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Research paper

New approach of delivering cytotoxic drugs towards CAIX expressing cells: A concept of dual-target drugs

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ABSTRACT

Carbonic anhydrase IX (CAIX) is a hypoxia-regulated and tumor-specific protein that maintains the pH balance of cells. Targeting CAIX might be a valuable approach for specific delivery of cytotoxic drugs, thereby reducing normal tissue side-effects. A series of dual-target compounds were designed and synthesized incorporating a sulfonamide, sulfamide, or sulfamate moiety combined with several different anti-cancer drugs, including the chemotherapeutic agents chlorambucil, tirapazamine, and temozolomide, two Ataxia Telangiectasia and Rad3-related protein inhibitors (ATRi), and the anti-diabetic biguanide agent phenformin. An ATRi derivative (**12**) was the only compound to show a preferred efficacy in CAIX overexpressing cells versus cells without CAIX expression when combined with radiation. Its efficacy might however not solely depend on binding to CAIX, since all described compounds generally display low activity as carbonic anhydrase inhibitors. The hypothesis that dual-target compounds specifically target CAIX expressing tumor cells was therefore not confirmed. Even though dual-target compounds remain an interesting approach, alternative options should also be investigated as novel treatment strategies.

1. Introduction

Solid tumors are characterized by a hypoxic microenvironment caused by their immature and inadequate vascular supply of oxygen and nutrients. These hostile hypoxic conditions result in a phenotype that is associated with a worse prognosis [1] and resistance to standard treatment options such as radiotherapy, chemotherapy, and surgery [2] [3], and [4]. Several different approaches are currently being investigated to target these hypoxic areas to make tumors more sensitive to standard treatment modalities [5] [6], and [7].

Carbonic anhydrase IX (CAIX) can be a valuable therapeutic target since it plays an important role in maintaining the intracellular pH homeostasis [8] and [9]. Furthermore its expression is

Abbreviations: ATRi, Ataxia Telangiectasia and Rad3 related inhibitor; CAIX, Carbonic anhydrase IX; CAIXi, Carbonic anhydrase IX inhibitor; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, Dimethylsulfoxide; FDA, Food and Drug Administration; HIF, Hypoxia-inducible factor; MDCK, Madin-Darby Canine Kidney; MTIC, 3-methyl-(triazen-1-yl)imidazole-4-carboxamide; OCR, Oxygen Consumption Rate.

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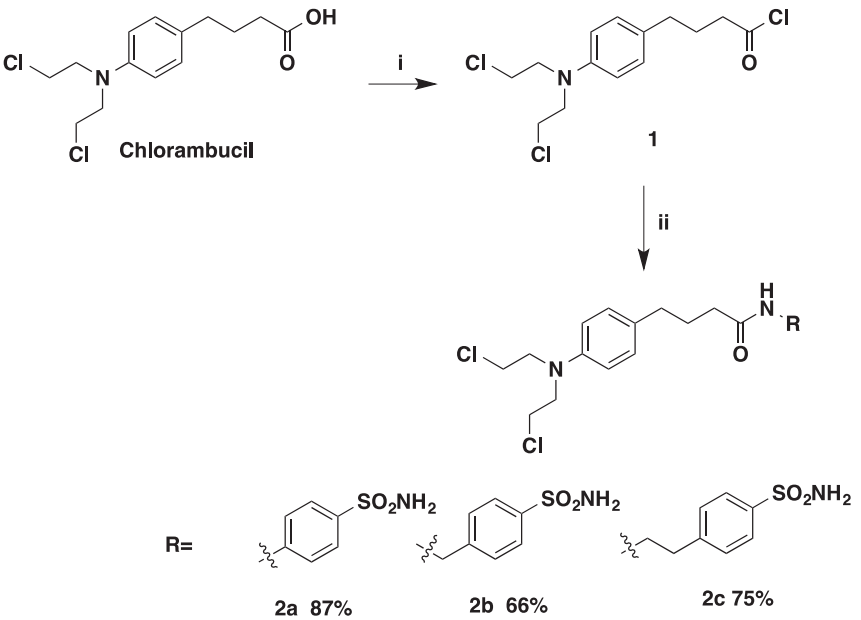
predominantly tumor specific [5] [8], and [10] and directly regulated via the hypoxia-inducible factor (HIF) pathway [11]. Even though alternative pathways are also able to modulate CAIX expression [12] [13], and [14], its significant prognostic value in many different tumor types [15] has promoted investigations in its use as an imaging agent for diagnostic and prognostic purposes [5] [16], [17] [18], and [19]. Together these characteristics of CAIX support investigations into the therapeutic targeting of CAIX to improve efficacy of standard treatments. The function of CAIX is evolutionary conserved and catalyzes the hydration of carbon dioxide to bicarbonate at the cell membrane. The bicarbonate is transported back intracellular from the extracellular space, whereas the free proton is extruded in the extracellular space. CAIX thereby maintains the balance between an acidic extracellular and alkaline intracellular pH of tumor cells, the latter of which would otherwise acidify due to the increased acid production resulting from their glycolytic metabolism [8] and [9]. Many different inhibitors are currently being developed to specifically target the tumor-associated CAIX isoform and have shown promise in reducing tumor cell survival, migration, invasion, and reduce tumor xenograft growth and metastases formation [20] [21], [22], and [23]. Furthermore, the combination therapy of CAIX inhibitors (CAIXi) with standard treatment options was previously found to increase the efficacy of radiotherapy [24] and of weakly basic chemotherapeutic agents such as doxorubicin [25].

The predominant expression of CAIX on hypoxic tumor cells can also be exploited to direct cytotoxic agents specifically to those CAIX expressing cancer cells thereby possibly minimizing normal tissue toxicity. This can be achieved by conjugating anti-cancer drugs with CAIX inhibiting molecules that bind to the Zn^{2+} active site of CAIX and hence inhibit its enzymatic function [8] [26], and [27], *i.e.* a so-called dual-targeting approach. Our group showed previously that such a dual-target approach with a sulfamide CAIXi moiety coupled to the radiosensitizing compound nitroimidazole to be a more effective radiosensitizer than an indanesulfonamide CAIXi [28]. Alternative novel dual-target compounds have been developed to investigate this strategy of dual-targeting further in the context of anti-cancer agents to target CAIX. Here we have designed five different classes of dual-target compounds conjugated to CAIXi (sulfonamide, sulfamide, or sulfamate), which included the chemotherapeutic anti-cancer agents chlorambucil, tirapazamine, and temozolomide, two ataxia telangiectasia and Rad3-related protein inhibitors (ATRi), and the biguanide agent phenformin, previously used in diabetes treatment. We hypothesize that these new dual-target compounds will have the ability to specifically target CAIX expressing cells and modulate their efficacy in a CAIX-dependent manner.

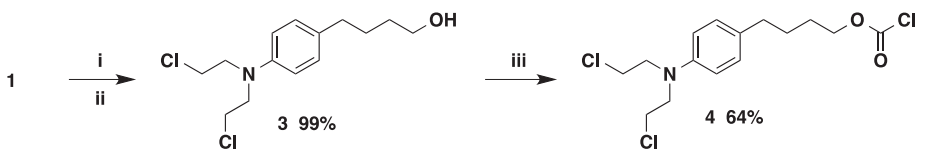
2. Results and discussion

2.1. Chemistry

Chlorambucil was converted to its acid chloride [29] **1** by using oxalyl chloride. This chlorambucil acid chloride reacted with different benzene sulfonamides under basic condition to obtain good yields of chlorambucil derivatives **2a**, **2b** and **2c**. Chlorambucil carbamate derivatives were obtained by converting compound **1** into a methyl ester [30] using methanol. This ester was reduced to alcohol [31], *i.e.* compound **3**, after treating with lithium aluminium hydride. Compound **3** was treated with triphosgene to obtain its respective chloroformate [32], *i.e.* compound **4** (Scheme 2). The reaction of chlorambucil chloroformate (**4**) with different benzene



Scheme 1. Reagents and conditions: (i) $(COCl)_2$, DMF, DCM, 0 °C–rt; (ii) DIPEA, THF, 0 °C–rt.



Scheme 2. Reagents and conditions: (i) MeOH, DCM; (ii) LAH, THF; (iii) Triphosgene, Na_2CO_3, Toluene, DMF.

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