ChemComm

COMMUNICATION

View Article Online
View Journal

Total synthesis of the cyanobacterial metabolite nostodione A: discovery of its antiparasitic activity against *Toxoplasma gondii*†

Cite this: DOI: 10.1039/c4cc03904a

Received 22nd May 2014,
Accepted 12th June 2014

DOI: 10.1039/c4cc03904a

www.rsc.org/chemcomm

J. McNulty,*[a] K. Keskar,[a] C. Bordón,[b] R. Yolken[b] and L. Jones-Brando[b]

A total synthesis of the cyanobacterial natural product nostodione A is reported involving a convergent, diversity-oriented route. A small assemblage of structural analogues were prepared and their cytotoxicity and anti-invasion activity against the protozoal parasite *Toxoplasma gondii* is reported for the first time.

Cyanobacteria (blue-green algae) isolated from marine, freshwater and terrestrial environments have proven to be a prolific source of biologically active secondary metabolites with a wide range of therapeutic potential.[1] The indole-containing natural product nostodione A (**1**) (Fig. 1) was first isolated from the terrestrial cyanobacterium *Nostoc commune* in 1994 and shown to inhibit mitotic spindle formation.[2] The same compound was subsequently isolated from the freshwater cyanobacteria *Scytonema hofmanni* and shown to possess proteasome inhibitory activity.[3] Nostodione A exists as a thermodynamic mixture of the (*E*)- and (*Z*)-*conformational* isomers shown (Fig. 1). The compound is believed to be biosynthesized from prenostodione,[4a] an oxidative coupling product of 4-hydroxyphenylpyruvic acid from L-tyrosine and L-tryptophan.[4b] Nostodione A belongs to a small family of alkaloids that have been isolated from cyanobacteria in recent years including the dimeric scytonemin (**2**), (Fig. 1).[5] In addition to the anti-mitotic and proteosomal activities described above, these highly UV-absorbing molecules are believed to serve a protective function against solar radiation within the cyanobacterial colony and are of interest as potential sunblock ingredients. The chemical synthesis of isoprenostodione was recently reported,[6a] while a single report of the synthesis of both nostodione A (**1**)[6b] and the dimeric scytonemin (**2**)[6c] have been reported by Mårtensson and co-workers. An enzymatic approach to the Scytonemin monomer has also recently been reported.[6d]

We became interested in the synthesis and biological evaluation of nostodione A (**1**) for several reasons. Our research groups recently initiated a joint program aimed at the discovery of novel small-molecules exhibiting biological activity against the parasite *Toxoplasma gondii*, the protozoan responsible for toxoplasmosis.[7] From a structural viewpoint, nostodione A resembles several known oxidized, condensed indole alkaloids such as indirubin, tryptanthrin[8] and the pyrroloiminoquinones,[9] examples of which display activity to *T. gondii*, Fig. 1.

Synthetic access to nostodione A would allow for a wider screening of its biological activity allowing a complete assessment of its therapeutic potential. In this paper we report a diversity-oriented approach towards the synthesis of nostodione A itself, as

[a] *Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario, Canada L8S 4M1. E-mail: jmcnult@mcmaster.ca; Tel: +1-905-525-9140 ext. 27393*

[b] *Stanley Division of Developmental Neurovirology, Department of Pediatrics, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, Maryland, 21287, USA*

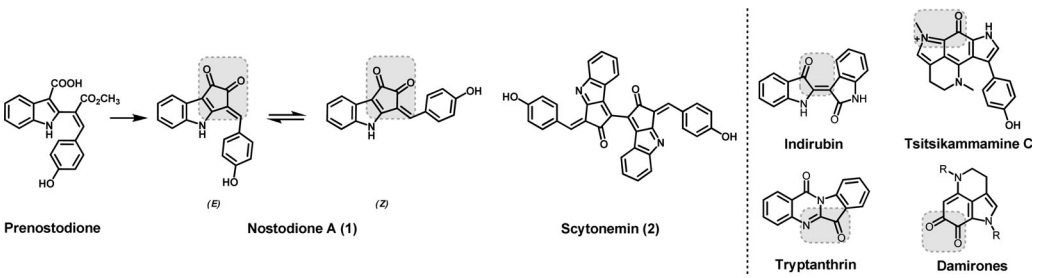


Fig. 1 Structure of the cyanobacterial secondary metabolite nostodione A (**1**) and related biosynthetic alkaloids.

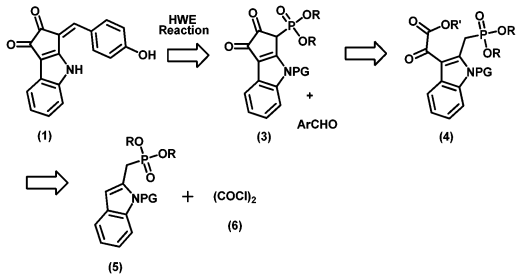


Fig. 2 Retrosynthetic analysis of nostodione A (**1**).

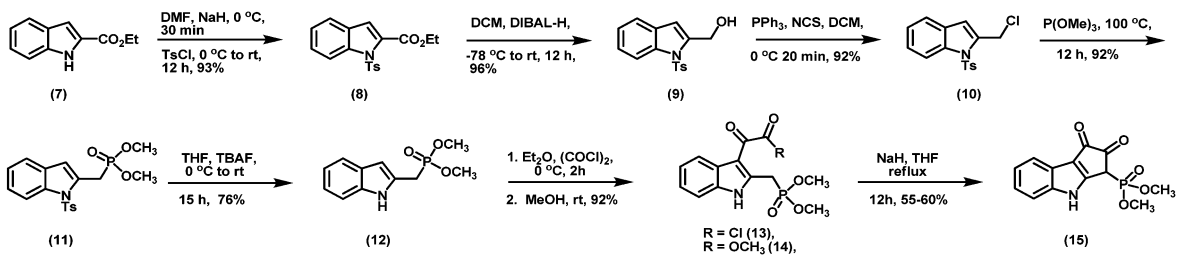
well as the synthesis of a mini-library of analogues and the discovery of cytotoxicity and anti-invasion activity of these derivatives to *T. gondii* infection.

Our retrosynthetic analysis of nostodione A (**1**) is outlined in Fig. 2. In order to conduct structure–activity evaluation of analogues of (**1**) we considered a Horner–Wadsworth–Emmons (HWE)-type disconnection of the 4-hydroxystyryl unit in (**1**) leading to the β-ketophosphonate (**3**) and 4-hydroxybenzaldehyde, or protected derivative thereof. In the synthetic direction, β-ketophosphonate (**3**) was envisioned as an ideal nexus that would allow construction of an assemblage of nostodione A analogues *via* a diversity-oriented HWE reaction (with ArCHO or RCHO) in the last step. It was envisioned that the β-ketophosphonate (**3**) could be accessed through an intramolecular α-phosphonate acylation-type reaction[10] from the 3-oxalylindole derivative (**4**), in turn, the product of acylation of the indole (**5**) with oxalyl chloride. Compound (**5**) was projected to be the product of a Michaelis–Arbuzov reaction of a 2-halomethylindole derivative, ultimately derived from commercially available indole-2-carboxylic acid derivatives.

The synthesis thus began with ethyl-indole-2-carboxylate (**7**), as shown in Scheme 1. In exploratory studies, we determined that formation of the necessary 2-halomethylindole derivatives was complicated in the presence of the free indole NH. Protection as the *N*-tosyl derivative (**8**) followed by reduction of the ester led efficiently to the desired alcohol (**9**). Conversion to the chloromethyl derivative (**10**) now occurred smoothly and this reactive intermediate underwent clean Michaelis–Arbuzov alkylation with trimethylphosphite yielding phosphonate (**11**). Attempts at C3-acylation on intermediate (**11**) with oxalyl chloride met with failure, which we attributed to *N*-tosyl-induced electronic deactivation at this position. Removal of the *N*-tosyl substituent was accomplished under mild conditions using TBAF.[11] Direct acylation on the deprotected phosphonate (**12**) now occurred smoothly with oxalyl chloride in diethyl ether yielding initially the monoacyl chloride derivative (**13**). Attempts at intramolecular acylation initially proved extremely challenging and a summary of select examples en-route to optimisation of this reaction is collected in Table S1 (ESI†). Attempted intramolecular acylation on the immediate acid chloride (**13**) proved futile with a number of bases and solvents (Table S1, entries 1 and 2, ESI†). The acid chloride was converted to the ester (**14**) in order to probe the intramolecular ester acylation.[10] A wide range of conditions were investigated for the conversion of (**14**) into the β-ketophosphonate (**15**) (see Table S1, ESI†). The use of sodium hydride (NaH) in refluxing THF proved optimal and compound (**15**) could be isolated in reasonable yields of 55–60%. With the β-ketophosphonate (**15**) now on hand, we began initial studies on the critical HWE reaction as summarized in Scheme 2. Protection of 4-hydroxybenzaldehyde with dihydropyran gave the unstable 4-tetrahydropyranyl ether. This was reacted with the dianion generated from (**15**) and the HWE reaction proceeded smoothly to give the THP-protected derivative of nostodione A (**16**) which was immediately deprotected, completing the synthesis of nostodione A in 68% isolated yield from (**15**). Synthetic nostodione A was isolated as a yellow pigment with m.p. 285 °C (decomp), lit. m.p. 280 °C (decomp)[2] and spectroscopic data in accord with the literature values including an (*E*):(*Z*) ratio of 4:1 (acetone) for both our synthetic material and the natural product.[2,6c] This synthesis constitutes the second reported total synthesis of nostodione A and was achieved in only 8 chemical steps and 21.6% overall yield from commercial ethyl indole-2-carboxylate (**7**).

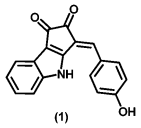
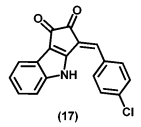
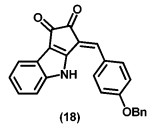
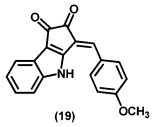
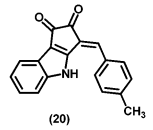
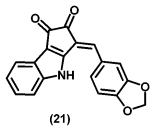
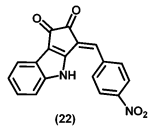
We were now positioned to exploit the diversity-oriented HWE reaction from (**15**) as described. The HWE reaction proved successful with a variety of substituted benzaldehydes and allowed for the assembly of the mini-library of aryl ring structural analogues (**17**) to (**22**) summarized in Table 1. NMR analysis of the series of analogues uncovered a prominent substituent effect on the (*E*):(*Z*) ratio of these nostodione A derivatives. The (*E*)-isomer content was observed to be predominant in all cases, including those containing either electron donating or withdrawing groups (ratio of (*E*)-isomer in DMSO-d_6: 4-CH_3 (88%), 3,4-methylenedioxy (95%), 4-NO_2 (>98%), 4-OBn (94%), 4-OCH_3 (94%), 4-Cl (83%)). These results indicate that the free phenolic group in (**1**) may play a unique role in stabilizing the (*Z*)-isomer of nostodione A.

With nostodione A itself and the mini-panel of synthetic analogues available, we now assessed their antiprotozoal activity. We employed a published colorimetric assay[12] to assess both *in vitro* cell cytotoxicity and anti-Toxoplasma capabilities of the

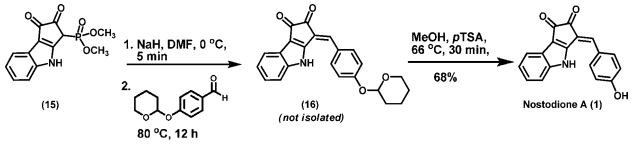


Scheme 1 Synthesis of the 3-oxalyl-indole-2-phosphonomethyl intermediates (**13**), (**14**) and ketophosphonate (**15**).

Table 1 Assembly of nostodione A mini-panel (**1**) and (**17**)–(**22**) *via* the HWE strategy and anti-Toxoplasma biological activity

Entry	Nostodione A and analogues	Isolated yield (%)	IC_{50} (μM)	IC_{90} (μM)	TD_{50} (μM)	TI
1	 (1)	68	85	183	108	1
2	 (17)	82	21.6	114	172	8
3	 (18)	68	4.6	30	≥320	70
4	 (19)	72	5.6	44	25	5
5	 (20)	55	27.8	103	161	6
6	 (21)	42	19	78	23	1
7	 (22)	44	50.6	166	219	4
8	Atovaquone		0.2	0.6	21	111

nostodione mini-library. Briefly, diluted compounds were added to human foreskin fibroblast (HFF; ATCC) cells growing in 96-well tissue culture plates. Beginning at 320 μM, the compounds were serially diluted across the plate by dilutions of 0.5 log 10. *T. gondii* RH-2F tachyzoites that constitutively express β-galactosidase (β-gal) were then added to most wells, leaving 2 wells in each column parasite-free for cytotoxicity testing. The substrate for β-gal, chlorophenol red-β-D-galactopyranoside (CPRG), was added to the Toxoplasma wells after 4 days of incubation at 37 °C/5% CO_2. Further incubation for 20 h was followed by addition of the cell viability reagent, CellTiter 96 Aqueous One Solution Reagent (Promega Corp., WI) to the uninfected cytotoxicity wells. Color reactions in all wells were then read in a Vmax microplate reader (Molecular Devices, CA) after 3 h of incubation. The amount of



Scheme 2 Completion of the synthesis of nostodione A (**1**) employing the HWE strategy.

absorbance (570–650 nm) in wells containing drug, Toxoplasma, and CPRG was compared to that in parasite control wells. In the cytotoxicity wells, the bioreduction of the cell viability reagent by viable cells into a soluble, colored formazan product was captured by reading the plates at 490–650 nm. The median and 90% inhibitory concentrations (IC_{50}, IC_{90} respectively) and the median cytotoxic dose (TD_{50}) were calculated using CalcuSyn software (Biosoft, Cambridge, U.K.). For each compound, a therapeutic index (TI) was calculated with the formula TI = TD_{50}/IC_{50}. This number reflects the specific activity of a compound against Toxoplasma. Atovaquone, a broad spectrum anti-parasitic drug used therapeutically to treat many parasitic protozoan diseases including malaria, was used as the assay positive control. The overall biological activity data is summarized in Table 1.

Nostodione A (**1**) proved to have quite low specific activity against *T. gondii* displaying an IC_{50} of 85 μM and a TI of 1. Of the structural analogues of nostodione A, the lowest IC_{50} values were displayed by the mono-substituted 4-alkoxy-substituted derivatives alone (Table 1, entries 3 and 4). The 4-benzyloxy- and 4-methoxy-derivatives exhibited IC_{50} values of 4.6 μM and of 5.6 μM respectively. In contrast, the 4-methyl- and 3,4-methylenedioxy derivatives were less potent, as were those containing electron withdrawing groups 4-chloro- and 4-nitro (entries 2 and 5–7). More importantly, the 4-benzyloxy-containing derivative (**18**) demonstrated low cytotoxicity with a therapeutic index of >70, significantly less cytotoxic than the methoxy-containing analog (**19**). The results also show clearly that the substituent effect on biological activity is not a simple electronic effect, demonstrating no discernible quantitative structure–activity correlation. The results point to the 4-benzyloxy substituent as a key fragment on the anti-Toxoplasma pharmacophore of nostodione A that is amenable to further optimisation of both potency and selectivity. A preliminary evaluation of direct effects of the compounds on extracellular tachyzoites was performed. The commonly used red/green invasion assay[12b] allowed us to evaluate nostodione A and the mini-panel of structural analogues for inhibition of host cell invasion by the tachyzoites using fluorescent labels to distinguish tachyzoites that had actively penetrated (green bars) the cells from those that were attached but unable to enter (red bars) the host cells. A decrease in the number of penetrated parasites (Fig. 3, green bars) relative to the vehicle [DMSO (VHL)] is indicative of invasion inhibition.

As shown in Fig. 3 all of the compounds tested (20 μM) significantly inhibited tachyzoite invasion of the host cell. Further, in this assay, an effect on attachment is defined as a difference in the total numbers of both penetrated and attached parasites relative to same of the vehicle.[12b] With the exception of the 4-methyl derivative (**20**), all of the test compounds significantly inhibited tachyzoite attachment. The inhibition of attachment and the inhibition of

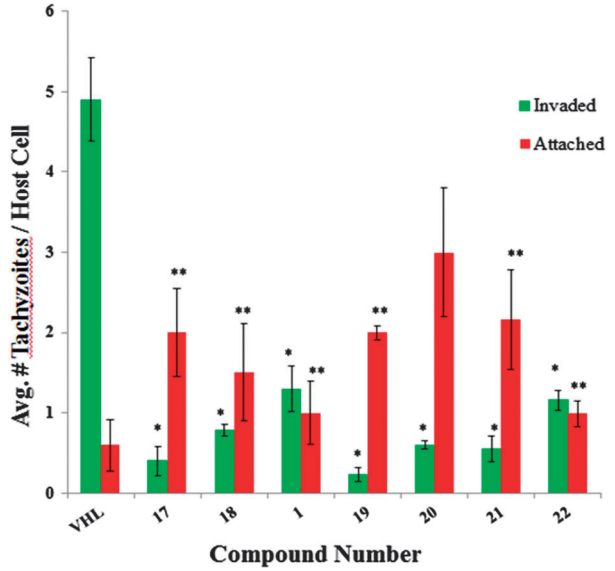


Fig. 3 Quantification of invasion inhibition of nostodione A and mini panel using red/green assay. Compounds (20 μM) were tested for activity directly on extracellular tachyzoites using an established method.[12b] Green bars represent invaded/intracellular parasites; red bars depict attached/extracellular parasites. Data are mean values ± SEM of three independent experiments. * Tachyzoite invasion was significantly lower ($P \leq 0.05$, two-tailed Student's *t*-test) than the VHL control. ** Tachyzoite attachment to host cell was significantly decreased ($P \leq 0.05$, two-tailed Student's *t*-test) relative to VHL control.

penetration are not necessarily interconnected. It is possible to inhibit invasion while not inhibiting attachment as is displayed by the 4-methyl derivative. Such activity has been reported for inhibitors of actin polymerization such as cytochalasin D.[12b]

In conclusion, we report the total synthesis of the cyanobacterial natural product nostodione A in 8 chemical steps and 21.6% overall yield from commercially available ethyl indole-2-carboxylate. The synthetic strategy employed a diversity-oriented late stage Horner–Wadsworth–Emmons olefination allowing for the assembly of a mini-panel of structural analogues. The antiparasitic biological activity of nostodione A and analogues is reported for the first time. The late stage HWE synthetic paradigm permitted the discovery of a valuable lead anti-Toxoplasma pharmacophore incorporating a 4-benzyloxy substituent on the nostodione A phenolic substituent. Further elaboration on this lead compound toward the development of a novel potent and selective anti-toxoplasmosis agent and investigation of related antiprotozoal activity of nostodione A and analogues from functionalised indole-2-carboxylates[13] is currently in progress. These preliminary results also indicate interesting diversity in the structure–activity relationships (SAR) of the compounds relevant to the inhibition of host cell attachment and invasion by *T. gondii* tachyzoites. Further biological studies to delineate the nature of such SARs in more detail are in progress.

We thank NSERC (JMcN, KK) and the Stanley Medical Research Institute (CB, RY, LJB, JMcN) for financial support of this work.

Notes and references

1 A. M. Burja, B. Banaigs, E. Abou-Mansour, J. G. Burgess and P. C. Wright, *Tetrahedron*, 2001, **57**, 9347.
2 A. Kobayashi, S. Kajiyama, K. Inawaka, H. Kanzaki and K. Kawazu, *Z. Naturforsch.*, 1994, **49c**, 464.
3 S. S. Hee, G. Chlipala and J. Orjala, *J. Microbiol. Biotechnol.*, 2008, **18**, 1655.
4 (*a*) A. Ploutno and S. Carmeli, *J. Nat. Prod.*, 2001, **64**, 544; (*b*) E. P. Balskus and C. T. Walsh, *J. Am. Chem. Soc.*, 2009, **131**, 14648.
5 P. J. Proteau, W. H. Gerwick, F. Garcia-Pichel and R. Castenholz, *Experientia*, 1993, **49**, 825.
6 (*a*) J. C. Badenock, J. J. Jordan and G. W. Gribble, *Tetrahedron Lett.*, 2013, **54**, 2759; (*b*) A. Ekebergh, A. Börje and J. Mårtensson, *Org. Lett.*, 2012, **14**, 6274; (*c*) A. Ekebergh, I. Karlsson, R. Mete, Y. Pan, A. Börje and J. Mårtensson, *Org. Lett.*, 2011, **13**, 4458; (*d*) S. Malla and M. O. A. Sommer, *Green Chem.*, 2014, **16**, 3255.
7 J. McNulty, R. Vemula, C. Bordón, R. Yolken and L. Jones-Brando, *Org. Biomol. Chem.*, 2014, **12**, 255.
8 B. Krivogorsky, P. Grundt, R. Yolken and L. Jones-Brando, *Antimicrob. Agents Chemother.*, 2008, **52**, 4466.
9 R. A. Davis, M. S. Buchanan, S. Duffy, V. M. Avery, S. A. Charman, W. N. Charman, K. L. White, D. M. Shackleford, M. D. Edstein, K. T. Andrews, D. Camp and R. J. Quinn, *J. Med. Chem.*, 2012, **55**, 5851.
10 (*a*) K. M. Maloney and J. Y. L. Chung, *J. Org. Chem.*, 2009, **74**, 7574; (*b*) T. Maegawa, K. Otake, K. Hirosawa, A. Goto and H. Fujioka, *Org. Lett.*, 2012, **14**, 4798; (*c*) A. Samarat, V. Fargeas, J. Villières, J. Lebreton and H. Amri, *Tetrahedron Lett.*, 2001, **42**, 1273.
11 (*a*) A. Yasuhara and T. Sakamoto, *Tetrahedron Lett.*, 1998, **39**, 595; (*b*) S. K. Jackson and M. A. Kerr, *J. Org. Chem.*, 2007, **72**, 1405; (*c*) S. Krishnan, J. T. Bagdanoff, D. C. Ebner, Y. K. Ramtohul, U. K. Tambar and B. M. Stoltz, *J. Am. Chem. Soc.*, 2008, **130**, 13745.
12 (*a*) L. Jones-Brando, E. F. Torrey and R. Yolken, *Schizophr. Res.*, 2003, **62**, 237; (*b*) C. P. Hencken, L. Jones-Brando, C. Bordón, R. Stohler, B. T. Mott, R. Yolken, G. H. Posner and L. E. Woodard, *J. Med. Chem.*, 2010, **53**, 3594.
13 J. McNulty and K. Keskar, *Eur. J. Org. Chem.*, 2011, 6902.

