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Synthesis of 6-arylvinyl analogues of the HIV drugs SJ-3366 and Emivirine

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Abstract—This paper reports the synthesis and the antiviral activities of a series of 6-arylvinyl substituted analogues of SJ-3366, a highly potent agent against HIV. The objective was to investigate whether substitution of the 6-arylketone with a 6-arylvinyl group could lead to an improved antiviral activity against HIV-1. The most active compounds 1-ethoxymethyl, 1-(2-propynyloxymethyl), and 1-(2-methyl-3-phenylallyloxymethyl) substituted 6-[1-(3,5-dimethylphenyl)vinyl]-5-ethyl-1*H*-pyrimidine-2,4-dione (**5b**, **16**, and **18**) showed activities against HIV-1 wild type in the range of Efavirenz, and moderate activities against Y181C and Y181C + K103N mutant strains were also observed.

1. Introduction

In the treatment of patients suffering from HIV/AIDS four classes of compounds are used, fusion inhibitors, protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The latter consist of many classes of compounds (reviewed in ref 1), and among them the most potent anti-HIV agents are found. The NNRTIs are highly specific as their binding site is a hydrophobic pocket located approximately 10 Å from the polymerase active site.[2] They bind allosterically forcing the RT-subunit into an inactive conformation.[3] However, the specificity results in NNRTI being vulnerable to mutations among the amino acids around the binding site,[4] which leads to drug resistance. A serious concern is the commonly found Y181C mutation which causes loss of antiviral activity among all NNRTIs including the FDA (US Food and Drug Administration) approved drugs, Nevirapine,[5] Delavirdine,[6] and Efavirenz.[7]

One of the first NNRTIs was 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)[8,9] which was synthesized in 1989. It was originally designed as a chain terminator in the viral enzyme Reverse Transcriptase (RT). But it was not acting as a NRTI as no activity against HIV-2 was measured. A new inhibitory mechanism was found and therefore HEPT was considered a lead compound for NNRTI synthesis, even though it was not very active itself. Among the many HEPT analogues synthesized in the last decade are Emivirine[10–12] (formerly known as MKC-442), GCA-186,[13] and SJ-3366[14] which all showed potent activity against HIV-1 (Fig. 1).

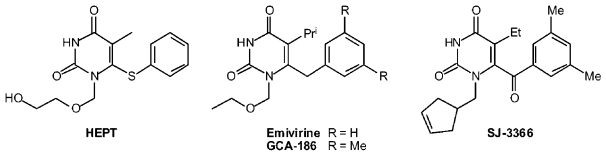

Figure 1.

Emivirine was chosen as a drug candidate for clinical trials[15] by Triangle Pharmaceuticals, but phase III studies was abandoned in January 2002, when comparative studies showed Emivirine to be less potent than other anti-HIV compounds.[16] Meanwhile it was reported that Emivirine triggers the liver enzyme Cytochrome P450, leading to drug interactions between Emivirine and protease inhibitors.[17]

GCA-186 differs structurally from Emivirine only in the introduction of two methyl substituents in 3- and 5-position at the C-6 benzyl group. This modification has improved the antiviral acitivity against the wild type

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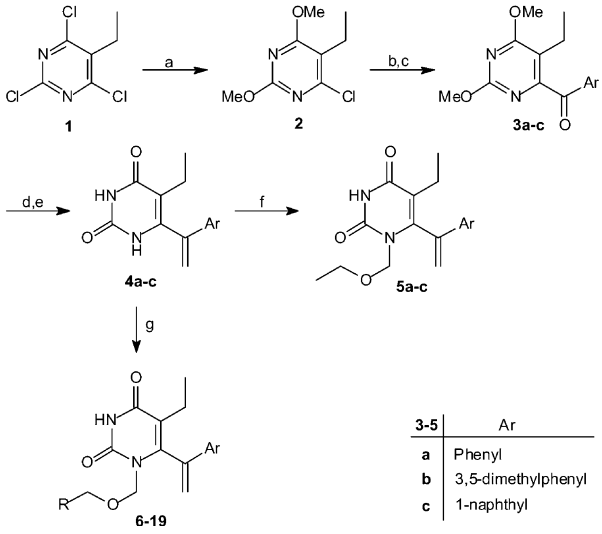
HIV-1, but more interesting also against the mutated HIV strains. The loss in activity towards the Y181C mutation is significantly lower for GCA-186 than for Emivirine.[13]

SJ-3366 is a highly potent inhibitor of HIV-1, that contrary to other NNRTIs; also shows activity against HIV-2. Besides the inhibitory effect on HIV-1 RT, SJ-3366 also inhibits the attachment of HIV-1 and HIV-2 to the target cells. The inhibitory concentration against HIV-1 has been reported to be approximately 1 nM in CEM-SS T cells.[14]

This paper reports a series of 6-arylvinyl substituted analogues of SJ-3366. The objective was to investigate whether substitution of the 6-arylketone with an isosteric 6-arylvinyl group could lead to an improved antiviral activity against HIV-1. In addition, the synthesis of new HEPT analogues would also provide information to the ongoing investigation of the NNRTI binding site.



Scheme 1. (a) Ref 18; (b) $ArCH_2CN$, NaH, DMF; (c) NaH, O_2, DMF, 66–93% (one-pot synthesis); (d) CH_3MgBr, THF; (e) HCl (aq), 69–86% (two steps); (f) BSA, $CH_3CH_2OCH_2Cl$, $CHCl_3$, 24–48%; (g) BSA, TMS-triflate, $RCH_2OCH_2OCH_2R$, CH_3CN, 10–99%.

2. Chemistry

It was believed that the SJ-3366 analogues could be synthesized using literature procedures[18,19] for the first two steps to get compound **3** followed by a Wittig reaction for the methylenation of the ketone. The ketones **3a–c** were prepared in 66–93% yield by a nucleofilic aromatic substitution with arylacetonitriles in a one-pot synthesis[19] where the intermediate nitrile was immediately oxidized to the ketone by passing pure oxygen through the reaction mixture. The Wittig reaction for obtaining the nucleobase **4** did not work when we did the reaction with methyltriphenylphosphonium bromide presumably because of steric hindrance of the ketone. Dollé et al.[20] tried to prepare a vinylic HEPT analogue from (3,5-dimethylphenyl) 5-ethyl-2-methoxy-6-methyl-3-pivaloylaminopyridin-4-yl ketone in a two step reaction using methyllithium to form a tertiary alcohol but failed as this alcohol could not be dehydrated under acidic conditions using *p*-toluenesulfonic acid, acetic anhydride nor hydrochloric acid.

Nevertheless, the compounds **3a–c** could be converted to the corresponding 6-arylvinyl substituted compounds **4a–c** in a two step reaction in 69–86% yield. First step was introduction of a methyl group and formation of a tertiary alcohol by a Grignard reaction with methylmagnesium bromide in dry THF as described by Boyd et al.[21] for a similar diaryl ketone. The tertiary alcohol was then dehydrated in refluxing 4M hydrochloric acid. The intermediate alcohol was isolated only for the phenyl compound **4a** in order to verify the intermediate product (Scheme 1).

A variety of N-1 alkylations on **4** were carried out to get a series of SJ-3366 and Emivirine analogues to reveal their biological potential. **4a–c** were N-1 alkylated according to the procedure described by Danel et al.[22] using *N,O*-bis(trimethylsilyl)acetamide (BSA) and chloromethyl ethyl ether to give **5a–c** in 24–48% yield. Futhermore N-1 alkylations on **4a–b** were carried out under the Vorbrüggen conditions[23] using BSA, TMS-triflate as a catalyst, and various substituted bis(allyloxy)methanes or bis(propynyloxy)methanes leading to target molecules **6–19**. The latter reagents have been developed recently in our group to produce active Emivirine analogues against HIV in the near picomolar range.[24,25] The yield for the alkylations reactions varied greatly from 10–99%. Especially the propynyloxymethyl substituents coupled badly, even though the reaction time was extended to 3 days. N-1 alkylations were verified by the NOE enhacement on NCH_2O-protons when vinylic protons were irradiated and vice versa.

^1H NMR spectra of the compounds **5–19** revealed diastereotopic protons in the methylene groups of the 5-ethyl and N-1 alkoxymethyl substituents. This is ascribed to hindered rotation of the exocyclic bond at the 6-position of the uracil ring which results in two enantiomeric rotamers with the phenylvinyl group perpendicular to the uracil ring.

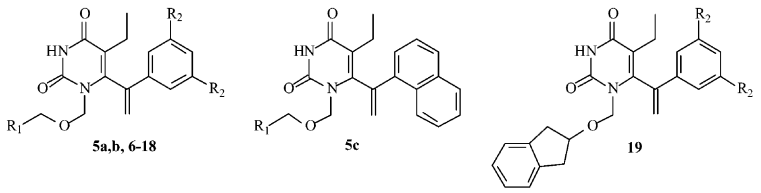
3. Biological results

Target compounds **5–19** were tested for biological activity against wild type HIV-1 strain IIIB in MT-4 cells. Results were compared with the anti-viral activity of Emivirine, a well examined HEPT analogue, and Efavirenz, the most active anti-HIV drug used in therapy. Compounds showing an antiviral activity against HIV-1 in the range of Emivirine (0.02 μM) were also tested against the HIV-1 strains with Y181C and Y181C+K103N mutations. All results are listed in Table 1.

4. Discussion

The biological results reveal that a 6-(1-naphthyl)vinyl group is too big a substituent for this type of analogues,

Table 1. Inhibitory and Cytotoxic Concentrations of the SJ-3366 and Emivirine analogues (compounds **5**–**19**). Data for Emivirine and Efavirenz are given for comparison



Compd	R_1	R_2	IC_{50} (μM)[a]	Wild type CC_{50} (μM)[b]	SI[c]	Y181C IC_{50} (μM)[a]	Y181C+K103N IC_{50} (μM)[a]
5a	CH_3	H	2.8	>100	>36	NT[d]	NT[d]
5b	CH_3	CH_3	0.035	>100	>2857	17	29
5c	CH_3		4.0	66	17	NT[d]	NT[d]
6	CH_2=CH	H	0.87	>100	>115	NT[d]	NT[d]
7	CH_2= $C(CH)_3$	H	3.4	>100	>29	NT[d]	NT[d]
8	$(CH_3)_2C$= CH	H	3.1	66	21	NT[d]	NT[d]
9	Ph-CH= CH	H	0.32	29	91	NT[d]	NT[d]
10	HC≡C	H	1.5	>100	>67	NT[d]	NT[d]
11	Ph-C≡C	H	3.8	28	7	NT[d]	NT[d]
12	CH_2= CH	CH_3	0.03	36	1200	>100	>100
13	CH_2= $C(CH)_3$	CH_3	0.10	34	358	NT[d]	NT[d]
14	$(CH_3)_2C$= CH	CH_3	0.15	28	187	NT[d]	NT[d]
15	Ph-CH= CH	CH_3	0.03	4	133	>100	>100
16	HC≡C	CH_3	0.03	>100	>3333	17	23
17	Ph-C≡C	CH_3	0.29	31	107	NT[d]	NT[d]
18	Ph-CH= $C(CH_3)$	CH_3	0.03	31	1033	7	>100
19		CH_3	0.30	34	113	NT[d]	NT[d]
Emivirine			0.02	>100	>5000	44	>100
Efavirenz			0.01	>100	>10,000	0.3	2.7

[a] Inhibitory concentration of compound required to achieve 50% inhibition of HIV-1 multiplication in MT-4-infected cells.
[b] Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%. The symbol (>) indicates that the CC_{50} was not reached at the highest concentration tested.
[c] Selectivity index: ratio CC_{50}/IC_{50}.
[d] NT: Not tested.

as **5c** was the least active among the synthesized compounds. A similar conclusion was reached in a recently published study[24] and therefore no further analogues with 6-(1-naphthyl)vinyl substituents were synthesized. The remaining compounds in Table 1 can be divided into two series with reference to their (phenyl)vinyl (**6**–**11**) or (3,5-dimethylphenyl)vinyl (**12**–**19**) substituents in the 6-position.

It is well known, that introduction of 3,5-dimethyl substituents on the aromatic ring in Emivirine leads to an up to 100-fold rise in biologic activity.[13,26] This is also the case in the present series of compounds, where the increase in activity rises up to 80-fold. In analogy with previous results,[13,26] the cytotoxicity for the series with 3,5-dimethyl substituents at the phenyl ring were higher than for the series without.

As seen from examination of the results listed in Table 1, the substitution pattern of the allyl group at the N-1 substituent should rather be unsubstituted (**6**,**12**) or phenylsubstituted (**9**,**15**) than methyl (**7**,**13**) or dimethylsubstituted (**8**,**14**) in order to achieve the more active compounds. The N-1 substituent of these analogues is believed to interact with the amino acids in the reverse transcriptase enzyme, but the exact binding still needs to be elucidated. It is thought that the allyl group interacts through π-stacking with RT-amino acid Phe227 or Pro236. Increasing the bulkyness around this allyl group result in a drop in activity which could be due to steric interactions with adjacent amino acids. However, introduction of a phenylsubstituent at the allyl group does not result in lower activity. Most likely, this region of the NNRTI binding site is flexible to larger aromatic rings with the ability to make π-interactions.

Emivirine analogues having a (2-propynyloxy)methyl substituent or phenylsubstituted allyloxymethyl at the N-1 position have shown high activities against HIV-1.[24,25] In continuation of this work a (3-phenyl-2-propynyloxy)methyl alkylating agent was prepared according to the method of Nazaretyan et al.[27] as described by El-Brollosy et al.[24] However, compounds **11** and **17** having this (3-phenyl-2-propynyloxy)methyl substituent in the N-1 position were the least active for R_2 = H and CH_3, respectively. In comparison, compounds **9** and **15** which instead of the triple bond have a double bond in the N-1 substituent were among the most active in the present series of analogues, but also the most toxic ones. The reason for the lower activity of **11** and **17** may be due to the low flexibility of the 3-phenyl-2-propynyl system to allocate the phenyl ring for stacking in the RT.

The five most potent compounds towards the wild type HIV-1 were also tested against HIV-1 strains with Y181C and Y181C+K103N mutations. Both strains are associated with high-level resistance to NNRTIs Emivirine, Nevirapine, Delavirdine and for the latter

also Efavirenz. **12** and **15** showed no activity at the highest concentration tested, while **5b**, **16** and **18** showed a marginally higher activity against the Y181C single mutated strain than Emivirine. **5b** and **16** were also active against the Y181C + K103N double mutated strain.

5. Experimental

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C with TMS as internal standard. MALDI spectra were recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Melting points were determined on a Büchi melting point apparatus. Elementary analyses were performed at H.C. Ørsted Institute, University of Copenhagen. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 F_{254} were purchased from Merck. UV-light or a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (50 g) and $Ce_2(SO_4)_3$ (1 g) in 5% sulfuric acid were used for visualization. Solvents used for column chromatography were distilled prior to use. Reagents were used as purchased.

5.1. General procedure for synthesis of 6-[1-(Aryl)vinyl]-5-ethylpyrimidine-2,4-diones (4a–c)

6-(Arylcarbonyl)-5-ethyl-2,4-dimethoxypyrimidine (**3a**–**c**) (3–17 mmol) was dissolved in dry THF (50–100 mL) under nitrogen atmosphere. The solution was cooled to 0 °C, and methylmagnesium bromide (2 equiv, 1.4 M in THF/Toluene) was added. The temperature was increased to rt and the solution was stirred at rt for 1–6 days until the colour of the solution turned yellow-reddish. Then the reaction was quenched with satd NH_4Cl (50 mL) and extracted with ethylacetate (3×100 mL). The combined organic phases were washed with water (2×100 mL) and satd NaCl (100 mL) and dried ($MgSO_4$), the solvent was evaporated off under reduced pressure yielding a yellow oil.

The oil was refluxed in 4M HCl (80 mL) overnight. Upon cooling to rt crystals precipitated. These were filtered off and dried in vacuo.

5.2. 5-Ethyl-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (4a)

Yield: 1.07 g (69%) as beige, needle formed crystals; mp 196 °C; R_f 0.45 (10% MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6): δ 0.84 (t, 3H, J = 7.2 Hz, CH_2CH_3), 2.07 (q, 2H, J = 7.2 Hz, CH_2CH_3), 5.46 (s, 1H, C=CH_{2a}), 6.09 (s, 1H, C=CH_{2b}), 7.36–7.47 (m, 5H, H_{arom}), 10.81 (s, 1H, NH) 11.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.53 (CH_3), 18.43 (CH_2), 111.31 (C-5), 117.77 (=CH_2), 125.78, 128.50, 128.68, 136.21 (C_{arom}), 140.05 (C=CH_2), 148.68 (C-6), 150.82 (C-2), 164.41 (C-4). HRMS (MALDI) m/z calcd for $C_{14}H_{14}N_2O_2Na^+$ (MNa^+) 265.0948, found 265.0946. Anal. calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.32; H, 5.94; N, 11.02.

5.3. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-Ethyl-1*H*-pyrimidine-2,4-dione (4b)

Yield: 3.85 g (86%); as beige, needle formed crystals; mp 246–247 °C; R_f 0.51 (10% MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6): δ 0.84 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.05 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.29 (s, 6H, 2×CH_3), 5.40 (s, 1H, C=CH_{2a}), 6.01 (s, 1H, C=CH_{2b}), 7.00 (s, 1H, H_{arom}), 7.05 (s, 2H, H_{arom}), 10.76 (s, 1H, NH), 11.11 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.51 (CH_3), 18.46 (CH_2), 20.83 (2×CH_3), 111.17 (C-5), 117.43 (=CH_2), 123.47, 129.99, 136.17, 137.67 (C_{arom}), 140.23 (C=CH_2), 148.89 (C-6), 150.81 (C-2), 164.44 (C-4). HRMS (MALDI) m/z calcd for $C_{16}H_{18}N_2O_3Na^+$ (MNa^+) 293.1261, found 293.1269. Anal. calcd for $C_{16}H_{18}N_2O_3$: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.90; H, 6.72; N, 10.31.

5.4. 5-Ethyl-6-[1-(1-naphthyl)vinyl]-1*H*-pyrimidine-2,4-dione (4c)

Yield: 0.78 g (80%) as brown, needle formed crystals; mp: decomposes at 244 °C; R_f 0.25 (25% EtOAc/Petroleum ether). ^{1}H NMR (DMSO-d_6): δ 0.42 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.91 (q, 2H, J = 7.2 Hz, CH_2CH_3), 5.89 (s, 1H, C=CH_{2a}), 5.97 (s, 1H, C=CH_{2b}), 7.49–8.06 (m, 7H, $H_{naphthyl}$) 11.01 (s, 1H, NH), 11.17 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 12.57 (CH_3), 18.03 (CH_2), 111.15 (C-5), 124.50, 124.83, 125.44, 125.99, 126.52, 126.78, 128.59, 128.73, 130.13, 133.39, 136.26 (=CH_2, 10×$C_{naphthyl}$), 140.05 (C=CH_2), 149.04 (C-6), 150.90 (C-2), 164.42 (C-4). HRMS (MALDI) m/z calcd for $C_{18}H_{17}N_2O_3^+$ (MH^+) 293.1284, found 293.1281.

5.5. General procedure for N-1 alkylation of 4a–c

5.5.1. Method A. *N,O*-bis(trimethylsilyl)acetamide (BSA) (3 equiv) was dissolved in dry $CHCl_3$ (100 mL) and the pyrimidine **4** was added. After 20 min, chloromethyl ethyl ether (2 equiv) was added and the solution was stirred at rt overnight. The reaction was quenched with satd $NaHCO_3$ (50 mL). The mixture was evaporated to dryness by co-evaporation with ethanol (2×50 mL) under reduced pressure. The solid, white compound was suspended in diethyl ether (200 mL) and stirred for 2 h, after which the ether was decanted. This procedure was repeated with more diethyl ether (2×200 mL) The combined ether phases were washed with satd. $NaHCO_3$ (2×50 mL) that was back-extracted with diethyl ether (2×100 mL). The combined ether phases were dried ($MgSO_4$) and evaporated under reduced pressure. The products were purified by silica column chromatography using ethyl acetate (**5a**) or 25% EtOAc/Petroleum ether (**5b** and **5c**) as eluents.

5.6. 1-Ethoxymethyl-5-ethyl-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (5a)

Yield: 69 mg (48%) as beige crystals; mp 80–83 °C; R_f 0.59 (EtOAc). ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.03 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 2.15

(dq, 1H, $J = 14.2$ Hz, 7.1 Hz, CH_{2a}CH$_3$), 2.44 (dq, 1H, $J = 14.2$ Hz, 7.1 Hz, CH_{2b}CH$_3$), 3.48 (dq, 2H, $J = 7.0$ Hz, OCH_2CH$_3$), 4.82 (d, 1H, $J = 10.3$ Hz, NCH$_{2a}$O), 5.30 (d, 1H, $J = 10.3$ Hz, NCH$_{2b}$O), 5.46 (s, 1H, C=CH$_{2a}$), 6.16 (s, 1H, C=CH$_{2b}$), 7.34–7.40 (m, 5H, H_{arom}), 9.47 (s, 1H, NH). ^{13}C NMR (CDCl$_3$): δ 13.76 (CH$_3$), 14.84 (*C*H$_3$CH$_2$O), 20.04 (CH$_2$), 64.56 (CH$_3$*C*H$_2$O), 73.59 (NCH$_2$O), 116.07 (C-5), 118.63 (=CH$_2$), 125.39, 129.00, 129.05, 135.74 (C_{arom}), 138.69 (*C*=*C*H$_2$), 150.37 (C-6), 151.68 (C-2), 163.41 (C-4). HRMS (MALDI) *m*/*z* calcd for $C_{17}H_{20}N_2O_3Na^+$ (MNa$^+$) 323.1366, found 323.1369. Anal. calcd for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33. Found: C, 67.93; H, 6.71; N, 9.18.

5.7. 6-[1-(3,5-Dimethylphenyl)vinyl]-1-ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (5b)

Yield: 78 mg (24%) as white crystals; mp 167–169 °C; R_f 0.53 (EtOAc). ^{1}H NMR (CDCl$_3$): δ 1.00 (t, 3H, $J = 7.3$ Hz, CH$_2$C*H*$_3$), 1.07 (t, 3H, $J = 7.4$ Hz, OCH$_2$C*H*$_3$), 2.14 (dq, 1H, $J = 14.6$ Hz, 7.3 Hz, CH_{2a}CH$_3$), 2.31 (s, 6H, 2×CH$_3$), 2.44 (dq, 1H, $J = 14.6$ Hz, 7.3 Hz, CH_{2b}CH$_3$), 3.48–3.53 (m, 2H, OCH_2CH$_3$), 4.76 (d, 1H, $J = 10.1$ Hz, NCH$_{2a}$O), 5.32 (d, 1H, $J = 10.1$ Hz, NCH$_{2b}$O), 5.42 (s, 1H, C=CH$_{2a}$), 6.12 (s, 1H, C=CH$_{2b}$), 6.97 (s, 2H, H_{arom}), 6.98 (s, 1H, H_{arom}), 9.45 (s, 1H, NH). ^{13}C NMR (CDCl$_3$): δ 13.79 (CH$_3$), 14.91 (*C*H$_3$CH$_2$O), 20.06 (CH$_2$), 21.32 (2×CH$_3$), 64.61 (CH$_3$*C*H$_2$O), 73.61 (NCH$_2$O), 115.93 (C-5), 118.33 (=CH$_2$), 123.16, 130.81, 135.60, 138.60 (C_{arom}), 138.70 (*C*=*C*H$_2$), 150.73 (C-6), 151.74 (C-2), 163.51 (C-4). HRMS (MALDI) *m*/*z* calcd for $C_{19}H_{25}N_2O_3^+$ (MH$^+$) 329.1859, found 329.1857, *m*/*z* calcd for (MNa$^+$) 351.1679, found 351.1664. Anal. calcd for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.36; H, 7.39; N, 8.51.

5.8. 1-Ethoxymethyl-5-ethyl-6-[1-(naphthyl)vinyl]-1*H*-pyrimidine-2,4-dione (5c)

Yield: 109 mg (31%) as white crystals; mp 58–60 °C; R_f 0.52 (EtOAc). ^{1}H NMR (CDCl$_3$): δ 1.01 (t, 3H, $J = 7.0$ Hz, CH$_2$C*H*$_3$), 1.09 (t, 3H, $J = 7.1$ Hz, OCH$_2$C*H*$_3$), 2.37 (dq, 1H, $J = 14.0$ Hz, 7.0 Hz, CH_{2a}CH$_3$), 2,63 (dq, 1H, $J = 14.0$ Hz, 7.0 Hz, CH_{2b}CH$_3$), 3.44 (q, 2H, $J = 7.1$ Hz, OCH_2CH$_3$), 4.83 (d, 1H, $J = 10.5$ Hz, NCH$_{2a}$O), 5.32 (d, 1H, $J = 10.5$ Hz, NCH$_{2b}$O), 6.00 (s, 1H, C=CH$_{2a}$), 6.09 (s, 1H, C=CH$_{2b}$), 7.24 (m, 1H, $H_{naphthyl}$), 7.42 (t, 1H, $J = 7.7$ Hz, $H_{naphthyl}$), 7.57 (m, 2H, $H_{naphthyl}$), 7.84 (d, 1H, $J = 8.2$ Hz, $H_{naphthyl}$), 7.92 (d, 1H, $J = 7.7$ Hz, $H_{naphthyl}$), 8.49 (d, 1H, $J = 8.2$ Hz, $H_{naphthyl}$), 9.34 (s, 1H, NH). ^{13}C NMR (CDCl$_3$): δ 14.23 (CH$_3$), 14.87 (*C*H$_3$CH$_2$O), 20.66 (CH$_2$), 64.46 (CH$_3$*C*H$_2$O), 73.55 (NCH$_2$O), 116.40 (C-5), 124.33, 124.96, 124.98, 125.43, 126.12, 127.03, 129.03, 129.24, 130.41, 134.35, 134.62 (=CH$_2$, 10×$C_{naphthyl}$), 136.44 (*C*=*C*H$_2$), 151.54, 151.84 (C-6, C-2), 163.52 (C-4). HRMS (MALDI) *m*/*z* calcd for $C_{21}H_{22}N_2O_3Na^+$ (MNa$^+$) 373.1522, found 373.1510. Anal. calcd for $C_{21}H_{22}N_2O_3{\cdot}0.25H_2O$: C, 71.07; H, 6.39; N, 7.89. Found: C, 71.29; H, 6.27; N, 7.92.

5.9. General procedure for N-1 alkylation of 4a,b

5.9.1. Method B. The pyrimidine **4a,b** (1 mmol) was suspended in dry acetonitrile (15 mL) under N$_2$ and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (0,87 mL, 3.5 mmol) was added. After a clear solution was obtained (10 min), the reaction mixture was cooled to −45 °C, and TMS triflate (0.18 mL, 1 mmol) was added followed by dropwise addition of the appropriate acetal (2 mmol). The temperature was slowly increased to rt and the reaction mixture stirred at rt overnight. (For bis(2-propynyloxy)methane and bis(3-phenyl-2-propynyloxy)methane the reaction time was 3 days). The reaction was quenched with cold satd. NaHCO$_3$ (5 mL) and the solvent evaporated to near dryness under reduced pressure. The residue was extracted with CH$_2$Cl$_2$ (3×75 mL), and the combined organic phases were dried (MgSO$_4$) and evaporated under reduced pressure yielding an oil, which was purified by silica column chromatography to afford the product using 2% MeOH/CH$_2$Cl$_2$ (**10**), chloroform (**6–8**, **12–14**) or 25% EtOAc/Petroleum ether (**9**,**11**, **15–19**) as eluents.

5.10. 1-Allyloxymethyl-5-ethyl-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (6)

Yield: 253 mg (80%) as yellow crystals; mp 105–107 °C; R_f 0.32 (CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ 0.99 (t, 3H, $J = 7.5$ Hz, CH$_2$C*H*$_3$), 2.16 (dq, 1H, $J = 15.0$ Hz, 7.5 Hz, CH_{2a}CH$_3$), 2.45 (dq, 1H, $J = 15.0$ Hz, 7.5 Hz, CH_{2b}CH$_3$), 3.00–4.03 (m, 2H, OCH_2CH=), 4.81 (d, 1H, $J = 10.5$ Hz, NCH$_{2a}$O), 5.09–5.20 (m, 2H, C=CH$_2$), 5.35 (d, 1H, $J = 10.5$ Hz, NCH$_{2b}$O), 5.48 (s, 1H, C=CH$_{2a}$), 5.68–5.74 (m, 1H, CH), 6.16 (s, 1H, C=CH$_{2b}$), 7.27–7.39 (m, 5H, H$_{arom}$), 9.57 (s, 1H, NH). ^{13}C NMR (CDCl$_3$): δ 13.75 (CH$_3$), 20.04 (CH$_2$), 70.27 (CH$_2$O), 73.38 (NCH$_2$O), 116.29 (C-5), 117.29 (=CH$_2$), 118.82 (=CH$_2$), 125.39, 129.05, 129.09 (C$_{arom}$), 133.66 (*C*H=CH$_2$), 135.66 (C$_{arom}$), 138.58 (Ph*C*=*C*H$_2$), 150.27 (C-6), 151.71 (C-2), 163.41 (C-4). HRMS (MALDI) *m*/*z* calcd for $C_{18}H_{20}N_2O_3Na^+$ (MNa$^+$) 335.1366, found 335.1361. Anal. calcd for $C_{18}H_{20}N_2O_3{\cdot}0.25H_2O$: C, 68.23; H, 6.52; N, 8.84. Found: C, 68.42; H, 6.38; N, 8.85.

5.11. 5-Ethyl-1-(2-methylallyloxymethyl)-6-[1-(phenyl)-vinyl]-1*H*-pyrimidine-2,4-dione (7)

Yield: 327 mg (99%) as yellow crystals; mp 104–105 °C; R_f 0.24 (CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ 0.99 (t, 3H, $J = 7.3$ Hz, CH$_2$C*H*$_3$), 1.64 (s, 3H, CH$_3$), 2.15 (dq, 1H, CH_{2a}CH$_3$), 2.45 (dq, 1H, CH_{2b}CH$_3$), 3.92 (s, 2H, CH_2O), 4.77 (d, 1H, $J = 10.1$ Hz, NCH$_{2a}$O), 4.82, 4.85 (2×s, 2H, C=CH$_2$), 5.34 (d, 1H, $J = 10.1$ Hz, NCH$_{2b}$O), 5.49 (s, 1H, C=CH$_{2a}$), 6.16 (s, 1H, C=CH$_{2b}$), 7.35–7.39 (m, 5H, H$_{arom}$), 9.36 (br s, 1H, NH). ^{13}C NMR (CDCl$_3$): δ 13.76 (CH$_2$*C*H$_3$), 19.36 (CH$_3$), 20.06 (CH$_2$), 73.42 (OCH$_2$), 73.60 (NCH$_2$O), 112.14 (C(CH$_3$)=*C*H$_2$), 116.05 (C-5), 118.89 (=CH$_2$), 125.40, 129.12, 129.09, 135.69 (C$_{arom}$), 138.58 (*C*=*C*H$_2$), 141.33 (*C*(CH$_3$)=CH$_2$), 150.32 (C-6), 151.59 (C-2), 163.34 (C-4). HRMS (MALDI) *m*/*z* calcd for $C_{19}H_{22}N_2O_3Na^+$

(MNa^+) 349.1522, found 349.1522. Anal. calcd for $C_{19}H_{22}N_2O_3 \cdot 0.25H_2O$: C, 68.97; H, 6.85; N, 8.47. Found: C, 69.07; H, 6.80; N, 8.49.

5.12. 5-Ethyl-1-(3-methyl-2-butenyloxymethyl)-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (8)

Yield: 162 mg (47%) as white crystals; mp 105–107 °C; R_f 0.37 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.62 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 2.15 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2a}CH_3$), 2.46 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2b}CH_3$), 3.98 (d, 2H, J = 7.0 Hz, CH_2O), 4.74 (d, 1H, J = 10.2 Hz, $NCH_{2a}O$), 5.14 (t, 1H, J = 7.0 Hz, C=CH), 5.34 (d, 1H, J = 10.2 Hz, $NCH_{2b}O$), 5.49 (s, 1H, $C=CH_{2a}$), 6.15 (s, 1H, $C=CH_{2b}$), 7.34–7.39 (m, 5H, H_{arom}), 9.31 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.78 (CH_2CH_3), 17.97 (CH_3), 20.03 (CH_2), 25.73 (CH_3), 65.74 (OCH_2), 73.34 (NCH_2O), 116.06 (C-5), 118.80 ($=CH_2$), 120.09 ($=C(CH_3)_2$), 125.38, 129.04, 135.71 (C_{arom}), 137.63 ($C=C(CH_3)_2$), 138.50 ($C=CH_2$), 150.34 (C-6), 151.54 (C-2), 163.32 (C-4). HRMS (MALDI) m/z calcd for $C_{20}H_{24}N_2O_3Na^+$ (MNa^+) 363.1679, found 363.1678. Anal. calcd for $C_{20}H_{24}N_2O_3 \cdot 0.25H_2O$: C, 69.64; H, 7.16; N, 8.12. Found: C, 69.48; H, 7.06; N, 8.02.

5.13. 5-Ethyl-1-((E)-3-phenylallyloxymethyl)-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (9)

Yield: 196 mg (49%) as white foam; mp 53–55 °C; R_f 0.08 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.16 (dq, 1H, J = 14.8 Hz, 7.4 Hz, $CH_{2a}CH_3$), 2.47 (dq, 1H, J = 14.8 Hz, 7.4 Hz, $CH_{2b}CH_3$), 4.17 (d, 2H, J = 6.0 Hz, CH_2O), 4.87 (d, 1H, J = 10.1 Hz, $NCH_{2a}O$), 5.39 (d, 1H, J = 10.1 Hz, $NCH_{2b}O$), 5.51 (s, 1H, $C=CH_{2a}$), 6.10 (td, 1H, J = 6.0 Hz, 15.9 Hz, PhCH=CHCH$_2$), 6.17 (s, 1H, $C=CH_{2b}$), 6.50 (d, 1H, J = 15.9 Hz, PhCH=CH), 7.24–7.39 (m, 10H, H_{arom}), 9.38 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.78 (CH_3), 20.08 (CH_2CH_3), 69.91 (CH_2O), 73.32 (NCH_2O), 116.17 (C-5), 118.79 ($=CH_2$), 124.78, 125.43, 126.46, 127.75, 128.49, 129.07, 132.83, 135.67 (7×C_{arom}, CH=), 136.43 (PhCH=), 138.69 ($C=CH_2$), 150.26 (C-6), 151.67 (C-2), 163.29 (C-4). HRMS (MALDI) m/z calcd for $C_{24}H_{24}N_2O_3Na^+$ (MNa^+) 411.1679, found 411.1670. Anal. calcd for $C_{24}H_{24}N_2O_3 \cdot 0.5H_2O$: C, 72.52; H, 6.34; N, 7.05. Found: C, 72.07; H, 5.92; N, 6.81.

5.14. 5-Ethyl-6-[1-(phenyl)vinyl]-1-(2-propynyloxymethyl)-1*H*-pyrimidine-2,4-dione (10)

Yield: 67 mg (22%) as beige crystals; mp 125–127 °C; R_f 0.32 (2% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J = 7.5 Hz, CH_2CH_3), 2.04 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2a}CH_3$) 2.29 (t, 1H, J = 2.3 Hz, ≡CH), 2.34 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2b}CH_3$), 4.09 (s, 2H, ≡CCH_2O), 4.69 (d, 1H, J = 10.5 Hz, $NCH_{2a}O$), 5.33 (d, 1H, J = 10.5 Hz, $NCH_{2b}O$), 5.42 (s, 1H, $C=CH_{2a}$), 6.05 (s, 1H, $C=CH_{2b}$), 7.15–7.28 (m, 5H, H_{arom}), 9.44 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.77 (CH_3), 20.03 (CH_2), 57.09 (≡CCH_2O), 73.39 (NCH_2O), 74.51 (≡ CH), 79.13 (–C≡),116.29 (C-5), 119.27 ($=CH_2$), 125.37, 129.11, 129.16, 135.49 (C_{arom}), 138.12 ($C=CH_2$), 150.12 (C-6), 151.79 (C-2), 163.37 (C-4). HRMS (MALDI) m/z calcd for $C_{18}H_{18}N_2O_3Na^+$ (MNa^+) 333.1209, found 333.1206. Anal. calcd for $C_{18}H_{18}N_2O_3 \cdot 0.5H_2O$: C, 67.70; H, 6.00; N, 8.77. Found: C, 67.68; H, 5.86; N, 8.47.

5.15. 5-Ethyl-1-(3-phenyl-2-propynyloxymethyl-6-[1-(phenyl)vinyl]-1*H*-pyrimidine-2,4-dione (11)

Yield: 39 mg (10%) as a colourless oil; R_f 0.08 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, J = 7.5 Hz, CH_2CH_3), 2.12 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2a}CH_3$), 2.44 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2b}CH_3$), 4.44 (d, 2H, ≡CCH_2O), 4.88 (d, 1H, J = 10.5 Hz, $NCH_{2a}O$), 5.51 (d, 1H, J = 10.5 Hz, $NCH_{2b}O$), 5.57 (s, 1H, $C=CH_{2a}$), 6.17 (s, 1H, $C=CH_{2b}$), 7.28–7.40 (m, 10H, H_{arom}), 9.24 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.66 (CH_3), 20.05 (CH_2), 57.84 (≡CCH_2O), 73.47 (NCH_2O), 84.46 (CH_2C≡), 86.35 (≡ CPh), 116.19 (C-5), 119.30 ($=CH_2$), 122.26 (≡C-C_{arom}), 125.41, 128.29, 128.58 129.11, 129.14, 131.74, 135.55 (C_{arom}), 138.18 ($C=CH_2$), 150.20 (C-6), 151.69 (C-2), 163.18 (C-4). HRMS (MALDI) m/z calcd for $C_{24}H_{22}N_2O_3Na^+$ (MNa^+) 409.1522, found 409.1507.

5.16. 1-Allyloxymethyl-6-[1-(3,5-dimethylphenyl)vinyl]-5-ethyl-1*H*-pyrimidine-2,4-dione (12)

Yield: 269 mg (77%) as a yellow solid; mp 133–134 °C; R_f 0.38 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.3 Hz, CH_2CH_3), 2.15 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2a}CH_3$), 2.32 (s, 6H, 2×CH_3), 2.46 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2b}CH_3$), 4.04 (m, 2H, OCH_2CH=), 4.77 (d, 1H, J = 10.2 Hz, $NCH_{2a}O$), 5.17 (m, 2H, $C=CH_2$), 5.37 (d, 1H, J = 10.2 Hz, $NCH_{2b}O$), 5.44 (s, 1H, $C=CH_{2a}$), 5.71–5.85 (m, 1H, CH), 6.13 (s, 1H, $C=CH_{2b}$), 6.97 (s, 2H, H_{arom}), 6.99 (s, 1H, H_{arom}), 9.61 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.79 (CH_3), 20.05 (CH_2), 21.31 (2×CH_3), 70.31 (CH_2O), 73.41 (NCH_2O), 116.00 (C-5), 117.20 ($=CH_2$), 118.51 ($=CH_2$), 123.16, 130.85, 133.75, 135.55 (C_{arom}), 138.64 (2×$C=CH_2$), 150.63 (C-6), 151.79 (C-2), 163.54 (C-4). HRMS (MALDI) m/z calcd for $C_{20}H_{24}N_2O_3Na^+$ (MNa^+) 363.1679, found 363.1675. Anal. calcd for $C_{20}H_{24}N_2O_3 \cdot 0.25H_2O$: C, 69.64; H, 7.16; N, 8.12. Found: C, 69.91; H, 7.07; N, 8.15.

5.17. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-(2-methylallyloxymethyl)-1*H*-pyrimidine-2,4-dione (13)

Yield: 146 mg (41%) as off-white crystals; mp 139–140 °C; R_f 0.34 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.5 Hz, CH_2CH_3), 1.66 (s, 3H, CH_3), 2.14 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2a}CH_3$), 2.32 (s, 6H, 2×CH_3), 2.45 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2b}CH_3$), 3.94 (s, 2H, CH_2O), 4.75 (d, 1H, J = 10.1 Hz, $NCH_{2a}O$), 4.83, 4.87 (2×s, 2H, $C=CH_2$), 5.36 (d, 1H, J = 10.1 Hz, $NCH_{2b}O$), 5.44 (s, 1H, $C=CH_{2a}$), 6.12 (s, 1H, $C=CH_{2b}$), 6.96 (s, 2H, H_{arom}), 6.99 (s, 1H, H_{arom}), 9.34 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.80 (CH_2CH_3), 19.36 (CH_3), 20.08 (CH_2), 21.33 (2×CH_3), 73.42 (OCH_2), 73.63 (NCH_2O), 112.08 ($C(CH_3)=CH_2$),

115.91 (C-5), 118.58 (=CH_2), 123.17, 130.87, 135.59, 138.64 (C_{arom}), 138.64 ($C=CH_2$), 141.40 ($C(CH_3)=CH_2$), 148.89 (C-6), 150.81 (C-2), 164.44 (C-4). HRMS (MALDI) *m/z* calcd for $C_{21}H_{26}N_2O_3Na^+$ (MNa^+) 377.1835, found 377.1819. Anal. calcd for $C_{21}H_{26}N_2O_3 \cdot 0.25H_2O$: C, 70.27; H, 7.44; N, 7.80. Found: C, 70.53; H, 7.42; N, 7.81.

5.18. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-(3-methyl-2-butenyloxymethyl)-1*H*-pyrimidine-2,4-dione (14)

Yield: 240 mg (65%) as yellow crystals; mp 134–136 °C; R_f 0.40 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.62 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 2.13 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2a}CH_3$), 2.31 (s, 6H, 2×CH_3), 2.42 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2b}CH_3$), 3.99 (d, 2H, J = 6.9 Hz, CH_2O), 4.70 (d, 1H, J = 10.3 Hz, $NCH_{2a}O$), 5.17 (t, 1H, J = 6.9 Hz, C=CH), 5.34 (d, 1H, J = 10.3 Hz, $NCH_{2b}O$), 5.44 (s, 1H, $C=CH_{2a}$), 6.11 (s, 1H, $C=CH_{2b}$), 6.96 (s, 2H, H_{arom}), 6.99 (s, 1H, H_{arom}), 9.39 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.81 (CH_2CH_3), 17.97 (CH_3), 20.05 (CH_2), 21.31 (2×CH_3), 25.73 (CH_3), 65.78 (OCH_2), 73.36 (NCH_2O), 115.93 (C-5), 118.50 (=CH_2), 120.20 (=$C(CH_3)_2$), 123.15, 130.81, 135.59, 137.52 (C_{arom}), 138.55 ($C=C(CH_3)_2$), 138.62 ($C=CH_2$), 150.68 (C-6), 151.63 (C-2), 163.48 (C-4). HRMS (MALDI) *m/z* calcd for $C_{22}H_{28}N_2O_3Na^+$ (MNa^+) 391.1992, found 391.1987. Anal. calcd for $C_{22}H_{28}N_2O_3 \cdot 0.25H_2O$: C, 70.85; H, 7.70; N, 7.51. Found: C, 70.90; H, 7.65; N, 7.61.

5.19. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-((*E*)-3-phenylallyloxymethyl)-1*H*-pyrimidine-2,4-dione (15)

Yield: 117 mg (28%) as white foam; mp 56–57 °C; R_f 0.10 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 1.02 (t, 3H, J = 7.3 Hz, CH_2CH_3), 2.16 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2a}CH_3$), 2.29 (s, 6H, 2×CH_3), 2.48 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2b}CH_3$), 4.19 (d, 2H, J = 6.1 Hz, CH_2O), 4.84 (d, 1H, J = 10.3 Hz, $NCH_{2a}O$), 5.41 (d, 1H, J = 10.3 Hz, $NCH_{2b}O$), 5.47 (s, 1H, $C=CH_{2a}$), 6.11 (m, 1H, PhCH = $CHCH_2$), 6.14 (s, 1H, $C=CH_{2b}$), 6.52 (d, 1H, J = 15.9 Hz, PhCH = CH), 6.97 (s, 3H, H_{arom}), 7.24–7.36 (m, 5H, H_{arom}), 9.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.82 (CH_3), 20.10 (CH_2CH_3), 21.34 (2×CH_3), 69.90 (CH_2O), 73.31 (NCH_2O), 116.17 (C-5), 118.79 (=CH_2), 124.78, 125.43, 126.46, 127.75, 128.49, 129.07, 132.74, 135.54 (7×C_{arom}, CH=), 136.45 (PhCH=), 138.69 ($C=CH_2$), 150.26 (C-6), 151.76 (C-2), 163.46 (C-4). HRMS (MALDI) *m/z* calcd for $C_{26}H_{28}N_2O_3Na^+$ (MNa^+) 439.1992, found 439.1982. Anal. calcd for $C_{26}H_{28}N_2O_3 \cdot 0.25H_2O$: C, 74.17; H, 6.82; N, 6.65. Found: C, 74.22; H, 6.75; N, 6.59.

5.20. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-(2-propynyloxymethyl)-1*H*-pyrimidine-2,4-dione (16)

Yield: 52 mg (15%) as a white powder; mp 116 °C; R_f 0.08 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.5 Hz, CH_2CH_3), 2.13 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2a}CH_3$) 2.32 (s, 6H, 2×CH_3), 2.42 (t, 1H, J = 2.3 Hz, ≡C*H*), 2.45 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2b}CH_3$), 4.22 (d, 2H, J = 2.3 Hz, $\equiv CCH_2O$), 4.79 (d, 1H, J = 10.3 Hz, $NCH_{2a}O$), 5.46 (d, 1H, J = 10.3 Hz, $NCH_{2b}O$), 5.49 (s, 1H, $C=CH_{2a}$), 6.13 (s, 1H, $C=CH_{2b}$), 6.97 (s, 2H, H_{arom}), 7.00 (s, 1H, H_{arom}), 9.24 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.83 (CH_3), 20.08 (CH_2), 21.34 (2×CH_3), 57.17 ($\equiv CCH_2O$), 73.48 (NCH_2O), 74.45 (≡CH), 79.23 (–C≡), 116.16 (C-5), 118.98 (=CH_2), 123.17, 130.95, 135.41, 138.74 (C_{arom}), 138.24 ($C=CH$), 150.53 (C-6), 151.76 (C-2), 163.35 (C-4). MS (MALDI) *m/z* calcd for $C_{20}H_{22}N_2O_3Na^+$ (MNa^+) 361.1522, found 361.1523. Anal. calcd for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 71.07; H, 6.72; N, 8.14.

5.21. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-(3-phenyl-2-propynyloxymethyl)-1*H*-pyrimidine-2,4-dione (17)

Yield: 42 mg (10%) as yellow crystals; mp 78 °C; R_f 0.13 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, J = 7.5 Hz, CH_2CH_3), 2.13 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2a}CH_3$), 2.44 (dq, 1H, J = 15.0 Hz, 7.5 Hz, $CH_{2b}CH_3$), 4.45 (s, 2H, $\equiv CCH_2O$), 4.85 (d, 1H, J = 10.5 Hz, $NCH_{2a}O$), 5.52 (d, 1H, J = 10.5 Hz, $NCH_{2b}O$), 5.52 (s, 1H, $C=CH_{2a}$), 6.14 (s, 1H, $C=CH_{2b}$), 6.96 (s, 2H, H_{arom}), 6.98 (s, 1H, H_{arom}), 7.27–7.41 (m, 5H, H_{arom}), 9.24 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.71 (CH_3), 20.09 (CH_2), 21.29 (2×CH_3), 57.81 ($\equiv CCH_2O$), 73.50 (NCH_2O), 84.52 ($CH_2C\equiv$), 86.30 (≡*C*Ph), 116.05 (C-5), 118.97 (=CH_2), 122.28 (≡C–C_{arom}), 123.14, 128.29, 128.56 130.92, 131.72, 135.43, 138.72 (C_{arom}), 138.23 ($C=CH_2$), 150.56 (C-6), 151.75 (C-2), 163.33 (C-4). HRMS (MALDI) *m/z* calcd for $C_{26}H_{26}N_2O_3Na^+$ (MNa^+) 437.1836, found 437.1821.

5.22. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-((*E*)-3-phenyl-2-methylallyloxymethyl)-1*H*-pyrimidine-2,4-dione (18)

Yield: 214 mg (49%) as colourless foam; mp 53 °C; R_f 0.12 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 1.03 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.81 (s, 3H, CH_3), 2.17 (dq, 1H, J = 14.4 Hz, 7.2 Hz, $CH_{2a}CH_3$), 2.30 (s, 6H, 2×CH_3), 2.48 (dq, 1H, J = 14.4 Hz, 7.2 Hz, $CH_{2b}CH_3$), 4.09 (s, 2H, CH_2O), 4.82 (d, 1H, J = 10.2 Hz, $NCH_{2a}O$), 5.41 (d, 1H, J = 10.2 Hz, $NCH_{2b}O$), 5.49 (s, 1H, $C=CH_{2a}$), 6.14 (s, 1H, $C=CH_{2b}$), 6.40 (s, 1H, C=CH), 6.98 (s, 3H, H_{arom}), 7.22–7.35 (m, 5H, H_{arom}), 9.56 (s, 1H, NH). HRMS (MALDI) *m/z* calcd for $C_{27}H_{30}N_2O_3Na^+$ (MNa^+) 453.2148, found 453.2146. Anal. calcd for $C_{27}H_{30}N_2O_3 \cdot 0.25H_2O$: C, 74.54; H, 7.07; N, 6.44. Found: C, 74.29; H, 6.93; N, 6.40.

5.23. 6-[1-(3,5-Dimethylphenyl)vinyl]-5-ethyl-1-(indan-2-yloxymethyl)-1*H*-pyrimidine-2,4-dione (19)

Yield: 262 mg (63%) as a white powder; mp 163–165 °C; R_f 0.14 (25% EtOAc/Petroleum ether). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 7.3 Hz, CH_2CH_3), 2.13 (dq, 1H, J = 14.6 Hz, 7.3 Hz, $CH_{2a}CH_3$) 2.30 (s, 6H, 2×CH_3), 2.44 (dq, 1H, J = 14.6 Hz, 7.3 Hz $CH_{2b}CH_3$),

2.81–3.11 (m, 4H, 2×CH_2), 4.45 (m, 1H, $(CH_2)_2CHO$), 4.80 (d, 1H, $J = 10.2$ Hz, $NCH_{2a}O$), 5.34 (s, 1H, $C{=}CH_{2a}$), 5.41 (d, 1H, $J = 10.2$ Hz, $NCH_{2b}O$), 6.06 (s, 1H, $C{=}CH_{2b}$), 6.95 (s, 2H, H_{arom}), 6.98 (s, 1H, H_{arom}), 7.12–7.18 (m, 4H, H_{arom}), 9.38 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 13.81 (CH_3), 20.07 (CH_2), 21.33 (2×CH_3), 39.03 (CH_2), 39.46 (CH_2), 72.20 (NCH_2O), 78.42 ($(CH_2)_2CHO$), 116.02 (C-5), 118.52 (=CH_2), 123.16, 124.53, 124.58, 126.48, 126.52, 130.82, 135.56, 138.61, 140.45, 140.61, (C_{arom}), 138.47 ($C{=}CH_2$), 150.74 (C-6), 151.67 (C-2), 163.35 (C-4). HRMS (MALDI) m/z calcd for $C_{26}H_{28}N_2O_3Na^+$ (MNa^+) 439.1992, found 439.1972. Anal. calcd for $C_{26}H_{28}N_2O_3$: C, 74.98; H, 6.78; N, 6.73. Found: C, 74.70; H, 6.81; N, 6.30.

6. Viruses and cells

The inhibitory activity against HIV-1 infection was evaluated using MT-4 cells[28] as target cells and the HIV-1 strain HTLV-IIIB[29] as infectious virus. The virus was propagated in H9[28] cells at 37 °C, 5% CO_2 using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquoted, and stored at −80 °C until use.

6.1. Inhibition of HIV-1 replication

Compounds were examined for possible antiviral activity against both strains of HIV-1 using MT4 cells as target cells. MT4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compounds for 6 days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was indirectly quantified using the MTT assay.[30] Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT4 cultures containing the test dilutions of compound as described above. A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentration (IC_{50}) and the 50% cytotoxic concentration (CC_{50}) were determined by interpolation from the plots of percent inhibition versus concentration of compound. The test for activity against HIV-1 was performed in MT-4 cell cultures infected with either wild-type HIV-1(strain IIIB[30]) or NNRTI resistant HIV-1 (strain N119,[31] strain A17,[31b,32]).

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