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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

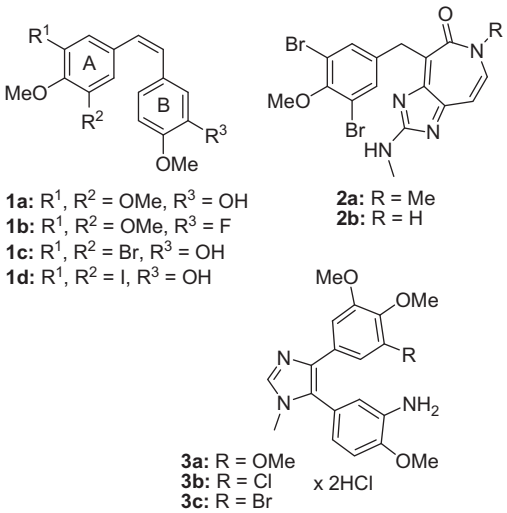

Figure 1. Structures of combretastatin A derivatives **1**, ceratamines **2**, and *N*-methyl-imidazoles **3**.

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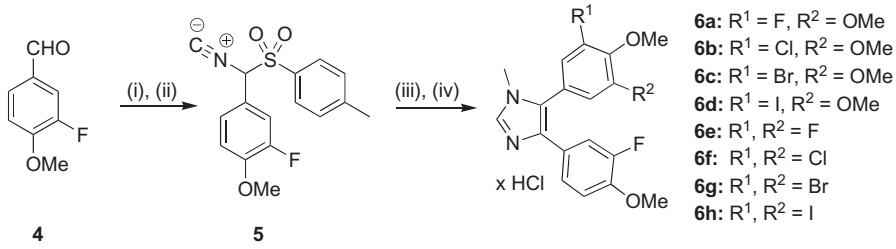
motif is also represented in natural anticancer compounds, for example, in the marine ceratamines **2**.[16] *N*-Methyl-4,5-diaryl imidazole analogs of **1a** are stable to *cis–trans* isomerization and are orally applicable in most cases.[17] Recently, we published their preparation by van Leusen reaction 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.

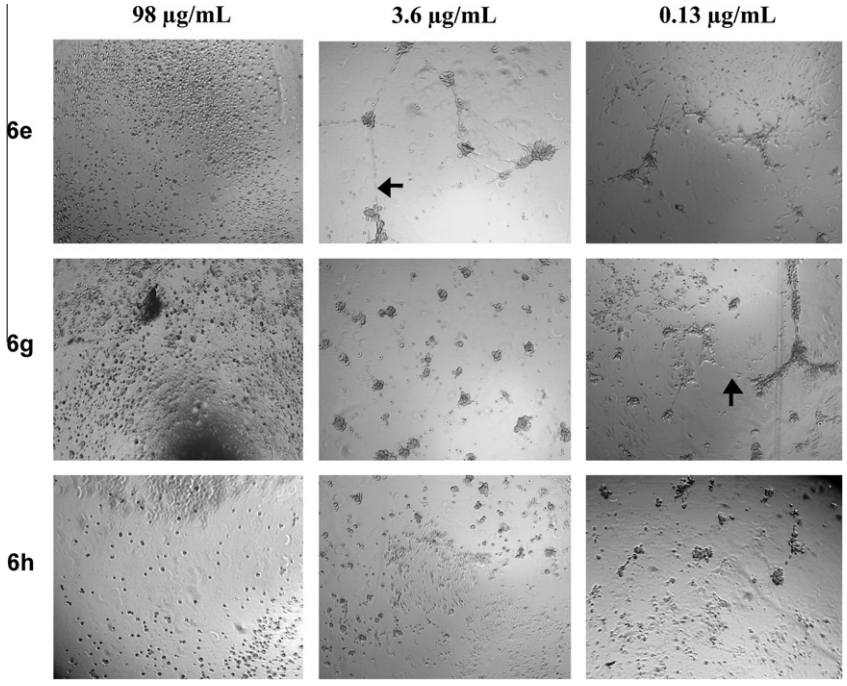


Figure 2. Micrographs showing the degree of tube formation by HUVEC after exposure for 20 h to various concentrations of compounds **6e**, **6g**, and **6h** which inhibited tube formation at minimal inhibitory concentrations of 11 µg/mL, 135 ng/mL, or 50 ng/mL, respectively. Black arrows indicate tubes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