European Journal of Medicinal Chemistry 95 (2015) 400–415

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and pharmacological evaluation of novel bisindole derivatives bearing oximes moiety: Identification of novel proapoptotic agents

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ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form
24 March 2015
Accepted 25 March 2015
Available online 27 March 2015

Keywords:
Bisindole
Oxime
Apoptosis
Anti-cancer
Cell cycle arrest

ABSTRACT

In an effort to develop potent anti-cancer chemopreventive agents, a novel series of bisindole derivatives bearing oxime moiety were synthesized. Structures of all compounds were characterized by NMR and HRMS. Anti-proliferative activities for all of these compounds were investigated by the method of MTT assay on 7 human cancer lines and the normal cell lines (HUVEC). Most of them showed a noteworthy anti-cancer activity *in vitro*, the half maximal inhibitory concentration (IC_{50}) value is 4.31 μM of **4e** against T24. The results from Hoechst 33258 and acridine orange/propidium iodide staining as well as annexinV-FITC assays provided evidence for an apoptotic cell death. The further mechanisms of compound **4e**-induced apoptosis in T24 cells demonstrated that compound **4e** induced the productions of ROS, and altered anti- and pro-apoptotic proteins, leading to mitochondrial dysfunction and activations of caspase-9 and caspase-3 for causing cell apoptosis. Moreover, the cell cycle analysis and western-blot analysis indicated that compound **4e** effectively arrested T24 cells in G1 stage and possibly has an effect on cell cycle regulatory proteins particularly cyclin D1.

1. Introduction

Cancer is characterized by uncontrolled cell proliferation and has become the second cause of mortality in the world. Traditionally prescribed chemotherapeutic agents have serious problems with toxicity, instability and poor water solubility. Even more unfortunately, narrow therapeutic index of anticancer compounds and the problem of multidrug resistance [1] are some of the major hurdles in the successful practice of chemotherapy. Therefore, development of potent and specific anti-cancer agents is urgently needed.

Indole, the most pharmacodynamic nucleus in nature, has been a major constituent of number of clinically effective agents and incorporated in various naturally occurring indole alkaloids, such as indirubin and serotonin [2,3]. Indole derivatives, especially the 3-substituted indoles [9–13], have been found to possess a wide range of pharmaceutical properties, such as anti-cancer [4], antibacterial [5], antiviral [6], anti-topoisomerase II [7] and anti-inflammatory [8] activities. Additionally, biindole scaffolds are important motifs in many natural products and the medicinal chemistry world [14].

Modern studies had reviewed bis-indole (Fig. 1) alkaloids show significant bioactivities comparing to monoindole alkaloids [15,16]. Among them, several bis-indoles are in clinical trials, such as indirubin which have been approved as anticancer agent for children with acute promyelocytic leukemia. In addition, indirubin and meisoindigo have been identified as a potent inhibitor for cyclin-dependent kinases (CDKs) and GSK-3β [17,18]. However, the poor aqueous solubility and bioavailability preclude the extensive clinical application of indirubin and its derivatives. Recently, flexibility

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http://dx.doi.org/10.1016/j.ejmech.2015.03.058
0223-5234/

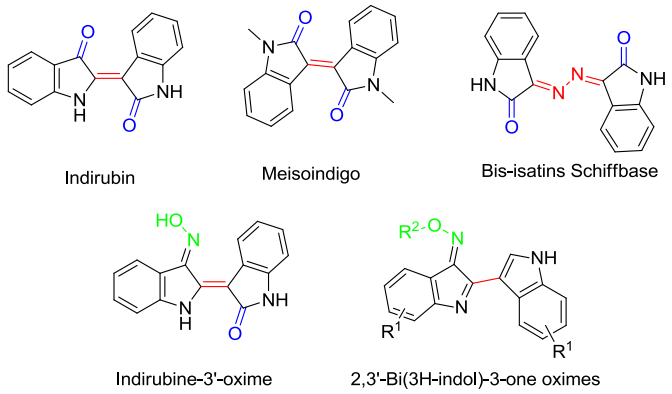

Fig. 1. A structural comparison of the designed 2, 3′-Bi (3H-indol)-3-one oximes with indirubin, indirubine-3′-oxime, bis-isatins schiffbase and meisoindigo.

and aqueous solubility bis-isatins schiffbase which was directly connected via a bis-Schiff base linker (Fig. 1) were reported [19]. In mechanism study, this bis-isatins schiffbase arrested the cell cycle at the G2/M phase in HepG2 cells by down-regulating the expression of cyclin B1 and CDC 2. Despite the fact that the bis-isatins are well studied compounds, new bis-indole derivatives are continually being discovered in terms of their biological activity.

In our previous works [20], we reported the synthesis of bis-indole which directly connected via a single bond by an efficient one-pot procedure. The results from the MTT assay for these compounds showed a noteworthy anti-cancer activity *in vitro*. Meanwhile, the introduction of an oxime function into an appropriate skeleton is a reasonable approach to the preparation of potent cytotoxic agents and many oxime derivatives have exhibited potent inhibition activities against human tumors [21,22]. In continuation of our search for pharmacologically active bis-indole derivatives, it seemed to be reasonable to prepare and evaluate 2, 3′-Bi (3H-indol)-3-one oximes (Fig. 1) for their biological properties.

It must be noted that a multitude of study was focused on the double bond coplanar skeleton. However, pharmacological activities of the single bond connecting bisindole were not reported. More over, in most previous studies, the oxime-containing bis-indole derivatives have not been thoroughly explored for their regulation of cellular signaling apoptosis inducing and growth arrest effects. In this paper, we further conducted the synthesis and antiproliferative evaluation of the oxime-containing bis-indole derivatives. Furthermore, the mechanism of apoptotic effects induced by the representative compound **4e** was also investigated.

2. Results and discussion

2.1. Chemistry

2.1.1. General procedure for the preparation of compounds

To a solution of indole (**1**, 0.50 mmol) and $NaNO_2$ (0.60 mmol) in CH_3CN (1 mL) was added $FeCl_3{\cdot}6H_2O$ (0.125 mmol) under atmosphere and the mixture was stirred at room temperature for 15–72 h (monitored by TLC). The reaction mitxure was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (eluant: EtOAc/PE = 1:1) to yield the corresponding product **4** (Scheme 1). To a solution of indole (**1**, 0.50 mmol), alkyl bromide (0.7 mmol) and $NaNO_2$ (1.0 mmol) in DMF (1 mL) was added $RuCl_3{\cdot}3H_2O$ (0.075 mmol) under atmosphere and the mixture was stirred at 40 °C for 8–26 h (monitored by TLC). The reaction mixture was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (eluant: EtOAc/PE = 1:4) to yield the corresponding product **5** and **6** (Scheme 1). All the target compounds were fully characterized by ^1H NMR, ^{13}C NMR and HRMS.

2.2. Biological evaluation

2.2.1. Cytotoxicity test

The inhibitory effect of these object compounds was evaluated by MTT assay against A549, MGC-803, HepG2, T24, SPCA-2, NCI-H460, SK-OV-3, HUVEC normal cells line, and compared the data with 2,3′-Bi(3*H*-indol)-3-one oxime (**4a**) and the positive control 5-fluorouracil (5-FU). The results were shown in Table 1 and Table 1S (Supplementary).

In all human cancer cell lines, almost all of the compounds showed an increased cytotoxic activity as compared to **4a**, and most of them even showed preferable cytotoxic activities than 5-FU. Compounds **4e**, **4i** and **5j** showed good inhibitory activity for all the tested carcinoma cell lines. Compound **4c** showed strong inhibitory activity selectivity for SK-OV-3 as well as HepG2, **4l** selectivity for SPCA-2 and **5g** selectivity for SK-OV-3. The others also showed good inhibitory except **4n** for SK-OV-3, **4o** for A549, NCI-H460 and SPCA-2, **6c** for NCI-H460 and SK-OV-3, **6d** for NCI-H460. In particular, the 5, 5′-Dimethoxy-2, 3′-bi (3*H*-indol)-3-one oxime (**4e)** showed a noteworthy anti-cancer activity *in vitro*, IC_{50} value is 4.31 μM of **4e** against T24.

The results from the MTT assay for compounds **4**, **5** and **6** using the human bladder carcinoma cell line T24 were presented in more detail in Fig. 2. The results revealed that the analogs **4d**, **4n** and **4o**, obtained by inserting the methyl moiety in the positions 5 of indole showed quite different antiproliferative potencies: compound **4d**, with IC_{50} value of 8.78 μM, was about 3-fold more active than derivatives **4n** and **4o**, and undoubtedly emerged as one of the most active compounds within this subset, thus suggesting that the C-5 insertion position plays a pivotal role. A beneficial effect was also observed with the modifications of the C-5 position: the derivative **4e** was 4-fold more active than **4j**. The presence of a weakly electron-withdrawing in position C_5 seemed to be associated with a general increase in activity. Moreover, the modifications of oxime moiety caused a loss of potency: compounds **4** showed an even better cytotoxicity. In addition, the inhibition activities of compounds **4**, **5** and **6** against HUVEC normal cell lines were also estimated in Table 1S (Supplementary). The results indicated that the cytotoxicity of most of compounds against cancer cells was much higher than HUVEC normal cells, making them good candidates as anti-cancer drugs. In summary, these modifications yield some small increase in cytotoxicity and a low but significant improvement for the selectivity of the compounds.

2.2.2. Induction of apoptosis

Apoptosis is characterized as programmed cell death which leads to characteristic changes including blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Inhibition of apoptosis is considered an essential step in tumorigenesis and is one of the hallmarks of cancer, allowing the survival of cells that accumulate oncogenic events that otherwise would have been removed by apoptosis [23]. It is therefore necessary to consider cell apoptosis as another effective approach in cancer treatment. Thus, in order to address the cell death caused by compounds **4e**, the mechanism of growth inhibition of T24 cell lines was evaluated.

The morphological character changing of T24 cells were investigated under fluorescence microscopy using acridine orange (AO)/ethidium bromide (EB), Hoechst 33258, JC-1 mitochondrial membrane potential staining to evaluate whether the growth inhibitory activity of the selected compound was related to the induction of