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Cytotoxic and antivascular 1-methyl-4-(3-fluoro-4-methoxyphenyl)-5-(halophenyl)-imidazoles

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ABSTRACT

A series of 1-methyl-4,5-diphenylimidazoles **6** with various patterns of *m*-halogen substitution at the 5-phenyl ring were tested for cytotoxicity in cancer and nonmalignant cell lines and for their capacity to prevent tube formation in HUVEC cultures. Unlike the monofluoro and difluoro derivatives **6a** and **6e**, the monobromo and diiodo analogs **6c** and **6h** were strongly cytotoxic and inhibited the polymerization of tubulin and the tube formation by HUVEC. The dibromo derivative **6g** displayed a unique selectivity for KB-3-1 cervix and PC-3 prostate cancer cells. It also inhibited the tube formation by HUVEC and the polymerization of tubulin which is indicative of its potential antiangiogenic activity in solid tumors.

Combretastatin A-4 (CA-4, **1a**, Fig. 1) was first isolated from the bark of the South African Cape Bushwillow (*Combretum caffrum*) and was later shown to have pronounced antivascular properties.[1] Prodrugs of **1a** with improved bioavailability such as the phosphate fosbretabulin have been investigated in a number of clinical trials which proved their selective impact on tumor vasculature.[2] Other CA-4 analogs are also being studied, for example, the serine amide ombrabulin which is currently in phase III trials for the treatment of NSCLC.[3] Vascular disrupting agents (VDA) are an intriguing alternative for the therapy of highly vascularized tumors or such no longer responding to conventional chemotherapy.[4] The mechanism of action of VDA is typically associated with destabilization of microtubules, activation of Rho signaling and reorganization of the cellular actin cytoskeleton. Morphologically, endothelial cells exposed to VDA get rounded and blebby which eventually leads to the collapse of the tumor blood vessels and thus to tumor necrosis.[5] However, due to their insufficient cytotoxicity, **1a** and its prodrugs have to be administered as part of combination regimens with other anticancer drugs such as carboplatin or bevacizumab to prevent tumor relapse.[6–8] In addition, they tend to isomerize to the biologically inactive *trans*-form.[9,10] Halogenated derivatives of **1a** seem to be less susceptible to biooxidative deactivation, yet retain their antivascularity only in certain cases. Several vascular disrupting fluoro derivatives, for example, **1b**[11] have been recently reported.[12–15] The nature of the halogen substituent was found to be particularly decisive for the activity of analogs with 3, 5-dihalo-substituted phenyl rings. The dibromo (**1c**) and diiodo (**1d**) derivatives showed a much higher activity in human umbilical vein endothelial cells (HUVEC) than the difluoro congener and when compared with **1a**.[15] The 3,5-dibromo-4-methoxyphenyl

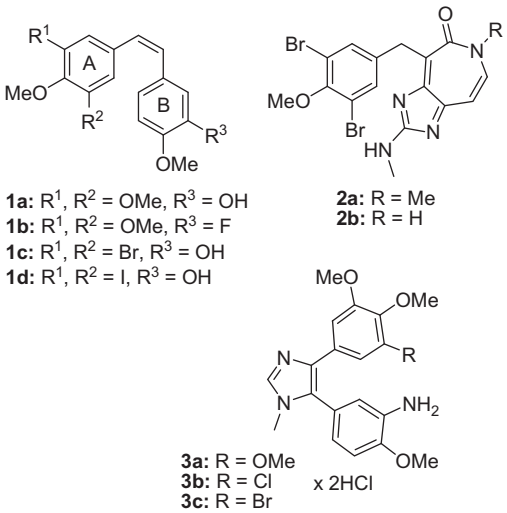

Figure 1. Structures of combretastatin A derivatives **1**, ceratamines **2**, and *N*-methyl-imidazoles **3**.

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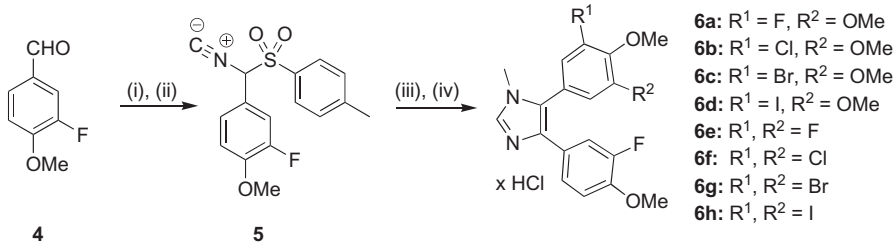
motif is also represented in natural anticancer compounds, for example, in the marine ceratamines **2**.[16] *N*-Methyl-4,5-diaryl imidazole analogs of **1a** are stable to *cis–trans* isomerization and are orally applicable in most cases.[17] Recently, we published their preparation by van Leusen reaction of *p*-toluenesulfonylmethyl isocyanide (TosMIC) with aryl aldehydes as an alternative to the palladium-mediated aryl coupling developed by Bellina et al.[18] We had also identified imidazoles **3** as examples of this type with



Scheme 1. Synthesis of 1-methyl-4-(3-fluoro-4-methoxyphenyl)-5-(halophenyl)-imidazoles **6**. Reagents and conditions: (i) $HCONH_2$, CSA, *p*-toluenesulfinic acid, 16 h, 60 °C; (ii) $POCl_3$, Et_3N, DME, 3 h, −5 °C, 31% (two steps); (iii) ArCHO, $MeNH_2$ (33% in EtOH), AcOH, EtOH, 2 h, reflux; then **5**, K_2CO_3, DME/EtOH, 6 h, reflux; (iv) 3 M HCl/dioxane, CH_2Cl_2, 10 min, rt, 25–91% (two steps).

Table 1
Inhibitory concentrations IC_{50} [nM] of **3** and **6** for the growth of cancer and nontumor derived cells[a] and for the formation of tubes by HUVEC[b]

Cell line/compound	L929	KB-3-1	PC-3	PtK2	NHDF	HUVEC
3a	220	70	90	450	140	290
3b	135	16	n/m[c]	70	970	70
6a	10,000	2000	1800	5000	7600	10,000
6b	220	190	n/m[c]	480	240	n/m[c]
6c	25	5	70	440	175	110
6d	40	90	n/m[c]	180	40	n/m[c]
6e	31,000	21,000	16,000	23,000	52,000	27,000
6f	2400	1150	n/m[c]	4300	7180	n/m[c]
6g	4400	14	10	1200	400	270
6h	18	3	3	330	3300	80

[a] Values are derived from dose–response curves obtained by measuring the percentual absorbance of viable cells relative to untreated controls (100%) after 5 days exposure of the cells to the test compounds in the MTT assay.
[b] Minimal inhibitory concentrations [nM] for HUVEC tube formation.
[c] Not measured.

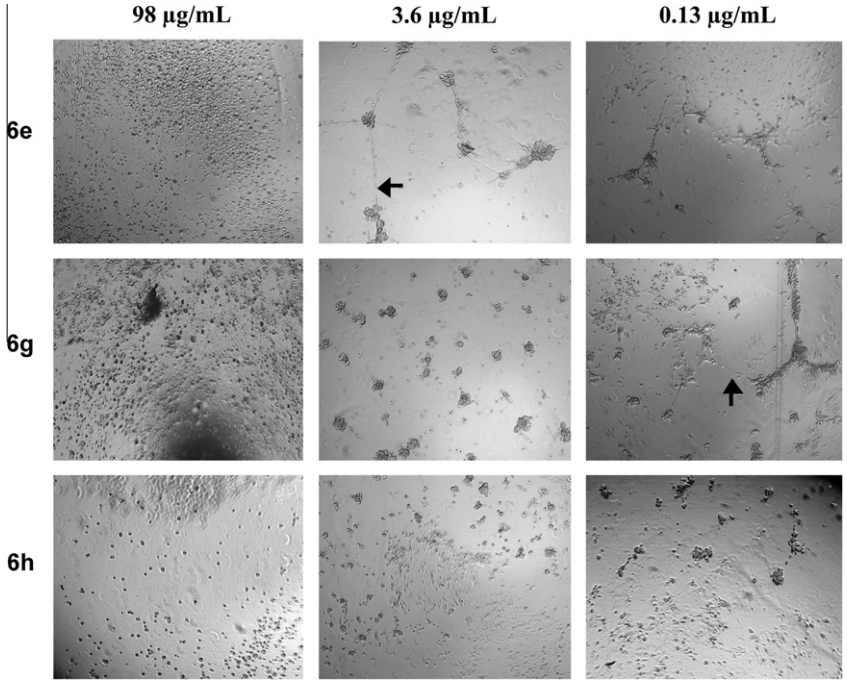


Figure 2. Micrographs showing the degree of tube formation by HUVEC after exposure for 20 h to various concentrations of compounds **6e**, **6g**, and **6h** which inhibited tube formation at minimal inhibitory concentrations of 11 µg/mL, 135 ng/mL, or 50 ng/mL, respectively. Black arrows indicate tubes formed by HUVEC.

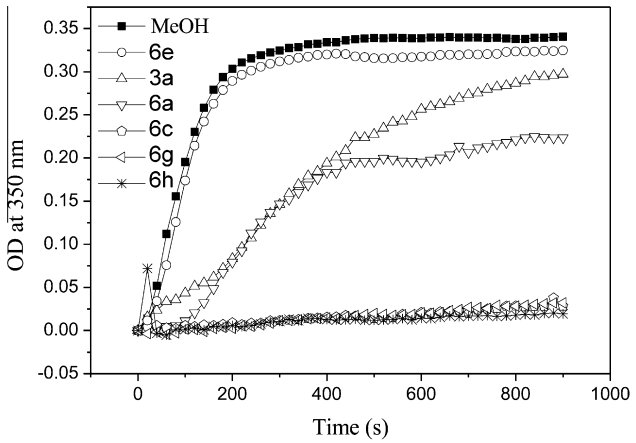


Figure 3. Tubulin polymerization in vitro at 37 °C in the presence of 25 μg/mL of compounds **3a** and **6a**, **6c**, **6e**, **6g**, and **6h** (control MeOH).

exceptional in vivo activity and tolerance.[19] Now we sought to further optimize the drug profiles of similar imidazoles by attaching halogen substituents to the tetrasubstituted A-ring.

The new 1-methyl-4-(3-fluoro-4-methoxyphenyl)-5-(halophenyl)imidazoles **6** feature A- and B-rings transposed with respect to **3** and were prepared from TosMIC **5**. 3-Fluoro-4-methoxybenzaldehyde **4** was reacted with *p*-toluenesulfinic acid and formamide in the presence of camphorsulfonic acid (CSA) to give a tosylmethylformamide intermediate, which was dehydrated to **5** by $POCl_3$. The halogenated benzaldehydes used for the van Leusen reaction with **5** were prepared according to known literature methods[12,15,20] and converted to their *N*-methylimines. Reaction of the latter with **5** and addition of HCl/dioxane finally afforded the imidazolium salts **6** (Scheme 1).

Growth inhibition of cells of L929 mouse fibroblasts, human KB-3-1 cervix carcinoma, PC-3 prostate cancer, PtK2 potoroo kidney cells and primary human dermal fibroblasts (NHDF) by compounds **3** and **6** was assessed by MTT assays (Table 1). The monobromo derivative **6c** and the diiodo compound **6h** proved most active. They were also distinctly more cytotoxic to cancer cells than the known trimethoxy derivative **3a** and also than **3b**, the best performer of our previously published imidazole series.[19] In contrast, the monofluoro (**6a**), difluoro (**6e**) and dichloro (**6f**) analogs were of moderate cytotoxicity. Compounds **6c** and **6h** were also most active with respect to suppressing tube formation in HUVEC cultures at minimal inhibitory concentrations of ca. 100 nM. The dibromide **6g** was selectively efficacious against the cervix and prostate cancer cells for yet unknown reason. The anticancer active compounds **6c**, **6g**, and **6h** were in almost all cases less harmful to the non-tumor derived L929, PtK2, and NHDF cells than to the cancer cells.

Figure 2 shows the inhibitory effects of selected test compounds on the tube formation propensity of HUVEC. While tube formation was observed even in the presence of ca. 4 μg/mL (=12 μM) of the tris-fluoro derivative **6e**, the dibromo analog **6g** was required at concentrations only slightly greater than 130 ng/mL (=270 nM) to prevent tube formation. The most effective compound, the diiodo derivative **6h**, suppressed tube formation even at this low concentration (cf. Supplementary data file for other compounds).

The capacities of compounds **3a**, **6a**, **6c**, **6e**, **6g**, and **6h** to inhibit the polymerization of tubulin as assessed by a standard turbidimetric method employing freshly isolated porcine microtubule protein[21] correlated well with their inhibitory effects on HUVEC tube formation and their cytotoxicities. The highly cytotoxic and HUVEC tube inhibiting compounds **6c**, **6g**, and **6h** were also strong inhibitors of tubulin polymerization (Fig. 3). The least cytotoxic and least effective HUVEC tube suppressor **6e** did not interfere with tubulin polymerization. Interestingly, the known trimethoxyphenyl compound **3a** while strongly cytotoxic, had but a minute effect on tubulin polymerization. Except for **6e**, a destruction of microtubules was observed for all test compounds when applied at 10 μg/mL to PtK2 potoroo (*Potorous tridactylis*) kidney epithelial cells which are easy to monitor due to their flat shape (Fig. 4). The greatest damage was caused by compounds **6h** and **6c** (not shown since similar to that of **6h**), which is in line with their high cytotoxicity in these cells.

In conclusion, we prepared eight new *N*-methylimidazole-bridged analogs **6a**–**h** of combretastatin A-4 (**1a**) featuring a 3-fluoro-4-methoxyphenyl B-ring and various *m*-halogenated A-rings. Some structure–activity relations already emerged in our tests for cytotoxicity and antiangiogenic activity. In both series of imidazoles **6** with mono- and dihalogenated A-rings the antiproliferative activity was lowest for the fluoro (**6a** and **6e**) and the chloro (**6b** and **6f**) derivatives. Substitution of one *m*-methoxy group of the A-ring by bromine or replacement of both *m*-methoxy groups by two iodine atoms led to strongly cytotoxic and antiangiogenic agents **6c** and **6h**. Their activities exceeded even that of the known trimethoxy derivative **3a**. This confirms recent findings by Myers et al.[15] who discovered that bigger bromo (**1c**) or iodo (**1d**), but not small fluoro substituents at both *meta*-positions of ring A of stilbenoid analogs of **1a** led to enhanced potency against HUVEC cells. The dibromophenyl substituted imidazole **6g** was a potent suppressor of HUVEC tube formation, of tubulin polymerization, and

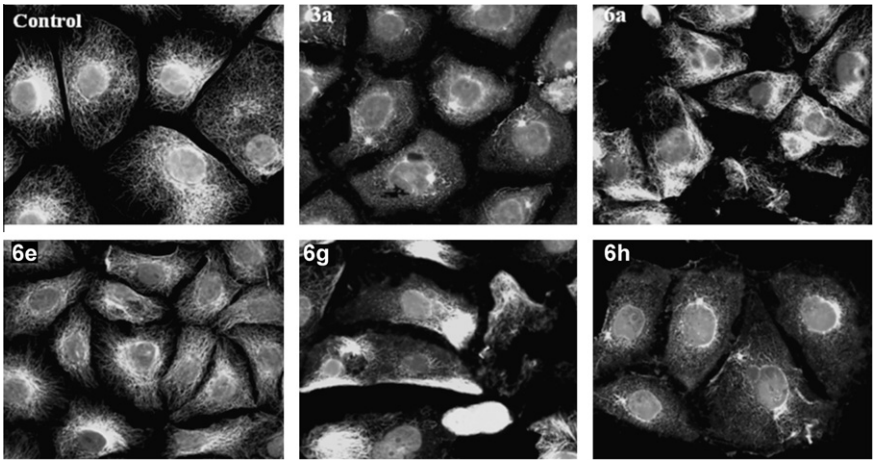


Figure 4. Effects on the microtubules network in PtK2 potoroo cells exposed for 16 h to 10 μg/mL of compounds **3a**, **6a**, **6e**, **6g**, or **6h** then fixed and stained with antitubulin antibodies.

of the growth of KB-3-1 cervix and PC-3 prostate cancer cells. For growth inhibition of the L929 mouse fibroblasts a residue larger than bromine, for example, iodine (**6h**) or methoxy (**6c**), has to be present in one of the *meta*-positions of the B-ring. It will be interesting to see whether the new imidazoles are as well tolerated in mice as the previously reported analogs **3**.

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Supplementary data

Supplementary data associated (details of the synthesis, characterization, and purity of compounds **6** and of the biological assays (MTT, HUVEC tube formation, tubulin polymerization, immunofluorescence staining of PtK2 cells)) with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.005.

References and notes

1. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
2. Mooney, C. J.; Nagaiah, G.; Fu, P.; Wasman, J. K.; Cooney, M. M.; Savvides, P. S.; Bokar, J. A.; Dowlati, A.; Wang, D.; Agarwala, S. S.; Flick, S. M.; Hartman, P. H.; Ortiz, J. D.; Lavertu, P. N.; Remick, S. C. *Thyroid* **2009**, *19*, 233.
3. Delmonte, A.; Sessa, C. *Expert. Opin. Invest. Drugs* **2009**, *18*, 1541.
4. Lippert, J. W. *Bioorg. Med. Chem.* **2007**, *15*, 605.
5. Kanthou, K.; Tozer, G. M. *Blood* **2002**, *99*, 2060.
6. Beauregard, D. A.; Thelwall, P. E.; Chaplin, D. J.; Hill, S. A.; Adams, G. E.; Brindle, K. M. *Br. J. Cancer* **1998**, *77*, 1761.
7. Bilenker, J. H.; Flaherty, K. T.; Rosen, M.; Davis, L.; Gallagher, M.; Stevenson, J. P.; Sun, W.; Vaughn, D.; Giantonio, B.; Zimmer, R.; Schnall, M.; O'Dowyer, P. J. *Clin. Cancer Res.* **2005**, *11*, 1527.
8. Garon, E.; Kabbinavar, F.; Balkisson, J.; Lu, S. P.; Neidhart, J.; Neidhart, J.; Gabrail, N.; Ribeiro de Oliveria, M. AACR-EORTC-NCI Molecular Targets and Cancer Therapeutics Conference, Boston, MA, November 15–18, **2009**.
9. Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R., et al *Clin. Cancer Res.* **2004**, *10*, 1446.
10. Aprile, S.; Del Grosso, E.; Tron, G. C.; Grosa, G. *Drug Metab. Dispos.* **2007**, *35*, 2252.
11. Gaukroger, K.; Hadfield, J. A.; Lawrence, N. J.; Nolan, S.; McGown, A. T. *Org. Biomol. Chem.* **2003**, *1*, 3033.
12. Pettit, G. R.; Minardi, M. D.; Rosenberg, H. J.; Hamel, E.; Bibby, M. C.; Martin, S. W.; Jung, M. K.; Pettit, R. K.; Cuthbertson, T. J.; Chapuis, J.-C. *J. Nat. Prod.* **2005**, *68*, 1450.
13. Hall, J. J.; Sriram, M.; Strecker, T. E.; Tidmore, J. K.; Jelinek, C. J.; Kumar, G. D. K.; Hadimani, M. B.; Pettit, G. R.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5146.
14. Lawrence, N. J.; Hepworth, L. A.; Rennison, D.; McGown, A. T.; Hadfield, J. A. *J. Fluorine Chem.* **2003**, *123*, 101.
15. Beale, T. M.; Myers, R. M.; Shearman, J. W.; Charnock-Jones, D. S.; Brenton, J. D.; Gergely, F. V.; Ley, S. V. *Med. Chem. Commun.* **2010**, *1*, 202.
16. Karjala, G.; Chan, Q.; Manzo, E.; Andersen, R. J.; Roberge, M. *Cancer Res.* **2005**, *65*, 3040.
17. Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y.-H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *J. Med. Chem.* **2002**, *45*, 1697.
18. (a) Bellina, F.; Cauteruccio, S.; Di Fiore, A.; Rossi, R. *Eur. J. Org. Chem.* **2008**, 5436; (b) Bonezzi, K.; Taraboletti, G.; Borsotti, P.; Bellina, F.; Rossi, R.; Giavazzi, R. *J. Med. Chem.* **2009**, *52*, 7906.
19. Schobert, R.; Biersack, B.; Dietrich, A.; Effenberger, K.; Knauer, S.; Mueller, T. *J. Med. Chem.* **2010**, *53*, 6595.
20. Clark, M. T.; Miller, D. D. *J. Org. Chem.* **1986**, *51*, 4072.
21. Gaskin, F.; Cantor, C. R.; Schelanski, M. *J. Mol. Biol.* **1974**, *98*, 737.