Developments of isoCombretastatin A-4 derivatives as highly cytotoxic agents

Abdallah Hamzé, Mouad Alami, Olivier Provot

Developments of *iso*Combretastatin A-4 Derivatives as Highly Cytotoxic Agents

Abdallah Hamze[a], Mouad Alami[a*], Olivier Provot[a*]

[a] *Université Paris-Saclay, CNRS, BioCIS, 92290, Châtenay-Malabry, France.*

**Corresponding authors. Email: olivier.provot@Universite-Paris-Saclay.fr and mouad.alami@Universite-Paris-Saclay.fr*

Summary

Combretastatin A-4 (CA-4) is a natural anti-cancer agent isolated in 1989 from the African willow tree, *Combretum caffrum*. Due to its chemical simplicity, this (*Z*)-stilbene has been the subject of many structural modifications mainly to improve its chemical and metabolic stability. Beside a large number of synthetic analogues, *iso*Combretastatin A-4 (*iso*CA-4), has proved to be a solution of choice since this non-natural isomer of CA-4 is stable, easier to synthesize and has equivalent antitumor properties as CA-4. In this review, we will present the structure-activity relationships (SARs) around *isoCA*-4 since its discovery in 2007. In a first part, we will describe some alternatives to replace the phenol B-ring of *iso*CA-4, then we will focus on the variations made on the 1,1-ethylene double bond and then, we will evocate very recent exiting results concerning the possible replacements of the 3,4,5-trimethoxyphenyl A-ring of *isoCA*-4 by suitable heterocycles.

Keywords: Combretastatin A-4, *iso*Combretastatin A-4, Heterocycles, Cytotoxicity, Tubulin, Cancer.

Contents

1. Introduction

Combretastatin A-4 (CA-4) a natural stilbene isolated by Pettit from the African willow tree *Combretum caffrum* was found to be a potent antitumor agent that strongly inhibited tubulin assembly by binding to the colchicine binding-site.[1] CA-4 displayed a nanomolar level of cytotoxicity against a wide range of human cancer cell lines including multi drugs resistant (MDR) cancer cells.[2,3] In 1997, it was demonstrated that CA-4 acted as a vascular disrupting agent (VDA), which selectively targeted the vascular network of tumors to induce irreversible shutdown of blood flow to neoplastic cells.[4] Since the understanding in its mode of action due in part to direct effects on endothelial cells, the interest on this molecule has raised considerably as evidenced by the increased number of publications and patents (Figure 1).

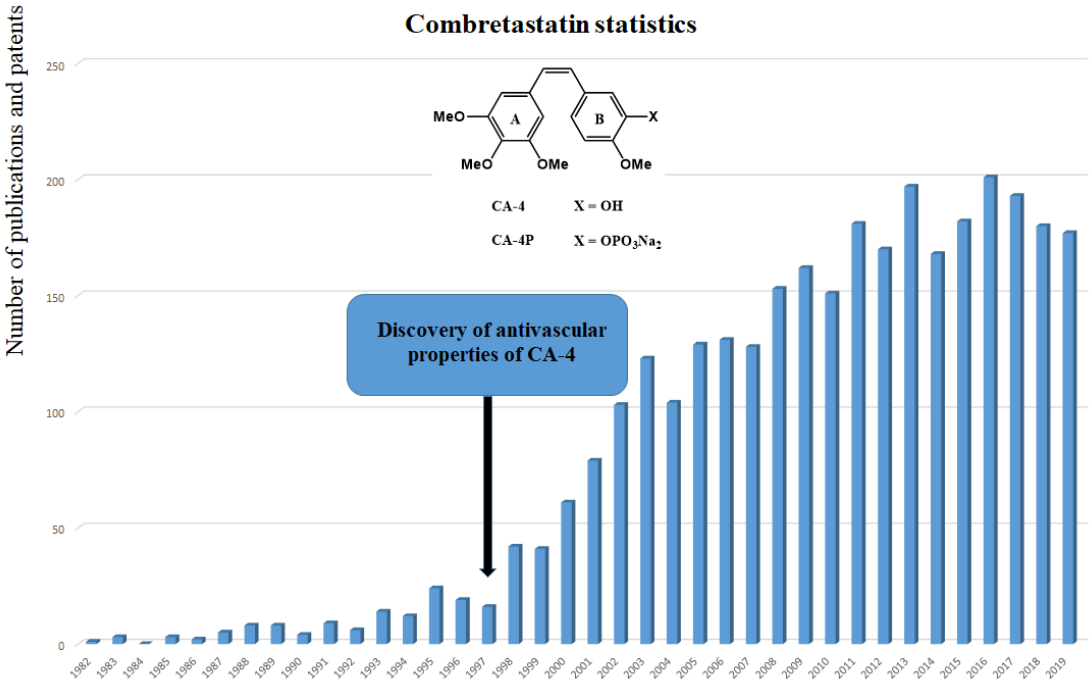
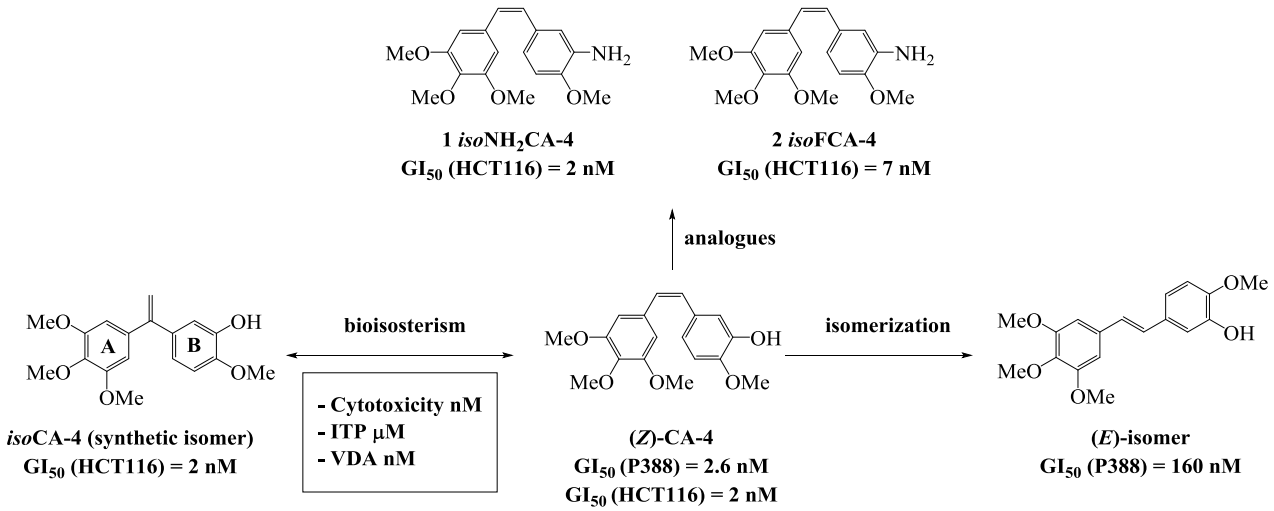


Figure 1. Combretastatin statistics until december 2019 (Scifinder)

In 2016, the water-soluble CA-4 prodrug CA-4P (fosbretabulin) has received the status of orphan drug in Europe and the USA for the treatment of ovarian cancers, neuroendocrine tumors, certain thyroid cancers and more recently, for multiform gliomas.[5] Several clinical trials are currently underway using CA-4P alone (*e.g.* unresectable or metastatic pancreatic neuroendocrine tumors (PNETs), gastrointestinal neuroendocrine tumors with elevated biochemical markers or in combination with (*i*) pazopanib, a tyrosine kinase inhibitor (in advanced recurrent ovarian cancers), (*ii*) paclitaxel and carboplatin (in anaplastic thyroid cancers), (*iii*) bevacizumab, a monoclonal antibody which inhibit angiogenesis by binding vascular endothelial growth factor (in central nervous system cancers), and others.[6] Despite its proven efficacy in therapeutics, the main handicap of CA-4 is the isomerization of its stilbene *Z*-double bond into the less active *E*-form[7] during storage, administration, and metabolism.[8,9] To avoid this isomerization problem, a large number of stable CA-4 analogs have been synthesized by including the double bond in heterocycle.[10,11,12] In 2005 our group initiated a medicinal chemistry program to prepare stable analogues of CA-4 by replacing the (Z)-ethylene double bond with various non-isomerizable spacers having different sizes (from 1 to 6 carbon atoms).[13,14,15,16] In 2007,[17] we resolved the stability problem of CA-4 by finding and demonstrating that *iso*CA-4,[18,19] the stable and non-natural isomer of CA-4 having a 1,1-diarylethylene structure, had the same biological properties as the natural product (Figure 2). In details, *iso*CA-4 (*i*) displayed antiproliferative activity with GI_{50} values ranging from 2 to 10 nM



HCT116: Human Colon Tumor; P388: Murine leukemia cell line

Figure 2. CA-4 and its isomers.

against different human cancer cell lines, (*ii*) inhibited tubulin polymerization (ITP) at a micromolar level, (*iii*) induced apoptosis in a variety of cancer cells and (*iv*) disrupted newly formed vascular tubes *in vitro*. We have also showed that *iso*CA-4 was stable in an aqueous HCl 12 N solution for a week, was easy to synthesize[20,21,22] and was metabolized much less than CA-4 (<5% in human liver microsomal fractions).[23]

To demonstrate the efficiency of *iso*CA-4, we have nano-precipitated the anticancer compound gemcitabine conjugated with squalene (SQ-gem) together with *iso*CA-4.[24] It was found that these two molecules spontaneously self-assembled as stable nanoparticles (SQ-gem/*iso*CA-4 NAs) of *ca*. 142 nm in a surfactant-free aqueous solution. The antitumor efficacy of these nanoparticles was investigated *in vivo* on a human colon vascularized (LS174-T) carcinoma xenograft model in mice. The SQgem/*iso*CA-4 nanoparticles showed considerable improvement in antitumor activity (93% tumor growth inhibition), as compared to SQ-gem NAs (73%), to SQ-gem NAs combined with *iso*CA-4 (71%), to gemcitabine (32%), or to an association gemcitabine/*iso*CA-4 (60%). As *iso*CA-4 is (*i*) very easy to prepare at a multi-gram scale, (*ii*) chemically and metabolically stable, (*iii*) highly cytotoxic at a nanomolar level, disrupting the tumor vascular network, and (*iv*) remarkably potent as antitumor agent. Herein, we are pleased to group together for the first time in this up-date, a selection of structural analogues and their structure-activity relationships since the discovery of *iso*CA-4. In the first part, we will present the main interesting chemical modifications of its phenol B-ring, then we will focus on the modifications made to the 1,1-ethene bridge. The last part will be dedicated to very recent advances concerning possible replacements of the 3,4,5-trimethoxyphenyl-A-ring (TMP) of *iso*CA-4 by appropriated heterocycles. In the B-ring modification section, possible changes on the 3' and 4' position of the A-ring will be first presented followed by efficient replacements of the A-ring by suitable heterocycles. Concerning the ethylene linker modifications, a selection of tri- and tetra-substituted ethylene derivatives will be showed, then restricted analogues (compounds having the ethylene double bond included into a cycle or heterocycle) will be presented and finally, we will showed that it is also possible to reduce with efficacy the ethylene double bond (*iso*erianin derivatives) or to replace it by a *N*-Me linker (*iso*azaerianin compounds). In the last section devoted to the possible replacements of the A-ring, we will show that quinazoline and quinoline rings are suitable bioisosteres of the traditional 3,4,5-trimethoxyphenyl A-ring. Again, compounds having an ethylene linker will be presented before *N*-methyl derivatives and, as before, compounds having an aromatic B ring will be presented first, followed by their heteroaromatic analogues.

For easier comparisons and the establishment of SARs, GI_{50} or IC_{50} values of *iso*CA-4 analogues will be preferentially reported on human colon cancer cell lines (HCT116), (HT29), (HT15) and (HCT8).

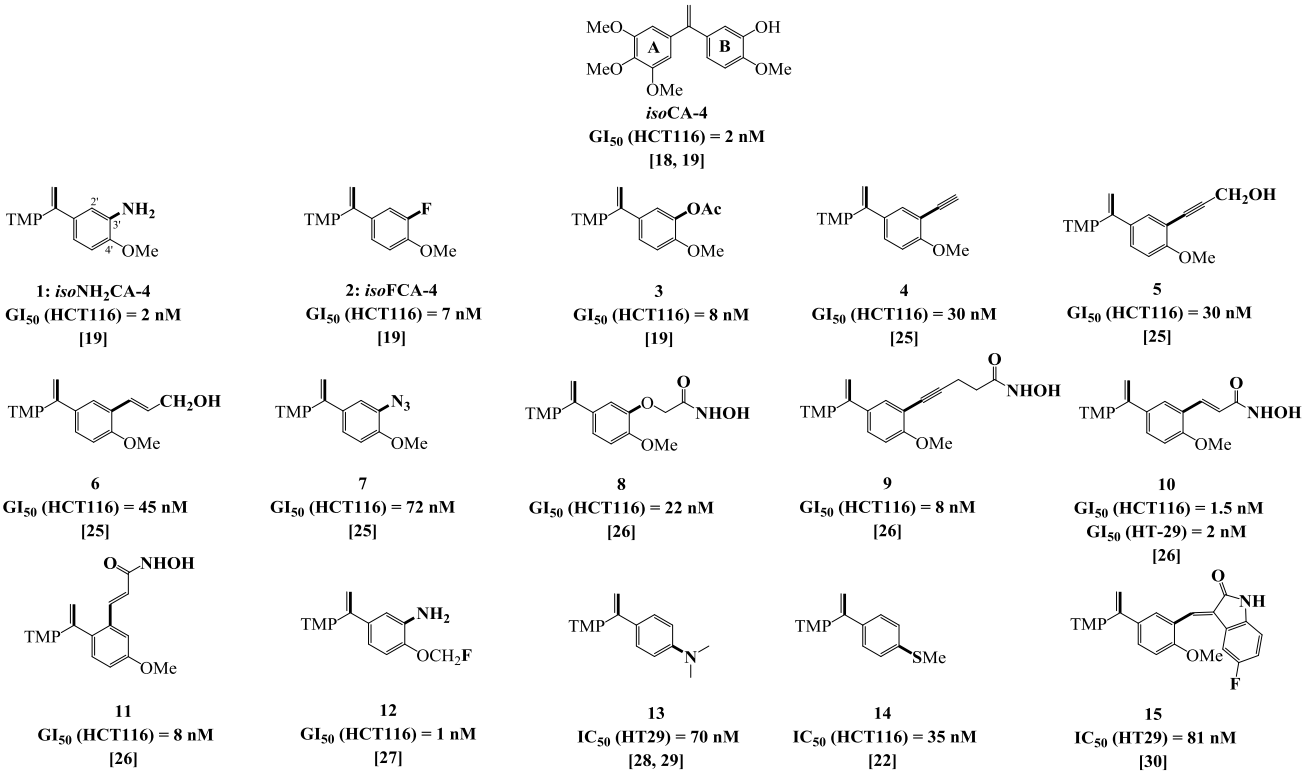


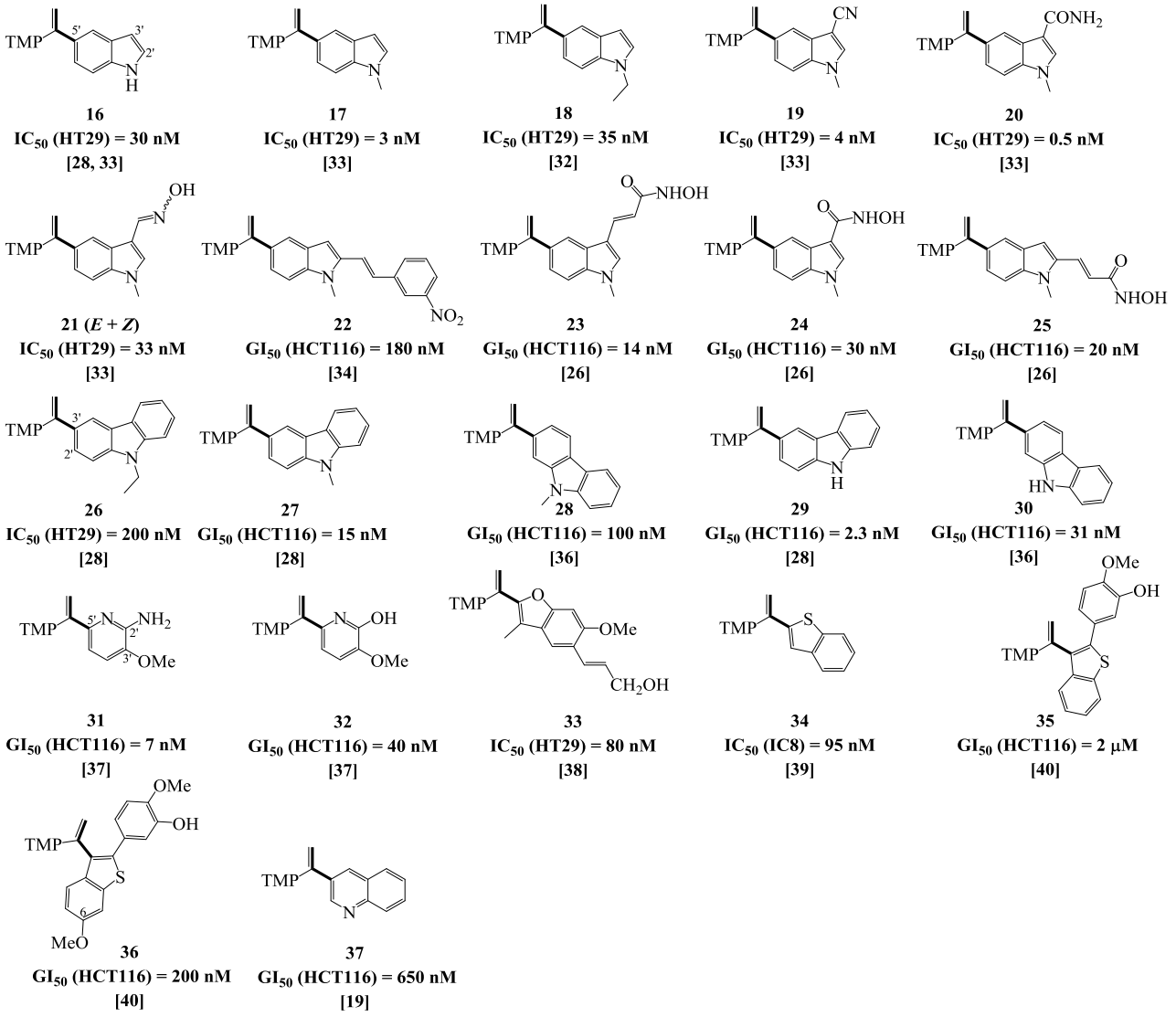
Figure 3. Replacement of the CA-4's B-ring by other substituted phenyl rings

Discussion

2. B-ring modifications

Due to the chemical simplicity of *iso*CA-4, its B-ring was the subject of various modifications. Figure 3 collects suitable substituents in the phenyl B-ring conferring interesting pharmacological properties to *iso*CA-4, as illustrated with the compounds **1-3**,[19] **4-7**,[25] **8-11**,[26] **12**,[27] **13**,[28,29] **14**[22] and **15**[30]. It was pointed out that the C3'-OH substituent is not essential and can be successfully replaced by a variety of polar and non-polar substituents, most of the time of small sizes (*e.g.*; NH_2, F, N_3,…). As expected, the corresponding acetate **3**, which can be seen as a prodrug of *iso*CA-4 displayed a similar cell growth inhibition as its parent molecule. Diarylethylenes **4-6** and **14** bearing C3'-alkynyl and C3'-alkenyl substituents on their B-ring exhibited nanomolar levels of cytotoxicity (GI_{50} values ranging from 30 to 81 nM). It is of note that diarylethylenes **8-11** having on C3' and C2' different chains with a hydroxamic acid function as a zinc chelator, were found to be very interesting cytotoxic dual molecules, targeting together β-tubulin and histone deacetylases 8 (HDAC8).[26] Compound **10**, a potent dual molecule inhibiting tubulin polymerization and HDAC also showed a marked cytotoxicity toward multi-drug resistance (MDR) HT-29 (human colon adenocarcinoma) cell line at a nanomolar level, whereas CA-4 and *iso*CA-4 were less effective (GI_{50} (HT29) = 9137 nM for CA-4).[31]

With respect to the best modifications identified on C4', it was found that SMe, -NMe_2 and -CHF_2 substituents can replaced with a certain success the traditional C4'-methoxy substituent of *iso*CA-4 leading for instance to highly cytotoxic compound **12**. Since the presence of a C4'-nitrogen atom is permitted (*e.g.*; dimethylaniline **13**) in place of a C4'-methoxy group, it has also been shown that indoles heterocycles may also replace the B-ring of *iso*CA-4 as well as other suitably functionalized heterocycles. Results showed that this strategy could lead to active compounds. Figure 4 highlighted relevant molecules bearijg these heterocycles, as well as their cytotoxicity levels. Indole nuclei[28,32,33,34,35] were welcome on the ethylene bridge as illustrated with free *N*H-indole **16** and *N*-substituted indole derivatives **17** and **18**. Interestingly, it was shown that substitutions on the C3'-position of the indole ring with



HCT116 and HT29: Human Colon Tumors; IC8: uterine sarcoma; TMP = 3,4,5-trimethoxyphenyl

Figure 4. Replacement of the CA-4's B-ring by heterocycles

polar groups increased the cytotoxicity as it can be observed with derivatives C3'-CN (**19**) and C3'-$CONH_2$ (**20**).[33] As has been observed in Figure 3 with various phenyl ring substitutions, an introduction on the indole of substituents bearing a hydroxamic acid function led to cytotoxic molecules **23**-**25**, which also selectively inhibited HDAC8.[26] The replacement of indole with a carbazole nucleus led to cytotoxic molecules **26**-**30**.[28,36] but that the position of the nitrogen atom with respect to the ethylene double bond plays a pivotal role (compare **27**, **28** and **29**, **30**). In fact, SARs studies for these compounds revealed that free NH-carbazole **29**[36] was the most anti-proliferative agent in this series. Among other heterocyclic B-ring explored, it has been reported that suitably substituted pyridine compounds **31** and **32** having on C2' -NH_2 or -OH substituents and on C3', a MeO-group, were highly cytotoxic agents.[37] Finally, benzofuran **33**,[38] benzothiophenes **34**-**36**[39,40] and quinoline **37**, in which the heterocyclic part is directly connected to the ethylene double bond, showed an average cytotoxicity on cancer cells (IC_{50} ~ 100 nM). Of note, that the installation of a naphthalene moiety instead of a quinoline (**37**) appears to induce a slightly increase in cytotoxicity.[19] From all these *iso*CA-4 analogues **1**-**37** having various B-rings, it appears, that in the benzenic series (compounds **1**-**14**), many modulations in C3'-position are allowed, on the contrary to what happens on C4'-position, where the methoxy group is often necessary. Moreover, with respect to the indole series, introducing into the C2' and C3'-position of substituents containing a hydroxamic acid function or a C3'-amido function was appropriated as illustrated with HDAC 8 inhibitors **23**-**25** and highly cytotoxic compound **20**. Concerning the replacement of the B-ring of *iso*CA-4 by

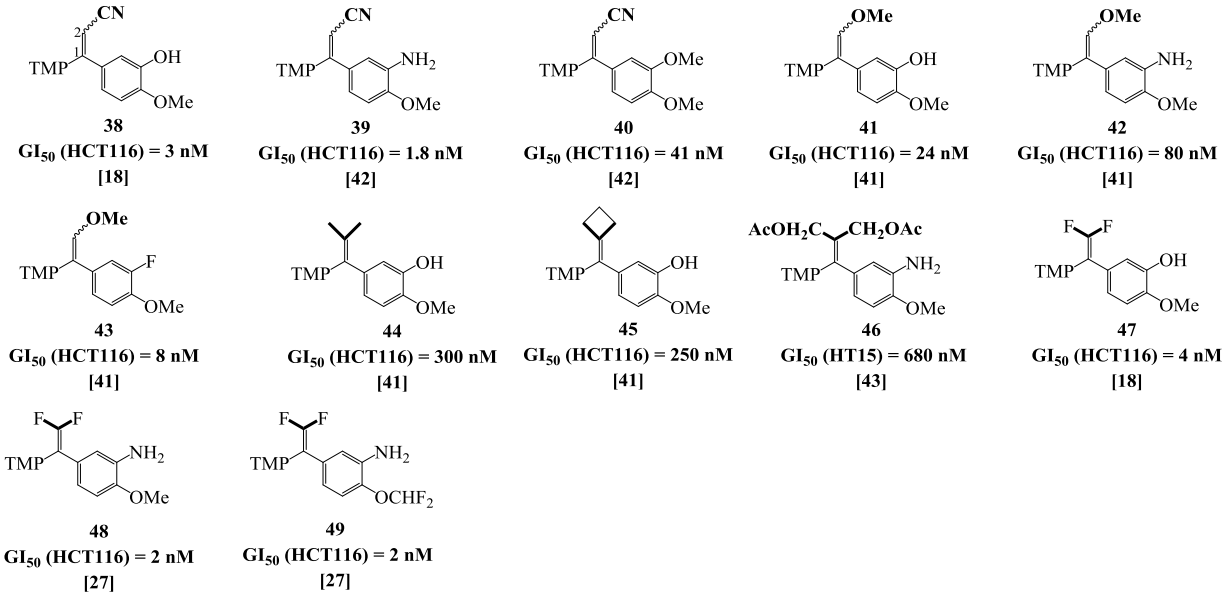


Figure 5. *Iso*CA-4 analogues having a tri- or tetra-substituted ethylene double bond

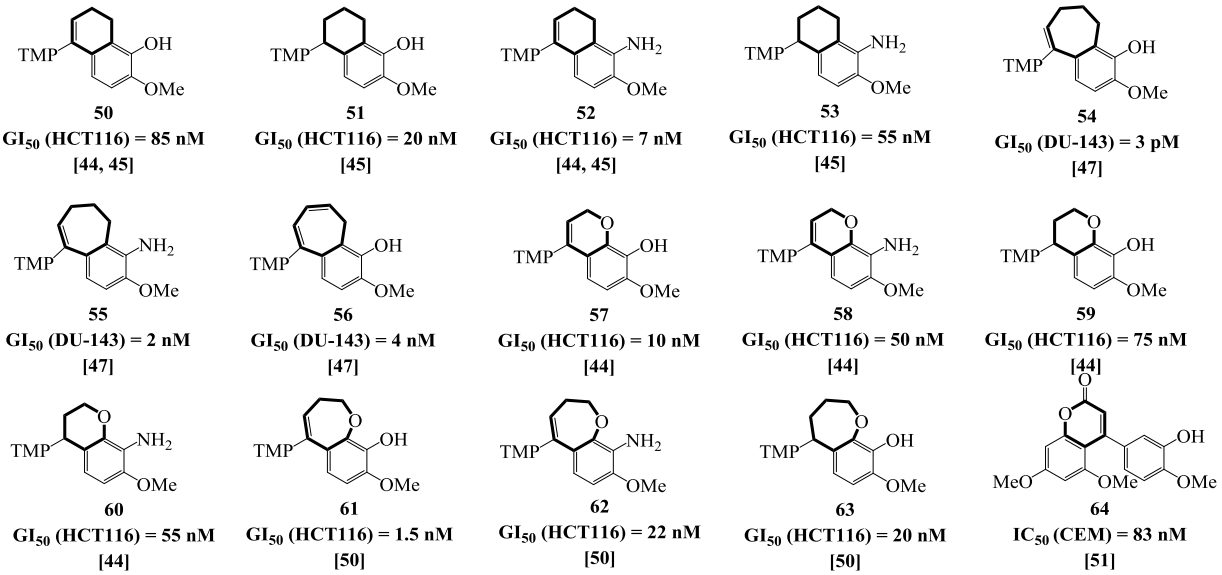
heterocycles (compounds **16**-**37**), it is clear that nitrogen-containing heterocycles such as indole, carbazole and pyridine were found to be the most appropriated heterocycles and some of them enhance the activity.

3. Ethylene linker modifications

In this part, we will highlight the most interesting variations achieved around the 1,1-ethylene double bond with compounds having a TMP A-ring and an appropriate B-ring. First of all, we will describe potent drugs having a tri- or a tetra-substituted ethylene double bond. Then, we will focus our attention with compounds in which the double bond was inserted into a cyclic system (constrained- or restricted-compounds), and finally, we will examine other convincing modifications of the 1,1-ethylene double bond, and notably its reduction or its replacement by a *N*-Me linker.

In Figure 5 are presented a selection of synthetic drugs having a tri-substituted- or a tetra-substituted ethylene linker (not included in a ring) which displayed an interesting level of cytotoxicity. As it could be seen, compounds **38**-**43**[18,40,41,42,] having a tri-substituted ethylene double bond were found to be cytotoxic agents against HCT116 cells at a nanomolar level. In the acrylonitrile series (**38**-**40**), prepared according to a Wittig olefination reaction (*E*/*Z* ~ 1:1), compounds having a C3'-OH (**38**) or -NH_2 (**39**) groups were found to be 15-fold more cytotoxic than their dimethoxy-congener **40** as it was previously observed in CA-4 and *iso*CA-4 series. Compounds **41**-**43**[41] having a small -OMe group on the ethylene double bond were prepared according to Barluenga coupling and displayed very interesting antiproliferative activities with fluorinated compound **43** as the lead compound in this series. On the exception of interesting compounds **47**-**49** having two fluorine atoms on the ethylene double bond,[18,27] tetra-substituted ethylene compounds **44**-**46** exhibited tedious cytotoxicity levels ranging from 250 to 680 nM.[41,43] One can note that the replacement of the C4' MeO-substituent in **48** by a CHF_2 led to an equipotent toxic drug **49**[27] which was found to be metabolically stable. As tri-substituted ethylene compounds **38**-**43** displayed a nanomolar level of cytotoxicity, the insertion of the ethylene double bond into carbocycles as well as heterocycles, leading to constraint *iso*CA-4 derivatives, was achieved mainly after relatively long syntheses with success. A selection of conformationally restricted *iso*CA-4 derivatives is described in Figure 6.

Dihydronaphthalenes **50** and **52**[21,44,45] and benzosuberenes **54**, **55**[46,47,48] in which the ethylene double bond has been inserted into 6 or 7-membered carbocycles were prepared in long and tedious syntheses. If dihydronaphtalene **50** displayed a relatively modest cytotoxicity level, the reduction of its double bond lead to compound **51** displaying a

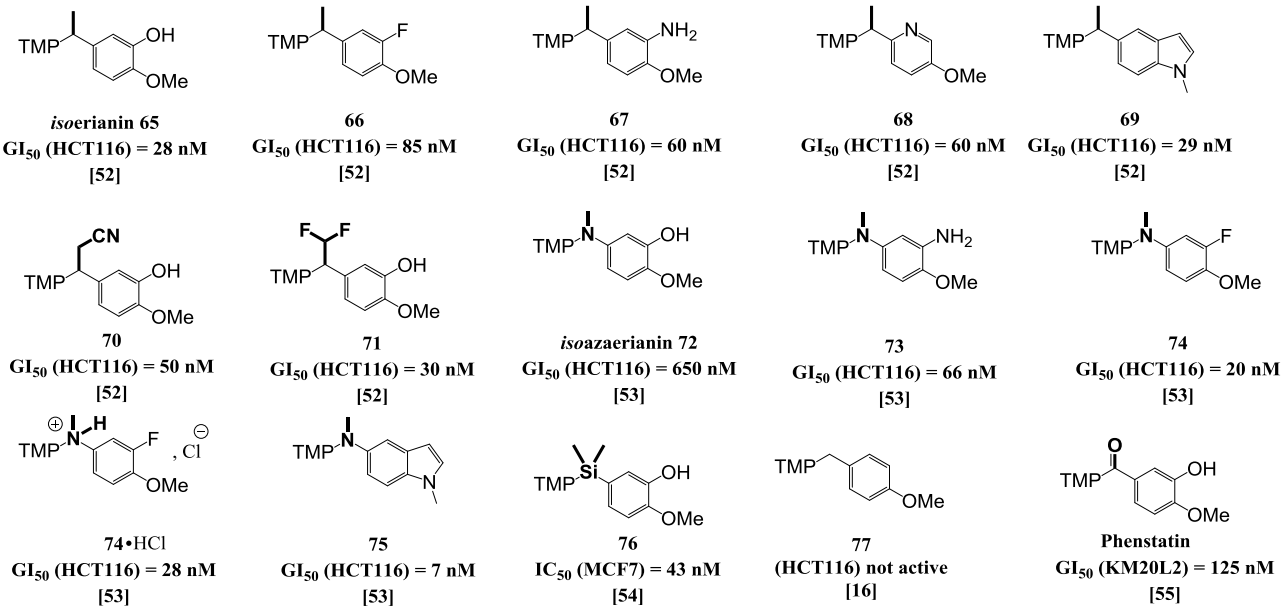


HCT116 : Human colon tumor; DU-143 Human prostate cancer cells; CEM : Human leukemia cancer cells; TMP = 3,4,5-trimethoxyphenyl

Figure 6. Constrained *iso*CA-4 analogues

4-fold stronger antiproliferative activity (GI_{50} = 20 nM for **51**). Interestingly in this dihydronaphthalene series, anilino-derivative **52** was found to be 12-fold more cytotoxic than phenol **50** but the reduction of the ethylene double bond led to a less cytotoxic derivative **53** (GI_{50} = 55 nM).[44] This observation does not apply to benzosuberenes **54** and **55** since phenol **54** which displayed a remarkable picomolar level of cytotoxicity (GI_{50} = 3 pM) has been shown to be more cytotoxic than its aniline counterpart **55** (GI_{50} = 11 nM). As it could be also seen in Figure 6, insertion of an additional double bond in **54**, led to annulenol **56** having nanomolar antiproliferative properties, but in this case a 1000-fold loss of cytotoxic activity was noticed when **56** was compared with benzosuberene **54**. Of note that inclusion of the ethylene double bond in 5- or 8-membered carbocycles resulted in an important decrease of cytotoxicity with GI_{50} values ranging from 100 and 360 nM, respectively.[49] Inserting the ethylene double bond into a chromene ring gave cytotoxic drugs **57** and **58** having respectively GI_{50} values of 10 and 50 nM and again, the reduction of their double bond was deleterious for activity (compare **57** and **58** with **59** and **60**).[44] The increase of the chromene size of one carbon unit proved to be interesting since benzoxepines **61** and **62** were found to be more effective than their chromenes counterparts **57** and **58**.[50] Finally, it can be observed that coumarin **64** showed promising antiproliferative activity with a GI_{50} value of 83 nM. [51] For all of these constrained analogues, if benzosuberene **54** appears to be the most cytotoxic and the most promising compound, it has been convincingly showed that the double bond of *iso*CA-4 may be inserted into 6- or 7-membered carbocycles or in heterocycles with success from a biological point of view but for all of these restricted *iso*CA-4 analogues, it seems to be difficult to predict if a OH-substituent close to the methoxy-substituent will be more appropriate than a -NH_2 group.

Next, as it was observed that the reduction of the ethylene double bond in carbocycles, as well as in heterocycles, led to interesting antiproliferative compounds, the reduction of the ethylene double bond in *iso*CA-4 giving *iso*erianin **65** was achieved.[52] Similarly, *iso*CA-4 analogues **66** and **67** having -F or -NH_2 suitable substituents on C3' or suitable heterocycles (*e.g*; pyridine (**68**) and indole (**69**)) were evaluated. In addition, the reduction of the tri- or tetra-substituted ethylene double bond of compounds **40** and **47** leading to **70** and **71** was achieved.[52] Moreover, in Figure 7, are also presented *iso*CA-4 derivatives for which the ethylene double bond was successfully replaced by (*i*) a NMe spacer to give *iso*azaerianin type derivatives **72**-**75**[53], (*ii*) a dimethylsilicon[54] or (*iii*) a methylene linker[16] to provide **76** and **77**. As it can be seen in Figure 7, the catalytic hydrogenation of *iso*CA-4 led to *iso*erianin **65** which was found to be 15-fold less toxic than CA-4. Similarly, modifications on C3' of the B-ring of *iso*erianin giving compounds **66** and **67** which were less cytotoxic than their ethylene analogues *iso*NH_2CA-4 **1** and *iso*FCA-4 **2** (see Fig. 3). In addition, the



HCT116 : Human colon tumor; MCF7 : Human breast cancer; KM20L2 : Colon cancer; TMP = 3,4,5-trimethoxyphenyl

Figure 7. Modifications of the ethylene linker

replacement of the B-ring of *iso*CA-4 by a substituted pyridine or by a *N*-methylindole led to compounds **68** and **69** which have proved to be potent cytotoxic agents (GI_{50} = 60 and 29 nM, respectively). One note that the reduction of compounds **40** and **47** having a tri- and a tetra-substituted ethylene bond lowered the toxicity of the reduced products **70** and **71** even if the level of activity remained appreciable.

In order to establish SARs, a series of diarylmethylamines **72-75** in which a *N*-Me linker replaced the 1,1-ethylene bridge were synthesized.[53] In this series of *iso*azaerianin derivatives, it is important to note that substituents on the B-ring played a crucial role on activity. Indeed, aza*iso*erianin **72** having on C3' an OH-group was found to be of low cytotoxicity on the contrary to its -NH_2 and F-congeners having GI_{50} values of 66 nM for **73** and 20 nM for **74**. As expected, the water-soluble hydrochloride salt **74**-HCl displayed also a high level of cytotoxicity against HCT116 cells (GI_{50} = 28 nM) comparable to that of its parent amine **74**. Finally, the replacement of the ethylene double bond by dimethyl-silicon or methylene bridges led to the less potent derivative **76** and a non-cytotoxic compound **77**. Finally note also, that the benzophenone phenstatin, which was serendipitously discovered by Pettit during of epoxidation of CA-4 by the Jacobsen chiral Mn (salen) complex,[55] displayed a heterogeneous level of cytotoxicity with respect to various cancerous lines. SARs concerning phenstatin and its analogues will not be presented in this review. From all of these analogues having a modified ethylene spacer depicted in Figure 7, it appears that the association of a *N*Me-linker with an indole nucleus as the B-ring is probably a promising combination (**75**) which may be possibly improved by the introduction of adequate substituents on the indole core. It will be also useful to study whether the 3,4,5-TMP A-ring of such compounds could be advantageously replaced (see next section).

4. A-ring modifications

As having reported that the ethylene double bond and the B-ring of *iso*CA-4 can be replaced while maintaining or increasing the cytotoxicity level, in this part will be described *iso*CA-4 related molecules having a suitable heterocycle as A-ring. The more potent molecules **78-101** having a modified TMP-A ring combined with an ethylene linker and an appropriated B-ring are described in Figure 8.

In 2015, we reported the synthesis and the biological properties of *iso*combretaquinazolines **78-83**[56] in which a quinazoline nuclei replaced the traditional TMP A-ring which was previously postulated as "crucial for activity". We demonstrated that compounds **78-81** were as potent as their TMP analogues (compare *iso*FCA-4 **2**; GI_{50} = 7 nM with **80** GI_{50} = 18 nM). In this series of highly potent molecules, we also showed that a chlorine atom, useful for coupling

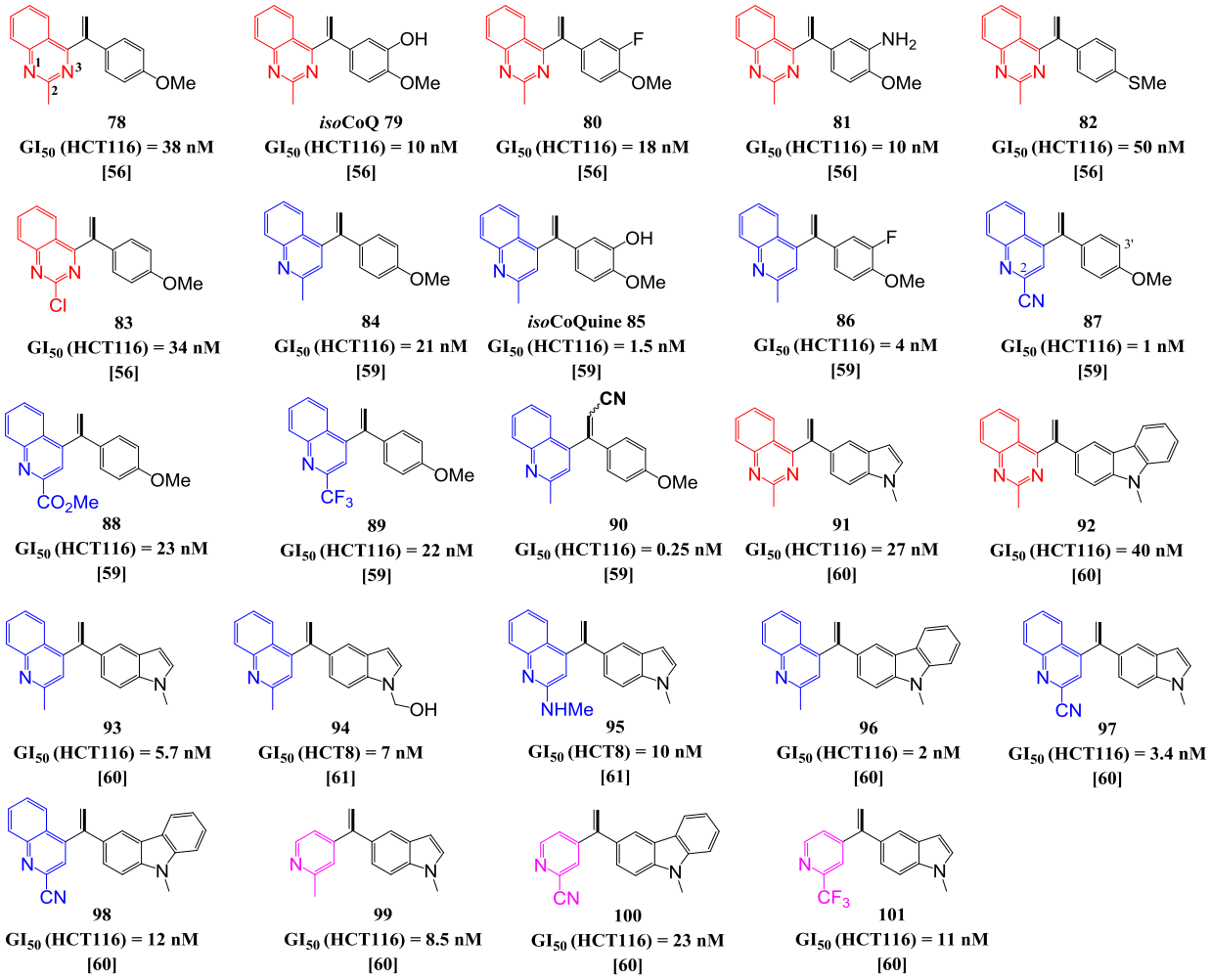
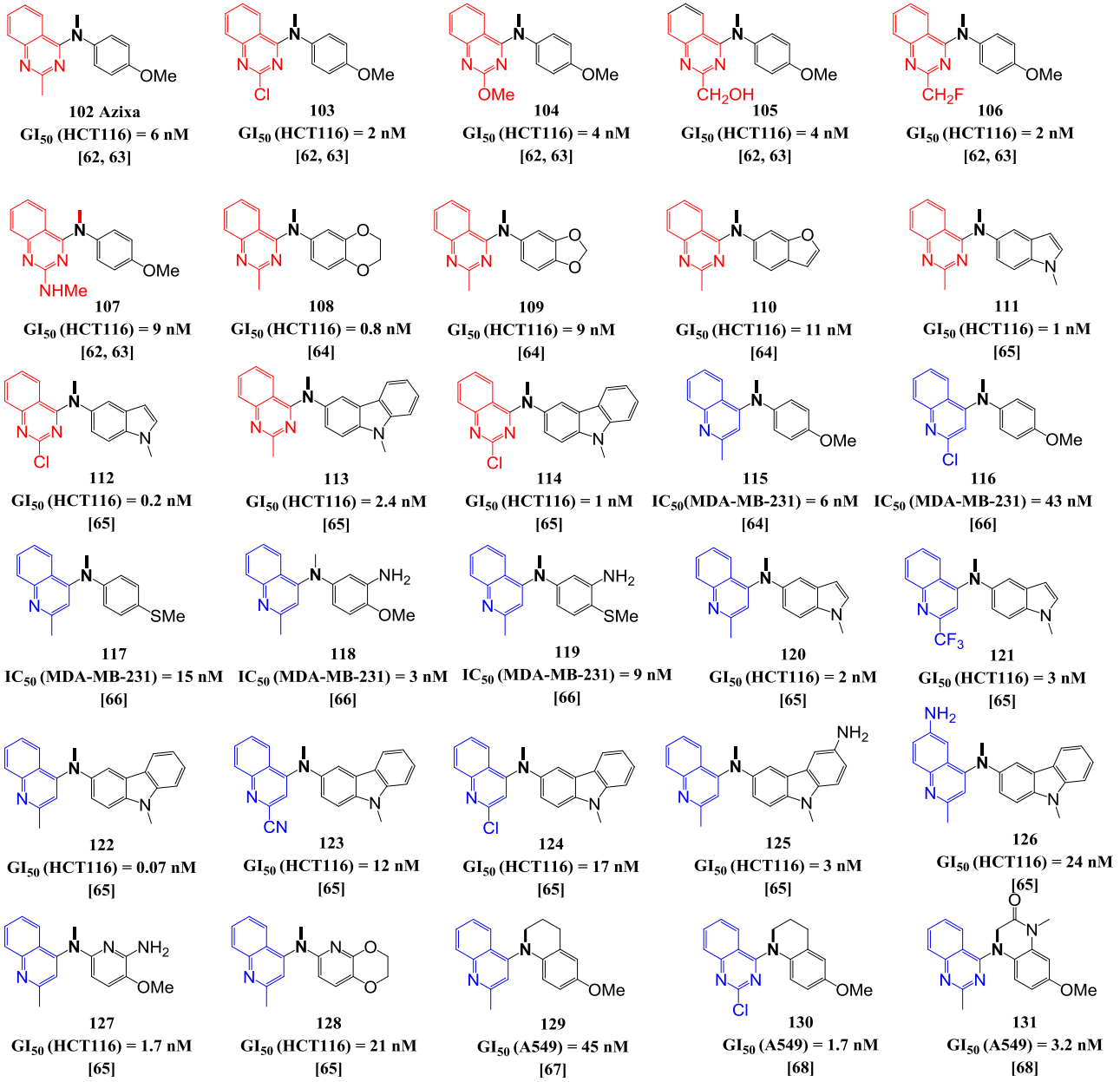


Figure 8. Replacements of traditional TMP A-ring by heterocycles in the ethylene series

reactions in view to establish SARs[57,58] and other substituents on the C2-position of the quinazoline nucleus may replace the Me-group on C2 (compare **78**; GI_{50} = 38 nM with **83**; GI_{50} = 34 nM). After docking studies showing that *N*1-atom in the quinazoline system, on the contrary to *N*3, was important to establish strong H-bonds with the cysβ231 residue of β-tubulin,[59] we synthesized a series of *iso*coQuines derivatives **84-90** in which a quinoline replaced the quinazoline nucleus.[59] As it can be seen in Figure 8, quinoline derivatives **84-86** were more cytotoxic than their quinazoline analogues **78-80** with low GI_{50} values ranging from 1.5 to 21 nM. Interestingly, the C2 position of quinolines tolerated variations since the Me-group on C2 of **84** was successfully replaced by small withdrawing groups as -CN (**87**; GI_{50} = 1 nM), -CO_2Me (**88**; GI_{50} = 23 nM) and -CF_3 groups (**89**; GI_{50} = 22 nM) with no significant loss of cytotoxicity. Compound **90**, (1/1 mixture of (*Z*)- and (*E*)-isomers) displayed a sub-nanomolar level of cytotoxicity which could be certainly lowered by introducing adequate substituents on C3' (**90**; GI_{50} = 0.25 nM). As observed with compounds **91-99**, in this series, heterocyclic systems are also well-tolerated as interesting B-rings.[60] Comparison of cytotoxicity levels of *bis*-heterocyclic derivatives indicated that quinoline **93** having an indolic B-ring (GI_{50} = 5.7 nM) displayed higher antiproliferative properties than its quinazoline congener **91** (GI_{50} = 27 nM). Moreover, it was recently reported that the replacement of the Me groups by polar substituents is possible since compounds **94** and **95** displayed a high cytotoxicity level (GI_{50} = 7 and 10 nM, respectively)[61] In this series of compounds having an ethylene linker, it was also showed that a carbazole B-ring is particularly adequate to promote highly cytotoxic derivatives as **96** (GI_{50} = 2 nM) and **98** (GI_{50} = 12 nM). It also should be noted that compound **96** showed a high human-microsomal stability when compared to *iso*CA-4. X-ray structure study demonstrate that compound **96** interacts mainly with the β-tubulin



HCT116 : Human colon tumor; MDA-MB-231 : Human breast cancer cells; A549 : lung carcinoma

Figure 9. Replacements of traditional TMP A-ring by heterocycles in the *N*Me series

intermediate domain, at the α-β interface. Its binding site overlaps with those of colchicine and CA-4.[60] Finally, the heterocyclic part of these quinoline nuclei is certainly sufficient to maintain an excellent cytotoxicity level since pyridine derivatives **99-101** having an indole or a carbazole B-ring were found as potent antiproliferative agents with GI_{50} values ranging from 8.5 to 23 nM.

Since we have shown in this series of compounds having an ethylenic double bond as a spacer, that the A-ring may be of heterocyclic nature, it is useful to compare the cytotoxicity of these drugs with their homologues having a *N*-methylamine link between the two (het)aromatic nuclei (Figure 9). Quinazoline derivatives [62,63] **102-107** having a *N*Me-linker and a non-heteroaromatic B-ring displayed low GI_{50} values (< 10 nM). As previously observed with quinolines **87-89** (Figure 8), few functional groups were well tolerated on C2 of the quinazoline nucleus leading to highly cytotoxic quinazoline derivatives **103-107**. On the other hand, it can be seen that various heterocyclic rings are welcome as B-rings leading to compounds **108-114** of very low cytotoxicity. Among them, indole and carbazole derivatives **111-114** having a *N*Me-linker and a 2-substituted quinazoline as A-ring displayed a nanomolar cytotoxicity level with GI_{50} values ranging from 0.2 to 2.4 nM.[64,65] Replacing the quinazoline nuclei in compounds **102** and **103**

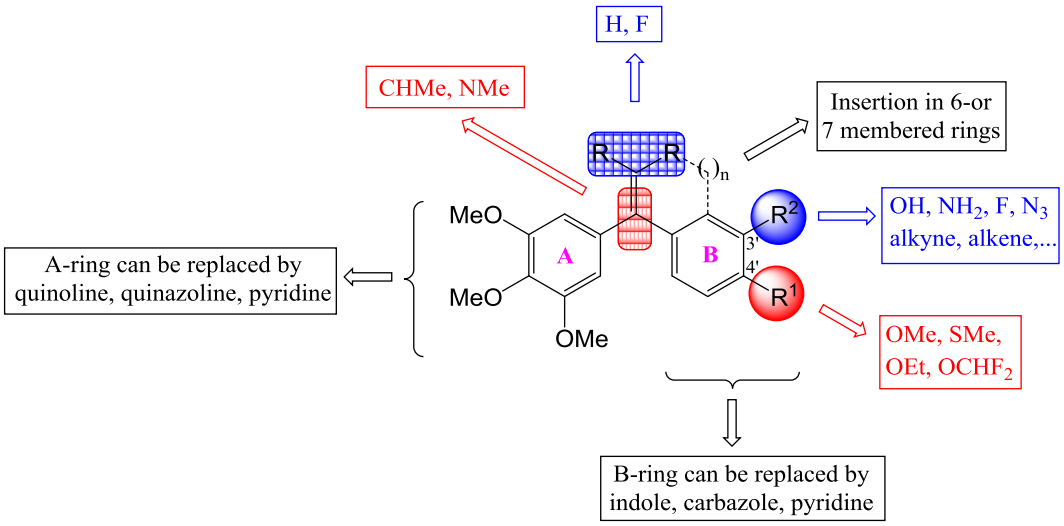


Figure 10. Summary of structure-activity relationships on *iso*CA-4

by a quinoline led to equipotent drugs **115** and **116** whose toxicity level can be enhanced by introducing in C3' a NH_2 group (**118**; IC_{50} = 3 nM).[66] In this quinoline series having a *N*Me spacer, carbazole **122** displayed the best antiproliferative properties with a remarkable GI_{50} value of 0.07 nM.[65] The toxicity level of all drugs in this series remained excellent with the introduction of substituents on one of the two heteroaromatic nuclei (quinoline and carbazole) to promote **125** and **126**. Other structure activity relationships (SARs) around compound **122** with the introduction of suitable functional groups at selected positions of heteroaromatic rings, may give access, in the future, to a selection of payloads anchorable to antibodies in an antibody-drug conjugates (ADC) strategy. As observed with quinaldines **127** and **128**, a substituted pyridine or a dihydrodioxinopyridine nuclei were satisfactory B-rings in this series, especially since compound **127** may be linked to an antibody and an appropriate linker *via* its -NH_2 group. Finally, *N*-aryl-6-methoxy-1,2,3,4-tetrahydroquinolines **129**[67] and **130**[68] (having respectively a quinaldine or a quinazoline A-ring) which could be seen as restricted analogues of quinaldine **115** and quinazoline **103** displayed an interesting nanomolar level of cytotoxicity. In this series of molecules having a constraint structure, Xie found that 3,4-dihydroquinoxalin-2(1*H*)-one **131** displayed a significant *in vivo* activity without obvious toxicity.[68]

5. Conclusion and perspectives

In summary, we have reported from the discovery of *iso*CA-4 in 2007, some efficient modifications achieved on this CA-4 non-natural isomer leading to highly cytotoxic compounds. Concerning the 1,1-ethylene double bond between the A- and B-rings of *iso*CA-4, very few changes are allowed except tri- or tetra-substitutions, reduction in some cases, incorporation into cycles ans heterocycles or replacement by a *N*Me-linker. The B-ring of *iso*CA-4 has been the subject of numerous modifications and, if the C3' MeO-substituent can be replaced by -OEt, -SMe or -CHF_2 groups, there are more possibility of changes on C4'-position (-NH_2, -F, -N_3,…) without being able to predict, *a priori*, which will be the most suitable substituent on this position. It is also interesting to replace the B-ring of *iso*CA-4 by selected heterocycles most of the time (indoles, carbazoles, pyridines) to improve the cytotoxicity level of compounds even if it is expected that the water-solubility of these synthetic heterocyclic compounds will be lowered.

Concerning the possibility of changes in the A-ring of *iso*CA-4, few modifications have been demonstrated as being effective and to date, only few substituted-heterocycles, quinolines, quinazolines and pyridines have shown a very interesting potential. All these considerations are summarized in Figure 10.

Finally, it is interesting to observe that quinoline-carbazole methylamine **122** discovered in 2019 in our group[69], displaying a picomolar level of cytotoxicity (GI_{50} = 70 pM), in which the A-ring, the B-ring as well as the ethylene bridge were successively changed since the discovery of *iso*CA-4 in 2007 and, cannot really be seen as a structural

*iso*CA-4 analogue. This observation indicates that there are certainly numerous possibilities to find other promising derivatives inserting in the large binding-site of colchicine.

Conflicts of interest

The authors report no conflict of interest.

Acknowledgments

The authors gratefully acknowledge support of this project by CNRS, University Paris-Sud and "La Ligue Contre le Cancer" throughout an "Equipe Labellisée 2014" grant.

References

[1] H.L. Lin, H. Ho, G.R. Pettit, E. Hamel. Antimitotic Natural Products Combretastatin A-4 and Combretastatin A-2: Studies on the Mechanism of Their Inhibition of the Binding of Colchicine to Tubulin. Biochemistry 28 (1989) 6984-6991.

[2] G.R. Pettit, S.B. Singh, E. Hamel, C.M. Lin, D.S. Alberts, D. Garcia-Kendall. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. Experientia 45 (1989) 209-211.

[3] A.T. Mc Gown, B.W. Fox. Differential cytotoxicity of combretastatins A1 and A4 in two daunorubicin-resistant P388 cell linesCancer Chemother. Pharmacol. 26 (1990) 79-81.

[4] G.G. Dark, S.A. Hill, V.E. Prise, G.M. Tozer, G.R. Pettit, D.J. Chaplin. Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. Cancer Res. 57 (1997) 1829-1834.

[5] http://investor.mateon.com/

[6] https://www.clinicaltrials.gov

[7] N.J. Lawrence, D. Rennison, M. Woo, A.T. McGown, J.A. Hadfield. Antimitotic and Cell Growth Inhibitory Properties of Combretastatin A-4-like Ethers. Bioorg. Med. Chem. Lett. 11 (2001) 51-54.

[8] K. Oshumi, T. Hatanaka, R. Fujita, R. Nakagawa, Y. Fukuda, Y. Nihei, Y. Suga, Y. Morinaga, Y. Akiyama, T. Tsuji. Syntheses and antitumor activity of cis-restricted combretastatins: 5-Membered heterocyclic analogues. Bioorg. Med. Chem. Lett. 8 (1998) 3153-3158.

[9] S. Aprile, E. Del Grosso, G.C. Tron, G. Grosa. In vitro metabolism study of combretastatin A-4 in rat and human liver microsomes. Drug Metab. Dispos. 35 (2007) 2252-2261.

[10] Z.S. Seddigi, M.M. Shaheer, S.A. Prasanth, A.S.A. Babalghith, A.O. Lamfon, H.A. Kamal, A. Recent advances in combretastatin based derivatives and prodrugs as antimitotic agents. MedChemComm 8 (2017), 1592-1603.

[11] A. Siebert, M. Gensicka, G. Cholewinski, K. Dzierzbicka. Synthesis of Combretastatin A-4 Analogs and their Biological Activities Anti-Cancer Agents in Medicinal Chemistry 18 (2016), 942-960.

[12] Y. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong. Developments of combretastatin A-4 derivatives as anticancer agents. Current Medicinal Chemistry 18 (2011), 523-538.

[13] O. Provot, A. Hamze, J.F. Peyrat, J.D. Brion, M. Alami. Discovery and Hit to Lead Optimization of Novel Combretastatin A-4 Analogues: Dependence of C-Linker Length and Hybridization. Anticancer Agents Med Chem. 13 (2013) 1614-1635.

[14] C. Mousset, A. Giraud, O. Provot, A. Hamze, J. Bignon J.M. Liu, S. Thoret J. Dubois J.D. Brion, M. Alami. Synthesis and anti-tumor activity of benzils related to combretastatin A-4. Bioorg Med Chem Lett 18 (2008) 3266-3271.

[15] O. Provot, A. Giraud, J.F. Peyrat, M. Alami, J.D. Brion. Synthetic approach to enyne and enediyne analogues of anticancer agents. Tetrahedron Lett. 46 (2005) 8547-8550.

[16] N. L'Hermite, A. Giraud, O. Provot, J.F. Peyrat, M. Alami, J.D. Brion. Disproportionation reaction of diarylmethylisopropyl ethers: a versatile access to diarylmethanes from diarylcarbinols speeded up by the use of microwave irradiation. Tetrahedron 62 (2006) 11994-12002.

[17] M. Alami, J.D. Brion, O. Provot, J.F. Peyrat, S. Messaoudi, A. Hamze, A. Giraud, J. Bignon, J. Bakala, J.M. Liu. IsoCA-4 et analogues: Puissants cytotoxiques, inhibiteurs de la polymérisation de la tubuline. Fr 0754280 4 Avril 2007, PCT WO 2008/122620 A1.

[18] S. Messaoudi, B. Tréguier, A. Hamze, O. Provot, J.F. Peyrat, J. Rodrigo De Losada, J.M. Liu, J. Bignon, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.D. Brion, M. Alami. Isocombretastatins A versus Combretastatins A: The Forgotten isoCA-4 Isomer as a Highly Promising Cytotoxic and Antitubulin Agent. J. Med. Chem. 52 (2009) 4538-4542.

[19] A. Hamze, A. Giraud, S. Messaoudi, O. Provot,. J.F. Peyrat, J. Bignon, J.M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.D. Brion, M. Alami. Synthesis, Biological Evaluation of 1,1-Diarylethylenes as a Novel Class of Antimitotic Agents. ChemMedChem 4 (2009) 1912-1924.

[20] A. Hamze, D. Veau, O. Provot, J.D. Brion, M. Alami. Palladium-catalyzed Markovnikov terminal arylalkynes hydrostannation: Application to the synthesis of 1,2-diarylethylenes. J. Org. Chem. 74 (2009) 1337–1340.

[21] E. Rasolofonjatovo, B. Tréguier, O. Provot, A. Hamze, E. Morvan, J.D. Brion, M. Alami. Palladium-catalyzed coupling of N-tosylhydrazones with ortho substituted aryl halides: Synthesis of 4-arylchromenes and related heterocycles. Tetrahedron Lett. 52 (2011) 1036-1040.

[22] A. Hamze, J.D. Brion, M. Alami. Synthesis of 1,1-Diarylethylenes via Efficient Iron/Copper Co-Catalyzed Coupling of 1-Arylvinyl Halides with Grignard Reagents. Org. Lett. 14 (2012) 2782–2785.

[23] M.A. Soussi, S. Aprile, S. Messaoudi, O. Provot, E. Del Grosso, J. Bignon, J. Dubois, J.D. Brion, G. Grosa, M. Alami. Metabolites of *iso*Combretastatin A-4 in Human Liver Microsomes: Identification, Synthesis and Biological Evaluation. ChemMedChem 6 (2011) 1781-1788.

[24] A. Maksimenko, M. Alami, F. Zouhiri, J.D. Brion, A. Pruvost, J. Mougin, A. Hamze, T. Boissenot, O. Provot, D. Desmaële, P. Couvreur. Therapeutic Modalities of Squalenoyl Nanocomposites in Colon Cancer: An Ongoing Search for Improved Efficacy. ACS Nano 8 (2014) 2018-2032.

[25] A. Hamze., E. Rasolofonjatovo, O. Provot, C. Mousset, D. Veau, J. Rodrigo., J. Bignon., J.M. Liu., J. Wdzieczak-Bakala., S. Thoret., J. Dubois, J.D. Brion, M. Alami. B-Ring Modified *iso*Combretastatin A-4 Analogues Endowed with Interesting Anticancer Activities. ChemMedChem 6 (2011) 2179-2191.

[26] D. Lamaa, H.P. Lin, L. Zig, C. Bauvais, G. Bollot, J. Bignon, H. Levaique, O. Pamlard, J. Dubois, M. Ouaissi, M. Souce, A. Kasselouri, F. Saller, D. Borgel, C. Jayat-Vignoles, H. Al-Mouhammad, J. Feuillard, K. Benihoud, M. Alami, A. Hamze. Design and Synthesis of Tubulin and Histone Deacetylase Inhibitor Based on iso-Combretastatin A-4. J. Med. Chem. 61 (2018) 6574-6591.

[27] T. Naret, G. Bernadat, M. Benchekroun, H. Levaique, C. Lenoir, J. Dubois, A. Pruvost, F. Saller, D. Borgel, B. Manoury, V. Leblais, R. Darrigand, S. Apcher, J.D. Brion, E. Schmitt, F.R. Leroux, M. Alami, A. Hamze. A fluorine scan of a tubulin polymerization inhibitor isocombretastatin A-4: Design, synthesis, molecular modelling, and biological evaluation. Eur. J. Med. Chem. 143 (2018) 473-490.
[28] C. Jimenez, Y. Ellahioui, R. Alvarez, L. Aramburu, A.Riesco, M. Gonzales, A. Vicente, A. Dahdouh, A. Ibn Mansour, C. Jimenez, D. Martin, R.G. Sarmiento, M. Medarde, E. Caballero, R. Pelaez. Exploring the size adaptability of the B ring binding zone of the colchicine site of tubulin with para-nitrogen substituted isocombretastatins. Eur. J. Med. Chem. 100 (2015) 210-222.
[29] M. González, Y. Ellahioui, R. Álvarez, L. Gallego-Yerga, E. Caballero, A. Vicente-Blázquez, L. Ramudo, M. Marín, C. Sanz, M. Medarde, R. Pelaéz. The Masked Polar Group Incorporation (MPGI) Strategy in Drug Design: Effects of Nitrogen Substitutions on Combretastatin and Isocombretastatin Tubulin Inhibitors. Molecules 24 (2019), 4319 https://doi.org/10.3390/molecules24234319.
[30] G.B. Kumar, V.L. Nayak, I.B. Sayeed, V.S. Reddy, A.B. Shaik, R. Mahesh, M.F. Baig, M.A. Shareef, A. Ravikumar, A. Kamal. Design, synthesis of phenstatin/isocombretastatin-oxindole conjugates as antimitotic agents. Bioorg. Med. Chem. 24 (2016) 1729-1740.
[31] L.M. Greene, N.M. O'Boyle, D.P. Nolan, M.J. Meegan, D.M. Zisterer. The vascular targeting agent Combretastatin-A4 directly induces autophagy in adenocarcinoma-derived colon cancer cells. Biochem. Pharmacol. 84 (2012) 612–624.
[32] R. Alvarez, C. Alvarez, F. Mollinedo, B.G. Sierra, M. Medarde, R. Pelaez. Isocombretastatins A: 1,1-Diarylethenes as potent inhibitors of tubulin polymerization and cytotoxic compounds. Bioorg. Med. Chem. 17 (2009) 6422-6431.
[33] R. Alvarez, P. Puebla, J.F. Diaz, A.C. Bento, R. Garcia-Navas, J. de la Iglesia-Vicente, F. Mollinedo, J.M. Andreu, M. Medarde, R. Pelaez Endowing Indole-Based Tubulin Inhibitors with an Anchor for Derivatization: Highly Potent 3‑Substituted Indolephenstatins and Indoleisocombretastatins. J. Med. Chem. 56 (2013) 2813-2827.
[34] G. Zhao, J. Bignon, H. Levaique, J. Dubois, M. Alami, O. Provot, O. Chloroenynes Chemistry: A Rapid and Versatile 2-Styrylindole one pot Synthesis. J. Org. Chem. 83 (2018) 15323-15332.
[35] G. Zhao, C. Roudaut, V. Gandon, M. Alami, O. Provot. Synthesis of 2-Substituted Indoles through Cyclization and Demethylation of 2-Alkynyldimethylanilines by Ethanol. Green Chem. 21 (2019) 4204-4210.
[36] T. Bzeih, T. Naret, A. Hachem, N. Jaber, A. Khalaf, J. Bignon, J.D. Brion, M. Alami, A. Hamze. A general synthesis of arylindoles and (1-arylvinyl)carbazoles via a one-pot reaction from N-tosylhydrazones and 2-nitro-haloarenes and their potential application to colon cancer. Chem. Commun. 52 (2016) 13027-130.
[37] M. Lawson, A. Hamze, J.F. Peyrat, J. Bignon, J. Dubois, J.D. Brion, M. Alami. An efficient coupling of N-tosylhydrazones with 2-halopyridines: synthesis of 2-α-styrylpyridines endowed with antitumor activity. Org. Biomol. Chem. 11 (2011) 3664-3673.
[38] A. Kamal, N.V. Subba Reddy, V.L. Nayak, V.S. Reddy, B. Prasad, V.D. Nimbarte, V. Srinivasulu, M.V.P.S. Vishnuvardhan, C.S. Reddy. Synthesis and Biological Evaluation of Benzo[b]furans asInhibitors of Tubulin Polymerization and Inducers of Apoptosis. ChemMedChem 9 (2014) 117-128.
[39] C.V. Do, A. Faouzi, C. Barette, A. Farce, M.O. Fauvargue, E. Colomb, L. Catry, O. Berthier-Vergnes, M. Haftek, R. Barret, T. Lomberget Synthesis and biological evaluation of thiophene and benzo[b]thiophene analogs of combretastatin A-4 and isocombretastatin A-4: A comparison between the linkage positions of the 3,4,5-trimethoxystyrene unit. Bioorg. Med. Chem. Lett. 26 (2016) 174-180.
[40] B. Treguier, M. Lawson, G. Bernadat, J. Bignon, J. Dubois, J.D. Brion, M. Alami, A. Hamze. Synthesis of a 3‑(α-Styryl)benzo[b]‑thiophene Library via Bromocyclization of Alkynes and Palladium-Catalyzed Tosylhydrazones Cross-Couplings: Evaluation as Antitubulin Agents. ACS Comb. Sci. 16 (2014) 702-710.
[41] J. Aziz, E. Brachet, A. Hamze, J.F. Peyrat, G. Bernadat, E. Morvan, J. Bignon, J. Wdzieczak, D. Desravines, M. Tueni, A. Yassine, J.D. Brion, M. Alami. Synthesis, biological evaluation, and structure–activity relationships of tri- and tetrasubstituted olefins related to isocombretastatin A-4 as new tubulin inhibitors. Org. Biomol. Chem. 11 (2013) 430-442.
[42] A.L. Ruchelman, H.W .Man, R. Chen, W. Liu, D. Cedzik, L. Zhang, J. Leisten, A. Collette, R.K. Narla, H.K. Raymon, H.W. Muller. 1,1-Diarylalkenes as anticancer agents: Dual inhibitors of tubulin polymerization and phosphodiesterase 4. Bioorg. Med. Chem. 19 (2011) 6356-6374.
[43] A. Novoa, N. Pelligrini-Moise, S. Bourg; S. Thoret, J. Dubois, G. Aubert, T. Cresteil, Y. Chapleur Design, synthesis and antiproliferative activities of biarylolefins based on polyhydroxylated and carbohydrate scaffolds. Eur. J. Med. Chem. 46 (2011) 3570-3580.
[44] D. Renko, O. Provot, E. Rasolofonjatovo, J. Bignon, J. Rodrigo, J. Dubois, J.D. Brion, A. Hamze, M. Alami. Rapid synthesis of 4-arylchromenes fromortho-substituted alkynols:A versatile access to restricted isocombretastatin A-4 analogues asantitumor agents. Eur. J. Med. Chem. 90 (2015) 834-844.
[45] K.G. Pinney, V.P. Mocharla, Z. Chen, C.M. Garner, A. Ghatak, M. Hadimani, J. Kessler, J.M. Dorsey, K. Edvardsen, D.J. Chaplin, J. Prezioso, U.R. Ghatak. U.S. Patent Appl. Publ. 20040043969 A1, 2004.
[46] M. Sriram, J.J. Hall, N.C. Grohmann, T.E. Strecker, T. Wootton, A. Franken, M.L. Trawick, K.G. Pinney. Design, synthesis and biological evaluation of dihydronaphthalene and benzosuberene analogs of the combretastatins as inhibitors of tubulin polymerization in cancer chemotherapy. Bioorg. Med. Chem. 16 (2008) 8161-8171.
[47] R.P. Tanpure, C.S. George, M. Siram, T.E. Strecker, J.K. Tidmore, E. Hamel, A.K. Charlton-Sevcik, D.J. Chaplin, M.L. Trawicka, K.G. Pinney. An amino-benzosuberene analogue that inhibits tubulin assembly and demonstrates remarkable cytotoxicity. Med. Chem. Commun. 3 (2012) 720-724.
[48] D. Mondal, H. Niu, K.G. Pinney. Efficient synthetic methodology for the construction of dihydronaphthalene and benzosuberene molecular frameworks. Tetrahedron Lett. 60 (2019) 397-401.
[49] C. Herdman, T.E. Strecker, R.P. Tanpure, Z. Chen, A. Winters, J. Gerberich, L. Liu, E. Hamel, R.P. Mason, D.J. Chaplin, M.L. Trawick, K.G. Pinney. Synthesis and biological evaluation of benzocyclooctene-based and indene-based anticancer agents that function as inhibitors of tubulin polymerization. Med. Chem. Comum. 7 (2016) 2418-2427.
[50] E. Rasolofonjatovo, O. Provot, A. Hamze, J. Rodrigo, J. Bignon, J. Wdzieczak, C. Lenoir, D. Desravines, J. Dubois, J.D. Brion, M. Alami. Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted isocombretastatin A-4 analogues. Eur. J. Med. Chem. 62 (2013) 28-39.
[51] C. Bailly, C. Bal., P. Barbier, S. Combes, J.P. Finet, M.P. Hildebrand, V. Peyrot, N. Wattez. Synthesis and Biological Evaluation of 4-Arylcoumarin Analogues of Combretastatins. J. Med. Chem. 46 (2003) 5437-5444.
[52] S. Messaoudi, A. Hamze, O. Provot, B. Treguier, J. Rodrigo De Losada, J. Bignon, J.M. Liu, J. Wdzieczak, S. Thoret, J. Dubois, J.D. Brion, M. Alami. Discovery of Isoerianin Analogues as Promising Anticancer Agents. ChemMedChem 6 (2011) 488-497.
[53] M.A. Soussi, O. Provot, G. Bernadat, J. Bignon, J. Wdzieczak-Bakala, D. Desravines, J. Dubois, J.D. Brion, S. Messaoudi, M. Alami. Discovery of Azarisoerianin Derivatives as Potential Antitumors Agents. Eur. J. Med. Chem. 78 (2014) 178-189.
[54] M. Nakamura, D. Kajita, Y. Matsumoto, Y. Hashimoto. Design and synthesis of silicon-containing tubulin polymerization inhibitors: Replacement of the ethylene moiety of combretastatin A-4 with a silicon linker. Bioorg. Med. Chem. 21 (2013) 7381-7391.
[55] G.R. Pettit, B. Toki, D.L. Herald, P. Verdier-Pinard, M.R. Boyd, E. Hamel, R.K. Pettit Antineoplastic Agents. 379. Synthesis of Phenstatin Phosphate. J. Med. Chem. 41 (1998) 1688-1695.
[56] M.A. Soussi, O. Provot, G. Bernadat, J. Bignon, D. Desravines, J. Dubois, J.D. Brion, S. Messaoudi, M. Alami. IsoCombretaQuinazolines: Novel Potent Antitumor Agents with Antitubulin Activity. ChemMedChem 10 (2015) 1392-1402.
[57] M. Dos Santos, X. Franck, R. Hocquemiller, B. Figadere, J.F. Peyrat, O. Provot, J.D. Brion, M. Alami. Iron-catalyzed coupling reaction between 1,1-dichloro-1-alkenes and Grignard reagents. Synlett 15 (2004) 2697-2700.

[58] M. Seck, X. Franck, R. Hocquemiller, B. Figadere, J.F. Peyrat, O. Provot, J.D. Brion, M. Alami. Synthesis of substituted quinolines by iron-catalyzed coupling reactions between chloroenynes and Grignard reagents. Tetrahedron Lett. 45 (2004) 1881-1884.
[59] I. Khelifi, T. Naret, D. Renko, A. Hamze, G. Bernadat, J. Bignon, C. Lenoir, J. Dubois, J.D. Brion, O. Provot, M. Alami. Design, synthesis and anticancer properties of IsoCombretaQuinolines as potent tubulin assembly inhibitors. Eur. J. Med. Chem. 127 (2017) 1025-1034.
[60] T. Naret, I. Khelifi, O. Provot, J. Bignon, H. Levaique, J. Dubois, M. Souce, A. Kasselouri, A. Deroussent, A. Paci, P.F. Varela, B. Gigant, M. Alami, A. Hamze. 1,1-Diheterocyclic Ethylenes Derived from Quinaldine and Carbazole as New Tubulin-Polymerization Inhibitors: Synthesis, Metabolism, and Biological Evaluation. J. Med. Chem. 62 (2019) 1902-1916.
[61] W. Li, W. Shuai, H. Sun, F. Xu, Y. Bi, J. Xu, C. Ma, H. Yao, Z. Zhu, S. Xu. Design, synthesis and biological evaluation of quinoline-indole derivatives as anti-tubulin agents targeting the colchicine binding site. Eur. J. Med. Chem. 163 (2019) 428-442.
[62] S. Kasibhata, V. Baichwal, S.X. Cai, B. Roth, I. Skvortsova, S. Skvortsov, P. Lukas, N.E. English, N. Sirisova, J. Drewe, A. Pervin, B. Tseng, R.O. Carlson, C.M. Pleiman. MPC-6827: A Small-Molecule Inhibitor of Microtubule Formation That Is Not a Substrate for Multidrug Resistance Pumps. Cancer Res. 67 (2007) 5865-5871.
[63] N. Sirisoma, A. Pervin, H. Zhang, S. Jiang, J.A.Willardsen, M.B. Anderson, G. Mather, C.M. Pleiman, S. Kasibhatla, B. Tseng, J. Drewe, S.X. Cai. Discovery of *N*-(4-Methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine, a Potent Apoptosis Inducer and Efficacious Anticancer Agent with High Blood Brain Barrier Penetration. J. Med. Chem. 52 (2009) 2341-2351.
[64] K. Mahal, M. Resch, R. Ficner, R. Schobert, B. Biersack, T. Mueller. Effects of the Tumor-Vasculature-Disrupting Agent Verubulin and Two Heteroaryl Analogues on Cancer Cells, Endothelial Cells, and Blood Vessels, ChemMedChem 9 (2014) 847-854.
[65] I. Khelifi, T. Naret, A. Hamze, J. Bignon, H. Levaique, M.C. Garcia Alvarez, J. Dubois, O. Provot, M. Alami. N,N-bis-heteroaryl methylamines: Potent anti-mitotic and highly cytotoxic agents. Eur. J. Med. Chem. 168 (2019) 176-188.
[66] Y. Zhou, W. Yan, D. Cao, M. Shao, D. Li; F. Wang, Z. Yang, Y. Chen, L. He, T. Wang, M. Shen, L. Chen. Design, synthesis and biological evaluation of 4-anilinoquinoline derivatives as novel potent tubulin depolymerization agents, Eur. J. Med. Chem. 138 (2017) 1114-1125.
[67] X.-F. Wang, S.-B. Wang, E. Ohkoshi, L.-T. Wang, E. Hamel, K. Qian, S.L. Morris-Natschke, K.-H. Lee, L. Xie. *N*-Aryl-6-methoxy-1,2,3,4-tetrahydroquinolines: A novel class of antitumor agents targeting the colchicine site on tubulin. Eur. J. Med. Chem. 67 (2013) 196-207.
[68] X.-F. Wang, F. Guan, E. Ohkoshi, W. Guo, D.-Q. Zhu, S.-B. Wang, L.-T. Wang, E. Hamel, D. Yang, L. Li; K. Qian, S.L. Morris-Natschke, S. Yuan, K.-H. Lee, L. Xie. Optimization of 4-(*N*-Cycloamino)phenylquinazolines as a Novel Class of Tubulin-Polymerization Inhibitors Targeting the Colchicine Site. J. Med. Chem. 57 (2014) 1390-1402.
[69] Compound **122** is currently undergoing biological studies in our laboratory.