*Ethiopian Journal of Science and Sustainable Development*

*e-ISSN 2663-3205* *Volume 8 (1), 2021*

Journal Home Page: www.ejssd.astu.edu.et

Review Paper

# Recent Trend in Synthesis of *Indenoisoquinoline Analoges* as *Topoisomerase I* Inhibitor and Cytotoxic Property: Review

Digafie Zeleke, Yadessa Melaku*

*Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, P. O. Box 1888, Adama, Ethiopia*

**Article Info**

**Article History**:
*Received 20 November 2020*
*Received in revised form 18 December 2020*
*Accepted 02 January 2021*

**Keywords:**
*Indenoisoquinolinediones*
*Cytotoxic*
*Antitumor*

**Abstract**

*Indenoisoquinolinediones* are a class of *non-camptothecin topoisomerase I* inhibitors that display marked cytotoxic and potent antitumor properties. Currently phase 2 clinical trial of three *indenoisoquinoline* derivatives LMP400 (indotecan, NSC724998), LMP776 (indimitecan, NSC725776) and LMP744 (MJ-III-65, NSC706744) is on progress. In the last two decades, various synthetic methodologies have been developed for syntethesis of *indenoisoquinolines* and its analogs. These compounds are of great research significance owing to their novel structures and broad biological activities including antitumor, cytotoxic and topomerase I inhibitory properties. This review address the recent trend in synthesis experimental protocols of *indenoisoquinolines* and their cytotoxicity, antitumor and topomerase I inhibitory efficacy during the period 2015-2020. The synthetic methodologies and bioactivities reviewed herein might serve as a reference for researchers who are interested to work in the area.

## 1. Introduction

Cancer is a major health problem projected to affect about 22 million people by 2030 and has become the second leading cause of morbidity and mortality after cardiac disease.(Bray et al., 2012) It is believed that one of the causes of caner is a genetic disease caused due to mutations in genes associated with cell proliferation and cell death that results in DNA damage.(Baikar and Malpathak, 2010) Among the variety of molecular targets for cancer therapy, DNA topoisomerases (topos) are well-characterized targets owing to their essential roles in triggering, controlling, and modifying a wealth of topological DNA problems during cell proliferation, differentiation and survival.(Chen et al., 2013; Hu et al. 2018) The human genome encodes six topoisomerases whereas *E. coli* encodes four.(Pommier et al., 2010)On the basis of their mechanisms, eukaryotic topoisomerases can be classified into two major classes, type I and type II, but there are subtypes under each class.(Delgado et al., 2018; Hevener et al., 2018; Liu et al., 1980).

### 1.1. DNA Topoisomerase I Inhibitors

Camptothecin (CPT) is a potent antitumor drug, an alkaloid isolated from the Chinese tree, *Camptotheca acuminate*, also known as the "tree of joy" by Monroe Wall and coworkers (Figure 1) (Baikar and Malpathak, 2010). Camptothecin carboxylate was tested clinically in the mid-1970s and showed anticancer activity, but was discontinued because of its side effects(Pommier, 2006; Wall et al., 1993; Yu et al., 2012).Two water-soluble camptothecin derivatives (irinotecan and topotecan) are presently approved by the Food and drug

* *Corresponding author, e-mail: yadessamelaku2010@gmail.com*

https://doi.org/10.20372/ejssdastu:v8.i1.2021.314

administration (FDA) for intravenously administration (Figure 1). Topotecan is used to treat ovarian cancers and small-cell lung cancers (SCLC), but yet with some side effects (Pommier, 2009). Irinotecan is approved by the FDA for colorectal tumors. It is a prodrug and needs to be converted to its active metabolite SN-38 by carboxylesterase (Figure 1) and it has also some undesired effects (Pommier, 2009). Two other newer camptothecin derivatives, gimatecan and belotecan are in clinical trials (Figure 1) (Beecham and Corp, 1995; Pommier, 2009; Zhang et al., 2005).

### 1.2. Indenoisoquinolines as topoisomerase I inhibitors

Though camptothecin derivatives are effective against previously resistant tumors and are the only class of topoisomerase I (Top1) inhibitors approved for cancer treatment in 1970s, yet, they have pharmacologic and clinical limitations that restrict the dose of active drug that can reach the tumor while sparing normal tissues (Pommier, 2012; Staker et al., 2005). A major limitation to the clinical efficacy of camptothecin-containing therapies is represented by drug resistance (Beretta et al., 2006). The "classical" mechanisms of resistance to CPTs have been extensively studied and include: i) pre-target events, which result in reduced accumulation or inadequate subcellular localization of drug in the cell (i.e. drug efflux, metabolism and intracellular drug distribution); ii) target related events, which result in reduced drug-target interaction (e.g., TopoI down-regulation or gene mutation); iii) post-target events, which result inalterations in the cellular response to DNA damage generated by the formation of the ternary complex (e.g., tyrosyl DNA phosphodiesterase 1, TDP-1) were typical (Beretta et al., 2013; Sharma et al., 2015). In 1978, Pommier and Cushman synthersized the first indenoisoquinolines (later named as NSC 314622) unexpectedly while treating a *cis* substituted isoquinolone with $SOCl_2$ afforded the aforementioned indenoisoquinolineinstead of its acid chloride (Figure 2). The topoisomerase I inhibitory activity of indenoisoquinolines was identified in 1998 when a COMPARE algorithm analysis was performed on NSC 314622, which indicated that it may act in a manner similar

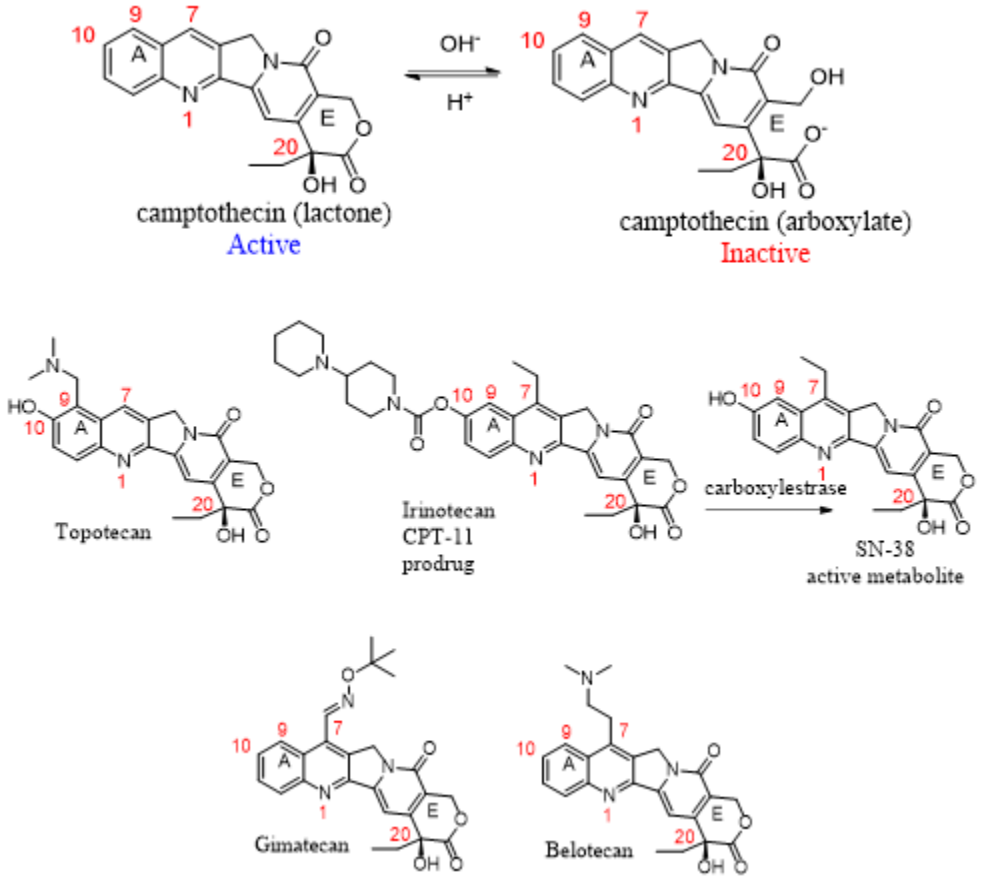



**Figure 1**: Camptothecin and its clinically approved derivatives

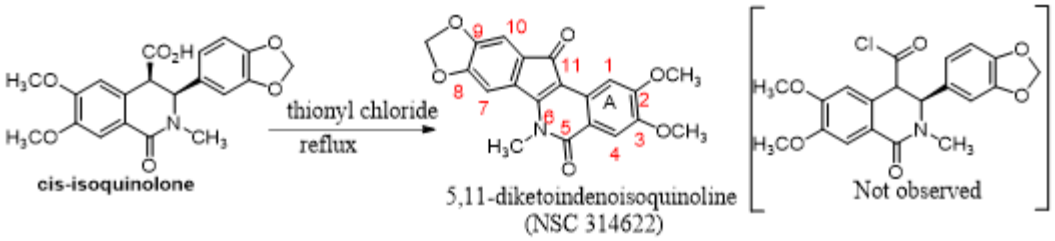



**Figure 2**: The first synthesized indenoisoquinoline

to that of camptothecin and derivatives. After being confirmed *in vitro* test, more potent indenoisoquinoline derivative was developed with an the maximal concentration of drug to cause 50% inhibition of biological activity of cancer cells ($IC_{50}$) of 20 µmol/L (Burke and Mi 1994; Cushman and Cheng 1978; Marzi et al. 2018; Morrell et al. 2006; Strumberg et al. 1999).

### 1.3. Biological activities of indenoispquinolines

Irinotecan and topotecan are the only current approved Top1 inhibitors approved by FDA for the treatment of cancer. However, these campotothecin derivatives are not ideal drug molecules owing to reversablity of the ToP-DNA cleavage complex and structurally they suffered from lactone ring opening to form a hydroxy acid that has a high affinity for human serum albumin. As a result of this pharmacokenetic problem, there is a great intrest in the development of non-campotothecin Top1 inhibitors among which indenoisoquinolinediones are one of them. Structurally, indenoisoquinolinediones are highly fused compounds which contain a planar tetracyclic heteroring system equipped with multifarious functionalities as exemplified by the lead compound (NCS 314622) (Figure 2) (Morrell et al., 2006). They have been demonstrated to inhibit topoisomerase I enzymes by intercalating between the DNA base pairs and to stabilize a ternary complex consisting of the drug molecule, DNA and topoisomerase I (Morrell et al., 2006). The indenoisoquinolines, like the camptothecins, stabilize DNA–Top1 cleavage complexes by intercalating at the DNA cleavage site, resulting in inhibition of the re-ligation reaction (Marzi et al., 2018). Motivated by this discovery, around 400 Indenoisoquinoline derivatives have been synthesized and evaluated for Top1 inhibition using recombinant enzyme and purified DNA substrates and cellular assays in the NCI-60 cell line panel by various scientists across the globe (Pommier and Cushman, 2009). Most of the synthesized indenoisoquinolines were assessed for antiproliferative activity against 55 different human cancer cell lines of diverse tumor origins at the USA National Cancer Institute screen. The results of topoisomerase I DNA cleavage experiments were reported semiquantitatively and provide a means of comparison with the biological activity of camptothecin (++++) and with the the lead compound (NSC 314622) (++) as follows: +: weak activity; ++: similar activity as the parent lead compound (NSC 314622); +++ and ++++: greater activity than the lead compound (NSC 314622); ++++: similar activity as 1 µM camptothecin (Morrell et al., 2006; Strumberg et al., 1999).

## 2. Synthesis and biological evaluation of Indenoisoquinolines

Because of the multiple disease nature of cancer, one chemotherapeutic agent does not work on all cancer types. Thus, specific biomolecule targeted therapies have become more popular which requires various effective anticancer agents (Foto et al., 2020). Since the discovery of indenoisoquinolines as a novel class of potential anticancer drug candidates, extensive structural modifications have been introduced by altering the substituent of the tetracyclic pharmacophore. Out of nearly 400 synthesized indenoisoquinoline derivatives, currently three of them namely, indotecan (LMP400), indimitecan (LMP744) and LMP776 (MJ-III-65, NSC706744)) were promoted to a Phase I clinical trial in 2010 (Figure 3) and their Phase 2 clinical trial is on progress (Marzi et al., 2019, 2020).

Indenoisoquinolines offer a number of potential advantages over the camptothecins, including greater chemical stability, formation of more persistent cleavage complexes, and induction of a unique pattern of DNA cleavage sites (Nagarajan et al., 2003). A varieties of reactions and synthetic methodologies that

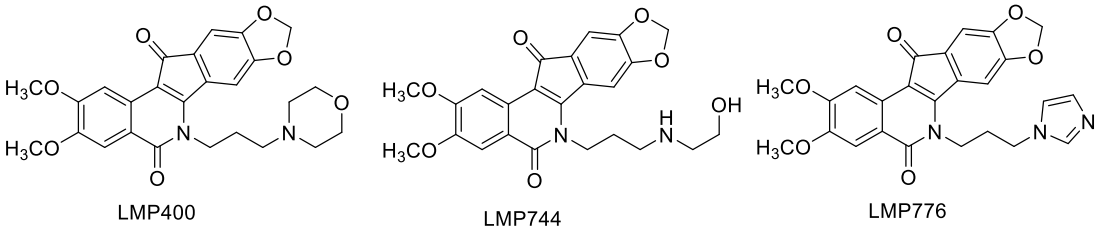


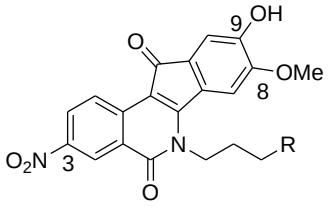

**Figure 3**: Indenoisoquinoline derivatives in the firstphase clinical trial

have been developed to construct indenoisoquinolines including condensation (Conda-Sheridan et al., 2013) of indenoisochromenone with a primary amine, Suzuki-Miyaura cross-coupling (Lebrun et al., 2011) reaction followed by ring-closing metathesis, (Liu et al., 2015) oxidative cyclization of *cis* acid produced by the condensation of a homophthalic anhydride and a Schiff base. Several other reactions such nucleophilic substitution, sandmeyer reaction, reduction and oxidation techniques have been employed to introduce and optimize varied functional groups on the scaffold (Kiselev et al., 2010; Lebrun et al., 2011; Nagarajan et al., 2003, 2004).

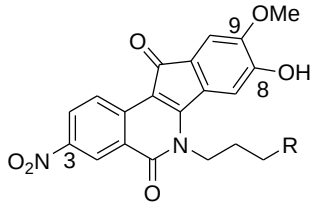
Among the latest synthetic efforts, Nguyen et al. (2015) synthesized eighteen nitrated 7-, 8-, 9-, and 10-hydroxyindenoisoquinolines bearing a 3-nitro substituent (Figure 4), all of which were potent dual Top1−TDP1 inhibitors, (Conda-Sheridan et al., 2013; Eun-jung et al., 2012; Morrell et al., 2007) using oxidative cyclization of cis acid produced by the condensation of a homophthalic anhydride and a schiff base (Scheme 1) (Nguyen et al., 2015).

## 2.1. Synthesis of 8-Hydroxy-9-methoxy-3-nitroindenoisoquinolines and 9-Hydroxy-8-methoxy-3-nitroindenoisoquinolines

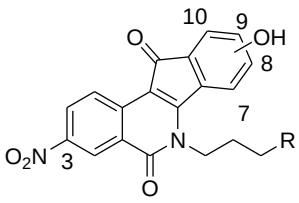
The synthesis of nitrated 9-hydroxy-8-methoxyindenoisoquinolines **10**, **12**, and **14**, (Scheme1 and 2) and the synthesis of 8-hydroxy-9-methoxyindenoisoquinolines **21**, **23**, and **25** (Scheme 3 and 4) were outlined. These synthesis began with commercially available homophthalic acid **1** which was nitrated with fuming $HNO_3$ to provide the diacid **2**, which underwent dehydration in AcCl to provide anhydride **3** (Scheme 1). The reactive hydroxyl groups in vanillin (**4**) and isovanillin (**15**) were protected with a benzylgroup. Benzylvanillin (**5**) and benzylisovanillin (**16**) reacted with 3-bromopropylamine hydrobromide to give Schiff bases **6** and 1**7**, which uponcondensation with anhydride **3** in $CHCl_3$ furnished cis acids **7** and **18**



**10** R = Morpholine
**12** R = imidazole
**14** R = $NH_2$



**22** R = Morpholine
**23** R = imidazole
**25** R = $NH_2$

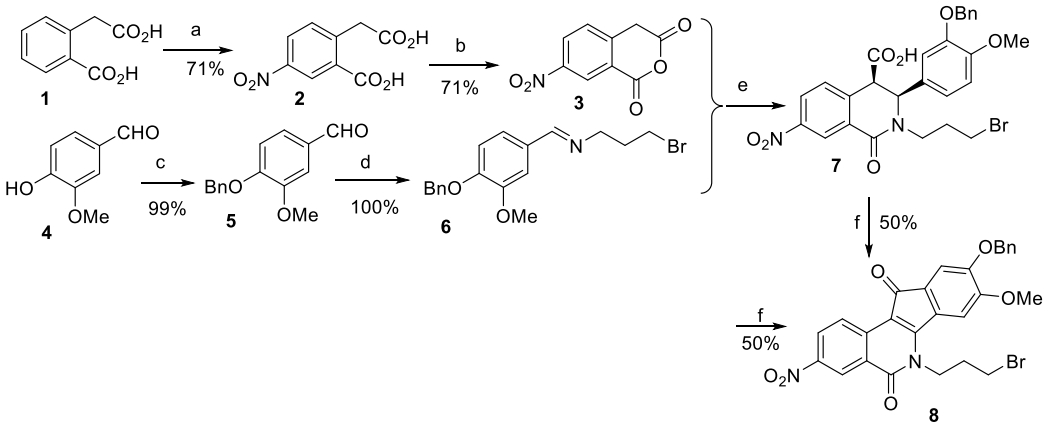


7-hydroxy { **31** R = Morpholine; **32** R = imidazole; **33** R = $NH_2$ }
9-hydroxy { **57** R = Morpholine; **58** R = imidazole; **59** R = $NH_2$ }

8-hydroxy { **43** R = Morpholine; **47** R = imidazole; **51** R = $NH_2$ }
10-hydroxy { **44** R = Morpholine; **48** R = imidazole; **52** R = $NH_2$ }

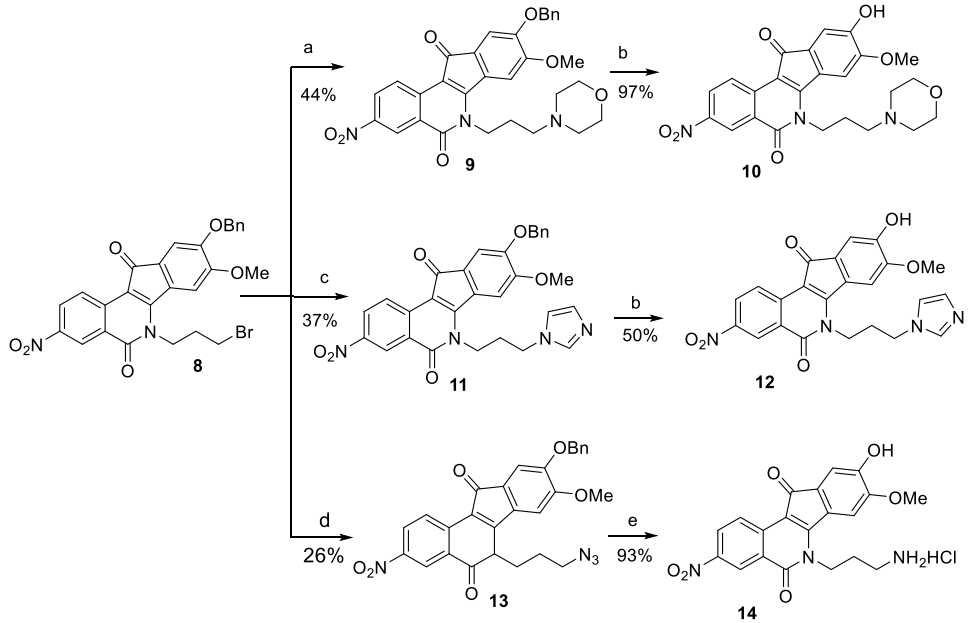
**Figure 4**: Proposed Top1−TDP1 inhibitors

in good yields with excellent diastereoselectivities. Treatment of **7** or **18** with $SOCl_2$ (neat) provided a mixture of indenoisoquinoline **8** or **19** and their regioisomers, which was confirmed by $^1H$ NMR spectroscopy. Isomers **8** and **19** appeared as the major products after column chromatography separation. The yields were low due tothe nitro group activating epimerization to the transdiastereomers, which exist in pseudodiaxial conformations and do not oxidize and cyclize in $SOCl_2$ (Nguyen et al., 2015). The displacement of the terminal bromide in **8** or **19** with morpholine or imidazole in 1,4-dioxane, or azide in DMSO, yielded the benzyl-protected compounds **9**, **11**, and **13** (from **8**) (Scheme 2), or **20**, **22**, and **24** (from **19**), (Scheme 4) (Morrell et al., 2007). The treatment of the benzyl-protected starting intermediates with aqueous HBr at 70 °C for 4−5 h, followed by dilution with acetone and then concentration (iterated three times), afforded a mixture that was suitable for vacuum filtration to provide the desired phenols **10**, **12**, **14**, **21**, **23**, and **25** in high yields (80−100%) and excellent purity (Nguyen et al., 2015).



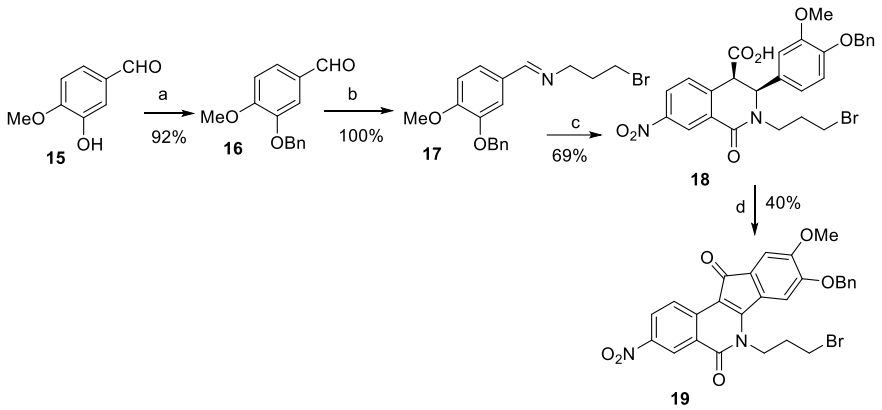
Reagents and conditions: (a) fuming $HNO_3$, 0−23°C; (b) AcCl; reflux; (c) BnCl, DMF, $K_2CO_3$, 23°C; (d) 3-bromopropylamine hydrobromide, Et3N, Na2SO4, $CHCl_3$, 23°C; (e) $CHCl_3$, 0−23°C; (f) SOCl2, 0−23°C;

**Scheme 1.** Synthesis of intermediate 8



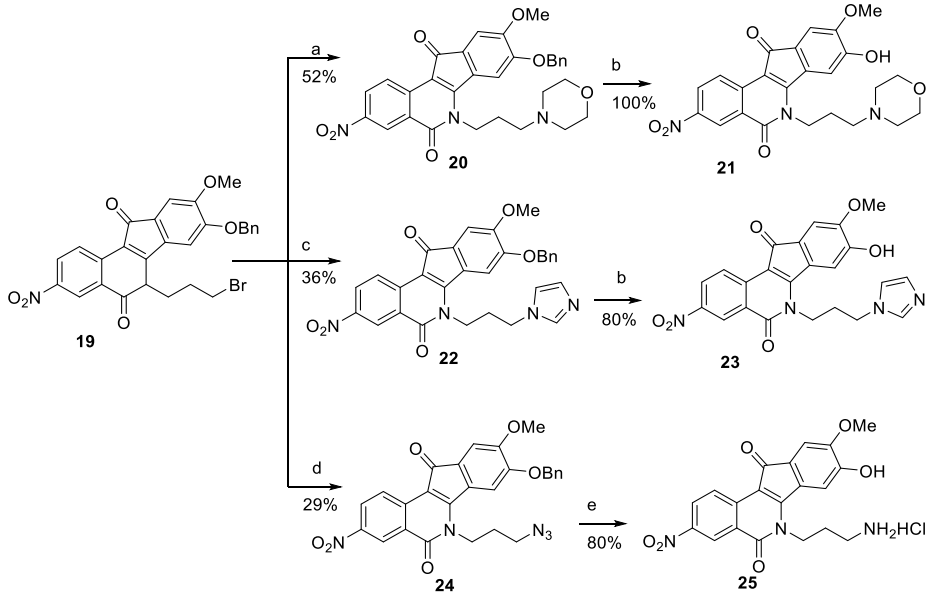
Reagents and conditions: (a) morpholine, 1,4-dioxane, 23°C; (b) aqueous HBr, 70°C; (c) imidazole, 1,4-dioxane, 70°C; (d) $NaN_3$, DMSO, 23°C; (e)(i)$P(OEt)_3$, benzene, reflux, (ii) aqueous HBr, 70°C.

**Scheme 2**. Synthesis of Nitrated 9-Hydroxy-8-methoxyindenoisoquinolines



Reagents and conditions: (a) BnCl, DMF, $K_2CO_3$, 23°C; (b) 3-bromopropylamine hydrobromide, $Et_3N$, $Na_2SO_4$, $CHCl_3$, 23°C; (c) anhydride **23c**, $CHCl_3$, 0−23°C; (d) $SOCl_2$, 0 to 23°C

**Scheme 3**. Synthesis of intermediate 19



Reagents and conditions: (a) morpholine, 1,4-dioxane, 23°C; (b) aqueous HBr, 70°C; (c) imidazole,1,4-dioxane, 70°C; (d) $NaN_3$, DMSO, 23°C; (e) (i) $P(OEt)_3$, benzene, reflux, (ii) aqueous HBr, 70°C.

**Scheme 4**. Synthesis of Nitrated 8-Hydroxy-9-methoxyindenoisoquinolines

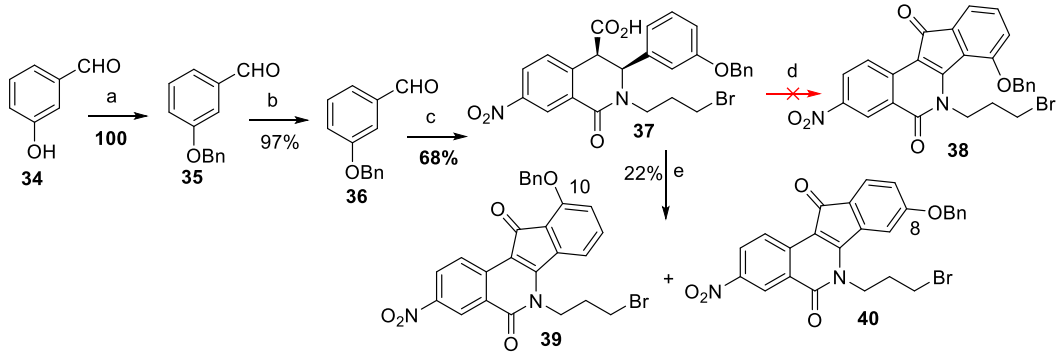
2.1.1. Synthesis of 7-Hydroxy-3-nitroindenoisoquinolines

Commercially available salicylaldehyde **26** was O-benzyl protected to give **27**, which reacted with 3-bromopropylamine to afford Schiff base **28** (Scheme 5). Condensation of **28** with anhydride **3** in $CHCl_3$ yielded cis acid **29**, which upon treatment with $SOCl_2$, followed by $AlCl_3$ in 1,2-dichloroethane, provided indenoisoquinoline bromide **30** in good yield (Kang et al., 2014; Mancuso at al., 1978). The displacement of the bromide in **23** with morpholine, imidazole, or $NaN_3$, followed by a Staudinger reduction of the azide intermediate and acidic hydrolysis with methanolic HCl, provided the desired amines **31**, **32**, and **23**, respectively. The pure products were isolated without chromatographic purification (Nguyen et al. 2015).

2.1.2. Synthesis of 8- and 10-Hydroxy-3-nitroindenoisoquinolines

A similar approach was implemented to prepare 8-hydroxy-3-nitroindenoisoquinolines as shown in scheme 6 (Conda-Sheridan et al., 2013)

The condensation of **36** with anhydride **3** provided a mixture of the desired cis acid **37** and its *trans* diastereomer. Boiling the mixture in $CHCl_3$, followed by filtration, helped to remove the unwanted *trans* acid and provide the pure cis acid **37** as a sole product. Unfortunately, the treatment of **37** with $SOCl_2$ 0 to 23°C,



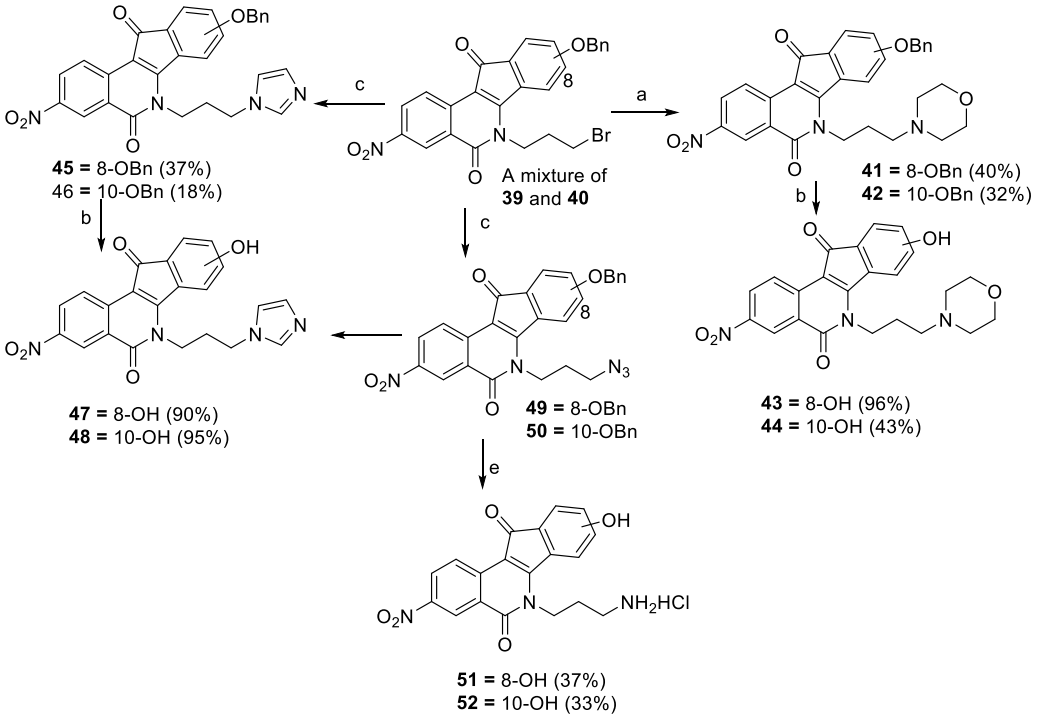
Reagents and conditions: (a) BnBr, DMF, $K_2CO_3$, 23°C; (b) 3-bromopropylaminehydrobromide, $Et_3N$, $Na_2SO_4$, $CHCl_3$, 23°C; (c) anhydride **3**, CHCl3, 0 to 23°C; (d) (i) $SOCl_2$, 0−23°C, (ii) $AlCl_3$, 1,2-dichloroethane, reflux; (e) $SOCl_2$, reflux.

**Scheme 6**. Synthesis of 8- and 10-Hydroxy-3-nitroindenoisoquinoline intermediates.

followed by $AlCl_3$ in refluxing 1,2-dichloroethane, did not yield the desired bromide **39**. However, transformation was observed when cis acid **37** was heated with $SOCl_2$ (neat) at reflux for 4 h, during which the solution turned to clear orange (Nguyen et al., 2015). The treatment of the mixture of bromides **39** and **40** with morpholine and imidazole, followed by chromatographic purification, allowed the isolation of each pure morpholinyl and imidazolyl 8-and 10-benzyloxy compounds **41**, **42**, **45**, and **46** (Scheme 7). After chromatographic separation, all of the benzylprotected materials were subjected to a 3 h debenzylation with aqueous HBr (48 wt %) to provide the desired 8- and 10-hydroxyindenoisoquinolines **44**, **44**, **47**and **48** in good yields and purities (Nguyen et al., 2015).

2.1.3. Synthesis of 9-Hydroxy-3-nitroindenoisoquinolines

The treatment of **53** with 3-bromopropylamine hydrobromide and $Et_3N$ provided Schiff base **54**, which upon condensation with anhydride **3** produced cis acid **55** in good yield and excellent purity (Scheme 8) (Nguyen et al., 2015). The treatment of **55** with morpholine



Reagents and conditions: (a) morpholine, 1,4-dioxane, 70°C; (b) HBr, $H_2O$, 70°C; (c) imidazole, 1,4-dioxane, 70°C; (d) $NaN_3$, DMSO, 23°C; (e) (i) $P(OEt)_3$, benzene, reflux, (ii) HBr, $H_2O$, 70°C.

**Scheme 7**. Synthesis of 8- and 10-Hydroxy-3-nitroindenoisoquinoline

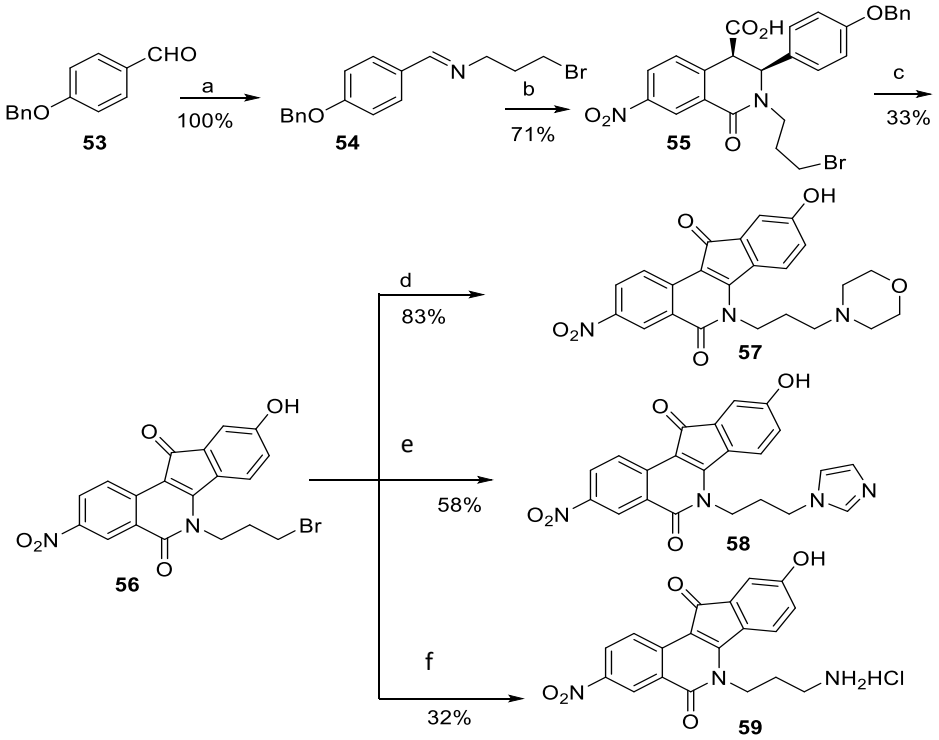
or imidazole in THF provided the corresponding displacement products, which were then stirred in freshly made methanolic HBr or HCl to afford the HBr and HCl salts **57** and **58**, respectively. The synthesis of amine **59** by the previous methodology involving reduction of the azide intermediate with $P(OEt)_3$ in benzene (Scheme 4) was not successful due to complications in purification and isolation of the compound in solid form. The Staudinger reaction was therefore reattempted by treating the azide intermediate, obtained from **56**, with $PPh_3$ in THF (instead of $P(OEt)_3$ in benzene), followed by 4 h acidic hydrolysis with methanolic HBr. This modification provided the desired amine **59** in 32% yield with excellent purity.

2.1.4. Top I inhibitory and Antiproferative properties of indenoisoquinolines

All of the synthesized compounds (collectively shown in Figure 4) were evaluated for Top1inhibitory, TDP1 inhibitory potencies and antiproliferative activities (Nguyen et al., 2015). The results revealed that the 3-nitro group seems to facilitate intercalation into free DNA so that **43**, **44**, **47,** and **48** with large substituents on the side chain, all act as Top1 suppressors at 0.1, 1.0, 10, and 100 μM, respectively (Nguyen et al., 2015). The nitrated compounds also displayed a significant improvement in terms of cytotoxicity when compared to their corresponding dimethoxy analogues, with the 9-hydroxy-8-methoxy series **10, 12** and **14** possessing low nanomolar antiproliferative potencies (MGM values of 16−21 nM). Indeed, the order of Top1 inhibition and cytotoxicity went from the 9-hydroxyl series **56**−**59** as the most active and cytotoxic (Top1 inhibition ++++ or more, MGM 14−117 nM) to the 8-hydroxyl series **43**, **47**, and **51** (+++ to ++++, 56−407 nM), and in the 7-hydroxyl series **31**−**33** and the 10-hydroxyl series **44**, **48**, and **52** (++ to +++ for both series, 234 to 3550 nM for the 10-hydroxyl) as the least active and least cytotoxic (Morrell et al., 2007).

## 2.2. Discovery of Potent Indenoisoquinoline Topoisomerase I Poisons Lacking the 3-NitroToxicophore

The NCI-60 screening service recently proposed a policy wherein submission of molecules containing "problematic" functionalities, (Morrell et al., 2006) including



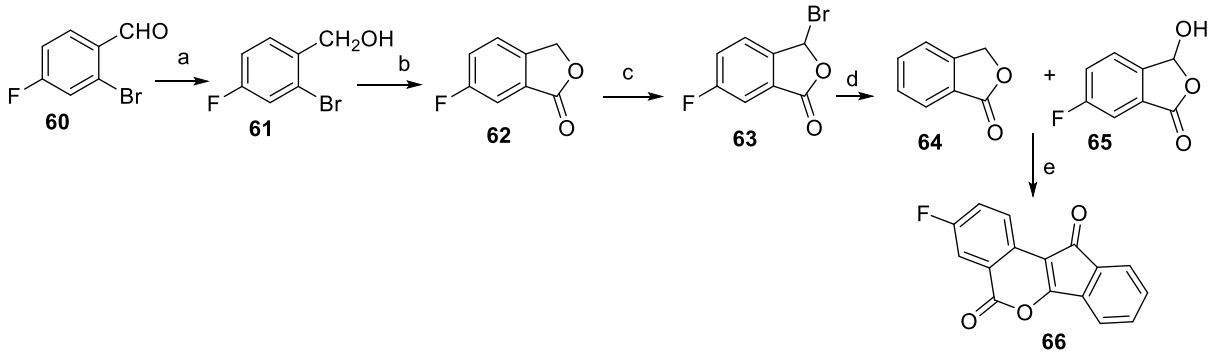

Reagents and conditions: (a) 3-bromopropylamine hydrobromide, $Et_3N$, $Na_2SO_4$, $CHCl_3$, 23°C; (b) anhydride **3**, $CHCl_3$, 0−23°C; (c) (i) SOCl2, 23°C, (ii) AlCl3, 1,2-dichloroethane, 0−23°C; (d) (i) morpholine, THF, 70°C, (ii) HBr, MeOH, 23°C; (e) (i) imidazole, THF, 70°C, ( HCl, MeOH, 23°C; (f) (i) $NaN_3$, DMSO, 23°C, (ii) $PPh_3$, THF, 70°C, (iii) HBr, MeOH, 70°C

**Scheme 8**. Synthesis of 9-Hydroxy-3- nitroindenoisoquinolines

nitro groups, are discouraged. Accordingly, Beck *et al.* (2015) adopted a research to discover a suitable bioisosteric replacement for the 3-nitro group on the indenoisoquinoline system that would maintain or improve Top1 poisoning activity and growth inhibitory potency (Beck et al., 2015). The synthesis was commenced with benzaldehyde **60**, which was reduced with $NaBH_4$ to provide the bromobenzylic alcohol **61** (Scheme 9). Rosenmund−von Braun reaction with CuCN and *in situ* hydrolysis and lactonization of the intermediate yielded compound **62**. Phthalide **62** was subjected to radical bromination, and the obtained 3-bromophthalide intermediate **63** was hydrolyzed to produce 3-hydroxyphthalide **65** which was condensed with phthalide (**64**) in refluxing methanol−EtOAc with NaOMe and then dehydratively cyclized in refluxing $Ac_2O$ to afford **66** (Beck et al., 2015)**.**

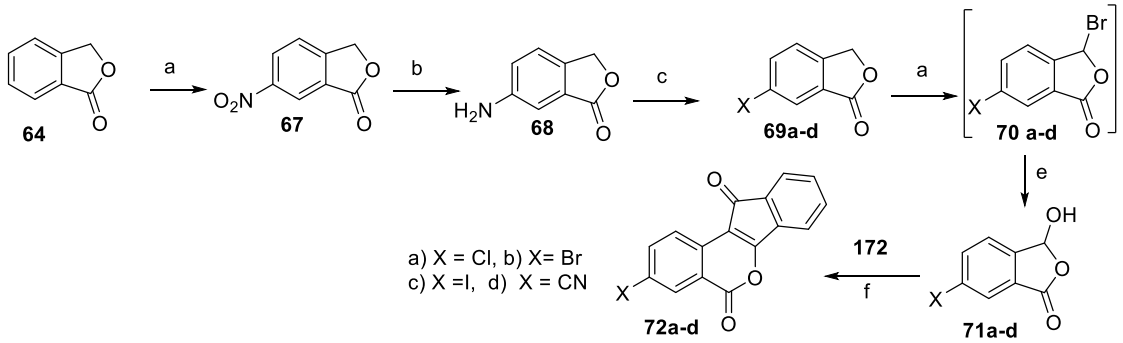
Indenobenzopyrans **72a-d** were synthesized by nearly identical synthetic routes, where the only point of difference was in step c (Scheme 10) (Conda-Sheridan et al., 2013; Robert et al., 1988).

Phthalic acid **73** was converted to its anhydride **74** in refluxing AcCl. Anhydride **74** was reduced with $NaBH_4$ in THF to give a 1:1 mixture of 5- and 6-(trifluoromethyl) phthalides **75a** and **75b** which were readily separated by silica gel column chromatography (Marzi et al., 2002)**.** Phthalides **75a** and **75b** were separately subjected to radical bromination and subsequent hydrolysis to provide 3-hydroxyphthalides **76a** and **76b**. Indenobenzopyrans **78a** and **78b** were obtained by the condensation of 3-hydroxyphthalide **77a** and **77b** with phthalide (**4**), and dehydrative cyclization of the unisolated intermediate was carried out as before in refluxing $Ac_2O$ (Scheme 11) (Beck et al., 2015).




Reagents and conditions: (a) $NaBH_4$, MeOH, 0°C to room temp; (b) (i) CuCN, DMF, reflux, (ii) $H_2O$, reflux; (c) NBS, AIBN, $CCl_4$, reflux; (d) KOH, $H_2O$, reflux; (e) (i) NaOMe, MeOH, EtOAc, reflux, (ii) $Ac_2O$, reflux.

**Scheme 9**. Synthesis of 3-Fluoroindenobenzopyran (**66)**




Reagents and conditions: (a) $KNO_3$, $H_2SO_4$, 0°C to room temp; (b) $H_2$, Pd/C, EtOAc; (c) (i) $NaNO_2$, 37% HCl, 0°C, (ii) CuCl, 37% HCl, 0°C to reflux (**69a**), or (i) $NaNO_2$, 48% HBr, 0°C, (ii) CuBr, 48% HBr, 0−80°C (**69b**), or (i) $NaNO_2$, 37% HCl, 0°C, (ii) KI, 0°C to room temp (**69c**), or (i) $NaNO_2$, 37% HCl, 0°C, (ii) NaCN, CuCN, 0°C to room temp (**69d**); (d) NBS, AIBN, $CCl_4$, reflux; (e) KOH, $H_2O$, reflux; (f) (i) NaOMe, MeOH, EtOAc, reflux, (ii) $Ac_2O$, reflux.

**Scheme 10**. Synthesis 3-substituted indenobenzopyran **72a-d**

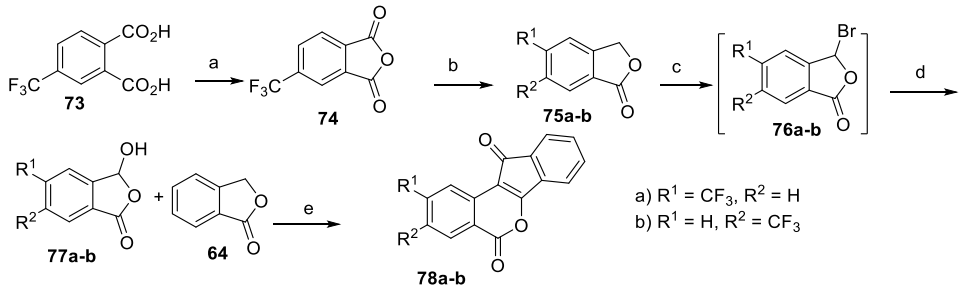
3-Hydroxyphthalide **65** was used to generate **81b** through nucleophilic substitution and condensation reaction **(**Scheme 12**).**

(S)-3-Amino-1, 2-propanediol (**82**) was condensed with indenobenzopyrans **66, 72a-d, 78a-b**, **80** and **81**, to produce indenoisoquinolines **83-86** (Scheme 13). 1-(3-Aminopropyl) imidazole (**87**) was condensed with the same indenobenzopyrans, to yield indenoisoquinolines **88-91**.

In the same way, 3-Morpholinopropylamine (**92**) was condensed with indenobenzopyrans **66**, **72a** and **72c** to produce indenoisoquinolines **93a-c** (Scheme 14).

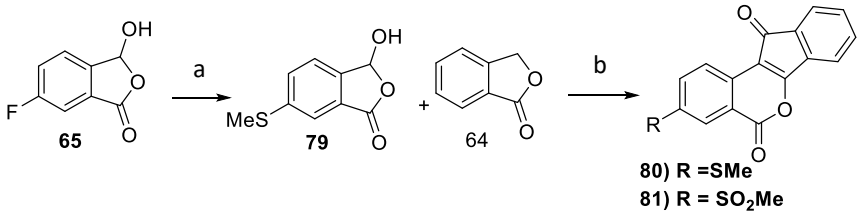
Anhydride **94** was reduced with $NaBH_4$ in PhMe-DMF and the reduction product was cyclized to provide phthalide **95** which was converted **98** in three steps (Scheme **15**).

In similar way to its carbocyclic analogues, lactone **98** was condensed with primary amines **87**, **92** and **10** to yield indenoisoquinolines **99**, **100**, and **102** (Scheme 16).



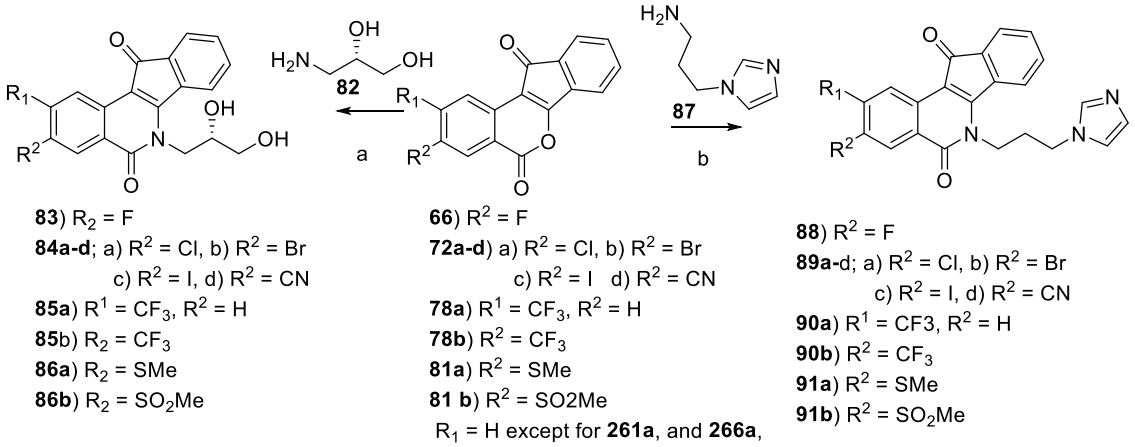

Reagents and conditions: (a) AcCl, reflux; (b) (i) $NaBH_4$, THF, 0°C, (ii) HCl; (c) NBS, AIBN, $CCl_4$, reflux; (d) KOH, $H_2O$, reflux; (e) (i) NaOMe, MeOH, EtOAc, reflux, (ii) $Ac_2O$, reflux.

**Scheme 11**. Synthesis of indenobenzopyran **78a, b**



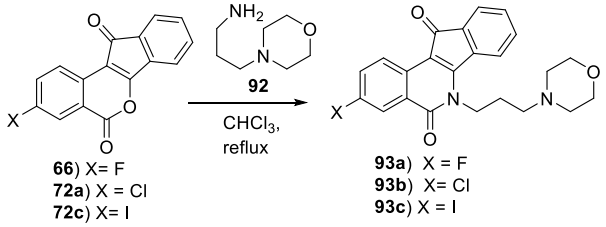

Reagents and conditions: (a) NaSMe, DMF, 120°C; (b) (i) NaOMe, MeOH, EtOAc, reflux, (ii) $Ac_2O$, reflux; (c) m-CPBA, $CHCl_3$, room temp

**Scheme 12**. Synthesis of indenobenzopyran **80** and **81.**

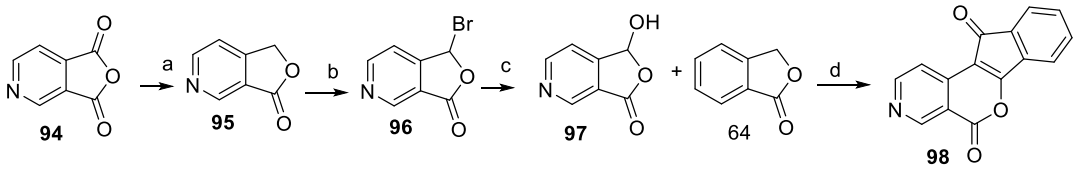



Reagents and conditions: (a) MeOH−$CHCl_3$, reflux; (b) $CHCl_3$, reflux.

**Scheme 13**. Synthesis of indenoisoquinolines **83-91**

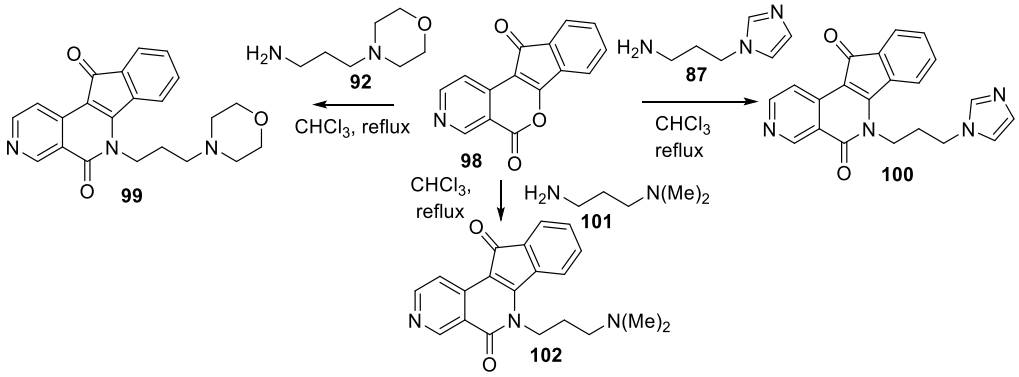



**Scheme 14**. Synthesis of indenoisoquinolines **93a-c**




Reagents and conditions: (a) (i) $NaBH_4$, PhMe, DMF, −20 to 35°C, (ii) 5 M HCl, reflux; (b) NBS, AIBN, $CCl_4$, reflux; (c) $H_2O$, reflux; (d) (i) NaOMe, MeOH, EtOAc, reflux, (ii) $Ac_2O$, reflux.

**Scheme 15**. Synthesis of indenobenzopyran **98**



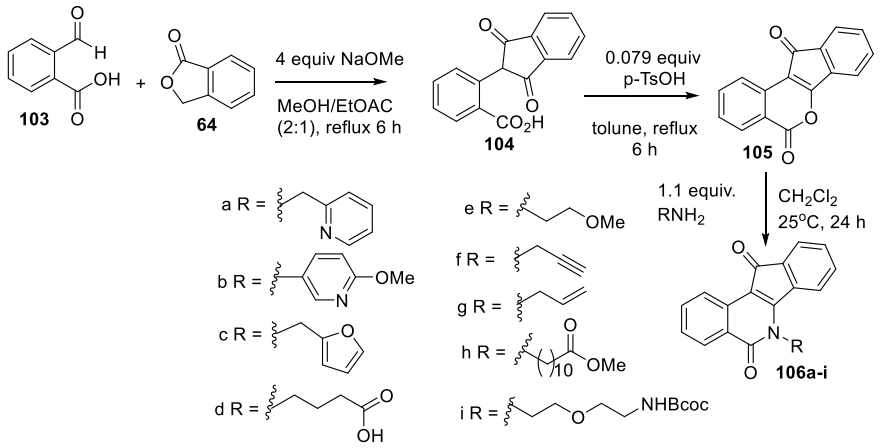

**Scheme 16**. Synthesis of indenoisoquinolines **99, 100**and **102**

2.2.1. Top I inhibitory and Antiproferative activities of indenoisoquinolines **88-102**

Among the bioisosteric compounds, **88**, **89a**, and **90a-c** display the best Top1 poisoning activities. The Top1-mediated DNA cleavage induced by these four compounds is between 75 and 95% that of 1 μM CPT (i.e. +++). MGM concentration of drug to cause 50% reduction in proliferation of cancer cells i.e. growth ($GI_{50}$) values were calculated to be 0.692 μM for **88**, 0.229 μM for **89**, and 2.75 μM for **93a**. Compounds **88**, **89a** and **93a** are substituted with the halogens F and Cl. The other potential bioisosteres (i.e. I, CN, $CF_3$, SMe and $SO_2Me$) did not display Top1-mediated DNA cleavage assay scores above ++. So the researchers had discosed that only fluorine and chlorine were identified as bioisosteres of nitro group on the basis of Top1 poisoning activities and growth inhibitory potencies (Antony et al., 2007; Beck et al., 2015; Pommier et al., 2014; Sirivolu et al., 2012).

## 2.3. Synthesis and Cytotoxic Evaluation of Novel Indenoisoquinoline-Propan-2-Ol Hybrids

Functionalized propanes are often part of biologically active agents. Particularly, the class of the β-amino propanols consists of multiple representatives with antimalarial, anticancer, *Src* kinase inhibiting, antimicrobial, and antifungal properties (Chennakesava et al., 2014; Pham et al., 2016; Robert et al., 1988). Inspired by above literature reports, Thi et al. (2016) developed a variety of novel indenoisoquinolines by combining the indenoisoquinoline scaffold with 2-hydroxypropane unit (Pham et al., 2016). In the process, the key starting material, benz[d]indeno[1,2-b]pyran-5,11-dione **105**, was synthesized using a two-step methodology. Condensation of 2-carboxybenzaldehyde **93** with phthalide **64** in the presence of sodium methoxide in methanol/ethyl acetate (2:1) under reflux furnished intermediate **104**, which could, after dehydrative acid-catalyzed lactonization in toluene, efficiently be




**Scheme 17**. Synthesis of indenoisoquinolines **106a–i.**

converted to indenobenzopyran **105** in 58% yield after recrystallization from ethyl acetate. Derivatives **106a-i** generated in high yields (81–96%) upon treatment of **105** with the appropriate primary amines, as shown in Scheme 17 (Morrell et al., 2006; Pham et al., 2016).

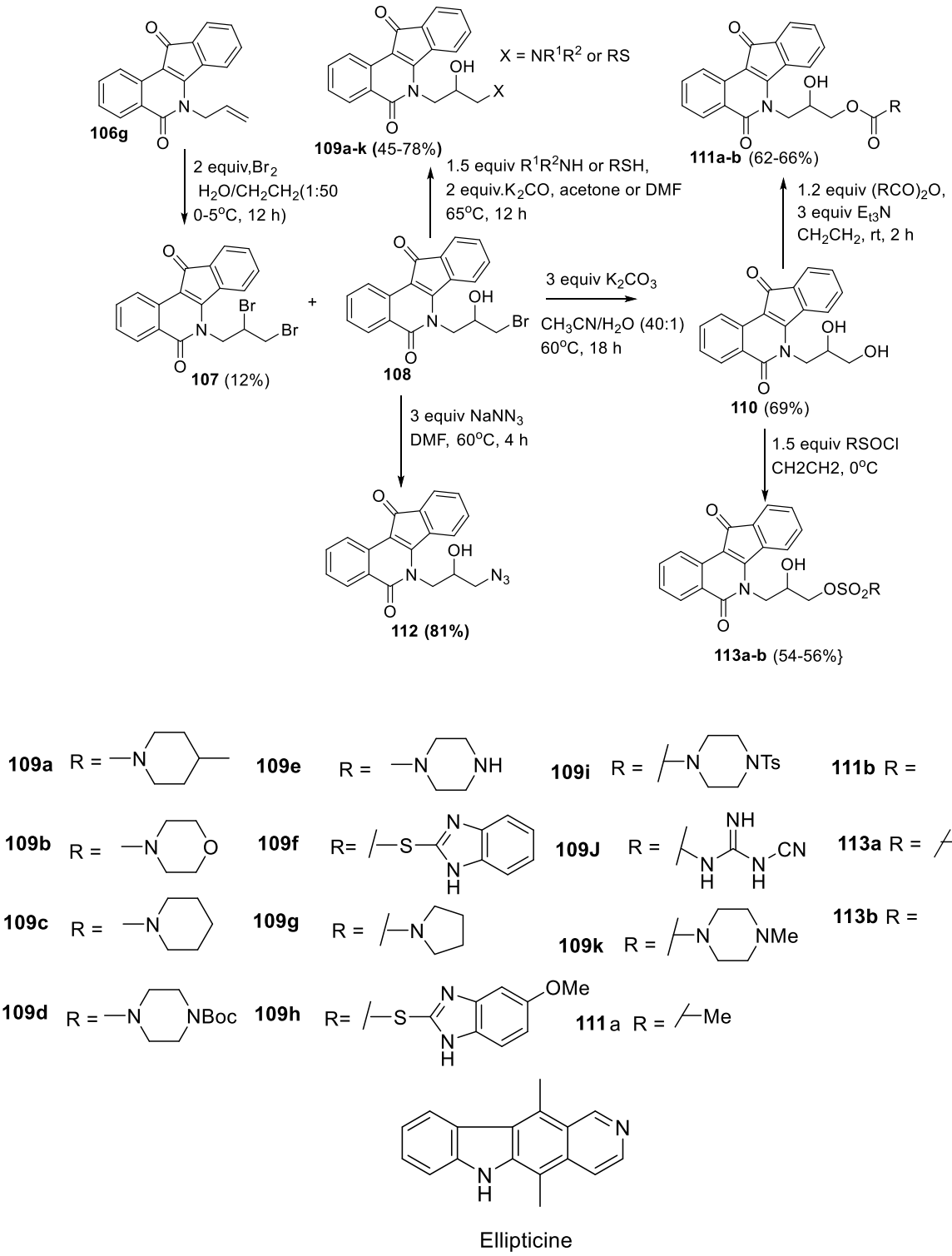
Multiple indenoisoquinoline derivatives SAR studies have demonstrated that the presence of hydrogen bonding groups (e.g., hydroxyl) on the lactam side chains correlates well with an increase in biological activity. To prove this principle emperically,the researchers derivatized N-allyl-substituted indenoisoquinoline **109g** via hydroxybromination (Pham et al., 2016).

Then **108** was converted into **109a-k** in moderate to good yields (45–78%) after base-catalyzed nucleophilic substitution of the primary bromide by a series of primary or secondary amines or primary thiols in acetone or DMF at 65°C (Scheme 18). Furthermore, intermediate **108** was converted to **110** via $K_2CO_3$-catalyzed nucleophilic substitution by water. Compound **110** was then further acylated using acetic and isobutyric anhydride in the presence of 3 equiv of triethylamine to provide esters **111a, b** in 62–65% yield. On the other hand, treatment of 2,3-propanediol **110** with tosyl or mesyl chloride resulted in the formation of the monosulfonylated diols **113** (54–58%) (Pham Thi et al. 2016). A final option involved the reaction of **108** with 3 equiv of sodium azide in order to furnish the corresponding azide **112** in good yield (81%) (Pham et al., 2016).

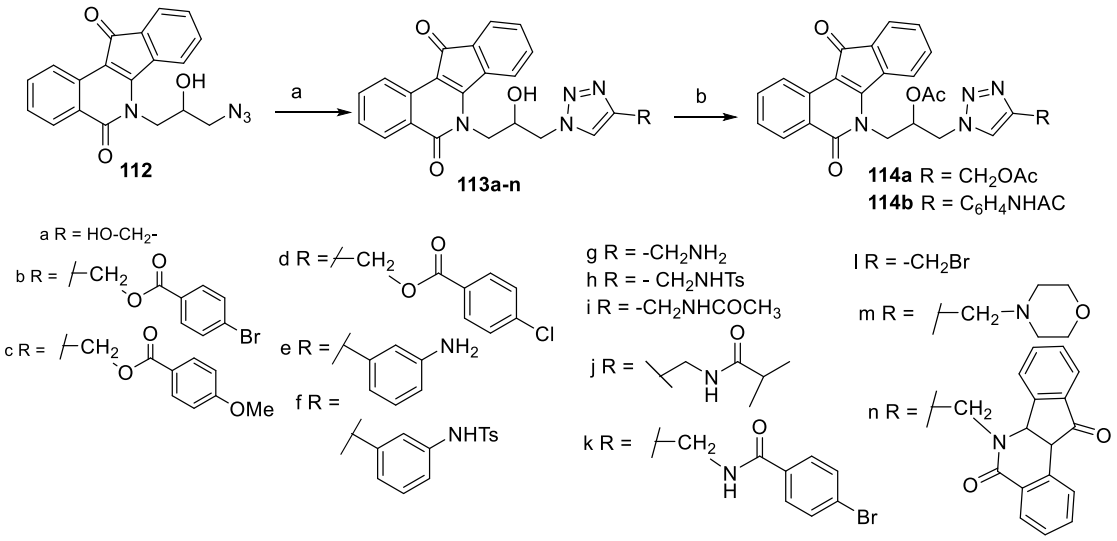
2.3.1. Top I inhibitory and antiproferative activities of indenoisoquinolines 106a-I, and 109-113

The synthesized indenoisoquinolines were evaluated in terms of their cytotoxicity profile against two human cancer cell lines, KB and Hep-G2 (Alley et al., 1988; Kiselev et al., 2011; Monks et al., 1991; Vann et al., 2016). Ellipticine was used as a reference compound. The results of this biological assessment, revealed the majority of the compounds exhibit at least moderate cytotoxicity against both cancer cell lines. **105i** and **109a**,**c**,**e**,**k** exhibited equal cytotoxic activity with the reference, **109a** and **109e** were the most promising ($IC_{50}$ values of 0.82 and 0.47 μM and 0.82 and 0.69 μM, respectively, against KB and Hep-G2) (Pham et al., 2016). In the same year, Thi et al. (2016) adopting another project wherein twenty three new indenoisoquinoline substituted triazole hybrids were prepared.(Pham et al., 2016). In recent years, it has been commonly accepted that agents containing more than one pharmacophore can have superior efficacy as compared to single-pharmacophore drugs (Solomon et al., 2010). They hypothesized that the introduction of triazole group into N-functionalized three-carbon side chain of indenoisoquinoline, especially indenoisoquinoline-propan-2-ols, could give potent biological compounds (Monks et al., 1991). To confirm their hypothesis, they synthesized and evaluated novel triazole-indenoisoquinolines hybrids (Monks et al., 1991). In their synthesis strategy, novel triazole-indenoisoquinoline hybrids were developedbased on a CuI-catalyzed 1,3-cycloaddition between propargyl-substituted derivatives and the azidecontaining indenoisoquinoline **112** which was prepared by previous four-step methodology (Haldón et al., 2015; Pham et al., 2016). Azidoindenoisoquinoline**112** was transformed to triazole–indenoisoquinolinehybrids **113a–n** in high

yields (60–80%) upon treatment with the appropriate 1-propargyl derivative (Scheme 19) (Pham et al., 2016).




**Scheme 18**. Synthesis of indenoisoquinoline **109-113** and Ellipticine.




Reagents and conditions: (a) 1.1 equiv ethynyl derivatives, 0.2 equiv DIPEA, 0.1 equiv CuI, THF, reflux, 24 h; (b) 3 equiv $Ac_2O$, 2 equiv $Et_3N$, DMF, rt, 24 h.

**Scheme 19**. Synthesis of triazole–indenoisoquinoline hybrids **113a–n** and **114a, b**.

2.3.2. Top I inhibitory and Antiproferative activities of indenoisoquinolines 113a-n and 114a, b

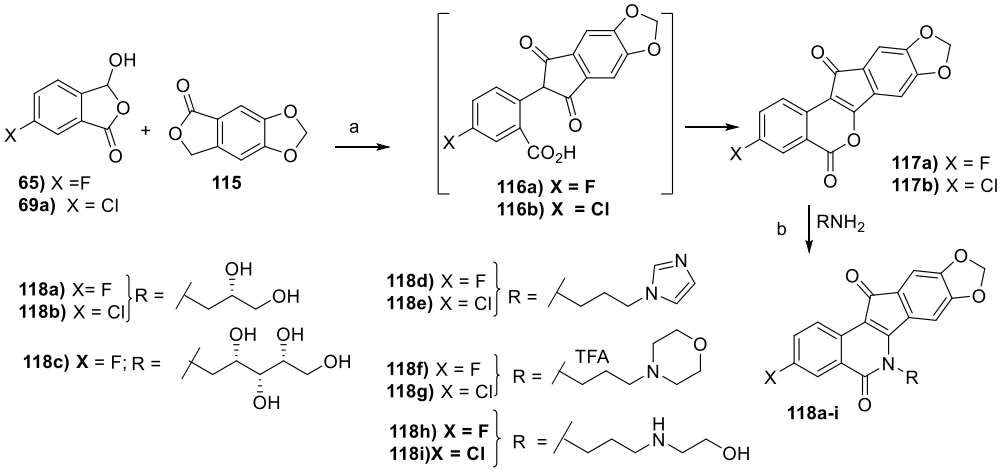
The *in vitro* biological assessment of compounds (Scheme 18 and 19) against two human cancer cell lines (KB-CCL-17, HepG2-HB-8065) (Pham et al., 2016) revealed that most of the derivatives possess at least moderate cytotoxic activity. It is important to note that these separate pharmacophores display considerably less potent cytotoxic activities as compared to the most promising conjugates **113a-n**.

## 2.4. Synthesis and Biological Evaluation of New Fluorinated and Chlorinated Indenoisoquinoline Topoisomerase I Poisons

In 2016, Beck D. *et al*. contined their effort to develop highly active analogs of the indenoisoquinolines in Phase 1 clinical trial drugs indotecan (LMP400) (Beck et al., 2014; Maris et al., 2012) and indimitecan (LMP776) (Maris at al., 2012; Pommier and Cushman, 2009), as well as the clinical trial candidate MJ-III-65 (LMP744) (Beck et al., 2016). In the process, structural modifications of fluorinated and chlorinated indenoisoquinolines (Pommier et al., 2014) were incoporatedto enhance their Top1 poisoning activities. They synthesized a series of pentacyclic lactone intermediates that could be used to probe the effects of having different A-ring substitution patterns and different side chains on the lactam nitrogen. In these Synthesis, 3-Hydroxyphthalides **65** (Pommier et al., 2014) and **69a** (Pommier et al., 2014) were each condensed with phthalide **64** under basic conditions (Scheme 20) (Shapiro et al., 1961). The 1,3-indanedione intermediates **116a** and **116b** were each cyclized in situ in refluxing $Ac_2O$ to yield lactones **117a** and **117b** (Shapiro et al., 1961) which were condensed with various primary amines (Antony et al., 2007; Beck et al., 2016; Strumberg et al., 1999) provided indenoisoquinolines **118a-i** (Beck et al., 2016)**.** Besides monofloro indenoisoquinoline derivatves, this team synthesized 2,3-diflouro and 2,3-diocloroindenoisoquinoline derives (**119a**-**b** and **120a**-**b**) by application related procedure discussed here (Figure 5 ).

2.4.1. Top I inhibitory and Antiproferative activities of indenoisoquinolines 118a-120b

The result of Top1 inhibitory assement revealed that out of new compounds, **118d** achieved a ++++ score, whereas **118h**, and **118i** achieved a +++ score (Beck et al., 2016). Among 3-fluoro- or 3-chloro-substituted analogs, two Top1 inhibitors (**118h** and **118i**) were also found to be active in both recombinant TDP1 and TDP2 inhibition assays (Beck et al., 2016). The most cytotoxic compound of the present series was the 3-fluoro derivative **118d** having an imidazole-containing side chain, which displayed a $GI_{50}$ MGM of 11 nM (Beck et al., 2016).




Reagents and conditions: (a) (i) NaOMe, MeOH, EtOAc, reflux; (ii) $Ac_2O$, reflux; (b) $CHCl_3$, MeOH, reflux.

**Scheme 20.** Synthesis of indenoisoquinolines **118a-i**

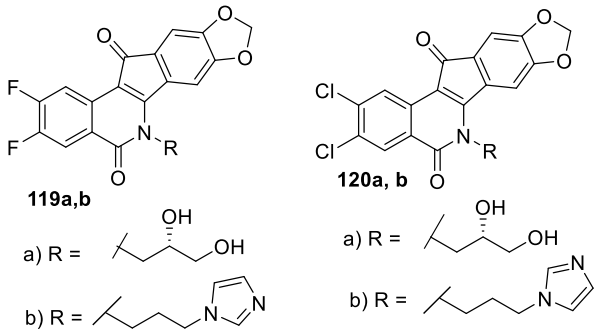



**Figure 5**: Diflouro and dicloroindenoisoquinolines by Beck et al. (2016).

## 2.5. Design and Synthesis of Chlorinated and Fluorinated 7-Azaindenoisoquinolines as Potent Cytotoxic Anticancer Agents That Inhibit Topoisomerase I

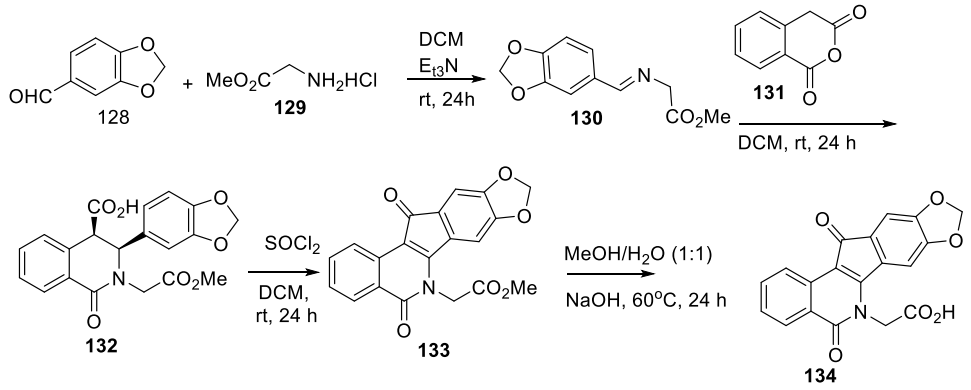
Motivated better bioactivity of 3-flouro and 3-chloro substituted analogs, Elsayed *et al*. (2017), synthesized eighteen chlorinated and fluorinated 7-Azaindenoisoquinolines by avoiding indenoisoquinolines with nitro groups on aromatic systems to mitigate the toxic effect of nitro groups (Elsayed et al., 2017). In their work, two strategies were involved to incorporate ring nitrogen and for the replacement of the 3-nitro group with halogens (Elsayed et al., 2017). The anhydrides **121a** and **121b** (Scheme **21**) were prepared by published literature procedures (Gwong-Jen, 2010; Kang et al., 2014). Bromination of 5-methoxy-3-methylpicolinonitrile in the presence of the radical initiator AIBN produced intermediate bromide **122** (Kiselev et al., 2011) which was used directly in the next step without additional purification. The condensation of **122** wit**h 121a** and **121b** in acetonitrile promoted by $Et_3N$ afforded compounds **123a** and **123b**. Oxidation of **123a** and **123b** with selenium dioxide provided azaindenoisoquinoline intermediates **124a** and **124b.** Treating compounds **124a** and **124b** with NaH in DMF at 0 °C, followed by reaction with 1-chloro-3-bromopropane, yielded the common intermediates **125a** and **125b**. The common intermediates **125a** and **125b** were used for the synthesis of the final compounds **126a−j** and **127a−i** by alkylation of the corresponding amines in DMF as shown in Scheme22 (Elsayed et al., 2017)**.**

## 2.6. Synthesis and Cytotoxic Evaluation of Carboxylic Acid-Functionalized Indenoisoquinolines

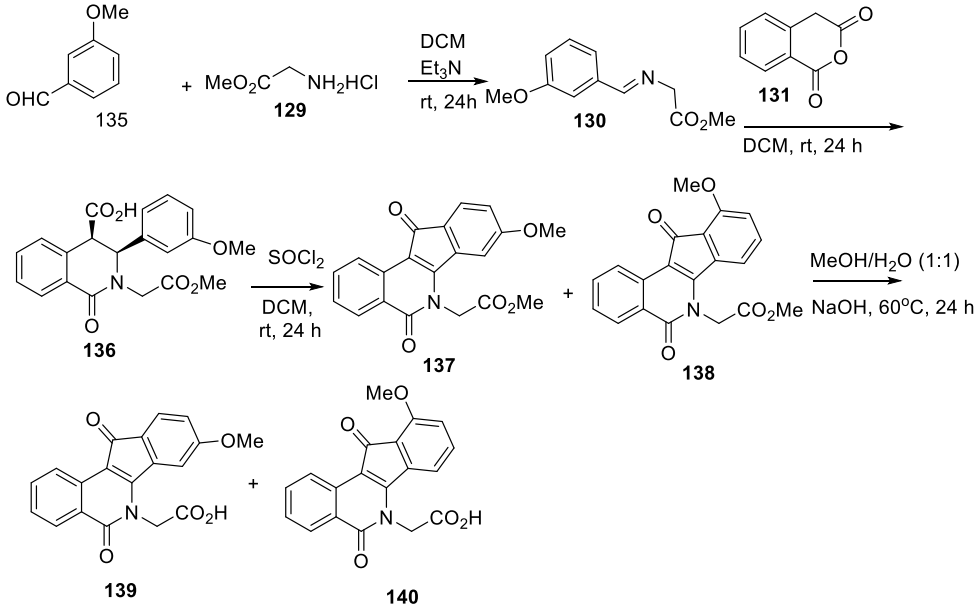
Functionalized carboxylic acids are often part of biologically active agents. They might provide a point of attachment for the synthesis of prodrugs so that the pharmacokinetics could be modulated and optimized. The importance of the carboxylic acid functional group

in drug design is illustrated by the fact that > 450 marketed drugs are containing carboxylic acid functional group (Ballatore et al., 2013; Dung et al., 2019). Inspired by this fact, Dung et al. (2019) synthesized a library of indenoisoquinoline acids in order to find out the influence of carboxylic acid functionalities in the N-lactam side chains of indenoisoquinolines on cytotoxic activities (Dung et al., 2019). Dung et al. (2019) first synthesized indenoisoquinoline acetic acid (Scheme 23). Piperonal **128** reacted with glycine methyl ester hydrochloride to give Schiff base **130**, which upon condensation with homophthalic acid anhydride furnished cis-acid **132** in good yield with excellent diastereoselectivity. The treatment of cis-acid **132** with thionyl chloride resulted in conversion to the acid chloride, dihydrogenation, and intramolecular Friedel-Crafts cyclization to provide the indenoisoquinoline ester **133** (Nguyen et al., 2015). The ester **133** was then subjected to hydrolysis by sodium hydroxide in MeOH/$H_2O$ (1:1) at 60°C to afford indenoisoquinoline acetic acid **134** in good yield.

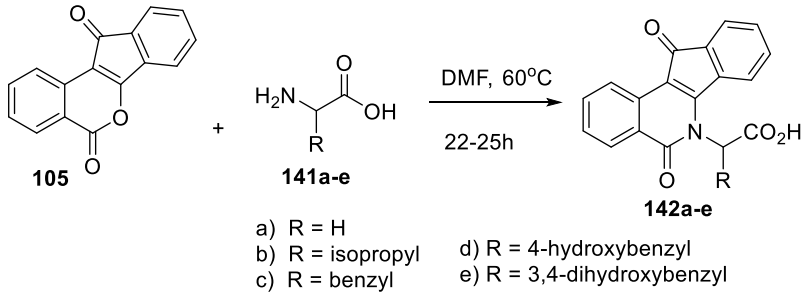
Similary, the indenoisoquinoline acetic acids containing methoxy group in D ring were synthesized by a procedure as illustrated in Scheme 24 (Kohlhagen et al. 1998; Thi et al. 2016). The cis-acid **136** reacted with thionyl chloride to provide a mixture of indenoisoquinoline esters **137** and **138**, which were separated by column chromatography. The latter were then hydrolyzed by sodium hydroxide to afford acids **139** and **140**, respectively (Dung et al. 2019).




**Scheme 23**. Synthesis of indenoisoquinoline acetic acid




**Scheme 24**. Preparation of indenoisoquinoline acids **139** and **140**.




**Scheme 25**. Synthetic route for the preparation of indenoisoquinolines **140a-e.**

A group of indenoisoquinolines containing carboxylic acid functionalized groups were also synthesized starting from indenobenzopyran **105** using the same protocol (Scheme 25) (Dung et al., 2019).

2.6.1. Cytotoxicity of 134, 139, 140 and 142a-e

All the synthesized compounds were subjected to *in vitro* biological assessment against two human cancer cell lines (KB, epidermoid carcinoma; HepG2, hepatoma carcinoma) and Ellipticine was as positive control. The results revealed that compounds constituting carboxylic acid groups on ring D (compounds **142a-e**) displayed moderate cytotoxic activity against KB cell line and high activity against HepG2 cell line with $IC_{50}$ values ranging from 10 to 32 μM with compound **142c** being the most active to KB and HepG2 cell lines ($IC_{50}$ value of 4.55 and 10.46 μM, respectively). However, the presence of the methylenedioxy or methoxy group on D-ring of indenoisoquinoline scaffold decreased the observed cytotoxicity activity.

## 4. Conclusion

A new class of Topisomerase I inhibitors, indenoisoquinolines, emerged with the accidental isolation of the first lead indenoisoquinoline (NSC 314622) as a byproduct of nitidine chloride synthesis in 1978. Since 1999 indenoisoquinolines were investigated extensively as a noncamptothecin topoisomerase I (Top1) inhibitors which later on succeeded to find the first lead indenoisoquinoline (NSC 314622) with prominent activity back in 1998. Over the years several synthesis reports have been developed leading to synthesis of indenoisoquinolines scaffold and it was pointed out that incorporating electron-donating alkoxy substituents on the A- and D-rings and including nitrogen hetrocycles on the nitrogen side chain of the lactam moeity of the core indenoisoquinolines improved cytotoxic properties. Significant cytotoxicity was observed for 3-nitro indenoisoquinolines compared to their corresponding dimethoxy analogues, in the synthesis reports of 9-hydroxy-8-methoxy series, worth mentioning among the recent achivements in the synthesis reports of the scaffold. The 7-azaindenoisoquinoline derivatives bearing a 3-nitro group and 9-methoxy group were able to partially overcome resistance in several drug-resistant cell lines, and they were not substrates for the ABCB1 drug efflux transporter. On comparative basis, 3-flourinated or 3-chlorinated indenoisoquinolines were found to be safer and better topoisomerase I inhibitors than their respectiive 3-nitro analogs with the most active being the one having immidazole ring on the nitogen side chain of the lactam moeity. Incorporating carboxylic acid moeity in the N-lactam side chain of 3-flourinated indenoisoquinolines afforded compound **142c** with significant activity towards KB and HepG2 cell lines ($IC_{50}$ value of 4.55 and 10.46 μM, respectively).

In this review efforts have been made to recap the synthetic methods, reactions and the biological results of various indenoisoquinoline derivatives during 2015-2020. In conclusion, through optimization of various substuents of indenoisoquinoline scaffold, there seems to be a great chance to develop more effective anticancer drugs in addition to those within various stages of clinical trials.

Finally we recommended additional research to be conducted particularly on flouro and chloro indenoisoquinoline analogs which are promising to develop safe anticancer drug candidates.

## Reference

Alley, Michael C., Dominic A. Scudiero, Anne Monks, Miriam Hursey, Maciej J. Czerwinski, Donald L. Fine, Betty J. Abbott, Joseph G. Mayo, Robert Shoemaker, and Michael R. Boyd. (1988). "Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay." *Cancer Research* 48(3):584–88.

Antony, Smitha, Keli K. Agama, Ze Hong Miao, Kazutaka Takagi, Mollie H. Wright, Ana I. Robles, Lyuba Varticovski, Muthukaman Nagarajan, Andrew Morrell, Mark Cushman, and Yves Pommier. (2007). "Novel Indenoisoquinolines NSC 725776 and NSC 724998 Produce Persistent Topoisomerase I Cleavage Complexes and Overcome Multidrug Resistance." *Cancer Research* 67(21):10397–405.

Baikar, Supriya, and Nutan Malpathak. (2010). "Secondary Metabolites as DNA Topoisomerase Inhibitors: A New Era towards Designing of Anticancer Drugs." *Pharmacognosy Reviews* 4(7):12–26.

Ballatore, Carlo, Donna M. Huryn, and Amos B. Smith. (2013). "Carboxylic Acid (Bio)Isosteres in Drug Design." *ChemMedChem* 8(3):385–95.

Beck, Daniel E., Monica Abdelmalak, Wei Lv, P. V.Narasimha Reddy, Gabrielle S. Tender, Elizaveta O'Neill, Keli Agama, Christophe Marchand, Yves Pommier, and Mark Cushman. (2015). "Discovery of Potent Indenoisoquinoline Topoisomerase i Poisons Lacking the 3-Nitro Toxicophore." *Journal of Medicinal Chemistry* 58(9):3997–4015.

Beck, Daniel E., Monica Abdelmalak, Wei Lv, P. V. Narasimha Reddy, Gabrielle S. Tender, Elizaveta O'Neill, Keli Agama, Christophe Marchand, Yves Pommier, and Mark Cushman. (2015). "Discovery of Potent Indenoisoquinoline Topoisomerase I Poisons Lacking the 3-Nitro Toxicophore." *Journal of Medicinal Chemistry* 58(9):3997–4015.

Beck, Daniel E., Keli Agama, Christophe Marchand, Adel Chergui, Yves Pommier, and Mark Cushman. (2014). "Synthesis and Biological Evaluation of New Carbohydrate-Substituted Indenoisoquinoline Topoisomerase I Inhibitors and Improved Syntheses of the Experimental Anticancer Agents Indotecan (LMP400) and Indimitecan (LMP776)." *Journal of Medicinal Chemistry* 57(4):1495–1512.

Beck, Daniel E., Wei Lv, Monica Abdelmalak, Caroline B. Plescia, Keli Agama, Christophe Marchand, Yves Pommier, and Mark Cushman. (2016). "Synthesis and Biological Evaluation of New Fluorinated and Chlorinated Indenoisoquinoline Topoisomerase i Poisons." *Bioorganic and Medicinal Chemistry* 24(7):1469–79.

Beecham, Smithkline, and W. Corp. (1995). "Oncologic, Endocrine & Metabolic: Process for Asymmetric Total Synthesis of Camptothecin." *Expert Opinion on Therapeutic Patents* 5(6):583–85.

Beretta, G., P. Perego, and F. Zunino. (2006). "Mechanisms of Cellular Resistance to Camptothecins." *Current Medicinal Chemistry* 13(27):3291–3305.

Bray, Freddie, Ahmedin Jemal, Nathan Grey, Jacques Ferlay, and David Forman. (2012). "Global Cancer Transitions According to the Human Development Index (2008-2030): A Population-Based Study." *The Lancet Oncology* 13(8):790–801.

Burke, Thomas G., and Zihou Mi. (1994). "The Structural Basis of Camptothecin Interactions with Human Serum Albumin: Impact on Drug Stability." *Journal of Medicinal Chemistry* 37(1):40–46.

Chen, Stefanie Hartman, Nei Li Chan, and Tao Shih Hsieh. (2013). "New Mechanistic and Functional Insights into DNA Topoisomerases." *Annual Review of Biochemistry* 82:139–70.

Chennakesava Rao, K., Y. Arun, K. Easwaramoorthi, C. Balachandran, T. Prakasam, T. Eswara Yuvaraj, and P. T. Perumal. (2014). "Synthesis, Antimicrobial and Molecular Docking Studies of Enantiomerically Pure N-Alkylated β-Amino Alcohols from Phenylpropanolamines." *Bioorganic and Medicinal Chemistry Letters* 24(14):3057–63.

Conda-Sheridan, Martin, P. V.Narasimha Reddy, Andrew Morrell, Brooklyn T. Cobb, Christophe Marchand, Keli Agama, Adel Chergui, Amélie Renaud, Andrew G. Stephen, Lakshman K. Bindu, Yves Pommier, and Mark Cushman. (2013). "Synthesis and Biological Evaluation of Indenoisoquinolines That Inhibit Both Tyrosyl-DNA Phosphodiesterase i (Tdp1) and Topoisomerase i (Top1)." *Journal of Medicinal Chemistry* 56(1):182–200.

Conda-Sheridan, Martin, P. V. Narasimha Reddy, Andrew Morrell, Brooklyn T. Cobb, Christophe Marchand, Keli Agama, Adel Chergui, Amélie Renaud, Andrew G. Stephen, Lakshman K. Bindu, Yves Pommier, and Mark Cushman. (2013). "Synthesis and Biological Evaluation of Indenoisoquinolines That Inhibit Both Tyrosyl-DNA Phosphodiesterase I (Tdp1) and Topoisomerase I (Top1)." *Journal of Medicinal Chemistry* 56(1):182–200.

Cushman, Mark, and Leung Cheng. (1978). "Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-c]Isoquinoline System." *Journal of Organic Chemistry* 43(19):3781–83.

Delgado, Justine L., Chao Ming Hsieh, Nei Li Chan, and Hiroshi Hiasa. (2018). "Topoisomerases as Anticancer Targets." *Biochemical Journal* 475(2):373–98.

Dung, Nguyen Tien, Le Nhat Thuy Giang, Pham Hoai Thu, Ngo Hanh Thuong, Dang Thi Tuyet Anh, Luc Quang Tan, Nguyen Ha Thanh, Le Thi Tu Anh, Nguyen Tuan Anh, Bach Long Giang, Nguyen Van Tuyen, and Phan Van Kiem. (2019). "Synthesis and Cytotoxic Evaluation of Carboxylic Acid-Functionalized Indenoisoquinolines." *Natural Product Communications* 14(5).

Elsayed, Mohamed S. A., Yafan Su, Ping Wang, Taresh Sethi, Keli Agama, Azhar Ravji, Christophe E. Redon, Evgeny Kiselev, Katharine A. Horzmann, Jennifer L. Freeman, Yves Pommier, and Mark Cushman. (2017). "Design and Synthesis of Chlorinated and Fluorinated 7-Azaindenoisoquinolines as Potent Cytotoxic Anticancer Agents That Inhibit Topoisomerase I." *Journal of Medicinal Chemistry* 60(13):5364–76.

Eun-jung, Evgeny Kiselev, Martin Conda-sheridan, Mark Cushman, and John M. Pezzuto. (2012). "Induction of Apoptosis by 3-Amino-6(3-Aminopropyl)-5,6-Dihydro-5,11-Dioxo-11H-Indeno[1,2-C)Isoquinoline." *Journal of Natural Product* 1069.

Foto, Egemen, Çigdem Özen, Fatma Zilifdar, Betül Tekiner-Gülbaş, İlkay Yıldız, Esin Akı-Yalçın, Nuran Diril, and İsmail

Yalçın. (2020). "Benzoxazines as New Human Topoisomerase I Inhibitors and Potential Poisons." *DARU Journal of Pharmaceutical Sciences* 28(1):65–73.

Gwong-Jen J., Fort Collins Co. (2010). "Flavivirus Immunogens and Methods of Use." 1(19).

Haldón, Estela, M. Carmen Nicasio, and Pedro J. Pérez. (2015). "Copper-Catalysed Azide-Alkyne Cycloadditions (CuAAC): An Update." *Organic and Biomolecular Chemistry* 13(37):9528–50.

Hevener, Kirk E., Tatsiana A. Verstak, Katie E. Lutat, Daniel L. Riggsbee, and Jeremiah W. Mooney. (2018). "Recent Developments in Topoisomerase-Targeted Cancer Chemotherapy." *Acta Pharmaceutica Sinica B* 8(6):844–61.

Hu, Wei, Xu Sheng Huang, Ji Feng Wu, Liang Yang, Yong Tang Zheng, Yue Mao Shen, Zhi Yu Li, and Xun Li. (2018). "Discovery of Novel Topoisomerase II Inhibitors by Medicinal Chemistry Approaches." *Journal of Medicinal Chemistry* 61(20):8947–80.

Kang, Bo Rui, Juan Wang, Huan Li, Yan Li, Qi Bing Mei, and San Qi Zhang. (2014). "Synthesis and Antitumor Activity Evaluation of 2-Arylisoquinoline-1,3(2H, 4H)-Diones in Vitro and in Vivo." *Medicinal Chemistry Research* 23(3):1340–49.

Kiselev, Evgeny, Sean Deguire, Andrew Morrell, Keli Agama, Thomas S. Dexheimer, Yves Pommier, and Mark Cushman. (2011). "7-Azaindenoisoquinolines as Topoisomerase i Inhibitors and Potential Anticancer Agents." *Journal of Medicinal Chemistry* 54(17):6106–16.

Kiselev, Evgeny, Thomas S. Dexheimer, Yves Pommier, and Mark Cushman. (2010). "Design, Synthesis, and Evaluation of Dibenzo[ c,h ][1,6]Naphthyridines as Topoisomerase i Inhibitors and Potential Anticancer Agents." *Journal of Medicinal Chemistry* 53(24):8716–26.

Kohlhagen, Glenda, Kenneth D. Paull, Mark Cushman, Pamela Nagafuji, and Yves Pommier. (1998). "Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison." *Molecular Pharmacology* 54(1):50–58.

L. Beretta, G., L. Gatti, P. Perego, and N. Zaffaroni. (2013). "Camptothecin Resistance in Cancer: Insights into the Molecular Mechanisms of a DNA-Damaging Drug." *Current Medicinal Chemistry* 20(12):1541–65.

Lebrun, Stéphane, Axel Couture, Eric Deniau, and Pierre Grandclaudon. (2011). "Suzuki-Miyaura Cross-Coupling and Ring-Closing Metathesis: A Strategic Combination to the Synthesis of Indeno[1,2-c]Isoquinolin-5,11-Diones." *Tetrahedron Letters* 52(13):1481–84.

Liu, Chuan Che, Jen Chieh Hsieh, Rajendra Prasad Korivi, and Chien Hong Cheng. (2015). "Cobalt-Catalyzed Dual Annulation of o-Halobenzaldimine with Alkyne: A Powerful Route toward Bioactive Indenoisoquinolinones." *Chemistry - A European Journal* 21(26):9544–49.

Liu, Leroy F., Chung Cheng Liu, and Bruce M. Alberts. (1980). "Type II DNA Topoisomerases: Enzymes That Can Unknot a Topologically Knotted DNA Molecule via a Reversible Double-Strand Break." *Cell* 19(3):697–707.

Mancuso, Anthony J., Shui Lung Huang, and Daniel Swern. (1978). "Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide 'Activated' by Oxalyl Chloride." *Journal of Organic Chemistry* 43(12):2480–82.

Maris A. Cinelli, P. V. Narasimha Reddy, Peng-Cheng Lv, Jian-Hua Liang, Lian Chen, Keli Agama, and and Mark Cushman Yves Pommier, Richard B. van Breemen. (2012). "Identification, Synthesis, and Biological Evaluation of Metabolites of the Experimental Cancer Treatment Drugs Indotecan (LMP400) and Indimitecan (LMP776) and Investigation of Isomerically Hydroxylated Indenoisoquinoline Analogues as Topoisomerase I Poiso." *J. Med. Chem* 314622(4).

Marzi, Elena, Andrea Spitaleri, Florence Mongin, and Manfred Schlosser. (2002). "Fluoro- or Trifluoromethyl-Substituted Benzyl and Phenethyl Alcohols: Substrates for Metal-Mediated Site-Selective Functionalization." *European Journal of Organic Chemistry* (15):2508–17.

Marzi, Laetitia, Keli Agama, Junko Murai, Simone Difilippantonio, Amy James, Cody J. Peer, William D. Figg, Daniel Beck, Mohamed S. A. Elsayed, Mark Cushman, and Yves Pommier. (2018). "Novel Fluoroindenoisoquinoline Non-Camptothecin Topoisomerase I Inhibitors." *Molecular Cancer Therapeutics* 17(8):1694–1704.

Marzi, Laetitia, Yilun Sun, Shar-yin N. Huang, Amy James, Simone Difilippantonio, and Yves Pommier. (2020). "The Indenoisoquinoline LMP517: A Novel Antitumor Agent Targeting Both TOP1 and TOP2." *Molecular Cancer Therapeutics* 19(8):1589–97.

Marzi, Laetitia, Ludmila Szabova, Melanie Gordon, Zoe Weaver Ohler, Shyam K. Sharan, Michael L. Beshiri, Moudjib Etemadi, Junko Murai, Kathleen Kelly, and Yves Pommier. (2019). "The Indenoisoquinoline TOP1 Inhibitors Selectively Target Homologous Recombinationdeficient and Schlafen 11-Positive Cancer Cells and Synergize with Olaparib." *Clinical Cancer Research* 25(20):6206–16.

Monks, Anne, Dominic Scudiero, Philip Skehan, Robert Shoemaker, Kenneth Paull, David Vistica, Curtis Hose, John Langley, Paul Cronise, Anne Vaigro-wolff, Marcia Gray-goodrich, Hugh Campbell, Joseph Mayo, and Michael Boyd. (1991). "Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines." *Journal of the National Cancer Institute* 83(11):757–66.

Morrell, Andrew, Smitha Antony, Glenda Kohlhagen, Yves Pommier, and Mark Cushman. (2006). "A Systematic Study of Nitrated Indenoisoquinolines Reveals a Potent Topoisomerase I Inhibitor." *Journal of Medicinal Chemistry* 49(26):7740–53.

Morrell, Andrew, Michael Placzek, Seth Parmley, Brian Grella, Smitha Antony, Yves Pommier, and Mark Cushman. (2007). "Optimization of the Indenone Ring of Indenoisoquinoline Topoisomerase I Inhibitors." *Journal of Medicinal Chemistry* 50(18):4388–4404.

Nagarajan, Muthukaman, Andrew Morrell, Brian C. Fort, Marintha Rae Meckley, Smitha Antony, Glenda Kohlhagen, Yves Pommier, and Mark Cushman. (2004). "Synthesis and Anticancer Activity of Simplified Indenoisoquinoline Topoisomerase I Inhibitors Lacking Substituents on the Aromatic Rings." *Journal of Medicinal Chemistry* 47(23):5651–61.

Nagarajan, Muthukaman, Xiangshu Xiao, Smitha Antony, Glenda Kohlhagen, Yves Pommier, and Mark Cushman. (2003). "Design, Synthesis, and Biological Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Featuring Polyamine Side Chains on the Lactam Nitrogen." *Journal of Medicinal Chemistry* 46(26):5712–24.

Nguyen, Trung Xuan, Monica Abdelmalak, Christophe Marchand, Keli Agama, Yves Pommier, and Mark Cushman. (2015). "Synthesis and Biological Evaluation of Nitrated 7-, 8-, 9-, and 10-Hydroxyindenoisoquinolines as Potential Dual Topoisomerase i (Top1)-Tyrosyl-DNA Phosphodiesterase i (TDP1) Inhibitors." *Journal of Medicinal Chemistry* 58(7):3188–3208.

Nitiss, John L., Eroica Soans, Anna Rogojina, Aman Seth, and Margarita Mishina. (2012). "Topoisomerase Assays." *Current Protocols in Pharmacology* (SUPPL.57):1–27.

Pham Thi, Tham, Thuy Giang Le Nhat, Thuong Ngo Hanh, Tan Luc Quang, Chinh Pham The, Tuyet Anh Dang Thi, Ha Thanh Nguyen, Thu Ha Nguyen, Phuong Hoang Thi, and Tuyen Van Nguyen. (2016). "Synthesis and Cytotoxic Evaluation of Novel Indenoisoquinoline-Substituted Triazole Hybrids." *Bioorganic and Medicinal Chemistry Letters* 26(15):3652–57.

Pommier, Yves. (2006). "Topoisomerase I Inhibitors: Camptothecins and Beyond." *Nature Reviews Cancer* 6(10):789–802.

Pommier, Yves. (2009). "ChemInform Abstract: DNA Topoisomerase I Inhibitors: Chemistry, Biology, and Interfacial Inhibition." *ChemInform* 40(46):2894–2902.

Pommier, Yves. (2012). "Drugging Topoisomerases: Lessons and Challenges."

Pommier, Yves, and Mark Cushman. (2009). "The Indenoisoquinoline Noncamptothecin Topoisomerase I Inhibitors: Update and Perspectives." *Molecular Cancer Therapeutics* 8(5):1008–14.

Pommier, Yves, Shar yin N. Huang, Rui Gao, Benu Brata Das, Junko Murai, and Christophe Marchand. (2014). "Tyrosyl-DNA-Phosphodiesterases (TDP1 and TDP2)." *DNA Repair* 19:114–29.

Pommier, Yves, Elisabetta Leo, Hongliang Zhang, and Christophe Marchand. (2010). "DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs." *Chemistry & Biology* 17(5):421–33.

Robert w.Irvine, Stephena A. KinLoch, Alison S. McCormix. (1988). "THE SYNTHEsls OF 2 - FLUORO AND 3 - FLUORO - 4-DEYETHOXYDAUNOYYCIN. A." *Tetrahedron* 44(14):4591–4604.

Shapiro, Seymour L., Karl Geiger, Joshua Youlus, and Louis Freedman. (1961). "Indandiones. II. A Modified Dieckmann Reaction." *Journal of Organic Chemistry* 26(9):3580–82.

Sharma, Nilesh K., Ashutosh Kumar, Amrita Kumari, Erik J. Tokar, Michael P. Waalkes, Carl D. Bortner, Jason Williams, Marilyn Ehrenshaft, Ronald P. Mason, and Birandra K. Sinha. (2015). "Nitric Oxide Down-Regulates Topoisomerase I and Induces Camptothecin Resistance in Human Breast MCF-7 Tumor Cells" edited by D. Jourd'heuil. *PLOS ONE* 10(11):e0141897.

Shoemaker, Robert H. (2006). "The NCI60 Human Tumour Cell Line Anticancer Drug Screen." *Natture Reviews* 6:813–23.

Sirivolu, Venkata Ramana, Sanjeev Kumar V. Vernekar, Christophe Marchand, Alena Naumova, Adel Chergui, Amelie Renaud, Andrew G. Stephen, Feng Chen, Yuk Y. Sham, Yves Pommier, and Zhengqiang Wang. (2012). "5-Arylidenethioxothiazolidinones as Inhibitors of Tyrosyl-DNA Phosphodiesterase I." *Journal of Medicinal Chemistry* 55(20):8671–84.

Solomon, V. Raja, Changkun Hu, and Hoyun Lee. (2010). "Design and Synthesis of Anti-Breast Cancer Agents from 4-Piperazinylquinoline: A Hybrid Pharmacophore Approach." *Bioorganic and Medicinal Chemistry* 18(4):1563–72.

Staker, Bart L., Michael D. Feese, Mark Cushman, Yves Pommier, David Zembower, Lance Stewart, and Alex B. Burgin. (2005). "Structures of Three Classes of Anticancer Agents Bound to the Human Topoisomerase I-DNA Covalent Complex." *Journal of Medicinal Chemistry* 48(7):2336–45.

Strumberg, Dirk, Yves Pommier, Kenneth Paull, Muthusamy Jayaraman, Pamela Nagafuji, and Mark Cushman. (1999). "Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons." *Journal of Medicinal Chemistry* 42(3):446–57.

Thi, Tham Pham, Lena Decuyper, Tan Luc Quang, Chinh Pham The, Tuyet Anh Dang Thi, Ha Thanh Nguyen, Thuy Giang Le Nhat, Tra Nguyen Thanh, Phuong Hoang Thi, Matthias D'Hooghe, and Tuyen Van Nguyen. (2016). "Synthesis and Cytotoxic Evaluation of Novel Indenoisoquinoline-Propan-2-Ol Hybrids." *Tetrahedron Letters* 57(4):466–71.

Vann, Kendra R., Yavuz Ergün, Sevil Zencir, Serkan Oncuoglu, Neil Osheroff, and Zeki Topcu. (2016). "Inhibition of Human DNA Topoisomerase IIα by Two Novel Ellipticine Derivatives." *Bioorganic and Medicinal Chemistry Letters* 26(7):1809–12.

Wall, Monroe E., Mansukh C. Wani, J. Allan W. Nicholas, Govindarajan Manikumar, Chhagan Telej, Linda Moore, Anne Truesdale, Peter Leitnerj, and Jeffrey M. Bestermant. (1993). "Synthesis and Structure Activity of Novel Camptothecin Analogs." *J. Med. Chem* 2689–2700.

Yu, Shanbao, Qing Qing Huang, Yu Luo, and Wei Lu. (2012). "Total Synthesis of Camptothecin and SN-38." *Journal of Organic*

*Chemistry* 77(1):713–17.

Zhang, Zhouen, Kazuhito Tanabe, Hiroshi Hatta, and Sei Ichi Nishimoto. (2005). "Bioreduction Activated Prodrugs of Camptothecin: Molecular Design, Synthesis, Activation Mechanism and Hypoxia Selective Cytotoxicity." *Organic and Biomolecular Chemistry* 3(10):1905–10.