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Synthesis of pyrimidine and quinolone conjugates as a scaffold for dual inhibitors of HIV reverse transcriptase and integrase

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Abstract—A series of conjugates combining a pyrimidine and a quinolone moiety were designed and synthesized. The assay results show that these compounds demonstrate both anti-RT activity and anti-IN activity and therefore provide a useful scaffold for identifying inhibitors with balanced dual activities.
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Human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS),[1,2] continues to be a major health threat worldwide despite FDA approval of around 30 anti-HIV drugs.[3] With the exception of two entry inhibitors, enfuvirtide[4] and maraviroc,[5] and an integrase (IN) inhibitor, raltegravir (MK-0518, **25**),[6] all FDA-approved anti-HIV drugs inhibit either reverse transcriptase (RT) or protease (PR). Singly dosed antivirals are rarely used in clinic due to the rapid emergence of resistant viral strains. This problem is countered by combining multiple mechanistically distinct drugs to form highly active antiretroviral therapy (HAART),[7] which can successfully suppress viral replication. However, the benefits of HAART as the standard AIDS chemotherapy are compromised by high cost and severe toxicity,[8] both resulting from using multiple drugs. Toxicity in particular imposes a huge barrier to excellent patient adherence, without which patients experience viral rebound, and even worse, multi-drug resistance (MDR).

Designed multiple ligands (DMLs)[9–11] are single structures engaging multiple biological targets, and therefore could incur lower cost and toxicity than HAART drug cocktails. Identification of multiple ligands through rational design has been a subject of growing interest in medicinal chemistry.[10] Compounds active against both RT and IN were reported,[12] though their usability is limited by toxicity and the lack of a general design strategy. Recently we disclosed the first rationally designed RT/IN dual ligands (**3**, Fig. 1).[13] Significantly, TNK-651 (**1**) of the 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) family is a potent non-nucleoside RT inhibitor of which the N-1 substituent extends from the NNRTI binding pocket to the protein/solvent interface.[14] This would tolerate structural modifications at the N-1 site of **1** and allow the incorporation of a second pharmacophore to generate activity against IN.[13]

As an example of our design, compound **3** contains a diketoacid (DKA) pharmacophore derived from the stereotypical DKA IN inhibitor **2**. Dual activities against RT and IN were observed[13] (**3**, Table 1), though the disparity between these two activities prompts us to continue searching for dual inhibitors with better balanced activities against these two enzymes. We were particularly interested in a scaffold where the DKA pharmacophore in compound **3** is replaced with an isosteric quinolone carboxylic acid moiety (**5**–**8**, Fig. 1). Quinolone compounds constitute one of the largest families of antimicrobial agents;[15,16] and GS-9137[17,18] (**4**, Fig. 1), which has a quinolone carboxylic acid core, is a very potent IN inhibitor currently under clinical development. Therefore, if dually active, conjugates combining quinolone pharmacophore with pyrimidine would provide a useful scaffold in our search for RT/IN dual inhibitors.

Inhibitors **6**–**8** were synthetically accessed through a convergent strategy featuring a classic Gould–Jacobs synthesis[19] of quinolone carboxylate as illustrated by the preparation of **8** (Scheme 1). In this event, a conju-

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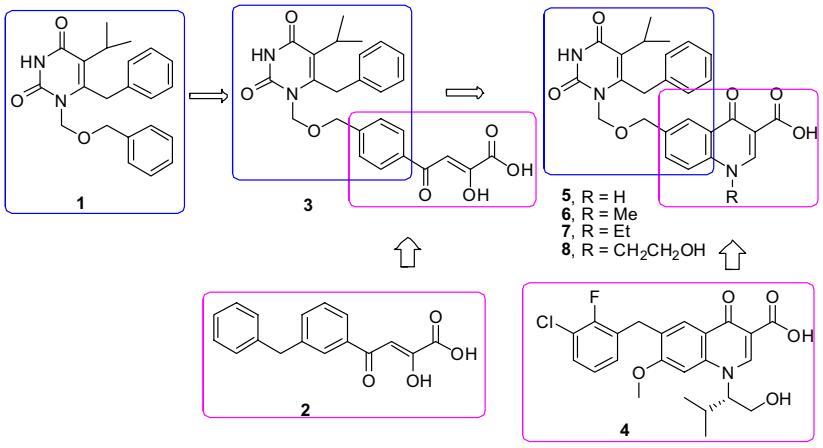
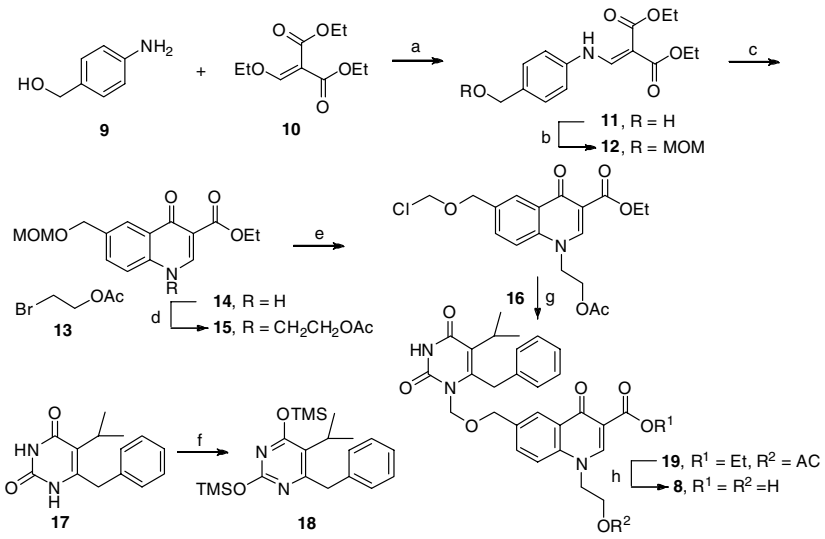
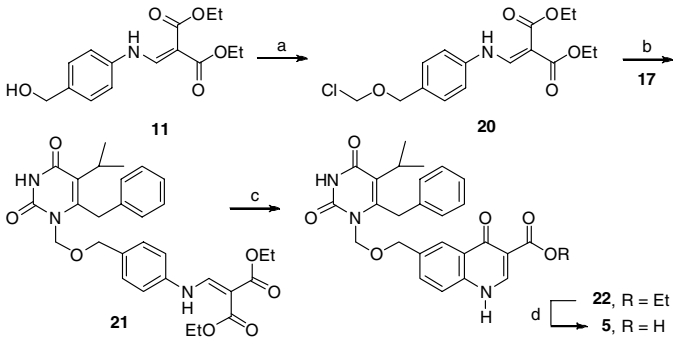


Figure 1. Design of dual inhibitors (**5**–**8**) based on RT inhibitor **1** and IN inhibitor **4**.



Scheme 1. Synthesis of inhibitor **8**. Reagents and conditions: (a) toluene, reflux, 3 h, 98%; (b) MOMCl, DIEA, rt, 15 h, 100%; (c) Ph_2O, 230 °C, 20 min, 65%; (d) **13**, K_2CO_3, DMF, 80 °C, 93%; (e) BCl_3, CH_2Cl_2; (f) BSA, CH_2Cl_2, rt, 20 min; (g) **18**, tetrabutylamonium iodide (TBAI), rt, 15 h; (h) NaOH, CH_2Cl_2/EtOH, rt, 29% over two steps.



Scheme 2. Synthesis of inhibitor **5**. Reagents and conditions: (a) $(HCHO)_n$, TMSCl; (b) **18**, TBAI, rt, 15 h, 82%; (c) Ph_2O, 230 °C, 20 min, 50%; (d) NaOH, CH_2Cl_2/EtOH, rt, 89%.

gate addition of the commercially available aniline **9** onto malonate **10** furnished enamine intermediate **11**. The protection of the free hydroxyl group with MOM then allowed a smooth thermal cyclization of intermediate **12** to produce quinolone ester **14**, which was N-alkylated with bromide **13** to give compound **15**. The direct generation of chloromethyl ether **16** from **15** was effected under the action of BCl_3.[20] The initial MOM

Table 1. Anti-RT, anti-IN and anti-HIV assay results for compounds **5–8**

Compound	RT IC_{50} (μM)	IN IC_{50} (μM)	HIV EC_{50} (μM)
3[13]	0.057	2.4	0.033
5	0.19	35	0.22
6	2.6	41	0.21
7	1.1	51	1.5
8	3.7	19	2.2

protection was designed based on this transformation. Compound **16** was then coupled with bissilylated pyrimidine **18** which was freshly prepared from pyrimidine **17**[21] to successfully produce key intermediate **19**. Finally, **19** was hydrolyzed to deliver the desired quinolone carboxylic acid **8**.[22]

Unfortunately, the direct generation of chloromethyl ether from MOM group failed with intermediate **14** (Scheme 1), presumably due to the influence of the free NH group. Alternatively, intermediate **11** was chloromethylated with paraformaldehyde in TMSCl,[13] and the resulting chloromethyl ether **20** was coupled with **18** to yield enamine **21** (Scheme 2). Gratifyingly, upon heating, **21** was smoothly cyclized to deliver the desired quinolone ester **22**, which after saponification produced inhibitor **5**. It is noteworthy that the strategy described in Scheme 2 is not suitable for the synthesis of inhibitors **6–8** as the N-alkylated analogues of enamine **21** failed to cyclize under thermal condition.

The assay results for compounds **5–8** are summarized in Table 1. Notably, these inhibitors demonstrate activity against RT at low to sub-micromolar range, which confirms that introducing a second pharmacophore at the N-1 pedant of HEPT compounds does not seriously diminish their binding affinity to RT. In addition, moderate activity against IN is also observed, which validates quinolone carboxylic acid as a pharmacophore choice in designing RT/IN dual inhibitors. The anti-HIV activity from cell-based assay falls into the same range as the anti-RT activity, implying that the contribution of IN activity to the overall activity might not be significant. Nevertheless, it was also found that the anti-RT and anti-IN activities are considerably more balanced in compound **8** than the original DKA dual inhibitor **3**. This is not trivial since achieving balanced activities against distinct biological targets remains the biggest challenge in designing dual inhibitors.[10]

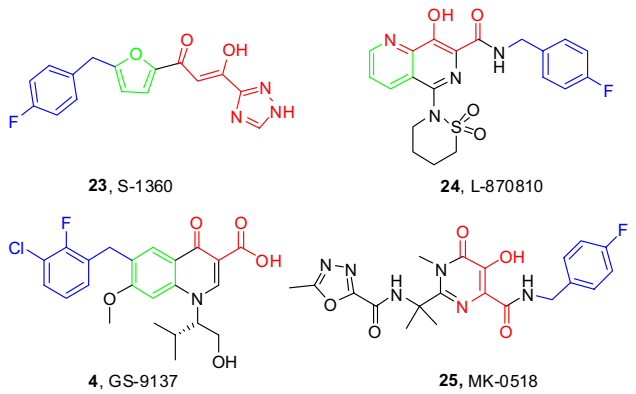


Figure 2. Structure of important IN inhibitors. Red, metal chelator; Green, hydrophobic aromatic ring; Blue, benzyl group.

We expect that the anti-IN activity can be further improved by structure–activity relationship (SAR) studies, as the quinolone carboxylic acid in these conjugates represents only the minimal structural requirement for binding to IN: the metal chelator and the hydrophobic aromatic ring (Fig. 2).[23,24] Interestingly, all IN inhibitors (Fig. 2) that have entered clinical trial bear a benzyl group that has a particular spatial relationship with the chelating functionality.[25] Such a benzyl group could be crucial to achieving optimal binding to IN. Studies are currently underway to determine the benzyl effect on RT/IN dual inhibitor scaffolds.

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22. Synthesis of inhibitor **8**: to a solution of MOM ether **15** (97 mg, 0.26 mmol) in dry CH_2Cl_2 (0.5 mL) at 0 °C was added a solution of BCl_3 in CH_2Cl_2 (0.086 mL, 0.086 mmol). The resulting orange solution was stirred at rt for 1 h and then added to freshly prepared **18** (0.17 mmol) in CH_2Cl_2 solution followed by the addition of a small amount of tetrabutylammonium iodide (TBAI) as catalyst. The resulting mixture was stirred for 15 h and quenched by adding 2 mL of a saturated aqueous solution of $NaHCO_3$. After standard workup, the crude residue was saponified with NaOH (H_2O/EtOH/CH_2Cl_2) and the final product was purified with HPLC to give inhibitor **8**. ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.87 (s, 1H), 8.48 (s, 1H), 7.83 (s, 2H), 7.59 (s, 1H), 7.34 (t, J = 7.8 Hz, 2H), 7.27(t,J = 7.8 Hz, 1H), 7.13 (d,J = 7.2 Hz, 2H), 5.30 (s, 2H), 4.85 (s, 2H), 4.57 (m, 2H), 4.24 (s, 2H), 4.00 (m, 2H), 3.39 (s, 1H), 2.86 (m, 1H), 1.22 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CD_3OD/CDCl_3$) δ 182.6, 172.3, 167.1, 156.2, 154.0, 152.7, 143.2, 140.4, 139.3, 137.6, 133.3, 131.4, 131.3, 130.4, 129.6, 123.9, 121.3, 116.1, 77.3, 74.7, 63.1, 60.6, 50.6, 37.6, 24.1; HRMS (ESI-) calcd for $C_{28}H_{28}N_3O_7$ $[M-H]^-$ 518.1927, found 518.1937.
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