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Benzochalcones bearing pyrazoline moieties show anti-colorectal cancer activities and selective inhibitory effects on aurora kinases

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ABSTRACT

Colorectal cancer is the third and fourth leading cause of cancer in males and females, respectively. Flavonoids, including chalcones, are secondary metabolites in plants that exhibit diverse biological activities, including antibacterial, antimalarial, and antitumor activities. In order to find potent and novel chemotherapy drugs for colorectal cancer, a series of benzochalcone derivatives, in which an α,β-unsaturated carbonyl group was replaced with a pyrazoline, was designed and synthesized. A clonogenic survival assay was performed with each derivative to evaluate antitumor activity. 1-(5-(2,4-Dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol (derivative **7**) had the most potent inhibitory effect on the long-term clonogenicity of HCT116 human colorectal cancer cells (IC_{50} = 2.4 μM). The results of Western blot and flow cytometric analyses suggested that derivative **7** could inhibit the proliferation of colorectal cancer cells through inhibition of cell cycle progression and induction of apoptosis. To elucidate its molecular mechanism, in vitro kinase binding assays were carried out, which demonstrated that derivative **7** inhibited aurora kinases A and B selectively. The binding modes between the compound and aurora kinases were interpreted using in silico docking experiments to explain the selective inhibitory effects on aurora kinases A and B. These findings will facilitate the design of potent novel benzochalcones as anticancer agents.

1. Introduction

The colon and rectum together form the large intestine. Colorectal cancer refers to metastatic cancer of the colon and rectum. A complete cure is difficult to achieve. Colorectal cancer is the third leading cause of cancer in males and the fourth leading cause in females, but its frequency varies around the world. Western diets tend to increase the incidence of colorectal cancer.[1] 5-Fluorouracil (5-FU) has been used to treat colorectal cancer for many years. It is typically used in combination with leucovorin to increase its effectiveness.[2] A pill form of 5-FU, Xeloda, is used to treat colorectal cancer that has spread to other organs.[3] Several new chemotherapy drugs, such as Camptosar, Eloxatin, Avastin, Erbitux, and Vectibix, have been developed and used for the treatment of colorectal cancer, but they affect the healthy cells as well as the target cancer cells.[4] Therefore, chemotherapy drugs for colorectal cancer are continually under development.

Chalcones, secondary metabolites in plants, have been reported to enhance tumor necrosis factor alpha (TNFα)-induced inhibition of NF-κB on HCT116 human colorectal cancer cells.[5] Compounds containing pyrazoline moieties are known to show activities against colon cancer.[6] In addition, 7,8-benzoflavone and 5,6-benzoflavone exhibited inhibitory effects on breast cancer resistance protein and on chemically induced mammary carcinogenesis, respectively.[7–9] Hence, we designed benzochalcones bearing pyrazoline moieties and synthesized 17 derivatives.[10]

Their inhibitory effects on HCT116 colorectal cancer cell lines were assessed using a long-term clonogenic survival assay. The relationships between their structures and inhibitory activities were elucidated and the structural conditions that result in good activities were derived. One of the benzochalcones, derivative **7**, exhibited a good half-maximal inhibitory concentration (IC_{50}), and so was subjected to further biological experiments. Because such experiments demonstrated that it induces apoptosis, its molecular mechanism was elucidated using in vitro kinase binding assays. Derivative **7** inhibited aurora kinases A and B selectively. To explain this selectivity, the binding modes of derivative **7** and aurora kinases were interpreted using in silico docking experiments.

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These findings will facilitate the design of potent novel benzochalcone anticancer agents.

2. Results and discussion

2.1. Biological activities

Seventeen benzochalcone derivatives bearing the pyrazoline moiety in place of the native α,β-unsaturated carbonyl group were synthesized and can be grouped as either 1-(1-(4-chlorophenyl)-5-methoxyphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ols (**1**–**4**, Fig. 1A), 1-(5-methoxyphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ols (**5**–**8**, Fig. 1B), or 2-(5-methoxyphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ols (**9**–**17**, Fig. 1C). The names of the 17 derivatives are listed in Table 1. Measuring cell viability in response to anticancer agents is widely used for screening biological activity. The most common methods for measuring short-term losses in cell viability, such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, may not be able to adequately express the activities of the aforementioned derivatives. Therefore, clonogenic cell survival assays (CSAs), which detect long-term cytostatic effects caused by anticancer agents, were adapted for measurement of the biological activities of the 17 derivatives.[11] CSAs can be used as dose-dependent indices of cell viability. In addition, CSAs are considered standards for measuring long-term cell viability because they reflect all modes of cell death or arrest.[12] Compounds exhibiting perfect inhibition in the clonogenic assay would yield zero cell viability.

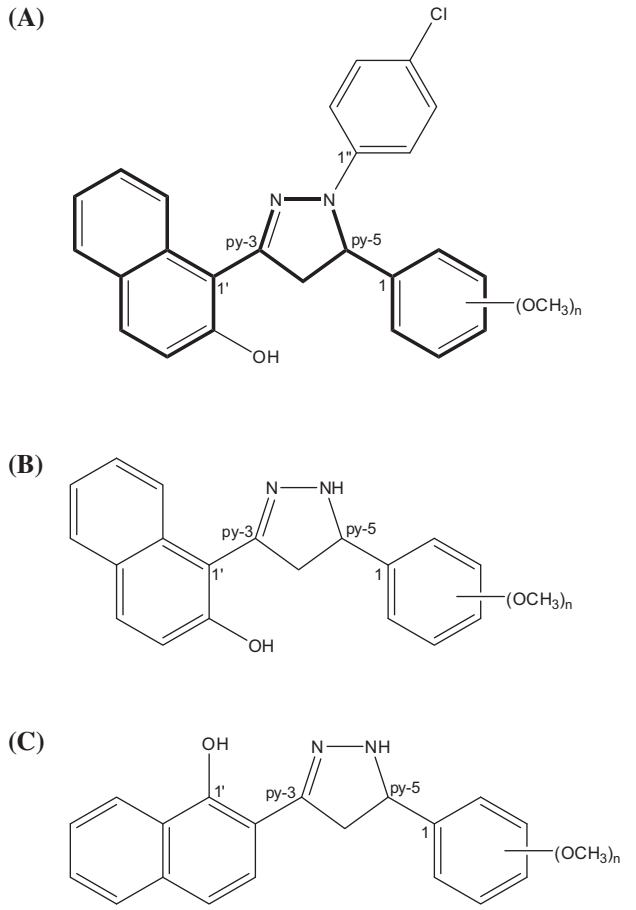

Figure 1. Structures and numbering of (A) 1-(1-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol where benzochalcone bearing pyrazoline moiety is marked in bold lines, (B) 1-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol, and (C) 2-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ol.

The half-maximal inhibitory concentrations (IC_{50}) of the 17 derivatives at four concentrations (0, 10, 20, and 40 μM) were determined (Table 1). Among the 17 benzochalcones tested, 1-(5-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ol (**7**) and 2-(5-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ol (**17**) showed the greatest and least inhibitory effects, with IC_{50} values of 2.4 and 32.5 μM, respectively (Fig. 2).

The only structural difference between the first group of derivatives, **1**–**4**, and the second group, **5**–**8**, is the presence or absence of a chlorophenyl group, respectively. Derivatives **1** and **5** bear the same substituent (the 4-methoxy group), and derivatives **2** and **7** both bear a 2,4-dimethoxy group. The IC_{50} values measured by clonogenic survival assay of derivatives **1** and **5** were 9.8 and 7.8 μM, respectively. Likewise, the IC_{50} values in response to derivatives **2** and **7** were 5.4 and 2.4 μM, respectively. In addition, while the average IC_{50} value of the first group was 7.38 μM, that of the second group was 5.55 μM. Therefore, the presence of the chlorophenyl group decreases the inhibitory effect. Likewise, the IC_{50} values of derivatives **6** (6.3 μM) and **7** (2.4 μM) in the second group were lower than those of **9** (15.2 μM) and **13** (8.8 μM) in the third group. The average IC_{50} values of the second and third groups were 5.55 and 14.77 μM, respectively. Regarding the IC_{50} values of derivatives **3** (6.5 μM) and **15** (11.3 μM), and **4** (7.8 μM) and **16** (9.0 μM), derivatives **3** and **4** (with a chlorophenyl group) exhibit lower IC_{50} values than derivatives **15** and **16** (without a chlorophenyl group). Therefore, the 1-pyrazolylnaphthalen-2-ol moiety yielded a greater inhibitory effect than did the 2-pyrazolylnaphthalen-1-ol moiety, and with regard to clonogenicity, the relative positions of the hydroxy and pyrazoline groups are more important than the presence of the chlorophenyl group.

To further examine the antitumor activity of derivative **7**, which showed the greatest inhibitory effect (IC_{50} = 2.4 μM) among the 17 benzochalcones tested, exponentially growing HCT116 cells were exposed to different concentrations of derivative **7** from 0 to 40 μM for durations of 24–48 h. Treatment with derivative **7** resulted in inhibition of the proliferation rate in a dose- and time-dependent fashion (Fig. 3). Many anticancer agents inhibit proliferation of tumor cells through the suppression of cell cycle progression. The cell cycle is tightly regulated by the timing of the expression of cell cycle-specific proteins such as cyclins. To determine the molecular mechanism by which derivative **7** inhibits cell proliferation, we examined its effect on the expression of cell cycle regulatory proteins in HCT116 cells. Western blotting showed that the level of p21, an endogenous inhibitor of cyclin-dependent protein kinase (CDK), increased gradually in a time-dependent manner, whereas cyclin B1 levels decreased rapidly after 24 h of treatment (Fig. 4). The level of cyclin D1 increased slightly within 12 h but then slowly returned to the level of the control. The level of cyclin A1 changed only slightly. These data suggest that derivative **7** has an inhibitory effect on the cell cycle progression through, at least in part, alteration of p21 and cyclin B1 expression. To corroborate this effect, HCT116 cell cycle profiles were measured following treatment with 40 μM derivative **7**. Flow cytometric analyses showed that the population of G1 cells increased slowly with concomitant decreases in the percentages of both S and G2/M phase cells (Fig. 5). After 48 h, apoptotic sub-G1 cells represented 21.3% of the total cells. Collectively, these data suggest that inhibition of clonogenicity by derivative **7** was due to dysregulation of cell cycle progression and induction of apoptosis.

2.2. Molecular binding modes based on in vitro kinase assays and in silico docking

To elucidate the mechanism underlying the above results at the molecular level, in vitro kinase assays were carried out using