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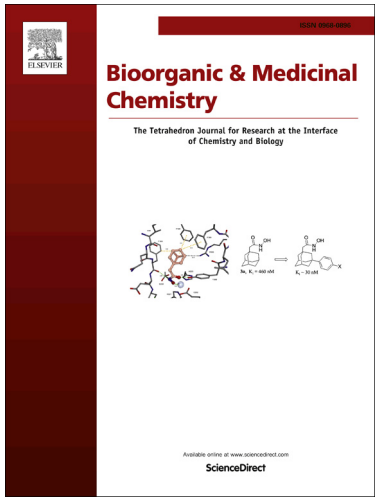

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Graphical Abstract

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Design and synthesis of N-methylpyrimidone derivatives as HIV-1 integrase inhibitors

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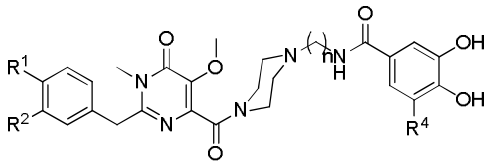

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ABSTRACT

A series of novel β-diketo derivatives which combined the virtues of dihydroxypyrimidine carboxamide derived from the evolution of DKA and polyhydroxylated aromatics moieties, were designed and synthesized as potential HIV-1 integrase (IN) inhibitors and evaluated their inhibition to the strand transfer process of HIV-1 integrase and anti-HIV-1 activity. The result indicates that 3,4,5-trihydroxylated aromatic derivatives exhibit good inhibition to HIV-1 integrase, but dihydroxylated aromatic derivatives appear little inhibition to HIV-1 integrase. In addition, the preliminary structure-activity relationship (SAR) of these new derivatives was rationalized by docking studies.

1. Introduction

The viral enzyme integrase (IN), which mediates integration of the viral cDNA into the host genome by catalytic reactions-first catalyzes removal of the terminal dinucleotide from each 3'-end of the viral DNA (3'-processing) and subsequently medicates joining of the 3'-end of the viral DNA to the host DNA (strand transfer) during the viral replication cycle[1,2]. Because of lack of a cellular homologue and its essential role in viral replication, it is an attractive target for the development of new anti-HIV-1 inhibitors with high selectivity and low toxicity[3,4]. In the past two decades, IN-targeted antiviral research has led to the identification of various inhibitor types, of which the most prominent scaffolds feature is a diketo acid (DKA) structure or its heterocyclic bioisosteres[5-9]. The dihydroxypyrimidine carboxamide derived from the evolution of DKA is a potent, reversible, and selective HIV-1 integrase strand transfer inhibitor[10]. Extensive research on these chemotypes culminated with raltegravir[11,12] (Fig. 1), it is the first drug approved for clinical use target to HIV integrase. The pharmacophoric center 5-hydroxypyrimidone carboxamide could bind to divalent metal ions (Mg^{2+} or Mn^{2+}) to block the strand transfer (ST) in the HIV-1 integrase catalytic site. Unfortunately, continuous mutation of viral genome leads to multi-drug resistant viral strains that are no longer susceptible to current therapy including raltegravir[13-15].

The polyhydroxylated aromatics were also the first HIV integrase inhibitor identified, but the early polyhydroxylated derivatives were later demonstrated to inhibit viral entry or to be too toxic to be pursued as therapeutic IN inhibitors[16,17]. In order to study possible interaction between the two main classes of inhibitors (polyphenols and DKAs), a kind of catechol or dicaffeoyltartaric-DKA hybrids have been designed and tested as IN inhibitors following the goals of refining the pharmacophore, enhancing antiviral potency and improving cellular membranes permeability[18,19]. The majority of the hybrid compounds exhibited submicromolar potency against strand transfer and modest antiviral activities, thus, there is a clear need to optimize the structure of compounds into the highly potent inhibitory activity derivatives to HIV integrase. Despite the high interest generated by these compounds, little number of the compounds results in a lake of structure-activity relationships information for the class of inhibitors.

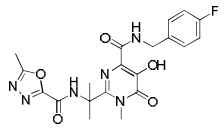

Figure 1. Structure of Raltegravire (MK-0518)

In our previous studies, a series of polyhydroxylated aromatics were designed and synthesized as potential HIV-1 integrase inhibitors and evaluated their inhibitory activity to the strand transfer process of HIV-1 integrase[17,20]. On the basis of the consideration as well as on our interest in development of new anti-IN agents, we planned to explore the effect of polyhydroxylated aromatics-dihydroxypyrimidine hybrids to gain new insight in the fundamental structural requirements for anti-

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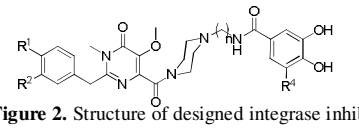


Figure 2. Structure of designed integrase inhibitors

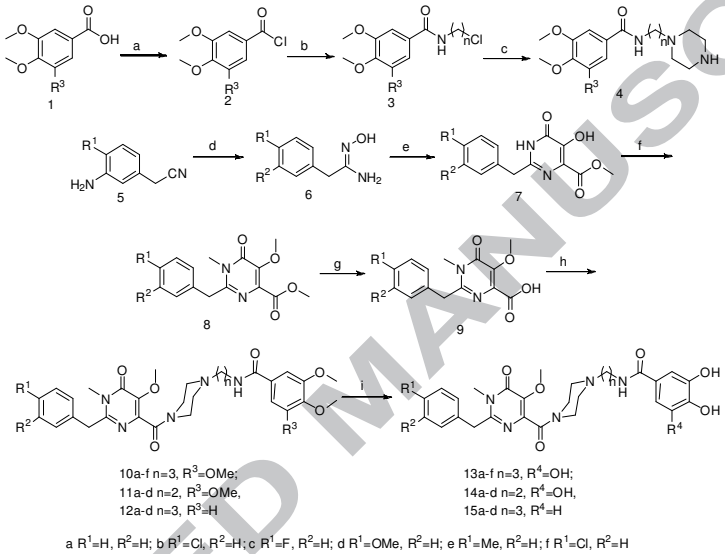
IN activity. Keeping in mind the pharmacophoric groups of hydroxyl-pyrimidone carboxamide and polyhydroxylated aromatics, adjusting length of carbon chain of linker between two pharmacophoric group, we designed and synthesized compound 3,4,5-polyhydroxy-N-(3-(4-(5-methoxy-1-methyl-2-(4-substitute-dbenzyl)-6-oxo-1,6-dihydropyrimidine-4-carbonyl)-piperazin-1-yl)alkyl)benzamide(Fig.2).

2. Results and discussion

2.1. Chemistry

Preparation of 3,4,5-polyhydroxy-N-(3-(4-(5-methoxy-1-methyl-2-(4-substitutedbenzyl)-6-oxo-1,6-dihydropyrimidine-4-carbonyl)-piperazin-1-yl)alkyl)benzamide is shown in Scheme 1. The synthesis started with the commercial available polymethoxy benzoic acid **1**, which reacted with thionyl chloride, to give benzoyl chloride **2** in excellent yield. It was treated with 3-chloropropylamine or 2-chloroethyamine to give benzamide **3**. Direct mono-amination of **3** with piperazine in chloroform obtain intermediate **4** in a good yield.

Furthermore, the commercially available phenylacetonitrile **5** firstly conversed to the corresponding amidoxime **6**, and followed by reaction with dimethyl acetylenedicarboxylate and cyclisation to desire pyrimidine methyl ester **7**. The ester **8** was prepared from **7** via K_2CO_3-promoted alkylation. Hydrolysis in standard conditions afforded the required acid **9**, which could then be progressed to amides **10**, **11** and **12** with variable amine **4** via pre-activation with CDI. Compounds **13**, **14** and **15** were obtained from compounds **10**, **11** and **12** by an exhaustive demethylation using boron tribromide in 50% yield.



Scheme 1. Reagant and conditions: (a) $SOCl_2$, reflux; (b) $Cl(CH_2)_nNH_2{\cdot}HCl$, Et_3N, DCM, rt; (c) piperazine, K_2CO_3, KI, $CHCl_3$, reflux; (d) $NH_2OH{\cdot}HCl$, KOH, reflux; (e) DMAD, $CHCl_3$, reflux; then xylene, reflux; (f) CH_3I, K_2CO_3, DMF, rt; (g) NaOH, H_2O/MeOH, rt; then 1N HCl(aq), rt; (h) **4**, CDI, DMF, rt; (i) 1N BBr_3, DCM, then MeOH

2.2. Inhibition of HIV IN activity

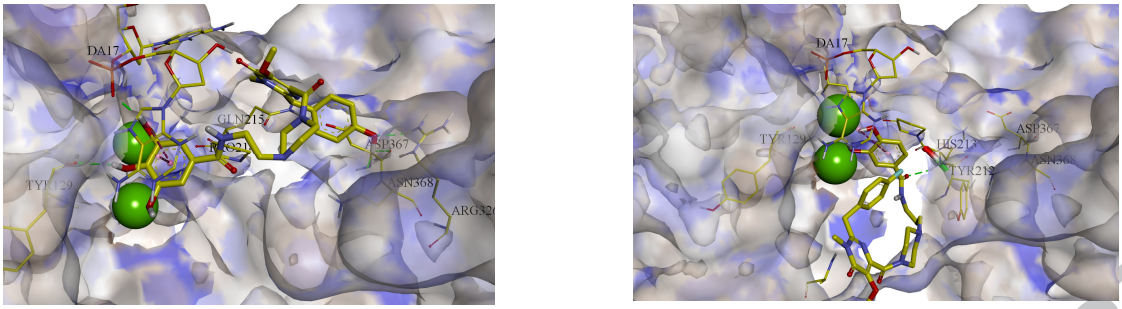
The inhibition effects of pyrimidone carboxamides **13-15** were measured by HIV-1 integrase strand transfer (ST) activity assay. Compounds **13-15** and reference compound raltegravir were tested in vitro for strand transfer in the enzyme inhibition assay, which was carried out as described previously and data were summarized in Table 1. 3,4-dihydroxylated aromatics derivatives **15** show less activity towards strand transfer, but 3,4,5-trihydroxylated aromatics derivatives **13** and **14** exhibit activities at micromolar concentrations. In comparing the potencies of **13**, **14** and **15** in the strand transfer assay, it was seen that introduction of a hydroxyl at 5-position of the caffeoyl in **15** gave **13** to improve IN inhibition activities. The results indicate the significance of polyhydroxyphenyl as a core pharmacophore. However, difference in the potencies between the compounds **13** and **14** is very low, which shows that the length of alkyl in linker domain isn't notable on their activities. In addition, in order to investigate the substituent effect on the phenyl ring in hydrophobic domain, different groups were utilized. But these compounds display significant potent inhibitory activities without any marked fluctuation. The HIV-IN inhibitory effect of compounds **13** and **14** are nearly as much as the reference compound raltegravir.

Table 1 Inhibition of HIV-1 integrase strand transfer catalytic activities[a]

Compounds	R^1	R^2	R^4	n	IC_{50}[b](μM)
13a	H	H	OH	3	0.9
13b	Cl	H	OH	3	2.4
13c	F	H	OH	3	18.9
13e	Me	H	OH	3	3.5
13f	Cl	Cl	OH	3	22.4
14a	H	H	OH	2	18.6
14c	F	H	OH	2	2.5
14d	OH	H	OH	2	0.7
15a	H	H	H	3	>100
15c	F	H	H	3	>100
15d	OH	H	H	3	>100
MK-0518					1.8

[a]HIV-1 IN inhibitory activities were measured according to the procedure described in Ref.18.

[b]Inhibition of strand transfer.



A **B**

Figure 3. A, B show the binding model of **14d** and **15c** respectively. Mg^{2+} ions (green sphere), oxygen atom (red stick), nitrogen atom (blue stick), H-bond (green dash line), metal bond (grey dash line), pi-pi stack (purple dash line).

2.3. Anti-HIV-1 activities

MAGI test, also called single life cycle, reflects only one round of infection. The test compound was added at different hours post the virus inoculation to observe which stage of the viral life cycle could be inhibited by the compound. The cells used were derived from HeLa cell line that both expresses high levels of CD4 and contains a single integrated copy of a β-galactosidase gene under the control of HIV-1 LTR. This cell line, called TZM-bl, can be used to determine quantitatively the titer of HIV wild type strains or HIV pseudoviruses. The inhibitory rate of the test compound could be calculated and the stage upon which the test compound acted could be determined. Compounds **13a**, **13b**, **13c** and **13e** were evaluated for their antiviral activity against HIV-1 replication in TZM-bl cells. TZM-bl cells were infected with HIV-1 and subsequently treated with increasing concentrations of drugs. The amount of virus was assayed by β-galactosidase assay, with HeLa-CD4-β-gal cells as reporting cells. Antiviral properties are report in Table 2.

Table 2 Antiviral activities of compounds **13a**, **13b**, **13c** and **13e**[a]

Compounds	EC_{50}(μM)
13a	1.13
13b	4.27
13c	4.39
13e	2.76

[a] Effictive concentration 50%

2.4. Docking Studies

To investigate the possible bonding mechanism of these compounds, an active compound and an inactive compound (**14d** and **15c**) were selected for docking studies. The binding mode of selected molecule was studied by Autodock 4.0 with the help of AutodockTools. The crystallographic structure of prototype foamy virus integrase (PFV-IN) with a double chains DNA and two magnesium ions (PDB:3OYA) was selected as the receptor for docking study[21], since it has been demonstrated that PFV-IN is a good model for HIV-1 integrase core domain[22].

In order to validate the docking protocol, the extracted ligand raltegravir (RAL, from 3OYA) was also re-docked into active site of the crystallographic structure of 3OYA. The best docking pose of RAL can higher superimposed with experimental crystallographic RAL with a calculated RMSD of 1.69.

The docking results are shown (Fig.3), the three hydroxylate ions of trihydroxybenzene in **14d** function to chelate with two Mg^{2+} ions and the benzene can form pi-pi stacking interactions with viral nucleotide DA17. In addition, the fourth hydroxyl in it can also bind to Asp367, Asn368 and Arg326 of integrase through H-bond. Howere, in **15c** there can only one hydroxyl which can function to Mg^{2+} as a metal acceptor and the benzyl just form pi-pi T-shaped stacking interactions with DA17. Moreover, the difference of AutoDock energy and AutoDock interaction energy of **14d** and **15c** can also well explain the difference of inhibiting activity.

3. Conclusion

In summary, a series of dihydropyrimidine carboxamides derivatives were facile synthesized and have been identified as HIV-1 integrase inhibitors. The biological results showed that the polyhydroxylated aromatic moiety plays an important role to inhibit HIV-1 integrase ST reaction. In particular, 3,4,5-trihydroxylated aromatics subunit is the most potential compounds. And further work based on these structures is in progress.

4. Experimental section

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and dried and purified by standard procedures. Melting points were determined on a Beijing Keyi elec-opti instrument factory melting point apparatus. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker Avance DRX400 spectrometer with $CDCl_3$, DMSO-d_6 or MeOH-d_4 as the solvent and tetramethyl-silane (TMS) as the internal standard. The chemical shifts were reported in δ (ppm). Mass spectra (MS) data were obtained using Esquire 6000 Mass Spectrometer. Petroleum ether used for column chromatography had a boiling range of 60-90^{o}C. Compounds **6** and **7**, as well as compounds **2**, **3**, and **4** were prepared according to the corresponding literature procedures[12,17].

*4.1.1. General procedure for the synthesis of dihydropyrimidine-4-carboxylate derivatives **8**.*

To a suspension of 5,6-dihydroxypyridine-4-carboxylate **7** (3.0mmol) in DMF (50mL) was added K_2CO_3 (9.0mmol) and CH_3I (9.0mmol) and the mixture was stirred overnight at room temperature. The mixture was extracted with EtOAc, and washed with H_2O and brine. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel eluting with petroleum ether/ether acetate (5:1).

*4.1.1.1. Methyl 2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydro pyrimidine-4-carboxylate (**8a**).*

Brown oil, yield: 30.0%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.36 (s, 3H), 3.92 (s, 3H), 3.95 (s, 3H), 4.13 (s, 2H), 7.15 (d, J = 7.2 Hz, 2H), 7.21 (m, 1H), 7.28 (t, J=6.8, 2H). MS (ESI): m/z 288.9 $[M+H]^+$.

*4.1.1.2. Methyl 2-(4-chlorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carboxylate (**8b**).*

Brown oil; yield: 31.2%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.42 (s, 3H), 3.98 (s, 3H), 4.01 (s, 3H), 4.15 (s, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 6.4 Hz, 2H). MS (ESI): m/z 322.9 $[M+H]^+$.

*4.1.1.3. Methyl 2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6 - dihydropyrimidine-4-carboxylate (**8c**).*

Brown oil; yield: 31.0%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.42 (s, 3H), 3.97 (s, 3H), 4.00 (s, 3H), 4.14 (s, 2H), 7.05-7.15 (m, 2H), 7.17-7.28 (m, 2H). MS (ESI): m/z 306.7 $[M+H]^+$.

*4.1.1.4. Methyl 2-(4-methoxybenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (**8d**).*

Brown oil; yield: 28.8%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.40 (s, 3H), 3.78 (s, 3H), 3.96 (s, 3H), 3.98 (s, 3H), 4.10 (s, 2H), 6.85(d, J = 8.8 Hz, 2H), 7.13(d, J = 8.4 Hz, 2H).

*4.1.1.5. Methyl 2-(4-methylbenzyl)-5-methoxy-1-methyl-6-oxo-1,6 -dihydropyrimidine-4-carboxylate (**8e**).*

Brown oil; yield: 29.6% ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 2.33 (s, 3H), 3.48 (s, 3H), 3.98 (s, 3H), 4.00 (s, 3H), 4.14 (s, 2H), 7.08 (d, J = 8.0 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H).

*4.1.1.6. Methyl 2-(3,4-dichlorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (**8f**).*

Brown oil; yield: 32.5%; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.42 (s, 3H), 3.98 (s, 3H), 4.01 (s, 3H), 4.15 (s, 2H), 7.04 (d, J = 7.2 Hz, 1H), 7.30 (s, 1H), 7.40 (d, J = 8.0 Hz, 1H).

*4.1.2. General procedure for the synthesis of dihydropyrimidine-4-carboxylic acid derivatives **9***

To a suspension of N-methylpyrimidone derivatives **8** (10.4mmol) in MeOH (50mL) was added 0.5N KOH (41.6mmol) at room temperature, the reaction mixture was acidified with 1N HCl after stirred one hour and then extracted with EtOAc. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated by rotary evaporation.

*4.1.2.1. 2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine -4-carboxylic acid (**9a**).*

Yellow solid; yield: 95%; m.p. 184-186 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.47 (s, 3H), 4.14 (s, 3H), 4.16 (s, 2H), 7.18 (d, J=6.8 Hz, 2H), 7.30-7.38 (m, 3H). MS (ESI): m/z 274.7 $[M+H]^+$.

*4.1.2.2. 2-(4-chlorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (**9b**).*

Yellow solid; yield: 96.2%; m.p. 155-157 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.49 (s, 3H), 4.15 (s, 3H), 4.16 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H). MS (ESI): m/z 308.8 $[M+H]^+$.

*4.1.2.3. 2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (**9c**).*

Yellow solid; yield: 93.1%; m.p. 137-138 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.50 (s, 3H), 4.15 (s, 3H), 4.15 (s, 2H), 7.07 (t, J = 8.4 Hz, 2H), 7.19(dd, J = 5.2 Hz and 8.4 Hz, 2H). MS (ESI): m/z 292.7 $[M+H]^+$.

*4.1.2.4. 2-(4-methoxybenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (**9d**).*

Yellow solid; yield: 96.3%; m.p. 133-134 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 3.47 (s, 3H), 3.79 (s, 3H), 4.09 (s, 3H), 4.10 (s, 2H), 6.87 (dd, J = 2.0 Hz and 6.8 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H).

*4.1.2.5. 2-(4-methylbenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (**9e**).*

Yellow solid; yield: 94.0%; m.p. 162-164 ^{o}C; ^{1}H NMR (400MHz, $CDCl_3$, δ ppm) δ: 3.49 (s, 3H), 4.15 (s, 3H), 4.16 (s, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.36(d, J = 8.0 Hz, 2H).

*4.1.2.6. 2-(3,4-dichlorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid (**9f**).*

Yellow solid; yield: 95.2%; m.p. 180-182 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 3.41 (s, 3H), 3.77 (s, 3H), 4.18 (s, 2H), 7.21(dd, J = 2.0 Hz and 8.4 Hz, 1H), 7.54 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H).

*4.1.3. General procedure for the synthesis of N-methylpyrimidone derivatives **10, 11, 12***

To suspension of CDI (2.38mmol) in dry DMF (10mL) was slowly added dihydropyrimidine-4-carboxylic acid derivatives **9** (1.83mmol) in dry DMF (25mL) at 0 ^{o}C and the mixture was stirred at room temperature for 1h, then compound **4** (1.83mL) was added. The mixture was stirred overnight at room temperature and the reaction was quenched by adding H_2O. The mixture was extracted with DCM and the organic layer was washed with H_2O, brine, and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and crude product was purified by column chromatography using ethyl acetate/ethanol (5:1).

*4.1.3.1. N-(3-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbobyl)pinperazin-1-yl)propyl)-3,4,5-trimethoxybenzamide (**10a**).*

Yellow solid; yield: 55.4%; m.p. 88-90 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.79-1.83 (m, 2H), 2.45 (t, J = 4.8 Hz, 2H), 2.54 (t, J = 6.8 Hz, 4H), 3.35 (t, J = 4.8 Hz, 2H), 3.42 (s, 3H), 3.55 (t, J = 6.0 Hz, 2H), 3.77 (t, J = 4.8 Hz, 2H), 3.87 (s, 3H), 3.89 (s, 6H), 3.94 (s, 3H), 4.12 (s, 2H), 7.00 (s, 2H), 7.10 (br, 1H), 7.17 (d, J = 6.8 Hz, 2H), 7.26-7.28 (m, 1H), 7.30-7.34 (m, 2H). MS (ESI): m/z 594.0 $[M+H]^+$.

*4.1.3.2. N-(3-(4-(2-(4-chlorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperzin-1-yl)propyl)-3,4,5-tri-methoxybenzamide (**10b**).*

White solid; yield: 53.0%; m.p. 85-88 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.81-1.84 (m, 2H), 2.43 (t, J = 4.8 Hz, 2H), 2.54 (t, J = 6.4 Hz, 4H), 3.34 (t, J = 4.8 Hz, 2H), 3.44 (s, 3H), 3.55 (t, J = 6.0 Hz, 2H), 3.77 (t, J = 4.8 Hz, 2H), 3.87 (s, 3H), 3.89 (s, 6H), 3.94 (s, 3H), 4.08 (s, 2H), 7.03 (s, 2H), 7.13 (d, J = 8.8 Hz, 2H), 7.21 (br, 1H), 7.29-7.32 (m, 2H). MS (ESI): m/z 628.0 $[M+H]^+$.

*4.1.3.3. N-(3-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trimethoxybenzamide (**10c**).*

Yellow solid; yield: 57.4%; m.p. 90-91 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.77-1.80 (m, 2H), 2.40 (t, J = 4.8 Hz, 2H), 2.51 (t, J = 6.8 Hz, 4H), 3.34 (t, J = 4.8 Hz, 2H), 3.41 (s,

3H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.74 (t, *J* = 4.8 Hz, 2H), 3.84 (s, 3H), 3.86 (s, 6H), 3.91 (s, 3H), 4.06 (s, 2H), 6.99 (s, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.24 (br, 1H). MS (ESI): m/z 612.0 $[M+H]^+$.

*4.1.3.4. N-(3-(4-(5-methoxy-1-methyl-2-(4-methylbenzyl)-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-tri-methoxybenzamide (**10e**).*

Yellow solid; yield: 55.2%; m.p. 86-88 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.81-1.85 (m, 2H), 2.33 (s, 3H), 2.47 (t, *J* = 4.8 Hz, 2H), 2.56 (t, *J* = 6.4 Hz, 4H), 3.37 (t, *J* = 4.8 Hz, 2H), 3.43 (s, 3H), 3.57 (t, *J* = 6.0 Hz, 2H), 3.79 (t, *J* = 4.8 Hz, 2H), 3.88 (s, 3H), 3.91 (s, 6H), 3.94 (s, 3H), 4.09 (s, 2H), 7.02 (s, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.14 (br, 1H). HRMS (ESI): m/z 608.3084 calcd for $C_{32}H_{42}N_5O_8$ $[M+H]^+$, found 608.3097.

*4.1.3.5. N-(3-(4-(2-(3,4-dichlorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4, 5-trimethoxybenzamide (**10f**).*

Yellow solid; yield: 59.3%; m.p. 105-108 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.78-1.84 (m, 2H), 2.39 (t, *J* = 4.4 Hz, 2H), 2.53 (t, *J* = 6.0 Hz, 4H), 3.31 (t, *J* = 4.8 Hz, 2H), 3.46 (s, 3H), 3.55 (t, *J* = 6.0 Hz, 2H), 3.76 (t, *J* = 4.8 Hz, 2H), 3.87 (s, 3H), 3.92 (s, 6H), 3.95 (s, 3H), 4.05 (s, 2H), 7.00 (s, 2H), 7.04 (dd, *J* = 2.0 Hz and 8.4 Hz, 1H), 7.05(br, 1H), 7.27(s, 1H), 7.40(d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) δ: 25.73, 31.22, 39.62, 40.83, 41.49, 46.59, 52.72, 53.21, 56.46, 57.04, 60.24, 60.89, 104.70, 128.00, 130.24, 130.52, 130.95, 131.82, 133.08, 134.40, 140.57, 141.11, 144.07, 153.20, 154.37, 159.35, 163.93, 167.11. HRMS (ESI): m/z 662.2148 calcd for $C_{31}H_{38}ClN_5O_7$ $[M+H]^+$, found 662.2147.

*4.1.3.6. N-(2-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)ethyl)-3,4,5-trimethoxybenzamide (**11a**).*

Yellow solid; yield: 62.1%; m.p. 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 2.51 (t, *J* = 4.8 Hz, 2H), 2.62 (t, *J* = 4.8 Hz, 2H), 2.66 (t, *J* = 6.0 Hz, 2H), 3.39 (t, *J* = 4.8 Hz, 2H), 3.44 (s, 3H), 3.57 (q, *J* = 6.4 Hz, 2H), 3.79-3.81 (m, 2H), 3.89 (s, 3H), 3.91 (s, 6H), 3.95 (s, 3H), 4.11 (s, 2H), 6.73 (br, 1H), 7.01 (s, 2H), 7.19 (d, *J* = 7.2 Hz, 2H), 7.26-7.28 (m, 1H), 7.33(d, *J* = 7.2 Hz, 2H). MS (ESI): m/z 580.1 $[M+H]^+$.

*4.1.3.7. N-(2-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)ethyl)-3,4,5-trimethoxybenzamide (**11c**).*

Yellow solid; yield: 65.7%; m.p. 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 2.51 (t, *J* = 4.8 Hz, 2H), 2.62 (t, *J* = 4.8 Hz, 2H), 2.66 (t, *J* = 6.0 Hz, 2H), 3.39 (t, *J* = 4.8 Hz, 2H), 3.43 (s, 3H), 3.57 (q, *J* = 6.4 Hz, 2H), 3.79-3.81 (m, 2H), 3.89 (s, 3H), 3.91(s, 6H), 3.95(s, 3H), 4.11(s, 2H), 6.73(br, 1H), 7.01(s, 2H), 7.19(d, *J* = 7.2 Hz, 2H), 7.26-7.28 (m, 1H), 7.33 (d, *J* = 7.2 Hz, 2H). HRMS (ESI): m/z 598.2677 calcd for $C_{30}H_{37}FN_5O_7$ $[M+H]^+$, found 598.2678.

*4.1.3.8. N-(2-(4-(5-methoxy-2-(4-methoxybenzyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)ethyl) benzamide (**11d**).*

Yellow solid; yield: 61.0%; m.p. 89-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 2.52 (t, *J* = 4.4 Hz, 2H), 2.63 (t, *J* = 4.4 Hz, 2H), 2.67 (t, *J* = 6.0 Hz, 2H), 3.39 (t, *J* = 4.4 Hz, 2H), 3.45 (s, 3H), 3.57 (q, *J* = 6.4 Hz, 2H), 3.80 (s, 3H), 3.80-3.82 (m, 2H), 3.90 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 4.07 (s, 2H), 6.64 (br, 1H), 6.87 (d, *J* = 8.4 Hz, 2H), 7.01 (s, 2H), 7.11 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) δ: 30.90, 31.24, 36.48, 41.38, 41.55, 46.66, 52.46, 52.92, 55.31, 56.40, 56.53, 60.30, 60.91, 104.52, 114.53, 125.96, 129.41, 130.03, 140.29, 141.10, 144.48, 153.24, 156.09, 158.97, 159.62, 164.18, 167.17. HRMS (ESI): m/z 610.2877 calcd for $C_{31}H_{40}N_5O_8$ $[M+H]^+$, found 610.2877.

*4.1.3.9. N-(3-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trimethoxybenzamide (**12a**).*

Yellow solid; yield: 54.5%; m.p. 77-80 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.77 (m, *J* = 6.0 Hz, 2H), 2.42 (t, *J* = 4.8 Hz, 2H), 2.49-2.53 (m, 4H), 3.32 (t, *J* = 4.8 Hz, 2H), 3.39 (s, 3H), 3.50 (q, *J* = 6.0 Hz, 2H), 3.74-3.75 (m, *J* = 4.8 Hz, 2H), 3.86(s, 3H), 3.87 (s, 3H), 3.91 (s, 3H), 4.09 (s, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 6.8 Hz, 2H), 7.22-7.30 (m, 3H), 7.28 (br, 1H), 7.43(s, 2H); HRMS (ESI): m/z 564.2822 calcd for $C_{30}H_{38}N_5O_6$ $[M+H]^+$, found 564.2825.

*4.1.3.10. N-(3-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4-dimethoxybenzamide (**12c**).*

Yellow solid; yield: 65.3%; m.p. 80-81 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.81-1.86 (m, 2H), 2.46 (t, *J* = 4.4 Hz, 2H), 2.56-2.59 (m, 4H), 3.35 (t, *J* = 4.8 Hz, 2H), 3.45 (s, 3H), 3.57 (q, *J* = 6.4 Hz, 2H), 3.80-3.82 (m, 2H), 3.93 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 4.10 (s, 2H), 6.87 (d, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 8.4 Hz, 2H), 7.16-7.18 (m, 2H), 7.19 (s, 1H), 7.37 (br, 1H), 7.48 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) δ: 25.33, 31.23, 39.86, 41.27, 41.53, 46.59, 52.80, 53.30, 56.01, 56.06, 57.47, 60.26, 110.20, 110.90, 115.93, 116.14, 118.92, 127.48, 129.79, 130.01, 140.37, 144.32, 149.07, 151.69, 155.44, 159.49, 160.86, 163.31, 164.03, 166.97. HRMS (ESI): m/z 582.2728 $C_{30}H_{37}FN_5O_6$, $[M+H]^+$, found 582.2726.

*4.1.3.11. N-(3-(4-(2-(4-methoxybenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4-dimethoxybenzamide (**12d**).*

Yellow solid; yield: 65.3%; m.p. 75-77 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 1.81 (m, *J* = 6.0 Hz, 2H), 2.48 (t, *J* = 4.4 Hz, 2H), 2.54-2.57 (m, 4H), 3.35 (t, *J* = 4.4 Hz, 2H), 3.42 (s, 3H), 3.55 (q, *J* = 6.4 Hz, 2H), 3.77 (s, 3H), 3.78-3.79 (m, *J* = 4.8 Hz, 2H), 3.91 (s, 6H), 3.91 (s, 3H), 4.05 (s, 2H), 6.85 (t, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.24-7.26 (m, 2H), 7.39 (br, 1H), 7.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) δ: 25.23, 31.24, 39.99, 41.38, 41.51, 46.59, 52.84, 53.36, 55.30, 56.03, 56.07, 57.61, 60.28, 110.20,110.93, 114.53, 118.84, 125.94, 127.49, 129.40, 140.29, 144.45, 149.11, 151.71, 156.09, 158.97, 159.61, 164.14, 166.97. HRMS (ESI): m/z 594.2928 calcd for $C_{31}H_{40}N_5O_7$, $[M+H]^+$, found 594.2915.

*4.1.4. General procedure for demethylation of N-methylpyrimidone derivative **13**, **14**, **15***

To a suspension of N-methylpyrimidone derivatives **10**, **11**, **12** (0.76mmol) in dry DCM (20mL) was added dropwise to a solution of BBr_3 (10mL, 1N) in dry DCM at 0 °C and the mixture was stirred overnight at room temperature, and then MeOH (30mL) was slowly added to above solution at 0 °C and the mixture was stirred continually for 1h. The solvent was evaporated under reduced pressure and crude product was purified by column chromatography using chloroform/methanol (3:1).

*4.1.4.1. N-(3-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trihydroxybenzamide (**13a**).*

White solid; yield: 50.1%; m.p. 200.2-203.2 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 2.04-2.07 (m, 2H), 2.70-2.80 (m, 2H), 3.00-3.10 (m, 2H), 3.15-3.30 (m, 2H), 3.30-3.40 (m, 2H), 3.40-3.60 (m, 4H), 3.52 (s, 3H), 3.90 (s, 3H), 4.20 (s, 2H), 6.90 (s, 2H), 7.17 (t, J = 7.2 Hz, 2H), 7.24-7.28 (m, 3H). MS (ESI): m/z 552.1 $[M+H]^+$.

*4.1.4.2. N-(3-(4-(2-(4-chlorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5–tr-ihydroxybenzamide (**13b**).*

White solid; yield: 55.0%; m.p. 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 1.55-1.75 (m, 2H), 2.10-2.45 (m, 4H), 3.10-3.30 (m, 4H), 3.30-3.40 (m, 2H), 3.50-3.70 (m, 2H), 3.43 (s, 3H), 3.77 (s, 3H), 4.16 (s, 2H), 6.81 (s, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 8.08 (br, 1H), 8.61 (s, 1H), 8.97 (s, 2H). MS (ESI): m/z 584.9 $[M-H]^-$.

*4.1.4.3. N-(3-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trihydroxybenzamide (**13c**).*

White solid; yield: 52.4%; m.p. 187-190 °C; ^{1}H NMR (400MHz, MeOH-d_4, δ ppm) δ: 2.05-2.08 (m, 2H), 2.80-2.90 (m, 2H), 3.00-3.15 (m, 2H), 3.15-3.20 (m, 2H), 3.30-3.35 (m, 2H), 3.40-3.55 (m, 4H), 3.57 (s, 3H), 3.92 (s, 3H), 4.18 (s, 2H), 6.89 (s, 2H), 7.08 (t, J = 8.4 Hz, 2H), 7.27 (q, J = 8.0 Hz, 2H). MS (ESI): m/z 567.9 $[M-H]^-$.

*4.1.4.4. N-(3-(4-(2-(4-methylbenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trihydroxybenzamide (**13e**).*

White solid; yield: 51.0%; m.p. 253-255 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 2.05-2.08 (m, 2H), 2.29 (s, 3H), 2.90-3.00 (m, 2H), 3.00-3.20 (m, 2H), 3.20-3.25 (m, 2H), 3.30-3.40 (m, 2H), 3.50-3.60 (m, 4H), 3.50 (s, 3H), 3.90 (s, 3H), 4.15 (s, 2H), 6.90 (s, 2H), 7.11 (d, J = 8.0 Hz, 2H), 7.15(d, J = 8.0 Hz, 2H). MS (ESI): m/z 563.9 $[M-H]^-$.

*4.1.4.5. N-(3-(4-(2-(3,4-dichlorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)-propyl)-3,4, 5-trihydroxybenzamide (**13f**).*

White solid; yield: 56.6%; m.p. 207-209 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 1.60-1.75 (m, 2H), 2.10-2.50 (m, 4H), 3.10-3.25 (m, 4H), 3.25-3.40 (m, 2H), 3.40-3.60 (m, 2H), 3.46 (s, 3H), 3.77 (s, 3H), 4.17 (s, 2H), 6.81 (s, 2H), 7.23 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 2H), 8.06 (br, 1H), 8.60 (s, 1H), 8.97 (s, 2H). MS (ESI): m/z 617.9 $[M-H]^-$.

*4.1.4.6. N-(2-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)ethyl)-3,4,5-trihydroxybenzamide (**14a**).*

White solid; yield: 55.7%; m.p. 193-194 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 3.10-3.20 (m, 2H), 3.20-3.40 (m, 2H), 3.45-3.60 (m, 8H), 3.50 (s, 3H), 3.91 (s, 3H), 4.22 (s, 2H), 6.93 (s, 2H), 7.27 (q, J = 7.2 Hz, 3H), 7.35(t, J = 7.2 Hz, 2H). MS (ESI): m/z 535.9 $[M-H]^-$.

*4.1.4.7. N-(3-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1, 6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4,5-trihydroxybenzamide (**14c**).*

White solid; yield: 52.8%; m.p. 210-213 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 3.15-3.25 (m, 2H), 3.25-3.40 (m, 2H), 3.45-3.65 (m, 8H), 3.48 (s, 3H), 3.90 (s, 3H), 4.19 (s, 2H), 6.91 (s, 2H), 7.27 (d, J = 8.0 Hz, 2H), 7.35 (d, J =8.0 Hz, 2H); MS (ESI): m/z 553.9 $[M-H]^-$.

*4.1.4.8. N-(2-(4-(2-(4-hydroxybenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)ethyl)-3,4,5-trihydroxybenzamide (**14d**).*

White solid; yield: 55.0%; m.p. 145-147 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 2.80-3.00 (m, 2H), 3.00-3.20 (m, 2H), 3.30-3.50 (m, 8H), 3.29 (s, 3H), 3.80 (s, 3H), 4.01 (s, 2H), 6.71 (d, J = 8.4 Hz, 2H), 6.84 (s, 2H), 7.01 (d, J = 8.4 Hz, 2H). MS (ESI): m/z 551.9 $[M-H]^-$.

*4.1.4.9. N-(3-(4-(2-benzyl-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4-dihydroxybenzamide (**15a**).*

White solid; yield: 57.5%; m.p. 162-164 °C; ^{1}H NMR (400MHz, MeOH-d_4, δ ppm) δ: 2.00-2.10 (m, 2H), 2.80-2.90 (m, 2H), 3.00-3.15 (m, 2H), 3.20-3.28 (m, 2H), 3.30-3.45 (m, 2H), 3.45-3.60 (m, 4H), 3.53 (s, 3H), 3.92 (s, 3H), 4.23 (s, 2H), 6.81 (d, J = 8.0 Hz, 1H), 7.25-7.30 (m, 4H), 7.30-7.37 (m, 3H); ^{13}C NMR (100 MHz, MeOH-d_4, δ ppm) δ: 22.83, 25.27, 29.33, 30.62, 37.35, 40.10, 40.93, 45.24, 51.87, 52.37, 55.34, 59.44, 114.40, 114.51, 119.30, 125.40, 126.98, 128.50, 128.63, 134.75, 140.41, 143.38, 144.96, 148.87, 156.92, 159.90, 164.65, 169.04; MS (ESI): m/z 536.2 $[M+H]^+$.

*4.1.4.10. N-(3-(4-(2-(4-fluorobenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)propyl)-3,4-dihydroxybenzamide (**15c**).*

White solid; yield: 53.0%; m.p. 156-158 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 2.07-2.12 (m, 2H), 2.75-2.95 (m, 2H), 3.05-3.15 (m, 2H), 3.20-3.30 (m, 2H), 3.30-3.40 (m, 2H), 3.50-3.65 (m, 4H), 3.56 (s, 3H), 3.91 (s, 3H), 4.19 (s, 2H), 6.80 (d, J = 8.0 Hz, 1H), 7.09 (t, J =8.8 Hz, 2H), 7.25-7.33 (m, 4H). ^{13}C NMR (100MHz, MeOH-d_4, δ ppm) δ: 22.84, 25.15, 29.35, 30.55, 37.24, 39.92, 40.13, 45.05, 51.83, 52.26, 55.26, 59.49, 78.11, 114.44, 114.55, 115.38, 119.37, 125.23, 125.33, 129.72, 140.42, 143.24, 144.95, 148.88, 156.35, 157.36, 159.95, 164.67, 169.09. MS (ESI): m/z 554.1 $[M+H]^+$.

*4.1.4.11. N-(3-(4-(2-(4-hydroxybenzyl)-5-methoxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carbonyl)piperazin-1-yl)-propyl)-3,4-dihydroxybenzamide (**15d**).*

White solid; yield: 53.6%; m.p. 162-165 °C; ^{1}H NMR (400 MHz, MeOH-d_4, δ ppm) δ: 2.05-2.10 (m, 2H), 2.70-2.90 (m, 2H), 3.00-3.10 (m, 2H), 3.15-3.25 (m, 2H), 3.30-3.40 (m, 2H), 3.45-3.55 (m, 4H), 3.49 (s, 3H), 2.89 (s, 3H), 4.09 (s, 2H), 6.08 (q, J = 8.4 Hz, 3H), 7.07 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.33 (s, 1H). ^{13}C NMR (100MHz, MeOH-d_4, δ ppm) δ: 22.84, 25.06, 29.36, 30.59, 37.16, 39.76, 40.03, 44.90, 51.76, 52.25, 55.17, 59.49, 60.16, 78.12, 114.54, 115.13, 119.39, 125.31, 140.51, 143.19, 144.94, 148.89, 156.75, 159.87, 160.79, 163.22, 164.64, 169.09. MS (ESI): m/z 549.9 $[M-H]^-$.

4.2. HIV-1 integrase inhibitory assay

Compounds diluted in DMSO were pre-incubated with 800ng integrase at 37.8 °C in the reaction buffer in the absence of Mn^{2+} for 10min. Subsequently, 1.5 pmol donor DNA and 9 pmol target DNA were added and the reaction was initiated by the addition of 10 mmol/L Mn^{2+} into the final reaction volumn. The reactions were carried out at 37.8 °C for 1h and subsequent detection procedure was applied to detect the assay signals. Raltegravir, current being clinical drug, was used as the positive control, whereas no compound but only DMSO in reaction mixture was set as the negative control. The inhibition effects of N-methylpyrimidone derivatives **13**, **14**, **15** were calculated based on the positive and negative controls.

4.3. Antiviral assay

The HeLa-CD4-LTR-b-gal indicator cells were plated in 96-well plates at 6000 cells per well. The highest concentration of the test compounds was 200 μg/mL, then diluted by four-fold serially. There were 5 dilutions and 4 duplicates for each dilution. The wells added only with virus-infected cells and without compounds were used as viral-control and the wells added only with mock-infected cells and without virus or compounds were used as cell-control. The supernatant in the test wells and the viral-control wells was discarded, and infected with 100 μL 2000 $TCID_{50}$ HIV-1 pseudoviruses. Then 100 μL diluted test compounds described above was added to the wells, making the final concentration of the test compounds decreased by half. The cell cultures were incubated at 37 ^{o}C in 5% CO_2 humidified atmosphere for 40–48 h, then fixed and stained. The blue cells were counted under an inverted microscope.

4.4. Molecular Docking Methods

4.4.1. HIV-1 integrase core initial model building

The HIV-1 integrase core model was generated by Discovery Studio 4.0 (DS4.0) based on the A, C and D chains of 3OYA crystallographic structure. Unwanted water and ligands were removed by the DS4.0, and the lost atoms such as hydrogen were added to build the initial receptor structure for docking [23].

4.4.2. Small molecular preparation

The structure of **14d** and **15c** was drawn by Gaussian03 software, then optimized the molecules to the minimum energy conformation used the semi-empirical AM1 method .

4.4.3. docking protocol

Docking procedure was performed by AutoDock 4 software with the help of Autodock Tools. The no-polar hydrogen of receptor and ligands were removed firstly, then, add the Computer Gasteiger charge for receptor and ligands. The grid maps were calculated using AutoGrid4 for three dockings, a grid map with 60×70×60 points and a grid-point spacing of 0.375 Å was applied. In order to fully explore the possible binding conformations, 100 conformations were generated using the Lamarckian Genetic Algorithm (LGA)[24]. For other docking parameters, standard values were used as software default. Cluster analysis was performed on the results using a root mean square (RMS) tolerance of 2.0 Å. The conformations and binding energy for further analysis were obtained from the average of the biggest cluster.

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