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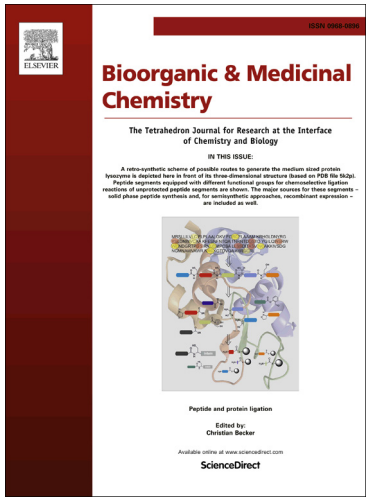

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1-Hydroxypyrido[2,3-d]pyrimidin-2(1H)-ones as Novel Selective HIV Integrase Inhibitors Obtained *via* Privileged Substructure-Based Compound Libraries

Ping Gao[a], Lingzi Zhang[a], Lin Sun[a], Tianguang Huang[a], Jing Tan[a], Jian Zhang[a], Zhongxia Zhou[a], Tong Zhao[a], Luis Menéndez-Arias[c], Christophe Pannecouque[b], Erik De Clercq[b], Peng Zhan[a,*], Xinyong Liu[a,*]

[a]*Department of Medicinal Chemistry, Key laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012, Jinan, Shandong, P. R. China*

[b]*Rega Institute for Medical Research, K.U.Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

[c]*Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas & Universidad Autónoma de Madrid), Madrid, Spain*

ABSTRACT:

A small library containing 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione and 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one scaffolds was obtained *via* the copper(I)-catalyzed azidealkyne cycloaddition (CuAAC) reaction and evaluated for their anti-HIV activity in MT-4 cells. Among the synthesized compounds, several 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one derivatives showed remarkable anti-HIV potency with EC_{50} values ranging from 0.92 to 26.85 μM. The most active one, **IIA-2**, also showed remarkable and selective potency against HIV type 1 integrase (IN). To the best of our knowledge, this is the first report showing that 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-ones are selective HIV IN inhibitors. Preliminary structure-activity relationship (SAR) studies suggested that the divalent metal ion chelators and the nature and position of substituents around the core are important for antiviral potency. Molecular modeling has been used to predict the binding site of the pyrido[2,3-d]pyrimidin-2(1*H*)-one core in HIV type 1 IN and suggestions are made for improvement of its inhibitory activity.

Keywords: HIV-1, CuAAC, HIV-1 integrase, HIV-1 RNase H, "privileged structure"-guided scaffold reposition.

1. Introduction

Acquired immunodeficiency syndrome (AIDS), mainly caused by the infection of human immunodeficiency virus type 1 (HIV-1), remains as one of the most serious public health problems throughout the world.[1] Up to now, approximately 30 drugs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV/AIDS, which target different steps in the viral life cycle such as viral entry, reverse transcription, integration, and viral maturation.[2] Although the clinical use of highly active antiretroviral therapy (HAART) has dramatically decreased the morbidity and mortality of HIV-1 infection, long-term successful treatments face major threats such as the emergence of drug-resistant strains,[3, 4] unwanted drug-drug interactions, and long-term toxicity (i.e., lipodystrophy, dyslipidemia, high cardiovascular risk, etc.).[2] Therefore, there is an urgent need for new anti-HIV drug candidates with increased potency, novel mechanisms of action, improved pharmacokinetic properties, and reduced side effects.

Over the past few decades, the identification of novel HIV-1 inhibitors was facilitated by traditional cellular phenotypic screening approaches (such as the MT4 cytoprotection assay), using large collections compounds, which in comparison with target-based screening, offered some advantages towards the identification of novel chemical structures with a unique mechanism of action, and the discovery of first-in class drugs. The high-throughput screening (HTS) campaigns using large diverse compound collections are time-consuming.[5, 6] On the other hand, the privileged substructure-based diversity-oriented synthesis (pDOS) strategy, has proven to be a fruitful tool to rapidly discover biologically active lead compounds by exploring the uncharted chemical space and constructing high-quality compound libraries.[7, 8, 9]

For the construction of pDOS libraries, it is crucial to select a privileged substructure with the potential for scaffold refining.[10] It is recognized that hydroxy-(iso)quinazoline-2,4(1,3)-dione and its analogues are featured in a number of molecules displaying a broad spectrum of biological activities. Among them, 2-hydroxyisoquinoline-1,3(*2H,4H*)-dione and

3-hydroxypyrimidine-2,4-dione derivatives are considered the core structures in many antiviral agents. Especially, they show potency against a wide range of metal-dependent enzymes, including dual inhibitors of HIV-1 ribonuclease H (RNase H, RNH) and integrase (IN) (e.g. **1**, **2**,[11] **3**,[12] **4**, **5**,[13]), HIV-1 RNase H active-site inhibitors (compounds **6** [14, 15, 16]), the HIV-1 IN active-site inhibitor **7**,[17] and the hepatitis C virus (HCV) NS5B polymerase inhibitor **8** [18] (**Figure 1**), suggesting that hydroxy-(iso)quinazoline-2,4(1,3)-diones represent a vast, untapped chemical space for further modification.

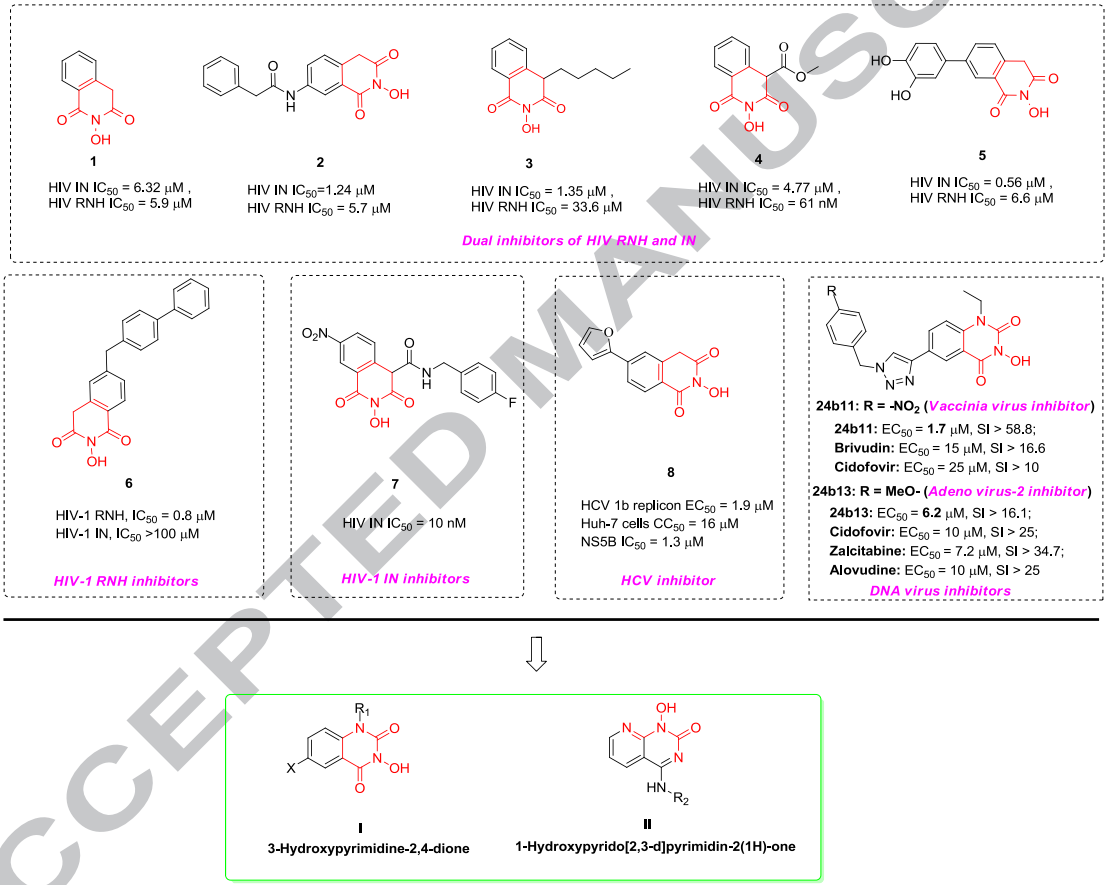


Figure 1. Representative antiviral agents containing 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione and 3-hydroxypyrimidine-2,4-dione, and further design of a privileged substructure-based library.

Very recently, our lab reported for the first time that novel 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones are specific drugs acting against DNA viruses such as vaccinia and adenovirus.[19] In particular, **24b11** displayed the most potent inhibitory activity against vaccinia virus with an EC_{50} value of 1.7 μM, 15 times more potent than the reference drug cidofovir (EC_{50} = 25 μM). On the other hand, **24b13** was the most potent compound against

adenovirus-2 with an EC_{50} value of 6.2 μM, lower than the EC_{50}s obtained with all the reference drugs tested in our assays. These findings warranted further investigations (library expansion and compound refinement) on this novel class of antiviral agents.

Continuing with this research, we have investigated the antiretroviral potential of the previously unexplored chemical space surrounding the 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-dione and its isosteres. Therefore, we constructed a small library of 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione and 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one scaffolds *via* the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction[20] and the biological significance of the novel compounds synthesized was determined after its evaluation in cell culture-based high-throughput screening (HTS) assays against HIV.

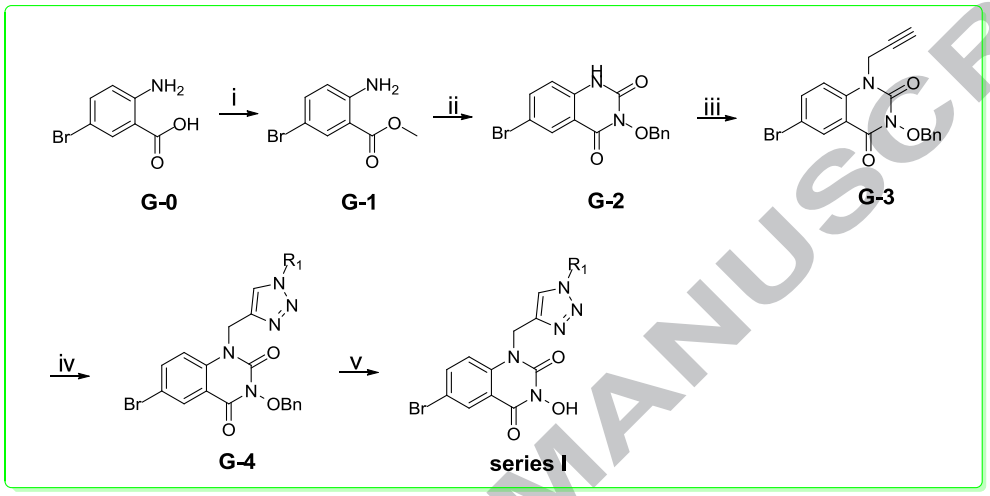
2. Results and discussion

2.1. Chemistry

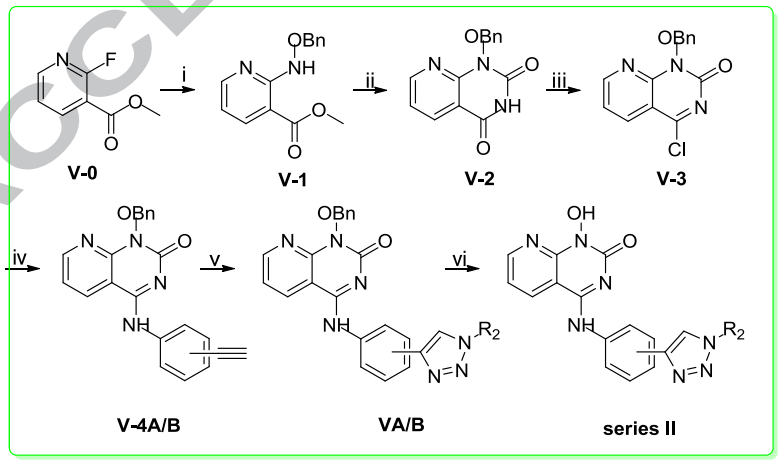
The library of 3-hydroxyquinolin-2(1*H*)-one compounds was constructed by following the synthetic route outlined in **Scheme 1**. The commercially available material 2-amino-5-bromobenzoic acid (**G-0**) was treated with methanol in the presence of concentrated H_2SO_4 to give the intermediate methyl 2-amino-5-bromobenzoate (**G-1**) *via* an esterification reaction. The intermediate 3-(benzyloxy)-6-bromoquinazoline-2,4(1*H*,3*H*)-dione (**G-2**) was obtained by ring closure of **G-1** with carbonyldiimidazole (CDI) and *O*-benzylhydroxylamine in a sodium hydroxide solution.[19] Then this heterocyclic scaffold was alkylated with 3-bromo-1-propyne in dimethylformamide (DMF) to afford N_1-substituted product **G-3**. CuAAC reaction of the alkyne key intermediate **G-4** with different azido substituent groups generated the corresponding key 1,2,3-triazole intermediates which were deprotected under basic conditions to render the target compounds 1,2,3-triazole-substituted 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones (series **I**).

The library of 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one compounds was synthesized as described in **Scheme 2**. The commercially available material methyl 2-fluoronicotinate (**V-0**) was treated with carbonyldiimidazole (CDI) and *O*-benzylhydroxylamine, followed by trichloroacetyl isocyanate to obtain the intermediate 1-(benzyloxy)pyrido[2,3-d]pyrimidine-2,4(1*H*,3*H*)-dione

(**V-2**).[21] Then this heterocyclic scaffold was treated with $POCl_3$ and the corresponding aniline to obtain the key intermediate **V-4**. The alkyne **V-4** reacted with different azido substituent groups *via* CuAAC reaction to generate the corresponding key 1,2,3-triazole intermediates which were then deprotected under trifluoroacetic acid to render the target 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-ones (series **II**).



Scheme 1. Synthesis of designed 3-hydroxyquinolin-2(1*H*)-one compounds. Reagents and conditions: i) CH_3OH , H_2SO_4 , reflux 8 h, yield 90.8%; ii) O-benzylhydroxylamine, CDI, tetrahydrofuran (THF), reflux; NaOH, EtOH, H_2O, reflux, yield 73.7%; iii) Propargyl bromide, K_2CO_3, DMF, room temperature, yield 67.8%; iv) Substituted azide, Sodium ascorbate, $CuSO_4{\cdot}5H_2O$, THF, H_2O, room temperature, yield 55.4%-89.2%; v) Acetic acid, HBr, reflux, yield 9.8%-31.0%.



Scheme 2. Synthesis of 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one compounds. Reagents and conditions: i) DIPEA, *O*-Benzylhydroxylamine, 140 °C, microwave, 120 min, yield: 34.3%; ii) Trichloroacetyl isocyanate, DCE,

TEA, MeONa, room temperature, yield: 21.1%; iii) DIPEA, $POCl_3$, 100 °C; iv) DMF, corresponding aniline, room temperature, yield over two steps, 17.4%-72.1%; v) Substituted azide, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, THF, H_2O, room temperature, yield 26.7%-100.0%; vi) Trifluoroacetic acid, 60 °C, overnight, yield 7.1%-100.0%.

2.2. Biological activity

2.2.1. Anti-HIV assay in MT-4 cells

The newly synthesized 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione and 1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one derivatives were evaluated for their anti-HIV activity against wild-type (WT) HIV-1 (IIIB) and HIV-2 (ROD) strains. Elvitegravir (EVG), raltegravir (RAL), and raltegravir-K (potassium salt), were used as reference drugs. The biological results are expressed as EC_{50}, CC_{50} and SI (selectivity index, given by the CC_{50}/EC_{50} ratio).

As shown in the **Table 1**, tested 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione derivatives were found to be inactive against HIV but showed remarkable cytotoxicity in MT-4 cells. In contrast, most of meta-triazole-benzene substituted 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one derivatives inhibited WT HIV-1 replication, with EC_{50} values in the range of 0.92 μM to 26.8 μM, indicating that activity is tightly associated with the structure of the scaffold. Among them, compound **IIA-2** was the most promising one and exhibited extremely potent inhibitory activity against HIV-1 strain IIIB with an EC_{50} of 0.92 μM. In addition, it showed inhibitory activity against HIV-2 strain ROD with an EC_{50} value of 2.82 μM.

The preliminary SAR analysis based on the results of the antiviral assays showed that: i) the compounds with *ortho*-substitution at the phenyl ring all exhibited higher cytotoxicity than those with *meta*-substitutions; ii) one or two carbon-containing linkers between the triazole and the terminal aromatic group may have a favorable effect in activity (exemplified by **IIA-2**, **IIA-1** and **IIA-7**), and the introduction of a carbanyl group has little influence in anti-HIV-1 potency (exemplified by **IIA-2** and **IIA-7**), but the structure modification had great influence versus HIV-2 activity; and iii) interestingly, the introduction of the NH group decreases their anti-HIV-1 activity, while losing their anti-HIV-2 activity (e.g. **IIA-5**, **IIA-6**, and **IIA-8**).

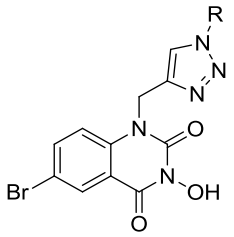
2.2.2. HIV-1 IN inhibitory assay

In an initial effort to identify the target of these 1-hydroxypyrido[2,3-d]pyrimidin-2(1*H*)-one derivatives, some representative compounds were also tested for their ability to inhibit HIV-1 IN and RNase H activities. The results are shown in Figure 1.

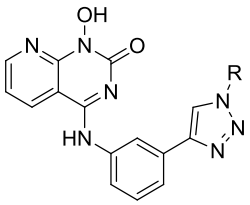
IIA-1 was tested in a HIV-1 IN inhibition assay at a concentration of 10 μg/ml (23.5 μM). Under these conditions, it produced a 93.1% inhibition of the IN strand transfer activity, a value similar to that obtained with the clinically approved drug EVG (97.8%). Based on this promising result, 5 μg/mL was selected as lower final concentration to test the inhibitory efficiency of several target compounds. As shown in **Figure 1**, all compounds exhibited >50% inhibitory activity, except compound **IIB-1**. The compounds **IIA-1, IIA-2** and **IIA-7** were the most potent, showing >80% inhibition of the strand transfer activity. These results were consistent with their potent anti-HIV activity in cell culture. Compound **IIA-2** was the most efficient compound, showing 86.2% inhibition of the HIV-1 IN activity, close to the percentages of 90.6% and 94.1% obtained with the reference drugs RAL and EVG, respectively.

A brief investigation of SARs in HIV-1 IN inhibitory assay revealed that i) the HIV-1 IN inhibition efficiency of compounds with *meta*-substitutions at the phenyl ring was superior to those of compounds with *ortho*-substitutions; ii) improved potency can be achieved by introducing one or two carbon-containing linkers between the triazole moiety and the terminal aromatic group (**IIA-2, IIA-1** and **IIA-7**); iii) the introduction of a carbonyl group has little influence in IN inhibition (compare **IIA-7** with **IIA-2**); and iv) the introduction of an NH group produces a significant decrease in the inhibitory efficiency of the derivatives (e.g. compare **IIA-5**, **IIA-6** and **IIA-8** with **IIA-7**). The above findings were generally consistent with the SAR conclusions regarding the anti-HIV potency of the tested compounds.

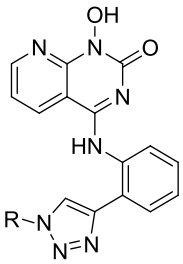
Table 1. Activity against HIV-1 (IIIB) and HIV-2 (ROD) strains, cytotoxicity and selectivity index in MT-4 cells.



series I



series IIA



series IIB

Compds	R	EC_{50} (μM)[a]		CC_{50} (μM)[b]	SI[c]	
		III_B	ROD		III_B	ROD
I-1	-CH_2-(4-F)Ph	>25.1	>25.1	25.1±1.9	<1	<1
I-2	-CH_2-(3-F)Ph	>24.9	>24.9	24.9±1.7	<1	<1
I-3	-CH_2-(3,4-diF)Ph	>24.7	>24.7	24.7±1.3	<1	<1
I-4	-CH_2-Ph	>23.7	>23.7	23.7±2.6	<1	<1
I-5	-CH_2-(4-Cl)Ph	>24.1	>24.1	24.1±1.6	<1	<1
I-6	-CH_2-(4-Br)Ph	>21.8	>21.8	21.8±1.8	<1	<1
I-7	-CH_2-(2-Cl)Ph	>22.3	>22.3	22.3±3.1	<1	<1
I-8	-CH_2-(4-NO_2)Ph	>22.4	>22.4	22.4±1.9	<1	<1
I-9	-CH_2-(2-CH_3)Ph	>17.8	>17.8	17.8±4.3	<1	<1
I-10	-CH_2-(3-CH_3)Ph	>24.	>24.0	24.0±0.86	<1	<1
IIA-1	-$(CH_2)_2$-Ph	**9.5±2.1**	**14.6±6.6**	>293.8	>31	>20
IIA-2	-CH_2-(4-F)Ph	**0.92±0.02**	**2.82±0.02**	>291.1	>318	>103
IIA-3	-$(CH_2)_3$-Ph	**26.1±8.2**	>239.2	239.2	9	<1
IIA-4	-1-naphthalene	**27.1±9.7**	**69.4±0.8**	>279.4	>10	>4
IIA-5	-CH_2-CONH-Ph	**26.8±0.3**	>275.1	>275.1	>10	X1

IIA-6	-CH_2-CONH-(4-F)Ph	**231.8±7.5**	>264.6	>264.6	>1	X1
IIA-7	-CH_2-CO-(4-F)Ph	**3.0±1.4**	>273.3	>273.3	>91	X1
IIA-8	-CH_2-CONH-(4-CF_3)Ph	>239.3	>239.3	>239.3	X1	X1
IIB-1	-CH_2-(4-F)Ph	>2.7	>2.7	**2.7±1.3**	<1	<1
IIB-2	-CH_2-(4-CH_3)Ph	>1.1	>1.1	**1.1±0.024**	<1	<1
Elvitegravir		(2.3±0.1) x 10^{-3}	(3.8±0.1) x 10^{-3}	6.3±2.5	2722	1661
Raltegravir		(7.8±0.2) x 10^{-3}	(11.0±0.8) x 10^{-3}	>55.8	>7146	>5123
Raltegravir-K		(7.7±0.5) x 10^{-3}	(16.0±0.4) x 10^{-3}	>51.8	>6812	>3214

[a]EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV-1-induced cytotoxicity, as determined by the MTT method.

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

[c]SI: selectivity index, the ratio of CC_{50}/EC_{50}.

X1: approximately equal to 1.

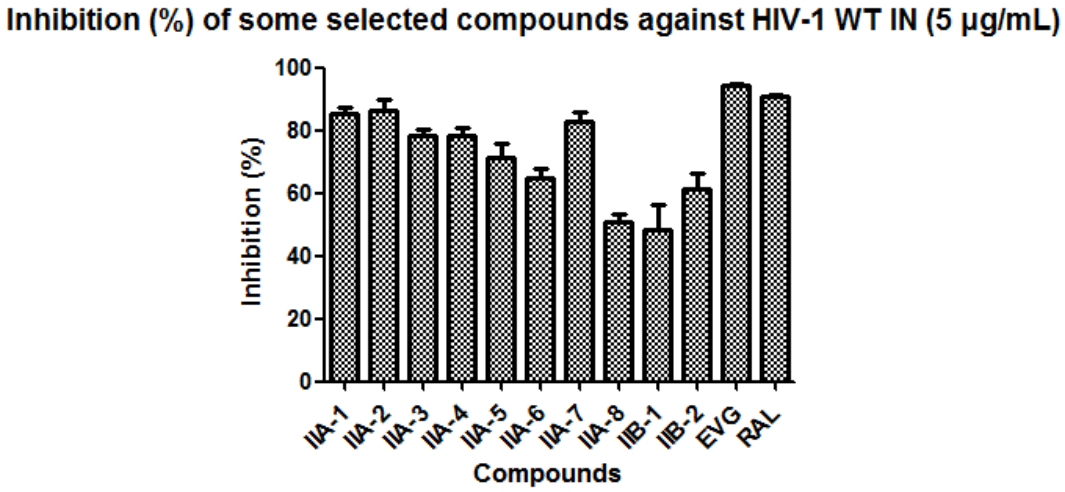


Figure 2. Inhibition of the HIV-1 IN strand transfer activity. Selected compounds were tested at a concentration of 5 µg/mL. Values represent an average ± standard deviation obtained from three

independent experiments.

2.2.3 HIV-1 RNase H inhibitory assay

As shown in Figure 3, the lack of activity in the HIV-1 RNase H inhibition assays indicated that the active compounds owed their anti-HIV activity, exclusively to their ability to inhibit the viral IN. Although HIV IN inhibitors and HIV RNase H inhibitors share similar metal-chelating capacities, the conformation of the metal-chelating backbone and the diversity of substituents in candidate drugs may influence target recognition. According to the latest report by GlaxoSmithKline in 2011, 1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one and its analogues were attractive structures as potential HIV-1 RNase H inhibitors[21]. However, our results with compounds of series II having modified substituents underline the importance of substituent diversity in the specificity of target recognition.

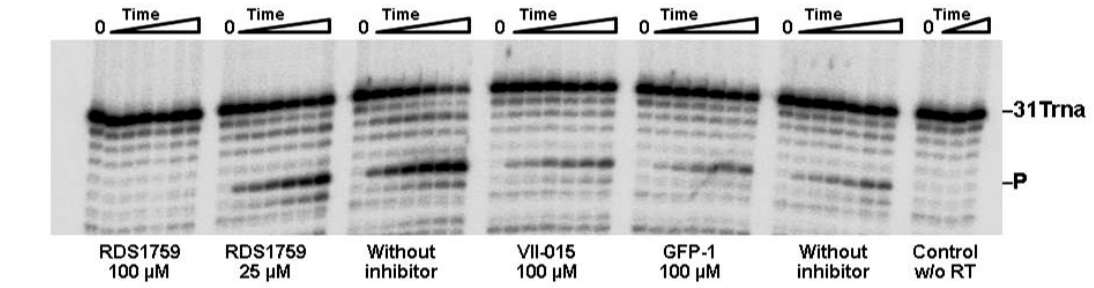


Figure 3. RNase H cleavage of heteropolymeric template-primer 31Trna/21P. RT-catalyzed cleavage of [^{32}P]RNA/DNA was carried out at 37ºC in the presence of different compounds at the indicated concentrations.[22] Reaction time points were taken at 0, 0.25, 0.5, 1, 2, 3 and 4 min. Samples on the right correspond to a control experiment carried out in the absence of RT and inhibitors, and show aliquots withdrawn at 0, 1, 2, and 4 min. 31Trna, stands for uncleaved RNA (31 nucleotides), and P indicates the cleavage product of 26 nucleotides.[22] GFP-1 and VII-015 refer to compounds **I-1** and **IIA-2**, respectively.

2.3. Molecular modeling analysis

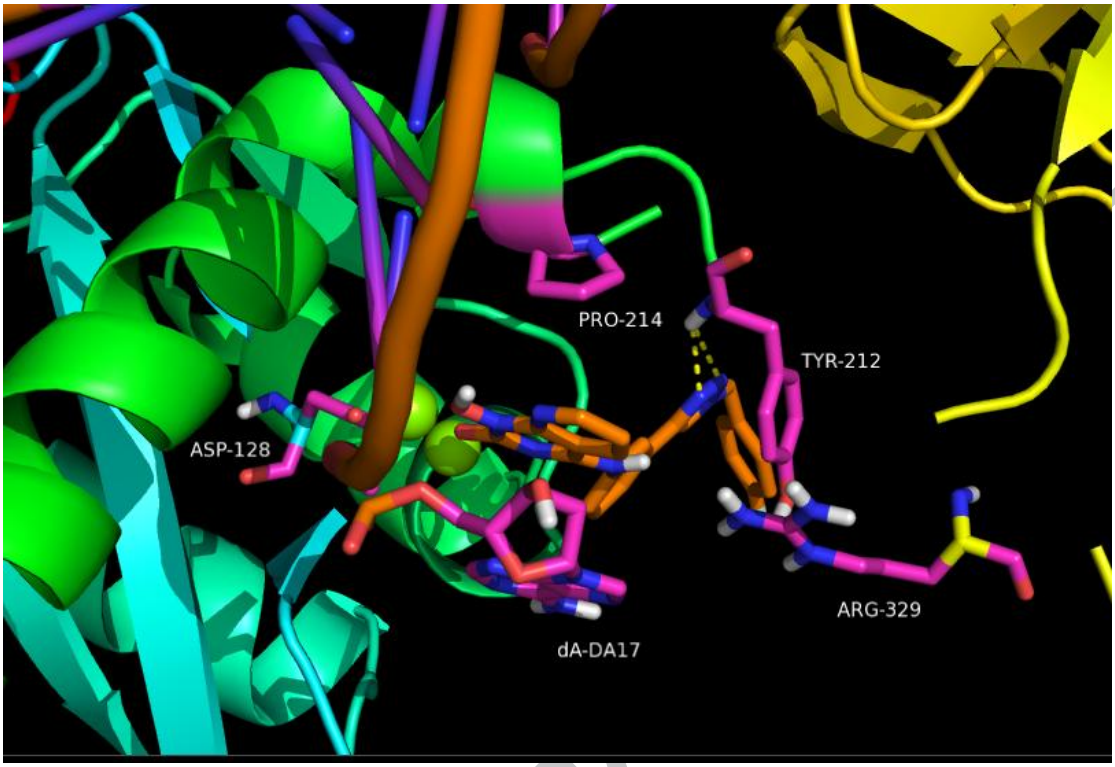


Figure 4. Predicted binding modes of **IIA-2** (orange) were explored using an HIV-1 IN model built on the basis of the crystal structure of prototype foamy virus (PFV) IN (PDB code: 3S3M). The hydrogen bonds between **IIA-2** and amino acids residues are indicated by dashed lines (yellow) and metal ions are shown as green spheres. DNA strands are represented with a stick model. A ribbon representation is used to depict the HIV-1 IN backbone and relevant amino acid residues are shown with sticks in magenta. All nonpolar hydrogens are hid. The figure was obtained with the Pymol visualization program.

To improve our understanding of structure-affinity relationships, the best compound of the series (i.e **IIA-2**) was docked into HIV-1 IN (PDB code: 3S3M) using the software SurflexeDock SYBYL-X 2.0. The results of the docking analysis showing its theoretical binding location and conformation are shown in Figure 4.

According to the docking analysis, **IIA-2** adopts a "Z" conformation in the active site of the IN, facilitated by the introduction of two turns in the inhibitor that coincide with NH and CH_2 groups. With this conformation, **IIA-2** could fit perfectly into the HIV-1 IN binding site by chelating the two Mg^{2+} ions found in the active site, making π-π interactions between the backbone of **IIA-2** and a nitrogen base, and establishing additional hydrogen bonds between the triazole and the main chain of Tyr212. Moreover, the terminal hydrophobic substituent could

interact with the benzene group of Tyr212. These findings could explain why **IIA-2** is a potent inhibitor of HIV-1 IN, and predictions based on the docking analysis will be taken into account in the next round of rational structural optimization.

3. Conclusion

The exploration of chemical space around privileged substructures remains one of the most challenging steps towards the identification of novel chemical entities valuable for drug discovery. The divalent metal ion chelators represent privileged, biologically relevant starting points for such explorations. We sought to approximate their structural diversity by focusing on two divalent metal ion chelators, 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione and 1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one, as frameworks. The focused compound collections based on these scaffolds were synthesized using CuAAC reaction which is widely applied in drug discovery[23, 24], and tested in anti-HIV bioassays to generate new promising leads for further development. In particular, some 1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one derivatives (exemplified by **IIA-2**) exhibited remarkable anti-HIV activity due to their ability to inhibit the viral IN, but their structures were different from those of other available IN inhibitors. Therefore, they could serve as good lead compounds for the development of a new generation of anti-HIV agents. All 3-hydroxyquinazoline-2,4(1H,3H)-dione derivatives didn't show anti-HIV potency, possibly because the triazole lack H-donor as other derivatives of 3-hydroxyquinazoline-2,4(1H,3H)-dione scaffold reported in the literature[17]. 1-Hydroxypyrido[2,3-d]pyrimidin-2(1H)-one and its analogues were attractive structures as potential HIV-1 RNase H inhibitors according to the latest report by GlaxoSmithKline in 2011, however, our results with compounds of series II having modified substituents didn't show activity in the HIV-1 RNase H inhibition assays but could selectively inhibit HIV-1 IN, which indicates the conformation of the metal-chelating backbone and the diversity of substituents in candidate drugs may influence target recognition. Preliminary SAR studies and molecular modeling analysis are expected to provide helpful guidance for further optimization. Furthermore, the application of CuAAC reaction in the construction of new pDOS libraries is being actively pursued in our laboratories.

4. Experimental section

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer using solvents as indicated (d^{6}-DMSO). Chemical shifts were reported in δ values (ppm) with tetramethylsilane as the internal reference, and *J* values were reported in hertz (Hz). Melting points (mp) were determined on a micromelting point apparatus. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapours or by irradiation with UV light (l ¼ 254 nm). Flash column chromatography was performed on column packed with Silica Gel60 (200-400 mesh). HPLC was performed on Agilent Infinity 1260/ G2260A.

Thin layer chromatography was performed on pre-coated HUANGHAI_HSGF254, 0.15-0.2 mm TLC-plates. Solvents were of reagent grade and were purified and dried by standard methods when necessary. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure. The key reactants including 2-amino-5-bromobenzoic acid, carbonyldiimidazole, methyl 2-fluoronicotinate, *O*-benzylhydroxylamine etc. were purchased from Adamas-beta Co. Ltd.

4.1.1. General procedure for synthesis of methyl 2-amino-5-bromobenzoate (**G-1**)

The commercially available material 2-amino-5-bromobenzoic acid (**G-0**) (0.2 g, 0.93 mmol) was dissolved in methanol (10 mL) and concentrated H_2SO_4 (0.2 ml, 3.72 mmol) was slowly added. The reaction mixture was refluxed for 6 h. After cooling, the solvent was evaporated mostly under reduced pressure and then aqueous $NaHCO_3$ was used to adjust the pH to 9-10. The residue was extracted with ethyl acetate (3×20 mL) and then the combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from ethyl acetate and petroleum ether or purified by column chromatography using ethyl acetate and petroleum ether (1:8) as an eluent to give **G-1** as white solid. yield 90.8%, mp:64-65℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 7.77 (s, 1H，PH-H), 7.38 (d, *J* = 8.6 Hz, 1H, Ph-H), 6.82 (s, 2H, NH_2-H), 6.77 (d, *J* = 8.9 Hz, 1H, Ph-H), 3.80 (s, 3H, CH_3-H). ^{13}C NMR (100 MHz, d^{6}-DMSO) δ 167.14, 150.86, 136.90, 132.76, 119.37, 110.65, 105.22, 52.15.

4.1.2 General procedure for synthesis of 3-(benzyloxy)-6-bromoquinazoline-2,4(1H,3H)-dione (**G-2**)

G-1 (0.4g, 1.74mmol) was refluxed with carbonyldiimidazole (CDI) (0.35 g, 3.17 mmol) in THF and the *O*-benzylhydroxylamine (0.32 g, 2.61 mmol) was added. The mixture was continued to reflux for 3 h. The solvent was evaporated mostly under reduced pressure and then ethyl alcohol (8 ml) was added to dissolve the residue. Sodium hydroxide (0.10 g, 2.61 mmol) in 5 ml H_2O was added then the mixture was reflux overnight. After cooling to room temperature, acetic acid was added until no more precipitate formed. The precipitate was filtrated and washed with MeOH to afford **G-2** as white solid. yield 73.7%, mp:238-240^o C.

^{1}H NMR (400 MHz, d^6-DMSO) δ 11.81 (s, 1H, N-H), 8.03 (d, *J* = 2.3 Hz, 1H, Ph-H), 7.85 (dd, *J* = 8.7, 2.3 Hz, 1H, Ph-H), 7.57 (dd, *J* = 7.5, 1.8 Hz, 2H, Ph-H), 7.42 (m, 3H, Ph-H), 7.17 (d, *J* = 8.7 Hz, 1H, Ph-H), 5.09 (s, 2H, CH_2).

4.1.3 General procedure for synthesis of 3-(benzyloxy)-6-bromo-1-(prop-2-yn-1-yl)quinazoline-2,4(1H,3H)-dione (**G-3**)

G-2 (0.1 g, 0.29 mmol), K_2CO_3 (0.05 g, 0.34 mmol) and 3-bromopropyne (0.04 g, 0.34 mmol) were added in 5 mL dimethyl formamide (DMF) and stirred under room temperature overnight. The solvent was evaporated mostly under reduced pressure and then water was added to wash the remaining DMF. The residue was extracted with ethyl acetate (3 ×20 mL) and then the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from ethyl acetate and petroleum ether to give **G-3** as white solid. yield 67.8%, mp:170-171℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.15 (d, *J* = 2.3 Hz, 1H, Ph-H), 8.02 (dd, *J* = 8.9, 2.3 Hz, 1H, Ph-H), 7.61 – 7.55 (m, 2H, Ph-H), 7.52 (d, *J* = 9.0 Hz, 1H, Ph-H), 7.44 (dd, *J* = 9.9, 4.8 Hz, 3H, Ph-H), 5.12 (s, 2H, CH_2), 4.98 (d, *J* = 1.8 Hz, 2H, CH_2), 3.43 (s, 1H, CH).

4.1.4 General procedure for synthesis of substituted 1-((1H-1,2,3-triazol-4-yl)methyl)-3-(benzyloxy)-6-bromoquinazoline-2,4(1H,3H)-dione (**G-4**)

G-3 (0.2 g, 0.52 mmol), corresponding azide (1.04 mmol), sodium ascorbate(0.03 g, 0.16 mmol) and copper sulfate pentahydrate (0.01 g, 0.05 mmol) were stirred together in THF:H_2O=1:1=6 mL overnight. The mixture was extracted with ethyl acetate (3×20 mL) and then the organic layer was dried over with Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from ethyl acetate and petroleum ether to give corresponding **G-4**.

3-(Benzyloxy)-6-bromo-1-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-1**): white solid, yield 73.7%, mp:183-185°C.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.17 (s, 1H, Ph-H), 8.13 (d, *J* = 2.0 Hz, 1H, Ph-H), 7.95 (dd, *J* = 8.9, 2.0 Hz, 1H, Ph-H), 7.59 (d, *J* = 8.9 Hz, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.44 – 7.32 (m, 5H, Ph-H), 7.19 (t, *J* = 8.8 Hz, 2H, Ph-H), 5.55 (s, 2H, CH2), 5.38 (s, 2H, CH_2), 5.12 (s, 2H, CH_2). $C_{25}H_{19}BrFN_5O_3$

3-(Benzyloxy)-6-bromo-1-((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-2**): white solid, yield 76.9%, mp:181-183℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.20 (s, 1H, Ph-H), 8.13 (d, *J* = 2.3 Hz, 1H, Ph-H), 7.94 (dd, *J* = 9.0, 2.3 Hz, 1H, Ph-H), 7.59 (d, *J* = 9.0 Hz, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.40 (m, 4H, Ph-H), 7.20 – 7.14 (m, 1H, Ph-H), 7.14 – 7.08 (m, 2H, Ph-H), 5.59 (s, 2H, CH2), 5.40 (s, 2H, CH2), 5.13 (s, 2H, CH2).

3-(Benzyloxy)-6-bromo-1-((1-(3,4-difluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-3**): white solid, yield 70.7%, mp:169-170℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.21 (s, 1H, Ph-H), 8.14 (d, *J* = 1.7 Hz, 1H, Ph-H), 7.99 – 7.91 (m, 1H, Ph-H), 7.59 (d, *J* = 9.1 Hz, 2H, Ph-H), 7.57 (s, 1H, triazole-H), 7.42 (d, *J* = 4.8 Hz, 5H, Ph-H), 7.16 (s, 1H, Ph-H), 5.57 (s, 2H, CH_2), 5.40 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-3-(benzyloxy)-6-bromoquinazoline-2,4(1H,3H)-dione (**G-4-4**): white solid, yield 87.9%, mp:192-194℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.17 (s, 1H, Ph-H), 8.13 (d, *J* = 2.3 Hz, 1H, Ph-H), 7.94 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.60 – 7.57 (m, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.44 – 7.38 (m, 3H, Ph-H), 7.37 – 7.25 (m, 5H, Ph-H), 5.56 (s, 2H, CH_2), 5.39 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

3-(Benzyloxy)-6-bromo-1-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-5**): white solid, yield 65.5%, mp:176-178℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.18 (s, 1H, Ph-H), 8.13 (d, *J* = 2.2 Hz, 1H, Ph-H), 7.95 (dd, *J* = 8.9, 2.3 Hz, 1H, Ph-H), 7.60 – 7.57 (m, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.45 – 7.37 (m, 5H, Ph-H), 7.30 (d, *J* = 8.3 Hz, 2H, Ph-H), 5.57 (s, 2H, CH_2), 5.39 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

3-(Benzyloxy)-6-bromo-1-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-6**): white solid, yield 62.7%, mp:197-198℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.18 (s, 1H, Ph-H), 8.13 (d, *J* = 2.4 Hz, 1H, Ph-H), 7.95 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.58 (m, 4H, Ph-H), 7.55 (s, 1H, triazole-H), 7.41 (m, 3H, Ph-H), 7.24 (d, *J* = 8.4 Hz, 2H, Ph-H), 5.55 (s, 2H, CH_2), 5.39 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

3-(Benzyloxy)-6-bromo-1-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-7**): white solid, yield 55.4%, mp:155-157℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.16 (s, 1H, Ph-H), 8.13 (d, *J* = 2.4 Hz, 1H, Ph-H), 7.94 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.59 – 7.57 (m, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.50 (dd, *J* = 7.8, 1.2 Hz, 1H, Ph-H), 7.43 – 7.33 (m, 5H, Ph-H), 7.17 (dd, *J* = 7.4, 1.6 Hz, 1H, Ph-H), 5.67 (s, 2H, CH_2), 5.41 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

3-(Benzyloxy)-6-bromo-1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-8**): yellow solid, yield 89.2%, mp:167-169° C.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.25 (s, 1H, Ph-H), 8.22 (d, *J* = 8.7 Hz, 2H, Ph-H), 8.14 (d, *J* = 2.4 Hz, 1H, Ph-H), 7.96 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.59 (d, *J* = 9.0 Hz, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.50 (d, *J* = 8.7 Hz, 2H, Ph-H), 7.44 – 7.37 (m, 3H, Ph-H), 5.75 (s, 2H, CH_2), 5.41 (s, 2H, CH_2), 5.12 (s, 2H, CH_2).

3-(Benzyloxy)-6-bromo-1-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-9**): white solid, yield 72.9%, mp:157-159℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.14 (d, *J* = 2.4 Hz, 1H, Ph-H), 8.08 (s, 1H, Ph-H), 7.95 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.58 (m, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.45 – 7.37 (m, 3H, Ph-H), 7.25 – 7.13 (m, 3H, Ph-H), 7.03 (d, *J* = 7.6 Hz, 1H, Ph-H), 5.57 (s, 2H, CH_2), 5.40 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 2.28 (s, 3H, CH_3).

3-(Benzyloxy)-6-bromo-1-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**G-4-10**): white solid, yield 62.4%, mp:164-166℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.16 (s, 1H, Ph-H), 8.13 (d, *J* = 2.4 Hz, 1H, Ph-H), 7.94 (dd, *J* = 9.0, 2.4 Hz, 1H, Ph-H), 7.58 (s, 2H, Ph-H), 7.56 (s, 1H, triazole-H), 7.41 (m, 3H, Ph-H), 7.23 (t, *J* = 7.8 Hz, 1H, Ph-H), 7.12 (d, *J* = 7.6 Hz, 1H, Ph-H), 7.07 (m, 2H, Ph-H), 5.51 (s, 2H, CH_2), 5.39 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 2.25 (s, 3H, CH_3).

4.1.5. General procedure for synthesis of substituted 1-((1H-1,2,3-triazol-4-yl)methyl)-6-bromo-3-hydroxyquinazoline-2,4(1H,3H)-dione (series **I**)

Corresponding **G-4** (0.2 g) was refluxed in a mixed solution of hydrogen bromide (2 mL) and acetic acid (2 mL) for 6-8 h. After cooling to room temperature, H_2O was added and the precipitate was recrystallized from MeOH to give corresponding series **I**.

6-Bromo-1-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dione (**I-1**): white solid, yield 13.6%, mp:270-272℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.98 (s, 1H, N-OH), 8.16 (s, 1H, Ph-H), 8.11 (d, *J* = 2.3 Hz, 1H, Ph-H), 7.91 (dd, *J* = 9.0, 2.3 Hz, 1H, Ph-H), 7.56 (d, *J* = 9.0 Hz, 1H, Ph-H), 7.36 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.19 (t, *J* = 8.8 Hz, 2H), 5.54 (s, 2H), 5.37 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 163.57, 161.14, 157.91, 149.50, 142.68, 138.34, 137.79, 132.61 (d, *J* = 12 Hz), 130.82, 130.73, 129.88, 124.12, 118.25, 117.47, 116.16, 115.95, 115.36, 52.50.

6-Bromo-1-((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dione (**I-2**): white solid, yield 24.8%, mp:268-270^o C.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.99 (s, 1H), 8.20 (s, 1H, triazole-H), 8.11 (d, *J* = 2.2 Hz, 1H), 7.91 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.41 (dd, *J* = 14.2, 7.9 Hz, 1H), 7.15 (m, 3H), 5.58 (s, 2H), 5.39 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 163.79 (s), 161.36 (s), 157.92 (s), 149.51 (s), 142.73 (s), 139.02 (d, *J* = 29.7 Hz), 138.33 (s), 137.77 (s), 131.31 (d, *J* = 32.8 Hz), 129.88 (s), 124.50 (d, *J* = 12.0 Hz), 124.38 (s), 118.25 (s), 117.48 (s), 115.60 (s), 115.37 (d, *J* = 1.9 Hz), 115.16 (s), 52.61 (s).

6-Bromo-1-((1-(3,4-difluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dione (**I-3**): white solid, yield 12.5%, mp:269-271℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.98 (s, 1H), 8.19 (s, 1H, triazole-H), 8.11 (d, *J* = 2.4 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.47 – 7.35 (m, 2H), 7.19 – 7.12 (m, 1H), 5.55 (s, 2H), 5.38 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.91, 150.95 (d, *J* = 49.5 Hz), 149.50, 148.50 (d, *J* = 51.9 Hz), 142.74, 138.33, 137.78, 133.92 (dd, *J* = 23.4, 15.8 Hz), 129.89, 125.67 (dd, *J* = 26.5, 13.9 Hz), 124.29, 118.44, 118.25 (d, *J* = 12 Hz), 117.95, 117.48, 117.46, 115.37, 52.12.

1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-6-bromo-3-hydroxyquinazoline-2,4(1H,3H)-dione (**I-4**): white solid, yield 11.4%, mp:269-271℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 10.97 (s, 1H), 8.16 (s, 1H), 8.11 (d, *J* = 2.3 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.39 – 7.22 (m, 5H), 5.55 (s, 2H), 5.38 (s, 2H). ^{13}C NMR (100 MHz, d^{6}-DMSO) δ 157.91, 149.51, 142.65, 138.33, 137.77, 136.35, 129.88, 129.21(2×C), 128.62, 128.42(2×C), 124.19, 118.25, 117.48, 115.35, 53.32.

6-Bromo-1-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dion e(**I-5**)：white solid, yield 19.3%, mp:272-273℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 10.97 (s, 1H), 8.17 (s, 1H), 8.11 (d, *J* = 2.4 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 5.56 (s, 2H), 5.38 (s, 2H).^{13}C NMR (100 MHz, d^{6}-DMSO) δ 157.91, 149.50, 142.70, 138.32, 137.78, 135.33, 133.37, 130.38(2×C), 129.89, 129.21(2×C), 124.27, 118.23, 117.47, 115.36, 52.51.

6-Bromo-1-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dion e(**I-6**)：white solid, yield 17.5%, mp:277-279℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 10.97 (s, 1H), 8.16 (s, 1H), 8.11 (d, *J* = 2.4 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 3H), 7.23 (d, *J* = 8.4 Hz, 2H), 5.54 (s, 2H), 5.38 (s, 2H).
^{13}C NMR (100 MHz, d^{6}-DMSO) δ 157.91, 149.50, 142.70, 138.32, 137.79, 135.74, 132.14(2×C), 130.68(2×C), 129.89, 124.28, 121.92, 118.23, 117.47, 115.36, 52.57.

6-Bromo-1-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-hydroxyquinazoline-2,4(1H,3H)-dion e (**I-7**): white solid, yield 31.0%, mp:274-276℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 10.97 (s, 1H), 8.13 (s, 1H), 8.11 (d, *J* = 2.3 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.53 – 7.46 (m, 1H), 7.42 – 7.31 (m, 2H), 7.17 (d, *J* = 7.3 Hz, 1H), 5.66 (s, 2H), 5.40 (s, 2H). ^{13}C NMR (100 MHz, d^{6}-DMSO) δ 157.91, 149.53, 142.51, 138.32, 137.75, 133.60, 133.08, 130.93, 130.73, 130.10, 129.88, 128.17, 124.60, 118.26, 117.48, 115.35, 51.10.

6-Bromo-3-hydroxy-1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dione (**I-8**): yellow solid, yield 9.8%, mp:238-240℃.

^{1}H NMR (400 MHz, d^{6}-DMSO) δ 10.98 (s, 1H), 8.24 – 8.21 (m, 3H), 8.11 (s, 1H), 7.91 (d, *J* = 5.6 Hz, 1H), 7.57 (d, *J* = 6.3 Hz, 1H), 7.50 (d, *J* = 6.8 Hz, 2H), 5.74 (s, 2H), 5.41 (s, 2H).^{13}C NMR (100 MHz, d^{6}-DMSO) δ 157.90, 149.50, 147.72, 143.71, 142.81, 138.31, 137.80, 129.90, 129.52(2×C), 124.69, 124.35(2×C), 118.23, 117.46, 115.38, 52.42.

6-Bromo-3-hydroxy-1-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dio ne (**I-9**): white solid, yield 18.6%, mp:282-284℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.96 (s, 1H), 8.11 (d, *J* = 2.3 Hz, 1H), 8.05 (s, 1H), 7.89 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.26 – 7.13 (m, 3H), 7.03 (d, *J* = 7.5 Hz, 1H), 5.55 (s, 2H), 5.38 (s, 2H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.91, 149.52, 142.52, 138.33, 137.74, 136.79, 134.45, 130.89, 129.87, 129.13, 128.81, 126.70, 124.20, 118.25, 117.48, 115.34, 51.48, 19.08.

6-Bromo-3-hydroxy-1-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)quinazoline-2,4(1H,3H)-dio ne (**I-10**): white solid, yield 22.4%, mp:264-266℃.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.97 (s, 1H), 8.14 (s, 1H), 8.11 (d, *J* = 2.4 Hz, 1H), 7.89 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 6.0 Hz, 2H), 5.50 (s, 2H), 5.38 (s, 2H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.90, 149.51, 142.62, 138.44, 138.31, 137.75, 136.24, 129.88, 129.24, 129.11, 128.94, 125.51, 124.17, 118.25, 117.49, 115.34 , 53.33, 21.36.

4.1.6. General procedure for synthesis of methyl 2-((benzyloxy)amino)nicotinate (**V-1**)

A mixture of **V-0** (1.0g, 6.45mmol) and *O*-benzylhydroxylamine (0.94g, 7.63mmol) in diisopropylethylamine (DIPEA, 12mL, 68.70mmol) was irradiated in microwave at 120℃ for 2h. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel chromatography using ethyl acetate and petroleum ether (1:10) as an eluent to provide **V-1**. Yellow oil. Yield: 34.3%. ESI-MS: m/z 259.0(M+1), 281.3(M+23). $C_{14}H_{14}N_2O_3$ [258.27].

4.1.7. General procedure for synthesis of 1-(benzyloxy)pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**V-2**)

A solution of **V-1** (0.18 g, 0.697 mmol) in 1,2-dichloroethane (DCE) (1.89 mL) was treated with Trichloroacetyl isocyanate (0.77 M in DCE) (1.93 mL, 1.49 mmol) and stirred t ambient temperature. After 30 minutes, triethylamine (TEA) (0.20 mL, 1.47 mmol) was added and the mixture was stirred at r.t. for 30 min. Then the solvent was removed under reduced pressure, and the residue was dissolved in 5ml MeOH and MeONa solution (25% wt. in MeOH) (0.76 mL, 3.5 mmol) was added. After 3 hours, the solvent was removed under under reduced pressure and 1 N

HCl was added to adjust the pH value to 3. Twenty milliliters of EA were then added and the aqueous phase was extracted with EA 3 times. The combined organic layer was washed with brine, dried over with Na_2SO_4 and purified by silica gel chromatography using ethyl acetate and petroleum ether (2:3) as an eluent to give **V-2**. White solid. Yield: 21.1%. mp:197-199℃。

^{1}H NMR (400 MHz, d^6-DMSO) δ 11.93 (s, 1H, NH), 8.79 (dd, *J* = 4.8, 1.7 Hz, 1H, C2-Py-H), 8.36 (dd, *J* = 7.7, 1.7 Hz, 1H, C4-Py-H), 7.70-7.56 (m, 2H, C5-Py-H, Ph-H), 7.49-7.33 (m, 4H, Ph-H), 5.18 (s, 2H, CH_2). ESI-MS: m/z 270.5(M+1), 292.5(M+23). $C_{14}H_{11}N_3O_3$ [269.26].

4.1.8. General procedure for synthesis of **V-4**

A solution of **V-2** (0.6 g, 2.23 mmol) in DIPEA was treated with $POCl_3$ (1.04 mL, 11.15 mmol) and heated to 100℃. After 45 min, the excess solvent was removed *in vacuo* and the black residue was dissolved in 10 mL DMF immediately. The corresponding amine (3.5 mmol) was added and then stirred at room temperature for 1 h. The mixture was poured into 200 mL H_2O and extracted with EA three times. The combined organic layers were dried over Na_2SO_4 and purified by silica gel chromatography to give corresponding compound **V-4**.

1-(benzyloxy)-4-((3-ethynylphenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**V-4a**): white solid, yield: 72.1%, mp:205-207°C

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.08 (s, 1H, NH), 8.82 (d, *J* = 5.4 Hz, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 7.86 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.65 (d, *J* = 6.5 Hz, 2H, Ar-H), 7.51-7.39 (m, 5H, Ar-H), 7.32 (d, *J* = 7.6 Hz, 1H, Ar-H), 5.15 (s, 2H, CH_2), 4.27 (s, 1H, C≡CH). ESI-MS: m/z 369.3(M+1), 391.4(M+23). $C_{22}H_{16}N_4O_2$ [368.39].

1-(benzyloxy)-4-((2-ethynylphenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**V-4b**): brown needles, yield: 17.4%，mp: 187-189°C.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.22 (s, 1H, NH), 8.83 (d, *J* = 3.9 Hz, 1H, C2-Py-H), 8.76 (d, *J* = 7.9 Hz, 1H, C4-Py-H), 7.62 (d, *J* = 7.3 Hz, 3H, Ar-H), 7.57-7.50 (m, 2H, Ar-H), 7.46-7.37 (m, 5H, Ar-H), 5.12 (s, 2H, CH_2), 4.33 (s, 1H, C≡CH). ESI-MS: m/z 369.2(M+1), 391.4(M+23). $C_{22}H_{16}N_4O_2$ [368.39].

4.1.9. General procedure for synthesis of series **II**

Corresponding **V-4** (0.52 mmol), corresponding azide (1.04 mmol), sodium ascorbate (0.03 g, 0.16 mmol) and copper sulfate pentahydrate (0.01 g, 0.05 mmol) were stirred together in THF:H_2O = 1:1 = 6 mL overnight. The residue was extracted with ethyl acetate (3×20 mL) and then the organic layer was dried over with Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from ethyl acetate and petroleum ether to give corresponding compound **V**.

1-(benzyloxy)-4-((3-(1-phenethyl-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-1**): white solid, yield 100%.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.16 (s, 1H), 8.87 (d, J = 7.8 Hz, 1H), 8.82 (d, J = 4.4 Hz, 1H), 8.53 (s, 1H), 8.21 (s, 1H), 7.89 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 6.5 Hz, 2H), 7.59 (d, J = 7.7 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.47-7.39 (m, 4H), 7.32-7.26 (m, 2H), 7.23 (dd, J = 7.6, 3.1 Hz, 3H), 5.16 (s, 2H), 4.68 (t, J = 7.3 Hz, 2H), 3.24 (t, J = 7.3 Hz, 2H). ESI-MS: m/z 516.7(M+1), 533.6(M+18), 538.6(M+23). $C_{30}H_{25}N_7O_2$ [515.57].

1-(benzyloxy)-4-((3-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-2**): light yellow solid, yield 21.4%. ESI-MS: m/z 520.5(M+1), 537.6(M+18), 542.5(M+23). $C_{29}H_{22}FN_7O_2$ [519.53].

1-(benzyloxy)-4-((3-(1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-3**): white solid, yield 85.7%.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.17 (s, 1H), 8.88 (d, *J* = 7.0 Hz, 1H), 8.85-8.80 (m, 1H), 8.63 (s, 1H), 8.26 (s, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 6.8 Hz, 3H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.48-7.39 (m, 4H), 7.30 (d, *J* = 7.3 Hz, 2H), 7.28-7.17 (m, 3H), 5.16 (s, 2H), 4.44 (t, *J* = 7.0 Hz, 2H), 2.70-2.55 (m, 2H), 2.31-2.15 (m, 2H). ESI-MS: m/z 530.4(M+1), 552.6(M+23). $C_{31}H_{27}N_7O_2$ [529.59].

1-(benzyloxy)-4-((3-(1-(naphthalen-1-yl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-4**): yellow solid, yield 53.3%.

^{1}H NMR (400 MHz, d^6-DMSO) δ 10.24 (s, 1H), 9.17 (s, 1H), 8.90 (d, *J* = 7.0 Hz, 1H), 8.83 (d, *J* = 4.7 Hz, 1H), 8.42 (s, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.16 (d, *J* = 7.3 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 7.2 Hz, 1H), 7.82-7.72 (m, 2H), 7.72-7.62 (m, 5H), 7.59 (dd, *J* = 16.3, 8.3 Hz,

1H), 7.49-7.39 (m, 4H), 5.17 (s, 2H). ESI-MS: m/z 538.6(M+1), 555.5(M+18), 560.5(M+23). $C_{32}H_{23}N_7O_2$ [537.57].

2-(4-(3-((1-(benzyloxy)-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)-N-phenylacetamide (**VA-5**): white solid, yield 26.7% .ESI-MS: m/z 545.5(M+1), 562.6(M+18), 567.6(M+23). $C_{30}H_{24}N_8O_3$ [544.56].

2-(4-(3-((1-(benzyloxy)-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorophenyl)acetamide (**VA-6**): white solid, yield 66.7%. ESI-MS: m/z 563.5(M+1), 580.5(M+18), 585.5(M+23). $C_{30}H_{23}FN_8O_3$ [562.55].

1-(benzyloxy)-4-((3-(1-(2-(4-fluorophenyl)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-7**): white solid, yield 33.3%. ESI-MS: m/z 548.6(M+1), 565.6(M+18), 570.6(M+23), 586.5(M+39). $C_{30}H_{22}FN_7O_3$ [547.54].

1-(benzyloxy)-4-((3-(1-(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VA-8**): white solid, yield 47.1%。 ESI-MS: m/z 613.5(M+1), 630.6(M+18), 635.4(M+23). $C_{31}H_{23}F_3N_8O_3$ [612.56].

1-(benzyloxy)-4-((2-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VB-1**): white solid, yield 21.4%. ESI-MS: m/z 520.7(M+1), 542.5(M+23). $C_{29}H_{22}FN_7O_2$[519.53].

1-(benzyloxy)-4-((2-(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**VB-2**): light yellow solid, yield 100%. ESI-MS: m/z 516.6(M+1), 538.5(M+23). $C_{30}H_{25}N_7O_2$ [515.57].

4.1.10. General procedure for synthesis of final product of series **II**.

Trifluoroacetic acid (TFA, 1 mL) was added to a solution of **V** in DCM and stirred at 60° C overnight. The excess solvent was removed in vacuo and the residue was washed with methanol or separated via HPLC to obtain the final product.

1-hydroxy-4-((3-(1-phenethyl-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one **(IIA-1)**: Grey solid, yield 37.5%. ^{1}H NMR (400 MHz, d^6-DMSO) δ10.56 (s, 1H, OH), 10.02

(s, 1H, NH), 8.83 (d, *J* = 6.7 Hz, 1H, C2-Py-H), 8.75 (d, *J* =3.9 Hz, 1H, C4-Py-H), 8.51 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.91 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.56 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.37 (s, 1H, CH), 7.26 (m, 5H, Ar-H), 4.68 (t, *J* = 7.0 Hz, 2H, N-CH2-C), 3.24 (t, *J* = 6.9 Hz, 2H, C-CH2-C). ^{13}C NMR (100 MHz, d^6-DMSO) δ 156.99, 154.69, 153.22, 151.76, 146.37, 139.39, 138.10, 134.13, 131.65, 129.57, 129.16(2×C), 128.92(2×C), 127.08, 122.95, 121.94, 119.93, 118.09, 106.40, 51.15, 36.06. ESI-MS: m/z 426.4 (M+1), 443.7 (M+18), 448.5 (M+23). $C_{23}H_{19}N_7O_2$ [425.44].

4-((3-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)-1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one **(IIA-2)**: Grey solid, yield 100.0%, HPLC purity: 97.5%. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.58 (s, 1H, OH), 10.03 (s, 1H, NH), 8.83 (d, *J* = 7.6 Hz, 1H, C2-Py-H), 8.75 (d, *J* = 4.0 Hz, 1H, C4-Py-H), 8.64 (s, 1H), 8.24 (s, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.48 – 7.41 (m, 3H), 7.37 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.24 (t, *J* = 8.8 Hz, 2H), 5.67 (s, 2H, CH_2). ^{13}C NMR (100 MHz, d^6-DMSO) δ 156.97, 154.69, 153.22, 151.75, 146.92, 139.39, 134.14, 130.82, 130.74, 129.58(2×C), 123.03, 122.08(2×C), 119.97, 118.11, 116.24(2×C), 116.03, 106.40, 52.74. ESI-MS: m/z 430.5(M+1), 447.5(M+18), 452.4(M+23). $C_{22}H_{16}FN_7O_2$ [429.41].

1-hydroxy-4-((3-(1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**IIA-3**): Yellow solid, yield 16.7%.mp: 215-217°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.18 (s, 1H), 10.05 (s, 1H), 8.84 (d, *J* = 7.7 Hz, 1H), 8.75 (d, *J* = 3.5 Hz, 1H), 8.62 (d, *J* = 7.5 Hz, 1H), 8.25 (s, 1H), 7.91 (d, *J* = 7.2 Hz, 1H), 7.62 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.37 (dd, *J* = 7.3, 4.8 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.26 – 7.20 (m, 3H), 4.44 (t, *J* = 7.1 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 2.21 (dt, *J* = 14.4, 7.1 Hz, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.00, 154.69, 153.26, 151.76, 146.62, 141.23, 139.38, 134.15, 131.70, 129.55, 128.88(2×C), 128.83, 126.49, 122.98, 122.10, 121.92, 120.02, 118.11, 106.41, 49.60, 32.39, 31.72. ESI-MS: m/z 440.5(M+1), 457.6(M+18). $C_{24}H_{21}N_7O_2$ [439.47].

1-hydroxy-4-((3-(1-(naphthalen-1-yl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**IIA-4**): Light yellow solid, yield 12.5%. mp: 263-265°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.60 (s, 1H), 10.11 (s, 1H), 9.17 (s, 1H), 8.87 (s, 1H), 8.76 (s, 1H), 8.42 (s, 1H), 8.24 (d, *J* = 8.3 Hz, 1H), 8.16 (d, *J* = 7.7 Hz, 1H), 7.98 (d, *J* = 7.4 Hz, 1H), 7.85 (d, *J* = 7.1 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.64 (d, *J* = 5.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.43 (dd, *J* = 15.1,

7.6 Hz, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 157.07, 154.72, 153.28, 151.77, 146.93, 139.51, 134.18, 133.76, 131.19, 130.86, 129.68, 128.85, 128.59, 128.39, 127.66, 125.95, 124.85, 124.43, 123.41, 122.58, 122.46, 120.32, 118.13, 106.43. ESI-MS: m/z 448.6(M+1), 465.5(M+18), 470.5(M+23). $C_{25}H_{17}N_7O_2$ [447.45]

2-(4-(3-((1-hydroxy-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)-N-phenylacetamide (**IIA-5**): Yellow solid, yield 100.0%. mp: 285-287°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.53 (s, 1H), 10.11 (d, *J* = 53.4 Hz, 1H), 8.93 – 8.73 (m, 2H), 8.61 (d, *J* = 4.5 Hz, 1H), 8.28 (s, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.63 (dd, *J* = 17.2, 7.6 Hz, 3H), 7.52 (dd, *J* = 15.9, 8.0 Hz, 1H), 7.47 – 7.40 (m, 1H), 7.36 (dd, *J* = 15.2, 7.6 Hz, 2H), 7.10 (t, *J* = 7.4 Hz, 1H), 5.42 (s, 2H), 5.16 (s, 1H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 164.64, 156.98, 154.68, 153.25, 151.76, 146.46, 139.46, 138.90, 134.18, 131.55, 129.95, 129.62(2×C), 129.39, 124.29, 123.67, 122.91, 122.03, 119.89, 119.76, 118.10, 106.44, 52.90. ESI-MS: m/z 455.5(M+1), 472.5(M+18), 477.4(M+23). $C_{23}H_{18}N_8O_3$ [454.44].

N-(4-fluorophenyl)-2-(4-(3-((1-hydroxy-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)acetamide (**IIA-6**): Yellow solid, yield 10.0%. mp: 181-183°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.60 (s, 1H), 10.12 (d, *J* = 50.9 Hz, 1H), 8.85 (dd, *J* = 23.6, 6.3 Hz, 1H), 8.61 (d, *J* = 4.4 Hz, 1H), 8.28 (s, 1H), 7.92 (d, *J* = 8.9 Hz, 1H), 7.66-7.60 (m, 3H), 7.52 (dd, *J* = 15.9, 8.0 Hz, 1H), 7.48-7.40 (m, 2H), 7.20 (t, *J* = 8.8 Hz, 2H), 5.42 (s, 2H), 5.16 (s, 1H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 164.60, 159.95, 157.56, 154.76, 146.47, 135.29, 131.55, 129.88(2×C), 129.65, 129.17, 128.78(2×C), 123.66, 122.30, 121.62, 121.54, 120.18, 119.87, 118.83, 116.12, 115.90, 52.82. ESI-MS: m/z 473.4(M+1), 490.6(M+18), 495.4(M+23). $C_{23}H_{17}FN_8O_3$ [472.43].

4-((3-(1-(2-(4-fluorophenyl)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)-1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one (**IIA-7**): Light yellow solid, yield 40.0%. mp: 261-263°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.60 (s, 1H), 10.06 (s, 1H), 8.85 (d, *J* = 7.5 Hz, 1H), 8.76 (d, *J* = 4.1 Hz, 1H), 8.53 (s, 1H), 8.30 (s, 1H), 8.26-8.16 (m, 2H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 7.4 Hz, 1H), 7.54-7.46 (m, 3H), 7.38 (dd, *J* = 7.6, 4.7 Hz, 1H), 6.29 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 191.34, 156.96, 154.69, 153.23, 152.38, 151.77, 139.48, 131.89, 131.79(2×C), 131.54, 129.65, 123.63, 122.89, 122.01, 119.88, 118.10, 116.73(2×C), 116.51, 106.43, 56.45. ESI-MS: m/z

458.6(M+1), 475.4(M+18), 480.4(M+23). $C_{23}H_{16}FN_7O_3$ [457.42].

2-(4-(3-((1-hydroxy-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)amino)phenyl)-1H-1,2,3-triazol-1-yl)-N-(4-(trifluoromethyl)phenyl)acetamide (**IIA-8**): Light yellow solid, yield 37.5%. mp> 300°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.92 (s, 1H), 10.06 (s, 1H), 8.85 (d, *J* = 12.8 Hz, 1H), 8.76 (s, 1H), 8.62 (s, 1H), 8.29 (s, 1H), 7.94 (s, 1H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 7.0 Hz, 2H), 7.59 – 7.47 (m, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 5.48 (s, 2H). ESI-MS: m/z 523.6(M+1), 540.6(M+18), 545.5(M+23). $C_{24}H_{17}F_3N_8O_3$ [522.44]

4-((2-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)-1-hydroxypyrido[2,3-d]pyrimidin-2(1H)-one (**IIB-1**): Yellow solid, yield 33.3%. mp: 233-235°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.94 (s, 1H, OH), 10.56 (s, 1H), 8.76 (s, 1H), 8.58 (s, 1H), 8.52 (s, 1H), 8.13 (s, 1H), 7.94 (d, *J* = 6.4 Hz, 1H), 7.64 (d, *J* = 6.7 Hz, 1H), 7.44 (d, *J* = 5.6 Hz, 1H), 7.42 (s, 1H, CH), 7.33 (s, 2H), 7.16 (t, *J* = 8.3 Hz, 2H), 5.63 (s, 2H). ^{13}C NMR (100 MHz, d^6-DMSO) δ 163.54, 161.12, 154.69, 151.76, 133.50, 132.41, 130.69, 130.61, 128.92(2×C), 128.35(2×C), 126.81, 126.36, 123.67, 118.32(2×C), 116.15(2×C), 115.94, 52.68. ESI-MS: m/z 430.5(M+1). $C_{22}H_{16}FN_7O_2$ [429.41].

1-hydroxy-4-((2-(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)phenyl)amino)pyrido[2,3-d]pyrimidin-2(1H)-one (**IIB-2**): Light yellow solid, yield 7.1%. mp: 225-227°C. ^{1}H NMR (400 MHz, d^6-DMSO) δ 10.97 (s, 1H, OH), 10.58 (s, 1H), 8.77 (d, *J* = 4.1 Hz, 1H), 8.60 (d, *J* = 7.8 Hz, 1H), 8.50 (d, *J* = 11.3 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.49 – 7.35 (m, 3H), 7.14 (dd, *J* = 16.4, 7.9 Hz, 4H), 5.58 (s, 2H), 2.26 (d, *J* = 7.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 157.50, 154.68, 153.26, 151.77, 145.50, 138.03, 135.48, 133.42, 133.08, 129.73, 128.87, 128.39(2×C), 128.32, 126.56, 126.26, 124.25, 123.58, 118.32, 106.57, 53.36, 21.18.. ESI-MS: m/z 426.4(M+1). $C_{23}H_{19}N_7O_2$ [425.44].

4.2. In vitro anti-HIV assay

By using the MTT method described previously,[25, 26] the synthesized compounds were evaluated for their activity against WT HIV-1 [27] (strain HIV-IIIB), and HIV-2 [28] (strain ROD) in MT-4 cells. At the beginning, stock solutions (10×final concentration) of test compounds were added at 25 μM volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock and HIV-infected cells. Serial five-fold dilutions of the test compounds were made directly

in flat-bottomed 96-well microtiter trays, including untreated control HIV-1 and mock-infected cells for each sample, using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). HIV-1 (IIIB), or HIV-2 (ROD) stocks (50 mL at 100-300 CCID50) (50% cell culture infectious dose) or culture medium were added to either the mock or HIV-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 their cytotoxicity. 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 transferred 50 mL volumes to the microtiter tray wells. Five days after infection the viability of mock-and HIV-infected cells was determined spectrophotometrically.

The MTT assay was based on the reduction of yellow colored 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland), at the wavelengths of 540 and 690 nm. All data were calculated using the median OD (optical density) value of two or three wells.

The 50% effective antiviral concentration (EC_{50}) was defined as the concentration of the tested compound achieving 50% protection from viral cytopathicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration that reduced the inability of mock-infected cells by 50%. The symbol '>' indicated the highest concentration at which the compound was tested and still found to be non-cytotoxic.

4.3. HIV-1 RNase H inhibition assay

Wild-type (WT) HIV-1 BH10 reverse transcriptase (RT) was expressed in *E. coli* and purified as previously described.[29, 30] Synthetic oligonucleotides 31Trna (5´-UUUUUUUUUAGGAUACAUAUGGUUAAAGUAU-3´) and 21P (5´-ATACTTTAACCATATGTATCC-3´) were obtained from Sigma. The RNase H inhibitor RD1759 [31, 32] was kindly provided by Drs. Roberto Di Santo (Sapienza University of Rome, Italy) and Enzo Tramontano (University of Cagliari, Italy).

The RNase H activity was determined using the template-primer 31Trna/21P.[22] The template RNA was labeled at its 5´ end with [γ-^{32}P]ATP (Perkin Elmer) and T4 polynucleotide kinase (New England Biolabs), and then purified with a mini Quick Spin™ column (Roche). The labeled template was annealed to the 21-nt primer (21P) in 50 mM Tris-HCl (pH 8.0) containing 50 mM NaCl, as previously described.[22] RNase H cleavage assays were carried out for 0 to 4 min at 37ºC with 20-40 nM HIV-1 RT in 30 µl of 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 25 nM ^{32}P-labeled template-primer (31Trna/21P), and 5% dimethyl sulfoxide (DMSO). The potential RNase H inhibitors were available in 50% DMSO. Appropriate dilutions of those drugs were preincubated with the RT for 5 min at 37ºC in the assay buffer, and reactions were initiated by adding the labeled template-primer. Aliquots were removed at appropriate times and quenched with an equal volume of sample loading buffer [10 mM EDTA in 90% formamide containing xylene cyanol FF (3 mg/mL) and bromophenol blue (3 mg/mL)] and analyzed by denaturing polyacrylamide gel electrophoresis.

4.4 HIV-1 IN inhibition assay

HIV-1 IN inhibition assay was carried out using an ELISA method [33] and the XpressBio HIV-1 Integrase Assay Kit EZ-1700 (purchased from Suzhou Xishan Biological technology co., LTD, China). Briefly, streptavidin-coated 96-well plates were coated with a double-stranded HIV-1 LTR U5 donor substrate (DS) DNA containing an end-labeled biotin for 30 min at 37ºC. Full-length recombinant HIV-1 IN protein was then added, and the mixture incubated for another 30 min at 37ºC. Test compounds were added to the enzyme reaction and incubated for 5 min at room temperature. Then a different double stranded target substrate (TS) DNA containing a 3'-end modification was added to the reaction mixture which was then incubated for 30 min at 37ºC. The HIV-1 IN cleaves the terminal two bases from the exposed 3'-end of the HIV-1 LTR DS DNA and then catalyzes a strand-transfer recombination reaction to integrate the DS DNA into the TS DNA. The products of the reaction are detected spectrophotometrically using a horse radish peroxidase (HRP)-labeled antibody directed against the TS 3'-end modification. Therefore, HRP antibody solutions were added to the reaction and incubated during 30 min at 37ºC. Following the incubation with the antibody, 100 µl of a 3,3',5,5'-tetramethylbenzidine (TMB) peroxidase substrate solution is added to each well and incubated for 10 minutes at room temperature. The

reaction is stopped by adding 100 μL of the commercial TMB stop solution and the absorbance of the product is determined at 450 nm using a microtiter plate ELISA reader. The percentage inhibition obtained with the tested compounds was calculated by using the formula:

% Inhibition= [OD value with IN but without inhibitors – OD value with IN and inhibitors]/[OD value with IN and inhibitors – OD value without IN and inhibitors]

4.5. Molecular docking

Molecular modeling of molecule **IIA-2** was performed using the Tripos molecular modeling packages Sybyl-X 2.0. **IIA-2** was optimized for 2000-generations till the maximum derivative of energy became 0.005 kcal/(mol*A) using the Tripos force field. The published three dimensional crystal structure of the IN complex was retrieved from the Protein Data Bank (PDB code: 3S3M).

The protein was prepared by using the Biopolymer application accompanying Sybyl. The dolutegravir (DTG) ligand was extracted from the complexes, water molecules were removed, hydrogen atoms were added, side chain amides and side chains bumps were fixed, and charges and atom types were assigned according to AMBER 99. After the protomol was generated, the optimized **IIA-2** was surflex-docked into the binding pocket of DTG.

Conflict of interest

The authors declare no conflict of interest.

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