

Research paper

Substituted 2-arylquinazolinones: Design, synthesis, and evaluation of cytotoxicity and inhibition of topoisomerases



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ABSTRACT

A series of 2-arylquinazolinones with structural homology to known 3-arylisoquinolines were designed and synthesized in order to develop safe, effective, and selective cytotoxic agents targeting topoisomerases (topos). 2-Arylquinazolinones with various substitutions on the aromatic rings were obtained by thermal cyclodehydration/dehydrogenation on reacting anthranilamides and benzaldehydes. The compounds had superior topo I-inhibitory activities but were generally inactive against topo II α . Among the 6-methyl-, 6-amino-, and 7-methylquinazolinones, 6-amino-substituted derivatives displayed potent cytotoxicity at submicromolar to nanomolar concentrations against human colorectal adenocarcinoma cells (HCT-15), human ductal breast epithelial tumor cells (T47D), and cervical cancer cells (HeLa). There was a good correlation between topo I inhibition and the cytotoxic effects of 6-aminoquinazolinones. Docking models demonstrated that topo I inhibition by these compounds is owing to intercalation and H-bond interactions with the DNA bases and amino acid residues at the enzymatic site.

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1. Introduction

One of the differences between cancer and normal cells observed during genomic and proteomic studies, is the expression of a specialized class of enzymes known as topoisomerases (topos) [1–5]. The elevated level of topos in cancer cells is expected because of their involvement in vital cellular processes required for rapidly growing and dividing cells, such as DNA transcription, replication, and recombination [6,7]. Topos are responsible for the resolution of topological issues in DNA including relaxation of supercoils, catenation, and knots. Enzymatic activities of topos basically involve transient breaks in the DNA strand(s), following which the DNA is rotated/strands are passaged and then the broken

strand(s) are religated [8,9]. Inhibition of either the DNA cleavage or topo resealing steps is fatal since transient cleavage is necessary to maintain the correct superhelical density of chromosomes and the lack of repair has direct consequences, transforming an essential enzyme into poison leading to the death of cell. Thus, topos (mainly topo I and II α) have been established as important cellular targets for cancer treatment.

Several topo I and II inhibitors, including camptothecin (CPT) derivatives (irinotecan and topotecan), etoposide, and doxorubicin (DOX) have been clinically prescribed for small-cell lung, testicular, and thyroid cancers, as well as for acute lymphoblastic and acute myelogenous leukemias (Fig. 1). However, these potent antitumor agents have several drawbacks that include chemical instability, poor water solubility, drug resistance in cells overexpressing drug efflux membrane transporters, and drug-related secondary malignancies [10,11]. The limitations are mainly attributable to the structural demerits of CPT derivatives, structural recognition of the drugs by drug efflux pumps, and non-selectivity of etoposide and DOX for different topo II α and β isoforms. Considering these

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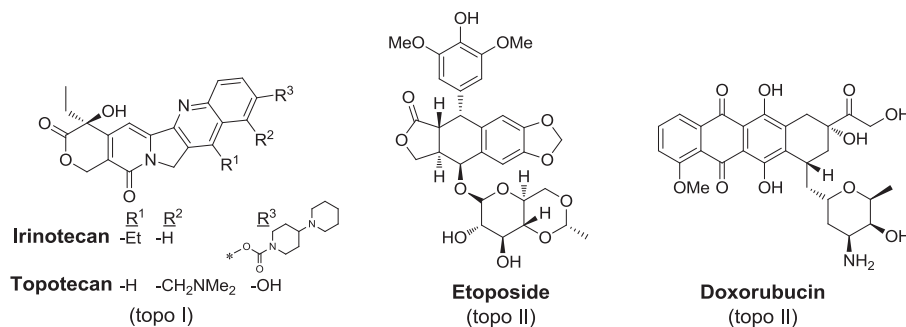


Fig. 1. Anticancer drugs targeting topos.

difficulties, several novel classes of compounds have been investigated for their potential to inhibit topos [12–14].

In spite of intense studies, only a few successful inhibitors of topos have been discovered. Development programs focused on non-CPT topo I inhibitors have yielded only four compounds suitable for entry into clinical trials—edotecarin, Genz-644282, and the indenoisoquinolines, (indotecan and indimitecan) (Fig. 2). Unfortunately, edotecarin failed in Phase III clinical trials while the remaining candidates are still in Phase I [15]. There is therefore an unmet need for an alternative class of topo inhibitors. Our research group has studied and developed 3-arylisquinolones [16] and their analogs, 4-amino-2-phenylquinazolines [17], as potent cytotoxic agents with topo-inhibitory activity (Fig. 3). In addition, previous studies have shown that 2-arylquinazolines act as bioisosteres of 3-arylisquinolones and that the additional N1 on the quinazoline ring may play an important role in establishing an H-bond with Arg364 of topo I. We now report the design and synthesis of 2-arylquinazolinones, with the essential structure maintained but with substitutions in the aromatic rings, together with the characterization of their cytotoxicity and inhibitory effects on topos (Fig. 3).

2. Chemistry

2-Aryl-4-quinazolinones were synthesized as shown in Scheme 1. 4-Methyl- **1a**, 5-methyl- **1b**, and 5-aminoanthranilamides **1c–1f** were prepared from 4-methyl-2-aminobenzonitrile [18], 5-methyl-2-nitrobenzoic acid [19], and 5-amino-2-nitrobenzoic acid [20], respectively. The reaction of these substituted anthranilamides with different benzaldehydes **2a–k** in the presence of *N,N*-

dimethylacetamide and NaHSO₃ at 150 °C yielded 2-aryl-4-quinazolinones **3–31** by thermal cyclodehydration/dehydrogenation, with moderate to excellent yields (50–95%). The reactions with 3-substituted-benzaldehydes were usually associated with better product yields than those obtained using 2-substituted-benzaldehydes. This may be owing to steric and electronic effects of the substituted group at the C2 position, which affects the quinazolinone ring formation. Indeed, no desired product was obtained from the reaction of 4-methylantranilamide with 2-trifluoromethylbenzaldehyde under similar conditions to those described above.

3. Results and discussion

3.1. Topos inhibition

The inhibitory activities of the 2-arylquinazolinones against topos were determined using DNA relaxation assay (Figs. 4 and 5) [21]. In this assay, the activities of topos I/II α were assayed by monitoring the relaxation of the supercoiled plasmid, pBR322, which has different electrophoretic mobility than the completely relaxed DNA. CPT and etoposide were used as positive controls for topo I and II α respectively. Table 1 shows the percentage inhibition of topos induced by the test compounds at concentrations of 100 μ M and 20 μ M. For a SAR study, the inhibitory activities of 2-arylquinazolinones were expressed 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 to CPT/etoposide), and >1.0 (higher activity than CPT/etoposide).

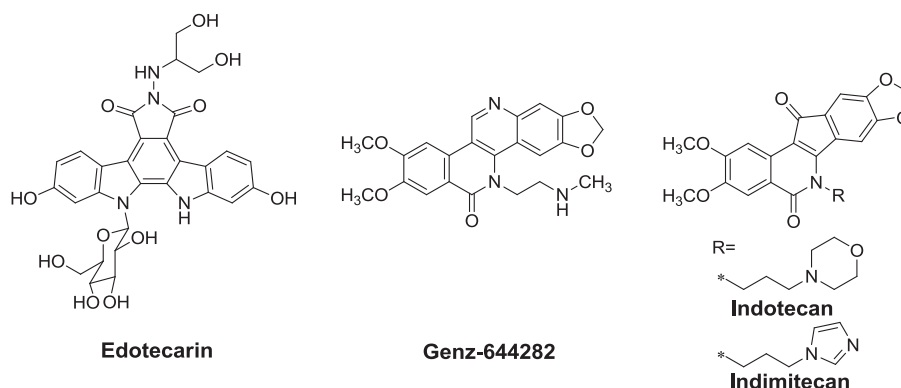


Fig. 2. Non-CPT topo I inhibitors in clinical trials.

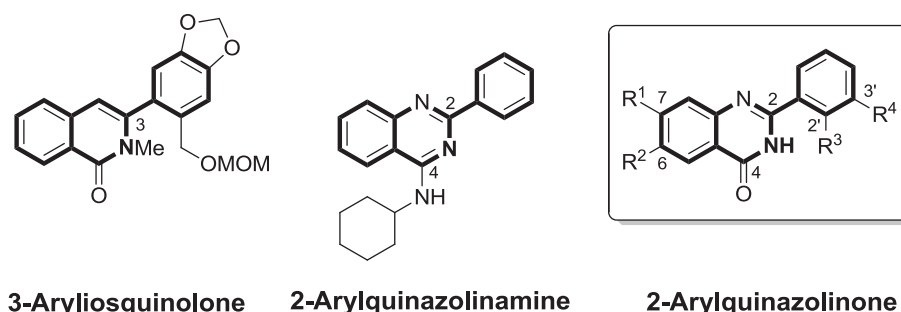
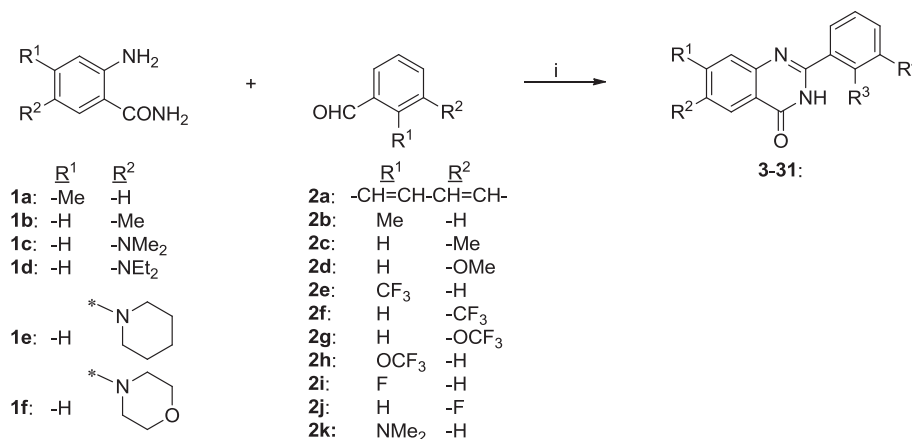


Fig. 3. Design of substituted 2-arylquinazolinone based on 3-arylisquinolone and 2-arylquinazolinamine.



Scheme 1. Synthesis of 2-arylquinazolinones **3–31**. Reagents and conditions: (i) NaHSO₃, *N,N*-dimethylacetamide, 150 °C.

2-Arylquinazolinones exhibited potent topo I-inhibitory activity (Fig. 4 and Table 1). Twenty-six 2-arylquinazolinones at a concentration of 100 μ M, were equipotent or more potent against topo I than CPT and potent topo I inhibition was maintained by ten of the derivatives at 20 μ M. Compound **8** had moderate topo I-inhibitory activity while the remaining two quinazolinones **3** and **4** possessed no activity.

2-Arylquinazolinones were relatively less active against topo II α compared to their activity against topo I (Figs. 4–6 and Table 1). The topo II α -inhibitory activity of 6-methylquinazolinone **6** at 100 μ M and 20 was similar to or lower than that of etoposide, respectively, while 6-morpholine- **21** and 7-methyl- **23** substituted derivatives showed moderate activities at both 100 μ M and 20 μ M. 7-Methyl compounds **24** and **25**

displayed poor topo II α -inhibitory activity. Interestingly, quinazolinone derivative **31** with a 2-aminophenyl moiety had a similar topo II α -inhibitory effect as etoposide at 20 μ M but low activity at 100 μ M.

6-Morpholine- **21** and 7-methyl- **23** substituted 2-arylquinazolinones which moderately inhibited DNA relaxation activity of topo II α were further subjected for kinetoplast DNA (kDNA) decatenation assay (Fig. 7). Decatenation is an activity specific to topo II [22]. The extent of inhibition of topo II α -mediated decatenation by **21** (83.8%) was similar to ellipticine (well-known topo II inhibitor, 88.3%) at 100 μ M. Meanwhile, at the same concentration, the inhibitory effect of **23** on decatenation by topo II α was low. Both of the compounds showed no marked effects at lower concentration (50 μ M).

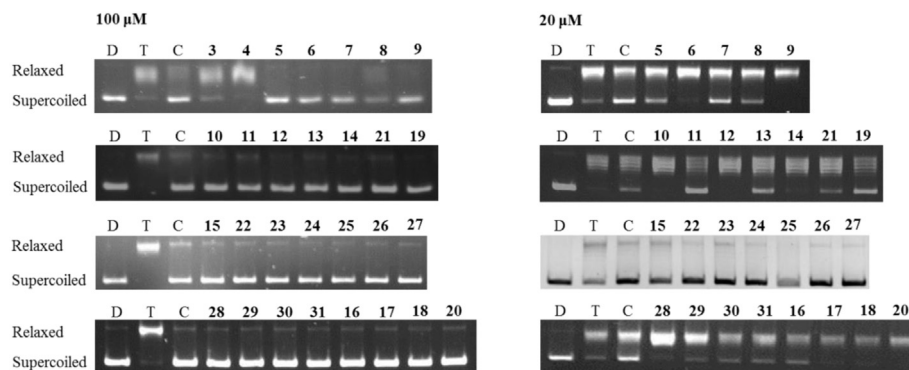


Fig. 4. Topo I inhibition by 2-arylquinazolinones. Lane D: pBR322 only; lane T: pBR322 + topo I; lane C: pBR322 + topo I + CPT; remaining lanes: pBR322 + topo I + the indicated 2-arylquinazolinones.

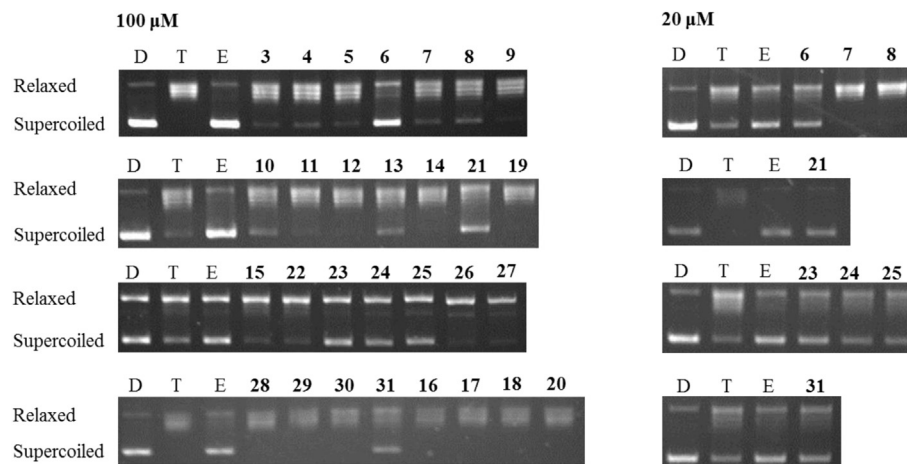


Fig. 5. Topo II α inhibition by 2-arylquinazolinones. Lane D: pBR322 only; lane T: pBR322 + topo II α ; lane E: pBR322 + topo II α + etoposide; remaining lanes: pBR322 + topo II α + the indicated 2-arylquinazolinones.

Table 1
Topo I and II α inhibition by 2-arylquinazolinones (**3–31**).

Compound	R ¹	R ²	R ³	R ⁴	Topo I (%) inhibition		Topo II α (%) inhibition	
					100 μ M	20 μ M	100 μ M	20 μ M
CPT	–	–	–	–	70.9 ^a /80.2 ^b / 77.0 ^c /82.9 ^d	32.3 ^a /17.2 ^b / 30.7 ^c /26.9 ^d	–	–
Etoposide	–	–	–	–	–	–	79.8	49.7
3	–H	–Me	–CH=CH–CH=CH–	–	4.1 (0.05) ^e	ND	5.5 (0.06)	ND
4	–H	–Me	–Me	–H	0.0	ND	0.0	ND
5	–H	–Me	–H	–Me	75.7 (1.06)	12.7 (0.39)	4.6 (0.05)	ND
6	–H	–Me	–H	–OMe	71.8 (1.01)	0.0	65.2 (0.81)	19.0 (0.38)
7	–H	–Me	–CF ₃	–H	72.0 (1.01)	21.1 (0.65)	7.2 (0.09)	0.0
8	–H	–Me	–H	–CF ₃	45.8 (0.64)	4.2 (0.13)	11.2 (0.14)	0.0
9	–H	–Me	–H	–OCF ₃	98.6 (1.39)	0.0	0.0	ND
10	–H	–Me	–OCF ₃	–H	66.9 (0.83)	0.0	5.47 (0.06)	ND
11	–H	–Me	–F	–H	67.9 (0.84)	36.3 (2.11)	0.0	ND
12	–H	–Me	–H	–F	82.9 (1.03)	0.2 (0.01)	0.0	ND
13	–H	–Me	–NMe ₂	–H	82.9 (1.03)	21.1 (1.22)	7.2 (0.09)	ND
14	–H	–NMe ₂	–CH=CH–CH=CH–	–	100.0 (1.24)	2.4 (0.14)	0.0	ND
15	–H	–NEt ₂	–CH=CH–CH=CH–	–	90.4 (1.17)	25.5 (0.83)	0.0	ND
16	–H	–NEt ₂	–CF ₃	–H	81.7 (0.98)	13.3 (0.49)	9.4 (0.11)	ND
17	–H	–NEt ₂	–NMe ₂	–H	82.1 (0.99)	0.0	0.0	ND
18	–H	–NEt ₂	–OCF ₃	–H	81.2 (0.98)	0.0	0.0	ND
19	–H		–CH=CH–CH=CH–	–	100.0 (1.24)	33.4 (1.94)	0.0	ND
20	–H		–OMe	–OMe	80.9 (0.97)	0.0	0.0	ND
21	–H		–CH=CH–CH=CH–	–	100.0 (1.24)	10.7 (0.62)	46.4 (0.58)	39.2 (0.78)
22	–Me	–H	–CH=CH–CH=CH–	–	100.0 (1.29)	60.1 (1.95)	0.0	ND
23	–Me	–H	–Me	–H	100.0 (1.29)	39.2 (1.27)	61.4 (0.77)	30.8 (0.62)
24	–Me	–H	–H	–Me	100.0 (1.29)	56.3 (1.83)	40.4 (0.5)	24.9 (0.5)
25	–Me	–H	–H	–OMe	100.0 (1.29)	80.0 (2.6)	43.6 (0.54)	25.2 (0.5)
26	–Me	–H	–H	–CF ₃	100.0 (1.29)	93.6 (3.04)	0.0	ND
27	–Me	–H	–OCF ₃	–H	100.0 (1.29)	100.0 (3.25)	0.0	ND
28	–Me	–H	–H	–OCF ₃	77.2 (0.93)	0.0	0.0	ND
29	–Me	–H	–F	–H	81.2 (0.98)	0.0	0.0	ND
30	–Me	–H	–H	–F	77.5 (0.93)	4.3 (0.16)	9.6 (0.12)	ND
31	–Me	–H	–NMe ₂	–H	82.1 (0.99)	5.3 (0.19)	28.1 (0.35)	41.9 (0.84)

Each value represents the mean $\pm$ S.D. of three separate experiments, performed in triplicate.

ND: Not determined.

^a Corresponding values when compounds **3–9** were tested.

^b Corresponding values when compounds **10–13**, **14**, **19**, and **21** were tested.

^c Corresponding values when compounds **15**, **22–27** were tested.

^d Corresponding values when compounds **16–18**, **20**, and **28–31** were tested.

^e Enclosed within parentheses is the relative topo-inhibitory activity; the topo-inhibitory activity of the test compound/the topo-inhibitory activity of CPT or etoposide.

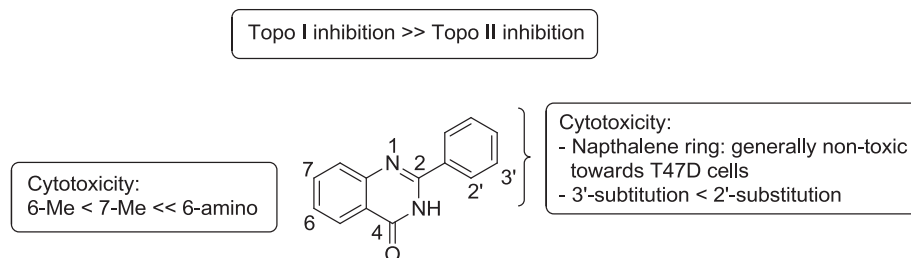


Fig. 6. Structure-activity relationship (SAR) of 2-arylquinazolinones.

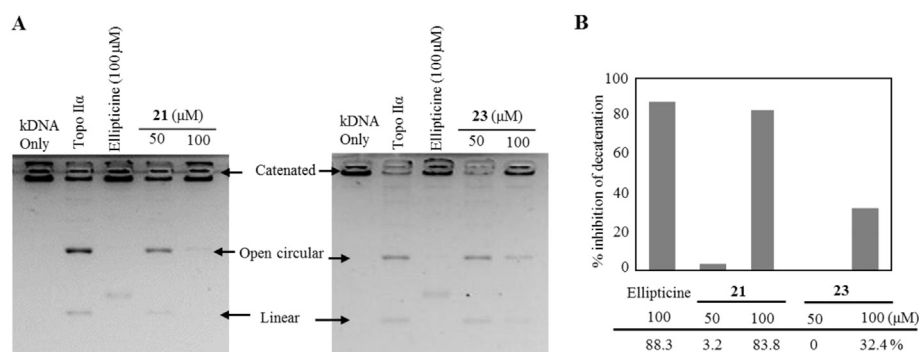


Fig. 7. (A) Inhibition of topo II α -mediated kDNA decatenation by 2-arylquinazolinones **21** and **23**. (B) Quantification of decatenated products.

3.2. Cytotoxicity

The cytotoxic effects of the synthesized 2-arylquinazolinones **3–31** were evaluated by MTT assay [21]. The assay was performed using a human colorectal adenocarcinoma cell line (HCT-15), a human ductal breast epithelial tumor cell line (T47D) and cervical cancer cell line (HeLa). Cytotoxicity results are summarized as IC₅₀ values (Table 2).

6-Methylquinazolinones were generally less toxic towards tested cancer cells than the 7-methyl- and 6-amino- substituted quinazolinones (Table 2 and Fig. 6). 2-Naphthyl- **3** and 2-(*o*-tolyl)- **4** substituted derivatives were more toxic against HeLa cells than etoposide. Quinazolinones **7** and **10** with CF₃ and OCF₃ substituents respectively at the *o*-position of the 2-aryl ring, were even more potent than **3** and **4**. The cytotoxicity was lost when the CF₃ and OCF₃ groups were shifted to the *m*-position of the 2-aryl ring as seen in compounds **8** and **9** (Fig. 6). Interestingly, compound **10** inhibited the proliferation of HCT-15 cells (IC₅₀: 2.79 ± 0.02 μM) better than the standards, CPT, etoposide, and DOX.

The antiproliferative effects of 7-methylquinazolinones were more pronounced than those of the 6-methyl derivatives. 7-Methylquinazolinones **24**, **25**, **29**, **30**, and **31** were more cytotoxic than their corresponding 6-methyl derivatives **5**, **6**, **11**, **12**, and **13**. The 2-(*m*-anisole) derivative **25** was cytotoxic to HCT-15 cells (IC₅₀: 4.85 ± 0.05 μM) compared to CPT and etoposide. It also exhibited moderate antiproliferative activity against HeLa cells (IC₅₀: 8.94 ± 0.1 μM). Among the 6-methyl- and 7-methylquinazolinones, the dimethylamino-substituted analog **31** is the only compound that is toxic to T47D cells. The 2'-F-substituted quinazolinone **29** was strongly cytotoxic against HeLa cells (IC₅₀: 0.83 ± 0.02 μM) and had moderate activity in HCT-15 cells although the activity decreased when the substitution was 3'-F as in case of compound **30**. Similar results were also observed for compounds **27** and **28** where a comparison of the cytotoxicity profile of 6-methyl- and 7-methylquinazolinones showed that substitution at C3' was detrimental.

6-Aminoquinazolinones displayed potent antiproliferative properties. Compounds **14–19** showed high cytotoxicity against HCT-15 cells (IC₅₀: 0.28 ± 0.02–4.2 ± 0.1 μM). The 2-naphthyl-substituted derivatives **14**, **19**, and **21** were inactive against T47D cells while the 6-diethylamino derivatives **15–18**, irrespective of the 2-aryl substituents, were all cytotoxic (IC₅₀: 1.67 ± 0.03–2.69 ± 0.16 μM). The 6-amino compounds were highly cytotoxic against HeLa cells. Compounds **14–19** and **21** had increased cytotoxicity (IC₅₀: 0.004 ± 0.000–0.33 ± 0.02 μM) compared to the standards, CPT, etoposide, and DOX. The 6-Morpholine derivative **21** was selectively cytotoxic against HeLa cells.

No apparent relationship between cytotoxicity and topoisomerase inhibition could be found upon detailed examination for several of the 2-arylquinazolinones. This discrepancy has also been previously observed for 3-heteroarylisoquinolinamines and 3,4-diarylisoquinolines [23,24]. The 2-arylquinazolinones **3** and **4** despite lacking topo-inhibitory activity, were potently cytotoxic, which may be attributed to interference with the function of other biological targets. Furthermore, the results of compounds **5**, **6**, **9**, **11**, **12**, **22**, **26**, and **28** were rather disappointing, since none of the compounds that inhibited topoisomerase were cytotoxic. This inconsistency may possibly be owing to problems related to low aqueous solubility and cellular uptake, or more general issues related to cellular distribution and metabolism.

There was strong correlation between cytotoxicity and topoisomerase inhibition for the compounds **7**, **10**, **25**, **29**, and **31**. In addition, all of the 6-amino-substituted quinazolinones **14–21** were both cytotoxic and inhibited topoisomerase. The increased cytotoxicity of these compounds is not surprising since the amino group improves the solubility of the compounds thereby affecting other pharmacological properties.

3.3. Molecular docking

Hypothetical binding modes of the topo I-DNA complex and 2-arylquinazolinones exhibiting both potent cytotoxicity and

Table 2
Cytotoxicity (IC₅₀) of 2-arylquinazolinones (**3–31**).

Compound	R ¹	R ²	R ³	R ⁴	IC ₅₀ (μM)		
					HCT-15 ^a	T47D ^b	HeLa ^c
CPT	—	—	—	—	6.08 ± 0.08	1.62 ± 0.11	1.59 ± 0.07
Etoposide	—	—	—	—	5.54 ± 0.38	9.28 ± 0.1	7.6 ± 0
Doxorubicin	—	—	—	—	3.33 ± 0.09	1.9 ± 0.01	0.52 ± 0
3	—H	—Me	—CH=CH—CH=CH—	—	>50	>50	5.36 ± 0.06
4	—H	—Me	—Me	—H	11.72 ± 0.07	>50	4.58 ± 0.03
5	—H	—Me	—H	—Me	>50	>50	>50
6	—H	—Me	—H	—OMe	31.97 ± 0.19	>50	>50
7	—H	—Me	—CF ₃	—H	>50	>50	2.45 ± 0.11
8	—H	—Me	—H	—CF ₃	>50	>50	>50
9	—H	—Me	—H	—OCF ₃	>50	>50	>50
10	—H	—Me	—OCF ₃	—H	2.79 ± 0.02	>50	0.85 ± 0.00
11	—H	—Me	—F	—H	>50	>50	>50
12	—H	—Me	—H	—F	>50	>50	>50
13	—H	—Me	—NMe ₂	—H	>50	26.71 ± 0.26	13.99 ± 0.10
14	—H	—NMe ₂	—CH=CH—CH=CH—	—	0.46 ± 0.01	>50	0.05 ± 0.00
15	—H	—NEt ₂	—CH=CH—CH=CH—	—	3.21 ± 0.16	2.69 ± 0.16	0.004 ± 0.000
16	—H	—NEt ₂	—CF ₃	—H	0.28 ± 0.02	2.55 ± 0.04	0.1 ± 0.00
17	—H	—NEt ₂	—NMe ₂	—H	4.2 ± 0.1	2.21 ± 0.09	0.07 ± 0.00
18	—H	—NEt ₂	—OCF ₃	—H	1.71 ± 0.09	1.67 ± 0.03	0.04 ± 0.01
19	—H		—CH=CH—CH=CH—	—	2.24 ± 0.25	>50	0.33 ± 0.02
20	—H		—OMe	—OMe	24.81 ± 0.18	10.41 ± 0.09	8.19 ± 0.09
21	—H		—CH=CH—CH=CH—	—	>50	>50	0.12 ± 0.00
22	—Me	—H	—CH=CH—CH=CH—	—	23.32 ± 0.02	>50	>50
23	—Me	—H	—Me	—H	11.87 ± 0.21	>50	>50
24	—Me	—H	—H	—Me	21.12 ± 0.07	>50	11.21 ± 0.13
25	—Me	—H	—H	—OMe	4.85 ± 0.05	>50	8.94 ± 0.10
26	—Me	—H	—H	—CF ₃	>50	>50	>50
27	—Me	—H	—OCF ₃	—H	15.87 ± 0.11	>50	18.3 ± 0.56
28	—Me	—H	—H	—OCF ₃	>50	>50	>50
29	—Me	—H	—F	—H	10.26 ± 0.63	>50	0.83 ± 0.02
30	—Me	—H	—H	—F	14.42 ± 0.35	>50	>50
31	—Me	—H	—NMe ₂	—H	12.81 ± 0.96	4.43 ± 0.25	43.8 ± 0.98

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

^a HCT-15: human colorectal adenocarcinoma cells.

^b T47D: human ductal breast epithelial tumor cells.

^c HeLa: cervical cancer cells.

topo I inhibition were generated using molecular docking tools in order to identify the potential interactions responsible for topo I inhibition [24]. The Surflex-Dock program available in Sybyl-X 2.0 was used to dock the selected 2-arylquinazolinones into the hydrated-pocket formed after removal of ligand from the 3-dimensional crystal structure of topo I-DNA-topotecan (PDB: 1K4T).

The potential interactions between topo I, DNA, and 2-arylquinazolinones based on the binding modes of the representative compounds **16–18** can be summarized as shown in Fig. 8. Hypothetical binding modes obtained after docking, suggested that the quinazolinone ring of the compounds was sandwiched between DNA bases (G11 and A113) of the scissile and intact strands in a similar manner to topotecan. The overlap of DNA bases and ligand indicates the possible π – π stacking interactions between the aromatic rings. The π – π stacking interactions of a topo I inhibitor placed between DNA base pairs at the site of DNA cleavage deforms the DNA structure and displaces the reactive 5'-OH of the –1 base away from the phosphotyrosine to prevent DNA ligation [25].

In addition to the π – π stacking interactions, the lactam NH and O of compound **16** interacted with DNA base C112 and Arg364 of topo I through H-bonds (Fig. 8B). The topo I inhibitors CPT, indenoisoquinoline (MJ238), and indolocarbazole (SA315F) have also been shown to be associated with Arg364 [26]. The

importance of the interaction between topo I inhibitors and Arg364 is supported by the fact that topo I with an Arg364His mutation is not inhibited by CPT and indolocarbazole. The lactam O of **18** also forms an H-bond with Arg364 (Fig. 8D) similar to **16**. The orientation of compound **17** however differed from that of **16** and **18**. The compound flipped vertically and had N1 in the same position as the lactam O of **16** and **18** and facilitated an H-bond association with Arg364 (Fig. 8C). The lactam O of **17** also formed an additional H-bond with the T10 (+1) base covalently attached to Tyr723 of topo I.

4. Conclusion

In conclusion, 2-arylquinazolinones were designed as heterocyclic analogs of 3-arylisoquinoline derivatives known to be cytotoxic and have potent topoisomerase inhibition. The quinazolinones were obtained in a single step by reacting substituted anthranilamides and benzaldehydes. 2-Arylquinazolinones, and particularly the 6-amino-substituted derivatives, exhibited excellent topo I-inhibitory activity as well as cytotoxicity. The potent biological activity and the similar interactions with topo I and DNA as known topo I inhibitors demonstrate that 2-arylquinazolinones can be established as a class of anti-cancer agents with potential to target topoisomerases activity.

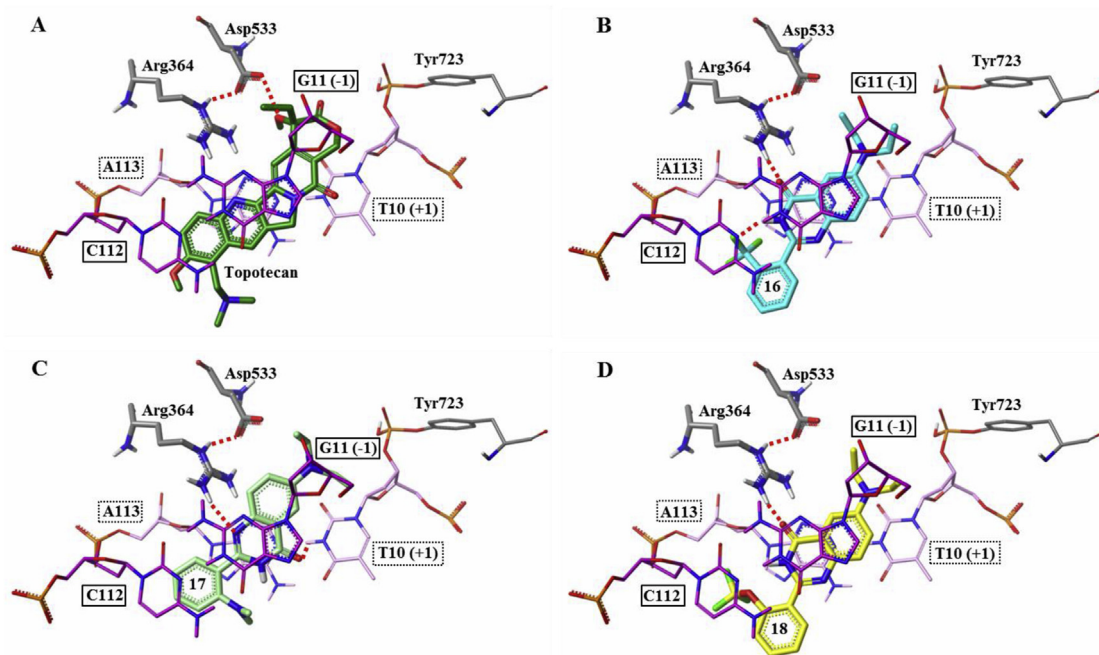


Fig. 8. Binding mode of topo I-DNA-topotecan (A). Hypothetical binding modes of compound **16** (B), **17** (C), and **18** (D) to topo I and DNA. H-bonds are illustrated as discontinuous red lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5. Experimental section

5.1. Chemistry

Melting points (mp) were determined by the capillary method with an EZ-Melt MPA 120 apparatus and were uncorrected. IR spectra were recorded on a Bruker FT-IR spectrophotometer using KBr disc. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance 500 MHz spectrometer. ^1H NMR spectral data are reported in the following order: chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, m: multiplet, dd: double of doublets, td: triplet of doublets), coupling constant and number of protons. The chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS, $\delta = 0$). Coupling constants (J) are reported in Hertz (Hz). Low resolution mass spectra were obtained on Agilent 1260 HPLC/MS system. High resolution mass spectra were obtained on FTICR MS Varian. Thin layer chromatography (TLC) was performed using analytical TLC plates precoated with silica gel 60 F₂₅₄ (Merck) and the spots were visualized by UV lamp (254 nm). Chemical reagents were purchased from Sigma–Aldrich, Acros and Merck and were used without further purification.

5.1.1. General procedure for the synthesis of 2-arylquinazolinones **3–31**

Sodium hydrogen sulfite (4 mmol) was added to a solution of anthranilamide **1** (2 mmol) and benzaldehyde **2** (2 mmol) in *N,N*-dimethylacetamide (5 mL). The mixture was heated under continuous stirring at 150 °C for 2–3 h and poured into ice water. The precipitate was then filtered, washed with water followed by ethanol, and dried to yield the 2-arylquinazolinones **3–31**.

5.1.2. 6-Methyl-2-(naphthalen-1-yl)quinazolin-4(3H)-one (**3**)

Yield 434 mg (76%). Light-yellow solid. Mp: 264–265 °C. IR (cm^{-1}): 3421 (NH), 3041, 1677 (C=O). ^1H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.5 (s, 1H); 8.15 (d, $J = 7.5$ Hz, 1H), 8.10 (d, $J = 8.5$ Hz, 1H), 8.05–8.02 (m, 2H); 7.78 (d, $J = 7.5$ Hz, 1H), 7.69–7.56 (m, 5H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.7, 152.7,

146.6, 136.4, 135.7, 133.0, 131.7, 130.2, 128.2, 127.5, 127.2, 126.9, 126.2, 125.16, 125.13, 125.0, 120.9, 20.8. HRMS (ESI): m/z 287.1181 ($M + \text{H}^+$) (calcd for C₁₉H₁₅N₂O, 287.1184).

5.1.3. 6-Methyl-2-(*o*-tolyl)quinazolin-4(3H)-one (**4**)

Yield 385 mg (77%). White solid. Mp: 215–217 °C. IR (cm^{-1}): 3420 (NH), 3041, 1682 (C=O). ^1H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.3 (s, 1H), 7.96 (s, 1H), 7.64 (dd, $J = 8.0, 1.5$ Hz, 1H); 7.58 (d, $J = 8.5$ Hz, 1H), 7.48 (d, $J = 7.5$ Hz, 1H), 7.41 (td, $J = 7.5, 1.0$ Hz, 1H), 7.33 (d, $J = 7.0$ Hz, 1H), 7.31 (d, $J = 7.0$ Hz, 1H), 2.46 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.6, 153.4, 146.7, 136.2, 136.0, 135.6, 134.2, 130.4, 129.7, 129.0, 127.1, 125.6, 125.0, 120.9, 20.8, 19.5. HRMS (ESI): m/z 251.1187 ($M + \text{H}^+$) (calcd for C₁₆H₁₅N₂O, 251.1184).

5.1.4. 6-Methyl-2-(*m*-tolyl)quinazolin-4(3H)-one (**5**)

Yield 450 mg (90%). White solid. Mp: 234–236 °C. IR (cm^{-1}): 3420 (NH), 3041, 1682 (C=O). ^1H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.3 (s, 1H); 8.00 (s, 1H); 7.95–7.94 (m, 2H), 7.65–7.37 (m, 4H), 2.45 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.1, 151.5, 146.7, 137.8, 136.2, 135.8, 132.6, 131.8, 128.4, 128.1, 127.3, 125.2, 124.7, 120.6, 20.9, 20.8. HRMS (ESI): m/z 251.1180 ($M + \text{H}^+$) (calcd for C₁₆H₁₅N₂O, 251.1184).

5.1.5. 2-(3-Methoxyphenyl)-6-methylquinazolin-4(3H)-one (**6**)

Yield 484 mg (91%). White solid. Mp: 213–215 °C. IR (cm^{-1}): 3420 (NH), 3041, 1682 (C=O). ^1H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.4 (s, 1H); 7.94 (s, 1H); 7.77 (d, $J = 7.5$ Hz, 1H); 7.72 (dd, $J = 2.5, 1.5$ Hz, 1H), 7.64 (s, 2H), 7.44 (t, $J = 8.0$ Hz, 1H), 7.13 (dd, $J = 8.0, 2.5$ Hz, 1H), 3.85 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.1, 159.3, 151.3, 146.8, 136.3, 135.9, 134.0, 129.7, 127.4, 125.2, 120.7, 120.0, 117.4, 112.4, 55.3, 20.8. HRMS (ESI): m/z 267.1127 ($M + \text{H}^+$) (calcd for C₁₆H₁₅N₂O, 267.1133).

5.1.6. 6-Methyl-2-(2-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (**7**)

Yield 304 mg (50%). White solid. Mp: 195–196 °C. IR (cm^{-1}):

3414 (NH), 3069, 1664 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.6 (s, 1H), 7.97 (s, 1H); 7.89 (d, $J = 7.5$ Hz, 1H), 7.80 (d, $J = 7.5$ Hz, 1H), 7.79–7.74 (m, 2H), 7.66 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.57 (d, $J = 7.5$ Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.2, 151.4, 146.3, 136.8, 135.8, 133.1, 132.3, 130.7, 130.4, 127.3, 127.0 ($^2J_{\text{C-F}} = 30.7$ Hz), 126.4 ($^3J_{\text{C-F}} = 4.7$ Hz), 125.1, 123.7 ($^1J_{\text{C-F}} = 272.1$ Hz), 120.9, 20.8. HRMS (ESI): m/z 305.0907 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_2\text{O}$, 305.0901).

5.1.7. 6-Methyl-2-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (8)

Yield 498 mg (82%). White solid. Mp: 297–299 °C. IR (cm^{-1}): 3414 (NH), 3069, 1664 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.6 (s, 1H); 8.53 (s, 1H); 8.47 (d, $J = 7.5$ Hz, 1H), 7.97 (s, 1H); 7.94 (d, $J = 7.5$ Hz, 1H), 7.79 (t, $J = 7.5$ Hz, 1H), 7.70–7.67 (m, 2H), 2.47 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.0, 150.1, 146.4, 136.8, 135.9, 133.7, 131.6, 129.8, 129.5 ($^2J_{\text{C-F}} = 30.7$ Hz), 127.6 ($^3J_{\text{C-F}} = 4.7$ Hz), 127.5, 125.2, 124.3, 123.8 ($^1J_{\text{C-F}} = 272.1$ Hz), 120.9, 20.8. HRMS (ESI): m/z 305.0905 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_2\text{O}$, 305.0901).

5.1.8. 6-Methyl-2-(3-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (9)

Yield 499 mg (95%). Light yellow solid. Mp: 201–202 °C. IR (cm^{-1}): 3420 (NH), 3022, 1676 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.6 (s, 1H); 8.23 (d, $J = 7.0$ Hz, 1H), 8.16 (s, 1H); 7.96 (s, 1H); 7.71–7.68 (m, 3H), 7.60 (d, $J = 8.0$ Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 162.0, 156.1, 149.8, 148.4, 136.7, 135.9, 135.0, 130.7, 127.5, 126.7, 125.2, 123.7, 120.1, 119.8 ($^1J_{\text{C-F}} = 256$ Hz), 109.5, 20.8. HRMS (ESI): m/z 321.0847 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$, 321.0850).

5.1.9. 6-Methyl-2-(2-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (10)

Yield 499 mg (78%). Light yellow solid. Mp: 245–246 °C. IR (cm^{-1}): 3420 (NH), 3022, 1676 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.5 (s, 1H); 7.97 (s, 1H); 7.79 (dd, $J = 8.0, 1.5$ Hz, 1H), 7.70–7.66 (m, 2H); 7.61 (d, $J = 8.5$ Hz, 1H), 7.56 (dd, $J = 7.5, 1.0$ Hz, 1H), 7.53 (d, $J = 8.0$ Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.3, 149.5, 146.6, 145.6, 136.9, 135.8, 132.1, 131.3, 128.2, 127.6, 127.3, 125.2, 121.5, 120.8, 119.9 ($^1J_{\text{C-F}} = 256$ Hz), 20.8. HRMS (ESI): m/z 321.0855 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_2$, 321.0850).

5.1.10. 2-(2-Fluorophenyl)-6-methylquinazolin-4(3H)-one (11)

Yield 441 mg (69%). White solid. Mp: 228–229 °C. IR (cm^{-1}): 3420 (NH), 3024, 1680 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.4 (s, 1H); 7.96 (s, 1H); 7.77 (td, $J = 7.5, 2.0$ Hz, 1H), 7.66 (dd, $J = 8.5, 2.0$ Hz, 1H), 7.63–7.59 (m, 2H), 7.40–7.34 (m, 2H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.3, 159.5 ($^1J_{\text{C-F}} = 248$ Hz), 149.0, 146.6, 136.3, 135.8, 132.7 ($^3J_{\text{C-F}} = 8.3$ Hz), 130.9, 127.3, 125.1, 124.5 ($^4J_{\text{C-F}} = 3.2$ Hz), 122.3 ($^3J_{\text{C-F}} = 12.8$ Hz), 120.8, 116.1 ($^2J_{\text{C-F}} = 21.2$ Hz), 20.8. HRMS (ESI): m/z 255.0931 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{15}\text{H}_{12}\text{FN}_2\text{O}$, 255.0933).

5.1.11. 2-(3-Fluorophenyl)-6-methylquinazolin-4(3H)-one (12)

Yield 570 mg (89%). White solid. Mp: 306–308 °C. IR (cm^{-1}): 3420 (NH), 3024, 1680 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.5 (s, 1H); 8.04 (d, $J = 8.0$ Hz, 1H), 7.99 (d, $J = 10$ Hz, 1H), 7.96 (s, 1H); 7.66 (s, 2H); 7.62–7.57 (m, 1H), 7.43 (t, $J = 8.0$ Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.3 ($^1J_{\text{C-F}} = 248$ Hz), 161.2, 150.1, 146.3, 136.6, 135.9 ($^3J_{\text{C-F}} = 8.7$ Hz), 135.0, 130.7 ($^3J_{\text{C-F}} = 8.7$ Hz), 127.4, 125.2, 123.7, 120.8, 118.0, 114.3 ($^2J_{\text{C-F}} = 23.8$ Hz), 20.8. HRMS (ESI): m/z 255.0939 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{15}\text{H}_{12}\text{FN}_2\text{O}$, 255.0933).

5.1.12. 2-(2-(Dimethylamino)phenyl)-6-methylquinazolin-4(3H)-one (13)

Yield 428 mg (77%). White solid. Mp: 204–206 °C. IR (cm^{-1}): 3414 (NH), 3069, 1664 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.7 (s, 1H); 7.94 (s, 1H); 7.83 (dd, $J = 8.0, 1.5$ Hz, 1H), 7.65–7.61 (m, 2H); 7.47 (td, $J = 7.5, 1.0$ Hz, 1H), 7.25 (d, $J = 8.0$ Hz, 1H), 7.13 (td, $J = 7.5, 1.0$ Hz, 1H), 2.66 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.1, 152.8, 151.8, 147.2, 136.2, 135.6, 131.6, 130.5, 127.3, 125.2, 122.1, 120.8, 119.1, 43.6, 20.8. HRMS (ESI): m/z 280.1442 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}$, 280.1449).

5.1.13. 6-(Dimethylamino)-2-(naphthalen-1-yl)quinazolin-4(3H)-one (14)

Yield: 446 mg (71%). Yellow solid. Mp: 251–253 °C. IR (cm^{-1}): 3420 (NH), 3041, 1682 (C=O). ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.3 (s, 1H); 8.17 (dd, $J = 7.0, 2.0$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 8.02 (dd, $J = 7.0, 2.5$ Hz, 1H), 7.74 (d, $J = 7.0$ Hz, 1H), 7.63–7.55 (m, 4H), 7.37 (dd, $J = 9.0, 3.0$ Hz, 1H), 7.28 (d, $J = 3.0$ Hz, 1H), 2.50 (s, 6H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.9, 149.08, 149.02, 139.6, 133.1, 131.9, 130.4, 129.9, 128.3, 128.2, 127.5, 126.8, 126.2, 125.2, 125.0, 122.8, 121.8, 120.4, 104.6, 40.1. MS (ESI): m/z 316.1 ($\text{M} + \text{H}$) $^+$.

5.1.14. 6-(Diethylamino)-2-(naphthalen-1-yl)quinazolin-4(3H)-one (15)

Yield 240 mg (70%). Yellow solid. Mp: 210–212 °C. ^1H NMR (500 MHz, DMSO- d_6) δ (ppm): 12.3 (s, 1H); 8.19 (dd, $J = 7.5, 2.0$ Hz, 1H), 8.05 (d, $J = 8.0$ Hz, 1H), 8.01 (dd, $J = 7.5, 2.5$ Hz, 1H), 7.43 (dd, $J = 7.5, 2.5$ Hz, 1H), 7.62 (d, $J = 7.5$ Hz, 1H), 7.60–7.55 (m, 3H), 7.30–7.25 (m, 2H), 3.47 (q, $J = 7$ Hz, 4H), 1.15 (t, $J = 7$ Hz, 6H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.9, 148.5, 146.2, 138.9, 133.1, 131.9, 130.4, 129.8, 128.6, 128.2, 127.4, 126.8, 126.1, 125.2, 125.0, 122.1, 119.9, 103.8, 43.9, 12.2. MS (ESI): m/z 344.1 ($\text{M} + \text{H}$) $^+$. HRMS (ESI): m/z 344.1758 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{22}\text{H}_{22}\text{N}_3\text{O}$, 344.1762).

5.1.15. 6-(Diethylamino)-2-(2-trifluoromethyl)phenyl)quinazolin-4(3H)-one (16)

Yield: 418 mg (58%). Brown solid. Mp: 195–197 °C. ^1H NMR (500 MHz, CDCl_3) δ (ppm): 9.62 (s, 1H), 7.80 (d, $J = 8$ Hz, 1H), 7.70–7.61 (m, 4H), 7.35 (d, $J = 3$ Hz, 1H), 7.19 (dd, $J = 9, 3$ Hz, 1H), 3.47 (q, $J = 7$ Hz, 4H), 1.22 (t, $J = 7$ Hz, 6H). ^{13}C NMR (125 MHz, CDCl_3) δ (ppm): 162.0, 147.1, 146.1, 138.9, 133.2, 132.1, 130.9, 130.2, 129.1, 128.6, 126.7, 123.6, 122.1, 120.1, 104.7, 44.9, 44.6, 12.4. MS (ESI): m/z 362.1 ($\text{M} + \text{H}$) $^+$. HRMS (ESI): m/z 362.1486 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{19}\text{H}_{19}\text{F}_3\text{N}_3\text{O}$, 362.1480).

5.1.16. 6-(Diethylamino)-2-(2-dimethylamino)phenyl)quinazolin-4(3H)-one (17)

Yield: 412 mg (62%). Yellow solid. Mp: 153–155 °C. ^1H NMR (500 MHz, CDCl_3) δ (ppm): 12.56 (s, 1H), 8.34 (dd, $J = 8, 1.5$ Hz, 1H), 7.67 (d, $J = 9$ Hz, 1H), 7.44–7.41 (m, 2H), 7.26–7.23 (m, 2H), 7.19 (dd, $J = 9, 3$ Hz, 1H), 3.47 (q, $J = 7$ Hz, 4H), 2.77 (s, 6H), 1.23 (t, $J = 7$ Hz, 6H). ^{13}C NMR (125 MHz, CDCl_3) δ (ppm): 162.0, 151.7, 147.8, 146.6, 140.0, 131.3, 130.9, 128.9, 125.5, 124.5, 122.8, 119.9, 119.8, 105.1, 44.9, 44.5, 12.1. MS (ESI): m/z 337.1 ($\text{M} + \text{H}$) $^+$. HRMS (ESI): m/z 337.2023 ($\text{M} + \text{H}$) $^+$ (calcd for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}$, 337.2028).

5.1.17. 6-(Diethylamino)-2-(2-trifluoromethoxy)phenyl)quinazolin-4(3H)-one (18)

Yield: 496 mg (66%). Yellow solid. Mp: 164–166 °C. ^1H NMR (500 MHz, CDCl_3) δ (ppm): 10.02 (s, 1H), 8.08 (dd, $J = 8, 1.5$ Hz, 1H), 7.67 (d, $J = 9$ Hz, 1H), 7.52 (td, $J = 7.5, 1.5$ Hz, 1H), 7.44 (t, $J = 8$ Hz, 1H), 7.38–7.35 (m, 2H), 7.19 (dd, $J = 9, 3$ Hz, 1H), 3.47 (q, $J = 7$ Hz, 4H), 1.15 (t, $J = 7$ Hz, 6H). ^{13}C NMR (125 MHz, DMSO- d_6) δ (ppm): 161.4, 146.3, 145.6, 145.3, 138.8, 131.6, 131.3, 128.7, 128.4, 127.5, 122.1,

121.5, 119.9, 119.8, 103.6, 43.9, 12.1. MS (ESI): m/z 378.1 ($M + H$)⁺. HRMS (ESI): m/z 378.1433 ($M + H$)⁺ (calcd for C₁₉H₁₉F₃N₃O₂, 378.1429).

5.1.18. 2-(Naphthalen-1-yl)-6-(piperidin-1-yl)quinazolin-4(3H)-one (19)

Yield 510 mg (72%). Brown solid. Mp: 231–233 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.4 (s, 1H); 8.16 (dd, $J = 7.5, 1.5$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 8.02 (dd, $J = 7.5, 2.0$ Hz, 1H), 7.74 (d, $J = 7.0$ Hz, 1H), 7.64–7.56 (m, 5H), 7.49 (d, $J = 2.0$ Hz, 1H), 3.29 (t, $J = 5.0$ Hz, 4H), 1.66–1.65 (m, 4H), 1.59–1.58 (m, 2H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.8, 150.0, 146.2, 138.9, 133.1, 131.8, 130.3, 129.9, 128.2, 127.5, 126.8, 126.2, 125.3, 125.2, 125.0, 124.0, 107.9, 49.2, 24.9, 23.7. MS (ESI): m/z 356.1 ($M + H$)⁺.

5.1.19. 2-(2,3-Dimethoxyphenyl)-6-(piperidin-1-yl)quinazolin-4(3H)-one (20)

Yield: 770 mg (67%). Brown solid. Mp: 182–184 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 10.91 (s, 1H); 7.96 (dd, $J = 8, 1.5$ Hz, 1H), 7.67 (d, $J = 9$ Hz, 1H), 7.64 (d, $J = 3$ Hz, 1H), 7.44 (dd, $J = 9, 3$ Hz, 1H), 7.20 (t, $J = 8$ Hz, 1H), 7.06 (dd, $J = 9, 3$ Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.32 (q, $J = 5.5$ Hz, 4H), 1.75–1.71 (m, 4H), 1.65–1.61 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 161.8, 152.9, 147.7, 147.3, 128.7, 125.4, 124.8, 124.4, 122.1, 121.9, 114.9, 109.3, 61.6, 56.1, 50.1, 25.5, 24.2. MS (ESI): m/z 366.1 ($M + H$)⁺. HRMS (ESI): m/z 366.1821 ($M + H$)⁺ (calcd for C₂₁H₂₄N₃O₃, 366.1817).

5.1.20. 6-Morpholino-2-(naphthalen-1-yl)quinazolin-4(3H)-one (21)

Yield 564 mg (79%). Yellow solid. Mp: 291–293 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.4 (s, 1H); 8.17 (dd, $J = 7.0, 2.0$ Hz, 1H), 8.09 (d, $J = 8.0$ Hz, 1H), 8.02 (dd, $J = 7.5, 2.0$ Hz, 1H), 7.76 (d, $J = 7.0$ Hz, 1H), 7.64–7.56 (m, 5H), 7.52 (d, $J = 2.5$ Hz, 1H), 3.79 (t, $J = 5.0$ Hz, 4H), 3.25 (t, $J = 5.0$ Hz, 4H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.8, 150.5, 149.7, 141.8, 133.1, 131.8, 130.3, 130.0, 128.3, 128.2, 127.5, 126.9, 126.2, 125.3, 125.0, 123.4, 121.7, 107.8, 65.9, 48.2. MS (ESI): m/z 358.1 ($M + H$)⁺.

5.1.21. 7-Methyl-2-(naphthalen-1-yl)quinazolin-4(3H)-one (22)

Yield 399 mg (70%). Light-yellow solid. Mp: 293–294 °C. IR (cm⁻¹): 3421 (NH), 3041, 1659 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.5 (s, 1H); 8.17 (d, $J = 8.0$ Hz, 1H), 8.12–8.09 (m, 2H), 8.04 (d, $J = 7.5$ Hz, 1H), 7.78 (d, $J = 7.0$ Hz, 1H), 7.66–7.54 (m, 4H), 7.40 (d, $J = 7.5$ Hz, 1H), 2.49 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.7, 153.6, 148.8, 144.9, 133.1, 131.6, 130.3, 130.2, 128.2, 128.1, 127.6, 127.0, 126.9, 126.3, 125.6, 125.1, 125.0, 118.7, 21.3. HRMS (ESI): m/z 287.1188 ($M + H$)⁺ (calcd for C₂₀H₁₇NO, 287.1184).

5.1.22. 7-Methyl-2-(*o*-tolyl)quinazolin-4(3H)-one (23)

Yield 290 mg (58%). White solid. Mp: 219–221 °C. IR (cm⁻¹): 3420 (NH), 3041, 1682 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.3 (s, 1H), 8.04 (d, $J = 8.0$ Hz, 1H), 7.49–7.48 (m, 2H); 7.42 (t, $J = 7.5$ Hz, 1H), 7.36–7.30 (m, 3H), 2.46 (s, 3H), 2.37 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.6, 154.3, 148.8, 144.8, 136.0, 134.2, 130.4, 129.7, 129.0, 127.9, 126.9, 125.64, 125.61, 118.5, 21.3, 19.5. HRMS (ESI): m/z 251.1189 ($M + H$)⁺ (calcd for C₁₆H₁₅N₂O, 251.1184).

5.1.23. 7-Methyl-2-(*m*-tolyl)quinazolin-4(3H)-one (24)

Yield 398 mg (80%). White solid. Mp: 241–243 °C. IR (cm⁻¹): 3414 (NH), 3036, 1682 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.3 (s, 1H), 8.02 (d, $J = 8.5$ Hz, 1H), 8.01 (s, 1H), 7.96 (d, $J = 7.5$ Hz, 1H), 7.54 (s, 1H); 7.43–7.38 (m, 2H), 7.33 (d, $J = 8.0$ Hz, 1H), 2.46 (s, 3H), 2.40 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.0, 152.3, 148.8, 144.9, 137.8, 132.6, 131.8, 128.4, 128.1,

127.8, 127.0, 125.6, 124.7, 118.5, 21.3, 20.9. HRMS (ESI): m/z 251.1187 ($M + H$)⁺ (calcd for C₁₆H₁₅N₂O, 251.1184).

5.1.24. 2-(3-Methoxyphenyl)-7-methylquinazolin-4(3H)-one (25)

Yield 441 mg (83%). White solid. Mp: 212–214 °C. IR (cm⁻¹): 3414 (NH), 3036, 1682 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.4 (s, 1H); 8.03 (d, $J = 8.0$ Hz, 1H), 7.77 (d, $J = 7.5$ Hz, 1H), 7.73 (s, 1H); 7.54 (s, 1H); 7.44 (t, $J = 7.5$ Hz, 1H), 7.32 (d, $J = 8.0$ Hz, 1H), 7.13 (d, $J = 8.0$ Hz, 1H), 3.85 (s, 3H), 2.46 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.0, 159.3, 151.9, 148.7, 145.0, 134.0, 129.6, 127.9, 127.1, 125.6, 120.0, 118.5, 117.5, 112.4, 55.3, 21.2. HRMS (ESI): m/z 267.1139 ($M + H$)⁺ (calcd for C₁₆H₁₅N₂O, 267.1133).

5.1.25. 7-Methyl-2-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (26)

Yield 452 mg (70%). White solid. Mp: 294–296 °C. IR (cm⁻¹): 3414 (NH), 3069, 1664 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.6 (s, 1H), 8.53 (s, 1H), 8.47 (d, $J = 7.5$ Hz, 1H), 8.05 (d, $J = 8$ Hz, 1H), 7.95 (d, $J = 8.0$ Hz, 1H), 7.80 (t, $J = 8.0$ Hz, 1H), 7.60 (s, 1H); 7.37 (d, $J = 8.0$ Hz, 1H), 3.85 (s, 3H), 2.51 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.9, 150.9, 148.5, 145.1, 133.7, 131.6, 129.7 (² $J_{C-F} = 31.8$ Hz), 129.4, 128.3, 127.4, 127.2, 125.6, 124.4 (³ $J_{C-F} = 3.8$ Hz), 123.9 (¹ $J_{C-F} = 271.1$ Hz), 118.7, 21.2. HRMS (ESI): m/z 305.0908 ($M + H$)⁺ (calcd for C₁₆H₁₂F₃N₂O, 305.0901).

5.1.26. 7-Methyl-2-(2-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (27)

Yield 441 mg (69%). Light yellow solid. Mp: 204–205 °C. IR (cm⁻¹): 3420 (NH), 3022, 1676 (C=O). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 10.3 (s, 1H); 8.17 (d, $J = 8.0$ Hz, 1H), 8.10 (dd, $J = 8.0, 1.0$ Hz, 1H), 7.61–7.58 (m, 2H); 7.49 (t, $J = 7.5$ Hz, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 7.34 (d, $J = 8.0$ Hz, 1H), 2.52 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 161.9, 149.2, 149.0, 146.4, 145.9, 132.5, 131.8, 128.9, 127.8, 127.6, 126.7, 126.3, 121.4, 120.1 (¹ $J_{C-F} = 256$ Hz), 118.6, 21.9. HRMS (ESI): m/z 321.0844 ($M + H$)⁺ (calcd for C₁₆H₁₂F₃N₂O₂, 321.0850).

5.1.27. 7-Methyl-2-(3-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (28)

Yield 454 mg (71%). Light yellow solid. Mp: 250–252 °C. IR (cm⁻¹): 3420 (NH), 3022, 1676 (C=O). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 12.5 (s, 1H); 8.30 (s, 1H); 8.29 (d, $J = 8.0$ Hz, 1H), 8.19 (d, $J = 8.0$ Hz, 1H), 7.61 (s, 1H); 7.58 (d, $J = 8.0$ Hz, 1H), 7.42 (d, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 8.0$ Hz, 1H), 2.53 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 162.2, 150.2, 149.7, 149.4, 146.1, 134.9, 130.2, 128.7, 127.8, 126.1, 125.9, 123.6, 120.6, 120.5 (¹ $J_{C-F} = 256$ Hz), 118.4, 21.9. HRMS (ESI): m/z 321.0856 ($M + H$)⁺ (calcd for C₁₆H₁₂F₃N₂O₂, 321.0850).

5.1.28. 2-(2-Fluorophenyl)-7-methylquinazolin-4(3H)-one (29)

Yield 356 mg (55%). White solid. Mp: 185–187 °C. IR (cm⁻¹): 3420 (NH), 3024, 1680 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.4 (s, 1H), 8.05 (d, $J = 8.0$ Hz, 1H), 7.78 (td, $J = 7.5, 1.5$ Hz, 1H), 7.65–7.60 (m, 1H), 7.54 (s, 1H), 7.41–7.36 (m, 3H), 2.48 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 161.3, 159.5 (¹ $J_{C-F} = 248$ Hz), 149.9, 148.7, 145.1, 132.8 (³ $J_{C-F} = 8.5$ Hz), 130.9, 128.4, 127.1, 125.6, 124.6 (⁴ $J_{C-F} = 3.3$ Hz), 122.3 (² $J_{C-F} = 13$ Hz), 118.6, 116.1 (² $J_{C-F} = 21.2$ Hz), 21.9. HRMS (ESI): m/z 255.0929 ($M + H$)⁺ (calcd for C₁₅H₁₂FN₂O, 255.0933).

5.1.29. 2-(3-Fluorophenyl)-7-methylquinazolin-4(3H)-one (30)

Yield 360 mg (71%). White solid. Mp: 291–293 °C. IR (cm⁻¹): 3420 (NH), 3024, 1680 (C=O). ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 12.5 (s, 1H), 8.05 (d, $J = 8.0$ Hz, 2H), 7.99 (d, $J = 8.5$ Hz, 1H), 7.62–7.57 (m, 2H), 7.78 (td, $J = 7.5, 2.5$ Hz, 1H), 7.37 (d, $J = 7.5$ Hz, 1H), 2.51 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.0 (¹ $J_{C-F} = 242.3$ Hz), 161.9, 151.0, 148.5, 145.1, 135.0 (³ $J_{C-F} = 8.5$ Hz),

130.7 ($^3J_{C-F}$ = 8.5 Hz), 128.3, 127.2, 125.7, 123.8, 118.3, 118.2, 114.4 ($^2J_{C-F}$ = 23.7 Hz), 21.3. HRMS (ESI): m/z 255.0939 ($M + H$)⁺ (calcd for C₁₅H₁₂FN₂O, 255.0933).

5.1.30. 2-(2-(Dimethylamino)phenyl)-7-methylquinazolin-4(3H)-one (31)

Yield 340 mg (61%). White solid. Mp: 214–216 °C. IR (cm⁻¹): 3414 (NH), 3069, 1664 (C=O). ¹H NMR (500 MHz, CDCl₃) δ (ppm): 12.92 (s, 1H), 8.44 (d, J = 8.0 Hz, 2H), 8.19 (d, J = 8.0 Hz, 1H); 7.60 (s, 1H); 7.49 (td, J = 7.5, 1.5 Hz, 1H), 7.31–7.26 (m, 3H); 2.79 (s, 6H), 2.52 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ (ppm): 162.0, 152.3, 149.6, 145.2, 132.3, 131.2, 128.0, 127.5, 126.2, 124.9, 124.7, 120.1, 119.2, 45.0, 21.9. HRMS (ESI): m/z 280.1442 ($M + H$)⁺ (calcd for C₁₇H₁₈N₃O, 280.1449).

5.2. Topoisomerases 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 test compounds at 37 °C for 30 min in relaxation buffer (10 mM Tris–HCl [pH 7.9], 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The 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 trans illumination with a UV light and the supercoiled DNA was quantified using Alphamager™ (Alpha Innotech Corporation).

Topo IIα inhibition by the test compounds was measured as follows. pBR322 (200 ng) and human DNA topo IIα (1 unit; Usb Corp., USA) were incubated with or without the test compounds in assay buffer (10 mM Tris–HCl [pH 7.9] containing 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 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 trans illumination with a UV light, and supercoiled DNA was quantified using Alphamager™ (Alpha Innotech Corporation).

5.3. kDNA decatenation assay

The assay was performed in a total reaction volume of 10 μL containing 50 mM Tris–HCl (pH 8.0), 120 mM KCl, 10 mM MgCl₂, 0.5 mM dithiothreitol, 0.5 mM ATP, 30 μg/mL bovine serum albumin, and 75 ng of kDNA. Compounds were then added to a final concentration of 50 or 100 μM. The final amount of DMSO added was 1%. Reactions were initiated by the addition of 2 units of topo IIα, and mixtures were incubated for 30 min at 37 °C. The reaction was terminated by the addition of 2.5 μL of stock solution (5% SDS, 25% ficoll, and 0.05% bromophenol blue) followed by treatment with 0.25 mg/mL proteinase K (Sigma, USA) at 37 °C for 30 min to eliminate the protein. Samples were resolved by electrophoresis on a 1.2% (w/v) agarose gel containing 0.5 μg/mL ethidium bromide in TAE buffer (100 mM Tris-acetate and 2 mM Na₂EDTA, pH 8.3). DNA bands were visualized by UV and photographed and documented with Alphamager™ (Alpha Innotech Corporation). For quantitation of the substrate (kDNA) and products of the topo IIα reaction (nicked, Nck; supercoiled, SC; and catenanes), the numerical values

of intensity profiles of the different lanes were obtained after background subtraction and exported to Microsoft Excel. For each lane, the overall intensity was used to obtain the fraction of intensity for the different bands (kDNA, Nck, and SC). Here, we measured topo IIα activity based on the final products (nicked plus supercoiled mini-circles). Positive control (1% DMSO, topo IIα) and negative control (1% DMSO) were used to set up the limit values (1 and 0, respectively). Ellipticine was used as a standard and the values of percentage of decatenation were averaged.

5.4. Cytotoxicity

The three cell lines used in this study (HCT-15, T47D and HeLa) were cultured according to the supplier's instructions. The cells were seeded in 96-well plates at a density of 2–4 × 10⁴ cells per well and incubated overnight in a 5% CO₂ incubator at 37 °C in 0.1 mL of media supplemented with 10% fetal bovine serum (Hyclone, USA). 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 IC₅₀ 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.

5.5. Molecular docking

The docking study was performed in Sybyl-X 2.0 (winnt_os5x) using the Surflex Dock program. The structure of topo I-DNA-topotecan was downloaded from the Protein Data Bank (PDB: 1K4T). The –SH group of the G11 nucleotide of the scissile DNA strand was changed to –OH. 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. Protomol, an idealized representation of a ligand that makes every potential interaction with the binding site, was generated on the basis of ligand mode. 2-Arylquinazolinones 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 as a reference molecule. The docking protomol could reproduce the position of topotecan (manually constructed and stored in the Sybyl database) in the binding site with 0.86 Å root-mean-square deviation (rmsd) of the heavy atoms of the extracted topotecan.

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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.2015.08.040>.

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