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Review

3-Arylisoquinolines as novel topoisomerase I inhibitors

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Topoisomerase I inhibitor

ABSTRACT

Topoisomerase I (topo I) is an essential enzyme for vital cellular processes. Inhibition of topo I activities is lethal and leads to cell death, thus establishing topo I as a promising target for cancer treatment. Camptothecin, a natural alkaloid, inhibits topo I. Topotecan and irinotecan, synthetic derivatives of camptothecin, are the most potent anticancer drugs in clinical use. However, several limitations of camptothecins such as solubility, toxicity, stability, resistance and the required high drug dose have encouraged the development of non-camptothecin topo I inhibitors. Natural alkaloid benzo[*c*]phenanthridines and synthetic indenoisoquinolines have been extensively studied as alternatives to camptothecin. Interestingly, these non-camptothecin topo I inhibitors share a common 3-arylisoquinoline scaffold. This review will describe the development of novel indeno[1,2-*c*]isoquinolines, isoindolo[2,1-*b*]isoquinolines, 12-oxobenzo[*c*]phenanthridines and benz[*b*]oxepines with a 3-arylisoquinoline nucleus as topo I inhibitors.

Contents

1. Introduction

DNA topoisomerase I (topo I) is a ubiquitous enzyme that resolves superhelical tension and other topological consequences that occur during separation of two DNA strands. Topo I relaxes DNA supercoil by relieving torsional stress due to various DNA metabolic processes, including replication, transcription, recombination, chromatin condensation and chromosome partitioning in cell division.[1–3] The catalysis of topo I consists of three main steps—single strand DNA cleavage, DNA relaxation and DNA religation. Topo I cleaves a single strand of DNA through transesterification of Tyr723 to form a 3′-phosphotyrosine linkage with DNA. The covalent enzyme-DNA structure is called the cleavage complex. After DNA break, the cleaved scissile strand rotates around the intact strand and removes DNA supercoils.[4] Ultimately, the broken DNA strand is resealed by a second transesterification reaction involving nucleophilic attack of the 5′-OH of the cleaved strand to the 3′-phosphotyrosine bond. The enzymatic action of topo I rewinds underwound negatively supercoiled DNA and unwinds overwound positively supercoiled DNA.[5]

Under normal physiological conditions, the covalent topo I-DNA cleavage complex is barely detectable as the DNA religation step is much faster than the DNA cleavage step.[2] However, several types of DNA alterations like single nucleotide gaps, basic sites, modified bases and modified sugars can lead to persistent accumulation of

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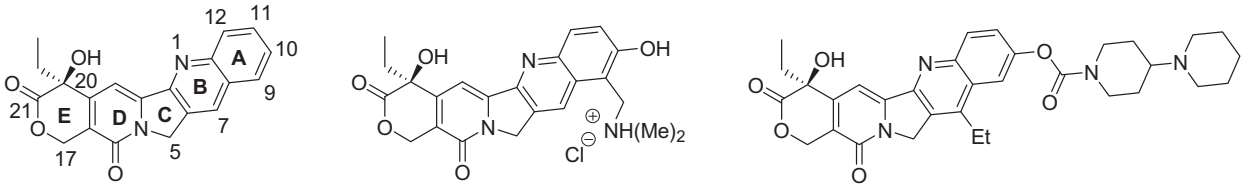


Figure 1. Camptothecin (**1**), topotecan (**2**), irinotecan (**3**).

topo I-DNA adducts.[6–9] In addition, natural products such as camptothecin (CPT) (**1**) can bind covalently to topo I-DNA complex, slowing the religation step and increasing the lifetime of the cleavage complex (Fig. 1).[10–14] Accumulation of cleavage complex increases the chance of collision of the replication fork with the cleaved DNA strand, which in turn leads to a permanent double-strand DNA break and ultimately cell death due to apoptosis.[15]

CPT is a pentacyclic alkaloid first isolated from extracts of *Camptotheca acuminate*.[16] It selectively poisons topo I[17] and exhibits strong antineoplastic activity against colorectal, breast, lung and ovarian cancers. Two water-soluble derivatives of CPT, topotecan (**2**) and irinotecan (**3**), have been used clinically for cancer treatment. Topotecan is prescribed for chemoresistant ovarian and small cell lung cancers, whereas irinotecan, a carboxylesterase-dependent prodrug, is administered with 5-fluorouracil and leucovorin for metastatic colorectal carcinomas.[18]

However, CPT and its clinical analogs have several limitations. They have relatively low water solubility. The camptothecin-DNA-topo I cleavage complex is reversible and requires a long infusion time. CPTs are also resisted by cells expressing drug efflux membrane transporters ABCG2 and ABCB1 (Pgp).[19–21] Above all, CPTs are unstable under physiological conditions. The α-hydroxy δ-lactone ring of camptothecin undergoes hydrolysis to carboxylate. The carboxylate form of camptothecin is inactive and readily binds to human serum albumin, making it less available for cellular uptake.[22] Moreover, the water-soluble sodium salt of camptothecin is cleared by the kidneys and causes hemorrhagic cystitis and myelotoxicity.[23,24]

As described, the essential role of topo I in vital processes of the cell cycle and its elevated level in solid tumors compared to normal tissue make topo I a promising target for the treatment of tumors. Because of the drawbacks of CPTs, other compounds have been screened as topo I inhibitors. Among many chemical entities, benzo[*c*]phenanthridines **4a**, 5*H*-dibenzo[*c*,*h*]1,6-naphthyridinones **5** and indeno[1,2-*c*]isoquinolines **6** have been studied extensively as non-camptothecin topo I-targeting agents (Fig. 2). Interestingly, these natural and synthetic compounds share a common 3-arylisoquinoline skeleton **7**. In fact, *N*-unsubstituted 3-arylisoquinolin-1-one has served as a precursor for the development of various topo I inhibitors with a 3-arylisoquinoline nucleus: 3-arylisoquinolinamines, novel indeno[1,2-*c*]isoquinolines, isoindolo[2,1-*b*]isoquinolines, 12-oxobenzo[*c*]phenanthridines and benz[*b*]oxepines (Scheme 1). In this review, synthesis, cytotoxicity and topo I inhibitory activity of 3-arylisoquinoline progeny are discussed.

2. 3-Arylisoquinolines

3-Arylisoquinolones are potent antitumor agents. The cytotoxicity of this class of compounds was revealed in the study of fagaridine derivative **8**. 8-Dimethoxy-2-methyl-3-(4,5-methylene-dioxy-2-vinylphenyl)isoquinoline-(2*H*)-one (**9**), a key intermediate of biomimetic transformation of protoberberine into benzo[*c*]phenanthridine, showed potent cytotoxicity (ED_{50} <0.10 μg/mL, SKMEL-2).[25]The styrene compound **9** is considered to be a bioisostere of benzo[*c*]phenanthridine via cleavage of the C–C bond of the aromatic B ring (Scheme 2).[26] In addition, 3-arylisoquinoline was identified as a bioisostere of 5-aryl-2,3-dihyroimidazo[2,1-*a*]isoquinolines (**10**, Fig. 3), which were reported to have antitumor activity.[27] Serendipitous discovery and continued studies have established 3-arylisoquinoline as a lead compound and precursor for development of anticancer agents specifically targeting human topo I.

Synthesis of 3-aryl 2-*N*-unsubstituted isoquinolin-1-one was initially accomplished by the action of ammonia on 3-phenylisocoumarin.[28] The yield was very low and this method did not receive much practical acceptance. 3-Arylisoquinolone was obtained with higher yield by benzoylation of homophthalic anhydride with benzoyl chloride followed by in situ amination and decarboxylation of the reaction intermediate, 4-benzyoylisochroman-1,3-dione.[29] 3-Aryl-2-*N*-unsubstituted isoquinolin-1-ones were also prepared by lithiated toluamide-benzonitrile cycloaddition method[30] and from 2,3-diarylacrylic acids by Eloy and Deryckere method.[31] Recently 3-aryl-2-*N*-unsubstituted isoquinolin-1-ones were synthesized via Pd/C-mediated Sonogashira coupling of 2-iodobenzoyl azide and terminal alkynes followed by intramolecular acetylenic Schmidt reaction of in situ generated *o*-alkynyl azido benzene.[32]

One-pot synthesis of 3-arylisoquinolone by lithiated toluamide-benzonitrile cycloaddition offers several advantages over other methods. The yield of 3-arylisoquinolone by this method is generally high. Diverse structures can be synthesized from various substituted starting materials that are commercially available or easily prepared. Furthermore, suitably substituted 3-arylisoquinolones generated by this method act as a starting point for synthesis

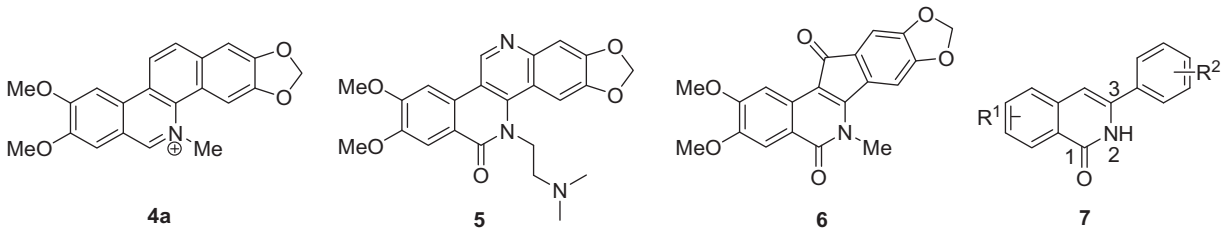
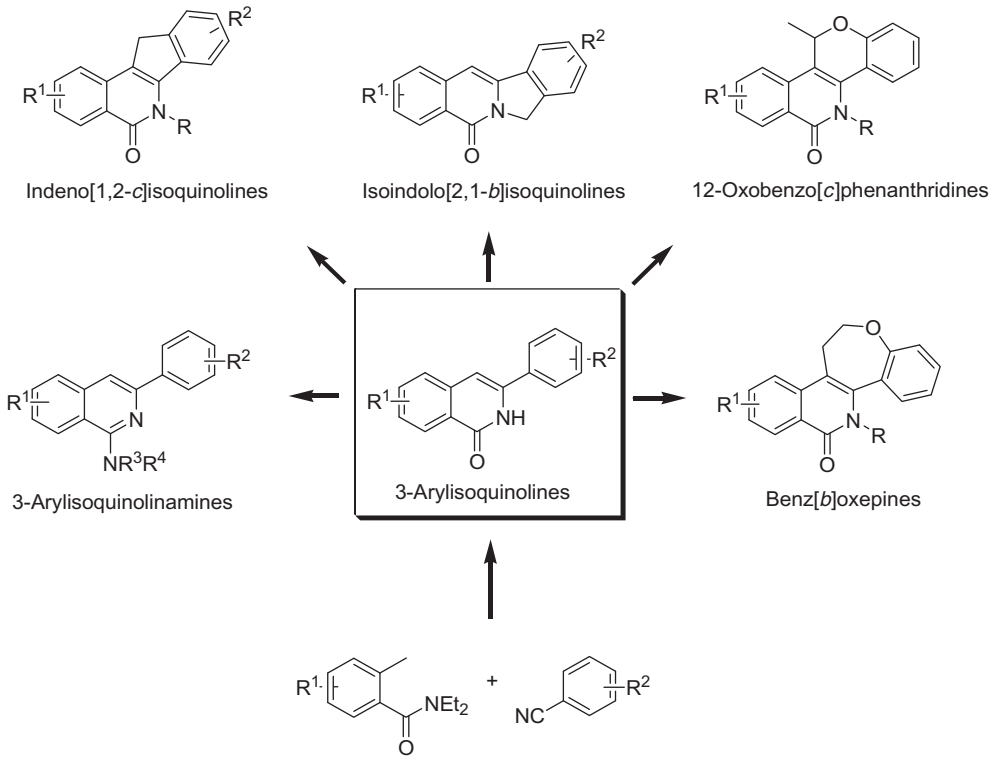
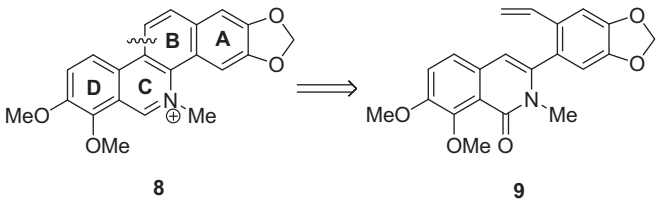


Figure 2. Benzo[*c*]phenanthridine **4a**, 5*H*-dibenzo[*c*,*h*]1,6-naphthyridinone **5**, indeno[1,2-*c*]isoquinoline **6**, 3-arylisoquinoline **7**.



Scheme 1. 3-Arylisoquinoline as precursor of different classes of topo I inhibitors.



Scheme 2. 3-Arylisoquinoline as bioisostere of benzo[*c*]phenanthridine.

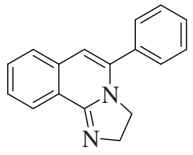


Figure 3. 5-Aryl-2,3-dihyroimidazo[2,1-*a*]isoquinoline **10**.

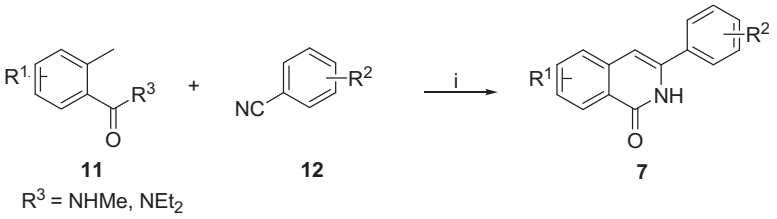
of varied classes of compounds.[26] Substituted *N*-methyl-*o*-toluamides or *N*,*N*-diethyl-*o*-toluamides **11** on treatment with 2 equiv of *n*-butyllithium form dimetalated, orange-red colored reaction intermediates (Scheme 3). The dilithiated species undergo intramolecular cycloaddition with substituted benzonitriles **12** to yield 3-arylisoquinolones **7**.

3. 3-Arylisoquinolinamines

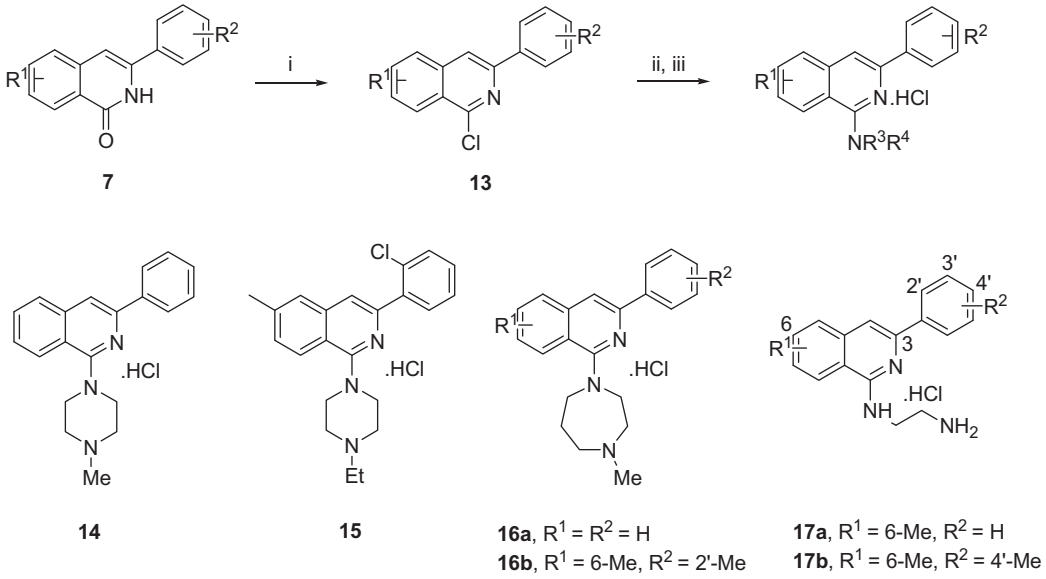
Structure–activity relationship (SAR) studies were performed for 3-arylisoquinoline to identify the active pharmacophore. During such SAR studies, water-soluble 3-arylisoquinolamine, 1-(4-methylpiperazinyl)-3-phenylisoquinoline hydrochloride (**14**) was developed with potent in vitro cytotoxicity, higher T/C (160%) and low toxicity.[33] The compound was stable in acidic conditions, lipophilic in nature and exhibited high oral bioavailability with a large volume of distribution.[34,35] Since the 3-arylisoquinolinamine exhibited properties essential for an oral drug, new 3-arylisoquinolinamines were synthesized and screened against topo I.

Compound **14** and related 3-arylisoquinolinamines were prepared by 1-amination of imine chlorides **13** obtained by reacting 3-arylisoquinolones (**7**) with phosphorus oxychloride (Scheme 4).[36] 3-Naphthylisoquinolinamine (**21**) was also synthesized in a similar way from 3-arylisoquinolone (**20**, Scheme 5).

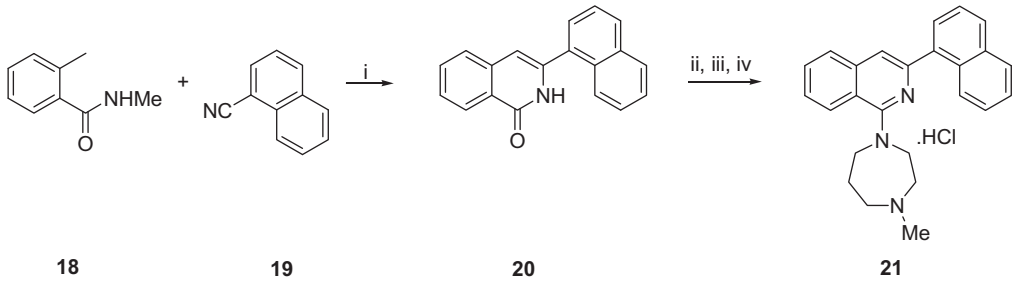
N-Methylhomopiperazine compound **16b** (IC_{50} = 0.44 μM, A549) and 3-naphthylisoquinolinamine (**21**) (IC_{50} = 0.52 μM, A549) exhibit cytotoxicity at submicromolar concentrations (Table 1).[36]



Scheme 3. Synthesis of 3-arylisoquinolone by lithiated toluamide-benzonitrile cycloaddition method. Reagents and conditions: (i) *n*-BuLi, THF.



Scheme 4. Synthesis of 3-arylisoquinolinamine and representative 3-arylisoquinolinamines. Reagents and conditions: (i) $POCl_3$; (ii) NHR^3R^4, K_2CO_3, DMF, reflux; (iii) HCl.



Scheme 5. Synthesis of 3-naphthylisoquinolinamine. Reagents and conditions: (i) *n*-BuLi, THF; (ii) $POCl_3$; (iii) *N*-methylhomopiperazine, K_2CO_3, DMF; (iv) HCl.

Table 1
IC_{50} cytotoxicity (μM) and topo I inhibition activity of 3-arylisoquinolinamines

Compound	A549	SK-OV-3	SK-MEL-2	HCT15	Topo I[a]
15	13.36	194.91	5.58	5.58	+++
16a	10.84	17.01	8.38	11.09	+++
16b	0.44	0.70	0.99	1.20	++
17a	>300	>300	>300	>300	+++
17b	>300	>300	>300	>300	+++
21	0.52	3.64	1.98	1.93	+
14	13.56	13.71	14.53	13.94	nt[b]
Doxorubicin	0.97	1.17	4.78	1.67	nt
CPT	—	—	—	—	++

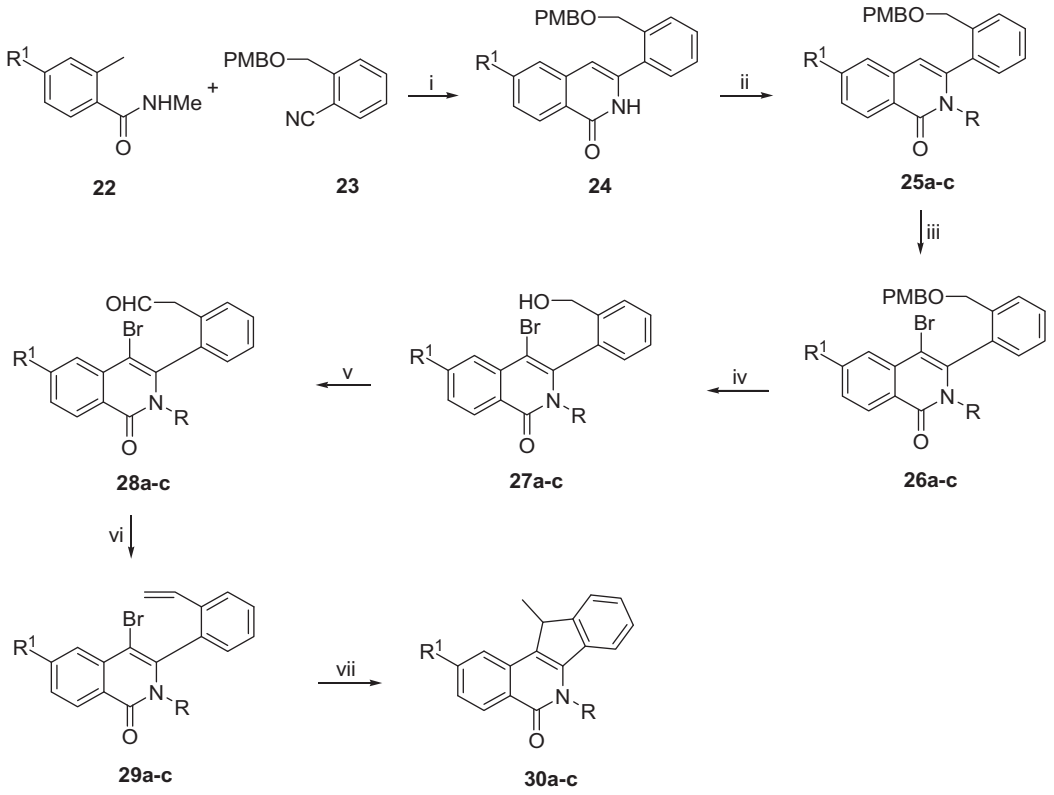
[a] Activity is expressed semi-quantitatively as follows: +, weak activity; ++, similar activity as CPT; +++, stronger activity than CPT.
[b] nt, not tested.

Isoquinolinamine **16b** has dimethyl substituents on aromatic rings, which confirms that methyl substituents on the aromatic rings of 3-arylisoquinoline enhance cytotoxicity.[37] 3-Arylisoquinolinamines with the ethylenediamine moiety (**15**, **16** and **17**) exhibit relatively potent topo I inhibition. Ethylenediamine analogues (**17a** and **17b**) show weak cytotoxicities (IC_{50} >300 μM), although their inhibition of topo I is greater than CPT. These unusual results may be due to differences in factors affecting cell cytotoxicity such as cell penetration and distribution within the cell. Topo I inhibition by compound **17b** is due to strong hydrogen bond between the ethylenediamine group and the carboxyl group of Asp 533 (2.221 Å), the phosphoester group of Guanine 12 (1.490 Å) and the amide carbonyl moiety of Gln 633 (2.739 Å) of topo I.

The bond linking the isoquinoline ring with the phenyl ring in the 3-arylisoquinoline is rotatable, but a flexible compound like 3-arylisoquinolamine lacks receptor specificity and efficiency whereas rigidified structures have little conformational entropy and can be more efficiently fitted into the active site of the receptor.[38] Based on this rationale, a constrained form of the 3-arylisoquinoline can be developed by linking the isoquinoline ring and the 3-aryl ring with variable bonds to form an additional ring. A similar approach has been applied to develop rigid indenoisoquinoline and isoindoloisoquinoline with an additional five-membered ring, 12-oxobenzo[*c*]phenanthridine with a new six-membered pyran ring, and benz[*b*]oxepine with an extra seven-membered oxepine ring.

4. Indenoisoquinolines

Because of various shortcomings of CPTs, a program for the development of novel non-CPT topo I inhibitors was initiated by the Cushman group, and indenoisoquinoline NSC 314622 (**6**) with a cytotoxicity profile comparable to CPT was discovered.[39] NSC 314622 cleaved DNA at micromolar concentrations at a different site from CPT. The cleavage complex trapped by the indenoisoquinoline was more stable than that induced by CPT. NSC 314622 was effective against CEM/C2, a CPT-resistant cell line with a point mutation in topo I. Based on these findings, various regions of the lead compound NSC 314622 were optimized by synthesizing and evaluating several derivatives. 11-Alkenyl-, 11-aminoalkenyl-, 11-diaminoalkenyl-, bisindenoisoquinolines and indenoisoquinoline



Scheme 6. Synthesis of 11-methylindenoisoquinoline. Reagents and conditions: (i) *n*-BuLi, THF; (ii) MeI, NaH or PMBCl, K_2CO_3; (iii) NBS, ACCN, CCl_4, *hv*; (iv) DDQ, H_2O, CH_2Cl_2; (v) PDC, CH_2Cl_2; (vi) $Ph_3P^+CH_3Br^-$, *n*-BuLi, THF; (vii) *n*-Bu_3SnH, ACCN, Toluene. (a) R = Me, R^1 = H; (b) R = R^1 = Me; (c) R = PMB, R^1 = Me.

with a nitro group in the isoquinoline aromatic ring, various substituents in the indenone ring or aromatic rings without substituents were studied.[40–45] In addition, the side chain of the lactam nitrogen with polyamine and different nitrogen heterocycles and varying chain lengths were used in the optimization of the lead.[46–48]

The indenoisoquinoline nucleus was obtained from indenopyran and 3-aryl-3,4-dihydro-isoquinolinone separately. Substituted and unsubstituted indenopyran were condensed with primary amines to yield various indenoisoquinolines with topo I inhibitory activity.[45,48–52] Alternatively, indenoisoquinolines were synthesized by oxidative Friedel–Craft acylation of the *cis* form of 3-aryl-3,4-dihydroisoquinolone-4-carboxylic acids with thionyl chloride. The diastereomeric mixture of *cis* and *trans* forms of the isoquinolones was formed by condensation of homophthalic anhydride and Schiff base.[53,54] In addition to these indenoisoquinolines, the development of novel 11-methyl, 11-alkoxy and indenoisoquinolines as cytotoxic agents targeting topo I is described below.

Table 2
IC_{50} cytotoxicity (μM) and topo I inhibition activity of 11-methylindenoisoquinolines and related precursors

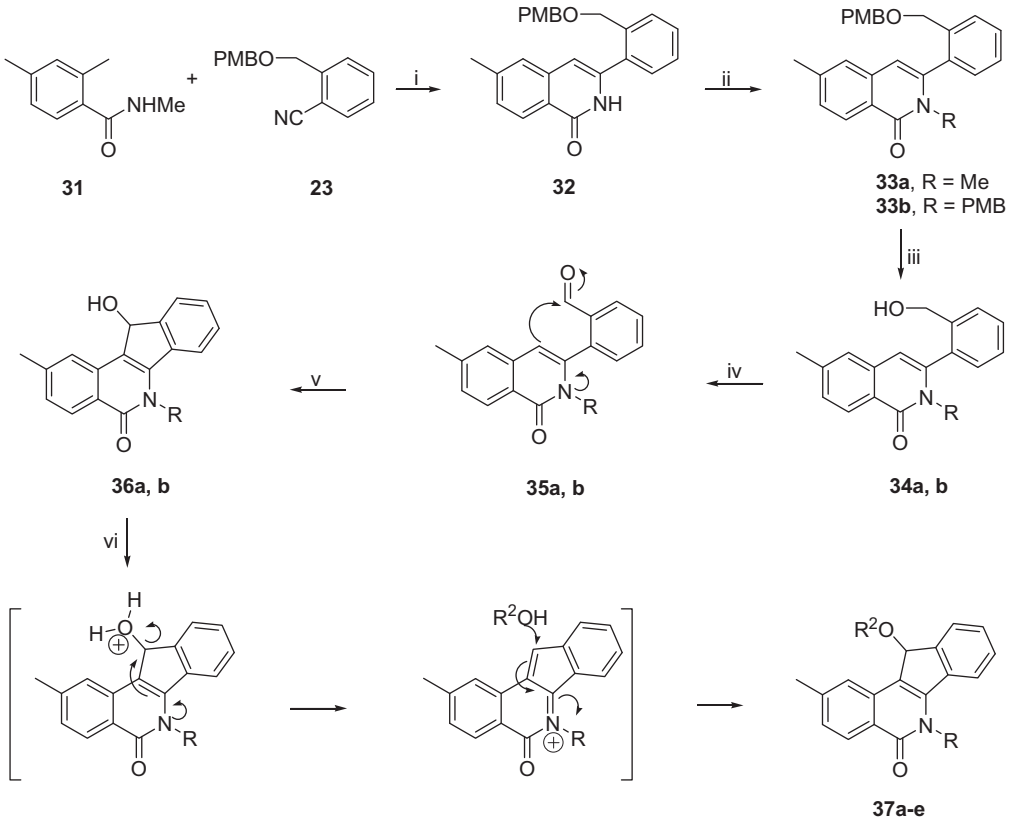
Compound	A549	Col2	SNU-638	HL60	HT1080	Topo I[a]
26a	77.64	>100	>100	76.7	>100	–
27c	43.37	23.4	16.4	>100	31.7	–
28a	60.67	67.4	16.4	6.0	11.4	–
29b	49.35	61.1	14.0	18.3	38.6	++
30a	2.58	4.90	7.40	1.70	5.80	–
30b	2.98	7.4	9.0	7.1	5.9	–
30c	3.84	4.40	6.60	8.50	4.20	–
Ellipticine	1.9	2.3	2.2	5.8	4.3	nt[b]
CPT	0.069	0.045	0.098	0.018	0.080	++++

[a] Activity is expressed semi-quantitatively as follows: –, very weak activity; ++, weak activity; ++++, similar activity as CPT.
[b] nt, not tested.

4.1. 11-Methylindenoisoquinolines

Synthesis of 11-methylindenoisoquinoline strategically involves formation of styrene derivatives (**29a–c**, Scheme 6).[55] Styrene compounds are prepared by formation of isoquinolines **24** by cycloaddition of lithiated toluamides **22** and benzonitriles **23**. The amide nitrogen of the isoquinoline is then alkylated by MeI or PMBCl in presence of base NaH and K_2CO_3 respectively. The alkylated isoquinoline is selectively brominated with subsequent oxidative deprotection of *p*-methoxybenzyl (PMB) by DDQ to yield allyl alcohols (**27a–c**). The allyl alcohols are further oxidized into aldehydes by pyridinium dichromate (PDC) and are subjected to the Wittig reaction with Ph_3PCH_3Br in the presence of *n*-BuLi to give the desired styrenes (**29a–c**). These styrenes undergo a ring closure reaction through the 5-*exo*-trig pathway to form 11-methylindenoisoquinolines (**30a–c**). Formation of 11-methylindenoisoquinoline is preferred to that of benzo[*c*]phenanthridine as the 5-*exo*-trig pathway is favored over the 6-*endo*-trig pathway, per Baldwin's rule.[56]

Indenoisoquinolines (**30a–c**) show potent cytotoxicity against five cancer cell lines (A549, Col2, SNU-638, HL60 and HT1080) compared with their respective 3-arylisoquinolines (Table 2). Although the indenoisoquinolines (**30a–c**) show potent in vitro cytotoxicity (IC_{50} = 1.70 μM, HL60; 2.58 μM, A549), they exhibit no significant topo I inhibition.[55] This discrepancy between potent cytotoxicity and absence of topo I inhibition has been identified for other indenoisoquinolines.[41,45,48] Similar observations have also been reported for anthracene and acridine polyamine conjugates inhibiting topoisomerase II (topo II).[57] The anomaly has been sug-



Scheme 7. Synthesis of 11-alkoxyindenoisoqinolines. (i) *n*-BuLi, THF, -70 °C; (ii) MeI, NaH or PMBCl, K_2CO_3; (iii) DDQ, H_2O, CH_2Cl_2; (iv) PDC, CH_2Cl_2; (v) 10% HCl, acetone; (vi) R^2OH, 10% HCl.

gested to be due to the interaction of the test compounds with unknown biological targets rather than the desired topo I or topo II.

4.2. 11-Alkoxyindenoisoquinolines

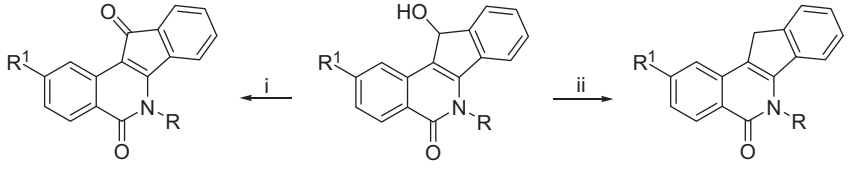
11-Alkoxyindenoisoquinoline has oxygen functionality at C11 that corresponds to the 11-carbonyl oxygen of 5,11-diketoindenoisoquinoline **6**. 11-Keto oxygen is essential for H-bonding with Arg 364 of topo I.[58] 11-Alkoxyindenoisoquinoline was designed to mimic 11-diketoindenoisoquinoline.

Synthesis of 11-hydroxyindenoisoquinoline and 11-alkoxyindenoisoquinoline is initiated by formation of isoquinolines (**32**, Scheme 7).[59] N-Alkylation by methyl and PMB groups followed by oxidative deprotection by DDQ gives allyl alcohols (**34a**, **34b**). Upon further oxidation by PDC, the allyl alcohols yield aldehydes (**35a**, **35b**), which act as precursors for the formation of 11-hydroxyindenoisoquinolines (**36a**, **36b**). The indeno ring of 11-hydroxyindenoisoquinolines (**36a**, **36b**) is formed by enamide aldehyde cyclization of the corresponding compounds (**35a**, **35b**). When reacted with various aliphatic alcohols in acidic conditions, 11-hydroxyindenoisoquinolines form the respective 11-alkoxyindenoisoquinolines (**37a**–**e**). The alkoxy derivatives are formed by

Table 3
IC_{50} cytotoxicity (μM) and topo I inhibition activity of 11-alkoxyindenoisoquinolines and related analogs

Compound	R	R^1	R^2	A549	HCT15	OV-3	MEL-2	Topo I[a]
36b	PMB	—	—	100.41	20.25	70.99	180.72	–
37a	Me	—	^{n}Pr	40.60	10.89	70.68	80.34	–
37b	Me	—	^{i}Bu	28.15	27.39	38.37	10.52	–
37c	PMB	—	^{n}Pr	3.45	1.42	5.76	6.26	+++
37d	PMB	—	^{i}Pr	6.22	0.91	1.21	2.43	+++
37e	PMB	—	^{i}Bu	1.87	9.92	1.63	2.07	+++++
38a	Me	Me	—	20.05	14.34	16.29	17.14	–
39b	PMB	Me	—	50.98	8.9	23.2	31.9	–
Doxorubicin	—	—	—	0.97	1.67	1.17	4.78	
CPT	—	—	—	0.067	0.080	0.024	0.075	+++

[a] Activity is expressed semi-quantitatively as follows: –, very weak activity; +++, similar activity as CPT; +++++, stronger activity than CPT.



Scheme 8. Oxidation and reduction of 11-hydroxyindenoisoquinolines. Reagents and conditions: (i) PDC, CH_2Cl_2; (ii) H_2, Pd/C, CH_3COOH, EtOH, 80 psi, rt.

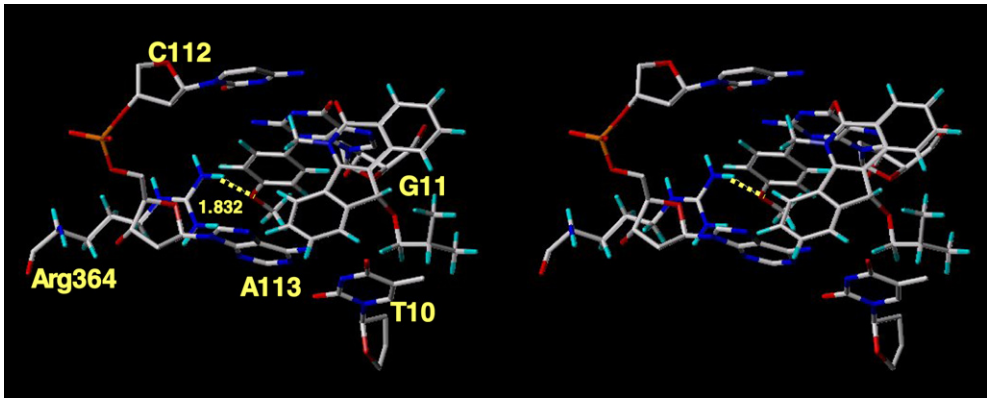


Figure 4. Wall-eyed view of docked model of **37e** in ternary complex with DNA and topo I.

acid-catalyzed dehydration and consecutive nucleophilic attack of aliphatic alcohols at C11 of (**36a**, **36b**). 11-Keto- (**38a**–**c**) and 11-dihydroindenoisoquinolines (**39a**–**c**) are formed by oxidation and reduction of 11-hydroxyindenoisoquinolines, respectively (Scheme 8).

11-Hydroxy-**36**, 11-keto-**38** and 11-dihydroindenoisoquinolines **39** do not exhibit potent cytotoxicity against four human tumor cell lines: A549, HCT15, SKOV-3 and SK-MEL-2 (Table 3).[59] Furthermore, none of these compounds inhibit topo I. However, 11-alkoxyindenoisoquinolines (**37c**–**e**) with amide group protected by PMB group exhibit potent cytotoxicity and topo I inhibition. 11-Propyl-**37c** and 11-isopropylindenoisoquinoline **37d** with PMB-protected amide group have strong cytotoxicity and topo I inhibition similar to CPT. 11-Isobutoxyindenoisoquinoline **37e** with PMB at the C2 nitrogen display potent cytotoxicity (IC_{50} = 1.87 μM, A549; 1.63 μM, OV-3; 2.07 μM, MEL-2) and greater topo I inhibition activity than camptothecin. The potent activity of **37e** is further supported by the docking study. Hypothetical binding model of the indenoisoquinoline with polished crystal structure of topo I and 22 base paired DNA (PDB ID: 1SC7) illustrates that the PMB group of **37e** intercalates between the −1 and +1 bases, parallel to the plane of base pairs. The oxygen of the PMB group has H-bond with Arg 364, which is considered an essential amino acid that binds the ligand in topo I-DNA cleavage complex (Fig. 4). In addition, the indenoisoquinoline ring lies in the cavity between DNA and topo I residues (Ala 351, Asn 352 and Lys 425), perpendicular to DNA base pairs.

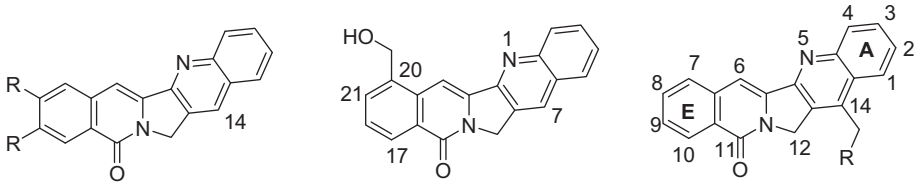
5. Isoindolo[2,1-*b*]isoquinolines

After hydrolysis in blood, the α-hydroxy δ-lactone ring of CPT forms an inactive carboxylate. The inactive carboxylate has high affinity for human serum albumin. Above all, the sodium carboxylate of CPT is highly toxic. Thus it can be seen that many problems are associated with the E ring of camptothecin. Thus, several modifications on the lactone ring have been made to stabilize camptothecin. One of these modifications is replacement of the lactone ring with an aromatic ring to afford a class of compounds called aromathecins.[60] Aromathecin is a hybrid structure of camptothecin and indenoisoquinoline.[61] Simple aromathecins such as rosettacin (**40a**) and dimethoxyrosettacin (**40b**), the rare natural product 22-hydroxyacuminate (**41**) and its AB-ring substituted analogs, are weak topo 1 inhibitors with low cytotoxicity profiles (Fig. 5).[60–62] The topo I inhibitory and antiproliferative activities of aromathecins have recently been improved by introducing mono and diamines, amino alcohols and nitrogenous heterocycles at C14.[63,64] Activities of C14-substituted aromathecins (**42**) are further improved by substituting various functional groups with steric, electronic and H-bonding properties on aromatic ring A.[65] Topo I-mediated DNA cleavage patterns induced by aromathecins resemble those induced by both camptothecin and indenoisoquinolines.[63,64]

The extent of structural similarities between camptothecin and indenoisoquinoline can be varied to broaden the diversity of topo I-targeted compounds. Aromathecin resembles camptothecin more than indenoisoquinoline with respect to structure. Strategically, a compound can be designed that shares more structural features with indenoisoquinoline than with camptothecin, as in the case of isoindolo[2,1-*b*]isoquinolines. Isoindolo[2,1-*b*]isoquinolines are synthesized as constrained forms of 3-arylisoquoline by intramolecular S_N2 reaction of mesyl intermediate (**47**, Scheme 9).[66]

Isoindolo[2,1-*b*]isoquinolines are synthesized in three steps. 3-Arylisoquinolines **45** are obtained by cycloaddition of lithiated toluamides **43** and benzonitriles (**44**, Scheme 9). Deprotection of hydroxyl group with DDQ and 10% HCl for PMB-protected and methoxymethyl (MOM)-protected compounds, respectively, yields hydroxymethyl compounds (**46a**–**i**). Finally, isoindolo[2,1-*b*]isoquinolines (**48a**–**i**) are obtained by reacting hydroxymethyl compounds with $MsCl/Et_3N$ in methylene chloride.

Isoindolo[2,1-*b*]isoquinolines (**48a**–**i**) exhibit potent cytotoxicity against A549, HCT15 and OV-3 cell lines (IC_{50} = 3.70–49.09 μg/mL, Table 4). Disappointingly, isoindolo[2,1-*b*]isoquinolines (**48e**–**i**) are

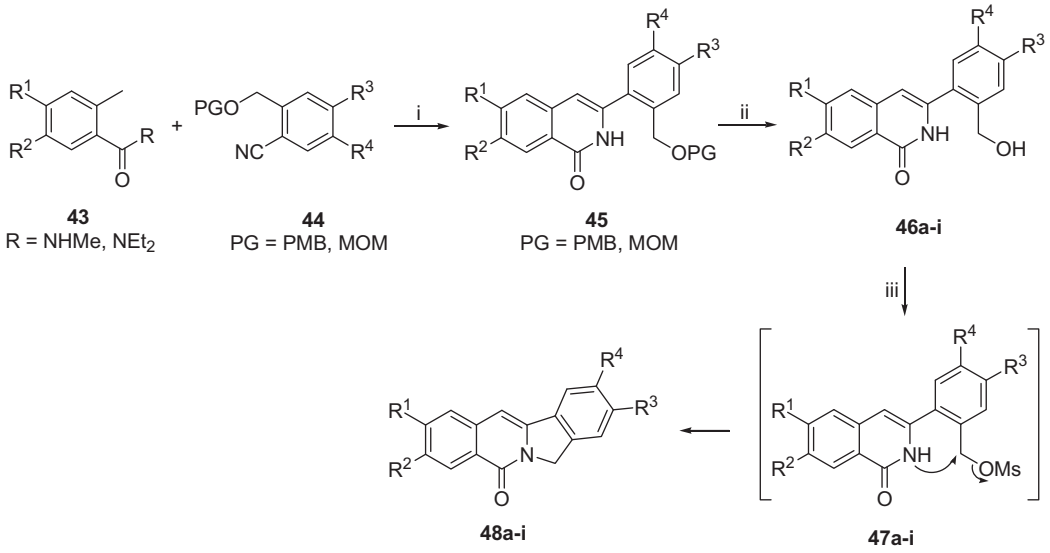


40a, R = H
40b, R = OMe

41

42

Figure 5. Rosettacin (**40a**), dimethoxyrosettacin (**40b**), 22-hydroxyacuminate (**41**), 14-substituted aromathcins **42**.



Scheme 9. Synthesis of isoindoloisoquinolines. Reagents and conditions: (i) *n*-BuLi, THF; (ii) DDQ or 10% HCl; (iii) MsCl, Et_3N, CH_2Cl_2. (a) $R^1 = R^2 = R^3 = R^4 = H$; (b) R^1 = Me, $R^2 = R^3 = R^4 = H$; (c) $R^1 = R^3 = R^4 = H$, R^2 = Me; (d) $R^1 = R^2 = R^4 = H$, R^3 = OMe; (e) $R^1 = R^2 = H$, $R^3 = R^4$ = OMe; (f) R^1 = Me, $R^2 = R^4 = H$, R^3 = OMe; (g) R^1 = Me, $R^2 = H$, $R^3 = R^4$ = OMe; (h) $R^1 = R^4 = H$, R^2 = Me, R^3 = OMe; (i) $R^1 = H$, R^2 = Me, $R^3 = R^4$ = OMe.

Table 4
IC_{50} cytotoxicity (μg/mL) and topo I inhibition activity of isoindoloisoquinolines

Compound	A549	HCT15	OV-3	Topo I[a]
48a	3.70	43.93	14.84	++++
48b	15.94	46.35	44.12	++
48c	41.44	35.27	48.92	++
48d	9.25	7.29	12.01	++++
48e	36.75	9.12	12.69	–
48f	5.95	8.78	5.52	+
48g	49.09	34.98	36.15	–
48h	45.12	34.33	15.34	+
48i	24.13	6.56	7.23	+
CPT	0.072	0.089	0.035	++++

[a] Activity is expressed semi-quantitatively as follows: –, very weak activity; +, ++, weak activity; ++++, similar activity as CPT.

poor topo I inhibitors.[66] Also, isoindoloisoquinolines (**48b**, **48c**) with methyl group in isoquinoline ring exhibit low topo I inhibition compared with unsubstituted compounds, possibly due to steric hindrance between methyl substituents and the side chain of Thr 718. Interestingly, compounds (**48a**, **48d**) had similar topo I inhibition potency as camptothecin. Topo I inhibition by **48d** can be explained by a docking model. The isoquinoline ring of compound **48d** intercalates between the +1 and −1 bases parallel to the plane of base pairs in a similar manner to indenoisoquinolines. Moreover, the amide carbonyl shares an H-bond with Asn 722, an amino acid playing a vital role in the interaction with ligand in topo I-DNA cleavage complex. In addition, stable conformers of **48d** superimposed with topotecan clarifies that the isoindoloisoquinolines share properties with both indenoisoquinolines and CPTs.

6. 12-Oxobenzo[*c*]phenanthridines & benz[*b*]oxepines

Chemical compounds with diverse structures have been reported as non-camptothecin topo I-targeting agents. Benzo[*c*]phenanthridines, benzo[*i*]phenanthridines and 5*H*-dibenzo[*c*,*h*]1,6-naphthyridinones have been extensively screened as topo I inhibitors. Natural benzo[*c*]phenanthridine alkaloids such as nitidine (**4a**) and fagaronine (**4b**) stabilize the covalent binary complex of topoisomerase and DNA (Fig. 6). They inhibit the topo I-mediated relaxation of supercoiled DNA more effectively than camptothecin.[67] Various benzo[*i*]phenanthridines have been studied as non-charged substitutes for nitidine, which has a charged iminium moiety.[68,69] Benzo[*i*]phenanthridine derivative **49** exhibits similar topo I inhibition and cytotoxicity as nitidine.[69] 5*H*-Dibenzo[*c*,*h*]1,6-naphthyridinones were developed as water-soluble analogues of benzo[*i*]phenanthridines as the later ones had low solubility in the preparation for cytotoxicity assessment in vivo.[70] 5*H*-Dibenzo[*c*,*h*]1,6-naphthyridinones share structural similarities with benzo[*c*]phenanthridines and benzo[*i*]phenanthridines. 5*H*-8,9-Dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]-naphthyridin-6-one (ARC-111) (**5**) exhibit strong topo I-targeting activity and cytotoxicity *in vitro* and reasonable antitumor activity *in vivo*.[71]

Interestingly, 5*H*-dibenzo[*c*,*h*]1,6-naphthyridinones have a 3-arylisoquinoline moiety. 12-Oxobenzo[*c*]phenanthridine with *O*12 instead of the *N*12 in 5*H*-dibenzo[*c*,*h*]1,6-naphthyridinone has been synthesized from 3-arylisoquinolone. Thus, 12-oxobenzo[*c*]phenanthridine can be viewed as a bioisostere of 5*H*-dibenzo[*c*,*h*]1,

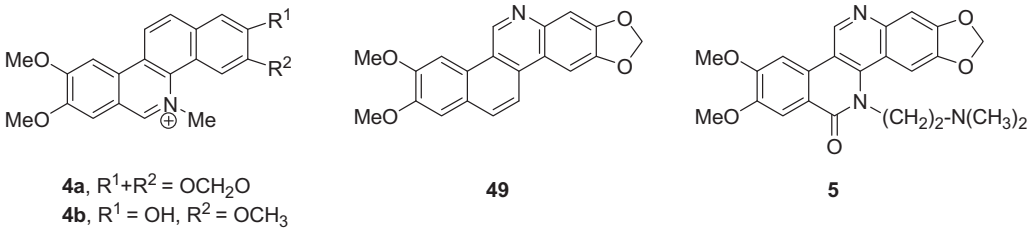
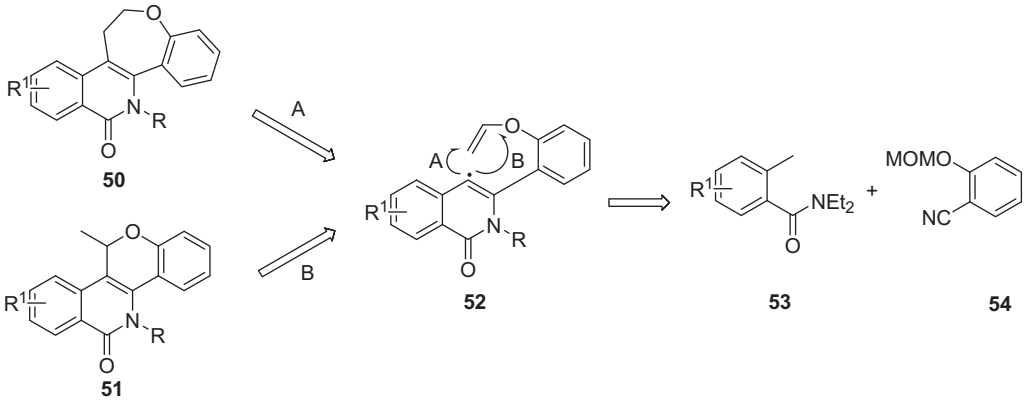
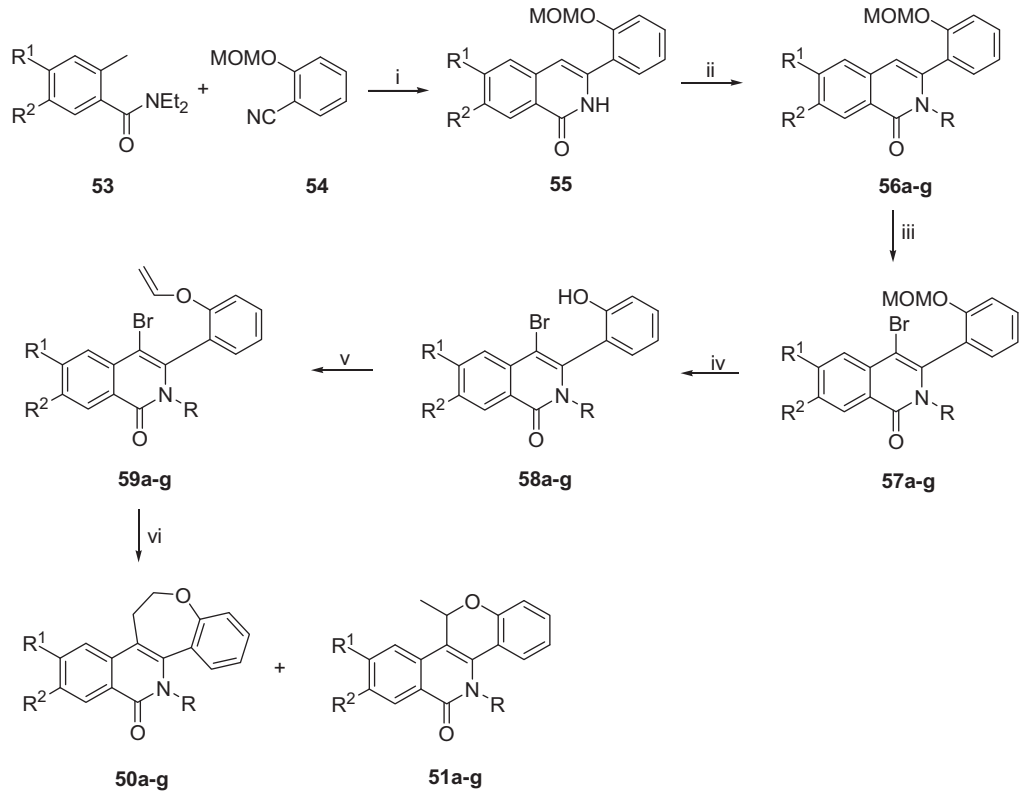


Figure 6. Nitidine (**4a**), fagaronine (**4b**), benzo[*i*]phenanthridine **49**, ARC-111 (**5**).



Scheme 10. Retrosynthesis of benz[*b*]oxepines and 12-oxobenzo[*c*]phenanthridines.



Scheme 11. Synthesis of benz[*b*]oxepines and 12-oxobenzo[*c*]phenanthridines. Reagents and conditions: (i) *n*-BuLi, THF; (ii) MeI, NaH or PMBCl, K_2CO_3; (iii) NBS, ACCN, CCl_4; (iv) 10% HCl, THF; (v) tetravinyl tin, $Cu(OAc)_2$, CH_3CN, O_2; (vi) *n*-Bu_3SnH, AIBN, benzene. (a) $R^1 = R^2 = H$, R = Me; (b) $R^1 = R = Me$, $R^2 = H$; (C) $R^1 = H$, $R^2 = R = Me$; (d) $R^1 = R^2 = OMe$, R = Me; (e)$R^1 = R^2 = H$, R = PMB; (f) $R^1 = Me$, $R^2 = H$, R = PMB; (g) $R^1 = H$, $R^2 = Me$, R = PMB.

6-naphthyridinone. Similarly, benz[*b*]oxepine has an oxepine ring instead of the pyan ring of 12-oxobenzo[*c*]phenanthridine.

Benz[*b*]oxepine (**50**) and 12-oxobenzo[*c*]phenanthridine (**51**) are formed through intramolecular radical cyclization. Benz[*b*]oxepine and 12-oxobenzo[*c*]phenanthridine are formed by endo type (A) and exo type (B) cyclization pathways (Scheme 10), respectively.[72] N-Alkylation, regioselective C4-bromination, and MOM deprotection of isoquinolines (**55**) formed by lithiated toluamide-benzonitrile coupling reaction give phenolic compounds (**58a**–**g**, Scheme 11). When reacted with tetravinyl tin and $Cu(OAc)_2$ in the presence of O_2, the phenols afford *O*-vinyl compounds (**59a**–**g**). Benz[*b*]oxepines (**50a**–**g**) and 12-oxobenzo[*c*]phenanthridines (**51a**–**g**) are synthesized through intramolecular radical cyclization of *O*-vinyl compounds with *n*-Bu_3SnH and azobisisobutyronitrile (AIBN). Formation of benz[*b*]oxepine by the 7-*endo-trig* pathway is favored over formation of 12-oxobenzo[*c*]phenanthridine. However, the ratio of formation of benz[*b*]oxepine and 12-oxobenzo[*c*]phenanthridine is mainly dependent upon substitution on the precursor **59** as shown: **50a**:**51a** (56:0), **50b**:**51b** (86:0), **50c**:**51c** (61:0), **50d**:**51d** (38:27), **50e**:**51e** (45:31), **50f**:**51f** (40:25), **50g**:**51g** (35:27). The compounds with *N*-PMB substitution (**59e**–**g**) yield only benz[*b*]oxepines.

12-Oxobenzo[*c*]phenanthridine analogs (**51a**–**d**) showed weak cytotoxicity toward A549, HL60, HELA cancer cell lines (Table 5).[72] Moreover, the topo I inhibitory activity of these compounds were very low (maximum 30%). In contrast, benz[*b*]oxepine showed relatively potent cytotoxicity and topo I inhibitory activity. *N*-PMB-substituted compounds (**50e**–**g**) were more cytotoxic than

Table 5
IC_{50} cytotoxicity (μM) and topo I inhibition activity (% inhibition) of benz[*b*]oxepines and 12-oxobenzo[*c*]phenanthridines

Compound	A549	HL60	HELA	Topo I
50a	66.76	37.59	19.66	–[a]
50b	61.32	33.57	41.57	–
50c	30.33	14.12	8.76	11.9
50d	72.98	34.82	38.06	98.2
50e	11.04	4.49	4.78	94.5
50f	>100	9.26	10.26	–
50g	4.56	4.40	4.54	–
51a	65.52	30.05	40.08	–
51b	46.00	29.13	28.32	30.4
51c	66.89	nt[b]	21.88	–
51d	13.76	8.54	9.02	5.3
CPT	0.058	0.089	0.072	86.2

[a] No inhibition activity.
[b] Not tested.

compounds (**50a**–**d**) with *N*-methyl substitution. Benz[*b*]oxepine **50e** exhibited both potent cytotoxicity and topo I inhibitory activity (94.5%). The oxepine ring of compound **50e** intercalated between +1 and −1 bases, parallel to the plane of base pairs. The oxygen of the oxepine ring had an H-bond with A113, a nucleotide essential for binding of ligand to topo I-DNA complex. In contrast to 11-alkoxyindenoisoquinoline, the oxepine group acted as an intercalator and the PMB group was located in the cavity between DNA and topo I.

7. Conclusion

Beside indeno[1,2-*c*]isoquinolines, isoindolo[2,1-*b*]isoquinolines, 12-oxobenzo[*c*]phenanthridines and benz[*b*]oxepines, natural alkaloids benzo[*c*]phenanthridines (including topo I poisons like nitidine and fagaronine)[73–78] and protoberberines[76,78–81] have been synthesized from 3-arylisoquinoline. Therefore, 3-arylisoquinoline could act as a potential source for novel anticancer benzo[*c*]phenanthridines and protoberberines targeting topo I. Furthermore, studies on free 3-arylisoquinolinamines and constrained compounds with new ring systems and diverse structures could be carried on the basis of receptor-based drug design for development of anticancer agents with more specificity and less toxicity.

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