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Tetrahydroxanthones by Sequential Pd-Catalyzed C−O and C−C Bond Construction and Use in the Identification of the "Antiausterity" Pharmacophore of the Kigamicins

Penelope A. Turner,[†] Ellanna M. Griffin,[†] Jacqueline L. Whatmore,[*,‡] and Michael Shipman[*,†]

Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, United Kingdom, and Peninsula Medical School, University of Exeter, St Luke's Campus, Heavitree, Exeter, EX1 2LU, United Kingdom

m.shipman@warwick.ac.uk; jackie.whatmore@pms.ac.uk

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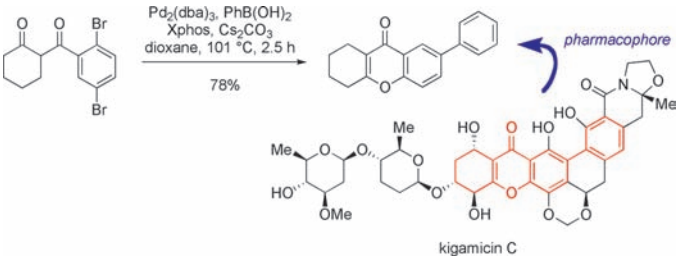
ABSTRACT



Readily available C-acylated cycloalkanones undergo efficient Pd catalyzed ring closure/cross-coupling providing 7-substituted tetrahydroxanthones in a single operation. One of the synthesized derivatives (depicted) is shown to selectively kill pancreatic cancer (PANC-1) cells under conditions of nutrient deprivation indicating that the tetrahydroxanthone is responsible, in part, for the "antiausterity" effects of the naturally occurring kigamicins.

Xanthones and partially hydrogenated di- and tetrahydroxanthones (THXs) are widely occurring classes of natural products (NPs) with considerable biological activity.[1] Of particular interest are the complex polyketide derived NPs including kigamicin C,[2] kibdelone A,[3] simaomicin α,[4] and puniceaside B[5] that display potent anticancer[2−4] or neuroprotective[5] activities (Figure 1). These molecules all possess THXs bearing an aryl substituent at C−7 (**1**, Scheme 1).[6] No detailed structure−activity relationships are available within these compound classes, nor are their modes of action well understood. That said, the fact that they all possess a common 7-arylated THX subunit suggests that this carbon framework may play an important role in the origin of their bioactivities.

To test this hypothesis, we sought to develop efficient, mild methods for the synthesis of 7-arylated THXs and related derivatives and evaluate their bioactivity. The presence of hydroxyl groups in the saturated rings of these NPs demands that any such methods must be nonacidic in

[†] University of Warwick.
[‡] University of Exeter.

(1) Chantarasriwong, O.; Batova, A.; Chavasiri, W.; Theodorakis, E. A. *Chem.—Eur. J.* **2010**, *16*, 9944. Krohn, K.; Kouam, S. F.; Kuigoua, G. M.; Hussain, H.; Cludius-Brandt, S.; Florke, U.; Kurtán, T.; Pescitelli, G.; DiBari, L.; Draeger, S.; Schulz, B. *Chem.—Eur. J.* **2009**, *15*, 12121. Na, Y. *J. Pharm. Pharmacol.* **2009**, *61*, 707. Pinto, M.; Sousa, M. E.; Nascimento, M. S. J. *Curr. Med. Chem.* **2005**, *12*, 2517 and references therein.

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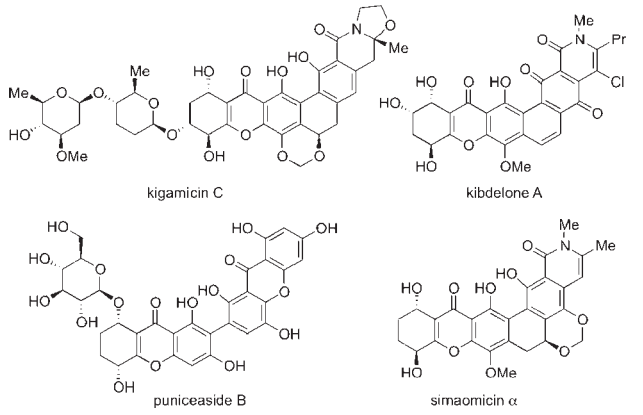
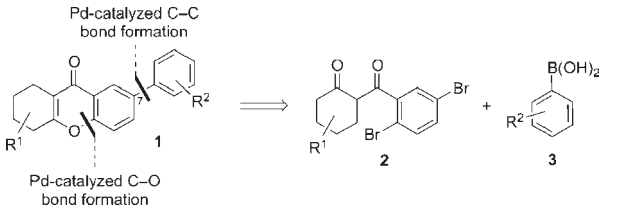


Figure 1. Bioactive polyketide derived natural products containing the 7-aryl tetrahydroxanthone (THX) nucleus.

nature to prevent facile aromatization to the corresponding xanthones through elimination of water.[7] In considering possible routes to these molecules, we were drawn to the idea of using palladium catalysis to construct the THX ring through selective C−O bond construction using the enolate derived from a bromoketone such as **2**.[8] Importantly, by careful orchestration of the reaction conditions, introduction of the 7-substituent might be realized concurrently by

Scheme 1. "One-Pot" Route to 7-Aryl THXs using Tandem Catalysis



Miyaura-Suzuki cross-coupling with boronic acid **3** using the same Pd catalyst (Scheme 1).

In this way, libraries of simple, "drug-like" 7-arylated THXs could be produced in a single operation through application of tandem catalysis.[9−11] In this communication, we successfully demonstrate the feasibility of this approach to 7-substituted THXs. Moreover, using one of the derived synthetic compounds, we confirm that this heterocyclic scaffold is indeed responsible, at least in part, for the bioactivity of one of these complex polyketide derived NPs.

To ascertain if THXs could be made by Pd catalyzed C−O bond construction, 2-(2-bromo-benzoyl)-cyclohexanone (**4a**) was synthesized in 65% yield in a single operation by deprotonation of cyclohexanone with LDA followed by C-acylation of the resulting lithium enolate with 2-bromobenzoyl chloride (Table 1).[12] The corresponding chloride and iodide based substrates **4b** and **4c** respectively, were made in the same way using the appropriate acid chlorides. Next, we investigated a range of catalytic conditions for ring closure to the THX nucleus.

Encouragingly, subjection of **4a** to $Pd_2(dba)_3$, Xantphos and Cs_2CO_3 in refluxing toluene furnished **5** in 76% yield (Table 1, entry 1), whose spectroscopic data matched those previously reported.[7a,13] Further improvements were achieved by screening a variety of phosphine ligands and solvents (Table 1, entries 2−10). The use of Xphos as ligand and dioxane as solvent proved most effective with **5** produced in an excellent 93% yield (Table 1, entry 10). The cyclization proceeded with similar efficiency when the bromide was replaced with a chloride, although appreciably lower yields were observed using the corresponding iodide (Table 1, entries 11−12).

The scope of this new route to THXs was investigated through the preparation of derivatives **6a**−**i** (Figure 2). In most cases, the reactions were conducted using bromide based substrates although for the preparation of **6h**, the corresponding chloride was used. Using the optimized conditions, good to excellent yields were observed and a variety of groups shown to be tolerated including acid sensitive functional groups (e.g., **6c**). The formation of

(2) (a) Kunimoto, S.; Lu, J.; Esumi, H.; Yamazaki, Y.; Kinoshita, N.; Honma, Y.; Hamada, M.; Ohsono, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiot.* **2003**, *56*, 1004. (b) Kunimoto, S.; Someno, T.; Yamazaki, Y.; Lu, J.; Esumi, H.; Naganawa, H. *J. Antibiot.* **2003**, *56*, 1012. (c) Lu, J.; Kunimoto, S.; Yamazaki, Y.; Kaminishi, M.; Esumi, H. *Cancer Sci.* **2004**, *95*, 547. (d) Someno, T.; Kunimoto, S.; Nakamura, H.; Naganawa, H.; Ikeda, D. *J. Antibiot.* **2005**, *58*, 56. (e) Masuda, T.; Ohba, S.; Kawada, M.; Ohsono, M.; Ikeda, D.; Esumi, H.; Kunimoto, S. *J. Antibiot.* **2006**, *59*, 209.

(3) Ratnayake, R.; Lacey, E.; Tennant, S.; Gill, J. H.; Capon, R. J. *Chem.—Eur. J.* **2007**, *13*, 1610.

(4) Koizumi, Y.; Tomoda, H.; Kumagai, A.; Zhou, X.-P.; Koyota, S.; Sugiyama, T. *Cancer. Sci.* **2009**, *100*, 322.

(5) Du, X.-G.; Wang, W.; Zhang, S.-P.; Pu, X.-P.; Zhang, Q.-Y.; Ye, M.; Zhao, Y.-Y.; Wang, B.-R.; Khan, I. A.; Guo, D.-A. *J. Nat. Prod.* **2010**, *73*, 1422.

(6) The numbering system adopted is based on that of the parent heterocycle, namely 1,2,3,4-tetrahydro-9H-xanthen-9-one.

(7) Existing routes to THXs rely on the use of strong acids, see: (a) Watanbe, T.; Katayama, S.; Nakashita, Y.; Yamauchi, M. *J. Chem. Soc., Perkin Trans. 1* **1978**, 726. (b) Singh, O. V; Kapil, R. S; Garg, C. P; Kapoor, R. P. *Tetrahedron Lett.* **1991**, *32*, 5619. (c) Kostakis, I. K.; Tenta, R.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L; Pratsinis, H.; Kletsas, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5057.

(8) For the synthesis of benzofurans using C−O bond formation, see: (a) Willis, M. C.; Taylor, D.; Gillmore, A. T. *Org. Lett.* **2004**, *6*, 4755. (b) Willis, M. C.; Taylor, D.; Gillmore, A. T. *Tetrahedron* **2006**, *62*, 11513.

(9) For a monograph, see: Tietze, L. F.; Brasche, G.; Gericke, K. *Domino Reactions in Organic Chemistry*; Wiley-VCH: Weinheim, 2006.

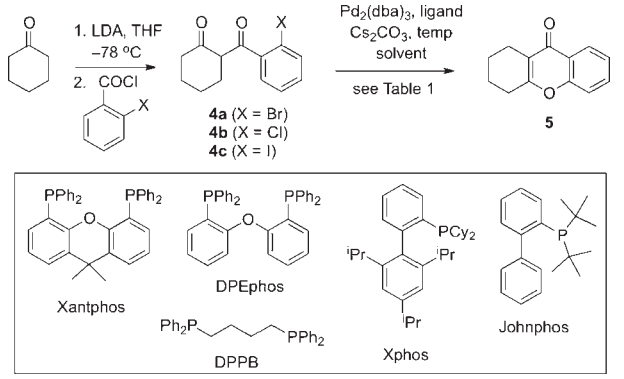
(10) For reviews, see: Bonne, D.; Coquerel, Y.; Constantieux, T.; Rodriguez, J. *Tetrahedron: Asymmetry* **2010**, *21*, 1085. Barluenga, J.; Rodriguez, F.; Fananas, F. J. *Chem. Asian. J.* **2009**, *4*, 1036. D'Souza, D. M.; Muller, T. J. J. *Chem. Soc. Rev.* **2007**, *36*, 1095. Chapman, C. J.; Frost, C. G. *Synthesis* **2007**, 1. Ajamian, A.; Gleason, J. L. *Angew. Chem. Int. Ed.* **2004**, *43*, 3754.

(11) For recent examples, see: Bryan, C. S; Braunger, J. A; Lautens, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 7064. Wu, X.-F; Neumann, H.; Beller, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 5284. Gandeepan, P.; Parthasarathy, K.; Cheng, C.-H. *J. Am. Chem. Soc.* **2010**, *132*, 8569. Kim, H.; Lee, K.; Kim, S.; Lee, P. H. *Chem. Commun.* **2010**, *46*, 6341. Spergel, S. H.; Okoro, D. R.; Pitts, W. *J. Org. Chem.* **2010**, *75*, 5316. Bararjanian, M.; Balalaie, S.; Rominger, F.; Movassagh, B.; Bijanzadeh, H. R. *J. Org. Chem.* **2010**, *75*, 2806.

(12) Although drawn as 1,3-dicarbonyl compounds, the substrates used in this study exist predominantly in the enol form in $CDCl_3$, as determined by ^1H NMR spectroscopy (see Supporting Information).

(13) Patonay, T.; Lévai, A.; Rimánm, E.; Varma, R. S. *Arkivoc* **2004**, 183.

Table 1. Optimization of Pd-Catalyzed C−O Bond Forming Conditions to THX (**5**)



entry	ketone	ligand[a]	solvent	temp (°C)	yield[b]
1	**4a**	Xantphos	toluene	111	76%
2	**4a**	Xantphos	THF	66	76%
3	**4a**	Xantphos	H_2O	100	<25%
4	**4a**	Xantphos	DMF	153	62%
5	**4a**	Xantphos	dioxane	101	87%
6	**4a**	DPEphos	toluene	111	79%
7	**4a**	DPEphos	dioxane	101	86%
8	**4a**	DPPB	dioxane	101	83%
9	**4a**	JohnPhos	dioxane	101	82%
10	**4a**	Xphos	dioxane	101	93%
11	**4b**	Xphos	dioxane	101	88%
12	**4c**	Xphos	dioxane	101	54%

[a] Reaction conditions: ketone (1 molar equiv), $Pd_2(dba)_3$ (2.5 mol %), ligand (6 mol %), Cs_2CO_3 (2.2 molar equiv) in solvent (0.41 M) for 18 h at stated temperature. [b] Yield of isolated product after column chromatography.

THX **6e** in good yield by selective insertion into the C−Br bond adjacent to the carbonyl group[14] was especially encouraging in the context of the proposed sequential C−O and C−C bond forming sequence (Scheme 1). Derivatives containing additional carbocyclic (e.g., **6a**) or heterocyclic rings (e.g., **6g**) are readily accessed. Moreover, the synthesis of ring expanded systems (e.g., **6b**) can be realized through use of cycloheptanone in place of cyclohexanone in the initial C-acylation reaction (*cf* Table 1). However, a poor yield was observed in the formation of ring contracted derivative **6d**.

The formation of THXs can be achieved using less expensive Cu (I) catalysis.[15] For example, treatment of **4a** with copper iodide (10 mol %), DMEDA (20 mol %) and Cs_2CO_3 (2 equiv) in refluxing THF for 18 h provided **5** in an unoptimized 54% yield. However, as this method was deemed to be less useful in the context of sequential bond forming sequences, further optimization of this reaction was not pursued.

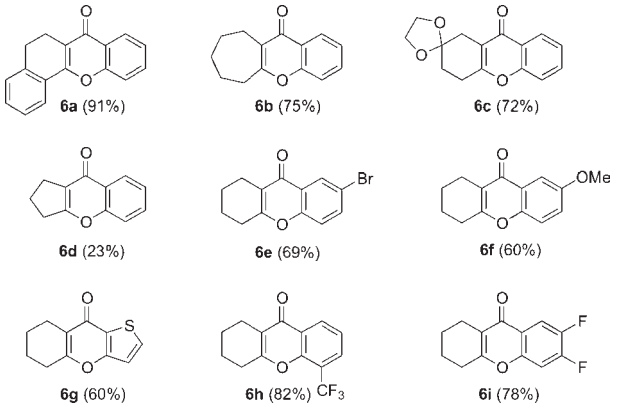
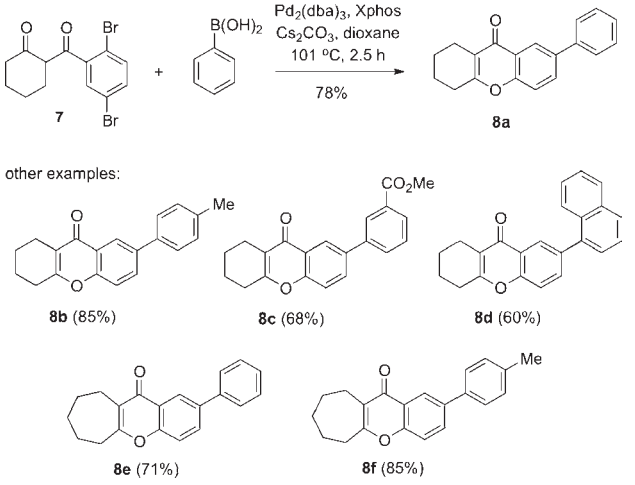


Figure 2. Evaluation of scope of Pd-catalyzed reaction for the synthesis of tetrahydroxanthones and related structures. Reaction conditions: haloketone (0.35 mmol), $Pd_2(dba)_3$ (2.5 mol %), Xphos (6 mol %), Cs_2CO_3 (2.2 molar equiv), 1,4-dioxane (1 mL), 101 °C, 18 h.

Scheme 2. Synthesis of THXs by Sequential Pd(0) Catalyzed C−O and C−C Bond Formation



Next, we explored the development of the tandem process. When dibromide **7** was subjected to Pd catalysis in the presence of $PhB(OH)_2$, **8a** was produced in 78% yield by sequential C−O and C−C bond construction (Scheme 2). Time-course GC−MS analysis revealed rapid consumption of **7** leading to **6e** by intramolecular C−O bond formation. Further conversion to **8a** was observed through subsequent intermolecular Miyaura-Suzuki cross-coupling. No evidence for products arising from C−C bond formation prior to ring closure could be detected by this method. A small library of arylated derivatives namely **8a**−**f** were made by use of different ketones and arylboronic acids in this sequential process. In the case of

(14) In related substrates bearing two C−Br bonds, the C−Br bond ortho to the carbonyl group has been shown to undergo regioselective Pd catalyzed reactions. For examples, see: (a) Ye, Y. Q.; Koshino, H.; Onose, J.-I.; Yoshikawa, K.; Abe, N.; Takahashi, S. *Org. Lett.* **2009**, *11*, 5074 (Miyaura-Suzuki). (b) Iwasawa, N.; Otsuka, M.; Yamashita, S.; Aoki, M.; Takaya, J. *J. Am. Chem. Soc.* **2008**, *130*, 6328 (Sonagashira). (c) Houpis, I. N.; Van Hoeck, J.-P.; Tilstam, U. *Synlett* **2007**, 2179 (Kumada).

(15) For copper catalyzed C−O bond construction of related benzopyrans see: Fang, Y.; Li, C. *J. Org. Chem.* **2006**, *71*, 6427.

8c, single crystal X-ray diffraction was used in conjunction with NMR spectroscopy to unambiguously confirm its identity.

With easy access to a range of 7-substituted THXs, we sought to ascertain if they exhibit useful biological effects. Esumi has recently pioneered a new anticancer strategy based upon "antiausterity" through the discovery of the naturally occurring kigamicins.[2] Five different kigamicins are known which vary only in the extent of glycosidation at C−14.[2b] Kigamicin D shows significant antitumor effects against a number of human pancreatic cancer xenografts in nude or scid mice through targeting cancer cells' tolerance to nutrient starvation.[2c,e] Cancer cell lines such as PANC-1 have adapted such that they normally survive for long periods of time under conditions of extreme nutrient starvation. However, they are selectively killed upon addition of kigamicins.[2a] In contrast, most conventional anticancer drugs show weaker cytotoxicity to cancer cells grown under nutrient-deprived conditions.[2c]

To ascertain if the 7−aryl THX nucleus is the pharmacophore responsible for the antiausterity effects of the kigamicins, we evaluated the effect of a representative member of the synthesized THXs against human pancreatic cancer (PANC-1) cells grown separately in nutrient rich media (NRM) and nutrient deprived media (NDM). Commercially sourced kigamicin C was used as a positive control.[16] Significantly, THX **8a** displays the same "antiausterity" activity as the NPs, inhibiting PANC-1 cells survival at >10 times lower concentrations in nutrient deprived conditions than in normal culture (Figure 3).[17] Indeed, synthetic **8a** demonstrated greater selectivity for cancer cells cultured in NDM than the NP itself.[18] That said it is clear that **8a** is appreciably less active (> 100 fold) than kigamicin C, suggesting that other structural elements of this class of NP have a role to play in eliciting the biological response.

In conclusion, we have developed a simple, efficient route to THXs that exploits palladium catalysis to construct the heterocyclic ring and simultaneously introduce the 7-aryl substituent in a controlled manner. Moreover, we have successfully established that the THX nucleus of **8a** is the minimum pharmacophore of the kigamicins. Future studies will be focused on: (i) using this methodology to optimize and refine the potency of these small-molecules with a view to producing medicinal agents that act by this new anticancer strategy and (ii) ascertaining if the bioactivity of related NPs[3−5] also resides in the 7-aryl THX nucleus.

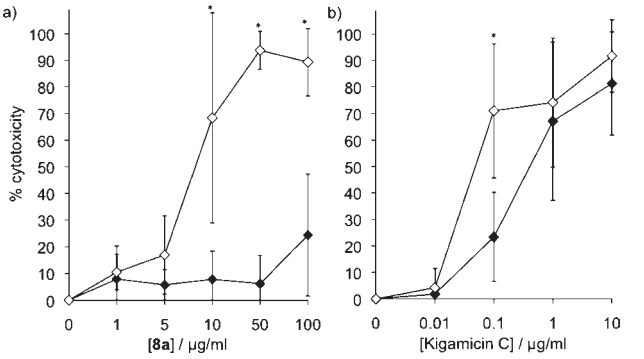


Figure 3. Effects of (a) synthetic THX (**8a**) and (b) kigamicin C on PANC-1 (pancreatic cancer) cells grown in nutrient deprived media (—◊—) and nutrient rich media (—♦—). Cell viability determined using LDH assay after incubation with agent (0−100 μg/mL) for 24 h. Measurements were performed at least in triplicate. Full details are in the Supporting Information. *p = ≤0.05, Mann−Whitney, n = 3−6.

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Supporting Information Available. Experimental procedures, characterization data, copies of NMR spectra for **4a**, **5**−**8**, and "antiausterity" assay. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Kigamicin C was purchased from bioaustralis fine chemicals (http://www.bioaustralis.com).

(17) IC_{50} = 24 μM in NDM, IC_{50} > 0.36 mM in NRM.

(18) Against PANC-1 cells, the IC_{50} value (0.089 μM in NDM) and selective cytotoxicity [selectivity = IC_{50} (in NRM)/IC_{50} (in NDM) = 9] determined in this study for kigamicin C are broadly consistent with previously published values (see refs 2a and 2e).