Drugs of the Future 2007, 32(12): 1025-1032

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.12.1158960

Combretastatin A-4 Phosphate

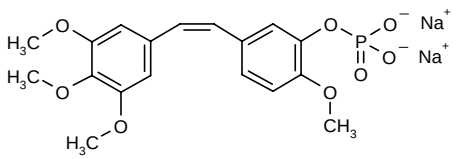
Vascular Disrupting Agent
Oncolytic
Treatment of Age-Related Macular Degeneration

CA4DP
CA4P
Zybrestat™

2-Methoxy-5-[2(*Z*)-(3,4,5-trimethoxyphenyl)vinyl]phenoxyphosphoric acid disodium salt

Disodium combretastatin A-4 3-*O*-phosphate

InChI=1/C18H21O8P.2Na/c1-22-14-8-7-12(9-15(14)26-27(19,20)21)5-6-13-10-16(23-2)18(25-4)17(11-13)24-3;;/h5-11H,1-4H3,(H2,19,20,21);;/q;2*+1/p-2/b6-5-;;



$C_{18}H_{19}Na_2O_8P$
Mol wt: 440.2920

CAS: 168555-66-6

CAS: 222030-63-9 (free acid)

EN: 225384

Abstract

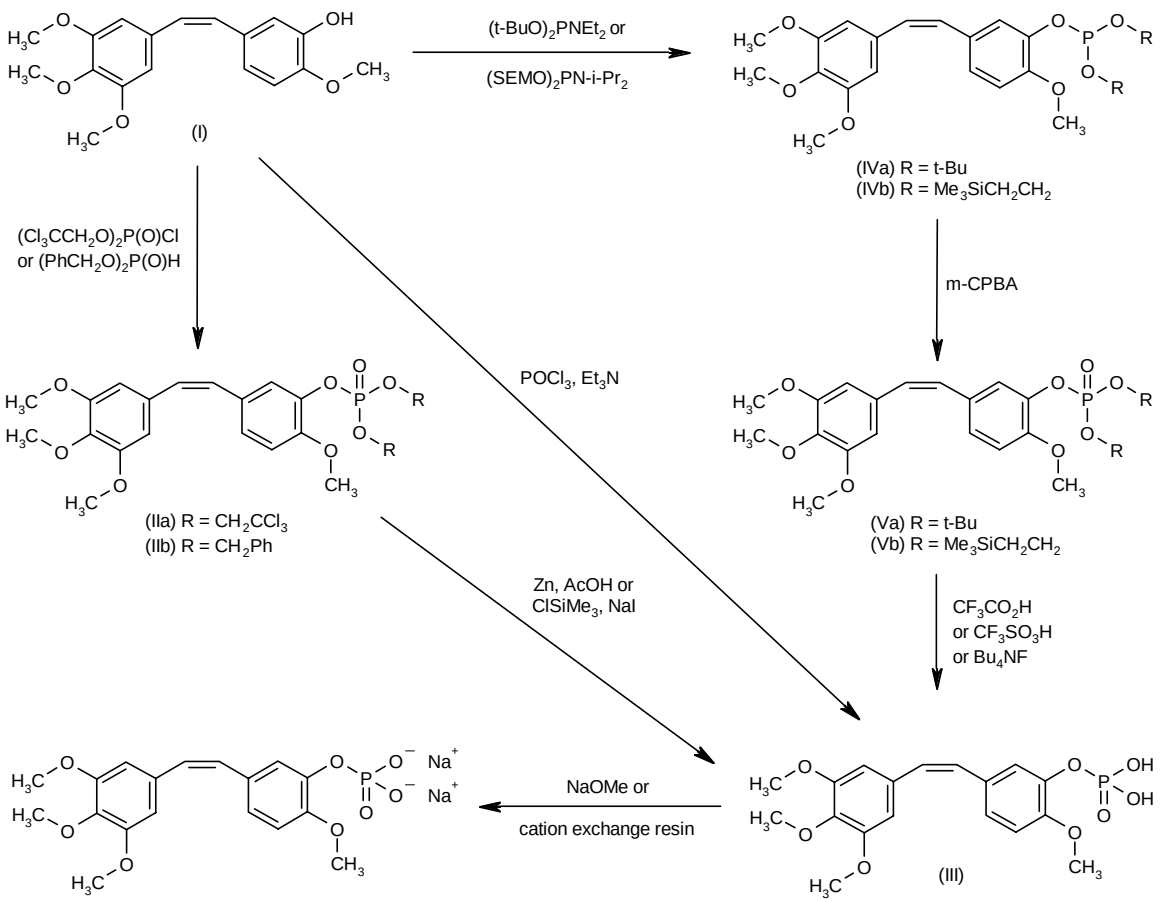
Combretastatin A-4 phosphate (CA4P, Zybrestat™) is a vascular disrupting agent (VDA) that binds to tubulin and selectively damages established tumor vasculature. Preclinical studies have shown that CA4P causes rapid vascular shutdown, leading to central tumor necrosis, although it leaves a rim of viable cells at the periphery, which requires additional antineoplastic treatment to enhance efficacy. Phase I monotherapy trials have shown the agent to be well tolerated, with tumor pain and mild transient cardiovascular effects being the most common side effects. Clinical trials of various CA4P combinations have been conducted or are ongoing with initially promising results. Preclinical work has shown CA4P to also be effective in disrupting ocular neovascularization, and human proof of concept was established for i.v. drug in patients with myopic macular degeneration; a topical formulation is in development as a potential treatment for age-related macular degeneration (AMD).

Synthesis[+]

Combretastatin A-4 phosphate can be synthesized by phosphorylation of combretastatin A-4 (I) following several related strategies. Direct phosphorylation of (I) employing either bis(2,2,2-trichloroethyl) phosphorochloridate in pyridine (1, 2) or dibenzyl phosphite in the presence of CCl_4 and DMAP (3-5) furnishes the corresponding bis-trichloroethyl (IIa) and dibenzyl (IIb) phosphate esters, which are subsequently deprotected to combretastatin A-4 phosphate (III) by either reductive trichloroethyl group cleavage with zinc dust and acetic acid (1, 2) or by debenzylation with chlorotrimethylsilane/sodium iodide (3-5). Alternatively, phosphitylation of (I) using di-*tert*-butyl *N,N*-diethylphosphoramidite (3, 4, 6) or bis(trimethylsilylethyl) *N,N*-diisopropylphosphoramidite (3-5) in the presence of tetrazole yields the phosphite esters (IVa) and (IVb), respectively, which are further oxidized to the corresponding phosphates (Va) and (Vb) using *m*-chloroperbenzoic acid in CH_2Cl_2/THF (3-6). Combretastatin A-4 phosphate (III) is then obtained by acidic cleavage of the *tert*-butyl ester (Va) with trifluoroacetic acid (3, 4, 6) or trifluoromethanesulfonic acid (6), or by tetrabutylammonium fluoride-promoted cleavage of the trimethylsilylethyl ester (Vb) (3-5). In a shorter procedure, phosphorylation of combretastatin A-4 (I) with $POCl_3$ in the presence of Et_3N in CH_2Cl_2 provides directly combretastatin A-4 phosphate (III) (7). Conversion of (III) to the title disodium salt is accomplished by treatment with methanolic NaOMe

Daniel M. Patterson*, Gordon J.S. Rustin. Department of Medical Oncology, The Clocktower, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, U.K. *Correspondence: danpatterson@doctors.org.uk. [+]Synthesis prepared by N. Serradell, E. Rosa, J. Bolós. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Combretastatin A-4 Phosphate



(3-5, 7) or by passage through a cation exchange resin (1, 2). Scheme 1.

The parent compound combretastatin A-4 (I) can be synthesized by bromination of 3,4,5-trimethoxybenzyl alcohol (VI) with lithium bromide and chlorotrimethylsilane, followed by condensation of the obtained benzylic bromide (VII) with triphenylphosphine to provide the phosphonium salt (VIII). Wittig reaction of (VIII) with 4-methoxy-3-(thexyldimethylsilyloxy)benzaldehyde (IX) affords the silyl-protected *cis*-stilbene (X), which is desilylated to (I) by treatment with tetrabutylammonium fluoride in THF (3, 4, 6). Scheme 2.

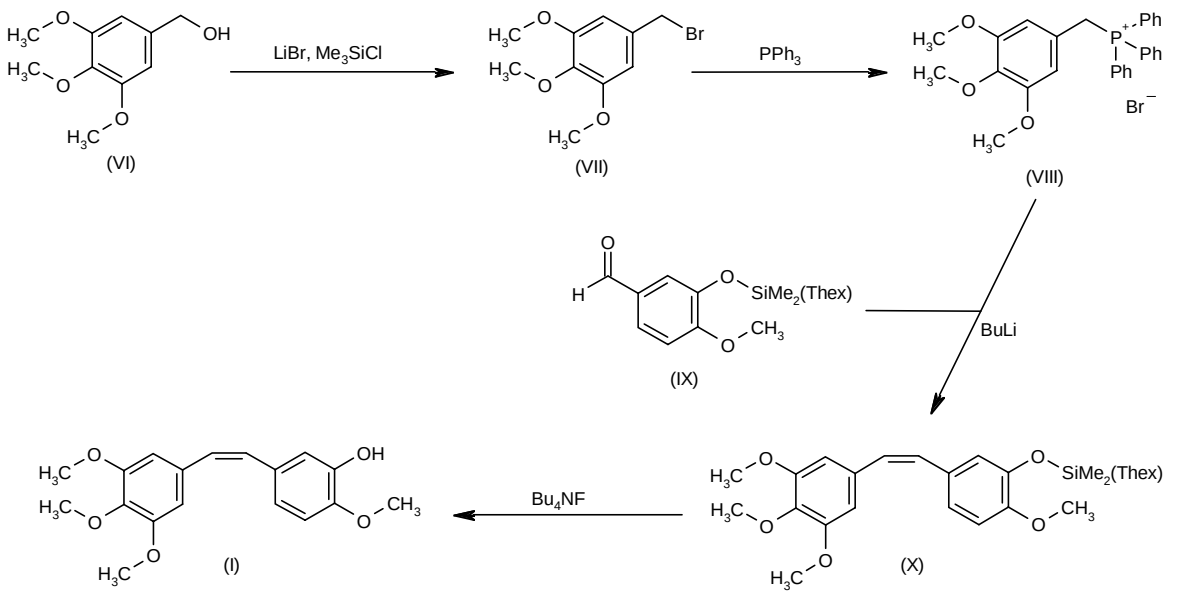
In a related strategy, the precursor di-*tert*-butyl combretastatin A-4 phosphate (Va) is prepared by protection of isovanillin (XI) as the corresponding imine (XII) with butylamine and *p*-toluenesulfonic acid, followed by phosphitylation with di-*tert*-butyl *N,N*-diethylphosphoramidite in the presence of tetrazole, and oxidation to phosphate (XIII) with *m*-chloroperbenzoic acid. Subsequent Wittig reaction of aldehyde (XIII) with 3,4,5-trimethoxybenzyl triphenylphosphonium bromide (VIII) produces the target stilbene phosphate derivative (Va) (6). Scheme 3.

Background

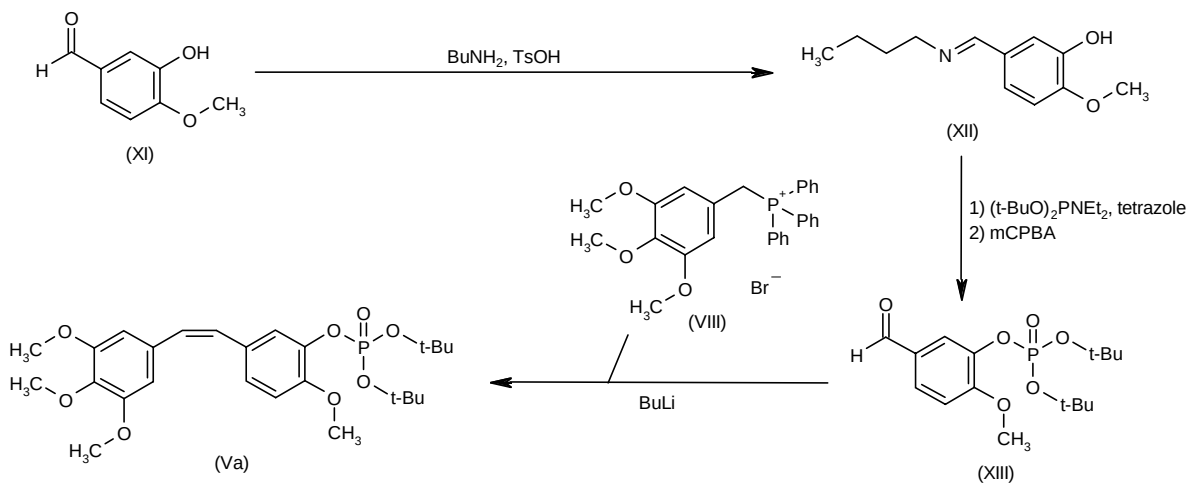
Vascular disrupting agents (VDAs) selectively damage established vasculature, leading to rapid vascular shutdown and subsequent ischemia and necrosis, and have generated much excitement in oncology and ophthalmology. It was reported by Walshe over 160 years ago that occasional tumor eradication could occur as a result of interrupting tumor blood supply either by torsion of the vascular pedicle or by thrombosis of a major feeding vessel (8). Woglum concluded in a 1923 review regarding theories of tumor regression and resistance that inhibition of tumor growth might best be achieved by inducing damage to the capillary system (9).

In the 1930s/1940s the tubulin inhibitor colchicine was found to cause hemorrhage and extensive tumor necrosis in experimental models and to preferentially affect tumor vasculature, but only at doses close to the maximum tolerated dose (MTD) (10-13). Unfortunately colchicine-induced deaths were seen in a subsequent clinical trial (14). Similarly, another tubulin-depolymerizing agent, podophyllotoxin (related to etoposide), also had VDA

Scheme 2: Synthesis of Combretastatin A-4 (I)



Scheme 3: Synthesis of Intermediate (Va)



effects near the MTD (15). Due to the narrow therapeutic range of these agents and the advent of 'conventional' cytotoxic agents, further development of VDAs did not occur until the early 1980s, when Denekamp demonstrated that the higher proliferation rate of tumor endothelial cells can be a selective target for treatment (16), with experiments showing tumor regressions by clamping the blood supply to implanted tumors (17). Towards the end of the 1980s, screening of new potential agents with VDA effects began, with the small molecule combretastatin A-4 phosphate (CA4P) producing the most exciting results (18). Combretastatin A-4 (CA4) was originally isolated from the bark of the African bush willow tree (*Combretum caffrum*) by Pettit *et al.* in the early 1980s and its powdered root bark is believed to be used by Zulus to harm enemies (19). Small-molecule VDAs in current development are summarized in Table I, with CA4P and DMXAA (AS-1404) at the most advanced stage of clinical development (20).

Table I: Small-molecule VDAs currently in clinical development.

Drug (Ref.)	Source/Highest development phase	Comments
Small-molecule cytokine inducers		
DMXAA/AS-1404 (49-52)	Antisoma/Phase II	Flavonoid
Tubulin-binding agents		
Combretastatin A-4 phosphate (21, 41, 42)	OXiGENE/Phase III	Prodrug of CA4
AZD-6126* (54)	Angiogene Pharmaceutical/Phase I/II	Prodrug of *N*-acetylcolchinol
AC-7700/AVE-8062 (55)	sanofi-aventis/Phase I	CA4 prodrug
ABT-751 (56)	Abbott/Phase II	Oral sulfonamide
Denibulin hydrochloride/MN-029 (57)	MediciNova/Phase I/II	Synthetic tubulin inhibitor
Combined cytotoxic and vascular targeting agents		
OXi-4503 (58)	OXiGENE/Phase I	Prodrug of CA1
Soblidotin/TZT-1027 (59-61)	ASKA Pharmaceutical, Yakult Honsha/Phase I/II	Synthetic derivative of dolastatin 10 (isolated from a marine mollusc)
NPI-2358 (62)	Nereus Pharmaceuticals/Phase I	Synthetic diketopiperazine
Others		
Arsenic trioxide (63)	Cephalon/Phase III	Launched for acute promyelocytic leukemia

*Also known as ZD-6126 and ANG-453. Angiogene reacquired rights to the product from AstraZeneca in 2006. Phase II studies were placed on hold pending additional laboratory studies, which the company reports have been successfully completed.

Preclinical Pharmacology

CA4P is a water-soluble prodrug that is dephosphorylated to the active drug CA4 by nonspecific endogenous phosphatases in plasma and on endothelial cells (21), which then undergoes glucuronidation to CA4 glucuronide (CA4G). CA4 is a tubulin-binding agent that binds to the colchicine binding site with greater affinity than colchicine, leading to inhibition of tubulin polymerization. CA4 has been shown to bind tightly to tubulin (K_d = 0.40 ± 0.06 μM) and inhibits tubulin polymerization with an IC_{50} of 2.4 ± 1.4 μM (22). CA4 also rapidly binds to and dissociates from tubulin over 100 times faster than colchicine, resulting in a shorter period of tubulin depolymerization, and thus a reduced likelihood for dose-limiting toxicities (DLTs) such as neuropathy, gastrointestinal toxicity and myelosuppression (23). Galbraith compared the effects of CA4P and colchicine on endothelial cell shape and found at 24 h postexposure that changes in endothelial cell shape recovered after CA4P but not after colchicine (24).

In vitro activity against several different cell lines was demonstrated for CA4P in 1993 (25). Studies assessing the effects of CA4P on human umbilical vein endothelial cells (HUVECs) demonstrated that it caused both disruption of the endothelial cytoskeleton (26) and preferential apoptosis in proliferating endothelial cells (27).

Vascular shutdown has been demonstrated in a number of *in vivo* studies. For example, using diffusible tracers, Chaplin *et al.* showed a > 90% loss of functional vascular volume in murine tumors 6 h after administration of 100 mg/kg (300 mg/m^2) CA4P, with a 50-60% reduction in tumor blood flow (28). Dark *et al.* found evidence for vascular shutdown at doses less than one-tenth the MTD (18). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has also shown reduced tumor perfusion in several preclinical studies (29-31) and is now routinely used as a pharmacodynamic endpoint in clinical trials of VDAs.

In terms of antitumor effects, CA4P caused rapid central hemorrhagic necrosis but left a rim of viable cells in the periphery that can rapidly regrow (32, 33). Subsequent preclinical work demonstrated enhanced antitumor effects when CA4P was combined with either chemotherapy or radiotherapy, which can kill the peripheral cells not affected by CA4P (34-36). Recent preclinical data have shown mobilization of circulating endothelial progenitor cells (CEPs) in response to the vascular disruptive effects of CA4P, which may lead to secondary angiogenesis at the peripheral rim that may be prevented by the prior administration of angiogenesis inhibitors, thus providing important evidence to justify trials of CA4P and other VDAs in combination with antiangiogenic agents (37).

An *in vivo* murine model of neovascularization using hyperplastic thyroid tissue has also demonstrated that CA4P disrupts neovascular tissue of non-neoplastic origin (38). Two studies in mice demonstrated that CA4P inhibits ocular neovascularization (39, 40), suggesting potential nononcological indications for CA4P.

Pharmacokinetics and Metabolism

Three phase I trials of single-agent CA4P performed in London (21), Philadelphia (41) and Cleveland (42)

Table II: Phase I trials of single-agent CA4P.

	UK CRC PH1/066 (21, 36)	UPENN CA4P-102 (41)	CWRU CA4P-101 (42)
Schedule	Weekly x 3every 28 days	Daily x 5 every 21 days	Every 3 weeks
Infusion time	10 min	10 min	10 min
Number of patients	34	37	25
CA4P dose range	5-114 mg/m^2	6-75 mg/m^2	18-90 mg/m^2
MTD	68 mg/m^2	75 mg/m^2	60 mg/m^2
DLT	Ataxia, motor neuropathy, syncope, diplopia, tumor pain, dyspnea	Tumor pain, sensorimotor neuropathy, syncope, dyspnea	Cardiac ischemia, dyspnea
RP2D	52-68 mg/m^2	52 mg/m^2	$\leq$ 60 mg/m^2
Number of doses of CA4P given	167	700	104
Terminal half-life CA4P (h)	0.489	0.36	0.47
Terminal half-life CA4 (h)	2.23	3.3	4.26

CA4P, combretastatin A-4 phosphate; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; RP2D, recommended phase II dose.

have been published using three different dose schedules in a total of 96 patients (Table II). Doses used ranged from 5 to 114 mg/m^2. All three trials showed dose-dependent linear pharmacokinetics (C_{max} and AUC) for both CA4P and CA4. Urine collection over 24 h following CA4P administration revealed that 58-67% of the dose was excreted as CA4G. The average terminal plasma half-lives of CA4P, CA4 and CA4G were 0.4, 3.8 and 4.5 h, respectively.

Safety

In the three phase I single-agent studies, most adverse events were fairly mild and tolerable (21, 41, 42). None of the traditional cytotoxic side effects were seen. Tumor pain was commonly reported, which may be related to tumor ischemia; however, no correlation with clinical response or MRI parameters was observed. Mild cardiovascular effects of tachycardia, bradycardia, hypertension and hypotension were also very common and self-limiting. Mild gastrointestinal and neurological side effects were quite common.

The MTDs for the three studies were comparable, ranging from 60 to 75 mg/m^2. DLTs were mainly neurological (reversible ataxia, diplopia, sensorimotor neuropathy) or cardiovascular. In the U.K. study (21), 1 patient died as a result of small bowel ischemia within an area of previous radical radiotherapy, which led to the exclusion criteria of previous radical radiotherapy in subsequent CA4P trials. However, dose-limiting cardiotoxicity was only seen in one of the three phase I trials in 2 patients with pre-existing cardiovascular disease where blood pressure control was not ideal. Data have also shown a dose-dependent prolongation of the Q-T_c interval (45). In general, patients with pre-existing coronary heart disease, uncontrolled hypertension or multiple cardiac risk factors should not receive VDAs.

Thrombocytopenia was the DLT in a phase Ib trial of CA4P and carboplatin (43). In the phase Ib/II trial of CA4P with carboplatin and/or paclitaxel, dose-limiting ataxia and hypertension were seen after CA4P 72 mg/m^2, which can be easily treated with prophylactic steroids and glyceryl trinitrate, respectively (46).

Clinical Studies

Signs of antitumor activity were seen in all three single-agent trials. In the U.K. trial, 1 unsustained partial response was obtained in a patient with metastatic adenocortical carcinoma (21). In the American studies, 1 patient each with anaplastic thyroid cancer and fibrosarcoma had complete and partial responses, respectively, and 17 other patients had stable disease (41, 42).

In the CA4P/carboplatin combination trial, 6 patients with solid tumors had stable disease (43). Partial responses were seen in esophageal, ovarian and small cell lung cancers in the CA4P/carboplatin/paclitaxel triplet study (46). Based on radiological or CA125 response criteria, 10 of the 15 relapsed ovarian cancer patients achieved a response; this triplet combination is currently being assessed in a phase II trial in platinum-resistant ovarian cancer patients. Phase I studies of CA4P in combination with bevacizumab (Avastin™; angiogenesis inhibitor) (47) are ongoing, as is a phase II/III trial of the drug in combination with paclitaxel and carboplatin in patients with anaplastic thyroid cancer (48).

Drug Interactions

A phase Ib trial evaluating carboplatin followed 60 min later by CA4P revealed an interesting pharmacokinetic interaction between the two drugs which led to higher carboplatin exposure than expected, resulting in dose-limiting thrombocytopenia (43). Analysis of the preclinical data and positron emission tomography (PET) data from one of the phase I trials of CA4P show that renal perfusion is reduced between 30 min and 4 h after CA4P (32). A reduction in renal blood flow would reduce the clearance of carboplatin and increase its myelotoxicity.

Acknowledgements

Daniel Patterson is a clinical research fellow funded by Cancer Research UK and OXiGENE; Gordon Rustin is on the OXiGENE Clinical Advisory Board.

Source

OXiGENE, Inc. (US) (licensed from Arizona State University).

References

1. Pettit, G.R., Temple, C. Jr., Narayanan, V.L. et al. *Antineoplastic agents 322. Synthesis of combretastatin A-4 prodrugs*. Anticancer Drug Des 1995, 10(4): 299-309.

2. Pettit, G.R. (Arizona State University). *Combretastatin A-4 prodrug*. US 5561122.

3. Pettit, G.R., Rhodes, M.R. *Antineoplastic agents 389. New syntheses of the combretastatin A-4 prodrug*. Anticancer Drug Des 1998, 13(3): 183-91.

4. Pettit, G.R., Rhodes, M.R. (Arizona State University). *Synthesis of combretastatin A-4 prodrugs and trans-isomers thereof*. CA 2314238, EP 1045853, US 7018987, WO 9935150.

5. Gale, J., Haider, R., Hoare, J., Seyedi, F. (OxiGene, Inc.). *Efficient method of synthesizing combretastatin A-4 prodrugs*. US 2002119951, WO 0206279.

6. Griffin, R.J., Quarterman, C.P., Rathbone, D.L., Slack, J.A. (Aston Molecules Ltd.). *Substd. diphenylethylenes and analogues of derivs. thereof*. WO 9216486.

7. Gill, G.S., Grobelny, D., Flynn, B. *A practical method for phosphorylation of combretastatin A-4 with phosphorus oxychloride*. Org Prep Proc Int 2006, 38(6): 604-8.

8. Walshe, W.W. (Ed.). *The Anatomy, Physiology, Pathology and Treatment of Cancer*. Ticknor and Company, Boston, 1844.

9. Woglom, W.H. *A critique of tumour resistance*. J Cancer Res 1923, 7: 283-311.

10. Boyland, E., Boyland, M.E. *Studies in tissue metabolism: The action of colchicine and B. typhosus extract*. Biochem J 1937, 31(3): 454-60.

11. Boyland, E., Boyland, M.E. *Studies in tissue metabolism: The action of colchicine on transplanted, induced and spontaneous mouse tumours*. Biochem J 1940, 34(3): 280-4.

12. Ludford, R.J. *Colchicine in the experimental chemotherapy of cancer*. J Natl Cancer Inst 1945, 6: 89-101.

13. Ludford, R.J. *Factors determining the action of colchicine on tumour growth*. Br J Cancer 1948, 2: 75-86.

14. Seed, L., Slaughter, D.P., Limarzi, L.R. *Effect of colchicine on human carcinoma*. Surgery 1940, 7: 696-709.

15. Leiter, J., Downing, V., Hartwell, J.L., Shear, M.J. *Damage induced in sarcoma 37 with podophyllin, podophyllotoxin alpha-peltatin, beta-peltatin, and quercetin*. J Natl Cancer Inst 1950, 10(6): 1273-93.

16. Denekamp, J., Hobson, B. *Endothelial-cell proliferation in experimental tumours*. Br J Cancer 1982, 46(5): 711-20.

17. Denekamp, J., Hill, S.A., Hobson, B. *Vascular occlusion and tumour cell death*. Eur J Cancer Clin Oncol 1983, 19(2): 271-5.

18. Dark, G.G., Hill, S.A., Prise, V.E., Tozer, G.M., Pettit, G.R., Chaplin, D.J. *Combretastatin A-4, an agent that displays potent and selective toxicity toward tumour vasculature*. Cancer Res 1997, 57(10): 1829-34.

19. Pettit, G.R., Cragg, G.M., Herald, D.L., Schmidt, J.M., Lohavanijaya, P. *Isolation and structure of combretastatin*. Can J Chem 1982, 60: 1374-6.

20. Patterson, D.M., Rustin, G.J. *Vascular damaging agents*. Clin Oncol (R Coll Radiol) 2007, 19(6): 443-56.

21. Rustin, G.J., Galbraith, S.M., Anderson, H. et al. *Phase I clinical trial of weekly combretastatin A4 phosphate: Clinical and pharmacokinetic results*. J Clin Oncol 2003, 21(15): 2815-22.

22. Lin, C.M., Singh, S.B., Chu, P.S., Dempcy, R.O., Schmidt, J.M., Pettit, G.R., Hamel, E. *Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin: A structure-activity study*. Mol Pharmacol 1988, 34(2): 200-8.

23. Lin, C.M., Ho, H.H., Pettit, G.R., Hamel, E. *Antimitotic natural products combretastatin A-4 and combretastatin A-2: Studies on the mechanism of their inhibition of the binding of colchicine to tubulin*. Biochemistry 1989, 28(17): 6984-91.

24. Galbraith, S.M., Chaplin, D.J., Lee, F., Stratford, M.R., Locke, R.J., Vojnovic, B., Tozer, G.M. *Effects of combretastatin A4 phosphate on endothelial cell morphology in vitro and relationship to tumour vascular targeting activity in vivo*. Anticancer Res 2001, 21(1A): 93-102.

25. el-Zayat, A.A., Degen, D., Drabek, S., Clark, G.M., Pettit, G.R., Von Hoff, D.D. *In vitro evaluation of the antineoplastic activity of combretastatin A-4, a natural product from Combretum caffrum (arid shrub)*. Anticancer Drugs 1993, 4(1): 19-25.

26. Grosios, K., Holwell, S.E., McGown, A.T., Pettit, G.R., Bibby, M.C. *In vivo and in vitro evaluation of combretastatin A-4 and its sodium phosphate prodrug*. Br J Cancer 1999, 81(8): 1318-27.

27. Iyer, S., Chaplin, D.J., Rosenthal, D.S., Boulares, A.H., Li, L.Y., Smulson, M.E. *Induction of apoptosis in proliferating human endothelial cells by the tumor-specific antiangiogenesis agent combretastatin A-4*. Cancer Res 1998, 58(20): 4510-4.

28. Chaplin, D.J., Pettit, G.R., Hill, S.A. *Anti-vascular approaches to solid tumour therapy: Evaluation of combretastatin A4 phosphate*. Anticancer Res 1999. 19(1A): 189-95.

29. Beauregard, D.A., Thelwall, P.E., Chaplin, D.J., Hill, S.A., Adams, G.E., Brindle, K.M. *Magnetic resonance imaging and spectroscopy of combretastatin A4 prodrug-induced disruption of tumour perfusion and energetic status*. Br J Cancer 1998, 77: 1761-7.

30. Galbraith, S.M., Maxwell, R.J., Lodge, M.A. et al. *Combretastatin A4 phosphate has tumour anti-vascular activity in rat and man as demonstrated by dynamic magnetic resonance imaging*. J Clin Oncol 2003, 21(15): 2831-42.

31. Maxwell, R., Wilson, J., Prise, V.E., Vojnovic, B., Rustin, G.J., Lodge, M.A., Tozer, G.M. *Evaluation of the anti-vascular effects of combretastatin in rodent tumours by dynamic contrast enhanced MRI*. NMR Biomed 2002, 15(2): 89-98.

32. Tozer, G.M., Prise, V.E., Wilson, J. et al. *Mechanisms associated with tumor vascular shut-down induced by combretastatin*

A-4 phosphate: Intravital microscopy and measurement of vascular permeability. Cancer Res 2001, 61(17): 6413-22.

33. Tozer, G.M., Prise, V.E., Wilson, J. et al. *Combretastatin A-4 phosphate as a tumor vascular-targeting agent: Early effects in tumors and normal tissues*. Cancer Res 1999, 59(7): 1626-34.

34. Horsman, M.R., Murata, R., Breidahl, T. et al. *Combretastatins novel vascular targeting drugs for improving anti-cancer therapy. Combretastatins and conventional therapy*. Adv Exp Med Biol 2000, 476: 311-23.

35. Nelkin, B.D., Ball, D.W. *Combretastatin A-4 and doxorubicin combination treatment is effective in a preclinical model of human medullary thyroid carcinoma*. Oncol Rep 2001, 8(1): 157-60.

36. Murata, R., Siemann, D.W., Overgaard, J., Horsman, M.R. *Interaction between combretastatin A-4 disodium phosphate and radiation in murine tumors*. Radiother Oncol 2001, 60(2): 155-61.

37. Shaked, Y., Ciarrocchi, A., Francoet, M. et al. *Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors*. Science 2006. 313(5794): 1785-7.

38. Griggs, J., Hesketh, R., Smith, G.A., Brindle, K.M., Metcalfe, J.C., Thomas, G.A., Williams, E.D. *Combretastatin-A4 disrupts neovascular development in non-neoplastic tissue*. Br J Cancer 2001, 84(6): 832-5.

39. Griggs, J., Skepper, J.N., Smith, G.A., Brindle, K.M., Metcalfe, J.C., Hesketh, R. *Inhibition of proliferative retinopathy by the anti-vascular agent combretastatin-A4*. Am J Pathol 2002, 160(3): 1097-103.

40. Nambu, H., Nambu, R., Melia, M., Campochiaro, P.A. *Combretastatin A-4 phosphate suppresses development and induces regression of choroidal neovascularization*. Invest Ophthalmol Vis Sci 2003, 44(8): 3650-5.

41. Stevenson, J.P., Rosen, M., Sun, W. et al. *Phase I trial of the antivascular agent combretastatin A4 phosphate on a 5-day schedule to patients with cancer: Magnetic resonance imaging evidence for altered tumor blood flow*. J Clin Oncol 2003, 21(23): 4428-38.

42. Dowlati, A., Robertson, K., Cooney, M. et al. *A phase I pharmacokinetic and translational study of the novel vascular targeting agent combretastatin A-4 phosphate on a single-dose intravenous schedule in patients with advanced cancer*. Cancer Res 2002, 62(12): 3408-16.

43. Bilenker, J.H., Flaherty, K.T., Rosen, M. et al. *Phase I trial of combretastatin A-4 phosphate with carboplatin*. Clin Cancer Res 2005, 11(4): 1527-33.

44. Anderson, H.L., Yap, J.T., Miller, M.P., Robbins, A., Jones, T., Price, P.M. *Assessment of pharmacodynamic vascular response in a phase I trial of combretastatin A4 phosphate*. J Clin Oncol 2003, 21(15): 2823-30.

45. Cooney, M.M., Radivoyevitch, T., Dowlati, A. et al. *Cardiovascular safety profile of combretastatin A4 phosphate in a single-dose phase I study in patients with advanced cancer*. Clin Cancer Res 2004, 10(1, Pt. 1): 96-100.

46. Rustin, G.J.S., Nathan, P.D., Boxall, J., Saunders, L., Ganeson, T.S., Wang, D., Gaya, A. *A dose escalation study combining combretastatin A-4 phosphate (CA4P) with carboplatin or paclitaxel in patients with advanced cancer*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst A12.

47. *Safety study of increasing doses of combretastatin in combination with bevacizumab (Avastin) in patients with advanced solid tumors (NCT00395434)*. ClinicalTrials.gov Web site, November 27, 2007.

48. *Study of combretastatin and paclitaxel/carboplatin in the treatment of anaplastic thyroid cancer (NCT00507429)*. ClinicalTrials.gov Web site, Novermber 27, 2007.

49. Jameson, M.B., Thompson, P.I., Baguley, B.C. et al. *Clinical aspects of a phase I trial of 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a novel antivascular agent*. Br J Cancer 2003, 88(12): 1844-50.

50. Rustin, G.J., Bradley, C., Galbraith, S. et al. *5,6-Dimethylxanthenone-4-acetic acid (DMXAA), a novel antivascular agent: Phase I clinical and pharmacokinetic study*. Br J Cancer 2003, 88(8): 1160-7.

51. McKeage, M., AS1404-201 Study Group Investigators. *Phase Ib/II study of DMXAA combined with carboplatin and paclitaxel in non-small cell lung cancer (NSCLC)*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 3-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 7102.

52. Gabra, H., AS1404-202 Study Group Investigators. *Phase II study of DMXAA combined with carboplatin and paclitaxel in recurrent ovarian cancer*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 3-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 5032.

53. Rustin, G.J.S., Nathan, P.D., Boxall, J., Saunders, L., Ganesan, T.S., Shreeves, G.E. *A phase Ib trial of combretastatin A-4 phosphate (CA4P) in combination with carboplatin or paclitaxel chemotherapy in patients with advanced cancer*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 3103.

54. Beerepoot, L.V., Radema, S.A., Witteveen, E.O., Thomas, T., Wheeler, C., Kempin, S., Voest, E.E. *Phase I clinical evaluation of weekly administration of the novel vascular-targeting agent, ZD6126, in patients with solid tumors*. J Clin Oncol 2006, 24(10): 1491-8.

55. Tolcher, A.W., Forero, L., Celio, P. et al. *Phase I, pharmacokinetic, and DCE-MRI correlative study of AVE8062A, an antivascular combretastatin analogue, administered weekly for 3 weeks every 28 days*. Proc Am Soc Clin Oncol (ASCO) 2003, 22: Abst 834.

56. Fox, E., Maris, J.M., Widemann, B.C. et al. *A phase 1 study of ABT-751, an orally bioavailable tubulin inhibitor, administered daily for 7 days every 21 days in pediatric patients with solid tumors*. Clin Cancer Res 2006, 12(16): 4882-7.

57. Ricart, A.D., Cooney, M., Sarantopoulos, J. et al. *A phase I pharmacokinetic (PK) and pharmacodynamic (PD) study of MN-029, a novel vascular disrupting agent (VDA), in patients (pts) with advanced solid tumors*. J Clin Oncol [42nd Annu Meet Am Soc Clin Oncol (ASCO) (June 3-6, Atlanta) 2006] 2006, 24(18, Suppl.): Abst 3096.

58. Patterson, D.M., Ross, P., Koetz, B. et al. *Phase I evaluation of OXi4503, a vascular disrupting agent, in patients with advanced solid tumours*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18. Suppl.): Abst 14146.

59. Tamura, K., Nakagawa, K., Kurata, T. et al. *Phase I study of TZT-1027, a novel synthetic dolastatin 10 derivative and inhibitor*

of tubulin polymerization, which was administered to patients with advanced solid tumors on days 1 and 8 in 3-week courses. Cancer Chemother Pharmacol 2007, 60(2): 285-93.

60. Patel, S., Keohan, M.L., Saif, M.W. et al. *Phase II study of intravenous TZT-1027 in patients with advanced or metastatic soft-tissue sarcomas with prior exposure to anthracycline-based chemotherapy*. Cancer 2006, 107(12): 2881-7.

61. Schoffski, P., Thate, B., Beutel, G. et al. *Phase I and pharmacokinetic study of TZT-1027, a novel synthetic dolastatin 10 derivative, administered as a 1-hour intravenous infusion every 3 weeks in patients with advanced refractory cancer*. Ann Oncol 2004, 15(4): 671-9.

62. Spear, M.A., LoRusso, P., Tolcher, A.W. et al. *A phase 1 dynamic accelerated titration dose escalation study of the vascular disrupting agent NPI-2358*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18. Suppl.): Abst 14097.

63. Soignet, S.L., Maslak, P., Wang, Z.G. et al. *Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide*. N Engl J Med 1998, 339(19): 1341-8.