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Antitrypanosomal pyridoacridine alkaloids from the Australian ascidian *Polysyncraton echinatum*

Yunjiang Feng, Rohan A. Davis, Melissa L. Sykes, Vicky M. Avery, Anthony R. Carroll, David Camp, Ronald J. Quinn *

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia

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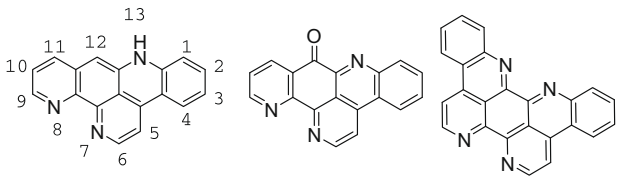
ABSTRACT

Bioassay-guided fractionation of the crude CH_2Cl_2/MeOH extract from the Australian ascidian *Polysyncraton echinatum* led to the isolation of a new pyridoacridine alkaloid, 12-deoxyascididemin (**1**), along with two known analogues, ascididemin (**2**) and eilatin (**3**). The structure of **1** was determined following extensive analysis of 1D/2D NMR and MS data. Biological evaluation showed that compounds **1–3** are potent antitrypanosomal agents with IC_{50} values of 0.077, 0.032, and 1.33 μM against *Trypanosoma brucei brucei*, respectively.

Human African Trypanosomiasis (HAT), also known as African Sleeping Sickness, is a fatal disease caused by two sub-species of a protozoan parasite, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. According to the latest statistics from the World Health Organization (WHO), African Sleeping Sickness threatens more than 60 million people in resource poor regions of Africa, and is the third most devastating parasitic disease.[1] Since the disease predominantly afflicts the very poor, it is designated as a neglected tropical disease. All the four main drugs (suramin, pentamidine, melarsorprol, and eflornithine) currently used to combat this disease have undesirable side-effects and new strains of *T. b. gambiense* are showing resistance to some of these agents.[2,3] There is an urgent need for the development of new, safer, and more effective drugs to fight African Sleeping Sickness. Historically, natural product research has not played a pivotal role in the search for antitrypanosomal therapeutics, however, in recent years a number of compounds from plants and marine organisms have emerged, which demonstrate promising activity against trypanosomes.[4–6]

In our ongoing search for bioactive natural products for neglected diseases,[7–9] a drug discovery program was established between Eskitis Institute and Drugs for Neglected Diseases Initiative (DNDi) in an attempt to identify novel trypanocidal compounds. A 384-well fluorescence-based whole cell high throughput screening (HTS) assay was developed against *Trypanosoma brucei brucei*[10] and used to screen the Eskitis prefractionated natural product library consisting of 202,983 fractions. *T. b. brucei* has been routinely used in screening for initial identification of antitrypanosomal lead compounds.[11] The library has been constructed by the fractionation of over 18,000 marine and terrestrial extracts enhanced for lead- and drug-likeness with 11 fractions collected per biota. Several active fractions were identified from the fractionated extract of the Australian ascidian *Polysyncraton echinatum* (4, 5, and 7 of 11). These fractions showed potent activity against *T. b. brucei*, and two of the fractions also had a desirable selectivity against the human embryonic kidney cell line, HEK293 (>10-fold). Bioassay-guided fractionation of the crude extract led to the isolation of a new pyridoacridine alkaloid, 12-deoxyascididemin (**1**), along with two known analogues, ascididemin (**2**) and eilatin (**3**).

Marine ascidians belonging to the family Didemnidae have proven to be a remarkable source of chemically diverse natural products with potent biological properties. Prominent among these are the cytotoxic didemnins,[12] the antibacterial shishididemniols,[13,14] and the anticancer patellazoles.[15] The Australian ascidian *P. echinatum* (Order: Enterogona, Family: Didemnidae) was collected from the North West of Farquharson Reef, Queensland, at the depth of 24 m by SCUBA diving in 2004. A voucher sample (G322053) is stored at the Queensland Museum, Brisbane, Australia.



1 **2** **3**

The freeze-dried and ground ascidian (10 g) was sequentially extracted with *n*-hexane, CH_2Cl_2/MeOH (4:1), and MeOH. The CH_2Cl_2/MeOH extracts were combined (1.67 g) and chromato-

* Corresponding author. Tel.:+61 7 3735 6000; fax: +61 7 3735 6001.
E-mail address: r.quinn@griffith.edu.au (R.J. Quinn).

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graphed using a C_{18}-bonded silica HPLC column (MeOH/H_2O/0.1% TFA). Following the biological evaluation, the active fractions were combined and further purified by C_{18} HPLC (MeOH/H_2O/0.28% NH_3) to yield a new pyridoacridine alkaloid, 12-deoxyascididemin (**1**, 1.06 mg, 0.011% dry weight), and two known analogues, ascidemin (**2**, 3.2 mg, 0.032% dry weight) and eilatin (**3**, 1.6 mg, 0.016% dry weight).

12-Deoxyascididemin (**1**) was obtained as a dark brown amorphous solid.[16] HRESIMS measurement: $[M+H]^+$ ion (*m/z* 270.1030), in combination with ^1H and ^{13}C NMR spectroscopic data (Table 1), supported the molecular formula of $C_{18}H_{11}N_3$ with 15 double bond equivalents. The ^1H NMR spectrum of **1** displayed an exchangeable proton at δ_H 12.35 along with ten aromatic signals between δ_H 7.00 and 9.00, including one singlet, four doublets, and five doublet of doublets (Table 1). The eighteen carbons of **1** were observed from the HSQC and HMBC experiments, and all carbons were shown to resonate between δ_C 99.4 and 147.2. The multiple UV absorptions at λ_{max} 219, 239, 271, 299, 342, and 400 nm suggested a highly conjugated aromatic system. These absorptions underwent hypsochromic shifts upon addition of acid. Compound **1** was a yellow solution under basic conditions and a purple solution under acidic conditions. This pH-dependent color change has been noted previously for a number of other pyridoacridine alkaloids.[17]

Analysis of the ^1H and ^{13}C NMR spectroscopic data with the aid of COSY and HSQC experiments (Table 1) established the H-bearing motif for **1**, including a disubstituted pyridine ring, a disubstituted phenyl ring, and a trisubstituted pyridine ring (Fig. 1). The arrangement of the remaining ring systems was afforded by detailed analysis of the HMBC correlations. The connectivity of C-4a and C-4b was established by the HMBC correlations from H-4 at δ_H 8.34 to C-4b at δ_C 147.2, and from H-5 at δ_H 8.23 to C-4a at δ_C 114.3. The HMBC correlations from the H-12 methine singlet at δ_H 7.35 to C-11 and C-11a at δ_C 135.8 and 131.1 indicated the connectivity of the methine group to C-11a. Correlations from the same methine proton to C-12a and C-12b at δ_C 135.4 and 120.2 established the connectivity of C-12 to C-12a, and C-12a to C-12b. Attachment of the exchangeable NH to C-12a and C-13a was elucidated by the HMBC correlations from the NH at δ_H 12.35 to C-12, C-12a, and C-12b at δ_C 99.4, 135.4, and 120.2, respectively, and from the same proton to C-1, C-4a, and C-13a at δ_C 116.6, 114.3, and 140.0, respectively. A weak $^4J_{CH}$ HMBC correlation observed between H-11 at δ_H 8.55 and C-7a at δ_C 137.9 confirmed the connection of C-7a to C-7b. The final structure of 12-deoxyascididemin was therefore elucidated as **1**.

Table 1
NMR data for 12-deoxyascididemin (**1**)[a]

Position	^{13}C	^1H mult. (*J* in Hz)	gCOSY	gHMBC
1	116.6	7.43 d (8.4)	2	3, 4, 4a, 4b
2	134.9	7.68 dd (7.2, 8.4)	1, 3	4, 13a
3	121.9	7.22 dd (7.2, 7.8)	2, 4	1, 2, 4, 4a
4	125.5	8.34 d (7.8)	3	2, 4a, 4b, 13a
4a	114.3	—	—	—
4b	147.2	—	—	—
5	111.3	8.23 d (6.0)	6	4a, 4b, 6, 12a, 12b
6	143.9	8.75 d (6.0)	5	4b, 5, 7a, 12b
7	—	—	—	—
7a	137.9	—	—	—
7b	130.6	—	—	—
8	—	—	—	—
9	145.4	8.90 dd (1.2, 4.2)	10, 11	7b, 10, 11
10	125.8	7.84 dd (4.2, 8.4)	9, 11	9, 11a
11	135.8	8.55 dd (1.2, 8.4)	9, 10	7a, 7b, 9, 12
11a	131.1	—	—	—
12	99.4	7.35 s	—	4b, 7b, 11, 12a, 12b
12a	135.4	—	—	—
12b	120.2	—	—	—
13	—	12.35 s	—	1, 4a, 12, 12a, 12b, 13a
13a	140.0		—	—

[a] Spectra were recorded at 600 MHz for ^1H and 150 MHz for ^{13}C in DMSO-d_6 at 30 °C.

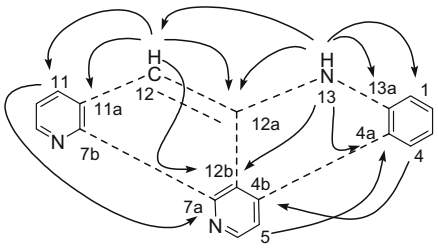

Figure 1. Key HMBC correlations for **1**.

Ascididemin (**2**) and eilatin (**3**) were previously isolated from the marine ascidians *Didemnum* sp.[18] and *Eudistoma* sp.,[19] respectively. Their ^1H and ^{13}C NMR data were identical to those reported in the literature.

The antitrypanosomal activities of **1**–**3** were evaluated *in vitro* against *T. b. brucei* (Table 2). Compounds **1**–**3** exhibited potent activity against *T. b. brucei* with IC_{50} values of 0.077, 0.032, and 1.33 μM, respectively. Preliminary toxicity studies using a human embryonic kidney cell line HEK293 indicated that compounds **1** and **2** had IC_{50} values of 7.63 and 1.48 μM against HEK293, thus showing a 99- and 46-fold selectivity toward *T. b. brucei*, respectively. Compound **3** exhibited a plateau of 62% inhibition against HEK293 at the top three concentrations screened (Table 2).

Pyridoacridines are a large group of marine alkaloids. The first reported example of this structure class was amphimedine, which was isolated from the marine sponge *Amphimedon* sp. in 1983.[20] Since then over 100 derivatives have been isolated and bioactivities such as antitumor, antibacterial, antiviral, and antiparasitic have been reported.[21] The prominent anticancer activity exhibited by pyridoacridines has prompted extensive research into the total synthesis of this class of natural product.[22] Comprehensive SAR and the mode of action have also been studied.[23,24] The mechanism of action studies have suggested that the cytotoxic properties of most of the pyridoacridines can be attributed to their nonspecific intercalations with DNA, as a result of the highly planar electron-deficient aromatic ring system of the pyridoacridine pharmacophore.[22] Previous studies have shown that the pyridoacridine alkaloids also display antitrypanosomal activity.[25,26] Our study showed that 12-deoxyascididemin (**1**) and ascididemin (**2**) were not only potent antitrypanosomal agents against *T. b. brucei*, but also exhibited a high degree of selectivity against HEK293. Although pyridoacridine alkaloids are well-known nonspecific DNA intercalators, the specific mode of action relating to their antitrypanosomal properties is unclear, and requires further investigation.

Table 2
Antitrypanosomal activity and cytotoxicity for compounds **1**–**3**

Compound	IC_{50} (μM) ± SD		Selectivity index
	T. b. brucei	HEK293	
1	0.077 ± 0.005	7.63 ± 0.75	99-fold
2	0.032 ± 0.004	1.48 ± 0.27	46-fold
3	1.33 ± 0.54	62.1 ± 0.5[a]	NA
Pentamidine[b]	0.028 ± 0.012	61.69 ± 6.44[c]	NA
Diminazene[b]	0.094 ± 0.011	>80	>850
Puromycin[b]	0.069 ± 5.40	0.541 ± 0.006	7.8-fold

[a] The activity reached a sub 100% plateau with a mean of 62.1% activity over three doses at 83, 42, and 21 μM.
[b] Reference compound activity for *T. b. brucei* and HEK293 assays.
[c] Percentage activity at a top screening dose of 70 μM. SD, standard deviation. NA, not available from doses screened.

In conclusion, we have isolated a new antitrypanosomal pyridoacridine alkaloid, 12-deoxyascididemin (**1**), along with two known analogues, ascididemin (**2**) and eilatin (**3**), from the Australian ascidian *P. echinatum*. Compounds **1** and **2** inhibit the growth of *T. b. brucei* with nM IC_{50} values.

Acknowledgments

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Supplementary data

Supplementary data (^{1}H and 2D NMR spectra for 12-deoxyascididemin (**1**), general experimental details, ascidian collection details, extraction and isolation procedure for **1**–**3**, antitrypanosomal and cytotoxicity assays) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.161.

References and notes

1. World Health Organization, http://www.who.int/mediacentre/factsheets/fs259/en/.
2. Milord, F.; Pepin, J.; Loko, L.; Ethier, L.; Mpia, B. *Lancet* **1992**, *340*, 652–655.
3. Mpia, B.; Pepin, J. *Trop. Med. Int. Health* **2002**, *7*, 775–779.
4. Salem, M. M.; Werbovetz, K. A. *Curr. Med. Chem.* **2006**, *13*, 2571–2598.
5. Kossuga, M. H.; Nascimento, A. M.; Reimao, J. Q.; Tempone, A. G.; Taniwaki, N. N.; Veloso, K.; Ferreira, A. G.; Cavalcanti, B. C.; Pessoa, C.; Moraes, M. O.; Mayer, A. M. S.; Hajdu, E.; Berlinck, R. G. S. *J. Nat. Prod.* **2008**, *71*, 334–339.
6. Rubio, B. K.; Tenney, K.; Ang, K.; Abdulla, M.; Arkin, M.; McKerrow, J.; Crews, P. *J. Nat. Prod.* **2009**, *72*, 218–222.
7. Buchanan, M. S.; Davis, R. A.; Duffy, S.; Avery, V. M.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 1541–1543.
8. Mueller, D.; Davis, R. A.; Duffy, S.; Avery, V. M.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 1538–1540.
9. Davis, R. A.; Duffy, S.; Avery, V. M.; Camp, D.; Hooper, J. N. A.; Quinn, R. J. *Tetrahedron Lett.* **2010**, *51*, 583–585.
10. Sykes, M. L.; Avery, V. M. *Am. J. Trop. Med. Hyg.* **2009**, *81*, 665–674.
11. Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. *Nat. Rev. Drug Disc.* **2005**, *4*, 727–740.
12. Vera, M. D.; Joullie, M. M. *Med. Res. Rev.* **2002**, *22*, 102–145.
13. Kobayashi, H.; Fujita, T.; Iwashita, T.; Nakao, Y.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **2007**, *72*, 1218–1225.
14. Kobayashi, H.; Miyata, Y.; Okada, K.; Fujita, T.; Iwashita, T.; Nakao, Y.; Fusetani, N.; Matsunaga, S. *Tetrahedron* **2007**, *63*, 6748–6754.
15. Richardson, A. D.; Aalbersberg, W.; Ireland, C. M. *Anticancer Drugs* **2005**, *16*, 533–541.
16. 12-Deoxyascididemin (**1**), isolated as a brown amorphous solid; UV (MeOH) λ_{max} (log ε) 219 (2.83), 239 (2.80), 271 (sh, 2.72), 299 (sh, 2.50), 342 (2.38), and 400 (2.10) nm; IR ν_{max} (KBr) 3684, 2924, 2854, 2360, 2341, 1383, 669, and 651 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Table 1; (+)-LRESIMS *m/z* 270 $[M+H]^+$; (+)-HRESIMS *m/z* 270.1030 ($C_{18}H_{12}N_3$ $[M+H]^+$ requires 270.1026).
17. Thale, Z.; Johnson, T.; Tenney, K.; Wenzel, P. J.; Lobkovsky, E.; Clardy, J.; Media, J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **2002**, *67*, 9384–9391.
18. Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y. *Tetrahedron Lett.* **1988**, *29*, 1177–1180.
19. Rudi, M.; Kashman, Y. *J. Org. Chem.* **1989**, *54*, 5331–5337.
20. Schmitz, F. J.; Agarwal, S. K.; Gunasedera, S. P.; Schmidt, P. G.; Shoolery, J. N. *J. Am. Chem. Soc.* **1983**, *105*, 4835–4836.
21. Molinski, T. F. *Chem. Rev.* **1993**, *93*, 1825–1838.
22. Labarbera, D. V.; Bugni, T. S.; Ireland, C. M. *J. Org. Chem.* **2007**, *72*, 8501–8505.
23. Belmont, P.; Bosson, J.; Godet, T.; Tiano, M. *Anticancer Agents Med. Chem.* **2007**, *7*, 139–169.
24. Dias, N.; Vezin, H.; Lansiaux, H.; Lansiaux, A.; Bailly, C. *Top. Curr. Chem.* **2005**, *253*, 89–108.
25. Marshall, K. M.; Barrows, L. R. *Nat. Prod. Rep.* **2004**, *21*, 731–751.
26. Copp, B. R.; Kayser, R.; Brun, R.; Kiderlen, A. F. *Planta Med.* **2003**, *69*, 527–531.