New oxazole-bridged combretastatin A-4 analogues as potential vascular-disrupting agents

Katharina Mahal, Bernhard Biersack and Rainer Schoberth

Chair of Organic Chemistry, University Bayreuth, Bayreuth, Germany

Introduction

The cis-stilbene combretastatin A-4 (CA-4) is a metabolite of the South-African bush willow *Combretum caffrum* with remarkable antitumoral properties. Its mode of action is based on its high affinity for the colchicine binding site of β-tubulin and the resulting destabilization of the microtubule cytoskeleton [1]. CA-4 is also known to target the vasculature of solid tumors and to induce blood vessel shutdown leading to secondary tumor cell death [2]. The metabolic conversion of CA-4 to its inactive trans-isomer and its poor solubility are drawbacks that limit its applicability in anticancer therapy. Recent efforts to stabilize the cis-configuration by integration into heterocycles led to CA-4 derivatives with imidazole and oxazole rings the activity of which is dependent on the pattern of substituents in the phenyl rings [3, 4, 5, 6]. Some halogen-substituted oxazoles of this type showed enhanced efficacy against resistant cancer cell lines and also exhibited anti-vascular properties [6]. The cytotoxicity and inhibition of tube formation, as well as the ability to interfere with the cell cycle of a second generation of chloro-substituted oxazoles with additional functional groups int the B-ring (1: -OMe, 2: -OEt, 3: -SMe) (Figure 1) has now been investigated using human endothelial cells.

Materials and methods

The synthesis of compounds 1 – 3 has been published or will be published elsewhere [6].

Cell culture conditions

Cells of resistant human HT-29 colon carcinoma, human 518A2 melanoma, and Ea.hy926 endothelial hybrid cells were cultivated in RPMI or DMEM (supplemented with 10% FBS, 1% Pen/Strep, 100X, Gibco) and incubated at 37 °C, 5% CO2 and 95% humidity. The cells were harvested by trypsinization and grown for 24 h prior to treatment with the compounds 1 – 3 (in DMSO).

MTT assay

The cytotoxic effect on the three cell lines upon exposure to the oxazoles 1 – 3 for 72 hours was determined by a standard MTT assay with 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.05% in 1X PBS acc. to ref. [6].

Cell cycle analysis

Ea.hy926 cells (0.1 × 10^6 cells/ml) grown on 6-well plates were treated with DMSO (control) or 1 – 3 (1 µM, 24 h), fixed (70% EtOH) and incubated with propidium iodide (PI, Carl Roth) staining solution (50 µg/ml PI, 0.1% sodium citrate, 1 µg/ml RNase A in 1X PBS) for 30 min at 37 °C. The fluorescence intensity of 10,000 single cells at λ_em = 570 nm (λ_ex = 488 nm) was recorded by a Beckman Coulter Cytomics FC 500 flow cytometer and analyzed (CXP software, Beckman Coulter) to render the percentage of cells in G1, S- and G2/M-phase of the cell cycle. Apoptotic cells were assessed from sub-G1-peaks.
**Tube formation assay**

The effect of oxazoles 1–3 on the propensity of stimulated Ea.hy926 cells to form vascular-like tubular networks in vitro was assessed by growing the cells \((0.5 \times 10^6\) cells/ml) on thin Matrigel (BD Biosciences) layers for 12 hours and then treating them with DMSO (control) or 100 nM of 1–3 for additional 24 hours. It was documented by light microscopy (10-fold magnification).

**Results and conclusions**

In growth inhibition MTT assays, the new oxazoles 1–3 were active with nanomolar IC\(_{50}(72\text{h})\) against drug sensitive 518A2 melanoma cells \((1: 3 \pm 2; 2: 2 \pm 1; 3: 50 \pm 15 \text{ nM})\), CA-4 resistant HT-29 colon carcinoma cells \((1: 6 \pm 1; 2: 11 \pm 1; 3: 76 \pm 3 \text{ nM})\), and the endothelial hybrid cell line Ea.hy926 \((1: 9 \pm 1; 2: 31 \pm 3; 3: 77 \pm 4 \text{ nM})\). CA-4 itself was efficacious against melanoma and endothelial cells while not affecting the growth of HT-29 cells \((\text{IC}_{50}(72\text{h}) > 1,000 \text{ nM})\), which feature MRP-1 transporters that expel xenobiotic phenols [5, 7]. Previous studies had already shown that the replacement or removal of the B-ring meta-hydroxy group resulted in a distinct increase of the cytotoxicity against HT-29 cells [5]. Likewise, the oxazoles 1–3 bearing a meta-chloro residue on the B-ring were much more efficacious against these resistant cells when compared with CA-4. Furthermore, the cytotoxicity of the oxazoles varied with the para-substituents. Compound 1 featuring a methoxy group was the most active one, either because of a higher affinity to the colchicine binding site at the tubulin heterodimer interface or due to an improved uptake via endocytosis. Docking studies lend some support to the first assumption (unpublished results).
By quantifying the DNA fragmentation after drug treatment, it could be shown that the cytotoxic effect of 1 – 3 was mediated by induction of apoptosis as assessed from slightly increased sub-G1-peaks of 10 – 15% when compared to untreated cells. The cell cycle profiles of Ea.hy926 cells treated with 1 µM of 1 – 3 (24 h) revealed an accumulation at the G2/M interphase which is typical of inhibitors of tubulin polymerization. Apparently, the compounds 1 – 3 prevent or delay cell division by disturbing the microtubule organization.

Next, we evaluated the antivascular effect of oxazoles 1 – 3 on the endothelial cells [8]. Ea.hy926 cells when grown on thin basement matrix layers (Matrigel) soon start to form tubular networks which are regarded to be suitable surrogates or mimics for blood vessels. These were significantly diminished and a neo-formation was inhibited by incubation with 100 nM of 1 – 3 for 24 hours (Figure 1) – a concentration that the cells tolerated well for at least 24 hours. This effect was most evident when cells were treated with the methoxy derivative 1 or the para-ethoxy congener 2. The methyl thioether 3 had a weaker inhibitory effect on tube formation, in line with its lower cytotoxicity. However, all new chloro oxazoles displayed antivascular activities without signs of toxicity. Taken together, the new chloro oxazoles 1 – 3 are interesting multi-modal drugs that combine vascular disrupting/antiangiogenic effects and strong cytotoxicity. Further investigations and in vivo studies are currently underway.

References


