Bioorganic & Medicinal Chemistry Letters 26 (2016) 174–180

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Cong Viet Do [a,†], Abdelfattah Faouzi [a,†], Caroline Barette [c,d,e], Amaury Farce [g,h], Marie-Odile Fauvarque [c,d,e], Evelyne Colomb [b], Laura Catry [a], Odile Berthier-Vergnes [f], Marek Haftek [b], Roland Barret [a], Thierry Lomberget [a,*]

[a] Université de Lyon, Université Lyon 1, Faculté de Pharmacie – ISPB, EA 4446 Biomolécules, Cancer et Chimiorésistances, SFR Santé Lyon-Est CNRS UMS3453 – INSERM US7, 8 avenue Rockefeller, F-69373 Lyon Cedex 08, France
[b] Université Lyon 1, Faculté de Médecine et de Pharmacie, EA 4169 «Fundamental, Clinical and Therapeutic Aspects of the Skin Barrier Function», 8 avenue Rockefeller, F-69373 Lyon Cedex 08, France
[c] Univ. Grenoble Alpes, iRTSV-BGE, F-38000 Grenoble, France
[d] CEA, iRTSV-BGE, F-38000 Grenoble, France
[e] INSERM U1038, BGE, F-38000 Grenoble, France
[f] Université de Lyon, Université Lyon 1, Centre de Génétique et de Physiologie Moléculaires et Cellulaires, CNRS UMR5534 – Université Lyon 1, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France
[g] Univ Lille Nord de France, F-59000 Lille, France
[h] Institut de Chimie Pharmaceutique Albert Lespagnol, EA GRIIOT (4481), IFR114, 3 Rue du Pr Laguesse, B.P. 83, F-59006 Lille, France

ARTICLE INFO

Article history:
Received 21 July 2015
Revised 3 November 2015
Accepted 4 November 2015
Available online 10 November 2015

Keywords:
Combretastatin A-4
Inhibitors of tubulin assembly
Antiproliferative agents
Heterocycles
Melanocyte

ABSTRACT

Combretastatin A-4 and isocombretastatin A-4 derivatives having thiophenes or benzo[*b*]thiophenes instead of the B ring were prepared and evaluated for their in cellulo tubulin polymerization inhibition (TPI) and antiproliferative activities. The presence of the benzo[*b*]thiophene ring proved to have a crucial effect as most of the thiophene derivatives, except those having one methoxy group, were inactive to inhibit tubulin polymerization into microtubules. The influence of the attachment position was also studied: benzo[*b*]thiophenes having *iso* or *cis* 3,4,5-trimethoxystyrenes at position 2 were 12–30-fold more active than the 3-regioisomers for the TPI activity. Some of the novel designed compounds exhibited interesting anti-proliferative effects on two different cell lines.

Cancer incidence is increasing worldwide[1] and, to face this ever-growing threat, scientists have stepped up efforts to find efficient treatments for all type of cancers.

Skin cancers are by far the most frequent forms of malignancy in man. Among these, malignant melanoma, which origin is in the pigment-producing cells (i.e., melanocytes) remains the most aggressive one. Indeed, although this disease comprises less than 5% of malignant skin tumors, it causes almost 60% of all deaths due to skin cancers.[2] This bad prognosis is partly due its strong tendency to give metastases. When possible, surgical exeresis is the first choice treatment and chemotherapy is used thereafter against metastases, according to several regimens.[3]

Nature has always been for the medicinal chemist an important source of inspiration for the development of many anti-cancer agents.[4] The inhibition of tubulin polymerization or microtubules depolymerization within cancer cells, thus leading to apoptosis,[5] was one of the most interesting approaches.[6]

A striking example of these tubulin-targeting agents[7] is combretastatin A-4 (CA-4, Fig. 1), which was isolated in the 90s from the bark of the south african willow tree *Combretum caffrum* by Pettit research group.[8,9]

This compound has shown remarkable tubulin polymerization inhibition activity and antiproliferative effects against a variety of cancer cells.[10]

* Corresponding author. Tel.: +33 478 777 082; fax: +33 478 777 082.
E-mail address: thierry.lomberget@univ-lyon1.fr (T. Lomberget).
† These two authors contributed equally.

http://dx.doi.org/10.1016/j.bmcl.2015.11.010
0960-894X/

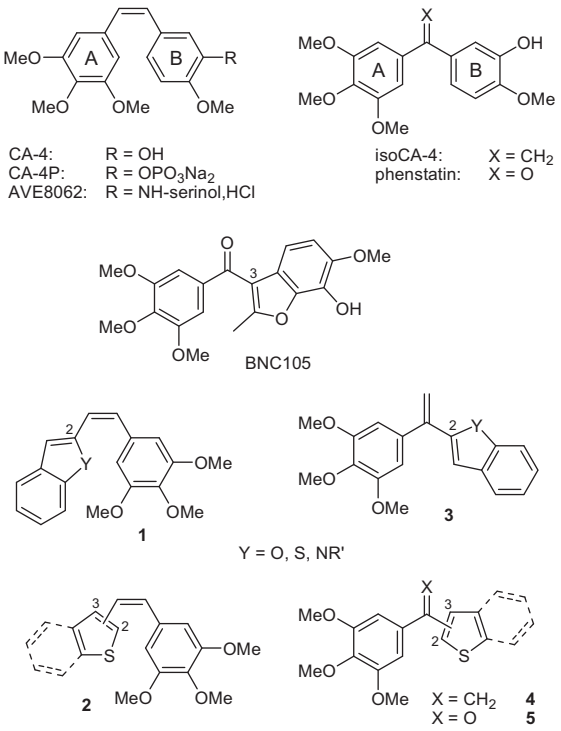


Figure 1. Combretastatin A-4 and its soluble derivatives, synthetic methylene and keto tubulin-targeting compounds.

Despite these potent activities, solubility parameters had to be improved and this was achieved with the preparation of several derivatives, having a phosphate group (R = OPO_3Na_2, fosbretabulin[10,11]) or an aminoalcohol side chain (R = NH-serinol hydrochloride salt, ombrabulin[12]). These compounds, which demonstrated Vascular Targeting or Disrupting properties,[13] actually undergo clinical evaluations for the treatment of various cancers but also diseases associated to vascularization disorders.[14]

Nevertheless, these compounds, known to interact with the colchicine binding site of tubulin,[15] were reported to isomerize in vivo,[16] thus leading to the less active *trans* isomers.[17] While this isomerization is not deleterious for the development of CA-4 derivatives as anticancer drugs (as shown by fosbretabulin and ombrabulin), the discovery of new tubulin-targeting agents based on this structure that also avoid the *Z* to *E* isomerization still remains a stimulating challenge.[18]

Besides the addition of a substituent onto the double bond,[19] one of the solutions to block this isomerization was to replace it by an (hetero)cycle[20] or by designing *iso* methylene derivatives, so-called isocombretastatins (e.g., *iso*CA-4, Fig. 1).[21,22] Other research groups also developed the isosteric version of *iso*CA-4, i.e., phenstatin[23] derivatives, and those ketones have also shown interesting tubulin-targeting properties. Based on this 3,4,5-trimethoxybenzophenone scaffold, several heterocyclic analogs were developed, including indoles,[24a] benzo[*b*]thiophenes[24b] or benzofurans such as BNC105[24c] (Fig. 1) which is currently undergoing clinical trials.

Our initial strategy for the development of new tubulin-targeting molecules aimed to evaluate the effect of the replacement of the B ring with different benzoheterocycles, attached to position 2, leading to compounds **1** (Fig. 1). This preliminary work demonstrated that the most active compound possessed a benzo[*b*]thiophene ring (Y = S).[25] One of the objectives of the present Letter was to evaluate the importance of the vicinal aromatic ring (thiophene versus benzo[*b*]thiophene) but also the effect of the grafting of the 3,4,5-trimethoxystyrene unit on these sulfur (benzo)heterocycles at different 2 and 3 positions (Fig. 1, compounds **2**).

Moreover, in parallel to our work, Alami and co-workers have described the preparation of *iso*CA-4 derivatives with substituted benzofurans **3a** (Y = O) and indole **3b** (Y = NR′) (Fig. 1) using a palladium-catalyzed coupling/ring closure domino reaction.[26]

So, the second goal of the present study was to assess the effect of the grafting of the 2-[1-(3,4,5-trimethoxy-phenyl)-vinyl] unit on sulfur heterocycles, at both positions 2 and 3, so extending the knowledge of the structure–activity relationships of this family (Fig. 1, compounds **4**).

The key step of our synthetic strategy to these new *iso*CA-4 derivatives **4** relied on the preparation of the methylene group by water elimination on methyl diarylcarbinols **6**: these compounds could be in turn obtained by trapping the appropriate aryl lithium species with 3,4,5-trimethoxyacetophenone **7a** (R′ = Me) or after addition of methyl magnesium iodide onto the diarylketo derivatives **5** (Fig. 2).

Since the first synthesis of phenstatin,[23] the preparation of biaryl keto derivatives were usually based on the addition of the aryl lithium onto an amide, derived from a benzoic acid. Recently, an alternative process which was described by Rigo and co-workers[27] and Petrov et al.[28] used an acylation process of a benzoic acid on the appropriate aromatic substrate, in Eaton's reagent (P_2O_5 in $MeSO_3H$).

In our synthetic approach, those compounds **5** could be prepared by the addition of the (benzo)thiophene lithium reagent, generated at the appropriate 2- or 3-position, onto 3,4,5-trimethoxy benzaldehyde **7b** (R′ = H), followed by an oxidation step of the obtained alcohol (Fig. 2).[29] By this way, the isosteric replacement of the exocyclic CH_2 in compounds **4** by an oxygen atom (phenstatin analogs **5**) could also be investigated.[30]

For the present study, in the thiophene series, 2- and 3-lithio species were prepared using halogen/lithium exchange with *n*-BuLi on commercially available 2- and 3-bromothiophene. The corresponding aryl lithium intermediate was then trapped by 3,4,5-trimethoxy acetophenone **7a** to obtain **6a**,**b**. These tertiary alcohols were submitted to an acid-catalyzed dehydration and the target compounds **4a**,**b** were isolated with good yields over these two steps (Scheme 1).[31]

For the preparation of benzoheterocyclic derivatives, 2- and 3-lithio reagents were obtained after deprotonation of benzo[*b*]thiophene itself or by bromine/lithium exchange on its 3-bromo derivative **8**[32] (Scheme 2).

After addition of aldehyde **7b**, alcohols **9a**,**b** (Scheme 2) were isolated and further oxidized into ketones **5a**,**b** using MnO_2 in acetonitrile (Scheme 3). These carbonyl compounds were thereafter

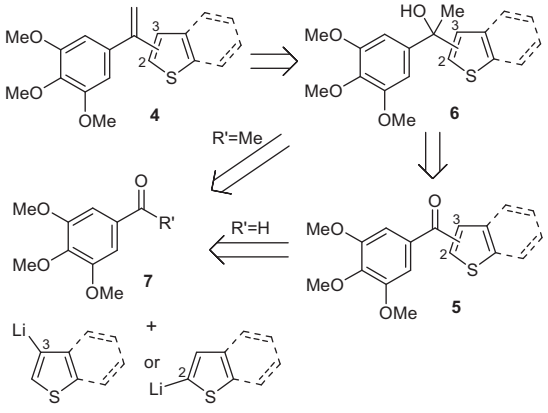
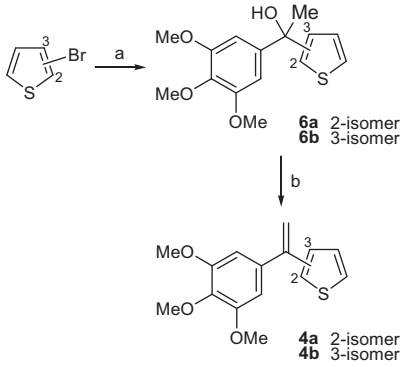
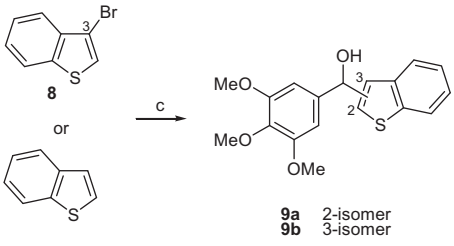


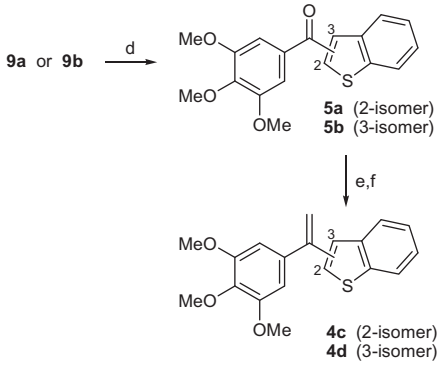
Figure 2. Synthetic strategy to *iso* methylene derivatives **4**.



Scheme 1. Reagents and conditions: (a) *n*-BuLi 1.5 equiv, THF for **6a** and Et_2O for **6b**, −70 °C, 1 h then addition of **7a** (1.2 equiv), −70 °C, 1 h then rt for 4 h, 67% for **6a** and 76% for **6b**. (b) *p*-TsOH 5 mol %, toluene, reflux, 2 h for **4a** and 1 h for **4b**, 98% for **4a** and 94% for **4b**.



Scheme 2. Reagents and conditions: (c) *n*-BuLi 1.3 equiv, Et_2O, 0 °C, 1.5 h then addition of **7b** (1.3 equiv), 0 °C, 3 h, 85% for **9a**, 82% for **9b**.



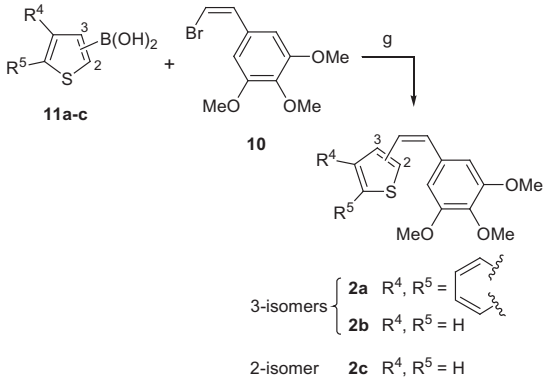
Scheme 3. Reagents and conditions: (d) MnO_2 6 equiv, CH_3CN, rt, 4 h, 99% for **5a** and 80% for **5b**. (e) MeMgI 3 equiv, THF, 0 °C, 30 min then rt, 15 min then 75 °C, 2 h, 95% for **6c** and 92% for **6d**. (f) *p*-TsOH 5 mol%, toluene, reflux, 2 h for **4c** and 1 h for **4d**; 93% for **4c** and 89% for **4d**.

submitted to the Grignard reagent addition/dehydration sequence to give benzo[*b*]thiophene *iso*CA-4 analogs **4c**,**d** (Scheme 3).

According to the same sequence and in order to complete our structure/activity relationship study, compound **3a**[26] (Fig. 1, Y = O) was prepared with a 62% overall yield, starting from benzofuran.

Among the different possibilities to get the *Z* isomer of methylene compounds **2**,[33] the Suzuki coupling[34] did not appear to be the most stereoselective reaction but one of the most direct strategy. Hence, the preparation of new (benzo)thiophene derivatives **2a**–**c** was carried out according to this palladium-catalyzed cross-coupling reaction between *Z*-monobromo derivative **10**[35] and the appropriate boronic acids **11a**–**c**[36] (Scheme 4).

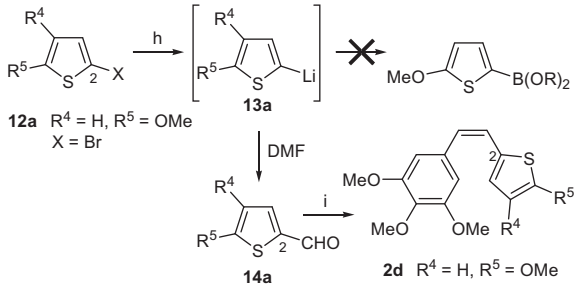
To prepare 2-methoxythiophene derivative **2d** (R^4 = H and R^5 = OMe) using a Suzuki coupling, the preparation of suitable



Scheme 4. Reagents and conditions: (g) $Pd(PPh_3)_4$ 5 mol %, Na_2CO_3 1.0 equiv, DME/water, 90 °C, 20 h, 28% for **2a**, 59% for **2b**, 38% for **2c**.

boron derivatives was necessary. Unfortunately, all our attempts based on a bromine/lithium exchange on **12a** followed by trapping of the aryl lithium intermediate **13a** by either trimethylborate (combined with an acidic hydrolysis) or bis-pinacolato diboron were unsuccessful to obtain the expected compounds (Scheme 5). It has been proved that the limitation step was not the formation of **13a** but its electrophilic trapping with organoboron reagents. Indeed, after the in situ formation, organolithium species **13a** was easily trapped by *N*,*N*-dimethylformamide (DMF) to obtain aldehyde **14a** (Scheme 5). Finally, a Wittig reaction[10,33] with this aldehyde allowed us to obtain the desired *Z* methylene methoxythiophene derivative **2d** with a low isolated yields, due to the poor stereoselectivity during the olefin formation (Scheme 5). The other 4-methoxy regioisomer **2e** (R^4 = OMe, R^5 = H) was obtained by using the same strategy on the known 4-methoxy-thiophene-2-carboxaldehyde[37] **14b** (Scheme 5).

All the prepared compounds, i.e., original *iso* derivatives **4b**–**d**, phenstatin **5b** and (*Z*) methylenes **2a**–**e**, known (*Z*)-**1b** and (*E*)-**1b**,[25] **3a**,[26] **4a**,[31] **5a**[38]) were then evaluated for their ability to inhibit tubulin assembly, using an in cellulo assay.[39] This test is based on parallel, discriminant fluorescent immunostainings of both tyrosinated and detyrosinated tubulin pools inside HeLa cells after a 2-hour exposure to compounds. A gentle cell permeabilization to eliminate free tyrosinated tubulin pool while preserving dynamic tyrosinated microtubules as well as detyrosinated stable microtubules was thereafter realized: this assay reflects thus the activity of either microtubules polymerization and depolymerization inhibitors.[39,40] The additional interest of this in cellulo tubulin polymerization inhibition (TPI) evaluation[41] is to know if the compound would be active within the cancer cell: this could be very interesting when developing a new drug as it could shorten the hit-to-lead transposition.



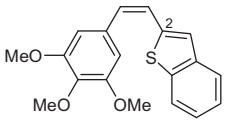
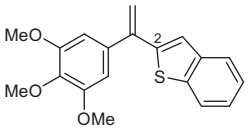
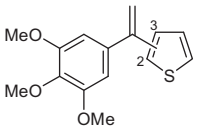
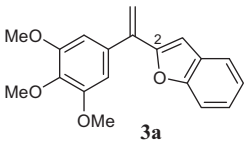
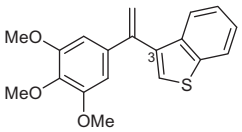
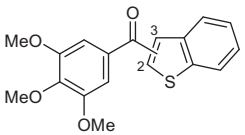
Scheme 5. Reagents and conditions: (h) *n*-BuLi 1.1 equiv, THF, −78 °C, 1 h then addition of DMF (1.2 equiv), −78 °C, 20 min then rt 2 h, 90% for **14a**, 37% for **14b**. (i) (1) 3,4,5-Trimethoxybenzyltriphenylphosphonium bromide 1.0 equiv, THF, *n*-Buli, −78 °C, 30 min, then rt 1 h. (2) Aldehyde addition, THF, −78 °C, 1 h then rt, 24 h, 14% for **2d**, 23% for **2e**.

Combretastatin A-4 was used as the reference compound (Table 1, entry 2), as well as (*Z*)-**1b**, which was formerly in vitro evaluated on purified tubulin and for antiproliferative effects on keratinocyte cells.[25]

This screening was helpful to draw several conclusions, as described hereafter.

One of the objectives of this Letter was to compare the tubulin polymerization modification using the known alkene derivative (*Z*)-**1b** with its isomethylene geometric isomer **4c**. In contrast to what was described in the literature for CA-4,[21] we have observed a significant loss of activity (EC_{50} value for tubulin polymerization inhibition drops from 80 to 321 nM) when including the isomethylene structural unit instead of the *cis* alkene (Table 1, entries 3 and 4).

Table 1
Inhibition of tubulin assembly and antiproliferative effects of colchicine, CA-4, (*Z*)-**1b**, synthesized *iso* **4** and keto **5** derivatives

Entry	Compound	EC_{50} for in cellulo TPI[a]	IC_{50} for anti-proliferative effects[c]
1	Colchicine	n.d.	IC8: 15 nM MES-SA: 15 nM
2	CA-4	9 nM	IC8: 4 nM MES-SA: 3 nM
3	 (*Z*)-**1b**	80 nM[b]	IC8: 17 nM MES-SA: 13 nM
4	 **4c**	321 nM	IC8: 101 nM MES-SA: 95 nM
5	 **4a** (2-isomer) **4b** (3-isomer)	**4a** > 10 μM **4b** > 10 μM	n.d. n.d.
6	 **3a**	>10 μM	n.d.
7	 **4d**	3.897 μM	n.d.
8	 **5a** (2-isomer) **5b** (3-isomer)	**5a** > 10 μM **5b** > 10 μM	n.d. n.d.

n.d.: Not determined, due to low TPI activities.
[a] EC_{50}: compound concentration required to decrease dynamic, tyrosinated microtubules content by 50%.
[b] Determined on isolated tubulin, TPI EC_{50} for compound (*Z*)-**1b** was equal to 2.6 μM.[40]
[c] IC_{50}: a sample's concentration which produces a 50% reduction in IC8 or MES-SA cell activity. For details, see Supplementary materials.

The position of the substitution was also crucial as the TPI activity was 12-fold lower for the 3-substituted *iso* compound **4d** (Table 1, entry 7) than its 2-regioisomer **4c** (Table 1, entry 4). The same tendency was observed for the *cis* alkene derivatives **1b** and **2a** (Table 1, entry 3 and Table 2, entry 2).

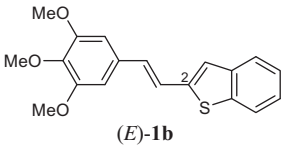
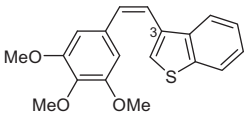
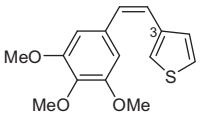
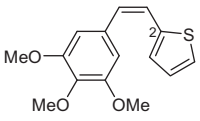
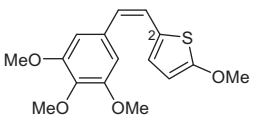
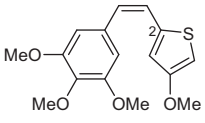
Structure simplification in the *iso* series, from benzo[*b*]thiophene to thiophene, resulted in the total loss of TPI activity, whatever the 2- or 3-substitution of the heterocycle (Table 1, entry 5).

Replacement of the sulfur with an oxygen atom in the benzoheterocycle (Table 1, entries 4 and 6) resulted also in a dramatic loss of activity. This last result is in accordance with a previous tendency for the methylene series.[25]

Finally, the isosteric replacement of the *iso* methylene group in **4c**,**d** with a ketone also led to a very low in cellulo TPI activity for **5**, whatever the position of the substitution (Table 1, entry 8).

After the in cellulo TPI screening, antiproliferative effects of the five most active compounds were then evaluated on two different cell lines, by using the MTT[42] or resazurin[43] assays (Tables 1 and 2, last column).

Table 2
Inhibition of tubulin assembly and antiproliferative effects for (*E*)-**1b** and newly synthesized (*Z*)-**2a–e**

Entry	Compound	EC_{50} for in cellulo TPI[a]	IC_{50} for anti-proliferative effect[b]
1	 (*E*)-**1b**	3.185 μM	n.d.
2	 (*Z*)-**2a**	2.430 μM	IC8: 288 nM MES-SA: 191 nM
3	 (*Z*)-**2b**	>10 μM	n.d.
4	 (*Z*)-**2c**	>10 μM	n.d.
5	 (*Z*)-**2d**	1.558 μM	IC8: 76 nM MES-SA: 53 nM
6	 (*Z*)-**2e**	81 nM	IC8: 27 nM MES-SA: 25 nM

n.d.: Not determined, due to low TPI activities.
[a] EC_{50}: compound concentration required to decrease dynamic, tyrosinated microtubules content by 50%.
[b] IC_{50}: a sample's concentration which produces a 50% reduction in IC8 or MES-SA cell activity. For details, see Supplementary materials.

IC8 line is a well-characterized human melanoma clone selected on the basis of its relatively low metastatic potential. The cells are robust in culture and therefore appropriate for pharmaco-toxicological tests.[44] MES-SA cell line has been developed from a uterine sarcoma[45] and is frequently used for anticancer drug sensitivity testing.

We have noticed a good correlation between the TPI and antiproliferative activities for all these compounds. The most active new *iso* derivative **4c** possesses an interesting IC_{50} value of 101 nM on IC8 and 95 nM on MES-SA (Table 1, entry 4), which is however almost 6–7-fold less active than our previously described alkene derivative (*Z*)-**1b** (Table 1, entry 3), having itself an antiproliferative activity on these two cell lines equivalent to that of colchicine (Table 1, entry 1).

As stated before, these first results on the isosteric modulations of the *cis* double bond of **1b** were rather disappointing and, if such *iso* compounds (e.g., **4c**) could be a solution to prevent the *cis* to *trans* in vivo isomerization, the anti-proliferative activity range appeared to be too low for a further development.

To improve our knowledge on structure/activity relationships on the *cis* 3,4,5-trimethoxystyrene derivatives, the biological evaluation of new compounds (*Z*)-**2a**–**e** was then carried out (Table 2).

First, it is important to notice that our in cellulo TPI screening reflects the compounds' real activity within the cell and a remarkable example is brought by (*E*)-**1b**, who has displayed a 40-fold lower TPI activity (Table 2, entry 1) than its stereomer (*Z*)-**1b** (Table 1, entry 3). Indeed, we have previously reported[25] that (*E*)-**1b** exhibited a 3-fold lower TPI activity on purified tubulin than (*Z*)-**1b**.[17]

The 3-regioisomer (*Z*)-**2a** (Table 2, entry 2) was less active than (*Z*)-**1b**, emphasizing the importance of the 2-substitution of the benzo[*b*]thiophene ring[46] for both TPI (EC_{50} = 2.430 μM) and antiproliferative activities (IC_{50} = 288 nM on IC8 cells and IC_{50} = 191 nM on MES-SA cells).

Ring simplification, from benzothiophene to thiophene, resulted in a dramatic loss of TPI activity, for both positions 3 (Table 2, entry 3) and 2 (Table 2, entry 4).

Though the presence of the fused aromatic ring seemed to be important, we wanted also to evaluate the influence of methoxy groups: introduction of this ether at different positions of the thiophene ring resulted in a significant improvement of the TPI. Indeed, (*Z*)-**2d** was at least 7-fold more potent than (*Z*)-**2c** (Table 2, entries 4 and 5) whereas EC_{50} for (*Z*)-**2e** was radically improved to reach 81 nM (Table 2, entry 6). Anti-proliferative activities of these two methoxy derivatives (*Z*)-**2d** and (*Z*)-**2e** appeared to be interesting: 76 nM/27 nM, respectively, on IC8 cell line and 53 nM/25 nM, respectively, on MES-SA cell line (Table 2, entries 5 and 6). Thiophene derivative (*Z*)-**2e** has shown similar activities than the benzo[*b*]thiophene analog (*Z*)-**1b** (Table 1, entry 3).

Lastly, combretastatin A-4 remains the most active compound, with a remarkable 4 nM IC_{50} value for IC8 cells and 3 nM IC_{50} value for MES-SA cells (Table 1, entry 2).

To bring some light on the observed biological activities, some docking experiments were carried out (Figs. 3 and 4).[47] 30 solutions were generated for each molecule and the consistency of the pose was assessed visually. In cases of several clusters of solutions, their relative population was taken into account to try to evaluate the stability of the binding mode.

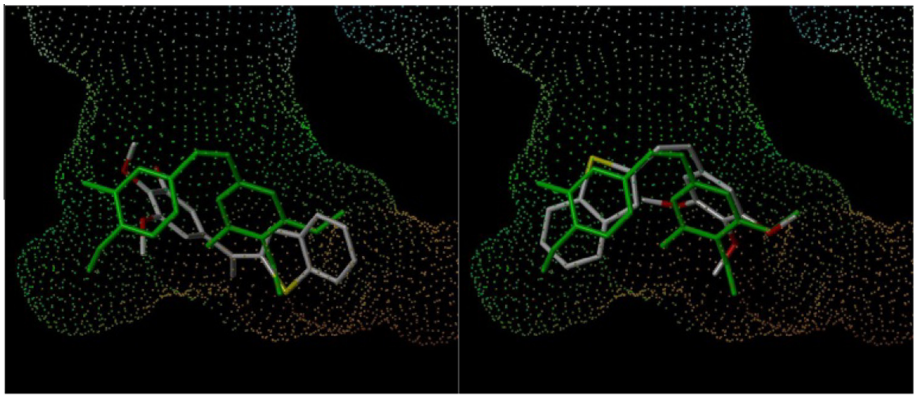


Figure 3. Docking experiments (1sa0.pdb) of combretastatin A-4 (green), *iso* derivative **4c** (left) and (*Z*)-**1b** (right).

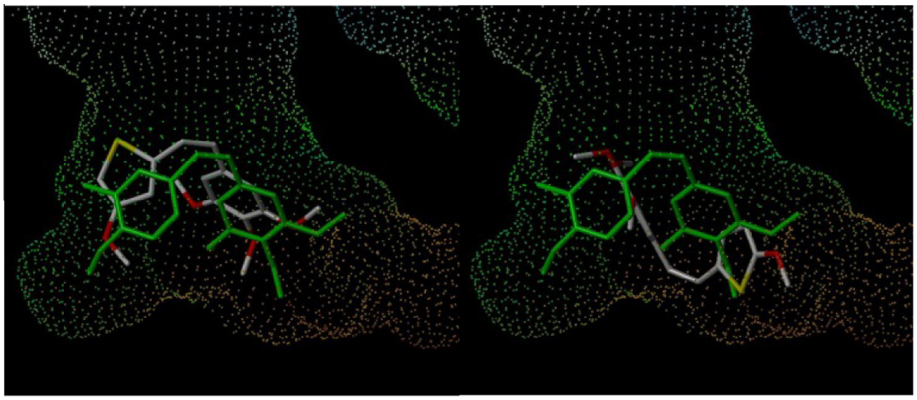


Figure 4. Docking experiments (1sa0.pdb) of combretastatin A-4 (green), (*Z*)-**2d** (left) and (*Z*)-**2e** (right).

The compounds of the *iso* series (Table 1) behave in a rather similar way, with multiple conformations emerging, none of which is really predominant. There is a discernable gradient of gathering though, with **4c** and **4d** having respectively, 29 conformations and about a half of the generated conformations gathered in a single cluster, hinting to a stronger affinity for the target. Interestingly, the near unique solution found for compound **4c** is occupying the pocket in a different manner than CA-4 (Fig. 3, on the left), which is common with one of the conformations not comprised in the main cluster of **4d**. The larger cluster of this compound has the benzothiophene left in the same position, but the trimethoxylated ring is pointing upward as the compound's orientation is inverted. Overall, oxygenated compounds in this series have difficulties to fit into the binding site due to an electrostatic mismatch with the surface of the pocket.

By contrast, (*Z*)-**1b**, with its different scaffold, adopts two major conformations. The less numerous is nearly identical to **4c**, while the other is reminiscent of CA-4, with the inversion of the trimethoxy ring (Fig. 3, on the right).

(*E*)-**1b** has several conformations. Hindered by its length and rigidity, it is unable to fit into the pocket, but adopts for about half of the solutions a straight conformation up the entry of the pocket, the benzothiophene being in the depth of the cavity: this binding mode perfectly matches with the observed biological evaluations.

Interestingly, the last four compounds of Table 2 can be easily divided in two groups. The two molecules without methoxy ((*Z*)-**2b**,**c**) are clearly too small and deprived of interaction to find a stable binding mode, with numerous conformations and several small size clusters of solutions. On the other hand, larger compounds behave much better. (*Z*)-**2a** has a full third of the solutions in a single cluster rather close to CA-4, with the planar benzothiophene occupying the position of the trimethoxylated cycle of CA-4, pulling away its own trimethoxybenzene in the entrance of the pocket. A less numerous conformations appear, with a placement identical to CA-4, with the rings inverted. The biological results clearly show that this last binding mode is predominant in cellular test. (*Z*)-**2d** and (*Z*)-**2e**, with a methoxy on the thiophene ring, have a different behavior than their congeners. (*Z*)-**2e** has a larger cluster of about two thirds of the solutions with the three trimethoxy groups in the middle of the pocket and the alkene linkage in contact with the pocket. The last third is again nearly identical to CA-4 (Fig. 4, on the right). (*Z*)-**2d** has the same conformations (Fig. 4, on the left), with an altered repartition between the two giving a larger cluster of solutions looking like CA-4, but this cluster is still the smallest and less well scored. The ranking is of these two compounds is in the reverse order, probably due to the fact that the sulfur atom of (Z)-**2e** is shielded by the adjacent methoxy and not so strongly disfavorably involved in scoring when not in contact with the pocket. In this particular case, with hindsight, the small size of the compound also resulted in an overevaluation of the fitness of conformations more closely packed against the pocket.

To conclude with this work, we have described the preparation of *cis* and *iso* methylene diaryl derivatives according to Suzuki/Wittig reactions and to a strategy using an alcohol formation/dehydration sequence. In cellulo TPI properties and antiproliferative effects were determined: *iso* derivatives could be of interest for the development of new tubulin-binding agents aiming to limit the *Z* to *E* in vivo isomerization process but were rather disappointing in terms of tubulin polymerization inhibition efficacy and antiproliferative effects to be further developed.

Moreover, we have shown that both the nature of the sulfur heterocycle, benzo[*b*]thiophene versus thiophene, and the position (2 or 3 position) of its grafting were crucial for the biological activities of *cis* and *iso* 3,4,5-trimethoxystyrene compounds. Finally, we have observed a significant improvement when adding a methoxy group on the appropriate position of the thiophene ring, as observed for compound (*Z*)-**2e**. The structure/activity relationships were confirmed by docking studies and these data could be helpful for the design of new (benzo)thiophene analogs of our hit compounds (*Z*)-**1b** and (*Z*)-**2e**.

Acknowledgments

C.V.D. would like to thank the University of Science and Technology of Hanoi (USTH) for a Ph.D. fellowship. Pr. Ahcène Boumendjel is also acknowledged for his suggestion concerning the preparation of compounds **6a,b**. R.B. is grateful to "La Ligue Contre le Cancer, comité Auvergne" for financial support.

Supplementary data

Supplementary data (these data include experimental procedures, docking poses of (*E*)-**1b**, (*Z*)-**2a** and **4d**, ^{1}H and ^{13}C NMR spectra of the thiophene and benzo[*b*]thiophene derivatives described in this article, LC–UV-MS chromatographic profiles of new evaluated compounds and antiproliferative effects (IC50 curves) of CA-4, colchicine, (*Z*)-**1b**, (*Z*)-**2a**, (*Z*)-**2d**,**e** and **4c** on IC8/MES-SA cell lines) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.010.

References and notes

1. Bray, F.; Ferlay, J.; Laversanne, M.; Brewster, D. H.; Gombe Mbalawa, C.; Kohler, B.; Piñeros, M.; Steliarova-Foucher, E.; Swaminathan, R.; Antoni, S.; Soerjomataram, I.; Forman, D. *Int. J. Cancer* **2015**, *137*, 2060.
2. Rigel, D. S. *Semin. Cutaneous Med. Surg.* **2010**, *29*, 204.
3. Saranga-Perry, V.; Ambe, C.; Zager, J. S.; Kudchadkar, R. R. *CA – Cancer J. Clin.* **2014**, *64*, 171.
4. Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012.
5. Dumontet, C.; Jordan, M. A. *Nat. Rev. Drug Disc.* **2010**, *9*, 790.
6. Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259.
7. Field, J. J.; Kanakkanthara, A.; Miller, J. H. *Bioorg. Med. Chem.* **2014**, *22*, 5050.
8. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
9. Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666.
10. Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Hamel, E.; Schmidt, J. M.; Pettit, R. K. *J. Med. Chem.* **2005**, *48*, 4087.
11. (a) Nabha, S. M.; Mohammad, R. M.; Dandashi, M. H.; Coupaye-Gerard, B.; Aboukameel, A.; Pettit, G. R.; Al-Katib, A. M. *Clin. Cancer Res.* **2002**, *8*, 2735; (b) Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W. I. I. I.; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446.
12. (a) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 3022; (b) Nihei, Y.; Suga, Y.; Morinaga, Y.; Ohishi, K.; Okano, A.; Ohsumi, K.; Hatanaka, T.; Nakagawa, R.; Tsuji, T.; Akiyama, Y.; Saito, S.; Hori, K.; Sato, Y.; Tsuruo, T. *Jpn. J. Cancer Res.* **1999**, *90*, 1016; (c) Hori, K.; Saito, S.; Kubota, K. *Br. J. Cancer* **2002**, *86*, 1604.
13. For reviews on vascular disrupting agents, see: (a) Gaya, A. M.; Rustin, G. J. S. *Clin. Oncol.* **2005**, *17*, 277; (b) Pilat, M. J.; LoRusso, P. M. *J. Cell. Biochem.* **2006**, *99*, 1021; (c) Marrelli, M.; Conforti, F.; Statti, G. A.; Cachet, X.; Michel, S.; Tillequin, F.; Menichini, F. *Curr. Med. Chem.* **2011**, *18*, 3035; (d) Mason, R. P.; Zhao, D.; Liu, L.; Trawick, M. L.; Pinney, K. G. *Integr. Biol.* **2011**, *3*, 375; (e) Porcù, E.; Bortolozzi, R.; Basso, G.; Viola, G. *Future Med. Chem.* **2014**, *6*, 1485.
14. A search with international non-proprietary names fosbretabulin and ombrabulin on http://www.clinicaltrials.gov/ web site returns clinical trials for the treatments of many cancers (alone or in combination with other drugs) but also diseases related to vascularization disorders (polypoidal choroidal vasculopathy, choroidal neovascularization secondary to pathologic myopia and neovascular age-related macular degeneration).
15. (a) Lin, M. C.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984; For a review of drugs interacting with the colchicine binding site, see: (b) Lu, Y.; Chen, J.; Xiao, M.; Li, W.; Miller, D. D. *Pharm. Res.* **2012**, *29*, 2943.
16. Aprile, S.; Del Grosso, E.; Tron, G. C.; Grosa, G. *Drug Metab. Dispos.* **2007**, *35*, 2252.
17. Previous work reported that (*Z*)-combretastatin A-4 was 30 times more potent than its (*E*) isomer for the in vitro inhibition of tubulin polymerization: see Ref. 10.
18. (a) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033; (b) Shan, Y.; Zhang, J.; Liu, Z.; Wang, M.; Dong, Y. *Curr. Med. Chem.* **2011**, *18*, 523.

19. For one example, see: Babu, B.; Lee, M.; Lee, L.; Strobel, R.; Brockway, O.; Nickols, A.; Sjoholm, R.; Tzou, S.; Chavda, S.; Desta, D.; Fraley, G.; Siegfried, A.; Pennington, W.; Hartley, R. M.; Westbrook, C.; Mooberry, S. L.; Kiakos, K.; Hartley, J. A.; Lee, M. *Bioorg. Med. Chem.* **2011**, *19*, 2359.
20. (a) Fürst, R.; Zupkó, I.; Berényi, Á.; Ecker, G. F.; Rinner, U. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6948; (b) O'Boyle, N. M.; Carr, M.; Greene, L. M.; Bergin, O.; Nathwani, S. M.; McCabe, T.; Lloyd, D. G.; Zisterer, D. M.; Meegan, M. J. *J. Med. Chem.* **2010**, *53*, 8569; (c) Theeramunkong, S.; Caldarelli, A.; Massarotti, A.; Aprile, S.; Caprioglio, D.; Zaninetti, R.; Teruggi, A.; Pirali, T.; Grosa, G.; Tron, G. C.; Genazzani, A. A. *J. Med. Chem.* **2011**, *54*, 4977; (d) Zheng, S.; Zhong, Q.; Mottamal, M.; Zhang, Q.; Zhang, C.; LeMelle, E.; McFerrin, H.; Wang, G. *J. Med. Chem.* **2014**, *57*, 3369.
21. (a) Messaoudi, S.; Tréguier, B.; Hamze, A.; Provot, O.; Peyrat, J.-F.; De Losada, J. R.; Liu, J.-M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J.; Brion, J.-D.; Alami, M. *J. Med. Chem.* **2009**, *52*, 4538; (b) Álvarez, R.; Álvarez, C.; Mollinedo, F.; Sierra, B. G.; Medarde, M.; Peláez, R. *Bioorg. Med. Chem.* **2009**, *17*, 6422.
22. For an example of constraint *iso*CA-4 derivatives: Rasolofonjatovo, E.; Provot, O.; Hamze, A.; Rodrigo, J.; Bignon, J.; Wdzieczak-Bakala, J.; Desravines, D.; Dubois, J.; Brion, J.-D.; Alami, M. *Eur. J. Med. Chem.* **2012**, *52*, 22.
23. Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **1998**, *41*, 1688.
24. (a) Hadimani, M. B.; MacDonough, M. T.; Ghatak, A.; Strecker, T. E.; Lopez, R.; Sriram, M.; Nguyen, B. L.; Hall, J. J.; Kessler, R. J.; Shirali, A. R.; Liu, L.; Garner, C. M.; Pettit, G. R.; Hamel, E.; Chaplin, D. J.; Mason, R. P.; Trawick, M. L.; Pinney, K. G. *J. Nat. Prod.* **2013**, *76*, 1668; (b) Pinney, K. G.; Bounds, A. D.; Dingeman, K. M.; Mocharla, V. P.; Pettit, G. R.; Bai, R.; Hamel, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1081; (c) Flynn, B. L.; Gill, G. S.; Grobelny, D. W.; Chaplin, J. H.; Paul, D.; Leske, A. F.; Lavranos, T. C.; Chalmers, D. K.; Charman, S. A.; Kostewicz, E.; Shackleford, D. M.; Morizzi, J.; Hamel, E.; Jung, M. K.; Kremmidiotis, G. *J. Med. Chem.* **2011**, *54*, 6014.
25. Nguyen, T. T. B.; Lomberget, T.; Tran, N. C.; Colomb, E.; Nachtergaele, L.; Thoret, S.; Dubois, J.; Guillaume, J.; Abdayem, R.; Haftek, M.; Barret, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7227.
26. Tréguier, B.; Rasolofonjatovo, E.; Hamze, A.; Provot, O.; Wdzieczak-Bakala, J.; Dubois, J.; Brion, J.-D.; Alami, M. *Eur. J. Org. Chem.* **2011**, 4868.
27. Ghinet, A.; Tourteau, A.; Rigo, B.; Stocker, V.; Leman, M.; Farce, A.; Dubois, J.; Gautret, P. *Bioorg. Med. Chem.* **2013**, *21*, 2932.
28. Petrov, O. I.; Gerova, M. S.; Chanev, C. D.; Petrova, K. V. *Synthesis* **2011**, 3711.
29. For a recent literature precedent of phenstatin synthesis using a PDC-mediated alcohol oxidation into a ketone, see: Chen, J.; Brown, D. P.; Wang, Y.-J.; Chen, Z.-S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5119.
30. (a) Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147; (b) Lima, L. M. A.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23.
31. During our studies, a research group published a 4 steps synthesis of compound **4a**, using a Negishi cross-coupling reaction: Malysheva, Y. B.; Buchvalova, S. Y.; Svirshchevskaya, E. V.; Fokin, V. V.; Fedorov, A. Y. *Synlett* **2013**, 1772. It is noteworthy that the TPI activity of this compound was still not described before our Letter..
32. Fournier Dit Chabert, J.; Joucla, L.; David, E.; Lemaire, M. *Tetrahedron* **2004**, *60*, 3221.
33. Nguyen, T. T. B.; Lomberget, T.; Tran, N. C.; Barret, R. *Tetrahedron* **2013**, *69*, 2336.
34. (a) Bazin, M.-A.; Jouanne, M.; El-Kashef, H.; Rault, S. *Synlett* **2009**, 2789; (b) Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135.
35. Herz, H.-G.; Queiroz, M. J. R. P.; Maas, G. *Synthesis* **1999**, 1013.
36. Boronic acids **11b**,**c** were commercially available whereas **11a** was prepared by trapping the appropriate heteroaryl lithium species by $B(OMe)_3$, followed by hydrolysis. See supporting information for details.
37. Jeong, Y.-C.; Ahn, D.-J.; Lee, W.-S.; Lee, S.-H.; Ahn, K.-H. *Bull. Korean Chem. Soc.* **2011**, *32*, 1063.
38. Although the synthesis (according to a different 4 steps approach) and antiproliferative effects of C2-derivative **5a** was reported in the following reference, its regioisomer **5b** (at position 3) was still not studied before our work: Romagnoli, R.; Baraldi, P. G.; Carrion, M. D.; Cruz-Lopez, O.; Tolomeo, M.; Grimaudo, S.; Di Cristina, A.; Pipitone, M. R.; Balzarini, J.; Brancale, A.; Hamel, E. *Bioorg. Med. Chem.* **2010**, *18*, 5114.
39. Vassal, E.; Barette, C.; Fonrose, X.; Dupont, R.; Sans-Soleilhac, E.; Lafanechère, L. J. *Biomol. Screening* **2006**, *11*, 377.
40. For a specified substance (e.g. (*Z*)-**1b**), the EC_{50} values between this TPI cellular evaluation and a turbidimetric test on purified tubulin are different: for comparison, see Table 1, entry 3 and Ref. 25.
41. For another method of the determination of microtubule content in the whole cell, see: Morrison, K. C.; Hergenrother, P. J. *Anal. Biochem.* **2012**, *420*, 26.
42. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
43. O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421.
44. Bechetoille, N.; Haftek, M.; Staquet, M. J.; Cochran, A. J.; Schmitt, D.; Berthier-Vergnes, O. *Melanoma Res.* **2000**, *10*, 427.
45. Harker, W. G.; MacKintosh, F. R.; Sikic, B. I. *Cancer Res.* **1983**, *43*, 4943.
46. For an example of benzothiophene acrylonitrile analogs targeting tubulin assembly, see: Penthala, N. R.; Sonar, V. N.; Horn, J.; Leggas, M.; Yadlapalli, J. S. K. B.; Crooks, P. A. *Med. Chem. Commun.* **2013**, *4*, 1073.
47. See Supporting information for details.