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

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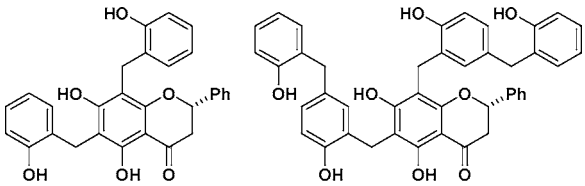
ABSTRACT



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 highly conserved 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 prokaryotic analogue 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).[2] FtsZ consumes GTP during Z-ring assembly, much like its eukaryotic analogue tubulin during mitosis. As such, FtsZ is susceptible to inactivation by compounds that interfere with the assembly-dependent GTPase activity of this protein.[3,4a]

Recent studies have revealed several compounds that inhibit the GTPase activity of FtsZ and kill bacteria,[3] and in

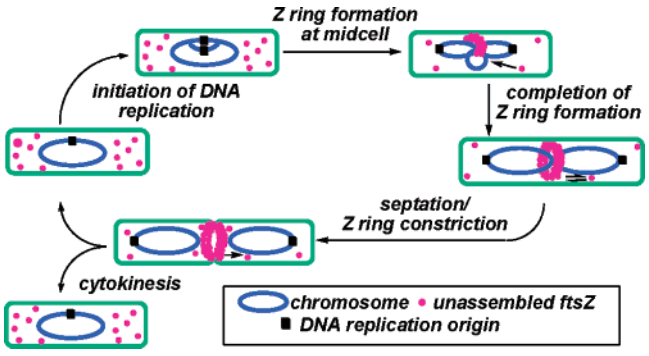


Figure 1. Bacterial cell division and the role of FtsZ.

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(1) (a) Walsh, C. *Nat. Rev. Microbiol.* **2003**, *1*, 65−70. (b) Walsh, C.; Wright, G. *Chem. Rev.* **2005**, *105*, 391−393.

(2) (a) Romberg, L.; Levin, P. A. *Annu. Rev. Microbiol.* **2003**, *57*, 125−154. (b) Margolin, W. *Curr. Biol.* **2003**, *13*, R16−R18. (c) Addinall, S. G.; Holland, B. *J. Mol. Biol.* **2002**, *318*, 219−236. (d) Weiss, D. S. *Mol. Microbiol.* **2004**, *54*, 588−597. (e) Errington, J.; Daniel, R. A.; Scheffers, D.-J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 52−65.

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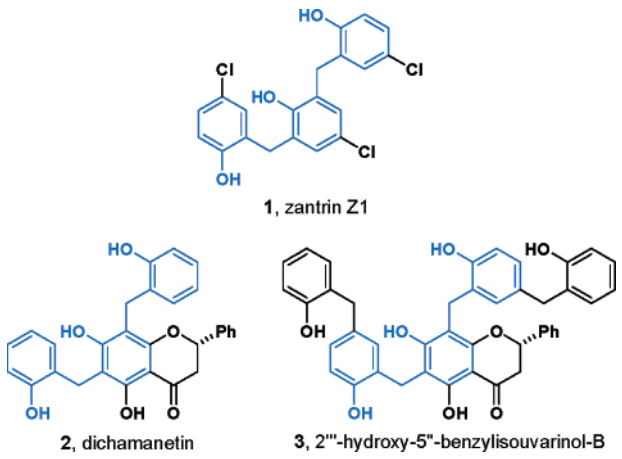


Figure 2. Structures of synthetic (**1**) and naturally occurring (**2** and **3**) polyphenolic compounds.

one case, several compounds were demonstrated to disrupt Z-ring formation.[4a] Zantrin Z1 (**1**, Figure 2), which was discovered in a high-throughput in vitro screen for inhibition of FtsZ GTPase activity,[4] possesses a polyphenolic structure reminiscent of several natural products that exhibit potent antimicrobial activity. Dichamanetin (**2**) and 2‴-hydroxy-5″-benzylisouvarinol-B (**3**), isolated independently by Hufford and Anam from *U. chamae* and *X. afticana* respectively, exhibited comparable MIC values to that seen with zantrin Z1 when evaluated against a variety of bacterial strains.[4a,5] It is notable that these compounds show a high level of activity against Gram-positive bacteria (e.g. *S. aureus*, *B. subtilis*; Table 1), and furthermore, the MIC values are comparable to those of 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. To test this hypothesis, we undertook the syntheses of compounds **2** and **3**. While naturally occurring flavanones have attracted

Table 1. Antimicrobial Activities (MICs, μM) of Compounds **1**−**3**

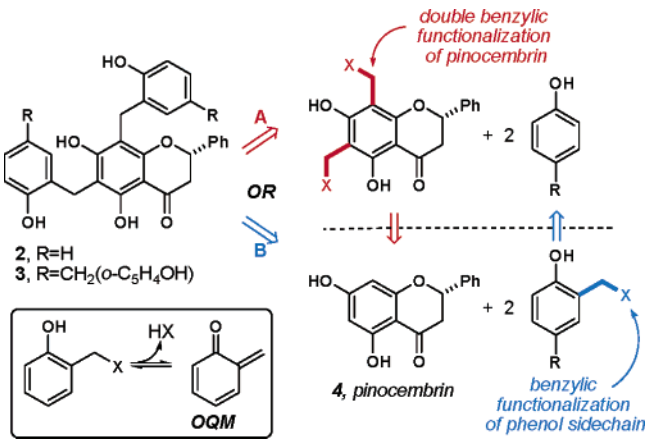
compd	*S. aureus*	*B. subtilis*	*M. smegmatis*	*E. coli*	*P. aeruginosa*
1[a]	2.5	1.25	*d*	20	40
2[b]	1.7	1.7	3.4	*e*	*e*
3[c]	10.7	2.6	3.8	2.3	15.4

[a] Ref 4a. [b] Ref 5a. [c] Ref 5b. [d] Not evaluated. [e] No significant activity.

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 analogues 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).

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



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

(3) (a) Wang, J.; Galgoci, A.; Kodali, S.; Herath, K. B.; Jayasuriya, H.; Dorso, K.; Vicente, F.; Gonzalez, A.; Cully, D.; Bramhill, D.; Singh, S. *J. Biol. Chem.* **2003**, *278*, 44424−44428. (b) Reynolds, R. C.; Srivastava, S.; Ross, L. J.; Suling, W. J.; White, E. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3161−3164. (c) White, E. L.; Suling, W. J.; Ross, L. J.; Seitz, L. E.; Reynolds, R. C. *J. Antimicrob. Chemother.* **2002**, *50*, 111−114.

(4) (a) Margalit, D. N.; Romberg, L.; Mets, R. B.; Hebert, A. M.; Mitchison, T. J.; Kirschner, M. W.; RayChaudhuri, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11821−11826. The antibacterial activity of **1** was first noted by Beaver: (b) Beaver, D. J.; Shumard, R. S.; Stoffel, P. J. *J. Am. Chem. Soc.* **1953**, *75*, 5579−5581. After Beaver, Hakimelahi made similar observations with **1** and related analogues: (c) Hakimelahi, G. H.; Moshfegh, A. A. *Helv. Chim. Acta* **1981**, *64*, 599−609. (d) Moshfegh, A. A.; Badri, R.; Hojjatie, M.; Kaviani, M.; Naderi, B.; Nazmi, A. H.; Ramezanian, M.; Roozpeikar, B.; Hakimelahi, G. H. *Helv. Chim. Acta* **1982**, *65*, 1221−1228.

(5) (a) Hufford, C. D.; Lasswell, W. L., Jr. *Lloydia* **1978**, *41*, 156−160. (b) Anam, E. M. *Ind. J. Chem., Sect. B* **1994**, *33B*, 1009−1011. See ref 4a for MIC values of **1**.

(6) The National Committee for Clinical Laboratory standards (NCCLS) defines the effective MICs of methicillin, erythromycin, and vancomycin as 0.8, 0.4, and 5.8 μM, respectively, against *S. aureus*. Higher MICs indicate that a strain is becoming resistant to the drug. Leegaard, T. M.; Caugant, D. A.; Frøholm, L. O.; Høiby, E. A. *Clin. Microbiol. Infect.* **2000**, *6*, 290−293.

(7) The synthesis of gericudranin, a related *p*-hydroxybenzylated 3-hydroxyflavanone has been reported: Choi, Y.-J.; Shim, P.-J.; Ko, K.-S.; Kim, H.-D. *Heterocycles* **1996**, *43*, 1223−1228.

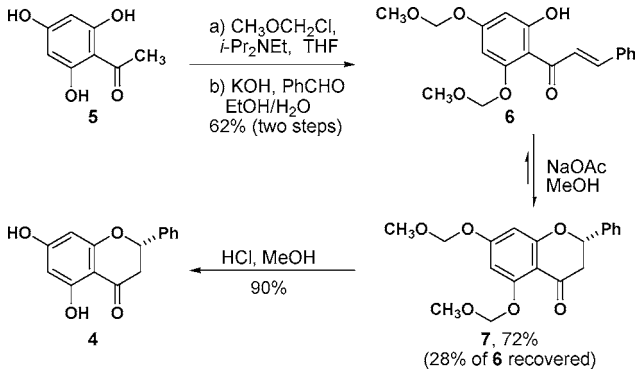
(8) Van De Water, R. W.; Pettus, T. R. R. *Tetrahedron* **2002**, *58*, 5367−5405.

(9) (a) Tabatabai, M.; Vogt, W.; Boehmer, V. *Tetrahedron Lett.* **1990**, *31*, 3295−3298. (b) Tabatabai, M.; Vogt, W.; Baehmer, V.; Ferguson, G.; Paulus, E. F. *Supramol. Chem.* **1994**, *4*, 147−152.

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 2). 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 multigram quantities of pinocembrin.[12] Trihydroxyacetophenone **5** is selectively bis-protected with methylchloromethyl ether and 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**.

Scheme 2. Synthesis of 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]

(10) (a) Lee, J.-M.; Tseng, T.-H.; Lee, Y.-J. *Synthesis* **2001**, 2247−2254. (b) Solladie, G.; Gehrold, N.; Maignan, J. *Eur. J. Org. Chem.* **1999**, *2309*, 9−2314. (c) Talapatra, B.; Deb, T.; Talapatra, S. *Ind. J. Chem., Sect. B* **1986**, *25B*, 1122−1125. (d) Suresh, R. V.; Iyer, C. S. R.; Iyer, P. R. *Heterocycles* **1986**, *24*, 1925−1930.

(11) Huzise, S.-I.; Tatsita, H. *Chem. Ber.* **1941**, *74B*, 275−278.

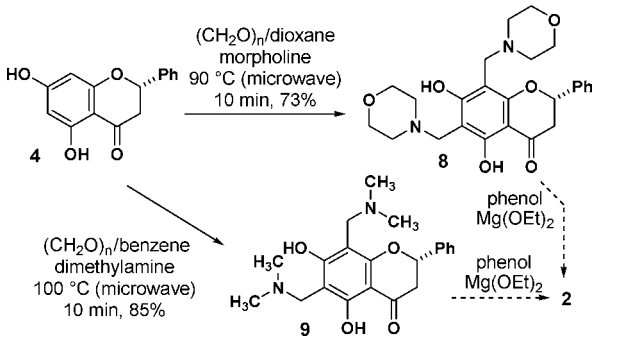
(12) Huang, C.; Li, S.; Hou, L.; Li, Y.; Li, Y. *Synth. Commun.* **1999**, *29*, 1383−1392. Zhao, L.; Li, Y. *Org. Prep. Proced. Int.* **1996**, *28*, 165−171.

(13) (a) Ghantwal, S. R.; Samant, S. D. *J. Indian Chem. Soc.* **2000**, *77*, 100−101. (b) Jerzmanowska, Z.; Jurkowska-Kowalczyk, E. *Rocz. Chem.* **1970**, *44*, 1395−1401.

(14) (a) Hellmann, H.; Pohlmann, J. L. W. *Liebigs Ann. Chem.* **1961**, *642*, 28−35. (b) Hellmann, H.; Pohlmann, J. L. W. *Liebigs Ann. Chem.* **1961**, *643*, 38−42.

(15) Mazzei, M.; Miele, M.; Nieddu, E.; Barbieri, F.; Bruzzo, C.; Alama, A. *Eur. J. Med. Chem.* **2001**, *36*, 915−923.

Scheme 3. Aminomethylation of **4** and Attempted Displacement with Phenol To Produce **2**



and *N*-oxide[18] 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 and Table 2). A survey of Lewis and protic acids revealed that $ZnCl_2$ in dioxane afforded bisalkylated product **11** in 49% yield.[21]

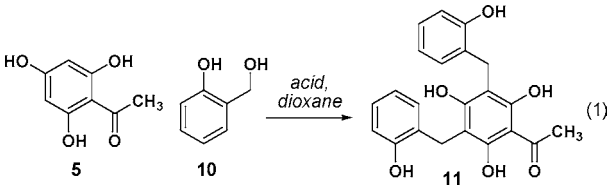
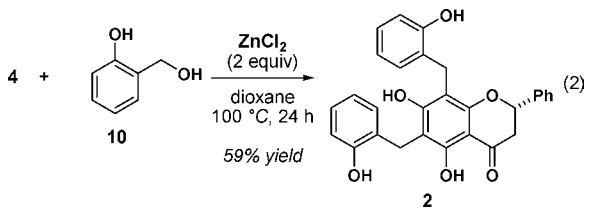


Table 2. Optimization of Benzylation Conditions (Eq 1)[a]

entry	T (°C)	acid (mol %)	yield (%)
1	130[b]	$Sc(OTf)_3$ (100)	<10[c]
2	60	$Sc(OTf)_3$ (100)	<20[c]
3	100	$AlCl_3$ (100)	21
4	100	$SnCl_4$ (25)	30
5	100	$SnCl_4$ (200)	33
6	130[b]	$Mg(OTf)_2$ (100)	18
7	100	*p*-TsOH (25)	24
8	100	CSA (100)	20
9	130[b]	$ZnCl_2$ (100)	49
10	130[b]	$ZnBr_2$ (100)	48
11	130[b]	ZnI_2 (100)	47
12	130[b]	$ZnCl_2$ (200)	49
13	60	$ZnCl_2$ (100)	<20[c]
14	23	$ZnCl_2$ (100)	0[c]

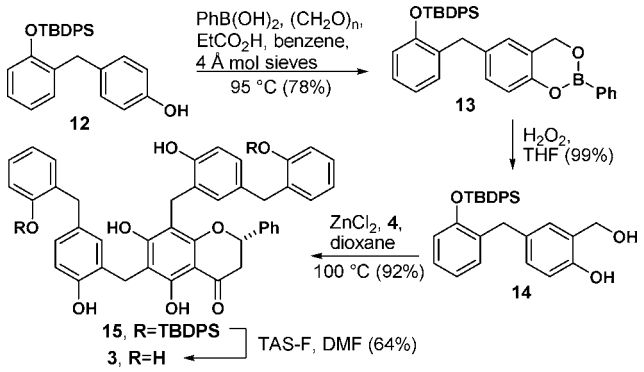
[a] Unless otherwise noted, reactions were heated to reflux under argon for 24 h. [b] Heated for 1 h under microwave irradiation. [c] Based on LCMS (product not isolated).

Similar reaction conditions converted pinocembrin **4** to dichamanetin **2** in 59% yield to complete the first selective synthesis of this natural product (eq 2).



We next addressed the synthesis of 2‴-hydroxy-5″-benzylisouvarinol B (**3**, Scheme 4). Phenol **12** is made by

Scheme 4. Synthesis of Compound **3**



selective monoprotection of commercially available 2,4′-dihydroxydiphenylmethane.[22] Phenol **12** was hydroxymethylated using phenylboronic acid and paraformaldehyde[23] 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 **2** and **3** has been documented, little is known about their mechanism of action. Our data demonstrate both **2** and **3** are potent inhibitors of *E. coli* FtsZ GTPase activity (Table 3), exhibiting IC_{50} values

Table 3. Inhibition of *E. coli* FtsZ GTPase Activity by **1**−**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

[a] See ref 4a.

similar to that of **1**. These data 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 structure-activity relationships can be studied in detail.

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Supporting Information Available: Experimental procedures for the preparation of all new compounds and determination of IC_{50} values for **2**, **3**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crisp, G. T.; Turner, P. D. *Tetrahedron* **2000**, *56*, 407−415.
(17) Sinhababu, A. K.; Borchardt, R. T. *Synth. Commun.* **1983**, *13*, 677−683.
(18) McCamley, K.; Ripper, J. A.; Singer, R. D.; Scammells, P. J. *J. Org. Chem.* **2003**, *68*, 9847−9850. While this oxidation may have yielded appreciable amounts of the desired *N*-oxide, this compound was difficult to handle due to its apparent high aqueous solubility.
(19) Baik, W.; Lee, H. J.; Yoo, C. H.; Jung, J. W.; Kim, B. H. *J. Chem. Soc., Perkin Trans. 1* **1997**, *5*, 587−589.
(20) Dichamanetin was produced in <2% yield using $BF_3{\cdot}OEt_2$ for this reaction: Lasswell, W. L., Jr.; Hufford, C. D. *J. Org. Chem.* **1977**, *42*, 1295−1302.
(21) A related process has been reported using phenol-derived, but not resorcinol-derived, substrates. The reported reaction was conducted under conditions using a domestic microwave, and while the temperature was not monitored, the wattage applied suggests that the reaction takes place at >200 °C: Khalafi-Nezhad, A.; Rad, M. N. S.; Hakimelahi, G. H. *Helv. Chim. Acta* **2003**, *86*, 2396−2403.
(22) De Bruyn, P. J.; Foo, L. M.; Lim, A. S. C.; Looney, M. G.; Solomon, D. H. *Tetrahedron* **1997**, *53*, 13915−13932.
(23) Nagata, W.; Okada, K.; Aoki, T. *Synthesis* **1979**, 365−368.
(24) The ^{1}H 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.