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# Concise syntheses of *N*-aryl-5,6,7-trimethoxyindoles as antimitotic and vascular disrupting agents: application of the copper-mediated Ullmann-type arylation†

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**In an attempt to mimic the 3,4,5-trimethoxyphenyl-*Z*-stilbene moiety of combretastatin A-4, a series of *N*-aryl-5,6,7-trimethoxyindoles were synthesized *via* copper-catalyzed Ullmann-type *N*-arylation through the corresponding 5,6,7-trimethoxyindole and aryl halides. These synthesized compounds demonstrated potent antiproliferative activity providing a novel skeleton for potent tubulin polymerization inhibitors.**

Halting the mitotic process of cancer cells through targeting microtubules has been a promising strategy to develop potent anticancer agents.[1] Natural products like colchicine (**1**) and combretastain A-4 (CA4, **2**) (Fig. 1) have been recognized as antimitotic agents interfering with the dynamics of tubulin polymerization. These compounds are known to interact with the colchicine binding site of microtubules resulting in mitotic arrest. Combretastain A-4 was discovered in 1988 and was further examined as a potent antimitotic agent.[2] Due to the simple structure and excellent potency of CA4, the distinguishable *Z*-stilbene and 3,4,5-trimethoxybenzene motif (A-ring) of CA4 attracted scientists' efforts to search for more potent anticancer agents.[3] In an attempt to increase the solubility, for instance, the phosphate prodrug CA4P (**3**) was synthesized and is currently in human clinical trails.[1,4] Compound **4** which has 3′-OH of CA4 replaced with 3′-$NH_2$ demonstrated potent antitubulin activity[5] and its serine prodrug (AVE8602, **5**) is undergoing human clinical trials as well.[1a]

The structural analysis of [6,7,7]-tricyclic colchicine shows that it also possesses a trimethoxyphenyl motif, thus attracting our attention to the synthesis of [6,5]-bicyclic 5,6,7-trimethoxyindoles as a surrogate of the A-ring of CA4. Based on CA4 structure and our experience, 3′-hydroxy-4′-methoxyphenyl, *N*-methylindolyl[6] and quinolinyl[7] groups were selected and attached to the N-1 position of 5,6,7-trimethoxyindole in this study. Therefore, a series of *N*-aryl-5,6,7-trimethoxyindoles were synthesized (**6–10**) and investigated for antiproliferative activity.

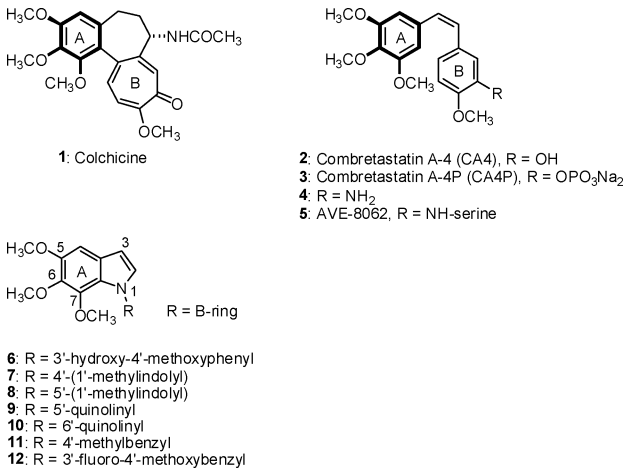


**Fig. 1**

Organometallic chemistry has been comprehensively applied to construct various heterocycles which serve as central structures in the field of medicinal chemistry.[8] In the lead optimization, organometallic chemistry played a significant role to carry out the functionalization as well.[9] *N*-Arylation of indoles from the corresponding aryl halides and indoles could be carried out through two approaches, transition metal-catalyzed coupling reactions and aromatic nucleophilic substitution reactions ($S_NAr$).[10] Palladium and copper are commonly exploited in transition metal-catalyzed coupling reactions. Compared to palladium-catalyzed reactions, however, copper provides milder conditions, lower cost reagents and broader applications.[11] To achieve our designed *N*-arylated 5,6,7-trimethoxyindoles, therefore, copper-catalyzed Ullmann-type *N*-arylation is the appropriate method of choice in this study.

The synthetic route to obtain the desired target compounds is as follows. A literature survey shows that the core

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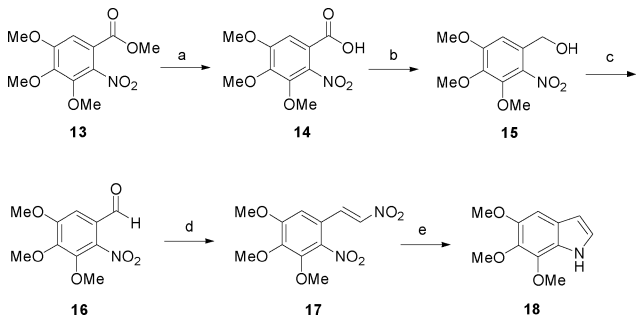
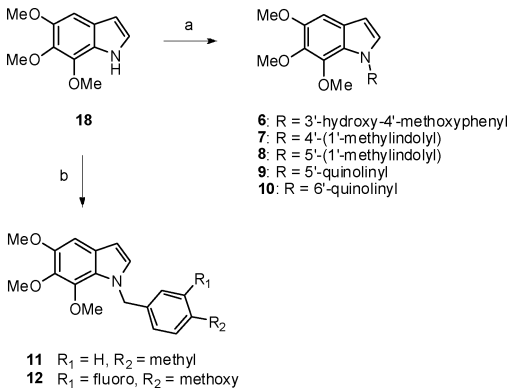
5,6,7-trimethoxyindole (**18**) had been synthesized by R. D. Morin *et al.* in 1957.[12] R. D. Morin and colleagues utilized reductive cyclization[13] a common approach to the indole ring to accomplish the synthesis of 5,6,7-trimethoxyindole from 2-nitro-3,4,5-trimethoxy-β-nitrostyrene in an overall yield of 4.6%. The low synthetic yield is because the nitration of 3,4,5-trimethoxy-β-nitrostyrene provided 2-nitro-3,4,5-trimethoxy-β-nitrostyrene in 9.3% yield only. In order to synthesize 5,6,7-trimethoxyindole efficiently, the commercially available compound **13** was used as a starting material (Scheme 1). The methyl ester of **13** was hydrolyzed to afford the corresponding carboxylic acid **14** followed by treatment with borane in boiling THF to furnish **15**. The primary alcohol **15** was oxidized by pyridinium dichromate to provide the corresponding aldehyde **16**. In order to achieve reductive cyclization for indole formation, compound **16** was treated with nitromethane in the presence of boiling acetic acid to obtain 2-nitro-3,4,5-trimethoxy-β-nitrostyrene (**17**) in an overall yield of 41.9% from starting material **13**. Compared with the literature, we have provided an efficient methodology to achieve the synthesis of 2-nitro-3,4,5-trimethoxy-β-nitrostyrene (**17**). Treatment of compound **17** with ferric powder in the presence of acetic acid afforded 5,6,7-trimethoxyindole (**18**).




**Scheme 1** Synthetic route to 5,6,7-trimethoxyindole (**18**): a. KOH, MeOH, reflux, 95%; b. $BH_3$–THF, reflux, 80%; c. pyridinium dichromate, molecular sieve, $CH_2Cl_2$, 65%; d. nitromethane, $NH_4OAc$, AcOH, 100 °C, 85%; e. Fe powder, acetic acid, EtOH, 100 °C, 30%.

To explore the structure–activity relationship of the substituent at the N-1 position, a series of *N*-aryl and heteroaryl-5,6,7-trimethoxyindoles (**6–12**) were prepared as shown in Scheme 2. To synthesize a series of *N*-heteroaryl-5,6,7-trimethoxyindoles, compound **18** was treated with various aryl halides and copper




**Scheme 2** Synthetic route to N-1 substituted 5,6,7-trimethoxyindoles (**6–12**): a. substituted aryl bromide, CuO, $K_2CO_3$ or $Cs_2CO_3$, DMF, reflux or microwave, 4.9%–9%; b. substituted benzyl chloride/bromide, KOH, KI, DMF, 35%–40%.

oxide in boiling ethanol to provide **6–10**. In addition, 5,6,7-trimethoxyindole analogues connected with various aryl rings with methylene linkage were investigated as well. Compound **18** was reacted with 4-methylbenzyl bromide and 3-fluoro-4-methoxybenzyl chloride in the presence of KOH to yield **11** and **12**, respectively.

## Biological evaluation. A. *in vitro* cell growth inhibitory activity

In an attempt to evaluate the effect of N-1 substitution on the cancer cell inhibitory ability, the synthesized *N*-aryl-5,6,7-trimethoxyindoles (**6–10**), and reference compounds CA4 and colchicine were evaluated for their antiproliferative activities against four human cancer cell lines, cervical carcinoma KB cells, colorectal carcinoma HT29 cells, non-small-cell-lung carcinoma H460 cells, and stomach carcinoma MKN45 cells (Table 1).

To examine whether 5,6,7-trimethoxyindole could provide an equivalent effect of A-ring of CA4, compound **6** containing 3′-hydroxy-4′-methoxybenzene (B-ring of CA4) was generated. In the antiproliferative evaluation, compound **6** demonstrated substantial activity comparable to the reference compound CA4. Compound **6** inhibited the growth of KB, HT29, MKN45 and H460 cancer cell lines with $IC_{50}$ values of 13.7, 10.2, 11.1 and 12.9 nM, respectively. Since the antiproliferative activity of compound **6** is comparable to CA4, 5,6,7-trimethoxyindole could

**Table 1** $IC_{50}$ values (nM ± SD[a]) of compounds **6–12**, colchicine and CA4

| | Cell Type ($IC_{50}$ ± SD[a] nM) | | | |
|---|---|---|---|---|
| Compd | KB | HT29 | MKN45 | H460 |
| **6** | 13.7 ± 4.8 | 10.2 ±3.1 | 11.1 ± 3.6 | 12.9 ± 5.2 |
| **7** | 1226.5 ± 125.2 | 3120.5 ± 959.5 | 1718.0 ± 816.0 | 4250.0 ± 458.7 |
| **8** | 48.9 ± 45.0 | 40.1 ± 2.1 | 35.4 ± 7.6 | 38.5 ± 5.5 |
| **9** | > 5000 | > 5000 | > 5000 | > 5000 |
| **10** | 31.6 ± 10.9 | 50.1 ± 10.6 | 38.6 ± 9.5 | 38.3 ± 8.4 |
| **11** | 946.3 ± 234.0 | 964.0 ± 62.2 | 1005.0 ± 24.0 | 1096.2 ± 316.5 |
| **12** | 751.7 ± 31.8 | 770.5 ± 35.1 | 490.5 ± 46.6 | 870.0 ± 89.1 |
| **CA4** | 2.0 ± 0.4 | 715.1 ± 32.6 | 8.5 ± 2.5 | 19.0 ± 3.5 |
| **Colchicine** | 10.3 ± 0.9 | 15.9 ± 4.9 | 17.9 ± 1.8 | 17.0 ± 5.1 |

[a] SD: standard deviation. All experiments were independently performed at least three times.

be considered to replace the core 3,4,5-trimethoxybenzene of CA4 revealing an avenue for future optimization.

In our previous studies of 3,4,5-trimethoxybenzoylindole[6] and 3,4,5-trimethoxybenzoylquinoline derivatives,[7] the attachment of *N*-methylindole and quinoline moieties to 3,4,5-trimethoxybenzoyl group resulted in an improved anticancer activity. Accordingly, *N*-methylindole and quinoline groups were selected and applied to the current study. Therefore, a series of 5,6,7-trimethoxyindoles with *N*-methylindolyl and quinolinyl groups were synthesized (**7–10**) as well. In the series of *N*-(*N*-methylindolyl)-5,6,7-trimethoxyindoles, compound **8** exhibited cancer cell inhibitory activity with $IC_{50}$ values in the range of 35 to 50 nM. A comparison between compounds **7** and **8** demonstrated that the *N*-methylindol-5-yl group of compound **8** contributed to a 25 to 110 fold increase in cytotoxicity as compared to **7** with an *N*-methylindol-4-yl group. This phenomenon was also observed when compounds **9** and **10** were compared. Compound **10** with a quinolin-6-yl group showed a 20 to 30 fold improved cytotoxicity as compared to **9** with an *N*-methylindol-5-yl group against several human cancer cell lines with $IC_{50}$ values of 30 to 50 nM. This observation shows that the regiospecificity played a significant role in improving the activity and the heteroatom at the *para* position to N-1 nitrogen is preferred. Compounds **11** and **12** were synthesized to explore the effect of distance between 5,6,7-trimethoxyindole and substitutions. Compounds **11** and **12** exhibited weak activities against a panel of human cancer cell lines as compared to compounds **6** to **10**. This result indicated that the methylene linkage between 5,6,7-trimethoxyindole and the aryl substituent is detrimental to cytotoxic activity.

## B. Inhibition of tubulin polymerization and colchicine binding activity

To investigate whether the activities of these *N*-aryl-5,6,7-trimethoxyindoles were related to interactions with the microtubule system, compounds **6–10** and reference compounds colchicine and CA4 were evaluated for their antitubulin activities and colchicine binding activities (Table 2). The results indicated that the compounds' antiproliferative activity correlated with the inhibition of tubulin polymerization. Compounds **6**, **8** and **10** were efficacious in inhibiting microtubulin assembly, with $IC_{50}$ values of 2.5, 2.5 and 3.2 μM, respectively. These values were comparable to reference compounds colchicine and CA4. In the [$^3$H]colchicine-binding assay, results demonstrated that **6**, **8** and **10** were bound to the colchicine binding site.

## C. Investigation of vascular disrupting activity

In addition to tubulin polymerization inhibitory activity, CA4P (**3**) has been shown to cause vascular shutdown within solid tumors as vascular disrupting agents (VDA).[16] Therefore, compound **6** which displayed the most potent antiproliferative activity was investigated for vascular disrupting activity. The HUVECs were plated on Matrigel and allowed to form capillary tubes in the presence of VEGF (20 ng mL$^{-1}$) followed by exposure to different concentrations of compound **6**.[17] As shown in Fig. 2, compound **6** is capable of disrupting formed capillaries in a concentration-dependent manner without affecting cell viability.

**Table 2** Inhibition of tubulin polymerization and colchicine binding by compounds **6–10**, colchicine and CA4

| Compd | Tubulin[a] $IC_{50}$ ± SD (μM) | Colchicine binding[b] (% ± SD) |
|---|---|---|
| **6** | 2.5 ± 0.2 | 82 ± 2 |
| **7** | > 10 | — |
| **8** | 2.5 ± 0.3 | 77 ± 3 |
| **9** | > 10 | — |
| **10** | 3.2 ± 0.2 | 78 ± 5 |
| **Colchicine** | 3.9 ± 0.4 | |
| **CA4** | 1.7 ± 0.2 | 95 ± 2 |

[a] Inhibition of tubulin polymerization.[14] [b] Inhibition of [$^3$H] colchicine binding.[15] Tubulin was at 1 μM; both [$^3$H]colchicine and inhibitor were at 5 μM.

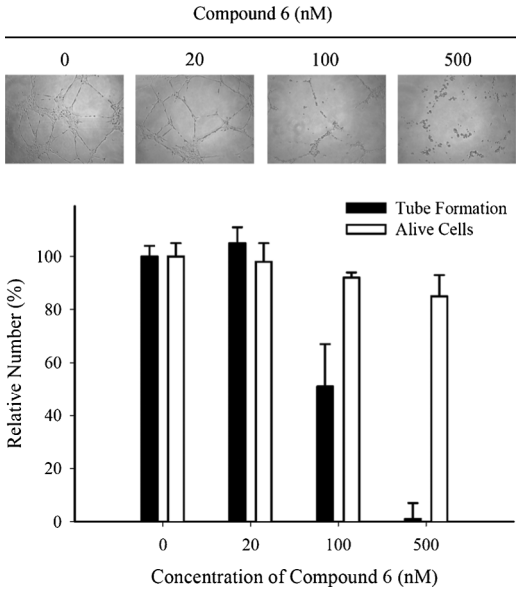



**Fig. 2** Investigation of vascular disrupting activity (VDA) of compound **6**. HUVECs were plated on Matrigel and allowed to form capillary tubes in the presence of VEGF (20 ng mL$^{-1}$) followed by exposure to different concentrations of compound **6**. Cultures were photographed and the number of capillary tube networks was determined by counting under a microscope (original magnification 100×). Data reflect the mean number of capillary tube networks relative to the to vehicle control group (DMSO) ± standard deviation (SD) from three separate experiments.

## Conclusions

In the analysis of several potent antimitotic natural products, the trimethoxyphenyl is a crucial scaffold and attracts our attention for optimization. Hence, a series of *N*-aryl-5,6,7-trimethoxyindoles (**6–12**) were synthesized *via* copper-mediated Ullmann-type *N*-arylation of 5,6,7-trimethoxyindole and investigated for cancer cell growth inhibitory activities. Among the synthesized *N*-aryl-5,6,7-trimethoxyindoles, compound **6** showed the best potency, which is comparable to CA4, against a panel of cancer cells ($IC_{50}$ = 10 to 14 nM). It also exhibited substantial inhibition of tubulin polymerization with $IC_{50}$ values of 2.5 μM which is comparable to those of reference compounds as well. The vascular disrupting activity assay indicated that compound **6** has a concentration-dependent action on the HUVECs. The relative position of the heteroatom to the N-1 nitrogen has significant impact on the antiproliferative activity in the series of *N*-heteroaryl-5,6,

7-trimethoxyindole analogues (**7–10**). The position of heteroatom on substituents, *N*-methylindolyl and quinonlinyl, is preferred to be *para* to the N-1 of 3,4,5-trimethoxyindole. In summary, the modification from 3,4,5-trimethoxyphenyl (A-ring of CA4) to 5,6,7-trimethoxyindole provides an opportunity for further optimization of CA4 structure based anitproliferative agents.

## Acknowledgements

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## Notes and references

1 (*a*) C. Dumontet and M. A. Jordan, *Nat. Rev. Drug Discovery*, 2010, **9**, 790; (*b*) A. Jordan and L. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 253.
2 (*a*) C. M. Lin, S. B. Singh, P. S. Chu, R. O. Dempcy, J. M. Schmidt, G. R. Pettit and E. Hamel, *Mol. Pharmacol.*, 1988, **34**, 200; (*b*) C. M. Lin, H. H. Ho, G. R. Pettit and E. Hamel, *Biochemistry*, 1989, **28**, 6984.
3 (*a*) G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca and A. A. Genazzani, *J. Med. Chem.*, 2006, **49**, 3033; (*b*) H. P. Hsieh, J. P. Liou and N. Mahindroo, *Curr. Pharm. Des.*, 2005, **11**, 1655; (*c*) Q. Li and H. L. Sham, *Expert Opin. Ther. Pat.*, 2002, **12**, 1663; (*d*) N. Mahindroo, J. P. Liou and J. Y. Chang, *Expert Opin. Ther. Pat.*, 2006, **16**, 647; (*e*) D. J. Chaplin, M. R. Horsman and D. W. Siemann, *Curr. Opin. Investig. Drugs*, 2006, **7**, 522.
4 G. R. Pettit, C. J. Temple, V. L. Narayanan, R. Varma, M. J. Simpson, M. R. Boyd, G. A. Rener and N. Bansal, *Anti-cancer Drug Des.*, 1995, **10**, 299.
5 K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama and T. Tsuji, *J. Med. Chem.*, 1998, **41**, 3022.
6 J. P. Liou, C. Y. Wu, H. P. Hsieh, C. Y. Chang, C. M. Chen, C. C. Kuo and J. Y. Chang, *J. Med. Chem.*, 2007, **50**, 4548.
7 C. Y. Nien, Y. C. Chen, C. C. Kuo, H. P. Hsieh, C. Y. Chang, J. S. Wu, S. Y. Wu, J. P. Liou and J. Y. Chang, *J. Med. Chem.*, 2010, **53**, 2309.
8 (*a*) D. M. D'Souza and T. J. J. Müller, *Chem. Soc. Rev.*, 2007, **36**, 1095; (*b*) J. J. Song, J. T. Reeves, F. Gallou, Z. Tan, N. K. Yee and C. H. Senanayake, *Chem. Soc. Rev.*, 2007, **36**, 1120.
9 (*a*) R. Hersperger, K. B. French, L. Mazzoni and T. Müller, *J. Med. Chem.*, 2000, **43**, 675; (*b*) H. C. Zhang, C. K. Derian, D. F. McComsey, K. B. White, H. Ye, L. R. Hecker, J. Li, M. F. Addo, D. Croll, A. J. Eckardt, C. E. Smith, Q. Li, W. M. Cheung, B. R. Conway, S. Emanuel, K. T. Demarest, P. A. Gordon, B. P. Damiano and B. E. Maryanoff, *J. Med. Chem.*, 2005, **48**, 1725.
10 H. Xu, *Mini-Rev. Org. Chem.*, 2009, **6**, 367.
11 I. P. Beletskaya and A. V. Cheprakov, *Coord. Chem. Rev.*, 2004, **248**, 2337.
12 R. D. Morin, F. Benington and L. C. Clark, Jr., *J. Org. Chem.*, 1957, **22**, 331.
13 (*a*) P. D. Rege, Y. Tian and E. J. Corey, *Org. Lett.*, 2006, **8**, 3117; (*b*) K. C. Nicolaou, U. Majumder, S. P. Roche and D. Y. K. Chen, *Angew. Chem., Int. Ed.*, 2007, **46**, 4715.
14 (*a*) J. P. Liou, K. S. Hsu, C. C. Kuo, C. Y. Chang and J. Y. Chang, *J. Pharmacol. Exp. Ther.*, 2007, **323**, 398; (*b*) J. Y. Chang, M. J. Lai, Y. T. Chang, H. Y. Lee, Y. C. Cheng, C. C. Kuo, M. C. Su, C. Y. Chang and J. P. Liou, *Med. Chem. Commun.*, 2010, **1**, 152.
15 C. C. Kuo, H. P. Hsieh, W. Y. Pan, C. P. Chen, J. P. Liou, S. J. Lee, Y. L. Chang, L. T. Chen and J. Y. Chang, *Cancer Res.*, 2004, **64**, 4621.
16 (*a*) G. G. Dark, S. A. Hill, V. E. Prise, G. M. Tozer, G. R. Pettit and D. J. Chaplin, *Cancer Res.*, 1997, **57**, 1829; (*b*) D. W. Siemann, M. C. Bibby, G. G. Dark, A. P. Dicker, F. A. L. M. Eskens, M. R. Horsman, D. Marme and P. M. LoRusso, *Clinical Cancer Res.*, 2005, **11**, 416; (*c*) G. M. Tozer, C. Kanthou and B. C. Baguley, *Nat. Rev. Cancer*, 2005, **5**, 423; (*d*) D. M. Patterson and G. J. S. Rustin, *Clin. Oncol.*, 2007, **19**, 443; (*e*) G. M. Tozer, S. Akerman, N. A. Cross, P. R. Barber, M. A. Björndahl, O. Greco, S. Harris, S. A. Hill, D. J. Honess, C. R. Ireson, K. L. Pettyjohn, V. E. Prise and C. C. Reyes-Aldasoro, *Cancer Res.*, 2008, **68**, 2301; (*f*) D. W. Siemann, D. J. Chaplin and P. A. Walicke, *Expert Opin. Invest. Drugs*, 2009, **18**, 189.
17 G. Kremmidiotis, A. F. Leske, T. C. Lavranos, D. Beaumont, J. Gasic, A. Hall, M. O'Callaghan, C. A. Matthews and B. Flynn, *Mol. Cancer Ther.*, 2010, **9**, 1562.