European Journal of Medicinal Chemistry 64 (2013) 603–612

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Cytotoxic activities of substituted 3-(3,4,5-trimethoxybenzylidene)-1,3-dihydroindol-2-ones and studies on their mechanisms of action

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ARTICLE INFO

Article history:
Received 21 January 2013
Received in revised form
14 March 2013
Accepted 20 March 2013
Available online 3 April 2013

Keywords:
Oxindole
Knoevenagel reaction
Antiproliferative activity
NADPH oxidase 4
Tubulin assembly

ABSTRACT

The synthesis of new trimethoxybenzylidene–indolinones is reported. Their cytotoxic activity was evaluated according to Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD, drug screen protocols. The study of the mechanism of action suggests that inhibition of Nox4 in B1647 cells (acute myeloid leukemia) could contribute to the antiproliferative effect of some compounds. Moreover, inhibition of tubulin assembly was observed for the most cytotoxic compound, and the structural basis for this activity was delineated by binding models.

1. Introduction

The 3,4,5-trimethoxyphenyl group is present, free or hindered, in well known antitumor agents, such as combretastatin A-4 (CSA4), colchicine and podophyllotoxin. With this in mind, we started to study the antiproliferative activity of Knoevenagel adducts in which the oxindole moiety is linked to a 3,4,5-trimethoxyphenyl group by means of a methine bridge, and in 2006 we published a paper describing an initial group of compounds [1]. Three derivatives (Chart 1) showed interesting activity profiles, encouraging us to develop new analogs (**3**–**15**, **18**, **19**, see Scheme 1) in which the variations concerned primarily the indolinone moiety.

Compounds **7**, **10** and **11** were prepared to evaluate the effect of the addition of a methyl group at the 6 position (**7**) or at the nitrogen (**10**, **11**), in order to compare their activity with those of NSC 736802 and NSC 134544. Moreover, considering that, as observed for compound NSC 736804, the presence a bulky substituent at the oxindole nitrogen is tolerated, substituted benzyl groups were introduced at the nitrogen (compounds **13**–**15**). This type of substitution gave good cytotoxicity results when performed in other indolinone derivatives that we had studied previously [2,3]. Meanwhile, studies examining introduction of new substituents, such as bromine, dimethylamino, or trifluoromethyl, and exploration of different positions for the oxindole ring were also undertaken (compounds **3**–**6**, **8** and **9**). Finally, we took into consideration the replacement of the methine bridge linking the oxindole and the 3,4,5-trimethoxybenzene with an imino group (compounds **18**, **19**).

The cytotoxic activities of all new compounds were evaluated according to Developmental Therapeutics Program (DTP), National Cancer Institute (NCI), Bethesda, MD, drug screen protocols. We

Abbreviations: DTP, Developmental Therapeutics Program; NCI, National Cancer Institute; CSA4, combretastatin A-4; Nox, NAD(P)H oxidase enzyme; ROS, reactive oxygen species; ECs, endothelial cells; NOE, nuclear Overhauser effect; DMSO, dimethylsulfoxide; GI, growth inhibition; TGI, total growth inhibition; LC, lethal concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPI, diphenyleneiodonium; DCFH-DA, 2′,7′-dichlorofluorescin-diacetate; DCF, 2′,7′-dichlorofluorescein; AML, human acute myeloid leukemia.

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http://dx.doi.org/10.1016/j.ejmech.2013.03.033

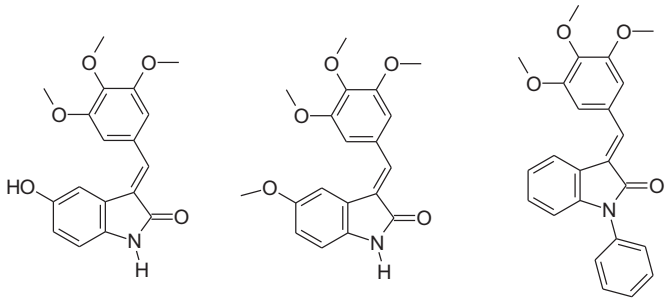


Chart 1. Most active derivatives described in Ref. [1].

also studied two possible mechanisms of action: inhibition of tubulin assembly and inhibition of NADPH oxidase 4 (Nox4).

It is well known that anticancer agents such as colchicine and combretastatin A-4 interfere with the dynamic equilibrium of microtubules by inhibition of tubulin polymerization. Moreover 3,4,5-trimethoxyphenylthioindoles, along with the corresponding ketone and methylene analogs, have been described as tubulin assembly inhibitors [4]. The structural analogy prompted us to test compounds **3**–**19** and derivatives reported in Chart 1 as inhibitors of tubulin polymerization.

Recently, the Nox4 inhibitory activity shown by some oxindole derivatives was described [5]. The chemical structure of the most active compound **9d** is shown in Chart 2. As in our 3-(3,4,5-trimethoxybenzylidene)-1,3-dihydroindol-2-ones, the indolinone moiety is linked to a substituted phenyl ring by means of a methine bridge. We therefore decided to determine whether Nox4 was a possible target for the compounds described here.

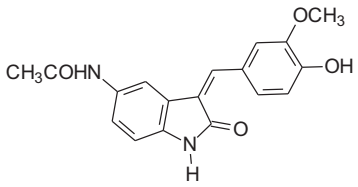
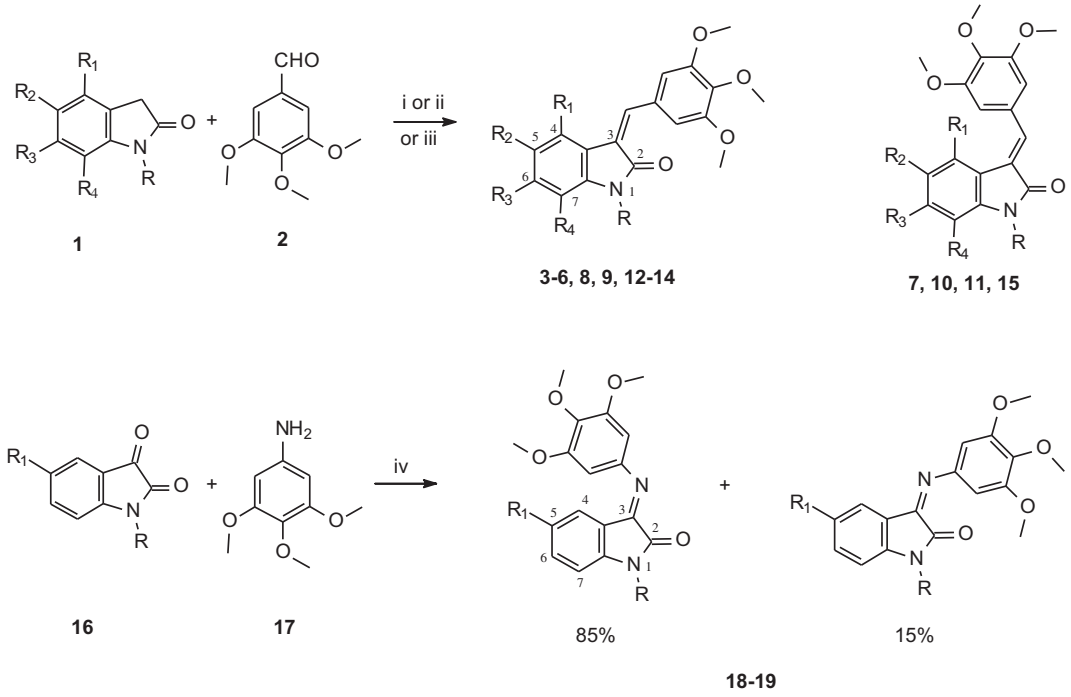


Chart 2. Potent Nox4 inhibitor described as **9d** in Ref. [5].

NAD(P)H oxidase enzymes (Nox) constitute a family of structural homologs of phagocytic Nox (Nox2 or gp91phox) and are one of the major sources of reactive oxygen species (ROS) in many cell types [6,7]. It is well known that ROS can act as messengers in cellular signaling transduction pathways and that an increase of ROS supports cellular growth and proliferation, contributing to cancer development [8]. In addition, accumulating evidence suggests that specific Nox isoforms, in particular Nox4, are associated with ROS production and proliferation of cancer cells. The ROS generated by this enzyme have been implicated in numerous biological functions, including signal transduction, cell differentiation and tumor cell growth. ROS produced by Nox4 in pancreatic cancer has been shown to transmit cell survival signals *via* the AKT-ASK1 pathway, while inhibition of Nox4 activates apoptosis [9]. It has been demonstrated that Nox4 overexpression plays an oncogenic role in breast tumorigenesis and that treatment of Nox4 overexpressing cells with catalase resulted in decreased tumorigenicity [10]. In glioblastoma multiforme, cycling hypoxia triggers ROS production *via* Nox4, and this is associated with increased tumor cell growth *in vitro* and in mouse xenografts. Inhibition of Nox4 with siRNA or antioxidant *in vitro* and Nox4 knockdown in mice were able to block cell growth or tumor progression [11]. Moreover, it has been shown that Nox4 derived ROS are required for



[a]Reagents and conditions: (i) methanol, piperidine; (ii) acetic acid, 37% HCl; (iii) anhydrous sodium acetate, acetic acid; (iv) ethanol.

Scheme 1. Synthesis of 3-(3,4,5-trimethoxybenzylidene)- and 3-[(3,4,5-trimethoxyphenyl)imino]-1,3-dihydroindol-2-ones derivatives.