Journal Pre-proof

Structure-Based Discovery of Novel Diarylpyrimidines as Potent and Selective Non-Nucleoside Reverse Transcriptase Inhibitors: From CH(CN)-Biphenyl-Diarylpyrimidines to C=NNH$_2$-Biphenyl-Diarylpyrimidines

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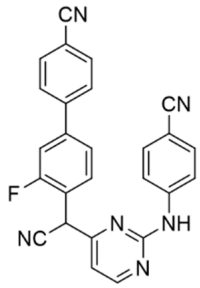
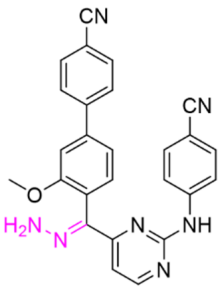
Structure-Based Discovery of Novel NNRTIs with Improved Potency and Selectivity



3

EC_{50}(nM) = 27 (WT)
1210 (L100I)
900 (K103N)
870 (Y181C)
9810 (Y188L)
170 (E138K)

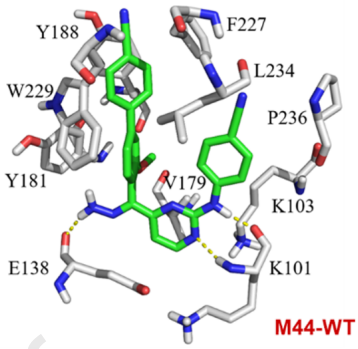
CC_{50} = 36 μM (WT)
SI = 1361



M44

EC_{50} (nM)= 5 (WT)
7 (L100I)
10 (K103N)
22 (Y181C)
148 (Y188L)
21 (E138K)

CC_{50} = 54 μM (WT)
SI = 10995



M44-WT

√ Good metabolic stability
$t_{1/2\ (plasma)}$ > 300 min
$t_{1/2\ (liver\ microsomes)}$ = 37.5 min
√ Favorable *in vivo* safety
LD_{50} > 2 g/kg

Structure-Based Discovery of Novel Diarylpyrimidines as Potent and Selective Non-Nucleoside Reverse Transcriptase Inhibitors: From CH(CN)-Biphenyl-Diarylpyrimidines to C=NNH$_2$-Biphenyl-Diarylpyrimidines

Xiao-Mei Chen[a], Christophe Pannecouque[e], Erik De Clercq[e], Yu-Xuan Lian[a], Angela Corona[f], Laura Dettori[f], Enzo Tramontano[f], Shuai Wang[b, c,*], Fen-Er Chen[a, b, c, d,*]

[a]Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, Chengdu 610041, China

[b]Engineering Center of Catalysis and Synthesis for Chiral Molecules, Department of Chemistry, Fudan University, Shanghai 200433, China

[c]Shanghai Engineering Center of Industrial Asymmetric Catalysis for Chiral Drugs, Shanghai 200433, China

[d]Institute of Flow Chemistry and Engineering, School of Chemistry and Materials, Jiangxi Normal University, Nanchang 330022, China

[e]Rega Institute for Medical Research, KU Leuven, Herestraat 49, Leuven B-3000, Belgium

[f]Dept Appl Sci Biosyst, University of Cagliari, I-09042 Monserrato, Italy.

*Corresponding author

Fen-Er Chen (rfchen@fudan.edu.cn)

Shuai Wang (shuaiwang@fudan.edu.cn)

ABSTRACT

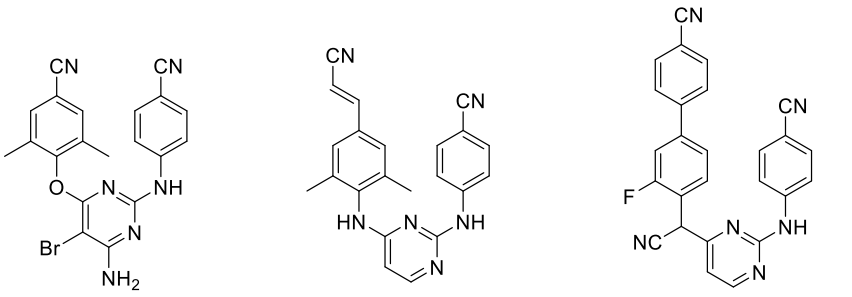
In order to enhance the anti-HIV-1 potency and selectivity of the previously reported compound **3** (EC_{50} = 27 nM, SI = 1361), a series of novel biphenyl-diarylpyrimidine derivatives were developed by employing structure-based drug design strategy. Among these derivatives, compound **M44** demonstrated the most potent inhibitory activity against wild-type (WT) HIV-1 as well as five drug-resistant mutants (EC_{50} = 5–148 nM), which were 5–173 times more potent than that of **3** (EC_{50} = 27–9810 nM). Furthermore, this analogue exhibited approximately 11-fold lower cytotoxicity (CC_{50} = 54 μM) than that of etravirine and rilpivirine. Concurrently, it possessed an improved selectivity index (SI) of 10995. Additionally, compound **M44** was characterized by favorable metabolic stability in human plasma and human liver microsomes. No acute toxicity or organ damage was observed at a dose of 2 g/kg. Overall, **M44** represents a highly promising lead compound that warrants further optimization efforts to identify potential anti-HIV-1 drug candidates.

Keywords: AIDS; HIV-1; NNRTIs; DAPYs

1. Introduction

The acquired immune deficiency syndrome (AIDS) is a major public health issue caused by human immunodeficiency virus (HIV) infection [1, 2]. Since it was first discovered in 1981, the number of people living with HIV-1 has continued to rise, reaching 39.9 million by 2023 [3, 4]. Combinational antiretroviral therapy (cART) is currently the most effective regimen for treating HIV, which has dramatically reduced the morbidity and mortality of HIV-infected patients through the combination of two or more classes of anti-HIV-1 agents [5-7]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are considered as important ingredients of cART owing to their high antiviral activity and low toxicity [8-11]. Until now, six NNRTIs have been approved by FDA, including the first generation delavirdine, nevirapine, efavirenz, and the second generation etravirine, rilpivirine, and doravirine [12]. Among them, etravirine (ETR, **1**), rilpivirine (RPV, **2**) are categorized as diarylpyrimidine (DAPY) derivatives and widely applied in the clinic due to their significant anti-HIV potency. However, rapid emergence of drug-resistant strains, severe adverse effects like hypersensitivity reactions and hepatotoxicity, plus with high cytotoxicity (CC_{50} = ~5 μM) and undesirable pharmacokinetics (PK) property limit their clinical use [13, 14].

Over the past two decades, the DAPY family has consistently led the development of novel anti-HIV-1 agent [12, 15, 16]. With the purpose of discovering new-generation anti-HIV-1 inhibitors, our research group, along with other esteemed counterparts, has made considerable efforts on the structural modification of ETR and RPV. Numerous series of novel NNRTIs have been identified, greatly expanding the DAPY family [17-22]. Among these series, CH(CN)-biphenyl-DAPYs were discovered using a molecular hybridization strategy [23]. Biological evaluation revealed that these derivatives exhibited decreased cytotoxicity compared to ETR and RPV. However, most of them exerted poor anti-HIV activity, especially against mutant strains, exemplified by the representative compound **3** (EC_{50} = 27 nM, CC_{50} = 36 μM). Hence, further optimization to identify ideal candidates with improved anti-resistance efficacy and low toxicity was triggered.



Etravirine (ETR, **1**)
EC_{50} = 3.2 nM (WT)
CC_{50} > 4.6 μM
SI > 1340

Rilpivirine (RPV, **2**)
EC_{50} = 1.0 nM (WT)
CC_{50} = 3.98 μM
SI = 3989

3
EC_{50} = 27 nM (WT)
CC_{50} = 36 μM
SI = 1361

Figure 1. Chemical structures of representative DAPYs.

This study was initiated by replacing the CH(CN) linker of compound **3** with several privileged functional groups, aiming to discover more potent NNRTIs and further enrich the SARs of DAPY derivatives. Molecular docking analysis of compound **3** revealed that the CH(CN) moiety was located in a hydrophobic subpocket, formed by V179, Y181 and E138, and adjacent to the entrance channel, providing more possibilities for structure expansion (Figure 2). To capitalize on this, the CH(CN) linker was sought to be replaced with an (alkylamino)methylene (CH(NHR)) motif, and some straight-chain, branched and substituted alkyl groups were incorporated to explore the tolerant region. Subsequently, the optimal terminal methyl group was retained. Building on the well-established use of double bonds (e.g., C=N, C=O) in linker modification of DAPYs [24, 25], compounds containing a $C{=}NCH_3$ linker, as intermediates of the $CH(NHCH_3)$-biphenyl-DAPYs, were further evaluated to probe the double bond effect on activity. Moreover, the E138 residue was reported to interact with some polar linkers such as NH, CH(OH) via H-bonds[14, 26]. Hydrazone, as a privileged pharmacophore, has been extensively utilized in drug design owing to its ability to serve as both hydrogen bond donor and acceptor, as well as relatively flexible structure [27]. Inspired by these, the hydrazone moiety ($C{=}NNH_2$) was also assessed. Herein, a series of novel biphenyl-DAPYs bearing CH(NHR), $C{=}NCH_3$ and $C{=}NNH_2$ linkers were developed and evaluated for their biological activity. Meanwhile, preliminary SAR analysis and molecular docking studies were conducted to gain deeper insights into these analogs.

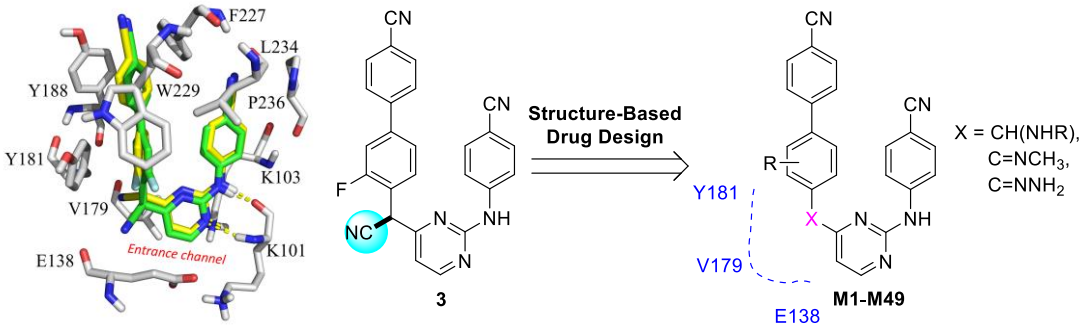
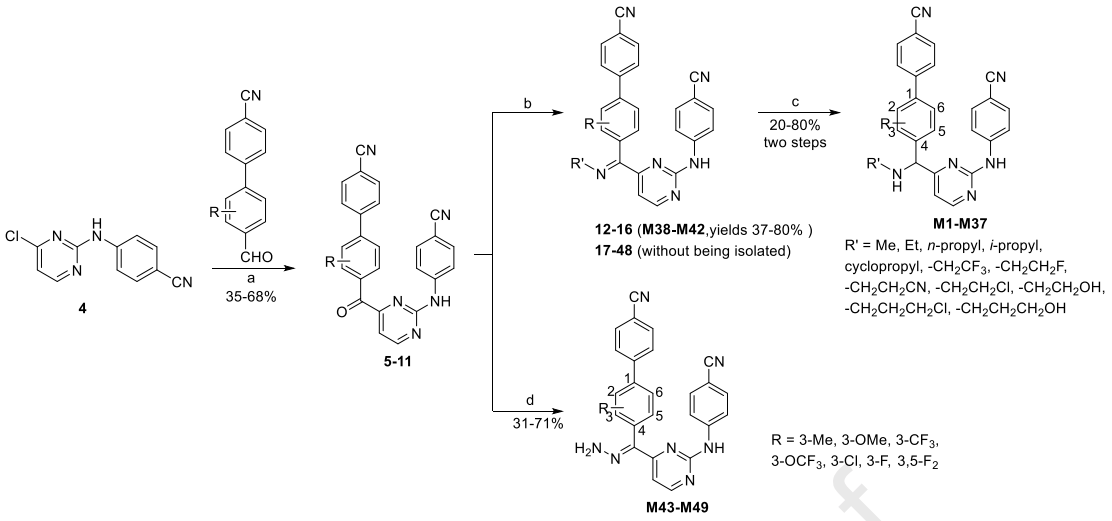


Figure 2. Structure-based drug design of biphenyl-DAPYs.

2. Results and discussion

2.1. Chemistry

The synthetic route of the target compounds **M1**–**M49** is depicted in scheme 1. The key intermediates **5**–**11** were synthesized through nucleophilic substitution of chloropyrimidine **4** with appropriate 4'-formyl-[1,1'-biphenyl]-4-carbonitrile, following our established procedure [26, 28, 29]. Subsequently, in the presence of acetic acid and anhydrous Na_2SO_4, compounds **7**–**12** were condensed with corresponding substituted amines, followed by direct reduction with $NaBH_3CN$, yielding **M1**–**M37** in 20%–80% yields. The target compounds **M38**–**M42** were obtained by isolating the imine intermediates **14**–**18** from the above reaction, with 37%–80% yields. Moreover, compounds **7**–**13** were reacted with $NH_2NH_2{\cdot}2HCl$ in the presence of pyridine to generate hydrazone derivatives **M43**–**M49** in the yields of 31%–71%. Notably, the imine compounds **M38**, **M42** and the hydrazones **M44**, **M45** were determined to be *E* isomers by X-ray crystallographic analysis (see Supporting Information). This result also established the absolute conformations of the other analogs in **M38**–**M49**.



Scheme 1. Reagents and condition: (a) 1,3-dimethylimidazolium iodide, NaH, DMSO, N_2, rt, 8-10 h; (b) R-NH_2, AcOH, Na_2SO_4, EtOH, reflux, 12–48 h; (c) $NaBH_3CN$, EtOH, 60 °C, 8–12 h. (d) NH_2NH_2·2HCl, pyridine, EtOH, reflux, 24–48.

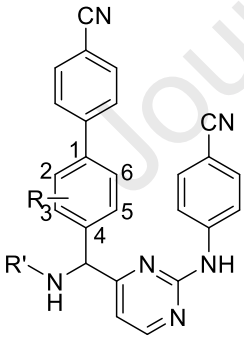
2.2. WT HIV-1 inhibitory activity and cytotoxicity

The inhibitory activity of these novel DAPYs against WT HIV-1 strain (IIIB) and their cytotoxicity were evaluated in MT-4 cells using MTT method. Nevirapine (NVP), efavirenz (EFV), etravirine (ETR) and rilpivirine (RPV) were selected as references. The results were determined by the values of EC_{50} (antiviral activity), CC_{50} (cytotoxicity), and SI (selectivity index, CC_{50}/EC_{50} ratio).

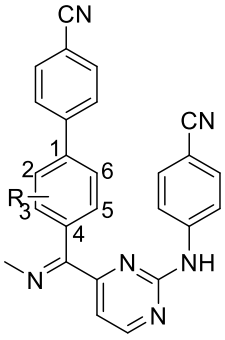
As depicted in Table 1, compounds **M1**–**M30**, possessing methyl, ethyl or propyl substituted CH(NHR) linkers, exhibited moderate activities against WT HIV-1 with EC_{50} values spanning from 0.011–1.19 μM. Most of them had lower cytotoxicity (CC_{50} > 20 μM) than ETR and RPV, but undesirable selectivity index (SI < 1000). The SAR analysis suggested that the alkyl groups at the linker with small size were more favorable for antiviral activity. For instance, **M1** exhibited an EC_{50} of 0.033 μM, the conversion of its methyl to ethyl results in a 2-fold decrease in activity (**M2**, EC_{50} = 0.063 μM), which was then changed to propyl, leading to a further reduced efficacy (**M3**–**M5**, EC_{50} = 0.146–0.166 μM). Several substituted alkyl groups were also assessed in **M31**–**M37**. However, all of these compounds were less active than the methyl substituted **M26**, though the introduction of electron withdrawing groups (-CF_3, -F, -CN, -Cl) resulted in a markedly improved safety (e.g., **M31**–**M34**, **M36**, CC_{50} > 200

μM). With the optimal terminal methyl group established, compounds **M38**–**M42** bearing a C=NCH$_3$ linker (*E*-isomer) were also evaluated. these derivatives exhibited remarkable anti-HIV-1 efficacy, particularly **M39**, **M42**, which demonstrated single nanomolar activity with EC$_{50}$ values of 8 nM and 6 nM, respectively, comparable to EFV and ETR. Notably, compared with compounds bearing a CH(NHCH$_3$) linker, these C=NCH$_3$-containing derivatives exhibited enhanced antiviral activity. For example, **M38** showed a 2.7-fold increase in activity compared to **M1**, while **M39** demonstrated 4.5-fold more active than **M6**. Moreover, they also displayed reduced cytotoxicity, as the CC$_{50}$ values of **M40**–**M42** (CC$_{50}$ = 45–251 μM) were markedly higher than those of **M11**, **M16** and **M26** (CC$_{50}$ = 7.8–18 μM). These results highlight the positive effects of the *E*-double bond. Compounds **M43**–**M49** containing a hydrazone (C=NNH$_2$) linker also exhibited good activity against WT HIV-1 with EC$_{50}$ values of 5–40 nM. Most of them were as potent as the corresponding C=NCH$_3$-containing derivatives, suggesting that the change of the terminal group from CH$_3$ to NH$_2$ had a minimal effect on activity against WT HIV-1.

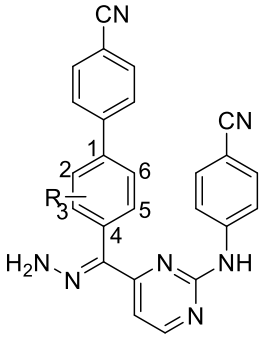
Table 1. WT HIV-1 inhibitory activity and cytotoxicity



M1-M37



M38-M42



M43-M49

Compd	R	R'	WT EC$_{50}$ (μM)	CC$_{50}$ (μM)	SI
M1	3-Me	Me	0.033 ± 0.009	12 ± 5.3	367
M2	3-Me	Et	0.063 ± 0.020	12 ± 4.0	188
M3	3-Me	*n*-propyl	0.146 ± 0.055	>273	>1872
M4	3-Me	*i*-propyl	0.166 ± 0.057	82 ± 72	495
M5	3-Me	cyclopropyl	0.156 ± 0.055	>274	>1755
M6	3-OMe	Me	0.036 ± 0.011	13 ± 7.1	354
M7	3-OMe	Et	0.326 ± 0.282	20 ± 6.9	60

M8	3-OMe	*n*-propyl	0.131 ± 0.032	14 ± 7.1	110
M9	3-OMe	*i*-propyl	0.211 ± 0.084	20 ± 5.8	94
M10	3-OMe	cyclopropyl	0.072 ± 0.013	123 ± 67	1683
M11	3-CF_3	Me	0.039 ± 0.014	15 ± 2.4	363
M12	3CF_3	Et	0.153 ± 0.048	22 ± 3.0	146
M13	3CF_3	*n*-propyl	1.01 ± 0.449	90 ± 69	87
M14	3CF_3	*i*-propyl	1.19 ± 0.000	29 ± 3.9	25
M15	3CF_3	cyclopropyl	0.294 ± 0.098	75 ± 1.2	250
M16	3-OCF_3	Me	0.056 ± 0.014	18 ± 5.9	313
M17	3-OCF_3	Et	0.505 ± 0.272	21 ± 4.2	43
M18	3-OCF_3	*n*-propyl	0.662 ± 0.303	27 ± 2.4	40
M19	3-OCF_3	*i*-propyl	0.927 ± 0.246	23 ± 5.8	24
M20	3-OCF_3	cyclopropyl	0.589 ± 0.190	73 ± 81	125
M21	3-F	Me	0.064 ± 0.037	13 ± 2.0	196
M22	3-F	Et	0.357 ± 0.201	31 ± 6.0	87
M23	3-F	*n*-propyl	0.259 ± 0.087	>270	>1056
M24	3-F	*i*-propyl	0.649 ± 0.130	45 ± 6.2	70
M25	3-F	cyclopropyl	0.630 ± 0.217	>271	>431
M26	3-Cl	Me	0.011 ± 0.005	7.8 ± 0.0	706
M27	3-Cl	Et	0.099± 0.058	19 ± 4.8	187
M28	3-Cl	*n*-propyl	0.184 ± 0.040	>261	>1424
M29	3-Cl	*i*-propyl	0.230 ± 0.042	30 ± 7.2	130
M30	3-Cl	cyclopropyl	0.147 ± 0.034	>262	>1798
M31	3-Cl	-CH_2CF_3	0.231 ± 0.077	>241	>1067
M32	3-Cl	-CH_2CH_2F	0.077 ± 0.033	>259	>3371
M33	3-Cl	-CH_2CH_2CN	0.071 ± 0.031	>255	>3604
M34	3-Cl	-CH_2CH_2Cl	0.042 ± 0.018	243 ± 6.0	5888
M35	3-Cl	-CH_2CH_2OH	0.092 ± 0.035	22 ± 7.5	238
M36	3-Cl	-$CH_2CH_2CH_2Cl$	0.195 ± 0.078	>244	>1264
M37	3-Cl	-$CH_2CH_2CH_2OH$	0.067 ± 0.030	23 ± 6.7	345
M38	3-Me		0.012 ± 0.004	>47	>3941
M39	3-OMe		0.008 ± 0.004	16 ± 5.3	1959
M40	3-CF_3		0.035 ± 0.021	119 ± 39	3275
M41	3-OCF_3		0.013 ± 0.007	>251	>19063
M42	3-Cl		0.006 ± 0.002	>45	>7143
M43	3-Me		0.007 ± 0.000	>291	>44964
M44	3-OMe		0.005 ± 0.002	54 ± 17	10995
M45	3-CF_3		0.031 ± 0.008	68 ± 23	2172
M46	3-OCF_3		0.040 ± 0.008	171 ± 15	4308
M47	3-Cl		0.006 ± 0.001	212 ± 7.6	33261

M48	3-F	0.007 ± 0.001	>46	>6211
M49	3,5-F_2	0.009 ± 0.005	23 ± 15	2480
3		0.027 ± 0.015	36 ± 11	1361
NVP		0.210 ± 0.090	15 ± 0.0	>72
EFV		0.004 ± 0.002	>6.3	>1425
ETR		0.003 ± 0.001	>4.6	>1340
RPV		0.001 ± 0.000	3.98	3989

2.3. Inhibitory activity toward HIV-1 mutants

In light of the promising activity of **M1**, **M6**, **M11**, **M16**, **M21**, **M26** and **M38**–**M49** against WT HIV-1, we further evaluated their potency against several clinically drug-resistant mutants. As shown in Table 2, most of the derivatives containing a $CH(NHCH_3)$ linker (**M1**, **M6**, **M11**, **M16**, **M21**, **M26**) exhibited micromolar efficacy against L100I, K103N, Y181C, Y188L. For E138K, they possessed relatively high potency with EC_{50} values in the range of 95–368 nM. Encouragingly, the switch from $CH(NHCH_3)$ to $C{=}NCH_3$ linker significantly enhanced the anti-resistance efficacy of **M38**–**M42**, as demonstrated by their increased activity toward L100I, K103N, Y181C and E138K (EC_{50} = 51–435 nM, except for **M38** against L100I). However, they were less active or inactive against Y188L. Notably, compounds **M43**–**M49**, possessing a $C{=}NNH_2$ linker, exhibited further improved potency against five mutant strains. For instance, the EC_{50} values of **M43** (R = 3-CH_3) toward L100I, K103N, Y181C, Y188L, E138K were 16, 17, 47, 442, 13 nM, respectively, which are about 96, 22, 8.9, 111 and 6.3-fold higher than that of compound **M38** ($C{=}NCH_3$, R = 3-CH_3). This trend was also observed in **M39**–**M42** versus **M44**–**M47**. Therefore, although the terminal -NH_2 had little effect on inhibition of WT HIV-1, it greatly enhanced the anti-resistance potency. Overall, **M44** demonstrated the most active with EC_{50} values of 7–148 nM. For L100I, Y181C, the potency of **M44** was comparable to that of ETR. For K103N and Y188L, **M44** exhibited 8.6- and 2-fold higher activity than EFV though inferior to ETR. For E138K, it was 7.5 times more effective than NVP. Moreover, compared to the lead compound **3**, **M44** showed an 8–173-fold increase in anti-resistance potency.

Table 2. Inhibitory activity of representative analogs toward clinically relevant HIV-1 mutants

Compd	EC_{50} (μM)				
	L100I	K103N	Y181C	Y188L	E138K
M1	2.439 ± 1.045	0.743 ± 0.070	1.092 ± 0.232	9.198 ± 0.441	0.279 ± 0.047
M6	2.329 ± 0.829	0.941 ± 0.246	1.299 ± 0.134	7.323 ± 0.291	0.224 ± 0.022
M11	2.436 ± 0.681	1.342 ± 0.557	1.177 ± 0.330	7.513 ± 4.314	0.184 ± 0.060
M16	1.938 ± 1.019	1.459 ± 0.779	2.218 ± 0.520	≥7.213	0.320 ± 0.080
M21	≥11.05	10.51 ± 0.691	1.910 ± 0.506	>12.7	0.368 ± 0.046
M26	0.821 ± 0.311	0.288 ± 0.022	0.444 ± 0.089	>7.762	0.095 ± 0.022
M38	1.540 ± 0.210	0.373 ± 0.047	0.420 ± 0.047	>46.7	0.082 ± 0.014
M39	0.293 ± 0.158	0.169 ± 0.155	0.293 ± 0.090	4.455 ± 2.497	0.083 ± 0.023
M40	0.352 ± 0.187	0.415 ± 0.042	0.435 ± 0.021	3.026 ± 0.477	0.290 ± 0.249
M41	0.301 ± 0.040	0.281 ± 0.060	0.361 ± 0.080	20.1 ± 16.9	0.122 ± 0.090
M42	0.149 ± 0.125	0.129 ± 0.076	0.192 ± 0.076	3.809 ± 1.158	0.051 ± 0.018
M43	0.016 ± 0.002	0.017 ± 0.003	0.047 ± 0.009	0.442 ± 0.140	0.013 ± 0.004
M44	0.007 ± 0.002	0.010 ± 0.005	0.022 ± 0.009	0.148 ± 0.135	0.021 ± 0.015
M45	0.058 ± 0.031	0.106 ± 0.068	0.134 ± 0.077	1.138 ± 0.310	0.068 ± 0.012
M46	0.180 ± 0.100	0.182 ± 0.088	0.260 ± 0.040	1.482 ± 0.180	0.340 ± 0.120
M47	0.022 ± 0.013	0.049 ± 0.027	0.067 ± 0.016	0.778 ± 0.111	0.076 ± 0.024
M48	0.069 ± 0.028	0.125 ± 0.095	0.210 ± 0.148	>46.1	0.194 ± 0.039
M49	0.016 ± 0.005	0.044 ± 0.013	0.064 ± 0.024	>23.1	0.038 ± 0.016
3	1.21 ± 0.21	0.9 ± 0.27	0.87 ± 0.23	9.81 ± 1.30	0.17 ± 0.03
NVP	2.065 ± 1.878	5.182 ± 2.704	5.783 ± 2.967	>15.0	0.158 ± 0.056
EFV	0.032 ± 0.016	0.086 ± 0.032	0.008 ± 0.003	0.301 ± 0.127	0.008 ± 0.003
ETR	0.007 ± 0.005	0.003 ± 0.001	0.016 ± 0.005	0.020 ± 0.008	0.009 ± 0.003

2.4. Inhibitory activity toward WT HIV-1 RT

To confirm the binding target of these newly synthesized compounds, the representative analogs **M1**, **M6**, **M11**, **M16**, **M21**, **M26** and **M38**–**M49** were evaluated for their efficacy against WT HIV-1 RT. As depicted in Table 3, the inhibitory activity of these derivatives follows the linker order of $CH(NHCH_3)$ < $C{=}NCH_3$ < $C{=}NNH_2$, which is consistent with their inhibition at the cellular level. With the exception of **M6**, **M11**,

M16 and **M21**, the tested compounds displayed significant activity with IC_{50} values ranging from 20 to 221 nM, approximately 3–37-fold higher than that of NVP. Among them, **M43** and **M47**–**M49** exhibited comparable potency to ETR. The most active compound **M44** in the cellular assay also kept a good enzymatic inhibition, possessing an IC_{50} value of 81 nM. These results suggested that these novel biphenyl-DAPYs belong to NNRTIs.

Table 3. Inhibitory activity of representative analogs toward WT HIV-1 RT

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
M1	0.153 ± 0.023	**M39**	0.061 ± 0.014	**M46**	0.072 ± 0.006
M6	0.963 ± 0.157	**M40**	0.180 ± 0.004	**M47**	0.036 ± 0.004
M11	0.495 ± 0.021	**M41**	0.221 ± 0.000	**M48**	0.020 ± 0.007
M16	1.998 ± 0.200	**M42**	0.069 ± 0.002	**M49**	0.031 ± 0.002
M21	0.783 ± 0.138	**M43**	0.023 ± 0.002	**NVP**	0.751 ± 0.150
M26	0.104 ± 0.020	**M44**	0.081 ± 0.007	**EFV**	0.012 ± 0.002
M38	0.058 ± 0.014	**M45**	0.058 ± 0.008	**ETR**	0.030 ± 0.005

2.5. Molecular docking

Molecular docking was performed on the most promising compound **M44** to preliminarily understand the binding pattern of this novel DAPY analog in the NNIBP. As shown in Figure 3A, **M44** bound to HIV-1 RT in a U-shaped conformation, akin to traditional NNRTIs such as ETR and RPV (Figure S1). The left biphenyl was locked in a hydrophobic channel, possessing π−π interactions and hydrophobic interactions with Y181, Y188 and W229. Two vital hydrogen bonds were observed, one formed by the N atom of pyrimidine and the NH of K101, and the other generated between the right linker NH and the O atom of K101. Besides, the introduced $C{=}NNH_2$ linker formed a direct hydrogen bonding with E138. Those extensive interactions contributed to the great antiviral efficacy of **M44**. Next, to elucidate the significant activity differences caused by linker alterations, especially in the case of L100I, Y188L, we selected **M6**, **M39** and **M44** (R = 3-OMe, bearing $C{=}NCH_3$, $CH(NHCH_3)$ and $C{=}NNH_2$ linker, respectively) to dock with L100I and Y188L RTs (Figures 3B–I). In contrast to the

freely rotating C-N single bond of **M6** (including *R*, *S*-enantiomers), the conformational limiting *E*-double bond of **M39** caused the terminal methyl group always being orientated towards the biphenyl side, probably forming sustained hydrophobic interaction with Y181. Moreover, this orientation may restrict the rotational freedom of the biphenyl aromatic ring, thereby favoring the stability of π−π interactions. In addition, the double bond extended the π−π conjugated system of the left wing and the central pyrimidine, which could contribute to stronger π−π interactions; this effect was absent in **M6** (Figures 3B–D, F–H). Therefore, **M39** exhibited higher potency than **M6**. Compared to **M6** and **M39**, compound **M44** not only benefited from the double bond effect, but also retained the hydrogen bonding with E138 (Figures 3E–I), which were responsible for its optimal efficacy. Additionally, comparing the docking modes of **M44** with WT, L100I and Y188L (Figures 3A, E, I), the change of L100 to I100 showed no significant effect on the binding affinity, while the conversion of Y188 to L188 led to a loss of π−π interaction with Y188. Thus, **M44** retained the activity against L100I but was 30-fold less active toward Y188L compared to its inhibition of WT HIV-1.

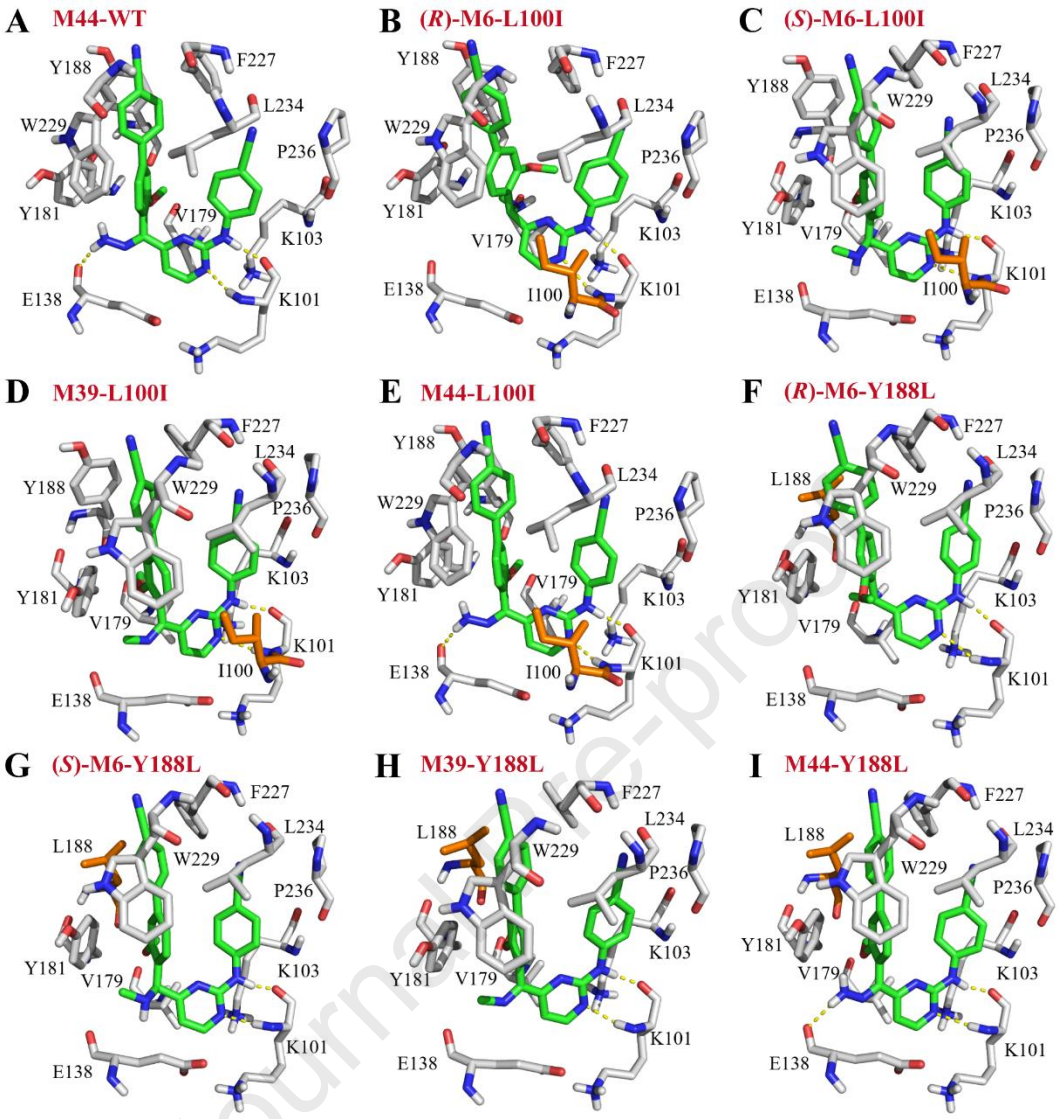


Figure 3. Predicted binding modes of representative compounds with WT HIV-1 RT and the mutants L100I, Y188L (PDB code: 2ZD1). (A) **M44** with WT; (B) (*R*)-**M6** with L100I; (C) (*S*)-**M6** with L100I; (D) **M39** with L100I; (E) **M44** with L100I; (F) (*R*)-**M6** with Y188L; (G) (*S*)-**M6** with Y188L; (H) **M39** with Y188L; (I) **M44** with Y188L.

2.6. Metabolic stability assay

Metabolic stability determines the sufficient active form of drugs *in vivo*, which is closely associated to drug efficacy, safety and PK profiles [30, 31]. Therefore, the optimal compound **M44** was evaluated for human plasma stability, using ETR and RPV as references. As shown in Table 4, **M44** exhibited good stability in human plasma, with a long half-life exceeding 300 min. Notably, after 300 min of incubation, **A44** showed no significant degradation, with residual amounts of 94.7%, which is comparable to that

of RPV (97.4%) and higher than that of ETR (78.5%).

Table 4. Human plasma stability

Compd	Percent remaining (%)						$t_{1/2}$ (min)
	0 min	15 min	30 min	60 min	120 min	300 min	
M44	100.0	103.6	100.6	101.3	98.2	94.7	> 300
ETR	100.0	101.6	95.4	97.0	92.1	78.5	> 300
RPV	100.0	101.5	99.4	99.3	95.3	97.4	> 300

Next, **M44** was further assessed for metabolic stability in human liver microsomes, and the results were illustrated in Table 5. **M44** displayed a medium half-life of 37.0 min, with clearance rates of 37 μL/min/mg in microsomes, and 33.3 mL/min/kg in liver. Compared with the reference drugs, **M44** had a higher stability than RPV ($t_{1/2}$ = 12.8 min), but inferior to ETR ($t_{1/2}$ = 65.5 min).

Table 5. Human liver microsomal stability

Compd	Human Liver microsomal stability[a]			
	R^2	$t_{1/2}$ (min)	$CL_{int(mic)}$ (μL/min/mg)	$CL_{int(liver)}$ (mL/min/kg)
M44	0.9776	37.5	37.0	33.3
ETR	0.9798	65.5	21.1	19.0
RPV	0.9544	12.8	108.4	97.6

[a]The data of ETR and RPV were obtained from the reference [32] with the same test method.

2.7. In vivo acute toxicity assay

To evaluate the safety of **M44** *in vivo*, a single-dose acute toxicity assay was conducted on healthy ICR mice (female and male mice). As shown in Figure 4, After being oral administrated **M44** at a single dose of 2 g/kg, all mice in the experimental group grew normally within 2 weeks, consistent with the control group (Figures 4A–B). No death and no toxic symptoms occurred. On the 14th days after administration, all mice underwent blood examinations and then were dissected for histopathological analysis. The blood biochemical parameters (ALT, AST, CREA, UREA, CKMB) of the experimental groups showed no marked differences compared to the control groups

(Figures 4C–D). Moreover, HE staining assay suggested that no significant histological lesions were observed in six vital organs (brain, heart, liver, spleen, lung, and kidney) (Figure 5E).

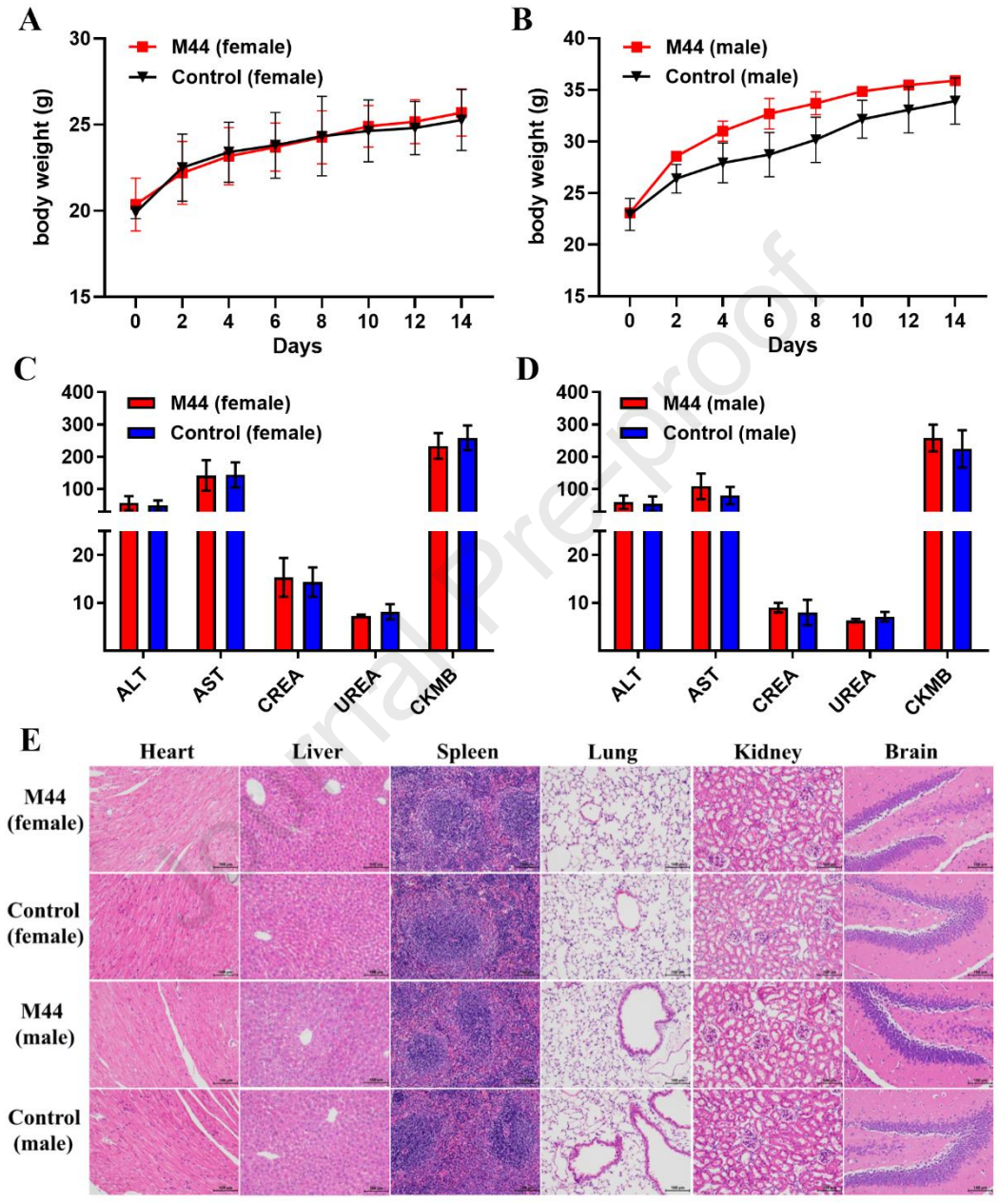


Figure 4. (A, B) Temporal progression of body weight. (C, D) The blood biochemical parameters. (E) HE staining of six vital organs (magnification: ×200).

3. Conclusion

In conclusion, a series of novel biphenyl-diarylpyrimidines containing CH(NHR), C=NCH$_3$ and C=NNH$_2$ linkers were developed via a structure-based drug design

strategy. Most of these analogues exhibited nanomolar activity against WT HIV-1 and lower cytotoxicity than ETR and RPV. For drug-resistant mutants, the representative analogues showed moderate to excellent efficacy, with EC_{50} values ranging from micromolar to nanomolar. SAR analysis indicated that these biphenyl-DAPYs with different linkers displayed significant differences in activity, following a trend: $CH(NHCH_3) < C{=}NCH_3 < C{=}NNH_2$, particularly against the mutant strains. Molecular docking preliminarily rationalized this activity discrepancy. The most promising compound **M44**, containing an (*E*)-$C{=}NNH_2$ linker, exhibited strong potency toward WT HIV-1 with an EC_{50} value of 5 nM, comparable to that of ETR. Also, it was highly effective against five mutant strains (EC_{50} = 7–148 nM), approximately 8–173 times more potent than the lead compound **3** (EC_{50} = 0.17–9.81 μM). Furthermore, **M44** exhibited lower cytotoxicity and higher selectivity (CC_{50} = 54 μM, SI = 10995) compared to ETR, RPV and compound **3**. *In vitro* metabolic stability assay indicated that **M44** has a suitable half-life in both human plasma ($t_{1/2}$ > 300 min) and human liver microsomes ($t_{1/2}$ = 37.5 min). No acute toxicity and organ damage were observed at a dose of 2 g/kg. Consequently, **M44** could serve as a novel lead compound to further guide the development of biphenyl-DAPY NNRTIs with stronger potency and better drug-like characteristics.

4. Experimental section

4.1. Chemistry

Chemical reagents and solvents were purchased from commercial sources and used as received. Column chromatography was performed on silica gel (200–300 mesh). TLC was carried out on 0.25 mm silica gel plates visualized with UV light (λ = 254, 365 nm). ^{1}H, ^{13}C and ^{19}F NMR were recorded on a Bruker Avance 400 MHz or 600 MHz spectrometer. Melting points were measured at 589 nm wavelength by a SRS-optic melting point apparatus. HRMS was obtained on a Waters Quattro Micromass instrument and Brukersolari X-70 FT-MS instrument, respectively, using electrospray ionization (ESI) technique. X-ray diffraction analysis was carried out by Dr. Meng Yang (Sichuan University). The purity of **M1**–**M49** was analyzed by HPLC (Agilent 1260 or

Thermo fisher U3000) using a C18 column (Eslipse XDB, 150×4.6 mm, 5 μm) with methanol/water at a flow rate of 0.8 mL/min: (a) 0–7 min, 50–85% MeOH; (b) 7–15 min, 85% MeOH; (c) 15–15.01 min, 85–50% MeOH; (c) 15.01–20 min, 50% MeOH. The purity of all target compounds **M1**–**M49** used in subsequent experiments was ≥ 95% as determined by HPLC.

*4.1.1. General procedure for preparation of **M1**–**M42***

The intermediate ketones **7**–**13** were synthesized following our previously disclosed procedure [26]. Next, ketones **7**–**12** (1.0 mmol, 1.0 equiv.), Na_2SO_4 (5.0 mmol, 5.0 equiv.) and appropriate substituted amine (15 mmol, 15 equiv.) were mixed in dry EtOH (30 ml), and then 5 drops of acetic acid were added. The reaction mixture was heated to reflux for 12–48 h until complete consumption of starting material as monitored by TLC. Subsequently, the solution was cooled to room temperature, $NaBH_3CN$ (5.0 mmol, 5.0 equiv.) was added, and the mixture was stirred at 60 °C for 8–12h (monitored by TLC). Until completion, the reaction was cooled, poured into 20 ml saturated $NaHCO_3$ aq., and extracted with ethyl acetate (30 mL×3). The organic layers were washed with saturated NaCl aq. (30mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuo. The obtained residue was purified by silica gel column chromatography, eluting with ethyl acetate/petroleum ether to afford **M1**–**M37**. Moreover, the imine intermediates **14**–**18** generated from the amination reaction were purified by recrystallisation in ethyl acetate/petroleum ether and then served as target compounds **M38**–**M42**.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M1**). Yield 32%, white solid, mp: 170 – 172 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.16 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.96 – 7.79 (m, 4H), 7.74 – 7.46 (m, 5H), 7.14 (d, J = 5.1 Hz, 1H), 5.04 (s, 1H), 2.57 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 173.3, 160.4, 159.4, 145.9, 145.7, 141.8, 138.6, 138.5, 133.7, 133.5, 130.0, 129.0, 128.5, 125.7, 120.0, 119.4, 119.3, 112.1, 111.6, 104.5, 66.4, 35.1, 20.1. HRMS (ESI): calcd for $C_{27}H_{23}N_6$ $[M + H]^+$, 431.1979, found, 431.1981. Purity: 97.88%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M2**). Yield 36%, white solid, mp: 152 – 154 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.14 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.08 – 7.99 (m, 2H), 7.93 – 7.80 (m, 4H), 7.69 – 7.52 (m, 5H), 7.14 (d, J = 5.0 Hz, 1H), 5.16 (s, 1H), 2.68 (q, J = 7.1 Hz, 2H), 2.57 (s, 3H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.4, 159.0, 158.5, 144.9, 144.4, 141.2, 137.0, 136.7, 132.9, 132.8, 128.9, 127.9, 127.4, 124.7, 119.6, 118.9, 118.2, 111.1, 109.8, 102.2, 63.1, 41.8, 19.5, 15.2. HRMS (ESI): calcd for $C_{28}H_{25}N_6$ $[M + H]^+$, 445.2135, found, 445.2134. Purity: 96.16%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M3**). Yield 24%, white solid, mp: 126 – 128 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.16 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.95 – 7.80 (m, 4H), 7.74 – 7.53 (m, 5H), 7.14 (d, J = 5.1 Hz, 1H), 5.14 (s, 1H), 2.61 (t, J = 7.1 Hz, 2H), 2.57 (s, 3H), 1.80 – 1.49 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 173.6, 160.4, 159.4, 146.0, 145.8, 142.2, 138.5, 138.3, 133.7, 133.5, 130.0, 129.1, 128.5, 125.7, 120.0, 119.4, 119.4, 112.2, 111.6, 104.5, 64.6, 50.9, 24.1, 20.1, 12.2. HRMS (ESI): calcd for $C_{29}H_{27}N_6$ $[M + H]^+$, 459.2292, found, 459.2296. Purity: 97.78%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M4**). Yield 34%, white solid, mp: 143 – 145 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 8.52 (d, J = 5.1 Hz, 1H), 8.09 – 7.81 (m, 6H), 7.74 – 7.47 (m, 5H), 7.17 (d, J = 5.1 Hz, 1H), 5.14 (s, 1H), 2.88 – 2.64 (m, 1H), 2.62 – 2.56 (m, 1H), 2.52 (s, 3H), 1.12 – 0.98 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.6, 159.0, 158.4, 144.9, 144.4, 141.3, 136.9, 136.7, 132.9, 132.8, 129.0, 128.0, 127.4, 124.7, 119.6, 118.9, 118.2, 111.4, 109.8, 102.2, 60.3, 46.0, 23.0, 22.8, 19.5. HRMS (ESI): calcd for $C_{29}H_{27}N_6$ $[M + H]^+$, 459.2292, found, 459.2294. Purity: 97.87%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M5**). Yield 20%, white solid, mp: 135 – 137 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.18 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.96 – 7.80 (m, 4H), 7.77 – 7.49 (m, 5H), 7.13 (d, J = 5.1 Hz, 1H), 5.25 (s, 1H), 3.07 (s, 1H), 2.57 (s, 3H), 2.39 – 2.12 (m, 1H), 0.41 (d, J = 5.2 Hz, 4H). ^{13}C NMR

(100 MHz, Acetone-d_6) δ: 173.3, 160.5, 159.3, 145.9, 145.8, 142.4, 138.5, 138.3, 133.7, 133.5, 129.9, 129.1, 128.5, 125.7, 120.0, 119.40, 119.37, 119.3, 112.6, 111.6, 104.5, 64.3, 20.0, 7.01, 6.99. HRMS (ESI): calcd for $C_{29}H_{25}N_6$ $[M + H]^+$, 457.2135, found, 457.2137. Purity: 96.76%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M6**). Yield 36%, white solid, mp: 134 – 136 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.13 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 7.97 – 7.86 (m, 6H), 7.65 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.0 Hz, 1H), 7.46 – 7.29 (m, 2H), 7.10 (d, J = 5.1 Hz, 1H), 5.04 (s, 1H), 3.89 (s, 3H), 2.73 (s, 1H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.2, 159.2, 158.5, 157.5, 145.1, 144.6, 138.6, 133.0, 132.9, 130.5, 128.9, 127.8, 119.7, 119.4, 119.0, 118.3, 111.4, 110.1, 109.9, 102.2, 62.2, 55.9, 34.6. HRMS (ESI): calcd for $C_{27}H_{22}N_6O$ $[M + H]^+$, 447.1928, found, 447.1929. Purity: 98.12%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M7**). Yield 63%, white solid, mp: 144 – 146 °C. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ: 8.46 (d, J = 5.1 Hz, 1H), 8.43 (s, 1H), 7.96 – 7.81 (m, 6H), 7.72 – 7.62 (m, 2H), 7.49 (d, J = 7.9 Hz, 1H), 7.41 – 7.27 (m, 2H), 6.80 (d, J = 5.1 Hz, 1H), 5.52 (s, 1H), 3.89 (s, 3H), 3.00 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ: 165.5, 160.4, 159.9, 158.9, 145.2, 144.9, 143.5, 134.2, 133.8, 132.3, 129.0, 123.0, 121.0, 120.2, 120.1, 119.6, 112.5, 112.4, 111.7, 105.5, 61.4, 56.8, 43.7, 12.1. HRMS (ESI): calcd for $C_{28}H_{25}N_6O$ $[M + H]^+$, 461.2084, found, 461.2085. Purity: 96.60%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M8**). Yield 75%, white solid, mp: 173 – 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.12 (s, 1H), 8.50 (d, J = 5.1 Hz, 1H), 8.19 – 7.83 (m, 6H), 7.65 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 7.9 Hz, 1H), 7.43 – 7.27 (m, 2H), 7.10 (d, J = 5.1 Hz, 1H), 5.15 (s, 1H), 3.90 (s, 3H), 2.61 (s, 1H), 2.49 – 2.42 (m, 2H), 1.60 – 1.38 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.4, 159.0, 158.3, 157.3, 145.0, 144.6, 138.5, 132.9, 132.8, 130.7, 128.8, 127.7, 119.6, 119.3, 118.9,

118.1, 111.2, 110.0, 109.9, 102.1, 60.2, 55.8, 49.5, 22.8, 11.8. HRMS (ESI): calcd for $C_{29}H_{27}N_6O$ $[M + H]^+$, 475.2241, found, 475.2244. Purity: 97.39%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M9**). Yield 76%, white solid, mp: 180 – 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.12 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.14 – 7.82 (m, 6H), 7.65 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 7.8 Hz, 1H), 7.41 – 7.28 (m, 2H), 7.12 (d, J = 5.1 Hz, 1H), 5.30 (s, 1H), 3.91 (s, 3H), 2.82 – 2.62 (m, 1H), 2.58 (s, 1H), 1.08 – 0.98 (m, , 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.6, 159.0, 158.2, 157.3, 145.0, 144.6, 138.4, 132.9, 132.8, 130.9, 128.9, 127.7, 119.6, 119.3, 118.9, 118.1, 111.4, 110.0, 109.9, 102.1, 57.3, 55.9, 45.8, 23.2, 22.7. HRMS (ESI): calcd for $C_{29}H_{27}N_6O$ $[M + H]^+$, 475.2241, found, 475.2243. Purity: 98.50%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M10**). Yield 80%, white solid, mp: 196 – 197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.12 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 8.02 – 7.85 (m, 6H), 7.65 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 7.9 Hz, 1H), 7.43 – 7.28 (m, 2H), 7.10 (d, J = 5.1 Hz, 1H), 5.22 (d, J = 7.5 Hz, 1H), 3.89 (s, 3H), 3.47 – 3.34 (m, 1H), 2.15 – 1.95 (m, 1H), 0.80 – 0.24 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.2, 159.0, 158.2, 157.2, 145.0, 144.6, 138.5, 132.9, 132.8, 130.8, 128.8, 127.7, 119.6, 119.2, 118.9, 118.1, 111.4, 110.0, 109.9, 102.1, 60.6, 55.8, 29.0, 6.53, 6.48. HRMS (ESI): calcd for $C_{29}H_{25}N_6O$ $[M + H]^+$, 473.2084, found, 473.2086. Purity: 98.95%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M11**). Yield 43%, white solid, mp: 141 – 142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.56 (d, J = 5.1 Hz, 1H), 8.20 – 8.05 (m, 2H), 8.04 – 7.90 (m, 5H), 7.81 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 5.1 Hz, 1H), 5.04 (s, 1H), 3.03 (s, 1H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 159.1, 159.0, 144.8, 142.8, 140.9, 137.7, 133.0, 132.8, 131.2, 130.9, 128.6 (q, J = 29.6 Hz), 127.9, 124.3 (q, J = 274.7 Hz), 124.0 (q, J = 5.5 Hz), 119.5, 118.7, 118.2, 111.6, 110.8, 102.3, 63.9, 34.4. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.3. HRMS (ESI): calcd for $C_{27}H_{20}F_3N_6$ $[M + H]^+$, 485.1696, found, 485.1698. Purity: 98.32%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M12**). Yield 20%, white solid, mp: 199 – 201 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.20 (s, 1H), 8.52 (d, J = 5.0 Hz, 1H), 8.14 – 8.04 (m, 2H), 8.04 – 7.94 (m, 5H), 7.95 – 7.88 (m, 2H), 7.78 – 7.49 (m, 2H), 7.10 (d, J = 5.1 Hz, 1H), 5.30 (s, 1H), 4.09 – 2.38 (m, 2H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 172.1, 160.5, 159.7, 145.6, 144.3, 142.3, 139.4, 133.74, 133.65, 132.1, 132.0, 130.0 (q, J = 29.8 Hz), 128.9, 125.5 (q, J = 274.1 Hz), 125.1 (q, J = 6.1 Hz), 119.9, 119.4, 119.2, 112.7, 112.6, 104.6, 63.2, 43.1, 15.6. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.4. HRMS (ESI): calcd for $C_{28}H_{22}F_3N_6$ $[M + H]^+$, 499.1853, found, 499.1856. Purity: 96.89%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M13**). Yield 46%, white solid, mp: 178 – 179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.30 – 8.07 (m, 1H), 8.09 – 8.03 (m, 1H), 8.03 – 7.91 (m, 5H), 7.88 – 7.78 (m, 2H), 7.73 – 7.55 (m, 2H), 7.11 (d, J = 5.1 Hz, 1H), 5.15 (s, 1H), 2.94 (s, 1H), 2.50 – 2.33 (m, 2H), 1.76 – 1.36 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 159.0, 158.9, 144.7, 142.8, 141.2, 137.6, 133.0, 132.7, 131.2, 131.0, 128.3 (q, J = 29.5 Hz), 127.9, 124.3 (q, J = 274.8 Hz), 124.0 (q, J = 5.8 Hz), 119.5, 118.7, 118.2, 111.6, 110.8, 102.3, 62.1, 49.5, 22.7, 11.7. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.4. HRMS (ESI): calcd for $C_{29}H_{24}F_3N_6$ $[M + H]^+$, 513.2009, found, 513.2011. Purity: 99.17%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M14**). Yield 21%, white solid, mp: 190 – 192 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.54 (d, J = 5.1 Hz, 1H), 8.47 – 7.88 (m, 7H), 7.81 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 5.1 Hz, 1H), 5.28 (d, J = 6.3 Hz, 1H), 2.89 (d, J = 6.1 Hz, 1H), 2.80 – 2.60 (m, 1H), 1.12 – 0.94 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 159.0, 158.8, 144.8, 142.8, 141.2, 137.6, 133.0, 132.7, 131.2, 131.1, 128.0 (q, J = 29.2 Hz), 127.9, 124.3 (q, J = 274.8 Hz), 124.1 (q, J = 6.1 Hz), 119.5, 118.7, 118.2, 111.8, 110.8, 102.3, 59.5,

46.1, 22.9, 22.5. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.7. HRMS (ESI): calcd for $C_{29}H_{24}F_3N_6$ $[M + H]^+$, 513.2009, found, 513.2010. Purity: 99.45%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M15**). Yield 25%, white solid, mp: 187 – 188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.14 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.25 – 8.03 (m, 2H), 8.04 – 7.91 (m, 5H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 5.1 Hz, 1H), 5.26 (d, *J* = 9.1 Hz, 1H), 4.07 – 3.66 (m, 1H), 2.26 – 1.86 (m, 1H), 0.65 – 0.12 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ: 170.8, 159.0, 158.8, 144.7, 142.8, 141.4, 137.5, 133.0, 132.7, 131.1, 128.2 (q, *J* = 29.6 Hz), 127.9, 124.3 (q, *J* = 274.8 Hz), 123.9 (q, *J* = 6.2 Hz), 119.5, 118.7, 118.2, 111.7, 110.8, 102.3, 62.1, 28.9, 6.6, 6.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.5. HRMS (ESI): calcd for $C_{29}H_{22}F_3N_6$ $[M + H]^+$, 511.1853, found, 511.1860. Purity: 96.86%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M16**). Yield 43%, white solid, mp: 132 – 134 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 7.98 – 7.91 (m, 4H), 7.89 – 7.80 (m, 4H), 7.75 – 7.68 (m, 1H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 5.1 Hz, 1H), 5.01 (s, 1H), 2.94 (s, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 159.1, 158.9, 147.3, 144.8, 142.6, 139.2, 134.7, 133.0, 132.8, 130.2, 127.8, 126.3, 120.1 (q, *J* = 257.4 Hz), 119.5, 119.0, 118.7, 118.2, 111.2, 110.8, 102.3, 62.3, 34.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -55.6. HRMS (ESI): calcd for $C_{27}H_{20}F_3N_6O$ $[M + H]^+$, 501.1645, found, 501.1648. Purity: 99.52%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M17**). Yield 73%, white solid, mp: 172 – 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.02 – 7.89 (m, 4H), 7.90 – 7.79 (m, 4H), 7.75 – 7.67 (m, 1H), 7.65 – 7.56 (m, 2H), 7.12 (d, *J* = 5.1 Hz, 1H), 5.13 (s, 1H), 2.86 (s, 1H), 2.66 – 2.51 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 170.9, 159.1, 158.9, 147.2, 144.9, 142.7, 139.2, 135.0, 133.0, 132.8, 130.3, 127.8, 126.3, 120.2 (q, *J* = 257.5 Hz), 119.5, 119.0, 118.7, 118.2, 111.2, 110.8, 102.3, 60.3, 41.7, 15.0. ^{19}F NMR (376 MHz, DMSO-d_6) δ:

-55.6. HRMS (ESI): calcd for $C_{28}H_{22}F_3N_6O$ [M + H]$^+$, 515.1802, found, 515.1803. Purity: 96.80%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M18**). Yield 64%, white solid, mp: 162 – 163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.02 – 7.91 (m, 4H), 7.90 – 7.79 (m, 4H), 7.73 – 7.65 (m, 1H), 7.64 – 7.55 (m, 2H), 7.13 (d, J = 5.1 Hz, 1H), 5.12 (s, 1H), 2.84 (s, 1H), 2.49 – 2.44 (m, 2H), 1.61 – 1.39 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 159.1, 158.9, 147.2, 144.8, 142.7, 139.2, 135.0, 133.0, 132.8, 130.3, 127.8, 126.3, 120.2 (d, J = 257.5 Hz), 119.5, 119.0, 118.7, 118.2, 111.2, 110.8, 102.3, 60.5, 49.4, 22.7, 11.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -55.6. HRMS (ESI): calcd for $C_{29}H_{24}F_3N_6O$ [M + H]$^+$, 529.1958, found, 529.1962. Purity: 96.86%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M19**). Yield 63%, white solid, mp: 131 – 132 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 7.99 – 7.90 (m, 4H), 7.90 – 7.79 (m, 4H), 7.70 (s, 1H), 7.61 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 5.1 Hz, 1H), 5.24 (d, J = 6.8 Hz, 1H), 2.79 (s, 1H), 2.73 – 2.64 (m, 1H), 1.07– 1.02 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 159.1, 158.8, 147.1, 144.8, 142.7, 139.2, 135.1, 133.0, 132.8, 130.5, 127.8, 126.3, 120.2 (d, J = 257.5 Hz), 119.5, 118.9, 118.7, 118.2, 111.4, 110.8, 102.3, 57.7, 46.0, 22.8, 22.6. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -55.5. HRMS (ESI): calcd for $C_{29}H_{24}F_3N_6O$ [M + H]$^+$, 529.1958, found, 529.1959. Purity: 98.65%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M20**). Yield 71%, white solid, mp: 166 – 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.04 – 7.89 (m, 4H), 7.90 – 7.78 (m, 4H), 7.77 – 7.69 (m, 1H), 7.61 (d, J = 8.6 Hz, 2H), 7.14 (d, J = 5.1 Hz, 1H), 5.20 (d, J = 9.4 Hz, 1H), 3.68 (d, J = 9.7 Hz, 1H), 2.24 – 1.90 (m, 1H), 0.56 – 0.16 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 159.1, 158.8, 147.1, 144.8, 142.7, 139.1, 135.1, 133.0, 132.8, 130.3, 127.8, 126.3, 120.2 (d, J = 257.6 Hz), 119.5, 119.0, 118.7, 118.2, 111.4, 110.8, 102.3, 60.6, 28.8, 6.5, 6.4. ^{19}F

NMR (376 MHz, DMSO-d_6) δ: -55.6. HRMS (ESI): calcd for $C_{29}H_{22}F_3N_6O$ $[M + H]^+$, 527.1802, found, 527.1807. Purity: 98.90%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M21**). Yield 21%, white solid, mp: 180 – 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.56 (d, J = 5.1 Hz, 1H), 8.23 – 7.81 (m, 6H), 7.76 – 7.55 (m, 5H), 7.17 (d, J = 5.1 Hz, 1H), 4.99 (s, 1H), 2.91 (s, 1H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 171.2, 160.6 (d, J = 245.1 Hz), 159.1, 158.8, 144.9, 142.9, 139.3 (d, J = 8.0 Hz), 132.9, 132.8, 129.8 (d, J = 4.5 Hz), 129.2 (d, J = 14.6 Hz), 127.6, 123.2 119.5, 118.7, 118.2, 113.9 (d, J = 23.5 Hz), 111.0, 110.6, 102.3, 61.8, 34.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -117.3. HRMS (ESI): calcd for $C_{26}H_{20}FN_6$ $[M + H]^+$, 435.1728, found, 435.1724. Purity: 97.33%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M22**). Yield 54%, white solid, mp: 163 – 164 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.17 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.03 – 7.84 (m, 6H), 7.74 – 7.58 (m, 5H), 7.18 (d, J = 5.1 Hz, 1H), 5.11 (s, 1H), 2.84 (s, 1H), 2.55 (q, J = 7.1 Hz, 2H), 1.08 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.4, 160.6 (d, J = 245.0 Hz), 159.1, 158.8, 144.9, 143.0 (d, J = 2.0 Hz), 139.3 (d, J = 8.2 Hz), 132.93, 132.89, 129.9 (d, J = 4.7 Hz), 129.5 (d, J = 14.5 Hz), 127.7, 123.2 (d, J = 3.1 Hz), 119.6, 118.8, 118.3, 113.9 (d, J = 23.9 Hz), 111.1, 110.6, 102.3, 59.8, 41.7, 15.1. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -117.4. HRMS (ESI): calcd for $C_{27}H_{22}FN_6$ $[M + H]^+$, 449.1884, found, 449.1886. Purity: 99.16%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M23**). Yield 25%, white solid, mp: 151 – 152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.70 (s, 1H), 8.10 (d, J = 5.0 Hz, 1H), 7.75 – 7.33 (m, 6H), 7.27 – 7.11 (m, 5H), 6.73 (d, J = 5.1 Hz, 1H), 4.65 (s, 1H), 2.35 (s, 1H), 2.04 – 2.00 (m, 2H), 1.18 – 0.95 (m, 2H), 0.43 (t, J = 7.4 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.5, 160.6 (d, J = 245.0 Hz), 159.1, 158.8, 144.9, 143.0 (d, J = 2.0 Hz), 139.3 (d, J = 8.2 Hz), 132.9, 132.9, 129.9 (d, J = 4.7 Hz), 129.6 (d, J = 14.6 Hz), 127.7, 123.2 (d, J = 2.8 Hz), 119.6, 118.8, 118.3, 113.9 (d, J = 23.7 Hz), 111.1, 110.6, 102.3, 60.0, 49.4,

22.7, 11.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -117.4. HRMS (ESI): calcd for $C_{28}H_{24}FN_6$ $[M + H]^+$, 463.2041, found, 463.2045. Purity: 97.50%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M24**). Yield 42%, white solid, mp: 106 – 108 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.20 – 7.84 (m, 6H), 7.78 – 7.53 (m, 5H), 7.20 (d, *J* = 5.1 Hz, 1H), 5.24 (d, *J* = 5.8 Hz, 1H), 2.77 (s, 1H), 2.73 – 2.65 (m, 1H), 1.10 – 1.00 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ: 171.6, 160.6 (d, *J* = 244.8 Hz), 159.0, 158.7, 144.9, 142.9 (d, *J* = 1.9 Hz), 139.2 (d, *J* = 7.9 Hz), 132.89, 132.86, 130.0 (d, *J* = 4.9 Hz), 129.7 (d, *J* = 14.8 Hz), 127.6, 123.2 (d, *J* = 2.8 Hz), 119.5, 118.8, 118.2, 113.9 (d, *J* = 24.0 Hz), 111.2, 110.6, 102.3, 57.1, 45.9, 23.0, 22.5. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -117.7. HRMS (ESI): calcd for $C_{28}H_{24}FN_6$ $[M + H]^+$, 463.2041, found, 463.2045. Purity: 98.20%.

4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M25**). Yield 26%, white solid, mp: 158 – 160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.24 – 7.84 (m, 6H), 7.81 – 7.58 (m, 5H), 7.18 (d, *J* = 5.1 Hz, 1H), 5.18 (d, *J* = 9.1 Hz, 1H), 4.53 – 3.52 (m, 1H), 2.32 – 1.95 (m, 1H), 1.04 – 0.28 (m, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 160.5 (d, *J* = 244.8 Hz), 159.1, 158.7, 144.9, 142.9 (d, *J* = 2.0 Hz), 139.2 (d, *J* = 8.1 Hz) , 132.89, 132.86, 129.9 (d, *J* = 4.7 Hz), 129.6 (d, *J* = 14.9 Hz), 127.6, 123.1 (d, *J* = 2.8 Hz), 119.5, 118.8, 118.2, 113.9 (d, *J* = 23.7 Hz), 111.2, 110.6, 102.2, 60.1, 28.9, 6.5, 6.3. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -117.3. HRMS (ESI): calcd for $C_{28}H_{22}FN_6$ $[M + H]^+$, 461.1884, found, 461.1885. Purity: 99.24%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylamino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M26**). Yield 69%, white solid, mp: 194 – 196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.56 (d, *J* = 5.1 Hz, 1H), 8.06 – 7.77 (m, 8H), 7.73 – 7.56 (m, 3H), 7.14 (d, *J* = 5.1 Hz, 1H), 5.14 (s, 1H), 2.33 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 170.7, 159.1, 158.8, 144.8, 142.7, 139.4, 138.8, 134.0, 132.9, 132.8, 130.0, 127.7, 127.6, 126.0, 119.5, 118.7, 118.2, 111.4, 110.6, 102.2, 65.0, 34.4. HRMS (ESI): calcd for $C_{26}H_{20}ClN_6$ $[M + H]^+$, 451.1432, found, 451.1434. Purity: 95.59%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(ethylamino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M27**). Yield 42%, white solid, mp: 168 – 170 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.56 (s, 1H), 8.03 – 7.84 (m, 7H), 7.82 – 7.68 (m, 2H), 7.66 – 7.56 (m, 2H), 7.14 (d, *J* = 5.1 Hz, 1H), 5.27 (s, 1H), 2.89 (s, 1H), 2.56 (q, *J* = 7.1 Hz, 2H), 1.08 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 159.1, 158.8, 144.8, 142.7, 139.8, 138.8, 133.9, 132.9, 132.8, 130.1, 127.7, 127.6, 126.1, 119.5, 118.7, 118.2, 111.4, 110.6, 102.2, 62.9, 41.7, 15.1. HRMS (ESI): calcd for $C_{27}H_{22}ClN_6$ $[M + H]^+$, 465.1589, found, 465.1592. Purity: 98.33%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(propylamino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M28**). Yield 65%, white solid, mp: 154 – 156 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.17 (s, 1H), 8.52 (d, *J* = 5.0 Hz, 1H), 8.06 – 7.97 (m, 2H), 7.96 – 7.91 (m, 2H), 7.91 – 7.84 (m, 2H), 7.84 – 7.75 (m, 3H), 7.66 – 7.56 (m, 2H), 7.15 (d, *J* = 5.1 Hz, 1H), 5.38 (s, 1H), 2.67 – 2.51 (m, 2H), 1.72 – 1.47 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 172.1, 160.5, 159.6, 145.7, 144.3, 141.0, 140.5, 135.3, 133.67, 133.65, 131.2, 128.8, 128.7, 126.9, 119.9, 119.4, 119.2, 112.6, 112.4, 104.5, 64.3, 50.7, 24.0, 12.1. HRMS (ESI): calcd for $C_{28}H_{24}ClN_6$ $[M + H]^+$, 479.1745, found, 479.1750. Purity: 96.26%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(isopropylamino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M29**). Yield 67%, white solid, mp: 119 – 121 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.17 (s, 1H), 8.51 (d, *J* = 5.1 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.96 – 7.92 (m, 2H), 7.90 – 7.82 (m, 3H), 7.81 – 7.74 (m, 2H), 7.68 – 7.56 (m, 2H), 7.17 (d, *J* = 5.0 Hz, 1H), 5.52 (s, 1H), 2.91 – 2.81 (m, 1H), 1.15 – 1.10 (m, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 172.3, 160.5, 159.6, 145.7, 144.3, 141.3, 140.5, 135.2, 133.7, 131.3, 128.8, 128.7, 126.9, 119.9, 119.4, 119.3, 119.2, 112.6, 112.4, 104.5, 61.6, 47.3, 23.7, 23.1. HRMS (ESI): calcd for $C_{28}H_{24}ClN_6$ $[M + H]^+$, 479.1745, found, 479.1743. Purity: 96.38%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(cyclopropylamino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M30**). Yield 33%, white solid, mp: 166 – 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H), 8.22 – 7.76 (m, 8H), 7.74 – 7.51 (m, 3H), 7.15 (d, *J* = 5.0 Hz, 1H), 5.33 (d, *J* = 8.0 Hz, 1H), 3.65 (s,

1H), 2.13 – 1.97 (m, 1H), 0.59 – 0.19 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 159.1, 158.7, 144.8, 142.7, 139.9, 138.7, 133.9, 132.9, 132.8, 130.1, 127.7, 127.6, 126.0, 119.5, 118.7, 118.2, 111.6, 110.6, 102.2, 63.3, 28.9, 6.6, 6.4. HRMS (ESI): calcd for $C_{28}H_{22}ClN_6$ $[M + H]^+$, 477.1589, found, 477.1581. Purity: 98.11%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)((2,2,2-trifluoroethyl)amino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M31**). Yield 37%, white solid, mp: 170 – 172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.58 (d, J = 5.1 Hz, 1H), 8.04 – 7.88 (m, 5H), 7.88 – 7.81 (m, 3H), 7.72 (d, J = 8.2 Hz, 1H), 7.67 – 7.57 (m, 2H), 7.15 (d, J = 5.1 Hz, 1H), 5.39 (d, J = 8.9 Hz, 1H), 4.11 – 3.69 (m, 1H), 3.45 – 3.34 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.9, 159.11, 159.07, 144.7, 142.6, 139.1, 139.0, 133.9, 132.9, 132.8, 130.0, 127.8, 127.7, 126.2, 126.0 (q, J = 278.6 Hz), 119.5, 118.7, 118.3, 111.4, 110.7, 102.3, 62.9, 47.7 (q, J = 30.8 Hz). ^{19}F NMR (376 MHz, DMSO-d_6) δ: -70.5. HRMS (ESI): calcd for $C_{27}H_{19}ClF_3N_6$ $[M + H]^+$, 519.1306, found, 519.1306. Purity: 99.31%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)((2-fluoroethyl)amino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M32**). Yield 25%, white solid, mp: 198 – 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.56 (d, J = 5.1 Hz, 1H), 8.09 – 7.92 (m, 4H), 7.92 – 7.79 (m, 4H), 7.72 (d, J = 8.2 Hz, 1H), 7.68 – 7.59 (m, 2H), 7.14 (d, J = 5.1 Hz, 1H), 5.32 (d, J = 7.7 Hz, 1H), 4.53 (dt, J = 47.6, 5.1 Hz, 2H), 3.19 – 3.12 (m, 1H), 3.00 – 2.76 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.5, 159.1, 158.9, 144.8, 142. 7, 139.4, 138.9, 133.9, 132.9, 132.8, 130.1, 127.7, 126.1, 119.5, 118.7, 118.3, 111.4, 110.6, 102.3, 83.6 (d, J = 164.0 Hz), 62.9, 47.3 (d, J = 19.8 Hz). ^{19}F NMR (376 MHz, DMSO-d_6) δ: -220.4. HRMS (ESI): calcd for $C_{27}H_{21}ClFN_6$ $[M + H]^+$, 483.1495, found, 483.1487. Purity: 97.57%.

3'-chloro-4'-(((2-cyanoethyl)amino)(2-((4-cyanophenyl)amino)pyrimidin-4-yl)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M33**). Yield 21%, white solid, mp: 173 – 175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.58 (d, J = 5.0 Hz, 1H), 8.15 – 7.79 (m, 8H), 7.73 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 5.1 Hz, 1H), 5.31 (d, J = 7.8 Hz, 1H), 3.31 – 3.25 (m, 1H), 3.07 – 2.73 (m, 2H), 2.68 (t, J = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.4, 159.1, 159.0, 144.8, 142.7, 139.3,

139.0, 133.9, 132.9, 132.8, 130.1, 127.7, 126.1, 120.0, 119.5, 118.7, 118.3, 111.4, 110.7, 102.3, 62.6, 43.1, 18.1. HRMS (ESI): calcd for $C_{28}H_{21}ClN_7$ $[M + H]^+$, 490.1541, found, 490.1536. Purity: 97.93%.

3'-chloro-4'-(((2-chloroethyl)amino)(2-((4-cyanophenyl)amino)pyrimidin-4-yl)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M34**). Yield 20%, white solid, mp: 166 – 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.57 (d, J = 5.1 Hz, 1H), 8.03 – 7.92 (m, 4H), 7.92 – 7.79 (m, 4H), 7.72 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 5.0 Hz, 1H), 5.33 (d, J = 7.0 Hz, 1H), 3.72 (t, J = 6.2 Hz, 2H), 3.28 – 3.10 (m, 1H), 3.04 – 2.81 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.4, 159.1, 158.9, 144.8, 142.6, 139.3, 139.0, 133.9, 132.9, 132.8, 130.1, 127.74, 127.71, 126.1, 119.5, 118.7, 118.3, 111.4, 110.7, 102.3, 62.6, 49.0, 44.5. HRMS (ESI): calcd for $C_{27}H_{21}Cl_2N_6$ $[M + H]^+$, 499.1199, found, 499.1201. Purity: 98.30%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)((2-hydroxyethyl)amino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M35**). Yield 23%, white solid, mp: 142 – 144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.00 – 7.77 (m, 8H), 7.77 – 7.56 (m, 3H), 7.12 (d, J = 5.1 Hz, 1H), 5.28 (d, J = 5.3 Hz, 1H), 4.59 (t, J = 5.3 Hz, 1H), 3.81 – 3.44 (m, 2H), 2.91 (s, 1H), 2.74 – 2.56 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.7, 159.1, 158.8, 144.8, 142.7, 139.6, 138.9, 133.9, 132.9, 132.8, 130.1, 127.70, 127.68, 126.1, 119.5, 118.7, 118.3, 111.4, 110.6, 102.3, 63.0, 60.4, 49.8. HRMS (ESI): calcd for $C_{27}H_{22}ClN_6O$ $[M + H]^+$, 481.1538, found, 481.1536. Purity: 97.20%.

3'-chloro-4'-(((3-chloropropyl)amino)(2-((4-cyanophenyl)amino)pyrimidin-4-yl)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M36**). Yield 35%, white solid, mp: 147 – 149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.56 (d, J = 5.0 Hz, 1H), 8.04 – 7.77 (m, 8H), 7.72 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 5.0 Hz, 1H), 5.25 (s, 1H), 3.75 (t, J = 6.4 Hz, 2H), 3.02 (s, 1H), 2.66 (t, J = 6.6 Hz, 2H), 2.06 – 1.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.8, 159.1, 158.9, 144.8, 142.7, 139.6, 138.9, 133.9, 132.9, 132.8, 130.1, 127.7, 126.1, 119.5, 118.7, 118.3, 111.4, 110.6, 102.3, 63.1, 44.4, 43.5, 32.4. HRMS (ESI): calcd for $C_{28}H_{23}Cl_2N_6$ $[M + H]^+$, 513.1356, found, 513.1360. Purity: 95.02%.

3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)((3-hydroxypropyl)amino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M37**). Yield 40%, white solid, mp: 112 – 113 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ: 8.45 (d, J = 5.1 Hz, 1H), 7.90 – 7.83 (m, 2H), 7.83 – 7.77 (m, 5H), 7.75 – 7.65 (m, 2H), 7.61 – 7.53 (m, 2H), 6.99 (d, J = 5.1 Hz, 1H), 5.48 (s, 1H), 4.05 – 3.58 (m, 2H), 2.99 – 2.65 (m, 2H), 2.18 – 1.76 (m, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 171.0, 161.0, 159.9, 146.1, 144.8, 141.6, 139.7, 135.9, 134.0, 133.9, 131.2, 129.3, 128.9, 127.3, 120.4, 119.8, 119.6, 112.9, 112.7, 104.7, 63.9, 61.7, 46.5, 32.9. HRMS (ESI): calcd for $C_{28}H_{24}ClN_6O$ $[M + H]^+$, 495.1695, found, 495.1697. Purity: 97.20%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylimino)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M38**). Yield 64%, white solid, mp: 259 – 261 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 8.68 (d, J = 5.1 Hz, 1H), 8.09 – 7.96 (m, 4H), 7.86 (d, J = 1.9 Hz, 1H), 7.77 (dd, J = 8.0, 1.9 Hz, 1H), 7.61 (d, J = 5.1 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.34 – 7.21 (m, 3H), 3.29 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.4, 163.4, 159.4, 159.1, 144.7, 144.1, 138.2, 136.2, 136.0, 133.0, 132.4, 128.5, 128.3, 127.5, 124.6, 119.3, 118.9, 118.0, 110.3, 109.2, 102.1, 41.5, 19.1. HRMS (ESI): calcd for $C_{27}H_{21}N_6$ $[M + H]^+$, 429.1822, found, 429.1824. Purity: 97.03%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylimino)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M39**). Yield 38%, pale yellow solid, mp: 234 – 236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.65 (d, J = 5.1 Hz, 1H), 8.11 – 8.06 (m, 2H), 8.02 – 7.98 (m, 2H), 7.59 – 7.50 (m, 5H), 7.32 – 7.24 (m, 3H), 3.77 (s, 3H), 3.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.9, 163.8, 159.1, 156.9, 144.7, 144.2, 140.2, 132.9, 132.4, 129.7, 128.1, 127.8, 124.5, 119.3, 118.8, 118.0, 110.4, 110.2, 109.2, 102.0, 55.9, 41.7. HRMS (ESI): calcd for $C_{27}H_{21}N_6O$ $[M + H]^+$, 445.1771, found, 445.1775. Purity: 96.61%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylimino)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M40**). Yield 54%, white solid, mp: 214 – 215 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.19 (s, 1H), 8.69 (d, J = 5.1 Hz, 1H), 8.40 – 8.23 (m, 2H), 8.15 (d, J = 8.3 Hz, 2H), 8.03 (d, J = 8.3 Hz, 2H), 7.69 – 7.54 (m, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H), 3.29 (s, 3H). ^{13}C NMR (100

MHz, DMSO-d_6) δ: 165.7, 163.2, 159.4, 158.9, 144.5, 142.3, 139.1, 134.5, 133.1, 132.3, 131.2, 130.7, 128.0, 127.7 (q, J = 30.6 Hz), 124.8 (q, J = 4.4 Hz), 123.6 (q, J = 274.1 Hz), 119.1, 118.6, 118.0, 111.2, 108.8, 102.2, 42.1.^{19}F NMR (376 MHz, DMSO-d_6) δ: -59.4. HRMS (ESI): calcd for $C_{27}H_{18}F_3N_6$ $[M + H]^+$, 483.1540, found, 483.1546. Purity: 95.02%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylimino)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M41**). Yield 37%, white solid, mp: 217 – 219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.20 (s, 1H), 8.69 (d, J = 5.1 Hz, 1H), 8.12 – 7.95 (m, 6H), 7.65 – 7.59 (m, 2H), 7.53 – 7.44 (m, 2H), 7.31 – 7.25 (m, 2H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.4, 162.9, 159.5, 159.0, 145.9 (q, J = 1.8 Hz), 144.5, 142.3, 140.9, 133.1, 132.4, 131.4, 128.8, 128.0, 126.3, 119.9 (q, J = 258.0 Hz), 119.7, 119.2, 118.7, 118.1, 111.2, 108.9, 102.2, 42.0. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.1. HRMS (ESI): calcd for $C_{27}H_{18}F_3N_6O$ $[M + H]^+$, 499.1489, found, 499.1490. Purity: 96.82%.

(*E*)-3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(methylimino)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M42**). Yield 80%, white solid, mp: 258 – 260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.20 (s, 1H), 8.69 (d, J = 5.1 Hz, 1H), 8.13 – 8.04 (m, 3H), 8.02 – 7.92 (m, 3H), 7.62 (d, J = 5.1 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.34 – 7.23 (m, 2H), 3.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.5, 162.7, 159.5, 159.1, 144.6, 142.5, 140.3, 134.9, 133.1, 132.4, 132.0, 130.4, 127.8, 127.6, 126.0, 119.2, 118.7, 118.1, 111.0, 109.1, 102.2, 41.7. HRMS (ESI): calcd for $C_{26}H_{18}ClN_6$ $[M + H]^+$, 449.1276, found, 449.1279. Purity: 98.69%.

*4.1.2. General procedure for preparation of **M43**–**M49***

The intermediate ketones **7**–**13** (1.0 mmol, 1.0 equiv.) and hydrazine dihydrochloride (10 mmol, 10 equiv.) were mixed in EtOH (30 ml), and then pyridine (25.00 mmol, 25.0 equiv.) was added. The reaction mixture was heated to reflux for 24–48 h, monitored by TLC. Until completion, the reaction was cooled to room temperature and yielded a pale yellow or white precipitate. The precipitate was collected by filtration and washed with water. After drying under vacuum, the obtained

residue was recrystallized with dimethyl sulfoxide/methanol to obtain the target compounds **M43**–**M49**.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3'-methyl-[1,1'-biphenyl]-4-carbonitrile (**M43**). Yield 31%, pale yellow solid, mp: 272 – 273 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.93 (s, 1H), 8.42 (d, *J* = 5.4 Hz, 1H), 8.12 – 7.94 (m, 4H), 7.87 (d, *J* = 1.9 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.45 – 7.36 (m, 3H), 7.28 – 7.20 (m, 3H), 2.13 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 164.3, 159.0, 157.3, 145.1, 144.3, 140.2, 138.3, 138.0, 133.2, 133.0, 132.3, 130.5, 128.8, 127.5, 125.0, 119.4, 118.9, 117.9, 110.2, 107.2, 101.5, 19.1. HRMS (ESI): calcd for $C_{26}H_{20}N_7$ $[M + H]^+$, 430.1775, found, 430.1768. Purity: 99.29%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3'-methoxy-[1,1'-biphenyl]-4-carbonitrile (**M44**).Yield 51%, pale yellow solid, mp: 281 – 283 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.92 (s, 1H), 8.39 (d, *J* = 5.4 Hz, 1H), 8.21 – 7.91 (m, 4H), 7.70 – 7.48 (m, 4H), 7.45 – 7.34 (m, 3H), 7.31 – 7.20 (m, 3H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.5, 159.0, 157.9, 157.0, 145.2, 144.4, 140.1, 137.9, 133.0, 132.3, 131.5, 127.7, 121.7, 119.7, 119.5, 118.9, 117.9, 110.5, 110.4, 107.3, 101.5, 55.7. HRMS (ESI): calcd for $C_{26}H_{20}N_7O$ $[M + H]^+$, 446.1724, found, 446.1716. Purity: 99.32%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonitrile (**M45**).Yield 52%, white solid, mp: 248 – 250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.91 (s, 1H), 8.40 (d, *J* = 5.3 Hz, 1H), 8.34 – 8.22 (m, 2H), 8.20 – 7.96 (m, 4H), 7.65 (s, 2H), 7.58 – 7.34 (m, 4H), 7.23 – 7.14 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.4, 158.8, 157.2, 145.0, 142.7, 139.0, 136.8, 133.2, 133.1, 132.7, 132.2, 131.7, 129.9 (q, *J* = 30.5 Hz), 127.9, 125.1 (q, *J* = 4.9 Hz), 123.7 (q, *J* = 274.4 Hz), 119.3, 118.7, 117.8, 111.1, 106.7, 101.6. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -60.5. HRMS (ESI): calcd for $C_{26}H_{17}F_3N_7$ $[M + H]^+$, 484.1492, found, 484.1489. Purity: 98.45%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbonitrile (**M46**). Yield 71%, white solid, mp: 223 – 225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.93 (s, 1H), 8.41 (d, *J* = 5.4 Hz, 1H),

8.09 – 7.92 (m, 6H), 7.79 (s, 2H), 7.68 – 7.46 (m, 3H), 7.37 (d, *J* = 5.4 Hz, 1H), 7.30 – 7.20 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 158.9, 157.2, 147.3, 145.0, 142.6, 140.5, 134.3, 133.2, 133.1, 132.3, 127.8, 126.8, 126.7, 120.1, 120.0 (q, *J* = 257.8 Hz), 119.4, 118.7, 117.9, 111.0, 106.8, 101.6. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -56.0. HRMS (ESI): calcd for $C_{26}H_{17}F_3N_7O$ $[M + H]^+$, 500.1441, found, 500.1437. Purity: 97.94%.

(*E*)-3'-chloro-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-[1,1'-biphenyl]-4-carbonitrile (**M47**). Yield 64%, pale yellow solid, mp: 299 – 301 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.90 (s, 1H), 8.41 (d, *J* = 5.3 Hz, 1H), 8.16 – 8.05 (m, 3H), 8.05 – 7.99 (m, 2H), 7.97 – 7.90 (m, 1H), 7.63 (s, 2H), 7.57 – 7.48 (m, 2H), 7.46 – 7.36 (m, 2H), 7.30 – 7.19 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.8, 158.9, 157.2, 145.0, 142.7, 140.1, 137.0, 134.3, 133.1, 132.5, 132.2, 127.9, 127.7, 126.4, 119.3, 118.7, 117.9, 110.9, 106.9, 101.6. HRMS (ESI): calcd for $C_{25}H_{17}ClN_7$ $[M + H]^+$, 450.1228, found, 450.1227. Purity: 98.20%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3'-fluoro-[1,1'-biphenyl]-4-carbonitrile (**M48**). Yield 66%, pale yellow solid, mp: 288 – 289 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H), 8.42 (d, *J* = 5.4 Hz, 1H), 8.23 – 7.98 (m, 4H), 7.94 – 7.81 (m, 2H), 7.79 (s, 2H), 7.63 – 7.52 (m, 2H), 7.49 – 7.42 (m, 1H), 7.38 (d, *J* = 5.4 Hz, 1H), 7.32 – 7.22 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.0, 160.4 (d, *J* = 245.1 Hz), 159.0, 157.3, 145.0, 142.9 (d, *J* = 2.1 Hz), 140.8 (d, *J* = 8.1 Hz), 134.1, 133.1, 132.5 (d, *J* = 5.0 Hz), 132.3, 127.7, 123.6 (d, *J* = 2.6 Hz), 120.5 (d, *J* = 18.9 Hz), 119.4, 118.7, 118.0, 114.6 (d, *J* = 23.4 Hz), 110.9, 107.0, 101.7. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -112.0. HRMS (ESI): calcd for $C_{25}H_{17}FN_7$ $[M + H]^+$, 434.1524, found, 434.1515. Purity: 96.22%.

(*E*)-4'-((2-((4-cyanophenyl)amino)pyrimidin-4-yl)(hydrazono)methyl)-3',5'-difluoro-[1,1'-biphenyl]-4-carbonitrile (**M49**). Yield 54%, pale yellow solid, mp: 300 – 301 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.98 (s, 1H), 8.43 (d, *J* = 5.4 Hz, 1H), 8.19 – 7.99 (m, 6H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 5.3 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6, 160.7 (dd, *J* = 246.4, 9.8 Hz), 159.1, 157.4, 145.0, 141.7, 141.6, 133.1, 132.4, 127.9, 127.7, 119.3, 118.6,

117.9, 111.4, 110.7 (d, J = 26.1 Hz), 109.5 (t, J = 23.8 Hz), 106.7, 101.8. ^{19}F NMR (376 MHz, DMSO-d_6) δ: -109.5. HRMS (ESI): calcd for $C_{25}H_{16}F_2N_7$ $[M + H]^+$, 452.1430, found, 452.1434. Purity: 97.72%.

4.2. Other protocols

Other experimental methods were illustrated in Supporting Information.

Declaration of competing interest

The authors declare no competing interests.

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Highlights:

1. A series of novel biphenyl-DAPYs containing CH(NHR), C=NCH$_3$ and C=NNH$_2$ linkers were developed by employing structure-based drug design strategy.
2. **M44** exhibited 5–173 times higher potency against WT and mutant strains (EC_{50} = 5–148 nM) than **3** (EC_{50} = 27–9810 nM).
3. **M44** showed improved cytotoxicity and selectivity (CC_{50} = 54 μM, SI = 10995).
4. **M44** demonstrated favorable metabolic stability in human plasma and human liver microsomes.
5. No acute toxicity was observed at a dose of 2 g/kg.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: