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Design, synthesis and biological evaluation of HIV-1 protease inhibitors with morpholine derivatives as P2 ligands in combination with cyclopropyl as P1′ ligand

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ABSTRACT

A series of novel HIV-1 protease inhibitors has been designed and synthesized, which contained morpholine derivatives as the P2 ligands and hydrophobic cyclopropyl as the P1′ ligand at the meantime in this study, with the aim of improving the interactions between the active sites of HIV-1 protease and the inhibitors. Twenty-eight compounds were synthesized and assessed, among which inhibitors **m18** and **m1** exhibited excellent inhibitory effect on the activity of HIV-1 protease with IC_{50} value of 47 nM and 53 nM, respectively. The molecular modeling of **m1** revealed possible hydrogen bondings or van der Waals between the inhibitor and the protease, worthy of in-depth study.

As a degenerative disease of immune system, acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), is a social and medical problem of staggering dimensions.[1] It is reported that there are approximately 1.7 million new infections and 770 thousand people died of AIDS-related illnesses in 2018. So effective chemotherapy drugs are urgently needed to combat the disease.

The highly active antiretroviral therapy treatment (HAART), consisting of HIV-1 protease inhibitors (PIs) and two or more reverse transcriptase inhibitors (RTIs), is certainly a blessing for patients with AIDS.[2] Ten HIV-1 PIs have been approved in clinical practice nowadays, which could prevent the cleavage of viral precursor peptides Gag and Gag-pol being enzymatic proteins and mature virions, so as to produce morphologically immature and noninfectious viral particles.[3] However, there still exists serious problems, such as genetic diversity, side effects, especially drug resistance.[4] Hence, there is an urgent need for novel HIV-1 PIs.

One of the most effective design strategies for eliminating the resistance is to increase the hydrogen bonds between inhibitor and the protease. The bis-THF of P2 ligand in Darunavir (DRV) forms strong hydrogen bonding interactions through cyclic ether oxygens with the backbone amide NH of the protease residues in the S2 subsite, which may explain the high resistance profile of DRV.[5,6] Inspired by the above, morpholine, with flexible ring including oxygen and nitrogen groups, was introduced as the P2 ligands instead of the bis-THF structural template in the lead compound DRV (see in Fig. 1), for the sake of improving hydrogen bonding interactions with amino acid residues of Asp29 or Asp30.[7,8] Furthermore, hydrophobic cyclopropyl group was introduced into P1′ ligand in order to improve backbone bindings or favorable van der Waals in the S1′ subsite,[9,10] as well as different phenylsulfonimide groups as the P2′ ligands.[11]

In Scheme 1, Intermediates **6–9** were prepared from the commercially available material (2*S*, 3*S*)-1,2-epoxy-3-(boc-amino)-4-phenylbutane (**1**), which was reacted with cyclopropanamine to provide the chiral secondary amine **2** in 91.4% yield, followed by nucleophilic substitution with 4-substituted-benzenesulfonyl chlorides to afford sulfonamide derivatives **3–5** in good yields (95.1–99.3%). Boc-group was effectively removed by trifluoroacetic acid to obtain compounds **6–8** in 74.1–80.6% yields.[12] Corresponding aminosulfonamide **9** was obtained by catalytic hydrogenation of **8** in MeOH with 5% Pd/C in yield of 97.2%.[13]

In Scheme 2, Commercially available 2-morpholinoethan-1-ol (**A1**) was reacted with 4-nitrophenyl carbonochloridate in ether to provide activated carbonate **B1** in 91.2% yield. Alkoxycarbonylation of amines **6–9** with 2-morpholinoethyl (4-nitrophenyl) carbonate (**B1**) provided inhibitors **m1-4** in yields of 67.6–89.3%.[14] *N*-Alkylation of **A2-A4** with

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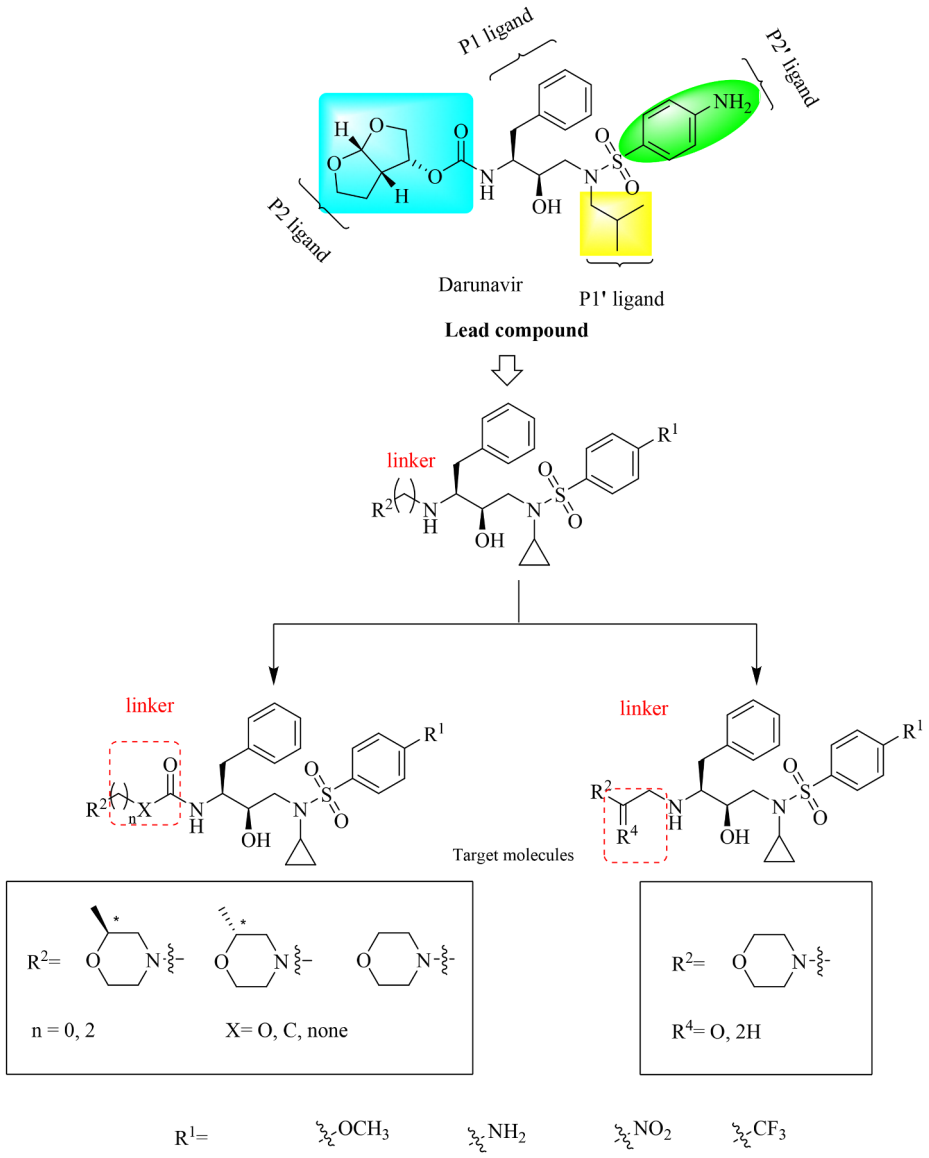
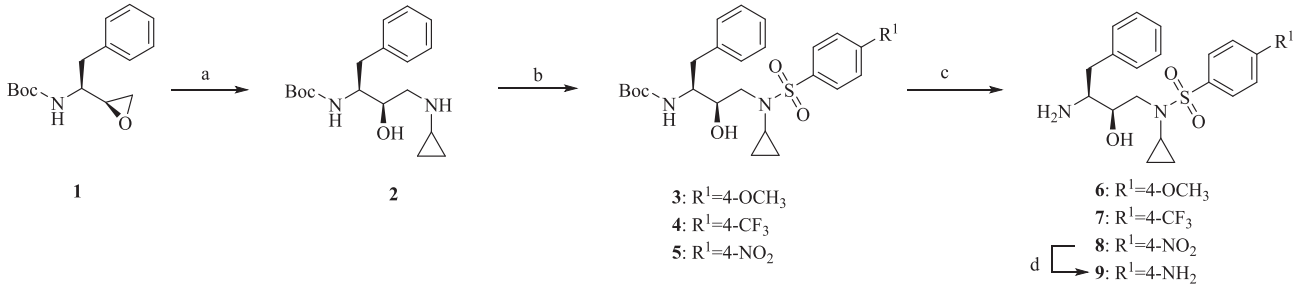


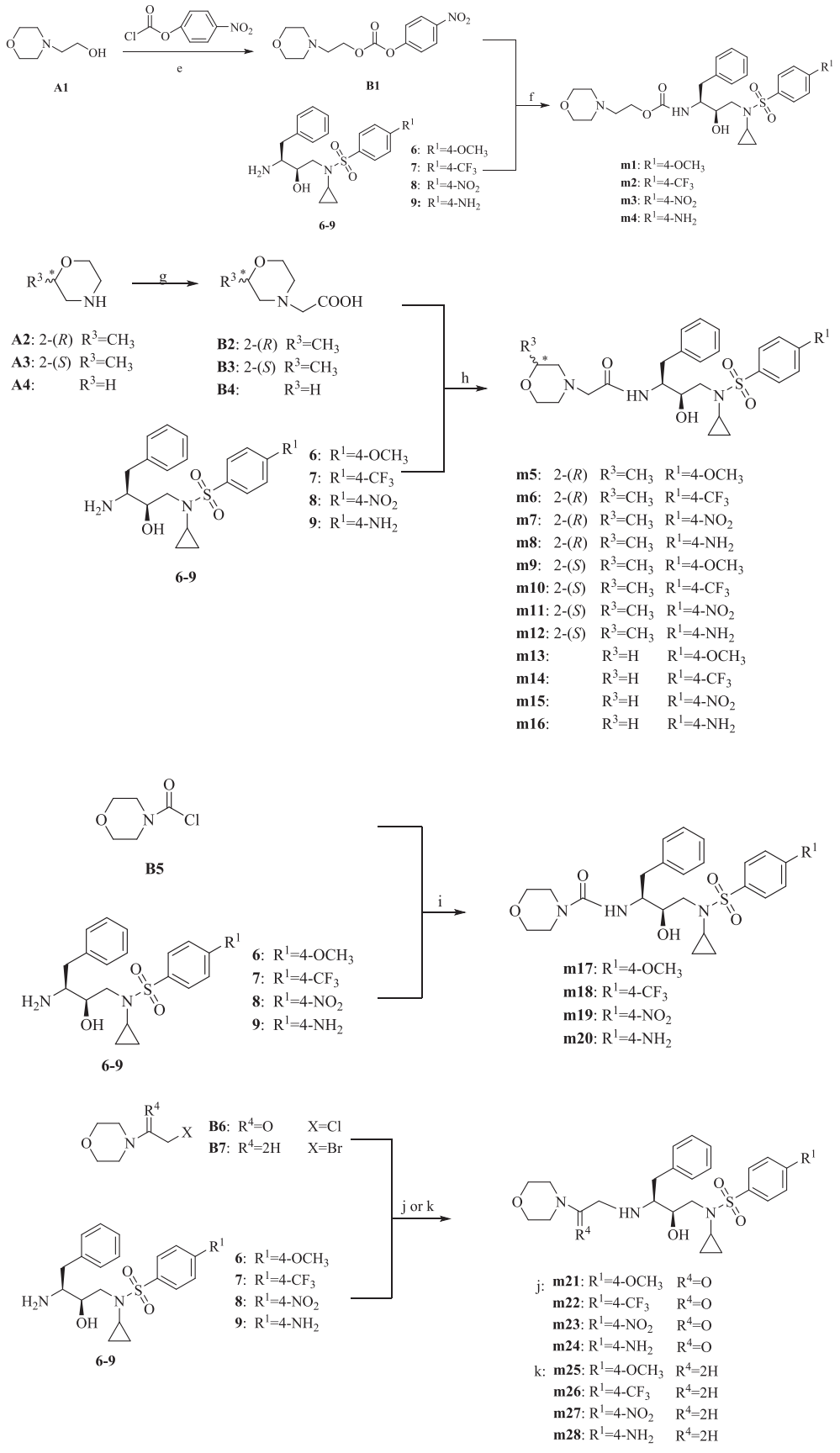
Fig. 1. Chemical structure of target molecules.



Scheme 1. Syntheses of Amines **6–9**. Reagents and conditions: (a) cyclopropanamine, CH_3CN, 80 ℃, 10 h, 91.4%; (b) Aryl sulfonyl chloride, DIEA, DMAP, THF, 0 ℃ ~ r.t, 7.5 h, 95.1–99.3%; (c) CH_2Cl_2-CF_3COOH (1:1), r.t, 5 h, 74.1–80.6%; (d) H_2 (gas), 50 psi, 5% Pd/C, MeOH-EA (2:1), r.t, 4 h, 97.2%.

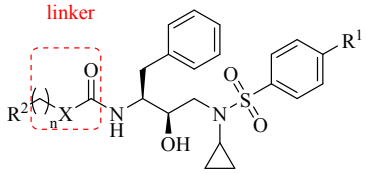
bromoacetic acid proceeded with K_2CO_3 as the acid binding agent in anhydrous DMF and then added 4 M HCl (aqueous) in dropwise to give corresponding acids **B2–B4** with yields of 85.3–88.2%. Treatment compounds **B2–B5** with amines **6–9** generated inhibitors **m5-20** under the condition of EDCI, HOBt and DMAP mediated coupling method in yields of 51.1–73.4%.[15] Inhibitors **m21-24** were synthesized by alkylation of amines **6–9** with compound **B6** under the catalytic condition of DIEA and DMAP in yields of 50.9–69.3%. Inhibitors **m25-28** were obtained by the reaction of compound **B7** and amines **6–9** with K_2CO_3 as the acid binder in yields of 60.3–72.8%.

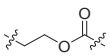
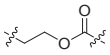
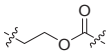
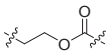
In this study, fluorescence resonance energy transfer (FRET) was adopted to assess the activity of the new HIV-1 PIs *in vitro*.[16] DRV, the most effective HIV-1 PI, was chosen as the positive control. The results were reported in Tables 1 and 2. Although not very potent, there still existed regularity. As a whole, inhibitors with amide chains (carbamate or carbamido) showed enhancement of HIV-1 protease inhibition over



Scheme 2. Syntheses of Inhibitors **m1-28**. Reagents and conditions: (e) ether, 0 ℃ ~ r.t, overnight, 91.2%. (f) DIEA, anhydrous DMF, Argon, 0 ℃ ~ r.t, 10 h, 67.6–89.3%. (g) (i) 2-Bromoacetic acid, K_2CO_3, anhydrous DMF, Argon, r.t, overnight; (ii) 4 M HCl, 0 ℃, 0.5 h, 85.3–88.2%. (h) EDCI, HOBt, DMAP, anhydrous DMF, Argon, 0 ℃ ~ r.t, 5 h, 51.1–65.8%. (i) EDCI, HOBt, DMAP, anhydrous DMF, Argon, 0 ℃ ~ r.t, 5 h, 55.8%–73.4%. (j) DIEA, DMAP, anhydrous EtOH, Argon, reflux, 7 h, 50.9–69.3%. (k) K_2CO_3, CH_3CN, 60 ℃, 1 h, 60.3–72.8%.

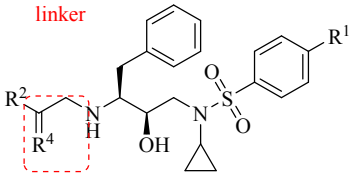
Table 1
Enzyme inhibitory activity of inhibitors with amide linker.



Compd.	R^1	R^2	Linker[a]	IC_{50}[b] (μM)
m1	4-OCH_3	Morpholine		0.054 ± 0.004
m2	4-CF_3	Morpholine		0.12 ± 0.011
m3	4-NO_2	Morpholine		0.080 ± 0.006
m4	4-NH_2	Morpholine		0.061 ± 0.005
m5	4-OCH_3	(*R*)-2-Methylmorpholine		0.19 ± 0.084
m6	4-CF_3	(*R*)-2 Methylmorpholine		2.99 ± 0.63
m7	4-NO_2	(*R*)-2-Methylmorpholine		0.93 ± 0.075
m8	4-NH_2	(*R*)-2-Methylmorpholine		1.53 ± 0.72
m9	4-OCH_3	(*S*)-2-Methylmorpholine		1.27 ± 0.39
m10	4-CF_3	(*S*)-2-Methylmorpholine		0.40 ± 0.032
m11	4-NO_2	(*S*)-2-Methylmorpholine		0.58 ± 0.053
m12	4-NH_2	(*S*)-2-Methylmorpholine		1.34 ± 0.95
m13	4-OCH_3	Morpholine		1.04 ± 0.21
m14	4-CF_3	Morpholine		0.40 ± 0.048
m15	4-NO_2	Morpholine		5.43 ± 1.77
m16	4-NH_2	Morpholine		> 10
m17	4-OCH_3	Morpholine		0.26 ± 0.083
m18	4-CF_3	Morpholine		0.047 ± 0.004
m19	4-NO_2	Morpholine		0.79 ± 0.037
m20	4-NH_2	Morpholine		0.64 ± 0.077
DRV (nM)	–	–	–	0.37 ± 0.070

[a] Linker was defined as chains connected backbone amino group to morpholine of the P2 ligand.
[b] All assays were conducted in triplicate, and the data shown represent mean values (± 1 standard deviation) derived from the results of three independent experiments.

Table 2
Enzyme inhibitory activity of inhibitors with aliphatic chain linkers.



Compd.	R^1	R^2	Linker[a]	IC_{50}[b] (μM)
m21	4-OCH_3	Morpholine		0.18 ± 0.099
m22	4-CF_3	Morpholine		1.26 ± 0.43
m23	4-NO_2	Morpholine		> 10
m24	4-NH_2	Morpholine		1.15 ± 0.76
m25	4-OCH_3	Morpholine		0.17 ± 0.090
m26	4-CF_3	Morpholine		1.69 ± 0.75
m27	4-NO_2	Morpholine		0.61 ± 0.087
m28	4-NH_2	Morpholine		0.94 ± 0.095
DRV (nM)	–	–	–	0.37 ± 0.070

[a] Linker was defined as chains connected backbone amino group to morpholine of the P2 ligand.
[b] All assays were conducted in triplicate, and the data shown represent mean values (± 1 standard deviation) derived from the results of three independent experiments

that with aliphatic chains (for instance, **m14** *vs* **m26**). The reason may be that amide chains mimic the peptide characteristics of HIV-1 protease substrate on the one hand. On the other hand, it may be that the carbonyl group in amide compounds could form hydrogen bonds with the backbone of the protease.[15,17–20] Furthermore, carbamate inhibitors exhibited the most potent activity with IC_{50} value in nanomolar order of magnitude, which might account for the degree of freedom and flexibility that can be better bound to protease. In addition, the morpholine ring located in S2 subsite and the chain forced the benzenesulfonyl group to extent inside S2′ subsite deeply. For acetamide compounds, the activity of compounds with (*R*)-2-methylmorpholine in R^3 fragment was better than that with (*S*)-2-methylmorpholine in general, which may be due to the difference of steric hindrance and conformation; besides, the van der Waals of (*R*)-2-methylmorpholine made it better matching the binding pocket of enzyme so as to improve the activity.

Compounds with the linker of non-substitution straight aliphatic chains exhibited better activity than the corresponding compounds with carbonyl substituted aliphatic chains such as **m27** *vs* **m23**, which may be due to the larger degree of freedom and the reduction of steric hindrance.

The functional groups in P2′ ligand also played important roles. Compounds with 4-methoxyl, 4-amino and 4-trifluoromethyl substituents showed higher potency than the corresponding 4-nitro substituent compounds, except for **m7, m11 and m27**. The oxygen atom in the 4-methoxyl group might form hydrogen bonds (O···H–N) with Asp30′ or Asp29′ in the protease S2′ subsite, which increased the binding ability of inhibitor and enzyme.[21] The 4-amino group could make weak hydrogen bonds involved directly interactions (N–H···N) with the main chain amides and water-mediated interactions (NH···H_2O···HOOC) with the side chain oxygen of Asp30′ or Asp29′ in S2′ active subsite.[19,20] The 4-trifluoromethyl group is a weak electron acceptor when connected with aromatic rings, and going forward, the introduction of trifluoromethyl group may enhance cell membrance permeability, which may be in favor of anti-HIV activity.[22] The 4-nitro group is a strong electron-withdrawing group, which would not only reduce the electron density of oxygen atom itself by inductive effects,

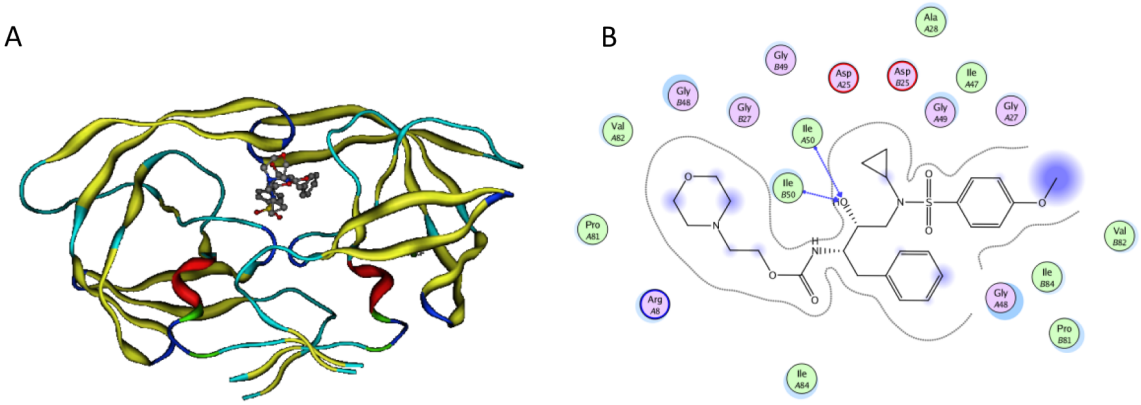


Fig. 2. The molecular modeling for compound **m1**. (A) Docked pose of **m1**. (B) Ligplot interaction of **m1**. Ligand exposures are described as purple spheres. Hydrogen bonding interactions between residues and ligands are represented as dashed arrows.

but also reduce the electron density of sulfonyl group by conjugative effects. That could reduce the ability of hydrogen to bond with amides of Asp30′ or Asp29′ in protease S2′ subsite.[23]

In order to explored molecular insight into the interactions of inhibitors and HIV-1 protease, we have measured the computational docking of **m1** with active sites using a HIV-1 protease crystal structure (PDB-ID: 4mc9) (Fig. 2).[24] The inhibitor **m1** fitted into the active site cavity and the oxygen atom of hydroxyethyl amine could form hydrogen bonds with amide of IIe50/IIe50′ in flap, as well as van der Waals were generated with the outer enzyme atoms. These interactions might be responsible for the HIV-1 protease inhibitory activity. Also as can be seen, oxygen atom of morpholine in P2 ligand could generate interactions with the active site while there was no clean evidence for interactions between the wrapped nitrogen atom and the active site, and this could account for why the inhibition of morpholine analogues were inferior to that of DRV containing two exposed oxygen atoms. Furthermore, modeling studies indicated decreased van der Waals interactions, although hydrophobic cyclopropyl of the P1′ ligand could fit into the S1′ subsite of HIV-1 protease.

In summary, a series of new HIV-1 PIs with morpholine derivatives as P2 ligands as well as cyclopropyl as P1′ ligand was reported in this study. The inhibitors were designed with the purpose of enhancing extensive hydrogen bonding interactions or favorable van der Waals with the protein skeleton of the active site of HIV-1 protease. Though not very satisfactory, there still exhibited potent inhibitors such as **m1**, **m3**, **m4** and **m18** containing carbamate or carbamido as linkers along with 4-methoxybenzenesulfonamide or 4-tifluoromethylbenzenesulfonamide as P2′ ligands, which displayed inhibitory effect on the activity of HIV-1 protease with IC_{50} value of 54 nM, 80 nM, 61 nM and 47 nM *in vitro*, respectively, and thus provided basis for further study. The rigid cyclopropyl system might decrease van der Waals interactions in the vicinity of the S1′ subsite, which is worthy of ulteriorly optimization. Further design and investigation of this kind of inhibitors, especially groups in P1′ ligands are in progress.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127019.

References

[1]. Gao BL, Zhang CM, Yin YZ, Tang LQ, Liu ZP. *Bioorg Med Chem Lett.* 2011;21:3730–3733.
[2]. Sun Y, Xu W, Fan N, et al. *Bioorg Med Chem.* 2017;25:1076–1084.
[3]. Lu XY, Wang LF, Jiang ZJ. *IEEE. 2018 5th International Conference on Systems and Informatics.* 2018; 2018:1299–1304.
[4]. Bonini C, Chiummiento L, Di Blasio N, et al. *Bioorg Med Chem.* 2014;22:4792–4802.
[5]. Johnson ECB, Malito E, Shen Y, et al. *J Mol Biol.* 2007;373:573–586.
[6]. Pawar S, Wang YF, Wong-Sam A, et al. *Biochem Biophys Res Commun.* 2019;514:974–978.
[7]. Yang ZH, Bai XG, Zhou L, Wang JX, Liu HT, Wang YC. *Bioorg Med Chem Lett.* 2015;25:1880–1883.
[8]. Aruksankunwong O, Hannongbua S, Wolschann P. *J Mol Struct.* 2006;790:174–182.
[9]. Vondrasek AWAJ. *Annu Rev Biophys Biomol Struct.* 1998;27:249–284.
[10]. Midde NM, Patters BJ, Rao P, Cory TJ, Kumar S. *Expert Opin Invest Drugs.* 2016;25:1189–1200.
[11]. Mitchell ML, Xu L, Newby ZE, Desai MC. *Tetrahedron Lett.* 2017;58:1123–1126.
[12]. Zhu M, Du XN, Li YG, Zhang GN, Wang JX, Wang YC. *Bioorg Med Chem Lett.* 2019;29:357–361.
[13]. Yan J, Huang N, Li S, et al. *Bioorg Med Chem Lett.* 2012;22:1976–1979.
[14]. Ghosh AK, Gemma S, Simoni E, et al. *Bioorg Med Chem Lett.* 2010;20:1241–1246.
[15]. Bai X, Yang Z, Zhu M, et al. *Eur J Med Chem.* 2017;137:30–44.
[16]. Hendrix J, Debyser Z, Hofkens J, Engelborghs Y. *Eur Biophys J Biophys Lett.* 2011;40:48.
[17]. Qiu X, Zhao GD, Tang LQ, Liu ZP. *Bioorg Med Chem Lett.* 2014;24:2465–2468.
[18]. Ghosh AK, Jadhav RD, Simpson H, et al. *Eur J Med Chem.* 2018;160:171–182.
[19]. Yeung CM, Klein LL, Flentge CA, et al. *Bioorg Med Chem Lett.* 2005;15:2275–2278.
[20]. Perez MA, Fernandes PA, Ramos MJ. *J Mol Graph Model.* 2007;26:634–642.
[21]. Ghosh AK, Brindisi M, Nyalapatla PR, et al. *Bioorg Med Chem.* 2017;25:5114–5127.
[22]. Adrian Meredith J, Wallberg H, Vrang L, et al. *Eur J Med Chem.* 2010;45:160–170.
[23]. Zhu M, Dong B, Zhang GN, Wang JX, Cen S, Wang YC. *Bioorg Med Chem Lett.* 2019;29:1541–1545.
[24]. Ganguly AK, Alluri SS, Wang CH, et al. *Tetrahedron.* 2014;70:2894–2904.