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Research paper

Discovery of potent molecular chimera (CM358) to treat human metastatic melanoma

Y. Gilad [a], H. Tuchinsky [b], G. Ben-David [c], R. Minnes [d], A. Gancz [b], H. Senderowitz [c], G. Luboshits [e], M.A. Firer [e], G. Gellerman [a, *]

[a] Department of Chemical Sciences, Ariel University, Ariel, 40700, Israel
[b] Department of Molecular Biology, Ariel University, Ariel, 40700, Israel
[c] Department of Chemistry, Bar Ilan University, Ramat Gan, 5290002, Israel
[d] Department of Physics, Ariel University, Ariel, 40700, Israel
[e] Department of Chemical Engineering, Ariel University, Ariel, 40700, Israel

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ABSTRACT

The resistance of cancer cells to chemotherapeutic agents, whether through intrinsic mechanisms or developed resistance, motivates the search for new chemotherapeutic strategies. In the present report, we demonstrate a facile synthetic strategy towards the discovery of new anti-cancer substances. This strategy is based on simple covalent coupling between known anti-cancer drugs, which results in novel 'chimeric' small molecules. One of these novel compounds, CM358, is the product of an amide bond formation between the known Topoisomerase II (Topo II) inhibitor amonafide (AM) and the known DNA mustard alkylator chlorambucil (CLB). It demonstrates significant enhanced cytotoxicity over an equimolar mixture of AM and CLB in various cancer cell lines and in a xenograft model of human metastatic melanoma. Topo II inhibition as well as in silico docking studies suggest that CM358 is a stronger Topo II binder than AM. This may be attributed, at least partially, to the placement of the CLB moiety in a favorable orientation with respect to DNA cross-linking with nearby guanines. In a human metastatic melanoma (WM 266-4) xenograft model, this compound was profoundly superior to a mixture of AM and CLB in reduction of tumor growth, maintenance of body weight and extension of overall survival.

1. Introduction

Chemotherapy is a well-established and potent method for cancer treatment. However, chemotherapy has several major pitfalls including lack of effectiveness against drug resistance clones. Administration of drug combinations with differing mechanisms of action is a promising therapeutic strategy to enhance the effectiveness of the treatment [1–4]. Yet, to date combination chemotherapy by simple co-administration of drugs did not profoundly inhibit the development of drug resistance in cancer cells [5,6]. Consequently alternative methods for combination therapies are actively sought [7,8]. One such approach is the use of chemical chimerasin which selected drug building blocks are covalently linked to form a single compound. Chimeras are expected to provide an efficient means to the discovery of novel drug candidates [9–11]. Yet to date there are only limited examples in the literature in which direct fusion of two small molecules resulted in more than an additive effect on anti-cancer activity. Ohsawa et al. synthesized bestrabucil by conjugating estradiol with CLB [12]. Bestrabucil was designed as a payload molecule with estradiol acting as the vehicle, directing the chimera to estrogen receptor positive tumors. This mutual prodrug exhibited an improved pharmacological profile [12,13]. In another study, Yan and coworkers performed direct conjugation between the hydrophilic irinotecan and the hydrophobic CLB to obtain an amphiphilic drug-drug conjugate which self assembles into nanoparticles [14]. The formulation resulted in both improved blood stability and tumor accumulation properties compared with the free drugs which led to enhanced anti-cancer activity. A somewhat related strategy to direct drug conjugation is the integration of pharmacophore elements from two different active compounds leading to a single entity with enhanced activity. Other examples for this approach were reported with small anti-cancer compounds [15–18] and with antimalarial chemotherapeutics [19–21]. To expend on the development of new chimeric

* Corresponding author.
E-mail address: garyg@ariel.ac.il (G. Gellerman).

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drug entities, we decided to directly conjugate pairs of known anti-cancer drugs and drug candidates. We anticipated that the resulting chimeras would possess improved therapeutic properties, such as potential multi-target interactions. We focused on five, mechanistically different anti-cancer agents (Fig. 1): (1) CLB – a DNA mustard alkylating drug; (2) amonafide (AM) – an intercalating agent which inhibits the activity of the Topoisomerase II (Topo II) enzyme; (3) camptothecin (CPT) – a Topoisomerase I (Topo I) inhibitor; (4) colchicine (COLCH) – a microtubulin poison; (5) cytarabine (CYT) – an anti-metabolic agent which interferes with DNA synthesis. These compounds were used to create several chimeric substances which were screened against a battery of cell lines from different cancer types. One chimera (CM358), a conjugate of CLB and AM, was the most cytotoxic and its ability to inhibit tumor growth *in* vivo was demonstrated in a metastatic human melanoma WM 266-4 xenograft model. In addition, docking simulations were performed to evaluate the Topo II binding properties of **C**M358. These studies suggested that the chimera binds the Topo II active site with higher affinity than the known Topo II inhibitor– etoposide. Taken together, our results indicate that the molecular chimera **CM358** is a promising new ant-cancer candidate.

2. Results and discussion

2.1. Chemistry

Several coupling methods were attempted to form an amide linkage between the carboxyl group of CLB and the amino groups of AM or (Cytarabine) CYT, in order to obtain chimeras **1**, 4-(4-(bis(2-chloroethyl)amino)phenyl)-N-(1-((2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)butanamide (**2a**) and ((2R,3S,4S,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoate (**2b**) [22]. These chimeras were prepared by most efficiently direct coupling between CLB and AM or CYT according to previously published procedures using nonsymmetrical anhydride coupling [23] (Scheme 1). CLB was pre-activated with isobutyl chloroformate (IBCF) in THF to form an asymmetric anhydride. The anhydride was then directly reacted with the primary aromatic amine of AM or CYT, pre-dissolved in DMF. The resulting amide conjugation between the drugs yielded **1** and a mixture of **2a** and **2b**in acceptable yields.

Regarding **2a** and **2b**, monitoring of the reaction by LCMS indicated two main products with the same mass, which could be separated by preparative HPLC. The reaction of CYT through its cytidine amine, resulted in the major product, **2a** (yield 47%) with an amide linkage between the drugs. On the other hand, the reaction of CYT through its primary hydroxyl group, resulted in the minor product, **2b** (yield 24%) with an ester linkage between the drugs (Scheme 1). The difference between these two structural isomers was confirmed by NMR spectroscopy: 2D NMR (HMBC) of substance **2a** showed a correlation between the amide hydrogen and its neighboring carbonyl carbon. For the **2b** isomer this cross interaction is absent (see supplementary info). In order to prepare chimera (S)-4-ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3′,4':6,7]indolizino[1,2-b]quinolin-4-yl((S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl) carbamate (**3**), deacetylatation of COLCH was first performed by a previously described procedure [24] to give a free primary amine on carbon 7 in compound **6** (Scheme 2). The second part of this chimera – CPT – was pre-activated by *para* nitrophenyl chloroformate to form compound **5** [25] with active carbonate functionality (Scheme 2). A one step reaction between the para nitrophenyl carbonate derivative of CPT and the de-acetylated COLCH in the presence of the organic base DIEA yielded the desired chimeric substance **3** (yield 58%) bearing carbamate linkage between the drug components (Scheme 2).

Notably, the deacetylation of COLCH did not affect its bioactivity and the deacetylated COLCH**6** is widely used as an active anticancer component in various anticancer applications [26,27]. Compound **4** was synthesized by stirring a DMF solution of AM with an excess of acetic anhydride [28] (Fig. 1). Overall, four different chimeric drug substances were prepared possessing different chemical functionalities between the drugs and each was subjected to biological evaluation.

2.2. In vitro evaluation of the chimeras

To assess the ability of the chimeras to inhibit the proliferation of cancer cells in comparison to their free drug components, dose response experiments were performed using five different human cancer cell lines from different tissues: non-small cell lung carcinoma cell line H-1299, prostate cancer cell line PC-3, glioblastoma cell line A-172, metastatic melanoma cell line WM 266-4 and breast cancer cell line MDA-MB-231 (Fig. 2). The results clearly show that in all cell lines compound **1** is more potent than either of the free drugs alone (Fig. 2 a–e). This result is further illustrated by a comparison of the GI_{50} values for chimera **1** versus AM and CLB as seen in Table S1 (supplementary information). Based on these data, we decided to test the efficacy of **1** in an animal model of melanoma. For this purpose the WM-266-4 cell line was selected due to its aggressive subcutaneous growth in mice. Thus, positive results obtained in this model could be considered as a reliable proof of concept of the compound's potential as an anti-cancer agent *in vivo*.

We also tested the kinetics of growth inhibition at the two lowest dosages tested previously, 1 and 5 μM. Prolonged exposure to 1 μM did not affect cell growth. However, exposure to 5 μM resulted in 100% growth inhibition by 72 h (Fig. 3).

Furthermore, in order to assess the importance of the CLB moiety in chimera **1**, we compared the growth inhibition effects induced by **1**, acetylated AM (**4**) and AM on WM 266-4 cells (Fig. 4). These *in-vitro* results clearly indicate that simple acylation of AM is not sufficient to bring about the desirable cytotoxicity on cancer

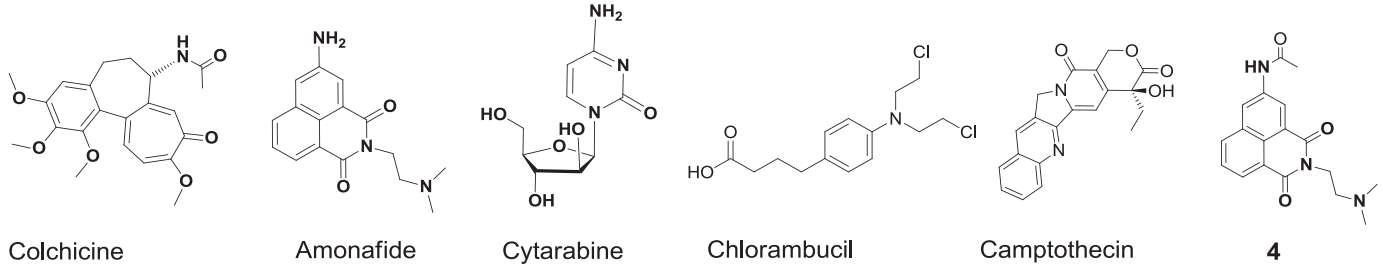

Fig. 1. Anticancer drugs and drug candidate building blocks used for the synthesis of the chimeras.