Organic LETTERS

Letter

pubs.acs.org/OrgLett

Cyano Group Removal from Cyano-Promoted Aza-Diels–Alder Adducts: Synthesis and Structure–Activity Relationship of Phenanthroindolizidines and Phenanthroquinolizidines

Chi-Fen Chang,[†] Chien-Fu Li,[‡] Chia-Chen Tsai,[‡] and Ta-Hsien Chuang*[,‡,§]

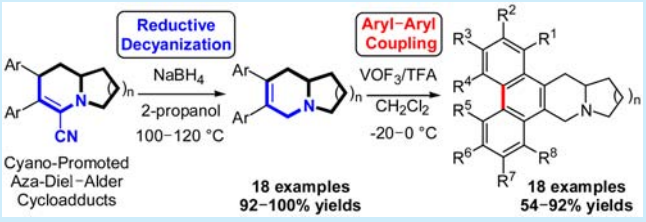
[†]Department of Anatomy, School of Medicine, China Medical University, Taichung 40402, Taiwan

[‡]School of Pharmacy, China Medical University, Taichung 40402, Taiwan

[§]Research Center for Chinese Herbal Medicine, China Medical University, Taichung 40402, Taiwan

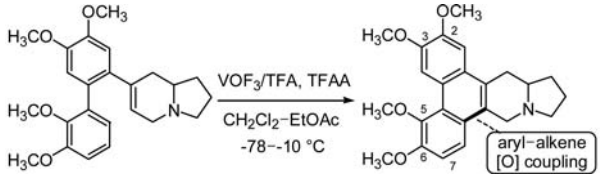
Supporting Information

ABSTRACT: Phenanthroindolizidines and phenanthroquinolizidines were concisely synthesized by the reductive decyanization of cyano-promoted intramolecular aza-Diels–Alder cycloadducts followed by aryl–aryl coupling. Cyano groups were removed from α-aminoacrylonitriles via treatment with sodium borohydride in 2-propanol in almost quantitative yields; a possible mechanism was proposed and examined using D-labeling experiments. A systematic study of the effects of the phenanthrene substitution pattern on the anticancer activity against three human cancer cell lines was discussed.



Intramolecular aza-Diels–Alder (IADA) reactions are useful for the synthesis of nitrogen heterocycles such as indolizidines and quinolizidines. However, it is difficult to react an unsubstituted 1-azadiene with an unreactive carbon–carbon double bond. In a normal ($HOMO_{diene}$-controlled) electron-demand IADA reaction, the introduction of a carbethoxy group on the dienophile would promote the IADA reaction.[1] Alternatively, the presence of an electron-withdrawing group (e.g., acyl, diethoxyphosphoryl, or cyano group) on 1-azadiene would also increase its reactivity toward enophiles by lowering the lowest unoccupied molecular orbital (LUMO) of 1-azadiene in the inverse electron-demand IADA reaction.[2−4] Although these functional groups promote the IADA reaction, their removal after the reaction presents a challenge that limits their applications in the total synthesis of natural products.

Phenanthroindolizidines and phenanthroquinolizidines exhibit several interesting biological activities (e.g., anticancer and antiamoebic activities, etc.), and several synthetic methods with access to these pentacyclic alkaloids for structure–activity relationship (SAR) studies have been developed.[5] Although several previously reported routes were concise, the syntheses and anticancer SARs focused only on specific positions (C-2, C-3, C-6, and C-7) of the phenanthrene ring owing to the limitations of the synthetic methods.[6] These limitations include commercially unavailable starting materials and sensitivity of the formation of the phenanthrene ring by intramolecular aryl–aryl coupling of stilbenes to the substituents on the phenyl rings and the double bond.[7] Notably, Niphakis and Georg reported a VOF_3-mediated aryl–alkene coupling to prepare C5-substituted phenanthroindolizidines (eq 1).[8] However, versatile



(1)

synthetic strategies to access other positions (especially C-1, C-4, and C-8) on phenanthroindolizidines and phenanthroquinolizidines for anticancer SAR studies regarding the phenanthrene substitution pattern are urgently needed.

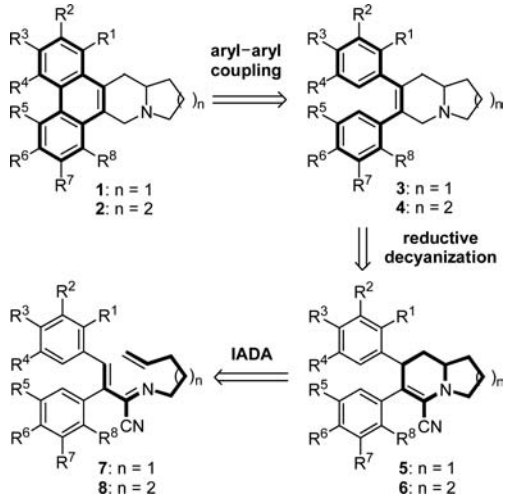
Herein, we report a concise strategy to construct pyrrolizidine and indolizidine systems by a cyano-group-promoted IADA reaction followed by reductive decyanization. A series of phenanthroindolizidines **1** and phenanthroquinolizidines **2** were synthesized from decyanization products **3** and **4** via aryl–aryl oxidative coupling reactions (Scheme 1). This reaction sequence provides a new synthetic approach to synthesize pentacyclic alkaloids with three to five methoxyl groups on the C-1 to C-8 positions of the phenanthrene ring.

First, (*E*)-2,3-diphenylacrylaldehydes **9a–i** were synthesized by the Knoevenagel condensation of commercially available benzaldehydes **11v–z** with phenylacetonitriles **12v–z**, followed by DIBAL-H reduction and hydrolysis under acidic conditions (for the yields of these two reactions see the Supporting Information).[9] Next, α-iminonitrile **7i** was synthesized in a low yield, probably because of poor solubility, by the one-pot reaction of acrylaldehyde **9i**, pent-4-enylamine, trimethylsilyl

Received: November 26, 2015
Published: February 2, 2016

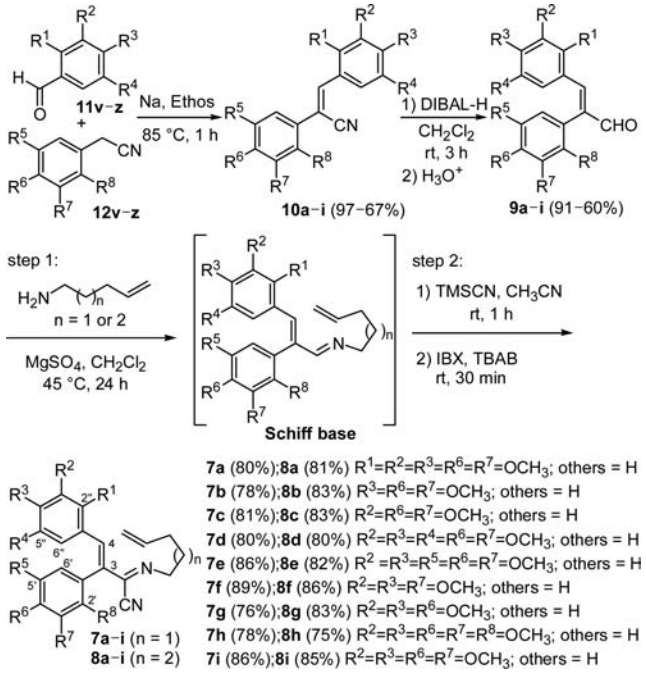
DOI: 10.1021/acs.orglett.5b03395
Org. Lett. 2016, 18, 638–641

Scheme 1. Strategy for Synthesis of Alkaloids 1 and 2



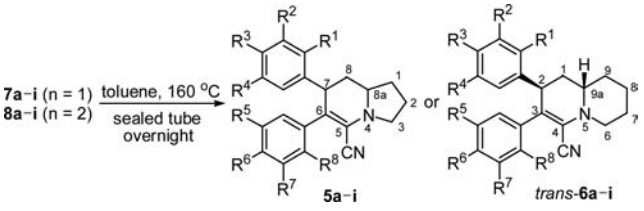
cyanide (TMSCN), 2-iodoxybenzoic acid (IBX), and tetrabutylammonium bromide (TBAB) in CH_3CN following the method described by Zhu et al.[10] Nevertheless, a two-step method was developed to synthesize 2-(alkenylimino)-3,4-diphenyl-(3*E*)-butenenitriles **7a−i** and **8a−i** in good yields via Schiff base formation (Scheme 2).

Scheme 2. Synthesis of IADA Precursors 7a−i and 8a−i



Subsequently, 3,4-bis(3,4-dimethoxyphenyl)-2-(4-pentenylimino)-(3*E*)-butenenitrile **7i** was selected as the initial model to investigate the feasibility of the IADA reaction. Conventional heating was selected over microwave heating, because the cycloaddition reaction of **7i** did not reach completion under microwave conditions. A 0.05 M solution of **7i** in toluene was heated in a sealed tube at 165 °C under the maximum power 250 W for 3 h using a focused microwave reactor. A solution of compound **7i** in toluene was refluxed for 72 h, affording cycloadduct **5i** in 58% yield. A similar result was obtained by heating the mixture in a sealed tube at 130 °C for 48 h. To our delight, the cycloaddition of **7i** could be carried out in a sealed tube by heating at 160 °C overnight, affording *trans*-**5i** (64% yield) and *cis*-**5i** (7% yield). The IADA reactions of 2-(alkenylimino)-3,4-diphenyl-(3*E*)-butenenitriles **7a−i** and **8a−i** were conducted under the above-mentioned optimized conditions, and the yields are shown in Table 1. The IADA

Table 1. Yields of IADA Cycloadducts 5 and *trans*-6

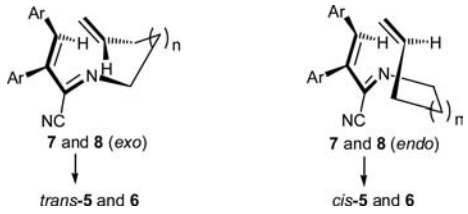


entry	7	8	substituent[a]	5 (%)[b]	6 (%)[c]
1	7a	8a	$R^1 = R^2 = R^3 = R^6 = R^7 = OCH_3$	5a 60	6a 84
2	7b	8b	$R^3 = R^6 = R^7 = OCH_3$	5b 73	6b 90
3	7c	8c	$R^2 = R^6 = R^7 = OCH_3$	5c 78	6c 91
4	7d	8d	$R^2 = R^3 = R^4 = R^6 = R^7 = OCH_3$	5d 67	6d 90
5	7e	8e	$R^2 = R^3 = R^5 = R^6 = R^7 = OCH_3$	5e 62	6e 86
6	7f	8f	$R^2 = R^3 = R^7 = OCH_3$	5f 70	6f 90
7	7g	8g	$R^2 = R^3 = R^6 = OCH_3$	5g 70	6g 92
8	7h	8h	$R^2 = R^3 = R^6 = R^7 = R^8 = OCH_3$	5h 78	6h 93
9	7i	8i	$R^2 = R^3 = R^6 = R^7 = OCH_3$	5i 71	6i 92

[a]The substituents not mentioned are hydrogens. [b]*Trans*/*cis* diastereomeric mixtures **5** were used in the next step. [c]Diastereomers *trans*-**6a−i** were obtained predominantly.

reactions of compounds **7b−i** with a three-atom spacer afforded cycloadducts **5b−i** in *trans*/*cis* ratios of ca. 10:1, as determined by their crude ^{1}H NMR spectra. Interestingly, when IADA precursors **8a−i** with four-atom spacers were used, only cycloadducts *trans*-**6a−i** were isolated in high yields. Thus, the efficiency of the IADA reactions and the *trans*/*cis* diastereomeric ratios of the IADA cycloadducts were affected by the spacer length. The IADA reactions of **7** and **8** could proceed through the more stable *exo* transition state with less steric hindrance, affording *trans*-cycloadducts **5** and **6** as the main products (Scheme 3).[3b]

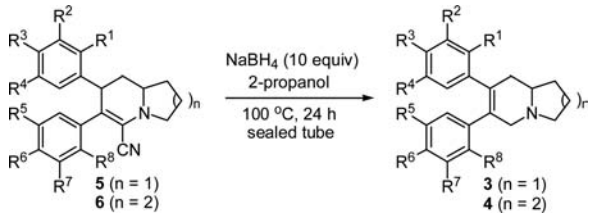
Scheme 3. *Exo* and *Endo* Transition States of IADA



With a series of 6,7-diphenylindolizine-5-carbonitriles (**5a−i**) and *trans*-2,3-diphenylquinolizine-4-carbonitriles (*trans*-**6a−i**) in hand, an efficient method was developed for the removal of the cyano group from the α-aminoacrylonitriles. A mixture of cycloadducts **5a−i** (and *trans*-**6a−i**) and 10 equiv $NaBH_4$ in 2-propanol was heated in a sealed tube at 100 °C for 24 h. The decyanization products, 6,7-diphenylindolizines **3a−i** and 7,8-diphenylquinolizines **4a−i**, were obtained in nearly quantitative yields (Table 2). The reductive decyanization of α-amino-acrylonitriles is of critical importance, because this key step helps to construct indolizidine and quinolizidine ring systems

DOI: 10.1021/acs.orglett.5b03395
Org. Lett. 2016, 18, 638−641

Table 2. Reductive Decyanization of Cycloadducts 5 and 6

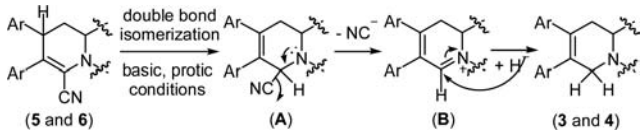


entry	5	6	substituent[a]	3 (%)	4 (%)
1[b]	5a	6a	$R^1 = R^2 = R^3 = R^6 = R^7 = OCH_3$	3a 92	4a 95
2	5b	6b	$R^3 = R^6 = R^7 = OCH_3$	3b 100	4b 100
3	5c	6c	$R^2 = R^6 = R^7 = OCH_3$	3c 100	4c 100
4	5d	6d	$R^2 = R^3 = R^4 = R^6 = R^7 = OCH_3$	3d 96	4d 98
5	5e	6e	$R^2 = R^3 = R^5 = R^6 = R^7 = OCH_3$	3e 100	4e 100
6	5f	6f	$R^2 = R^3 = R^7 = OCH_3$	3f 100	4f 100
7	5g	6g	$R^2 = R^3 = R^6 = OCH_3$	3g 100	4g 100
8[b]	5h	6h	$R^2 = R^3 = R^6 = R^7 = R^8 = OCH_3$	3h 94	4h 97
9	5i	6i	$R^2 = R^3 = R^6 = R^7 = OCH_3$	3i 100	4i 100

[a]The substituents not mentioned are hydrogens. [b]Reactions were carried out at 120 °C.

via the formation of a cyano-group-promoted IADA adduct. A possible mechanism for the reductive decyanization is shown in Scheme 4. First, allylic deprotonation of **5** and **6** under basic,

Scheme 4. A Possible Reductive Decyanization Mechanism



protic conditions could result in the shift of the double bound into stilbene **A**. Then, elimination of a cyanide ion from **A** would lead to the formation of iminium ion **B**, followed by a hydride attack on the iminium carbon to produce diphenyltetrahydropyridine derivatives **3** and **4**.

The mechanism was evaluated using three D-labeling experiments. Reduction of **5i** with $NaBH_4$ in 2-propanol-d_8 (99+ atom% D) and $NaBD_4$ (98 atom % D) in 2-propanol under the same conditions (100 °C, 24 h) afforded one-deuterium product ***d*-3i**, indicating that one of the methylene hydrogens of the allylamine in **3i** was derived from the protic solvent and the other hydrogen came from the reductive agent. Again, only ***d*$_2$-3i** was obtained via treatment of $NaBD_4$ in 2-propanol-d_8 at 100 °C for 24 h (Figure 1).

Next, an efficient oxidizing agent, vanadium oxytrifluoride (VOF_3), was chosen to examine the oxidative aryl−aryl coupling of 6,7-diphenylindolizines **3a−i** and 7,8-diphenylquinolizines **4a−i**. Phenanthroindolizidines **1b**, **1c**, **1f**, **1g**, and **1i** as well as phenanthroquinolizidines **2b**, **2c**, **2f**, **2g**, and **2i**

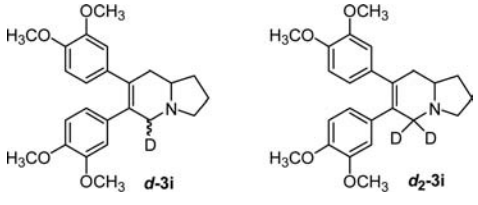
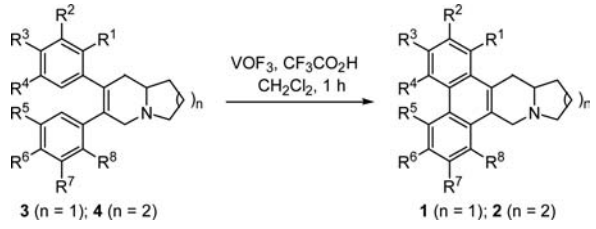


Figure 1. Products of D-labeling reductive decyanization.

with three or four methoxyl groups could be smoothly synthesized under Park's conditions (method A, entries 2, 3, 6, 7, and 9 in Table 3).[11] However, an attempt to prepare

Table 3. Aryl−Aryl Coupling of *cis*-Stilbenes 3 and 4



entry	3	4	Substituent[c]	1 (%)	2 (%)
1[b]	3a	4a	$R^1 = R^2 = R^3 = R^6 = R^7 = OCH_3$	1a 70	2a 77
2[a]	3b	4b	$R^3 = R^6 = R^7 = OCH_3$	1b 82	2b 88
3[a]	3c	4c	$R^2 = R^6 = R^7 = OCH_3$	1c 86	2c 84
4[b]	3d	4d	$R^2 = R^3 = R^4 = R^6 = R^7 = OCH_3$	1d 89	2d 90
5[b]	3e	4e	$R^2 = R^3 = R^5 = R^6 = R^7 = OCH_3$	1e 85	2e 88
6[a]	3f	4f	$R^2 = R^3 = R^7 = OCH_3$	1f 85	2f 86
7[a]	3g	4g	$R^2 = R^3 = R^6 = OCH_3$	1g 86	2g 88
8[b]	3h	4h	$R^2 = R^3 = R^6 = R^7 = R^8 = OCH_3$	1h 54	2h 71
9[a]	3i	4i	$R^2 = R^3 = R^6 = R^7 = OCH_3$	1i 85	2i 92

[a]Method A: a 0.04 M solution of **3** or **4** (0.2 mmol) in anhydrous CH_2Cl_2 (5 mL) was added to VOF_3 (1.0 mmol) at 0 °C and the mixture was stirred for 15 min. TFA (2.8 mmol) was added and the mixture was stirred at 0 °C for 1 h. [b]Method B: a 0.04 M solution of **3** or **4** (0.2 mmol) in anhydrous CH_2Cl_2 (5 mL) was added to VOF_3 (0.4 mmol) at −20 °C and the mixture was stirred for 15 min. TFA (2.8 mmol) was added and the mixture was stirred at −20 °C for 1 h. [c]The substituents not mentioned are hydrogens.

phenanthroquinolizidine **2a** with five methoxyl groups under the same conditions resulted in extensive oxidative decomposition. To our delight, phenanthroindolizidines **1a**, **1d**, **1e**, and **1h** as well as phenanthroquinolizidines **2a**, **2d**, **2e**, and **2h** with five methoxyl groups could be obtained in good to moderate yields (entries 1, 4, 5, and 8 in Table 3) with complete regiospecificity under mild conditions (method B: 2 equiv VOF_3, −20 °C). The total synthesis of phenanthroindolizidines **1a−i** and phenanthroquinolizidines **2a−i** from benzaldehydes with phenylacetonitriles was achieved in six steps in 8.8−42.1% and 19.3−63.5% overall yields, respectively.

Finally, the cytotoxic activities of 18 compounds, **1a−i** and **2a−i**, were evaluated against three human cancer cell lines, breast carcinoma (MCF-7), lung carcinoma (H1299), and prostate carcinoma (DU145), by using tylophorine (**1i**) and 7-methoxycryptopleurine (**2i**) for comparison. The cytotoxic activities of phenanthroindolizidines **1a−i** and phenanthroquinolizidines **2a−i** are shown in Table 4. Phenanthroquinolizidines **2a−i** were more active than the corresponding phenanthroindolizidines **1a−i**.[6h] In the phenanthroindolizidine series, the IC_{50} ratio of compounds **1a−h** to tylophorine (**1i**) followed the order **1a** ∼ **1h** ∼ **1c** > **1f** ≫ **1b** ∼ **1e** > **1d** > **1g**; however, in the phenanthroquinolizidine series, the IC_{50} ratio of compounds **2a−h** to 7-methoxycryptopleurine (**2i**) followed the order **2c** ∼ **2a** ∼ **2h** ∼ **2b** ≫ **2f** ∼ **2d** > **2e** > **2g**. The data indicate that when the C-1 and C-8 substituents were converted from methoxy to hydrogen and the C-3 substituent was converted from hydrogen to methoxy, the cytotoxicity increased dramatically (entries 1, 3, and 8 vs entry 9). Interestingly, C-6 methoxylation was more important than C-2 methoxylation for cytotoxicity of the phenanthroindolizidine

DOI: 10.1021/acs.orglett.5b03395
Org. Lett. 2016, 18, 638−641

Table 4. IC_{50} Values of 1a−i and 2a−i against Three Human Cancer Cell Lines

	compd	IC_{50} (nM)[a]		
entry	1 {2}	MCF-7	H1299	DU145
1	1a {2a}	>2500 {276.4}	>2500 {251.9}	>2500 {271.9}
2	1b {2b}	304.9 {272.4}	235.2 {210.6}	175.6 {184.5}
3	1c {2c}	2481.0 {392.5}	>2500 {314.4}	2152.0 {278.9}
4	1d {2d}	61.2 {61.9}	54.1 {54.4}	58.8 {35.7}
5	1e {2e}	223.9 {19.3}	165.3 {27.6}	150.1 {23.8}
6	1f {2f}	1909.0 {68.7}	2017.0 {57.3}	1510.0 {41.8}
7	1g {2g}	42.4 {11.3}	32.5 {9.0}	30.3 {4.6}
8	1h {2h}	>2500 {277.4}	>2500 {232.4}	>2500 {214.9}
9	1i {2i}	41.1 {10.7}	30.9 {7.1}	28.3 {4.5}

[a]The values in the brackets show the IC_{50} of compounds **2**.

series, whereas a reverse trend was observed in the phenanthroquinolizidine series (entries 2 and 6 vs entry 9). Moreover, C-4 and C-5 methoxylation showed only slightly deleterious effects on the cytotoxicity (entries 4 and 5 vs entry 9). Notably, the absence of a methoxy group at the C-7 position did not significantly affect the cytotoxicity in the two series of alkaloids (entry 7 vs entry 9).

In summary, we developed a concise synthetic strategy for the construction of phenanthroindolizidines and phenanthroquinolizidines by the reductive decyanization of α-aminoacrylonitriles, followed by an efficient aryl−aryl coupling. The reductive decyanization was investigated using D-labeling experiments. The development of more polar and water-soluble phenanthroquinolizidines to minimize the central nervous system toxicity by decreasing diffusion through the blood−brain barrier is underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03395.

Full experimental and characterization data for all compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: thchuang@mail.cmu.edu.tw.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was financially supported by the Ministry of Science and Technology, Taiwan (MOST 104-2113-M-039-001), China Medical University (CMU 104-N-01), and, in part, by a grant from the Chinese Medicine Research Center, China Medical University (the Ministry of Education, the Aim for the Top University Plan).

■ REFERENCES

(1) (a) Hurst, T. E.; Miles, T. J.; Moody, C. J. *Tetrahedron* **2008**, *64*, 874. (b) Ihara, M.; Kirihara, T.; Kawaguchi, A.; Fukumoto, K.; Kametani, T. *Tetrahedron Lett.* **1984**, *25*, 4541.

(2) Sisti, N. J.; Zeller, E.; Grierson, D. S.; Fowler, F. W. *J. Org. Chem.* **1997**, *62*, 2093.

(3) (a) Hwang, Y. C.; Fowler, F. W. *J. Org. Chem.* **1985**, *50*, 2719. (b) Cheng, Y. S.; Lupo, A. T., Jr.; Fowler, F. W. *J. Am. Chem. Soc.* **1983**, *105*, 7696. (c) Cheng, Y. S.; Fowler, F. W.; Lupo, A. T., Jr. *J. Am. Chem. Soc.* **1981**, *103*, 2090.

(4) Whitesell, M. A.; Kyba, E. P. *Tetrahedron Lett.* **1984**, *25*, 2119.

(5) (a) de Fatima Pereira, M.; Rochais, C.; Dallemagne, P. *Anti-Cancer Agents Med. Chem.* **2015**, *15*, 1080. (b) Burtoloso, A. C. B.; Bertonha, A. F.; Rosset, I. G. *Curr. Top. Med. Chem.* **2014**, *14*, 191. (c) Chemler, S. R. *Curr. Bioact. Compd.* **2009**, *5*, 2. (d) Li, Z.; Jin, Z.; Huang, R. *Synthesis* **2001**, *16*, 2365. (e) Gellert, E. *J. Nat. Prod.* **1982**, *45*, 50. (f) Ralph, I.; Bick, C.; Sinchai, W. *Alkaloids* **1981**, *19*, 193. (g) Govindachari, T. R.; Viswanathan, N. *Heterocycles* **1978**, *11*, 587.

(6) (a) Lee, Y. Z.; Yang, C. W.; Hsu, H. Y.; Qiu, Y. Q.; Yeh, T. K.; Chang, H. Y.; Chao, Y. S.; Lee, S. J. *J. Med. Chem.* **2012**, *55*, 10363. (b) Wang, Z.; Wu, M.; Wang, Y.; Li, Z.; Wang, L.; Han, G.; Chen, F.; Liu, Y.; Wang, K.; Zhang, A.; Meng, L.; Wang, Q. *Eur. J. Med. Chem.* **2012**, *51*, 250. (c) Ikeda, T.; Yaegashi, T.; Matsuzaki, T.; Yamazaki, R.; Ueno, S.; Hashimoto, S.; Sawada, S. *Lett. Drug Des. Discovery* **2012**, *9*, 447. (d) Lv, H.; Ren, J.; Ma, S.; Xu, S.; Qu, J.; Liu, Z.; Zhou, Q.; Chen, X.; Yu, S. *PLoS One* **2012**, *7*, e30342. (e) Su, C. R.; Damu, A. G.; Chiang, P. C.; Bastow, K. F.; Morris-Natschke, S. L.; Lee, K. H.; Wu, T. S. *Bioorg. Med. Chem.* **2008**, *16*, 6233. (f) Gao, W.; Bussom, S.; Grill, S. P.; Gullen, E. A.; Hu, Y. C.; Huang, X.; Zhong, S.; Kaczmarek, C.; Gutierrez, J.; Francis, S.; Baker, D. C.; Shishan, Y.; Cheng, Y. C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4338. (g) Fu, Y.; Lee, S. K.; Min, H. Y.; Lee, T.; Lee, J.; Cheng, M.; Kim, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 97. (h) Chuang, T. H.; Lee, S. J.; Yang, C. W.; Wu, P. L. *Org. Biomol. Chem.* **2006**, *4*, 860.

(7) Jin, Z.; Wang, Q.; Huang, R. *Synth. Commun.* **2004**, *34*, 119.

(8) Niphakis, M. J.; Georg, G. I. *Org. Lett.* **2011**, *13*, 196.

(9) Chuang, T. H.; Chang, W. Y.; Li, C. F.; Wen, Y. C.; Tsai, C. C. *J. Org. Chem.* **2011**, *76*, 9678.

(10) Fontaine, P.; Chiaroni, A.; Masson, G.; Zhu, J. *Org. Lett.* **2008**, *10*, 1509.

(11) Xu, X.; Liu, Y.; Park, C. M. *Angew. Chem., Int. Ed.* **2012**, *51*, 9372.

DOI: 10.1021/acs.orglett.5b03395
Org. Lett. 2016, 18, 638−641