Review

A Mini Review of Novel Topoisomerase II Inhibitors as Future Anticancer Agents

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Abstract: Several reviews of inhibitors of topoisomerase II literature have been published covering research before 2018. Therefore, this review is focused primarily on more recent publications with relevant points from the earlier literature. Topoisomerase II is an established target for anticancer drugs, that are further subdivided into poisons and catalytic inhibitors. Whereas most of the topoisomerase II-based drugs in clinical use are mostly topoisomerase II poisons, their mechanism of action has posed severe concern due to DNA damaging potential, including development of multi drug resistance. As a result, we are beginning to see a gradual paradigm shift towards a non-DNA damaging agents, such as the lesser studied topoisomerase II catalytic inhibitors. In addition, this review will describe some novel selective catalytic topoisomerase II inhibitors. The ultimate goal is to bring researchers up to speed by curating and delineating new scaffolds as leads for optimization and development to new potent, safe and selective agents for the treatment of cancer.

Keywords: topoisomerase; anticancer; cancer; anticancer drugs; enzyme

1. Introduction

Cancer is a word that creates deep-seated fear because we immediately associate it with grave illness and high mortality rate. Almost all of us know someone whose life has been blighted by cancer diagnosis, and who has suffered the prolonged pain of the illness. Cancer patients are forced to tolerate a tough treatment regime with all the accompanying side effects. Few are fortunate to escape the distress of cancer over their lifetime since the frightening statistics would suggest that the vast majority of us will either experience it first hand or have a loved one afflicted. As a result of the above and in order to improve the survival and quality of life of cancer patients, medicinal chemists are actively searching for novel effective, safe and selective anticancer drugs. This review deals with an overview of chemical structures and bioactivities of recent agents that target the nuclear enzyme, topoisomerase.

Topoisomerase is an established target for anticancer drugs and is known to be responsible for regulating the topological constraints in DNA, such as under-winning, over-winning, knotting, and tangling. In particular, under-winning (negatively supercoiled) is important because the two strands of DNA must be separated in order for replication to start. Thus, one of the physiological roles of topoisomerase is to relax both positive and negative supercoiled DNA [1]. Topoisomerase fall into two major classes in human eukaryotes, namely Topo I and Topo II. Topo I is a monomeric protein that doesn't need cofactors for biological activity. They regulate the topological state of DNA by breaking a single strand and re-ligating it after the strand passage reaction. Topo II is a homodimer that requires Mg^{2+} and ATP for its catalytic activity. Topo II breaks double strand and passes an intact DNA through the break.

Topo II inhibitors are classically divided into catalytic inhibitors and topo II poisons, according to their mechanism of action. The mechanisms of action of catalytic inhibitors include interfering with DNA binding, DNA cleavage or ATP hydrolysis, or binding to

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the ATP binding site, while the topo II poisons alter topoisomerases into cellular toxins which act by stabilizing the normally transient covalent topo II–DNA complex, leading to permanent breaks in DNA and, subsequently, apoptosis, and they include drugs such as etoposide **1**, doxorubicin **2**, and *m*-amsacrine **3** [2,3,4], as shown in **Figure 1**. Most of the first line agents for treating cancer are Topo II poisons, such as etoposide, that binds to ATP competitive binding site [4-5], doxorubicin and its analogs, that bind to DNA and inhibits religation [6,7], and m-amsacrine (m-amsa) is considered to be an intercalative Topo II poison [8,9]. Although, a study by Ketron et al [9], indicated that the activity and specificity of m-amsa lies in the head group 4′-amino-methanesulfon-m-anisidide. The DNA intercalation of m-amsa is used primarily to enhance the affinity of the drug for Topo II-DNA cleavage complex.

Topo II is further divided into Topo IIαand Topo IIβ. Topo IIα is highly expressed in proliferating cells, while Topo IIβ is dispensable during proliferation. Although the two isoforms are structurally similar the precise role of the β-isoform is unclear. There is speculation that the B-isoform is responsible for the occurrence of acute MLL in patients treated with topoisomerase inhibitor. Topoisomerase II has four domains: (i) N gate, (ii) DNA gate, (iii) C gate and (iv) C-terminal domain that is responsible for DNA recognitaion [10,11].

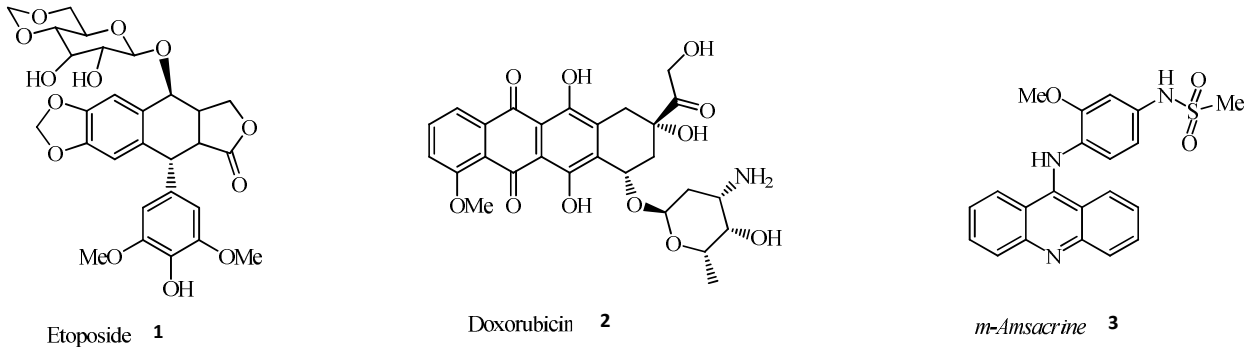

Figure 1. Structure of topoisomerase II poisons.

Topoisomerase II catalytic cycle consists of six sequential steps as follows: (i) recognition and binding of the enzyme to DNA helix 1, (ii) trapping of DNA helix 2 through the ATPase domain dimerization, (iii) double strand break that results in covalent bond formation between the enzyme and the 5′ phosphodiester of DNA, (iv) another segment of DNA is passed through break facilitated by ATP hydrolysis, (v) relegation of the DNA strand break mediated by the release of ADP, (vi) the enzyme and DNA are each restored and ready to start another catalytic cycle. It is worthy of note that DNA cleavage by either Topo I or Topo II is transient and rate determining, while the relegation process is fast and well tolerated by cells. The ability of small organic molecules to modulate topoisomerase activity is an effective method for identifying new cancer therapeutics. On the following pages we delineate recent inhibitors that are still in various stages of development.

A study that used virtual high-throughput screening (VHTS) of ZINC database, showed that four zinc compounds: **4-7** could be potent inhibitors of TopoIIα based on their better docking score than standard drug etoposide, as well as suitable predicted ADME/Tox properties [12]. Similarly, an *in silico* study conducted in Nigeria by Adeniran et al. [13], that used VHTS, three-dimensional quantitative structure activity and relationship (3D-QSAR) and molecular docking approaches, has reported the potential of 20-betaecdysone **8** and andropanoside **9** as better inhibitors of topoisomerase IIα (TopoIIα) than a standard drug, etoposide. This study need further investigation as these compounds are phytochemicals and could be structural optimized to deliver efficient anticancer activity. Additionally, in Slovenia, Skok et al. [14] used *in silico* screening of bacterial topoisomerase inhibitors with *invitro* assay to identify ATP-competitive inhibitors of human DNA

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TopoIIα, and they reported *N-(4-Carbamoyl-2-isopropoxyphenyl)-3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamide*, as a potential active inhibitor of TopoIIα. Further investigation of these computationally screened compounds is necessary to validate their biological activity as anticancer agent.

Ellipticine, an alkaloid from *Ochrosia elliptica* labil has been previously indicated for the treatment of metastatic breast cancer, and several carbazole derivatives based on Ellipticine have shown inhibitory activities against TopoI and TopoII [15,16,17]. In Italy, Saturnino et al. [18] reported *2-(4-((3-Chloro-9H-carbazol-9-yl)pentyl)piperazin-1-yl)-N,N,N-trimethylethanammonium iodide* **10** as a good inhibitor of TopoII and that it showed anti-proliferative activity on breast cancer cells, causing apoptosis by activation of the caspases pathway. Additionally, *7-((2-(dimethylamino)ethyl)amino)indolo[2,1-b]quinazoline-6,12-dione* **26**, which was derived from trypthantrin, a natural alkaloidal compound containing a basic indoloquinazoline moiety, has been reported for inhibitory activity against TopoII, and showed properties such as high water solubility and antiproliferative activity on acute leukemia, colon, and breast cancer cell lines [19]. Garcinol, a polyisoprenylated benzophenone isolated from *Garcinia* genus, has been reported to inhibit eukaryotic TopoI and TopoII at concentrations comparable to that of standard drug etoposide [20]. Mai et al. [21] in China, investigated *9-bromo-2,3-diethylbenzo[de]chromene-7,8-dione* (MSN54) **(16)**, a derivative of Mansonone F (MsF) [22], and they found it to be non-intercalative catalytic inhibitor of TopoIIα. MsF (**17**) is a phytoalexin obtained from *Helicteres Angustifolia* L. with a rare sesquiterpene o-quinone structure, that possessed anti-tumour activity, and its derivatives have shown strong inhibitory activity on TopoII [23].

A study in Italy that make used of biochemical assays along with molecular docking and molecular dynamics methods, has reported that the derivatives of garcinol were acting as catalytic inhibitors of TopoII via mixed inhibition of ATP hydrolysis; in which guttiferone M showed the most significant effects against TopoIIα and TopoIIβ, whereas oxyguttiferones K and M showed activity against TopoIIβ that was slightly better than that of the parent garcinol compound [24]. A collaborative work by Zidar et al. [25], in Italy and Slovenia, have reported that *N-phenoxylypropyl-3-methyl-2-phenyl-1H-indoles* **20, 21**, had potent antiproliferative and antitopoisomerase II activities against three selected human tumor cell lines: cervix adenocarcinoma (HeLa), ovarian carcinoma (A2780), and biphasic mesothelioma (MSTO-211H), and it was capable of inducing the apoptosis pathway. This compound has structural similarities with some naturally occurring flavonoids that inhibit topoisomerase II (TopoII), such as quercetin and luteolin. Thus, it is scientifically okay and could have broader application in anticancer drug development.

Zhou et al. [26], in China, studied a novel perimidine *o*-quinone derivatives, and found that *2-(4-Chlorophenyl)-1-methyl-1H-perimidine-5,6-dione*, showed the best anti-proliferative activity (IC50 ≤ 1 μM) against four cancer cell lines (HL-60, Huh7, Hct116, and Hela) by inducing apoptosis in a dose- dependent manner, and exhibited potent topoisomerase IIα (TopoIIα) inhibitory activity (IC_{50} = 7.54 μM). They provided evidence that compound 21 did not intercalate into DNA and suggested that it may act as an ATP competitive inhibitor by blocking the ATP-binding site of the enzyme; this was also tested by molecular docking of compound 21 in the ATP-binding domain of human TopoIIα (PDB code: 1ZXM). Overall, the study is well-robust and may provide advanced opportunities for the design and development of new cancer chemotherapy agents.

Sakr et al. [27], in Egypt, reported that *N-Cyclohexyl-2-(3-methyl-[1,2,4]-triazolo[3,4-a]phthalazin-6-yl)-hydrazine-1-carboxamide* **11**, a derivative of triazolophthalazine, showed slightly high cytotoxic activity than doxorubicin when tested against human cancer cell line (HepG2, MCF-7, and HCT-116 cells), induced apoptosis in HepG2 cells and G2/M phase cell cycle arrest, and showed Topo II inhibitory activity. However, they reported that compound 22 showed TopoII poisoning effects at 2.5 μM and Topo II catalytic inhibitory effects at 5 and 10 μM; and these results indicate that this compound could serve as a two-edge chemotherapeutic agent, thus require further validation. Previous study has reported compound that contain the 1,4-diaminobenzo[g]phthalazine nucleus, had a promising binding affinity against DNA by intercalation [28]. Also, Arencibia et al. [29],

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reported *6-Hydroxy-4-oxo-1,3-diphenyl-2-thioxo-N-(3-(trifluoromethoxy)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide* **24** as the most promising drug-like candidate that act as TopoII poison and exhibit good solubility, metabolic (microsomal) stability, and promising cytotoxicity in three cancer cell lines (DU145, HeLa, A549).

Additionally, four compounds from a new series of [1,2,4]triazolo[4,3-*a*]quinoxaline and bis([1,2,4]triazolo)[4,3-*a*:3',4'-*c*]quinoxaline derivatives, showed cytotoxic activities against three tumor cell lines (HePG-2, Hep-2 and Caco-2), of which *2-(Bis[1,2,4]triazolo[4,3-a:3',4'-c]quinoxalin-3-ylsulfanyl)-N-(4-fluorophenyl) acetamide* was the most effective inhibitor against TopoII, intercalates DNA, and caused cell cycle arrest at G2/M phase and induced apoptosis in Caco-2 cells [30]. Also, El-Adl et al. [31], in Egypt, worked on twenty-four novel [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives, and they reported that *2-{4-([1,2,4]Triazolo[4,3-a]quinoxalin-4-ylamino)benzoyl}-N-cyclohexylhydrazine-1-carboxamide* **12**, *2-{4-([1,2,4]Triazolo[4,3-a]quinoxalin-4-ylamino)benzoyl}-N-cyclohexylhydrazine-1-carbothioamide*, and *4-(Diethylamino)-[1,2,4]triazolo[4,3-a]quinoxaline-1-thiol* **11b**, were the most potent derivatives against the tested three HepG2, HCT116 and MCF-7 cancer cell lines, and that these three compounds also displayed very good to moderate DNA-binding affinities, and exhibited very good inhibitory activities against TopoII enzyme. However, no in vitro differentiation was made between TopoIIα and TopoIIβ, but molecular docking was carried out on DNA-Topo II receptor (PDB code: 4G0U). Previous study has reported several quinoxaline compounds such as *1-(2-Bromoethyl)-1,4-dihydroquinoxaline-2,3-dione; 4-Amino-N'-(3-chloroquinoxalin-2-yl) benzohydrazide; N'-(3-chloroquinoxalin-2-yl)-isonicotinohydrazide;* and *3-mercaptoquinoxalin-2-yl carbamimidothioate*, from the series of quinoxaline derivatives were DNA intercalator, effective inhibitor of TopoII, and showed anti-proliferative activities against HePG-2, MCF-7 and HCT-116 cell lines [32-35].

Bruno et al [36], in USA, have explored CRISPR datasets (19Q3 DepMap Public data) together with biochemical and cell biological assays to showed that CX-5461 **25** which is structurally similar to ciprofloxacin and voreloxin, exerts its primary cytotoxic activity through topoisomerase II poisoning. This study was holistic in its approach, and it could be applied to other investigational small molecules. They attempted to rethink the verdict by Lin et al [37], which stated that off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. They further suggested that the mechanism of cell death induced by CX-5461 is critical for rational clinical development in patients with relapse/refractory hematopoietic tumors based on its previous clinical indication as an inhibitor of RNA polymerase I [38,39]. Recent study has suggested that CX-5461 could stabilize G-quadruplex DNA and cause DNA damage [40], and that BMH-21 which is an inhibitor of RNA polymerase I, suppresses the nucleolar translocation of both TopoIIα and TopoIIβ in ATP-depleted cells [41]. Similarly, in United Kingdom, Cowell et al [42] explored PR-619 [*2,6-Diaminopyridine-3,5-bis(thiocyanate)*] **31**, a broad-spectrum deubiquitinating enzyme (DUB) inhibitor [43], and they found it to be a potent TopoII poison, inducing both TopoIIα and TopoIIβ covalent DNA complexes with an efficiency equal to that of etoposide.

On prostate cancer (PCa) targeted TopoII inhibition, Jeon et al [44], investigated the mechanism by which AK-I-190 [*2-(3-trifluorophenyl)-4-(3-hydroxyphenyl)-5H-indeno[1,2-b]pyridin-6-ol*], inhibits TopoII by using various types of biological and spectroscopic evaluations, and they found that its inhibitory activity was through intercalating into DNA without stabilizing the DNA-enzyme cleavage complex, which result in significantly less DNA toxicity than etoposide; and inhibits the growth of AR-negative PCa cell. This work served as a therapeutic strategy against castration-resistant prostate cancer (CRPC), resulting from androgen independence in cellular growth [45]. The lead compound, T60 (PubChem CID: 36589274) **13**, was reported as potent inhibitors of both TopoIIα and TopoIIβ enzymatic activities as well as dual inhibitory activity on androgen receptor (AR) (an oncogenic transcriptional factor that require TopoIIβ) and AR-positive PCa cell growth [46]. Matias-Barrios et al [47] in Canada, investigated the derivatives of T60 in order to improve the pharmacokinetic properties and further enhance its efficacy to inhibit TopoII proteins; and they found that T638 an amino derivative on the centre benzene

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ring of T60), retained the TopoII inhibitory activities, and shown improved solubility and better metabolic stability, with possibility of high dosage administration due to low cytotoxicity [47]. In like manner, heteronemin (a marine sponge *Hyrtios* sp. Sesterterpene) promoted apoptosis and autophagy through the inhibition of TopoIIα and HSP90 as well as protein tyrosine phosphatase (PTP) activation in PCa cells [48], and inhibited TNFα-induced NF-κB activation through proteasome and induced apoptotic cell death [49].

Ortega et al [50] in Italy, studied a novel class of 6-amino-tetrahydroquinazoline derivatives, and they pinpointed *N⁴-[4-(Dimethylamino)phenyl]-2-(4-pyridyl)-5,6,7,8-tetrahydroquinazoline-4,6-diamine* **15** as the main lead compound for inhibition of DNA relaxation, which possessed excellent metabolic stability and solubility than etoposide, and that this compound showed about 100-fold selectivity for TopoIIα over Topoβ, with a broad antiproliferative activity against cultured human cancer cells, satisfactory *invivo* pharmacokinetic profile, and penetrability of the blood–brain barrier. These excellent properties applauded this compound 36 as a highly promising lead for the development of novel and potentially safer TopoII-targeted anticancer drugs. Also in China, Chen et al [51], have evaluated the derivatives of acridine hydroxamic acid, and they found *7-(4-(4-(Acridin-9-ylamino)-phenyl)-1H-1,2,3-triazol-1-yl)-N-hydroxyheptanamide* **27**, showing best inhibitory activities for TopoII and histone deacetylase (HDAC), and it could intercalate into DNA and induce U937 apoptosis. Combination of Topo and HDAC inhibitors have been found showing synergistic anticancer effects with enhanced cytotoxicity [52,53]. These dual inhibitory compounds are promising drug candidates that could serve as double-edge sword to effectively inhibit tumour growth and progression.

In USA, Oyedele et al [54], worked on a novel series of novel acridone derivatives, of which five 7-chloro-3-phenyl-3,4-dihydroacridin-1(2H)-one], 7-bromo-3-phenyl-3,4-dihydroacridin-1(2H)-one, 7-methoxy-3-(trifluoromethyl)-3,4-dihydroacridin-1(2H)-one, 7-methoxy-3-phenyl-3,4-dihydroacridin-1(2H)-one, and 5,7-dibromo-3-phenyl-3,4-dihydroacridin-1(2H)-one, showed excellent invitro antiproliferative activities against 60 human cancer cell lines. Overall, 5,7-dibromo-3-phenyl-3,4-dihydroacridin-1 (2H)-one was found to be the most active and sensitive agent in all the nine cancer panels in an order of: prostate > leukemia > non-small cell lung cancer > colon cancer > CNS cancer > melanoma > renal cancer > ovarian cancer > breast cancer, and they suggested possible inhibition of TopoIIα based on molecular binding interaction with the active site ATPase domain [54]. The limitation to this work was that no standard drug was used to compared against the activity of the synthesized compounds on the 9 cancer panels, and actual assay for TopoIIα was not conducted. Moreover in China, Li et al [55], reported that newly developed acridone derivatives, 1-((3-(dimethylamino)propyl)amino)-7-hydroxy-4-nitroacridin-9(10H)-one, could inhibit TopoIIα, intercalates with DNA, and showed significant and long-term anti-proliferative activity at relatively high concentrations. Previous studies have identified several acridone derivatives as TopoII inhibitors and DNA intercalator with cell cycle arrest and apoptosis [56,57]. Furthermore, in Egypt, Nemr et al [58], and Nemr and AboulMagd [59], worked on a novel series of thiazolopyrimidines and fused thiazolopyrimidines, screened for anticancer activity against 60 human cancer cell lines. They found Ethyl 4-(4-bromophenyl)-2-imino-9-(3,4,5-trimethoxyphenyl)-7-phenyl-1,2-dihydro-9H-pyrimido[4',5':4,5]thiazolo[3,2-a]pyrimidine-8-carboxylate, and Ethyl 3-(4-chlorophenyl)-5-(4-chlorophenyl)-7-phenyl-5H-thiazolo-[3,2-a] pyrimidine-6-carboxylate hydrobromide, to be the potent inhibitors on renal cell line (A-498) and induce cell cycle arrest at G2/M phase leading to cell proliferation inhibition and apoptosis, and that their fused derivative both showed potent TopoII inhibitory activity with IC_{50} slightly higher than that of standard drug, doxorubicin.

Moreover, in Egypt, *2-(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbonyl)-N-(m-tolyl)-hydrazinecarbothioamide* a derivative of nalidixic acid (**14**), has been shown to be a potent inhibitor of TopoIIα and TopoIIβ, and induced cell cycle arrest at G2-M phase leading to inhibition of cell proliferation and apoptosis [60]. According to Jiang et al [61], four compounds from a series of carbazole-rhodanine conjugates, was found possessing topoisomerase II inhibitory activity, with potency at 20 μM. However,

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the study did not use any human cell lines but plasmid (pBR322 DNA), not extensively explored biologically and failed to differentiate between TopoIIα and TopoIIβ. Shrestha et al [62] in Korea, investigated a series of new benzofuro[3,2-b]pyridin-7-ols derivatives, and their results showed chemical structure named compound 11, that has *meta*-OH positions in 2,4-diphenol moieties of benzofuro[3,2-b]pyridin-7-ol ring, as the most selective and potent TopoII inhibition, with sturdiest antiproliferative activity in HeLa cell line. Although, the work could differentiate between Topo I and TopoII activity, it failed to classify the inhibition of TopoII whether as IIα and IIβ. A collaborative work by Oviatt et al [63] in Italy and USA, reported that etoposide derivatives in which the C4 sugar moiety was replaced with a variety of polyamine tails induce higher levels of DNA cleavage with human topoisomerase IIα and IIβ than does the parent drug. Although some of the hybrid compounds showed better cleavage on TopoIIα than etoposide, the interaction of all these derivatives on TopoIIβ in more fold cleavage than etoposide, implicated Gln778 and limit their further clinical usefulness.

In China, Song et al [64] worked on a novel series of pyrazoline **22, 23** derivatives, and they reported 8-chloro-3-(1H-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydrothiochromeno[4,3-c] pyrazole and 6,8-dichloro-3-(1H-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydrothiochromeno-[4,3-c]pyrazole, for antiproliferative activity in four human cancer cell lines (MGC-803, Hela, MCF-7 and Bel-7404) and a low cytotoxicity on normal cell line L929 in vitro. Both were nonintercalative Topo II catalytic inhibitors, and able to induce G2/M cell cycle arrest and apoptosis in MGC-803 cells. Moreover, the derivatives of 4,5′-bithiazoles were reported acting as catalytic inhibitors of TopoIIα via competitive inhibition of ATP hydrolysis, and they were able to reduce cell proliferation and stop the cell cycle mainly in the G1 phase [65]. Li et al [66] in China, reported that N-(3-(4-Methylpiperazin-1-yl)propyl)-50-methyl-10H-ursa-2,12-dieno[3,2-b]indol-28-carboxamide, a new indole derivative of ursolic acid, exhibits the most effective activity against two human hepatocarcinoma cell lines (SMMC-7721 and HepG2) and normal hepatocyte cell line (LO2) via MTT assay. The results showed that the compound significantly inhibited TopoII activity, and elevate the intracellular ROS levels, decrease mitochondrial membrane potential, and caused apoptosis of SMMC-7721 cells.

Moreover, Legina et al [67] in Austria, have used biological assays and molecular dynamic simulations to showed that thiomaltol-containing ruthenium (Ru^{II})-, osmium (Os^{II})-, rhodium (Rh^{III})- and iridium (Ir^{III})-based organometallic complexes bearing 1-methylimidazole or chloride as leaving group, possessed cytotoxic and DNA-damaging activity in human mammary carcinoma cell lines. A study on the anticancer properties of novel Ru^{II}, Os^{II}, Rh^{III}, and Ir^{III} thiomaltol complexes showed that they act as inhibitors of TopoII catalytic activity and have a significantly higher enzyme inhibitory capacity than the free ligand [68]. In 2014, Bau, Kang and their group [69] reported that salicylate **29** showed selectivity for topo IIα-isoform in DNA cleavage assay, thus acting as a catalytic inhibitor. However, further studies are needed to confirm the basis for its isoform selectivity. In their studies, they reported that salicylate did not intercalate DNA and did not prevent the enzyme from interacting with DNA. Furthermore, salicylate did not stabilize the cleavable "complex".

Ziga *et al.* [70] reported that **30** as a novel ATP-competitive inhibitor of hDNA topo IIα containing pyrrolamide pharmacophore. The compound showed high kinetic ATPase activity (IC_{50} 0.43 μM). In another studies, Kamila, Anna, Krzysztof, and Agnieszka [71] reported that **32**, a quinolone derivative is in phases I and II clinical trials in combination with azacitidine and infusional cytabarin. In a recent report Sisodiya and co-workers [72] reported the synthesis of benzo-fused carbazolequinone derivatives, that contain both indole and quinone moieties found in numerous drugs, including natural products. Compound **28** displayed significant apoptotic antiproliferation in cancer cells with cell cycle arrest at S-phase. It also inhibited topoIIα with more efficiency compared to etoposide. The structures of topoisomerase II inhibitors (4-17, and 18-32) are shown in **Figure 2 and 3**.

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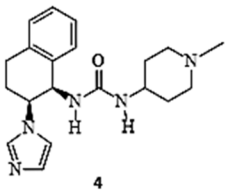

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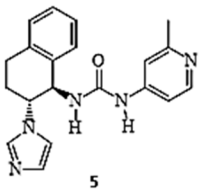

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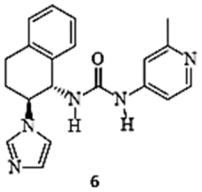

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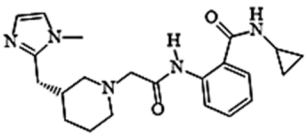

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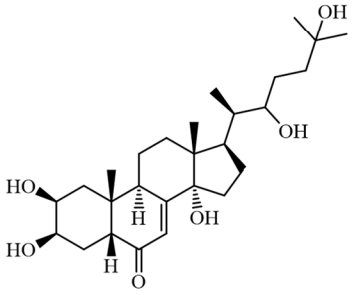
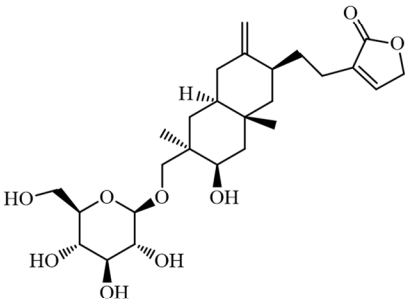
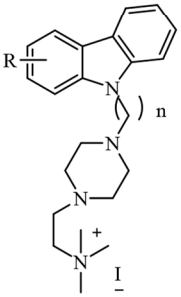


20-beta-ecdysone, **8**



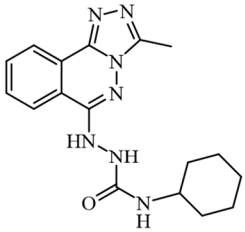
Adropanoside, 9



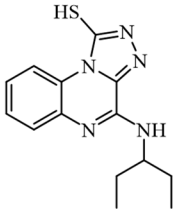
R = H: n = 3,4,5,6,7

R = Cl: n = 5,6

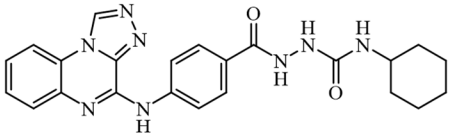
N-Alkylcarbazole, **10**



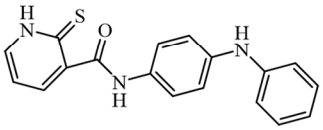
Triazolo-phthalazine derivative, IX_b **11**,



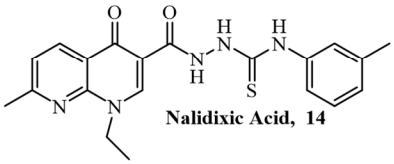
11, topo II = 0.384 micromolar



12, topo II = 0.379 micromolar



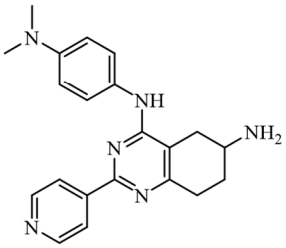
T60, Catalytic Topo 2 inhibitor, **13**



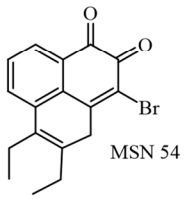
Nalidixic Acid, 14

topo II-alpha IC_{50} = 1.30 micromolar

topo II-beta IC_{50} = 0.017 micromolar

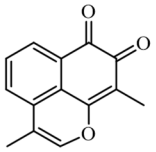


Selective catalytic inhibitor of Topo II, **15**



MSN 54

16



Mansanone F, **17**

Figure 2. Structures of topoisomerase II inhibitors 1.

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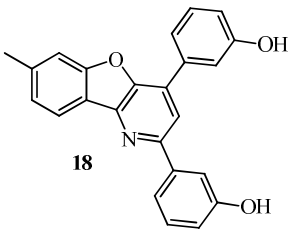
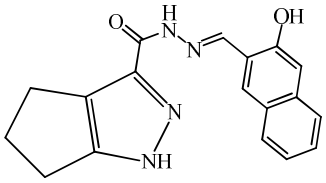
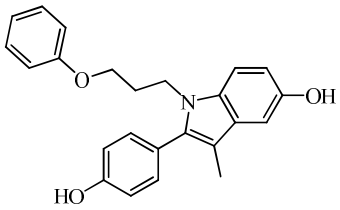


18

Topo II: IC50 = 0.86 micromolar in HeLa cell line
topo II, 100% inhibition at 100 micromolar



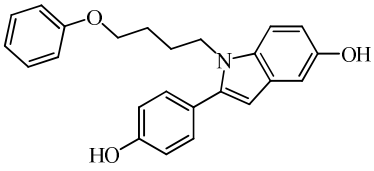
19



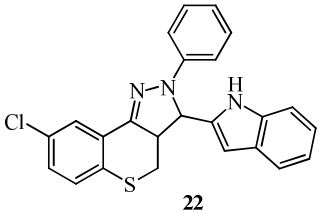
20

inhibitory effect on topo II relaxation; induction of apoptosis

GI50: HeLa 4.4 micromolar
A2780 2.2 micromolar
MSTO-211H 2.4 micromolar



21



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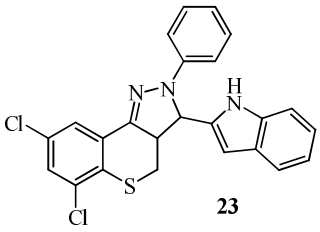
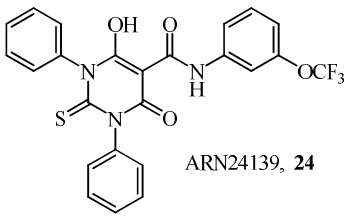
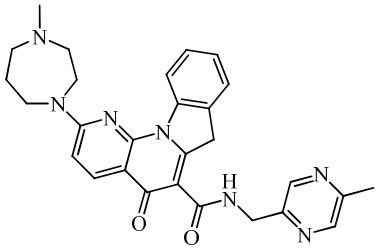


23

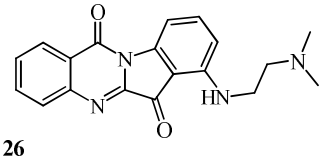
Pyrazoline-containing indole skeleton



ARN24139, **24**

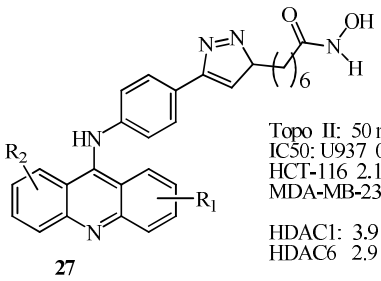


CX-5461, **25**



26

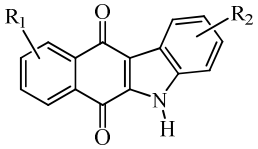
Topo II-alpha: IC50 = 26.6 micromolar



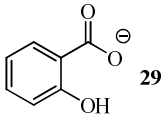
27

Topo II: 50 micromolar inhibition
IC50: U937 0.90 micromolar
HCT-116 2.11
MDA-MB-231 7.09

HDAC1: 3.9 nm
HDAC6 2.9 nm

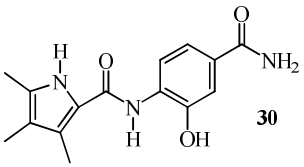


Benzo[b]carbazole-6,11 diones **28**



29

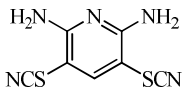
Salicylate, selective catalytic inhibitor of Topo II



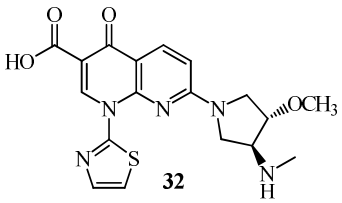
30

pyrrolamide pharmacophore

Topo II-alpha , 3.2 micromolar
kinetic ATpase assy, 0.43 micromolar



31, PR-619
potent DNA Topo II poison, DUB



32

Novel quinolone Der

Figure 3. Structures of topoisomerase II inhibitors 2.

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2. Conclusion

In this review we see significant number of reports of small molecule inhibitors of topoisomerase II. Topoisomerase II poisons continue to dominate the literature despite reports of cardiotoxicity, and multi-drug resistance, including secondary malignancy. In the past three years we have also seen a gradual increase in the number of catalytic inhibitors, that appear more attractive from safety standpoint. However, no catalytic inhibitor has received FDA approval. The large number of reports of topoisomerase II inhibitors in the recent literature reflect the high level of interest in topoisomerase II inhibitors as therapeutic target. Several reports have confirmed the structural similarity between topo IIα and topo IIβ. The two isoforms are similar except in the C-terminus. The above calls for structure-based drug design beyond molecular docking. Docking simulations are prone to inaccuracy because the scoring functions used make estimates of the binding energy. In addition, docking often excludes hydrogens and solvents. On the other hand, molecular dynamic simulation of the drug-protein complex gives a more accurate binding energy since it considers protonation and solvation. Thus, docking and MD simulation would undoubtedly reveal the structural differences that exist at the C-terminus. In addition, structural modification of natural products and hit molecules will continue to be an integral part of drug discovery of novel topoisomerase II inhibitors.

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