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Anti-AIDS agents 87. New bio-isosteric dicamphanoyl-dihydropyranochromone (DCP) and dicamphanoyl-khellactone (DCK) analogues with potent anti-HIV activity

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ABSTRACT

Six 3′*R*,4′*R*-di-*O*-(*S*)-camphanoyl-2′,2′-dimethyldihydropyrano[2,3-*f*]chromone (DCP) and two 3′*R*,4′*R*-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) derivatives were designed, synthesized, and evaluated for inhibition of HIV-1_{NL4-3} replication in TZM-bl cells. 2-Ethyl-2′-monomethyl-1′-oxa- and -1′-thia-DCP (**5a**, **6a**), as well as 2-ethyl-1′-thia-DCP (**7a**) exhibited potent anti-HIV activity with EC_{50} values of 30, 38 and 54 nM and therapeutic indexes of 152.6, 48.0 and 100.0, respectively, which were better than or comparable to those of the lead compound 2-ethyl-DCP in the same assay. 4-Methyl-1′-thia-DCK (**8a**) also showed significant inhibitory activity with an EC_{50} of 128 nM and TI of 237.9.

In our previous research, 3′*R*,4′*R*-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**, Fig. 1) demonstrated extremely potent inhibitory activity against HIV-1 replication in H9 lymphocytic cells.[1] Subsequently, hundreds of DCK and some of its ring-A positional isomer DCP (3′*R*,4′*R*-di-*O*-(*S*)-camphanoyl-2′,2′-dimethyl-dihydropyrano[2,3-*f*]chromone, **2**, Fig. 1) derivatives have been designed, synthesized and screened for anti-HIV activity in H9 lymphocytes, MT-2 cell lines, and MT-4 cell lines.[2–8] 4-Methyl-DCK (**3**, Fig. 1) and 2-ethyl-DCP (**4**, Fig. 1) showed the most promising anti-HIV results in these two series. Structure–activity relationship (SAR) studies found that DCP derivatives exhibited better anti-HIV activity than the corresponding DCKs;[8] 2′-α-monomethyl-4-methyl DCK derivatives were more potent than 2′-*gem*-dimethyl DCKs;[9] bio-isosteric analogues with a sulfur rather than oxygen in the ring-C of DCK exhibited remarkable inhibitory effects on HIV-1 replication;[9,10] and a 3′,4′-dicamphanoyl moiety is indispensable for anti-HIV activity.[11] Considering these SAR research results, we have now designed and synthesized 2′-monomethyl-DCP (**5**, 1′-oxa; **6**, 1′-thia), 2-ethyl-1′-thia-DCP (**7**), and 4-methyl-1′-thia-DCK (**8**) analogues to further explore the pharmacophores of the 2′-position and the bio-isosteric effect at the 1′-position. This paper reports their synthesis and anti-HIV bioassay data.

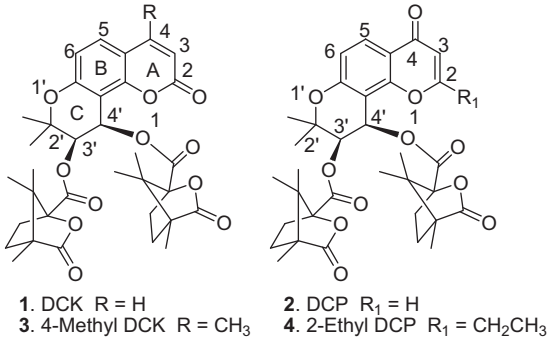

Figure 1. Structures of previously synthesized DCK and DCP analogues (**1**–**4**).

The synthetic routes to **5a**, **5b**, **6a** and **6b** are shown in Scheme 1. The intermediate 2-ethyl-7-mercapto-4*H*-chromen-4-one (**12**) was obtained by reacting 2-ethyl-7-hydroxy-4*H*-chromen-4-one (**9**) with dimethylthiocarbamoyl chloride in EtOH in

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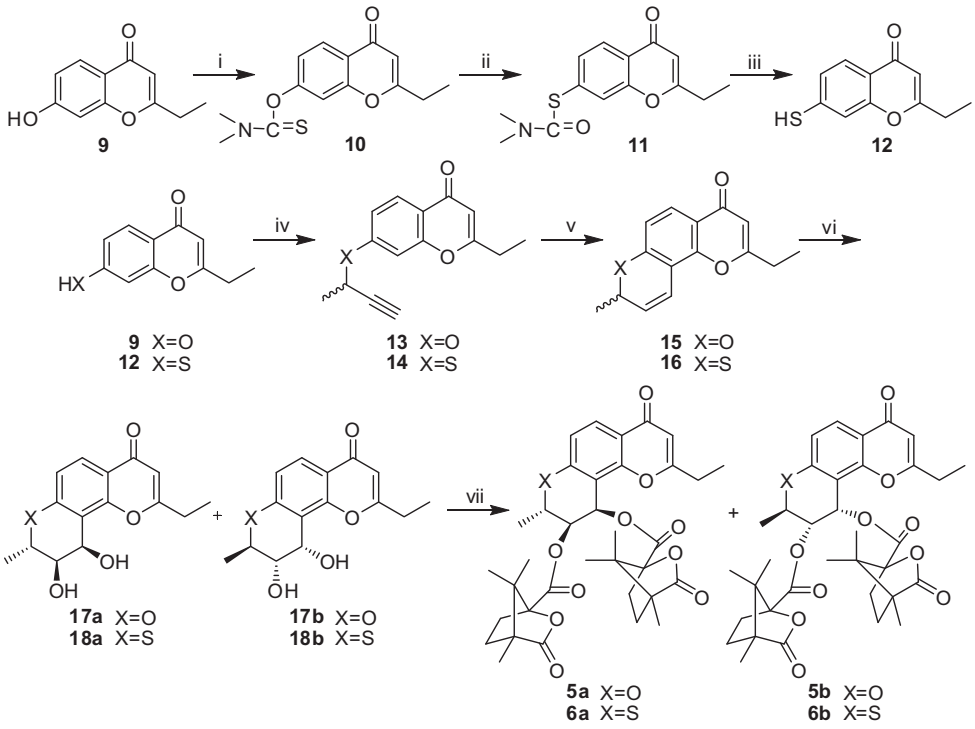
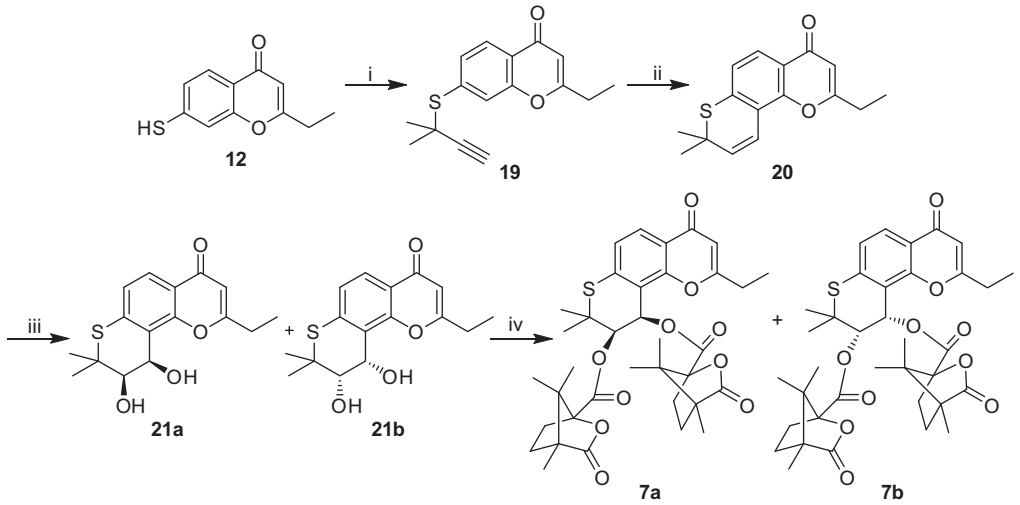


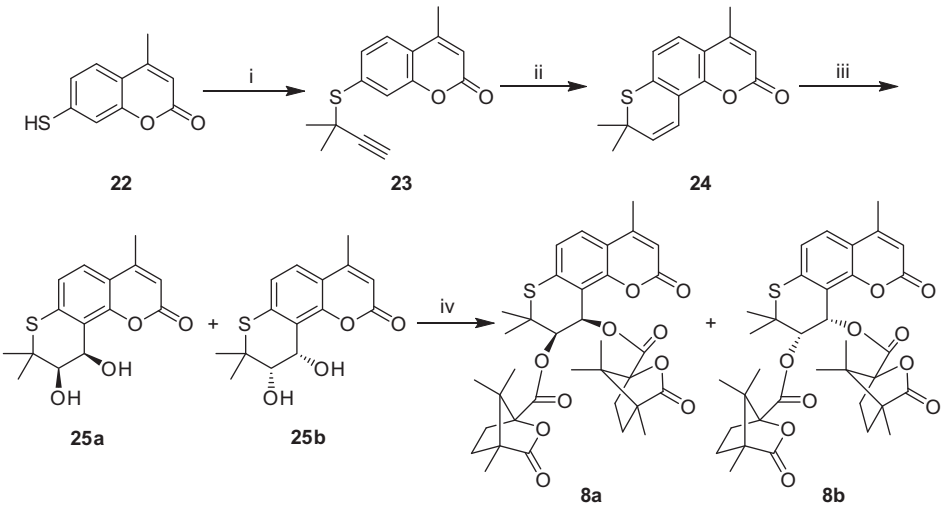
Scheme 1. Reagents and conditions: (i) Dimethylthiocarbamoyl chloride, EtOH, K_2CO_3, rt; (ii) 240 °C, N_2; (iii) KOH, CH_3OH, N_2, reflux; (iv) 3-chloro-1-butyne, K_2CO_3, KI in DMF or acetone, rt; (v) *N*,*N*-diethylaniline, reflux; (vi) $K_2OsO_2(OH)_4$, $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 in *t*-butanol/H_2O (v/v = 1:1), ice bath; (vii) (*S*)-camphanic chloride, DMAP in CH_2Cl_2, rt.

the presence of anhydrous potassium carbonate, followed by a rearrangement at 240 °C, then hydrolysis with methanolic KOH and acidification with HCl. Compounds **9** and **12** were treated with 3-chloro-1-butyne in dimethyl formamide (DMF) or acetone in the presence of anhydrous potassium carbonate and potassium iodide at room temperature to produce the propargyl ethers **13** and **14**, followed by thermal rearrangement in refluxing *N*,*N*-diethylaniline to form intermediates **15** and **16**. Sharpless dihydroxylation (AD) of **15** and **16** afforded dihydroxy derivatives **17a**/**17b** and **18a**/**18b**, respectively, as diastereoisomeric mixtures. Target compounds **5a** and **5b** were obtained by acylation of **17a** and **17b** with (*S*)-(−)-camphanic chloride in CH_2Cl_2 at room temperature with 4-dimethylaminopyridine (DMAP) as acid scavenger. Compounds **6a** and **6b** were synthesized by the same procedure from **18a** and **18b**. The pure diastereoisomers **5a**, **5b**, **6a**, and **6b** were obtained by separation with column chromatography on silica gel [petroleum ether/ethyl acetate, 3:1 (v/v)].

The preparation of **7a** and **7b** is illustrated in Scheme 2. 2-Ethyl-7-mercapto-4*H*-chromen-4-one (**12**) was treated with 3-chloro-3-methyl-1-butyne in EtOH/H_2O (v/v = 1:1) in the presence of potassium hydroxide at room temperature to produce the propargyl ether **19**, followed by thermal rearrangement in refluxing *N*,*N*-diethylaniline to form intermediate **20**. Sharpless AD of **20** afforded dihydroxy derivatives **21a** and **21b**. Target compounds



Scheme 2. Reagents and conditions: (i) 3-Chloro-3-methyl-1-butyne, KOH, N_2, EtOH/H_2O (v/v = 1:1), rt; (ii) *N*,*N*-diethylaniline, reflux; (iii) $K_2OsO_2(OH)_4$, $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 in *t*-butanol/H_2O (v/v = 1:1), ice bath; (iv) (*S*)-camphanic chloride, DMAP in CH_2Cl_2, rt.



Scheme 3. Reagents and conditions: (i) 3-Chloro-3-methyl-1-butyne, KOH in EtOH, N_2; (ii) *N*,*N*-diethylaniline, reflux; (iii) $K_2OsO_2(OH)_4$, $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 in *t*-butanol/H_2O (v/v = 1:1), ice bath; (iv) (*S*)-camphanic chloride, DMAP in CH_2Cl_2.

7a and **7b** were obtained by acylation of **21a** and **21b** with (*S*)-(−)-camphanic chloride in CH_2Cl_2 at room temperature with DMAP as an acid scavenger. Diastereoisomers **7a** and **7b** could be separated by column chromatography on silica gel [petroleum ether/ethyl acetate, 3:1 (v/v)].

The synthesis of **8a** and **8b** was accomplished by a similar four-step sequence, as depicted in Scheme 3. Diastereoisomers **8a** and **8b** were separated by HPLC on an Alltima column (2.1 mm × 150 mm, C-18) with acetonitrile/water 70:30 (v/v) as eluant.

The eight newly synthesized compounds **5**–**8**[12] were evaluated for anti-HIV activity in TZM-bl cells in parallel with 2-ethyl-DCP.[13] The bioassay data are summarized in Table 1. Compounds **5a**, **6a**, and **7a** showed significant anti-HIV activity with EC_{50} values of 30, 38 and 54 nM, which were better than the reference compound (2-ethyl-DCP, EC_{50}: 120 nM), and had good therapeutic index (TI) values of 152.6, 48.0 and 100.0, respectively. With a two-fold lower EC_{50} value, 2-ethyl-1′-thia-DCP (**7a**) was more potent than 4-methyl-1′-thia-DCK (**8a**). This result was coincident with the previous activity comparison between the DCP and DCK series, for example, 2-ethyl DCP was more active than 4-methyl DCK.[8] 2′-Monomethyl-2-ethyl-1′-oxa- (**5a**) and -1′-thia-DCP derivatives (**6a**) exhibited better anti-HIV activity than the corresponding 2′-*gem*-dimethyl substituted compounds 2-ethyl-DCP and **7a**. Interestingly, **5b**, **6b**, **7b** and **8b** exhibited remarkably reduced or even completely abolished anti-HIV activity, consistent with the results from prior compounds. This finding suggested that, just as in the DCK series, the spatial orientations of the 2′-methyl group and the 3′,4′-dicamphanoyls are also crucial to anti-HIV activity in DCP analogues.

Table 1
Anti-HIV-1 $_{NL4-3}$ data of analogues **5**–**8** in TZM-bl cells [a]

Compound	CC_{50} (μM)	EC_{50} (μM)	TI
5a	4.55	0.030	153
5b	—	—	NS
6a	1.84	0.038	48.0
6b	3.83	0.184	20.8
7a	5.4	0.054	100
7b	—	—	NS
8a	>30.6	0.128	>238
8b	>30.6	8.59	>3.6
2-Ethyl-DCP	14.3	0.12	119

[a] All data presented in this table were averaged from at least three independent experiments. EC_{50}: concentration that inhibits NL4-3 replication by 50%. CC_{50}: concentration that inhibits uninfected TZM-bl cell growth by 50%. TI = CC_{50}/EC_{50}. NS: there was no inhibition at concentrations below the CC_{50}.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.105.

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12. Analytical data of target compounds **5**–**8**: Configuration assignments of isomeric compound pairs were based on prior data in Ref.[9].
Compound **5a**: Mp 138–141 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.95–1.12 (18H, m, 6 × –CH_3 in camphanoyl), 1.67–2.50 (8H, m, 4 × –CH_2 in camphanoyl), 1.24 (3H, t, –CH_3 in ethyl), 1.48 (3H, d, *J* = 6.3 Hz, 2′-CH_3), 2.56 (2H, m, –CH_2 in ethyl), 4.55 (1H, m, 2′-CH), 5.17 (1H, m, 3′-H), 6.14 (1H, s, 3-H), 6.80 (1H, d, *J* = 3.1 Hz, 4′-H), 6.93 (1H, d, *J* = 9.0 Hz, 6-H), 8.12 (1H, d, *J* = 9.0 Hz, 5-H). $[\alpha]_D$ −37 (*c* 0.1, $CHCl_3$). HRMS(MALDI-DHB): calcd for $C_{35}H_{40}O_{11}$: 636.2571; found: 637.2643 [M+H$^+$]. Compound **5b**: Mp 203–206 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, s, –CH_3 in camphanoyl), 1.02–1.13 (15H, m, –CH_3 × 5 in camphanoyl), 1.68–2.41 (8H, m, 4 × –CH_2 in camphanoyl), 1.29 (3H, t, –CH_3

in ethyl), 1.47 (3H, d, J = 6.3 Hz, 2′-CH_3), 2.47 (2H, m, –CH_2 in ethyl), 4.66 (1H, m, 2′-CH), 5.29 (1H, m, 3′-H), 6.14 (1H, s, 3-H), 6.81 (1H, d, J = 3.5 Hz, 4′-H), 6.92 (1H, d, J = 8.6 Hz, 6-H), 8.11 (1H, d, J = 9.0 Hz, 5-H). $[\alpha]_D$ +81 (c 0.1, $CHCl_3$). HRMS (MALDI-DHB): calcd for $C_{35}H_{40}O_{11}$: 636.2571; found: 637.2643 [M+H$^+$]. Compound **6a**: Mp 175–178 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98–1.13 (18H, m, 6 × –CH_3 in camphanoyl), 1.68–2.59 (8H, m, 4 × –CH_2 in camphanoyl), 1.25 (3H, t, –CH_3 in ethyl), 1.37 (3H, d, J = 6.7 Hz, 2′-CH_3), 2.47 (2H, m, –CH_2 in ethyl), 3.87 (1H, m, 2′-CH), 5.32 (1H, m, 3′-H), 6.15 (1H, s, 3-H), 6.97 (1H, d, J = 2.7 Hz, 4′-H), 7.12 (1H, d, J = 8.6 Hz, 6-H), 8.05 (1H, d, J = 8.6 Hz, 5-H). $[\alpha]_D$ −272 (c 0.1, $CHCl_3$). HRMS (MALDI-DHB): calcd for $C_{35}H_{40}O_{10}S$: 675.2240 [M+Na$^+$]; found: 675.2234 [M+Na$^+$]. Compound **6b**: mp 152–155 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) 0.82–1.13 (18H, m, –CH_3 × 6 in camphanoyl), 1.68–2.66 (8H, m, 4 × –CH_2 in camphanoyl), 1.29 (3H, t, –CH_3 in ethyl), 1.38 (3H, d, J = 6.7 Hz, 2′-CH_3), 2.44 (2H, m, –CH_2 in ethyl), 3.99 (1H, m, 2′-CH), 5.44 (1H, m, 3′-H), 6.16 (1H, s, 3-H), 6.97 (1H, d, J = 2.4 Hz, 4′-H), 7.12 (1H, d, J = 8.7 Hz, 6-H), 8.05 (1H, d, J = 8.3 Hz, 5-H). $[\alpha]_D$ +135 (c 0.1, $CHCl_3$). HRMS (MALDI-DHB): calcd for $C_{35}H_{40}O_{10}S$: 675.2240 [M+Na$^+$]; found: 675.2234 [M+Na$^+$]. Compound **7a**: mp 249–252 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96–1.76 (24H, 6 × –CH_3 in camphanoyl, 2 × 2′-CH_3), 1.25 (3H, t, –CH_3 in ethyl), 1.70–2.60 (10H, m, –CH_2 in ethyl, 4 × –CH_2 in camphanoyl), 5.62 (1H, d, J = 4.3 Hz, 3′-CH), 6.16 (1H, s, 3-H), 6.96 (1H, d, J = 4.3 Hz, 4′-H), 7.11 (1H, d, J = 8.2 Hz, 6-H), 8.06 (1H, d, J = 8.6 Hz, 5-H). $[\alpha]_D$ −130 (c 0.1, $CHCl_3$). HRMS (MALDI-DHB): calcd for $C_{36}H_{42}O_{10}S$: 689.2396 [M+Na$^+$]; found: 689.2391 [M+Na$^+$]. Compound **7b**: mp 188–189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88–1.76 (24H, 6 × –CH_3 in camphanoyl, 2 × 2′-CH_3), 1.26 (3H, t, –CH_3 in ethyl), 1.60–2.66 (10H, m, –CH_2 in ethyl, 4 × –CH_2 in camphanoyl), 5.71 (1H, d, J = 4.3 Hz, 3′-CH), 6.15 (1H, s, 3-H), 6.92 (1H, d, J = 4.3 Hz, 4′-H), 7.10 (1H, d, J = 8.6 Hz, 6-H), 8.04 (1H, d, J = 8.2 Hz, 5-H). $[\alpha]_D$ +27 (c 0.1, $CHCl_3$). HRMS (MALDI-DHB): calcd for $C_{36}H_{42}O_{10}S$: 689.2396 [M+Na$^+$]; found: 689.2391 [M+Na$^+$]. Compound **8a**: mp 135–137 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.11–1.13 (18H, 6 × –CH_3 in camphanoyl), 1.56–2.53 (8H, m, 4 × –CH_2 in camphanoyl), 1.38 (3H, s, 2′-CH_3), 1.66 (3H, s, 2′-CH_3), 2.40 (3H, s, 4-CH_3), 5.63 (1H, d, J = 4.5 Hz, 3′-H), 6.18 (1H, d, J = 0.9 Hz, 3-H), 6.76 (1H, d, J = 4.5 Hz, 4′-H), 7.04 (1H, d, J = 8.7 Hz, 6-H), 7.48 (1H, d, J = 8.4 Hz, 5-H). HRMS (MALDI-DHB) calcd mass for $C_{35}H_{40}O_{10}S$ [M$^+$−H] 651.2269, found 651.2270. Compound **8b**: mp 151–153 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87–1.15 (18H, 6 × –CH_3 in camphanoyl), 1.64–2.64 (8H, m, 4 × –CH_2 in camphanoyl), 1.38 (3H, s, 2′-CH_3), 1.73 (3H, s, 2′-CH_3), 2.40 (3H, d, J = 1.5 Hz, 4-CH_3), 5.69 (1H, d, J = 4.5 Hz, 3′-CH), 6.17 (1H, d, J = 1.5 Hz, 3-H), 6.92 (1H, d, J = 4.5 Hz, 4′-H), 7.04 (1H, d, J = 8.1 Hz, 6-H), 7.48 (1H, d, J = 8.4 Hz, 5-H). HRMS (MALDI-DHB) calcd mass for $C_{35}H_{40}O_{10}S$ [M$^+$−H] 651.2269, found 651.2273.

13. *HIV-1 infectivity assay*: Anti-HIV-1 activity was measured as reductions in Luc reporter gene expression after a single round of virus infection of TZM-bl cells. HIV-1 at 200 $TCID_{50}$ and various dilutions of test samples (eight dilutions, four-fold stepwise) were mixed in a total volume of 100 μL growth medium in 96-well black solid plates (Corning-Costar). After 48-h incubation, culture medium was removed from each well and 100 μL of Bright Glo luciferase reagent was added to each culture well. The luciferase activity in the assay wells was measured using a Victor 2 luminometer. The 50% inhibitory dose (EC_{50}) was defined as the sample concentration that caused a 50% reduction in Relative Luminescence Units (RLU) compared to virus control wells after subtraction of background RLU.