Published in final edited form as:
Expert Opin Ther Pat. 2011 September ; 21(9): 1285–1292. doi:10.1517/13543776.2011.604314.

Tyrosyl-DNA Phosdiesterase 1 (Tdp1) Inhibitors

Shar-yin N. Huang[Post-Doctoral Fellow], **Yves Pommier*[Lab Chief]**, and **Christophe Marchand[Staff Scientist]**
Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892

Abstract

Inhibitors of topoisomerase I (Top1) that result in stalled Top1 cleavage complexes (Top1cc) are commonly employed against cancer. Combination chemotherapy with DNA repair inhibitors can potentially improve response to these widely used chemotherapeutics. One line of inquiry focuses on inhibitors of tyrosyl-DNA phosphodiesterase 1 (Tdp1), a repair enzyme for Top1cc. Tdp1 catalyzes the hydrolysis of DNA adducts covalently linked to the 3′-phosphate of DNA, including Top1-derived peptides and also 3′-phosphoglycolates. Tdp1 inhibitors should synergize not only with Top1-targeting drugs (camptothecins, indenoisoquinolines), but also with bleomycin, topoisomerase II (Top2) inhibitors (etoposide, doxorubicin) and DNA alkylating agents. Here, we summarize the structural-activity relationship obtained from the reported Tdp1 inhibitors. Better understanding of Top1cc repair *in vivo* coupled with detailed structural studies on Tdp1-inhibitor interaction will be crucial in guiding the rational design of Tdp1 inhibitors.

1. Introduction

Inhibitors of DNA topoisomerase I (Top1) are established effective anti-cancer chemotherapeutic agents (for reviews see[1, 2]). Camptothecin (CPT) and its derivatives reversibly bind and trap the Top1-DNA cleavage complex (Top1cc), which is normally a transient intermediate of the Top1 enzymatic reaction[3]. When replication or transcription machineries subsequently encounter the trapped Top1cc, DNA double-strand breaks (DSBs) are generated and cell death occurs as a consequence of DNA damage (Top1-DNA adducts and DSBs). In addition to the current Top1 inhibitors commercially available for cancer chemotherapy, several promising new derivatives with improved pharmacokinetic properties are currently in clinical trials[1, 2, 4].

The repair mechanisms of Top1cc prove to be complex because several pathways are involved (for review see[5]). One of the pathways involves tyrosyl-DNA phosphodiesterase 1 (Tdp1), which catalyzes hydrolysis of the Top1 tyrosine residue covalently linked to the 3′-phosphate of DNA. The unique enzymatic activity of Tdp1 removes the covalent-linked Top1 from the DNA 3′-end after Top1 has been denatured or proteolysed[6, 7]. The discovery of a Tdp1 mutation responsible for the rare neurodegenerative disease, spinocerebellar ataxia with axonal neuropathy (SCAN1), further emphasizes the physiological importance of Tdp1 in central nervous system tissues[8]. While loss of Tdp1 does not appear to cause apparent cell dysfunctions, Tdp1 knockout mice and human cells deficient in Tdp1 or harboring the SCAN1 mutation all show hypersensitivity to CPT[9-12]. Conversely, human cells overexpressing Tdp1 show reduction in CPT- and etoposide-induced DNA

*Correspondence: Laboratory of Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, Phone: +1-301-496-5944, Pommier@nih.gov.

Declaration of interest The authors declare no conflict of interest and have received no payment in preparation of this manuscript.

damage[13, 14]. Therefore, chemotherapy combining Top1- or Top2-targeting drugs with Tdp1 inhibitor could potentially be more effective.

In addition to Tdp1, alternative pathways such as DNA repair (XPF/ERCC1, Mre11, CtIP), homologous recombination (BRCA1, BRCA2, CtIP, Mre11, Rad52) and cell cycle checkpoint signaling (Rad9, BRCA1, BRCA2, p53) are involved in the repair of CPT-induced DNA damage[5]. When these alternative pathways are inactivated, cells most likely rely more on the Tdp1-dependent pathway for repairing CPT-induced DNA damage[15-25]. Since many cancer cells are deficient in one or more DNA damage repair pathways, inhibiting Tdp1-dependent pathway have the potential to selectively sensitize cancer cells over normal cells to Top1 poisons[2, 26].

Mechanistic studies of Tdp1 activity have been enormously important in guiding the search for effective Tdp1 inhibitors. As a member of phospholipase D (PLD) superfamily, Tdp1 has two conserved HKD motifs located on different domains but clustering together in tertiary arrangement to form an active site[27] (Figure. 1A and C). These two HKD motifs are responsible for two sequential hydrophilic attacks on the phosphodiester bond at the active site (Figure. 1D). Tdp1 can act on DNA substrates linked to a wide-range of 3′-blocking lesions via a phosphodiester linkage[28, 29]. The relative K_m values of Tdp1 for different substrates should be helpful in rational design of Tdp1 inhibitors.

Co-crystal structures of Tdp1 with a transition-state substrate mimic further illustrate the geometry of the Tdp1 active sites and the basis of Tdp1 catalytic activity (Figure. 1B and C)[30]. Assembled from vanadate, single-strand DNA, and a Top1-derived peptide, the three-part substrate mimic binds in a deep substrate-binding cleft[30]. The center of the cleft bottom is the active site formed by the two HKD motifs, covalently linked to the vanadate. On one side of the active site is a long and narrow cleft occupied by the single-strand DNA and on the opposite side a larger binding pocket occupied by the Top-derived peptide (Figure. 1B)[30]. The surface charge/contour of the substrate-binding site, as well as the conformation of the complex in transition-state revealed by the crystal structures should all be informative in the search for Tdp1 inhibitors.

Here we provide a brief summary of the available findings toward identifying specific Tdp1 inhibitors and address the issues that remain to be examined in this line of research.

2. Aminoglycoside Antibiotics and Ribosome Inhibitors

Because Tdp1 belongs to the PLD superfamily, known inhibitors for the other members of the family, such as neomycin (Figure 2), were tested for Tdp1 inhibitory activity. *In vitro* biochemical assays using recombinant Tdp1 enzyme demonstrate that neomycin inhibits Tdp1 but requires high concentrations (in the low millimolar range)[31, 32]. The inhibitory potency of neomycin is slightly weaker than that of vanadate, a known general inhibitor of phosphatases. Two other derivatives of neomycin tested, paromomycin and lividomycin (Figure 2), also inhibit Tdp1 activity at slightly higher concentrations[31, 32]. Given that these aminoglycoside compounds are established inhibitors of bacterial ribosomes, other bacterial ribosomal inhibitors were also screened using the same *in vitro* biochemical assay. All of the aminoglycoside and non-aminoglycoside antibiotics tested show weak inhibition against recombinant Tdp1 in the millimolar range[31, 32]. While the inhibitors identified in the initial screen were only weak Tdp1 inhibitors with uncharacterized mechanism, a reliable *in vitro* assay system was established for validation of other Tdp1 inhibitors.

3. Furamidine

A high-throughput electrochemiluminescent assay was subsequently developed to screen the 2000-compound “diversity set” chemical library of the NCI-Developmental Therapeutics Program. Furamidine, a compound in phase III clinical trials against trypanosomiasis, a common parasitic disease prevalent in Africa, was identified as a positive hit in the screen (Figure 2). *In vitro* biochemical assays also confirmed that furamidine inhibits Tdp1 at low micromolar concentrations[33, 34]. Furamidine is known to bind to duplex DNA in the minor groove selectively at AT-rich sites, as well as intercalating between GC base pairs. Since no duplex DNA was present in the screen, the mode of action of furamidine is likely a novel one. Further investigation by surface plasmon resonance (SPR) analysis revealed that furamidine binds not only to DNA duplex, but also to single-stranded DNA, albeit to a lesser degree. Similar analysis also showed that furamidine interacts with Tdp1. These results raised the possibility of an inhibitory mode of action by combined interaction to the DNA substrate and to Tdp1, which is reminiscent of the interfacial inhibition of Top1cc by Top1 inhibitors [3, 35].

Two other diamidine compounds, berenil and pentamidine, are much less effective than furamidine at inhibiting Tdp1 (Figure 2)[33, 34]. Both compounds share the overall curved structure of furamidine, but substitute the furan ring linker in furamidine with other linking groups (Figure 2). This suggests that the furan linker is important in inhibiting Tdp1 activity, potentially by directly interacting with the DNA or Tdp1 or both, or by stabilizing the overall curvature of the compound.

4. Tetracyclines

A number of tetracycline compounds including rolitetracycline (Figure 2) were also identified as positive hits in two separate high-throughput screens for Tdp1 inhibitors. The preliminary results on tetracyclines showed micromolar inhibitory effect against Tdp1[36] but lacked apparent structure-activity relationship. Nevertheless, these compounds potentially warrant further investigation.

5. Phosphotyrosine Mimetics

The strongest Tdp1 inhibitors identified to date are Tdp1 phosphotyrosine substrate mimetics. Identified in two separate high through-put screens as positive hits, methyl-3,4-dephostatin[37, 38] and the steroid compound NSC88915[36, 39] both share similar structural characteristics as the phosphotyrosine substrate (Figure 2 & 3). Methyl-3,4-dephostatin is an aromatic amine derivative with a hydroxyl-substituted benzyl ring, bearing close resemblance to the phosphate group linked to the tyrosyl-moiety in the Tdp1 substrate (Figure 2). NSC88915 and its derivatives contain an aromatic sulfonyl ester group linked to a steroid, simulating the phosphate group covalently linked to the tyrosyl moiety on one side and the DNA oligonucleotide on the other (Figure 3).

Close examination of the structure-activity relationship derived from methyl-3,4-dephostatin and NSC88915 and their respective derivatives is informative. For example, the only difference between dephostatin and methyl-3,4-dephostatin is the position of one hydroxyl substitution group on the aromatic ring (Figure 2). However, while methyl-3,4-dephostatin inhibits Tdp1 at sub-micromolar concentrations, dephostatin displays almost no activity in the same concentration range[37, 38]. Similarly, NSC88915 and its five derivatives only differ by the nature of the substitution at the para position of the aromatic ring (Figure 3). While the difference in activity for these six derivatives are within 10-fold of each other, another derivative lacking the aromatic ring all together lost its potency[36, 39]. Molecular dynamics of these seven steroid derivatives docking in the Tdp1 active site were simulated. Indeed the

calculated free energy of binding for each compound closely correlates to its respective inhibitory activity against Tdp1[36, 39]. All together, these results suggest that the aromatic ring linked to a non-hydrolysable phosphate mimic could potentially be active at inhibiting Tdp1. Furthermore, both the nature and position of the substitution groups could influence the potency of such inhibitors.

6. Expert Opinion

The effort to identify inhibitors of Tdp1 activity has led to development of a few high-throughput screen formats and several promising leads. The current efforts are largely on refining the structure-activity relationship of the existing inhibitors as well as searching for additional lead compounds. The biochemical activity of Tdp1 and various mutants is also an active area of research, which continues to guide the search for inhibitors of Tdp1. Further studies on the mode of actions of these lead inhibitors are warranted.

To better correlate inhibitor efficacies obtained from *in vitro* and *in vivo* screens, the expression and activity of Tdp1 in tumor tissues need to be further investigated. For example, non-small cell lung cancer tissues have been reported to show high level of Tdp1 expression and activity[40]. The relative contribution of the different DNA repair pathways in human cells also needs to be better understood. As discussed before, many cancer cells are deficient in one or more DNA damage response pathways. Thus, it will be important to develop clinical assays that can characterize tumors and identify deficiencies in specific DNA repair pathways. Tumors deficient in cell cycle signaling pathways (Chk2, ATM, p53), DNA repair (XPF, ERCC1, Mre11, Nbs1, CtIP) and/or homologous recombination (CtIP, BRCA1, BRCA2) should be prime candidates for combination chemotherapies with Top1 and Tdp1 inhibitors. Most notable examples might be colon cancers with Mre11 mutations[41, 42] and lung cancers with ERCC1 mutations[25, 43]. It remains to be determined whether BRCA1- or BRCA2-deficient tumors will be selectively sensitized by Tdp1 inhibitors because, in budding yeast, Rad52 is epistatic with Tdp1[16, 20].

Tdp1 is involved in the repair of other types of lesions besides Top1cc, such as DNA strand breaks with phosphoglycolate adducts at 3′-ends[28, 44, 45]. The repair of those lesions by Tdp1 has been invoked to account for the sensitivity of Tdp1-deficient cells to bleomycin[12]. Moreover, the possible involvement of Tdp1 in the repair of Top2-induced DNA damage[46] suggests that Tdp1 inhibitors may have value in wide range of combination therapies with DNA-targeting agents including, not only Top1 inhibitors but also bleomycin, Top2 inhibitors and potentially DNA alkylating agents.

The size of Top1cc-derived substrates *in vivo* remains elusive. While proteasome processing of Top1cc and poly(ADP-ribosylation) are required for the Tdp1-dependent repair pathway[25], the extent of proteasome processing *in vivo* is not known[7]. The high-throughput screens to date employed a substrate that contains a single-tyrosine amino acid covalently linked to the 3′-phosphate of DNA. It should be noted that Tdp1 processes this particular substrate more efficiently than any substrates containing longer peptides, which are expected to be closer to the physiological Top1cc-derived substrates. The use of such an optimal substrate may underestimate the efficacy of inhibitors against more physiological substrates. Therefore, it is crucial to cross-validate any hits using both *in vitro* and *in vivo* systems. *In vivo* screening taking advantage of the differential sensitivity to Tdp1 inhibitors in Tdp1 knockout *vs*. wild-type cell lines are ongoing. Such efforts have the added benefits of potentially discovering prodrugs that would not be active in *in vitro* screens alone.

It is not surprising that the most potent Tdp1 inhibitors to date are non-hydrolysable Tdp1 substrate mimetics. Since the catalytic activity of Tdp1 is targeted towards a specific group of DNA lesions with well-defined structures, substrate mimetics potentially can inhibit Tdp1

with high degree of specificity. Another line of inquiry is to screen for small molecules that would act as interfacial inhibitors by stabilizing and trapping the Tdp1 intermediate covalently linked to DNA[3, 47]. Co-crystal structures of Tdp1 with lead compounds from either class or other detailed structural studies will provide valuable insights into their protein-drug interactions. Efforts on that front should pay handsome dividend to successful rational design of Tdp1 inhibitors.

References

1. Pommier Y, Leo E, Zhang H, et al. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol. May 28; 2010 17(5):421–33. [PubMed: 20534341]
2. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer. Oct; 2006 6(10):789–802. [PubMed: 16990856]
3. Marchand C, Antony S, Kohn KW, et al. A novel norindenoisoquinoline structure reveals a common interfacial inhibitor paradigm for ternary trapping of the topoisomerase I-DNA covalent complex. Mol Cancer Ther. Feb; 2006 5(2):287–95. [PubMed: 16505102]
4. Teicher BA. Next generation topoisomerase I inhibitors: Rationale and biomarker strategies. Biochem Pharmacol. Mar 15; 2008 75(6):1262–71. [PubMed: 18061144]

**5. Pommier Y, Barcelo JM, Rao VA, et al. Repair of topoisomerase I-mediated DNA damage. Prog Nucleic Acid Res Mol Biol. 2006; 81:179–229. [PubMed: 16891172] Comprehensive review on the repair of Top-1 mediated DNA damage

6. Debethune L, Kohlhagen G, Grandas A, et al. Processing of nucleopeptides mimicking the topoisomerase I-DNA covalent complex by tyrosyl-DNA phosphodiesterase. Nucleic Acids Res. Mar 1; 2002 30(5):1198–204. [PubMed: 11861912]
7. Interthal H, Champoux JJ. Effects of DNA and protein size on substrate cleavage by human tyrosyl-DNA phosphodiesterase (TDP1). Biochem J. Apr 4.2011

*8. Takashima H, Boerkoel CF, John J, et al. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. Nat Genet. Oct; 2002 32(2):267–72. [PubMed: 12244316] Study showing that a mutation in Tdp1 is responsible for the SCAN1 neurodegenerative disease

9. El-Khamisy SF, Katyal S, Patel P, et al. Synergistic decrease of DNA single-strand break repair rates in mouse neural cells lacking both Tdp1 and aprataxin. DNA Repair (Amst). Jun 4; 2009 8(6): 760–6. [PubMed: 19303373]
10. Das BB, Antony S, Gupta S, et al. Optimal function of the DNA repair enzyme TDP1 requires its phosphorylation by ATM and/or DNA-PK. The EMBO Journal. 2009; 28(23):3667–80. [PubMed: 19851285]
11. Katyal S, el-Khamisy SF, Russell HR, et al. TDP1 facilitates chromosomal single-strand break repair in neurons and is neuroprotective in vivo. EMBO J. Nov 14; 2007 26(22):4720–31. [PubMed: 17914460]
12. Hirano R, Interthal H, Huang C, et al. Spinocerebellar ataxia with axonal neuropathy: consequence of a Tdp1 recessive neomorphic mutation? EMBO J. November 11; 2007 26(22):4732–43. [PubMed: 17948061]

*13. Barthelmes HU, Habermeyer M, Christensen MO, et al. TDP1 overexpression in human cells counteracts DNA damage mediated by topoisomerases I and II. J Biol Chem. Dec 31; 2004 279(53):55618–25. [PubMed: 15494395] Study implicating Tdp1 in the repair of Top1 and Top2-mediated DNA lesions.

14. Nivens MC, Felder T, Galloway AH, et al. Engineered resistance to camptothecin and antifolates by retroviral coexpression of tyrosyl DNA phosphodiesterase-I and thymidylate synthase. Cancer Chemother Pharmacol. Feb; 2004 53(2):107–15. [PubMed: 14605862]

*15. Pouliot JJ, Yao KC, Robertson CA, et al. Yeast gene for a Tyr-DNA phosphodiesterase that repairs topo I covalent complexes. Science. 1999; 286:552–5. [PubMed: 10521354] *Discovery of the TDP1 gene*.

16. Pouliot JJ, Robertson CA, Nash HA. Pathways for repair of topoisomerase I covalent complexes in Saccharomyces cerevisiae. Genes Cells. 2001; 6(8):677–87. [PubMed: 11532027]

*17. Liu C, Pouliot JJ, Nash HA. Repair of topoisomerase I covalent complexes in the absence of the tyrosyl-DNA phosphodiesterase Tdp1. Proc Natl Acad Sci U S A. Nov 12; 2002 99(23):14970–5. [PubMed: 12397185] Genetic studies on alternative DNA repair pathways.

18. Eng WK, Faucette L, Johnson RK, et al. Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. Mol Pharmacol. 1988; 34:755–60. [PubMed: 2849043]

19. Vance JR, Wilson TE. Repair of DNA strand breaks by the overlapping functions of lesion-specific and non-lesion-specific DNA 3′ phosphatases. Mol Cell Biol. Nov; 2001 21(21):7191–8. [PubMed: 11585902]

20. Vance JR, Wilson TE. Yeast Tdp1 and Rad1-Rad10 function as redundant pathways for repairing Top1 replicative damage. Proc Natl Acad Sci U S A. Oct 15; 2002 99(21):13669–74. [PubMed: 12368472]

21. Deng C, Brown JA, You D, et al. Multiple Endonucleases Function to Repair Covalent Topoisomerase I Complexes in Saccharomyces cerevisiae. Genetics. Apr 16.2005 170:591–600. [PubMed: 15834151]

22. Sartori AA, Lukas C, Coates J, et al. Human CtIP promotes DNA end resection. Nature. Nov 22; 2007 450(7169):509–14. [PubMed: 17965729]

23. Nakamura K, Kogame T, Oshiumi H, et al. Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. PLoS Genet. Jan.2010 6(1):e1000828. [PubMed: 20107609]

24. Hartsuiker E, Neale MJ, Carr AM. Distinct Requirements for the Rad32(Mre11) Nuclease and Ctp1(CtIP) in the Removal of Covalently Bound Topoisomerase I and II from DNA. Mol Cell. Jan 16; 2009 33(1):117–23. [PubMed: 19150433]

25. Zhang YW, Regairaz M, Seiler JA, et al. Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells. Nucleic Acids Res. Jan 11.2011

**26. Dexheimer TS, Antony S, Marchand C, et al. Tyrosyl-DNA phosphodiesterase as a target for anticancer therapy. Anticancer Agents Med Chem. May; 2008 8(4):381–9. [PubMed: 18473723] First review on Tdp1 as a target for anticancer therapy.

27. Davies DR, Interthal H, Champoux JJ, et al. Insights into substrate binding and catalytic mechanism of human tyrosyl-DNA phosphodiesterase (Tdp1) from vanadate and tungstate-inhibited structures. J Mol Biol. December 12; 2002 324(5):917–32. [PubMed: 12470949]

28. Interthal H, Chen HJ, Champoux JJ. Human Tdp1 cleaves a broad spectrum of substrates, including phosphoamide linkages. J Biol Chem. October 10; 2005 280(43):36518–28. [PubMed: 16141202]

29. Dexheimer TS, Stephen AG, Fivash MJ, et al. The DNA binding and 3′-end preferential activity of human tyrosyl-DNA phosphodiesterase. Nucleic Acids Res. April; 2010 38(7):2444–52. [PubMed: 20097655]

*30. Davies DR, Interthal H, Champoux JJ, et al. Crystal structure of a transition state mimic for Tdp1 assembled from vanadate, DNA, and a topoisomerase I-derived peptide. Chem Biol. February; 2003 10(2):139–47. [PubMed: 12618186] *Co-crystal structure of Tdp1-substrate complex*

*31. Liao Z, Thibaut L, Jobson A, et al. Inhibition of human tyrosyl-DNA phosphodiesterase by aminoglycoside antibiotics and ribosome inhibitors. Mol Pharmacol. Jul; 2006 70(1):366–72. [PubMed: 16618796] *First report on Tdp1 inhibitors*.

32. Pommier, Y.; inventor The Government of the United States of America, assignee. Aminoglycosides and ribosome inhibitors as inhibitors of tyrosyl-DNA-phosphodiesterase. USA patent. 60/661,306. 2005.

33. Antony S, Marchand C, Stephen AG, et al. Novel high-throughput electrochemiluminescent assay for identification of human tyrosyl-DNA phosphodiesterase (Tdp1) inhibitors and characterization of furamidine (NSC 305831) as an inhibitor of Tdp1. Nucleic Acids Res. 2007; 35(13):4474–84. [PubMed: 17576665]

34. Pommier, Y.; Marchand, C.; Thibaut, L.; inventors; The Government of the United States of America, assignee. Diamidine derivatives as inhibitors of human tyrosyl-DNA-phosphodiesterase (Tdp1). USA patent. 60/786,604. 2006.

35. Pommier Y, Cherfils J. Interfacial protein inhibition: a nature's paradigm for drug discovery. Trends Pharmacol Sci. 2005; 28:136–45.

36. Pommier, Y.; Marchand, C.; Antony, S.; et al. inventors; The Government of the United States of America, assignee. Steroid derivatives as inhibitors of human tyrosyl-DNA phosphodiesterase (Tdp1). USA patent. 60/921,980. 2007.

*37. Marchand C, Lea WA, Jadhav A, et al. Identification of phosphotyrosine mimetic inhibitors of human tyrosyl-DNA phosphodiesterase I by a novel AlphaScreen high-throughput assay. Mol Cancer Ther. Jan; 2009 8(1):240–8. [PubMed: 19139134] *Report on the first robotic high througput screen*.

38. Pommier, Y.; Marchand, C.; Dexheimer, T.; et al. inventors; The Government of the United States of America, assignee. Protein-Tyrosine Phosphatase Inhibitors as Inhibitors of TDP1 and Methods of Treating Disorders. USA patent. 61/042,706. 2008.

39. Dexheimer TS, Gediya LK, Stephen AG, et al. 4-Pregnen-21-ol-3,20-dione-21-(4-bromobenzenesulfonate) (NSC 88915) and related novel steroid derivatives as tyrosyl-DNA phosphodiesterase (Tdp1) inhibitors. J Med Chem. Nov 26; 2009 52(22):7122–31. [PubMed: 19883083]

40. Liu C, Zhou S, Begum S, et al. Increased expression and activity of repair genes TDP1 and XPF in non-small cell lung cancer. Lung Cancer. Mar; 2007 55(3):303–11. [PubMed: 17118488]

41. Giannini G, Rinaldi C, Ristori E, et al. Mutations of an intronic repeat induce impaired MRE11 expression in primary human cancer with microsatellite instability. Oncogene. Apr 8; 2004 23(15): 2640–7. [PubMed: 15048091]

42. Takemura H, Rao VA, Sordet O, et al. Defective Mre11-dependent Activation of Chk2 by Ataxia Telangiectasia Mutated in Colorectal Carcinoma Cells in Response to Replication-dependent DNA Double Strand Breaks. J Biol Chem. Oct 13; 2006 281(41):30814–23. [PubMed: 16905549]

43. Knez L, Sodja E, Kern I, et al. Predictive value of multidrug resistance proteins, topoisomerases II and ERCC1 in small cell lung cancer: A systematic review. Lung Cancer. Jun; 2011 72(3):271–9. [PubMed: 21440950]

44. Zhou T, Akopiants K, Mohapatra S, et al. Tyrosyl-DNA phosphodiesterase and the repair of 3′-phosphoglycolate-terminated DNA double-strand breaks. DNA Repair (Amst). Jun 6; 2009 8(8): 901–11. [PubMed: 19505854]

45. Das BB, Dexheimer TS, Maddali K, et al. Role of tyrosyl-DNA phosphodiesterase (TDP1) in mitochondria. Proc Natl Acad Sci U S A. Nov 16; 2010 107(46):19790–5. [PubMed: 21041670]

*46. Nitiss KC, Malik M, He X, et al. Tyrosyl-DNA phosphodiesterase (Tdp1) participates in the repair of Top2-mediated DNA damage. Proc Natl Acad Sci U S A. Jun 13; 2006 103(24):8953–8. [PubMed: 16751265] Study implicating Tdp1 in the repair of Top2-mediated DNA lesions.

47. Pommier Y, Cherfils J. Interfacial inhibition of macromolecular interactions: nature's paradigm for drug discovery. Trends Pharmacol Sci. Mar; 2005 26(3):138–45. [PubMed: 15749159]

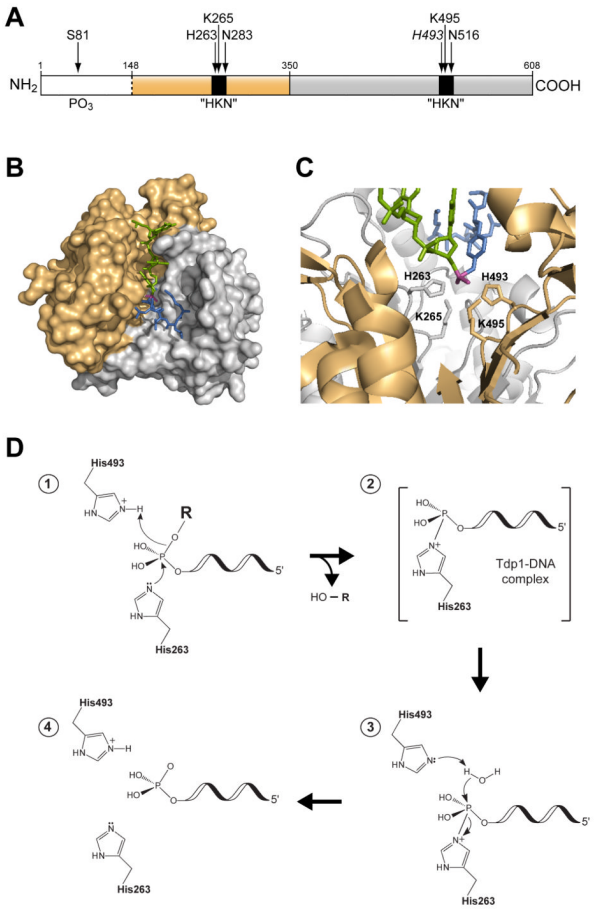

Figure 1.

A) Scheme of the domain structure of human Tdp1. The N-terminal and C-terminal domains correspond to residues 1-350 and 351-608, respectively. Positions of the "HKN" motifs and phosphorylation site (serine 81) are shown in black, with the position of the SCAN1 mutation (H493) shown in italics. B) Crystal structure of the quaternary complex consisting of truncated Tdp1 (Δ1-148), vanadate, a Top1 peptide, and single-strand DNA (PDB: 1NOP). Shown as surface models, the N-terminal and C-terminal domains of Tdp1 are in light brown and gray, respectively [*see* (A)]. Shown in stick structures is the substrate transition-state mimic consisting of single-strand DNA in green, vanadate in magenta, and the peptide in blue. C) The active site residues of Tdp1 are shown in stick structures with the rest of the protein shown in ribbon diagram; the domain colors correspond to those shown in (A) and (B). The substrate transition-state mimic structures are in the same colors as in (B), seen here from the bottom of the binding cleft projecting outwards. For clarity, two loops in the N-terminal domain have been removed from the view. D) Proposed two-step catalytic mechanism of human Tdp1. (1) In the first step, His263 acts as a nucleophile, attacking the phosphorus atom in the phosphodiester bond between the 3′-lesion and the DNA 3′-oxygen. His493 donates a proton to the leaving group (HO-R). (2) A Tdp1-DNA intermediate forms wherein His263 is attached covalently to the 3′-end of the DNA via a phosphoamide bond. (3) A second nucleophilic attack by a water molecule activated by His493 hydrolyzes the phosphohistidine intermediate (4) The Tdp1 active site is regenerated and the 3′-phosphate DNA end is released.

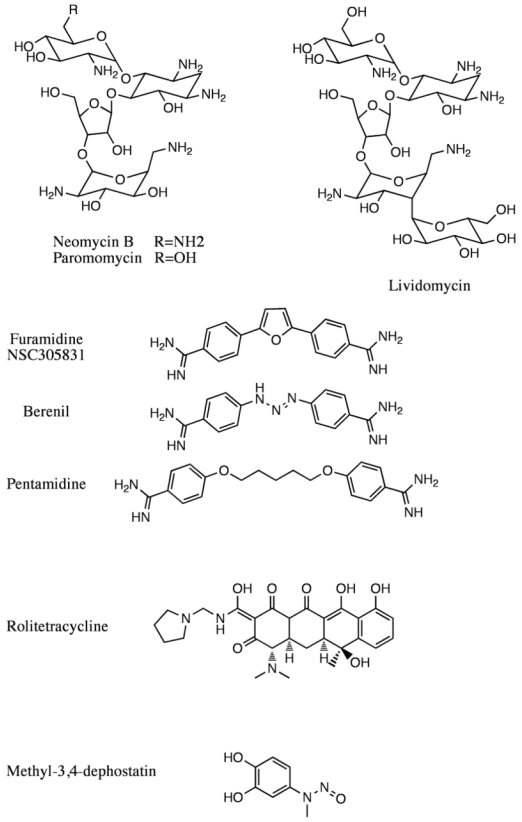

Figure 2.
Chemical structures of the presented compounds

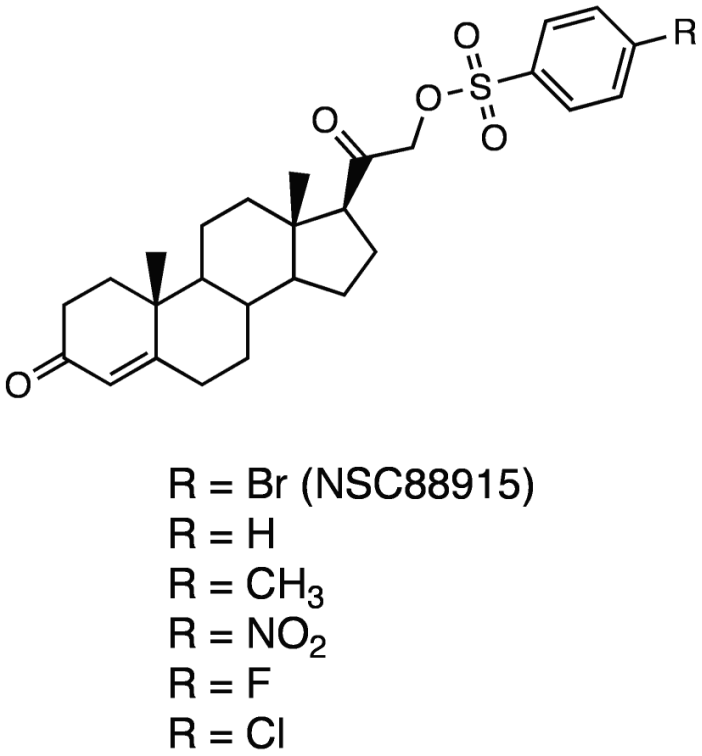

Figure 3.
Chemical structures of the steroid derivatives