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Semisynthetic Derivatives of Sesquiterpene Lactones by Palladium-Catalyzed Arylation of the α-Methylene-γ-lactone Substructure

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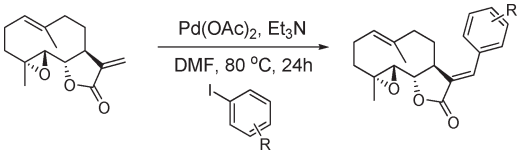

The palladium-catalyzed arylation of different α-methylene-γ-lactone-containing sesquiterpene lactones was shown to produce *E*-olefin coupling products selectively in moderate to excellent yields. Biological evaluation of these semisynthetic sesquiterpene lactone derivatives in HeLa cells showed interesting antiproliferative profiles and provided initial structure−activity data.

The conversion of α-methylene-γ-butyrolactone into α-alkylidene-γ-butyrolactones via metal-catalyzed processes has recently attracted attention to develop efficient routes to biologically active natural products containing these functional groups.[1−5] Specifically, the palladium-catalyzed arylation of α-methylene-γ-butyrolactone was reported to provide a mixture of 3-benzylfuran-2(5*H*)-ones and α-benzylidene-γ-butyrolactones with the *Z*-olefin geometry (Figure 1).[1] In contrast, ruthenium-catalyzed cross-metathesis protocols from Cossy[2] and Howell[3] provided the α-alkylidene-γ-butyrolactones with excellent selectivity for the *E*-olefin geometry. Thus far, no investigations have extended these studies to highly complex molecules containing the α-methylene-γ-lactone substructure.[5] Such work would provide valuable details about the scope of these synthetic methods.

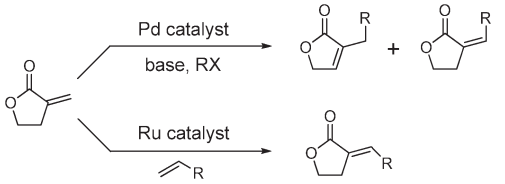

FIGURE 1. Products reported from Heck[1] and cross-metathesis reactions[2,3] on α-methylene-γ-butyrolactone.

The sesquiterpene lactone class of natural products has a diverse range of biological activities, including anticancer, anti-inflammatory, and antiviral properties, and many contain the α-methylene-γ-lactone motif.[6] Recently, interest in these natural products has dramatically increased due to reports of anticancer stem cell activity.[7] Although some structural modifications to these natural products have been made,[6c,8−10] the direct homologation of the α-methylene-γ-lactone substructure has not been explored. Such derivatives would provide additional structure−activity data for this class of natural products, because the electrophilic α-methylene-γ-lactone is known to react with nucleophilic intracellular thiols.[6] Herein, we report our studies on the palladium-catalyzed arylation of sesquiterpene lactones to provide coupled products with exclusively the *E*-olefin geometry. Although this stereochemical result conflicted with prior work of Arcadi and co-workers,[1] our data supports that the

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(2) Moise, J.; Arseniyadis, S.; Cossy, J. *Org. Lett.* **2007**, *9*, 1695–1698.

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(5) (a) One example of an intramolecular Heck reaction has been reported. Ishibashi, H.; Ito, K.; Hirano, T.; Tabuchi, M.; Ikdea, M. *Tetrahedron* **1993**, *49*, 4173–4182. (b) Combinatorial libraries have recently been assembled from some substituted α-methylene-γ-butyrolactones. Elford, T. G.; Ulaczyk-Lesanko, A.; De Pascale, G.; Wright, G. D.; Hall, D. G. *J. Comb. Chem.* **2009**, *11*, 155–168.

(6) (a) Recent reports of anti-cancer activity: Liu, Z.; Liu, S.; Xie, Z.; Pavlovicz, R. E.; Wu, J.; Chen, P.; Aimiuwu, J.; Pang, J.; Bhasin, D.; Neviani, P.; Fuchs, J. R.; Plass, C.; Li, P.-K.; Li, C.; Huang, T. H. M.; Wu, L.-C.; Rush, L.; Wang, H.; Perrotti, D.; Marcucci, G.; Chan, K. K. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 505–514. Gopal, Y. N. V.; Arora, T. S.; Van Dyke, M. W. *Chem. Biol.* **2007**, *14*, 813–823. Steele, A. J.; Jones, D. T.; Ganeshaguru, K.; Duke, V. M.; Yogashangary, B. C.; North, J. M.; Lowdell, M. W.; Kottaridis, P. D.; Mehta, A. B.; Prentice, A. G.; Hoffbrand, A. V.; Wickremasinghe, R. G. *Leukemia* **2006**, *20*, 1073–1079. Lindenmeyer, M. T.; Hrenn, A.; Kern, C.; Castro, V.; Murillo, R.; Muller, S.; Laufer, S.; Schulte-Monting, J.; Siedle, B.; Merfort, I. *Bioorg. Med. Chem.* **2006**, *14*, 2487–2497. (b) Anti-inflammatory activity: Wagner, S.; Hofmann, A.; Siedle, B.; Terfloth, L.; Merfort, I.; Gasteiger, J. *J. Med. Chem.* **2006**, *49*, 2241–2252. Siedle, B.; Garcia-Pineres, A. J.; Murillo, R.; Schulte-Monting, J.; Castro, V.; Rungeler, P.; Klaas, C. A.; Da Costa, F. B.; Kisiel, W.; Merfort, I. *J. Med. Chem.* **2004**, *47*, 6042–6054. (c) Anti-viral activity: Hwang, D.-R.; Wu, Y.-S.; Chang, C.-W.; Lien, T.-W.; Chen, W.-C.; Tan, U.-K.; Hsu, J. T. A.; Hsieh, H.-P. *Bioorg. Med. Chem.* **2006**, *14*, 83–91.

(7) Guzman, M. L.; Rossi, R. M.; Karnischky, L.; Li, X.; Peterson, D. R.; Howard, D. S.; Jordan, C. T. *Blood* **2005**, *105*, 4163–4169.

(8) Recent examples: Nasim, S.; Crooks, P. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3870–3873. Srivastava, S. K.; Abraham, A.; Bhat, B.; Jaggi, M.; Singh, A. T.; Sanna, V. K.; Singh, G.; Agarwal, S. K.; Mukherjee, R.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4195–4199. Macias, F. A.; Velasco, R. F.; Alvarez, J. A.; Castellano, D.; Galindo, J. C. G. *Tetrahedron* **2004**, *60*, 8477–8488. Barrero, A. F.; Rosales, A.; Cuerva, J. M.; Oltra, J. E. *Org. Lett.* **2003**, *5*, 1935–1938. (e) Kwok, B. H. B.; Koh, B.; Ndubuisi, M. I.; Elofsson, M.; Crews, C. M. *Chem. Biol.* **2001**, *8*, 759–766.

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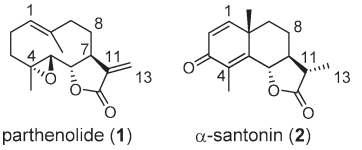
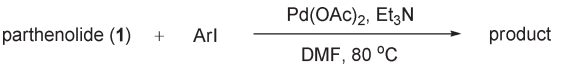


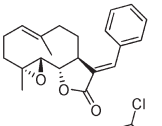
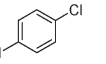
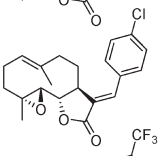
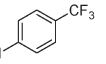
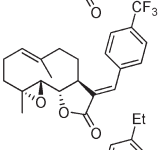
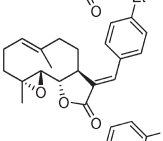
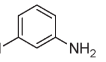
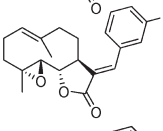
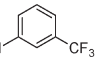
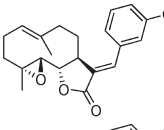
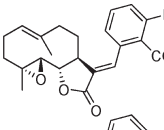
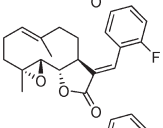
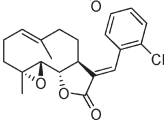
FIGURE 2. Structures of two sesquiterpene lactones.

preferential selectivity for the *E*-isomer in the Heck reaction extends to α-methylene-γ-lactones as well. Growth inhibition studies on cancer cells were subsequently performed to evaluate the biological activity of these semisynthetic sesquiterpene derivatives. Also, an analysis of the structure−activity data for these arylated α-methylene-γ-lactones is described.

We began our investigations to determine the results of palladium-catalyzed arylation reactions with substituted α-methylene-γ-lactones. Using sesquiterpene lactone, parthenolide (**1**), as a representative structure (Figure 2), we conducted a series of reactions with readily available aryl iodides using the previously reported Heck reaction conditions[1] (Table 1). We hypothesized that only an exocyclic olefin would be produced because the C11−C13 insertion should occur opposite to the C7 proton which renders this proton unavailable for β-hydride elimination.[11] Indeed, when 5 mol % of $Pd(OAc)_2$ with Et_3N in DMF at 80 °C was used, a single product with an exocyclic olefin was isolated in each case after purification in moderate to good yields (57%−85%). The substitution pattern on the aromatic ring and the presence of electron-donating or electron-withdrawing substituents did not affect the yields or the preference for a single olefin geometry in the isolated product. We also strategically selected aryl iodides that contained primarily fluorine substituents as well as a variety of election-donating and -withdrawing groups in order to understand the structure−activity relationships of substituents on the aromatic ring of the analogues. Fluorinated compounds can have enhanced biological profiles and serve as metabolic probes. The assignment of the C11−C13 olefin geometry (parthenolide numbering) of **3** was determined using ^1H, COSY, and NOESY NMR experiments to be the *E*-olefin. Specifically, NOESY crosspeaks were readily apparent between the phenyl ring protons and the protons attached to C7 and C8 on the macrocycle. Also, the C13 vinyl proton had a chemical shift of 7.68 ppm that supports the assignment as an *E*-olefin. For **4**−**11**, the assignment of the *E*-olefin geometry was accomplished by the diagnostic chemical shift of the C13 proton.[12] The assignment of the *E*-olefin for the compounds **8** and **11** was further verified following determination of an X-ray crystal structure of each (Figure 3).[13] These data support that the preferential selectivity for the *E*-isomer (over the *Z*-isomer) from the Heck reaction[11] extends to α-methylene-γ-lactones and renders the prior assignment of the *Z*-olefin-containing products questionable.[1,14] The conformation of the macrocycle of **8** and **11** did not change under the reaction conditions and is similar to the reported X-ray structure of **1**.[15] Also, no structural reorganizations of the bicyclic ring system in the parthenolide derivatives were observed.[9]

TABLE 1. Heck Couplings with Parthenolide and Aryl Iodides[a]



entry	ArI	product	yield[b]
1		**3** 	60%
2		**4** 	81%
3		**5** 	73%
4		**6** 	57%
5		**7** 	85%
6		**8** 	80%
7		**9** 	72%
8		**10** 	85%
9		**11** 	58%

[a]All reactions were carried out for 24 h. [b]All yields refer to isolated, pure products.

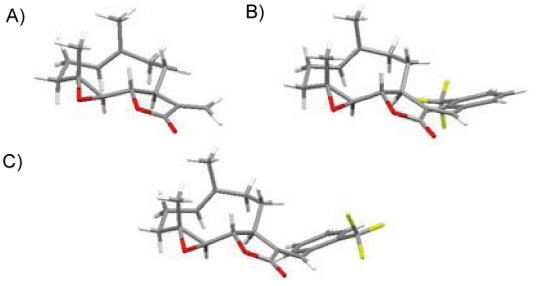
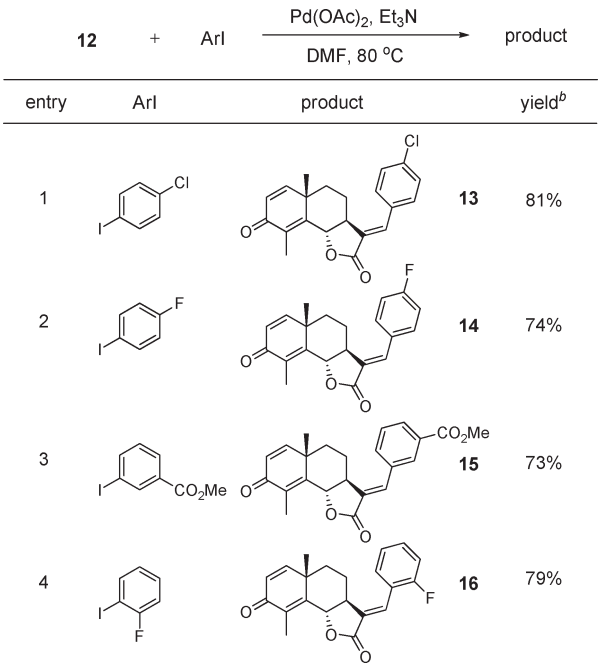


FIGURE 3. X-ray structures of sesquiterpene lactones: (A) parthenolide[15] (**1**), (B) compound **8**, (C) compound **11**.

To explore the scope of this arylation protocol further, the sesquiterpene lactone, α-santonin (**2**), was modified to install

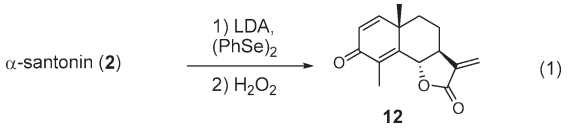
(11) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009–3066.
(12) See Table S1 in the Supporting Information.
(13) See the Supporting Information.
(14) See Table S2 in the Supporting Information.
(15) Quick, A.; Rogers, D. *J. Chem. Soc., Perkins Trans. 2* **1976**, 465–469.

TABLE 2. Heck Couplings with 12 and Aryl Iodides[a]



[a]All reactions were carried out for 20−24 h. [b]All yields refer to isolated, pure products.

the requisite α-methylene-γ-lactone, according literature precedent (eq 1).[16]



The 11,13-dehydrosantonin **12** presents three α,β-unsaturated carbonyl systems for the investigation of chemoselectivity under the Heck reaction conditions. The reaction proceeded exclusively at the *exo*-methylene functional group of **12** and, similar to **1**, the *E*-olefin products were isolated in good yields (73%−81%) with several aryl iodides (Table 2). Again, the substituents on the aromatic ring did not affect the reaction yields. The assignment of *E*-olefin geometry was analogously accomplished by the diagnostic C13 vinyl proton.[12] Similar to the arylation of **1**, no structural reorganization of the tricyclic ring system was observed in the derivatives of **2**.[17]

Because the sesquiterpene lactones are bioactive natural products, we conducted growth inhibition assays with HeLa (cervical cancer) cells[18] on sesquiterpene lactones **3**−**16** to determine the antiproliferative action (Table 3). We selected HeLa cells because they have been widely studied with sesquiterpene lactones.[6a,19] Although the electrophilic α-methylene-γ-lactone is known to trap nucleophilic intracellular thiols, such as cysteine residues,[6] the biological effect

TABLE 3. Antiproliferative Assay in HeLa Cells[a]

compd	IC_{50}, μM	compd	IC_{50}, μM
1	7.8 ± 1.3	**10**	> 200
3	> 200	**11**	> 200
4	21.6 ± 1.3	**12**	12.1 ± 1.5
5	49.8 ± 1.7	**13**	> 200
6	32.7 ± 1.5	**14**	> 200
7	> 200	**15**	> 200
8	15.2 ± 1.5	**16**	> 200
9	> 200		

[a]Antiproliferative assays were conducted in the HeLa (cervical cancer) cell line. All values represent the average of $n = 3 \pm$ standard deviation.

of α-arylation of this functional group is not known. In our assay, parthenolide **1** shows activity similar to previously reported values in HeLa cells ($IC_{50} = 8\ \mu M$) and served as a positive control.[19] The 11,13-dehydrosantonin **12** is 2-fold less active than parthenolide. The most potent arylated derivatives of **1** or **12** is the parthenolide analogue **8** containing the *m*-trifluoromethyl substituent with an $IC_{50} = 15.2\ \mu M$. This semisynthetic derivative **8** is 2-fold less potent than the parent compound, despite the presumed steric hindrance on the electrophilic α-methylene-γ-lactone caused by the α-aryl group during a thiol approach. Analysis of structure−activity data shows the parthenolide analogues **4**−**6** containing a para substituent on the aryl ring retain some activity. However, all derivatives (**9**−**11**) containing ortho-substituents show no activity in the assay. Also, the arylated α-santonin derivatives **13**−**16** were inactive. In contrast, the semisynthetic parthenolide derivatives **7**−**9** with a meta substituent displayed a dramatic range of activity. Overall, the analogues with electron-withdrawing substituents at the meta- and para-positions retain activity, and this observation may imply that analogues with multiple electron-withdrawing substituents at these sites will provide additional improvements in biological activity.

In summary, we have demonstrated the utility of the Heck reaction to generate α-benzylidene-γ-lactones with selectivity for the *E*-olefin geometry from sesquiterpene lactones with α-methylene-γ-lactone substructures. This strategy provides products in good yields and is amenable to assembling derivatives of molecules containing the α-methylene-γ-lactone substructure. A preliminary biological evaluation of these new semisynthetic sesquiterpene lactones in HeLa cells led to the identification of the novel *m*-trifluoromethyl compound **8** and provided structure−activity data about the role of substituents on the α-benzylidene-γ-lactone functional group. Additional studies to improve the biological activity of **8** through the incorporation of multiple substituents on the aromatic ring and by replacing the phenyl ring with heteroaromatic rings are underway.

Experimental Section

General Procedure for Palladium-Catalyzed Arylation. Synthesis of (*E*)-13-Phenylparthenolide (3). A mixture of parthenolide (10 mg, 0.04 mmol), triethylamine (17 μL, 0.12 mmol), and iodobenzene (5 μL, 0.045 mmol) in DMF (200 μL) was treated with palladium(II) acetate (0.5 mg, 0.002 mmol) and then heated at 80 °C under air. After 24 h, the reaction mixture was allowed to cool to rt, water (2 mL) was added, and the resultant mixture was extracted with Et_2O (2 mL × 5). The organics were dried over Na_2SO_4 and concentrated under reduced pressure. SiO_2 flash

(16) Rossi, C.; Ambrogi, V.; Grandolini, G.; Scarcia, V. *J. Pharm. Sci.* **1986**, *75*, 784–786.
(17) Barton, D. H. R. *Helv. Chim. Acta* **1959**, *42*, 2604–2616.
(18) Sun, H.-X.; Zheng, Q.-F.; Tu, J. *Bioorg. Med. Chem.* **2006**, *14*, 1189–1198.
(19) Fonrose, X.; Ausseil, F.; Soleilhac, E.; Masson, V.; David, B.; Pouny, I.; Cintrat, J.-C.; Rousseau, B.; Barette, C.; Massiot, G.; Lafanechere, L. *Cancer Res.* **2007**, *67*, 3371–3378.

chromatography (19:1−7:3 hexanes/EtOAc) afforded the (*E*)-13-phenylparthenolide product **3** as a solid (7.8 mg) in 60% yield: mp 196−198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, J = 3.6 Hz, 1H), 7.46−7.35 (m, 5H), 5.29 (d, J = 11.5 Hz, 1H), 3.96 (dd, J = 8.8, 6.8 Hz, 1H), 3.32 (m, 1H), 2.84 (d, J = 8.9 Hz, 1H), 2.42 (m, 1H), 2.27−2.09 (m, 5H), 1.68 (s, 3H), 1.42 (m, 1H), 1.31 (s, 3H), 1.27 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 138.2, 134.8, 133.5, 129.8 (2), 129.7, 129.0, 128.5 (2), 125.0, 83.0, 66.5, 61.6, 46.8, 41.8, 36.1, 30.1, 24.3, 17.5, 17.4; IR (film) ν_{max} 2928, 1753, 1644, 1193 cm^{-1}; HRMS (EI) m/z calcd for $C_{21}H_{24}O_3$ $(M)^+$ 324.1725, found 324.1732; $[\alpha]^{25}_D$ +142 (c 0.73, $CHCl_3$).

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Supporting Information Available: Additional experimental details, full characterization data, ^{1}H and ^{13}C spectral reproductions, and X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.