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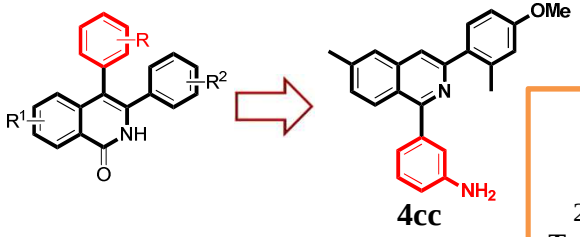

4cc

Topo I catalytic inhibitor

IC_{50} (μM):
0.74±0.05 (T47D)
5.06±0.04 (HeLa)
2.77±0.38 (HCT-15)
Topo I inhibition > CPT

Design, synthesis, and biological evaluation of 1,3-diarylisoquinolines as novel topoisomerase I catalytic inhibitors

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ABSTRACT

With a goal of identifying potent topoisomerase (topo) inhibitor, the C4-aromatic ring of the anticancer agent, 3,4-diarylisoquinolone, was strategically shifted to design 1,3-diarylisoquinoline. Twenty-two target compounds were synthesized in three simple and efficient steps. The 1,3-diarylisoquinolines exhibited potent anti-proliferative effects on cancer cells but few compounds spared non-cancerous cells. Inhibition of topo I/IIα-mediated DNA relaxation by several derivatives was greater than that by camptothecin (CPT)/etoposide even at low concentration (20 μM). In addition, these compounds had little or no effect on polymerization of tubulin. A series of biological evaluations performed with the most potent derivative **4cc** revealed that the compound is a non-intercalative topo I catalytic inhibitor interacting with free topo I. Collectively, the potent cytotoxic effect on cancer cells including the drug resistance ones, absence of lethal effect on normal cells, and different mechanism of action than topo I poisons suggest that the 1,3-diarylisoquinolines might be a promising class of anticancer agents worthy of further pursuit.

Keywords:
Antitubulin activity, Topoisomerase catalytic inhibitor, 1,3-Diarylisoquinoline, Suzuki coupling, Topoisomerase

1. Introduction

Deregulated cell proliferation and suppressed cell death are two main characteristics of cancer [1]. The factors that determine the efficacy of an anticancer therapy include identification and targeting of the molecular anatomy responsible for the relentless proliferation of tumor cells and the compensatory mutations necessary for their survival. The success of the anticancer drugs, etoposide, doxorubicin (DOX), and the camptothecin (CPT) derivatives irinotecan and topotecan, is primarily attributable to the exploitation of cancer cell mechanisms that differ from those of normal cells. These anticancer agents interfere with the activity of a specialized class of enzymes called topoisomerase (topo), which are overexpressed in cancer cells [2-6]. Topo is involved in resolving the topological consequences of DNA like supercoils, knots, and catenation during cellular processes, including transcription, recombination, and replication of rapidly proliferating cells [7].

Six topos are expressed in human cells [8-10]. On the basis of function they are classified as type I and type II. Type I topos cleave single strand of DNA while type II affect both strands during their enzymatic activities. Topos are further divided into A and B subtypes based on structure. Among the type IA (topo IIIα and β), type IB (topo I and Imt), and type IIA (topo IIα and IIβ) enzymes, topo I and II are established molecular targets of anticancer drugs. The catalytic function of topo I(II) involves transient DNA strand(s) cleavage, DNA swivel (strands passage), and resealing of broken strand(s) [11]. Topo cleaves phosphodiester backbone of DNA by nucleophilic attack from catalytic tyrosine to form short-lived covalent topo-DNA complex, commonly referred as “topo-DNA cleavage/cleavable complex” (topocc; Fig. 1). Topo I forms tyrosyl-DNA covalent link at the 3′ end whereas topo II at the 5′ ends of broken strand(s) at the cleavage site. Rotation of broken strand around its intact complement and passage of intact DNA duplex through double strand break of another DNA duplex are the main events that relax, unknot, and decatenate DNA.

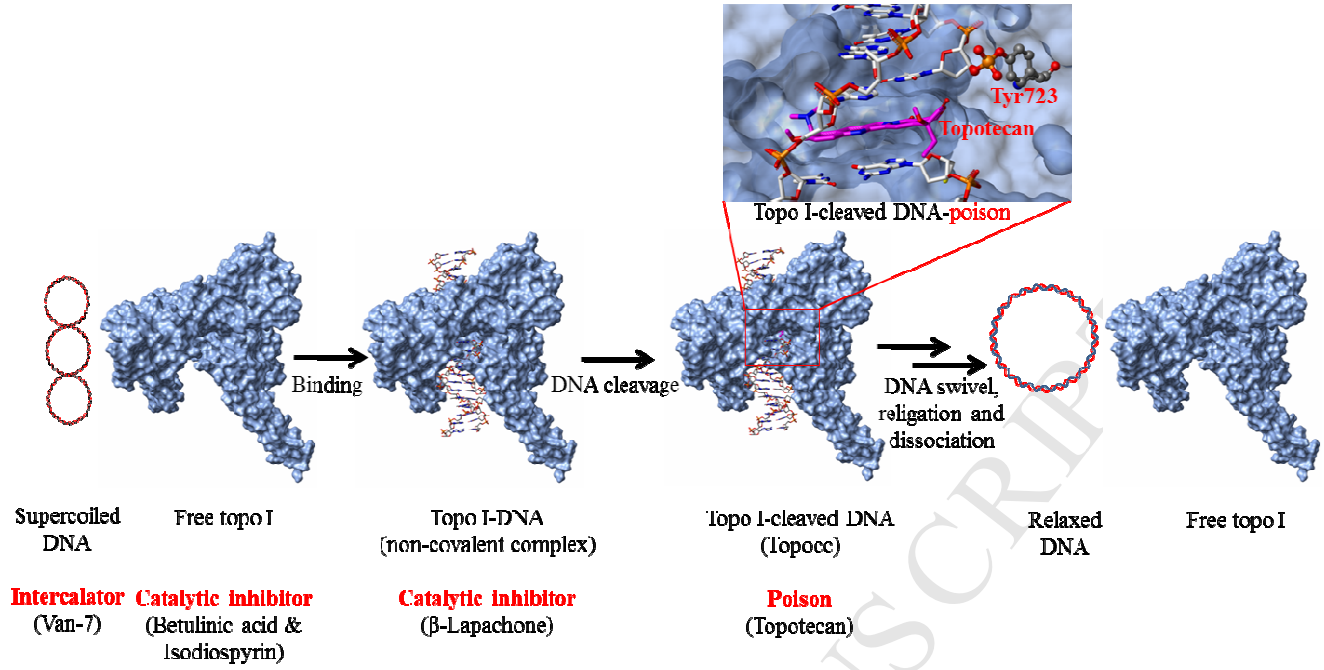


Fig. 1. Schematic diagram showing different stages of topo I enzymatic processes and inhibitors involved in each stage.

The enzymatic activities of topo can be obstructed at various stages. Inhibition of DNA break resealing and stabilization of topocc are the most important mechanisms exploited for cancer treatment. Topocc, when trapped during replication and transcription, causes permanent DNA breakage due to collision with replication or transcription machineries, eventually leading to DNA damage, cell cycle arrest, or apoptotic cell death [12]. Anticancer drugs like irinotecan and topotecan can interact and freeze topo Icc. These topo inhibitors that permit topo I-mediated DNA cleavage but prevent DNA resealing convert functional topo I into a lethal component and are called topo I poisons (Fig. 1).

Apart from preventing DNA resealing, anticancer agents like the vanadium compound, Van-7 [13], intercalates between DNA bases to distort and inhibit association of DNA with topo I. In addition, betulinic acid [14] and isodiospyrin [15] bind with topo I and prevent interaction with DNA; β-lapachone [16] interacts with topo I and allows assembly of topo I and DNA but inhibits the formation of topo Icc. The latter types of topo I inhibitors are collectively known as topo I catalytic inhibitors/suppressors [17, 18] (Fig. 1). Similarly, several topo II poisons and catalytic inhibitors act at different points of the enzymatic reaction cycle [19, 20].

The topo I poisons prescribed clinically, irinotecan and topotecan, are chemically unstable (the lactone ring of the drugs opens in physiological neutral pH into the inactive carboxylate form) and are cross-resistant to cells expressing the drug efflux membrane transporters, ABCG2 and

ABCB1 [8]. The topo II poison, etoposide, is associated with higher incidences of secondary malignancies and most notably with the development of myeloid leukemia [21]. Etoposide-induced carcinogenesis is linked with drug-related DNA double-strand breaks and DNA sequence rearrangements. It may be possible to overcome the drawbacks and limitations of these drugs by eliminating chemically liable groups, using a novel scaffold to avoid extrusion from the cell, and developing drug candidates that affect a suitable target with different mode of activity to avoid the development of secondary malignancies. We therefore report the design, synthesis, and biological evaluation of cytotoxic 1,3-diarylisoquinolines, non-intercalative topo I catalytic inhibitors.

2. Results and discussion

2.1. Design of 1,3-diarylisoquinoline

Subtle changes in the bridge length and relative positions between aromatic rings of multi-arylated compound comprise the general strategy utilized during drug design. The inhibition of tubulin polymerization by 2,3-diarylpyridine **1** was restored with a comparable potency as that of combretastatin A-4 (CA-4) when the bridge length between the two aromatic rings was increased to 3 atoms and the aromatic rings were placed between pyridine *N*, as in 2,6-diarylpyridine **2** (Fig. 2A) [22, 23]. Thus, it can be expected that the shift of the 4-aryl group of 3,4-diarylisoquinolone **3** (inhibitor of topo activity) [24] to the C1 position to form the isomeric 1,3-diarylisoquinoline **4** may be advantageous in terms of its pharmacological properties.

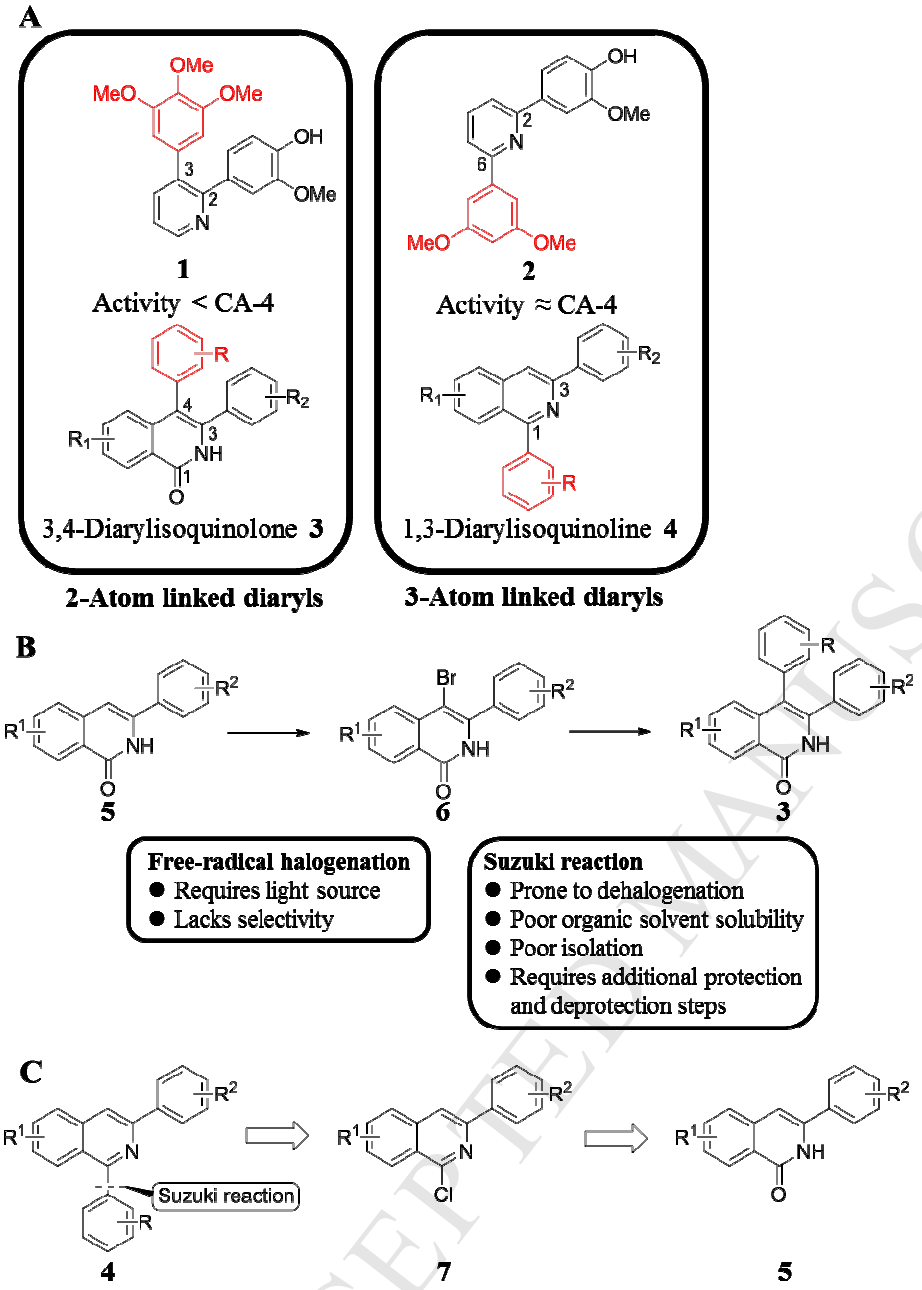


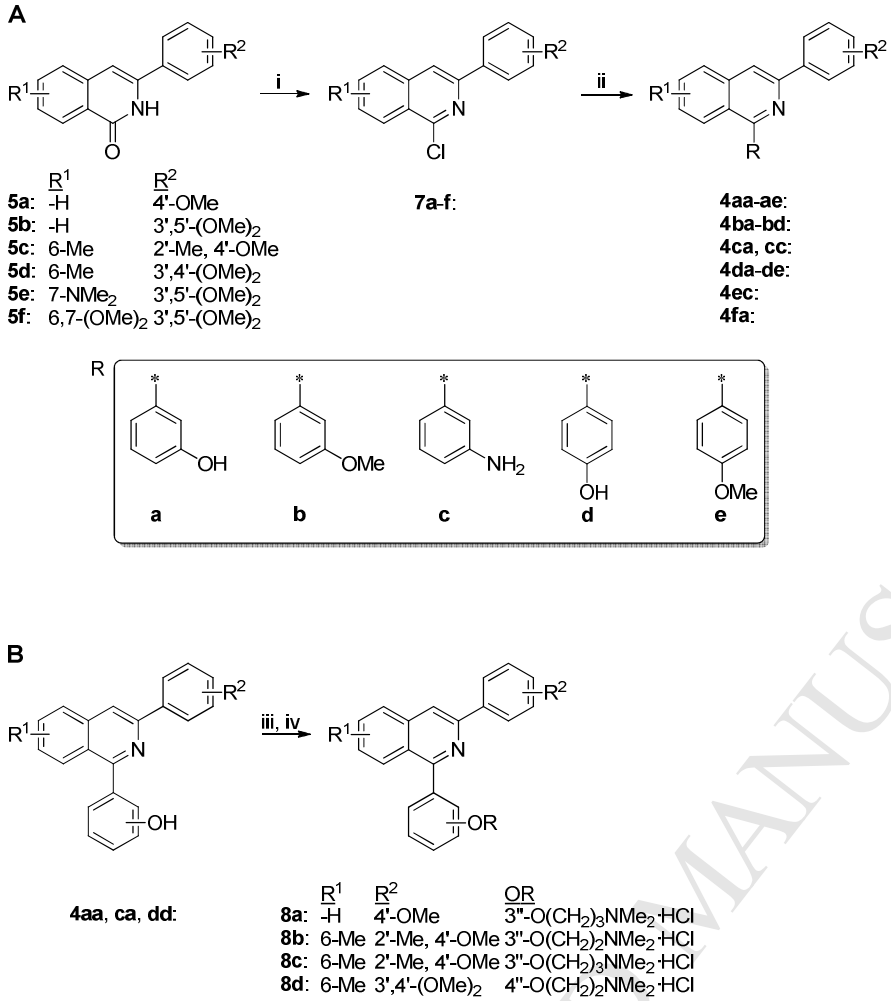
Fig. 2. Design of 1,3-diarylisoquinoline. A) 2- and 3-atom linked diaryls. B) Synthetic drawbacks of 3,4-diarylisoquinolones. C) Retrosynthesis of 1,3-diarylisoquinolines. CA-4: combretastatin A-4.

Besides improving biological activity, synthesis of 1,3-diarylisoquinoline via an efficient pathway is desirable; the synthetic pathway used to obtain 3,4-diarylisoquinolone has several drawbacks and limitations (Fig. 2B). Free-radical halogenation of 3-arylisoquinolone **5** lacks selectivity. Dihalogenation occurs when an activating group (e.g. -OMe) is present in the C3-aryl ring (Scheme S2, Supplementary data) [24]. Moreover, the Suzuki reaction of 4-brominated isoquinolin-1(2*H*)-one **6** with aryl boronic acid is always accompanied by a debrominated byproduct,

which interferes with isolation procedures and requires additional protection and deprotection steps. Meanwhile, 1,3-diarylisoquinoline **4** can be obtained in two reaction steps via Suzuki reaction of monohalogenated isoquinoline **7**, obtained simply by heating isoquinolone **5** with $POCl_3$ (Fig. 2C). Moreover, based on our previous experience, Suzuki coupling of 1-chloroisoquinoline and boronic acid results in no detectable dehalogenated byproduct. The 1,3-diarylisoquinoline formed by the reaction has a satisfactory solubility in organic solvents, which eases purification and isolation steps.

2.2. Chemistry

Synthesis of 1,3-diarylisoquinolines **4** involved two steps, starting from 3-arylisoquinolones **5** (Scheme 1A; refer to Supplementary data for the synthesis of 3-arylisoquinolones, Scheme S1). Isoquinolones **5** were converted to imine chlorides **7** on treatment with $POCl_3$. The isoquinolinyl chlorides thus obtained were coupled with aryl boronic acids **a-e** under Suzuki reaction conditions to form 1,3-diarylisoquinolines **4** with high yield (quantitative–62%). Phenol substituted 1,3-diarylisoquinolines (**4aa**, **ca**, and **dd**) and alkyl halide with amino terminal were further reacted to afford compounds **8** with an amino alkyl tail (Scheme 1B). Free amines **8** were converted to **8**·HCl salts with *c*-HCl.



Scheme 1. Synthesis of 1,3-diarylisoquinolines **4** (A) and 1,3-diarylisoquinolines with amino alkyl chain **8** (B). Reagents and conditions: (i) $POCl_3$, 75 °C; (ii) $ArB(OH)_2$, $Pd(PPh_3)_4$ (0), Na_2CO_3, $(MeOCH_2)_2$, 90 °C. (iii) RCl, K_2CO_3, DMF, 130 °C; (iv) *c*-HCl, acetone, room temperature (r.t.).

2.3. Cytotoxicity

The *in vitro* cytotoxicity of the synthesized 1,3-diarylisoquinolines (**4** and **8**) was evaluated using Adriamycin® (DOX·HCl) as positive control. The assay was performed using non-cancerous (human breast epithelial, MCF-10A) and tumor (human ductal breast epithelial tumor, T47D; human prostate cancer, DU145; human colorectal adenocarcinoma, HCT-15; and human cervical cancer, HeLa) cells [25]. Cytotoxicity results are summarized as IC_{50} (concentration that inhibits 50% of cell proliferation) values (Tables 1 and 2).

Initially, eight 1,3-diarylisoquinolines were tested to assess their toxicity in non-cancerous (MCF-10A) and cancerous (T47D, DU145, and HCT-15) cells (Table 1). Compounds **4ab**, **4db**, and **4de,** with an anisole ring at C1, are significant, as they were non-toxic to normal MCF-10A cells but had potent cytotoxicity against cancerous T47D cells (Fig. 3). Moreover, these compounds had no

effect on prostate cancer (DU145) cells. The hydroxylated derivatives **4aa**, **4da**, **4dd** and **4fa** were, however, toxic to both MCF-10A and DU145 cells. The phenolic compound **4ad** exhibited a unique cytotoxicity profile; it selectively suppressed the proliferation of T47D cells with no cytotoxic effect on MCF-10A, DU145, and HCT-15 cells. Similarly, isoquinoline **4de** had minimal anti-proliferative effect on HCT-15 cells. Besides **4ad** and **4de**, the isoquinolines compiled in Table 1 exhibited more potent antitumor activity than the standard, Adriamycin, in HCT-15 cells (IC_{50}: 0.37±0.02–2.52±0.1 μM).

Table 1. Cytotoxicity (IC_{50}) of 1,3-diarylisoquinolines **4**.

S. No.	Compound	R^1	R^2	R	IC_{50} (μM) MCF-10A[a]	T47D[b]	DU145[c]	HCT-15[d]
1.	Adriamycin	/	/	/	0.93±0.03	0.84±0.04	4.88±0.12	3.76±0.12
2.	**4aa**	-H	4′-OMe	3″-OH	7.83±0.06	0.58±0.01	5.36±0.01	1.2±0.07
3.	**4ab**	-H	4′-OMe	3″-OMe	>50	0.86±0.01	>50	1.3±0.01
4.	**4ad**	-H	4′-OMe	4″-OH	>50	1.22±0.03	>50	>50
5.	**4da**	6-Me	3′,4′-$(OMe)_2$	3″-OH	13.48±0.11	4.34±0.04	6.51±0.04	0.37±0.02
6.	**4db**	6-Me	3′,4′-$(OMe)_2$	3″-OMe	>50	7.86±0.15	>50	1.84±0.05
7.	**4dd**	6-Me	3′,4′-$(OMe)_2$	4″-OH	18.1±0.24	4.42±0.05	5.98±0.29	2.52±0.1
8.	**4de**	6-Me	3′,4′-$(OMe)_2$	4″-OMe	>50	3.34±0.18	>50	28.69±0.35
9.	**4fa**	6,7-$(OMe)_2$	3′,5′-$(OMe)_2$	3″-OH	5.9±0.15	3.92±0.16	8.12±0.08	0.54±0.03

Each value represents the mean ± S.D. from three different experiments performed in triplicate.

[a] MCF-10A: non-cancerous human breast epithelial cells.

[b] T47D: human ductal breast epithelial tumor cells.

[c] DU145: human prostate cancer cells.

[d] HCT-15: human colorectal adenocarcinoma cells.

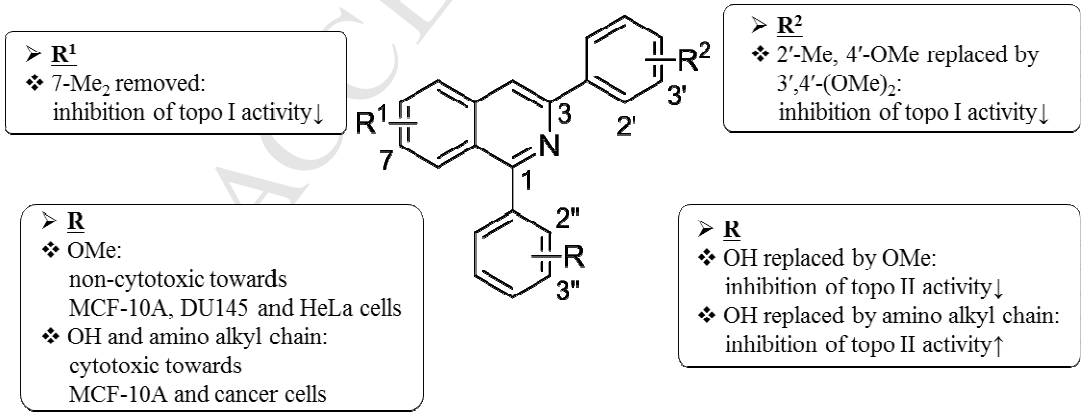


Fig. 3. Structure-activity relationship (SAR) of 1,3-diarylisoquinolines.

The preliminary cytotoxicity profile of 1,3-diarylisoquinolines proves that this class of compounds may be useful in the development of safer anticancer drugs. Encouraged by the initial results, newer derivatives of 1,3-diarylisoquinolines were synthesized and further tested for anti-proliferative activity against MCF-10A, T47D, HeLa, and HCT-15 cells (Table 2). In accordance with the earlier result, anisoles **4ae** and **4bb** of the second batch of compounds were not toxic to non-cancerous MCF-10A cells. Similarly, anilines **4bc** and **4ec** had no effect on these cells. However, phenols and aminoalkyl substituted derivatives had detrimental effect on the proliferation of MCF-10A cells at concentrations > 5 μM. On the other hand, 1,3-Diarylisoquinolines of Table 2 exhibited potent antitumor activity against T47D cells at lower concentrations (IC_{50} < 1 μM). The notable difference in cytotoxicity of 1,3-diarylisoquinolines between MCF-10A and T47D confers that they are safer anticancer agents. C1-anisole substituted derivatives **4ae** and **4bb** were inactive against HeLa cells, while the remaining compounds exhibited cytotoxicity at micromolar concentrations (IC_{50}: 3.06±0.01–13.19±0.85 μM). Importantly, the diarylisoquinolines listed in Table 2 exhibited significant inhibitory effects on HCT-15 cell proliferation (IC_{50}: 0.84±0.01–4.08±0.11 μM). The potent cytotoxicity of 1,3-diarylisoquinolines against HCT-15 indicates that their cellular concentration is not affected by overexpressed multidrug resistance protein 1 (MDR1; also known as P-glycoprotein, P-gp, or as ATP-binding cassette sub-family B member 1, ABCB1) [8, 26-28].

Table 2. Cytotoxicity (IC_{50}) of 1,3-diarylisoquinolines **4** and **8**.

S. No.	Compound	R^1	R^2	R	IC_{50} (μM) MCF-10A[a]	T47D[b]	HeLa[c]	HCT-15[d]
1.	Adriamycin	/	/	/	0.72±0.06	1.15±0.01	1.51±0.22	2.03±0.03
2.	**4ac**	-H	4′-OMe	3″-NH_2	18.76±1.12	0.95±0.04	8.61±0.68	2.35±0.16
3.	**4ae**	-H	4′-OMe	4″-OMe	>50	0.85±0.06	>50	2.79±0.09
4.	**4ba**	-H	3′,5′-$(OMe)_2$	3″-OH	6.77±0.24	0.76±0.03	3.87±0.01	0.84±0.01
5.	**4bb**	-H	3′,5′-$(OMe)_2$	3″-OMe	>50	1.12±0.03	>50	1.17±0.10
6.	**4bc**	-H	3′,5′-$(OMe)_2$	3″-NH_2	>50	0.98±0.04	13.19±0.85	4.08±0.11
7.	**4bd**	-H	3′,5′-$(OMe)_2$	4″-OH	7.28±0.1	0.78±0.02	4.58±0.09	2.78±0.00
8.	**4ca**	6-Me	2′-Me, 4′-OMe	3″-OH	5.05±0.06	0.56±0.1	5.51±0.06	1.75±0.06
9.	**4cc**	6-Me	2′-Me, 4′-OMe	3″-NH_2	5.74±0.15	0.74±0.05	5.06±0.04	2.77±0.38
10.	**4dc**	6-Me	3′,4′-$(OMe)_2$	3″-NH_2	5.6±0.1	0.45±0.33	4.19±0.03	2.70±0.24
11.	**4ec**	7-NMe_2	3′,5′-$(OMe)_2$	3″-NH_2	42.04±0.15	3.59±0.39	7.33±0.34	2.53±0.02
12.	**8a**	-H	4′-OMe	3″-$O(CH_2)_3NMe_2$·HCl	10.15±0.03	0.16±0.03	6.86±0.46	1.75±0.09
13.	**8b**	6-Me	2′-Me, 4′-OMe	3″-$O(CH_2)_2NMe_2$·HCl	6.35±0.07	0.6±0.18	4.92±0.11	2.2±0.07
14.	**8c**	6-Me	2′-Me, 4′-OMe	3″-$O(CH_2)_3NMe_2$·HCl	6.07±0.04	0.29±0.02	3.06±0.01	0.87±0.02
15.	**8d**	6-Me	3′,4′-$(OMe)_2$	4″-$O(CH_2)_2NMe_2$·HCl	5.31±0.04	2.33±0.04	3.48±0.09	0.97±0.04

Each value represents the mean ± S.D. from three different experiments performed in triplicate.

[a] MCF-10A: non-cancerous human breast epithelial cells.

[b] T47D: human ductal breast epithelial tumor cells.

[c] HeLa: human cervical cancer cells.

[d] HCT-15: human colorectal adenocarcinoma cells.

2.4. Inhibition of topo-mediated DNA relaxation

Inhibition of topo activity is a principle mechanism of cell cytotoxicity of a huge library of 3-arylisoquinoline derivatives [24, 29-31], analogs [32-34], and compounds bearing the 3-arylisoquinoline skeleton [10]. The inhibition of topo activity by the 1,3-diarylisoquinolines **4** and **8** was determined using the "DNA relaxation assay" (Fig. 4) [25]. In the DNA relaxation assay, topo relaxes supercoiled plasmid, which has a different electrophoretic mobility from that of completely relaxed DNA. Inhibition of topo activity by a chemical compound in the assay system is measured by determining the extent of supercoiled plasmid that was prevented from relaxing. The DNA relaxation assay was conducted using human topo I/IIα, pBR322 with CPT (as topo I positive control), and etoposide (as topo IIα positive control). The assay was performed with 100 and 20 μM concentrations of positive controls and test compounds. The inhibition of topo activity by the test compounds has been represented as % inhibition (Tables S1–S4). Furthermore, to ease the structure-activity relationship (SAR) study, the relative topo inhibition potency of various diarylisoquinolines was determined as the ratio of topo inhibition by individual compounds to that by CPT/etoposide (Table 3).

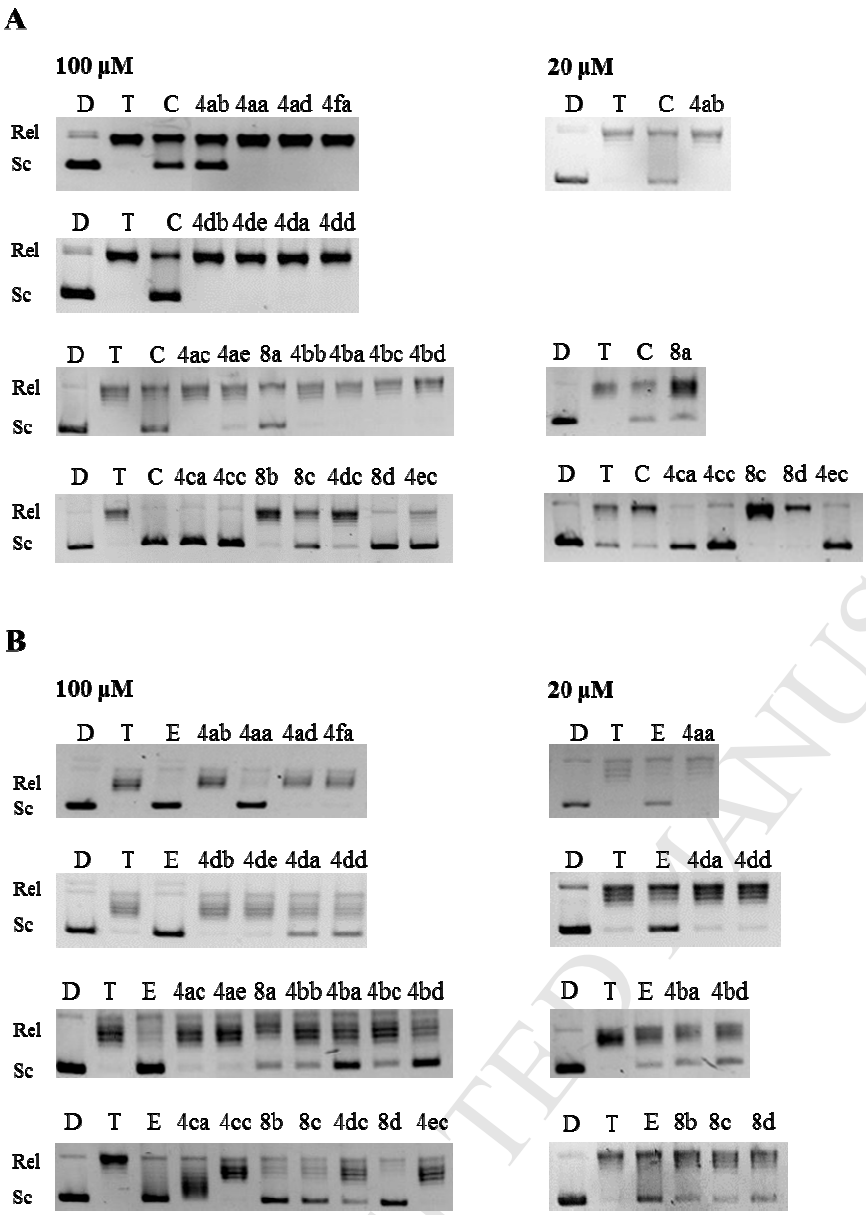


Fig. 4. Inhibition of topo I (A) and topo IIα (B) activity by 1,3-diarylisoquinolines **4** and **8** at 100 µM and 20 µM (DNA relaxation assay). Lane D: pBR322 only, lane T: pBR322 + topo I/IIα, lane C: pBR322 + topo I + camptothecin (CPT), lane E: pBR322 + topo IIα + etoposide, remaining lanes: pBR322 + topo I/IIα + the indicated 1,3-diarylisoquinolines. Rel: relaxed, Sc: supercoiled.

Table 3. Relative inhibition of topo activity by 1,3-diarylisoquinolines **4** and **8**.

S. No.	Compound	R^1	R^2	R	Topo I		Topo IIα	
					100 µM	20 µM	100 µM	20 µM
1.	**4aa**	-H	4′-OMe	3″-OH	0.12	-[a]	1.07	0.0
2.	**4ab**	-H	4′-OMe	3″-OMe	0.58	0.0	0.0	-
3.	**4ac**	-H	4′-OMe	3″-NH_2	0.0	-	0.12	-

4.	**4ad**	-H	4′-OMe	4″-OH	0.09	-	0.0	-
5.	**4ae**	-H	4′-OMe	4″-OMe	0.0	-	0.11	-
6.	**4ba**	-H	3′,5′-$(OMe)_2$	3″-OH	0.0	-	0.59	0.9
7.	**4bb**	-H	3′,5′-$(OMe)_2$	3″-OMe	0.0	-	0.26	-
8.	**4bc**	-H	3′,5′-$(OMe)_2$	3″-NH_2	0.0	-	0.37	-
9.	**4bd**	-H	3′,5′-$(OMe)_2$	4″-OH	0.0	-	0.82	1.17
10.	**4ca**	6-Me	2′-Me, 4′-OMe	3″-OH	1.13	2.46	0.2	-
11.	**4cc**	6-Me	2′-Me, 4′-OMe	3″-NH_2	1.22	2.53	0.06	-
12.	**4da**	6-Me	3′,4′-$(OMe)_2$	3″-OH	0.03	-	0.38	0.01
13.	**4db**	6-Me	3′,4′-$(OMe)_2$	3″-OMe	0.05	-	0.0	-
14.	**4dc**	6-Me	3′,4′-$(OMe)_2$	3″-NH_2	0.11	-	0.11	-
15.	**4dd**	6-Me	3′,4′-$(OMe)_2$	4″-OH	0.005	-	0.4	0.0
16.	**4de**	6-Me	3′,4′-$(OMe)_2$	4″-OMe	0.04	-	0.0	-
17.	**4ec**	7-NMe_2	3′,5′-$(OMe)_2$	3″-NH_2	1.12	2.5	0.04	-
18.	**4fa**	6,7-$(OMe)_2$	3′,5′-$(OMe)_2$	3″-OH	0.06	-	0.0	-
19.	**8a**	-H	4′-OMe	3″-$O(CH_2)_3NMe_2\cdot HCl$	0.81	0.38	0.32	-
20.	**8b**	6-Me	2′-Me, 4′-OMe	3″-$O(CH_2)_2NMe_2\cdot HCl$	0.05	-	0.81	0.35
21.	**8c**	6-Me	2′-Me, 4′-OMe	3″-$O(CH_2)_3NMe_2\cdot HCl$	0.6	0.0	0.67	0.28
22.	**8d**	6-Me	3′,4′-$(OMe)_2$	4″-$O(CH_2)_2NMe_2\cdot HCl$	1.22	0.0	1.24	0.41

Relative inhibition of topo activity = inhibition of topo activity by compound/inhibition of topo activity by camptothecin (CPT) or etoposide.

[a] Not determined.

Semi quantitative representation: 0.0–0.25 (no potency), 0.25–0.5 (low potency), 0.5–0.8 (moderate potency), 0.8-1.0 (potency similar to CPT/etoposide) and >1.0 (greater potency than CPT/etoposide).

The inhibition of topo I activity by 1,3-diarylisoquinoline **4ec** was slightly and about 2.5-fold greater than that by CPT at 100 and 20 μM, respectively (Table 3). The potency was lost when 7-Me_2 was removed as in **4bc** (Fig. 3). Similar to **4ec**, diarylisoquinolines **4ca** and **4cc** also had strong interference on topo I activity. The efficacy of the derivatives was lost when 2′-Me, 4′-OMe groups were replaced by 3′,4′-$(OMe)_2$ (**4da** and **4dc**). The potency of phenolic compound **4dd** at 100 μM increased after substitution with amino alkyl chain (**8d**). In addition, amino alkyl substituted compounds **8a** and **8c** exhibited significant potency at 100 μM.

The phenolic derivative **4aa** had similar topo IIα inhibition potency as that of etoposide at 100 μM (Table 3). Likewise, phenols **4ba** and **4bd** had a considerable inhibitory effect on topo IIα activity at both 100 and 20 μM. Conversion of 3″-OH (**4aa** and **4ba**) to 3″-OMe (**4ab** and **4bb**) had detrimental effect on potency to inhibit topo II activity (Fig. 3). On the other hand, the potency of phenol **4ca** was elevated by aminoalkyl substitution (**8b** and **8c**). Importantly, compound **8d** inhibited

DNA relaxation by both topo I and IIα.

A positive correlation was found between inhibition of topo I/IIα activity and cytotoxicity for 8 1,3-diarylisoquinolines **4aa**, **4bd**, **4ca**, **4cc**, **4ec**, **8a**, **8b**, and **8d**. Furthermore, it can be concluded that the cytotoxic effect of 3 compounds **4ab**, **4ba**, and **8c** is partially due to inhibition of topos. However, no obvious relationship between inhibition of enzyme activity and cytotoxicity was observed with remaining diarylisoquinolines. The potent antiproliferative effect of these compounds on cancer cells might be due to interference with function of other biological targets.

2.5. Inhibition of tubulin polymerization

1,3-Diaryliosquinolines **4** are structurally similar to 2,6-diarylpyridine **2**, inhibitors of tubulin polymerization. Thus, to investigate the effect of compounds **4** on microtubule dynamics the tubulin polymerization assay was performed using porcine brain tubulin. 1,3-Diarylisoquinolines **4ba**, **4bd**, **4ca**, **4cc** and **4ec** that showed potent inhibition of topo activity even at low concentration (20 µM) were considered for the test (Table 3). Paclitaxel (PTX) and DMSO were used as positive control and vehicle, respectively. PTX has been known to enhance tubulin polymerization and inhibit dissociation of microtubules [35]. As shown in Figure 5A, diarylisoquinolines **4ba**, **4bd**, **4ca**, **4cc** and **4ec** at 10 µM did not increase the absorbance unlike PTX, which reflects that compounds neither enhanced nor stabilized tubulin polymerization. However, compared to vehicle, **4ca** appeared to slightly inhibit tubulin polymerization. Similarly, compound **4cc** exhibited low potency at even higher concertation (15 µM, Fig. 5B). The inhibition of tubulin polymerization by **4ac** and **4cc** is very weak in comparison to CA-4 which nearly completely blocks tubulin polymerization at 10 µM [23].

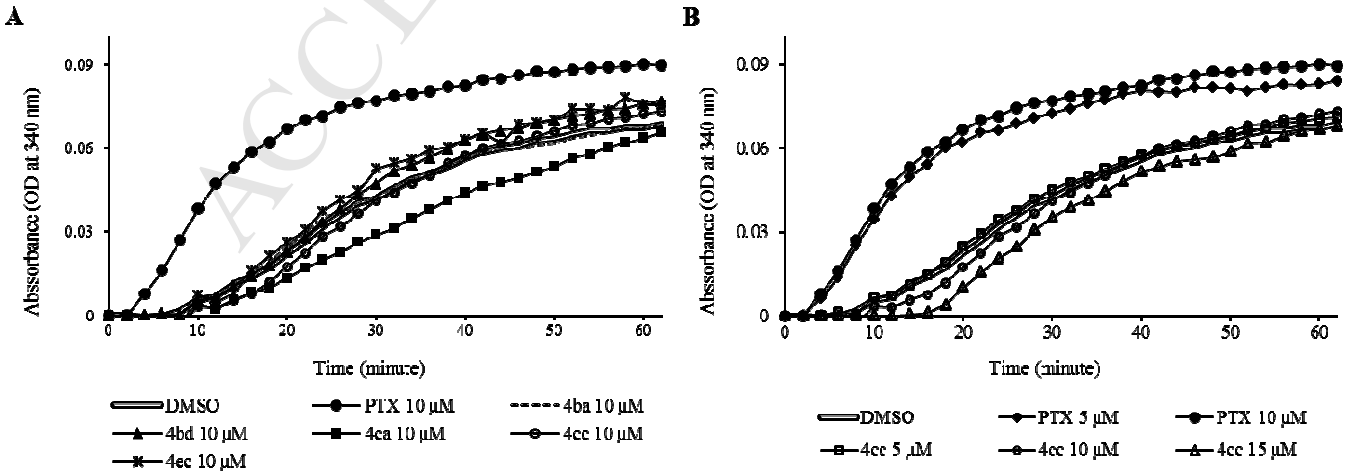


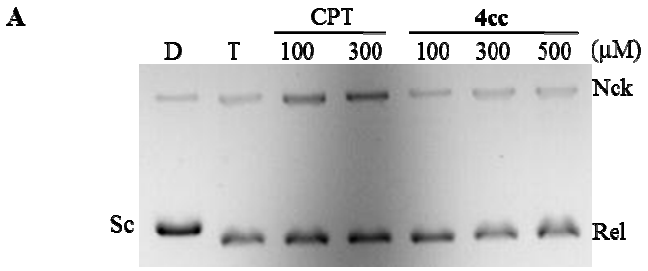
Fig. 5. Effect of 3,4-diarylisoquinolines on microtubule dynamics (tubulin polymerization assay).

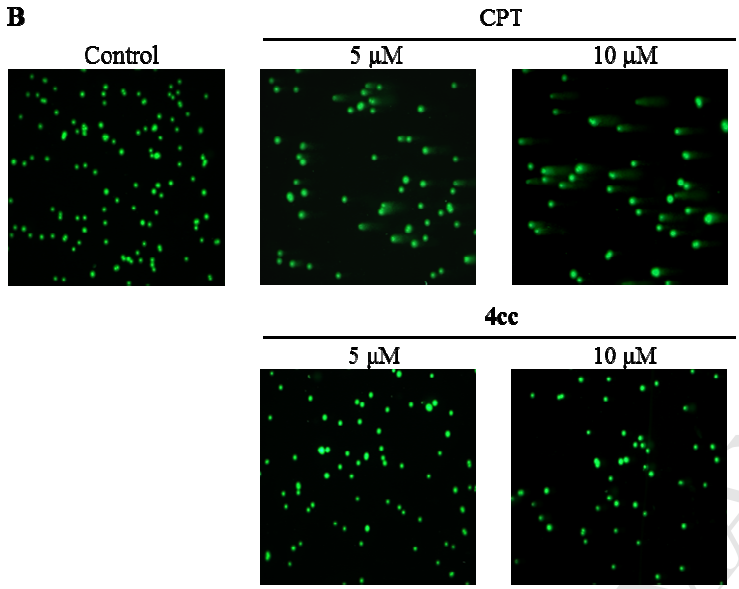
(A) The absorbance of diarylisoquinolines **4ba**, **4bd**, **4ca**, **4cc**, and **4ec**- (10 μM), PTX- (10 μM) and DMSO-treated tubulin solution at 340 nm. (B) The absorbance of diarylisoquinoline **4cc**- (5, 10, and 15 μM) and PTX-treated solution (5 and 10 μM) at 340 nm. OD: optical density.

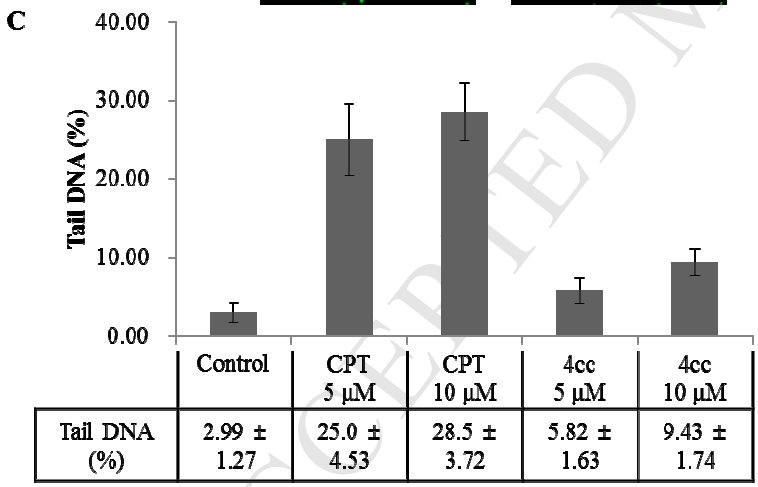
Among the tested 1,3-diarylisoquinolines, compound **4cc** had the most potent inhibitory effect on topo I activity, was cytotoxic to cancer cells at low micromolar concentration, and had no significant inhibitory effect on tubulin polymerization. Thus, further tests were performed to determine the mode of inhibition of topo I activity by the compound.

2.6. Topo I-DNA cleavage complex assay

The well-known mechanism of topo I inhibition is stabilization of topo Icc and prevention of DNA break resealing by topo I poison. This mechanism of topo I inhibition can be investigated via the topo I-DNA cleavage complex assay. In the cleavage complex assay, supercoiled plasmid pBR322 was incubated with topo I and the test compounds, followed by treatment with sarcosyl and proteinase K to remove any covalently bound topo I and electrophoresis in a system containing ethidium bromide (DNA intercalator). Ethidium bromide inserts between DNA bases of relaxed closed circular DNA, unwinds double-helical structure, and increases its electrophoretic mobility [36]. In contrast, nicked-DNA is partially unaffected. Nicked-DNA is a consequence of inhibition of DNA break resealing by the topo I poison, CPT. In fact, CPT increased nicked-DNA compared to the negative control (treated with only DMSO, Fig. 6A). Upon treatment with the isoquinoline **4cc**, however, there was no change in the level of nicked-DNA, even at a higher concentration (500 μM). This observation indicates that compound **4cc** does not act as a topo I poison.







	Control	CPT 5 μM	CPT 10 μM	4cc 5 μM	4cc 10 μM
Tail DNA (%)	2.99 ± 1.27	25.0 ± 4.53	28.5 ± 3.72	5.82 ± 1.63	9.43 ± 1.74

Fig. 6. 1,3-diarylisoquinoline **4cc** is not a topo I poison. A) Topo I-DNA cleavage complex assay. B & C) Assessment of DNA damage/repair by comet assay. CPT: camptothecin, Nck: nicked, Sc: supercoiled, Rel: relaxed.

2.7. Comet assay

The comet assay confirms the degree of DNA damage induced by a chemical compound. Debris (fragments) of broken or damaged DNA form comet tails (tail DNA) [37]. DNA damage and comet formation are characteristic properties of topo I poison. The assay is therefore useful to study

the mode of topo I inhibition. In this assay, CPT-treated T47D cells showed severe DNA damage with 8 times more tail DNA than untreated control, even at a low concentration of CPT (25.0±4.53% at 5 μM, Fig. 6B, C). However, isoquinoline **4cc** induced less tail DNA (5.82±1.63% at 5 μM and 9.43±1.74% at 10 μM), somewhat comparable to that of the untreated control (2.99±1.27%). This result is consistent with that of cleavage complex assay and confirms that the 1,3-diarylisoquinoline **4cc** is not a topo I poison.

2.8. DNA unwinding assay

Besides topo Icc stabilization and prevention of DNA break resealing, DNA intercalation is also a mode of inhibition of topo I activity. A DNA intercalator unwinds relaxed plasmid DNA into a compact form. The DNA unwinding assay, performed with amsacrine (*m*-AMSA), one of the eminent intercalative topo II poisons [38] and compound **4cc**, shows that *m*-AMSA recovered the fully relaxed pHOT1 DNA to its supercoiled form, while isoquinoline **4cc** failed to do so even at a higher concentration (1 mM, Fig. 7A).

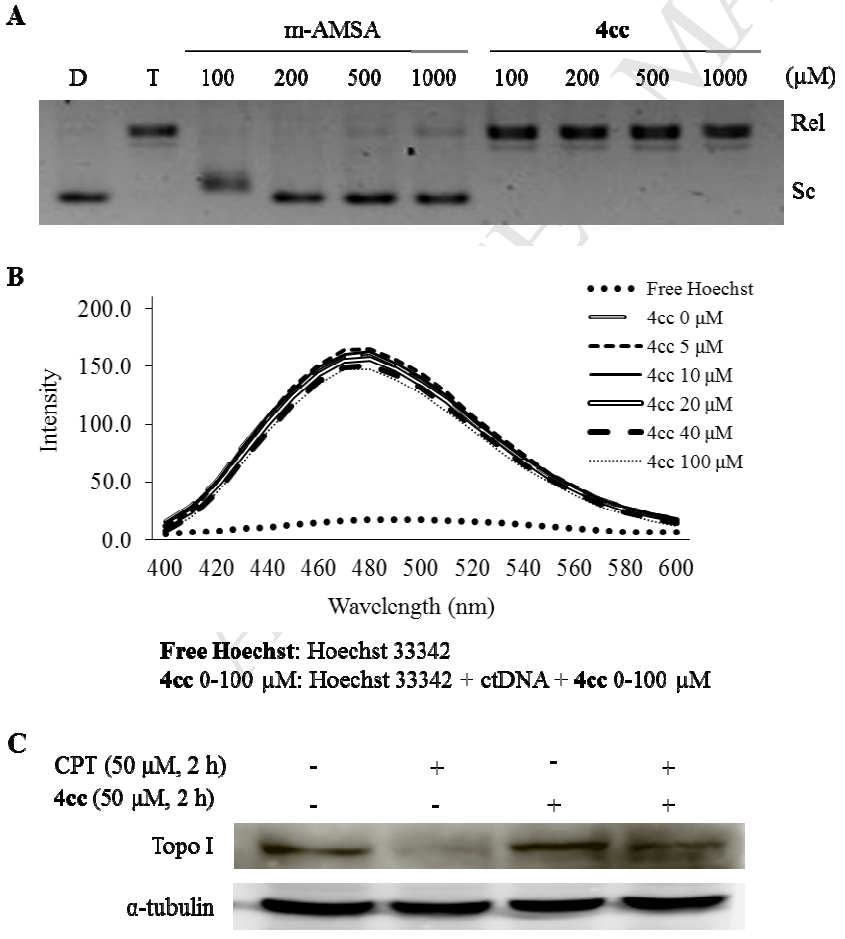


Fig. 7. A) DNA unwinding assay. B) Hoechst assay. Fluorescence spectra of Hoechst-ctDNA in absence and presence of **4cc**. Free Hoechst 33342 (1 μg/mL) in 10 mM Tris-HCl (pH 7.2) showed very weak emission intensity, while when Hoechst interacted with ctDNA, the fluorescence intensity was strong. The addition of various concentrations of **4cc** (0–100 μM) to the pre-reacted Hoechst-ctDNA did not cause change in the florescence intensity. C) Band depletion assay. Rel: relaxed, Sc: supercoiled, CPT: camptothecin.

2.9. Hoechst Assay

Hoechst assay was further performed to investigate the effect of compound **4cc** on DNA (Fig. 7B). Hoechst 33342 is a well-known DNA minor groove binder and the Hoechst–DNA complex is known to be excited at 361 nm [39, 40]. To determine whether compound **4cc** is a DNA minor groove binder, Hoechst and circulating tumor DNA (ctDNA) were pre-reacted and then treated with **4cc** in a series of concentrations (0–100 μM). It was observed that fluorescence intensity did not decrease even when 100 μM of **4cc** was treated. The results of DNA-related experiments confirmed that the compound **4cc** is neither a DNA intercalator nor a DNA ancillary groove binder but may act as topo I catalytic inhibitor (i.e. it may interact with free topo I, prevent association with supercoiled DNA and stop the consequent processes or may stabilize non-covalent topo I-DNA complex and prevent DNA break).

2.10. Band depletion assay

To further decipher the mechanism of topo I inhibition by diarylisoquinoline **4cc**, a band depletion assay was performed. Following treatment with CPT (50 μM) for 2 h at 37 °C, free topo I in T47D cells decreased, indicating the formation of topo Icc (Fig. 7C). In contrast, free topo I remained unaffected in presence of compound **4cc**. This result infers that the isoquinoline **4cc** binds with topo I and inhibits the harbor of DNA. Furthermore, treatment with compound **4cc** before CPT treatments restored free topo I in T47D cells. This indicates that compound **4cc** binds with free topo I and can competitively sequester topo I from being incorporated into the cleavage complex by co-treated topo I poison. Taken together, 1,3-diarylisoquinoline **4cc** functions as a non-intercalative topo I catalytic inhibitor, interacting with free topo I.

3. Conclusion

3,4-Diarylisoquinolone (with 2 Cs spacer between aryl rings) was modified to 1,3-diarylisoquinoline (separated by two covalent bonds) and synthesized in three simple steps with high

yield. It was found that most of the 1,3-diarylisoquinolines showed potent anti-proliferative effects against cancer cells. The potent cytotoxic effects of 1,3-diarylisoquinoline against HCT-15 cells suggest decreased risk of drug resistance due to overexpressed drug efflux membrane transporters. Several of the compounds tested had no effect on normal (non-cancerous) cells. Furthermore, 1,3-diarylisoquinolines inhibited topo I/IIα-mediated DNA relaxation. The inhibition of topo I/IIα activity by several compounds including the most potent derivative, **4cc**, at 20 μM was greater than that of CPT and had weak or no antitubulin activity. Topo I-DNA cleavage complex, comet, DNA unwinding, Hoechst, and band depletion assays revealed that the diarylisoquinoline **4cc** acts as a non-intercalative topo I catalytic inhibitor that binds with free topo I to stop the downward steps and prevent DNA damage. Altogether, this study might provide valuable information for the further research related to isoquinoline and topo I catalytic inhibitor.

4. Experimental section

4.1. Chemistry

Solvents were distilled prior to use; THF was distilled from sodium/benzophenone. The reaction temperature mentioned, except for the lithiated toluamide-benzonitrile cycloaddition reaction, is the set temperature of an oil bath. Column chromatography and medium performance liquid chromatography (MPLC, flow rate: 10 mL/min, make: Yamazen) were performed with Merck silica gel 60 (70–230 mesh). Thin-layer chromatography (TLC) was performed using plates coated with silica gel 60 F_{254} (Merck). Melting points were determined by the capillary method with a MEL-TEMP® capillary melting point apparatus and were uncorrected. IR spectra were obtained on a JASCO FT/IR 300E Fourier transform infrared spectrometer using KBr pellets. ^{1}H NMR, and ^{13}C NMR spectra were obtained on Varian Unity Plus 300 MHz, Varian Unity Inova 500 MHz and Bruker Ascend 400 spectrometers at the Korea Basic Science Institute. The chemical shift (δ) is reported in parts per million (ppm) downfield to tetramethylsilane (δ = 0). The coupling constant, *J*, is given in Hertz (Hz). The data are reported in the following order: chemical shift, multiplicity, coupling constant, number of protons, and proton assignment where applicable. The multiplicity of proton signals is reported as s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, b: broad, bs: broad singlet, and dd: doublet of doublets, dt: doublet of triplets, ddd: doublet of doublet of doublets. Mass spectra were obtained on a Shimadzu LCMS-2010 EV liquid chromatograph mass spectrometer and Quattro micro API Tandem Quadrupole System (Waters) using the electron spray ionization (ESI) method.

*4.1.1. 1-Chloro-3-(4-methoxyphenyl)isoquinoline (**7a**)*

A solution of compound **5a** (1.46 g, 5.83 mmol) and $POCl_3$ (20 mL) was heated using an oil bath set at 75 °C. Upon completion of the reaction, excess $POCl_3$ and volatile substances were evaporated by vacuum distillation. Saturated (sat.) $NaHCO_3$ solution (100 mL) was added to the residue obtained and was extracted with CH_2Cl_2. The organic extract was further washed with water, dried over anhydrous Na_2CO_3 and concentrated under reduced pressure. The residue was then purified by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **7a** as a white floppy solid (1.54 g, 98%). R_f = 0.45 (*n*-hexane:EtOAc = 3:1). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.41 (s, 1H, 4-H), 8.25 (dd, J = 8.4, 0.9 Hz, 8-H), 8.15–8.07 (m, 3H, 2′,6′-$(H)_2$, Ar-H), 7.90–7.85 (m, 1H, Ar-H), 7.78–7.73 (m, 1H, Ar-H), 7.12–7.07 (m, 2H, 3′,5′-$(H)_2$), 3.32 (s, 3H, OCH_3).

*4.1.2. 1-Chloro-3-(3,5-dimethoxyphenyl)isoquinoline (**7b**)*

The procedures described for **7a** were used with **5b** (894 mg, 3.17 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, EtOAc) to obtain compound **7b** as a white solid (925 mg, 97%). R_f = 0.32 (*n*-hexane:EtOAc = 4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36–8.32 (m, 1H, 8-H), 7.97 (d, *J* = 0.3 Hz, 1H, 4-H), 7.90–7.87 (m, 1H, 5-H), 7.77–7.72 (m, 1H, Ar-H), 7.69–7.63 (m, 1H, Ar-H), 7.27 (d, *J* = 2.1 Hz, 2H, 2′,6′-$(H)_2$), 6.54 (t, *J* = 2.4 Hz, 1H, 4′-H), 3.90 (s, 6H, 3′,5′-$(OCH_3)_2$). MS (ESI): *m/z* 300 $(M+H)^+$.

*4.1.3. 1-Chloro-3-(4-methoxy-2-methylphenyl)-6-methylisoquinoline (**7c**)*

The procedures described for **7a** were used with a mixture of **5c**, **S3** and polar byproducts (798 mg) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **7c** as a white solid (810 mg). R_f = 0.65 (*n*-hexane:EtOAc = 4:1). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.16 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.86 (s, 2H, Ar-H), 7.63 (dd, *J* = 8.7, 1.5 Hz, 1H, Ar-H), 7.47–7.44 (m, 1H, Ar-H), 6.91–6.87 (m, 2H, Ar-H), 3.80 (s, 3H, OCH_3), 2.54 (s, 3H, Ar-CH_3), 2.39 (s, 3H, Ar-CH_3).

*4.1.4. 1-Chloro-3-(3,4-dimethoxyphenyl)-6-methylisoquinoline (**7d**)*

The procedures described for **7a** were used with **5d** (1.41 g, 4.8 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **7d** as an off-white solid (1.45 g, 96%). R_f = 0.38 (*n*-hexane:EtOAc = 3:1). ^{1}H NMR (500 MHz, DMSO-*d6*): δ 8.33 (s, 1H, Ar-H), 8.13 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.75–7.71 (m, 2H, Ar-H), 7.58 (dd, *J* = 8.5, 1.5 Hz, 1H, Ar-H), 7.10 (d, *J* = 9.0 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 2.53 (s, 3H, 6-CH_3).

*4.1.5. 1-Chloro-3-(3,5-dimethoxyphenyl)-N,N-dimethylisoquinolin-7-amine (**7e**)*

The procedures described for **7a** were used with **5e** (800 mg, 2.46 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 4:1, 2:1, EtOAc) to obtain compound **7e** as a yellow solid (243 mg, 28%). R_f = 0.59 (*n*-hexane:EtOAc = 2:1). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.33 (s, 1H, Ar-H), 7.93 (d, *J* = 9.3 Hz, 1H, Ar-H), 7.57 (dd, *J* = 9.3, 2.7 Hz, 1H, Ar-H), 7.25 (d, *J* = 2.1 Hz, 2H, 2′,6′-$(H)_2$), 7.04 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.52 (t, *J* = 2.4 Hz, 1H, 4′-H), 3.83 (s, 6H, 3′,5′-$(OCH_3)_2$), 3.11 (s, 6H, $N(CH_3)_2$). MS (ESI): *m/z* 343 $(M+H)^+$.

*4.1.6. 1-Chloro-3-(3,5-dimethoxyphenyl)-6,7-dimethoxyisoquinoline (**7f**)*

The procedures described for **7a** were used with **5f** (607 mg, 1.77 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, EtOAc) to obtain compound **7f** as a light yellow solid (627 mg, 98%). R_f = 0.23 (*n*-hexane:EtOAc = 3:1). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.36 (s, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.27 (d, *J* = 2.1 Hz, 2H, 2′,6′-$(H)_2$), 6.56 (t, *J* = 2.4 Hz, 1H, 4′-H), 3.98 (s, 3H, OCH_3), 3.97 (s 3H, OCH_3), 3.33 (s, 6H, $(OCH_3)_2$).

*4.1.7. 3-(3-(4-Methoxyphenyl)isoquinolin-1-yl)phenol (**4aa**)*

A solution of 3-hydroxyphenylboronic acid (143 mg, 1.03 mmol) in MeOH (5 mL) was added to a solution of compound **7a** (200 mg, 0.74 mmol) in 1,2-dimethoxyethane (5 mL) followed by $Pd(PPh_3)_4$ (0) (43 mg, 0.03 mmol) and sat. Na_2CO_3 solution (2 mL). The reaction mixture was heated using an oil bath set at 90 °C. Upon completion of the reaction, water (150 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (100 mL x 8); the organic extract was washed with water (100 mL) and concentrated under reduced pressure. The residue thus obtained was purified by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **4aa** as an off-white solid (220 mg, 90%). R_f = 0.27 (*n*-hexane:EtOAc = 3:1). Melting point (mp): 146–148 °C. IR: 3236 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.68 (bs, 1H, OH), 8.32 (s, 1H, Ar-H), 8.21–8.18 (m, 2H, 2′,6′-$(H)_2$), 8.07–8.02 (m, 2H, Ar-H), 7.79–7.74 (m, 1H, Ar-H), 7.61–7.55 (m, 1H, Ar-H), 7.41–7.35 (m, 1H, Ar-H), 7.15–7.12 (m, 2H, Ar-H), 7.10–7.06 (m, 2H, 3′, 5′-$(H)_2$), 6.96–6.93 (m, 1H, 2″-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 160.1, 155.8, 150.1, 140.5, 137.8, 132.1, 130.3, 129.3, 128.5, 127.7, 127.1, 126.7, 125.5, 122.4, 117.6, 116.1, 115.5, 114.1, 55.3. MS (ESI): *m/z* 328.12 $(M+H)^+$, 329.50 $(M+2H)^+$, 327.33 $(M)^+$, 325.97 $(M-H)^-$.

*4.1.8. 1-(3-Methoxyphenyl)-3-(4-methoxyphenyl)isoquinoline (**4ab**)*

The procedures described for **4aa** were used with **7a** (200 mg, 0.74 mmol), a solution of 3-methoxyphenylboronic acid (146 mg, 0.96 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (43 mg, 0.03 mmol),

sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (CH_2Cl_2, *n*-hexane:EtOAc = 3:1) to obtain compound **4ab** as a transparent semi-solid, which solidified upon storage (260 mg, quantitative). R_f = 0.33 (*n*-hexane:EtOAc = 5:1). Mp: 117–120 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.34 (s, 1H, Ar-H), 8.22–8.19 (m, 2H, 2′,6′-$(H)_2$), 8.07 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.01 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.08–7.74 (m, 1H, Ar-H), 7.61–7.55 (m, 1H, Ar-H), 7.51 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.30–7.26 (m, 1H, Ar-H), 7.15–7.11 (m, 1H, 2″-H), 7.10–7.05 (m, 2H, 3′,5′-$(H)_2$), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1, 160.0, 159.5, 149.8, 141.2, 137.9, 132.2, 129.9, 129.2, 128.3, 127.5, 127.2, 126.5, 125.4, 122.7, 115.6, 114.6, 114.3, 114.0, 55.4, 55.3. MS (ESI): *m/z* 343.37 $(M+2H)^+$, 341.39 $(M)^+$.

*4.1.9. 3-(3-(4-Methoxyphenyl)isoquinolin-1-yl)aniline (**4ac**)*

The procedures described for **4aa** were used with **7a** (200 mg, 0.74 mmol), a solution of 3-aminophenylboronic acid monohydrate (161 mg, 1.03 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (43 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (8 mL), followed by column chromatography (*n*-hexane:EtOAc = 4:1) to obtain compound **4ac** as a light yellow semi-solid, which solidified upon prolonged heating and vacuum pumping (193 mg, 79%). R_f = 0.61 (*n*-hexane:EtOAc = 1:1). Mp: 119–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (m, 3H, Ar-H), 7.97 (d, *J* = 0.9 Hz, 1H, 4-H), 7.88 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.67–7.61 (m, 1H, Ar-H), 7.48–7.43 (m, 1H, Ar-H), 7.34–7.29 (m, 1H, Ar-H), 7.16–7.11 (m, 2H, Ar-H), 7.04–6.99 (m, 2H, 3′, 5′-$(H)_2$), 6.85–6.81 (m, 1H, 2″-H), 3.87 (s, 3H, OCH_3), 3.81 (bs, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 160.0, 149.8, 146.4, 140.9, 137.8, 132.3, 129.9, 129.0, 128.3, 127.7, 127.1, 126.3, 125.4, 120.7, 116.8, 115.3, 114.5, 114.0, 55.3. MS (ESI): *m/z* 327 $(M+H)^{+\cdot}$ MS (ESI): *m/z* 327.03 $(M+H)^+$, 328.44 $(M+2H)^+$, 326.41 $(M)^+$.

*4.1.10. 4-(3-(4-Methoxyphenyl)isoquinolin-1-yl)phenol (**4ad**)*

The procedures described for **4aa** were used with **7a** (200 mg, 0.74 mmol), a solution of 4-hydroxyphenylboronic acid (143 mg, 1.03 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (43 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 7:1) to obtain compound **4ad** as a white solid (151 mg, 62%). R_f = 0.48 (*n*-hexane:EtOAc = 3:1). Mp: 160 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.82 (bs, 1H, OH), 8.26 (s, 1H, 4-H), 8.22–8.18 (m, 2H, Ar-H), 8.09–8.02 (m, 2H, Ar-H), 7.77–7.72 (m, 1H, Ar-H), 7.62–7.54 (m, 3H, Ar-H), 7.10–7.06 (m, 2H, Ar-H), 6.99–6.96 (m, 2H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.3, 159.7, 158.5, 149.0, 138.1, 131.8, 130.67, 130.64, 128.3, 127.9, 127.44, 127.41, 125.0, 115.6, 114.6, 114.0, 55.7. MS (ESI): *m/z* 327.98 $(M+H)^+$, 329.50 $(M+2H)^+$,

327.37 $(M)^+$, 326.00 $(M-H)^-$.

*4.1.11. 1,3-Bis(4-methoxyphenyl)isoquinoline (**4ae**)*

The procedures described for **4aa** were used with **7a** (200 mg, 0.74 mmol), a solution of 4-methoxyphenylboronic acid (146 mg, 0.96 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (43 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by MPLC (*n*-hexane:CH_2Cl_2 = 3:1, 1:1) to obtain compound **4ae** as a white solid (208 mg, 82%). R_f = 0.5 (*n*-hexane:EtOAc = 4:1). Mp: 131–133 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19–8.12 (m, 3H, Ar-H), 7.96 (d, *J* = 0.9 Hz, 1H, 4-H), 7.88 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.80–7.75 (m, 2H, Ar-H), 7.67–7.62 (m, 1H, Ar-H), 7.50–7.44 (m, 1H, Ar-H), 7.11–7.06 (m, 2H, Ar-H), 7.04–6.99 (m, 2H, Ar-H), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1, 160.0, 159.8, 149.8, 138.0, 132.5, 132.3, 131.6, 129.9, 128.3, 127.6, 127.3, 126.4, 125.4, 114.2, 114.0, 113.7, 55.42, 55.36. MS (ESI): *m/z* 343.41 $(M+2H)^+$, 341.40 $(M)^+$, 327.79 $(M-CH_3+2H)^+$.

*4.1.12. 3-(3-(3,5-Dimethoxyphenyl)isoquinolin-1-yl)phenol (**4ba**)*

The procedures described for **4aa** were used with **7b** (200 mg, 0.66 mmol), a solution of 3-hydroxyphenylboronic acid (129 mg, 0.93 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (38 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by MPLC (*n*-hexane:EtOAc = 7:1) to obtain compound **4ba** as a transparent semi-solid, which solidified into a white solid upon prolonged heating (243 mg, quantitative). R_f = 0.68 (*n*-hexane:EtOAc = 2:1). Mp: 140–142 °C. IR: 3265 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.71 (bs, 1H, OH), 8.45 (s, 1H, 4-H), 8.11–8.05 (m, 2H, Ar-H), 7.82–7.77 (m, 1H, Ar-H), 7.66–7.60 (m, 1H, Ar-H), 7.41–7.36 (m, 3H, Ar-H), 7.17–7.14 (m, 2H, Ar), 6.97–6.93 (m, 1H, 2″-H), 6.58 (t, *J* = 2.2 Hz, 1H, 4′-H), 3.84 (s, 6H, 3′,5′-$(OCH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.0, 160.6, 156.0, 150.1, 141.6, 140.1, 137.6, 130.4, 129.2, 127.7, 127.3, 127.1, 126.0, 122.1, 117.5, 117.0, 116.0, 105.4, 100.9 55.5. MS (ESI): *m/z* 358 $(M+H)^+$, 356 $(M-H)^-$. MS (ESI): *m/z* 355.91 $(M-H)^-$.

*4.1.13. 3-(3,5-Dimethoxyphenyl)-1-(3-methoxyphenyl)isoquinoline (**4bb**)*

The procedures described for **4aa** were used with **7b** (200 mg, 0.66 mmol), a solution of 3-methoxyphenylboronic acid (142 mg, 0.93 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (38 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL), and 1,2-dimethoxy ethane (5 mL), followed by MPLC (*n*-hexane:EtOAc = 7:1) to obtain compound **4bb** as a transparent semi-solid, which solidified upon storage (186 mg, 75%). R_f = 0.61 (*n*-hexane:EtOAc = 3:1). Mp: 95–98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (dd, *J* = 8.4, 0.9 Hz, 1H, Ar-H), 8.04 (s, 1H, 4-H), 7.93 (d, *J* = 8.1 Hz, 1H, Ar-H),

7.71–7.65 (m, 1H, Ar-H), 7.54–7.48 (m, 1H, Ar-H), 7.46–7.43 (m, 1H, Ar-H), 7.38–7.34 (m, 4H, Ar-H), 7.08–7.04 (m, 1H, 2″-H), 6.53(t, *J* = 2.2 Hz, 1H, 4′-H), 3.89 (s, 9H, 3′,5′, 3″-$(OCH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.1, 160.0, 159.5, 149.8, 141.7, 141.1, 137.7, 130.1, 129.2, 127.5, 127.4, 127.0, 125.9, 122.7, 116.1, 115.6, 114.3, 105.2, 100.7, 55.5, 55.4. MS (ESI): *m/z* 372 $(M+H)^+$. MS (ESI): *m/z* 373.26 $(M+2H)^+$, 371.38 $(M)^+$, 357.93 $(M-CH_3+2H)^+$.

*4.1.14. 3-(3-(3,5-Dimethoxyphenyl)isoquinolin-1-yl)aniline (**4bc**)*

The procedures described for **4aa** were used with **7b** (200 mg, 0.66 mmol), a solution of 3-aminophenylboronic acid monohydrate (145 mg, 0.93 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (38 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (10 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **4bc** as a light yellow transparent semi-solid, which solidified upon storage (212 mg, 89%). R_f = 0.22 (*n*-hexane:EtOAc = 2:1). Mp: 58–62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.01 (s, 1H, 4-H), 7.91 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.69–7.64 (m, 1H, Ar-H), 7.52–7.47 (m, 1H, Ar-H), 7.36–7.29 (m, 3H, Ar-H), 7.16–7.11 (m, 2H, Ar-H), 6.85–6.81 (m, 1H, 2″-H), 6.52 (t, *J* = 2.2 Hz, 1H, 4′-H), 3.89 (s, 6H, 3′,5′-$(OCH_3)_2$), 3.81 (s, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.1, 160.5, 149.8, 146.4, 141.9, 140.8, 137.6, 130.0, 129.0, 127.8, 127.4, 126.9, 126.0, 120.7, 116.9, 116.0, 115.3, 105.3, 100.6, 55.5. MS (ESI): *m/z* 357 $(M+H)^+$. MS (ESI): *m/z* 356.93 $(M+H)^+$.

*4.1.15. 4-(3-(3,5-Dimethoxyphenyl)isoquinolin-1-yl)phenol (**4bd**)*

The procedures described for **4aa** were used with **7b** (200 mg, 0.66 mmol), a solution of 4-hydroxyphenylboronic acid (129 mg, 0.93 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (38 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (7 mL), followed by MPLC (*n*-hexane:EtOAc = 7:1) to obtain compound **4bd** as a transparent semi-solid, which solidified into a white solid upon prolonged heating and vacuum pumping (69 mg, 28%). R_f = 0.62 (*n*-hexane:EtOAc = 2:1). Mp: 185–188 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (dd, *J* = 8.4, 0.9 Hz, 1H, Ar-H), 8.00 (s, 1H, 4-H), 7.91 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.72–7.65 (m, 3H, 2″,6″-$(H)_2$, Ar-H), 7.54–7.48 (m, 1H, Ar-H), 7.36 (d, *J* = 2.4 Hz, 2H, 2′,6′-$(H)_2$), 6.99–6.95 (m, 2H, 3″,5″-$(H)_2$), 6.53 (t, *J* = 2.2 Hz, 1H, 4′-H), 5.34 (s, 1H, OH), 3.89 (s, 6H, 3′,5′-$(OCH_3)_2$). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 161.3, 159.7, 158.6, 148.7, 141.5, 138.0, 131.8, 130.7, 130.5, 128.2, 127.9, 127.4, 125.6, 115.68, 115.64, 105.1, 100.9, 55.8. MS (ESI): *m/z* 358 $(M+H)^+$, 356 $(M-H)^-$. MS (ESI): *m/z* 357.98 $(M+H)^+$, 355.95 $(M-H)^-$.

*4.1.16. 3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)phenol (**4ca**)*

The procedures described for **4aa** were used with **7c** (350 mg, 1.17 mmol), a solution of 3-

hydroxyphenylboronic acid (227 mg, 1.64 mmol) in 5 mL MeOH, $Pd(PPh_3)_4$ (0) (68 mg, 0.05 mmol), sat. Na_2CO_3 solution (3 mL) and 1,2-dimethoxy ethane (10 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **4ca** as a transparent semi-solid, which solidified into a white solid upon prolonged heating and vacuum pumping (394 mg, 94%). R_f = 0.28 (*n*-hexane:EtOAc = 3:1). Mp: 104–107 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.65 (s, 1H, OH), 7.97 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 7.50–7.44 (m, 2H, Ar-H), 7.35 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.10–7.08 (m, 2H, Ar-H), 6.93–6.86 (m, 3H, Ar-H), 3.80 (s, 3H, OCH_3), 2.51 (≈3H, Ar-CH_3, overlapped with DMSO peaks), 2.42 (s, 3H, Ar-CH_3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.48–7.45 (m, 1H, Ar), 7.35–7.28 (m, 2H, Ar-H), 7.22–7.19 (m, 1H, Ar-H), 7.12–7.11 (m, 1H, Ar-H), 6.86–6.80 (m, 3H, Ar-H), 6.08 (bs, 1H, OH), 3.83 (s, 3H, OCH_3), 2.55 (s, 3H, Ar-CH_3), 2.44 (s, 3H, Ar-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9, 159.3, 156.1, 152.2, 140.8, 139.9, 137.7, 133.1, 131.3, 129.2, 129.0, 127.7, 125.8, 123.7, 121.8, 119.4, 117.8, 116.4, 116.1, 111.2, 55.2, 21.9, 20.8. MS (ESI): *m/z* 356 $(M+H)^+$, 354 $(M-H)^-$. MS (ESI): *m/z* 356.04 $(M+H)^+$, 353.95 $(M-H)^-$.

*4.1.17. 3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)aniline (**4cc**)*

The procedures described for **4aa** were used with **7c** (395 mg, 1.32 mmol), a solution of 3-aminophenylboronic acid monohydrate (288 mg, 1.85 mmol) in 5 mL MeOH, $Pd(PPh_3)_4$ (0) (77 mg, 0.06 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (8 mL), followed by column chromatography (*n*-hexane:EtOAc = 7:1) to obtain compound **4cc** as a brown semi-solid (368 mg, 78%). R_f = 0.28 (*n*-hexane:EtOAc = 3:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.50–7.47 (m, 1H, Ar-H), 7.34–7.31 (m, 1H, Ar-H), 7.29–7.25 (m, ≈1H. Ar-H, partially overlapped with $CHCl_3$ peak), 7.09 (dt, *J* = 7.6, 1.2 Hz, 1H, Ar-H), 7.05 (t, *J* = 1.8 Hz, 1H, Ar-H), 6.83–6.81 (m, 2H, Ar-H), 6.77 (ddd, *J* = 8, 2.4, 0.8 Hz, 1H, Ar-H), 3.83 (s, 3H, OCH_3), 3.30 (bs, 2H, NH_2), 2.54 (s, 3H, Ar-CH_3), 2.46 (s, 3H, Ar-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 159.3, 152.4, 146.3, 140.7, 140.4, 137.8, 137.7, 133.5, 131.3, 129.0, 127.6, 125.9, 123.5, 120.7, 118.9, 116.8, 116.1, 115.3, 111.2, 55.3, 21.8, 21.0. MS (ESI): *m/z* 355 $(M+H)^+$. MS (ESI): *m/z* 354.99 $(M+H)^+$, 356.54 $(M+2H)^+$, 354.39 $(M)^+$.

*4.1.18. 3-(3-(3,4-Dimethoxyphenyl)-6-methylisoquinolin-1-yl)phenol (**4da**)*

The procedures described for **4aa** were used with **7d** (200 mg, 0.63 mmol), solution of 3-hydroxyphenylboronic acid (132 mg, 0.95 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (37 mg, 0.03 mmol), sat. Na_2CO_3 solution (3 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, 1:1) to obtain compound **4da** as a light green semi-solid, which solidified

upon prolonged heating and vacuum pumping (260 mg, quantitative). R_f = 0.55 (*n*-hexane:EtOAc = 1:1). Mp: 96–99 °C. IR: 3411 cm^{-1}. ^1H NMR (300 MHz, DMSO-*d6*): δ 9.66 (bs, 1H, OH), 8.25 (s, 1H, Ar-H), 7.93 (b, *J* = 8.7 Hz, 1H, Ar-H), 7.82–7.79 (m, 3H, Ar-H), 7.44–7.35 (m, 2H, Ar-H), 7.14–7.07 (m, 3H, Ar), 6.96–6.92 (m, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.52 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 156.1, 150.3, 149.4, 149.0, 140.9, 140.0, 138.0, 132.6, 129.2, 127.6, 125.9, 124.1, 121.8, 119.9, 117.6, 116.1, 115.8, 111.1, 110.5, 55.9, 21.9. MS (ESI): *m/z* 371.93 $(M+H)^+$.

*4.1.19. 3-(3,4-Dimethoxyphenyl)-1-(3-methoxyphenyl)-6-methylisoquinoline (**4db**)*

The procedures described for **4aa** were used with **7d** (200 mg, 0.63 mmol), a solution of 3-methoxyphenylboronic acid (145 mg, 0.95 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (37 mg, 0.03 mmol), sat. Na_2CO_3 solution (3 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (CH_2Cl_2, *n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **4db** as a transparent semi-solid, which solidified into a white solid upon storage (248 mg, quantitative). R_f = 0.23 (*n*-hexane:EtOAc = 3:1). Mp: 58–61 °C. ^1H NMR (300 MHz, DMSO-*d6*): δ 8.28 (s, 1H, Ar-H), 7.92 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.83–7.79 (m, 3H, Ar-H), 7.53–7.48 (m, 1H, Ar-H), 7.42 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-H), 7.30–7.26 (m, 2H, Ar-H), 7.14–7.07 (m, 2H, Ar-H), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.52 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 159.5, 149.9, 149.5, 149.1, 141.4, 140.3, 138.2, 132.7, 129.1, 128.9, 127.3, 126.1, 123.9, 122.7, 119.5, 115.6, 114.5, 114.2, 111.1, 110.2, 56.0, 55.9, 55.4. MS (ESI): *m/z* 385.94 $(M+H)^+$, 387.58 $(M+2H)^+$, 385.37 $(M)^+$.

*4.1.20. 3-(3-(3,4-Dimethoxyphenyl)-6-methylisoquinolin-1-yl)aniline (**4dc**)*

The procedures described for **4aa** were used with **7d** (200 mg, 0.63 mmol), a solution of 3-aminophenylboronic acid monohydrate (148 mg, 0.95 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (37 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by MPLC (*n*-hexane:EtOAc = 5:1) to obtain compound **4dc** as a yellow semi-solid, which solidified upon prolonged heating and vacuum pumping (202 mg, 85%). R_f = 0.51 (*n*-hexane:EtOAc = 1:1). Mp: 122–126 °C. ^1H NMR (300 MHz, DMSO-*d6*): δ 8.22 (s, 1H, Ar-H), 7.94 (d, *J* = 9 Hz, 1H, Ar-H), 7.82–7.79 (m, 3H, Ar-H), 7.42–7.38 (m, 1H, Ar-H), 7.23–7.18 (m, 1H, Ar-H), 7.08 (d, *J* = 9.3 Hz, 1H, Ar-H), 6.92–6.91 (m, 1H, Ar-H), 6.83–6.79 (m, 1H, Ar-H), 6.74–6.70 (m, 1H, 2″-H), 5.27 (s, 2H, NH_2), 3.87 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.51 (≈3H, Ar-CH_3, overlapped with DMSO peaks). ^1H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.78–7.72 (m, 2H, Ar-H), 7.65 (s, 1H, Ar-H), 7.34–7.26 (m, 2H, Ar-H), 7.16–7.10 (m, 2H, Ar-H), 6.98 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.85–6.81 (m, 1H, 2″-H), 4.01 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.79 (bs, 2H, NH_2),

2.54 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.1, 149.9, 149.5, 149.1, 146.3, 141.0, 140.2, 138.1, 132.8, 129.0, 128.8, 127.6, 126.0, 123.9, 120.7, 119.6, 116.8, 115.2, 114.5, 111.1, 110.2, 56.0, 55.9, 21.8. MS (ESI): *m/z* 371 $(M+H)^+$. MS (ESI): *m/z* 371.01 $(M+H)^+$, 372.42 $(M+2H)^+$, 370.39 $(M)^+$.

*4.1.21. 4-(3-(3,4-Dimethoxyphenyl)-6-methylisoquinolin-1-yl)phenol (**4dd**)*

The procedures described for **4aa** were used with **7d** (200 mg, 0.63 mmol), a solution of 4-hydroxyphenylboronic acid (114 mg, 0.82 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (37 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (CH_2Cl_2, *n*-hexane:EtOAc = 4:1) to obtain compound **4dd** as a light hay-colored semi-solid, which solidified upon storage (236 mg, 99%). R_f = 0.22 (*n*-hexane:EtOAc = 2:1). Mp: 190–193 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.79 (bs, 1H, OH), 8.19 (s, 1H, Ar-H), 7.97 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.81–7.78 (m, 3H, Ar-H), 7.61–7.58 (m, 2H, 2″,6″-$(H)_2$), 7.42–7.39 (m, 1H, Ar-H), 7.08 (d, *J* = 9 Hz, 1H, Ar-H), 6.98–6.95 (m, 2H, 3″,5″-$(H)_2$), 3.87 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.51–2.48 (3H, 6-CH_3, overlapped with DMSO peaks). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.78 (d, *J* = 2 Hz, 1H, Ar-H), 7.72 (dd, *J* = 8.4, 2 Hz, 1H, Ar-H), 7.69–7.66 (m, 3H, 2″,6″-$(H)_2$, Ar-H, overlapped), 7.32–7.29 (m, 1H, Ar-H), 7.00–6.95 (m, 3H, Ar-H, 3″,5″-$(H)_2$, overlapped), 5.35 (bs, 1H, OH), 4.01 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 2.55 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 159.3, 158.5, 149.9, 149.3, 149.2, 140.4, 138.4, 132.2, 131.8, 130.7, 129.5, 127.3, 126.7, 123.5, 119.7, 115.5, 113.8, 112.2, 110.5, 56.06, 56.02, 21.8. MS (ESI): *m/z* 371.92 $(M+H)^+$, 369.93 $(M-H)^-$.

*4.1.22. 3-(3,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-6-methylisoquinoline (**4de**)*

The procedures described for **4aa** were used with **7d** (200 mg, 0.63 mmol), a solution of 4-methoxyphenylboronic acid (126 mg, 0.82 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (37 mg, 0.03 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (CH_2Cl_2, *n*-hexane:EtOAc = 5:1) to obtain compound **4de** as a transparent semi-solid, which solidified into an off-white solid upon storage (231 mg, 94%). R_f = 0.32 (*n*-hexane:EtOAc = 3:1). Mp: 84–88 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.22 (s, 1H, Ar-H), 7.95 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.82–7.79 (m, 3H, Ar-H), 7.73–7.69 (m, 2H, 2″,6″-$(H)_2$), 7.42 (dd, *J* = 8.4, 1.5 Hz, 1H, Ar-H), 7.17–7.13 (m, 2H, 3″,5″-$(H)_2$), 7.09 (d, *J* = 9.3 Hz, 1H, Ar-H), 3.87 (s, 6H, $(OCH_3)_2$), 3.82 (s, 3H, OCH_3), 2.52 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.0, 159.4, 149.9, 149.5, 149.1, 140.1, 138.3, 132.9, 132.6, 131.5, 128.8, 127.4, 126.3, 123.9, 119.5, 114.0, 113.7, 111.2, 110.2, 56.03, 56.0, 55.4, 21.8. MS (ESI): *m/z* 386.08 $(M+H)^+$, 387.62 $(M+2H)^+$, 385.34 $(M)^+$.

*4.1.23. 1-(3-Aminophenyl)-3-(3,5-dimethoxyphenyl)-N,N-dimethylisoquinolin-7-amine (**4ec**)*

The procedures described for **4aa** were used with **7e** (197 mg, 0.57 mmol), a solution of 3-aminophenylboronic acid monohydrate (125 mg, 0.80 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (33 mg, 0.02 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (10 mL), followed by MPLC (*n*-hexane:EtOAc = 5:1) to obtain compound **4ec** as a yellow semi-solid, which solidified upon prolonged heating (157 mg, 68%). Mp: 101–103 °C. IR: 3473 cm^{-1}. ^1H NMR (300 MHz, DMSO-*d6*): δ 8.21 (s, 1H, Ar-H), 7.91 (d, *J* = 9 Hz, 1H, Ar-H), 7.51–7.47 (m, 1H, Ar-H), 7.34 (d, *J* = 2.4 Hz, 2H, 2′, 6′-$(H)_2$), 7.19 (t, *J* = 7.8 Hz, 1H, Ar-H), 7.13 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.99 (t, *J* = 1.9 Hz, 1H, Ar-H), 6.90–6.87 (m, 1H, Ar-H), 6.70–6.66 (m, 1H, 2″-H), 6.51 (t, *J* = 2.2 Hz, 1H, 4′-H), 5.25 (s, 2H, NH_2), 3.82 (s, 6H, 3′,5′-$(OCH_3)_2$), 2.97 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.0, 158.0, 149.1, 146.4, 146.3, 142.3, 141.5, 130.5, 128.8, 128.2, 127.5, 120.4, 119.9, 116.8, 115.9, 115.0, 105.4, 104.8, 99.9, 55.5, 40.6. MS (ESI): *m/z* 400 $(M+H)^+$. MS (ESI): *m/z* 399.97 $(M+H)^+$.

*4.1.24. 3-(3-(3,5-Dimethoxyphenyl)-6,7-dimethoxyisoquinolin-1-yl)phenol (**4fa**)*

The procedures described for **4aa** were used with **7f** (200 mg, 0.55 mmol), a solution of 3-hydroxyphenylboronic acid (100 mg, 0.72 mmol) in 3 mL MeOH, $Pd(PPh_3)_4$ (0) (32 mg, 0.02 mmol), sat. Na_2CO_3 solution (2 mL) and 1,2-dimethoxy ethane (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **4fa** as a semi-solid, which solidified into a white solid upon prolonged heating and vacuum pumping (147 mg, 63%). R_f = 0.46 (*n*-hexane:EtOAc = 1:1). Mp: 174–176 °C. IR: 3302 cm^{-1}. ^1H NMR (300 MHz, DMSO-*d6*): δ 9.65 (bs, 1H, OH), 8.28 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.40–7.35 (m, 4H, Ar-H), 7.21–7.19 (m, 2H, Ar-H), 6.93–6.89 (m, 1H, 2″-H), 6.55 (t, *J* = 2.1 Hz, 4′-H), 3.97 (s, 3H, OCH_3), 3.83 (s, 6H, $(OCH_3)_2$), 3.79 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9, 158.4, 156.6, 153.1, 150.1, 149.4, 141.9, 140.2, 134.5, 129.2, 121.9, 121.3, 117.7, 116.3, 116.1, 105.7, 105.3, 105.2, 100.8, 56.1, 55.9, 55.4. MS (ESI): *m/z* 417.97 $(M+H)^+$, 419.38 $(M+H)^+$, 417.33 $(M)^+$, 415.88 $(M-H)^-$.

*4.1.25. 3-(3-(3-(4-Methoxyphenyl)isoquinolin-1-yl)phenoxy)-N,N-dimethylpropan-1-amine (**8a**)*

Compound **4aa** (149 mg, 0.45 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF, 7 mL). Potassium carbonate (378 mg, 2.73 mmol) and 96% 3-dimethylamino-1-propylchloride hydrochloride (216 mg, 1.31 mmol) were added to the solution. The reaction mixture was heated using an oil bath set at 100 °C. Water (100 mL) was poured into the reaction mixture; the product was extracted with CH_2Cl_2 (100 mL x 9), washed with water (100 mL) and concentrated under reduced pressure. The residue thus obtained was purified by MPLC (*n*-hexane:EtOAc = 3:1, EtOAc,

MeOH) to obtain **8a** as a transparent semi-solid (71 mg, 37%). The free amine **8a** was dissolved in acetone (5 mL). *c*-HCl (11 drops) was added, and the reaction mixture was stirred for several hours. The solvent and volatile substances were evaporated by vacuum distillation in presence of heat to obtain **8a**·HCl salt as a yellow semi-solid, which solidified upon prolonged heating. R_f = 0.34 (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19–8.14 (m, 2H, 2′,6′-$(H)_2$), 8.10 (dd, *J* = 8.4, 0.9 Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.89 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.68–7.62 (m, 1H, Ar-H), 7.49–7.41 (m, 2H, Ar-H), 7.35–7.32 (m, 2H, Ar-H), 7.07–6.99 (m, 3H, 3′,5′,2″-$(H)_3$), 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 2.47 (t, *J* = 7.3 Hz, 2H, CH_2N), 2.25 (s, 6H, $N(CH_3)_2$), 2.00 (quintet, 2H, $OCH_2C\underline{H}_2$).

*4.1.26. 3-(3-(3-(4-Methoxyphenyl)isoquinolin-1-yl)phenoxy)-N,N-dimethylpropan-1-amine hydrochloride (**8a**·HCl)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 12.03 (bs, 1H, NH), 8.17–8.05 (m, 6H, Ar-H), 7.82–7.77 (m, 1H, Ar-H), 7.57–7.49 (m, 2H, Ar-H), 7.26–7.21 (m, 2H, Ar-H, partially overlapped with $CHCl_3$ peak), 7.08 (d, *J*= 6.7 Hz, 2H, Ar-H), 4.39 (s, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 3.37 (s, 2H, CH_2N), 2.86 (s, 6H, $N(CH_3)_2$), 2.45 (s, 2H, $OCH_2C\underline{H}_2$). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.5, 159.3, 158.7, 148.3, 138.3, 131.7, 130.6, 130.0, 128.8, 128.2, 128.1, 127.5, 125.1, 123.0, 116.5, 116.0, 115.7, 114.6, 65.6, 55.7, 54.4, 42.4, 24.3. MS (ESI): *m/z* 412.89 $(M+H)^+$, 328.21 $(M-(CH_2)_3NMe_2+2H)^+$.

*4.1.27. 2-(3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylethanamine (**8b**)*

The procedures described for compound **8a** were used with compound **4ca** (143 mg, 0.41 mmol), 2-chloro-*N*,*N*-dimethylamine hydrochloride (90 mg, 0.62 mmol), potassium carbonate (174 mg, 1.25 mmol) and DMF (7 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **8b** as a transparent semi-solid (125 mg, 69%). The free amine **8b** was converted to **8b**·HCl salt, a yellow semi-solid, which solidified upon prolonged heating and vacuum pumping. R_f = 0.39 (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.61 (d, *J* = 0.9 Hz, 1H, Ar-H), 7.51–7.48 (m, 1H, Ar), 7.40 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.35–7.29 (m, 3H, Ar), 7.05–7.01 (m, 1H, 2″-H), 6.84–6.82 (m, 2H, Ar-H), 4.13 (t, *J* = 5.8 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.75 (t, *J* = 5.8 Hz, 2H, CH_2N), 2.55 (s, 3H, Ar-CH_3),2.48 (s, 3H, Ar-CH_3), 2.34 (s, 6H, $N(CH_3)_2$). MS (ESI): *m/z* 427 $(M+H)^+$.

*4.1.28. 2-(3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylethanamine hydrochloride (**8b**·HCl)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 12.44 (bs, 1H, NH), 8.18 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.87 (s, 2H, Ar-H), 7.69–7.53 (m, 4H, Ar-H), 7.28–7.26 (1H, Ar-H, partially overlapped with $CHCl_3$ peak), 6.91–6.88 (m, 3H, Ar-H), 4.87 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2N), 2.96 (s, 6H, $N(CH_3)_2$), 2.70 (s, 3H, Ar-CH_3), 2.45 (s, 3H, Ar-CH_3). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 160.6, 157.8, 157.5, 139.1, 138.7, 132.5, 132.4, 130.3, 128.6, 126.9, 123.9, 123.4, 117.2, 116.3, 111.9, 63.2, 55.8, 55.4, 43.1, 22.2, 20.7. MS (ESI): *m/z* 426.85 $(M+H)^+$.

*4.1.29. 3-(3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylpropan-1-amine (**8c**)*

The procedures described for compound **8a** were used with compound **4ca** (175 mg, 0.51 mmol), 96% 3-dimethylamino-1-propylchloride hydrochloride (127 mg, 0.76 mmol), potassium carbonate (212 mg, 1.53 mmol) and DMF (7 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **8c** as a transparent semi-solid (221 mg, 97%). The free amine **8c** was converted to **8c**·HCl salt, a semi-solid, which solidified into a yellow solid upon prolonged heating. R_f = 0.19 (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.51–7.48 (m, 1H, Ar-H), 7.43–7.26 (m, 4H, Ar-H, partially overlapped with $CHCl_3$ peak), 7.03–6.99 (m, 1H, 2″-H), 6.84–6.81 (m, 2H, Ar-H), 4.07 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.55 (s, 3H, Ar-CH_3), 2.48 (s, 3H, Ar-CH_3, overlapped), 2.45 (t, *J* = 7.8 Hz, 2H, CH_2N, overlapped), 2.24 (s, 6H, $N(CH_3)_2$), 1.97 (quintet, *J* = 6.8 Hz, 2H, $OCH_2C\underline{H}_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 159.2, 158.6, 152.6, 141.3, 140.3, 137.8, 137.7, 133.6, 131.3, 129.2, 129.1, 127.3, 126.0, 123.4, 122.8, 119.0, 116.16, 116.12, 114.6, 111.2, 65.6, 55.7, 55.3, 43.8, 25.9, 21.8, 21.0.

*4.1.30. 3-(3-(3-(4-Methoxy-2-methylphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylpropan-1-amine hydrochloride (**8c**·HCl)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 11.88 (bs, 1H, NH), 8.16 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.86 (s, 2H, Ar-H), 7.67 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.57–7.50 (m, 3H, Ar-H), 7.26–7.21 (m, 2H, Ar-H, partially overlapped with $CHCl_3$ peak), 6.91–6.88 (m, 2H, Ar-H), 4.40 (s, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.37 (s, 2H, CH_2N), 2.83 (s, 3H, Ar-CH_3), 2.70 (s, 3H, Ar-CH_3), 2.45 (s, 6H, $N(CH_3)_2$), 2.12 (s, 2H, $OCH_2C\underline{H}_2$). MS (ESI): *m/z* 441.15 $(M+H)^+$.

*4.1.31. 2-(4-(3-(3,4-dimethoxyphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylethanamine (**8d**)*

The procedures described for compound **8a** were used with compound **4dd** (123 mg, 0.33

mmol), 2-chloro-*N*,*N*-dimethylamine hydrochloride (72 mg, 0.49 mmol), potassium carbonate (137 mg, 0.99 mmol) and DMF (7 mL), followed by MPLC (*n*-hexane:EtOAc = 5:1, 3:1, EtOAc, MeOH) to obtain compound **8d** as a rust colored semi-solid (108 mg, 73%). The free amine **8d** was converted to **8c**·HCl salt, a yellow solid. R_f = 0.26 (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.80 (d, *J* = 2.1 Hz, 1H, Ar), 7.76–7.72 (m, 3H, Ar-H), 7.66 (s, 1H, Ar-H), 7.30 (dd, *J* = 8.7, 1.5 Hz, 1H, Ar-H), 7.12–7.07 (m, 2H, 3″,5″-$(H)_2$), 6.98 (d, *J* = 8.4 Hz, 1H, Ar-H), 4.18 (t, *J* = 5.7 Hz, 2H, OCH_3), 4.01 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 2.79 (t, *J* = 5.7 Hz, 2H, CH_2N), 2.54 (s, 3H, 6-CH_3),2.38 (s, 6H, $N(CH_3)_2$).

*4.1.32. 2-(4-(3-(3,4-Dimethoxyphenyl)-6-methylisoquinolin-1-yl)phenoxy)-N,N-dimethylethanamine hydrochloride (**8d**·HCl)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 12.70 (bs, 1H, NH), 12.70 (bs, 1H, NH), 8.04 (s, 2H, Ar-H), 7.84–7.55 (m, 6H, Ar-H), 7.20 (s, 2H, Ar-H), 7.02 (s, 1H, Ar-H), 4.67 (s, 2H, OCH_2), 4.05 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.56 (s, H, CH_2N), 2.97 (s, 6H, $N(CH_3)_2$), 2.66 (s, 3H, 6-CH_3). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 159.1, 158.4, 150.4, 149.3, 139.0, 132.3, 130.9, 127.9, 126.9, 123.6, 120.6, 115.2, 112.3, 111.3, 63.1, 56.18, 56.12, 55.5, 43.2, 22.0. MS (ESI): *m/z* 443.05 $(M+H)^+$.

4.2. Cytotoxicity

Five lines of cells, MCF-10A, T47D, DU145, HCT-15, and HeLa were cultured according to the supplier's instructions. The cells were seeded in 96-well plates at a density of 2×10^5 or 1×10^5 cells per well and incubated overnight in 0.1 mL of media supplemented with 10% fetal bovine serum (FBS, Hyclone, USA) in a 5% CO_2 incubator at 37 °C. After FBS starvation for 4 h, the culture medium in each well was replaced with 0.1 mL aliquots of medium containing graded concentrations of the test compounds followed by additional incubation for 72 h . On day 4, 5 μL cell counting kit-8 solution (Dojindo, Japan) was added to each well and incubated for an additional 4 h under the same conditions. The absorbance of each well was determined using an Automatic ELISA Reader System (Bio-Rad3550) at a wavelength of 450 nm. To determine the IC_{50} values, the absorbance readings at 450 nm were fitted to a four-parameter logistic equation using Table Curve 2D (SPSS Inc., USA). The positive control, Adriamycin, was purchased from Sigma (USA).

4.3. Topo I-mediated DNA relaxation assay

A mixture of pBR322 (100 ng) and recombinant human DNA topo I (0.4 units; Topo-GEN Inc., USA) was incubated with and without the test compounds at 37 °C for 30 min in relaxation buffer [10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine,

5% glycerol]. The reaction was quenched by adding 2.5 μL of stop solution containing 5% sarcosyl, 0.0025% bromophenol blue and 25% glycerol in a final volume of 10 μL. The DNA samples were electrophoresed on 1% agarose gel at 15V for 7 h with TAE (Tris-acetate-EDTA) as the running buffer. The gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). The DNA bands were visualized by transillumination with UV light, and the supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

4.4. Topo IIα-mediated DNA relaxation assay

A mixture of supercoiled plasmid DNA pBR322 (200 ng) and human DNA topo IIα (1 unit; Usb Corp., USA) was incubated with and without the test compounds in assay buffer [10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 mg/mL bovine serum albumin] for 30 min at 30 °C. The reaction was quenched by adding 3 mL of 7 mM EDTA in a final volume of 20 mL. The DNA samples were electrophoresed on 1% agarose gel at 25 V for 4 h with TAE as the running buffer. The gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). The DNA bands were visualized by transillumination with UV light; the supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

4.5. Tubulin polymerization assay

Purified porcine brain tubulin and PEM buffer [80 mM Na-PIPES (pH 6.9), 1 mM $MgCl_2$, 30 % glycerol and 1 mM EGTA], PTX and GTP required for *in vitro* tubulin polymerization assay were purchased from Cytoskeleton (Denver, CO). The assay was performed according to the protocol by manufacturer and previously published method [41]. Porcine brain tubulin (final concentration 1.5 mg/mL) was dissolved in cold PEM buffer containing 1 mM GTP (G-PEM buffer). Each compound and PTX were dissolved in DMSO and diluted by PEM buffer to attain desired concentration. Each diluted compound solution was placed in pre-warmed 96-well plate and incubated at 37 °C for 2 min before addition of tubulin solution. DMSO- and PTX-treated tubulin solutions (1.5 mg/mL) were used as vehicle and tubulin polymerization control, respectively. The degree of tubulin polymerization was evaluated spectroscopically. The change in absorbance at 340 nm was measured every kinetic cycle (per minute) for 61 min at 37 °C using a multimode microplate reader (TECAN™, Switzerland).

4.6. Topo I-DNA cleavage complex assay

Cleavage complex assay was carried out according to the previously reported methods[42]

with minor modifications. Supercoiled DNA (pBR322, 100 ng; Thermo, USA) was reacted with 3 units topo I (TopoGen, USA) for 10 min before addition of the test compounds. The reaction mixture was incubated at 37 °C for 20 min; the reaction was stopped by the addition of 0.5% sarcosyl, followed by digestion with proteinase K at 45 °C for 30 min. After the addition of loading buffer, the reaction mixture was heated for 2 min at 70 °C and electrophoresed on a 1.5% agarose gel in TAE buffer containing 0.5 μg/mL ethidium bromide, followed by destaining the gel with water for 20 min. The gel was visualized using an AlphaImager™ (Alpha Innotech Corporation).

4.7. Comet assay

To evaluate DNA damage, the comet assay was performed using single-cell gel electrophoresis with a Trevigen kit (Gaithersburg, USA) according to the method previously reported[43]. Briefly, T47D cells, seeded at a density of 1 x 10^5 cells per well in six-well plates, were treated with CPT and compound **4cc** at 5 μM and 10 μM, respectively, for 24 h and harvested by trypsinization, followed by resuspension of cells in 1 mL of ice-cold PBS. Then, 8 mL resuspended cells were mixed with 80 μL low melting agarose at 37 °C, spread on slides and solidified in the dark for 40 min at 4 °C. Slides were lysed in ice-cold lysis solution in the dark for 30 min at 4 °C and then submerged in a fresh alkaline solution (pH > 13) at room temperature (r.t.) for 30 min to allow alkaline unwinding. Electrophoresis was performed under alkaline conditions for 20 min at 15 V. Slides were rinsed twice with distilled water, once with 70% ethanol and stained with SYBR Green (Trevigen INC., USA) in TE buffer for 5 min in the dark at 4 °C. Comet images were obtained using an inverted fluorescence microscope (Zeiss, Axiovert 200) at 10 magnification; percent DNA in tail was analyzed by Komet 5.0 software (Kinetic imaging Ltd, UK). Data were represented both by imaging and graphically by randomly selecting comet lengths of T47D cells.

4.8. DNA unwinding assay

The DNA unwinding capacity of compound **4cc** was analyzed using a DNA unwinding kit (TopoGEN, Port Orange, FL, USA) according to the manufacturer's instructions. Briefly, 100 ng pHOT1 plasmid DNA was treated with 3 units topo I (TopoGEN) in 20 mL of reaction buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) for 30 min at 37 °C. Relaxed plasmids were then incubated in the presence of compound **4cc** at 37 °C for an additional 30 min. Amsacrine (100, 200, 500, and 1000 μM) was used as a positive control. The reaction was terminated by adding 1% SDS and loading dye at the end of the incubation; the reaction product was resolved on 1% agarose gels at 15 V/cm for 12–15 h. After electrophoresis, the gels were stained in TAE buffer with ethidium bromide

for 30 min and visualized using an AlphaImager™ (Alpha Innotech Corporation).

4.9. Competitive Hoechst assay

ctDNA (30 μM) was mixed with Hoechst 33342 (1 μg/mL) in final concentrations in the 10 mM tris buffer solution (pH 7.2), followed by incubation for 30 min at r.t. with shaking. Diverse concentration of compound **4cc** was added to the white 96 well plate (NuncTM, Denmark) containing the mixture of ctDNA and Hoechst 33342. Untreated control was treated with same amount of DMSO instead of compound and the final DMSO amount was equally 1% for all samples. After addition of compound, the plate was continuously incubated for 30 min at r.t. The fluorescence intensity was measured by multimode microplate reader (TECAN^{TM}, Switzerland) at 361 nm excitation. The emission spectrum was collected at 400–600 nm.

4.10. Band depletion assay

The transient covalent adduct of topo I with DNA in cells were evaluated in the absence and presence of compounds (CPT and **4cc**) according to the previously reported method[44]. T47D cells were seeded in a 60-mm cell culture plate at a density of 2 x 10^5 cells per well. On day 2, cells were treated with the test compounds for 2 h at 37 °C. The cells were then harvested by trypsinization and suspended in 1 mL phosphate-buffered saline (PBS) spiked with the same concentration of each test compound to prevent rapid dissociation of the cleavable complex. The suspended cells were centrifuged, and cell pellets were lysed in denaturing buffer (62.5 mM Tris-HCl (pH 6.8), 1 mM EDTA and 2% SDS) with sonication (20 bursts, 2 sec each) followed by centrifuge at 4 °C and 12,000 rpm for 30 min. The lysate was added to 2x SDS loading buffer (1 M Tris-HCl (pH 6.8), 10% SDS, 0.04% bromophenol blue, 20% glycerol, 10% β-mercaptoethanol) and then separated on 8% SDS-PAGE gels for Western blot analysis. 50 μg of protein per sample was resolved by SDS-PAGE and transferred to a PVDF membrane (Millipore, USA). The membranes were blocked with 5% skim milk or BSA in Tris buffered saline containing 0.1% tween 20 (TBST) and probed with primary antibodies in a dilution ratio of 1:1000 for 3 h at r.t. The blots were washed, exposed to HRP-conjugated anti-rabbit or mouse IgG (Cell Signaling Technology Inc. USA) in a dilution ratio of 1:5000 for 1 h, and detected with ECL western blotting detection reagent (GE healthcare, Denmark). Western blot images were taken by LAS-3000 (Fuji Photo Film Co., Ltd, Japan) and analyzed using Multi-Gauge Software (Fuji Photo Film Co. Ltd., Japan).

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at ……..

References

[1] G.I. Evan, K.H. Vousden, Nature, 411 (2001) 342-348.

[2] I. Husain, J.L. Mohler, H.F. Seigler, J.M. Besterman, Cancer Res., 54 (1994) 539-546.

[3] T.D. Pfister, W.C. Reinhold, K. Agama, S. Gupta, S.A. Khin, R.J. Kinders, R.E. Parchment, J.E. Tomaszewski, J.H. Doroshow, Y. Pommier, Mol. Cancer Ther., 8 (2009) 1878-1884.

[4] W.C. Reinhold, J.L. Mergny, H. Liu, M. Ryan, T.D. Pfister, R. Kinders, R. Parchment, J. Doroshow, J.N. Weinstein, Y. Pommier, Cancer Res., 70 (2010) 2191-2203.

[5] R. Kim, N. Hirabayashi, M. Nishiyama, T. Yorishima, T. Toge, K. Okada, Jpn. J. Surg., 21 (1991) 587-589.

[6] G. Giaccone, J. van Ark-Otte, G. Scagliotti, G. Capranico, P. van der Valk, G. Rubio, O. Dalesio, R. Lopez, F. Zunino, J. Walboomers, et al., Biochim. Biophys. Acta, 1264 (1995) 337-346.

[7] J.C. Wang, Nat. Rev. Mol. Cell Biol., 3 (2002) 430-440.

[8] Y. Pommier, Chemical Rev., 109 (2009) 2894-2902.

[9] Y. Pommier, E. Leo, H. Zhang, C. Marchand, Chem. Biol., 17 (2010) 421-433.

[10] D.B. Khadka, W.J. Cho, Expert Opin. Ther. Pat., 23 (2013) 1033-1056.

[11] P.J. McKinnon, Nat. Rev. Neurosci., 17 (2016) 673-679.

[12] Y. Pommier, Nat. Rev. Cancer, 6 (2006) 789-802.

[13] X.M. Mo, Z.F. Chen, X. Qi, Y.T. Li, J. Li, Bioinorg. Chem. Appl., 2012 (2012) 756374.

[14] A.R. Chowdhury, S. Mandal, B. Mittra, S. Sharma, S. Mukhopadhyay, H.K. Majumder, Med. Sci. Monit., 8 (2002) BR254-265..

[15] C.Y. Ting, C.T. Hsu, H.T. Hsu, J.S. Su, T.Y. Chen, W.Y. Tarn, Y.H. Kuo, J. Whang-Peng, L.F. Liu, J. Hwang, Biochem. Pharmacol., 66 (2003) 1981-1991.

[16] C.J. Li, L. Averboukh, A.B. Pardee, J. Biol. Chem., 268 (1993) 22463-22468.

[17] Y. Pommier, P. Pourquier, Y. Fan, D. Strumberg, Biochim. Biophys. Acta, 1400 (1998) 83-105.

[18] C. Bailly, Curr. Med. Chem., 7 (2000) 39-58.

[19] A.K. Larsen, A.E. Escargueil, A. Skladanowski, Pharmacol. Ther., 99 (2003) 167-181.

[20] J.L. Nitiss, Nat. Rev. Cancer, 9 (2009) 338-350.

[21] A.M. Azarova, Y.L. Lyu, C.P. Lin, Y.C. Tsai, J.Y. Lau, J.C. Wang, L.F. Liu, Proc. Nat. Acad. Sci. U.S.A., 104 (2007) 11014-11019.

[22] D. Simoni, G. Grisolia, G. Giannini, M. Roberti, R. Rondanin, L. Piccagli, R. Baruchello, M. Rossi, R. Romagnoli, F.P. Invidiata, S. Grimaudo, M.K. Jung, E. Hamel, N. Gebbia, L. Crosta, V. Abbadessa, A. Di Cristina, L. Dusonchet, M. Meli, M. Tolomeo, J. Med. Chem., 48 (2005) 723-736.

[23] S. Zheng, Q. Zhong, M. Mottamal, Q. Zhang, C. Zhang, E. Lemelle, H. McFerrin, G. Wang, J. Med. Chem., 57 (2014) 3369-3381.

[24] D.B. Khadka, H. Woo, S.H. Yang, C. Zhao, Y. Jin, T.N. Le, Y. Kwon, W.J. Cho, E. J. Med. Chem., 92 (2015) 583-607.

[25] S. Park, T.B. Thapa Magar, T.M. Kadayat, H.J. Lee, G. Bist, A. Shrestha, E.S. Lee, Y. Kwon, E. J. Med. Chem., 127 (2017) 318-333.

[26] T. Nakumura, T. Sakaeda, N. Ohmoto, Y. Moriya, C. Komoto, T. Shirakawa, A. Gotoh, M. Matsuo, K. Okmura, Pharm. Res., 20 (2003) 324-327.

[27] G. Zhao, J. Huang, K. Xue, L. Si, G. Li, E. J. Pharm. Sci., 50 (2013) 429-439.

[28] F. Shen, S. Chu, A.K. Bence, B. Bailey, X. Xue, P.A. Erickson, M.H. Montrose, W.T. Beck, L.C. Erickson, J. Pharmacol. Exp. Ther., 324 (2008) 95-102.

[29] D.B. Khadka, W.J. Cho, Bioorg. Med. Chem., 19 (2011) 724-734.

[30] H.T. Van, D.B. Khadka, S.H. Yang, T.N. Le, S.H. Cho, C. Zhao, I.S. Lee, Y. Kwon, K.T. Lee, Y.C. Kim, W.J. Cho, Bioorg. Med. Chem., 19 (2011) 5311-5320.

[31] H.T. My Van, H. Woo, H.M. Jeong, D.B. Khadka, S.H. Yang, C. Zhao, Y. Jin, E.S. Lee, K. Youl Lee, Y. Kwon, W.J. Cho, E. J. Med. Chem., 82 (2014) 181-194.

[32] D.B. Khadka, Q.M. Le, S.H. Yang, H.T. Van, T.N. Le, S.H. Cho, Y. Kwon, K.T. Lee, E.S. Lee, W.J. Cho, Bioorg. Med. Chem., 19 (2011) 1924-1929.

[33] T.N. Le, S.H. Yang, D.B. Khadka, H.T. Van, S.H. Cho, Y. Kwon, E.S. Lee, K.T. Lee, W.J. Cho, Bioorg. Med. Chem., 19 (2011) 4399-4404.

[34] D.B. Khadka, G.H. Tran, S. Shin, H.T. Nguyen, H.T. Cao, C. Zhao, Y. Jin, H.T. Van, M.V. Chau, Y. Kwon, T.N. Le, W.J. Cho, E. J. Med. Chem., 103 (2015) 69-79.

[35] J. Parness, S.B. Horwitz, J. Cell Biol., 91 (1981) 479-487.

[36] C. Bailly, Meth. Enzymol., 340 (2001) 610-623.

[37] X. Pan, J.M. Hartley, J.A. Hartley, K.N. White, Z. Wang, S.W. Bligh, Phytomedicine, 19

(2012) 618-624.

[38] A.C. Ketron, W.A. Denny, D.E. Graves, N. Osheroff, Biochemistry, 51 (2012) 1730-1739.

[39] L. Li, L. Yang, R.M. Kotin, J. Gene Med., 7 (2005) 420-431.

[40] C.F. Brunk, K.C. Jones, T.W. James, Anal. Biochem., 92 (1979) 497-500.

[41] F. Loganzo, C.M. Discafani, T. Annable, C. Beyer, S. Musto, M. Hari, X. Tan, C. Hardy, R. Hernandez, M. Baxter, T. Singanallore, G. Khafizova, M.S. Poruchynsky, T. Fojo, J.A. Nieman, S. Ayral-Kaloustian, A. Zask, R.J. Andersen, L.M. Greenberger, Cancer Res., 63 (2003) 1838-1845.

[42] A.D. Sai Krishna, G. Panda, A.K. Kondapi, Arch. Biochem. Biophys., 438 (2005) 206-216.

[43] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Environ. Mol. Mutagen., 35 (2000) 206-221.

[44] K. Umemura, T. Mizushima, H. Katayama, Y. Kiryu, T. Yamori, T. Andoh, Mol. Pharmacol., 62 (2002) 873-880.

Highlights

- 1,3-Diarylisoquinoline was designed based on structure of 3,4-diarylisoquinoline.
- 1,3-Diarylisoquinolines were cytotoxic against cancer cells.
- Several derivatives inhibited topo I/IIα activity even at low concentration.
- 1,3-Diarylisoquinolines had weak or no antitubulin activity.
- 1,3-Diarylisoquinoline **4cc** is a non-intercalative topo I catalytic inhibitor.