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Synthesis of a 3-(α-Styryl)benzo[b]-thiophene Library via Bromocyclization of Alkynes and Palladium-Catalyzed Tosylhydrazones Cross-Couplings: Evaluation as Antitubulin Agents

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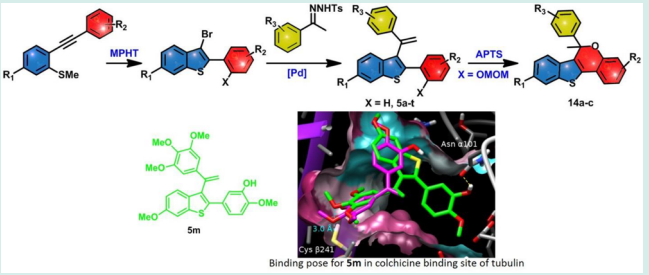
Supporting Information

ABSTRACT: A library of functionalized 3-(α-styryl)-benzo-[b]thiophenes, endowed with a high level of molecular diversity, was efficiently synthesized by applying a synthetic sequence that allowed introduction of various substituents on aromatic A, B, and C-rings. The strategy developed involves the synthesis of 3-bromobenzo[b]thiophene derivatives through a bromocyclization step of methylthio-containing alkynes using *N*-methylpyrrolidin-2-one hydrotribromide reagent (MPHT). Further coupling of 3-bromobenzothiophenes under palladium-catalysis with *N*-tosylhydrazones efficiently furnished 2-aryl-3-(α-styryl)benzo[b]thiophene derivatives. The antiproliferative properties of target compounds were studied. Among them, compound **5m** has demonstrated submicromolar cytotoxic activity against HCT-116 cell line, and inhibited the polymerization of tubulin at micromolar level comparable to that of CA-4.



KEYWORDS: *α-styrylbenzo[b]thiophene, bromocyclization, palladium, migratory insertion, N-tosylhydrazone, antimitotic agents*

INTRODUCTION

Microtubules are dynamic structures that undergo continual assembly and disassembly within the cell. Behind their function to determine cell shape, they are essential in a variety of cellular processes, including cell movements, the intracellular transport of organelles, and the separation of duplicated chromosomes during mitosis.[1] As microtubules are important regulators of endothelial cell biology, it is not surprising that tubulin binding compounds have the potential to target the tumor vasculature.[2] Tubulin binding agents can damage the existing tumor vasculature to shut down the blood supply to tumors, which lead to central necrosis of the tumor tissue.[3] Three binding sites are present in tubulin: the vinca-binding site,[4] the taxane-binding site,[5] and the colchicine binding site.[6] Among the compounds that bind to the colchicine site, by far combretastatin A-4 (CA-4), a *Z*-stilbene isolated by Pettit from an African tree, *Combretum caffrum*,[7] is the most studied example of vascular disrupting agents (VDAs), with established vascular disrupting activity at nontoxic doses (Figure 1).[8]

Moreover, it has been demonstrated that the water-soluble phosphate prodrug CA-4P and AVE-8062, a serinamido derivative, are currently in clinical trials (phase II/III) for the treatment of anaplastic thyroid and advanced cancers.[9] The discovery of CA-4 has led to a diverse library of antitubulin agents designed to mimic the simple stilbenoid structure. It is important to note that the *Z* olefinic bridge of CA-4 is subject to rapid *Z*−*E* isomerization under the influence of light, heat, and protic media, resulting in a dramatic loss on antitumor activity.[8c] In recent years, our interest in the 1,1-diarylethylene unit synthesis[10] combined with our efforts to discover nonisomerizable CA-4 analogues,[11] led us to identify *iso*combretastatin A-4 (*iso*CA-4) as well as *iso*NH_2CA-4[12] as lead compounds that exhibit potent antineoplastic and antivascular properties (Figure 1). Since heterocycles are prevalent as a core molecular component in a variety of tubulin assembly inhibitors, we recently synthesized a series of compounds, where the *iso*CA-4 B-ring was replaced by a pyridine (**1**),[13] benzofurane (**2**), or indole (**3**) nucleus.[14]

As a privileged structure, benzothiophenes constitute an important class of heterocycles and are present in a variety of natural products and in numerous pharmaceutically compounds.[15] In addition, many of benzothiophene derivatives are potent inhibitors of tubulin polymerization. A specific example is the tubulin binding agent **4**, an analogue of CA-4.[16] In the continuation of the structure activity relationships study of benzoheterocycle-based *iso*CA-4, 2-aryl-3-(α-styryl)-benzo[b]-thiophenes **5** were designed as novel, highly potent inhibitors of tubulin polymerization. Herein, we report a convergent

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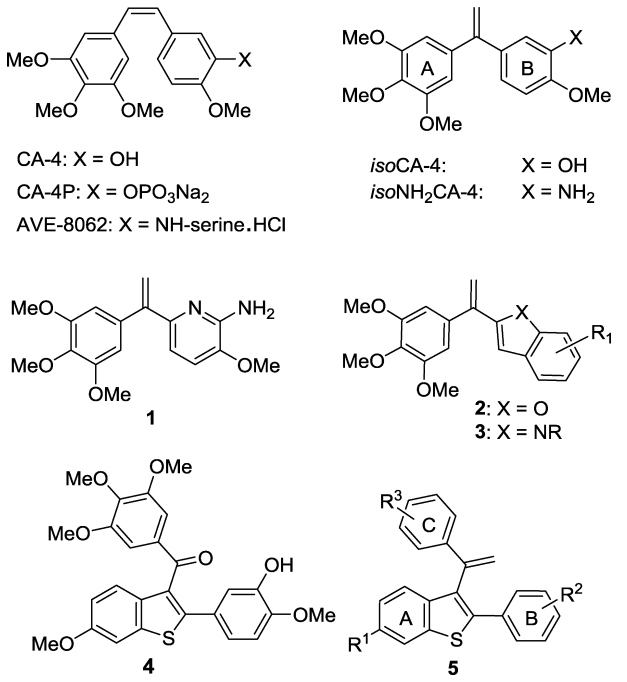


Figure 1. Structure of antitubulin agents and target structure **5**.

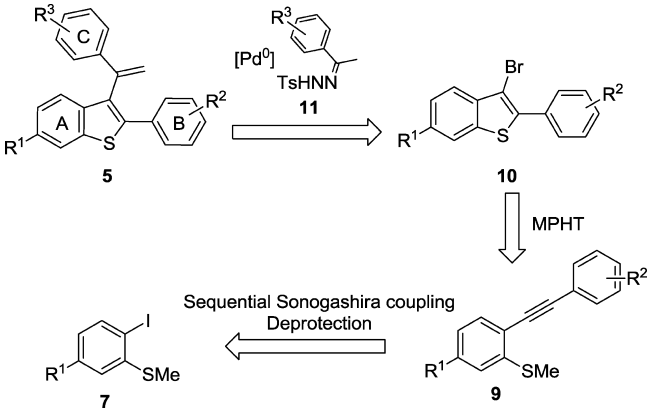
protocol for rapid access to benzo[*b*]thiophenes **5**, which is based on a two-step sequence involving sequential bromocyclization reactions and palladium-mediated coupling of *N*-tosylhydrazones from diarylalkynes. The scope of this new coupling reaction has been studied in the context of a structure−activity relationship study of benzothiophenes **5** analogues. New compounds were then evaluated in vitro to assess their tubulin assembly and growth inhibitory activity.

RESULTS AND DISCUSSION

Although the synthesis of 2,3-disubstituted benzothiophenes is well documented,[17] to our knowledge, only one report deals with the synthesis of 2-aryl-3-(α-styryl)benzothiophenes through a Wittig reaction.[18] We have recently shown that the electrophilic cyclization of 2-(1-alkynyl)thioanisoles in the presence of *N*-methylpyrrolidin-2-one hydrotribromide (MPHT) provides a mild, high yielding synthesis of 3-bromo-benzothiophenes.[19] On the other hand, *N*-tosylhydrazones have emerged as a new type of cross-coupling partner in transition-metal-catalyzed reactions and have attracted increasing attention in the past five years.[20] Our interest in this chemistry[10a,c,21] led us to evaluate the scope of this coupling reaction for the preparation of the target 2-aryl-3-(α-styryl)-benzothiophenes **5**.

Using our previous benzothiophene methodology, we envisioned an efficient strategy that would lead to a library of disubstituted benzo[*b*]thiophenes with multiple points of diversity present in each aromatic ring (A, B, and C). Our strategy is outlined in Scheme 1, retrosynthetically, we planned that the target products **5** could be prepared according to a convergent route with the introduction of the C-ring at the final step of the synthesis. Ortho-substituted diarylalkynes **9** will be formed by iterative sequence Sonogashira Pd-couplings[22]/desilylation from readily available 2-iodothioanisole derivatives. The key intermediate 3-bromobenzo[*b*]thiophene **10** can be efficiently prepared using our alkyne bromocyclization chemistry and then its coupling with various *N*-tosylhydrazones

Scheme 1. Synthetic Strategy to Target 2-Aryl-3-(α-styryl)benzo[*b*]thiophenes 5 via Bromocyclization and Pd-catalyzed *N*-Tosylhydrazones Coupling



of acetophenone **11** under palladium catalysis to furnish the target molecules **5** variously substituted at the indicated positions.

As outlined in Scheme 2, the precursor alkynes **9** were prepared according to two paths. The first one (path a) involves the use of 2-iodothioanisole **6** in a Sonogashira coupling,[22] C−Si bond cleavage and a second Sonogashira reaction furnishing diarylalkynes **9a−e** in good overall yields. Starting from ortho-dihalogenated derivative **8b**, the second path (path b) is based on Sonogashira coupling, and subsequent lithiation followed by methylthiolation with dimethyl disulfide.[23] Accordingly derivatives **9j−m** were isolated with yields up to 77%.

To achieve the synthesis of 3-bromobenzothiophene derivatives **10a−i**, the key step of our strategy is the electrophilic cyclization of methylthio-containing alkynes **9** using solid and easily handled MPHT (Table 1). The cyclization takes place rapidly under mild conditions (room temperature), leading to their respective 3-bromobenzo[*b*]-thiophenes **10a−i** in good to excellent yields.

Having 3-bromobenzo[*b*]thiophenes **10** in hand, the experimental conditions for the coupling of **10a** and *N*-tosylhydrazone **11a** were carefully examined by screening, the palladium sources, phosphine ligands, the base and the solvent (Table 2). At the beginning of this optimization study, we tested the initial conditions reported by Barluenga et al.[20a] (entry 1) and those developed in our laboratory (entry 2).[10c] The coupling was inefficient and resulted in concomitant formation of the desired product **5a**, and the byproduct 1,2,3-trimethoxy-5-vinylbenzene, the Bamford−Stevens degradation product of **11a** along with starting material **10a**. This complicated mixture makes the purification difficult and not adapted for combinatorial library synthesis. Based on our experience in other coupling reactions with tosylhydrazones, led us in the development of a more robust protocol for improving this cross-coupling in the context of our medicinal chemistry program.

Next, we compared the effect of two bases, Cs_2CO_3 and LiO*t*Bu and found that the use of LiO*t*Bu provided the desired product **5a** in a slightly better yield (60%, entry 3). With LiO*t*Bu as the base, we then screened the ligand effect on this transformation (entries 3−10). We found that *t*BuX-Phos, and XantPhos were ineffective ligands in this reaction (entries 6−7). The use of other diphenylphosphino-based ligand such as

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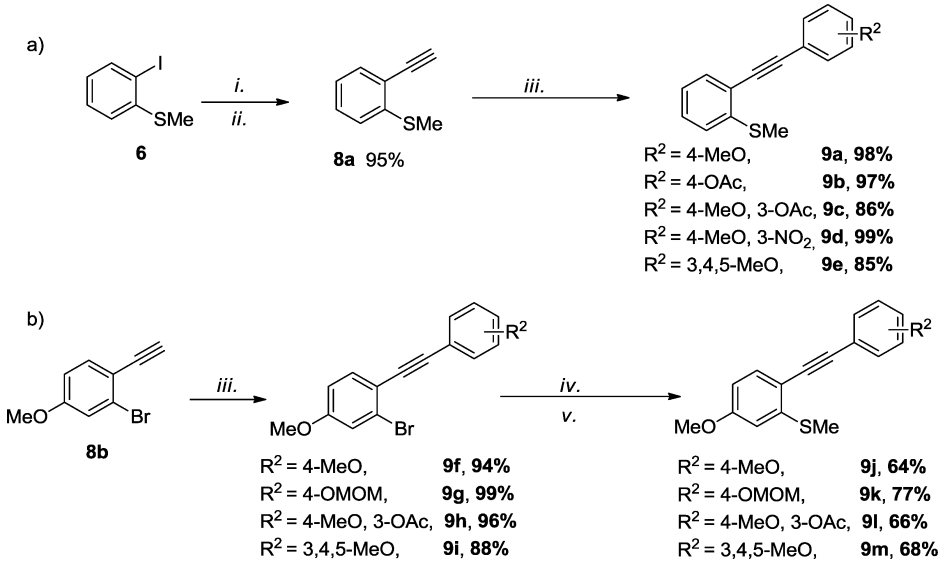
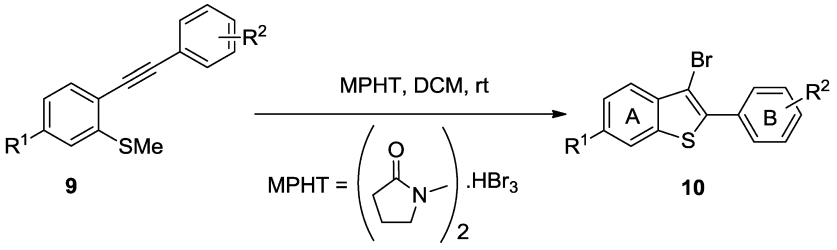
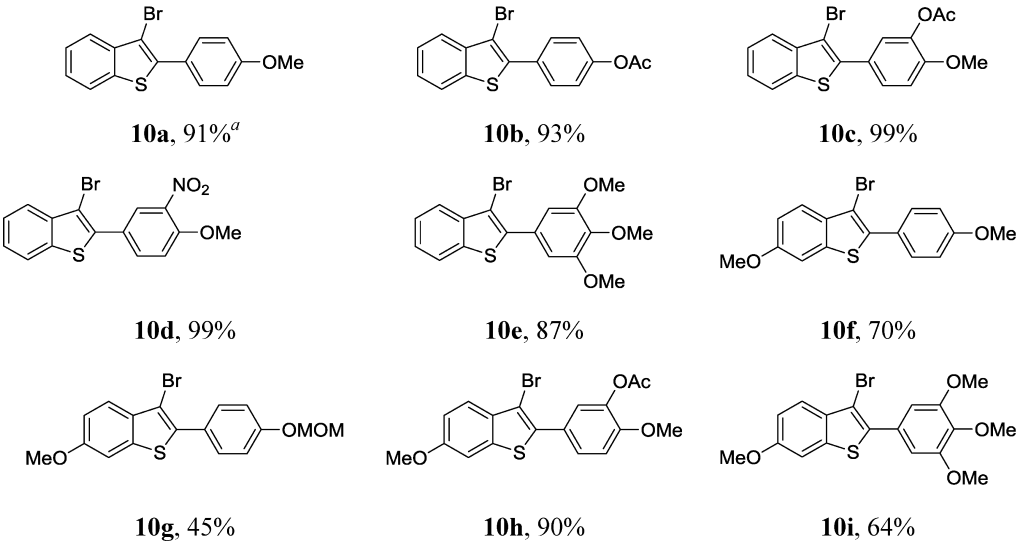
Scheme 2. (a) Synthesis of *ortho*-(1-Alkynyl)thioanisoles 9a−e by a Sequential Sonogashira Coupling/Deprotection from *ortho*-Iodothioanisole and (b) Synthesis of Alkynes 9j−m by a Subsequent Lithiation, Followed by Methylthiolation[a]



[a]Reagents and conditions: (i) 1) $PdCl_2(PPh_3)_2$ 2 mol %, CuI 2 mol %, TMSA, THF, Et_3N, 50 °C; (ii) K_2CO_3, MeOH; (iii) $PdCl_2(PPh_3)_2$ 2 mol %, CuI 2 mol %, Ar−I, THF, Et_3N, 50 °C; (iv) *n*BuLi, −78 °C, THF ; (v) Me_2S_2, −78 °C to rt.

Table 1. Preparation of 3-Bromobenzo[*b*]thiophenes 10 Library





[a]Isolated yield after column chromatography.

dppb leads to a decrease in the yield of **5a**. DPEPhos, X-Phos gave a similar result as for dppp. Further modification of the substituents on the monodentate ligand led us to find that DavePhos was a more efficient ligand, and the desired product **5a** was isolated in a nearly quantitative 96% isolated yield (entry 9). One can note that dppf ligand was also effective, albeit furnishing **5a** in a 78% yield (entry 10).

With the optimized reaction conditions, the scope of this Pd-catalyzed coupling reaction was then examined by using a series of *N*-tosylhydrazones and 3-bromobenzo[*b*]thiophene derivatives. As illustrated in Table 3, this coupling proceeds smoothly

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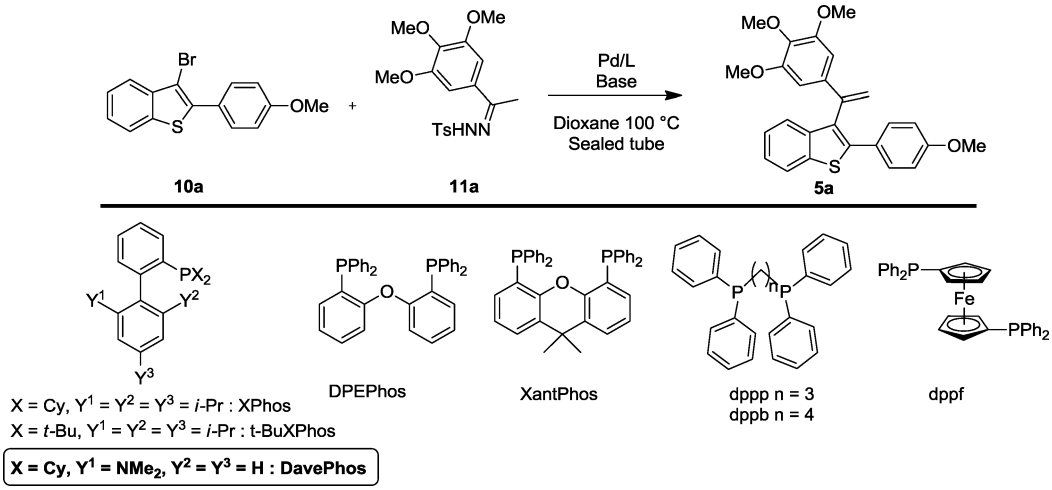
Table 2. Optimization of Reaction Parameters between 3-Bromobenzo[*b*]thiophene 10a and *N*-Tosylhydrazone Partner 11a.[a]



entry	Pd sources	ligand	base	yield of **5a** (%)[b]
1	$Pd_2dba_3{\cdot}CHCl_3$	X-Phos	LiOtBu	50[c]
2	$PdCl_2(MeCN)_2$	dppp	Cs_2CO_3	52[c]
3	$PdCl_2(MeCN)_2$	dppp	LiO*t*Bu	60
4	$PdCl_2(MeCN)_2$	dppb	LiO*t*Bu	40[d]
5	$PdCl_2(MeCN)_2$	X-Phos	LiO*t*Bu	57
6	$PdCl_2(MeCN)_2$	*t*BuX-Phos	LiO*t*Bu	20[d]
7	$PdCl_2(MeCN)_2$	XantPhos	LiO*t*Bu	0[d]
8	$PdCl_2(MeCN)_2$	DPEPhos	LiO*t*Bu	58
9	**$PdCl_2(MeCN)_2$**	**DavePhos**	**LiO*t*Bu**	**96**[e]
10	$PdCl_2(MeCN)_2$	dppf	LiO*t*Bu	78

[a]The reactions were carried out in a sealed tube with **10a** (1 mmol), **11a** (1.2 mmol), [Pd] source 4 mol %, ligand 8 mol %, and base (2.2 equiv) at 100 °C in dioxane (3.0 mL). [b]Isolated yield of **5a.** [c]Reaction was performed at atmospheric pressure. [d]Bamford Stevens product 1,2,3-trimethoxy-5-vinylbenzene was isolated, along with **11a**. [e]Performing the reaction at atmospheric pressure led to a 55% of **5a**.

over a wide range of substrates, providing the 2-aryl-3-(α-styryl)benzothiophenes with good to excellent yields.

Compounds bearing a phenol group (**5c**−**f**, **5i**−**j**, and **5m**−**o**) were obtained in a single synthetic operation from the corresponding acetylated benzothiophene derivatives in good yields for the two steps (coupling/deprotection). Both electron-donating and electron-withdrawing groups such as OMe, OAc, and NO_2 present on the 3-bromobenzothiophenes or tosylhydrazones were well tolerated. Variations with respect to the 3-bromobenzothiophenes partner **10** were next examined. To our satisfaction, the reaction proceeded well with 6-methoxybenzothiophene derivatives, affording 2-aryl-3-(α-styryl)benzothiophenes **5k**−**o** in good yields (Table 3).

As shown in Scheme 3, the key step in this palladium-catalyzed cross-coupling reaction with carbenes, generated in situ from *N*-tosylhydrazones, is the migratory insertion step of the Pd-carbene species **12**. Such insertion is well-known in the case of aromatic carbon ring,[20b] this work represent the first example of migratory insertion of the benzothiophenyl nucleus in the Pd-carbene species **12**.

To increase the scope of this synthetic method, and thus enlarge the molecular diversity, we decided to take advantage from the presence of the double bond on the skeleton of compounds **5** and to use it in further transformations (Scheme 4). First, *ortho*-substituted 3-bromobenzo[*b*]thiophenes derivatives (**10j**-**k**) were prepared in excellent yields by a sequence of Sonogashira/bromocyclization reactions; in these cases NBS was used instead of MPHT to avoid premature cleavage of MOM protection. Then coupling with hydrazones **11** under our optimized conditions gave the corresponding 2-aryl-3-(α-styryl)benzo[*b*]thiophenes (**5p**-**r**) in good yields (66−83%). Finally, treatment with PTSA led to the formation of benzo[4,5]thieno[3,2-*c*]chromenes derivatives (**13a**-**c**) in excellent yields.

To obtain the amino derivatives of our analogues, the nitro group of compound **5g** was subjected to a reduction step by iron powder, producing compound **5s** in an excellent yield (Scheme 5).[24] Finally, deprotection of phenolic methoxymethyl (MOM) ether of compound **5l** was efficiently achieved using PTSA in EtOH in a nearly quantitative yield.

Biological Evaluation. Inhibition of tubulin polymerization (ITP) and in vitro cellular growth inhibitory activity.

Selected 3-(α-styryl)-benzo[*b*]thiophenes (Table 4), prepared above were evaluated for their capacity to inhibit tubulin polymerization. The newly synthesized compounds were compared in contemporaneous experiments to the potent tubulin polymerization inhibitor CA-4.[25] As presented in Table 4, compounds **5a**−**d**, **5f**−**k**, **5n**−**t**, **14b**, and **14c** showed no effect on tubulin assembly at concentrations as high as 20 μM. Compounds **5e** and **5m** exhibit similar inhibition of tubulin assembly as CA-4 (IC_{50} = 4 and 3 μM, respectively). In terms of SAR information, no inhibition of tubulin assembly was found for similar derivatives **5a** and **5k** where the OH group on the B-ring was omitted (compare **5a** vs **5e** and **5m** vs **5k**). This can be explained by the participation of this group (phenol) to the formation of a hydrogen bond at the active site of tubulin (see below, the molecular modeling section). Switching of the trimethoxy group from C-ring to B-ring (compare **5e** and **5i**)

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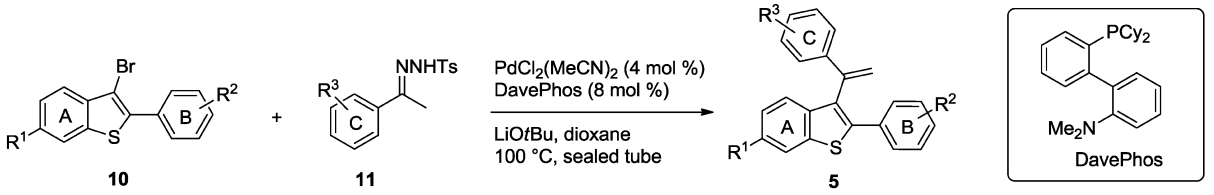
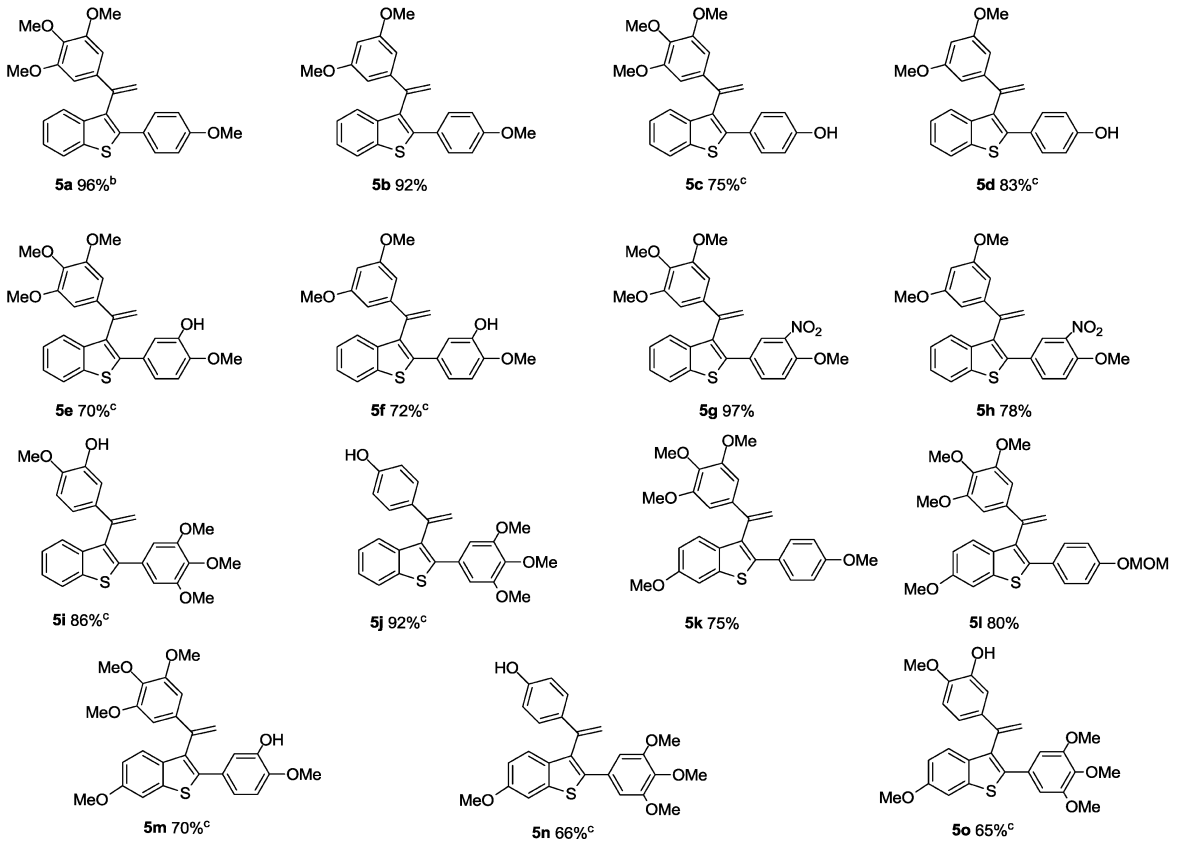
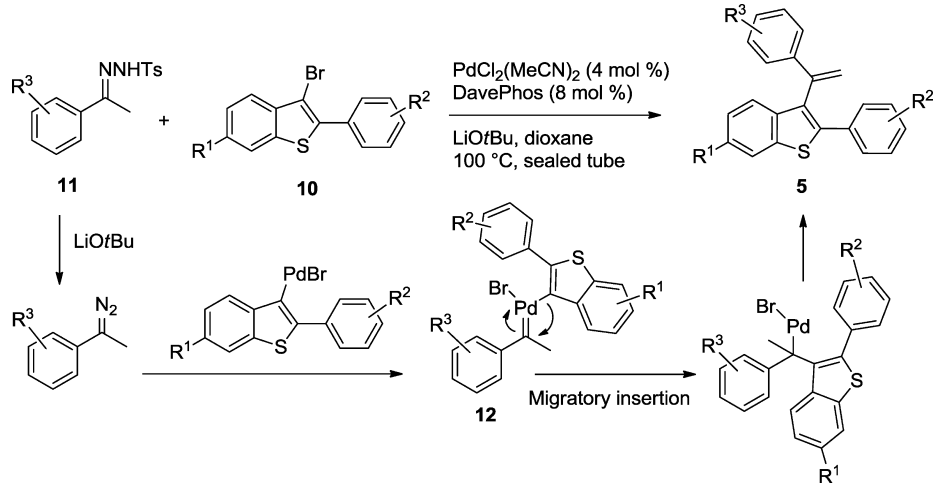
Table 3. Synthesis of 2-Aryl-3-(α-styryl)benzothiophenes via the Coupling of *N*-Tosylhydrazones 11 with 3-Bromobenzothiophenes 10 under Palladium Catalysis.[a]





[a]Reactions were performed with *N*-tosylhydrazone **11** (1.2 equiv), 3-bromobenzo[*b*]thiophenes **10** (1 equiv), $PdCl_2(MeCN)_2$ (4 mol %), DavePhos (8 mol %), LiO*t*Bu (2.2 equiv), 1,4-dioxane (3 mL) in a sealed tube at 100 °C for 3 h. [b]Yield of isolated product after column chromatography. [c]Additional 1 equiv of LiO*t*Bu was added to complete deprotection of the acetyl group.

Scheme 3. Mechanism of the Pd-Catalyzed Synthesis of (α-Styryl)benzo[*b*]thiophenes



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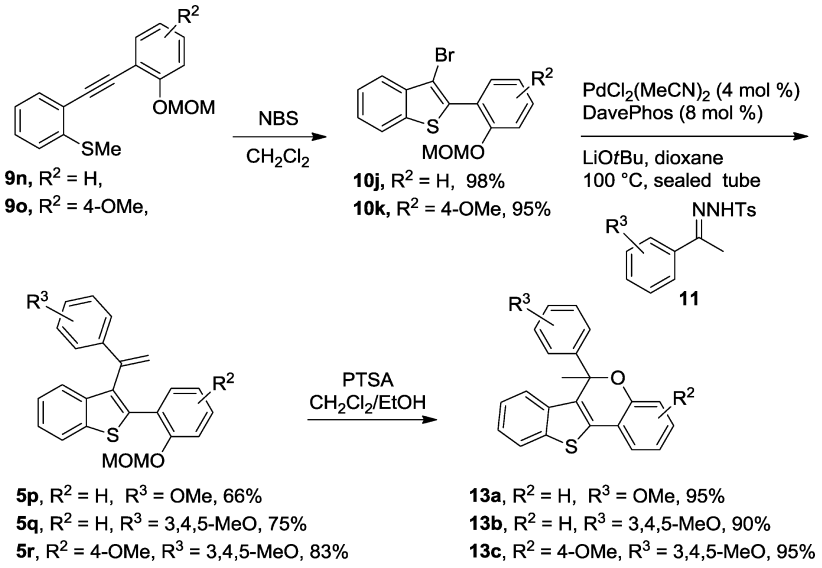
Scheme 4. Synthesis of 6-Methyl-6-phenyl-6H-benzo[4,5]thieno[3,2-*c*]chromenes Derivatives



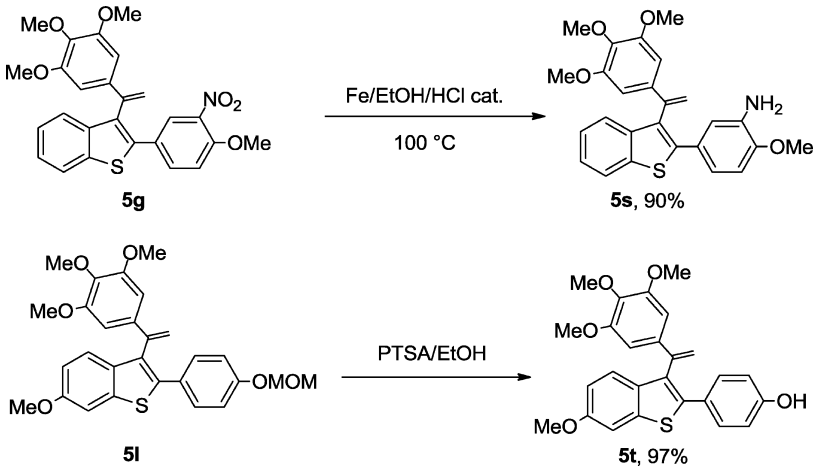
9n, R^2 = H,
9o, R^2 = 4-OMe,

10j, R^2 = H, 98%
10k, R^2 = 4-OMe, 95%

5p, R^2 = H, R^3 = OMe, 66%
5q, R^2 = H, R^3 = 3,4,5-MeO, 75%
5r, R^2 = 4-OMe, R^3 = 3,4,5-MeO, 83%

13a, R^2 = H, R^3 = OMe, 95%
13b, R^2 = H, R^3 = 3,4,5-MeO, 90%
13c, R^2 = 4-OMe, R^3 = 3,4,5-MeO, 95%

Scheme 5. Synthesis of Amino and Phenol Analogues of Compounds 5



5g

5s, 90%

5l

5t, 97%

led also to no inhibition of tubulin polymerization. Fusion of the C-ring to the double bond was unfavorable for the activity (compounds **13b–c**).

Next, the cytotoxic activity was evaluated against the human colon carcinoma cell line (HCT-116). Best cytotoxicity were observed for compounds **5m** and **5t**, which displayed interesting antiproliferative effects on the growth of HCT-116 cells (IC_{50} < 1 μM). In all tested compounds, we observed a good correlation between cytotoxicity and inhibition of tubulin polymerization except for compound **5t**. This indicates that the latter compound has a different mechanism of action independent of tubulin polymerization. Compound **5m** was found three time more active on the proliferation of colon carcinoma cell in comparison with the reference compound **4**. Compounds such as **5e** that inhibit tubulin polymerization at micromolar concentrations, but do not exhibit a significant cytotoxic effect, may prove useful as selective vascular disrupting agents. In principle it should be desirable to maximize the difference between the dose required to affect cell proliferation and/or viability and that required to disrupt other cellular cytoskeletal function.[26]

Molecular Modeling Study. The molecular docking study was performed to elucidate the interactions of this class of compounds with tubulin. In order to provide a possible explanation of the crucial role played by the O–H moiety on B ring for tubulin polymerization inhibition (Table 4), we performed docking experiments with derivatives **5e**, **5k**, and **5m** within the colchicine binding site in tubulin. These calculations suggested that these 3 compounds could share a common binding mode, in which A-ring inherited from *iso*CA-4 would remain in its usual location, namely the lipophilic pocket belonging to the β subunit including Cys β241. On one hand, the side-chain of the Cys β241would establish in every case the already described hydrogen bond-type interaction with the trimethoxy pattern on A ring[27] (at the price of a slight reorientation of compound **5e**, where the methoxy group is missing from the thiophene ring). On the other hand, an auxiliary hydrogen bond could be formed between the hydroxyl group borne by B ring in compounds **5e** and **5m** and Asn α101, whereas this interaction would be missing in derivative **5k**, contributing to decrease the potency of this compound.

CONCLUSIONS

In summary, we have developed an efficient method to synthesize a variety of 3-(α-styryl)-benzo[*b*]thiophene deriva-

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Table 4. Cytotoxicity and Inhibition of Tubulin Polymerization (ITP) of Selected Benzothiophenes 5 Against Colon Carcinoma cells (HCT-116)

compound	GI_{50} (μM)[a] HCT-116	ITP IC_{50} (μM)[b]	compound	GI_{50} (μM)[a] HCT-116	ITP IC_{50} (μM)[b]
5a	>20	NC[c]	**5m**	0.2	3.0 ± 0.2
5b	>20	NC[c]	**5n**	>20	NC[c]
5c	8	NC[c]	**5s**	>20	NC[c]
5d	>20	NC[c]	**5t**	0.8	NC[c]
5e	2	4 ± 0.9	**13b**	>20	NC[c]
5f	>20	NC[c]	**13c**	>20	NC[c]
5i	>20	NC[c]	**4**[d]	0.7	3.5 ± 0.4
5j	>20	NC[c]	***iso*CA4**	0.003	2.5
5k	>20	NC[c]	**CA4**[e]	0.003	2.4

[a]GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). [b]ITP, inhibition of tubulin polymerization; IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [c]IC_{50} value not calculated owing to the low activity of the compound. [d]Compound **4** was prepared according to the ref 17d. [e]The GI_{50} and IC_{50} values for *iso*CA-4 and CA-4 were determined in this study.

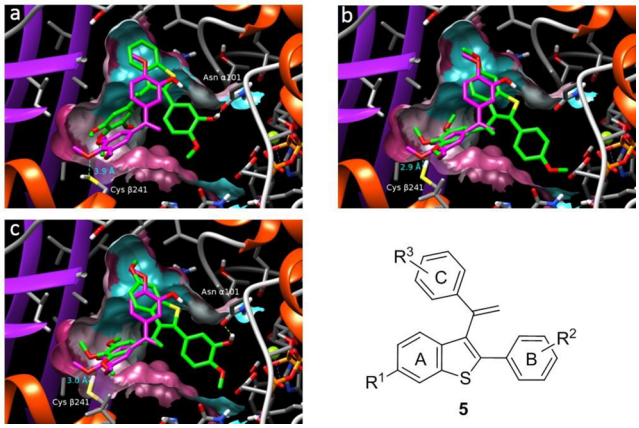

Figure 2. Calculated binding modes for compounds **5e** (a), **5k** (b), and **5m** (c) (green color) in the colchicine binding site of tubulin and superimposition of the latter with that of *iso*CA-4 (magenta color). Solvent-accessible surface is colored accordingly to its local polarity (maroon color means hydrophobic, cyan color means polar).

tives via a bromocyclization of methylthio-containing alkynes, followed by a palladium cross-coupling reaction with *N*-tosylhydrazones. To improve the molecular diversity, benzo[4,5]thieno[3,2-*c*]chromene derivatives were also obtained through a cyclization step under acidic conditions. This method is featured with mild reaction conditions, simple operation, good substrate scope and good yields. By using this methodology, we have identified compounds **5m** and **5e** that inhibit tubulin polymerization at micromolar concentrations. It was shown that the binding of these compounds to β-tubulin is directed by their hydroxy group borne by B-ring and is stabilized by hydrogen bonding of Asn α101. Compounds **5m** and **5t** display cytotoxic activity against HCT-116 cells in submicromolar range, and **5m** was found to be slightly more active than control product **4**. Such agents interfering with microtubules may prove useful as selective vascular disrupting agents.

EXPERIMENTAL PROCEDURES

General Procedure for Preparation of *N*-Tosylhydrazones.[28] To a rapidly stirred suspension of *p*-toluenesulphonohydrazide (5 mmol) in dry methanol (10 mL) at 60 °C, the ketone (5 mmol) was added dropwise. Within 5−60 min the *N*-tosylhydrazone began to precipitate. The mixture was cooled to 0 °C, and the product was collected on a Büchner funnel, washed by petroleum ether, and then was dried in vacuo to afford the pure product.

General Procedure for Sonogashira Coupling. To a solution of aryl iodide (1 mmol) in THF, were added CuI (2% mol), $PdCl_2(PPh_3)_2$ (2% mol), Et_3N (4 mmol), and the terminal alkynes. The mixture was stirred and heated at 50 °C and follow by TLC. After 4 h, THF was evaporated and the crude product was dissolved by Et_2O and washed two times by a saturated solution of NH_4Cl. The layers are separated and the aqueous layers were extracted by Et_2O. Organic layers were washed by a saturated solution of NaCl and dried dry by anhydrous $MgSO_4$. The crude product was concentrated under reduced pressure and purified by silica gel chromatography.

Typical Procedure for the Formation of 3-Bromobenzo[*b*]thiophene derivatives. *Method A.* To a solution of diarylalkyne compound (1.6 mmol, 1 equiv) in DCM (3.5 mL/mmol) are added in several portions at room temperature *N*-methylpyrrolidin-2-one hydrotribromide (MPHT, 1.9 mmol, 1.2 equiv) The mixture was stirred at room temperature and follow by TLC. After full conversion, the mixture was hydrolyzed by a saturated solution of $Na_2S_2O_3$ and extract by AcOEt. Layers are separated and aqueous layer was extracted three times by EtOAc. Organic layers were washed by a saturated solution of NaCl, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.

Method B. The same protocol was used as in method A, but MPHT has been replaced by *N*-bromsuccinimide.

Typical Procedure for Coupling between *N*-Tosylhydrazones and Bromobenzo[*b*]thiophenes. *Method C.* In a sealed tube, under inert atmosphere, the *N*-tosylhydrazone (1.2 mmol), $PdCl_2(MeCN)_2$ (4 mol %), DavePhos (8 mol %), and 3 mL of dioxane were mixed under argon for 5 min at rt. LiO*t*Bu (2.2 mmol) was then added, the reaction mixture was stirred for an additional 1 min at rt, and finally, bromobenzo[*b*]thiophene (1.0 mmol) was added. The resulting was stirred at 100 °C in a sealed tube during 3 h. The crude reaction mixture was allowed to cool to rt. EtOAc was added, and the mixture was filtrated through Celite. Solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography on silica gel.

Method D. The same protocol was used as in method A, but when the reaction was completed 1 equiv of LiO*t*Bu was added and the mixture was stirred at room temperature during 1 h. EtOAc was added that the mixture was filtrated on Celite. Solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography on silica gel.

General Procedure for the Preparation of 6*H*-Benzo[4,5]thieno[3,2-*c*]chromenes,. *Method E.* To a solution of alkene **5** (1 mmol) in EtOH 5 mL was added PTSA (3 mmol). The mixture was stirred overnight at 60 °C. The mixture was treated by a statured solution of $NaHCO_3$ and extract by EtOAc. The layers were separated. Organic layer was washed with a saturated solution of NaCl, dried by anhydrous $MgSO_4$

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and concentrated under reduced pressure. The crude product was purified by silica gel chromatography.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details about synthesis and characterization of compounds, biological and computational methods, copies of ^{1}HNMR, and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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