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Expeditious Synthesis of 1,1-Diarylethylenes Related to Isocombretastatin A-4 (*isoCA4*) via Palladium-Catalyzed Arylation of *N*-Tosylhydrazones with Aryl Triflates

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Abstract— A quick and efficient entry to 1,1-diarylethylenes *via* the reaction of poly-oxygenated aryl *N*-tosylhydrazones with aryl triflates is described. The reaction employs the catalytic system Pd(OAc)₂/XPhos, *t*BuOLi as the base and dioxane as the solvent. A variety of substituents on both coupling partner's hydrazones and triflates are tolerated. This procedure provides a complementary route to the existing methods for the access to 1,1-diarylethylenes of biological interest.

Combretastatins CA-4 and CA-1 are a very interesting class of cytotoxic agents of natural origin, which have received much attention due to their simple structures, their high potency as cytotoxic and antimitotic agents as well as their ability to selectively damage tumor neovasculature¹ Despite their remarkable anticancer activities, these *Z*-stilbene compounds are prone to double bond isomerization during storage and administration.² The *E*-isomers display dramatically reduced inhibition of cancer cell growth and tubulin assembly.³ A number of structure-activity relationships (SARs) have been reported for the combretastatins.⁴ These studies revealed that the 3,4,5-trimethoxyphenyl unit as well as the *cis* orientation of the two aromatic rings is a prerequisite for significant biological activities. Therefore, extensive studies have been conducted to prepare various *cis* restricted analogues by inserting mainly the *cis*-olefin in a five-membered heterocyclic ring (e.g.; pyrazoles, thiazoles, triazoles, imidazoles, ...).⁵

Our interest in 1,1-diarylethylene unit synthesis,^{6,7} combined with our efforts to discover novel potent tubulin assembly inhibitors, related to CA-4,⁸ led us to identify a promising class of substances with strong anticancer activities, simply by switching the trimethoxyphenyl nucleus from the C(1) to the C(2) position of the ethylene bridge.⁹ In contrast to their natural parent combretastatins A, these synthetic isomers, named *isocombretastatins* A (*isoCA*), are easy to synthesize without the need to control the olefin geometry and constitute the simplest isomers of *isoCA*. The most active compound *isoCA-4*

appears to elicit its tumor cytotoxicity in a fashion similar to CA-4, *via* inhibition of tubulin polymerization, which then leads to cell cycle arrest in G2/M. As the replacement of the 1,2-ethylene bridge by the 1,1-ethylene one resulted in retention of biological activity, our finding encouraged us to use this bioisostere¹⁰ in future structure activity relationship studies. To this end, a set of compounds derived from the general structures **1** were considered (Figure 1), and a versatile synthesis that would allow the incorporation of a variety of aryl substituents was therefore needed.

Figure 1. Combretastatins A-1, A-4, synthetic tubulin polymerization inhibitor *isoCA-4* and target structure **1**.

Typically, the synthesis of the most active compound *isoCA-4* was performed by the reaction of 3,4,5-trimethoxyacetophenone *N*-tosylhydrazone¹¹ **2a** with 2-*t*-butyldimethylsilyloxy-4-iodoanisole. Thus, when using Pd₂(dba)₃ (10 mol%) as a catalyst and Xphos (20 mol%) as a ligand, the coupling reaction gave the desired product *isoCA-4* in a 75% yield.¹² This synthetic approach proved to be suitable for the synthesis of the others

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isocombretastatins A-1 to A-5^{9a} since no stoichiometric organometallic reagent was employed and also, in view of the simple transformation of the acetophenones into their corresponding *N*-tosylhydrazones. Although this reaction has been investigated with aryl halides,^{9a,11} there is no report employing the use of aryl triflates. Extension of this coupling reaction to include aryl triflates as electrophiles¹³ is especially important since these substrates can be easily synthesized from phenol sources and consequently can be revealed at a late stage in a synthetic sequence. The commercial availability of phenol derivatives will make this approach sufficiently diversity oriented, thus fulfilling the recent demand for the generation of large combinatorial chemical libraries. Herein we report our studies in the synthesis of 1,1-diarylethylenes **1** from the coupling of polyoxygenated *N*-tosylhydrazone **2** with aryl triflates.

Initial studies were performed by coupling 3,4,5-trimethoxyacetophenone *N*-tosylhydrazone (**2a**) and aryl triflate **3a**, as model substrates using Pd₂(dba)₃ (5 mol%), Xphos (10 mol%) and *t*BuOLi (2 equiv) in dioxane at 90 °C. Under these conditions (conventional atmospheric pressure reaction), **1a** was formed in a modest 54% yield (entry 1). Fortunately, a much higher yield was obtained under pressure by running the reaction in a sealed tube (84%, entry 2). As summarized in Table 1, the nature of the base has an influence on the outcome of the reaction. *t*BuOLi appeared the base of choice to secure the 1,1-diarylethylene **1a** formation since performing the same reaction with *t*BuOK, Cs₂CO₃ or K₃PO₄ lead to lower yields (entries 3-6). The screening was continued with respects to palladium source and ligands. We were pleased to find that the catalytic activity of Pd(OAc)₂ or PdCl₂(MeCN)₂ proved to be similar to Pd₂(dba)₃ leading to **1a** with excellent yields (89-91%, entries 9 and 10). Pd/C or Pd(OH)₂ however, showed moderate catalytic activities (entries 7 and 8) since **1a** was formed with lower yields than those obtained with Pd(OAc)₂ or PdCl₂(MeCN)₂. Evaluation of ligands revealed that Xphos (**L3**) and CyJohnPhos (**L1**) have a similar efficiency and are superior to all other choices (entries 11-13). Finally we examined the effect of the leaving group (LG) of the electrophilic coupling partner. A comparative study revealed that the reactivity of triflate **3a** is superior to that of imidazolylsulfonate **3b** (entry 14) and nonaflate **3c** (entry 15) whereas the coupling failed when using tosylate **3d** (entry 16). Considering its high catalytic activity Pd(OAc)₂ was our choice for further experimentation. In summary, the best conditions were found to require: **2a** (1 equiv), **3a** (1 equiv), Pd(OAc)₂ (5 mol%), Xphos (10 mol%), *t*BuOLi (2 equiv), dioxane in a sealed tube at 90 °C for 3 h.¹⁴ It should be noted that microwave heating is also effective for this reaction. However, if the reaction time is reduced (~0.5 h),¹⁵ a slightly lower yield (78%) was obtained.

Table 1. Initial studies for Pd-Catalyzed Barluenga Coupling of *N*-tosylhydrazone **2a** with **3**.^a

3a: LG = OTf
3b: LG = Im (Im = Imidazolylsulfonate)
3c: LG = ONfF
3d: LG = OTs

Entry	[Pd] / Ligand	LG	Base	Yield ^b (%)
1	Pd ₂ (dba) ₃ / L3	OTf	<i>t</i> BuOLi	54 ^c
2	Pd ₂ (dba) ₃ / L3	OTf	<i>t</i> BuOLi	84
3	Pd ₂ (dba) ₃ / L3	OTf	<i>t</i> BuOK	34
4	Pd ₂ (dba) ₃ / L3	OTf	Cs ₂ CO ₃	23
5	Pd ₂ (dba) ₃ / L3	OTf	K ₃ PO ₄	50
6	Pd ₂ (dba) ₃ / L3	OTf	Li ₂ CO ₃	0
7	Pd/C / L3	OTf	<i>t</i> BuOLi	13
8	Pd(OH) ₂ /C / L3	OTf	<i>t</i> BuOLi	30
9	PdCl ₂ (MeCN) ₂ / L3	OTf	<i>t</i> BuOLi	89
10	Pd(OAc) ₂ / L3	OTf	<i>t</i> BuOLi	91
11	Pd(OAc) ₂ / L1	OTf	<i>t</i> BuOLi	82
12	Pd(OAc) ₂ / L2	OTf	<i>t</i> BuOLi	55
13	Pd(OAc) ₂ / L4	OTf	<i>t</i> BuOLi	19
14	Pd(OAc) ₂ / L3	OSO ₂ Im	<i>t</i> BuOLi	70
15	Pd(OAc) ₂ / L3	ONfF	<i>t</i> BuOLi	52
16	Pd(OAc) ₂ / L3	OTs	<i>t</i> BuOLi	5

^a All reactions were heated in a sealed tube in dioxane using **2a** (1 equiv), **3** (1 equiv), [Pd] (5 mol%), Ligand (10 mol%) and base (2 equiv) at 90 °C for 3 h.

^b Isolated yield of **1a**.

^c Reaction was performed at 90 °C at atmospheric pressure.

Table 2. Synthesis of 1,1-diarylethylene **1a**

Entry	Hydrazone 2	ArOTf 3	Product 1	Yield ^b (%)
1				91

2				90
3				75
4				69
5				94
6				97
7				90
8				74
9				75
10				66
11				52 ^c

^a All reactions were heated in a sealed tube in dioxane using **2a** (1 equiv), **3** (1 equiv), Pd(OAc)₂ (5 mol%), XPhos (10 mol%) and *t*BuOLi (2 equiv) at 90 °C for 3 h.

^b Isolated yield of **1**.

^c Not optimized.

After these optimization studies, we applied this catalytic system for the coupling of various aryl triflates and polyoxygenated tosylhydrazones **2** to assess the scope of the developed reaction conditions (Table 2). First, 3,4,5-trimethoxyacetophenone *N*-tosylhydrazone **2a** was reacted with various aryl triflates **3** to afford the corresponding 1,1-diarylethenes **1a-c** (entries 1-3). Running the reaction from aryl triflate **3g** bearing a para sp²-carbon-chlorine bond, the coupling reaction gave

selectively **1d** in a 69% yield (entry 4). Among these substrates, the coupling reaction worked efficiently even in the case of alkaline sensitive tosylhydrazones **2b** and **2c** bearing silyloxy groups in good to excellent yields (entries 5-8). Heteroaromatic triflates **3f-e** also were coupled successfully with hydrazone **2a** and provided the desired coupling products **1i-k** in a satisfactory yields (entries 9-11), despite the fact that the reaction conditions with these heterocyclic substrates had never been optimized.

In conclusion, we have described an efficient and general method for cross coupling of different aryl triflates with polyoxygenated hydrazones catalyzed by the combination of Pd(OAc)₂ or PdCl₂(MeCN)₂ and Xphos ligand in the presence of *t*BuOLi as base in a sealed tube. In our opinion, this approach seems to be a suitable method for the synthesis of others *isocombrestatin A* analogues. The design of antitumor agents based on the 1,1-diarylethylene scaffold in future structure-activity relationship studies is currently underway; the results of synthetic and biological studies will be reported in due course.

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- (a) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119–131. (b) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666–1672. (d) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209–211.
- Pettit, G. R.; Toki, B.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Chapuis, G. C. *J. Med. Chem.* **1999**, *42*, 1459–1465.
- (a) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579–2588. (b) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 2293–2306.
- For a review, see: (a) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Dusacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033–3044. For some recent works, see: (b) Ty, N.; Kaffy, J.; Arrault, A.; Thoret, S.; Pontikis, R.; Dubois, J.; Morin-Allory, L.; Florent, J.-C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1318–1322. (c) Ty, N.; Dupeyre, G.; Chabot, G. G.; Seguin, J.; Tillequin, F.; Scherman, D.; Michel, S.; Cachet, X. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 7494–7503. (d) Lai, M.-J.; Kuo, C.-Y.; Yeh, T.-K.; Hsieh, H.-P.; Chen, L.-T.; Pan, W.-Y.; Hsu, K.-Y.; Chang, J.-Y.; Liou, J.-P. *ChemMedChem* **2009**, *4*, 588–593.
- (a) Nam, N. H. *Curr. Med. Chem.* **2003**, *10*, 1697–1722. (b) Odlo, K.; Hentzen, J.; Fournier dit Chabert, J.; Ducki, S.; Gani, O. A. B. S. M.; Sylte, I.; Skrede, M.; Flørenes, V. A.; Hansen, T. V. *Bioorg. Med. Chem.* **2008**, *16*, 4829–4838. (c) Congiu, C.; Cocco, M. T.; Onnis, V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 989–993. (d) Xue, N.; Yang, X.; Wu, R.; Chen, J.; He, Q.; Yang, B.; Lu, X.; Hu, Y. *Bioorg. Med. Chem.* **2008**, *16*, 2550–2557. (e) Zhang, Q.; Peng, Y.; Wang, X. I.; Keenan, S. M.; Arora, S.; Welsh, W. J. *J. Med. Chem.* **2007**, *50*, 749–754.
- (a) Hamze, A.; Veau, D.; Provot, O.; Brion, J.-D.; Alami, M. *J. Org. Chem.* **2009**, *74*, 1337–1340. (b) Liron, F.; Gervais, M.; Peyrat, J.-F.; Alami, M.; Brion, J.-D. *Tetrahedron Lett.* **2003**, *44*, 2789–2794.
- For some recent preparations of 1,1-diarylethenes see: (a) Bhilare, S. V.; Darvatkar, N. B.; Deorukhkar, A. R.; Raut, D. G.; Trivedi, G. K.; Salunkhe, M. M. *Tetrahedron Lett.* **2009**, *50*, 893–896. (b) Yadav, J. S.; Reddy, B. V. S.; Sengupta, S.; Biswas, S. K.

- Synthesis* **2009**, 1301-1304. (c) Sabarre, A.; Love, J. *Org. Lett.* **2008**, *10*, 3941-3944. (d) Yoshida, K.; Shishikura, Y.; Takahashi, H.; Imamoto, T. *Org. Lett.* **2008**, *10*, 2777-2780. (e) Yuan, D.-Y.; Tu, Y.-Q.; Fan, C.-A. *J. Org. Chem.* **2008**, *73*, 7797-7799. (f) Li, J.-H.; Li, J.-L.; Xie, Y.-X. *Synthesis* **2007**, 984-988. (g) Buettner, M. W.; Naetscher, J. B.; Burschka, C.; Tacke, R. *Organometallics* **2007**, *26*, 4835-4838. Hansen, A. L.; Ebran, J. P.; Gogsig, T. M.; Skrydstrup, T. *Chem. Commun.* **2006**, 4137-4139. (h) Caldwell, S. T.; Quin, C.; Edge, R.; Hartley, R. C. *Org. Lett.* **2007**, *9*, 3499-3502.
- 8 (a) Provot, O.; Giraud, A.; Peyrat, J.-F.; Alami, M.; Brion, J.-D. *Tetrahedron Lett.* **2005**, *46*, 8547-8550. (b) Mousset, C.; Giraud, A.; Provot, O.; Hamze, A.; Bignon, J.; Liu, J. M.; Thoret, S.; Dubois, J.; Brion, J.-D.; Alami, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3266-3271. (c) Mousset, C.; Provot, O.; Hamze, A.; Bignon, J.; Brion, J.-D.; Alami, M. *Tetrahedron* **2008**, *64*, 4287-4294. (d) Giraud, A.; Provot, O.; Hamze, A.; Brion, J.-D.; Alami, M. *Tetrahedron Lett.* **2008**, *49*, 1107-1110.
- 9 (a) Messaoudi, S.; Tréguier, B.; Hamze, A.; Provot, O.; Peyrat, J.-F.; Rodrigo De Losada, J. R.; Liu, J.-M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J.; Brion, J.-D.; Alami, M. *J. Med. Chem.* **2009**, *52*, 4538-4542. (b) Alami, M.; Brion, J.-D.; Provot, O.; Peyrat, J.-F.; Messaoudi, S.; Hamze, A.; Giraud, A.; Bignon, J.; Bakala, J.; Liu, J.-M. WO 122620 A1, 2008.
- 10 Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147-3176.
- 11 (a) Barluenga, J.; Moriel, P.; Valdés, C.; Aznar, F. *Angew. Chem. Int. Ed.* **2007**, *46*, 5587-5590. (b) Barluenga, J.; Tomas-Gamasa, M.; Moriel, P.; Aznar, F.; Valdés, C. *Chem. Eur. J.* **2008**, *14*, 4792-4795.
- 12 Only 50% of the desired product was obtained when running the coupling reaction with Pd₂(dba)₃ (1 mol%) and Xphos (2 mol%).
- 13 For excellent reviews, see: (a) Ritter, K. *Synthesis* **1993**, 735-762. (b) Baraznenok, I. L.; Nenajdenko, V. G.; Balenkova, E. S. *Tetrahedron* **2000**, *56*, 3077-3119.
- 14 **General procedure for the synthesis of 1.** To a dioxane (6 mL) solution of *N*-tosylhydrazone **2** (0.5 mmol), *t*BuOLi (1 mmol), Pd(OAc)₂ (0.025 mmol) and X-Phos (0.05 mmol) was added the aryl triflate **3** (0.5 mmol) in dioxane (2 mL). The reaction vessel was sealed, and then heated at 90 °C for 3 h. The resulting suspension was cooled to room temperature and filtered through a pad of Celite eluting with AcOEt, and the inorganic salts were removed. The filtrate was concentrated and purification of the residue by silica gel column chromatography gave the desired product.
- Diarylethylene **1a**: Yield: 91%. *R_f* (cyclohexane/EtOAc : 6/4) = 0.60. IR (cm⁻¹): 1579, 1507, 1454, 1411, 1346, 1299, 1233, 1174, 1122, 1030, 1004. ¹H RMN: (δ ppm, CD₃COCD₃, 300 MHz): 3.75 (s, 3H, OCH₃), 3.78 (s, 6H, OCH₃), 3.82 (s, 3H, OCH₃), 5.34 (m, 2H, CH₂), 6.60 (s, 2H), 6.92 (d, 2H, *J* = 8.7 Hz), 7.29 (d, 2H, *J* = 8.7 Hz). ¹³C NMR (δ ppm, 75 MHz, CD₃COCD₃): 55.5, 56.4 (2), 60.5, 106.8 (2), 112.7, 114.4 (2), 130.1 (2), 134.4, 138.2 (2), 150.6, 154.1 (2), 160.5.
- Diarylethylene **1d**: Yield: 69%. *R_f* (cyclohexane/EtOAc : 9/1) = 0.58. IR (cm⁻¹): 1579, 1504, 1462, 1410, 1344, 1235, 1125, 1007. ¹H RMN: (δ ppm, CDCl₃, 300 MHz): 3.73 (s, 6H, OCH₃), 3.80 (s, 3H, OCH₃), 5.34 (d, 2H, *J* = 1.0 Hz, CH₂), 6.44 (s, 2H, CH), 7.22 (s, 4H, CH). ¹³C NMR (δ ppm, 75 MHz, CDCl₃): 56.1 (2), 60.9, 105.5 (2), 114.3, 128.4 (2), 129.6 (2), 133.7, 136.8, 137.8, 139.7, 149.0, 153.0 (2).
- Diarylethylene **1i**: Yield: 75 %.. *R_f* (cyclohexane/EtOAc : 7/3) = 0.28. Mp: 143-145 °C. IR (cm⁻¹): 1719, 1574, 1508, 1453, 1411, 1350, 1221, 1177, 1124, 996. ¹H RMN: (δ ppm, CDCl₃, 300 MHz): 3.82 (s, 6H), 3.89 (s, 3H), 5.54 (s, 1H), 5.56 (s, 1H), 6.42 (d, 1H, *J* = 9.5 Hz), 6.51 (s, 2H), 7.27 (dd, 1H, *J* = 1.6 Hz, *J* = 8.0 Hz), 7.35 (d, 1H, *J* = 1.5 Hz), 7.44 (d, 1H, *J* = 8.0 Hz), 7.71 (d, 1H, *J* = 9.5 Hz). ¹³C NMR (δ ppm, 75 MHz, CDCl₃): 56.0 (2), 60.8, 105.6 (2), 116.1, 116.2, 116.4, 118.2, 124.3, 127.4, 136.0, 138.1, 142.9, 145.1, 148.6, 153.0 (2), 153.9, 160.6.
- 15 The reaction was conducted according to the general method described above. The reaction vessel was sealed, then placed in the Emrys Optimizer and exposed to microwave irradiation according to the following specifications: temperature: 120 °C; time: 30 min; fixed hold time: on; sample absorption: high; pre-stirring: 60 s.