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Research paper

Design, synthesis, and biological evaluation of (2E)-(2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene)acetate derivatives as anti-proliferative agents through ROS-induced cell apoptosis

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ABSTRACT

A novel class of (2E)-(2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene)acetate derivatives were designed and synthesized as potent anti-proliferative agents. Most of these compounds showed potent anti-proliferative activity against some tumor cell lines, including SK-BR-3, MDA-MB-231, HCT-116, SW480, Ovcar-3, HL-60, Saos-2 and HepG2. Compounds **8c** and **11h** were identified as the most potent ones, while HL-60, HCT116 and MDA-MB-231 were the most sensitive cell lines. Mechanistic study revealed that compound **8c** enhanced reactive oxygen species level by inhibiting TrxR and then induced apoptosis by activating apoptosis proteins, bax and cleaved-caspase 3 in HCT116 cells. Preliminary SAR analysis indicated that modifications of the double bond and ester group made great effects on the anti-proliferative activity. Our findings suggested that it was worth further studies on the antitumor potency of (2E)-(2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene)acetates.

1. Introduction

Compounds based on indolinone-fragment, which are widespread in natural products and synthetic small molecules, have a wide range of biological activities (Fig. 1). For example, Indirubin (**1**), the major active component of a traditional Chinese herbal medicine used as an anticancer agent [1,2], is an inhibitor of protein kinases such as cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3β (GSK-3β) [3,4]. Spirotryprostatin B (**2**), an indolic alkaloid found in the *Aspergillus fumigatus* fungus, is a novel mammalian cell cycle inhibitor in the G2/M phase [5,6]. Spiro-oxindole compound **3** is an inhibitor of p53-MDM2 interaction and prevents the ubiquitination and degradation of p53, thus promoting cancer cell apoptosis and preventing cell cycle progression through p53 as the key tumor suppressor [7–11].

Previous studies mainly focused on the 3-spiro-oxindole skeleton construction and related biological evaluation. However, the rarely studied 3-ylideneoxindole skeleton also showed great bioactivity. For example, Nintedanib (Ofev) (**4**), a kinases inhibitor [12–14], has launched for the treatment of idiopathic pulmonary fibrosis (IPF) and cancer. Oxindole compound **5** and related compounds also showed inhibitory effects on CDKs [15,16]. Recently, 3-ylideneoxindole acetamides (**6**) have been reported as antitumor agents, displaying a similar profile to that of roscovitine [17].

In this study, a new series of (2E)-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene)acetate derivatives (Chart 1) have been designed, synthesized and evaluated for their inhibitory activity against several cancer cell lines. Besides, we also attempted to delineate their mechanism of action. In the presence of compound **8c** with the best *in vitro* anti-proliferative activity, cell apoptosis and cell cycle distribution were investigated. The apoptosis proteins were also analyzed. We preliminarily proved that their anti-proliferative activity was attributed to the fact that the enhanced reactive oxygen species (ROS) level induced cell apoptosis by inhibiting thioredoxin reductase (TrxR). Moreover, structure-activity relationship (SAR) was also analyzed for further study of this series of compounds.

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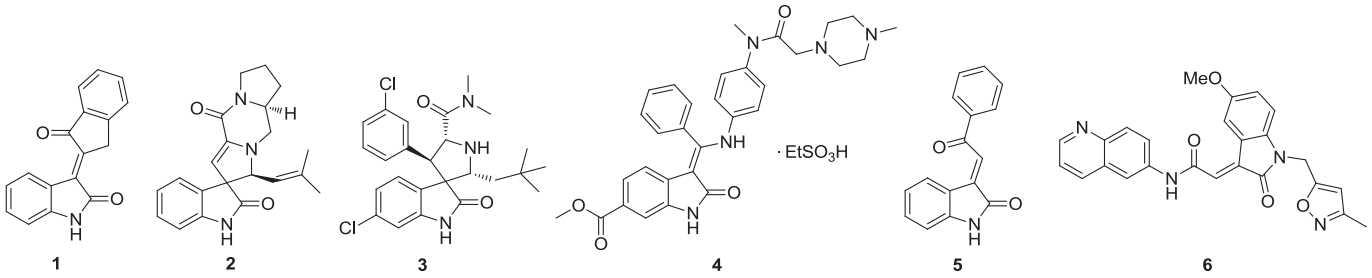


Fig. 1. Indolinone-fragment based compounds.

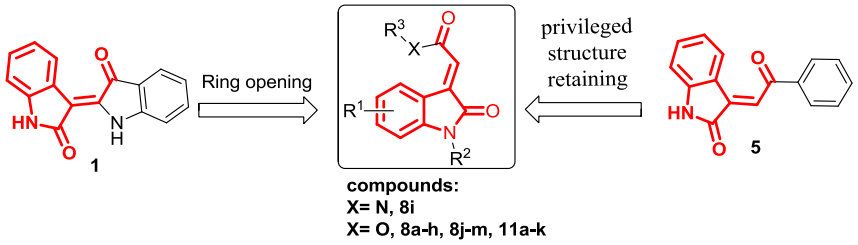
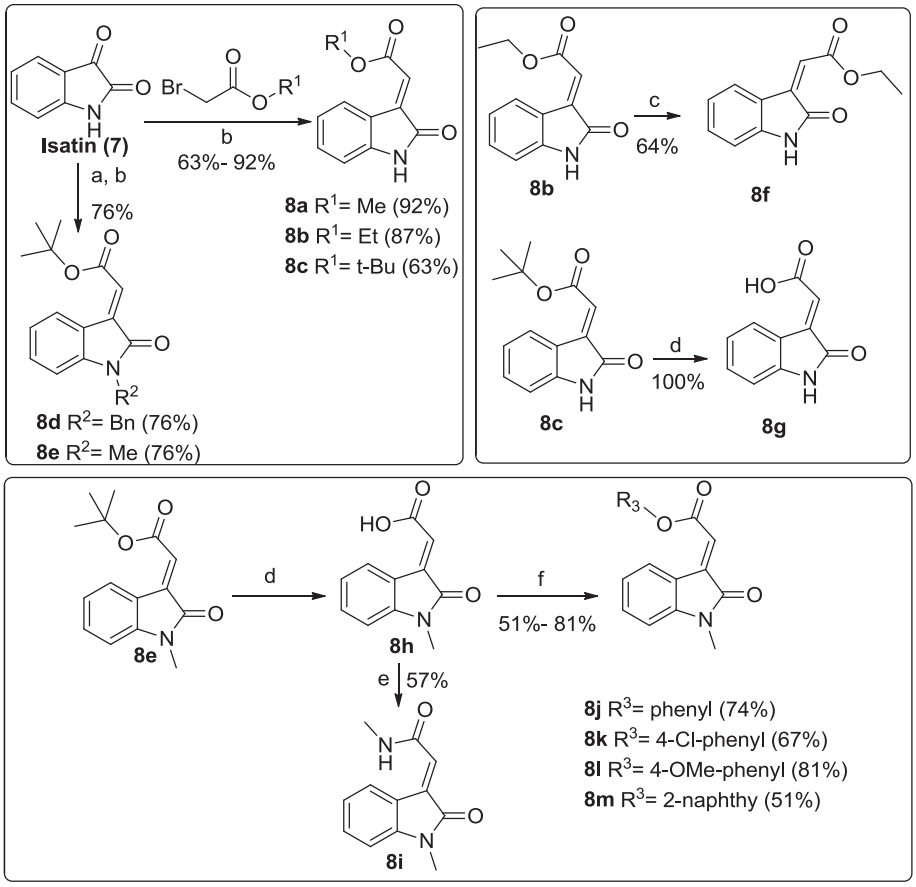


Chart 1. The SAR analysis of (2E)-(2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene)acetate derivatives.

2. Results and discussion

2.1. Synthesis of compounds *8a-m*, *11a-k* and *12a-d*

Compounds **8a-m**, **11a-k** and **12a-d** were prepared according to the procedure depicted in Schemes 1 and 2 [18,19]. Isatin (**7**) was reacted with three different substituted 2-bromoacetates to get the corresponding products **8a-c** through a rapid and efficient one-pot methodology [18], and the *trans*-type of **8c** was confirmed by ^{1}H NMR, ^{13}C NMR, ESI-MS and NOESY (see the Supplementary material). Firstly, compound **8c** was respectively alkylated at –NH by benzyl bromide and iodomethane to give the corresponding N-



Scheme 1. Synthesis of compounds **8a-m.** Reagents and conditions: (a) CH_3I, K_2CO_3, MeCN, 0°C- r.t. overnight or $PhCH_2Br$, NaH, THF, 0°C- r.t.; (b) Ph_3P (1.2eq), morpholine (1.2eq), 80 °C; (c) Butadiene sulfone (1.1eq), phenol (0.02eq), EtOH, 150 °C, 12 h; (d) CF_3COOH, CH_2Cl_2, r.t. 12 h; (e) EDC (1.2eq), HoBt (1.3eq), DIEA (3eq), CH_2Cl_2, r.t. 1 h; then methylamine hydrochloride (1.2eq), −2.5°C- r.t. overnight; (f) DCC (1.5eq), DMAP (1.3eq), substituted phenol (1.2eq), CH_2Cl_2, r.t. 1 h–10 h.