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Original article

Design, synthesis and systematic evaluation of cytotoxic 3-heteroarylisoquinolinamines as topoisomerases inhibitors

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ABSTRACT

A series of 3-heteroarylisoquinolinamines were designed, synthesized and evaluated for cytotoxicity, topoisomerases (topos) inhibitory activities and cell cycle inhibition. Several of the 3-heteroarylisoquinolines exhibited selective cytotoxicity against human ductal breast epithelial tumor (T47D) cells over non-cancerous human breast epithelial (MCF-10A) and human prostate cancer (DU145) cells. Most of the derivatives showed greater cytotoxicity in human colorectal adenocarcinoma (HCT-15) cells than camptothecin (CPT), etoposide and doxorubicin (DOX). Generally, 3-heteroarylisoquinolinamines displayed greater affinity for topo I than topo II. 3-Heteroarylisoquinolinamines with greater topo I inhibitory effect exhibited potent cytotoxicity. Piperazine-substituted derivative, **5b**, with potent topo I and moderate topo II activities intercalated between DNA bases and interacted with topos through H-bonds at the DNA cleavage site of a docking model. Moreover, flow cytometry indicated that cytotoxic 3-heteroarylisoquinolinamines led to accumulation of human cervical (HeLa) cancer cells in the different phases of the cell cycle before apoptosis. Taken together, 3-heteroarylisoquinolinamines possessed potent cytotoxicity with topos and cell cycle inhibitory activities.

1. Introduction

DNA topological consequences such as supercoils, knots and catenation arise during cellular processes like transcription, replication, recombination, and chromosome condensation and segregation in fast-growing and dividing cells [1–3]. These problems need to be addressed for the vitality of cells. In fact, nature has reserved a specialized class of enzymes called topoisomerases (topos) to handle DNA supercoiling and entanglements. The human genome encodes six topos [4,5]. Among them, topo I and topo II (α, β) are established as important targets for cancer treatment.

The enzymatic function of topos involves DNA strand(s) cleavage, DNA swivel/strands passage and religation of broken strand(s). DNA scission and resealing take place through reversible transesterifications between the tyrosyl unit of topos and the DNA phosphate group. During this activity, a short-lived covalent topo-DNA complex with scissile end(s) is formed and is termed 'topoisomerase cleavage complex' (topocc). The topocc can be trapped by chemical entities to inhibit the DNA resealing activity of topos. Stabilization of topocc in turn causes permanent DNA damage and triggers apoptotic cell death. Thus, these topo inhibitors convert functional topo into a fatal component of the cell. Apart from this, topo inhibitors can also interfere with functions of topos at other steps [5].

Topos are highly expressed in tumors. Increased levels of topos have been reported to be found in colorectal, prostate and breast cancers, and leukemia [6–8]. The difference in expression of topos between normal and tumor tissues makes topos inhibitors reliable chemotherapeutic agents. Topos inhibitors hinder vital activities necessary for rapidly growing and dividing cells. In addition, topos

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inhibitors that specifically freeze topocc cause DNA damage and have deleterious effects in cancer cells versus normal cells. Therefore, intensive work has been done in developing antitumor agents that target topos. Among the large number of chemical classes evaluated as topos inhibitors, 3-arylisoquinolinamine-based compounds are the most promising (Fig. 1).

6-Amino-substituted benzo[*c*]phenathridines (**1**) represent the constrained form of 3-arylisoquinolinamines. Benzo[*c*]phenanthridin-6-ylamines were basically developed as water-soluble derivatives of the natural alkaloids, fagaronine and nitidine [9]. Unlike the parent compounds, they lack the iminium charge responsible for acute toxicity and low antitumor activity in few cases. Amino-substituted nitidine (**1**) displays potent cytotoxicity and inhibitory effects towards both topo I and II. Likewise, as part of our work to develop water-soluble non-camptothecin (CPT) topo I inhibitors with 3-arylisoquinolinamine scaffold, 5-amino-substituted indeno [1,2-*c*]isoquinolines (**2**) were principally designed to preserve the essential tetracyclic planar scaffold and C11 keto group necessary for topo I inhibition and to incorporate amino alkyl tail or heterocyclic amines to improve their aqueous solubility [10,11].

In addition to the rigidified isoquinolinamines, flexible 3-arylisoquinolinamines (**3**) have been developed by our research groups as potent cytotoxic agents with topo I inhibitory activity and the appropriate physiochemical properties and pharmacokinetic profile necessary for oral drug use [12–15]. Apart from this, we have also developed 4-amino-2-phenylquinazoline (**4**) with an additional N in the isoquinoline ring (Fig. 2) [16]. Replacement of the isoquinoline ring by quinazoline is advantageous as the tricyclic compound attains planar conformation to intercalate between DNA bases. Considering this fact, it can be expected that 3-pyrrole- and 3-thiophene-substituted isoquinolinamines (**5**) may attain a flat structure and be potential topos inhibitors. Based on these rationales, we herein describe the synthesis and systematic evaluation of 3-heteroarylisoquinolinamines as a novel class of topos inhibitors.

2. Chemistry

3-Heteroarylisoquinolinamines **5** were mainly synthesized according to the synthetic pathway reported earlier [13]. Synthesis was initiated by cycloaddition of lithiated toluamides **6** and heteroaryl nitriles **7** to obtain 3-heteroarylisoquinolones **8** (Scheme 1). In the next step, isoquinolones **8** were aromatized into isoquinolinyl chlorides **9** by phosphorus oxychloride ($POCl_3$). Finally, 1-amination of imine chlorides **9** with heterocyclic (piperidine, piperazines, morpholine and homopiperazine) and aliphatic (benzyl) amines produced the desired isoquinolinamines **5a**–**e** and **5h**–**t** (Table 1 and Fig. 3). Piperidinyl, piperazinyl and morpholinyl derivatives were further treated with concentrated HCl to afford HCl salts. Treatment of **9b** with sodium azide afforded the corresponding azidoisoquinoline **5g**. Primary isoquinolinamine **5f** was obtained on heating isoquinolinyl chloride **9a** with ammonia in presence of $NaHCO_3$ and DMF (Scheme 2). Alternatively, **5f** was also obtained by coupling of lithiated tolunitrile **7c** and thiophenenitrile **7a** assisted by hexamethylphosphoramide (HMPA).

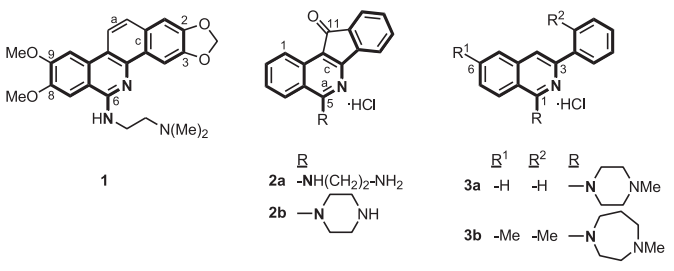


Fig. 1. 3-Arylisoquinolinamine-based topos inhibitors: 6-amino-substituted benzo[*c*] phenanthridine (**1**), 5-amino-substituted indeno[1,2-*c*]isoquinolines (**2**) and 3-arylisoquinolinamines (**3**).

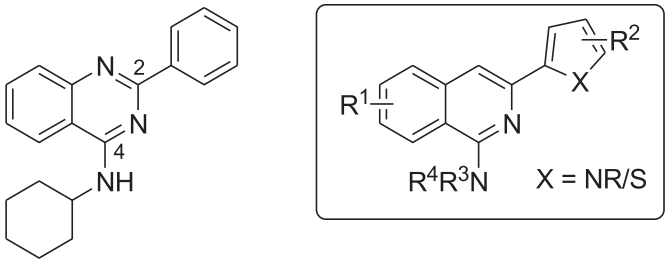


Fig. 2. 4-Amino-2-phenylquinazoline (**4**) and 3-heteroarylisoquinolinamine (**5**).

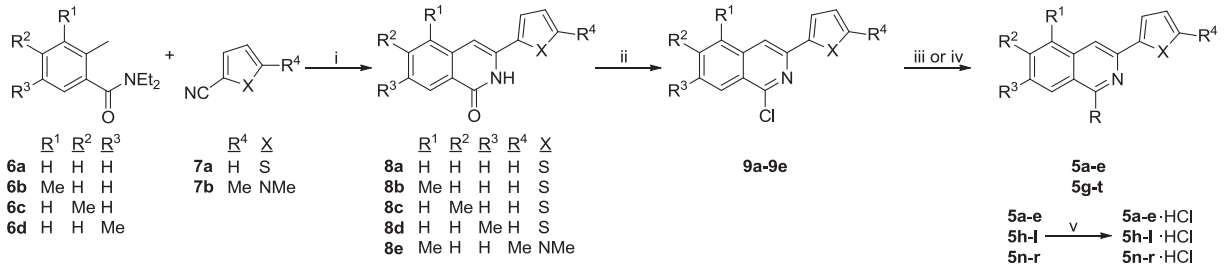
3. Results and discussion

3.1. Cytotoxicity

Cytotoxicity of 3-heteroarylisoquinolinamines (**5**) was assessed using non-cancerous (human breast epithelial, MCF-10A) and tumor (human ductal breast epithelial tumor, T47D; human prostate cancer, DU145; and human colorectal adenocarcinoma, HCT-15) cells [17]. Cytotoxicity results are summarized as IC_{50} values in Table 2, and structure–cytotoxicity relationships of heterocyclic amine-substituted isoquinolines are summarized in Table 3. Piperidine (**5a**, **5h** and **5n**), morpholine (**5e**, **5l** and **5r**), –NHBn (**5m**) and $-NH_2$ (**5f**) substituted 3-heteroarylisoquinolinamines and their corresponding HCl salts exhibited selective cytotoxicity towards tumorigenic T47D cells and non-toxic effects in normal MCF-10A cells. In contrast, doxorubicin (DOX), a chemotherapeutic agent approved as adjuvant therapy for breast cancer, was cytotoxic to both malignant and non-malignant cell populations. Beside MCF-10A, the aforementioned compounds had no effect on the viability of prostate cancer DU145 cells. Among the set of 3-heteroarylisoquinolinamines inactive towards MCF-10A and DU145, piperidine substituted analogs (**5a**, **5h**, **5n** and their HCl salts) showed most potent activity towards T47D (IC_{50}: 2.56 ± 0.12–8.03 ± 0.35 μM).

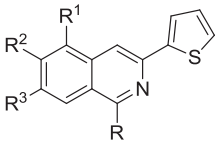
In contrast, piperazine- and homopiperazine-substituted analogs lacked remarkable selectivity for MCF-10A, T47D and DU145 cells. N4′-unsubstituted piperazinyl isoquinolines (**5b**, 5**i** and 5**o**) and their corresponding salts were more toxic to MCF-10A, T47D and DU145 cells than N4′-alkylated piperazines. Among the piperazine analogs, compounds **5p**·HCl and **5q**·HCl are worth mentioning as they were less toxic towards MCF-10A and DU145 (IC_{50} > 20 μM) and represent the most potent 3-heteroarylisoquinolinamines against T47D (IC_{50}: 1.02 ± 0.04, 2.84 ± 0.1 μM, respectively). Similarly, homopiperazine derivative **5s** was more toxic towards T47D (IC_{50}: 1.32 ± 0.02 μM) than MCF-10A and DU145.

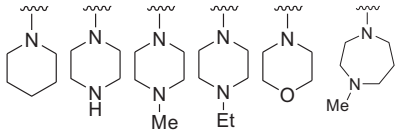
3-Heteroarylisoquinolinamines showed potent cytotoxic effects against human colorectal adenocarcinoma (HCT-15) cells. Piperidine (**5a**, **5h** and **5n**), N4′-unsubstituted piperazine (**5b**, **5i** and **5o**), N4′-methyl piperazine (**5c**, **5j** and **5p**), N4′-ethyl piperazine (**5k** and **5q**), homopiperazine (**5s**) and $-NH_2$ (**5f**) substituted 3-heteroarylisoquinolinamines or their HCl salts exhibited greater cytotoxicity than CPT, etoposide and DOX (Table 3). The low activity of the positive control drugs against HCT-15 cells is due to multiple

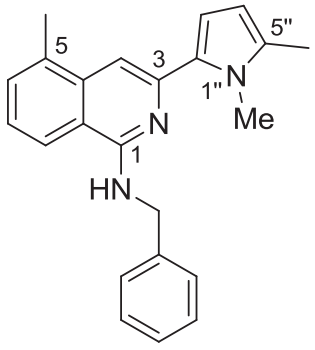


Scheme 1. Synthesis of 3-heteroarylisoquinolinamines **5**. Reagents and conditions: (i) *n*-BuLi, dry THF, −78 °C; (ii) $POCl_3$, 50 °C; (iii) Amine, K_2CO_3, DMF, 110 °C; (iv) NaN_3, DMF, 100 °C; (v) *c*-HCl, acetone.

Table 1
Synthesis of 3-thiophenylisoquinolinamines **5a**-**s**.



R^1, R^2, R^3	R								
							-NHBn	$-N_3$	$-NH_2$
H, H, H	**5a**	**5b**	**5c**	**5d**	**5e**	–	–	–	**5f**
Me, H, H	–	–	–	–	–	–	–	**5g**	–
H, Me, H	**5h**	**5i**	**5j**	**5k**	**5l**	–	**5m**	–	–
H, H, Me	**5n**	**5o**	**5p**	**5q**	**5r**	**5s**	–	–	–



5t

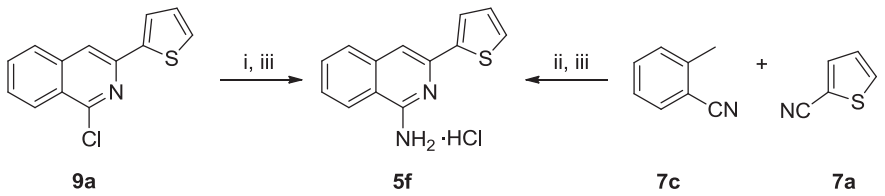
Fig. 3. 3-Pyrrolylisoquinolinamines **5t**.

drug resistance. The drug efflux membrane transporter, multidrug resistance protein 1 (MDR1; also known as p-glycoprotein, P-gp or as ATP-binding cassette sub-family B member 1, ABCB1), which is overexpressed in HCT-15 cells, reduces intracellular accumulation of CPT, etoposide and DOX by actively extruding them from the cell [4,18–20]. In addition, the fraction of the drug (DOX) absorbed into the cell is further metabolized by cytochrome P450 3A (CYP3A) [19]. Despite these hurdles, the potent cytotoxicity profile of the above-mentioned 3-heteroarylisoquinolinamines suggests that they are unlikely to be substrates of MDR1 and CYP3A. N4′-Ethyl piperazine (**5d**), morpholine (**5l** and **5r**) and benzyl (**5m**) derivatives and their HCl salts were less toxic than DOX but showed greater or similar activity to CPT and etoposide on HCT-15. Compound **5e** showed no significant cytotoxicity (IC_{50} = 21.26 ± 0.43 μM). The azide-substituted derivative **5g** is the only compound that had no effects in all of the cells used.

3.2. Topoisomerases inhibition

The topoisomerases inhibitory activities of the 3-heteroarylisoquinolinamines were determined by 'DNA relaxation assay' [17]. The topos inhibitory activities of HCl salts were generally greater than those of free amines, probably due to improved affinity of the positively charged chemical species for negatively charged DNA (Table 2, Figs. 4 and 5). Topos inhibition by 3-thiophenylisoquinolinamines showed a distinct pattern in relation to chemical structures (Table 4). The activities were mainly dependent upon the position of the methyl group in the isoquinoline ring and upon the type of heterocyclic amine at C1.

Piperidine-substituted analogs (**5a**, **5a**·HCl) were inactive against topo I while they showed moderate activity against topo II. In contrast, their 7-Me derivatives (**5n**, **5n**·HCl) showed superior activity only against topo I. Piperidine derivative **5h** and its HCl salt displayed unique topos inhibition properties. **5h** was selective against topo II, while the selectivity of its HCl salt switched to topo I. N4′-unsubstituted piperazinyl isoquinolines (**5b**·HCl and **5i**·HCl) showed superior activity against topo I and moderate to low activity against topo II. However, 7-Me derivative **5o** lacked significant activities towards both topos. N4′-methyl-substituted piperazine derivatives were inactive toward topo II. In addition to



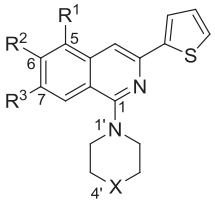
Scheme 2. Synthesis of 3-heteroarylisoquinolinamine **5f**. Reagents and conditions: (i) NH_4OH, $NaHCO_3$, DMF, 50 °C; (ii) $LiNMe_2$, HMPA, dry THF, −78 °C; (iii) *c*-HCl, acetone.

Table 2
Topos (I and II) inhibitory activities and cytotoxicity (IC_{50}) of 3-heteroarylisoquinolinamines **5** and their corresponding HCl salts.

Compound	Topo I (%) inhibition		Topo II (%) inhibition		IC_{50} (μM)			
	100 μM	20 μM	100 μM	20 μM	MCF-10A[d]	T47D[e]	DU145[f]	HCT-15[g]
Camptothecin	74.0/74.5[a]/ 62.6[b]/72.3[c]	35.5/23.8[a]/ 34.7[b]/41.7[c]			0.27 ± 0.1	0.28 ± 0.01	4.01 ± 2.45	10.07 ± 0.58
Etoposide			86.9/78.6[a]/ 87.9[b]/86.2[c]	31.1/49.9[a]/ 42.5[b]	12.23 ± 1.47	2.57 ± 0.08	13.85 ± 1.75	9.45 ± 0.28
Doxorubicin					0.93 ± 0.03	0.84 ± 0.04	4.88 ± 0.12	3.76 ± 0.12
5a	0.0	ND	48.1	1.8	>50	3.22 ± 0.05	>50	3.61 ± 0.1
5a·HCl	0.0	ND	42.9	0.5	>50	7.26 ± 0.25	>50	1.04 ± 0.06
5b	73.0	0.6	3.7	ND	44.12 ± 0.69	9.53 ± 0.44	17.6 ± 0.26	1.25 ± 0.04
5b·HCl	100.0	4.8	56.0	0.0	18.1 ± 0.46	4.51 ± 0.17	8.25 ± 0.01	5.45 ± 0.29
5c	97.5	18.2	0.0	ND	18.1 ± 0.84	15.44 ± 0.43	40.25 ± 1.26	2.44 ± 0.09
5c·HCl	80.8	76.6	0.0	ND	38.78 ± 0.22	16.08 ± 0.5	44.45 ± 0.61	7.9 ± 0.39
5d	2.6	ND	9.1	ND	49.01 ± 0.32	17.41 ± 0.42	>50	7.42 ± 0.23
5d·HCl	15.0	ND	3.2	ND	23.77 ± 0.15	8.08 ± 0.39	46.61 ± 0.08	9.29 ± 0.44
5e	0.0	ND	21.8	0.0	>50	24.41 ± 0.05	>50	21.26 ± 0.43
5e·HCl	0.0	ND	61.3	0.0	>50	14.77 ± 0.31	47.03 ± 0.82	14.74 ± 0.3
5f	0.0	ND	14.6	ND	>50	7.93 ± 0.18	>50	1.2 ± 0.06
5f·HCl	0.0	ND	22.5	0.0	>50	17.48 ± 0.57	>50	24.39 ± 1.18
5g	2.1	ND	4.8	ND	>50	>50	>50	>50
5h	0.0	ND	42.8	1.1	>50	2.56 ± 0.12	>50	5.45 ± 0.24
5h·HCl	45.9	0.0	2.9	ND	>50	8.03 ± 0.35	>50	1.88 ± 0.08
5i	59.0	0.0	14.8	ND	7.25 ± 0.02	8.74 ± 0.29	8.31 ± 0.17	1.31 ± 0.06
5i·HCl	59.6	0.0	40.9	0.0	8.18 ± 0.05	7.57 ± 0.22	11.7 ± 0.27	0.94 ± 0.04
5j	11.2	ND	0.0	ND	13.29 ± 0.37	9.35 ± 0.08	32.97 ± 0.1	4.37 ± 0.23
5j·HCl	5.1	ND	0.0	ND	14.91 ± 0.2	18.38 ± 0.27	39.98 ± 0.36	3.19 ± 0.14
5k	0.0	ND	0.0	ND	33.94 ± 0.02	8.45 ± 0.22	17.21 ± 0.03	2.41 ± 0.14
5k·HCl	0.0	ND	0.0	ND	28.85 ± 0.25	17.74 ± 0.19	22.11 ± 0.27	4.4 ± 0.25
5l	0.0	ND	11.3	ND	>50	6.76 ± 0.34	>50	7.58 ± 0.35
5l·HCl	0.0	ND	66.3	1.7	48.7 ± 0.38	7.88 ± 0.14	>50	7.79 ± 0.39
5m	0.0	ND	68.9	1.1	>50	6.57 ± 0.35	>50	8.62 ± 0.14
5n	89.6	0.0	13.7	ND	>50	7.38 ± 0.25	>50	3.74 ± 0.18
5n·HCl	77.5	0.0	3.6	ND	>50	3.02 ± 0.05	>50	0.89 ± 0.07
5o	10.7	0.0	22.1	ND	5.54 ± 0.17	1.14 ± 0.06	4.15 ± 0.08	1.29 ± 0.05
5p	29.6	ND	0.0	ND	11.58 ± 0.21	1.96 ± 0.06	16.25 ± 0.56	1.64 ± 0.02
5p·HCl	35.9	0.0	0.0	ND	20.87 ± 0.02	1.02 ± 0.04	24.7 ± 0.17	1.04 ± 0.02
5q	22.8	ND	0.0	ND	43.14 ± 0.13	4.15 ± 0.14	29.4 ± 0.14	3.63 ± 0.13
5q·HCl	59.5	6.5	0.0	ND	42.9 ± 0.06	2.84 ± 0.1	31.07 ± 0.02	7.71 ± 0.26
5r	77.9	1.1	0.0	ND	35.62 ± 0.47	6.38 ± 0.32	47.32 ± 0.13	7.58 ± 0.2
5r·HCl	71.9	3.7	5.1	ND	49.06 ± 1.38	7.63 ± 0.26	48.27 ± 0.11	9.52 ± 0.44
5s	18.3	ND	8.0	ND	20.59 ± 0.36	1.32 ± 0.02	14.69 ± 0.6	1.5 ± 0.05

Each data represents mean ± S.D. from three different experiments performed in triplicate.
ND: Not determined.
[a] Values for compound **5g**.
[b] Values for compounds **5h**–**m** and their corresponding HCl salts.
[c] Values for compounds **5n**–**s** and their corresponding HCl salts.
[d] MCF-10A: non-cancerous human breast epithelial cells.
[e] T47D: human ductal breast epithelial tumor cells.
[f] DU145: human prostate cancer cells.
[g] HCT-15: human colorectal adenocarcinoma cells.

Table 3
Structure–cytotoxicity relationships of piperidine-, piperazine- and morpholine-substituted 3-thiophenylisoquinolinamines.



X	MCF-10A	T47D	DU145	HCT-15
CH_2	✗[a]	✓[b]	✗	<DOX[c]
NH, NMe, NEt	✓	✓	✓	<DOX
O	✗	✓	✗	>DOX[d]

[a] IC_{50} > 50 μM.
[b] IC_{50} < 50 μM.
[c] IC_{50} < 3.76±0.12 μM (IC_{50} of doxorubicin).
[d] IC_{50} > 3.76±0.12 μM (IC_{50} of doxorubicin).

topo II, 6-Me and 7-Me derivatives of this subset were also inactive or had minimal effect on topo I. However, **5c**·HCl exhibited greater topo I inhibitory activity at both 100 and 20 μM. Similar to N4′-methyl-substituted piperazines, N4′-ethyl-substituted piperazines were also inactive against topo II. Moreover, **5d**, **5k** and their HCl salts had minimal or no effect on topo I. 7-Me analog **5q** showed minimal topo I activity whereas **5q**·HCl showed similar activity to CPT at 100 μM. Morpholine derivatives (**5e**·HCl and **5l**·HCl) were inactive against topo I but exhibited moderate activity against topo II. 7-Me analogs **5r** and **5r**·HCl showed superior and similar activity to CPT against topo I, respectively. Benzylamine-substituted derivative (**5m**) showed moderate activity only against topo II. Homopiperazine (**5s**), azide (**5g**) and $-NH_2$ (**5f**) derivatives had no significant activities against both topos.

3-Heteroarylisoquinolinamines like **5b**, **5c**, **5i**, **5n**, **5q**, **5r** and their HCl salts with topo I inhibitory activity similar to or greater than CPT showed potent cytotoxicity, indicating that topo I is major target for such antitumor agents. Moderate topo I or II activities but

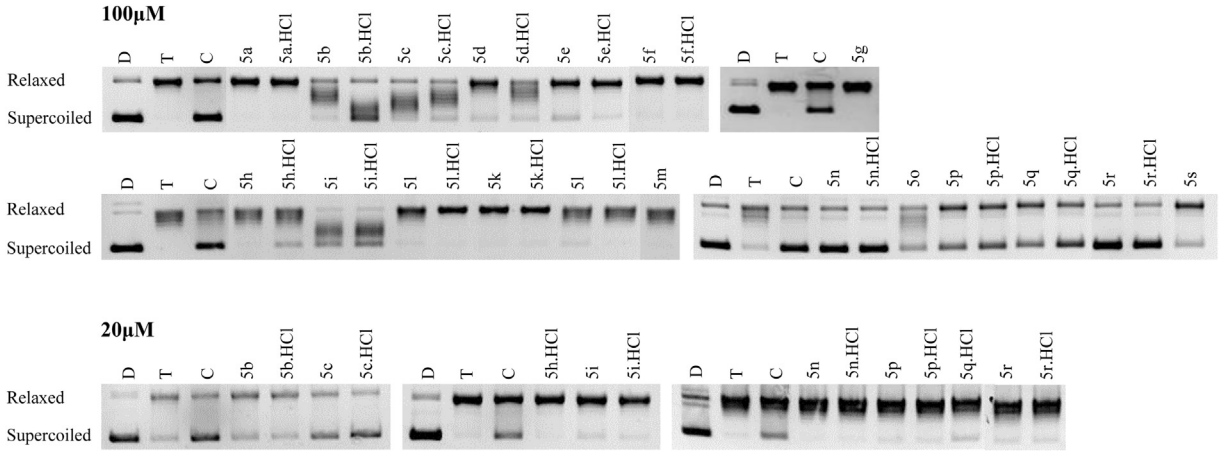


Fig. 4. Topoisomerase I inhibitory activity of 3-heteroarylisoquinolinamines **5** and their corresponding HCl salts. Lane D: pBR322 only, lane T: pBR322 + topo I, lane C: pBR322 + topo I + CPT, remaining lanes: pBR322 + topo I + 3-heteroarylisoquinolinamines and their corresponding salts.

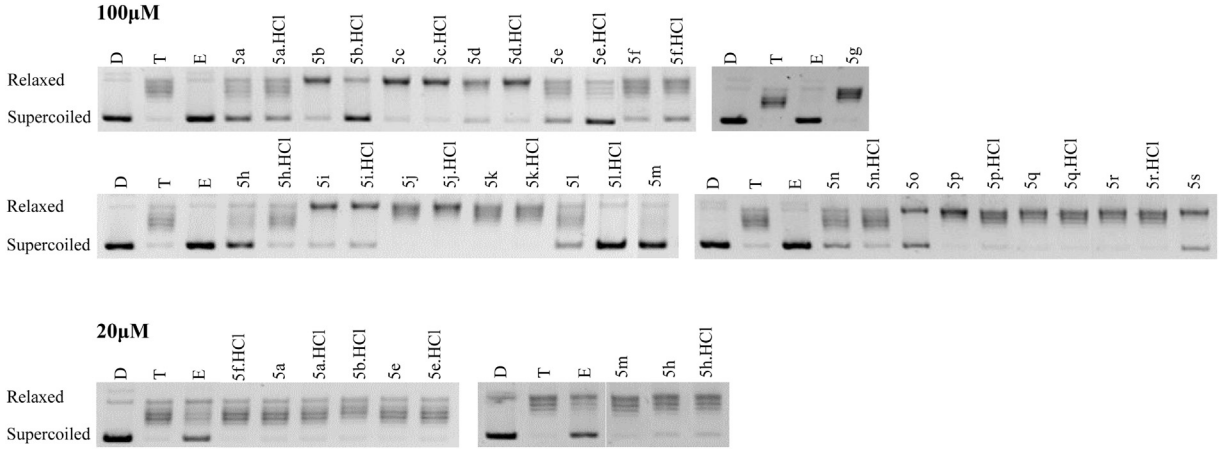
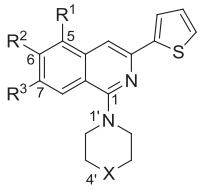


Fig. 5. Topoisomerase II inhibitory activity of 3-heteroarylisoquinolinamines **5** and their corresponding HCl salts. Lane D: pBR322 only, lane T: pBR322 + topo II, lane E: pBR322 + topo II + etoposide, remaining lanes: pBR322 + topo II + 3-heteroarylisoquinolinamines and their corresponding salts.

Table 4
Structure and topoisomerases inhibition relationships of piperidine, piperazine and morpholine-substituted 3-thiophenylisoquinolinamines.



R^1, R^2, R^3	X	Topo I[a]	Topo II[b]
H, H, H	CH_2	0	++
H, H, Me	CH_2	++++	0
H, H/Me, H	NH	+++/++++	+/++
H, H, Me	NH	0	0
H, H/Me, H/Me	NMe	0 - ++++	0
H, H/Me, H/Me	NEt	0 - +++	0
H, H/Me, H	O	0	++
H, H, Me	O	++++	0

[a, b] Semi quantitative representation of topos inhibition. The ratios of topos inhibition by 100 μM of the individual compounds to CPT and etoposide are assigned as follows: 0.0–0.25, 0 (inactive); 0.25–0.5, + (low activity); 0.5–0.8, ++ (moderate activity); 0.8–1.0, +++ (similar activity as CPT or etoposide); >1.0, ++++ (greater activity than CPT or etoposide).

strong cytotoxic effects of compounds such as **5a**, **5h**·HCl, **5l**·HCl and **5m** indicate that targets other than topos may also be responsible for cell death. On the other hand, compounds such as **5d**, **5f**, **5j**, **5k**, **5o**, **5p**, **5s** and their HCl salts are likely to exert toxic effects on cells through different mechanisms. Moreover, low cytotoxic effects of **5e**·HCl despite moderate topo II inhibitory activity may be due to the physiochemical properties of the compound that prevent cell penetration or cause wide distribution within cells, or may be due to cellular metabolic factors that degrade the compound before reaching the designated target.

3.3. Molecular docking

Hypothetical binding models of DNA-topo I/II complexes and 3-heteroarylisoquinolinamines (**5b** and **5b**·HCl) exhibiting potent topo I and moderate topo II inhibition were constructed by molecular docking tools to understand the possible interactions responsible for topos inhibition. The Surflex-Dock program available in *Sybyl-X 2.0 (winnt_os5x)* was used to dock the selected 3-heteroarylisoquinolinamines into the pockets formed after removal of ligands from the 3D crystal structures of topotecan-DNA-topo I (PDB: 1K4T) [21] and amsacrine-DNA-topo IIβ (PDB: 4G0U) [22] ternary complexes. The **5b**-DNA-topo I docking model shows that the adenine base (A113) of the non-scissile DNA strand

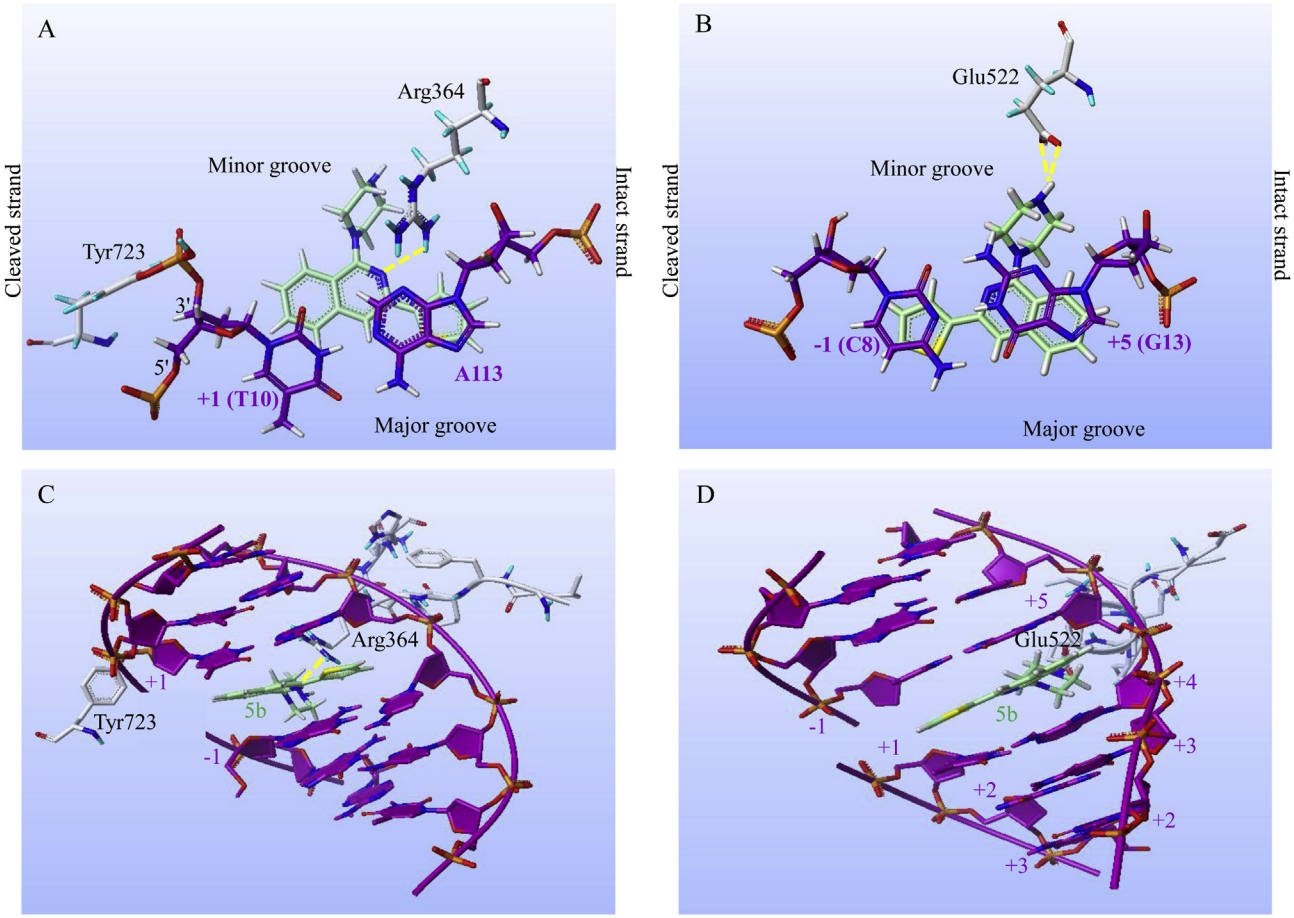


Fig. 6. Hypothetical binding models of 3-heteroaylisoquinolinamine **5b** with (A and C) DNA-topo I and (B and D) DNA-topo IIβ complexes. Carbon units are light green in **5b**, purple in nucleotides and gray in amino acids. H-bonds are illustrated as yellow discontinuous lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

overlapped the thiophene ring of **5b** and indicates possible π–π stacking interactions between the aromatic rings (Fig. 6A, C). In addition, the isoquinoline N of **5b** was associated with Arg364 of topo I by H-bond.

Intercalation of a chemical species between DNA base pairs at the cleavage site is a major factor that inhibits topos function [21]. This topos inhibitor displaces the reactive –OH of the downstream (−1) base and prevents transesterification with a phosphotyrosine unit of the upstream base (+1) to join the cleaved DNA stand and release topos. The stacking interactions between the polycyclic topos inhibitors and DNA bases are governed by multiple factors as dispersion forces, electrostatic attraction and charge transfer interactions. The stacking interaction energies calculated by an *ab initio* quantum mechanics (at the MP2/6-31g(d) level of theory) has been reported to highly correlate with actual binding orientation of CPT at the cleavage site [23]. Thus, the *ab initio* quantum mechanics calculation can be a valuable tool for validation of hypothetical molecular models of chemical species (e.g. **5b**) generated by docking studies. Besides intercalation, the H-bond interaction with Arg364 amino acid of topo I may be essential for topo I inhibitory activity as seen in CPT, indenoisoquinoline (MJ238) and indolocarbazole (SA315F) [24]. The importance of contact with Arg364 is further supported by decreased affinity of CPT and indolocarbazole toward topo I with Arg364His mutation. Considering these facts, the docking model of **5b** with DNA-topo I complex supports its potent topo I inhibitory activity.

The docking model of **5b** with DNA-topo IIβ complex shows that the compound was bound to DNA and topo IIβ by both π–π stacking and H-bond interactions. The aromatic rings of **5b** attained planar conformation and intercalated between DNA bases, parallel to the plane of the bases, at the cleavage site (Fig. 6B, D). The piperazine moiety protruded into the minor groove and was associated with Glu522 by H-bond. Identical intercalative and H-bond associations with DNA and topo IIβ have been displayed by the anticancer drugs amsacrine and mitoxantrone, and the anticancer agent ametantrone [22]. The similarity in binding modes of **5b** with well-known topo IIβ inhibitors proves the topo II inhibitory activity of **5b**·HCl.

3.4. Cell cycle inhibition

To gain better understanding of the preliminary mechanism of cytotoxicity of 3-heteroarylisoquinolinamines, the interference of cell cycle progression by the compounds was studied. The N4′-unsubstituted piperazinyl isoquinolines (**5b**, **5b**·HCl, **5i**, **5i**·HCl and **5o**), N4′-ethyl-substituted piperazines (**5d**, **5d**·HCl and **5k**) and primary isoquinolinamines (**5f** and its corresponding HCl salt) were selected for cell cycle analysis on the basis of their toxic effects in human breast adenocarcinoma (MCF-7), human gastric cancer (MKN-28), human lung adenocarcinoma epithelial (A549), human cervical cancer (HeLa) and mouse melanoma (B16) cells (Fig. 7). The cell viability of at least 2 types of cancer cells due to the selected analogs was below 50%.

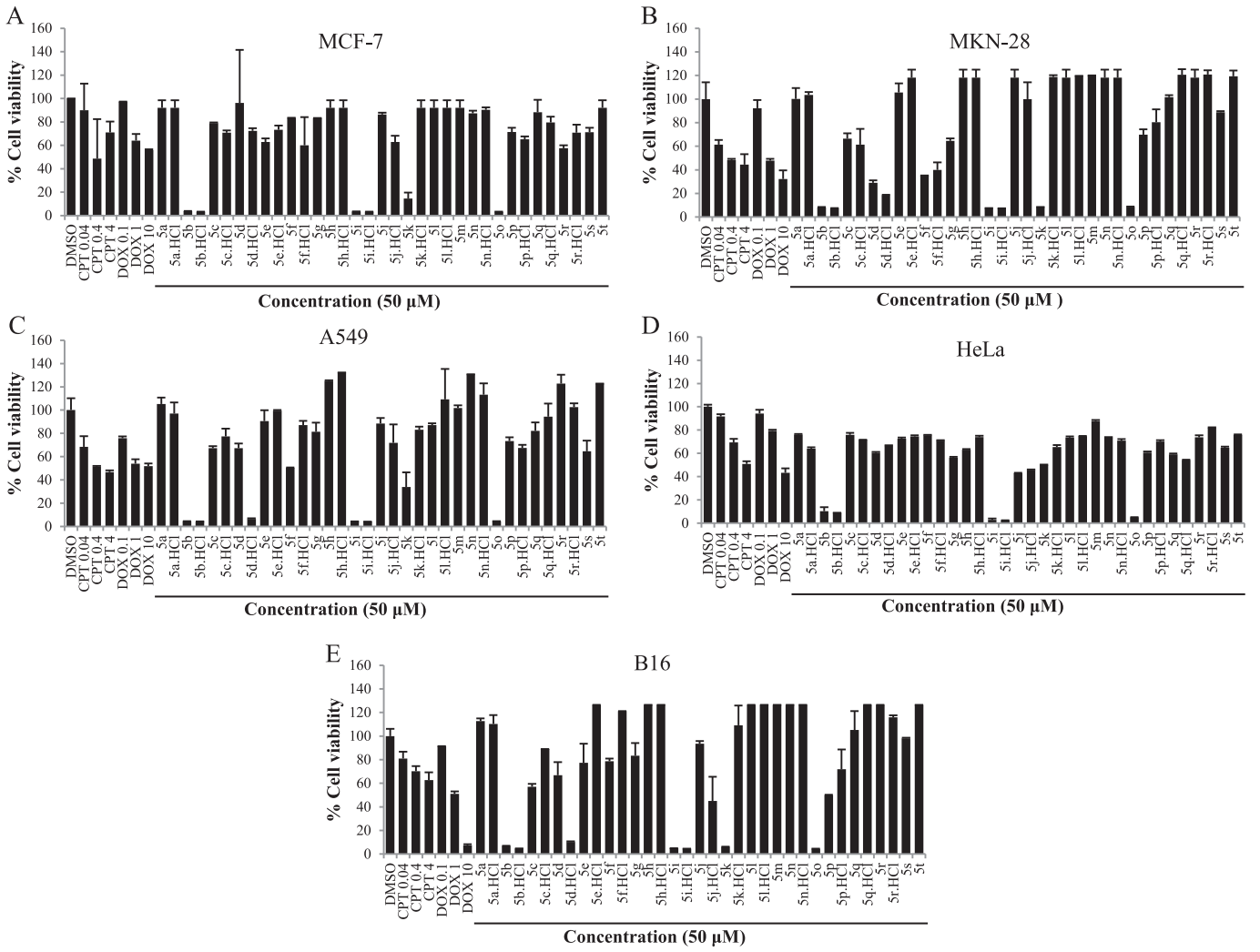


Fig. 7. Effect of 3-heteroarylisoquinolinamines on the viability of (**A**) human breast adenocarcinoma (MCF-7), (**B**) human gastric cancer (MKN-28), (**C**) human lung adenocarcinoma epithelial (A549), human cervical cancer (HeLa) and (**D**) mouse melanoma (B16) cells. Cell viability was determined after 48 h by MTT assay. CPT was used as a positive control at 0.04, 0.4 and 4 μM. Similarly, DOX was also used as a positive control at 0.1, 1 and 10 μM. Each column is the mean percent of viable cells from two independent experiments.

The effects of different concentrations of the selected derivatives on the cell cycle of HeLa cells were investigated by determining the cell population in each phase of the cell cycle by flow cytometry. The number of cells in the G0/G1 phase increased by 2, 4 and 10% upon treatment with compound **5b** at 5, 10 and 25 μM, respectively (Fig. 8). However, the cells underwent apoptosis upon increasing the concentration to 50 μM. Likewise, the HCl salt of **5b** led to HeLa cell accumulation in G0/G1 phase. The HCl salt was more effective than the free base **5b** to inhibit cell cycle progression and to induce apoptosis. 10% rise in the number of G0/G1 phase cells was attained at 10 μM and 85% of sub-G0/G1 phase cells (apoptotic debris) piled up at 25 μM. Similarly, N4′-unsubstituted piperazines (**5i**, **5i**·HCl and **5o**) slightly accumulated the cells in G0/G1 phase before apoptosis (Fig. S1). N4′-ethyl piperazine derivative **5d** showed no definite pattern of cell cycle inhibition, whereas its HCl salt and **5k** induced accumulation in S phase at 25 μM and lower concentrations.

In contrast to piperazines, the primary isoquinolinamine **5f**, which lacked topos inhibitory activities, arrested the cells in G2/M phase. The number of cells in G2/M phase increased significantly by 43% at 50 μM. The amount of cell accumulation at G2/M phase is concentration-dependent and is largely at the expense of G0/G1 phase. Accordingly, apoptotic cells also increased in a dose-dependent manner. Similar observations have previously been reported for structurally alike 6,7-dimethoxy-3-(3-methoxyphenyl) isoquinolin-1-aime (**10**, CWJ-082), which cause α-tubulin polymerization, induce mitotic arrest and subsequent caspase-dependent apoptosis (Fig. 9) [25]. These findings further suggest that the cytotoxic effects of primary isoquinolinamine **5f** are principally related to targets other than topos. **5f**·HCl (50 μM) also inhibited cell cycle progression at G2/M phase with a 6% rise in cell number compared to the control.

4. Conclusion

3-Heteroarylisoquinolinamines were designed and synthesized under the presumption that heteroaromatic rings retain the planar structure necessary for expressing topos inhibitory activities. The cytotoxic effect of 3-heteroarylisoquinolinamines was highly dependent upon the type of amine substituted at C1. Piperidine, morpholine and benzyl amines-substituted derivatives were cytotoxic to T47D tumor cells, while they were ineffective towards non-cancerous breast epithelial MCF-10A and cancerous prostate DU145 cells. Generally, 3-heteroarylisoquinolinamines showed superior

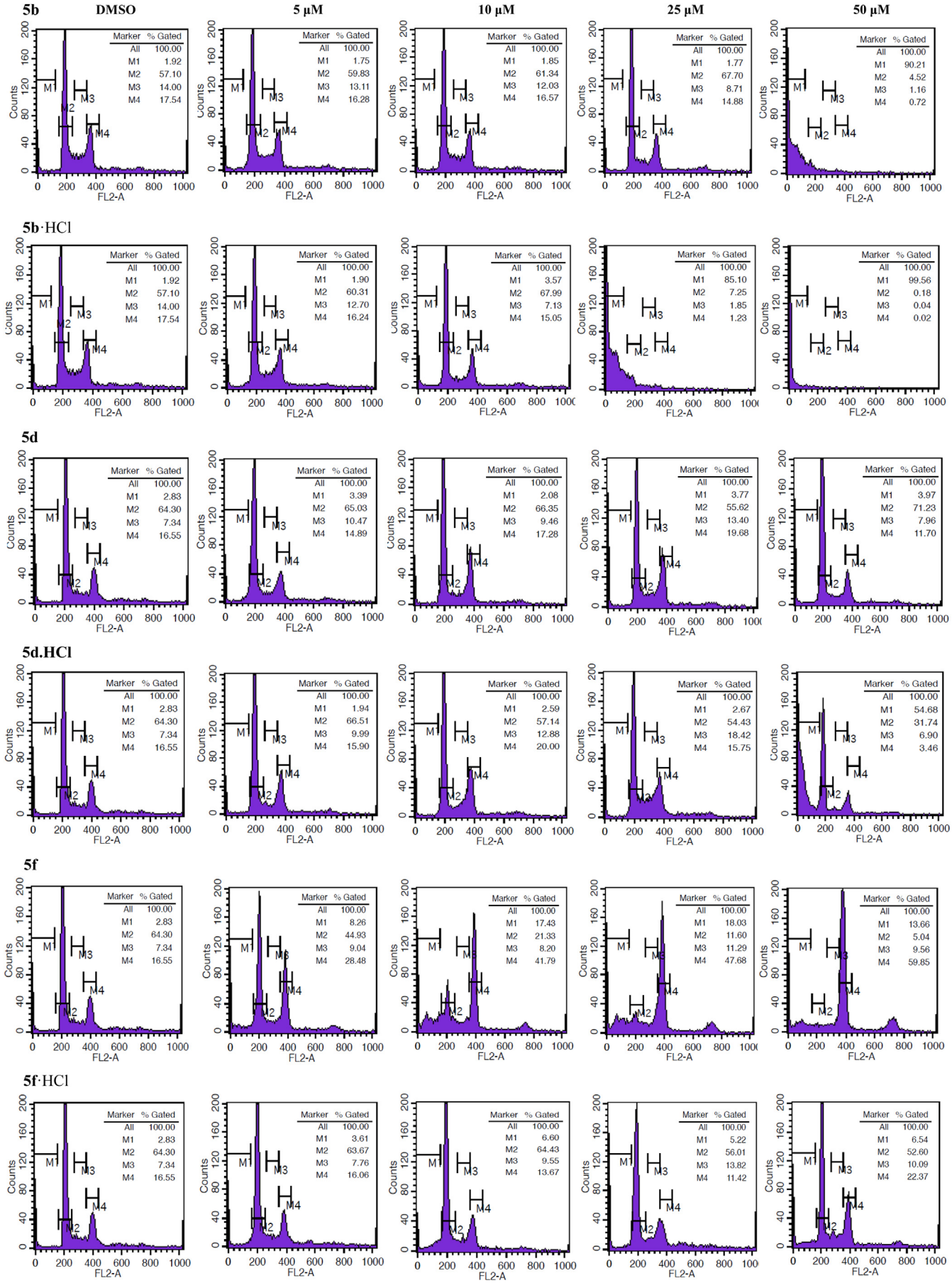


Fig. 8. Effects of 3-heteroarylisoquinolinamines (**5b**, **5b**·HCl, **5d**, **5d**·HCl **5f** and **5f**·HCl) on cell cycle progression and apoptosis in human cervical cancer (HeLa) cells. M1, apoptotic cells (sub-G0/G1); M2, G0/G1 phase cells; M3, S phase cells; M4, G2/M phase cells.

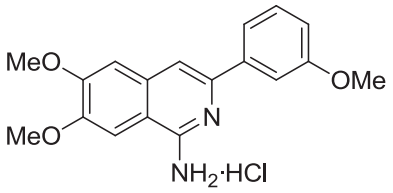


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Fig. 9. 6,7-Dimethoxy-3-(3-methoxyphenyl)isoquinolin-1-aime (**10**, CWJ-082).

cytotoxicity against human colorectal adenocarcinoma HCT-15 cells compared with the established anticancer drugs used as positive control. These observations suggest that 3-heteroarylisoquinolinamines can be developed into oral drugs for treatment of breast cancer. Topos inhibitory activities were also largely dependent upon the position of the methyl group and the type of amine substituted to the isoquinoline ring. HCl salts showed greater topos inhibition than free bases. 3-Heteroarylisoquinolinamines were more effective toward topo I inhibition than topo II. Hypothetical binding mode of compound **5b** with topo I/II-DNA showed that the aromatic ring(s) intercalated between DNA bases and associated with Arg364 and Glu522 amino acids of topo I and II, respectively, through H-bonds. Cell cycle inhibition experiments showed that 3-heteroarylisoquinolinamines arrested cell cycle and caused apoptotic cell death. All these findings highlight that 3-heteroaylisoquinolinamines might be developed into new therapeutic agents to fight cancer.

5. Experimental section

5.1. Chemistry

Melting points were determined by the capillary method with a MEL-TEMP® capillary melting point apparatus and were uncorrected. IR spectra were obtained on a JASCO FT/IR 300E Fourier transform infrared spectrometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian Unity Plus 300 MHz and Varian Unity Inova 500 MHz spectrometers at the Korea Basic Science Institute. The chemical shifts are reported in parts per million (ppm) downfield to TMS ($\delta = 0$). The coupling constants J are given in Hertz. The data are reported in the following order: chemical shift, multiplicity, coupling constant, and number of protons. Multiplicity of proton signals are reported as s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, dd: double doublet. Mass spectra were obtained on Shimadzu LCMS-2010 EV liquid chromatograph mass spectrometer using the electron spray ionization (ESI) method. High resolution mass spectrum was obtained on Waters Synapt HDMS mass spectrometer. Elemental analyses were performed using Thermo Fischer Flash 2000 elemental analyzer and the values are within ±0.4% of the theoretical values. Column chromatography was performed with Merck silica gel 60 (70–230 mesh). 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; THF was distilled from sodium/benzophenone. All reactions were conducted under an atmosphere of nitrogen in oven-dried glassware with magnetic stirring.

5.1.1. 3-Thiophen-2-yl-2H-isoquinolin-1-one (*8a*)

A solution of *N,N*-diethyl-2-methyl-benzamide **6a** (955 mg, 5 mmol) and thiophene-2-carbonitrile **7a** (681 mg g, 6.25 mmol) in dry THF (5 mL) was added drop-wise to a mixture of *n*-butyllithium (7.5 mL of 1.6 M in hexane, 12 mmol) in dry THF (20 mL) at −78 °C. The reaction mixture was then stirred at the same temperature for 6 h. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography with *n*-hexane-ethyl acetate (2:1) to afford compound **8a** as yellow solid (400 mg, 35%). Mp: 198–200 °C. IR (cm^{-1}): 3451 (N–H), 1647 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 11.16 (s, 1H, NH), 8.42 (d, $J = 8.1$ Hz, 1H, 8-H), 7.86 (d, $J = 3.6$ Hz, 1H, Ar), 7.66 (t, $J = 6.9$ Hz, 1H, Ar), 7.56 (d, $J = 7.5$ Hz, 1H, Ar), 7.48 (t, $J = 6.9$ Hz, 1H, Ar), 7.39 (d, $J = 5.1$ Hz, 1H, Ar), 7.19 (t, $J = 5.1$ Hz, 1H, Ar), 6.82 (s, 1H, 4-H).

5.1.2. 5-Methyl-3-thiophen-2-yl-2H-isoquinolin-1-one (*8b*)

The procedure described for **8a** was used with *N,N*-diethyl-2,3-dimethyl-benzamide **6b** (2.05 g, 10 mmol), thiophene-2-carbonitirle **7a** (1.36 g, 12.5 mmol), and *n*-butyllithium (9.6 mL of 2.5 M in hexane, 24 mmol) in dry THF to afford compound **8b** (1.3 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 11.85 (s, 1H, NH), 8.28 (d, $J = 7.8$ Hz, 1H, 8-H), 7.98 (d, $J = 2.7$ Hz, 1H, Ar), 7.48–7.46 (m, 1H, Ar), 7.38–7.32 (m, 2H, Ar), 7.20–7.17 (m, 1H, Ar), 6.89 (s, 1H, 4-H), 2.56 (s, 1H, 5-CH_3).

5.1.3. 6-Methyl-3-thiophen-2-yl-2H-isoquinolin-1-one (*8c*)

The procedure described for compound **8a** was used with *N,N*-diethyl-2,4-dimethyl-benzamide **1e** (4.52 g, 22 mmol), thiophene-2-carbonitrile **7a** (3 g, 27.5 mmol) and *n*-butyllithium (33 mL of 1.6 M in hexane, 52.9 mmol) in dry THF (50 mL) to afford compound **8c** as yellow solid (2.14 g, 40%). Mp: 224–225 °C. IR (cm^{-1}): 3434 (N–H), 1652 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.8 (s, 1H, NH), 8.30 (d, $J = 8.1$ Hz, 1H, 8-H), 7.80–7.79 (m, 1H, Ar), 7.39–7.35 (m, 2H, Ar), 7.30 (d, $J = 8.1$ Hz, 1H, Ar), 7.19–7.16 (m, 1H, Ar), 6.75 (s, 1H, 4-H), 2.49 (s, 3H, 6-CH_3).

5.1.4. 7-Methyl-3-thiophen-2-yl-2H-isoquinolin-1-one (*8d*)

The procedure described for compound **8a** was used with *N,N*-diethyl-2,5-dimethyl-benzamide **6d** (5.27 g, 25.7 mmol), thiophene-2-carbonitrile **7a** (3.5 g, 32.1 mmol) and *n*-butyllithium (39 mL of 1.6 M in hexane, 61.7 mmol) in dry THF (50 mL) to afford compound **8d** as yellow solid (3.18 g, 50%). Mp: 219–222 °C. IR (cm^{-1}): 3439 (N–H), 1639 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.78 (s, 1H, NH), 8.21 (s, 1H, 8-H), 7.77–7.75 (m, 1H, Ar), 7.51–7.45 (m, 2H, Ar), 7.37 (dd, $J = 5.1$, 1.2 Hz, 1H, Ar), 7.19–7.16 (m, 1H, Ar), 6.79 (s, 1H, 4-H), 2.50 (s, 3H, 7-CH_3).

5.1.5. 3-(1,5-Dimethyl-1H-pyrrol-2-yl)-5-methyl-2H-isoquinolin-1-one (*8e*)

The procedure described for compound **8a** was used with *N,N*-diethyl-2,3-dimethyl-benzamide **6b** (1.02 g, 4.99 mmol), 1,5-dimethyl-1H-pyrrole-2-carbonitrile **7b** (750 mg, 6.24 mmol) and *n*-butyllithium (5 mL of 2.5 M in hexane, 12 mmol) in dry THF to afford compound **8e** (1 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.12 (s, 1H, NH), 8.24 (d, $J = 8.1$ Hz, 1H, 8-H), 7.47 (d, $J = 6.9$ Hz, 1H, 6-H), 7.32 (t, $J = 7.8$ Hz, 1H, 7-H), 6.58 (s, 1H, 4-H), 6.45 (d, $J = 3.6$ Hz, 1H, 3″-H), 6.02 (dd, $J = 3.6$, 0.6 Hz, 1H, 4″-H), 3.66 (s, 3H, NCH_3), 2.53 (s, 3H, 5-CH_3), 2.32 (s, 3H, 5″-CH_3).

5.1.6. 1-Chloro-3-thiophen-2-yl-isoquinoline (*9a*)

Compound **8a** (300 mg, 1.32 mmol) and $POCl_3$ (10 mL) were stirred at 50 °C overnight. Excess $POCl_3$ and volatile byproducts were removed by vacuum distillation. The residue was then carefully treated with ice-water, neutralized with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*.

The crude product was purified by column chromatography with *n*-hexane-ethyl acetate to afford **9a** as yellow solid (213 mg, 66%). Mp: 106–107 °C. IR (cm^{-1}): 3030 (Ar–-H). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.27 (d, $J = 8.4$ Hz, 1H, Ar), 7.83–7.78 (m, 2H, Ar), 7.71–7.66 (m, 2H, Ar), 7.61–7.55 (m, 1H, Ar), 7.40–7.38 (m, 1H, Ar), 7.14–7.11 (m, 1H, Ar).

*5.1.7. 1-Chloro-5-methyl-3-thiophen-2-yl-isoquinoline (**9b**)*

The procedure described for **9a** was used with compound **8b** (1.1 g, 4.55 mmol) and $POCl_3$ to afford compound **9b** (800 mg, 67%). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.99 (d, $J = 9.0$ Hz, 1H, Ar), 7.78 (s, 1H, 4-H), 7.62 (d, $J = 3.7$ Hz, 1H, Ar), 7.40–7.32 (m, 3H, Ar), 7.07 (dd, $J = 4.9$, 3.7 Hz, 1H, Ar), 2.57 (s, 3H, 5-CH_3).

*5.1.8. 1-Chloro-6-methyl-3-thiophen-2-yl-isoquinoline (**9c**)*

The procedure described for compound **9a** was used with compound **8c** (2 g, 8.3 mmol) and $POCl_3$ (20 mL) to afford compound **9c** as yellow solid (1.9 g, 88%). Mp: 88–89 °C. IR (cm^{-1}): 3023 (Ar–-H). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.14 (d, $J = 8.7$ Hz, 1H, Ar), 7.75 (s, 1H, Ar), 7.68 (dd, $J = 4.8$, 1.2 Hz, 1H, Ar), 7.55 (s, 1H, Ar), 7.42–7.37 (m, 2H, Ar), 7.14–7.11 (m, 1H, Ar), 2.53 (s, 3H, 6-CH_3).

*5.1.9. 1-Chloro-7-methyl-3-thiophen-2-yl-isoquinoline (**9d**)*

The procedure described for compound **9a** was used with compound **8d** (2 g, 8.3 mmol) and $POCl_3$ (20 mL) to afford compound **9d** as yellow solid (1.95 g, 85%). Mp: 90–92 °C. IR (cm^{-1}): 3048 (Ar–-H). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): ^1H NMR (300 MHz, $CDCl_3$) δ: 8.02–8.01 (m, 1H, Ar), 7.79 (s, 1H, Ar), 7.70–7.65 (m, 2H, Ar), 7.51 (dd, $J = 9.4$, 1.6 Hz, 1H, Ar), 7.37 (dd, $J = 5.0$, 1.1 Hz, 1H, Ar), 7.13–7.10 (m, 1H, Ar), 2.54 (s, 3H, 7-CH_3).

*5.1.10. 1-Chloro-3-(1,5-dimethyl-1H-pyrrol-2-yl)-5-methyl-isoquinoline (**9e**)*

The procedure described for compound **9a** was used with compound **8e** (1 g, 3.96 mmol) and $POCl_3$ to afford compound **9e** (600 mg, 55%). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.08 (d, $J = 8.0$ Hz, 1H, Ar), 7.79 (s, 1H, 4-H), 7.45–7.36 (m, 2H, Ar), 6.51 (d, $J = 3.6$ Hz, 1H, 3″-H), 5.98 (dd, $J = 3.5$, 0.6 Hz, 1H, 4″-H), 3.84 (s, 3H, NCH_3), 2.61 (s, 3H, 5-CH_3), 2.29 (s, 3H, 5″-CH_3).

*5.1.11. 1-Piperidin-1-yl-3-thiophen-2-yl-isoquinoline (**5a**)*

A mixture of compound **9a** (100 mg, 0.4 mmol), piperidine (52 mg, 0.6 mmol) and potassium carbonate (283 g, 2.05 mmol) in DMF was refluxed overnight. The reaction mixture was cooled to room temperature, diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography with CH_2Cl_2–MeOH (15:1) to afford compound **5a** as dark green solid (95 mg, 79%). The free base was dissolved in acetone and then carefully treated with concentrated HCl dropwise. The solid obtained was filtered off and dried to obtain **5a**·HCl salt (light brown solid). Mp: 84–85 °C. IR (cm^{-1}): 3071 (Ar–-H), 2927, 2846. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.98 (d, $J = 8.2$ Hz, 1H, Ar), 7.69–7.66 (m, 1H, Ar), 7.62 (dd, $J = 3.6$, 1.1 Hz, 1H, Ar), 7.56–7.48 (m, 2H, Ar), 7.40–7.35 (m, 1H, Ar), 7.30 (dd, $J = 1.1$, 5.1 Hz, 1H, Ar), 7.09–7.06 (m, 1H, Ar), 3.46–3.42 (m, 4H, $N(CH_2)_2$), 1.86–1.79 (m, 4H, 3′-CH_2, 5′-CH_2), 1.77–1.65 (m, 2H, 4′-CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 161.5 (1-C), 146.3 (3-C), 143.9 (2″-C), 138.9 (4a-C), 129.6 (6-C), 127.8, 127.2, 126.2, 125.8, 125.2, 123.2, 120.8 (8a-C), 108.6 (4-C), 52.4 (2′-C, 6′-C), 26.1 (3′-C, 5′-C), 24.9 (4′-C). ^{13}C NMR (**5a**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 160.2 (1-C), 145.0 (3-C), 142.9 (2″-C), 138.6 (4a-C), 130.5 (6-C), 128.4, 127.5, 127.3, 126.1, 125.5, 124.0, 119.8 (8a-C), 108.9 (4-C), 52.2 (2′-C, 6′-C), 25.4 (3′-C, 5′-C), 24.2 (4′-C). MS (ESI) *m/z* 295 $(M+H)^+$. Anal. Calcd. for $C_{18}H_{18}N_2S \cdot 0.1C_4H_8O_2$: C, 72.88; H, 6.25; N, 9.24; S, 10.57. Found C, 73.29; H, 6.09; N, 9.40; S, 10.17.

*5.1.12. 1-Piperazin-1-yl-3-thiophen-2-yl-isoquinoline (**5b**)*

The procedure described for compound **5a** was used with compound **9a** (270 mg, 1.1 mmol), piperazine (142 mg, 1.6 mmol) and K_2CO_3 (761 mg, 5.5 mmol) in DMF to afford compound **5b** as ivory solid (236 mg, 72%). The free base was converted to **5b**·HCl salt (brown solid). Mp: 152–154 °C. IR (cm^{-1}): 3403 (N–H), 3066(Ar–-H), 2947, 2832. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.02 (d, $J = 8.4$ Hz, 1H, Ar), 7.74 (d, $J = 8.1$ Hz, 1H, Ar), 7.65 (d, $J = 2.8$ Hz, 1H, Ar), 7.59–7.54 (m, 2H, Ar), 7.46–7.43 (m, 1H, Ar), 7.35–7.33 (m, 1H, Ar), 7.12–7.09 (m, 1H, Ar), 3.61–3.48 (m, 4H, 1′-$N(CH_2)_2$), 3.22–3.20 (m, 4H, 4′-$N(CH_2)_2$), 2.96 (s, 1H, NH). ^{13}C NMR (**5b**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.3 (1-C), 144.9 (3-C), 143.1 (2″-C), 138.6 (4a-C), 130.7 (6-C), 128.5, 127.5, 127.4, 126.5, 125.4, 124.0, 119.6 (8a-C), 109.7 (4-C), 47.6 (2′-C, 6′-C), 42.5 (3′-C, 5′-C). MS (ESI) *m/z* 296 $(M+H)^+$. Anal. Calcd. for $C_{17}H_{17}N_3S \cdot 0.45CH_2Cl_2$: C, 62.82; H, 5.41; N, 12.6. Found C, 62.86; H, 5.45; N, 12.64.

*5.1.13. 1-(4-Methyl-piperazin-1-yl)-3-thiophen-2-yl-isoquinoline (**5c**)*

The procedure described for compound **5a** was used with compound **9a** (500 mg, 2.04 mmol), *N*-methylpiperazine (306 mg, 3.06 mmol) and K_2CO_3 (1.4 g, 10.2 mmol) in DMF to afford compound **5c** as brown solid (600 mg, 95%). The free base was converted to **5c**·HCl salt (brownish yellow solid). Mp: 64–66 °C. IR (cm^{-1}): 3050 (Ar–-H), 2969, 2885, 2931, 2832. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.02 (d, $J = 8.2$ Hz, 1H, Ar), 7.73 (d, $J = 8.2$ Hz, 1H, Ar), 7.64 (dd, $J = 3.6$, 1.1 Hz, 1H, Ar), 7.55–7.52 (m, 2H, Ar), 7.44–7.39 (m, 1H, Ar), 7.33 (dd, $J = 5.0$, 1.1 Hz, 1H, Ar), 7.10 (dd, $J = 5.1$, 3.7 Hz, 1H, Ar), 3.58–3.55 (m, 4H, 1′-$N(CH_2)_2$), 2.72–2.68 (m, 4H, 4′-$N(CH_2)_2$), 2.40 (s, 3H, NCH_3). ^{13}C NMR (**5c**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 158.8 (1-C), 144.9 (3-C), 143.1 (2″-C), 138.6 (4a-C), 130.7 (6-C), 128.5, 127.6, 127.4, 126.5, 125.3, 124.1, 119.5 (8a-C), 109.7 (4-C), 52.1 (3′-C, 5′-C), 47.5 (2′-C, 6′-C), 42.2 (4′-NCH_3). MS (ESI) *m/z* 310 $(M+H)^+$. Anal. Calcd. for $C_{18}H_{19}N_3S \cdot 0.1C_4H_8O_2 \cdot 0.1H_2O$: C, 69.05; H, 6.30; N, 13.13; S, 10.02. Found C, 69.01; H, 6.16; N, 13.13; S, 10.10.

*5.1.14. 1-(4-Ethyl-piperazin-1-yl)-3-thiophen-2-yl-isoquinoline (**5d**)*

The procedure described for compound **5a** was used with compound **9a** (250 mg, 1.02 mmol), 1-ethylpiperazine (175 mg, 1.53 mmol) and K_2CO_3 (704 mg, 5.1 mmol) in DMF to afford compound **5d** as dark green liquid (260 mg, 79%). The free base was converted to **5d**·HCl salt (yellow solid). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.01 (s, 1H, Ar), 7.74–7.72 (m, 1H, Ar), 7.64 (dd, $J = 3.6$, 1.1 Hz, 1H, Ar), 7.58–7.53 (m, 2H, Ar), 7.44–7.39 (m, 1H, Ar), 7.33 (dd, $J = 5.0$, 1.1 Hz, 1H, Ar), 7.10 (dd, $J = 5.0$, 3.6 Hz, 1H, Ar), 3.58 (t, $J = 4.7$ Hz, 4H, 1′-$N(CH_2)_2$), 2.74 (t, $J = 4.7$ Hz, 4H, 4′-$N(CH_2)_2$), 2.54 (q, $J = 7.2$ Hz, 2H, NCH_2CH_3), 1.17 (t, $J = 7.2$ Hz, 3H, NCH_2CH_3). ^{13}C NMR (**5d**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 158.9 (1-C), 144.9 (3-C), 143.1 (2″-C), 138.6 (4a-C), 130.7 (6-C), 128.5, 127.6, 127.4, 126.5, 125.3, 124.1, 119.5 (8a-C), 109.7 (4-C), 50.7 (3′-C, 5′-C), 50.0 (4′-NCH_2CH_3), 47.5 (2′-C, 6′-C), 8.8 (4′-NCH_2CH_3). MS (ESI) *m/z* 324 $(M+H)^+$.

*5.1.15. 1-Morpholin-4-yl-3-thiophen-2-yl-isoquinoline (**5e**)*

The procedure described for compound **5a** was used with compound **9a** (400 mg, 1.6 mmol), morpholine (428 mg, 4.9 mmol) and K_2CO_3 (1.1 g, 8.2 mmol) in DMF to afford compound **5e** as ash colored solid (386 mg, 80%). The free base was converted to **5e**·HCl salt (light yellow solid). Mp: 125–126 °C. IR (cm^{-1}): 3063 (Ar–-H), 2955, 2844. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.03 (d, $J = 8.3$ Hz,

1H, Ar), 7.75 (d, J = 7.9 Hz, 1H, Ar), 7.65 (dd, J = 3.7, 1.1 Hz, 1H, Ar), 7.60–7.53 (m, 2H, Ar), 7.44 (t, J = 7.6 Hz, 1H, Ar), 7.34 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.12 (dd, J = 5.0, 3.7 Hz, 1H, Ar), 4.00–3.97 (m, 4H, $O(CH_2)_2$), 3.53–3.50 (m, 4H, $N(CH_2)_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.3 (1-C), 145.9 (3-C), 143.9 (2″-C), 138.9 (4A-C), 129.9 (6-C), 127.9, 127.4, 126.4, 125.6, 125.4, 123.4, 120.4 (8a-C), 109.4 (4-C), 66.9 (3′-C, 5′-C), 51.6 (2′-C, 6′-C). ^{13}C NMR (**5e**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.9 (1-C), 145.2 (3-C), 143.2 (2″-C), 138.6 (4a-C), 130.4 (6-C), 128.4, 127.5, 127.3, 126.2, 125.4, 123.9, 119.6 (8a-C), 109.1 (4-C), 66.1 (3′-C, 5′-C), 51.4 (2′-C, 6′-C). MS (ESI) m/z 297 $(M+H)^+$. Anal. Calcd. for $C_{17}H_{16}N_2OS$: C, 68.89; H, 5.44; N, 9.45; S, 10.82. Found C, 68.72; H, 5.48; N, 9.36; S, 10.74.

5.1.16. 3-Thiophen-2-yl-isoquinolin-1-ylamine (**5f**)

A mixture of compound **9a** (100 mg, 0.4 mmol), ammonium hydroxide (28% NH_3 content, 77 mg), sodium bicarbonate (172 mg, 2.04 mmol) was heated at 50 °C for 48 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography to afford compound **5f** as dark semi-solid (23 mg, 24%). The free base was then converted to **5f**·HCl salt (ivory solid).

Alternatively, a solution of *o*-tolunitrile (351 mg, 3 mmol) and thiophene-2-carbonitrile (654 mg, 6 mmol) in dry THF was added dropwise to a mixture of $LiNMe_2$ (5 mL, 3.3 mmol) and hexamethylphosphoramide (596 mg, 3.3 mmol) in dry THF at −78 °C. The reaction was maintained at the same temperature overnight. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography to afford compound **5f** as dark semi-solid (140 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.64–7.58 (m, 3H, Ar), 7.49 (m, 1H, Ar), 7.35 (s, 1H, 4-H), 7.30–7.28 (m, 2H, Ar), 7.06 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 5.35 (s, 2H, NH_2). ^{13}C NMR (**5f**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 155.6 (1-C), 137.2 (3-C), 134.9 (2″-C), 132.5 (4a-C), 129.3, 128.7, 128.2, 127.8, 127.7, 125.5, 116.6 (8a-C), 107.0 (4-C). MS (ESI) m/z 268 $(M+CH_3CN+H)^+$, 227 $(M+H)^+$.

5.1.17. 1-Azido-5-methyl-3-thiophen-2-yl-isoquinoline (**5g**)

To a solution of compound **9b** (500 mg, 1.92 mmol) in DMF, sodium azide (187 mg, 2.88 mmol) was added and the reaction mixture was heated at 100 °C for overnight. The reaction mixture was cooled, diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography to afford compound **5g** as ivory solid (450 mg, 87%). Mp: 172–174 °C. IR (cm^{-1}): 3104 (Ar–-H), 2972, 2863. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.62–8.59 (m, 1H, Ar), 8.31 (dd, J = 3.8, 1.1 Hz, 1H, Ar), 7.77 (s, 1H, 4-H), 7.65–7.61 (m, 2H, Ar), 7.58 (dd, J = 5.1, 1.1 Hz, 1H, Ar), 7.28–7.25 (m, 1H, Ar), 2.76 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 148.5 (1-C), 134.7 (3-C), 132.5 (2″-C), 132.4 (4a-C), 130.8, 130.1, 128.8, 128.5, 128.4, 128.2, 122.8, 118.5, 110.5 (4-C), 19.5 (5-CCH_3). Anal. Calcd. for $C_{14}H_{10}N_4S$: C, 63.14; H, 3.78; N, 21.04; S, 12.04. Found C, 62.94; H, 3.87; N, 20.74; S, 12.28.

5.1.18. 6-Methyl-1-piperidin-1-yl-3-thiophen-2-yl-isoquinoline (**5h**)

The procedure described for compound **5a** was used with compound **9c** (600 mg, 2.3 mmol), piperidine (587 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5h** as light yellow solid (632 mg, 89%). The free base was then converted to **5h**·HCl salt (light yellow solid). Mp: 106–108 °C. IR (cm^{-1}): 3062 (Ar–-H), 2927, 2853. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.91 (d, J = 8.5 Hz, 1H, Ar), 7.62 (dd, J = 3.6, 1.1 Hz, 1H, Ar), 7.48–7.45 (m, 2H, Ar), 7.31 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.25–7.22 (m, 1H, Ar), 7.11–7.08 (m, 1H, Ar), 3.46–3.43 (m, 4H, $N(CH_2)_2$), 2.48 (s, 3H, 6-CH_3), 1.87–1.80 (m, 4H, 3′-CH_2, 4′-CH_2), 1.74–1.67 (m, 2H, 4′-CH_2). ^{13}C NMR (**5h**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 160.0 (1-C), 144.9 (3-C), 142.8 (2″-C), 140.4 (6-C), 138.9 (4a-C), 128.3, 128.1, 127.2, 126.3, 125.4, 123.9, 118.0 (8a-C), 108.6 (4-C), 52.2 (2′-C, 6′-C), 25.4 (3′-C, 5′-C), 24.2 (4′-C), 21.3 (6-CCH_3). MS (ESI) m/z 309 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{20}N_2S$: C, 73.99; H, 6.54; N, 9.08. Found C, 73.86; H, 6.44; N, 9.45.

5.1.19. 6-Methyl-1-piperazin-1-yl-3-thiophen-2-yl-isoquinoline (**5i**)

The procedure described for compound **5a** was used with compound **9c** (600 mg, 2.3 mmol), piperazine (594 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5i** as brownish green solid (495 mg, 70%). The free base was then converted to **5i**·HCl salt (light yellow). Mp: 72–74 °C. IR (cm^{-1}): 3418 (N–H), 3067(Ar–-H), 2933, 2836. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.91 (d, J = 8.5 Hz, 1H, Ar), 7.63 (dd, J = 3.6, 1.0 Hz, 1H, Ar), 7.51–7.49 (m, 2H, Ar), 7.32 (dd, J = 5.0, 1.0 Hz, 1H, Ar), 7.25 (dd, J = 8.5, 1.6 Hz, 1H, Ar), 7.10 (dd, J = 5.0, 3.7 Hz, 1H, Ar), 3.51–3.47 (m, 4H, 1′-$N(CH_2)_2$), 3.19–3.16 (m, 4H, 4′-$N(CH_2)_2$), 2.59 (s, 1H, NH), 2.49 (s, 3H, 6-CH_3). ^{13}C NMR (**5i**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.1 (1-C), 145.0 (3-C), 143.2 (2″-C), 140.5 (6-C), 138.9 (4a-C), 128.5, 128.4, 127.3, 126.4, 125.2, 123.9, 117.8 (8a-C), 109.4 (4-C), 47.5 (2′-C, 6′-C), 42.5 (3′-C, 5′-C), 21.4 (6-CCH_3). MS (ESI) m/z 310 $(M+H)^+$. Anal. Calcd. for $C_{18}H_{19}N_3S \cdot 0.85H_2O$: C, 66.58; H, 6.42; N, 12.94; S, 9.87. Found C, 66.28; H, 6.02; N, 12.67; S, 9.47.

5.1.20. 6-Methyl-1-(4-methyl-piperazin-1-yl)-3-thiophen-2-yl-isoquinoline (**5j**)

The procedure described for compound **5a** was used with compound **9c** (600 mg, 2.3 mmol), *N*-methylpiperazine (690 mg, 6.9 mmol) and K_2CO_3 (1.6 g, 11.5 mmol) in DMF to afford compound **5j** as light brown solid (592 mg, 80%). The free base was then converted to **5j**·HCl salt (yellow solid). Mp: 85–87 °C. IR (cm^{-1}): 3067 (Ar–-H), 2969, 2882, 2935, 2839. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.90 (d, J = 8.6 Hz, 1H, Ar), 7.63 (d, J = 3.7 Hz, 1H, Ar), 7.49–7.47 (m, 2H, Ar), 7.33–7.31 (m, 1H, Ar), 7.26–7.22 (m, 1H, Ar), 7.11–7.08 (m, 1H, Ar), 3.56 (m, 4H, 1′-$N(CH_2)_2$), 2.70–2.67 (m, 4H, 4′-$N(CH_2)_2$), 2.48 (s, 3H, 6-CH_3), 2.40 (s, 3H, NCH_3). ^{13}C NMR (**5j**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 158.7 (1-C), 145.0 (3-C), 143.2 (2″-C), 140.5 (6-C), 138.9 (4a-C), 128.5, 128.4, 127.3, 126.5, 125.1, 124.0, 117.8 (8a-C), 109.4 (4-C), 52.1 (3′-C, 5′-C), 47.5 (2′-C, 6′-C), 42.2 (4′-NCH_3), 21.4 (6-CCH_3). MS (ESI) m/z 324 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{21}N_3S \cdot 0.05C_4H_8O_2 \cdot 0.05C_3H_7NO$: C, 70.11; H, 6.61; N, 12.89; S, 9.67. Found C, 69.94; H, 6.58; N, 12.79; S, 9.82.

5.1.21. 1-(4-Ethyl-piperazin-1-yl)-6-methyl-3-thiophen-2-yl-isoquinoline (**5k**)

The procedure described for compound **5a** was used with compound **9c** (600 mg, 2.3 mmol), 1-ethylpiperazine (787 mg, 6.9 mmol) and K_2CO_3 (1.6 g, 11.5 mmol) in DMF to afford compound **5k** as dark green liquid (700 mg, 90%). The free base was then converted to **5k**·HCl salt (yellow solid). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.90 (d, J = 8.5 Hz, 1H, Ar), 7.62 (dd, J = 3.6, 1.1 Hz, 1H, Ar), 7.48–7.46 (m, 2H, Ar), 7.31 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.23 (dd, J = 8.5, 1.6 Hz, 1H, Ar), 7.09 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 3.57–3.54 (m, 4H, 1′-$N(CH_2)_2$), 2.73–2.70 (m, 4H, 4′-$N(CH_2)_2$), 2.52 (q, J = 7.2 Hz, 2H, NCH_2CH_3), 2.47 (s, 3H, 6-CH_3), 1.15 (t, J = 7.2 Hz, 3H, NCH_2CH_3). ^{13}C NMR (**5k**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 145.0 (1-C), 143.2 (3-C), 140.6 (2″-C), 138.9 (6-C), 128.5 (4a-C), 128.4, 127.3, 126.5, 125.1, 124.0, 117.8 (8a-C), 109.4 (4-C), 50.7 (3′-C, 5′-C), 50.0 (4′-NCH_2CH_3), 47.5 (2′-C, 6′-C), 21.4 (6-CCH_3), 8.8 (4′-NCH_2CH_3). MS (ESI) m/z 338 $(M+H)^+$.

*5.1.22. 6-Methyl-1-morpholin-4-yl-3-thiophen-2-yl-isoquinoline (**5l**)*

The procedure described for compound **5a** was used with compound **9c** (600 mg, 2.3 mmol), morpholine (600 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5l** as off white solid (460 mg, 64%). The free base was then converted to **5l**·HCl salt (yellow solid). Mp: 117–119 °C. IR (cm^{-1}): 3066 (Ar–-H), 2965, 2878, 2910, 2828. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.91 (d, J = 8.5 Hz, 1H, Ar), 7.64–7.62 (m, 1H, Ar), 7.52–7.51 (m, 2H, Ar), 7.33 (dd, J = 3.9, 1.1 Hz, 1H, Ar), 7.27–7.25 (m, 1H, Ar), 7.12–7.09 (m, 1H, Ar), 3.99–3.96 (m, 4H, $O(CH_2)_2$), 3.52–3.48 (m, 4H, $N(CH_2)_2$), 2.50 (s, 3H, 6-CH_3). ^{13}C NMR (**5l**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.8 (1-C), 145.2 (3-C), 143.1 (2″-C), 140.3 (6-C), 138.9 (4a-C), 128.3, 128.2, 127.2 (8a-C), 126.4 (4-C), 125.3, 123.9, 117.9, 108.8, 66.1 (3′-C, 5′-C), 51.4 (2′-C, 6′-C), 21.3 (6-CCH_3). MS (ESI) *m/z* 311 $(M+H)^+$. Anal. Calcd. for $C_{18}H_{18}N_2OS$: C, 69.65; H, 5.84; N, 9.02; S, 10.33. Found C, 70.22; H, 5.98; N, 9.02; S, 10.41.

*5.1.23. Benzyl-(6-methyl-3-thiophen-2-yl-isoquinolin-1-yl)-amine (**5m**)*

The procedure described for compound **5a** was used with compound **9c** (1.4 g, 5.4 mmol), benzylamine (4.62 g, 43.2 mmol) and K_2CO_3 (3.72 g, 27 mmol) in DMF to afford compound **5m** as gray solid (1.4 g, 78%). Mp: 102–104 °C. IR (cm^{-1}): 3462 (N–H), 3064, 2914. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.73 (s, 1H, Ar), 7.61–7.59 (m, 2H, Ar), 7.51–7.40 (m, 6H, Ar), 7.36 (s, 1H, Ar), 7.22–7.16 (m, 2H, Ar), 5.49 (t, J = 4.8 Hz, 1H, NH), 4.99 (d, J = 5.1 Hz, 1H, NCH_2), 2.50 (s, 3H, 6-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 154.1 (1-C), 146.5 (3-C), 144.5 (2″-C), 139.99, 139.94, 138.1, 128.5, 128.2, 127.7, 127.3, 127.2, 126.6, 125.9, 122.9, 121.3, 115.4 (8a-C), 104.8 (4-C), 45.7 (NCH_2), 21.6 (6-CCH_3). MS (ESI) *m/z* 331 $(M+H)^+$. Anal. Calcd. for $C_{21}H_{18}N_2S$: C, 76.33; H, 5.49; N, 8.48; S, 9.70. Found C, 76.07; H, 5.53; N, 8.26; S, 9.85.

*5.1.24. 7-Methyl-1-piperidin-1-yl-3-thiophen-2-yl-isoquinoline (**5n**)*

The procedure described for compound **5a** was used with compound **9d** (600 mg, 2.3 mmol), piperidine (587 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5n** as light yellowish green solid (528 mg, 74%). The free base was then converted to **5n**·HCl salt (yellow solid). Mp: 116–117 °C. IR (cm^{-1}): 3050 (Ar–-H), 2924, 2845. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (s, 1H, Ar), 7.64–7.60 (m, 2H, Ar), 7.50 (s, 1H, Ar), 7.38 (dd, J = 8.4, 1.6 Hz, 1H, Ar), 7.30 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.09 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 3.45–3.42 (m, 4H, $N(CH_2)_2$), 2.51 (s, 3H, 7-CH_3), 1.89–1.81 (m, 4H, 3′-CH_2, 5′-CH_2), 1.74–1.68 (m, 2H, 4′-CH_2). ^{13}C NMR (**5n**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.6 (1-C), 145.0 (3-C), 142.1 (2″-C), 136.7 (4a-C), 135.7 (7-C), 132.5 (6-C), 128.3, 127.4, 127.0, 124.3, 123.7, 120.0 (8a-C), 109.1 (4-C), 52.2 (2′-C, 6′-C), 25.3 (3′-C, 5′-C), 24.1 (4′-C), 21.6 (7-CCH_3). MS (ESI) *m/z* 309 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{20}N_2S \cdot 0.1C_4H_8O_2 \cdot 0.1H_2O$: C, 73.03; H, 6.63; N, 8.78; S, 10.05. Found C, 72.73; H, 6.53; N, 8.84; S, 10.32.

*5.1.25. 7-Methyl-1-piperazin-1-yl-3-thiophen-2-yl-isoquinoline (**5o**)*

The procedure described for compound **5a** was used with compound **9d** (600 mg, 2.3 mmol), piperazine (594 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5o** as brown solid (545 mg, 76%). The free base was then converted to **5o**·HCl salt. Mp: 128–130 °C. IR (cm^{-1}): 3418 (N–H), 3047 (Ar–-H), 2979, 2843, 2910, 2817. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80 (s, 1H, Ar), 7.67–7.61 (m, 2H, Ar), 7.54 (s, 1H, Ar), 7.41 (dd, J = 8.4, 1.6 Hz, 1H, Ar), 7.32 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.10 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 3.48–3.45 (m, 4H, 1′-$N(CH_2)_2$), 3.19–3.15 (m, 4H, 4′-$N(CH_2)_2$), 2.52 (s, 3H, 7-CH_3). ^{13}C NMR (125 MHz, DMSO-*d6*) δ (ppm): 160.1 (1-C), 145.6 (3-C), 142.5 (2″-C), 136.6 (4a-C), 135.5 (7-C), 132.2 (6-C), 128.2, 127.3, 126.8, 124.3, 123.3, 119.9 (8a-C), 108.6 (4-C), 52.2 (2′-C, 6′-C), 45.5 (3′-C, 5′-C), 21.6 (7-CCH_3). MS (ESI) *m/z* 310 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{20}N_2S \cdot 0.05C_4H_8O_2 \cdot 0.4H_2O$: C, 68.09; H, 6.34; N, 13.09; S, 9.99. Found C, 67.99; H, 6.14; N, 13.01; S, 10.15.

*5.1.26. 7-Methyl-1-(4-methyl-piperazin-1-yl)-3-thiophen-2-yl-isoquinoline (**5p**)*

The procedure described for compound **5a** was used with compound **9d** (600 mg, 2.3 mmol), *N*-methylpiperazine (690 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5p** as dark solid (700 mg, 94%). The free base was then converted to **5p**·HCl salt (yellow solid). Mp: 78–80 °C. IR (cm^{-1}): 3047 (Ar–-H), 2972, 2839, 2925, 2821. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.78 (s, 1H, Ar), 7.65–7.61 (m, 2H, Ar), 7.53 (s, 1H, Ar), 7.39 (dd, J = 8.4, 1.7 Hz, 1H, Ar), 7.31 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.09 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 3.55 (m, 4H, 1′-$N(CH_2)_2$), 2.72–2.69 (m, 4H, 4′-$N(CH_2)_2$), 2.51 (s, 3H, 7-CH_3), 2.41 (s, 3H, NCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 159.9 (1-C), 146.2 (3-C), 143.1 (2″-C), 137.0 (4a-C), 135.3 (7-C), 131.8 (6-C), 127.8, 127.2, 126.0, 124.5, 123.0, 120.6 (8a-C), 109.1 (4-C), 55.0 (3′-C, 5′-C), 50.9 (2′-C, 6′-C), 46.2 (4′-NCH_3), 22.0 (7-CCH_3). MS (ESI) *m/z* 324 $(M+H)^+$. Anal. Calcd. for $C_{19}H_{21}N_3S \cdot 0.2H_2O$: C, 69.78; H, 6.6; N, 12.85; S, 9.8. Found C, 69.70; H, 6.50; N, 12.75; S, 10.00.

*5.1.27. 1-(4-Ethyl-piperazin-1-yl)-7-methyl-3-thiophen-2-yl-isoquinoline (**5q**)*

The procedure described for compound **5a** was used with compound **9d** (600 mg, 2.3 mmol), 1-ethylpiperazine (787 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5q** as brown solid (760 mg, 97%). The free base was then converted to **5q**·HCl salt (yellow solid). Mp: 90–91 °C. IR (cm^{-1}): 3042 (Ar–-H), 2972, 2832, 2914, 2815. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.78 (s, 1H, Ar), 7.62–7.59 (m, 2H, Ar), 7.50 (s, 1H, Ar), 7.37 (dd, J = 6.7, 1.7 1H, Ar), 7.30 (dd, J = 3.9, 1.1 Hz, 1H, Ar), 7.09–7.06 (m, 1H, Ar), 3.56–3.53 (m, 4H, 1′-$N(CH_2)_2$), 2.74–2.71 (m, 4H, 4′-$N(CH_2)_2$), 2.56–2.49 (m, 5H, NCH_2CH3, 7-CH_3), 1.16 (t, J = 7.2 Hz, 3H, NCH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 159.9 (1-C), 146.3 (3-C), 143.2 (2″-C), 137.0 (4a-C), 135.3 (7-C), 131.8 (6-C), 127.8, 127.2, 126.0, 124.5, 122.9, 120.7 (8a-C), 109.0 (4-C), 52.8 (3′-C, 5′-C), 52.5 (4′-NCH_2CH_3), 50.9 (2′-C, 6′-C), 22.0 (7-CCH_3), 12.0 (4′-NCH_2CH_3). MS (ESI) *m/z* 338 $(M+H)^+$. Anal. Calcd. for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45; S, 9.50. Found C, 70.84; H, 6.90; N, 12.34; S, 9.44.

*5.1.28. 7-Methyl-1-morpholin-4-yl-3-thiophen-2-yl-isoquinoline (**5r**)*

The procedure described for compound **5a** was used with compound **9d** (600 mg, 2.3 mmol), morpholine (594 mg, 6.9 mmol) and K_2CO_3 in DMF to afford compound **5r** as gray solid (590 mg, 82%). The free base was then converted to **5r**·HCl salt (cream colored solid). Mp: 124–126 °C. IR (cm^{-1}): 3052 (Ar–-H), 2965, 2862, 2911, 2833. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80–7.79 (m, 1H, Ar), 7.67 (d, J = 8.3 Hz, 1H, Ar), 7.63 (dd, J = 3.6, 1.1 Hz, 1H, Ar), 7.57 (s, 1H, Ar), 7.42 (dd, J = 8.3, 1.5 Hz, 1H, Ar), 7.33 (dd, J = 5.0, 1.1 Hz, 1H, Ar), 7.11 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 4.01–3.98 (m, 4H, $O(CH_2)_2$), 3.52–3.48 (m, 4H, $N(CH_2)_2$), 2.52 (s, 3H, 7-CH_3). ^{13}C NMR (**5r**·HCl) (125 MHz, DMSO-*d6*) δ (ppm): 159.4 (1-C), 145.3 (3-C), 142.4 (2″-C), 136.7 (4a-C), 135.8 (7-C), 132.4 (6-C), 128.3, 127.4, 126.9, 124.2, 123.5, 119.8 (8a-C), 109.1 (4-C), 66.1 (3′-C, 5′-C), 51.3 (2′-C, 6′-C), 21.5 (7-CCH_3). MS (ESI): *m/z* 311 $(M+H)^+$. HRMS (ESI): *m/z* 311.1218 $(M+H)^+$ (calcd for $C_{18}H_{19}N_2OS$, 311.1218).

*5.1.29. 7-Methyl-1-(4-methyl-[1,4]diazepan-1-yl)-3-thiophen-2-yl-isoquinoline (**5s**)*

The procedure described for compound **5a** was used with compound **9d** (550 mg, 2.1 mmol), 1-methylhomopiperazine

(718 mg, 6.3 mmol) and K_2CO_3 (1.45 g, 10.5 mmol) in DMF to afford compound **5s** as dark semi-solid (570 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.80 (s, 1H, Ar), 7.60–7.57 (m, 2H, Ar), 7.41 (s, 1H, Ar), 7.36 (dd, J = 8.4, 1.6 Hz, 1H, Ar), 7.30–7.28 (m, 1H, Ar), 7.08 (dd, J = 5.0, 3.6 Hz, 1H, Ar), 3.92 (t, J = 4.8 Hz, 2H, 1′-NCH_2), 3.86 (t, J = 6.0 Hz, 2H, 1′-NCH_2), 2.92–2.89 (m, 2H, 4′-NCH_2), 2.76–2.72 (m, 2H, 4′-NCH_2), 2.48 (s, 3H, 7-CH_3), 2.44 (s, 3H, NCH_3), 2.16–2.08 (m, 2H, 6′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 159.4 (1-C), 146.3 (3-C), 142.4 (2″-C), 137.4 (4a-C), 134.1 (7-C), 131.3 (6-C), 127.6, 126.9, 125.6, 124.9, 122.5 (8-C), 119.6 (8a-C), 107.1 (4-C), 57.8 (3′-C), 57.3 (5′-C), 51.8 (2′-C), 51.2 (7′-C), 46.2 (4′-C), 27.7 (6′-C), 21.8 (7-C$\underline{C}H_3$). MS (ESI) m/z 338 $(M+H)^+$.

5.1.30. Benzyl-[3-(1,5-dimethyl-1H-pyrrol-2-yl)-5-methyl-isoquinolin-1-yl]-amine (**5t**)

The procedure described for compound **5a** was used with compound **9e** (500 mg, 1.84 mmol), benzylamine (990 mg, 9.23 mmol) and K_2CO_3 (1.27 g, 9.23 mmol) in DMF to afford compound **5t** as dark brown semi-solid (454 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.51 (d, J = 8.2 Hz, 1H, Ar), 7.38–7.16 (m, 8H, Ar), 6.46 (d, J = 3.5 Hz, 1H, 3″-H), 5.94 (dd, J = 3.5, 0.6 Hz, 1H, 4″-H), 5.50 (s, 1H, NH), 4.83 (d, J = 5.3 Hz, 2H, NCH_2), 3.64 (s, 3H, NCH_3), 2.57 (s, 3H, 5-CH_3), 2.23 (s, 3H, 5″-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ(ppm): 154.2 (1-C), 145.0 (3-C), 139.9, 137.3, 133.9, 133.4, 131.7, 130.0, 128.5, 127.5, 127.0, 124.3, 119.2, 115.7, 108.5, 106.3, 104.9 (4-C), 45.8 (NCH_2), 32.3 (1″-NCH_3), 19.4 (5-C$\underline{C}H_3$), 12.7 (5″-C$\underline{C}H_3$).

5.2. Cytotoxicity

Four lines of cancer cells, 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 × 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 graded concentration of the compounds. On day 4, 5 μL of the 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.

5.3. Topoisomerases inhibition

Topo I inhibitory activity was determined by assessing the relaxation of supercoiled DNA pBR322. A mixture of pBR322 (100 ng) and recombinant human DNA topo I (0.4 units; Topo-GEN INC., USA) was incubated with and without the compounds at 37 °C for 30 min in relaxation buffer [10 mM Tris–HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol]. The reaction in a final volume of 10 μL was quenched by adding 2.5 μL of stop solution containing 5% sarcosyl, 0.0025% bromophenol blue and 25% glycerol. The DNA samples were electrophoresed on 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 UV light and the supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

The DNA topo IIa inhibitory activity of the compounds was measured as follows. A mixture of supercoiled plasmid DNA pBR322 (200 ng) and human DNA topo IIa (1 unit; Usb Corp., USA) was incubated with and 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 reaction in a final volume of 20 mL was quenched by adding 3 mL of 7 mM EDTA. The DNA samples were electrophoresed on 1% agarose gel at 25 V for 4 h with TAE as the running buffer. The gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). The DNA bands were visualized by transillumination with UV light and the supercoiled DNA was quantified using AlphaImager™ (Alpha Innotech Corporation).

5.4. Molecular docking

The docking study was performed in Sybyl-X 2.0 (winnt_os5x) using the Surflex Dock program. The structure of topotecan-DNA-topo I was downloaded from Protein Data Bank (PDB: 1K4T). An atom of Hg, a molecule of PEG and 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, using a conjugate gradient method, distance-dependent dielectric constant and converging to 0.01 kcal/mol·Å. The −SH group of G11 nucleotide of 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. 3-Heteroarylisoquinolinamine **5b** and topotecan were constructed in Sybyl, energy 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 protomol constructed earlier. The extracted topotecan was considered a reference molecule. The docking protocol 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 amsacrine-DNA-topo IIβ was downloaded from Protein Data Bank (PDB: 4G0U). A topo IIβ momomer, a molecule of amsacrine and 3 Mg^{2+} associated with the monomer were deleted. Rest of the procedures was performed in a similar manner as explained for topo I. The docking protocol was able to reproduce the position of manually constructed amsacrine in binding site with 0.61 Å rmsd of the heavy atoms of the extracted amsacrine.

5.5. Cell viability

Cell viability was determined by MTT assay. MCF-7, MKN-28, A549, HeLa and B16 cancer cells were cultured in 96-well plates and treated with compounds under test for 48 h. MTT (0.5 μg/mL) was added 4 h before the end of culture. Finally, 100 μL DMSO was added to each well and the absorbance was measured after 30 min at 570 nm using a microplate spectrophotometer.

5.6. Cell cycle inhibition

Cell cycle phase distribution analysis of HeLa cells was performed by flow cytometry after DNA staining. Cells were incubated with various concentrations of compounds under test for 24 h before they were harvested by centrifugation. Harvested cells were washed twice with PBS, fixed in 70% ethanol (in PBS) on ice overnight and then resuspended in PBS containing 10 μg/mL propidium iodide (PI), 0.5 mg/mL RNase, and 0.1% Triton X-100. The cells were then incubated at 37 °C in the dark for 30 min and analyzed using

flow cytometer (Becton Dickinson Immunocytometry System, San Jose, CA, USA).

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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.2014.05.047.

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