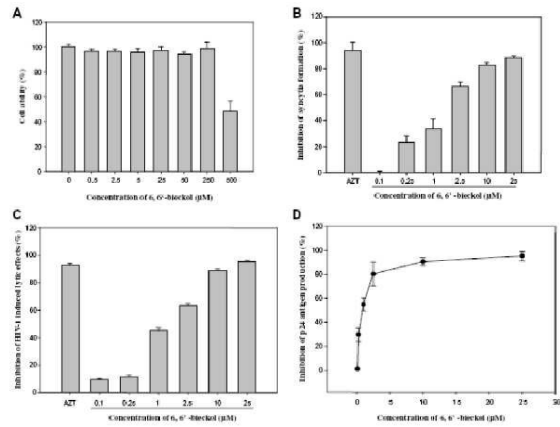


	(19) (12)	(KR) (A)	(11) (43)	10-2010-0039103 2010 04 15
(51)	Int. Cl.		(71)	
	<i>A61K 36/03</i> (2006.01) <i>A61P 31/18</i> (2006.01)			100
(21)	10-2008-0098327		(72)	
(22)	2008 10 07			
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			207 2401 ()	
			(74)	
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(54)	-1
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(57)

6,6'-
6,6'-
HIV-1
HIV-1
p24
HIV-1
HIV-1
RT
HIV-1
HIV-1
1



(72)

3 599-1

125-1402

2

3 599-1

1

HIV-1

2

1

6, 6' -

•

[0001] -HIV-1

HIV-1

6, 6' -

[0002] -1(Human Immunodeficiency virus type-1; HIV-1)

3

3		(AIDS)	
2007	210	AIDS	250
UNAIDS	6,800	HIV	5,700

[0003]	1980	AIDS	, 1	-HIV	AIDS	.
	-		HIV	50%	-AIDS	.

HIV

[0004]

[0005] , -HIV
 , -HIV

. HIV

HIV

70%

-HIV

-N(cyanovirin-N)

gp120

11-kDa
HIV

(viral entry stage)

HIV

-HIV

[0006] , HIV-1 RT
HIV-1 , MT-4 HIV-1
 , 1.9 μM IC₅₀ HIV-1 RT
1,3,4- -0- 3,5- -0-
-
-MMP (Ahn
et al.) 8,8'- 8',4'''- in vitro HIV-1 RT
[0007] (*Ecklonia cava*)
(30,000 /),
[0008] (10-
2000-0048933), (10-2001-0025580),
(10-2006-0093939)
[0009] 10-2002-
83555 " " 10%
15 10-2004-3912 "
" , , ,
[0010] 10-0518179 " " ACE(ANGIOTENSIN CONVERTING ENZYME)
(EC) NMR
-HIV-1 6,6'-
[0012] HIV-1
[0013] HIV-1 (syncytia formation), (lytic effect) p24 6,6'-
HIV-1 (cytopathic effect) , RT
HIV-1 , 6,6'- HIV-1

- [0014] HIV-1
- [0015] 6,6'-
- [0016] , " " ()
- [0017] 6,6'- 500 μ M , CC₅₀ 484 μ M
 (1A). C8166 CEM-SS
 HIV-1 . 6,6'- HIV-1 (syncytia formation)
 , HIV-1
 (syncytia formation) 6,6'- EC₅₀ 1.72 μ M (1B). 6,6'-
 (25 μ M), 88% . 6,6'- in vitro HIV-1
 6,6'- (25 μ M), 96% (1C). 6,6'-
 (therapeutic index; TI) 393 . HIV-1
 p24 . HIV-1 p24 6,6'- EC₅₀ 1.26 μ M
 M (1D). HIV-1 p24 6,6'-
 (2A). HIV-1 RT 6,6'-
 (1 μ M 50.46%). 6,6'- 10 μ M 96.33%
 HIV-1 RT (1C).
- [0018] HIV-1IIIB C8166 H9 - , 6,6'-
 HIV-1 . 6,6'- - (1B) - (3A)
 . - 6,6'- , 80% .
- [0019] , 6,6'- -HIV-1 . 6,6'- CEM-22 C8166
 HIV-1 in vitro HIV-1 .
 RT RNA HIV-1 .
- [0020] 1B 3B , HIV-1 RT HIV-1
 HIV-1 RT . 6,6'- HIV-1 HIV-1
 . 6,6'- HIV-1
- [0021] HIV-1 6,6'- . C8166 H9/HIV-1IIIB
 6,6'- HIV-1 gp41 6-
 (gp41 six-helix bundle formation) HIV-1
 . 6,6'- , HIV-1
 , HIV-1
- [0022] p24 p24 ELISA (p24 antigen capture ELISA and
 Western blot analysis) . ELISA
 (1D), 6,6'- 10 μ M p24 90% . 2C , 25
 μ M 6,6'- p24 AZT(5 μ M) . 8,8'- 6,6'-
 , HIV-1 RT 0.51 μ M 81.5 μ M IC₅₀
 . 6,6'- 8,8'- .
- [0023] , p24 p55 gag (p55, p41 p24)
 , 6,6'- . 6,6'-
 (2A 2B). , - 6,6'- ,
 p24

- [0024] , 6,6'- HIV-1
6,6'- HIV-1 RT
- [0025] HIV-1 6,6'- HIV-1 , p24
HIV-1 , RT HIV-1 , HIV-1
- [0026]
- [0027]
- [0028] ^1H NMR (400 MHz) ^{13}C NMR (100 MHz) (internal reference standard)
DMSO- d_6 (^1H NMR d 2.50 ppm ^{13}C NMR d 39.5 ppm) JEOL JNM-ECP 400 NMR
(JEOL, Japan)
- [0029] MS JEOL JMS-700 (JEOL, Japan) EC (Extraction
Unit; Dongwon Scientific Co., Korea) silica gel 60 (230-
400 mesh, Merck, Germany), (Sephadex) LH-20 (Sigma, St. Louis, MO, USA) TLC
(precoated Merck Kieselgel) 60 F254 (0.25 mm) , TLC
CHCl₃/MeOH/H₂O/ (65:25:4:3, v/v) UV (254 365
nm) , -I H₂SO₄
- [0030] AZT, (Mw 8000 Da) (Sigma Chemical Co.; St.
Louis, MO, USA) 1 2 (SantaCruz
Biotechnology; CA, USA) HIV-1 p24 ELISA -
(Perkin-Elmer Life Sciences; Boston, MA, USA)
(InvitroGen; CA, USA) (RPMI 1640), / ,
(FBS), BRL (Gibco BRL, Life Technology; NY, USA)
(Sigma Chemical Co.; St. Louis, MO, USA)
- [0031] H9 H9/HIV-1IIIB ATCC(American Type of Culture Collection; Manassas, VA, USA)
(Dr. P. Nara) CEM-SS (Dr. G. Farrar) C8166 EU
Programme EVA Centre for AIDS Reagents, NIBSC, UK CEM-SS, C8166, H9 H9/HIV-1IIIB
10% FBS, mL 100 µg mL 100U RPMI 1640
H9/HIV-1IIIB HIV-1IIIB
0.22 ?m
80

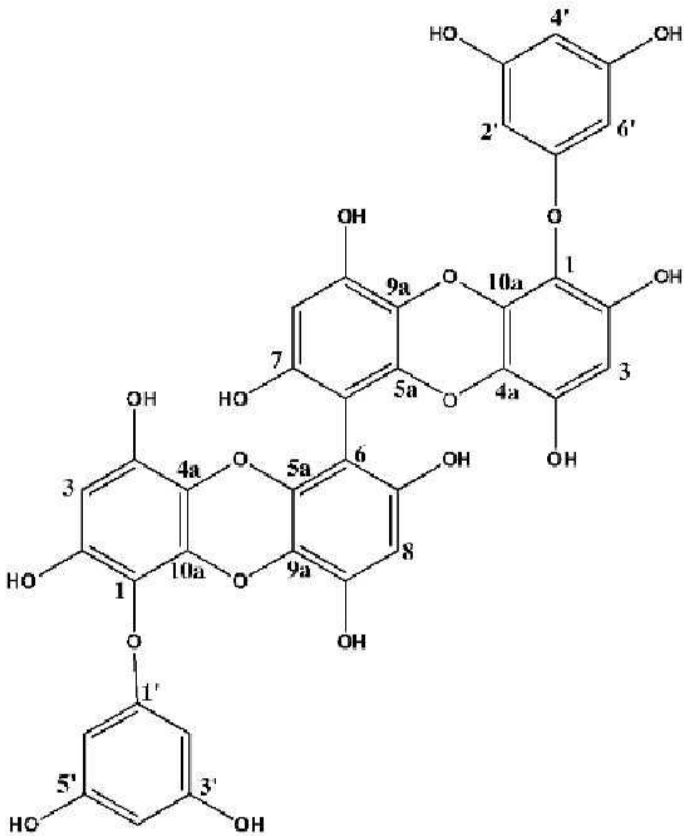
[0032] 3 , ± .

[0033] 1 : (Ecklonia cava; EC)

[0034] EC 2004 10 2005 3 .
EC 3 EC EC
(10 kg) 10 MeOH (35 L) (273 g)
, n- (35.92 g), CH₂Cl₂ (20.49 g), EtOAc (24.87 g) n-BuOH H9
H9/HIV-1IIIB -HIV EtOAc (24.87 g)
/EtOAc/MeOH F1 F10 10

[0035] -HIV F5 (378.39 mg) MeOH LH-20
1 (102.85 mg)

[0036] < 1>



[0037]

[0038] 1 LREIMS, ¹H, ¹³C, ¹³C DEPT C₃₆H₂₂O₁₈ 1
¹H NMR d 5.75 (2H, J = 2.2 Hz), 5.80 (1H, J = 2.2 Hz) AB₂ d 6.05
(2H, s) 6.09 (2H, s) d 9.29 (2H, s), 9.16 (4H, s), 9.15 (2H,
s), 9.09 (2H, s), 8.65 (2H, s) 12 OH .

[0039] ¹³C NMR 10 24 0- 2 4
1
(742 vs 372). 1 6

6,6'- 8,8'- C-8 13C 6.4 ppm

[0040] 1 : () ; ¹H NMR (DMSO-d₆, 400 MHz) δ 9.29 (1H, s, OH-9), 9.16 (2H, s, OH-30, 50), 9.15 (1H, s, OH-2), 9.09 (1H, s, OH-4), 8.65 (1H, s, OH-7), 6.09 (1H, s, H-3), 6.05 (1H, s, H-8), 5.80 (1H, d, J = 2.2 Hz, H-40), 5.75 (2H, d, J = 2.2 Hz, H-20, 60); ¹³C NMR (DMSO-d₆, 100 MHz) δ 123.5 (s, C-1), 145.4 (s, C-2), 97.7 (d, C-3), 141.4 (s, C-4), 121.9 (s, C-4a), 141.3 (s, C-5a), 99.7 (d, C-6), 151.3 (s, C-7), 97.8 (d, C-8), 144.5 (s, C-9), 122.7 (s, C-9a), 137.2 (s, C-10a), 160.4 (s, C-10), 93.7 (d, C-20), 158.8 (s, C-30), 96.1 (d, C-40), 158.8 (s, C-50), 93.7 (d, C-60); LREIMS m/z 742.10 [M]⁺.

[0041] 2 :

[0042] 3-(4,5- 6,6'- 48- -2-)-2,5- (MTT) MTT
CEM-SS H9 400 μL 105 cell/mL
500 μg/mL MTT 100 μL
540 nm
100%

[0043] 6,6'- 500 μM , CC₅₀ 484 μM (1A).

[0044] 3 : -HIV

[0045] 3-1 :

[0046] 300 μL 1X10⁵ C8166 100 μL 48-
200 CCID₅₀ HIV-1IIIIB
100 μL 37 72

[0047] C8166 CEM-SS HIV-1 (syncytia formation)
6,6'- HIV-1 (syncytia formation)
6,6'- EC₅₀ 1.72 μM (1B). 6,6'- (25 μM), 88%

[0048] 3-2 : HIV-1

[0049] CEM-SS 6,6'- -HIV-1 , MTT-
(log-growth phase) , 100 μL
48- 1X10⁵ 300 μL 3 HIV-
1IIIIB (7 90%) , 100
μL 37 7 , 7 MTT

[0050] 6,6'- in vitro HIV-1 6,6'-
(25 μM), 96% (1C). 6,6'- (therapeutic

index; TI) 393 . HIV-1 p24 . HIV-1
p24 6,6'- EC₅₀ 1.26 μ M (1D).

[0051] 3-3 :

[0052] 1X10⁶ cells/mL H9 . , 2
 , 200 CCID₅₀ HIV-1IIIIB . 37 96 , 10 1000 rpm
 , PBS 3 , 50 mM Tris-HCl (pH 7.5), 0.4%
Nonidet P-40, 120 mM NaCl, 1.5 mM MgCl₂, 2 mM , 80 μ g/mL , 3 mM NaF,
1 mM DTT 500 μ L . 100 μ g
(immunoblot analysis) .

[0053] 0.22 μ M , 30% (PEG) (50% v/v)
0.4 M NaCl , 15000 rpm 45 .
 , SDS , (20 μ L) SDS .
25 mM Tris, 192 mM , 4% 0.1% SDS 10% SDS-AGE .
 , 0.1% (v/v) Tween 20 (TBS-T) 5%
Tris . -p24
(horseradish peroxidase; HRP)- - IgG 2 (1:5000, SantaCruz)
 . (chemiluminescence; Fujifilm Life Science, Tokyo, Japan) .

[0054] HIV-1 p24 6,6'-
(2A). HIV-1 RT 6,6'-
(1 μ M 50.46%). 6,6'- 10 μ M 96.33% HIV-1 RT
(1C).

[0055] 3-4 :

[0056] HIV-1 RT (fluorescence RT assay kit; InvitroGe
n) . , / ,
poly(A)/d(T)16, dTTP 20 μ L
(microtiter plate) , 6,6'- . 37
1 , 200 mM EDTA 2 μ L . - (single-
stranded) sDNA DNA-RNA (heteroduplexes)
(PicoGreenreagent) 173 μ L ,
(GENiosmicroplate reader; Tecan Austria GmbH, Austria) 480 nm () 520 nm ()
 .

[0057] , 6,6'- -HIV-1 . 6,6'- CEM-22 C8166
HIV-1 in vitro HIV-1 .
RT RNA HIV-1 .
 . (Kilkuskie et al.)
 , 1 .
HIV RT , , ,
HIV .

[0058] 1B 3B , HIV-1 RT HIV-1
HIV-1 RT 6,6'- HIV-1
HIV-1 RT 6,6'- HIV-1

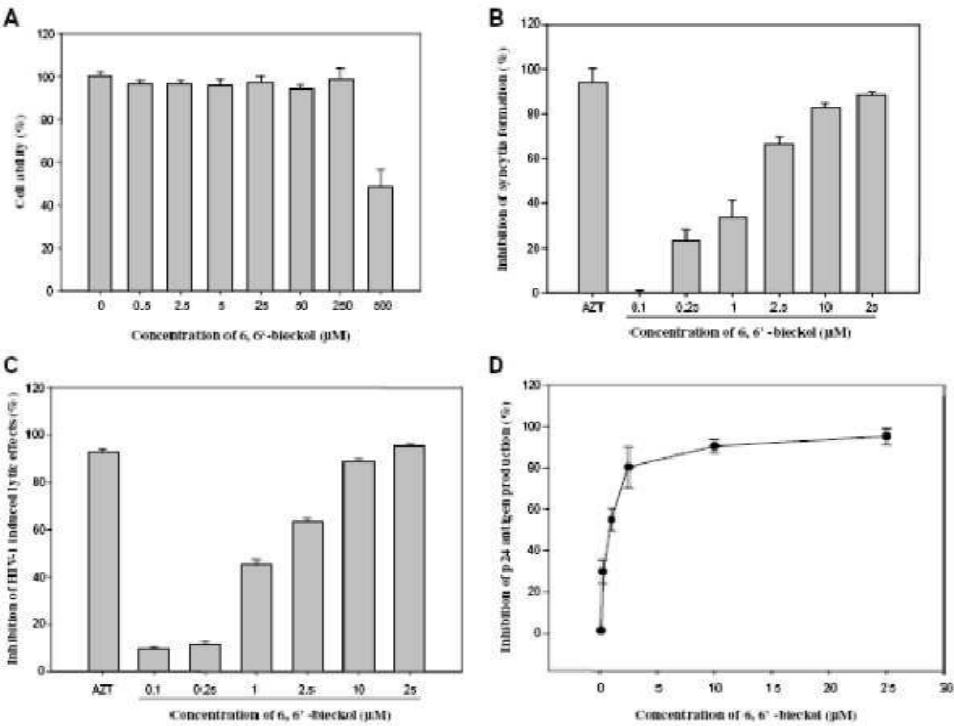
- [0059] HIV-1 6,6'- (Nonaka et al.) HIV-1 C8166 H9/HIV-1IIIB gp41 6- (gp41 six-helix bundle formation) HIV-1 HIV-1 , HIV-1 , HIV-1 .
- [0060] **3-5 : p24 ELISA**
- [0061] H9 (3X10⁶ cells/ml) 37 1 HIV-1IIIB 3X10⁵ cells/mL 1 mL 6,6'- 24- AZT (Perkin-Elmer Life Sciences, Boston, MA) HIV-1 p24 ELISA
- [0062] p24 p24 ELISA (p24 antigen capture ELISA and Western blot analysis) ELISA (1D), 6,6'- 10 μM p24 90% 2C , 25 μM 6,6'- p24 AZT(5 μM) 8,8'- 6,6'- , HIV-1 RT 0.51 μM 81.5 μM IC₅₀ 6,6'- 8,8'-
- [0063] , p24 p55 gag (p55, p41 p24) 6,6'- p24 (2A 2B). , - 6,6'- ,
- [0064] **3-6 : -**
- [0065] 3X10⁴ C8166 6,6'- 2 , 5% CO₂ 37 HIV-1IIIB 3X10³ H9 - 72 ,
- [0066] HIV C8166 H9 - , 6,6'- HIV-1IIIB HIV-1 (1B) - (3A) - 6,6'- 80% ,
- [0067] 1 6,6' - HIV-1 ± SE(n=3) (A) MTT CEM-SS 6,6' - , (B) HIV-1 (C) MTT HIV-1 CEM-SS (D) ELISA p24
- [0068] 2 6,6' - HIV-1 p24 H9 HIV-1IIIB , 0.1, 0.25, 1.25, 10 25 μM 6,6' - AZT(5 μM) 4 (A) (B) .

± SE(n=3) , - (C) p24 % .

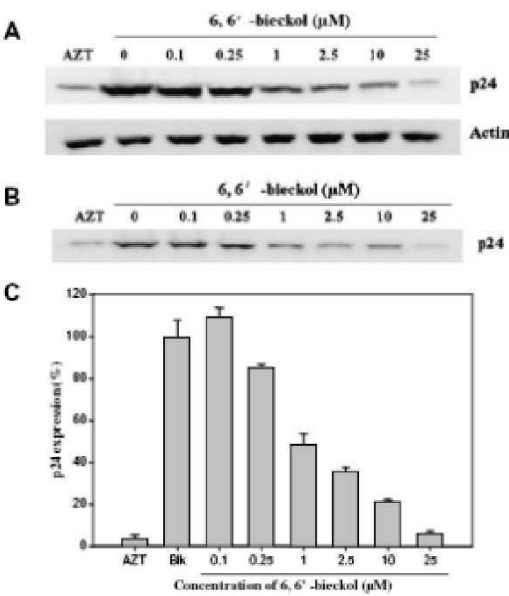
3 6,6'-HIV-1 HIV- RT . ± SE(n=3)

AZT(5 μM) (100 μg/mL) . (A) HIV-1 C8166 H9/HIV-1_{IIIB} (B) 6,6'-HIV-1 RT .

1



2



3

