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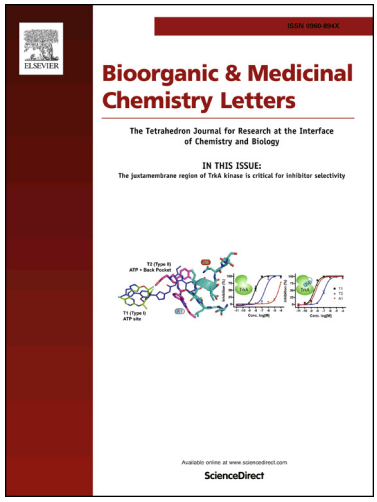

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Design and synthesis of hybrids of diarylpyrimidines and diketo acids as HIV-1 inhibitors

Ping Xue [a], Huan-Huan Lu [a], Yuan-Yuan Zhu [b,*], Xiu-Lian Ju [a], Christophe Pannecouque [c], Xiao-Jiao Zheng [a], Gen-Yan Liu [a], Xiu-Lan Zhang [a], Shuang-Xi Gu [a,*]

[a] *Key Laboratory for Green Chemical Process of Ministry of Education, School of Chemical Engineering & Pharmacy, Wuhan Institute of Technology, Wuhan 430205, China*

[b] *School of Chemistry and Environmental Engineering, Wuhan Institute of Technology, Wuhan 430205, China*

[c] *KU Leuven, Department of Microbiology and Immunology, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, B-3000 Leuven, Belgium*

[*]Corresponding Authors:

E-mail: yyzhu531@163.com (Y.-Y. Zhu); shuangxigu@163.com (S.-X. Gu).

Abstract: Based on the strategy of molecular hybridization, diketo acid fragment as a classical phamacophore of integrase inhibitors was introduced to reverse transcriptase inhibitors diarylpyrimidines to design a series of diarylpyrimidine-diketo acid hybrids (DAPY-DKAs). The target molecules **10b** and **11b** showed inhibitory activities against WT HIV-1 with EC_{50} values of 0.18 μM and 0.14 μM, respectively. And the results of molecular docking demonstrated the potential binding mode and revealed that the DKA moiety and its ester could both be tolerated in the nonnucleoside binding site (NNBS) of HIV-1 RT.

Key word: HIV-1 inhibitors; AIDS; reverse transcriptase; integrase; molecular hybridization; diarylpyrimidines; diketo acids

Reverse transcriptase (RT) and integrase (IN) both play vital roles in the process of HIV-1 replication.[1] NNRTIs has become a mainstay of highly active antiretroviral therapy (HAART)[2] and been used as the first-line drugs against HIV-1 due to their unique antiviral potency, high specificity and low cytotoxicity.[3, 4, 5] Among them, TMC125 and TMC278 (Fig. 1) as NNRTIs characterized by their DAPY structures, have been paid extensive attention for their excellent activities against both WT HIV-1 and mutant strains.[6, 7, 8] And in recent years, some compounds were discovered to exhibit potent activities against a recombinant IN, however, the majority of these compounds did not show antiviral activity in cellular level, and the remaining compounds generally exhibited high cytotoxicity.[9, 10] Among them, β-diketo acid derivatives (DKAs) displayed excellent inhibitory activities against both recombinant IN and HIV-1 strains.[11] The three FDA-approved INIs, raltegravir, dolutegravir and elvitegravir, are all derived from classical diketo acids.[9] So, it is rational to design a series of diarylpyrimidine-diketo acids hybrids to get the better HIV-1 inhibitions.

In this work, we adopted molecular hybridization strategy[12, 13] to design a series of

diarylpyrimidine-diketo acids hybrids (Fig. 2), in which the introduction of DKA fragment was expected to improve the anti-HIV-1 activities. The reported crystal structures of the HIV-1 RT/DAPY complexes [6] have verified the existence of π-π stacking interaction between the left aryl ring and aromatic amino acid residues such as Tyr181, Tyr188, Phe227 and Trp229 in hydrophoic pockets. Because strong electron withdrawing ability of the fragment of diketo acid will reduce the left wing aromatic ring electron density and π-π stacking interaction between the left aryl ring and aromatic amino acid residues. Therefore, the diketo acid esters **10a** and **10b** were designed in order to better keep the hydrophobicity of target molecules.

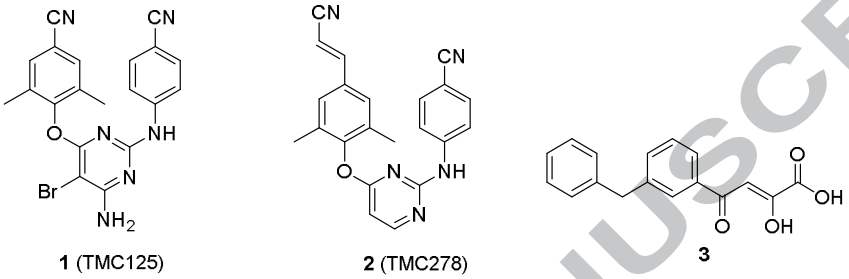


Figure 1. The structure of DAPYs inhibitors (**1** and **2**) and DKAs inhibitor (**3**)

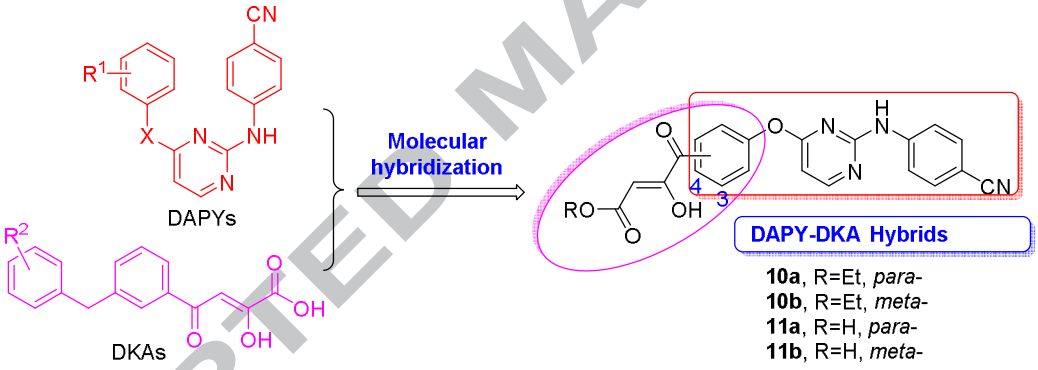
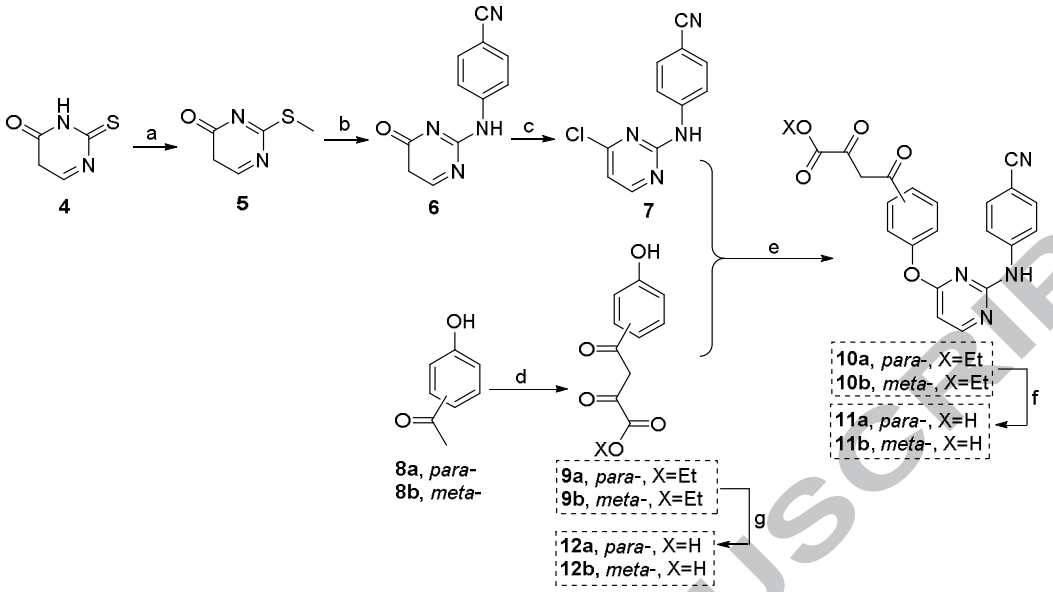


Figure 2. Design of diarylpyrimidine-diketo acid hybrids through molecular hybridization

The newly designed compounds were synthesized by an expeditious method as depicted in Scheme 1. The intermediate **7** was synthesized through a three-step reaction according to our previously reported steps.[14, 15] And intermediates **9a-b** were prepared from **8a-b** and diethyl oxalate in the presence of THF and NaH, and the **10a-b** were obtained via a nucleophilic substitution of **7** with **9a-b** using Cs_2CO_3 as base in DMSO. Finally, **9a-b** and **10a-b** were subject to a simple hydrolysis to get the corresponding compounds **11a-b** and **12a-b**.



Scheme 1. Synthesis of target molecules Reagents and conditions: (a) MeI, NaOH, H_2O, rt, 24 h; (b) 4-cyanoaniline, 180–190℃, 10 h; (c) $POCl_3$, reflux, 0.5 h; (d) diethyl oxalate, NaH, THF, reflux, 20 min; (e) Cs_2CO_3, DMSO, 110℃, 40 min; (f) THF, CH_3OH, NaOH, rt, 2.5 h; (g) THF, CH_3OH, NaOH, rt, 2 h

Table 1. Activities against WT HIV-1 and mutants in MT-4 cell and HIV-1 RT inhibitory activities

Compd	HIV-1 EC_{50} (μM)			CC_{50} (μM)	SI	IC_{50} (μM)
	WT	K103N	RES056	HIV-1 WT	HIV-1 WT	RT
9a	>466	-	-	466	<1	-
9b	>356	-	-	356	<1	-
10a	0.19	10	>18	18	95	5.864
10b	0.18	>14	>14	14	78	6.059
11a	0.23	>42	>42	42	183	25.208
11b	0.14	38	>9	10	71	5.930
12a	>600	-	-	>600	1	-
12b	>528	-	-	528	<1	-
Nevirapine	0.084	>15	>15	>15	>179	1.528[a]
Efavirenz	0.0054	0.17	0.17	>9	>1717	0.022[a]
Etravirine	0.0033	0.004	0.03	>5	>1391	0.011[a]
Azidothymidine	0.0077	0.016	0.01	>7	>971	-
3[b]	0.16	-	-	>10	>61	>100

[a] Ref. [15]

[b] Ref. [16]

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method was used for biological evaluation.[17, 18] Compounds **10a-b**, **11a-b**, **12a-b** were evaluated for their

cytotoxicity and anti-HIV activities in MT-4 cells infected with wild-type (WT) HIV-1 (strain IIIB), common HIV-1 mutants [L100I, K103N, Y181C, Y188L, E138K, F227L+V106A, K103N+Y181C (RES056)], as well as HIV-2 (strain ROD), along with four FDA-approved drugs: nevirapine, efavirenz, etravirine (TMC125), azidothymidine as reference drugs. And also, the activities of DKA inhibitor **3**was also cited as reference[16]. The results were summarized in Table 1, which was expressed by CC_{50} (cytotoxic concentration that reduces 50% normal cell), EC_{50} (concentration requires to attain 50% HIV-1 replication), SI (selectivity index, CC_{50}/EC_{50} ratio) and IC_{50} (concentration requires to inhibit 50% HIV-1 RT polymerase activity) values. Intermediates **9a-b** and their hydrolysis products **12a-b** were inactive against WT and drug-resistant mutant strains. All hybrid molecules showed activities against WT HIV-1 strain closed to the activities of DKA inhibitor **3** with EC_{50} values ranged from 0.14 to 0.23 μM. Among them, the most potent compound **11b** displayed good activity with an EC_{50} of 0.14 μM. And the trend of the potency seems to be as follows: *meta*-substitution > *para*-substitution (**10b > 10a** and **11b > 11a**). Unfortunately, none of these inhibitors exhibited activities against double mutant strain RES056 at a subtoxic concentration. And also, nearly all compounds are devoid of activities against common HIV-1 mutants such as L100I, Y181C, Y188L, E138K, F227L+V106A (see supporting information). Therefore, our further efforts are aimed at improving activities against both WT HIV-1 and HIV-1 mutants. Moreover, expectedly, the compounds lack the HIV-2 inhibitory activities (see supporting information), demonstrating that non-nucleoside reverse transcriptase inhibitors (NNRTIs) are specific for human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and do not inhibit HIV-2.[19]

HIV-1 RT inhibitory assay was also performed via an ELISA assay using a commercial kit (using poly rA: Dt as the template/primer). All hybrid inhibitors displayed moderate inhibitory activities against HIV-1 RT with EC_{50} values ranged from 5.864 μM to 25.208 μM. Although the activities of all targets compounds were not as good as etravirine (IC50 = 0.011 μM). But it is worth mention that DKAs inhibitor **3** showed no RT inhibitor activities[16]. It suggests that the newly synthesized DAPY-DKAs bind to HIV-1 RT and belong to the NNRTIs. It is notable that **10a**, **10b** and **11b** exhibited similar activity against HIV-1 RT, while the anti-HIV-1 activity of **11a** was distinctly inferior to them, which might be ascribed to that the *para*-substitution was not conducive to binding between DKA and RT.

To investigate the potential binding mode, the molecular docking was performed using the software SYBYL Surflex-Dock program and crystal structure of WT HIV-1 RT/TMC125 (PDB code: 3MEC) to take the docking studies.[6] Ester **10b** and acid **11b** were both docked into the HIV-1 RT nonnucleoside binding site (NNBS) (Fig. 3). The docking studies showed that the molecules could be well located in the NNBS and verified the existence the π-π stacking interactions between the left wing of **10b** (or **11b**) and the aromatic amino acid residues Tyr188, Trp229 as well as Phe227. It shows the fragment of diketo acid (ester) seems to have no significant effect on π-π stacking interaction between the left aryl ring and aromatic amino acid

residues, which might be the carboxylic (ester) group has no obvious effect on the left wing aromatic ring electron density because it far away from the benzene. It also account for that acid **11a (11b)** displayed the similar WT HIV-1 inhibitory activity with correponding ester **10a (10b)**.

In conclusion, a series of DAPY-DKA hybrids were designed and synthesized as HIV-1 inhibitors. All target molecules showed activities against WT HIV-1 at submicromolar conentrations and HIV-1 RT at micromolar concentrations. It illustrated that the introduction of DKA or DKA ester fragment is tolerable in the modification of DAPYs. Compounds **10b** and **11b** showed inhibitory activities against WT HIV-1 with EC_{50} values of 0.18 μM and 0.14 μM, respectively. This work enriched the SAR of DAPYs, and provided a potential scaffold for the development of new anti-HIV compounds.

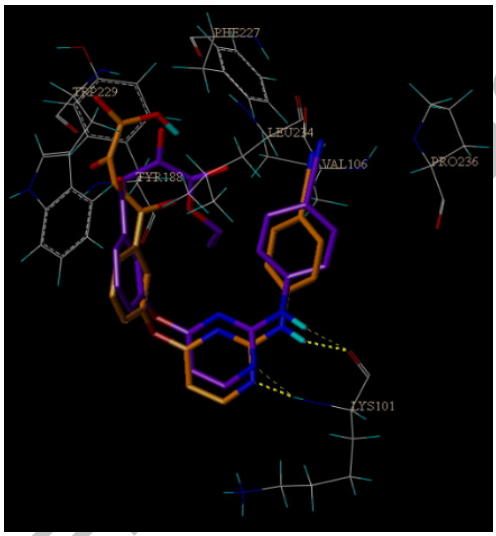


Figure 3. Docking studies of **10b** (purple) and **11b** (organge) into NNBS

Acknowledgments

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Supplementary data

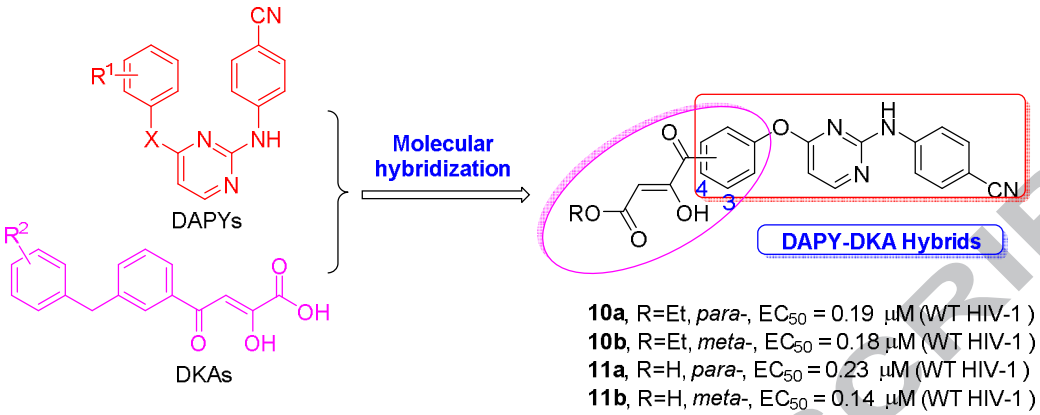
Supplementary data (^{1}H NMR, ^{13}C NMR, and part of biological data) associated with this article can be found, in the online version, at http://dx.doi.org/********.

References and notes

1. Engelman, A.; Cherepanov, P. *Nat. Rev. Microbiol.* **2011**, *10*, 279.
2. Yeni, P. *J. Hepatol.* **2006**, *44*, 100.
3. Li, D.; Zhan, P.; De, Clercq E; Liu, X. *J. Med. Chem.* **2012**, *55*, 3595.

4. Huang, B.; Liang, X.; Li, C.; Chen, W.; Liu, T.; Li, X.; Sun, Y.; Lu, F.; Liu, H.; De Clercq, Erik. *Eur. J. Med. Chem.* **2015**, *93*, 330.
5. Li, X.; Chen, W.; Tian, Y.; Liu, H.; Zhan, P.; De, Clercq E; Pannecouque, C; Balzarini, J; Liu, X. *Eur. J. Med. Chem.* **2014**, *80*, 112.
6. Lansdon, E. B.; Brendza, K. M.; Hung, M. ; Wang, R.; Mukund, S.; Jin, D.; Birkus, G.; Kutty, N.; Liu, X. *J. Med. Chem.* **2010**, *53*, 4295.
7. Janssen, P. A.; Lewi, P. J.; Arnold E; Daeyaert, F; De, Jonge M; Heeres, J; Koymans, L; Vinkers, M; Guillemont, J; Pasquier, E; Kukla, M. *J. Med. Chem.* **2005**, *48*, 1901.
8. Chen, X.; Zhan, P.; Li, D.; De, Clercq E.; Liu, X. *Curr. Med. Chem.* **2011**, *18*, 359.
9. Di Santo R. *J. Med. Chem.* **2013**, *57*, 539.
10. Gu, S. X; Zhu, Y. Y.; Chen, F. E; De Clercq, E.; Balzarini, J.; Pannecouque, C. *Med. Chem. Res.* **2015**, *24*, 220.
11. Sechi, M; Bacchi, A; Carcelli, M; Compari, C; Duce, E; Fisicaro, E; Rogolino, D; Gates, P; Derudas, M; Al-Mawsawi, L. Q. *J. Med. Chem.* **2006**, *49*, 4248.
12. Viegasjunior, C; Danuello, A; Da, Silva Bolzani V; Barreiro, E. J.; Fraga, C. A. *Curr. Med. Chem.* **2007**, *14*, 1829.
13. Gu, S. X; Xue, P.; Ju, X.-L.; Zhu, Y.-Y. *Bioorg. Med. Chem.* **2016**, *24*, 5007.
14. Gu, S. X; Yang, S. Q.; He, Q. Q.; Ma, X. D.; Chen, F. E.; Dai, H. F.; De Clercq, E.; Balzarini, J.; Pannecouque, C. *Bioorg. Med. Chem.* **2011**, *19*, 7093.
15. Gu, S. X; Qiao, H.; Zhu, Y. Y.; Shu, Q. C.; Liu, H.; Ju, X. L.; De Clercq, E.; Balzarini, J.; Pannecouque, C. *Bioorg. Med. Chem.* **2015**, *23*, 6587.
16. Wai, J. S.; Egbertson, M.S.; Payne, L. S.; Fisher, T. E.; Embrey, M.W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Naylorolsen, A. M.; Hazuda, D. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S.D. *J. Med. Chem.* **2000**, *43*, 4923.
17. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
18. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protocols* **2008**, *3*, 427.
19. Auwerx, J.; North, T. W.; Preston, B. D.; Klarmann, G. J.; De Clercq, E.; Balzarini, J. *Mol. Pharmacol.* **2002**, *61*, 400.

Graphical abstract



10a, R=Et, *para*-, EC_{50} = 0.19 μM (WT HIV-1)
10b, R=Et, *meta*-, EC_{50} = 0.18 μM (WT HIV-1)
11a, R=H, *para*-, EC_{50} = 0.23 μM (WT HIV-1)
11b, R=H, *meta*-, EC_{50} = 0.14 μM (WT HIV-1)