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Research paper

A new series of 2-phenol-4-aryl-6-chlorophenyl pyridine derivatives as dual topoisomerase I/II inhibitors: Synthesis, biological evaluation and 3D-QSAR study

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ABSTRACT

As a continuous effort to develop novel antitumor agents, a new series of forty-five 2-phenol-4-aryl-6-chlorophenyl pyridine compounds were synthesized and evaluated for cytotoxicity against four different human cancer cell lines (DU145, HCT15, T47D, and HeLa), and topoisomerase I and II inhibitory activity. Several compounds (**10**–**15**, **20, 22, 24, 28, 42,** and **49**) displayed strong to moderate dual topoisomerase I and II inhibitory activity at 100 μM. It was observed that hydroxyl and chlorine moiety at *meta* or *para* position of phenyl ring is favorable for dual topoisomerase inhibitory activity and cytotoxicity. Most of the compounds displayed stronger cytotoxicities than those of all positive controls against the HCT15 and T47D cell lines. For investigation of the structure-activity relationships, a 3D-QSAR analysis using the method of comparative molecular field analysis (CoMFA) was performed. The generated 3D contour maps can be used for further rational design of novel terpyridine derivatives as highly selective and potent cytotoxic agents.

1. Introduction

For the past several years, our research group has been seeking to identify and optimize low molecular weight molecules capable of inhibiting dual topoisomerase I and II activity and cancer cell proliferation. A series of mono, di and tri-hydroxylated 2,4,6-triphenyl pyridine derivatives have recently been reported with significant topoisomerase inhibitory activity and cytotoxicity against several human cancer cell lines [1,2]. The importance of hydroxyl moieties has also been reported in natural polyphenolic compounds such as resveratrol, curcumin, and epigallocatechin for several biological activities including cytotoxicity and dual topoisomerase I and II inhibition [3–6]. Similarly, formation of halogen bond with protein plays important role to increase the protein-ligand binding affinity, and therefore insertion of halogen atoms is widely practiced in drug design and discovery area to improve potency, blood brain barrier permeability, and metabolic and chemical stability. Moreover, for the lead optimization of drug, many researchers in medicinal chemistry are attracted to develop chlorinated compounds as chlorine can accommodate in tight and deep cavities, as well as in hydrophobic pockets of the biological targets that contribute to the stability and specificity of the binding site [7,8].

Human DNA topoisomerases (topo I and topo II) are expressed at different level in different cancer types [9–12]. For instance, topo I is overexpressed in colon cancer cell lines, while topo II is overexpressed in breast and ovarian cancer cell lines [11,13]. Camptothecin and etoposide, topo I and topo II inhibitor, respectively, are clinically approved anticancer drugs [14,15]. Recent studies have reported that use of single topo I enzyme inhibitor can develop its resistance by inducing concomitant increase in expression level of

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topo II enzyme, and vice versa [16,17]. Though this concept is still not very clear, some new class of compounds exhibited strong dual inhibitory activity in preclinical studies [18]. Furthermore treatment of dual topo I and II inhibitors may exhibit synergistic effect to result in better efficacy, lower side effects and lower resistance. Therefore, dual inhibition of topo I and II, which are involved in solving the topological problems associated with nuclear processes, may be a promising strategy for the design of anticancer agents [19,20]. Previously reported 2,4,6-triaryl pyridines containing chlorophenyl and phenolic moiety at 2- and 4-position of central pyridine, respectively, have shown dual topo I and II inhibitory activity [21]. In addition, several reports on importance of *meta* or *para* hydroxyl groups on 2-phenyl ring and thienyl or furyl moiety on 4-position of central pyridine [22,23] motivated us to synthesize a new series of 2-phenol-4-aryl-6-chlorophenyl pyridines as potential antitumor agents.

The aim of the present work was to incorporate the chlorine moiety at 6-phenyl position and aryl group at 4-position of central pyridine and to examine their biological effects. Herein, we report the synthesis and three-dimensional quantitative structure-activity relationship (3D-QSAR) study using the method of comparative molecular field analysis (CoMFA) for a series of novel 2-phenol-4-aryl-6-chlorophenyl pyridines which inhibit both topo I and II activity and show significant cytotoxicity. The substitution of hydroxyl and chlorine group allows us to investigate their combined effect on cytotoxicity and dual topo inhibition with respect to the position (*ortho*, *meta*, *para*) on phenyl ring.

2. Design and synthesis

Previously, we reported 2-phenol-4,6-diphenyl pyridines, **A, B** and **C** (Fig. 1) for the topo inhibitory activity and cytotoxicity against several human cancer cell lines [1]. We were interested to observe the combined effect on biological results by incorporating hydroxyphenyl moiety on 2-positon, phenyl, thienyl or furyl moieties on 4-position and chlorophenyl moiety on 6-positon. Design of 2-phenol-4-aryl-6-chlorophenyl pyridines allowed us to investigate whether the change in the aryl moiety at 4-position and chlorophenyl moiety at 6-position of central pyridine has any effect on the dual topo I and II inhibitory activity and cytotoxicity. Subsequently, the *ortho*, *meta* and *para* substitution of chlorine moiety will help us to further confirm the effect of chlorine position on biological activity with respect to the previous data. Total forty-five 2-phenol-4-aryl-6-chlorophenyl pyridine compounds (**7**–**51**) were systematically designed in five different series as shown in Fig. 2. All the designed compounds contain one hydroxyl and one chlorine moiety at *ortho*, *meta* or *para* position of 2- and 6- phenyl ring, respectively, attached to the central pyridine. The 4-position of central pyridine was substituted with various aryl groups (phenyl, 2/3-thienyl, 2/3-furyl).

The synthetic route with three different steps for the target compounds **7**–**51** is outlined in Scheme 1. In the first step, fifteen different hydroxylated chalcones were synthesized as intermediates using *Claisen-Schmidt* condensation reaction [24]. In this method, 4 M aqueous solution of NaOH was added to the solution of equimolar amounts of aryl ketone **1** (R^1 = a-c) and aryl aldehyde **2** (R^2 = d-h) in ethanol to obtain compounds **3** (R^1 = a-c, R^2 = d-h) in 47–98% yield. In the second step, three pyridinium iodide salts **5** (R^3 = i-k) were synthesized in a quantitative yield by the treatment of aryl ketone **4** (R^3 = i-k) with iodine in pyridine. Finally using previously reported synthetic methods [25–29], final compounds (**7**–**51**) were synthesized by the reaction of appropriate hydroxylated chalcones **3** (R^1 = a-c, R^2 = d-h) with pyridinium iodide salt **5** (R^3 = i-k) in the presence of ammonium acetate and glacial acetic acid in 36–95% yield (Fig. 3). The yields (%), purities (%) measured by HPLC, retention time, and melting points of the synthesized compounds are listed in Table S1 (Supplementary Data).

3. Results and discussion

All the synthesized 2-phenol-4-aryl-6-chlorophenyl pyridine compounds (**7**–**51**) were evaluated for topo I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. The conversion of supercoiled plasmid DNA to relaxed DNA by topo I and II was examined in the presence of synthesized 2-phenol-4-aryl-6-chlorophenyl pyridine compounds (**7**–**51**). Camptothecin and etoposide, well known topo I and II inhibitors, respectively, were used as positive controls. Figs. 4 and 5 and Table 1 illustrate the topo I and II inhibitory activity and cytotoxicity of synthesized compounds **7**–**51**.

3.1. In vitro cytotoxicity of the compounds

We performed cytotoxicity assay using four different human cancer cell lines: human prostate tumor cell line (DU145), human colorectal adenocarcinoma cell line (HCT15), human ductal breast epithelial tumor cell line (T47D), and human cervix tumor cell line (HeLa). Adriamycin, etoposide (topo II inhibitor) and camptothecin (topo I inhibitor), widely used anticancer drugs, were used as positive controls. The inhibitory activity (IC_{50}) is expressed as micromolar concentration as illustrated in Table 1. Several compounds displayed significant cytotoxicity against the tested cell lines at low micromolar concentration compared to the positive controls.

Compounds **10**–**15** (from Series A) containing phenyl moiety at 4-position of central pyridine showed significant cytotoxicity (<2 μM) against the cell lines DU145, HCT15 and T47D, and considerable cytotoxicity (<8 μM) against HeLa. Compound **14** possessed stronger cytotoxicity (0.83 μM) than that of all the positive controls against HCT15. Compound **7**–**9** belonging to the same series exhibited moderate to weak cytotoxicity against the tested cell lines. Similar type of cytotoxicity pattern was observed with compounds containing 2-thienyl moiety at 4-position of central pyridine (**16**–**24**, Series B). Compounds **19**–**24** exhibited significant cytotoxicity (<2 μM) against the cell lines DU145, HCT15 and T47D.

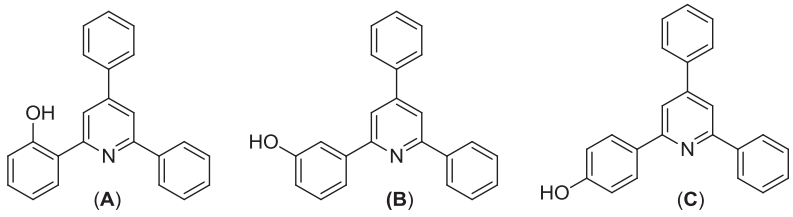

Fig. 1. Structures of previously synthesized 2,4,6-trisubstituted pyridines.