Natural Compounds as Therapeutic Agents: The Case of Human Topoisomerase IB

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Abstract: Natural products are widely used as source for drugs development. An interesting example is represented by natural drugs developed against human topoisomerase IB, a ubiquitous enzyme involved in many cellular processes where several topological problems occur due the formation of supercoiled DNA. Human topoisomerase IB, involved in the solution of such problems relaxing the DNA cleaving and religating a single DNA strand, represents an important target in anticancer therapy. Several natural compounds inhibiting or poisoning this enzyme are under investigation as possible new drugs. This review summarizes the natural products that target human topoisomerase IB that may be used as the lead compounds to develop new anticancer drugs. Moreover, the natural compounds and their derivatives that are in clinical trial are also commented on.

Keywords: topoisomerase; cancer; natural products

1. Introduction

Humankind has faced numerous challenges for its survival, even when the challenge was an invisible enemy such as viruses, bacteria and other pathogens. Nature has always provided help in order to allow its survival, and natural products (NPs) have been one solution of human health problems [1,2]. Even nowadays, despite the advent of the pharmaceutical industry, the availability of synthetic compound libraries and the power of the high-throughput screening, scientists find it useful to look at nature as a source of drugs [3,4].

The understanding of the power of NPs as a source of drugs started in the 19th century with the isolation of morphine from Papaver somniferum, used as an analgesic and sleep-inducing agent that, today, has developed into codeine, a painkiller [5,6]. The most famous drug of natural origin is probably salicylic acid, initially called salicin, extracted from the bark of the willow tree Salix alba [4,7]. Salicylic acid was the first NP produced in a large scale by chemical synthesis in 1853 and gave rise to the famous drug aspirin [8]. Additional examples are anti-malaria compounds such as artemisinin from the Chinese herb Artemisia annua, used to treat the malaria-causing parasites Plasmodium falciparum, and quinine, used since 2004 when it was approved by the US Food and Drug Administration (FDA), isolated from the bark of Cinchona succirubra [4,7].

Several NPs have been found to display antitumor activity [9]. From bacterial sources, we can list daunorubicin, an anthracycline from Streptomyces peucetius [10,11], and its semi-synthetic derivate doxorubicin, which acts intercalating in DNA and blocking human topoisomerase II [12,13]. From plants, we can find vincristine and vinblastine, two terpenes extracted from Catharanthus roseus [14,15], that inhibit the mitosis, binding to microtubules [16–18]. Another important antitumor agent is camptothecin (CPT), extracted from the bark of the Chinese tree Camptotheca acuminata, and the soluble derivatives irinotecan and topotecan, both efficient topoisomerase I poisons [19,20].
DNA topoisomerases are a class of ubiquitous enzymes identified for the first time in 1971 in *Escherichia coli* by James C. Wang [21]. Subsequently, this enzyme was found in nuclear extracts from eukaryotic mouse embryo cells by Champoux and Dulbecco [22]. The enzyme is able to relax supercoiled DNA to introduce negative or positive supercoils into DNA and to decatenate circular DNA. Indeed, DNA topoisomerases deal with all the cellular processes that involve DNA topological issues and, in human cells, are involved in regulating several fundamental processes: DNA replication, transcription and chromosome segregation [23]. Human topoisomerases (hTops) are grouped into class I (hTopI) and II (hTopII), according to their ability to cut one or both DNA strands to release the constrains and unwind supercoiled DNA [24]. In the hTopI enzyme, catalysis occurs through a tyrosine residue, located in the catalytic pocket at the C-terminal, which undergoes a nucleophilic attack on the phosphodiester bond of DNA, forming a transient phosphotyrosyl bond with the 3' or 5' DNA break (Figure 1). These two different types of bonds define two subclasses of the enzyme named A and B when they bind the 3' or 5', respectively. Once the rotation has been completed, the religation step can occur, bringing the reconstitution of the phosphodiester backbone and the consequent release of the enzyme from the DNA (Figure 1) [25–28]. It is worth noting that DNA unwinding is driven by torsional strain, rather than powered by ATP hydrolysis [28,29].

![Figure 1. Schematic representation of the catalytic cycle of hTopI. Once the enzyme binds a supercoiled DNA (1), the cleavage step occurs (2), followed by the controlled rotation of the cleaved strand (3) and by a religation event (4) and the release of the unwound substrate (5).](image)

**2. Human DNA Topoisomerase IB as the Tumor Target**

hTopIB is a 91-KDa protein, made up of 765 amino acids, divided into 4 domains: The N-terminal, the core, the linker and the C-terminal domain (Figure 2 Top). The N-terminal domain (1–214) allows the enzyme nuclear localization [30] and is involved in the modulation of the noncovalent enzyme–DNA interactions [31]. The core domain (215–635) is highly conserved and is directly involved in the binding of the DNA substrate [32,33]. Single mutations in this domain, such as glutamine 418, induce a different DNA-binding specificity and modulate the enzyme–drug interactions [34]. The linker domain (636–712) has a fundamental role in the catalytic mechanism controlling the rotation of the free DNA strand around the cleavage site [35,36]. Indeed, mutations that alter the flexibility of the linker perturb the enzyme sensitivity to the drugs targeting the enzyme [37–40]. The C-terminal domain (713–765) contains Tyr 723, which undergoes the nucleophilic attack to the substrate and forms together with Arg 488, Lys 532, Arg 590 and His 632 the...
catalytic site [23,41,42]. The mutation of Gly 717, located in this domain, causes a slight rearrangement of the active site and perturbs the drug binding site [43].

Figure 2. Structure of hTopIB. Top panel schematic representation of the hTopIB domains. The N-terminal domain in yellow (1–214), the core in red (215–635), the linker in green (636–712) and the C-terminal domain in light blue (713–765). The arrows represent the amino acids forming the active site. Bottom panel is the 3D structure of the enzyme, where the domains are represented in the same color.

There are two different types of drugs that can affect hTopIB catalysis: poisons and inhibitors [44–46]. The poisons are compounds that lead to the stabilization of a ternary complex between the enzyme, DNA and drug itself, turning the enzyme into a poison. In detail, the catalytic cycle consists in the cutting of a single DNA strand, strand rotation and, finally, religation of the relaxed substrate. In the presence of a poisoning drug that intercalates DNA in correspondence to the cleavage site, the enzyme is inhibited to undergo the religation step. The persistence of hTopIB on the nicked DNA leads to the stalling and collapse of the replication fork and to the formation of DNA double-stranded breaks on the enzyme cleavage site activating apoptosis and inducing cell death [47]. The inhibitors work in a simpler manner; they inhibit the cleavage of the DNA by the enzyme or prevent the binding to DNA. In this case, the persistence of supercoiled regions during cell replication lead to the stall of the replication fork, the formation of DNA single-stranded breaks and a consequent genomic damage that brings the cell to its death.

Poisons have clinical relevance, and their efficient cytotoxic effect is demonstrated by the use of CPT, the first discovered hTopIB poison [19,48,49]. CPT is an E-ring lactone that reversibly interacts with both DNA and hTopIB, intercalating between the DNA bases after the DNA cleavage has occurred, trapping the enzyme on the DNA and bringing the cells to death. The CPT derivatives, irinotecan and topotecan, are in clinical use, but they have several side effects [44].
The arising importance of hTopIB as a tumor target has pushed researchers to look for novel natural sources to be used as lead compounds to selectively poison hTopIB without the side effects observed for the CPT derivatives [20]. In a previous manuscript, we reviewed the natural compounds targeting hTops up to 2012 [50]; here, we review the natural compounds reported to target hTopIB from 2012 to now and the modified natural compounds that are in clinical trials.

3. Natural Compounds with In Vitro and In Vivo Activity on hTopIB

Natural compounds with in vitro and/or in vivo antitumor activity targeting hTopIB are listed in Table 1.

| Compound Name     | Source                | Type of Inhibitor         | Reference |
|-------------------|-----------------------|---------------------------|-----------|
| EGCG              | Camellia sinensis     | Not studied               | [51]      |
| Kakuol            | Asarum sieboldii      | Catalytic Inhibitor       | [52]      |
| Berberine         | Coptis chinensis and Berberis vulgaris | Catalytic Inhibitor | [53] |
| Pinostrobin       | Honey and dietary vegetables | Poison              | [54]      |
| SQDG              | Azadirachta indica    | Catalytic Inhibitor       | [55]      |
| Benzoxazines      | Capparis sikimensis   | Catalytic Inhibitor       | [56]      |
| Evodiamine        | Evodia rutaecarpa      | Poison                    | [57]      |
| Cytosporolide C   | Cytospora sp           | Not studied               | [58]      |

3.1. Epigallocatechin-3-Gallate

Epigallocatechin-3-gallate (EGCG) (Figure 3A) is a major polyphenolic constituent of green tea extracted from Camellia sinensis leaves [59]. Over the years, this plant has received a lot of attention for the health benefits associated with green tea consumption, such as antioxidant effects, cancer chemoprevention, cardiovascular health improvement, weight loss enhancement and skin protection from the damage caused by ionizing radiation. Nowadays, several laboratories have demonstrated that EGCG possess cancer therapeutic effects, and Mukhtar’s group at the University Hospitals of Cleveland has shown that this compound has a dose-dependent inhibitory effect on several human carcinoma cell lines [60]. In normal cell lines, EGCG does not have any cytotoxic effect, as tested by a viability assay [60]. EGCG has a complex mechanism of action, and it has several targets, such as nuclear factor kappa light-chain enhancer of activated B cells (NF-kB) [61], vascular endothelial growth factor (VEGF) [62] and hTopIB [51]. A significant inhibition of hTopoIB activity but not hTopII was observed through a relaxation assay [51].

3.2. Kakuol

Kakuol (Figure 3B) is a metabolic oxidation product isolated from the rhizomes of Asarum sieboldii. Extracts from this plant, have antalgic, anti-inflammatory, anticonvulsive, antitussive, antiallergic and antitumoral activities [63]. Studies of interactions of this compound and its derivatives with hTopIB demonstrate that kakuol is a catalytic inhibitor of this enzyme. The compound inhibits a cleavage reaction, and the effect is enhanced preincubating the drug with the enzyme. The effect is due to the inhibition of the catalytic activity and not to the prevention of DNA binding, as shown by the EMSA assay [64].

3.3. Berberine

Coptis chinensis and Berberis vulgaris are two Chinese plants that produce a natural quaternary alkaloid called berberine [65]. This compound (Figure 3C) can be found in roots, rhizome and the stem bark of plants, and it has been used since ancient times in Chinese medicine. Berberine has several positive effects, such as antimicrobial [66], anti-inflammatory [67], anti-arrhythmic [68] and antitumor activity [52]. Regarding antitumor activity, it has been observed that berberine and its derivatives are able to inhibit hTopIB in
a dose-dependent manner. The inhibition is more evident when hTopIB is preincubated with the compound [69]. The drug works as a catalytic inhibitor, since the enzyme is able to bind, but not to cleave, the DNA in the presence of berberine.

![Structures of natural products with in vivo and in vitro activity against hTopIB.](image)

**Figure 3.** Structures of natural products with in vivo and in vitro activity against hTopIB. (A) Epigallocatechin-3-gallate, (B) Kakuol, (C) Berberine, (D) Pinostrobin, (E) Sulfonoquinovosyl diacylglyceride SQDG, (F) Benzoxacin, (G) Evodiamine and (H) Cytosporolide C. Structures are represented by MarvinSketch, as reported on PubChem.

### 3.4. Pinostrobin

Pinostrobin (Figure 3D) is a flavonoid found in honey and in some dietary plants and is used as a natural food supplement. The compound has shown antimicrobial [70], anti-inflammatory [71], antioxidant [72] and antiproliferative properties [73]. Pinostrobin has been studied by Jadaun et al., who suggested that the compound forms a ternary complex with hTopIB and DNA [53]. The in vitro catalytic assay and in silico analysis indicate that the binding of pinostrobin occurs at the interface of hTopIB and DNA in a CPT-like manner. The authors propose that the compound can be used as the lead compound to develop new hTopIB poisons.
3.5. Sulfonoquinovosyl Diacylglyceride

Sulfonoquinovosyl diacylglyceride (SQDG), identified for the first time by Benson and coworkers in photosynthetic bacteria and higher plants [74], is a plant sulfolipid isolated from *Azadirachta indica*, showing antibacterial, antiviral [75] and antileukemic activity [76]. SQGD (Figure 3E) is able to inhibit the hTopIB enzyme, as evaluated by the relaxation assay and by a cleavage assay on a radiolabeled oligonucleotide [76]. The results indicated that the compound acts as a catalytic inhibitor. The in vitro test on acute lymphoblastic/lymphocytic leukemia cell lines overexpressing hTopIB and in vivo experiments in nude mice demonstrate that SQDG treatment delays tumor growth and reduces the expression of cell proliferation markers.

3.6. Benzoxazines

Benzoxazines, such as 1,4-benzoxazin-3-ones and 2,4-Dihydroxy-1,4-benzoxazin-3-one (Figure 3F), present in maize [77], wheat and rye [54] are a group of molecules showing antimicrobial and antitumor activity [78,79]. In a recent work, Foto et al. demonstrated that benzoxazines and their derivatives act as hTopIB inhibitors, interfering with the binding of the enzyme to the DNA [55]. According to relaxation assay and EMSA experiments, the DNA-binding capacity of hTopoIB is reduced by benzoxazines in a dose-dependent concentration, suggesting a possible use of these molecules as a lead compound to develop new drugs for cancer treatment.

3.7. Evodiamine

The *Evodia rutaecarpa* fruit, officially listed in the Chinese Pharmacopoeia, has been used as an analgesic, anti-inflammatory and in the treatment of hypertension, suggesting a beneficial use for a variety of therapeutic applications [80,81]. Researchers have isolated from this fruit an alkaloid named evodiamine (EVO) reported in Figure 3G, that has shown in vitro anticancer properties [82]. EVO inhibits hTopIB, as shown by a relaxation assay on the supercoiled DNA [83]. The proposed mechanism of action is an inhibition of the enzyme in a CPT-like manner. Chan et al. [56] reported that this compound is able to trap hTopIB on DNA to form a ternary covalent complex. 3H-thymidine-labeled cells were treated with EVO, and the cell extracts were subjected to KCl/SDS that induces protein but not DNA precipitation, except when it is linked to a protein. The amount of precipitated DNA, evaluated by autoradiography, is proportional to the amount of the hTopIB-EVO-DNA complex, indicating a DNA EVO-trapping activity. The authors suggest that the compound acts by stabilizing the covalent complex between hTopIB and DNA, forming a barrier to the DNA replication fork and converting the ternary covalent complex into a cell poison.

3.8. Cytosporolide C

Cytosporolide C (Cyto-C) (Figure 3H) is a NP isolated from the fungus *Cytospora* spp. [84]. This compound has antimicrobial activity [85], but a novel bioactivity as anti-proliferative compound has been recently demonstrated, suggesting a potential use as an anticancer drug [86]. The results demonstrate that Cyto-C inhibits the hTopIB relaxation of a supercoiled DNA substrate and has an antiproliferative activity against A549 (non-small-cell lung cancer cells), HCT-116 (human colon cancer cells) and MCF-7 (breast cancer cells) cell lines. Cyto-C is an interesting hTopIB-specific inhibitor and a promising lead compound for the development of new drugs for cancer treatment.

4. HTopIB Inhibition by Natural Compounds Coordinated with Metals

Some NPs display positive properties only upon metal coordination [87], and among them, there are some hTopIB inhibitors, reported in Figure 4 and Table 2.
Figure 4. Structures of natural compounds coordinated with metals with in vitro activity against hTopIB. (A) Polyhydroxybenzaldehyde thiosemicarbazones zinc complex, (B) chalcone thiosemicarbazones copper(II) complex and (C) silibinin oxidovanadium(IV) complex. All structures are represented by Marvinsketch.

Table 2. Natural compounds coordinated with metals showing an anti-hTopIB activity.

| Compound Name                                      | Source                      | Type of Inhibitor                  | Reference |
|----------------------------------------------------|-----------------------------|-----------------------------------|-----------|
| Zinc complexes of polyhydroxybenzaldehyde thiosemicarbazones | Plants natural product          | Catalytic Inhibitor                   | [88]      |
| Chalcones-Thiosemicarbazone copper(II) complex     | Piper methysticum, Boesen-bergia rotunda, Lophira alata | Catalytic Inhibitor                    | [89]      |
| Silibinin oxidovanadium (IV)                       | Silybum marianum            | Catalytic Inhibitor                  | [90]      |

Thiosemicarbazones are fundamental compounds for regulating plants growth [57]. Zinc complexes of polyhydroxybenzaldehyde thiosemicarbazones (Figure 4A) interact with hTopIB [91]. Incubation of the metal complex with hTopIB and DNA gives rise to two different modes of actions: one concerns the binding of the metal complex to DNA, while the other one involves the binding to the enzyme. When the drug is incubated with hTopIB before adding the supercoiled DNA substrate, there is an inhibition of the enzyme activity stronger than preincubating the compound with DNA. These experiments are an indication that the binding of the metal complex to hTopIB is the main inhibition mechanism.
Chalcones are intermediate products in flavonoids synthesis [92], found mainly in *Piper methysticum* [58], *Boesen-bergia rotunda* [93] and *Lophira alata* [94]. Chalcones-derived thiosemicarbazones (Figure 4B) are efficient in inhibiting hTopoIB only when coordinated to a copper atom [88]. This complex, when preincubated with the enzyme, prevent the binding to DNA, as demonstrated by the EMSA and relaxation assay, indicating that the copper complex acts as an inhibitor and not as a poison.

A similar behavior is observed with the flavonoid silibinin, extracted from *Silybum marianum* [95,96] and chrysin from *Passiflora caerulea* [97]. The two flavonoids did not show any effect on hTopIB, but silibinin inhibits the enzyme when forming oxidovanadium(IV) complexes [98]. The results demonstrate that the silibinin oxidovanadium(IV) complex (Figure 4C) acts by preventing the formation of the enzyme–DNA complex. The compound has a positive effect on human colon cancer cell line HT-29, as tested by a cell viability assay, suggesting its possible use in antitumor treatments.

### 5. Natural Compounds from Marine Organism

Due to their different growing environments, marine and, in particular, Antarctic organisms have developed NPs with novel characteristics that deserve to be investigated. Hereafter, the effects of these NPs targeting hTopIB are presented in Table 3.

#### Table 3. Natural products of marine origins inhibiting hTopIB.

| Compound Name          | Source                                | Type of Inhibitor  | Reference |
|------------------------|---------------------------------------|--------------------|-----------|
| Bacillosporin C        | *Penicillium purpurogenum* species    | Not studied        | [67]      |
| α-Methoxylated Δ5,9 fatty acids | *Asteropus niger*                      | Catalytic Inhibitor | [99]      |
| Lamellarin D           | *Leathesia nana*                      | Poison             | [100]     |
| BDDE                   | *Rhodomela confervoides*              | Catalytic Inhibitor | [101]     |
| Deoxyvariolin B        | *Kirckpatrickia variolosa*            | Not studied        | [102]     |
| Discorhabdins          | *Latrunculia bifornis*                | Not studied        | [103]     |

#### 5.1. Bacillosporin C

Bacillosporin C (Bac-C) (Figure 5A) is an oxaphenalenone, an important class of phenolic natural products, isolated from fungi, such as *Penicillium purpurogenum* [89]. *P. purpurogenum* has the ability to synthesize a variety of substances with antibacterial activity and inhibitory effects on several human cancer cell lines. The strains with large biotechnological potential are mutants resistant to antibiotics [104]. An example is the marine G59 strain producing Bac-C. Bac-C can target hTopIB, as shown by an experimental bioassay and docking simulation [51]. The screening of 128 compounds, by docking them on the hTopI–DNA complex, permitted the selection of compounds found to be hTopIB inhibitors through a relaxation assay on a supercoiled DNA substrate. The researchers did not investigate the mechanism of action; thus, the compounds cannot be classified as catalytic inhibitors or poisons.

#### 5.2. Alpha-Methoxylated δ5,9 Fatty Acids

Sponges are the source of new phospholipid fatty acids, having long chains (C23–C30) with no counterpart in the terrestrial world [105]. α-Methoxylated δ5,9 fatty acids (Figure 5B) were extracted and isolated from the Caribbean sponge *Asteropus niger* [106]. The compound is able to inhibit hTopIB with a mechanism of action different from that displayed by CPT. Indeed, the compound does not bind to the DNA-hTopIB complex but directly interacts with the enzyme, preventing the catalytic tyrosine to do the nucleophilic attack on the DNA phosphate bond [106].
and tested as an hTopIB inhibitor. This compound partially affects hTopIB activity, inhibiting the relaxation of supercoiled DNA [115]. This result suggests the possibility of developing new variolin B derivatives with improved antitumor efficacy.

5.6. Discorhabdins

Discorhabdins (Figure 5F) are a subclass of pyrroloiminoquinone alkaloids [116] associated with the chemical defense of the Antarctic sponge *Latrunculia biformis*, turning its color from green to brown to deter predators such as sea stars [101]. These NPs have shown a strong anticancer activity in different cancer types, such as human colon cancer, adenocarcinoma and leukemia, but its mechanism of action is still unknown [117]. Li et al. suggested hTopIB as the possible target applying a structure-based docking approach [118]. This result comes from a computational study but appears promising and suggests that discorhabdins can be experimentally tested against hTopIB.

Figure 5. Structures of marine natural products with in vitro and/or in silico activity against hTopIB. (A) Bacillosporin C, (B) alpha-methoxylated δ5,9 fatty acids, (C) Lamellarin D, (D) Bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (BDDE), (E) Variolin B and (F) Discorhabdin C. The structures are represented by Marvinsketch, as reported on PubChem.
5.3. Lamellarin D

Lamellarin D (LAM-D) (Figure 5C) is a hexacyclic marine alkaloid isolated for the first time from a mollusk of the genus Lamellaria [90], with a cytotoxic effect on the tumor cell lines [107,108]. On the basis of its chemical structure and on a molecular modeling analysis, it has been suggested that LAM-D can bind to DNA and interact with hTopIB, affecting its catalytic mechanism [109]. LAM-D inhibits the relaxation of supercoiled DNA in a dose-dependent manner. The researchers, investigating the cleavage/religation reaction, demonstrated that LAM-D stabilizes the DNA–enzyme complex, turning the enzyme into a poison, acting like CPT. LAM-D has appeared as a new potent hTopIB poison [99] that should be further investigated to develop a new non-CPT derivatives drug.

5.4. Bis(2,3-dibromo-4,5-dihydroxybenzyl) Ether

Marine bromophenols, found in sponges and algae have been always attracted food and pharmaceutical company due to their multiple bioactivities, such as antioxidant [110], antimicrobials [111] and antidiabetic activity [112]. Among marine bromophenols, bis (2,3-dibromo-4,5-dihydroxybenzyl) ether (BDDE) (Figure 5D), isolated from marine algae Leathesia nana and Rhodomela confervoides, has been shown to inhibit the proliferation of several tumor cells lines and induce apoptosis in human myelogenous leukemia cell line K562 [113]. A relaxation assay of a supercoiled DNA in the presence of different amounts of BDDE indicates that the compound inhibits hTopIB in a dose-dependent manner. BDDE is not able to trap the enzyme–DNA cleavable complex, and no nicked DNA is observed [113]. These data suggest that BDDE behave as a catalytic inhibitor rather than a poison.

5.5. Variolin B

Variolin B (Figure 5E) is a NP from the Antarctic sponge Kirckpatrickia variolosa and is reported to have antitumor and antiviral properties [100]. Due to its planar structure and the presence of a central aromatic ring, a cytotoxic effect through DNA intercalation has been proposed [114]. The more soluble derivative deoxyvariolin B has been developed and tested as an hTopIB inhibitor. This compound partially affects hTopIB activity, inhibiting the relaxation of supercoiled DNA [115]. This result suggests the possibility of developing new variolin B derivatives with improved antitumor efficacy.

5.6. Discorhabdins

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6. HTTopIB Poisons Derived from Natural Compounds in Preclinical and Clinical Trial

The best characterized NP against hTopoIB is CPT [44,49,102]. Two water-soluble derivatives, both approved by the FDA in 1996, are currently used in clinics, irinotecan for colon carcinomas [119,120] and topotecan for ovarian cancers [121]. Topotecan has been subsequently also approved for small cell lung cancer and, in combination with cisplatin, for stage IV-B cervical carcinoma [103,122]. Another interesting CPT derivative is belotecan [123], approved in South Korea in 2003 for the treatment of non-small cell lung cancer [124,125] and ovarian cancer [126]. New drugs, targeting hTopIB, are also under development, because some tumors show resistance to the currently in use CPT derivatives [127,128].

Drugs having a completed or ongoing clinical trial are listed in Table 4 [129].
Table 4. Drugs targeting hTopIB under clinical trial [129].

| Study Purpose | Time Frame | Sample Size | NCT |
|---------------|------------|-------------|-----|
| Describe the dose limiting toxicities and adverse event profile of Camptothecin-20-O-Propionate hydrate (CZ48) administered orally every day for 4 weeks | July 2008–February 2020 | 65 participants | NCT02575638 |
| Establish the safety, tolerability and the maximum tolerated dose (MTD) of LMP744 administered intravenously (IV) in patients with refractory solid tumors and lymphomas | February 2017–ongoing (estimated completion October 2022) | 53 participants | NCT03030417 |
| Evaluate the efficacy and safety of Lipotecan® monotherapy in subjects with poorly differentiated neuroendocrine carcinomas. Only those subjects who have failed to first line chemotherapy | July 2015–ongoing (last update 3 April 2019) | 23 participants | NCT02457273 |
| Estimate the objective response rate (Complete Response or Partial Response, as measured by RECIST version 1.1) for patients with metastatic or recurrent NSCLC being treated with etirinotecan pegol after failure of second-line therapy. | January 2013–ongoing (last update April 2020) | 40 participants | NCT01773109 |
| Evaluate the efficacy and safety of LY01610 in subjects with extensive small cell lung cancer that progressed after first-line anti-tumor therapy | November 2019–ongoing (estimated completion September 2022) | 90 participants | NCT04381910 |

Camptothecin-20(S)-O-propionate hydrate (CZ48) [130], obtained reacting CPT with propionic anhydride, is in Phase 1 clinical trials to evaluate its dose-limiting toxicities profile. The drug has been administered for the treatment of malignant lymphoma of extranodal and/or solid organ sites and solid tumors.

LMP744, an indenoisoquinoline with improved characteristics over CPT derivatives [131], is in phase 1 for the treatment of solid tumors and lymphomas. Indenoisoquinolines have a chemical stability larger than CPT derivatives, produce stable DNA-hTopIB cleavage complexes and exhibit a sequence preference for the DNA cleavage sites. The drug has activity against CPT-resistant cell lines and produces irreversible DNA–protein crosslinks. LMP744 exhibits antitumor activity with lower toxicity than other agents in preclinical studies. The treatment of patients with LMP744 is expected to reduce the tumor burden at doses that are well-tolerated. Among indenoisoquinoline derivatives, CYB-L10 is not yet in clinical trial, but preclinical studies indicated an interesting cytotoxicity profile and an hTopIB inhibition higher than CPT [132]. CYB-L10 is active in vitro against 60 clinical cancer cell lines and displays an antitumor efficiency in an HCT-116 xenograft nude mice model with no obvious loss of weight of the body of the mice at a 20-mg/kg dose [132].
Lipotecan®, which is the trade name of TLC388, is on a phase II trial to evaluate the drug efficacy and safety in subjects with poorly differentiated neuroendocrine carcinomas [133]. The Abramson Cancer Center of the University of Pennsylvanian is testing erinotecan pegol (NKTR-102), a hTopIB inhibitor polymer conjugate, made up of irinotecan conjugated with polyethylene glycol (PEG), administered to subjects with metastatic and recurrent NSCLC, after the failure of second-line therapy. Luye Pharma Group Ltd. is testing in a phase II trial, irinotecan hydrochloride liposome (LY01610), in patients with extensive-stage small cell lung cancer (SCLC) that progressed after first-line antitumor therapy. Irinotecan liposome is formulated with irinotecan into a liposomal dispersion. The liposome is a unilamellar lipid bilayer vesicle that encapsulates an aqueous space containing irinotecan in a gelled or precipitated state as sucrose octasulfate salt. Administration via a liposomal formulation results in prolonged intratumor exposure at levels above the threshold for antitumor activity.

7. Conclusions

Analysis of the literature indicates that several natural compounds are targeting hTopIB, a ubiquitous enzyme involved in several fundamental cellular processes [23, 134]. The development of these molecules into selective and efficient antitumor drugs still requires several passages. However, besides the CPT derivatives already in clinical use, there are interesting indenoisoquinoline compounds and additional CPT derivatives that are in clinical trials [129,135]. We believe that attention must be paid to additional natural compounds, such as NPs coming from the marine and Antarctic worlds (Table 3 and Figure 5). This type of environment has selected organisms adapted to extreme life conditions, producing NPs with no counterparts in the terrestrial world [14]. These compounds, chemically different from CPT, may have the potential to overcome the diffuse drug resistance caused by intense and long-lasting treatments of CPT derivatives and offer a more personalized patient treatment [127].

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