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Short communication

Synthetic cannabinoid quinones: Preparation, in vitro antiproliferative effects and in vivo prostate antitumor activity

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ABSTRACT

Chromenopyrazolediones have been designed and synthesized as anticancer agents using the multi-biological target concept that involves quinone cytotoxicity and cannabinoid antitumor properties. In cell cytotoxicity assays, these chromenopyrazolediones have antiproliferative activity against human prostate cancer and hepatocellular carcinoma. It has been shown that the most potent, derivative **4** (PM49), inhibits prostate LNCaP cell viability (IC_{50} = 15 μM) through a mechanism involving oxidative stress, PPARγ receptor and partially CB_1 receptor. It acts on prostate cell growth by causing G_0/G_1 phase arrest and triggering apoptosis as assessed by flow cytometry measurements. In the in vivo treatment, compound **4** at 2 mg/kg, blocks the growth of LNCaP tumors and reduces the growth of PC-3 tumors generated in mice. These studies suggest that **4** is a good potential anticancer agent against hormone-sensitive prostate cancer.

1. Introduction

Prostate cancer is the second most common cancer worldwide for males. The rates have increased in recent years as its detection has improved in the younger men and as life expectancy is longer [1]. Therefore, there are extensive, ongoing efforts to develop new therapeutic strategies to treat prostate cancer [2].

A large diversity of biological signaling-pathways is implicated in the pathogenesis of cancer. Appropriate treatment of neoplasms often depends on pharmaceutical intervention at multiple pathways, with a combination of different drugs. In this context, targeting different anticancer modes of action in a single molecule is a significant challenge [3,4]. Our interest in cannabinoid ligands and antiproliferative agents has led to the development of molecules which includes in one entity cannabinoid and quinone features.

In the last decade, cannabinoids (Sativex® and Marinol®) have been clinically used as palliative treatment in chemotherapy [5]. They reduce emesis, stimulate appetite and relieve pain. More recently, increasing biochemical and pharmacological data have showed that cannabinoids can modulate tumor growth, apoptosis and angiogenesis in various types of cancer [6–13]. Evidences have been recently reported on the dysregulation of the endocannabinoid system in prostate tumor [14]. Thus, this system provides a new therapeutic target for this type of cancer. Interestingly, its modulation by endocannabinoid hydrolysis inhibitors produces antiproliferation of prostate carcinoma [15,16].

On the other hand, the well-established antitumor properties of quinones are still the focus of much research. Nowadays cytotoxic quinones represent an important group of antineoplastic drugs [17]. They are mainly involved in oxidative phosphorylation and electron transport processes. Therefore, their cytotoxicity activity

Abbreviations: LNCaP, lymph node carcinoma prostate; PC-3, prostate cancer; HepG2, heptoblastoma; HT-29, human colon adenocarcinoma; ROS, reactive oxygen species; EBNA, Epstein–Barr virus nuclear antigen; TBAP, tetrabutylammonium perchlorate; PBS, phosphate buffered saline; S.D., standard deviation; S.E., standard error; FITC, fluorescein isothiocyanate; PI, propidium iodide.

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can be accounted for by their bioreductive and/or Michael acceptor properties that produce oxidative stress.

Few years ago, Kogan et al. reported the antiproliferative activity of three quinone derivatives of phytocannabinoid (Fig. 1: HU-331, HU-336, HU-345) [18]. The most effective, HU-331, reduced growth of human colon carcinoma HT-29 cells in nude mice. Its antiproliferative effect was not attributed to a mechanism involving cannabinoid receptors since it does not have any affinity for these receptors [19]. Accordingly, developing new antitumoral agents with dual mechanism, cannabinoid and oxidative stress, is highly valued to improve the efficacy and overcome side-effects related to quinones.

Recently, we published the cannabinoid properties of a new family of chromenopyrazoles [20], fully selective CB_1 receptor ligand lacking psychoactive effects. Following with chromenopyrazole as scaffold we report herein the synthesis of the corresponding quinone related derivatives (**4**–**6**). Their ability to bind to CB_1 and CB_2 cannabinoid receptors, to generate reactive oxygen species, and to produce antiproliferative properties in vitro and in vivo will be discussed. Thus, we report the first antitumoral quinone which antiproliferative activity proceeds through various mechanisms including cannabinoid receptors.

2. Results

2.1. Chemistry and electrochemistry

The starting 7-(1′,1′-dimethylheptyl)-dihydro-4,4-dimethylchromeno[4,3-*c*]pyrazol-9-ols **1**–**3** were prepared according to a synthetic procedure previously reported by us [20]. Scheme 1 outlines the synthesis of the cannabinoid quinones **4**–**6**. The reagent [bis(trifluoroacetoxy)iodo]benzene [$(CF_3CO_2)_2IC_6H_5$] (BTIB) was used to oxidize the chromenopyrazoles **1**–**3** to the corresponding quinones **4**–**6**. This oxidative reagent is a hypervalent iodine compound that allows regio-controlled oxidations of phenol to *para*-quinones under mild conditions [21,22]. The 7-(1′,1′-dimethylheptyl)-dihydro-4,4-dimethylchromeno[4,3-*c*]pyrazol-6,9-diones **4**–**6** were obtained in moderate yields (21–36%).

Since most of the biological functions of quinones are associated with their redox activity, the electrochemical properties of 7-(1′,1′-dimethylheptyl)-1,4-dihydro-4,4-dimethylchromen[4,3-*c*]pyrazol-6,9-dione (**4**; PM49) and 7-(1′,1′-dimethylheptyl)-2-ethyl-2,4-dihydro-4,4-dimethylchromen[4,3-*c*]pyrazol-6,9-dione (**6**) have been studied by cyclic voltammetry. Chromenopyrazoledione **5** is structurally close enough to derivative **6** to consider that both compounds show similar electrochemical behavior.

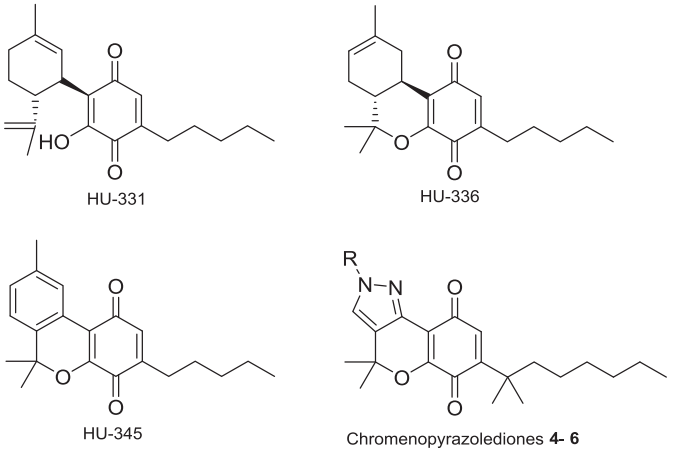

Fig. 1. Quinone structures related to cannabinoids.

Chromenopyrazolediones **4** and **6** displayed comparable voltammetric behavior, showing two well-defined reduction waves in DMSO. The first wave for both quinones studied corresponded to a cuasi-reversible one-electron transfer. The reverse scan showed the anodic counterpart of the reduction waves (Fig. 2). According to the standard reversibility criteria, this couple corresponds to a cuasi-reversible diffusion-controlled one-electron transfer. It is attributable to the reduction of quinone to semiquinone that involves a stable anion radical at room temperature. The second couple is irreversible over the whole range of sweep rates used (0.1 at 2.0 V/s). We can attribute this wave to the production of a hydroquinone derivative.

Electron spin resonance (ESR) experiments were carried out in order to correlate the cytotoxicity to the formation of radicals. The semiquinone free radicals were prepared in situ by electrochemical reductions in DMSO, applying a potential corresponding to the first wave for **4** or **6** obtained from the cyclic voltammetric experiments. The interpretation of the ESR spectra by means of a simulation process has led to the determination of the coupling constants for all the magnetic nuclei, confirmed by theoretical calculations. The ESR spectrum of **4** was analyzed and simulated in terms of one doublet from hydrogen nuclei of the quinone moiety; the hyperfine constant was 2.635 G. Fig. 3 shows the ESR experimental and simulation spectra. Similar hyperfine pattern was found for **6** with hydrogen hyperfine constant value of 2.925 G.

The results obtained with these electrochemical experiments showed that the redox potentials of **4** and **6** are low enough to generate reactive oxygen species (ROS). Therefore, these quinone radical entities should be able to produce oxidative stress.

2.2. Biology

2.2.1. Cannabinoid receptor affinity

The binding affinity of the chromenopyrazolediones **4**–**6** was assessed through radioligand competition binding experiments. The ability of **4**–**6** to displace [^{3}H]CP55940 from human cannabinoid CB_1 or CB_2 receptors transfected into HEK293 EBNA cells was evaluated. Standard cannabinoid ligand WIN55,212-2 was also tested for comparison with the new compounds. The experimental binding affinities of **4**–**6** and WIN55,212-2 are reported in Table 1. Interestingly, **4** showed significant affinity in the nanomolar (submicromolar) range both CB_1 and CB_2 receptors. This is a noteworthy feature since the only cannabinoid quinones reported so far did not show affinity for the cannabinoid receptors, as already mentioned [18].

2.2.2. In vitro antiproliferative effect on cancer cells

The antiproliferative effect of chromenopyrazolediones **4**–**6** was evaluated against the cancer cell lines HepG2, LNCaP and PC-3. HepG2 is a hepatocellular carcinoma-derived cell line, LNCaP is an androgen-dependent prostate cancer-derived cell line and PC-3 is an androgen-refractory prostate cancer-derived cell line. Different doses of **4**–**6** were added to cell cultures for 48 h and cell viability was assayed by colorimetric measurements using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a dye. As showed in Fig. 4, **4**–**6** were more effective in prostate than in hepatocellular carcinoma cells, being **4** the most potent with an IC_{50} of 30 μM for HepG2 cells and 15 μM for prostate cancer LNCaP and PC-3 cells. Therefore, the chromenopyrazoledione **4** (PM49) was selected for following investigations in prostate cancer cells.

2.2.3. Mechanism of antiproliferative action

To study the effect of the chromenopyrazoledione **4** on the cell cycle of prostate cells, flow cytometry analysis were carried out. Results shown in Fig. 5 demonstrate that **4** increased the amount of