Published in final edited form as:

Bioorg Med Chem Lett. 2019 January 15; 29(2): 257–261. doi:10.1016/j.bmcl.2018.11.044.

Triazolopyrimidine and triazolopyridine scaffolds as TDP2 inhibitors

Carlos J. A. Ribeiro^{a,§}, Jayakanth Kankanala^{a,§}, Jiashu Xie^a, Jessica Williams^a, Hideki Aihara^b, and Zhengqiang Wang^{a,*}

^aCenter for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, United States

^bDepartment of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455, United States

Abstract

Tyrosyl-DNA phosphodiesterase 2 (TDP2) repairs topoisomerase II (TOP2) mediated DNA damages and causes cellular resistance to clinically used TOP2 poisons. Inhibiting TDP2 can potentially sensitize cancer cells toward TOP2 poisons. Commercial compound P10A10, to which the structure was assigned as 7-phenyl triazolopyrimidine analogue **6a**, was previously identified as a TDP2 inhibitor hit in our virtual and fluorescence-based biochemical screening campaign. We report herein that the hit validation through resynthesis and structure elucidation revealed the correct structure of P10A10 (Chembridge ID 7236827) to be the 5-phenyl triazolopyrimidine regioisomer **7a**. Subsequent structure-activity relationship (SAR) via the synthesis of a total of 47 analogues of both the 5-phenyl triazolopyrimidine scaffold (**7**) and its bioisosteric triazolopyridine scaffold (**17**) identified four derivatives (**7a**, **17a**, **17e**, and **17z**) with significant TDP2 inhibition ($IC_{50} < 50 \mu M$), with **17z** showing excellent cell permeability and no cytotoxicity.

Graphical Abstract

Keywords

Tyrosyl-DNA phosphodiesterase 2 (TDP2); Anti-cancer; Triazolo-pirimidines; Triazolo-pyridines

Topoisomerase II (TOP2) resolves DNA catenanes and knots and relax both positive and negative supercoils generated during normal physiological processes, such as transcription and replication.¹ TOP2 manages these topological transactions via producing transient TOP2

*Corresponding author. wangx472@umn.edu (Z. Wang).

§These authors contributed equally to this work

Appendix A. Supplementary data

Experimental procedures, NMR characterization, as well as crystal data and structure refinement for compound **11**, can be found in the supporting information. Supplementary data associated with this article can be found online at

cleavage complexes (TOP2cc) which feature a unique covalent bond between its active site tyrosine and the 5'-phosphate terminus of the DNA backbone at the break sites.¹ In the presence of TOP2 poisons, such as anticancer drugs etoposide and doxorubicin, these transient TOP2cc can be stabilized and become abortive, resulting in DNA double-strand breaks (DSBs) and ultimately leading to cell death.^{2–3} Tyrosyl-DNA phosphodiesterase 2 (TDP2) repairs these DSBs by specifically cleaving the 5'-phosphotyrosine bond that links TOP2 to DNA to create competent DSB termini ready for ligation through the non-homologous end joining (NHEJ).^{4–7} By virtue of its cellular function TDP2 allows cancer cells to become resistant toward TOP2 poisons,⁶ a major class of anticancer drugs widely used in clinic. Evidence of TDP2 function in cancer cell damage repair also includes the observation that TDP2-deleted chicken lymphoma DT40 cells were hypersensitive to TOP2 poison etoposide, but not to the TOP1 poison camptothecin.⁸ Therefore, TDP2 represents an attractive anticancer drug target since its inhibition can sensitize cancer cells against TOP2 poisons. In addition, since treatment with TOP2 poisons is associated with non-specific toxicity due to limited tumor selectivity, co-administration with TDP2 inhibitors will potentially allow therapeutic efficacy with much lower doses of TOP2 poisons.^{5,9}

Interestingly, TDP2 is also implicated in the genome repair of certain viruses. TDP2 was identified as the host VPg-unlinkase enzyme necessary to cleave VPg-RNA bonds during picornavirus replicative cycle.^{10–12} In addition, TDP2 could also play a role in hepatitis B virus (HBV) cccDNA formation by facilitating the release of P-protein from viral DNA.¹³

Phosphodiesterase activity of TDP2 was only discovered in 2009,⁸ and since then only a small number of scaffolds have been reported with TDP2 inhibitory activity (Fig. 1).^{9, 14–23} In addition, most of these scaffolds, such as compound **1** (NSC111041)²³ are poorly characterized, moderately active and lacking drug-like properties. Deazaflavin (e.g. **2**, Fig. 1),^{19–21} is the only inhibitor type with nanomolar biochemical potency¹⁹ and well characterized mechanism of inhibition,²¹ yet these inhibitors did not exhibit strong efficacy in cells²⁰ due to their poor cellular permeability.¹⁹ As part of our own efforts in developing drug like small molecule TDP2 inhibitors, we have previously identified an attractive inhibitor core isoquinoline-1,3-diones (e.g. **3**).²²

Recently we also developed the first fluorescence-based high throughput assay¹⁴ and identified new scaffolds with TDP2 inhibitory activity (e.g. compound **4**, **5**, and P10A10, Fig.1) during HTS of small library of 1600 compounds curated via virtual screening. In particular, [1,2,4]triazolo[1,5-*a*]pyrimidine compound P10A10 was found to inhibit 14M_zTDP2, a validated surrogate protein for hTDP2. Triazolopyrimidine is a highly privileged core in medicinal chemistry, with several biological activities reported just in the past three years: anti-Alzheimer's disease,^{24–25} antibacterial,^{26–27} anticancer,^{28–29} antileishmanial,³⁰ antimalarial,^{31–32} antiviral,^{33–34} CB2 cannabinoid receptor inverse agonists,³⁵ and phosphodiesterase 2 (PDE2a) inhibition.³⁶ Similar core is also featured in various herbicidal compounds.³⁷ In the present report, we describe our hit confirmation efforts including resynthesis and structural elucidation of hit P10A10, and the subsequent SAR of the triazolopyrimidine scaffold as TDP2 inhibitors.

The synthesis of triazolopyrimidines is well documented.^{38–40} In our studies, [1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)benzamides **6** and **7** (scheme 1) were synthesized in two steps starting with phenylpropene-2-one (**8**) or phenylpropan-2-one (**9**) to obtain 7-phenyl isomer **6** or 5-phenyl isomer **7**, respectively. In both cases, compounds **8** or **9** were condensed with diaminotriazole **10** in the presence of acetic acid under MW conditions to yield [1,2,4]triazolo[1,5-*a*]pyrimidin-2-amine intermediates **11** and **12**, respectively. Acylation with different acid chlorides, followed by treatment with ammonia solution 7N in methanol, provided the desired final compounds in good yields (73 – 83%). [1,2,4]Triazolo[1,5-*a*]pyridin-2-amine scaffold **13a** was constructed by reacting 2-amino-5-bromopyridine (**14**) with ethoxycarbonyl isothiocyanate in dichloromethane at room temperature to form thiourea **15**, which was cyclized by reacting with hydroxylamine under basic conditions at reflux in ethanol/methanol. Intermediate **13** then underwent microwave-irradiated Suzuki coupling with different boronic acids in the presence of Pd(PPh₃)₄ to afford [1,2,4]triazolo[1,5-*a*]pyridin-2-amines **16** in moderate yields (46 – 81%). Compounds **17** and **18** were obtained by acylation of **16** in the same manner as described for compounds **6** and **7** (45–96% yield). Compound **19** was synthesized via reductive amination involving compound **16a** and 3-chlorobenzaldehyde. Treating compound **16a** with 3-chlorobenzenesulphonyl chloride in methanol at room temperature for 2 h afforded compound **20** in 53% yield.

Our hit validation started with resynthesizing hit compound P10A10 (**6a**)⁴¹ and closely related analogues **6b-c** to confirm the inhibitory capability of this scaffold (Scheme 1, Table 1). Surprisingly, all three analogues were found to be completely inactive at 100 µM, indicating that the structural assignment of compound P10A10 as **6a** could be questionable. In further structural analysis, although mass spectrometry showed the same mass for P10A10 and **6a**, from ¹H-NMR spectra the proton chemical shifts, and specially the coupling constant, for the pyrimidine hydrogens differed significantly (*J* = 7.1 versus *J* = 4.7 Hz, respectively, Figure 2) between P10A10 and **6a**. In addition, literature search revealed that reacting aminoazoles with α,β-unsaturated compounds can generate different triazolopyrimidine regioisomers depending on the reaction conditions employed.^{42–43} These observations suggest that either our synthesized compound **6a** or the commercial P10A10 could be the 5-phenyl regioisomer **7a**. To further elucidate the structure, we obtained the crystal structure for an advanced intermediate **11** (step a, Scheme 1), which confirmed that the phenyl ring is indeed on the 7 position (Figure 2) as reported in literature for the same reaction.⁴⁴ In the meantime, we synthesized compound **7a**, the 5-phenyl isomer of compound **6a**. Comparison of **7a** to commercial compound P10A10 showed identical ¹H-NMR spectrum and same potency inhibiting TDP2. With all these, we corrected the structure for P10A10 (Chembridge ID 7236827) to be **7a**, not **6a** as assigned by the vendor.

All 47 final compounds were evaluated in a fluorescence-based biochemical assay¹⁴ measuring the catalytic activity of TDP2. In addition, permeability of selected derivatives was assessed by parallel artificial membrane permeability assay (PAMPA) and cytotoxicity was determined for the two overall best derivatives.

With the confirmation of **7a** as a TDP2 inhibitor hit, we started exploring the SAR to see if we could improve the hit. We were particularly interested in replacing the nitro group as nitroarenes are associated with potential toxicity in medicinal chemistry with negative impact to the pharmacokinetic and physicochemical profile.^{45–46} To probe the importance of *meta*-nitro group of the benzamide ring to activity, we first synthesized analogues without nitro (**7b**), with nitro at *para* position (**7c**), or with nitro replaced with another electron withdrawing group, such as nitrile (**7d**), trifluoromethyl, (**7e**) and bromo (**7f**). As shown in Table 1, these substitutions had a dramatic impact on TDP2 inhibition as significant activity was not observed with compounds **7b–e** whereas compound **7f** exhibited only weak inhibitory activity (IC_{50} = 80.9 μ M). One of the challenges we had technically with these compounds was their poor solubility in DMSO. In an attempt to improve solubility and to further probe the SAR, we decided to synthesize the 4-deaza bioisosteric core, the triazolopyridine (**17**). Interestingly, compound **17a** which bears the same *meta*-nitrophenyl moiety as hit **7a** was found to be slightly more active (IC_{50} = 16.6 μ M vs. 22.0 μ M) with a better solubility profile in both DMSO and buffer. Therefore, we decided to continue our study focusing on the triazolopyridine scaffold **17** instead. Successful nitro bioisosters⁴⁷ described in literature include nitrile,^{48–50} pyridine,^{51–52} and halogenated analogues.^{53–54} In addition, nitroarenes can behave as halo- or alkylarenes when noncovalently interacting with proteins.⁴⁶ Unfortunately, all these bioisosteric replacements led to less active compounds (**17b–o**), with only 3-chloro derivative (**17e**) exhibiting an IC_{50} lower than 50 μ M. Moreover, changing chloro to *para* and *ortho* positions (**17l–m**) or adding a second chloro (**17o**) further reduced activity.

Another SAR trend was that the phenyl group at different positions in the main core favored position 7 (**17a**, IC_{50} = 16.6 μ M vs. **18**, IC_{50} = 77.3 μ M vs. **6a**, IC_{50} > 100 μ M). Although the potency of compound **17e** was found to be 2-fold lower than the hit compound (IC_{50} = 43.8 μ M vs 22.0 μ M), its permeability in PAMPA assay was 6-fold higher (Table 3), and therefore we decided to maintain a 3-chlorobenzamide moiety when probing substitutions at position 7 of the pyridine ring (**17p–ac**, Table 2). Only **17s** with 4-methoxyphenyl group was able to retain almost the same activity (IC_{50} = 59.0 μ M), with pyridine-3-yl substitution leading to a two-fold improve of potency (**17z**, IC_{50} = 21.0 μ M vs. **17e**, 43.8 μ M) and 7-fold increase in permeability (Table 3). The increase in potency observed for derivative **17z** led us to further test substitution at meta position with potential H bond acceptor properties (3-methylsulfonyl, **17ad**; 3-acetyl, **17ae**; 3-carboxylate **17af**) and also additional derivatives with aromatic nitrogens (pyridine-4-yl, **17ag**; quinoline-3-yl, **17ah**; pyrimidin-5-yl, **17ai**). However, all modifications led to a decrease in potency.

Lastly, we synthesized compounds in which the carboxamide moiety of **17** is replaced by an amine (**19**) and or a sulfonamide (**20**). Both substitutions led to inactive derivatives, highlighting the importance of the amide group to activity.

Since the goal of developing TDP2 inhibitors is to sensitize cancer cells toward TOP2 poisons to ultimately overcome drug resistance and potentially also allow TOP2 therapeutic efficiency at lower and less toxic concentrations, it is imperative that these inhibitors are cell permeable and non-cytotoxic. Toward these ends, selected active compounds (**7a**, **17a**, **17e**, and **17z**) were first tested in the PAMPA permeability assay along with the most potent

deazaflavin inhibitor **1** (Table 3). From this assay, excellent permeability was observed only with our compound **17z**. Another analogue **17e** also exhibited borderline P_e value which is about 10 times higher than that of **1**. These findings are consistent with the report that deazaflavin compounds lacked cellular permeability. We then assessed the two cell-permeable compounds **17e** and **17z** for cytotoxicity in two different cell lines (HepG2 and HeLa), from which no cytotoxicity was observed at concentrations up to 100 μ M (Table 3). These results indicate that despite the relatively moderate TDP2 inhibitory activity, scaffold **17** has the potential to be developed into cell permeable and noncytotoxic chemical probes for studying cellular functions.

From a previous screening campaign against TDP2, compound P10A10, reported as 7-phenyl triazolopyrimidine **6a**, was identified as a hit. The present study on hit validation via resynthesis and structure elucidation revealed that the correct structure for P10A10 (Chembridge ID 7236827) should be 5-phenyl isomer **7a**. To probe TDP2 inhibition potentialities of this scaffold we synthesized a total of 47 compounds: 11 derivatives inhibited TDP2 ($IC_{50} < 100 \mu$ M), with four derivatives (**7a**, **17a**, **17e**, and **17z**) reaching potencies below 50 μ M. Although we were unable to increase significantly the potency of the original hit, compounds **17e** and **17z**, particularly the latter, demonstrated good cellular permeability and no cytotoxicity and can be further developed into useful chemical probes for studying cellular functions of TDP2 in host DNA repair as well as virus genome repair.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Academic Health Center Faculty Research Development Grant Program (FRD #14.23), University of Minnesota, and partially by the Center for Drug Design, University of Minnesota, and NIH grant GM118047 to HA. We acknowledge Professor Bert Semler at University of California, Irvine and Professor Haitao Guo at Indiana University School of Medicine, for providing HeLa and HepG2 Cells, respectively. We also acknowledge Victor G. Young, Jr. and the X-Ray Crystallographic Laboratory for the X-ray crystallography studies. The Bruker-AXS D8 Venture diffractometer was purchased through a grant from NSF/MRI (#1224900) and the University of Minnesota.

References

1. Pommier Y; Sun Y; Huang SN; Nitiss JL, Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. *Nat. Rev. Mol. Cell Biol* 2016, 17 (11), 703–721. [PubMed: 27649880]
2. Pommier Y, Drugging topoisomerases: lessons and challenges. *ACS Chem. Biol* 2013, 8 (1), 82–95. [PubMed: 23259582]
3. Fortune JM; Osheroff N, Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice. *Prog. Nucleic Acid Res. Mol. Biol* 2000, 64, 221–53. [PubMed: 10697411]
4. Menon V; Povirk LF, End-processing nucleases and phosphodiesterases: An elite supporting cast for the non-homologous end joining pathway of DNA double-strand break repair. *DNA Repair (Amst)* 2016, 43, 57–68. [PubMed: 27262532]
5. Pommier Y; Huang S.-y. N.; Gao R; Das BB; Murai J; Marchand C, Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). *DNA Repair* 2014, 19, 114–129. [PubMed: 24856239]

6. Do PM; Varanasi L; Fan S; Li C; Kubacka I; Newman V; Chauhan K; Daniels SR; Bocchetta M; Garrett MR; Li R; Martinez LA, Mutant p53 cooperates with ETS2 to promote etoposide resistance. *Genes Dev* 2012, 26 (8), 830–45. [PubMed: 22508727]
7. Zeng Z; Cortés-Ledesma F; El Khamisy SF; Caldecott KW, TDP2/TTRAP is the major 5'-tyrosyl DNA phosphodiesterase activity in vertebrate cells and is critical for cellular resistance to topoisomerase II-induced DNA damage. *J. Biol. Chem* 2011, 286 (1), 403–409. [PubMed: 21030584]
8. Cortes Ledesma F; El Khamisy SF; Zuma MC; Osborn K; Caldecott KW, A human 5'-tyrosyl DNA phosphodiesterase that repairs topoisomerase-mediated DNA damage. *Nature* 2009, 461 (7264), 674–8. [PubMed: 19794497]
9. Laev SS; Salakhutdinov NF; Lavrik OI, Tyrosyl-DNA phosphodiesterase inhibitors: Progress and potential. *Bioorg. Med. Chem* 2016, 24 (21), 5017–5027. [PubMed: 27687971]
10. Virgen-Slane R; Rozovics JM; Fitzgerald KD; Ngo T; Chou W; van Noort GJV; Filippov DV; Gershon PD; Semler BL, An RNA virus hijacks an incognito function of a DNA repair enzyme. *Proc. Natl. Acad. Sci. U. S. A* 2012, 109 (36), 14634–14639. [PubMed: 22908287]
11. Maciejewski S; Nguyen JHC; Gomez-Herreros F; Cortes-Ledesma F; Caldecott KW; Semler BL, Divergent requirement for a DNA repair enzyme during enterovirus infections. *Mbio* 2016, 7 (1).
12. Maciejewski S; Ullmer W; Semler BL, VPg unlinkase/TDP2 in cardiovirus infected cells: Re-localization and proteolytic cleavage. *Virology* 2018, 516, 139–146. [PubMed: 29353210]
13. Koniger C; Wingert I; Marsmann M; Rosler C; Beck J; Nassal M, Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc. Natl. Acad. Sci. U. S. A* 2014, 111 (40), E4244–E4253. [PubMed: 25201958]
14. Ribeiro CJA; Kankanala J; Shi K; Kurahashi K; Kiselev E; Ravji A; Pommier Y; Aihara H; Wang Z, New fluorescence-based high-throughput screening assay for small molecule inhibitors of tyrosyl-DNA phosphodiesterase 2 (TDP2). *Eur. J. Pharm. Sci* 2018, 118, 67–79. [PubMed: 29574079]
15. Thomson G; Watson A; Caldecott K; Denny O; Depledge P; Hamilton N; Hopkins G; Jordan A; Morrow C; Raoof A; Waddell I; Ogilvie D, Generation of assays and antibodies to facilitate the study of human 5'-tyrosyl DNA phosphodiesterase. *Anal. Biochem* 2013, 436 (2), 145–150. [PubMed: 23416181]
16. Kossmann BR; Abdelmalak M; Lopez S; Tender G; Yan C; Pommier Y; Marchand C; Ivanov I, Discovery of selective inhibitors of tyrosyl-DNA phosphodiesterase 2 by targeting the enzyme DNA-binding cleft. *Bioorg. Med. Chem. Lett* 2016, 26 (14), 3232–6. [PubMed: 27262595]
17. Beck DE; Lv W; Abdelmalak M; Plescia CB; Agama K; Marchand C; Pommier Y; Cushman M, Synthesis and biological evaluation of new fluorinated and chlorinated indenoisoquinoline topoisomerase I poisons. *Bioorg. Med. Chem* 2016, 24 (7), 1469–1479. [PubMed: 26906474]
18. Wang P; Elsayed MSA; Plescia CB; Ravji A; Redon CE; Kiselev E; Marchand C; Zeleznik O; Agama K; Pommier Y; Cushman M, Synthesis and biological evaluation of the first triple inhibitors of human topoisomerase 1, tyrosyl-DNA phosphodiesterase 1 (tdp1), and tyrosyl-DNA phosphodiesterase 2 (tdp2). *J. Med. Chem* 2017, 60 (8), 3275–3288. [PubMed: 28418653]
19. Raoof A; Depledge P; Hamilton NM; Hamilton NS; Hitchin JR; Hopkins GV; Jordan AM; Maguire LA; McGonagle AE; Mould DP; Rushbrooke M; Small HF; Smith KM; Thomson GJ; Turlais F; Waddell ID; Waszkowycz B; Watson AJ; Ogilvie DJ, Toxoflavins and deazaflavins as the first reported selective small molecule inhibitors of tyrosyl-DNA phosphodiesterase II. *J. Med. Chem* 2013, 56 (16), 6352–6370. [PubMed: 23859074]
20. Marchand C; Abdelmalak M; Kankanala J; Huang SY; Kiselev E; Fesen K; Kurahashi K; Sasanuma H; Takeda S; Aihara H; Wang Z; Pommier Y, Deazaflavin inhibitors of tyrosyl-DNA phosphodiesterase 2 (TDP2) specific for the human enzyme and active against cellular TDP2. *ACS Chem. Biol* 2016, 11 (7), 1925–33. [PubMed: 27128689]
21. Hornyak P; Askwith T; Walker S; Komulainen E; Paradowski M; Pennicott LE; Bartlett EJ; Brissett NC; Raoof A; Watson M; Jordan AM; Ogilvie DJ; Ward SE; Atack JR; Pearl LH; Caldecott KW; Oliver AW, Mode of action of DNA-competitive small molecule inhibitors of tyrosyl DNA phosphodiesterase 2. *Biochem. J* 2016, 473, 1869–1879. [PubMed: 27099339]

22. Kankanala J; Marchand C; Abdelmalak M; Aihara H; Pommier Y; Wang Z, Isoquinoline-1,3-diones as selective inhibitors of tyrosyl DNA phosphodiesterase II (TDP2). *J. Med. Chem* 2016, 59 (6), 2734–2746. [PubMed: 26910725]
23. Kont YS; Dutta A; Mallisetty A; Mathew J; Minas T; Kraus C; Dhopeswarkar P; Kallakury B; Mitra S; Uren A; Adhikari S, Depletion of tyrosyl DNA phosphodiesterase 2 activity enhances etoposide-mediated double-strand break formation and cell killing. *DNA Repair* 2016, 43, 38–47. [PubMed: 27235629]
24. Kumar J; Meena P; Singh A; Jameel E; Maqbool M; Mobashir M; Shandilya A; Tiwari M; Hoda N; Jayaram B, Synthesis and screening of triazolopyrimidine scaffold as multi-functional agents for Alzheimer's disease therapies. *Eur. J. Med. Chem* 2016, 119, 260–277. [PubMed: 27227482]
25. Kovalevich J; Cornec AS; Yao Y; James M; Crowe A; Lee VMY; Trojanowski JQ; Smith AB; Ballatore C; Brunden KR, Characterization of Brain-Penetrant Pyrimidine-Containing Molecules with Differential Microtubule-Stabilizing Activities Developed as Potential Therapeutic Agents for Alzheimer's Disease and Related Tauopathies. *J. Pharmacol. Exp. Ther* 2016, 357 (2), 432–450. [PubMed: 26980057]
26. Jung IP; Ha NR; Lee SC; Ryoo SW; Yoon MY, Development of potent chemical antituberculosis agents targeting Mycobacterium tuberculosis acetohydroxyacid synthase. *Int. J. Antimicrob. Agents* 2016, 48 (3), 247–258. [PubMed: 27451857]
27. Wang H; Lee M; Peng Z; Blázquez B; Lastochkin E; Kumarasiri M; Bouley R; Chang M; Mobashery S, Synthesis and Evaluation of 1,2,4-Triazolo[1,5-a]pyrimidines as Antibacterial Agents Against Enterococcus faecium. *J. Med. Chem* 2015, 58 (10), 4194–4203. [PubMed: 25923368]
28. Wang S; Zhao L-J; Zheng Y-C; Shen D-D; Miao E-F; Qiao X-P; Zhao L-J; Liu Y; Huang R; Yu B; Liu H-M, Design, synthesis and biological evaluation of [1,2,4]triazolo[1,5-a]pyrimidines as potent lysine specific demethylase 1 (LSD1/KDM1A) inhibitors. *Eur. J. Med. Chem* 2017, 125, 940–951. [PubMed: 27769034]
29. Arenas-Gonzalez A; Mendez-Delgado LA; Merino-Montiel P; Padron JM; Montiel-Smith S; Vega-Baez JL; Meza-Reyes S, Synthesis of monomeric and dimeric steroids containing 1,2,4 triazolo 1,5-a pyrimidines. *Steroids* 2016, 116, 13–19. [PubMed: 27692994]
30. da Silva ER; Boechat N; Pinheiro LCS; Bastos MM; Costa CCP; Bartholomeu JC; da Costa TH, Novel Selective Inhibitor of Leishmania (Leishmania) amazonensis Arginase. *Chem. Biol. Drug Des* 2015, 86 (5), 969–978. [PubMed: 25845502]
31. Phillips MA; White KL; Kokkonda S; Deng X; White J; El Mazouni F; Marsh K; Tomchick DR; Manjulanagara K; Rudra KR; Wirjanata G; Noviyanti R; Price RN; Marfurt J; Shackelford DM; Chiu FCK; Campbell M; Jimenez-Diaz MB; Bazaga SF; Angulo-Barturen I; Martinez MS; Lafuente-Monasterio M; Kaminsky W; Silue K; Zeeman A-M; Kocken C; Leroy D; Blasco B; Rossignol E; Rueckle T; Matthews D; Burrows JN; Waterson D; Palmer MJ; Rathod PK; Charman SA, A Triazolopyrimidine-Based Dihydroorotate Dehydrogenase Inhibitor with Improved Drug-like Properties for Treatment and Prevention of Malaria. *ACS Infectious Diseases* 2016, 2 (12), 945–957. [PubMed: 27641613]
32. Kokkonda S; Deng XY; White KL; Coteron JM; Marco M; de las Heras L; White J; El Mazouni F; Tomchick DR; Manjulanagara K; Rudra KR; Chen G; Morizzi J; Ryan E; Kaminsky W; Leroy D; Martinez-Martinez MS; Jimenez-Diaz MB; Bazaga SF; Angulo-Barturen I; Waterson D; Burrows JN; Matthews D; Charman SA; Phillips MA; Rathod PK, Tetrahydro-2-naphthyl and 2-Indanyl Triazolopyrimidines Targeting Plasmodium falciparum Dihydroorotate Dehydrogenase Display Potent and Selective Antimalarial Activity. *J. Med. Chem* 2016, 59 (11), 5416–5431. [PubMed: 27127993]
33. Massari S; Nannetti G; Desantis J; Muratore G; Sabatini S; Manfroni G; Mercorelli B; Cecchetti V; Palu G; Cruciani G; Loregian A; Goracci L; Tabarrini O, A Broad Anti-influenza Hybrid Small Molecule That Potently Disrupts the Interaction of Polymerase Acidic Protein-Basic Protein 1 (PA-PB1) Subunits. *J. Med. Chem* 2015, 58 (9), 3830–42. [PubMed: 25856229]
34. Huang B; Li C; Chen W; Liu T; Yu M; Fu L; Sun Y; Liu H; De Clercq E; Pannecouque C; Balzarini J; Zhan P; Liu X, Fused heterocycles bearing bridgehead nitrogen as potent HIV-1 NNRTIs. Part 3: Optimization of [1,2,4]triazolo[1,5-a]pyrimidine core via structure-based and

- physicochemical property-driven approaches. *Eur. J. Med. Chem* 2015, 92, 754–765. [PubMed: 25626145]
35. Aghazadeh Tabrizi M; Baraldi PG; Ruggiero E; Saponaro G; Baraldi S; Poli G; Tuccinardi T; Ravani A; Vincenzi F; Borea PA; Varani K, Synthesis and structure activity relationship investigation of triazolo[1,5-a]pyrimidines as CB2 cannabinoid receptor inverse agonists. *Eur. J. Med. Chem* 2016, 113, 11–27. [PubMed: 26922225]
36. Gomez L; Massari ME; Vickers T; Freestone G; Vernier W; Ly K; Xu R; McCarrick M; Marrone T; Metz M; Yan YG; Yoder ZW; Lemus R; Broadbent NJ; Barido R; Warren N; Schmelzer K; Neul D; Lee D; Andersen CB; Sebring K; Aertgeerts K; Zhou X; Tabatabaei A; Peters M; Breitenbucher JG, Design and Synthesis of Novel and Selective Phosphodiesterase 2 (PDE2a) Inhibitors for the Treatment of Memory Disorders. *J. Med. Chem* 2017, 60 (5), 2037–2051. [PubMed: 28165743]
37. Billington R; Gehen SC; Hanley TR, Chapter 87 - Toxicology of Triazolopyrimidine Herbicides In *Hayes' Handbook of Pesticide Toxicology* (Third Edition), Krieger R, Ed. Academic Press: New York, 2010; pp 1865–1885.
38. Elashry ESH; Rashed N, 1,2,4-Triazolo- and Tetrazolo[x,y-z]pyrimidines In *Adv. Heterocycl. Chem*, Katritzky AR, Ed. Academic Press: 1998; Vol. 72, pp 127–224.
39. Fischer G, Recent Progress in 1,2,4-Triazolo[1,5-a]pyrimidine Chemistry In *Adv. Heterocycl. Chem*, Katritzky AR, Ed. Academic Press: 2007; Vol. 95, pp 143–219.
40. Fizer M; Slivka M, Synthesis of 1,2,4 triazolo 1,5-a pyrimidine (microreview). *Chem. Heterocycl. Compds* 2016, 52 (3), 155–157.
41. <https://www.molinstincts.com/chemical-structure/Cambridge-id-7236827-cstr-CT1067003447.html> (accessed 9/19/2018).
42. Allen CFH; Beilfuss HR; Burness DM; Reynolds GA; Tinker JF; VanAllan JA, The Structure of Certain Polyazaindenes. IV. Compounds from β -Keto Acetals and β -Methoxyvinyl Ketones I. *J. Org. Chem* 1959, 24 (6), 796–801.
43. Massari S; Desantis J; Nannetti G; Sabatini S; Tortorella S; Goracci L; Cecchetti V; Loregian A; Tabarrini O, Efficient and regioselective one-step synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-a]pyrimidine derivatives. *Org. Biomol. Chem* 2017, 15 (37), 7944–7955. [PubMed: 28902220]
44. Menet CJ; Fletcher SR; Van Lommen G; Geney R; Blanc J; Smits K; Jouannigot N; Deprez P; van der Aar EM; Clement-Lacroix P; Lepescheux L; Galien R; Vayssiere B; Nelles L; Christophe T; Brys R; Uhring M; Ciesielski F; Van Rompaey L, Triazolopyridines as selective JAK1 inhibitors: from hit identification to GLPG0634. *J. Med. Chem* 2014, 57 (22), 9323–42. [PubMed: 25369270]
45. Boelsterli UA; Ho HK; Zhou S; Leow KY, Bioactivation and hepatotoxicity of nitroaromatic drugs. *Curr. Drug Metab* 2006, 7 (7), 715–27. [PubMed: 17073576]
46. Dörwald FZ, *Lead Optimization for Medicinal Chemists: Pharmacokinetic Properties of Functional Groups and Organic Compounds* Wiley-VCH: Weinheim, Germany: 2012.
47. Meanwell NA, Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem* 2011, 54 (8), 2529–2591. [PubMed: 21413808]
48. Abeywardane A; Caviness G; Choi Y; Cogan D; Gao A; Goldberg D; Heim-Riether A; Jeanfavre D; Klein E; Kowalski JA; Mao W; Miller C; Moss N; Ramsden P; Raymond E; Skow D; Smith-Keenan L; Snow RJ; Wu F; Wu J-P; Yu Y, N-Arylsulfonyl- α -amino carboxamides are potent and selective inhibitors of the chemokine receptor CCR10 that show efficacy in the murine DNFB model of contact hypersensitivity. *Bioorg. Med. Chem. Lett* 2016, 26 (21), 5277–5283. [PubMed: 27692854]
49. Tang H; Walsh SP; Yan Y; de Jesus RK; Shahripour A; Teumelsan N; Zhu Y; Ha S; Owens KA; Thomas-Fowlkes BS; Felix JP; Liu J; Kohler M; Priest BT; Bailey T; Brochu R; Alonso-Galicia M; Kaczorowski GJ; Roy S; Yang L; Mills SG; Garcia ML; Pasternak A, Discovery of Selective Small Molecule ROMK Inhibitors as Potential New Mechanism Diuretics. *ACS Med. Chem. Lett* 2012, 3 (5), 367–372. [PubMed: 24900480]
50. Cottyn B; Acher F; Ramassamy B; Alvey L; Lepoivre M; Frapart Y; Stuehr D; Mansuy D; Boucher J-L; Vichard D, Inhibitory effects of a series of 7-substituted-indazoles toward nitric oxide

synthases: Particular potency of 1H-indazole-7-carbonitrile. *Bioorg. Med. Chem* 2008, 16 (11), 5962–5973. [PubMed: 18502134]

51. Gneuwuch CT; Friedman HL, Pyridine isosteres of the .beta.-adrenergic antagonists, 2-(p-nitrophenyl)-1-isopropylamino-2-ethanol and 3-(p-nitrophenoxy)-1-isopropylamino-2-propanol. *J. Med. Chem* 1972, 15 (12), 1321–1324. [PubMed: 4404591]
52. Julémont F; de Leval X; Michaux C; Damas J; Charlier C; Durant F; Pirotte B; Dogné J-M, Spectral and Crystallographic Study of Pyridinic Analogues of Nimesulide: Determination of the Active Form of Methanesulfonamides as COX-2 Selective Inhibitors. *J. Med. Chem* 2002, 45 (23), 5182–5185. [PubMed: 12408728]
53. Yu L-F; Li Y-Y; Su M-B; Zhang M; Zhang W; Zhang L-N; Pang T; Zhang R-T; Liu B; Li J-Y; Li J; Nan F-J, Development of Novel Alkene Oxindole Derivatives As Orally Efficacious AMP-Activated Protein Kinase Activators. *ACS Med. Chem. Lett* 2013, 4 (5), 475–480. [PubMed: 24900695]
54. Nagarathnam D; Miao SW; Lagu B; Chiu G; Fang J; Murali Dhar TG; Zhang J; Tyagarajan S; Marzabadi MR; Zhang F; Wong WC; Sun W; Tian D; Wetzel JM; Forray C; Chang RSL; Broten TP; Ransom RW; Schorn TW; Chen TB; O'Malley S; Kling P; Schneck K; Bendesky R; Harrell CM; Vyas KP; Gluchowski C, Design and Synthesis of Novel α 1a Adrenoceptor-Selective Antagonists. 1. Structure–Activity Relationship in Dihydropyrimidinones. *J. Med. Chem* 1999, 42 (23), 4764–4777. [PubMed: 10579840]

Figure 1.
Representative reported TDP2 inhibitors

Figure 2.

Comparison of pyrimidine hydrogens NMR coupling constant from both isomers **6a** and **7a**, and X-ray crystallography studies of intermediate **11** (CCDC 1869530) reveals that P10A10 is not isomer **6a**, but **7a** instead.

Scheme 1.

Reagents and conditions: (a) AcOH, reflux, 4h, 72%; (b) (i) acid chloride (2.5 equiv), pyridine, 0°C → r.t., 1–2 h, (ii) 7N NH₃ in MeOH, o.n., 45 – 96% (c) (i) AcOH, reflux, 6h; (ii) NBS, EtOH, reflux, 5h, 29% (d) ethoxycarbonylisothiocyanate (1 equiv), dioxane, under argon, 0°C to r.t., 12 h; (e) NH₂OH·HCl (5 equiv), DIPEA (3 equiv), EtOH/MeOH 1:1, 2 h r.t. then 60°C, 3 h, 65% (over 2 steps); (f) boronic acid, Pd(PPh₃)₄, K₂CO₃, EtOH, H₂O (1:1), M.W., 150°C, 30 min, 46 – 81% (g) 3 chlorobenzaldehyde (1.1 equiv), acetic acid (cat.), MeOH, r.t., 2 h; then sodium cyanoborohydride (2.0 equiv), r.t., 2 h, 76 %; (h) 3-chlorobenzenesulfonyl chloride (2.5 equiv), pyridine, 0°C → r.t., 2 h, 53%.

Table 1.TDP2 inhibitory activities of derivatives **6a-c**, **7 a-f**, **17a-o** and **18**.

Compds	R ¹	IC ₅₀ (μM) ^a
6a	3-NO ₂	>100
6b	H	>100
6c	4-NO ₂	>100
7a	3-NO ₂	22.0 ± 2.5
7b	H	>100
7c	4-NO ₂	>100
7d	3-CN	>100
7e	3-CF ₃	>100
7f	3-Br	80.9 ± 4.0
17a	3-NO ₂ Ph	16.6 ± 0.4
17b	3-OCH ₃ Ph	>100
17c	3-CH ₃ Ph	>100
17d	3-FPh	>100
17e	3-ClPh	43.8 ± 3.9
17f	pyridin-3-yl	>100
17g	pyridin-4-yl	>100
17h	4-OCH ₃ Ph	>100
17i	4-N(CH ₃) ₂	>100
17j	3,4-FPh	>100
17k	furan-2-yl	>100
17l	4-ClPh	>100
17m	2-ClPh	>100
17n	3,5-ClPh	>100
17o	2-Cl, 4-FPh	>100
18	-	77.3 ± 7.5

^aIC₅₀ values are the mean of three independent experiments performed in triplicate.

Table 2.

TDP2 inhibitory activities of derivatives **17p-ai**, **19–20**.

Compds	R ¹	IC ₅₀ (μM) ^a
17p	4-ClPh	80.8 ± 7.5
17q	4-OHPh	>100
17r	4-CF ₃ Ph	>100
17s	4-OCH ₃ Ph	59.0 ± 3.7
17t	4-FPh	85.2 ± 4.6
17u	3-ClPh	>100
17v	3-OCH ₃ Ph	>100
17w	3-CF ₃ Ph	>100
17x	2-OCH ₃ Ph	>100
17y	2-ClPh	>100
17z	pyridin-3-yl	21.0 ± 2.1
17aa	furan-2-yl	94.5 ± 6.8
17ab	thiophen-2-yl	>100
17ac	furan-3-yl	>100
17ad	3-SO ₂ CH ₃ Ph	>100
17ae	3-COCH ₃ Ph	>100
17af	3-COOHPh	>100
17ag	pyridin-4-yl	>100
17ah	quinolin-3-yl	>100
17ai	pyrimidin-5-yl	>100
Compds	X	IC ₅₀ (μM)
19	CH ₂	>100
20	SO ₂	>100

^aIC₅₀ values are the mean of three independent experiments performed in triplicate.

Table 3.

TDP2 inhibition, permeability and cytotoxicity of the four best derivatives

Compds	TDP2 IC ₅₀ (μM) ^a	P _e (10 ⁻⁶ cm/s) ^b	HepG2 CC ₅₀ (μM) ^c	HeLa CC ₅₀ (μM) ^c
7a	22.0 ± 2.5	0.16	--	--
17a	16.6 ± 0.4	0.15	--	--
17e	43.8 ± 3.9	0.9	>100	>100
17z	21.0 ± 2.1	6.3	>100	>100
1	0.040	0.095	--	--

^a IC₅₀ values are the mean of three independent experiments performed in triplicate;^b permeability is considered high when Pe > 1.5;^c compounds were tested at 50 μM and 100 μM (two independent experiments were performed in triplicate).