Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3741–3744
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological evaluation of analogues of the anti-tumor alkaloid naamidine A

Nicholas Aberle,[a,b] Jenny Catimel,[c] Edouard C. Nice[c] and Keith G. Watson[a,*]

[a]*The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Vic. 3050, Australia*
[b]*Department of Medical Biology, The University of Melbourne, Parkville, Vic. 3010, Australia*
[c]*Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, Vic. 3050, Australia*

Received 13 March 2007; revised 4 April 2007; accepted 5 April 2007
Available online 10 April 2007

Abstract—A small series of derivatives of the alkaloid naamidine A was synthesized and tested in vitro for their ability to inhibit mitogenesis in BaF/ERX cells. Replacement of the imidazole core with a thiazole was found to have only a minor effect on potency, and the 4-methoxybenzyl substituent of the natural product was shown to be unnecessary for activity.

Naamidine A (**1**, Fig. 1) is a 2-aminoimidazole alkaloid from the calcareous sponge *Leucetta chagosensis*.[1] An initial report detailing its biological activity indicated the ability of **1** to inhibit epidermal growth factor-dependent mitogenesis (IC_{50} = 11 μM), as determined by a [^{3}H]thymidine incorporation assay.[2] A subsequent report from the same group revealed that treatment of A431 cells with **1** resulted in cell cycle arrest in the G_1 phase, which was attributed to sustained activation of the extra-cellular signal regulated kinase, ERK.[3] This proposed mechanism of action is previously unknown for small molecules, thus raising the potential for **1** being useful either as a molecular probe to learn more about the function of the MAPK signal transduction pathway, or as an anti-cancer therapeutic.

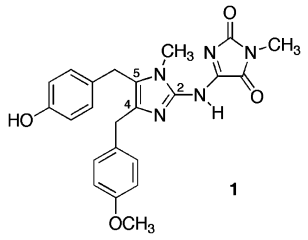
Evaluation of the structure of **1** provides some clear opportunities for the development of a structure–activity relationship. Specifically, we were interested in determining the importance to activity of the three major substituents of the central 2-aminoimidazole ring: the unusual C-2 dehydrohydantoin; the C-4 *p*-methoxybenzyl unit; and the C-5 *p*-hydroxybenzyl unit. Variation of the core heterocycle was also identified as a potential source of more potent analogues and, in particular, replacing the imidazole ring with a thiazole ring appeared to be worth pursuing, given the prevalence of 2-aminothiazole-containing molecules that display useful biological activities.

Keywords: Naamidine A; Structure–activity relationship; 2-Aminoimidazole; 2-Aminothiazole; Anti-tumor; Dehydrohydantoin.
* Corresponding author. E-mail: aberle@wehi.edu.au

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.017

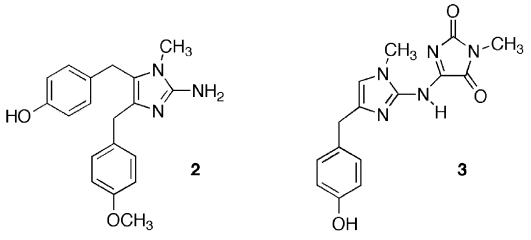
This change from imidazole to thiazole has been performed on another bioactive natural product, girolline, in an effort to minimize undesired side effects of the anti-tumor alkaloid.[4,5] In that instance, the thiazole analogues did not possess the same cytotoxicity.

The IC_{50} values detailed below were obtained by [^{3}H]thymidine incorporation assay, performed on BaF/ERX cells according to the procedure described in Ellis et al.[6]



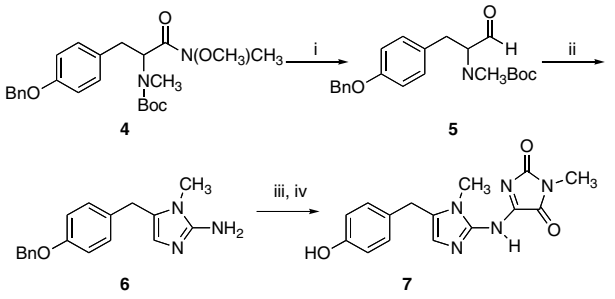
1

Figure 1. Structure of naamidine A (**1**).

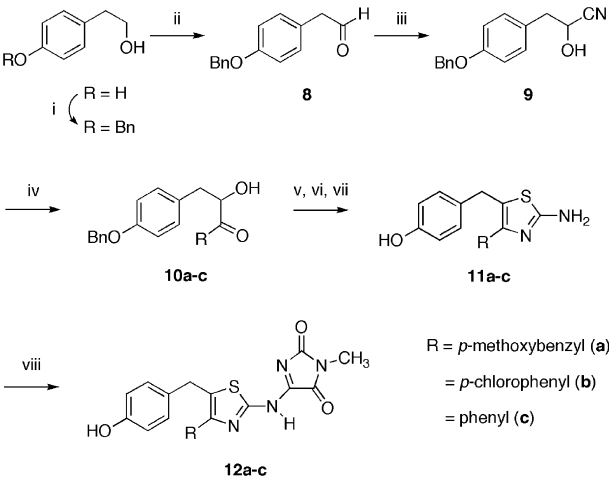


2 **3**

Figure 2. Structures of naamine A (**2**) and clathridine C (**3**).

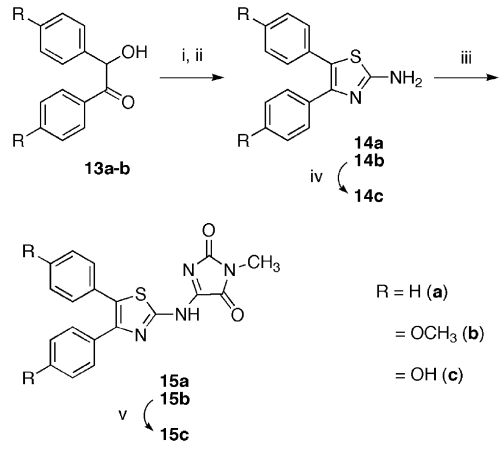


Scheme 1. Synthesis of C-4 deletion analogue. Reagents and conditions: (i) $LiAlH_4$, THF, 0 °C, 30 min, 75%; (ii) a—4 M HCl in Et_2O, 30 min; b—cyanamide, H_2O, pH 4, 90 °C, 45 min, 61%; (iii) H_2, Pd/C (10 wt %), MeOH, 18 h, 95%; (iv) 1-methyl-3-trimethylsilylparabanic acid, toluene, Δ, 18 h, 48%.

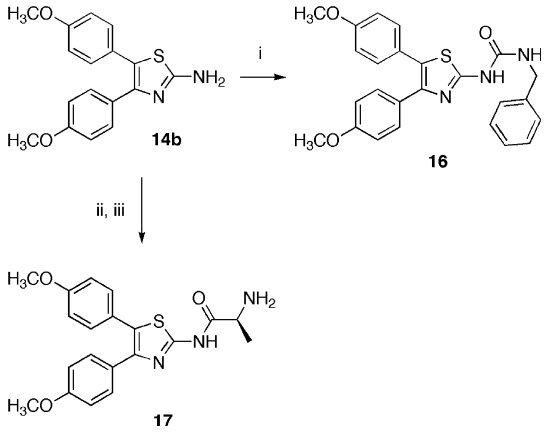


Scheme 2. Synthesis of 2-aminothiazole analogues. Reagents and conditions: (i) BnCl, K_2CO_3, DMF, 65 °C, 24 h, 99%; (ii) Dess-Martin periodinane, CH_2Cl_2, 80 min, 97%; (iii) $NaHSO_3$, EtOAc, H_2O, 1 h, then KCN in H_2O, 48 h, 89%; (iv) TMSOTf, NEt_3, Et_2O, then RMgX, 61–70%; (v) $SOBr_2$, pyridine, toluene, 80 °C, 4 h, 54–69%; (vi) thiourea, dioxane, 70 °C, 4 h, 57–99%; (vii) BCl_3, CH_2Cl_2, 47–52%; (viii) 1-methyl-3-trimethylsilylparabanic acid, toluene, Δ, 18 h, 41–63%.

Naamidine A was obtained via a total synthesis that was previously completed in our laboratory.[7] Analysis in the mitogenic assay revealed an IC_{50} of 3 μM for EGF-dependent mitogenesis and 6 μM for IL3-dependent mitogenesis. The similarity between these values provides an indication that the site of action of **1** is downstream of the cellular receptors, consistent with the proposed mechanism of action.

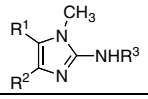


Scheme 3. Synthesis of symmetrical thiazole derivatives. Reagents and conditions: (i) $SOCl_2$, DMF, CH_2Cl_2, 5 h; (ii) thiourea, EtOH, Δ, 18 h, 83–95% over two steps; (iii) 1-methyl-3-trimethylsilylparabanic acid, toluene, Δ, 18 h, 54–83%; (iv) BBr_3, CH_2Cl_2, −78 °C, 1 h, 80%; (v) BBr_3, CH_2Cl_2, −78 °C to rt, 1 h, 8%.



Scheme 4. Variation at 2-position. Reagents and conditions: (i) benzyl isocyanate, toluene, 80 °C, 18 h, 56%; (ii) Boc-L-Ala, HOBt, EDCI, NEt_3, CH_2Cl_2, 18 h, 48%; (iii) TFA, CH_2Cl_2, 25 min, quant.

Table 1. SAR for deletion analogues of naamidine A



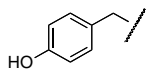
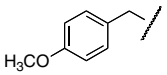
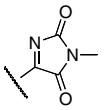
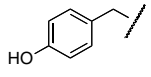
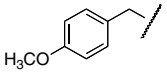
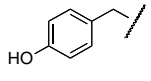
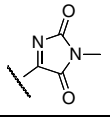
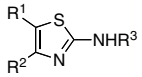
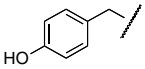
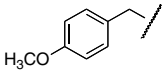
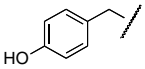
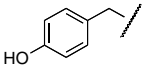
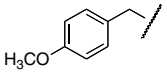
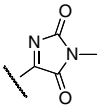
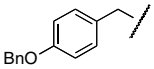
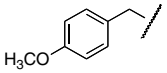
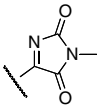
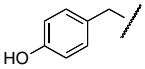
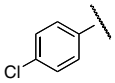
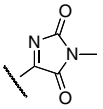
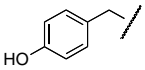
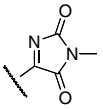
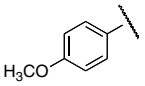
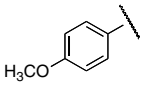
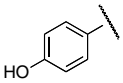
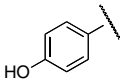
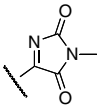
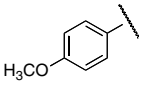
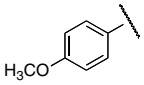
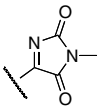
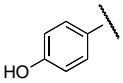
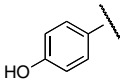
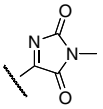
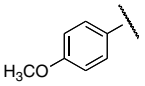
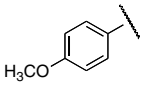
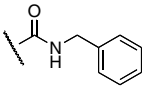
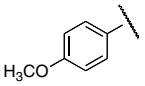
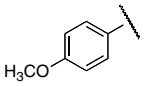
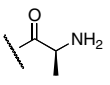
Compound	R^1	R^2	R^3	IC_{50} (μM)
1				3
2			H	75
7		H		4

Table 2. SAR for thiazole analogues of naamidine A



Compound	R^1	R^2	R^3	IC_{50} (μM)
11a			H	18
11c			H	50
12a				10
OBn-12a[a]				>75
12b				15
12c				25
14a			H	>75
14b			H	>75
14c			H	>75
15a				>75
15b				EGF: 50 IL3: 10
15c				5
16				>75
17				12

[a] Obtained as per Scheme 2, without the deprotection step (vii).

A natural deletion analogue of **1** was obtained in the course of the total synthesis. Naamine A (**2**, Fig. 2), the presumed biosynthetic precursor of **1**, lacks the dehydrohydantoin moiety at the 2-amino position of the core heterocycle and was found to be essentially inactive in our assay, with IC_{50} values of 75 μM for both EGF- and IL3-dependent mitogenesis.

Clathridine C (**3**, Fig. 2) is very close to a natural deletion analogue of **1**—the C-5 *p*-hydroxybenzyl has been removed and the C-4 substituent contains a *p*-hydroxy

group rather than the *p*-methoxy. This molecule has been reported to possess only weak cytotoxicity toward a variety of cell lines,[8] indicating the importance of the C-5 substituent, and thus the exact C-5 deletion analogue was not prepared here.

No natural equivalent to the C-4 deletion analogue is known. Its synthesis was achieved from an intermediate in the synthesis of **1**,[7] as shown in Scheme 1. Reduction of Weinreb amide **4** with $LiAlH_4$ provided the aminoaldehyde **5**. Following deprotection of the amine, exposure of the resulting hydrochloride salt to a large excess of cyanamide resulted in formation of the 2-aminoimidazole **6**. Hydrogenolysis of the benzyl ether and installation of the dehydrohydantoin unit through condensation with 1-methyl-3-trimethylsilylparabanic acid[9] provided the C-4 deletion analogue of naamidine A, **7**. Interestingly, the IC_{50} for this structure was found to be 4 μM, indicating that the *p*-methoxybenzyl group at C-4 of the imidazole ring does not contribute to the activity of **1** (Table 1).

To investigate the importance of the 2-aminoimidazole core, as well as to obtain structure–activity relationship data on other elements of the molecule, a small series of 2-aminothiazole derivatives was synthesized. The direct thiazole analogue of **1**, along with a number of other derivatives, was prepared according the sequence shown in Scheme 2, where the choice of Grignard reagent defines the C-4 position of the final heterocycle. The phenol of 4-hydroxyphenethyl alcohol was first protected as the benzyl ether, then the primary alcohol was oxidized to the aldehyde **8** using Dess-Martin periodinane. Treatment with potassium cyanide in the presence of sodium hydrogen sulfite resulted in the cyanohydrin **9**. Conversion of **9** to the α-hydroxyketones **10a**–**c** was achieved through in situ silylation of the hydroxyl group using TMSOTf, followed by addition of the appropriate Grignard reagent.[10,11] After bromination with $SOBr_2$, the α-bromoketones were condensed with thiourea to afford the 2-aminothiazoles, which were deprotected using BCl_3 to give **11a**–**c**. Installation of the dehydrohydantoin unit was performed as above to give the naamidine analogues **12a**–**c**.

A range of symmetrical 2-aminothiazoles was also synthesized using similar methodology, starting from the commercially available α-hydroxyketones benzoin (**13a**) and anisoin (**13 b**, Scheme 3) and proceeding via the α-chloroketones rather than the α-bromoketones.

The final analogues that were prepared in this series are shown in Scheme 4. From the anisoin-derived 2-aminothiazole **14b**, two different side chains were incorporated into the 2-amino position to examine the effect of replacing the dehydrohydantoin.

Each of the 2-aminothiazole derivatives prepared above was subjected to the [^{3}H]thymidine incorporation assay to determine its effect on both EGF- and IL3-dependent mitogenesis. In all cases, except for **15b**, the IC_{50} values for each stimulus were within 5 μM and the listed value is the average (Table 2).

The results presented above demonstrate that replacing the 2-aminoimidazole core of naamidine A with a 2-aminothiazole does not have a major influence on the potency of the molecule: the direct thiazole analogue of **1**, **12a**, was found to have an IC_{50} of 10 μM, which is very similar to the natural product (3 μM), indicating that the $N–CH_3$ component of the heterocycle is not engaged in any interaction with the target. Consistent with the results obtained for **1** and **2**, the dehydrohydantoin moiety was also found to be essential for activity in the thiazole series, compared to the derivatives with unsubstituted amines at C-2. The alanine-derived **17** suggests, however, that a small cluster of heteroatoms at this position may be sufficient, but this effect is lost upon introduction of a larger substituent, such as **16**.

In summary, we have demonstrated that the ability of **1** to inhibit EGF- and IL3-dependent DNA synthesis is not impaired by replacement of the imidazole core by a thiazole. While none of the analogues demonstrated greater potency than the natural product, there is scope for optimization of the side chains based on the initial observations made here. Also, the discovery that the C-4 chain does not contribute to the activity of **1** provides the option of using an affinity reagent in further mechanism of action studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.017.

References and notes

1. Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003.
2. Copp, B. R.; Fairchild, C. R.; Cornell, L.; Casazza, A. M.; Robinson, S.; Ireland, C. M. *J. Med. Chem.* **1998**, *41*, 3909.
3. James, R. D.; Jones, D. A.; Aalbersberg, W.; Ireland, C. M. *Mol. Cancer Ther.* **2003**, *2*, 747.
4. Schiavi, B.; Ahond, A.; Al-Mourabit, A.; Poupat, C.; Chiaroni, A.; Gaspard, C.; Potier, P. *Tetrahedron* **2002**, *58*, 4201.
5. Nay, B.; Schiavi, B.; Ahond, A.; Poupat, C.; Potier, P. *Synthesis* **2005**, 97.
6. Ellis, A. G.; Doherty, M. M.; Walker, F.; Weinstock, J.; Nerrie, M.; Vitali, A.; Murphy, R.; Johns, T. G.; Scott, A. M.; Levitzki, A.; McLachlan, G.; Webster, L. K.; Burgess, A. W.; Nice, E. C. *Biochem. Pharmacol.* **2006**, *71*, 1422.
7. Aberle, N. S.; Lessene, G.; Watson, K. G. *Org. Lett.* **2006**, *8*, 419.
8. Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 1229.
9. Nakamura, S.; Kawasaki, I.; Yamashita, M.; Ohta, S. *Heterocycles* **2003**, *60*, 583.
10. Krepski, L. R.; Heilmann, S. M.; Rasmussen, J. K. *Tetrahedron Lett.* **1983**, *24*, 4075.
11. Gill, M.; Kiefel, M. J.; Lally, D. A. *Tetrahedron Lett.* **1986**, *27*, 1933.