Tetrahedron Letters 51 (2010) 485–488

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Modification of poorly bioactive sinomenine into more potent immunosuppressive agents by embedding of drug-like fragments

Yang-Tong Lou [a], Hai-Bin Zhou [a], Jia Zou [b], Ling-Chen Yan [b], En-Guan Bi [b], Bing Sun [b,*], Zhu-Jun Yao [a,*]

[a] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China
[b] Shanghai Institute of Biochemistry and Cell Biology, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 219 Yueyang Road, Shanghai 200031, China

ARTICLE INFO

Article history:
Received 23 September 2009
Revised 27 October 2009
Accepted 6 November 2009
Available online 4 December 2009

Keywords:
Sinomenine
Alkaloid
Drug fragment
Modification
Immunomodulating activity

ABSTRACT

Embedment of drug-like heterocyclic moieties was successfully employed in the novel modification of the readily available but poorly bioactive natural alkaloid sinomenine. Application of the newly proposed approach afforded a number of more potent sinomenine-like molecules with a significantly high hit rate. Among these new analogous, up to 500-fold increase of in vitro immunosuppressive activity was achieved. Further biological experiments of representative compound **4b** indicated that it might inhibit NF-κB activation induced by TNF-α in a dose dependent way and showed remarkable in vivo treatment effects against the mouse experimental autoimmune uveoretinitis (EAU) disease models.

For many decades, numerous bioactive products isolated from various natural resources have influenced modern drug discovery across the therapeutic spectrum.[1] However, a significant portion of these agents, which are naturally abundant and economically available, exhibit very weak biological potency and usually lack the qualities needed for further pharmaceutical development. Sinomenine (**1**, Fig. 1), an alkaloid isolated from the Chinese medicinal plant *Sinomenium acutum*, is a typical example of these cases. It has been used in China and other Asian countries as an immunomodulating and anti-inflammatory ingredient of 'Feng-Tong-Ning' (loosely translated from the Chinese as 'Ache-free')[2] with application for treating rheumatoid arthritis disease (RA) for thousands of years. However, sinomenine exhibits unsatisfactory immunomodulating activity and its mechanism in treating rheumatoid arthritis is ambiguous.[3–5] As a useful strategy for enriching the chemical entities with pharmaceutical properties, it would be of great value to convert the large number of less potent natural products, including sinomenine, to more biologically potent compounds with appropriate chemical modifications.

Though quite a number of modifications upon the skeleton of sinomenine have been attempted previously, limited successes have been achieved (Fig. 1).[6] In order to open a new route to successful modification on sinomenine, new insights and chemistries are needed. It is believed that plant-derived metabolites are not naturally 'made' for human chemotherapeutic uses. Instead, they are more likely produced for the physiological demands of their own originating organisms or for meeting the requirement of their environment changes. To merge such a gap between the disparate targets of plant-derived natural product and human drugs, fine tuning of the natural structure of sinomenine with appropriate chemistry could potentially prove to be highly productive. In order to improve biological activities and drug-like properties, we under-

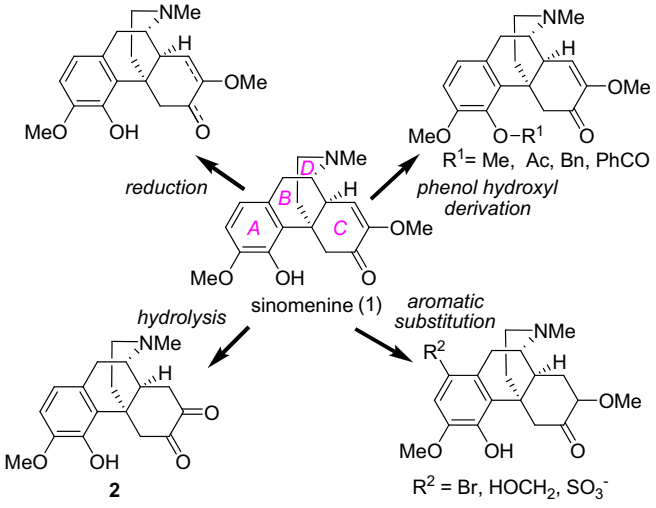


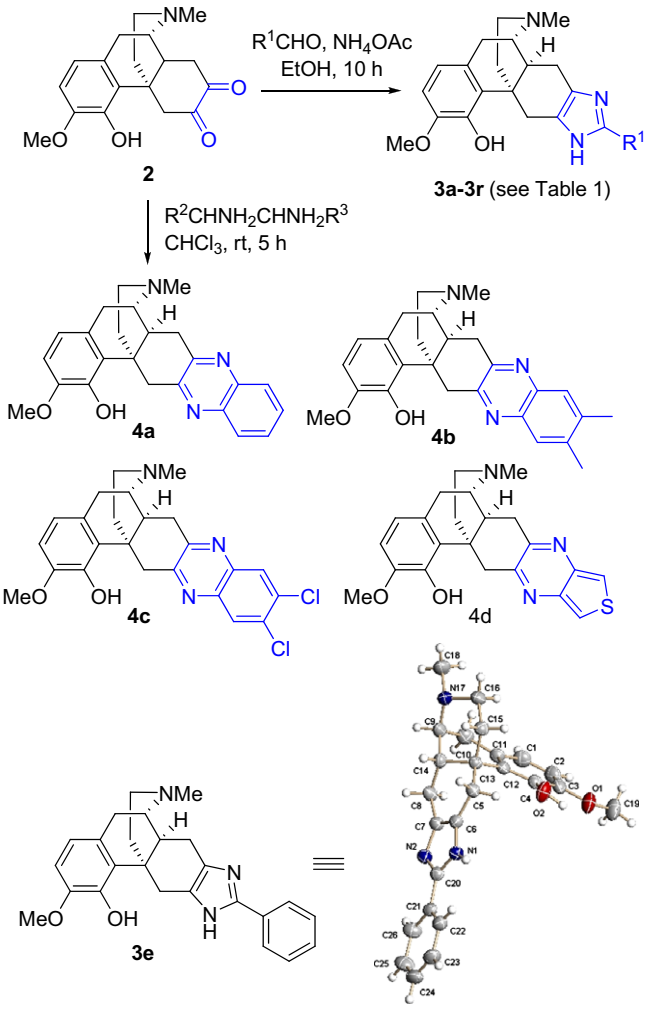
Figure 1. Previous representative modifications of sinomenine.

* Corresponding authors. Tel.: +86 021 54925133; fax: +86 21 54925124 (Z.-J.Y.).
E-mail addresses: bingsun@sib.ac.cn (B. Sun), yaoz@mail.sioc.ac.cn (Z.-J. Yao).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.11.019

took embedding into sinomenine certain fragments, such as small nitrogen-containing heterocycles, which are frequently employed in modern drug design. In this work, we wish to report the results of our recent efforts with the above principles,[7] including parallel preparation of new sinomenine derivatives, examination of the immunosuppressive activities, and primary assessment of a representative compound in treatment of autoimmune diseases using an animal model.

After comprehensive analysis of the natural structure of sinomenine and its previous modifications, we devised a synthetic protocol directed at the C-ring of **1** that could potentially yield new derivatives with 'human-friendly' heterocyclic fragments. To circumvent previously patented derivatives, vicinal diketone **2**,[8] a hydrolysis product of sinomenine, was employed as the starting material for the current study. Starting from diketone **2**, two classes of sinomenine derivatives **3** (fusing with an imidazole-ring to the C-ring) and **4** (fusing with a pyrazine-ring to the C-ring) were efficiently prepared in parallel (Scheme 1, Table 1). Three-component reactions of **2** with a variety of aldehydes and ammonium acetate in EtOH[9] gave the products **3**, whose structure was confirmed by an X-ray single crystallography study of the representative compound **3e**, in satisfactory yields. The pyrazine derivatives **4** were synthesized by the direct reaction of **2** with the symmetrical vicinal diamines under mild conditions.[10] Attempts using unsymmetrical vicinal diamines (**4**, R^2/R^3) gave poor regioselectivity. Most of the resulting regiomeric products were not separable using the chromatographic methods.



Scheme 1. Synthesis of imidazole (**3**) and pyrazine (**4**) derivatives of sinomenine.

Using natural sinomenine as a comparative control, immunosuppressive activities of newly synthesized derivatives were examined using in vitro ConA-induced T lymphocyte proliferation and the LPS-induced B lymphocyte proliferation models. In these assays, cyclosporine A (CsA) served as a positive control.[11] Inhibitory effects on cell proliferation by sinomenine derivatives were summarized in Table 1. The IC_{50} data show that all the newly synthesized derivatives exhibit more potent immunosuppressive activities as compared with the natural parent sinomenine (IC_{50} = 600 μM for T cells, and IC_{50} = 538 μM for B cells). Some analogues (**3g**, **3h**, **3n**, **3q**, **3r**, **4b**, and **4c**) showed dramatic increases in their immunosuppressive activities with IC_{50} in low micromolar range (1–10 μM). These data clearly indicate that our concept and strategy worked well in the modification of sinomenine. Among the most potent compounds, some exhibited similar inhibitory effects against the proliferation of both B cells and T cells (**3g**, **3n,** and **4c**), and several were found to be more effective in inhibiting the proliferation of T cells (**3l**, **3o,** and **3q**). This meant that they might have different pathways leading to their immunomodulating activities. The best T/B selectivity (42.6 times) is afforded by the furan compound **3o**, although it presents only moderate activity against the proliferation of T cells (IC_{50} 15.2 μM). Imidazole derivative **3q**, bearing 3-pyridinyl functionality, was identified as the most potent compound in this study, with an IC_{50} value of 1.17 μM against the proliferation of T cells. This compound represents over a 500-fold increase and shows a greatly improved safety index (SI = 47.9, based on its actions on T cells) compared to sinomenine (SI = 1.39).

The high hit rate undoubtedly suggests that the strategy of embedding a rigid lipophilic drug-like hetereocyclic moiety into the C-ring of sinomenine generally improves the immunosuppressive activity. In this work, introduction of an additional aromatic ring significantly enhances the bioactivity (**3e**–**r**, **4c,** and **4e**), although more complicated substituent effects were also observed. For derivatives having an aliphatic substituent, quite different inhibitory effects were exhibited. For example, compounds **3b** and **3d** are much more potent than compounds **3a** and **3c**. As compared with the dimethyl compound **4b**, the dichloro compound **4c** acquires only a small increase in its immunosuppressive activity. More interestingly, minor differences in the structures of compounds **3k**, **3l**, and **3m** result in a reversal of action selectivity between the two types of lymphocyte cells.

Since TNF-α mediated signaling pathways are highly associated with many inflammatory diseases (including RA, etc.),[12,13] we further examined whether these sinomenine derivatives could inhibit the TNF-α signaling pathways or not. Hela cells seeded in six-well flat-bottomed plates were treated with 0.4 ng/mL TNF-α with or without small molecules (after comprehensive consideration of biological potency, water solubility and cytotoxicity, compounds **4b**, **3q**, and **3h** were chosen). Cells were harvested for western blot analysis. As showed in Figure 2A, compound **4b** clearly blocks the degradation of Iκb-α in a dose dependent way, while compound **1** and **3q** showed little effect on it and compound **3h** had only slight improvement on it. Although compound **3q** exhibited the highest potency in the experiments of T cells inhibition (Table 1), it did not present satisfactory effects to interact with TNF-α. Such a result mentions that **3q** might take an alternative pathway to inhibit the growth of T cells. To explain these complicated results, further biological work will be required in the future.

Unambiguously, among the above examined compounds, compound **4b** could most effectively inhibit the NF-κB activation induced by TNF-α. The inhibitory effect of **4b** was then further confirmed by experimental autoimmune uveoretinitis (EAU), an in vivo inflammatory eye disease model.[14] EAU is an organ-specific, Th1-cell-mediated disease model that targets the neural retina.[15] It is characterized by ocular changes. To the best of our knowledge,

Table 1
In vitro inhibitory effects of sinomenine and its derivatives on spleen lymphocyte proliferation induced by mitogens[a,b,c,d,e]

Compounds		Yields (%)	IC_{50} (10^{-6} M)		CC_{50} (10^{-6} M)
			T cells	B cells	
1	—	—	600	538	835
2	—	—	253	149	280
3a	$R^1 = CH_3$	67	384	352	130
3b	$R^1 = CH_3CH_2$	75	22.3	81.0	43.1
3c	$R^1 = (CH_3)_2CHCH_2$	76	476	111	138
3d	$R^1 = CH_3(CH_2)_5CH_2$	80	11.4	70	13.5
3e[f]	$R^1 = C_6H_5$	79	10.7	15.4	15.9
3f	$R^1 = p$-Et-C_6H_4	74	16.7	67.5	12.3
3g	$R^1 = p$-Cl-C_6H_4	75	5.29	5.96	15.0
3h	$R^1 = o$-Br-C_6H_4	84	6.98	7.73	35.8
3i	$R^1 = m$-NO_2-C_6H_4	82	18.8	191	45.3
3j	$R^1 = o$-CF_3-C_6H_4	71	28.7	48.2	36.3
3k	R^1 = 3,4-$(MeO)_2C_6H_3$	85	32.7	16.7	42.5
3l	R^1 = 2,4-$(MeO)_2C_6H_3$	75	17.8	80.0	20.9
3m	R^1 = 4-HO-3,5-$(MeO)_2C_6H_2$	72	143	26.0	ND
3n	R^1 = 1-naphthyl	75	7.15	5.10	14.3
3o	R^1 = 2-furanyl	85	15.2	648	275
3p	R^1 = 2-thiophenyl	78	41.9	41.1	32.5
3q	R^1 = 3-pyridinyl	86	1.17	20.5	56.0
3r	R^1 = 2-quinolinyl	68	5.47	6.10	17.4
4b	—	79	8.91	14.0	24.6
4c	—	75	3.16	2.73	11.3
4d	—	49	50.6	63.7	155

[a] Cyclosporin A (CsA) was used as positive control with IC_{50} 7.15 nM (T cells) and 34.1 nM (B cells).
[b] Inhibition of ConA-induced T lymphocyte proliferation.
[c] Inhibition of LPS-induced B lymphocyte proliferation.
[d] Fresh spleen cells obtained from C57BL/6 mice (6–8 weeks old) were used as cytotoxicity assay (MTT method). For the details, see Supplementary data.
[e] All the compounds were assayed in the form of free amine.
[f] The structure was confirmed by the X-ray single crystallography data (see Supplementary data); CC: cytotoxicity; ND = not determined.

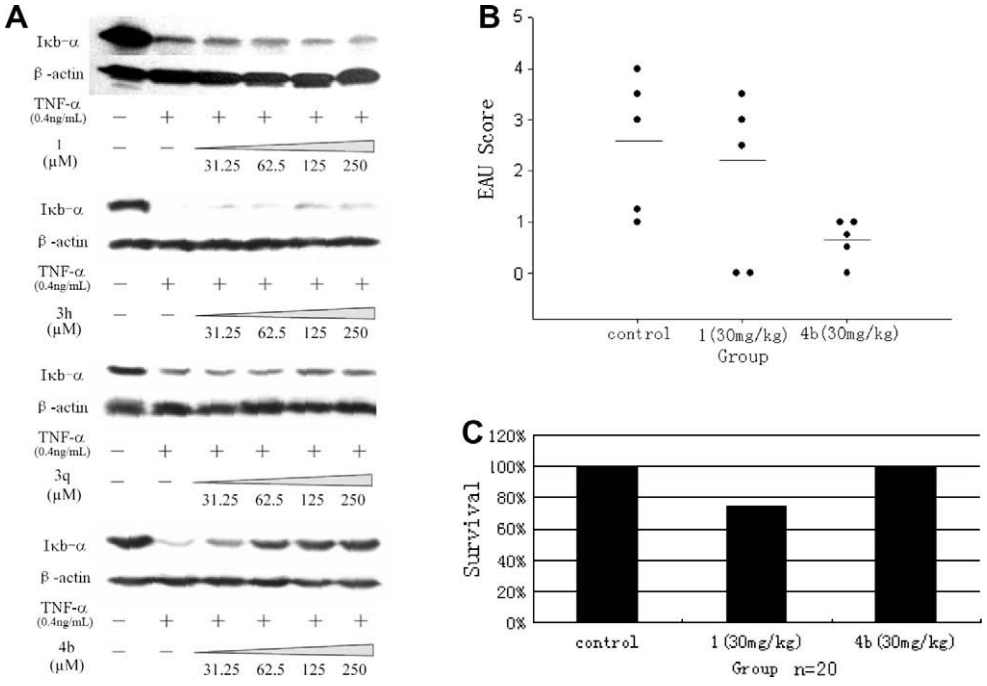


Figure 2. Treatment effects of **4b** in an EAU mouse model. (A) Compound **4b** blocks the TNF-α induced Iκb-α degradation. Hela cells were treated with TNF-α 0.4 ng/mL and cpds **1**, **3h**, **3q**, **4b** were added as indicated. **4b** blocks the degradation of Ikb-a in a dose dependent way, while **1**, **3h**, **3q** showed little effect on it. (B) EAU disease model was induced and the treatment effect of **1** and **4b** was evaluated. The EAU score was significantly reduced in **4b** treated mice. (C) Survival rate of mice after treatment. All the compounds were assayed in the form of HCl salt.

very few small-molecule drugs have been developed for the amelioration of EAU, an animal model for panuveitis. In this assay, compound **1** and **4b** were chosen to examine effects against EAU in vivo (Fig. 2B). As shown, the EAU score was significantly reduced in **4b** treated mice (at 30 mg/kg dosage) as compared with that treated with sinomenine **1**. Obviously, treatment with **4b** greatly improved the conditions of EAU mice, thereby showing therapeutic potential for further drug development.

In summary, embedment of drug-like heterocyclic moieties was successfully employed in the novel modification of the readily

available but poorly bioactive natural product sinomenine. The relatively simple chemistry employed by the newly proposed approach afforded a number of more potent sinomenine-like molecules with a significantly high hit rate. Among the analogues, up to 500-fold increase of in vitro immunosuppressive activity was achieved. Biological experiments also indicated that compound **4b** might inhibit NF-κB activation induced by TNF-α in a dose dependent way and showed remarkable in vivo treatment effects in mouse EAU disease models. Further optimization of the potent sinomenine derivatives and development of their potential therapeutic applications, as well as detailed mechanisms of action, are currently underway in our laboratory and will be reported in due course.

Acknowledgments

This project is financially supported by NSFC (90713044), MOH (2009ZX09501), CAS (KSCX2-YW-R23, KJCX2-YW-H08) and SHMCST (08431903000). The authors also thank Dr. Kevin Pan and Dr. Terrence R. Burke, Jr. for their helpful discussions and comments for this work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.019.

References and notes

1. Wermuth, C. G. *The Practice of Medicinal Chemistry*, 2nd ed.; Academic Press: San Diego, 2003.
2. Knorr, L.; Horlein, H. *Chem. Ber.* **1907**, *40*, 4883.
3. Wang, Y.; Fang, Y.; Huang, W.; Zhou, X.; Wang, M.; Zhong, B.; Peng, D. *J. Ethnopharmacol.* **2005**, *98*, 37–43.
4. Zhao, Y.; Li, J.; Yu, K. Q.; Liu, Y.; Chen, X. G. *Int. J. Immunopharmacol.* **2007**, *7*, 637–645.
5. Ou, Y. Q.; Chen, L. H.; Li, X. J.; Lin, Z. B.; Li, W. D. *Acta Pharmacol. Sinica* **2009**, *30*, 435–441.
6. Previous modifications on sinomenine, see: (a) Wu, F., Feng, X., Wu, K., Cheng, G., Huang, Y., Ye, X., Qiu, P., Zheng, X., CN1876634-A.; (b) Luo, J., Zeng, H., Li, Y., Yin, S., CN1948291-A.; (c) Whittall, J., Mather, P., WO2004022564-A3 (AU2003263301-A8).; (d) Qin, G., Tang, X., Wang, R., Zhou, T., Lestage, P., Caignard, D., Renard, P., WO2004048340-A1 (CN1504469-A).; (e) Kaken, S., JP8301778-A.; (f) Ibuka, T.; Kitano, M. *Chem. Pharm. Bull. (Tokyo)* **1967**, *15*, 1944–1947; (g) Shionogi & Co. Ltd. JP64021845-B.; (h) Shionogi & Co. Ltd. JP65010153-B.; (i) Shionogi & Co. Ltd. JP65010217-B.; (j) Wu, F., Zheng, X., Qiu, P., Qiu, S., CN1995019-A.; (k) Pan, Y., Li, Y., Bu, Q., Huang, L., Wang, J., Li, J., CN1785977-A.; (l) Pan, Y., Li, Y., Bu, Q., Huang, L., Wang, J., Li, J., CN1785976-A.; (m) Christians, U., Kaever, V. W., WO9942105-A (US6372756-B1).
7. (a) Yao, Z. J., Zhou, H. B., CN1687065-A, CN1298718-C (ZL 2005 1 0024478.9).; (b) Yao, Z. J., Zhou, H. B., CN1687070-A, CN1298720-C (ZL 2005 1 0024479.3).
8. Goto, K.; Shishido, H. *Bull. Chem. Soc. Jpn.* **1935**, *10*, 597–600.
9. (a) Debus, H. *Ann. Chem. Pharm.* **1858**, *107*, 199–208; (b) Wolkenberg, S. E.; Wisnoski, D. D.; Leister, W. H.; Wang, Y.; Zhao, Z.; Lindsley, C. W. *Org. Lett.* **2004**, *6*, 1453–1456.
10. Derivative **4a** was firstly reported in the literature work: Fleischhacker, W.; Viebock, F.; Zeidler, F. *Monatsh. Chem.* **1970**, *101*, 1215–1222.
11. Chan, C. C.; Caspi, R. R.; Leake, W.; Wiggert, C. B.; Chader, G. J.; Nussenblatt, R. B. *J. Autoimmun.* **1990**, *3*, 247–255.
12. Saxne, T.; Palladino, M. A.; Heinegard, D., Jr.; Talal, N.; Wollheim, F. A. *Arthritis Rheum.* **1988**, *31*, 1041–1045.
13. (a) Kitamura, M.; Iwabuchi, K.; Kitaichi, N.; Kon, S.; Kitamei, H.; Namba, K.; Yoshida Denhardt, K. D. T.; Rittling, S. R.; Ohno, S.; Uede, T.; Onoe, K. *J. Immunol.* **2007**, *178*, 6567–6572; (b) Liu, X.; Lee, Y. S.; Yu, C. R.; Egwuagu, C. E. *Immunology* **2008**, *180*, 6070–6076.
14. (a) Caspi, R. R.; Roberge, F. G.; Chan, C. C.; Wiggert, B.; Chader, G. J.; Rozenszajn, L. A.; Lando, Z.; Nussenblatt, R. B. *J. Immunol.* **1988**, *140*, 1490–1495; (b) Caspi, R. R.; Chan, C. C.; Wiggert, B.; Chader, G. J. *Curr. Eye Res.* **1990**, *9*, 169–174.
15. Fox, G. M.; Kuwabara, T.; Wiggert, B.; Redmond, T. M.; Hess, H. H.; Chader, G. J.; Gery, I. *Clin. Immunol. Immunopathol.* **1987**, *43*, 256–264.