Accepted Manuscript

Design and synthesis of a new series of modified *CH*-diarylpyrimidines as drug-resistant HIV non-nucleoside reverse transcriptase inhibitors

Ge Meng , Yang Liu , Aqun Zheng , Fener Chen , Wenxue Chen , Erik De Clercq , Christophe Pannecouque , Jan Balzarini

PII: S0223-5234(14)00483-8

DOI: 10.1016/j.ejmech.2014.05.059

Reference: EJMECH 7017

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 17 February 2014

Revised Date: 11 May 2014

Accepted Date: 25 May 2014

Please cite this article as: G. Meng, Y. Liu, A. Zheng, F. Chen, W. Chen, E. De Clercq, C. Pannecouque, J. Balzarini, Design and synthesis of a new series of modified *CH*-diarylpyrimidines as drug-resistant HIV non-nucleoside reverse transcriptase inhibitors, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.05.059.

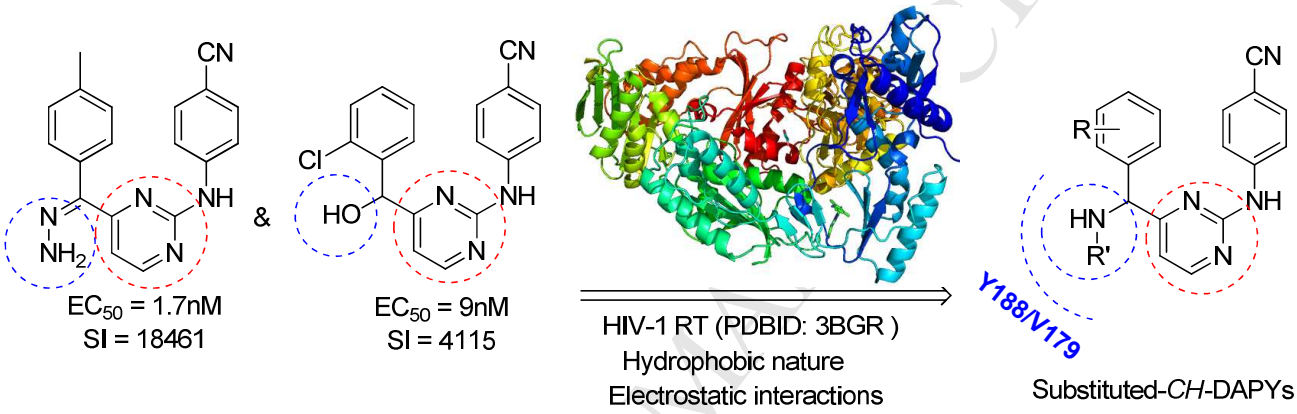
Graphic abstract

Design and synthesis of a new series of modified *CH*-diarylpyrimidines as drug -resistant HIV non-nucleoside reverse transcriptase inhibitors

Ge Meng[a,*], Yang Liu[a], Aqun Zheng[b], Fener Chen[c,*], Wenxue Chen[c], Erik De Clercq[d], Christophe Pannecouque[d], Jan Balzarini[d]

[a] *School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P. R. China*
[b] *School of Science, Xi'an Jiaotong University, Xi'an, Shaanxi, 710049, P. R. China*
[c] *Department of Chemistry, Fudan University, Shanghai, 200433, P. R. China*
[d] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

A series of new diarylpyrimidine analogues featuring a functional substituted amino group between the pyrimidine scaffold and the aryl wing have been designed and synthesized, which were also evaluated as anti-drug-resistant HIV reverse transcriptase inhibitors.



Highlights

- 18 new compounds were designed, synthesized as the anti-drug resistant HIV NNRTIs.
- All the new molecules were evaluated for their biological activities against HIV.
- Compound **1d** displayed anti HIV-1 activities 47 fold than that of AZT.
- Compound **1d** also showed activities against double mutated HIV-1 and HIV-2 strain.
- SAR study including the docking analysis was also investigated deeply.

Design and synthesis of a new series of modified *CH*-diarylpyrimidines as drug-resistant HIV non-nucleoside reverse transcriptase inhibitors

Ge Meng[a,*], Yang Liu[a], Aqun Zheng[b], Fener Chen[c,*], Wenxue Chen[c], Erik De Clercq[d], Christophe Pannecouque[d], Jan Balzarini[d]

[a] *School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P. R. China*
[b] *School of Science, Xi'an Jiaotong University, Xi'an, Shaanxi, 710049, P. R. China*
[c] *Department of Chemistry, Fudan University, Shanghai, 200433, P. R. China*
[d] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Non-nucleoside HIV RT inhibitors
Diarylpyrimidines
Cyanide group
Double mutant HIV-1
Docking analysis
SAR

ABSTRACT

This article reports the design, synthesis and antiviral evaluation of a new series of non-nucleoside reverse transcriptase inhibitors (NNRTIs). The basic skeleton of these target 18 molecules is diarylpyrimidine featuring a substituted amino group between the pyrimidine scaffold and the aryl wing. All of the new compounds have been characterized by spectra analysis. The entire target molecules were evaluated for their *in vitro* anti-HIV activity with controlling group of FDA approved drugs. Most of them showed good to potent activities against wild-type (WT) HIV-1 with IC_{50} values in the range of 0.0175-69.21 μM. 2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydrazonomethyl)pyrimidine (**1d**) displayed potent anti-HIV-1 activity against WT HIV-1 with a selectivity index (SI) of 106367 and an IC_{50} value of 1.75 nM, which was 47 fold lower than that of AZT. Compound **1d** also showed a broad-spectrum inhibitory activity, with an IC_{50} value of 5.33 μM and 5.05 μM against both HIV-1 double-mutated (K103N/Y181C) strain and HIV-2 strain, respectively. The preliminary structure-activity relationship (SAR) was also investigated. The binding modes with HIV-1 RT for both the wild type and mutant type have also been discussed.

1. Introduction[1]

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is one of the key enzymes in the HIV life cycle[1], which represents one of the main targets for designing selective agents for the treatment of HIV/AIDS[2]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) against HIV-1 are the main and important drugs to combating with the plague of AIDs[3]. Among the classes of compounds to which the US FDA-approved RT inhibitors belong [4], the three most important main types of NNRTIs are aromatic heterocylclic compounds, including HEPTs[5, 6], DABOs[7, 8], diarylpyrimidines (DAPYs) [9-11], *etc*[12]. All these therapeutic agents shared the pyrimidine ring as the main structural skeleton (Fig. 1) [6], which could directly bind with the allosteric site, located 10-15 Å away from the catalytic site of RT [13-15]. While the emergence of RT mutations (such as Y181C, K103N, *etc*.) rapidly confers resistance to the first-generation NNRTIs, highly potent second-generation NNRTIs have also been identified[1, 16, 17], such as the Y181C mutation-associated diarylpyrimidine (DAPY) analogs[18], represented by TMC125[19-21] and TMC278[22-24], which have been corroborated by many crystallographic data[25-27].

The crystal structures of DAPY/HIV-1 RT complexes and the relative molecular modeling results have revealed some important features of enzyme-ligand interactions, which are crucial for maintaining the antiviral activity against a wide range of resistance mutations[27, 28]. DAPYs shared the similar pharmacophore with the first-generation NNRTIs, including the hydrophobic center, the hydrogen bond donor and acceptors, the active binding conformation of DAPYs resembled a horseshoe[29] or "U" shape when bound in the non-nucleoside inhibitor binding pocket (NNIBP)[25, 27]. For example, TMC125 could be taken as an example to delineate this interaction mechanism in details as following: the ether and amino linkages of the two cyanophenyl groups of the TMC125 could provide sufficient flexibility to allow strong π-π stacking interactions with the aromatic ring of Y181, Y188, F227 and W229[27, 30], respectively. It was also suggested that DAPY NNRTIs could bind to RT through torsional flexibility ("Wiggling") and repositioning flexibility ("Jiggling")[25]. Based on the above concepts, a general strategy for designing flexible drugs against a variety of drug-resistant mutants HIV-1 strains has been proposed.

Considerable efforts made on the structural modifications of the linker group of CH_2-DAPYs might end up with various effects on the biological activity *via* the assistance of the different substituted groups on both sides of the aromatic rings[31, 32]. It has already been shown that there are still some possibilities to achieve the anti-drug resistance effects by modifying the linker group between the left benzene ring and the central pyrimidine ring[33]. In order to identify more potent DAPYs as possible NNRTIs, we have modified DAPY derivatives with cyclopropylamino groups attached to the CH_2- linker between the left benzene ring and the central pyrimidine ring[34]. The results have shown that both the cyano and the cyanovinyl groups are beneficial for enhancement of the anti-HIV activity.

* Abbreviations: NNRTIs, non-nucleoside reverse transcriptase inhibitors; HEPTs, 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio) thymines; DABOs, 3,4-dihydro-2-alkyl-6-benzyl-pyrimidin-4(3*H*)-ones; DAPYs, diarylpyrimidine derivatives; NVP, nevirapine; DLV, delavirdine; HIV-1 RT, Human immunodeficiency virus type 1 reverse transcriptase; US FDA, United States Food and Drug Administration; NNRTIs, non-nucleoside RT inhibitors; EFV, efavirenz; TMC125, etravirine; TMC278, rilpivirine; NNIBP, nonnucleoside inhibitor binding pocket; wt, wild-type; BPs, benzophenone derivatives; SI, selectivity index; AZT, zidovudine; ESI, electrospray ionization; TMS, tetramethylsilane; MTT, 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $CCID_{50}$, 50% cell culture infectious dose; CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; Kd, dissociation constant; IC_{50}, 50% inhibitory concentration.

*Corresponding author. Tel./fax: +86 29 82656327-801.
E-mail address: mengge@mail.xjtu.edu.cn (G. Meng),
rfchen@fudan.edu.cn (F.-E. Chen).

We therefore proposed that it might be a short cut to start from fixing the cyano or cyanovinyl group while changing the attached group on the CH_2 linker. On the other hand, either a hydrazine[35] (**2**, Fig. 2) or hydroxyl[36, 37] (**3**, Fig. 2) groups has also been proven to be very beneficial for improving the antiviral activity, especially increasing the drug resistance activities, which have been also verified by many biological assays[37].

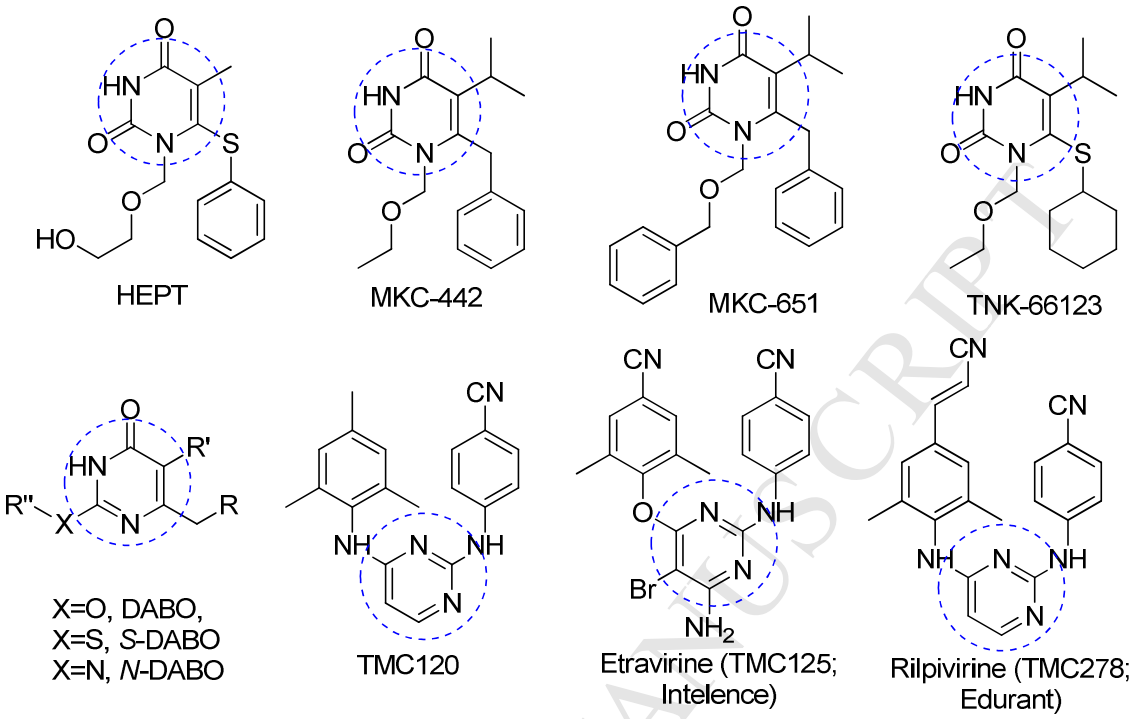


Fig. 1. Structure of some important NNRTIs including HEPT, DABCO and DAPYs.

Guided by the mechanism of interaction between DAPY and HIV-1 RT as well as the related SAR analysis of these analogues, we wanted to find more drug candidates against the drug-resistant HIV-1 strains. In this paper, we therefore have designed and synthesized another series of new 4-(substituted-amino)arylmethyl DAPYs derivatives (**1**, Fig. 2) accordingly. The target molecules shared the pyrimidine skeleton and the hydrazine or the hydroxyl-CH_2 linker, as well as the cyano and the cyanovinyl groups on both of the aromatic rings. The chemical structures of all the new target molecules have been characterized by spectra analysis data. The anti-HIV screening tests show that almost all of the compounds show moderate to excellent activity against HIV, of which compound **1d** is the best one. The relative SAR discussions was also investigated together with the comparative docking analysis.

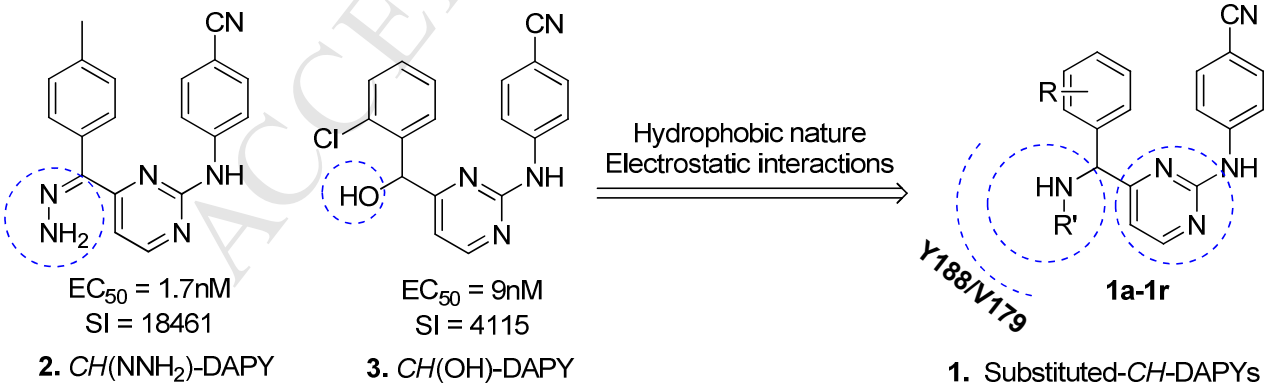
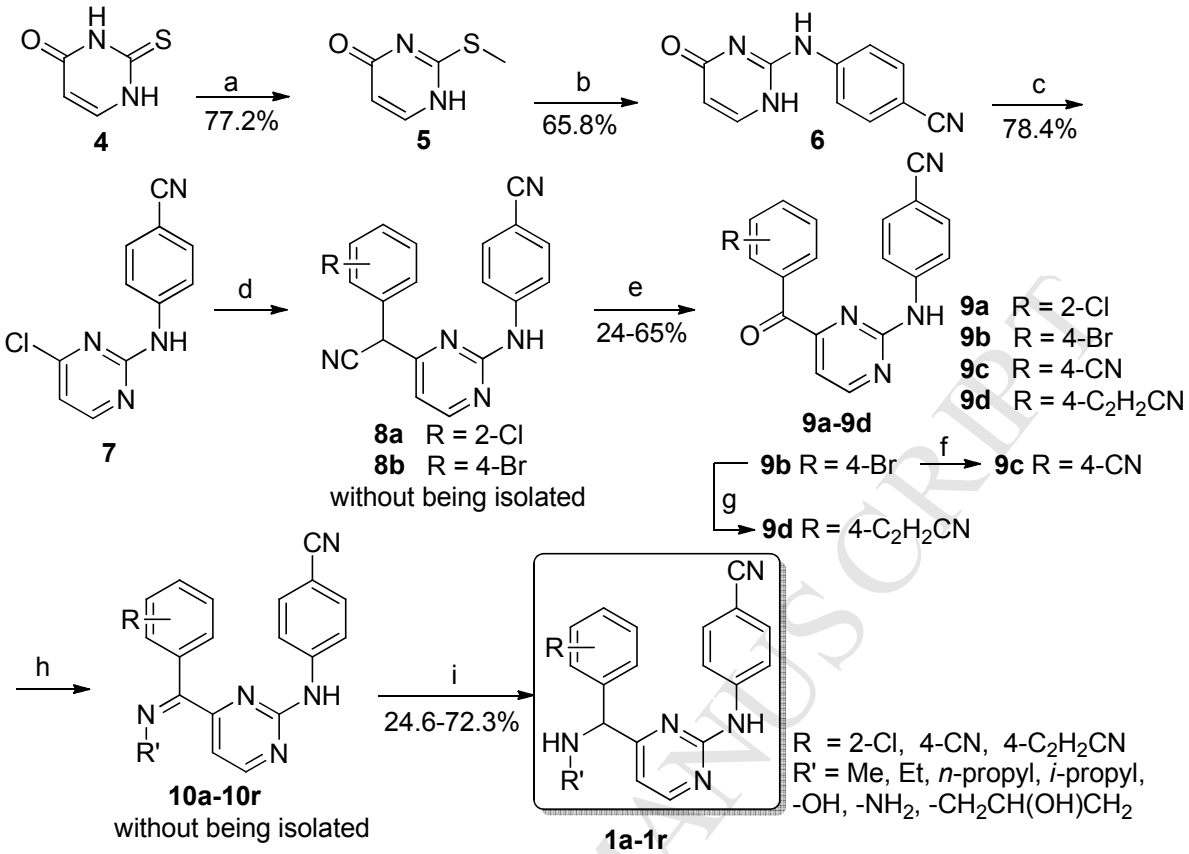


Fig. 2. Molecular design of new substituted *CH*-DAPYs.



10, 1	R	R'
a	4-CN	OH
b	4-CH=CHCN	OH
c	4-CN	NH_2-NH
d	4-CH=CHCN	NH_2-NH
e	4-CN	Me
f	4-CH=CHCN	Me

10, 1	R	R'
g	2-Cl	Me
h	4-CN	Et
i	4-CH=CHCN	Et
j	2-Cl	Et
k	4-CN	*n*-Pr
l	4-CH=CHCN	*n*-Pr

10, 1	R	R'
m	2-Cl	*n*-Pr
n	4-CN	*i*-Pr
o	4-CH=CHCN	*i*-Pr
p	2-Cl	*i*-Pr
q	4-CN	2-Hydroxylpropyl
r	2-Cl	2-Hydroxylpropyl

Scheme 1. Synthetic route of the new DAPYs derivatives (**1a-1r**). Reagents and conditions: (a) MeI, NaOH, H_2O, r. t.; (b) 4-cyanoaniline, 180 ℃; (c) $POC1_3$, reflux; (d) R (R=2-Cl or 4-Br)-phenylacetonitrile, 60 % NaH, Ar, DMF, -20℃ to r. t., 48-72 h; (e) NaH, air, DMF, r. t., 36-72 h; (f) CuCN, DMF, 150℃, 10h; (g) acrylonitrile, $Pd(OAc)_2$, P(*o*-Tol)$_3$, NaOAc, DMA, 150℃, overnight; (h) R'-NH_2, EtOH, Na_2SO_4, reflux, 4-8 h; (i) $NaBH_3CN$, EtOH, 60 ℃, 4 h

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **1a-1r** was shown in Scheme 1. Starting from commercially available material 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**4**) and iodomethane, 2-(methylthio)-2,3-dihydropyrimidin-4(1*H*)-one (**5**) were obtained *via* methylation. Methylthio ether **5** was transformed into 4-((4-oxo-1, 2, 3, 4-tetrahydropyrimidin-2-yl)amino) benzonitrile (**6**) through the reaction with excess amount of 4-cyanoniline at high temperature of 180℃. The purification of **6** was achieved by washing with organic solvent. The important intermediates 4-(4-chloropyrimidin-2-yl-amino) benzonitriles (**7**) was easily prepared from intermediate **6** *via* refluxing with $POC1_3$. The key intermediates, oxo-CH_2-DAPYs (**9a-9d**) were conveniently synthesized from benzonitriles **7** in two steps through our previously reported modified methods[31, 38]. The cyanation of **9b** with CuCN at 150 ℃ for 10 h in anhydrous DMF gave the corresponding 4-cyano intermediate **9c**[34]. The acrylonitrile derivative **9d** was prepared *via* the coupling reaction, which was conducted on **9b** with classical Heck conditions using palladium (II) diacetate as a catalyst in the presence of sodium acetate in DMA[34]. Imino compounds **10a-10r** were synthesized by refluxing **9a-9d** with various substituted amines in ethanol with the assistance of small amount of acetic acid and anhydrous sodium sulfate, respectively. Reductions of **10a-10r** with sodium cyanoborohydride in ethanol provided the target compounds **1a-1r** (Scheme 1).

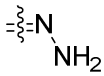
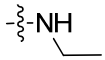
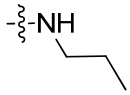
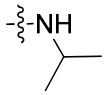
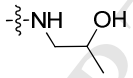
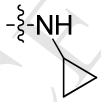
2.2. Biological activity

The activity and cytotoxicity of these newly synthesized DAPY analogues were evaluated in MT-4 cells for their ability to inhibit HIV-1 and HIV-2 induced cytopathogenicities. The results, expressed as the cytotoxicity (CC_{50}), half maximal (50%) inhibitory concentration IC_{50} and selective index (SI = CC_{50}/IC_{50}), which indicates the specificity of the antiviral effect, are illustrated in Table 1 together with those of the five

FDA-approved drugs: Nevirapine, Zidovudine, Efavirenz, Delavirdine and Etravirine as the reference standards. The cells were infected with HIV-1 wild-type virus (LAI) or double RT mutant (K103N/ Y181C) strain derived from wild-type LAI, and HIV-2 strain (ROD).

Table 1

Anti-HIV activities and cytotoxicity of compounds **1a-1r** MT-4 cells.

Compd	R	R'	IC_{50} (μM)			CC_{50}	SI
			IIIB	RES056	ROD		
1a	4-CN		7.90±1.45	>218.73	>218.73	218.73±23.01	28
1b	4-C_2H_2CN		0.0163±0.0012	>160.946	35.66±7.20	160.946±40.217	9891
1c	4-CN		0.234±0.019	5.95	2.92±1.45	40.175±4.907	172
1d	4-C_2H_2CN		0.00175	5.33	5.05±0.79	186.308±15.58	106367
1e	4-CN		15.17±7.29	>189.13	>189.13	189.13±6.99	12
1f	4-C_2H_2CN		0.108±0.049	>41.414	>41.414	41.414±11.741	385
1g	2-Cl		0.063±0.015	>36.09	>6.09	36.09±1.873	571
1h	4-CN		21.06±9.16	>157.52	>157.52	157.52±11.34	7
1i	4-C_2H_2CN		0.192±0.041	>37.324	>37.324	37.324±1.955	194
1j	2-Cl		0.35±0.01	>39.44	>39.44	39.44±0.91	113
1k	4-CN		≥9.45	>34.94	>34.94	34.94±3.19	≤4
1l	4-C_2H_2CN		0.107±0.063	>37.324	>37.324	33.970±3.457	319
1m	2-Cl		0.048±0.003	>68.476	>68.476	68.476±17.664	1422
1n	4-CN		>69.21	>69.21	>69.21	69.21±55.00	na*
1o	4-C_2H_2CN		0.080±0.003	>32.322	>32.322	32.322±2.589	405
1p	2-Cl		0.040±0.012	>35.462	>35.462	35.462±0.779	879
1q	4-CN		23.87±1.45	>65.03	>65.03	>65.03	>3
1r	2-Cl		0.33±0.04	>34.66	>34.66	34.66±1.35	105
*	4-CN		>34.71	>34.71	>34.71	34.71±1.08	<1
**	4-C_2H_2CN		0.196±0.03	>56.108	>56.108	56.108±9.788	287
***	2-Cl		0.12±0.01	>37.89	>37.89	37.89±1.98	317
	Nevirapine		0.101±0.015	0.90		>15.021	>148
	Lamivudine		3.89±2.89		15.53±11.17	>87.24	>22
	Zidovudine		0.0066±0.0006	0.0077	0.0017	>93.5489	>14124
	Efavirenz		0.0226	0.16		6.3356	>280
	Delavirdine		0.81			43.84	>54

*na: The data are not available or calculated.

*, **, ***: The data for the preparation of the compounds have been described in another manuscript.

In general, most of the tested compounds were active against the wild type of HIV-1 infected cell lines except for the compounds with 4-cyanophenyl as the left wing of DAPYs. The activities of these compounds are comparable to that of Nevirapine. Some of the compounds show the similar biological activity to that of Efavirenz. From all the tested compounds, 2-(4-cyanophenylamino)-4-(2-cyanovinylphenylhydrazonomethyl)pyrimidine (**1d**) was found to be the most promising one with the lowest IC_{50} of 1.75 nM, 4 time as the reference compound AZT. The toxicity of compound **1d** is also very low with the SI

of **1d** being 106367 = 7 times than that of AZT.

As shown in Table 1, the substitution pattern on the phenyl ring of DAPYs might play a determinant role in their HIV-1 inhibitory potency. In the case of the 4-cyanovinyl or 2-chloro substituted analogues (**1b**, **1d**, **1f**, **1g**, **1i**, **1j**, **1l**, **1m**, **1o**, **1p** and **1r**), all of the compounds show very high activity. But as for the 4-cyano substitution, the majority of these compounds are inactive against HIV-1 with an IC_{50} above 10 μM, except for the compound (**1c**) with a hydrazine group on the methylene linker. The activity of **1c** was not as high as what has been expected from the SAR analysis. The reasons for the ordinary activity of these compounds with similar substitutions can be explained as follows.

It was well-known that the introduction of a cyano group into DAPYs could elevate both the biological activity and the anti-drug resistance ability *via* an interaction with the conserved amino acid W229 in NNIBP. This kind of interaction can be assisted by the 2-substituted group on the phenyl ring of DAPYs with increased π-π stacking between the ligand and Y181 or Y188 in the hydrophobic pocket of NNIBP by the restriction on the flexible rotation of the left phenyl ring of DAPYs[39]. It was also observed that most of the active DAPY derivatives shared both 4-cyano group and 2-substituted groups. For example, TMC125 contains two methyl groups on the 2, 6-positions of the left phenyl ring (Fig. 1)[30].

The possibility for the insertion of 4-substituted group into the W229-containing narrow pocket of NNIBP is depended on both the physical properties (including the steric effects *etc*.) of the 4-substituted group and the synergistic effect of the *C*2-sustituted groups on the phenyl ring. In some circumstance, the function of the *C*2-sustituted groups might exert much more assisting effect than the 4-substituted group *via* fixing the suitable conformations of the 4-substituted group. The 4-cyanovinyl could adjust its conformation to adapt with the hydrophobic channel by σ-bond rotation, while the cyano group could exert a similar effect only by the assistance of the 2-substituted groups. The biological result of the former series of DAPYs with cyclopropyl attached to the linker also supported these suggestions[34].

Replacing cyclopropyl with a smaller or more flexible alkyl amino group could inevitably result in the enhancement of the biological activities to 2-8 folds as the mother skeletons including the less active cyano substituted molecules. It is quite interesting that even the sequence of the activity for different substitution groups are quite similar in the more active 4-cyanovinyl and 2-chloro series, which could be in the following order: *iso*-propylamino > *n*-propylamino > methylamino > ethylamino ≈ cyclopropylamino. The result also showed that the flexibility of these substituted groups can play a very important role in enhancing the activity together with the steric effects. These alkyl groups are less effective than the hydrazino group, which is probably related to the fact that the unsubstituted NH_2 group could easily form an H-bond with the V179 residue, while the substituted NH structures are less beneficial for the H-bond formation, although the alkyl group could occupy more space in the V179 pocket.

Interesting results were also observed in considering the hydrazino group substitution on the NH-linker. There are two compounds showed a micromolar activity on double mutant strains (K103N/Y181C), comparable to Nevirapine but less active than Efavirenz and Zidovudine. Anyway, the anti-drug resistant activities of these two compounds were still similar to that of the hydroxyl or hydrazino substituted DAPY derivatives even if the cyanovinyl group was introduced into the DAPY skeletons. This result might be explained by the disappearance of the preferred fixing conformation due to lacking the assistance of the 2-substituted groups on the aromatic rings. The introduction of the cyanovinyl group could enhance the biological potency against wild-type virus strain, while the same group could also decrease the cytotoxicity, which again verified the cyanovinyl group at 4-position to be superior to other substituents.

In addition, all the title compounds were also evaluated for their capability to inhibit the HIV-2 (ROD) replication in MT-4 cells. The result showed that compound **1b**, **1c**, **1g** also have some activity against HIV-2, with the potency less than that of AZT (Table 1). It is also interesting to note that the 4-cyano group was the best one in keeping potency against HIV-2. This similar sequence in enhancing ability of the different groups might imply some similar mechanism in the different circumstances of both the HIV-1 and HIV-2. These findings showed that this new series of DAPY derivatives was active against both HIV-1 and HIV-2, which might be lead to a new way to broaden the anti-virus spectrum.

3. Molecular docking analysis

The docking analysis about the possible binding mode has been compared between the most potential compound **1d** and TMC278 with HIV-1 RT^{wt} and double mutant HIV-1 $RT^{K103N/Y181C}$, respectively. The results are shown in Fig. 3. The following features of the docking models are worthy to be outlined in detail.

The torsion angles of different HIV-1 RTIs are very important parameters and correlated with the beneficial interaction between the ligand and the HIV-1 RT, which is supposed to be further related with the anti-HIV-1 biological activities[40]. Therefore, the torsion angles in the binding conformations of TMC278 and the most potent compound **1d** were calculated and are further shown in Table 2 for comparison.

As shown in Fig. 3 (b), the binding mode between compound **1d** and wild type RT is illustrated as follow. The pyrimidine ring of compound **1d** is located in the space between L100 and V179. The torsional rotation initiated by the methylene group could lead the pyrimidine ring of **1d** not to be paralleled with the pyrimidine plane of TMC278. While moving upward for 0.5Å, the pyrimidine ring of **1d** might get close to E138 for about 0.5Å. Formed by both the K101 main chain carbonyl and the NH group, the two hydrogen bonds in the crystal structure of TMC278-RT^{wt} (PDB ID: 2ZD1[25], Fig.3 (a)) still exist between **1d** and K101 in the RT-**1d** docking model as a similar fashion to that of TMC278, while the bond lengths of both of the two hydrogen bond are 0.1Å longer than that of TMC278. The right cyanophenyl rings of both compound **1d** and TMC278 are almost overlapping with each other by comparison of (a) and (b) in Fig. 3. Although the methylene group between the left wing and pyrimidine ring moves over a distance from the NH of TMC278 on account of the variation in τ, but the distance between the CH_2 linker and E138 did not change markedly.

It is quite interesting to note that another three hydrogen bonds are also formed between the NH_2 of the hydrazino group of **1d** and the oxygen of Y188 (2.547 Å, 2.734 Å), the oxygen of V179 (2.477Å), respectively. While the left phenyl ring of **1d** might rotate to some extent comparing with that of TMC278 with the variations in τ1 and τ3, it could still adopt the parallel position to the phenyl ring of Y181 so as to keep the π-π stacking interactions. Because of the remarkable changes in τ5, the cyanovinyl group of compound **1d** moved closer to W229 for 0.3 Å more than that of TMC278, which could explain the enhanced biological activity.

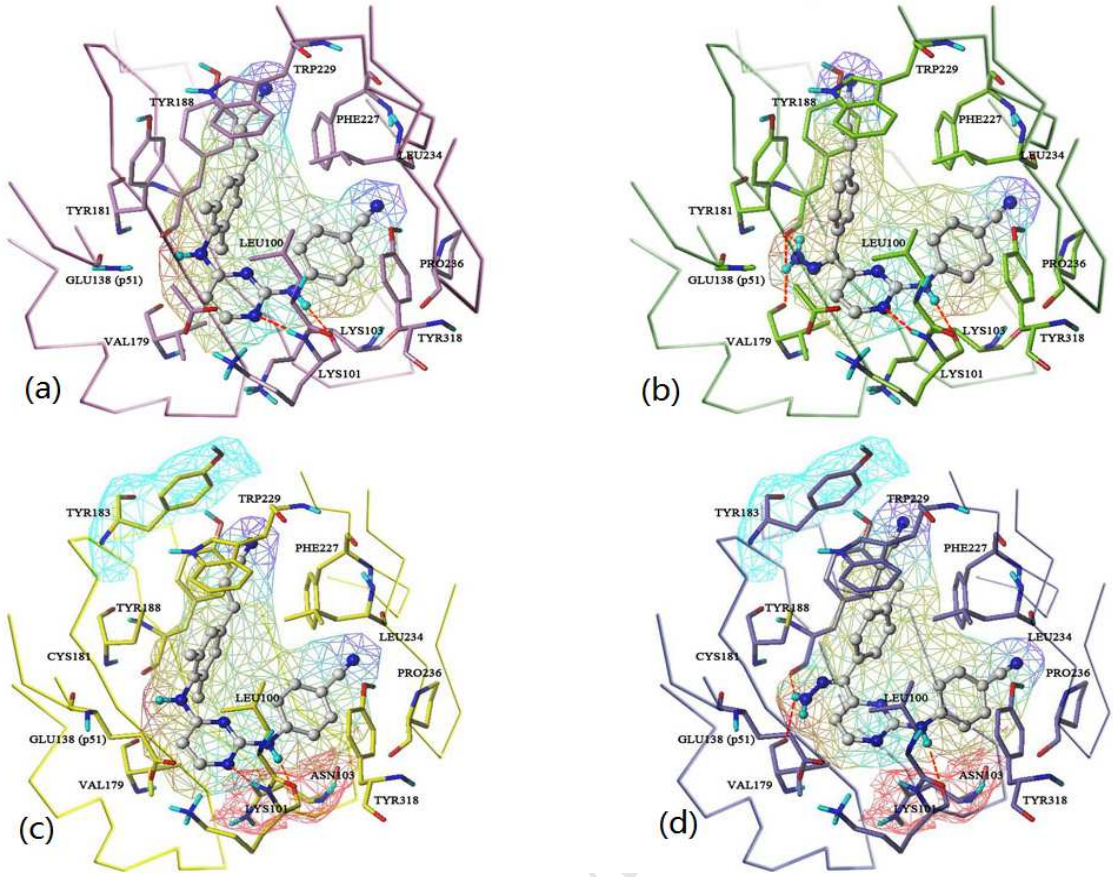


Fig. 3. Comparison of the binding modes between the representative potential compound **1d** and TMC278 in NNRTBP of wild type of HIV-1 RT (PDB code: 2ZD1[25]) and K103N/Y181C double mutated HIV-1 RT (PDB code: 3BGR[25]). (a) **T**MC278/RT^{WT}, (b) **1d**/RT^{WT}, (c) TMC278/RT$^{K103N/Y181C}$, (d) **1d**/RT$^{K103N/Y181C}$. The figures are generated by the software of SYBYL-X 2.0. (For interpretation of the references to color in these figures legend, the reader is referred to the web version of this article.) Possible hydrogen bonds are indicated with dashed lines in red. The amino acid residues are shown in liner frame and the docked conformations of the small molecules are shown in ball and stick both for **1d** and TMC278, respectively.

Table 2

Comparison of the torsion angles of the binding conformations of TMC278 and compound **1d**.

Compound and RT complex	Torsion angles (°)				
	τ1	τ2	τ3	τ4	τ5
TMC278/RT^{WT}	85.0	16.1	-11.5	-12.0	-49.6
1d/ RT^{WT}	-35.1	28.0	-48.9	0.6	-150.4
TMC278/RT$^{K103N/Y181C}$	-98.4	16.88	-6.0	-9.6	-49.2
1d/RT$^{K103N/Y181C}$	-79.6	37.0	-33.7	0.65	-67.5

TMC278

The K103N mutant and Y181C mutant are two of the most commonly known mutants relevant to clinical NNRTI-resistance. Generally speaking, the mutation of aromatic tyrosine to nonaromatic cysteine would cause a dramatic change from a hydrophobic environment to a hydrophilic environment of the binding pocket, so to abolish most of the possible hydrophobic contacts[41]. The IC_{50} of TMC278 against K103N, Y181C and K103N+Y181C double mutant strains are less than 1 nm. The cocrystal structure for the complex of TMC278 and K103N/Y181C double mutant RT is therefore retrieved from PDB (PDB ID: 3BGR[25]) and is shown in Fig. 3 (c). The interaction mode show that the conformation of TMC278 in mutant RT complex did not change significantly compared with that in the wild type RT-complex. The torsion angle τ3 in TMC278 might change by a slight outside angle of 5° away from C181. The mutation would lead to the disappearance of the aromatic interaction between the phenyl ring and the aromatic ring of the primary Y181. At this point, the Y_{183}MDD sequence at the active site of this polymerase would move for a distance of 1.5 Å, so as to lead the amino residue Y183 moving close to the cyanovinyl group of TMC278, inducing another new kind of

interaction to compensate the lose in π-interaction caused by Y181C. Although this kind of compensation effect caused by Y183 is serendipitous, it could still be relied on in the further designing new potent HIV-1 RT inhibitors since the YMDD is a highly conserved sequence. This kind of new interaction with Y183 in mutant strain of HIV-1 RT is caused by slight change in the conformation of TMC278, which is contributed by rotation of the cyanovinyl group.

As for another mutant site N103, the interaction mode did not change very much compared to that of K103 in wild type of RT. N103 is situated below both the pyrimidine and phenyl ring, forming a hydrogen bond (3.0 Å) with the carbonyl group of the main chain of K101, without hampering the formation of the hydrogen bond between K101 and TMC278.

As shown in Fig. 3 (d), generally speaking, the interaction mode of **1d** with mutant type of HIV-1 RT is similar to that of TMC278, but the whole skeleton of the molecule of **1d** moved distinctively upward. The pyrimidine ring moved for 1.5 Å, the distances between E138 and V179 increased for about 1 Å and 0.3 Å, respectively. These changes would inevitably lead to the result that the original conserved hydrogen bond in the TMC278-mutant RT complex could not be formed between the N1 of **1d** and K101. The bond length of the hydrogen bond between the secondary amine of **1d** and K101 is 0.6 Å longer than that on TMC278-complex, the result of which is not beneficial for enhancing the biological activity. The cyano group on the right wing of **1d** moved upward for about 0.8 Å, which might be beneficial for the biological activity. The bond lengths of the hydrogen bonds between the hydrazino group of **1d** and Y188 and V179 are 2.070 Å and 2.571 Å, respectively.

The effect of Y181C mutation on this skeleton of **1d** shall be discussed as follows. The losing of π-π stacking effect in the interactions might be crucial for drug resistance. TMC278 show the high anti-drug resistance effect for the newly formed interaction with Y183 instead of the original π-π stacking in wild type. The changing in torsion angle τ5 could lead the cyanovinyl group of **1d** to get deepen into the hydrophobic channel composed of W229, Y188 and L234 and get much closer to Y188. From theoretical speculation, compound **1d** should have excellent anti-drug resistance, but the experimental result show that **1d** is only a moderate drug-resistant inhibitor. The result could be explained by the different interaction with other positions of the molecule of **1d**, especially by the disappearance of the conserved hydrogen bond between the pyrimidine ring of **1d** and K101, which might influence the biological activity significantly. Quite similar to that of TMC278, the variation in the interaction between **1d** with N103 is not very distinct, for most of the DAPYs derivatives are active against the K103N mutant strain.

4. Conclusions

In summary, a series of new 4-(substituted-amino) arylmethyl DAPYs were successfully synthesized as anti-HIV RT agents against the drug resistant virus strains. Biological evaluation indicated that most of these derivatives showed good to potent activities against wild-type (WT) HIV-1 with IC_{50} values in the range of 69.21-0.0175 μM. Compound **1d** displayed potent anti-HIV-1 activity against WT HIV-1 with an IC_{50} value of 1.75 nM and a SI of 106367, 47 fold lower than that of AZT. The compound **1d** also showed a broad-spectrum inhibitory activity, with an IC_{50} value of 5.33 μM and 5.05 μM against HIV-1 double -mutated (K103N/Y181C) strain and HIV-2 strain, respectively. These attributes earmark compound **1d** as a potential new lead for developing more therapeutically applicable inhibitors against both HIV-1 and HIV-2 with better resistance profile. A preliminary SAR analysis of these target molecules highlighted a preference for the smaller or more flexible groups substitution in the linker between the left ring and the pyrimidine skeleton for enhancing the anti-HIV activity. It was also demonstrated that 4-cyano group substitution was also the best one in keeping potency against HIV-2. The subsequent studies undertaken to evaluate the different interaction modes of the most potential compound **1d** with both the wild type and mutant type of HIV-1 RT have identified the similar interaction modes with the FDA-approved drug TMC278. These results of this paper might provide the useful indicators for guiding further rational design of new DAPYs analogues as novel HIV-1 NNRT inhibitors that are more active against drug resistant HIV-strains.

5. Experimental section

5.1. Chemistry

Chemical reagents and solvents, purchased from commercial sources, were of analytical grade and were used without further purification. Melting points were measured on a SGW X-1 microscopic melting point apparatus. TLC analyses were carried on precoated silica gel G plates at 254 nm under a UV lamp using a variety of solvent systems. Column chromatography separations were performed with silica gel (300-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a Waters Quattro Micromass instrument using electrospray ionization (ESI) techniques.

5.1.1. Preparation of 1-aminopropan-2-ol

Propylene epoxide (5.80 g, 100 mmol) was added slowly into a concentrated ammonium hydroxide (50.0 mL) with stirring in an ice bath. The reaction mixture was kept under 0℃ for 1 h, and the evaporated under *vacuo* to get rid of the water to give the product as the colorless oil (6.2 g), the GC-MS analysis showed that the content percentage of 1-amino-2-propanol is 35.0 %, which could be stoichiometrically used in the final step in the synthesis of the target compounds (**1q** and **1r**).

5.1.2. Synthesis of 2-(methylthio)-2,3-dihydropyrimidin-4(1H) -one(5)

NaOH (12.6 g, 315 mmol) was dissolved in water (300 mL), to which 2-thiouracil (**3**, 38.4 g, 300 mmol) was added with stirring to make the materials to dissolve completely. Methyl iodide (53.3 g, 375 mmol) was then added to the above solution. The reaction mixture was then kept stirring at r. t. for 24 h with TLC monitoring. A large amount of white solid appeared from the reaction mixture while being adjusted the pH to 7 with acetic acid. The product was obtained by filtration and washed with small amount of water, dried *in vacuo* to afford the intermediate **5** with the yield of 77.2%.

5.1.3. Synthesis of 2-(4-cyanophenylamino)pyrimidin-4(1H) -one(6)

Intermediate **5** (28.4 g, 200 mmol) was mixed well together with 4-aminobenzonitrile (59.0 g, 500 mmol). The reaction temperature was rise to 160 ℃ to melt the solids, and was then allowed to be raised to 180-190 ℃ with stirring for 10 h. The reaction mixture was cooled down a little bit before adding acetonitrile to smash the solid with ultrasonic conditions. The product was obtained by filtration and washed with a small amount of acetonitrile and methylene chloride. It was then dried *in vacuo* to give the product **6** as yellow solid with the yield of 65.8%.

5.1.4. Synthesis of 2-(4-cyanophenylamino)-4-chloropyrimidine (7)

Compound **6** (32.1 g, 150 mmol) was mixed with phosphorus oxychloride (40.0 mL) and then heating to refluxing for 0.5 h. The reaction mixture was then slowly added to icy water (250 mL) with vigorously stirring. The yellow solid was precipitated and filtrated to give a filtrate cake, which was then again added into water (50.0 mL) and was adjusted to pH = 7 with sodium hydroxide. The solid was obtained by filtration and dried under *vacuo* to provide the intermediate **7** as pale yellow solids with the yield of 78.4%.

5.1.5. General procedure for the synthesis of 2-(4-cyano phenylamino)-4-(2-chlorobenzoyl)pyrimidine (9a) and 2-(4- cyanophenylamino)-4-(4-bromobenzoyl)pyrimidine (9b)

To dried DMF (30.0 mL) was added the intermediate **7** (2.00

mmol) and the halogenated phenylacetonitrile (**a**, R = 2-Cl, 755 mg; **b**, 4-Br, 975 mg; 5.00 mmol). The reaction mixture was cooled down to -20 ℃ within an ethanol cooling bath. NaH (60.0 %, 184 mg, 4.60 mmol) was added under nitrogen atmosphere and was continued with stirring for 1 h. The reaction mixture was raised to r. t. for 24-72 h with the monitoring by TLC. The intermediate **8** (**8a** or **8b**) was then obtained without further purifications. The reaction mixture was then kept for another 24-72 h without N_2 protection until the TLC analysis showed the disappearance of compound **8**. The reaction mixture was then poured into water (300 mL) and acidified with HCl to pH = 7. The obtained mixture was extracted with ethyl acetate (150 mL × 3), dried over anhydrous sodium sulfate, and the solvent was evaporated to give the solid, which was recrystallized with acetonitrile to afford the important intermediate **9a** or **9b**, with a yield of 24-65%, respectively.

5.1.6. Synthesis of 2-(4-cyanophenylamino)-4-(4-cyanobenzoyl) pyrimidine (9c)

A mixture of **9b** (756 g, 2.00 mmol), CuCN (178 mg, 2.00 mmol) and DMF (2.00 mL) was heated at 150℃ for 10 h with stirring, then cooled. The suspension was a solution of iron (III) chloride (1.00 g), concentrated HCl (0.20 mL), and water (20.0 mL). The mixture was stirred at 70℃ for 20 min and extracted with CH_2Cl_2 (20.0 mL×3). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (silica gel; CH_2Cl_2) gave **9c**.

5.1.7. Synthesis of 2-(4-cyanophenylamino)-4-(2-cyanovinyl) benzoyl)pyrimidine (9d)

A mixture of **9b** (756 mg, 2.00 mmol.), acrylonitrile (1.06 g, 20.0 mmol), $Pd(OAc)_2$ (89.8 mg, 400 mmol), tri-*O*-tolylphosphine (0.60 g, 2.00 mmol) and sodium acetate (656 mg, 8.00 mmol.) in anhydrous DMA (30.0 mL) was stirred in a sealed tube at 150℃ overnight. The reaction mixture was hydrolyzed with water and extracted with CH_2Cl_2 (20.0 mL×3). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product **9d** was recrystallized from acetonitrile to provide the pure product, which could be used directly in the next step.

5.1.8. Synthesis of target compounds 1a-1r

1) General procedure for the synthesis of hydroxyl substituted CH_2-DAPYs target molecules (1a, 1b)

The intermediate **9** (**9c**, 975 mg; or **9d**, 1.05 mg; 3.00 mmol) was dissolved in methanol (30.0 mL). KBH_4 (108 mg, 2.00 mmol) was then added into the above solution while cooling down in an ice bath. The reaction mixture was kept stirring at this temperature for 2 h before pouring into water (100 mL) under stirring. The mixture was extracted with ethyl acetate (50.0 mL × 3), dried over anhydrous Na_2SO_4, and was then concentrated to evaporate the excess solvent. The residue was purified with column chromatography (EtOAc/petroleum ether = 1 : 4) to give the pure product **1a** and **1b**, respectively. The yields, the physical properties and the related spectra data of these two compounds were as follows:

2-(4-Cyanophenylamino)-4-(4-cyanophenylhydroxymethyl) pyrimidine (1a) Yield: 67.6%; gray white solid; mp 177.9-179.7 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H, N*H*), 8.56 (d, J = 4.9 Hz, 1H, pyrimidine H_6), 7.85 (d, d, J = 8.6 Hz, 4H, 2-Ar$H_{3,5}$+ 4-Ar'$H_{3',5'}$), 7.66 (d, d, J = 8.3 Hz, 4H, 2-Ar$H_{2,6}$+ 4-Ar'$H_{2',6'}$), 7.18 (d, J = 4.9 Hz, 1H, pyrimidine H_5), 6.53 (d, J = 4.1 Hz, 1H, O*H*), 5.69 (d, J = 3.9 Hz, 1H, C*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.85, 159.59, 159.22, 148.98, 145.27, 133.38 (2C), 132.71 (2C), 128.18 (2C), 120.03, 119.28, 118.75 (2C), 110.69, 110.11, 102.84, 74.64; MS (ESI+) m/z 328 $(M+H)^+$; HR-MS: m/z Calcd for $C_{19}H_{13}N_5O$: 327.1120. Found: 327.1129.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydroxymethyl)pyrimidine (1b) Yield: 58.7%; yellow solid; mp 193.5-195.1 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, N*H*), 8.55 (t, J = 4.5 Hz, 1H, pyrimidine H_6), 7.88 (d, J = 8.5 Hz, 2H, Ar$H_{3,5}$), 7.80-7.33 (m, 7H, Ar'*H*+alkenyl *H*+ Ar$H_{2,6}$), 7.18 (d, J = 5.1 Hz, 1H, pyrimidine H_5), 6.53-6.26 (m, 2H, alkenyl *H*+O*H*), 5.63 (d, J = 4.1 Hz, 1H, C*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.41, 159.41, 159.20, 150.75, 146.57, 145.34, 133.48, 133.36 (2C), 128.14 (2C), 127.78 (2C), 120.05, 119.28, 118.74 (2C), 110.03, 102.77, 97.08, 74.95; MS (ESI+) m/z 354 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{15}N_5O$: 353.1277. Found: 353.1285.

2) General procedure for the synthesis of hydrazino substituted CH_2-DAPYs target molecules (1c, 1d)

The intermediate **9** (**9c**, 975 mg; or **9d**, 1.05 g; 3.00 mmol) and the hydrazine hydrochloride (411 mg, 6.00 mmol) were dissolved in ethanol (30.0 mL), to which pyridine (1.94 mL, 1.90 g, d= 0.98 g/mL, 24.0 mmol) was added. The reaction mixture was refluxed for 2 h before cooling down to r. t.. The obtained mixture was then poured into water (100 mL) and extracted with ethyl acetate. The extraction was dried over anhydrous sodium sulfate and was then concentrated under *vacuo* to get rid of the excess solvent. The residue was purified with column chromatography to give the pure product **1c** and **1d**, respectively. The yields, the physical properties and the related spectra data of these two compounds were as follows:

2-(4-Cyanophenylamino)-4-(4-cyanophenylhydrazonomethyl)pyrimidine (1c) Yield: 49.0%; yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H, N*H*), 8.40 (d, J = 5.4, 1H, pyrimidine H_6), 8.04 (d, J = 8.0, 2H, Ar$H_{3,5}$), 7.59 (s, 2H, NH_2), 7.54-7.44 (d,d, 4H, Ar$H_{2,6}$+ Ar'$H_{2,6}$), 7.43-7.32 (m, 3H, Ar'*H*+ pyrimidine H_5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.54, 159.24, 157.75, 145.42, 138.96, 138.38, 133.34 (2C), 132.85 (2C), 131.44 (2C), 120.04, 119.37, 118.41 (2C), 111.48, 107.74, 102.27; MS (ESI+) m/z 340 $(M+H)^+$; HR-MS: m/z Calcd for $C_{19}H_{13}N_7$: 339.1232. Found: 339.1245.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydrazonomethyl)pyrimidine (1d) Yield: 38.2%; yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H, N*H*), 8.39 (d, J = 5.3 Hz, 1H, pyrimidine H_6), 8.16-7.22 (m, 12H, Ar*H* +Ar'*H* +pyrimidine H_5+NH_2+alkenyl *H*), 6.60 (d, J = 16.8 Hz, 1H, alkenyl *H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.83, 159.26, 157.70, 150.84, 145.44, 140.16, 135.71, 134.19, 132.86 (2C), 130.80 (2C), 128.75 (2C), 120.04, 119.31, 118.46 (2C), 107.91, 102.19, 97.67; MS (ESI+) m/z 366 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{15}N_7$: 365.1389. Found: 365.1401.

3) General procedure for the synthesis of rest target molecules (1e-1r)

To a mixture of **9** (1.00 mmol), Na_2SO_4 (213 mg, 1.50 mmol) and ethanol (20.0 mL) was added various substituted amine (5.00 mmol) and 5 drops of acetic acid. The mixture was heated to reflux for 4-8 h monitored by TLC, then cooled. Sodium cyanoborohydride was added at r. t. and subsequently the mixture was heated at 60 ℃ for 4 h, then poured into saturated sodium bicarbonate and extracted with CH_2Cl_2 (20.0 mL × 3). The organic layers were combined together, dried over anhydrous $MgSO_4$, and concentrated under *vacuo*. The residue was then purified by silica gel column chromatography (silica gel; EtOAc/petroleum ether, 1:5 to 1:3). The yields, the physical properties and the related spectra data of these compounds were as follows:

2-(4-Cyanophenylamino)-4-(4-cyanophenylmethylaminomethyl)pyrimidine (1e) Yield: 24.6%; gray yellow solid; mp 71.0-72.4 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H, N*H*), 8.50 (d, J = 5.0, 1H, pyrimidine H_6), 7.85 (d, J = 8.6, 2H, Ar$H_{3,5}$), 7.80 (d, J = 8.1, 2H, Ar'*H*), 7.71-7.56 (d, d, 4H, Ar'$H_{2',6'}$ +, Ar$H_{2,6}$), 7.10 (d, J = 5.0, 1H, pyrimidine H_5), 4.75 (s, 1H, C*H*), 2.25 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.61, 159.53, 159.36, 147.77, 145.21, 133.42 (2C), 132.83 (2C), 129.14 (2C), 120.05, 119.26, 118.79 (2C), 111.42, 110.58, 102.83, 68.98, 34.57; MS (ESI+) m/z 341 $(M+H)^+$; HR-MS: m/z Calcd for $C_{20}H_{16}N_6$: 340.1436. Found: 340.1449.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylmethylamino methyl)pyrimidine (1f) Yield: 26.7%; yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, N*H*), 8.50 (t, *J* = 6.0 Hz, 1H, pyrimidine H_6), 7.89 (d, *J* = 9.0 Hz, 2H, Ar$H_{3,5}$), 7.83-7.45 (m, 7H, Ar$H_{2,6}$+Ar'*H*+alkenyl *H*), 7.11 (dd, *J* = 9.8, 4.6 Hz, 1H, pyrimidine H_5), 6.10 (dd, *J* = 14.5 Hz, 1H, alkenyl *H*), 4.79-4.52 (m, 1H, C*H*), 2.26 (d, *J* = 5.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.58, 159.56, 159.48, 150.72, 145.66, 145.35, 133.37 (2C), 133.34, 128.61 (2C), 128.28 (2C), 120.05, 119.32, 118.77 (2C), 111.36, 102.75, 96.95, 67.25, 32.8; MS (ESI+) m/z 367 (M+H)$^+$; HR-MS: m/z Calcd for $C_{22}H_{18}N_6$: 366.1593. Found: 366.1607.

2-(4-Cyanophenylamino)-4-(2-chlorophenylmethylaminomethyl)pyrimidine (1g) Yield: 78.5%; earth yellow solid; mp 130.5-131.6 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H, N*H*), 8.52 (d, *J* = 4.8 Hz, 1H, pyrimidine H_6), 7.84 (d, *J* = 8.3 Hz, 2H, Ar$H_{3,5}$), 7.61 (d, *J* = 8.3 Hz, 2H, Ar$H_{2,6}$), 7.56 (d, *J* = 7.6 Hz, 1H, Ar'*H*), 7.44 (d, *J* = 7.9 Hz, 1H, Ar'*H*), 7.37 (t, *J* = 7.4 Hz, 1H, Ar'*H*), 7.29 (t, *J* = 7.5 Hz, 1H, Ar'*H*), 7.08 (d, *J* = 4.9 Hz, 1H, pyrimidine H_5), 5.08 (s, 1H, C*H*), 2.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.42, 159.55, 159.14, 145.33, 139.46, 133.66, 133.26 (2C), 129.81, 129.76, 129.35, 127.84, 120.04, 118.70 (2C), 111.85, 102.70, 65.59, 34.86; MS (ESI+) m/z 350 (M+H)$^+$; HR-MS: m/z Calcd for $C_{19}H_{16}ClN_5$: 349.1094. Found: 349.1109.

2-(4-Cyanophenylamino)-4-(4-cyanophenylethylaminomethyl) pyrimidine (1h) Yield: 49.6%; yellow solid; mp 51.0-52.8 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H, N*H*), 8.50 (d, *J* = 5.1 Hz, 1H, pyrimidine H_6), 7.88 (d, *J* = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.80 (d, *J* = 8.2 Hz, 2H, Ar'*H*), 7.73-7.59 (d, d, 4H, Ar'$H_{2',6'}$,+Ar$H_{2,6}$), 7.12 (d, *J* = 5.1 Hz, 1H, pyrimidine H_5), 4.87 (s, 1H, C*H*), 2.49-2.40 (m, 2H, CH_2), 1.05 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.06, 159.54, 159.25, 148.29, 145.28, 133.39 (2C), 132.80 (2C), 129.09 (2C), 120.04, 119.27, 118.76 (2C), 111.43, 110.51, 102.82, 67.15, 42.02, 15.52; MS (ESI+) m/z 355 (M+H)$^+$; HR-MS: m/z Calcd for $C_{23}H_{23}N_5O$: 354.1593. Found: 354.1605.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylethylaminomethyl)pyrimidine (1i) Yield: 50.7%; yellow solid; mp 68.2-69.9 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, N*H*), 8.48 (d, *J* = 5.1 Hz, 1H, pyrimidine H_6), 7.90 (d, *J* = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.66 (d, *J* = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.63-7.54 (m, 3H, Ar'*H* + alkenyl *H*), 7.49 (d, *J* = 8.1 Hz, 2H, Ar'*H*), 7.10 (d, *J* = 5.1 Hz, 1H, pyrimidine H_5), 6.38 (d, *J* = 16.7 Hz, 1H, alkenyl *H*), 4.80 (s, 1H, C*H*), 2.49-2.42 (m, *J* = 7.3 Hz, 2H, CH_2), 1.04 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.48, 159.50, 159.08, 150.71, 145.63, 145.32, 133.39 (2C), 133.33, 128.62 (2C), 128.29 (2C), 120.06, 119.30, 118.76 (2C), 111.35, 102.76, 96.96, 67.24, 42.03, 15.45; MS (ESI+) m/z 381 (M+H)$^+$; HR-MS: m/z Calcd for $C_{23}H_{20}N_6$: 380.1749. Found: 380.1755.

2-(4-Cyanophenylamino)-4-(2-chlorophenylethylaminomethyl) pyrimidine (1j) Yield: 64.4%; gray white solid; mp 117.8-119.3 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H, N*H*), 8.50 (d, *J* = 5.0 Hz, 1H, pyrimidine H_6), 7.83 (d, *J* = 8.6 Hz, 2H, Ar$H_{3,5}$), 7.58 (d, d, *J* = 8.1 Hz, 3H, Ar$H_{2,6}$+Ar'$H_{2',6'}$), 7.42 (d, *J* = 7.8 Hz, 1H, Ar'*H*), 7.36 (t, *J* = 7.3 Hz, 1H, Ar'*H*), 7.29 (d, *J* = 7.3 Hz, 1H, Ar'*H*), 7.07 (d, *J* = 5.0 Hz, 1H, pyrimidine H_5), 5.20 (s, 1H, C*H*), 2.54-2.46 (m, 2H, CH_2), 1.04 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.61, 159.51, 159.11, 145.31, 139.73, 133.53, 133.25 (2C), 129.85, 129.74, 129.32, 127.85, 120.04, 118.70 (2C), 111.83, 102.70, 63.47, 42.10, 15.48; MS (ESI+) m/z 364 (M+H)$^+$; HR-MS: m/z Calcd for $C_{20}H_{18}ClN_5$: 363.1251. Found: 363.1259.

2-(4-Cyanophenylamino)-4-(4-cyanophenylpropylaminomethyl)pyrimidine (1k) Yield: 35.5%; yellow solid; mp 39.2-41.0 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H, N*H*), 8.51 (d, *J* = 4.4 Hz, 1H, pyrimidine H_6), 7.89 (d, *J* = 8.3 Hz, 2H, Ar$H_{3,5}$), 7.81 (d, *J* = 7.6 Hz, 2H, Ar'*H*), 7.75-7.56 (d, d, 4H, Ar'$H_{2',6'}$,+Ar$H_{2,6}$), 7.12 (d, *J* = 4.5 Hz, 1H, pyrimidine H_5), 4.85 (s, 1H, C*H*), 2.46-2.34 (m, 2H, CH_2), 1.55-1.37 (m, 2H, CH_2), 0.84 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.08, 159.55, 159.24, 148.33, 145.29, 133.38 (2C), 132.79 (2C), 129.09 (2C), 120.03, 119.26, 118.76 (2C), 111.46, 110.52, 102.83, 67.26, 49.73, 23.16, 12.21; MS (ESI+) m/z 369 (M+H)$^+$; HR-MS: m/z Calcd for $C_{22}H_{20}N_6$: 368.1749. Found: 368.1761.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylpropylaminomethyl)pyrimidine (1l) Yield: 34.0%; yellow solid; mp 60.7-61.5 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H, N*H*), 8.46 (d, *J* = 4.7 Hz, 1H, pyrimidine H_6), 7.89 (d, *J* = 8.5 Hz, 2H, Ar$H_{3,5}$), 7.79-7.40 (m, 7H, Ar$H_{3,5}$+ Ar'*H*+ alkenyl *H*), 7.08 (d, *J* = 7.0 Hz, 1H, pyrimidine H_5), 6.07 (dd, *J* = 14.5 Hz, 1H, alkenyl *H*), 4.78 (s, *J* = 8.6 Hz, 1H, C*H*), 2.45-2.34 (m, *J* = 7.0 Hz, 2H, CH_2), 1.44 (dd, *J* = 14.3, 7.2 Hz, 2H, CH_2), 0.81 (t, *J* = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.46, 159.49, 159.05, 150.69, 145.67, 145.30, 133.36 (2C), 133.30, 128.62 (2C), 128.28 (2C), 120.05, 119.29, 118.76 (2C), 111.39, 102.77, 96.91, 67.33, 49.71, 23.09, 12.18; MS (ESI+) m/z 395 (M+H)$^+$; HR-MS: m/z Calcd for $C_{24}H_{22}N_6$: 3394.1906. Found: 394.1918.

2-(4-Cyanophenylamino)-4-(2-chlorophenylpropylaminomethyl)pyrimidine (1m) Yield: 72.3%; light yellow solid; mp 125.5-126.8 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H, N*H*), 8.52 (d, *J* = 5.0 Hz, 1H, pyrimidine H_6), 7.84 (d, *J* = 8.8 Hz, 2H, Ar$H_{3,5}$), 7.61 (d, *J* = 8.7 Hz, 2H, Ar$H_{2,6}$), 7.59-7.55 (m, 1H, Ar'*H*), 7.44 (d, *J* = 7.7 Hz, 1H, Ar'*H*), 7.36 (d, *J* = 7.4 Hz, 1H, Ar'*H*), 7.33-7.26 (m, 1H, Ar'*H*), 7.09 (d, *J* = 5.0 Hz, 1H, pyrimidine H_5), 5.20 (s, 1H, C*H*), 2.45 (t, *J* = 7.0 Hz, 2H, CH_2), 1.55-1.37 (m, *J* = 7.2 Hz, 2H, CH_2), 0.85 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.68, 159.53, 159.12, 145.35, 139.81, 133.57, 133.27 (2C), 129.90, 129.76, 129.32, 127.86, 120.03, 118.69 (2C), 111.84, 102.70, 63.68, 49.87, 23.15, 12.23; MS (ESI+) m/z 378 (M+H)$^+$; HR-MS: m/z Calcd for $C_{21}H_{20}ClN_5$: 377.1407. Found: 377.1419.

2-(4-Cyanophenylamino)-4-(4-cyanophenyl*iso*propylaminomethyl)pyrimidine (1n) Yield: 46.8%; yellow solid; mp 60.9-62.6 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H, N*H*), 8.50 (d, *J* = 4.8 Hz, 1H, pyrimidine H_6), 7.88 (d, *J* = 8.2 Hz, 2H, Ar$H_{3,5}$), 7.80 (d, *J* = 8.0 Hz, 2H, Ar'*H*), 7.72-7.62 (d, d, 4H, Ar'$H_{2',6'}$,+Ar$H_{2,6}$), 7.14 (d, *J* = 4.8 Hz, 1H, pyrimidine H_5), 5.00 (s, 1H, C*H*), 2.66-2.56 (m, 1H, C*H*), 1.01 (d, *J* = 5.7 Hz, 6H, 2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.22, 159.54, 159.15, 148.58, 145.29, 133.38 (2C), 132.77 (2C), 129.12 (2C), 120.02, 119.27, 118.76 (2C), 111.74, 110.45, 102.84, 64.31, 46.25, 23.37, 23.10; MS (ESI+) m/z 369 (M+H)$^+$; HR-MS: m/z Calcd for $C_{22}H_{20}N_6$: 368.1749. Found: 368.1763.

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenyl*iso*propyl aminomethyl)pyrimidine (1o) Yield: 39.8%; yellow solid; mp 58.7-59.0 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (d, *J* = 8.0 Hz, 1H, N*H*), 8.60-8.32 (m, 1H, pyrimidine H_6), 7.87 (t, *J* = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.78-7.39 (m, 7H, Ar$H_{3,5}$+ Ar'*H*+ alkenyl *H*), 7.10 (dd, *J* = 8.8, 5.1 Hz, 1H, pyrimidine H_5), 6.16 (dt, *J* = 9.0 Hz, 1H, alkenyl *H*), 4.93 (d, *J* = 8.8 Hz, 1H, C*H*), 2.59 (dt, *J* = 12.2, 6.0 Hz, 1H, C*H*), 0.99 (d, *J* = 6.0 Hz, 6H, 2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.55, 159.49, 158.96, 150.67, 145.85, 145.29, 133.35 (2C), 133.23, 128.63 (2C), 128.27 (2C), 120.04, 119.28, 118.77 (2C), 111.68, 102.80, 96.89, 64.39, 46.19, 23.29, 22.97; MS (ESI+) m/z 395 (M+H)$^+$; HR-MS: m/z Calcd for $C_{24}H_{22}N_6$: 394.1906. Found: 394.1921.

2-(4-Cyanophenylamino)-4-(2-chlorophenyl*iso*propylaminomethyl)pyrimidine (1p) Yield: 64.3%; light yellow solid; mp 143.6-144.9 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H, N*H*), 8.51 (d, *J* = 5.1 Hz, 1H, pyrimidine H_6), 7.84 (d, *J* = 8.7 Hz, 2H, Ar$H_{3,5}$), 7.61 (d, d, *J* = 8.2 Hz, 3H, Ar'$H_{5'}$+Ar$H_{2,6}$), 7.45 (d, *J* = 7.8 Hz, 1H, Ar'$H_{2'}$), 7.36 (t, *J* = 7.2 Hz, 1H, Ar'$H_{3'}$), 7.30 (t, 1H, Ar'$H_{4'}$), 7.10 (d, *J* = 5.1 Hz, 1H, pyrimidine H_5), 5.34 (s, 1H, C*H*), 2.68-2.60 (m, 1H, C*H*), 1.02 (d, *J* = 5.8 Hz, 6H, 2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.90, 159.49, 159.07, 145.33, 140.00, 133.48, 133.26 (2C), 130.01, 129.78, 129.30, 127.86, 120.02, 118.69 (2C), 111.87, 102.72, 60.91, 46.36, 23.57, 22.95; MS (ESI+)

m/z 378 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{20}ClN_5$: 377.1407. Found: 377.1419.

2-(4-Cyanophenylamino)-4-(4-cyanophenyl-2-hydroxypropylaminomethyl)pyrimidine (1q) Yield: 38.5%; brown solid; mp 48.6-50.0 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (d, J = 2.6 Hz, 1H, N*H*), 8.50 (d, J = 5.0 Hz, 1H, pyrimidine H_6), 7.89 (d, J = 8.7, 2H, Ar$H_{3,5}$), 7.81 (d, J = 8.2, 2H, Ar'$H_{3',5'}$), 7.63 (d, d, J = 8.4 Hz, 4H, Ar'$H_{2',6'}$+Ar$H_{2,6}$), 7.09 (dd, J = 5.0 Hz, 1H, pyrimidine H_5), 4.89 (d, J = 5.3 Hz, 1H, C*H*), 4.62 (d, J = 7.6 Hz, 1H, O*H*), 3.74 (s, 1H, C*H*), 2.44-2.33 (m, 2H, CH_2), 1.04 (d, J = 6.0 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.80, 159.59 (d, J = 5.5 Hz), 159.33, 148.16, 145.25, 133.39 (2C), 132.84 (2C, d, J = 2.9 Hz), 129.12 (2C), 120.03, 119.26, 118.82 (2C, d, J = 3.9 Hz), 111.60 (d, J = 16.9 Hz), 110.56, 102.84, 67.04, 65.99 (d, J = 18.6 Hz), 55.55 (d, J = 28.4 Hz), 21.95 (d, J = 4.7 Hz); MS (ESI+) m/z 385 $(M+H)^+$; HR-MS: m/z Calcd for $C_{22}H_{20}N_6O$: 384.1699. Found: 384.1707.

2-(4-Cyanophenylamino)-4-(2-chlorophenyl-2-hydroxypropylaminomethyl)pyrimidine (1r) Yield: 41.8%; brown solid; mp 54.6-56.0 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (d, J = 6.2 Hz, 1H, N*H*), 8.51 (t, J = 4.0 Hz, 1H, pyrimidine H_6), 7.85 (t, J = 8.4 Hz, 2H, Ar$H_{3,5}$), 7.68-7.51 (d, d, 3H, Ar'$H_{5'}$+Ar$H_{2,6}$), 7.49-7.34 (m, 2H, Ar'*H*), 7.30 (t, J = 7.4 Hz, 1H, Ar'*H*), 7.05 (dd, J = 4.9 Hz, 1H, pyrimidine H_5), 5.21 (d, J = 13.7 Hz, 1H, C*H*), 4.61 (s, 1H, O*H*), 3.74 (d, J = 5.2 Hz, 1H, C*H*), 2.47-2.35 (m, J = 8.1 Hz, 2H, CH_2), 1.04 (d, J = 4.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.39 (d, J = 17.0 Hz), 159.58 (d, J = 6.6 Hz), 159.20, 145.31, 139.59, 133.52 (d, J = 9.1 Hz), 133.27 (2C), 129.82 (d, J = 4.8 Hz), 129.79, 129.41, 127.92, 120.04, 118.74 (2C, d, J = 4.7 Hz), 111.90 (d, J = 11.1 Hz), 102.75, 65.99 (d, J = 20.3 Hz), 63.78 (d, J = 16.8 Hz), 55.80 (d, J = 26.7 Hz), 21.97; MS (ESI+) m/z 394 $(M+H)^+$; HR-MS: m/z Calcd for $C_{21}H_{20}ClN_5O$: 393.1356. Found: 393.1368.
7.61 (d, d, J = 8.2 Hz, 3H, Ar'$H_{5'}$+Ar$H_{2,6}$),

5.2. Anti-HIV activity assay

The anti-HIV activity and cytotoxicity of the compounds **1a-1r**, were evaluated against wild-type HIV-1 strain IIIB, a double RT mutant (K103N + Y181C) HIV-1 strain and HIV-2 strain ROD in MT-4 cell cultures using the 3-(4,5 -dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method[42, 43]. Briefly, stock solutions (10 × final concentration) of test compounds were added in 25 mL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock-and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control HIV-and mock-infected cell samples were included for each sample. Virus stock (50.0 mL) at 100-300 $CCID_{50}$ (50.0 % cell culture infectious dose) or culture medium was added to either the virus-infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compounds on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL and 50.0 mL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored MTT by mitochondrial dehydrogenase activity of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Infinite M1000, Tecan), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) values of tree wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

Reverse transcriptase assay: Recombinant wild type p66/p51 HIV-1 RT was expressed and purified as the previously described procedure[44]. The RT assay was performed with the EnzCheck Reverse Transcriptase Assay kit (Molecular Probes, Invitrogen), as described by the manufacturer. The assay was based on the dsDNA quantitation reagent PicoGreen. This reagent showed a pronounced increase in fluorescence signal upon binding to dsDNA or RNA-DNA heteroduplexes. Single-stranded nucleic acids generate only minor fluorescence signal enhancement when a sufficiently high dye: base pair ratio was applied[45].

A poly(rA) template of approximately 350 bases long, and an oligo$(dT)_{16}$ primer, were annealed in a molar ratio of 1:1.2 (60 min, at r. t.). 52 ng of the RNA/DNA was brought into each well of a 96-well plate in a volume of 20 mL polymerization buffer (60 mM Tris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 mM dTTP, pH 8.1). 5.0 mL of RT enzyme solution, diluted to a suitable concentration in enzyme dilution buffer (50 mM Tris-HCl, 20% glycerol, 2m MDTT, pH = 7.6), was added. The reaction mixture were incubated at 25 ℃ for 40 min and then stopped by the addition of EDTA (15 mM). Heteroduplexes were then detected by addition of PicoGreen. Signals were read using an excitation wavelength of 490 nm and emission detection at 523 nm using a spectrofluorometer (Safire2, Tecan). To test the activity of compounds against RT, 1.0 mL of compound in DMSO was added to each well before the addition of RT enzyme solution. Control wells without compound contained the same amount of DMSO. Results were expressed as relative fluorescence, i. e. the fluorescence signal of the reaction mixed with compound divided by the signal of the same reaction mixed without compound.

5.3. Molecular simulation

Molecular modeling was carried out with the Tripos molecular modeling packages Sybyl-X2.0. All the molecules for docking were built using standard bond lengths and angles from Sybyl-X2.0/base Builder and were then optimized using the Tripos force field for 2000 generations two times or more, until the minimized conformers of the ligand were the same. The flexible docking method, called Surflex-Dock[46], docks the ligand automatically into the ligand binding site of the receptor by using a protocol-based approach and an empirically derived scoring function[47]. The protocol is a computational representation of a putative ligand that binds to the intended binding site and is a unique and essential element of the docking algorithm[48]. The scoring function in Surflex-Dock, which contains hydrophobic, polar, repulsive, entropic, and salvation terms, was trained to estimate the dissociation constant (Kd) expressed in -log $(Kd)^2$.

Prior to docking, the protein was prepared by removing water molecules, the ligands (TMC278), and other unnecessary small molecules from the crystal structure of the ligands-HIV-1 RT complex (PDB code: 2ZD1[25] and 3BGR[25]); and then the hydrogen atoms were added to the protein also. Surflex-Dock default settings were used for other parameters, such as the number of starting conformations per molecule (set to 0), the size to expand search grid (set to 8Å), the maximum number of rotatable bonds per molecule (set to 100), and the maximum number of poses per ligand (set to 20).

During the docking procedure, all of the single bonds in residue side chains inside the defined RT binding pocket were regarded as rotatable or flexible, and the ligand was allowed to rotate on all single bonds and move flexibly within the tentative binding pocket. The atomic charges were recalculated using the Kollman all-atom approach for the protein and the Gasteiger Hückel approach for the ligand. The binding interaction energy was calculated to include *van* der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 20,000 generations using a genetic algorithm, and the 20-best-scoring ligand-protein complexes were kept for further analyses. The -log $(Kd)^2$ values of the 20-best-scoring complexes, which represented the binding affinities of ligand with RT, ranged over a wide scope of functional classes (10^{-2}~10^{-9}). Therefore, only the highest-scoring 3D structural model of the ligand-bound RT was chosen to define the

binding interaction[49].

Acknowledgments

The authors are grateful to the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, P. R. China (2011), the Shaanxi Province Science and Technology Research and Development Program of China, International Cooperation (2013KW31-04) and the National Natural Science Foundation of China (Grant No. 81172918).

References

[1] M.-P. de Béthune, Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989-2009), Antiviral Res., 85 (2010) 75-90.

[2] C. Reynolds, C.B. de Koning, S.C. Pelly, W.A. van Otterlo, M.L. Bode, In search of a treatment for HIV-current therapies and the role of non-nucleoside reverse transcriptase inhibitors (NNRTIs), Chem. Soc. Rev., 41 (2012) 4657-4670.

[3] E. De Clercq, The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection, Antiviral Res., 38 (1998) 153-179.

[4] P. Zhan, X. Chen, D. Li, Z. Fang, E. De Clercq, X. Liu, HIV-1 NNRTIs: structural diversity, pharmacophore similarity, and implications for drug design, Medicinal research reviews, 33 (2011) E1–E72.

[5] G. Meng, F. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Nonnucleoside HIV-1 reverse transcriptase inhibitors: part I. synthesis and structure-activity relationship of 1-alkoxymethyl-5-alkyl-6-naphthylmethyl uracils as HEPT analogues, Chem. Pharm. Bull. (Tokyo), 51 (2003) 779-789.

[6] C. Sahlberg, X.-X. Zhou, Development of non-nucleoside reverse transcriptase inhibitors for anti-HIV therapy, Anti-Infective Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Infective Agents), 7 (2008) 101-117.

[7] Y.-P. Wang, F.-E. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Synthesis and in vitro anti-HIV evaluation of a new series of 6-arylmethyl-substituted *S*-DABOs as potential non-nucleoside HIV-1 reverse transcriptase inhibitors, Eur. J. Med. Chem., 44 (2009) 1016-1023.

[8] Y. He, F. Chen, G. Sun, Y. Wang, E. De Clercq, J. Balzarini, C. Pannecouque, 5-Alkyl-2-[(aryl and alkyloxylcarbonylmethyl)thio]-6-(1-naphthylmethyl) pyrimidin-4(3H)-ones as an unique HIV reverse transcriptase inhibitors of S-DABO series, Bioorg. Med. Chem. Lett., 14 (2004) 3173-3176.

[9] D.W. Ludovici, B.L. De Corte, M.J. Kukla, H. Ye, C.Y. Ho, M.A. Lichtenstein, R.W. Kavash, K. Andries, M.-P. de Béthune, H. Azijn, Evolution of anti-HIV drug candidates. part 3: diarylpyrimidine (DAPY) analogues, Bioorg. Med. Chem. Lett., 11 (2001) 2235-2239.

[10] H. Zhang, F. Qin, W. Ye, Z. Li, S. Ma, Y. Xia, Y. Jiang, J. Zhu, Y. Li, J. Zhang, Revealing the drug-resistant mechanism for diarylpyrimidine analogue inhibitors of HIV-1 reverse transcriptase, Chem. Biol. Drug Des., 78 (2011) 427-437.

[11] S.-Q. Yang, C. Pannecouque, D. Daelemans, X.-D. Ma, Y. Liu, F.-E. Chen, E. De Clercq, Molecular design, synthesis and biological evaluation of BP-*O*-DAPY and *O*-DAPY derivatives as non-nucleoside HIV-1 reverse transcriptase inhibitors, Eur. J. Med. Chem., 65C (2013) 134-143.

[12] E. De Clercq, Non-nucleoside reverse transcriptase inhibitors (NNRTIs): past, present, and future, Chem. Biodivers., 1 (2004) 44-64.

[13] J. Ren, R.M. Esnouf, A.L. Hopkins, J. Warren, J. Balzarini, D.I. Stuart, D.K. Stammers, Crystal structures of HIV-1 reverse transcriptase in complex with carboxanilide derivatives, Biochemistry, 37 (1998) 14394-14403.

[14] A. Spallarossa, S. Cesarini, A. Ranise, M. Ponassi, T. Unge, M. Bolognesi, Crystal structures of HIV-1 reverse transcriptase complexes with thiocarbamate non-nucleoside inhibitors, Biochem. Biophys. Res. Commun., 365 (2008) 764-770.

[15] J. Ren, P.P. Chamberlain, A. Stamp, S.A. Short, K.L. Weaver, K.R. Romines, R. Hazen, A. Freeman, R.G. Ferris, C.W. Andrews, L. Boone, J.H. Chan, D.K. Stammers, Structural basis for the improved drug resistance profile of new generation benzophenone non-nucleoside HIV-1 reverse transcriptase inhibitors, J. Med. Chem., 51 (2008) 5000-5008.

[16] D.L. Romero, R.A. Morge, M.J. Genin, C. Biles, M. Busso, L. Resnick, I.W. Althaus, F. Reusser, R.C. Thomas, W.G. Tarpley, Bis(heteroaryl)piperazine (BHAP) reverse transcriptase inhibitors: structure-activity relationships of novel substituted indole analogues and the identification of 1-[(5-methanesulfonamido-1*H*-indol-2-yl)-carbonyl]-4-[3-[(1-methylethyl)amino]-pyridinyl]piperazine monomethanesulfonate (U-90152S), a second generation clinical candidate, J. Med. Chem., 36 (1993) 1505-1508.

[17] O.M. Klibanov, R.L. Kaczor, IDX-899, an aryl phosphinate-indole non-nucleoside reverse transcriptase inhibitor for the potential treatment of HIV infection, Current Opinion in Investigational Drugs, 11 (2010) 237-245.

[18] J. Guillemont, E. Pasquier, P. Palandjian, D. Vernier, S. Gaurrand, P.J. Lewi, J. Heeres, M.R. de Jonge, L.M. Koymans, F.F. Daeyaert, M.H. Vinkers, E. Arnold, K. Das, R. Pauwels, K. Andries, M.P. de Béthune, E. Bettens, K. Hertogs, P. Wigerinck, P. Timmerman, P.A. Janssen, Synthesis of novel diarylpyrimidine analogues and their antiviral activity against human immunodeficiency virus type 1, J. Med. Chem., 48 (2005) 2072-2079.

[19] A. Lazzarin, T. Campbell, B. Clotet, M. Johnson, C. Katlama, A. Moll, W. Towner, B. Trottier, M. Peeters, J. Vingerhoets, G. de Smedt, B. Baeten, G. Beets, R. Sinha, B. Woodfall, Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial, Lancet, 370 (2007) 39-48.

[20] J.V. Madruga, P. Cahn, B. Grinsztejn, R. Haubrich, J. Lalezari, A. Mills, G. Pialoux, T. Wilkin, M. Peeters, J. Vingerhoets, G. de Smedt, L. Leopold, R. Trefiglio, B. Woodfall, Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial, Lancet, 370 (2007) 29-38.

[21] A.M. Geretti, Shifting paradigms: the resistance profile of etravirine, The Journal of antimicrobial chemotherapy, 62 (2008) 643-647.

[22] X. Chen, P. Zhan, D. Li, E. De Clercq, X. Liu, Recent advances in DAPYs and related analogues as HIV-1 NNRTIs, Curr. Med. Chem., 18 (2011) 359-376.

[23] P.A. Janssen, P.J. Lewi, E. Arnold, F. Daeyaert, M. De Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, M. Kukla, D. Ludovici, K. Andries, M.P. de Béthune, R. Pauwels, K. Das, A.D. Clark, Jr., Y.V. Frenkel, S.H. Hughes, B. Medaer, F. De Knaep, H. Bohets, F. De Clerck, A. Lampo, P. Williams, P. Stoffels, In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine), J. Med. Chem., 48 (2005) 1901-1909.

[24] L. Baert, G. van 't Klooster, W. Dries, M. Francois, A. Wouters, E. Basstanie, K. Iterbeke, F. Stappers, P. Stevens, L. Schueller, P. Van Remoortere, G. Kraus, P. Wigerinck, J. Rosier, Development of a long-acting injectable formulation with nanoparticles of rilpivirine (TMC278) for HIV treatment, European Journal of Pharmacetical Biopharmaceutical, 72 (2009) 502-508.

[25] K. Das, J.D. Bauman, A.D. Clark, Y.V. Frenkel, P.J. Lewi, A.J. Shatkin, S.H. Hughes, E. Arnold, High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: strategic flexibility explains potency against resistance mutations, Proceedings of the National Academy of Sciences, 105 (2008) 1466-1471.

[26] J.D. Bauman, K. Das, W.C. Ho, M. Baweja, D.M. Himmel, A.D. Clark, D.A. Oren, P.L. Boyer, S.H. Hughes, A.J. Shatkin, Crystal engineering of HIV-1 reverse transcriptase for structure-based drug design, Nucleic Acids Res., 36 (2008) 5083-5092.

[27] E.B. Lansdon, K.M. Brendza, M. Hung, R. Wang, S. Mukund, D. Jin, G. Birkus, N. Kutty, X. Liu, Crystal structures of HIV-1 reverse transcriptase with etravirine (TMC125) and rilpivirine (TMC278): implications for drug design, J. Med. Chem., 53 (2010) 4295-4299.

[28] S.R. Ribone, M.A. Quevedo, M. Madrid, M.C. Briñón, Rational approaches for the design of effective human immunodeficiency virus type 1 nonnucleoside reverse transcriptase inhibitors, J. Chem. Inf. Model., 51 (2010) 130-138.

[29] X. Tian, B. Qin, Z. Wu, X. Wang, H. Lu, S.L. Morris-Natschke, C.H. Chen, S. Jiang, K.-H. Lee, L. Xie, Design, synthesis, and evaluation of diarylpyridines and diarylanilines as potent non-nucleoside HIV-1 reverse transcriptase inhibitors, J. Med. Chem., 53 (2010) 8287-8297.

[30] K. Das, A.D. Clark, Jr., P.J. Lewi, J. Heeres, M.R. De Jonge, L.M. Koymans, H.M. Vinkers, F. Daeyaert, D.W. Ludovici, M.J. Kukla, B. De Corte, R.W. Kavash, C.Y. Ho, H. Ye, M.A. Lichtenstein, K. Andries, R. Pauwels, M.P. de Béthune, P.L. Boyer, P. Clark, S.H. Hughes, P.A. Janssen, E. Arnold, Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants J. Med. Chem., 47 (2004) 2550-2560.

[31] Z.-S. Zeng, Y.-H. Liang, X.-Q. Feng, F.-E. Chen, C. Pannecouque, J. Balzarini, E. De Clercq, Lead optimization of diarylpyrimidines as non-nucleoside Inhibitors of HIV-1 reverse transcriptase, ChemMedChem, 5 (2010) 837-840.

[32] X.-Q. Feng, Z.-S. Zeng, Y.-H. Liang, F.-E. Chen, C. Pannecouque, J. Balzarini, E. De Clercq, Synthesis and biological evaluation of 4-(hydroxyimino) arylmethyl diarylpyrimidine analogues as potential non-nucleoside reverse transcriptase inhibitors against HIV, Bioorg. Med. Chem., 18 (2010) 2370-2374.

[33] C. Mordant, B. Schmitt, E. Pasquier, C. Demestre, L. Queguiner, C. Masungi, A. Peeters, L. Smeulders, E. Bettens, K. Hertogs, Synthesis of novel diarylpyrimidine analogues of TMC278 and their antiviral activity against HIV-1 wild-type and mutant strains, Eur. J. Med. Chem., 42 (2007) 567-579.

[34] Y. Liu, G. Meng, A. Zheng, F. Chen, W. Chen, E. De Clercq, C. Pannecouque, J. Balzarini, Design and synthesis of a new series of cyclopropylamino-linking diarylpyrimidines as HIV non-nucleoside reverse transcriptase inhibitors, Manucript, (2014).

[35] X.-D. Ma, S.-Q. Yang, S.-X. Gu, Q.-Q. He, F.-E. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Synthesis and anti-HIV activity of aryl-2-[(4-cyanophenyl) amino]-4-pyrimidinone hydrazones as potent non-nucleoside reverse transcriptase inhibitors, ChemMedChem, 6 (2011) 2225-2232.

[36] S.-X. Gu, Z.-M. Li, X.-D. Ma, S.-Q. Yang, Q.-Q. He, F.-E. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Chiral resolution, absolute configuration assignment and biological activity of racemic diarylpyrimidine *CH*(*OH*)-DAPY as potent nonnucleoside HIV-1 reverse transcriptase inhibitors, Eur. J. Med. Chem., 53 (2012) 229-234.

[37] S.-X. Gu, Q.-Q. He, S.-Q. Yang, X.-D. Ma, F.-E. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Synthesis and structure-activity relationship of novel diarylpyrimidines with hydromethyl linker (*CH*(*OH*)-DAPYs) as HIV-1 NNRTIs, Bioorg. Med. Chem., 19 (2011) 5117-5124.

[38] X.-Q. Feng, Y.-H. Liang, Z.-S. Zeng, F.-E. Chen, J. Balzarini, C. Pannecouque, E. De Clercq, Structural modifications of DAPY analogues with potent anti-HIV-1 activity, ChemMedChem, 4 (2009) 219-224.

[39] A. Thakur, M. Thakur, A. Bharadwaj, S. Thakur, SAR and QSAR studies: modelling of new

DAPY derivatives, Eur. J. Med. Chem., 43 (2008) 471-477.
conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors, Prog. Biophys. Mol. Biol., 88 (2005) 209-231.
[41] A. Ranise, A. Spallarossa, S. Cesarini, F. Bondavalli, S. Schenone, O. Bruno, G. Menozzi, P. Fossa, L. Mosti, M. La Colla, Structure-based design, parallel synthesis, structure-activity relationship, and molecular modeling studies of thiocarbamates, new potent non-nucleoside HIV-1 reverse transcriptase inhibitor isosteres of phenethylthiazolylthiourea derivatives, J. Med. Chem., 48 (2005) 3858-3873.
[42] C. Pannecouque, D. Daelemans, E. De Clercq, Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: revisited 20 years later, Nature Protocols, 3 (2008) 427-434.
[43] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds, J. Virol. Methods, 20 (1988) 309-321.
[44] J. Auwerx, T.W. North, B.D. Preston, G.J. Klarmann, E. De Clercq, J. Balzarini, Chimeric human immunodeficiency virus type 1 and feline immunodeficiency virus reverse transcriptases:
[40] K. Das, P.J. Lewi, S.H. Hughes, E. Arnold, Crystallography and the design of anti-AIDS drugs: role of the subunits in resistance/sensitivity to non-nucleoside reverse transcriptase inhibitors, Mol. Pharmacol., 61 (2002) 400-406.
[45] V.L. Singer, L.J. Jones, S.T. Yue, R.P. Haugland, Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation, Anal. Biochem., 249 (1997) 228-238.
[46] R. Spitzer, A.N. Jain, Surflex-Dock: docking benchmarks and real-world application, J. Comput. Aided Mol. Des., 26 (2012) 687-699.
[47] A.N. Jain, Surflex-Dock 2.1: robust performance from ligand energetic modeling, ring flexibility, and knowledge-based search, J. Comput. Aided Mol. Des., 21 (2007) 281-306.
[48] A.N. Jain, Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine, J. Med. Chem., 46 (2003) 499-511.
[49] J. Ruppert, W. Welch, A.N. Jain, Automatic identification and representation of protein binding sites for molecular docking, Protein Sci., 6 (1997) 524-533.

A list of captions should be provided as the following:

Fig. 1. Structure of some important NNRTIs including HEPT, DABCO and DAPYs.

Fig. 2. Molecular design of new substituted *CH*-DAPYs.

Fig. 3. Comparison of the binding modes between the representative potential compound **1d** and TMC278 in NNRTBP of wild type of HIV-1 RT (PDB code: 2ZD1[25]) and K103N/Y181C double mutated HIV-1 RT (PDB code: 3BGR[25]). (a) **T**MC278/RT^{WT}, (b) **1d**/RT^{WT}, (c) TMC278/$RT^{K103N/Y181C}$, (d) **1d**/$RT^{K103N/Y181C}$. The figures are generated by the software of SYBYL-X 2.0. (For interpretation of the references to color in these figures legend, the reader is referred to the web version of this article.) Possible hydrogen bonds are indicated with dashed lines in red. The amino acid residues are shown in liner frame and the docked conformations of the small molecules are shown in ball and stick both for **1d** and TMC278, respectively.

Scheme 1. Synthetic route of the new DAPYs derivatives (**1a-1r**). Reagents and conditions: (a) MeI, NaOH, H_2O, r. t.; (b) 4-cyanoaniline, 180 ℃; (c) $POC1_3$, reflux; (d) R (R=2-Cl or 4-Br)-phenylacetonitrile, 60 % NaH, Ar, DMF, -20℃ to r. t., 48-72 h; (e) NaH, air, DMF, r. t., 36-72 h; (f) CuCN, DMF, 150℃, 10h; (g) acrylonitrile, $Pd(OAc)_2$, P(*o*-Tol)$_3$, NaOAc, DMA, 150℃, overnight; (h) R'-NH_2, EtOH, Na_2SO_4, reflux, 4-8 h; (i) $NaBH_3CN$, EtOH, 60 ℃, 4 h

Table 1 Anti-HIV activities and cytotoxicity of compounds **1a-1r** MT-4 cells.

Table 2 Comparison of the torsion angles of the binding conformations of TMC278 and compound **1d**.

Supplementary material for the following article

Design and synthesis of a new series of modified *CH*-diarylpyrimidines as the drug resistant HIV non-nucleoside reverse transcriptase inhibitors

Ge Meng[a]*, Yang Liu[a], Aqun Zheng[b], Fener Chen*[c], Wenxue Chen[c], Erik De Clercq[d], Christophe Pannecouque[d], Jan Balzarini[d]

[a] *School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P. R. China*
[b] *School of Science, Xi'an Jiaotong University, Xi'an, Shaanxi, 710049, P. R. China*
[c] *Department of Chemistry, Fudan University, Shanghai, 200433, P. R. China*
[d] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

The representative ^{1}H and ^{13}C NMR spectra and MS of the new compounds reported in this article are shown as follow.

2-(4-Cyanophenylamino)-4-(4-cyanophenylhydroxymethyl) pyrimidine (1a)

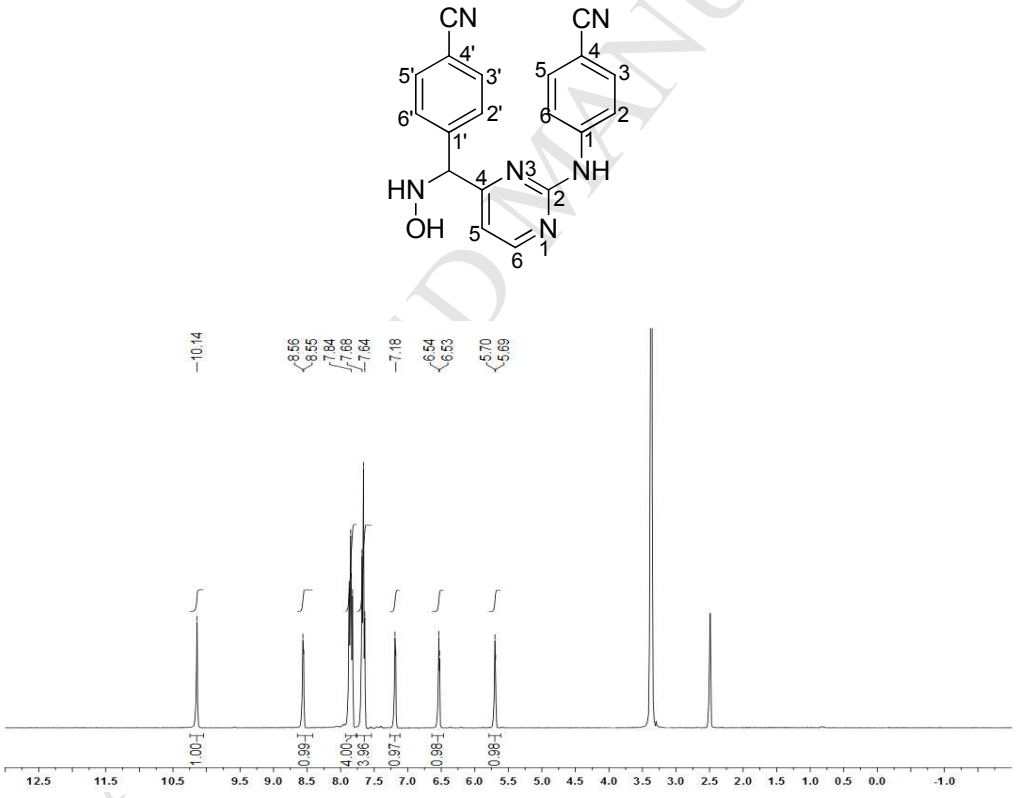


Fig. 1. ^{1}H NMR spectrum of **1a**

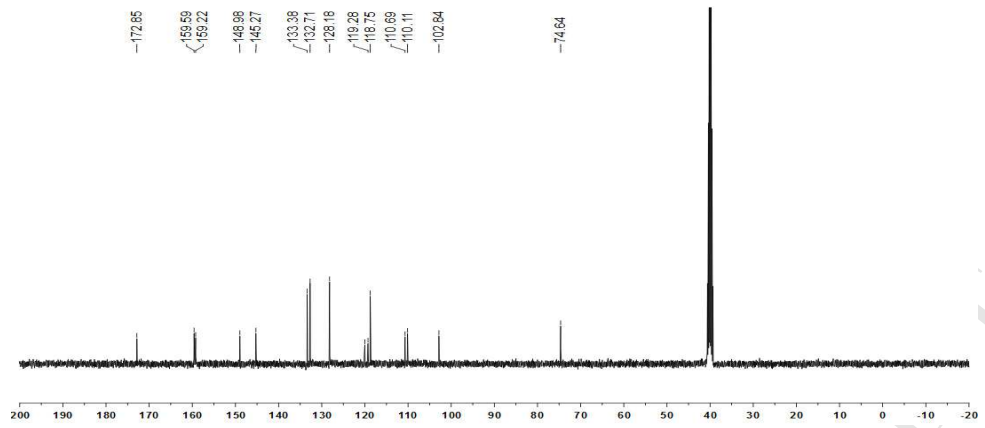


Fig. 2.[13]C NMR spectrum of **1a**

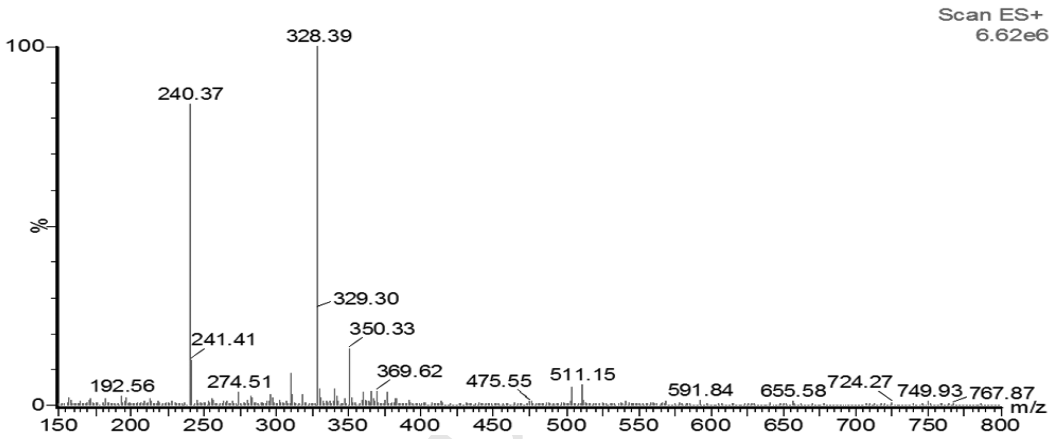


Fig. 3. MS spectrum of **1a**

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydroxymethyl)pyrimidine (1b)

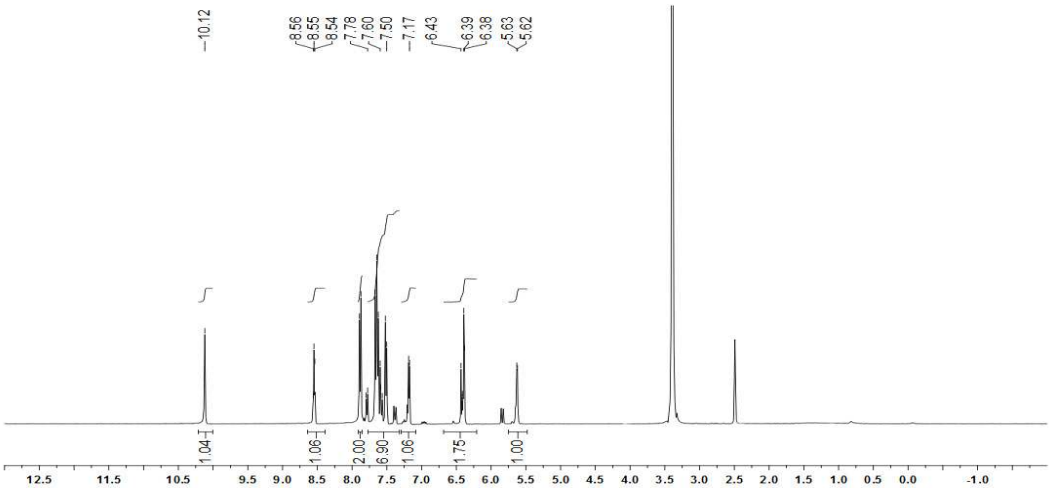


Fig. 4. [1]H NMR spectrum of **1b**

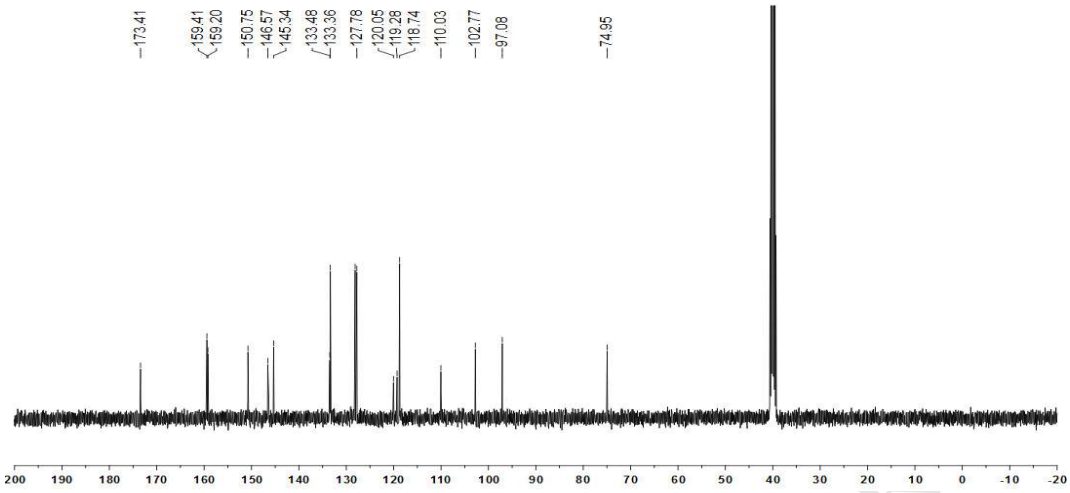


Fig. 5. ^{13}C NMR spectrum of **1b**

2-(4-Cyanophenylamino)-4-(4-cyanophenylhydrazonomethyl)pyrimidine (1c)

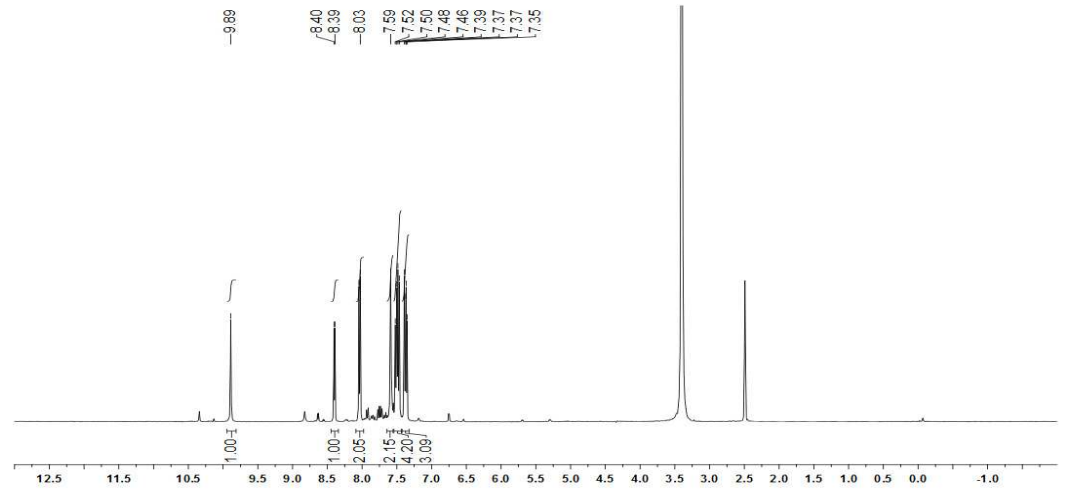


Fig. 6. ^{1}H NMR spectrum of **1c**

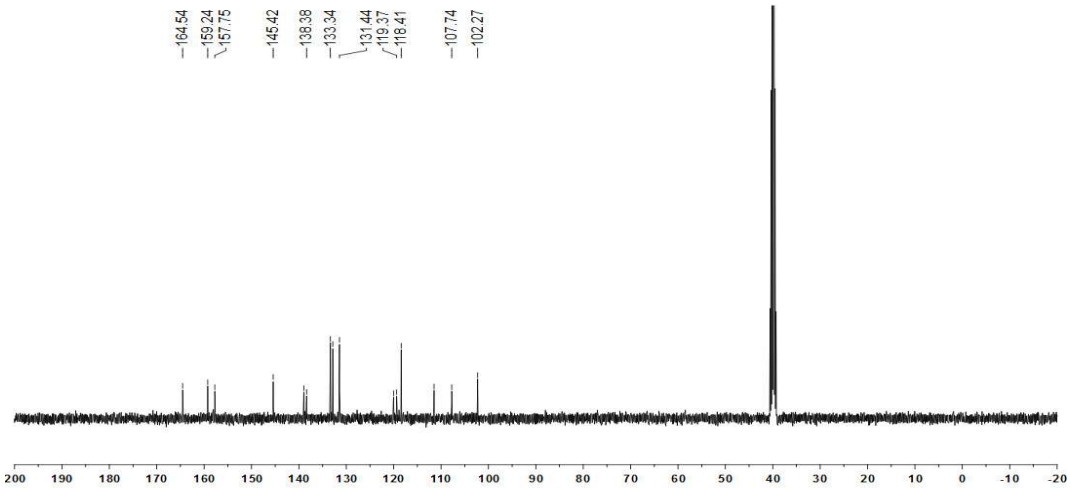


Fig. 7. ^{13}C NMR spectrum of **1c**

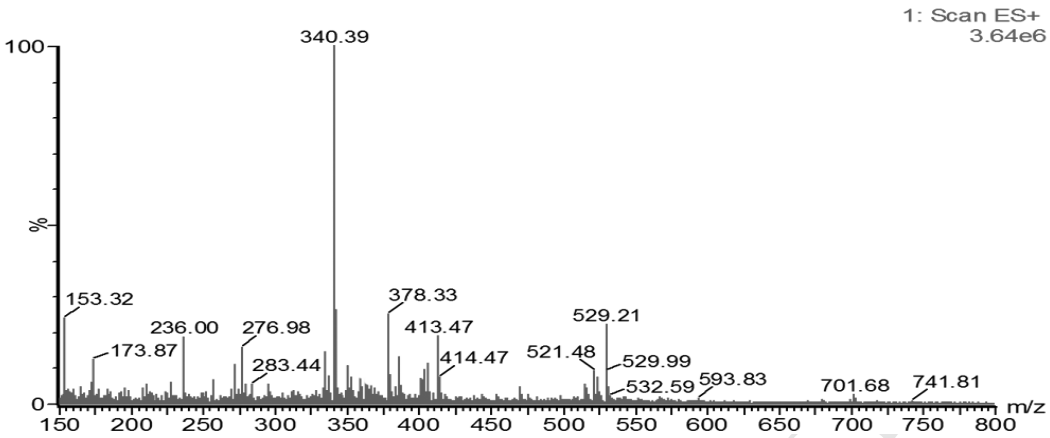


Fig. 8. MS spectrum of **1c**

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydrazonomethyl)pyrimidine (1d)

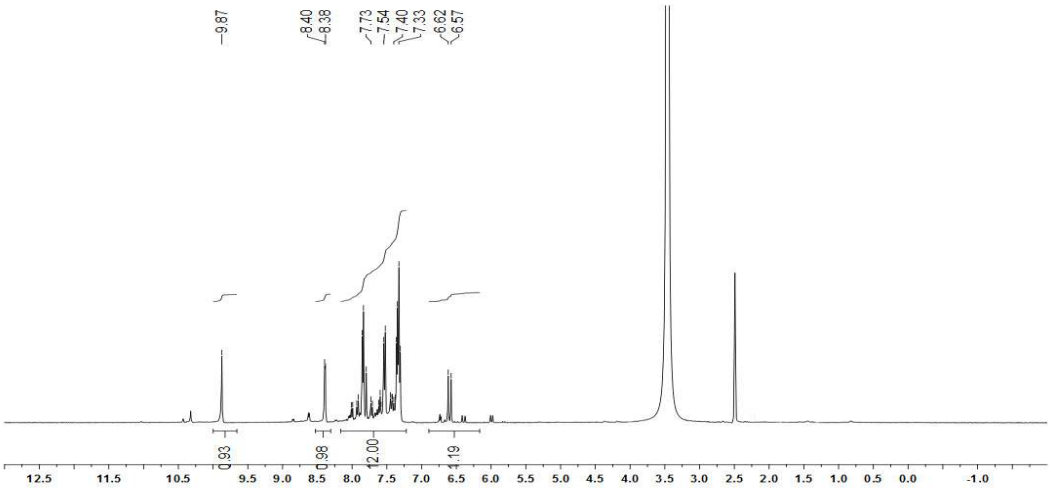


Fig. 9. ^{1}H NMR spectrum of **1d**

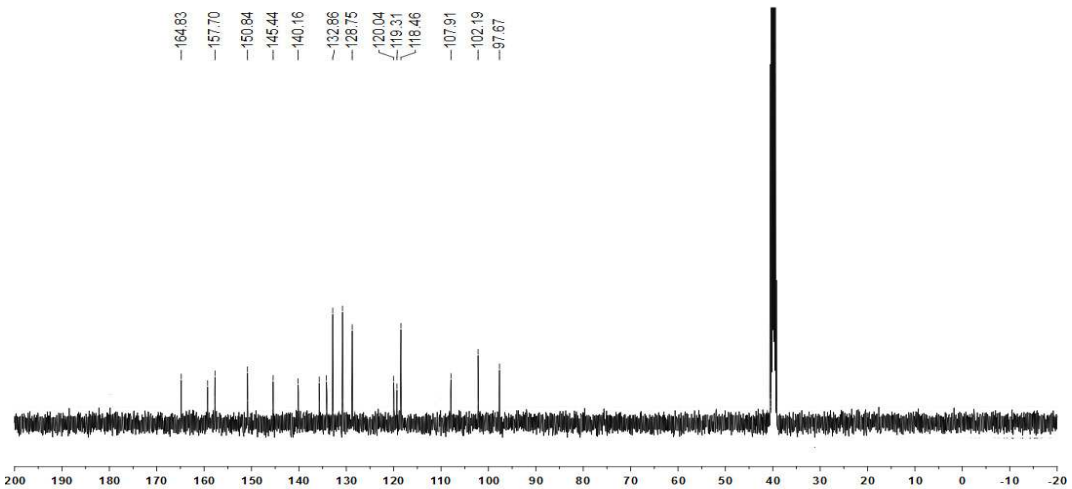


Fig. 10. ^{13}C NMR spectrum of **1d**

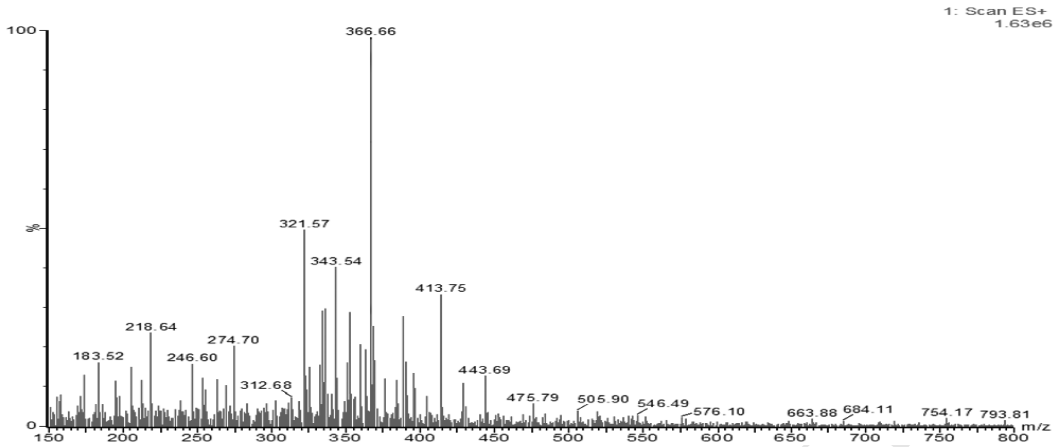


Fig. 11. MS spectrum of **1d**

2-(4-Cyanophenylamino)-4-(4-cyanophenylmethylaminomethyl)pyrimidine (1e)

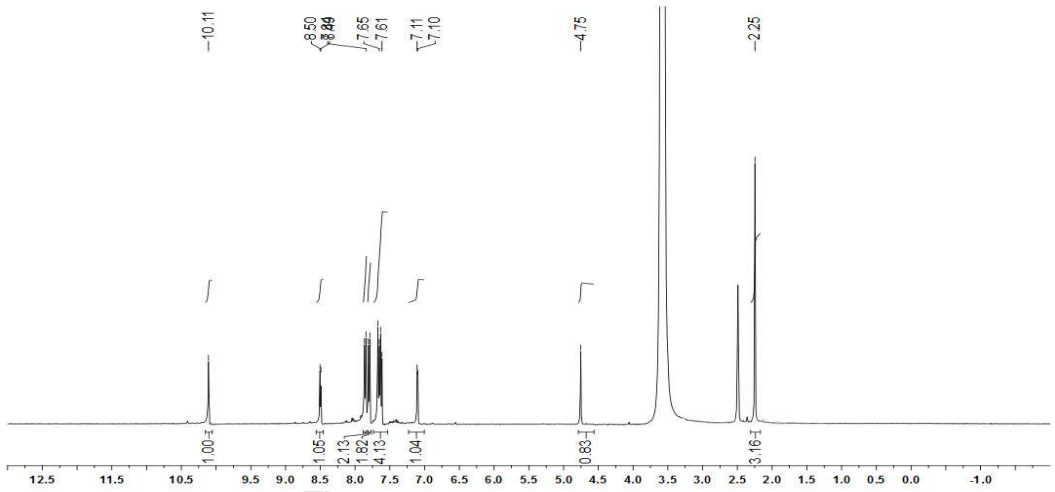


Fig. 12. ^{1}H NMR spectrum of **1e**

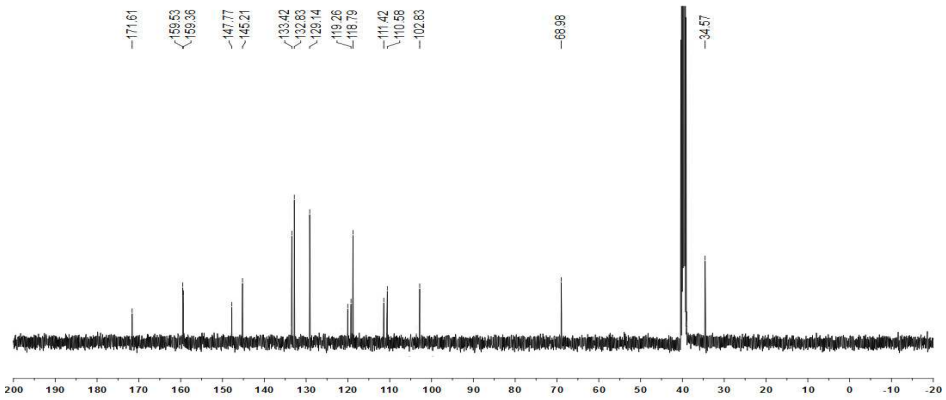


Fig. 13. ^{13}C NMR spectrum of **1e**

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylmethylaminomethyl)pyrimidine (1f)

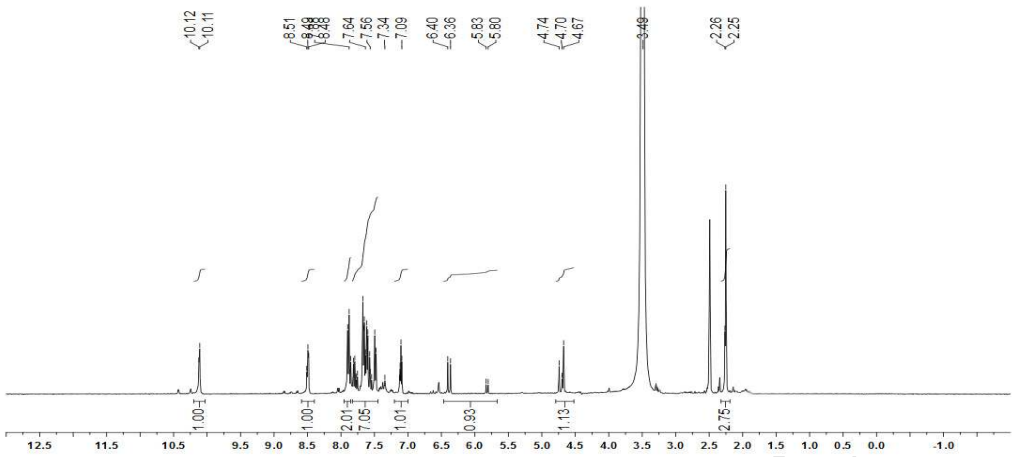


Fig. 14. ^{1}H NMR spectrum of **1f**

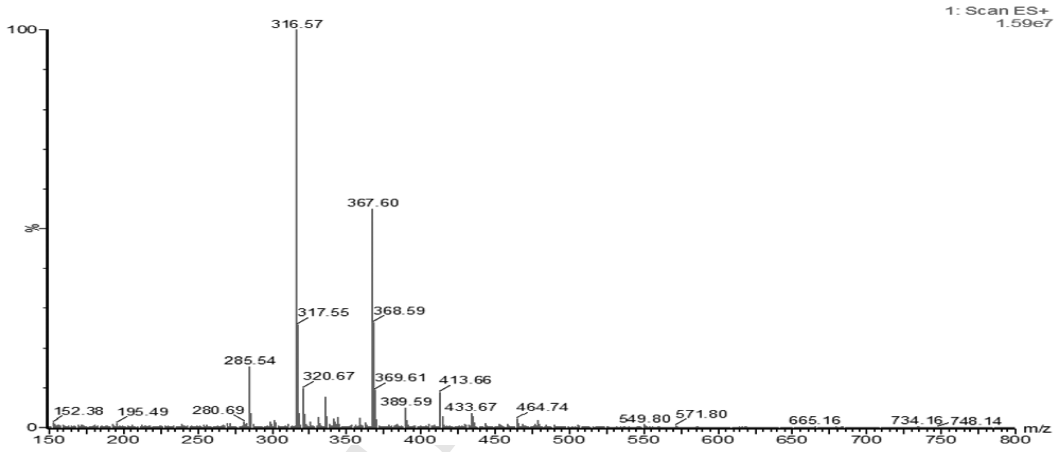


Fig. 15. MS spectrum of **1f**

2-(4-Cyanophenylamino)-4-(2-chlorophenylmethylaminomethyl)pyrimidine (1g)

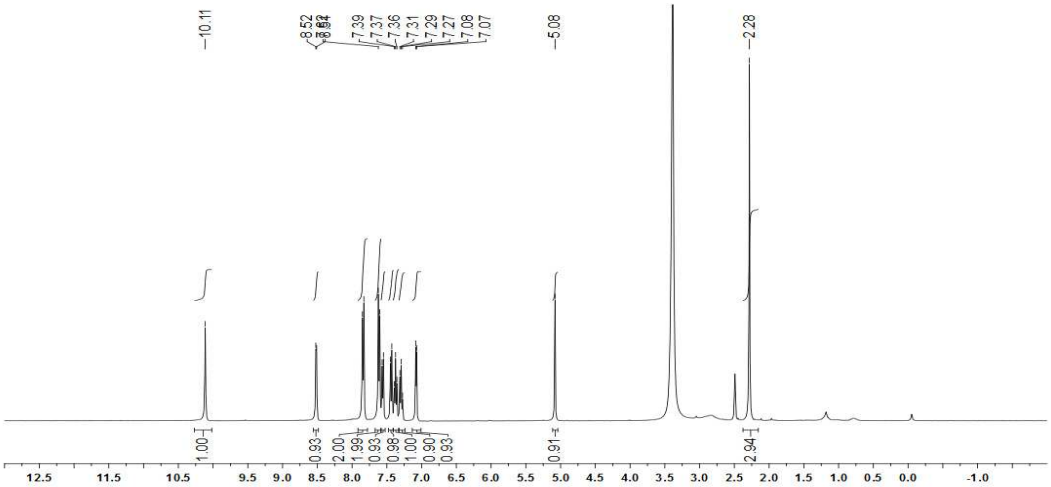


Fig. 16. ^{1}H NMR spectrum of **1g**

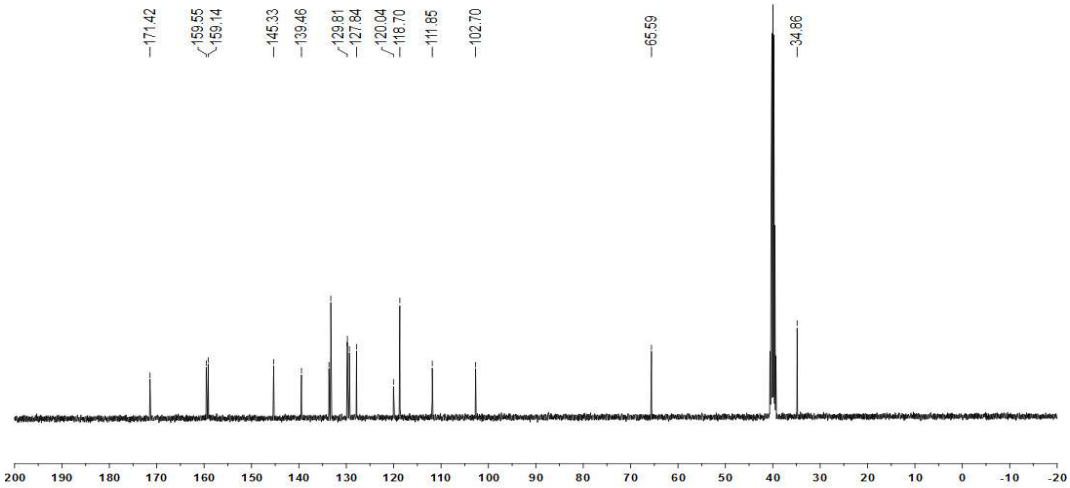


Fig. 17. ^{13}C NMR spectrum of **1g**

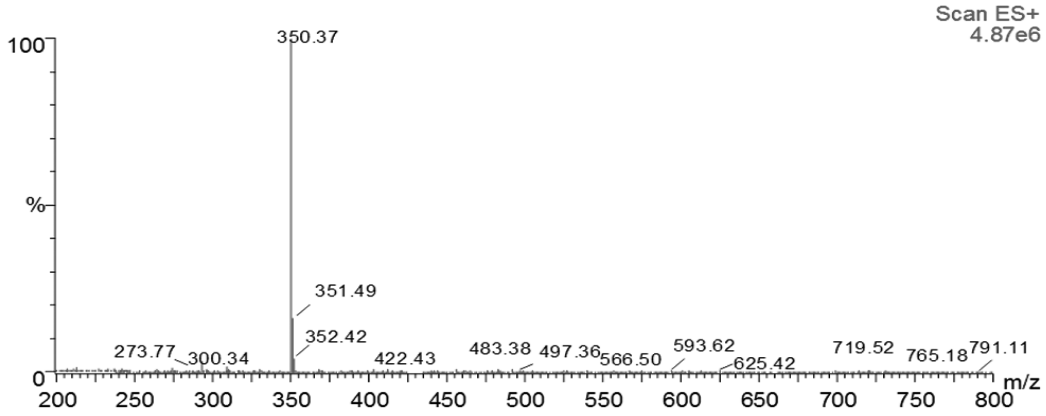


Fig. 18. MS spectrum of **1g**

2-(4-Cyanophenylamino)-4-(4-cyanophenylethylaminomethyl)pyrimidine (1h)

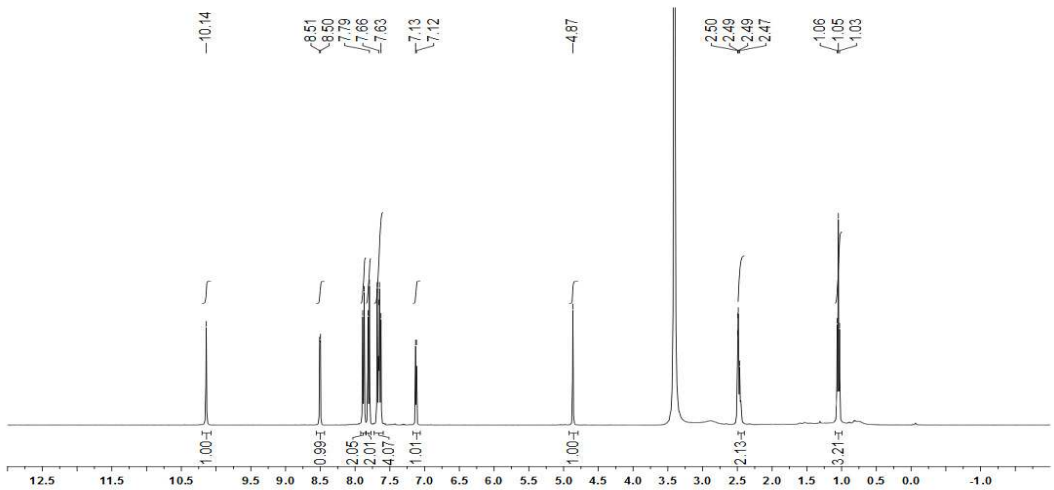


Fig. 19. ^{1}H NMR spectrum of **1h**

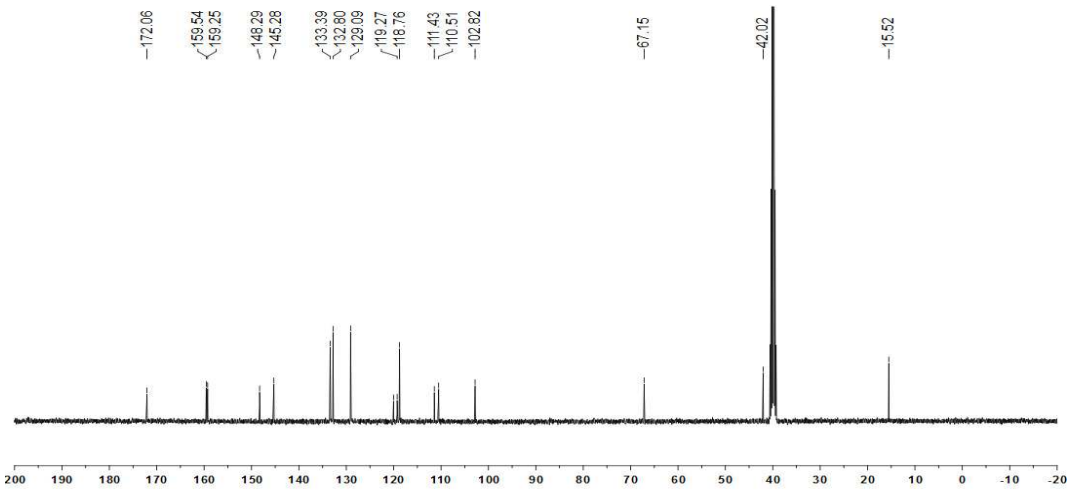


Fig. 20. ^{13}C NMR spectrum of **1h**

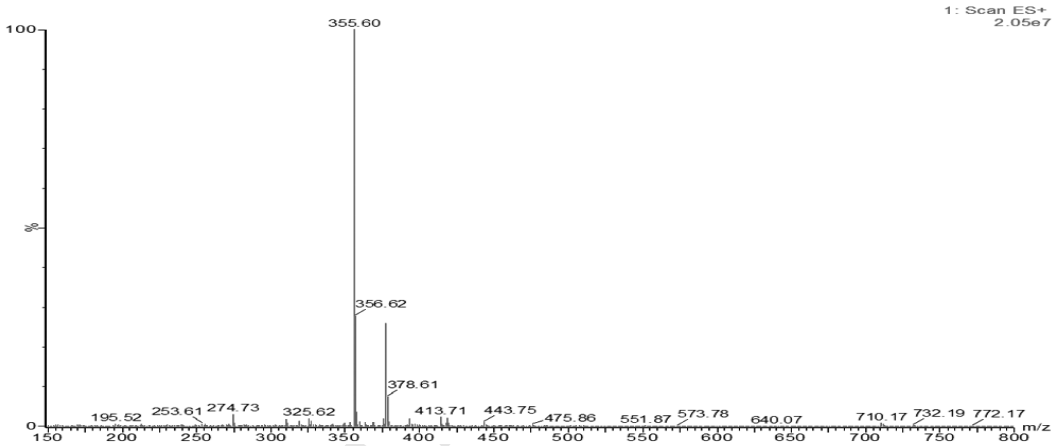


Fig. 21. MS spectrum of **1h**

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylethylaminomethyl)pyrimidine (1i)

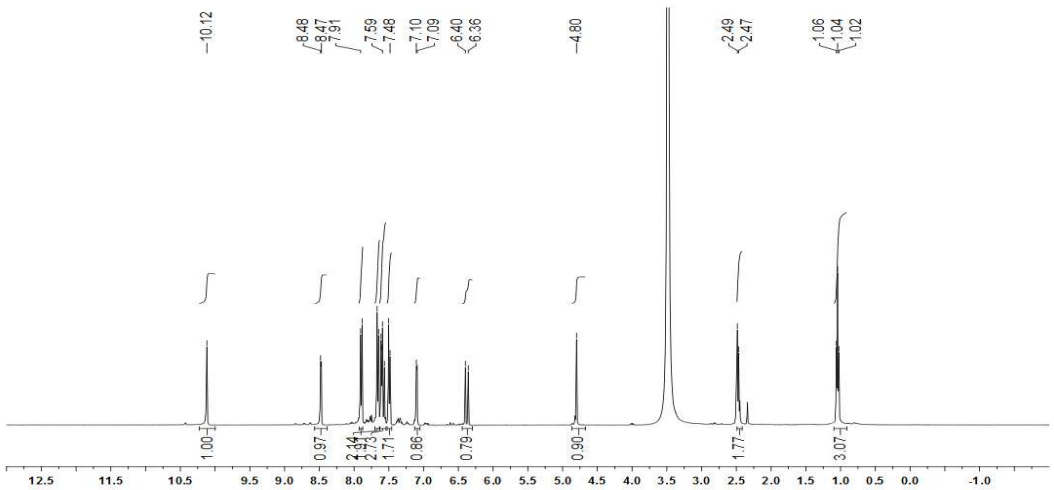


Fig. 22. ^{1}H NMR spectrum of **1i**

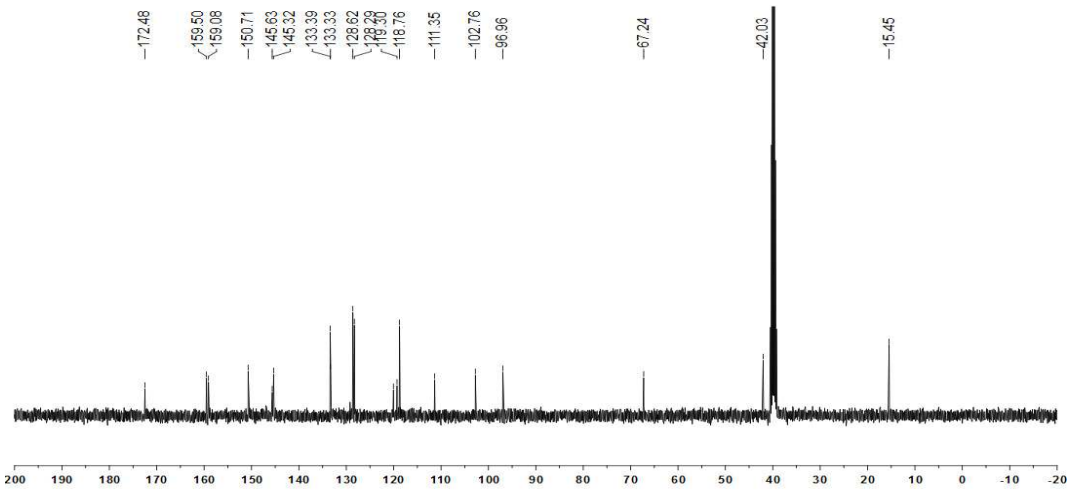


Fig. 23. ^{13}C NMR spectrum of **1i**

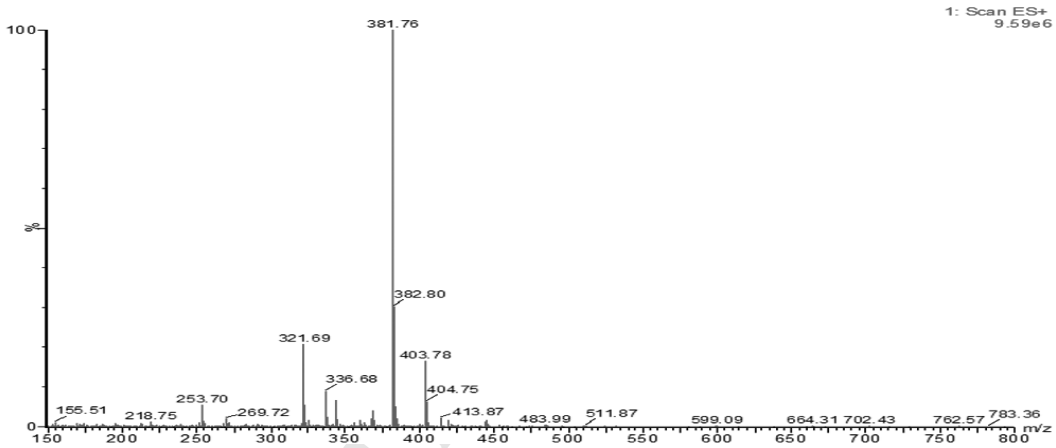


Fig. 24. MS spectrum of **1i**

2-(4-Cyanophenylamino)-4-(2-chlorophenylethylaminomethyl)pyrimidine (1j)

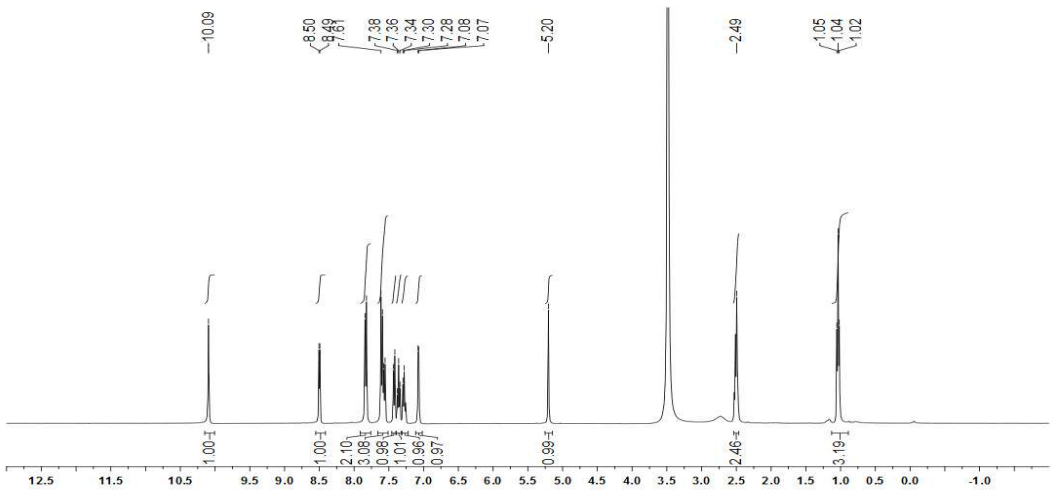


Fig. 25. ^{1}H NMR spectrum of **1j**

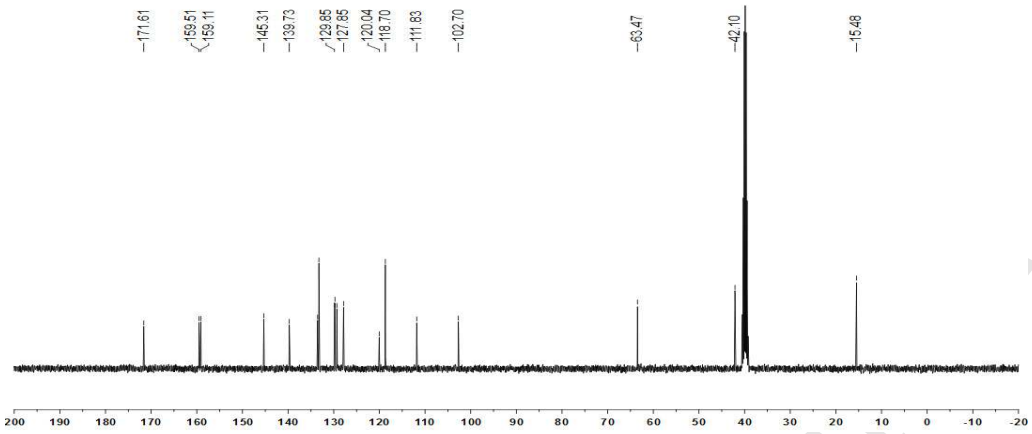


Fig. 26. ^{13}C NMR spectrum of **1j**

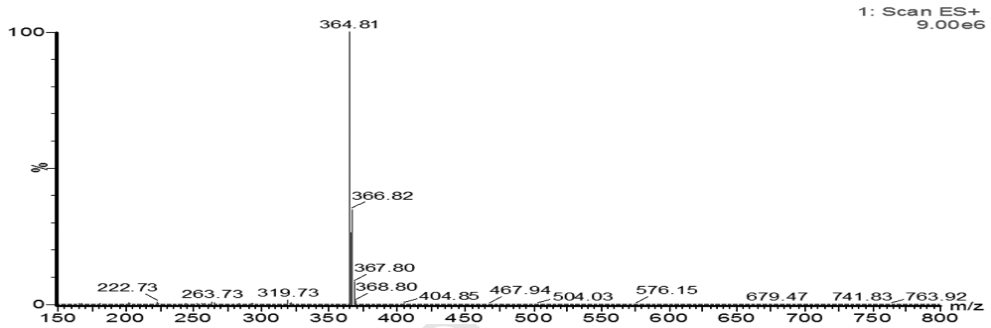


Fig. 27. MS spectrum of **1j**

2-(4-Cyanophenylamino)-4-(4-cyanophenylpropylaminomethyl)pyrimidine (1k)

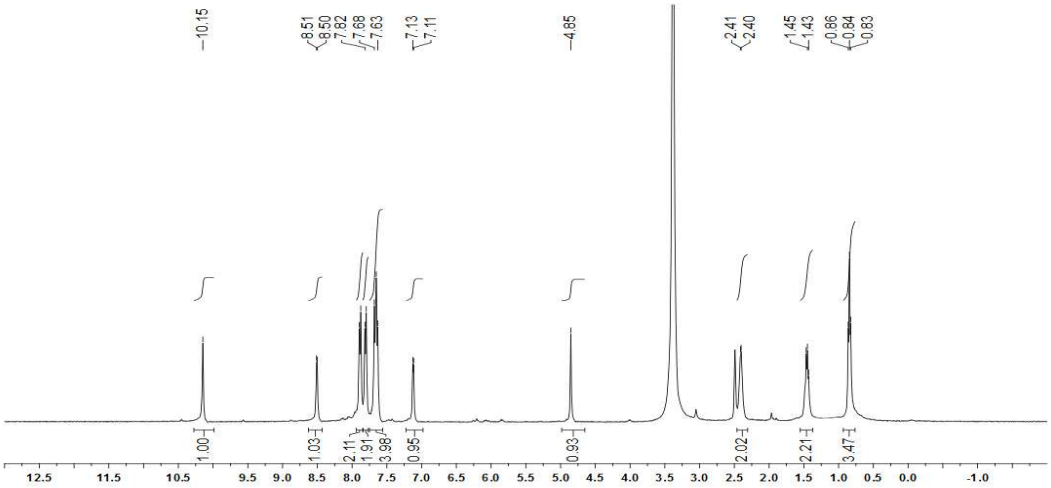


Fig.28. ^{1}H NMR spectrum of **1k**

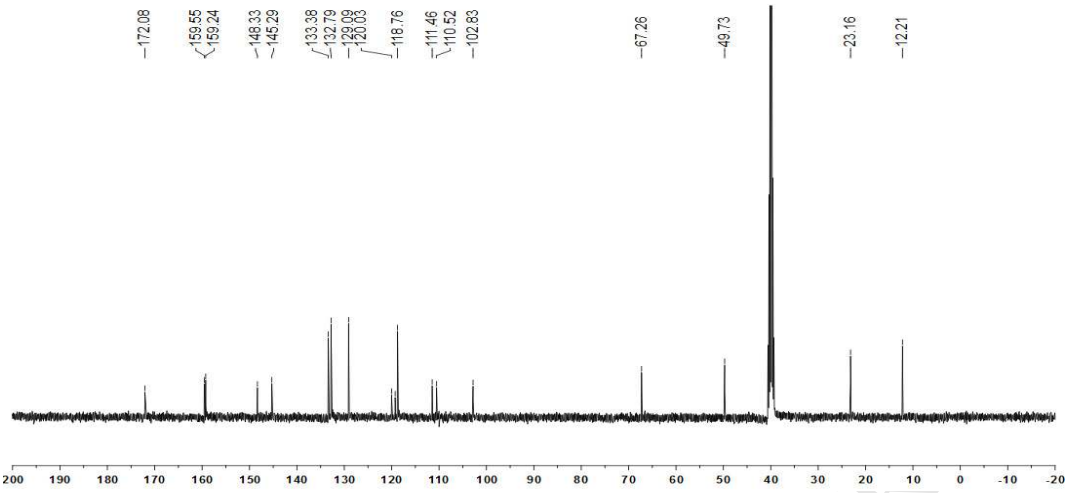


Fig. 29. ^{13}C NMR spectrum of **1k**

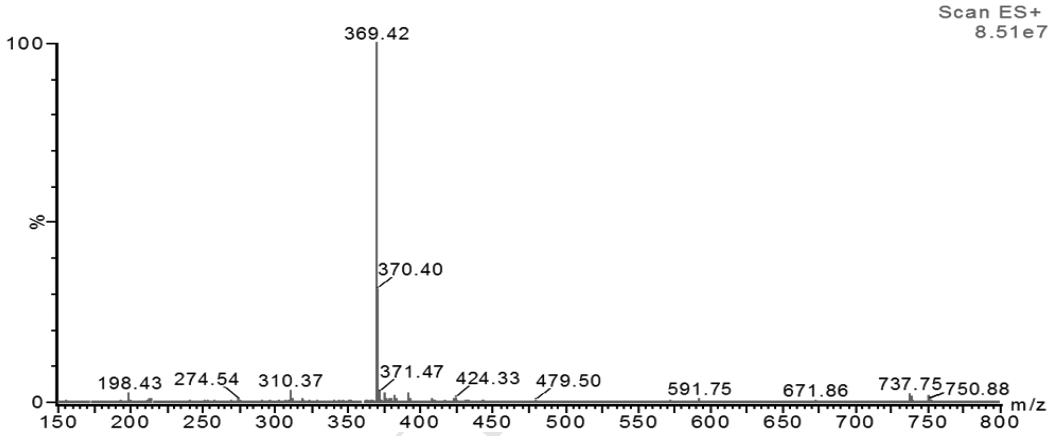


Fig. 30. MS spectrum of **1k**

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylpropylaminomethyl)pyrimidine (1l)

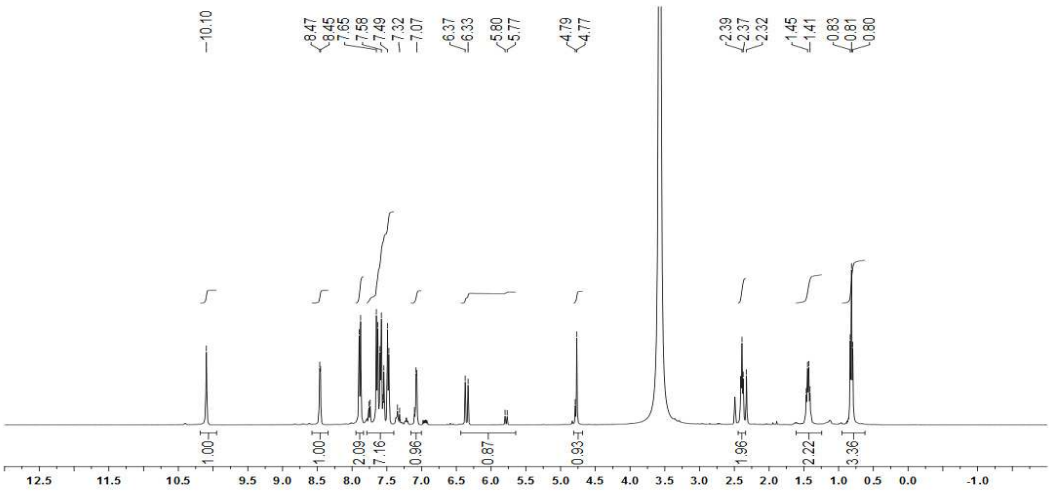


Fig. 31. ^{1}H NMR spectrum of **1l**

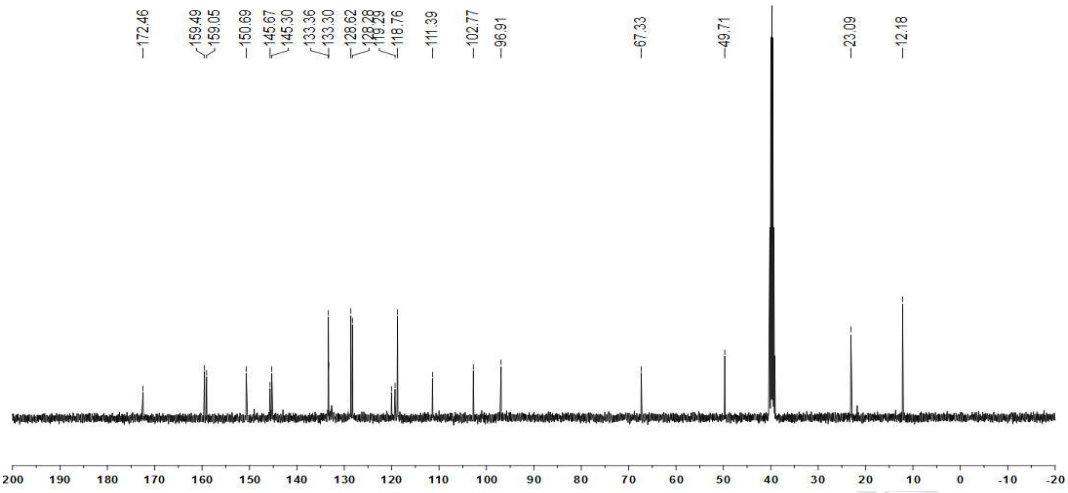


Fig. 32. ^{13}C NMR spectrum of **1l**

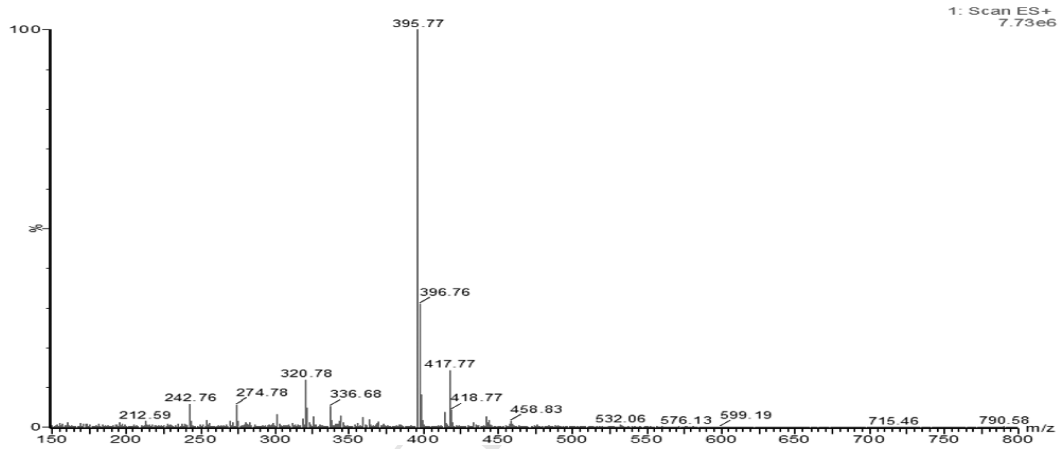


Fig. 33. MS spectrum of **1l**

2-(4-Cyanophenylamino)-4-(2-chlorophenylpropylaminomethyl)pyrimidine (1m)

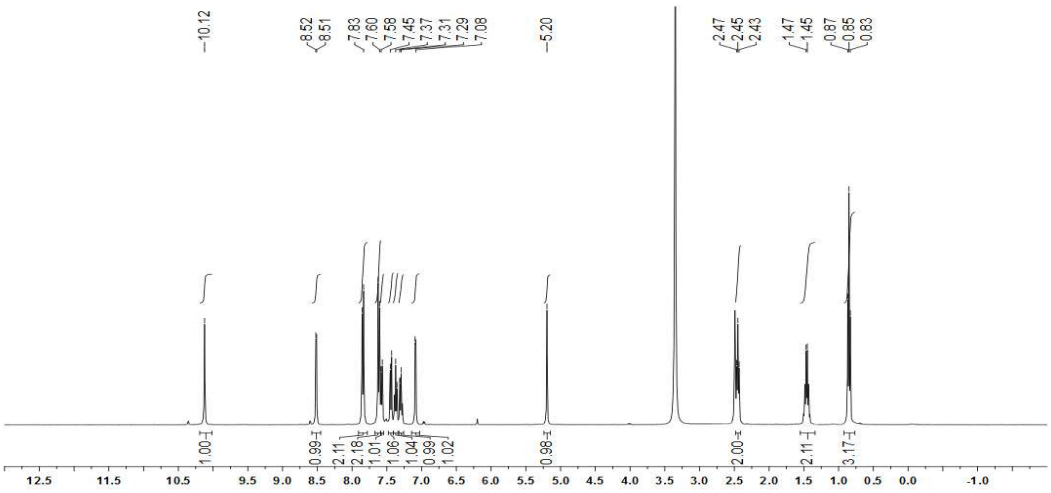


Fig. 34. ^{1}H NMR spectrum of **1m**

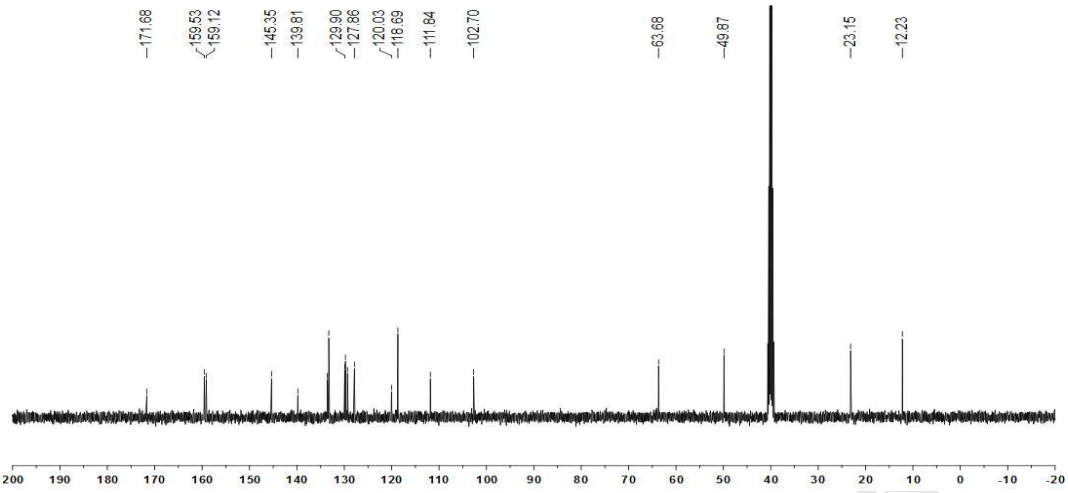


Fig. 35. ^{13}C NMR spectrum of **1m**

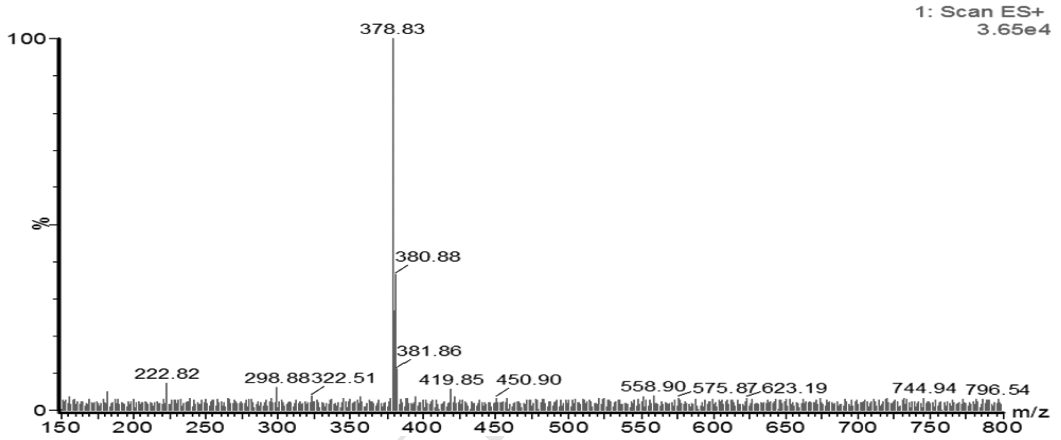


Fig. 36. MS spectrum of **1m**

2-(4-Cyanophenylamino)-4-(4-cyanophenylisopropylaminomethyl)pyrimidine (1n)

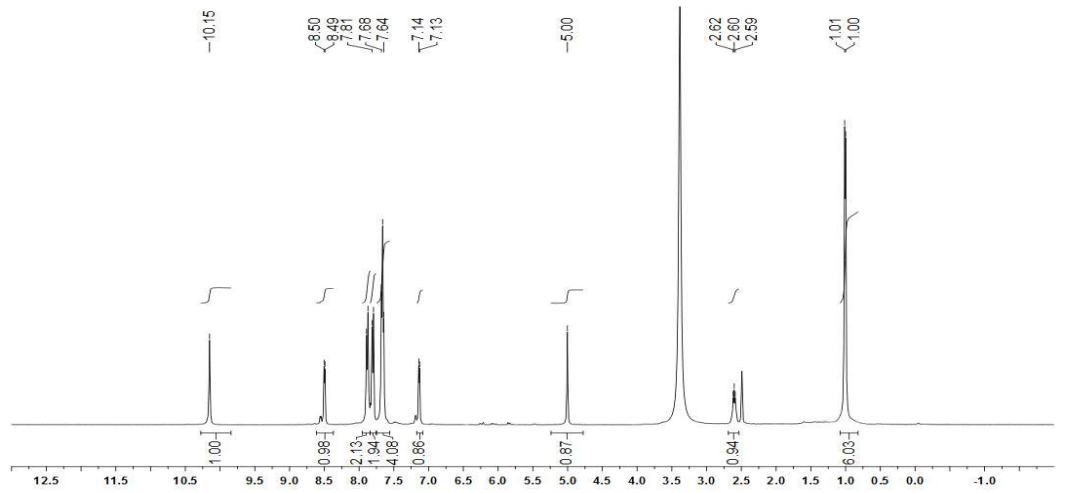


Fig. 37. ^{1}H NMR spectrum of **1n**

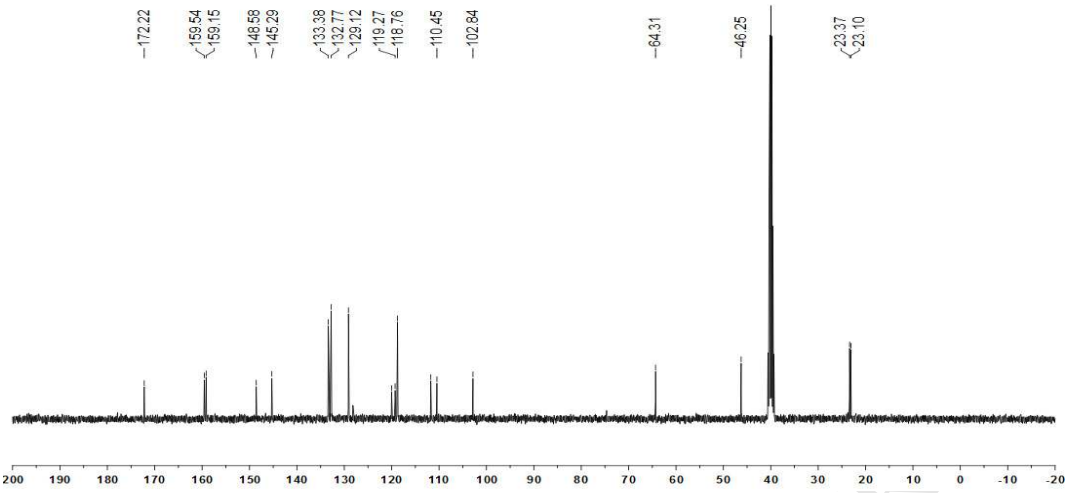


Fig. 38. ^{13}C NMR spectrum of **1n**

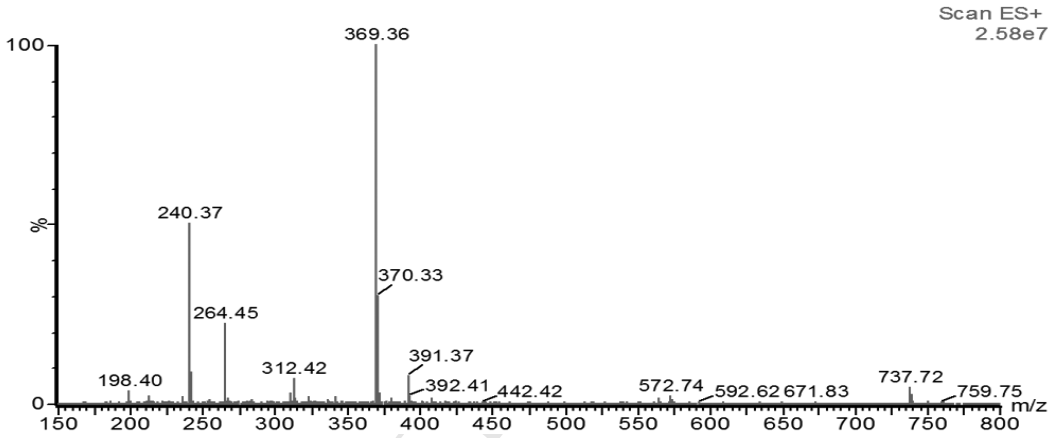


Fig. 39. MS spectrum of 1n

2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylisopropyl aminomethyl)pyrimidine (1o)

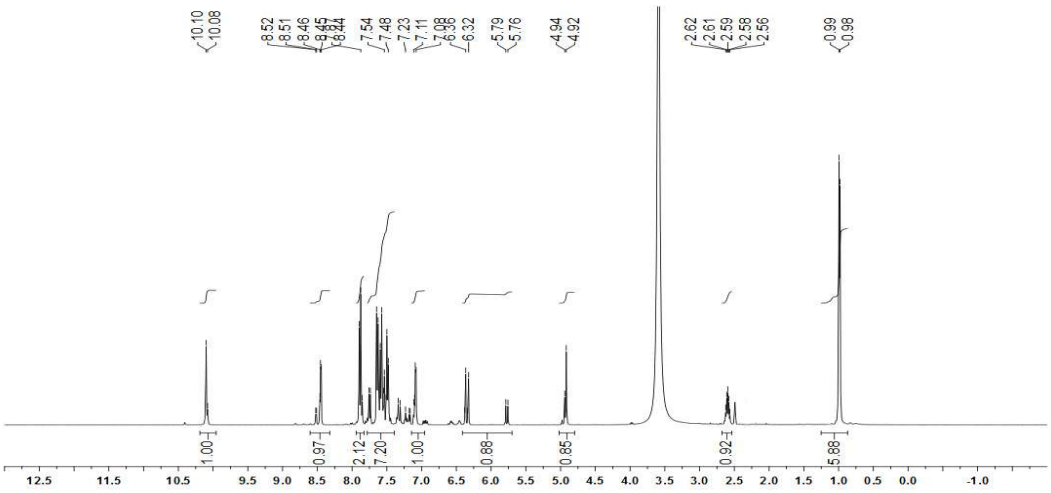


Fig. 40. ^{1}H NMR spectrum of **1o**

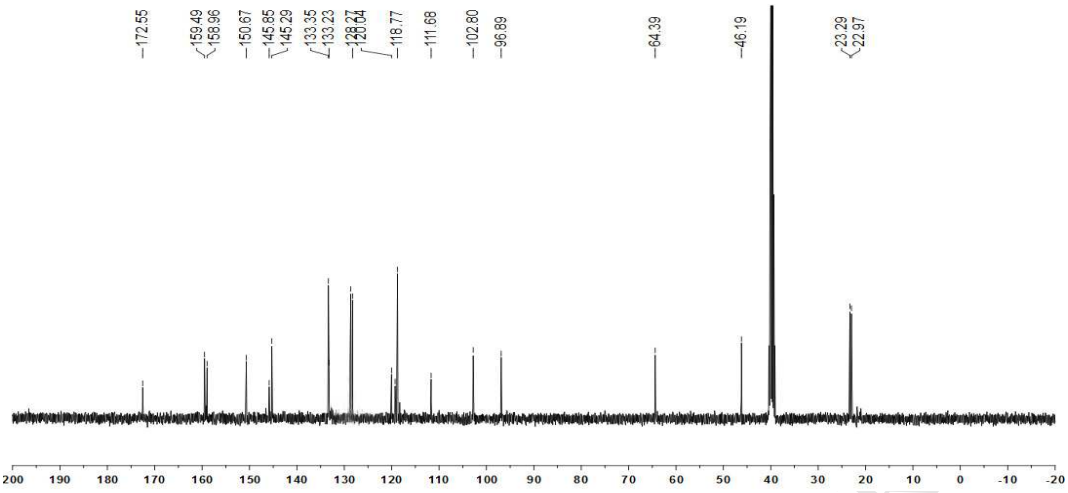


Fig. 41. ^{13}C NMR spectrum of **1o**

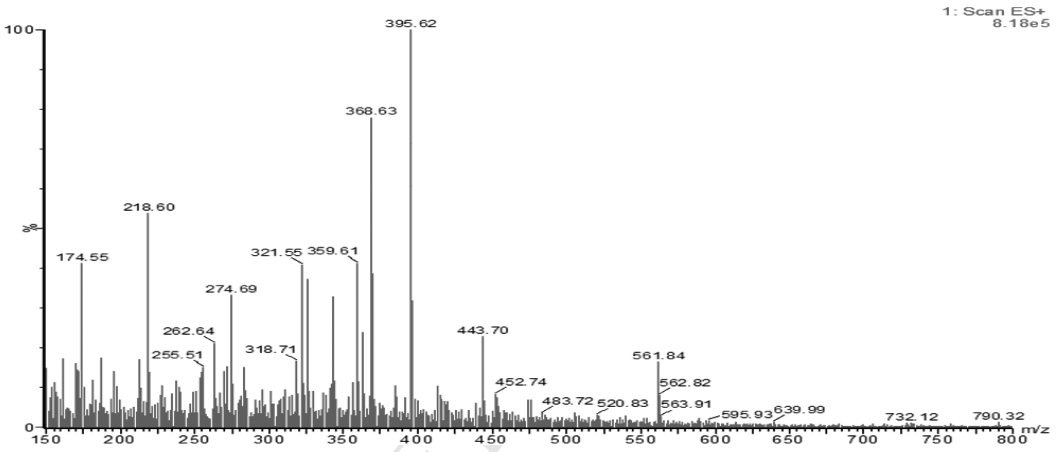


Fig. 42. MS spectrum of **1o**

2-(4-Cyanophenylamino)-4-(2-chlorophenylisopropylaminomethyl)pyrimidine (1p)

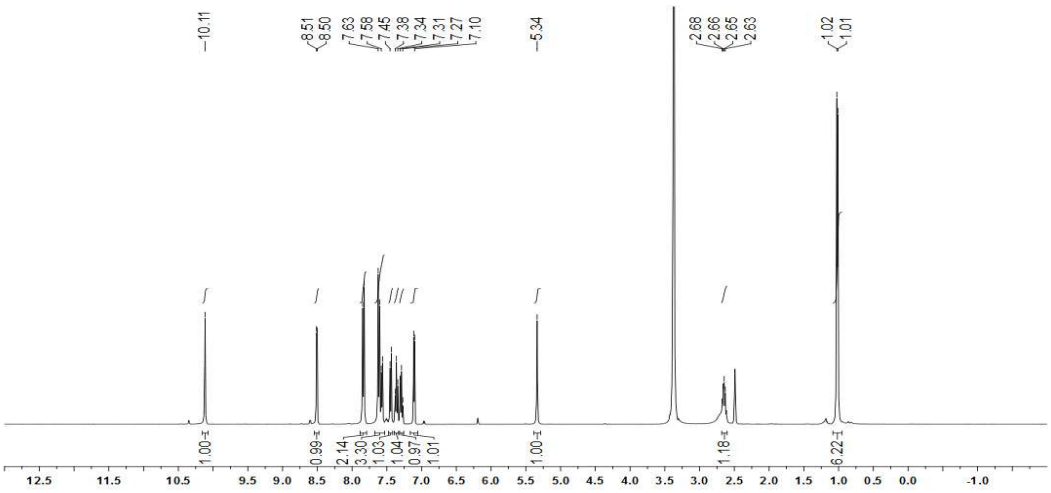


Fig. 43 ^{1}H NMR spectrum of **1p**

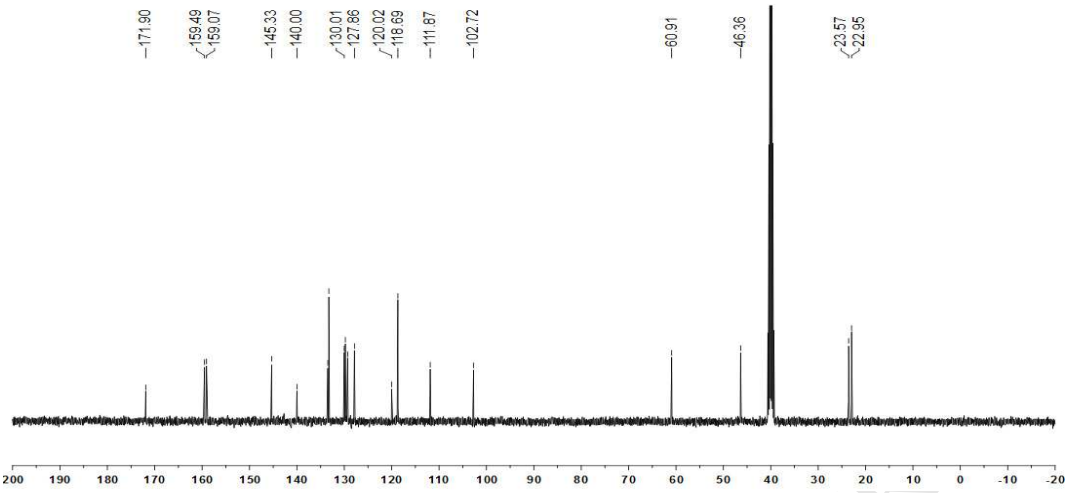


Fig. 44. ^{13}C NMR spectrum of **1p**

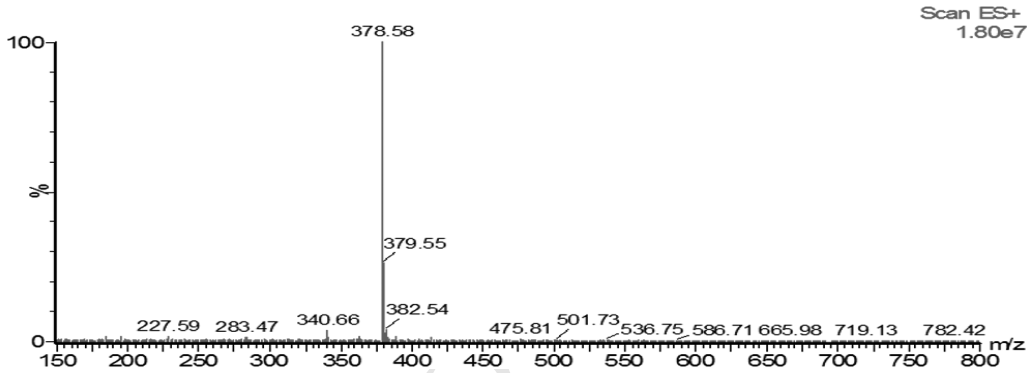


Fig. 45. MS spectrum of **1p**

2-(4-Cyanophenylamino)-4-(4-cyanophenyl-2-hydroxypropylaminomethyl)pyrimidine (1q)

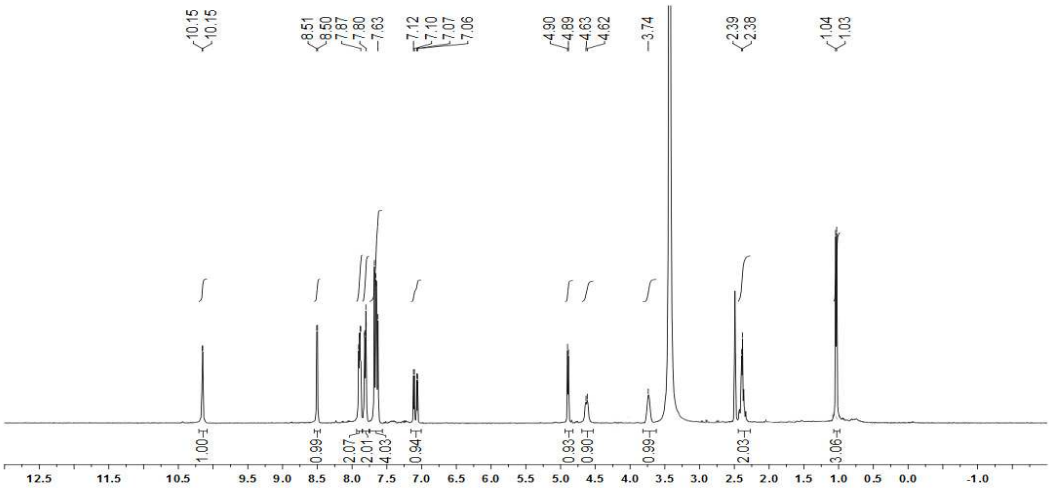


Fig. 46. ^{1}H NMR spectrum of **1q**

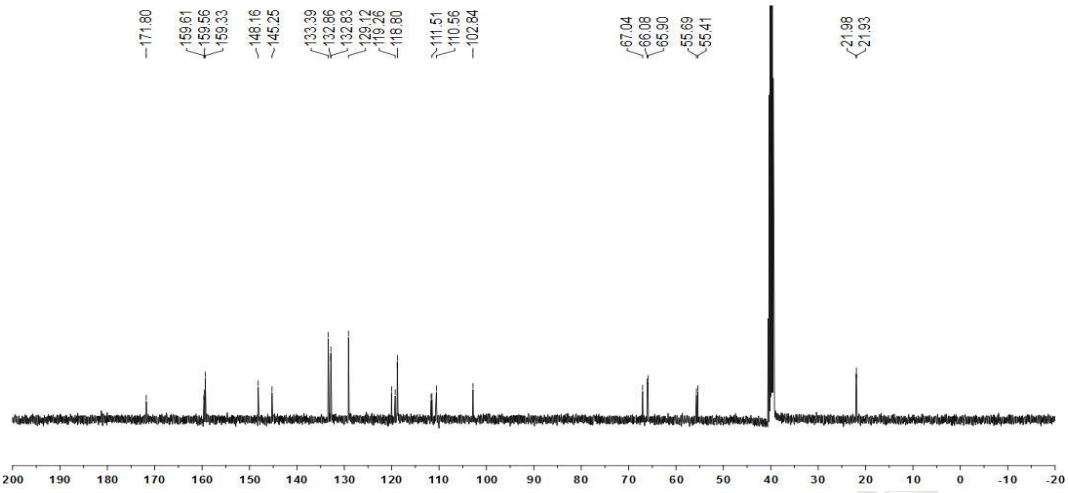


Fig. 47. ^{13}C NMR spectrum of **1q**

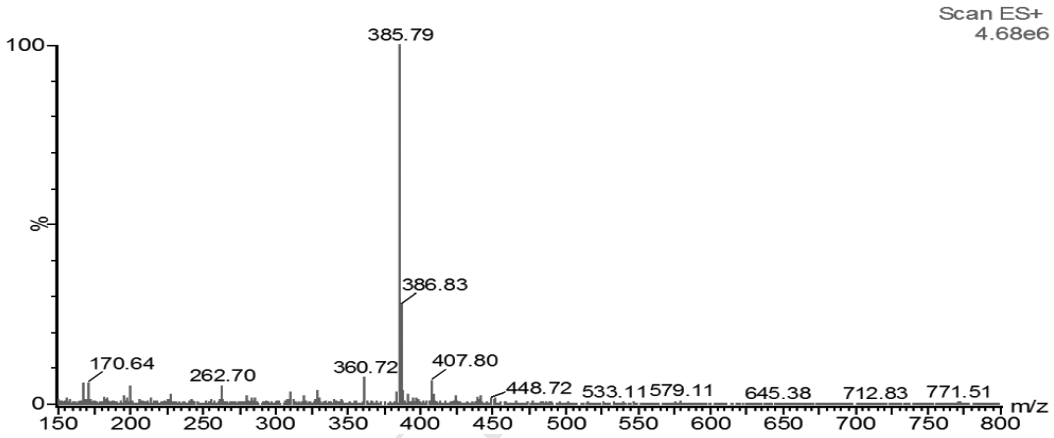


Fig. 48. MS spectrum of **1q**

2-(4-Cyanophenylamino)-4-(2-chlorophenyl-2-hydroxypropylaminomethyl)pyrimidine (1r)

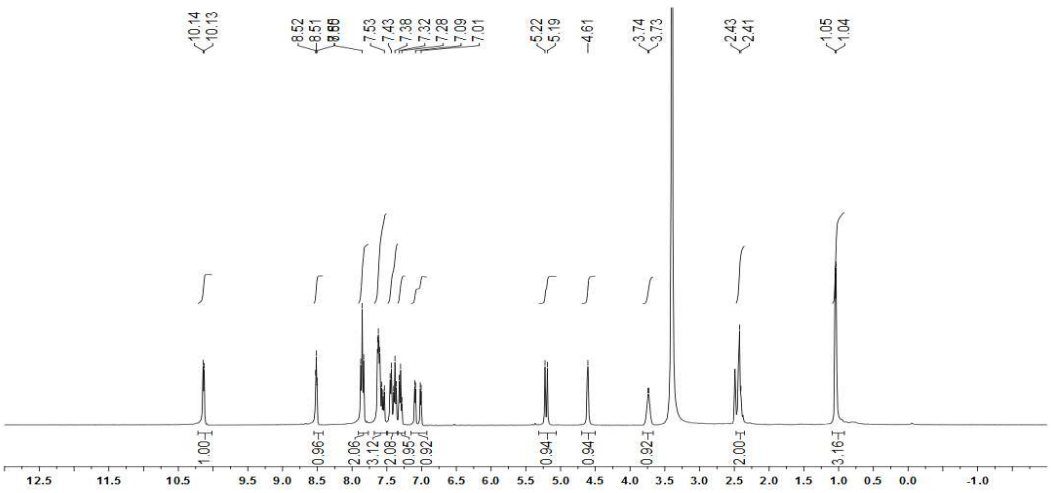


Fig. 49. ^{1}H NMR spectrum of **1r**

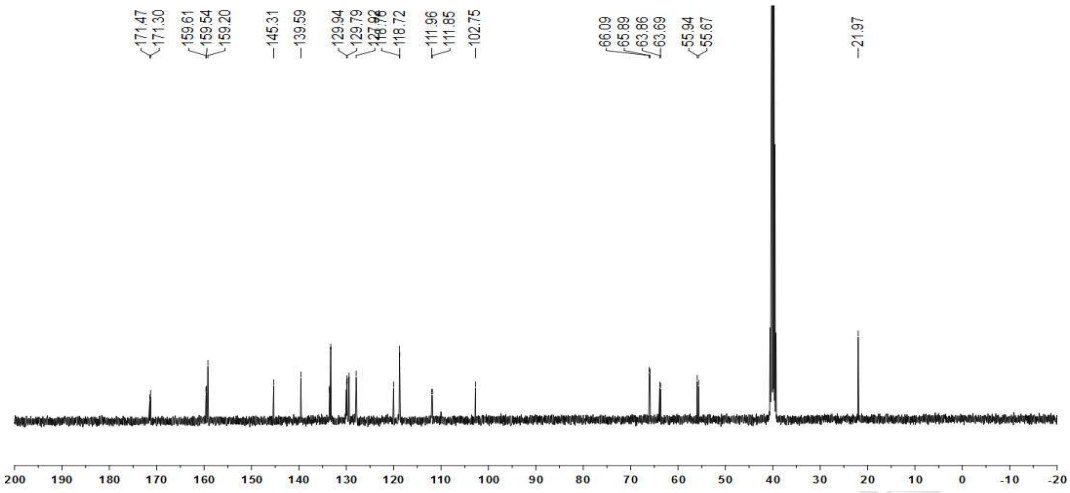


Fig. 50. ^{13}C NMR spectrum of **1r**

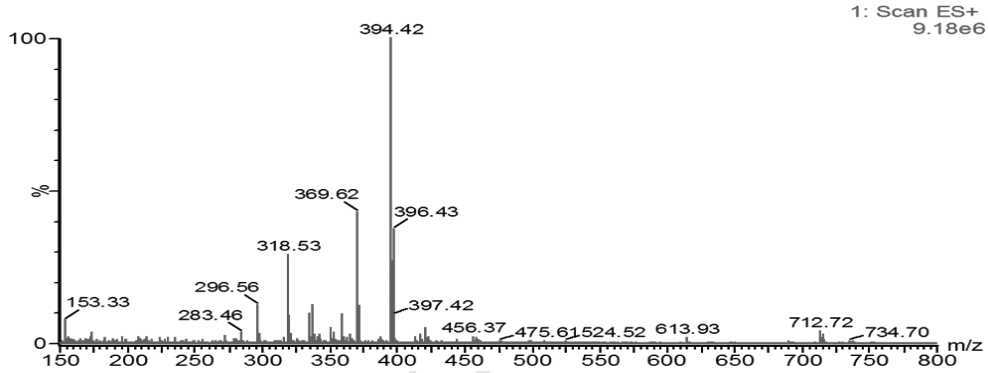


Fig. 51. MS spectrum of **1r**