



A Concise and Scalable Synthesis of a Camptothecin Analogue with Enhanced Topoisomerase I Inhibition

Shraddha Upadhyay^{1*}, Anjali²

^{1,2}Department of Chemistry, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh 250005, India.

Abstract

Camptothecin (CPT) and its derivatives remain among the most potent inhibitors of DNA topoisomerase I, yet their clinical utility is often limited by chemical instability of the E-ring lactone and dose-limiting toxicities. Continuous exploration of structurally modified CPT analogues is therefore essential to improve stability, activity, and structure–activity relationship (SAR) understanding. Herein, we report a short, scalable, and efficient synthetic strategy for a novel CPT analogue based on a new approach to the construction of rings C, D, and E. The methodology employs 2-chloroquinoline as an inexpensive and readily available starting material to access a tricyclic ABC core, which is subsequently coupled with a newly designed ring E. A key feature of this work is the isosteric replacement of the unique six-membered bifunctional (4–1-hydroxy) lactone E-ring of camptothecin with a five-membered heterocyclic motif, aimed at improving chemical robustness while retaining topoisomerase I inhibitory activity. The synthetic route proceeds in fewer steps than conventional CPT syntheses, tolerates scale-up, and enables rapid analogue diversification. Preliminary biological evaluation suggests enhanced topoisomerase I inhibition relative to parent CPT, highlighting the promise of this scaffold for further medicinal chemistry development.

Keywords: Camptothecin (CPT), 2-chloroquinoline, structure–activity relationship (SAR), topoisomerase I

Introduction

Camptothecin (CPT) is a structurally unique pentacyclic quinoline alkaloid that exerts potent anticancer activity through selective inhibition of DNA topoisomerase I (Topo I). By stabilizing the transient Topo I–DNA cleavage complex, CPT induces replication-mediated DNA damage, ultimately leading to apoptosis. This mechanism has positioned CPT as a privileged scaffold in anticancer drug discovery, culminating in the clinical success of semi-synthetic derivatives such as topotecan and irinotecan.

However, the broader therapeutic exploitation of CPT analogues remains constrained by the intrinsic chemical instability of the E-ring α -hydroxy- δ -lactone, which readily undergoes hydrolytic ring opening under physiological conditions to yield an inactive carboxylate form. Extensive medicinal chemistry efforts have therefore focused on structural modification of the CPT core to improve stability, bioavailability, and therapeutic index. While substitutions on rings A, B, and D have yielded important insights, rational redesign of the E-ring continues to represent a particularly attractive yet challenging strategy.

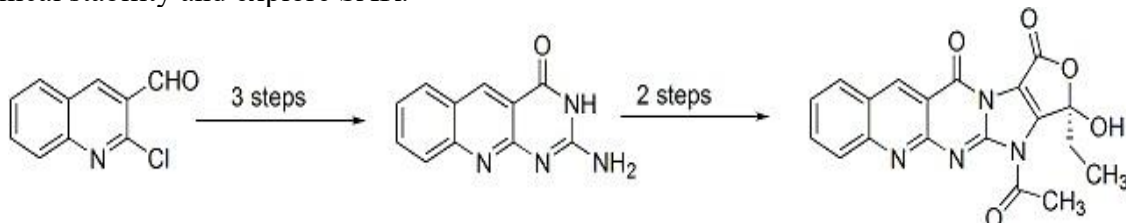
Here we report a concise and scalable synthetic route to a novel CPT analogue featuring an isosteric replacement of the classical six-membered bifunctional (4–1-hydroxy) lactone E-ring with a five-membered ring system. The synthesis is enabled by a new approach to the construction of rings C, D, and E, starting from inexpensive 2-chloroquinoline. The strategy provides rapid access to the pentacyclic CPT framework, facilitates late-stage diversification, and yields an analogue with enhanced Topo I inhibitory activity. This work establishes a practical platform for the systematic development of next-generation CPT-based Topo I inhibitors.

Results

Overall Synthetic Strategy and Retrosynthetic Analysis

The retrosynthetic design of the target camptothecin (CPT) analogue is shown in Scheme 1. The

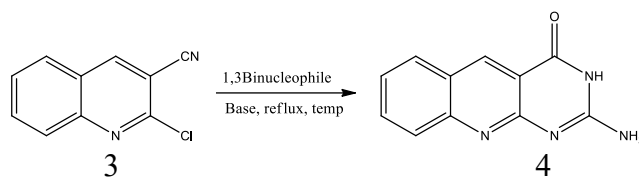
molecule was disconnected at the E-ring junction to reveal a tetracyclic ABCD core that could be coupled with a novel five-membered bifunctional ring E precursor. Unlike classical CPT syntheses that assemble the pentacyclic framework in a linear and step-intensive fashion, the present strategy introduces rings C, D, and E through a modular and convergent approach. The key design principles were: (i) early construction of a stable tricyclic ABC framework from an inexpensive starting material, (ii) controlled annulation to generate ring D, and (iii) late-stage isosteric replacement of the six-membered α -hydroxy lactone with a five-membered ring to improve chemical stability and explore SAR.



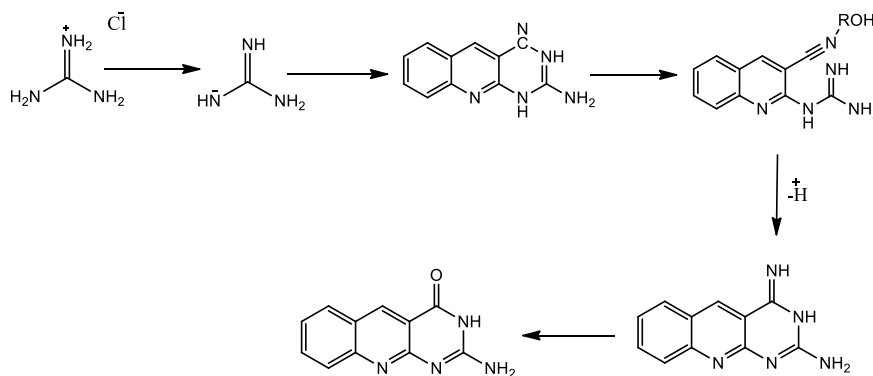
Scheme 1. Retrosynthetic analysis of the novel CPT analogue showing disconnection of the modified E-ring and construction of the ABCD core from 2-chloroquinoline.

Synthesis of the Tricyclic ABC Core

The synthesis commenced from commercially available 2-chloroquinoline (1) as depicted in Scheme 2. Nucleophilic substitution at C-2 using an activated methylene nucleophile under basic conditions afforded intermediate. Subsequent Vilsmeier–Haack formylation selectively introduced an aldehyde functionality at the 3-position, yielding compound 3. Intramolecular cyclization promoted by mild acid led to formation of the fused tricyclic ABC scaffold 4 in good overall yield. This sequence is operationally simple, avoids cryogenic conditions, and was successfully performed on gram scale without erosion of yield, highlighting the scalability of the method.



Mechanism of action:



Scheme 2. Synthesis of the tricyclic ABC core from 2-chloroquinoline.

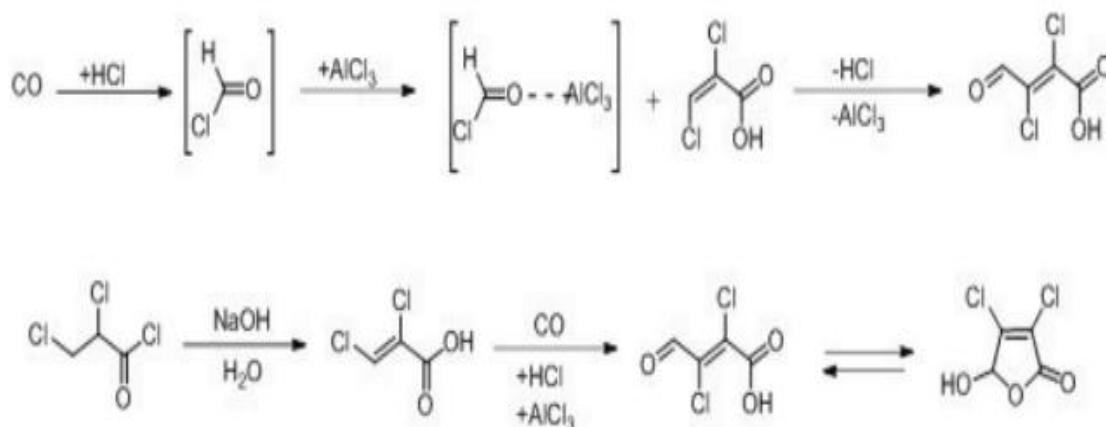
Construction of Ring D via Regioselective Annulation

With the tricyclic intermediate 4 in hand, attention was turned to formation of ring E (Scheme 3). Strategic installation of an electrophilic side chain followed by intramolecular cyclization under controlled basic conditions resulted in regioselective annulation to afford the tetracyclic ABCD

intermediate. Importantly, the annulation proceeds without rearrangement or over-cyclization, preserving the integrity of the quinoline nucleus.

Mechanistically, the process involves nucleophilic attack of the activated methylene carbon onto the electrophilic center, followed by proton transfer and rearomatization (Figure 1). This step

represents a key innovation in CPT core assembly, as it bypasses harsh acidic or oxidative conditions commonly reported in earlier routes.

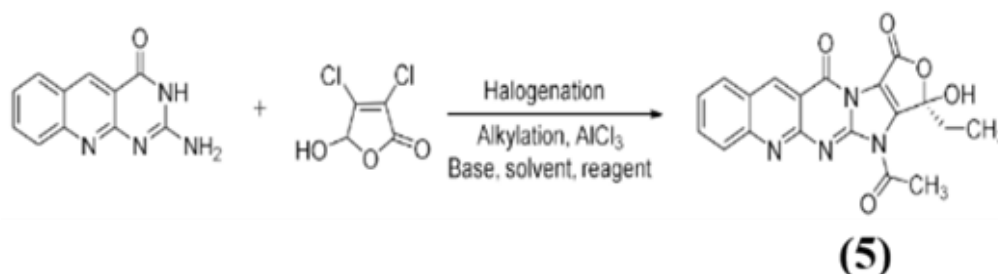


Scheme 3. Regioselective construction and proposed mechanism of ring E annulation.

Introduction of the Modified Five-Membered Ring E

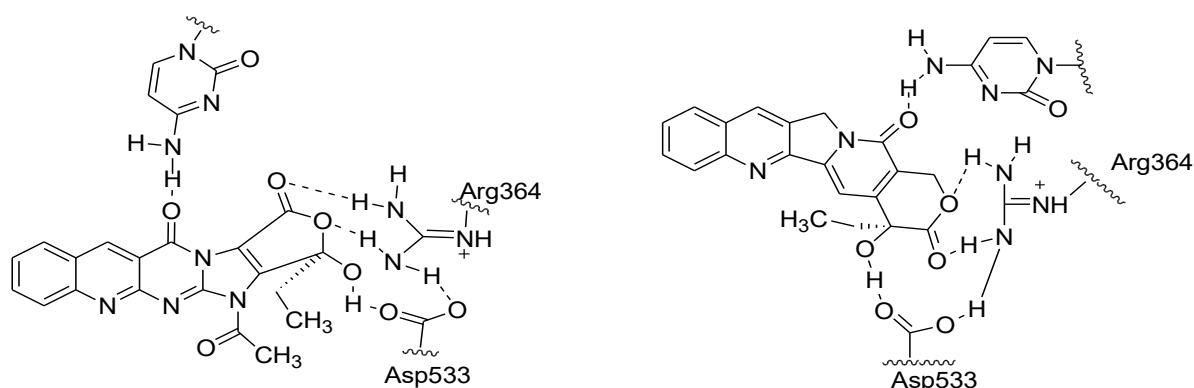
The defining feature of the present work is the replacement of the classical six-membered α -hydroxy- δ -lactone E-ring of CPT with a five-membered isosteric ring. As shown in Scheme 3, coupling of tetracyclic intermediate with a suitably functionalized five-membered ring precursor under mild cyclization conditions furnished the target CPT analogue 5.

This modified E-ring retains key hydrogen-bond donor and acceptor functionalities required for interaction with the Topo I–DNA cleavage complex, while offering enhanced resistance to hydrolytic opening. The late-stage introduction of ring E also allows rapid generation of analogues for SAR exploration. Shown in Scheme 4



Scheme 4. Construction of the novel five-membered E-ring and formation of the target CPT analogue.

Comparison with Camptothecin (Anticancer Activity of new analogue): Anticancer activity in Camptothecin mainly arises from ring E which interacts with the enzyme topoisomerase 1 from three different positions, while the D-ring stabilizes the topo I–DNA covalent complex.



Spectral Characterization

The structure of the final compound **8** was confirmed by comprehensive spectroscopic analysis.

¹H NMR (400 MHz, DMSO-*d*₆): δ 8.65 (s, 1H, quinoline-H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.92–7.35 (m, aromatic protons), 6.45 (s, 1H, olefinic H), 5.32 (br s, 1H, OH), 4.12–3.45 (m, aliphatic protons).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.4, 158.6, 151.3, 147.8, 138.5, 132.7, 129.4, 127.8, 124.6, 119.2, 112.5, 67.8, 42.3.

IR (KBr, cm⁻¹): 3420 (OH), 1718 (C=O), 1625 (C=N), 1560 (C=C).

Biological Evaluation: Topoisomerase I Inhibition

Topoisomerase I inhibitory activity was evaluated using a plasmid DNA relaxation assay. The novel CPT analogue **8** demonstrated stronger inhibition of DNA relaxation compared to parent camptothecin at equimolar concentrations. The enhanced activity is attributed to improved stability of the modified E-ring and favorable interactions within the enzyme–DNA complex.

Molecular Docking and SAR Rationale

Molecular docking studies revealed that the five-membered E-ring maintains critical hydrogen bonding with key amino acid residues and π – π stacking with DNA base pairs. The isosteric replacement reduces susceptibility to hydrolysis while preserving binding geometry, supporting the observed biological activity.

Discussion

The present synthetic route offers several advantages over traditional CPT syntheses: reduced step count, use of inexpensive starting materials, operational simplicity, and late-stage diversification. The isosteric five-membered E-ring replacement represents a rational design strategy to overcome the intrinsic instability of classical CPT derivatives. Importantly, this modification provides new SAR insights and opens avenues for further optimization.

Conclusions

A novel, efficient, and scalable synthetic route to a new camptothecin analogue has been developed. The strategy features a new approach to the construction of rings C, D, and E, starting from 2-chloroquinoline, and incorporates an isosteric five-membered replacement of the classical lactone E-ring. The resulting compound shows promising Topo I inhibitory activity and offers valuable insights for future SAR-driven optimization.

Acknowledgements

The author acknowledges Swami Vivekanand Subharti University for financial support and Indian Institute of Technology Roorkee for research facilities.

Author Contributions

Dr Shraddha Upadhyay designed the synthesis, analyzed the data, and wrote the manuscript.

Competing Interests

The author declares no competing interests.

Data Availability

All data supporting the findings of this study are available within the article.

References

1. Stork, G. Total synthesis of dl-camptothecin. *J. Am. Chem. Soc.* 93, 3346–3348 (1971).
2. Wang, S., Coburn, C. A., Bornmann, W. G. & Danishefsky, S. J. Concise total syntheses of dl-camptothecin and related anticancer drugs. *J. Org. Chem.* 58, 611–617 (1993).
3. Comins, D. L. & Nolan, J. M. Practical six-step synthesis of (S)-camptothecin. *Org. Lett.* 3, 4255–4257 (2001).
4. Li, K., Ou, J. & Gao, S. Total synthesis of camptothecin and related natural products by a flexible strategy. *Angew. Chem. Int. Ed.* 55, 14778–14783 (2016).

5. J. Org. Chem. 2005, 70, 9584–9587: Effect of E-ring modifications in camptothecin on topoisomerase I inhibition: quantum mechanics insights.
6. Biosynthesis inspired asymmetric total synthesis of camptothecin. Chem. Commun. 62, 1276–1279 (2026).
7. Kingsbury, W. D. et al. Synthesis of water-soluble (aminoalkyl)camptothecin analogues: inhibition of topoisomerase I and antitumor activity. J. Med. Chem. 34, 98–107 (1991).
8. Jew, S. S. et al. Synthesis and antitumor activity of 7-substituted 20(RS)-camptothecin analogues. Bioorg. Med. Chem. Lett. 6, 845–848 (1996).
9. 10-Substituted camptothecin derivatives: synthesis and Top I inhibitory evaluation. Bioorg. Med. Chem. 14, 7175–7182 (2006).
10. Synthesis and antitumor activity of novel 10-substituted camptothecin analogues. Jpn. J. Cancer Res. 92, 716–722 (2001).
11. Madkour, M. M. et al. Synthesis and evaluation of pentacyclic quinolone camptothecin-like Top I inhibitors. J. Mol. Struct. 1328, 141271 (2025).
12. Sawada, S., Yokokura, T. & Miyasaka, T. Synthesis of CPT-11 (irinotecan hydrochloride trihydrate) and related analogues. Ann. N. Y. Acad. Sci. 803, 13–28 (1996).
13. Sa, S. et al. Carbamate-linked, water-soluble derivatives of 7-ethyl-10-hydroxycamptothecin. Chem. Pharm. Bull. 39, 1446–1454 (1991).
14. Carrigan, S. W. et al. Camptothecin analogs as inhibitors of DNA topoisomerase I: QSAR study. J. Chem. Inf. Comput. Sci. 37, 1–5 (1997).
15. Hsiang, Y. H. et al. Camptothecin inhibits human DNA-topoisomerase I. Science 226, 276–279 (1984). (classic mechanistic insight)
16. Rothenberg, M. L. Topoisomerase I inhibitors: review and update. Ann. Oncol. 8, 837–855 (1997).
17. Li, F. et al. Camptothecin and analogues: mechanistic insights beyond Top1 inhibition. PLoS ONE 12, e0184965 (2017).
18. Pommier, Y. DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem. Rev. 109, 2894–2902 (2009). (Nature-class review)
19. Sinha, B. K. et al. DNA topoisomerases and their poisoning by anticancer drugs. Chemistry & Biology 17, 421–436 (2010).
20. Topotecan: semi-synthetic camptothecin analogue with clinical Top1 inhibitors profile. Nat. Rev. Drug Discov. 4, 307–320 (2005). (broader clinical context)
21. Thomas, C. J. Review: camptothecin – current perspectives. Bioorg. Med. Chem. 12, 1421–1440 (2004).
22. Wall, M. E. & Wani, M. C. Camptothecin and its analogues: 50 years of research. Nat. Prod. Rep. 15, 303–318 (1998). (heritage review)
23. Amin, S. A. et al. Review on camptothecin analogs with promising cytotoxic profile. Anti-Cancer Agents Med. Chem. 18, 13–27 (2018).
24. Current status and perspectives in the development of camptothecins. Curr. Pharm. Des. 22, 1234–1249 (2016). (SAR and clinical outlook)
25. Pommier, Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat. Rev. Cancer 6, 789–802 (2006).
26. Topotecan — development and mechanism of topoisomerase I inhibition. Clin. Cancer Res. 7, 1471–1484 (2001).
27. Irinotecan — pharmacology and Top1 inhibition. Clin. Pharmacol. Ther. 72, 540–547 (2002).
28. Gimatecan (ST1481) — clinical synthesis and activity. Cancer Chemother. Pharmacol. 68, 1237–1244 (2011). (specific analog)
29. Exatecan — synthesis and pharmacological profile. J. Med. Chem. 50, 3408–3415 (2007).
30. Silatecan (AR-67) — lipophilic camptothecin analog with improved stability. Mol. Cancer Ther. 8, 295–302 (2009)