(+)-7-Bromotrypargine: an antimalarial β-carboline from the Australian marine sponge *Ancorina* sp.

Rohan A. Davis,[a] Sandra Duffy,[a] Vicky M. Avery, [a] David Camp, [a] John N. A. Hooper,[b] Ronald J. Quinn[a,*]

[a]*Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia*

[b]*Queensland Museum, South Brisbane, QLD 4101, Australia*

Abstract— Mass-directed isolation of the CH_2Cl_2/MeOH extract from the Australian marine sponge *Ancorina* sp. resulted in the purification of a new antimalarial β-carboline, (+)-7-bromotrypargine **1**, along with the previously isolated natural product, 6-bromotryptamine **2**. The structure of **1** is determined by extensive 1D/2D NMR, and MS data analyses. Comparison of the chiro-optical data for **1** with literature values of related natural products is used to determine the absolute stereochemistry of (+)-7-bromotrypargine as 1*R*. Antimalarial activity data for **1** and **2** against a chloroquine-resistant (Dd2) and chloroquine-sensitive (3D7) *Plasmodium falciparum* strain are also provided.

Malaria is an infectious disease caused by protozoan parasites belonging to the genus *Plasmodium*. Each year up to 600 million infections occur worldwide, and over 1 million people die from this disease.[1] Approximately 3.2 billion people – mostly in the poorest countries – are at risk of contracting malaria.[1] Despite the presence of commercially available antimalarial drugs, the control of this ancient disease is increasingly limited by the emergence of drug-resistant strains of the *Plasmodium* parasite.[2] Historically, natural products have played a major role in the treatment of this infectious disease. [3-5] For centuries the indigenous people from South America have used the bark from the "fever tree", *Cinchona succiruba,* for the treatment of malaria.[3-5] The Chinese medicinal plant, *Artemisia annua*, has likewise been used as an antimalarial herbal remedy for hundreds of years.[3-5] Chemical investigations of *Cinchona succiruba* and *Artemisia annua*, identified the major active metabolites to be quinine and artemisinin, respectively.[3-5] This research has subsequently led to the development of numerous antimalarial drugs based on these two important plant natural products.[5] Unfortunately, resistance of the malaria parasite to current small molecule therapies and subsequent lack of efficacy, has highlighted the need for the discovery and development of new antimalarials.

As part of our continuing research[6,7] into the discovery of new antimalarial leads, we undertook high-throughput screening (HTS) of a prefractionated natural product extract library. From the HTS data we identified one fraction derived from the sponge *Ancorina* sp. (Ancorinidae) that showed parasitic growth inhibition in the antimalarial imaging assay, and no cytotoxicity towards a human embryonic kidney cell line (HEK293). MS analysis of the active fraction identified ions in the (+)-LRESIMS at *m/z* 350/352, that were predicted to correspond to the bioactive natural product(s). Mass-directed fractionation on the large-scale CH_2Cl_2/MeOH extract resulted in the isolation of a new β-carboline alkaloid, (+)-7-bromotrypargine **1**, along with the previously reported natural product, 6-bromotryptamine **2** (Figure 1). Herein we report the isolation, structure elucidation and antimalarial activity of (+)-7-bromotrypargine **1**.

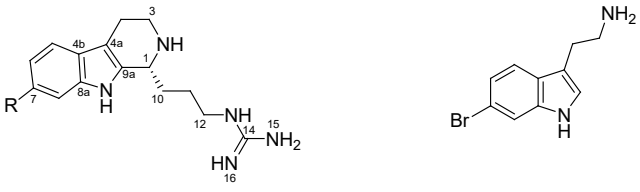

Figure 1. Chemical structures of compounds **1**-**3**.

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

The freeze-dried and ground *Ancorina* sp. was extracted with *n*-hexanes, CH_2Cl_2:MeOH (4:1), and MeOH. All CH_2Cl_2/MeOH extracts were combined and chromatographed using C_{18} bonded silica HPLC (MeOH/H_2O/0.1% TFA) to yield (+)-7-bromotrypargine (**1**, 2.7 mg, 0.050% dry wt) and a less-polar fraction. This later material required further purification using C_{18} bonded silica HPLC (MeOH/H_2O/0.1% TFA) to ultimately yield 6-bromotryptamine (**2**, 2.0 mg, 0.040% dry wt).

The *bis*-TFA salt of (+)-7-bromotrypargine **1** was isolated as an optically active brown gum.[8] The (+)-LRESIMS of **1** displayed two pseudo-molecular ions of equal relative intensity at *m/z* 350 and 352, which indicated the molecule contained one bromine atom. The ^{1}H NMR spectrum (Table 1) of **1** displayed five exchangeable signals (δ_H 7.10 (brs, 4H), 9.09 (brs, 1H), 9.62 (brs, 1H) and 11.34 (s, 1H), 7.81 (t, J = 5.4 Hz, 1H), three aromatic resonances indicative of a tri-substituted ABX benzene system [δ_H 7.54 (d, J = 1.8 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H) and 7.16 (dd, J = 8.4, 1.8 Hz, 1H)] and eight unique aliphatic signals [δ_H 4.69 (brm, 1H), 3.34 (m, 1H)/3.57 (dt, J = 12.0, 4.8 Hz, 1H), 3.19 (m, 2H), 2.93 (m, 2H), 2.13 (m, 1H)/1.92 (m, 1H) and 1.69 (m, 2H)], several of which appeared to be adjacent to heteroatoms. HSQC spectral analysis enabled all the protons to be attached to their respective carbons. The gCOSY spectrum of **1** supported the presence of a tri-substituted benzene system, and also allowed the structure elucidation of two aliphatic spins systems, -CH-CH_2-CH_2-CH_2-NH-, and –CH_2-CH_2-. The exchangeable proton singlet at δ_H 11.34 showed HMBC correlations with 4 downfield carbons at δ_C 131.0, 106.5, 125.0 and 137.3. HMBC correlations from both the *para*-substituted aromatic methine protons δ_H 7.44 and 7.54 to carbons at δ_C 125.0 and 137.3 allowed the construction of an indole system. A strong ROESY correlation between δ_H 7.54 and the indole proton at δ_H 11.34 further supported this substructure. The two contiguous methylene groups were attached to C-4a of the indole system based on HMBC correlations from δ_H 2.93 (H-4) to the carbons resonating at δ_C 131.0, 106.5 and 125.0. A ROESY correlation between δ_H 2.93 and 7.44 confirmed this assignment. The methine proton from the remaining aliphatic spin system, -CH-CH_2-CH_2-CH_2-NH-, also showed one $^2J_{CH}$ correlation with the C-9a indole carbon at δ_C 131.0. A 1,2,3,4-tetrahydro-β-carboline system was established based on HMBC correlations from the nitrogen substituted methylene protons, δ_H 3.57/3.34 (H-3a/H-3b), to the aliphatic methine carbon at δ_C 52.1. The heteronuclear correlations observed for both the methylene signal at δ_H 3.19 (H-12) and the exchangeable proton at δ_H 7.81 (H-13) to a carbon at δ_H 157.2 (C-14) indicated the presence of a guanidinium moiety[9] at the end of the pendant spin system. The bromine atom present in **1** was attached to C-7 (δ_C 114.7) of the indole system on the basis of the ^{13}C NMR chemical shift[17,18] and the ^{1}H-^{1}H coupling constants observed for the three aromatic methines, and their HMBC correlations. Hence the planar structure **1** was assigned to (+)-7-bromotrypargine.

Table 1. NMR data for the *bis*-TFA salt of (+)-7-bromotrypargine **1**.[a]

position	^{13}C	^{1}H (mult., J in Hz, int.)	gCOSY	gHMBC
1	52.1	4.69 (brm, 1H)	10a, 10b	9a, 10
2		7.10[b] (brs, 2H[c])		
3	40.3	3.34 (m, 1H)	3b, 4	1
		3.57 (dt, 12.0, 4.8, 1H)	3a, 4	1, 4, 4a
4	18.6	2.93 (m, 2H)	3a, 3b	3, 4a, 4b, 9a
4a	106.5			
4b	125.0			
5	119.1	7.44 (d, 8.4, 1H)	6	4a, 4b, 7, 8a
6	122.2	7.16 (dd, 8.4, 1.8, 1H)	5, 8	4b, 7, 8
7	114.7			
8	114.0	7.54 (d, 1.8, 1H)	6	4b, 6, 7, 8a
8a	137.3			
9		11.34 (s, 1H[c])	8	4a, 4b, 8a, 9a
9a	131.0			
10	28.0	2.13 (m, 1H)	1, 10b, 11	1, 9a, 11, 12
		1.92 (m, 1H)	1, 10a, 11	1, 9a, 11, 12
11	23.9	1.69 (m, 2H)	10a, 10b, 12	1, 10, 12
12	40.3	3.19 (m, 2H)	11, 13	10, 11, 14
13		7.81 (t, 5.4, 1H[c])	12	11, 12, 14
14	157.2			
15		7.10[b](brs, 2H[c])		
16		9.09[b] (brs, 1H[c])		
		9.62[b] (brs, 1H[c])		

[a] Spectra were recorded in DMSO-d_6 at 30 °C; ^{1}H (600 MHz), ^{13}C (150 MHz). [b] Interchangeable signals. [c] Total number of exchangeable protons equals eight, since **1** was purified as its *bis*-TFA salt.

Several natural products that are structurally related to **1** have been previously reported in the literature. For example, (-)-trypargine was originally isolated from the skin of the African frog *Kassina senegalensis*, and was shown to display lethality towards mice on intravenous administration (10 mg/kg), causing death within two min by respiratory failure and paralysis.[10] Both enantiomers of trypargine have been subsequently synthesized by a number of different research groups, and the absolute stereochemistry at C-1 determined by X-ray crystallographic and CD studies.[11-14] Other related guanidinium β-carboline natural products include the neurotoxin, 6-hydroxytrypargine, purified from the venom of the spider *Parawixia bistriata*,[15] and trypargimine and 1-carboxytrypargine, which were both isolated from the marine ascidian, *Eudistoma* sp.[16]

In order to determine the absolute stereochemistry of **1** we compared the optical rotation data for (+)-7-bromotrypargine with literature values of the related natural products, whose absolute stereochemistry had been unequivocally determined.[11-14] The optical rotation for the HCl salt of synthetic (-)-trypargine was reported as $[\alpha]_D^{21}$ -37.3 (*c* 1.00, MeOH),[13] while that of (+)-trypargine **3** has been reported as $[\alpha]_D^{15}$ +37.2 (*c* 0.54, MeOH)[13] and $[\alpha]_D^{23}$

+34.3 (*c* 1.00, MeOH).[11] We recorded $[\alpha]_D^{22}$ +20 (*c* 0.025, MeOH) for the *bis*-TFA salt of (+)-7-bromotrypargine **1**. These data suggest that (+)-7-bromotrypargine has the same absolute stereochemistry as (+)-trypargine and hence we have assigned *R* absolute stereochemistry to C-1 of **1**. Interestingly, this marine-derived trypargine analogue (+)-**1** has the opposite absolute stereochemistry to that of the naturally occuring terrestrial metabolite, (-)-trypargine. Trypargine has also been isolated from the marine environment, and was determined to be a racemic mixture based on optical rotation data, $[\alpha]_D^{25}$ 0 (*c* 1.00, MeOH).[16]

Compound **2** was determined to be 6-bromotryptamine following spectroscopic data comparison with literature values.[17,18]

Compounds **1** and **2** were tested against both a chloroquine-resistant (Dd2) and chloroquine-sensitive (3D7) *Plasmodium falciparum* strain.[6,7] Preliminary toxicity towards human cells was investigated using a human embryonic kidney cell line (HEK293).[6,7] (+)-7-Bromotrypargine **1** was shown to display IC_{50} values of 5.4 µM (Dd2) and 3.5 µM (3D7), and was inactive against the HEK293 cell line up to 80 µM. 6-Bromotryptamine **2** was inactive against both malarial strains and HEK293 when tested at several concentrations up to and including 80 µM.

Malaria-focussed biodiscovery has identified hundreds of antimalarial plant secondary metabolites over the past 50 years.[19,5,20,21] In comparison, marine natural products have been under investigated, although there is growing interest in researching marine organisms for new small molecules that might have applications as antiplasmodial agents.[22-25]

Examples of antimalarial marine-derived metabolites include manzamine A,[26-28] lepadins D-F,[29] 6-bromoaplysinopsin,[30] and venturamides A and B.[31] The above-listed natural products, in conjunction with these current studies, further supports the continued research into marine natural products and their potential application as new antimalarial leads or drugs.

In conclusion, we have isolated a new antimalarial β-carboline, (+)-7-bromotrypargine **1**, from the marine sponge *Ancorina* sp., and shown that this compound inhibits the growth of two *Plasmodium falciparum* strains (Dd2 and 3D7) with single digit µM IC_{50} values.

Acknowledgements

The authors would like to acknowledge Medicines for Malaria Venture for financial support. We thank H. Vu (Eskitis Institute) for acquiring the HRESIMS measurements. The authors thank C. Lewis and K. Watts (Eskitis Institute) for their assistance in the preparation of the screening library. We thank B. Aldred and R. Lang (Eskitis Institute) for technical assistance with the HTS. We also acknowledge the Australian Red Cross Blood Service for the provision of Type O+ erythrocytes and K. T. Andrews from the Queensland Institute for Medical Research for the *P. falciparum* strains. Finally, we would also like to thank Aquenal Pty Ltd (Tasmania, Australia) for collection of the marine sponge.

Supplementary Material

Supplemental material available: ^{1}H and 2D NMR spectra for the *bis*-TFA salt of (+)-7-bromotrypargine **1**, general experimental details, sponge description and collection details, extraction and isolation procedure for **1** and **2**, antimalarial and cytotoxicity assays. Supplementary data associated with this article can be found in the online version, at doi

References and notes

1. *Medicines for Malaria Venture, http://www.mmv.org/.*
2. Craft, J. C. *Curr. Opin. Microbiol.* **2008**, *11*, 428-433.
3. Williams, D. A.; Lemke, T. L., Eds. *Foye's Principles of Medicinal Chemistry*, 5th ed.; Lippincott Williams & Wilkins, 2002.
4. Kajimoto, T. *Kagaku to Kyoiku* **2007**, *55*, 418-419.
5. Kayser, O.; Kiderlen, A. F.; Croft, S. L. *Stud. Nat. Prod. Chem.* **2002**, *26*, 779-848.
6. Buchanan, M. S.; Davis, R. A.; Duffy, S.; Avery, V. M.; Quinn, R. J. *J. Nat. Prod.*, **2009**, *72*, 1541-1543.
7. Mueller, D.; Davis, R. A.; Duffy, S.; Avery, V. M.; Camp, D.; Quinn, R. J. *J. Nat. Prod.*, **2009**, *72*, 1538-1540..
8. Brown gum; $[\alpha]_D^{22}$ +20 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.72), 253 sh (4.36), 280 (3.97), 295 (3.98), 325 nm (3.67); IR ν_{max} (KBr) 1655, 1432, 1315, 1204, 1142, 1142, 1052 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Table 1; (+)-LRESIMS *m/z* (rel. int.) 350 (100), 352 (100); (+)-HRESIMS *m/z* 350.0966 ($C_{15}H_{21}{}^{79}BrN_5$ $[M\text{-}C_4HF_6O_4]^+$ requires 350.0975).
9. Buchanan, M. S.; Carroll, A. R.; Wessling, D.; Jobling, M.; Avery, V. M.; Davis, R. A.; Feng, Y.; Xue, Y.; Oster, L.; Fex, T.; Deinum, J.; Hooper, J. N. A.; Quinn, R. J. *J. Med. Chem.* **2008**, *51*, 3583-3587.
10. Akizawa, T.; Yamazaki, K.; Yasuhara, T.; Nakajima, T.; Roseghini, M.; Erspamer, G. F.; Erspamer, V. *Biomed. Res.* **1982**, *3*, 232-234.
11. Czarnocki, S. J.; Wojtasiewicz, K.; Jozwiak, A. P.; Maurin, J. K.; Czarnocki, Z.; Drabowicz, J. *Tetrahedron* **2008**, *64*, 3176-3182.
12. Shimizu, M.; Ishikawa, M.; Komoda, Y.; Matsubara, Y.; Nakajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 4529-4533.
13. Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 909-914.
14. Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T.; Yamaguchi, K.; Sakai, S. *Chem. Pharm. Bull.* **1984**, *32*, 1313-1325.
15. Cesar, L. M. M.; Tormena, C. F.; Marques, M. R.; Silva, G. V. J.; Mendes, M. A.; Rittner, R.; Palma, M. S. *Helv. Chim. Acta* **2005**, *88*, 796-801.
16. Van Wagoner, R. M.; Jompa, J.; Tahir, A.; Ireland, C. M. *J. Nat. Prod.* **1999**, *62*, 794-797.
17. Fahy, E.; Potts, B. C. M.; Faulkner, D. J.; Smith, K. *J. Nat. Prod.* **1991**, *54*, 564-569.
18. Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1994**, *59*, 6600-6605.
19. Kaur, K.; Jain, M.; Kaur, T.; Jain, R. *Bioorg. Med. Chem.* **2009**, *17*, 3229-3256.

20. Schwikkard, S.; van Heerden, F. R. *Nat. Prod. Rep.* **2002**, *19*, 675-692.
21. Dictionary of Natural Products on CD-ROM, version 18.1; Chapman and Hall / CRC Press: London, UK, 2009.
22. Ioset, J.-R. *Curr. Org. Chem.* **2008**, *12*, 643-666.
23. Laurent, D.; Pietra, F. *Mar. Biotechnol.* **2006**, *8*, 433-447.
24. Mancini, I.; Guella, G.; Defant, A. *Mini Rev. Med. Chem.* **2008**, *8*, 1265-1284.
25. Mayer, A. M. S.; Rodriguez, A. D.; Berlinck, R. G. S.; Hamann, M. T. *Biochim. Biophys. Acta, Gen. Subj.* **2009**, *1790*, 283-308.
26. Ang, K. K.; Holmes, M. J.; Higa, T.; Hamann, M. T.; Kara, U. A. *Antimicrob. Agents Chemother.* **2000**, *44*, 1645-9.
27. Rao, K. V.; Kasanah, N.; Wahyuono, S.; Tekwani, B. L.; Schinazi, R. F.; Hamann, M. T. *J. Nat. Prod.* **2004**, *67*, 1314-1318.
28. Rao, K. V.; Santarsiero, B. D.; Mesecar, A. D.; Schinazi, R. F.; Tekwani, B. L.; Hamann, M. T. *J. Nat. Prod.* **2003**, *66*, 823-828.
29. Wright A. D.; Goclik, E.; Konig G. M.; Kaminsky, R. *J Med Chem* **2002**, *45*, 3067-3072.
30. Hu, J.-F.; Schetz, J. A.; Kelly, M.; Peng, J.-N.; Ang, K. K. H.; Flotow, H.; Leong, C. Y.; Ng, S. B.; Buss, A. D.; Wilkins, S. P.; Hamann, M. T. *J. Nat. Prod.* **2002**, *65*, 476-480.
31. Linington, R. G.; Gonzalez, J.; Urena, L. D.; Romero, L. I.; Ortega-Barria, E.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 397-401.