Tetrahedron Letters 50 (2009) 3031–3034

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Design and synthesis of novel pinacolylboronate containing combretastatin 'antimitotic agent' analogues

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ARTICLE INFO

Article history:
Received 25 February 2009
Revised 31 March 2009
Accepted 1 April 2009
Available online 7 April 2009

Keywords:
Antimitotic agent
Combretastatin
Stilbene
Pinacolatoborane
TGF-beta

ABSTRACT

We developed a procedure to synthesize pinacolyl boronate containing stilbene derivatives and used this procedure to synthesize boron-containing combretastatin analogues. The key step involves the Wittig reaction of the ylide 4-(4,4,5,5-tetramethyl-1,3,2-dioxaboratophenyl)-methyl triphenylphosphonium bromide **11** with 3,4,5-trimethoxy benzaldehyde in the presence of ^{t}BuONa in DMF, providing 88% yield. We are now in a position to evaluate the biological activity of these derivatives as modulators of TGF-beta signaling pathways.

For an ongoing chemical biology project involving the study of the TGF-beta (Transforming Growth Factor-beta) pathways using developing zebrafish embryos, we synthesized a small library of 2-substituted 2*H*-chromene derivatives, and after screening, we identified a lead molecule **BT7** capable of modulating a specific relevant pathway, namely p-SAPK/JNK, that is known to be downstream of TGF-beta and can mediate Smad-independent signaling.[1,2] To increase the activity and potency of our lead molecule, and for structure/activity relationship studies, we envisioned developing a boron-based stilbene-containing small molecule library (Fig. 1) based on a hypothesis that (a) introducing chromene isosteres like stilbene may increase potency and (b) introducing a boron atom into a biologically active framework, might allow interaction with a target protein not only through hydrogen bonds but also through covalent bonds, and this interaction would produce potent biological activity, and (c) from a literature search we also found that combretastatin A modulates TGF-beta signaling pathway. Therefore, we considered if different CA4 analogues could provide additional insight into TGF-beta signaling pathways to identify new gene targets or co-factors. In this context we undertook a project to develop synthetic methodology to synthesize boron-containing stilbene derivatives, and use those methods to synthesize CA4 analogues.

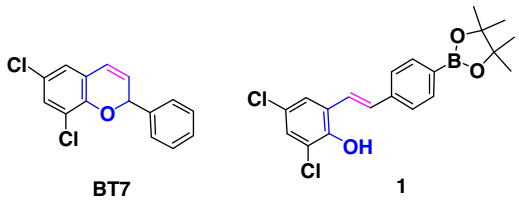

Figure 1. Structure of BT7.

Stilbene (1,2-diphenylethene) represents a prototype of a 1,2-disubstituted olefin. Stilbene itself does not occur in nature, but substituted stilbenes (Fig. 2) such as pinosilvin **2**, resveratrol **3**,[3,4] diethylstilbestrol **4**,[5] 4,4-diaminostilbene-2,2-disulfonic acid,[6] nitro-substituted stilbene boronate pinacol esters,[7] arotinoids[8], combretastatin **5**, **6**[9] (Fig. 2) are among the many synthetic and natural stilbene derivatives that are biologically active compounds.

The use of boron atoms in pharmaceutical drug design possesses a high potential for discovery of new biological activity.[10–13] Among various boron compounds synthesized, much attention has been paid to boronic acid containing peptides such as Velcade and DPP-IV inhibitors.[12] In these boropeptides, a carboxylic acid has been replaced by a boronic acid group.

Combretastatin A-4 (CA-4) has been found to be a potent inhibitor of tubulin polymerization through binding to the colchicine binding site. It is also a cytotoxic agent toward a wide variety of human cancer cell lines and also modulates TGF-beta signaling

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0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.04.003

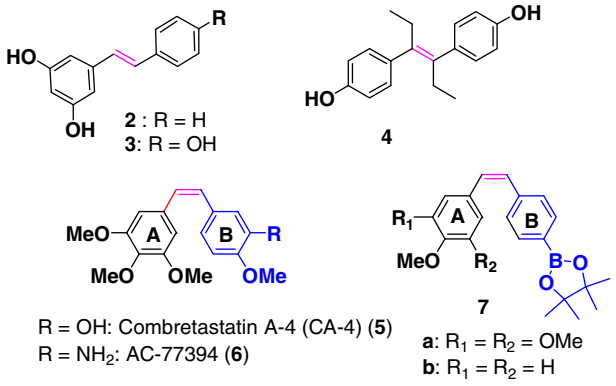


Figure 2. Biologically important stilbene derivatives.

pathways.[14] From a structure/activity relationship point of view, CA-4 belongs to the class of natural compounds related to biphenyls and contains, as a key structural feature, the cis-stilbene motif.

Due to the strong biological significance of stilbene-containing compounds, a general applicable synthetic protocol to synthesize these highly demanding molecules with boronic acid groups would be useful. The protocol should tolerate a large number of functional groups in different positions on the aromatic ring. The most familiar and general strategy for synthesis of boron-containing stilbenes **8** is based on disconnection **A** (Fig. 3) and involves the Wittig reaction of the various substituted benzyl phosphonium ylide **9** with the pinacol ester of boronate aldehyde **10**.[15]

Although Wittig and Horner–Wadsworth–Emmons reactions have been carried out on aldehyde derivatives of boronate esters[16,17] the problem with this approach (**A**) for the synthesis of a library of boron-containing stilbene derivatives is the need to utilize various substituted benzyl phosphonium ylides and boron-containing aldehydes. Boron-containing aldehydes are very prone to self-dimerization and oxidation and are unstable in air, so this method is limited in scope. To overcome this problem, we undertook the disconnection **B** approach (Fig. 3), envisaging the use of the pinacolylboronate phosphonium ylide **11**, which has not previously been explored at all. However, organotrifluoroborato phosphonium ylides are used to synthesize alkene derivatives by Wittig reactions.[18]

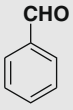
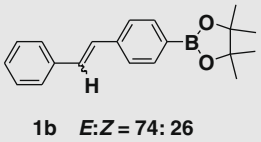
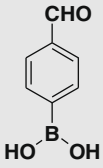
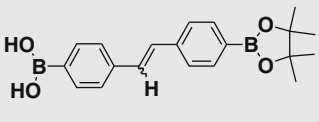
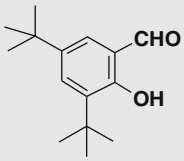
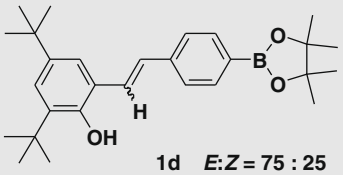
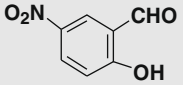
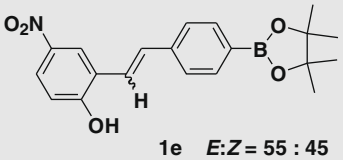
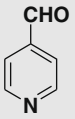
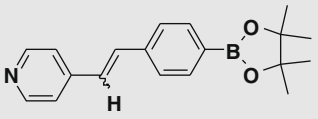
To synthesize our model compound **1**, we first synthesized compound **11**. 4-(4,4,5,5-tetramethyl-1,3,2 dioxaboratophenyl)-methyl triphenylphosphonium bromide **11** was synthesized from 2-[4′-(bromomethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **13** in the presence of 1.01 equiv of triphenylphosphine in acetonitrile at reflux conditions. The minor excess PPh_3 was removed from the product by trituration with ether 2–3 times and the product is stable under normal atmospheric conditions (Scheme 1).

Compound **13**, was synthesized by refluxing **14**, NBS (N-bromosuccinimide) and AIBN (azobisisobutyronitrile) in carbon tetrachloride for six hours to provide the intermediate **13** (85% yield).[19]

In this study, we found that 4-(4,4,5,5-tetramethyl-1,3,2-dioxaboratophenyl)-methyl triphenylphosphonium bromide **11**, when treated with 2-hydroxy-3,5-dichloro benzaldehyde as a model substrate in the presence of 3 equiv of sodium tert-butoxide in DMF as solvent at room temperature produced the 2,4-dichloro-6-{2-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl]-vinyl}-phenol **1** in 86% yield with a mixture of *E* and *Z* isomers (*E*:*Z* = 42:58) (Scheme 2).

After successfully synthesizing our target compound **1**, next we examined the scope of the Wittig reaction for the synthesis of pinacolylboronate-substituted stilbenes using various aryl aldehydes.[20] The results are summarized in Table 1. The reaction proved tolerant of both electron-withdrawing (NO_2; Table 1, entry 4) and electron-donating groups ($B(OH)_2$, di-*t*-butyl; Table 1, en-

Table 1
Wittig reaction of boronate ylide (**11**) with various aldehydes[a]

Entry	Aldehyde	Product	Yield[b] (%)
1		 **1b** *E*:*Z* = 74: 26	86
2		 **1c** *E*:*Z* = 64 : 36	72
3		 **1d** *E*:*Z* = 75 : 25	71
4		 **1e** *E*:*Z* = 55 : 45	76
5		 **1f** *E*:*Z* = 100 : 0	78

[a] All reactions were performed using 1 equiv of aldehyde and 3 equiv of ^tBuONa in DMF for 2–12 h.
[b] Isolated yield (mixture of *E* and *Z*) refers to aldehyde and the *E*/*Z* ratios were determined by ^1H NMR.

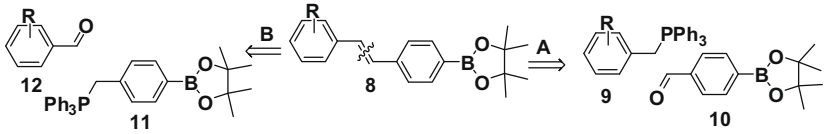


Figure 3. Retrosynthetic analysis.

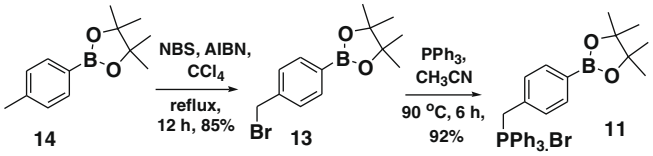
tries 2 and 3) on the phenyl ring of the aldehyde. For the Wittig reaction of aryl aldehydes containing a heteroatom such as nitrogen (Table 1, entry 5) gave the product **1f** with good yield with high *E* selectivity.

Next we focussed on target compound CA4 analogues **7a** and **7b**, bearing a boronic acid system that replaces the OMe group of the natural CA-4 in ring B (Fig. 2). To synthesize **7a** and **7b**, the ylide **11** was reacted with aldehydes **12a** and **12b**. Wittig reaction between the aldehyde **12a** and ylide **11** in the presence of ^{t}BuONa, afforded the corresponding products **7a** and **7b** with 88% and 82% yields, respectively. The products were a mixture of *E* and *Z* isomers (Scheme 3). As R_f value is very close to each other, it was very difficult to separate them, but from TLC we found R_f 0.69 is considerably more fluorogenic than R_f 0.76 (1:1 ethyl acetate/hexane; solvent system).

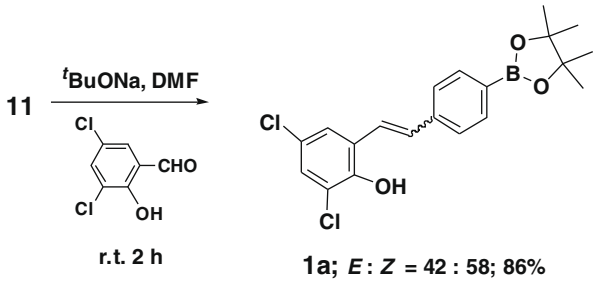
Moreover, we have also developed a one-pot Wittig reaction for synthesizing the compound **7a**. In this case, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaboratophenyl)methyltriphenylphosphonium bromide **11**, the intermediate generated by reaction of **13** with triphenylphosphine, was reacted directly with 3,4,5-trimethoxy benzaldehyde **12a** in the presence of ^{t}BuONa as a base, and the desired boron-containing combretastatin analogue **7a** was isolated in 74% overall yield as a one-pot Wittig reaction (Scheme 4).

To compare our approach B with the approach A in (Fig. 3), we synthesized the **7a** starting from **16** using aldehyde **17** via the Wittig ylide **15** (Scheme 5), and found our approach gives higher yields with high stereoselectivity.

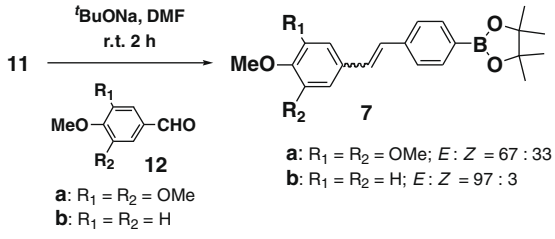
In summary, we developed a procedure to synthesize pinacolylboronate containing stilbene derivatives and used this procedure to synthesize boron-containing combretastatin analogues. Experiments are currently underway to separate the E and *Z* isomers and test the biological activity of these derivatives and to determine their utility as modulators of TGF-beta signaling pathways.



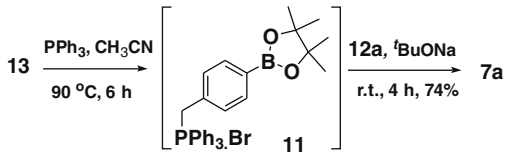
Scheme 1. Synthesis of boronate ylide **11**.



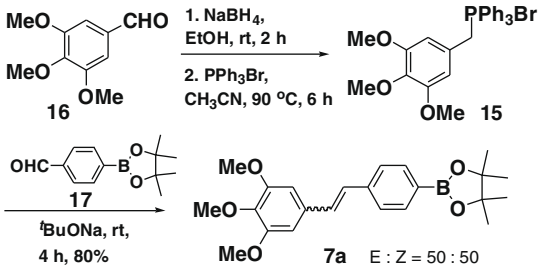
Scheme 2. Synthesis of model compound **1**.



Scheme 3. Synthesis of boronate containing combretastatin analogues **7a**–**b**.



Scheme 4. One-pot synthesis of **7a**.



Scheme 5. Synthesis of **7a** using approach A.

Acknowledgments

The author B.C.D. is thankful to AECOM for start up funding. T.E. is supported by grants from the NIH (HL56182 and HL64282). S.M.M. is thankful to K. Hema for initiating this project. The instrumentation in the AECOM Structural NMR Resource is supported by the Albert Einstein College of Medicine and in part by Grants from the NSF (DBI9601607 and DBI0331934), the NIH (RR017998) and the HHMI Research Resources for Biomedical Sciences.

Supplementary data

Supplementary data (experimental procedures and copies of ^{1}H, ^{13}C NMR) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.003.

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20. *General procedure for the synthesis of stilbenes* (Table 1): A flask was equipped with a magnetic stirring bar, a septum inlet, charged with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaboratophenyl)methyltriphenylphosphonium bromide (**11**) (1 mmol), dry DMF (5 mL), and ^{t}BuONa (3 mmol) under nitrogen. The mixture was stirred at room temperature for 5–10 min. To this solution was added aldehyde (1 mmol) and the resulting mixture was then stirred at room temperature for 4–6 h. The reaction mixture was treated with water (20 mL) and was neutralized with 1 M HCl and the product was extracted with ethyl acetate (3 × 10 mL) washed with brine and dried over Na_2SO_4. The product was isolated by chromatography over silica gel.