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Graphical Abstract

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Synthesis and biological evaluation of dihydroquinazoline-2-amines as potent non-nucleoside reverse transcriptase inhibitors of wild-type and mutant HIV-1 strains

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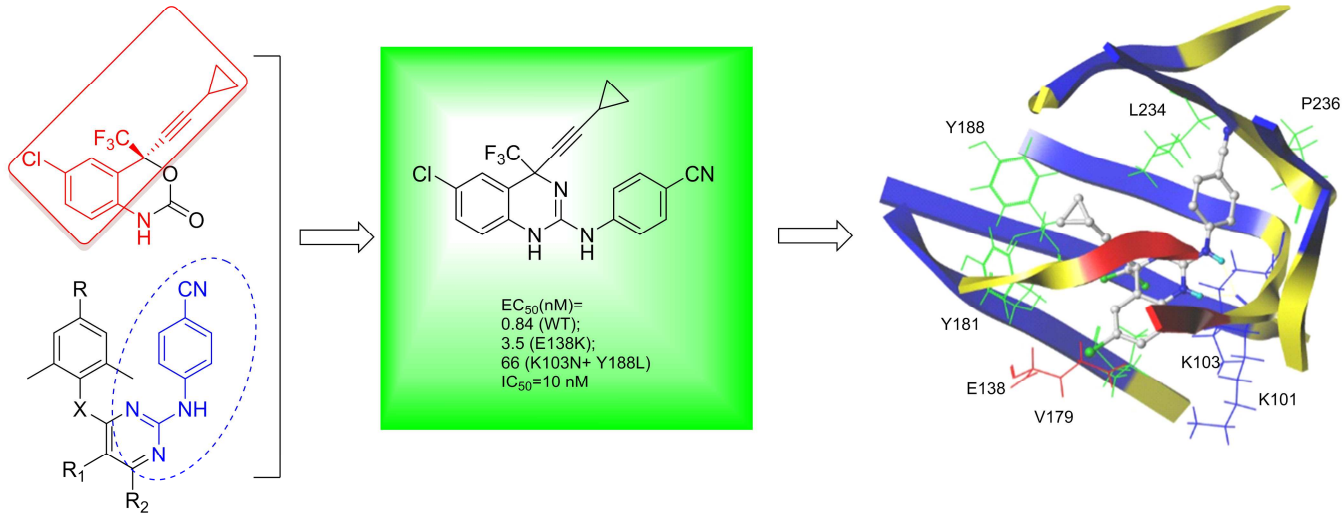

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ABSTRACT

A novel series of dihydroquinazolin-2-amine derivatives were synthesized and evaluated for their anti-HIV-1 activity in MT-4 cell cultures. All of the molecules were active against wild-type HIV-1 with EC_{50} values ranging from 0.61 μM to 0.84 nM. The most potent inhibitor, compound **4b**, had an EC_{50} value of 0.84 nM against HIV-1 strain IIIB, and thus was more active than the reference drugs efavirenz and etravirine. Moreover, most of the compounds maintained high activity (low-micromolar EC_{50} values) against strains bearing the reverse transcriptase (RT) E138K mutation. Compound **4b** had EC_{50} values of 3.5 nM and 66 nM against non-nucleoside reverse transcriptase inhibitor-resistant strains bearing the RT E138K and RES056 mutations. In enzyme activity assays, compound **4b** exhibited an IC_{50} value of 10 nM against HIV-1 RT. Preliminary SARs and molecular docking studies provide valuable insights for further optimization.

1. Introduction

The life cycle of human immunodeficiency virus type 1 (HIV-1) involves reverse transcription of its RNA genome to cDNA followed by integration of DNA into the host cell genome. The HIV-1 reverse transcriptase (RT) is critical for this process and is a major target for antiretroviral therapies [1]. RT inhibitors fall into two main classes: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [2, 3]. Despite their high chemical diversity, most NNRTIs interact with RT in a noncompetitive manner at the same hydrophobic pocket (the NNRTI binding pocket or NNIBP) located about 10 Å from the catalytic site of RT. Due to their high specificity and low toxicity, NNRTIs are key components of highly active antiretroviral therapy(HAART) used to treat HIV infection[4]. Efavirenz (**1**, EFV), the first-generation NNRTI, is widely used in the clinic due to its favorable pharmacological properties and high potency. The activity of EFV relies heavily on formation of ring-stacking interactions with RT K103, and its efficacy is strongly affected by the most common NNRTI resistance-associated mutation, K103N[5]. Etravirine (**2**, ETV) and rilpivirne (**3**, RPV) are second-generation NNRTIs (Figure 1); both are diarylpyrimidine analogues (DAPYs). Although ETV and RPV showed a broad spectrum of activities against wild-type (WT) and mutant HIV-1 strains, resistance mutations still emerged and some adverse effects have been reported[6, 7].

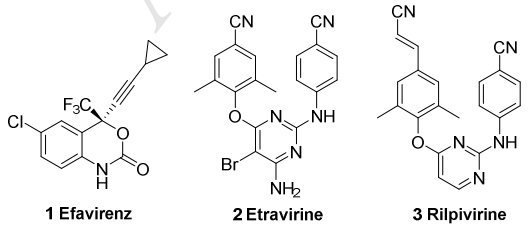


Figure 1. Chemical structures of FDA-approved NNRTIs **1-3**.

To varying degrees, use of NNRTIs results in the emergence of resistance mutations, which has required the continuous development of novel agents active against resistant HIV-1 strains. Therefore, the design and synthesis of NNRTIs with high efficiency using novel skeletons has become an urgent task in drug design.

Molecular hybridization (MH) is an important method in the efficient design of new drugs, and might be of benefit for discovering novel active site or allosteric inhibitors of RT with distinct mechanisms[8, 9]. In our previous modifications of the above NNRTI structures, we used MH successfully to develop several series of NNRTIs, some of which showed potency against the WT HIV-1 strain IIIB at low nanomolar concentrations (1.8nM)[10-13]. Inspired by these results, we made further attempts using this strategy to develop new NNRTIs with novel chemical structures [12, 14, 15].

Analyses of structure-activity relationships (SARs) showed that the cyclopropylacetylene and trifluoromethyl groups are the most important pharmacophores in EFV (**1**), while the 4-cyano aniline group is essential for the biological activities of ETV (**2**) and RPV (**3**) [16-18]. Molecular modeling of EFV and ETV in the binding pocket of HIV-1 RT (**Fig. 2**) suggested that their binding sites overlap and that they might interact with HIV-1 RT in a similar fashion. Therefore, a new series of dihydroquinazoline derivatives (**3a**-**3z**) sharing the structure features of EFV (**1**) and DAPYs was designed and synthesized by fusing the basic skeletons of both **1** and **2**, in the hope that this approach would furnish analogues with high potency and resilience to RT mutations (**Fig.3**). Preliminary SARs and molecular modeling results were used to understand the biological activities of these novel NNRTIs.

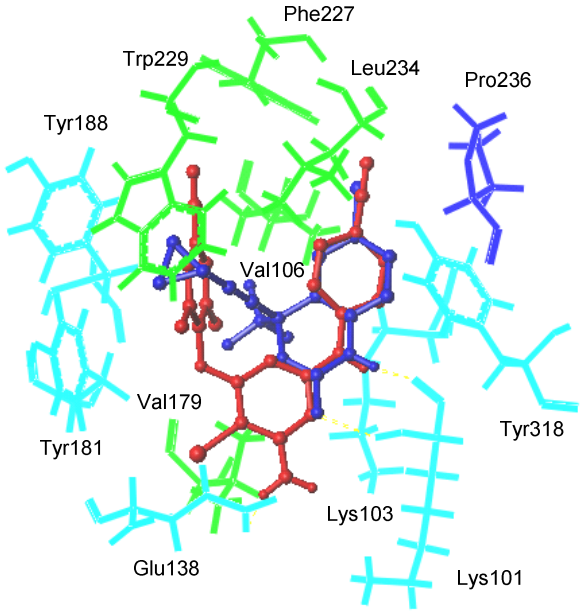


Figure 2. Superposition of lower-energy docking conformations of EFV (blue) and ETV (red) in the binding pocket of HIV-1 reverse transcriptase.

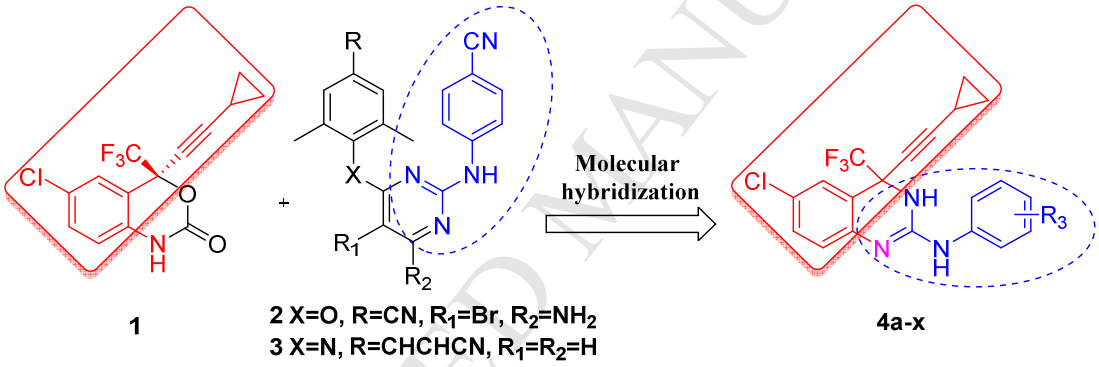
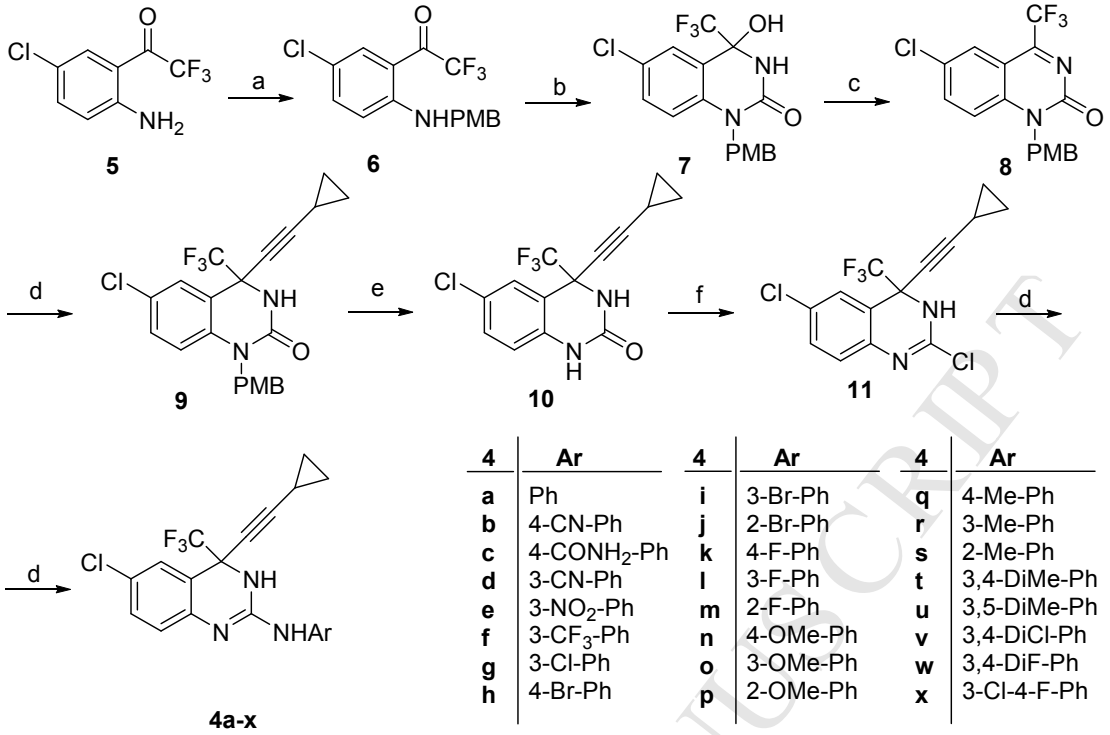


Figure 3. Molecular design strategy of dihydroquinazoline derivatives using molecular hybridization.

2. Results and discussion

2.1. Chemistry

The target compounds designed in the present study all had the general structure shown in Scheme 1. The synthetic route for compounds **4a-x** is also depicted in Scheme 1. Briefly, 1-(2-amino-5-chlorophenyl)-2, 2, 2-trifluoroethan-1 -one (**5**) was converted to intermediate **6** by reaction with 4-methoxy-benzenemethanol at 60 °C for 8h. Subsequently, intermediate **6** was reacted with potassium cyanate at 60°C for 5h to give intermediate **7**, which was then refluxed in xylene for 8 h to give compound **8**. Compound **8** was reacted with cyclopropylacetylene in tetrahydrofuran under *n*-BuLi at -50°C for 1h to give intermediate **9**, then deprotected using ceric ammonium nitrate at room temperature for 4h to give compound **10**. Compound **10** was refluxed in $POCl_3$ for 9 h to give the key intermediate **11**[19-21]. Finally, intermediate**11**was treated with the indicated anilines **12** under reflux in *n*-butanol for 5–8 h to give the desired target compounds **4a-x** with 65–80% yield [22].



Scheme 1. Reagents and conditions: (a) 4-methoxy-benzenemethanol, *p*-Toluenesulfonic acid, acetonitrile, 60°C, 8 h, 80%; (b) Potassium cyanate, AcOH, H_2O, 60°C, 5 h, 68%; (c) Xylene, reflux, 8h, 60%; (d) Cyclopropylacetylene, *n*-BuLi, tetrahydrofuran, -50°C, 1 h, 65%; (e) ceric ammonium nitrate, acetonitrile, H_2O, room temperature, 4 h, 45%; (f) $POCl_3$, reflux, 9 h, 40%; (g) $ArNH_2$ (**12**), *n*-BuOH, reflux, 5–8 h, 65–80%.

2.2. *In vitro* anti-HIV-1 activity and cytotoxicity

The newly synthesized compounds were evaluated for their anti-HIV-1 activities in MT-4 cell cultures infected with one of three HIV-1 strains: (i) the WT strain IIIB; (ii) the single RT mutant E138K; or (iii) the double RT mutant K103N + Y181C (RES056).The cytotoxicity of the compounds was also assessed using the MTT method. The results were expressed as EC_{50} values (anti-HIV-1 activity), CC_{50} values (cytotoxicity) and SI values (selectivity index, given by the CC_{50}/EC_{50} ratio). The FDA-approved drugs nevirapine (NVP), delavirdine (DLV), EFV and ETV were used as references (Table **1**).

As shown in Table **1**, the newly synthesized compounds **4a-x** exhibited moderate to excellent potency against WT HIV-1 with EC_{50} values ranging from 0.61 to 8.4 × 10^{-4} μM. Five compounds showed a low nanomolar EC_{50}: compound **4f** (EC_{50} = 51 nM), compound **4j** (EC_{50} = 88 nM), compound **4o** (EC_{50} = 63 nM), compound **4v** (EC_{50} = 30 nM), and the most active derivative, compound **4b** (EC_{50} = 0.84 nM). Compound **4b (**EC_{50} = 0.84 nM**)** was about nine times more active than ETV (EC_{50} = 6.4 nM) andtwice as active as EFV (EC_{50} = 1.6 nM). Interestingly, all of the hybrid derivatives showed high activity against the single RT mutantE138K, with EC_{50} values in the low-micromolar to single-digit nanomolar range (3.4μM to 3.5 nM). Notably, compound **4b** displayed outstanding potency against the E138K mutant strain (EC_{50} = 3.5 nM); its potency was about twice that of ETV (EC_{50} = 6.3 nM) and nearly reached that of EFV (EC_{50} =2 nM). This finding might be useful as a guide for drug discovery to overcome drug resistance associated with the E138K mutation. Unfortunately, except for compound **4b** (EC_{50} = 66nM), none of the new hybrid compounds were active at sub-cytotoxic concentrations against the double mutant strain RES056.

Table 1

Anti-HIV-1 activity and cytotoxicity of the derivative compounds **4a-x** in MT-4 cells[a].

Compounds	EC_{50}(μM)[b]			CC_{50}(μM)[c]	SI (□B)[d]
	□B	E138K	RES056		
4a	0.11 ± 0.05	0.38 ± 0.03	> 2.4	2.4	21
4b	8.4×10^{-4} ± 6.0×10^{-5}	3.5×10^{-3} ± 1.0×10^{-4}	6.6×10^{-2} ± 4.0×10^{-4}	1.9	2304
4c	0.22 ± 0.11	0.16 ± 0.05	> 2.9	12	58
4d	0.44 ± 0.06	0.65 ± 0.03	> 2.2	2.2	5
4e	0.12 ± 0.06	0.36 ± 0.05	> 1.6	1.6	14
4f	5.1×10^{-2} ± 2.1×10^{-2}	0.35 ± 0.02	> 3.2	3.3	64
4g	0.21 ± 0.10	0.48 ± 0.09	> 2.0	2.0	9
4h	0.45 ± 0.04	>3.4	> 3.4	>3.4	8
4i	0.15 ± 0.06	0.42 ± 0.03	> 2.1	2.1	14
4j	8.8×10^{-2} ± 2.4×10^{-2}	0.16 ± 0.01	1.8 ± 0.28	11	133
4k	0.25 ± 0.04	0.46 ± 0.06	> 2.3	2.4	9
4l	0.16 ± 0.06	0.34 ± 0.02	> 2.0	2.0	12
4m	0.10 ± 0.04	0.14 ± 0.04	> 3.1	3.1	31
4n	0.26 ± 0.09	0.41 ± 0.01	> 8.4	8.4	32
4o	6.3×10^{-2} ± 2.3×10^{-2}	0.29 ± 0.00	> 2.6	2.7	44
4p	0.15 ± 0.05	0.19 ± 0.02	> 8.6	8.6	58
4q	0.25 ± 0.13	0.40 ± 0.06	> 4.9	5.0	20
4r	0.21 ± 0.10	0.36 ± 0.05	> 2.1	2.1	10
4s	0.14 ± 0.03	0.40 ± 0.13	> 4.1	4.1	30
4t	0.61 ± 0.12	0.56 ± 0.06	> 5.8	5.9	8
4u	0.36 ± 0.03	0.60 ± 0.14	> 2.1	2.1	6
4v	3.0×10^{-2} ± 1.9×10^{-2}	0.17 ± 0.01	>2.1	2.1	70
4w	0.36 ± 0.03	>1.8	> 1.8	1.8	5
4x	0.19 ± 0.10	0.46 ± 0.01	> 1.8	1.9	10
EFV	1.6×10^{-3}± 6.0×10^{-4}	2.0×10^{-3}± 6.0×10^{-4}	5.5×10^{-2} ± 2.0×10^{-2}	>2.0	>1269
ETV	2.2×10^{-3}± 5.0×10^{-4}	6.3×10^{-3} ± 4.3×10^{-3}	1.5×10^{-2} ± 9.0×10^{-4}	>2.0	>923

[a] All data represent the mean values from three independent experiments.

[b]EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV-1-induced cytotoxicity, presented as the mean ± standard deviation (SD), determined by the MTT method.

[c] CC_{50}: concentration required to reduce the viability of mock-infected cell cultures by 50%, as determined by the MTT method.

[d] SI: selectivity index, ratio of CC_{50}/EC_{50} (WT).

Preliminary SAR analyses revealed that the nature and position of aniline substitution of the hybrids compounds **4** plays an important role in modulating their anti-HIV-1activity.Comparedwith

the unsubstituted compound **4a** (EC_{50} = 0.11 μM), it is more favorable when introducing bromine atom in the *ortho* position of the benzene ring (**4j** *vs* **4m**, **4p**, **4s**); In the *meta* position of the benzene ring, trifluoromethyl is more favorable than other substitutions (**4f** *vs* **4d**, **4e**, **4g**, **4i**, **4l**, **4o** and **4r**). Surprisingly, a nitrile group in the *para*-position of the benzene ring to obtain the most active compound **4b**, indicating that the scaffold of4-cyano-aniline is very important for enhancing the potency of NNRTIs; this result is consistent with the previous studies of NNRTIs. In the case of di-substitution *vs* mono-substitution of the phenyl ring, although the 3,4-dimethylsubstituted compound **4t** (EC_{50} = 0.61 μM) and 3,5-dimethyl-substituted compound **4u** (EC_{50} = 0.36μM) showed no enhanced in activity compared withthe4-Me-substituted compound **4q** (EC_{50} = 0.25 μM), the 3-Me-substituted compound **4r** (EC_{50} = 0.21 μM) or the 2-Me-substituted compound **4s** (EC_{50} = 0.14μM). However, the 3,4-dichlorosubstituted compound **4v** (EC_{50} = 30nM) was more potent than the 3-Cl-substituted compound **4g** (EC_{50} = 21 μM). It appeared that the potencies of *ortho*-substituted derivatives (compounds **4j**, **4m** and **4s**) were higher than *meta*-substituted derivatives (**4i**, **4l** and **4r**) and the *para*-substituted congeners (**4h**, **4k** and **4q**). In addition, compounds **4f** (EC_{50} = 51 nM) and **4o** (EC_{50}= 63nM) had opposite electrical properties, but their activities were almost the same, this shows that liposolubility and water solubility of the substituents had a great influence on the activity besides steric hindrance and electrical properties. These results and SAR conclusions are consistent with our original hypothesis in designing the target molecules.

2.3. HIV-1 RT inhibition assay

In order to evaluate their effects on HIV-1 RT, compounds **4b**,**4f**, **4j**, **4o** and **4v** were tested in enzyme activity assays using purified recombinant HIV-1 RT. Poly(A)•oligo$(dT)_{15}$ was used as the template primer for reverse transcription (RT kit, Roche), and NVP and EFV were used as the reference drugs in this assay (Table **2**). All five compounds displayed inhibitory activity against RT at low micromolar concentrations (0.448–0.010 μM), and compounds **4b** (IC_{50} = 10 nM) and **4o** (IC_{50} = 0.11 μM) showed superior inhibition compared with NVP (IC_{50} = 0.19 μM). Notably, compound**4a** showed the excellent inhibition of HIV-1 RT (IC_{50} = 10 nM) and was nearly equipotent to EFV (IC_{50} = 6nM). These results suggested that the target compounds could potently inhibit the activity of WT HIV-1 RT, and belong to a new group of HIV-1 NNRTIs.

Table 2 Inhibitory activity of representative dihydroquinazoline-2-amines against WT HIV-1 reverse transcriptase[a].

Compounds	IC_{50}[b](μM)
4b	$1.0\times 10^{-2} \pm 2.0 \times 10^{-3}$
4f	$0.45 \pm 1.3 \times 10^{-2}$
4j	$0.34 \pm 5.1 \times 10^{-2}$
4o	$0.11 \pm 2.5 \times 10^{-2}$
4v	$0.37 \pm 7.1 \times 10^{-2}$
NVP	$0.19 \pm 4.2 \times 10^{-2}$
EFV	$6.0 \times 10^{-3} \pm 2.0 \times 10^{-3}$

[a] Data represent the mean values of two independent experiments.

[b] IC_{50}: inhibitory concentration of test compound required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation intoHIV-1 (WT) RT by 50%, presented as the mean ± standard deviation (SD).

2.4. Molecular modeling analysis

To better understand the binding modes of the newly synthesized hybrid compounds and account for the SAR conclusions, compound **4a** was docked with the NNIBP of WT HIV-1 RT (PDB code: 3MEC) using Surflex-Docking Sybyl-X 2.0[23]. The theoretical binding models are shown in Fig.**4**

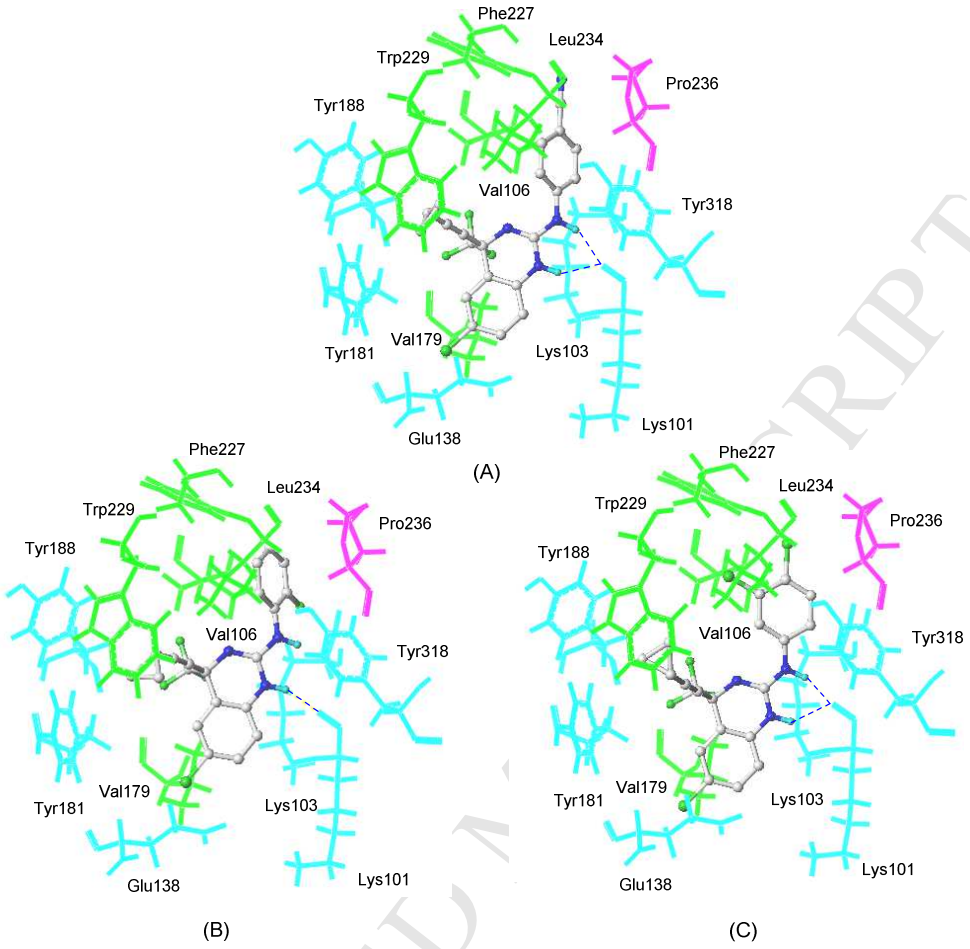


Figure 4. A): The predicted binding modes of compounds **4b** (A), **4m** (B) and **4v** (C) with WT HIV-1 RT (PDB ID: 3MEC).

The model indicated that the representative compound **4b** adopted a similar binding orientation and horseshoe conformation within the NNIBP compared with ETV. The trifluoromethyl and cyclopropyl acetylene groups were accommodated within the aromatic-rich sub-pocket consisting of residues Y181, Y188, F226 and W229, forming weak π-π interaction with these residues. The benzonitrile moiety extended into a solvent-exposed region which was surrounded by residues V106, P236 and Y318; the para-substituted cyan group extended towards L234, forming a dipole-dipole interaction. Moreover, the fused central dihydroquinazoline ring targeted the entrance channel, forming Van der Waals interactions with residues E138 and V179; this might explain the high activity of this compound against strains bearing the E138K mutation. The backbone nitrogen of the dihydroquinazoline ring and the NH linker between the central dihydroquinazoline ring and the benzonitrile ring formed two hydrogen bonds with the backbone carbonyl group of K101, improving the binding affinity between the analogue and RT. Compounds **4m** and **4v** showed similar conformations within the NNIBP as compound **4b**. The chlorine group in compound **4v** also formed dipole-dipole interaction with RT residue L234, while such kind of interaction did not occur in compound **4m**. On the other hand, compound **4m** only formed one hydrogen bonds with Lys101, which might explain the higher activities of compounds **4b** and **4v** compared to compound **4m**.

In summary, the docking analyses revealed the binding modes of the EFV-ETV hybrid compounds to WT HIV-1 RT and provided critical

insights for further optimization of these new lead drugs.

3. Conclusion

In conclusion, to extend the range of NNRTI chemicalstructures and overcomethe issue of resistance, we designed a series of dihydroquinazolin-2-amine derivatives as potent new HIV-1 NNRTIs using MH of the lead compounds EFV and ETV. The results showed that all of the target compounds exhibited high potency against WT HIV-1. Five compounds (**4b**, **4f**, **4j**, **4o** and **4v**) showed excellent activity in the low nanomolar concentration range. Furthermore, most of the compounds maintained high activity against HIV-1 strains bearing the RT mutation E138K. On this basis, compound **4b** might represent a potential drug candidate for further development to address the issue of drug resistance. Additionally, preliminary SARs were analyzed in detail based on the anti-HIV-1 activities of the lead molecules. Molecular modeling studies were carried out to investigate the structure-function relationships between the hybrid compounds and HIV-1 RT. Further optimization of this novel chemical skeleton to improve its biological activity is ongoing in our lab.

4. Experiment section

4.1. Chemistry

Chemical reagents and solvents, purchased from commercial sources, were of analytical grade and were used without further purification. All air-sensitive reactions were run under a nitrogen atmosphere. All the reactions were monitored by TLC on the re-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/n-hexane as the eluent. Flash column chromatography was performed on glass column packed with silica gel (200-300 mesh) using ethyl acetate/n-hexane as the eluent. Melting points were measured on a SGW X-1 microscopic melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400 MHz spectrometer in DMSO-d_6. Chemical shifts were reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra and HRMS were obtained on a Waters Quattro Micromass instrument and Bruker solari X-70 FT-MS instrument, respectively, using electrospray ionization (ESI) techniques. The purities of the target compounds were ≥ 95%, measured by HPLC, performed on an Agilent 1200 HPLC system with UV detector and Agilent Eclipse Plus C18 column (150 ×4.6 mm, 5 mm), eluting with a mixture of solvents H_2O (A) and CH_3CN (B) from VA:VB=90:10 to 10:90. Peaks were detected at λ 254 nm with a flow rate of 1.0 mL/min.

4.1.1 General procedure to prepare the intermediates of **6**, **7**, **8**, **9** and **10**.

The intermediates including **6**, **7**, **8**, **9** and **10** were obtained easily according to the improved procedure published previously [19-21].

4.1.2 Synthesis of 2,6-dichloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazoline (**11**).

A solution of 6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-3,4-dihydroquinazolin-2(1*H*)-one (**10**) (3.14 g, 10 mmol) in $POCl_3$ (10 mL) was refluxed for 10 h. The reaction process was monitored by TLC analysis until its completion. The reaction mixture was then poured into brine (50 mL) after being cooled and stirred for approximately 15 min, filtered and the filter cake was diluted with ethyl acetate (30 mL×3). The organic layer was then dried with anhydrous Na_2SO_4. The mixture was then concentrated under the reduced pressure. The crude product was purified by column chromatography to afford the target compound **11** with the yield of 45%[22].

4.1.3 General procedure for **4a**-**x**.

To a solution of **11** (1.0eq) in *n*-BuOH (5 mL) was added $ArNH_2$ (1.5eq). The resulting mixture was heated at 110 °C for 6-8 h and was then cooled to r.t. and concentrated *in vacuo*. The crude product was purified by column chromatography to afford the target compound **4a**-**x**, with the yield of 60-80%, respectively.

4.1.3.1.6-chloro-4-(cyclopropylethynyl)-*N*-phenyl-

4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4a**)

The title product was synthesized following the general procedure, starting from **11** and aniline (**12a**). Yield 77%, white solid, m.p. 131-133 °C (EA(ethyl acetate)/PE (petroleum ether));^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.12 (s, 2H, N*H*), 7.63 (d, J = 8.6 Hz, 1*H*), 7.56 (s, 1H, Ph*H*), 7.49 (m, 2H, Ph*H*), 7.40-7.33 (m, 4H, Ph*H*), 1.60-1.58 (m, 1H, C*H*), 0.95-0.93 (m, 2H, CH_2), 0.79 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.32(N=*C*-(NH)$_2$), 135.31 (Ar*C*), 132.51 (Ar*C*),132.20 (Ar*C*), 130.27 (Ar*C*, 4C), 129.58 (Ar*C*), 127.67 (Ar*C*), 124.57 (Ar*C*), 123.75 (d, J_{CF} = 287 Hz), 119.89 (Ar*C*), 117.35 (Ar*C*), 94.46 (*C*≡C), 66.64 (*C*≡C), 57.48 (d, J_{CCF}= 34 Hz), 8.90 (*C*H$_2$), 8.85 (*C*H$_2$), -0.71 (*C*C≡C); IR: υ 2842, 2250, 1639, 1546, 1483, 1168 cm^{-1}; HRMS calcd for $C_{20}H_{15}ClF_3N_3$ [M+H]$^+$: 390.0979, found: 390.0973.

4.1.3.2.

4-((6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-yl) amino)benzonitrile (**4b**)

The title product was synthesized following the general procedure, starting from **11** and 4-cyanoaniline (**12b**). Yield 65%, white solid, m.p. 180-182°C (EA/PE);^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.16 (s, 2H, N*H*), 7.73 (s, 4H, Ph*H*), 7.42-7.37(m, 2H, Ph*H*), 7.01 (d, J = 8.2 Hz, 1H, Ph*H*), 1.49 (m, 1H, C*H*), 0.87 (m, 2H, CH_2), 0.69 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 151.19(N=*C*-(NH)$_2$), 137.22 (Ar*C*), 133.61 (Ar*C*), 131.41 (Ar*C*, 2C), 127.58 (Ar*C*), 125.52 (Ar*C*), 123.73 (d, J_{CF} = 286 Hz), 119.91 (Ar*C*), 118.93 (Ar*C*), 118.86 (Ar*C*), 116.78 (2C), 115.43 (Ar*C*), 91.96 (*C*≡C), 68.68 (*C*≡C), 58.79 (d, J_{CCF}= 32 Hz), 8.75 (*C*H$_2$), 8.70 (*C*H$_2$),-0.70 (*C*C≡C); IR: υ 2971, 2226, 1646, 1588, 1485, 1243 cm^{-1}; HRMS calcd for $C_{21}H_{14}ClF_3N_4$ [M+H]$^+$: 415.0932, found:415.0934.

4.1.3.3.

4-((6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-yl) amino)benzamide (**4c**)

The title product was synthesized following the general procedure, starting from **11** and 4-aminobenzamide (**12c**). Yield 60%, white solid, m.p. 178-179 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 11.06 (s, 2H, N*H*), 8.05–7.97 (m, 3H, Ph*H*), 7.64-7.56 (m, 2H, Ph*H*), 7.48–7.41 (m, 3H, Ph*H*), 7.33 (s, 1H, Ph*H*), 1.59 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.79 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d$_6$) δ: 167.47 (*C*=O), 149.00 (N=*C*-(NH)$_2$), 132.14 (Ar*C*), 129.50 (Ar*C*), 127.71 (Ar*C*), 117.53 (Ar*C*), 8.89 (*C*H$_2$), 8.84 (*C*H$_2$), -0.70 (*C*C≡C); IR: υ 3172, 2971, 2249, 1651, 1487, 1170 cm^{-1}; HRMS calcd for $C_{21}H_{16}ClF_3N_4O$ [M+H]$^+$: 433.1037, found: 433.1033.

4.1.3.4.

3-((6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-yl) amino)benzonitrile (**4d**)

The title product was synthesized following the general procedure, starting from **11** and 3-aminobenzonitrile (**12d**). Yield 75%, white solid, m.p. 183-185 °C (EA/PE);^{1}H NMR (400 MHz, DMSO-d_6) δ:11.06 (s, 2H, NH), 7.96 (s, 1H, Ph*H*), 7.76-7.73 (m, 2H, Ph*H*), 7.65 (m,, Ph*H*), 7.55 (s, 1H, Ph*H*), 7.35 (d, J = 8.4 Hz, 1H, Ph*H*), 1.60–1.58 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.80 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.10(N=*C*-(NH)$_2$), 137.20 (Ar*C*), 132.95(Ar*C*), 132.10(Ar*C*), 131.46 (Ar*C*), 130.24 (Ar*C*), 129.53(Ar*C*), 128.88(Ar*C*), 127.67 (Ar*C*), 127.45 (Ar*C*), 123.81 (d, J_{CF} = 286 Hz), 119.97 (Ar*C*), 118.76 (Ar*C*), 117.50 (Ar*C*), 112.86 (Ar*C*), 94.23 (*C*≡C), 67.02 (*C*≡C), 57.78 (d, J_{CCF}= 32 Hz), 8.88 (*C*H$_2$), 8.83 (*C*H$_2$), -0.71(*C*C≡C); IR: υ 3368, 2804, 2253, 1612, 1576, 1164 cm^{-1}; HRMS calcd for $C_{21}H_{14}ClF_3N_4$ [M+H]$^+$: 415.0932, found: 435.0925.

4.1.3.5.6-chloro-4-(cyclopropylethynyl)-*N*-(3-nitrophenyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4e**)

The title product was synthesized following the general procedure, starting from **11** and 3-nitroaniline (**12e**). Yield 66%, white solid, m.p. 183-185 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.11 (s, 2H, N*H*), 8.40 (s, 1H, Ph*H*),

8.11 (d, *J* = 7.6 Hz, 1H, Ph*H*), 7.84 (d, *J* = 7.9 Hz, 1H, Ph*H*), 7.74 (t, *J* = 8.1 Hz, 1H, Ph*H*), 7.62 (d, *J* = 8.6 Hz, 1H, Ph*H*), 7.55 (s, 1H, Ph*H*), 7.33 (d, *J* = 7.5 Hz, 1H, Ph*H*), 1.59 (m, 1H, C*H*), 0.94 (m, 2H, CH_2), 0.79 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 149.09(N=*C*-(NH)$_2$), 148.87(*C*NO$_2$), 147.16(Ar*C*), 137.84 (Ar*C*), 132.04(Ar*C*), 131.40(Ar*C*), 129.25 (Ar*C*), 127.70 (Ar*C*), 123.86 (d, J_{CF} = 286 Hz), 122.38 (Ar*C*), 120.77 (Ar*C*), 119.90 (Ar*C*), 118.43 (Ar*C*), 117.59 (Ar*C*), 93.94 (*C*≡C), 67.35 (*C*≡C), 57.73 (q,J_{CCF} = 21 Hz), 8.87 (*C*H$_2$), 8.81(*C*H$_2$), -0.69 (*C*C≡C); IR: υ 3357, 2906, 2246, 1658, 1538, 1171 cm^{-1}; HRMS calcd for $C_{20}H_{14}ClF_3N_4O_2$ $[M+H]^+$: 435.0830, found: 435.0830.

4.1.3.6.

6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenyl)-1,4-dihydroquinazolin-2-amine (**4f**)

The title product was synthesized following the general procedure, starting from **11** and 3-(trifluoromethyl)aniline (**12f**). Yield 68%, white solid, m.p. 123-125°C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 10.62 (s, 2H, N*H*), 7.75 (d, *J*= 8.2 Hz, 2H, Ph*H*), 7.69 (m, 2H, Ph*H*), 7.57-7.46 (m, 2H, Ph*H*), 7.17 (s, 1H, Ph*H*), 1.61-1.50 (m, 1H, C*H*), 0.92 (m, 2H, CH_2), 0.75 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 148.15(N=*C*-(NH)$_2$), 141.55(Ar*C*), 134.85(Ar*C*), 131.58(Ar*C*), 128.49(Ar*C*), 127.63(Ar*C*), 126.90 (Ar*C*, 2C), 126.03(Ar*C*), 124.16(d, J_{CF} = 286 Hz), 123.88(d, J_{CF} = 232 Hz), 122.72(Ar*C*), 121.86(Ar*C*), 119.94(Ar*C*), 117.51(Ar*C*), 92.75(*C*≡C), 68.71(*C*≡C), 58.47(d, J_{CCF}= 33 Hz), 8.73(*C*H$_2$), 8.68(*C*H$_2$), -0.81(*C*C≡C); IR: υ 2953, 2248, 1651, 1486, 1321, 1164 cm^{-1}; HRMS calcd for $C_{25}H_{20}ClN_5$ $[M+H]^+$: 458.0853, found: 458.0852.

4.1.3.7

6-chloro-*N*-(3-chlorophenyl)-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydro quinazolin-2-amine (**4g**)

The title product was synthesized following the general procedure, starting from **11** and *m*-chloroaniline (**12g**). Yield 72%, white solid, m.p. 196-198 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.18 (s, 2H, N*H*), 7.63 (d, *J* = 8.6 Hz, 1H, Ph*H*), 7.56 (s, 2H, Ph*H*), 7.49 (t, *J* = 8.0 Hz, 1H, Ph*H*), 7.37 (s, 3H, Ph*H*), 1.60 (dd, *J* = 8.4, 3.7 Hz, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.79 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.12 (N=*C*-(NH)$_2$), 137.47 (Ar*C*), 134.23 (Ar*C*), 132.79 (Ar*C*), 132.15 (Ar*C*), 131.73 (Ar*C*), 129.57 (Ar*C*), 127.68 (Ar*C*), 126.72 (Ar*C*), 124.04 (Ar*C*), 123.78 (d, J_{CF}= 286 Hz), 122.77 (Ar*C*), 119.88 (Ar*C*), 117.49 (Ar*C*), 94.32 (*C*≡C), 66.86 (*C*≡C), 57.69 (d, J_{CCF}= 33 Hz), 8.88 (*C*H$_2$), 8.83 (*C*H$_2$), -0.71(*C*C≡C); IR: υ 2809, 2243, 1647, 1589, 1474, 1170 cm^{-1}; HRMS calcd for $C_{20}H_{14}Cl_2F_3N_3$ $[M+H]^+$: 424.0590, found: 424.0588.

4.1.3.8.

N-(4-bromophenyl)-6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4h**)

The title product was synthesized following the general procedure, starting from **11** and 4-bromoanilines (**12h**). Yield 75%, white solid, m.p. 133-135 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.13 (s, 2H, N*H*), 7.69-7.62 (m, 2H, Ph*H*), 7.52 (m, 2H, Ph*H*), 7.45-7.34 (m, 3H, Ph*H*), 1.59 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.80 (m, 2H,CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 148.96(N=*C*-(NH)$_2$), 135.85 (Ar*C*), 133.65 (Ar*C*), 132.86 (Ar*C*, 3C), 132.45(Ar*C*), 131.93(Ar*C*), 129.00 (Ar*C*), 127.67(Ar*C*), 125.81(Ar*C*), 123.93 (d, J_{CF}= 288 Hz), 119.82 (Ar*C*), 117.40 (Ar*C*), 93.67 (*C*≡C), 67.55 (*C*≡C), 57.89 (d, J_{CCF}= 38 Hz), 8.85 (*C*H$_2$), 8.80 (*C*H$_2$),-0.70 (*C*C≡C); IR: υ 2790, 2246, 1645, 1484, 1239, 1167 cm^{-1}; HRMS calcd for $C_{20}H_{14}BrClF_3N_3$ $[M+H]^+$: 468.0084, found: 468.0082.

4.1.3.9.

N-(3-bromophenyl)-6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4i**)

The title product was synthesized following the general procedure, starting from **11** and 3-bromoaniline (**12i**). Yield 70%, white solid, m.p.

193-195 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.12 (s, 2H, N*H*), 7.62 (m, 4H, Ph*H*), 7.37 (m, 3H, Ph*H*), 1.58 (m, 1H, C*H*), 0.94 (m, 2H, CH_2), 0.79 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.14 (N=*C*-(NH)$_2$), 148.96 (Ar*C*), 137.57 (Ar*C*), 132.77 (Ar*C*), 132.07 (Ar*C*, 2C), 129.57 (Ar*C*), 127.68 (Ar*C*), 126.87 (Ar*C*), 123.76 (d, J_{CF}= 287 Hz), 123.19 (Ar*C*), 122.45 (Ar*C*), 119.88 (Ar*C*), 117.47 (Ar*C*), 94.33 (*C*≡C), 66.83 (*C*≡C), 57.67 (d, J_{CCF}= 32 Hz), 8.89 (*C*H$_2$), 8.83 (*C*H$_2$), -0.71 (*C*C≡C); IR: υ 2807, 2243, 1648, 1587, 1473, 1184 cm-1; HRMS calcd for $C_{20}H_{14}BrClF_3N_3$ $[M+H]^+$: 468.0084, found: 468.0079.

4.1.3.10.

N-(2-bromophenyl)-6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,

4-dihydroquinazolin-2-amine (**4j**)

The title product was synthesized following the general procedure, starting from **11** and 4-methylbenzeneboronic acid (**12j**). Yield 74%, white solid, m.p. 215-217 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.85 (s, 2H, N*H*), 7.81 (s, 1H, Ph*H*), 7.58 (m, 4H, Ph*H*), 7.39 (s, 1H, Ph*H*), 7.31 (s, 1H, Ph*H*), 1.58 (m, 1H, C*H*), 0.96 (m, 2H, CH_2), 0.80 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.85 (N=*C*-(NH)$_2$), 145.38 (Ar*C*), 134.03 (Ar*C*), 133.35 (Ar*C*), 132.64 (Ar*C*), 132.31 (Ar*C*), 129.70 (Ar*C*), 128.77 (Ar*C*), 127.75 (Ar*C*), 123.67 (d, J_{CF}= 287 Hz), 119.61 (Ar*C*), 118.53 (Ar*C*), 117.00 (Ar*C*), 116.50 (Ar*C*), 108.50 (*C*≡C), 66.76 (*C*≡C), 57.53 (d, J_{CCF}= 34 Hz), 8.93 (*C*H$_2$), 8.89 (*C*H$_2$), -0.70 (*C*C≡C); IR: υ 2747, 2249, 1659, 1581, 1474, 1167 cm^{-1}; HRMS calcd for $C_{20}H_{14}BrClF_3N_3$ $[M+H]^+$: 468.0084, found: 468.0085.

4.1.3.11.6-chloro-4-(cyclopropylethynyl)-*N*-(4-fluorophenyl)-4-(trifluoromethyl)-1, 4-dihydroquinazolin-2-amine (**4k**)

The title product was synthesized following the general procedure, starting from **11** and 4-fluoroaniline (**12k**). Yield 69%, white solid, m.p. 141-143 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.91 (s, 2H, N*H*), 7.59 (d, *J* = 8.5 Hz, 1H, Ph*H*), 7.49 (m, 3H, Ph*H*), 7.31 (m, 3H, Ph*H*), 1.59-1.57 (m, 1H, C*H*), 0.94 (m, 2H, CH_2), 0.78 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 160.74 (d, *J* = 242 Hz), 149.39 (N=*C*-(NH)$_2$), 133.55 (Ar*C*), 132.22 (Ar*C*), 131.96 (Ar*C*), 128.97 (Ar*C*), 127.65 (Ar*C*), 126.54 (Ar*C*), 123.91 (d, J_{CF}= 287 Hz), 119.81 (Ar*C*), 117.29 (Ar*C*), 116.96 (Ar*C*), 116.73 (Ar*C*), 93.8 (*C*≡C), 67.55 (*C*≡C), 57.74 (d, J_{CCF}= 32 Hz), 55.39 (*C*H$_2$), 8.86 (*C*H$_2$), 8.81 (*C*H$_2$), -0.71 (*C*C≡C); IR: υ 2891, 2249, 1654, 1505, 1482, 1182 cm^{-1}; HRMS calcd for $C_{20}H_{14}ClF_4N_3$ $[M+H]^+$: 408.0885, found: 408.0882.

4.1.3.12.

6-chloro-4-(cyclopropylethynyl)-*N*-(3-fluorophenyl)-4-(trifluoromethyl)-1, 4-dihydroquinazolin-2-amine (**4l**)

The title product was synthesized following the general procedure, starting from **11** and 3-fluoroaniline (**12l**). Yield 76%, white solid, m.p. 145-147 °C (EA/PE);^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.24 (s, 2H, N*H*), 7.63 (d, *J* = 8.3 Hz, 1H, Ph*H*), 7.51 (m, 2H, Ph*H*), 7.48-7.35 (m, 2H, Ph*H*), 7.19 (m, 2H, Ph*H*), 1.59 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.80 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ:162.85 (d, *J* = 242 Hz), 148.48 (N=*C*-(NH)$_2$), 131.58-131.29 (Ar*C*, 4), 128.28 (Ar*C*), 127.95 (Ar*C*), 127.63 (Ar*C*), 124.19 (Ar*C*, d, J_{CF}= 287 Hz), 119.89 (Ar*C*), 118.07 (Ar*C*), 117.45 (Ar*C*), 92.85 (*C*≡C), 68.87 (*C*≡C), 58.40 (d, J_{CCF}= 32 Hz), 8.78 (*C*H$_2$), 8.72 (*C*H$_2$), -0.72 (*C*C≡C); IR: υ 2815, 2245, 1651, 1603, 1481, 1170 cm^{-1}; HRMS calcd for $C_{20}H_{14}ClF_4N_3$ $[M+H]^+$: 408.0885, found: 408.0879.

4.1.3.13.6-chloro-4-(cyclopropylethynyl)-*N*-(2-fluorophenyl)-4-(trifluoromethyl)-1,

4-dihydroquinazolin-2-amine (**4m**)

The title product was synthesized following the general procedure, starting from **11** and 2-fluoroaniline (**12m**). Yield 70%, white solid, m.p. 206-208 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.02 (s, 2H, N*H*), 7.64 (d, *J* = 8.5 Hz, 1H, Ph*H*), 7.56 (s, 2H, Ph*H*), 7.42-7.34 (m, 3H, Ph*H*), 7.32 (t, *J* = 7.3 Hz, 1H, Ph*H*), 1.59-1.57 (m, 1H, CH), 0.94 (m, 2H, CH_2), 0.80 (m, 2H, CH_2);

^{13}C NMR (101 MHz, DMSO-d_6) δ: 156.74 (d, J_{CF}= 248 Hz), 149.89 (N=*C*-(NH)$_2$), 132.32 (Ar*C*, 2C), 129.68 (Ar*C*), 128.43 (Ar*C*), 127.76 (Ar*C*), 125.87 (Ar*C*), 125.63 (d, J_{CF}= 287 Hz), 122.59 (Ar*C*), 119.64 (Ar*C*), 117.34 (Ar*C*), 117.15 (Ar*C*, 2C), 94.56 (*C*≡C), 66.63 (*C*≡C), 57.57 (d, J_{CCF}= 33 Hz), 8.90 (*C*H$_2$), 8.85 (*C*H$_2$), -0.70 (*C*C≡C); IR: υ 2723, 2248, 1648, 1488, 1182, 1166 cm^{-1}; HRMS calcd for $C_{20}H_{14}ClF_4N_3$ $[M+H]^+$: 408.0885, found: 408.0881.

4.1.3.14.

6-chloro-4-(cyclopropylethynyl)-*N*-(4-methoxyphenyl)-4-(trifluoromethyl)-1,

4-dihydroquinazolin-2-amine (**4n**)

The title product was synthesized following the general procedure, starting from **11** and 4-aminoanisole (**12n**). Yield 78%, white solid, m.p. 239-242 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.11 (s, 2H, N*H*), 7.49-7.40 (m, 4H, Ph*H*), 7.12 (d, *J* = 8.0 Hz, 1H, Ph*H*), 6.97 (m, 2H, Ph*H*), 3.77 (s, 3H, OC*H*$_3$), 1.55-1.53 (m, 1H, C*H*), 0.99-0.85 (m, 2H, C*H*$_2$), 0.74 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 157.01 (Ar*C*O), 148.81 (N=*C*-(NH)$_2$), 135.76 (Ar*C*), 131.38 (Ar*C*), 130.16 (Ar*C*), 127.63 (Ar*C*), 127.53 (Ar*C*), 124.44 (Ar*C*), 124.29 (d, J_{CF}= 287 Hz), 122.59 (Ar*C*), 119.85 (Ar*C*), 117.33 (Ar*C*), 114.91 (Ar*C*, 2C), 92.28 (*C*≡C), 69.15 (*C*≡C), 58.37 (d, J_{CCF}=32 Hz), 55.75 (O*C*H$_3$), 8.72 (*C*H$_2$), 8.66 (*C*H$_2$), -0.79 (*C*C≡C); IR: υ 2968, 2244, 1655, 1509, 1245, 1166 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3O$ $[M+H]^+$: 420.1085, found: 420.1086.

4.1.3.15.

6-chloro-4-(cyclopropylethynyl)-*N*-(3-methoxyphenyl)-4-(trifluoromethyl)-1,

4-dihydroquinazolin-2-amine (**4o**)

The title product was synthesized following the general procedure, starting from **11** and 3-methoxyaniline (**12o**). Yield 69%, white solid, m.p. 142-144 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 2H, N*H*), 7.64-7.56 (m, 2H, Ph*H*), 7.38 (m, 2H,Ph*H*), 7.03 (s, 1H, Ph*H*), 6.93 (m, 2H, Ph*H*), 3.79 (s, 3H, OC*H*$_3$), 1.58 (m, 1H, C*H*), 0.99-0.89 (m, 2H, C*H*$_2$), 0.79 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 160.62 (Ar*C*O), 149.21 (N=*C*-(NH)$_2$), 136.51 (Ar*C*), 132.65 (Ar*C*), 132.17 (Ar*C*), 131.02 (Ar*C*), 129.50 (Ar*C*), 127.66 (Ar*C*), 123.76 (d, J_{CF}= 287 Hz), 119.89 (Ar*C*), 117.35 (Ar*C*), 116.40 (Ar*C*), 113.11 (Ar*C*), 110.01 (Ar*C*), 94.38 (*C*≡C), 66.67 (*C*≡C), 57.53 (q, J_{CCF}= 34 Hz), 55.78 (O*C*H$_3$), 8.89 (*C*H$_2$), 8.84 (*C*H$_2$), -0.71 (*C*C≡C); IR: υ 2829, 2248, 1652, 1607, 1488, 1157 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3O$ $[M+H]^+$: 420.1085, found: 420.1090.

4.1.3.16.6-chloro-4-(cyclopropylethynyl)-*N*-(2-methoxyphenyl)-4-(trifluoromethyl)-1,

4-dihydroquinazolin-2-amine (**4p**)

The title product was synthesized following the general procedure, starting from **11** and 2-methoxyaniline (**12p**). Yield 72%, white solid, m.p. 225-227 °C (EA/PE);^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.81 (s, 1H, N*H*), 10.74 (s, 2H, Ph*H*), 7.63 (d, *J* = 8.5 Hz, 1H, Ph*H*), 7.55-7.50 (m, 1H, Ph*H*), 7.40 (m, 3H, Ph*H*), 7.21 (d, *J* = 8.2 Hz, 1H, Ph*H*), 7.06 (t, *J* = 7.5 Hz, 1H, Ph*H*), 3.80 (s, 3H, OC*H*$_3$), 1.58 (m, 1H, C*H*), 0.94 (m, 2H, C*H*$_2$), 0.79 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 154.10 (Ar*C*O), 149.89 (N=*C*-(NH)$_2$), 132.33 (Ar*C*), 129.91 (Ar*C*), 129.38 (Ar*C*), 127.75 (Ar*C*), 123.65 (d, J_{CF}= 286 Hz), 122.40 (Ar*C*), 121.42 (Ar*C*), 119.36 (Ar*C*), 116.91 (Ar*C*), 113.15 (Ar*C*), 94.43 (*C*≡C), 66.71 (*C*≡C), 57.42 (q, J_{CCF}= 29 Hz), 56.29 (O*C*H$_3$), 8.90 (*C*H$_2$), 8.85 (*C*H$_2$), -0.71 (*C*C≡C); IR: υ 2790, 2249, 1666, 1491, 1182, 1167 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3O$ $[M+H]^+$: 420.1085, found: 420.1087.

4.1.3.17.

6-chloro-4-(cyclopropylethynyl)-*N*-(*p*-tolyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4q**)

The title product was synthesized following the general procedure, starting from **11** and 4-toluidine (**12q**). Yield 76%, white solid, m.p. 173-175 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.88 (s, 2H, N*H*), 7.62 (d, J = 8.6 Hz, 1H, Ph*H*), 7.55 (s, 1H, Ph*H*), 7.34-7.26 (m, 5H, Ph*H*), 2.35 (s, 3H, C*H*$_3$), 1.60-1.58 (m, 1H, C*H*), 0.95 (m, 2H, C*H*$_2$), 0.80 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 149.22 (N=*C*-(NH)$_2$),

137.21 (Ar*C*), 132.39 (Ar*C*), 132.22 (Ar*C*), 130.78 (Ar*C*, 3C), 129.65 (Ar*C*), 127.66 (Ar*C*), 124.85 (Ar*C*, 2C), 123.72 (d, J_{CF}= 286 Hz), 119.76 (Ar*C*), 117.39 (Ar*C*), 94.63 (*C*≡C), 66.43 (*C*≡C), 57.48 (q, J_{CCF}= 33 Hz), 21.01 (*C*H_3), 8.87 (*C*H_2), 8.81 (*C*H_2), -0.79 (*C*C≡C); IR: υ 2830, 2248, 1654, 1490, 1186, 1166 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3$ $[M+H]^+$: 404.1136, found: 404.1139.

4.1.3.18.

6-chloro-4-(cyclopropylethynyl)-*N*-(*m*-tolyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4r**)

The title product was synthesized following the general procedure, starting from **11** and *m*-toluidine (**12r**). Yield 73%, white solid, m.p. 182-184 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.08 (s, 2H, N*H*), 7.63 (dd, *J* = 8.6, 1.9 Hz, 1H, Ph*H*), 7.56 (s, 1H, Ph*H*), 7.38 (m, 2H, Ph*H*), 7.19 (d, *J*= 6.6 Hz, 3H, Ph*H*), 2.35 (s, 3H, C*H*$_3$), 1.60-1.58 (m, 1H, C*H*), 0.95 (m, 2H, C*H*$_2$), 0.80 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 149.35(N=*C*-(NH)$_2$), 139.81 (Ar*C*), 135.11(Ar*C*), 132.51 (Ar*C*), 132.21 (Ar*C*), 130.10 (Ar*C*, 2C), 129.54 (Ar*C*), 128.09 (Ar*C*), 127.67 (Ar*C*), 123.75 (d, J_{CF}= 286 Hz), 121.71 (Ar*C*), 119.90 (Ar*C*), 117.30 (Ar*C*), 94.44 (*C*≡C), 66.67 (*C*≡C), 57.44 (q, J_{CCF}= 33 Hz), 21.41 (*C*H_3), 8.91 (*C*H_2), 8.86 (*C*H_2), -0.71 (*C*C≡C); IR: υ 2818, 2245, 1652, 1479, 1184, 1166 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3$ $[M+H]^+$: 404.1136, found: 404.1130.

4.1.3.19.

6-chloro-4-(cyclopropylethynyl)-*N*-(*o*-tolyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4s**)

The title product was synthesized following the general procedure, starting from **11** and 2-toluidine (**12s**). Yield 80%, white solid, m.p. 169-171 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.27 (m, 3H, N*H*), 7.63-7.56(m, 2H, Ph*H*), 7.37 (m, 5H, Ph*H*), 2.27 (s, 3H, C*H*$_3$), 1.61-1.59 (m, 1H, C*H*), 0.96-0.94 (m, 2H, C*H*$_2$), 0.80 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 150.07 (N=*C*-(NH)$_2$), 135.14 (Ar*C*), 135.10 (Ar*C*), 132.92 (Ar*C*), 132.28 (Ar*C*), 131.72 (Ar*C*), 129.45 (Ar*C*), 128.87 (Ar*C*), 127.86 (Ar*C*), 127.72 (Ar*C*), 127.36 (Ar*C*), 123.74 (d, J_{CF}= 287 Hz), 119.64 (Ar*C*), 116.94 (Ar*C*), 94.45 (*C*≡C), 66.67 (*C*≡C), 57.38 (q, J_{CCF} = 33 Hz), 17.86 (*C*H_3), 8.93 (*C*H_2), 8.88 (*C*H_2), -0.71 (*C*C≡C); IR: υ 2716, 2251, 1647, 1488, 1181, 1166 cm^{-1}; HRMS calcd for $C_{21}H_{17}ClF_3N_3$ $[M+H]^+$: 404.1136, found: 404.1132.

4.1.3.20.

6-chloro-4-(cyclopropylethynyl)-*N*-(3,4-dimethylphenyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4t**)

The title product was synthesized following the general procedure, starting from **11** and 3,4-xylidine (**12t**). Yield 77%, white solid, m.p. 161-163 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 10.99 (s, 2H, N*H*), 7.63 (d, *J* = 8.7 Hz, 1H, Ph*H*), 7.55 (s, 1H, Ph*H*), 7.36 (d, J = 8.6 Hz, 1H, Ph*H*), 7.25 (d, *J* = 8.0 Hz, 1H, Ph*H*), 7.16 (s, 1H, Ph*H*), 7.10 (d, *J* = 7.9 Hz, 1H, Ph*H*), 2.26 (s, 6H, 2 × C*H*$_3$), 1.59 (m, 1H, C*H*), 0.96-0.94 (m, 2H, C*H*$_2$), 0.80 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 149.51 (N=*C*-(NH)$_2$), 138.35 (Ar*C*), 135.96 (Ar*C*), 132.47 (Ar*C*), 132.40 (Ar*C*), 132.22 (Ar*C*), 131.15 (Ar*C*, 2C), 129.47 (Ar*C*), 127.66 (Ar*C*), 126.11 (Ar*C*), 123.83 (d, J_{CF}= 269 Hz), 119.85 (Ar*C*), 117.17 (Ar*C*), 94.42 (*C*≡C), 66.66 (*C*≡C), 57.37 (q, J_{CCF}= 32 Hz), 19.89 (*C*H_3), 19.40 (*C*H_3), 8.91 (*C*H_2), 8.86 (*C*H_2), -0.72 (*C*C≡C); IR: υ 2830, 2247, 1654, 1489, 1185, 1168 cm^{-1}; HRMS calcd for $C_{22}H_{19}ClF_3N_3$ $[M+H]^+$: 418.1292, found: 418.1290.

4.1.3.21.

6-chloro-4-(cyclopropylethynyl)-*N*-(3,5-dimethylphenyl)-4-(trifluoromethyl)-1,
4-dihydroquinazolin-2-amine (**4u**)

The title product was synthesized following the general procedure, starting from **11** and 3,5-Dimethylaniline (**12u**). Yield 76%, white solid, m.p. 225-227 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.06 (s, 2H, N*H*), 7.63 (d, *J* = 8.6 Hz, 1H, Ph*H*), 7.56 (s, 1H, Ph*H*), 7.38 (d, *J* = 8.6 Hz, 1H, Ph*H*), 6.99 (s, 3H, Ph*H*), 2.31 (s, 6H, 2 × C*H*$_3$), 1.61-1.58(m, 1H, C*H*), 0.95 (m, 2H, C*H*$_2$), 0.80 (m, 2H, C*H*$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 149.05(N=*C*-(NH)$_2$), 139.89 (Ar*C*, 2C), 134.78 (Ar*C*), 132.23 (Ar*C*), 132.13 (Ar*C*), 129.90 (Ar*C*),

129.15 (Ar*C*), 127.68 (Ar*C*), 125.09 (d, J_{CF}= 286 Hz), 122.13 (Ar*C*,2C), 119.64 (Ar*C*), 117.48 (Ar*C*), 94.86 (*C*≡C), 66.15 (*C*≡C), 57.48 (q, J_{CCF}= 33 Hz), 21.19 (CH_3, 2C), 8.83 (CH_2), 8.77 (CH_2), -0.88 (*C*C≡C). IR: υ 3230, 3101, 2250, 1694, 1500, 1168 cm^{-1}; HRMS calcd for $C_{22}H_{19}ClF_3N_3$ $[M+H]^+$: 418.1292, found: 418.1290.

4.1.3.22.6-chloro-4-(cyclopropylethynyl)-*N*-(3,4-dichlorophenyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4v**)

The title product was synthesized following the general procedure, starting from **11** and 3,4-dichloroaniline (**12v**). Yield 69%, white solid, m.p. 136-138 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 10.84 (s, 2H, N*H*), 7.86 (s, 1H, Ph*H*), 7.66 (d,*J* = 8.4 Hz, 1H, Ph*H*), 7.60-7.47 (m, 2H, Ph*H*), 7.43 (d, *J*= 8.5 Hz, 1H, Ph*H*), 7.24 (d, *J* = 7.0 Hz, 1H, Ph*H*), 1.56 (m, 1H, C*H*), 0.92 (m, 2H, CH_2), 0.77 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 148.74 (N=*C*-(NH)$_2$), 137.42 (Ar*C*), 131.97 (Ar*C*), 131.75 (Ar*C*), 131.60 (Ar*C*, 2C), 128.71 (Ar*C*), 128.22 (Ar*C*), 127.66 (Ar*C*, 2C), 124.07 (d, J_{CF}= 286 Hz), 123.25 (Ar*C*), 119.79 (Ar*C*), 117.42 (Ar*C*), 93.20 (*C*≡C), 68.13 (*C*≡C), 58.40 (q, J_{CCF} = 33 Hz), 8.83 (CH_2), 8.77 (CH_2), -0.69 (*C*C≡C); IR: υ 2929, 2250, 1637, 1473, 1242, 1167 cm^{-1}; HRMS calcd for $C_{20}H_{13}Cl_3F_3N_3$ $[M+H]^+$: 458.0200, found: 458.0192.

4.1.3.23.

6-chloro-4-(cyclopropylethynyl)-*N*-(3,4-difluorophenyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4w**)

The title product was synthesized following the general procedure, starting from **11** and 3,4-difluoroaniline (**12w**). Yield 74%, white solid, m.p. 200-202 °C (EA/PE);^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 10.94 (s, 2H, N*H*), 7.69-7.52 (m, 5H, Ph*H*), 7.35 (d,*J* = 7.8 Hz, 1H, Ph*H*), 7.27 (d, *J* = 7.9 Hz, 1H, Ph*H*), 1.61-1.59 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.80 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 150.05 (d, *J* =244 Hz), 149.91 (d, *J* =245 Hz), 149.39 (N=*C*-(NH)$_2$), 148.83 (Ar*C*), 148.69 (Ar*C*, 2C), 132.98 (Ar*C*), 132.48 (Ar*C*), 132.11 (Ar*C*), 129.43 (Ar*C*), 127.65 (Ar*C*), 123.76 (d, J_{CF}= 287 Hz), 119.89 (Ar*C*), 118.77 (Ar*C*, 2C), 117.35 (Ar*C*), 94.27 (*C*≡C), 66.94 (*C*≡C), 57.64 (q, J_{CCF}= 34 Hz), 8.88 (CH_2), 8.83 (CH_2), -0.72 (*C*C≡C); IR: υ 2892, 2248, 1654, 1482, 1239, 1173 cm^{-1}; HRMS calcd for $C_{20}H_{13}ClF_5N_3$ $[M+H]^+$: 427.0791, found: 427.0796.

4.1.3.24.

6-chloro-*N*-(3-chloro-4-fluorophenyl)-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydroquinazolin-2-amine (**4x**)

The title product was synthesized following the general procedure, starting from **11** and 3-Chloro-4-fluoroaniline (**12x**). Yield 72%, white solid, m.p.197-198 °C (EA/PE); ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 11.03 (s, 2H, NH), 7.76 (d, *J* = 6.3 Hz, 1H, N*H*), 7.62 (d, *J* = 8.6 Hz, 1H, N*H*), 7.55-7.51 (m, 2H, Ph*H*), 7.45-7.42 (m, 1H, Ph*H*), 7.36 (d, *J* = 8.6 Hz, 1H, Ph*H*), 1.61-1.58 (m, 1H, C*H*), 0.95 (m, 2H, CH_2), 0.80 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 156.36 (d, *J* =245 Hz), 149.40 (N=C-(NH)$_2$), 132.65 (ArC), 132.17 (ArC), 129.59 (ArC), 127.66 (ArC), 127.37 (ArC), 125.90 (ArC), 123.72 (d, J_{CCF} = 287 Hz), 120.79 (ArC), 120.60 (ArC), 119.77 (ArC), 118.38 (ArC), 118.16 (ArC), 117.35 (ArC), 94.41 (C≡C), 66.77 (C≡C), 57.46 (d, J_{CCF} = 33 Hz), 8.87 (CH_2), 8.82 (CH_2), -0.74 (CC≡C); IR: υ 2819, 2244, 2220, 1497, 1254, 1169 cm^{-1}; HRMS calcd for $C_{20}H_{13}Cl_2F_4N_3$ $[M+H]^+$: 442.0495, found: 442.0493.

4.2. *In vitro* anti-HIV assay

The anti-HIV activity and cytotoxicity of the newly synthesized compounds were evaluated with WT HIV-1 (strain HIV-IIIB), double RT mutant strains of HIV-1 IIIB (RES056 and F227L/V106A), five single RT mutant strains of HIV-1 IIIB (L100I, K103N, E138K, Y181C, Y188L), and HIV-2 (strain ROD) in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as described previously[24, 25].At the beginning of each experiment, stock solutions (10× final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells in order to allow simultaneous evaluation of their effects on

mock- and HIV-infected cells. Using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA), serial 5-fold dilutions of the test compounds (final 200 μL volume per well) were made directly in flat-bottomed 96-well microtiter trays, including untreated control HIV-1 and mock-infected cell samples for each sample. HIV-1 (IIIB) and mutant HIV-1 strains (RES056, F227L/V106A, L100I, K103N, E138K, Y181C, and Y188L) or HIV-2 (ROD) stock (50 μL at 100−300 CCID50) (50% cell culture infectious dose) or culture medium was added to either the 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 compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 μL aliquots were transferred to the microtiter tray wells. At 5 days after infection, the viability of mock- and HIV-infected cells was determined spectro-photometrically by means of the MTT assay.

The MTT assay is based on the reduction of yellow-colored MTT (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to form a blue-purple formazan that can be measured spectro-photometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Lab systems, Helsinki, Finland) at the wavelengths of 540 and 690 nm. All data were calculated using the median optical density (OD) value of three wells. The 50% effective antiviral concentration (EC_{50}) led to 50% protection from viral cytopathogenicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration that reduced the absorbance (OD_{540}) of mock-infected cells by 50%.

4.3. HIV-1 RT inhibition assay

The HIV-1 RT inhibition assay was conducted by using an RT assay kit produced by Roche. All the reagents for performing the RT reaction were contained in the kit and the procedure for assayingHIV-1 RT inhibition (recombinant WT and K103N/Y181C double mutant RT enzymes) was performed as described in the kit protocol[26].

Briefly, the reaction mixture containing HIV-1 RT enzyme, reconstituted template, and viral nucleotides [digoxigenin (DIG)-dUTP, biotin-dUTP, and dTTP] in the incubation buffer with or without inhibitors was incubated for 1 h at 37 °C. Then the reaction mixture was transferred to a streptavidin-coated microtiter plate (MTP) and incubated for another 1 h at 37 °C. The biotin-labeled dNTPs that were incorporated into the cDNA chain in the presence of RT were bound to streptavidin. The unbound dNTPs were washed with washing buffer, and anti-DIG-POD was added to the MTPs.

After incubation for 1 h at 37 °C, the DIG-labeled dNTPs incorporated in cDNA were bound to the anti-DIG-POD antibody. The unbound anti-DIG-PODs were washed out, and the peroxide substrate (ABST) solution was added to the MTPs. The reaction mixture was incubated at 25 °C until the green color was sufficiently developed for detection. The absorbance of the sample was determined at OD_{405} nm using a microtiter plate ELISA reader. The percentage inhibitory activity of RT inhibitors was calculated according to the following formula: % inhibition = [O.D. value with RT but without inhibitors −O.D. value with RT and inhibitors]/[O.D. value with RT and inhibitors− O.D. value without RT and inhibitors]. TheIC_{50} values correspond to the concentrations of the inhibitors required to inhibit biotin-dUTP incorporation by 50%.

4.4. Molecular docking

Molecular modeling studies was performed with the Tripos molecular modelling software packages (Sybyl-X 1.2). All the molecules for docking analysis were built using the standard bond lengths and angles from Sybyl-X 1.2/base Builder before being optimized using the Tripos force field for 10000 generations two times or more, until the minimized conformers of the ligand were

the same. The flexible docking method, called Surflex-Dock[27], docks the ligand automatically into the ligand binding site of the receptor by using a protocol-based approach and an empirically-derived scoring function[28]. 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[29]. The scoring function in Surflex-Dock, which contains hydrophobic, polar, repulsive, entropic, and solvation terms, was trained to estimate the dissociation constant (Kd) expressed in -log $(Kd)^2$. The scoring function in Surflex-Dock, which contains hydrophobic, polar, repulsive, entropic and solvation terms, was trained to estimate the binding energy. Prior to docking, the protein was prepared by removing water molecules, the ligand ETV, and other unnecessary small molecules from the crystal structure of the HIV-1 RT complex (PDB code:3MEC)[23]; simultaneously, hydrogen atoms were added to the protein. 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 8 Å), the maximum number of the 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 amino acid residue side-chains inside the defined RT binding pocket were regarded as rotatable or flexible, and the ligand was allowed to rotate at 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, including van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for more than 10,000 generations using a genetic algorithm, and the 20-best-scoring ligand-protein complexes were kept for the further analyses. The -log$(Kd)^2$ values of the 20-best-scoring complexes, which represented the binding affinities of the ligand with RT, encompassed a wide scope of the functional classes (10^{-2} - 10^{-9}). Only the highest scoring 3D structural model of the ligand-bound RT was chosen to define the binding interaction[30, 31].

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Supplementary Material

Supplementary data related to this article can be found in the online version at the web site.

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Highlights

1. Twenty-four new compounds were synthesized as new HIV-1 NNRTIs.
2. Five out 24 compounds exhibited EC_{50} in the low nanomolar range.
3. Compound **4b** showed the best result against the single and double mutant strain of HIV-1.
4. The preliminary SAR and docking studies with HIV-1 RT were also investigated.