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Synthesis of Antimicrobial Natural Products Targeting FtsZ: (±)-Dichamanetin and (±)-2'''-Hydroxy-5''-benzylisouvarinol-B

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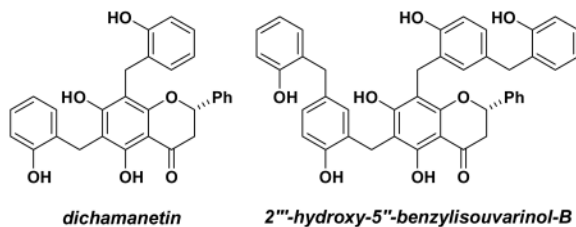
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Abstract



Two natural products have been synthesized using a $ZnCl_2$ -mediated benzylic coupling reaction. Both are potent inhibitors of the GTPase activity of FtsZ, a protein that is essential for bacterial cytokinesis.

The emergence of bacterial strains that are resistant to current drugs has prompted a renewed effort to discover new methods for fighting infectious disease.¹ One promising new target is FtsZ, the bacterial analog of tubulin, which mediates bacterial cell division. During bacterial cytokinesis, FtsZ monomers polymerize at mid-cell to form the Z-ring, which eventually constricts, leading to septation and formation of daughter cells (Figure 1). FtsZ consumes GTP during Z-ring assembly, much like its eukaryotic analog tubulin during mitosis. As such, FtsZ is susceptible to inactivation by compounds that interfere with the GTPase activity of this protein.²

Recent studies have revealed several compounds that inhibit the GTPase activity of FtsZ and kill bacteria,³ and in one case several compounds were demonstrated to disrupt Z-ring

formation.⁴ Zantrin Z1 (**1**; Figure 2), which was discovered in a high throughput *in vitro* screen for inhibition of GTPase activity,⁴ possesses a polyphenolic structure reminiscent of several natural products that exhibit potent antimicrobial activity. Dichamanetin (**2**) and 2''-hydroxy-5''-benzyliso-uvarinol-B (**3**), isolated independently by Hufford and Anam from *U. chamae* and *X. afticana* respectively, exhibit comparable MIC values to zantrin Z1 when evaluated against a variety of bacterial strains.⁵ It is notable that these compounds show a high level of activity against gram positive bacteria (e.g. *S. aureus* and *B. subtilis*), and furthermore the MIC values are comparable to clinically relevant antibiotics.⁶

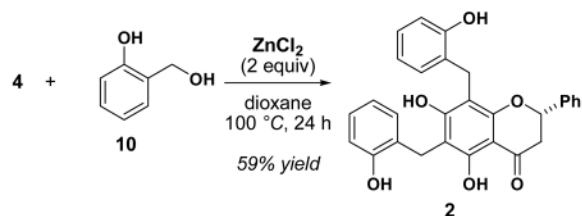
The structural similarity between polyphenolic compounds **1-3** suggested that they might all derive their antimicrobial activity by inhibiting the GTPase activity of FtsZ. In order to test this hypothesis, we undertook the syntheses of compounds **2** and **3**. While naturally-occurring flavanones have attracted the attention of synthetic chemists and biologists alike, benzylated flavanones are quite rare, and as such no efficient syntheses of compounds related to **2** and **3** have been reported.⁷ A straightforward synthesis would allow us to evaluate the origin of their biological activity and prepare analogs that may be more potent.

The substituent symmetry of **2** and **3** suggested that a common core could be elaborated to provide both molecules. The formation of benzylic carbon-carbon bonds with electron-rich arenes is often achieved *via* ortho quinone methide (OQM) intermediates, which can be accessed by a variety of routes.⁸ Pinocembrin (**4**) could be converted to the OQM precursor by benzylic functionalization (Scheme 1, path A). We initially planned to explore halomethylation, hydroxymethylation, and aminomethylation, since all of these processes take place under neutral or acidic conditions. While all of these processes are well-established for phenols, the analogous transformations using resorcinols are almost unknown.⁹ Furthermore, the base sensitivity of the flavanone would limit the conditions that could be employed for the formation of the OQM intermediate. An alternate synthetic approach would involve functionalization of the incoming phenolic side-chain (Scheme 1, path B).

Our synthesis began with the development of an efficient route to pinocembrin (Scheme 1). Flavanones related to pinocembrin have been prepared in high yield from the reaction of phenols with cinnamoyl chlorides through a Friedel-Crafts/cyclization sequence.¹⁰ Since this process is known to be low yielding for pinocembrin,¹¹ we developed an aldol condensation/cyclization route that rapidly provides multi-gram quantities of pinocembrin.¹² Trihydroxyacetophenone **5** is selectively bis-protected with methylchloromethyl ether, then converted to chalcone **6** under standard conditions. Cyclization with sodium acetate provided an equilibrium mixture of the cyclized product and chalcone starting material. Acidic hydrolysis of the MOM groups provided pinocembrin **4**.

We explored several methods of benzylic functionalization of pinocembrin in an effort to prepare a suitable intermediate that would eventually lead to **2** and **3**. We were able to produce both the morpholine (**8**, Scheme 3) and dimethylamine (**9**) Mannich bases from pinocembrin in high yield, though these reactions are not well established for complex resorcinol substrates.¹³ We made several unsuccessful attempts to convert diamines **8** and **9** directly to our desired product **2** using catalytic amounts of magnesium ethoxide, which has proven effective for dicarbonyl compounds.¹⁴ Attempts to convert Mannich bases **8** and **9** to the more reactive acetoxymethyl,¹⁵ hydroxymethyl,¹⁶ chloromethyl,¹⁷ and *N*-oxide¹⁸ derivatives were unsuccessful. Quaternization of the amino groups and subsequent displacement with phenol under Lewis acid-mediated conditions was unsuccessful.¹⁹ These experiments demonstrate that resorcinol-derived OQM intermediates are significantly more difficult to access than their phenol-derived counterparts, and are thus not suited to the synthesis of **2** and **3**.

We next turned our attention to the use of benzylic side-chain substrates (Scheme 1, path B). This process was known to be extremely inefficient for the synthesis of dichamanetin.²⁰ We employed trihydroxyacetophenone **5** as a model system to investigate reaction conditions for the aryl alkylation reaction with *o*-hydroxybenzyl alcohol (eq 1, Table 2). A survey of Lewis and protic acids revealed that ZnCl₂ in dioxane afforded bisalkylated product **11** in 49% yield.²¹ Similar reaction conditions converted pinocembrin **4** to dichamanetin **2** in 59% yield to complete the first selective synthesis of this natural product (eq 2).



(2)

We next addressed the synthesis of 2''-hydroxy-5''-benzyl-isouvarinol-B (**3**, Scheme 4). Phenol **12** is made by selective mono-protection of commercially available 2,4'-dihydroxydiphenylmethane.²² Phenol **12** was hydroxyl-methylated using phenylboronic acid and paraformaldehyde²³ to produce boronate ester **13**. Hydrolysis of **13** with hydrogen peroxide provided the requisite benzylic alcohol **14** which was converted to **3**²⁴ in high yield after benzylation and deprotection. The increased yield for the benzylation reaction relative to model compound **5** might be due to the decreased propensity of **4** to enolize and participate in undesirable side reactions.

While the antimicrobial activity of these compounds has been documented, little is known about their mechanism of action. Both **2** and **3** are potent inhibitors of *E. coli* GTPase activity (Table 3) and they exhibit IC₅₀ values similar to **1**. These experiments indicate that the bacterial cell division protein FtsZ is a target of these compounds. Compound **11**, which lacks the flavanone core structure, is much less potent.

In summary, we have developed the first efficient route to hydroxybenzylated flavanone natural products. We synthesized dichamanetin and 2''-hydroxy-5''-benzylisouvarinol-B from a common core structure using a new zinc chloride-mediated benzylic coupling reaction. The efficient synthesis described in this paper will allow the preparation of a panel of derivatives so that the mechanism of action can be studied in more detail.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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24. The ^1H NMR spectrum of synthetic **3** is consistent with the published data (ref. 5b), but no ^{13}C NMR is reported and we were unable to obtain an authentic sample for comparison.

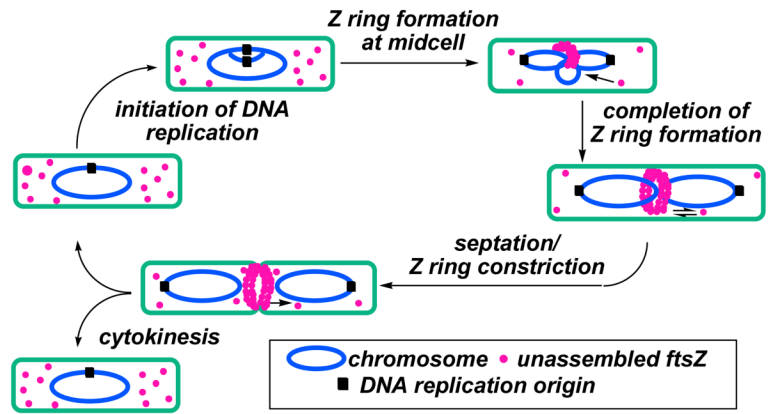


Figure 1.
Bacterial cell division and the role of FtsZ

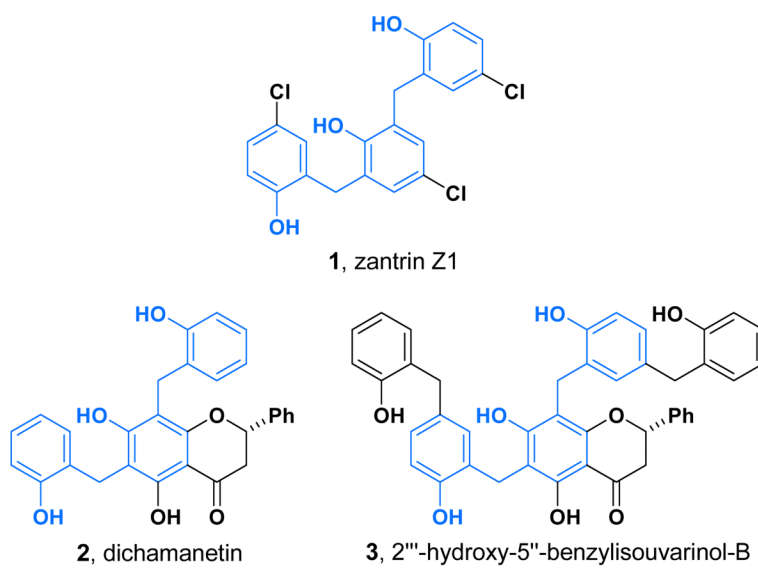
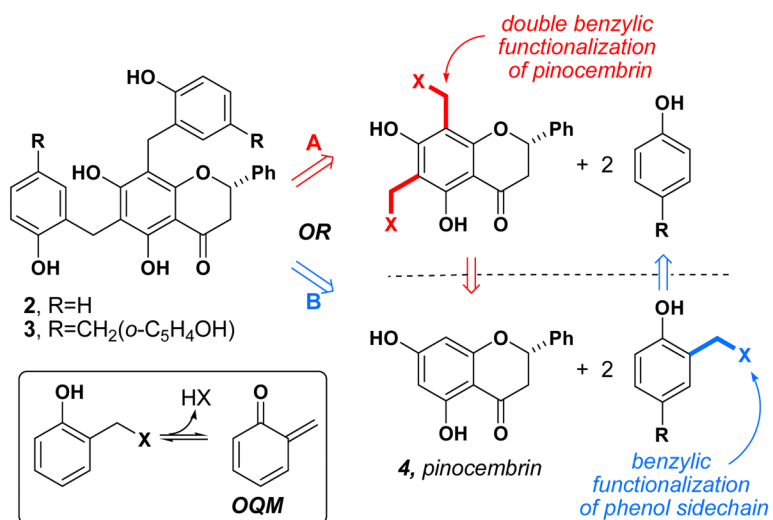
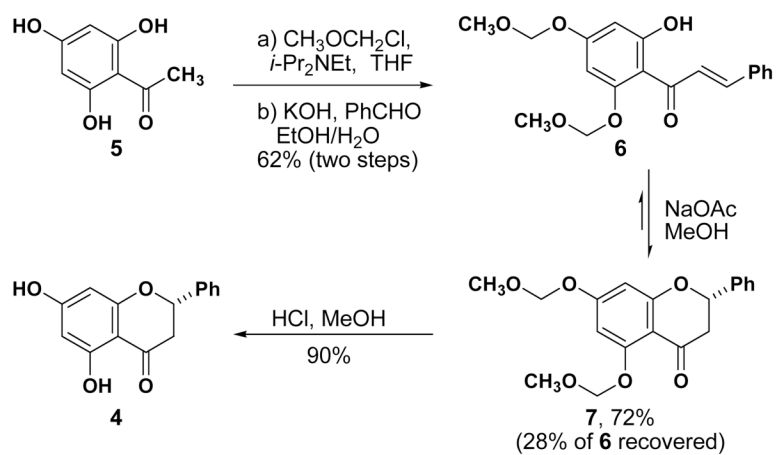


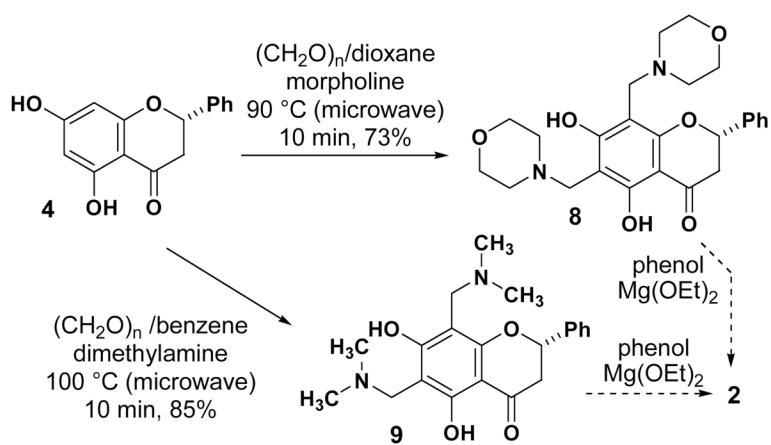
Figure 2. Structures of synthetic (**1**) and naturally occurring (**2** and **3**) polyphenolic compounds



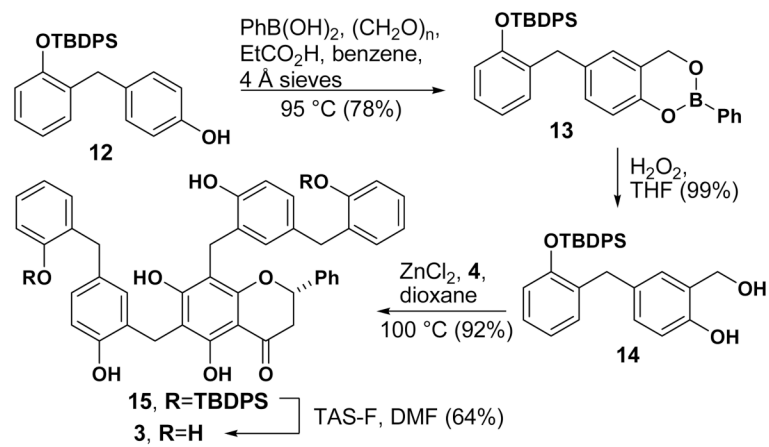
Scheme 1.
Retrosynthetic analysis of **2** and **3**



Scheme 2.
Synthesis of pinocembrin (4)



Scheme 3.
Aminomethylation of 4 and attempted displacement with phenol to produce 2



Scheme 4.
Synthesis of compound 3

Table 1

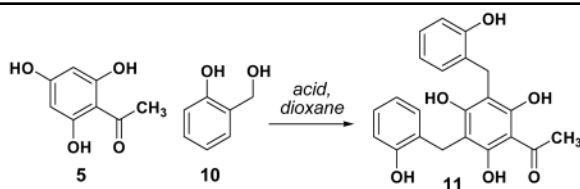
Antimicrobial activities (MICs, μ M) of compounds 1-3

compound	S. aureus	B. subtilis	M. smegmatis	E. coli	P. aeruginosa
1	2.5	1.25	^a	20	40
2	1.7	1.7	3.4	^b	^b
3	10.7	2.6	3.8	2.3	15.4

^a not evaluated.^b no significant activity.

Table 2

Optimization of benzylation conditions (Eq 1)



entry	temperature	acid (mol %)	yield
1	130 °C ^a	Sc(OTf) ₃ (100)	<10% ^b
2	60 °C	Sc(OTf) ₃ (100)	<20% ^b
3	100 °C	AlCl ₃ (100)	21%
4	100 °C	SnCl ₄ (25)	30%
5	100 °C	SnCl ₄ (200)	33%
6	130 °C ^a	Mg(OTf) ₂ (100)	18%
7	100 °C	<i>p</i> -TsOH (25)	24%
8	100 °C	CSA (100)	20%
9	130 °C ^a	ZnCl ₂ (100)	49%
10	130 °C ^a	ZnBr ₂ (100)	48%
11	130 °C ^a	ZnI ₂ (100)	47%
12	130 °C ^a	ZnCl ₂ (200)	49%
13	60 °C	ZnCl ₂ (100)	<20% ^b
14	23 °C	ZnCl ₂ (100)	-

Unless otherwise noted, reactions were heated to reflux under argon for 24 h.

^aHeated for 1 h under microwave irradiation.

^bBased on LCMS (product not isolated)

Table 3Inhibition of *E. coli* FtsZ GTPase activity by **1**, **2**, **3**, and **11**

compound	IC ₅₀ (μM)
1 (zantrin Z1) ^a	5.0 ± 0.5
2 (dichamanetin)	12.5 ± 0.5
3 (2''-hydroxy-5''-benzylisouvarinol-B)	8.3 ± 0.5
11	60.4 ± 2.2

^aSee reference 4a.