Polish J. Chem., **78**, 323–341 (2004) **REVIEW ARTICLE**

Combretastatin A-4 and Its Analogues as Antineoplastic Agents

by K. Dzierzbicka and A.M. Kołodziejczyk

Department of Organic Chemistry, Gdańsk University of Technology, 11/12 G. Narutowicza Street, 80-952 Gdańsk, Poland

(Received October 1st, 2003; revised manuscript November 11th, 2003)

Combretastatin A-4 is a potent antineoplastic and antiangiogenesis natural substance isolated from the South African tree *Combretum caffrum*. This article concerns synthesis and biological activity of combretastatin A-4 and its analogues which are considered as prodrugs.

Key words: combretastatin A-4, angiogenesis inhibitors, synthesis, biological activity, antimitotic agents, antineoplastic agent

Since 1971, when Folkman formulated a hypothesis concerning the role of angiogenesis during the tumour process [1], many researchers have directed their efforts to develop an effective non-toxic anticancer therapy basing on inhibiting of tubulin polymerization. The role of angiogenesis in tumour growth and metastasis is discussed in [2–5]. The process of angiogenesis is nowadays intensively preclinically and clinically investigated as a new potential target of antitumour therapy. So far, 35 bioactivators, 18 endogeneous inhibitors, and 35 pharmacological antagonists of tumour angiogenesis have been described [6,7]. Combretastatin A-4 **1** (Figure 1) not only belongs to the group of angiogenesis inhibitors, but it is also a leading compound in the development of anticancer drugs.

Cancerogenic diseases cause a significant number of deaths and are very difficult in treatment. Moreover, there is supposition that the number of cancerous patients will grow in XXI century, and the cost of the anticancer therapy will rise significantly. Taking into consideration the enormous developments in the research on chemotherapeutic agents where screening techniques, combinatorial synthesis, processing data, computerization and considerable financial support are involved, research centres and pharmaceutical companies have achieved relatively small success in cancer therapy. Most of the anticancer drugs exhibit high toxicity, undesired side effects and cause hypersensitive reaction. A serious drawback of the drugs, which are in use, is the frequent occurrence of multidrug resistance. The search for new drugs and effort toward their modification continually attracts the interest of scientists and pharmaceutical companies. Chemical modification of the molecule of a known drug may lead to compounds exhibiting new properties, which can be advantageous from the clinical point of view. New sulphonamides, semisynthetic penicillin and cephalosporin analogues and riphamycin derivatives are good examples of such achievements of pharmacology. Thus, researchers all over the world undertake the challenge of modifying

existing drugs to prepare therapeutic agents, which could act not only more effectively, but also safer and more selectively on the patient organism. Cancer patients eagerly await their success because so far the drugs, which are in use as anticancer agents, are very often unselective, cause destruction of quickly reproducing cells, *e.g.* the hematopoietic system, epithelium alimentary tract, other mucous membranes, skin, hair, and inhibit ovarian and testis function. Antimitotic agents, one of the major classes of cytotoxic drugs for cancer treatment, have recently received increasing attention, partially due to the clinical success of Taxol **2** [8–10]. One of the last achievement is discovery, that there are at least three binding regions on the microtubules for antimitotic agent action: the taxane binding site, the vinca alkaloid binding site, and the colchicine binding site. Taxol **2** (Figure 1), for example, stabilizes microtubules and prevents tubulin depolymerization [11], whereas vincristine **3** (Figure 1) interacts with tubulins to inhibit their polymerization [12]. Although antimitotic drugs, such as vincristine and taxol, have gained wide clinical use in the treatment of various cancers, they have serious drawbacks: undesired side effects, difficulty in the dosing schedule, and lack of efficacy against multidrug resistance (MDR) cancer cell lines. Moreover, they are extremely expensive. Thus the search for better antimitotic agents is justifiable.

In this paper we describe combretastatin A-4 (CA-4) **1** (Figure 1), a very promising antimitotic natural product and its analogues, their synthesis and biological activity.

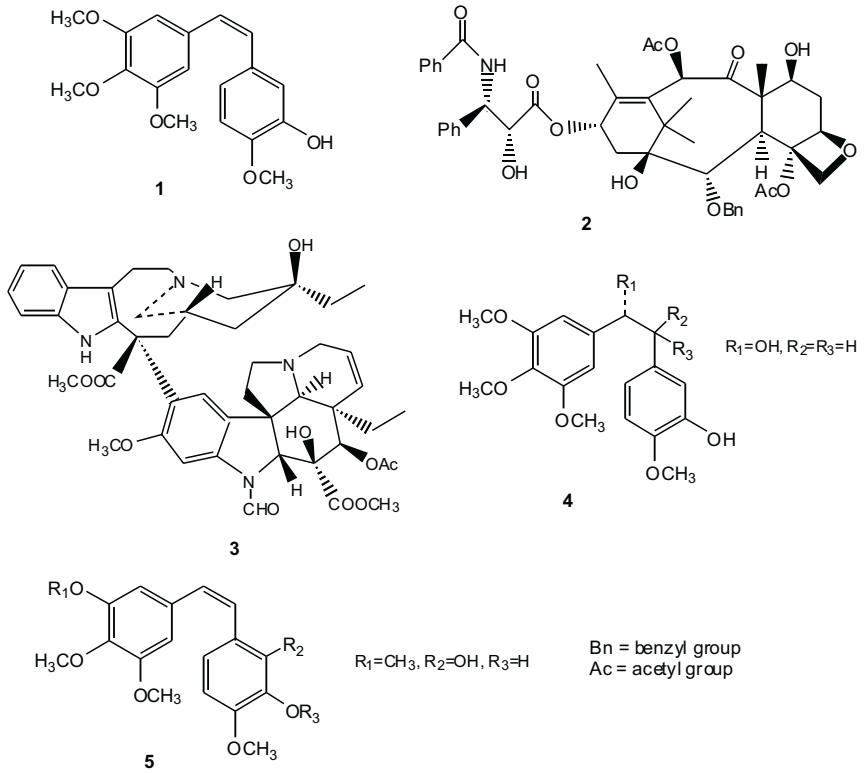


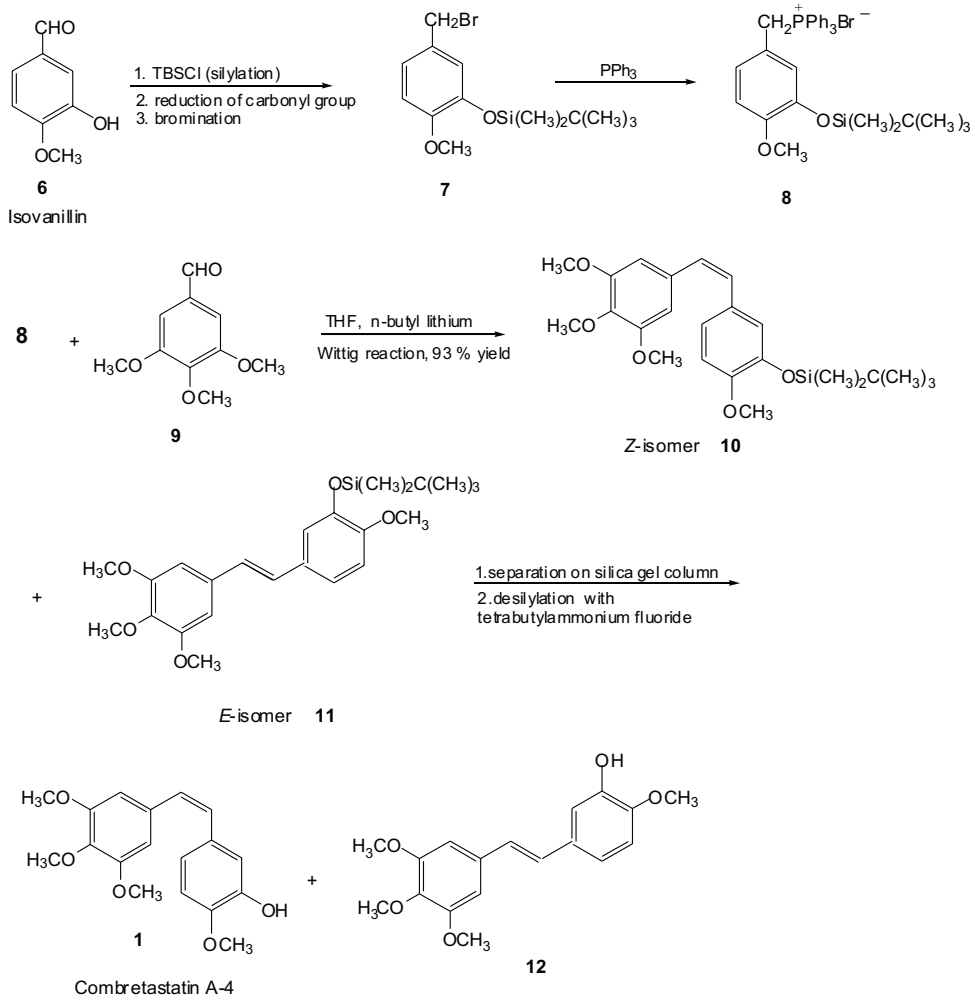
Figure 1.

(*Z*)-1-(3-HYDROXY-4-METHOXYPHENYL)-2-(3,4,5-TRIMETHOXYPHENYL) ETHENE (COMBRETASTATIN A-4, CA-4, **1**)

The isolation and a structural determination of a series of active phenanthrenes, stilbenes, and bibenzyls [13–15] was a result of widespread research started in 1979. Cancer cell growth inhibitors, constituents of the African willow tree *Combretum caffrum* were intensively tested. It was found that bibenzyl combretastatin **4** (Figure 1) causes substantial astrocyte reversal in the 9ASK system, inhibition of the P388 lymphocytic leukaemia cell line (PS cell line), and inhibition of tubulin polymerization. The discovery of related compounds combretastatins A-1 **5** (Figure 1) [13] and A-4 ((*Z*)-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene; CA-4) **1** (Figure 1) [15] as very potent cell growth and tubulin inhibitors was recognized as a great success. Both compounds proved to be the strongest currently known inhibitors of colchicines binding to tubulin and to be exceptionally strong inhibitors of tubulin polymerization (IC_{50} values of 2–3 μM) [15]. Furthermore, CA-4 was found to inhibit markedly growth of a selection of colon cancer cell lines.

The isolation of combretastatin A-4 was a rather complicated process [13–15]. The dichloromethane-methanol extract of *Combretum caffrum* stem wood was fractionated (monitoring by PS bioassay was involved) using a solvent partition sequence followed by gel filtration through Sephadex LH-20 (methanol as eluent). Following partition chromatography of the active fraction on Sephadex LH-20 using hexane-toluene-methanol (3:1:1) as the mobile phase, further separation by ambient column and finally high-performance liquid silica gel chromatography yielded a very active fraction. However, high-resolution ^{1}H-NMR and ^{13}C-NMR spectral data suggested that the fraction was a mixture of at least three substituted stilbenes **1**, **4** and **5**. Hydrogenation gave the mixture of two bibenzyl derivatives, which indicated that two of the original stilbenes were geometrical isomers, while the third was a positional isomer. The mixture of stilbene isomers was separated, using preparative thin layer chromatography on silica gel.

A very efficient synthesis of combretastatin A-4 was achieved by the Wittig reaction (Scheme 1) [16–18]. Phosphonium bromide **8** was prepared by silylation of isovanillin **6** followed by reduction of the carbonyl group, bromination and reaction of **7** with triphenylphosphine. The ylide formed in the reaction of bromide **8** with one equiv of butyllithium was treated with 3,4,5-trimethoxybenzaldehyde **9** to give a mixture of *Z* and *E* stilbenes **10** and **11** in 93% yield; ^{1}H-NMR analysis indicated a *Z/E* ratio of 1:1.5. The compounds were separated by silica gel column chromatography, and *Z*-isomer **10** was shown to be identical (by spectroscopic and chromatographic comparisons) to the silyl ether of natural combretastatin A-4. Both *Z*- and *E*-isomers were desilylated in the presence of tetrabutylammonium fluoride to give combretastatin A-4 **1** (ED_{50} 3.4×10^{-3} μg/mL) and its *E*-isomer **12** (ED_{50} 5.0×10^{-2} μg/mL) in 43% and 42% yield, respectively.



Scheme 1. Steps of synthesis of (*E*) and (*Z*)-combretastatins A-4 [16–18].

CA-4, a strong anticancer agent *in vitro*, does not show *in vivo* activity against murine colon 26 adenocarcinoma, which is in part due to the poor pharmacokinetics of this compound resulting from its excessive lipophilicity and low aqueous solubility [19]. The presence of a *cis* double bond is necessary for anticancerogenic activity of CA-4. However, the *cis* double bond is prone to isomerization to the more stable *trans* isomer, completely devoid of cytotoxicity [19]. Despite these drawbacks, CA-4 is still a very interesting compound due to its strong cytotoxicity and structural simplicity.

Numerous efforts have been undertaken to modify CA-4 in order to improve its *in vivo* activity. A series of water-soluble derivatives (Figure 2) have been recently reported [20–22]. Among these, phosphate disodium salt **13c** [23–26] has proved to be the most attractive, but its original synthesis is rather complicated. Phosphorylation

sequence employing reaction with bis-(2,2,2-trichloroethyl) phosphorochloridate, followed by reduction (Zn, CH_3CO_2H) and isolation by ion-exchange chromatography is not well suited for synthesis of a prodrug on a large scale. An improved synthesis of analogues of CA-4 has been proposed by Pettit and Rhodes [27]. The phosphorylation step is considerably easier using in situ-generated dibenzyl chlorophosphite (Scheme 2). Cleavage of the benzyl esters employing a trimethylchlorosilane/sodium iodide procedure, followed by treatment with sodium methoxide, led to the water-soluble prodrug **13c** in high yield.

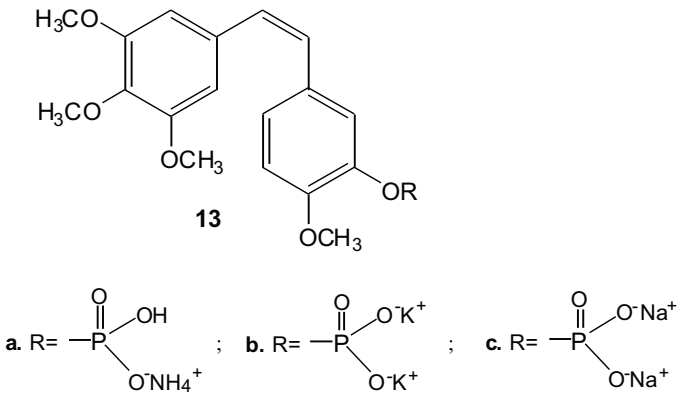
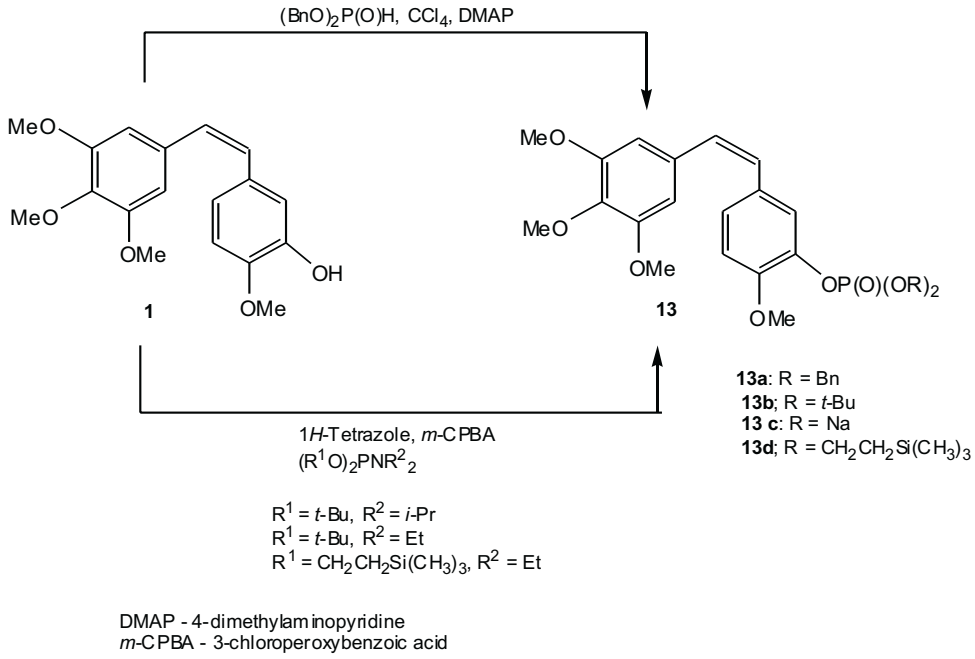


Figure 2.



Scheme 2. Synthesis of combretastatin A-4 as the phosphate esters [27].

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ANALOGUES OF COMBRETASTATIN A-4

The design of inhibitors of tubulin polymerization is an attractive strategy for the development of powerful drugs useful in cancer chemotherapy. Ligands binding in the colchicine binding site of tubulin represent an array of antimitotic agents that inhibit cancer cell proliferation. Such compounds, including colchicines **14** [28–31], podophyllotoxin **15** [32–34], steganacin **16** (Figure 3) [35,36], and their synthetic analogues, inhibit tubulin polymerization. Structurally related biphenyls [37], diphenylmethanes [38], benzopyrans [39], and chalcones **17** [40] (Figure 3) have also been prepared and found to display similar activity. A series of natural products termed combretastatins and their synthetic analogues were added to the list of substances which interact with the colchicine binding site of tubulin [41].

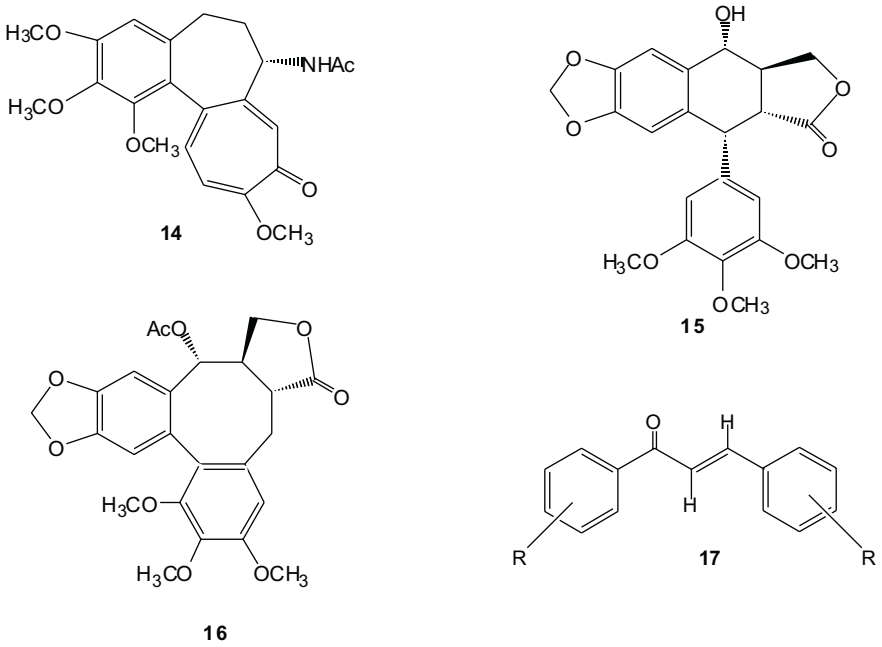
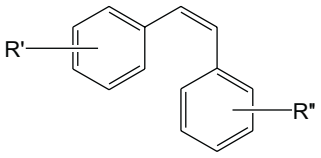


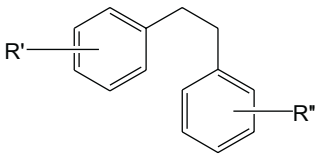
Figure 3.

Cushman *et al.* [42] described a group of *cis*- **18**, *trans*- **19**, and dihydrostilbenes **20** and some *N*-arylbenzylamines **21** (Figure 4), and evaluated their cytotoxicity in five cancer cell cultures: A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. Several *cis*-stilbenes, structurally similar to CA-4, were highly cytotoxic in all five cell lines, and were also found to be active as inhibitors of tubulin polymerization. One of them, (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene **18a**, is very potent both as a tubulin polymerization inhibitor and as a cytotoxic agent. This substance is almost as potent as CA-4 as a tubulin polymerization inhibitor and even more with respect to cytotoxic activity [42]. It was ascertained to be approximately 140 times



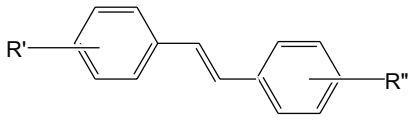
18

e.g. **a.** R' = 3,4,5-$(OMe)_3$; R'' = 4-OMe
b. R' = 3,4,5-$(OMe)_3$; R" = 3-OMe
c. R' = 3,4,5-$(OMe)_3$; R" = 2-OMe
d. R' = 3,4,5-$(OMe)_3$; R" = 4-OMe
e. R' = 3,4,5-$(OMe)_3$; R" = 2-Cl-4-OMe
f. R' = 3,4,5-$(OMe)_3$; R" = 4-OH
g. R' = 3,4,5-$(OMe)_3$; R" = 4-OAc
h. R' = 3,4-$(OMe)_3$; R" = 4-NO_2



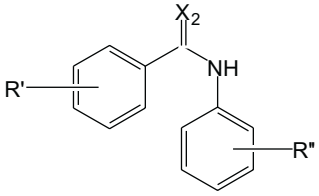
20

e.g. **a.** R' = 3,4,5-$(OMe)_3$; R'' = 4-OMe
b. R' = 3,4,5-$(OMe)_3$; R" = 3-OMe
c. R' = 3,4,5-$(OMe)_3$; R" = 2-OMe
d. R' = 3,4,5-$(OMe)_3$; R" = H
e. R' = 3,4,5-$(OMe)_3$; R" = 4-$NHCOCH_3$
f. R' = 3,4,5-$(OMe)_3$; R" = 4-OH
g. R' = 3,4,5-$(OMe)_3$; R" = 4-OAc
h. R' = 3,4-$(OMe)_3$; R" = 4-NMe_2
i. R' = 3,4-$(OMe)_3$; R" = H
j. R' = 2,3,4-$(OMe)_3$; R" = H
k. R' = 3,4,5-$(OMe)_3$; R" = 4-NH_2



19

e.g. **a.** R' = 3,4,5-$(OMe)_3$; R'' = 4-OMe
b. R' = 3,4,5-$(OMe)_3$; R" = 3-OMe
c. R' = 3,4,5-$(OMe)_3$; R" = 2-OMe
d. R' = 3,4,5-$(OMe)_3$; R" = H
e. R' = 3,4,5-$(OMe)_3$; R" = 2-Cl-4-OMe
f. R' = 3,4,5-$(OMe)_3$; R" = 4-OH
g. R' = 3,4,5-$(OMe)_3$; R" = 4-OAc
h. R' = 3,4-$(OMe)_3$; R" = H
i. R' = 2,3,4-$(OMe)_3$; R" = H
j. R' = 3,4,5-$(OMe)_3$; R" = 4-NMe_2
k. R' = 3,4,5-$(OMe)_3$; R" = 4-NH_2



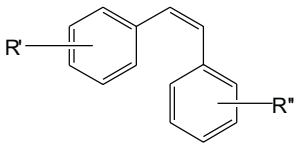
21

e.g. **a.** R' = 3,4,5-$(OMe)_3$; R" = 4-OMe; X_2 = O
b. R' = 3,4-$(OMe)_3$; R" = 3,4,5-$(OMe)_3$; X_2 = O
c. R' = 4-OMe; R" = 3,4,5-$(OMe)_3$; X_2 = O
d. R' = 3,4,5-$(OMe)_3$; R" = 4-OMe; X_2 = H_2
e. R' = 4-OMe; R" = 3,4,5-$(OMe)_3$; X_2 = H_2
f. R' = 4-OMe; R" = 3,5-$(OMe)_2$; X_2 = H_2

Figure 4.

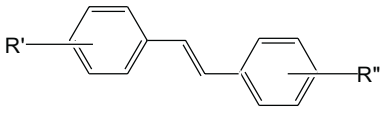
more toxic against HT-29 colon adenocarcinoma cells and about 10 times more toxic against MCF-7 breast carcinoma cells than CA-4.

In 1992, Cushman *et al.* [43] prepared a novel series of stilbenes (Figure 5) and tested them for cytotoxicity in five human cancer cell lines: A-549 non-small cell lung, MCF-7 breast, HT-29 colon, SKMEL-5 melanoma, and MLM melanoma. The *cis* stilbenes **22** proved to be cytotoxic in all five cell lines, with activity comparable to that of combretastatin A-4. These cytotoxic compounds were all potent inhibitors of tubulin polymerization. The corresponding *trans* stilbenes **23** were inactive as tubulin polymerization inhibitors and were significantly less cytotoxic in these five cancer cell lines. In the dihydro series, **24b**, **24c**, and **24f** were inactive as tubulin polymerization inhibitors, while **24a**, **24d,** and **24e** were active but much less than the corresponding *cis* isomers. The inactive compounds included the benzylisoquinoline series **26, 27** as well as the protoberberines **28** and **29**. All of the phenylcinnamic acid derivatives **25a–c** and **25d–f** were inactive in the tubulin polymerization inhibition assay.



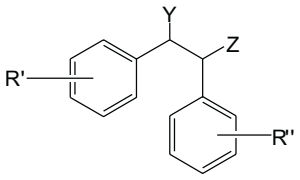
22

e.g.
- **a.** R' = 3,4,5-$(OMe)_3$; R" = 4-OEt
- **b.** R' = 3,4,5-$(OMe)_3$; R" = 4-OPr^n
- **c.** R' = 3,4,5-$(OMe)_3$; R" = 4-Me
- **d.** R' = 2,3,4-$(OMe)_3$; R" = 4-Et
- **e.** R' = 3,4,5-$(OMe)_3$; R" = 4-Bu
- **f.** R' = 3,4-$(OMe)_2$; R" = 4-OMe
- **g.** R' = 3,5-$(OMe)_2$; R" = 4-OMe
- **h.** R' = 3,4,5-$(OMe)_3$; R" = 4-OMe



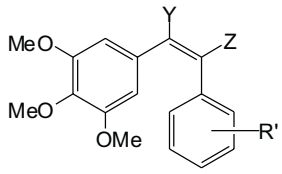
23

e.g.
- **a.** R' = 3,4,5-$(OMe)_3$; R" = 4-OEt
- **b.** R' = 3,4,5-$(OMe)_3$; R" = 4-SMe
- **c.** R' = 3,4,5-$(OMe)_3$; R" = 4-Me
- **d.** R' = 3,4,5-$(OMe)_3$; R" = 4-Et
- **e.** R' = 3,5-$(OMe)_2$; R" = 4-OMe
- **f.** R' = 3,4-$(OMe)_2$; R" = 4-OMe
- **g.** R' = 3,5-$(OMe)_2$; 4-OBn; R" = 4-OMe



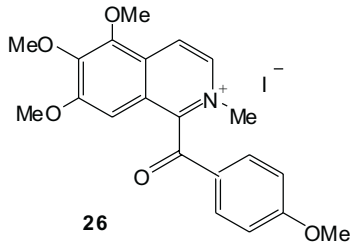
24

e.g.
- **a.** R' = 3,4,5-$(OMe)_3$; R" = 4-OEt; Y = H; Z = H
- **b.** R' = 3,4,5-$(OMe)_3$; R" = 4-OPr; Y = H; Z = H
- **c.** R' = 3,4,5-$(OMe)_3$; R" = 4-SMe; Y = H; Z = H
- **d.** R' = 3,4,5-$(OMe)_3$; R" =4-Me; Y = H; Z = H
- **e.** R' = 3,4,5-$(OMe)_3$; R" = 4-Et; Y = H; Z = H
- **f.** R' = 3,4,5-$(OMe)_3$; R" = 4-$O(CH_2)_2NMe_2$; Y = H; Z = H

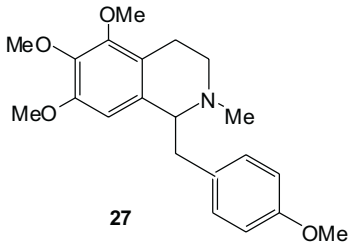


25

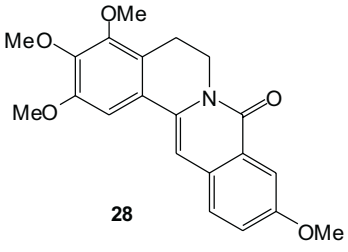
e.g.
- **a.** R' = 4-OMe; Y = COOH; Z = H
- **b.** R' = 3-OMe; Y = COOH; Z = H
- **c.** R' = 4-OMe; Y = H; Z = COOH
- **d.** R' = 4-OMe; Y = COOMe; Z = H
- **e.** R' = 3-OMe; Y = COOMe; Z = H
- **f.** R' = 4-OMe; Y = CONHMe; Z = H



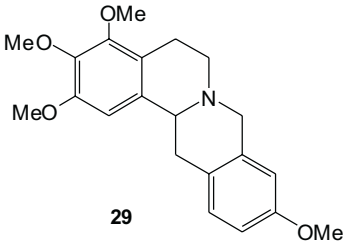
26



27



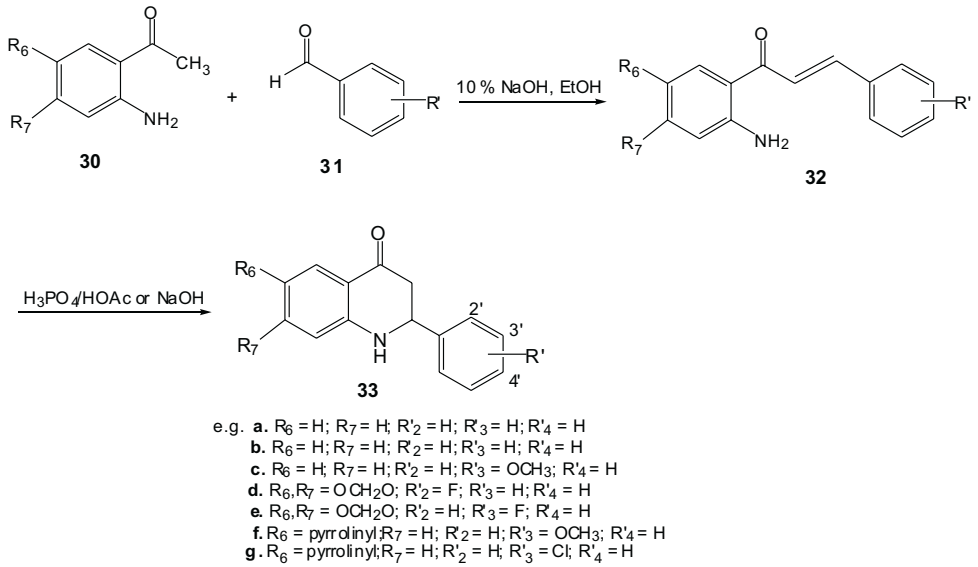
28

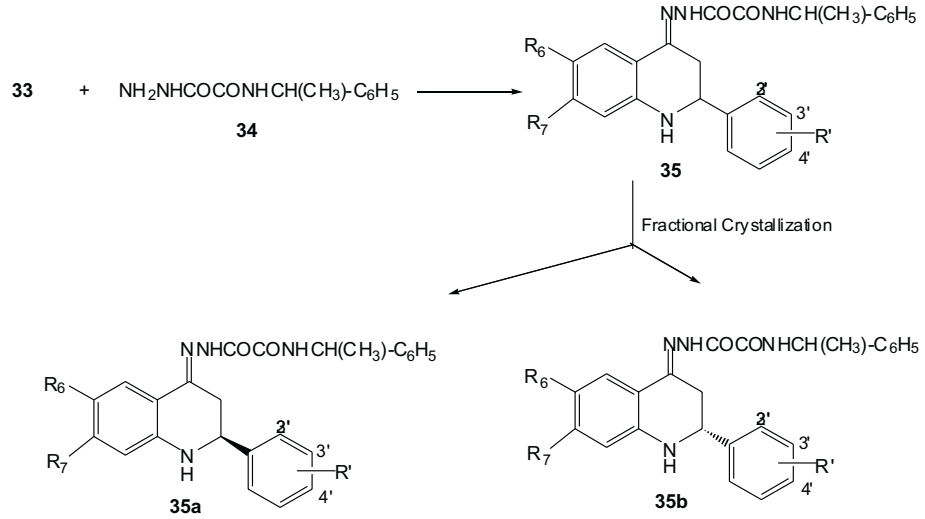


29

Figure 5.

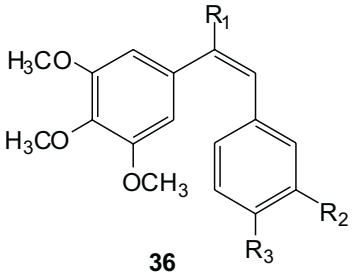
Xia *et al.* [44] synthesized a series of 6,7,2′,3′,4′-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones **33** and **35a,b** (Scheme 3), and the cytotoxic activity of these compounds was checked against human tumour cell lines, including ileocecal carcinoma (HCT-8), breast cancer (MCF-7), lung carcinoma (A-549), epidermoid carcinoma of the nasopharynx (KB), renal cancer (CAKI-1), and melanoma cancer (SKMEL-2). Most of the compounds **33a–f** showed potent cytotoxic and antitubulin effects. The most active individuals **33d**, **33e**, **33f** have activities comparable to those of colchicines, podophyllotoxin, and combretastatin A-4.





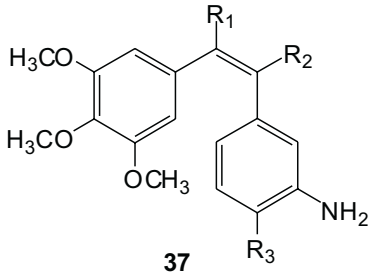
Scheme 3. General synthetic routes to 6,7,2′,3′,4′-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones derivatives [44].

Ohsumi *et al.* [45] described the synthesis of novel analogues of CA-4 and their cytotoxic effect against murine Colon 26 adenocarcinoma and inhibitory effect on tubulin polymerization. These analogues were modified both in the olefin part – **36**, **37** (Figure 6), and by replacement of the aryl substituents – **38** and **39**. Since CA-4 has limited solubility in water, its analogues were designed to improve solubility by introduction of an amine group to the molecule. Derivatives of CA-4 in which the phenolic hydroxy group was replaced by the amino function showed *in vitro* potent antitubulin activity and cytotoxicity against murine Colon 26 adenocarcinoma. Some of these compounds which were potent *in vitro* were evaluated in the murine tumour Colon 26 *in vivo*. Among these, **36aHCl**, **37aHCl**, and **37bHCl** (Figure 6) revealed significant antitumour activity in the animal model, while CA-4 was ineffective in the test. Two of the compounds, **36aHCl** and **37aHCl**, have been selected for further evaluation in two murine tumour models (Colon 38 and 3LL) and human xenografts HCT-15. These compounds also showed potent antitumour activity, comparable or superior to that of Cisplatin. Cisplatin did not show antitumour activity against Colon 38, 3LL, or



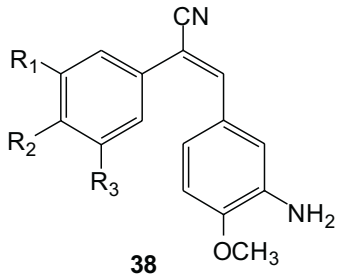
36

e.g. **a.** R_1 = CN; R_2 = NH_2; R_3 = OCH_3
b. R_1 = CN; R_2 = H; R_3 = OCH_3
c. R_1 = CH_2OH; R_2 = H; R_3 = OCH_3



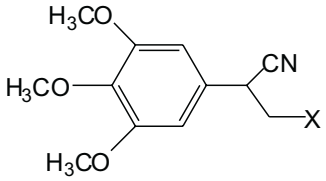
37

e.g. **a.** R_1 = H; R_2 = H; R_3 = OCH_3
b. R_1 = H; R_2 = H; R_3 = CH_3
c. R_1 = H; R_2 = $CONH_2$; R_3 = OCH_3



38

e.g. **a.** R_1 = H; R_2 = H; R_3 = H
b. R_1 = H; R_2 = OCH_3; R_3 = H
c. R_1 = OCH_3; R_2 = OCH_3; R_3 = OCH_3



39

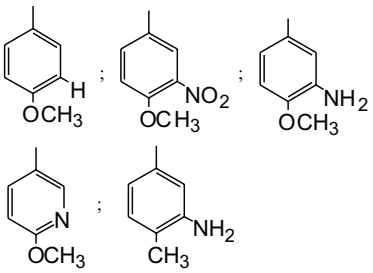
X = 

Figure 6.

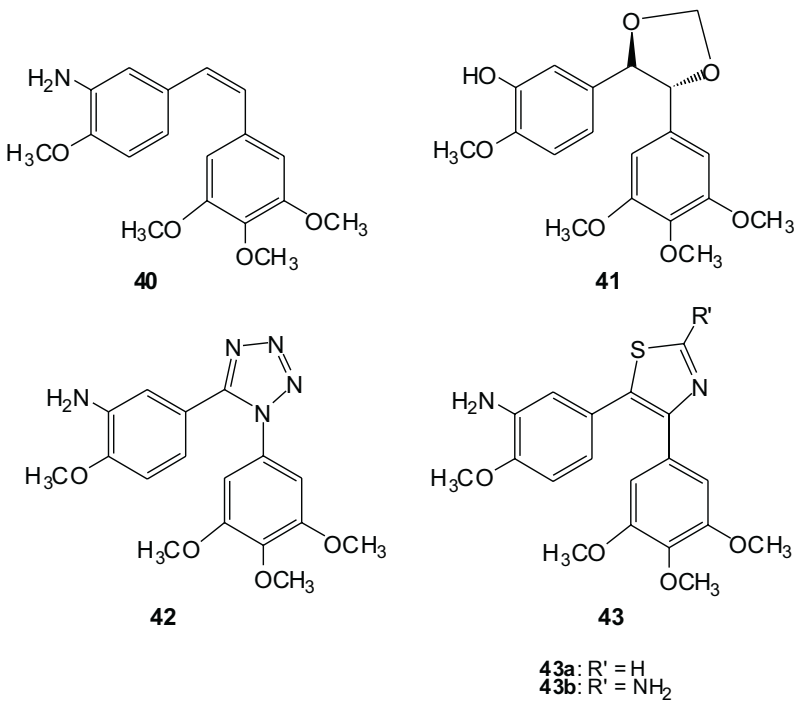


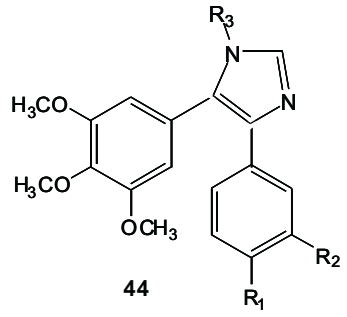
Figure 7.

HCT-15. Ohsumi *et al.* [45] reported that replacement of the methoxy group in CA-4 with the amino group **40** (Figure 7) resulted in a significant increase in water solubility and *in vivo* antitumour activity against murine solid tumors when it was administered intravenously.

Shirai *et al.* [46] have reported that the *cis* carbon-carbon double bond in CA-4 could be replaced by dioxolane in **41** (Figure 7) without loss of biological activity. Ohsumi *et al.* [19] also demonstrated that a tetrazole **42** or a thiazole **43a** and **43b** ring could replace the *cis* double bond to maintain potent cytotoxicity. All three compounds **42**, **43a** and **43b** showed antitumour activity against the colon 26 murine tumour when given intravenously (Figure 7). Medarde *et al.* [47] reported the synthesis and cytotoxic evaluation of indole-bridged CA-4 analogues. Gwaltney *et al.* [48] have proved that the *cis* double bond in CA-4 could also be replaced with a sulfonate without losing antitubulin properties. Recently Wang *et al.* [49] described a series of compounds with heterocycles such as imidazole **44**, pyrazole **45**, and oxazole **46** in place of the *cis* double bond in CA-4 (Figure 8). Substituted tosylmethyl isocyanides were found to be the key intermediates in the construction of these heterocycles. Cytotoxicities of the heterocycle-based CA-4 analogues were evaluated against NCI-H460 and HCT-15 cancer cell lines. It appears that the replacement of the 3-hydroxy-4-methoxyphenyl in CA-4 by 3-amino-4-methoxyphenyl and *N*-methyl-indol-5-yl is the most promising modification of CA-4. Insertion of 4,5-disubstituted imidazole residue instead of the *cis* double bond in CA-4 is also a good way of improving the phar-

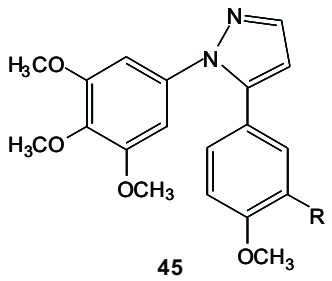
macological properties of combretastatin. Compounds **44a** and **44b** (Figure 8) were active in mice against murine M5076 reticulum sarcoma cell line with ILS values of 38.5 and 40.5%, respectively.

In the course of a systematic investigation of the secondary metabolites from South African plants, the researchers isolated a phenolic glucosides **47–49** (Figure 9) which possess promising biological activity [50]. The phenolic glucosides **47** (combretastatin A-1 2′-β-D-glucoside) and **48** (combretastatin B-1 2′-β-D-glucoside), extracted from *Combretum kraussi*, have shown antimitotic activity. The phenolic glucoside **49** (resveratrol 3-β-D-glucoside), isolated from *Erythrophleum lasianthum*, displayed antiplatelet aggregation activity. Since these compounds are available from natural sources only in small amounts, the synthesis of glucosides **47–49** and related compounds has been undertaken to provide larger quantities for further biological evaluation. Orsini *et al.* [50] reported the synthesis of biologically active polyphenolic glycosides **47–49**. These compounds were synthesized *via* Wittig reactions followed by glucosylation under phase-transfer catalysis conditions. Several of the synthesized compounds were tested with respect to different types of biological activities (cytostatic, cytotoxic, antimitotic, neurotoxic and antiplatelet aggregation activity) [50].



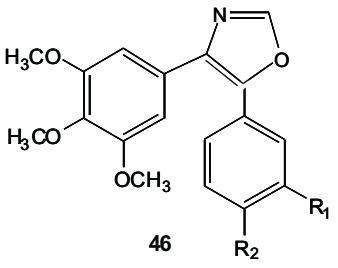
44

a: R_1, R_2 = CHCHN(CH_3), R_3 = CH_3
b: R_1 = OCH_3, R_2 = NH_2, R_3 = CH_3



45

R = NH_2



46

e.g. **a:** R_1 = NH_2, R_2 = OCH_3
b: R_1,R_2 = CHCHN(CH_3)
c: R_1 = H, R_2 = $N(CH_3)_2$

Figure 8.

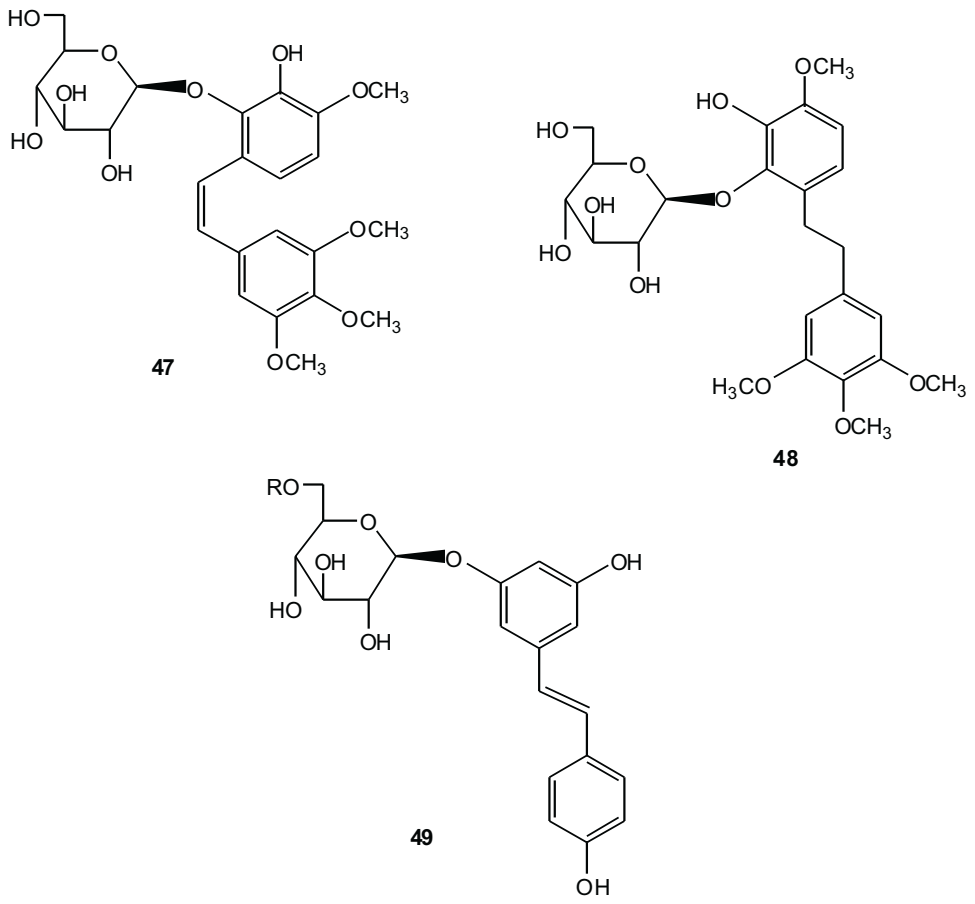
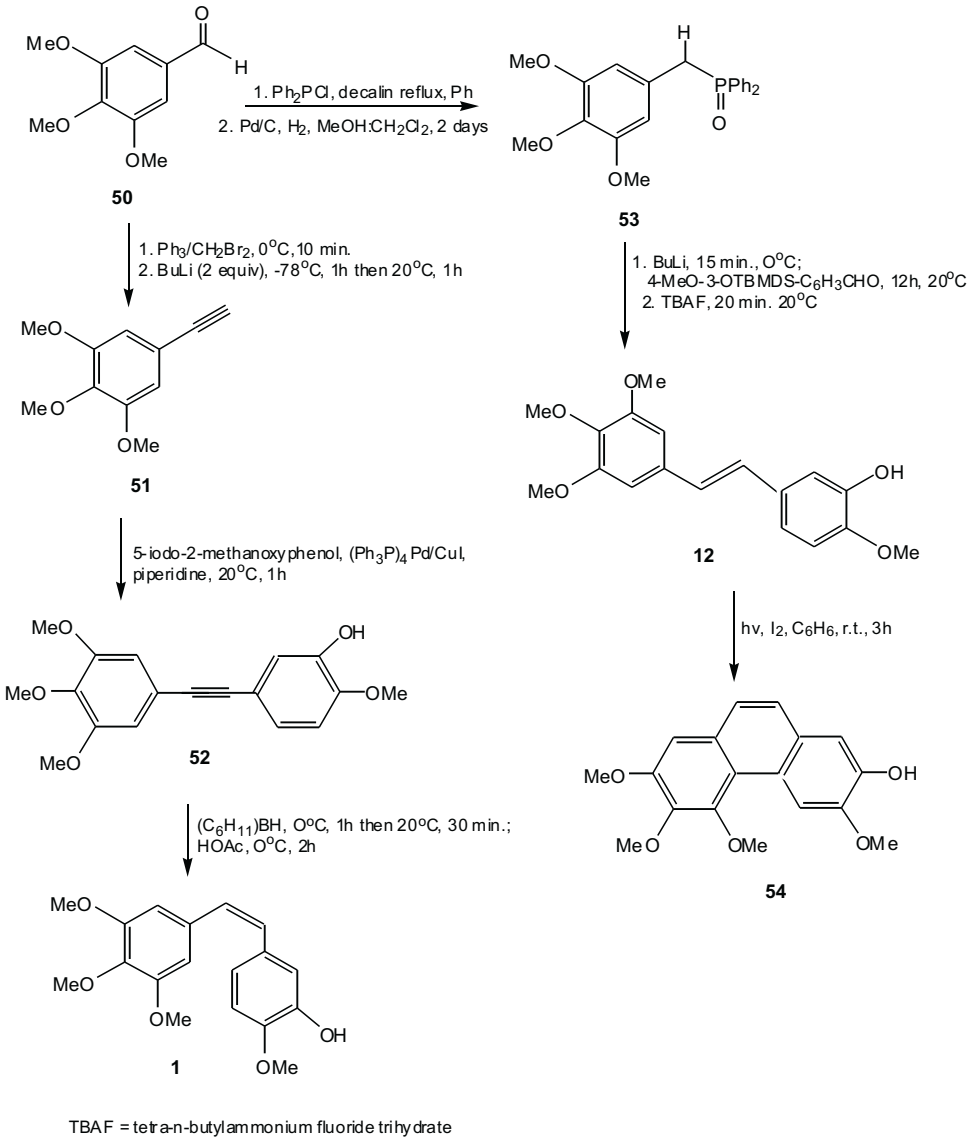


Figure 9.

Lawrence *et al.* [51] described a convenient method of the synthesis of (*E*) **12** and (*Z*)-combretastatin A-4 **1** and phenanthrene **54** from *Combretum caffrum* (Scheme 4). The synthesis of the *trans* stilbene *E*-combretastatin A-4 has been achieved *via* a Horner-Wittig reaction of (3,4,5-trimethoxybenzyl)diphenylphosphine oxide, and its isomer *Z*-combretastatin A-4 was prepared by the hydroboration/protonation of a diaryl-alkyne.

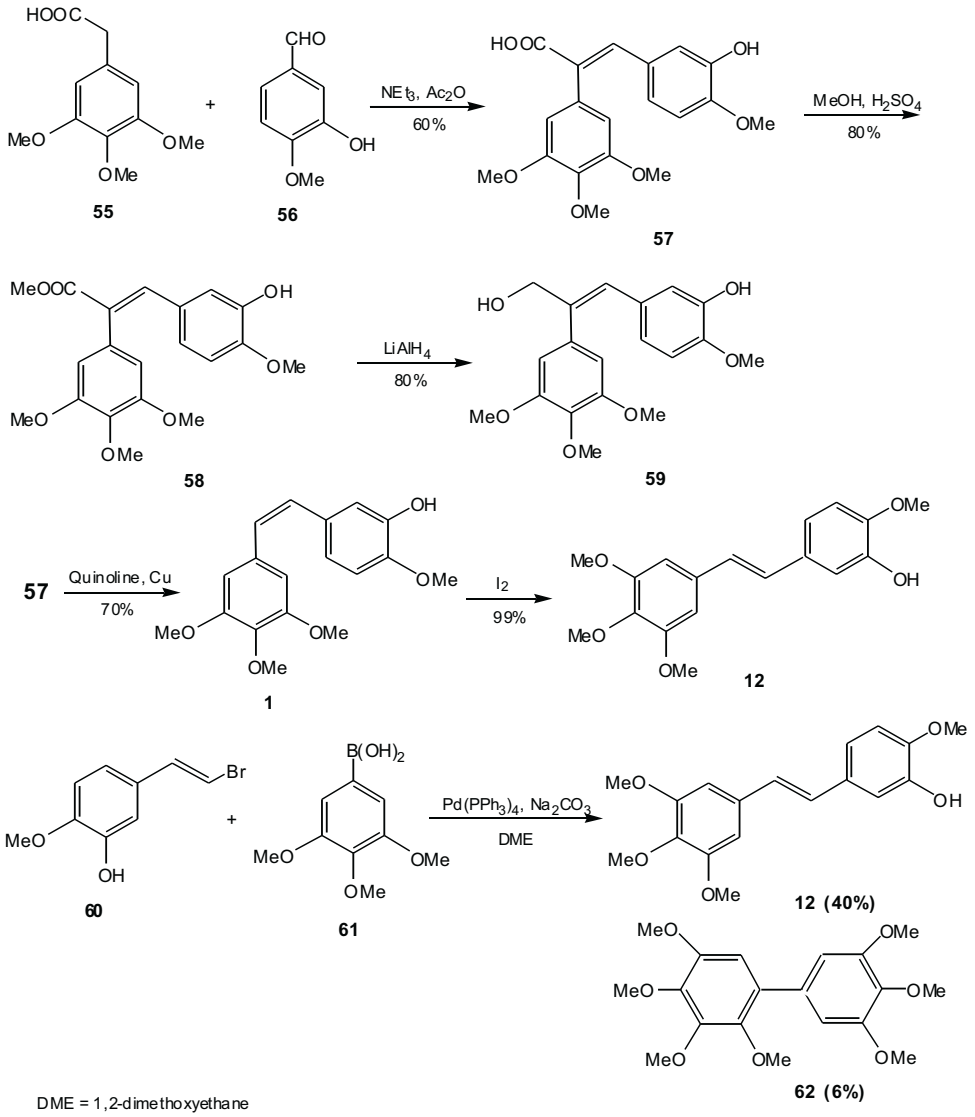
Most of the chemical ways of the combretastatin and its analogue preparation utilize the Wittig reaction. The route developed by Pettit *et al*. is representative [13]. The Wittig method has, however, serious inconveniences. Firstly, owing to the poor stereoselectivity, the yield of the desired *Z* isomer is reduced, and, secondly, the crude product as a multicomponent mixture require separation and careful purification. The isomers of combretastatin A-4 are difficult to separate, even by chromatography. Furstner *et al.* [52] have developed a stereoselective synthesis of analogues of CA-4 based on the Lindlar-type semihydrogenation of an alkyne precursors (assembled by 9-MeO-9-BBN-mediated Suzuki-type cross-coupling). This reaction was *Z* selective



Scheme 4. Synthesis of (*E*) and (*Z*)-combretastatins A-4 and a phenanthrene from *Combretum caffrum* [51].

and only a small amount of the corresponding alkane occurred (*Z*-combretastatin A-4: alkane, 86:14). This methodology has been developed further, eliminating the use of protecting groups and constructing the alkene moiety with greater stereoselectivity [51]. However, both these methods [51,52] have also shortcomings and are not ideal for large-scale applications.

In 2001 Gaukroger *et al.* [53] described novel stereoselective synthesis of *cis* and *trans* isomers of combretastatin A-4. The method uses the Perkin condensation of



Scheme 5. Novel syntheses of *cis* and *trans* isomers of combretastatin A-4 [53].

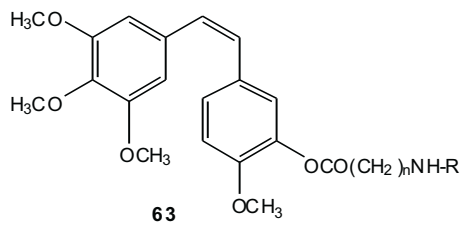
3,4,5-trimethoxyphenylacetic acid **55** and 3-hydroxy-4-methoxybenzaldehyde **56** followed by decarboxylation of the cinnamic acid intermediate using copper and quinoline. The iodine-catalyzed isomerization of the *Z* isomer **1** results in complete conversion to the *E* isomer **12**. The Suzuki cross-coupling of an aryl boronic acid **61** and vinyl bromide **60** has also been successfully employed to produce both *Z* and *E* isomers of combretastatin A-4 stereoselectively (Scheme 5). Both methods are far superior to the five-step Wittig synthesis in which both isomers are produced nonstereoselectively.

CONCLUSIONS

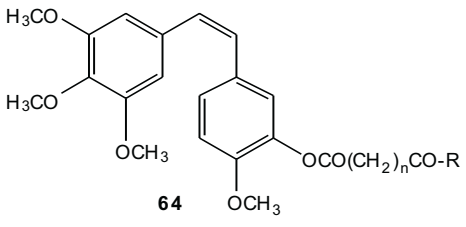
Combretastatin A-4 (CA-4), a derivative of *Z*-stilbene, a natural product isolated from the South African tree *Combretum caffrum*, exhibits a strong antitubulin activity by binding to the colchicine binding site of tubulin. The IC_{50} of CA-4 against tubulin polymerization ranges from 0.53 to 3.0 μM [54]. It exhibits a potent cytotoxicity against a broad spectrum of human cancer lines, including those that are multidrug resistance (MDR) pump, a cellular pump which rapidly transports out foreign molecules. Combretastatin A-4 shows potent antiangiogenic activity [55–60] and the derived sodium phosphate prodrug **13c** is also a powerful *in vivo* inhibitor of tumour vascularization. CA-4 is now under second phase clinical trials.

However, the very limited water solubility of this compound complicates the drug application. Some improved derivatives of the combretastatin A-4 at 3′-phenol group, *e.g.* the sodium salt, potassium salt and hemisuccinic acid ester are still poorly soluble in water [23]. Transformation of phosphate ester into ammonium **13a**, potassium **13b**, and sodium salts **13c** increased solubility in water. Currently, the prodrug **13c** [27,57] is under clinical evaluation; the first phase of human cancer clinical trials started in 1998.

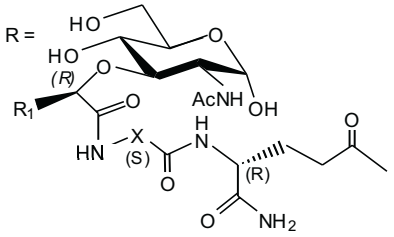
Higher efficiency of the cancer therapy could be achieved when the antiangiogenic agents were assisted with other antitumor treatments, *e.g.* chemotherapy, irradiation, surgery or photodynamic therapy. A very important problem concerns the introduction of the antiangiogenic drugs into clinical practice. Unlike cytostatics, antiangiogenic drugs do not exhibit tumour growth inhibition. Therefore the evaluation of their effect in early clinical treatment is very difficult. Nevertheless, a proper combination of drugs with different action of mechanisms is able to improve the efficiency of tumour therapy.



63



64



R_1 = H,CH_3; X = Ala,Val

R = Thr-Lys-Pro-Arg ; Arg-Pro-Lys-ThrOCH_3 ;
Thr-Lys(Val)-Pro-Arg ; Arg-Pro-Lys(Val)-ThrOCH_3 ;
Arg-Pro-Lys(Ala)-ThrOCH_3

Figure 10.

Continuing our search for potential anticancer drug candidates [61–64], we synthesized new combretastatin A-4 analogues containing chemically bonded immunomodulators such as muramyl dipeptide or tuftsin and its derivatives. We hope that the conjugation of immunomodulators like muramyl dipeptide (MDP) **63** or tuftsin **64** with combretastatin A-4 (CA-4) (Figure 10) could not only increase the anticancer activity of both compounds comprising such conjugates, but also improve the therapeutical properties of combretastatin A-4. The synthesis of these conjugates and their biological results will be reported in due course.

Acknowledgments

This work was supported by the Polish State Committee for Scientific Research (Grant No. 3 P05F 014 25).

REFERENCES

1. Folkman J., *N. Engl. J. Med.*, **285**, 1182 (1971).
2. O'Reilly M.S., Boehm T., Shing Y., Fukai N., Vasios G., Lane W.S., Flynn E., Birkhead J.R., Olsen B.R. and Folkamn J., *Cell*, **88**, 277 (1997).
3. Harris A.L., *Lancet*, **351**, 1598 (1998).
4. Gourley M. and Williamson J.S., *Curr. Pharmacol. Design*, **6**, 417 (2000).
5. Aiello L.P., *Nature Med.*, **6**, 379 (2000).
6. Liekens S., De Clercq E. and Neyts J., *Biochemical Pharmacology*, **61**, 253 (2001).
7. Opolski A. and Wietrzyk J., *Post. Hig. Med. Dośw.*, **55**, 369 (2001).
8. Rowinsky E.K., Donehower R.C., In *Cancer, Principle and Practice of Oncology*, 5th ed.; DeVita V.T., Jr, Hellman S., Rosenberg S.A., eds.: Lippincott-Raven: Philadelphia, pp. 467–483 (1997).
9. Iwasaki S., *J. Pharmacol. Soc. Jpn*., **118**, 111 (1998).
10. Li Q., Sham H.L., Rosenberg S.H., Antimitotic Agents. In *Annual Reports in Medicinal Chemistry*; Doherty A.M., Ed.; Academic Press: San Diego, Vol. 34, pp. 139–148 (1999).
11. Shi Q., Chen K., Morris-Natschke S.L. and Lee K.H., *Curr. Pharm. Des.,* **4**, 219 (1998).
12. Jordan A., Hadfield J.A., Laurence N.J. and McGown A.T., *Med. Chem. Rev.,* **18**, 259 (1998).
13. Pettit G.R., Singh S.B., Niven M.L., Hamel E. and Schmidt J.M.,*J. of Natural Products*, **50**, 119 (1987).
14. Pettit G.R. and Singh S.B., *Can. J. Chem.*, **65**, 2390 (1987).
15. Pettit G.R., Singh S.B., Hamel E., Lin C.M., Alberts D.S. and Garcia-Kendall D., *Experientia*, **45**, 209 (1989).
16. Pettit G.R., Singh S.B. and Cragg G.M., *J. Org. Chem.*, **50**, 3404 (1985).
17. Singh S.B. and Pettit G.R., *J. Org. Chem.*, **54**, 4105 (1989).
18. Pettit G.R., Singh S.B., Boyd M.R., Hamel E., Pettit R.K., Schmidt J.M. and Hogan F., *J. Med. Chem.*, **38**, 1666 (1995).
19. Ohsumi K., Nakagawa R., Fukuda Y., Hatanaka T., Morinaga Y., Nihei Y., Ohishi K., Suga Y., Akiyama Y. and Tsuji T., *J. Med. Chem.*, **41**, 3022 (1998).
20. Brown R.T., Fox B.W., Hadfield J.A., McGown A.T., Mayalarp S.P., Pettit G.R. and Woods J.A., *J. Chem. Soc. Perkin Trans. I*, **5**, 577 (1995).
21. Woods J.A., Hadfield J.A., Pettit G.R., Fox B.W. and McGown A.T., *Br. J. Cancer*, **71**, 705 (1995).
22. Bedford S.B., Quarterman C.P., Rathbone D.L., Slack J.A., Griffin B.J. and Stevens M.F.G., *Bioorg. Med. Chem. Lett*., **6**, 157 (1996).
23. Pettit G.R., Temple C., Jr, Narayanan V.L., Varma R., Simpson M.J., Boyd M.R., Rener G.A. and Bansal N., *Anti-Cancer Drug Design*, **10**, 299 (1995).
24. Chaplin D.J., Pettit G.R. and Hill S.A., *Anticancer Res.,* **19**, 189 (1999).

25. Dziba J.M., Marcinek R., Venkataraman G., Robinson J.A. and Ain K.B., *Thyroid*, **12**, 1063 (2002).
26. Hua J., Sheng Y., Pinney K.G., Garner C.M., Kane R.R., Prezioso J.A., Pettit G.R., Chaplin D.J. and Edvardsen K., *Anticancer Res.,* **23**, 1433 (2003).
27. Pettit G.R. and Rhodes M.R., *Anti-Cancer Drug Design*, **13**, 183 (1998).
28. Kerekes P., Sharma P.N., Brossi A., Chignell C.F. and Quinn F.R, *J. Med. Chem*., **28**, 1204 (1985).
29. Dumont R., Brossi A., Chignell C.F., Quinn F.R. and Suffness M.A., *J. Med. Chem*., **30**, 732 (1987).
30. Hamel E., Ho H.H., Kang G.J. and Lin C.M., *Biochem. Pharmacol.*, **37**, 2445 (1988).
31. Muzaffar A., Brossi A., Lin C.M. and Hamel E.,*J. Med. Chem.*, **33**, 567 (1990).
32. Zavala F., Guenard D., Robin J.P. and Brown E., *J. Med. Chem*., **23**, 546 (1980).
33. Van der Eycken J., Bosmans J.P., Van Haver D., Vandewalle M., Hulkenberg A.,Veerman W. and Nieuwenhuizen R., *Tetrahedron Lett.*, **30**, 3873 (1989).
34. Klein L.L., Yeung C.M., Chu D.T., McDonald E.J., Clementy J.J. and Plattner J.J.,*J. Med. Chem.*, **34**, 984 (1991).
35. Wang R.W.J., Rebhun L.I. and Kupchan S.M., *Cancer Res.*, **37**, 3071 (1977).
36. Tomioka K., Ishiguro T., Mizuguchi H., Komeshima N., Koga K., Tsukagoshi S., Tsuruo T., Tashiro T., Tanida S. and Kishi T., *J. Med. Chem*., **34**, 54 (1991).
37. Itoh Y., Brossi A., Hamel E. and Lin C.M., *Helv. Chim. Acta*, **71**, 1199 (1988).
38. Batra J.K, Jurd L. and Hamel E., *Mol. Pharmacol.*, **27**, 94 (1985).
39. Batra J.K., Kang G.J., Jurd L. and Hamel E., *Biochem. Pharmacol.*, **37**, 2595 (1988).
40. Edwards M.L., Stemerick D.M. and Sunkara P.S., *J. Med. Chem*., **33**, 1948 (1990).
41. Lin C.M., Singh S.B., Chu P.S., Dempcy R.O., Schmidt J.M., Pettit GR. and Hamel E.,*Mol. Pharmacol.*, **34**, 200 (1988).
42. Cushman M., Nagarathnam D., Gopal D., Chakraborti A.K., Lin Ch.M. and Hamel E., *J. Med. Chem.*, **34**, 2579 (1991).
43. Cushman M., Nagarathnam D., Gopal D., Hu-Ming He., Lin Ch.M. and Hamel E.,*J. Med. Chem.*, **35**, 2293 (1992).
44. Xia Y., Yang Z.Y., Xia P., Bastow K.F., Tachibana Y., Kuo S.Ch., Hamel E., Hackl T. and Lee K.H., *J. Med. Chem*., **41**, 1155 (1998).
45. Ohsumi K., Hatanaka T., Fujita K., Nakagawa R., Fukuda Y., Niher Y., Suga Y., Morinaga Y., Akiyama Y. and Tsuji T., *Bioorg. Med. Chem. Lett.*, **8**, 3153 (1998).
46. Shirai R., Takayama H., Nishikawa A., Koiso Y. and Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **8**, 1997 (1998).
47. Medarde M., Ramos A.C., Caballero E., de Clairac R.P.L., Lopez J.L., Gravalos D.G. and Feliciano A.S., *Bioorg. Med. Chem. Lett.*, **9**, 2303 (1999).
48. Gwaltney S.L., Imade H.M., Barr K.J., Li Q., Gehrke L., Credo R.B., Warner R.B., Lee J.Y., Kovar P., Wang J., Nukkala M.A., Zieliński N.A., Frost D., Ng S.C. and Sham H.L., *Bioorg. Med. Chem. Lett.*, **11**, 871 (2001).
49. Wang L., Woods K.W., Li Q., Barr K.J., McCroskey R.W., Hannick S.M., Gherke L., Credo R.B., Hui Y.H., Marsh K., Warner R., Lee J.Y., Zieliński-Mozng N., Frost D., Rosenberg S.H. and Sham H.L., *J. Med. Chem*., **45**, 1697 (2002).
50. Orsini F., Pelizzoni F., Bellini B. and Miglierini G., *Carbohydr. Res*., **301**, 95 (1997).
51. Lawrence N.J., Ghani F.A., Hepworth L.A., Hadfield J.A., McGown A.T. and Pritchard R.G., *Synthesis*, 1656 (1999).
52. Furstner A. and Nikolakis K., *Liebigs Ann. Chem*., 2107 (1996).
53. Gaukroger K., Hadfield J.A., Hepworth L.A., Lawrence N.J. and McGown A.T.,*J. Org. Chem*.,**66**, 8135 (2001).
54. Pettit G.R., Lippert J.W., Herald D.L., Hamel E. and Pettit R.K., *J. Nat. Prod.*, **63**, 969 (2000).
55. Dark G.G., Hill S.A., Prise V.E., Tozer G.M., Pettit G.R. and Chaplin D.J.,*Cancer Res.*, **57**, 1829 (1997).
56. Lin C.M., Ho H.H., Pettit G.R. and Hamel E., *Biochemistry*, **28**, 6984 (1989).
57. Pettit G.R., Toki B., Herald D.L., Verdier-Pinard P., Boyd M.R. and Hamel E.,*J. Med. Chem.*, **41**, 1688 (1998).
58. Iyer S., Chaplin D.J., Rosenthal D.S., Boulares A.H., Li L.Y. and Smulson M.E., *Cancer Res.*, **58**, 4510 (1998).
59. Dark G.G., Hill S.A., Prise V.E., Tozer G.M., Pettit G.R. and Chaplin D.J.,*Cancer Res.*, **57**, 1829 (1997).

60. Tozer G.M., Prise V.E., Wilson J., Locke R.J., Vojnovic B., Stratford M.R., Dennis M.F. and Chaplin D.J., *Cancer Res.,* **59**, 1626 (1999).
61. Dzierzbicka K., Kołodziejczyk A.M., Wysocka-Skrzela B., Myśliwski A. and Sosnowska D., *J. Med. Chem.*, **44**, 3606 (2001).
62. Sosnowska D., Myśliwski A., Dzierzbicka K. and Kołodziejczyk A., *Biotherapy*, **10**, 161 (1997).
63. Dzierzbicka K. and Kołodziejczyk A.M., *J. Med. Chem.*, **46**, 183 (2003).
64. Dzierzbicka K., Trzonkowski P., Sewerynek P. and Myśliwski A., *J. Med. Chem.*, **46**, 978 (2003).