Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, antiviral activity, and SARs of 13a-substituted phenanthroindolizidine alkaloid derivatives

Bo Su [†], Chunlong Cai [†], Meng Deng, Demin Liang, Lizhong Wang, Qingmin Wang [*]

State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 24 February 2014
Revised 11 April 2014
Accepted 23 April 2014
Available online xxxx

Keywords:
Phenanthroindolizidine alkaloid
Antiviral activity
Tobacco mosaic virus
Structure–activity relationship
Anti-TMV

ABSTRACT

On the basis of our previous structure–activity relationship (SAR) and antiviral mechanism studies, a series of 13a-substituted phenanthroindolizidine alkaloid analogues (**3a**–**16a**, **3b**, **4b**, **6b**, **7b**, **10b**, and **14b**) were designed targeting tobacco mosaic virus (TMV) RNA, synthesized, and evaluated for their antiviral activity against TMV for the first time. The bioassay results showed that most of the synthesized compounds (such as **4a**, **6a**, **7a**, **11a**, **14a**, **6b**, and **14b**) exhibited good to excellent antiviral activity against TMV both in vitro and in vivo. Especially, for inactivation effect and curative effect, compounds **4a**, **6a**, **7a**, **11a**, **14a**, and **14b** showed higher activity at both concentrations (500 μg mL^{-1} and 100 μg mL^{-1}) than commercial Ningnanmycin. Preliminary SARs showed that the substituted groups with hydrogen donor at 13a position were found to be favorable for keeping high antiviral activity. The present work demonstrates that 13a-substituted phenanthroindolizidines can be used as possible lead compounds for developing anti-TMV agents.

Tobacco mosaic virus (TMV), one of the well-studied plant viruses in the world, is known to infect members of 9 plant families and at least 125 individual species, which include tobacco, pepper, cucumber, tomato, and many ornamental flowers.[1] Because TMV is very difficult to control, it is also known as 'plant cancer'. It was reported that the loss caused by TMV is up to U.S. $100 million worldwide each year.[2]

Ningnanmycin (Fig. 1) is perhaps the most effective registered plant viral inhibitor, which displayed 56.0% in vivo curative effect at 500 μg mL^{-1}. Ribavirin (Fig. 1) is another widely used anti-plant viral agent, whose inhibitory effect is always less than 50% at 500 μg mL^{-1}. Because of great economic loss caused by TMV and the unsatisfactory inhibitory effect (usually 30–60%) of these antiviral agents, much effort has been paid on the development of novel and high effective plant virucide. Although some chemicals, such as pyrazole derivatives,[3] nucleotides,[4] α-aminophosphonate derivatives,[5] 3-acetonyl-3-hydroxyoxindole,[6] triazolyl compounds,[7] oxidized polyamines,[8] and substituted phenylureas,[9] were reported possessing antiviral activities, few of which has been applied successfully in agriculture.

Compared with synthetic chemicals, natural product-based antiviral agents have many advantages, which include low mammalian toxicity, easy decomposition, friendly to environment, specific to a target species, and unique mode of action etc.[10,11] Phenanthroindolizidine alkaloids are a group of pentacyclic natural products isolated mainly from the *Cynanchum*, *Pergularia*, *Tylophora* species.[12] Diverse biological activities of these alkaloids range from cancer cell growth inhibition and anti-inflammatory activity to antiamoebic and antilupus effects.[13–17] In a program aimed at screening of plants for biologically active natural products as alternatives to conventional synthetic antiviral agents, we first found

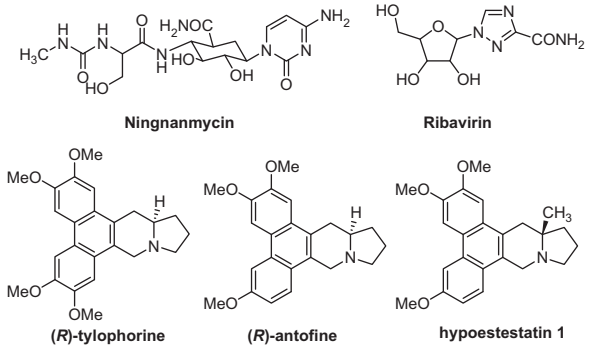

Figure 1. Chemical structures of Ningnanmycin, Ribavirin and representative phenanthroindolizidine alkaloids.

* Corresponding author. Tel./fax:+86 022 23503952.
E-mail addresses: wang98h@263.net, wangqm@nankai.edu.cn (Q. Wang).
† These authors contributed equally.

http://dx.doi.org/10.1016/j.bmcl.2014.04.101
0960-894X/

that the alcohol extract of *Cynanchum komarovii* exhibited moderate antiviral activity against TMV.[18] Further investigation demonstrated that the main active compound was (*R*)-antofine (Fig. 1). Antiviral mechanism studies revealed that antofine has a favorable interaction with the origin of TMV RNA (oriRNA), exhibiting its virus inhibition by binding to oriRNA and interfering with virus assembly initiation.[19] Moreover, the structure–activity relationship studies showed that most compounds of the antofine-based library with structural diversity displayed inhibitory effect against TMV. Hypoestestatin 1 was a structurally novel

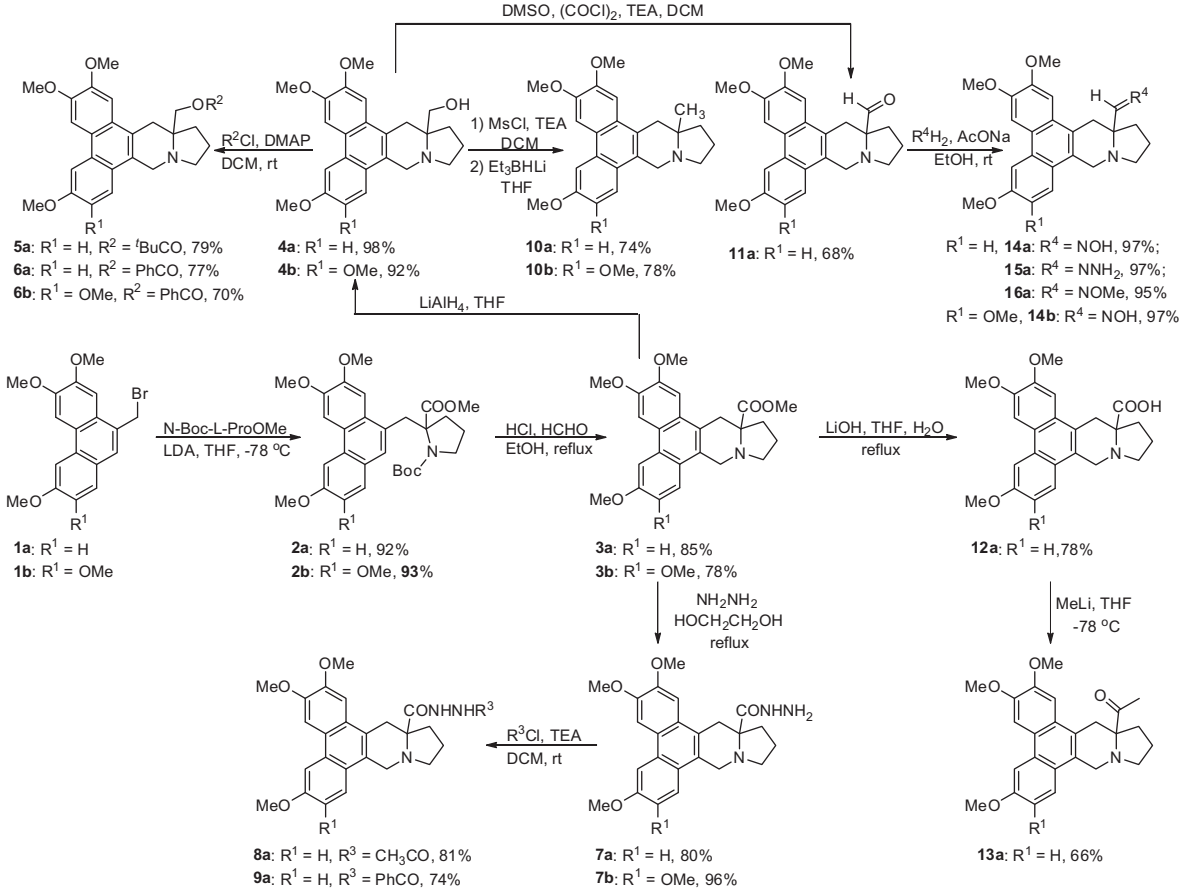

Figure 2. Synthetic route for compounds **2a**–**16a**, **3b**, **4b**, **6b**, **7b**, **10b**, and **14b**.

Table 1
In vitro antiviral activity of compounds **3a**–**16a**, **3b**, **4b**, **6b**, **7b**, **10b**, and **14b** against TMV. Bold values are used to attract readers's attention. These values of the corresponding compounds indicating high bioactivities.

Compd	Concn (μg mL^{-1})	Inhibitory effect (%)	Compd	Concn (μg mL^{-1})	Inhibitory effect (%)
3a	500	54.6	**15a**	500	50.3
	100	30		100	16.8
4a	500	**77.6**	**16a**	500	**60**
	100	**55.6**		100	**28.1**
5a	500	55.8	**3b**	500	40.3
	100	30.4		100	20
6a	500	**65.8**	**4b**	500	38.6
	100	**39.1**		100	21.3
7a	500	**62.6**	**6b**	500	68.2
	100	**35**		100	29.7
8a	500	45.6	**7b**	500	40
	100	20		100	21.6
9a	500	**57.2**	**10b**	500	42.5
	100	**27.6**		100	17.8
10a	500	47.1	**14b**	500	59.6
	100	22.4		100	33.3
11a	500	**55.5**	Ribavirin	500	38
	100	**33.3**		100	12.5
12a	500	43.3	Ningnan mycin	500	69.3
	100	21.3		100	26.8
13a	500	45	(+/−)-Tylophorine	500	50
	100	26.6		100	15.9
14a	500	**66.6**			
	100	**38.1**			

phenanthroindolizidine alkaloid, which has a methyl group at 13a position. It was found that hypoestestatin 1 exhibited distinguished cytotoxic activity ($ED_{50} = 10^{-5}$ μg/mL against murine P-388 cell line),[20] but its anti-virus activity has not been investigated.

In the previous work, the SAR studies mainly focused on the substituted positon and number of methoxyl group on the phenanthrene ring and C-14 substituted devivatives.[21,22] However, modification on the phenanthrene ring or at C-14 position won't change the spatial configuration of the molecules dramatically compared with the 13a-H phenanthroindolizidines. C-13a was positioned at the bridgehead of D/E ring, which has a large effect on the molecular spatial configuration. To study the influence of the substituted group at the C-13a position, aiming at optimizing phenanthroindolizidine alkaloids as antiviral agents, a series of 13a-substituted phenanthroindolizidines, synthesized, and evaluated for their antiviral activity against TMV. The structure–activity relationship study of these compounds against TMV is also discussed.

As shown in Figure 2, pentacyclic ester **3** was prepared via a one-pot deprotection and Pictet–Spengler cyclization from intermediate **2**, which was obtained from readily available N-Boc-L-ProOMe and phenanthryl bromide **1**.[23] After reduction by $LiAlH_4$, compound **3** was converted to alcohol **4** in nearly quantitative yield. The alcohol **4** underwent acylation, dehydroxylation, and oxidation to give compound **5** and **6**, **10**, and **11**, respectively. It was noteworthy that such a seemingly easy transformation from alcohol **4** to compound **10** proved to be difficult, and methods such as Barton–McCombie radical deoxygenation, transforming **4** to bromide followed by reduction, and transforming **4** to aldehyde then deoxygenation under Wolff–Kishner conditions, did not work well. After extensive investigation, the angular methyl group was installed efficiently via a sequential methanesulfonylation and super hydride reduction procedure. Hydrazide **7** was prepared from hydrazinolysis of ester **3**, and high boiling point ethylene glycol was found to be optimal solvent for this reaction. Hydrazide **7** was then converted to bishydrazides **8** and **9** when it was subjected to the corresponding acylating reagents. Ester **3** was hydrolyzed to acid **12** under alkaline conditions, which was then transformed to ketone **13** when treated with 2 equiv of MeLi. Aldehyde **11** was prepared from alcohol **4** under standard Swern-oxidation conditions, but other oxidation reagents, such as Dess–Martin

Table 2
In vivo antiviral activity of compounds **3a**–**16a**, **3b**, **4b**, **6b**, **7b**, **10b**, and **14b** against TMV. Bold values are used to attract readers's attention. These values of the corresponding compounds indicating high bioactivities.

Compd	Concn (μg/mL)	Protection effect (%)	Inactivation effect (%)	Curative effect (%)
3a	500	49.6	52.3	51
	100	28.3	20	22.2
4a	500	**68.4**	**75.3**	**76.2**
	100	**35.1**	**50**	**53.6**
5a	500	53.2	51.6	53.8
	100	32.5	26.3	19.6
6a	500	**67.5**	**66.3**	**62.4**
	100	**34**	**30.4**	**32.8**
7a	500	**60.2**	**62.1**	**61.5**
	100	**31.7**	**30**	**28.4**
8a	500	40.6	43.7	38.3
	100	12.3	16.9	17.5
9a	500	52.3	50	55.9
	100	25.8	22.6	25.3
10a	500	45.4	41.3	43.6
	100	19.8	17.5	23.7
11a	500	**64.2**	**62.7**	**60.3**
	100	**35.1**	**28.4**	**32.8**
12a	500	41.7	37.8	39.8
	100	15.5	16.5	20.1
13a	500	50.6	49.4	53.5
	100	20.2	21.1	17
14a	500	**61.4**	**58.3**	**63.5**
	100	**34.2**	**31.5**	**30.7**
15a	500	47.1	48.5	44.6
	100	14.4	21.4	16.5
16a	500	58.4	56.2	53.8
	100	26.4	32.3	30.6
3b	500	39.2	42.1	41.3
	100	9.5	18.5	15.4
4b	500	43.4	40	45
	100	17.5	15.7	18.2
6b	500	**60.8**	**58.7**	**55.9**
	100	**37.2**	**33.3**	**30**
7b	500	43.5	46.1	41.4
	100	12.2	16.3	12.9
10b	500	40.7	38.9	42.6
	100	6.7	12.4	10.8
11b	500	58.9	55	52.3
	100	25.4	28.1	27.3
14b	500	**64.3**	**62.8**	**60**
	100	**34.1**	**32.4**	**27.6**
Ribavirin	500	37.2	36.5	38.5
	100	13.1	15.2	16.7
Ningnan mycin	500	57.9	54.2	58.7
	100	38.4	20.0	23.1
(+/−)-Tylophorine	500	52.6	48.2	44.8
	100	20.4	18.1	19.7

Please cite this article in press as: Su, B.; et al. *Bioorg. Med. Chem. Lett.* (2014), http://dx.doi.org/10.1016/j.bmcl.2014.04.101

reagent, PCC etc., proved to be less efficient. Compounds **14**, **15** and **16** were prepared by condensation of aldehyde **11** with the corresponding nucleophile hydroxylamine, hydrazine, and methoxylamine in high yields.

To make a judgement of the antiviral potency of 13a-substituted phenanthroindolizidines (**3a–16a**, **3b**, **4b**, **6b**, **7b**, **10b**, and **14b**), the commercial plant virucide Ningnanmycin, Ribavirin, and (+/−)-tylophorine were used as the controls. The in vitro antiviral assay results of all of the synthesized compounds were listed in Table 1. As the control, Ribavirin exhibited 38% and 12.5% inhibitory effect at 500 μg mL^{-1} and 100 μg mL^{-1}, respectively. All of the synthesized compounds showed higher antiviral activities at both concentrations than Ribavirin.

Compared with Ningnanmycin, perhaps the most successful registered plant virucide, compound **4a** exhibited higher activity at both concentrations. Interestingly, although compounds **3a**, **5a**, **6a**, **7a**, **9a**, **11a**, **14a**, **16a**, **6b**, and **14b** showed slightly lower activity than Ningnanmycin at 500 μg mL^{-1}, they displayed higher inhibitory effect at 100 μg mL^{-1}. In other words, these compounds could still keep much potent antiviral activities in low concentration. Although the other synthesized 13a-substitueted compounds **8a**, **10**a, **12a**, **13a**, **15a**, **3b**, **4b**, **7b**, and **10b** were less effective than Ningnanmycin, they showed higher or equivalent inhibitory effect compared with that of representative 13a-H phenanthroindolizidine alkaloid (+/−)-tylophorine. Because all of the synthesized 13a-substitueted phenanthroindolizidines displayed distinguished in vitro antiviral activity against TMV, further investigates of their antiviral activity in vivo were deserved.

The in vivo antiviral activity was assayed in three models (protection, inactivation and curative), and the detailed results were shown in Table 2. All of the synthesized compounds displayed good to excellent in vivo inhibitory effect, especially for compounds **4a**, **6a**, **7a**, **11a**, **14a**, **6b**, and **14b**, which exhibited higher or comparable antiviral activity at three models compared with Ningnanmycin. For inactivation effect and curative effect, compounds **4a**, **6a**, **7a**, **11a**, **14a**, and **14b** showed higher activities at both concentrations (500 μg mL^{-1} and 100 μg mL^{-1}) than Ningnanmycin. At these two models, **6b** was also more active than Ningnanmycin at both concentrations, except for curative effect at 500 μg mL^{-1}. In the protection model, compounds **4a**, **6a**, **7a**, **11a**, **14a**, **6b**, and **14b** displayed higher antiviral activities (from 60.8% to 68.4%) than Ningnanmycin (57.9%) at 500 μg mL^{-1}, but slightly lower at 100 μg mL^{-1}.

Some important structure and activity relationships were also observed. 13a-Substituted phenanthroindolizidine analogues **4a**, **6a**, **7a**, **11a** and **14a** showed much potent inhibitory effect than the corresponding 13a-H member tylophorine, demonstrating that the spatial configuration of 13a-H phenanthroindolizidine alkaloids is not optimal. Compound **4a**, with methylene hydroxyl group at 13a position, displayed much higher antiviral activity than compounds **5a**, **6a** and **10a**. The same tendency was also demonstrated in other compounds (antiviral effect: **7a** > **3a**, **14a** > **15a**, **14a** > **16a**, and **14a** > **11a**), which indicated that a hydrogen donor at 13a position may play an important role for keeping high inhibitory effect. When a methoxyl group was introduced at the C8 position, the activities decreased for most compounds (antiviral effect: **3a** > **3b**, **4a** > **4b**, **7a** > **7b**, and **10a** > **10b**), but some showed considerable or slightly higher activities, for example, compound **14b** displayed higher effect than **14a** at 500 μg mL^{-1}. The results indicated that the methoxy substituent on the phenanthrene unit can also influence the activity and deserves further investigation in the future structure modification.

In summary, on the basis of our previous SAR and antiviral mechanism studies, a series of 13a-substituted phenanthroindolizidines were synthesized and evaluated for their antiviral activity against TMV systematically for the first time. The bioassay results indicated that most of these compounds exhibited good to excellent in vivo anti-TMV activities, of which compounds **4a**, **6a**, **7a**, **11a**, **14a**, **6b**, and **14b**, displayed higher or comparable both in vitro and in vivo antiviral activity compared with Ningnanmycin, thus emerging as potential inhibitors of plant-virus. The substituted group at 13a position and the phenanthrene substitution pattern have great effect on the anti-TMV activity, demonstrating that the spatial configuration of 13a-H phenanthroindolizidine alkaloids is not optimal against TMV and structure optimization at this position is deserving. It was noteworthy that a hydrogen donor at 13a position may play an essential role for keeping high activities. Further studies on structural optimization and mode of action are currently underway in our laboratory.

Acknowledgments

This work was supported by the National Key Project for Basic Research (2010CB126106), the National Natural Science Foundation of China (21132003, 21121002, 21372131), and the Specialized Research Fund for the Doctoral Program of Higher Education (20130031110017).

Supplementary data

Supplementary data (experimental details and compound characterization for all synthesized compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.101.

References and notes

1. Hari, V.; Das, P. Ultra Microscopic Detection of Plant Viruses and Their Gene Products. In *Plant Disease Virus Control*; Hadidi, A., Khetarpal, R. K., Koganezawa, H., Eds.; APS Press: St. Paul, MN, 1998; pp 417–427.
2. Bos, L. *Trends Microbiol.* **2000**, *8*, 82.
3. Ouyang, G. P.; Cai, X. J.; Chen, Z.; Song, B. A.; Bhadury, P. S.; Yang, S.; Jin, L. H.; Xue, W.; Hu, D. Y.; Zeng, S. *J. Agric. Food Chem.* **2008**, *56*, 10160.
4. Reichman, M.; Devash, Y.; Suhadolnik, R. J.; Sela, I. *Virology* **1983**, *128*, 240.
5. Chen, M. H.; Chen, Z.; Song, B. A.; Bhadury, P. S.; Yang, S.; Cai, X. J.; Hu, D. Y.; Xue, W.; Zeng, S. *J. Agric. Food Chem.* **2009**, *57*, 1383.
6. Li, Y. M.; Zhang, Z. K.; Jia, Y. T.; Shen, Y. M.; He, H. P.; Fang, R. X.; Chen, X. Y.; Hao, X. J. *Plant Biotechnol. J.* **2008**, *6*, 301.
7. Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705.
8. Bachrach, U. *Amino Acids* **2007**, *33*, 267.
9. De Meester, C. *Mutat. Res.* **1995**, *339*, 139.
10. Qian, X. H.; Lee, P. W.; Cao, S. *J. Agric. Food Chem.* **2010**, *58*, 2613.
11. Seiber, J. N. *J. Agric. Food Chem.* **2011**, *59*, 1.
12. Gellert, E. *J. Nat. Prod.* **1982**, *45*, 50.
13. Gellert, E.; Rudzats, R. *J. Med. Chem.* **1964**, *7*, 361.
14. Damu, A. G.; Kuo, P. C.; Shi, L. S.; Li, C. Y.; Kuoh, C. S.; Wu, P. L.; Wu, T. S. *J. Nat. Prod.* **2005**, *68*, 1071.
15. Yang, C. W.; Chen, W. L.; Wu, P. L.; Tseng, H. Y.; Lee, S. J. *Mol. Pharmacol.* **2006**, *69*, 749.
16. Baumgartner, B.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O. *Phytochemistry* **1990**, *29*, 3327.
17. Choi, J. Y.; Gao, W.; Odegard, J.; Shiah, H. S.; Kashgarian, M.; McNiff, J. M.; Baker, D. C.; Cheng, Y. C.; Craft, J. *Arthritis Rheum.* **2006**, *54*, 3277.
18. An, T. Y.; Huang, R. Q.; Yang, Z.; Zhang, D. K.; Li, G. R.; Yao, Y. C.; Gao, J. *Phytochemistry* **2001**, *58*, 1267.
19. Xi, Z.; Zhang, R. Y.; Yu, Z. H.; Ouyang, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4300.
20. Pettit, G. R.; Goswami, A.; Cragg, G. M.; Schmit, J. M.; Zou, J. C. *J. Nat. Prod.* **1984**, *47*, 913.
21. Wang, K.; Su, B.; Wang, Z.; Wu, M.; Li, Z.; Hu, Y.; Fan, Z.; Mi, N.; Wang, Q. *J. Agric. Food Chem.* **2009**, *58*, 2703.
22. Wang, Z.; Wang, L.; Ma, S.; Liu, Y.; Wang, L.; Wang, Q. *J. Agric. Food Chem.* **2012**, *60*, 5825.
23. Su, B.; Cai, C.; Wang, Q. *J. Org. Chem.* **2012**, *77*, 7981.