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A tryptophanol-derived oxazolopiperidone lactam is cytotoxic against tumors via inhibition of p53 interaction with murine double minute proteins

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ABSTRACT

Inactivation of the p53 tumor suppressor protein by interaction with murine double minute (MDM) proteins, MDM2 and MDMX, is a common event in human tumors expressing wild-type p53. In these tumors, the simultaneous inhibition of these interactions with MDMs, for a full p53 reactivation, represents a promising anticancer strategy. Herein, we report the identification of a dual inhibitor of the p53 interaction with MDM2 and MDMX, the (*S*)-tryptophanol derivative OXAZ-1, from the screening of a small library of enantiopure tryptophanol-derived oxazolopiperidone lactams, using a yeast-based assay. With human colon adenocarcinoma HCT116 cell lines expressing wild-type p53 (HCT116 $p53^{+/+}$) and its p53-null isogenic derivative (HCT116 $p53^{-/-}$), it was shown that OXAZ-1 induced a p53-dependent tumor growth-inhibitory effect. In fact, OXAZ-1 induced p53 stabilization, up-regulated p53 transcription targets, such as MDM2, MDMX, p21, Puma and Bax, and led to PARP cleavage, in $p53^{+/+}$, but not in $p53^{-/-}$, HCT116 cells. In addition, similar tumor cytotoxic effects were observed for OXAZ-1 against MDMX-overexpressing breast adenocarcinoma MCF-7 tumor cells, commonly described as highly resistant to MDM2-only inhibitors. In HCT116 $p53^{+/+}$ cells, the disruption of the p53 interaction with MDMs by OXAZ-1 was further confirmed by co-immunoprecipitation. It was also shown that OXAZ-1 potently triggered a p53-dependent mitochondria-mediated apoptosis, characterized by reactive oxygen species generation, mitochondrial membrane potential dissipation, Bax translocation to mitochondria, and cytochrome *c* release, and exhibited a p53-dependent synergistic effect with conventional chemotherapeutic drugs.

Collectively, in this work, a novel selective activator of the p53 pathway is reported with promising antitumor properties to be explored either alone or combined with conventional chemotherapeutic drugs. Moreover, OXAZ-1 may represent a promising starting scaffold to search for new dual inhibitors of the p53–MDMs interaction.

Abbreviations: $\Delta\psi_m$, mitochondrial membrane potential; CFU, colony-forming units; co-IP, co-immunoprecipitation; cyt *c*, cytochrome *c*; DMSO, dimethyl sulfoxide; FCCP, p-triflouromethoxyphenylhydrazone; IgG, immunoglobulin G; PI, propidium iodide; MDM2, murine double minute 2; MDMX, murine double minute X; MMP, mitochondrial membrane permeabilization; ROS, reactive oxygen species; SRB, sulforhodamine B; wt, wild-type.

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1. Introduction

The p53 transcription factor is a major regulator of several cellular processes involved in tumor progression, such as cell cycle arrest and apoptosis [1,2]. The inactivation of p53 oncosuppressor function is a common event in human cancers either by mutation in the *TP53* gene [1], or by interaction of the p53 protein with endogenous inhibitors [2]. In fact, in tumors retaining a wild-type (wt) p53 status, the activity of this protein is suppressed due to interaction with two structurally related negative regulators, murine double minute (MDM) proteins, MDM2 and MDMX. Disruption of the p53–MDMs regulatory network has therefore clear implications in tumorigenesis and presents exciting opportunities for cancer therapy [2]. However, to date, most of the current efforts have been focused on the p53–MDM2 interaction [2]. The role of MDMX in the fine regulation of p53 is still emerging, but it is well-established that even normal levels of MDMX can partially silence activated p53. Additionally, MDMX overexpression (namely in retinoblastomas and melanomas) renders cancer cells highly resistant to MDM2-only inhibitors, such as nutlin-3a, since they are unable to liberate p53 from MDMX [3]. This clearly indicates that dual inhibition of the p53 interaction with MDM2 and MDMX may substantially improve the outcome of this p53 activation strategy due to the full p53 activation [2]. In spite of this, to date, just few small molecule dual inhibitors of the p53–MDMs interaction have been identified, namely a pyrrolopyrimidine [4], an indolylhydantoin (RO-5963; [3]) and a cis-imidazoline derivative (H109; [5]).

Recently, our group developed a new approach, based on yeast cells co-expressing human p53 and MDM2 or MDMX, for the targeted screening of inhibitors of the p53 interaction with MDM2 [6] or MDMX [7], respectively. The efficacy of these cell systems for the screening of p53–MDMs interaction inhibitors was demonstrated by the identification of two new small molecule inhibitors of the p53–MDM2 interaction, a pyranoxanthone [7] and an oxazoloisoindolinone [8].

In this work, we report the identification of a new small molecule dual inhibitor of the p53–MDMs interaction, the (*S*)-tryptophanol derivative OXAZ-1 (Fig. 1), from the screening of a small library of enantiopure tryptophanol-derived oxazolopiperidone lactams using a yeast-based approach. OXAZ-1 exhibits a p53-dependent *in vitro* tumor cytotoxic effect via p53 activation with dual inhibition of the p53 interaction with MDM2 and MDMX. The promising antitumor activity of OXAZ-1 is reinforced by its ability to trigger a p53-dependent mitochondria-mediated apoptosis, as well as by its p53-dependent synergistic effect with conventional chemotherapeutic drugs.

2. Materials and methods

2.1. Compounds

OXAZ-2, OXAZ-3, OXAZ-4, OXAZ-5, and OXAZ-6, whose molecular structures are shown in Fig. 1, were synthesized according to the described procedures [9–11]. Nutlin-3a was from Alexis Biochemicals (Grupo Taper, Sintra, Portugal); doxorubicin and SJ-172550 were from Sigma-Aldrich (Sintra, Portugal); etoposide was from Calbiochem (VWR, Carnaxide, Portugal). All tested compounds were dissolved in dimethyl sulfoxide (DMSO) from Sigma-Aldrich (Sintra, Portugal).

2.2. Synthesis of OXAZ-1

A solution of (*S*)-tryptophanol-derived oxazolopiperidone lactam OXAZ-5 (0.2 g, 0.548 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C. *p*-Toluenesulphonyl chloride (0.133 g, 0.701 mmol) and tetrabutylammonium chloride (cat., 10% mmol) were added and the mixture was stirred for 10 min. Then, an aqueous solution of NaOH (30% m/v, 0.5 v CH_2Cl_2) was added and the reaction was allowed to stand at room temperature for 24 h. After this period, the reaction was diluted with CH_2Cl_2 (10 mL) and the phases were separated. The organic phase was washed with HCl (1 M, 10 mL) and dried over Na_2SO_4. After solvent evaporation the crude compound was purified by flash chromatography (EtOAc/*n*-hexane 1:1). The desired compound was obtained as a white crystal solid after recrystallization from dichoromethane. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.1 Hz, 1H), 7.72 (m, 3H), 7.36 (s, 1H), 7.31 (t, J = 7.7 Hz, 1H), 7.25 (m, 1H), 7.21 (m, 2H), 4.70 (dd, J = 9.9, 2.5 Hz, 1H), 4.23 (m, 1H), 3.95 (d, J = 9.4 Hz, 1H), 3.74 (t, J = 7.8 Hz, 1H), 3.70 (s, 3H), 3.58 (d, J = 14.1 Hz, 1H), 2.68 (dd, J = 14.0, 9.8 Hz, 1H), 2.60 (dd, J = 17.6, 4.6 Hz, 1H), 2.37 (m, 3H), 2.33 (s, 3H), 2.28 (d, J = 12.0 Hz, 1H), 2.10 (dd, J = 17.6, 10.2 Hz, 1H), 1.21 (m, 1H). Elemental Anal. calcd. for $C_{26}H_{28}N_2O_6S$: C 62.89, H 5.68, N 5.64; found: C 62.98, H 5.84, N 6.00. The ^{1}H NMR spectra was found to be identical to the one described in Ref. [11].

2.3. Yeast targeted screening assay

Saccharomyces cerevisiae (strain CG379) expressing human wt p53 alone and combined with human MDM2 or MDMX were obtained in previous works [6,7]. For expression of human proteins (routinely grown in minimal selective medium), cells were diluted to 0.05 OD_{600} in selective induction medium containing 2% (w/w) galactose, 1% (w/w) raffinose, 0.7% (w/w) yeast nitrogen base without amino acids from Difco (Quilaban, Sintra, Portugal) and

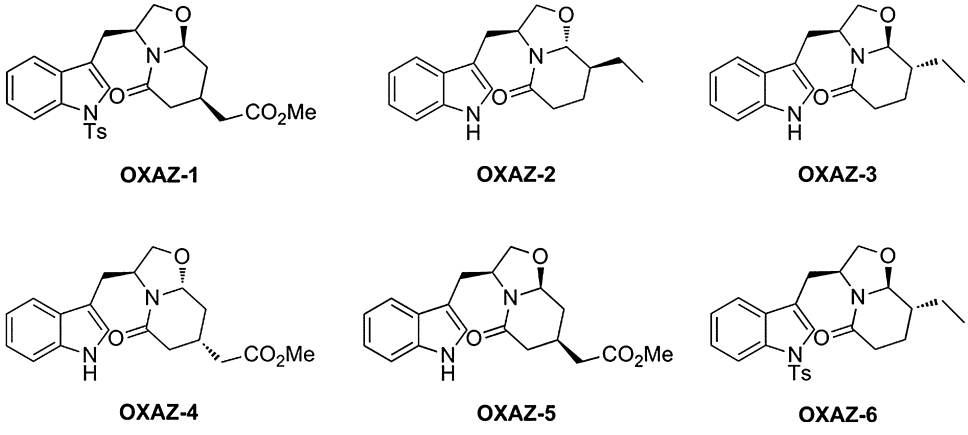

Fig. 1. Chemical structure of tryptophanol-derived oxazolopiperidone lactams evaluated in yeast.