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Design, synthesis, biological evaluation, structure-activity relationship study, and mode of action of 2-phenol-4,6-dichlorophenyl-pyridines

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ABSTRACT

Human DNA topoisomerases (Topos) are essential nuclear enzyme whose level of expression is potential indicator for prediction of responsive result of chemotherapy. Topos has become a key cellular target for most of the anticancer agents that regulates topological problems of DNA during cellular metabolic processes such as replication, transcription, and recombination. Inspired by previous studies of 2,4,6-trisubstituted pyridines to find out safer and effective topoisomerase targeted anticancer agent, twenty-seven 2-phenol-4,6-dichlorophenyl-pyridines were designed, synthesized, and tested for their topo I and IIα inhibitory and anti-proliferative activity. Most of the dichlorinated *meta*- and *para*-phenolic series compounds (**1**–**18**) exhibited potent and selective topo IIα inhibition along with significant anti-proliferative activity in the HCT-15 and T47D cell lines compared to the positive control, etoposide. Interestingly, dichlorinated *ortho*-phenolic series compounds (**19**–**27**) exhibited potent and dual topo inhibition but very weak anti-proliferative activity in the tested cancer cell lines. Structure-activity relationship with previously synthesized compounds revealed the importance of chlorine moiety to improve the potency of topo inhibitory activity. Further mechanistic study confirmed that compounds **2** and **12** acted as non-intercalative specific topo IIα catalytic inhibitor with less DNA damage, and induced G1 arrest and apoptosis in HCT-15 and T47D cell lines, respectively.

1. Introduction

Human DNA topoisomerases (Topos), which solve the inherent challenges of cells during various DNA metabolic processes, are considered important biological targets for the development of cancer therapeutics [1–4]. Human topoisomerases are classified mainly into two types: topoisomerase I (topo I), which cleaves only one DNA strand; and topoisomerase II (topo II), which, in the presence of Mg (II) and ATP hydrolysis, cleaves both strands of the DNA double helix to complete their catalytic functions [5,6]. Moreover, topo II also plays an important role in chromosome segregation and proper organization [7]. Topo II has two isoforms: topo IIα and topo IIβ. Both homodimer isoforms have 70% similarity in the amino acid sequence but are distinguished by different genes located at the 17q21–22 and 3p24 chromosomal band, respectively [8–10]. Although they share a similar function, the expression of topo IIα depends on cellular growth and is essential for cell proliferation, whereas topo IIβ is independent of the cell cycle [11,12]. In addition, topo IIβ is a key mediator of anthracycline-associated cardiotoxicity, and etoposide-associated t-AMLs [13–16].

Topo inhibitors are classified into two types based on their mode of action. Topo poisons, such as etoposide and anthracycline, act by stabilizing the topo-DNA cleavable complex, blocking the religation step leading to cell death, whereas topo catalytic inhibitors, such as novobiocin and aclarubicin, interfere with at least one step of the catalytic cycle of the topo enzymes [6,17,18]. Human DNA topo inhibitors are still frontline interventions that are used widely as anticancer drugs in clinical use. On the other hand, their use is limited by their drug insolubility, drug resistance, poor bioavailability, and various side effects that appear to be inherent to drug action. Therefore, the search for novel chemical structures is being pursued actively to develop selective topo IIα catalytic inhibitors as safer and effective anticancer agents. Previously, our research group reported the synthesis of flexible tetracyclic compounds (Fig. 1) containing a central pyridine rings with three substitutions as the topo inhibitory and anti-proliferative agents.

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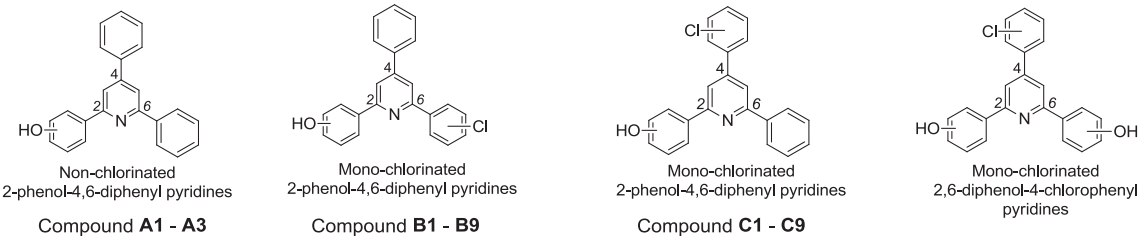

Fig. 1. Previously synthesized non & mono-chlorinated 2-phenol-4,6-diphenyl pyridines [19–22].

Various studies disclosed the importance of the incorporation of hydroxyl and chlorine group in 2,4,6-trisubstituted phenyl ring of central pyridine for moderate to strong anti-proliferative activity and topo inhibitory activity [19–22]. Similarly, many phenolic compounds also contribute to cell cycle arrest by apoptosis, regulation of the carcinogen metabolism, and ontogenesis expression [23–25]. A molecular modeling study of clinically used drugs, such as camptothecin and etoposide, which are topo I and II inhibitors, respectively, also highlighted the importance of the hydroxyl group in stabilizing the ternary cleavable complex by forming hydrogen bonds [16,26,27]. Moreover, the presence of a chlorine group in drug molecules was found to be crucial for their anticancer activity. In recent years, many natural and synthetic products, such as pericosine, simocyclinone D8, parthenin, and imidazo[1,2-*a*]pyridines/pyrazines, having a chlorine group, were found to exhibit both topo inhibitory activity and antitumor activity [28]. These promising findings encouraged us to study whether dichlorinated 4,6-diphenyl 2-phenol pyridine enhance potency and topo IIα selective anti-proliferative activity.

Herein, we report design, synthesis, structure-activity relationship (SAR) and biological evaluation of tri-substituted pyridine scaffolds containing a phenol group at the 2-position and chlorine groups at the 4- and 6-phenyl ring of the central pyridine. Compounds (**1**–**27**) synthesized in *para*-, *meta*-, and *ortho*- phenolic series (Fig. 2) were examined for their effects on topoisomerase inhibition and anti-proliferative activity.

2. Results and discussion

2.1. Chemistry

As outlined in Scheme 1, compounds **1**–**27** were synthesized in three steps. The synthetic procedure was simple according to previously reported methods [29–32]. In the beginning, based on the *Claisen Schmidt* condensation reaction, nine prop-2-enone intermediates **III** (R = **a-c**, R^1 = **d-f**) were prepared [29]. Three different hydroxy-acetophenone **I** (R = **a-c**) reacted with three different chlorobenzaldehydes **II** (R^1 = **d-f**) in the presence of aqueous NaOH using methanol as a solvent to give a 52–83% yield of **III** (R = **a-c**, R^1 = **d-f**). In parallel, based on the *Ortoleva King* reaction, three different chloroacetophenones **IV** (R^2 = **d-f**) were heated under reflux with iodine and pyridine for 3 h at 140 °C to prepare three pyridinium iodide salts **V** (R^2 = **d-f**) in 66–94% yield. In the final step, based on the modified *Kröhnke* synthesis [30,31], pyridinium iodide salts **V** (R^2 = **d-f**) and prop-2-enone intermediates **III** (R = **a-c**, R^1 = **d-f**) were heated to 95–120 °C under reflux for 6–24 h under nitrogen in the presence of anhydrous ammonium acetate and glacial acetic acid to synthesize the final compounds (**1**–**27**) in 20–60% yield. Fig. 3 shows the structures of the synthesized compounds. Table S1 (Supplementary Data) lists all the prepared compounds that were characterized by the melting point (°C), purity by HPLC (%), and yield (%).

2.2. Topo I and IIα inhibitory activity of compounds

Table 1 and Fig. 4 summarize the topo I and IIα inhibitory activity of the synthesized compounds **1**–**27** at 100 and 20 μM. All the compounds with the *para*-phenolic series, **1**–**9**, exhibited weak topo I inhibition at 100 μM compared to camptothecin. Only compound **8** displayed stronger topo I inhibition (40.6%) than camptothecin (33.6%) at 20 μM. Interestingly, all the *para*-phenolic series compounds **1**–**9** possessed significantly stronger topo IIα inhibitory activity than etoposide (64.8%) at 100 μM. Compounds **7**–**9** exhibited 100% topo IIα inhibitory activity at 100 μM. With the exception of compounds **4** and **9**, all showed potent topo IIα inhibitory activity, ranging from 52.4% to 72.7% compared to the positive control, etoposide (40.8%) at 20 μM. Compound **8** exhibited the strongest topo IIα inhibitory activity at both 100 μM (100%) and 20 μM (72.7%). None of the compounds with the *meta*-phenolic series, **10**–**18**, showed topo I inhibition, indicating that all the compounds exhibited selective and potent topo IIα inhibition. These compounds at 20 μM displayed topo IIα inhibition, ranging from 45.9% to 75.7%, which were higher than that of etoposide (41%). Compounds **10** (74.4%), **11** (75.9%), **12** (80.8%), **17** (83.5%), and **18** (87.7%) showed stronger IIα inhibition than etoposide (73.4%) at 100 μM. Interestingly, all the *ortho*-phenolic series compounds **19**–**27** displayed dual topo I and topo IIα inhibitory activity. With the exception of compounds **19** and **20**, the other

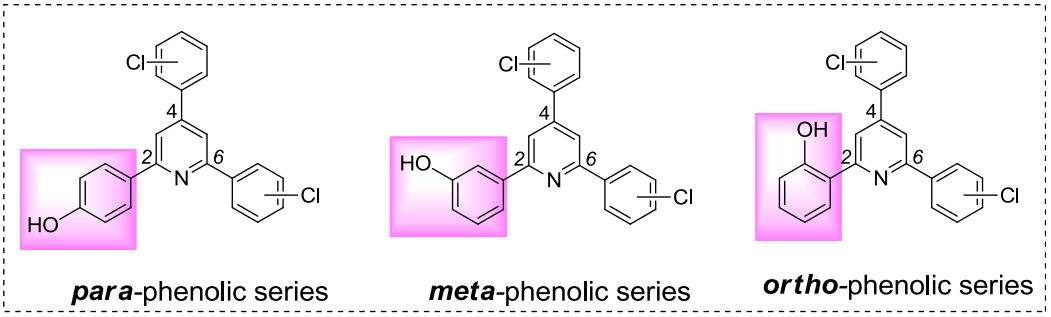

Fig. 2. Strategic series of 2-phenol-4,6-dichlorophenyl pyridines.