약학석사 학위논문

Dual-targeting 전략을 이용한 잠재적 항암 활성 물질의 개발

Toward the development of potent anti-cancer agents using dual targeting strategies

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ABSTRACT

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The insulin-like growth factor 1 receptor (IGF-1R) is a membrane receptor tyrosine kinase over-expressed in various human cancers, such as lung cancer, breast cancer, prostate cancer and colorectal cancer. Several compounds targeting IGF-1R have been discovered but rapidly acquired resistance in patients. The resistance to IGF-1R inhibitors is thought to be related to the high activation of Src. However, the combination therapy of IGF-1R and Src inhibitors may lead to toxicity problems.

In this study, a new class of 3-substituted-pyrazolo[3,4-d]pyrimidin-4-amine derivatives was designed and synthesized toward the development of dual-targeting anti-cancer agents. In addition, their anti-proliferative activities on A549 and MCF-7 cancer cell lines were evaluated using cell viability test. Structure-activity relationship of this series was explored based on the modification of linkers as well as substituents. Several compounds containing triazole or alkyne linker exhibited significant inhibitory effects on both cell lines.

Our results demonstrated that dual targeting Src/IGF-1R compounds offered the advantages over individual treatment of Src or IGF-1R inhibitor. In particular, linker modification was found to play an important role in the design of dual-targeting anti-cancer agents. Further biochemical and *in vivo* studies need to be followed, but this study provided valuable design strategies toward the development of dual targeting inhibitors, which may be used to overcome anti-cancer drug resistance.

Keywords: dual target, anti-cancer, resistance

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LIST OF ABBREVIATIONS

DCM	Dichloromethane
DIEA	*N*,*N*-Diisopropylethylamine
DMSO	Dimethyl sulfoxide
DMF	*N*,*N*-dimethylformamide
Et	Ethyl
i-Pr	isopropyl
MsCl	Methanesulfonyl chloride
NaAsc	Sodium ascorbate
NMR	Nuclear magnetic resonance
rt	room temperature
*t*BuOH	*tert*-butyl alcol
THF	tetrahydrofuran
TLC	thin layer chromotography

INTRODUCTION

Cancer is a major public health problem worldwide. There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide.[1] Cancer is also the second leading cause of death in the United States.[2] Lung, prostate, colorectal, stomach, and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, uterine cervix, and stomach cancer are the most common among women.[1]

The era of chemotherapy for treatment of cancer began in the 1940s with the uses of nitrogen mustards, antifolate drugs, alkylating agents and antimetabolites, etc.[3] This type of therapy which is based on the different cell division rate between normal and cancer cells is associated with significant negative side-effects including nausea, vomiting, hair loss, loss of appetite, peripheral neuropathy and anemia. It may also disrupt central nervous system function such as memory loss, decreased information processing speed, reduced attention, anxiety, depression and fatigue.[4]

In the late 1980s, the targeted-therapy revolution has been developed against specific signalling pathways driving tumor growth with limited side effects. Phosphorylation by protein kinase is one of the most common and important mechanism in signal transduction pathways. Many protein kinases (PKs) have been found to be intimately involved in the processes leading to tumour cell proliferation and survival.[5]

At the beginning of the development of protein kinase inhibitors (PKI), the highly selective agents targeting a single PK was identified as promising therapy. However, recent studies suggested that multi-targeted PKI may exhibit more effective than single target molecules.[6,7,8] Because individual molecular targets cannot usually combat multigenic diseases such as cancer.[9] For specific cancer types which several

kinases are frequently overexpressed, multi-kinase inhibitors will show better efficacy than single targeted molecule. The use of PKI may associate with significant problem in drug resistance. Because tumor cells rapidly develop resistance to individual inhibitors through mutation of PK which decrease the affinity of PK to the inhibitor or activation of other signalling pathways. To create effective therapy that overcomes or avoids drug resistance, it would be better to use multi-kinase targeting inhibitors.[10]

The insulin-like growth factor 1 receptor (IGF-1R) is a membrane receptor tyrosine kinase over-expressed in various human cancers such as breast cancer, lung cancer, colorectal cancer and prostate cancer.[11] IGF-1R consists of two extracellular α-subunits disulphide-bonded to two transmembrane-spanning β-subunits containing cytoplasmic tyrosine kinase activity (**Figure 1**).[12]

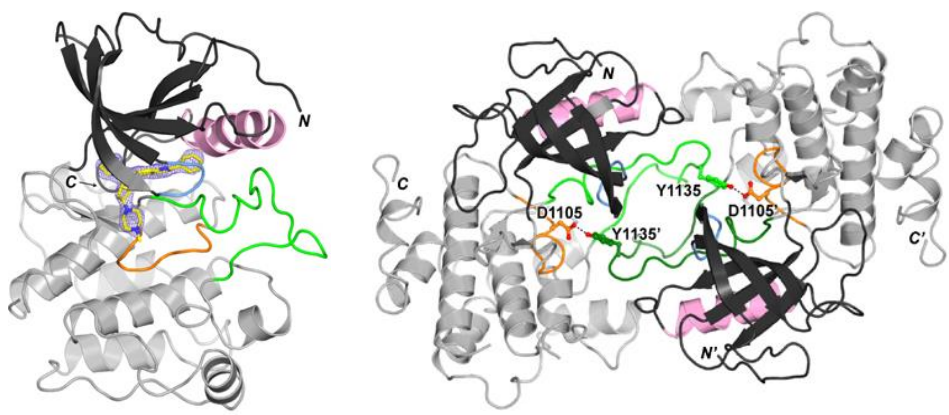


Figure 1. Crystal structure of IGF-1R

Several compounds targeting IGF-1R have been developed but rapidly acquired resistance in patients.[13,14,15,16] The resistance to IGF-1R inhibitors is thought to be related to the high activation of Src.[17,18] In particular, IGF-1 which failed to bind to IGF-1R due to the IGF-1R blockade by cixutumumab – a monoclonal antibody against IGF-1R, bound to integrin β3 and induced concomitant activation of integrin

signalling through FAK and Src and subsequent stimulation of EGFR and Akt (**Figure 2**).[19]

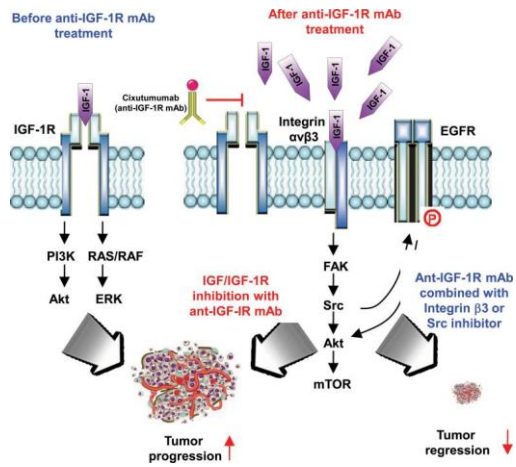


Figure 2. Schematic model of adaptive primary resistant responses by tumors to anti–IGF-1R monoclonal antibodies.

The non-receptor tyrosine kinase Src plays a vital role in cell division, motility, adhesion and survival.[20] Also, combined inhibition of Src and IGF-1R has been demonstrated to enhance anti-tumour effects in several cancers.[17,21] However, the combination therapy of two inhibitors may lead to toxicity problems because the biological systems need to compensate for the simultaneous action of two drugs.[9]

Moreover, the multi-targeted single agent has more advantages than combination therapy based on pharmacokinetics. The pharmacokinetics of a drug is defined by its absorption, distribution, metabolism and excretion. Single molecule has greater clinical benefits than combination therapy if one of the two drugs is a substrate for a drug transport pump or is metabolised. The administration of two inhibitors depends on their clearance metabolism. Possible problem is that the metabolism of the one drug can interfere with the metabolism of the other. Although the pharmacokinetics of PKI are known but it can be changed in particular patients. It is not possible to predict which types of inhibitor will show the optimal pharmacokinetics in

combination. In addition, to identify and compare the pharmacokinetic properties of two molecules are difficult in clinical trials because of the impact of other factors like smoking, dietary habits, etc. Thus, combination therapy might need to align pharmacokinetics or pharmacodynamics of component agents in co-formulated product. In addition, compared to combination therapy, multi-targeted single agent approach has the advantage of no risks related to drug-drug interactions and simplifies clinical trial design. Furthermore, manufacture and formulation of an individual active molecule are easier than a mixture.[9,10]

Recently, various multi-targeted protein kinase inhibitors have also been developed (**Figure 3**). Nordihydroguaiaretic acid (NDGA or INSM18) is a phenolic compound isolated from the creosote bush *Larrea divaricatta* that effectively inhibit the function of IGF-1R and the c-erbB2/HER2/neu receptor in *in vitro* and tumor *in vivo*. NDGA is in clinical trials and may represent a potential new class of agents for the treatment of breast and other cancers where the IGF-1R and/or HER2/neu play a role in the oncogenic process.[22] XL228 is a protein kinase inhibitor targeting IGF-1R, the Aurora kinases, FGFR1-3, Abl and Src family kinases. This compound is derived from a 2,4-disubstituted pyrimidine series and is in phase I clinical trials.[23] RBx10080307 was discovered as a dual Epidermal growth factor receptors (EGFR)/IGF-1R kinase inhibitor to limit the resistance development of selective EGFR inhibitor. The xenograft study with RBx10080307 showed significant tumor growth inhibition that was comparable to erlotinib – a selective EGFR inhibitor. RBx10080307 has also been found to be effective in the EGFR mutant cell line.[24]

In 2004, Bristol-Myers Squibb discovered Dasatinib (BMS-354825) as a dual Src/Abl inhibitor. Dasatinib can inhibit Bcr-Abl activation loop mutations that are found in some chronic myelogenous leukemia (CML) patients with acquired clinical resistance to imatinib.[25,26] In 2010, Dasatinib has been approved by FDA for

treatment of newly diagnosed adult patients with CML.[27] In 2011, Stefanie Schmidt et al. established dual IGF-1R/Src inhibitors with the efforts to interfere with two targets of the same signalling axis and lower the resistance development. In particular, the cell growth of A549/ATCC in the presence of 10 μM of the most potent compound is about 7.38%.[28] In 2013, Kristin S. Ko et al. developed chimeric c-Src and HDAC inhibitors on the basis of their synergism. HDAC inhibitor and Src inhibitor were appended by varied hydrophobic linkers using click chemistry. The most active compound has improved efficacy in cellular experiments compared to two individual inhibitors while not possessing significant toxicity to primary human cells.[29]

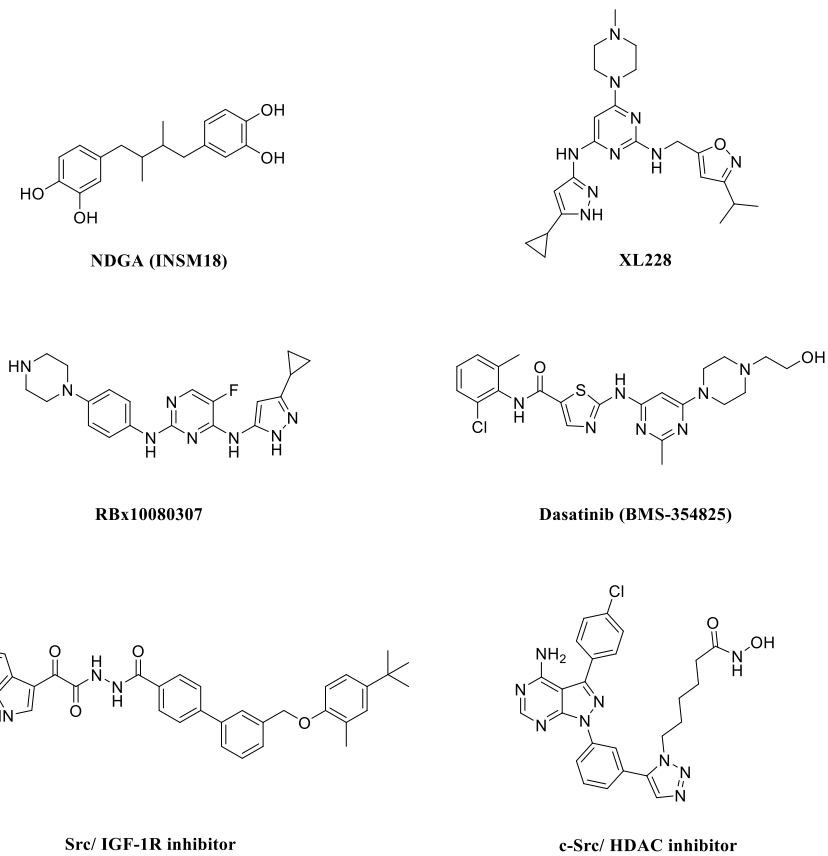


Figure 3. Structure of several multi-kinase inhibitors

In this study, we described the design strategy and synthesis of single molecules that could serve as Src and IGF-1R inhibitor. We also discussed the structure-activity relationship (SAR) of synthesized compounds based on the growth inhibition against cancer cell lines.

RESULTS

1. Design

The 4-aminopyrazolo[3,4-d]pyrimidines PP1 and PP2 were the first Src family kinase (SFK) inhibitors and were reported by Pfizer researchers in 1996. *In vitro* kinase assays showed that these compounds inhibit SFK members with IC_{50} values in the low nanomolar range.[30]

A diaryl urea compound PQ401 inhibited autophosphorylation of the IGF-1R in cultured human MCF-7 cells with an IC_{50} of 12 μM.[31] More importantly, through a mouse xenograft model, administration of PQ401 on mice led to suppression of glioma tumour growth. At lower doses, PQ401 in large inhibits cell migration, while at higher doses, PQ401 synergistically suppresses cell migration and proliferation.[32]

A new class of multi-target compounds **1-2** was synthesized by linking Src inhibitor PP1/PP2 to an IGF-1R inhibitor (PQ401) (**Figure 4**). The biological evaluation showed that these kinds of compounds have K_d value range from 1.9-2.3 μM against Src/Abl respectively. However, they displayed very weak inhibitory activity against A549 and MCF-7 cell lines in WST assay.

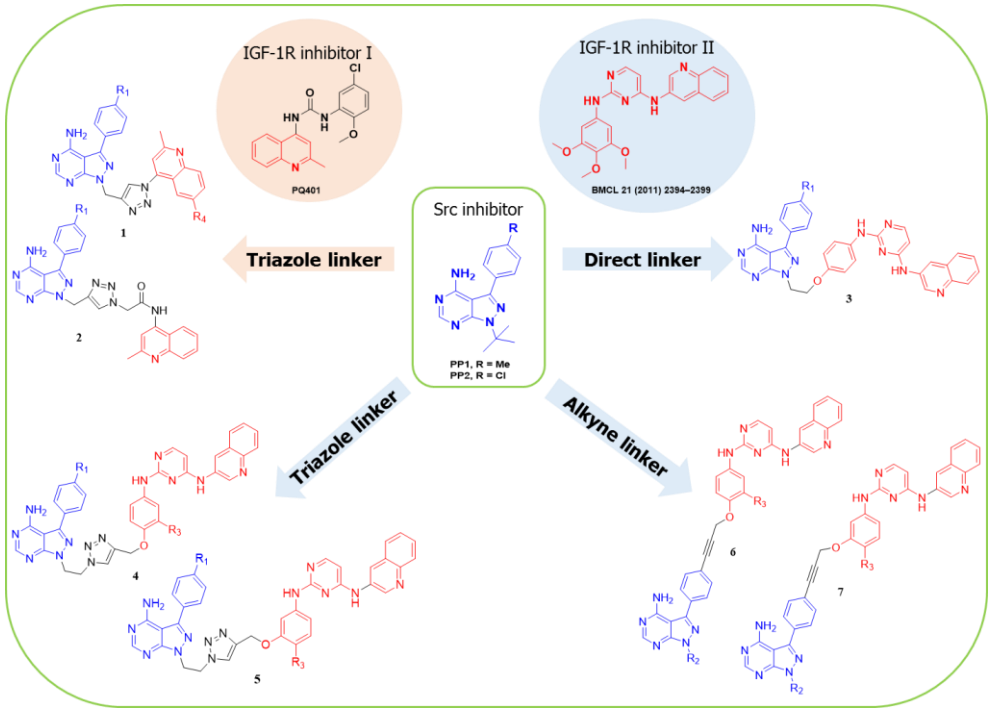


Figure 4. Design strategy of dual-targeting Src/ IGF-1R inhibitors

To enhance potency toward IGF-1R protein, we attempted to incorporate 2-arylamino-1,3-pyrimidine into quinoline ring to get second IGF-1R module. We conjugated the IGF-1R and Src modules directly via *N*-alkylation reaction to give a series of compounds **3**. To establish the structure-activity relationship (SAR) and optimize the potency, we primarily focused our modifications on the linker. Triazole-based derivatives have been known as one of the most active areas in the developments of new drugs with strong pharmacological activity, low toxicity, fewer multi-drug resistances, high bioavailability, good pharmacokinetic property.[33] Hence, the copper(I)-catalyzed alkyne-azide cycloaddition was applied to get triazole linker compounds **4**-**5**. Based on SAR study of second IGF-1R module, pyrimidine and quinoline ring were not modified to maintain the highest potency.[34] However, we introduce different functional groups to aminophenol ring at R_3 to determine the substituent effects. Surprisingly**,** this modification resulted in significant loss of potency, suggesting those substituents caused steric clash. We hypothesized that slim

linker as alkyne may avoid steric clash. This linker is the key factor for ponatinib – a Bcr-Abl inhibitor to prevent steric clash.[35] Recently, alkyne linker has applied successfully in several PKI (**Figure 5**).[36,37] Therefore, a new series of compounds **6-7** were synthesized by Sonogashira cross-coupling reaction. Interestingly, addition of any groups, especially methoxy to the aminophenol ring increased overall potency on A549 cancer cell line compared to unsubstituted compounds.

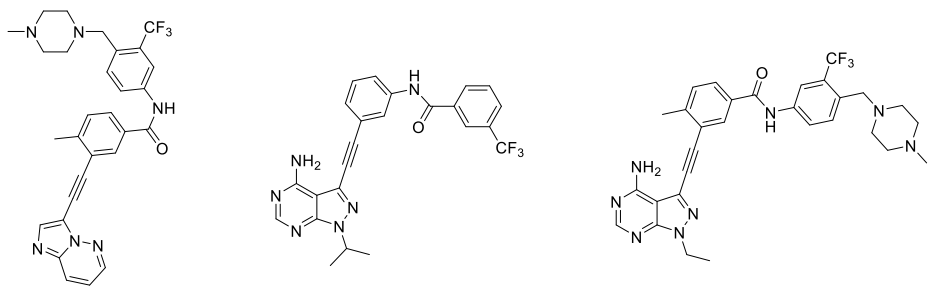
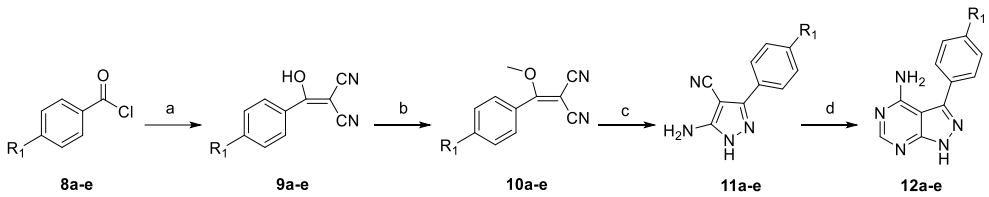


Figure 5. Structure of several PKI containing alkyne linker

2. Synthesis

Initially, the scaffold of pyrazolo[3,4-d]pyrimidine from Src inhibitor (PP1, PP2) was obtained from the reported procedure with modified conditions (**Scheme 1**).[38] Various commercial reagents **8a-e** reacted with malononitrile in the presence of sodium hydride in anhydrous THF gave **9a-e**. Subsequent methylation with dimethyl sulfate in the presence of sodium bicarbonate in dioxane-water mixtures afforded **10a-e**, which were cyclized by treatment with hydrazine hydrochloride in the presence of triethylamine generated **11a-e**. Reaction of **11a-e** with formamide at 160 °C for 12 h afforded key intermediates **12a-e**.

Scheme 1. Synthesis of 3-substituted-pyrazolopyrimidine

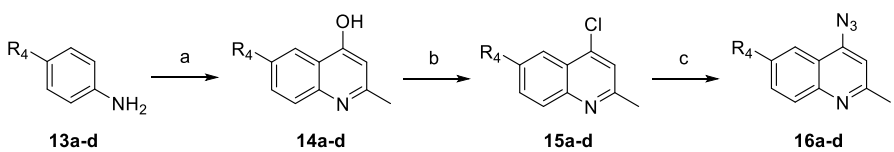


8a - 12a: R_1 = Me, **8b - 12b**: R_1 = H, **8c - 12c**: R_1 = Cl, **8d - 12d**: R_1 = CF_3, **8e - 12e**: R_1 = I

Reagents and conditions: a) Malononitrile, NaH, THF anhydrous, 0 °C to rt, 1 h, 65-93%; b) Me_2SO_4, $NaHCO_3$, 1,4-dioxane:H_2O = 5:1, 80 °C, 3 h, 50-65%; c) NH_2NH_2·HCl, TEA, EtOH, 120 °C, 30 min, 75-83%; d) $HCONH_2$, 160 °C, 12 h, 36-83%.

Next, the synthesis of the first IGF-1R module is illustrated in **Scheme 2** based on the previously described route.[39,40] As shown in this scheme, **14a-d** were synthesized starting from the commercially available substituted anilines **13a-d** and ethylacetoacetate in the presence of polyphosphoric acid. Subsequent chlorination using phosphoryl chloride produced **15a-d** which was easily transformed to the corresponding azide **16a-d** by the addition of sodium azide.

Scheme 2. Synthesis of IGF-1R module I (substituted-quinoline)



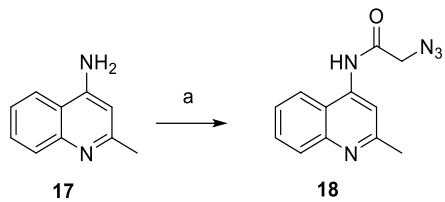
13a-16a: R_4 = H, **13b-16b**: R_4 = F, **13c-16c**: R_4 = I, **13d-16d**: R_4 = OMe

Reagents and conditions: a) Ethylacetoacetate, Polyphosphoric acid, 120 °C, 16 h, 80-94%; b) $POCl_3$, Acetone 105 °C, 3 h, 86-96%; c) NaN_3, 100 °C, DMF, 4 h, 79-92%.

In order to have an extended linkage with acetyl group, the commercially available 2-methylquinolin-4-amine **17** was transformed to **18** by adding 2-

azidoacetic acid in the presence of 1-ethyl-3-(3 dimethylaminopropyl)carnodiimide hydrochloride (EDC) and *N*,*N*-diisopropylethylamine (DIEA). (**Scheme 3**).

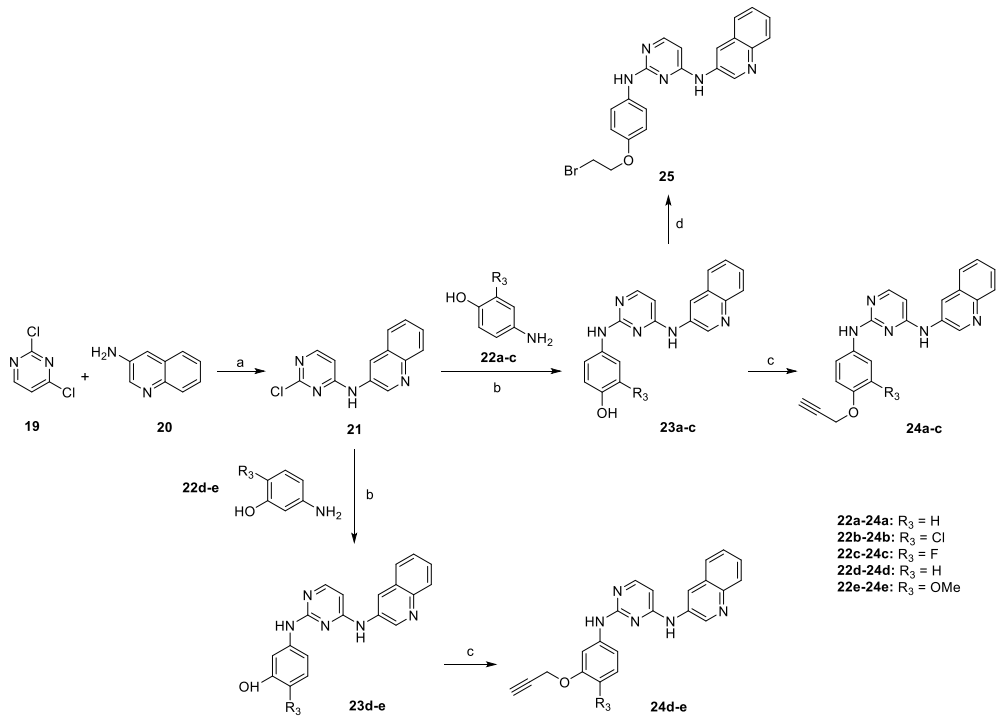
Scheme 3. Synthesis of IGF-1R module I (2-azido-N-(2-methylquinolin-4-yl)acetamide)



Reagents and conditions: a) 2-azidoacetic acid, EDC, DIEA, DMF:DCM, 60 °C, 8 h, 66%.

The synthesis of the second IGF-1R module is depicted in **Scheme 4**. Reaction of 2,4-dichloropyrimidine **19** with 3-aminoquinoline **20** in the presence of DIEA produced **21**, which reacted with the commercially available aminophenol derivatives **22a-e** to form **23a-e**. *O*-alkylation of phenol with propargyl bromide or 1,2-dibromoethane under basic conditions gave **24a-e** or **25** respectively.

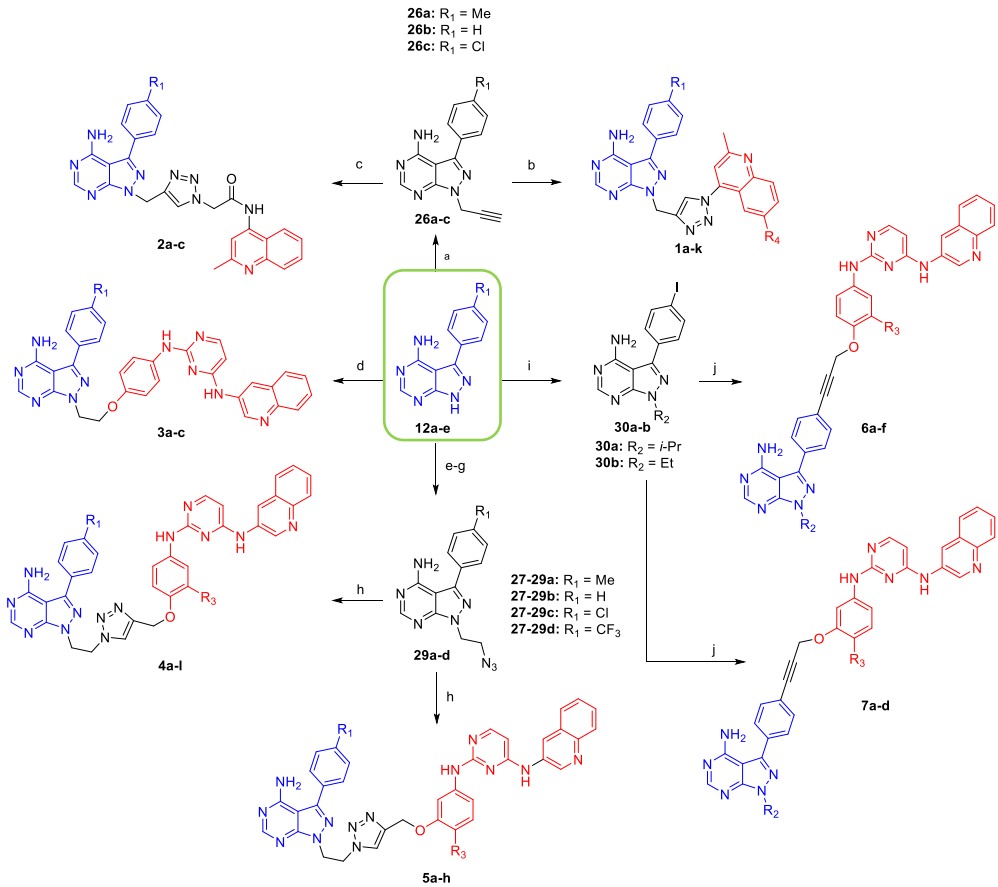
Scheme 4. Synthesis of IGF-1R module II



Reagents and conditions: a) DIEA, *i*-PrOH, refluxed 100 °C, 24 h, 70%; b) DMSO, 90 °C, 2 h, 88-99%; c) Propargyl bromide, Cs_2CO_3, DMF, rt, 2 h, 59-67%; d) 1,2-dibromoethane, K_2CO_3, DMF, rt, 12 h, 49%.

As shown in **Scheme 5**, key intermediates **12a-e** were *N*-alkylated using propargyl bromide or 2-bromoethanol, 2-iodopropane, iodoethane in the presence of base in DMF to give **26a-c** or **27a-d**, **30a, 30b** respectively. Subsequent mesylation of **27a-d** with mesylate chloride in pyridine, followed by addition of sodium azide provided **29a-d**.

Scheme 5. Synthesis of target compounds

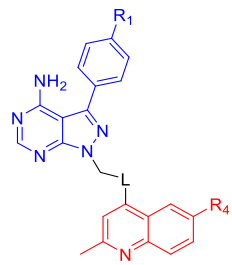


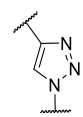
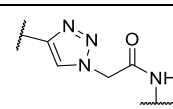
Reagents and conditions: a) Propargyl bromide, K_2CO_3, DMF, rt, 3 h, 45-61%; b) **16a-d**, $CuSO_4.5H_2O$, NaAsc, *t*BuOH:H_2O, 80 °C, 3 h, 43-94%; c) **18**, $CuSO_4.5H_2O$, NaAsc, *t*BuOH:H_2O, 80 °C, 3 h, 45-55%; d) **25**, Cs_2CO_3, DMF, 80 °C, 3 h, 36-48%; e) 2-bromoethanol, Cs_2CO_3, DMF, 80 °C, 3 h, 50-63%; f) MsCl, Pyridine, rt, 5 h, 45-76%, g) NaN_3, DMF, 60 °C, 8 h, 80-88%; h) **24a-e**, $CuSO_4.5H_2O$, NaAsc, *t*BuOH:H_2O:DMF, 80 °C, 3 h, 39-80%; i) R_2I, K_2CO_3, DMF, 60 °C, 3 h, 51-56%; j) **24a-e**, $Pd(PPh_3)_2Cl_2$, CuI, TEA, DMF, rt, 3 h, 40-71%.

Next, the first series of compounds **1a-k** and **2a-c** were synthesized by copper(I)-catalyzed alkyne-azide cycloaddition between 2**6a-c** and **16a-d**/**18** (**Table 1**). *N*-alkylation reaction gave direct linker compounds **3a-c**. The cycloaddition between alkyne containing quinoline **24a-e** and various azide containing pyrazolopyrimidine **29a-d** under copper(I) catalyst afforded the triazole linker compounds **4a-l**, **5a-h**.

Finally, the Sonogashira cross-coupling of *N*-substitued pyrazolopyrimidine derivatives **30a-b** with various alkyne **24a-e** yielded **6a-f** and **7a-d** possessing alkyne linker.

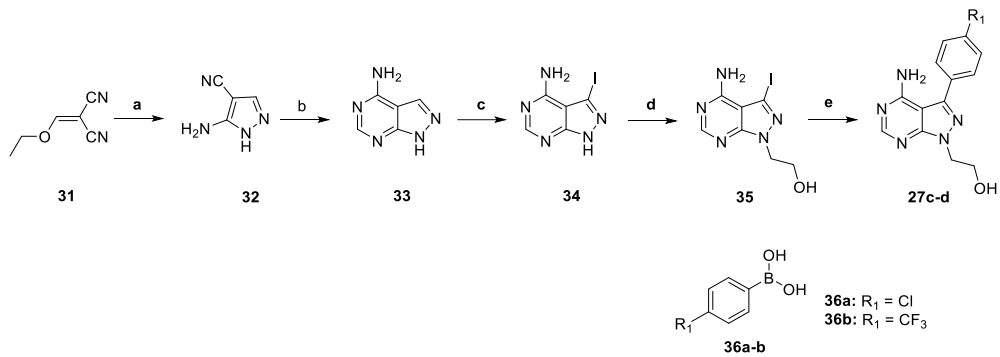
Table 1. Structure of target compounds **1-2**



Compd	R_1	R_4	L (linker)
1a	Me	H	
1b	H	H	
1c	Cl	H	
1d	Me	F	
1e	H	F	
1f	Me	I	
1g	H	I	
1h	Cl	I	
1i	Me	OMe	
1j	H	OMe	
1k	Cl	OMe	
2a	Me	H	
2b	H	H	
2c	Cl	H	

In order to obtain pyrazolo[3,4-d]pyrimidin-4-amine as a common intermediate which will be subsequently derivatized to furnish aryl analogs with R_1, we also established the more efficient synthetic scheme starting from the common commercial reagents based on the previously described route (**Scheme 6**).[41,42]

Scheme 6. Efficient synthetic scheme for Src module



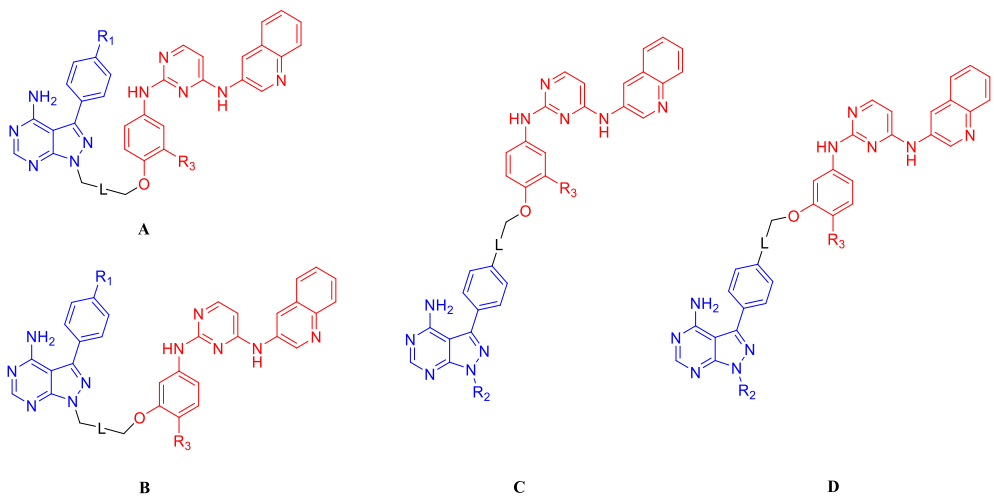
Reagents and conditions: a) $NH_2NH_2 \cdot HCl$, TEA, EtOH, 100 °C, 30 min, 57%; b) $HCONH_2$, 160 °C, 12 h, 80%; c) NIS, DMF, 60 °C, 12 h, 80%; d) 2-bromoethanol, Cs_2CO_3, DMF, 80 °C, 3 h, 60%; e) **36a-b**, $Pd(PPh_3)_4$, Cs_2CO_3, DMF:H_2O, 100 °C, 3h, 81-92%.

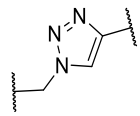
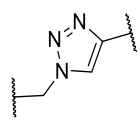
Condensation of 2-(ethoxymethylene)malononitrile **31** with hydrazine hydrochloride gave **32**, followed by treatment with formamide to generate **33**. Iodination of **33** with *N*-iodosuccinimide produced **34** which was subsequently *N*-alkylated with 2-bromoethanol in the presence of base afforded key intermediate **35**. **35** was coupled with **36a-b** under Suzuki coupling reaction condition to yield **27c-d**. In the case of Iodine at R_1 position, Suzuki coupling was not successful. However, we were able to obtain compound **27c-d** as common intermediates in a large scale.

3. Preliminary *in vitro* cytotoxic assay

In order to provide a broader assessment of cytotoxicity, all compounds were tested in WST assay for growth inhibitory activity against human non-small cell lung cancer A549 and human breast cancer cell MCF-7. Bosutinib was also included in the assay as a control. To identify whether the dual targeting compounds would be better than single targeting molecule, two key intermediates **12a**, **23a** were also examined in WST assay. (**Table 2**)

Table 2. Anti-proliferation efficacies against cancer cell lines of compounds **3-7**



Compd	Structure	R_1/R_2	R_3	L (linker)	% cells growth at 10 μM	
					A549	MCF-7
3a	A	Me	H		98	108
3b		H	H		96	100
3c		Cl	H		82	95
4a	A	Me	H		82	74
4b		H	H		20	21
4c		Cl	H		6.4	1.3
4d		CF_3	H		26	13
4e		Me	Cl		101	99
4f		H	Cl		103	86
4g		Cl	Cl		99	96
4h		CF_3	Cl		27	37
4i		Me	F		88	90
4j		H	F		96	71
4k		Cl	F		94	61
4l		CF_3	F		47	18
5a	B	Me	H		68	51
5b		H	H		35	70
5c		Cl	H		52	38
5d		CF_3	H		76	77
5e		Me	OMe		65	47
5f		H	OMe		91	55
5g		Cl	OMe		100	100
5h		CF_3	OMe		106	100
6a	C	*i*-Pr	H		34	27
6b		*i*-Pr	Cl		16	53
6c		*i*-Pr	F		28	58
6d		Et	H		87	84
6e		Et	Cl		95	94
6f		Et	F		99	90
7a	D	*i*-Pr	H		68	71
7b		*i*-Pr	OMe		9.7	15
7c		Et	H		87	82
7d		Et	OMe		6.2	6.4
Bosutinib					46	75
12a					98	92
23a					69	60

Several active compounds were selected to determine IC_{50} value against two cancer cell lines A549 and MCF-7. (**Table 3**)

Table 3. IC_{50} value of selected compounds

Compd	IC_{50} (μM)	
	A549	**MCF-7**
4b	5.77	8.07
4c	2.59	2.16
4d	4.87	3.00
4l	8.24	2.51
5b	5.51	6.79
5c	7.15	5.73
6a	3.69	4.62
6b	5.06	7.57
6c	7.25	7.87
7b	3.57	3.98
7d	4.28	3.54

4. Structure activity relationship (SAR)

Our preliminary screening results showed that **12a** from Src module did not exhibit inhibitory effects on both cancer cell lines while **23a** from IGF-1R module showed weak inhibition. Likewise, three compounds with direct linker **3a-c** did not show any inhibition on two cell lines at 10 μM. In contrast, several target compounds with triazole linker **4b-d**, **4h**, **4l**, **5b-c** or alkyne linker **6a-c**, **7b**, **7d** displayed significant anticancer activity. These findings suggested that: 1) Dual targeting Src/IGF-1R compounds offered the advantages over the individual treatment of Src or IGF-1R inhibitor and 2) Linker modification played an important role in design of potent anti-cancer agents.

About unsubstituted compounds, para isomers **4b-d** showed greater potency against both cell lines than meta isomers **5b-d**.

To determine whether functional groups are more critical for activities, chlorine, florine, methoxy groups were introduced to the phenol ring and the compounds **4e-l, 5e-h, 6b-c, 6e-f, 7b, 7d** were tested. Changes made to this ring in triazole linker compounds resulted in significant loss of potency, implying that the large substituents caused steric clash. In contrast, in the case of alkyne linker compounds, this modification maintained high potency on A549 **6b-c, 7b**, **7d**. These data are consistent with our hypothesis. We concluded that 1) Slim alkyne linker can avoid steric clash caused by substituents, 2) Substituent on phenol ring are critical for activity.

We further analysed the influences of substituents at R_2 on bioactivities. Replacement of isopropyl in **6a-c**, **7a-b** with an ethyl group **6d-f**, **7c** led to the decrease of inhibitory effects indicating that bulky group at R_2 is required for cytotoxicity. This requirement has been previously recognized and appears to be a general feature for kinase inhibition with this pyrazolopyrimidine scaffold.[43,44,45,46] However, in the presence of methoxy group on phenol ring, compound **7d** containing ethyl at R_2 also showed good cytotoxicity. This suggested that optimal activity of alkyne linker compounds is achieved with methoxy group.

CONCLUSION

In this study, we have developed novel pyrazolopyrimidine-based inhibitors which showed good inhibitory effects on two human cancer cell lines, A549 and MCF-7. The structure-activity relationship of dual-targeting inhibitors was also established based on cell viability assay.

Our results demonstrated that dual targeting Src/IGF-1R compounds offered the advantages over individual treatment of Src or IGF-1R inhibitor. In particular, linker modification was found to play an important role in the design of dual-targeting anti-cancer agents. Triazole linker and alkyne linker compounds displayed greater inhibitory effects on cancer cell lines than direct linker. While substituent on aminophenol ring is not important for triazole linker compounds because of steric clash, it exhibited promising activity in alkyne linker compounds. This suggested that alkyne linker may avoid the steric clash caused by substituents.

Our cell viability assay showed that the most potent compound in triazole linker series **4c** has IC_{50} value of 2.59 and 2.16 μM against A549 and MCF-7 respectively. Also, compound **7b** containing alkyne linker and methoxy group on aminophenol ring has IC_{50} value of 3.57 and 3.98 μM against A549 and MCF-7 respectively.

Further biochemical and *in vivo* studies need to be followed, but this work provided valuable design strategies toward the development of dual targeting inhibitors, which may be used to overcome anti-cancer drug resistance.

EXPERIMENTAL SECTION

1. General method

1.1. Solvents and reagents

All reagents and solvents were purchased from commercial suppliers and used without further purification.

1.2. Chromatography

TLC analyses were performed using Merck precoated TLC plate (silica gel 60 F_{254}). Column chromatography was carried out on Zeochem silica gel (Zeo prep 60, 40-63 μm)

1.3. Spectra data

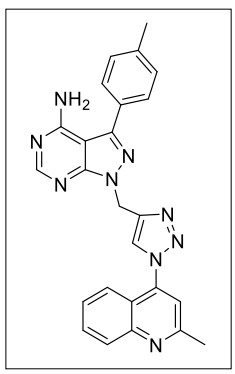
^{1}H NMR spectra were acquired on a 300, 400, 500, 600, 800 MHz spectrometer, and ^{13}C NMR spectra were acquired on 75, 100, 125, 150, 200 MHz spectrometer. Chemical shift (δ) are reported in parts per million (ppm) and coupling constants (*J*) are given in hertz (Hz).

All ESI-MS were undertaken on 6130 Single Quadrupole LC/MS (Agilent Technologies, CA, USA). High-resolution mass spectra (HRMS) were acquired under fast atom bombardments (FAB) condition on a JMS-700 MStation (JEOL, Germany).

2. General procedure

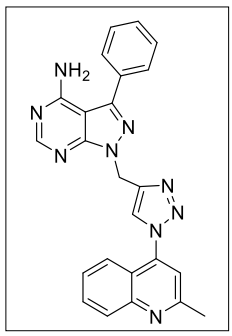
2.1. General procedure for the copper(I)-catalyzed alkyne-azide cycloaddition between alkyne 26a-c and azide 16a-d/18

1-((1-(2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1a)



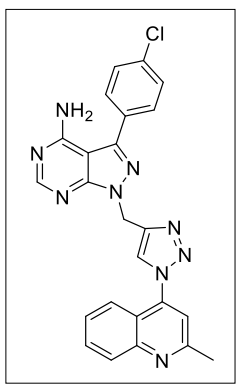
To a solution of **16a** (21 mg, 0.11 mmol) and an alkyne **26a** (30 mg, 0.11 mmol) in *t*BuOH:H_2O (1:1, 5 mL) were added sodium ascorbate (4.5 mg, 0.022 mmol) and copper(II) sulfate pentahydrate (2.8 mg, 0.011 mmol). The mixture was stirred at 80 °C for 3 h. The resulting mixture was concentrated *in vacuo* and then extracted with DCM (3 X 100 mL). The organic phase was dried with anhydrous sodium sulfate, the solvent was evaporated, and the crude product was purified using silica gel chromatography with DCM/methanol gradient to give a white solid (28 mg, 0.063 mmol, yield = 54.9%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.31 (s, 1H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.86-7.78 (m, 2H), 7.71 (s, 1H), 7.63 (d, *J* = 6.9 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 5.79 (s, 2H), 2.71 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.44, 158.15, 155.99, 154.36, 148.53, 144.32, 143.35, 140.23, 138.14, 130.52, 129.91, 129.69 (2C), 128.73, 128.17 (2C), 127.36, 125.97, 122.67, 120.21, 117.71, 97.37, 41.59, 24.77, 20.92 ppm. LC-MS (ESI) *m/z* 448.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{25}H_{21}N_9$ $[M + H]^+$: 448.1920, found: 448.1994.

1-((1-(2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1b)



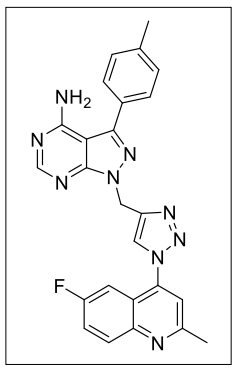
This compound was synthesized using a similar procedure, yield = 46%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.32 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.86-7.78 (m, 2H), 7.71-7.45 (m, 7H), 5.80 (s, 2H), 2.71 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.43, 158.15, 155.99, 154.42, 148.53, 144.30, 143.31, 140.22, 132.73, 130.51, 129.10 (2C), 128.72, 128.25 (2C), 127.34, 125.99, 122.67, 120.19, 117.69, 97.40, 41.63, 24.76 ppm. LC-MS (ESI) *m/z* 434.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{24}H_{19}N_9$ $[M + H]^+$: 434.1763, found: 434.1843.

3-(4-chlorophenyl)-1-((1-(2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1c)



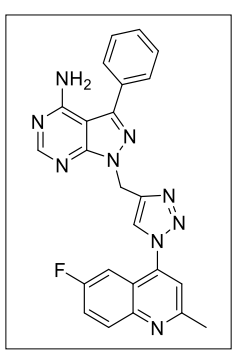
This compound was synthesized using a similar procedure, yield = 89%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.32 (s, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.86-7.78 (m, 2H), 7.71 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 8.4 Hz, 2H), 5.80 (s, 2H), 2.71 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.42, 158.17, 156.02, 154.52, 148.55, 143.24, 140.22, 133.41, 131.52, 130.49, 130.06 (2C), 129.06 (2C), 128.72, 127.33, 125.96, 122.64, 120.19, 117.65, 97.38, 41.65, 24.71 ppm. LC-MS (ESI) *m/z* 468.10 $[M + H]^+$. HRMS (FAB) calculated for $C_{24}H_{18}ClN_9$ $[M + H]^+$: 468.1374, found: 468.1446.

1-((1-(6-fluoro-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1d)



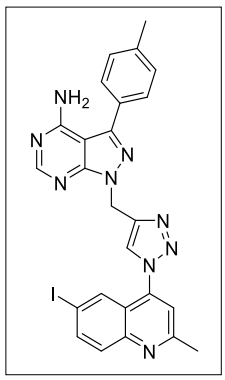
This compound was synthesized using a similar procedure, yield = 86%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.31 (s, 1H), 8.16 (dd, J = 9.15 Hz, 5.6 Hz, 1H), 7.81-7.75 (m, 2H), 7.60 (s, 1H), 7.57 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 5.79 (s, 2H), 2.71 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.76, 158.98 (d, $J_{C\text{-}F}$ = 2.55 Hz), 158.50, 158.15, 155.93, 154.36, 145.83, 144.32, 143.48, 139.83 (d, $J_{C\text{-}F}$ = 5.4 Hz), 138.14, 131.80 (d, $J_{C\text{-}F}$ = 8.625 Hz), 129.89, 129.66 (2C), 128.15 (2C), 125.85, 120.75 (d, $J_{C\text{-}F}$ = 10.2 Hz), 120.37, 118.20, 97.38, 41.59, 24.61, 20.91 ppm. LC-MS (ESI) *m/z* 466.11 $[M + H]^+$. HRMS (FAB) calculated for $C_{25}H_{20}FN_9$ $[M + H]^+$: 466.1826, found: 466.1911.

1-((1-(6-fluoro-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1e)



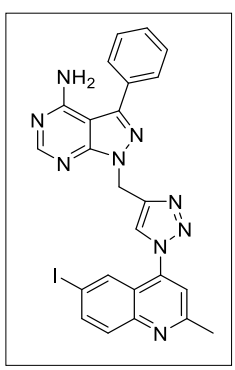
This compound was synthesized using a similar procedure, yield = 58%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.32 (s, 1H), 8.15 (dd, J = 9.3 Hz, 5.4 Hz, 1H), 7.81-7.74 (m, 2H), 7.68 (d, J = 7.8 Hz, 2H), 7.59-7.45 (m, 4H), 5.80 (s, 2H), 2.71 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.75, 158.98 (d, $J_{C\text{-}F}$ = 2.55 Hz), 158.49, 158.14, 155.96, 154.41, 145.82, 144.30, 143.43, 139.83 (d, $J_{C\text{-}F}$ = 5.4 Hz), 132.71, 131.80 (d), 129.09 (2C), 128.70, 128.24 (2C), 125.85, 120.75 (d, $J_{C\text{-}F}$ = 10.125 Hz), 120.37, 118.22, 97.397, 41.63, 24.61 ppm. LC-MS (ESI) *m/z* 452.10 $[M + H]^+$. HRMS (FAB) calculated for $C_{24}H_{18}FN_9$ $[M + H]^+$: 452.1669, found: 452.1742.

1-((1-(6-iodo-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1f)



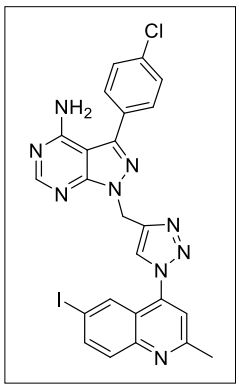
This compound was synthesized using a similar procedure, yield = 94%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.32 (s, 1H), 8.18 (d, *J* = 1.8 Hz, 1H), 8.10 (dd, *J* = 8.7 Hz, 1.8 Hz, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.74 (s, 1H), 7.58 (d, *J* = 8.1 Hz, 2H) 7.35 (d, *J* = 8.1 Hz, 2H), 5.79 (s, 2H), 2.69 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.22, 158.15, 155.97, 154.37, 147.45, 144.34, 143.50, 138.85 (2C), 138.13, 131.14, 130.61, 129.89, 129.66 (2C), 128.16 (2C), 125.89, 121.74, 118.13, 97.40, 93.68, 41.57, 24.74, 20.85 ppm. LC-MS (ESI) *m/z* 574.10 $[M + H]^+$. HRMS (FAB) calculated for $C_{25}H_{20}IN_9$ $[M + H]^+$: 574.0886, found: 574.0966.

1-((1-(6-iodo-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1g)



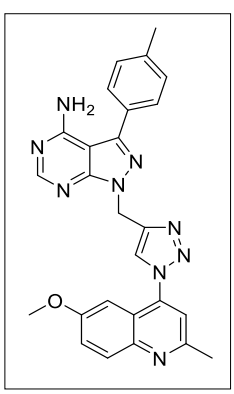
This compound was synthesized using a similar procedure, yield = 89%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.33 (s, 1H), 8.18 (d, *J* = 1.5 Hz, 1H), 8.10 (dd, *J* = 8.7 Hz, 1.8 Hz, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.75 (s, 1H), 7.69 (d, *J* = 6.6 Hz, 2H), 7.57-7.48 (m, 3H), 5.81 (s, 2H), 2.69 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.24, 158.16, 156.01, 154.43, 147.46, 144.33, 143.46, 138.87, 132.72, 131.13, 130.63, 129.10 (2C), 128.69, 128.26 (2C), 125.93, 121.77, 118.18, 97.42, 93.70, 41.61, 24.75 ppm. LC-MS (ESI) *m/z* 560.10 $[M + H]^+$. HRMS (FAB) calculated for $C_{24}H_{18}IN_9$ $[M + H]^+$: 560.0730, found: 560.0802.

3-(4-chlorophenyl)-1-((1-(6-iodo-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1h)



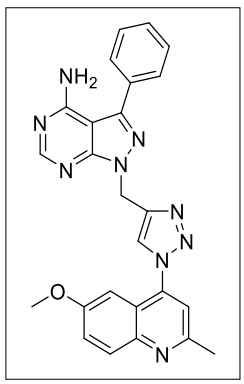
This compound was synthesized using a similar procedure, yield = 44%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.33 (s, 1H), 8.17 (d, *J* = 1.5 Hz, 1H), 8.10 (dd, *J* = 8.7 Hz, 1.8 Hz, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.75 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 5.81 (s, 2H), 2.69 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.24, 158.17, 156.07, 154.50, 147.44, 143.43, 143.27, 138.86 (2C), 133.42, 131.50, 131.11, 130.64, 130.09 (2C), 129.09 (2C), 125.94, 121.75, 118.21, 97.38, 93.83, 41.67, 24.80 ppm. LC-MS (ESI) *m/z* 594.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{24}H_{17}ClIN_9$ $[M + H]^+$: 594.0340, found: 594.0417.

1-((1-(6-methoxy-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1i)



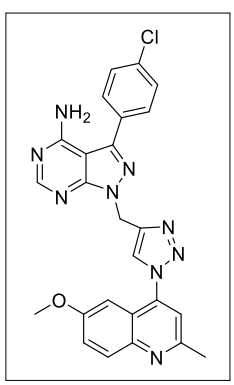
This compound was synthesized using a similar procedure, yield = 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.30 (s, 1H), 7.98 (d, *J* = 9.3 Hz, 1H), 7.65 (s, 1H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.47 (dd, *J* = 9.3 Hz, 2.7 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.05 (d, *J* = 2.7 Hz, 1H), 5.79 (s, 2H), 3.69 (s, 3H), 2.67 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.15, 157.77, 156.43, 155.94, 154.37, 144.72, 144.32, 143.39, 139.22, 138.14, 130.43, 129.91, 129.65 (2C), 128.13 (2C), 125.77, 122.60, 121.04, 117.79, 100.61, 97.40, 55.23, 41.597, 24.30, 20.85 ppm. LC-MS (ESI) *m/z* 478.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{26}H_{23}N_9O$ $[M + H]^+$: 478.2026, found: 478.2097.

1-((1-(6-methoxy-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1j)



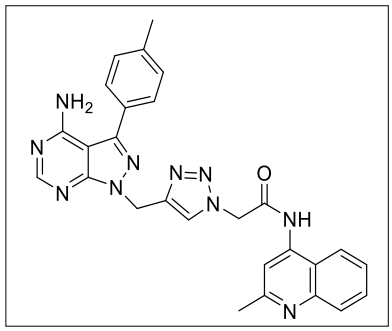
This compound was synthesized using a similar procedure, yield = 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.31 (s, 1H), 7.98 (d, J = 9.15 Hz, 1H), 7.67 (d, J = 6.78 Hz, 2H), 7.65 (s, 1H), 7.56-7.45 (m, 4H), 7.04 (d, J = 2.76 Hz, 1H), 5.81 (s, 2H), 3.68 (s, 3H), 2.66 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.16, 157.76, 156.45, 155.99, 154.42, 144.72, 144.32, 143.38, 139.22, 132.74, 130.45, 129.12 (2C), 128.72, 128.25 (2C), 125.86, 122.68, 121.04, 117.86, 100.57, 97.41, 55.25, 41.67, 24.37 ppm. LC-MS (ESI) *m/z* 464.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{25}H_{21}N_9O$ $[M + H]^+$: 464.1869, found: 464.1946.

3-(4-chlorophenyl)-1-((1-(6-methoxy-2-methylquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1k)



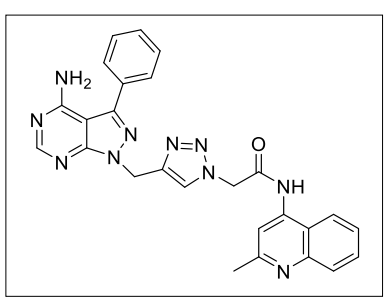
This compound was synthesized using a similar procedure, yield = 62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.31 (s, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.65 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.48 (dd, J = 9.3 Hz, 2.7 Hz, 1H), 7.05 (d, J = 2.7 Hz, 1H), 5.80 (s, 2H), 3.69 (s, 3H), 2.67 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.17, 157.77, 156.43, 156.01, 154.51, 144.72, 143.30, 143.23, 139.21, 133.40, 131.53, 130.43, 130.04 (2C), 129.05 (2C), 128.79, 125.78, 122.60, 121.03, 117.79, 100.63, 55.23, 41.69, 24.31 ppm. LC-MS (ESI) *m/z* 498.10 $[M + H]^+$. HRMS (FAB) calculated for $C_{25}H_{20}ClN_9O$ $[M + H]^+$: 498.1479, found: 498.1551.

2-(4-((4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-methylquinolin-4-yl)acetamide (2a)



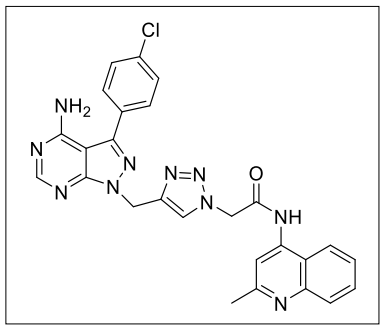
This compound was synthesized using a similar procedure, yield = 54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (brs, 1H), 8.31 (d, J = 11.4 Hz, 2H), 8.13 (s, 1H), 7.91 (d, J = 11.4 Hz, 2H), 7.73 (t, J = 7.65 Hz, 1H), 7.61-7.54 (m, 3H), 7.34 (d, J = 8.4 Hz, 2H), 5.66 (s, 2H), 5.55 (s, 2H), 2.57 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.86, 158.98, 158.13, 155.91, 154.18, 144.14, 142.53, 138.12, 129.92, 129.67 (3C), 128.53, 128.12 (3C), 125.37, 125.20, 121.99, 119.04, 112.36, 97.30, 52.34, 41.71, 24.98, 20.85 ppm. LC-MS (ESI) *m/z* 505.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{27}H_{24}N_{10}O$ $[M + H]^+$: 505.2135, found: 505.2218.

2-(4-((4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-methylquinolin-4-yl)acetamide (2b)



This compound was synthesized using a similar procedure, yield = 44%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (brs, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.31(s, 1H), 8.13 (s, 1H), 8.00 (s, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.79 (t, J = 7.2 Hz, 1H), 7.69-7.61 (m, 3H), 7.56-7.48 (m, 3H), 5.67 (s, 2H), 5.57 (s, 2H), 2.61 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.78, 158.98, 149.65, 148.15, 144.74, 140.74, 132.77, 129.51, 129.08 (3C), 128.67 (2C), 128.19 (3C), 125.14, 121.94, 52.41, 41.72, 25.07 ppm. LC-MS (ESI) *m/z* 491.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{26}H_{22}N_{10}O$ $[M + H]^+$: 491.1978, found: 491.2053.

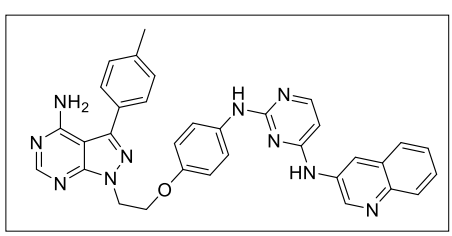
2-(4-((4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-methylquinolin-4-yl)acetamide (2c)



This compound was synthesized using a similar procedure, yield = 43%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.61 (brs, 1H), 8.31 (d, J = 7.8 Hz, 2H), 8.14 (s, 1H), 7.90 (d, J = 9.0 Hz, 2H), 7.23 (t, J = 7.5 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.1 Hz, 3H), 5.66 (s, 2H), 5.54 (s, 2H), 2.57 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.84, 159.02, 158.14, 154.30, 148.19, 143.03, 142.41, 140.73, 133.37, 131.52, 130.02 (2C), 129.53, 129.08 (2C), 128.74, 125.41, 125.16, 121.99, 119.05, 112.36, 97.27, 52.35, 41.80, 25.15 ppm. LC-MS (ESI) *m/z* 525.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{26}H_{21}ClN_{10}O$ $[M + H]^+$: 525.1588, found: 525.1673.

2.2. General procedure for the *N*-alkylation between 12a-e and 25

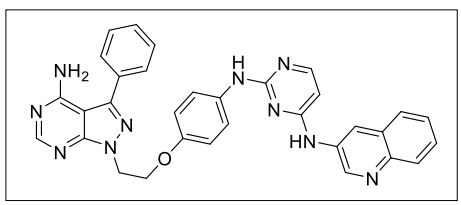
N^2-(4-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (3a)



To a solution of **12a** (10 mg, 0.044 mmol) and **25** (19.4 mg, 0.044 mmol) in anhydrous DMF (2 mL) under a N_2 atmosphere was added cesium carbonate (57.9 mg, 0.178 mmol) and the mixture was stirred at 80 °C for 3h. The resulting mixture was partitioned between water and ethyl acetate (3 X 100 mL). The organic layer was dried with sodium sulfate and evaporated *in vacuo*. The crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a white solid (12.4 mg, 0.021mmol, 48.1% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.08 (s, 1H), 8.90 (brs, 1H), 8.87 (s, 1H), 8.27 (s,

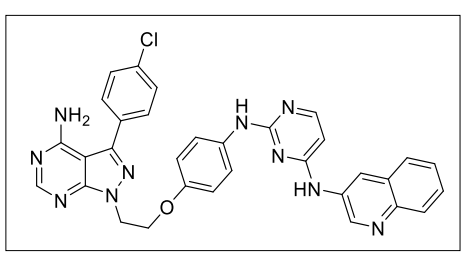
1H), 8.05 (d, *J* = 5.7 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.68 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.47-7.44 (m, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 6.83 (d, *J* = 9.3 Hz, 2H), 6.27 (d, *J* = 5.4 Hz, 1H), 4.72 (t, *J* = 5.1 Hz, 2H), 4.46 (t, *J* = 5.1 Hz, 2H), 2.37 (s, 3H) ppm. LC-MS (ESI) *m/z* 581.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{33}H_{28}N_{10}O$ $[M + H]^+$: 581.2448, found: 581.2522.

N^2-(4-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (3b)



This compound was synthesized using a similar procedure, yield 46.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.08 (s, 1H), 8.91 (brs, 1H), 8.87 (s, 1H), 8.29 (s, 1H), 8.05 (d, *J* = 5.4 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.69-7.66 (m, 3H), 7.56-7.41 (m, 7H), 6.84 (d, *J* = 9 Hz, 2H), 6.27 (d, *J* = 5.4 Hz, 1H), 4.74 (t, *J* = 5.1 Hz, 2H), 4.47 (t, *J* = 4.95 Hz, 2H) ppm. LC-MS (ESI) *m/z* 567.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{32}H_{26}N_{10}O$ $[M + H]^+$: 567.2291, found: 567.2365.

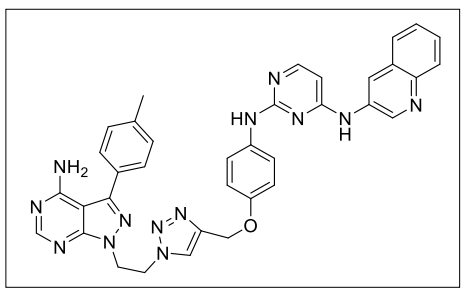
N^2-(4-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (3c)



This compound was synthesized using a similar procedure, yield 35.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.08 (s, 1H), 8.90 (brs, 1H), 8.88 (s, 1H), 8.28 (s, 1H), 8.05 (d, *J* = 5.4 Hz, 1H), 7.89 (d, *J* = 8.7 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 3H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.53-7.44 (m, 4H), 6.83 (d, *J* = 9 Hz, 2H), 6.27 (d, *J* = 5.4 Hz, 1H), 4.73 (t, *J* = 5.1 Hz, 2H), 4.47 (t, *J* = 4.8 Hz, 2H) ppm. LC-MS (ESI) *m/z* 601.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{32}H_{25}ClN_{10}O$ $[M + H]^+$: 601.1901, found: 601.1974.

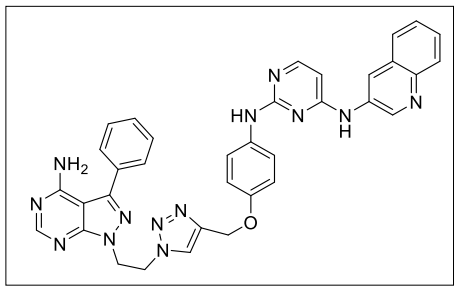
2.3. General procedure for the copper(I)-catalyzed alkyne-azide cycloaddition between alkyne 24a-e and azide 29a-d

N^2-(4-((1-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4a)



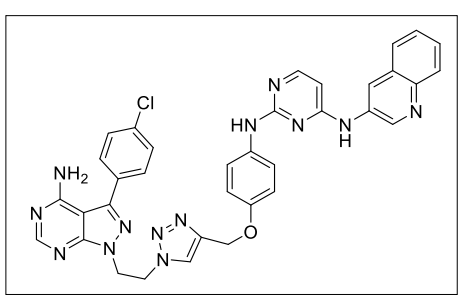
To a solution of **29a** (15 mg, 0.051 mmol) and **24a** (18.7 mg, 0.051 mmol) in a mixture of DMF, *t*BuOH and water (2:2:1, 5 mL) were added sodium ascorbate (2.0 mg, 0.010 mmol) and copper(II) sulfate pentahydrate (1.2 mg, 0.005 mmol). The mixture was stirred at 70 °C for 3 h. The resulting mixture was concentrated *in vacuo* and then extracted with DCM (3 X 100 mL). The organic phase was dried with anhydrous sodium sulfate, the solvent evaporated, and the crude product was purified using silica gel chromatography with DCM/methanol gradient to give a white solid (18.5 mg, 0.028 mmol, 54.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.09 (s, 1H), 8.93 (brs, 2H), 8.19 (s, 1H), 8.12 (s, 1H), 8.08 (d, *J* = 5.68 Hz, 1H), 7.92 (d, *J* = 7.56 Hz, 1H), 7.76 (d, *J* = 6.28 Hz, 1H), 7.59–7.54 (m, 4H), 7.50 (d, *J* = 7.96 Hz, 2H), 7.32 (d, *J* = 7.88 Hz, 2H), 6.94 (d, *J* = 8.96 Hz, 2H), 6.30 (d, *J* = 5.76 Hz, 1H), 5.05 (s, 2H), 4.91 (t, *J* = 5.48 Hz, 2H), 4.82 (t, *J* = 5.46 Hz, 2H), 2.35 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 159.85, 158.04, 156.43, 155.73, 154.58, 153.15, 145.06, 144.16, 143.25, 142.82, 138.06, 134.01, 133.98, 129.84, 129.61 (2C), 128.47, 128.15, 128.08 (2C), 127.25, 126.88, 126.76, 124.69, 121.69, 121.08, 114.59 (2C), 98.69, 97.29, 61.32, 48.52, 46.26, 20.82 ppm. LC-MS (ESI) *m/z* 662.30 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{31}N_{13}O$ $[M + H]^+$: 662.2775, found: 662.2849

N^2-(4-((1-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4b)



This compound was synthesized using similar procedure, yield = 66.6%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.11 (s, 1H), 8.92 (brs, 2H), 8.21 (s, 1H), 8.14 (s, 1H), 8.08 (d, *J* = 5.94 Hz, 1H), 7.92 (d, *J* = 8.28 Hz, 1H), 7.76 (s, 1H), 7.62 (d, *J* = 7.32 Hz, 2H), 7.59-7.56 (m, 3H), 7.54-6.51 (m, 3H), 7.46 (t, *J* = 7.35 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.30 (d, *J* = 5.94 Hz, 1H), 5.05 (s, 2H), 4.92 (t, *J* = 5.73 Hz, 2H), 4.84 (t, *J* = 5.73 Hz, 2H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.39, 159.86, 158.05, 156.45, 155.77, 154.64, 153.17, 145.06, 144.15, 143.25, 142.82, 134.00, 132.68, 129.05 (3C), 128.65, 128.49, 128.20 (3C), 128.16, 127.26, 126.91, 126.78, 124.74, 121.72, 121.09, 114.60 (2C), 98.72, 97.31, 61.32, 48.54, 46.32 ppm. LC-MS (ESI) *m/z* 648.30 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{29}N_{13}O$ $[M + H]^+$: 648.2618, found: 648.2701.

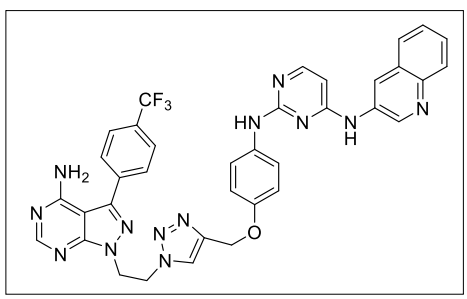
N^2-(4-((1-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4c)



This compound was synthesized using similar procedure, yield = 49.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.11 (s, 1H), 8.93 (brs, 1H), 8.91 (s, 1H), 8.19 (s, 1H), 8.13 (s, 1H), 8.07 (d, *J* = 5.7 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.74 (s, 1H), 7.61-7.53 (m, 8H), 6.93 (d, *J* = 9.3 Hz, 2H), 6.29 (d, *J* = 5.7 Hz, 1H), 5.04 (s, 2H), 4.90 (t, *J* = 5.1 Hz, 2H), 4.82 (t, *J* = 5.1 Hz, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.90, 160.43, 159.85, 158.09, 156.40,

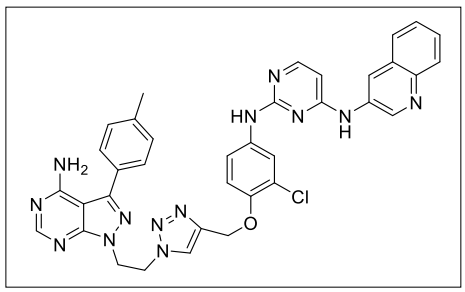
155.84, 154.75, 153.19, 145.08, 143.27, 143.09, 142.85, 134.04, 133.98, 133.40, 131.48, 129.99 (2C), 129.06 (2C), 128.51, 128.19, 127.30, 126.95, 126.82, 124.79, 121.74, 121.13, 114.64 (2C), 98.77, 97.30, 61.31, 48.56, 46.39 ppm. LC-MS (ESI) *m/z* 682.22 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}ClN_{13}O$ $[M + H]^+$: 682.2228, found: 682.2305.

N^2-(4-((1-(2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4d)



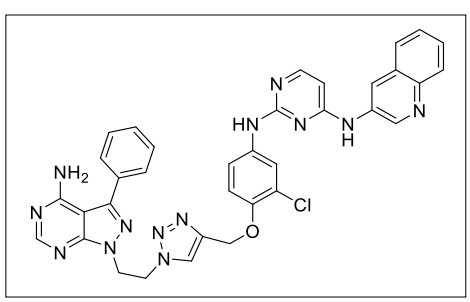
This compound was synthesized using similar procedure, yield = 73.3%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.11 (s, 1H), 8.94 (brs, 1H), 8.92 (s, 1H), 8.22 (s, 1H), 8.15 (s, 1H), 8.08 (d, *J* = 5.52 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.85 (d, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 8.28 Hz, 2H), 7.76 (s, 1H), 7.59-7.53 (m, 4H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.30 (d, *J* = 6 Hz, 1H), 5.05 (s, 2H), 4.93 (t, *J* = 5.73 Hz, 2H), 4.86 (t, *J* = 5.73 Hz, 2H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.42, 159.87, 158.09, 156.46, 155.89, 154.88, 153.17, 145.07, 143.26, 142.85 (d, $J_{C\text{-}F}$ = 3.59 Hz), 136.58, 134.03 (d, $J_{C\text{-}F}$ = 5.03 Hz), 128.96 (3C), 128.78, 128.57, 128.49, 128.18, 127.28, 126.91, 126.80, 125.88 (d, $J_{C\text{-}F}$ = 3.6 Hz), 125.16, 124.79, 123.36, 121.71, 121.09, 114.61 (3C), 98.76, 97.38, 61.31, 48.56, 46.48 ppm. LC-MS (ESI) *m/z* 715.98 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{28}F_3N_{13}O$ $[M + H]^+$: 716.2492, found: 716.2572.

N^2-(4-((1-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4e)



This compound was synthesized using similar procedure, yield = 49.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.27 (s, 1H), 8.94 (s, 1H), 8.92 (brs, 1H), 8.17 (s, 1H), 8.13 (s, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.88 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.60-7.53 (m, 3H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 8.7 Hz, 1H), 6.34 (d, *J* = 6 Hz, 1H), 5.11 (s, 2H), 4.93 (t, *J* = 6 Hz, 2H), 4.80 (t, 2H), 2.34 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.46, 159.38, 158.09, 156.38, 155.79, 154.63, 148.05, 145.20, 144.23, 143.39, 142.48, 138.12, 135.07, 133.98, 129.88, 129.69 (2C), 128.53, 128.18, 128.14 (2C), 127.48, 127.10, 126.87, 124.94, 121.31, 121.24, 120.91, 119.65, 119.11, 99.45, 97.30, 62.43, 48.61, 46.32, 20.92 ppm. LC-MS (ESI) *m/z* 696.20 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}ClN_{13}O$ $[M + H]^+$: 696.2385, found: 696.2468.

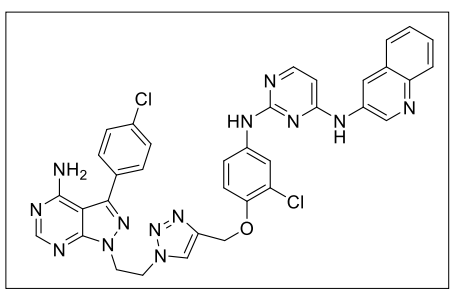
N^2-(4-((1-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4f)



This compound was synthesized using similar procedure, yield = 71.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.27 (s, 1H), 8.96 (s, 1H), 8.92 (brs, 1H), 8.19 (s, 1H), 8.15 (s, 1H), 8.12 (d, *J* = 5.94 Hz, 1H, 7.93 (d, *J* = 8.28 Hz, 1H), 7.89 (brs, 1H), 7.82 (d, *J* = 6.9 Hz, 1H), 7.62 (d, *J* = 7.59 Hz, 2H), 7.60-7.56 (m, 3H), 7.54-7.51 (m, 2H), 7.47 (t, *J* = 7.32 Hz, 1H), 7.21

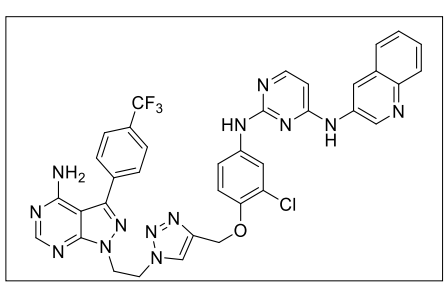
(d, *J* = 8.7 Hz, 1H), 6.35 (d, *J* = 5.46 Hz, 1H), 5.12 (s, 2H), 4.94 (t, *J* = 5.73 Hz, 2H), 4.84 (t, *J* = 5.73 Hz, 2H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.43, 159.36, 158.04, 156.34, 155.77, 154.66, 148.05, 145.19, 144.17, 143.38, 142.45, 135.06, 133.92, 132.68, 129.04 (2C), 128.66, 128.48, 128.19 (2C), 128.13, 127.42, 127.03, 126.79, 124.86, 121.34, 121.27, 120.92, 119.11, 114.84, 99.36, 97.31, 62.48, 48.54, 46.31 ppm. LC-MS (ESI) *m/z* 681.97 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}ClN_{13}O$ $[M + H]^+$: 682.2228, found: 682.2310.

N^2-(4-((1-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4g)



This compound was synthesized using similar procedure, yield = 39.2%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.26 (s, 1H), 8.94 (s, 1H), 8.91 (brs, 1H), 8.18 (s, 1H), 8.14 (s, 1H), 8.11 (d, *J* = 5.1 Hz, 1H), 7.93-7.88 (m, 2H), 7.79 (s, 1H), 7.60-7.55 (m, 7H), 7.20 (d, *J* = 8.4 Hz, 1H), 6.34 (d, *J* = 5.1 Hz, 1H), 5.11 (s, 2H), 4.92 (t, 2H), 4.81 (t, 2H) ppm. LC-MS (ESI) *m/z* 715.90 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{27}Cl_2N_{13}O$ $[M + H]^+$: 716.1839, found: 716.1920.

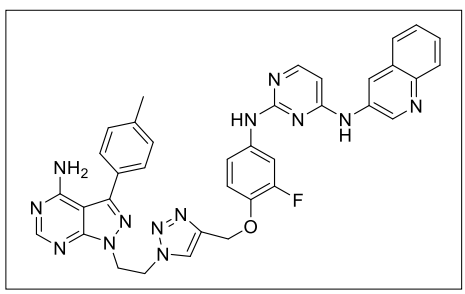
N^2-(4-((1-(2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4h)



This compound was synthesized using similar procedure, yield = 68.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.26 (s, 1H), 8.94 (s, 1H), 8.91 (brs, 1H), 8.20 (s, 1H), 8.16 (s, 1H), 8.11 (d, *J* = 5.4 Hz, 1H), 7.92

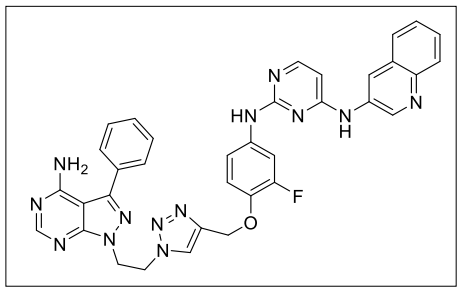
(d, *J* = 7.8 Hz, 1H), 7.88 (s, 1H), 7.85-7.78 (m, 5H), 7.58-7.54 (m, 3H), 7.21 (d, *J* = 9 Hz, 1H), 6.34 (d, *J* = 6 Hz, 1H), 5.12 (s, 2H), 4.93 (t, *J* = 6 Hz, 2H), 4.84 (t, 2H) ppm. LC-MS (ESI) *m/z* 749.90 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{27}ClF_3N_{13}O$ $[M + H]^+$: 750.2102, found: 750.2174.

N^2-(4-((1-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4i)



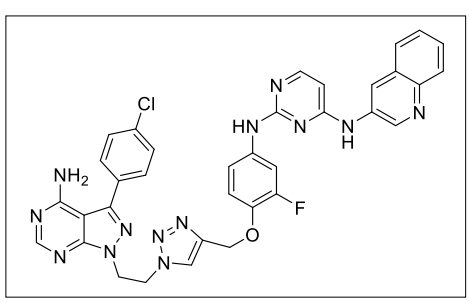
This compound was synthesized using similar procedure, yield = 72.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.32 (s, 1H), 8.95 (s, 1H), 8.93 (brs, 1H), 8.18 (s, 1H), 8.17 (s, 1H), 8.12 (d, *J* = 5.72 Hz, 1H), 7.94 (d, *J* = 7.96 Hz, 1H), 7.81 (t, *J* = 6.5 Hz, 1H), 7.76 (s, 1H), 7.61-7.54 (m, 2H), 7.49 (d, *J* = 7.92 Hz, 2H), 7.35 (s, 1H), 7.31 (d, *J* = 8.04 Hz, 2H), 7.18 (t, *J* = 9.3 Hz, 1H), 6.35 (d, *J* = 5.72 Hz, 1H), 5.11 (s, 2H), 4.92 (t, *J* = 5.8 Hz, 2H), 4.82 (t, *J* = 5.6 Hz, 2H), 2.35 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.42, 159.35, 158.07, 156.38, 155.75, 154.59, 152.66, 150.26, 145.20, 144.18, 143.38, 142.45, 140.04, 138.07, 135.05, 134.96, 133.90, 129.85, 129.64 (2C), 128.51, 128.13, 128.10 (2C), 127.33, 127.07, 126.86, 124.98, 121.33, 116.04, 115.09, 97.29, 62.53, 48.58, 46.29, 20.87 ppm. LC-MS (ESI) *m/z* 680.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}FN_{13}O$ $[M + H]^+$: 680.2680, found: 680.2755.

N^2-(4-((1-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4j)



This compound was synthesized using similar procedure, yield = 74.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.31 (s, 1H), 8.96 (d, *J* = 2.32 Hz, 1H), 8.92 (s, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 8.12 (d, *J* = 5.68 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.81 (t, *J* = 7.64 Hz, 1H), 7.76 (s, 1H), 7.63-7.57 (m, 3H), 7.56-7.44 (m, 4H), 7.35 (d, *J* = 8.64 Hz, 1H), 7.19 (t, *J* = 9.3 Hz, 1H), 6.35 (d, *J* = 5.8 Hz, 1H), 5.11 (s, 2H), 4.93 (t, *J* = 5.54 Hz, 2H), 4.83 (t, *J* = 5.48 Hz, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.42, 159.35, 158.56, 156.37, 155.77, 154.64, 152.66, 150.26, 145.21, 144.16, 143.39, 142.44, 140.16 (d), 134.97 (d), 133.89, 132.68, 129.05 (2C), 128.67, 128.51, 128.20 (2C), 128.13, 127.32, 127.07, 128.86, 124.99, 121.35, 116.08 (d, $J_{C\text{-}F}$ = 2.3 Hz), 115.09, 99.33, 97.31, 62.56, 48.57, 46.33 ppm. LC-MS (ESI) *m/z* 666.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}FN_{13}O$ $[M + H]^+$: 666.2524, found: 666.2603.

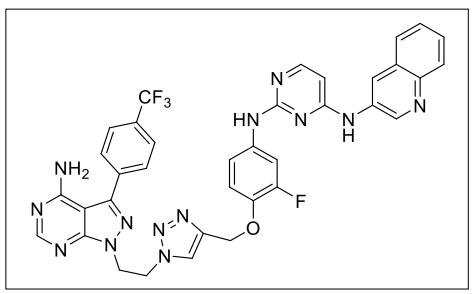
N^2-(4-((1-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4k)



This compound was synthesized using similar procedure, yield = 78.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.31 (s, 1H), 8.94 (s, 1H), 8.92 (brs, 1H), 8.18 (s, 1H), 8.16 (s, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.79-7.74 (m, 2H), 7.60-7.52 (m, 6H), 7.33 (d, *J* = 9.3 Hz, 1H), 7.18 (t, *J* = 9.3 Hz, 1H), 6.34 (d, *J* = 6 Hz, 1H), 5.10 (s, 2H), 4.90 (t, *J* = 6

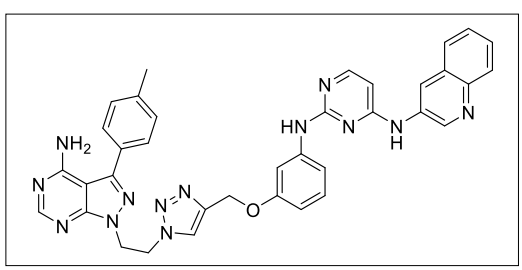
Hz, 2H), 4.81 (t, *J* = 5.7 Hz, 2H) ppm. LC-MS (ESI) *m/z* 699.91 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{27}ClFN_{13}O$ $[M + H]^+$: 700.2134, found: 700.2217.

N^2-(4-((1-(2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (4l)



This compound was synthesized using similar procedure, yield = 76.0%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.30 (s, 1H), 8.94 (s, 1H), 8.91 (brs, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 8.10 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.85-7.74 (m, 6H), 7.60-7.55 (m, 2H), 7.34 (d, *J* = 9.3 Hz, 1H), 7.18 (t, 9.45 Hz, 1H), 6.34 (d, *J* = 6 Hz, 1H), 5.10 (s, 2H), 4.92 (t, *J* = 6 Hz, 2H), 4.84 (t, *J* = 5.7 Hz, 2H) ppm. LC-MS (ESI) *m/z* 733.91 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{27}F_4N_{13}O$ $[M + H]^+$: 734.2398, found: 734.2463.

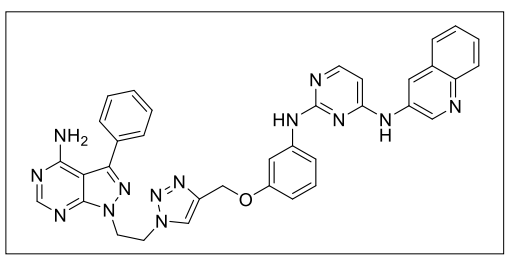
N^2-(3-((1-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5a)



This compound was synthesized using similar procedure, yield = 48.1%. ^{1}H NMR (800 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.29 (s, 1H), 8.99 (brs, 1H), 8.96 (d, *J* = 2.24 Hz, 1H), 8.19 (s, 1H), 8.13 (d, *J* = 5.6 Hz, 1H), 8.02 (s, 1H), 7.92 (d, *J* = 8.16 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 7.48 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 7.92 Hz, 2H), 7.47 (s, 1H), 7.34 (d, *J* = 8.12 Hz, 1H), 7.32 (d, *J* = 7.84 Hz, 2H), 7.16 (t, *J* = 8.12 Hz, 1H), 6.64 (d, *J* = 8.04 Hz, 1H), 6.36 (d, *J* = 5.68 Hz, 1H), 5.01 (s, 2H), 4.89 (t, *J* = 5.84

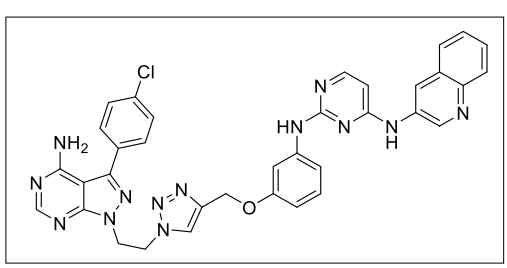
Hz, 2H), 4.80 (t, *J* = 5.80 Hz, 2H), 2.34 (s, 3H) ppm. ^{13}C NMR (200 MHz, DMSO-d_6) δ 160.40, 159.43, 158.34, 158.06, 156.32, 155.74, 154.56, 145.11, 144.16, 143.31, 142.66, 141.85, 138.06, 133.95, 129.84, 129.64 (2C), 129.14, 128.47, 128.17, 128.08 (2C), 127.36, 126.97, 126.82, 124.68, 121.16, 112.15, 107.09, 106.09, 99.49, 97.31, 60.86, 48.52, 46.26, 20.84 ppm. LC-MS (ESI) *m/z* 662.30 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{31}N_{13}O$ $[M + H]^+$: 662.2775, found: 662.2855.

N^2-(3-((1-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5b)



This compound was synthesized using similar procedure, yield = 61.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.30 (s, 1H), 8.99 (s, 1H), 8.96 (d, *J* = 2.16 Hz, 1H), 8.20 (s, 1H), 8.12 (d, *J* = 5.64 Hz, 1H), 8.04 (s, 1H), 7.92 (d, *J* = 7.92 Hz, 1H), 7.84 (d, *J* = 7.44 Hz, 1H), 7.61-7.44 (m, 8H), 7.34 (d, *J* = 8.04 Hz, 1H), 7.16 (t, *J* = 8.1 Hz, 1H), 6.63 (dd, *J* = 8.08 Hz, 1.64 Hz, 1H), 6.36 (d, *J* = 5.68 Hz, 1H), 5.00 (s, 2H) 4.89 (t, *J* = 5.68 Hz, 2H), 4.82 (t, *J* = 5.52 Hz, 2H) ppm. LC-MS (ESI) *m/z* 648.30 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{29}N_{13}O$ $[M + H]^+$: 648.2618, found: 648.2689.

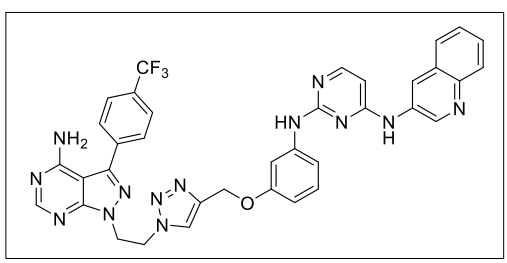
N^2-(3-((1-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5c)



This compound was synthesized using similar procedure, yield = 54.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.31 (s, 1H), 9.00 (s, 1H), 8.96 (d, *J* = 2.24 Hz, 1H), 8.20 (s, 1H), 8.13 (d,

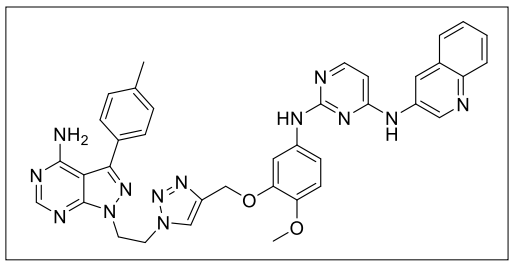
J = 5.64 Hz, 1H), 8.03 (s, 1H), 7.92 (d, *J* = 7.88 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.61-7.51 (m, 7H), 7.35 (d, *J* = 8.04 Hz, 1H), 7.17 (t, *J* = 8.12 Hz, 1H), 6.63 (dd, *J* = 8.06 Hz, 1.78 Hz, 1H), 6.37 (d, *J* = 5.68 Hz, 1H), 5.01 (s, 2H), 4.89 (t, *J* = 5.18 Hz, 2H), 4.81 (t, *J* = 5.56 Hz, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.42, 159.43, 158.36, 158.10, 156.33, 155.84, 154.72, 145.13, 143.32, 143.07, 142.69, 141.89, 133.98, 133.39, 131.46, 129.98 (2C), 129.17, 129.06 (2C), 128.49, 128.20, 127.39, 126.99, 126.84, 124.74, 121.18, 112.15, 107.03, 106.06, 99.52, 97.30, 60.87, 48.53, 46.38 ppm. LC-MS (ESI) *m/z* 681.95 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}ClN_{13}O$ $[M + H]^+$: 682.2228, found: 682.2306.

N^2-(3-((1-(2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5d)



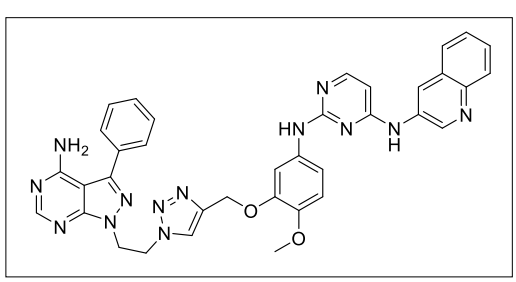
This compound was synthesized using similar procedure, yield = 57.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.29 (s, 1H), 8.98 (s, 1H), 8.95 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 8.04 (s, 1H), 7.91 (d, *J* = 9 Hz, 1H), 7.85-7.77 (m, 5H), 7.59-7.53 (m, 2H), 7.49 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.15 (t, *J* = 7.95 Hz, 1H), 6.62 (d, *J* = 10.2 Hz, 1H), 6.35 (d, *J* = 5.7 Hz, 1H), 4.99 (s, 2H), 4.88 (t, 2H), 4.82 (t, *J* = 4.5 Hz, 2H) ppm. LC-MS (ESI) *m/z* 715.94 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{28}F_3N_{13}O$ $[M + H]^+$: 716.2492, found: 716.2573.

N^2-(3-((1-(2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5e)



This compound was synthesized using similar procedure, yield = 79.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.09 (s, 1H), 8.96 (s, 1H), 8.94 (s, 1H), 8.19 (s, 1H), 8.09 (d, *J* = 5.64 Hz, 1H), 8.03 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 6.2 Hz, 1H), 7.59-7.52 (m, 2H), 7.49 (d, *J* = 7.96 Hz, 2H), 7.45 (s, 1H), 7.33-7.28 (m, 3H), 6.90 (d, *J* = 8.8 Hz, 1H), 6.30 (d, *J* = 5.68 Hz, 1H), 4.96 (s, 2H), 4.89 (t, *J* = 5.5 Hz, 2H), 4.80 (t, *J* = 5.44 Hz, 2H), 3.72 (s, 3H), 2.36 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 159.77, 158.08, 156.45, 155.78, 154.58, 147.26, 145.06, 144.27, 144.19, 143.23, 142.53, 138.08, 134.08, 129.85, 129.65 (3C), 128.50, 128.18, 128.10 (3C), 127.29, 126.91, 126.78, 124.87, 121.02, 112.59, 107.47, 98.87, 97.31, 61.63, 55.87, 48.50, 46.26, 20.88 ppm. LC-MS (ESI) *m/z* 692.30 $[M + H]^+$. HRMS (FAB) calculated for $C_{37}H_{33}N_{13}O_2$ $[M + H]^+$: 692.2880, found: 692.2854.

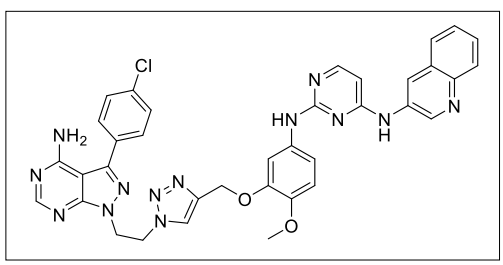
N^2-(3-((1-(2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5f)



This compound was synthesized using similar procedure, yield = 72.4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.07 (s, 1H), 8.94 (brs, 2H), 8.21 (s, 1H), 8.10 (s, 1H), 8.04 (s, 1H), 7.91 (d, *J* = 8.28 Hz, 1H), 7.77 (brs, 1H), 7.62 (d, *J* = 8.01 Hz, 2H), 7.57 (t, *J* = 6.87 Hz, 1H), 7.54-7.51 (m, 3H), 7.47 (t, *J* = 7.35 Hz, 1H), 7.44 (s, 1H), 7.29 (d, *J* = 8.76 Hz,

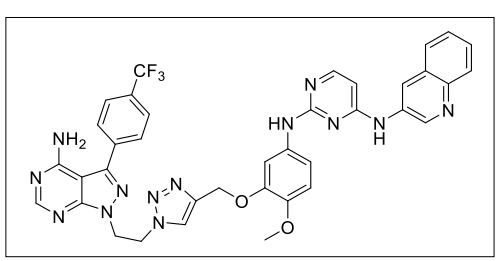
1H), 6.90 (t, *J* = 8.7 Hz, 1H), 6.31 (d, *J* = 5.52 Hz, 1H), 4.96 (s, 2H), 4.90 (t, *J* = 5.73 Hz, 2H), 4.81 (t, *J* = 5.73 Hz, 2H), 3.72 (s, 3H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.37, 159.78, 158.06, 156.40, 155.78, 154.63, 147.28, 145.05, 144.28, 144.16, 143.24, 142.55, 134.08, 132.67, 129.04 (3C), 128.65, 128.49, 128.19 (3C), 127.27, 126.89, 126.76, 124.83, 121.04, 112.91, 112.64, 107.54, 98.87, 97.35, 61.66, 55.89, 48.46, 46.28 ppm. LC-MS (ESI) *m/z* 678.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{31}N_{13}O_2$ $[M + H]^+$: 678.2724, found: 678.2792.

N^2-(3-((1-(2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5g)



This compound was synthesized using similar procedure, yield = 46.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.07 (s, 1H), 8.94 (brs, 1H), 8.93 (s, 1H), 8.19 (s, 1H), 8.07 (d, *J* = 6 Hz, 1H), 8.03 (s, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.75 (s, 1H), 7.61-7.52 (m, 6H), 7.44 (s, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 9 Hz, 1H), 6.29 (d, *J* = 5.4 Hz, 1H) 4.94 (s, 2H), 4.86 (t, 2H), 4.79 (t, 2H), 3.71 (s, 3H) ppm. LC-MS (ESI) *m/z* 711.92 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}ClN_{13}O_2$ $[M + H]^+$: 712.2334, found: 712.2403.

N^2-(3-((1-(2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (5h)

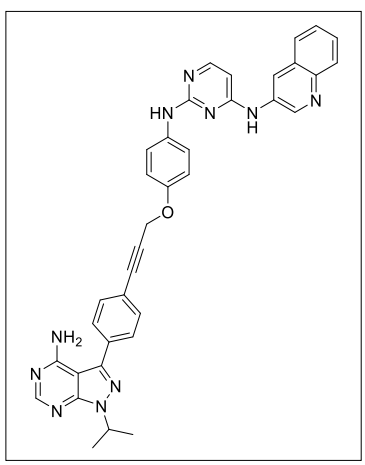


This compound was synthesized using similar procedure, yield = 80.0 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.07 (s, 1H), 8.95 (brs, 1H), 8.93 (s,

1H), 8.21 (s, 1H), 8.07 (d, *J* = 5.7 Hz, 1H), 8.05 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.75 (s, 1H), 7.58-7.49 (m, 2H), 7.44 (s, 1H), 7.29 (d, *J* = 9 Hz, 1H), 6.89 (d, *J* = 9 Hz, 1H), 6.29 (d, *J* = 6 Hz, 1H), 4.95 (s, 2H), 4.89 (t, *J* = 5.1 Hz, 2H), 4.82 (t, *J* = 5.1 Hz, 2H), 3.70 (s, 3H) ppm. LC-MS (ESI) *m/z* 745.93 $[M + H]^+$. HRMS (FAB) calculated for $C_{37}H_{30}F_3N_{13}O_2$ $[M + H]^+$: 746.2598, found: 746.2668.

2.4. General procedure for Sonogashira cross-coupling reaction between 30a-b and alkyne 24a-e

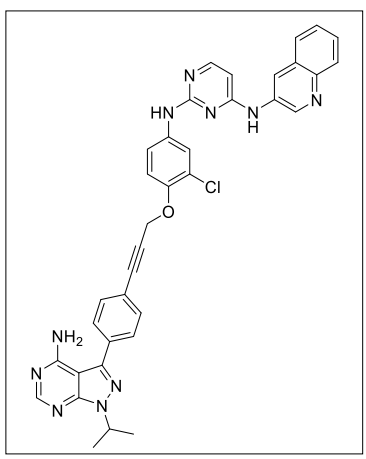
N^2-(4-((3-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6a)



Solid components including **30a** (20 mg, 0.053 mmol), $Pd(PPh_3)_2Cl_2$ (1.1 mg, 0.0016 mmol), and CuI (0.3 mg, 0.0016 mmol) were first added to a two necks flask (10 mL) under a N_2 atmosphere. This was followed by addition of anhydrous DMF (1 mL), alkyne **24a** (21.3 mg, 0.058 mmol), TEA (0.074 mL, 0.53 mmol) which was degassed for 15 minutes with N_2. The reaction flask was covered with aluminum foil, and the mixture was stirred for 3h at room temperature. Distilled water was added and the resulted mixture was extracted with ethyl acetate (3 X 100 mL). The organic phases were dried with anhydrous sodium sulfate, the solvent was evaporated, and the crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to give a light yellow solid (19 mg, 0.031 mmol, 58.2% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.17 (s, 1H), 8.94 (brs, 2H), 8.24 (s, 1H), 8.08 (d, *J* = 5.65 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* =

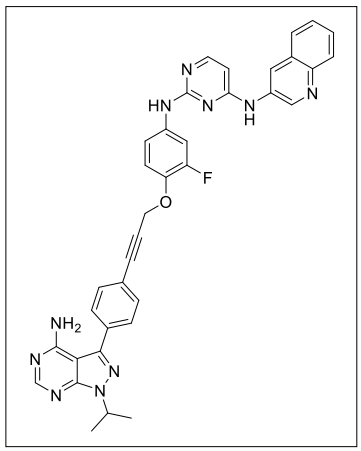
5.45 Hz, 1H), 7.67-7.65 (m, 4H), 7.59-7.55 (m, 4H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.31 (d, *J* = 5.7 Hz, 1H), 5.09-5.04 (m, 3H), 1.48 (d, *J* = 6.7 Hz, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.43, 159.79, 158.07, 156.44, 155.44, 153.46, 152.34, 145.15, 143.31, 142.40, 134.48, 134.01, 133.47, 132.14 (3C), 128.52, 128.44 (3C), 128.18, 127.28, 126.97, 126.82, 121.48, 121.23, 115.04 (2C), 98.82, 97.42, 86.34, 86.06, 56.43, 48.18, 21.73 ppm. LC-MS (ESI) *m/z* 619.01 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}N_{10}O$ $[M + H]^+$: 619.2604, found: 619.2675.

N^2-(4-((3-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6b)



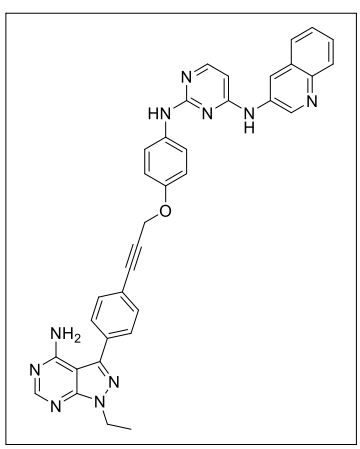
This compound was synthesized using similar procedure, yield = 71%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.33 (s, 1H), 8.97 (d, *J* = 2.28 Hz, 1H), 8.92 (brs, 1H), 8.24 (s, 1H), 8.13 (d, *J* = 5.94 Hz, 1H), 7.96 (s, 1H), 7.94 (d, *J* = 8.28 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 8.22 Hz, 2H), 7.64 (dd, *J* = 8.91 Hz, 2.55 Hz, 1H), 7.61-7.55 (m, 4H), 7.27 (d, *J* = 9.18 Hz, 1H), 6.36 (d, *J* = 5.52 Hz, 1H), 5.15 (s, 2H), 5.06 (sep, 1H), 1.48 (d, *J* = 6.9 Hz, 6H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.45, 159.31, 158.05, 156.31, 155.42, 153.44, 147.23, 145.26, 143.42, 142.37, 135.60, 133.88, 133.56, 132.17 (2C), 128.49, 128.44 (2C), 128.13, 127.40, 127.07, 126.78, 121.70, 121.45, 121.31, 120.84, 118.90, 115.48, 99.43, 97.41, 86.54, 85.76, 57.59, 48.17, 21.70 (2C) ppm. LC-MS (ESI) *m/z* 653.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{29}ClN_{10}O$ $[M + H]^+$: 653.2214, found: 653.2297.

N^2-(4-((3-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6c)



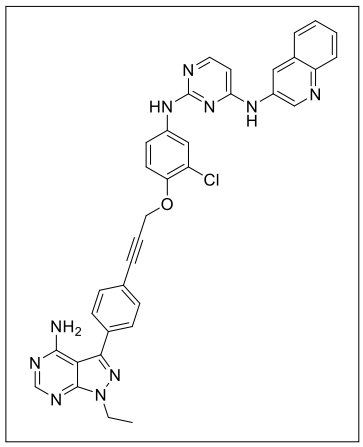
This compound was synthesized using similar procedure, yield = 45.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.36 (s, 1H), 8.95 (d, *J* = 2.1 Hz, 1H), 8.92 (brs, 1H), 8.23 (s, 1H), 8.17 (d, *J* = 5.7 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.85-7.80 (m, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.62-7.56 (m, 4H), 7.40 (d, *J* = 10.2 Hz, 1H), 7.25 (t, *J* = 9.3 Hz, 1H), 6.35 (d, *J* = 6 Hz, 1H), 5.11 (s, 2H), 5.07-5.03 (m, 1H), 1.47 (d, *J* = 6.3 Hz, 6H) ppm. LC-MS (ESI) *m/z* 637.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{29}FN_{10}O$ $[M + H]^+$: 637.2510, found: 637.2578.

N^2-(4-((3-(4-(4-amino-1-ethyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6d)



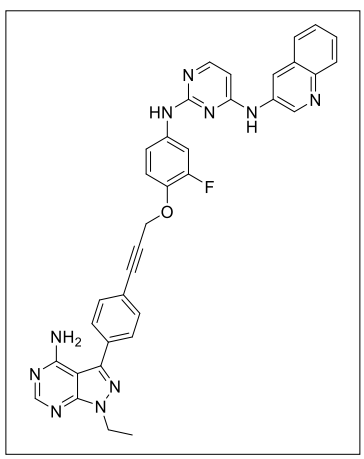
This compound was synthesized using similar procedure, yield = 60.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.15 (s, 1H), 8.92 (s, 2H), 8.24 (s, 1H), 8.07 (d, *J* = 6 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.79 (s, 1H), 7.67-7.62 (m, 4H), 7.58-7.55 (m, 4H), 7.01 (d, *J* = 9 Hz, 2H), 6.29 (d, *J* = 5.4 Hz, 1H), 5.05 (s, 2H), 4.36 (q, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.2 Hz, 3H) ppm. LC-MS (ESI) *m/z* 605.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}N_{10}O$ $[M + H]^+$: 605.2448, found: 605.2526.

N^2-(4-((3-(4-(4-amino-1-ethyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-3-chlorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6e)



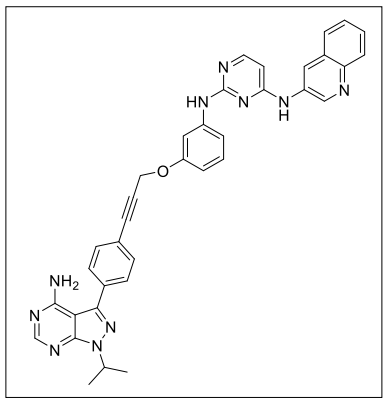
This compound was synthesized using similar procedure, yield = 57.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.32 (s, 1H), 8.95 (s, 1H), 8.91 (s, 1H), 8.24 (s, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 9.3 Hz, 2H), 7.82 (d, 1H), 7.67-7.56 (m, 7H), 7.26 (d, *J* = 8.7 Hz, 1H), 6.34 (d, *J* = 5.7 Hz, 1H), 5.14 (s, 2H), 4.36 (d, *J* = 7.5 Hz, 2H), 1.39 (t, *J* = 7.2 Hz, 3H) ppm. LC-MS (ESI) *m/z* 638.99 [M + H]$^+$. HRMS (FAB) calculated for $C_{35}H_{27}ClN_{10}O$ [M + H]$^+$: 639.2058, found: 639.2137.

N^2-(4-((3-(4-(4-amino-1-ethyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-3-fluorophenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (6f)



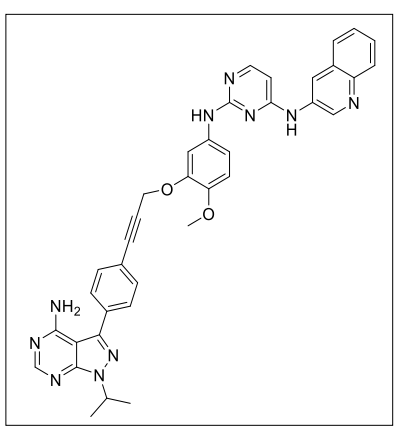
This compound was synthesized using similar procedure, yield = 50%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.36 (s, 1H), 8.95 (d, *J* = 2.4 Hz, 1H), 8.92 (brs, 1H), 8.24 (s, 1H), 8.11 (d, *J* = 5.4 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.85-7.80 (m, 2H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.62-7.54 (m, 4H), 7.40 (d, *J* = 9.3 Hz, 1H), 7.25 (t, *J* = 9.3 Hz, 1H), 6.35 (d, *J* = 6 Hz, 1H), 5.11 (s, 2H), 4.36 (q, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.35 Hz, 3H) ppm. LC-MS (ESI) *m/z* 623.00 [M + H]$^+$. HRMS (FAB) calculated for $C_{35}H_{27}FN_{10}O$ [M + H]$^+$: 623.2353, found: 623.2435.

N^2-(3-((3-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (7a)



This compound was synthesized using similar procedure, yield = 51.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.37 (s, 1H), 9.01 (d, *J* = 1.88 Hz, 1H), 8.95 (d, *J* = 2.52 Hz, 1H), 8.24 (s, 1H), 8.14 (d, *J* = 5.72 Hz, 1H), 7.95-7.86 (m, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.62-7.56 (m, 5H), 7.36 (dd, *J* = 8.12 Hz, 1.04 Hz, 1H), 7.23 (t, *J* = 8.12 Hz, 1H), 6.72 (dd, *J* = 8.04 Hz, 2.24 Hz, 1H), 6.37 (d, *J* = 5.72 Hz, 1H), 5.06 (sep, 1H), 4.50 (s, 2H), 1.48 (d, *J* = 6.68 Hz, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.44, 159.46, 158.08, 157.73, 156.36, 155.45, 153.46, 145.12, 143.34, 142.42, 141.94, 133.94, 133.47, 132.16 (2C), 129.21, 128.50, 128.43 (2C), 128.20, 127.43, 127.04, 126.87, 121.49, 121.20, 112.63, 107.27, 106.43, 99.56, 97.42, 86.17, 86.04, 55.98, 48.18, 21.75 (2C) ppm. LC-MS (ESI) *m/z* 619.01 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}N_{10}O$ $[M + H]^+$: 619.2604, found: 619.2683.

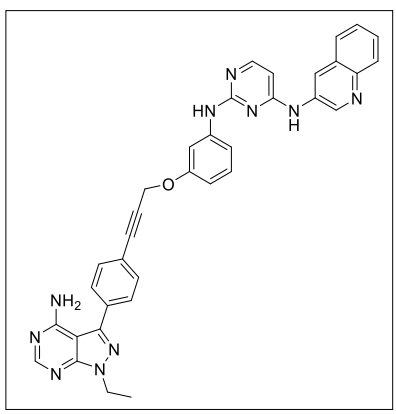
N^2-(3-((3-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (7b)



This compound was synthesized using similar procedure, yield = 58.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.14 (s, 1H), 8.94 (s, 1H), 8.89 (s, 1H), 8.24 (s, 1H), 8.08 (d, *J* = 5.94 Hz, 1H), 7.92 (d, *J* = 8.28 Hz, 1H), 7.76 (s, 1H), 7.63 (d, *J* = 8.22 Hz, 2H), 7.59-7.53 (m, 3H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 7.32 Hz, 1H),

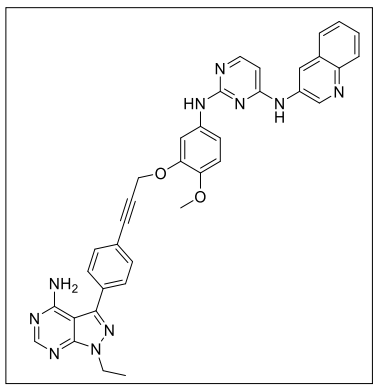
6.96 (d, *J* = 8.76 Hz, 1H), 6.30 (d, *J* = 5.52 Hz, 1H), 5.06 (sep, 1H), 4.94 (s, 2H), 3.81 (s, 3H), 1.48 (d, *J* = 6.42 Hz, 6H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.38, 159.88, 158.06, 156.41, 155.42, 153.45, 146.51, 144.99, 144.73, 143.21, 142.38, 134.03, 133.39, 132.08 (2C), 128.46, 128.32 (2C), 128.17, 127.29, 126.89, 126.73, 121.47, 121.06, 113.91, 112.58, 108.70, 98.86, 97.39, 86.15, 86.11, 57.00, 55.92, 48.16, 21.71 (2C) ppm. LC-MS (ESI) *m/z* 649.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{37}H_{32}N_{10}O_2$ $[M + H]^+$: 649.2710, found: 649.2795.

N^2-(3-((3-(4-(4-amino-1-ethyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)phenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (7c)



This compound was synthesized using similar procedure, yield = 40.3%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.36 (s, 1H), 9.00 (s, 1H), 8.94 (s, 1H), 8.24 (s, 1H), 8.12 (d, *J* = 5.67 Hz, 1H), 7.93-7.86 (m, 3H), 7.65 (d, *J* = 8.43 Hz, 2H), 7.57-7.55 (m, 4H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 8.06 Hz, 1H), 6.71 (dd, *J* = 7.77 Hz, 2.28 Hz, 1H), 6.36 (d, *J* = 5.88 Hz, 1H), 4.99 (s, 2H), 4.36 (q, *J* = 7.14 Hz, 2H), 1.39 (t, *J* = 7.14 Hz, 3H) ppm. LC-MS (ESI) *m/z* 605.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{35}H_{28}N_{10}O$ $[M + H]^+$: 605.2448, found: 605.2518.

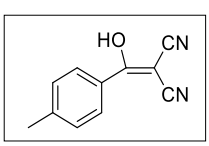
N^2-(3-((3-(4-(4-amino-1-ethyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)prop-2-yn-1-yl)oxy)-4-methoxyphenyl)-N^4-(quinolin-3-yl)pyrimidine-2,4-diamine (7d)



This compound was synthesized using similar procedure, yield = 42.9%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.17 (s, 1H), 8.95 (brs, 1H), 8.89 (s, 1H), 8.25 (s, 1H), 8.08 (d, *J* = 5.52 Hz, 1H), 7.92 (d, *J* = 8.22 Hz, 1H), 7.76 (s, 1H), 7.63 (d, *J* = 8.28 Hz, 2H), 7.59-7.54 (m, 3H), 7.51 (d, *J* = 8.22 Hz, 2H), 7.30 (d, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.30 (d, *J* = 6 Hz, 1H), 4.94 (s, 2H), 4.37 (q, *J* = 7.18 Hz, 2H), 3.81 (s, 3H), 1.40 (t, *J* = 7.35 Hz, 3H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.40, 159.91, 158.11, 156.45, 155.65, 153.84, 146.50, 144.99, 144.72, 143.20, 142.63, 134.05, 134.03, 133.25, 132.10 (2C), 128.47, 128.30 (2C), 128.19, 127.31, 126.89, 126.75, 121.52, 121.05, 113.91, 112.57, 108.71, 98.87, 97.27, 86.14, 56.99, 55.92, 41.42, 14.64 ppm. LC-MS (ESI) *m/z* 635.00 $[M + H]^+$. HRMS (FAB) calculated for $C_{36}H_{30}N_{10}O_2$ $[M + H]^+$: 635.2553, found: 635.2625.

2.5. General procedure for synthesis of Src module

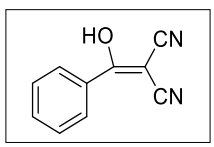
2-(hydroxy(p-tolyl)methylene)malononitrile (9a)



Sodium hydride 57-63% oil dispersion (1.55 g, 38.8 mmol) was dried in vacuum for 15 minutes and then suspended in dry THF (4 mL) and the mixture was cooled to 0 °C under a N_2 atmosphere. Malononitrile (1.47 g, 22.3 mmol) was dissolved in dry THF (15 mL) and added dropwise to the stirring sodium hydride mixture in THF over 20 minutes. *p*-Toluoyl chloride (3 g, 19.4 mmol) was dissolved in dry THF (15 mL) and added dropwise to the stirring mixture over 30 minutes. The reaction was allowed to warm to room

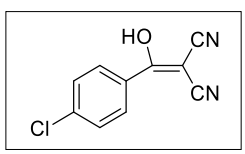
temperature and stirred for an additional 30 minutes. Isopropanol was added slowly to quench sodium hydride and the reaction mixture was evaporated. The mixture was extracted with ethyl acetate (3 X 200 mL) and dried over anhydrous sodium sulfate. The ethyl acetate was evaporated under reduced pressure and the compound was purified by silica gel column chromatography using ethyl acetate as the eluent to afford a white solid (3.1 g, 16.8 mmol, yield = 86.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.45 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 7.2 Hz, 2H), 2.29 (s, 3H) ppm.

2-(hydroxy(phenyl)methylene)malononitrile (9b)



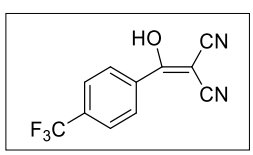
This compound was synthesized by using benzoyl chloride, yield = 64.5 %. ^{1}H NMR (300 MHz, CD_3OD) δ 7.64 (dd, J = 8.4 Hz, 1.8 Hz, 2H), 7.44-7.34 (m, 3H) ppm.

2-((4-chlorophenyl)(hydroxy)methylene)malononitrile (9c)



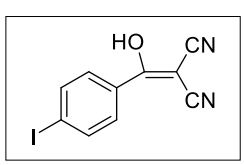
This compound was synthesized using 4-chlorobenzoyl chloride, yield = 74.4%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.63 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H) ppm.

2-(hydroxy(4-(trifluoromethyl)phenyl)methylene)malononitrile (9d)



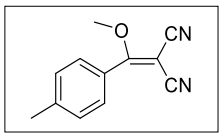
This compound was synthesized using 4-(trifluoromethyl)benzoyl chloride, yield = 90.3%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.81 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H) ppm.

2-(hydroxy(4-iodophenyl)methylene)malononitrile (9e)



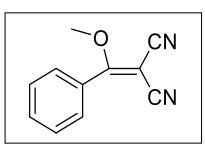
This compound was synthesized using 4-iodobenzoyl chloride, yield = 92.5%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.86 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H) ppm.

2-(methoxy(p-tolyl)methylene)malononitrile (10a)



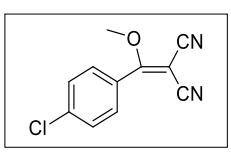
To a solution of **9a** (1 g, 5.4 mmol) in dioxane (10 mL) and water (2 mL) was added sodium bicarbonate (3.6 g, 43.4 mmol) slowly. Dimethyl sulfate (3.0 mL, 32.5 mmol) was then added to the reaction mixture and the solution was refluxed at 80 °C for 3h. The mixture was cooled, diluted with water and extracted with ethyl acetate (3 X 200 mL). The organic layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane/ethyl acetate to afford a light yellow solid (0.7 g, 3.5 mmol, yield = 65.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.56 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.7 Hz, 2H), 3.87 (s, 3H), 2.40 (s, 3H) ppm.

2-(methoxy(phenyl)methylene)malononitrile (10b)



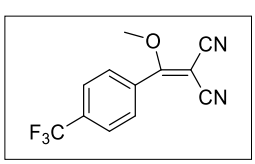
This compound was synthesized using **9b**, yield = 62.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66-7.46 (m, 5H), 3.89 (s, 3H) ppm.

2-((4-chlorophenyl)(methoxy)methylene)malononitrile (10c)



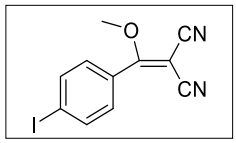
This compound was synthesized using **9c**, yield = 50.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (dd, J = 8.7 Hz, 1.8 Hz, 2H), 7.47 (dd, J = 8.7 Hz, 1.8 Hz, 2H), 3.97 (s, 3H) ppm.

2-(methoxy(4-(trifluoromethyl)phenyl)methylene)malononitrile (10d)



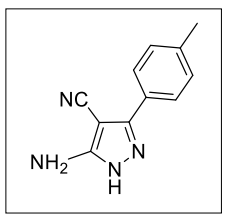
This compound was synthesized using **9d**, yield = 58.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 7.8 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 3.99 (s, 3H) ppm.

2-((4-iodophenyl)(methoxy)methylene)malononitrile (10e)



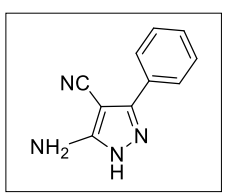
This compound was synthesized using **9e**, yield = 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.7 Hz, 0.9 Hz, 2H), 7.23 (dd, *J* = 6.9 Hz, 0.9 Hz, 2H), 3.97 (s, 3H) ppm.

5-amino-3-(p-tolyl)-1H-pyrazole-4-carbonitrile (11a)



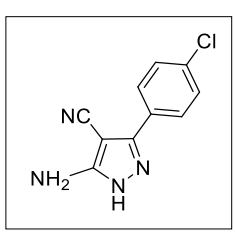
To a solution of **10a** (2.1 g, 10.6 mmol) in ethanol (15 mL) were added triethylamine (4.4 mL, 31.8 mmol) and hydrazine hydrochloride (0.76 g, 11.1 mmol) and the mixture was refluxed at 120 °C for 30 minutes. Ethanol was evaporated and the residue was purified by silica gel column chromatography with ethyl acetate/ hexane gradient to give a yellow solid (1.74 g, 8.8 mmol, yield = 82.9%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (brs, 1H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.25 (d, *J* = 7.5 Hz, 2H), 6.43 (s, 2H), 2.33 (s, 3H) ppm. LC-MS (ESI) *m/z* 199.10 $[M + H]^+$.

5-amino-3-phenyl-1H-pyrazole-4-carbonitrile (11b)



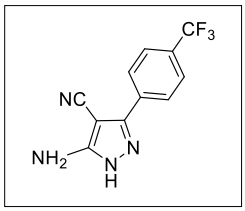
This compound was synthesized using **10b**, yield = 77.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.15 (brs, 1H), 7.78 (d, *J* = 7.8 Hz, 2H), 7.44 (m, 3H), 6.46 (s, 2H) ppm.

5-amino-3-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile (11c)



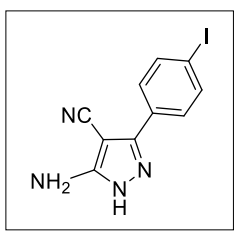
This compound was synthesized using **10c**, yield = 79.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.23 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 6.54 (brs, 2H) ppm.

5-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazole-4-carbonitrile (11d)



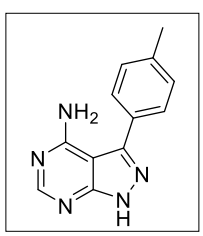
This compound was synthesized using **10d**, yield = 75%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.35 (brs, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.83 (d, J = 7.8 Hz, 2H), 6.57 (brs, 2H) ppm.

5-amino-3-(4-iodophenyl)-1H-pyrazole-4-carbonitrile (11e)



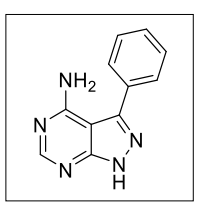
This compound was synthesized using **10e**, yield = 79.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.53 (brs, 1H), 7.82 (d, J = 7.8 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 6.53 (brs, 2H) ppm.

3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12a)



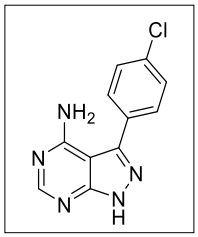
Compound **11a** (1.74 g, 8.8 mmol) was suspended in formamide (8 mL) and heated at 160 °C for 12 h. The reaction was cooled to rt, water was added and the resulting precipitate was filtered and dried to give a sand color solid (1.65 g, 7.3 mmol, yield = 83.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.49 (brs, 1H), 8.20 (s, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 2.38 (s, 3H) ppm. LC-MS (ESI) *m/z* 226.10 $[M + H]^+$.

3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12b)



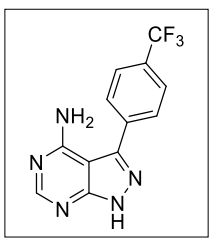
This compound was synthesized using **11b**, yield = 52.9%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.61 (s, 1H), 8.21 (s, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.56-7.47 (m, 3H) ppm.

3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12c)



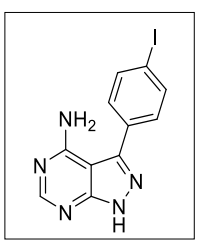
This compound was synthesized using **11c**, yield = 35.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.65 (brs, 1H), 8.21 (s, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H) ppm. LC-MS (ESI) m/z 246.00 $[M + H]^+$.

3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12d)



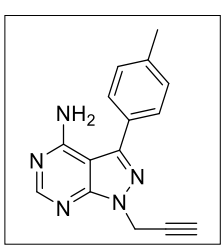
This compound was synthesized using **11d**, yield = 67.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.75 (brs, 1H), 8.23 (s, 1H), 7.87 (s, 4H) ppm.

3-(4-iodophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (12e)



This compound was synthesized using **11e**, yield = 64.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.65 (s, 1H), 8.20 (s, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H) ppm.

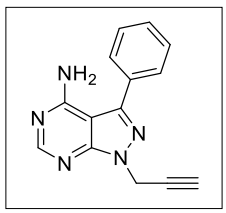
1-(prop-2-yn-1-yl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (26a)



To a solution of **12a** (400 mg, 1.78 mmol) in anhydrous DMF (5 mL) were added potassium carbonate (614 mg, 4.4 mmol) and propargyl bromide (0.17 mL, 1.95 mmol). The mixture was stirred at rt for 3h. DCM was added and the mixture was filter to remove potassium carbonate and partitioned between water and DCM (3 X 150 mL). The organic layer was dried with sodium sulfate and evaporated *in vacuo*. The crude product was purified using silica gel chromatography with DCM/methanol gradient to afford the desired product as a yellow solid (250 mg, 0.95 mmol, 53.5% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.60 (d, J = 7.8 Hz, 2H), 7.34 (d,

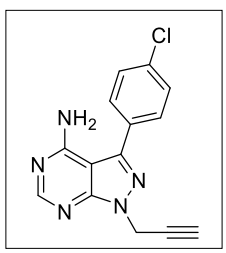
J = 8.1 Hz, 2H), 5.52 (brs, 2H), 5.24 (d, *J* = 2.4 Hz, 2H), 2.44 (s, 3H), 2.38 (t, *J* = 2.55 Hz, 1H) ppm. LC-MS (ESI) *m/z* 264.10 $[M + H]^+$.

3-phenyl-1-(prop-2-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (26b)



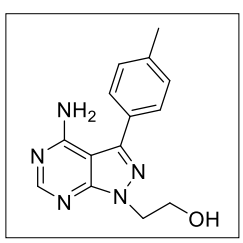
This compound was synthesized using **12b**, yield = 44.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.72 (dd, *J* = 7.95 Hz, 1.65 Hz, 2H), 7.57-7.49 (m, 3H), 5.57 (brs, 2H), 5.25 (d, *J* = 2.7 Hz, 2H), 2.38 (t, *J* = 2.55 Hz, 1H) ppm.

3-(4-chlorophenyl)-1-(prop-2-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (26c)



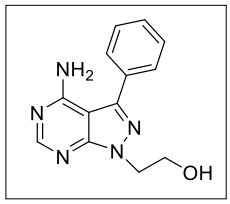
This compound was synthesized using **12c**, yield = 60.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 5.60 (brs, 2H), 5.24 (d, *J* = 2.4 Hz, 2H), 2.39 (t, *J* = 2.55 Hz, 1H) ppm.

2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27a)



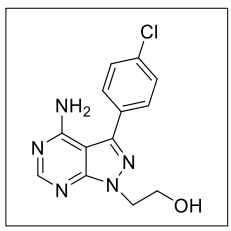
To a solution of **12a** (1 g, 4.4 mmol) in anhydrous DMF (7 mL) under a N_2 atmosphere were added Cs_2CO_3 (2.1 g, 6.6 mmol) and 2-bromoethanol (0.47 mL, 6.6 mmol) and the mixture was stirred at 80 °C for 3 h. The mixture was extracted with ethyl acetate (3 X 200 mL). The combined organic phase was separated, dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated to dryness under reduced pressure. The crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a white solid (0.6 g, 2.2 mmol, 50.2% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 4.88 (t, *J* = 5.3 Hz, 1H), 4.36 (t, *J* = 5.9 Hz, 2H), 3.82 (q, *J* = 6.3 Hz, 2H), 2.38 (s, 3H) ppm.

2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27b)



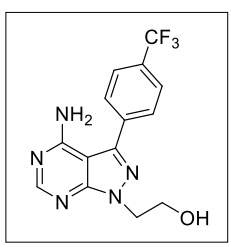
This compound was synthesized using **12b**, yield = 50.3%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.67 (dd, J = 8.4 Hz, 1.5 Hz, 2H), 7.57-7.45 (m, 3H), 4.88 (t, J = 5.55 Hz, 1H), 4.38 (t, J = 6 Hz, 2H), 3.84 (q, J = 5.8 Hz, 2H) ppm.

2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27c)



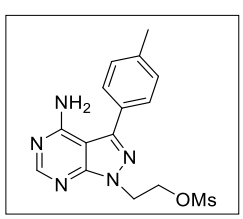
This compound was synthesized using **12c**, yield = 62.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 4.88 (t, J = 5.55 Hz, 1H), 4.37 (t, J = 6 Hz, 2H), 3.83 (q, J = 5.8 Hz, 2H) ppm.

2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27d)



This compound was synthesized using **12d**, yield = 53%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.88 (s, 4H), 4.90 (t, J = 5.7 Hz, 1H), 4.40 (t, J = 5.7 Hz, 2H), 3.84 (q, J = 5.8 Hz, 2H) ppm.

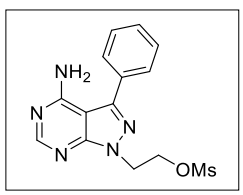
2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl methanesulfonate (28a)



To a suspension of **27a** (600 mg, 2.2 mmol) in pyridine (3 mL) under a N_2 atmosphere was added methanesulfonyl chloride (0.2 mL, 2.6 mmol) and the mixture was stirred at rt for 12h. The resulting mixture was concentrated *in vacuo* and then partitioned between water and ethyl acetate. The organic layer was dried with sodium sulfate and evaporated under reduced pressure. The crude product was purified using

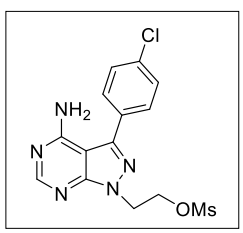
silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a white solid (405 mg, 1.17 mmol, 52.3% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 5.59 (brs, 2H), 4.79-4.73 (m, 4H), 2.94 (s, 3H), 2.44 (s, 3H) ppm.

2-(4-amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl methanesulfonate (28b)



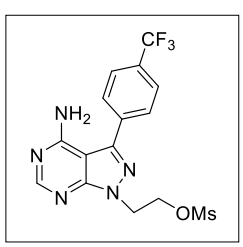
This compound was synthesized using **27b**, yield = 75.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.69-7.67 (m, 2H), 7.57-7.46 (m, 3H), 5.90 (brs, 2H), 4.77-4.73 (m, 4H), 2.93 (s, 3H) ppm.

2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl methanesulfonate (28c)



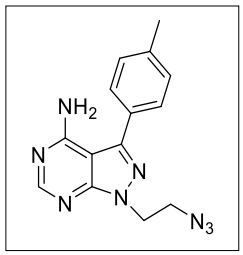
This compound was synthesized using **27c**, yield = 45.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.64 (dd, *J* = 6.9 Hz, 1.8 Hz, 2H), 7.53 (dd, *J* = 8.4 Hz, 1.5 Hz, 2H), 5.55 (brs, 2H), 4.79-4.73 (m, 4H), 2.95 (s, 3H) ppm.

2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl methanesulfonate (28d)



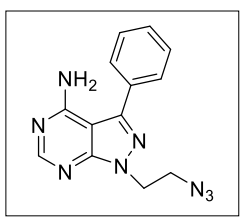
This compound was synthesized using **27d**, yield = 68.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.87-7.80 (m, 4H), 5.53 (brs, 2H), 4.82-4.74 (m, 4H), 2.96 (s, 3H) ppm.

1-(2-azidoethyl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (29a)



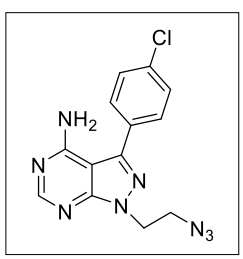
To a solution of **28a** (405 mg, 1.17 mmol) in anhydrous DMF (4 mL) under a N_2 atmosphere was added sodium azide (152 mg, 2.3 mmol) and the mixture was stirred at 70 °C for 8 h. The mixture was extracted with ethyl acetate (3 X 150 mL). The combined organic phase was separated, dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated to dryness under reduced pressure. The crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a white solid (300 mg, 1.02 mmol, yield = 87.4 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 5.49 (brs, 2H), 4.62 (t, *J* = 6 Hz, 2H), 3.85 (t, *J* = 5.7 Hz, 2H), 2.44 (s, 3H) ppm. LC-MS (ESI) *m/z*: 295.10 $[M + H]^+$.

1-(2-azidoethyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (29b)



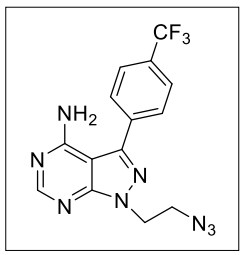
This compound was synthesized using **28b**, yield = 83.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.71 (dd, *J* = 8.4 Hz, 1.8 Hz, 2H), 7.53 (m, 3H), 5.55 (brs, 2H), 4.63 (t, *J* = 5.85 Hz, 2H), 3.86 (t, *J* = 5.7 Hz, 2H) ppm.

1-(2-azidoethyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (29c)



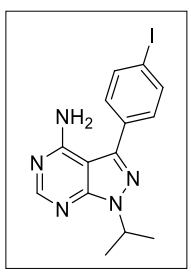
This compound was synthesized using **28c**, yield = 80.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.65 (dd, *J* = 6.9 Hz, 1.8 Hz, 2H), 7.52 (dd, *J* = 8.55 Hz, 1.65 Hz, 2H), 5.84 (brs, 2H), 4.62 (t, *J* = 5.85 Hz, 2H), 3.84 (t, *J* = 5.85 Hz, 2H) ppm.

1-(2-azidoethyl)-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (29d)



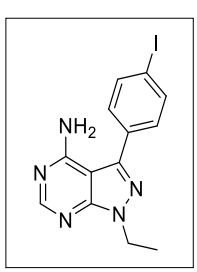
This compound was synthesized using **28d**, yield = 88.3 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.84 (q, *J* = 8.7 Hz, 4H), 5.48 (br, 2H), 4.65 (t, *J* = 5.85 Hz, 2H), 3.87 (*J* = 5.85 Hz, 2H) ppm.

3-(4-iodophenyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (30a)



To a solution of **12e** (400 mg, 1.19 mmol) in anhydrous DMF (5 mL) under a N_2 atmosphere were added potassium carbonate (656 mg, 4.7 mmol) and 2-iodopropane (0.13 mL, 1.3 mmol) and the mixture was stirred at 60 °C for 3 h. The mixture was diluted with ethyl acetate and filtered. The filtrate was then partitioned between water and ethyl acetate. The organic layer was dried with sodium sulfate and concentrated *in vacuo*. The crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a yellow solid (250 mg, 0.66 mmol, 55.6% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.88 (dd, *J* = 8.7 Hz, 0.9 Hz, 2H), 7.46 (dd, *J* = 8.7 Hz, 0.9 Hz, 2H), 5.44 (brs, 2H), 5.18 (sep, 1H), 1.59 (dd, *J* = 6.6 Hz, 0.9 Hz, 6H) ppm.

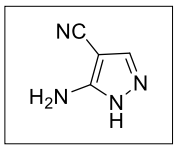
1-ethyl-3-(4-iodophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (30b)



This compound was synthesized using iodoethane, 50.8% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 6.9 Hz, 2H), 5.49 (brs, 2H), 4.50 (q, *J* = 7.2 Hz, 2H), 1.54 (t, *J* = 7.35 Hz, 3H) ppm.

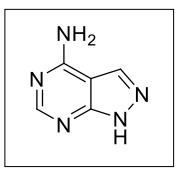
2.6. General procedure for efficient synthesis of Src module

5-amino-1H-pyrazole-4-carbonitrile (32)



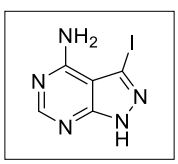
To a solution of 2-(ethoxymethylene)malononitrile **31** (5g, 40.9 mmol) in ethanol (8 mL) were added hydrazine monohydrochloride (2.86 g, 41.7 mmol) and triethylamine (0.34 mL, 122.8 mmol) and the mixture was refluxed at 100 °C for 30 minutes. The resulting mixture was evaporated and the crude product was purified using silica gel chromatography with hexane/ethyl acetate gradient to afford the desired product as a yellow solid (2.6 g, 24.1 mmol, yield = 58.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (brs, 1H), 7.48 (s, 1H), 6.30 (brs, 2H) ppm. LC-MS (ESI) *m/z* 109.10 $[M + H]^+$.

1H-pyrazolo[3,4-d]pyrimidin-4-amine (33)



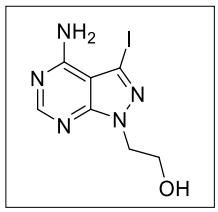
32 (2.6 g, 24.1 mmol) was mixed with formamide (5 mL) and heated to 160 °C overnight under N_2. The mixture was cooled to rt and distilled water was added, the resulting precipitate was filtered to give **33** as sand colour solid (2.575 g, 19.1 mmol, yield = 79.2%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.32 (brs, 1H), 8.12 (s, 1H), 8.05 (s, 1H), 7.58 (brs, 2H) ppm.

3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (34)



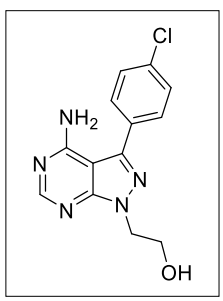
To a solution of **33** (2.575 g, 19.1 mmol) in anhydrous DMF (10 mL) was added *N*-iodosuccinimide (6.43 g, 28.6 mmol) and the mixture was stirred at 80 °C for 12 h. The resulting mixture was cooled to rt and DCM was added and the precipitate was filtered to give yellow solid (4.8 g, 18.4 mmol, yield = 96.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.79 (brs, 1H), 8.16 (s, 1H) ppm. LC-MS (ESI) *m/z* 261.80 $[M + H]^+$.

2-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (35)



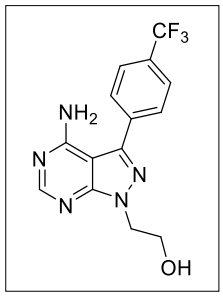
To solution of **34** (2 g, 7.7 mmol) in anhydrous DMF (8 mL) under N_2 atmosphere were added 2-bromoethanol (0.81 mL, 11.5 mmol) and cesium carbonate (3.7 g, 11.5 mmol) and the mixture was stirred at 80 °C for 3 h. The resulting mixture was cooled to rt and distilled water was added and the resulting precipitate was filtered to give yellow solid (1.4 g, 4.6 mmol, yield = 59.9%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (s, 1H), 4.84 (t, *J* = 5.7 Hz, 1H), 4.29 (d, *J* = 5.85 Hz, 2H), 3.76 (q, *J* = 5.6 Hz, 2H) ppm.

2-(4-amino-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27c)



Solid components including **35** (1 g, 3.3 mmol), tetrakis(triphenylphosphine)palladium(0) (189 mg, 0.16 mmol), cesium carbonate (1.6 g, 4.9 mmol) were first added to a flask under N_2. This was followed by addition of 4-chlorophenylboronic acid (768 mg, 4.9 mmol) and a mixture of DMF:water (2:1, 6 mL). The mixture was then refluxed at 100 °C for 3 h and extracted with EA and water. The crude product was purified using silica gel chromatography with ethyl acetate/methanol gradient to afford the desired product as a white solid (770 mg, 2.7 mmol, yield = 81.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 4.88 (t, *J* = 5.55 Hz, 1H), 4.37 (t, *J* = 6 Hz, 2H), 3.83 (q, *J* = 5.8 Hz, 2H) ppm.

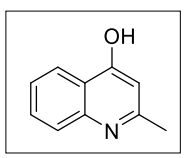
2-(4-amino-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethan-1-ol (27d)



This compound was synthesized by using 4-(trifluoromethyl)phenylboronic acid, yield = 92.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.88 (s, 4H), 4.89 (t, J = 5.7 Hz, 1H), 4.40 (t, J = 5.85 Hz, 2H), 3.85 (q, J = 5.8 Hz, 2H) ppm.

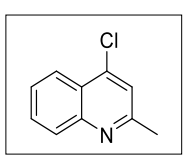
2.7. General procedure for synthesis of IGF-1R module I

2-methylquinolin-4-ol (14a)



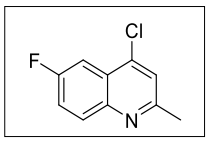
Aniline (100 mg, 1 mmol) and polyphosphoric acid (6 mL) was heated to 80 °C, ethylacetoacetate (0.15 mL, 1.2 mmol) was added drop wise and heated to 120 °C for 16 h. The mixture was poured over crushed ice and neutralized with aqueous ammonia, the resulting precipitate was filtered, washed with diethyl ether and to give a solid (140 mg, 0.9 mmol, 81.9% yield).

4-chloro-2-methylquinoline (15a)



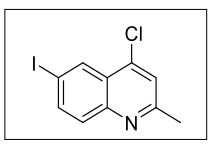
To the solution of **14a** (50 mg, 1.0 mmol) in acetone (0.15 mL), was added phosphorous oxycholoride (5 mL) carefully and the mixture was heated to 105 °C for 3 h. The excess of phosphorous oxycholoride was removed under reduce pressure. The residue was quenched into crushed ice. The reaction mixture was neutralized using saturated sodium bicarbonate and the resulting precipitate was filter to give a yellow solid (48 mg, 0.27 mmol, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.75 (t, J = 7.65 Hz, 1H), 7.57 (t, J = 7.65 Hz, 1H), 7.39 (s, 1H), 2.72 (s, 3H) ppm.

4-chloro-6-fluoro-2-methylquinoline (15b)



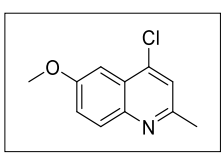
This compound was synthesized using **14b**, yield = 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 9.0 Hz, 5.4 Hz, 1H), 7.67 (dd, *J* = 9.3 Hz, 2.7 Hz, 1H), 7.37 (td, *J* = 8.1 Hz, 2.7Hz, 1H), 7.29 (s, 1H), 2.59 (s, 3H) ppm. LC-MS (ESI) *m/z* 196 $[M + H]^+$.

4-chloro-6-iodo-2-methylquinoline (15c)



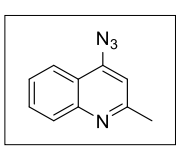
This compound was synthesized **14c**, yield = 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 1.8 Hz, 1H), 8.00 (dd, *J* = 8.7 Hz, 1.8 Hz, 1H), 7.83 (brs, 1H), 7.42 (s, 1H), 2.74 (s, 3H). LC-MS (ESI) *m/z* 303 $[M + H]^+$.

4-chloro-6-methoxy-2-methylquinoline (15d)



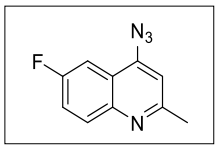
This compound was synthesized using **14d,** yield = 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.7 Hz, 1H), 7.41-7.38 (m, 2H), 7.36 (s, 1H), 3.96 (s, 3H), 2.68 (s, 3H) ppm. LC-MS (ESI) *m/z* 208.10 $[M + H]^+$.

4-azido-2-methylquinoline (16a)



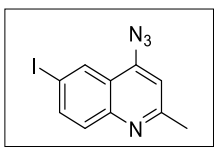
To a solution of **15a** (48 mg, 0.27 mmol) in DMF was added sodium azide (88 mg, 1.35 mmol) and the mixture was heated to 100 °C for 4 h. The resulting mixture was cooled to rt, extracted with DCM and brine. The organic phase was dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated to dryness under reduced pressure. The crude product was purified using silica gel chromatography with hexane/ethyl acetate gradient to afford the desired product as a brown solid (44 mg, 0.24 mmol, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.7 Hz, 2H), 7.68 (t, *J* = 8.25 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 6.96 (s, 1H), 2.70 (s, 3H) ppm. LC-MS (ESI) *m/z* 185.10 $[M + H]^+$.

4-azido-6-fluoro-2-methylquinoline (16b)



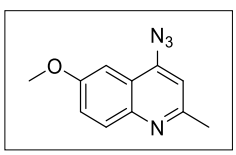
This compound was synthesized using **15b**, yield = 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (brs, 1H), 7.63 (dd, *J* = 9.3 Hz, 2.7 Hz, 1H), 7.48 (dt, *J* = 10.8 Hz, 2.7 Hz, 1H), 7.06 (s, 1H), 2.59 (s, 3H) ppm. LC-MS (ESI) *m/z* 203.00 $[M + H]^+$.

4-azido-6-iodo-2-methylquinoline (16c)



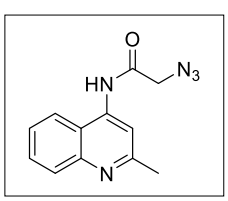
This compound was synthesized using **15c**, yield = 79%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, *J* = 2.1 Hz, 1H), 7.92 (dd, *J* = 9.0 Hz, 2.4 Hz, 1H), 7.67 (d, *J* = 9.0 Hz, 1H), 7.01 (s, 1H), 2.69 (s, 3H). LC-MS (ESI) *m/z* 311.00 $[M + H]^+$.

4-azido-6-methoxy-2-methylquinoline (16d)



This compound was synthesized using **15d**, yield = 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 9.6 Hz, 1H), 7.35 (dd, *J* = 9.3 Hz, 2.7 Hz, 1H), 7.24 (d, *J* = 2.7 Hz, 1H), 7.00 (s, 1H), 3.92 (s, 3H), 2.70 (s, 3H).

2-azido-N-(2-methylquinolin-4-yl)acetamide (18)

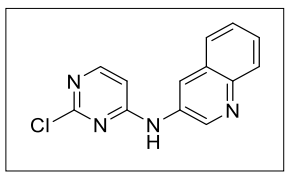


To a solution of 4-aminoquinaldine **17** (100 mg, 0.63 mmol) in a mixture of DMF and DCM (4 mL) under a N_2 atmosphere were added 2-azidoacetic acid (0.05 mL, 0.63 mmol), 1-ethyl-3-(3 dimethylaminopropyl)carnodiimide hydrochloride (EDC) (182 mg, 0.95 mmol), DIEA (0.17 mL, 0.95 mmol) and the mixture was stirred at 60 °C for 8 h. The mixture was partitioned with DCM, water and the crude product was purified using silica gel chromatography with hexane/ethyl acetate gradient to afford the desired product as a brown solid (100 mg, 0.4 mmol, yield = 65.6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (brs, 1H), 8.22 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.82 (d,

J = 8.1 Hz, 1H), 7.73 (t, *J* = 7.65 Hz, 1H), 7.57 (t, *J* = 7.65 Hz, 1H), 4.34 (s, 2H), 2.80 (s, 3H) ppm. LC-MS (ESI) *m/z* 242.10 $[M + H]^+$.

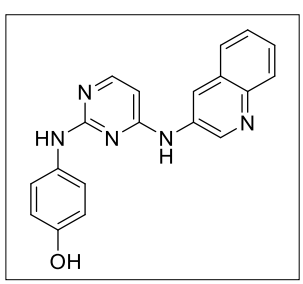
2.8. General procedure for synthesis of IGF-1R module II

N-(2-chloropyrimidin-4-yl)quinolin-3-amine (21)



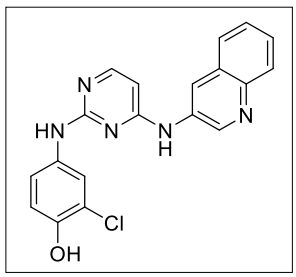
To a solution of 2,4-dichloropyrimidine **19** (1 g, 6.71 mmol) and 3-aminoquinoline **20** (968 mg, 6.71 mmol) in 2-propanol (12 mL) under a N_2 atmosphere was added DIEA (1.75 mL, 10.07 mmol) and the mixture was refluxed for 24h. The reaction mixture was concentrated *in vacuo* and methanol was added, the resulting precipitate was filtered and dried to give a white solid (1.2 g, 4.67 mmol, 69.6% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.47 (brs, 1H), 8.98 (d, *J* = 2.4 Hz, 1H), 8.63 (d, *J* = 2.4 Hz, 1H), 8.26 (d, *J* = 6 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 6.9 Hz, 1H), 7.66 (t, *J* = 6.8 Hz, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 5.7 Hz, 1H) ppm.

4-((4-(quinolin-3-ylamino)pyrimidin-2-yl)amino)phenol (23a)



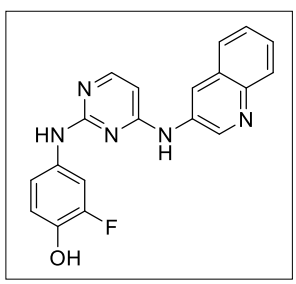
To a solution of **21** (500 mg, 1.9 mmol) in DMSO (1 mL) was added 4-aminophenol **22a** (233 mg, 2.1 mmol) and the mixture was stirred at 90 °C under a N_2 atmosphere for 2 h. The mixture was cooled to rt, and DCM was added, the resulting precipitate was filtered and dried to give a grey solid (580 mg, 1.8 mmol, 90.4% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.12 (s, 1H), 8.96 (s, 1H), 8.94 (brs, 1H), 8.89 (s, 1H), 8.04 (dd, *J* = 5.7 Hz, 2.4 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.75 (s, 1H), 7.58-7.56 (m, 2H), 7.42 (d, *J* = 6.9 Hz, 2H), 6.72 (d, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 5.7 Hz, 1H) ppm.

2-chloro-4-((4-(quinolin-3-ylamino)pyrimidin-2-yl)amino)phenol (23b)



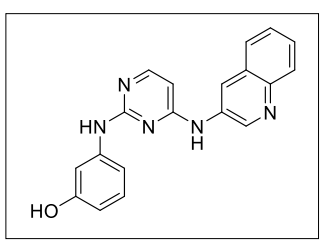
This compound was synthesized using 2-chloro-4-aminophenol **22b** (yield = 98.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.74 (s, 1H), 9.13 (s, 1H), 8.92 (s, 2H), 8.08 (d, *J* = 6 Hz, 1H), 7.93 (d, *J* = 9 Hz, 1H), 7.78 (d, 1H), 7.72 (s, 1H), 7.61-7.55 (m, 2H), 7.42 (dd, *J* = 8.85 Hz, 2.55 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.30 (d, *J* = 5.7 Hz, 1H) ppm.

2-fluoro-4-((4-(quinolin-3-ylamino)pyrimidin-2-yl)amino)phenol (23c)



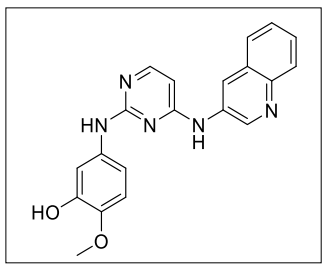
This compound was synthesized using 4-amino-2-fluorophenol **22c**, yield = 96%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.38 (s, 1H), 9.15 (s, 1H), 8.92 (s, 2H), 8.08 (d, *J* = 6 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 7.2 Hz, 1H), 7.65-7.55 (m, 3H), 7.22 (d, *J* = 8.7 Hz, 1H), 6.86 (t, *J* = 9.45 Hz, 1H), 6.30 (d, *J* = 5.7 Hz, 1H) ppm.

3-((4-(quinolin-3-ylamino)pyrimidin-2-yl)amino)phenol (23d)



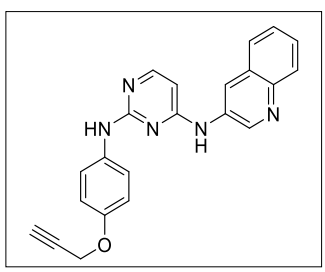
This compound was synthesized using 3-aminophenol **22d**, yield = 87.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.25 (s, 1H), 9.18 (s, 1H), 9.06 (s, 1H), 8.92 (s, 1H), 8.10 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.61-7.55 (m, 2H), 7.24 (s, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.04 (t, *J* = 7.95 Hz, 1H), 6.40 (d, *J* = 8.1 Hz, 1H), 6.34 (d, *J* = 6 Hz, 1H) ppm.

2-methoxy-5-((4-(quinolin-3-ylamino)pyrimidin-2-yl)amino)phenol (23e)



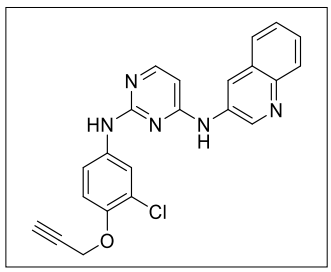
This compound was synthesized using 2-methoxy-5-aminophenol **22e**, yield = 95.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H), 9.14 (s, 1H), 9.05 (s, 1H), 8.97 (s, 1H), 8.88 (s, 1H), 8.03 (d, J = 5.7 Hz, 1H), 7.87 (d, J = 9 Hz, 1H), 7.68 (d, 1H), 7.50 (m, 2H), 7.04 (s, 1H), 6.96 (d, 1H), 6.89 (d, J = 8.7 Hz, 1H), 6.20 (d, J = 5.7 Hz, 1H), 3.78 (s, 3H) ppm.

N_2-(4-(prop-2-yn-1-yloxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (24a)



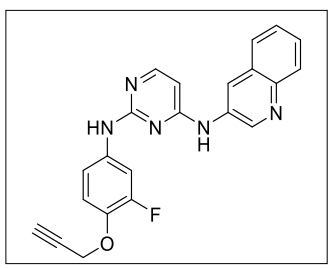
To a solution of **23a** (200 mg, 0.6 mmol) in anhydrous DMF (3 mL), were added cesium carbonate (396 mg, 1.2 mmol) and propargyl bromide (0.06 mL, 0.7 mmol). The reaction mixture was stirred at rt for 2 h. The distilled water was added and the precipitate was filtered and washed with water to give a grey solid (150 mg, 0.4 mmol, 67.2% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.13 (s, 1H), 8.94 (brs, 1H), 8.91 (d, J = 2.4 Hz, 1H), 8.07 (d, J = 6 Hz, 1H), 7.92 (d, J = 9 Hz, 1H), 7.77 (s, 1H), 7.62-7.53 (m, 4H), 6.93 (d, J = 9 Hz, 2H), 6.29 (d, J = 5.4 Hz, 1H), 4.76 (d, J = 2.4 Hz, 2H), 3.56 (t, J = 2.4 Hz, 1H) ppm. LC-MS (ESI) calculated for $C_{22}H_{17}N_5O$ $[M + H]^+$: 368.14, found: 368.20.

N_2-(3-chloro-4-(prop-2-yn-1-yloxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (24b)



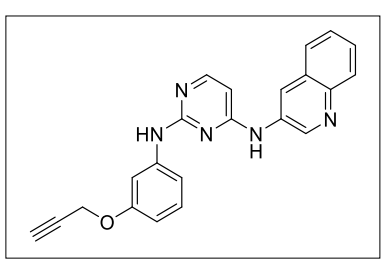
This compound was synthesized by using **23b**, yield = 59%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.30 (s, 1H), 8.94 (d, *J* = 2.7 Hz, 1H), 8.92 (brs, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.94-7.91 (m, 2H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.62-7.53 (m, 3H), 7.14 (d, *J* = 9 Hz, 1H), 6.35 (d, *J* = 6 Hz, 1H), 4.85 (d, *J* = 2.7 Hz, 2H), 3.61 (t, *J* = 2.25 Hz, 1H) ppm.

N_2-(3-fluoro-4-(prop-2-yn-1-yloxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (24c)



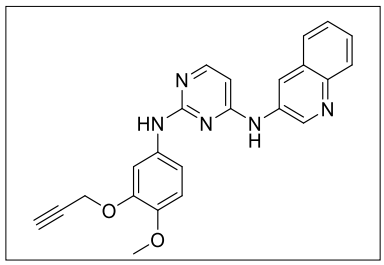
This compound was synthesized by using **23c**, yield = 61%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.34 (s, 1H), 8.94 (d, *J* = 2.4 Hz, 1H), 8.92 (s, 1H), 8.11 (d, *J* = 5.7 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.84-7.75 (m, 2H), 7.62-7.54 (m, 2H), 7.37 (d, *J* = 8.7 Hz, 1H), 7.14 (t, *J* = 9.3 Hz, 1H), 6.35 (d, *J* = 6 Hz, 1H), 4.82 (d, *J* = 2.4 Hz, 2H), 3.60 (t, *J* = 2.4 Hz, 1H) ppm.

N_2-(3-(prop-2-yn-1-yloxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (24d)



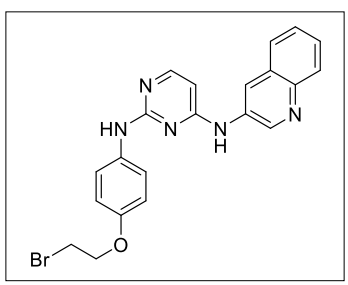
This compound was synthesized by using **23d**, yield = 62.8%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.31 (s, 1H), 8.99 (d, *J* = 2.4 Hz, 1H), 8.94 (d, *J* = 2.7 Hz, 1H), 8.12 (d, *J* = 5.7 Hz, 1H), 7.94-7.83 (m, 2H), 7.62-7.54 (m, 2H), 7.46 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.19 (t, *J* = 8.25 Hz, 1H), 6.62 (dd, *J* = 8.1 Hz, 1.8 Hz, 1H), 6.36 (d, *J* = 5.7 Hz, 1H), 4.70 (d, *J* = 2.4 Hz, 2H), 3.54 (t, *J* = 2.4 Hz, 1H) ppm.

N_2-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (24e)



This compound was synthesized by using **23e**, yield = 60.3%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.81 (s, 1H), 9.10 (s, 1H), 8.94 (s, 2H), 8.08 (d, *J* = 6 Hz, 1H), 7.92 (d, *J* = 8.25 Hz, 1H), 7.78 (d, *J* = 6.6 Hz, 1H), 7.60-7.52 (m, 2H), 7.38 (s, 1H), 7.32 (dd, *J* = 8.7 Hz, 2.4 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.30 (d, *J* = 5.7 Hz, 1H), 4.64 (d, *J* = 2.1 Hz, 2H), 3.76 (s, 3H), 3.50 (t, *J* = 2.25 Hz, 1H) ppm.

N_2-(4-(2-bromoethoxy)phenyl)-N_4-(quinolin-3-yl)pyrimidine-2,4-diamine (25)



To a solution of **23a** (100 mg, 0.3 mmol) in anhydrous DMF (3 mL), were added potassium carbonate (300 mg, 1.5 mmol) and 1,2-dibromoethanol (0.029 mL, 0.33 mmol) and the mixture was stirred at rt for 12 h. The resulting mixture was diluted with ethyl acetate and filtered. The filtrate was then extracted with ethyl acetate (3 X 100 mL) and water. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and the filtrate was evaporated under reduced pressure. The crude was purified by column chromatography with ethyl acetate/methanol gradient give **25** as a white solid (65 mg, 0.15 mmol, 49% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.14 (s, 1H), 8.90 (d, 2H), 8.07 (d, *J* = 6 Hz, 1H), 7.93 (d, 1H), 7.75 (s, 1H), 7.59-7.57 (m, 4H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.28 (d, *J* = 6 Hz, 1H), 4.30 (t, *J* = 5.4 Hz, 2H), 3.82 (t, *J* = 5.4 Hz, 2H) ppm.

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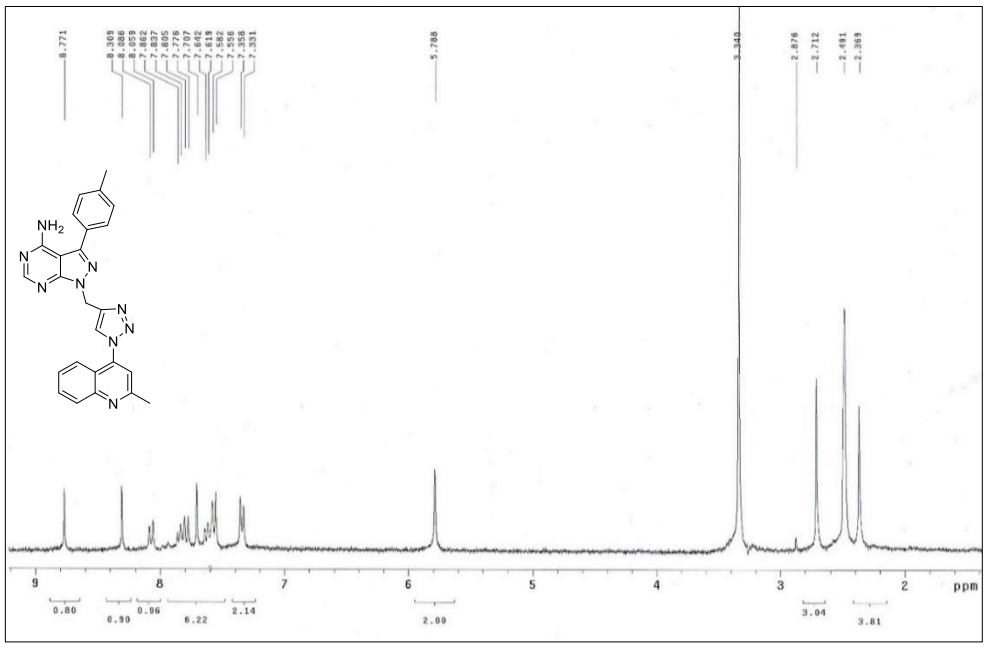
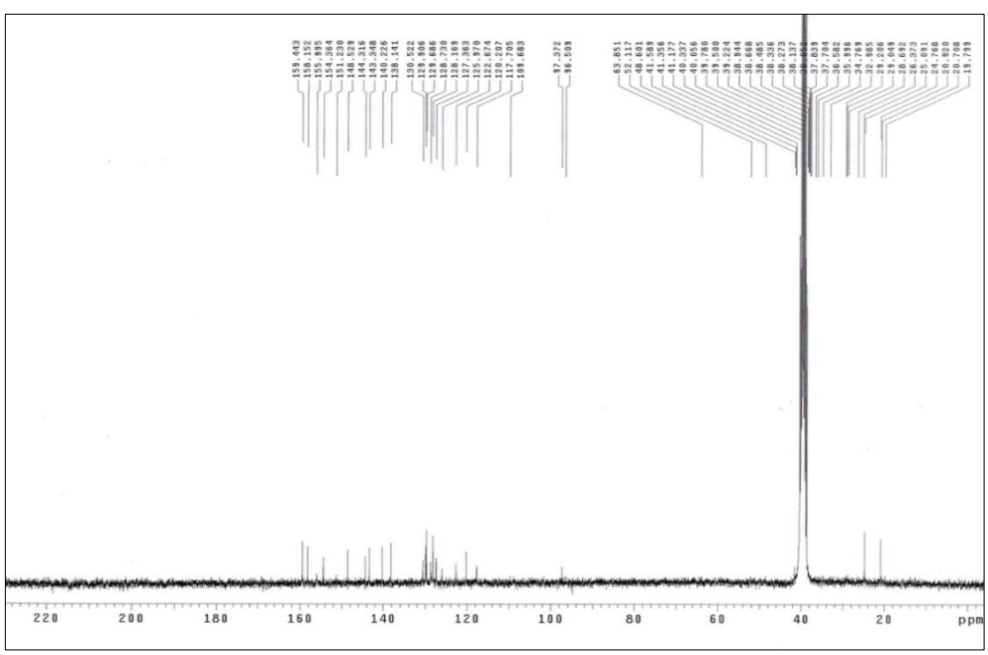
APPENDIX

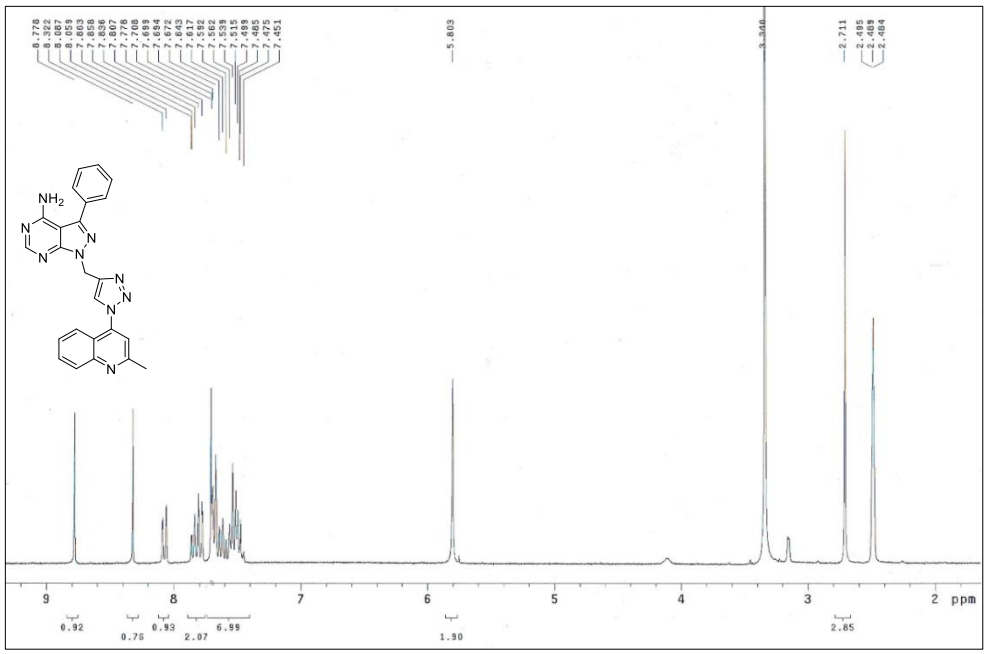
^{1}H NMR of compound **1a** (300 MHz, DMSO-d_6)



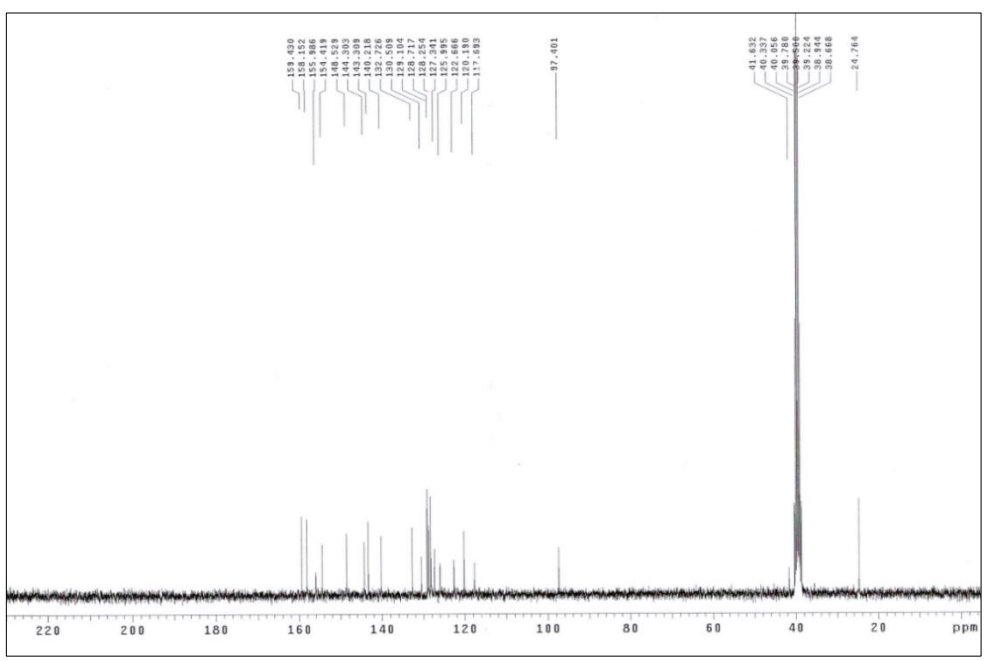
^{13}C NMR of compound **1a** (75 MHz, DMSO-d_6)



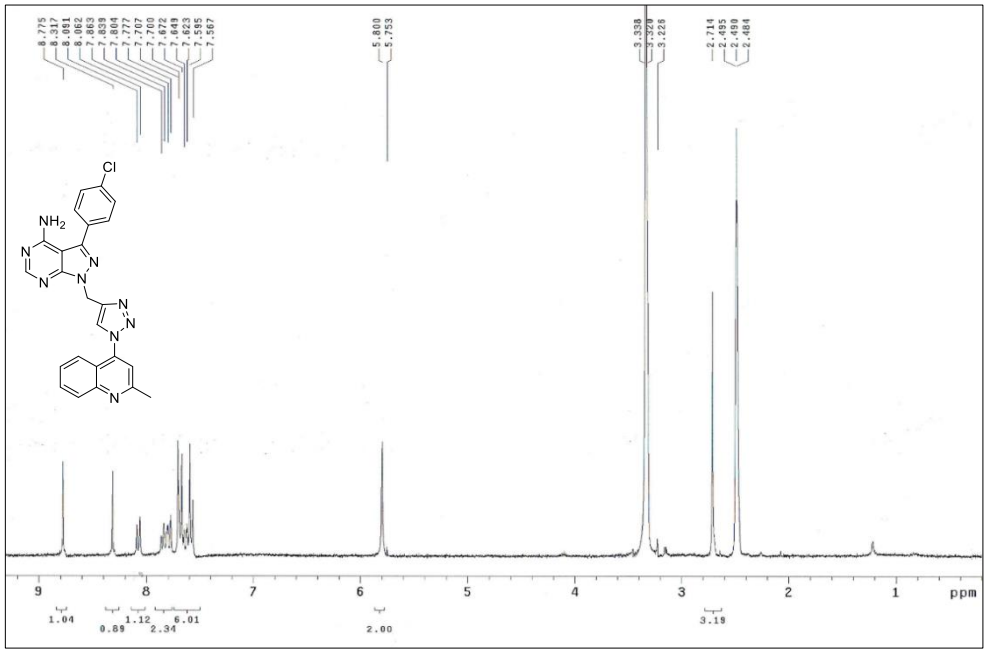
^{1}H NMR of compound **1b** (300 MHz, DMSO-d_6)



^{13}C NMR of compound **1b** (75 MHz, DMSO-d_6)



^{1}H NMR of compound **1c** (300 MHz, DMSO-d_6)



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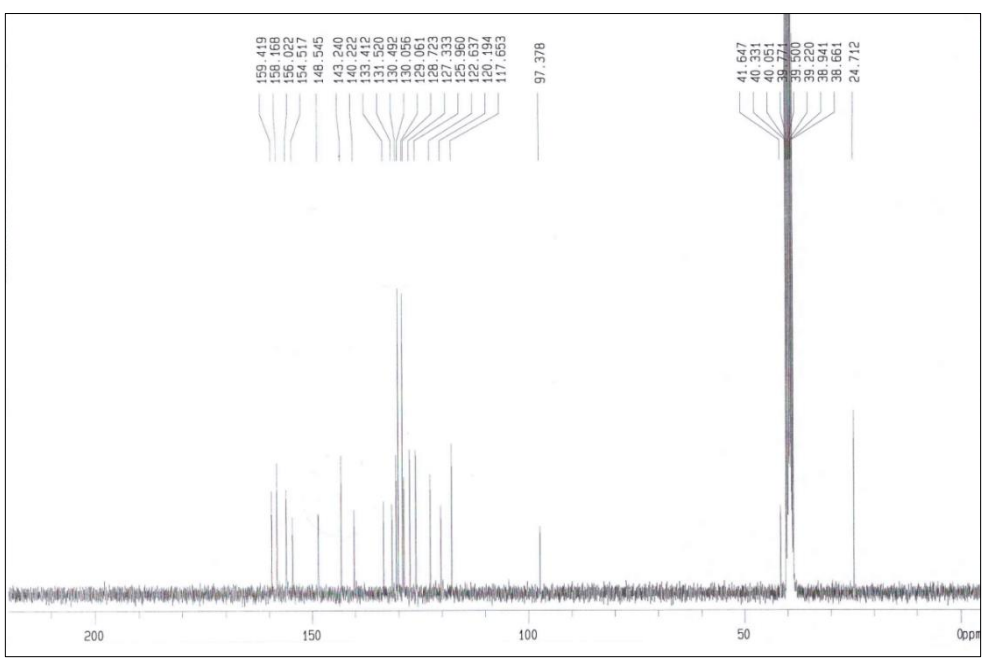
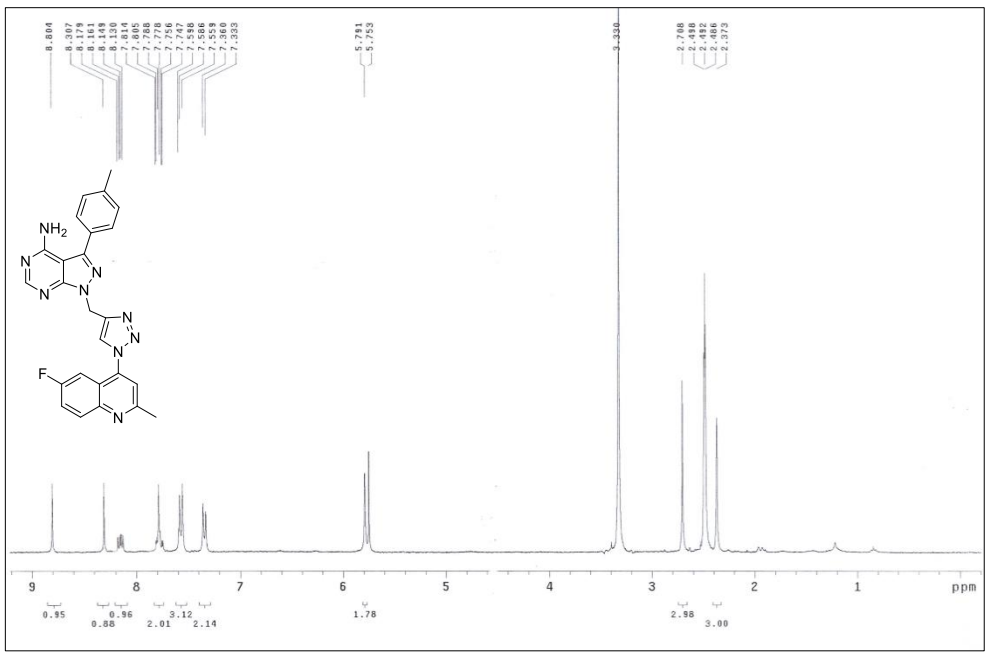
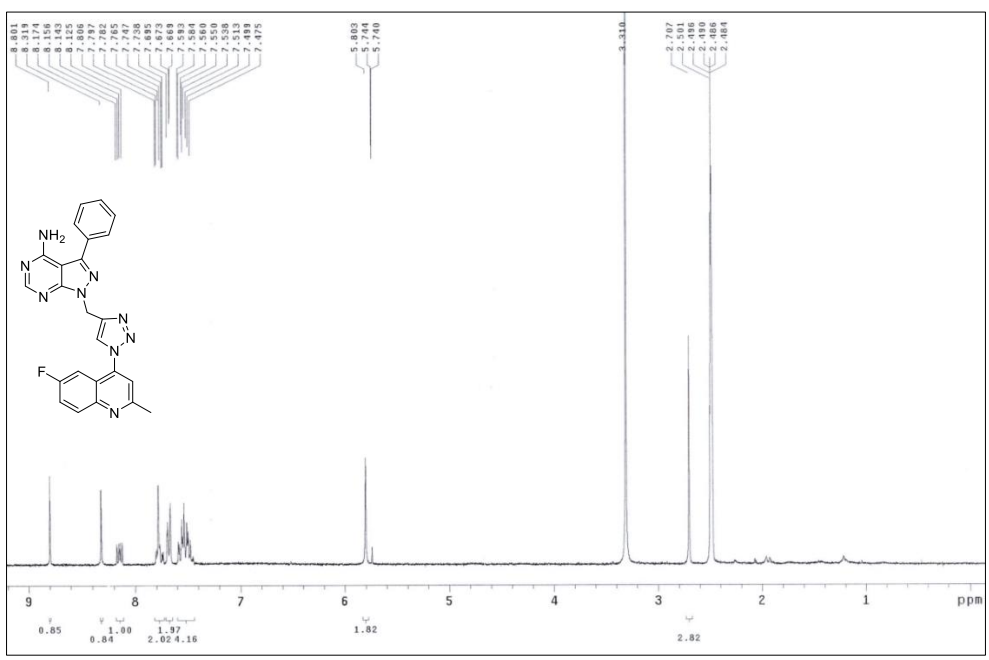


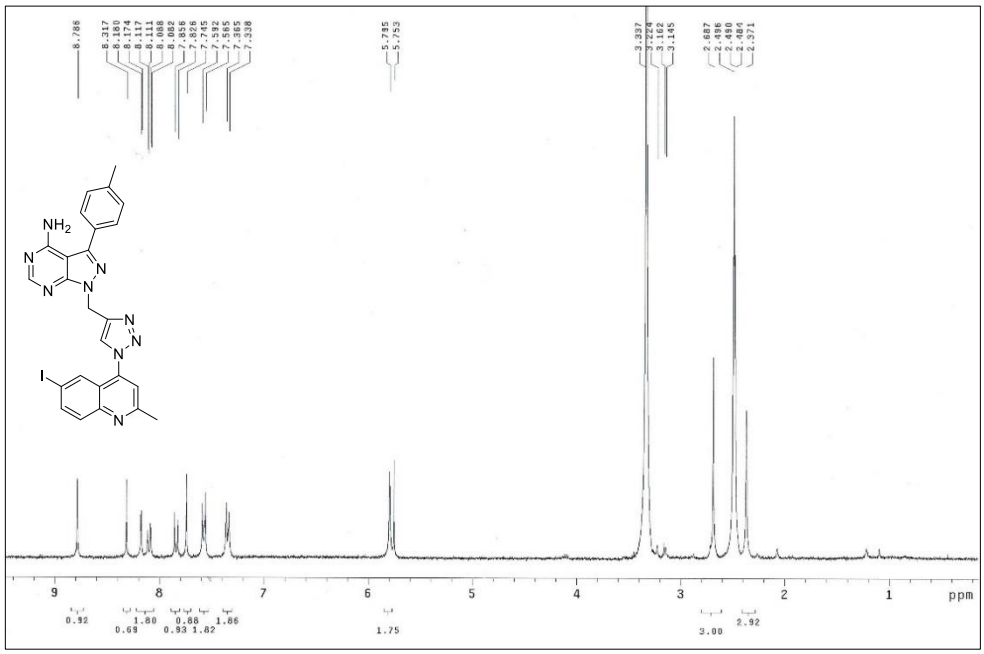
^{1}H NMR of compound **1d** (300 MHz, DMSO-d_6)



^{1}H NMR of compound **1e** (300 MHz, DMSO-d_6)



^{1}H NMR of compound **1f** (300 MHz, DMSO-d_6)



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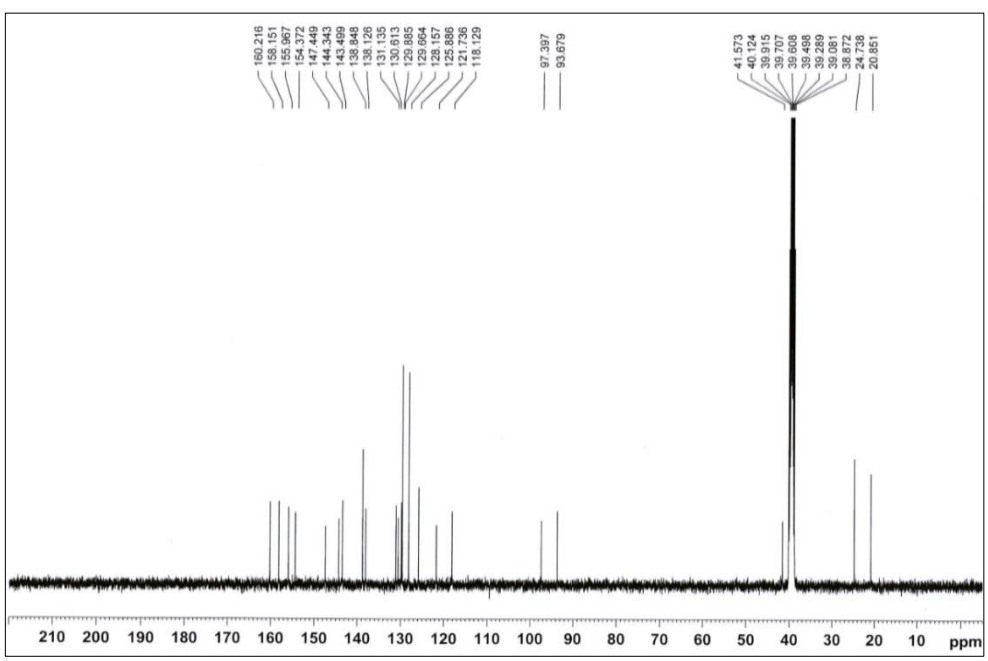

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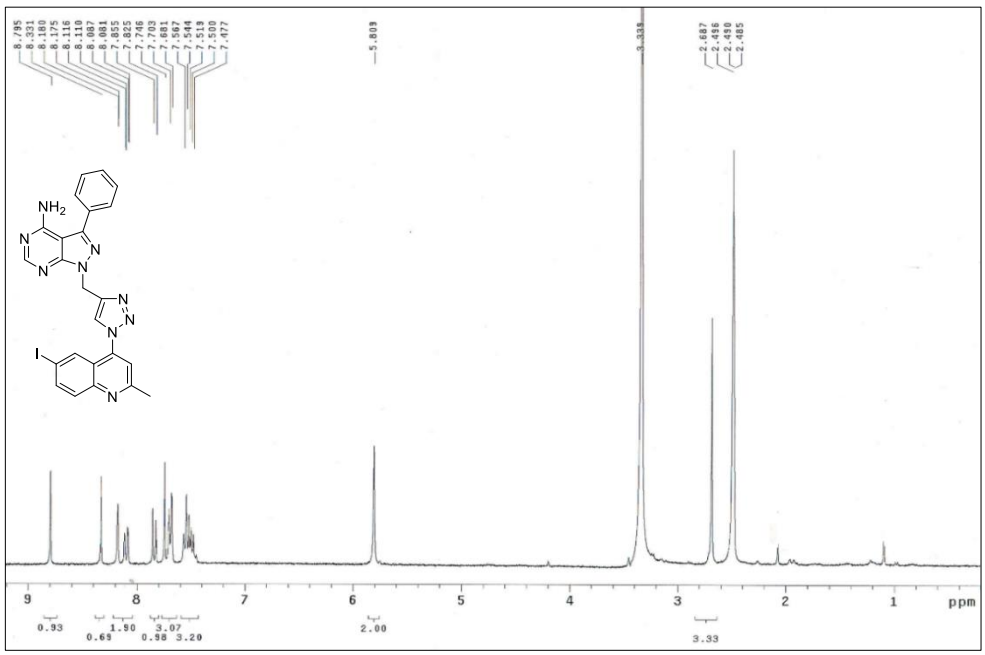

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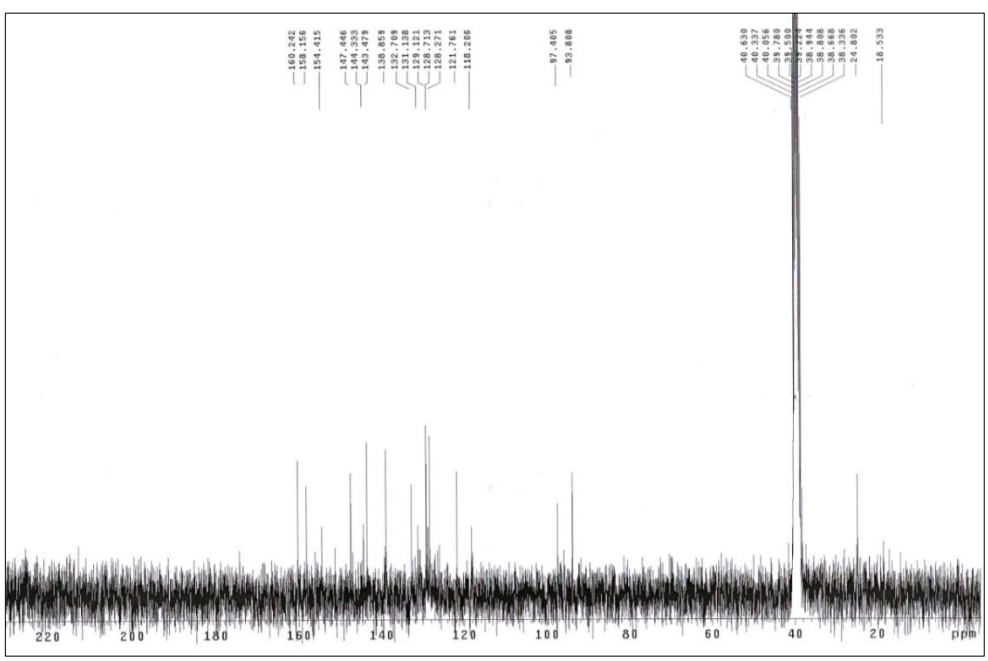

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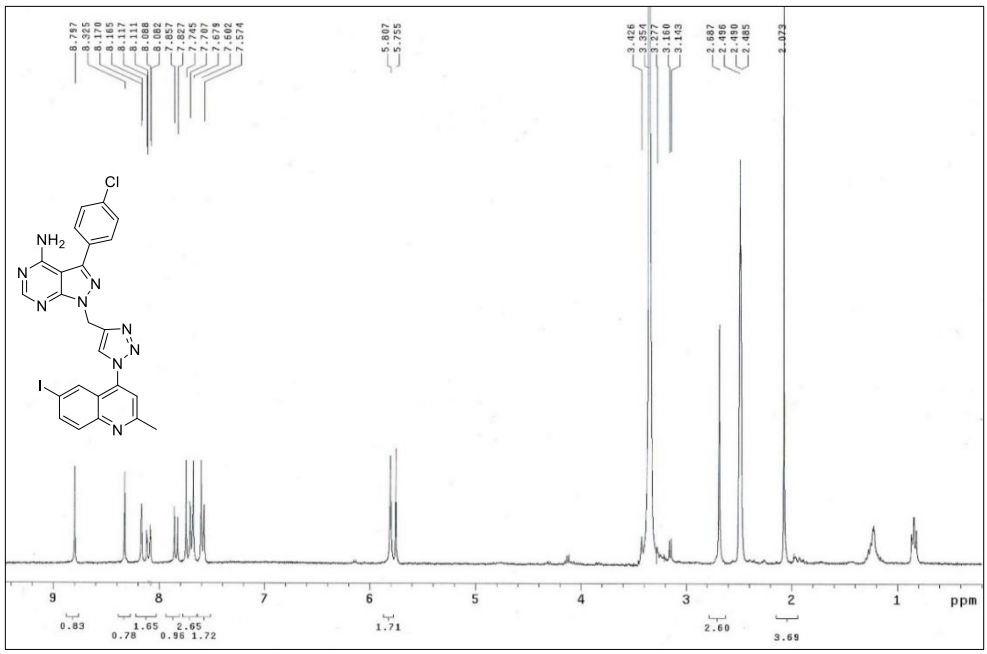

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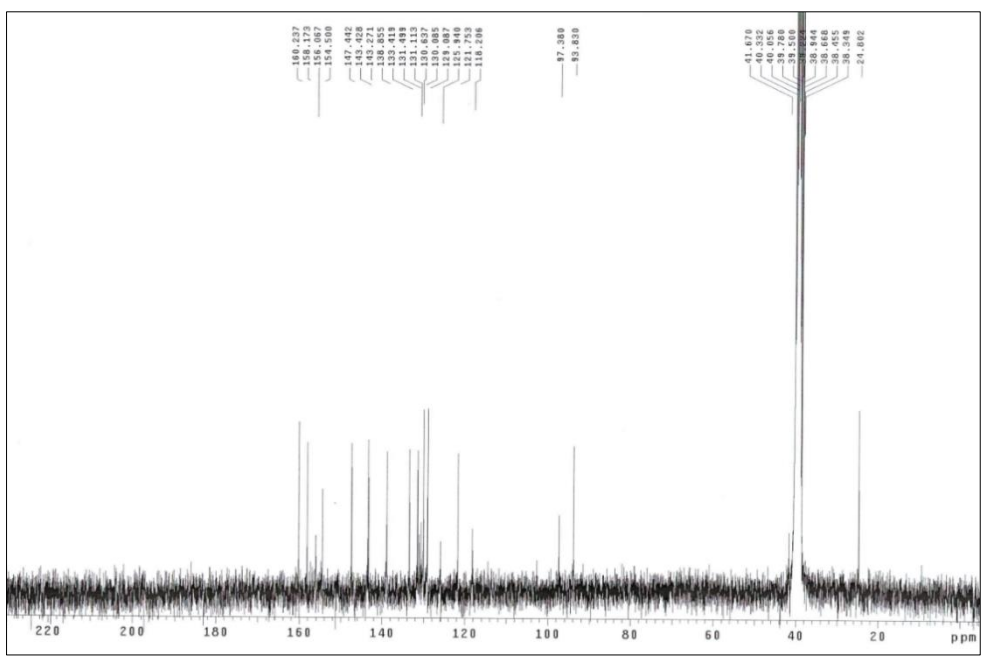
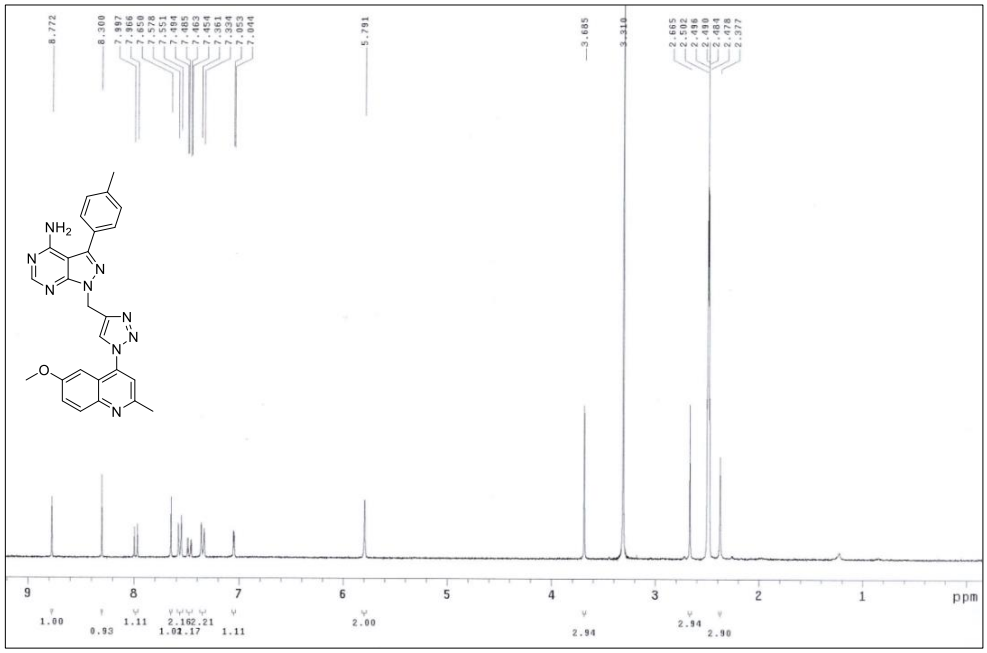


^{1}H NMR of compound **1i** (300 MHz, DMSO-d_6)



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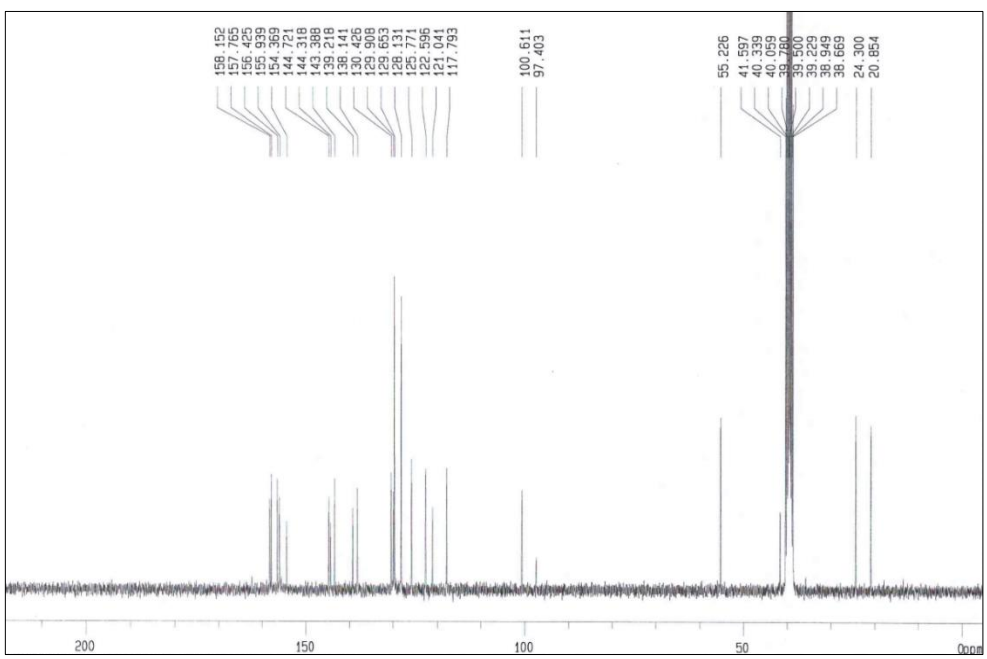

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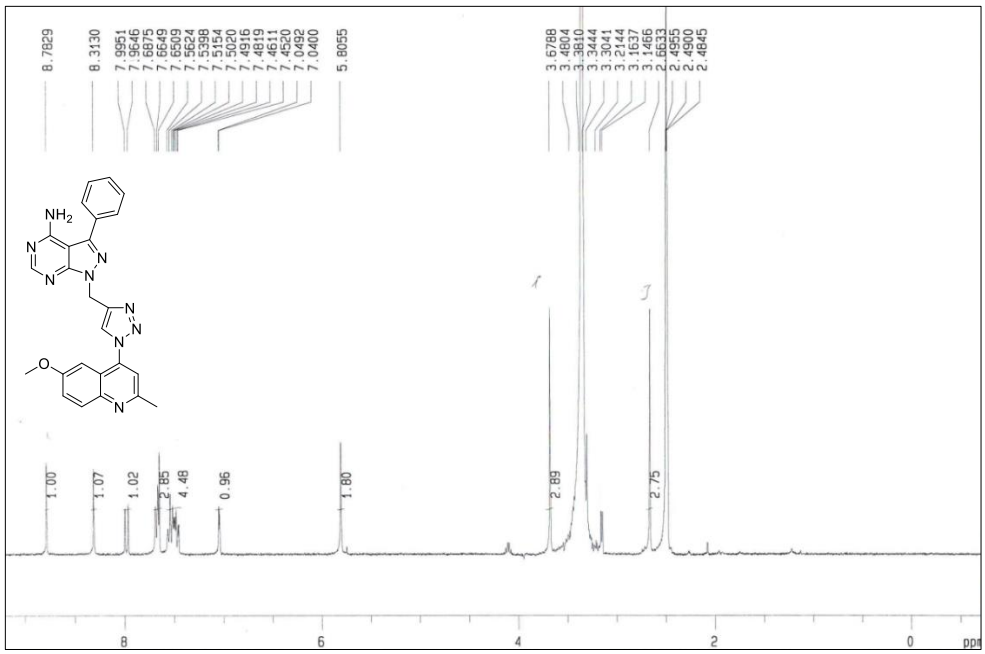

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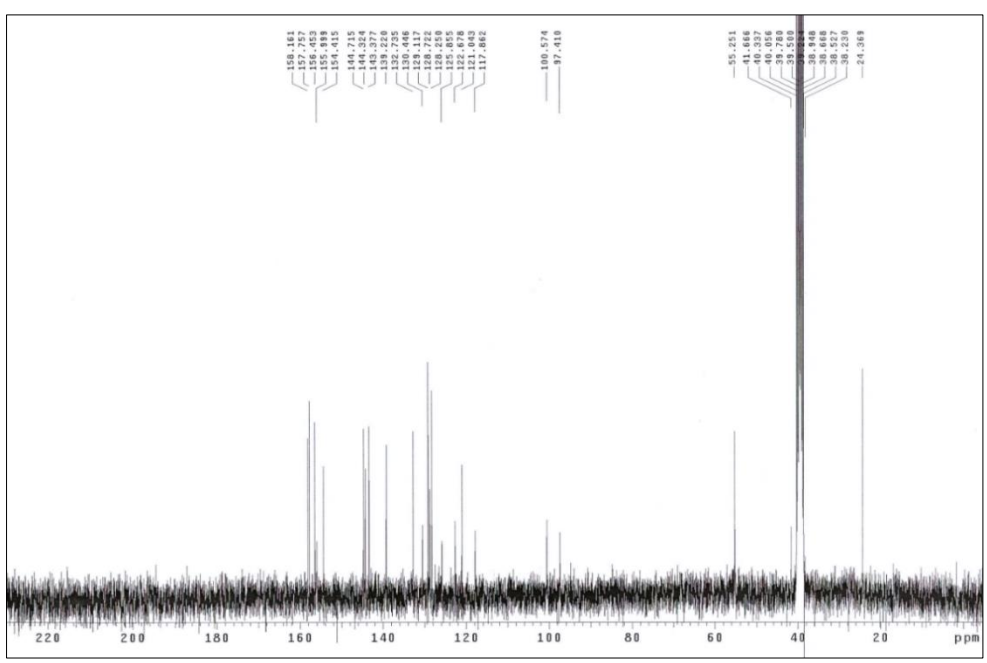

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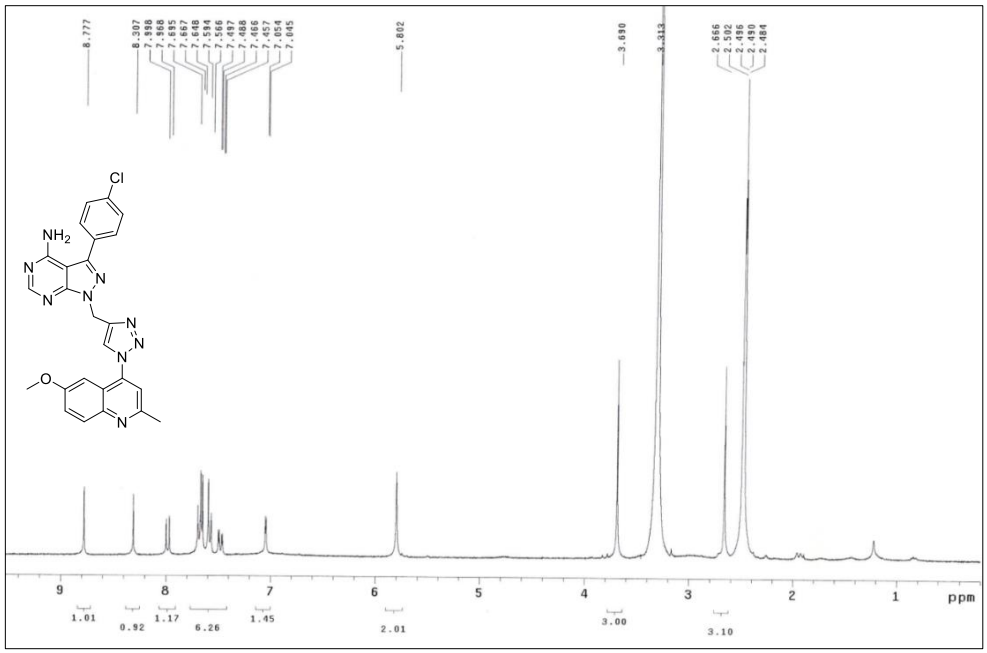

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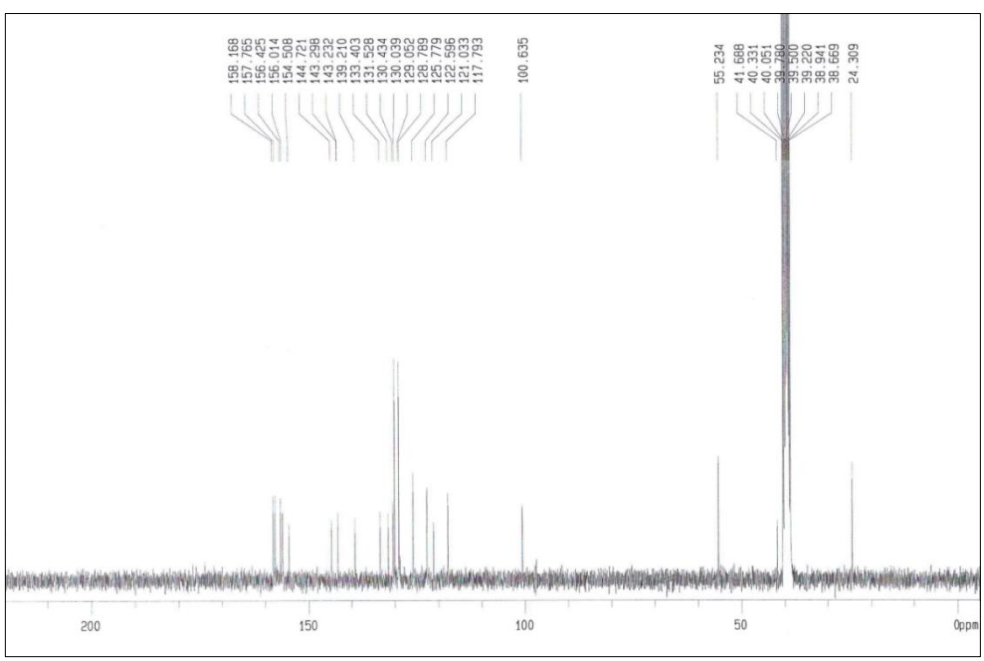
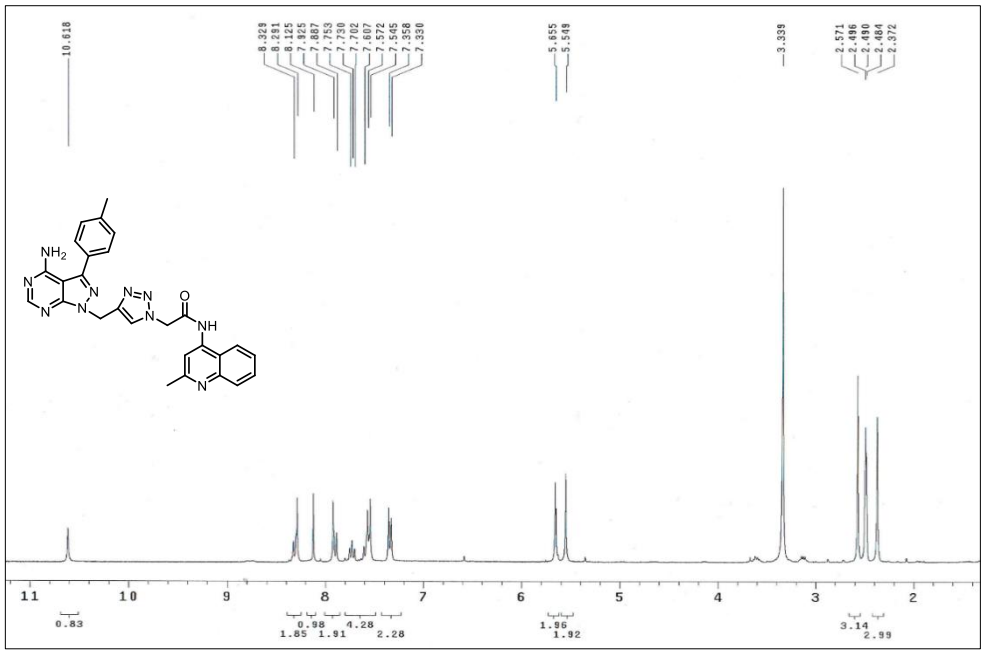


^{1}H NMR of compound **2a** (300 MHz, DMSO-d_6)



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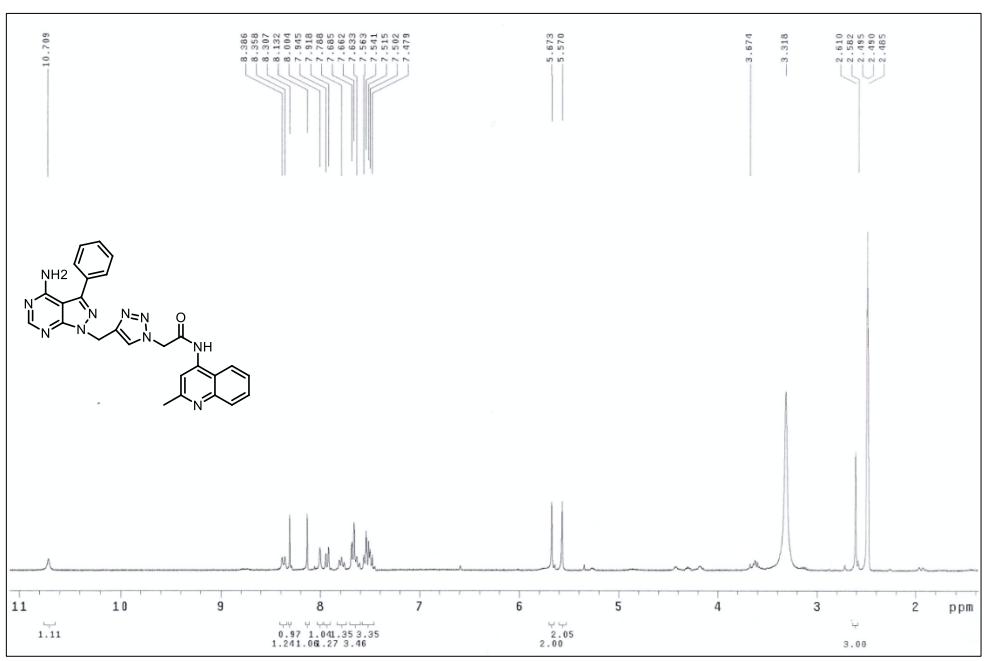
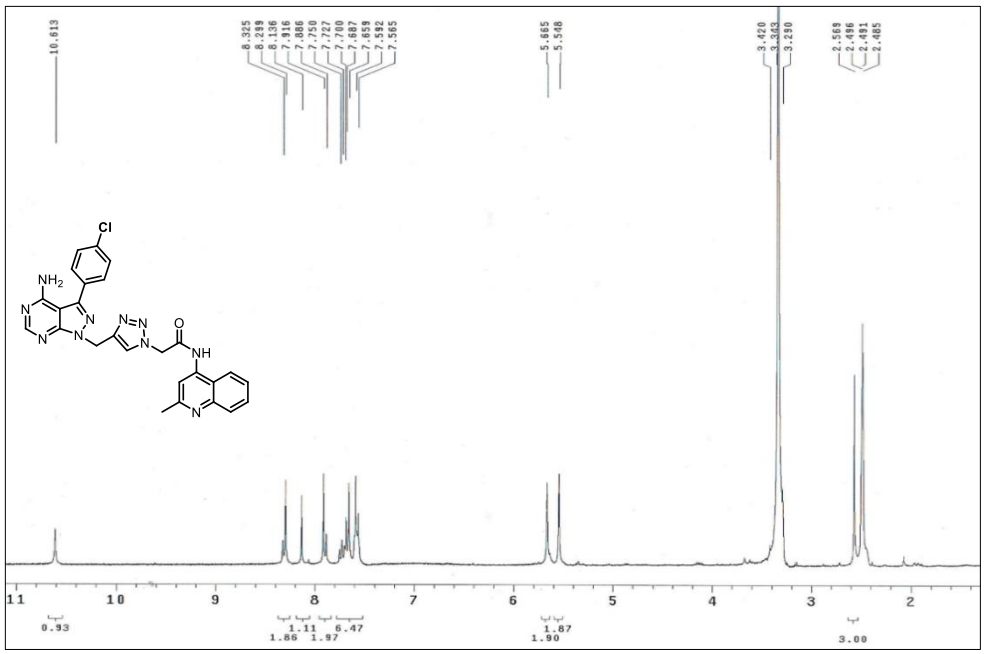
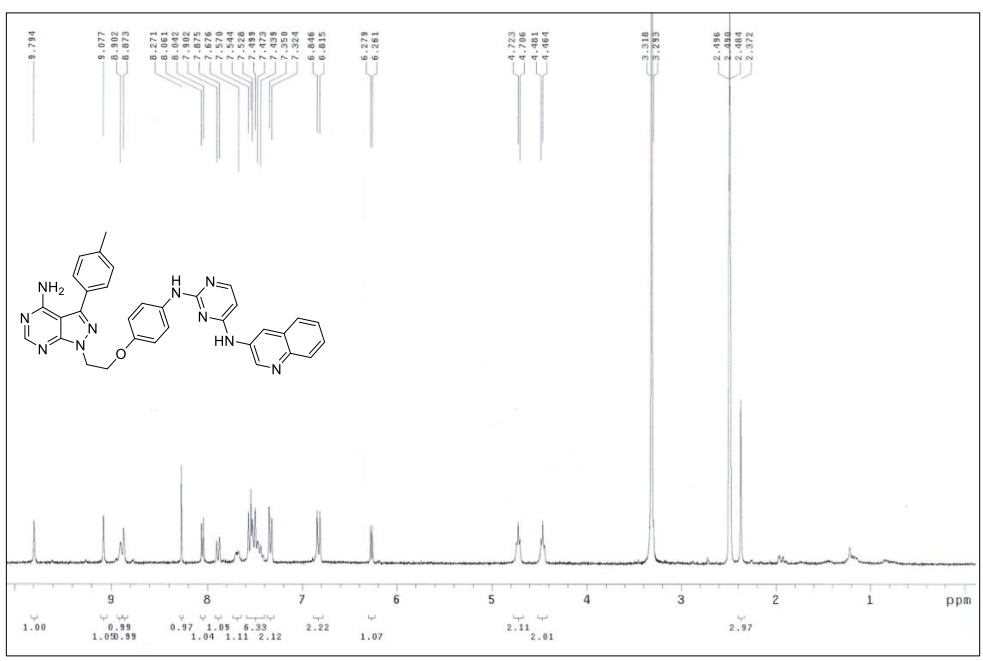


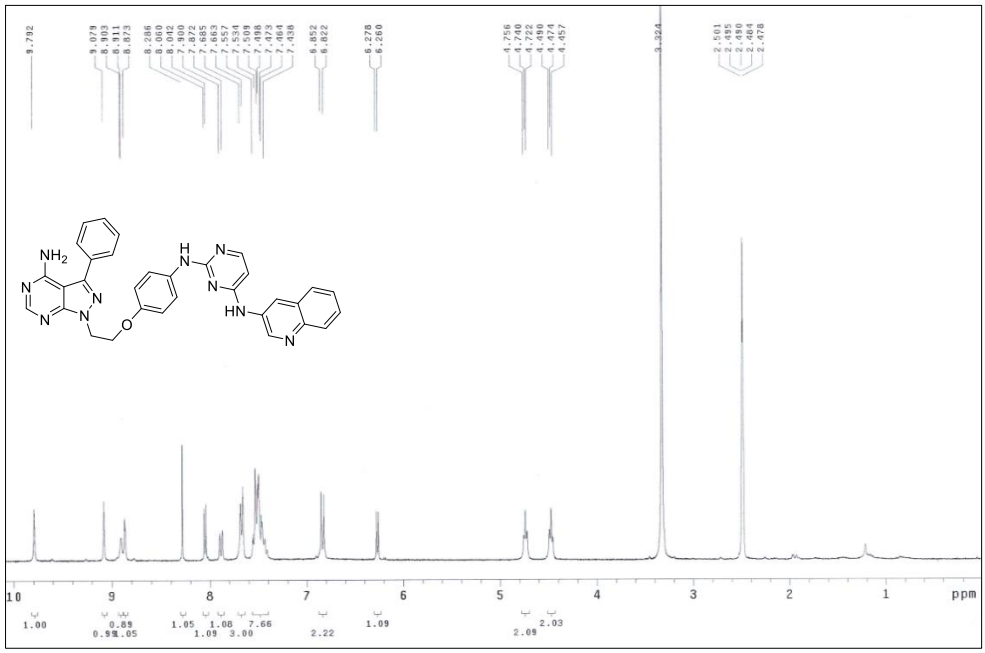
^{1}H NMR of compound **2c** (300 MHz, DMSO-d_6)



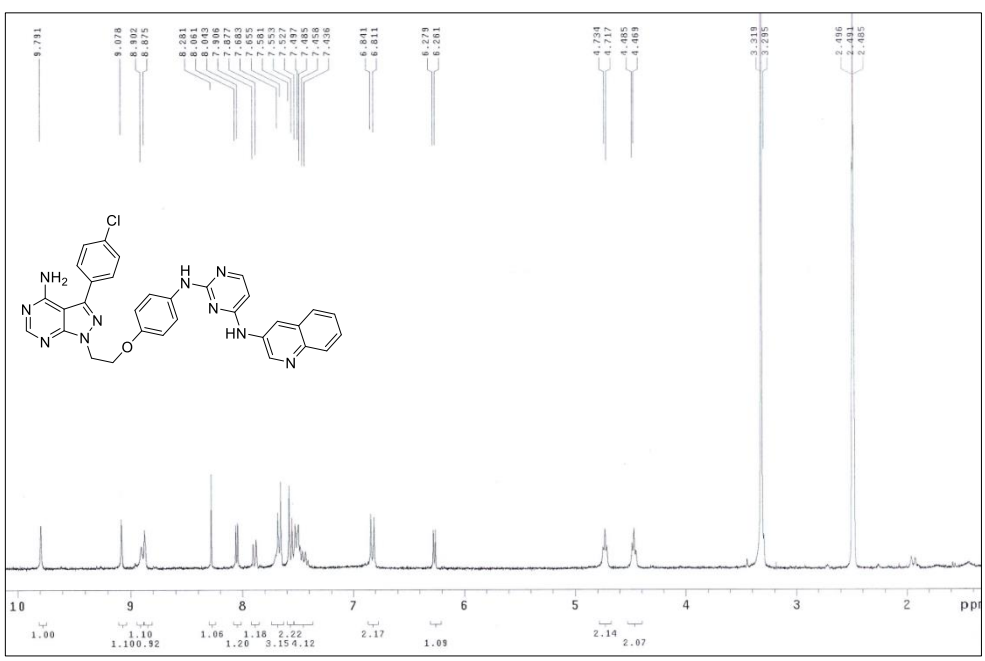
^{1}H NMR of compound **3a** (300 MHz, DMSO-d_6)



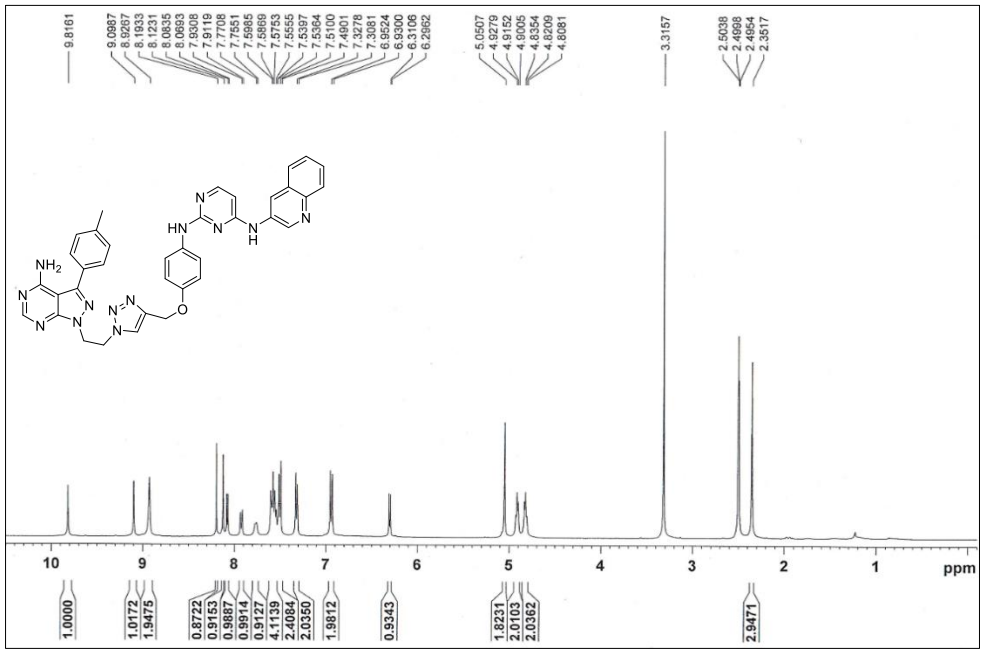
^{1}H NMR of compound **3b** (300 MHz, DMSO-d_6)



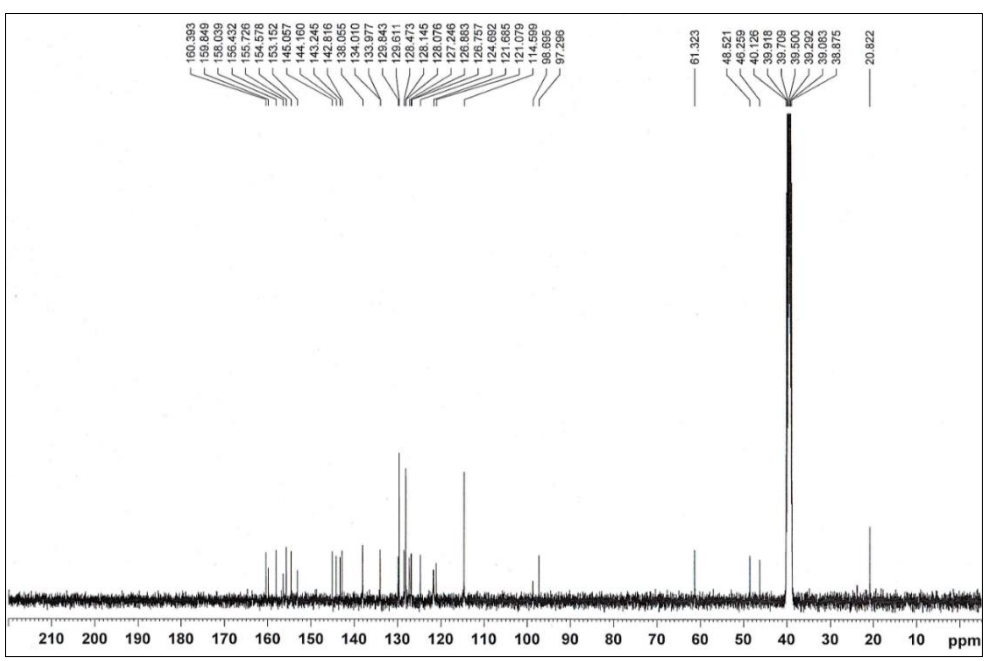
^{1}H NMR of compound **3c** (300 MHz, DMSO-d_6)



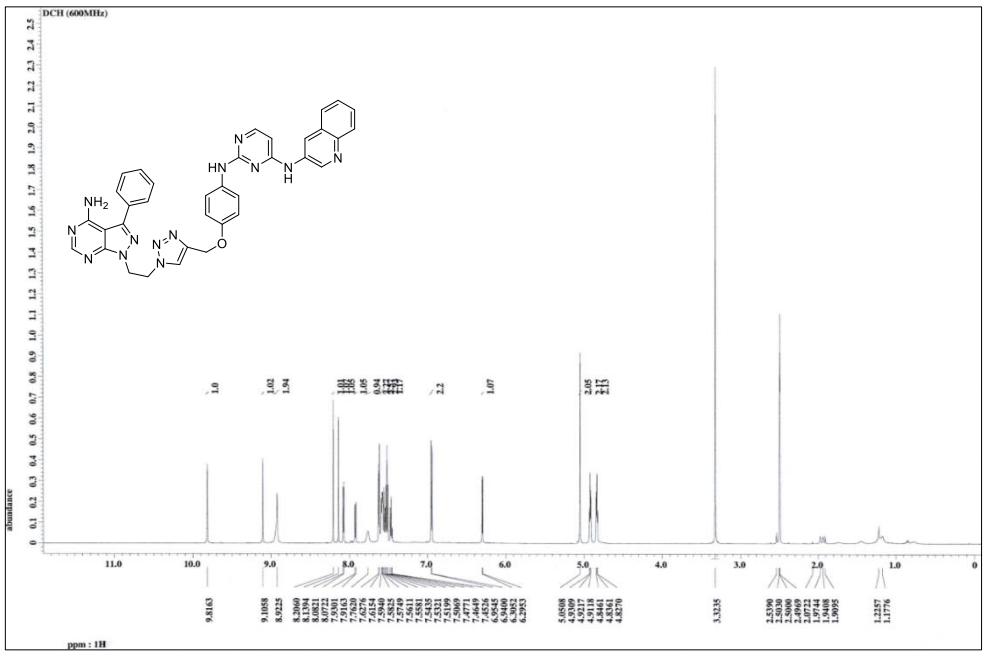
^{1}H NMR of compound **4a** (400 MHz, DMSO-d_6)



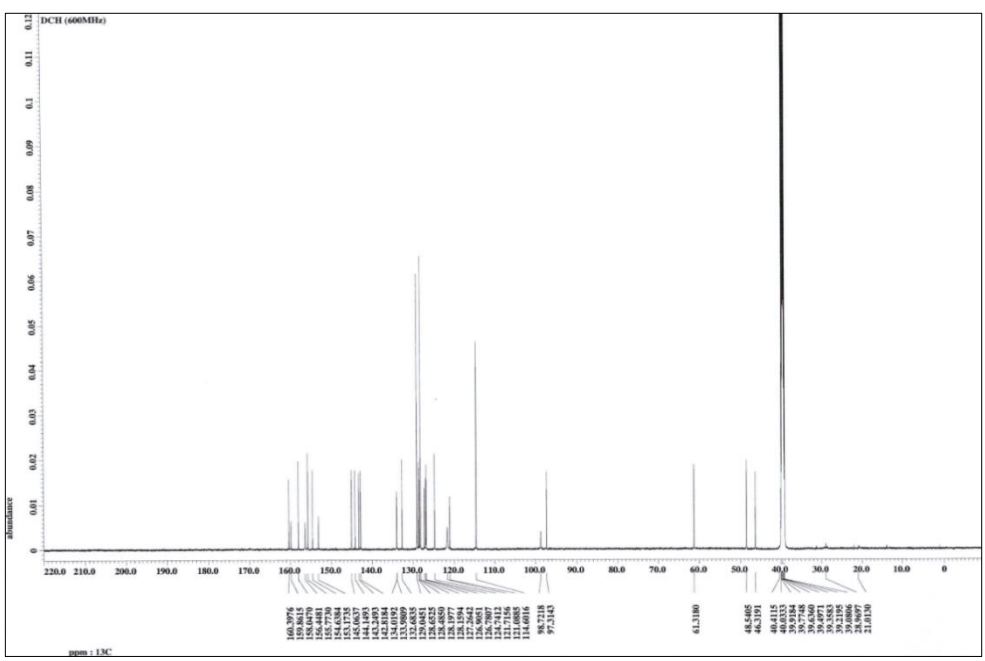
^{13}C NMR of compound **4a** (100 MHz, DMSO-d_6)



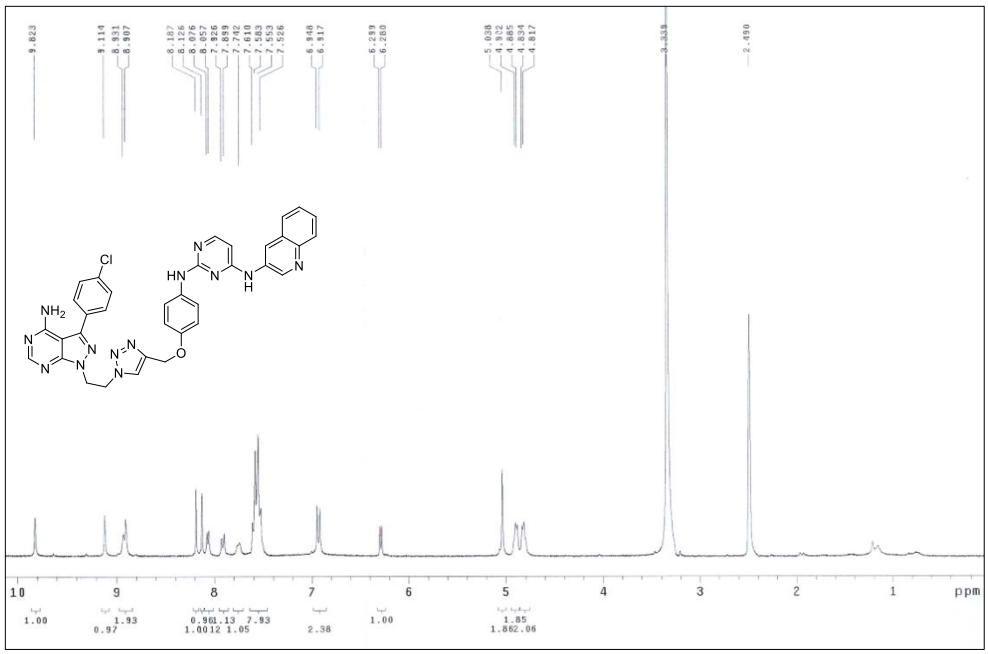
^{1}H NMR of compound **4b** (600 MHz, DMSO-d_6)



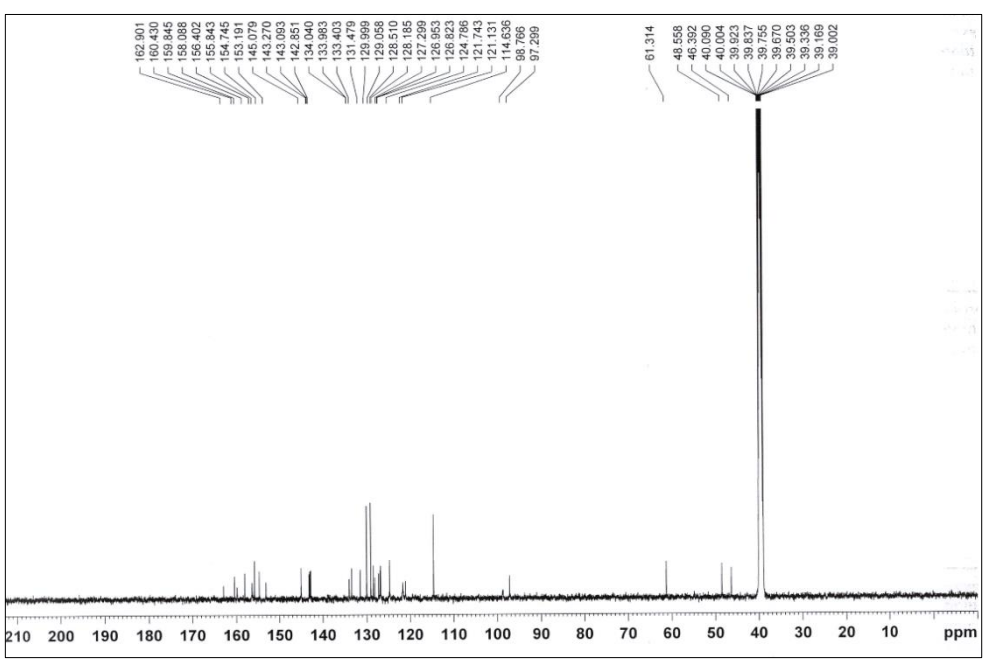
^{13}C NMR of compound **4b** (150 MHz, DMSO-d_6)



^{1}H NMR of compound **4c** (300 MHz, DMSO-d_6)

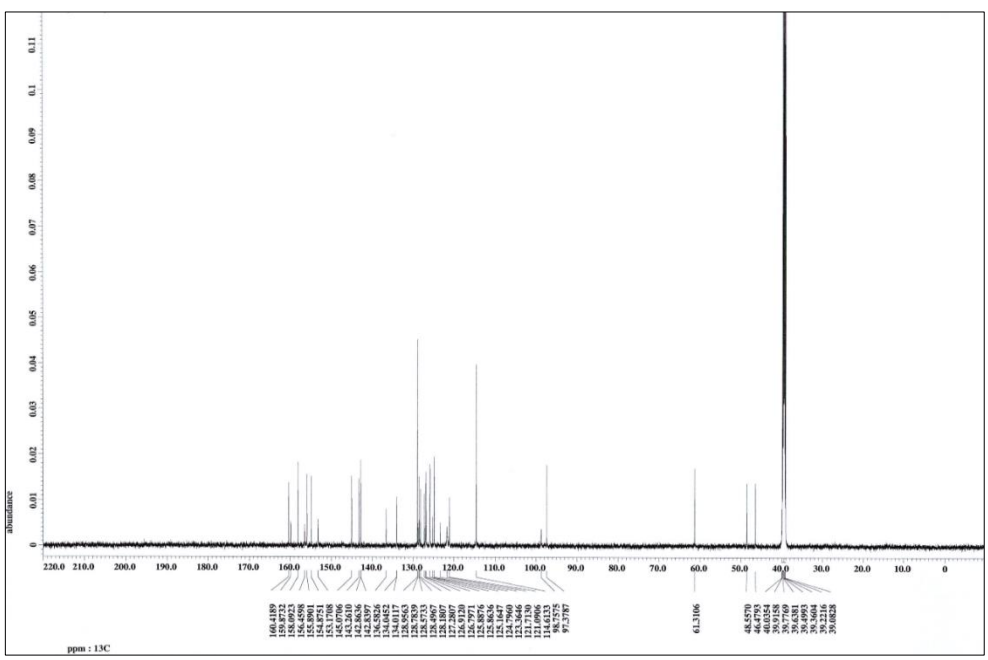


^{13}C NMR of compound **4c** (125 MHz, DMSO-d_6)

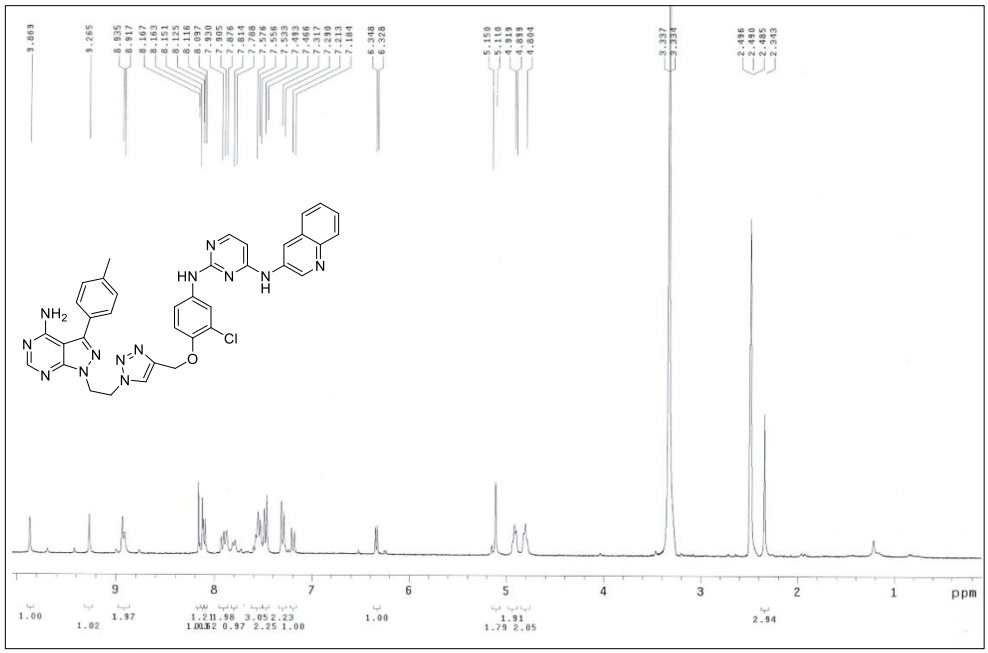


^{1}H NMR of compound **4d** (600 MHz, DMSO-d_6)

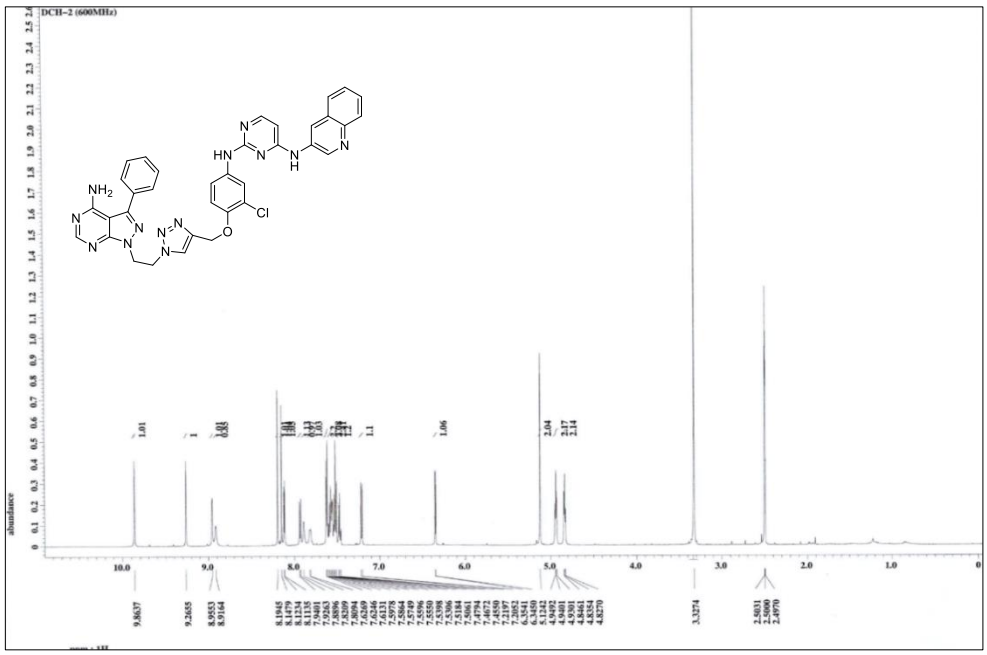
^{13}C NMR of compound **4d** (150 MHz, DMSO-d_6)



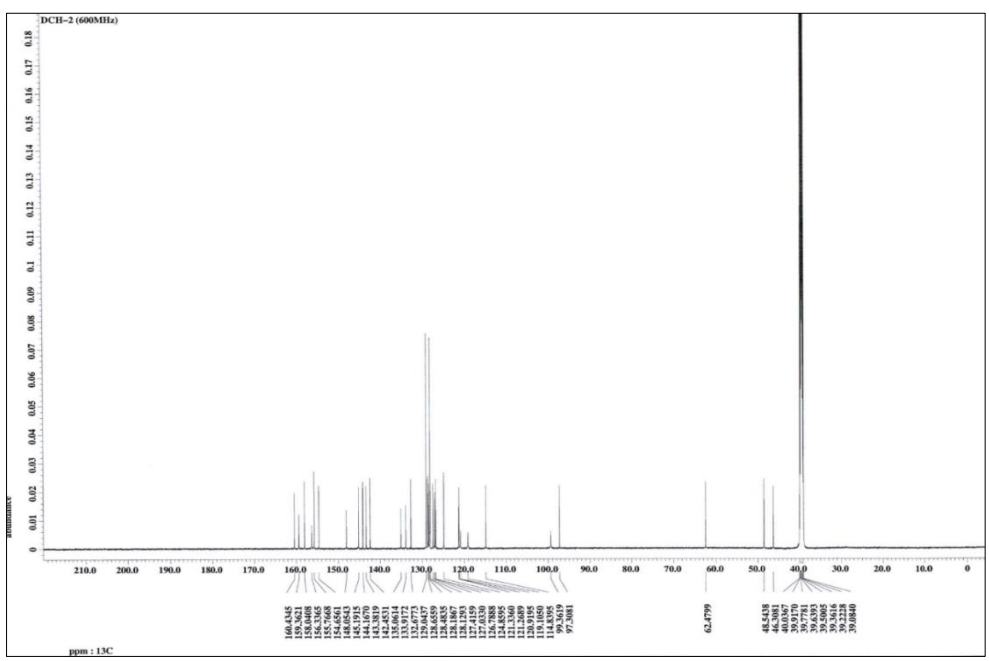
^{1}H NMR of compound **4e** (300 MHz, DMSO-d_6)



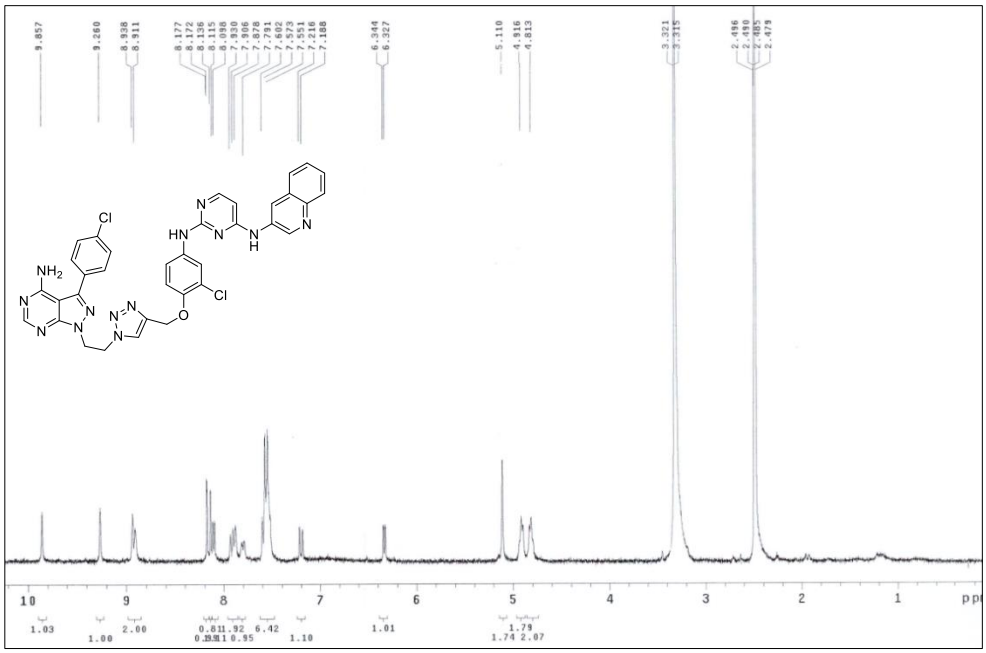
^{1}H NMR of compound **4f** (600 MHz, DMSO-d_6)



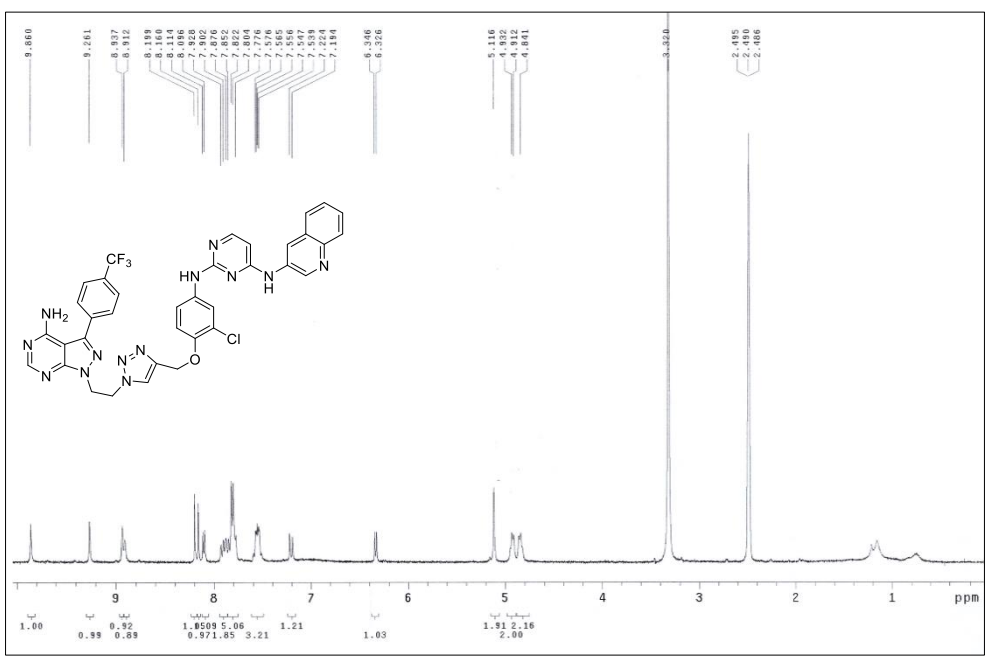
^{13}C NMR of compound **4f** (150 MHz, DMSO-d_6)



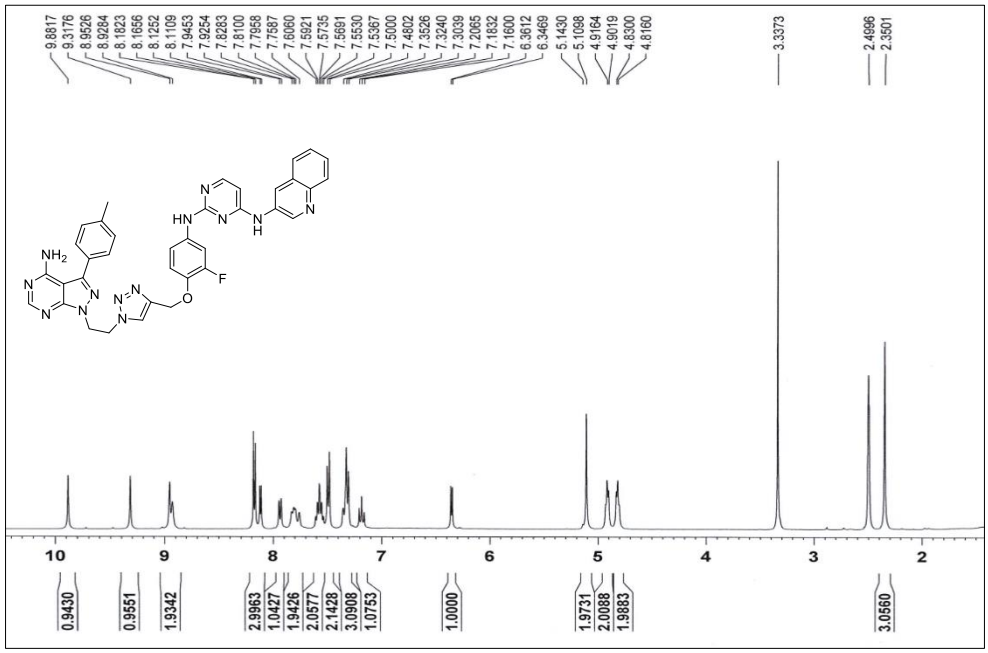
^{1}H NMR of compound **4g** (300 MHz, DMSO-d_6)



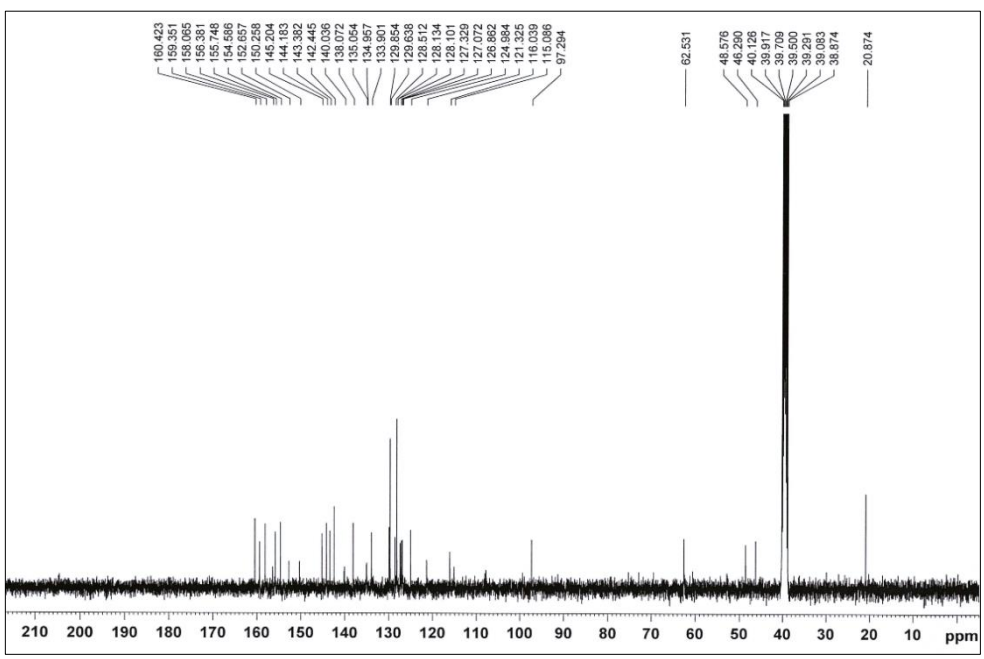
^{1}H NMR of compound **4h** (300 MHz, DMSO-d_6)



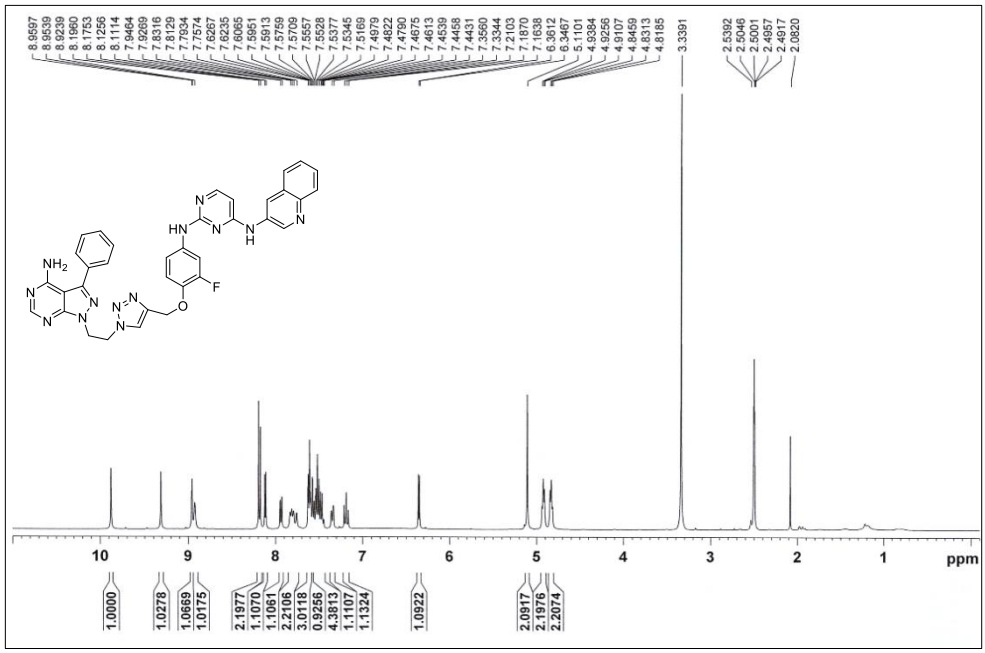
^{1}H NMR of compound **4i** (400 MHz, DMSO-d_6)



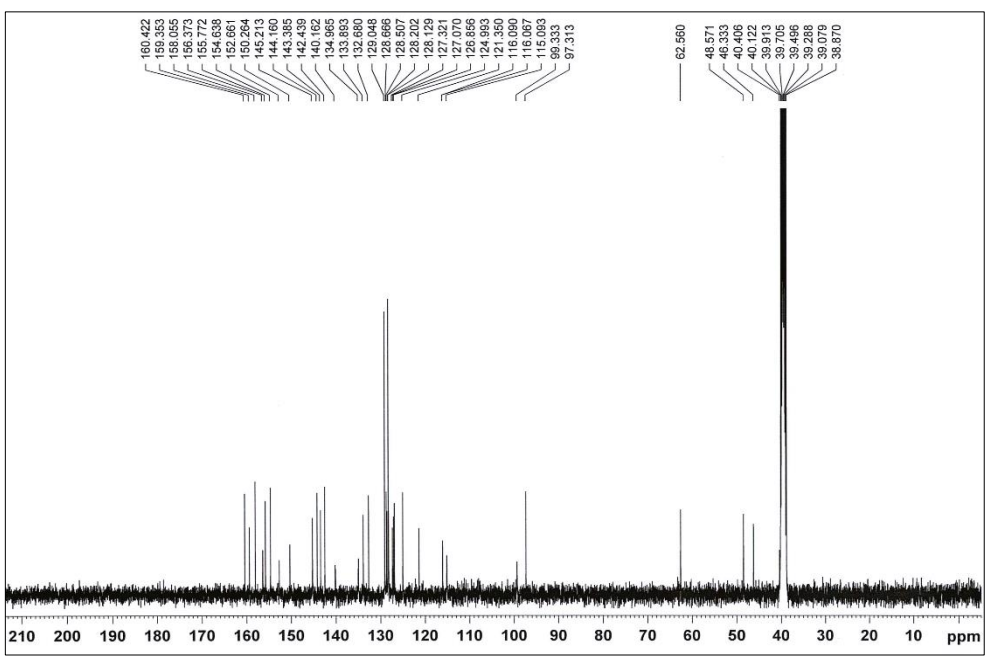
^{13}C NMR of compound **4i** (100 MHz, DMSO-d_6)



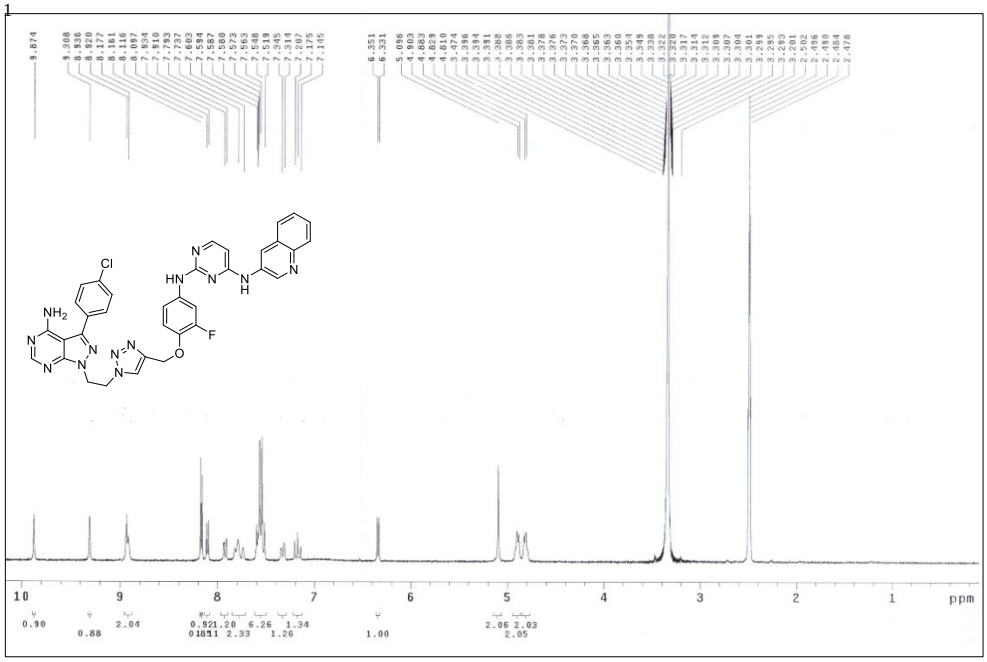
^{1}H NMR of compound **4j** (400 MHz, DMSO-d_6)



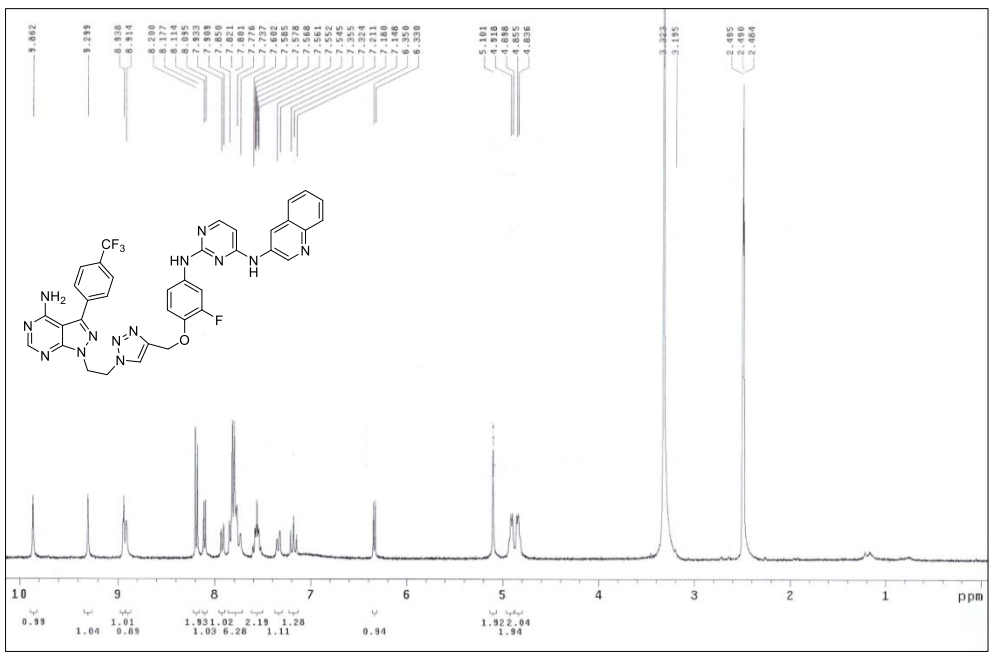
^{13}C NMR of compound **4j** (100 MHz, DMSO-d_6)



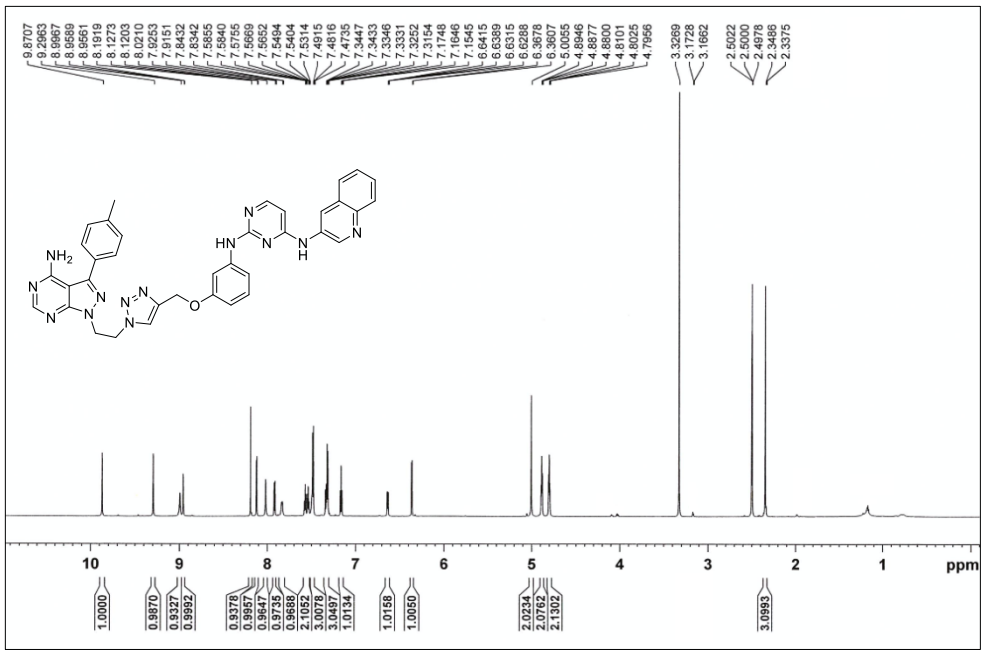
^{1}H NMR of compound **4k** (300 MHz, DMSO-d_6)



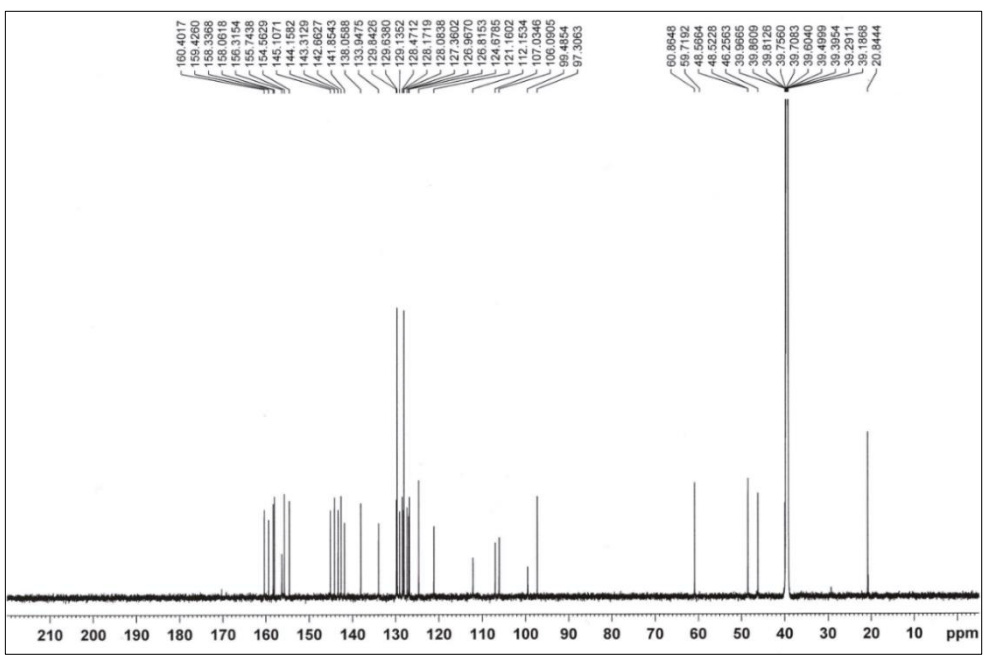
^{1}H NMR of compound **4l** (300 MHz, DMSO-d_6)



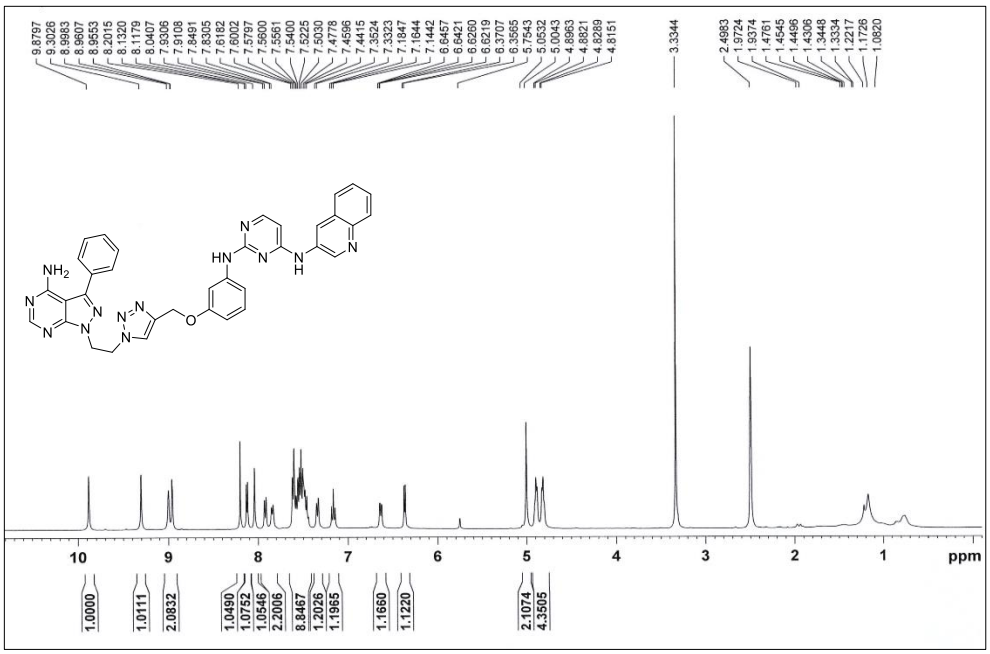
^{1}H NMR of compound **5a** (800 MHz, DMSO-d_6)



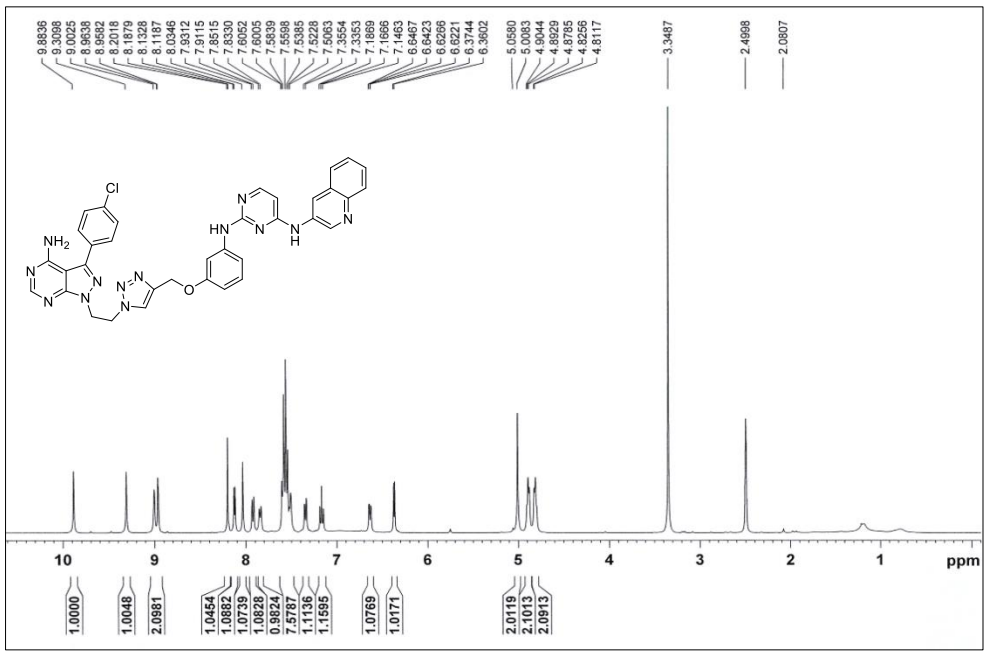
^{13}C NMR of compound **5a** (200 MHz, DMSO-d_6)



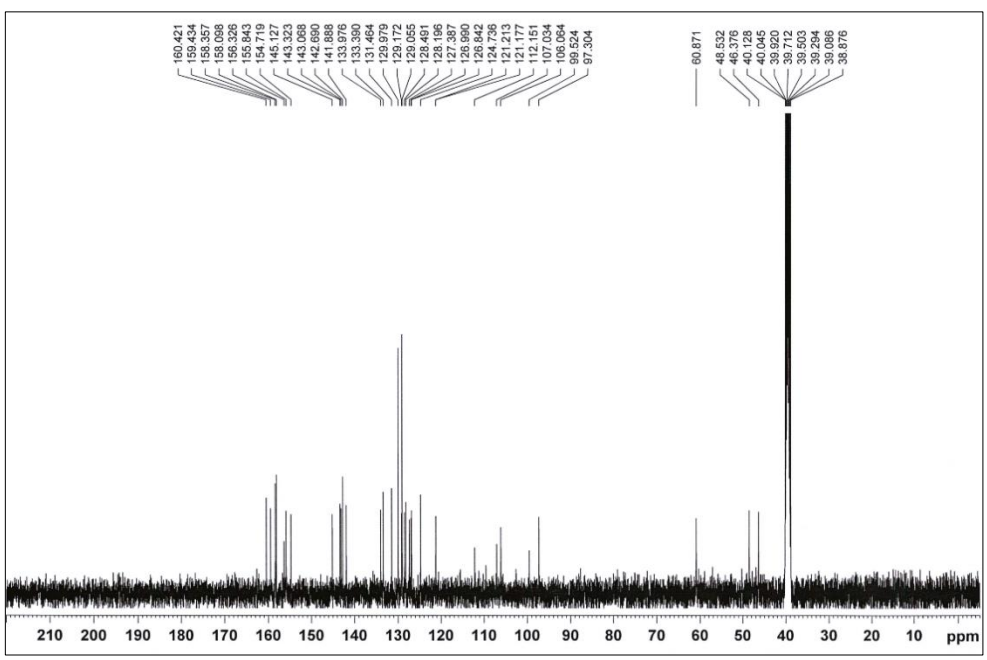
^{1}H NMR of compound **5b** (400 MHz, DMSO-d_6)



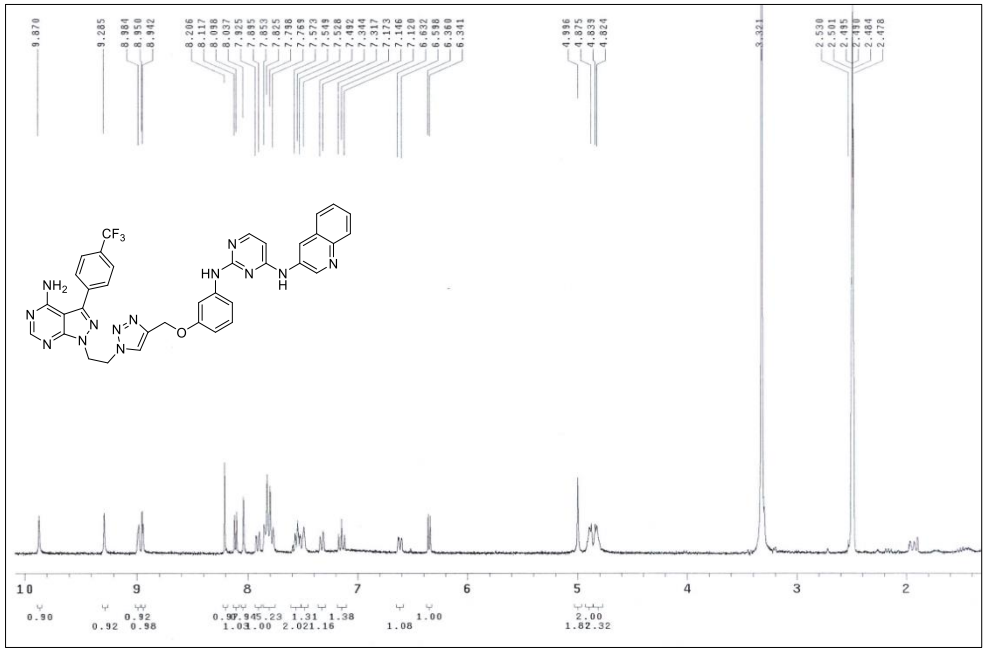
^{1}H NMR of compound **5c** (400 MHz, DMSO-d_6)



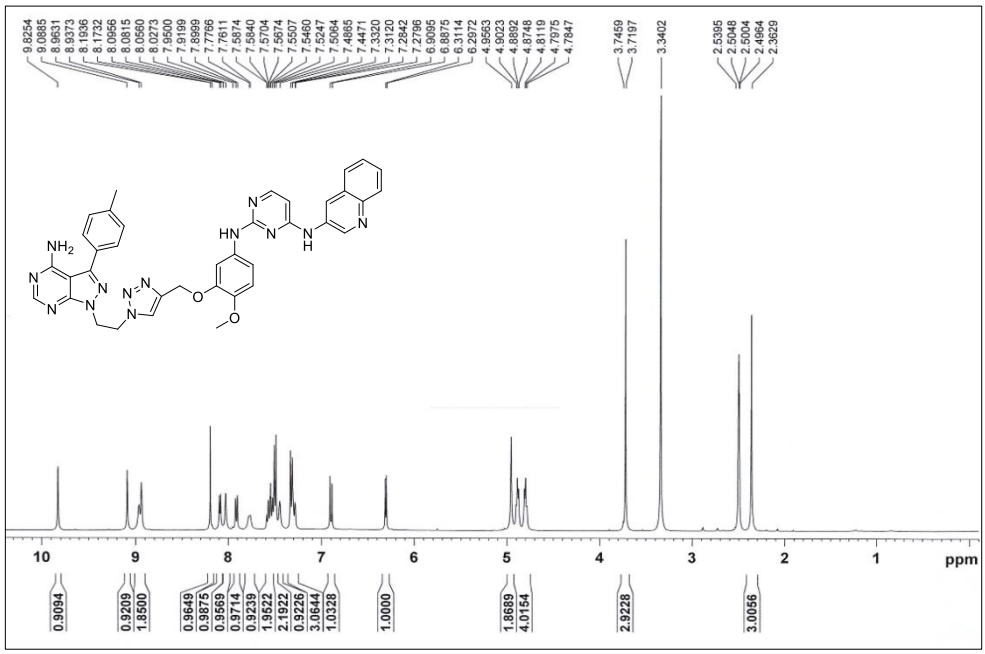
^{13}C NMR of compound **5c** (100 MHz, DMSO-d_6)



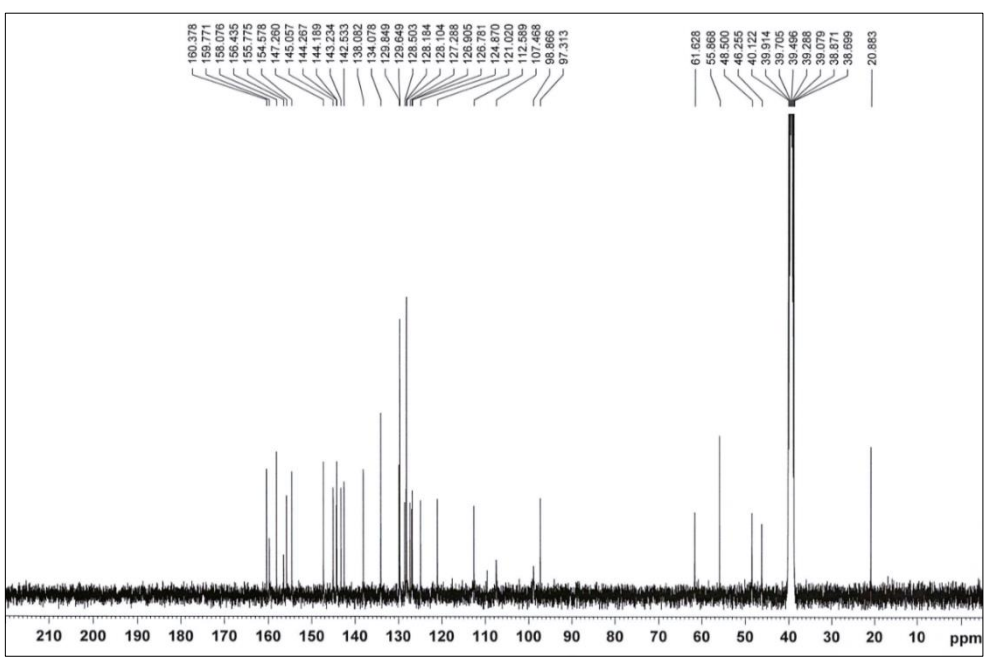
^{1}H NMR of compound **5d** (300 MHz, DMSO-d_6)



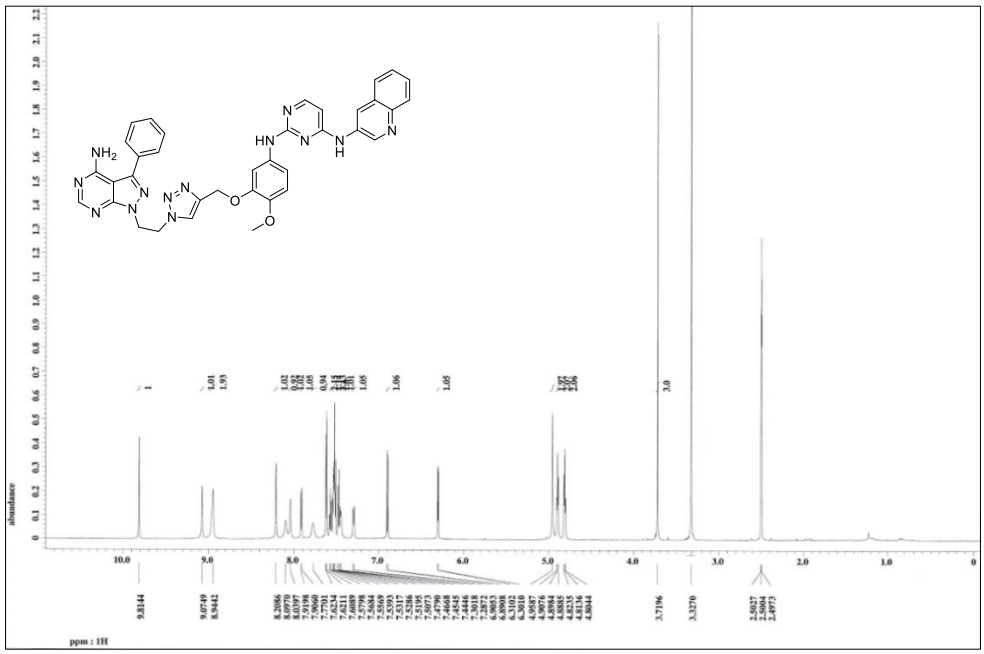
^{1}H NMR of compound **5e** (400 MHz, DMSO-d_6)



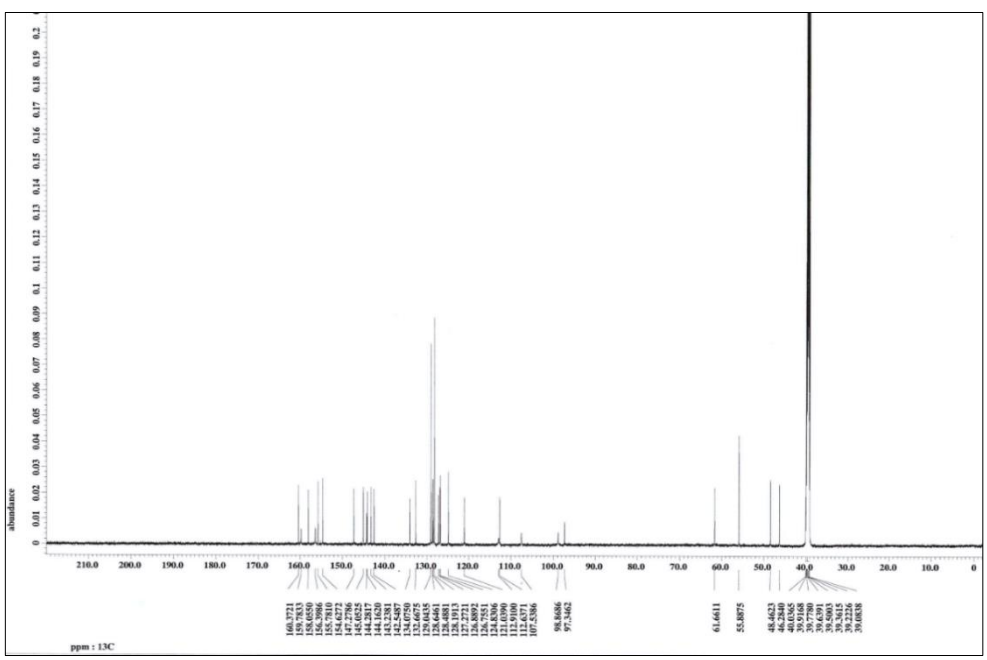
^{13}C NMR of compound **5e** (100 MHz, DMSO-d_6)



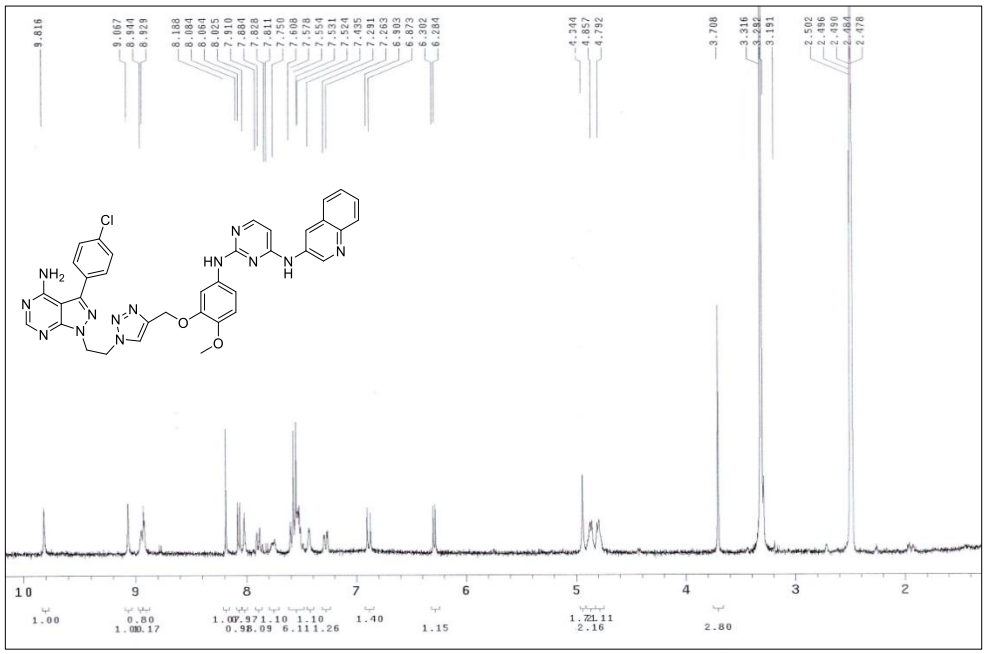
^{1}H NMR of compound **5f** (600 MHz, DMSO-d_6)



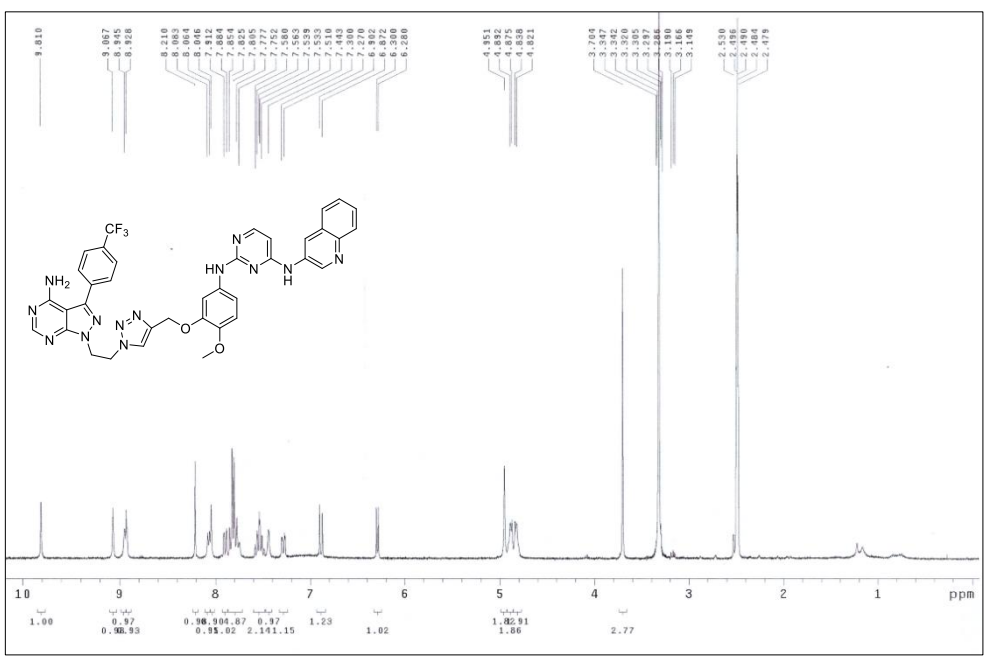
^{13}C NMR of compound **5f** (150 MHz, DMSO-d_6)



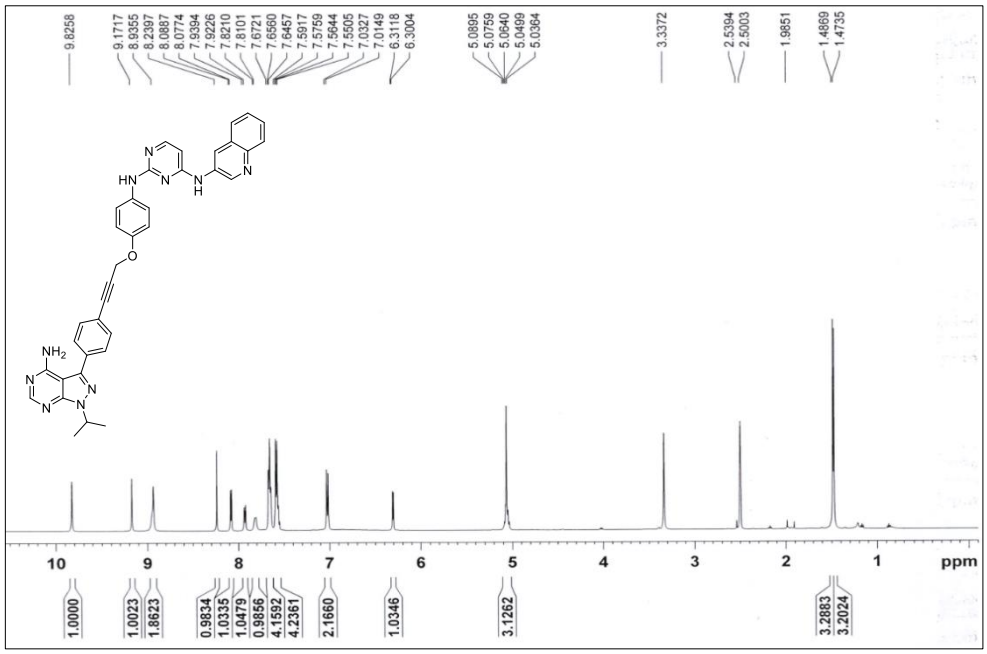
^{1}H NMR of compound **5g** (300 MHz, DMSO-d_6)



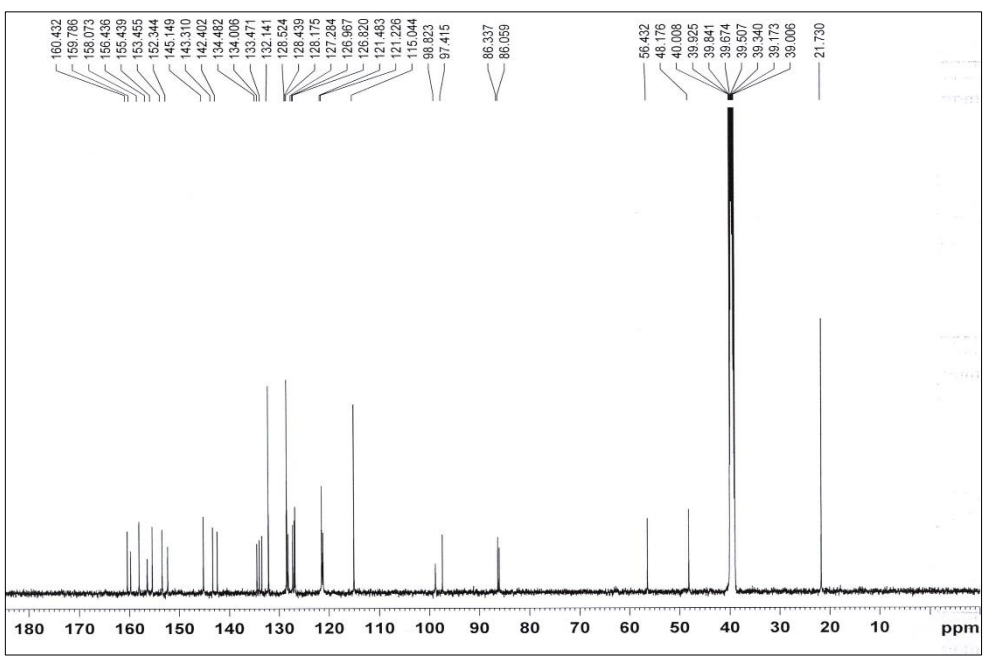
^{1}H NMR of compound **5h** (300 MHz, DMSO-d_6)



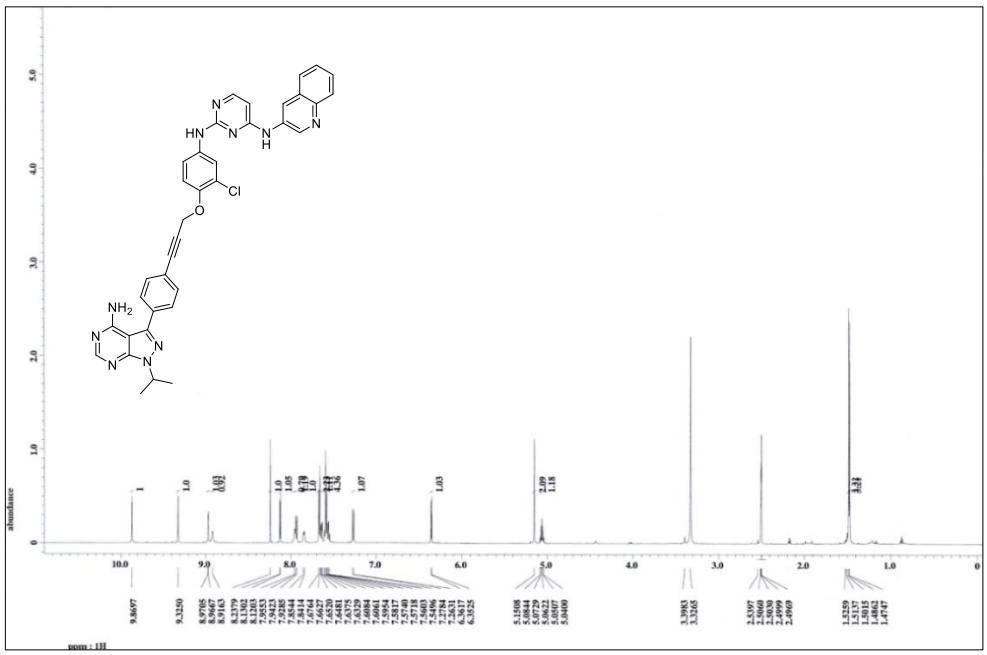
^{1}H NMR of compound **6a** (600 MHz, DMSO-d_6)



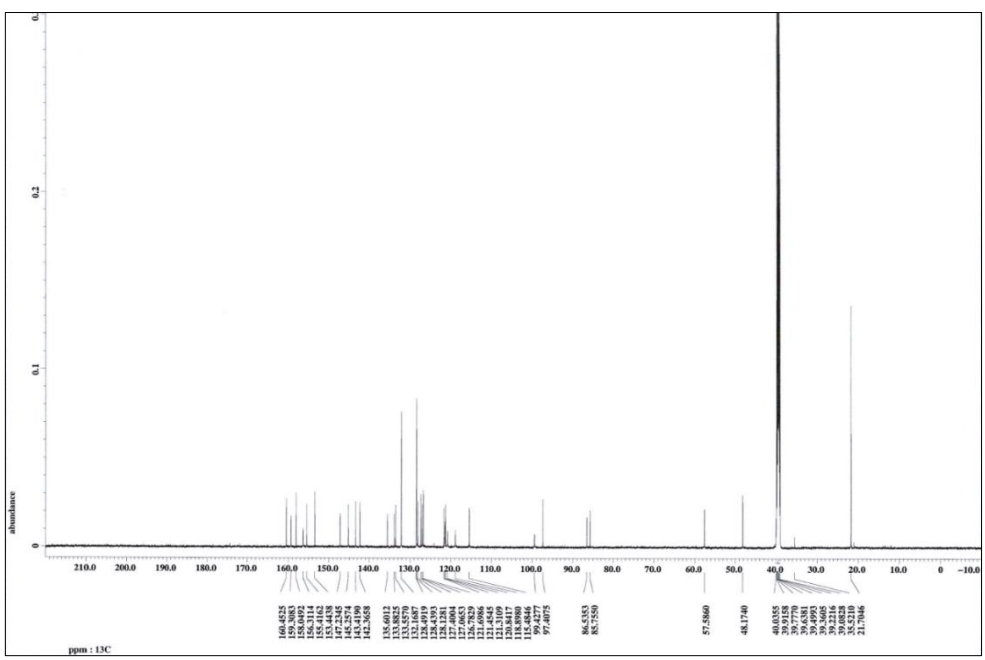
^{13}C NMR of compound **6a** (150 MHz, DMSO-d_6)



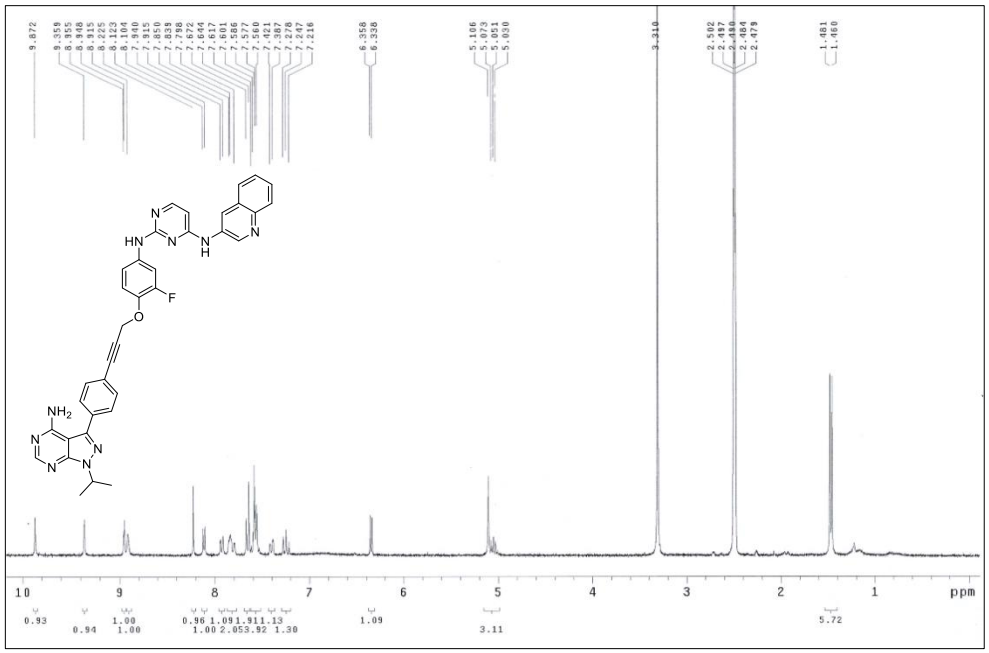
^{1}H NMR of compound **6b** (600 MHz, DMSO-d_6)



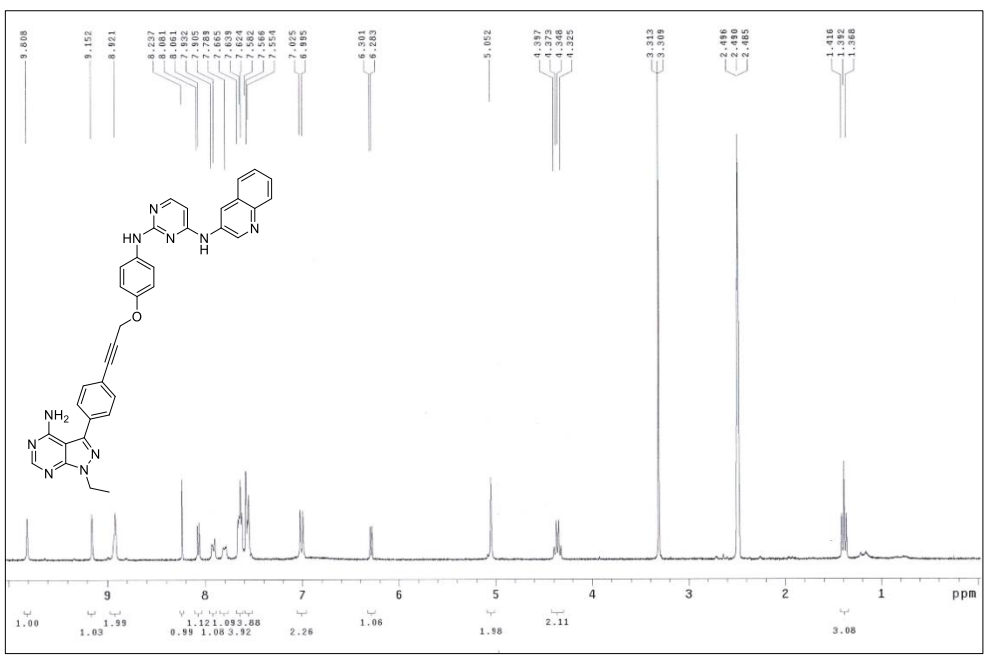
^{13}C NMR of compound **6b** (150 MHz, DMSO-d_6)



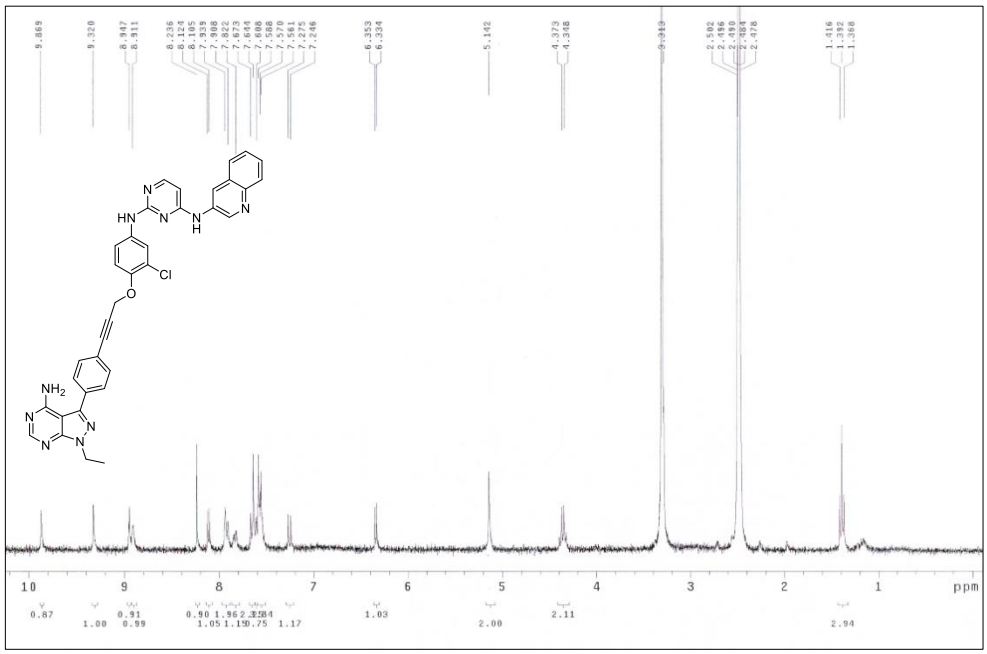
^{1}H NMR of compound **6c** (300 MHz, DMSO-d_6)



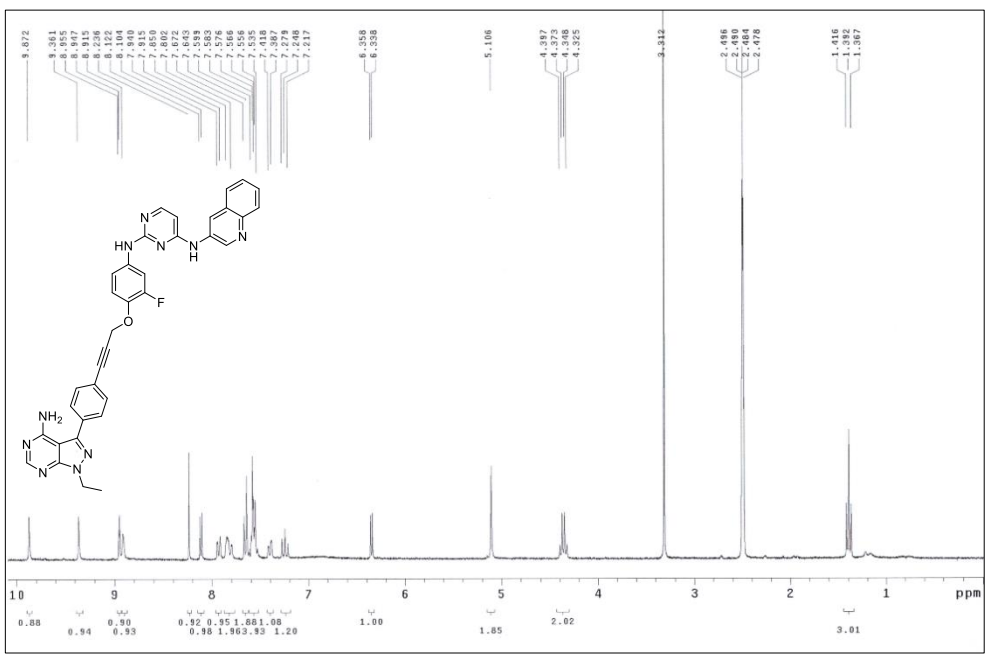
^{1}H NMR of compound **6d** (300 MHz, DMSO-d_6)



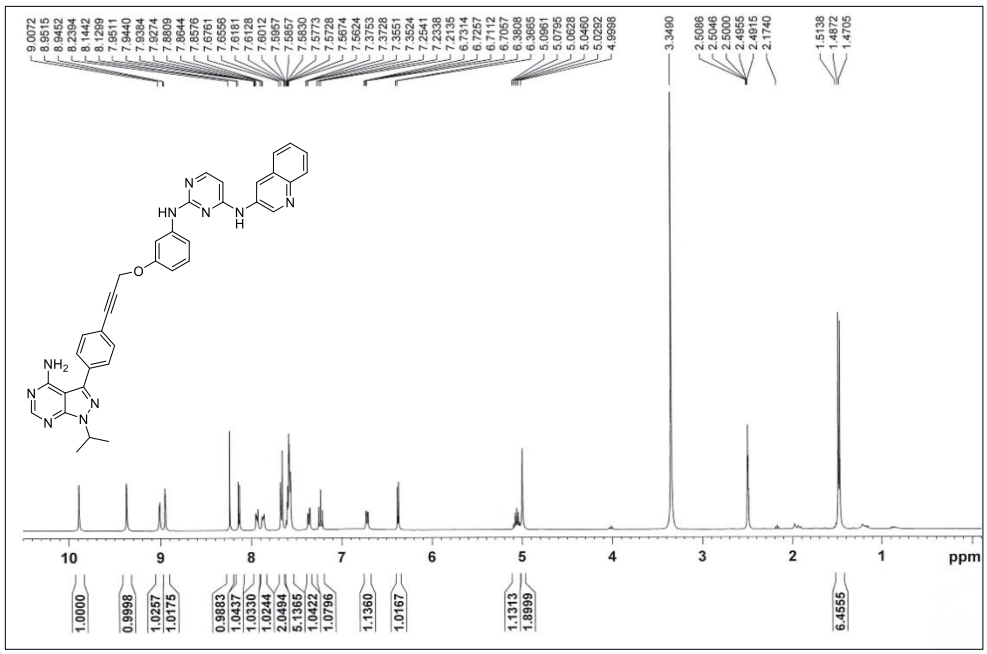
^{1}H NMR of compound **6e** (300 MHz, DMSO-d_6)



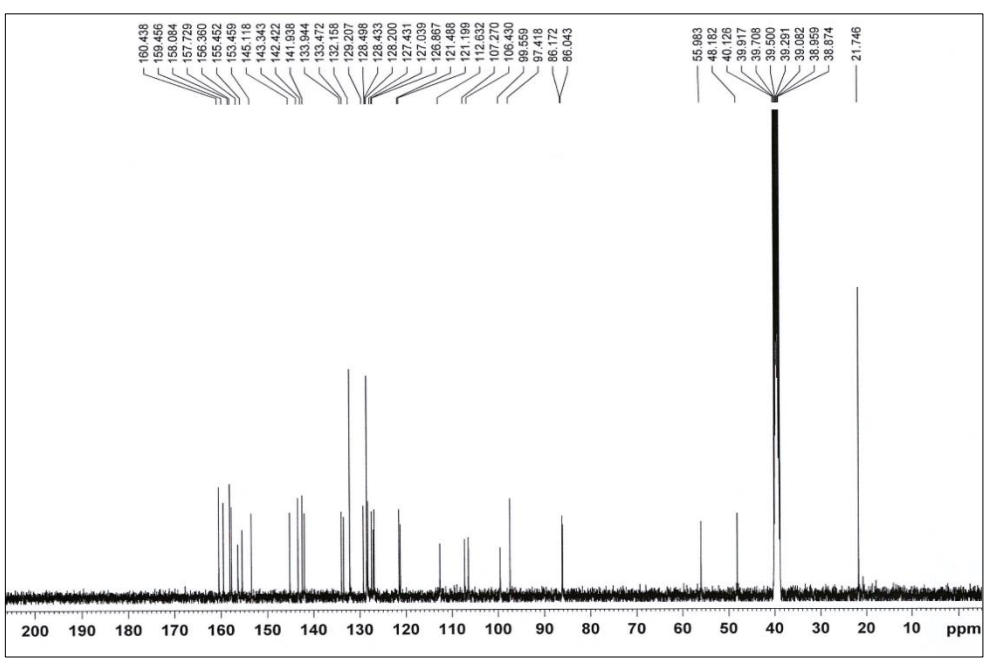
^{1}H NMR of compound **6f** (300 MHz, DMSO-d_6)



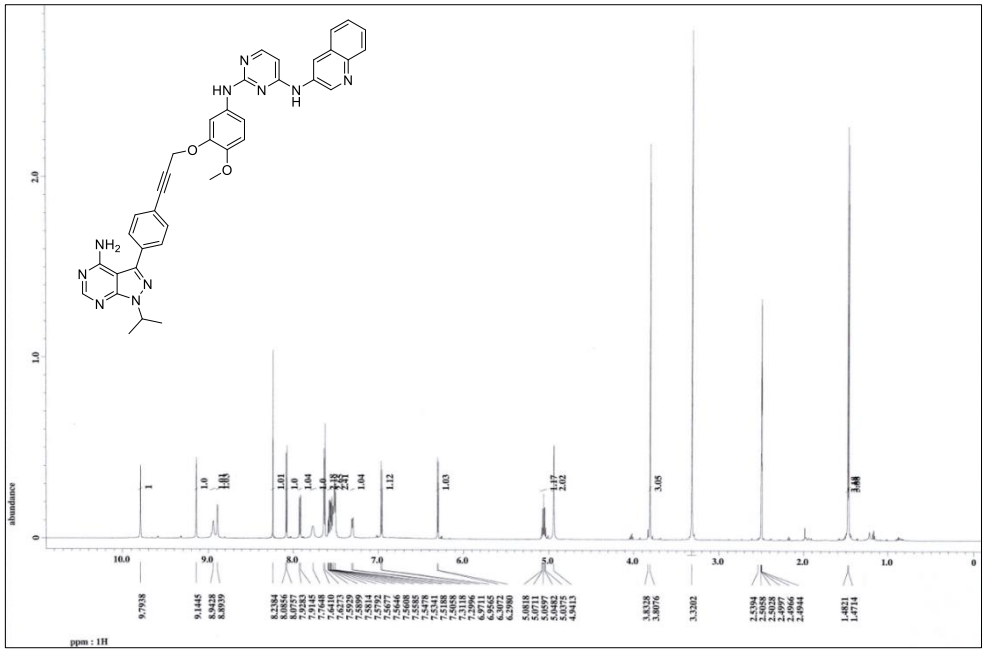
^{1}H NMR of compound **7a** (400 MHz, DMSO-d_6)



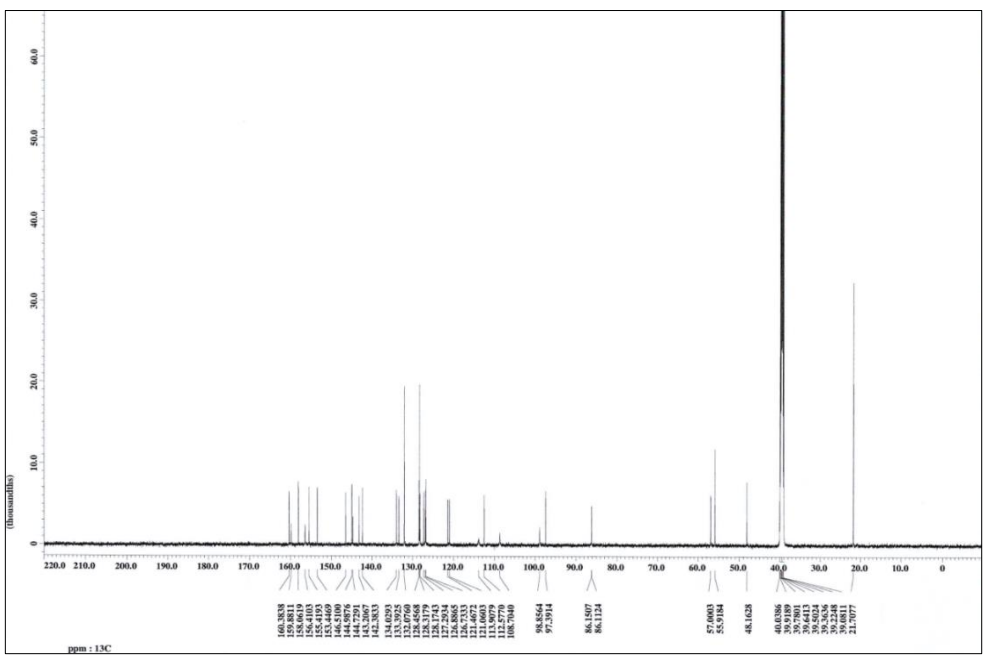
^{13}C NMR of compound **7a** (100 MHz, DMSO-d_6)



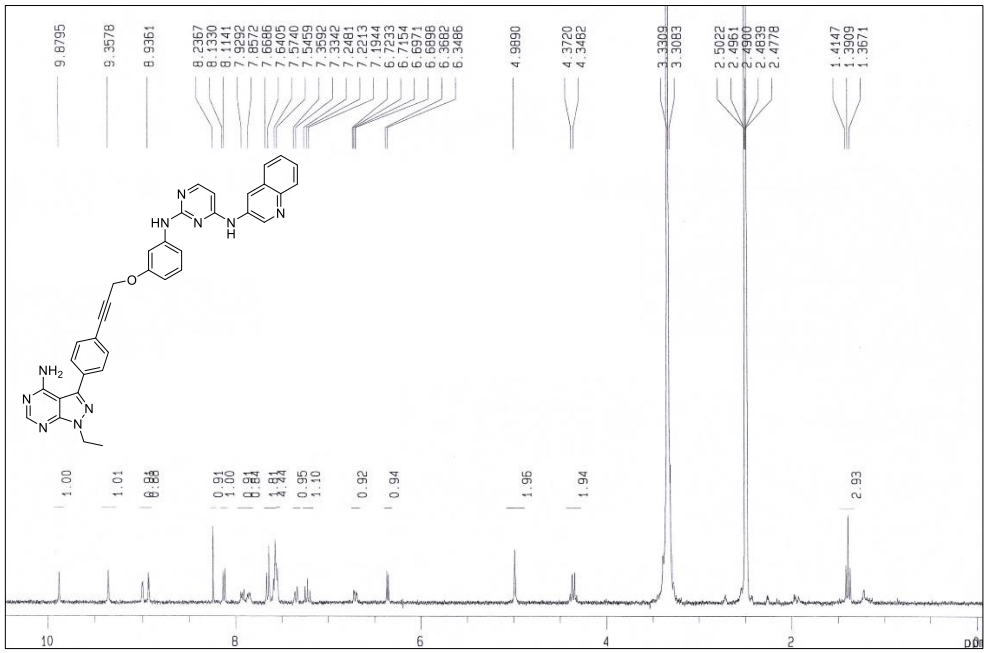
^{1}H NMR of compound **7b** (600 MHz, DMSO-d_6)



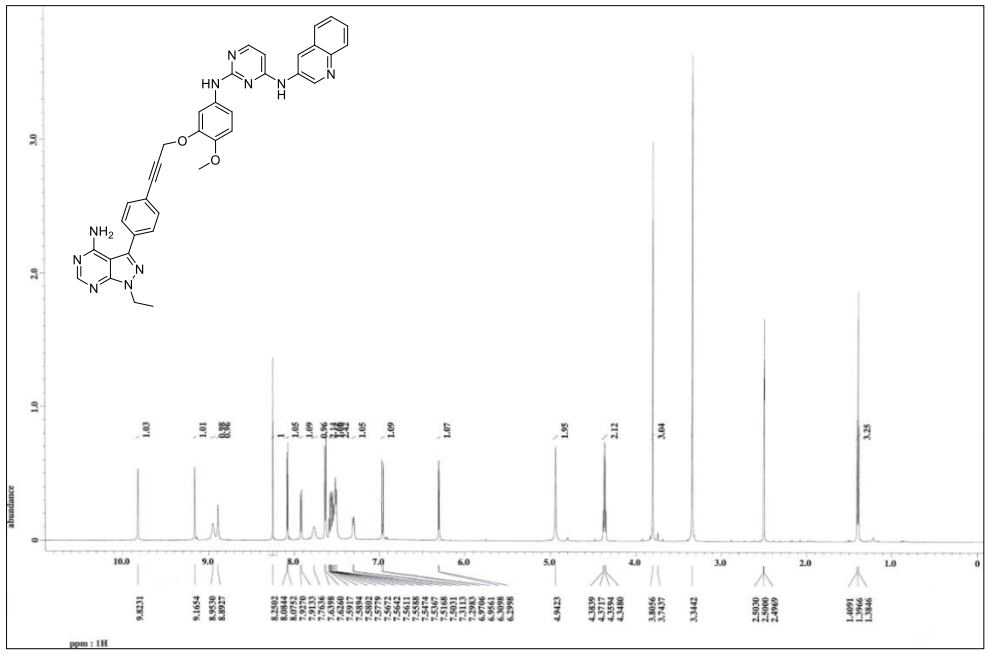
^{13}C NMR of compound **7b** (150 MHz, DMSO-d_6)



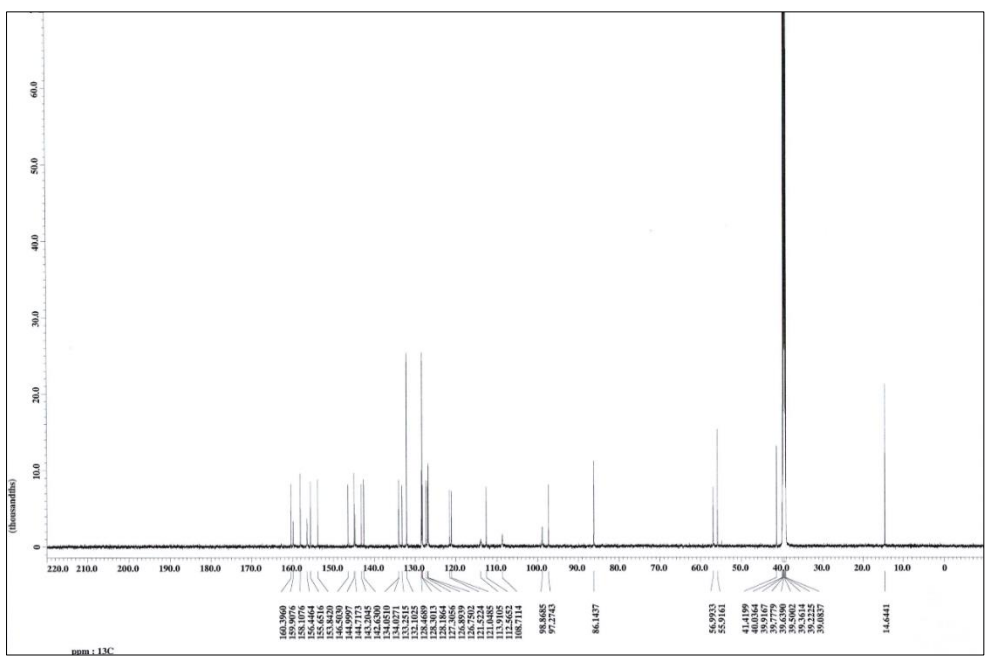
^{1}H NMR of compound **7c** (300 MHz, DMSO-d_6)



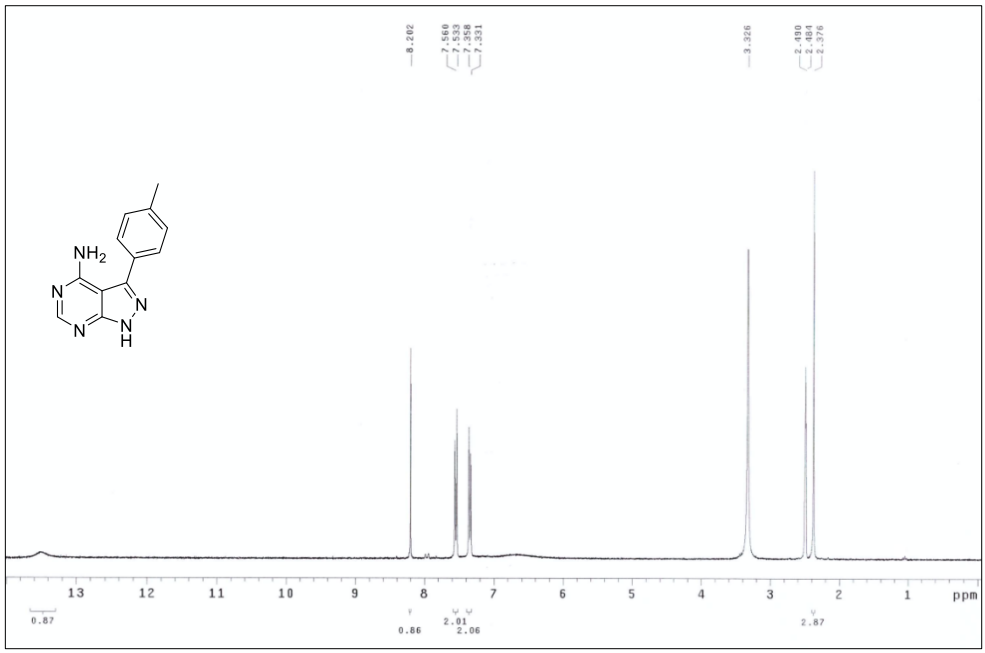
^{1}H NMR of compound **7d** (600 MHz, DMSO-d_6)



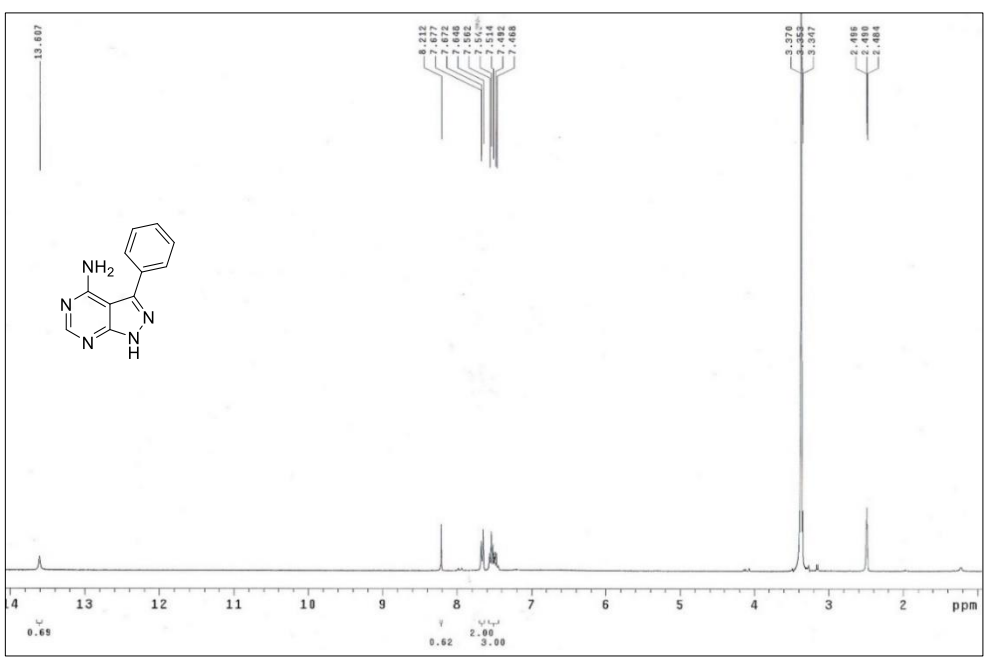
^{13}C NMR of compound **7d** (150 MHz, DMSO-d_6)



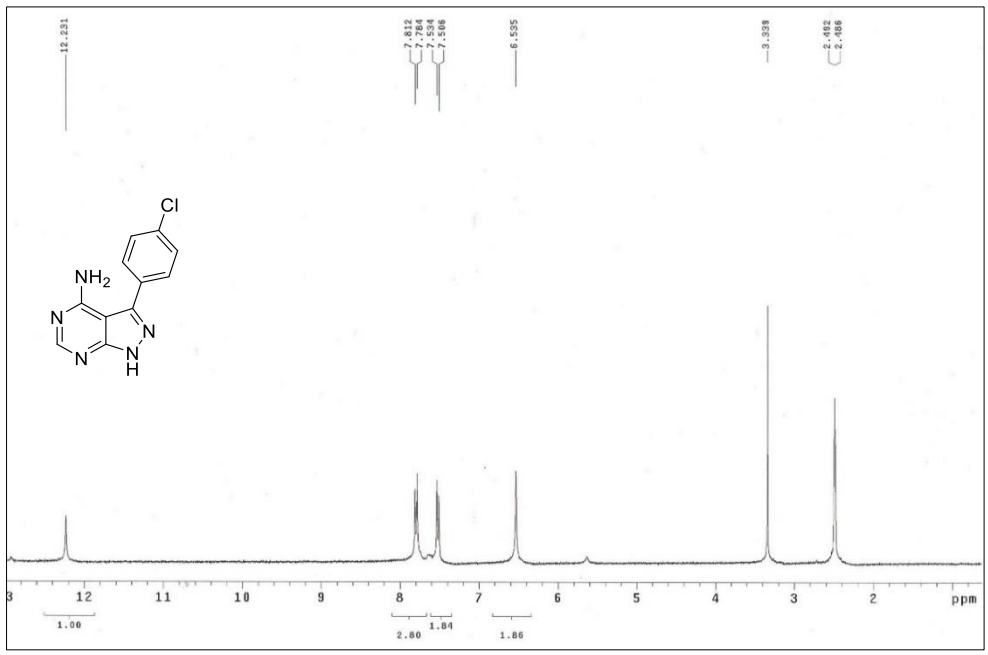
^{1}H NMR of compound **12a** (300 MHz, DMSO-d_6)



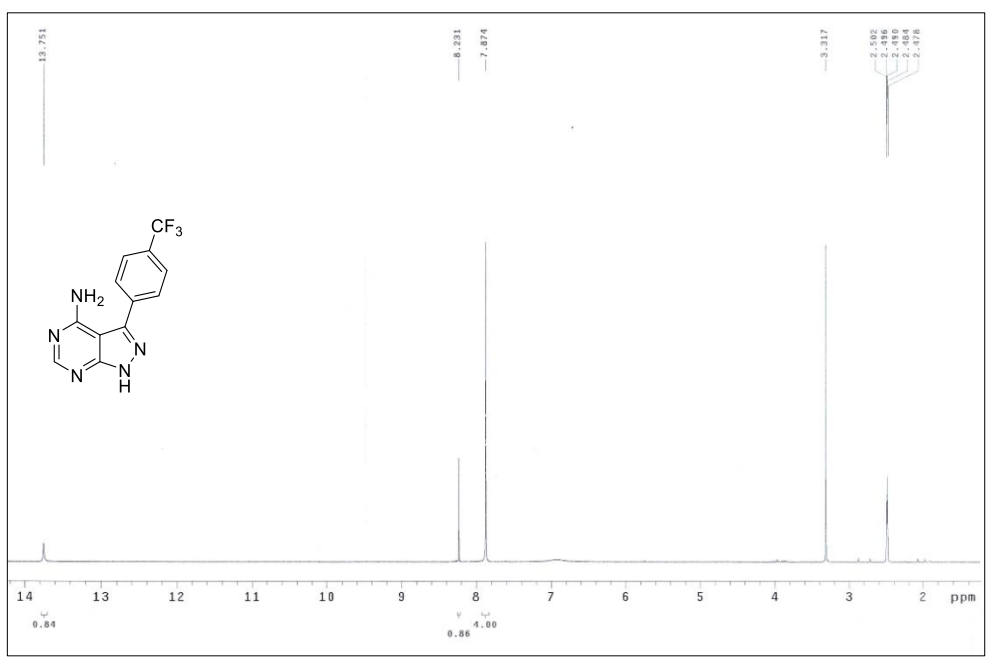
^{1}H NMR of compound **12b** (300 MHz, DMSO-d_6)



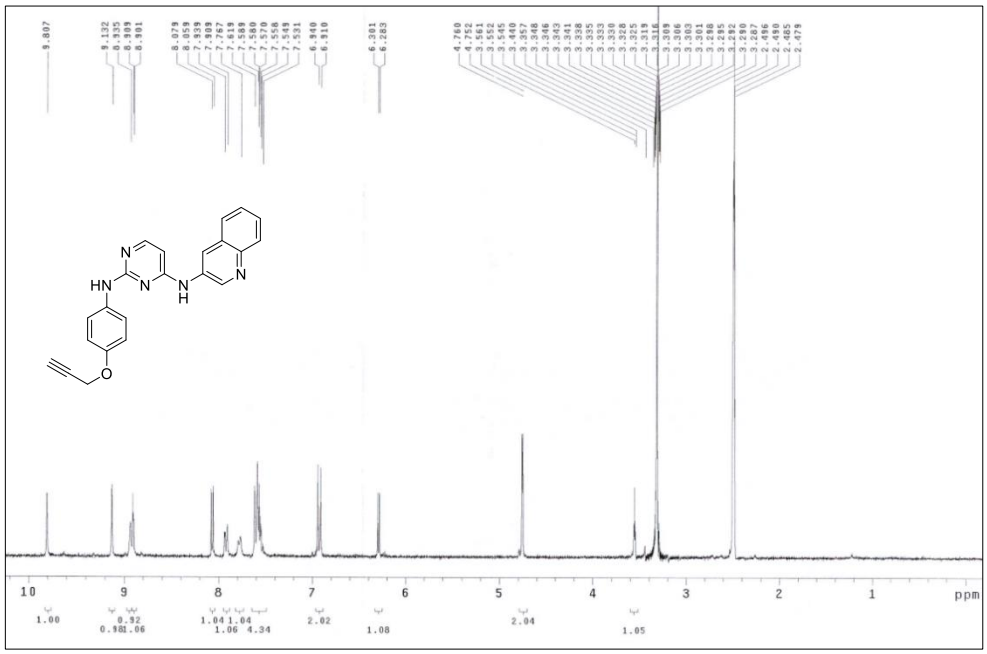
^{1}H NMR of compound **12c** (300 MHz, DMSO-d_6)



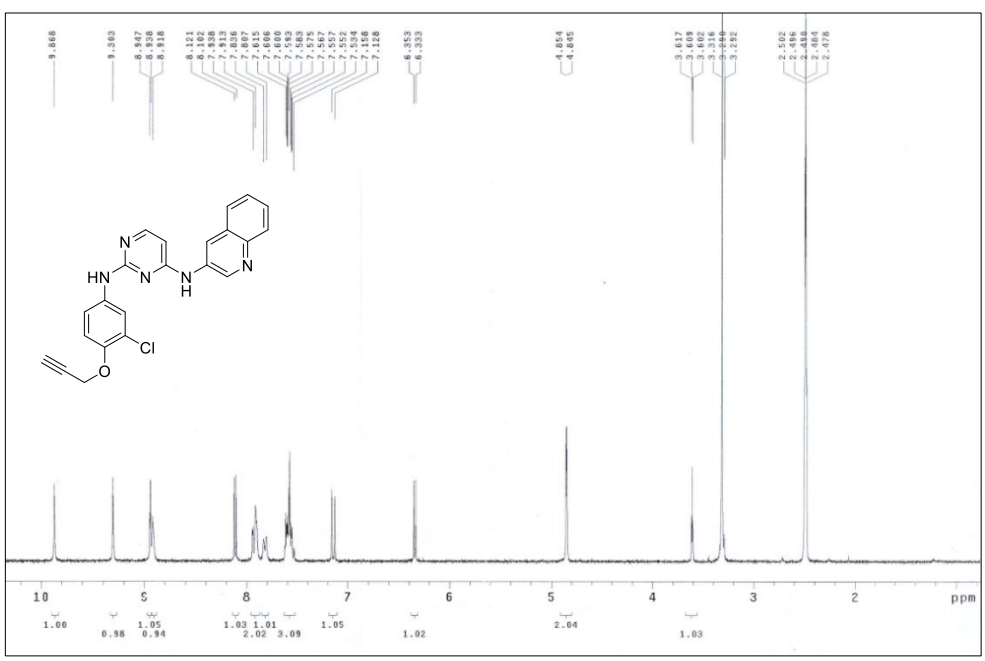
^{1}H NMR of compound **12d** (300 MHz, DMSO-d_6)



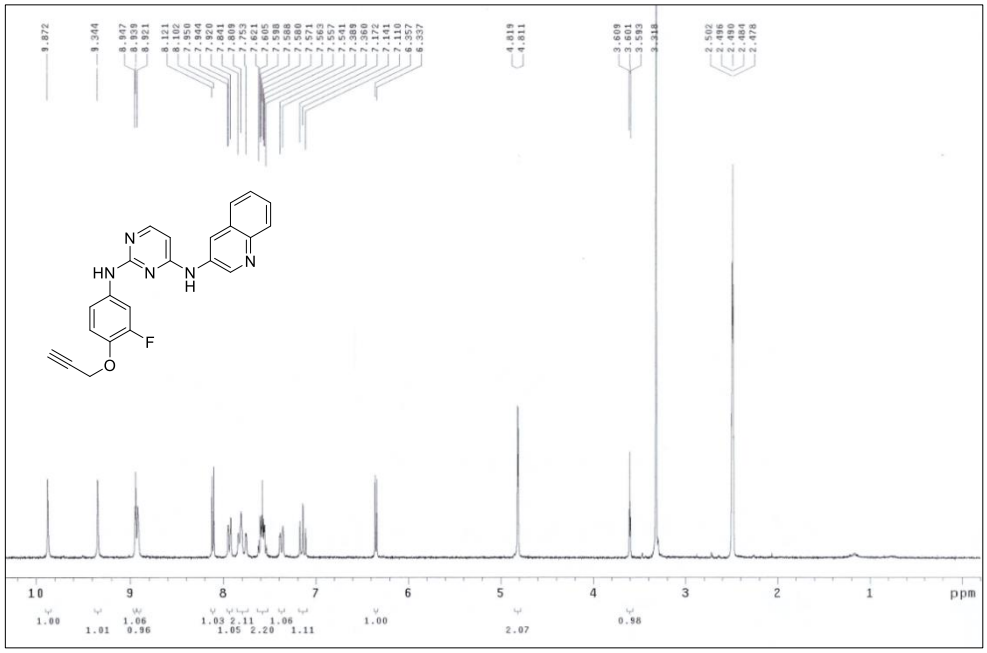
^{1}H NMR of compound **24a** (300 MHz, DMSO-d_6)



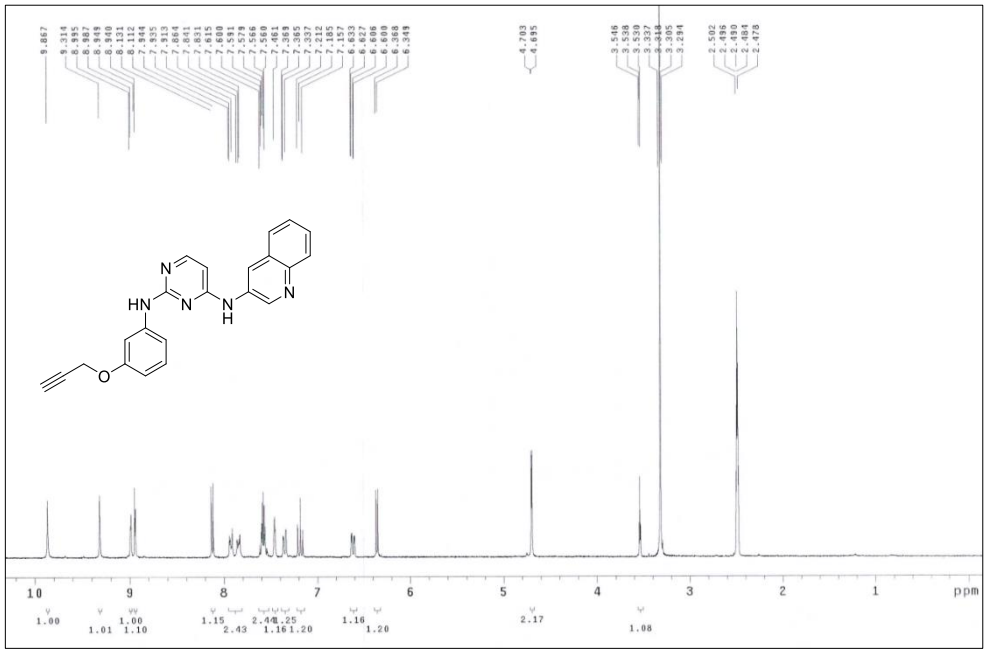
^{1}H NMR of compound **24b** (300 MHz, DMSO-d_6)



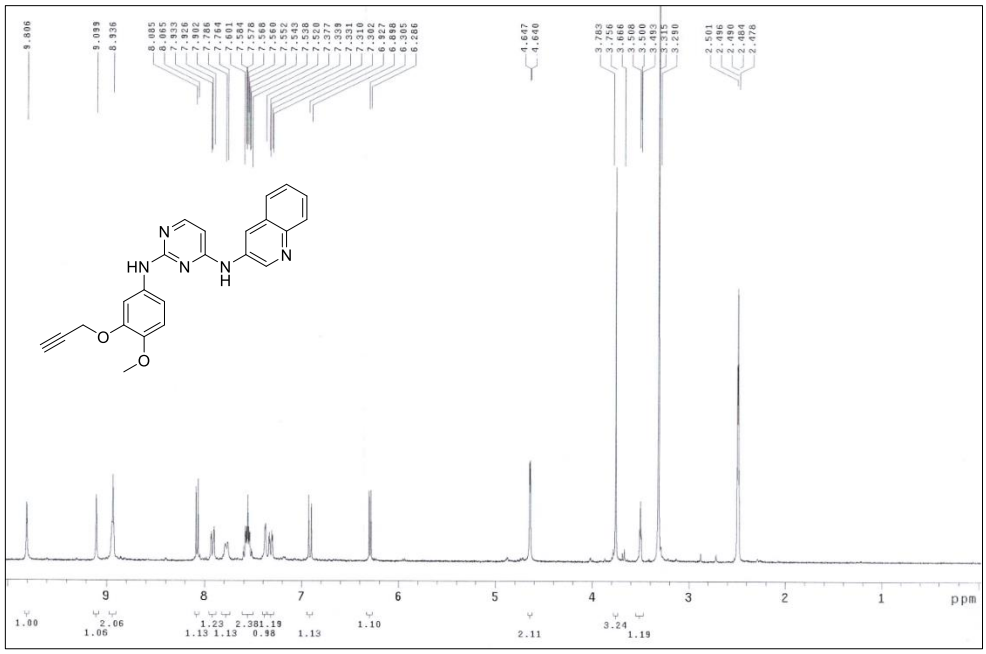
^{1}H NMR of compound **24c** (300 MHz, DMSO-d_6)



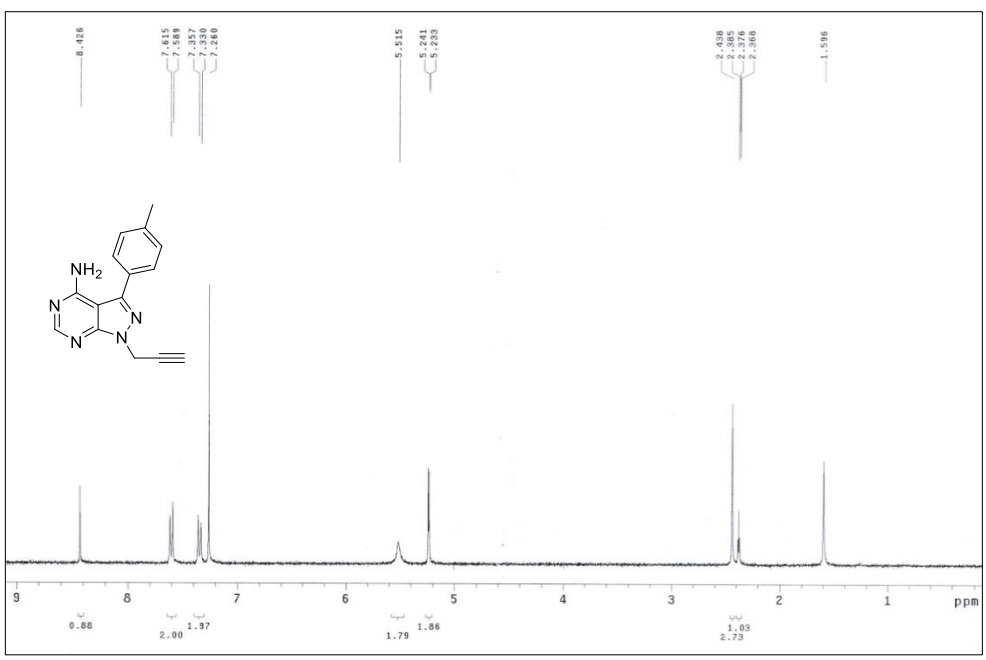
^{1}H NMR of compound **24d** (300 MHz, DMSO-d_6)



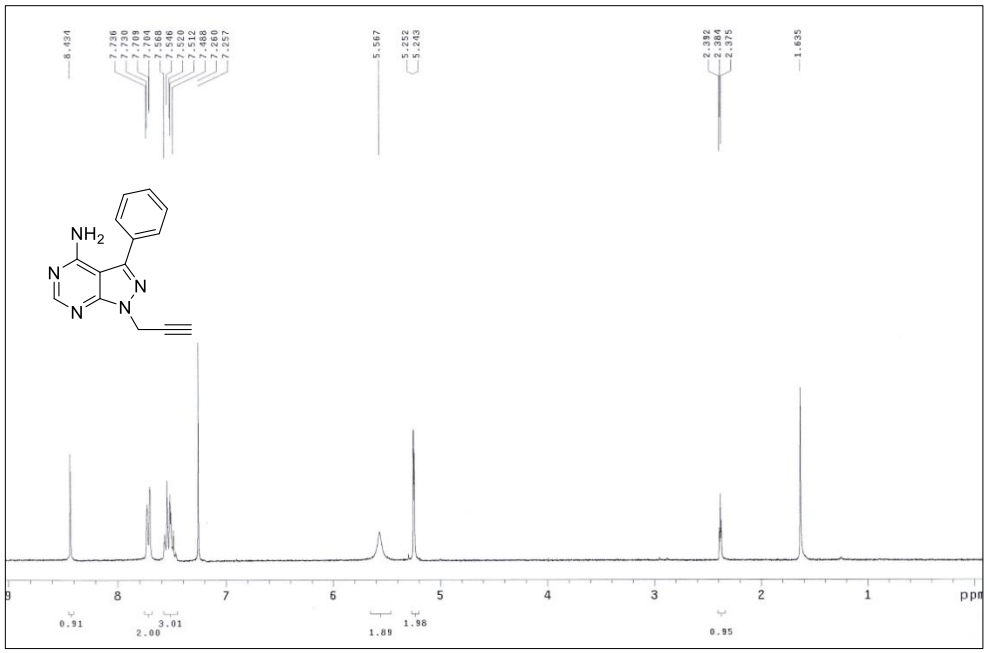
^{1}H NMR of compound **24e** (300 MHz, DMSO-d_6)



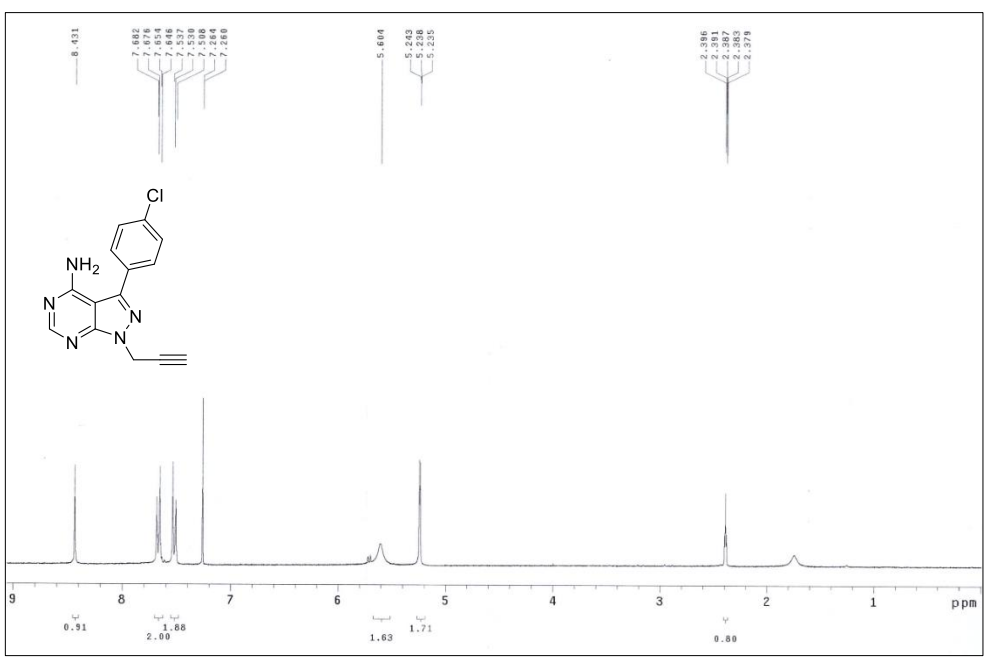
^{1}H NMR of compound **26a** (300 MHz, $CDCl_3$)



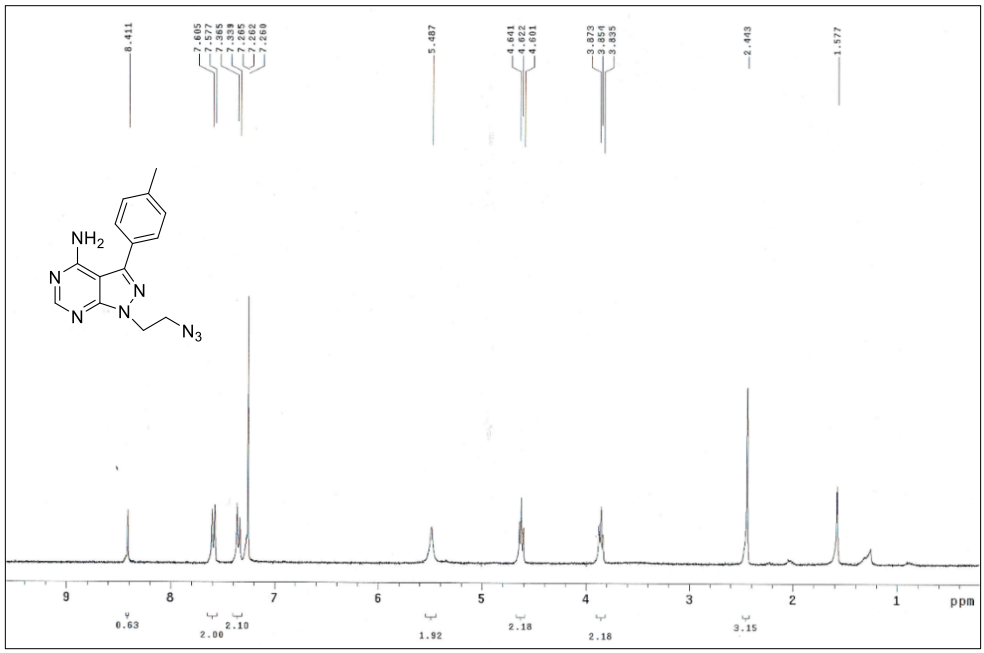
^{1}H NMR of compound **26b** (300 MHz, $CDCl_3$)



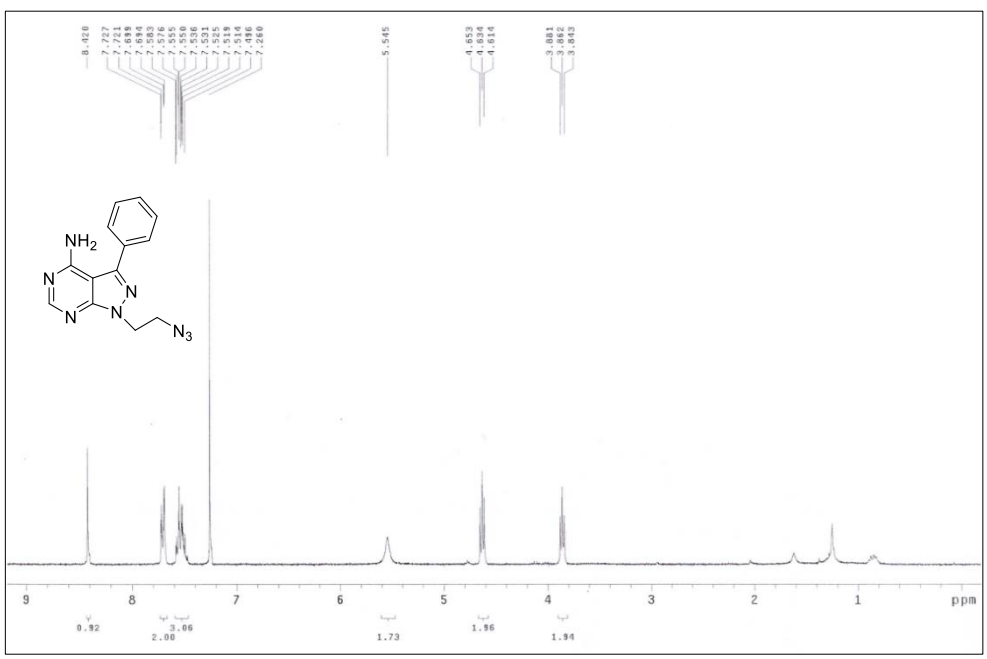
^{1}H NMR of compound **26c** (300 MHz, $CDCl_3$)



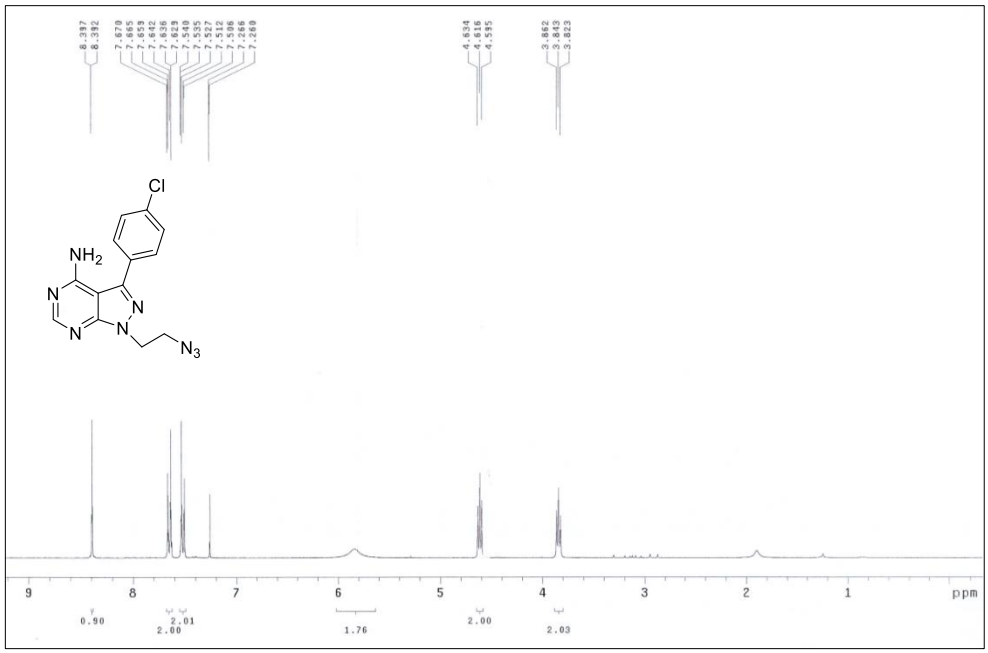
^{1}H NMR of compound **29a** (300 MHz, $CDCl_3$)



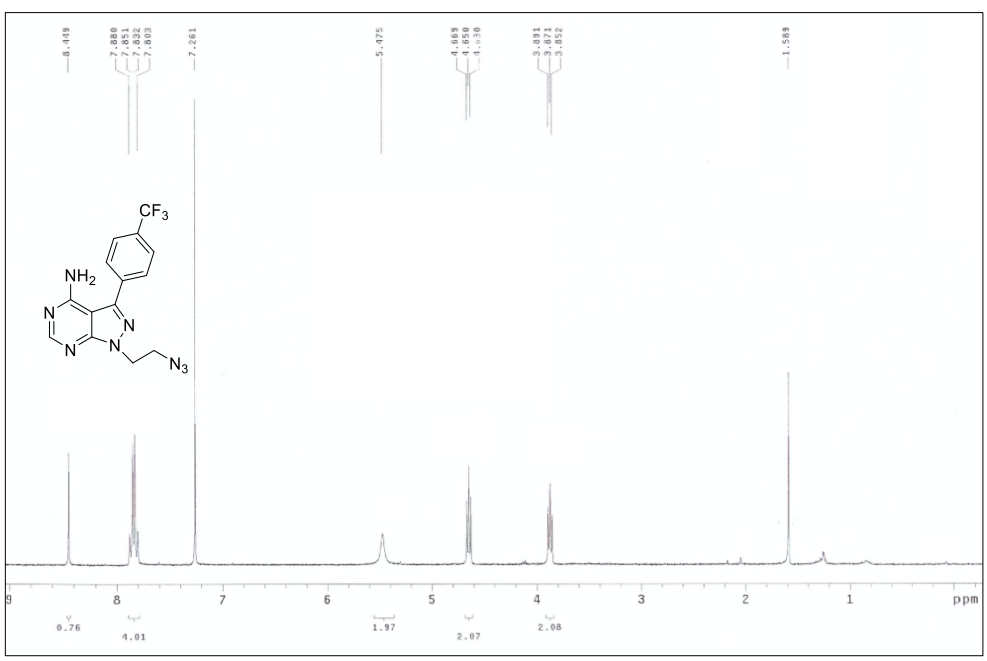
^{1}H NMR of compound **29b** (300 MHz, $CDCl_3$)



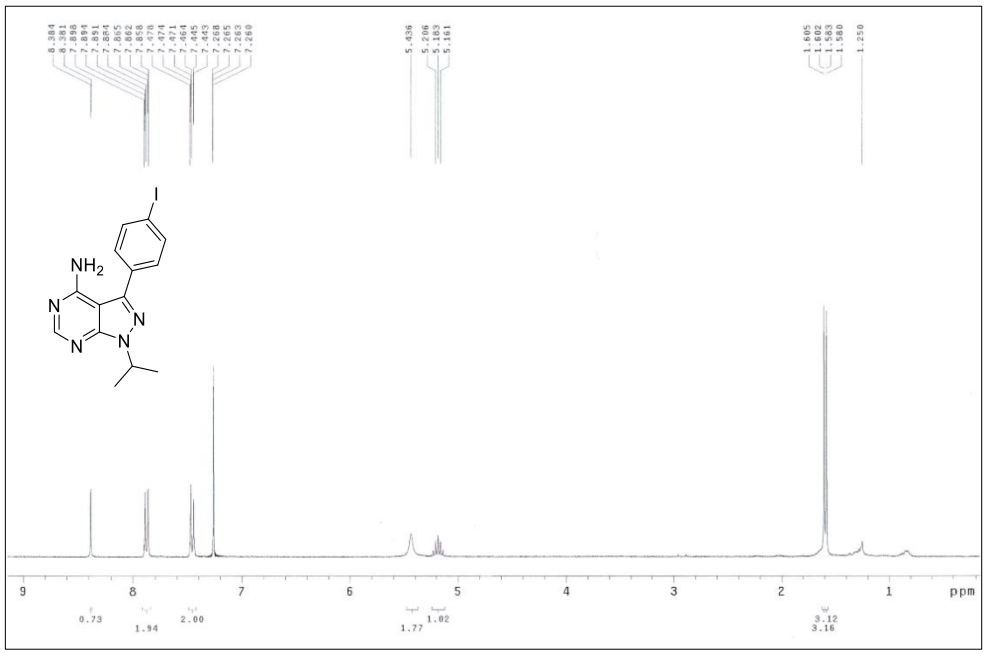
^{1}H NMR of compound **29c** (300 MHz, $CDCl_3$)



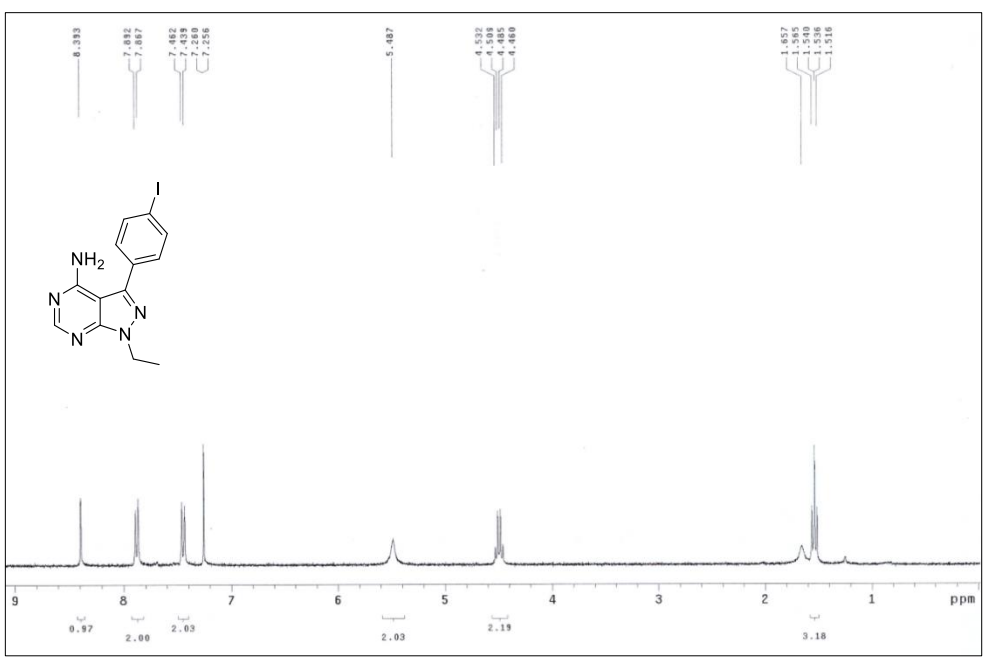
^{1}H NMR of compound **29d** (300 MHz, $CDCl_3$)



^{1}H NMR of compound **30a** (300 MHz, $CDCl_3$)



^{1}H NMR of compound **30b** (300 MHz, $CDCl_3$)



논문초록

Dual-targeting 전략을 이용한 잠재적 항암 활성 물질의 개발

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Insulin-like growth factor 1 receptor (IGF-1R)은 폐암, 유방암, 전립선암, 그리고 직장암 등에서 과발현 된 membrane receptor tyrosine kinase이다. IGF-1R을 타겟으로 합성된 여러 약물을 처리한 환자에게서 획득 저항성이 발견되었다. 이와 같은 IGF-1R 저항성이 생기는 이유는 Src의 높은 활성 때문과 관련이 되어있다고 생각된다. 그러나, IGF-1R과 Src 저해제를 병용투여 하는 경우 독성 문제를 야기할 수 있다.

본 연구에선, dual-target 항암제로서 새로운 3-substituted-pyrazolo[3,4-d]pyrimidin-4-amine 유도체를 디자인하고 합성하였다. 합성된 약물의 항암세포 증식억제 효과를 A549와 MCF-7 암세포주에서 확인하였다. 합성된 물질의 구조-활성 상관관계를 분석한 결과 triazole 또는 alkyne linker를 가지는 물질이 두 가지 cell line에서 뛰어난 저해 활성을 보였다.

본 연구의 결과는 합성된 dual-targeting 물질이 Src와 IGF-1R 단일

저해제를 능가하는 장점을 보여주고 있다. 또한, dual-targeting anti-cancer 물질을 디자인하는데 있어서 linker의 다양화가 중요한 역할을 한다는 것도 밝혀졌다. 앞으로 추가적인 *in vivo* 연구들이 필요하겠지만, 본 연구는 기존의 단일 저해제의 저항성을 극복 할 수 있는 dual-targeting 저해제를 개발하는데 있어서 중요한 디자인 전략을 제공한다는 것에 가치가 있다.

주 요 어 : dual target, anti-cancer (항암), resistance (저항성, 내성)

학 번 : 2014-25215