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One drug for two targets: Biological evaluation of antiretroviral agents endowed with antiproliferative activity

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ABSTRACT

AIDS-related cancer diseases are malignancies with low incidence on healthy people that affect mostly subjects already immunocompromised. The connection between HIV/AIDS and these cancers has not been established yet, but a weakened immune system is certainly the main cause. We envisaged the possibility to screen a small library of compounds synthesized in our laboratory against opportunistic tumors mainly due to HIV infection like Burkitt's Lymphoma. From cellular assays and gene expression analysis we identified two promising compounds. These derivatives have the dual action required inhibiting HIV replication in human TZM-bl cells infected with HIV-1 NL4.3 and showing cytotoxic activity on human colon HT-29 and breast adenocarcinoma MCF-7 cells. In addition, preclinical *in vitro* adsorption, distribution, metabolism, and excretion studies highlighted a satisfactory pharmacokinetic profile.

Kaposi's Sarcoma (KS), Hodgkin's Lymphoma (HL) and Burkitt's Lymphoma (BL), are orphan AIDS-related cancers diseases.[1] These cancers are rare among the healthy population but have high incidence in immune-compromised patients (e.g. persons infected by HIV).[2] The current therapy for KS, BL and HL still require the use of surgery, debilitating radiotherapy, and anticancer compounds such as Taxol® and Doxorubicin for which heavy side effects are well known.[3] Moreover, the treatment of these cancers is complicated by the concomitant intake of antiretroviral drugs.

The development of a preclinical drug candidates active both against rare AIDS-related cancers and against HIV replication (one drug for two targets) will reduce the total number of drugs that a patient should assume. Moreover the use of a drug with a dual mode of action has also a clear benefit in terms of possible side effects and multi-drug interactions.[4]

Recently, we reported the discovery and synthesis of a series of compounds endowed with anti-retroviral activity which proved to be strongly active against HIV virus.[5] The general structure of these compounds is shown in Fig. 1 (general structure A). The antiretroviral mode of action of these molecules resides in the inhibition of the gp120-CD4 protein-protein interaction, detrimental for the entry process of the viral infection. A block at this stage of the infection results in the inability for the virus to infect healthy cells. Two compounds of that series (compounds **3** and **4**, Fig. 2), combined with another small library synthesized in our laboratory (general structures **B** and **C**, Fig. 1), were screened against human MCF-7 breast cancer cells, HT-29 colon cancer cells and Ramos Human Caucasian Burkitt's Lymphoma cells.

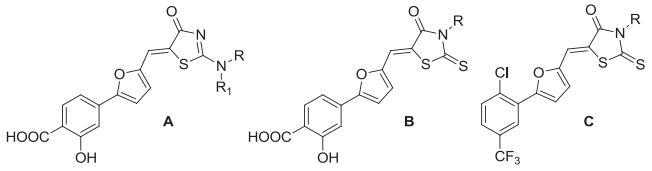

Fig. 1. General structures of the compounds of the library.

To obtain compounds with general structure **A** we used a one pot microwave assisted multicomponent reaction protocol (Scheme 1).[6,7] This microwave-assisted procedure allowed us to obtain, in short reaction time and easy workup, different final

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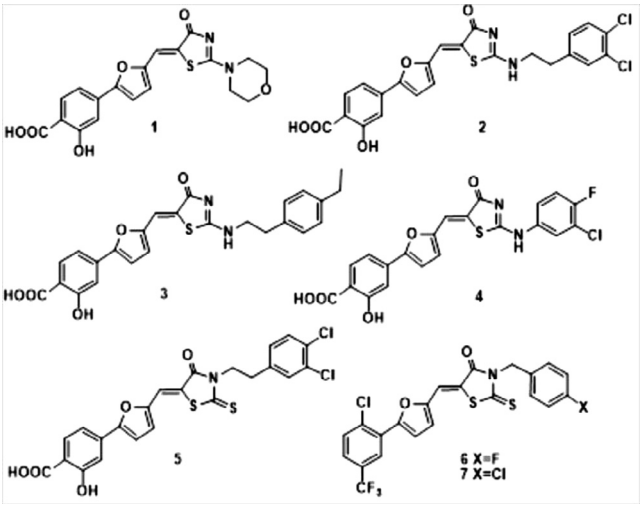
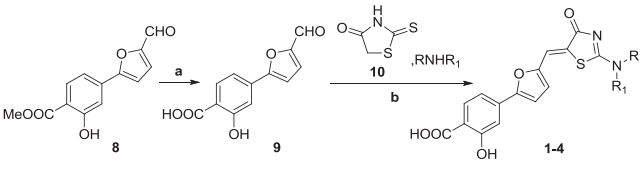


Fig. 2. Structure of compounds synthesized and tested.

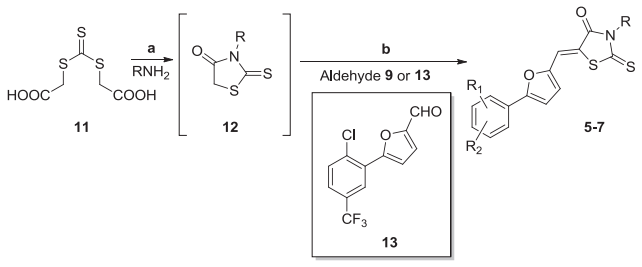
products by changing only the amine employed. To generate compound **1** morpholine was used as secondary amine whilst 2-(3,4-dichlorophenyl)ethanamine, 2-(4-ethylphenyl)ethanamine and 3-chloro-4-fluoroaniline were used as primary amines to generate compounds **2**–**4**, respectively.

The other series of substituted rhodanine derivatives with general structures **B** (compound **5**) and **C** (compounds **6** and **7**) were obtained by exploiting a practical and rapid procedure developed by us and showed in Scheme 2.[8] This methodology consists of a sequential, one-pot, two-steps microwave-assisted process with the formation of the desired final compounds in few minutes and high purity (Scheme 2).

All the synthesized compounds were tested *in vitro* to evaluate their ability to inhibit HIV replication in human TZM-bl cells infected with HIV-1 NL4.3 (a CXCR4-tropic strain) and their biological results are listed in Table 1. All these derivatives showed antiviral activity in the low micromolar range, with compounds **3** and **4** being the more potent of the series (see Table 1).[5] Furthermore, we carried out a measurement of the cytotoxic activity of our synthetic compounds **1**–**7** using three tumoral cells, the human



Scheme 1. Synthesis of derivatives **1**–**4** (a) 1 M NaOH aq., THF/MeOH, reflux, 2 h (99%). (b) 2-thioxothiazolidin-4-one **10**, amine $RNHR_1$, EtOH, MW (300 W), 150 °C, 20 min. Yields: **1** (49%); **2** (39%); **3** (80%); **4** (54%).



Scheme 2. Synthesis of derivatives **5**–**7** (a) DME, Et_3N, MW (300 W), 90 °C, 10 min. (b) aldehyde **9** or **13**, MW (300 W), 110 °C, 5 min. Yields: **5** (29%); **6** (18%); **7** (54%).

colon HT-29, the breast adenocarcinoma MCF-7 and the Human Caucasian Burkitt's Lymphoma Ramos, as well as two non-tumoral cell lines, the human embryonic kidney cell line HEK-293 and the bovine aortic endothelial cells BAE.[9] Table 1 shows the cytotoxicity values for compounds **1**–**7**, expressed as the compound concentration (μM) that causes 50% inhibition of cell growth (IC_{50}). Table 1further shows the selectivity indexes, named here as α, β, γ, δ, ε and ζ obtained by dividing the IC_{50} values of the non-tumoral cell lines HEK-293 or BAE by those of HT-29, MCF-7 or Ramos cell lines respectively (see footnote in Table 1). The higher the value of either coefficients, the higher the therapeutic safety margin of the compound in the corresponding cell line.

The cytotoxicity of compounds **1**, **3**, **4**, **6** and **7** is in the low micromolar range. Regarding the HT-29 line, compounds **1** and **4** showed promising inhibitory activity and as a consequence high α and γ values. For MCF-7 cells, compounds **6** and **7** showed the lowest IC_{50} values, having β and δ values reasonably high. Moreover, compounds **1**, **4**, **6**, **7** showed good IC_{50} values on Ramos cell line. Among all these compounds, derivatives **1** and **4** are the ones that combine high cytotoxicity towards HT-29 cell line, acceptable inhibitory activity against MCF-7 and Ramos and low cytotoxicity towards non-tumoral cell lines HEK-293 and BAE.

Moreover, all these compounds were further investigated for the inhibition of gene expressions. Most of the viruses responsible for the AIDS-related cancers aforementioned, in fact, promote the up-regulation of proto-oncogenes like c-*Myc* and genes like *hTERT*.[10] These two genes are overexpressed as a result of the infection from oncoviruses like the Epstein Barr virus (EBV) and the human virus 8 (HHV-8), responsible for BL, HL and KS.[11] To study the ability of our compounds to inhibit *hTERT* and c-*Myc* gene expression, HT-29 cells were incubated with a non-cytotoxic concentration of each compound (concentration lower than their IC_{50} value), then the RNA was extracted and retrotranscripted to cDNA to quantify the amount of gene expressed.

In order to determine whether the synthesized compounds were able to downregulate the expression of *hTERT* and c-Myc genes, we have performed a reverse transcription quantitative PCR (RT-qPCR) analysis using HT-29 tumoral cells (see Experimental Section).[12]

For these measurements compounds **2** and **5** were not selected because they were not cytotoxic towards HT-29 cells (IC_{50} values higher than 100 μM). In these assays, concentrations lower than the IC_{50} values towards the HT-29 cell line were used. Accordingly, concentrations were always 1 μM except for compound **6**, which was used at a concentration of 5 μM, and compound **3**, which was used at a concentration of 20 μM, because of their lower cytotoxicity on HT-29 cells.

Results for the selected compounds are depicted in Fig. 3 which shows the percentage of *hTERT* gene expression after 48 h of incubation in the presence of DMSO (control experiment) and in the presence of each of the compounds investigated at a concentration of 1 μM (lower than their IC_{50} values). All values were standardized (100%) to control (DMSO) and to β-actin.

It is worth noting that all selected compounds are able to significantly downregulate *hTERT* gene expression around **50%**.

The most remarkable compound is **7**, which has the strongest inhibitory activity on the *hTERT* gene expression, downregulating *hTERT* gene expression to 37%.

In order to determine whether the studied compounds were able to regulate the expression of the *c-Myc* gene, we have performed a RT-qPCR analysis using again HT-29 tumoral cells. The cells were incubated for 48 h in the presence of DMSO (control) and, as above, 1 μM of each of the studied compounds (for **6**, 5 μM and for **3**, 20 μM) were used. Results, standardized (100%) to the control (DMSO) and to β-actin, are depicted in Fig. 4.