Synthesis of Anti-tumor Combretastatin Analogues

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Abstract

Since the isolation and characterization of the anti-tumor agent combretastatin A-4 from the African bushwillow tree Combretum caffrum (Combretaceae), a large family of synthetic combretastatin analogues has been produced that show great potential for use as chemotherapy drugs. This study has produced novel combretastatin analogues that contain functional groups new to the combretastatin family of compounds. The ethene group that bridges the two phenyl rings of the combretastatins was replaced with an aziridine group.

Keywords: drug design, combretastatin, aziridine

1. Introduction

Many anticancer agents currently in clinical use are focused on the disruption of microtubules, which leads to cellular death. Tubulin--the protein whose $\alpha$- and $\beta$-subunits dimerize and then polymerize to form microtubules--contains three main sites for attack: (1) the vinca alkaloid domain; (2) the colchicine site; and (3) the taxoid site. The combretastatins are a family of anti-tumor drugs originally modeled on the naturally occurring compound combretastatin A-4 (CA-4), which binds at the colchicine site of tubulin, disrupting the formation of microtubules.\textsuperscript{1,2} Colchicine and CA-4 have similarities structurally, as seen in figure 1, implicating the trimethoxyphenyl ring as a major component of the compounds' binding ability.\textsuperscript{3} Because of the complexity of colchicine's structure, it's difficult synthesis makes the combretastatin ring system an appealing alternative.\textsuperscript{4}

The introduction of heterocycle functional groups (rings containing elements other than carbon) at the \textit{cis} double bond of combretastatin analogues typically increases the bioactivity of the molecule.\textsuperscript{3} Heterocyclic analogues such as (3)\textsuperscript{3} and (4)\textsuperscript{5} actually display increased reactivity and potency compared to CA-4. When designing the compounds tested in this study (Figure 2), it was theorized that substituting an aziridine ring at the \textit{cis} $\pi$ bond of combretastatins would preserve the rigidity of the ring system since three membered rings are locked in place and do not rotate freely, as well as increase water solubility and overall reactivity.
2. Results and Discussion

2.1 Methodology

The choice of replacing the cis-olefin bridge of the combretastatins with aziridine and azirine functionalities was made with two factors in mind: (1) reactivity and (2) water solubility. The azirine group in particular was theorized to produce an activity similar to, if not better than, CA-4 because the carbon-nitrogen pi-system relatively preserves the rigidity of CA-4 while increasing its general reactivity. Two main bond formations were key when designing the synthesis of the analogues in this study: (1) the aziridine ring formation and (2) the joining of the A and B rings.

2.1 Synthesis

The combretastatin analogues produced in this study were synthesized through the Wittig reaction typical of combretastatin synthesis. The triphenylphosphine bromide (9) was reacted with the corresponding aldehyde (13-16) to produce the trans-olefins (17-20). Compound (9) was prepared by first treating 3,4,5-trimethoxybenzyl alcohol with phosphorous tribromide, then with triphenylphosphine.
In order to ensure that the hydroxyl group present in aldehyde (10) did not interfere with the Wittig or subsequent reactions, it was protected with the SEM group. The \textit{trans}-analogues (17-20) were generated by Wittig reactions, utilizing \textit{tert}-butoxide as a base, which was chosen because the SEM protecting group is removed under hydride conditions, which was used previously. The next series of transformations involved production of the alkyne functionality present in (21-24). First, the \textit{trans}-olefins were brominated using pyridinium bromide perbromide, a mild source of bromine. The dibromo-compounds were then subjected to potassium \textit{tert}-butoxide to perform the double elimination, yielding the alkyne. Currently, compounds (22) and (23) have been produced, while \( R = H \) and \( NO_2 \) analogues have just begun synthesis.

Reduction of the alkyne moiety to the \textit{cis}-alkene is to be achieved using disamylborane followed by acetic acid instead of the traditional Lindlar's Catalyst partial hydrogenation. This method was chosen because the conditions should not affect the nitro functionality on compound (24), in addition to its high yield and comparatively low reaction time. Once the \textit{cis}-alkenes have been accessed, they are to be aziridinated using PhINNs, a nitrrenoid source.

\section*{4. Experimental Section}

All reagents were used as purchased from Sigma-Aldrich or Acros Organics. Solvents (THF, DMF, benzene) were dried according to literature procedures. Chemical shifts (\( \delta \)) are given in ppm referenced to CDCl\(_3\) and were recorded on a Varian 200 MHz NMR.

\subsection*{4.1 3,4,5-trimethoxybenzyl bromide (8).}

To a stirring solution of 3,4,5-trimethoxybenzylalcohol in \( \text{Et}_2\text{O} \) (0.75 M) at -40°C was added dropwise 1.1 equivalents of PBr\(_3\). The mixture was stirred at -40°C for 3 hours then washed once with \( \text{H}_2\text{O} \) and extracted with \( \text{Et}_2\text{O} \). The ethereal layer was dried with Na\(_2\)SO\(_4\) and solvent was removed under reduced pressure. The pale yellow solid was obtained in 93\% yield and used for further reactions without purification. \(^1\)H-NMR \( \delta \): 6.61 (2H, s); 4.45 (2H, s); 3.90 (6H, s); 3.86 (3H, s).

\subsection*{4.2 3,4,5-trimethoxybenzyltriphenylphosphine (9).}

To a 0.9 M solution of 3,4,5-trimethoxybenzyl bromide in benzene was added 1.1 equivalents of PPh\(_3\) and was stirred for 36 hours at room temperature. The white solid precipitate was vacuum filtered and washed with benzene to give pure product in 83.2\% yield.
4.3 Protection with SEMCl (11).

A solution of 3-hydroxy-4-methoxybenzaldehyde (0.5 M/CH₂Cl₂), 4 eq. ethyldiisopropylamine and 3 eq. 2-(trimethoxysilyl)ethoxymethyl chloride was heated to 40°C and stirred for 12 hours. The solution was poured into water, extracted with CH₂Cl₂ and dried over Na₂SO₄. The organic solvent was removed under reduced pressure and column chromatography was performed on the resulting mixture using 50:50 (hexane:ethyl acetate) as eluent. \(^{1}\)H-NMR δ: 9.88 (1H, s); 7.70 (1H, s); 7.55 (1H, d); 7.04 (1H, d); 5.32 (2H, s); 3.98 (3H, s); 3.82 (2H, t); 0.92 (2H, t); 0.00 (9H, s).
4.4 General procedure for Wittig Reactions.

To a 0.5 M solution of (9) in THF was added 1.1 eq. KOtBu and the mixture was stirred at room temperature for 3 hours. Next 0.9 eq. of the corresponding aldehyde in THF was added dropwise and the solution was refluxed for 24-36 hours. The product mix was washed twice with water and extracted with dichloromethane. The organic layer was dried and removed under reduced pressure to yield crude product that was purified by gravity column chromatography (50:50 hexane:ethyl acetate).

4.5 3,4,5-trimethoxyphenyl-2-(3-(2-(trimethylsilyl)ethoxymethoxy)-4-methoxyphenyl)ethane (18).

Yield = 67%. 1H-NMR δ: 7.72 (1H, s); 7.54 (1H, s); 7.41 (1H, s); 6.89 (1H, d); 6.67 (1H, s); 6.58 (1H, s); 6.40 (1H, s); 5.31 (2H, s); 3.91 (3H, s); 3.83 (9H, s); 2.38 (2H, t); 0.96 (2H, t); 0.00 (9H, s).

4.6 3,4,5-trimethoxyphenyl-2-(3,4-dimethoxyphenyl)ethane (19).

Yield = 74%. 7.78 (1H, d); 7.54 (1H, d); 7.35 (1H, s); 7.01 (2H, d); 6.41 (2H, s); 4.02 (3H, s); 3.96 (6H, s); 3.91 (6H, d).

4.7 3,4,5-trimethoxyphenyl-2-(3-nitro-4-methoxyphenyl)ethene.

Yield = 63%. 1H-NMR δ: 7.78 (1H, d); 7.71 (2H, s); 7.63 (1H, s); 7.50 (1H, d); 6.40 (1H, s); 5.38 (1H,s); 4.01 (3H, s); 3.78 (6H, s); 3.52 (3H, s).

4.8 General procedure for bromination of (E)-stilbenes.

A 0.4 M solution of alkene in acetic acid was refluxed until the solid dissolved (about 5 min.). To the warm solution, 1.1 eq. pyridinium bromide perbromide was added and stirred for an additional 5-6 min. After the solution had cooled, water was added and the solution was placed in an ice bath to induce precipitation of product. The product was isolated by vacuum filtration and washed with cold water.

4.9 General procedure for didehydrohalogenation.

A 0.5 M solution of dibromo compound (in THF) and 2.2 eq. KOtBu were refluxed for 2 hours. The product mix was washed with water and extracted with chloroform. The organic layer was dried and solvent was removed under reduced pressure to afford solid products.

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6. References