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Original article

Synthesis and anti-HIV activity evaluation of 1-[(alkenyl or alkynyl or alkyloxy)methyl]-5-alkyl-6-(1-naphthoyl)-2,4-pyrimidinediones as novel non-nucleoside HIV-1 reverse transcriptase inhibitors

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Abstract

The synthesis and anti-HIV activity evaluation of a new series of 2,4-pyrimidinediones bearing a 6-(1-naphthoyl) group are described. In general, it was found that most of the title compounds showed good activities against human immunodeficiency virus type-1 (HIV-1). In particular, compound **26** displayed the most potent anti-HIV-1 activity ($IC_{50} = 0.11 \pm 0.05$ μM), inhibiting HIV-1 replication in MT-4 cells more effectively than HEPT (by 45-fold) and DDI (by 50-fold).

Keywords: Non-nucleoside HIV-1 reverse transcriptase inhibitors; 2,4-Pyrimidinediones; 6-(1-Naphthoyl); Synthesis; Anti-HIV activity

1. Introduction

The currently available drugs for acquired immunodeficiency syndrome (AIDS) therapy are divided into four classes: fusion inhibitors, protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs are highly specific for HIV-1 and comprise more than 30 structurally different classes of molecules endowed with potent activity and low toxicity [1–3]. However, a number of problems still remain with these agents, in particular, the therapeutic potential of this class of drugs has been compromised by the rapid development of resistance, but their use in combination therapy has been encouraging and has revived interest in the search for novel and potent NNRTIs [3–5].

Among the representatives of the NNRTIs, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-thymine (HEPT, **1**) (Fig. 1) has an interesting structure and constitutes an important inhibitor which has induced many kinds of structural modifications on the skeleton of thymine as a lead compound [6–9]. Initially, we developed a series of 6-(1-naphthylmethyl) and 6-(1-naphthylthio) substituted HEPT analogues (HEPTs) (**2**–**3**, Fig. 1), which exhibited significant anti-HIV-1 activities [10,11]. Inspired by these promising results and in continuation of our work on the research of novel NNRTIs, we thought it is worthwhile to synthesize new compounds of HEPTs having 6-(1-naphthoyl) moiety, with the aim to improve the interaction between the inhibitors and reverse transcripts (RT) and to obtain new biologically active compounds.

In the present paper, we described the synthesis and bio-logical evaluation of a series of 1-[(alkenyl or alkynyl or alkyloxy)methyl]-5-alkyl-6-(1-naphthoyl)-2,4-pyrimidinediones (**4**, Fig. 1) as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. It is a further object of this paper to develop our understanding of the structure–activity relationship among

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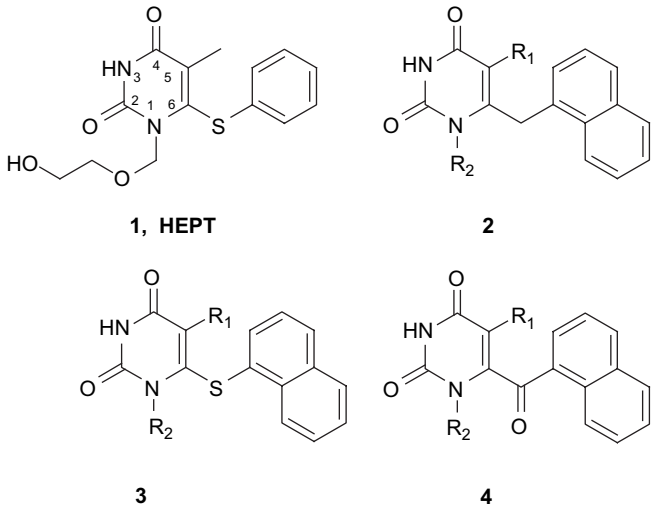


Fig. 1. Structure of HEPT and HEPT analogues.

HEPTs and to afford information to the ongoing investigation of the NNRTI binding site.

2. Results and discussion

2.1. Chemistry

The synthesis of the newly designed compounds is described in Scheme 1. The required 5-alkyl-6-chloro-2,4-dimethoxypyrimidines **5a–c** were synthesized according to the method of Baddiley and Topham [12] and Merkatz [13] from the corresponding trichloropyrimidines prepared by reaction of the appropriate barbituric acid derivatives with $POCl_3$. The resulting 5-alkyl-2,4,6-trichloropyrimidines were reacted with NaOMe to afford the 5-alkyl-6-chloro-2,4-dimethoxypyrimidines **5a–c**.

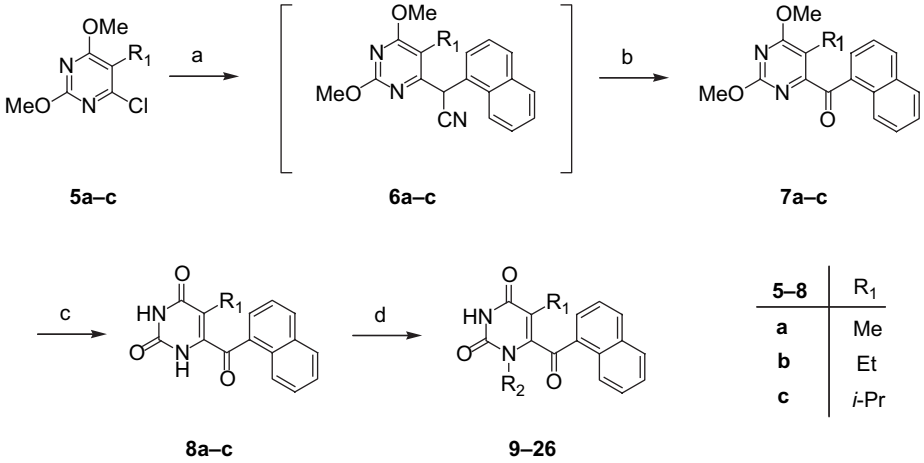
Condensation of **5a–c** with 1-naphthylacetonitrile in the presence of 60% NaH in anhydrous DMF for 48 h at room temperature under N_2 gave the corresponding nitriles **6a–c**, which, without separation, were immediately oxidized to ketones **7a–c** by passing air into the reaction mixture at room temperature. Demethylation of **7a–c** with conc. HCl in refluxing MeOH for 24–48 h afforded the 5-alkyl-6-(1-naphthoyl)-2,4-pyrimidinediones **8a–c**, which were subjected to *N*-1 alkylation with various halides in the presence of K_2CO_3 in anhydrous DMF to afford the desirable target compounds **9–26**.

All the target compounds **9–26** were characterized by NMR, MS and elemental analysis. And *N*-1 substitution was further confirmed by the two-dimensional $^1H-^{13}C$ heteronuclear multiple-bond correlation (HMBC). The *N*-3 regioisomer was not observed. Both analytical and spectral data of all the compounds are in full agreement with the proposed structures.

2.2. Anti-HIV activity

Title compounds were evaluated for cytotoxicity and inhibitory activity against wild type HIV-1 strain III_B and HIV-2 ROD in MT-4 cells (Table 1) in comparison with HEPT and 2,3-dideoxyinosine (DDI) used as reference drugs. The results, expressed as CC_{50} (cytotoxicity), IC_{50} (anti-HIV activity) and SI (selectivity, given by the CC_{50}/IC_{50} ratio) values, are summarized in Table 1.

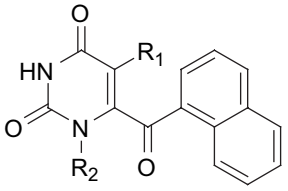
In general, it was found that the majority of the synthesized compounds showed good activity against wild type HIV-1 with a wide range of IC_{50} values from 7.69 ± 2.20 μM to 0.11 ± 0.05 μM, except for compounds **9**,**10**. In contrast, most of the title compounds exhibited more potent activities than the reference compounds. It is worth noting that compounds **21**, **23** and **26** bearing 1-benzyloxymethyl or 1-allyloxymethyl substituents were found to be the most active compounds against wild type HIV-1. In particular, compound **26** is the most potent among the newly discussed HEPTs ($IC_{50} = 0.11 \pm 0.05$ μM and SI = 233), inhibiting HIV-1 replication in MT-4 cells more effectively than HEPT (by 45-fold) and DDI (by 50-fold).



Reagents and conditions: (a) 1-naphthylacetonitrile, 60% NaH, DMF, N_2; (b) 60% NaH, DMF, air, 80–90% (one-pot synthesis); (c) conc. HCl, MeOH, reflux 24-48 h; (d) R_2X (X=Br, Cl), K_2CO_3, DMF, rt 36–48 h, 30.4–63.7%.

Scheme 1. Synthesis of the target compounds **9–26**.

Table 1
Anti-HIV activity in MT-4 cells of compounds **9**–**26**



9–26

Compound	R_1	R_2	IC_{50} (μM)[a]		CC_{50} (μM)[b]	SI[c]
			HIV-1 III_B	HIV-2 ROD		
9	Me	$(CH_3)_2C{=}CHCH_2$	>44.63	>130.83	44.63 ± 4.54	<1
10	Me	$CH_3COOCH_2CH{=}C(CH_3)CH_2$	41.38 ± 5.91	>200.00	200.00 ± 4.73	5
11	Me	$PhC{\equiv}CCH_2$	2.79 ± 0.91	>29.85	29.85 ± 8.91	11
12	Me	$CH_3CH_2OCH_2$	4.02 ± 1.12	>51.66	51.66 ± 15.80	13
13	Me		7.69 ± 2.20	>77.58	77.58 ± 14.34	10
14	Me	$PhCH_2OCH_2$	1.60 ± 0.53	>45.55	45.55 ± 13.75	29
15	Me	$CH_2{=}CHCH_2OCH_2$	2.11 ± 0.77	>131.31	131.31 ± 41.06	62
16	Et	*trans*-$CH_3OOCCH{=}CHCH_2$	0.71 ± 0.28	>38.62	38.62 ± 6.43	54
17	Et	$PhC{\equiv}CCH_2$	0.21 ± 0.13	>16.96	16.96 ± 8.63	83
18	Et		0.22 ± 0.03	>18.56	18.56 ± 5.48	84
19	Et	$CH_3CH_2OCH_2$	0.28 ± 0.03	>23.01	23.01 ± 8.58	78
20	Et		0.93 ± 0.26	>45.34	45.34 ± 16.01	48
21	Et	$PhCH_2OCH_2$	0.15 ± 0.08	>38.55	38.55 ± 9.69	257
22	Et	$(4\text{-}NO_2)PhCH_2OCH_2$	0.54 ± 0.20	>30.54	30.54 ± 10.28	55
23	Et	$CH_2{=}CHCH_2OCH_2$	0.19 ± 0.08	>45.60	45.60 ± 12.86	243
24	Et	$(CH_3)_2C{=}CHCH_2OCH_2$	6.89 ± 2.50	>36.35	36.35 ± 3.90	5
25	*i*-Pr	$(CH_3)_2C{=}CHCH_2$	2.42 ± 0.21	>30.82	30.82 ± 7.79	13
26	*i*-Pr	$PhCH_2OCH_2$	0.11 ± 0.05	>26.85	26.85 ± 8.93	233
HEPT			5.06 ± 0.00	>405.8	405	80
DDI			5.37	2.71	≥529	≥98

[a] Concentration required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[b] Concentration that reduces the normal uninfected MT-4 cell viability by 50%.
[c] Selectivity index: ratio CC_{50}/IC_{50}, a higher SI means a more selective compound.

As far as the substituents of the *N*-1 side chain are concerned, compounds **14** and **21** having benzyloxymethyl group were generally more potent than the ethoxymethyl substituted compounds **12** and **19**. Interestingly, the introduction of NO_2 at the phenyl ring leads to a decrease in potency and an increase in cytotoxicity in the case of compound **22**, which was 3-fold less potent than the unsubstituted compound **21**. It is thought that the nitro group as an electron withdrawing substituent decreases the electron density of the phenyl ring and thus might be not benefit the *N*-1 substituent to interact through π-stacking with the amino acids in the reverse transcriptase enzyme. In the *N*-1 alkenyloxymethyl substituted series, increasing the bulkiness around the double bond of compound **23** with two methyl moieties to **24** leads to significant decrease in anti-HIV-1 activity together with selectivity (35-fold and 49-fold, respectively). These results suggest that the bulky substituent may weaken π-stacking to the NNRTI binding site.

According to our results, it can be seen that some *N*-1 side chains are suitable to improve the anti-HIV-1 activity, e.g. *trans*-methoxycarbonylallyl, 3-phenyl-2-propynyl, (cyclopent-3-en-1-yl)methyl, (4-nitrobenzyloxy)methyl, cyclopropylmethoxymethyl, allyloxymethyl, ethoxymethyl and benzyloxymethyl. In particular, the benzyloxymethyl substituent generally was the best. Most of our newly synthesized title compounds were more potent than the lead compound HEPT and the reference drug DDI. The factors responsible for the improved inhibitory potency of these compounds remain to be elucidated. One explanation may be that these *N*-1 substituted groups have suitable length to make interactions with the RT. On the other hand, these groups could interact with RT-amino acid Tyr 318 or Pro 236 through π-stacking.

When the C-5 substituents are changed from Me, Et to *i*-Pr, the anti-HIV-1 activity increases. Especially, the *i*-Pr is the most potent in improving the biological activity. This conclusion is in agreement with the previous relative QSAR studies [14,15].

All title compounds were also evaluated for their capability to inhibit the HIV-2 multiplication in MT-4 cells, but none was found effective (Table 1). These findings show that our synthesized compounds are specific for HIV-1 and belong to typical NNRTIs.

3. Conclusion

In summary, we designed, synthesized and evaluated a series of 1-[(alkenyl or alkynyl or alkyloxy)methyl]-5-alkyl-6-(1-naphthoyl)-2,4-pyrimidinediones as novel NNRTIs on the basis of our previous studies concerning the development of

NNRTIs. The results showed that most of the title compounds have potent activities against HIV-1. In particular, compound **26** displayed the most potent anti-HIV-1 activity ($IC_{50} = 0.11 \pm 0.05$ μM), which is more potent than the reference compounds HEPT (by 45-fold) and DDI (by 50-fold). Further structural modification around the *N*-1 and C-6 positions is still required with an aim to develop novel and more potent NNRTIs. Our resulting studies provided useful indicators for guiding further rational design of novel agents for the treatment of AIDS. And it also further afforded information to the development of the structure–activity relationship among HEPTs and to the ongoing investigation of the NNRTI binding site.

4. Experimental protocols

4.1. Chemistry

Melting points were measured on a WRS-1 digital melting point instrument and are uncorrected. ^{1}H NMR, ^{13}C NMR and HMBC spectra were recorded in dimethylsulfoxide-d_6 (DMSO-d_6) or chloroform ($CDCl_3$) on a Brucker DMX 400 MHz spectrometer. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a MAT-95 mass spectrometer. Elemental analyses were performed on a Carlo Erba 1106 instrument and the results of elemental analyses for C, H and N were within ±0.4% of the theoretical values. All chemicals and solvents used were of reagent grade and were purified and dried by standard methods before use. All air-sensitive reactions were run under a nitrogen atmosphere. All reactions were monitored by TLC on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/*n*-hexane as eluents. Silica column chromatography separations were obtained on silica gel (300–400 mesh).

4.1.1. General procedure for preparation of 5-alkyl-2,4-dimethoxy-6-(1-naphthoyl)pyrimidine (**7a–c**)

To a solution of 5-alkyl-6-chloro-2,4-dimethoxypyrimidine **5a–c** (200 mmol) in anhydrous DMF (500 ml), 1-naphthylacetonitrile (40.08 g, 240 mmol) was added with stirring at room temperature. After the mixture was stirred for 0.5 h, NaH (9.60 g, 240 mmol) (60% in paraffin) was portion wise added at −15 °C under a nitrogen atmosphere. The whole was stirred at the same temperature for 1 h, warmed to room temperature and then reacted for 48 h to give the corresponding intermediate nitriles **6a–c**. Then without separation, air was immediately passed into the reaction mixture at room temperature for 48–72 h. The resulting mixture was neutralized with glacial acetic acid under ice cooling and concentrated under reduced pressure to afford the crude product **7a–c** to be used in the next step without further purification.

4.1.2. General procedure for preparation of 5-alkyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**8a–c**)

To a suspension of **7a–c** (90 mmol) in methanol (450 ml), 36% HCl (225 ml) was added. The resulting mixture was refluxed for 24–48 h. A yellow precipitate, which formed, was filtered, washed with H_2O, and recrystallized from MeOH/$CHCl_3$ (5:1).

*4.1.2.1. 5-Methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**8a**).* Yield: 21.5 g, 85.2%, m.p. 259.9–261.0 °C; ^{1}H NMR (DMSO-d_6) δ 1.63 (s, 3H, CH_3), 7.64–9.10 (m, 7H, naphthyl), 11.23 (s, 1H, NH).

*4.1.2.2. 5-Ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**8b**).* Yield: 21.7 g, 82.0%, m.p. 270.9–272.0 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, $J = 7.3$ Hz, 3H, CH_3), 2.03 (q, $J = 7.2$ Hz, 2H, CH_2), 7.66–9.00 (m, 7H, naphthyl), 11.20 (s, 1H, NH).

*4.1.2.3. 5-Isopropyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**8c**).* Yield: 19.3 g, 69.6%, m.p. 228.8–231.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (d, $J = 6.4$ Hz, 6H, CH_3), 2.49 (sep, $J = 6.4$ Hz, 1H, CH), 7.69–9.22 (m, 7H, naphthyl), 11.25 (s, 1H, NH), 11.38 (s, 1H, NH).

4.1.3. General procedure for preparation of compounds (**9–26**)

Anhydrous K_2CO_3 (2 mmol) was added to a solution of **8a–c** (2 mmol) in dry DMF (8 ml), and stirring was continued at room temperature for 0.5 h. Then, appropriate halides (2.2 mmol) were added. The reaction mixture was stirred for additional 36–48 h. Following filtration, the filtrate was concentrated in vacuo to give a brown residue, which was purified by silica column chromatography (ethyl acetate/*n*-hexane = 1:2).

*4.1.3.1. 5-Methyl-1-(3-methyl-2-butenyl)-6-(1-naphthoyl)-2,4-pyrimidinedione (**9**).* Yield: 0.32 g, 45.5%, m.p. 104.2–106.0 °C; ^{1}H NMR (DMSO-d_6) δ 1.07 (s, 3H, =C(CH_3)$_2$), 1.29 (s, 3H, =C(CH_3)$_2$), 1.56 (s, 3H, CH_3), 4.17 (d, $J = 5.8$ Hz, 2H, NCH_2), 4.94 (t, $J = 5.7$ Hz, 1H, =CH), 7.65–9.18 (m, 7H, naphthyl), 11.63 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.06 (CH_3), 17.75 (=C(CH_3)$_2$), 25.41 (=C(CH_3)$_2$), 43.38 (NCH_2), 106.99 (C-5), 120.10 (=CH), 125.23, 125.79, 127.64, 129.71, 129.74, 130.28, 130.31, 134.12, 136.19, 136.63, 137.19 (naphthyl, =C), 148.24 (C-6), 150.97 (C-2), 164.10 (C-4), 192.07 (C=O). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N; ESIMS *m/z*: 349 ([M + H]$^+$).

*4.1.3.2. 1-(4-Acetate-2-methyl-2-butenyl)-5-methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**10**).* Yield: 0.28 g, 33.9%, m.p. 177.2–177.9 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 3H, =CCH_3), 1.60 (s, 3H, CH_3), 1.88 (s, 3H, $COCH_3$), 4.15 (m, 4H, NCH_2, OCH_2), 5.00 (m, 1H, =CH), 7.62–9.08 (m, 7H, naphthyl), 11.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.14 (CH_3), 14.18 (=CCH_3), 21.02 ($COCH_3$), 50.40 (NCH_2), 60.14 (OCH_2), 107.63 (C-5), 121.08 (=CH), 125.24,

125.73, 127.64, 129.70, 129.90, 130.11, 130.22, 134.19, 135.33, 136.00, 137.10 (naphthyl, =C), 147.99 (C-6), 151.14 (C-2), 164.08 (C-4), 170.40 (OC=O), 191.74 (C=O). Anal. ($C_{23}H_{22}N_2O_5$) C, H, N; ESIMS *m/z*: 407 ($[M+H]^+$).

*4.1.3.3. 5-Methyl-6-(1-naphthoyl)-1-(3-phenyl-2-propynyl)-2,4-pyrimidinedione (**11**).* Yield: 0.49 g, 62.1%, m.p. 202.9–203.8 °C; ^{1}H NMR (DMSO-d_6) δ 1.59 (s, 3H, CH_3), 4.67 (bs, 2H, NCH_2), 7.06–7.34 (m, 5H, phenyl), 7.60–9.18 (m, 7H, naphthyl), 11.81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.27 (CH_3), 35.14 (NCH_2), 84.24 (≡C), 84.64 (≡C), 108.29 (C-5), 121.70, 125.30, 125.81, 127.68, 128.76, 128.88, 129.11, 129.33, 129.71, 130.20, 130.25, 130.31, 131.74, 134.18, 136.50, 137.15 (naphthyl, phenyl), 147.13 (C-6), 150.63 (C-2), 163.99 (C-4), 191.70 (C=O). Anal. ($C_{25}H_{18}N_2O_3$) C, H, N; ESIMS *m/z*: 395 ($[M+H]^+$).

*4.1.3.4. 1-Ethoxymethyl-5-methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**12**).* Yield: 0.32 g, 47.2%, m.p. 181.0–181.9 °C; ^{1}H NMR (DMSO-d_6) δ 0.73 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.56 (s, 3H, CH_3), 3.32 (m, 2H, CH_2CH_3), 5.06–5.09 (bs, 2H, NCH_2), 7.64–9.09 (m, 7H, naphthyl), 11.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.12 (CH_3), 14.67 (CH_2CH_3), 63.71 (OCH_2), 73.05 (NCH_2), 108.68 (C-5), 125.35, 125.66, 127.50, 129.57, 129.95, 130.27, 130.52, 134.09, 135.64, 136.61 (naphthyl), 146.59 (C-6), 151.16 (C-2), 163.99 (C-4), 191.91 (C=O). Anal. ($C_{19}H_{18}N_2O_4$) C, H, N; ESIMS *m/z*: 339 ($[M+H]^+$).

*4.1.3.5. 1-Cyclopropylmethoxymethyl-5-methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**13**).* Yield: 0.24 g, 33.8%, m.p. 167.1–168.5 °C; ^{1}H NMR (DMSO-d_6) δ −0.04–(−0.05) (m, 2H, cyclopropyl), 0.23–0.24 (m, 2H, cyclopropyl), 0.64 (m, 1H, cyclopropyl), 1.55 (s, 3H, CH_3), 3.15 (m, 2H, OCH_2), 5.09 (bs, 2H, NCH_2), 7.64–9.09 (m, 7H, naphthyl), 11.69 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 3.09 (cyclopropyl), 3.31 (cyclopropyl), 10.26 (CH_3), 11.12 (cyclopropyl), 72.86 (NCH_2), 73.03 (OCH_2), 108.78 (C-5), 125.40, 125.68, 127.48, 129.54, 129.90, 130.29, 130.58, 134.10, 135.69, 136.55 (naphthyl), 146.64 (C-6), 151.14 (C-2), 164.00 (C-4), 191.84 (C=O). Anal. ($C_{21}H_{20}N_2O_4$) C, H, N; ESIMS *m/z*: 365 ($[M+H]^+$).

*4.1.3.6. 1-Benzyloxymethyl-5-methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**14**).* Yield: 0.34 g, 42.3%, m.p. 150.0–151.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.57 (s, 3H, CH_3), 4.42 (s, 2H, OCH_2), 5.17 (bs, 2H, NCH_2), 7.00–7.19 (m, 5H, phenyl), 7.55–9.07 (m, 7H, naphthyl), 11.73 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.15 (CH_3), 70.26 (OCH_2), 73.13 (NCH_2), 108.77 (C-5), 125.34, 125.70, 127.50, 127.97, 128.06 (2C), 128.42 (2C), 129.56, 129.96, 130.25, 130.44, 134.10, 135.86, 136.62, 137.25 (naphthyl, phenyl), 146.67 (C-6), 151.24 (C-2), 164.03 (C-4), 191.90 (C=O). Anal. ($C_{24}H_{20}N_2O_4$) C, H, N; ESIMS *m/z*: 401 ($[M+H]^+$).

*4.1.3.7. 1-Allyloxymethyl-5-methyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**15**).* Yield: 0.35 g, 50.1%, m.p. 117.7–118.8 °C; ^{1}H NMR (DMSO-d_6) δ 1.56 (s, 3H, CH_3), 3.86 (m, 2H, OCH_2), 4.93–4.99 (m, 2H, $=CH_2$), 5.10 (bs, 2H, NCH_2), 5.47–5.54 (m, 1H, =CH), 7.62–9.10 (m, 7H, naphthyl), 11.72 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 11.12 (CH_3), 69.08 (OCH_2), 72.81 (NCH_2), 108.71 (C-5), 117.62 ($=CH_2$), 125.33, 125.73, 127.49, 129.57, 129.96, 130.26, 130.47, 133.89, 134.09, 135.79, 136.61 (naphthyl, =CH), 146.64 (C-6), 151.19 (C-2), 164.01 (C-4), 191.91 (C=O). Anal. ($C_{20}H_{18}N_2O_4$) C, H, N; ESIMS *m/z*: 351 ($[M+H]^+$).

*4.1.3.8. 5-Ethyl-1-(trans-methoxycarbonylallyl)-6-(1-naphthoyl)-2,4-pyrimidinedione (**16**).* Yield: 0.29 g, 36.9%, m.p. 109.2–110.3 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 1.98–2.07 (m, 2H, CH_2CH_3), 3.62 (s, 3H, OCH_3), 4.04–4.19 (m, 2H, NCH_2), 5.67 (d, $J = 15.9$ Hz, 1H, =CHCO), 6.63–6.67 (m, 1H, =CH), 7.62–9.10 (m, 7H, naphthyl), 11.72 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.62 (CH_2CH_3), 19.27 (CH_2CH_3), 46.40 (NCH_2), 51.87 (OCH_3), 112.74 (C-5), 122.05, 125.20, 125.57, 127.76, 129.73, 129.76, 130.22, 130.43, 134.18, 136.89, 137.50 (naphthyl, =CHCO), 142.98 (=$CHCH_2$), 148.12 (C-6), 150.84 (C-2), 163.72 (C-4), 165.79 (COO), 191.70 (C=O). Anal. ($C_{22}H_{20}N_2O_5$) C, H, N; ESIMS *m/z*: 393 ($[M+H]^+$).

*4.1.3.9. 5-Ethyl-6-(1-naphthoyl)-1-(3-phenyl-2-propynyl)-2,4-pyrimidinedione (**17**).* Yield: 0.52 g, 63.7%, m.p. 179.4–180.7 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.99 (m, 2H, CH_2CH_3), 4.63 (bs, 2H, NCH_2), 7.10–7.35 (m, 5H, phenyl), 7.61–9.17 (m, 7H, naphthyl), 11.79 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.62 (CH_2CH_3), 19.30 (CH_2CH_3), 35.27 (NCH_2), 84.16 (≡C), 84.59 (≡C), 113.61 (C-5), 121.74, 125.29, 125.57, 127.71, 128.92 (2C), 129.34, 129.74, 130.20, 130.27, 130.32, 131.78 (2C), 134.13, 136.82, 137.28 (naphthyl, phenyl), 147.36 (C-6), 150.57 (C-2), 163.51 (C-4), 191.54 (C=O). Anal. ($C_{26}H_{20}N_2O_3$) C, H, N; ESIMS *m/z*: 409 ($[M+H]^+$).

*4.1.3.10. 1-[(Cyclopent-3-en-1-yl)methyl]-5-ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**18**).* Yield: 0.23 g, 30.4%, m.p. 175.8–176.9 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.95–2.15 (m, 4H, CH_2CH_3, $2 \times CH_{2a}$), 2.17–2.22 (m, 2H, $2 \times CH_{2b}$), 2.51–2.59 (m, 1H, CH), 3.01 (m, 1H, =CH), 3.75 (bs, 1H, =CH), 5.56 (bs, 2H, NCH_2), 7.66–9.08 (m, 7H, naphthyl), 11.65 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.45 (CH_2CH_3), 16.70 (CH_2CH_3), 32.14 (CH), 39.07 (2C, $2 \times CH_2$), 53.11 (NCH_2), 114.75 (C-5), 125.12, 125.27, 127.49, 129.56, 129.89 (2C), 130.00, 130.23, 130.59, 134.09, 135.98, 136.46 (naphthyl, CH=CH), 147.01 (C-6), 151.23 (C-2), 163.48 (C-4), 191.52 (C=O). Anal. ($C_{23}H_{22}N_2O_3$) C, H, N; ESIMS *m/z*: 375 ($[M+H]^+$).

*4.1.3.11. 1-Ethoxymethyl-5-ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**19**).* Yield: 0.32 g, 46.0%, m.p. 145.4–146.6 °C; ^{1}H NMR (DMSO-d_6) δ 0.74–0.82 (m, 6H, CH_3), 1.99 (m, 2H, CH_2CH_3), 3.38 (m, 2H, OCH_2), 5.09 (bs, 2H, NCH_2), 7.65–9.10 (m, 7H, naphthyl), 11.69 (s, 1H, NH). ^{13}C NMR

(DMSO-d_6) δ 13.56 (CH_2CH_3), 14.70 (CH_2CH_3), 19.10 (OCH_2CH_3), 63.80 (OCH_2), 73.13 (NCH_2), 114.08 (C-5), 125.32, 125.39, 127.50, 129.59, 130.00, 130.28, 130.61, 134.04, 136.00, 136.69 (naphthyl), 146.84 (C-6), 151.13 (C-2), 163.51 (C-4), 191.75 (C=O). Anal. ($C_{20}H_{20}N_2O_4$) C, H, N; ESIMS *m/z*: 353 ($[M+H]^+$).

*4.1.3.12. 1-Cyclopropylmethoxymethyl-5-ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**20**).* Yield: 0.31 g, 40.9%, m.p. 162.2–163.6 °C; ^{1}H NMR ($CDCl_3$) δ −0.01–(−0.02) (m, 2H, cyclopropyl), 0.32–0.33 (m, 2H, cyclopropyl), 0.72 (m, 1H, cyclopropyl), 0.91 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 2.04–2.23 (m, 2H, CH_2CH_3), 3.19 (m, 2H, OCH_2), 5.01–5.31 (bs, 2H, NCH_2), 7.53–8.41 (m, 7H, naphthyl), 9.18 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 3.11 (cyclopropyl), 3.33 (cyclopropyl), 10.29 (cyclopropyl), 13.56 (CH_2CH_3), 19.11 (CH_2CH_3), 72.94 (NCH_2), 73.11 (OCH_2), 114.18 (C-5), 125.36, 125.43, 127.50, 129.57, 129.96, 130.29, 130.65, 134.05, 136.04, 136.64 (naphthyl), 146.88 (C-6), 151.11 (C-2), 163.52 (C-4), 191.69 (C=O). Anal. ($C_{22}H_{22}N_2O_4$) C, H, N; ESIMS *m/z*: 379 ($[M+H]^+$).

*4.1.3.13. 1-Benzyloxymethyl-5-ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**21**).* Yield: 0.33 g, 39.9%, m.p. 158.2–159.0 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 1.99 (m, 2H, CH_2CH_3), 4.48–4.64 (s, 2H, OCH_2), 5.15 (m, 2H, NCH_2), 7.23–9.08 (m, 11H, naphthyl, phenyl), 11.72 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.56 (CH_2CH_3), 19.15 (CH_2CH_3), 70.38 (OCH_2), 73.23 (NCH_2), 114.14 (C-5), 125.31, 125.45, 127.52, 127.97, 128.07 (2C), 128.44 (2C), 129.59, 130.02, 130.26, 130.51, 134.05, 136.21, 136.71, 137.30 (naphthyl, phenyl), 146.90 (C-6), 151.20 (C-2), 163.54 (C-4), 191.74 (C=O). Anal. ($C_{25}H_{22}N_2O_4$) C, H, N; ESIMS *m/z*: 415 ($[M+H]^+$).

*4.1.3.14. 5-Ethyl-6-(1-naphthoyl)-1-(4-nitrobenzyloxymethyl)-2,4-pyrimidinedione (**22**).* Yield: 0.34 g, 37.4%, m.p. 199.0–201.5 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.99 (m, 2H, CH_2CH_3), 4.48–4.64 (m, 2H, OCH_2), 5.16 (bs, 2H, NCH_2), 7.23–9.08 (m, 11H, naphthyl, phenyl), 11.72 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.54 (CH_2CH_3), 19.18 (CH_2CH_3), 69.08 (OCH_2), 73.45 (NCH_2), 114.07 (C-5), 123.47 (2C), 125.26, 125.42, 127.54, 128.48 (2C), 129.60, 130.09, 130.23, 130.36, 134.03, 136.35, 136.83 (naphthyl, phenyl), 145.31 (phenyl), 146.78 (C-6), 147.09 (phenyl), 151.23 (C-2), 163.52 (C-4), 191.80 (C=O). Anal. ($C_{25}H_{21}N_3O_6$) C, H, N; ESIMS *m/z*: 460 ($[M+H]^+$).

*4.1.3.15. 1-Allyloxymethyl-5-ethyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**23**).* Yield: 0.38 g, 51.6%, m.p. 122.5–123.9 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 2.17 (m, 2H, CH_2CH_3), 3.90 (m, 2H, OCH_2), 5.00–5.31 (m, 4H, $=CH_2$, NCH_2), 5.53–5.58 (m, 1H, =CH), 7.51–8.45 (m, 7H, naphthyl), 9.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.54 (CH_2CH_3), 19.11 (CH_2CH_3), 69.17 (OCH_2), 72.90 (NCH_2), 114.08 (C-5), 117.60 ($=CH_2$), 125.29, 125.49, 127.52, 129.61, 130.03, 130.26, 130.55, 133.96, 134.04, 136.17, 136.72 (naphthyl, =CH), 146.87 (C-6), 151.16 (C-2), 163.52 (C-4), 191.76 (C=O). Anal. ($C_{21}H_{20}N_2O_4$) C, H, N; ESIMS *m/z*: 365 ($[M+H]^+$).

*4.1.3.16. 5-Ethyl-1-(3-methyl-2-butenyloxymethyl)-6-(1-naphthoyl)-2,4-pyrimidinedione (**24**).* Yield: 0.41 g, 52.3%, m.p. 146.8–148.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, $J = 7.4$ Hz, 3H, CH_2CH_3), 1.34 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.91–1.99 (m, 2H, CH_2CH_3), 3.33–3.70 (m, 3H, OCH_2, =CH), 5.03 (bs, 2H, NCH_2), 7.64–9.11 (m, 7H, naphthyl), 11.73 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.70 (CH_2CH_3), 19.27 (CH_2CH_3), 30.89 ($=C(CH_3)_2$), 31.77 ($=C(CH_3)_2$), 65.48 (OCH_2), 73.63 (NCH_2), 114.19 (C-5), 125.27, 125.49, 125.62, 127.56, 127.64, 129.65, 130.12, 130.24, 130.51, 134.16, 135.90, 136.98 (naphthyl, CH=C), 147.03 (C-6), 151.29 (C-2), 163.51 (C-4), 191.57 (C=O). Anal. ($C_{23}H_{24}N_2O_4$) C, H, N; ESIMS *m/z*: 393 ($[M+H]^+$).

*4.1.3.17. 5-Isopropyl-1-(3-methyl-2-butenyl)-6-(1-naphthoyl)-2,4-pyrimidinedione (**25**).* Yield: 0.36 g, 47.3%, m.p. 173.6–174.9 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (d, $J = 6.2$ Hz, 3H, $CH(CH_3)_2$), 1.05 (s, 3H, $=C(CH_3)_2$), 1.12 (d, $J = 6.2$ Hz, 3H, $CH(CH_3)_2$), 1.31 (s, 3H, $=C(CH_3)_2$), 2.30 (sep, $J = 6.2$ Hz, 1H, $CH(CH_3)_2$), 4.06–4.16 (bs, 2H, NCH_2), 4.95 (m, 1H, =CH), 7.68–9.26 (m, 7H, naphthyl), 11.51 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 17.71 ($=C(CH_3)_2$), 19.57 ($CH(CH_3)_2$), 20.58 ($CH(CH_3)_2$), 25.41 ($=C(CH_3)_2$), 28.88 ($CH(CH_3)_2$), 43.45 (NCH_2), 114.93 (C-5), 119.99 (=CH), 125.29, 125.56, 127.67, 129.57, 129.76, 130.25, 130.44, 134.10, 135.97, 137.32, 137.48 (naphthyl, =C), 147.92 (C-6), 150.81 (C-2), 162.97 (C-4), 192.53 (C=O). Anal. ($C_{23}H_{24}N_2O_3$) C, H, N; ESIMS *m/z*: 377 ($[M+H]^+$).

*4.1.3.18. 1-Benzyloxymethyl-5-isopropyl-6-(1-naphthoyl)-2,4-pyrimidinedione (**26**).* Yield: 0.47 g, 55.1%, m.p. 197.4–199.0 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (d, $J = 6.6$ Hz, 3H, $CH(CH_3)_2$), 1.13 (d, $J = 6.8$ Hz, 3H, $CH(CH_3)_2$), 2.32 (sep, $J = 6.6$ Hz, 1H, $CH(CH_3)_2$), 4.35–4.46 (m, 2H, OCH_2), 5.03–5.21 (bs, 2H, NCH_2), 6.97–7.17 (m, 5H, phenyl), 7.58–9.17 (m, 7H, naphthyl), 11.60 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 19.37 (CH_3), 20.61 (CH_3), 28.84 (CH), 70.31 (OCH_2), 73.26 (NCH_2), 116.43 (C-5), 125.36, 125.48, 127.53, 127.91, 127.98 (2C), 128.39 (2C), 129.64, 130.19, 130.20, 130.57, 134.07, 136.80, 136.94, 137.34 (naphthyl, phenyl), 146.60 (C-6), 151.12 (C-2), 162.85 (C-4), 192.40 (C=O). Anal. ($C_{26}H_{24}N_2O_4$) C, H, N; ESIMS *m/z*: 429 ($[M+H]^+$).

4.2. Biological activity

The anti-HIV activity and cytotoxicity were evaluated against wild type HIV-1 strain III_B and HIV-2 ROD in MT-4 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [16]. Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/ml and infected with HIV at

a multiplicity of infection of 0.02. Immediately after viral infection, 100 μl of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in DMSO at 50 mM or higher. After four-day incubation at 37 °C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

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