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Original article

Synthesis and structure-activity relationship study of 8-hydroxyquinoline-derived Mannich bases as anticancer agents

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ABSTRACT

To continue our early study on the structural modifications of clioquinol, more 8-hydroxyquinoline-derived Mannich bases were synthesized and examined for growth-inhibitory effect. Taken Mannich base **1** as our lead compound, upon replacement of either sulfonyl group with methylene group or piperazine ring with ethylenediamine group resulted in an appreciable increase in potency. On the other hand, as 8-hydroxyquinoline was replaced with phenol, 3-hydroxypyridine and 1-naphthol, a dramatic decrease in activity was observed, indicating that 8-hydroxyquinoline is a crucial scaffold for activity. Further 3D-QSAR analysis on HeLa cells revealed that both steric and electronic effects contributed equally to growth inhibition. Taken together, the structure-activity relationships obtained from both *in vitro* data and CoMFA model warrant a valuable reference for further study.

1. Introduction

In addition to applications in the design of chemosensors and optical devices [1–4], 8-hydroxyquinoline has been synthesized with a variety of biological activities, such as inhibitors of catechol *O*-methyltransferase [5], inhibitors of HIF-1α prolyl hydroxylase [6], inhibitors of HIV-1 integrase [7], antibacterial [8,9], antimalarial [10], and antitumor agents [11–13]. Similar to carbonyl compounds with keto-enol tautomerism that enables a multiple-component Mannich reaction, 8-hydroxyquinoline can be carried out to generate the corresponding Mannich bases [10,14,15]. As a consequence, several Mannich bases of 8-hydroxyquinoline derivatives have been synthesized to show several biological activities [16,17].

On the other hand, clioquinol (5-chloro-7-iodo-8-hydoxyquinoline, Fig. 1) was clinically used as an antibiotic for the treatment of diarrhea and skin infection. Recently, clioquinol has demonstrated to exhibit anti-Alzheimer's disease in a mouse model via the reduction or prevention of amyloid plaque accumulation in the brain [18,19]. Apart from its antibiotic and anti-Alzheimer's disease efficacies, clioquinol also showed a moderate antiproliferative effect on cancer cells. The mechanistic study revealed that clioquinol-induced antiproliferative effect is attributed to caspase-dependent apoptotic pathway. Moreover, antiproliferative effect mediated by clioquinol could be enhanced in the presence of metal ions thanks to its metal-binding property [20,21].

Our early study showed that upon the elongation of 8-hydroxyquinoline appended by an arylsulfonylpiperazine moiety (**1** and **25**, Fig. 1) through Mannich-type reaction resulted in dramatically improved growth-inhibitory effect. We further demonstrated that growth inhibition induced by 8-hydroxyquinoline-derived Mannich bases was attributed to caspase-dependent apoptotic pathway and generation of oxygen reactive species. The synergistic effect of growth inhibition mediated by Mannich bases was also observed in the presence of copper ion, which was in accordance with that of clioquinol [22]. Herein, we would like to report the structural modifications of 8-hydroxyquinoline-derived Mannich bases with aim to examine their growth-inhibitory effect. Moreover, 3D-QSAR analysis employed by CoMFA model on HeLa cell line will be presented in this paper as well.

2. Chemistry

As shown in Fig. 2, a series of 8-hydroxyquinoline-derived Mannich bases were prepared in a systematic manner. In Route A,

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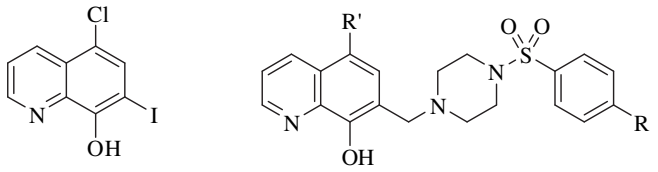

Fig. 1. Chemical structures of clioquinol and its Mannich bases **1** and **25**.

we replaced a variety of substituents on benzene ring to obtain **2**–**17** (Scheme 1, Fig. 3). In Route B, the sulfonyl group was replaced by either a carbonyl group or a methylene group to generate **18** and **19**, respectively (Scheme 2, Fig. 3). In Route C, the piperazine moiety in **1** was replaced by an ethylenediamine linker to synthesize **20** (Scheme 3, Fig. 3). In Route D, Mannich-type reaction of hydroxyarenes such as phenol, 3-hydroxypyridine, 1-naphthol and 5-subsituted 8-hydroxyquinoline were utilized to afford **21**–**27** (Scheme 4, Fig. 3, Table 1). Accordingly, a mixture of hydroxyarene, along with formaldehyde and amine was stirred in ethanol at reflux for 16–22 h that successfully prepared the corresponding Mannich bases [10,14,15,22].

3. Results and discussion

All tested compounds were screened on a panel of human carcinoma cell lines for growth-inhibitory activities, including HeLa (cervical epithelioid carcinoma cell), BT483 (mammary gland adenocarcinoma cell), SKHep (hepatocellular carcinoma cell) and CE81T (esophageal carcinoma cell). The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay [23] was employed for the growth inhibition studies and the GI_{50} values are summarized in Tables 2 and 3. The compound concentration causing a 50% cell growth inhibition (GI_{50}) was determined by interpolation from dose-response curves.

As compared to **1** (phenyl, GI_{50}, 6.8 μM), **5** (4-methylphenyl, GI_{50}, 2.5 μM), **6** (4-isoprpylphenyl, GI_{50}, 6.1 μM), **7** (4-*tert*-butylphenyl, GI_{50}, 4.7 μM) and **8** (4-biphenyl, GI_{50}, 4.5 μM), both **16** and **17** bearing 1-naphthyl and 2-naphthyl moieties exhibited higher growth-inhibitory effect against HeLa cells with GI_{50} values of 1.8 and 1.7 μM, respectively. These results indicate a certain degree of planar geometry in aromatic system connected to the sulfonyl group is favorable for growth-inhibitory activity. On the other hand, data showed that either electron-donating substituents such as **5** (4-CH_3, GI_{50}, 2.5 μM) and **10** (3-OMe, GI_{50}, 2.4 μM) or electron-withdrawing substituents like **9** (4-CF_3, GI_{50}, 2.2 μM) and **15** (4-NO_2, GI_{50}, 2.3 μM) revealed better activities than the counterpart **1** (GI_{50}, 6.8 μM) against HeLa cells. Interestingly, **10** (GI_{50}, 2.4 μM) bearing 3-methoxy group exhibited higher activity than the counterparts **11** (4-OMe, GI_{50}, 5.0 μM), **12** (3, 4-*di*-OMe, GI_{50}, 5.0 μM) and **13** (4-OCF_3, GI_{50}, 4.3 μM) against HeLa cells. Likewise, the abovementioned structure-activity relationship was also found in both BT483 and SKHep cells. As shown, **10** (3-OMe, GI_{50}, 4.1 μM) showed 3-fold more potent than **12** (GI_{50}, 17.4 μM) against SKHep cells. In addition, the growth inhibition of *mono*-substituted benzene ring in Mannich bases such as **10** (3-OMe, GI_{50}, 7.2 μM) and **11** (4-OMe, GI_{50}, 5.2 μM) revealed higher activity than the *di*-substituted counterpart **12** (3, 4-*di*-OMe, GI_{50}, 12.1 μM) against CE81T cells. These findings suggest a distinct steric effect stemmed from the substituted benzene ring that counts for the growth-inhibitory activity. Nevertheless, the electron-withdrawing substituent such as **14** and **15** bearing a nitro group on the benzene ring exhibited a comparable growth-inhibitory activity against all four cell lines. On the other hand, only SKHep showed a higher sensitivity in response to halogen-substituted Mannich bases **2** (4-F, GI_{50}, 4.8 μM), **3** (4-Cl, GI_{50}, 5.0 μM) and **4** (4-Br, GI_{50}, 12.7 μM) as compared to their counterpart **1** (GI_{50}, 14.6 μM). Among analogs modified in Route A, **13** (4-OCF_3) exhibited the most potent growth-inhibitory activity with a GI_{50} value of 2.9 μM against CE81T cell line.

As shown in Scheme 2, upon replacement of the sulfonyl group in **1** with a carbonyl group (**18**) and a methylene group (**19**) resulted in an interesting structure-activity correlation. For example, **19** showed 2- to 10-fold more potent than the counterparts **1** and **18** against four carcinoma cells (Table 3), suggesting the flexibility originated from the rotational methylene group plays a significant role for activity. In addition, **19** exhibited higher potency with a GI_{50} value of 2.6 μM against SKHep cells while both **1** and **18** merely showed moderate activities with GI_{50} values of 14.6 and 26.6 μM, respectively. Unlike **19** bearing a methylene group for free rotation, both **1** (sulfonyl group) and **18** (carbonyl group) are devoid of the rotation capability for activity. Interestingly, as the piperazine ring in **1** was replaced with an ethylenediamine group to generate **20**, an improved growth-inhibitory effect was also observed. Together, these results indicate that the flexible fragments in both **19** and **20**

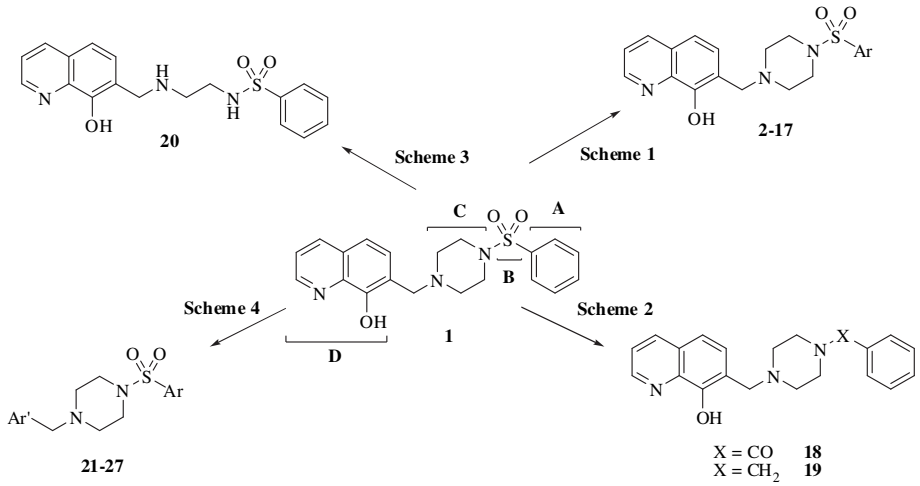

Fig. 2. Overall synthetic routes of structure-activity relationship study of 8-hydroxyquinoline-derived Mannich bases.