European Journal of Medicinal Chemistry 118 (2016) 9–20

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Combretastatin A-4 derived 5-(1-methyl-4-phenyl-imidazol-5-yl) indoles with superior cytotoxic and anti-vascular effects on chemoresistant cancer cells and tumors

Katharina Mahal [a], Bernhard Biersack [a], Sebastian Schruefer [a], Marcus Resch [b], Ralf Ficner [b], Rainer Schobert [a, *], Thomas Mueller [c]

[a] Organic Chemistry Laboratory, University Bayreuth, 95440 Bayreuth, Germany
[b] Department of Molecular Structural Biology, Georg-August-University Göttingen, 37077 Göttingen, Germany
[c] Department of Internal Medicine IV, Oncology/Hematology, Martin-Luther-University Halle-Wittenberg, 06120 Halle-Saale, Germany

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
15 April 2016
Accepted 16 April 2016
Available online 19 April 2016

Keywords:
Imidazole
Indole
Anticancer drugs
Combretastatin A-4
Vascular-disrupting agents
Mouse tumor xenografts

ABSTRACT

5-(1-Methyl-4-phenyl-imidazol-5-yl)indoles **5** were prepared and tested as analogs of the natural vascular-disrupting agent combretastatin A-4 (CA-4). The 3-bromo-4,5-dimethoxyphenyl derivative **5c** was far more active than CA-4 with low nanomolar IC_{50} concentrations against multidrug-resistant KB-V1/Vbl cervix and MCF-7/Topo mamma carcinoma cells, and also against CA-4-resistant HT-29 colon carcinoma cells. While not interfering markedly with the polymerization of tubulin *in vitro*, indole **5c** completely disrupted the microtubule cytoskeleton of cancer cells at low concentrations. It also destroyed real blood vessels, both in the chorioallantoic membrane (CAM) of fertilized chicken eggs and within tumor xenografts in mice, without harming embryo or mouse, respectively. Indole **5c** was less toxic than CA-4 to endothelial cells, fibroblasts, and cardiomyocytes. In highly vascularized xenograft tumors **5c** induced distinct discolorations and histological features typical of vascular-disrupting agents, such as disrupted vessel structures, hemorrhages, and extensive necrosis. In a first preliminary therapy trial, indole **5c** retarded the growth of resistant xenograft tumors in mice.

1. Introduction

The natural tubulin binding agent combretastatin A-4 (CA-4, Fig. 1) was isolated from the bark of the South African Cape Bush-willow (*Combretum caffrum*). Its water soluble phosphate prodrug Zybrestat™ (fosbretabulin) has entered advanced clinical trials that revealed its tumor-selective angiotoxicity, even in multidrug-resistant tumors [1,2]. However, due to its insufficient *in vivo* cytotoxicity it had to be applied in combination with other drugs such as carboplatin, paclitaxel or the anti-angiogenic agent bevacizumab [3,4]. The related combretastatin A-1 and its bisphosphate prodrug OXi4503 also proved efficacious against certain tumor cells in *in vivo* models for which their redox-active catechol moiety was believed to be responsible [5,6]. A drawback of combretastatins is the tendency of their *Z*-alkene to isomerize to a biologically inactive *E*-isomer in protic solvents. So far, the search for chemically more stable derivatives has led to compounds with the alkene adopting a fixed *Z*-configuration, e.g., by being part of a heterocycle [7]. Some 4,5-diaryloxazoles, -imidazoles, and -1,2,3-triazoles of this type actually retained the cytotoxicity and tubulin affinity of the parent combretastatins [8,9]. Among these were orally applicable, water soluble derivatives with advantageous pharmacokinetics and distinct *in vivo* activity such as the imidazole **1** [8]. In another study 3-halo- and 3-aminostilbenes **2**, structurally reminiscent of combretastatin A-3, showed increased affinity for tubulin and a more selective activity profile against tumor cells with potential to overcome drug resistance [10]. Yet, both structure variants have their shortcomings. Derivative **1** is inferior to combretastatin A-4 with respect to cytotoxicity while the analogous oxazoles are hampered with unsatisfactory pharmacological properties [8]. The halo combretastatins **2** prepared by Pettit et al. were quite active yet chemically unstable and required formulations as phosphate prodrugs in order to reach sufficient water solubility and uptake rates [10].

* Corresponding author.
E-mail address: rainer.schobert@uni-bayreuth.de (R. Schobert).

http://dx.doi.org/10.1016/j.ejmech.2016.04.045
0223-5234/

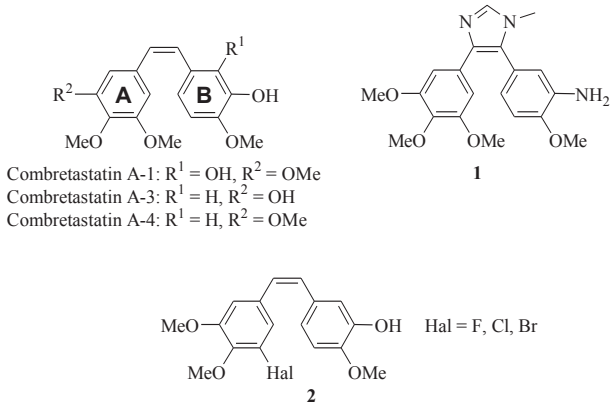
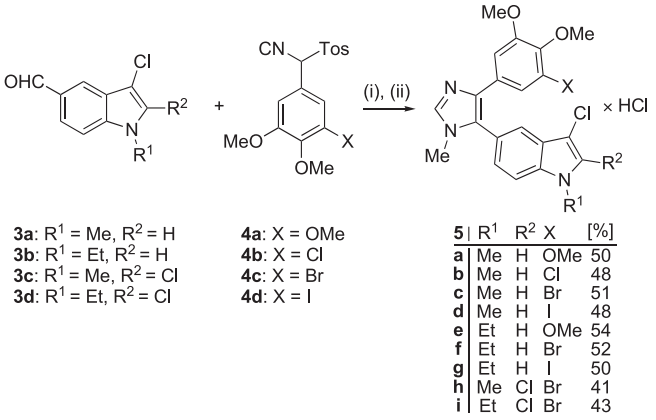


Fig. 1. Combretastatins A-1, A-3, and A-4 and known analogs **1** and **2**: potent inhibitors of tubulin polymerization. Designation of A- and B-ring in the natural drugs combretastatin A.



Scheme 1. Reagents and conditions: (i) TosMIC reagent **4**, $MeNH_2$, AcOH, EtOH, reflux, 2 h, then **3**, K_2CO_3, EtOH, reflux, 3 h; (ii) 3 M HCl/dioxane, CH_2Cl_2, r.t., 15 min.

CA-4 fragments were also covalently combined with other tubulin modifier motifs such as indoles. For instance, Dalton et al. disclosed the antitumoral 2-(trimethoxybenzoylphenyl) substituted indole I-387 [11]. Romagnoli et al. reported a 2-aroyl-3-amino derivative which inhibited the growth of breast cancer cells [12]. Simoni et al. prepared an (*N*-methylindol-5-yl)stilbene which caused growth inhibition of triple-negative MDA-MB-231 breast cancer cells [13]. Welsh et al. reported a triazole-bridged indole analog of CA-4 with high tubulin affinity and anticancer activity [14,15]. Pinney et al. developed and optimized 2-aryl-3-aroylindoles such as OXi8006, a highly cytotoxic vascular-disrupting agent [16]. Zhang et al. reported 3-(trimethoxyphenylseleninyl)-1*H*-indoles that bind to tubulin in a similar orientation as CA-4 [17]. Based on the work of Wang et al. our group developed a water-soluble hydrochloride of an *N*-methyl-(3-chloroindol-5-yl)imidazole analog of combretastatin A-4 which was far more cytotoxic against topotecan-resistant (BCRP-positive) MCF-7 breast cancer cells than CA-4 [8,18]. We also prepared CA-4 analogous imidazoles with *meta*-halo-substituted A-rings that had an improved anticancer activity [18,19]. Herein, we continue this work and report on new CA-4 analogous imidazoles with 5-indole residues and their superior anticancer and antivascular properties *in vitro* and *in vivo* when compared with the lead compound CA-4 and with previously published indole **5a** that had been tested only on two cancer cell lines before [8].

2. Results and discussion

2.1. Chemistry

The 5-formylindole derivatives **3a-d** and the TosMIC reagents **4a-d** were prepared according to literature procedures [8,18]. The aldehydes **3a-d** were treated with $MeNH_2$ to give the respective imines, which were reacted with the TosMIC reagents **4a-d** under basic conditions to give the corresponding new *N*-methyl imidazoles **5b-i** (Scheme 1). The known compound **5a** was prepared analogously [8]. All compounds **5** were finally converted with 3 M HCl in dioxane to the corresponding hydrochlorides, which were purified by recrystallization. 5-Formylindoles that lacked a 3-chloro substituent gave no stable hydrochlorides but underwent a rapid degradation with dark red discoloration.

2.2. Biological evaluation

2.2.1. Cytotoxicity

The growth inhibitory activity of the compounds **5a-i** against a panel of cancer cell lines was tested via MTT assays and compared with that of CA-4 (Table 1). Though the lead compound CA-4 displayed the greatest cytotoxicity against highly proliferative 518A2 melanoma cells, it showed only moderate activity against the multidrug-resistant cervix and breast carcinoma cell lines KB-V1/Vbl and MCF-7/Topo. HT-29 colon carcinoma cells were resistant to CA-4 by overexpression of the MRP-1 (multidrug-resistance protein) type ABC (ATP-binding cassette) transporter which detoxifies cells from CA-4 [20]. In contrast, the indoles **5a-5h** were active against the resistant cell lines with nanomolar IC_{50} values. The *N*-methyl indoles **5a-5d** were particularly active at IC_{50} values in the two-digit nanomolar range. Compounds **5a** bearing the original trimethoxy A-ring motif, and **5c** featuring a 3-bromo-4,5-dimethoxyphenyl A-ring, were slightly more active against the resistant cancer cells than the chloro- and iodo-derivatives **5b** and **5d**. Within the triad of *N*-ethyl substituted indoles **5e-5g**, only the trimethoxyphenyl derivative **5e** showed a comparable efficacy. Any further substitution at the indole moiety resulted in an attenuated growth inhibition. We hypothesize that the cell growth inhibiting effect of the compounds **5a-i** might be correlated to their ability to bind to tubulin as reported for CA-4.

The best indole compounds **5a** and **5c** were additionally tested against HCT-116 colon carcinoma cells with either a functional tumor suppressor protein p53 (wildtype, wt) or a knocked-out p53 (HCT-116 *p53*−/−). While the IC_{50} values of CA-4 and **5c** were similar against both cell lines, the trimethoxyphenyl derivative **5a** was more cytotoxic against p53-wildtype cells (Table 2).

To assess the selectivity of the highly active derivatives **5a** and **5c** for cancer over non-malignant cells, we also determined their IC_{50} values against primary fibroblasts (CHF) and cardiomyocytes (CCM) that were explanted from chicken embryos (Table 3). All values for CHF were in the micromolar range with compound **5c** being least cytotoxic. It also affected cardiomyocytes to a lesser extent than CA-4 and derivative **5a**. The distinct *in vitro* cytotoxicity of the anticancer drug doxorubicin against cardiomyocytes is correlated with its severe cardiotoxic side-effects *in vivo* [22]. We therefore conclude that the bromo substituted indole **5c** with the best selectivity profile and the least toxic effects on chicken fibroblasts and cardiomyocytes will probably be the one with the lowest general toxicity in subsequent *in vivo* experiments. It is worth noting that the endothelial cell line included in our growth inhibition tests (Table 1) was less affected by **5a** and **5c** than by CA-4 and that endothelial damage was also reported to be responsible for cardiotoxic side-effects of some tubulin-binding agents [23]. However, Ea.hy926 is a hybrid cell line that does not fully reflect the