The design and synthesis of 9-phenylcyclohepta[*d*]pyrimidine-2,4-dione derivatives as potent non-nucleoside inhibitors of HIV reverse transcriptase

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Novel compounds, which can be considered as conformationally restricted analogues of MKC-442, have been synthesized and tested as inhibitors of the reverse transcriptase of human immunodeficiency virus type-1 (HIV-1). Reaction of urea with a β-ketoester furnished 6,7,8,9-tetrahydro-9-phenyl-1*H*-cyclohepta[*d*]pyrimidine-2,4-(3*H*,5*H*)-dione (**6a**) and 6,7,8,9-tetrahydro-9-*p*-tolyl-1*H*-cyclohepta[*d*]pyrimidine-2,4-(3*H*,5*H*)-dione (**6b**) which were then alkylated at the N-1 position with chloromethyl ether, allyl bromide and benzyl bromide to afford the target compounds **7a**–**b**, **8a**–**b**, **9** and **10**, respectively. The seven-membered, annelated compounds have a relatively rigid structures and can lock the orientation of the aromatic ring. Chemical modification at N-1 of the pyrinidine ring and the 9-phenyl ring was attempted, with the aim of improving the antiretroviral activity. In particular, replacement of the aliphatic group with the phenyl moiety at the terminus of N-1 side chain can enhance the activity. The most active compounds showed activity in the low micromolar range with IC_{50} values comparable to that of nevirapine. The biological activity results are in accordance with the docking results.

Introduction

Reverse transcriptase (RT), being the pivot in HIV replication, is one of the most attractive targets for the development of new antiretroviral agents.[1–3] Two functionally-distinct classes of HIV-1 RT inhibitors have been described so far: nucleoside reverse transcriptase inhibitors (NRTIs)[4–9] and non-nucleoside reverse transcriptase inhibitors (NNRTIs).[10–16] In recent years, NNRTIs have gained an increasingly important role in the therapy of HIV infection. To date, more than 30 different classes of NNRTIs have been reported. Among the representatives of the NNRTIs, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT,[17–18] Fig. 1) was considered as an interesting lead compound for the synthesis of new analogues, among them the 6-benzyl-1-(ethoxymethyl)-5-isopropyl pyrimidine-2,4-dione (emivirine, formerly known as MKC-442)[19–21] and 6-benzyl-1-(benzyloxymethyl)-5-isopropyl pyrimidine-2,4-dione (TNK-651)[22] showed high activity against HIV-1, and MKC-442 was chosen as a candidate for clinical trials with AIDS patients.[23] However, Triangle Pharmaceuticals halted development of emivirine in January 2002 for being less potent than other antiretrovirals.[24] Like almost all NNRTIs, the HEPT analogues rapidly develop drug resistance by mutation of residues that line the NNRTI binding pocket of the viral RT and reduce drug binding. Their clinical use is limited, even though they are more active against HIV-1 RT with generally low toxicity and favorable pharmacokinetic properties. Therefore, in the NNRTIs field, interest is focused on finding new analogues with higher binding affinity and the capability of inhibiting clinically resistant mutants.[25–28]

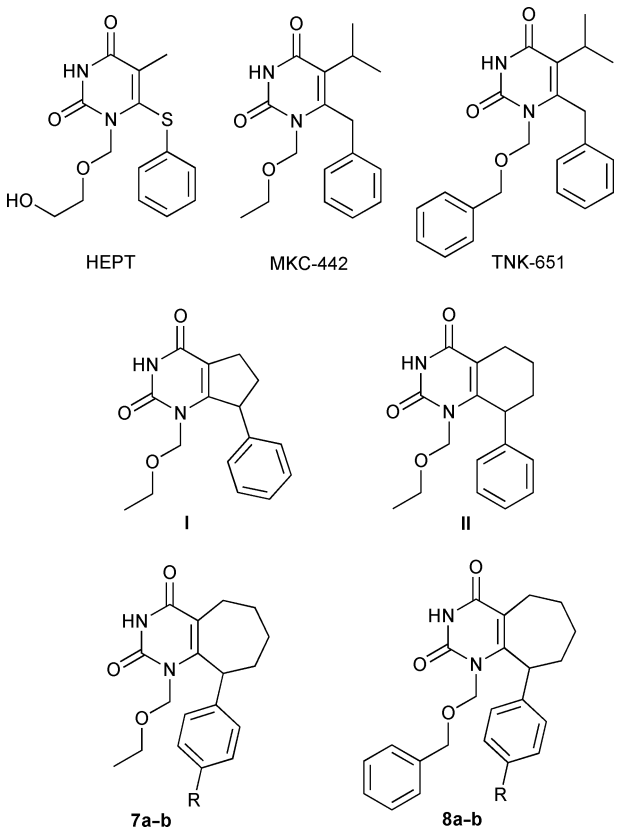

Fig. 1 Structure of HEPT, MKC-442, TNK-651. Compounds **I** and **II**: structures of previously synthesized by Claus Nielsen *et al.*; compounds **7a**–**b** and **8a**–**b**: structures of target molecules.

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Results and discussions

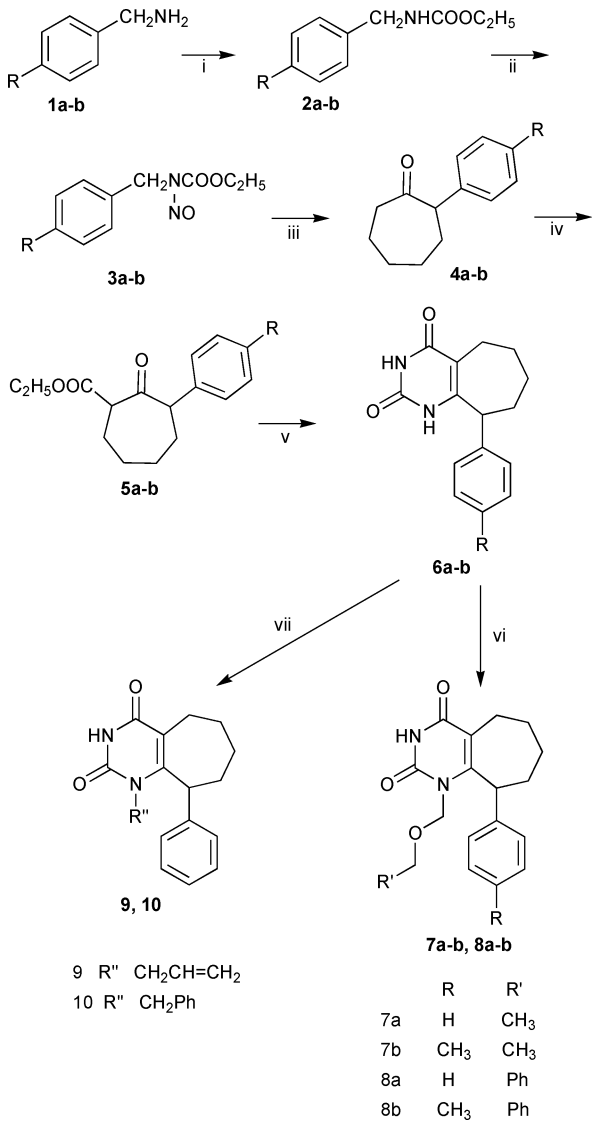
Design

According to structure–activity relationships (SARs), studies of several crystal structures of the RT complex with inhibitors suggest that NNRTIs share a common mode of action and interact with a hydrophobic pocket that is distinct from the NRTI binding site, the polymerase catalytic site.[29–34] Upon binding, NNRTIs distort the polymerase active site and alter the conformation of Tyr181, Tyr183 and Tyr188 residues with respect to that of the unliganded RT. Interestingly, the two HEPT analogues (MKC-442, TNK-651), with their bulky isopropyl groups, force Tyr181 into a position where strong interactions with the 6-benzyl group of the inhibitor can take place ("trigger" action).[22,31,33] On the contrary, HEPT does not produce any "trigger" effect. Its weak anti-HIV-1 activity can be explained by a slight perturbation of the Tyr181 side-chain conformation produced by the C-5 methyl group. This revealed that the C-5 substituent of the pyrimidine ring is very importance for antiviral activity.[22]

Although extensive SARs for non-cyclic analogues (such as MKC-442 and TNK-651) have been obtained, little or no information was available about the annelated series. In the year 2000, Claus Nielsen *et al.* synthesized the five- and six-annelated compounds **I** and **II** (Fig. 1), the conformation of which was restricted by locking the orientation of the aromatic ring, but the activity was *ca.* 10^3-fold lower than MKC-442.[35] Later, they synthesized compounds with extra methyl groups next to the C5 position of the uracil to increase the steric bulkiness, but the biological activities were only slightly better than those for **I** and **II**[36] (Fig. 1). In addition, Macchia *et al.* have reported that tetrahydrobenzocycloheptenuracils do not have higher anti HIV-1 virus activities.[37]

After inspection of the reports mentioned above, we decided that the key point for the development of such compounds is the right size of the annelated rings, which should not only posses conformationally flexibility, but should also restrict effectively the rotation and position of the aromatic ring to the plane of the pyrimidine ring. The cycloheptyl group represents just these two characteristics.

To support our design, seven-membered ring analogues **7a**–**b**, **8a**–**b** (Fig. 1), **9** and **10** (Scheme 1) were designed and synthesized. These compounds were flexibly docked into the binding site of HIV-1 RT complexed with TNK-651, using the AutoDock 3.0 program.[38] The first-ranking docked conformations are shown in Fig. 2. It can be seen that the binding modes proposed by Autodock for **7a** and **8a** (with the R configuration) are fairly close to each other and have approximately the same conformation as in the TNK-651-RT complex. The docking results revealed that the target compounds bind to a hydrophobic pocket in the RT with an conformation forming hydrogen bonds with the main-chain of Lys103, while the 9-phenyl ring of **7a** and **8a** adopts a fashion similar to that of the TNK-651, making favorable π–π interactions with the residues of the RT allosteric pocket formed by the side chains of Try181, Try188, Phe227, and Trp229. The N-1 side chain of **8a** has an extended conformation and occupies a more hydrophobic pocket. Thus the bulky substituted groups on the side chain of N-1 may be accommodated and improve the activity.



Scheme 1 Reagents and conditions: (i) $ClCOOC_2H_5$, NaOH–H_2O, 10–15 °C (ii) $NaNO_2$, H_2SO_4, rt.; (iii) freshly distilled cyclohexanone, anhydrate K_2CO_3 in dry CH_3OH, 20 °C; (iv) $CO(OC_2H_5)_2$, NaH, benzene, reflux 5 h; (v) NH_2CONH_2, EtONa–EtOH, reflux 6 h; (vi) BSA, chloromethyl ether, $CHCl_3$, rt.; (vii) allyl bromide (benzyl bromide), anhydrate K_2CO_3 in DMF, rt.

Furthermore, we have synthesized compounds **7b** and **8b** which have a methyl substituent on the 9-phenyl ring. This substitution pattern is similar to that of GCA-186. The structural change of GCA-186 tolerates the presence of Y181C or K103N RT mutations better than MKC-442 itself.[39] The target compounds (**7a**–**b**, **8a**–**b**) with a locked torsion angle may decrease the toxicity.

Chemistry

A new conventional strategy of synthesizing seven-membered, annelated analogues is described in Scheme 1, in which the β-ketoesters **5a**–**b** are the key intermediates. Compound **5a** was synthesized in four steps from benzylamine **1a**.[40] First **1a** was converted easily to the ethyl *N*-nitrosocarbarnate **3a** by treatment with ethyl chlorocarbonate followed by nitrosation with nitrous

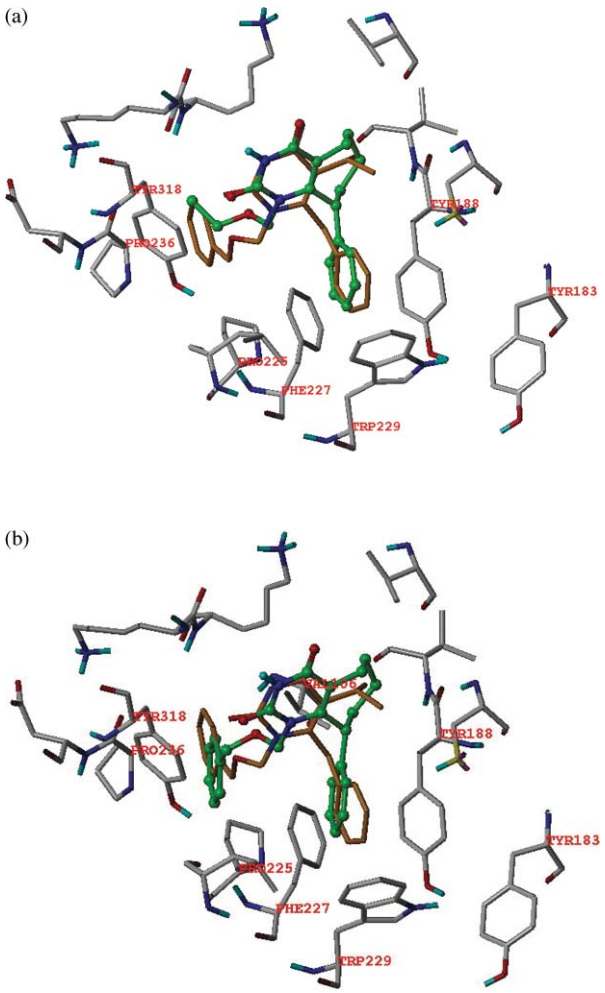


Fig. 2 AutoDock-predicted binding mode of compounds **7a** (a), **8a** (b) (green) compared to the crystallized position of TNK-651 (yellow). Only closely interacting residues are shown.

acid in a quantitative yield. Reaction of **3a** with freshly distilled cyclohexanone in the presence of methanol and potassium carbonate was then carried out, and the product was chromatographed by silica gel column to give **4a** in a 59.0% yield, which is higher than that of the literature reported 41%. The side product was methyl benzyl ether, the yield of which was affected by the conditions. The preparation of several medium- and large-sized β-ketoesters has been accomplished by treatment of the cycloalkanone with sodium triphenylmethyl, followed by carbonation with dry ice, and esterification with diazomethane. The procedure is laborious. Another synthetic method, *via* the Dieckmann cyclization of dimethyl azelate with sodium hydride, yields 48% of this product. Using potassium *t*-butoxide and diethyl carbonate in benzene, the preparation of 2-carbethoxycycloheptanone has been reported only in 40% yield. With a modified method of Paul Krapcho *et al.*,[41] we prepared the intermediate cyclic β-ketoester **5a** by reaction of 2-phenylcycloheptanone **4a** (racemate) with diethyl carbonate in the presence of sodium hydride under reflux, followed by hydrolysis (with dilute HCl preferred to glacial acetic acid) after which the yield was increased to 94.9%.

Claus Nielsen *et al.*[35] reported the preparation of annelated analogues **I** and **II**, using the Dannel *et al.* method, the β-ketoester reacted with thiourea in the presence of sodium ethoxide to give a very low yield (4%). All efforts were made to improve the yield, but without any success. Then they looked for an alternative synthetic route in three steps by reaction of 2-(*s*-methylthio)isourea with the β-ketoester in aqueous KOH to afford the 2-amino-1,3-oxazine-4(3*H*)-ones and under acidic conditions to give the 7-phenyl-6,7-dihydrocyclopenta[*e*][1,3]oxazine-2,4(3*H*,5*H*)-dione which was then converted to the corresponding pyrimidine with concentrated aqueous ammonia.

We thought the key problem may be the ring strain. Therefore, application of the Danel method to our compounds with a seven-numbered ring would be feasible. In order to achieve the uracil ring, we have investigated a one-step process to synthesize cyclohepta[*d*]pyrimidine-2,4-dione (**6**). In our knowledge, the use of a one-step process to synthesize pyrimidine rings is infrequent. The β-ketoester **5** condensed directly with urea in the presence of sodium methoxide and the corresponding pyrimidine **6** was precipated by acidifying with dilute hydrochlororide in an aqueous solution. and recrystalized from ethanol in yields of 46.0–52.0%. The advantage of the above-mentioned reaction pathway was its simplicity, because the procedures were undertaken conveniently and the materials were commercially available.

The preparation of N-1 substituted uracils deserves some comment. When **6a**–**b** were treated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in $CHCl_3$ at room temperature, reaction with alkyl chloromethyl ether gave **7a**–**b** or **8a**–**b** in good yield. In contrast, a similar reaction with other alkyl halides, such as allyl bromide and benzyl bromide, gave the corresponding products in low yield (<10%).

The yields were improved by applying an alternative route. Treatment of **6a**–**b** with allyl bromide and benzyl bromide, respectively, using potassium carbonate as an alkali in anhydrous DMF, gave the mixture of N-1, N-3 substituted and disubstituted products. Because N-1 is more nucleophilic than N-3, the N-1 substituted compound can be made the major product by controlling the ratio of reagents and the reaction temperature and time. The addition of a catalytic amount of KI can shorten the time and improve the yield. The structure assignments of these compounds were identified by NMR and mass spectral data as well as a single crystal X-ray structure analysis for compound **7a** (Fig. 3).

The N-1 alkylated compound was confirmed by ^{1}H-nuclear Overhauser effects (NOE) (Fig. 4), as irradiation of 9-H resulted in NOE in the N–CH_2–R′ groups. Furthermore, being affected by the 9-phenyl ring and the 9-chiral carbon, the ^{1}H NMR spectrum showed N–CH_aH_b–R′ with different chemical shifts at δ 4.74, 5.54 (d, each, J = 11.4 Hz).

Biological activity

The compounds **6a**–**b**, **7a**–**b**, **8a**–**b**, **9** and **10** were tested for their activity against HIV in RT assay, using a poly(rA)/oligo(dT)$_{15}$ homopolymer template with the HIV antigen detection ELISA method[42] and nevirapine as a reference compound. It is particularly noteworthy that compound **8a** (IC_{50} = 1.51 μM) was more active than nevirapine (IC_{50} = 3.67 μM) at concentrations 2-fold and 10^2-fold compared to **7a** (IC_{50} = 100.52 μM) by introducing the terminal phenyl moiety at the N-1 side chain. However,

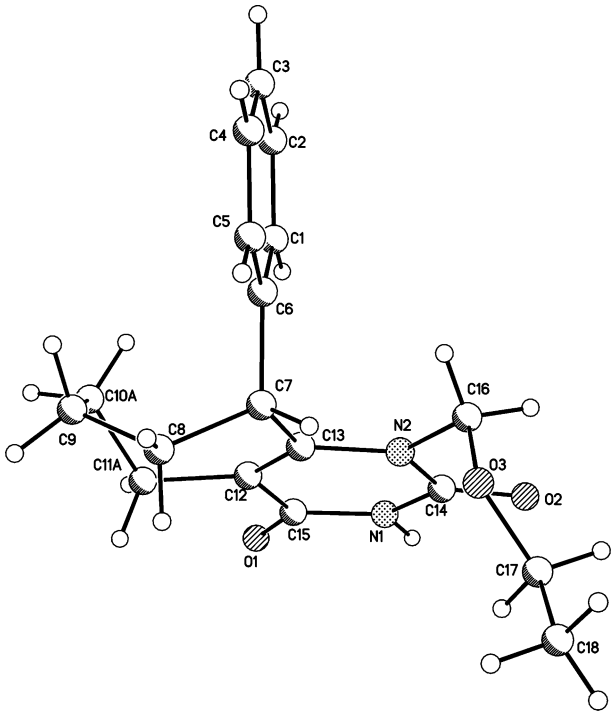


Fig. 3 X-Ray crystal structure of **7a**.

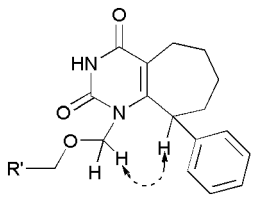


Fig. 4 NOE between 9-H with the N–CH_2–R′ groups.

an increase of the steric bulkyness at the 9-phenyl ring with 4-methyl substitution showed lower activity for **8b** (IC_{50} = 9.31 μM) compared to **8a**.

Experimental

Melting points were determined with an electrothermal capillary melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a JEOL-AL-300 FT spectrometer with TMS as the internal standard. The ESI-TOF spectra were taken on a Liner Scieneific LDI-1700 mass spectrometer. IR spectra were recorded with Avatar 360 FT-IR and reported in cm^{-1}. Elemental analyses were performed on VarVario EL III. Silica gel (0.040–0.064 mm) was used for column chromatography. Some reagents were purchased from Aldrich.

General procedure for the preparation of compounds 2a–b

Compound **1** (37.3 mmol) was mixed with 2 mL H_2O and 6 g chopped ice. To the stirred mixture 40 mmol of ethyl chlorocarbonate was added dropwise. Simultaneously, an ice-cold solution of 41 mmol of sodium hydroxide in 5.2 mL water was added dropwise, at the same rate with that of the ethyl chlorocarbonate in order to maintain the temperature at 10–15 °C. The reaction mixture was stirred for an additional 20 min and then filtered. The solid residue was washed with cold water and obtained the white needles solid.

Ethyl *N*-benzylcarbamate (2a). Yield 96.7% mp 44–45 °C (H_2O) (lit.[40] 45–47 °C).

(4-Methyl-benzyl)carbamic acid ethyl ester (2b). Yield 90.0%, mp 55–56 °C (H_2O) (lit.[43] 55–57 °C).

General procedure for the preparation of compounds 3a–b

A mixture of 20 mmol of **2** in 20 mL ethyl ether and 174 mmol of sodium nitrite in 20 mL H_2O was stirred, and then treated with another solution of 10 ml concentrated nitric acid and water for each. The dropping rate was adjusted to keep the aqueous phase green. The reaction mixture was allowed to stand for another 20 min. The ether layer was separated and the resulting solution was washed with 5 mL 10% potassium carbonate solution, dried and evaporated under reduced pressure to give the nitroso product as orange–red oil in a nearly quantitative yield.

Ethyl *N*-nitroso-*N*-benzylcarbamate (3a). Yield 98.0%.

Ethyl *N*-nitroso-*N*-(4′-methyl)benzylcarbamate (3b). Quantitative yield.

General procedure for the preparation of compounds 4a–b

To a stirred mixture which include 40 mmol of freshly distilled cyclohexanone, 0.3 g of finely powdered anhydrous potassium carbonate and 8 mL absolute methanol, was added, over a period of 10 min, 20 mmol of the ethyl *N*-nitroso compound **3** during which time the temperature was maintained at 20–25 °C by means of a cold water bath. The reaction mixture was then allowed to stand by overnight. The potassium carbonate was removed by filtration, the solvents were evaporated under reduced pressure, an oily residue was obtained which was purified by silica gel column chromatography with EtOAc–petroleum ether (60–90 °C) to give the products.

2-Phenylcycloheptanone (4a). Yield 59.0%, light yellow oil, (lit.[40] 41%).

2-*p*-Tolylcycloheptanone (4b). Yield 31.5%, mp 52–54 °C (EtOAc–petroleum ether) (lit.[43] 26%, 57–58 °C).

General procedure for the preparation of compounds 5a–b

A 8.5 mmol amount of sodium hydride was washed with freshly distilled hexane. After most of the mineral oil had been removed, 8 mL of benzene and 6.0 mmol of diethyl carbonate were added. The mixture was heated to reflux and a solution of 3.0 mmol **4** in 1 mL benzene was added dropwise. After addition, this mixture was refluxed for 5 h, cooled to room temperature and hydrolyzed with dilute HCl. The benzene layer was separated and the aqueous layer was extracted with ethyl ether (3 × 10 mL). The combined organic layer was washed with cold water, dried and evaporated *in vacuo* to afford the crude product which was purified by silica gel column chromatography with EtOAc–petroleum ether (60–90 °C) as eluent.

Ethyl-2-oxo-3-phenylcycloheptanecarboxylate (5a). Yield (741 mg, 94.9%), yellow oil; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (3H, t, CH_2CH_3), 1.46–2.18 (8H, m, $CH_2CH_2CH_2CH_2$),

3.67 (1H, m, COC*H*CO), 3.94 (1H, m, ArC*H*CO), 4.13 (q, 2H OCH_2CH$_3$), 7.23–7.32 (5H, m, Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH$_2$*C*H$_3$), 26.3, 27.9, 28.7, 32.7 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 57.2 (CO*C*HAr), 58.4 (CO*C*HCO), 61.0 (COO*C*H$_2$CH$_3$), 126.9, 127.0, 127.9, 128.2, 128.3, 139.7 (Ar), 170.2 (*C*OOC$_2$H$_5$), 207.9 (*C*O); ESI-TOF$^+$: 261.1748 (M + H)$^+$.

Ethyl-2-oxo-3-*p*-tolylcycloheptanecarboxylate (5b). Yield (740 mg, 89.9%); yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, t, CH$_2$C*H*$_3$), 1.35–2.23 (8H, m, CH_2CH_2CH_2CH_2), 2.32 (3H, s, ArCH_3), 3.65 (1H, m, COC*H*CO), 3.91 (1H, m, ArC*H*CO), 4.15 (q, 2H, OCH_2CH$_3$), 7.10–7.25 (4H, m, Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (CH$_2$*C*H$_3$), 21.0 (Ar*C*H$_3$), 26.2, 27.7, 28.3, 32.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 56.9 (CO*C*HAr), 58.2 (CO*C*HCO), 61.1 (COO*C*H$_2$CH$_3$), 127.7, 128.2, 128.9, 129.1, 136.6,136.7 (Ar), 170.2 (*C*OOC$_2$H$_5$), 208.1 (*C*O); ESI-TOF$^+$ 275.1558 (M+ H)$^+$.

General procedure for the preparation of compounds 6a–b

Urea (3.3 mmol) and compound **5** (3.0 mmol) were added to a solution of sodium metal (4.0 mmol) in 9 mL of absolute ethanol, and the mixture was heated at reflux for 6 h. After evaporation *in vacuo* at 40–50 °C until nearly dryness, the residue was dissolved in 5 mL water and then precipitated by addition of dilute aqueous HCl to pH 4. The crude product was recrystalized from ethanol to afford pure **6a**–**b**.

6,7,8,9-Tetrahydro-9-phenyl-1*H*-cyclohepta[*d*]pyrimidine-2,4-(3*H*,5*H*)-dione (6a). Yield (400 mg, 52.0%); mp 230–232 °C (EtOH), white solid; (found: C, 70.31; H, 6.25; N, 10.87%, $C_{15}H_{16}N_2O_2$, requires C, 70.29; H, 6.29; N, 10.93%); ν_{max}/cm^{-1} 3452.17, 3229.26, 1716.58 and 1634.28;^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.35–2.83 (8H, m, CH_2CH_2CH_2CH_2), 4.05 (1H, m, C*H*Ar), 7.19–7.39 (5H, m, Ar), 10.53 (1H, s, NH1), 11.10 (1H, s, NH3); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 21.5, 24.0, 25.6, 29.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 46.7 (Ar*C*H), 111.1 (C5), 126.5, 127.1, 127.1, 128.7, 128.7, 138.5 (Ar), 150.8 (C6), 153.4 (C2), 164.8 (C4); ESI-TOF$^+$ 257.1845 (M + H)$^+$.

6,7,8,9-Tetrahydro-9-*p*-tolyl-1*H*-cyclohepta[*d*]pyrimidine-2,4-(3*H*,5*H*)-dione (6b). Yield (373 mg, 46.0%); mp 236–238 °C (EtOH), white solid; (found: C, 71.11; H, 6.75; N, 10.30%, $C_{16}H_{18}N_2O_2$, requires C, 71.09; H, 6.71; N, 10.36%); ν_{max}/cm^{-1} 3410.89, 3219.36, 1709.85 and 1640.31; ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.22–2.83 (8H, m, CH_2CH_2CH_2CH_2), 2.28 (3H, s, ArCH_3), 4.00 (1H, m, C*H*Ar), 7.05–7.17 (4H, m, Ar), 10.52 (1H, s, NH1), 11.10 (1H, s, NH3); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 20.6 (Ph*C*H$_3$), 21.5, 24.1, 25.7, 29.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 46.3 (Ar*C*H), 111.5 (C5), 127.0, 127.0, 129.3, 129.3, 131.5, 135.4 (Ar), 151.0 (C6), 153.7 (C2), 164.8 (C4); ESI-TOF$^+$ 271.1654 (M + H)$^+$.

General procedure for the preparation of compounds 7a–b and 8a–b

To a suspension of **5** (0.11 mmol) in 2 mL dry $CHCl_3$ was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) 0.24 mmol and the stirring was continued to obtain a clear solution. Then chloromethyl ether (0.13 mmol) was added and the reaction mixture was stirred until TLC showed no change in amount of starting material. After evaporation of the solvent *in vacuo*, the residue was chromatographed on silica gel with EtOAc–petroleum ether as eluent to afford the pure N-1 alkylated product.

1-(Ethoxymethyl)-6,7,8,9-tetrahydro-9-phenyl-1*H*-cyclohepta-[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (7a). Yield (32 mg, 92.6%); mp 180–182 °C (10% EtOAc–petroleum ether), white solid; (found: C, 68.73; H, 7.10; N, 9.00%, $C_{18}H_{22}N_2O_3$, requires C, 68.77; H, 7.05; N, 8.91); ν_{max}/cm^{-1} 3349.99, 1669.80 and 1608.77; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (3H, t, CH$_2$C*H*$_3$), 1.42–2.98 (8H, m, CH_2CH_2CH_2CH_2), 3.60 (2H, m, OCH_2CH$_3$), 4.71(1H, m, ArC*H*), 4.74, 5.54 (2H, 2 × d, *J* 11.4, NCH_2O), 7.17–7.38 (5H, m, Ar), 8.51 (1H, s, NH3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1 (CH$_2$*C*H$_3$), 21.7, 23.6, 24.7, 30.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 44.5 (Ar*C*H), 64.9 (OCH_2CH$_3$), 72.8 (N*C*H$_2$O), 116.0 (C5),126.9, 127.0, 127.0, 129.1, 129.1, 138.5 (Ar), 151.6 (C6), 154.8 (C2), 163.2 (C4); ESI-TOF$^+$ 315.1571 (M + H)$^+$.

1-[(Benzyloxy)methyl]-6,7,8,9-tetrahydro-9-phenyl-1*H*-cyclohepta[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (8a). Yield (29 mg, 70.5%); mp 50–52 °C (10% EtOAc–petroleum ether), white solid; (found: C, 73.40; H, 6.47; N, 7.49%, $C_{23}H_{24}N_2O_3$, requires C, 73.38; H, 6.43; N, 7.44%); ν_{max}/cm^{-1} 3340.18, 1670.23 and 1617.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60–2.95 (8H, m, CH_2CH_2CH_2CH_2), 4.69(1H, m, ArC*H*), 4.75(2H, m, PhCH_2O), 4.79, 5.66 (2 H, 2 × d, *J* 11.1, NCH_2O), 7.25–7.37 (10H, m, 2 × Ar), 9.2 (1H, s, NH3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 23.5, 24.6, 30.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 44.7 (Ar*C*H), 65.2 (O*C*H$_2$Ph), 72.7 (N*C*H$_2$O), 116.0 (C5),126.8, 126.8, 126.9, 126.9, 127.5, 127.9, 128.5, 128.5, 129.1, 129.1, 137.2, 138.4 (2 × Ar), 152.0 (C6), 154.5 (C2), 163.6 (C4); ESI-TOF$^+$: 377.1569 (M + H)$^+$.

1-(Ethoxymethyl)-6,7,8,9-tetrahydro-9-*p*-tolyl-1*H*-cyclohepta[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (7b). Yield (32 mg, 88.2%); mp 136–138 °C (10% EtOAc–petroleum ether), white solid; (found: C, 69.44; H, 7.41 N, 8.60%, $C_{19}H_{24}N_2O_3$, requires C, 69.49; H, 7.37; N, 8.53%); ν_{max}/cm^{-1} 3338.15, 1668.54, 1600.98; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (3H, t, CH$_2$C*H*$_3$), 1.45–2.98 (8H, m, CH_2CH_2CH_2CH_2), 2.34 (3 H, s, ArCH_3), 3.61 (2H, m, OCH_2CH$_3$), 4.66 (1 H, m, PhC*H*), 4.73, 5.56 (2 H, 2 × d, *J* 11.4, NCH_2O), 7.04–7.17 (4H, m, Ar), 8.94 (1H, s, NH3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1 (CH$_2$*C*H$_3$), 21.0 (Ph*C*H$_3$), 21.7, 23.6, 24.7, 30.9 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 44.2 (Ar*C*H), 64.8 (O*C*H$_2$CH$_3$), 72.7 (N*C*H$_2$O), 115.8 (C5), 126.8, 126.8, 129.8, 129.8, 135.4, 136.6 (Ar), 151.8 (C6), 155.0 (C2), 163.4 (C4); ESI-TOF$^+$: 329.1687 (M + H)$^+$.

1-[(Benzyloxy)methyl]-6,7,8,9-tetrahydro-9-*p*-tolyl-1*H*-cyclohepta[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (8b). Yield (30 mg, 70.0%); mp 60–62 °C (10% EtOAc–petroleum ether), white solid; (found: C, 73.79; H, 6.68; N, 7.12%, $C_{24}H_{26}N_2O_3$, requires C, 73.82; H, 6.71; N, 7.17%); ν_{max}/cm^{-1} 3343.28, 1675.36 and 1619.21; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60–2.95 (8H, m, CH_2CH_2CH_2CH_2), 2.33 (3H, s, ArCH_3), 4.64(1H, m, ArC*H*), 4.70 (2H, m, PhCH_2O), 4.77, 5.66 (2 H, 2 × d, *J* 11.1, NCH_2O), 7.00–7.38 (9H, m, 2 × Ar), 8.89 (1H, s, NH3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 (Ar*C*H$_3$), 21.6, 23.5, 24.6, 31.0 (*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 44.4 (Ar*C*H), 71.5 (O*C*H$_2$Ph), 72.7 (N*C*H$_2$O), 115.9 (C5), 126.8, 127.9, 127.9, 128.0, 128.0, 128.4, 128.4, 129.8, 129.8, 135.3, 136.6, 137.2 (2 × Ar), 151.8 (C6), 154.7 (C2), 163.3 (C4); ESI-TOF$^+$: 391.1543 (M + H)$^+$.

General procedure for the preparation of compounds 9 and 10

A mixture of **6** (0.12 mmol), proper alkyl bromide (0.14 mmol), potassium carbonate (20 mg, 0.15 mmol) and catalytic amount of KI in 1 mL of anhydrous *N*,*N*-dimethyl formamide (DMF) was stirred at room temperature for 8 h. After treatment with cold water (10 mL), the solution was extracted with ethyl acetate (3 × 15 mL). The organic layers were collected, dried and evaporated to furnish the crude product, which was purified by column chromatography on silica gel (eluent: 20% EtOAc–petroleum ether).

1-Allyl-6,7,8,9-tetrahydro-9-phenyl-1*H*-cyclohepta[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (9). Yield (32 mg, 90%); mp 175–177 °C (10% ethyl acetate–petroleum ether), light yellow solid; (found: C, 73.35; H, 6.90; N, 9.50%. $C_{18}H_{20}N_2O_2$, requires C, 72.95; H, 6.80; N, 9.45%); ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.25–3.18 (8H, m, CH_2CH_2CH_2CH_2), 4.05 (1H, m, ArC*H*), 4.50 (2H, d, *J* 6.0, NCH_2CH=), 5.17, 5.24 (2H, 2 × d, *J* 10.5 and 18.0 CH=CH_2), 5.87 (1H, m, CH_2C*H*=CH_2), 7.37–7.54 (5H, m, Ar), 8.87 (1H, s, NH3); ^{13}C NMR (75 MHz; $CDCl_3$) δ 21.5, 24.1, 25.7, 29.9 (*C*H_2*C*H_2*C*H_2*C*H_2), 46.3 (Ar*C*H), 47.8 (N*C*H_2CH=), 111.1 (C5), 117.2 (CH=*C*H_2), 126.0, 127.2, 127.2, 129.3, 129.3, 135.4 (Ar), 135.6 (*C*H=CH_2), 150.8 (C6), 153.7 (C2), 164.8 (C4); ESI-TOF$^+$ 297.1496 $[M + H]^+$.

1-Benzyl-6,7,8,9-tetrahydro-9-phenyl-1*H*-cyclohepta[*d*]pyrimidine-2,4(3*H*,5*H*)-dione (10). Yield (36 mg, 87%); mp 162–164 °C (10% ethyl acetate–petroleum ether), light yellow solid; (found: C, 76.35; H, 6.58; N, 8.28%. $C_{22}H_{22}N_2O_2$, requires C, 76.28; H, 6.40; N, 8.09%); ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.42–3.14 (8H, m, CH_2CH_2CH_2CH_2), 4.03 (1H, m, ArC*H*), 5.06 (2H, s, NCH_2Ph) 7.20–7.47 (10H, m, 2 × Ar), 8.91 (1H, s, NH); ^{13}C NMR (75 MHz; $CDCl_3$) δ 23.6, 25.8, 30.1, 32.3 (*C*H_2*C*H_2*C*H_2*C*H_2), 44.2 (Ph*C*H), 48.2 (NH*C*H_2Ph), 111.9 (C5), 127.5, 128.3, 128.3, 128.4, 128.4, 128.4, 128.4, 129.2, 129.7, 129.7, 136.9, 138.4 (2 × Ar), 150.7, 151.5 (C6 and C2), 163.6 (C4); ESI-TOF$^+$ 347.1481 $[M + H]^+$.

Crystal data for 7a†

$C_{18}H_{22}N_2O_3$, $M = 314.38$, triclinic, space group P-1, $a = 8.2013$ (12), $b = 10.5938$ (16), $c = 10.9160$ (17) Å, unit-cell volume = 810.1(2), at 297(2) K, $Z = 2$, absorption coefficient $\mu = 0.088$, total reflections = 3286, refined final R values for all = 0.0509. Two of the carbon atoms (C10, C11) of the cycloheptene ring are disordered unequally over two adjacent sites with occupancies 0.732(7) and 0.268(7).

Conclusions

In the course of synthesizing target molecules (TMs) **7a**–**b**, **8a**–**b**, **9** and **10**, we have developed a new synthetic route in reaching a C5 and C6 annelated structure of uracil with a seven-membered ring. These include (a) using a modified method of Paul Krapcho *et al.*, the intermediate cyclic β-ketoester **5** was prepared with high yield (90–95%); (b) with a one-step process, the cyclohepta[*d*]pyrimidine-2,4-diones (**6a**–**b**) were condensed directly from a β-ketoester with urea; (c) for diverse alkyl agents, different methods were used in forming the side chain of N-1.

As seen from examination of the results, structural variation in the TMs have resulted in a wide range of biological activities with IC_{50} values from 1 to 200 μM against wild-type HIV-1 RT. When comparing trends among the series of compounds, we found that **8a** ($IC_{50} = 1.51$ μM) substituted at R′ by a phenyl group was 2 times more potent than nevirapine ($IC_{50} = 3.67$ μM) and 100 times more active than **7a** ($IC_{50} = 100.52$ μM). From the factors responsible for the significant activity of compound **8a**, it can be elucidated that the terminal phenyl moiety at the N-1 side chain of **8a** extends deeper into and occupies a more hydrophobic pocket, comprised of Tyr318 and Pro236. Particularly, a Tyr318 situated within a short distance of the N-1 group provides the π–π interaction with the phenyl ring. The bulky N-1 substituents may be accommodated because of the flexibility of the Pro236 loop region. To confirm the above-mentioned findings, we shortened the side chain of N1 with allyl and benzyl moieties to **9** and **10**, respectively, which led to a significant drop in activity compared to **8a**. However, the activity of compound **10** ($IC_{50} = 101.28$ μM) is better than that of **9** ($IC_{50} > 200$ μM). Thus, an appropriately substituted benzyloxy methyl ether may improve the activity.

We next investigated the influence of the substituent at the 9-phenyl ring for activity against HIV-1. It is known from the literature that the corresponding GCA-186 with 3,5-dimethyl substituents on its phenyl ring was better able to tolerate the presence of Y181C or K103N mutations than MKC-442. In our study compounds, **7b** and **8b**, the increase in steric bulkiness at the 9-phenyl ring with 4-methyl substitution seems to be not particularly favorable; the biological activity obtained for **8b** ($IC_{50} = 9.31$ μM) is lower than that of **8a**. The 4-methyl group probably forced the 9-phenyl ring away from the hydrophobic pocket formed by the side chains of Try181, Try188, Phe227, Trp229 and reduced the π–π interactions. In contrast, the 3,5-dimethyl groups of GCA-186 could correlate with an overall tighter binding in the hydrophobic pocket, and thus enhance the affinity to the RT. Encouraged by these results, more detailed SAR studies on the annelated compounds are underway, with a focus on exploring the important role of this unique structure feature.

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