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# Modification of 3-arylisoquinolines into 3,4-diarylisoquinolines and assessment of their cytotoxicity and topoisomerase inhibition

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ABSTRACT

Inspired by the initial success of the monoarylisoquinolines and the quest to identify more potent and selective anticancer agents with topoisomerase (topo) inhibitory activity, series of diarylisoquinolines (3,4-diarylisoquinolones and 3,4-diarylisoquinolinamines) were designed and synthesized. Synthesis of these compounds primarily involved lithiated toluamide–benzonitrile cycloaddition, Suzuki coupling, and nucleophilic aromatic substitution reactions. Eight of the derivatives were selectively toxic against human ductal breast epithelial tumor cells (T47D), human prostate cancer cells (DU145), and human colorectal adenocarcinoma cells (HCT-15), but had no effect on normal human breast epithelial cells (MCF10A). The topo inhibitory activities of the diarylisoquinoline compounds were relatively dependent upon their chemical structure. 3,4-Diarylisoquinolones generally did not inhibit topo I and only showed moderate inhibition of topo II. In contrast, several 3,4-diarylisoquinolinamines showed superior topo I inhibitory activity. Isoquinolinamine derivatives had greater affinity for topo I than for topo II. Topo inhibition by 3,4-diarylisoquinolines was further supported by docking models showing intercalative and/or H-bond interactions between these compounds and the DNA/topo(s). An analysis of the correlation between the cytotoxicity and topo inhibition of these compounds indicated that the primary biological target of derivatives with potent cytotoxicity was topo, which in turn establishes diaryl-substituted isoquinolines as a novel class of potential anticancer drugs.



## 1. Introduction

Topoisomerases (topos) are enzymes essential for cell growth and proliferation. They are involved in resolving the topological consequences of DNA like supercoils, knots, and catenation during cellular processes as transcription, recombination, and replication [1]. Because topos play crucial role in activities necessary for cell growth and division, they are often overexpressed in cancer cells [2–6]. Therefore, topo inhibition is an important mechanism for cancer treatment.

Six topos are encoded in the human genome and topo I and II are of therapeutic importance. Several topo I and II inhibitors, topotecan (a camptothecin (CPT) derivative), etoposide, and doxorubicin (DOX) have been approved by the United States Food and Drugs Administration for treatment of ovarian, small-cell lung, testicular, and thyroid cancers, as well as acute lymphoblastic and acute myelogenous leukemias (Fig. 1). These topo inhibitors are potent antitumor agents but they also have well-known drawbacks that include chemical instability, low water solubility, drug resistance by cells overexpressing drug efflux membrane transporters, and drug-related secondary malignancies [7,8].

The most common strategies employed to overcome the shortcomings of clinically prescribed drugs are the removal of chemically susceptible functional groups, the introduction of functional (e.g. amino) group(s) to improve aqueous solubility, and the use of a chemical species with a novel scaffold to reduce the chance of it being a substrate for the drug efflux membrane

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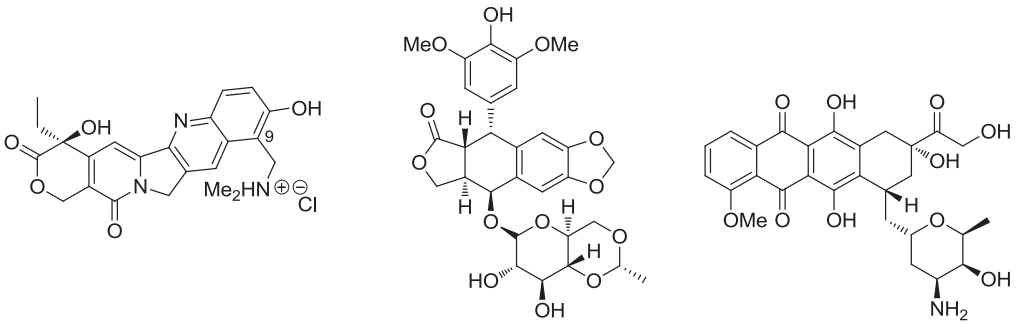



**Fig. 1.** Anticancer drugs targeting topo.

transporter. Considering these facts, 3-arylisoquinoline derivatives have been investigated in our laboratory as alternative classes of topo inhibitors (Fig. 2). 3-Arylisoquinolones **1** [9], 3-arylisoquinolinamines **2** [10], 4-amino-2-phenylquinazolines **3** [11], and 3-heteroarylisoquinolinamines **4** [12] have been developed by our research group as potent cytotoxic agents with topo inhibitory activity and the appropriate physiochemical and pharmacokinetic properties necessary for oral drug administration [13,14].

Structure–activity relationship (SAR) studies of 3-arylisoquinolines show that their cytotoxicity and topo inhibition are dependent upon the structure of the compound. Subtle changes in the structure result in remarkable changes in its pharmacological activities. Thus, to explore the possible effects of an additional aromatic ring on cytotoxicity and topo inhibition, 3,4-diarylisoquinolones **5** were designed (Fig. 3). 3,4-Diarylisoquinolones were further considered for modification into 3,4-diarylisoquinolinamines **6** in order to improve aqueous solubility. The details of the synthetic pathways employed, the cytotoxicity, topo inhibitory activities of these compounds, and docking studies using these new diaryl-substituted isoquinoline derivatives will be discussed in the following sections.

## 2. Chemistry

### 2.1. Retro synthesis

3,4-Diarylisoquinolinamine **6** was planned to be synthesized by nucleophilic aromatic substitution reactions ($S_NAr$) between various amines and isoquinolinyl chloride **7** as reported earlier (Scheme 1) [10]. 3,4-Diarylisoquinolone **5** was expected to be obtained by the Suzuki reaction on 4-brominated 3-arylisoquinolone **8**. 4-Brominated isoquinolone **8** can be produced by free radical halogenation of 3-arylisoquinolone **9**, obtained by cycloaddition of lithiated toluamide **10** and benzonitrile **11** [15].

### 2.2. Attempts to synthesize 3,4-diarylisoquinolones

The first attempt to synthesize 3,4-diarylisoquinolones was initiated by coupling of toluamide **10a** and *o*-tolunitrile **11a** in the presence of *n*-BuLi to afford 3-arylisoquinolone **9a** (Scheme 2, refer Supplementary data for the purification of isoquinolone substituted with *o*-tolyl ring at C3). Regioselective bromination of **9a** at C4 was accomplished by treatment with *N*-bromosuccinimide (NBS) and 1,1′-azobis(cyclohexanecarbonitrile) (ACCN) in the presence of a light source, to obtain compound **8a**. Suzuki reaction of 4-brominated isoquinolone **8a** with aryl boronic acids in the presence of the palladium catalyst, tetrakis(triphenylphosphine) palladium (0) ($Pd(PPh_3)_4$ (0)), resulted in the corresponding diaryl products, **5a′** and **5a** (Scheme 3). Unfortunately, the desired diarylisoquinolone was always accompanied by a debrominated byproduct, **9a**. Isolation of these compounds was complicated because of their low organic solvent solubility and similar retention factors ($R_f$).

The low solubility of isoquinolones in organic solvents prompted a search for an alternative pathway to achieve the desired 3,4-diarylisoquinolones. 4-Brominated isoquinolones **8a-b** were converted to relatively non-polar 4-bromo-3-chloro isoquinolines **12a-b**, which were then subjected to the Suzuki reaction (Scheme 4). However, this method was not satisfactory either, because the major products obtained following Suzuki coupling were 1,3,4-triarylisoquinolines **13a-b**.

Following these unsuccessful attempts, a new synthetic method was designed. Conversion of the lactam group of **8** to lactim, instead of imine chloride, was expected to increase its solubility in organic solvent and leave a single site for Suzuki coupling (Scheme 5). The desired 3,4-diarylisoquinolones **5** can be obtained by acid-catalyzed hydrolysis of lactim **14**.

### 2.3. Synthesis of 3,4-diarylisoquinolones

Synthesis was reinitiated according to the revised synthetic strategy. *O*-Methylation of isoquinolone **8** was predominantly achieved in the presence of $Ag_2CO_3$ base to afford 3-aryl-4-bromo-1-methoxyisoquinolines **15** at a yield of 33–89% (Scheme 2, Table S1). Compounds **15** were then coupled with aryl boronic acids under Suzuki reaction conditions to give 3,4-diarylisoquinolines **14**. Formation of byproducts **16a–b**, possibly by deboronation and homocoupling of aryl boronic acids, usually affected the purification procedures (Fig. 4). In such cases, a mixture of the 3,4-diarylisoquinolines **14** and the byproducts was subjected to the next step after isolating enough amount of **14** to enable structural characterization. 3,4-Diarylisoquinolones **5** were ultimately obtained after acid-catalyzed hydrolysis of lactim to lactam.

Free radical bromination of 3-arylisoquinolones generally resulted into selective bromination at C4. However, isoquinolone **9e**, under similar conditions, yielded a mixture of the mono- and dibrominated products, **8e** and **8g**. This mixture was subjected to further reaction steps and was ultimately separated after Suzuki reaction.

### 2.4. Synthesis of 3,4-diarylisoquinolinamines

3,4-Diarylisoquinolones **5a–g** were further modified into 3,4-

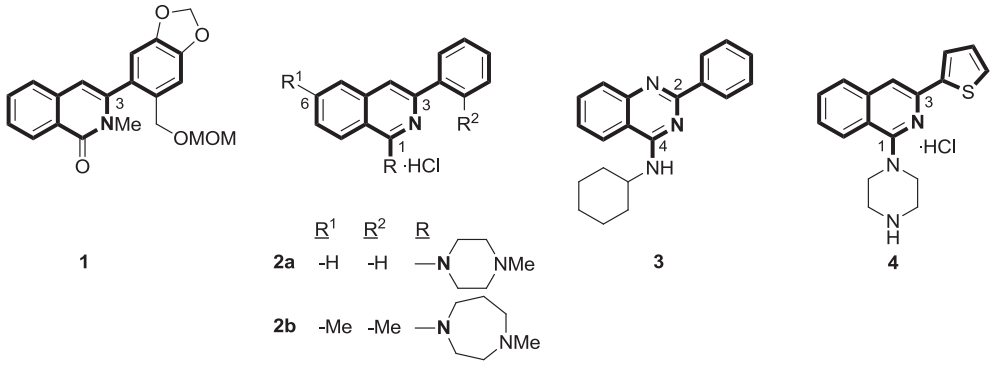



**Fig. 2.** 3-Arylisoquinoline-based cytotoxic agents with topo inhibitory activity: 3-arylisoquinolone **1**, 3-arylisoquinolinamines **2**, 4-amino-2-phenylquinazoline **3**, and 3-heteroarylisoquinolinamine **4**.

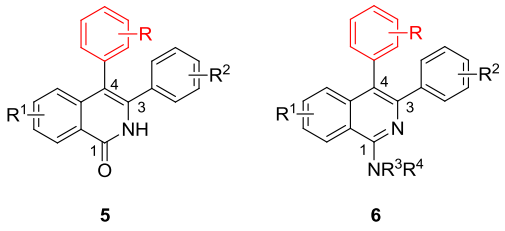
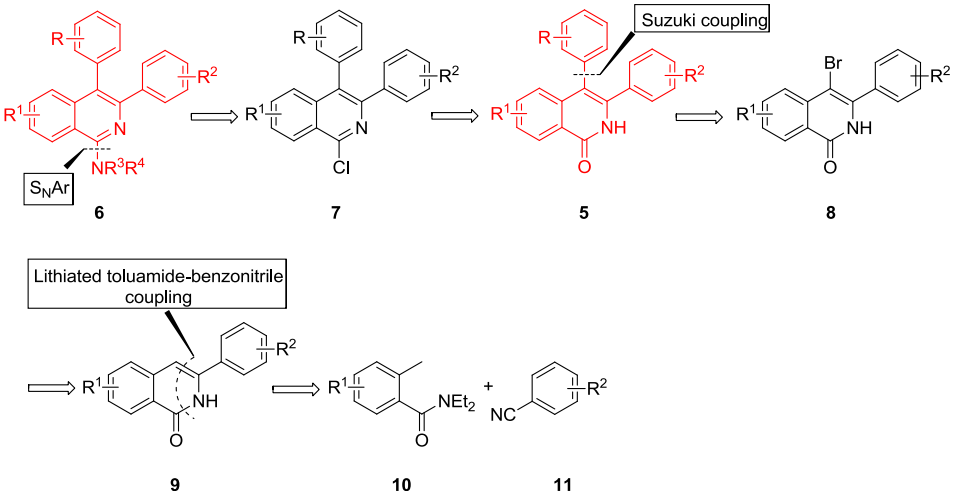



**Fig. 3.** 3,4-Diarylisoquinolones **5** and 3,4-diarylisoquinolinamines **6**.

## 3. Biological evaluation

### 3.1. Cytotoxicity

The cytotoxic effects of the synthesized 3,4-diarylisoquinolones (**5** and **19**) and the 3,4-diarylisoquinolinamines (**6** and **17**) were evaluated by MTT assay. The assay was performed using a non-cancerous human breast epithelial cell line (MCF10A), a human ductal breast epithelial tumor cell line (T47D), a human prostate cancer cell line (DU145), and a human colorectal adenocarcinoma cell line (HCT-15) [16]. Cytotoxicity results are summarized as $IC_{50}$ values (Tables 1,3–5).



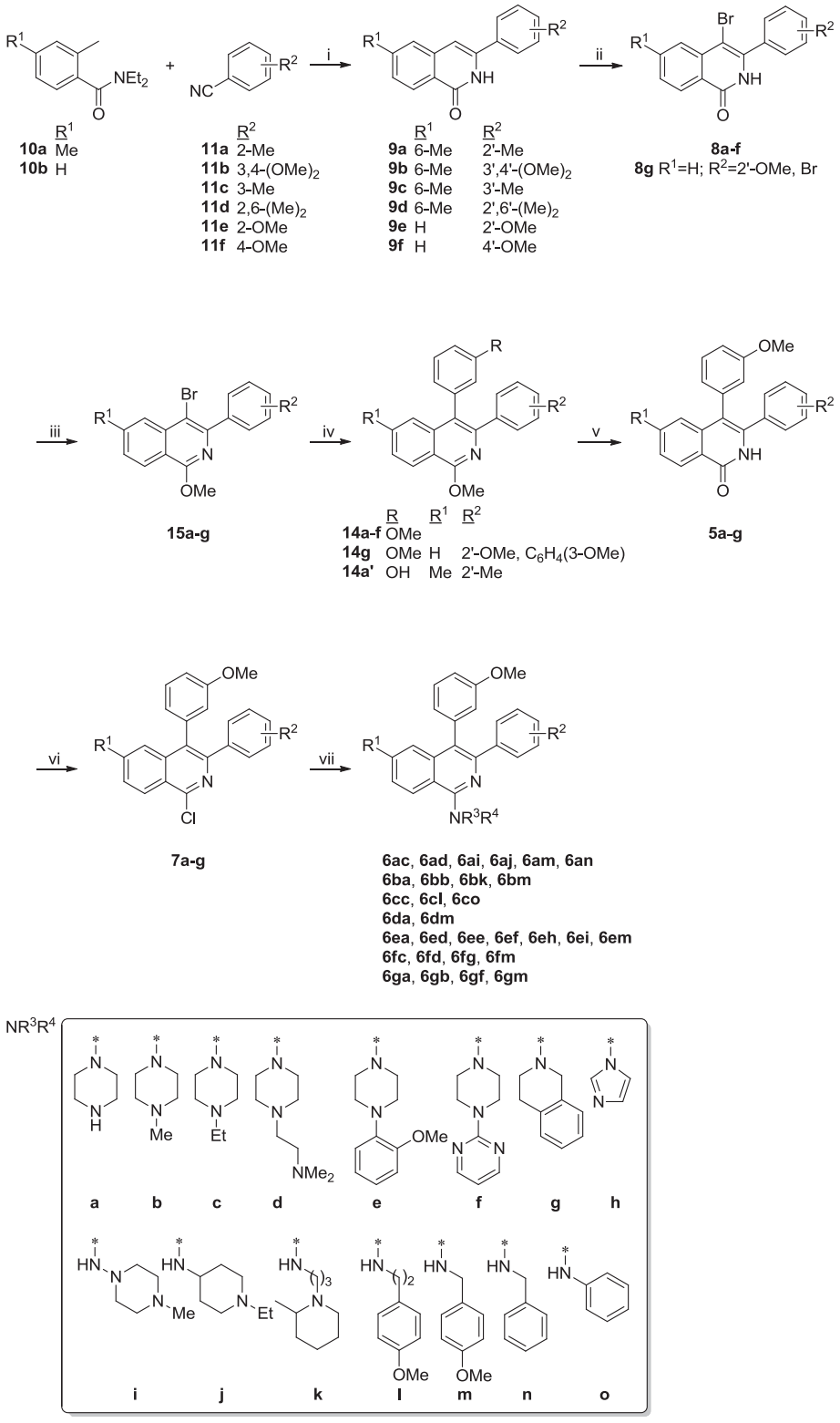

**Scheme 1.** Initial synthetic plan of 3,4-diarylisoquinolines **5** and **6**.

diarylisoquinolinamines **6** (Scheme 2). Aromatization of isoquinolones **5** into isoquinolinyl chlorides **7**, followed by 1-amination with heterocyclic (**a**–**g**), aromatic (**h**) and primary amines (**i**-**o**), produced the desired 3,4-diarylisoquinolinamines **6**. Primary isoquinolinamines **17** were obtained by deprotection of the *p*-methoxybenzyl group of **6am**–**bm** and **6dm**–**fm** with trifluoroacetic acid (Scheme 6).

Furthermore, hydroxyphenyl-substituted 3,4-diarylisoquinoline **14a′** was reacted with alkyl halide containing an amino terminal in the presence of $K_2CO_3$ as a base, to afford 3,4-diarylisoquinoline **18** (Scheme 7). The lactim group of **18** was finally hydrolyzed to lactam under acidic conditions to afford isoquinolone **19**.

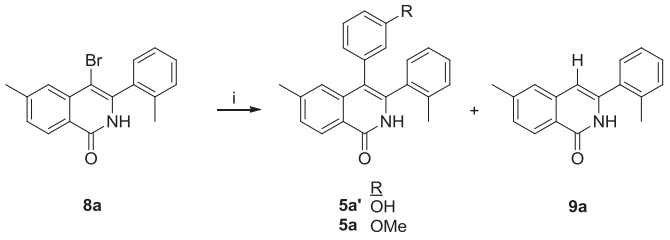
### 3.2. Topo inhibition

The topo inhibitory activities of the 3,4-diarylisoquinolones and 3,4-diarylisoquinolinamines were determined by DNA relaxation assay (Figs. 5, 6, 8 and 9) [16]. In this assay, topo relaxes a supercoiled plasmid, which has a different electrophoretic mobility than the completely relaxed DNA. Topo inhibitory activity of the test compounds was measured by determining the extent of supercoiled plasmid prevented from relaxation. DNA relaxation assays were conducted using human topo I/IIα and the plasmid, pBR322, with CPT and etoposide as positive controls for topo I and II




**Scheme 2.** Synthesis of 3,4-diarylisoquinolines **5** and **6**. Reagents and conditions: (i) *n*-BuLi, dry THF, −78 °C; (ii) NBS, ACCN, $CHCl_3/CCl_4$, hν, reflux; (iii) MeI, $Ag_2CO_3$, toluene, dark, 90 °C; (iv) $ArB(OH)_2$, $Pd(PPh_3)_4$ (0), $Na_2CO_3$, $(MeOCH_2)_2$, 90 °C; (v) AcOH:$H_2O$ = 9:1, reflux; (vi) $POCl_3$, 75 °C; (vii) $NHR^3R^4$, $K_2CO_3$, *N*,*N*-dimethylformamide (DMF), 150 °C.

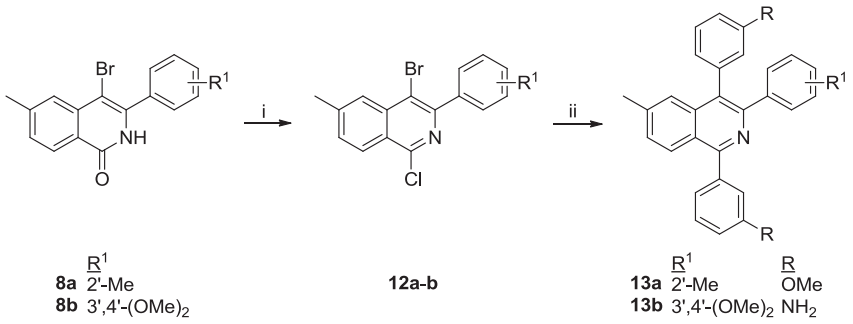
inhibition respectively. The assays were performed in the presence of 20 μM or 100 μM of the positive control or test compounds. The topo inhibition induced by the test compounds are presented as % inhibition (Tables 2,6–8). The relative topo inhibitory activities of diarylisoquinolines were further determined for SAR study as the ratio of topo inhibition of each individual compound to that of CPT/etoposide. Each compound is categorized as follows: 0.0–0.25 (no activity), >0.25–0.5 (low activity), >0.5–0.8 (moderate activity), >0.8–1.0 (similar activity as CPT/etoposide), and >1.0 (greater activity than CPT/etoposide).



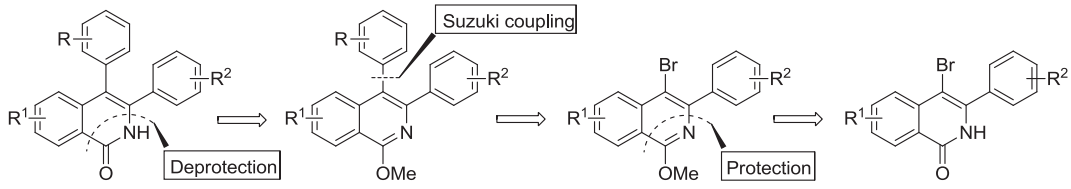

**Scheme 3.** Attempt to synthesize 3,4-diarylisoquinolone **5**. Reagents and conditions: (i) $ArB(OH)_2$, $Pd(PPh_3)_4$ (0), $Na_2CO_3$, $(MeOCH_2)_2$, 90 °C.

### 3.3. Docking

Hypothetical binding models of DNA-topo I/II complexes and diaryl-substituted isoquinoline derivatives exhibiting potent topo I and II inhibition were constructed using molecular docking tools in order to explore the potential interactions responsible for topo inhibition. The Surflex-Dock program available in *Sybyl-X 2.0 (winnt_os5x)* was used to dock the selected diarylisoquinolines into the pocket formed after removal of ligand from the 3-dimensional crystal structure of DNA-topo-ligand. The complex of DNA-topo I-




**Scheme 4.** Synthesis of 1,3,4-triarylisoquinolines **13a-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.




**Scheme 5.** Improved retrosynthetic approach to production of 3,4-diarylisoquinolones **5**.

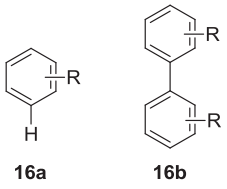
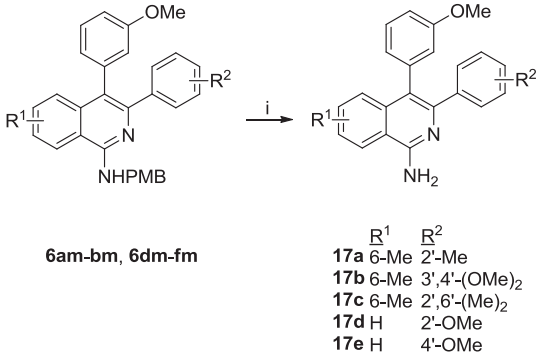



**Fig. 4.** Possible byproducts derived from aryl boronic acids following the Suzuki reaction.



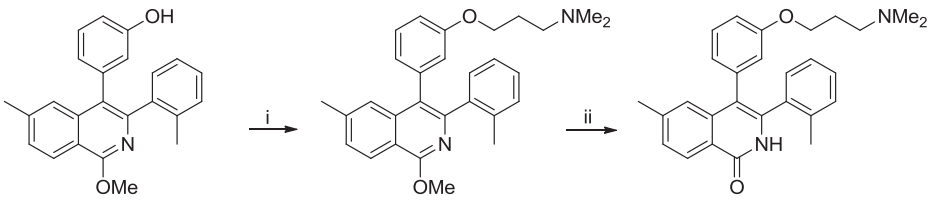

**Scheme 6.** Synthesis of primary 3,4-diarylisoquinolinamines **17**. Reagents and conditions: (i) $F_3CCOOH$, $CH_2Cl_2$, reflux.

topotecan (PDB: 1K4T) was used for studying topo I inhibition [17]. However, due to the absence of crystal structure of DNA-topo IIα-ligand, the available DNA-topo IIβ-etoposide (PDB: 3QX3) ternary complex was modified for docking [18]. Only two amino acids differ in the etoposide-binding catalytic pocket of topo IIα and IIβ [19]. Met762 and Ser800, in topo IIα, are replaced by Gln778 and Ala816, respectively, in the β isoform. Thus, Gln778 and Ala816 of 3QX3 were modified into Met and Ser as per the parameters available in crystal structure of DNA-topo IIα (PDB: 4FM9) [19].

## 4. Results and discussion

### 4.1. 3,4-Diarylisoquinolones

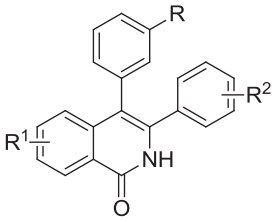
The 3,4-Diarylisoquinolones, **5a**, **5c**, and **5f**, exhibited selective cytotoxicity toward the tumorigenic T47D cells and were not toxic in the normal MCF-10A cell line (Table 1). Likewise, compounds **5b**, **5e**, and **19** were more toxic to T47D than to MCF-10A cells. This cancer cell-specific isoquinolone toxicity represented an advantage over DOX, a chemotherapeutic agent approved as adjuvant therapy for breast cancer, as DOX was cytotoxic to both malignant and non-malignant cells. Apart from MCF-10A, compounds **5a** and **5f** had no effect on the viability of the prostate cancer DU145 cells. The **5a**′ analog was cytotoxic towards both MCF10A and T47D cells, but had no effect on DU145 cells.

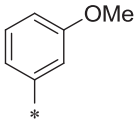



14a' 18 19

**Scheme 7.** Modification of hydroxyphenyl-substituted 3,4-diarylisoquinoline **14a′**. Reagents and conditions: (i) $Cl(CH_2)_3NMe_2 \cdot HCl$, $K_2CO_3$, DMF, 130 °C; (ii) $AcOH:H_2O = 9:1$, reflux.

**Table 1**
Cytotoxicity ($IC_{50}$) of 3,4-diarylisoquinolones (**5** and **19**).




| Compound | $R^1$ | $R^2$ | R | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|---|---|
| | | | | MCF-10A[a] | T47D[b] | DU145[c] | HCT-15[d] |
| CPT | – | – | – | 0.27 ± 0.1 | 0.28 ± 0.01 | 4.01 ± 2.45 | 10.1 ± 0.58 |
| Etoposide | – | – | – | 12.2 ± 1.47 | 2.57 ± 0.08 | 13.8 ± 1.75 | 9.45 ± 0.28 |
| Doxorubicin | – | – | – | 0.93 ± 0.03 | 0.84 ± 0.04 | 4.88 ± 0.12 | 3.76 ± 0.12 |
| **5a′** | 6-Me | 2′-Me | OH | 2.67 ± 0.08 | 5.35 ± 0.13 | >50 | 43.9 ± 1.41 |
| **5a** | 6-Me | 2′-Me | OMe | >50 | 0.45 ± 0.03 | >50 | >50 |
| **5b** | 6-Me | 3′4′-$(OMe)_2$ | OMe | 20.8 ± 1.24 | 0.11 ± 0.002 | 0.95 ± 0 | 0.56 ± 0.02 |
| **5c** | 6-Me | 3′-Me | OMe | >50 | 0.24 ± 0.01 | 6.7 ± 0.96 | 0.3 ± 0.01 |
| **5d** | 6-Me | 2′,6′-$(Me)_2$ | OMe | >50 | >50 | >50 | 3.29 ± 0.04 |
| **5e** | H | 2′-OMe | OMe | 25.9 ± 0.09 | 0.35 ± 0.02 | 1.39 ± 0 | 0.02 ± 0.003 |
| **5f** | H | 4′-OMe | OMe | >50 | 0.1 ± 0.001 | >50 | 0.06 ± 0.002 |
| **5g** | H | 2′-OMe,  | OMe | >50 | >50 | >50 | 28.4 ± 0.92 |
| **19** | 6-Me | 2′-Me | $O(CH_2)_3NMe_2$ | 20.7 ± 0.04 | 11.7 ± 0.26 | 6.59 ± 0.12 | 36.6 ± 1.48 |



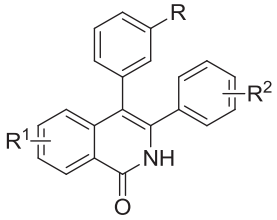
Each value represents the mean ± S.D. from three different experiments, performed in triplicate.
[a] MCF-10A: non-cancerous human breast epithelial cells.
[b] T47D: human ductal breast epithelial tumor cells.
[c] DU145: human prostate cancer cells.
[d] HCT-15: human colorectal adenocarcinoma cells.

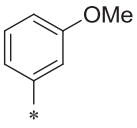
3,4-Diarylisoquinolones showed potent cytotoxic effects against human colorectal adenocarcinoma (HCT-15) cells. Compounds **5b**, **5c**, **5d**, **5e**, and **5f** were more potent than CPT, etoposide, and DOX. The low activity of the positive control drugs against HCT-15 cells is due to drug efflux from these cells which overexpress multidrug resistance protein 1 (MDR1; also known as P-glycoprotein, P-gp, or as ATP-binding cassette sub-family B member 1, ABCB1) [7,20–22], and due to metabolism of intracellular DOX by cytochrome P450 3A (CYP3A) [21]. The potent cytotoxicity profiles of the 3,4-diarylisoquinolones inferred that they were unaffected by MDR1 or CYP3A. Moreover, of the four cell lines tested, compound **5d** was only active in HCT-15 cells. Interestingly, the **5a′**, **5a**, and **19** analogs, which had different C4 aromatic ring substitutions, were inactive towards HCT-15. The triaryl-substituted isoquinolone **5g** was the only isoquinolone that was ineffective in the entire human cell lines tested. Similarly, 1,3,4-Triarylisoquinoline **13a** also had no cytotoxic effects ($IC_{50}$: >50 μM).

3,4-Diarylisoquinolones did not generally inhibit topo I (Table 2, Figs. 5 and 6). Compound **19** showed low topo I inhibitory activity at 100 μM, whereas **5f** produced moderate inhibition of both topo I and topo II. **5a** and **5d** also showed moderate inhibitory activity towards topo II. The multi aryl-substituted isoquinolone **5g** possessed topo inhibitory activity that was superior to that produced by CPT and etoposide. Increased interaction of this compound's aromatic rings with DNA bases, possibly by π–π interaction, may explain its superior activity. However, even though 1,3,4-triarylisoquinoline **13a** had more aromatic rings, it showed no topo inhibitory activity. The non-planar conformation of this compound may have been the chief factor hindering its interaction with planar DNA bases.

Despite its potent topos inhibitory activity, **5g** had a low cytotoxic effect; this may indicate that the physiochemical properties of the compound prevented cell penetration or caused wide distribution within the cell, or that cellular metabolic factors degraded

**Table 2**
Topo I and II inhibition by 3,4-diarylisoquinolones (**5** and **19**).




| Compound | $R^1$ | $R^2$ | R | Topo I (%) inhibition | | Topo II (%) inhibition | |
|---|---|---|---|---|---|---|---|
| | | | | 100 μM | 20 μM | 100 μM | 20 μM |
| CPT | – | – | – | 53.0[a]/57.3[b]/65.4[c]/81.1[d]/63.4[e] | 44.5[a]/40.3[b]/45.2[c]/24.6[d]/36.6[e] | – | – |
| Etoposide | – | – | – | – | – | 90.5[a]/70.7[b]/84.1[c]/90.6[d]/85.8[e] | 38.0[a]/35.0[b]/ND[c]/37.8[d]/35.5[e] |
| **5a′** | 6-Me | 2′-Me | OH | 6.7 (0.12)[f] | ND | 28.4 (0.31) | 3.0 (0.07) |
| **5a** | 6-Me | 2′-Me | OMe | 3.8 (0.07) | ND | 48.9 (0.54) | 0.0 |
| **5b** | 6-Me | 3′4′-$(OMe)_2$ | OMe | 2.8 (0.04) | ND | 1.1 (0.01) | ND |
| **5c** | 6-Me | 3′-Me | OMe | 0.0 | ND | 0.0 | ND |
| **5d** | 6-Me | 2′,6′-$(Me)_2$ | OMe | 4.0 (0.07) | ND | 51.0 (0.72) | 0.8 (0.02) |
| **5e** | H | 2′-OMe | OMe | 12.2 (0.15) | ND | 0.0 | ND |
| **5f** | H | 4′-OMe | OMe | 37.0 (0.58) | 3.4 (0.09) | 59.9 (0.69) | 3.4 (0.09) |
| **5g** | H | 2′-OMe,  | OMe | 87.5 (1.07) | 0.0 | 81.1 (0.89) | 5.8 (0.15) |
| **19** | 6-Me | 2′-Me | $O(CH_2)_3NMe_2$ | 20.1 (0.37) | 1.2 (0.02) | 0.0 | ND |



Each value represents the mean ± S.D. from three different experiments, performed in triplicate.
ND: Not determined.
[a] Corresponding values when compounds **5a′**, **5a**, and **19** were tested.
[b] Corresponding values when compounds **5b** and **5d** were tested.
[c] Corresponding values when compound **5c** was tested.
[d] Corresponding values when compounds **5e** and **5g** were tested.
[e] Corresponding values when compound **5f** was tested.
[f] Enclosed within parentheses is the relative topoisomerase activity; the topo inhibitory activity of the compound/the topo inhibitory activity of CPT or etoposide.

the compound before it reached the designated target (topo). On the other hand, cytotoxic compounds such as **5a′**, **5b**, **5c**, **5e**, and **19** are likely to exert effects on cells through different mechanisms. The moderate topo inhibitory activity and potent cytotoxicity of **5a**, **5d**, and **5f** indicated that their effects on topos may be partially responsible for the cell death observed.

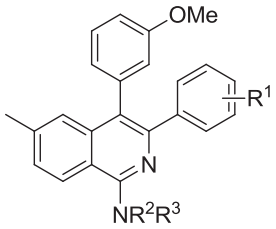
Hypothetical binding model of modified DNA-topo IIβ complex and 3,4-diarylisoquinolone **5f**, which exhibited moderate topo I and II inhibition and potent toxicity in tumor cells, was constructed using molecular docking tools. The DNA-topo II**-5f** docking model indicated that this compound interacted with the amino acid Asp479 of topo II via H-bonds, in a similar manner to etoposide (Fig. 7). In addition, +5 guanine base of the intact strand overlapped the 4-aryl group of **5f** possibly by π–π interaction.

### 4.2. 3,4-Diarylisoquinolinamines

The antiproliferative activity of 3,4-diarylisoquinolinamines in human cell lines (MCF-10A, T47D, DU145, and HCT-15) was largely dependent on the type of amine substituted at C1 (Tables 3 and 4). Isoquinolones with aromatic amines (**h**), and heterocyclic (**e**, **f**, and **g**) and secondary amines (**l**, **m**, **n**, and **o**) with aromatic moieties, generally did not affect cell viability. Close examination of the cytotoxicity profiles indicated that **6an**, **6ef**, and **6gf** isoquinolinamines were not toxic to all four cell lines. Similarly, *p*-methoxybenzylamine-substituted isoquinolines (**6bm**, **6em**, **6fm**, and **6gm**) did not show cytotoxicity. However, the **6am** and **6dm** derivatives did show potent toxicity in T47D cells. Furthermore, compound **6ee** was selectively toxic towards T47D cells ($IC_{50}$: 11.7 ± 0.4 μM). Likewise, compound **6cl** showed selective toxicity towards HCT-15 cells ($IC_{50}$: 6.48 ± 0.34). Aniline-(**6co**), imidazole-(**6eh**), and isoquinoline-substituted (**6fg**) derivatives were not toxic toward MCF-10A and DU145 cells, but possessed moderate to potent antiproliferative activity in T47D and HCT-15 cells ($IC_{50}$: 13.5 ± 0.22–2.06 ± 0.1 μM). A secondary 3,4-diarylisoquinolinamine **6ei** also showed selective toxicity towards T47D and HCT-15 cell lines. In contrast, compounds **6ai**, **6aj**, and **6bk** were not toxic to T47D cells, but were toxic towards DU145, HCT-15, and MCF-10A cell lines. Isoquinolinamines substituted with piperazines (**a**–**d**) were generally more toxic than those compounds substituted with other amines. Piperazinylethanamine **6ad** represented the most cytotoxic compound of all the isoquinolines tested in T47D ($IC_{50}$: 0.05 ± 0.003 μM) and in HCT-15 ($IC_{50}$: 0.08 ± 0.001 μM) cells. Unfortunately, these compounds were also toxic to normal MCF-10A cells.

Primary 3,4-diarylisoquinolinamines **17** demonstrated moderate or low cytotoxicity towards MCF-10A cells ($IC_{50}$: 14.9 ± 0.08–42.0 ± 0.1, Table 5). Surprisingly, primary isoquinolinamines (with the exception of **17e**) had no toxic effects on cancerous T47D cells. Likewise, isoquinolinamines **17** had moderate and low antiproliferative effects on DU145 cells ($IC_{50}$: 10.8 ± 0.03–44.8 ± 0.89). The important aspect of this series of compounds was their greater cytotoxic potency against HCT-15 ($IC_{50}$: 2.8 ± 0.05–0.78 ± 0.02) than that of CPT, etoposide, and DOX.

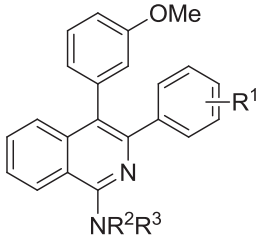
**Table 3**
Cytotoxicity ($IC_{50}$) of 3,4-diaryl-6-methylisoquinolinamines (**6a**, **6b**, **6c** and **6d** series).




| Compound | $R^1$ | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | MCF-10A[a] | T47D[b] | DU145[c] | HCT-15[d] |
| CPT | – | 0.27 ± 0.1 | 0.28 ± 0.01 | 4.01 ± 2.45 | 10.1 ± 0.58 |
| Etoposide | – | 12.2 ± 1.47 | 2.57 ± 0.08 | 13.8 ± 1.75 | 9.45 ± 0.28 |
| Doxorubicin | – | 0.93 ± 0.03 | 0.84 ± 0.04 | 4.88 ± 0.12 | 3.76 ± 0.12 |
| **6ac** | 2′-Me | 7.51 ± 0.04 | 3.91 ± 0.05 | 13.1 ± 0.49 | 2.9 ± 0.13 |
| **6ad** | 2′-Me | 6.1 ± 0.24 | 0.05 ± 0.003 | 4.64 ± 0.03 | 0.08 ± 0.001 |
| **6ai** | 2′-Me | 5.2 ± 0.01 | >50 | 10.2 ± 0.02 | 2.94 ± 0.13 |
| **6aj** | 2′-Me | 6.94 ± 0.06 | >50 | 11.2 ± 0.06 | 1.97 ± 0.08 |
| **6am** | 2′-Me | >50 | 10.3 ± 0.47 | >50 | 24.4 ± 0.73 |
| **6an** | 2′-Me | >50 | >50 | >50 | >50 |
| **6ba** | 3′,4′-$(OMe)_2$ | 8.36 ± 0.01 | >50 | 3.72 ± 0.01 | 0.77 ± 0.01 |
| **6bb** | 3′,4′-$(OMe)_2$ | 6.92 ± 0.08 | 0.69 ± 0.01 | 5.23 ± 0.01 | 1.08 ± 0.02 |
| **6bk** | 3′,4′-$(OMe)_2$ | 6.15 ± 0.06 | >50 | 4.55 ± 0.01 | 0.63 ± 0.03 |
| **6bm** | 3′,4′-$(OMe)_2$ | >50 | 23.9 ± 0.94 | >50 | >50 |
| **6cc** | 3′-Me | 19.2 ± 1.23 | >50 | 11.5 ± 0.2 | 0.98 ± 0.03 |
| **6cl** | 3′-Me | >50 | >50 | >50 | 6.48 ± 0.34 |
| **6co** | 3′-Me | >50 | 2.06 ± 0.1 | >50 | 10.4 ± 0.38 |
| **6da** | 2′,6′-$(Me)_2$ | 5.78 ± 0.03 | >50 | 8.49 ± 0.05 | 1.05 ± 0.05 |
| **6dm** | 2′,6′-$(Me)_2$ | >50 | 1.33 ± 0.05 | >50 | >50 |

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.

**Table 4**
Cytotoxicity ($IC_{50}$) of 3,4-diarylisoquinolinamines (**6e**, **6f**, and **6g** series).




| Compound | $R^1$ | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | MCF-10A[a] | T47D[b] | DU145[c] | HCT-15[d] |
| CPT | – | 0.27 ± 0.1 | 0.28 ± 0.01 | 4.01 ± 2.45 | 10.1 ± 0.58 |
| Etoposide | – | 12.2 ± 1.47 | 2.57 ± 0.08 | 13.8 ± 1.75 | 9.45 ± 0.28 |
| Doxorubicin | – | 0.93 ± 0.03 | 0.84 ± 0.04 | 4.88 ± 0.12 | 3.76 ± 0.12 |
| **6ea** | 2′-OMe | 13.3 ± 0.19 | 4.46 ± 0.18 | 10.3 ± 0.02 | >50 |
| **6ed** | 2′-OMe | 12.1 ± 0.12 | 0.56 ± 0.03 | 9.56 ± 0.03 | >50 |
| **6ee** | 2′-OMe | >50 | 11.7 ± 0.4 | >50 | >50 |
| **6ef** | 2′-OMe | >50 | 40.1 ± 1.62 | >50 | >50 |
| **6eh** | 2′-OMe | 30.8 ± 0.02 | 13.5 ± 0.22 | 49.5 ± 0 | 7.88 ± 0.38 |
| **6ei** | 2′-OMe | 36.3 ± 0.44 | 9.6 ± 0.8 | 49.4 ± 0 | 1.96 ± 0.09 |
| **6em** | 2′-OMe | >50 | 38.8 ± 0.66 | >50 | >50 |
| **6fc** | 4′-OMe | 12.2 ± 0.06 | 0.57 ± 0.02 | 11.1 ± 0.02 | 1.22 ± 0.02 |
| **6fd** | 4′-OMe | 6.59 ± 0.02 | 0.29 ± 0.01 | 10.1 ± 0.02 | 0.18 ± 0.01 |
| **6fg** | 4′-OMe | >50 | 2.85 ± 0.05 | >50 | 4.19 ± 0.09 |
| **6fm** | 4′-OMe | >50 | 37.7 ± 0.72 | >50 | 29.7 ± 0.23 |
| **6ga** | 2′-OMe, $-C_6H_4$(3-OMe) | 6.47 ± 0.05 | >50 | 8.9 ± 0.02 | >50 |
| **6gb** | 2′-OMe, $-C_6H_4$(3-OMe) | 9.61 ± 0.06 | 2.87 ± 0.1 | 10.7 ± 0.01 | 1.46 ± 0.01 |
| **6gf** | 2′-OMe, $-C_6H_4$(3-OMe) | >50 | 28.5 ± 1.08 | >50 | >50 |
| **6gm** | 2′-OMe, $-C_6H_4$(3-OMe) | >50 | >50 | >50 | 22.4 ± 0.95 |

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.

3,4-Diarylisoquinolinamines exhibited potent topo I inhibitory activities (Tables 6 and 7, Fig. 8). Piperazinyl-(**6ad**, **6ba**, **6ed**, **6fd**, and **6ga**), methoxyphenylpiperazinyl-**6ee**, pyrimidinylpiperazinyl-**6ef**, isoquinolinyl-**6fg** and benzyl-**6fm** derivatives were superior or equipotent to CPT with respect to topo I inhibition. Among these derivatives, compounds **6ee** and **6fd** are worth mentioning as they inhibited topo I, even at a relatively low concentration (20 μM). 3,4-Diarylisoquinolinamines did not inhibit or moderately inhibited topo II (Tables 6 and 7, Fig. 9). A multiaryl isoquinolinamine **6gm** showed similar topo II inhibitory activity as etoposide, and moderate topo I inhibition. The topo II inhibitory activity of **6dm** was 86% that of etoposide at 100 μM. Primary isoquinolinamines **17c** and **17e** inhibited topo I, while the remaining derivatives did not inhibit either topo I or topo II (Table 8, Figs. 8 and 9).

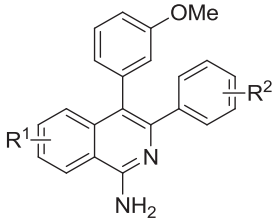
3,4-Diarylisoquinolinamines (**6ef** and **6fm**) were non-toxic to the human cell lines tested, despite their potent inhibition of topo I activity. However, a strong correlation between cytotoxicity and topo inhibition was observed in the remaining topo inhibitors. Binding models generated by docking showed that the isoquinoline ring of compound **6ba** overlapped the DNA bases of the scissile strand, indicating possible π−π stacking interactions between the aromatic rings (Fig. 10B, E). The compound **6ba** was associated with Arg364 and Thr718 of topo I by H-bonding. Interactions similar to those of **6ba** were also observed between compound **6fd**, DNA bases, and Arg364 of topo I (Fig. 10C, F). Additionally, **6fd** had H-bond with cytosine base of DNA. Secondary isoquinolinamine **6dm** interacted with Asp479 of topo II by H-bond. However, it lacked (π−π stacking/H-bonding) with DNA (Fig. 11).

The π−π stacking interaction of a chemical species intercalated between DNA base pairs at the cleavage site is a special event that inhibits topo activity [17]. Intercalation of a topo inhibitor between nucleotides distorts the DNA structure and prevents the reactive −OH of the −1 base (of the scissile DNA strand) from detaching topo covalently attached to the +1 base and joining the cleaved DNA strand by a transesterification reaction. In addition to intercalation, the H-bond interaction with the Arg364 amino acid of topo I may be essential for the topo I inhibitory activity because it is common in known topo I inhibitors like CPT, indenoisoquinoline (MJ238), and indolocarbazole (SA315F) [23]. The importance of this interaction with Arg364 is further supported by the observation that CPT and indolocarbazole show decreased affinity towards topo I with an Arg364His mutation. Taken together, the docking models for **6ba**, **6fd** and **6dm** with the DNA-topo complex validated their potent topo inhibitory activity.

### 4.3. SAR of 3,4-diarylisoquinolines

The cytotoxicity and topo inhibitory activities of the 3,4-diarylisoquinolones and 3,4-diarylisoquinolinamines were dependent upon certain structural features of these compounds (Fig. 12). The additional aromatic ring at the 3-aryl group of 3,4-

**Table 5**
Cytotoxicity ($IC_{50}$) of primary 3,4-diarylisoquinolinamines **17**.




| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|---|
| | | | MCF-10A[a] | T47D[b] | DU145[c] | HCT-15[d] |
| CPT | – | – | 0.27 ± 0.1 | 0.28 ± 0.01 | 4.01 ± 2.45 | 10.1 ± 0.58 |
| Etoposide | – | – | 12.2 ± 1.47 | 2.57 ± 0.08 | 13.8 ± 1.75 | 9.45 ± 0.28 |
| Doxorubicin | – | – | 0.93 ± 0.03 | 0.84 ± 0.04 | 4.88 ± 0.12 | 3.76 ± 0.12 |
| **17a** | 6-Me | 2′-Me | 21.0 ± 0.14 | >50 | 44.8 ± 0.89 | 1.46 ± 0.06 |
| **17b** | 6-Me | 3′4′-$(OMe)_2$ | 42.0 ± 0.1 | >50 | 10.8 ± 0.03 | 0.86 ± 0.02 |
| **17c** | 6-Me | 2′,6′-$(Me)_2$ | 21.3 ± 0.21 | >50 | 15.2 ± 0.11 | 2.8 ± 0.05 |
| **17d** | H | 2′-OMe | 15.9 ± 0.08 | >50 | 12.9 ± 0.09 | 0.99 ± 0.04 |
| **17e** | H | 4′-OMe | 14.9 ± 0.08 | 1.22 ± 0.04 | 20.2 ± 0.06 | 0.78 ± 0.02 |

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.

diarylisoquinolones produced potent inhibition of both topo I and topo II. Replacement of the 3″-OMe of 4-aryl by −OH and $-O(CH_2)_3NMe_2$ was not beneficial, as these substitutions transformed derivatives that were non-toxic to non-cancerous MCF-10A cells into compounds that were strongly or moderately toxic. 3,4-Diarylisoquinolones with 6,2′-$(Me)_2$ substituents did not reduce proliferation of HCT-15 cells.

Conversion of isoquinolones to isoquinolinamines had a large impact on their cytotoxicity and topo inhibitory activities. Generally, 3,4-diarylisoquinolinamines were more cytotoxic and inhibited topo more effectively than did the 3,4-diarylisoquinolones. Interestingly, 3,4-diarylisoquinolinamines that lacked a methyl substituent at C6 showed superior inhibition of topo I activity than did 6-Me derivatives, irrespective of amine and other substituents (Tables 6 and 7). Piperazine-substituted analogs showed the highest cytotoxicity profiles of all the isoquinolines tested. Primary isoquinolinamines were more toxic towards normal MCF-10A cells than were isoquinolones. However, it was surprising that they were generally not cytotoxic towards T47D cells. Conversion of isoquinolones to primary isoquinolinamines was advantageous in that it increased their inhibition of topo I. Unfortunately, isoquinolinamines substituted with aromatic amine (imidazole) and amines containing an aromatic moiety had low cytotoxicity profiles. Likewise, 1,3,4-triaylisoquinolinamine was non-toxic towards all the human cell lines tested in this study, and did not inhibit topo activity.

## 5. Conclusion

The limitations of established anticancer drugs which target topo necessitate the development of alternative classes of topo inhibitors. Among a large number of chemical entities investigated as topo inhibitors, 3-arylisoquinoline derivatives are promising. Based on SAR studies of monoaryl-substituted isoquinolines, 3,4-diarylisoquinolones and 3,4-diarylisoquinolinamines were designed, synthesized, and evaluated for their cytotoxicity and ability to inhibit topo activity. The present study found that some of these compounds were cytotoxic at sub-μM concentrations, showed selective toxicity towards cancerous cells with no effects on normal cells, and showed a positive correlation between their cytotoxicity and topo inhibition, indicating that the diaryl-substituted isoquinolines have the potential to be developed into safer drugs for cancer treatment.

## 6. Experimental section

### 6.1. Chemistry

Melting point (mp) was determined by the capillary method with a MEL-TEMP® 3.0 apparatus and was expressed as the uncorrected value. $^1$H NMR and $^{13}$C NMR spectra were recorded by Varian Unity Plus 300 MHz and Varian Unity Inova 500 MHz spectrometers at the Korea Basic Science Institute. The spectral data are reported in the following order: chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, b: broad, bs: broad singlet, dd: doublet of doublets, dt: doublet of triplets, td: triplet of doublets, ddd: doublet of doublet of doublets), coupling constant, number of protons, and proton assignment where applicable. The chemical shifts are reported in parts per million (ppm) downfield to TMS (tetramethylsilane, $\delta = 0$). The coupling constants, *J*, are presented in Hertz (Hz). Mass spectra were obtained on a Shimadzu LCMS-2010 EV liquid chromatograph mass spectrometer using the electron spray ionization (ESI) method. High resolution mass specta were obtained on Waters Synapt G2 High Definition Mass Spectrometer. Column chromatography (gravity) and medium performance liquid chromatography (MPLC; Yamazen) were performed using Merck silica gel 60 (70–230 mesh) at a flow rate of 10 mL/min. Thin-layer chromatography (TLC) was performed using plates coated with silica gel 60 $F_{254}$ (Merck). Chemical reagents were purchased from Sigma–Aldrich and Tokyo Chemical Industry Co., Ltd. and were used without further purification. Solvents were distilled prior to use; tetrahydrofuran (THF) was distilled from sodium/benzophenone. All reactions were conducted under an atmosphere of nitrogen in oven-dried glassware with magnetic stirring. The reaction temperature mentioned, with the exception of the lithiated toluamide–benzonitrile cycloaddition reaction, was the external temperature of the oil bath.

#### 6.1.1. 6-Methyl-3-(o-tolyl)isoquinolin-1(2H)-one (**9a**)

An oven-dried, three-necked flask was sealed with septa and evacuated/backfilled with $N_2$ three times before starting the reaction. To a mixture of 2.5 M *n*-BuLi in hexane (38 mL, 95.9 mmol) and dry THF (35 mL) maintained at below −70 °C, *N*,*N*-diethyl-2,4-dimethylbenzamide (**10a**; 7.88 g, 38.4 mmol) and *o*-tolunitrile (**11a**; 6.74 g, 57.5 mmol) were added maintaining the temperature below −60 °C. The reaction mixture was stirred at −78 °C for 14 h. The reaction mixture was then warmed to room temperature, quenched with water and extracted with $CHCl_3$. The organic layer was washed with water and concentrated under reduced pressure. The EtOAc-insoluble fraction was filtered off and further washed with EtOAc to obtain compound **9a** as an off-white solid (3.98 g, 41%). The filtrate was further subjected to column chromatography (*n*-hexane:EtOAc = 5:1, 1:1) to obtain an impure mixture of **9a** as a yellow mass (2.79 g; refer Supplementary data for further purification) $^1$H NMR (300 MHz, DMSO-*d6*) $\delta$ (ppm): 11.30 (s, 1H, NH), 8.08 (d, $J$ = 8.1 Hz, 1H, 8-H), 7.45 (s, 1H, 5-H), 7.39–7.25 (m, 5H, Ar–H), 6.39 (s, 1H, 4-H), 2.43 (s, 3H, Ar–$CH_3$), 2.29 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 250 $(M+H)^+$, 272 $(M+Na)^+$.

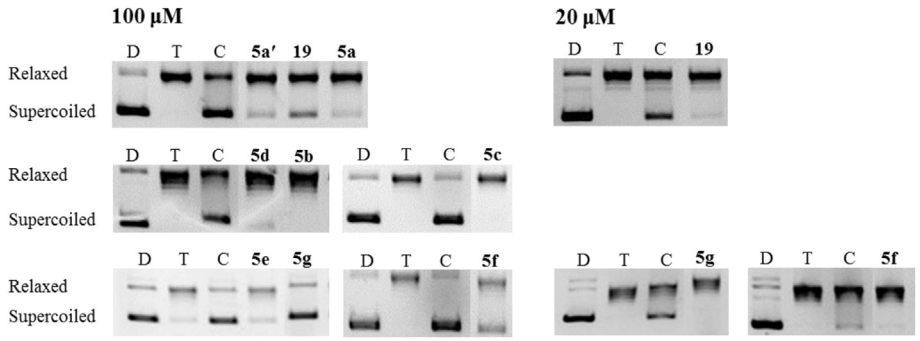



**Fig. 5.** The topo I inhibitory activities of 3,4-diarylisoquinolones. Lane D: pBR322 only; lane T: pBR322 + topo I; lane C: pBR322 + topo I + CPT; remaining lanes: pBR322 + topo I + the indicated 3,4-diarylisoquinolone compound.

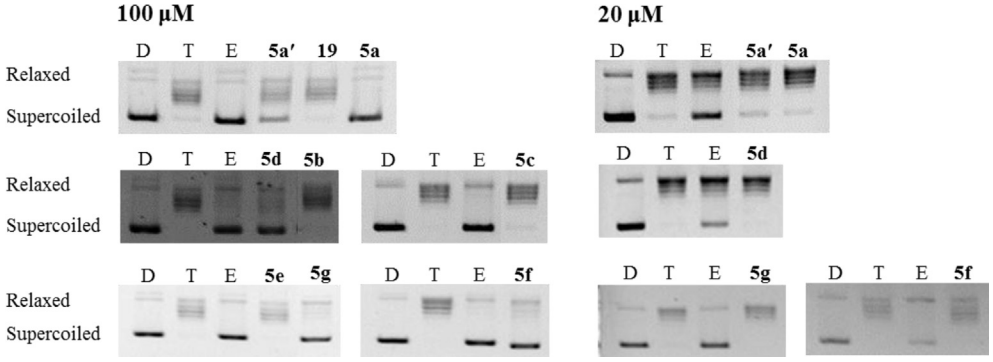



**Fig. 6.** The topo II inhibitory activities of 3,4-diarylisoquinolones. Lane D: pBR322 only; lane T: pBR322 + topo II; lane E: pBR322 + topo II + etoposide; remaining lanes: pBR322 + topo II + the indicated 3,4-diarylisoquinolone compound.

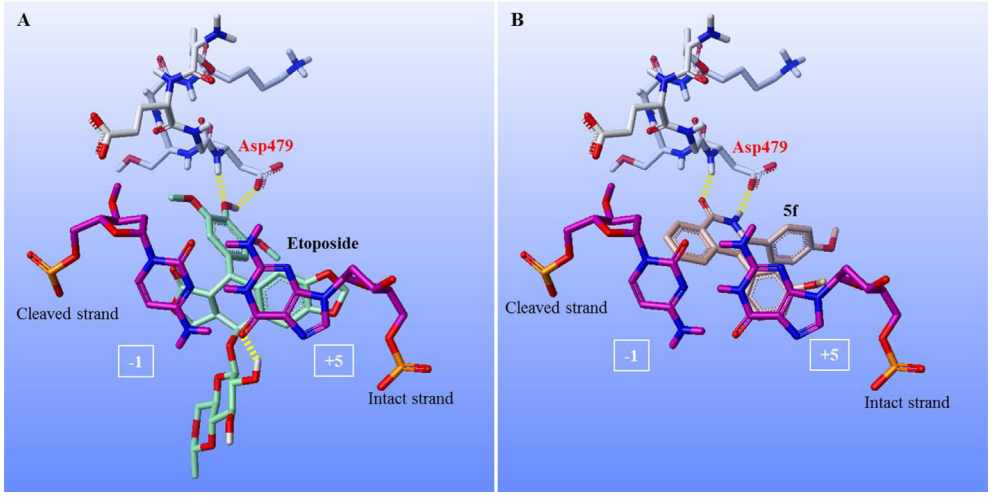



**Fig. 7.** (**A**) Binding model of etoposide with modified DNA-topo IIβ complex. (**B**) Hypothetical binding model of **5f** with modified DNA-topo IIβ complex. Carbon units are light green and brown in etoposide and **5f** respectively, purple in nucleotides, and gray in amino acids. H-bonds are illustrated as discontinuous yellow lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*6.1.2. 4-Bromo-6-methyl-3-(o-tolyl)isoquinolin-1(2H)-one (**8a**)*

NBS (1.07 g, 6.01 mmol) and 98% ACCN (250 mg, 1 mmol) were added to compound **9a** (1 g, 4.01 mmol) in $CCl_4$ (10 mL). The reaction mixture was refluxed for 2 h using a sand bath in the presence of a light source. Water was poured into the reaction mixture and it was extracted with $CH_2Cl_2$. The organic layer was dried using brine (saturated (sat.) NaCl solution (sol.)) and anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was then purified by column chromatography (*n*-hexane:EtOAc = 4:1) to obtain compound **8a** as a yellow solid (1.11 g, 84%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 8.66 (s, 1H, NH), 8.30 (d, $J$ = 8.1 Hz, 1H, 8-H), 7.80 (s, 1H, 5-H), 7.44–7.29 (m, 5H, Ar–H), 2.56 (s, 3H, Ar–$CH_3$), 2.27 (s, 3H, Ar–$CH_3$).

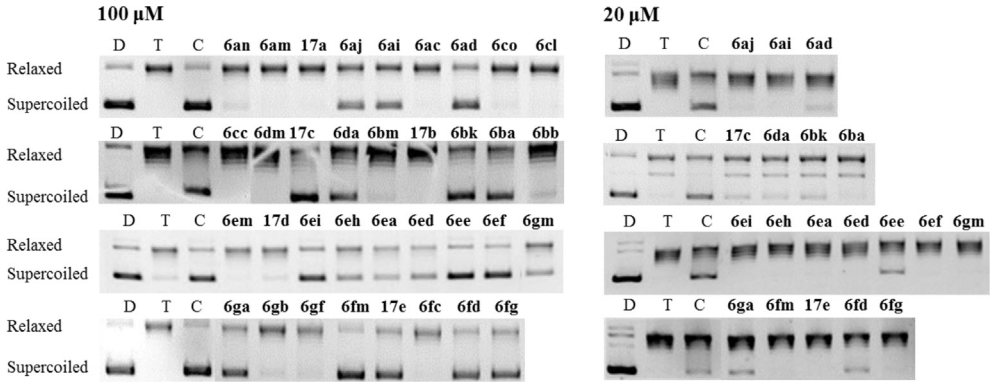



**Fig. 8.** Topo I inhibitory activities of 3,4-diarylisoquinolinamines. Lane D: pBR322 only; lane T: pBR322 + topo I; lane C: pBR322 + topo I + CPT; remaining lanes: pBR322 + topo I + the indicated 3,4-diarylisoquinolinamine.

#### 6.1.3. 4-(3-Hydroxyphenyl)-6-methyl-3-(o-tolyl)isoquinolin-1(2H)-one (**5a′**)

Compound **8a** (200 mg, 0.61 mmol) was dissolved in 1,2-dimethoxyethane (10 mL) and a solution of 3-hydroxyphenyl boronic acid (168 mg, 1.22 mmol) in MeOH (5 mL) was added, followed by $Pd(PPh_3)_4$ (0) (35 mg, 0.03 mmol) and sat. $Na_2CO_3$ sol. (5 mL). The reaction mixture was heated using an oil bath set at 90 °C for 23 h. The reaction mixture was then filtered through a bed of celite. The filtrate was extracted with EtOAc, dried using brine and anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue obtained was purified by MPLC (*n*-hexane:$CH_2Cl_2$ = 5:1) to obtain **5a′** as a yellow solid (70 mg, 33%). Mp: 182 °C (decompose; dec.). IR ($cm^{-1}$): 3212 (N–H), 1646 (C=O). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.31 (s, 1H, NH), 9.31 (bs, 1H, OH), 8.18 (d, *J* = 8.1 Hz, 1H, 8-H), 7.35–7.32 (m, 1H, Ar–H), 7.19–6.93 (m, 6H, Ar–H), 6.65–6.57 (m, 2H, Ar–H), 6.43–6.40 (m, 1H, Ar–H), 2.32 (s, 3H, Ar–$CH_3$), 2.18 (d, *J* = 4.8 Hz, 3H, Ar–$CH_3$). $^{13}$C NMR (75 MHz, DMSO-*d6*) δ (ppm): 161.5, 156.9, 142.4, 138.2, 137.0, 136.2, 134.5, 130.4, 129.7, 129.2, 129.0, 128.5, 127.7, 126.9, 125.1, 124.6, 123.0, 118.6, 117.1, 115.7, 114.1, 21.7, 19.6. MS (ESI): *m*/*z* 405 $(M+Na+CH_3CN)^+$, 342 $(M+H)^+$, 364 $(M+Na)^+$. Anal. calcd for $C_{23}H_{19}NO_2 \cdot 0.1C_4H_8O_2$: C, 80.25; H, 5.7; N, 4. Found C, 80.33; H, 5.52; N, 3.84.

#### 6.1.4. 3-(3,4-Dimethoxyphenyl)-6-methylisoquinolin-1(2H)-one (**9b**)

An oven-dried, three-necked flask was sealed with septa and evacuated/backfilled with $N_2$ three times before starting the reaction. To a mixture of 2.5 M *n*-BuLi in hexane (35 mL, 87.6 mmol) and dry THF (20 mL), maintained at below −70 °C, solution of *N*,*N*-diethyl-2,4-dimethylbenzamide (**10a**; 6 g, 29.2 mmol) and 3,4-dimethoxybenzonitrile (**11b**; 7.15 g, 43.8 mmol) in dry THF (7 mL) were added maintaining the temperature below −60 °C. The reaction mixture was stirred at −78 °C for 38.5 h. The reaction mixture was then warmed to room temperature and quenched with water. The precipitate was filtered off and washed with water and EtOAc to obtain **9b** as an off-white solid. The filtrate was extracted with $CH_2Cl_2$, washed with water, and concentrated under reduced pressure. The EtOAc-insoluble fraction was again filtered off and washed with EtOAc to obtain **9b** (6.46 g, 74%). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.34 (s, 1H, Ar–H), 8.07 (d, *J* = 8.1 Hz, 1H, 8-H), 7.46 (s, 1H, Ar–H), 7.37–7.34 (m, 2H, Ar–H), 7.29–7.26 (m, 1H, Ar–H), 7.05 (d, *J* = 9.0 Hz, 1H, Ar–H), 6.81 (s, 1H, 4-H), 3.87 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 2.43 (s, 3H, 6-$CH_3$). MS (ESI): *m*/*z* 296 $(M+H)^+$, 337 $(M+CH_3CN+H)^+$.

#### 6.1.5. 4-Bromo-3-(3,4-dimethoxyphenyl)-6-methylisoquinolin-1(2H)-one (**8b**)

The procedures described for compound **8a** were used with compound **9b** (5.52 g, 18.7 mmol), NBS (4.99 g, 28.1 mmol), 98% ACCN (1.16 g, 4.67 mmol), and $CHCl_3$ (40 mL), followed by filtration and column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **8b** a light yellow solid (5.73 g, 81%). $R_f$ = 0.19 (*n*-hexane:EtOAc = 1:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.63 (s, 1H, NH), 8.15 (d, *J* = 8.1 Hz, 1H, 8-H), 7.73 (s, 1H, Ar–H), 7.42 (d, *J* = 8.1, 1.2, 1H, Ar–H), 7.11 (s, 1H, Ar–H), 7.09–7.04 (m, 2H, Ar–H), 3.82 (s, 3H, $OCH_3$), 3.80 (s, 3H, $OCH_3$), 2.51 (≈3H, 6-$CH_3$, overlapped with the DMSO peaks). MS (ESI): *m*/*z* 376 $(M+H)^+$ ($^{81}$Br), 374 $(M+H)^+$ ($^{79}$Br), 417 $(M+CH_3CN+H)^+$ ($^{81}$Br), 415 $(M+CH_3CN+H)^+$ ($^{79}$Br), 372 $(M-H)^-$ ($^{79}$Br), 374 $(M-H)^-$ ($^{81}$Br).

#### 6.1.6. 4-Bromo-1-chloro-6-methyl-3-(o-tolyl)isoquinoline (**12a**)

A solution of compound **8a** (1.21 g, 3.68 mmol) in $POCl_3$ (20 mL) was heated using an oil bath set at 75 °C. Upon completion of the reaction, excess $POCl_3$ and volatile materials were evaporated by vacuum distillation. Sat. $NaHCO_3$ sol. (100 mL) was added to the resulting residue and then extracted with EtOAc. The organic layer was further washed with water, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was then purified by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **12a** as a brown solid (847 mg, 66%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.28 (d, *J* = 8.7 Hz, 1H, Ar–H), 8.10 (s, 1H, 5-H), 7.60 (dd, *J* = 8.7, 1.5 Hz, 1H, Ar–H), 7.37–7.27 (m, 4H, Ar–H), 2.64 (s, 3H, Ar–$CH_3$), 2.17 (s, 3H, Ar–$CH_3$).

#### 6.1.7. 4-Bromo-1-chloro-3-(3,4-dimethoxyphenyl)-6-methylisoquinoline (**12b**)

The procedures described for compound **12a** were used with compound **8b** (660 mg, 1.84 mmol) and $POCl_3$ (15 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **12b** as a light yellow solid (649 mg, 93%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.24 (d, *J* = 8.7 Hz, 1H, Ar–H), 8.14 (s, 1H, Ar–H), 7.56 (dd, *J* = 8.7 Hz, 1H, Ar–H), 7.36–7.32 (m, 1H, Ar–H), 7.28 (d, *J* = 1.8 Hz, 1H, Ar–H), 6.97 (d, *J* = 8.4 Hz, 1H, Ar–H), 3.95 (s, 6H, $OCH_3$), 2.64 (s, 3H, 6-$CH_3$).

#### 6.1.8. 1,4-Bis(3-methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinoline (**13a**)

The procedures described for compound **5a′** were used with compound **12a** (785 mg, 2.26 mmol), a solution of 3-methoxyphenyl boronic acid (688 mg, 4.54 mmol) in MeOH (5 mL), $Pd(PPh_3)_4$ (0) (131 mg, 0.11 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (15 mL), followed by MPLC (*n*-

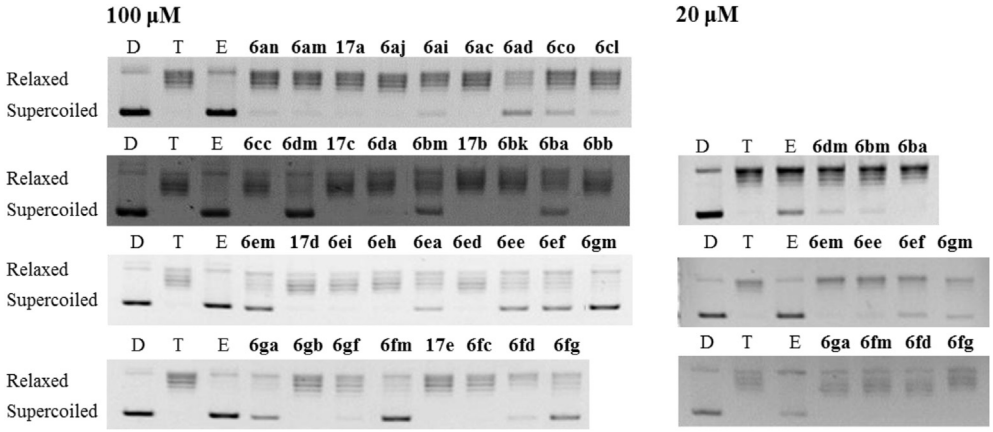



**Fig. 9.** Topo II inhibitory activities of 3,4-diarylisoquinolinamines. Lane D: pBR322 only; lane T: pBR322 + topo II; lane E: pBR322 + topo II + etoposide; remaining lanes: pBR322 + topo II + the indicated 3,4-diarylisoquinolinamine.

hexane:$CH_2Cl_2$ = 3:1, *n*-hexane:EtOAc = 5:1) to obtain **13a** as a light yellow viscous gel (822 mg, 81%). $R_f$ = 0.5 (*n*-hexane:EtOAc = 4:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.04 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.53–7.46 (m, 2H, Ar–H), 7.37 (s, 1H, Ar–H), 7.29–7.20 (m, 3H, Ar–H), 7.15–6.99 (m, 5H, Ar–H), 6.86–6.83 (m, 2H, Ar–H), 6.77 (s, 1H, Ar–H), 3.82 (s, 3H, $OCH_3$), 3.65 (s, 3H, $OCH_3$), 2.41 (s, 3H, Ar–$CH_3$), 2.11 (s, 3H, Ar–$CH_3$).

### *6.1.9. 3,3′-(3-(3,4-Dimethoxyphenyl)-6-methylisoquinoline-1,4-diyl)dianiline (**13b**)*

The procedures described for compound **5a′** were used with compound **12b** (200 mg, 0.53 mmol), a solution of 3-aminiphenylboronic acid monohydrate (206 mg, 1.32 mmol) in 5 mL MeOH, $Pd(PPh_3)_4$ (0) (31 mg, 0.02 mmol), sat. $Na_2CO_3$ sol. (2 mL), and 1,2-dimethoxy ethane (12 mL), followed by MPLC (*n*-hexane:EtOAc = 5:1, 4:1, 3:1, 1:1) to obtain compound **13b** as a brown semi-solid which foamed out into a solid on pumping (115 mg, 46%). $R_f$ = 0.45 (*n*-hexane:EtOAc = 1:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.01 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.44–7.40 (m, 1H, Ar–H), 7.38 (s, 1H, Ar–H), 7.25–7.10 (m, 3H, Ar–H), 6.97–6.92 (m, 2H, Ar–H), 6.87–6.81 (m, 2H, Ar–H), 6.74–6.70 (m, 1H, Ar–H), 6.62–6.59 (m, 1H, Ar–H), 6.47–6.45 (m, 2H, Ar–H), 5.25 (s, 2H, $NH_2$), 5.14 (s, 2H, $NH_2$), 3.71 (s, 3H, $OCH_3$), 3.46 (s, ≈3H, $OCH_3$, overlapped with the DMSO peak).

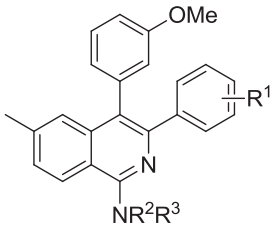
### *6.1.10. 6-Methyl-3-(m-tolyl)isoquinolin-1(2H)-one (**9c**)*

The procedures described for compound **9b** were used with *N*,*N*-diethyl-2,4-dimethylbenzamide **10a** (4.66 g, 22.7 mmol), *m*-tolunitrile **11c** (3.99 g, 34.1 mmol), 2.5 M *n*-BuLi in hexane (23 mL, 56.8 mmol), and dry THF (30 mL), followed by filtration and column chromatography (*n*-hexane:EtOAc = 3:1) to obtain compound **9c** as an off-white solid (4.6 g, 81%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.54 (bs, 1H, NH), 8.29 (d, *J* = 8.4 Hz, 1H, 8-H), 7.52–7.47 (m, 2H, Ar–H), 7.42–7.37 (m, 2H, Ar–H), 7.32–7.26 (m, ≈2H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.70 (s, 1H, 4-H), 2.50 (s, 3H, Ar–$CH_3$), 2.46 (s, 3H, Ar–$CH_3$). MS (ESI) *m/z* 250 $(M+H)^+$.

### *6.1.11. 3-(2,6-Dimethylphenyl)-6-methylisoquinolin-1(2H)-one (**9d**)*

The procedures described for compound **9b** were used with *N*,*N*-diethyl-2,4-dimethylbenzamide **10a** (2 g, 9.74 mmol), 2,6-dimethylbenzonitrile **11d** (1.91 g, 14.6 mmol), 2.5 M *n*-BuLi in hexane (10 mL, 24.3 mmol), and dry THF (35 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain an impure mixture of compound **9d** as a yellow mass (2.63 g; Refer Supplementary data for further purification of the impure mixture). **9d** was white solid. $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.23 (s, 1H, NH), 8.08 (d, *J* = 8.1 Hz, 1H, 8-H), 7.42 (s, 1H, 5-H), 7.31 (dd, *J* = 8.1, 1.2 Hz, 1H, Ar–H), 7.27–7.22 (m, 1H, Ar–H), 7.15–7.12 (m, 2H, Ar–H), 6.29 (d, *J* = 0.9 Hz, 1H, 4-H), 2.43 (s, 3H, 6-$CH_3$), 2.14 (s, 6H, 2′,6′-$(CH_3)_2$).

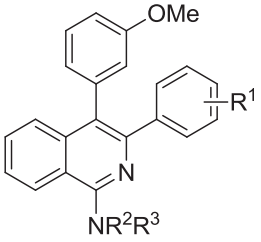
**Table 6**
Topo I and II inhibition by 3,4-diaryl-6-methylisoquinolinamines (**6a**, **6b**, **6c**, and **6d** series).




| Compound | $R^1$ | Topo I (%) inhibition | | Topo II (%) inhibition | |
|---|---|---|---|---|---|
| | | 100 μM | 20 μM | 100 μM | 20 μM |
| CPT | – | 65.4[a]/57.3[b] | 45.2[a]/40.3[b] | – | – |
| Etoposide | – | – | – | 84.1[a]/70.7[b] | ND[a]/35.0[b] |
| **6ac** | 2′-Me | 0.0 | ND | 0.0 | ND |
| **6ad** | 2′-Me | 62.4 (0.95)[c] | 9.4 (0.2) | 29.9 (0.35) | ND |
| **6ai** | 2′-Me | 34.4 (0.52) | 1.2 (0.02) | 2.4 (0.02) | ND |
| **6aj** | 2′-Me | 31.9 (0.48) | 1.2 (0.02) | 0.0 | ND |
| **6am** | 2′-Me | 0.0 | ND | 0.0 | ND |
| **6an** | 2′-Me | 4.6 (0.07) | ND | 0.0 | ND |
| **6ba** | 3′,4′-$(OMe)_2$ | 60.8 (1.06) | 0.0 | 30.8 (0.43) | 0.0 |
| **6bb** | 3′,4′-$(OMe)_2$ | 10.4 (0.18) | ND | 0.4 | ND |
| **6bk** | 3′,4′-$(OMe)_2$ | 54.1 (0.94) | 6.3 (0.15) | 2.0 (0.02) | ND |
| **6bm** | 3′,4′-$(OMe)_2$ | 5.0 (0.08) | ND | 29.5 (0.41) | 2.0 (0.05) |
| **6cc** | 3′-Me | 0.5 | ND | 0.0 | ND |
| **6cl** | 3′-Me | 0.0 | ND | 0.0 | ND |
| **6co** | 3′-Me | 0.0 | ND | 8.5 (0.10) | ND |
| **6da** | 2′,6′-$(Me)_2$ | 44.3 (0.77) | 5.2 (0.13) | 8.3 (0.11) | ND |
| **6dm** | 2′,6′-$(Me)_2$ | 6.6 (0.11) | ND | 61.5 (0.86) | 3.7 (0.1) |

Each value represents the mean ± S.D. from three different experiments, performed in triplicate.
ND: Not determined.
[a] Corresponding values when compounds of **6a** series, **6co**, and **6cl** were tested.
[b] Corresponding values when compounds of **6b** and **6d** series, and **6cc** were tested.
[c] Enclosed within parentheses is the relative topoisomerase activity; the topo inhibitory activity of the compound/the topo inhibitory activity of CPT or etoposide.

**Table 7**
Topo I and II inhibition by 3,4-diarylisoquinolinamines (**6e**, **6f**, and **6g** series).




| Compound | $R^1$ | Topo I (%) inhibition | | Topo II (%) inhibition | |
|---|---|---|---|---|---|
| | | 100 μM | 20 μM | 100 μM | 20 μM |
| CPT | – | 81.1[a]/63.4[b] | 24.6[a]/ 36.6[b] | – | – |
| Etoposide | – | – | – | 90.6[a]/ 85.8[b] | 37.8[a]/ 35.5[b] |
| **6ea** | 2′-OMe | 50.8 (0.62)[c] | 0.0 | 21.7 (0.23) | ND |
| **6ed** | 2′-OMe | 83.4 (1.02) | 0.0 | 0.5 | ND |
| **6ee** | 2′-OMe | 100.0 (1.23) | 30.0 (1.21) | 38.0 (0.41) | 0.0 |
| **6ef** | 2′-OMe | 73.1 (0.9) | 0.0 | 35.2 (0.38) | 17.5 (0.46) |
| **6eh** | 2′-OMe | 41.6 (0.51) | 0.0 | 0.0 | ND |
| **6ei** | 2′-OMe | 53.1 (0.65) | 0.0 | 0.0 | ND |
| **6em** | 2′-OMe | 0.0 | ND | 50.0 (0.55) | 0.0 |
| **6fc** | 4′-OMe | 8.4 (0.13) | ND | 1.2 (0.01) | ND |
| **6fd** | 4′-OMe | 76.1 (1.2) | 27.2 (0.74) | 20.5 (0.23) | 5.3 (0.14) |
| **6fg** | 4′-OMe | 73.3 (1.15) | 4.9 (0.13) | 58.4 (0.68) | 2.7 (0.07) |
| **6fm** | 4′-OMe | 101.3 (1.59) | 3.8 (0.1) | 58.3 (0.67) | 1.9 (0.05) |
| **6ga** | 2′-OMe, $-C_6H_4$(3-OMe) | 67.3 (1.06) | 20.9 (0.57) | 55.9 (0.65) | 3.6 (0.1) |
| **6gb** | 2′-OMe, $-C_6H_4$(3-OMe) | 7.2 (0.11) | ND | 0.6 | ND |
| **6gf** | 2′-OMe, $-C_6H_4$(3-OMe) | 10.8 (0.17) | ND | 6.7 (0.07) | ND |
| **6gm** | 2′-OMe, $-C_6H_4$(3-OMe) | 57.2 (0.7) | 0.0 | 87.8 (0.96) | 16.4 (0.43) |

Each value represents the mean ± S.D. from three different experiments, performed in triplicate.
ND: Not determined.
[a] Corresponding values when compounds of the **6e** series and **6gm** were tested.
[b] Corresponding values when compounds of the **6f** series, **6ga**, **6gb**, and **6gf** were tested.
[c] Enclosed within parentheses is the relative topoisomerase activity; the topo inhibitory activity of the test compound/the topo inhibitory activity of CPT or etoposide.

#### 6.1.12. 3-(2-Methoxyphenyl)isoquinolin-1(2H)-one (*9e*)

The procedures described for compound **9b** were used with *N*,*N*-diethyl-2-methylbenzamide **10b** (7 g, 36.6 mmol), 2-methoxybenzonitrile **11e** (7.31 g, 54.8 mmol), 2.5 M of *n*-BuLi in hexane (44 mL, 109.8 mmol), and dry THF (30 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1) to obtain compound **9e** as an off-white solid (6.5 g, 70%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.46 (s, 1H, NH), 8.63 (td, *J* = 8.1, 0.6 Hz, 1H, 8-H), 7.68–7.56 (m, 3H, Ar–H), 7.50–7.40 (m, 2H, Ar–H), 7.08 (dt, *J* = 7.5, 1.2 Hz, 1H, Ar–H), 7.03 (d, *J* = 8.4 Hz, 1H, Ar–H), 6.71 (s, 1H, 4-H), 3.93 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 251 $(M+H)^+$, 293 $(M+CH_3CN+H)^+$.

#### 6.1.13. 3-(4-Methoxyphenyl)isoquinolin-1(2H)-one (*9f*)

The procedures described for compound **9b** were used with *N*,*N*-diethyl-2-methylbenzamide **10b** (3.29 g, 17.2 mmol), 4-methoxybenzonitrile **11f** (3.43 g, 25.8 mmol), 2.5 M of *n*-BuLi in hexane (17 mL, 43 mmol), and dry THF (35 mL) to obtain compound **9f** as a white solid (2.86 g, 66%). Compound **9f** has been reported previously [24].

#### 6.1.14. 4-Bromo-6-methyl-3-(m-tolyl)isoquinolin-1(2H)-one (*8c*)

The procedures described for compound **8a** were used with compound **9c** (1.98 g, 7.95 mmol), NBS (2.12 g, 11.9 mmol), 98% ACCN (496 mg, 1.99 mmol), and $CHCl_3$ (30 mL), followed by recrystallization from MeOH to obtain compound **8c** as an off-white solid (2.02 g, 77%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.87 (bs, 1H, NH), 8.30 (d, *J* = 8.1 Hz, 1H, 8-H), 7.84 (s, 1H, 5-H), 7.40–7.38 (m, 4H, Ar–H), 7.32–7.30 (m, 1H, Ar–H), 2.56 (s, 3H, Ar–$CH_3$), 2.44 (s, 3H, Ar–$CH_3$).

#### 6.1.15. 4-Bromo-3-(2,6-dimethylphenyl)-6-methylisoquinolin-1(2H)-one (*8d*)

The procedures described for compound **8a** were used with compound **9d** (2.32 g, 8.80 mmol), NBS (2.35 g, 13.2 mmol), 98% ACCN (549 mg, 2.20 mmol), and $CHCl_3$ (30 mL), followed by filtration and column chromatography (*n*-hexane:EtOAc = 5:1, 1:1) to obtain compound **8d** as a light orange solid (2.97 g, 98%). $R_f$ = 0.5 (*n*-hexane:EtOAc = 1:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.64 (s, 1H, NH), 8.16 (d, *J* = 8.1 Hz, 1H, 8-H), 7.68 (s, 1H, 5-H), 7.44 (ddd, *J* = 8.1, 1.5, 0.6 Hz, 1H, Ar–H), 7.30–7.25 (m, 1H, Ar–H), 7.18–7.16 (m, 2H, Ar–H), 2.51 (≈3H, overlapped with the DMSO peaks), 2.09 (s, 6H, 2′,6′-$(CH_3)_2$).

#### 6.1.16. 4-Bromo-3-(2-methoxyphenyl)isoquinolin-1(2H)-one (*8e*) and 3-aryl-4-bromoisoquinolin-1(2H)-one (*8g*)

The procedures described for compound **8a** were used with compound **9e** (5.80 g, 23.1 mmol), NBS (6.16 g, 34.6 mmol), 98% ACCN (1.44 g, 5.77 mmol), and $CHCl_3$ (25 mL) to obtain a mixture of compounds **8e** and **8g** as a white solid (5.72 g). $R_f$ = 0.37 (*n*-hexane:EtOAc = 1:1). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.19 (bs, 1H, NH), 8.89 (bs, 1H, NH), 8.43–8.38 (m, 2H, Ar–H), 8.06–8.01 (m, 2H, Ar–H), 7.82–7.76 (m, 2H, Ar–H), 7.60–7.54 (m, 4H, Ar–H), 7.52–7.40 (m, 2H, Ar–H), 7.11–7.06 (m, 1H, Ar–H), 7.03 (d, *J* = 8.4 Hz, 1H, Ar–H), 6.91 (d, *J* = 8.7 Hz, 1H, Ar–H), 3.81 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$). **8e**: MS (ESI) *m*/*z* 330 $(M-H)^-$ ($^{81}$Br), 328 $(M-H)^-$ ($^{79}$Br). **8g**: MS (ESI): *m*/*z* 408 $(M-H)^-$ ($^{79}$Br, $^{81}$Br), 406 $(M-H)^-$ ($^{79}$Br), 410 $(M-H)^-$ ($^{81}$Br).

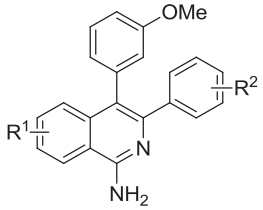
#### 6.1.17. 4-Bromo-3-(4-methoxyphenyl)isoquinolin-1(2H)-one (*8f*)

The procedures described for compound **8a** were used with compound **9f** (3.44 g, 13.7 mmol), NBS (3.65 g, 20.5 mmol), 98% ACCN (837 mg, 3.35 mmol), and $CHCl_3$ (40 mL), followed by filtration and column chromatography (*n*-hexane:EtOAc = 4:1, 1:1) to obtain compound **8f** as a white solid (4.21 g, 93%). Mp: 199–204 °C. $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.73 (s, 1H, NH), 8.28–8.25 (m, 1H, 8-H), 7.95–7.84 (m, 2H, Ar–H), 7.66–7.57 (m, 1H, Ar–H), 7.49–7.45 (m, 2H, 2′-H and 6′-H), 7.08–7.03 (m, 2H, 3′-H and 5′-H), 3.82 (s, 3H, $OCH_3$).

#### 6.1.18. 4-Bromo-1-methoxy-6-methyl-3-(o-tolyl)isoquinoline (*15a*)

To a mixture of compound **8a** (1.47 g, 4.49 mmol) in toluene (25 mL), silver carbonate (1.11 g, 4.04 mmol) and iodomethane (765 mg, 5.39 mmol) were added. The reaction mixture was heated in the dark (by covering the reaction flask with aluminum foil) using an oil bath set at 90 °C. Upon completion of the reaction, water was poured into the reaction mixture and it was then extracted with EtOAc. The organic layer was dried using brine and anhydrous $Na_2CO_3$, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **15a** as a yellow solid

**Table 8**
Topo I and II inhibition by primary 3,4-diarylisoquinolinamines **17**.




| Compound | $R^1$ | $R^2$ | Topo I (%) inhibition | | Topo II (%) inhibition | |
|---|---|---|---|---|---|---|
| | | | 100 μM | 20 μM | 100 μM | 20 μM |
| CPT | – | – | 65.4[a]/57.3[b] 81.1[c]/63.4[d] | 45.2[a]/40.3[b] 24.6[c]/36.6[d] | | |
| Etoposide | – | – | – | – | 84.1[a]/70.7[b] 90.6[c]/85.8[d] | ND[a]/35.0[b] 37.8[c]/35.5[d] |
| **17a** | 6-Me | 2′-Me | 0.0 | ND | 0.0 | ND |
| **17b** | 6-Me | 3′4′-$(OMe)_2$ | 4.4 (0.07)[e] | ND | 0.3 | ND |
| **17c** | 6-Me | 2′,6′-$(Me)_2$ | 72.8 (1.27) | 9.6 (0.23) | 1.4 (0.02) | ND |
| **17d** | H | 2′-OMe | 0.0 | ND | 0.0 | ND |
| **17e** | H | 4′-OMe | 79.0 (1.24) | 1.9 (0.05) | 1.1 (0.01) | ND |

Each value represents the mean ± S.D. from three different experiments, performed in triplicate.
ND: Not determined.
[a] Corresponding values when compound **17a** was tested.
[b] Corresponding values when compounds **17b** and **17c** were tested.
[c] Corresponding values when compound **17d** was tested.
[d] Corresponding values when compound **17e** was tested.
[e] Enclosed within parentheses is the relative topoisomerase activity; the topo inhibitory activity of the test compound/the topo inhibitory activity of CPT or etoposide.

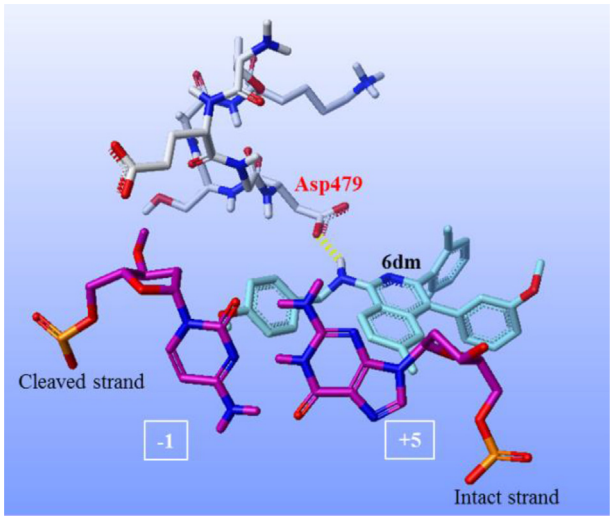



**Fig. 11.** Hypothetical complex of DNA, modified topo IIβ and **6dm**. Carbon units are light blue in **6dm**, purple in nucleotides, and gray in amino acids. H-bonds are illustrated as discontinuous yellow lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1.16 g, 75%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.17 (d, $J$ = 8.4 Hz, 1H, 8-H), 7.97 (s, 1H, 5-H), 7.44 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.34–7.28 (m, 4H, Ar–H), 4.06 (s, 3H, $OCH_3$), 2.59 (s, 3H, Ar–$CH_3$), 2.20 (s, 3H, Ar–$CH_3$).

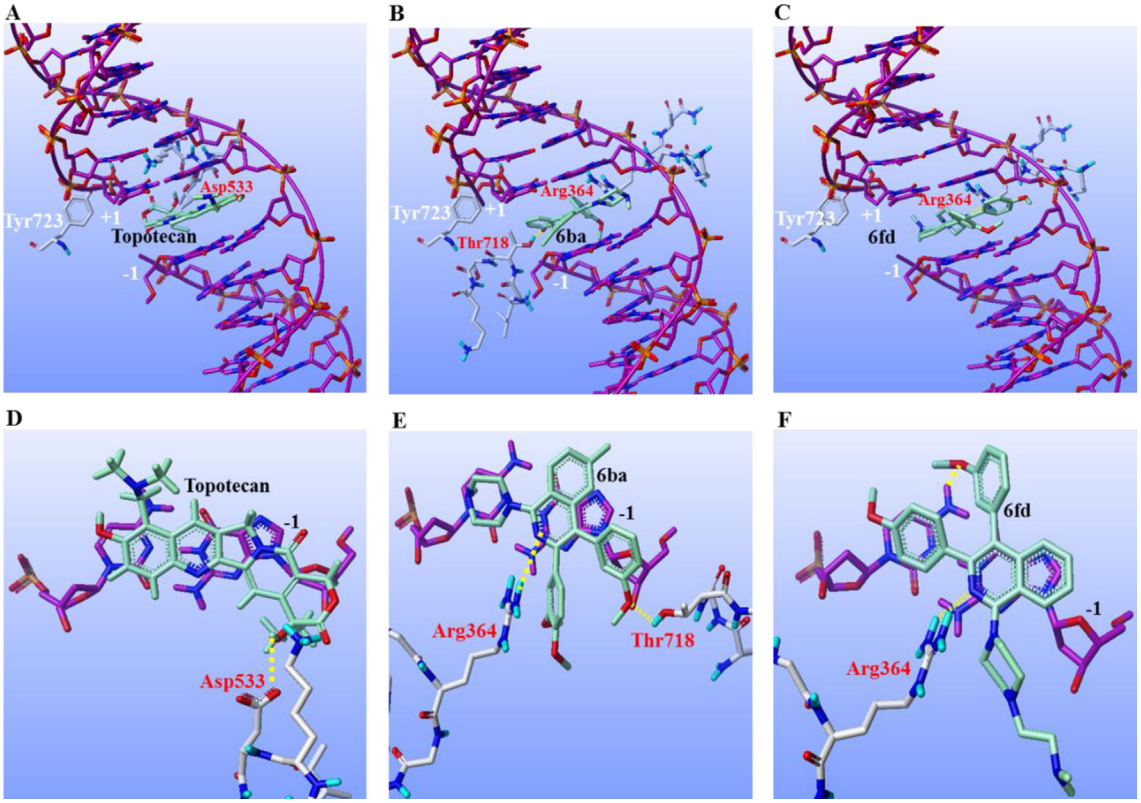



**Fig. 10.** (**A**, **D**) Binding model showing topotecan with DNA and topo I. (**B**, **E**) Hypothetical binding model of **6ba** with DNA and topo I. (**C**, **F**) Hypothetical binding model of **6fd** with DNA and topo I. Carbon units are light green in topotecan, **6ba**, and **6fd**, purple in nucleotides, and gray in amino acids. H-bonds are illustrated as discontinuous yellow lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

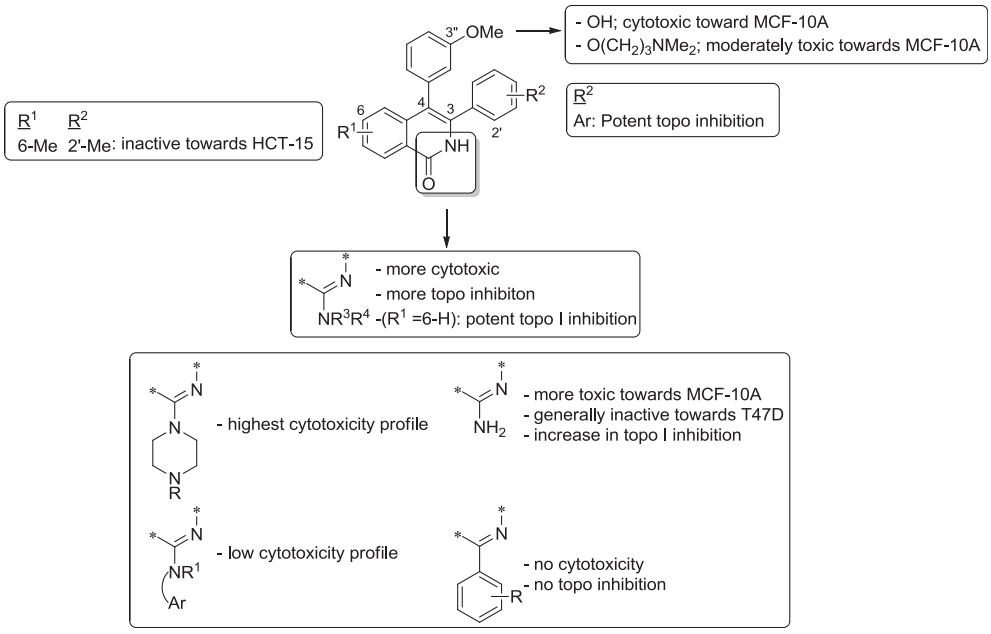



**Fig. 12.** SAR of 3,4-diarylisoquinolines.

*6.1.19. 4-Bromo-3-(3,4-dimethoxyphenyl)-1-methoxy-6-methylisoquinoline (**15b**)*

The procedures described for compound **15a** were used with compound **8b** (4.93 g, 13.2 mmol), iodomethane (2.8 g, 19.7 mmol), silver carbonate (3.27 g, 11.8 mmol), and toluene (30 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **15b** as a white solid (4.1 g, 80%). $R_f$ = 0.4 (*n*-hexane:EtOAc = 5:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.12 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.97 (s, 1H, Ar–H), 7.56 (dd, *J* = 8.4, 1.2 Hz, 1H, Ar–H), 7.33–7.29 (m, 2H, Ar–H), 7.07 (d, *J* = 8.1 Hz, 1H, Ar–H), 4.07 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 2.58 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 390 $(M+H)^+$ ($^{81}$Br), 388 $(M+H)^+$ ($^{79}$Br).

*6.1.20. 4-Bromo-1-methoxy-6-methyl-3-(m-tolyl)isoquinoline (**15c**)*

The procedures described for compound **15a** were used with compound **8c** (1.44 g, 4.41 mmol), iodomethane (939 mg, 6.61 mmol), silver carbonate (1.09 g, 3.97 mmol), and toluene (20 mL), followed by MPLC (*n*-hexane:EtOAc = 5:1) to obtain compound **15c** as a transparent viscous gel which solidified into a white solid on storage (784 mg, 51%). $R_f$ = 0.78 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.14 (d, *J* = 8.4 Hz, 1H, 8-H), 8.01 (s, 1H, 5-H), 7.56–7.54 (m, 2H, Ar–H), 7.41 (dd, *J* = 8.4, 1.5 Hz, 1H, Ar–H), 7.36 (t, *J* = 7.8 Hz, 1H, 3′-H), 7.22 (d, *J* = 7.5 Hz, 1H, Ar–H), 4.11 (s, 3H, $OCH_3$), 2.58 (s, 3H, Ar–$CH_3$), 2.44 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 344 $(M+H)^+$ ($^{81}$Br), 342 $(M+H)^+$ ($^{79}$Br).

*6.1.21. 4-Bromo-3-(2,6-dimethylphenyl)-1-methoxy-6-methylisoquinoline (**15d**)*

The procedures described for compound **15a** were used with compound **8d** (2.89 g, 8.46 mmol), iodomethane (1.8 g, 12.6 mmol), silver carbonate (2.09 g, 7.61 mmol), and toluene (25 mL), followed by column chromatography (*n*-hexane:$CH_2Cl_2$ = 3:1) to obtain compound **15d** as white crystals (2.66 g, 88%). $R_f$ = 0.75 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.18 (d, *J* = 8.4 Hz, 1H, 8-H), 7.96 (pentet, *J* = 0.7 Hz, 1H, 5-H), 7.44 (ddd, *J* = 8.4, 1.8, 0.6 Hz, 1H, Ar–H), 7.26–7.21 (m, ≈1H, Ar–H, partially overlapped with $CHCl_3$ peak), 7.15–7.12 (m, 2H, Ar–H), 4.04 (s, 3H, $OCH_3$), 2.59 (s, 3H, 6-$CH_3$), 2.06 (s, 6H, 2′,6′-$(CH_3)_2$). MS (ESI): *m*/*z* 358 $(M+H)^+$ ($^{81}$Br), 356 $(M+H)^+$ ($^{79}$Br).

*6.1.22. 4-Bromo-1-methoxy-3-(2-methoxyphenyl)isoquinoline (**15e**) and 3-aryl-4-bromo-1-methoxyisoquinoline (**15g**)*

The procedures described for compound **15a** were used with the mixture of compounds **8e** and **8g** (4.99 g), iodomethane (3.22 g, 22.7 mmol), silver carbonate (3.75 g, 13.6 mmol), and toluene, followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain a mixture of **15e** and **15g** as a white solid (4.7 g). $R_f$ = 0.68 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.28–8.25 (m, 2H, Ar–H), 8.21–8.17 (m, 2H, Ar–H), 7.80–7.73 (m, 2H, Ar–H), 7.63–7.56 (m, 2H, Ar–H), 7.50 (dd, *J* = 8.7, 2.7 Hz, 1H, Ar–H), 7.46–7.44 (m, 1H, Ar–H), 7.43–7.34 (m, 2H, Ar–H), 7.11–7.06 (m, 1H, Ar–H), 7.03 (d, *J* = 8.4 Hz, 1H, Ar–H), 6.89 (d, *J* = 8.7 Hz, 1H, Ar–H), 4.09 (s, 3H, $OCH_3$), 4.09 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 3.79 (s, 3H, $OCH_3$). **15e**: MS (ESI): *m*/*z* 346 $(M+H)^+$ ($^{81}$Br), 344 $(M+H)^+$ ($^{79}$Br). **15g**: 424 $(M+H)^+$ ($^{79}$Br, $^{81}$Br), 422 $(M+H)^+$ ($^{79}$Br), 426 $(M+H)^+$ ($^{81}$Br).

*6.1.23. 4-Bromo-1-methoxy-3-(4-methoxyphenyl)isoquinoline (**15f**)*

The procedures described for compound **15a** were used with compound **8f** (3.41 g, 10.3 mmol), iodomethane (2.2 g, 15.5 mmol), silver carbonate (1.68 g, 6.1 mmol), and toluene (30 mL), followed by column chromatography (*n*-hexane:EtOAc = 4:1, 3:1) to obtain compound **15f** as a white solid (3.19 g, 89%). $R_f$ = 0.28 (*n*-hexane:$CH_2Cl_2$ = 5:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.25–8.17 (m, 2H, Ar–H), 7.97–7.91 (m, 1H, Ar–H), 7.75–7.68 (m, 3H, Ar–H, 2′-H and 6′-H), 7.07–7.02 (m, 2H, 3′-H and 5′-H), 4.07 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$).

*6.1.24. 1-Methoxy-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl) isoquinoline (**14a**)*

The procedures described for compound **5a**′ were used with compound **15a** (891 mg, 2.60 mmol), a solution of 3-methoxyphenylboronic acid (791 mg, 5.20 mmol) in MeOH (4 mL), $Pd(PPh_3)_4$ (0) (150 mg, 0.13 mmol), sat. $Na_2CO_3$ sol. (3 mL), and 1,2-dimethoxy ethane (15 mL), followed by column

chromatography (*n*-hexane:$CH_2Cl_2$ = 5:1) to obtain compound **14a** as an off-white solid (942 mg, 97%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.22 (d, *J* = 9.0 Hz, 1H, Ar–H), 7.39–7.37 (m, 2H, Ar–H), 7.19–7.06 (m, 3H, Ar–H), 7.02–6.96 (m, 2H, Ar–H), 6.77–6.74 (m, 2H, Ar–H), 6.66 (s, 1H, Ar–H), 4.11 (s, 3H, $OCH_3$), 3.66 (s, 3H, $OCH_3$), 2.43 (s, 3H, Ar–$CH_3$), 2.20 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 370 $(M+H)^+$.

### 6.1.25. 3-(3,4-Dimethoxyphenyl)-1-methoxy-4-(3-methoxyphenyl)-6-methylisoquinoline (**14b**)

The procedures described for compound **5a**′ were used with compound **15b** (4.03 g, 10.4 mmol), a solution of 3-methoxyphenylboronic acid (3.16 g, 20.8 mmol) in MeOH (8 mL), $Pd(PPh_3)_4$ (0) (352 mg, 0.3 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (30 mL), followed by MPLC (*n*-hexane:$CH_2Cl_2$ = 3:1, 1:1) to obtain compound **14b** as a transparent viscous gel which solidified on pumping (2.59 g, 60%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.19 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.35 (s, 1H, Ar–H), 7.32–7.30 (m, 2H, Ar–H), 7.27–7.23 (m, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.92–6.86 (m, 3H, Ar–H), 6.80–6.77 (m, 2H, Ar–H), 4.21 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 3.73 (s, 3H, $OCH_3$), 3.56 (s, 3H, $OCH_3$), 2.41 (s, 3H, 6-$CH_3$). MS (ESI): *m*/*z* 416 $(M+H)^+$.

### 6.1.26. 1-Methoxy-4-(3-methoxyphenyl)-6-methyl-3-(m-tolyl) isoquinoline (**14c**)

The procedures described for compound **5a**′ were used with compound **15c** (1.73 g, 5.07 mmol), a solution of 3-methoxyphenylboronic acid (1.6 g, 10.5 mmol) in MeOH (5 mL), $Pd(PPh_3)_4$ (0) (305 mg, 0.26 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (20 mL), followed by column chromatography (*n*-hexane:$CH_2Cl_2$ = 5:1, *n*-hexane:EtOAc = 5:1) to isolate compound **14c** as a transparent gel (85 mg, 4%) and to obtain an impure **14c** (1.6 g). This **14c**-containing mixture was used in the next step, without further purification. $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.20 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.36–7.34 (m, 2H, Ar–H), 7.30–7.25 (m, ≈2H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.18 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.05 (t, *J* = 7.5 Hz, 1H, Ar–H), 6.98 (d, *J* = 7.2 Hz, 1H, Ar–H), 6.89–6.85 (m, 1H, Ar–H), 6.84–6.81 (m, 1H, Ar–H), 6.76–6.75 (m, 1H, Ar–H), 4.20 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$), 2.41 (s, 3H, Ar–$CH_3$), 2.24 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 370 $(M+H)^+$.

### 6.1.27. 3-(2,6-Dimethylphenyl)-1-methoxy-4-(3-methoxyphenyl)-6-methylisoquinoline (**14d**)

The procedures described for compound **5a**′ were used with compound **15d** (2.61 g, 7.32 mmol), a solution of 3-methoxyphenylboronic acid (2.22 g, 14.6 mmol) in MeOH (10 mL), $Pd(PPh_3)_4$ (0) (423 mg, 0.36 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (25 mL), followed by column chromatography (*n*-hexane:$CH_2Cl_2$ = 5:1, *n*-hexane:EtOAc = 5:1) to obtain compound **14d** as a transparent semi-solid which foamed out into white solid upon pumping (118 mg, 4%), and an impure **14d** (3.19 g). The **14d**-containing mixture was used in the next step without further purification. $^1$H NMR (500 MHz, DMSO-*d6*) δ (ppm): 8.18 (d, *J* = 8.5 Hz, 1H, Ar–H), 7.50–7.48 (m, 1H, Ar–H), 7.25 (s, 1H, Ar–H), 7.21 (t, *J* = 8.0 Hz, 1H, Ar–H), 7.02 (t, *J* = 7.5 Hz, 1H, Ar–H), 6.95 (d, *J* = 7.5 Hz, 1H, Ar–H), 6.89 (d, *J* = 7.5 Hz, 1H, Ar–H), 6.80 (dd, *J* = 8.0, 2.5 Hz, 1H, Ar–H), 6.77 (d, *J* = 7.5 Hz, 1H, Ar–H), 6.62 (t, *J* = 2.0 Hz, 1H, Ar–H), 4.00 (s, 3H, $OCH_3$), 3.60 (s, 3H, $OCH_3$), 2.39 (s, 3H, Ar–$CH_3$), 2.02 (s, 3H, Ar–$CH_3$), 1.90 (s, 3H, Ar–$CH_3$).

### 6.1.28. Methoxy-3-(2-methoxyphenyl)-4-(3-methoxyphenyl) isoquinoline (**14e**)

The procedures described for **5a**′ were used with the mixture of compounds **15e** and **15g** (4.62 g), a solution of 3-methoxyphenylboronic acid (4.08 g, 26.86 mmol) in MeOH (10 mL), $Pd(PPh_3)_4$ (0) (776 mg, 0.67 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (35 mL), followed by MPLC (*n*-hexane:$CH_2Cl_2$ = 12:1, 10:1, 5:1) to obtain compounds **14e** as a white solid (2.4 g) and **14g** as an egg yolk-colored viscous liquid which solidified on storage (1.89 g). $R_f$ = 0.56 (*n*-hexane:EtOAc = 5:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.28–8.25 (m, 1H, Ar–H), 7.74–7.60 (m, 2H, Ar–H), 7.48–7.45 (m, 1H, Ar–H), 7.24–7.16 (m, 3H, Ar–H), 6.87–6.77 (m, 3H, Ar–H), 6.72 (d, *J* = 7.5 Hz, 1H, Ar–H), 6.67 (s, 1H, 2″-H), 4.04 (s, 3H, $OCH_3$), 3.61 (s, 3H, $OCH_3$), 3.50 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 372 $(M+H)^+$.

### 6.1.29. 3-Aryl-1-methoxy-4-(3-methoxyphenyl)isoquinoline (**14g**)

$R_f$ = 0.43 (*n*-hexane:EtOAc = 5:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.30–8.27 (m, 1H, Ar–H), 7.74–7.62 (m, 2H, Ar–H), 7.53–7.47 (m, 3H, Ar–H), 7.30 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.22 (t, *J* = 7.8 Hz, 1H, Ar–H), 7.09 (d, *J* = 8.1 Hz, 1H, Ar–H), 7.01 (t, *J* = 2.1 Hz, 1H, Ar–H), 6.94 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.86–6.76 (m, 4H, Ar–H), 4.06 (s, 3H, $OCH_3$), 3.78 (s, 3H, $OCH_3$), 3.60 (s, 3H, $OCH_3$), 3.57 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 478 $(M+H)^+$.

### 6.1.30. 1-Methoxy-4-(3-methoxyphenyl)-3-(4-methoxyphenyl) isoquinoline (**14f**)

The procedures described for **5a**′ were used with compound **15f** (3.06 g, 8.89 mmol), a solution of 3-methoxyphenylboronic acid (1.81 g, 11.9 mmol) in MeOH (5 mL), $Pd(PPh_3)_4$ (0) (514 mg, 0.44 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to recover **15f** (1.83 g, 59%), to isolate compound **14f** as a transparent viscous liquid which solidified on storage (265 mg, 8%), and to obtain an impure **14f**. The procedures described for **5a**′ were further used with the recovered **15f** (1.83 g, 5.31 mmol), a solution of 3-methoxyphenylboronic acid (1.61 g, 10.6 mmol) in MeOH (5 mL), $Pd(PPh_3)_4$ (0) (307 mg, 0.26 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (20 mL). An impure **14f** (2.16 g) obtained from these two reactions was used in the next step, without further attempts at isolation. $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.31–8.28 (m, 1H, Ar–H), 7.60–7.47 (m, 3H, Ar–H), 7.44–7.39 (m, 2H, 2′-H and 6′-H), 7.32–7.27 (m, 1H, Ar–H), 6.91–6.78 (m, 3H, Ar–H), 6.76–6.71 (m, 2H, 3′-H and 5′-H), 4.21 (s, 3H, $OCH_3$), 3.77 (s, 3H, $OCH_3$), 3.74 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 372 $(M+H)^+$.

### 6.1.31. 3-(1-Methoxy-6-methyl-3-(o-tolyl)isoquinolin-4-yl)phenol (**14a**′)

The procedures described for compound **5a**′ were used with compound **15a** (986 mg, 2.88 mmol), a solution of 3-hydroxyphenylboronic acid (795 mg, 5.76 mmol) in MeOH (5 mL), $Pd(PPh_3)_4$ (0) (166 mg, 0.14 mmol), sat. $Na_2CO_3$ sol. (5 mL), and 1,2-dimethoxy ethane (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **14a**′ as free-flowing white crystals (650 mg, 63%). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 9.34 (bs, 1H, OH), 8.16 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.49–7.46 (m, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.15–7.04 (m, 3H, Ar–H), 7.00–6.94 (m, 2H, Ar–H), 6.63–6.54 (m, 3H, Ar–H), 4.01 (s, 3H, $OCH_3$), 2.38 (s, 3H, Ar–$CH_3$), 2.14 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 356 $(M+H)^+$.

### 6.1.32. 4-(3-Methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinolin-1(2H)-one (**5a**)

A mixture of compound **14a** (904 mg, 2.44 mmol) and AcOH:$H_2O$ = 9:1 (10 mL) was refluxed for 39 h. Excess AcOH was neutralized with sat. $NaHCO_3$ sol. (100 mL) followed by extraction with $CH_2Cl_2$. The organic layer was washed with water,

concentrated under reduced pressure, and purified by re-crystallization from MeOH to obtain compound **5a** as a white solid (617 mg, 70%). Mp: 239 °C (dec.). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.39 (d, $J$ = 8.1 Hz, 1H, 8-H), 8.32 (s, 1H, NH), 7.35 (d, $J$ = 8.1 Hz, 1H, 7-H), 7.22–7.08 (m, 6H, Ar–H), 6.78–6.74 (m, 2H, Ar–H), 6.65 (s, 1H, Ar–H), 3.67 (s, 3H, $OCH_3$), 2.39 (s, 3H, Ar–$CH_3$), 2.18 (s, 3H, Ar–$CH_3$). MS (ESI): $m/z$ 356 $(M+H)^+$, 419 $(M+Na+CH_3CN)^+$. HRMS (ESI): $m/z$ 378.1466 $(M+Na)^+$ (calcd for $C_{24}H_{21}NNaO_2$, 378.1470), 356.1645 $(M+H)^+$ (calcd for $C_{24}H_{22}NO_2$, 356.1651).

### 6.1.33. 3-(3,4-Dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1(2H)-one (**5b**)

The procedures described for compound **5a** were used with compound **14b** (973 mg, 2.34 mmol) and AcOH:$H_2O$ = 9:1 (20 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, EtOAc) to obtain compound **5b** as a white solid (825 mg, 87%). $R_f$ = 0.51 (EtOAc). Mp: 164–165 °C. $^1$H NMR (300 MHz, DMSO-$d6$) δ (ppm): 11.35 (s, 1H, NH), 8.19 (d, $J$ = 8.1 Hz, 1H, Ar–H), 7.33–7.30 (m, 1H, Ar–H), 7.26 (t, $J$ = 7.9 Hz, 1H, Ar–H), 6.97 (s, 1H, Ar–H), 6.88–6.72 (m, 6H, Ar–H), 3.70 (s, 3H, $OCH_3$), 3.66 (s, 3H, $OCH_3$), 3.53 (s, 3H, $OCH_3$), 2.31 (s, 3H, 6-$CH_3$). $^{13}$C NMR (75 MHz, DMSO-$d6$) δ (ppm): 161.7, 159.1, 148.5, 147.4, 142.4, 138.3, 137.7, 129.4, 127.5, 126.8, 126.8, 124.4, 124.0, 122.6, 122.3, 117.4, 114.7, 113.6, 112.5, 110.7, 55.3, 55.1, 55.0, 21.6. MS (ESI): $m/z$ 402 $(M+H)^+$, 443 $(M+CH_3CN+H)^+$, 400 $(M-H)^-$. HRMS (ESI): $m/z$ 424.1517 $(M+Na)^+$ (calcd for $C_{25}H_{23}NNaO_4$, 424.1525), 402.1698 $(M+H)^+$ (calcd for $C_{25}H_{24}NO_4$, 402.1705).

### 6.1.34. 4-(3-Methoxyphenyl)-6-methyl-3-(m-tolyl)isoquinolin-1(2H)-one (**5c**)

The procedures described for compound **5a** were used with the impure **14c** (1.60 g) and AcOH:$H_2O$ = 9:1 (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc) to obtain compound **5c** as a free flowing white solid (1.01 g, 56% yield from **15c**). Mp: 203–204 °C. $^1$H NMR (300 MHz, DMSO-$d6$) δ (ppm): 11.36 (s, 1H, NH), 8.19 (d, $J$ = 8.1 Hz, 1H, 8-H), 7.33 (dd, $J$ = 8.1, 1.2 Hz, 1H, Ar–H), 7.23 (t, $J$ = 7.9 Hz, 1H, Ar–H), 7.11–7.04 (m, 3H, Ar–H), 6.99–6.96 (m, 2H, Ar–H), 6.85–6.81 (m, 1H, Ar–H), 6.74–6.71 (m, 1H, Ar–H), 6.68–6.67 (m, 1H, Ar–H), 3.64 (s, 3H, $OCH_3$), 2.32 (s, 3H, Ar–$CH_3$), 2.19 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 162.5, 159.4, 143.3, 138.6, 138.1, 137.2, 137.0, 135.0, 129.4, 129.3, 129.2, 128.1, 127.4, 126.2, 125.3, 124.3, 122.7, 117.2, 116.7, 112.8, 55.2, 22.1, 21.3. MS (ESI): $m/z$ 419 $(M+Na+CH_3CN)^+$, 378 $(M+Na)^+$. HRMS (ESI): $m/z$ 378.1463 $(M+Na)^+$ (calcd for $C_{24}H_{21}NNaO_2$, 378.1470), 356.1644 $(M+H)^+$ (calcd for $C_{24}H_{22}NO_2$, 356.1651).

### 6.1.35. 3-(2,6-Dimethylphenyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1(2H)-one (**5d**)

The procedures described for compound **5a** were used with the two preparations of impure **14d** (1.48 + 1.6 g) and AcOH:$H_2O$ = 9:1 (20 + 20 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc) to obtain compound **5d** as a white solid (1.19 + 1.44 g). The net yield of compound **5d**, starting from **15d**, was 97%. $R_f$ = 0.12 ($n$-hexane:EtOAc = 3:1). Mp: 266–268 °C. IR ($cm^{-1}$): 3115 (N–H), 1621 (C=O). $^1$H NMR (500 MHz, DMSO-$d6$) δ (ppm): 11.32 (s, 1H, NH), 8.19 (d, $J$ = 8.0 Hz, 1H, 8-H), 7.35 (dd, $J$ = 8.5, 1.0 Hz, 1H, Ar–H), 7.18 (t, $J$ = 8.0 Hz, 1H, Ar–H), 7.07 (t, $J$ = 7.7 Hz, 1H, Ar–H), 7.00–6.97 (m, 2H, Ar–H), 6.92 (d, $J$ = 7.5 Hz, 1H, Ar–H), 6.77–6.76 (m, 1H, Ar–H), 6.73 (d, $J$ = 7.5 Hz, 1H, Ar–H), 6.59–6.58 (m, 1H, Ar–H), 3.59 (s, 3H, $OCH_3$), 2.32 (s, 3H, Ar–$CH_3$), 2.17 (s, 3H, Ar–$CH_3$), 2.05 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 162.6, 159.0, 143.3, 138.4, 136.7, 136.6, 136.5, 135.9, 134.0, 128.9, 128.8, 128.1, 127.5, 127.4, 127.3, 125.1, 123.1, 122.6, 117.2, 115.4, 113.1, 55.0, 22.0, 20.1, 20.0. Anal. calcd for $C_{25}H_{23}NO_2$: C, 81.27; H, 6.27; N, 3.79. Found C, 80.93; H, 6.27; N, 3.55.

### 6.1.36. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)isoquinolin-1(2H)-one (**5e**)

The procedures described for compound **5a** were used with compound **14e** (2.36 g, 6.35 mmol) and AcOH:$H_2O$ = 9:1 (20 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc) to obtain compound **5e** as a white solid (2.03 g, 89%). $R_f$ = 0.71 (EtOAc). Mp: 187–191 °C. $^1$H NMR (300 MHz, DMSO-$d6$) δ (ppm): 11.42 (s, 1H, NH), 8.30–8.27 (m, 1H, 8-H), 7.66–7.60 (m, 1H, Ar–H), 7.53–7.47 (m, 1H, Ar–H), 7.27–7.14 (m, 3H, Ar–H), 7.07–7.04 (m, 1H, Ar–H), 6.94 (d, $J$ = 8.4 Hz, 1H, Ar–H), 6.80–6.65 (m, 4H, Ar–H), 3.69 (s, 3H, $OCH_3$), 3.61 (s, 3H, $OCH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 162.3, 159.1, 156.7, 138.5, 137.2, 134.8, 132.3, 131.8, 130.4, 128.9, 127.4, 126.4, 125.5, 125.2, 123.8, 123.3, 120.2, 118.0, 116.6, 112.8, 110.7, 55.3, 55.1. MS (ESI): $m/z$ 358 $(M+H)^+$, 399 $(M+CH_3CN+H)^+$. HRMS (ESI): $m/z$ 380.1257 $(M+Na)^+$ (calcd for $C_{23}H_{19}NNaO_3$, 380.1263), 358.1436 $(M+H)^+$ (calcd for $C_{23}H_{20}NO_3$, 358.1443).

### 6.1.37. 4-(3-Methoxyphenyl)-3-(4-methoxyphenyl)isoquinolin-1(2H)-one (**5f**)

The procedures described for compound **5a** were used with the impure **14f** (2.16 g) and AcOH:$H_2O$ = 9:1 (20 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, 1:1) to obtain compound **5f** as a white solid (1.73 g, 54% yield from **15f**). Mp: 217–219 °C. IR ($cm^{-1}$): 3154 (N–H), 1653 (C=O). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.73 (bs, 1H, NH), 8.49–8.46 (m, 1H, 8-H), 7.59–7.55 (m, 1H, Ar–H), 7.51–7.46 (m, 1H, Ar–H), 7.39–7.35 (m, 1H, Ar–H), 7.27–7.22 (m, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.18–7.15 (m, 2H, 2′-H and 6′-H), 6.86–6.72 (m, 5H, Ar–H, 3′-H and 5′-H), 3.78 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 162.8, 159.5, 138.6, 137.3, 136.8, 132.5, 130.4, 129.3, 127.3, 127.2, 126.3, 125.4, 124.8, 124.3, 117.2, 116.6, 113.7, 112.8, 55.1. MS (ESI) $m/z$ 358 $(M+H)^+$, 399 $(M+CH_3CN+H)^+$. Anal. calcd for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found C, 76.87; H, 5.34; N, 3.5.

### 6.1.38. 3-Aryl-4-(3-methoxyphenyl)isoquinolin-1(2H)-one (**5g**)

The procedures described for compound **5a** were used with compound **14g** (1.83 g, 3.84 mmol) and AcOH:$H_2O$ = 9:1 (20 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, EtOAc) to obtain compound **5g** as a transparent semi-solid which foamed out into a white solid upon pumping (1.73 g, 97%). $R_f$ = 0.25 ($n$-hexane:EtOAc = 1:1). Mp: 113–116 °C. IR ($cm^{-1}$): 3175 (N–H), 1648 (C=O). $^1$H NMR (300 MHz, DMSO-$d6$) δ (ppm): 11.48 (s, 1H, NH), 8.31 (dd, $J$ = 8.1, 1.2 Hz, 1H, 8-H), 7.68–7.62 (m, 1H, Ar–H), 7.57–7.49 (m, 2H, Ar–H), 7.40 (d, $J$ = 2.7 Hz, 1H, Ar–H), 7.28 (t, $J$ = 7.8 Hz, 1H, Ar–H), 7.23–7.17 (m, 2H, Ar–H), 7.04–7.00 (m, 2H, Ar–H), 6.92 (t, $J$ = 1.9 Hz, 1H, Ar–H), 6.84 (dd, $J$ = 8.1, 1.8 Hz, 1H, Ar–H), 6.79–6.76 (m, 3H, Ar–H), 3.78 (s, 3H, $OCH_3$), 3.74 (s, 3H, $OCH_3$), 3.61 (s, 3H, $OCH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 162.4, 159.8, 159.3, 156.3, 141.4, 138.5, 137.4, 134.8, 133.1, 132.3, 131.0, 129.6, 129.0, 128.9, 127.4, 126.4, 125.5, 125.2, 123.9, 123.2, 119.1, 118.1, 116.9, 112.9, 112.3, 112.1, 111.1, 55.5, 55.2, 55.1. MS (ESI): $m/z$ 464 $(M+H)^+$, 462 $(M-H)^-$. Anal. calcd for $C_{30}H_{25}NO_4$: C, 77.74; H, 5.44; N, 3.02. Found C, 77.3; H, 5.42; N, 2.71.

### 6.1.39. 1-Chloro-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl) isoquinoline (**7a**)

The procedures described for **12a** were used with **5a** (442 mg, 1.24 mmol) and $POCl_3$ (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1) to obtain compound **7a** as a white solid (465 mg, quantitative). $^1$H NMR (300 MHz, DMSO-$d6$) δ (ppm): 8.29 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.72–7.68 (m, 1H, Ar–H),

7.34 (s, 1H, Ar–H), 7.27–7.22 (m, 1H, Ar–H), 7.15–7.13 (m, 2H, Ar–H), 7.10–6.99 (m, 2H, Ar–H), 6.86–6.74 (m, 3H, Ar–H), 3.63 (s, 3H, $OCH_3$), 2.44 (s, 3H, Ar–$CH_3$), 2.06 (s, 3H, Ar–$CH_3$).

*6.1.40. 1-Chloro-3-(3,4-dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methylisoquinoline (**7b**)*

The procedures described for compound **12a** were used with compound **5b** (2.02 g, 5.05 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **7b** as a transparent semi-solid which solidified into a white solid on storage (1.8 g, 84%). $R_f$ = 0.18 (*n*-hexane:EtOAc = 1:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.25 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.66–7.63 (m, 1H, Ar–H), 7.41–7.36 (m, 1H, Ar–H), 7.32 (s, 1H, Ar–H), 7.07 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar–H), 7.02–6.98 (m, 1H, Ar–H), 6.89–6.80 (m, 4H, Ar–H), 3.72 (s, 3H, $OCH_3$), 3.70 (s, 3H, $OCH_3$), 3.44 (s, 3H, $OCH_3$), 2.43 (s, 3H, 6-$CH_3$). MS (ESI): *m*/*z* 420 $(M+H)^+$.

*6.1.41. 1-Chloro-4-(3-methoxyphenyl)-6-methyl-3-(m-tolyl) isoquinoline (**7c**)*

The procedures described for **12a** were used with **5c** (983 mg, 2.88 mmol) and $POCl_3$ (15 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 4:1) to obtain compound **7c** as a free-flowing white solid (954 mg, 88%). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.27 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.67 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.37–7.32 (m, 2H, Ar–H), 7.22 (s, 1H, Ar–H), 7.08–7.05 (m, 3H, Ar–H), 6.99–6.95 (m, 1H, Ar–H), 6.85–6.80 (m, 2H, Ar–H), 3.68 (s, 3H, $OCH_3$), 2.44 (s, 3H, Ar–$CH_3$), 2.21 (s, 3H, Ar–$CH_3$). MS (ESI): *m*/*z* 374 $(M+H)^+$.

*6.1.42. 1-Chloro-3-(2,6-dimethylphenyl)-4-(3-methoxyphenyl)-6-methylisoquinoline (**7d**)*

The procedures described for **12a** were used with **5d** (1.12 g, 3.05 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **7d** as a transparent semi-solid which foamed out into a white solid on pumping (1.19 g, quantitative). $R_f$ = 0.64 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.30 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.73–7.69 (m, 1H, Ar–H), 7.37 (s, 1H, Ar–H), 7.28–7.23 (m, 1H, Ar–H), 7.09–7.04 (m, 1H, Ar–H), 6.99–6.91 (m, 2H, Ar–H), 6.88–6.80 (m, 2H, Ar–H), 6.69 (dd, *J* = 2.4, 1.5 Hz, 1H, Ar–H), 3.62 (s, 3H, $OCH_3$), 2.45 (s, 3H, Ar–$CH_3$), 1.98 (s, 3H, Ar–$CH_3$), 1.87 (s, 3H, Ar–$CH_3$).

*6.1.43. 1-Chloro-3-(2-methoxyphenyl)-4-(3-methoxyphenyl) isoquinoline (**7e**)*

The procedures described for **12a** were used with compound **5e** (1.98 g, 5.54 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **7e** as a white solid (1.93 g, 92%). $R_f$ = 0.51 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.40–8.35 (m, 1H, Ar–H), 7.88–7.82 (m, 2H, Ar–H), 7.62–7.58 (m, 1H, Ar–H), 7.27–7.16 (m, 3H, Ar–H), 6.89–6.82 (m, 3H, Ar–H), 6.78–6.74 (m, 2H, Ar–H), 3.62 (s, 3H, $OCH_3$), 3.53 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 376 $(M+H)^+$.

*6.1.44. 1-Chloro-4-(3-methoxyphenyl)-3-(4-methoxyphenyl) isoquinoline (**7f**)*

The procedures described for **12a** were used with **5f** (1.64 g, 4.61 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:$CH_2Cl_2$ = 3:1, 1:1, *n*-hexane:EtOAc = 5:1) to obtain compound **7f** as a transparent semi-solid which foamed out into a white solid on pumping (1.26 g, 72%). $R_f$ = 0.45 (*n*-hexane:EtOAc = 5:1). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.41–8.37 (m, 1H, Ar–H), 7.69–7.62 (m, 3H, Ar–H), 7.38–7.29 (m, 3H, 2′-H and 6′-H, Ar–H), 6.94–6.90 (m, 1H, Ar–H), 6.85–6.82 (m, 1H, Ar–H), 6.78–6.72 (m, 3H, Ar–H, 3′-H and 5′-H), 3.77 (s, 3H, $OCH_3$), 3.74 (s, 3H, $OCH_3$). MS (ESI): *m*/*z* 376 $(M+H)^+$.

*6.1.45. 3-Aryl-1-chloro-4-(3-methoxyphenyl)isoquinoline (**7g**)*

The procedures described for **12a** were used with **5g** (1.66 g, 3.59 mmol) and $POCl_3$ (20 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1) to obtain compound **7g** as an off-white semi-solid which foamed out into an off-white solid on pumping (1.68 g, 97%). $R_f$ = 0.43 (*n*-hexane:EtOAc = 3:1). $^1$H NMR (300 MHz, $CD_3OD$) δ (ppm): 8.49–8.42 (m, 1H, Ar–H), 7.83–7.70 (m, 3H, Ar–H), 7.51–7.47 (m, 1H, Ar–H), 7.38 (d, *J* = 1.8 Hz, 1H, Ar–H), 7.28–7.22 (m, 2H, Ar–H), 7.03–7.00 (m, 1H, Ar–H), 6.96–6.93 (m, 2H, Ar–H), 6.87–6.79 (m, 4H, Ar–H), 3.80 (s, 3H, $OCH_3$), 3.67 (s, 3H, $OCH_3$), 3.65 (s, 3H, $OCH_3$).

*6.1.46. 1-(4-Ethylpiperazin-1-yl)-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinoline (**6ac**)*

Compound **7a** (265 mg, 0.71 mmol) was dissolved in DMF (5 mL) prior to the addition of 1-ethylpiperazine (324 mg, 2.83 mmol) and $K_2CO_3$ (882 mg, 6.38 mmol). The reaction mixture was heated using an oil bath set at 150 °C. Upon completion of the reaction, water (50 mL) was poured into the reaction mixture and it was then extracted with EtOAc. The organic layer was washed with water, dried using brine and anhydrous $Na_2CO_3$, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1, EtOAc, MeOH) to obtain compound **6ac** as a brown semi-solid (288 mg, 90%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.06 (d, *J* = 8.4 Hz, 1H, Ar–H), 7.40 (s, 1H, Ar–H), 7.35–7.31 (m, 1H, Ar–H), 7.19–7.04 (m, 3H, Ar–H), 6.97–6.90 (m, 2H, Ar–H), 6.77–6.73 (m, 2H, Ar–H), 6.64 (s, 1H, Ar–H), 3.65 (s, 3H, $OCH_3$), 3.53 (m, 4H, 1″-$N(CH_2)_2$), 2.77 (m, 4H, 4″-$N(CH_2)_2$), 2.58 (q, 2H, $CH_2CH_3$), 2.41 (s, 3H, Ar–$CH_3$), 2.18 (s, 3H, Ar–$CH_3$), 1.17 (t, *J* = 7.2 Hz, 3H, $CH_2CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 158.9, 149.5, 139.6, 139.0, 138.1, 136.4, 132.1, 131.9, 130.3, 129.6, 128.6, 128.5, 128.4, 127.5, 126.9, 125.2, 125.0, 124.6, 123.7, 118.3, 116.5, 112.5, 55.0, 52.9, 52.5, 51.2, 22.0, 20.2, 11.9. MS (ESI): *m*/*z* 452 $(M+H)^+$.

*6.1.47. 2-(4-(4-(3-Methoxyphenyl)-6-methyl-3-(o-tolyl) isoquinolin-1-yl)piperazin-1-yl)-N,N-dimethylethanamine (**6ad**)*

The procedures described for compound **6ac** were used with compound **7a** (220 mg, 0.58 mmol), 1-[2-(dimethylamino)ethyl] piperazine (370 mg, 2.35 mmol), $K_2CO_3$ (732 mg, 5.29 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **6ad** as a yellow solid (239 mg, 82%). Mp: 81–83 °C. $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.07 (d, *J* = 8.7 Hz, 1H, Ar–H), 7.40 (s, 1H, Ar–H), 7.34–7.31 (m, 1H, Ar–H), 7.18–7.04 (m, 3H, Ar–H), 6.97–6.91 (m, 2H, Ar–H), 6.77–6.73 (m, 2H, Ar–H), 6.64 (s, 1H, Ar–H), 3.65 (s, 3H, $OCH_3$), 3.48 (m, 4H, 1″-$N(CH_2)_2$), 2.75 (t, *J* = 4.6 Hz, 4H, 4″-$N(CH_2)_2$), 2.63–2.58 (m, 2H), 2.53–2.48 (m, 2H), 2.41 (s, 3H, Ar–$CH_3$), 2.28 (s, 6H, $N(CH_3)_2$), 2.18 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 158.9, 149.3, 140.8, 139.9, 138.8, 138.1, 136.3, 130.3, 129.6, 128.6, 127.8, 126.9, 126.2, 125.1, 124.7, 123.6, 118.4, 116.4, 112.5, 55.0, 54.3, 54.1, 53.7, 53.2, 44.0, 21.9, 20.2. MS (ESI): *m*/*z* 495 $(M+H)^+$. HRMS (ESI): *m*/*z* 495.3129 $(M+H)^+$ (calcd for $C_{32}H_{39}N_4O$, 495.3124), 517.2947 $(M+Na)^+$ (calcd for $C_{32}H_{38}N_4NaO$, 517.2943).

*6.1.48. 4-(3-Methoxyphenyl)-6-methyl-N-(4-methylpiperazin-1-yl)-3-(o-tolyl)isoquinolin-1-amine (**6ai**)*

The procedures described for compound **6ac** were used with compound **7a** (192 mg, 0.51 mmol), 97% 1-amino-4-methylpiperazine (244 mg, 2.05 mmol), $K_2CO_3$ (639 mg, 4.62 mmol), and DMF (7 mL), followed by column chromatography (*n*-hexane:EtOAc = 1:1, EtOAc, MeOH) to obtain compound **6ai** as a

yellow semi-solid (193 mg, 83%). IR ($cm^{-1}$): 3407 (N–H). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 9.06 (bs, 1H, NH), 8.75 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.36–7.31 (m, 2H, Ar–H), 7.18–7.06 (m, 3H, Ar–H), 7.03–6.96 (m, 2H, Ar–H), 6.76–6.65 (m, 3H, Ar–H), 3.65 (s, 3H, $OCH_3$), 2.98 (bs, 4H, $N(CH_2)_2$), 2.58 (bs, 4H, $N(CH_2)_2$), 2.41 (s, 3H, $NCH_3$), 2.33 (s, 3H, Ar–$CH_3$), 2.11 (s, 3H, Ar–$CH_3$). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ (ppm):159.2, 140.8, 136.2, 130.4, 130.2, 128.9, 127.7, 125.4, 125.0, 124.1, 116.8, 112.8, 55.3, 55.0, 45.9, 22.2, 20.2. MS (ESI): $m/z$ 453 $(M+H)^+$.

### 6.1.49. *N-(1-ethylpiperidin-4-yl)-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinolin-1-amine (**6aj**)*

The procedures described for compound **6ac** were used with compound **7a** (271 mg, 0.72 mmol), 97% 3-amino-*N*-ethylpiperidine (383 mg, 2.89 mmol), $K_2CO_3$ (902 mg, 6.52 mmol), and DMF (7 mL), followed by column chromatography (*n*-hexane:EtOAc = 1:1, EtOAc, MeOH) to obtain compound **6aj** as a yellow semi-solid (361 mg, quantitative). IR ($cm^{-1}$): 3341 (N–H). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80 (d, $J$ = 7.8 Hz, 1H, Ar–H), 7.35–7.29 (m, 2H, Ar–H), 7.14–6.90 (m, 5H, Ar–H), 6.77–6.60 (m, 3H, Ar–H), 4.58 (bs, 1H, NH), 3.66 (s, 3H, $OCH_3$), 2.60 (bs, 2H), 2.45–2.39 (m, 5H), 2.28–2.20 (m, 4H), 1.81–1.63 (m, 6H), 1.10 (t, $J$ = 7.3 Hz, 3H, $NCH_2\underline{CH}_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm):158.9, 152.7, 141.6, 139.6, 137.2, 136.1, 132.1, 132.0, 131.9, 130.2, 129.4, 128.5, 128.4, 127.3, 126.7, 124.9, 124.6, 115.1, 112.2, 57.7, 55.0, 53.5, 52.5, 21.9, 20.2, 11.3, 7.6. MS (ESI): $m/z$ 466 $(M+H)^+$.

### 6.1.50. *N-(4-methoxybenzyl)-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinolin-1-amine (**6am**)*

The procedures described for compound **6ac** were used with compound **7a** (314 mg, 0.84 mmol), 98% 4-methoxybenzylamine (461 mg, 3.36 mmol), $K_2CO_3$ (1.04 g, 7.55 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:$CH_2Cl_2$ = 3:1, 1:1, *n*-hexane:EtOAc = 5:1) to obtain compound **6am** as a light green viscous liquid (325 mg, 81%). IR ($cm^{-1}$): 3365 (N–H). $^1H$ NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.27 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.94 (t, $J$ = 5.8 Hz, 1H, NH), 7.35 (dd, $J$ = 8.7, 1.5 Hz, 1H, Ar–H), 7.27–7.24 (m, 2H, 2″-H and 6″-H), 7.18–7.14 (m, 2H, Ar–H), 7.04–7.01 (m, 2H, Ar–H), 6.93 (m, 2H, Ar–H), 6.84–6.81 (m, 2H, 3″-H and 5″-H), 6.74–6.70 (m, 2H, Ar–H), 6.57 (bs, 1H, Ar–H), 4.63 (m, 2H, $NCH_2$), 3.70 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 2.33 (s, 3H, Ar–$CH_3$), 1.96 (s, 3H, Ar–$CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 158.9, 158.7, 153.2, 141.4, 139.7, 139.4, 137.1, 136.3, 131.9, 130.2, 129.4, 128.5, 127.4, 126.8, 125.2, 124.6, 121.5, 121.4, 121.1114.8, 113.9, 112.2, 55.3, 55.0, 45.3, 21.9, 20.1. MS (ESI): $m/z$ 475 $(M+H)^+$.

### 6.1.51. *N-benzyl-4-(3-methoxyphenyl)-6-methyl-3-(o-tolyl) isoquinolin-1-amine (**6an**)*

The procedures described for compound **6ac** were used with compound **7a** (110 mg, 0.29 mmol), benzylamine (126 mg, 1.17 mmol), $K_2CO_3$ (366 mg, 2.64 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane, $CH_2Cl_2$) to obtain compound **6an** as a yellow semi-solid which foamed out into a solid on pumping (43 mg, 32%). Mp: 127–129 °C. $^1H$ NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.29 (d, $J$ = 8.4 Hz, 1H, Ar–H), 8.05 (t, $J$ = 6.0 Hz, 1H, NH), 7.39–7.23 (m, 5H, Ar–H), 7.21–7.12 (m, 3H, Ar–H), 7.01–6.99 (m, 2H, Ar–H), 6.90–6.89 (m, 2H, Ar–H), 6.73–6.70 (m, 2H, Ar–H), 6.56 (bs, 1H, Ar–H), 4.69 (s, 2H, $NCH_2$), 3.58 (s, 3H, $OCH_3$), 2.34 (s, 3H, Ar–$CH_3$), 1.90 (s, 3H, Ar–$CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 158.9, 153.2, 150.1, 141.4, 140.0, 139.7, 139.4, 137.2, 136.3, 130.2, 129.5, 128.5, 128.0, 127.4, 127.1, 126.8, 125.3, 124.6, 121.6, 121.1, 114.8, 112.2, 55.0, 45.8, 21.9, 20.1. MS (ESI): $m/z$ 445 $(M+H)^+$. Anal. calcd for $C_{31}H_{28}N_2O \cdot 0.35C_4H_8O_2$: C, 81.86; H, 6.53; N, 5.89. Found C, 81.78; H, 6.31; N, 5.88.

### 6.1.52. *3-(3,4-Dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methyl-1-(piperazin-1-yl)isoquinoline (**6ba**)*

The procedures described for compound **6ac** were used with compound **7b** (300 mg, 0.71 mmol), anhydrous piperazine (246 mg, 2.85 mmol), $K_2CO_3$ (889 mg, 6.43 mmol), and DMF (7 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1, EtOAc, MeOH) to obtain compound **6ba** as a transparent semi-solid which foamed out into an off-white solid on pumping (283 mg, 84%). $R_f$ = 0.11 (MeOH). Mp: 82–85 °C. IR ($cm^{-1}$): 3411 (N–H). $^1H$ NMR (300 MHz, $CD_3OD$) δ (ppm): 8.09 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.39–7.31 (m, 3H, Ar–H), 7.16 (dd, $J$ = 8.4, 2.1 Hz, 1H, Ar–H), 6.95–6.92 (m, 1H, Ar–H), 6.89 (d, $J$ = 2.1 Hz, 1H, Ar–H), 6.83–6.81 (m, 2H, Ar–H), 6.74 (dd, $J$ = 2.7, 1.5 Hz, 1H, Ar–H), 3.79 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 3.51 (s, 3H, $OCH_3$), 3.46–3.43 (m, 4H, 1″-$N(CH_2)_2$), 3.15–3.12 (m, 4H, 4″-$N(CH_2)_2$), 2.38 (s, 3H, 6-$CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 159.7, 158.4, 148.0, 147.4, 146.7, 140.1, 139.7, 138.8, 133.2, 129.4, 128.0, 125.36, 125.31, 124.3, 123.9, 122.8, 117.8, 116.8, 113.4, 112.7, 110.3, 55.7, 55.4, 55.2, 48.7, 43.4, 22.0. MS (ESI): $m/z$ 470 $(M+H)^+$. HRMS (ESI): $m/z$ 470.2442 $(M+H)^+$ (calcd for $C_{29}H_{32}N_3O_3$, 470.2444), 492.2260 $(M+Na)^+$ (calcd for $C_{29}H_{31}N_3NaO_3$, 492.2263).

### 6.1.53. *3-(3,4-Dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methyl-1-(4-methylpiperazin-1-yl)isoquinoline (**6bb**)*

The procedures described for compound **6ac** were used with compound **7b** (200 mg, 0.47 mmol), 1-methylpiperazine (191 mg, 1.9 mmol), $K_2CO_3$ (592 mg, 4.28 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 1:1, EtOAc, MeOH) to obtain compound **6bb** as a light hay-colored semi-solid (216 mg, 93%). $R_f$ = 0.48 (MeOH). $^1H$ NMR (300 MHz, $CD_3OD$) δ (ppm): 8.09 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.41–7.32 (m, 3H, Ar–H), 7.17 (dd, $J$ = 8.3, 2.0 Hz, 1H, Ar–H), 6.96–6.92 (m, 1H, Ar–H), 6.89 (d, $J$ = 2.0 Hz, 1H, Ar–H), 6.84–6.80 (m, 2H, Ar–H), 6.74 (dd, $J$ = 2.5, 1.4 Hz, 1H, Ar–H), 3.79 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$), 3.56 (b, 4H, 1″-$N(CH_2)_2$), 3.51 (s, 3H, $OCH_3$), 2.92 (t, $J$ = 4.6 Hz, 4H, 4″-$N(CH_2)_2$), 2.53 (s, 3H, $NCH_3$), 2.39 (s, 3H, Ar–$CH_3$). MS (ESI): $m/z$ 484 $(M+H)^+$.

### 6.1.54. *3-(3,4-Dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methyl-N-(3-(2-methylpiperidin-1-yl)propyl)isoquinolin-1-amine (**6bk**)*

The procedures described for compound **6ac** were used with compound **7b** (356 mg, 0.84 mmol), 96% 1-(3-aminopropyl)-2-piperazine (552 mg, 3.39 mmol), $K_2CO_3$ (1.05 g, 7.63 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, EtOAc, $CH_2Cl_2$:MeOH = 1:1) to obtain compound **6bk** as a yellow semi-solid (424 mg, 92%). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.10 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.31–7.20 (m, ≈5H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.91–6.74 (m, 4H, Ar–H), 3.84 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 3.54 (s, 3H, $OCH_3$), 3.34–3.25 (m, 1H), 3.22–3.16 (m, 1H), 2.96–2.86 (m, 1H), 2.81–2.71 (m, 1H), 2.64–2.54 (m, 1H), 2.38 (s, 3H, Ar–$CH_3$), 2.15–2.05 (m, 4H), 1.88–1.64 (m, 5H), 1.52–1.38 (m, 1H), 1.24 (d, $J$ = 6.6 Hz, 3H, $NCHC\underline{H}_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 177.1, 159.6, 154.3, 147.6, 147.2, 147.1, 140.7, 139.6, 137.5, 134.5, 129.2, 127.3, 113.7, 112.1, 110.1, 55.6, 55.4, 55.1, 39.0, 23.3, 23.2, 23.1, 22.4, 21.9. MS (ESI): $m/z$ 540 $(M+H)^+$.

### 6.1.55. *3-(3,4-Dimethoxyphenyl)-N-(4-methoxybenzyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1-amine (**6bm**)*

The procedures described for compound **6ac** were used with compound **7b** (350 mg, 0.83 mmol), 98% 4-methoxybenzylamine (467 mg, 3.33 mmol), $K_2CO_3$ (1.03 g, 7.50 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **6bm** as a green solid (289 mg, 66%). $R_f$ = 0.17 (*n*-hexane:EtOAc = 3:1). Mp: 172–175 °C. $^1H$ NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.24 (d, $J$ = 8.4 Hz, 1H, Ar–H), 8.04 (t, $J$ = 5.8 Hz,

1H, NH), 7.37–7.29 (m, 4H, Ar–H), 7.11 (s, 1H, Ar–H), 6.97–6.85 (m, 4H, Ar–H), 6.80–6.68 (m, 4H, Ar–H), 4.71 (d, $J$ = 5.4 Hz, 2H, $NCH_2$), 3.70 (s, 3H, $OCH_3$), 3.68 (s, 3H, $OCH_3$), 3.67 (s, 3H, $OCH_3$), 3.38 (s, ≈3H, $OCH_3$, partially overlapped with the DMSO peak), 2.32 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.7, 158.8, 153.4, 147.8, 147.4, 147.3, 140.6, 139.7, 137.7, 134.1, 132.1, 129.3, 127.2, 125.3, 124.5, 122.8, 121.0, 120.1, 117.3, 114.6, 113.9, 113.7, 112.3, 110.1, 55.7, 55.38, 55.31, 55.2, 45.3, 21.9. MS (ESI): $m/z$ 521 $(M+H)^+$, 559 $(M+K)^+$.

#### 6.1.56. 1-(4-Ethylpiperazin-1-yl)-4-(3-methoxyphenyl)-6-methyl-3-(m-tolyl)isoquinoline (**6cc**)

The procedures described for compound **6ac** were used with compound **7c** (200 mg, 0.53 mmol), 1-ethylpiperazine (244 mg, 2.139 mmol), $K_2CO_3$ (665 mg, 4.81 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, 1:1, EtOAc) to obtain compound **6cc** as a brown semi-solid (141 mg, 58%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.05 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.38 (s, 1H, Ar–H), 7.32–7.24 (m, ≈3H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.15 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.03 (t, $J$ = 7.5 Hz, 1H, Ar–H), 6.96 (d, $J$ = 7.2 Hz, 1H, Ar–H), 6.89–6.85 (m, 1H, Ar–H), 6.84–6.80 (m, 1H, Ar–H), 6.76–6.74 (m, 1H, Ar–H), 3.71 (s, 3H, $OCH_3$), 3.56 (M, 4H, 1″-$N(CH_2)_2$), 2.76 (m, 4H, 4″-$N(CH_2)_2$), 2.56 (q, $J$ = 7.2 Hz, 2H, $NCH_2CH_3$), 2.40 (s, 3H, Ar–$CH_3$), 2.22 (s, 3H, Ar–$CH_3$), 1.17 (t, $J$ = 7.2 Hz, 3H, $NCH_2CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.4, 147.6, 141.0, 139.6, 138.5, 136.6, 132.0, 131.0, 129.1, 128.5, 128.4, 127.5, 127.2, 127.1, 125.2, 125.0, 124.8, 124.2, 55.2, 52.9, 52.4, 51.0, 22.0, 21.4, 11.8. MS (ESI): $m/z$ 452 $(M+H)^+$.

#### 6.1.57. N-(3-methoxyphenethyl)-4-(3-methoxyphenyl)-6-methyl-3-(m-tolyl)isoquinolin-1-amine (**6cl**)

The procedures described for compound **6ac** were used with compound **7c** (200 mg, 0.53 mmol), 2-(3-methoxyphenyl)ethylamine (324 mg, 2.14 mmol), $K_2CO_3$ (665 mg, 4.81 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:$CH_2Cl_2$ = 5:1, 3:2, $n$-hexane:EtOAc = 7:1) to obtain compound **6cl** as a transparent semi-solid (238 mg, 91%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.57 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.35–7.18 (m, 6H, Ar–H), 7.04 (t, $J$ = 7.6 Hz, 1H, Ar–H), 6.97–6.74 (m, 7H, Ar–H), 5.31 (t, $J$ = 5.4 Hz, 1H, NH), 3.97 (q, $J$ = 6.5 Hz, 2H, $NCH_2$), 3.77 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 3.07 (t, $J$ = 6.9 Hz, 2H, $NCH_2CH_2$), 2.38 (s, 3H, Ar–$CH_3$), 2.23 (3H, Ar–$CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.7, 159.3, 153.6, 148.4, 141.5, 140.2, 139.5, 137.5, 136.5, 131.0, 129.5, 129.0, 127.4, 127.2, 127.0, 121.3, 120.9, 120.4, 117.4, 114.8, 114.4, 112.3, 111.7, 55.2, 55.1, 42.8, 35.9, 21.9, 21.4. MS (ESI): $m/z$ 489 $(M+H)^+$.

#### 6.1.58. 4-(3-Methoxyphenyl)-6-methyl-N-phenyl-3-(m-tolyl) isoquinolin-1-amine (**6co**)

The procedures described for compound **6ac** were used with compound **7c** (211 mg, 0.56 mmol), aniline (210 mg, 2.25 mmol), $K_2CO_3$ (702 mg, 5.08 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:$CH_2Cl_2$ = 5:1, 3:1, 1:1, $n$-hexane:EtOAc = 5:1) to obtain compound **6co** as a yellow semi-solid (114 mg, 46%). IR ($cm^{-1}$): 3426 (N–H). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 9.24 (s, 1H, NH), 8.53 (d, $J$ = 8.4 Hz, 1H, Ar–H), 8.00–7.96 (m, 2H, Ar–H), 7.49–7.46 (m, 1H, Ar–H), 7.34–7.27 (m, 3H, Ar–H), 7.24–7.23 (m, 2H, Ar–H), 7.09–6.90 (m, 5H, Ar–H), 6.79 (d, $J$ = 7.8 Hz, 1H, Ar–H), 6.73–6.71 (m, 1H, Ar–H), 3.67 (s, 3H, $OCH_3$), 2.38 (s, 3H, Ar–$CH_3$), 2.18 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.4, 150.6, 148.0, 141.0, 140.9, 140.0, 139.7, 137.9, 136.7, 131.0, 129.1, 128.8, 128.0, 127.5, 127.2, 127.1, 125.6, 124.4, 123.0, 121.9, 121.2, 119.3, 117.3, 115.6, 112.5, 55.2, 21.9, 21.4. MS (ESI): $m/z$ 431 $(M+H)^+$.

#### 6.1.59. 3-(2,6-Dimethylphenyl)-4-(3-methoxyphenyl)-6-methyl-1-(piperazin-1-yl)isoquinoline (**6da**)

The procedures described for compound **6ac** were used with compound **7d** (200 mg, 0.51 mmol), anhydrous piperazine (177 mg, 2.06 mmol), $K_2CO_3$ (641 mg, 4.64 mmol), and DMF (7 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **6da** as an off-white semi-solid (180 mg, 79%). $R_f$ = 0.65 (EtOAc). IR ($cm^{-1}$): 3496 (N–H). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.08 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.46–7.43 (m, 1H, Ar–H), 7.26 (s, 1H, Ar–H), 7.19 (t, $J$ = 7.9 Hz, 1H, Ar–H), 7.03–6.86 (m, 3H, Ar–H), 6.80–6.74 (m, 2H, Ar–H), 6.60 (m, 1H, Ar–H), 3.59 (s, 3H, $OCH_3$), 3.22 (m, ≈4H, 1″-$N(CH_2)_2$, partially overlapped with the DMSO peak), 2.93 (m, 4H, 4″-$N(CH_2)_2$), 2.37 (s, 3H, Ar–$CH_3$), 1.97 (s, 3H, Ar–$CH_3$), 1.86 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm):159.7, 158.7, 148.5, 140.5, 139.6, 138.3, 138.1, 135.77, 135.7, 128.4, 127.9, 127.1, 126.9, 126.8, 126.2, 125.1, 124.9, 122.9, 118.4, 115.4, 113.0, 55.0, 51.0, 44.7, 22.0, 20.4, 20.2.

#### 6.1.60. 3-(2,6-Dimethylphenyl)-N-(4-methoxybenzyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1-amine (**6dm**)

The procedures described for compound **6ac** were used with compound **7d** (400 mg, 1.03 mmol), 98% 4-methoxybenzylamine (577 mg, 4.12 mmol), $K_2CO_3$ (1.28 g, 9.28 mmol), and DMF (7 mL), followed by column chromatography ($CH_2Cl_2$, $n$-hexane:EtOAc = 5:1) to obtain compound **6dm** as a transparent semi-solid (208 mg, 41%). $R_f$ = 0.67 ($n$-hexane:EtOAc = 5:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.26 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.97 (t, $J$ = 5.7 Hz, 1H, NH), 7.37–7.34 (m, 1H, Ar–H), 7.24–7.21 (m, 2H, 2″-H and 6″-H), 7.18–7.12 (m, 2H, Ar–H), 6.97–6.87 (m, 2H, Ar–H), 6.83–6.79 (m, 3H, Ar–H, 3″-H and 5″-H), 6.74–6.69 (m, 2H, Ar–H), 6.55–6.54 (m, 1H, Ar–H), 4.61–4.59 (m, 2H, $NCH_2$), 3.69 (s, 3H, $OCH_3$), 3.57 (s, 3H, $OCH_3$), 2.33 (s, 3H, Ar–$CH_3$), 1.94 (s, 3H, Ar–$CH_3$), 1.84 (s, 3H, Ar–$CH_3$).

#### 6.1.61. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-1-(piperazin-1-yl)isoquinoline (**6ea**)

The procedures described for compound **6ac** were used with compound **7e** (200 mg, 0.53 mmol), anhydrous piperazine (183 mg, 2.12 mmol), $K_2CO_3$ (662 mg, 4.79 mmol), and DMF (7 mL), followed by column chromatography ($n$-hexane:EtOAc = 4:1, EtOAc, MeOH) to obtain compound **6ea** as a transparent semi-solid (184 mg, 81%). $R_f$ = 0.16 (MeOH). IR ($cm^{-1}$): 3454 (N–H). $^1$H NMR (300 MHz, $CD_3OD$) δ (ppm): 8.26–8.21 (m, 1H, Ar–H), 7.58–7.54 (m, 3H, Ar–H), 7.22–7.13 (m, 3H, Ar–H), 6.87–6.74 (m, 4H, Ar–H), 6.66 (s, 1H, Ar–H), 3.63 (s, 3H, $OCH_3$), 3.53 (s, 3H, $OCH_3$), 3.42–3.38 (m, 4H, 1″-$N(CH_2)_2$), 3.13–3.09 (M, 4H, 4″-$N(CH_2)_2$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.2, 158.7, 156.3, 146.5, 138.8, 137.7, 131.5, 130.6, 129.6, 128.7, 128.4, 127.6, 126.2, 125.8, 124.8, 123.7, 120.1, 120.0, 116.1, 112.7, 110.6, 55.08, 55.0, 50.1, 44.1. MS (ESI): $m/z$ 426 $(M+H)^+$.

#### 6.1.62. 2-(4-(3-(2-Methoxyphenyl)-4-(3-methoxyphenyl) isoquinolin-1-yl)piperazin-1-yl)-N,N-dimethylethanamine (**6ed**)

The procedures described for compound **6ac** were used with compound **7e** (200 mg, 0.53 mmol), 1-[2-(dimethylamino)ethyl] piperazine (335 mg, 2.12 mmol), $K_2CO_3$ (662 mg, 4.79 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **6ed** as a yellow semi-solid (229 mg, 86%). $R_f$ = 0.14 (MeOH). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.18–8.15 (m, 1H, Ar–H), 7.71–7.68 (m, 1H, Ar–H), 7.34–7.45 (m, 2H, Ar–H), 7.28–7.27 (m, ≈2H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.21–7.13 (m, 2H, Ar–H), 6.92–6.87 (m, 1H, Ar–H), 6.82–6.66 (m, 3H, Ar–H), 3.62 (s, 3H, $OCH_3$), 3.54–3.50 (m, 4H, 1″-$N(CH_2)_2$), 3.45 (s, 3H, $OCH_3$), 2.77 (t, $J$ = 4.5 Hz, 4H, 3″,5″-$(CH_2)_2$), 2.65–2.60 (m, 2H, $CH_2CH_2$ $N(CH_3)_2$), 2.54–2.49 (m, 2H, $CH_2CH_2$ $N(CH_3)_2$), 2.29 (s, 6H, $N(CH_3)_2$). $^{13}$C NMR

(125 MHz, $CDCl_3$) δ (ppm): 159.9, 158.7, 156.3, 146.5, 139.1, 137.5, 131.6, 131.0, 129.3, 128.6, 128.1, 126.7, 125.9, 125.3, 125.2, 123.8, 120.2, 120.0, 116.1, 112.6, 110.7, 56.8, 55.0, 53.8, 51.2, 45.9. MS (ESI): *m*/*z* 497 $(M+H)^+$. HRMS (ESI): *m*/*z* 497.2916 $(M+H)^+$ (calcd for $C_{31}H_{37}N_4O_2$, 497.2917), 519.2737 $(M+Na)^+$ (calcd for $C_{31}H_{36}N_4NaO_2$, 519.2736).

#### 6.1.63. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-1-(4-(2-methoxyphenyl)piperazin-1-yl)isoquinoline (**6ee**)

The procedures described for compound **6ac** were used with compound **7e** (200 mg, 0.53 mmol), 1-(2-methoxypheynyl)piperazine (409 mg, 2.12 mmol), $K_2CO_3$ (662 mg, 4.79 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **6ee** as an off-white solid (249 mg, 88%). $R_f$ = 0.35 (*n*-hexane:EtOAc = 3:1). Mp: 178–182 °C. $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.26–8.22 (m, 1H, Ar–H), 7.73–7.70 (m, 1H, Ar–H), 7.55–7.48 (m, 2H, Ar–H), 7.30–7.26 (m, ≈2H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.21–7.14 (m, 2H, Ar–H), 7.08–6.88 (m, 5H, Ar–H), 6.86–6.67 (m, 3H, Ar–H), 3.88 (s, 3H, $OCH_3$), 3.71–3.68 (m, 4H, 4″-$N(CH_2)_2$), 3.63 (s, 3H, $OCH_3$), 3.46 (s, 3H, $OCH_3$), 3.36 (t, *J* = 4.8 Hz, 4H, 1″-$N(CH_2)_2$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 160.0, 158.7, 156.3, 152.3, 146.6, 141.5, 139.2, 137.6, 131.6, 131.1, 129.3, 128.6, 128.1, 126.7, 125.9, 125.3, 123.8, 122.8, 121.0, 120.3, 120.0, 118.3, 116.1, 112.6, 111.4, 110.7, 55.4, 55.0, 51.6, 50.8. MS (ESI): *m*/*z* 532 $(M+H)^+$. Anal. calcd for $C_{34}H_{33}N_3O_3 \cdot 0.35C_4H_8O_2 \cdot 0.1H_2O$: C, 76.19; H, 6.32; N, 7.75. Found C, 76.17; H, 6.27; N, 7.72.

#### 6.1.64. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)isoquinoline (**6ef**)

The procedures described for compound **6ac** were used with compound **7e** (200 mg, 0.53 mmol), 1-(2-pyrimidyl)piperazine (349 mg, 2.12 mmol), $K_2CO_3$ (662 mg, 4.79 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 3:1) to obtain compound **6ef** as a yellow solid (219 mg, 81%). $R_f$ = 0.24 (*n*-hexane:EtOAc = 3:1). Mp: 187–189 °C. $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.34 (d, *J* = 4.8 Hz, 2H, 10″-H and 12″-H), 8.27–8.23 (m, 1H, Ar–H), 7.73–7.69 (m, 1H, Ar–H), 7.57–7.51 (m, 2H, Ar–H), 7.28–7.25 (m, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.21–7.14 (m, 2H, Ar–H), 6.89 (td, *J* = 7.3, 1.2 Hz, 1H, Ar–H), 6.86–6.66 (m, 4H, Ar–H), 6.51 (t, *J* = 4.8 Hz, 1H, 11″-H), 4.11–4.08 (m, 4H, $N(CH_2)_2$), 3.63 (s, 3H, $OCH_3$), 3.57–3.54 (m, 4H, $N(CH_2)_2$), 3.45 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 161.9, 160.0, 158.7, 157.6, 156.3, 146.5, 139.0, 137.6, 131.6, 130.8, 129.5, 128.6, 128.1, 127.2, 126.0, 125.6, 125.1, 123.7, 120.4, 120.0, 116.2, 112.7, 110.6, 109.9, 55.0, 51.2, 43.9. MS (ESI): *m*/*z* 504 $(M+H)^+$. Anal. calcd for $C_{31}H_{29}N_5O_2 \cdot 0.35C_4H_8O_2 \cdot 0.05H_2O$: C, 72.69; H, 6.01; N, 13.08. Found C, 72.23; H, 5.73; N, 13.52.

#### 6.1.65. 1-(1H-imidazol-1-yl)-3-(2-methoxyphenyl)-4-(3-methoxyphenyl)isoquinoline (**6eh**)

The procedures described for compound **6ac** were used with compound **7e** (110 mg, 0.29 mmol), imidazole (29 mg, 0.44 mmol), $K_2CO_3$ (23 mg, 0.58 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, 1:1, EtOAc) to obtain compound **6eh** as an off-white solid (67 mg, 56%). $R_f$ = 0.32 (EtOAc). Mp: 174–177 °C. $^1H$ NMR (500 MHz, $CDCl_3$) δ (ppm): 8.15 (s, 1H, 2″-H), 8.06 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.84 (d, *J* = 8.5 Hz, 1H, Ar–H), 7.71–7.60 (m, 3H, Ar–H), 7.30–7.21 (m, ≈5H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.92–6.82 (m, 3H, Ar–H), 6.80–6.72 (m, 1H, Ar–H), 3.67 (s, 3H, $OCH_3$), 3.53 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 158.9, 156.3, 147.8, 138.0, 137.6, 132.9, 131.3, 130.9, 129.4, 129.1, 128.5, 127.9, 126.3, 124.2, 123.2, 121.7, 120.2, 115.9, 113.3, 110.6, 55.1, 54.9. MS (ESI): *m*/*z* 408 $(M+H)^+$, 449 $(M+CH_3CN+H)^+$. Anal. calcd for $C_{26}H_{21}N_3O_2 \cdot 0.35C_4H_8O_2$: C, 75.08; H, 5.47; N, 9.59. Found C, 74.62; H, 5.09; N, 10.05.

#### 6.1.66. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-N-(4-methylpiperazin-1-yl)isoquinolin-1-amine (**6ei**)

The procedures described for compound **6ac** were used with compound **7e** (200 mg, 0.53 mmol), 97% 1-amino-4-methylpiperazine (253 mg, 2.12 mmol), $K_2CO_3$ (662 mg, 4.79 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 5:1, EtOAc, MeOH) to obtain compound **6ei** as an orange colored semi-solid (143 mg, 58%). $R_f$ = 0.5 (MeOH). IR ($cm^{-1}$): 3451 (N–H). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.16–8.12 (m, 1H, Ar–H), 7.71–7.67 (m, 1H, Ar–H), 7.54–7.45 (m, 2H, Ar–H), 7.27–7.13 (m, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 7.21–7.13 (m, 2H, Ar–H), 6.89 (td, *J* = 7.5, 1.2 Hz, 1H, Ar–H), 6.80–6.67 (m, 4H, Ar–H), 3.62 (s, 3H, $OCH_3$), 3.60–3.56 (m, 4H, $N(CH_3)_2$), 3.46 (s, 3H, $OCH_3$), 2.79–2.76 (m, 4H, $N(CH_2)_2$), 2.45 (s, 3H, $NCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 159.7, 158.7, 156.3, 146.5, 139.1, 137.6, 131.6, 130.9, 129.3, 128.6, 128.1, 126.8, 126.0, 125.3, 125.2, 123.7, 120.2, 120.0, 116.1, 112.6, 110.7, 55.0, 50.8, 45.9. MS (ESI): *m*/*z* 408 $(M+H-15)^+$.

#### 6.1.67. N-(4-methoxybenzyl)-3-(2-methoxyphenyl)-4-(3-methoxyphenyl)isoquinolin-1-amine (**6em**)

The procedures described for compound **6ac** were used with compound **7e** (310 mg, 0.82 mmol), 98% 4-methoxybenzylamine (462 mg, 3.29 mmol), $K_2CO_3$ (1.02 g, 7.42 mmol), and DMF (5 mL), followed by MPLC (*n*-hexane:EtOAc = 10:1) to obtain compound **6em** as a light brown viscous semi-solid (231 mg, 58%). $R_f$ = 0.37 (*n*-hexane:EtOAc = 3:1). $^1H$ NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.34–8.31 (m, 1H, Ar–H), 7.95 (t, *J* = 6.0 Hz, 1H, NH), 7.58–7.46 (m, 2H, Ar–H), 7.39–7.36 (m, 1H, Ar–H), 7.35–7.32 (m, 2H, 2″-H and 6″-H), 7.17–7.12 (m, 2H, Ar–H), 7.06 (d, *J* = 6.6 Hz, 1H, Ar–H), 6.87–6.83 (m, 2H, 3″-H and 5″-H), 6.81–6.78 (m, 2H, Ar–H), 6.74–6.60 (m, 3H, Ar–H), 4.65–4.62 (m, 2H, $NCH_2$), 3.71 (s, 3H, $OCH_3$), 3.58 (s, 3H, $OCH_3$), 3.46 (s, 3H, $OCH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 158.8, 158.7, 156.3, 153.7, 136.7, 131.5, 129.6, 129.4, 128.5, 128.0, 126.0, 125.3, 124.1, 122.4, 121.2, 119.9, 116.8, 116.5, 113.9, 112.3, 110.5, 55.3, 55.0, 45.5. MS (ESI): *m*/*z* 477 $(M+H)^+$.

#### 6.1.68. 1-(4-Ethylpiperazin-1-yl)-4-(3-methoxyphenyl)-3-(4-methoxyphenyl)isoquinoline (**6fc**)

The procedures described for compound **6ac** were used with compound **7f** (211 mg, 0.56 mmol), 1-ethylpiperazine (256 mg, 2.24 mmol), $K_2CO_3$ (698 mg, 5.05 mmol), and DMF (5 mL), followed by column chromatography (*n*-hexane:EtOAc = 3:1, 1:1, EtOAc, MeOH) to obtain compound **6fc** as a brown semi-solid (211 mg, 82%). $R_f$ = 0.11 (EtOAc). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.16–8.12 (m, 1H, Ar–H), 7.61–7.58 (m, 1H, Ar–H), 7.50–7.44 (m, 2H, Ar–H), 7.43–7.38 (m, 2H, 2′-H and 6′-H), 7.29 (t, *J* = 7.8 Hz, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.91–6.82 (m, 2H, Ar–H), 6.79–6.78 (m, 1H, Ar–H), 6.75–6.70 (m, 2H, 3′-H and 5′-H), 3.76 (s, 3H, $OCH_3$), 3.73 (s, 3H, $OCH_3$), 3.60–3.56(m, 4H, 1″-$N(CH_2)_2$), 2.79–2.76 (m, 4H, 4″-$N(CH_2)_2$), 2.57 (q, *J* = 7.2 Hz, 2H, $NCH_2CH_3$), 1.18 (t, *J* = 7.2 Hz, 3H, $NCH_2CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ (ppm): 159.5, 158.5, 146.8, 139.7, 138.4, 133.4, 132.0, 8, 132.0, 131.9, 131.4, 129.4, 129.3, 128.5, 128.4, 126.0, 125.2, 125.1, 125.2, 125.1, 124.5, 124.0, 119.6, 116.9, 112.8, 112.6, 55.1, 52.7, 52.3, 50.8, 11.6. MS (ESI): *m*/*z* 454 $(M+H)^+$.

#### 6.1.69. 2-(4-(4-(3-Methoxyphenyl)-3-(4-methoxyphenyl)isoquinolin-1-yl)piperazin-1-yl)-N,N-dimethylethanamine (**6fd**)

The procedures described for compound **6ac** were used with compound **7f** (210 mg, 0.55 mmol), 1-[2-(dimethylamino)ethyl] piperazine (351 mg, 2.23 mmol), $K_2CO_3$ (695 mg, 5.02 mmol), and DMF (5 mL), followed by column chromatography (*n*-

hexane:EtOAc = 3:1, EtOAc, MeOH) to obtain compound **6fd** as a hay-colored semi-solid (193 mg, 69%). $R_f$ = 0.1 (MeOH). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.15–8.12 (m, 1H, Ar–H), 7.61–7.58 (m, 1H, Ar–H), 7.52–7.44 (m, 2H, Ar–H), 7.43–7.38 (m, 2H, 2′-H and 6′-H), 7.29 (t, $J$ = 8.1 Hz, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.90–6.78 (m, 3H, Ar–H), 6.74–6.70 (m, 2H, 3′-H and 5′-H), 3.76 (s, 3H, $OCH_3$), 3.73 (s, 3H, $OCH_3$), 3.58–3.54 (m, 4H, 1″-$N(CH_2)_2$), 2.81–2.78 (m, 4H, 4″-$N(CH_2)_2$), 2.66–2.61 (m, 2H, $C\underline{H}_2CH_2$ $N(CH_3)_2$), 2.55–2.50 (m, 2H, $CH_2C\underline{H}_2N(CH_3)_2$), 2.29 (s, 6H, $N(CH_3)_2$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.5, 158.5, 146.7, 139.5, 138.3, 131.3, 129.5, 129.3, 126.1, 125.4, 124.9, 123.9, 119.8, 116.8, 112.8, 112.6, 61.5, 55.1, 55.0, 54.1, 53.2, 53.0, 50.9, 50.8, 43.9. MS (ESI): $m/z$ 497 $(M+H)^+$.

### 6.1.70. 4-(3-Methoxyphenyl)-3-(4-methoxyphenyl)-3′,4′-dihydro-1′H-1,2′-biisoquinoline (**6fg**)

The procedures described for compound **6ac** were used with compound **7f** (213 mg, 0.56 mmol), 1,2,3,4-tetrahydroisoquinoline (302 mg, 2.26 mmol), $K_2CO_3$ (705 mg, 5.1 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:$CH_2Cl_2$ = 3:1, $CH_2Cl_2$) to obtain compound **6fg** as a brown solid (197 mg, 73%). $R_f$ = 0.7 ($n$-hexane:EtOAc = 3:1). Mp: 158–161 °C. $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.20–8.17 (m, 1H, Ar–H), 7.64–7.60 (m, 1H, Ar–H), 7.54–7.45 (m, 2H, Ar–H), 7.45–7.40 (m, 2H, 2′-H and 6′-H), 7.30 (t, $J$ = 7.9 Hz, 1H, Ar–H), 7.26–7.20 (m, ≈4H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.91–6.80 (m, 3H, Ar–H), 6.77–6.73 (m, 2H, 3′-H and 5′-H), 4.76 (s, 2H, 1″-H), 3.82–3.78 (m, 5H, 3″-H, $OCH_3$), 3.74 (s, 3H, $OCH_3$), 3.25 (t, $J$ = 5.5 Hz, 2H, 4″-H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.8, 159.5, 158.6, 146.8, 139.7, 138.4, 135.3, 134.7, 133.5, 131.4, 129.5, 129.3, 128.9, 126.7, 126.1, 126.0, 125.8, 125.2, 125.0, 124.4, 124.1, 119.9, 116.9, 112.9, 112.6, 55.2, 55.1, 52.5, 50.4. MS (ESI): $m/z$ 473 $(M+H)^+$. HRMS (ESI): $m/z$ 473.2229 $(M+H)^+$ (calcd for $C_{32}H_{29}N_2O_2$, 473.2229), 495.2043 $(M+Na)^+$ (calcd for $C_{32}H_{28}N_2NaO_2$, 495.2048).

### 6.1.71. N-(4-methoxybenzyl)-4-(3-methoxyphenyl)-3-(4-methoxyphenyl)isoquinolin-1-amine (**6fm**)

The procedures described for compound **6ac** were used with compound **7f** (313 mg, 0.83 mmol), 98% 4-methoxybenzylamine (466 mg, 3.33 mmol), $K_2CO_3$ (1.03 g, 7.49 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:$CH_2Cl_2$ = 1:1, $n$-hexane:EtOAc = 5:1) to obtain compound **6fm** as a white solid (281 mg, 75%). $R_f$ = 0.22 ($n$-hexane:EtOAc = 5:1). Mp: 162–164 °C. IR ($cm^{-1}$): 3374 (N–H). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.76 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.59–7.56 (m, 1H, Ar–H), 7.49–7.36 (m, 6H, Ar–H), 7.26 (t, $J$ = 7.9 Hz, ≈1H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.92–6.78 (m, 5H, Ar–H), 6.73–6.68 (m, 2H, Ar–H), 5.47 (t, $J$ = 5.1 Hz, 1H, NH), 4.85 (d, $J$ = 5.1 Hz, 2H, $NCH_2$), 3.81 (s, 3H, $OCH_3$), 3.75 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.5, 158.8, 158.4, 153.5, 147.5, 140.2, 137.5, 134.0, 132.0, 131.4, 129.4, 129.2, 126.1, 125.1, 124.5, 121.0, 120.3, 117.3, 116.4, 113.9, 112.7, 112.3, 55.3, 55.17, 55.1, 45.4. Anal. calcd for $C_{31}H_{28}N_2O_3$: C, 78.13; H, 5.92; N, 5.88. Found C, 78.34; H, 5.95; N, 5.74.

### 6.1.72. 3-Aryl-4-(3-methoxyphenyl)-1-(piperazin-1-yl)isoquinoline (**6ga**)

The procedures described for compound **6ac** were used with compound **7g** (200 mg, 0.41 mmol), anhydrous piperazine (143 mg, 1.66 mmol), $K_2CO_3$ (516 mg, 3.37 mmol), and DMF (7 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc, MeOH) to obtain compound **6ga** as an off-white solid (103 mg, 46%). $R_f$ = 0.17 (MeOH). Mp: 99–102 °C. IR ($cm^{-1}$): 3451 (N–H). $^1$H NMR (500 MHz, DMSO-*d6*) δ (ppm): 8.18 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.65–7.59 (m, 2H, Ar–H), 7.51–7.46 (m, 3H, Ar–H), 7.29 (t, $J$ = 8.0 Hz, 1H, Ar–H), 7.20 (t, $J$ = 7.5 Hz, 1H, Ar–H), 7.09 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.01 (s, 1H, Ar–H), 6.91 (d, $J$ = 8.5 Hz, 1H, Ar–H), 6.84 (dd, $J$ = 9.0, 2.0 Hz, 1H, Ar–H), 6.79–6.76 (m, 3H, Ar–H), 3.78 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 3.52 (s, 4H, 1″-$N(CH_2)_2$), 3.25 (s, 4H, 4″-$N(CH_2)_2$), 2.96 (s, 3H, $OCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.8, 158.9, 156.2, 146.3, 142.3, 138.8, 137.7, 132.8, 130.6, 130.4, 129.7, 129.55, 129.51, 128.4, 127.8, 127.3, 126.3, 125.9, 125.4, 124.7, 123.7, 120.1, 119.2, 116.2, 112.9, 112.3, 111.9, 111.0, 55.2, 55.0, 49.5, 43.7. HRMS (ESI): $m/z$ 532.2600 $(M+H)^+$ (calcd for $C_{34}H_{34}N_3O_3$, 532.2600), 554.2417 $(M+Na)^+$ (calcd for $C_{34}H_{33}N_3NaO_3$, 554.2420).

### 6.1.73. 3-Aryl-4-(3-methoxyphenyl)-1-(4-methylpiperazin-1-yl) isoquinoline (**6gb**)

The procedures described for compound **6ac** were used with compound **7g** (200 mg, 0.41 mmol), 1-methylpiperazine (166 mg, 1.66 mmol), $K_2CO_3$ (516 mg, 3.73 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc, MeOH) to obtain compound **6gb** as a brown semi-solid (180 mg, 79%). $R_f$ = 0.58 (MeOH). $^1$H NMR (300 MHz, $CD_3OD$) δ (ppm): 8.25–8.22 (m, 1H, Ar–H), 7.63–7.56 (m, 2H, Ar–H), 7.44 (dd, $J$ = 8.5, 2.4 Hz, 1H, Ar–H), 7.37 (d, $J$ = 2.3 Hz, 1H, Ar–H), 7.28–7.17 (m, 2H, Ar–H), 7.03–7.00 (m, 1H, Ar–H), 6.94–6.88 (m, 2H, Ar–H), 6.83–6.72 (m, 5H, Ar–H), 3.80 (s, 3H, $OCH_3$), 3.63 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 3.53 (s, 4H, 1″-$N(CH_2)_2$), 2.87 (t, $J$ = 4.4 Hz, 4H, 4″-$N(CH_2)_2$), 2.48 (s, 3H, $NCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.8, 159.6, 158.8, 156.2, 146.3, 142.4, 139.0, 137.6, 132.8, 130.9, 130.6, 129.53, 129.5, 128.3, 127.2, 127.1, 126.1, 125.5, 125.1, 123.8, 120.2, 119.2, 116.2, 112.8, 112.3, 111.9, 111.1, 55.3, 55.2, 55.0, 54.5, 50.3, 45.3.

### 6.1.74. 3-Aryl-4-(3-methoxyphenyl)-1-(4-(pyrimidin-2-yl) piperazin-1-yl)isoquinoline (**6gf**)

The procedures described for compound **6ac** were used with compound **7g** (200 mg, 0.41 mmol), 1-(2-pyrimidyl)piperazine (272 mg, 1.66 mmol), $K_2CO_3$ (516 mg, 3.73 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1) to obtain compound **6gf** as a transparent semi-solid which foamed out into an off-white solid on pumping (184 mg, 72%). $R_f$ = 0.6 ($n$-hexane:EtOAc = 1:1). Mp: 100–103 °C. $^1$H NMR (500 MHz, DMSO-*d6*) δ (ppm): 8.39 (d, $J$ = 4.5 Hz, 2H, 10″-H and 12″-H), 8.30–8.28 (m, 1H, Ar–H), 7.70–7.64 (m, 2H, Ar–H), 7.53–7.49 (m, 3H, Ar–H), 7.29 (t, $J$ = 7.7 Hz, 1H, Ar–H), 7.21 (m, 1H, Ar–H), 7.09 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.01 (s, 1H, Ar–H), 6.91 (d, $J$ = 8.5 Hz, 1H, Ar–H), 6.85–6.83 (m, 1H, Ar–H), 6.81–6.77 (m, 3H, Ar–H), 6.66 (t, $J$ = 4.5 Hz, 1H, 11″-H), 4.03–4.00 (m, 4H, $N(CH_2)_2$), 3.77 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 3.52 (s, 3H, $OCH_3$), 3.41 (s, 4H, $N(CH_2)_2$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 161.9, 160.0, 159.8, 158.8, 157.6, 156.2, 146.4, 142.4, 139.0, 137.6, 132.8, 130.9, 130.6, 129.56, 129.53, 128.3, 127.3, 126.1, 125.7, 125.2, 123.8, 120.5, 119.2, 116.2, 112.8, 112.3, 111.9, 111.1, 109.9, 55.3, 55.2, 55.0, 51.2, 44.0. Anal. calcd for $C_{38}H_{35}N_5O_3 \cdot 0.35C_4H_8O_2 \cdot 0.05H_2O$: C, 73.77; H, 5.96; N, 10.92. Found C, 73.59; H, 5.75; N, 11.11.

### 6.1.75. 3-Aryl-N-(4-methoxybenzyl)-4-(3-methoxyphenyl) isoquinolin-1-amine (**6gm**)

The procedures described for compound **6ac** were used with compound **7g** (300 mg, 0.62 mmol), 98% 4-methoxybenzylamine (348 mg, 2.49 mmol), $K_2CO_3$ (774 mg, 5.6 mmol), and DMF (5 mL), followed by column chromatography ($n$-hexane:EtOAc = 7:1) to obtain compound **6gm** as a light green semi-solid (163 mg, 44%). $R_f$ = 0.45 ($n$-hexane:EtOAc = 2:1). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.34 (d, $J$ = 7.8 Hz, 1H, Ar–H), 8.03 (t, $J$ = 5.5 Hz, 1H, NH), 7.59–7.27 (m, 8H, Ar–H, 2″-H and 6″-H), 7.17 (t, $J$ = 7.5 Hz, 1H, Ar–H), 7.06–7.00 (m, 2H, Ar–H), 6.88–6.79

(m, 4H, Ar–H, 3″-H and 5″-H), 6.73 (dd, $J$ = 8.1, 2.7 Hz, 2H, Ar–H), 6.68 (s, 1H, Ar–H), 4.63 (t, $J$ = 5.2 Hz, 2H, $NCH_2$), 3.78 (s, 3H, $OCH_3$), 3.69 (s, 3H, $OCH_3$), 3.57 (s, 3H, $OCH_3$), 3.49 (s, 3H, $OCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.8, 158.8, 156.1, 153.7, 142.5, 139.6, 136.7, 132.7, 130.5, 129.6, 129.5, 128.1, 127.1, 126.0, 125.4, 124.1, 122.5, 121.2, 119.2, 116.8, 113.8, 112.3, 111.8, 110.9, 55.25, 55.21, 55.0, 45.4. MS (ESI): $m/z$ 583 $(M+H)^+$, 581 $(M-H)^-$.

### 6.1.76. 4-(3-Methoxyphenyl)-6-methyl-3-(o-tolyl)isoquinolin-1-amine (**17a**)

Compound **6am** (358 mg, 0.75 mmol) was dissolved in $CH_2Cl_2$ (15 mL), trifluoroacetic acid (4 mL) was added, and the reaction mixture was refluxed for 19 h. Excess trifluoroacetic acid was neutralized by sat. $NaHCO_3$ sol. (100 mL). The reaction mixture was then extracted with $CH_2Cl_2$. The organic layer was further washed with water and concentrated under reduced pressure. The resulting residue was purified by column chromatography ($n$-hexane:EtOAc = 4:1, 1:1) to obtain compound **17a** as an off-white solid (227 mg, 84%). Mp: 212–215 °C. IR ($cm^{-1}$): 3135 ($NH_2$). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.78 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.39–7.32 (m, 2H, Ar–H), 7.19–7.13 (m, 1H, Ar–H), 7.08–6.97 (m, 4H, Ar–H), 6.78–6.72 (m, 2H, Ar–H), 6.66 (s, 1H, Ar–H), 5.20 (s, 2H, $NH_2$), 3.65 (s, 3H, $OCH_3$), 2.42 (s, 3H, Ar–$CH_3$), 2.13 (s, 3H, Ar–$CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 158.9, 154.7, 149.8, 140.6, 140.5, 138.8, 137.4, 135.7, 129.9, 129.7, 128.5, 127.7, 127.1, 125.1, 124.9, 122.8, 122.4, 116.6, 114.7, 112.4, 55.0, 22.0, 19.9. Anal. calcd for $C_{24}H_{22}N_2O \cdot 0.1C_4H_8O_2 \cdot 0.05H_2O$: C, 80.48; H, 6.34; N, 7.69. Found C, 80.6; H, 6.29; N, 7.53.

### 6.1.77. 3-(3,4-Dimethoxyphenyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1-amine (**17b**)

The procedures described for compound **17a** were used with compound **6bm** (273 mg, 0.52 mmol), trifluoroacetic acid (5 mL), and $CH_2Cl_2$ (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, 1:1, EtOAc) to obtain compound **17b** as a white solid (186 mg, 88%). $R_f$ = 0.31 (EtOAc). Mp: 194–196 °C. IR ($cm^{-1}$): 3427 ($NH_2$). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.75 (d, $J$ = 8.4 Hz, 1H, Ar–H), 7.35–7.25 (m, ≈3H, Ar–H partially overlapped with the $CHCl_3$ peak), 7.07–7.04 (m, 1H, Ar–H), 6.87–6.82 (m, 3H, Ar–H), 6.76–6.73 (m, 2H, Ar–H), 5.15 (s, 2H, $NH_2$), 3.83 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 3.60 (s, 3H, $OCH_3$), 2.40 (s, 3H, 6-$CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm): 159.5, 154.9, 148.1, 147.8, 147.6, 140.3, 139.9, 137.7, 133.7, 129.1, 127.6, 125.2, 124.3, 122.5, 122.3, 121.8, 117.3, 114.4, 113.4, 112.3, 110.3, 55.7, 55.4, 55.1, 22.0. MS (ESI): $m/z$ 401 $(M+H)^+$, 442 $(M+CH_3CN+H)^+$. Anal. calcd for $C_{25}H_{24}N_2O_3 \cdot 0.7H_2O$: C, 72.69; H, 6.2; N, 6.78. Found C, 72.47; H, 5.93; N, 6.62.

### 6.1.78. 3-(2,6-Dimethylphenyl)-4-(3-methoxyphenyl)-6-methylisoquinolin-1-amine (**17c**)

The procedures described for compound **17a** were used with compound **6dm** (263 mg, 0.53 mmol), trifluoroacetic acid (5 mL), and $CH_2Cl_2$ (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, 1:1) to obtain compound **17c** as a yellow solid (171 mg, 86%). $R_f$ = 0.56 (EtOAc). Mp: 245–247 °C. IR ($cm^{-1}$): 3126 ($NH_2$). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.17 (d, $J$ = 8.7 Hz, 1H, Ar–H), 7.33 (dd, $J$ = 8.4, 1.5 Hz, 1H, Ar–H), 7.19–7.14 (m, 2H, Ar–H), 6.98–6.82 (m, 5H, Ar–H & $NH_2$), 6.75–6.71 (m, 2H, Ar–H), 6.59–6.57 (m, 1H, Ar–H), 3.58 (s, 3H, $OCH_3$), 2.33 (s, 3H, Ar–$CH_3$), 2.02 (s, 3H, Ar–$CH_3$), 1.89 (s, 3H, Ar–$CH_3$). Anal. calcd for $C_{25}H_{24}N_2O \cdot 0.55\ CH_2Cl_2 \cdot 0.05C_4H_8O_2$: C, 73.71; H, 6.13; N, 6.68. Found C, 73.73; H, 5.98; N, 6.54.

### 6.1.79. 3-(2-Methoxyphenyl)-4-(3-methoxyphenyl)isoquinolin-1-amine (**17d**)

The procedures described for compound **17a** were used with compound **6em** (200 mg, 0.42 mmol), trifluoroacetic acid (5 mL), and $CH_2Cl_2$ (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 5:1, EtOAc) to obtain **17d** as a transparent semi-solid which foamed out into a white solid on pumping (130 mg, 86%). $R_f$ = 0.23 (EtOAc). Mp: 145–149 °C. IR ($cm^{-1}$): 3109 ($NH_2$). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.88–7.85 (m, 1H, Ar–H), 7.65–7.62 (m, 1H, Ar–H), 7.57–7.47 (m, 2H, Ar–H), 7.20–7.12 (m, 3H, Ar–H), 6.88–6.69 (m, 5H, Ar–H), 5.21 (s, 2H, $NH_2$), 3.64 (s, 3H, $OCH_3$), 3.54 (s, 3H, $OCH_3$). $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 8.25 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.58–7.45 (m, 2H, Ar–H), 7.35 (d, $J$ = 7.5 Hz, 1H, Ar–H), 7.17–7.04 (m, 3H, Ar–H), 6.86–6.66 (m, 7H, $NH_2$ & Ar–H), 3.59 (s, 3H, $OCH_3$), 3.51 (s, 3H, $OCH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$) δ (ppm):158.7, 156.3, 154.9, 147.0, 139.1, 136.9, 131.0, 130.4, 130.0, 128.7, 128.2, 126.0, 125.6, 124.1, 123.8, 122.4, 120.1, 116.6, 116.3, 112.5, 110.4, 55.0. MS (ESI): $m/z$ 357 $(M+H)^+$, 398 $(M+CH_3CN+H)^+$. Anal. calcd for $C_{23}H_{20}N_2O_2 \cdot 0.15C_4H_8O_2$: C, 76.69; H, 5.78; N, 7.58. Found C, 76.72; H, 5.66; N, 7.49.

### 6.1.80. 4-(3-Methoxyphenyl)-3-(4-methoxyphenyl)isoquinolin-1-amine (**17e**)

The procedures described for compound **17a** were used with compound **6 fm** (239 mg, 0.53 mmol), trifluoroacetic acid (5 mL), and $CH_2Cl_2$ (15 mL), followed by column chromatography ($n$-hexane:EtOAc = 3:1, 1:1) to obtain compound **17e** as an off-white solid (181 mg, 94%). Mp: 163–169 °C. IR ($cm^{-1}$): 3122 ($NH_2$). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.87–7.83 (m, 1H, Ar–H), 7.62–7.45 (m, 3H, Ar–H), 7.32–7.22 (m, ≈3H, 2′-H and 6′-H, Ar–H, partially overlapped with the $CHCl_3$ peak), 6.86–6.79 (m, 2H, Ar–H), 6.76–6.70 (m, 3H, Ar–H, 3′-H and 5′-H), 5.20 (s, 2H, $NH_2$), 3.75 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm):159.3, 158.5, 155.0, 148.2, 139.6, 137.5, 133.5, 131.1, 130.0, 129.1, 126.1, 125.5, 124.4, 122.3, 122.2, 117.2, 116.2, 112.9, 112.4, 55.16, 55.14. MS (ESI): $m/z$ 357 $(M+H)^+$. Anal. calcd for $C_{23}H_{20}N_2O_2 \cdot 0.25C_4H_8O_2 \cdot 0.05\ H_2O$: C, 75.99; H, 5.87; N, 7.38. Found C, 76.32; H, 5.66; N, 7.03.

### 6.1.81. 3-(3-(1-Methoxy-6-methyl-3-(o-tolyl)isoquinolin-4-yl) phenoxy)-N,N-dimethylpropan-1-amine (**18**)

Potassium carbonate (713 mg, 5.15 mmol) was added to a solution of compound **14a**′ (611 mg, 1.72 mmol) in DMF (15 mL), followed by 96% 3-(dimethylamino)propyl chloride·HCl (425 mg, 2.57 mmol). The reaction mixture was heated using an oil bath set at 130 °C. Water (100 mL) was poured into the reaction mixture and it was then extracted with EtOAc. The organic layer was further washed with water and concentrated under reduced pressure. The residue thus obtained was purified by column chromatography ($n$-hexane:EtOAc = 5:1, 3:1, EtOAc, MeOH) to obtain compound **18** as an egg yolk-colored semi-solid (542 mg, 71%). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.22 (d, $J$ = 9.3 Hz, 1H, Ar–H), 7.39–7.36 (m, 2H, Ar–H), 7.17–7.05 (m, 3H, Ar–H), 7.02–6.94 (m, 2H, Ar–H), 6.77–6.66 (m, 3H, Ar–H), 4.11 (s, 3H, $OCH_3$), 3.94–3.76 (m, 2H, $OCH_2$), 2.42–2.36 (m, 5H, Ar–$CH_3$, $C\underline{H}_2(NCH_3)_2$), 2.23 (s, 6H, $N(CH_3)_2$), 2.20 (s, 3H, Ar–$CH_3$), 1.86 (quintet, $J$ = 6.9 Hz, 2H, $OCH_2C\underline{H}_2$). MS (ESI): $m/z$ 441 $(M+H)^+$.

### 6.1.82. 4-(3-(3-(Dimethylamino)propoxy)phenyl)-6-methyl-3-(o-tolyl)isoquinolin-1(2H)-one (**19**)

The procedures described for compound **5a** were used with compound **18** (466 mg, 1.05 mmol) and AcOH:$H_2O$ = 9:1 (10 mL), followed by column chromatography ($n$-hexane:EtOAc = 2:1 EtOAc, MeOH) to obtain compound **19** as an off-white semi-solid which foamed out into a light yellow solid on pumping (345 mg, 76%). Mp: 160–164 °C. $^1$H NMR (300 MHz, DMSO-*d6*) δ (ppm): 11.35 (bs, 1H, NH), 8.19 (d, $J$ = 8.4 Hz, 1H, 8-H), 7.36–7.32 (m, 2H, Ar–H), 7.22–6.95 (m, 5H, Ar–H), 6.82–6.50 (m, 3H, Ar–H), 3.95–3.73 (m,

2H, $OCH_2$), 2.32–2.24 (m, 5H, Ar–$CH_3$, $C\underline{H}_2N(CH_3)_2$), 2.11 (s, 6H, $N(CH_3)_2$), 2.09 (s, 3H, Ar–H), 1.78–1.67 (m, 2H, $OCH_2C\underline{H}_2$). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ (ppm): 162.4, 158.4, 143.3, 138.4, 137.1, 136.8, 134.4, 130.2, 128.9, 128.2, 127.4, 125.5, 125.2, 122.9, 117.5, 113.6, 65.9, 55.9, 44.7, 26.7, 22.0, 19.6. HRMS (ESI): *m/z* 427.2384 $(M+H)^+$ (calcd for $C_{28}H_{31}N_2O_2$, 427.2386), 449.2202 $(M+Na)^+$ (calcd for $C_{28}H_{30}N_2NaO_2$, 449.2205).

### 6.2. Cytotoxicity

The four cell lines employed in this study (MCF-10A, T47D, DU145, and HCT-15) were cultured according to the supplier's instructions. The cells were seeded in 96-well plates at a density of $2–4 \times 10^4$ cells per well and incubated overnight in 0.1 mL of media supplied with 10% fetal bovine serum (Hyclone, USA) in a 5% $CO_2$ incubator at 37 °C. On day 2, the culture medium in each well was replaced with 0.1-mL aliquots of medium containing a range of concentrations of the compounds. On day 4, 5 μL of 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. The positive controls (CPT, etoposide and DOX) were purchased from Sigma.

### 6.3. Topoisomerase inhibition

Topo I inhibitory activity was determined by assessing the relaxation of supercoiled plasmid DNA, pBR322. A mixture of pBR322 (100 ng) and recombinant human DNA topo I (0.4 units; Topo-GEN INC., USA) was incubated with or without the 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 final reaction volume of 10 μL was quenched by adding 2.5 μL stop solution containing 5% sarcosyl, 0.0025% bromophenol blue and 25% glycerol. The DNA samples were then electrophoresed on a 1% agarose gel at 15 V 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 a UV light and the supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

Topo IIα inhibitory activity of the compounds was measured as follows. pBR322 (200 ng) and human DNA topo IIα (1 unit; Usb Corp., USA) were incubated with or without the 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 final reaction volume of 20 μL was quenched by adding 3 μL of 7 mM EDTA. The DNA samples were electrophoresed on a 1% agarose gel at 25 V for 4 h with TAE as the running buffer. The gels were then stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). The DNA bands were visualized by transillumination with a UV light and supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

### 6.4. Molecular docking

The docking study was performed in *Sybyl-X 2.0 (winnt_os5x)* using the Surflex Dock program. The structure of DNA-topo I-topotecan was downloaded from the Protein Data Bank (PDB: 1K4T). An atom of Hg, a molecule of polyethylene glycol (PEG) and an open carboxylate form of topotecan were deleted. The ligand (topotecan) was extracted. Hydrogens were added and minimization was performed using the MMFF94s force field with MMFF94 charges, by using a conjugate gradient method, distance-dependent dielectric constant and converging to 0.01 kcal/mol·Å. The –SH group of the G11 nucleotide of the scissile DNA strand was changed to –OH. Protomol, an idealized representation of a ligand that makes every potential interaction with the binding site, was generated on the basis of ligand mode. Diarylisoquinolines and topotecan were constructed in Sybyl; energy was minimized with MMFF94s force field and MMFF94 charges and stored in a Sybyl database. The compounds in the Sybyl database were docked into the binding site by Surflex Dock on the basis of the protomol constructed earlier. The extracted topotecan was considered to represent a reference molecule. The docking protomol was able to reproduce the position of topotecan (manually constructed and stored in the Sybyl database) in the binding site with 0.61 Å root-mean-square deviation (rmsd) of the heavy atoms of the extracted topotecan.

Similarly, the structure of DNA-topo IIβ-etoposide was downloaded from the Protein Data Bank (PDB: 3QX3). A topo IIβ monomer, a molecule of etoposide, and 3 $Mg^{2+}$ associated with the monomer were deleted. Gln778 and Ala816 of the remaining monomer were modified into Met and Ser respectively. The bond length, bond angle and torsional angle of the redrawn side chains were adjusted to match the Met762 and Ser800 of DNA-topo IIα (PDB: 4FM9). The remaining procedures were performed in a similar manner to that described above for topo I. The docking protomol was able to reproduce the position of manually constructed etoposide in the binding site with 0.54 Å rmsd of the heavy atoms of the extracted etoposide.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.01.016.

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