Tetrahedron Letters 50 (2009) 2932–2935

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Studies toward the duocarmycin prodrugs for the antibody prodrug therapy approach

Lian-Sheng Li, Subhash C. Sinha *

Department of Molecular Biology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

ARTICLE INFO

Article history:
Received 5 February 2009
Revised 24 February 2009
Accepted 30 March 2009
Available online 5 April 2009

ABSTRACT

A tricyclic precursor for the synthesis of the prodrugs of *pro*-1,2,9,9a-tetrahydrocyclopropa[*c*]benz-[*e*]indole-4-one tetramethoxyindolecarboxamide (CBI-TMI) was prepared using the ring-closing metathesis approach. The tricyclic intermediate was converted to an advanced precursor of a CBI-TMI prodrug equipped with a linker presumably suitable for activation using the aldolase catalytic antibody 38C2. An attempted 38C2-catalyzed two-step activation of the hydroxy-*pro*-CBI intermediate involving *retro*-aldol and the β-elimination reactions was also examined.

Selective delivery of cytotoxins to tumor and cancer-associated cells without affecting the normal cells remains one of the important unmet goals.[1] We are developing several alternative approaches, including the chemical programing[2] of the catalytic aldolase antibody (Ab) 38C2[3] using inhibitors of the cancer-associated antigens/receptors, and use of the chemically programmed Ab 38C2 (*cpAb* or *cp38C2*)[4] and inhibitors to selectively deliver a cytotoxin to tumor site. In one approach, we are using chemically labeled Ab 38C2 (*clAb* or *cl38C2*), that possess both the catalytic and the cell-targeting properties for a selective prodrug activation at the tumor site.[5] The other counterpart of this approach is a prodrug that should be nontoxic and efficiently activated using Ab 38C2 or the *cl38C2* to afford the therapeutically effective cytotoxins for the desired outcome. Unfortunately, all prodrugs available so far lack these criteria, mainly due to a combination of the drugs examined and that the prodrugs could not be activated efficiently, and they cannot be advanced to preclinical studies.[6,7] With new doxorubicin prodrugs, we have made some improvements with respect to their toxicities and the activation rates, but they might also not meet the desired criteria. We argue that the desired prodrugs should (1) have sub- or low nanomolar cytotoxic drug counterpart, (2) be at least 1000–10,000-fold less toxic than the drug used, (3) have low nanomolar binding to the catalyst, viz. *cl38C2*, and (4) be activated rapidly. With these hypotheses, we are seeking new prodrugs from a highly potent cytotoxin, such as CBI-TMI (**1**).[8]

CBI-TMI (**1**) is a synthetic analog[9] of the naturally occurring antitumor antibiotic, duocarmycin SA (**2**)[10] (Fig. 1). CBI (1,2,9,9a-tetrahydro-cyclopropa[*c*]-benz[*e*]indole-4-one) derivatives are both chemically stable, and also synthetically easily accessible. Like the parent molecule, CBI-TMI selectively binds and alkylates duplex DNA at the N-3 position of adenines in the minor groove.[11] Figure 1 also shows structures of several *seco*-CBI-TMI derivatives (**3–8**), some of which were previously prepared and evaluated.[12] These *seco*-CBI-TMIs are indeed the prodrug form of the corresponding active CBI-TMI drugs, as the latter are produced in situ before reacting with DNA. In fact, most duocarmycin analogs and prodrugs were developed on this principle in that the cyclopropane ring was generated by Winstein cyclization[13] of the chloromethyl group or an analogous reactive function and facilitated by the free phenolic hydroxy or amine functional groups in **3–5**. Similarly, the isomeric compounds **6–8** could also undergo cyclization reaction affording **1** or its corresponding imine analogs. Because these com-

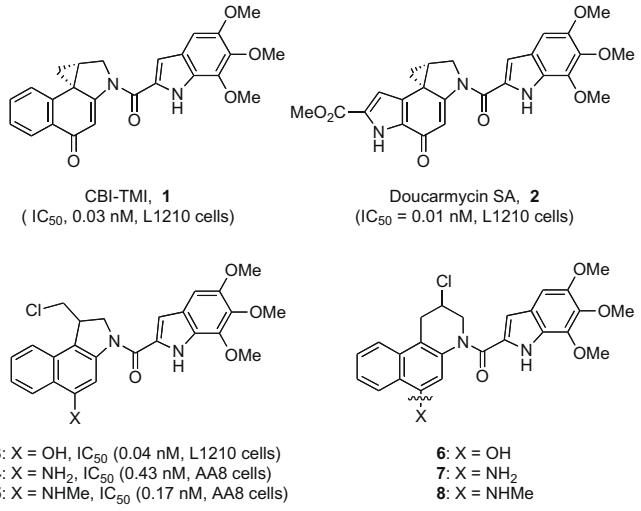


Figure 1. Structure of duocarmycin, its non-natural analog, CBI-TMI and its precursors.

* Corresponding author. Tel.: +1 858 784 8512; fax: +1 858 784 8732.
E-mail address: subhash@scripps.edu (S.C. Sinha).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.03.205

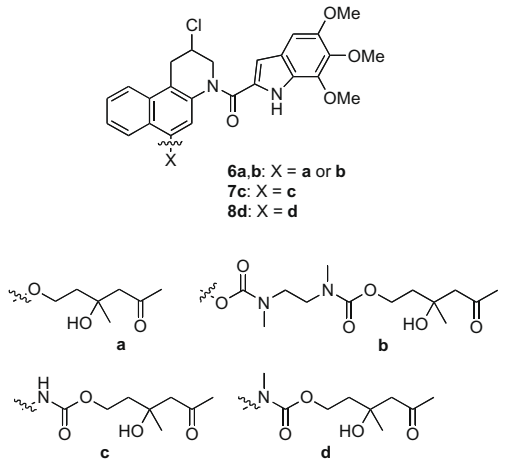
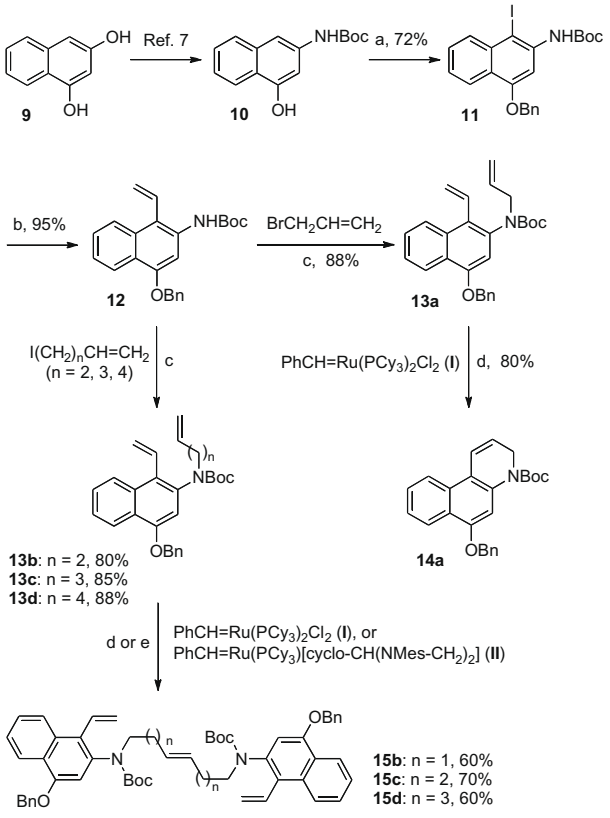


Figure 2. Structure of the proposed prodrugs of CBI-TMI precursors that may be activated using antibody 38C2-catalyzed retro-aldol and β-elimination reactions.

pounds could cause indiscriminate toxicity to normal cells, we decided to explore a prodrug approach for their delivery.[14] In this strategy, a prodrug functionalized with a linker can be selectively activated using tumor-associated proteases (TAPs)[15] or a non-endogenous enzyme, including catalytic Ab.[16] Here, we report our preliminary study toward the synthesis of a CBI-TMI prodrug **6a** and its activation using Ab 38C2.[17]

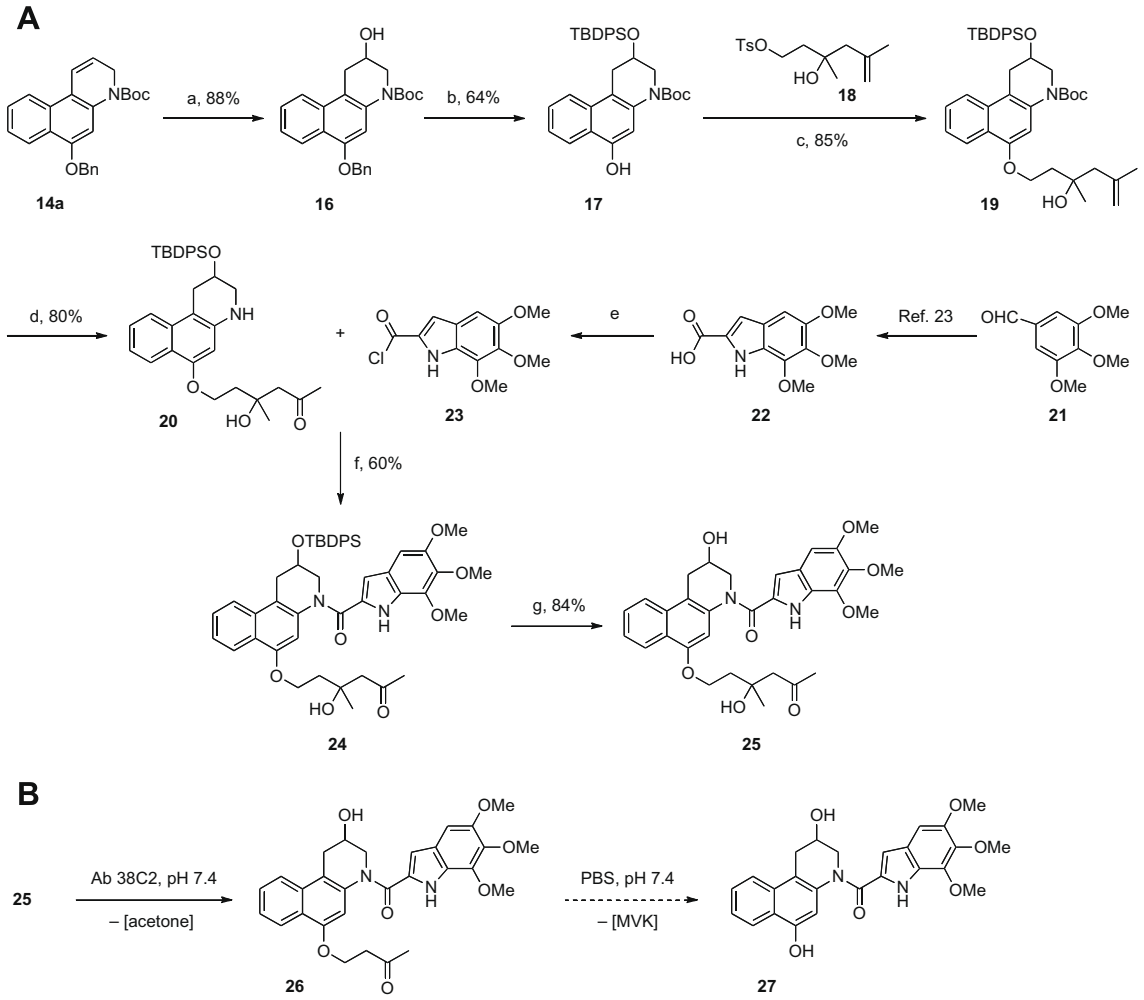
Figure 2 shows structures of the CBI-TMI prodrug, **6a**, as well as several designed analogs, **6b**, **7c**, and **8d**, as the viable candidates of the Ab 38C2-catalyzed activation. Like the previously studied prodrugs of doxorubicin, etoposide, camptothecin, and enediynes, these prodrugs also possessed an 'aldol-Michael' linker, which on treatment with Ab 38C2 would start the activation by the *retro*-aldol reaction. The resultant labile intermediates would undergo β-elimination to give **6** from **6a**. Other prodrugs would continue subsequent transformation, including the decarboxylation reaction and urea formation, under the physiologic conditions to give **6**, **7**, or **8**, which should serve as the precursor of compound **1** or its imine analogs.



Scheme 1. Synthesis of tricyclic intermediate (**14a**) by RCM approach. Reagents and conditions: (a) (i) BnBr, K_2CO_3, Bu_4NI, DMF, (ii) NIS, *p*-TsOH, THF. MeOH; (b) $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_4$ (5 mol %), 2,6-di-*tert*-butyl-4-methyl phenol, toluene; (c) NaH, DMF; (d) for compound **14a**: Grubbs cat **I** (10 mol %), CH_2Cl_2; (e) for compounds **15b–d**: Grubbs cat II (10 mol %), CH_2Cl_2.

We anticipated that prodrug **6a** could be readily prepared by modifying the previously described synthesis of **1** via a tricyclic intermediate, **14a**.[18] However, we designed an alternative route using the Grubbs ring-closing metathesis (RCM) reaction[19] for the synthesis of **14a** (Scheme 1). Thus, the required diene precursor **13a** for the RCM reaction was prepared starting with the readily available phenol **10**.[20] The latter was synthesized from a commercial compound, 1,3-dinaphthol, **9**, in three steps, including reaction of compound **9** with 4-methoxybenzyl amine (PMB-amine) to give 3(4-methoxybenzyl)amino-1-naphthol, deprotection of the latter compound under acidic conditions using H_2SO_4 and TFA, and Boc protection of the resulting free amine, 2-amino-1-naphthol, using Boc anhydride, as described by Gieseg et al. Compound **10** was next protected using benzylbromide and K_2CO_3 in the presence of tetrabutyl-ammonium iodide, and the resulting product was iodinated using NIS[21] to yield the iodonaphthalene derivative **11**. The latter product underwent Pd-catalyzed Stille coupling reaction[22] with tributylvinylstannane to afford the vinylnaphthalene derivative **12**. N-Alkylation of compound **12** with allylbromide using NaH in DMF provided the requisite diene **13a** for the RCM step that was accomplished using the Grubbs catalyst **I** affording the tricyclic compound **13a** in good yield. Encouraged by this result, we examined whether the above RCM approach could be utilized to prepare the ring expanded analogs of **14a** (7-, 8-, and 9-membered cyclic alkenes) and their corresponding prodrugs. Accordingly, dienes **13b–d** were prepared by treatment of **13** with 4-iodo-1-butene, 5-iodo-1-pentene, and 6-iodo-1-hexene, respectively, and then submitted to the RCM reactions using Grubbs catalysts **I** or **II**. Somewhat surprisingly, only the dimerization products **15b–d** were obtained in good yields under the attempted conditions presumably due to the increased ring strain in 7- to 9-membered tricyclic olefin products.

With the intermediate **14a** in hand, synthesis of the desired precursor **25** of the prodrug **6a** progressed as shown in Scheme 2, via a coupling of amine **20** with the acid chloride **23**. Here, amine **20** was prepared from the intermediate **14a** and the acid chloride **23** was obtained from the commercially available aldehyde **21**. Thus, intermediate **14a** was hydroborated using $BH_3{\cdot}SMe_2$ and the hydroborated product was oxidized with H_2O_2/NaOH giving alcohol **16**. The free hydroxyl group in **16** was protected as TBDPS ether, and the benzyl group was removed by the Pd-catalyzed hydrogenolysis affording intermediate **17**. Compound **17** was alkylated with tosylate **18** and cesium carbonate in the presence of 18-Crown-6 to give compound **19**. The latter product underwent OsO_4-catalyzed dihydroxylation and subsequent oxidative cleavage of the resulting diol with $Pb(OAc)_4$ to afford an aldol product. The Boc group in the latter compound was removed using TFA/CH_2Cl_2 to give a free amine compound **20**. Separately, chlorotrimethoxyindolecarboxylate, **23**, was prepared by a usual acyl chloride forming reaction of the readily available acid **22** (prepared from aldehyde **21**)[23] with oxalyl chloride. Next amine **20** was coupled with acid chloride **23** affording amide **24**, and the TBDPS protecting group in the latter product was removed using HF·Py giving the desired alcohol precursor, **25**, of the prodrug **6a**. We expected that compound **25** should be converted to **6a** using CCl_4 and PPh_3, however to our surprise, no such reaction took place.



Scheme 2. (A) Synthesis of an immediate precursor of the CBI-TMI prodrug **6a**. Reagents and conditions: (a) $BH_3.SMe_2$, THF, 0 °C then H_2O_2, NaOH, 60 °C; (b) (i) TBDPSCl, imidazole, DMF, 0 °C to rt, (ii) H_2, Pd/C (10%), MeOH, rt; (c) Cs_2CO_3, 18-crown-6, CH_3CN, rt; (d) (i) OsO_4, NMO, acetone, H_2O, rt, (ii) $Pb(OAc)_4$, CH_2Cl_2, rt; (iii) TFA, CH_2Cl_2, 0 °C; (e) $(COCl)_2$, DMF (cat.), benzene; (f) Et_3N, THF, 0 °C to rt; (g) HF·Py, THF, 0 °C to rt. (B) Attempted Ab 38C2-catalyzed activation of the prodrug analog **25**.

While we were yet to develop a suitable method for the conversion of **25** to **6a**, we examined the 38C2-catalyzed activation of the former as a model to give phenol **27** via a Michael-type adduct, **26**. Thus, compound **25** (100 μM) was incubated with a catalytic amount of antibody 38C2 (5 μM) at 37 °C overnight, and was then analyzed by LCMS analysis. The LCMS showed the formation of the retro-aldol intermediate **26** along with the remaining **25** in approximately 3:2 ratio. However, the subsequent β-elimination of **26** to give **27** was very slow, which was surprising because the β-ketoalkylether of a phenolic drug was reported to undergo β-elimination reaction to produce free drug under the physiologic conditions. Moreover, antibody 38C2 was also known to catalyze β-elimination reaction of β-alkoxy-ketones and aldehydes to give free alcohol or phenol and the α,β-unsaturated aldehydes or ketones. Whereas there is no clear explanation why intermediate **26** does not undergo β-elimination reaction to produce **27** at pH 7.4 (PBS buffer), the inertness of the former intermediate to antibody 38C2 can be due to the steric hindrance of the substrate. Presumably, the ketone linker in intermediate **26** is very close to the bulky drug molecule, and out of the reach of the reactive lysine residues in the antibody 38C2 binding sites. Therefore the antibody could not catalyze β-elimination reaction in intermediate **26**. This result may not be surprising, because a compound bearing a longer linker is activated faster than one coupled to an analogous shorter linker, as found in our recent studies on doxorubicin prodrugs. These observations suggested that new CBI prodrugs equipped with longer or more active linker should be conceived for an effective release of the free drug when antibody 38C2 will be used as a catalyst. In particular, the linker should be conjugated to parent molecule **6** through the carbamate function rather than the ethereal function. Therefore, now we are focusing on new prodrugs of **6–8**, such as **6b**, **7c**, and **8d**, which are more likely to be activated by Ab 38C2.

In conclusion, we have developed an efficient RCM approach to the synthesis of the CBI skeleton, which can be applied to synthesize a wide variety of CBI analogs and CBI-derived prodrugs. In our approach, the prodrugs would possess appropriate linkers that are susceptible to aldolase antibodies or a tumor-associated protease. The preliminary activation study with the precursor of our CBI-TMI prodrug showed that the ethereal linker was not suitable for an effective release of the drug using Ab 38C2. Further study along this line as well as development of new prodrugs that can be activated using Ab 38C2 or TAPs are in progress and will be described in a due course.

Acknowledgments

We thankfully acknowledge the Skaggs Institute for Chemical Biology and National Cancer Institute (R01CA120289) for the financial support.

Supplementary data

Supplementary data (spectroscopic data and experimental procedures) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.205.

References and notes

1. Singh, Y.; Palombo, M.; Sinko, P. J. *Curr. Med. Chem.* **2008**, *15*, 1802.
2. Rader, C.; Sinha, S. C.; Popkov, M.; Lerner, R. A.; Barbas, C. F., III *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5396.
3. Wagner, J.; Lerner, R. A.; Barbas, C. F., III *Science* **1995**, *270*, 1797.
4. Guo, F.; Das, S.; Mueller, B. M.; Barbas, C. F., III; Lerner, R. A.; Sinha, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11009.
5. Abraham, S.; Guo, F.; Li, L.-S.; Rader, C.; Liu, C.; Barbas, C. F., III; Lerner, R. A.; Sinha, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5584.
6. Sinha, S. C.; Li, L.-S.; Miller, G. P.; Dutta, S.; Rader, C.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3095.
7. Shamis, M.; Lode, H. N.; Shabat, D. *J. Am. Chem. Soc.* **2004**, *126*, 1726.
8. Boger, D. L.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 7996.
9. MacMillan, K. S.; Nguyen, T.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 1187.
10. Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037.
11. Boger, D. L.; Garbaccio, R. M. *Acc. Chem. Res.* **1999**, *32*, 1043.
12. Atwell, G. J.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1493.
13. Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567.
14. Tietze, L. F.; Schuster, H. J.; Schmuck, K.; Schuberth, I.; Alves, F. *Biorg. Med. Chem.* **2008**, *16*, 6312.
15. de Groot, V. F. M. H.; Scheeren, H. W. *Actualite Chim.* **2004**, 17.
16. Wojcik, T.; Kiec-Kononowicz, K. *Curr. Med. Chem.* **2008**, *15*, 1606.
17. Shabat, D.; Rader, C.; List, B.; Lerner, R. A.; Barbas, C. F., III *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6925.
18. Boger, D. L.; McKie, J. A.; Boyce, C. W. *Synlett* **1997**, 515.
19. Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117.
20. Gieseg, M. A.; Matejovic, J.; Denny, W. A. *Anticancer Drug Des.* **1999**, *14*, 77.
21. Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271.
22. McKean, D. R.; Parrinello, G.; Renaldo, A. F.; Stille, J. K. *J. Org. Chem.* **1987**, *52*, 422.
23. Fukuda, Y.; Itoh, Y.; Nakatani, K.; Terashima, S. *Tetrahedron* **1994**, *50*, 2793.