Pergamon

Tetrahedron Letters 43 (2002) 1617–1620

TETRAHEDRON LETTERS

Discovery, structure and HIV-1 integrase inhibitory activities of integracins, novel dimeric alkyl aromatics from *Cytonaema* sp.

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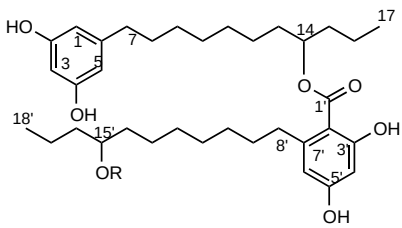
Received 5 December 2001; revised 6 January 2002; accepted 9 January 2002

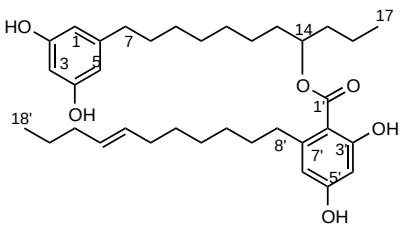
Abstract—Integrase is a critical viral enzyme for HIV-1 replication and is a novel target for therapeutic intervention against HIV infections. Integracins A–C are three novel dimeric alkyl aromatic inhibitors of HIV-1 integrase discovered from the screening of fungal extracts using an in vitro assay. These compounds inhibit both coupled and strand transfer activity of HIV-1 integrase with IC_{50} values of 3.2–6.1 and 17–88 μM, respectively. The discovery, structure and activity of these compounds are described. © 2002 Published by Elsevier Science Ltd.

HIV-1 causes AIDS and in the last decade, significant progress has been made in development of new drugs, which control the progression of HIV-1 infection. Specifically various protease and reverse transcriptase inhibitors are available, however, rapid viral mutations render many of these drugs ineffective and drugs with new mechanisms are needed to combat HIV-1 infections. Integration is an essential step in the life cycle of HIV-1 and is a three-step process that includes assembly of proviral DNA onto integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA into host cell DNA.[1] This is a distinctive process by which the virus proliferates and the entire reaction is catalyzed by a single viral enzyme, HIV-1 integrase. This enzyme is absent in the host and therefore presents a safe target for development of anti-HIV therapy either used alone or in combination with existing therapies. The recent discovery of diketo acid (DKA) based inhibitors helped validate this target for potential anti-HIV-1 therapy.[2] Unfortunately, the clinical outcome of a given class of compounds is uncertain and therefore new classes of inhibitors are needed to further elucidate and define this as a clinically effective target.

Natural product extracts have long been used for the discovery of novel leads for numerous biological targets. Screening of such extracts against recombinant HIV-1 integrase led to the discovery of several natural product inhibitors including equisetin,[3] integric acid[4] and complestatin.[5] Continued screening of fungal extracts led to the discovery of three compounds named herein integracins A (**1a**), B (**1b**), and C (**2**), novel dimeric alkyl aromatics of polyketide origin which inhibited recombinant HIV-1 integrase coupled reaction with an IC_{50} value of 3.2, 6.1, and 3.5μM, respectively. The isolation, structure, and the biological activities of integracins A–C are herein described.



1a: R = $COCH_3$ (Integracin A)
1b: R = H (Integracin B)



2: Integracin C

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0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00083-7

Isolation. The producing fungus *Cytonaema* sp. (MF6253, ATCC74413) was isolated from living twigs of *Quercus ilex* collected in El Pardo, province of Madrid, Spain and grown in a medium containing (g/L), sucrose (75), tomato paste (10), malt extract (5), soy flour (1), $(NH_4)_2SO_4$ (1), K_2HPO_4 (9) for 7 days at 25°C in a rotatory shaker (220 rpm). The broth was extracted with 1.2 volumes of methyl ethyl ketone and chromatographed on sephadex LH 20 in methanol to produce three zones of activities eluting between 1–2 column volumes consisting of the same three compounds, presumably present in different salt forms. The combined active fractions were pooled, cooled (0°C) and acidified (pH 2.0) with dil. HCl and was purified either by reversed phase HPLC (Zorbax RX C-8) or by silica gel chromatography to give integracins A[6] (260 mg/L), B (285 mg/L), and C (1.5 mg/L), all as gums.

Integracin A (1a): HREIMS analysis of integracin A afforded a molecular ion at m/z 628 that yielded a molecular formula $C_{37}H_{56}O_6$. The ^{13}C NMR spectrum of **1a** (Table 1) supported the molecular formula and revealed the presence of 35 signals for 37 carbons that included three methyls, 18 methylenes, two oxy-methines, four signals for five aromatic methines, three shielded aromatic quaternaries, three signals for four oxygen bearing deshielded aromatic quaternaries and two ester carbonyl carbons. The UV spectrum of **1a** showed absorption bands for benzoate type system and the IR spectrum showed the absorption bands characteristic of hydroxy (3352 cm^{-1}), two type of ester group (1737 and 1702 cm^{-1}) and aromatic (1604 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) of **1a** showed two methyl triplets [δ 0.87 ($J=6.8$ Hz) and δ 0.90 ($J=7.2$ Hz)] typical of ω methyl groups and an acetyl methyl singlet (δ 1.95), two oxy-methine protons, two *meta*-coupled aromatic protons located in a tetra-substituted phenyl ring, three aromatic protons present in 1,3,5-trisubstituted phenyl ring, chelated phenolic group (δ 11.76) and methylene protons. In addition to two sets

Table 1. 400 MHz NMR assignments of integracins A (**1a**), B (**1b**) and C (**2**) in CD_3CN

Position	**1a** δC	**1b** δC	**1a** δH, mult, J in Hz	**1b** δH, mult, J in Hz	**2**[b] δH, mult, J in Hz
1	108.0	108.0	6.12, d, 2.4	6.13, d, 2.4	5.98, d, 2.0
2	158.9	158.9	–	–	–
3	100.7	100.7	6.06, t, 2.4	6.05, t, 2.4	5.94, t, 2.4
4	158.9	158.9	–	–	–
5	108.0	108.0	6.12, d, 2.4	6.13, d, 2.4	5.98, d, 2.0
6	146.6	146.5	–	–	–
7	36.5	36.5	2.40, t, 8.0	2.40, t, 8.0	2.27, t, 8.0
8	32.0	32.0	1.50, m	1.50, m	1.37, m
9	30.0	30.0	1.22, m	1.22, m	1.14, m
10	[a]30.1	[a]30.1	1.22, m	1.22, m	1.14, m
11	[a]30.2	[a]30.3	1.22, m	1.22, m	1.14, m
12	26.3	26.3	1.22, m	1.22, m	1.14, m
13	35.0	35.0	1.65, m	1.65, m	1.50, m
14	76.8	76.8	5.21, pent, 6.0	5.21, pent, 6.4	5.07, m
15	37.2	37.2	1.62, m	1.62, m	1.50, m
16	19.7	19.7	1.30, m	1.30, m	1.14, m
17	14.3	14.4	0.87, t, 6.8	0.87, t, 6.8	0.78, t, 7.2
1′	172.5	172.5	–	–	–
2′	105.5	105.5	–	–	–
3′	166.3	166.3	–	–	–
4′	101.9	101.9	6.19, d, 2.4	6.19, d, 2.4	6.04, d, 2.4
5′	162.8	162.8	–	–	–
6′	111.8	111.8	6.23, d, 2.4	6.23, d, 2.4	6.04, d, 2.4
7′	148.7	149.7	–	–	–
8′	37.6	37.6	2.78, m	2.81, m	2.67, t, 8.8
9′	33.2	33.3	1.50, m	1.50, m	1.37, m
10′	30.7	30.8	1.22, m	1.22, m	1.14, m
11′	[a]30.4	[a]30.6	1.22, m	1.22, m	1.14, m
12′	[a]30.3	[a]30.6	1.22, m	1.22, m	1.14, m
13′	26.1	26.6	1.22, m	1.22, m	1.84, m
14′	35.0	35.0	1.45, m	1.45, m	5.28, dt, 15.2, 5.6
15′	74.6	71.8	4.82, pent, 6.4	3.49, m	5.22 dt, 15.2, 5.6
16′	37.2	38.3	1.45, m	1.45, m	1.84, m
17′	19.4	19.6	1.35, m	1.35, m	1.14, m
18′	14.3	14.6	0.90, t, 7.2	0.90, t, 7.2	0.80, t, 7.2
1″	171.5	–	–	–	–
2′	21.4	–	1.95, s	–	–
3″-OH	–	–	11.76, s	11.77, s	11.77, s
3 × OH	–	–	6.8, brs	7.08, brs	6.27, 7.14, brs

[a] Chemical shifts could be interchanged.
[b] Spectrum recorded in a mixture of CD_3CN+$CDCl_3$.

of *meta*-substituted aromatic protons, the COSY and TOCSY spectrum revealed the presence of two-spin systems each for the two-alkyl aromatics as shown in Fig. 1. It indicated that the oxygen substitutions were located at ω-3 carbons on both alkyl chains, which was confirmed by the corresponding HMBC correlations (Fig. 1). The HMBC experiment was critical in establishing the substitution pattern of both aromatic rings. For example, H-1 showed HMBC correlations to C-2, C-3, C-5, C-6 and C-7; H-3 to C-2, C-4, and C-5 thus establishing the 11-carbon alkyl chain at C-6 of the 3,5-dihydroxy phenyl ring. Similarly, the HMBC correlations of the two aromatic protons of the other phenyl ring established it to be 2,4-dihydroxy-5-alkyl-benzoate. The HMBC correlation of H-14 to the benzoate carbonyl (δ 166.3) confirmed the connectivity of the two units through benzoate ester at C-14. Accordingly, the corresponding HMBC correlations established the location of acetate group at C-15′. The EIMS fragmentation (Fig. 2) of **1a** corroborated these structural assignment. It showed a number of fragment ions with 14 or 28 Da apart due to successive cleavages of C–C methylene bonds of the chain that terminated at both end with fragment ion at m/z 124 for 3,5-dihydroxytoluene due to the cleavage of C7–C8 bond and at m/z 237 due to the cleavage of C14–C15. The molecule was split into two halves in the mass spectrum due to the cleavage of the benzoate ester bond producing fragment ions at m/z 280 and 349. The benzoate half loses the CO followed by acetic acid to afford a fragment ion at m/z 262 which is also obtained by the dehydration of the m/z 280 ion of the upper half, thus confirming the equal alkyl chain lengths in the two halves. This was further confirmed by formation of a single product **3**[7] upon basic (4N NaOH in MeOH–H_2O) hydrolysis of **1a** obtained after concomitant facile decarboxylation of

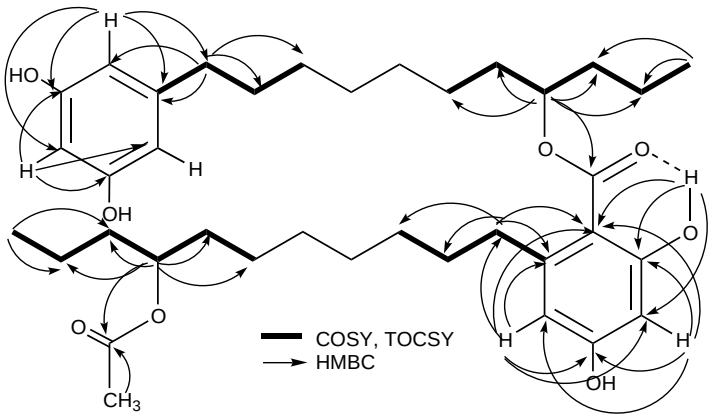


Figure 1. HMBC correlations of integracin A (**1a**).

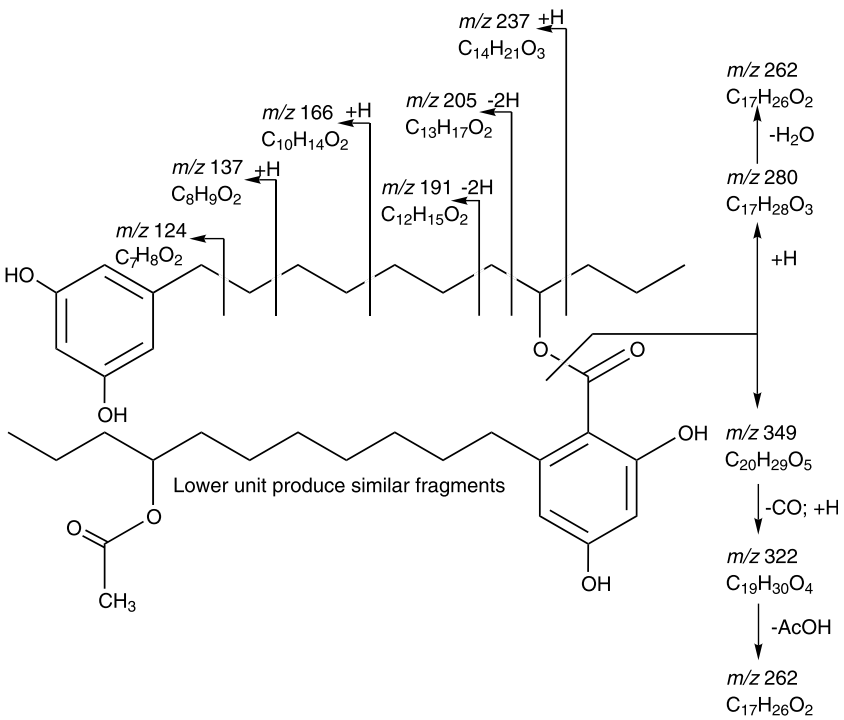
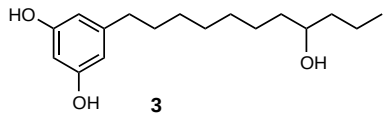


Figure 2. EIMS fragmentation of integracin A (**1a**).

ortho-hydroxy benzoic acid produced from the lower half of the molecule.



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Integracin B (1b) and C (2): HREIMS spectra of **1b** and **2** gave molecular ions at m/z 586.3867 and 568.3737 and affording formulae of $C_{35}H_{54}O_7$ and $C_{35}H_{52}O_6$, respectively. Comparison of these formulae with **1a** indicated that **1b** lacked the acetate group and **2** had lost a molecule of acetic acid that resulted in $\Delta^{14'}$ olefin. These structural assignments were confirmed by respective ^1H and ^{13}C NMR spectral analyses of these two compounds (Table 1). In addition, mild acid (dil. HCl) hydrolysis of **1a** produced exclusively **1b**.

Biological activity: All compounds reported in this paper were evaluated in HIV-1 integrase coupled and strand transfer assays.[2] Integracins A, B and C inhibited coupled reaction with IC_{50} values of 3.2, 6.1 and 3.5 μM, respectively. Like many other phenolics, these compounds were 10–30 fold less active in strand transfer assay and exhibited IC_{50} values of 32, 17 and 88 μM, respectively. The monomeric compound **3** was completely inactive in both assays at 100 μM.

In conclusion, we described in this paper the discovery, structure and HIV-1 integrase activities of three novel dimeric alkyl aromatic compounds integracins A–C isolated from *Cytonaema* sp.

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6. Compound **1a**: $[\alpha]_D^{22}=-1$ ($c=0.6$, MeOH); UV (MeOH) λ_{max}: 208 ($\varepsilon=42260$), 220 (sh), 265 (15330), 301 (6908) nm; IR (ZnSe) ν_{max}: 3352, 2930, 2856, 1737, 1702, 1642, 1604, 1451, 1381, 1310, 1257, 1202, 1157, 1102, 1067, 1023, 998, 928, 838, 763, 738, 713, 696 cm^{-1}; HREIMS (m/z): 628.3996 (M^+, calcd for $C_{37}H_{56}O_8$: 628.3974). Compound **1b**: $[\alpha]_D^{22}=-5.3$ ($c=1$, MeOH); UV (MeOH) λ_{max}: 225 ($\varepsilon=16924$), 265 (13483), 302 (5684) nm; IR (ZnSe) ν_{max}: 3333, 2930, 2856, 1702, 1604 (br), 1456, 1311, 1259, 1205, 1158, 1101, 998, 928, 839, 737, 696 cm^{-1}; HREIMS (m/z): 586.3867 (M^+, calcd for $C_{35}H_{54}O_7$: 586.3869).
7. Gum, $[\alpha]_D^{22}=-2.9$ ($c=0.28$, MeOH); HREIMS (m/z): 280.2035 (M^+, calcd for $C_{17}H_{28}O_3$: 280.2038).