0	100.0	1.6	100.0
HIPK1	76.0	71.0	100.0	44.0	82.0
HIPK2	100.0	100.0	100.0	19.0	34.0
HIPK3	78.0	87.0	100.0	57.0	89.0
HIPK4	86.0	57.0	100.0	91.0	86.0
HPK1	66.0	89.0	100.0	12.0	67.0
HUNK	100.0	83.0	100.0	91.0	64.0
ICK	100.0	100.0	100.0	100.0	100.0
IGF1R	67.0	93.0	100.0	31.0	100.0
IKK-alpha	100.0	100.0	100.0	94.0	64.0
IKK-beta	100.0	100.0	100.0	100.0	100.0
IKK-epsilon	100.0	96.0	100.0	51.0	85.0
INSR	86.0	80.0	100.0	7.3	100.0
INSRR	92.0	96.0	100.0	19.0	72.0
IRAK1	100.0	100.0	100.0	78.0	100.0
IRAK3	68.0	56.0	100.0	61.0	79.0
IRAK4	100.0	100.0		100.0	99.0
ITK	88.0	91.0	100.0	19.0	84.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
JAK1(JH1domain-catalytic)	80.0	76.0	100.0	96.0	100.0
JAK1(JH2domain-pseudokinase)	20.0	56.0	100.0	0.0	100.0
JAK2(JH1domain-catalytic)	88.0	96.0	100.0	7.4	100.0
JAK3(JH1domain-catalytic)	63.0	58.0	100.0	8.7	83.0
JNK1	74.0	100.0	100.0	47.0	100.0
JNK2	89.0	100.0	100.0	95.0	100.0
JNK3	83.0	100.0	100.0	93.0	100.0
KIT	29.0	46.0	100.0	2.0	81.0
KIT(A829P)	85.0	93.0		49.0	97.0
KIT(D816H)	81.0	82.0		57.0	94.0
KIT(D816V)	94.0	73.0	100.0	40.0	84.0
KIT(L576P)	6.5	10.0	100.0	3.0	98.0
KIT(V559D)	21.0	36.0	100.0	1.1	94.0
KIT(V559D,T670I)	64.0	66.0	100.0	5.2	99.0
KIT(V559D,V654A)	45.0	59.0	100.0	7.6	87.0
KIT-autoinhibited	17.0	28.0		43.0	100.0
LATS1	96.0	81.0	100.0	59.0	39.0
LATS2	100.0	74.0	100.0	96.0	28.0
LCK	93.0	90.0	100.0	9.5	93.0
LIMK1	97.0	73.0	100.0	92.0	87.0
LIMK2	62.0	91.0	100.0	100.0	100.0
LKB1	92.0	91.0	100.0	100.0	79.0
LOK	72.0	88.0	100.0	28.0	76.0
LRRK2	91.0	96.0		98.0	100.0
LRRK2(G2019S)	80.0	90.0		82.0	93.0
LTK	86.0	87.0	100.0	10.0	76.0
LYN	85.0	100.0	100.0	43.0	97.0
LZK	97.0	100.0	100.0	20.0	95.0
MAK	79.0	81.0	100.0	91.0	93.0
MAP3K1	100.0	100.0	100.0	93.0	100.0
MAP3K15	100.0	94.0	100.0	96.0	100.0
MAP3K2	100.0	100.0	100.0	13.0	80.0
MAP3K3	79.0	97.0	100.0	10.0	100.0
MAP3K4	68.0	86.0	100.0	95.0	71.0
MAP4K2	90.0	100.0	100.0	9.8	90.0
MAP4K3	71.0	85.0	100.0	51.0	92.0
MAP4K4	99.0	80.0	100.0	78.0	83.0
MAP4K5	89.0	86.0	100.0	93.0	100.0
MAPKAPK2	80.0	100.0	100.0	96.0	85.0
MAPKAPK5	89.0	100.0	100.0	75.0	64.0

KINOMEscan Gene Symbol	PQR309	BKM120	GDC0941	GDC0980	PKI-587
	%	%	%	%	%
MARK1	75.0	87.0	100.0	67.0	97.0
MARK2	87.0	100.0	100.0	54.0	85.0
MARK3	78.0	100.0	100.0	67.0	97.0
MARK4	76.0	94.0	100.0	77.0	100.0
MAST1	100.0	100.0	100.0	69.0	94.0
MEK1	72.0	94.0	100.0	26.0	100.0
MEK2	98.0	92.0	100.0	7.0	100.0
MEK3	100.0	79.0	100.0	99.0	100.0
MEK4	100.0	100.0	100.0	100.0	100.0
MEK5	100.0	63.0		0.4	100.0
MEK6	70.0	75.0	100.0	68.0	60.0
MELK	64.0	64.0	100.0	73.0	76.0
MERTK	100.0	56.0	100.0	37.0	87.0
MET	60.0	91.0	100.0	63.0	95.0
MET(M1250T)	93.0	63.0	100.0	83.0	98.0
MET(Y1235D)	100.0	98.0	100.0	68.0	74.0
MINK	86.0	93.0	100.0	24.0	64.0
MKK7	100.0	100.0		89.0	91.0
MKNK1	100.0	100.0	100.0	100.0	100.0
MKNK2	91.0	61.0	100.0	100.0	65.0
MLCK	94.0	90.0	100.0	74.0	62.0
MLK1	87.0	94.0	100.0	3.2	97.0
MLK2	59.0	93.0	100.0	41.0	100.0
MLK3	100.0	97.0	100.0	17.0	89.0
MRCKA	100.0	91.0	100.0	88.0	74.0
MRCKB	89.0	82.0	100.0	94.0	83.0
MST1	83.0	94.0	100.0	87.0	84.0
MST1R	69.0	86.0	100.0	81.0	91.0
MST2	72.0	58.0	100.0	92.0	91.0
MST3	92.0	85.0	100.0	93.0	96.0
MST4	56.0	73.0	100.0	97.0	100.0
MTOR	0.0	1.8		0.1	0.0
MUSK	100.0	92.0	100.0	90.0	74.0
MYLK	95.0	63.0	100.0	73.0	37.0
MYLK2	97.0	74.0	100.0	87.0	100.0
MYLK4	77.0	75.0	100.0	96.0	95.0
MYO3A	86.0	68.0	100.0	94.0	64.0
MYO3B	100.0	94.0	100.0	80.0	83.0
NDR1	71.0	83.0	100.0	26.0	54.0
NDR2	93.0	80.0	100.0	44.0	62.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
NEK1	76.0	94.0	100.0	85.0	62.0
NEK10	100.0	100.0		5.0	61.0
NEK11	100.0	82.0		50.0	82.0
NEK2	100.0	95.0	100.0	24.0	100.0
NEK3	100.0	65.0		100.0	100.0
NEK4	100.0	100.0		9.2	100.0
NEK5	100.0	97.0	100.0	100.0	100.0
NEK6	100.0	73.0	100.0	84.0	95.0
NEK7	100.0	94.0	100.0	87.0	100.0
NEK9	100.0	91.0	100.0	90.0	90.0
NIK	100.0	100.0		73.0	99.0
NIM1	92.0	98.0	100.0	100.0	98.0
NLK	99.0	67.0	100.0	75.0	68.0
OSR1	100.0	98.0	100.0	100.0	100.0
p38-alpha	98.0	92.0	100.0	92.0	98.0
p38-beta	95.0	86.0	100.0	79.0	92.0
p38-delta	55.0	80.0	100.0	72.0	73.0
p38-gamma	74.0	43.0	100.0	83.0	100.0
PAK1	94.0	86.0	100.0	53.0	93.0
PAK2	88.0	86.0	100.0	65.0	100.0
PAK3	86.0	98.0	100.0	51.0	100.0
PAK4	93.0	88.0	100.0	3.9	100.0
PAK6	83.0	95.0	100.0	47.0	92.0
PAK7	86.0	91.0	100.0	0.7	87.0
PCTK1	100.0	100.0	100.0	100.0	100.0
PCTK2	98.0	64.0	100.0	95.0	76.0
PCTK3	100.0	100.0	100.0	98.0	100.0
PDGFRA	85.0	76.0	0.0	73.0	100.0
PDGFRB	97.0	79.0	100.0	8.9	75.0
PDPK1	53.0	65.0	100.0	96.0	80.0
PFCDPK1(P.falciparum)	100.0	100.0		67.0	84.0
PFPK5(P.falciparum)	78.0	100.0		99.0	100.0
PFTAIRE2	100.0	100.0	100.0	86.0	93.0
PFTK1	93.0	91.0	100.0	96.0	100.0
PHKG1	91.0	84.0	100.0	82.0	98.0
PHKG2	89.0	75.0	100.0	87.0	67.0
PIK3C2B	41.0	64.0	22.0	31.0	0.0
PIK3C2G	7.0	27.0	27.0	12.0	0.2
PIK3CA	0.1	0.0	0.2	0.0	0.0
PIK3CA(C420R)	0.0	0.0	0.4	0.0	0.2

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
PIK3CA(E542K)	0.2	0.0	0.5	0.1	0.0
PIK3CA(E545A)	0.1	0.0	0.4	0.0	0.2
PIK3CA(E545K)	0.2	0.0	0.3	0.1	0.0
PIK3CA(H1047L)	14.0	0.6	9.8	2.0	2.3
PIK3CA(H1047Y)	1.4	0.8	14.0	0.4	1.8
PIK3CA(I800L)	0.4	0.0		0.1	0.8
PIK3CA(M1043I)	13.0	2.4	19.0	0.7	3.5
PIK3CA(Q546K)	0.5	0.1	0.4	0.1	0.2
PIK3CB	2.6	0.7	0.1	0.3	0.3
PIK3CD	2.6	2.1	5.2	0.0	4.8
PIK3CG	1.0	2.2	5.3	0.0	0.1
PIK4CB	95.0	100.0	100.0	100.0	95.0
PIM1	85.0	80.0	100.0	82.0	100.0
PIM2	86.0	75.0	100.0	87.0	66.0
PIM3	78.0	86.0	100.0	85.0	100.0
PIP5K1A	82.0	53.0	100.0	8.9	27.0
PIP5K1C	100.0	100.0		27.0	80.0
PIP5K2B	94.0	96.0	100.0	84.0	99.0
PIP5K2C	96.0	100.0		96.0	83.0
PKAC-alpha	67.0	83.0	100.0	70.0	84.0
PKAC-beta	83.0	82.0	100.0	72.0	94.0
PKMYT1	66.0	90.0	100.0	98.0	87.0
PKN1	73.0	65.0	100.0	71.0	93.0
PKN2	100.0	83.0	100.0	93.0	100.0
PKNB(M.tuberculosis)	100.0	95.0		100.0	66.0
PLK1	49.0	100.0	100.0	100.0	95.0
PLK2	65.0	64.0	100.0	67.0	89.0
PLK3	96.0	79.0	100.0	69.0	97.0
PLK4	81.0	72.0	100.0	57.0	100.0
PRKCD	100.0	86.0	100.0	62.0	76.0
PRKCE	42.0	95.0	100.0	26.0	73.0
PRKCH	100.0	100.0	100.0	45.0	86.0
PRKCI	92.0	85.0		100.0	100.0
PRKCQ	100.0	99.0	100.0	63.0	97.0
PRKD1	100.0	90.0	100.0	100.0	100.0
PRKD2	90.0	54.0	100.0	100.0	89.0
PRKD3	85.0	70.0	100.0	98.0	84.0
PRKG1	100.0	68.0	100.0	85.0	68.0
PRKG2	92.0	90.0	100.0	91.0	89.0
PRKR	72.0	69.0	100.0	20.0	98.0

KINOMEscan Gene Symbol	PQR309	BKM120	GDC0941	GDC0980	PKI-587
	%	%	%	%	%
PRKX	75.0	99.0	100.0	59.0	73.0
PRP4	79.0	100.0	100.0	75.0	84.0
PYK2	100.0	95.0	100.0	58.0	100.0
QSK	84.0	89.0	100.0	94.0	100.0
RAF1	92.0	56.0	100.0	100.0	100.0
RET	80.0	72.0	100.0	68.0	76.0
RET(M918T)	84.0	80.0	100.0	62.0	99.0
RET(V804L)	86.0	83.0	100.0	63.0	92.0
RET(V804M)	86.0	95.0	100.0	57.0	87.0
RIOK1	97.0	94.0	100.0	24.0	63.0
RIOK2	90.0	85.0	100.0	2.1	67.0
RIOK3	84.0	92.0	100.0	79.0	61.0
RIPK1	84.0	72.0	100.0	100.0	100.0
RIPK2	100.0	97.0	100.0	76.0	72.0
RIPK4	100.0	100.0	100.0	99.0	53.0
RIPK5	98.0	79.0		53.0	78.0
ROCK1	100.0	89.0	100.0	76.0	65.0
ROCK2	100.0	94.0	100.0	75.0	58.0
ROS1	65.0	56.0	100.0	26.0	96.0
RPS6KA4(Kin.Dom.1-N-terminal)	86.0	93.0	100.0	89.0	96.0
RPS6KA4(Kin.Dom.2-C-terminal)	88.0	93.0	100.0	92.0	91.0
RPS6KA5(Kin.Dom.1-N-terminal)	92.0	72.0	100.0	91.0	93.0
RPS6KA5(Kin.Dom.2-C-terminal)	95.0	67.0	100.0	83.0	91.0
RSK1(Kin.Dom.1-N-terminal)	74.0	69.0	100.0	25.0	100.0
RSK1(Kin.Dom.2-C-terminal)	86.0	86.0	100.0	92.0	91.0
RSK2(Kin.Dom.1-N-terminal)	96.0	84.0	100.0	14.0	84.0
RSK2(Kin.Dom.2-C-terminal)	96.0	100.0	100.0	100.0	100.0
RSK3(Kin.Dom.1-N-terminal)	100.0	100.0	100.0	51.0	100.0
RSK3(Kin.Dom.2-C-terminal)	100.0	70.0		88.0	95.0
RSK4(Kin.Dom.1-N-terminal)	100.0	65.0	100.0	66.0	77.0
RSK4(Kin.Dom.2-C-terminal)	100.0	71.0	100.0	99.0	99.0
S6K1	100.0	86.0		73.0	91.0
SBK1	100.0	68.0	100.0	75.0	89.0
SGK	93.0	94.0		100.0	100.0
SgK110	76.0	82.0	100.0	57.0	100.0
SGK2	96.0	99.0		100.0	100.0
SGK3	89.0	100.0		69.0	85.0
SIK	91.0	72.0	100.0	49.0	94.0
SIK2	97.0	76.0	100.0	98.0	70.0
SLK	96.0	97.0	100.0	14.0	86.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
SNARK	100.0	100.0	100.0	52.0	97.0
SNRK	100.0	100.0		89.0	85.0
SRC	77.0	62.0	100.0	1.6	100.0
SRMS	94.0	85.0	100.0	27.0	97.0
SRPK1	78.0	75.0	100.0	66.0	93.0
SRPK2	58.0	100.0	100.0	80.0	58.0
SRPK3	77.0	68.0	100.0	64.0	64.0
STK16	78.0	70.0	100.0	22.0	73.0
STK33	66.0	73.0	100.0	61.0	93.0
STK35	85.0	81.0	100.0	12.0	87.0
STK36	86.0	98.0	100.0	92.0	84.0
STK39	100.0	100.0	100.0	71.0	100.0
SYK	100.0	93.0	100.0	1.8	71.0
TAK1	100.0	100.0	100.0	35.0	91.0
TAOK1	86.0	79.0	100.0	100.0	2.0
TAOK2	100.0	75.0	100.0	75.0	0.6
TAOK3	87.0	100.0	100.0	100.0	12.0
TBK1	76.0	61.0	100.0	71.0	90.0
TEC	80.0	100.0	100.0	8.4	67.0
TESK1	83.0	94.0	100.0	86.0	87.0
TGFBR1	100.0	93.0	100.0	100.0	89.0
TGFBR2	90.0	82.0	100.0	89.0	100.0
TIE1	100.0	86.0	100.0	80.0	64.0
TIE2	100.0	83.0	100.0	86.0	100.0
TLK1	93.0	86.0	100.0	97.0	94.0
TLK2	74.0	89.0	100.0	88.0	88.0
TNIK	75.0	100.0	100.0	27.0	73.0
TNK1	90.0	56.0	100.0	92.0	100.0
TNK2	75.0	60.0	100.0	81.0	87.0
TNNI3K	100.0	100.0	100.0	62.0	72.0
TRKA	88.0	79.0	100.0	32.0	88.0
TRKB	84.0	80.0	100.0	30.0	100.0
TRKC	92.0	92.0	100.0	51.0	96.0
TRPM6	85.0	89.0		32.0	66.0
TSSK1B	90.0	59.0	100.0	49.0	75.0
TTK	80.0	81.0	100.0	56.0	80.0
TXK	74.0	83.0	100.0	30.0	94.0
TYK2(JH1domain-catalytic)	91.0	100.0	100.0	56.0	98.0
TYK2(JH2domain-pseudokinase)	100.0	100.0	100.0	0.1	92.0
TYRO3	68.0	100.0	100.0	56.0	95.0

KINOMEscan Gene Symbol	**PQR309**	**BKM120**	**GDC0941**	**GDC0980**	**PKI-587**
	%	%	%	%	%
ULK1	100.0	83.0	100.0	100.0	100.0
ULK2	91.0	100.0	100.0	100.0	100.0
ULK3	100.0	80.0	100.0	6.6	100.0
VEGFR2	100.0	100.0	100.0	30.0	100.0
VRK2	100.0	100.0		97.0	97.0
WEE1	84.0	79.0	100.0	100.0	100.0
WEE2	100.0	97.0	100.0	88.0	100.0
WNK1	100.0	100.0		46.0	38.0
WNK3	100.0	100.0		24.0	31.0
YANK1	100.0	100.0		100.0	100.0
YANK2	76.0	72.0	100.0	79.0	89.0
YANK3	79.0	84.0	100.0	64.0	94.0
YES	76.0	66.0	100.0	15.0	100.0
YSK1	69.0	86.0	100.0	95.0	70.0
YSK4	100.0	100.0	100.0	1.8	6.7
ZAK	83.0	87.0	100.0	96.0	99.0
ZAP70	87.0	93.0	100.0	35.0	100.0

Table S3: Anti-proliferative action of compound PQR309 in four independent tumor cell line panels

Cell Lines	Log GI_{50cell}- Log $GI_{50,}$ mean	GI_{50} NCI60	GI_{50} [a]NTRC	GI_{50} Horizon	GI_{50} CLOVIS
SHP-77	0.981		6.701		
K-562	0.624	3.389	2.510		
SW480	0.565		2.574		
Ishikawa	0.435			1.909	
MDA-MB-231	0.425	1.720			2.010
SNB-19	0.406	1.784			
HEC-6	0.383			1.690	
KM12	0.380	1.680			
UWB1.289	0.340				1.532
CAL 27	0.337		1.523		
U-87 MG	0.326		1.483		
MDA-MB-436	0.314			0.537	2.348
SW-620	0.287	0.936	1.774		
786-0	0.284	0.620	2.076		
SR	0.279	2.375	0.289		
FaDu	0.278		1.329		
DLD-1	0.277		1.326		
CCRF-CEM	0.260	1.698	0.849		
MDA-MB-468	0.246	0.148		1.209	2.348
HCC1395	0.244			0.827	1.628
UWB1.289+BRCA11	0.233				1.199
A-172	0.229		1.188		
A375	0.216		1.153		
769-P	0.203		1.119		
HCC-2998	0.197	1.102			
C-33 A	0.192		1.091		
MDA-MB-453	0.192			1.089	
SF-268	0.175	1.047			
MeWo	0.173		1.043		
HCT-116	0.167	0.733	1.324		
YD-8	0.157			1.004	
J82	0.149		0.988		
LS 174T	0.136		0.957		
SK-N-AS	0.122		0.927		
NCI-H82	0.114		0.911		
OVCAR-8	0.107	0.685		1.109	
BT-549	0.096	0.422	1.324		
NCI-H23	0.089	0.859			
RPMI-7951	0.044		0.775		
PC-3	0.037	0.226			1.298
A-427	0.031		0.753		
EFO-27	0.031			0.751	
HL-60(TB)	0.029	0.748			
DU-4475	0.017			0.728	
Jurkat E6.1	0.012		0.720		
SK-MEL-5	0.008	0.714			
PA-1	-0.001		0.612	0.785	
NCI/ADR-RES	-0.005	0.692			
MDA-MB-157	-0.007			0.690	
SCC-9	-0.012			0.682	
HSC-4	-0.013			0.680	
KYM-1	-0.019			0.670	
SNU-1041	-0.027			0.658	
DU145	-0.030	0.653			
NCI-H322M	-0.038	0.642			

Cell Lines	Log GI_{50cell}- Log GI_{50}, mean	GI_{50} NCI60	GI_{50} [a]NTRC	GI_{50} Horizon	GI_{50} CLOVIS
RPMI-8226	-0.075	0.589			
LOX IMVI	-0.080	0.582			
NCI-H2452	-0.084			0.577	
U251	-0.110	0.544			
SJCRH30	-0.114		0.539		
MG-63	-0.116		0.536		
U-2 OS	-0.116		0.536		
SF-539	-0.119	0.532			
A549/ATCC	-0.121	0.437	0.624		
HCT-15	-0.123	0.343	0.713		
HuH-28	-0.133			0.516	
BT-20	-0.139		0.509		
OVCAR-4	-0.155	0.490			
SN12C	-0.170	0.473			
Caov-4	-0.176			0.468	
M14	-0.180	0.463			
SK-MEL-2	-0.188	0.454			
CAKI.1	-0.200	0.442			
MOLT-4	-0.203	0.484	0.393		
MPP-89	-0.205			0.437	
MCF7	-0.214	0.103			0.753
MDA-MB-435	-0.215	0.427			
OC 316	-0.219			0.423	
BICR 6	-0.226			0.416	
NCI-H226	-0.227	0.415			
BxPC-3	-0.228		0.414		
Hs 578T	-0.241	0.198	0.817	0.192	
YD-10B	-0.251			0.393	
NCI-H460	-0.256	0.300	0.477		
Caov-3	-0.260			0.385	
BICR 18	-0.263			0.382	
ACHN	-0.265	0.392	0.369		
NCI-H522	-0.268	0.378			
HOP-62	-0.271	0.375			
MDA-MB-361	-0.279				0.369
BICR 16	-0.287			0.362	
HEC-1-A	-0.292			0.358	
BHY	-0.304			0.348	
AGS	-0.305			0.347	
LoVo	-0.324		0.332		
YD-15	-0.325			0.331	
OVCAR-3	-0.327	0.430	0.229		
RL95-2	-0.329			0.329	
SK-MEL-28	-0.329	0.328			
JHUEM-1	-0.332			0.326	
COLO 205	-0.335	0.324			
AU-565	-0.340		0.320		
SK-OV-3	-0.350	0.202		0.424	
HT29	-0.355	0.309			
A-2780	-0.363			0.157	0.451
A498	-0.364	0.076	0.530		
AN3 CA	-0.378		0.293		
OVCAR-5	-0.387	0.293		0.281	
SF-295	-0.387	0.287			
NUGC-3	-0.411			0.272	
RXF.393	-0.425	0.263			
NCI-H28	-0.427			0.262	
NCI-SNU-5	-0.442			0.253	

Cell Lines	Log GI_{50cell}- Log GI_{50}, mean	GI_{50} NCI60	GI_{50} [a]NTRC	GI_{50} Horizon	GI_{50} CLOVIS
TGBC11TKB	-0.482			0.231	
UACC-62	-0.484	0.230			
MSTO-211H	-0.541			0.202	
UACC-257	-0.555	0.195			
KLE	-0.578			0.185	
SW48	-0.602		0.175		
CAL-33	-0.633			0.163	
T47D	-0.645	0.052		0.265	
BICR 31	-0.647			0.158	
MALME-3M	-0.664	0.152			
TK-10	-0.669	0.150			
IST-MES1	-0.683			0.145	
UO.31	-0.693	0.142			
HCC1187	-0.710			0.137	
HCC2218	-0.740			0.128	
SNB-75	-0.763	0.121			
OVISE	-0.794			0.113	
IGROV1	-0.796	0.112			
A-204	-0.837		0.102		
MDA-MB-175-VII	-0.905			0.087	
HOP-92	-1.146	0.050			
RERF-GC-1B	-1.327			0.033	

[a]The dataset used here for GI_{50} determination is represented as IC_{50} calculations in Ref. 11.

Table S4: In Vitro Pharmacology I – Ligand Binding Assays with PQR309 at 10 µM

GPCRs, Ion channels, Nuclear Receptors and Transporters, [% of control]			
A1*, aRL	94.3	M2*, ant-RL	106.1
A2A*, aRL	100.3	M3*, ant-RL	100.1
A2B*, ant-RL	98.9	M4*, ant-RL	111.5
A3*, aRL	69.1	NK1*, aRL	93.1
alpha 1A*, ant-RL	99.1	NK2*, aRL	104.6
alpha 1B*, ant-RL	109.2	Y1*, aRL	113.2
alpha 2A*, ant-RL	102.2	N neuronal alpha4, beta2*, aRL	99.4
alpha 2B*, ant-RL	124.1	N muscle-type*, ant-RL	85.5
alpha 2C*, ant-RL	112.4	delta2 (DOP)*, aRL	100.1
beta1*, aRL	104.1	kappa (KOP), aRL	120.7
beta2*, aRL	103.9	mu (MOP)*, aRL	96.8
beta3*, ant-RL	103.3	NOP (ORL1)*, aRL	98.7
AT1*, ant-RL	103.7	PPARgamma *, aRL	96.3
AT2*, aRL	101.5	PAF*, aRL	92.9
APJ (apelin)*, aRL	103.2	PCP, ant-RL	100.0
BZD (central), aRL	132.0	EP2*, aRL	88.8
BB3*, aRL	103.4	FP*, aRL	109.5
B2*, aRL	100.6	IP (PGI2)*, aRL	105.9
CB1*, aRL	77.7	LXRbeta *, aRL	103.5
CB2*, aRL	107.8	5-HT1A*, aRL	93.1
CCK1 (CCKA)*, aRL	103.0	5-HT1B, ant-RL	113.5
CCK2 (CCKB)*, aRL	118.9	5-HT1D, aRL	85.5
CRF1*, aRL	94.6	5-HT2A*, aRL	96.6
D1*, ant-RL	96.1	5-HT2B*, aRL	111.8
D2S*, aRL	106.6	5-HT2C*, aRL	106.0
D3*, ant-RL	94.8	5-HT3*, ant-RL	107.0
ETA*, aRL	103.2	5-HT4e*, ant-RL	103.3
ETB*, aRL	91.9	5-HT6*, aRL	104.7
GABAA1* (alpha1, beta2, gamma2), aRL	108.8	5-HT7*, aRL	93.0
GABAB(1b)*, ant-RL	108.0	sigma (non-selective)*, aRL	100.2
glucagon*, aRL	88.0	sst1*, aRL	86.7
AMPA, aRL	134.5	sst4*, aRL	88.5
kainate, aRL	75.3	GR*, aRL	87.7
NMDA, ant-RL	94.8	ERalpha * (agonist fluoligand)	103.2
glycine (strychnine-insensitive), ant-RL	134.2	AR*, aRL	97.3
TNF-alpha*, aRL	97.0	TR (TH), aRL	121.1
CCR2*, aRL	85.3	UT*, aRL	94.3
H1*, ant-RL	105.4	VPAC1 (VIP1)*, aRL	101.1
H2*, ant-RL	99.9	V1a*, aRL	95.6
H3*, aRL	107.3	V2*, aRL	92.0
H4*, aRL	99.4	Ca2+ channel (L, dihydropyridine site), ant-RL	162.1
BLT1 (LTB4)*, aRL	95.0	Ca2+ channel (L, diltiazem site) (benzothiazepines), ant-RL	101.6
CysLT1 (LTD4)*, aRL	91.9	Ca2+ channel (L, verapamil site) (phenylalkylamine), ant-RL	96.8
MCH1*, aRL	92.9	Ca2+ channel (N), ant-RL	109.6
MC1, aRL	109.2	SKCa channel, ant-RL	107.5
MC3*, aRL	90.2	Na+ channel (site 2), ant-RL	103.1
MC4*, aRL	109.4	Cl- channel (GABA-gated), ant-RL	92.7
MT1 (ML1A)*, aRL	59.8	norepinephrine transporter*, ant-RL	91.5
MT3 (ML2), aRL	72.1	dopamine transporter*, ant-RL	74.1
MAO-A, ant-RL	109.2	GABA transporter, ant-RL	101.9
motilin*, aRL	98.8	choline transporter (CHT1)*, ant-RL	106.7
M1*, ant-RL	122.9	5-HT transporter*, ant-RL	96.7

*human target; aRL: agonist radioligand; ant-RL: antagonist radioligand; For further information see Cerep.fr P22-p.

Table S5: In Vitro Pharmacology II – Enzyme Assays with PQR309 at 10 µM

Enzymes tested	% of control
COX1*	94.0
COX2*	116.7
inducible NOS	121.5
PDE2A1*	87.3
PDE3B*	88.1
PDE4D2*	90.6
PDE5* (non-selective)	85.8
PDE6 (non-selective)	87.4
ACE*	90.4
ACE-2*	95.2
BACE-1* (beta -secretase)	97.0
caspase-3*	95.9
HIV-1 protease*	98.5
neutral endopeptidase*	100.3
MMP-1*	95.2
MMP-2*	98.2
MMP-9*	115.2
Abl kinase*	98.5
CaMK2alpha *	99.9
CDK2* (cycA)	81.9
ERK2* (P42mapk)	99.1
FLT-1 kinase* (VEGFR1)	92.0
Fyn kinase*	95.6
IRK* (InsR)	113.6
Lyn A kinase*	98.3
p38alpha kinase*	99.5
ZAP70 kinase*	87.8
acetylcholinesterase*	84.7
COMT (catechol- O-methyl transferase)	92.9
xanthine oxidase/superoxide O_2^- scavenging	101.5
ATPase (Na^+/K^+)	94.8
guanylyl cyclase* (activator effect; controls effect = 100)	0.95

*human target protein

Table S6: Safety Profile of Compound 1

Studies	Results
Toxicity tests in vivo	
28-day oral toxicity studies (GLP) in Wistar rats and Beagle dogs with 4-week treatment-free recovery (based on 4-day dose-range finding and 28-day pilot studies)	Steep dose-response; body weight loss was dose-limiting. Otherwise mild toxicity (organ weight and/or histopathology) in the hemato-lymphoid system (mainly rats), reproductive system (rats and dogs), and the salivary glands (rats only). Reversible with exception of an atrophy of the sublingual salivary glands. Rats: Severely toxic dose > 8 (♂), > 4 (♀) mg/kg. No Observed Adverse Effect Level (NOAEL) < Low dose (♂ 2 and ♀ 1 mg/kg). Dogs: NOAEL = 4 mg/kg for both genders. Highest-Non-Severely-Toxic-Dose 8 (♂), 6 (♀) mg/kg.
Safety pharmacology testing (part of the 28-day toxicity studies below)	Neuropharmacology profiling and respiratory function in rats as well as cardiovascular function in male dogs: no relevant findings; (GLP)
Dog telemetry, cardiovascular safety pharmacology	Single oral dose. No relevant findings; (GLP)
Toxicity tests in vitro	
hERG patch clamp assays	No inhibition of hERG tail current; (GLP)
Phototoxicity test	No phototoxicity; (non-GLP)
Ames test	No mutagenicity; (non-GLP)
PK and metabolism in vivo	
Toxicokinetic investigations in 28 day oral toxicity studies (rats, dogs)	C_{max} after 0.5 to 2 hrs; dose-proportional exposure with dose-normalized $AUC_{24\ hrs}$ in rats: ~ 1000 (♂) to 2000 (-3500) (♀) hr·kg·ng/mL/mg, in dogs: ~ 300 – 800 hr·kg·ng/ml/mg; with exception of 2-fold accumulation in ♀ at 6 mg/kg. Terminal $t_{1/2}$ in rats: ♂ 3.3 – 5.1 hrs, ♀ 4.8 – 16.0 hrs and in dogs (♂ and ♀): 5.9 to 9.3 hrs
Pharmacokinetic (PK) and bioavailability (BAV) in male dogs	Single oral dose and i.v. dose: BAV 23%, t_{max} ~ 1-2 hrs, $t^1/_2$ ~ 8 hrs; non-GLP
Single oral dose ADME study in rats	BAV 24-32%; t_{max} 4 hrs, highly distributed; no binding to melanin; extensively metabolized: 5 major metabolites, mainly by hydrolysis and hydroxylation; rapidly excreted. Performed with radioactive PQR309; (GLP)
PK and metabolism in vitro	
Metabolism in hepatocytes	In rat (R), dog (D) and human (Hu): high metabolic stability, 25 unsuspicious metabolites in small quantities; (GLP)
Blood/plasma distribution	In male Wistar Han rat (R), Beagle dog (D) and human (Hu) plasma (GLP): up to 6050 ng/ml, 43.4% binding in R; 43.9% in D; and 32.5% in Hu; concentration independent.
Plasma protein binding	In male Wistar Han rat, Beagle dog and human plasma (GLP) 74.0-75.0% in R, 78.7-80.0% in D and 86.7-86.9% in Hu
Inhibition of CYP450 isoenzymes in human hepatocytes	At > 30 µM some inhibition of CYP 2C19 and 3A4 and tendency for inhibition of CYP1A2, 2C8, 2C9. No inhibition of CYP2B6, 2D6. Effects occur at > 10x of expected human therapeutic plasma levels. Drug-drug interactions cannot be excluded.
Induction of CYP450 isoenzymes in human hepatocytes	mRNA levels: slight increase for CYP2B6 at 0.5 and 1 µM in donor 1 (not at 5 and 10 µM) and CYP3A4 at 10 µM in donor 2; Drug-drug interactions cannot be excluded; (GLP)

Table S7. Solubility of PI3K inhibitors and reference compounds

Compounds*	Solubility [µM], Mean ± SD		Range	
at 200 µM	pH 7.4	pH 4.0	pH 7.4	pH 4.0
PQR309	206.0 ± 8.0	206.0 ± 0.1	>=200	>=200
2	63.0 ± 0.1	76.0 ± 1.0	<200	<200
BKM120	99.0 ± 0.1	182.0 ± 18.0	<200	<=200
ZSTK474	3.0 ± 0.1	4.0 ± 0.1	<<<200	<<<200
BEZ235	15.0 ± 0.1	57.0 ± 10.0	<<200	<200
Diclofenac	195.0 ± 2.0	10.0 ± 1.0	>=200	<<<200
Ketoconazole	135.0 ± 10.0	197.0 ± 7.0	<200	<=200
Phenytoin	186.0 ± 17.0	149.0 ± 5.0	<200	<200

*Compounds were diluted in aqueous solutions (PBS for pH 7.4, citrate for pH 4.0, 1% DMSO) at 200 µM, and soluble compounds were analyzed by LC/MS.

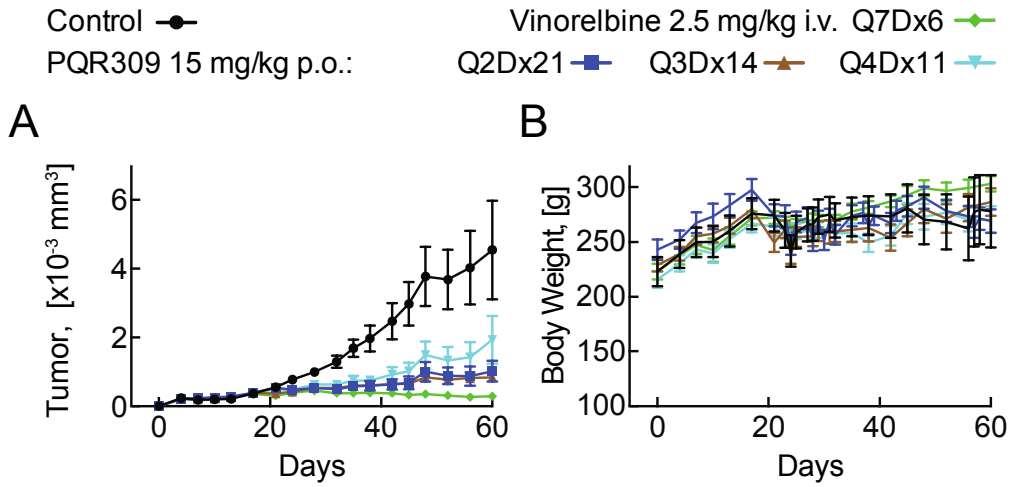
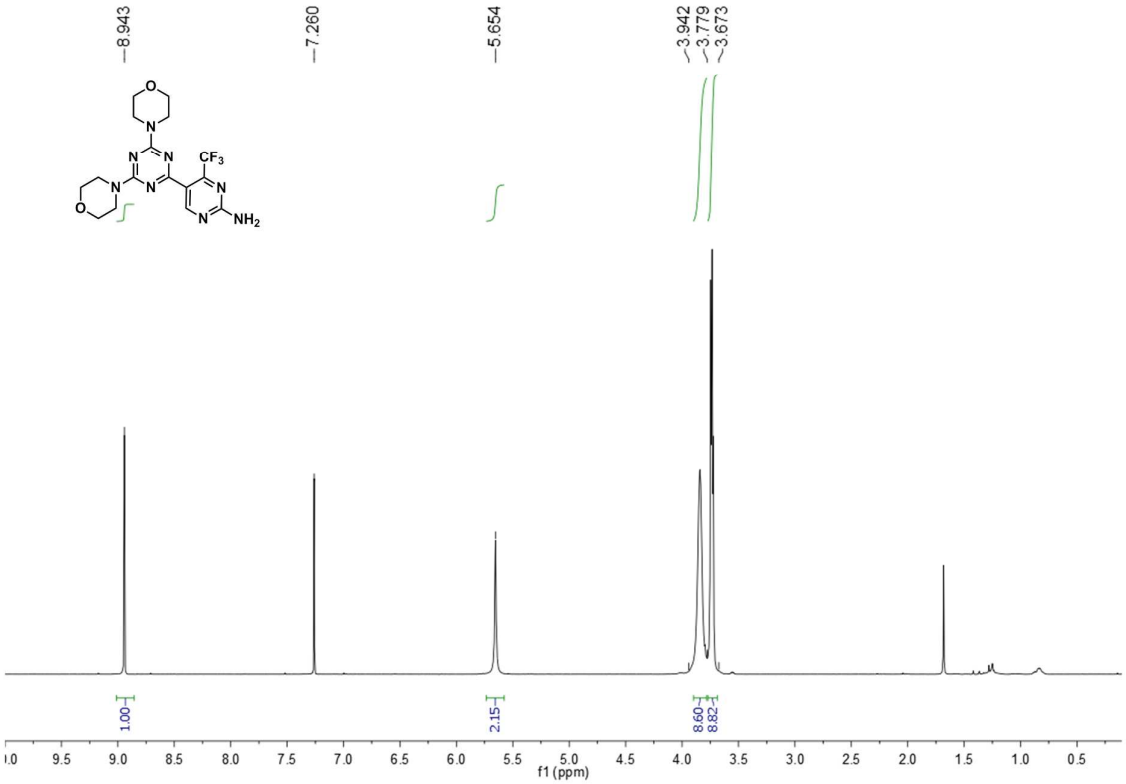


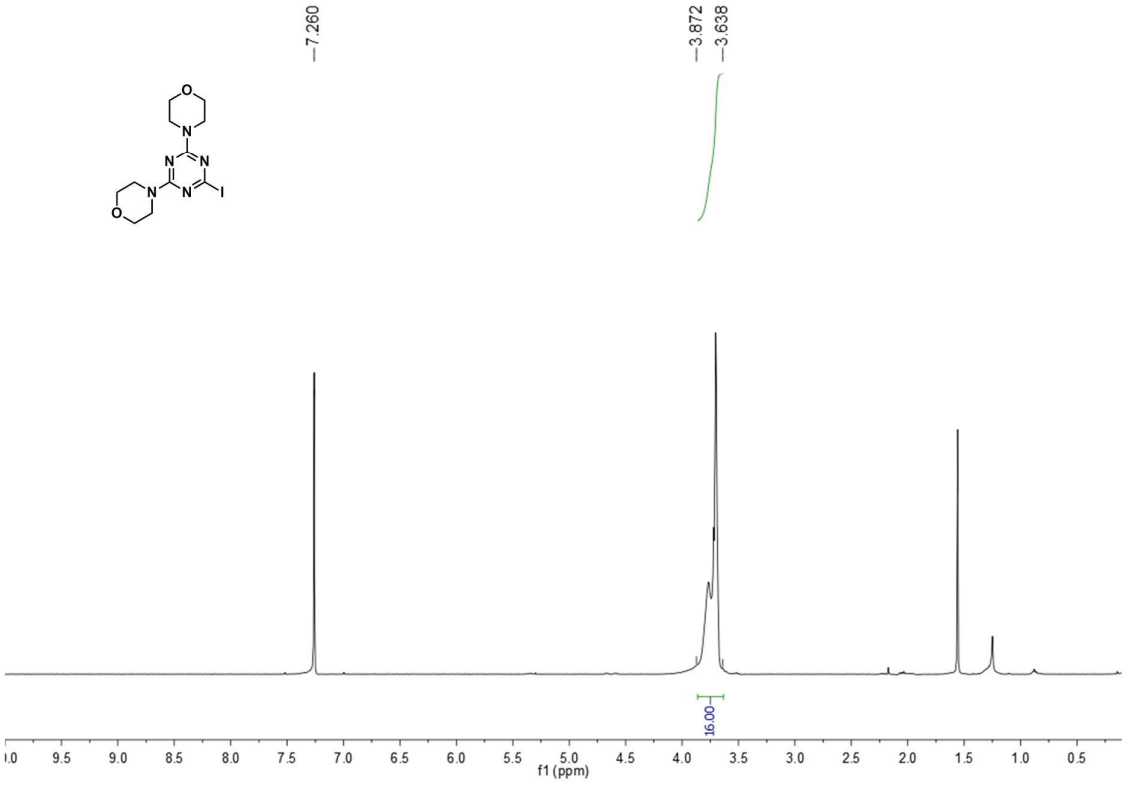
Figure S2. Compound PQR309 intermittent dosing efficacy model in PC3 xenografts in nude rats. **A)** Tumor volume: PQR309 was given every second day (Q2D), every third day (Q3D) or every fourth day (Q4D) p.o. at a dose of 15 mg/kg. Vinorelbine was given weekly (Q7D; 2.5 mg/kg i.v.), and control animals received vehicle only (Q2D). **B)** Body weight changes in nude rats during the treatment as mentioned in A). Data are depicted as mean ± SEM (Control n=6, Vinorelbine n=6, compound PQR309 each dose n=8).

^{1}H NMR Spectra:

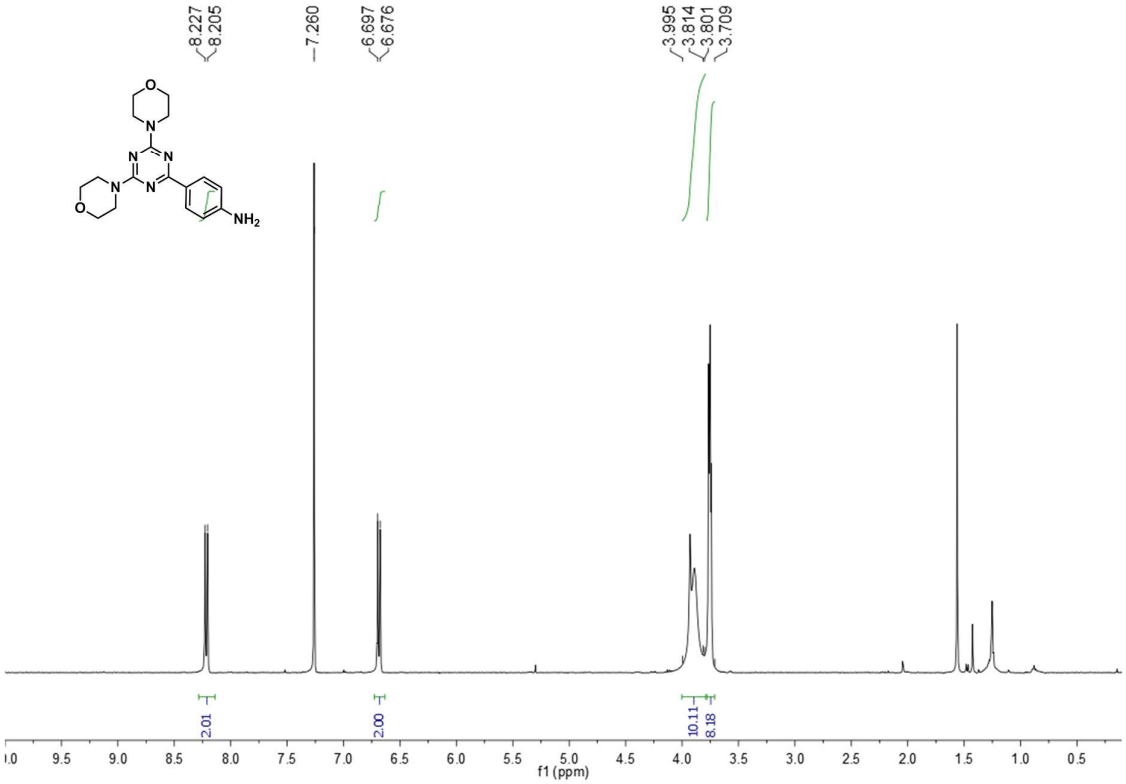
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyrimidin-2-amine (**2**):



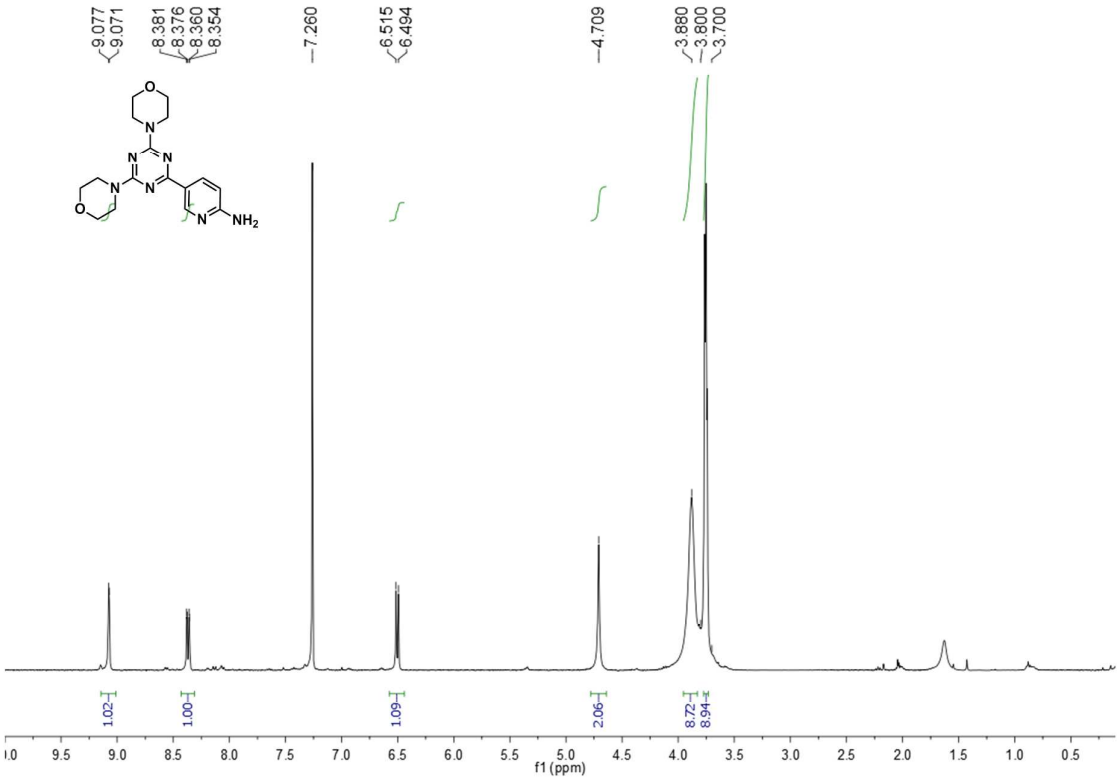
4,4'-(6-Iodo-1,3,5-triazine-2,4-diyl)dimorpholine (**8**):



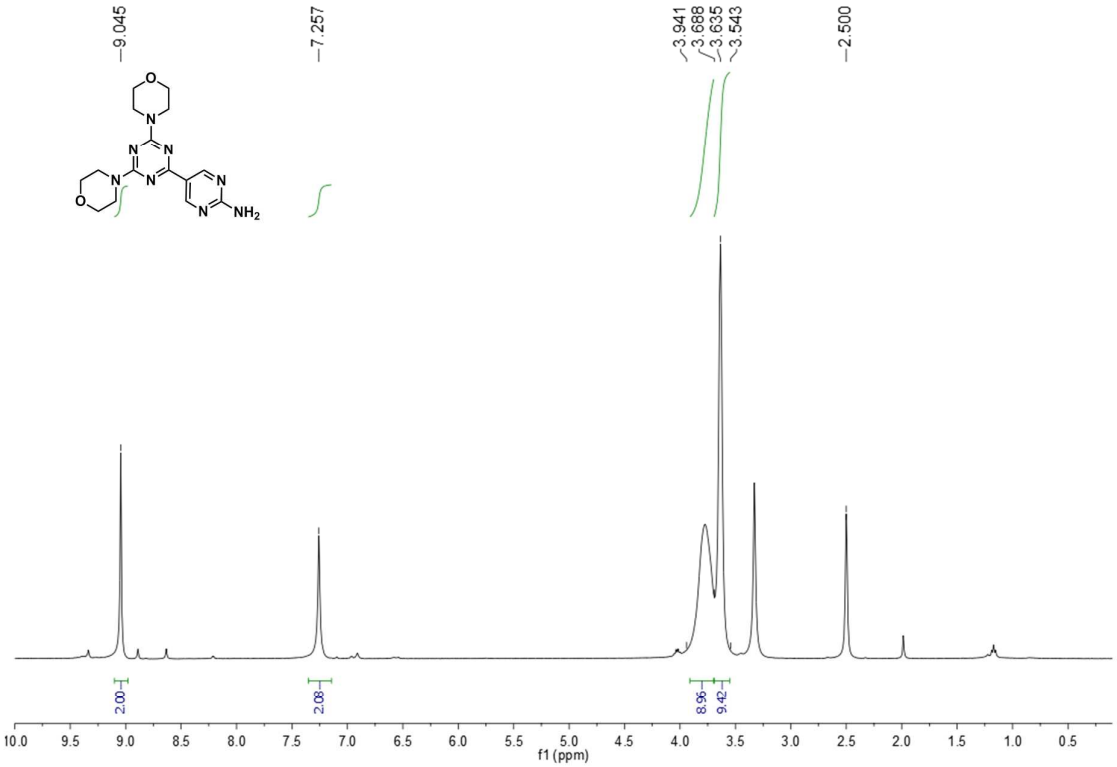
4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)aniline (**9**):



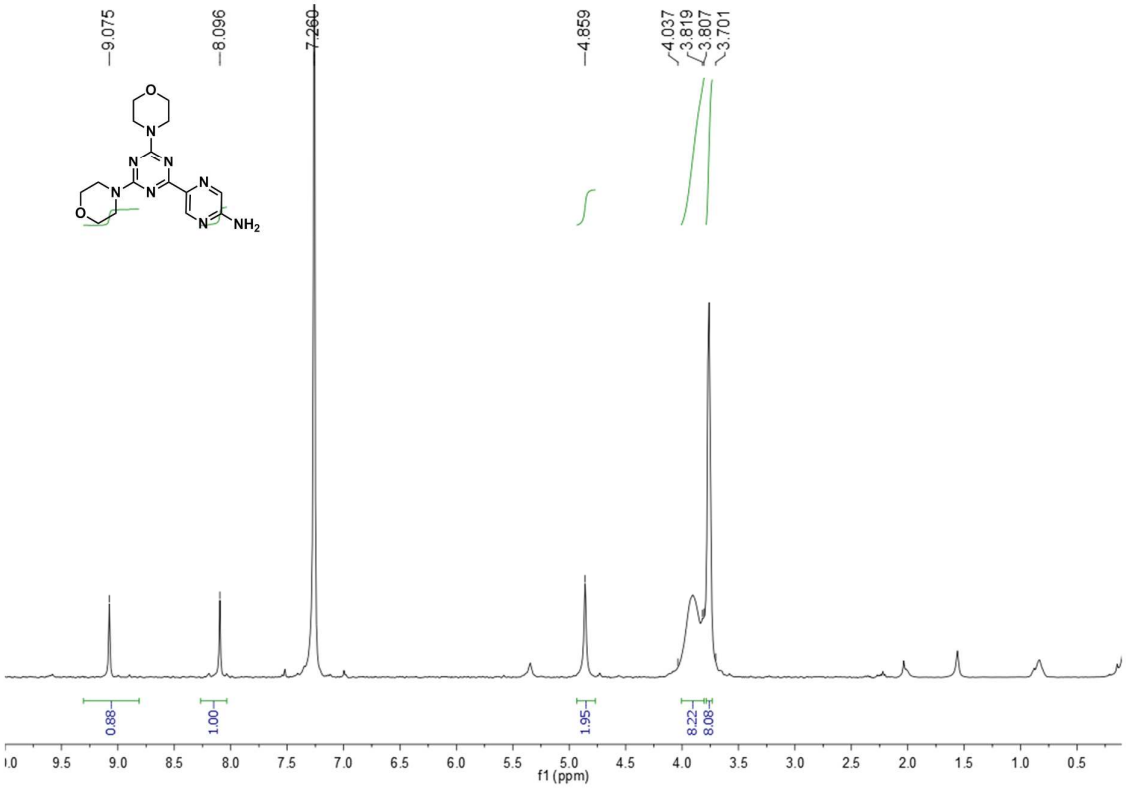
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyridin-2-amine (**10**):



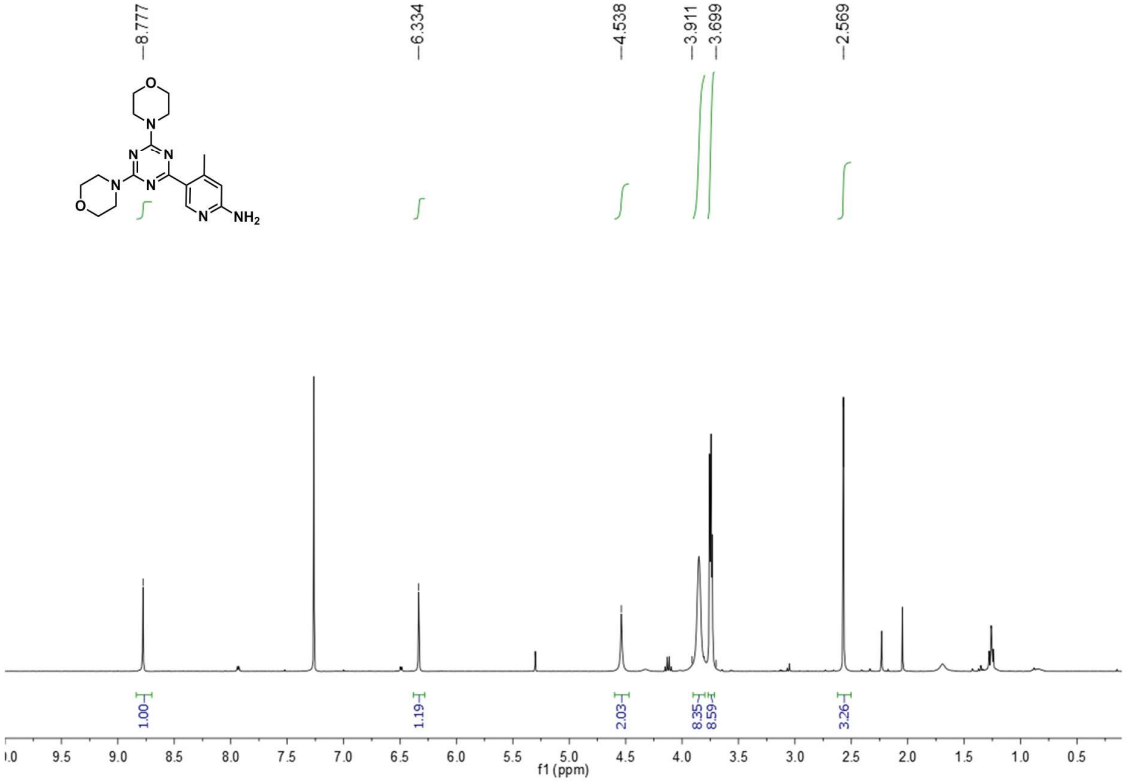
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrimidin-2-amine (**11**):



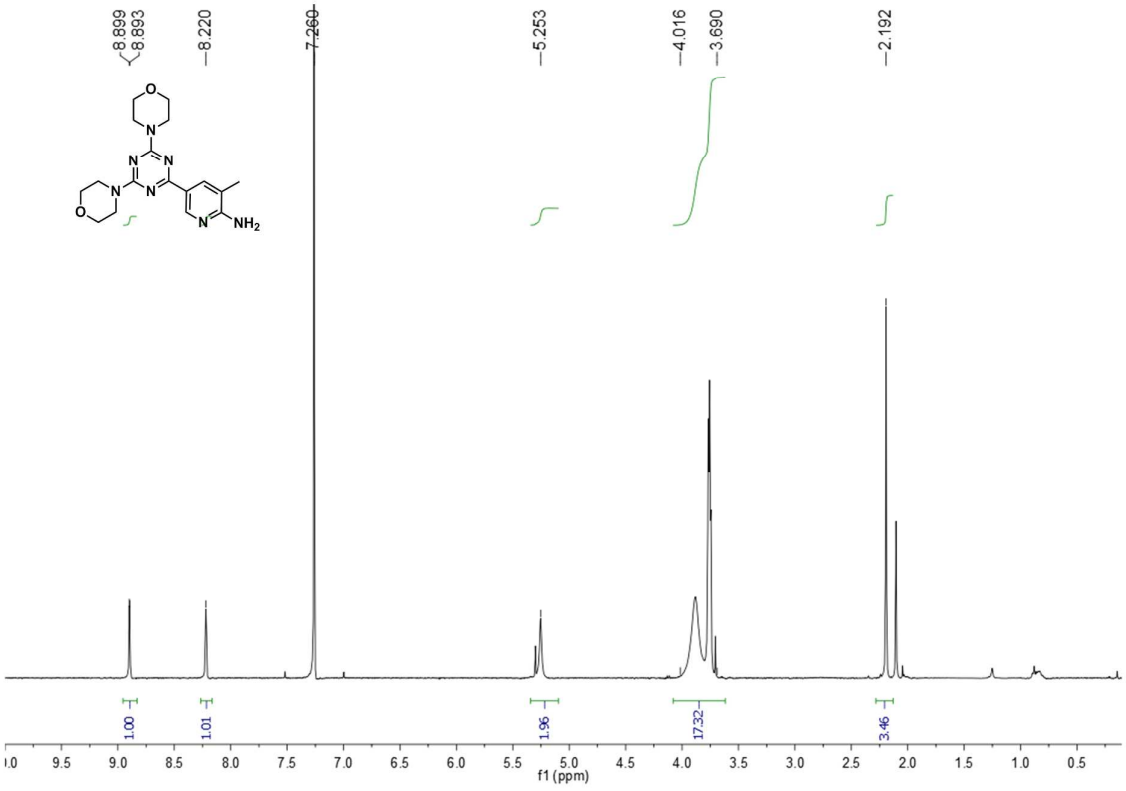
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrazin-2-amine (**12**):



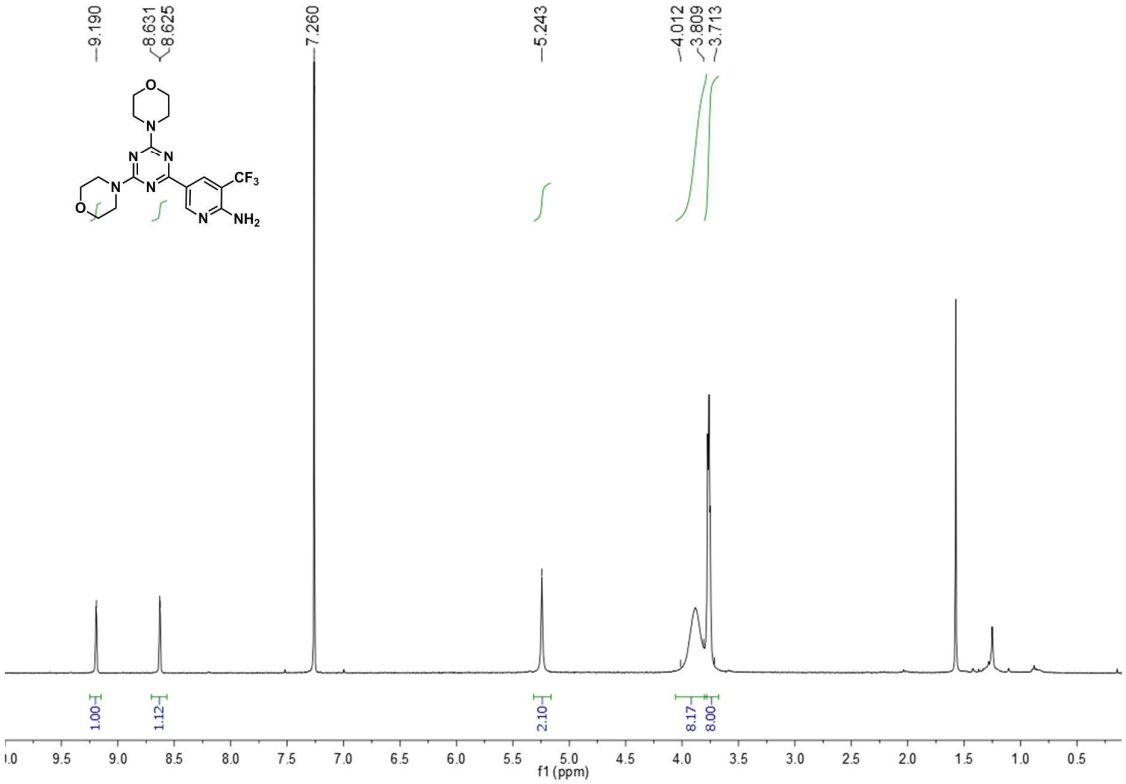
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyridin-2-amine (**13**):



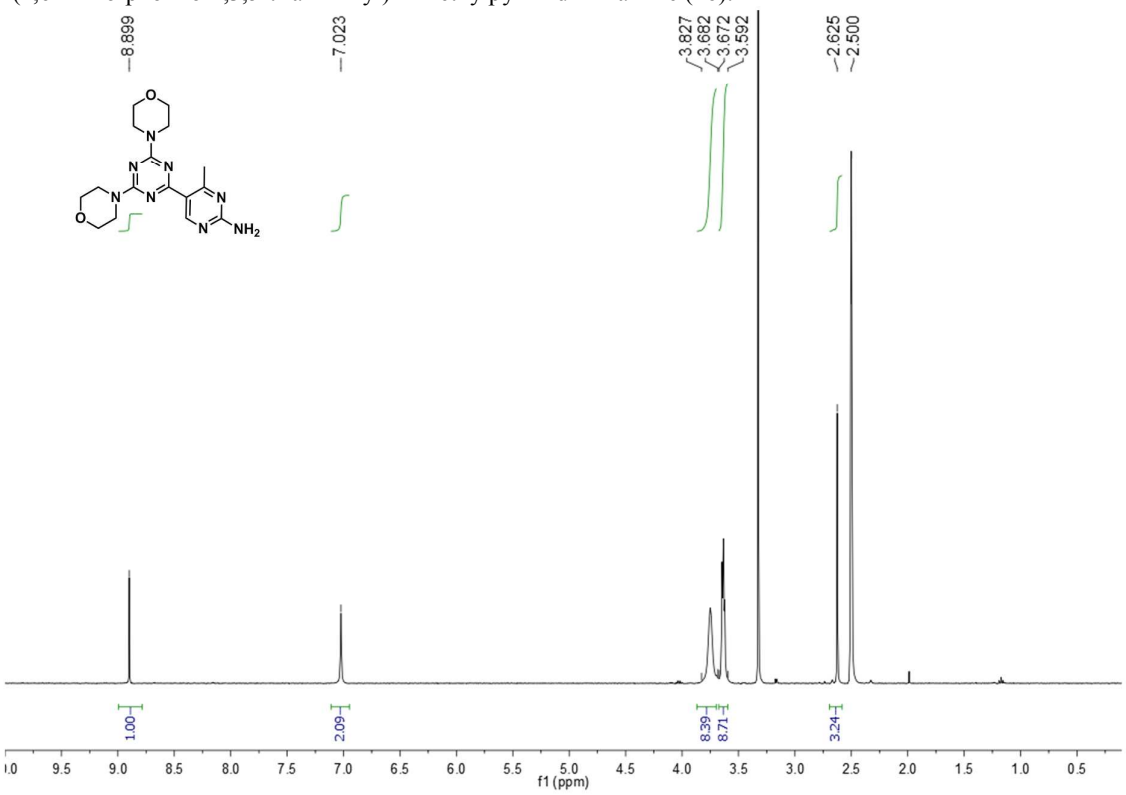
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-methylpyridin-2-amine (**14**):



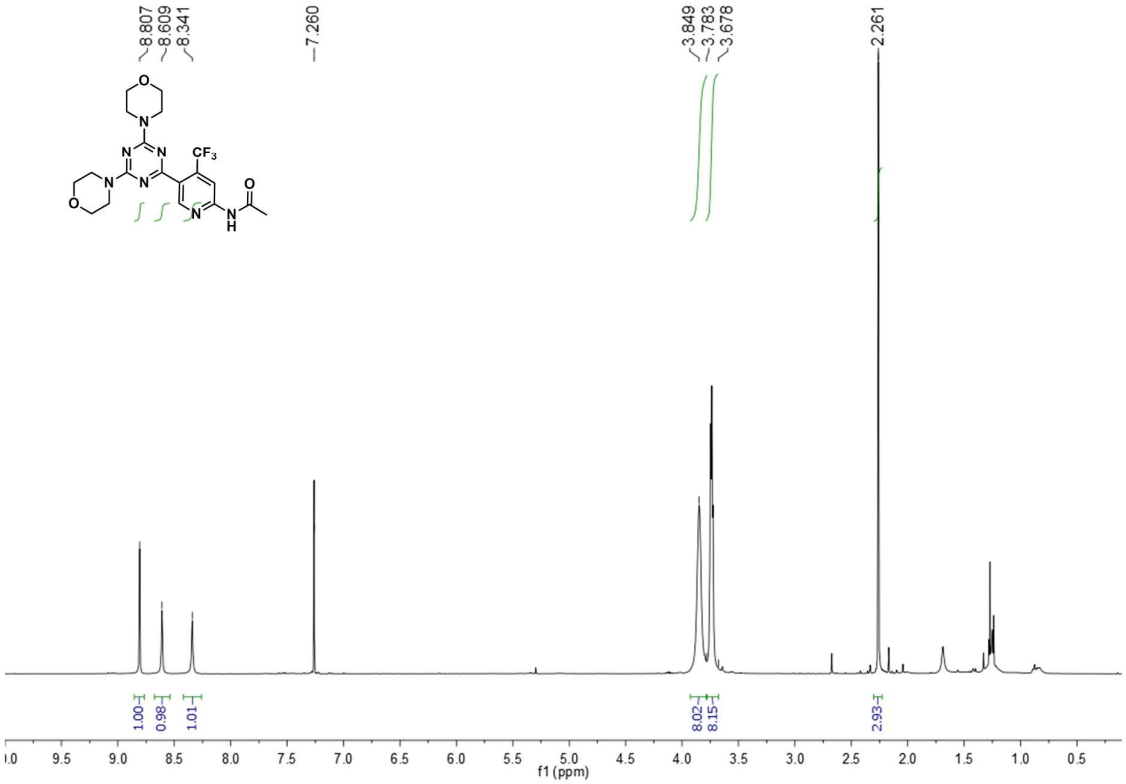
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-(trifluoromethyl)pyridin-2-amine (**15**):



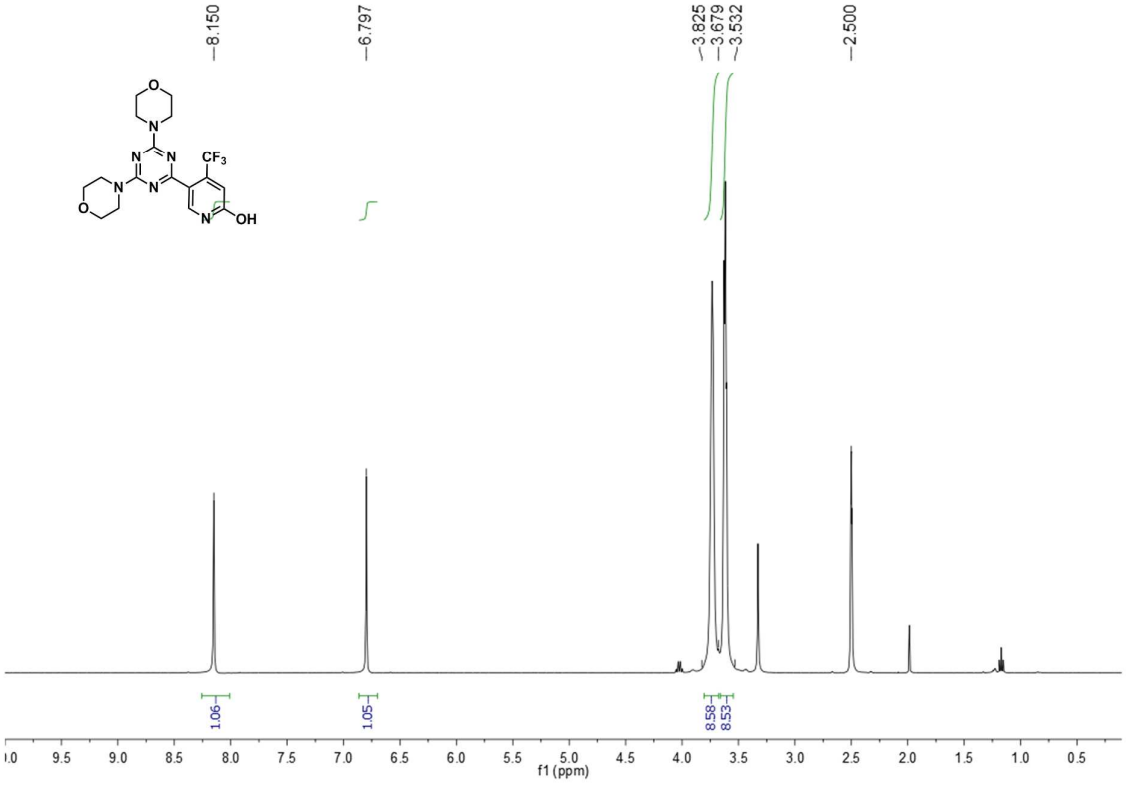
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyrimidin-2-amine (**16**):



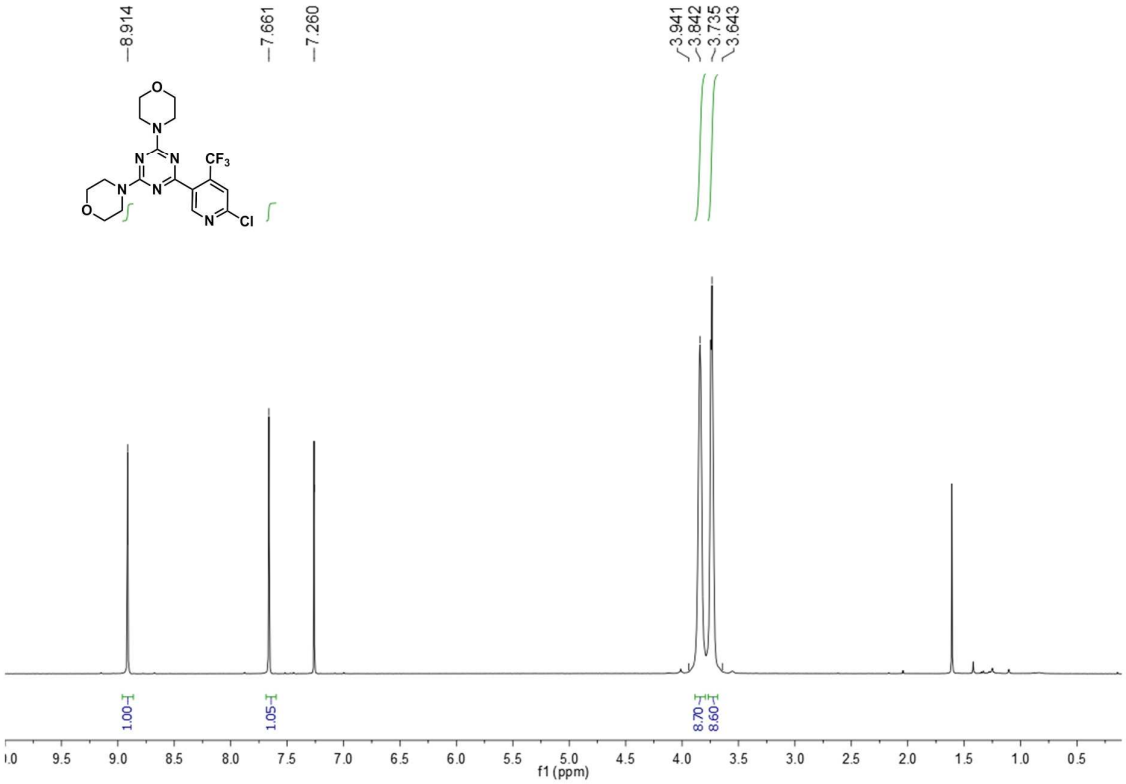
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)acetamide (**17**):



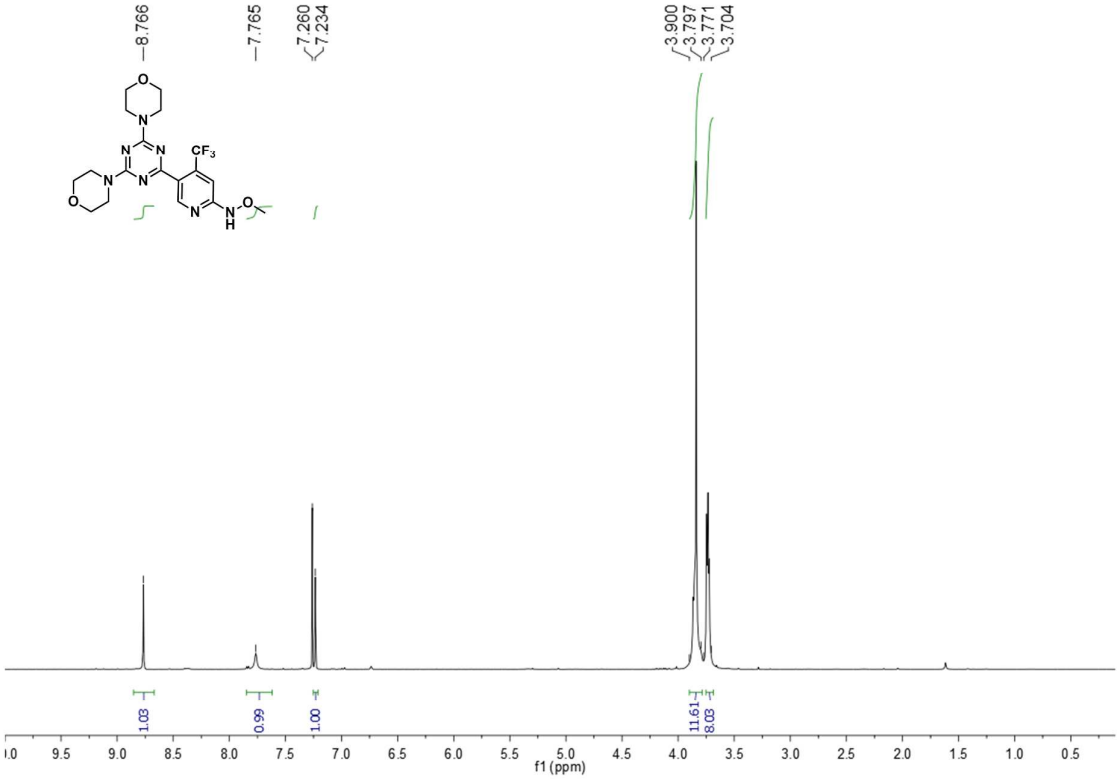
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-ol (**18**):



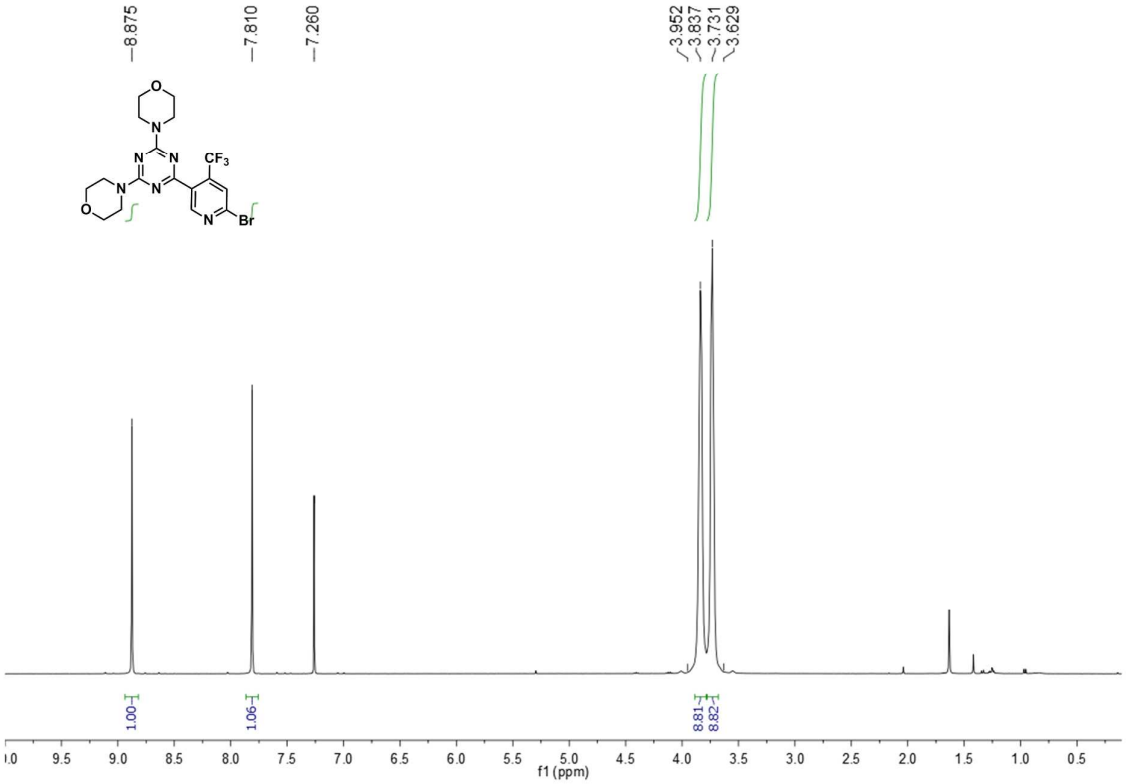
4,4'-(6-(6-Chloro-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**19**):



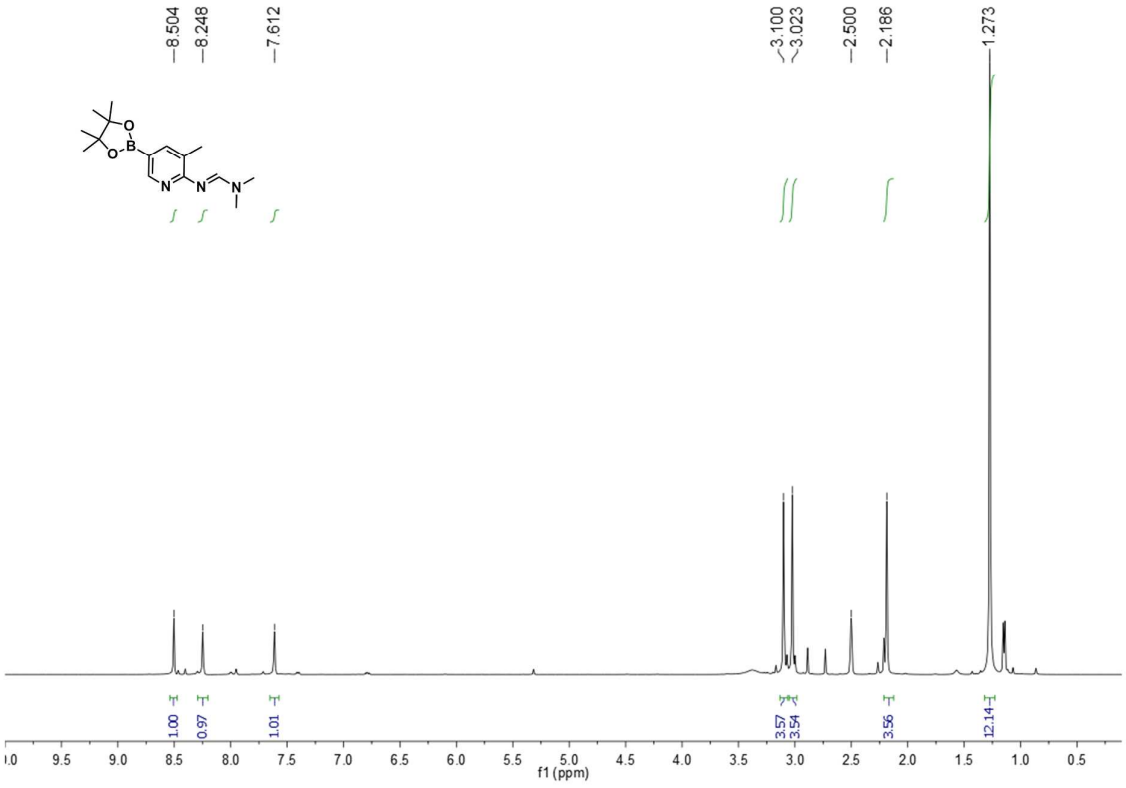
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine (**20**):



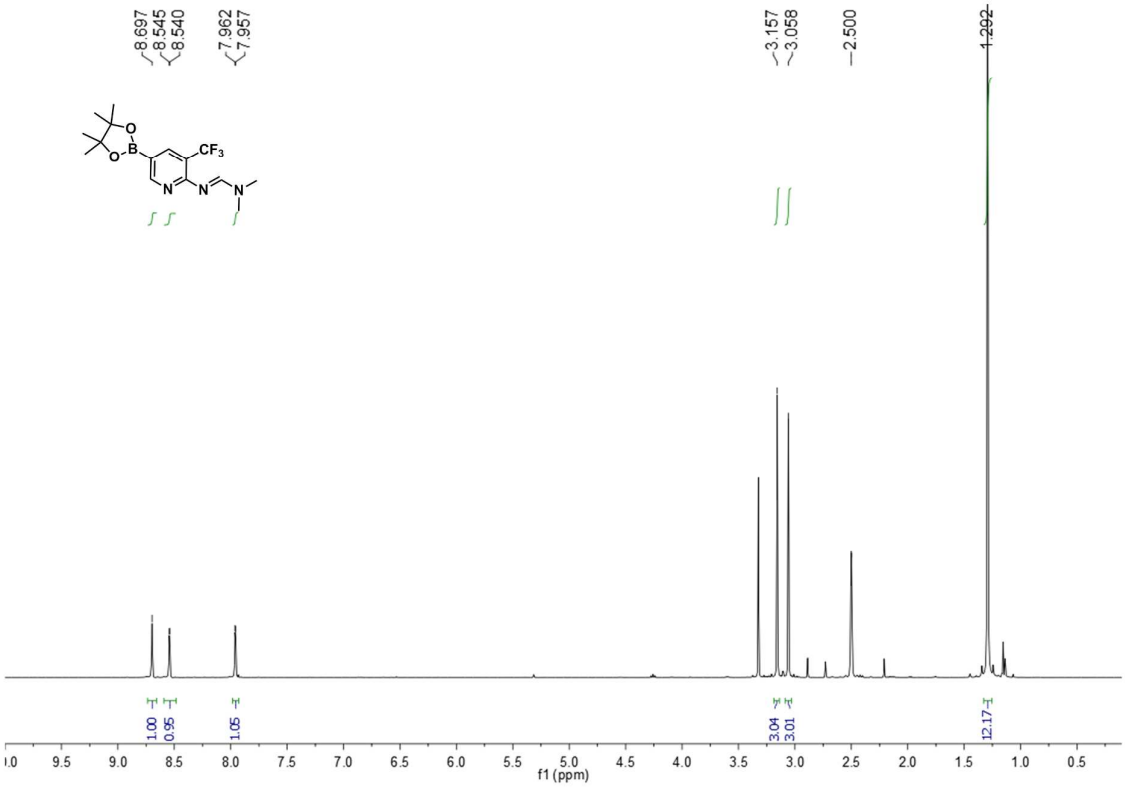
4,4'-(6-(6-Bromo-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**21**):



N,*N*-Dimethyl-*N*′-(3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)formimidamide (**23**):

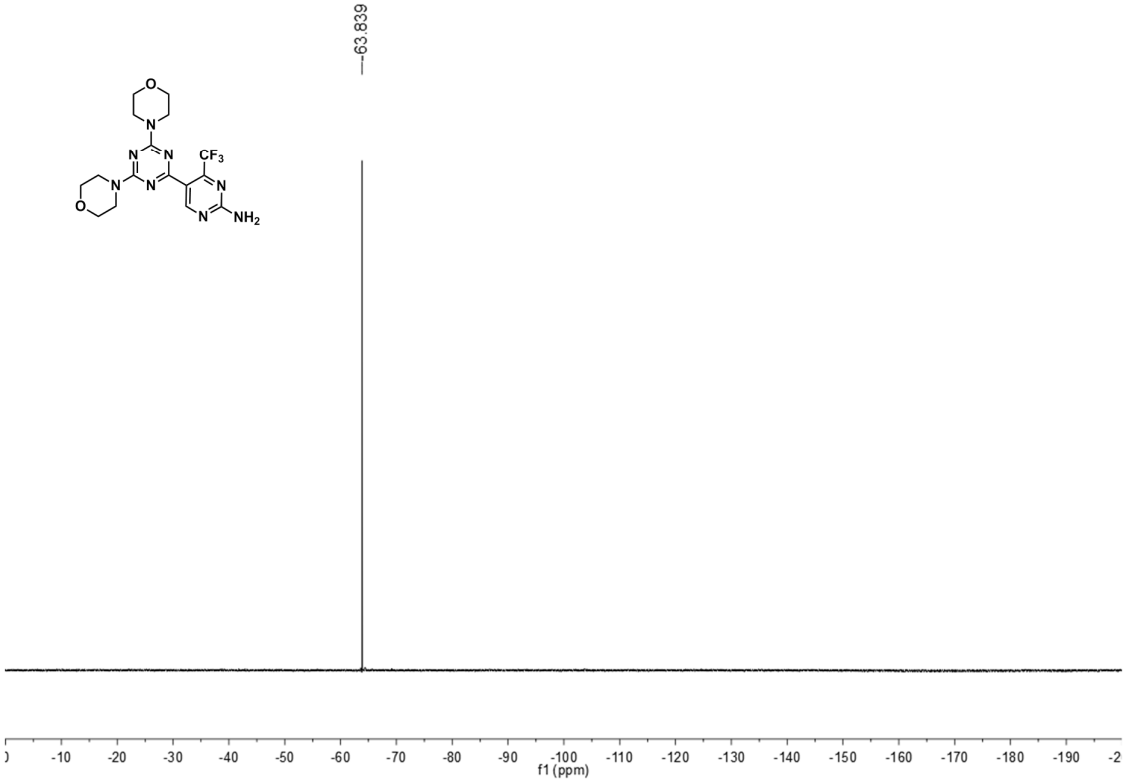


N,*N*-Dimethyl-*N*′-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)pyridin-2-yl)formimidamide (**24**):

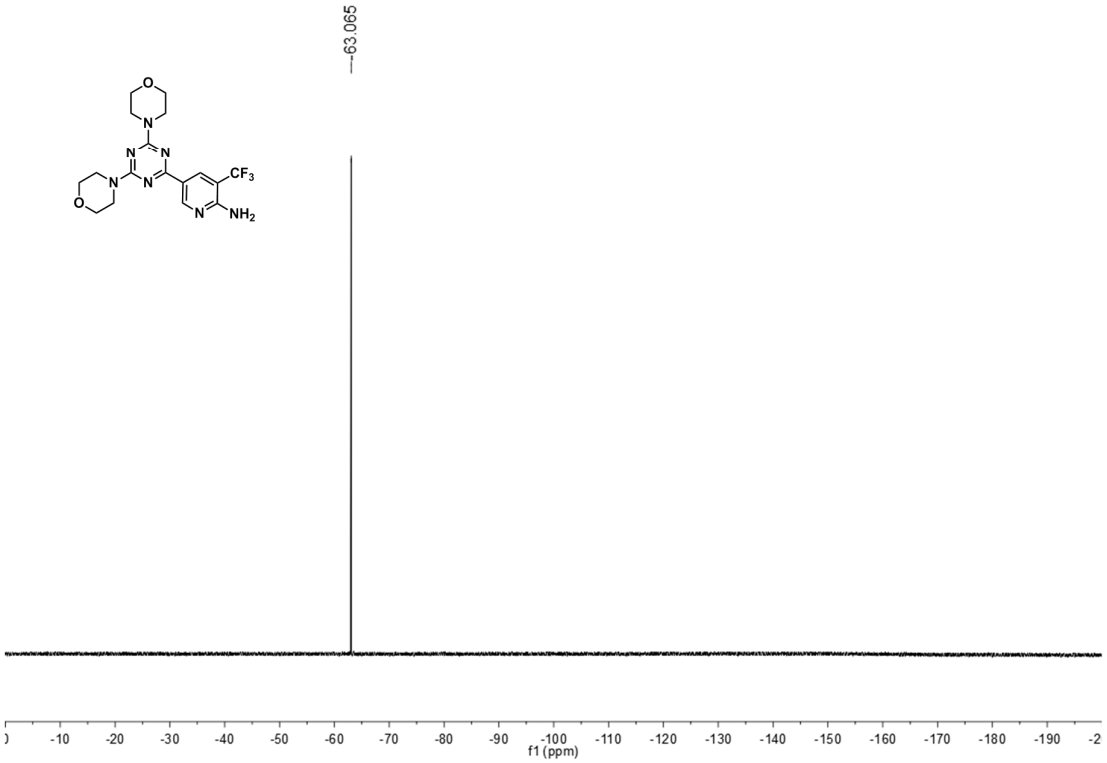


$^{19}F\{^{1}H\}$ NMR Spectra:

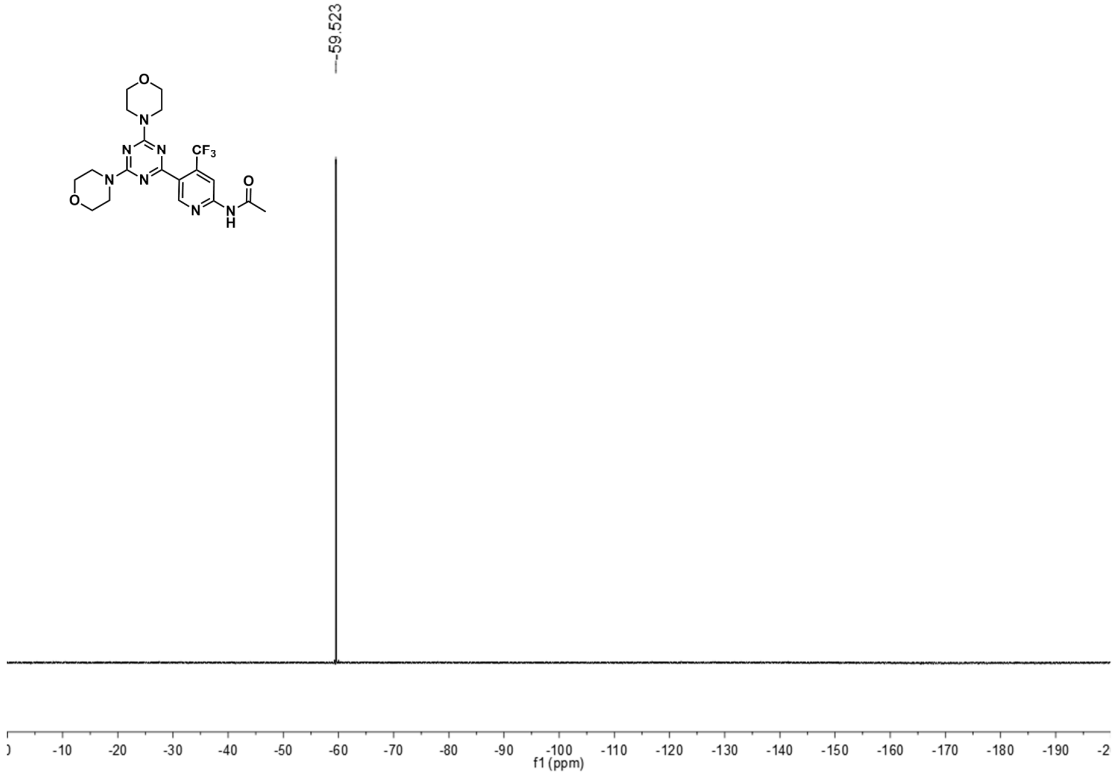
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyrimidin-2-amine (**2**):



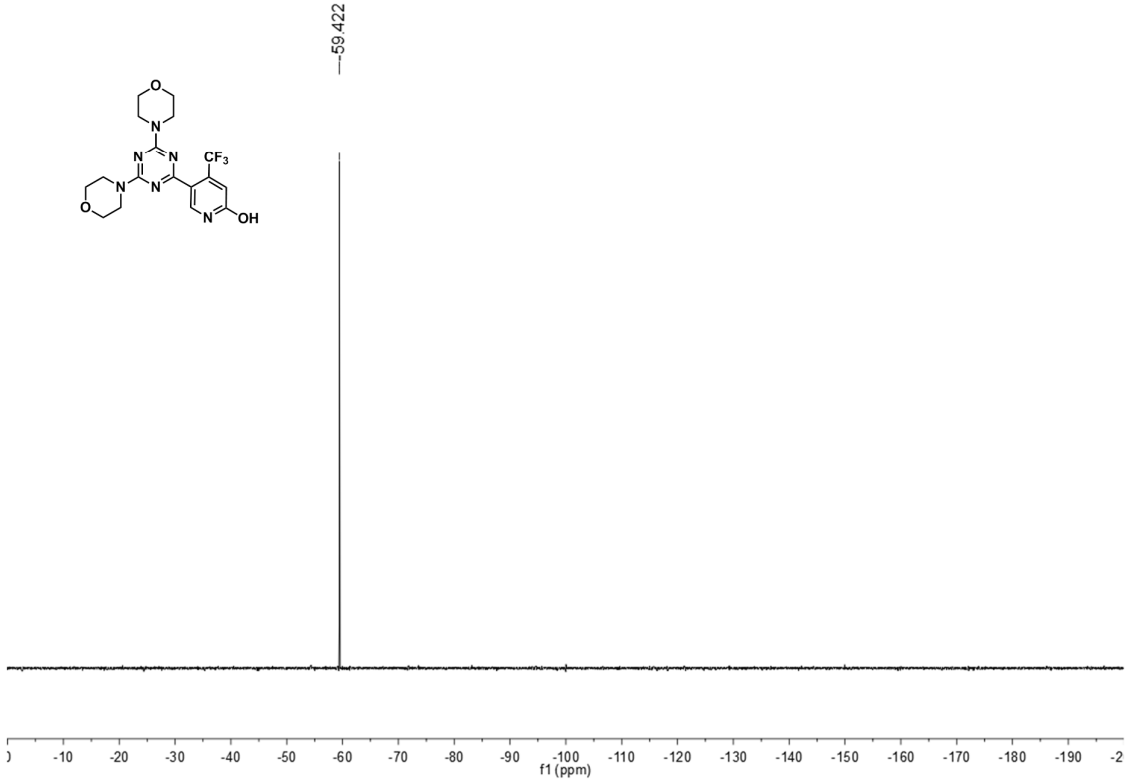
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-(trifluoromethyl)pyridin-2-amine (**15**):



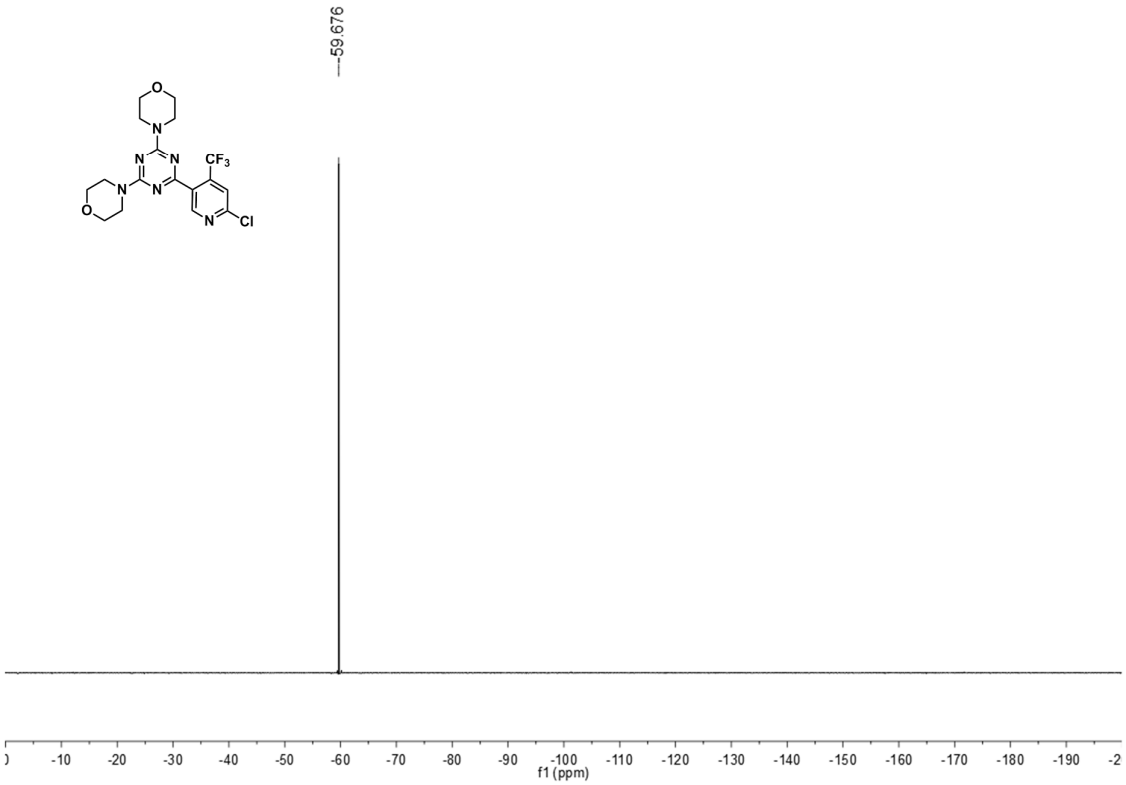
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)acetamide (**17**):



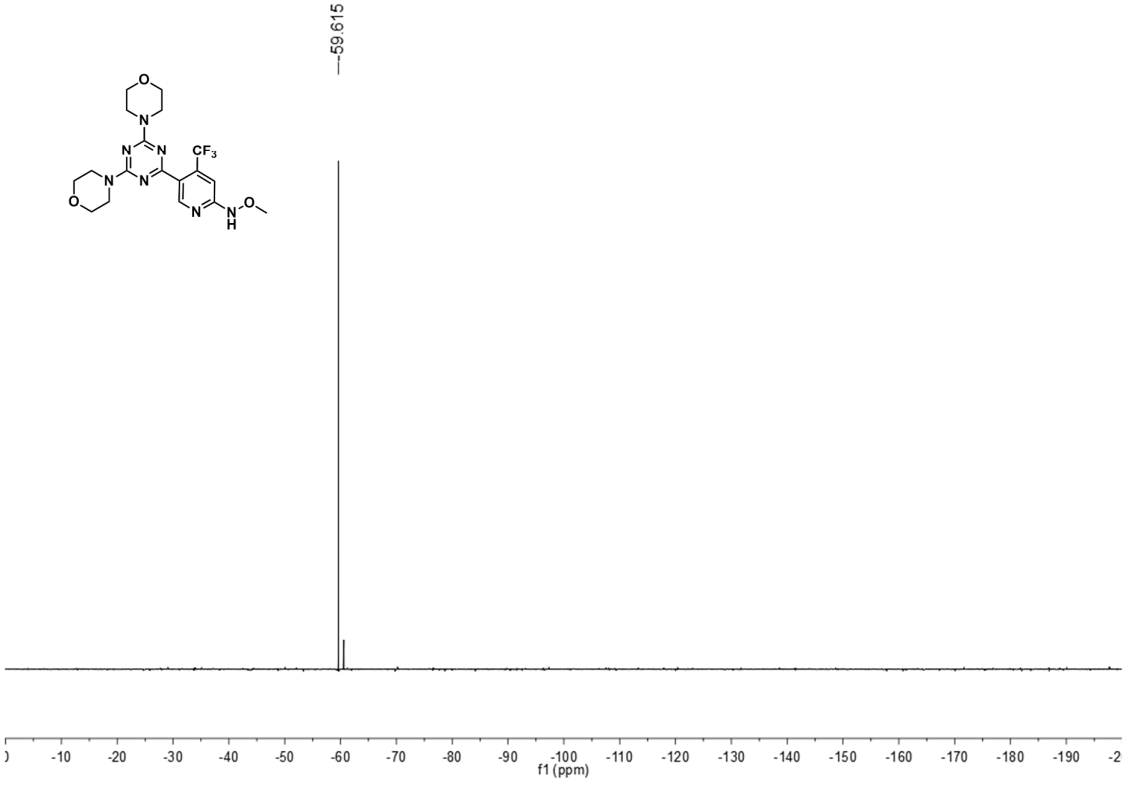
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-ol (**18**):



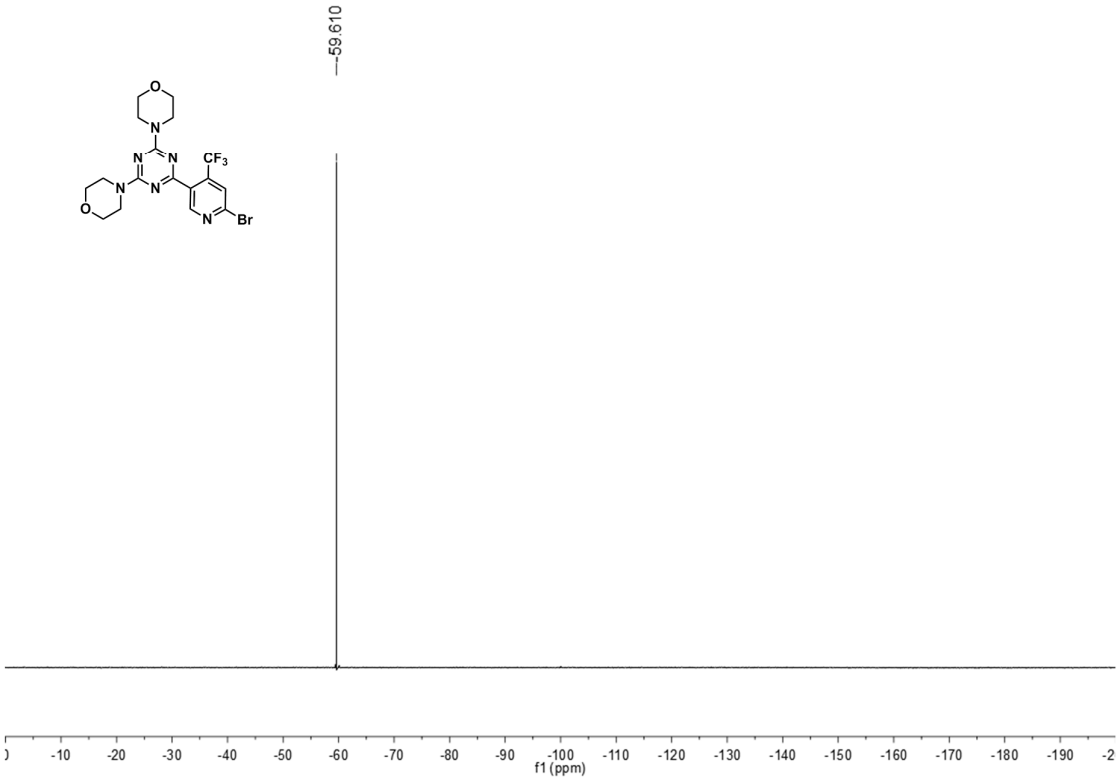
4,4'-(6-(6-Chloro-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**19**):



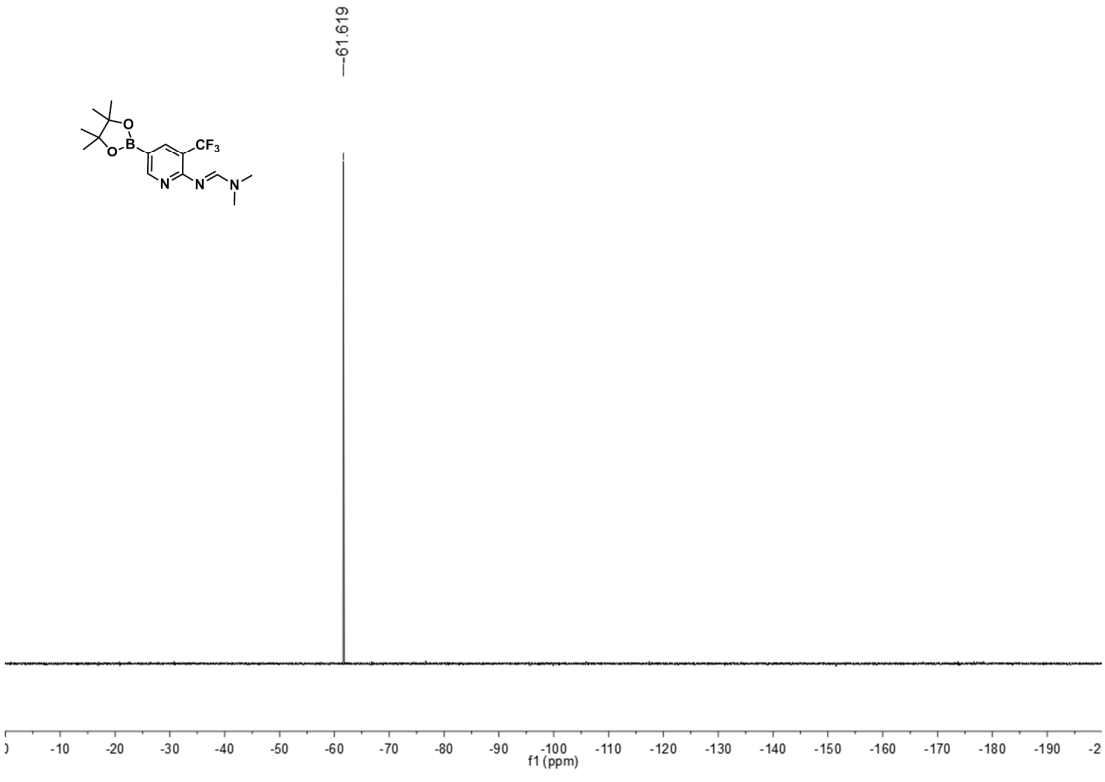
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine (**20**):



4,4'-(6-(6-Bromo-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**21**):

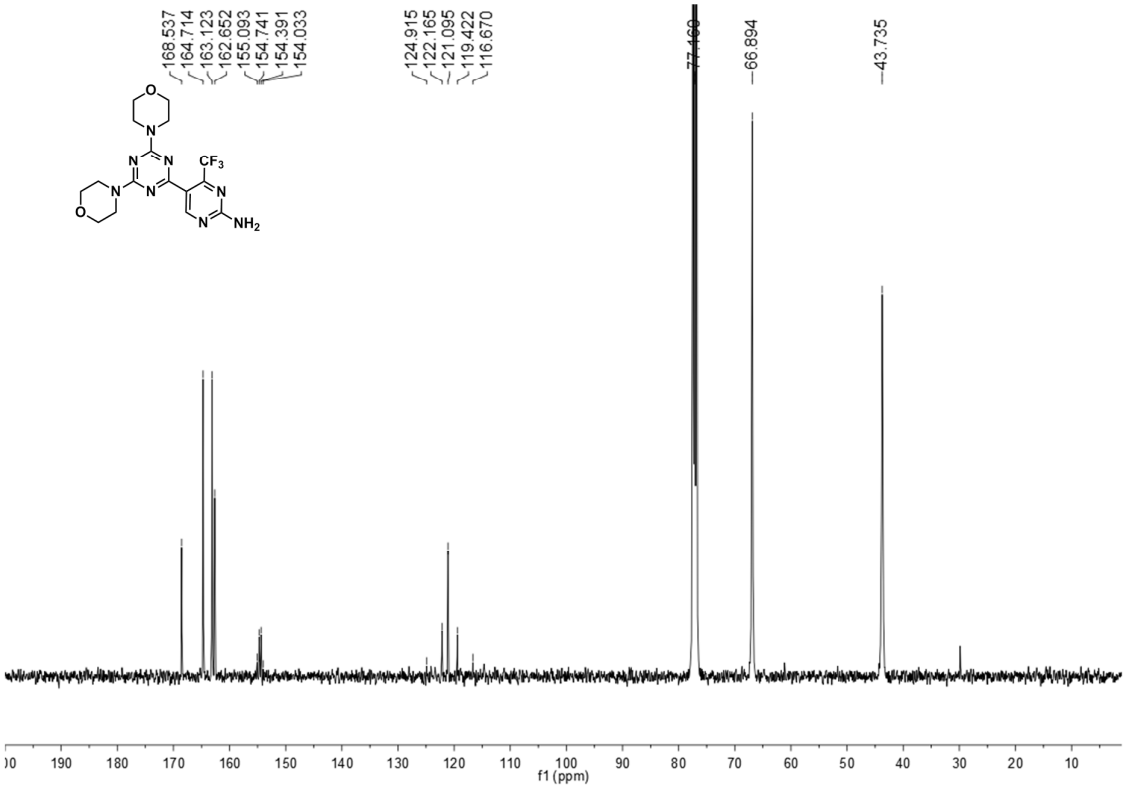


N,*N*-Dimethyl-*N'*-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)pyridin-2-yl)formimidamide (**24**):

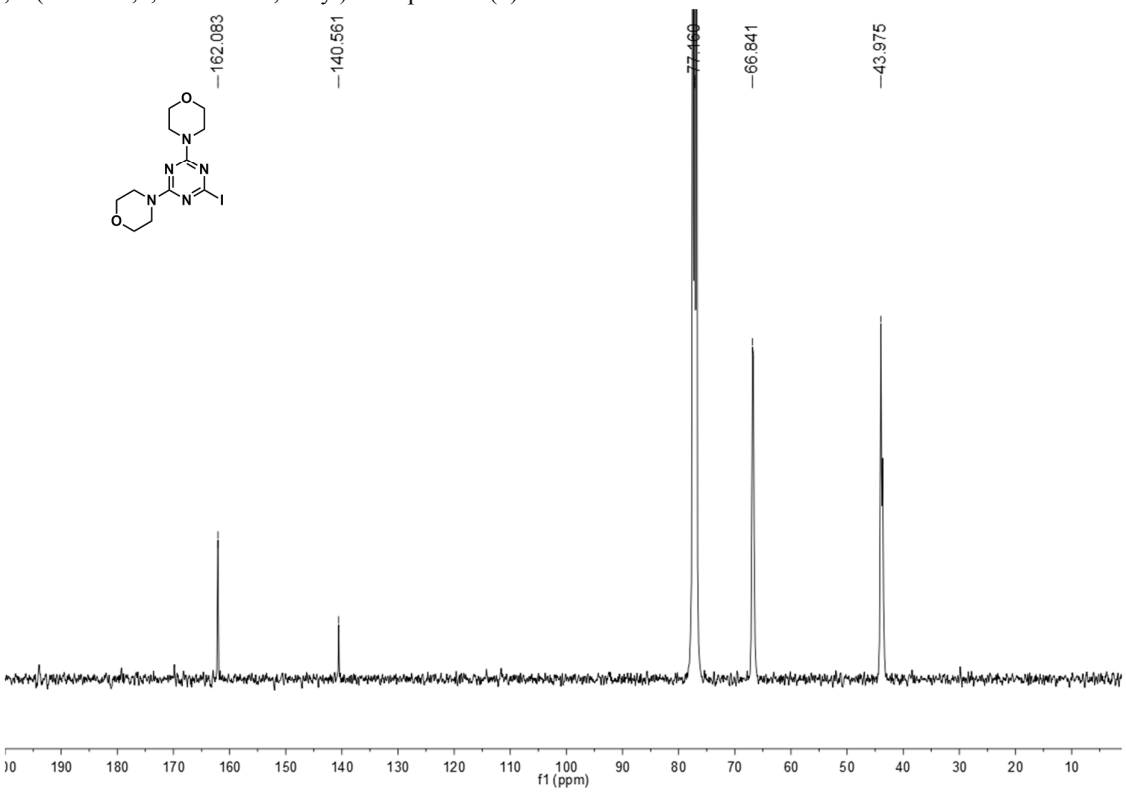


$^{13}C\{^{1}H\}$ NMR Spectra.

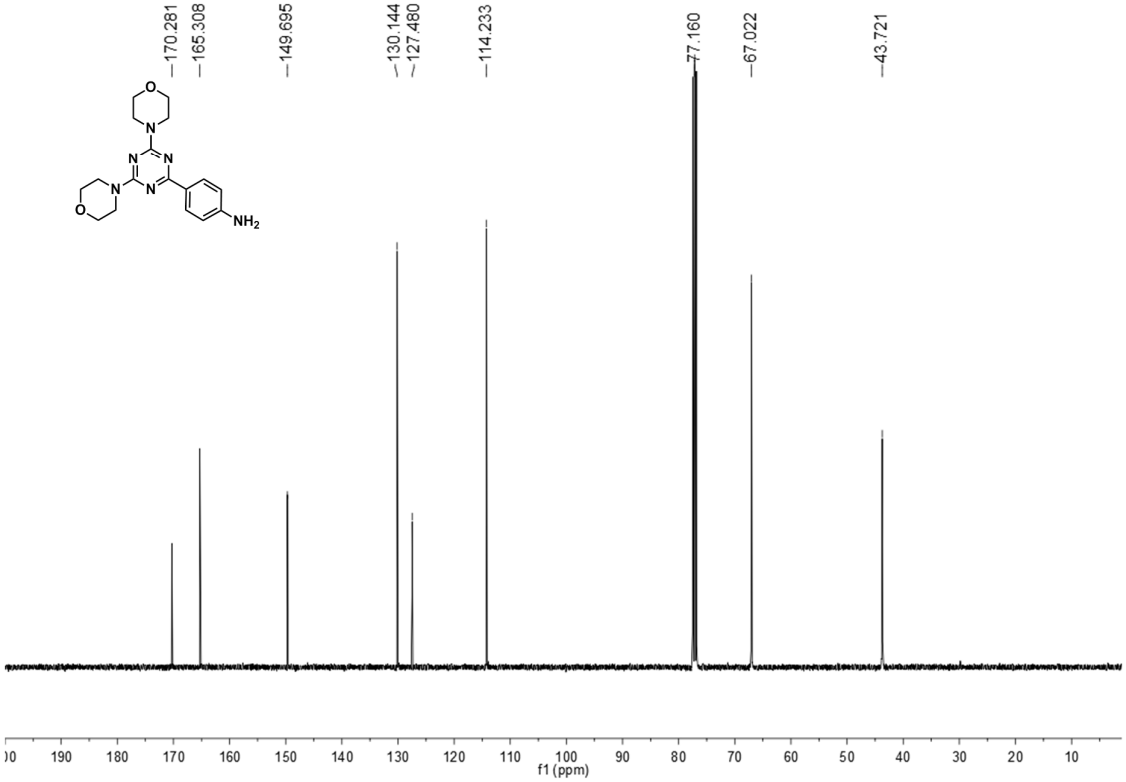
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyrimidin-2-amine (**2**):



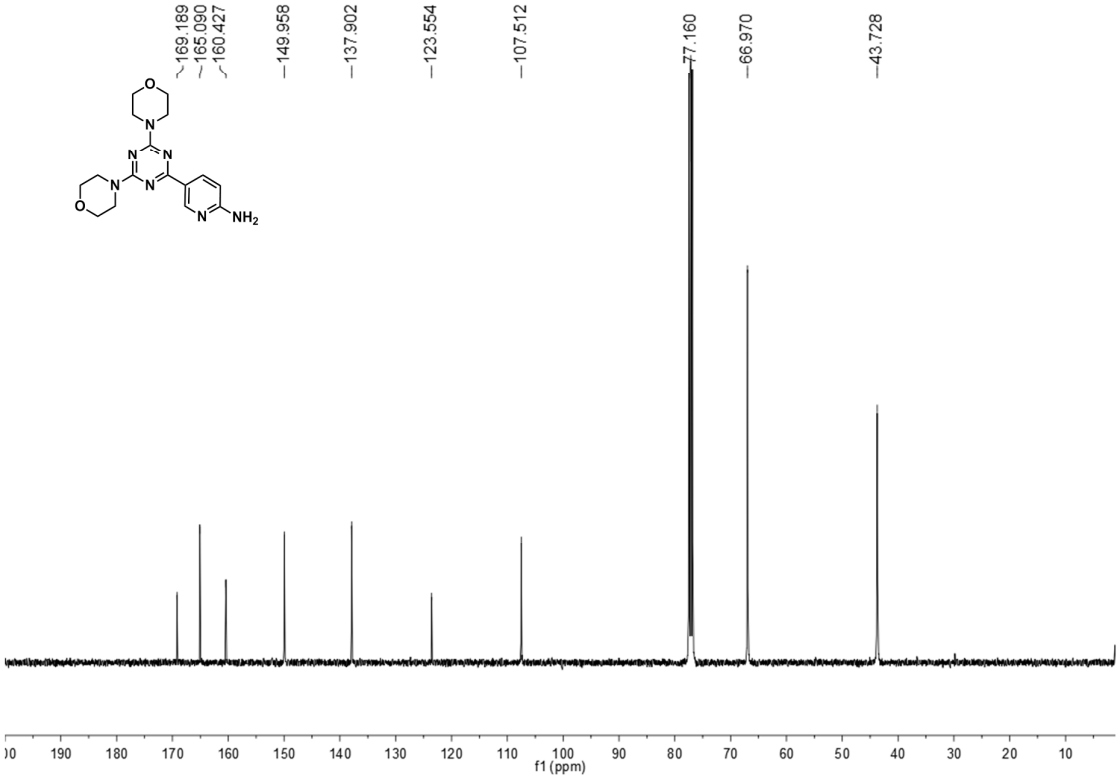
4,4'-(6-Iodo-1,3,5-triazine-2,4-diyl)dimorpholine (**8**):



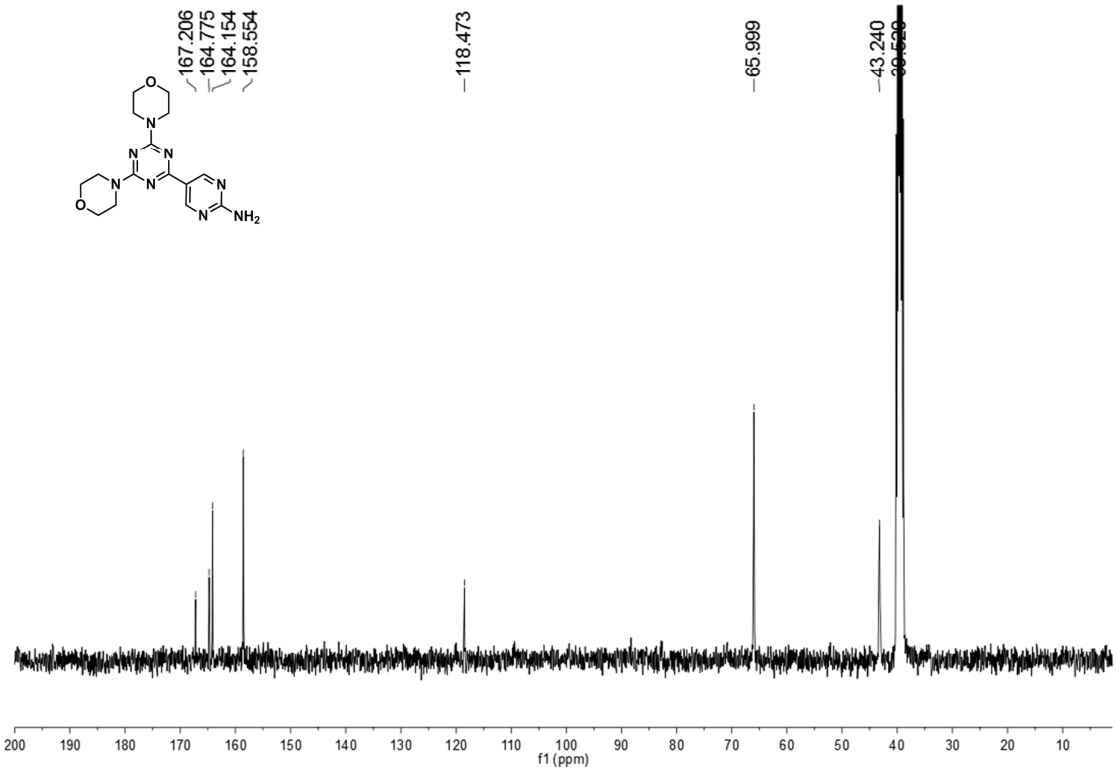
4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)aniline (**9**):



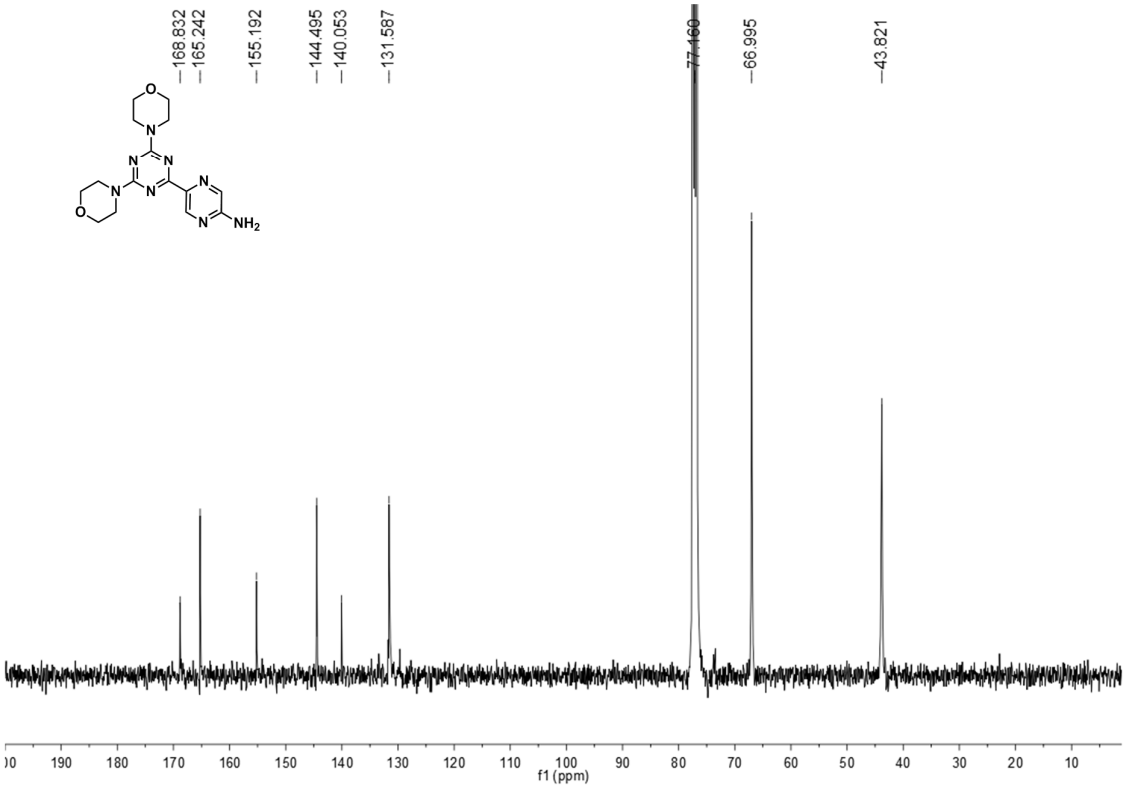
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyridin-2-amine (**10**):



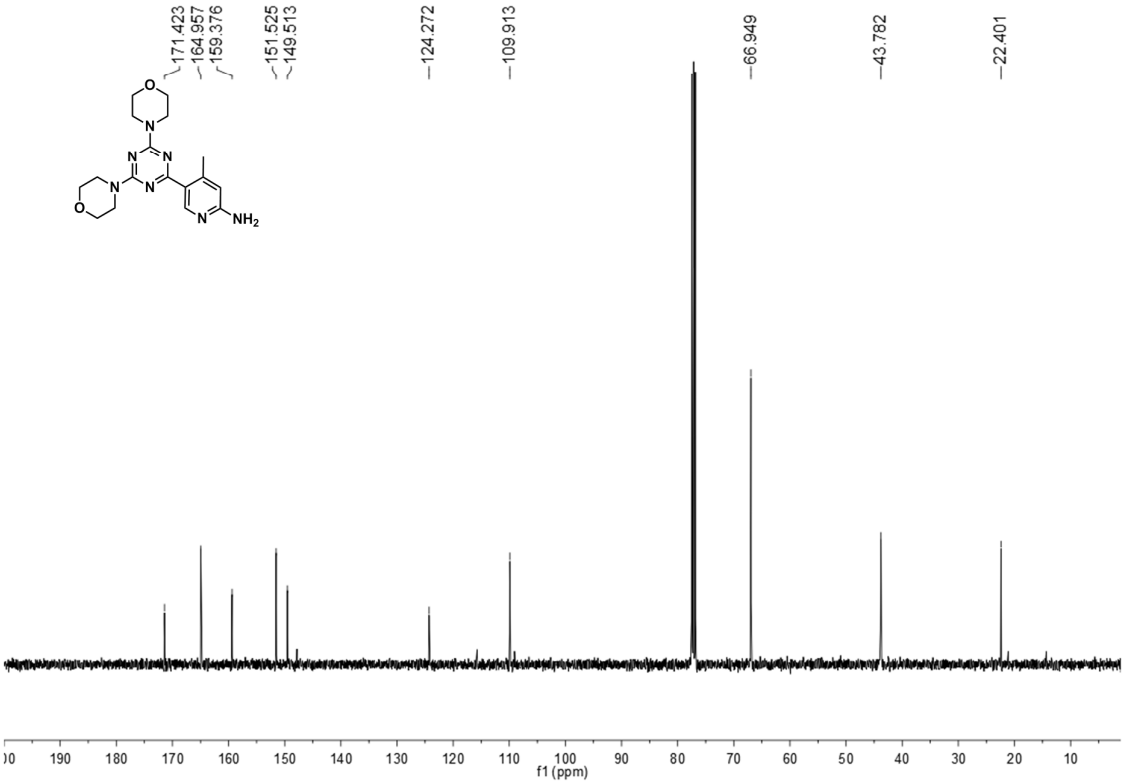
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrimidin-2-amine (**11**):



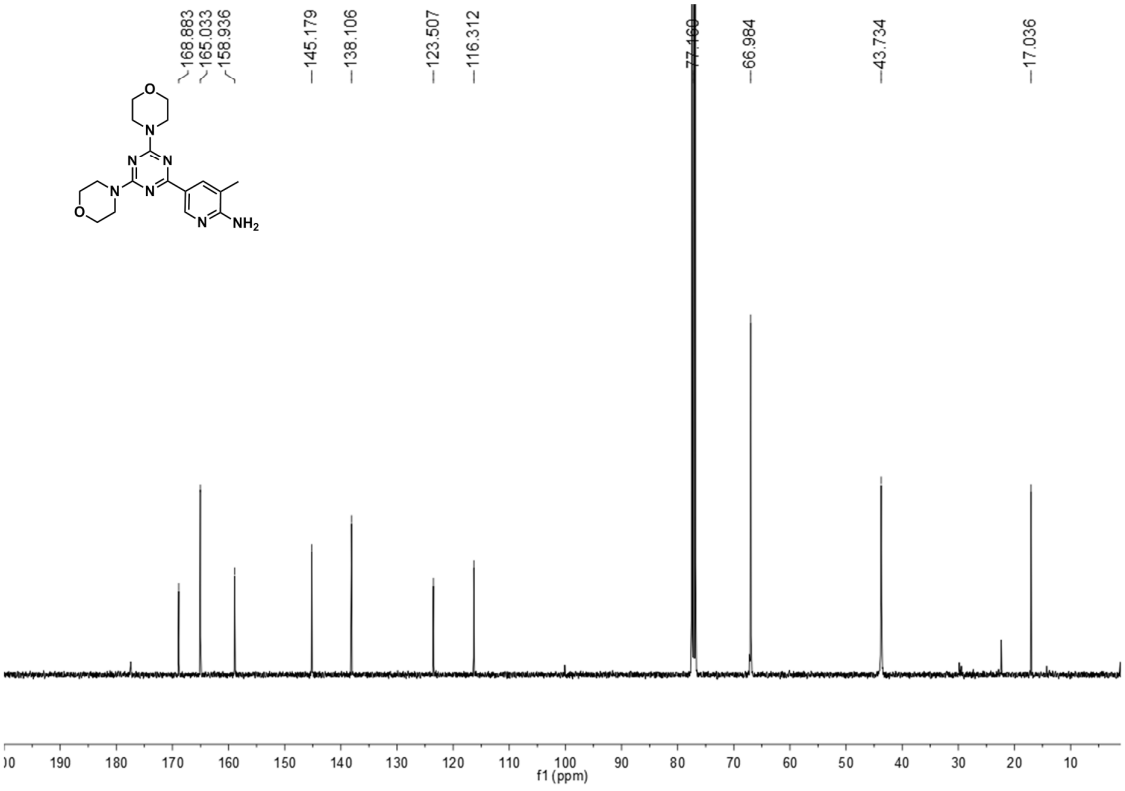
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrazin-2-amine (**12**):



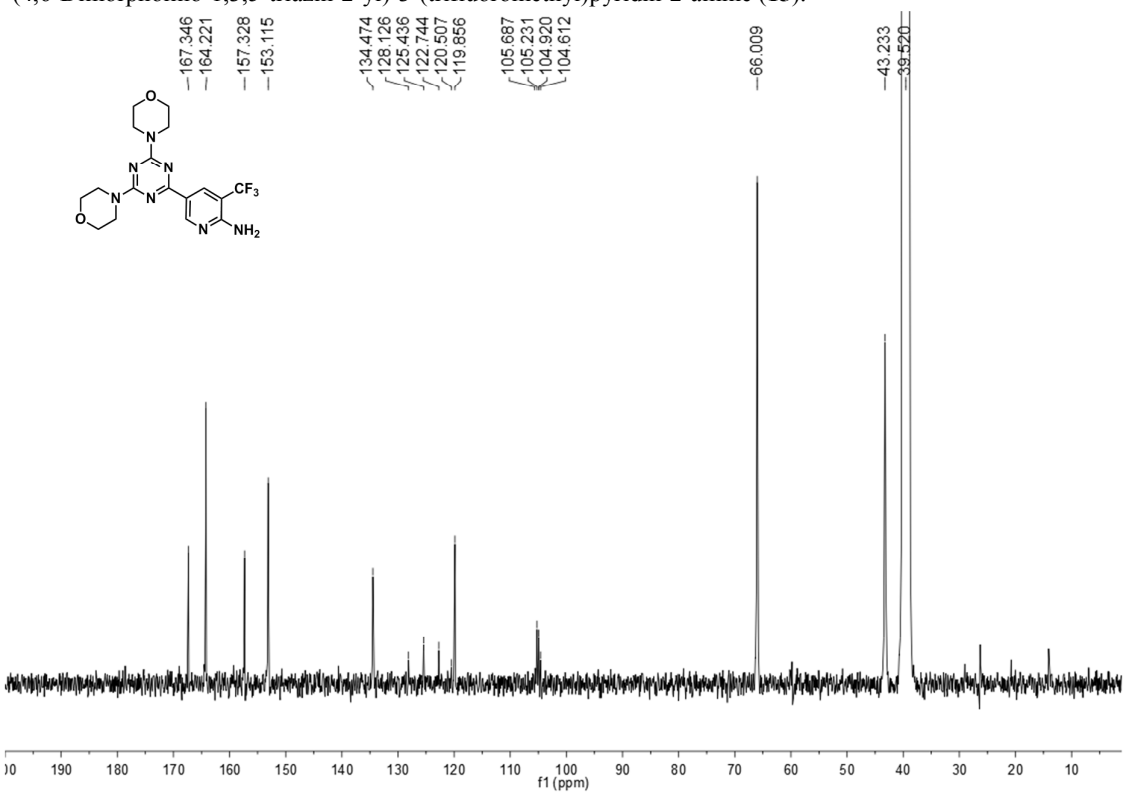
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyridin-2-amine (**13**):



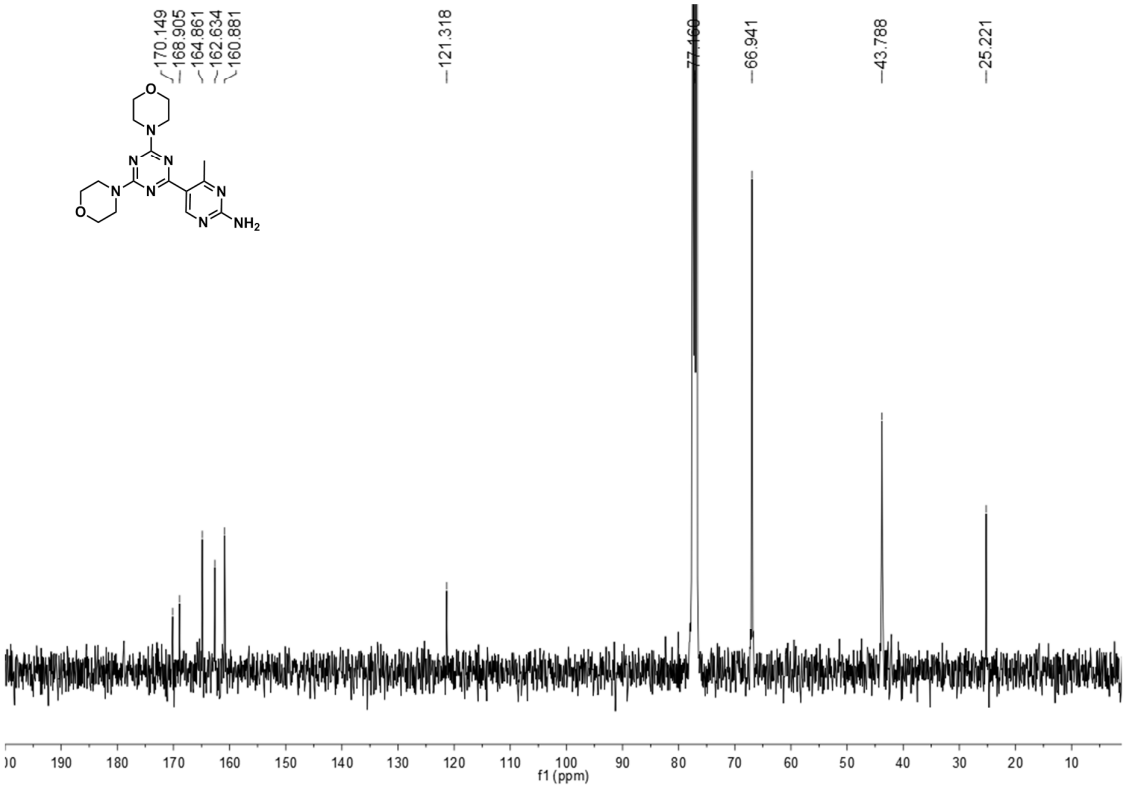
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-methylpyridin-2-amine (**14**):



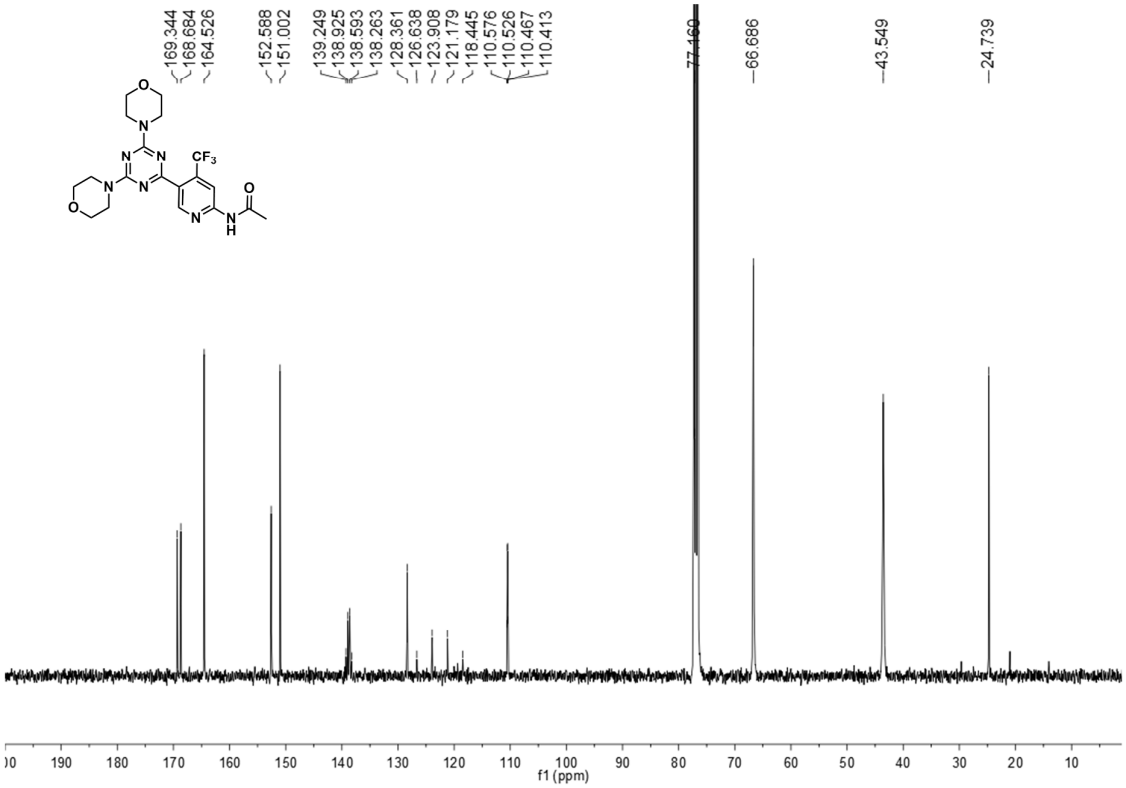
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-(trifluoromethyl)pyridin-2-amine (**15**):



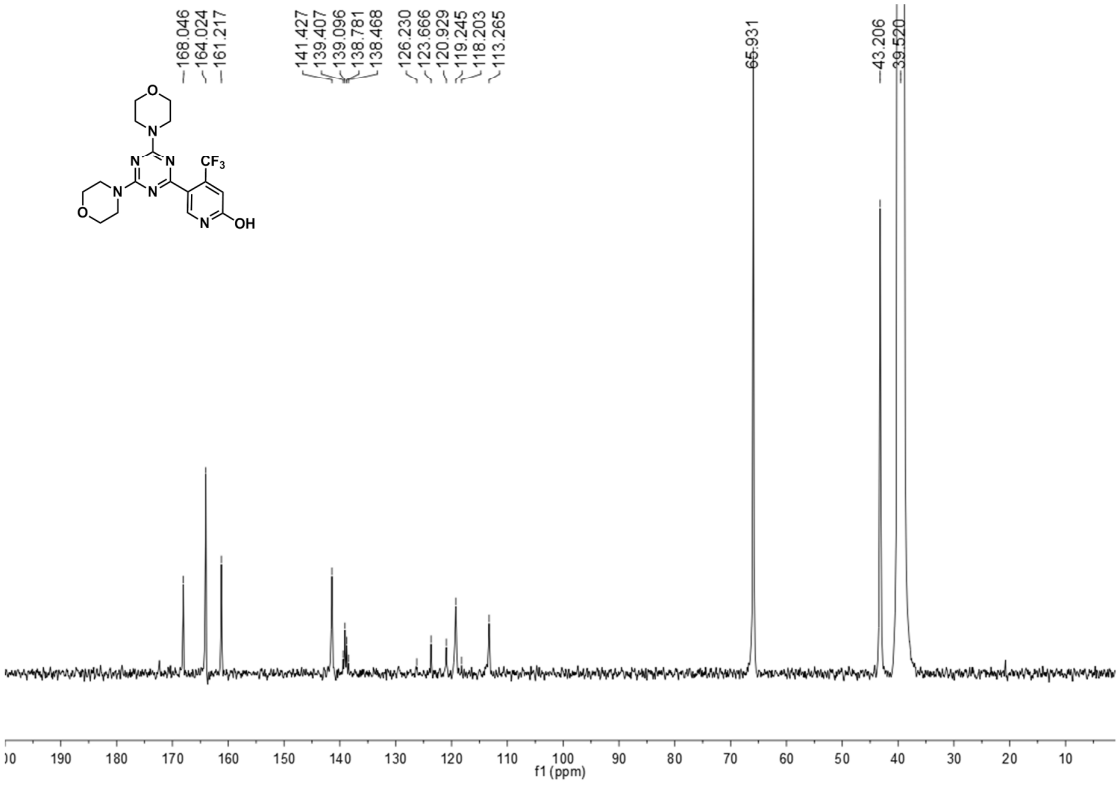
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyrimidin-2-amine (**16**):



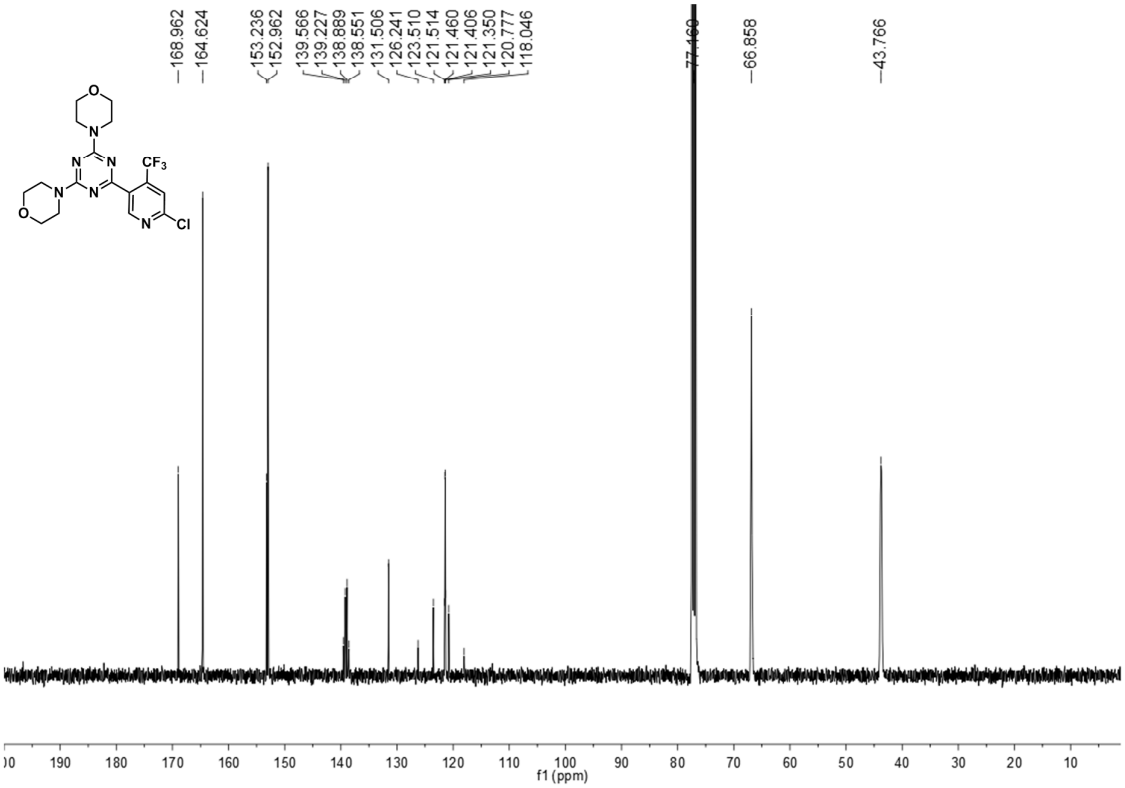
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)acetamide (**17**):



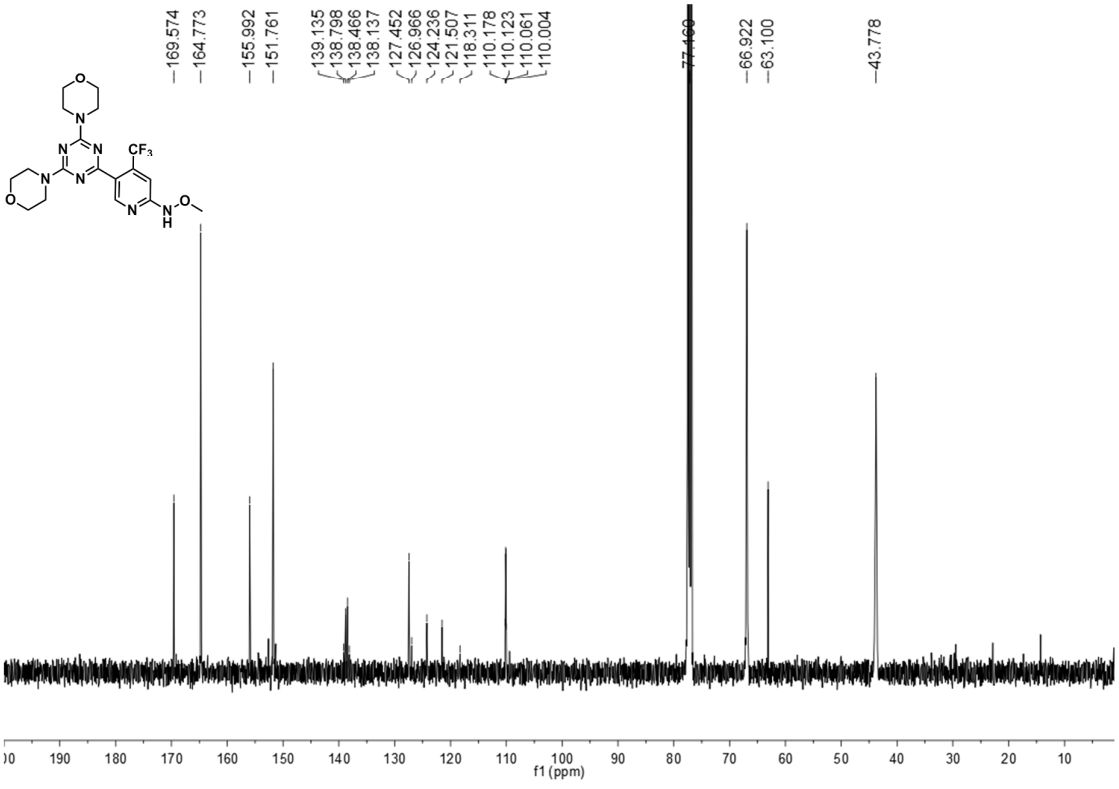
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-ol (**18**):



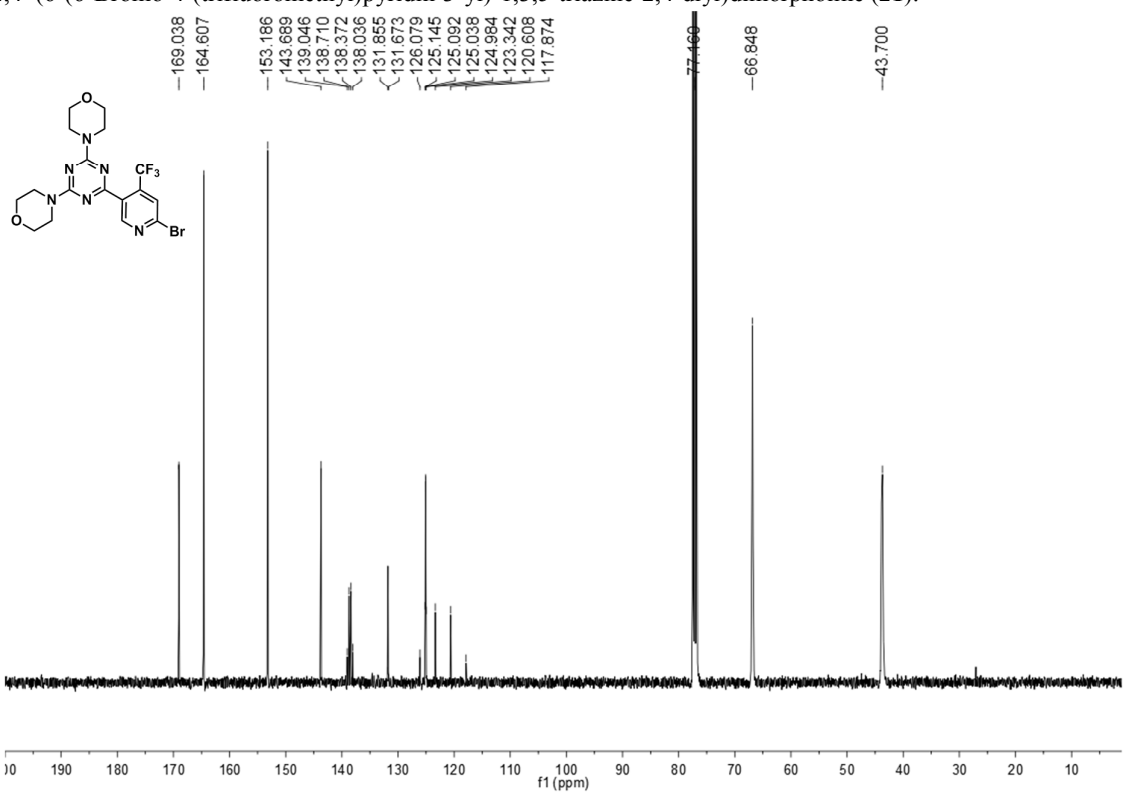
4,4'-(6-(6-Chloro-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**19**):



N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine (**20**):

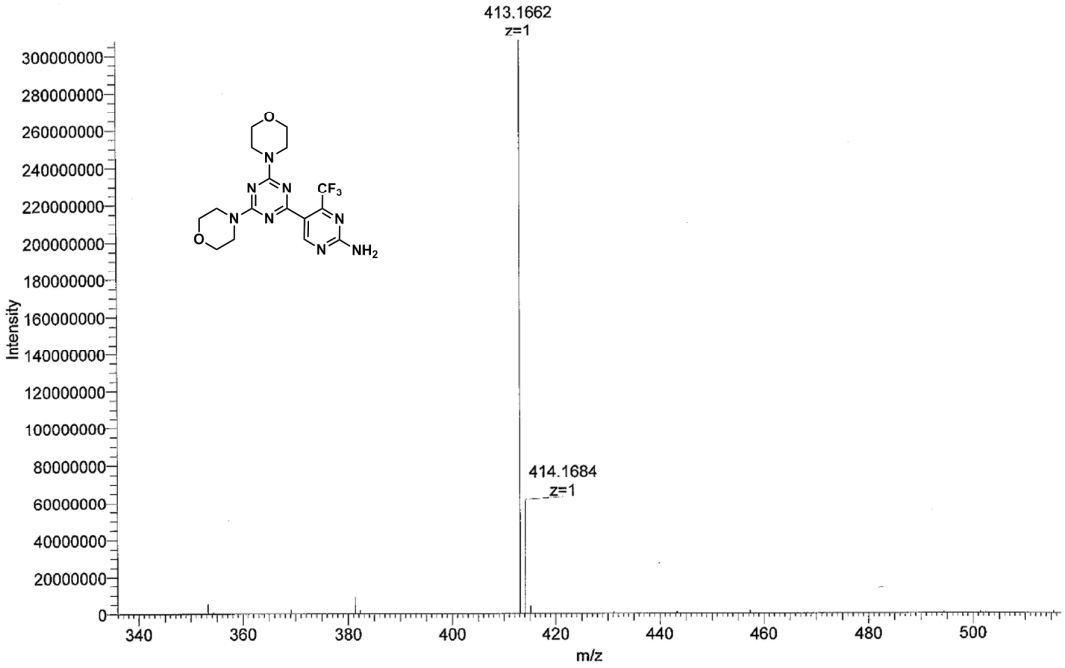


4,4'-(6-(6-Bromo-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**21**):

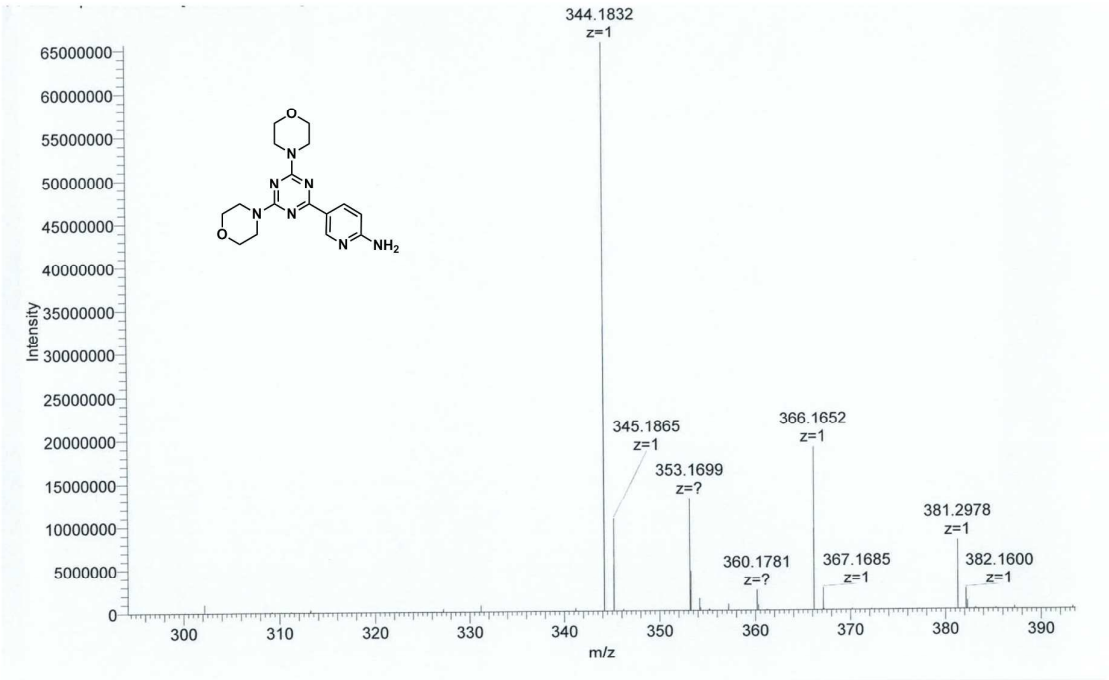


ESI-HRMS Spectra:

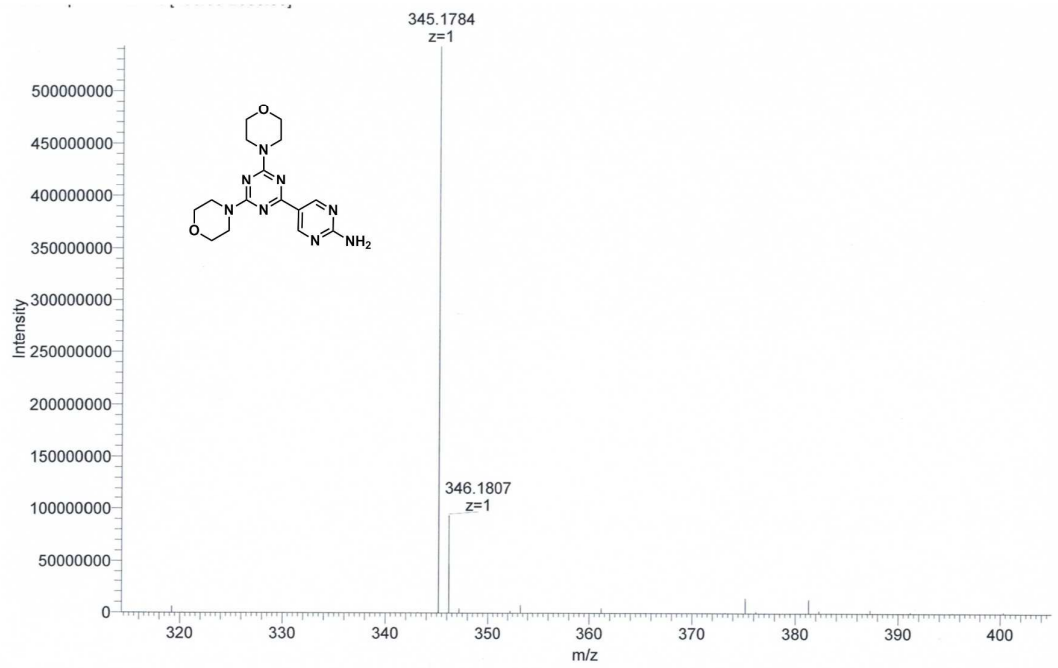
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyrimidin-2-amine (**2**):



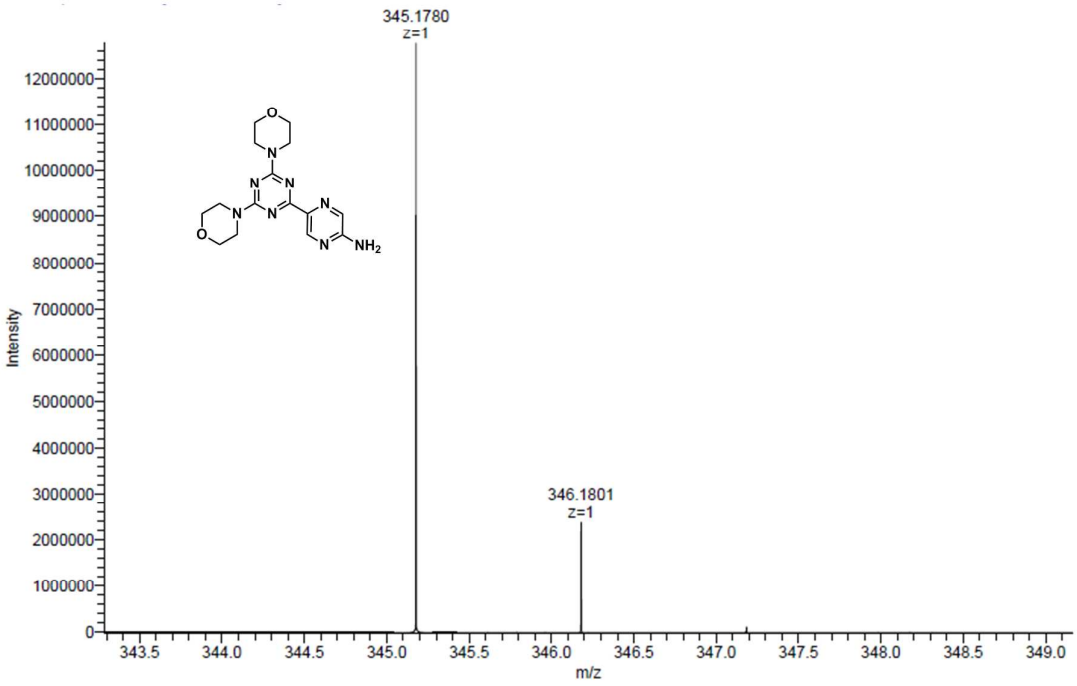
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyridin-2-amine (**10**):



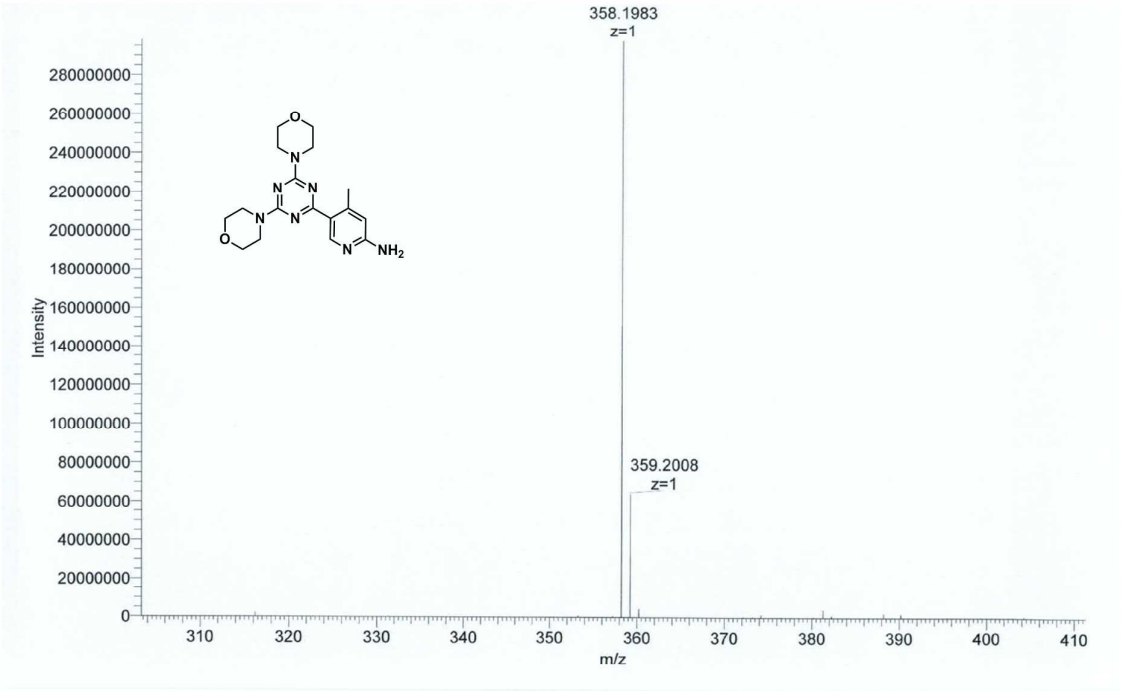
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrimidin-2-amine (**11**):



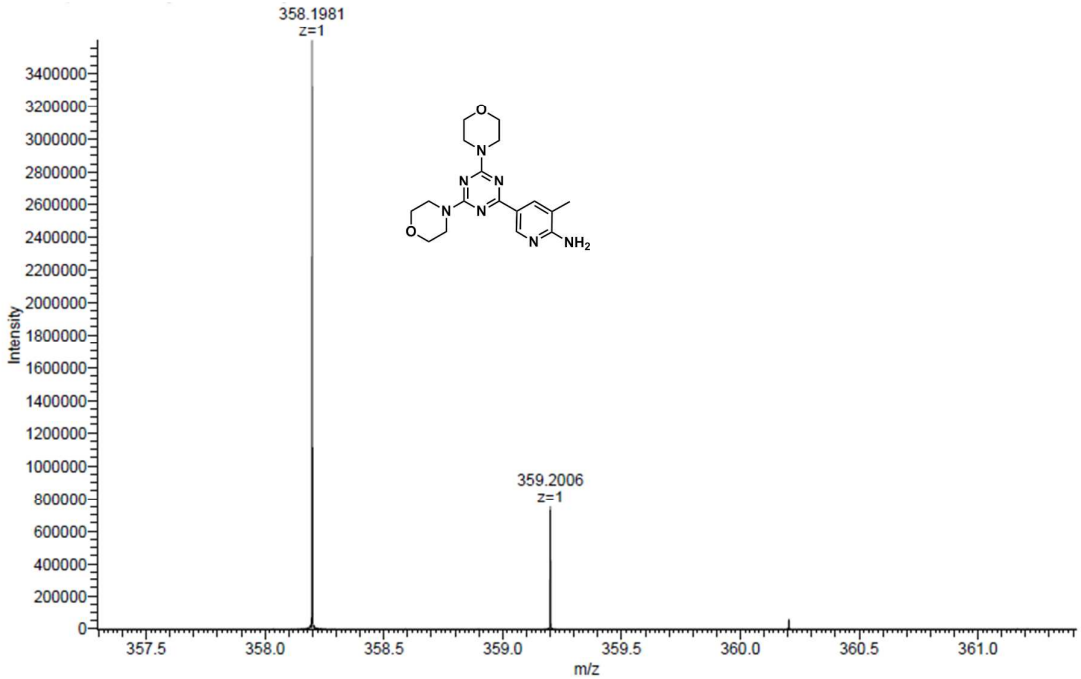
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrazin-2-amine (**12**):



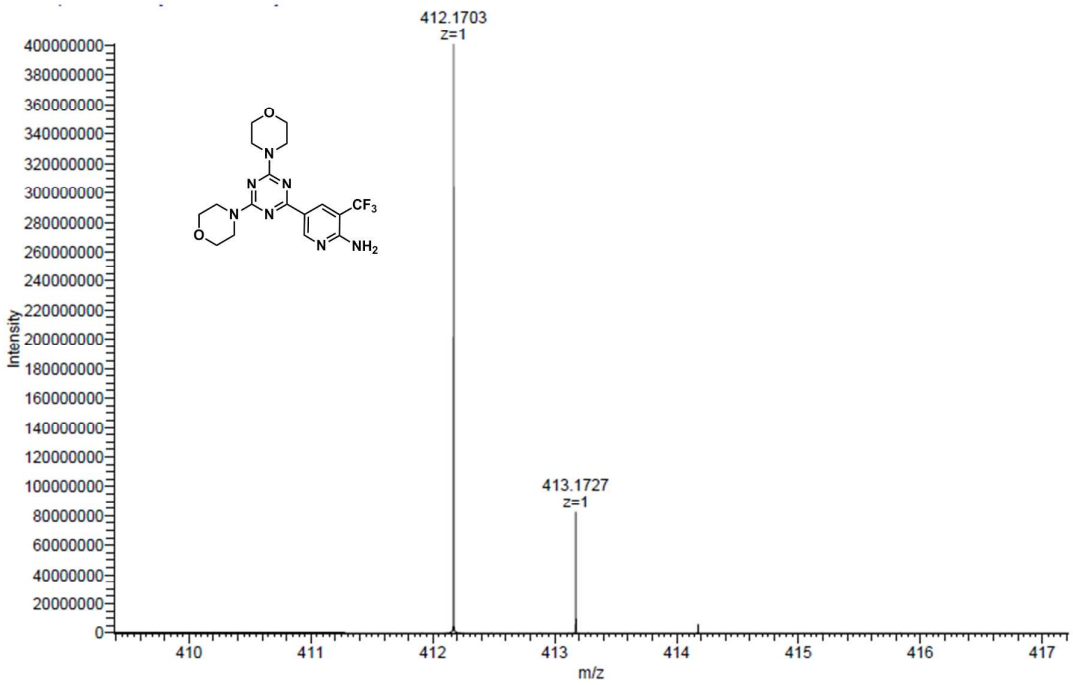
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyridin-2-amine (**13**):



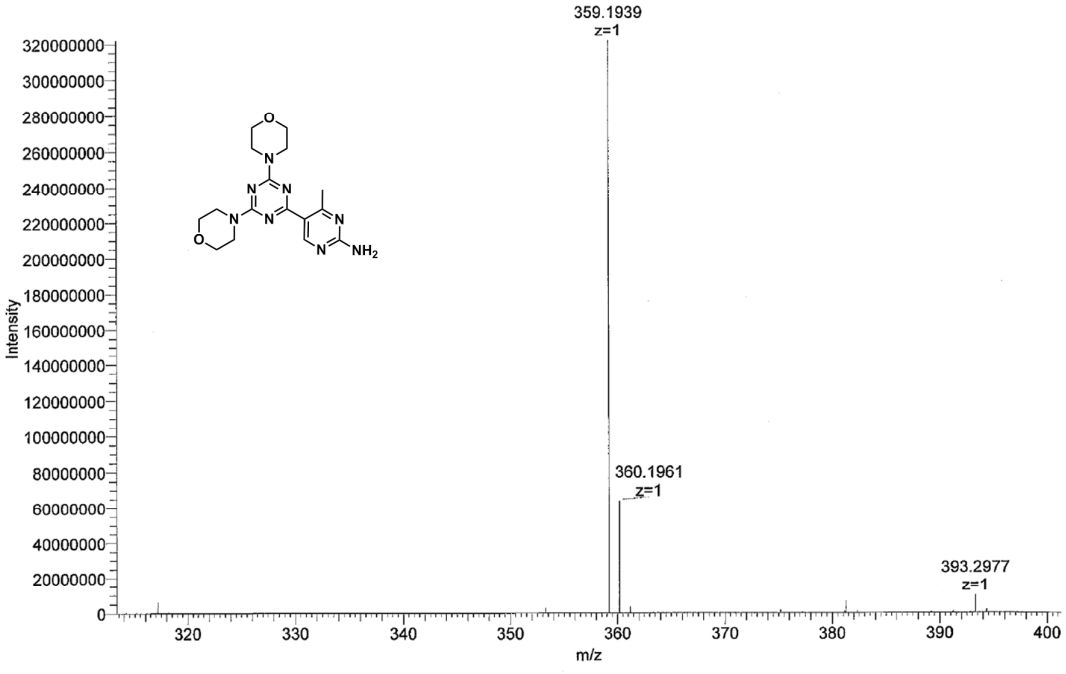
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-methylpyridin-2-amine (**14**):



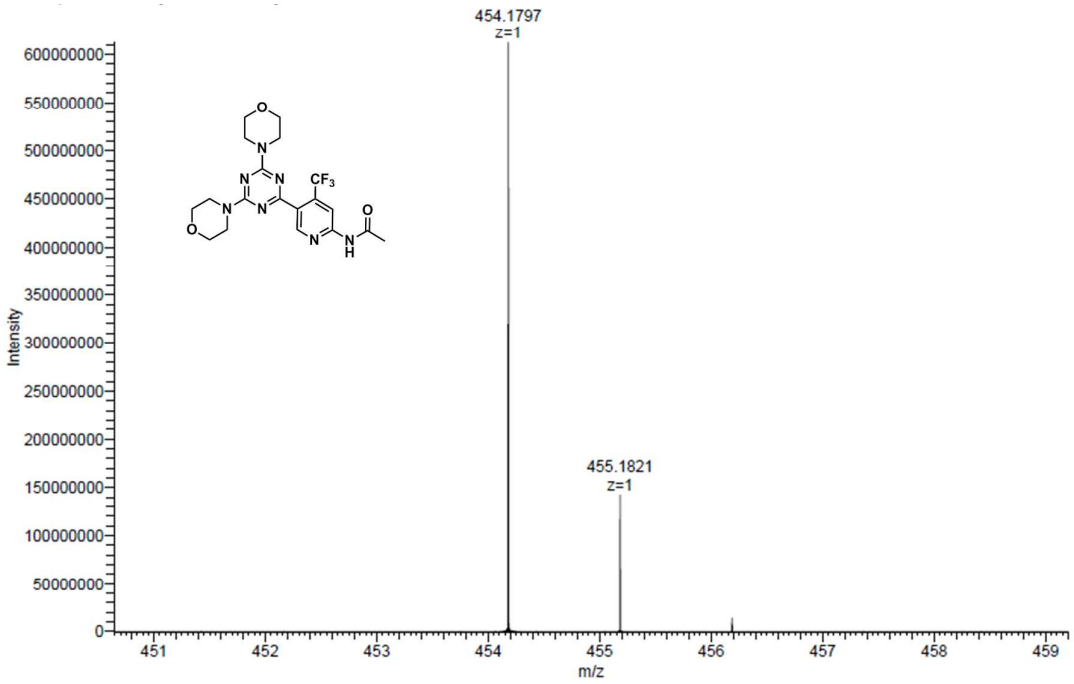
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-(trifluoromethyl)pyridin-2-amine (**15**):



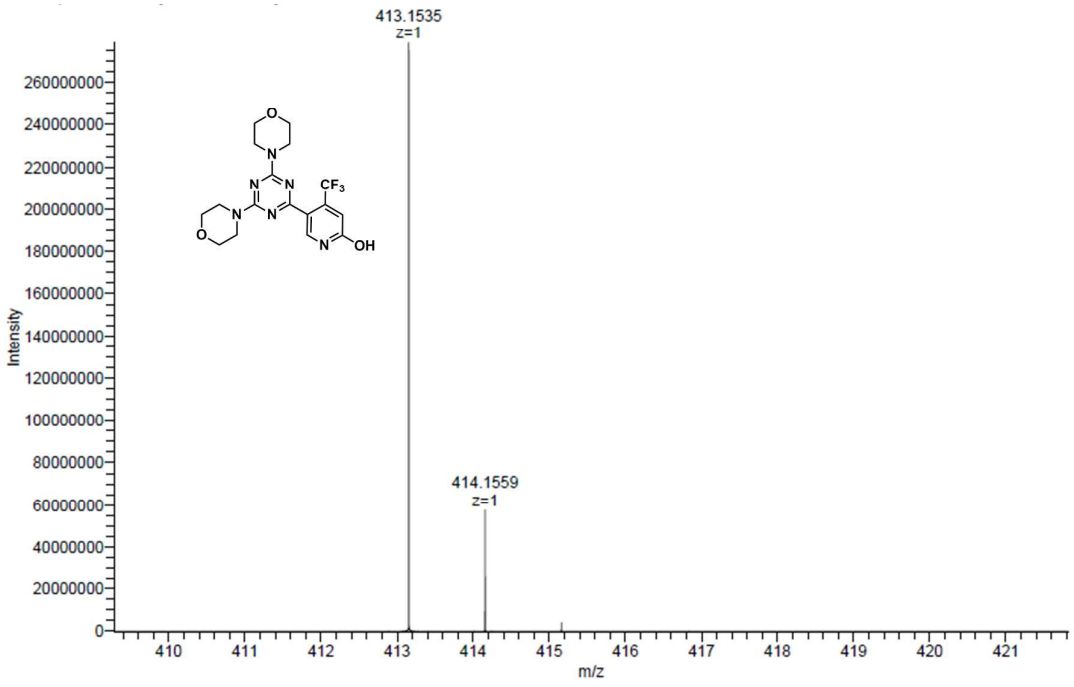
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyrimidin-2-amine (**16**):



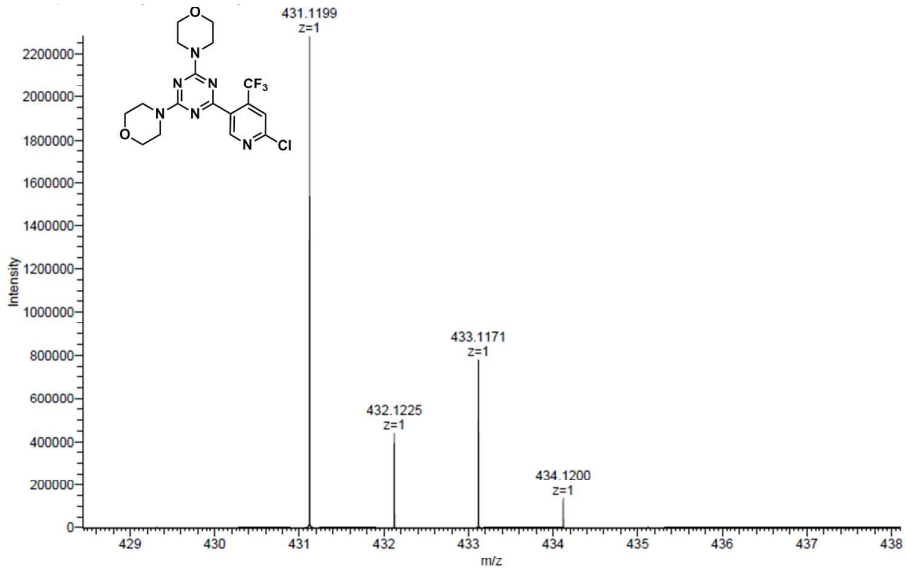
N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)acetamide (**17**):



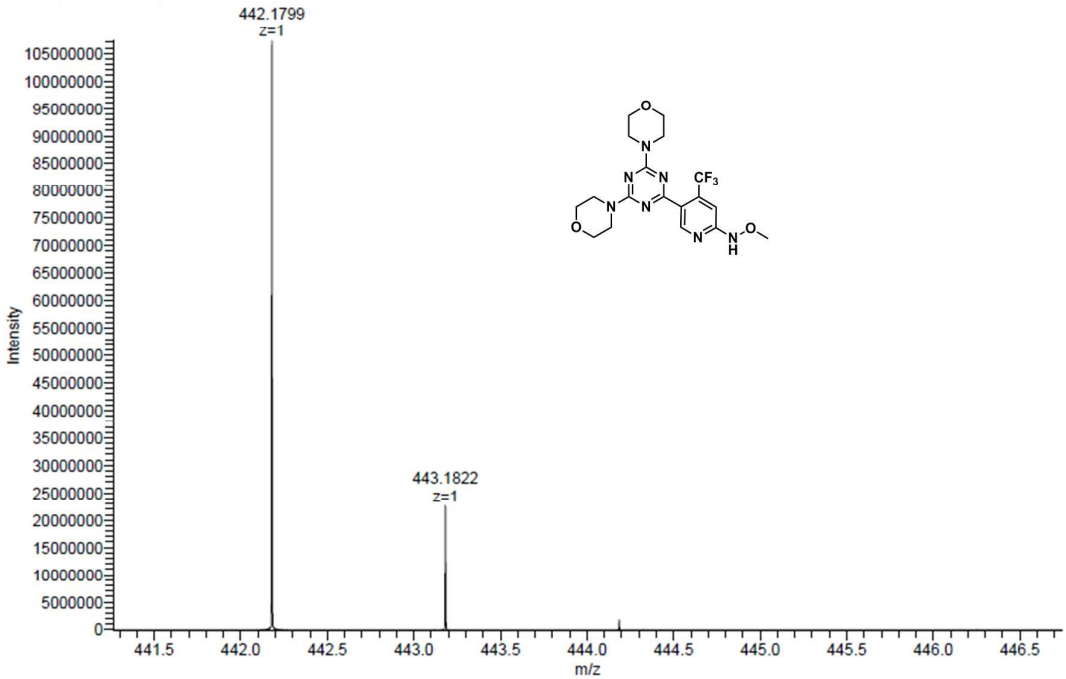
5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-ol (**18**):



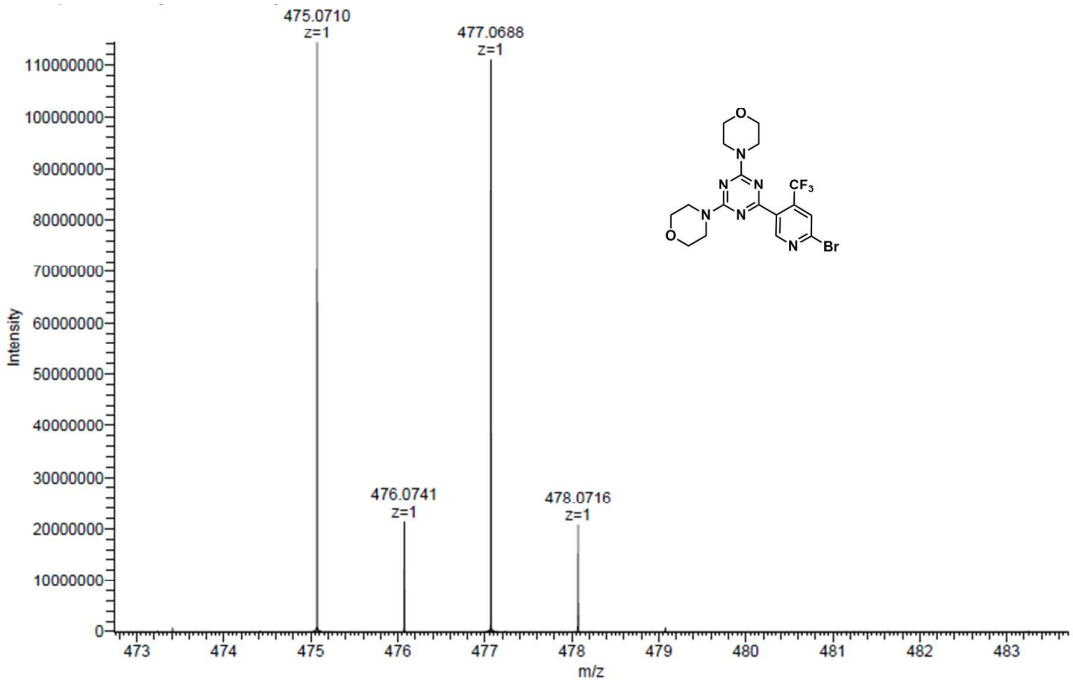
4,4'-(6-(6-Chloro-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**19**):



N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine (**20**):

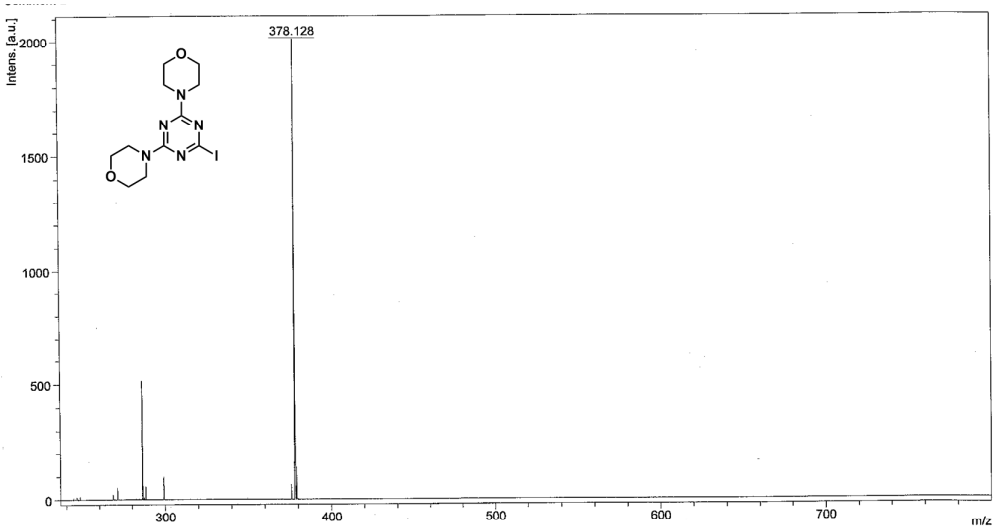


4,4'-(6-(6-Bromo-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**21**):

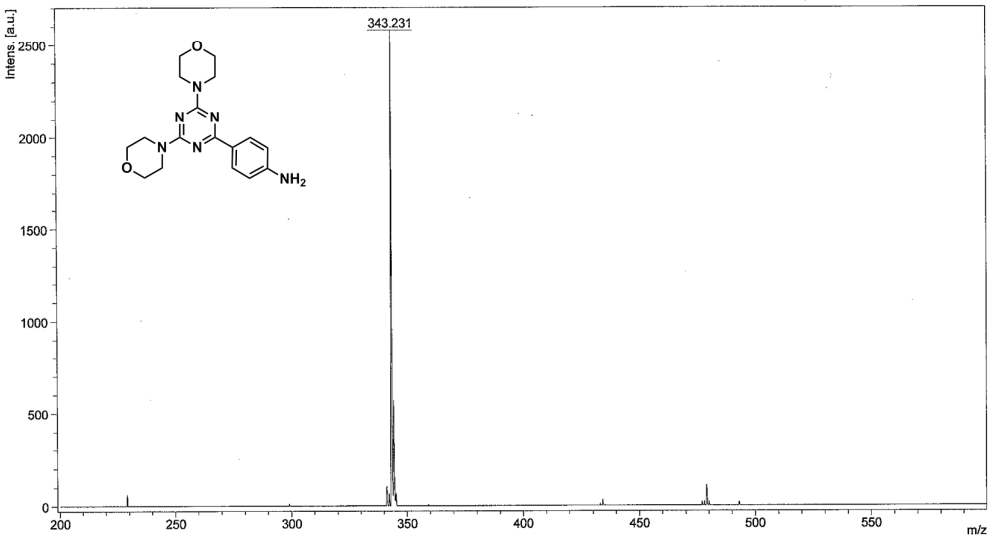


MALDI-MS Spectra.

4,4'-(6-Iodo-1,3,5-triazine-2,4-diyl)dimorpholine (**8**):

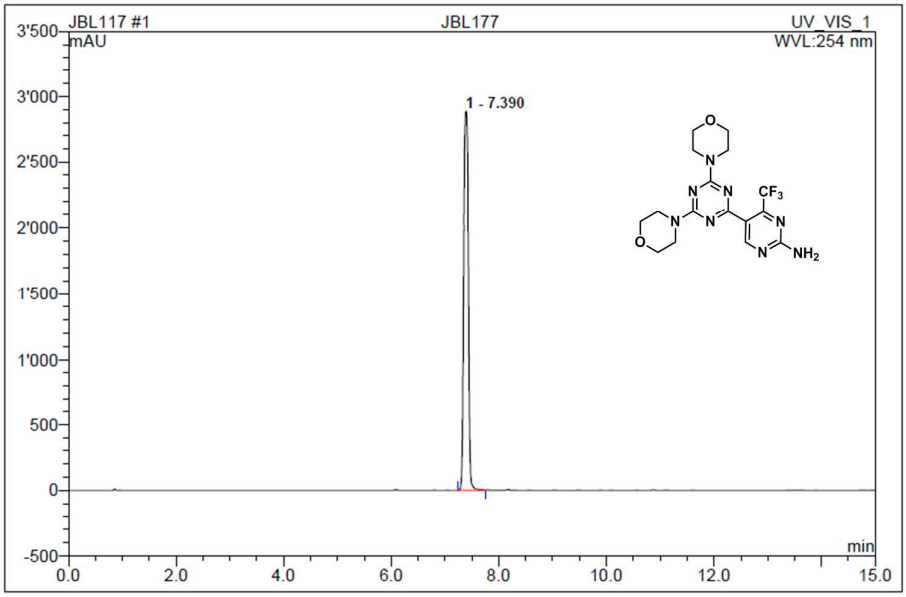


4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)aniline (**9**):



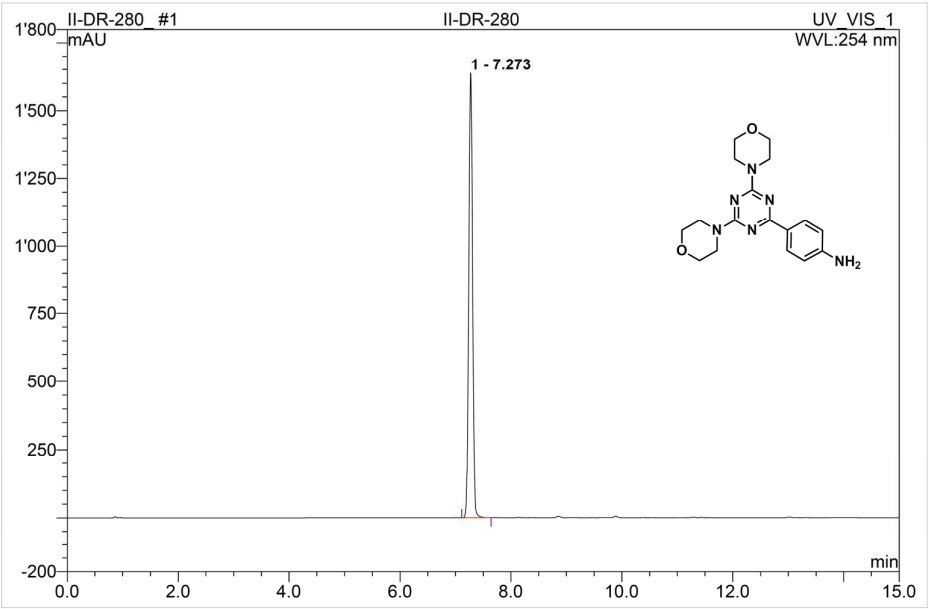
HPLC Chromatograms.

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyrimidin-2-amine (**2**):



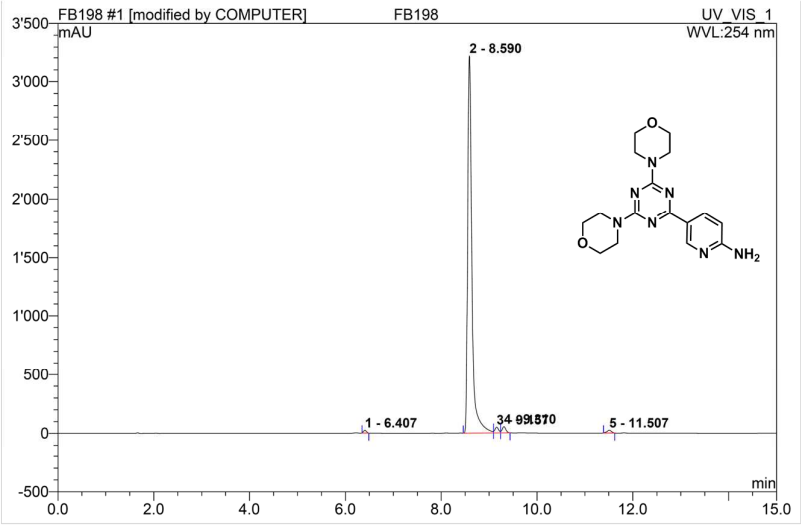
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	7.39	n.a.	2888.195	287.191	100.00	BMB
Total:			2888.195	287.191	100.00	#REF!

4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)aniline (**9**):



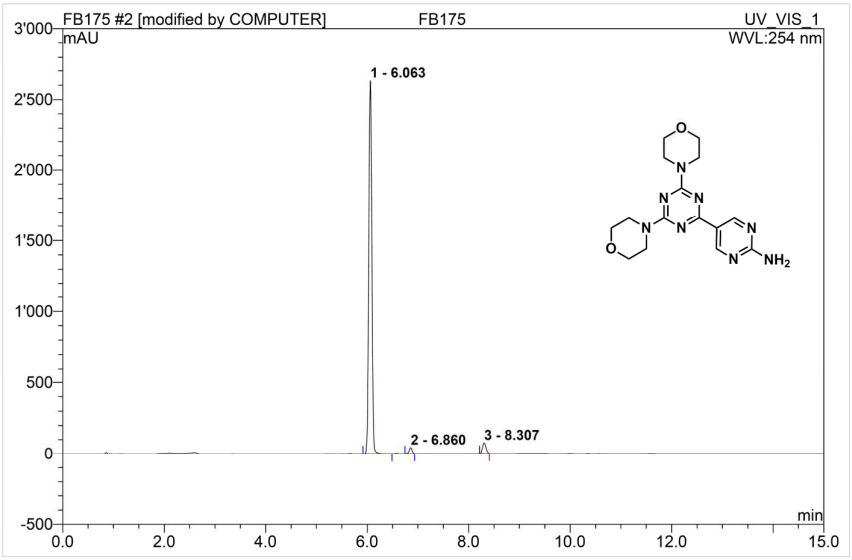
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	7.27	n.a.	1637.555	129.426	100.00	BMB
Total:			1637.555	129.426	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyridin-2-amine (**10**):



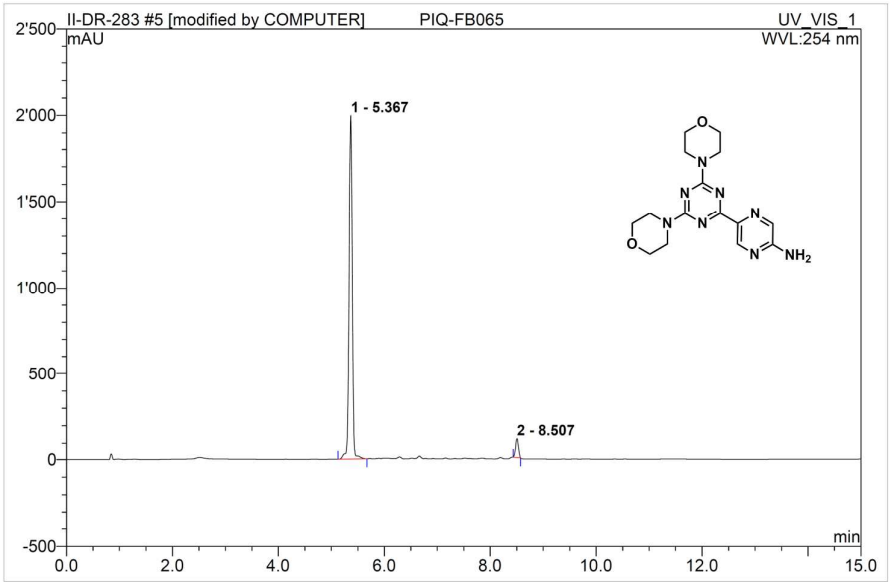
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	6.41	n.a.	21.807	1.574	0.46	BMB*
2	8.59	n.a.	3223.180	328.869	96.20	BM *
3	9.16	n.a.	48.055	4.301	1.26	M *
4	9.31	n.a.	51.803	4.665	1.36	MB*
5	11.51	n.a.	22.519	2.445	0.72	BMB*
Total:			3367.363	341.855	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrimidin-2-amine (**11**):



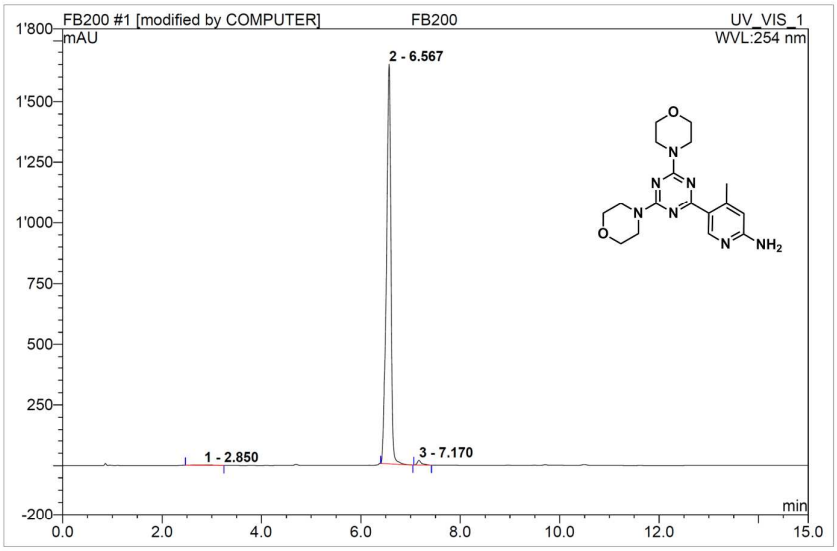
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	6.06	n.a.	2629.845	182.817	95.37	BMB
2	6.86	n.a.	42.345	2.857	1.49	BMB*
3	8.31	n.a.	75.155	6.013	3.14	BMB*
Total:			2747.345	191.688	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)pyrazin-2-amine (**12**):



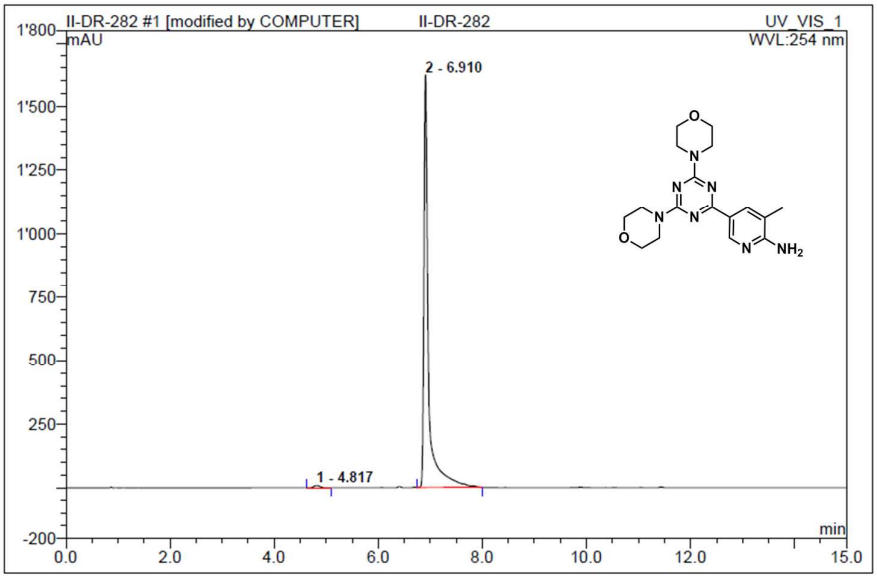
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	5.37	n.a.	1996.681	142.003	95.24	BMB
2	8.51	n.a.	115.274	7.102	4.76	BMB*
Total:			2111.956	149.105	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyridin-2-amine (**13**):



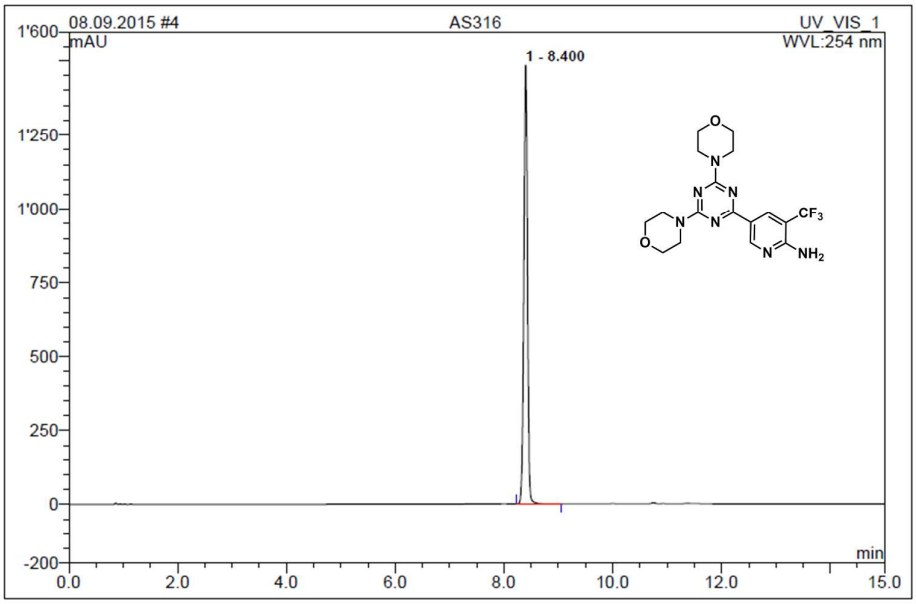
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	2.85	n.a.	1.404	0.651	0.37	BMB*
2	6.57	n.a.	1645.160	171.658	98.52	BMB
3	7.17	n.a.	18.749	1.926	1.11	BMB
Total:			1665.312	174.236	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-methylpyridin-2-amine (**14**):



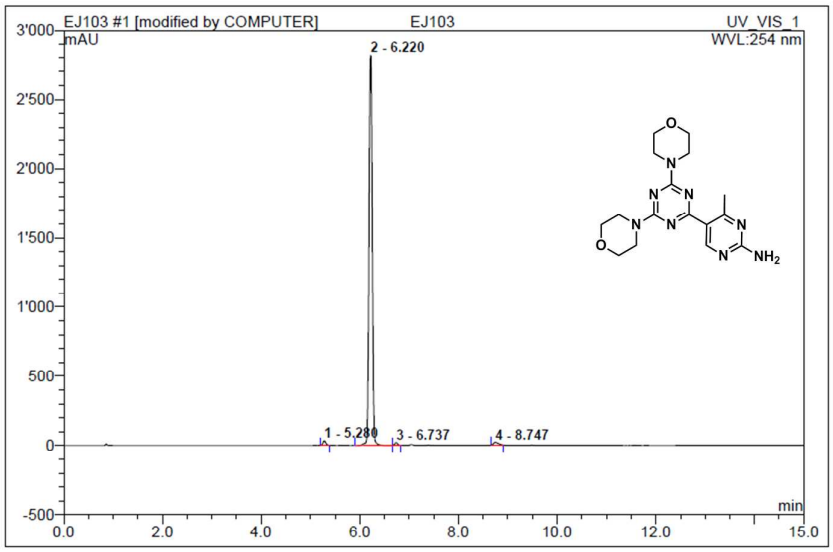
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	4.82	n.a.	8.347	1.155	0.70	BMB
2	6.91	n.a.	1620.594	164.284	99.30	BMB*
Total:			1628.940	165.439	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-3-(trifluoromethyl)pyridin-2-amine (**15**):



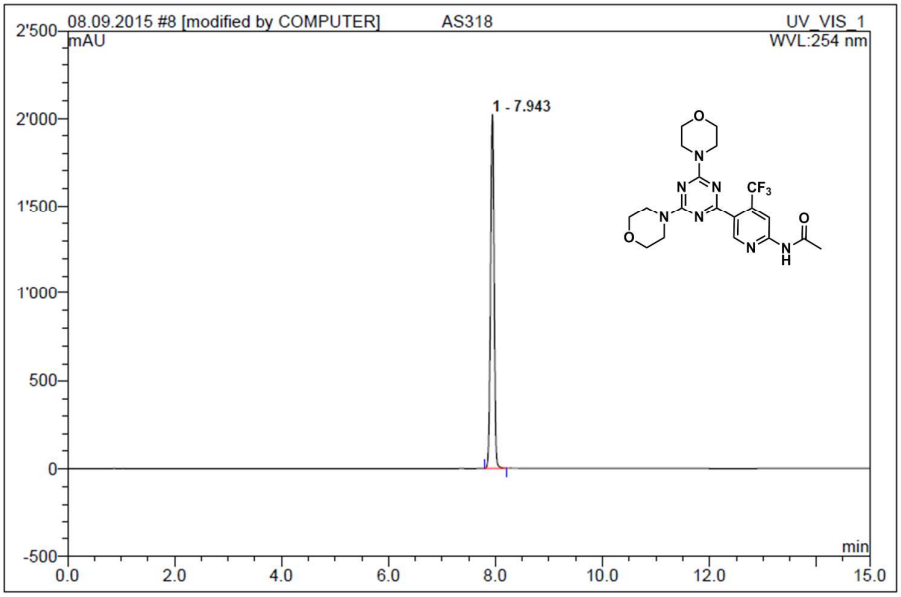
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	8.40	n.a.	1484.296	115.716	100.00	BMB
Total:			1484.296	115.716	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-methylpyrimidin-2-amine (**16**):



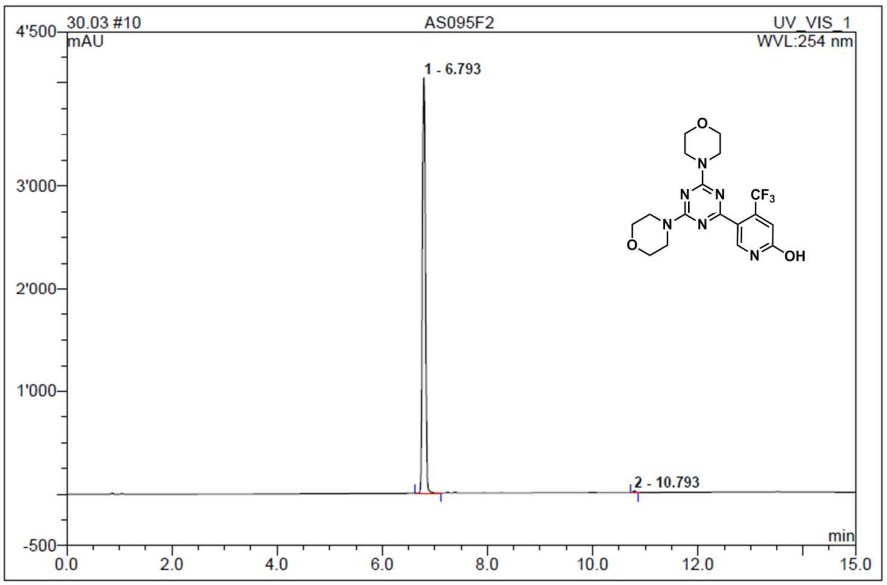
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	5.28	n.a.	32.626	2.234	1.00	BMB*
2	6.22	n.a.	2821.225	216.587	97.34	BM *
3	6.74	n.a.	20.354	1.341	0.60	MB*
4	8.75	n.a.	19.730	2.336	1.05	BMB*
Total:			2893.935	222.498	100.00	#REF!

N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)acetamide (**17**):



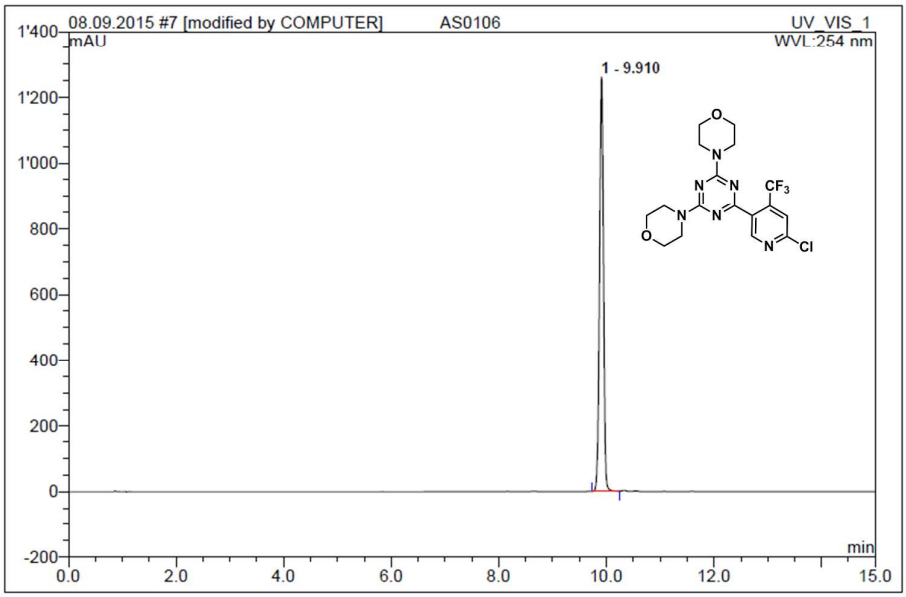
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	7.94	n.a.	2022.288	157.304	100.00	BMB
Total:			2022.288	157.304	100.00	#REF!

5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-ol (**18**):



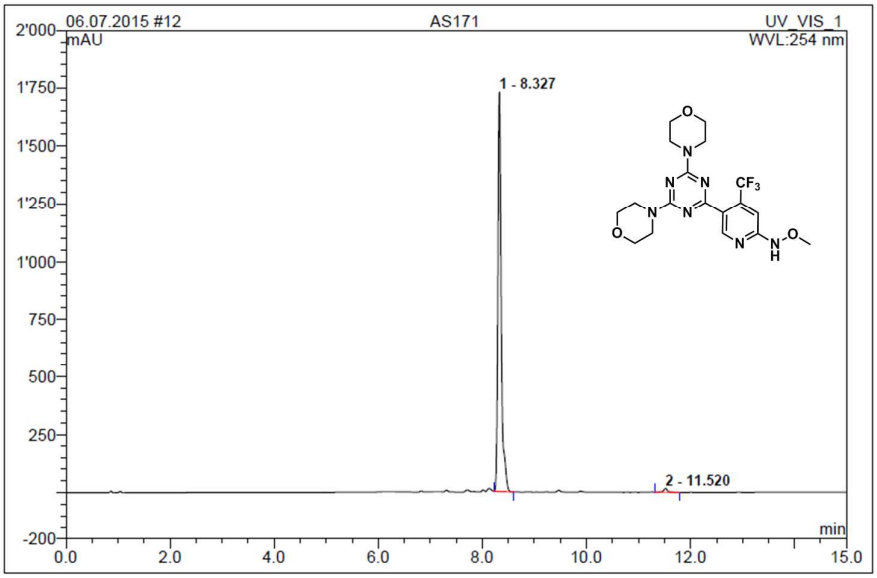
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	6.79	n.a.	4038.876	274.306	99.63	BMB
2	10.79	n.a.	17.907	1.016	0.37	BMB
Total:			4056.783	275.322	100.00	#REF!

4,4'-(6-(6-Chloro-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**19**):



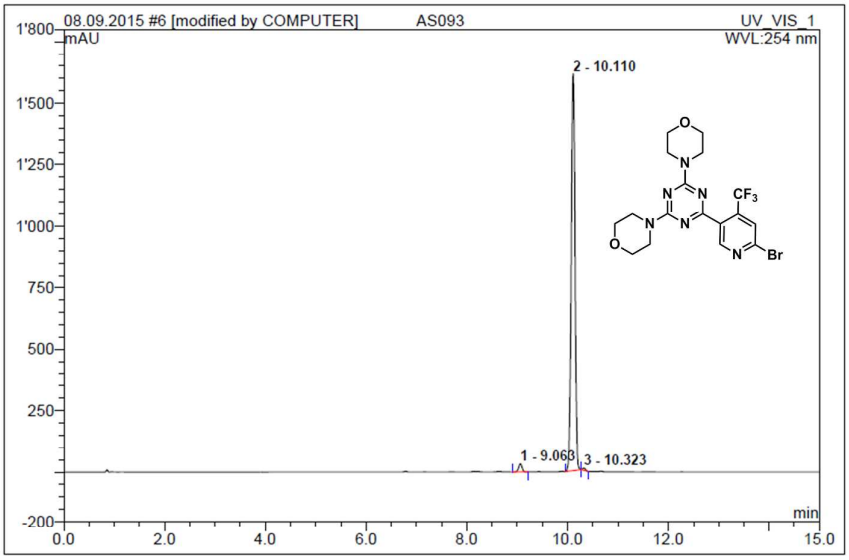
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	9.91	n.a.	1260.721	108.798	100.00	BMB
Total:			1260.721	108.798	100.00	#REF!

N-(5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)-*O*-methylhydroxylamine (**20**):



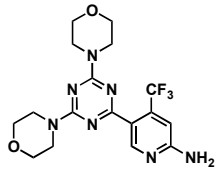
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	8.33	n.a.	1727.149	128.359	98.89	BMB
2	11.52	n.a.	16.504	1.435	1.11	BMB
Total:			1743.653	129.795	100.00	#REF!

4,4'-(6-(6-Bromo-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**21**):

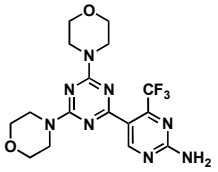


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	9.06	n.a.	32.610	2.563	1.81	BMB
2	10.11	n.a.	1611.287	138.839	97.78	BMB
3	10.32	n.a.	8.916	0.592	0.42	BMB*
Total:			1652.812	141.995	100.00	#REF!

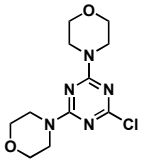
Appendix 1: collected chemical formulas



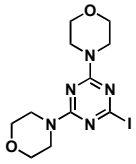
1 (PQR309)



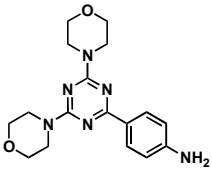
2



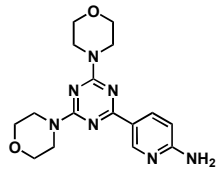
7



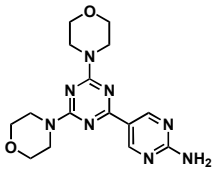
8



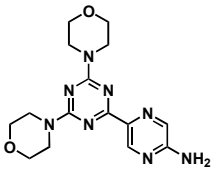
9



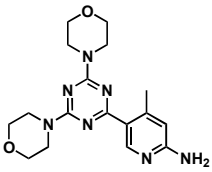
10



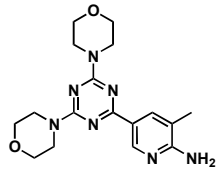
11



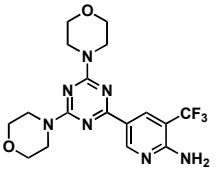
12



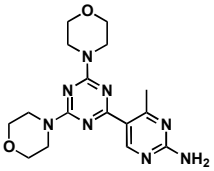
13



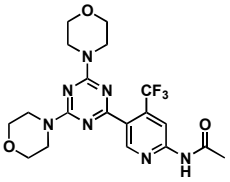
14



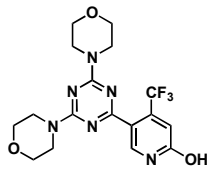
15



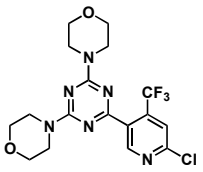
16



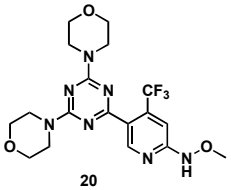
17



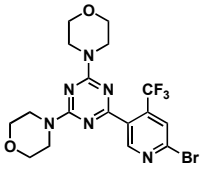
18



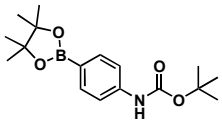
19



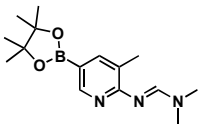
20



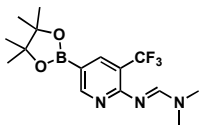
21



22



23



24