Nitric Oxide-Releasing Selective Cyclooxygenase-2 Inhibitors as Promising Radiosensitizers in Melanoma Cells

In Vitro

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Abstract

Expression of cyclooxygenase-2 (COX-2) and subsequent higher availability of eicosanoids are important modulators of tumor radioresistance. Additionally, elevated COX-2 protein is closely associated with hypoxia, which itself is a key promoter of tumor radioresistance. In this regard, selective COX-2 inhibitors (coxibs) containing a nitric oxide (NO)-releasing moiety (NO-coxibs) are hypothesized to act as bi-functional radiosensitizers. Therefore, novel NO-coxibs with a (pyrazolyl) benzenesulfonamide lead were investigated in vitro. As model, two human melanoma cell lines were exposed to several doses of X-ray in presence or absence of the novel NO-coxibs or the corresponding coxib during irradiation. Cells were examined in clonogenic cell survival assays to determine radiosensitizing effects under both normoxic and hypoxic conditions. COX-2 protein expression of two melanoma cell lines with a dissimilar baseline COX-2 synthesis was increased by irradiation and, furthermore, by experimental hypoxia. Radiosensitivity of both cell lines was significantly enhanced by the novel NO-coxibs and, to a lesser extent, also by the corresponding coxib. Moreover, the most potent NO-coxib 5 significantly increased the radiosensitivity of both cell lines also under hypoxic conditions. By administration of 5 the required radiation dose for 10% survival could be reduced from 6.6 Gy (DMSO control) to 5.2 Gy (p<0.01) for the ‘COX-2-positive’ A2058 cells and from 4.2 Gy (DMSO control) to 3.2 Gy for the ‘COX-2-negative’ Mel-Juso cells. The results confirm the auspicious bifunctional approach of the novel NO-coxibs as potential adjuvant radiosensitizers under normoxic and hypoxic conditions in vitro. Further studies are necessary to confirm the promising findings in vivo.

Keywords: Chemoradiotherapy; Malignant melanoma; Radiosensitization; Multi-target approach; Carbonic anhydrase-IX, CoCl2-induced hypoxia

Introduction

Cyclooxygenase (COX)-1 and COX-2 are key enzymes in the conversion of arachidonic acid into prostaglandin H₂ (PGH₂). PGH₂ afterwards is converted to a multitude of eicosanoids, e.g., other prostaglandins like PGE₂, prostacyclin and thromboxanes, depending on definite downstream isomerase pathways present in various cell types. These eicosanoids act as potent para- and endocrine mediators of metabolic processes in homeostasis, but also in inflammatory and neoplastic processes, via G-protein-coupled receptors. In particular, the inducible isoenzyme COX-2 is involved in pathophysiological situations. Consequently, COX-2-initiated signaling cascades have been recognized as potential targets for therapeutic interventions in various diseases, including chronic inflammatory disorders and cancer [1]. In the latter regard, COX-2 overexpression has been demonstrated in various tumor entities, like head and neck squamous cell carcinoma, mamma carcinoma, glioblastoma and also malignant melanoma, and is positively correlated with progression and poor patient’s survival [1,2]. COX-2 overexpression also contributes to radioresistance [3,4] and, particularly, COX-2-derived PGE₂ seems to be a key mediator for the radioreponse [5-8]. Therefore, studies on the use of selective COX-2 inhibitors (coxibs) as targeted chemotherapeutic agents and potential radiosensitizers were initiated. In this regard, there is experimental and clinical evidence that coxibs, like celecoxib, valdecoxib, and NS-398 (Figure 1), exhibit promising radiosensitizing properties [9-11].

An auspicious approach is the development of multi-targeted or bifunctional drugs,
hypothesizing that a balanced modulation of several targets can provide a superior therapeutic effect and side effect profile compared to the action of a selective ligand [12,13]. In this regard, a nitric oxide-releasing coxib (NO-coxib) [14,15] would target two ‘hallmarks’ of cancer radioresistance [16], tumor inflammation [17] and hypoxia [18-20], concurrently. The free radical Nitric Oxide (NO) provides an additional reaction partner for the formation of reactive oxygen/nitrogen species, like peroxynitrite (ONOO) [21], which contribute, e.g., to fixation of radiation-induced DNA-lesions even in highly radioresistant hypoxic tumor areas [22]. Moreover, NO enhances radiosensitivity via reduction of hypoxic conditions by increasing tumor perfusion [23,24].

We have developed two novel coxibs based on a (pyrazolyl) benzenesulfonylamine lead with a NO-releasing moiety (Figure 1) using a direct NO-coupling strategy [25]. To our knowledge, data on NO-coxibs regarding their potential radiosensitizing properties in tumor cells are scarce. This prompted us to investigate the radiosensitizing effects of the novel compounds in human malignant melanoma cell lines with high and very low baseline COX-2 protein levels.

Materials and Methods

All reagents were of highest purity available from Sigma-Aldrich GmbH (Germany), VWR International GmbH (Germany), Carl Roth GmbH & Co. KG (Germany), Merck KGaA (Germany), or Thermo Fisher Scientific Inc. (Germany), if not indicated otherwise. The synthesis of coxib 1 and NO-coxibs 2 and 5 was described in detail elsewhere [25].

Cell culture

The human A2058 melanoma cell line was acquired from Sigma-Aldrich GmbH (Germany), VWR International GmbH (Germany), Carl Roth GmbH & Co. KG (Germany), Merck KGaA (Germany), or Thermo Fisher Scientific Inc. (Germany), if not indicated otherwise. The synthesis of coxib 1 and NO-coxibs 2 and 5 was described in detail elsewhere [25].

Western blot analysis

Protein synthesis of COX-2, CA IX and β-actin was detected by Western blot analysis. Moreover, detection of stabilized HIF-1α was used as an independent hypoxia marker. Cells were cultured at reduced oxygen partial pressure (≤ 1% O2) in a special incubator equipped with an oxygen-sensor (Gasboy, Labotec, Germany). This approach (extrinsic hypoxia model) was applied for Western blot analysis only. A second approach (chemical hypoxia model) was used for clonogenic cell survival assays and Western blot analysis, too. Therefore, cells were treated with different concentrations of CoCl2 × H2O for 24 hours [26,27]. In all cases cells were cultured at 37°C, 5% CO2 and 95% humidity. VenorGeM Mycoplasma Detection Kit (Minerva Biolabs, Germany) was used for testing cells to be mycoplasma free. After trypsinization (trypsin/EDTA; 0.05%/0.02%) cells were diluted with complete medium (DMEM with 10% FCS and 1% Pen-Strep) and counted by CASY® cell counter (model TT, Schaerfe System, Germany). Regarding standardization all recultivated cells were passaged at least one times and it was ensured that cells reached subconfluency to confluency at the time point of each individual experiment.

Radiation exposure

Irradiation was executed at room temperature using single doses of 200 kV X-ray irradiation filtered with 0.5 mm copper (Maxishot, XYLON International). The absorbed dose was measured using UNIDOS dosimeter (PTW) and the dose rate amounted to ~1.1 Gy/min at 20 mA. Doses of 0, 2, 4, 6 and 10 Gy were applied. Non-irradiated cells were synchronized with the cells irradiated at 10 Gy to obtain comparable conditions.

Figure 1: Chemical structure (A) of selective COX-2 inhibitors used as potential radiosensitizers and (B) of the novel NO-coxibs 2 and 5 as well as the corresponding coxib 1 formed after NO-release (adapted from [25]).
secondary antibodies as shown in Table S1. Densitometric analysis was performed with TotalLab (TotalLab Limited, UK) as published elsewhere [29].

### Viability assay

To investigate the effect of coxib 1, NO-coxibs 2/5 and celecoxib on cell viability the CellTiter-Blue® Cell Viability Assay (Promega, Germany) was used. In analogy to manufacturer’s instructions both cell lines were seeded in 96-well plates and incubated for 24 hours with different concentrations of the compounds. After 4 hours incubation at 37°C with CellTiter-Blue® reagent the supernatants were transferred in a black 96-well microplate and the fluorescent signal was measured at 560 Ex/590 Em by Synergy HT plate reader (BioTek Instruments, Germany). Half maximal effective concentration (EC50) was calculated from dose-response curve (OriginPro 8.6 G, dose-response fit).

### Clonogenic cell survival assay under normoxia

Clonogenic cell survival assays were used to determine the radiosensitizing effects of the NO-coxibs 2 and 5 and the corresponding coxib 1 on melanoma cells. Dependent on the radiation exposure an optimized cell number (Table S2) was plated with complete medium in each well of a 6-well plate. After 24 hours the plated cells were treated with 10 µM coxib 1 or NO-coxib 2 and 5. DMSO (0.1%) was used as control. After 1 hour incubation cells were exposed to different doses of X-ray radiation (sham, 2, 4, 6 and 10 Gy). Additionally, incubation with 500 nM PGE 2 1 hour before irradiation was performed. After irradiation, cells were cultured for 8 (A2058) or 10 (Mel-Juso) days depending on the growth pattern. After cultivation cells were washed with PBS and fixed in methanol/ PBS (1/1; v/v) for 5 min followed by methanol for 10 min. Cells were stained with 0.5% crystal violet, 20% methanol in PBS for 15 min under agitation. After removal of the staining solution plates were washed three times with water and air-dried. Colonies larger than 50 cells were counted under a stereo-microscope (Stemi 2000-c, Carl Zeiss Microscopy). If not indicated otherwise, colonies formed by Mel-Juso cells post irradiation at 10 Gy were excluded from the analysis, because of the very low number of colonies arisen.

Data were normalized to the non-irradiated/untreated control (detailed description is given in supplementary data). Moreover, radiation dose necessary for 10% or 50% clonogenic survival (ID10 or ID50) was estimated by using a Linear-Quadratic (LQ) model (S(D)/ S(0)=exp(-αD-βD2)) of biological response to radiation as described in detail in the supplementary material.

### Clonogenic cell survival assay under hypoxia

In order to investigate the radiosensitizing potential of NO-coxib 5 under hypoxic conditions by clonogenic cell survival assays chemically induced hypoxia was applied as model. Therefore, 0.3 x 10^6 cells/well was seeded in 6-well plates. After 24 hours cells were treated with 50 µM or 100 µM cobalt (II) chloride hexahydrate. After 24 hours incubation the treated cells were exposed to several doses of X-ray radiation (sham, 2, 4, 6 and 10 Gy) with and without pretreatment with 10 µM NO-coxib 5 (1 hour before irradiation). Medium was removed 1 hour after irradiation and clonogenic cell survival was determined.

![Figure 2](image-url)
survival assays were performed as described above.

Statistical analysis
Statistical analysis was performed using OriginPro 8.6G software package. If not indicated otherwise, data were analyzed by one-way analysis of variance (ANOVA) using the post hoc Bonferroni method with p<0.05 or p<0.01 defined as significant.

Results and Discussion
COX-2 in melanoma cells
The relative COX-2 level (vs. β-actin) in A2058 cells was significantly higher compared to Mel-Juso cells (Figure 2A). Derived from this finding, the terms ‘COX-2-positive’ (A2058) and ‘COX-2-negative’ (Mel-Juso) cells were used to characterize the baseline level of COX-2 protein expression under normoxic conditions. Following irradiation ‘COX-2-positive’ A2058 cells showed an upregulation of COX-2 depending on the irradiation dose and the time point after irradiation (Figure 2B and 2C). Of interest, ‘COX-2-negative’ Mel-Juso cells also showed a light upregulation of COX-2 after irradiation (Figure 2B and 2C). Moreover, extrinsic hypoxia resulted in an upregulation of COX-2 in both cell lines (Figure 3A). The hypoxic marker carbonic anhydrase-IX (CA-IX) was considerably upregulated under extrinsic hypoxia in both cell lines. Chemically induced hypoxia via CoCl₂ showed only a slight influence on both CA-IX and COX-2 expression (Figure 3B). Similar to celecoxib, treatment with compounds 1, 2 and 5 showed no influence on the COX-2 protein synthesis in both cell lines (Figure S3). At baseline (normoxic) conditions the ‘COX-2-positive’ A2058 cells showed a higher radioresistance than the ‘COX-2-negative’ Mel-Juso cells (plating efficiency (PE), 0.098 vs. 0.026) (Figure 2D).

Influence of (NO-) coxibs on melanoma cell viability and plating efficiency
Treatment with the non-NO-releasing (corresponding) coxib 1 showed no influence on cell viability (Figure 4A). The NO-coxibs 2 and 5 decreased cell viability in a micromolar range, with the chlorinated NO-coxib 5 showing higher potency (EC₅₀ (A2058): 22 μM, EC₅₀ (Mel-Juso): 18 μM). Similar results could be observed
regarding their influence on the number of proliferating cells (Figure S4). Additionally, the effect of the novel compounds on the relative plating efficiency (PE_rel) was analyzed by clonogenic cell survival assays (Figure 4B and 4C; Tables S3 and S4). Administration of all compounds resulted in a lower PE_rel of 'COX-2-negative' A2058 cells. The corresponding compounds 1 and 2 showed an effect on the PE_rel of A2058 cells similar to celecoxib, whereas NO-coxib 5 reduced the PE_rel to a substantially higher extent (Figure 4B). Interestingly, the PE_rel of 'COX-2-negative' Mel-Juso cells was not affected by coxib 1 and celecoxib, but treatment with the NO-coxibs 2 and 5 resulted in a significant reduction of the PE_rel. To investigate effects potentially mediated by PGE_2 (500 nM) on 'COX-2-positive' A2058 cells PGE_2 was added to cell culture medium. PGE_2 had no influence on the PE_rel of A2058 cells and the effect of the NO-coxibs (Figure 4C).

**Influence of (NO-) coxibs on melanoma cell radiosensitivity under normoxic conditions**

Treatment with the novel compounds enhanced the radiosensitivity of A2058 cells significantly (Figure 5A and Table S3). Interestingly, treatment with the NO-coxibs resulted in a significant radiosensitization of the 'COX-2-negative' Mel-Juso, too (Figure 5C and Table S4). Again, effects of NO-coxibs 2 and 5 were higher than those of the reference coxib 1 and celecoxib. Already at a dose of 10 µM the radiosensitivity of both cell lines was significantly affected by the novel compounds. By administration of 5 the required radiation dose for 10% survival (ID_{10}) could be reduced from 6.6 Gy (DMSO control) to 5.2 Gy (p<0.01) for the 'COX-2-positive' A2058 cells and from 4.2 Gy (DMSO control) to 3.2 Gy for the 'COX-2-negative' Mel-Juso cells. Coxib 1 and celecoxib affected the radiosensitivity of the A2058 cells at higher doses to a comparable extent, but treatment with coxib 1 reduced, moreover, the necessary dose for 50% survival from 2.7 Gy (DMSO) to 1.8 Gy (p<0.001). The radiosensitizing potential of celecoxib was controversially discussed in recent publications [30,31].

Our results are in accordance with those findings by showing that celecoxib protects 'COX-2-negative' Mel-Juso cells against radiation, whereas 'COX-2-positive' A2058 cells were only slightly sensitized for radiation (Figure S5). Treatment with 2 resulted in a significant down-regulation of PGE_2 in A2058 cells (Figure S7). To clarify if the radiosensitizing properties of the NO-coxibs are PGE_2-dependent, 'COX-2-positive' A2058 cells were incubated with PGE_2 and NO-coxib 2 or 5 1 hour before irradiation (Figure 5B and Table S3). Clonogenic cell survival assays in presence of PGE_2, also resulted in a radiosensitization of the 'COX-2-positive' cells by the NO-coxibs. Of note, the results indicate that the influence on cell viability and the radiosensitizing potential of the novel NO-coxibs are dependent on the COX-2 affinity (NO-coxib 2 vs. 5) [25] and on the additional °NO release (NO-coxib 2 vs. corresponding coxib 1). To investigate the influence of the additional °NO release on the bioavailability of °NO in cell culture, the stable products of °NO, nitrite and S-nitrosothiol, were analyzed in cell culture supernatants (Figure S6). Treatment with NO-coxib 2 and, especially, 5 resulted in a concentration dependent increase of the bioavailable °NO 24 hours after incubation. Also in combination with irradiation a slight upregulation of bioavailable °NO was verifiable (90 min and 24 hours after irradiation). These data support the additive effect of the bifunctional approach used.

**Influence of NO-coxib 5 on melanoma cell radiosensitivity under hypoxic conditions**

Tumor hypoxia is clearly associated with an increased radioresistance [32]. Therefore, the radiosensitizing capacity of the most potent NO-coxib 5 was analyzed also under hypoxic conditions. Here, the chemically induced hypoxia model was used. The radiosensitivity of both cell lines was diminished by CoCl_2, concentration-dependently (Figure 6 and Table S5). Especially, at low doses (2 Gy) CoCl_2 treatment induced radioresistance. Importantly, treatment with NO-coxib 5 resulted in a significant decrease of the

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**Figure 4:** Influence of coxib 1, NO-coxibs 2/5 and celecoxib on (A) cell viability and (B) relative plating efficiency (PE_rel) of A2058 and Mel-Juso cells. (C) Influence of the NO-coxibs on the PE_rel in the presence of 500 nM PGE2. To determine the influence of coxib 1, NO-coxibs 2 and 5, and celecoxib on the relative plating efficiency of A2058 and Mel-Juso cells a concentration of 10 µM was used. Three or five independent experiments were performed (n=9-15). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, *p<0.05 or, **p<0.01, or vs. coxib 1, #p<0.05 or, ##p<0.01, or vs. NO-coxib 2, $$$p<0.01, or vs. celecoxib, §§§p<0.01.
CoCl₂-induced radioresistance in A2058 cells. After pretreatment with NO-coxib 5 the CoCl₂-induced radioresistance was reduced below the basal radiosensitivity of the cells under normoxic conditions. Radiosensitivity of the 'COX-2-negative' Mel-Juso cells also was significantly increased by treatment with 5. This reveals NO-coxib 5 to be a potent radiosensitizer under normoxic as well as hypoxic conditions in human melanoma cell lines employed as in vitro model.

This study demonstrated the radiosensitizing potential of novel (pyrazolyl) benzenesulfonamide-based NO-coxibs in vitro. As model, two human melanoma cell lines with dissimilar COX-2 expression were used. As expected, a close correlation between COX-2 synthesis and radiosensitivity was observed. Of interest, in both cell lines irradiation contributed to an enhancement of COX-2 protein expression. Consequently, increased synthesis and secretion of PGE₂ should result in a decrement of radiosensitivity as demonstrated previously [5,7]. Moreover, in both cell lines COX-2 expression was induced by experimental hypoxia. Tumor hypoxia itself is associated with a higher radioresistance of tumors and modification of tumor hypoxia should improve the effect of radiotherapy [32]. In this regard, COX-2 expression also can be considered as a surrogate marker of hypoxia [33,34]. This coincides with the observation that CA-IX, a well-characterized hypoxic marker, was affected equally [35]. Moreover, CA-IX is associated with a poor prognosis, tumor aggressiveness and progression [36]. A further rationale to determine CA-IX is based on the observation that various sulfonamides also bind to carbonic anhydrases [37]. Coxib 1 was previously described as CA inhibitor with a slight CA-IX selectivity (K_{\text{huma}} [nM]: CA-I: 1509; CA-II: 176; CA-IX: 22) [38]. Also for the aryl sulfonamides celecoxib and valdecoxib, a nanomolar inhibition of CA was documented [39]. Perhaps, the novel (pyrazolyl) benzenesulfonamide-based NO-coxibs modified the cellular response additionally in a CA dependent manner. Expression of CA-IX under normoxic conditions could be demonstrated in both melanoma cell lines (Figure 2A).

At baseline conditions treatment with coxib 1 led to a COX-2-independent decrease of proliferating cells, which could be amplified by the additional NO-release of NO-coxib 2 and 5. The cellular response to "NO depends on a finely tuned "NO homeostasis; whereby lower concentrations favor cell proliferation and survival, and higher
concentrations promote cell cycle arrest and apoptosis [40]. For the treatment with NO-NSAIDs a "NO-dependent" stagnation of cells in the G2/M transition stage and the induction of apoptosis could be observed [41-44].

Under the experimental conditions the radiosensitization of the melanoma cells mediated by (NO-) coxibs was independent of their COX-2 synthesis. Moreover, we could demonstrate a PGE2-independent radiosensitization of 'COX-2-positive' A2058 cells, which, as expected, was enhanced by the additional NO-release of NO-coxib 2 and 5 compared to reference coxib 1. The novel coxibs with and without a NO-releasing moiety resulted in a greater radiosensitization than celecoxib. COX-2-independent radiosensitization by coxibs was described previously [30,45]. For example, Dittmann et al. [30] showed a COX-2-independent radiosensitization of different tumor cell lines by celecoxib and described the inhibition of an Epidermal Growth Factor Receptor (EGFR)-mediated mechanisms responsible for the radiosensitizing properties. Moreover, Che et al. [46] demonstrated a PGE1-independent radiosensitization of radioresistant esophageal cancer cells by NS-398, and showed an inhibition of protein kinase B (AKT) activation and the induction of apoptosis by use of 100 µM of the coxib. On the other hand, radiosensitization by coxibs seems to be dependent on the coxib itself and the time point of administration [45]. Moreover, dimethyl-celecoxib, a derivative of celecoxib that lacks COX-2 inhibitory activity, showed similar anti-tumorigenic and anti-proliferative effects like celecoxib [47]. As a result, in the current experimental setting inhibition of COX-2 and, subsequently, lowering of PGE, secretion also seemed not to contribute to higher radiosensitivity of melanoma cells.

The (pyrazolyl) benzenesulfonamides, as other coxibs, show low aqueous solubility and high log Ps, allocating them, beside other factors, to the low-solubility, high-permeability BCS Class II compounds, strongly suggesting a natural predisposition for increased binding and transport in lipid compartments [48]. In monolayer cell culture such compounds to a large extent will be unspecifically bound and taken up by at least all cells and, in the present case, exert the intracellular release of "NO also in cells with low COX-2 protein. This, of course, has to be considered as a limitation of this in vitro approach. From a pharmacological point of view targeting of COX-2 by these highly affine and selective NO-coxibs under in vivo conditions should, on the other hand, result in a targeted release of "NO exclusively in COX-2 expressing cells and tumors. Normal tissues, on the other hand, will not be specifically addressed due to absent or very low COX-2 expression. Normal tissues, on the other hand, will not be specifically addressed due to absent or very low COX-2 expression. Unspecific uptake of such compounds in normal tissue, e.g., in adipose tissue, will be counterbalanced by back-diffusion into the circulation. In turn, this contributes to bioavailability of the compound and will foster the targeting of COX-2 expressing tumors [48].

Introduction of an additional chloride on the pyrazole ring in NO-coxib 5 resulted in a higher COX-2 affinity [25] and, moreover, the anti-proliferative effects and radiosensitizing properties could be increased compared to the non-chlorinated NO-coxib 2. Moreover, NO-coxib 5 sensitized radioresistant melanoma cells under CoCl2-induced hypoxia for X-ray irradiation. CoCl2 triggers transcriptional changes, for example the induction of HIF-1a, that mimic the hypoxic response [26,27]. Interestingly, CoCl2 treatment induced radioresistance in the melanoma cells mainly at low irradiation dose.

Figure 6: Influence of CoCl2-induced hypoxia with and without pretreatment of NO-coxib 5 on the radiosensitivity of (A-C) A2058 and (D-F) Mel-Juso cells. The radioresistance of (A) A2058 and (D) Mel-Juso cells was enhanced by the treatment with cobalt (II) chloride. ID10/50 values only were calculated, by the fit in consideration of the error, for the A2058 cells (model estimations are shown in Supplementary material). Three or four independent experiments were performed (n=9-12). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO, *p<0.05 or **p<0.01, or vs. CoCl2 treatment alone, $ p<0.05, or $$ p<0.01. P-values were calculated in comparison to (A) DMSO control, (B) 50 µM CoCl2 or (C) 100 µM CoCl2 by z-test.
Further experiments under, e.g., intrinsic hypoxia (multicellular spheroids) have to be performed to evaluate the radiosensitizing potential of NO-couxibs under hypoxia.

This work showed (pyrazolyl) benzenesulphonamide-derived NO-couxibs to be potent radiosensitizing agents in vitro. Of importance, radiosensitizing effects of these NO-couxibs were observed at substantially lower concentrations (10 µM vs. 10 µM to 400 µM) compared to other couxibs reported in the literature [10,30,45,46]. As discussed above, suitability as adjuvants in external radiation and/or endoradionucleide therapy has to be confirmed by preclinical experiments in vivo. The present bi-functional approach allows targeted accumulation of these compounds in radioresistant tumors that are characterized by basically high (‘constitutive’) and/or hypoxia/radiation-induced expression of COX-2.

Acknowledgements

The excellent technical assistance of Mareike Barth, Catharina Heining, Sonja Lehnhert, Aline Morganegg, Johanna Pufe, and Peggy Wecke is greatly acknowledged. The authors also are grateful to Birgit Belter, Ph.D., Jörg van den Hoff, Ph.D., Markus Laube, Ph.D., and Martin Ulrich, Ph.D. for their expert advice and many fruitful discussions. The published work has been funded in the frame of the intramural research initiative “Radiation-Induced Vascular Dysfunction (RIVAD)”. NB and JP also are thankful to the Deutsche Forschungsgemeinschaft (DFG) for supporting this work within the intramural research initiative “Radiation-Induced Vascular Dysfunction (RIVAD)”. NB and JP also are thankful to the Deutsche Forschungsgemeinschaft (DFG) for supporting this work within the Collaborative Research Center Transregio205 “The Adrenal: Central Relay in Health and Disease” (CRC/TRR 205/1). NB and SH (née Ullm) were recipients (2011-2014) of a fellowship by Europäischer Sozialfonds (ESF).

Author’s Contributions

T.K. and J.P. jointly conceived and supervised the study. N.B., S.H., and F.H. performed the chemical syntheses, cell experiments, and analyzed the data. T.K. provided analytical tools and supported the supply of reagents. N.B., S.H., F.H., and J.P. interpreted the data and wrote the paper. All authors discussed the results and implications and commented on the manuscript at all stages. All authors read and approved the final manuscript.

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