Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Antiretroviral (HIV-1) activity of azulene derivatives

Julia Peet, Anastasia Selyutina, Aleksei Bredihhin *

Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

ARTICLE INFO

Article history:
Received 18 November 2015
Revised 17 February 2016
Accepted 27 February 2016
Available online xxxx

Keywords:
Azulene
Antiviral
HIV
HIV-1 inhibition

ABSTRACT

The antiretroviral activity of azulene derivatives was detected for the first time. A series of eighteen diversely substituted azulenes was synthesized and tested in vitro using HIV-1 based virus-like particles (VLPs) and infectious HIV-1 virus in U2OS and TZM-bl cell lines. Among the compounds tested, the 2-hydroxyazulenes demonstrated the most significant activity by inhibiting HIV-1 replication with IC_{50} of 2–10 and 8–20 μM for the VLPs and the infectious virus, respectively. These results indicate that azulene derivatives may be potentially useful candidates for the development of antiretroviral agents.

1. Introduction

The human immunodeficiency virus 1 (HIV-1)[1] is a pandemic virus, and the number of people infected with HIV-1 is constantly increasing. In 2014, about 37 million people were living with HIV and 1.2 million have died from HIV-associated diseases.[2] Currently, there are about 30 anti-HIV drugs of different classes available; however, the search for new highly active and safe compounds targeting the HIV remains a very important task, which could potentially affect lives of millions of people.

Azulene derivatives have been shown to have anti-inflammatory,[3,4] antibacterial,[5–7] anti-ulcer,[8–10] anti-cancer[11–14] and other activities,[15–18] but to the best of our knowledge, their antiviral effect has not been reported before. However, strong antiviral activity against the *Herpes simplex* virus was demonstrated by reiswigin diterpenes that have similar structures to azulene but with a non-aromatic skeleton.[19] In the current study, we screened azulene and its derivatives for compounds with high anti-HIV activity.

2. Results and discussion

2.1. Chemistry

The series of diversely substituted azulenes that was used in this study is shown in Figure 1. We incorporated a variety of substituents including halogeno, carboxyethyl, hydroxy, phenyl, aryl and heteroaryl groups to maximize the scope of this study. Polar and hydrophilic compounds were preferred because of their superior solubility and bioavailability. To study the structure–activity relationship (SAR) and possible mechanisms of action of the investigated compounds, we included well-studied and documented compounds with biological activity such as guaiazulene (**2**) and sodium gualenate (**3**) in the list of tested compounds.

The synthesis of azulene derivatives is often a complicated task, and in some cases requires multistep procedures or even the construction of an azulene core that already bears the desired substituents. Recently, our research group has developed several methods that significantly simplified the synthesis of azulene derivatives, and most of the tested compounds in this study were prepared according to the procedures described in our previous publications.[20,21] Thus, compounds **5**, **6** and **12** were easily obtained starting with azulene (**1**) (see Scheme 1), and compounds **7**, **10**, **11**, **17** and **18** were obtained using the Negishi cross-coupling of bromoazulenes with corresponding organozinc reagents.[20] In addition, compounds **4** and **16** were synthesized via azulenyl zinc intermediates.[21] Compound **14** was prepared according to the procedure described by Zhang[22] and further decarboxylated to produce **13**.[23] Compound **15** can be obtained by decarboxylation of **14**,[24] however, we have obtained it more conveniently by the direct boronation of azulene followed by oxidation with hydrogen peroxide as was described by Kurotobi.[25] Sodium gualenate (**3**) was easily prepared from guaiazulene (**2**) and sulfuric acid; however, compound **9** was obtained only with a low yield using the Buchwald–Hartwig amination. Compound **8** was prepared by the hydrolysis of ethyl 4-(3-chloroazulen-1-yl) benzoate.

* Corresponding author.
E-mail address: aleksei.bredihhin@ut.ee (A. Bredihhin).

http://dx.doi.org/10.1016/j.bmc.2016.02.038
0968-0896/

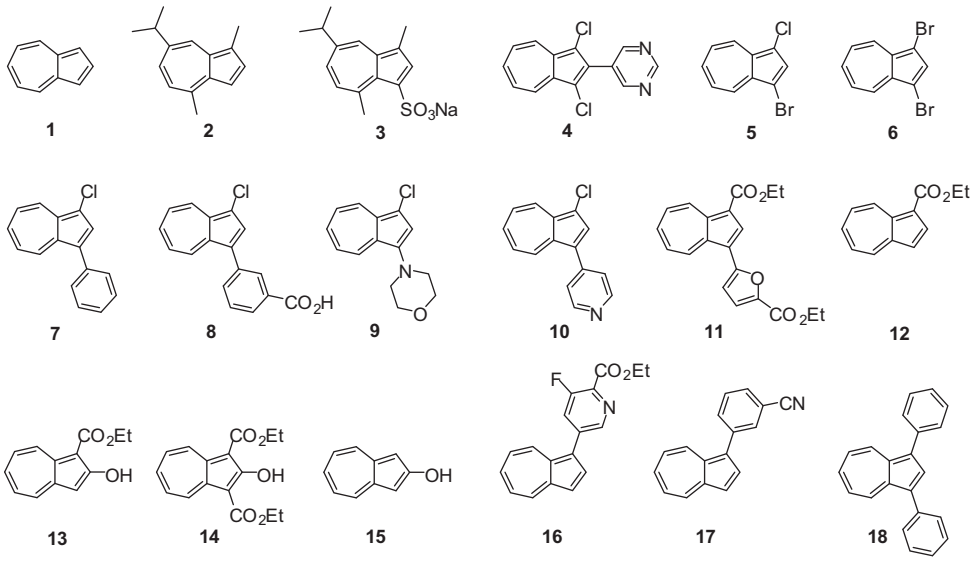
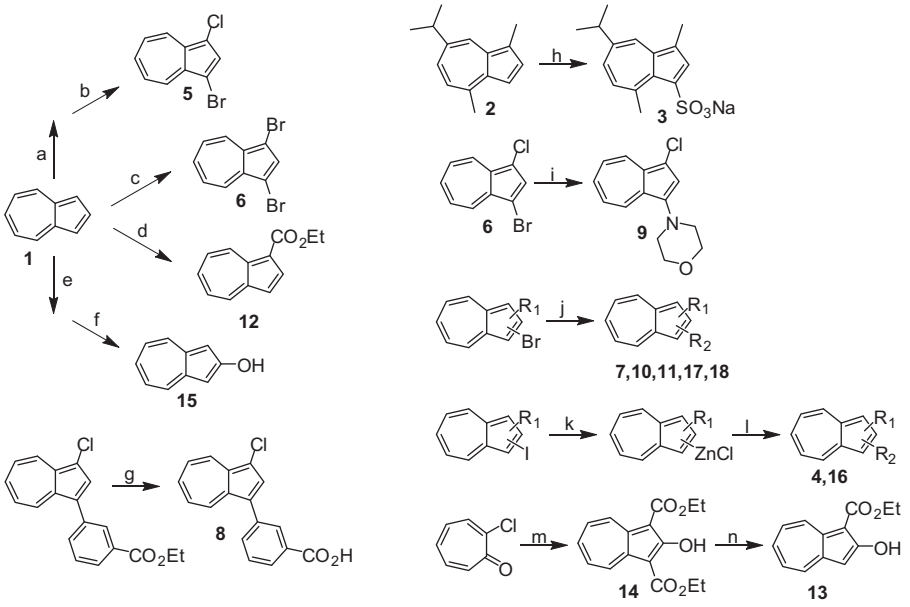


Figure 1. Structure of compounds investigated in this study.



Scheme 1. Synthesis of substituted azulenes. Reagents and conditions: (a) NCS, DCM, 0 °C to rt, 25 min; (b) NBS, DCM, 0 °C to rt, 25 min; (c) 2.0 equiv NBS, DCM, 0 °C to rt, 25 min; (d) $AlCl_3$, $ClCO_2Et$, rt,1 h; (e) pin_2B_2, $[IrCl(cod)]_2$, bpy, cyclohexane, reflux, 14 h; (f) H_2O_2 (aq 30%), EtOH, 0 °C, 2 h; (g) NaOH (aq 10%), MeOH/1,2-dimethoxyethane, reflux, 2 h; (h) H_2SO_4, Ac_2O, 0 °C to rt, 4 h; (i) $Pd_2(dba)_3$, BINAP, *t*BuONa, morpholine, PhMe, 100 °C, 2 h; (j) $Pd(dba)_2$, SPhos, RZnCl, THF, rt, 0.5–5 h; (k) (1) *i*PrMgCl·LiCl, 20 min; (2) $ZnCl_2$, 15 min, THF, −30 °C; (l) $Pd(dba)_2$, SPhos, RHal, THF, rt or 50 °C, 0.5–2 h; (m) EtONa, diethyl malonate, EtOH, 72 h, rt; (n) KOH (2 M in EtOH), reflux, 3 h.

2.2. Evaluation of antiretroviral activity

First, the cytotoxicity of the compounds was tested using the xCELLigence RTCA DP instrument and U2OS cell line. The studied compounds were applied in several concentrations, and viability of the cells was compared with viability of the cells, treated with 0.5% dimethyl sulphoxide (DMSO (served as a vehicle control)). The highest non-toxic concentration of the compound was determined as the concentration that reduced cell viability by less than 20% (Table 1).

The first screening for antiretroviral activity was performed using HIV-1-based virus-like particles (VLPs). This assay is fast, safe and sensitive, and therefore, allowed the convenient preliminary evaluation of the antiretroviral activity of test compounds. VLP particles encode the Gaussia luciferase (Gluc) reporter gene in their genome RNA, and thus Gluc activity in the infected cells correlates with the efficiency of infection.[26] To estimate the antiretroviral activity, we have infected the U2OS cells with VLPs in the presence of the test compounds. In the first screening, the compounds were applied at their corresponding highest non-toxic concentrations (Table 1) while 0.5% DMSO (used for dilution of compounds) and nevirapine (an HIV-1 non-nucleoside reverse transcriptase inhibitor) were used as the negative and positive controls, respectively. Among the tested substances, guaiazulene (**2**) and compounds **5**, **6**, **10**, **12**, **13**, **14** and **15** inhibited Gluc activity by 48% or more compared to the negative control (Table 1). Guaiazulene (**2**) and compounds **5**, **6** and **12** showed moderate antiretroviral activity and inhibited Gluc activity to 48%, 32%, 36% and 52%, respectively; however, since these compounds were applied at a relatively high concentrations (75 μM), we have assumed that their IC_{50} values

Table 1
Toxicity and antiretroviral activity of tested compounds

Compound	Highest non-toxic concentration (μM)	Toxicity assay: cell viability[a]	Antiretroviral activity: normalized Gluc activity[a]
DMSO	0.5%	100	100
1	150	104	76
2	75	106	48
3	500	91	154
4	0.5	80	141
5	75	101	32
6	75	96	36
7	75	88	94
8	50	92	103
9	5	80	98
10	18.75	80	36
11	150	83	70
12	75	85	52
13	150	89	6.4
14	50	80	3.5
15	5	80	51
16	50	86	80
17	150	80	63
18	150	80	124
Nevirapine	50	NT	0.01

[a] Percentage of negative control (DMSO); NT—not tested.

would be well above physiological concentrations and, therefore, they were excluded from further analysis. Interestingly, compound **10** showed slightly better results than compounds **2**, **5**, **6** and **12** did; however, its activity was also considered too low for further analysis.

The most prominent inhibitory effect was demonstrated by compounds **13**, **14** and **15**, which all bear a hydroxyl (OH) group at position 2. Compound **15** inhibited Gluc activity only to 51%, which was similar to the effect exhibited by guaiazulene (**2**) and compound **12**; however, this occurred at a concentration that was 15-fold lower (5 vs 75 μM). The most promising results were shown by compounds **13** and **14**, which suppressed Gluc activity to 6.4% and 3.5% (at 150 and 50 μM, respectively) and, therefore, were selected for further studies.

The CC_{50} and IC_{50} values for compounds **13**, **14** and **15** (Table 2) were determined using U2OS cells and HIV-1-based VLPs. The most significant activity, as shown in Table 2, was demonstrated by compound **15** with IC_{50} = 2.2 ± 0.5 μM, but due to its relatively high toxicity (CC_{50} = 26.8 ± 7 μM) its selectivity index (SI, ratio of CC_{50}/IC_{50}) was 12. Compound **14** was less toxic (CC_{50} = 72.6 ± 10 μM) but also less active (IC_{50} = 4.4 ± 1 μM) compared to **15** (Table 2). Therefore, the selectivity index (SI = 16) of **14** was not significantly different from that of compound **15**. Compound **13** showed moderate anti-HIV activity with IC_{50} = 10.1 ± 1 μM while its CC_{50} = 344.2 ± 40 μM, which resulted in the highest selectivity index (SI = 34.1, Table 2).

The investigated compounds (**13**, **14** and **15**) have several similar structural features including the presence of an OH group at position 2 in all of them while compounds **13** and **14** have an additional one or two ethoxycarbonyl groups. In the same time, compound **12**, which has only one ethoxycarbonyl group and no OH group, showed much lower antiretroviral activity (IC_{50} > 75 μM). That led us to the conclusion, that the presence of the OH group in the second position is the key structural element required for demonstrating a high antiretroviral activity.

Because compounds **13**, **14** and **15** showed a positive effect in the first screening for anti-HIV activity (performed using HIV-1-based VLPs), we have further tested their ability to inhibit infectious HIV-1 virus replication using TZM-bl cells (Table 2). The toxicity of the compounds in the experiments with TZM-bl cells was comparable with their toxicity against U2OS cells (CC_{50} of **13**, **14** and **15** was 260 ± 30, 65 ± 15, and 60 ± 7 μM, respectively, Table 2). Surprisingly, compound **13** was not active against HIV-1 virus (data not shown). In contrast, compounds **14** and **15** were active with of IC_{50} of 20 ± 7 and 8.5 ± 3 μM, respectively, and their SI values were 3 and 7, respectively (Table 2).

We have supposed, that antiretroviral activity of compounds **13**, **14**, and **15** can be explained by their inhibition of an important HIV-1 enzyme—reverse transcriptase. In order to test this hypothesis we tested compounds **13**, **14**, and **15** for their ability to inhibit reverse transcriptase activity in the cell-free assay. These compounds were tested at the high concentration (500 μM) and only compound **14** inhibited the reaction. IC_{50} (**14**) in the cell-free assay was 104.2 ± 4 μM which indicates that compound **14** acts as a reverse transcriptase inhibitor.

There are two types of reverse transcriptase inhibitors—nucleoside (nucleotide) and non-nucleoside. Compounds from the first group mimic natural substrates of reverse transcriptase (nucleotides). We assume that compound **14** is a non-nucleoside reverse transcriptase inhibitor because it does not resemble nucleoside or nucleotide structure. Non-nucleoside inhibitors can have very diverse chemical structures, but all of them bind reverse transcriptase allosterically which leads to the change in the enzyme conformation and inhibition of its activity.

Despite we have found that compound **14** is a reverse transcriptase inhibitor, the mechanism of action of compound **15** is still unclear. It may directly target some HIV-1 enzyme or function, but it can also act as host-targeting inhibitor. This suggests that compound **15** may inhibit certain cellular enzyme or processes, which is of immense importance for inhibiting viral replication. Host-targeting inhibitors are a very promising group of drugs because resistant forms of the virus cannot be easily developed. However, a serious drawback of host-targeting inhibitors is their toxicity to the host organism and for this reason, a careful optimization of such compounds is required.

3. Conclusions

In summary, a series of azulene derivatives were synthesized, and their antiretroviral activity was tested using a two-stage process. The first screening was performed using HIV-1-based VLPs, and three compounds (**13**, **14** and **15**) showed the highest activity in this analysis.

Table 2
Inhibition of HIV-1 VLP and infectious HIV-1 by azulenes

Compounds	MW	First screen (VLP)			Test with HIV-1		
		CC_{50}, μM (U2OS)	IC_{50}, μM	SI	CC_{50}, μM (TZM-bl)	IC_{50}, μM	SI
13	216.23	344.2 ± 40	10.1 ± 1	34.1	260 ± 30	NA	–
14	288.30	72.2 ± 10	4.4 ± 1	16.4	65 ± 15	20 ± 7	3
15	144.16	26.8 ± 7	2.2 ± 0.5	12.2	60 ± 7	8.5 ± 3	7

Abbreviations used: MW—molecular weight; CC_{50}—half-maximal cytotoxic concentration; IC_{50}—half-maximal inhibitory concentration; SI—selectivity index; VLP—virus-like particles; NA—not available.

Please cite this article in press as: Peet, J.; et al. *Bioorg. Med. Chem.* (2016), http://dx.doi.org/10.1016/j.bmc.2016.02.038

The compounds, that were selected based on their performance in the first screen, were further tested for their activity against the infectious HIV-1 virus. One compound (**13**) was unable to inhibit HIV-1 infection, whereas the other two were active, with compound **15** being the most active (IC_{50} = 8.5 ± 3 μM and SI = 7). Additionally, we have found that compound **14** can act as a non-nucleoside reverse transcriptase inhibitor with IC_{50} = 104.2 ± 4 μM. Thus, the present study demonstrated that compounds **14** and **15** can be regarded as promising compounds for development as novel HIV inhibitors.

4. Experimental section

4.1. Chemistry

All reagents were purchased from Sigma–Aldrich and used without further purification. All reactions with organometallic reagents were carried out under an argon atmosphere using dried glassware. The syringes used to transfer anhydrous solvents or reagents were purged with argon prior to use. The tetrahydrofuran was continuously refluxed and freshly distilled from sodium benzophenone ketyl under nitrogen. The $ZnCl_2$ solution was freshly prepared and stored under argon for not more than one week. Petroleum ether (PE) refers to the fraction boiling at a temperature range of 40–65 °C. NMR spectroscopy was performed on a Bruker AVANCE II 400 MHz spectrometer using the residual solvent peak ($CDCl_3$, 7.26 ppm for ^1H and 77.16 for ^{13}C spectra) as the internal standard. The infrared (IR) spectra were measured on Shimadzu IRAffinity-1 FTIR spectrometer using ATR module with a ZnSe crystal. The reactions were monitored using thin-layer chromatography (TLC) and visualized with ultraviolet (UV) light. The products were purified using flash chromatography and silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM).

4.1.1. Sodium gualenate (3)

Sulfuric acid (370 μL, 7 mmol) was added dropwise to a solution of guaiazulene (198 mg, 1 mmol) in acetic anhydride (2 mL, 20 mmol) at 0 °C, then cooling was removed and the resulting mixture was stirred at room temperature for 4 h. Then, a 40% aqueous solution of sodium hydroxide (10 g) was added dropwise, and the resulting mixture was extracted with dichloromethane and the precipitated product was quickly separated using a glass filter to give **3** as dark purple colored crystals (176 mg, 57%). Mp 159–161 °C (dec.). IR (ATR, neat): 3410, 3086, 2955, 2924, 1578, 1447, 1373, 1192, 1142, 1119, 1049, 1011, 637. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.28 (s, 1H), 8.03 (s, 1H), 7.56 (d, J = 10.8 Hz, 1H), 7.30 (d, J = 10.8 Hz, 1H), 3.26 (s, 3H), 3.03 (dquin, J = 13.7 Hz, 1H), 2.51 (s, 3H), 1.24 (d, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.3, 144.1, 139.6, 138.8, 137.3, 135.5, 132.5, 131.0, 124.7, 123.1, 37.1, 26.9, 23.6, 23.3, 11.9.

4.1.2. 3-(3-Chloroazulen-1-yl) benzoic acid (8)

To a solution of ethyl 4-(3-chloroazulen-1-yl) benzoate[20] (700 mg, 2.25 mmol) in a mixture of methanol (55 mL) and 1,2-dimethoxyethane (20 mL) 10% aqueous solution of NaOH (28 mL) was added and the reaction mixture was heated under reflux for 2 h. Then, the organic solvents were evaporated, and the aqueous residue was treated with 10% hydrochloric acid to obtain a solution pH 2–3, and finally extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over $MgSO_4$, and evaporated in vacuo to give **8** as a green solid (564 mg, 87%). Mp 186–187 °C (dec.). IR (ATR, neat): 2959, 2874, 2661, 2546, 1697, 1686, 1574, 1454, 1412, 1393, 1346, 1300, 1265, 910, 837, 732, 682. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.48 (d, J = 9.7 Hz, 1H), 8.39 (d, J = 9.7 Hz, 1H), 8.15 (s broad, 1H), 8.09 (s, 1H), 7.97 (d, J = 7.5 Hz, 1H), 7.81–7.77 (m, 2H), 7.60 (t, J = 7.6 Hz, 1H), 7.37–7.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.6, 136.5, 135.5, 134.9, 134.3, 134.0, 133.9, 144.1, 130.1, 128.9, 127.6, 125.0, 124.0, 115.0. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{11}ClO_2$: 283.0520; found: 283.0514.

4.1.3. 4-(3-Chloroazulen-1-yl) morpholine (9)

A mixture of 1-Cl-3-Br-azulene (242 mg, 1 mmol), $Pd_2(dba)_3$, (12 mg, 2 mol%), 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (19 mg, 3 mol%), sodium *tert*-butoxide (240 mg, 2.5 mmol), and morpholine (174 mg, 2 mmol) in toluene (2 mL) was heated to 100 °C under argon atmosphere. After stirring for 2 h, the reaction mixture was poured into water and extracted with toluene. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (eluent: EtOAc/PE, 1:4) to give **9** as a green solid (23 mg, 10%). Mp 107–108 °C. IR (ATR, neat): 3047, 3024, 2974, 2940, 2835, 1574, 1501, 1447, 1350, 1261, 1150, 1111, 907, 741. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.26 (d, J = 9.5 Hz, 1H), 8.20 (d, J = 9.5 Hz, 1H), 7.50–7.45 (m, 2H), 6.94 (q, J = 9.2 Hz, 2H), 3.96–3.94 (m, 4H), 3.11–3.08 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.8, 135.1, 134.0, 130.1, 129.8, 124.6, 121.7, 121.4, 114.3, 67.2, 54.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{14}ClO$: 248.0837; found: 248.0834.

4.2. Biological assays

4.2.1. Materials

DMSO, polybrene, phorbol 12-myristate 13-acetate (PMA), nevirapine and trichloracetic acid (TCA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). $Na_4P_2O_7$ was purchased from Applichem (Germany). HIV-1 reverse transcriptase was purchased from Calbiochem (USA). All reagents and media used for cell cultivation were purchased from Naxo OÜ (Estonia).

4.2.2. Cells and growth media

The ACH-2 and TZM-bl cell lines were obtained through the National Institutes for Health (NIH) AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), NIH: ACH-2[27,28] was from Dr. Thomas Folks and TZM-bl[29–32] was from Dr. John C. Kappes, Dr. Xiaoyun Wu, and Tranzyme Inc.

The U2OS (human osteosarcoma) cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum (FBS), 100 U penicillin, and 100 μg/mL streptomycin (Pen/Strep).

The ACH-2 cell line was maintained in Roswell Park Memorial Institute medium 1640 (RPMI 1640), supplemented with 25 mM HEPES, 0.3 g/L L-glutamine, 10% heat-inactivated FBS, and Pen/Strep. The TZM-bl cell line was maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS and Pen/Strep. All cells were maintained at 37 °C in the presence of 5% CO_2.

4.2.3. Cytotoxicity assay

The cytotoxicity of all the test compounds was measured using the xCELLigence RTCA DP Instrument (ACEA Biosciences, Inc.) as described previously.[26]

4.2.4. Analysis of the antiretroviral activity of compounds using HIV-1-based VLPs

The antiretroviral activity of the test compounds was tested using a ViraPower Lentiviral Expression System (Invitrogen) as described previously.[26]

4.2.5. Analysis of the antiretroviral activity of compounds infectious HIV-1

4.2.5.1. HIV-1 production. The HIV-1 was produced using the ACH-2 cell line with a T cell clone caring an HIV-1 provirus in its genome. To induce HIV-1 production, 6×10^6 ACH-2 cells were seeded in 10 mL of full media with 100 nM PMA and incubated for 48 h. Then, the virus-containing media was collected, filtered through a 0.45-μM filter (Sarstedt) and the amount of viral p24 protein was measured using an enzyme-linked immunosorbent assay (ELISA) developed in house.

4.2.5.2. Tests for antiretroviral activity. TZM-bl cell line was used for this assay. Its genome contains the firefly luciferase (Luc) reporter gene under the control of the HIV-1 LTR. The HIV-1 Tat protein, which is produced after HIV-1 integration into the host genome, is required for effective reporter gene expression. Therefore, the firefly luciferase activity in cell lysate correlates with the efficiency of infection. First, 5×10^4 TZM-bl cells per well were seeded in a 24-well plate and incubated for 24 h. The cells were washed with PBS, infected with 150 μL of media containing 30 ng of the HIV-1 protein p24, and incubated for 2 h in the presence of the test compounds at the indicated concentrations. Then, the infectious media was replaced with full growth media, containing the test compounds and incubated for another 48 h after which the cells were lysed, and the firefly luciferase activity was measured using Luciferase Assay System and Glomax 20/20 Luminometer (Promega). The firefly luciferase activity was normalized to the total protein concentration (measured using the Bio-Rad protein assay).

4.2.6. Cell-free assay for HIV-1 reverse transcriptase activity

The test for inhibition of DNA polymerase activity was performed using RNA template (234 nt long irrelevant RNA), DNA primer (18 nt long DNA) and recombinant HIV-1 reverse transcriptase (Calbiochem). First, 1.25 μL of tested compounds or nevirapine in required concentrations (or DMSO as a negative control) were mixed with 21.75 μL of the reaction mixture containing 50 mM Tris–HCl, pH 7.8, 1 mM DTT, 6 mM $MgCl_2$, 80 mM KCl, 0.3% PEG 4000, 50 μM of dATP, dGTP and dTTP, 5 μM dCTP, 1 unit of HIV-1 reverse transcriptase and 1 μCi (0.33 pmol) $\alpha[P^{33}]$dCTP (Perkin Elmer). Then, tested compounds and HIV-1 reverse transcriptase were incubated together for 10 min at room temperature to allow enzyme-inhibitor complex to form and then DNA synthesis was initiated by addition of 2 μL of template-primer annealed (containing 2 pmol of template and 4 pmol of primer, 10 mM Tris–HCl, pH 7.5, 1 mM EDTA). Samples were incubated for 40 min at 37 °C. The reaction was stopped by addition of 1 mL of buffer A (10% TCA, 0.5% $Na_4P_2O_7$) and 100 μg of salmon sperm carrier DNA. Synthesized DNA was precipitated for 30 min on ice and then mixtures were filtered through GF/C Whatman filter and washed twice with 5 mL buffer B (1% TCA, 0,1% $Na_4P_2O_7$). Filters were dried, 5 mL of scintillation cocktail ScintiSafe 3 (Fisher Scientific) was added and radioactivity bound to the filter was measured with Liquid Scintillation Analyzer Tri-Carb 2810 TR (Perkin Elmer).

4.2.7. Statistical analysis

The statistical analysis was performed using the GraphPad Prism 5 software.

Acknowledgments

The authors would like to thank the Estonian Science Foundation (grant ETF9198), the Archimedes Foundation (Project No. 3.2.0501.10-0004) and the European Union through the European Regional Development Fund via the Center of Excellence in Chemical Biology for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.02.038. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. For review on HIV infection see: Maartens, G. M.; Celum, C.; Lewin, S. R. *Lancet* **2014**, *384*, 258.
2. WHO Global summary of the AIDS epidemic 2014 http://www.who.int/hiv/data/epi_core_july2015.png [accessed 2.11.15].
3. Ramadan, M.; Goeters, S.; Watzer, B.; Krause, E.; Lohmann, K.; Bauer, R.; Hempel, B.; Imming, P. *J. Nat. Prod.* **2006**, *69*, 1041.
4. Rekka, E.; Chrysselis, M.; Siskou, I.; Kourounakis, A. *Chem. Pharm. Bull.* **2002**, *50*, 904.
5. Billow, J. A.; Tully, J.; Speaker, T. J. J. *Pharm. Sci.* **1975**, *64*, 862.
6. Wang, D.-L.; Xu, J.; Han, S.; Gu, Z. *Chin. J. Org. Chem.* **2016**, *2008*, 28.
7. Toyama, Y.; Miyazawa, S.; Yokota, M. JP 2004217602 A, 2004; *Chem. Abstr.* **2004**, *141*, 150961.
8. Zhang, L.-Y.; Yang, F.; Shi, W.-Q.; Zhang, P.; Li, Y.; Yin, S.-F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5722.
9. Yanagisawa, T.; Kosakai, K.; Tomiyama, T.; Yasunami, M.; Takase, K. *Chem. Pharm. Bull.* **1990**, *38*, 3355.
10. Yanagisawa, T.; Wakabayashi, S.; Tomiyama, T.; Yasunami, M.; Takase, K. *Chem. Pharm. Bull.* **1988**, *36*, 641.
11. Sekine, T.; Takahashi, J.; Nishishiro, M.; Arai, A.; Wakabayashi, H.; Kurihara, T.; Kobayashi, M.; Hashimoto, K.; Kikuchi, H.; Katayama, T.; Kanda, Y.; Kunii, S.; Motohashi, N.; Sakagami, H. *Anticancer Res.* **2007**, *27*, 133.
12. Wakabayashi, H.; Hashiba, K.; Yokoyama, K.; Hashimoto, K.; Kikuchi, H.; Nishikawa, H.; Kurihara, T.; Satoh, K.; Shioda, S.; Sato, S.; Kusano, S.; Nakashima, H.; Motohashi, N.; Sakagami, H. *Anticancer Res.* **2003**, *23*, 4747.
13. Asato, A. E.; Peng, A.; Hossain, M. Z.; Mirzadegan, T.; Bertram, J. S. *J. Med. Chem.* **1993**, *36*, 3137.
14. Ishihara, M.; Wakabayashi, H.; Motohashi, N.; Sakagami, H. *Anticancer Res.* **2011**, *31*, 515.
15. Löber, S.; Hübner, H.; Buschauer, A.; Sanna, F.; Argiolas, A.; Melis, M. R.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7151.
16. Löber, S.; Tschammer, N.; Hübner, H.; Melis, M. R.; Argiolas, A.; Gmeiner, P. *ChemMedChem* **2009**, *4*, 325.
17. Ikegai, K.; Imamura, M.; Suzuki, T.; Nakanishi, K.; Murakami, T.; Kurosaki, E.; Noda, A.; Kobayashi, Y.; Yokota, M.; Koide, T.; Kosakai, K.; Ohkura, Y.; Takeuchi, M.; Tomiyama, H.; Ohta, M. *Bioorg. Med. Chem.* **2013**, *21*, 3934.
18. Chen, C.-H.; Lee, O.; Yao, C. N.; Chuang, M. Y.; Chang, Y. L.; Chang, M. H.; Wen, Y. F.; Yang, W. H.; Ko, C. H.; Chou, N. T.; Lin, M. W.; Lai, C. P.; Sun, C. Y.; Wang, L. M.; Chen, Y. C.; Hseu, T. H.; Chang, C. N.; Hsu, H. C.; Lin, H. C.; Chang, Y. L.; Shih, Y. C.; Chou, S. H.; Hsu, Y. L.; Tseng, H. W.; Liu, C. P.; Tu, C. M.; Hu, T. L.; Tsai, Y. J.; Chen, T. S.; Lin, C. L.; Chiou, S. J.; Liu, C. C.; Hwang, C. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6129.
19. Kashman, Y.; Hirsch, S.; Koehn, F.; Cross, S. *Tetrahedron Lett.* **1987**, *28*, 5461.
20. Dubovik, J.; Bredihhin, A. *Synthesis* **2015**, *47*, 538.
21. Dubovik, J.; Bredihhin, A. *Synthesis* **2015**, *47*, 2663.
22. Zhang, J.; Petoud, S. *Chem. Eur. J.* **2008**, *14*, 1264.
23. Nozoe, T.; Takase, K.; Shimazaki, N. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 1644.
24. Koch, M.; Blacque, O.; Venkatesan, K. *Org. Lett.* **2012**, *14*, 1580.
25. Kurotobi, K.; Miyauchi, M.; Takakura, K.; Murafuji, T.; Sugihara, Y. *Eur. J. Org. Chem.* **2003**, 3663.
26. Ilisson, M.; Tomson, K.; Selyutina, A.; Türk, S.; Mäeorg, U. *Synth. Commun.* **2015**, *45*, 1367.
27. Clouse, K. A.; Powell, D.; Washington, I.; Poli, G.; Strebel, K.; Farrar, W.; Barstad, P.; Kovacs, J.; Fauci, A. S.; Folks, T. M. *J. Immunol.* **1989**, *142*, 431.
28. Duh, E. J.; Maury, W. J.; Folks, T. M.; Fauci, A. S.; Rabson, A. B. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5974.
29. Platt, E. J.; Bilska, M.; Kozak, S. L.; Kabat, D.; Montefiori, D. C. *J. Virol.* **2009**, *83*, 8289.
30. Takeuchi, Y.; McClure, M. O.; Pizzato, M. *J. Virol.* **2008**, *82*, 12585.
31. Derdeyn, C. A.; Decker, J. M.; Sfakianos, J. N.; Wu, X.; O'Brien, W. A.; Ratner, L.; Kappes, J. C.; Shaw, G. M.; Hunter, E. *J. Virol.* **2000**, *74*, 8358.
32. Platt, E. J.; Wehrly, K.; Kuhmann, S. E.; Chesebro, B.; Kabat, D. *J. Virol.* **1998**, *72*, 2855.