European Journal of Medicinal Chemistry 137 (2017) 558–574

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Selective photocytotoxicity of anthrols on cancer stem-like cells: The effect of quinone methides or reactive oxygen species

Lidija Uzelac [a, 1], Đani Škalamera [b, 1], Kata Mlinarić-Majerski [b], Nikola Basarić [b, *], Marijeta Kralj [a, **]

[a] Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia
[b] Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 20 April 2017
Received in revised form
29 May 2017
Accepted 31 May 2017
Available online 3 June 2017

Keywords:
Antiproliferative activity
Cancer stem cells
Photodehydration
Quinone methides
Reactive oxygen species
Singlet oxygen

ABSTRACT

Cancer stem cells (CSCs) are a subpopulation of cancer cells that share properties of embryonic stem cells like pluripotency and self-renewal and show increased resistance to chemo- and radiotherapy. Targeting CSC, rather than cancer cells in general, is a novel and promising strategy for cancer treatment. Novel therapeutic approaches, such as photodynamic therapy (PDT) have been investigated. A promising group of phototherapeutic agents are reactive intermediates - quinone methides (QMs). This study describes preparation of QM precursor, 2-hydroxy-3-hydroxymethylanthracene (**2**) and a detailed photochemical and photobiological investigation on similar anthracene derivatives **3** and **4**. Upon photoexcitation with near visible light at $\lambda > 400$ nm **1** and **2** give QMs, that were detected by laser flash photolysis and their reactivity with nucleophiles has been demonstrated in the preparative irradiation experiments where the corresponding adducts were isolated and characterized. **3** and **4** cannot undergo photodehydration and deliver QM, but lead to the formation of phenoxyl radical and singlet oxygen, respectively. The activity of **1**–**4** was tested on a panel of human tumor cell lines, while special attention was devoted to demonstrate their potential selectivity towards the cells with CSC-like properties (HMLEshEcad). Upon the irradiation of cell lines treated with **1**–**4**, an enhancement of antiproliferative activity was demonstrated, but the DNA was not the target molecule. Confocal microscopy revealed that these compounds entered the cell and, upon irradiation, reacted with cellular membranes. Our experiments demonstrated moderate selectivity of **2** and **4** towards CSC-like cells, while necrosis was shown to be a dominant cell death mechanism. Especially interesting was the selectivity of **4** that produced higher levels of ROS in CSC-like cells, which forms the basis for further research on cancer phototherapy, as well as for the elucidation of the underlying mechanism of the observed CSC selectivity based on oxidative stress activation.

1. Introduction

Cancer stem cells (CSCs) are a subpopulation of cancer cells that share properties of embryonic stem cells like pluripotency and self-renewal. Importantly, CSCs show increased resistance to chemo- and radiotherapy. The molecular pathways contributing to drug resistance are not clear, but are related to enhanced survival pathways, elevated expression of drug transporters (e.g. P-gp) or depletion of oxygen radicals. CSCs surviving primary treatment frequently cause relapse; thus, attacking the CSC should abolish the tumor's ability to recur or metastasize. Targeting CSCs, rather than cancer cells in general, is a novel and highly promising strategy for cancer treatment [1,2].

CSCs are difficult to propagate outside of the tumor environment, because CSCs generally comprise only small minorities within cancer cell populations. Therefore, standard high-throughput cell viability assays applied to bulk cancer cells populations cannot identify agents with CSC-specific toxicity. Accordingly, screening for agents that preferentially kill CSCs depends on the ability to propagate stable, highly enriched populations of CSCs in vitro, or on using specific model cell lines enriched with cells that have stem-like properties [3]. Recently, a model of CSCs that experimentally induces the epithelial-mesenchymal transition (EMT) in breast epithelial cells was established and a large library of compounds was screened for selective activity against the CSCs. The model consists of HMLE control cells and HMLE cells with

* Corresponding author.
** Corresponding author.
E-mail addresses: nbasaric@irb.hr (N. Basarić), marijeta.kralj@irb.hr (M. Kralj).
[1] The first two authors contributed equally to the preparation of this manuscript.

http://dx.doi.org/10.1016/j.ejmech.2017.05.063
0223-5234/

knocked-down E-cadherin (HMLEshEcad), which display characteristics of cancer stem cells, such as: mesenchymal phenotype, ability to form mammospheres, $CD44^{high}/CD24^{low}$ marker profile, resistance to chemotherapeutic drugs and others [3].

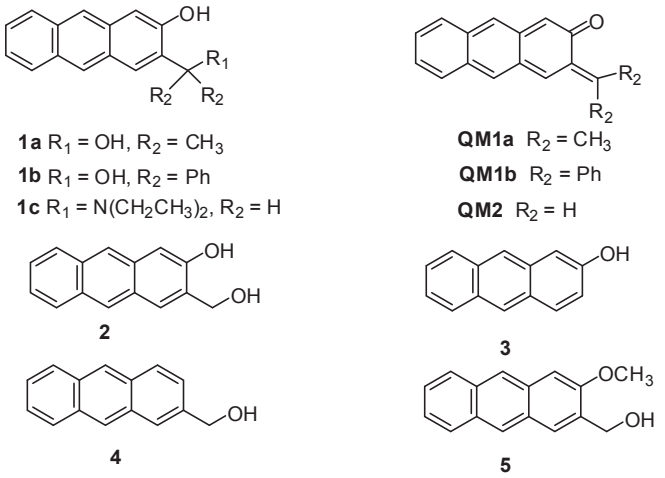
As said, it was demonstrated that CSCs possess an enhanced ROS defense capability compared to their non-stem cell counterparts. Therefore, strategies to abrogate ROS defense in CSCs or the discovery of non-toxic molecules that selectively upregulate ROS in malignant cells might result in the eradication of these ROS-resistant cells and thereby provide a basis for the development of efficient cancer therapies [4,5].

The lack of significant progress in the CSC-targeted treatments reveals the need for new therapeutic approaches, such as photodynamic therapy (PDT). PDT has been investigated as a promising alternative non-invasive method for cancer treatment [6]. The method is based on the sensitization of oxygen to the singlet excited state by dyes [7]. Compounds that are mostly used in the treatment are sensitizers with porphyrine structure [8]. However, a progress has been made recently in the use of nanoparticles [9]. Indeed, nanomedicine has great potential in the development of CSC-targeting drugs, controlled drug delivery and release, and the design of novel gene-specific drugs and diagnostic modalities [10]. Nevertheless, for the further development of the clinical method and search for new targets and compound *leads*, it is of pivotal importance to find new phototherapy mechanisms and get their full understanding on molecular level. One promising group of the phototherapeutic agents are quinone methides (QMs), reactive intermediates encountered in the chemistry and photochemistry of phenols [11]. It is widely accepted that biological action of QMs stems from their reactivity with DNA, since antineoplastic antibiotics such as mitomycin exhibit antiproliferative action on metabolic formation of QMs and subsequent alkylation of DNA [12–16]. However, QMs react with other biologically relevant nucleophiles, and particularly with amino acids [17] and proteins [18]. We have recently demonstrated that antiproliferative activity of photogenerated QMs stems from their reaction with intracellular proteins rather than with DNA [19].

QMs can be formed under mild conditions in photochemical reactions [14,15], such as photodeamination from the Mannich salts of the corresponding phenols [17,20], particularly applicable to biological systems [21,22]. Moreover, photodehydration of hydroxybenzyl alcohols [23], is a convenient method for the QM generation. Although dehydration takes place less efficiently then the elimination of ammonium salts, it is more appealing due to the fact that the thermal back-hydration regenerates the starting material, so there is no loss of an active molecule inside the cell due to the competing reaction of a QM with H_2O. In addition to simple phenol derivatives [23], the photodehydration has been demonstrated on naphthols [24], or anthrol derivatives [25,26]. Adequately substituted anthrols **1** can be excited with near visible light at $\lambda > 400$ nm delivering **QM1** in photodehydration or photodeamination [26]. The QMs react with nucleophiles giving adducts. Preliminary biological investigation indicated enhancement of antiproliferative activity for the human cancer cells which were irradiated, suggesting that the effect is due to intracellular photochemical formation of QMs [25,26].

Dichotomy of photochemical pathways leading to QMs or singlet oxygen generation has recently been described as a function the QM-precursor substituents by Freccero et al. [27], but their biological effects were not evaluated. Herein we perform a detailed photochemical and photobiological investigation on a series of anthracene derivatives **2**–**5**. The molecules were strategically designed to probe for the effect of molecular structure to biological effect. Whereas excitation of **2** can give QMs, **3**–**5** cannot undergo photodehydration and deliver QM. Furthermore, **2** and **3** can both give phenoxyl radicals in the photochemical reaction, but **4** and **5** cannot. The highest yield of singlet oxygen formation is anticipated from anthracene derivative **4** without strong electron donating substituent. Phenoxyl radicals and singlet oxygen are anticipated to lead to oxidative stress. Freccero et al. demonstrated phototoxicity of Mannich bases due to the generation of reactive phenoxyl radicals [28]. Photochemical reactivity of **2**–**4** has been investigated by performing preparative irradiations in the de-aerated and aerated solutions where the differences are expected for molecules that can sensitize oxygen. In addition, irradiations of **2** have been conducted in the presence of nucleophiles to demonstrate the ability of photochemically formed QMs to give adducts. Photophysical properties were investigated by fluorescence spectroscopy, whereas formation of QM, phenoxyl radicals and other potential reactive intermediates that could result in biological effects was probed by laser flash photolysis (LFP).

Biological investigations included antiproliferative assessment on four human cancer cell lines, HCT 116 (colon), MCF-7, SUM 159 (breast), H 460 (lung). We additionally used a breast epithelial stem-like cell model described above [3], in order to test their potential CSC-selectivity. The cells were kept in dark or irradiated. Interestingly, all anthracene derivatives **2**–**4** showed enhanced antiproliferative activity on exposure to irradiation. We additionally tried to explain their biological effects, demonstrate their localization within the cells and also validate their selectivity.

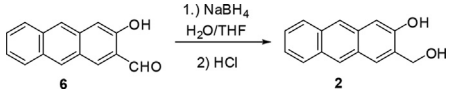


2. Results

2.1. Synthesis and photochemistry

Anthrols **1** and **3** were prepared according to the described procedures, whereas **4** can be purchased. Synthesis of anthrol **2** was accomplished in five steps, starting from the commercially available 2-aminoanthraquinone which was converted to carbaldehyde **6** following the procedure in literature precedent [26]. Reduction of the aldehyde with $NaBH_4$ afforded anthrol **2** in almost quantitative yield (Scheme 1).

Synthesis of methoxy derivative **5** started from aldehyde **6** which was methylated to methyl ether **7**, and then reduced to



Scheme 1. Synthesis of **2**.