European Journal of Medicinal Chemistry 125 (2017) 14–28

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

# Novel 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridines as potent DNA non-intercalative topoisomerase catalytic inhibitors

Seojeong Park [a, 1], Tara Man Kadayat [b, 1], Kyu-Yeon Jun [a], Til Bahadur Thapa Magar [b], Ganesh Bist [b], Aarajana Shrestha [b], Eung-Seok Lee [b, **], Youngjoo Kwon [a, *]

[a] College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Republic of Korea
[b] College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea

ARTICLE INFO

*Article history:*
Received 14 June 2016
Received in revised form
5 September 2016
Accepted 6 September 2016
Available online 8 September 2016

*Keywords:*
Anticancer agents
2-Aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-b]pyridine
Antiproliferative activity
Docking study
Dual topoisomerase I and II inhibitors

ABSTRACT

On the basis of previous reports on the importance of thienyl, furyl or phenol group substitution on 5*H*-indeno[1,2-*b*]pyridine skeleton, a new series of rigid 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridine derivatives were systematically designed and synthesized. Topoisomerase inhibitory activity and antiproliferative activity of all the synthesized compounds were determined using human colorectal (HCT15), breast (T47D), prostate (DU145) and cervix (HeLa) cancer cells. Compounds **9**, **10**, **12**, **13**, **15**, **16**, **18** and **19** with thienyl or furyl moiety at the 2-position and hydroxyl group at the *meta* or *para* positions of 4-phenyl ring displayed strong to moderate topoisomerase IIα (topo IIα) inhibitory activity and significant antiproliferative activity. The evaluation of compound **16** to determine its mechanism of action was performed with topo IIα-DNA cleavable complex, topo IIα-mediated ATPase assay, DNA unwinding and *in vitro* and *ex vivo* topo IIα relaxation assay. Compound **16** functioned as a DNA non-intercalative topo IIα catalytic inhibitor with better potency than etoposide in T47D breast cancer cells. Molecular docking study revealed that compound **16** cannot intercalate into regularly stacked base-pairs of DNA duplex but can interact or intercalate to topo IIα-bound DNA.



## 1. Introduction

Despite several rationale strategies for the treatment of cancer, chemotherapy remains as one of the well-established approach for cancer therapy [1]. The human DNA topoisomerases consisting of two subtypes, I (topo I) and II (topo II), have been vitally important molecular targets for the development of anticancer drugs [2,3]. Human topo II has been considered as more important molecular target than topo I for designing anticancer agents because of its ability to cleave both strands of one DNA duplex (G-segment) to make the other DNA duplex (T-segment) pass through transient break of G-segment and thus to solve the DNA topological problems. This simultaneous cleavage function of topo II on DNA double strand endows topo II to play an essential role for DNA chromosome condensation and segregation in mitosis [4,5].

Several compounds containing indenopyridine skeleton showed important biological activities such as anticancer, anti-inflammatory, antidepressant and coronary dilating activities [6–9]. There are more reports that some similar pharmacore compounds consisting of imidazo-pyridine [10,11], 2-arylquinazolinone [12], thiophenylmethylene-thiohydantoin [13], 2-phenylnaphthalenoids and 2-phenylbenzofuranoids [14] have human topo I and/or II catalytic inhibitory activity. In our previous studies, conformationally constrained 2-hydroxyphenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines (aryl = 2- or 3-furyl or 2- or 3-thienyl) (Fig. 1a) showed dual topo I and II inhibition [15]. In addition, subtle modification in the position of hydroxyl group on the phenyl ring and aryl group attached to central pyridine altered the mechanism of action of compounds, for instance, compound **Y** (Fig. 1c) functioned as a topo II poison [16] whereas compound **Z** (Fig. 1c) was defined to be a topo II catalytic inhibitor [17]. Compounds containing thienyl or furyl groups at the 4-position of 5*H*-indeno[1,2-*b*]pyridine (Fig. 1b) exhibited strong topo II inhibitory activity [18]. Thus, we put efforts continuously to design and synthesize a new series of rigid analogs of 2-aryl-4-(4′-hydroxyphenyl)-5*H*-indeno[1,2-*b*]pyridines with strategy described in Fig. 3. Hydroxylated rigid compounds **8**–**28** (Fig. 4) were newly synthesized in the

* Corresponding author.
** Corresponding author.
*E-mail addresses:* eslee@yu.ac.kr (E.-S. Lee), ykwon@ewha.ac.kr (Y. Kwon).
[1] Authors are equal contributors.

http://dx.doi.org/10.1016/j.ejmech.2016.09.019
0223-5234/

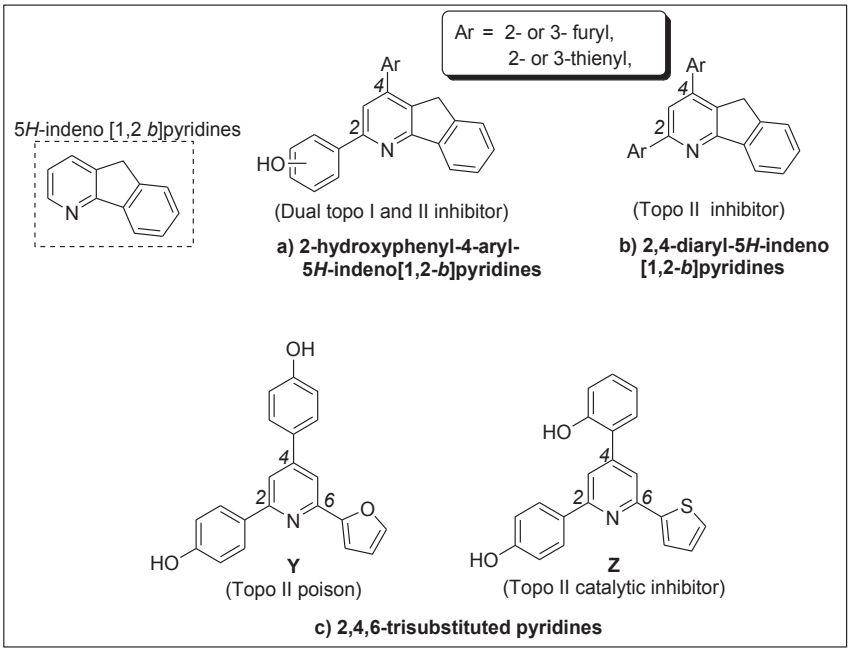


**Fig. 1.** Structures of previously synthesized a) 2-hydroxyphenyl-4-aryl-5*H*-indeno[1,2-*b*]pyridines, b) 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines, and c) 2,4,6-trisubstituted pyridines.

current study and their biological activities were examined. In addition, structure-activity relationship study of compounds **8**–**28** was compared with non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno [1,2-*b*]pyridines (Fig. 2, compounds **1**–**7**). The most active compound **16** in *in vitro* and *ex vivo* anticancer efficacy was further carried out for molecular docking study to clarify its mechanism of action, and turned out to be a novel DNA non-intercalative topo catalytic inhibitor.

## 2. Results and discussion

### 2.1. Design and synthesis

Twenty-eight compounds (**1**–**28**) in seven different series were prepared (Fig. 3). Compounds **1**–**7** (Fig. 2) are non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno[1,2-*b*]pyridines whereas each of compounds **8**–**28** has a hydroxyl group at *ortho, meta* or *para* position of the 4-phenyl ring (Fig. 4). Synthesis of compounds **1**–**3, 5, 6,** and **8**–**10** was reported earlier [12,19]. The synthetic route for compounds **4, 7**–**28** is outlined in Scheme 1. In the first step (i), 1-indanone (**I**) was condensed with aryl aldehydes **II** (R = **a-d)** in the presence of 5% NaOH in ethanol (EtOH) to obtain indanone intermediates **III** (R = **a-d)** using *Clasien-Schmidt* condensation reaction [13,20]. In the second step (ii), acetophenones **IV** ($R^1$ = **e-k)** were refluxed with iodine in pyridine to yield seven pyridinium iodide salts **V** ($R^1$ = **e-k)**. In the last step (iii), indanone intermediates **III** (R = **a-d)** were reacted with pyridinium iodide salts **V** ($R^1$ = **e-k)** in the presence of ammonium acetate in methanol or acetic acid using modified Kröhnke synthesis [21,22] to obtain final compounds **4, 7**–**28** in the yields of 20–83%. The yields (%), melting points (°C), purities (%), and retention time of the prepared compounds are listed in Table S1 (Supplementary Data).

On the basis of the previously reported biological results, the present investigation was undertaken in order to determine the effect of thienyl or furyl moieties at the 2-position and phenol moiety at the 4-position of 5*H*-indeno[1,2-*b*]pyridine skeleton

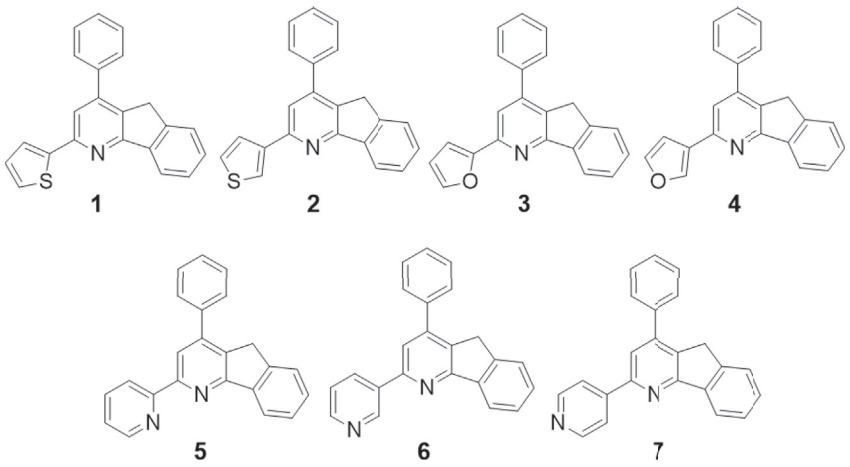


**Fig. 2.** Structures of non-hydroxylated 2-aryl-4-phenyl-5*H*-indeno[1,2-*b*]pyridines.