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Study of novel anticancer 4-thiazolidinone derivatives

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ABSTRACT

4-Thiazolidinones are a known class of prospective drug-like molecules, especially in the design of new anticancer agents. Two of the most prominent subtypes of these compounds are 5-ene-2-amino(amino)-4-thiazolidinones and thiopyrano[2,3-*d*]thiazoles. The latter are considered to be cyclic mimetics of biologically active 5-ene-4-thiazolidinones with similar pharmacological profiles. Therefore, the aim of this study was to evaluate the impact of 4-thiazolidinone-based compounds on cytotoxicity, the apoptotic process, and metabolism in the human squamous carcinoma (SCC-15) cell line. The SCC-15 cells were cultured in phenol red-free DMEM/F12 medium supplemented with 10% FBS, hydrocortisone, and exposed to rising concentrations (1 nM–100 μM) of the studied compounds for 6, 24 and 48 h. Afterwards, reactive oxygen species (ROS) formation, cell viability, caspase-3 activity, and cell metabolism were measured. The obtained results showed that all of the studied compounds in a wide range of concentrations (1 nM–100 μM) increased DCF fluorescence which suggests a stimulation of ROS production. Nevertheless, these new compounds showed cytotoxic and proapoptotic properties only at high (10–100 μM) concentrations. Our studies are the first to be carried out on these compounds and require further investigation to clarify the mechanism of action of their anticancer potential.

1. Introduction

4-Thiazolidinone-based compounds have been of special interest in the field of medicinal chemistry as sources of new drug-like molecules in the last few years [1,2]. Following achievements with the use of 4-thiazolidinone and the introduction of glitazones (peroxisome proliferator-activated receptor gamma (PPARγ)-agonists) onto the market, the main directions are the search for new antidiabetic, antiviral, and antimicrobial agents [3,4]. However, most papers have been devoted to the search for a 4-thiazolidinone-based anticancer agent [5,6]. Despite the variety of compounds bearing thiazolidinone and related cores (rhodanine, 2,4-thiazolidinedione, 2,3-disubstituted-4-thiazolidinone derivatives, etc.), most of the attention has been focused on 5-substituted-2-amino(imino)thiazolidinones (namely 5-ene derivatives) [7,8]. Numerous data obtained in the screening assays have confirmed the anti-cancer potency of the above-mentioned compounds, with the half maximal inhibitory concentration (IC_{50}) of the lead compounds up to a nanomolar level. This was outlined in a thesis on the crucial role of the presence/nature of the C5 substituent of the main core for the pharmacological effect [9–12]. Anticancer activity is often related to a reactive oxygen species (ROS)-dependent mode of action and to the proapoptotic effect of 4-thiazolidinones, and it has been demonstrated in various cancer cells [7,13–15]. ROS are an effective weapon used by anticancer chemotherapeutics against tumor cells, and the anticancer effect of well-known anticancer drugs (cisplatin and doxorubicin) is due to an increase in the intracellular level of ROS that contributes to their therapeutic effect. The increase in ROS level was detected under the influence of 5-ene-4-thiazolidinones in the human colorectal adenocarcinoma (HT29) and leukemic cell line (CEM) and it was inhibited by ascorbic acid [12,15]. Similarly, in human leukemia (HL-60) and HL-60/ADR cells, another 4-thiazolidinone was found to induce ROS accumulation and caused an antitumor effect under,

Abbreviations: DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; PBS, phosphate-buffered saline; ROS, reactive oxygen species; LDH, lactate dehydrogenase.

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most probably, mitochondria-mediated apoptosis. Moreover, this cytotoxic effect was blocked by well-known ROS scavengers such as *N*-acety-L-cysteine and ascorbic acid [7]. Compounds increasing ROS production also represent an alternative option among the chemotherapeutic strategies against cancer cells [16].

Given the pharmacological effects of 5-ene-4-thiazolidinones, their reactivity (combination of several reaction centers), and their belonging to the privileged heterocycles [17], they are considered to be an efficient tool in "privileged substructure-based diversity-oriented synthesis" [18]. 5-Ene-4-thiazolidinones are attractive reagents for the design of thiopyrano[2,3-*d*][1,3]thiazoles. The latter are considered to be cyclical bioactive mimetics of 5-ene-4-thiazolidinediones without the Michael acceptor functionality and open up new perspectives in the design of new anticancer agents [19–21]. Screening studies of 4-thiazolidinones and related thiopyrano[2,3-*d*]thiazoles within DTP (NCI, USA, https://dtp.cancer.gov) have led to the identification of compounds with significant antitumor activity that is interesting for further study of some certain types of cancer and for establishing the mechanisms of action [20,22,23].

Squamous cell carcinoma (SCC) is a cancer of the epithelial cells which is a very diverse cancer in terms of histology. SCC encompasses at least 90% of all oral malignancies, and The World Health Organization expects worldwide rising incidence of oral squamous cell carcinomas [24]. According to statistical data, the main group to be at high risk of this disease are smokers and alcohol drinkers [25,26]. It has been confirmed that in squamous carcinoma cells, PPARγ mRNA and protein are present [27]. Additionally, it has been shown that the PPARγ ligands significantly and dose-dependently inhibit the proliferation of SCC lines [27].

Therefore, the aim of this study was to evaluate the anticancer activity of thiazolidinones and their impact on cytotoxicity, the apoptotic process, and metabolism in the human squamous carcinoma cell line SCC-15. The selected compounds used in our study belong to thiopyrano[2,3-*d*][1,3]thiazoles (Les-2194, Les-3377) and 5-ene-2-amino-4-thiazolidinones (Les-3640) (Fig. 1).

2. Materials and methods

2.1. Reagents

Trypsin, penicillin, streptomycin, neutral red solution, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}, hydrocortisone, sodium pyruvate, sodium bicarbonate, fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), and resazurin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The DMEM/F12 (1:1) medium was purchased from ATCC (Manassas, VA, USA). The caspase-3 substrate was purchased from Calbiochem (Merck Corporation, Darmstadt, Germany). The LDH-based cytotoxicity detection kit was purchased from Roche Applied Science (Mannheim, Germany).

Synthesis and physicochemical data of the compounds used in this study were described previously: Les-2194 – *rel-N*-(2,4-dichlorophenyl)-2-[(5aR,11bR)-2-oxo-5a, 11b-dihydro-2*H*,5*H*-chromeno[4′,3':4,5]thiopyrano[2,3-*d*][1,3]thiazol-3(6*H*)-yl]acetamide [22]; Les-3377 –5,10-dihydro-2*H*-benzo[6,7]thiochromeno[2,3-*d*][1,3]thiazole-2,5,10-trione [20]; and Les-3640 – 3-{2-[5-(4-dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydro-1,3-thiazol-5-ylidene}-2,3-dihydro-1H-indol-2-one [23]. All stock solutions were prepared by dissolving the compounds in DMSO. The final concentration of DMSO in the culture medium was always 0.1%.

2.2. Cell culture and treatment

Human squamous carcinoma cell line SCC-15 (ATCC CRL-1623) was obtained from the American Type Culture Collection (ATCC, distributor: LGC Standards, Łomianki, Poland). The SCC-15 cells were maintained in DMEM/F12 1:1 medium containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, and 0.5 mM sodium pyruvate supplemented with 400 ng/mL hydrocortisone and 10% fetal bovine serum (FBS). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells were seeded in 96-well culture plates (Costar, St. Louis, MO, USA) at a density of 10×10^3 (for the 3 h treatment), 9×10^3 (for the 24 h treatment), or 8×10^3 (for the 48 h treatment) per well and initially cultured before the experiment for 24 h. Subsequently, the medium was changed to a fresh one by raising the concentrations of the studied compounds (1, 10, 50, 100 nM and 1, 10, 50, 100 μM).

2.3. Measurement of DCF fluorescence

Fluorogenic dye H_2DCFDA was used to detect intracellular ROS. After diffusion into the cell, H_2DCFDA is deacetylated by cellular esterases into a non-fluorescent compound that is subsequently oxidized by ROS into 2′,7′-dichlorofluorescein (DCF) [28]. A total of

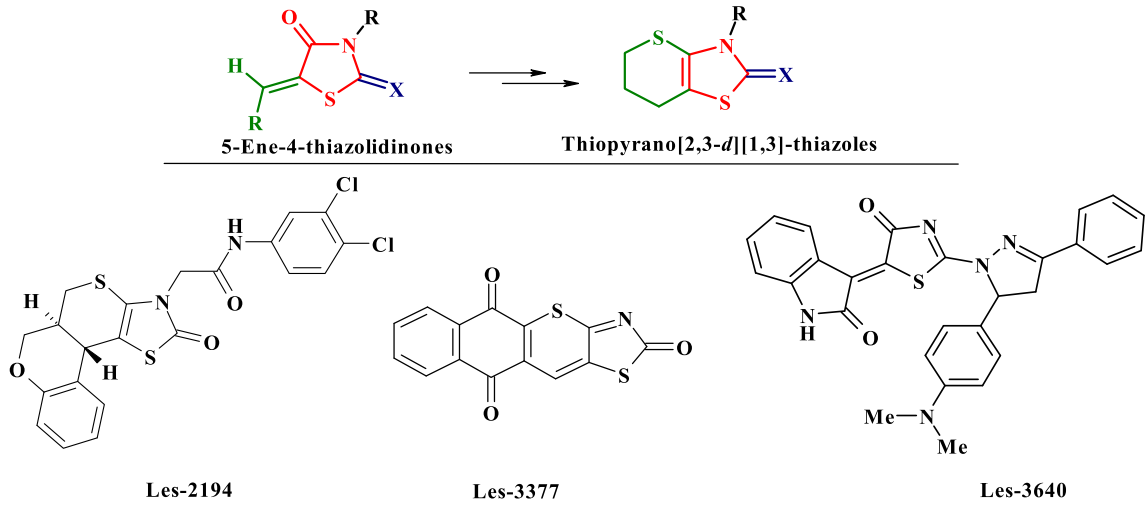

Fig. 1. Structure of the tested 4-thiazolidinone derivatives.