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# Synthesis and biological evaluation of new HIV-1 protease inhibitors with purine bases as P2-ligands

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**Abstract:** Introducing purine bases to P2-ligands might enhance the potency of Human Immunodeficiency Virus-1 (HIV-1) protease inhibitory because of the carbonyl and NH groups promoting the formation of extensive H-bonding interactions. In this work, thirty-three compounds are synthesized and evaluated, among which inhibitors **16a**, **16f** and **16j** containing *N*-2-(6-substituted-9*H*-purin-9-yl)acetamide as the P2-ligands along with 4-methoxylphenylsulfonamide as the P2'-ligand, display potent inhibitory effect on the activity of HIV-1 protease with $IC_{50}$ 43 nM, 42 nM and 68 nM *in vitro*, respectively.

**Keywords:** purine bases; HIV-1 protease inhibitors; biological evaluation

## 1. Introduction

AIDS is one of the most challenging problems in medicine and causes severe perniciousness situations for human health. More than thirty kinds of drugs have thus far been approved for the treatment of HIV/AIDS for targeting different steps of the HIV viral life cycle. [1] It has become evident that combination chemotherapy is significantly more effective than dosing drugs sequentially. [2] The appearance of HIV-1 protease inhibitors (PIs) in the mid-1990's and their combination with reverse transcriptase inhibitors marked the beginning of highly active antiretroviral therapy (HAART). [3,4] However, there still exist severe problems, such as the emergence of extensively cross-resistant strains of HIV-1, in addition to adverse effects. [5-7] Thus, there is an urgent need for new anti-HIV drug candidates with increased potency, improved pharmacokinetic properties, and reduced side effects.

HIV-1 protease inhibitors (HIV-1 PIs) play an important role in the treatment of HIV/AIDS. [8,9] Although approximate ten HIV-1 PIs have been approved by the FDA, the rapid emergence of multi-drug-resistant (MDR) strains of HIV-1 protease (PR) has severely limited long-term treatment options. [10-13] Thus, the design of novel HIV-1 PIs is urgent.

As is already known, nucleoside drugs are important antitumor, antifungal and especially antiviral agents. [14-19] We introduced purine bases into P2 moieties of HIV-1 protease inhibitors in this work, aiming at increasing inhibitory activity, according to the strategy to overcome drug resistance through increasing interactions between inhibitors and protease, carbonyl and/or NH groups involved in newly introduced P2 moieties can promote extensive H-bonding interactions involved directly or

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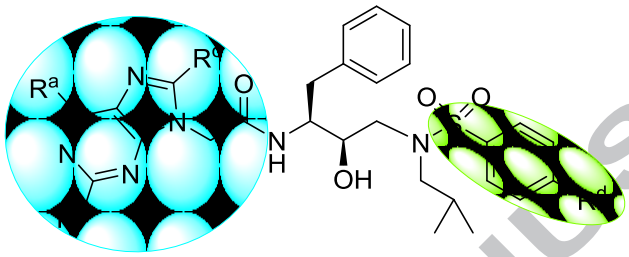
water-mediated with the backbone NH groups of residues Asp29 and Asp30 of PR in the corresponding S2 subsite (**Fig. 1**); these interactions may be responsible for the impressive activities against MDR PR mutants and antiviral activities in cellular assays. [20-25] According to the literatures and our previous studies, 4-substitutied phenylsulfonamide on P2'-ligands showed better inhibitory effect on the activity of HIV-1 protease than other substitutied phenylsulfonamide. [26-29] Both modifications of the substitution pattern on the phenylsufonamide P2'-ligands of all series, and the configuration of purine bases on P2-ligands, were synthesized for the structure-activity relationship (SAR) study in this work.




**Target molecules**

$\mathbf{R^a}$ = H, $NH_2$, OH, $OCH_3$, $CH_3$, Cl; $\mathbf{R^b}$ = H, $NH_2$; $\mathbf{R^c}$ = H, $CH_3$

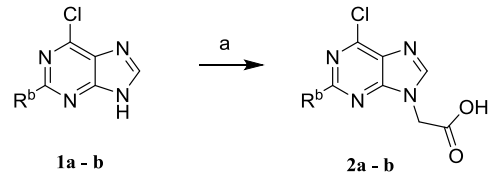
$\mathbf{R^d}$ = $OCH_3$, $NO_2$, $NH_2$

**Fig. 1.** Design and general structure of target molecules

## 2. Results and Discussion

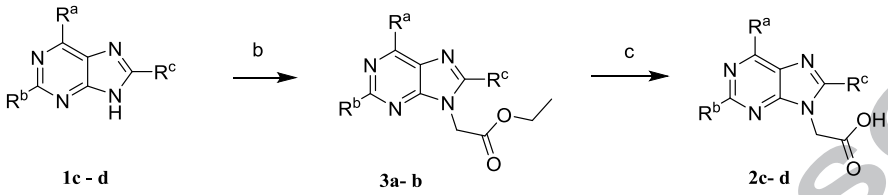
The syntheses of intermediates of substituted 2-(9*H*-purin-9-yl) acetic acids **2a−k** are outlined in **Scheme 1**. All starting materials are commercially available. The acids **2a** and **2b** were readily accomplished by substitution with bromoacetic acid using potassium carbonate as base from corresponding 6-chloro-9*H*-purines (**1a** and **1b**) in moderate yields. [30] *N*-Alkylation of **1c** and **1d** with ethyl bromoacetate proceeded using sodium hydride as the base in anhydrous DMF to give corresponding esters **3a** and **3b** in yields of 52% and 59%, respectively. Subsequent saponification was finished by sodium hydroxide to give acetic acids **2c** and **2d** in 55% and 45% yields, respectively. However, acids **2e−g** were synthesized directly by saponification using a "one−pot" method after the *N*-alkylation of **1e−g** in 43-62% yields. The 2-(6-methoxy-9*H*-purin-9-yl) acetic acids **2h−i** were accomplished firstly by refluxing **1a−b** with sodium methylate in methanol as 6-methoxy purines **4a−b**, followed by *N*-alkylation and then hydrolysis using the same method as described in **2c−e**. For synthesis of 6-hydroxy purines acetic acids **2j−k**, 6-chloro-9*H*-purines (**1a−b**) were heated at 130 ℃ with sodium benzyloxide in benzyl alcohol to give 6-benzyloxy-9*H*-purines (**5a−b**), followed by treatment of ethyl bromoacetate to afford purine acetates (**6a−b**). The benzyl protecting group was removed by hydrogenolysis under 25 bar of hydrogen using lead(Ⅱ) acetate as a catalyst in methanol for 12 hours in a moderate yield of 6-hydroxy purine esters **7a−b**.

Saponification of the ester moiety permitted conversion to acids **2j−k**.



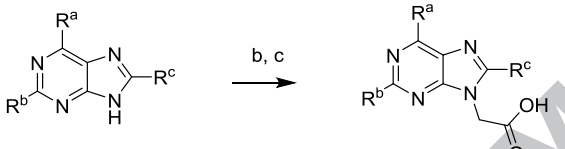

**1a - b** **2a - b**

**1a** and **2a: $R^b$ = H;**
**1b** and **2b: $R^b$ = $NH_2$**



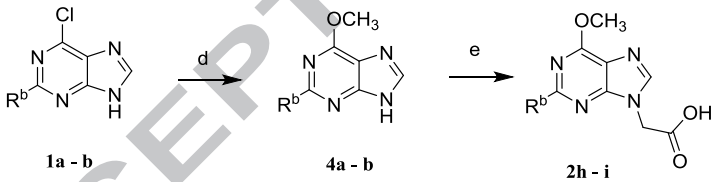

**1c - d** **3a- b** **2c- d**

**1c**, **3a** and **2c: $R^a$ = H, $R^b$ = H, $R^c$ = H;**
**1d**, **3b** and **2d: $R^a$ = $NH_2$, $R^b$ = H, $R^c$ = H;**



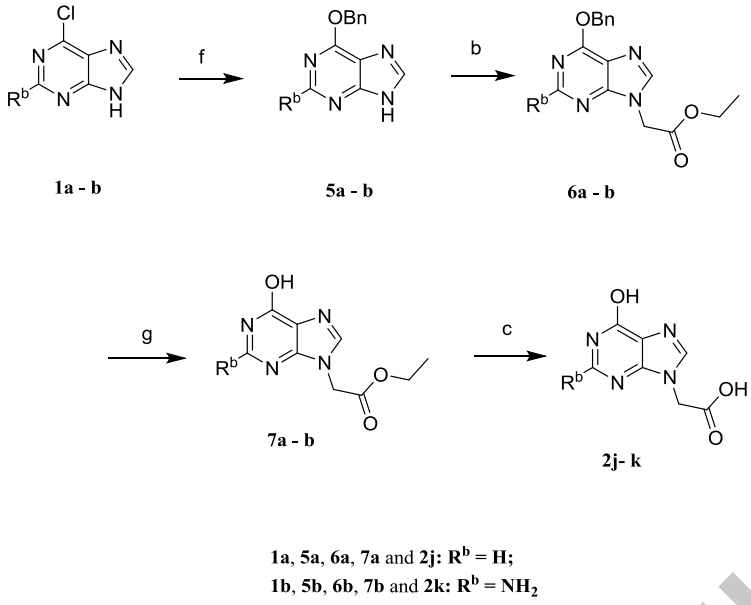

**1e - g** **2e- g**

**1e** and **2e: $R^a$ = H, $R^b$ = NH2, $R^c$ = H;**
**1f** and **2f: $R^a$ = $CH_3$, $R^b$ = H, $R^c$ = H;**
**1g** and **2g: $R^a$ = H, $R^b$ = H, $R^c$ = $CH_3$**



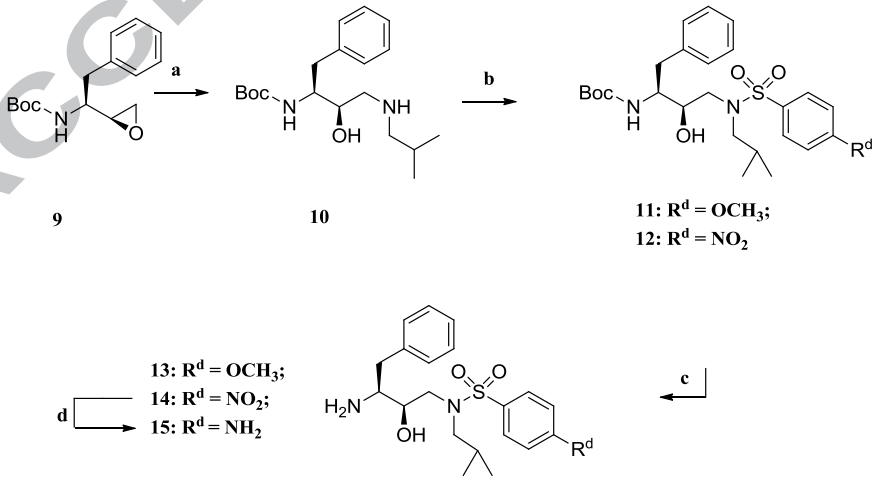

**1a - b** **4a - b** **2h - i**

**1a**, **4a** and **2h: $R^b$ = H;**
**1b**, **4b** and **2i: $R^b$ = $NH_2$**




**Scheme 1.** Syntheses of substituted 2-(9*H*-purin-9-yl) acetic acids **2a-k**. Reagents and conditions: (a) Bromoacetic acid, $K_2CO_3$, anhydrous DMF, Ar, r.t, overnight; (b) Ethyl bromoacetate, NaH, anhydrous DMF, Ar, 0 ℃~ r.t, 3 h; (c) (i) NaOH, $H_2O$, r.t, 1 h; (ii) 4 M HCl, 0 ℃, 0.5 h; (d) $CH_3ONa$, $CH_3OH$, reflux, 12 h; (e) (i) Ethyl bromoacetate, NaH, anhydrous DMF, Ar, 0 ℃~ r.t, 3 h; (ii) NaOH, $H_2O$, r.t, 1 h; (iii) 4 M HCl, 0 ℃, 0.5 h; (f) (i) $PhCH_2OH$, Na, 130 ℃, 2 h; (ii) $PhCH_2OH$, 130 ℃, 4 h; (g) $H_2$ (gas), 25 bar, $Pb(OAc)_2$, $CH_3OH$, r.t, 12 h.

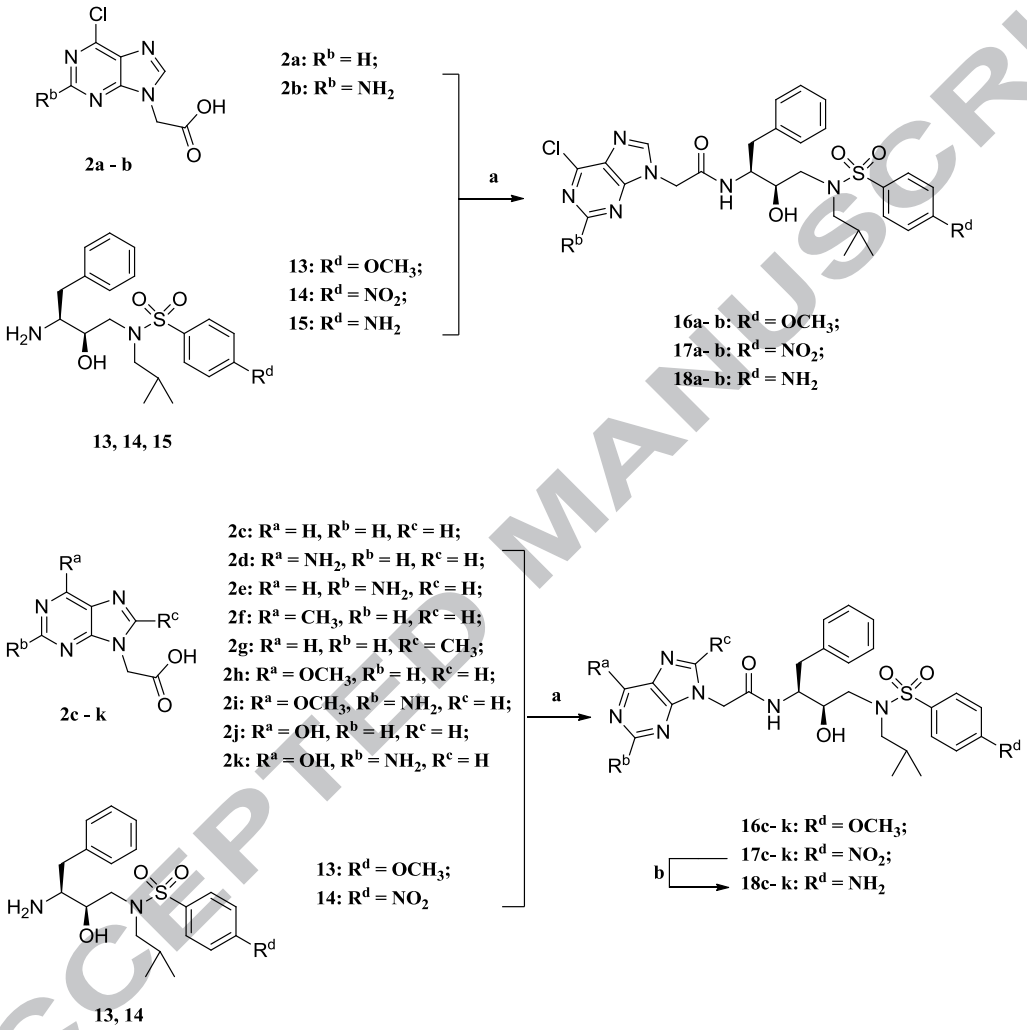
Compounds **13-15** were prepared from the commercially available material (2*S*, 3*S*)-1,2-epoxy-3-(boc-amino)-4-phenylbutane (**9**), as reported in the literature and shown in **Scheme 2**. [26-28] Catalytic hydrogenation of **14** over 10% Pd/C in methanol afforded the corresponding aminosulfonamide derivatives **15** [29].




**Scheme 2.** Syntheses of amines **13-15**. Reagents and conditions: (a) *i*-$BuNH_2$, $CH_3CN$, 80 ℃, 6 h; (b) Aryl sulfonyl chloride, DIEA, DMAP(Cat.), THF, 0 ℃ ~ r.t, 3- 5 h; (c) $CH_2Cl_2$-$CF_3COOH$

(1:1), 0 ℃~ r.t, 3 h; (d) $H_2$ (gas), 50 psi, 10% Pd/C, $CH_3OH$, r.t, 2 h.

The syntheses of inhibitors **16−18** shown in **Scheme 3** were carried out by coupling acids **2a−k** with amines **13−15** under an EDCI/HOBt/DMAP-mediated coupling method. [26-29] Catalytic hydrogenation of **17c−k** over 10% Pd/C in methanol afforded the corresponding aminosulfonamide derivatives**18c−k**.




**Scheme 3.** Syntheses of inhibitors **16−18**. Reagents and conditions: (a) EDCI, HOBt, DMAP, anhydrous DMF, Ar, 0 ℃~ r.t, 3 h; (b) $H_2$ (gas), 50 psi, 10% Pd/C, $CH_3OH$, r.t, 2 h.

The inhibitory activities of the synthetic compounds against HIV-1 wild-type protease were evaluated *in vitro* using a fluorescence resonance energy transfer (FRET) method. [31,32] As a reference of the best marketed compound, darunavir (DRV) has also been included. Purine bases as P2-ligands were investigated in combination with other phenylsulfonamide substituents as P2 '-ligands in the protease S2 ' subsite. The results are presented in **Table 1**. Most of the inhibitors with purine base amine-acetamide P2-ligands displayed micromolar potency. Inhibitors **16a**, **16f** and **16j** containing *N*-2-(6-substituted-9*H*-purin-9-yl)acetamide as the P2-ligands along

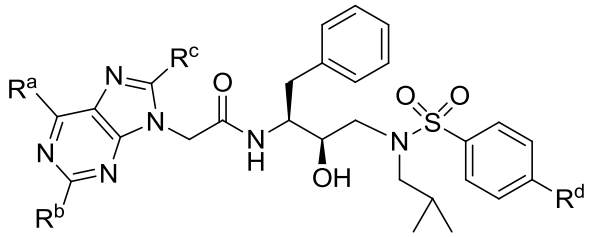
with 4-methoxylphenylsulfonamide as the P2'-ligand, displayed potent inhibitory activity of HIV-1 protease with $IC_{50}$ 43 nM, 42 nM and 68 nM *in vitro*, respectively.

As shown in **Table 1**, most of the series of purine derivatives exhibited micromolar inhibitory potency. However, functional 2'-phenylsulfonamide ligand still plays a vital role in this series of inhibitors, as reported in previously results. For example, phenylsulfonamide derivatives with a 4-methoxy substituent displayed generally higher potency in sub-micromolar potency than that with 4-amino and 4-nitro substituents. The P2' oxygen atom of OMe is likely to form hydrogen bonds (O⋯H-N) with the main-chain amide of Asp30' in the protease S2 ' subsite, similar to inhibitors GRL-0489A, TMC-126, GRL-0467 and GRL-0519A. [6,23] Moreover, the strong electron-withdrawing group 4-nitro would reduce the electron density of not only the oxygen atom itself via inductive effects but also the oxygen atom on the sulfonyl group via conjugative effects, reducing the ability of hydrogen to bond with the amide of Asp30' (phenyl-$O_{inh}$⋯N-H) or water-mediated interactions with the amide of Ile50' ($SO_{2inh}$⋯$H_2O$⋯N-H) in the generally conserved protease S2' subsite. [20-22] Among these compounds, inhibitors with chloride atom at $R^a$ and methoxy group at $R^d$ showed better potency than those with electron-donating groups at $R^a$ and methoxy group at $R^d$, i.e., **16a** *vs* **16b**, **16d**, **16e**, **16f**, **16g**, **16h**, **16i**, **16j** and **16k**. It also appears that more substituted groups at the purine ring such **16a** *vs* **16b**, **16h** and **16i** were detrimental to the inhibitory potency. This observation suggests that higher electron density or bigger space volume on the purine ring might reduce the ability of nitrogen atoms to act as hydrogen bond-accepting groups to form hydrogen bonds with the amide of possible residues in the generally conserved protease S2 subsite. Furthermore, methyl substituent in hexa-heterocycle of purine with a 4-methoxy substituent in P2'-ligand shows better potency than that of methyl substituent in penta-heterocycle of purine such **16f** *vs* **16g**, which might give a hint that the location of the substituent might affect the combination of compounds with the protease S2 subsite. In addition, the purine ring might be improper to adapt to the cavity in the protease S2 subsite very well, the inhibitory potency of which was inferior to that with the pyrimidine ring in our later work. The reason might be that massive and rigid structures were not matched with the residues of the protease S2 subsite very well, just as previously reported that inhibitors with flexible heterocyclic moieties at P2 were considerably more potent than their inflexible and acyclic counterparts in both enzyme inhibitory and antiviral assays. [25]

In summary, we have reported the structure-based design of novel HIV-1 protease inhibitors incorporating purine bases as P2-ligands and phenylsulfonamides as P2'-ligands. The inhibitors were designed with the purpose of making extensive hydrogen binding interactions with the protein backbone of HIV-1 protease active sites by introducing heterocyclic moieties, carbonyl groups, and amino groups on purine bases. Both P2- and P2'-ligands were involved in hydrogen bonding interactions with the backbone of both S2 and S2' subsites. It was found that inhibitors **16a**, **16f** and **16j** containing *N*-2-(6-substituted-9*H*-purin-9-yl)acetamide as the P2-ligands along with 4-methoxylphenylsulfonamide as the P2'-ligand, display potent inhibitory effect on the activity of HIV-1 protease with $IC_{50}$ 43 nM, 42 nM and 68 nM *in vitro*, respectively. Further study on the evaluation of another nucleobases,

pyrimidine bases are currently underway.

**Table 1** Enzymatic inhibitory activities of inhibitors with purine base amine-acetamide P2-ligands



| Compd. | $R^a$ | $R^b$ | $R^c$ | $R^d$ | $IC_{50}$[a](μM) | Compd. | $R^a$ | $R^b$ | $R^c$ | $R^d$ | $IC_{50}$[a](μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16a | Cl | H | H | OMe | 0.043±0.007 | 18f | Me | H | H | $NH_2$ | 3.76±0.90 |
| 17a | Cl | H | H | $NO_2$ | 0.31±0.11 | 16g | H | H | Me | OMe | 0.15±0.091 |
| 18a | Cl | H | H | $NH_2$ | 7.02±5.96 | 17g | H | H | Me | $NO_2$ | 0.57±0.18 |
| 16b | Cl | $NH_2$ | H | OMe | 0.57±0.19 | 18g | H | H | Me | $NH_2$ | 0.64±0.26 |
| 17b | Cl | $NH_2$ | H | $NO_2$ | 0.96±0.42 | 16h | OMe | H | H | OMe | 0.18±0.08 |
| 18b | Cl | $NH_2$ | H | $NH_2$ | 11.7±8.57 | 17h | OMe | H | H | $NO_2$ | 2.60±0.25 |
| 16c | H | H | H | OMe | 0.24±0.16 | 18h | OMe | H | H | $NH_2$ | 1.51±0.24 |
| 17c | H | H | H | $NO_2$ | 2.58±1.72 | 16i | OMe | $NH_2$ | H | OMe | 0.46±0.21 |
| 18c | H | H | H | $NH_2$ | 0.57±0.52 | 17i | OMe | $NH_2$ | H | $NO_2$ | 1.73±0.80 |
| 16d | $NH_2$ | H | H | OMe | 1.98±0.55 | 18i | OMe | $NH_2$ | H | $NH_2$ | 0.19±0.069 |
| 17d | $NH_2$ | H | H | $NO_2$ | 3.58±1.27 | 16j | OH | H | H | OMe | 0.068±0.029 |
| 18d | $NH_2$ | H | H | $NH_2$ | 1.24±0.62 | 17j | OH | H | H | $NO_2$ | 2.43±0.51 |
| 16e | H | $NH_2$ | H | OMe | 0.36±0.17 | 18j | OH | H | H | $NH_2$ | 1.81±0.34 |
| 17e | H | $NH_2$ | H | $NO_2$ | 3.68±1.12 | 16k | OH | $NH_2$ | H | OMe | 0.080±0.020 |
| 18e | H | $NH_2$ | H | $NH_2$ | 6.79±3.25 | 17k | OH | $NH_2$ | H | $NO_2$ | 0.79±0.43 |
| 16f | Me | H | H | OMe | 0.042±0.027 | 18k | OH | $NH_2$ | H | $NH_2$ | 4.73±2.80 |
| 17f | Me | H | H | $NO_2$ | 2.53±1.99 | DRV(nM) | - | - | - | - | 4.01±1.35 |

[a] Values were means of three independent experiments.

**Acknowledgements**

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**Conflicts of Interest**

The authors declare no competing financial interests.

**Supplementary data**

Supplementary data associated with this article can be found in the online version. The data include MOL files of the compounds described in this article.

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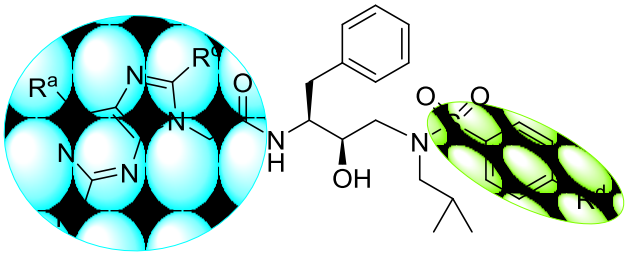


**Target molecules**

$\mathbf{R^a}$ = H, $NH_2$, OH, $OCH_3$, $CH_3$, Cl; $\mathbf{R^b}$ = H, $NH_2$; $\mathbf{R^c}$ = H, $CH_3$

$\mathbf{R^d}$ = $OCH_3$, $NO_2$, $NH_2$

$IC_{50}$ = 43 nM, 42 nM and 68 nM (*in vitro*)