Bioorganic & Medicinal Chemistry 25 (2017) 3206–3214

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Ortho group activation of a bromopyrrole ester in Suzuki-Miyaura cross-coupling reactions: Application to the synthesis of new microtubule depolymerizing agents with potent cytotoxic activities

John T. Gupton [a,*], Scott Yeudall [a], Nakul Telang [a], Megan Hoerrner [a], Ellis Huff [a], Evan Crawford [a], Katie Lounsbury [a], Michael Kimmel [a], William Curry [a], Andrew Harrison [a], Wen Juekun [a], Alex Shimozono [a], Joe Ortolani [a], Kristin Lescalleet [a], Jon Patteson [a], Veronica Moore-Stoll [a], Cristina C. Rohena [b], Susan L. Mooberry [b], Ahmad J. Obaidullah [c], Glen E. Kellogg [c], James A. Sikorski [d]

[a] Department of Chemistry, University of Richmond, Richmond, VA 23173, USA
[b] Department of Pharmacology and Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
[c] Department of Medicinal Chemistry & Institute of Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, VA 23298, USA
[d] Chemistry and Drug Discovery, Chesterfield, MO 63005, USA

ARTICLE INFO

Article history:
Received 24 February 2017
Revised 30 March 2017
Accepted 5 April 2017
Available online 11 April 2017

Keywords:
Suzuki-Miyaura cross-coupling
Pyrrole
Microtubule inhibitors
Marine natural products
Cytotoxic activity

ABSTRACT

New microtubule depolymerizing agents with potent cytotoxic activities have been prepared with a 5-cyano or 5-oximino group attached to a pyrrole core. The utilization of ortho activation of a bromopyrrole ester to facilitate successful Suzuki-Miyaura cross-coupling reactions was a key aspect of the synthetic methodology. This strategy allows for control of regiochemistry with the attachment of four completely different groups at the 2, 3, 4 and 5 positions of the pyrrole scaffold. Biological evaluations and molecular modeling studies are reported for these examples.

1. Introduction

The efficient and regiocontrolled preparation[1] of highly functionalized pyrroles continues to be a very active area of research as the result of biologically important pyrrole[2] containing natural products and their synthetic counterparts. Suzuki-Miyaura cross-coupling reactions offer an excellent synthetic opportunity to functionalize brominated pyrroles regiochemically, but such reactions are known to be problematic when the pyrrole nitrogen is unsubstituted as demonstrated by Handy and coworkers[3] (Scheme 1). In this example, reduction of the organometallic intermediate becomes a significant side reaction.

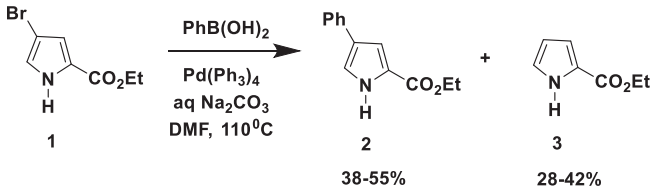
Handy and coworkers have also shown[3] that when the nitrogen is substituted with various nitrogen protecting groups, much better yields (70–80% range) are obtained for these cross-coupling transformations. Buchwald and co-workers[4] have reported the successful use of two new precatalysts for such cross-coupling reactions as applied to various nitrogen unsubstituted heterocycles (indoles and pyrazoles). However, no examples of *N*-unsubstituted pyrroles were presented.

We recently reported[5] a partial solution to this problem by the use of a 5-formyl group to activate such 4-bromo-2-carbethoxypyrroles (Scheme 1, **1**) for successful Suzuki-Miyaura cross-coupling reactions. Additionally, our research groups have a long term interest[6] in exploring highly functionalized pyrroles as colchicine site microtubule depolymerizers and we now report the application of this ortho activation methodology to the synthesis of some new and uniquely functionalized, unsymmetrical pyrroles, which exhibit significant cytotoxic actions.

The biological activities of compounds **4a** and **4b** (Fig. 1) were recently reported.[7] Compound **4a** was particularly interesting, since it displayed potent activity as a colchicine site microtubule depolymerizer with low nanomolar antiproliferative and cytotoxic activities against multiple cancer cell lines. In contrast, compound **4b** was much less potent as both a microtubule depolymerizer and cytotoxin.[7] This comparison suggests that introducing smaller substituents than bromine at the 3- and 5-positions could weaken the

* Corresponding author.
E-mail address: jgupton@richmond.edu (J.T. Gupton).

http://dx.doi.org/10.1016/j.bmc.2017.04.012
0968-0896/



Scheme 1. Suzuki-Miyaura Cross-Coupling studies of 4-bromo-2-carbethoxypyrrole.

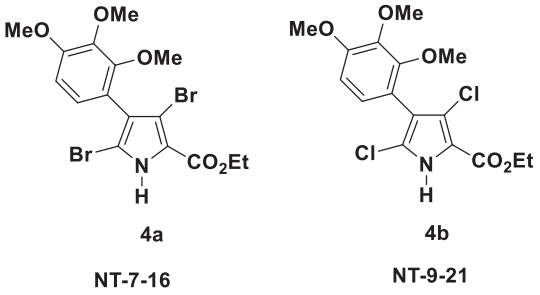


Fig. 1. Examples of two bioactive, synthetic pyrroles.

overall fit of these molecules within the colchicine binding site on tubulin.

However, there has been some concern in the literature[8] regarding the toxicity of certain dibrominated pyrroles related to the discovery of the drug atorvastatin. The dibromo-pyrrole, PD 123244-15, caused severe toxicities in rats related to induction of liver HMG-CoA reductase and localized esophageal and forestomach irritations when administered by oral gavage. These toxicities were associated with high plasma drug concentrations.[8] Compound **4a** is also quite hydrophobic and for these reasons appropriate modifications of compound **4a** seemed reasonable to pursue. In addition, we previously established that the 4-(2,3,4-trimethoxyphenyl) group and the 2-carbethoxy group of the pyrrole scaffold[6,9] are optimum for the inhibition of microtubule formation and cancer cell proliferation. Consequently, we have focused our attention on the introduction of selective functional group changes at the 5-position of compound **4a** in anticipation of minimizing potential toxicity issues while maximizing pharmacodynamic and pharmacokinetic properties. Our initial choice for substituent replacements at the 5-position were small cyano or oximino groups given their compact size, lower molecular weight relative to bromine, greater polarity and the additional SAR insights, which would be provided by their preparation and bioassay. We now report both the synthetic and biological studies to this end.

2. Results and discussions

The synthesis of 4-bromo-5-cyano-2-carbethoxypyrrole (**7**, Scheme 2) was accomplished by first converting our previously reported formylpyrrole building block[5] (**5**, Scheme 2) to a mixture of oxime isomers (**6**, Scheme 2). This was followed by treatment of the crude oxime mixture with phosphorous oxychloride at room temperature in chloroform, producing the desired cyanopyrrole (**7**, Scheme 2) in an overall 71% yield from the formylpyrrole building block.

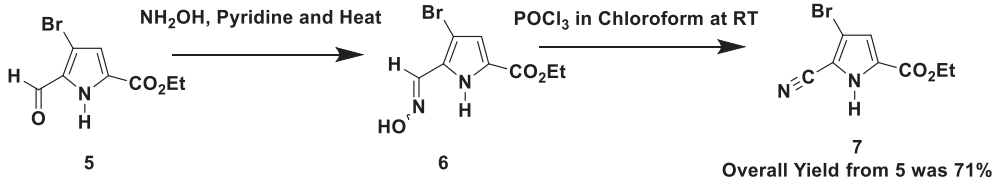
As part of our broad based synthetic studies we also wanted to determine the ability of the cyano group to activate an ortho bromine on our pyrrole scaffold in the Suzuki-Miyaura cross-coupling reaction as it relates to the inability of non-activated, non-nitrogen protected systems to effectively accomplish this end. With the desired starting material (**7**) in hand, we examined a variety of aryl and heteroaryl boronic acids under our standard cross-coupling conditions[5] and these results are described in Scheme 3 and Table 1.

The isolated yields for the various cross-coupled pyrroles were quite good irrespective of the electron releasing or electron withdrawing properties of the substituents attached to the aromatic group of the boronic acid. Heterocyclic groups such as furan and thiophene were also efficiently accommodated by our standard cross-coupling conditions. In comparison to the earlier studies regarding formyl group activation of the 4-bromo-2-carbethoxypyrrole (Scheme 5and **5**) towards Suzuki-Miyaura cross-coupling reactions, the cyano analog (Scheme 3 and **7**) gave very similar results. Compound **8n** in Table 1 was the required precursor to obtain the 5-cyano analog of NT-7-16 (Fig. 1, **4a**) and this transformation was accomplished by treatment of **8n** with KOH and NBS in DMF at room temperature (Scheme 4), in which case an 87% yield of the desired product (**9**) was obtained. It should also be noted that this sequence, which leads to KL-3-95 (**9**), provides a very unique tetrasubstitued pyrrole with four completely different substituents and with complete control of regiochemical specificity.

The preparation of the oxime analog (NT-7-45, **12**) of NT-7-16 (**4a**) was accomplished by a slightly different sequence as illustrated in Scheme 5. The 5-formyl pyrrole building block (**5**) utilized in Scheme 2 was crossed-coupled with 2,3,4-trimethoxyphenyl boronic acid under our standard ortho activation conditions[5] to yield a 2,4,5-trisubstituted pyrrole (**10**) in good yield (75%), which was then treated with KOH, NBS in DMF at room temperature. The resulting 3-bromo analog (**11**, 96% yield) was then reacted with hydroxylamine in pyridine with heating and the desired oxime analog NT-7-45 (**12**) was obtained as a mixture of Z (**12a**) and E (**12b**) isomers in good overall yield (68%). The stereoisomeric mixture was separated by flash chromatography into the pure Z (**12a**) and pure E (**12b**) isomers.

As was indicative for the preparation of the 5-cyano analog (**9**) of NT-7-16, the oxime analogs (**12a** and **12b**, Scheme 5) represent 2,3,4,5-tetrasubstitued pyrroles with four different groups attached and were prepared with regiochemical specificity.

The biological evaluations of KL-3-95 (**9**) and the NT-7-45 isomers (**12a** and **12b**) were conducted using standard protocols[7,10,11] and are presented in Table 2. These results show that KL-3-95 (**9**), NT-7-45 (Z) (**12a**) and NT-7-45 (E) (**12b**) exhibit low nanomolar potency in a panel of cancer cell lines including MDA-MB-435 melanoma cells, HeLa cervical carcinoma cells and SK-OV-3 ovarian cancer cells. The ability of **9**, **12a** and **12b** to overcome clinically relevant drug resistance mechanisms including the expression of



Scheme 2. Preparation of 4-bromo-5-cyano-2-carbethoxypyrrole building block.