Published in final edited form as:

Medchemcomm. 2016 December 1; 7(12): 2418–2427. doi:10.1039/C6MD00459H.

Synthesis and Biological Evaluation of Benzocyclooctene-based and Indene-based Anticancer Agents that Function as Inhibitors of Tubulin Polymerization[†]

Christine A. Herdman^a, Tracy E. Strecker^a, Rajendra P. Tanpure^a, Zhi Chen^a, Alex Winters^b, Jeni Gerberich^b, Li Liu^b, Ernest Hamel^c, Ralph P. Mason^b, David J. Chaplin^{a,d}, Mary Lynn Trawick^a, and Kevin G. Pinney^a

^aDepartment of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, Texas 76798-7348, United States

^bPrognostic Imaging Research Laboratory, Department of Radiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9058, United States

^cScreening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD 21702, United States

^dMateon Therapeutics, Inc., 701 Gateway Boulevard, Suite 210, South San Francisco, California 94080, United States

Abstract

The natural products colchicine and combretastatin A-4 (**CA4**) have been inspirational for the design and synthesis of structurally related analogues and spin-off compounds as inhibitors of tubulin polymerization. The discovery that a water-soluble phosphate prodrug salt of **CA4** (referred to as **CA4P**) is capable of imparting profound and selective damage to tumor-associated blood vessels paved the way for the development of a new therapeutic approach for cancer treatment utilizing small-molecule inhibitors of tubulin polymerization that also act as vascular disrupting agents (VDAs). Combination of salient structural features associated with colchicine and **CA4** led to the design and synthesis of a variety of fused aryl-cycloalkyl and aryl-heterocyclic compounds that function as inhibitors of tubulin polymerization. Prominent among these compounds is a benzosuberene analogue (referred to as **KGP18**), which demonstrates sub-nM

[†]A significant portion of the work presented in this manuscript was funded through Mateon Therapeutics Inc. (formerly OXiGENE Inc.), and this relationship is properly indicated in the acknowledgement section. One Mateon employee and shareholder Dr. David Chaplin, is included as a co-author on this manuscript for his valuable contributions to the overall project. In addition, one of the authors (KGP) is a paid consultant and shareholder with Mateon. We are pleased with this productive and useful long-term scientific collaboration and funding relationship with Mateon, and it is important to note that there is absolutely no actual conflict of interest associated with the science presented in this manuscript.

5. Supplementary Data

Supplementary data including experimental procedures (synthesis, SRB assay, colchicine binding assay, inhibition of tubulin polymerization, *in vivo* BLI, and histology), ¹H NMR, ¹³C NMR, ³¹P NMR, HPLC, HRMS for target compounds and intermediates (¹H NMR, ¹³C NMR, only) and X-ray crystallography for compounds **20** and **23** associated with this article can be found in the online Supplementary data file. All animal procedures were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee.

cytotoxicity against human cancer cell lines and functions (when administered as a water-soluble prodrug salt) as a VDA in mouse models. Structure activity relationship considerations led to the evaluation of benzocyclooctyl [6,8 fused] and indene [6,5 fused] ring systems. Four benzocyclooctene and four indene analogues were prepared and evaluated biologically. Three of the benzocyclooctene analogues were active as inhibitors of tubulin polymerization ($IC_{50} < 5 \mu M$), and benzocyclooctene phenol **23** was comparable to **KGP18** in terms of potency. The analogous indene-based compound **31** also functioned as an inhibitor of tubulin polymerization ($IC_{50} = 11 \mu M$) with reduced potency. The most potent inhibitor of tubulin polymerization from this group was benzocyclooctene analogue **23**, and it was converted to its water-soluble prodrug salt **24** to assess its potential as a VDA. Preliminary *in vivo* studies, which utilized the MCF7-luc-GFP-mCherry breast tumor in a SCID mouse model, demonstrated that treatment with **24** (120 mg/kg) resulted in significant vascular shutdown, as evidenced by bioluminescence imaging at 4 h post administration, and that the effect continued at both 24 and 48 h. Contemporaneous studies with **CA4P**, a clinically relevant VDA, were carried out as a positive control.

Graphical Abstract

Synthesis of benzocyclooctene and indene analogues inspired by colchicine and combretastatin A-4.

Keywords

benzocyclooctene analogues; indene analogues; vascular disrupting agents (VDAs); inhibitors of tubulin polymerization; small-molecule synthesis; bioluminescence imaging (BLI)

1. Introduction

The colchicine site located on the β -subunit of the α,β -tubulin heterodimer continues to serve as a rich and productive target for a wide-range of structurally diverse small-molecule anticancer agents. Through a direct binding interaction at the colchicine site, these compounds inhibit the polymerization of the tubulin heterodimer into microtubules. Disruption of the inherent dynamic relationship between microtubules and tubulin heterodimers leads to two mechanistically distinct, yet complementary, cancer treatment strategies. One approach centers on the well known cytotoxic effect that results when disruption of the tubulin-microtubule system, caused by treatment with small-molecule inhibitors of tubulin polymerization or depolymerization, impacts the ability of cancer cells to divide. A wide-range of small-molecule therapeutic agents has been discovered and developed that function as antiproliferative agents through this mechanism (Fig. 1).

This approach, while productive as an anticancer strategy, has limitations and challenges since both neoplastic and healthy cells are impacted. Targeting strategies such as the use of

antibody-drug conjugates (ADCs) bearing active payloads have been successful with two FDA approved ADCs currently available.^{17–19} In another example, the targeting of tumor hypoxia with bioreductively activatable prodrug conjugates (BAPCs) has been explored^{20,21} with recent clinical trials involving PR-104^{22,23} and TH-302^{24–26} serving as representative examples (Fig. 2).

PR-104 is hydrolyzed to its alcohol parent compound by available phosphatases and, under hypoxic conditions, is activated by NADPH-cytochrome P450 reductases to the reactive nitrogen mustards that crosslink to DNA and ultimately cause tumor cytotoxicity.²² TH-302 utilizes a similar hypoxia reduction strategy in order to release its 2-nitroimidazole trigger leaving the bromo-isophosphoramidate mustard to cross link with DNA.²⁶ The targeting of tumor hypoxia has also been examined with BAPCs that incorporate the tubulin-active natural product combretastatin A-4 (**CA4**).²⁷ These strategies, along with others, continue to represent areas of ongoing research effort.

A second mechanistic approach to cancer therapy involving inhibition of the tubulin-microtubule protein system centers on selective disruption of existing vasculature feeding tumors, leading to limitation of oxygen and nutrient delivery to the cells and impedence of the ability of these cells to clear cellular waste products. This ultimately leads to tumor necrosis. This approach to targeting existing tumor-feeding vasculature utilizes anticancer agents referred to as vascular disrupting agents (VDAs) and is unique and mechanistically distinct from the fairly well-established and more commonly described therapeutic approach employing angiogenesis inhibiting agents (AIAs) that impede the formation of new vessels.^{28–30} Rapid neovascularization occurs when tumors grow larger than about 1 mm³, as they can no longer acquire sufficient nutrients from the surrounding vasculature.³¹ Such tumors require their own vascular network to supply oxygen and nutrients and remove waste products.^{28,29,32} Because of their rapid growth and development, these vessels feature irregular branching and diameter, poor wall structure, abnormal bulges, and blind ends.^{31,33–36} This immature vasculature provides an attractive target for cancer therapy. Therapeutic agents that target tumor vasculature are referred to as vascular targeting agents, which can be divided into AIAs and VDAs.^{28,33,30} AIAs inhibit the growth of new vasculature to the tumor, while VDAs damage already sprouted vessels.^{35–40} VDAs are subdivided into biological agents and small-molecule therapeutics (Fig. 3),⁴⁰ and treatment with either of these entities leads to tumor necrosis.

Colchicine, a natural product originally isolated from the autumn crocus, *Colchicum autumnale*, and the namesake for the colchicine site on tubulin,^{52,53} is a potent inhibitor of tubulin polymerization, but colchicine has a narrow therapeutic window limiting its development as an anticancer agent.^{35,54,55} Another natural product binding to the colchicine site on tubulin is **CA4** (Fig. 1).⁴ Originally isolated from the African bushwillow tree, *Combretum cafferum*, and synthesized by Pettit and co-workers, **CA4** binds tightly to the colchicine site on tubulin and functions as a VDA.^{55–58} Combretastatin A-4P [(**CA4P**), Fig. 3] was synthesized as a water-soluble prodrug salt for therapeutic use,^{41,55,56,58} and both **CA4** and **CA4P** are now being evaluated in clinical trials.^{42,45,59–62}

Drawing upon the key structural motifs of colchicine and **CA4**, the Pinney Research Group (Baylor University) has a long-standing interest in the discovery and development of new small-molecule anticancer agents that function as highly cytotoxic agents and/or as potent VDAs. Representative examples within this library include combretastatin,^{63–66} benzo[*b*]thiophene,⁶⁷ dihydronaphthalene,⁹ benzosuberene,^{9–12} and indole-based analogues.^{14,68} The Pinney Research Group had previously shown that expansion of the fused alkyl ring of the dihydronaphthalene anticancer agent known as **OXi6196** (Fig. 1) by one carbon to the fused seven-membered ring benzosuberene anticancer agent known as **KGP18** (Fig. 1) was an effective design paradigm, since **KGP18** was highly cytotoxic against human cancer cell lines and strongly inhibits tubulin polymerization.

To further elucidate the structure activity relationship (SAR) considerations associated with functionalized fused aryl-carbocyclic ring systems that mimic colchicine and **CA4** and their interaction with the tubulin-microtubule system, benzocyclooctene (fused 6,8 ring system) analogues and corresponding indene (fused 6,5 ring system) analogues were prepared by chemical synthesis and subjected to preliminary biological evaluation.

2. Results and Discussion

2.1 Synthesis

Four benzocyclooctene analogues and four indene analogues were synthesized to further investigate SAR considerations associated with fused aliphatic ring structures and corresponding cytotoxicity and inhibition of tubulin polymerization. These analogues were prepared utilizing a synthetic strategy reminiscent of that employed for a variety of benzosuberene analogues developed in the Pinney Research Group.^{10,11} The synthesis of each benzocyclooctene analogue was initiated with a Wittig olefination followed by catalytic hydrogenation to afford carboxylic acids **3** and **4** (Scheme 1). A Friedel-Crafts intramolecular annulation was accomplished using Eaton's reagent (7.7 weight percent P₂O₅ in CH₃SO₃H),⁶⁹ and the reaction was diluted and cooled in order to facilitate the construction of benzocyclooctanones **5** and **6** (Scheme 1). Without dilution and cooling, the desired product was not formed and only starting material remained.

A similar catalytic reduction was utilized with starting material *trans*-2,3-dimethoxycinnamic acid to afford carboxylic acids **9** and **10**, which were cyclized with Eaton's reagent to their corresponding indanone analogues **11** and **12** (Scheme 2).

Benzocyclooctanone **5** and indanone **11** bearing the *ortho* dimethoxy motif were each subjected to a selective demethylation^{10,11,70} to afford phenolic analogues **13** and **15**, which were subsequently protected with TBS to yield **14** and **16** (Scheme 3).

Trimethoxyphenyllithium (prepared from the corresponding aryl bromide) underwent a 1,2-addition reaction to benzocyclooctanone analogues **5**, **6** and **14** to afford the corresponding tertiary alcohols **17**, **18**, and **19**. Subsequent dehydration afforded benzocyclooctene analogues **20** and **21** and TBS-protected analogue **22**, which underwent deprotection to afford phenolic benzocyclooctene **23** (Scheme 4).

Phenolic analogue **23** was converted to its corresponding phosphate prodrug salt **24**, in order to increase water solubility for *in vivo* studies (Scheme 5).

Analogous aryl addition reactions were carried out on indanone intermediates **11**, **12**, and **16** to generate the corresponding tertiary alcohols **25**, **26**, and **27** (Scheme 6). Subsequent dehydration afforded indene analogues **28**, **29**, and **30**. Removal of the TBS group afforded phenolic indene **31**.

The indene phenol **31** was also converted to its corresponding phosphate salt **32** (Scheme 7). While TEA proved to be an acceptable base (due to ease of removal) for use in the synthesis of the eight membered ring phosphate salt **24**, in the synthesis of the indene phosphate salt **32** TEA proved to be hard to remove during purification. Altering the base to pyridine solved this problem and allowed the water-soluble phosphate salt **32** to be synthesized in high purity.

Due to their increased size, eight membered rings are the first in the homologous series (3, 4, 5, 6, 7 – membered rings) that can accommodate both *Z* and *E* double bond configurations.^{71,72} However, in our hands only the *Z* configurations were synthesized. X-ray crystal structures were obtained for benzocyclooctene analogues **20** and **23** to confirm their *Z* double bond configuration (see Supplementary Data).

2.2 Biological Evaluation

The four target benzocyclooctene and four indene analogues (Fig. 4) were evaluated for their cytotoxicity against human cancer cell lines and for their abilities to inhibit tubulin polymerization and colchicine binding to tubulin (Table 1).

Among the eight compounds synthesized for this study and analyzed biologically, only the target benzocyclooctene analogues (**20**, **21**, and **23**) had activities as inhibitors of tubulin polymerization similar to those of **CA4** and **KGP18**. Benzocyclooctene phenol **23** demonstrated the lowest IC₅₀ value (1.2 μM) among the compounds evaluated in this study. A binding study utilizing radiolabeled colchicine demonstrated that at a concentration of 5 μM, phenol **23** inhibited colchicine binding by 78%, 20% less than the activity obtained with 5 μM **CA4** (used as a positive control). Considering the four indene analogues, only phenolic indene **31** demonstrated modest inhibition of tubulin polymerization, with an IC₅₀ value of 11 μM.

Benzocyclooctene phenol **23** and its corresponding phosphate prodrug salt **24** were the most cytotoxic analogues in these series against the three human cancer cell lines examined (for example, GI₅₀ = 0.105 and 0.0410 μM against DU-145 (prostate) for analogues **23** and **24**, respectively). While structurally similar to **KGP18**, differing by the addition of one carbon to the aliphatic ring, the phenolic benzocyclooctene analogue is dramatically less cytotoxic than its benzosuberene counterpart. Compounds **23** and **24** had GI₅₀ values in the submicromolar range, while the values obtained with **KGP18** were all subnanomolar.

2.3 Dynamic bioluminescence imaging

Bioluminescence imaging (BLI) is a highly valuable modality with diverse applications in a wide range of biological models and systems.^{74–76} BLI is a particularly useful tool for assessing and quantifying *in vivo* blood flow reduction following treatment with a VDA.^{54,76–79} In the current study MCF7 human breast cancer cells, which had been previously transfected stably to express the enzyme luciferase, as well as two fluorescent proteins, as designated by MCF7-luc-GFP-mCherry, were implanted in SCID mice, as described previously.⁷⁸ Since the luciferase substrate luciferin can be delivered to the tumor via the blood stream, damage to the vessels by a VDA can be quantified by measurement of reduced bioluminescence upon injection of luciferin.⁵⁴ In the study shown in Fig. 5, three mice bearing the MCF7-luc-GFP-mCherry breast tumor were injected ip with analogue **24** (120 mg/kg), and BLI assessment was performed at baseline (0 h, pre-administration) and at 4, 24, and 48 h after administration of **24**. In a contemporaneous study, one mouse was treated with **CA4P** (120 mg/kg) as a positive control, and an additional mouse was treated with saline alone. A limited dose escalation study with analogue **24** in non-tumor bearing SCID mice (60–150 mg/kg, unpublished data) suggested that a dose of 120 mg/kg was tolerated and likely below the maximum tolerated dose (MTD), which has not yet been determined. The optimal **CA4P** dose in SCID mice for evaluation of VDA efficacy has been previously established at 120 mg/kg.⁷⁶ Single dose treatment with both analogue **24** and **CA4P** resulted in a significant reduction in bioluminescence (approximately 80% decrease as compared to baseline or saline control; $p < 0.05$) at 4 h after compound injection. While bioluminescence intensity in the mouse treated with compound **24** recovered somewhat at 24 and 48 h, it remained significantly below baseline values ($p < 0.005$). In this study, the BLI data for both analogue **24** and **CA4P** at 4 h were statistically different from the saline control, but not from each other or from the data obtained at other time points. Following the 48 h time point BLI mice were sacrificed and tumors excised and prepared for histology. Routine staining using H&E indicated much more extensive necrosis throughout the tumors following administration of **24** or **CA4P** each at 120 mg/kg, as compared with saline control (Fig. 6). Thus, analogue **24** is a good candidate for further assessment and may be a therapeutically useful VDA.

3. Conclusions

These studies, which focused on indene and benzocyclooctene ring systems, expanded the known SAR associated with the effect that fused aliphatic ring size plays in regard to cytotoxicity and inhibition of tubulin polymerization. From the group of eight analogues prepared by chemical synthesis, three benzocyclooctene analogues were strong inhibitors of tubulin polymerization and one indene analogue was a modest inhibitor. The most promising compound (benzocyclooctene analogue **23**) was prepared and evaluated as its corresponding water-soluble phosphate salt **24**. It demonstrated significant *in vivo* reduction of blood flow (as evidenced by BLI) in an MCF7-luc-GFP-mCherry tumor model in SCID mice, and the results indicated that **24** should undergo further evaluation as a VDA for potential cancer treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to the National Cancer Institute of the National Institutes of Health (Grant No. 5R01CA140674 to K.G.P, M.L.T, and R.P.M), the Cancer Prevention and Research Institute of Texas (CPRIT, Grant No. RP140399 to K.G.P, M.L.T., and R.P.M.), and Mateon Therapeutics, Inc. (grant to K.G.P. and M.L.T.) for their financial support of this project. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health. The authors would also thank Dr. Craig Moehnke and Dr. Michelle Nemec (Director) for the use of the shared Molecular Biosciences Center at Baylor University, Dr. Alejandro Ramirez (Mass Spectrometry Core Facility, Baylor University) and Dr. Kevin Klausmeyer and Dr. Marissa Penney (X-ray analysis). The authors are grateful to Mr. Jack Littlejohn and Mr. Jake Lofman (Baylor University) for their contributions to the synthesis of certain analogues. John Shelton (JAR Molecular Pathology core, UTSW) prepared the H&E stained sections. Imaging was facilitated with the assistance of resources of the Harold C. Simmons Cancer Center supported through a National Institutes of Health National Cancer Institute Cancer Center Support Grant [Grant 1P30 CA142543], specifically, the Southwestern Small Animal Imaging Resource, and Live Cell Imaging Resource. The IVIS Spectrum was purchased with support of 1S10RR024757.

References

1. Ludford RJ. J Natl Cancer Inst. 1945; 6:89–101.
2. Schiff PB, Horwitz SB. Proc Natl Acad Sci. 1980; 77:1561–1565. [PubMed: 6103535]
3. Schiff PB, Horwitz SB. Biochemistry (Mosc). 1981; 20:3247–3252.
4. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendal D. Experientia. 1989; 45:209–211. [PubMed: 2920809]
5. Gorman M, Neuss N, Svoboda GH, Barnes AJ, Cone NJ. J Am Pharm Assoc. 1959; 48:256–257.
6. Owellen RJ, Owens AH, Donigian DW. Biochem Biophys Res Commun. 1972; 47:685–691. [PubMed: 5026289]
7. Pinney, KG., Sriram, M., George, C., Tanpure, RP. WO2012068284 (A2). 2012.
8. Pinney, KG., Mocharla, VP., Chen, Z., Garner, CM., Ghatak, A., Hadimani, M., Kessler, J., Dorsey, JM. WO2001068654 (A2). 2001.
9. Sriram M, Hall JJ, Grohmann NC, Strecker TE, Wootton T, Franken A, Trawick ML, Pinney KG. Bioorg Med Chem. 2008; 16:8161–8171. [PubMed: 18722127]
10. Tanpure RP, George CS, Strecker TE, Devkota L, Tidmore JK, Lin C-M, Herdman CA, MacDonough MT, Sriram M, Chaplin DJ, Trawick ML, Pinney KG. Bioorg Med Chem. 2013; 21:8019–8032. [PubMed: 24183586]
11. Herdman CA, Devkota L, Lin C-M, Niu H, Strecker TE, Lopez R, Liu L, George CS, Tanpure RP, Hamel E, Chaplin DJ, Mason RP, Trawick ML, Pinney KG. Bioorg Med Chem. 2015; 23:7497–7520. [PubMed: 26775540]
12. Tanpure RP, George CS, Sriram M, Strecker TE, Tidmore JK, Hamel E, Charlton-Sevcik AK, Chaplin DJ, Trawick ML, Pinney KG. Med Chem Comm. 2012; 3:720–724.
13. Devkota L, Lin C-M, Strecker TE, Wang Y, Tidmore JK, Chen Z, Guddneppanavar R, Jelinek CJ, Lopez R, Liu L, Hamel E, Mason RP, Chaplin DJ, Trawick ML, Pinney KG. Bioorg Med Chem. 2016; 24:938–956. [PubMed: 26852340]
14. Hadimani MB, MacDonough MT, Ghatak A, Strecker TE, Lopez R, Sriram M, Nguyen BL, Hall JJ, Kessler RJ, Shirali AR, Liu L, Garner CM, Pettit GR, Hamel E, Chaplin DJ, Mason RP, Trawick ML, Pinney KG. J Nat Prod. 2013; 76:1668–1678. [PubMed: 24016002]
15. Flynn BL, Gill GS, Grobelny DW, Chaplin JH, Paul D, Leske AF, Lavranos TC, Chalmers DK, Charman SA, Kostewicz E, Shackelford DM, Morizzi J, Hamel E, Jung MK, Kremmidiotis G. J Med Chem. 2011; 54:6014–6027. [PubMed: 21774499]
16. Kremmidiotis G, Leske AF, Lavranos TC, Beaumont D, Gasic J, Hall A, O'Callaghan M, Matthews CA, Flynn B. Mol Cancer Ther. 2010; 9:1562–1573. [PubMed: 20515948]

17. Lambert JM. Br J Clin Pharmacol. 2013; 76:248–262. [PubMed: 23173552]
18. Prota AE, Bargsten K, Diaz JF, Marsh M, Cuevas C, Liniger M, Neuhaus C, Andreu JM, Altmann K-H, Steinmetz MO. Proc Natl Acad Sci. 2014; 111:13817–13821. [PubMed: 25114240]
19. Ma D, Hopf CE, Malewicz AD, Donovan GP, Senter PD, Goeckeler WF, Maddon PJ, Olson WC. Clin Cancer Res. 2006; 12:2591–2596. [PubMed: 16638870]
20. Hay, MP., Hicks, KO., Wang, J. Tumor Microenvironment and Cellular Stress. Koumenis, C.Hammond, E., Giaccia, A., editors. Springer; New York: 2014. p. 111-145.
21. Wilson WR, Hay MP. Nat Rev Cancer. 2011; 11:393–410. [PubMed: 21606941]
22. McKeage MJ, Jameson MB, Ramanathan RK, Rajendran J, Gu Y, Wilson WR, Melink TJ, Tchekmedyan NS. BMC Cancer. 2012; 12:1–10. [PubMed: 22212211]
23. Abbattista MR, Jamieson SMF, Gu Y, Nickel JE, Pullen SM, Patterson AV, Wilson WR, Guise CP. Cancer Biol Ther. 2015; 16:610–622. [PubMed: 25869917]
24. Chawla SP, Cranmer LD, Tine BAV, Reed DR, Okuno SH, Butrynski JE, Adkins DR, Hendifar AE, Kroll S, Ganjoo KN. J Clin Oncol. 2014;1–11.
25. Liu Q, Sun JD, Wang J, Ahluwalia D, Baker AF, Cranmer LD, Ferraro D, Wang Y, Duan J-X, Ammons WS, Curd JG, Matteucci MD, Hart CP. Cancer Chemother Pharmacol. 2012; 69:1487–1498. [PubMed: 22382881]
26. Meng F, Evans JW, Bhupathi D, Banica M, Lan L, Lorente G, Duan J-X, Cai X, Mowday AM, Guise CP, Maroz A, Anderson RF, Patterson AV, Stachelek GC, Glazer PM, Matteucci MD, Hart CP. Mol Cancer Ther. 2012; 11:740–751. [PubMed: 22147748]
27. Thomson P, Naylor MA, Everett SA, Stratford MRL, Lewis G, Hill S, Patel KB, Wardman P, Davis PD. Mol Cancer Ther. 2006; 5:2886–2894. [PubMed: 17121936]
28. Siemann DW, Bibby MC, Dark GG, Dicker AP, Eskens FA, Horsman MR, Marmé D, LoRusso PM. Clin Cancer Res. 2005; 11:416–420. [PubMed: 15701823]
29. Horsman MR, Bohn AB, Busk M. Exp Oncol. 2010; 32:143–148. [PubMed: 21403608]
30. Thorpe PE. Clin Cancer Res. 2004; 10:415–427. [PubMed: 14760060]
31. Denekamp J. Cancer Metastasis Rev. 1990; 9:267–282. [PubMed: 2292139]
32. Siemann DW, Chaplin DJ, Horsman MR. Cancer. 2004; 100:2491–2499. [PubMed: 15197790]
33. Fens MH, Storm G, Schiffelers RM. Expert Opin Investig Drugs. 2010; 19:1321–1338.
34. Denekamp J. Br J Cancer. 1982; 45:136–139. [PubMed: 7059456]
35. Tozer GM, Kanthou C, Baguley BC. Nat Rev Cancer. 2005; 5:423–435. [PubMed: 15928673]
36. Siemann DW. Cancer Treat Rev. 2011; 37:63–74. [PubMed: 20570444]
37. Vasudev NS, Reynolds AR. Angiogenesis. 2014; 17:471–494. [PubMed: 24482243]
38. Jain RK. Cancer Cell. 2014; 26:605–622. [PubMed: 25517747]
39. Folkman J. Nat Rev Drug Discov. 2007; 6:273–286. [PubMed: 17396134]
40. Pilat MJ, LoRusso PM. J Cell Biochem. 2006; 99:1021–1039. [PubMed: 16927308]
41. Pettit GR, Rhodes MR. Anticancer Drug Des. 1998; 13:183–191. [PubMed: 9595032]
42. Kretschmann VK, Fürst R. Phytochem Rev. 2014; 13:191–206.
43. Ji Y-T, Liu Y-N, Liu Z-P. Curr Med Chem. 2015; 22:1348–1360. [PubMed: 25620094]
44. Nathan P, Zweifel M, Padhani AR, Koh D-M, Ng M, Collins DJ, Harris A, Carden C, Smythe J, Fisher N, Taylor NJ, Stirling JJ, Lu S-P, Leach MO, Rustin GJS, Judson I. Clin Cancer Res Off J Am Assoc Cancer Res. 2012; 18:3428–3439.
45. Baguley BC, McKeage MJ. Clin Investig. 2012; 2:985–993.
46. Pettit GR, Lippert JW. Anticancer Drug Des. 2000; 15:203–216. [PubMed: 11049088]
47. Rischin D, Bibby DC, Chong G, Kremmidiotis G, Leske AF, Matthews CA, Wong S, Rosen M, Desai J. Clin Cancer Res. 2011; 17:5152–5160. [PubMed: 21690571]
48. Hori K, Saito S, Nihei Y, Suzuki M, Sato Y. Jpn J Cancer Res. 1999; 90:1026–1038. [PubMed: 10551334]
49. Hori K, Saito S. Br J Cancer. 2003; 89:1334–1344. [PubMed: 14520469]

50. Blay J-Y, Pápai Z, Tolcher AW, Italiano A, Cupissol D, López-Pousa A, Chawla SP, Bompas E, Babovic N, Penel N, Isambert N, Staddon AP, Saâda-Bouid E, Santoro A, Franke FA, Cohen P, Le-Guennec S, Demetri GD. *Lancet Oncol.* 2015; 16:531–540. [PubMed: 25864104]
51. Davis PD, Dougherty GJ, Blakey DC, Galbraith SM, Tozer GM, Holder AL, Naylor MA, Nolan J, Stratford MRL, Chaplin DJ, Hill SA. *Cancer Res.* 2002; 62:7247–7253. [PubMed: 12499266]
52. Lee RM, Gewirtz DA. *Drug Dev Res.* 2008; 69:352–358.
53. Ludford J. *J Natl Cancer Inst.* 1945; 6:89–101.
54. Mason RP, Zhao D, Liu L, Trawick ML, Pinney KG. *Integr Biol.* 2011; 3:375–387.
55. Dark GG, Hill SA, Prise VE, Tozer GM, Pettit GR, Chaplin DJ. *Cancer Res.* 1997; 57:1829–1834. [PubMed: 9157969]
56. Kirwan IG, Loadman PM, Swaine DJ, Anthoney DA, Pettit GR, Lippert JW, Shnyder SD, Cooper PA, Bibby MC. *Clin Cancer Res.* 2004; 10:1446–1453. [PubMed: 14977848]
57. Grosios K, Holwell SE, McGown AT, Pettit GR, Bibby MC. *Br J Cancer.* 1999; 81:1318–1327. [PubMed: 10604728]
58. Iyer S, Chaplin DJ, Rosenthal DS, Boulares AH, Li L-Y, Smulson ME. *Cancer Res.* 1998; 58:4510–4514. [PubMed: 9788591]
59. Cooney MM, Ortiz J, Bukowski RM, Remick SC. *Curr Oncol Rep.* 2005; 7:90–95. [PubMed: 15717941]
60. Dowlati A, Robertson K, Cooney M, Petros WP, Stratford M, Jesberger J, Rafie N, Overmoyer B, Makkar V, Stambler B, Taylor A, Waas J, Lewin JS, McCrae KR, Remick SC. *Cancer Res.* 2002; 62:3408–3416. [PubMed: 12067983]
61. Rustin GJS, Galbraith SM, Anderson H, Stratford M, Folkes LK, Sena L, Gumbrell L, Price PM. *J Clin Oncol.* 2003; 21:2815–2822. [PubMed: 12807934]
62. Nathan P, Zweifel M, Padhani AR, Koh D-M, Ng M, Collins DJ, Harris A, Carden C, Smythe J, Fisher N, Taylor NJ, Stirling JJ, Lu S-P, Leach MO, Rustin GJS, Judson I. *Clin Cancer Res.* 2012; 18:3428–3439. [PubMed: 22645052]
63. Pinney KG, Mejia MP, Villalobos VM, Rosenquist BE, Pettit GR, Verdier-Pinard P, Hamel E. *Bioorg Med Chem.* 2000; 8:2417–2425. [PubMed: 11058036]
64. Siles R, Ackley JF, Hadimani MB, Hall JJ, Mugabe BE, Guddneppanavar R, Monk KA, Chapuis J-C, Pettit GR, Chaplin DJ, Edvardsen K, Trawick ML, Garner CM, Pinney KG. *J Nat Prod.* 2008; 71:313–320. [PubMed: 18303849]
65. Tanpure RP, Harkrider AR, Strecker TE, Hamel E, Trawick ML, Pinney KG. *Bioorg Med Chem.* 2009; 17:6993–7001. [PubMed: 19733085]
66. Tanpure RP, Nguyen BL, Strecker TE, Aguirre S, Sharma S, Chaplin DJ, Siim BG, Hamel E, Lippert JW, Pettit GR, Trawick ML, Pinney KG. *J Nat Prod.* 2011; 74:1568–1574. [PubMed: 21718055]
67. Pinney KG, Bounds AD, Dingeman KM, Mocharla VP, Pettit GR, Bai R, Hamel E. *Bioorg Med Chem Lett.* 1999; 9:1081–1086. [PubMed: 10328289]
68. MacDonough MT, Strecker TE, Hamel E, Hall JJ, Chaplin DJ, Trawick ML, Pinney KG. *Bioorg Med Chem.* 2013; 21:6831–6843. [PubMed: 23993969]
69. Eaton PE, Carlson GR, Lee JT. *J Org Chem.* 1973; 38:4071–4073.
70. Kemperman GJ, Roeters TA, Hilberink PW. *Eur J Org Chem.* 2003; 2003:1681–1686.
71. Neuenschwander U, Hermans I. *J Org Chem.* 2011; 76:10236–10240. [PubMed: 22077196]
72. Cossy, J., Arseniyadis, S., Meyer, C. *Metathesis in Natural Product Synthesis: Strategies, Substrates and Catalysts.* John Wiley & Sons; 2011.
73. Pettit GR, Grealish MP, Herald DL, Boyd MR, Hamel E, Pettit RK. *J Med Chem.* 2000; 43:2731–2737. [PubMed: 10893310]
74. Paley MA, Prescher JA. *Med Chem Comm.* 2014; 5:255–267.
75. Ma X, Hui H, Shang W, Jia X, Yang X, Tian J. *Curr Drug Targets.* 2015; 16:542–548. [PubMed: 25557258]
76. Zhao D, Richer E, Antich PP, Mason RP. *FASEB J.* 2008; 22:2445–2451. [PubMed: 18263704]

77. Alhasan MK, Liu L, Lewis MA, Magnusson J, Mason RP. PLoS ONE. 2012; 7:e46106. [PubMed: 23029403]
78. Liu L, Beck H, Wang X, Hsieh H-P, Mason RP, Liu X. PloS One. 2012; 7:e43314. [PubMed: 22937031]
79. Zhou H, Hallac RR, Lopez R, Denney R, MacDonough MT, Li L, Liu L, Graves EE, Trawick ML, Pinney KG, Mason RP. Am J Nucl Med Mol Imaging. 2015; 5:143–153. [PubMed: 25973335]

Figure 1.

Representative small-molecule anti-proliferative agents including colchicine,¹ paclitaxel,^{2,3} CA4,⁴ vinblastine,^{5,6} OXi6196,^{7,8} KGP18,^{9–11} KGP156,^{10,12,13} OXi8006,¹⁴ and BNC105.^{15,16}

PR-104 **TH-302**

Figure 2.
Structures of PR-104 and TH-302.

Figure 3. Several clinically relevant VDAs including **CA4P**,^{41–45} **CA1P**,^{43,46} **BNC105**,^{15,16,43,45,47} **AVE8062**,^{43,48–50} and **ZD6126**.^{42,43,51}

Figure 4.
Compilation of synthesized benzocyclooctene analogues **20**, **21**, **23**, and **24** and indene analogues **28**, **29**, **31**, and **32**.

Figure 5.

Dynamic bioluminescence with respect to vascular disruption. A) Graphs show evolution of light emission from individual MCF7-luc-GFP-mCherry breast tumors following administration of luciferin substrate subcutaneously in the fore-back region of each mouse at time 0. Respective curves are baseline (blue), 4 h (red), 24 h (green) and 48 h (purple) post administration of VDA. Left hand panel shows a representative mouse with 120 mg/kg analogue **24** and right hand panel shows **CA4P** (120 mg/kg). B) Photographs show selected images at the 10 min. time point. Left hand group for analogue **24** and right hand group for **CA4P** for the same mice used to generate the curves for part A. Respective images are each scaled to same values.

Figure 6.

Histological assessment of tumor necrosis. H&E stained tumor cross sections showing necrotic (pink) and viable (purple) regions at 48 h post treatment for A: Analogue **24** (120 mg/kg) B: **CA4P** (120 mg/kg) C: Saline.

Scheme 1.
Synthesis of benzocyclooctanone analogues **5** and **6**.

Scheme 2.
Synthesis of indanone analogues **11** and **12**.

Scheme 3. Synthesis of TBS-protected benzocyclooctanone **14** and indanone **16**.

Scheme 4.

Synthesis of target benzocyclooctene analogues **20**, **21**, and **23**.

Scheme 5.

Conversion of analogue **23** to its corresponding phosphate prodrug salt **24**.

Scheme 6.
Synthesis of target indene compounds **28**, **29**, and **31**.

Scheme 7.
Synthesis of target indene water-soluble analogue **32**.

Inhibition of tubulin polymerization [expressed as half maximal inhibitory concentration (IC₅₀)] and cytotoxicity [expressed as growth inhibition of 50% (GI₅₀)] of the eight target analogues synthesized.

Table 1

Compound	Inhibition of tubulin polymerization IC ₅₀ (μ M) $\pm$ SD	% Inhibition of colchicine binding $\pm$ SD	GI ₅₀ (μ M) SRB assay ^a		
			NCL-H460	DU-145	SK-OV-3
CA4	1.0 ^b	98 $\pm$ 0.007	0.00500 ^{c,d}	0.00602 ^{c,d}	0.00506 ^d
CA4P	>20 ^b	nr ^e	0.00282 ^c	0.00336 ^c	0.00190
KGP18	1.4 ^f	nr ^e	0.0000418 ^g	0.0000249 ^g	0.0000543 ^g
20	4.5 $\pm$ 0.5	31 $\pm$ 0.6	0.395	0.448	0.512
21	3.0 $\pm$ 0.1	29 $\pm$ 5	0.431	0.570	0.400
23	1.2 $\pm$ 0.1	78 $\pm$ 4	0.107	0.105	0.0811
24	>20	nd ^h	0.0260	0.0410	0.0366
28	>20	nd ^h	42.2	34.6	9.46
29	>20	nd ^h	4.02	4.66	6.15
31	11 $\pm$ 2	17 $\pm$ 5	0.388	0.362	0.704
32	>20	nd ^h	1.50	3.34	0.334

^a Average of n = 3 independent determinations

^b Data from ref. 73

^c For additional data, see ref. 73

^d Data from ref. 13

^e nr = not reported

^f For additional data, see ref. 12

^g For additional data, see ref. 9

^h nd = not determined