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Synthesis of Combretastatin A-4 analogs and their biological activities

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Abstract

Combretastatin A-4 (CA-4) is a natural product, which consists of two phenyl rings, linked by an ethylene bridge. CA-4, inhibitor of polymerization of tubulin to microtubules, possesses a strong antitumor and anti-vascular properties both *in vitro* and *in vivo*. Previous studies showed that disodium phosphate salt of CA-4, a water-soluble prodrug is well tolerated at therapeutically useful doses. However, it should be noted that the *cis*-configuration of the double bond and the 3,4,5-trimethoxy group on ring A is necessary for the biological activity of CA-4. While, structure of CA-4 renders the compound readily susceptible to isomerization, which reduces the potency and bioavailability. To circumvent this problem, a lot of scientists in the world synthesized a series of *cis*-restricted CA-4 analogues, where the double bond have been replaced by introduction of non-heterocyclic groups or heterocyclic groups like β-lactam and oxadiazole. This paper reviews the most important approaches in analogs of combretastatin synthesis and presents structure-reactivity relationships for these compounds.

Key Words:

Combretastatin A-4; CA-4; Inhibitors of angiogenesis; Synthesis; Biological activity; Cancer therapy.

1. INTRODUCTION

In the last two decades many anticancer compounds were received, however, a special attention is paid to the ones that cause the reorganization of microtubules [1]. Microtubules are fibrous, cylindrical tubes having a diameter of 25-26 nm. They are formed by polymerization of tubulin proteins and represent one of the components of the cytoskeleton [2]. Microtubules Play a central role in the functioning of cells, influence cell division, motility, intracellular transport direction, to maintain cell shape, arrangement and movement of organelles, and vesicles and cytosolic proteins. For the maintenance of normal structure they are responsible for polymerization and depolymerization processes that extend over the ends of the filaments. Some biologically active compounds is influence the poles of tubulin, which can cause excessive microtubule polymerization or its inhibition. Microtubules are attractive for pharmacological target killing of tumor cells [3,4].

Colchicine **1** (Fig. **1**) was the first tubulin-binding agent noted to have some antivascular action, producing hemorrhagic necrosis in experimental tumours that resembled that produced by bacterial toxins [3]. Furthermore, it was noted that the endothelial cells of growing capillaries appeared sensitive to its toxic actions [4].

Combretastatin 2 (Fig. 1) was isolated from the bark of an African willow Combretum caffrum. Although it exists as two isomers, only the cis isomer exhibits a biological activity and it is a potent inhibitor of tumor cell growth. Combretastatin has antiangiogenic effects by inhibiting tubulin polymerization leading to the breakdown of microtubules [5-7]. CA-4 induces apoptosis of proliferating endothelial cells of the tumor [5,8].

Pettit *et al.* [9-19], during their many years of research, they have isolated compounds from the group of combretastatins **2**, **3-5**, **6**, **7-10** (Fig. **1**) and demonstrated their anti-cancer properties. The strongest activity was characterized by the CA-4 **2**. Combretastatin A-4 is made up of two aryl rings connected by an ethylene bridge [8].

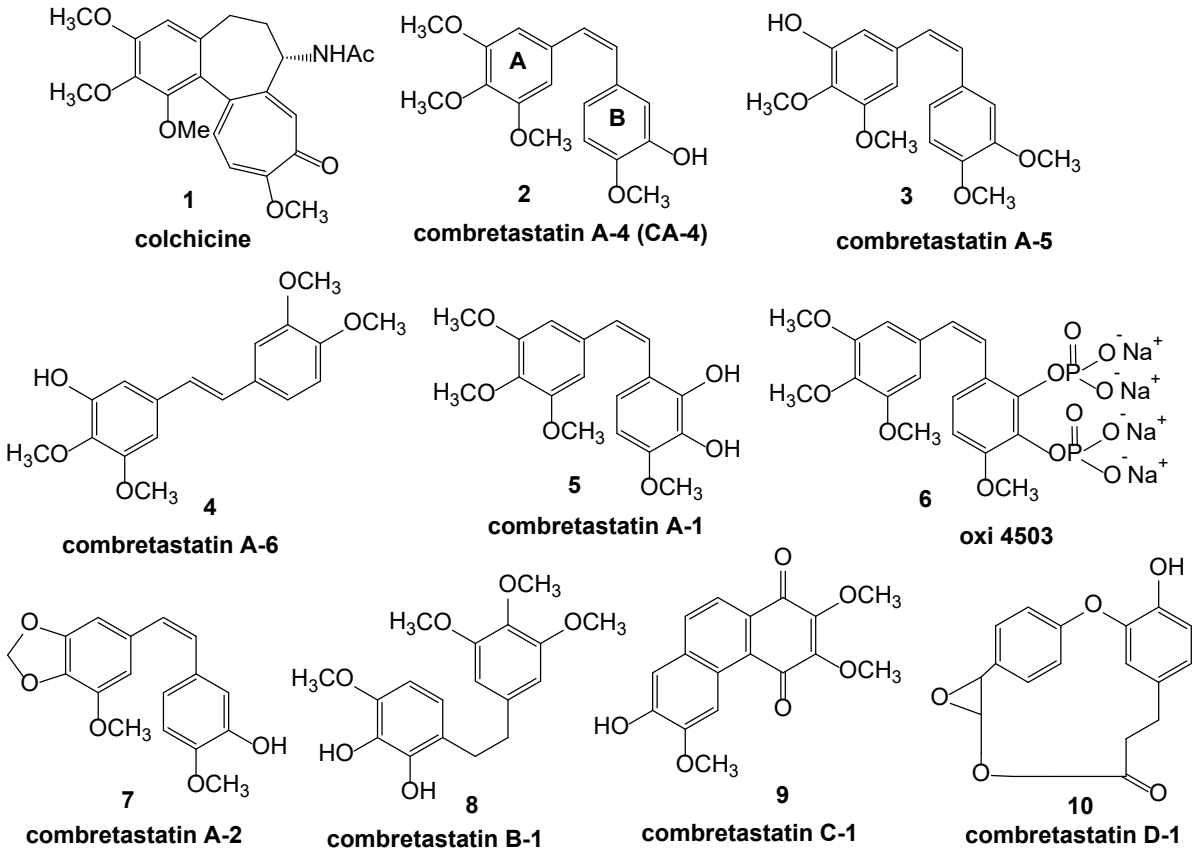


Fig. (1). Combretastatin and related compounds isolated from the bark of the South African tree *Combretum caffrum* [20].

Combretastatin despite the fact that showed significant biological activity *in vitro* was not acceptable for clinical studies because of low solubility in water, which reduces the efficacy of the compound *in vivo* [20].

2. COMBRETASTATIN A-4 ANALOGS

2.1. Modification of double bond of combretastatin A-4

Lee and co-workers [21] presented the synthesis of hydroxyethyl-analogs of combretastatin A-4 (CA-4) **11** that contain the 1-(1'-hydroxyethyl)-1-(3",4",5"-trimethoxyphenyl)-2-(substituted phenyl)ethene (Fig. **2**). Derivatives **11** were prepared in two steps from the respective benzaldehyde [21].

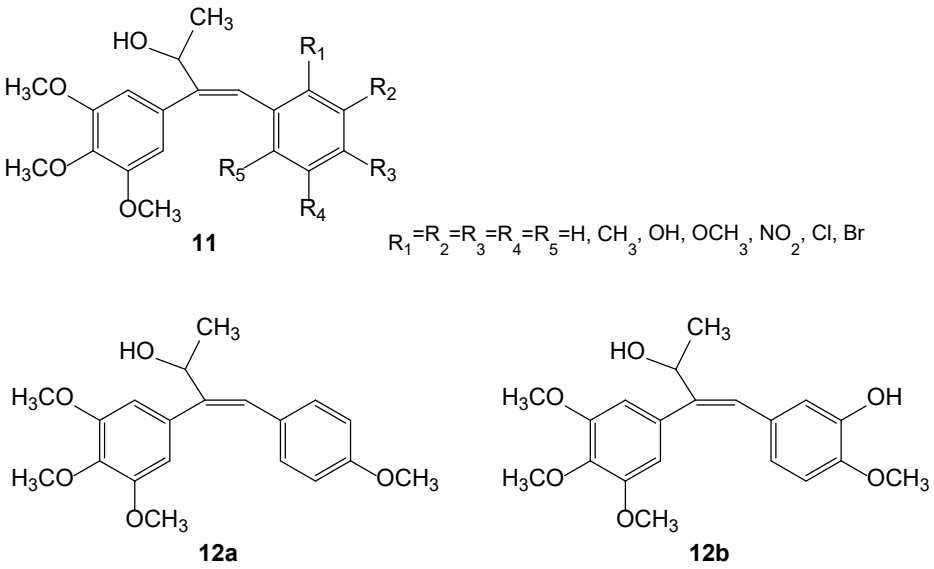


Fig (2). Structure of hydroxyethyl-analogs of combretastatin A-4 (CA-4) [21].

All synthesized compounds **11** were tested on biological activities against L1210 and B16 cells (murine lymphoma and melanoma respectively) using a 72h continuous exposure MTT assay. The most active were derivatives **12a** (IC_{50} = 3.9 μM for L1210 and IC_{50} = 17,5 μM for B16) and **12b** (IC_{50} = 4.1 μM for L1210 and IC_{50} = 16.1 μM for B16). Other analogs exhibited lower activity or were inactive. Studies revealed that substitution at the 4-position is significant, both size and electronic characteristic strongly influenced potency, and the highest cytotoxicity gave methoxy group. Additionally, substitution on the 3-position further affected the potency of the compounds. For instance, compound **12b** containing a hydroxy group on the 3-position and a methoxy group on the 4-position, was similarly active as compound **12a**. But if the 3-position contained bulkier substituent such as nitro or methoxy one, activity decreased clearly. Derivative **12b**, which hold a substitution pattern the closest to CA-4, was tested also in terms of its mechanism of action, aqueous solubility, and tested *in vivo* using DBA2 female mice that were inoculated with L1210 mouse lymphocytic leukemia cells.

Analogue **12b** demonstrated promising antitumor activity in mice with no toxicity. In addition, compound **12b** showed a much greater aqueous solubility than CA-4 [21].

Babu group [22] synthesized new acetyl-CA-4 analogs **15a-d** (Fig. **3**), which contained 3,4,5-trimethoxyphenyl group, and a variety of aromatic moieties instead of ring B. The compounds were prepared by Claisen-Schmidt condensation using 3,4,5-trimethoxy-phenylacetone **13**, aldehydes (**14a-d**) and suitable catalysts such as piperidine and benzoic acid (Scheme **1**) [22].

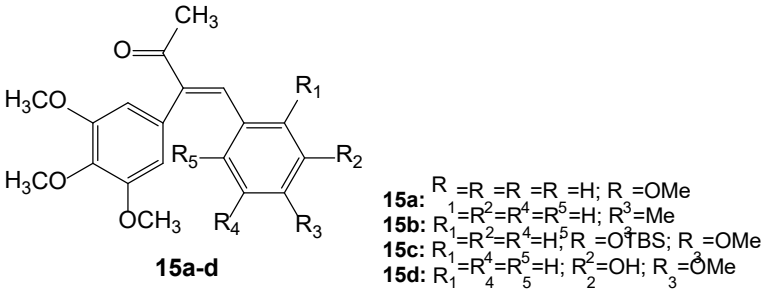
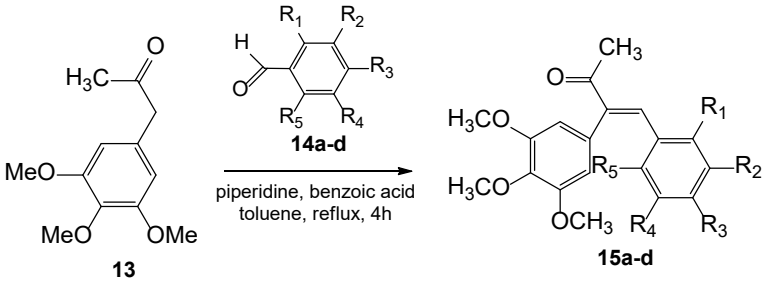


Fig. (3). Novel acetyl combretastatin analogs reported herein **15a-d** [22].



Scheme 1. Synthesis of acetyl-CA-4 analogs **15a-d** [22].

It was found that the conformation of the obtained compounds was similar to those observed in the X-ray structure and molecular formulas of combretastatin A-4, suggested that the products **15a-d** were able to demonstrate similar tubulin-targeting mechanism. During the tests it was also found that the introduction of a methyl group to CA-4 increased the water

solubility of the obtained compounds, for example analog **15a** characterized by solubility in water gave: 319 μM, **15b** 443 μM and **15d** 456 μM. The resulting compounds were tested for cytotoxic activity against leukemia L1210 cells and the murine B16 melanoma. Studies revealed that the compounds **15a**-**d** showed a high cytotoxic activity against leukemia cells (IC_{50} was sequentially 0.38 μM for **15a**, 0.36 μM for **15b**, 0.18 μM for **15c** and 0,45 ± 0,1 μM for **15d**). Furthermore, the compounds **15b** (IC_{50}=2.9μM) and **15d** (IC_{50}=3.5 ± 1.4 μM) gave high antiproliferative activity in relation to B16. Because the compounds **15b** and **15d** provided the strongest anticancer effect, they were subjected to further testing *in vivo*. The resulting compound **15d** was examined by the National Cancer Institute against a panel of 60 human cancer cell lines. In conducting research on cell growth inhibition concentration of 50% (GI_{50}) showed selectivity relationship **15d** against leukemia, colon, melonoma, ovarian and renal cancer target lines. Incredibly potent activity **15d** exhibited against MDA-MB 435 melanoma cell line. During the tests TGI (the concentration for total growth inhibition), it was found that **15d** is highly active against colon, ovarian, renal, breast, non-small lung cancer, CNS, prostate and the most potent against MDA-MB-435. Concentration range was investigated for compounds **15a**, **15b**, and **15d** and the EC_{50} values (concentration required to cause 50% loss of cellular microtubules) which were successively 18.6, 5.6 and 1.8 μM. Then, studies indicated that acetyl-analog **15d** acted with the most similar mechanism of action to CA-4. Compound **15d** administered five times at a dose of 75 mg/kg for 19 days was found to be non-toxic to mice and showed to act antitumor leukemia L1210. Investigations on the other groups of mice after 23 days of treatment with the compound **15d** provided average 35% reduction of the tumor compared to control animals. This results confirmed that the compound **15d** worked anti-cancer *in vivo* [22].

According to literature data, isomerisation of combretastatin and its analogs to *trans*-forms considerably diminishes their activities. Recently, maleimide derivatives were reported, which are examples of conformationally restricted *cis*-structures (Fig. **4**).

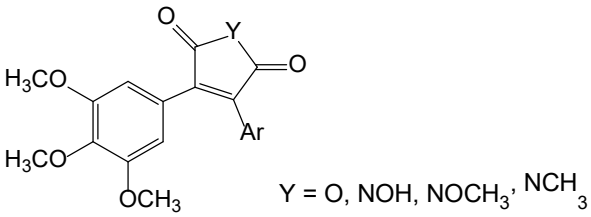


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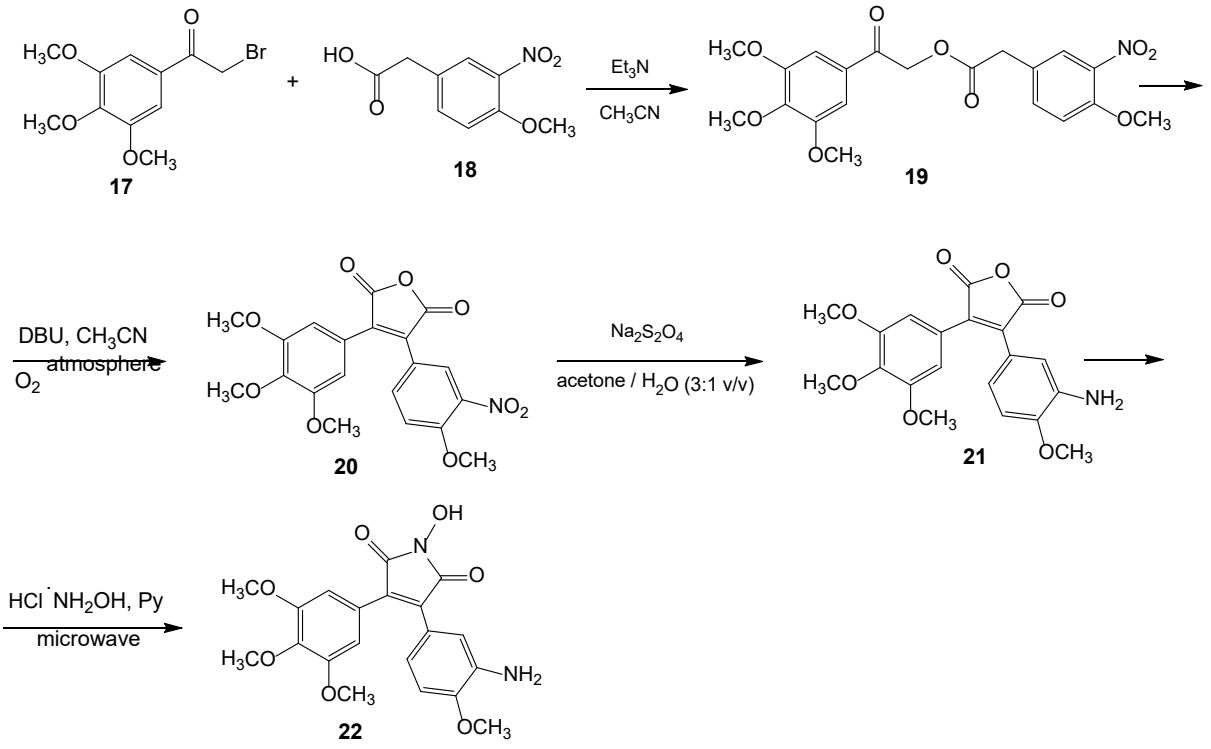
Fig. (4). Maleic anhydride and maleimide analogs of combratastatin CA-4.

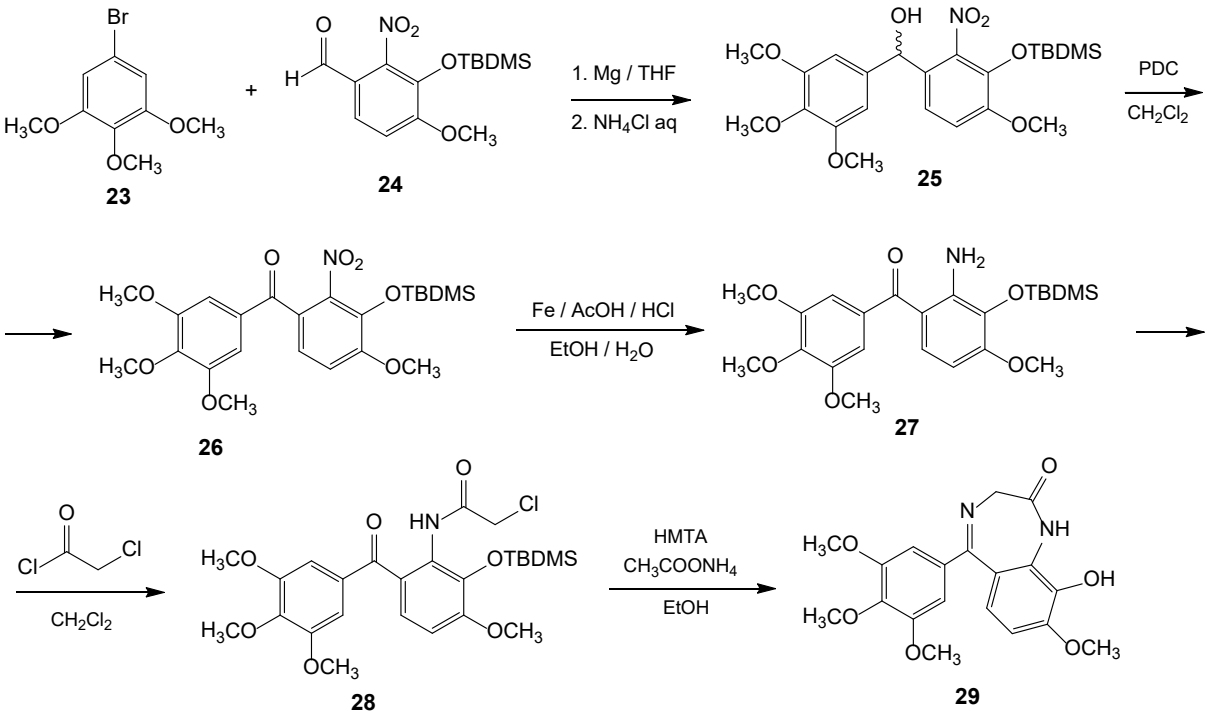
Potency of the designed compounds was measured with MTT test against three human tumor cell lines (SGC-7901, HT-1080 and KB). The most promising one occurred to be *N*-hydroxymaleimide with 3-amino-4-methoxy groups at ring B (Scheme **2**). Molecular modeling studies revealed, that this derivative interacts with colchicine binding site of tubulin similarly to combretastatin CA-4, where amino group and *N*-hydroxyl maleimide moiety are involved in hydrogen bonds with docking site.

The synthetic pathway of this compound included the reaction of α-bromo-3,4,5-trimethoxy-acetophenone **17** with arylacetic acid **18** in the presence of triethylamine (Scheme **2**). In the next stage ester **19** underwent cyclization to 3,4-diaryl maleic anhydride **20** upon DBU and oxygene atmosphere. Reduction of nitro group to amine **21** was performed with $Na_2S_2O_4$, and maleic anhydride moiety was converted to maleimide **22** in microwave assisted reaction with hydroxylamine [23].



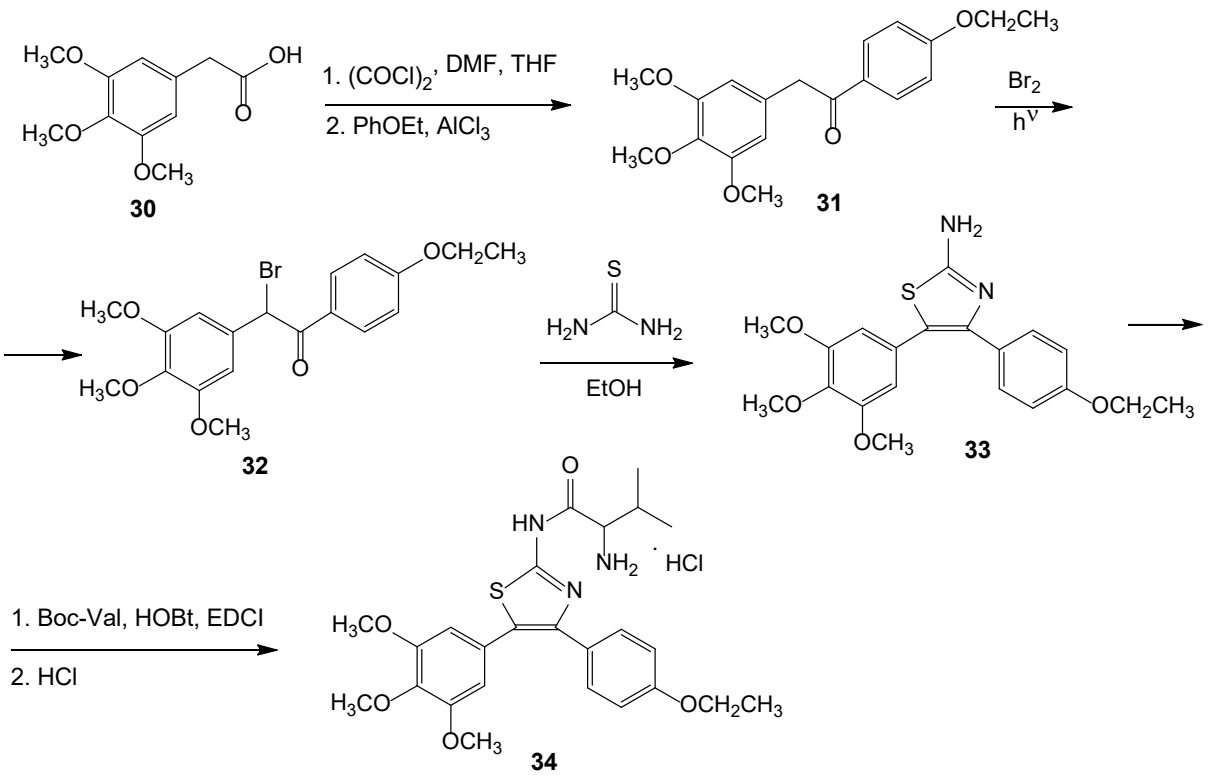
Scheme 2. Synthesis of maleimide analogs of combretastatin.

In search of combretastatin analogs with improved pharmacokinetic properties, benzodiazepine analogs were designed and their cytotoxicity against neuroblastoma cells, docking with tubulin, metabolic stability investigated. Synthesis of the most active compound in this series is depicted in Scheme **3**. First, 5-bromo-1,2,3-trimethoxybenzene **23** was converted to Grignard reagent, and treated with respective aldehyde **24**. Then, alcohol **25** was oxidized to ketone **26** with pyridinium dichromate (PDC), followed by reduction of nitro group. Obtained amine **27** underwent acylation with chloroacetyl chloride to produce 2-chloroacetamide **28**, which cyclized in the presence of ammonium acetate and hexamethylenetetramine (HMTA) to adequate combretabenzodiazepine **29**. This compound exhibited better metabolic stability in comparison to combretastatin CA-4 [24].



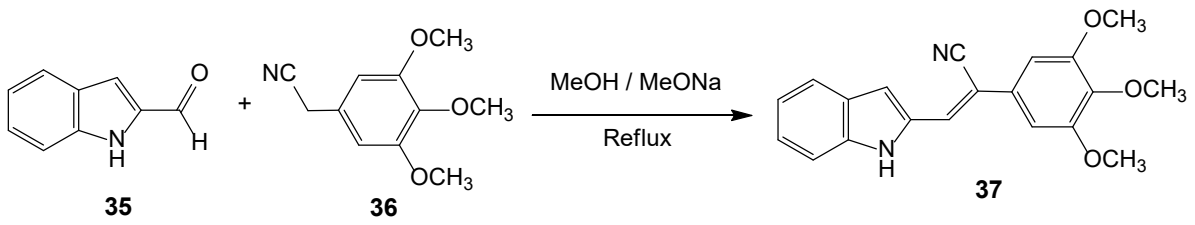
Scheme 3. Synthesis of 9-hydroxy-8-methoxy-5-(3,4,5-trimethoxyphenyl)-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one.

To improve pharmacological properties Yu *et al.* [25] developed water-soluble amino acid derivatives of combretastatin CA-4 (Scheme **4**). Designed compounds were examined against HepG2, H460 and SKOV-3 cells, and prodrugs containing glycine, D-leucine, valine, α-alanine gave the best cytotoxicity. However, valine analog **34** provided the highest inhibition ratio in murine tumor model, and was selected to further investigations. This compound was obtained from 2-(3,4,5-trimethoxyphenyl)acetic acid **30**, which was converted to respective acyl chloride and used in Friedel-Crafts reaction. Ketone **31** underwent bromination and **32** cyclization with thiourea. Subsequently, amine **33** was condensed with Boc-valine in the presence HOBt and EDCI as coupling reagent, followed by deprotection with HCl to **34**.



Scheme 4. Synthesis of amino acid derivative of combretastatin A.

One of the structural modification of combretastatin CA-4 is replacing of the phenyl ring by heterocyclic moiety, where in some cases anticancer activities can be improved. Penthala *et al.* [26] reported (*Z*)-cyanocombretastatin analogs possessing 2- and 3-indolyl, 2- and 3-benzofuranyl, 2-benzothiophenyl, and 2-benzothiazolyl units instead of 3-hydroxy-4-methoxyphenyl group. Previously, this research group described also benzothiophene cyanocombretastatin derivatives, which overcome cell-associated P-glycoprotein (P-gp)-mediated resistance in tumor cells [27]. Designed cyanocombretastatins were tested on numerous cancer cell lines and (*Z*)-2-indolyl analog **37** was an example exhibiting high growth inhibition activity (e.g. $GI_{50} <$ 0,01 μM for K-562). The key step of synthesis was a condensation of indole-2-carbaldehyde **35** with 3,4,5-trimethoxyphenylacetonitrile **36** (Scheme **5**).

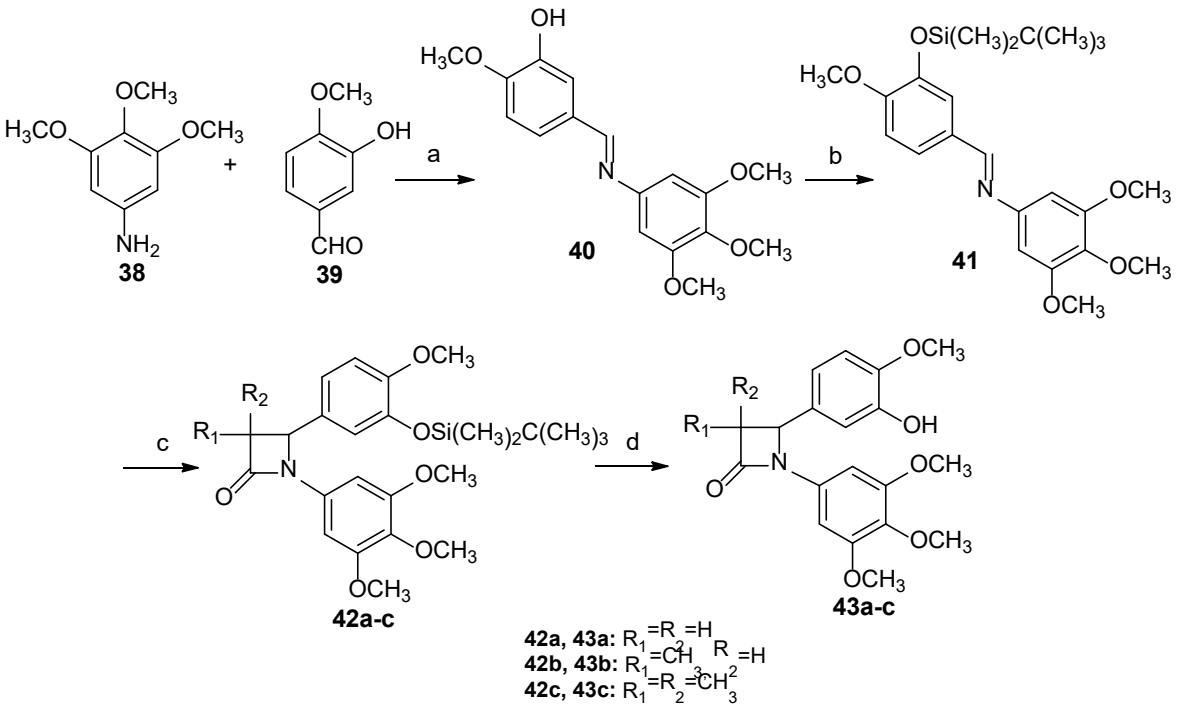


Scheme 5. Synthesis of (*Z*)-indol-2-yl cyanocombretastatin analog **37.**

Noteworthy, designed compounds revealed considerable activity despite of fact, that both aryl groups were *trans* positioned. The authors presented molecular modeling studies, where (*Z*)-indol-2-yl cyanocombretastatin analog, similarly to benzofuran and benzothiophene derivatives, occupied hydrophobic colchicine pocket of tubulin within numerous Van der Waal's interactions.

Carr and co-workers [28] presented the synthesis of analogues of combretastatin A-4 containing the 1,4-diaryl-2-azetidinone (β-lactam) ring system in place of the usual ethylene bridge of CA-4. The procedure for the preparation of the target compounds **43a-c** is presented in Scheme **6**. In a first step were obtained Schiff base **40** by condensation of the appropriate amine **38** and aldehyde **39**. Subsequent protected of the hydroxyl group by treatment with *tert*-butyldimethylchlorosilane to obtain silyl ether **41**, which in the reaction with ethylbromoacetate or ethyl-2-bromopropionate or ethyl-2-bromoisobutyrate in the presence of zinc and trimethylchlorosilane yielded the racemic β-lactam compound **42a-c**. The final products **43a-c** obtained by removing of the silyl protecting group by using TBAF to afford compounds **43a-c** (Scheme **6**). Derivative **43a** was the most potent compound having low nanomolar activity in both MCF-7 (IC_{50} = 0.017 μM) and MDA-MB-231 breast cancer cells (IC_{50} = 0.054 μM) and was able to arrest cells in the G2/M phase of the cell cycle. Moreover,

analog **84a** inhibited the polymerisation of tubulin with improved efficacy when compared with combretastatin CA-4 [28].



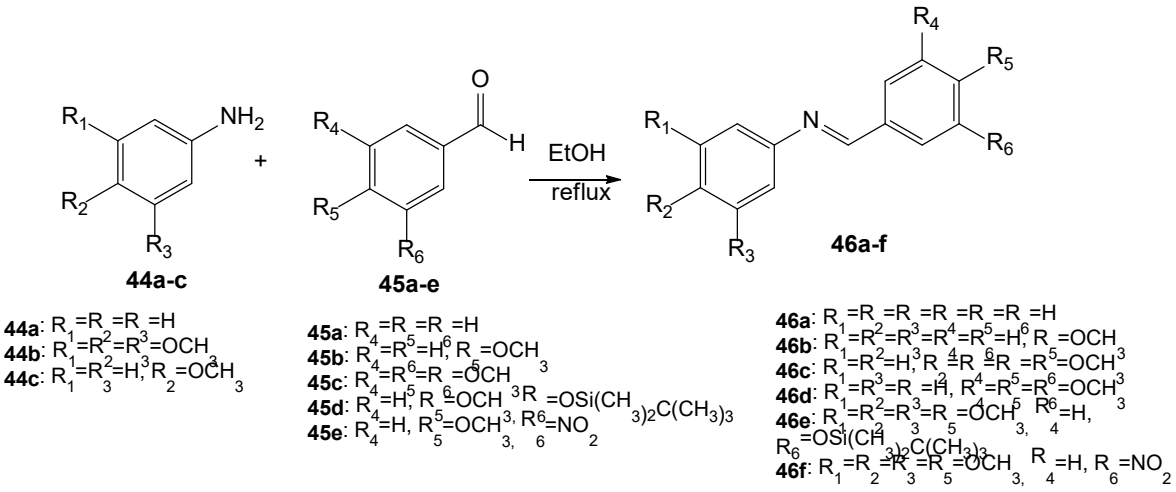
Scheme 6. Reagents and conditions: (a) EtOH, reflux, 2.5h; (b) $(CH_3)_3C(CH_3)_2SiCl$, K_2CO_3, CH_2Cl_2, DBU, 20°C; (c) $BrCH_2CO_2Et$, $BrCH(CH_3)CO_2Et$ or $Br(CH_3)_2CCO_2Et$, Zn, $(CH_3)_3SiCl$, C_6H_6, reflux; (d) $(CH_3CH_2CH_2CH_2)_4NF$, THF, 0°C [28].

O'Boyle *et al.* [29] synthesized further derivatives of combretastatin A-4 which contain the 1,4-diaryl-2-azetidinone (β-lactam). These compounds were substituted at position C-3 of the β-lactam ring with aryl rings. Synthesis of β-lactam was carried for using Staudinger cycloaddition reaction between appropriate ketene and imine under basic conditions. Intermediate compounds imines **46a-f** were prepared by condensation reaction of benzaldehydes **45a-e** with anilines **44a-c** (Scheme **7**). The intermediates acid chlorides **47a-d** were obtained by reaction of the appropriately substituted acetic acids and thionyl chloride (Scheme **8**). The desired β-lactam products **48-55** were prepared by the reaction of the imines

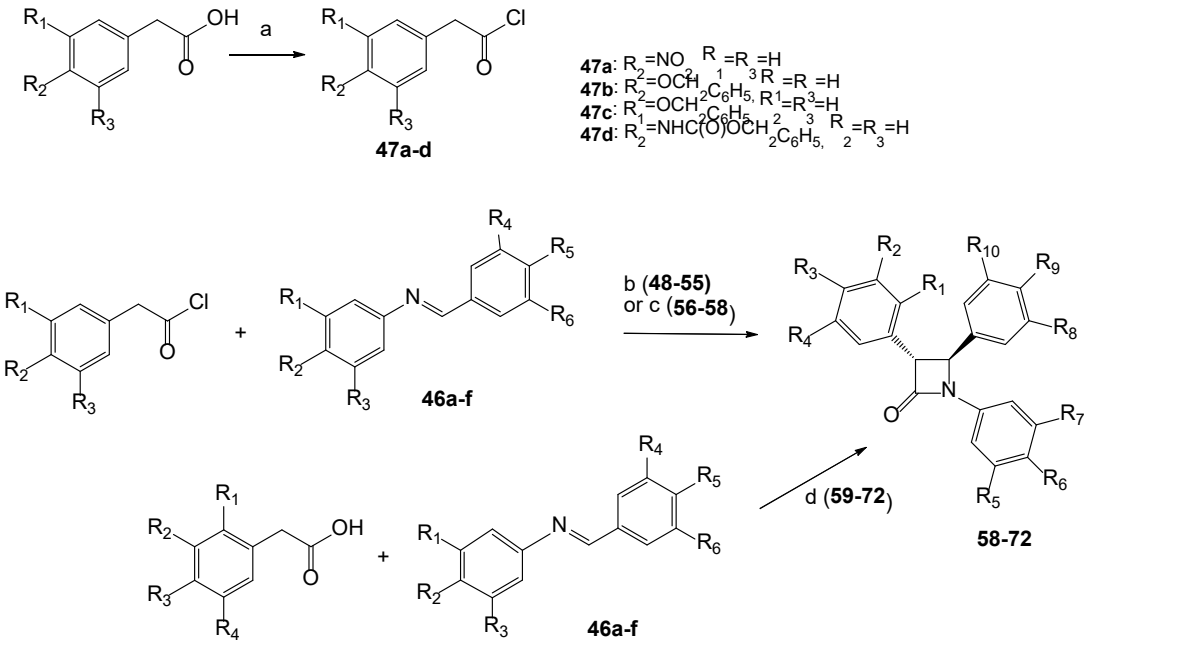
48a-f with the appropriate acid chloride in the presence of triethylamine at reflux in anhydrous dichloromethane (Scheme **8**). Derivatives **48-58** were obtained with imines and the appropriate acid chloride in the presence of triethylamine in anhydrous dichloromethane like compounds **48-59**, but at room temperature (Scheme **8**). Analogues **59-72** were synthesized in direct reaction the appropriate phenylacetic acid with imine **46a-f** in the presence of triphosgene and triethylamine at reflux in anhydrous dichloromethane (Scheme **8**). Following Reformatsky reaction between ethyl 2-bromo-2-phenylacetate with imines **46a**, **46c**, **46e** in the presence of zinc, trimethylchlorosilane and benzene under microwave afforded derivatives **73-75** (Scheme **9**). As a result of treatment of the silyl ethers **54**, **70**, **71**, and **74** with tetrabutylammonium fluoride at 0°C in THF obtained phenolic products **75-78** (Scheme **10**) [29].

Derivatives of combretastatin A-4 which contain the 1,4-diaryl-2-azetidinone were tested for antiproliferative activity. Compounds **73** and **75** inhibited the polymerization of tubulin with the better efficacy when compared to CA-4 [29].

Subsequently, compounds **43a** and **75** were tested with respect their the anti-vascular effects directly on primary HUVECs and indirectly on the release of pro-angiogenic VEGF from tumour cells. In addition, analogs **43a**, **75** were assessed of the effect of the tumour cell migration. These derivatives **43a** and **75** exerted both anti-endothelial effects and anti-angiogenic effects. Moreover, derivative **75** abrogated the migration of MDA-MB-231 cells indicating an anti-metastatic function for these compounds [30].



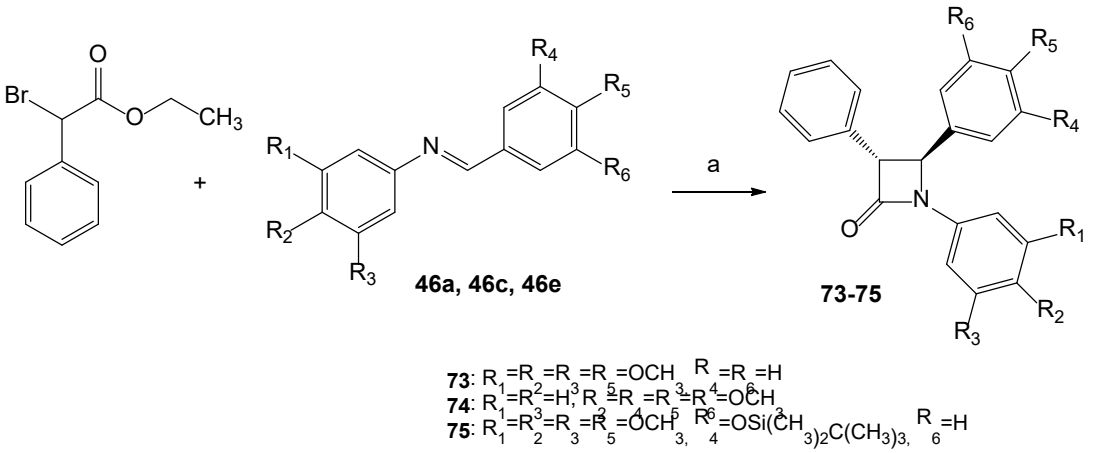
Scheme 7. Synthesis of imines **46a-f** [29].



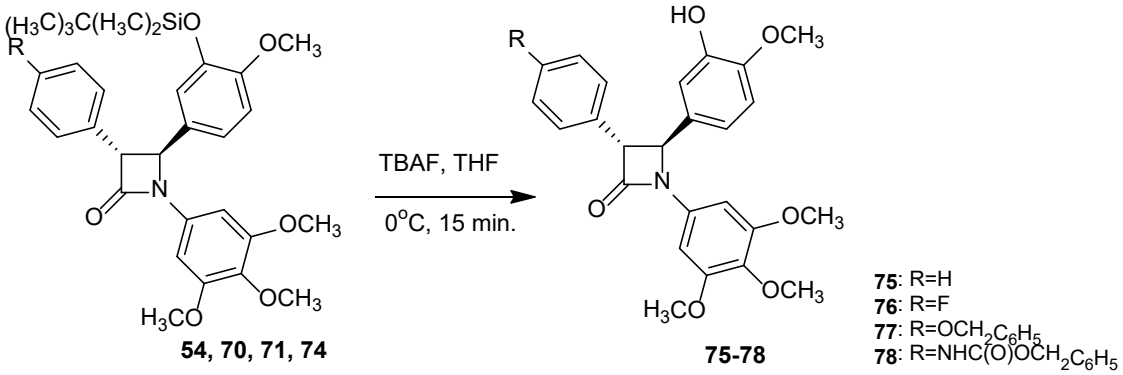
48: R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=R_9=R_{10}=H
49: R_1=R_2=R_4=R_5=R_6=R_7=R_8=R_9=R_{10}=OCH_3
50: R_1=R_3=R_4=R_8=R_{10}=H, R_2=R_5=R_6=R_7=R_9=OCH_3
51: R_1=R_2=R_4=R_8=R_{10}=H, R_3=R_5=R_6=R_7=R_9=OCH_3
52: R_1=R_4=R_8=R_{10}=H, R_3=Cl, R_2=R_5=R_6=R_7=R_9=OCH_3
53: R_1=R_2=R_4=R_8=R_{10}=H, R_3=$OCH_2C_6H_5$, R_5=R_6=R_7=R_9=R_2=OCH_3
54: R_1=R_2=R_4=R_8=R_{10}=H, R_3=F, R_5=R_6=R_7=R_9=OCH_3, R_8=$OSi(CH_3)_2C(CH_3)_3$
55: R_1=R_2=R_4=R_{10}=H, R_3=R_5=R_6=R_7=R_9=OCH_3, R_8=NO_2
56: R_1=R_2=R_3=R_4=R_{10}=H, R_5=NO_2, R_6=R_7=R_9=R_3=OCH_3
57: R_1=R_2=R_4=R_8=H, R_{10}=$NHC(O)OCH_2C_6H_5$, R_7=R_9=R_3=OCH_3, R_8=NO_2
58: R_1=R_2=R_4=R_{10}=H, R_3=F, R_5=R_6=R_7=R_9=OCH_3, R_8=NO_2
59: R_1=R_2=R_4=R_{10}=R_3=H, R_5=R_6=R_7=R_9=OCH_3
60: R_2=R_3=R_4=R_8=R_{10}=H, R_1=R_5=R_6=R_7=R_9=OCH_3

61: R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=R_9=R_{10}=H
62: R_1=R_8=R_{10}=R_2=H, R_4=SCH_3, R_5=R_6=R_7=R_9=R_3=OCH_3
63: R_1=R_2=R_4=R_8=R_{10}=H, R_3=CH_3, R_5=R_6=R_7=R_9=OCH_3
64: R_1=R_2=R_4=R_8=R_{10}=H, R_3=Br, R_5=R_6=R_7=R_9=OCH_3
65: R_1=R_2=R_4=R_8=R_{10}=H, R_3=F, R_5=R_6=R_7=R_9=OCH_3
66: R_1=R_2=R_4=R_8=H, R_{10}=R_3=Cl, R_5=R_6=R_7=R_9=OCH_3
67: R_1=R_4=R_8=R_{10}=H, R_2=R_3=F, R_5=R_6=R_7=R_9=OCH_3
68: R_1=R_4=R_8=R_{10}=R_2=H, R_3=CF_3, R_5=R_6=R_7=R_9=OCH_3
69: R_1=R_2=R_4=R_8=R_{10}=H, R_3=OCH_3, R_5=R_6, R_7=R_9=R_3=OCH_3
70: R_1=R_3=R_4=R_8=H, R_{10}=$OCH_2C_6H_5$, R_5=R_6=R_7=OCH_3, R_9=$OSi(CH_3)_2C(CH_3)_3$
71: R_1=R_2=R_4=R_{10}=H, R_3=$NHC(O)OCH_2C_6H_5$, R_5=R_6=R_7=R_9=R_3=OCH_3, R_8=$OSi(CH_3)_2C(CH_3)_3$
72: R_1=R_2=R_4=R_{10}=H, R_3=$OCH_2C_6H_5$, R_5=R_6=R_7=R_9=OCH_3, R_8=NO_2

Scheme 8. Synthesis of azetidinones **48-72**. Reagents and conditions: (a) $SOCl_2$, $CHCl_3$, reflux, 3h; (b) NEt_3, anhydrous CH_2Cl_2, reflux, 3h; (c) NEt_3, anhydrous CH_2Cl_2, 20°C, 18h; (d) triphosgene, NEt_3, anhydrous CH_2Cl_2, reflux, 5h, 20°C, stirred 18h [29].



Scheme 9. Synthesis of azetidinones **73-75**. Reagents and conditions: (a) zinc, trimethylchlorosilane, benzene, microwave [29].

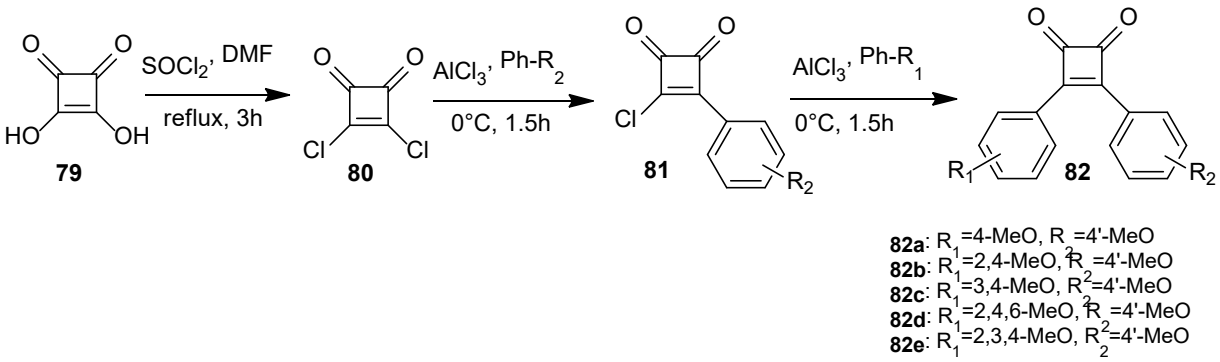


Scheme 10. Synthesis of azetidinones **75-78** [29].

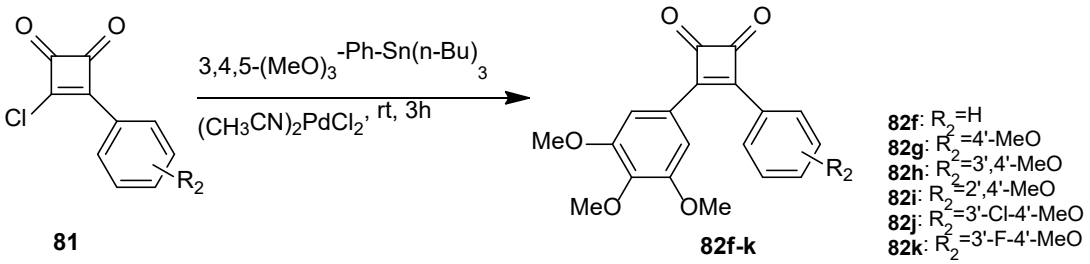
Liu group [31] synthesized a series of novel 3,4-diaryl squaric acid analogs **82a-r** related to combretastatin A-4 (CA-4). Derivatives **82a-e** containing electron-donating groups on the aromatic rings were prepared by reaction squaric acid **79** in thionyl dichloride with DMF to obtain 3,4-dichloro-3-cyclobutene-1,2-dione **80**. Compound **80** on treatment with substituted benzenes under Friedel-Crafts conditions gave 3-chloro-4-R_2Ph-3-cyclobutene-1,2-dione **81**. As a result, treatment of **81** with substituted benzenes under Friedel-Crafts conditions yielded the desired compounds **82a-e** (Scheme **11**). Derivatives **82f-k** also contained electron-

donating groups on the aromatic ring, they employed an alternate synthesis to previous one. Compound **82f-k** were prepared under Stille cross-coupling conditions by reaction compound **81** with (3,4,5-trimethoxyphenyl)tri-*n*-butylstannane (Scheme **12**). Derivatives **82l-r** containing electron-poor aromatic rings were prepared by reaction of compound **80** with 0.5 equiv. of *p*-methoxybenzenethiol with triethylamine to obtain 3-chloro-4-(4-methoxyphenylthio)-3-cyclobutene-1,2-dione **83**. Following reaction compound **83** with (3,4,5-trimethoxyphenyl)tri-*n*-butylstannane under Stille cross-coupling conditions afforded the corresponding compound **84**. Reaction of **84** with appropriate arylboronic acids under Liebeskind-Srogl cross-coupling conditions yielded corresponding compound **82l-m**, **82o** and **82q-r** (Scheme **13**). Analog **82n** was prepared from derivative **82m** in the presence of $NaHCO_3$ in refluxing methanol (Scheme **14**). As a result of reduction of the nitro group of compound **82o** with Pd,C/H_2 in ethanol and EtOAc gave compound **82p** (Scheme **15**) [31].

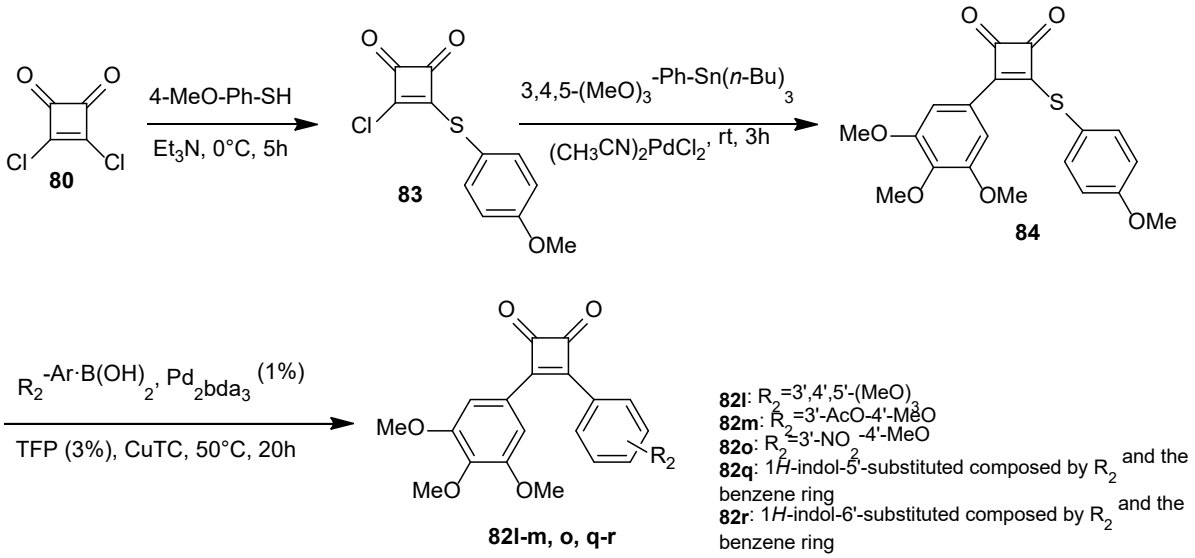
All compounds **82a-r** were evaluated for their *in vitro* anticancer activities against several cell lines. Derivatives **82g**, **82k**, **82m**, **82n**, **82p**, **82q** and **82r** exhibited strong activities against human leukemia cells with IC_{50} values of <20 nM. Compounds **82n**, **82p**, **82k** showed potent cytotoxicity against the human liver cancer cells Bel-7402, HepG2, SMMC-7221, human breast cancer cells MCF-7, human pancreatic cancer cells SW-1990, human colon adenocarcinoma cells HCT116 and human leukemia cells CEM. The highest cytotoxicity for both compound **82n** and **82p** was observed against CEM with IC_{50} <2 nM. Moreover, derivatives **82n** and **82p** exhibited also high activities against human liver cancer cell HepG2 with IC_{50} values of less than 14 nM. Furthermore, the cytotoxicity of analog **82n** against human liver cancer cells Bel-7402 and human breast cancer cells MCF-7 was 5- to 6-fold stronger than that of positive control CA-4 and the cytotoxicity of derivative **82p** against human liver cancer cells Bel-7402 was 122-fold stronger than that of positive control CA-4 [31].



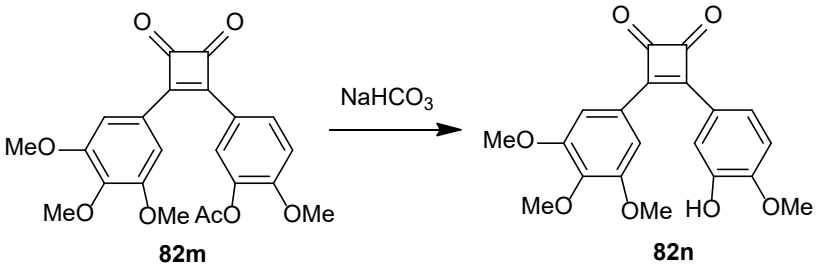
Scheme 11. Synthesis of derivatives **82a-e** [31].



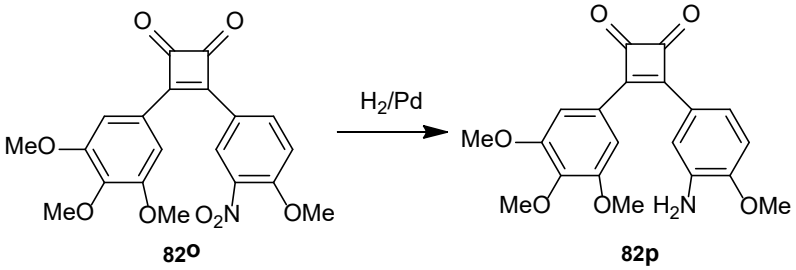
Scheme 12. Synthesis of derivatives **82f-k** [31].



Scheme 13. Synthesis of derivatives **82l-m**, **o**, **q-r** [31].



Scheme 14. Synthesis of compound **82n** [31].



Scheme 15. Synthesis of compound **82p** [31].

Zhou *et al.* [32] designed and synthesized a new CA-4 analogs with 4-metoxy-1*H*-benzo[*d*]-imidazole as the B ring and oxazole ring in place of the connector between rings A and B (Fig. **5**) [32].

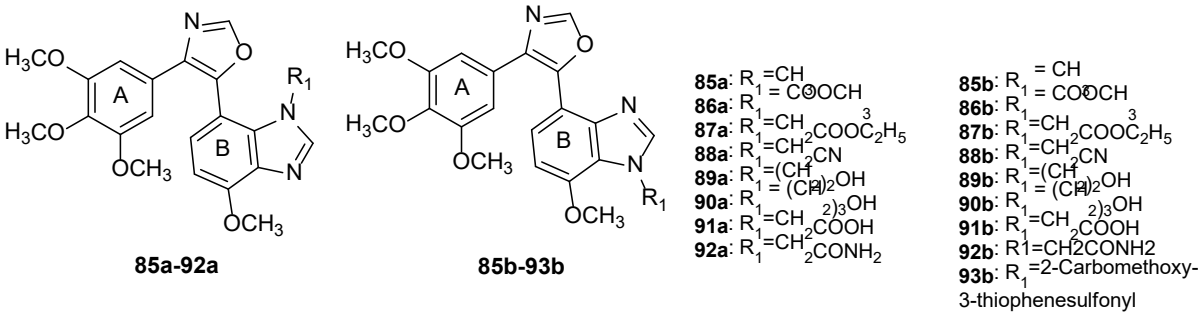
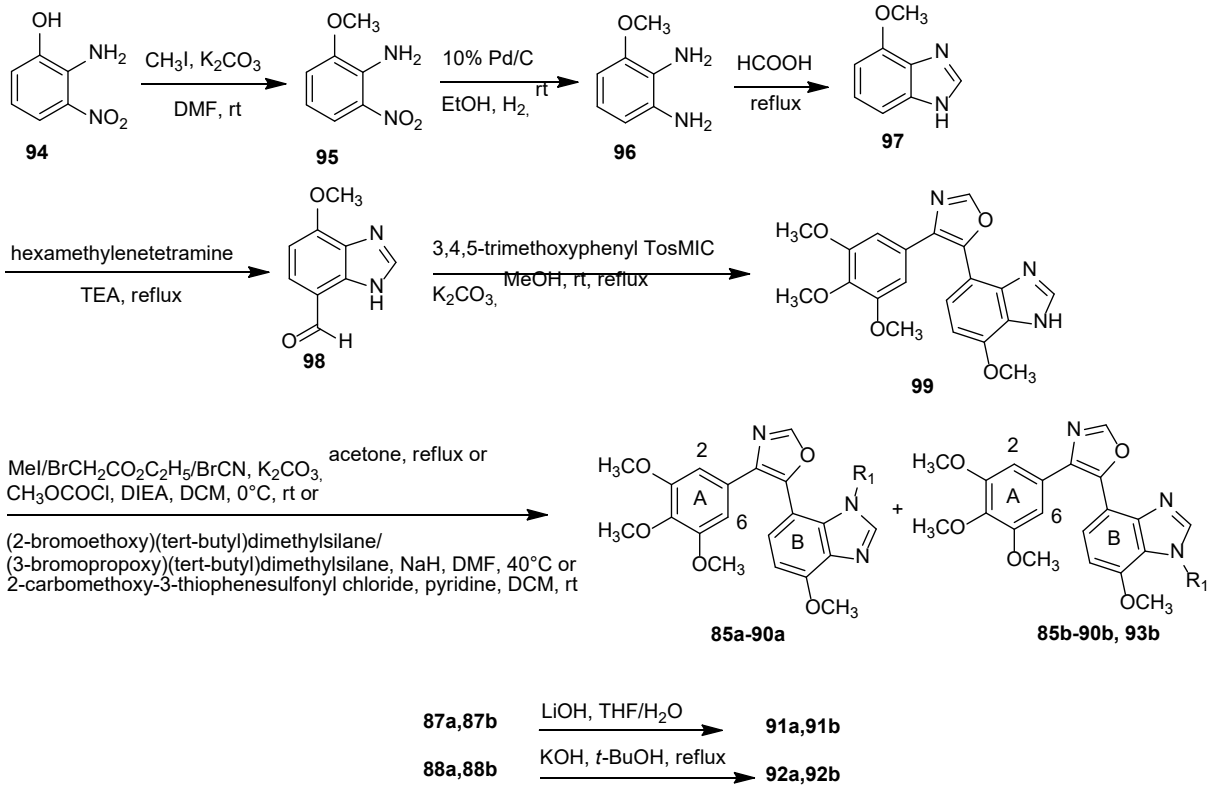


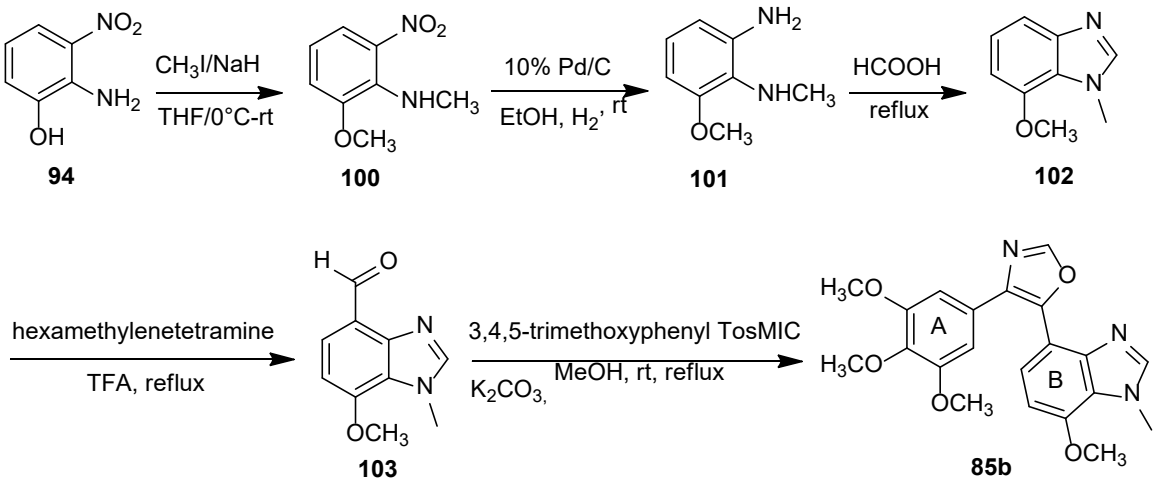
Fig. (5). Structures of designed oxazole derivatives **85a-92a** and **85b-93b** [32].

A ring or 3,4,5-trimethoxybenzene was preserved. While benzo[*d*]-imidazole was used as the B ring to mimic the 2- and 3-hydroxyl groups in the CA-1 and CA-4, the aim was to enhance the metabolic stability and physicochemical properties. Using data SAR studies of ring B created a 4-methoxy group. In addition, the connector in the form of oxazole was included to block *cis* orientation of A and B rings. Novel benzimidazoles-contained oxazol-bridges analogs of combretastatin A-4 were synthesized on the basis of the Scheme **16**.



Scheme 16. Synthesis of oxazole derivatives **85a-92a** and **85b-93b** [32].

Due to the tautomerization of benzimidazoles ring of compound **99** alkylation or acylation on nitrogen resulted in formation of a pair of regioisomers **85a-92a** and **85b-93b**. In most compounds formed **85b-93b** and in a minority **85a-92a**. To confirm the chemical structure of regioisomers **85a** and **85b** additionally, the compound **85b** was prepared using alternative synthetic route presented in Scheme **17** [32].



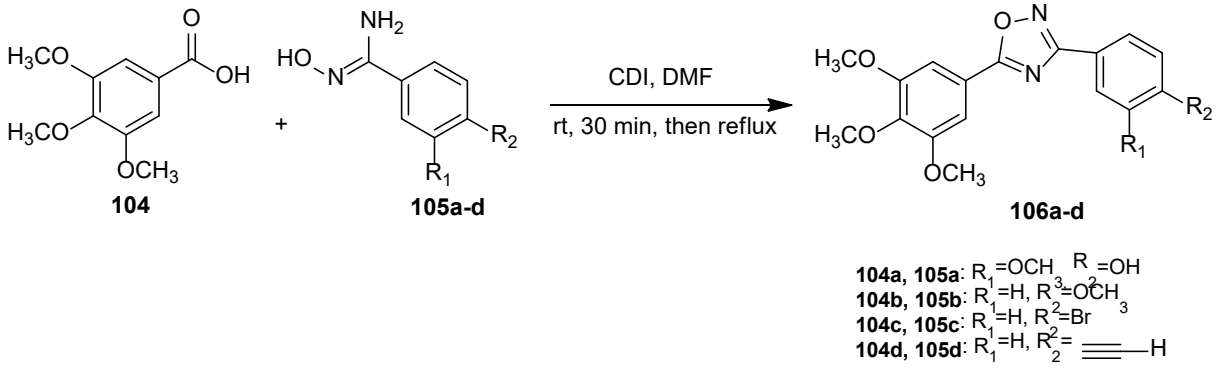
Scheme 17. Synthesis of oxazole derivatives **85b** [32].

Antiproliferative activity *in vitro* of the obtained compounds was evaluated using the MTT assay of five human tumor cell lines: MCF-7, A549, HT29, HepG2 and BxPC3, where VCR, CA-4 were chosen as references. Compounds **99**, **85b**, **86a** and **86b** showed an excellent cytotoxic activity IC_{50} values in the nanomolar level in the range of 3.0 – 56 nM. The most active compounds of **99**, **85b**, **86a** and **86b**, and moderately active compounds **90a** and **90b** were further assessed using the MTT assay against the tumor cell KB, vincristine resistant KB, KBV, MX-1 and MX-resistant taxol 1 (MX-1/T). Compounds **99**, **85b**, **86a** and **86b** greatly inhibited vincristine resistant KB cells with IC_{50} in the double-digit nanomolar range respectively 16, 41, 27 and 107 nM, although, unfortunately, are less active than the CA-4. The resulting compounds excluding **90a** and **90b** showed moderate growth inhibitory activity. Compound **99** and **86a** were more active than the compounds **85b** and **86b** in relation to tumor cells MX-1/T [32].

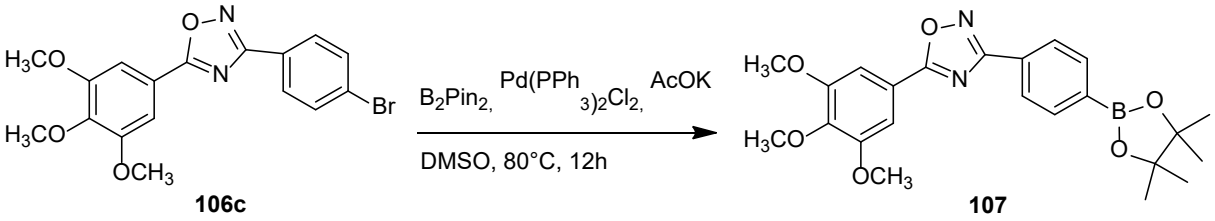
CA-4 interact with tubulin and inhibit tubulin assembly [32, 34] and therefore the compound **99** was evaluated in terms of inhibition of tubulin polymerization. Studies suggested that compound **99** provided higher inhibitory activity of tubulin with an IC_{50} value 0.39 mM, than the CA-4 for which the IC_{50} value was 2.7 mM. It was demonstrated that the

compound **99** is capable of binding to tubulin and to transform the dynamic tubulin polymerization process leads to cell death. During the study it was also found that the compound **99** caused arrest G2/M in a concentration dependent manner and this was done in accordance with the behavior of tubulin-binding agents. Compound **99** was also tested *in vivo* using the H22 mice xenograft model KM. When mice were treated with either 15mg/kg of compound **99** on days 1 and 4, tumor growth was significantly lowered with the inhibition of 66%. This is comparable to a VCR dose of 0.5mg/kg [32].

Das and co-workers [35] synthesized the derivatives of 3,5-disubstituted-1,2,4-oxadiazole which contained CA-4 analogs. The oxadiazole moiety probably give an optimal conformational geometry for interaction with the colchicine site on tubulin as well as increasing the number of heteroatoms in the core structure. The point was an increase in the polarity of the molecule, thus have improve water solubility. Compounds **106a-d** were synthesized by a coupling reaction between the respective amidoxime **105a-d** and carboxylic acid **104** in DMF solvent, and utilizing CDI as a coupling reagent (Scheme **18**). The compound **107** synthesized by a Suzuki coupling reaction using bromide compound **106c** and B_2Pin_2 (*bis*-pinocolatodiboron) to give the boronic ester containing compound **107** (Scheme **19**) [35].

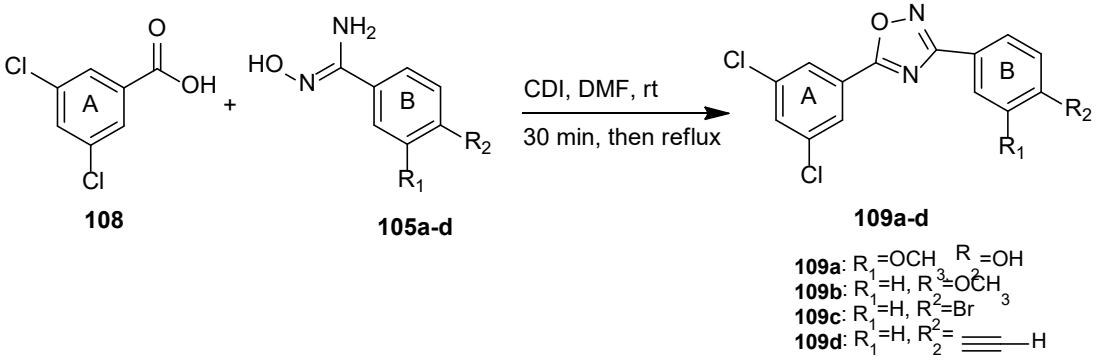


Scheme 18. Synthesis of compounds **106a-d** [35].

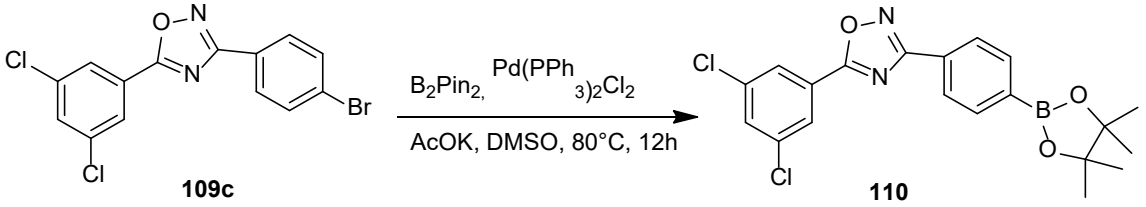


Scheme 19. Synthesis of compound **107** [35].

The same research group [35] have developed a few CA-4 analogs by substituting the trimethoxy group in ring A with more hydrophobic chloro derivatives. Compounds **109a-d** were synthesized by the protocol described above, by using the respective amidoxime **105a-d** and a carboxylic acid **108** (Scheme **20**). Compound **110** was prepared by a Suzuki coupling reaction between the bromide compound **109c** and B_2Pin_2 (Scheme **21**) [35].

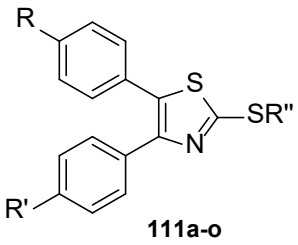


Scheme 20. Synthesis of compounds **109a-d** [35].



Scheme 21. Synthesis of compounds **110** [35].

Salehi *et al.* [36] designed and synthesized a new series of 4,5-diarylthiazol-2-thialkyl analogs (Fig. **6**) of combretastatin A-4.

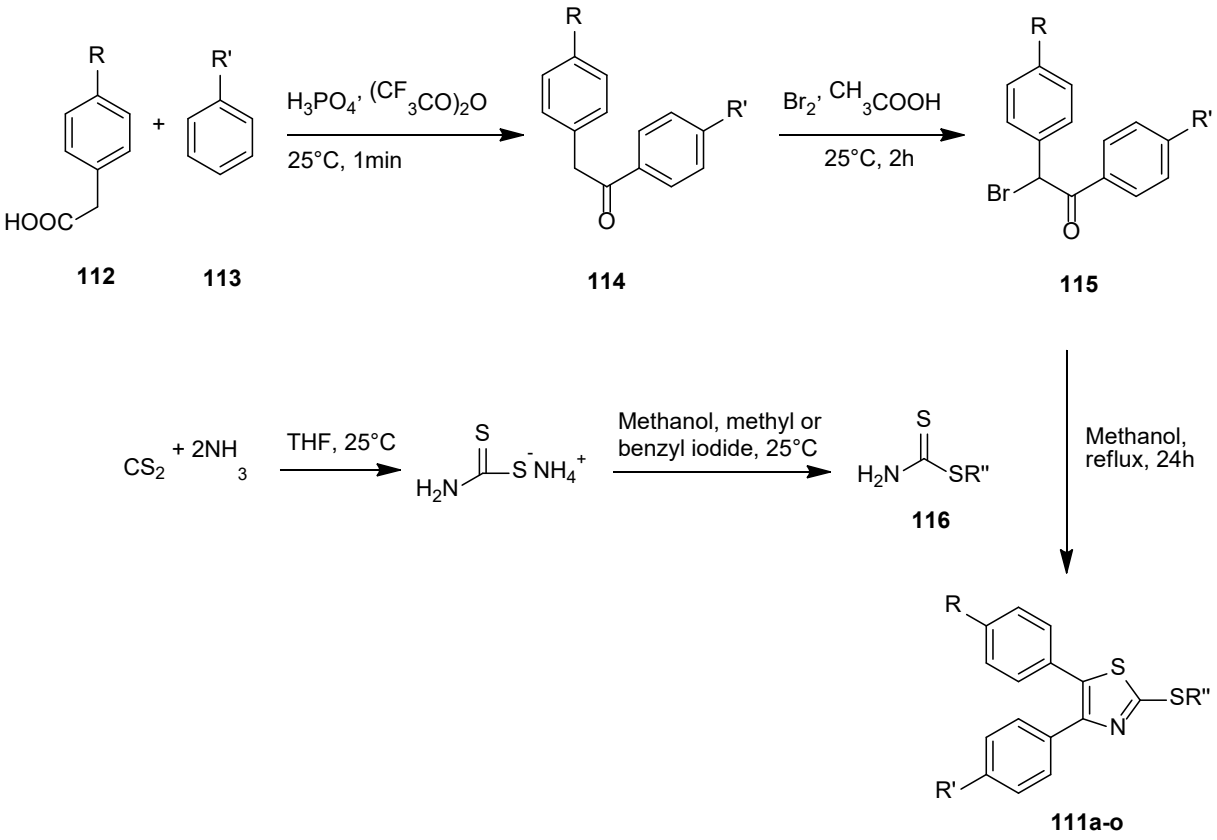


111a-o

111a: R=H; R'=H; R"=CH_3
111b: R=H; R'=H; R"=CH_2Ph
111c: R=H; R'=OCH_3; R"=CH_3
111d: R=H; R'=OCH_3; R"=CH_2Ph
111e: R=H; R'=SCH_3; R"=CH_3
111f: R=H; R'=SCH_3; R"=CH_2Ph
111g: R=Cl; R'=OCH_3; R"=CH_3
111h: R=Cl; R'=OCH_3; R"=CH_2CH_3
111i: R=Cl; R'=OCH_3; R"=CH_2Ph
111j: R=Cl; R'=SCH_3; R"=CH_2Ph
111k: R=F; R'=OCH_3; R"=CH_3
111l: R=F; R'=OCH_3; R"=CH_2Ph
111m: R=F; R'=SCH_3; R"=CH_2Ph
111n: R=OCH_3; R'=OCH_3; R"=CH_3
111o: R=OCH_3; R'=OCH_3; R"=CH_2Ph

Fig. (6). Structure of 4,5-diarylthiazol-2-thiones (**111a-o**) [36].

The synthetic pathway for heterocyclic derivatives of CA-4 **111a-o** was depicted in Scheme **22** [36]. Compound **113** underwent acylation with **112** to ketone **114**, followed by bromination and acylation with dithiocarbaminates **116** to **111a-o**.

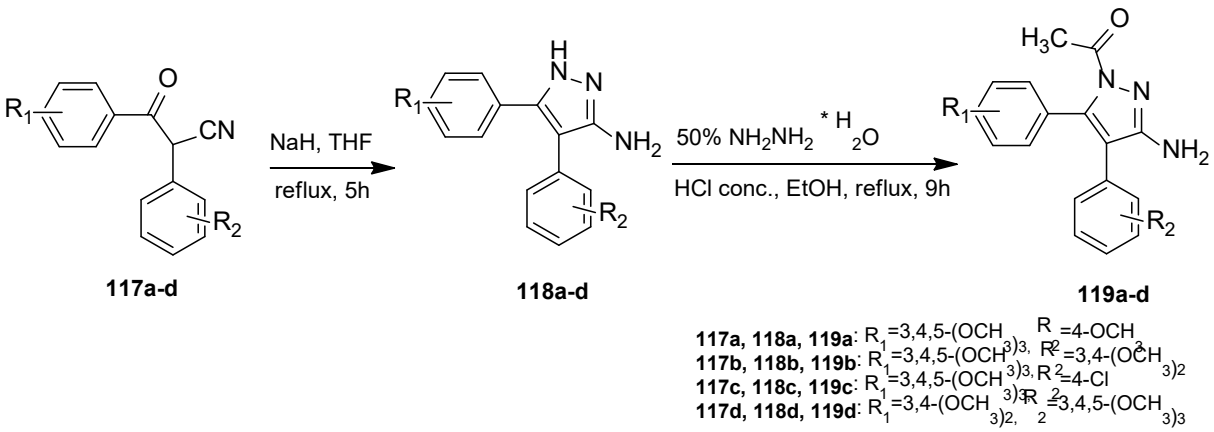


Scheme 22. Synthesis of compounds **111a-o** [36].

All received analogs were investigated on three cancer cell lines: AGS, MCF-7 and HT-29, and also mouse NIH-3T3 using the MTT assay. Studies revealed that none of the obtained compounds was not effective enough to sufficiently inhibit HT-29. Compound **111j** exhibited the best cytotoxic activity against HT-29 of the respondents. The great antiproliferative activity relative to the cell line MCF-7 showed again **111j** (IC_{50} = 7.1 ± 0.6 μM) with 4-chloro and 4-thiomethyl on substituted phenyl ring. The compound **111o** with methoxy group on two phenyl rings and 2-(benzylthio) group had an average antiproliferative activity against cell lines MCF-7 and AGS. These studies indicated, that of all tested compounds, **111j** was the most promising, even though this activity was not remarkable in comparison with the reference. The study inhibition of tubulin polymerization selected three compounds: **111h**, **111j** and **111o**. Studies showed that **111h** was ineffective in the test with tubulin. In contrast,

activity of microtubule polymerization, the compounds **111j** and **111o** was significantly lower than the control experiment. However, the level of inhibitory activity of CA-4 in final concentration of 10 μM was more than received **111j** and **111o** compounds. Docking studies exhibited that the compounds **111j** and **111o** could be successfully docked in the colchicine binding site of α, β-tubulin [36].

Liu *et al.* [37] synthesized a series of restricted *cis*-4,5-diaryl-3-aminopyrazole derivatives analogs of combretastatin A-4. Synthesis of the obtained compounds **119a-d** is shown in Scheme **23** [37].

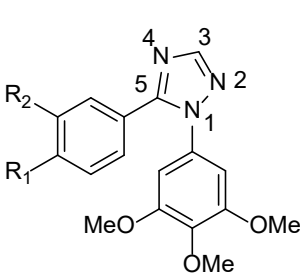


Scheme 31. Synthesis of analogs **119a-d** [37].

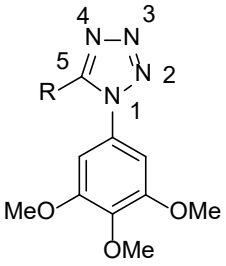
Compounds **118a-d** and **119a-d** were tested for *in vitro* cytotoxicity on five human cancer cell lines: ECA-10, SMMC-772, K562, PC-3 and A549 cells using MTT assay. Analyzing the results, it was found that 5-diaryl-3-aminopyrazole **118a-d** possess a potent activity *in vitro* than an *N*-acetylated 4,5-diaryl-3-aminopyrazoles **119a-d** for most of the tested line. The compounds **118a-c** and **119c** showed the greatest opportunity for inhibition against all tested cancer cell lines. The most active compound against K562, A549 and SMMC-7721 proved to be **118a** (IC_{50} = 0.08 ± 0.04; IC_{50} = 1.38 ± 0.94; IC_{50} = 12.07 ± 2.66), against the ECA-109 compound **118b** (IC_{50} = 1.56 ± 0.37) and against PC-3 compound

118c (IC_{50} = 0.61 ± 0.53). SAR studies revealed that the 3,4,5-trimethoxyphenyl (ring A) located on the 5-position of pyrazole ring (near N1-position) promotes the cytotoxic activity and the introduction of an acetyl group at the N1 position of the pyrazole ring causes injurious cytotoxicity. Flow cytometry analysis showed that the compound **118a** was inhibitor potent tubulin polymerization and stop the cell cycle in G2/M phase. Compound **118a** was evaluated for inhibitory effects on tubulin polymerization. Investigations suggested that the compound **118a** shows a potential inhibitory effect against microtubule compared with combretastatin A-4 in sequence (IC_{50} = 2.4 μM for **118a** and IC_{50} = 1.2 μM CA-4). The docking study revealed that compound **118a** shows similar binding posture as CA-4 in the crystallized protein complex, which indicates that, the 4,5-diaryl-3-aminopyrazole derivatives well mimic of CA-4 [37].

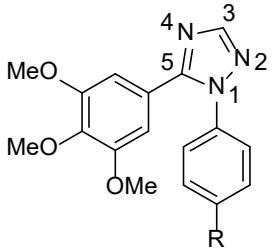
In 2010 Romagnoli group [38] described a number of analogs of 1,5-diaryl-1,2,4-triazole as potent inhibitors of cell growth and possessing antimitotic properties. The most active compounds proved **120a** and **120b**. Next time in 2012 Romagnoli *et al.* [39] received two series of 1,5-diaryl substituted 1,2,3,4-tetrazoles **121a-p**; **122a-b** (Fig. **7**).



120a: R_1=OCH_2CH_3, R =H
120b: R1=OCH_2CH_3, R_2=Cl



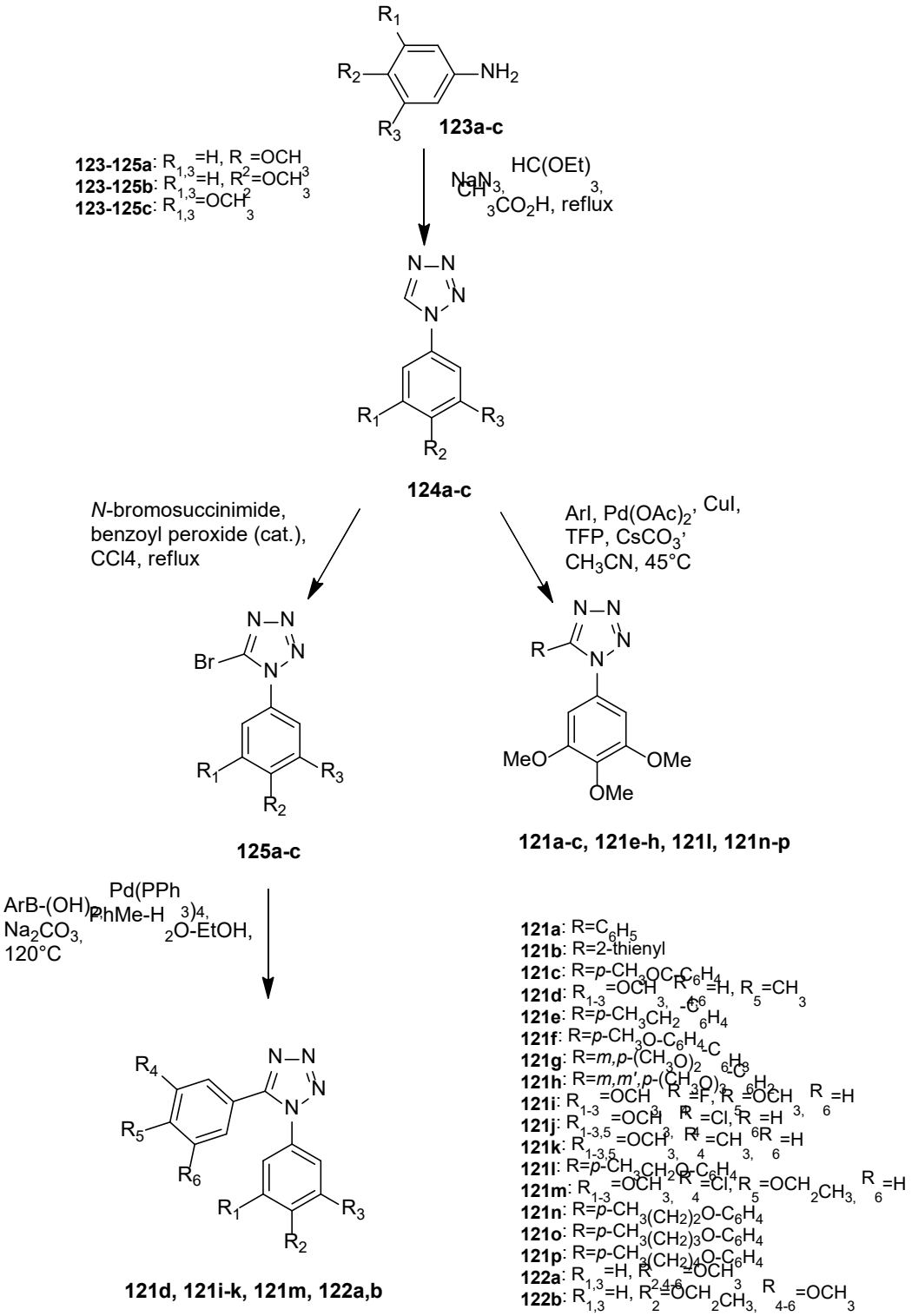
121a: R=C_6H_5
121b: R=2-thienyl
121c: R=*p*-CH_3OC-C_6H_4
121d: R=*p*-CH_3-C_6H_4
121e: R=*p*-CH_3CH_2-C_6H_4
121f: R=*p*-CH_3O-C_6H_4-C
121g: R=*m,p*-$(CH_3O)_2$-C_6H_3
121h: R=*m,m',p*-$(CH_3O)_3$-C_6H_2
121i: R=*p*-CH_3O, *m*-F-C_6H_4
121j: R=*p*-CH_3O, *m*-Cl-C6H4
121k: R=*p*-CH_3O, *m*-CH_3-C_6H_4
121l: R=*p*-CH_3CH_2O-C_6H_4
121m: R=*p*-CH_3CH_2O, *m*-Cl-C_6H_4
121n: R=*p*-$CH_3(CH_2)_2O$-C_6H_4
121o: R=*p*-$CH_3(CH_2)_3O$-C_6H_4
121p: R=*p*-$CH_3(CH_2)_4O$-C_6H_4



122a: R=OCH_3
122b: R=OCH_2CH_3

Fig. (7). Received by Romagnoli *et al.* [39] potential inhibitors of tubulin polymerization.

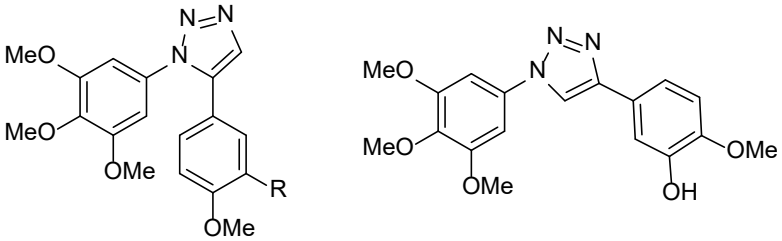
These compounds were synthesized from anilines **123a-c** using tetrazoles **124a-c** formation and the reaction of a palladium catalyzed cross-coupling (Scheme **24**) [39].



Scheme 24. Synthesis of tetrazole derivatives **121a-c**, **d**, **e-h**, **i-k**, **l**, **m**, **n-p**, **122a**,**b** [39].

Antiproliferative studies *in vitro* were performed by assessing the inhibition of growth of six different human tumor cell lines (HeLa, AS49, HL-60, Jurkat, MCF-7, HT-29) and comparing the obtained compounds **121a-p** and **122a-b**. As a control was used a derivative of 1,2,4-

triazole **120a** and CA-4. The most cytotoxic for all cancer cell lines proved to be compounds **121l**, **121m** and **122b** in the range 0.16 – 28.8 nM . IC_{50} values for these compounds showed a lower value than the reference substances. It was found that the antiproliferative activity depended on the substitution pattern on the phenyl at the 5-position of the tetrazole ring. Situated in the *para* position methoxy and ethoxy groups increased the biological activity of the compound. Introduction to the *meta* position of F, Cl or Me substituent to 4'-methoxy phenyl ring cause a slight increase in activity, while a group of *m*-methoxy caused a significant reduction in potency. Compound **121l** occured to be a potent inhibitor of tubulin polymerization (IC_{50} = 1.1 mM), and strongly inhibited colchicine binding to tubulin (78%). Furthermore, the compounds **121l**, **121m** and **122b** stopped the cell cycle at the G2/M phase and induce apoptosis through the mitochondrial pathway. It was also examined the antitumor efficacy of the compound **121l** on xenograft-bearing mouse. It was found that the inhibition of tumor growth needed only three doses of the compounds **121l**, without a significant decreasing in weight of the animal [39].



126: R=OH
127: R=H

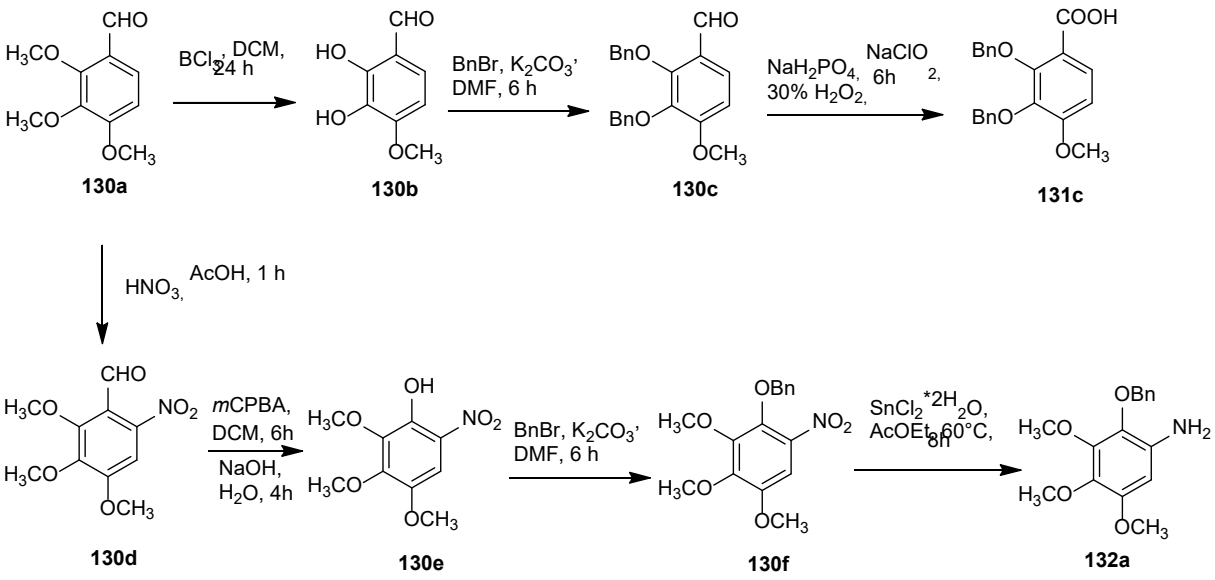
128

Fig. (8). Structures of combretastatin A-4 triazole analogues; Ana-2 **126**; Ana-3 **127**; Ana-4 **128** [40].

Aziz *et al.* [40] investigated the structural requirements for reactive oxygen species production by CA-4 and the triazole analogues Ana-2 **126**, Ana-3 **127** and Ana-4 **128** (Fig. **8**). Authors reported that combretastatin A-4 caused cell death in PC12 cells in a caspase-3 and

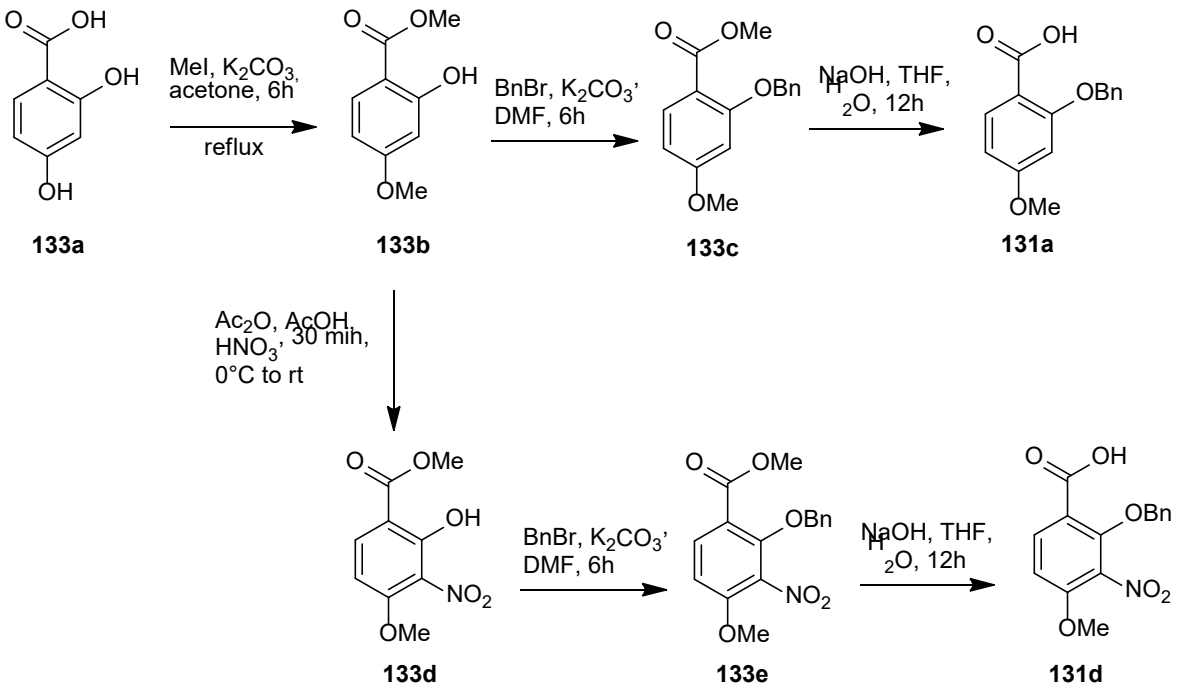
caspase-9 dependent pathway, involving peroxynitrite as an early trigger and also a caspase-independent pathway involving superoxide. The former way was influenced by the phenolic group and the latter not. Ana-2 **126** mimics the CA-4, because this compound activated caspase-3, produced reactive oxygen species and cause cell death. In the case of Ana-3 **127**, which did not have the phenolic group and there was no activation of caspase-3 nor early dihydrorhodamine oxidation, supporting these effects are linked. Ana-4 **128** *trans*-configuration between the two aryl rings also did not produce caspase-3, reactive oxygen species or cell death [41-43]. The results confirm that the CA-4 and Ana-2 **126** produce superoxide, as well as peroxynitrite which is linked to caspase-3 activation. Ana 3 **127** only produce superoxide and exclude cell death which is dependent on the activation of caspase-3. Further studies on mechanisms revealed that caspase-9 is also involved in cell death (Caspase-8 not), caspase-3 activation and dihydrorhodamine-related reactive oxygen species induced by CA-4 and Ana-2 **126**. In summary studies suggested that CA4 and Ana-2 **126** was highly toxic, Ana-3 **127** less toxic and Ana-4 **128** did not show toxicity [40].

Jedhe and co-workers [44] received a series of 1,5-disubstituted tetrazole analogs **129** (Scheme **27**) with an extended hydrogen-bond donors at the *ortho*-position as potential *cis*-restricted combretastatin derivatives. The synthesized compounds are then tested for antitubulin and antiproliferative activity. In the early stages of the synthesis was prepared substrates designed to produce tetrazole-tethered combretastatin analogues. Scheme **25** presents the synthesis of substrate **131c** from 2,3,4-trimethoxybenzaldehyde **130a**, as well as using another set of experiments towards aniline derivative **132a** [44].



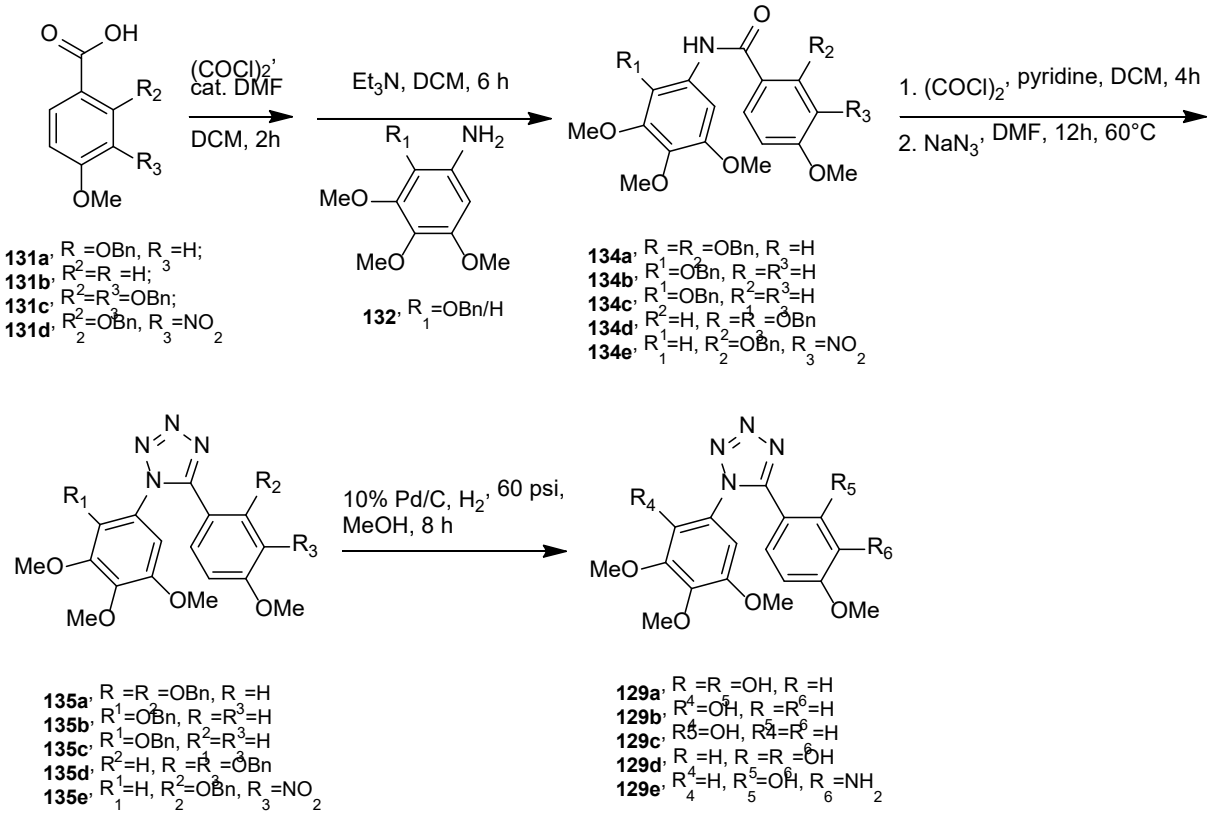
Scheme 25. Synthesis of derivatives **131c**, **132a**.

Preparation of further substrates **131a** and **131d** from 2,4-dihydroxybenzoic acid **133a** was depicted in Scheme **26** [44].



Scheme 26. Synthesis of compounds **131a**, **131d** [44].

Synthesis of tetrazole CA-4 derivatives**129a-e**, described by Jedhe *et al.* [44] is shown in Scheme **27**. From the previously synthesized carboxylic acids **131a-d** and the aniline derivative **132** were obtained amides **134a-e**, then converted to the corresponding benzyloxy-protected tetrazoles **135a-e**. In the last stage, tetrazoles were subjected to hydrogenolysis to give the target compounds **129a-e** [44].



Scheme 27. Synthesis of tetrazole derivatives **129a-e** [44].

The compounds **129a-e** were tested on inhibition of four different human cancer cell lines such as HeLa, A549, H1299 and MCF-7. Compounds **129a** and **129b**, of which the hydroxyl group in ring A was in the *ortho*-position, exhibited weaker antiproliferative activity against all tested cell lines exhibited an IC_{50} > 45 mM. The greatest antiproliferative activity exhibited compound **129e** in relation to all tested cell lines (for HeLa $IC_{50} = 0.9 \pm 0.0016$ for AS49 $IC_{50} = 0.52 \pm 0.0009$, for MCF-7 $IC_{50} = 2.9 \pm 0.07$ and for the H1299 $IC_{50} = 4.0 \pm 0.4$).

None of the compounds proved to be more active than the CA-4. During the tests on the inhibition of tubulin polymerization and colchicine binding by the compounds **129a-e** it was found that analogs **129a** and **129b** were inactive for as inhibitors of tubulin polymerization. Compounds of **129c-e** showed a similar activity in the inhibition of tubulin polymerization (sequence: IC_{50} = 2.7 ± 0.2, IC_{50} = 2.5 ± 0.1, IC_{50} = 2.2 ± 0.1). It was found in the docking studies, that compound **129e** nicely overlaps with all structural features of colchicine in the binding site (69 ± 0.3%) [44].

Nkepang *et al.* [45] reported prodrug strategy, that combretastatin CA-4 is locally released by visible / near IR light (Fig. **9**). The designed conjugates **136** consisted of CA-4 attached *via* acrylic acid moiety to photosensitizer (phthalocyanine or porphyrin). Folic acid was used as a delivery vector to provide selectivity towards overexpressing cancer cells and tumors. In the course of the photodynamic process, aminoacrylate is singlet oxygen cleavable linker and enables drug release. The authors observed influence of the length of the PEG spacer on the partition coefficients and efficacy, received promising results in selective damage of colon 26 tumors in Balb/c mice.

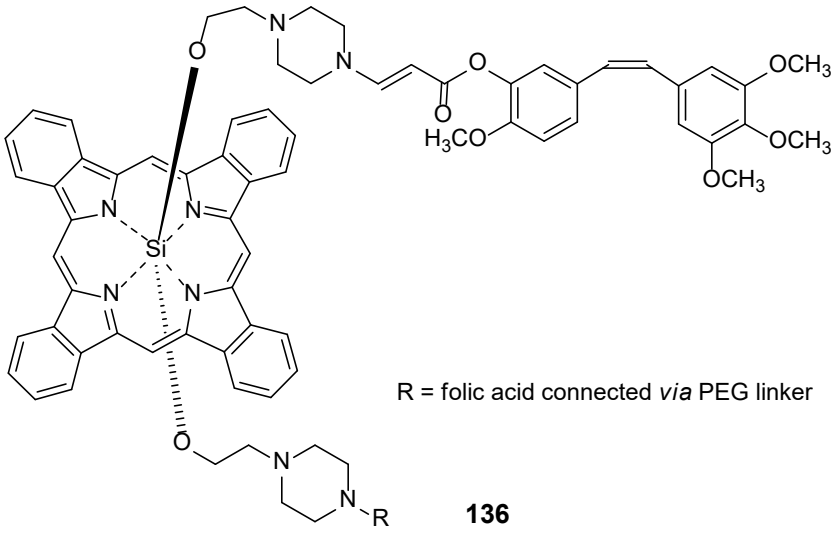


Fig. (9). Targeted prodrugs **136** developed by Nkepang *et al.* [45].

2.2. Modifications of ring B in combretastatin A-4

Torijano-Gutierrez *et al.* [46] in their work described a series of hybrid molecules containing a CA-4 analogue moiety and pironetin fragment linked by an ester linker of varying length. Examples of these compounds are shown in Fig. **10**.

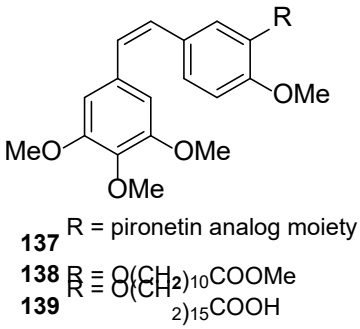
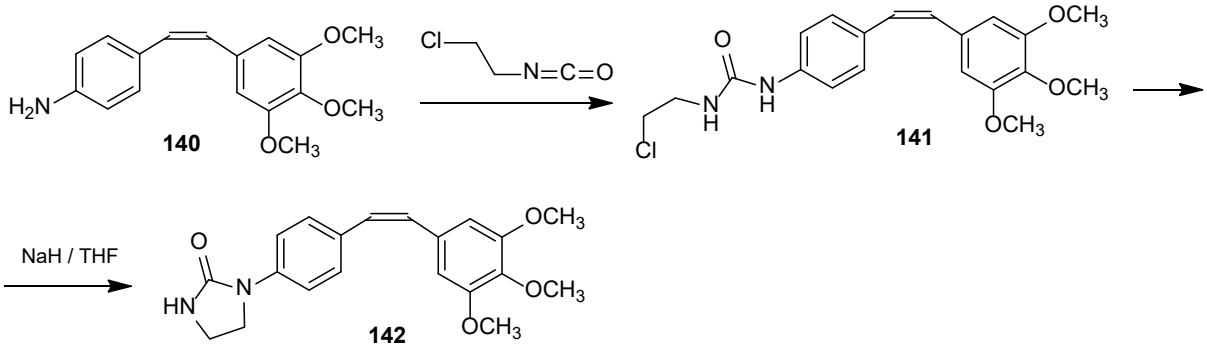


Fig. (10). Structures of the pironetin analog/combretastatin A-4 hybrids used in Torijano-Gutierrez *et al.* [46].

The resulting derivatives **137-139** were tested for their cytotoxic activity using two cancer cell lines: human colon adenocarcinoma (HT-29) and breast adenocarcinoma (MCF-7) and one normal human embryonic kidney cell line (HEK-293). These analogs provided lower than the CA-4, IC_{50} parameter cell line HT-29. The most cytotoxic compound was **139** (IC_{50} = 1.9 ± 0.3). In the case of MCF-7 some high activities were obtained, but none of them showed a higher cytotoxicity than the CA-4. During research on HEK-293 was found that some of the compounds exhibited a high cytotoxicity to cancer cells, and characterized by a low cytotoxicity to normal embryonic kidney cell line. This was a very desirable feature. An example may be the compound **138**, having for HT-29: IC_{50} = 8 ± 1, for MCF-7: IC_{50} = 3 ± 0.6 and for HEK-293: IC_{50}> 300 [46].

Styrylphenylimidazolidin-2-one (Scheme **28**) is one of the active combretastatin CA-4 analogs designed by Gagné-Boulet *et al*. [47]. Nanomolar tumor cell growth inhibition for

compound **142** was comparable to CA-4 against M21 (IC_{50} = 2.4 nM), MCF7 (IC_{50} = 2.6 nM) and HT-1080 (IC_{50} = 2.3 nM), and significantly higher against HT-29 human cancer cell line (IC_{50} = 1.7 nM). The key step of synthesis of this compounds is reaction of amine with 2-chloroethylisocyanate, followed by cyclization in the presence of sodium hydride.

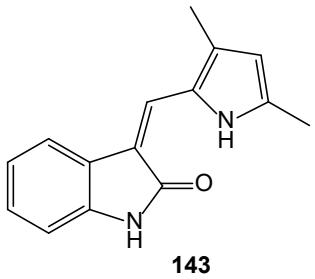


Scheme 28. Synthesis of (*Z*)-1-(4-(3,4,5-trimethoxystyryl)phenyl)imidazolidin-2-one **142**.

The developed derivatives revealed antimicrotubule properties, bound to the colchicine-binding site, blocked the cell cycle in G2/M phase, disrupted the cytoskeleton of cancer cells. These promising features exhibited both (*Z*)-styrylphenylimidazolidin-2-ones and (*Z*)-styryl-*N*-phenyl-*N*'-(2-chloroethyl)ureas.

2.3. Analogs of combretastatin A-4 derived from Semaxanib

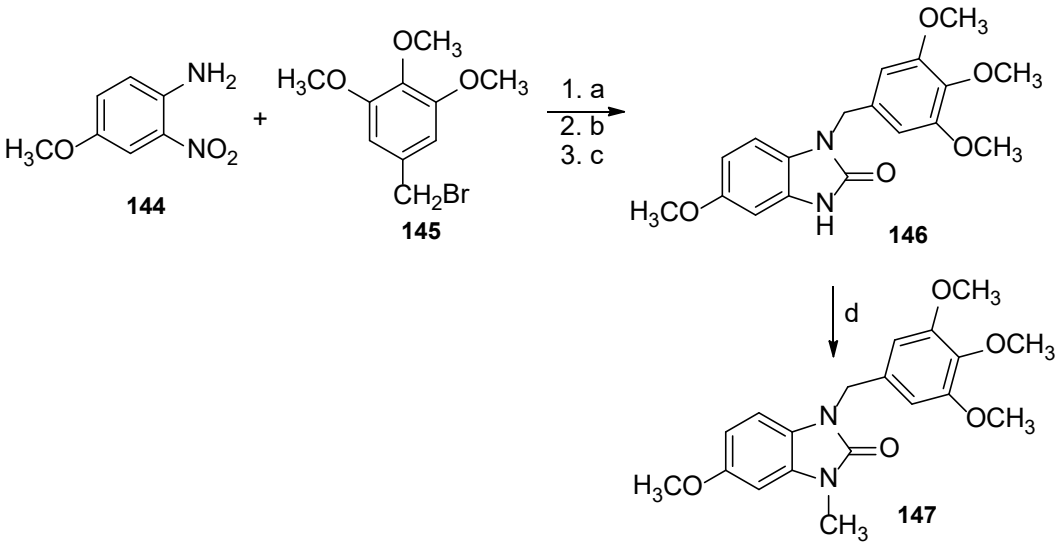
Sun's group [48] synthesized and evaluated analogs of combretastatin A-4 derived from Semaxanib **143** (SU5416) (Fig. **11**), a tyrosine kinase inhibitor.



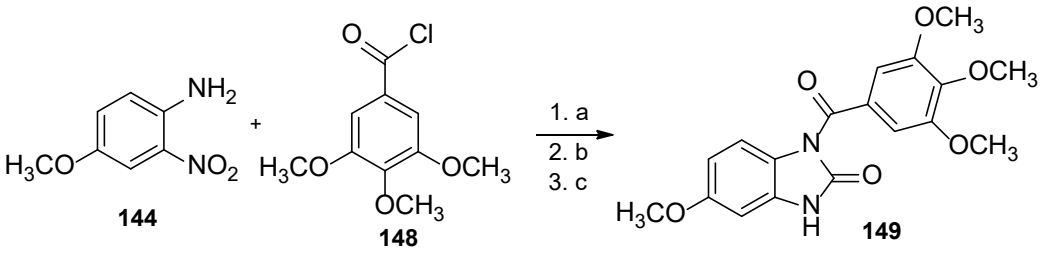
143

Fig. (11). Structure of Semaxanib **143** [48a].

The procedure for the preparation of the compounds **146** and **147** is presented in Scheme **30**. First, 4-methoxy-2-nitroaniline **144** was alkylated with 3,4,5-trimethoxybenyl bromide **145**, potassium carbonate and potassium iodide in DMF, followed by reduction of the nitro group in the presence of Zn/HOAc to give respective amine, which was converted by cyclization with CDI to the derivative **146**. Compound **146** was reacted with methyl iodide to obtain the analog **147** (Scheme **29**). Derivative **149** (Scheme **30**) was synthesized by using a similar procedure including acylation with 3,4,5-trimethoxybenzyl chloride **148** in the first stage. Other Semaxanib-related structures **150-153** are depicted in Fig. **12**. [48].



Scheme 29. Synthesis of **146** and **147**: (a) K_2CO_3, KI, DMF, rt. 4h; (b) Zn, HOAc, 110°C, 2h; (c) CDI, THF, overnight; (d) MeI, K_2CO_3, KI, DMF, rt, 24h [48].



Scheme 30. Synthesis of compound **149**: (a) Et_3N, CH_2Cl_2, rt, 2h; (b) $SnCl_2x2H_2O$, HCl, EtOH, 80-90°C, 30 min; (c) CDI, THF, rt, overnight [48].

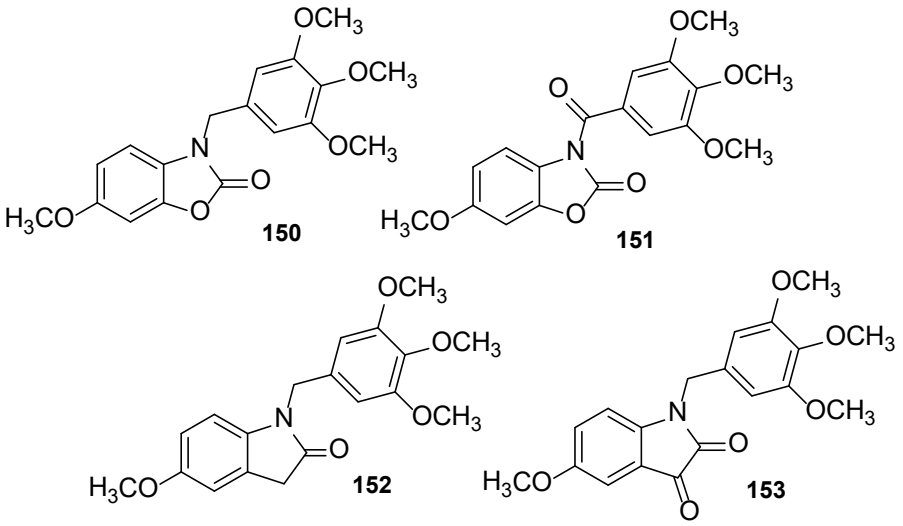


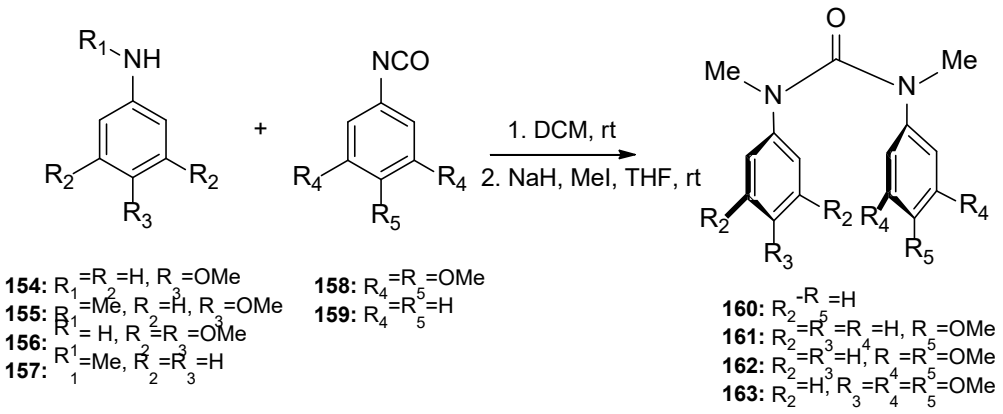
Fig. (12). Structures of Semaxanib – related derivatives **150-153** [48].

Compounds **146**, **147**, **149**, **150**, **151**, **152**, **153** were investigated on their inhibition of cancer cell proliferation (PC-3 and MDA-MB-231 cancer cells) and tubulin polymerization. The most active one occurred to be compound **146**, which structurally resembles the combretastatin A-4. Analog **146** inhibited activities on both PC-3 and MDA-MB-231 cells with IC_{50} values of 44.25 and 52.75 nM, respectively. Moreover derivative **146** was the most potent inhibitor of tubulin polymerization, while compounds **150** and **151** were inactive [48].

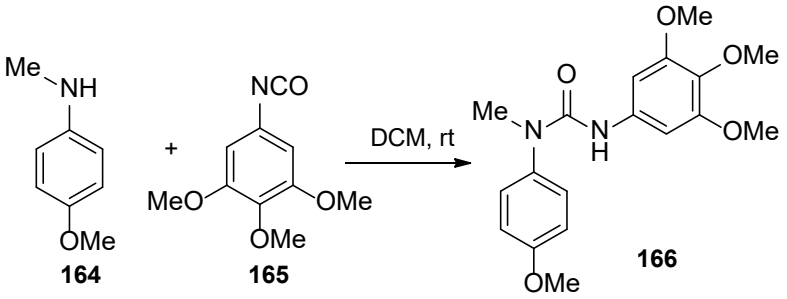
2.4. Derivatives of combretastatin C-4 where the double bond between the aryl rings is replaced by introduction of noncyclic groups

Snape and co-workers [49] reported the synthesis of series of *N,N'*-dimethyl-*N,N'*-diarylureas as a new analogues of combretastatin A-4. The aromatic urea were prepared by reaction substituted aniline **154–157** with an appropriate isocyanate **158** or **159**, then by *N*-methylation to obtain the (*cis*, *cis*)-*N,N'*-dimethyl-*N,N'*-diarylureas **160–163** (Scheme **31**). While (*cis*, *trans*)-urea **166** was synthesized by the reaction between 4-methoxy-*N*-methyl-aniline **164** and isocyanate **165** (Scheme **32**).

Compounds **160-163,166** were tested for inhibition of tubulin polymerisation (TPI). The most active inhibitor compared to the DMSO was CA-4P, which was used as a reference. Studies indicated that the relative TPI activity of derivatives **160-163,166** reflects their shape and predicted activity. Thus, compounds **162** and **163**, which are most like CA-4P and CA-4 in shape and also oxygenation, inhibited tubulin polymerisation by 34% and 31%, respectively. While, analogs **160** and **161**, which lacking some or all –OMe groups, were less active by 23% and 25%, respectively. Compound **166** possessing a completely different shape, was inactive. Moreover, all derivatives **160-163,166** were evaluated in 2 GBM short-term cell cultures (IN1472 and IN1760) and the established GBM cell line U251MG at 10 μM of the test compound. However, CA-4P exhibited a higher activity than the derivatives **160-163,166** in all the *in vitro* assays [49].



Scheme 31. Synthesis of ureas **160-163** [49].



Scheme 32. Synthesis of compound **166** [49].

Santos *et al.* [50] developed the synthesis of new derivatives of combretastatin A-4 containing sulfur **167** and selenium **168** atoms as separated group between aromatic rings (Fig. **13**) [50].

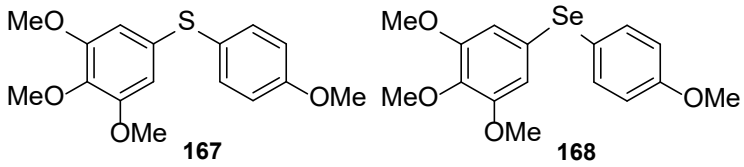
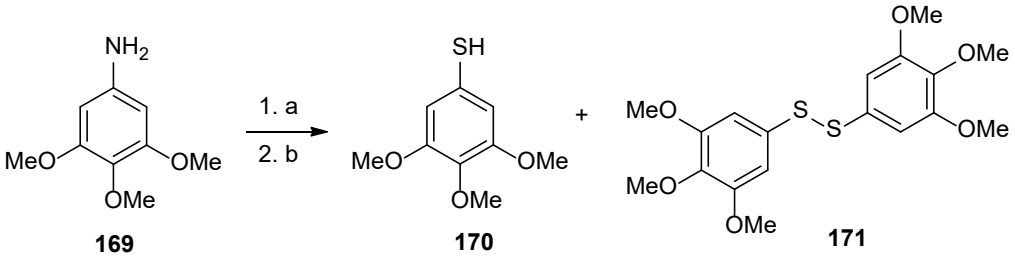


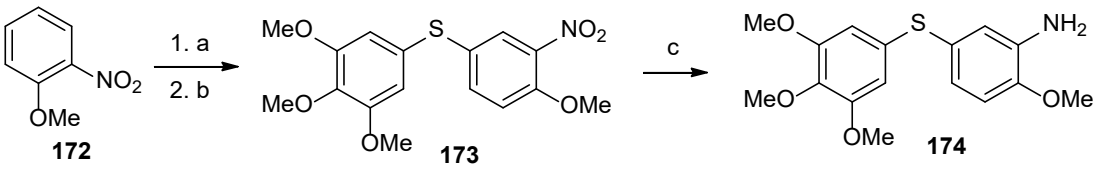
Fig. (13). New derivative of combretastatin A-4 containing selenium as a separated group between aromatic rings [50].

The first step in the synthesis of sulfur derivative of CA-4 was to obtain sulfide **170**. In this step was also generated a small amount of disulfide **171** (Scheme **33**).



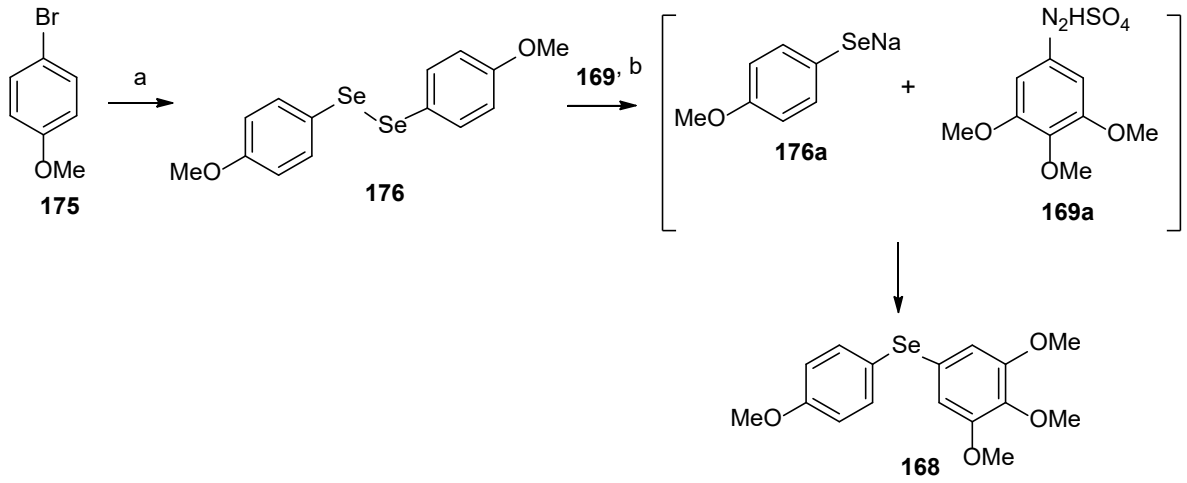
Scheme 33. Reagents and conditions: (a) (1) $NaNO_2$, $HCl_{(conc.)}$, H_2O, 0°C, 10 min., (2) $EtOCS_2K$, 50-55°C, 40 min; (b) EtOH, NaOH, 65°C, 1.5 h [50].

In the second step commercially available compound 1**72** was converted to sulfide 1**73** *via* iodination and coupling with **169**. Reduction of **172** led to amine **174**. (Scheme **34**) [50].



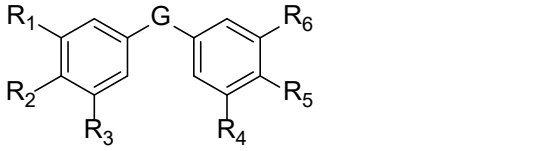
Scheme 34. Reagents and conditions: (a) NIS, H_2SO_4(conc), 0°C-rt, 20 min; (b) **169**, Neocuproine, CuI, *t*-NaOBu, toluene, Δ, N_2, 17h; (c) $SnCl_2$, HCl (36%), AcOH, rt, 2h [50].

Synthesis of new CA-4 analogs containing selenium **168** is depicted in Scheme **35** [50], where 4-bromoanizole **175** was converted to diselenide **176**, followed by reduction to selenol **176a**. Nucleophile **176a** reacted with diazonium salt **169a** to produce **168**.



Scheme 35. Reagents and conditions: (a) (1) Mg, THF (dry), N_2, 1h, (2) Se, Δ, N_2, 3h; (b) (1) $NaNO_2$, $H_2SO_{4(aq)}$ 6%, 0°C, 1h (the diazonium salt **169a** formation); (2) $NaBH_4$, $THF_{(aq)}$, 0°C, 10 min, (3) the diazonium salt of the amine, 50°C-rt, 17h [50].

The resulting compounds **173**, **174**, **177**, **178**, **179** and **180** (Fig. **14**) were tested on four human tumor cell lines (MCF-7 (breast cancer), 786 (kidney), HT-29 (colon), PC-3 (prostate), and tubulin polymerization and the inhibition of binding of colchicine [50].



(CA-4): G=*cis*-CH=CH, R_1=R_2=R_3=R_5=OCH_3, R_4=H, R_6=OH
173: G=S, R_1=R_2=R_3=R_5=OCH_3, R_4=H, R_6=NO_2
174: G=S, R_1=R_2=R_3=R_5=OCH_3, R_4=H, R_6=NH_2
177: G=Se, R_1=R_2=R_3=R_5=OCH_3, R_4=R_6=H
178: G=S, R_1=R_2=OCH_3
179: G=Se, R_1=R_6=R_2=H, R_2=R_5=OCH_3
180: G=Se_2, R_1=R_3=R_4=R_6=H, R_2=R_5=OCH_3

Fig. (14). Structures of received new analogues combretastatin A-4 containing sulfur or selenium [50].

Diaryl sulfides **173** and **174** exhibited a high ability to inhibit tubulin polymerization (IC_{50} = 2.8 ± 0.3μM for the compound **173** and IC_{50} = 0.74 ± 0.04 μM for the compound **174**). In

addition, compound **174** was found to be also a potent inhibitor of colchicine binding (95 ± 0.1%), which indicates that a substitution at the *meta*-position of the amino group enhances the ability of the sulfides to interact with tubulin than substitution at the *meta*-position nitro group. CA-4 derivative comprising selenium **177** was characterized by the highest ability to inhibit tubulin (IC_{50} = 0.62 ± 0.08 μM) and was found to be a potent inhibitor of colchicine binding (94 ± 1%). The compound **180** also significantly inhibited tubulin polymerization (IC_{50} = 1.7 ± 0.06 μM). During investigations of cytotoxicity on the cell line MCF-7 was found, that compounds **173**, **174**, **179** and **180** are inactive. Only derivatives **174** and **177** showed similar activities as CA-4 (IC_{50} = 0.008 ± 0.003 for **173** and IC_{50} = 0.010 ± 0 in case of **174**). The best results in the study of cytotoxic activity against human caner header lines 786, HT-29 and PC-3 were obtained for the selenium derivative of CA-4 **177** (IC_{50} = 0.68 ± 0.09 μM for 786, IC_{50} = 0.28 ± 0.08 μM for HT-29 and IC_{50} = 0.08 ± 0.003 μM). Sulfides **173** and **174** gave very similar activities. Compounds **178**, **179** and **180** exhibited the lowest cytotoxicity. Molecular modeling studies indicated that substitution sulfur of selenium resulted in deeper and more "colchicine-like" binding conformation and increased the hydrophobicity of the molecule [50].

CONCLUSION

In summary, combretastatin A-4 is one of the strongest natural antimitotic compounds. CA-4 possesses a potent cytotoxicity in the low nanomolar concentrations against a variety of tumor cells, is an inhibitor of proliferation and migration of endothelial cells. Its limited water solubility gives rise to searching analogs with improved pharmacological properties. Results received by different research groups, which are presented in this review, show that many new combretastatin A-4 analogues possess promising pharmacological features, including

increased aqueous solubility compared to CA-4. Moreover, new derivatives retain high antitumor activity and inhibit the polymerization of tubulin with the better efficacy than CA-4. Thus, combretastatin A-4 moiety can still serve as a lead structure and can be further modified to increase the specificity of new analogs toward tumor cells.

CONFLICT OF INTEREST

None declared.

ABBREVIATION

A549 = Human non-small lung cancer cells

AGS = Human stomach adenocarcinoma

CA-4 = Combretastatin A-4

CA-4P = Combretastatin A-4 phosphate

CDI = *N,N*-carbonyldiimidazole

combretastatin A-4 = (Z)-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl) ethene

*m*CPBA = *m*-Chloroperoxybenzoic acids

DCM = Dichloromethane

DDQ = 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone

DMF = *N,N*-Dimethylformamide

DMSO = Dimethyl sulfoxide

ECA-10 = Human esophageal carcinoma cells

H1299 = Human non-small cell lung carcinoma

HeLa = Human cervix carcinoma

HT-29 = Human colon adenocarcinoma

IC_{50}	=	Half maximal inhibitory concentration
K562	=	Human myeloid leukemia cells
MCF-7	=	Human adenocarcinoma breast
MTT	=	Colorimetric assay for assessing cell metabolic activity
NIH-3T3	=	Mouse embryonic fibroblast cell line
NMO	=	*N*-Methylmorpholine *N*-oxide
PC-3	=	Human prostate carcinoma cells b
PPTS	=	Pyridinum *p*-toluenesulfonate
SMMC-7721	=	Human hepatocellular carcinoma cells
TBAF	=	Tetra-*n*-butylammonium fluoride
TBS	=	*tert*-Butyldimethylsilyl
Tf	=	Trifuoromethanesulfonyl
THF	=	Tetrahydrofuran
VCR	=	Vincristine

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