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Design, Synthesis, and SAR Studies of 4-Substituted Methoxylbenzoyl-aryl-thiazoles Analogues as Potent and Orally Bioavailable Anticancer Agents

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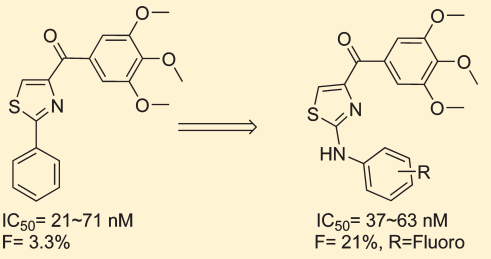
Supporting Information

ABSTRACT: In a continued effort to improve upon the previously published 4-substituted methoxybenzoyl-aryl-thiazole (SMART) template, we explored chemodiverse "B" rings and "B" to "C" ring linkage. Further, to overcome the poor aqueous solubility of this series of agents, we introduced polar and ionizable hydrophilic groups to obtain water-soluble compounds. For instance, based on in vivo pharmacokinetic (PK) studies, an orally bioavailable phenyl-amino-thiazole (PAT) template was designed and synthesized in which an amino linkage was inserted between "A" and "B" rings of compound **1**. The PAT template maintained nanomolar (nM) range potency against cancer cell lines via inhibiting tubulin polymerization and was not susceptible to P-glycoprotein mediated multidrug resistance in vitro, and markedly improved solubility and bioavailability compared with the SMART template (**45a**−**c** (PAT) vs **1** (SMART)).



INTRODUCTION

Microtubules are cytoskeletal filaments consisting of αβ-tubulin heterodimers and are involved in a wide range of cellular functions, including shape maintenance, vesicle transport, cell motility, and division. Tubulin is the major structural component of the microtubules and a well verified target for a variety of highly successful anticancer drugs. Anticancer drugs like paclitaxel and vinblastine that are able to interfere with microtubule−tubulin equilibrium in cells are extensively used in cancer chemotherapy.[1] There are two major classes of antimitotic agents: microtubule-stabilizing agents, which inhibits the microtubule depolymerization by binding to and stabilizing microtubule, are represented by taxanes and epothilones. Another class is microtubule-destabilizing agents, such as vinca alkaloids and colchicine, which cause microtubule disassembling and inhibit tubulin polymerization into microtubules. Vinblastine represents one of vinca alkaloids. Colchicine and colchicine-site binders are all defined as microtubule-destabilizing antimitotic agents.[2] While no colchicine-site binders are currently approved for cancer chemotherapy, both the taxanes and vinca alkaloids are widely used to treat human cancers. However, colchicine binding agents like combretastatin A-4 (CA-4) and *N*-(2-(4-hydroxy phenylamino)pyridin-3-yl)-4-methoxybenzenesulfonamide (ABT-751, **47**) (Figure 1) are now under clinical investigation as potential new chemotherapeutic agents.[2,3,4]

Unfortunately, microtubule-interacting anticancer drugs in clinical use share two major problems, resistance and high lipophilicity. A common mechanism of multidrug resistance

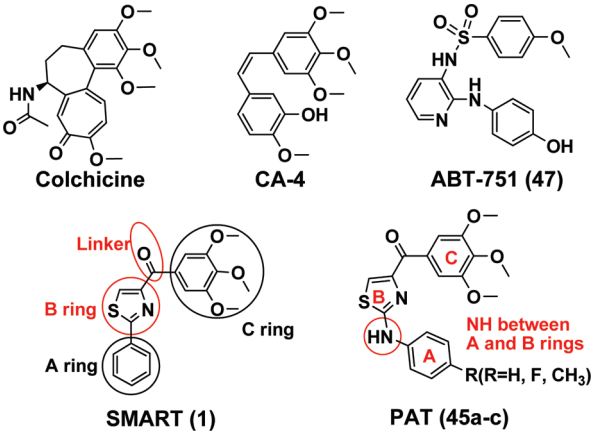


Figure 1. Structures of Colchicine-Binding Site Tubulin Inhibitors.

(MDR), namely ATP binding cassette (ABC) transporter protein-mediated drug efflux, limits their efficacy.[5−7] P-glycoproteins (P-gp, encoded by the MDR1 gene) is an important member of the ABC superfamily.[8] P-gp prevents the intracellular accumulation of many cancer drugs by increasing their efflux out of cancer cells as well as contributing to hepatic, renal, or intestinal clearance pathways. Attempts to coadminister P-gp

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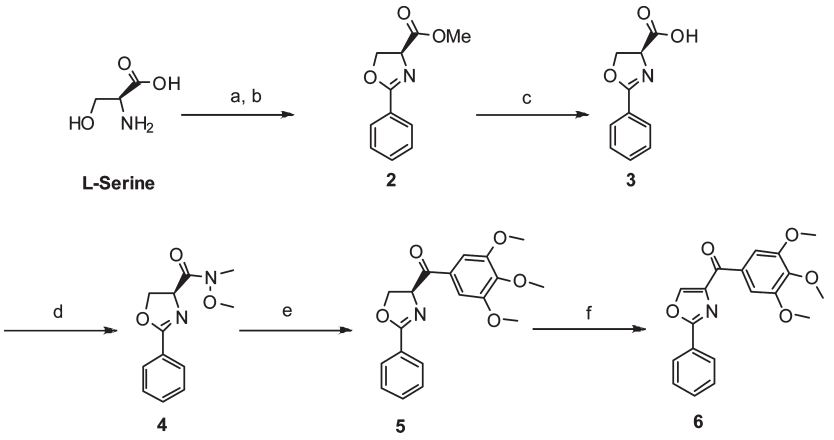
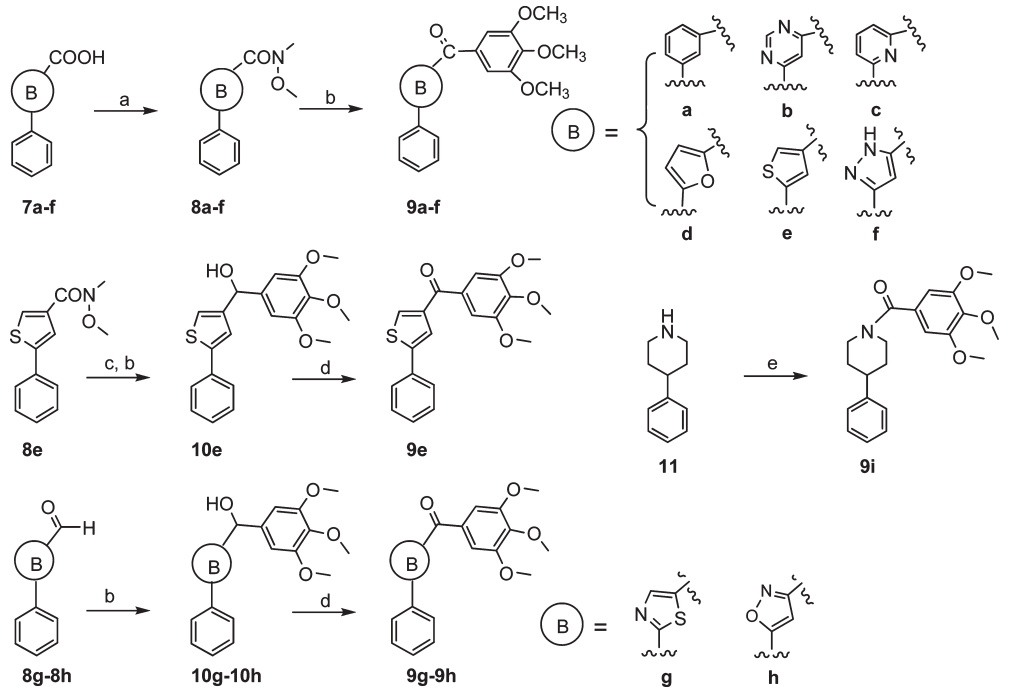
Scheme 1[a]



[a] (a) MeOH, CH_3COCl, 83%; (b) ethyl benzimidate hydrochloride, CH_2Cl_2, Et_3N, 96%; (c) LiOH, MeOH, H_2O, 65%; (d) EDCI, HOBt, NMM, $CH_3OCH_3NH \cdot HCl$, 61%; (e) 3,4,5-trimethoxyphenylmagnesium bromide, THF, 48−71%; (f) $CBrCl_3$, DBU, CH_2Cl_2, 56%.

Scheme 2[a]



[a] (a) EDCI, HOBt, NMM, $CH_3OCH_3NH \cdot HCl$, CH_2Cl_2, 51−95%; (b) 3,4,5-trimethoxyphenyl-magnesium bromide, THF, 48-78%; (c) LAH, −78 °C, THF, 85%; (d) Dess−Martin reagent, CH_2Cl_2, 81%; (e) EDCI, HOBt, NMM, 3,4,5-trimethoxybenzoic acid, CH_2Cl_2, 58%.

modulators or inhibitors to increase cellular availability by blocking the actions of P-gp have met with limited success.[8,9] The other major problem with taxanes, as with many biologically active natural products, is its high lipophilicity and lack of solubility in aqueous systems. This leads to the use of emulsifiers like Cremophor EL and Tween 80 in clinical preparations. A number of biologic effects related to these drug formulation vehicles have been described, including acute hypersensitivity reactions and peripheral neuropathies.[10,11] Compared to compounds binding the paclitaxel- or vinca alkaloid-binding site, colchicine-binding agents usually exhibit relatively simple structures. Thus, it provides a better opportunity for oral bioavailability via structural optimization, as reported herein for compounds with improved solubility and pharmacokinetic (PK) parameters. In addition, most of these new agents appear to circumvent P-gp-mediated MDR. Therefore, these novel colchicine binding site targeted compounds hold great promise as therapeutic agents, particularly because they have improved aqueous solubility and overcome P-gp mediated MDR.

4-Substituted methoxybenzoyl-aryl-thiazole (SMART, **1**, Figure 1) is a potential anticancer agent that was discovered recently in our laboratory which targets tubulin by binding to its

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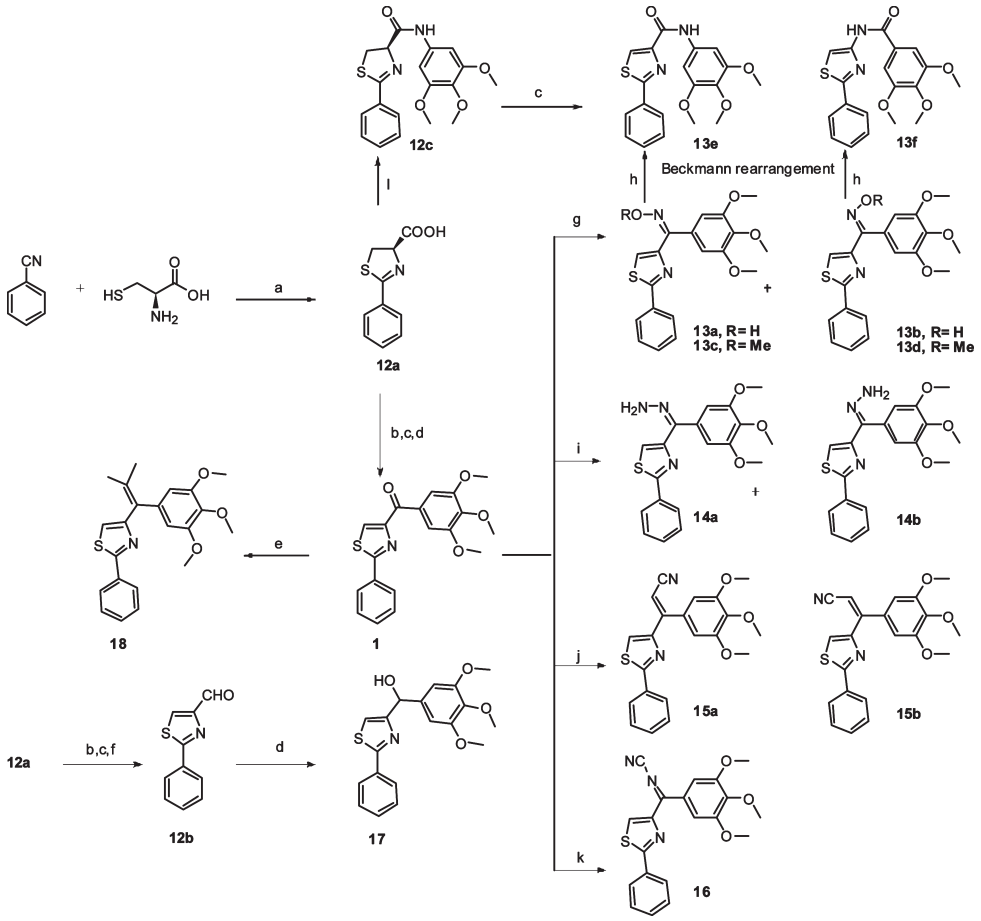
Scheme 3[a]



[a] (a) MeOH/pH = 6.4 phosphate buffer, RT; (b) EDCI, HOBt, NMM, $HNCH_3OCH_3$; (c) $CBrCl_3$, DBU, CH_2Cl_2; (d) 3,4,5-trimethoxyphenylmagnesium bromide, THF; (e) isopropyl triphenylphosphonium iodide, *n*-BuLi, THF; (f) LAH, THF; (g) for **13a** and **13b**, $NH_2OH \cdot HCl$, C_2H_5OH, H_2O, NaOH; for **13e** and **13f**, $NH_2OMe \cdot HCl$, pyridine; (h) TsCl, NaH, basic Al_2O_3; (i) $NH_2NH_2 \cdot xH_2O$, CH_2Cl_2, C_2H_5OH; (j) diethyl cyanomethylphosphonate, *n*-BuLi, THF; (k) bis-trimethylsilylcarbodiimide, $TiCl_4$, CH_2Cl_2; (l) EDCI, HOBt, Et_3N, 3,4,5-trimethoxyaniline, CH_2Cl_2.

colchicine binding site.[12] The structure−activity-relationship (SAR) studies in the "A" and "C" rings were discussed based on synthesized analogues. Whereas, alternatives to the thiazole "B" ring and carbonyl linker were not investigated. In this article, we synthesized and evaluated the biological properties of "B" ring and carbonyl linker modified derivatives to extend the SAR studies beyond just the SMART template and found some more potent compounds. "A" ring modifications and introducing a NH linkage between "A" and "B" rings produced potent water-soluble compounds including the phenyl amino thiazole (PAT) compounds **45a**−**c**. We demonstrated that these compounds can effectively inhibit cancer cell growth in vitro by interfering with tubulin polymerization. In experiments described herein, we have examined the antiproliferative effects of these novel compounds on a drug-sensitive ovarian cancer cell line (OVCAR-8) and its P-gp-overexpressing drug-resistant counterpart (NCI/ADR-RES). Tested compounds did not demonstrate susceptibility to P-gp mediated drug resistance. We also compared the oral bioavailability of the PAT compounds in rats. Compounds **45a** and **45c** showed a significant improvement in bioavailability compared with SMART compound **1**. Thus, the new PAT compounds and other molecules reported herein represent new families of compounds that may be very useful in the treatment of cancer with improved PK properties.

RESULTS AND DISCUSSION

Chemistry. Design of novel chemotypes of SMART agents that target tubulin polymerization were investigated mainly by preparing three series of modifications based upon compound **1**. The first series of derivatives (**5**, **6**, **9a**−**9h**) were characterized by the replacement of the thiazole in SMART molecules with a panel of different B rings as illustrated in Schemes 1−2. L-Sertine methyl ester hydrochloride was obtained from L-serine stirred in acetyl chloride/MeOH solution. Condensation of the methyl ester with ethyl benzimidate hydrochloride led to oxazoline methyl ester **2** in excellent yield (Scheme 1).[13] Hydrolyzation of the methyl ester gave carboxylic acid **3** that was in turn coupled to *N*,*O*-dimethylhydroxylamine to provide Weinreb amide **4**. Compound **4** was reacted with appropriate Grignard reagents in anhydrous THF to give the oxazoline **5**. Oxidation of **5** with $BrCCl_3$/DBU gave the oxazole product **6**.[14] Other B ring variants **9a**−**9f** (except **9e**) were obtained from different acids **7a**−**7f** with a similar method as described above (Scheme 2). Pure

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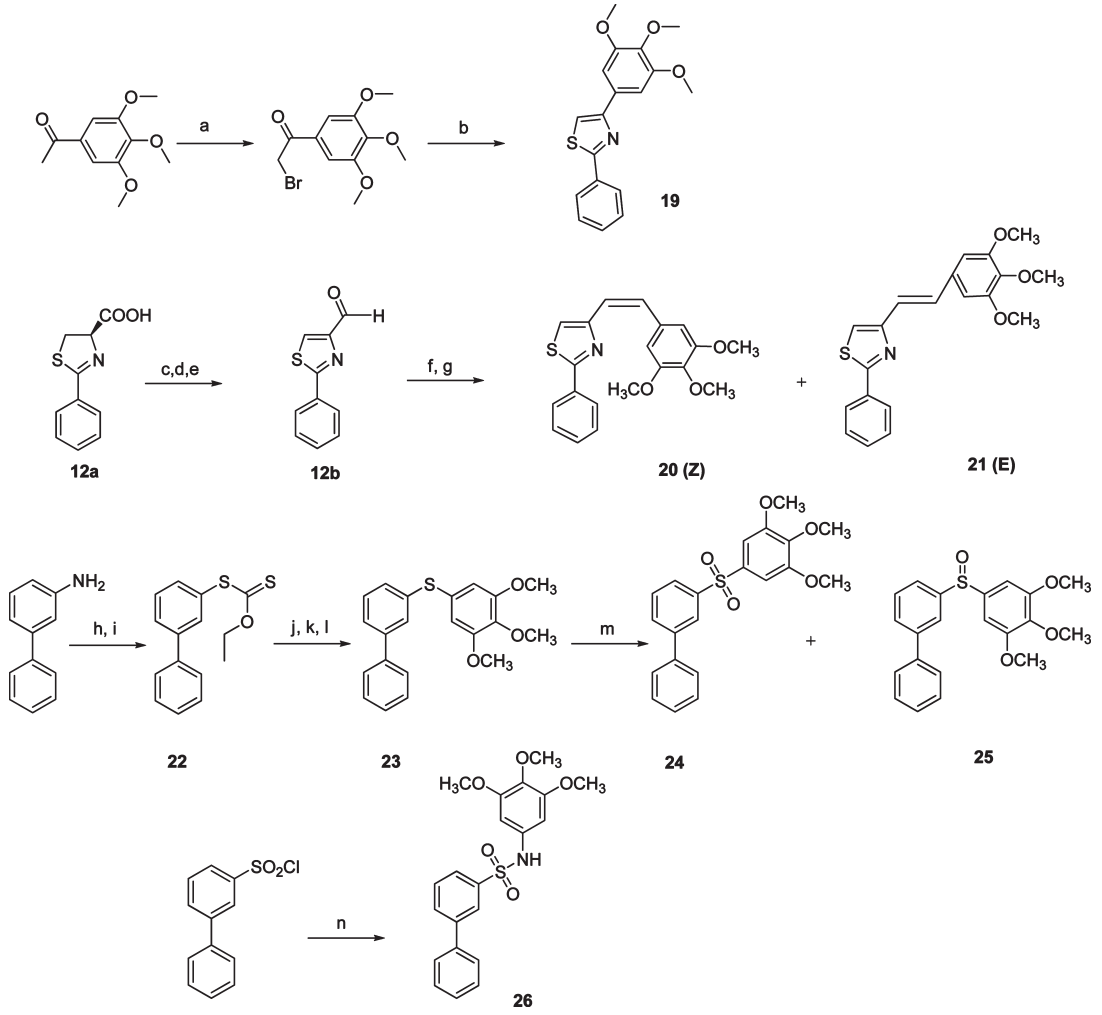
Scheme 4[a]



[a] (a) Bromine, EtOH; (b) benzothioamide, EtOH, reflux; (c) EDCI, HOBt, NMM, $HNCH_3OCH_3$, CH_2Cl_2; (d) $CBrCl_3$, DBU, CH_2Cl_2; (e) LAH, THF; (f) 5-(bromomethyl)-1,2,3-trimethoxybenzene, Ph_3P, THF; (g) *n*-BuLi, THF; (h) (1) HCl, H_2O; (2) $NaNO_2$, H_2O, 0 °C; (i) ethyl potassium xanthate; (j) KOH/EtOH; (k) H_2O, HCl; (l) 5-iodo-1,2,3-trimethoxybenzene, CuI, *t*-BuONa; (m) 2 equiv or 1 equiv *m*-CPBA, CH_2Cl_2; (n) 3,4,5-trimethoxyaniline, NEt_3, DMF.

compound **9e** with thiophene in B ring position can not be separated from the mixture of **9e** and a Grignard reagent coupling byproduct 3,4,5,3′,4′,5′-hexamethoxybiphenyl using chromatography. So we used an alternative method to prepare **9e**: Weinreb amide **8e** was converted into an aldehyde and then reacted with 3,4,5-trimethoxyphenylmagnesium bromide to afford the alcohol **10e**, which can be easily separated from 3,4,5,3′,4′,5′-hexamethoxybiphenyl after flash column purification. Oxidation with pyridinium dichromate (PDC) or DMSO did not afford **9e** from secondary alcohol **10e** with good yields, but using Dess−Martin periodinane reagent as oxidant successfully formed the desired ketone compound **9e** with 81% yield.[15] **9g** and **9h** were prepared from alcohol **10g**−**10h** using a similar method. Compound **9i** was obtained via a coupling reaction from piperidine **11** and 3,4,5-trimethoxybenzoic acid.

In the second series of novel templates, structure modifications focused on alternatives to the carbonyl group to avoid potential metabolic problems caused by ketone reduction (Scheme 3 and Scheme 4).[16] SMART compound **1** was synthesized from 2-phenyl-4,5-dihydro-thiazole-4-carboxylic acid **12a** through three steps described previously.[17] **1** was converted to oxime isomers (**13a**−**d**) upon reaction with hydroxylamines, NH_2OH or NH_2OCH_3. Assignments were made on the basis of chemical and spectral data as described infra. An improved Beckmann rearrangement[18] readily produced the rearranged amides **13e** and **13f** from the two geometric stereoisomers **13a** and **13b** via their reaction with tosyl chloride and subsequent basic aluminum oxide column. Hydrazide derivatives **14a** and **14b** were prepared by mixing **1** with hydrazine hydrate in ethanol/CH_2Cl_2 and refluxing for 24 h. Acrylonitriles **15a**−**15b** were obtained from Wittig reaction of **1** with diethyl cyanomethylphosphonate.[19] Cyanoimine **16** was prepared using the procedure described by Cuccia.[20] The carbonyl group in compound **1** was also reduced to a secondary alcohol (**17**) or converted to an alkene (**18**) as illustrated. We also tried to remove the carbonyl group between B and C rings in compound **1** and thus obtained compound **19** in Scheme 4. Introducing *cis*- and *trans*- double bonds into the carbonyl position formed compounds **20** and **21**, which were synthesized from a Wittig reaction with 2-phenylthiazole-4-carbaldehyde. We also

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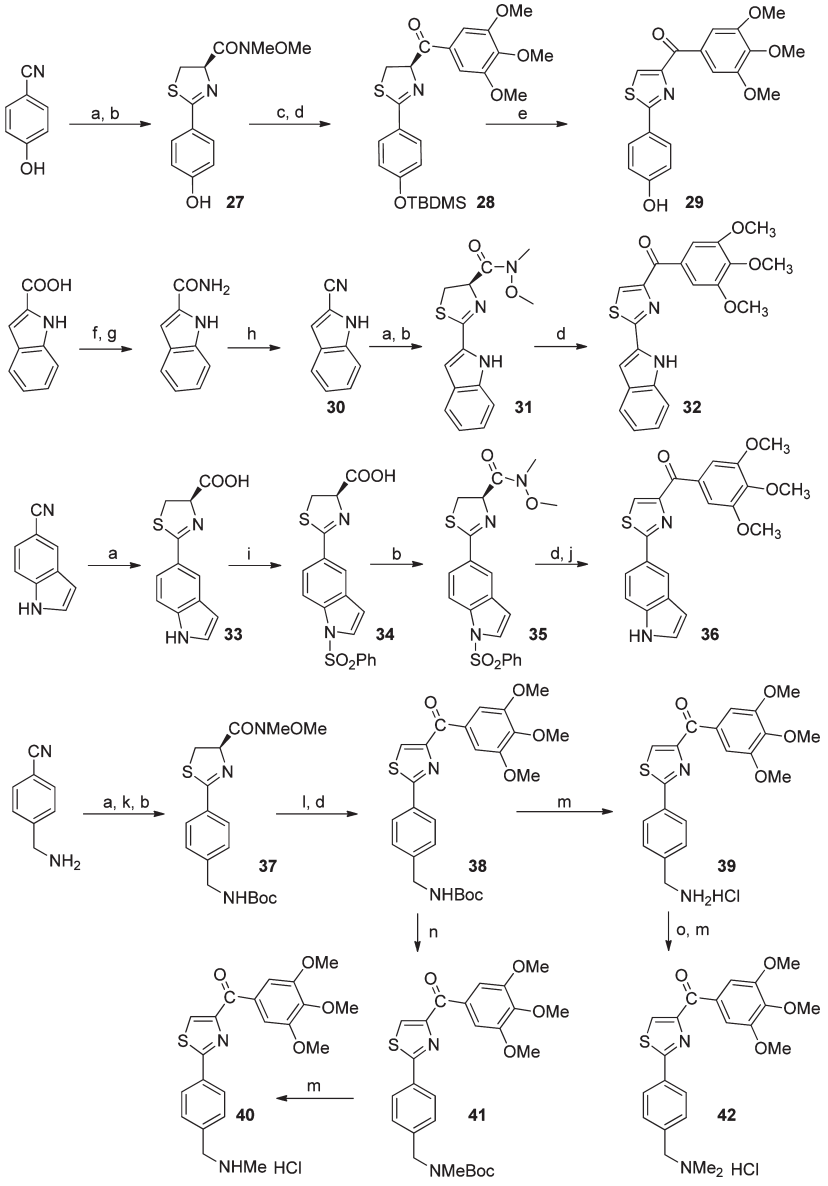
Scheme 5[a]



[a] (a) L-Cysteine, EtOH, 65 °C; (b) EDCI, HOBt, NMM, $HNCH_3OCH_3$, CH_2Cl_2; (c) TBDMSCl, imidazole, THF; (d) 3,4,5-trimethoxyphenylbromide, BuLi, THF; (e) TBAF, THF; (f) $SOCl_2$, Et_2O; (g) NH_3, MeOH; (h) $POCl_3$; (i) $PhSO_2Cl$, Bu_4NHSO_4, toluene, 50% NaOH; (j) 1N NaOH, EtOH, reflux; (k) Boc_2O, 1N NaOH, 1,4-dioxane; (l) $CBrCl_3$, DBU, CH_2Cl_2; (m) 2N HCl in 1,4-dioxane; (n) NaH, DMF, MeI; (o) HCHO, $NaBH_3CN$, Et_3N.

prepared sulfide compound **23**, sulfone **24** and sulfoxide **25** using 3-aminobiphenyl as starting material through an initial Sandmeyer reaction to yield carbonodithioate **22**, followed by CuI catalyzed coupling reaction and *m*-CPBA oxidation.[21] Sulfonamide linked compound **26** was prepared from reaction of 3-biphenylsulfonyl chloride with 3,4,5-trimethoxyaniline in the presence of NEt_3 in DMF.

A third aim was to improve aqueous solubility and oral bioavailability of these colchicine site targeted agents. As illustrated in Scheme 5, we introduced hydroxyl and aminomethyl at the *para*-position of the phenyl A-ring, as well as replacing phenyl with 5-indolyl and 2-indolyl rings. Weinreb amides **27**, **31**, **35**, and **37** were prepared by the procedure described before using aryl nitriles as starting materials.[12] 2-Cyano-indole **30** was prepared with reported method.[22] Protections of hydroxyl (TBDMSCl), indolyl ($PhSO_2Cl$), and amino (Boc_2O) groups were used in preparations. Deprotection of TBDMS and oxidation from thiazoline (**28**) to thiazole (**29**) were finished in one-step using TBAF/THF solution. We reported this thiazoline–thiazole oxidation can happen spontaneously in the reaction of thiazoline Weinreb amide and Grignard reagent.[12] We also observed the same phenomena during preparing indole compounds **32** and **36**. Compound **32** was separated as a pure thiazole compound after reaction with 3,4,5-trimethoxphenyllithium and needed no further oxidation. Compound **36** was obtained after removing phenylsulfonyl protecting groups in hot NaOH ethanol solution. *para*-OH and NH_2 on the A ring of **29** and **39** were obtained from similar Grignard reactions from

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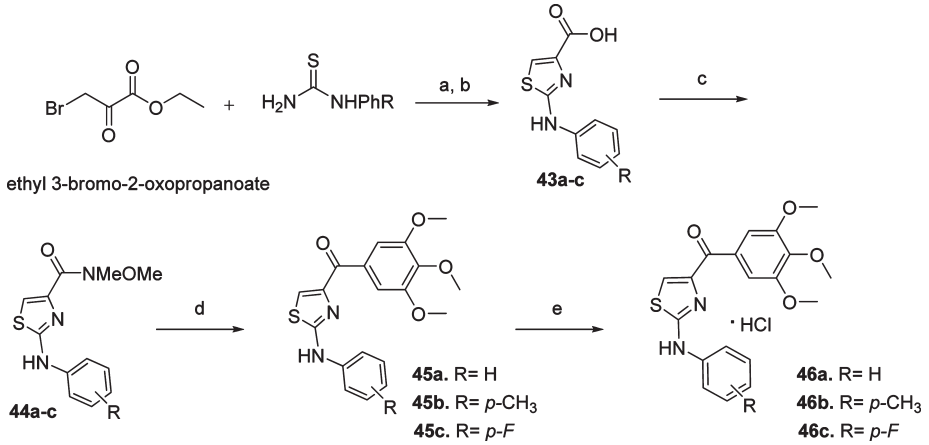
Scheme 6[a]



[a] (a) EtOH, 65 °C; (b) NaOH, C_2H_5OH, refluxing; (c) EDCI, HOBt, NMM, $HNCH_3OCH_3$, CH_2Cl_2; (d) 3,4,5-trimethoxyphenylbromide, BuLi, THF; (e) 2 N HCl in 1, 4-dioxane.

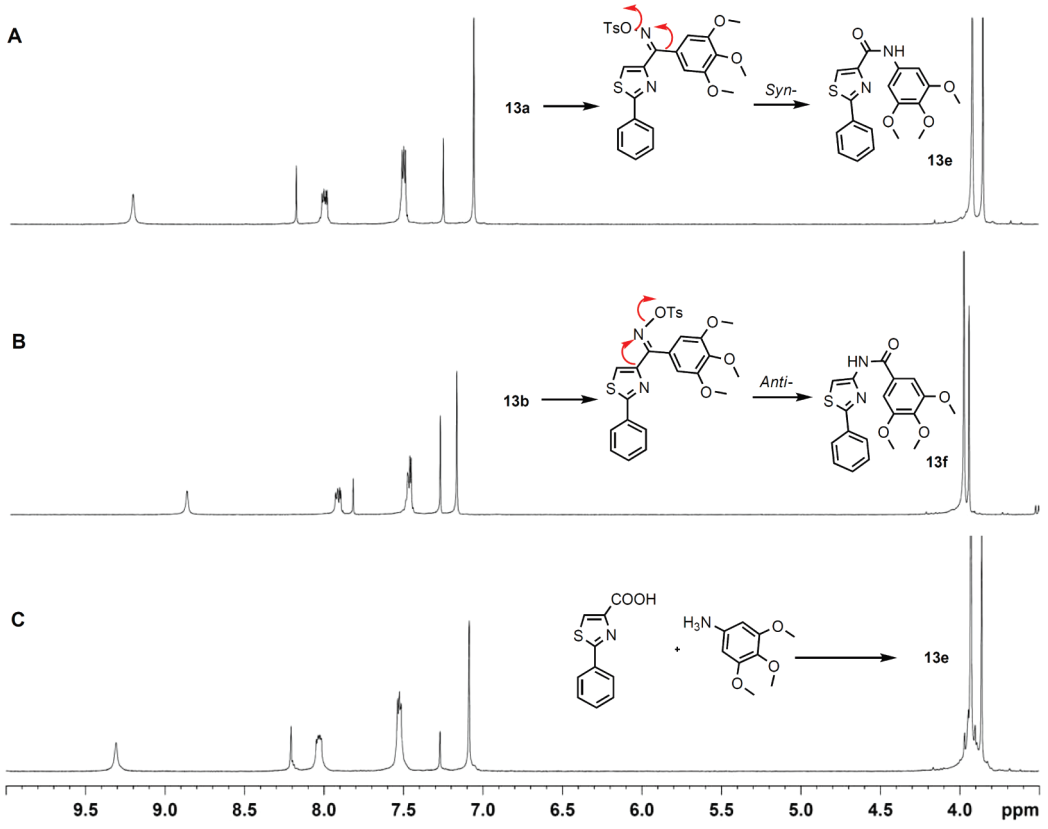


Figure 2. ^{1}H NMR of Beckmann rearrangement product from **13a** isomer (A) is the same with previous prepared 4-carbonyl amide **13e** (C) while rearrangement product from isomer **13b** showed reversed 4-amino amide NMR signals (B).

Weinreb amides **27** and **37**. Compound **38** was further converted to HCl salt of monomethyl amine **40** via NaH/MeI conditions, and compound **39** was converted to HCl salt of dimethylamine **42** under HCHO/$NaBH_3CN$ conditions. To improve bioavailability, we introduced an NH linker between A phenyl and B thiazole rings. We synthesized this new series of compounds as shown in Scheme 6. Reaction of 3-bromo-2-oxopropanoic acid ethyl ester and arylthiourea in ethanol under 65 °C produced 2-(arylamino)-thiazole-4-carboxylic acids **43a**−**c** with high yields. Then these acids were converted to Weinreb amides **44a**−**c**, followed by reactions with 3,4,5-trimethoxphenyllithium yielded aniline linked free bases **45a**−**c**, which can be converted into HCl salts **46a**−**c**.

Structural Identification of *syn*-/*anti*- Isomers. Oximes **13a**−**13b**, hydrazides **14a**−**14b**, and acrylonitriles **15a**−**15b** were obtained from flash column as separated isomer pairs.

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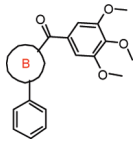
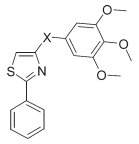
Table 1. SAR of Alternate B ring Compounds



	B ring	$IC_{50} \pm SEM$ (μM)					
		B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
1	2,4-thiazole	0.055 ± 0.005	0.028 ± 0.005	0.071 ± 0.004	0.021 ± 0.001	0.028 ± 0.004	0.043 ± 0.005
5	2,4-oxazoline	6.5 ± 0.8	0.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
6	2,4-oxazole	0.6 ± 0.2	0.3 ± 0.1	0.292 ± 0.01	0.292 ± 0.02	0.331 ± 0.06	0.343 ± 0.04
9a	1,3-phenyl	0.5 ± 0.2	0.087 ± 0.015	0.171 ± 0.01	0.124 ± 0.02	0.052 ± 0.01	0.080 ± 0.01
9b	4,6-pyrimidine	>30	>30	6.9 ± 5.2	8.3 ± 4.7	7.0 ± 2.9	3.7 ± 0.2
9c	2,6-pyridine	0.039 ± 0.012	0.030 ± 0.014	0.033 ± 0.003	0.032 ± 0.002	0.027 ± 0.002	0.025 ± 0.001
9d	2,5-furan	0.151 ± 0.024	0.027 ± 0.008	0.035 ± 0.004	0.021 ± 0.002	0.023 ± 0.001	0.020 ± 0.001
9e	2,4-thiophene	0.072 ± 0.015	0.015 ± 0.006	0.026 ± 0.005	0.012 ± 0.001	0.017 ± 0.001	0.015 ± 0.001
9f	3,5-pyrazol	0.245 ± 0.032	0.100 ± 0.018	0.145 ± 0.01	0.101 ± 0.02	0.101 ± 0.01	0.084 ± 0.01
9g	2,5-thiazole	12.5 ± 5.2	13.6 ± 3.8	>10	>10	>10	>10
9h	3,5-isoxazole	>30	>30	>10	>10	>10	>10
9i	1,4-piperidine	>30	>30	>20	>20	>20	>20

Table 2. SAR of Alternative to the Carbonyl Linker



	X linker	$IC_{50} \pm SEM$ (μM)						
		B16-F1	A375	WM-164	DU 145	PC-3	LNCaP	PPC-1
1	C═O	0.055 ± 0.005	0.028 ± 0.005	0.064 ± 0.004	0.071 ± 0.004	0.021 ± 0.001	0.028 ± 0.004	0.043 ± 0.005
13a	*syn*-C═N-OH	0.3 ± 0.1	0.2 ± 0.1	ND[a]	0.103 ± 0.04	0.120 ± 0.05	0.169 ± 0.06	0.144 ± 0.01
13b	*anti*-C═N-OH	11.4 ± 2.1	7.8 ± 1.2	ND	>10	>10	>10	>10
13c	*syn*-C═N-OMe	3.8 ± 1.6	2.9 ± 1.2	3.4 ± 1.8	>10	>10	>10	>10
13d	*anti*-C═N-OMe	>10	>10	>10	>10	>10	>10	>10
13e	CONH	>30	>30	ND	>10	>10	>10	>10
13f	NHCO	>30	>30	ND	>10	>10	>10	>10
14a	*syn*-C═N-NH2	2.0 ± 0.8	0.9 ± 0.3	ND	1.210	1.120	1.800	0.872
14b	*anti*-C═N-NH_2	1.8 ± 0.7	0.6 ± 0.2	ND	1.210	1.040	1.300	0.966
15a	*syn*-C═C-CN	5.4 ± 2.1	4.6 ± 1.5	4.9 ± 1.3	2.28	0.89 ± 0.34	0.58 ± 0.12	0.90 ± 0.1
15b	*anti*-C═C-CN	1.2 ± 0.3	1.2 ± 0.4	1.0 ± 0.2	~10	~10	1.99	~10
16	C═N-CN	0.060 ± 0.021	0.028 ± 0.012	0.027 ± 0.013	0.042 ± 0.002	0.027 ± 0.001	0.023 ± 0.002	0.020 ± 0.001
17	CHOH	>30	>30	ND	>10	>10	>10	>10
18	C═CMe2	3.8 ± 1.3	1.9 ± 0.8	3.7 ± 1.2	2.65	2.47	1.39 ± 0.39	2.04
19	none	>10	>10	>10	>10	>10	>10	>10
20	*cis*-C═C	11.0 ± 2.8	46.5 ± 23.3	10.6 ± 5.8	>10	>10	>10	>10
21	*trans*-C═C	32.8 ± 13	>100	30.8 ± 12	>10	>10	>10	>10
23	S	2.4 ± 0.9	1.6 ± 0.4	2.0 ± 1.2	>10	>10	2.3 ± 0.2	2.3 ± 0.1
24	SO_2	>10	>10	>10	>10	>10	>10	>10
25	SO	>10	>10	>10	>10	>10	>10	>10
26	$SONH_2$	>10	>10	>10	>10	>10	>10	>10

[a] ND = not determined.

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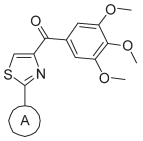
Table 3. Antiproliferative Activity of Modified Compounds with Improved Aqueous Solubility



		$IC_{50} \pm SEM$ (μM)					
	apart	B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
28	4-OTBDMSPh	0.5 ± 0.2	0.7 ± 0.3	0.434 ± 0.030	0.183 ± 0.024	0.549	0.246 ± 0.008
29	4-OHPh	0.11 ± 0.02	0.10 ± 0.01	0.116 ± 0.014	0.087 ± 0.005	0.103 ± 0.009	0.076 ± 0.002
32	2-indolyl	0.043 ± 0.021	0.019 ± 0.009	0.032 ± 0.001	0.024 ± 0.004	0.028 ± 0.003	0.028 ± 0.002
36	5-indolyl	0.025 ± 0.013	0.008 ± 0.001	0.013 ± 0.001	0.007 ± 0.001	0.010 ± 0.001	0.008 ± 0.001
38	4-BocNHCH$_2$Ph	2.9 ± 0.4	7.9 ± 0.5	4.3 ± 3.7	3.1 ± 1.7	2.6 ± 0.9	2.7 ± 1.5
39	4-NH$_2$CH$_2$Ph	0.038 ± 0.011	0.041 ± 0.013	0.025 ± 0.001	0.080 ± 0.007	0.013 ± 0.001	0.034 ± 0.001
40	4-NHMeCH$_2$Ph	>10	>10	~10	>10	1.14 ± 0.08	~10
42	4-NMe$_2$CH$_2$Ph	>10	>10	>10	>10	1.025 ± 0.2	>10

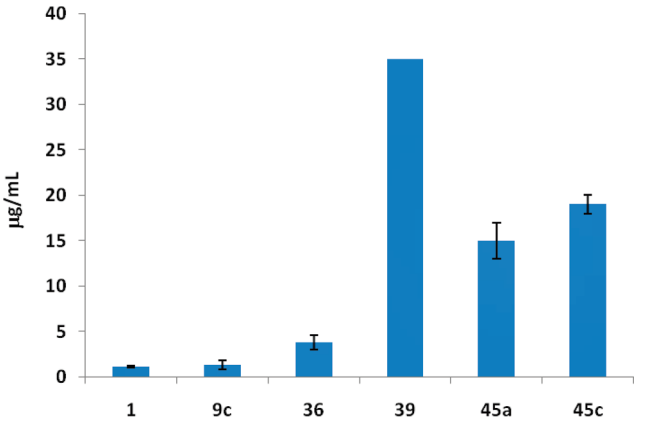


Figure 3. Aqueous solubility of novel anti-tubulin compounds. Compounds **45a** and **45c** showed improved solubility compared with compound **1**. Compound **39** is soluble at 35 μg/mL concentration.

Their configurations were identified using a chemistry method, NMR NOE spectrum and quantum chemical shift calculations. Improved Beckmann rearrangement was used to distinguish oxime isomers **13a** and **13b** as the group *anti-* position to the departing OTs that migrates to nitrogen (Figure 2). The *syn-* isomer **13a** rearranged by a 1,2- shift of 3,4,5-trimethoxyphenyl (TMP) to yield amide **13e**, whereas the *anti-*isomer **13b** underwent a 1, 2-thiazolyl shift to yield **13f**. An alternative coupling method was also used to prepare compound **13e** via intermediate **12c** (see Scheme 3),[12] which was characterized and compared with two rearrangement products **13e** and **13f**. The NMR spectra showed that alternatively prepared **13e** was identical with Beckmann rearrangement product of *syn-* isomer **13a**. Quantum chemical calculations based on ^{1}H NMR also corroborated the conformations of these two isomers.

2D NOESY NMR and 1D NOE NMR were used to identify *syn/anti-* isomers of hydrazides **14a**−**14b**, and acrylonitriles **15a**−**15b**, respectively. The ^{1}H NMR, 2D NOESY, and 1D NOE NMR spectra of **14a**−**14b**, **15a**−**15b** are shown in Supporting Information . From chemical structures, we can see the distance between amino in *syn-* isomer (**14a**) hydrazine is far away from C ring protons while in *anti-* isomer (**14b**) is short, the 2D NOESY spectrum of compound **14b** showed strong NOE correlations between NH_2 and C ring protons and demonstrated **14b** is *anti-* confirmation. Compound **14a** which has a *syn-* conformation did not show any NOE on 2D NOESY. Acrylonitriles **15a** and **15b** were identified with the similar 1D NOE spectrum. There are no NOE correlations observed among protons from B ring (H_b)/C ring (H_c) and acrylonitrile linker (H_a), thus compound **15a** is an *anti-* isomer. Compound **15b** showed NOE between H_a and H_c protons, which corresponding to a *syn-* confirmation (Supportin Information).

Biological Evaluation: In Vitro Cell Growth Inhibtions. All of the reported compounds were first evaluated for cytotoxicity in a mouse melanoma cell line B16-F1, human melanoma cell lines (A375 and WM-164), and prostate cancer cell lines (DU145, PC-3, LNCaP, and PPC-1). Compounds **1** (Tables 1, 2, and 4) and **47** (E7010, Abbott Laboratories/Eisai Co Ltd., Table 4), which has entered phase II clinical studies in treating patients with different cancers, were included in the assays as examples of colchicine-site binding agents. IC_{50} values for cell growth inhibition are shown in Tables 1−4.

SAR of Alternative "B" Ring Molecules. The first series was targeted to alternatives to the thiazole "B" ring. Accordingly, a series of heterocyclic "B" rings was examined. As shown in Table 1, the successful replacements of the thiazole were pyridine **9c**, furan **9d**, and thiophene **9e**. The IC_{50}s (12−35 nM against prostate cancer cells) are close to the thiazole compound **1**. Introducing oxazoline (**5**), oxazole (**6**), phenyl (**9a**), and pyrazole (**9f**) maintained activity in the hundreds of nanomolar range. But introduction of pyrimidine (**9b**, IC_{50} 3.7−8.3 μM), a reversed 2,5-thiazole or 3,5-isoxazole (**9g** and **9h**, IC_{50} > 10 μM) caused obvious losses of potency. Modification of "B" ring to the saturated ring of piperidine (**9i**) also totally abolished activity (**9c**, IC_{50} >20 μM).

SAR of Alternative Linkers between "B" and "C" Rings. In vitro hepatic metabolic stability studies revealed that the carbonyl linker between "B" and "C" rings in SMART compounds caused short half-lives (5−17 min) primarily due to carbonyl reduction.[16] For the sake of blocking this carbonyl reduction to the inactive hydroxyl linker compound **17**, we modified the carbonyl linker in the second series of compounds (Table 2).

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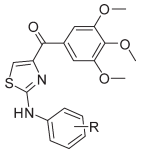
Table 4. Antiproliferative Activity of Phenyl Amino Thiazole Compounds



		$IC_{50} \pm SEM$ (μM)					
	R	B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
45a	H	0.065 ± 0.012	0.045 ± 0.008	0.070 ± 0.004	0.057 ± 0.003	0.051 ± 0.001	0.054 ± 0.001
46b	4-CH_3	ND[a]	ND	0.035 ± 0.001	0.038 ± 0.002	0.035 ± 0.001	0.036 ± 0.001
45c	4-F	ND	ND	0.063 ± 0.001	0.043 ± 0.001	0.041 ± 0.001	0.037 ± 0.001
1		0.055 ± 0.005	0.028 ± 0.005	0.071 ± 0.004	0.021 ± 0.001	0.028 ± 0.004	0.043 ± 0.005
47		2.127 ± 0.351	1.111 ± 0.108	0.839 ± 0.719	0.786 ± 0.089	0.658 ± 0.117	0.701 ± 0.307

[a] ND = not determined.

Table 5. Pharmacokinetic Parameters for Compounds Tested in Vivo

	1		**39**		**46a**		**46c**	
route	IV	PO	IV	PO	IV	PO	IV	PO
N[a]	4	3	3	3	3	3	3	3
dose (mg/kg)	2.5	10	2.5	4	5	10	5	10
CL[b] (mL/min/kg)	7.7 ± 1.0		22 ± 13		17 ± 3		13 ± 2	
Vss[c] (L/kg)	4.9 ± 1.9		0.33 ± 0.25		1.4 ± 0.2		1.4 ± 0.2	
AUC[d] (min·μg/mL)	279 ± 53	37 ± 20	139 ± 77	0.4	296 ± 46	65 ± 20	381 ± 65	160 ± 13
C_{max}[e] (ng/mL)	3816 ± 509	212 ± 65	3794 ± 1580	3.2 ± 1.6	4198 ± 438	814 ± 255	3349 ± 686	1262 ± 362
F[f] (%)	3.3		0.2		11		21	

[a] Numbers of rats. [b] Systemic clearance. [c] Volume of distribution following intravenous dosing. [d] Area under the curve following intravenous dosing, integrated drug concentration with respect to time and integrated drug concentration with respect to time following oral dosing. [e] Maximum plasma concentration following intravenous dosing. [f] Percent oral bioavailability.

The carbonyl linker was replaced with double bonds (**18**, **20**, **21**), amides (**13e**, **13f**), oximes (**13a**−**13d**), hydrazide (**14a**, **14b**), acrylonitriles (**15a**, **15b**), cyanoimine (**16**), sulfonyl amide (**26**), sulfur ether (**23**), and sulfonyl and sulfinyl compounds (**24**, **25**). A direct link compound **19** without any linker between "B" and "C" rings was also prepared. Among these linker modifications, only cyanoimine linkage (**16**) showed promising activity (20−60 nM) compared with carbonyl compound **1**, but an in vitro metabolism study showed that the half-life of **16** in human liver microsome was less than 5 min (data not shown). This result suggested that although we blocked the ketone reduction, it might introduce another new metabolic liability in compound **16**. We separated the isomer pairs of compounds containing double bonds, oximes, and hydrazides. Compound **20** was designed to mimic the structure of CA-4, which contain a *cis*-C═C between two aryl rings, unfortunately **20**(*Z*) and other isomer **21**(*E*) lost activity after replacing the C═O linker. One interesting phenomenon is *syn*-isomer of **13a** (0.1−0.3 μM) showed 10-fold more activity than its *anti* isomer **13b** (>10 μM). The half-life of **13a** in human liver microsomes is extended to 35 min, while the half-lives of compounds **14a**−**b** were prolonged to 55 min, however, activities were much lower (∼1 μM) than compound **1**.

Introducing Polar and Ionizable Groups into the SMART Agents. One major limitation of the SMART agents was low aqueous solubility. We used surfactant formulation strategies like Captex200/Tween80 (1/4, IP) to study in vivo SMART behavior and obtained favorable results.[23] But these surfactants are biologically active and are responsible for many side effects.[10] In addition, it was thought[24] that low aqueous solubility of **1** resulted in low oral bioavailability (3.3%, in Table 5). In our third series of compounds, we successfully increased aqueous solubility without impacting the potency by introducing polar groups like hydroxyl and indolyls. In addition, we also designed ionizable groups like amino and alkylamino groups into "A" ring *para*-position. As shown in Scheme 5 and Table 3, introducing indolyl groups to the "A" ring, especially 5-indolyl (**36**, 7−25 nM), increased the potency compared with the 4-OH compound **29** (76−116 nM). Aminomethyl at the "A" ring *para* position also maintained potency (**39**, 13−80 nM), but *p*-NHMe (**40**) or *p*-NMe_2 (**42**) abrogated activity. As shown in Figure 3, analytical measurement to estimate aqueous solubility showed that indolyl compound **36** increased solubility in PBS from 1.1 μg/mL (compound **1**) to 3.8 μg/mL. Aminomethyl compound **39** was converted to the HCl salt, which increased solubility over 35-fold (>35 μg/mL). Although compound **39** showed satisfactory aqueous solubility, the pharmacokinetic studies showed this compound still had very poor bioavailability (F = 0.2%, Table 5).

Modifications to Improve Oral Bioavailability. Many established tubulin targeting anticancer drugs like taxanes and

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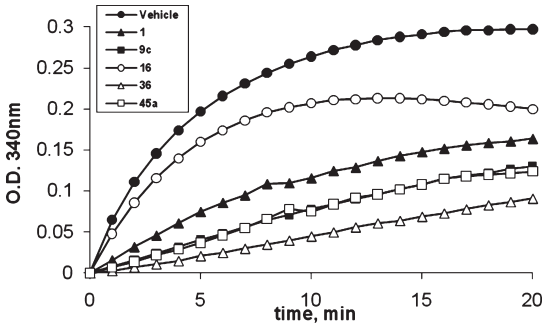

Figure 4. Compounds inhibit tubulin polymerization in vitro.

Table 6. Antiproliferative Activity of Selected Compounds Against P-gp Overexpressed MDR Cell Lines

	IC_{50} (nM)		
compd	OVCAR-8	NCI/ADR-RES	resistance factor
9c	33 ± 3	13 ± 0.8	0.4
16	34 ± 2	14 ± 1	0.4
36	10 ± 3	4 ± 2	0.4
39	26 ± 2	11 ± 2	0.4
45a	46 ± 6	27	0.6
45b	28	21	0.8
45c	44 ± 3	25 ± 6	0.6
1	35 ± 2	13 ± 1	0.4
paclitaxel[23]	4.7 ± 0.1	6263 ± 634	1333
vinblastine	3.9 ± 0.1	582 ± 57	149
colchicine	17 ± 1	1113 ± 79	65

vinblastine require intravenous administration because of low oral bioavailability. Oral bioavailability is a complex parameter involving many chemical and physiological processes, such as solubility, permeability, and metabolic stability. Our initial thought was that the low bioavailability of **1** might be caused by poor solubility. However, the water-soluble compound **39** failed to improve oral bioavailability. We further improved the solubility of these tubulin inhibitors by inserting an amino linker between the "A" and "B" rings (phenyl amino thiazole, PAT) as in **45a**−**c** (Scheme 6), which is similar to the reported orally active colchicine binding anticancer agent **47** (Figure 1, clinical phase II trial).[3,25] IC_{50} values (Table 4) demonstrate that these compounds (**45a**, **46b**, and **45c**) had similar potency (35−65 nM) as **1** with increased solubility (15 and 19 μg/mL for **45a** and **45c**, respectively (Figure 3), and they are over 20-fold more active than **47**.

Rat pharmacokinetic studies were performed to study whether these new compounds exhibited improved bioavailability compared to compound **1** (Table 5). The data clearly showed that **46c** (HCl salt of **45c**) exhibited more than 4.3-fold increased exposure (AUC, 160 vs 37 min·μg/mL) by the oral route as compared to **1**, suggesting that improved aqueous solubility by the amino linker successfully improved oral bioavailability. In addition, the maximal concentration (C_{max}) of **46a** and **46c** by oral administration were 814 and 1262 ng/mL, respectively, while C_{max} of **1** was only 212 ng/mL. Overall, the bioavailability of **46a** and **46c** were increased from 3.3% of **1** to 11% and 21%, respectively (Table 5). Compound **46c** exhibited moderate clearance, moderate volume of distribution, and acceptable oral bioavailability. This data suggested that these new synthesized amino linked compounds have appropriate potency and PK profiles to be developed as a new class of orally bioavailable anticancer agents.

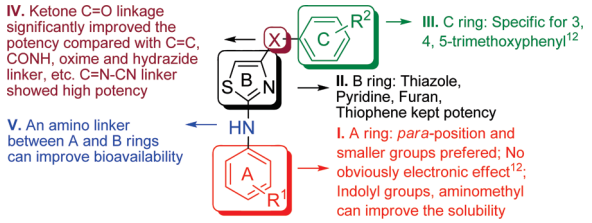

Figure 5. Structure−activity relationship of novel anti-tubulin compounds.

Compounds Inhibit in Vitro Tubulin Polymerization. We investigated the inhibition of tubulin polymerization of selected potent compounds **9c**, **16**, **36**, and **45a** from all three design strategies (alternative B-rings, novel linkers, and solubilizing moieties) and compared them with **1**. Bovine brain tubulin (>97% pure) was incubated with the individual compounds (5 μM) to test their effect on tubulin polymerization (Figure 4). After 20 min incubation, tubulin polymerization was inhibited 45% by **1**, as compared to vehicle. Compound **16** inhibited 33% of polymerization at 20 min with different inhibition patterns. Compounds **9c** and **45a** provided similar inhibitions of 56% and 58%, respectively. Compound **36**, which showed low average IC_{50} of 10.1 nM, inhibited 69% of tubulin polymerization. These data suggest that these compounds exhibit strong antitubulin polymerization activity that corresponds well with their antiproliferative potency.

Compounds Overcome P-Glycoprotein Mediated Multidrug Resistance. The P-glycoprotein (P-gp) system appears to be a primary physiological mechanism of multidrug resistance (MDR) which acts as an ATP-dependent drug efflux pump, actively removing a variety of structurally diverse cytotoxic compounds.[8,26] Enhanced efflux of these compounds reduces their intracellular accumulation and so reduces their cytotoxicity. Therefore, novel compounds which are not susceptible to drug resistance could be of high therapeutic and economic value. In addition to P-gp, clinically used antitubulin agents have other resistance mechanisms such as changes in microtubule dynamics[27] and mutations in β-tubulin which are known to limit sensitivity to the taxanes.[28] We tested our selected compounds against an ovarian cancer cell line OVCAR-8 (parent) and P-gp overexpressing NCI/ADR-RES cell line (Table 6). Notably, tested compounds demonstrated equipotent antiproliferative effects against OVCAR-8 and NCI/ADR-RES cell lines, suggesting that they are not P-gp substrates and that they function in a P-gp-independent manner. This feature is distinct from that of paclitaxel, vinblastine, and colchicine in NCI/ADR-RES cells which demonstrate 1333-, 149-, and 65-fold resistance.

CONCLUSION

A new series of tubulin polymerization inhibitors with acceptable oral bioavailability and equipotent activity in multidrug resistant tumor cell lines has been discovered. Medicinal chemistry efforts improved upon SMART compound **1**. Chemical modifications were include alternative "B" ring and alternative linkages between "B" and "C" rings with regard to in vitro cytotoxicity against cancer cells (Figure 5) based on biological

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evaluation against cancer cells in vitro. SAR studies revealed that optimal "B" rings include pyridine (**9c**), thiophene (**9e**), and furan (**9d**), which maintain excellent in vitro potency. Replacing carbonyl linker with cyanoimine (**16**) between "B" and "C" ring also increased the activity. Structure modifications to increase aqueous solubility and bioavailability were performed. Introducing an amino between "A" and "B" rings gave us PAT compounds **45a**−**c**, which showed similar in vitro antiproliferative potency against tested cancer cells as well as resistant cancer cell lines, furthermore, the solubility and in vivo bioavailability were improved greatly over those of **1**. Therefore, these new compounds represent a new family of antimitotic agents that may be very useful in the treatment of cancer.

EXPERIMENTAL SECTION

General. All reagents were purchased from Sigma-Aldrich Chemical Co., Fisher Scientific (Pittsburgh, PA), AK Scientific (Mountain View, CA), Oakwood Products (West Columbia, SC), etc. and were used without further purification. Moisture-sensitive reactions were carried under an argon atmosphere. **47** was prepared according methods reported by Yoshino et al.[29] Routine thin layer chromatography (TLC) was performed on aluminum backed Uniplates (Analtech, Newark, DE). Melting points were measured with Fisher-Johns melting point apparatus (uncorrected). NMR spectra were obtained on a Bruker AX 300 (Billerica, MA) spectrometer or Varian Inova-500 (Vernon Hills, Illinois) spectrometer. Chemical shifts are reported as parts per million (ppm) relative to TMS in $CDCl_3$. Mass spectral data was collected on a Bruker ESQUIRE electrospray/ion trap instrument in positive and negative ion modes. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Unless specified, all the tested compounds described in the article present >95% purity established through combustion analysis.

*(2R)-(2-Phenyl-4,5-dihydro-oxazol-4-yl)-(3,4,5-trimethoxy-phenyl)-methanone (**5**).* To a solution of *n*-BuLi (1.6 M, 0.713 mL) in 8 mL of THF was added a solution of 3,4,5-trimethoxybromobenzene (1.09 mmol) in 3 mL of THF under −78 °C. The mixture was allowed to stir for 2 h, and a solution of Weinreb amide **4** (1.14 mmol) in 3 mL of THF was charged. The temperature was allowed to increase at RT and stirred overnight. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **5** as a white solid (47.9%); mp 60−62 °C. ^{1}H NMR ($CDCl_3$) δ 7.97 −7.94 (m, 2 H), 7.62 (s, 2 H), 7.54−7.37 (m, 3 H), 5.61 (q, 1 H, J = 7.5 Hz, 9.9 Hz), 5.12 (t, 1 H, J = 7.5 Hz), 4.57 (q, 1 H, J = 7.8 Hz, 9.9 Hz), 3.96 (s, 6 H), 3.95 (s, 3 H). MS (ESI) m/z 364.1$(M + Na)^+$, 340.1 $(M - H)^-$. Anal. ($C_{19}H_{19}NO_4S$) C, H, N.

*(2R)-(2-Phenyl-oxazol-4-yl)-(3,4,5-trimethoxy-phenyl)-methanone (**6**).* A mixture of **5** (1.48 mmol), $CBrCl_3$ (2.59 mmol), and DBU (2.97 mmol) in CH_2Cl_2 (20 mL) was stirred overnight. The reaction mixture was absorbed on silica gel and purified by column chromatography to yield pure compound **6** as desired (61.6%); mp 138−139 °C. ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1 H), 8.14−8.12 (m, 2 H), 7.74 (s, 2 H), 7.52−7.49 (m, 3 H), 3.97 (s, 9 H). MS (ESI) m/z 362.1 $(M + Na)^+$. Anal. ($C_{19}H_{17}NO_5$) C, H, N.

*Biphenyl-3-yl(3,4,5-trimethoxyphenyl)methanone (**9a**).* To a solution of **8a** (0.174 g, 0.72 mmoL) in 5 mL of THF was added a THF solution of 3,4,5-trimethoxyphenylmagnesiumbromide (0.5 N, 1.08 mmol) at 0 °C. The mixture was allowed to stir for 30 min and quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **9a** as a white solid (43.8%); mp 87−89 °C. ^{1}H NMR ($CDCl_3$) δ 8.02 (t, 1 H), 7.84−7.74 (m, 2 H), 7.64−7.38 (m, 6 H), 7.11 (s, 2 H), 3.95 (s, 3 H), 3.88 (s, 6 H). MS (ESI) m/z 371.1 $(M + Na)^+$. Anal. ($C_{22}H_{20}O_4$) C, H, N.

*(6-Phenylpyrimidin-4-yl)(3,4,5-trimethoxyphenyl)methanone (**9b**).* To a solution of **8b** (0.243 g, 1 mmoL) in 5 mL of THF was added a THF solution of 3,4,5-trimethoxyphenylmagnesiumbromide (0.5 N, 5.6 mL, 1.4 mmol) at 0 °C. The mixture was allowed to stir for 30 min and quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **9b** (52.3%); mp 132−133 °C. ^{1}H NMR ($CDCl_3$) δ 9.40 (d, 1 H, J = 1.5 Hz), 8.29 (d, 1 H, J = 1.5 Hz), 8.22−8.18, 7.57−7.54 (m, 5 H), 7.46 (s, 2 H), 3.96 (s, 3 H), 3.91 (s, 6 H). MS (ESI) m/z 351.1 $(M + H)^+$. Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

*(6-Phenylpyridin-2-yl)(3,4,5-trimethoxyphenyl)methanone (**9c**).* To a solution of **8c** (0.210 g, 0.86 mmoL) in 5 mL of THF was added a THF solution of 3,4,5-trimethoxyphenylmagnesiumbromide (0.5 N, 3.5 mL, 1.73 mmol) at 0 °C. The mixture was allowed to stir for 30 min and quenched with water, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **9c** as white needle crystals (78%); mp 116−117 °C. ^{1}H NMR ($CDCl_3$) δ 8.10 (d, br, 2 H), 8.02−8.00 (m, 1 H), 7.97−7.96 (m, 2 H), 7.66 (s, 2 H), 7.49−7.43 (m, 3 H), 3.97 (s, 3 H), 3.89 (s, 6 H). MS (ESI) m/z 372.6 $(M + Na)^+$. Anal. ($C_{21}H_{19}NO_4$) C, H, N.

*(5-Phenylfuran-2-yl)(3,4,5-trimethoxyphenyl)methanone (**9d**).* To a solution of **8d** (0.231 g, 1 mmoL) in 5 mL of THF was added a THF solution of 3,4,5-trimethoxyphenylmagnesiumbromide (0.5 N, 4.0 mL, 2 mmol) at 0 °C. The mixture was allowed to stir for 30 min and quenched with water, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **9d** as white crystals (35.5%); mp 114−116 °C. ^{1}H NMR ($CDCl_3$) δ 7.85−7.82 (m, 1 H), 7.48−7.36 (m, 4 H), 7.35 (s, 2 H), 7.25 (d, 1 H, J = 4.0 Hz), 6.86 (d, 1 H, J = 4.2 Hz), 3.96 (s, 3 H), 3.95 (s, 6 H). MS (ESI) m/z 339.1 $(M + H)^+$. Anal. ($C_{20}H_{18}O_5$) C, H.

*(5-Phenylthiophen-3-yl)(3,4,5-trimethoxyphenyl)methanone (**9e**).* To a solution of **10e** (0.260 g, 0.73 mmoL) in 20 mL of anhydrous CH_2Cl_2 was added Dess−Martin reagent (0.465 g, 1.36 mmol). The mixture was allowed to stir for 30 min and quenched with satd $Na_2S_2O_3$ solution, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to give pure compound **9e** as light-yellow crystals (81.0%); mp 140−141 °C. ^{1}H NMR ($CDCl_3$) δ 7.97 (d, 1 H, J = 1.5 Hz), 7.82 (d, 1 H, J = 1.5 Hz), 7.59−7.57 (m, 2 H), 7.45−7.34 (m, 3 H), 7.19 (s, 2 H), 3.95 (s, 3 H), 3.93 (s, 6 H). MS (ESI) m/z 355.1 $(M + H)^+$. Anal. ($C_{20}H_{18}O_4S$) C, H.

*(3-Phenyl-1H-pyrazol-5-yl)(3,4,5-trimethoxyphenyl)methanone (**9f**).* Compound **9f** was prepared using the same method as used of compound **9c** from 3-phenyl-1*H*-pyrazole-5-carboxylic acid **7f** via **8f** as intermidiate. ^{1}H NMR (500M, $CDCl_3$) δ 10.97 (br, 1 H), 7.77 (s, br, 2 H), 7.48−7.38 (m, 5 H), 7.14 (s, br, 1 H), 3.96 (s, 3 H), 3.94 (s, 6 H). MS (ESI) m/z 361.1 $(M + Na)^+$, 337.0 $(M - H)^-$. Anal. ($C_{19}H_{18}N_2O_4$) C, H, N.

*(2-Phenylthiazol-5-yl)(3,4,5-trimethoxyphenyl)methanone (**9g**).* To a solution of **10g** (0.357 g, 1 mmoL) in 40 mL of anhydrous CH_2Cl_2 was added Dess−Martin reagent (0.848 g, 2 mmol). The mixture was allowed to stir for 30 min and quenched with satd $Na_2S_2O_3$ solution, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to give pure compound 9 g (80.1%); mp 147−148 °C. ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1 H), 8.04 (m, 2 H), 7.51 (m, 3 H), 7.18 (s, 2 H), 3.96 (s, 3 H), 3.93 (s, 6 H). MS (ESI) m/z 378.1 $(M + H)^+$. Anal. ($C_{19}H_{17}NO_4S$) C, H, N.

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*(5-Phenylisoxazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**9h**).* To a solution of **10h** (0.110 g, 0.73 mmoL) in 8 mL of anhydrous CH_2Cl_2 was added Dess−Martin reagent (0.274 g, 0.645 mmol). The mixture was allowed to stir for 30 min and quenched with satd $Na_2S_2O_3$ solution, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to give pure compound **9h** (70.1%); mp 143−144 °C. ^{1}H NMR ($CDCl_3$) δ 7.87−7.85 (m, 2 H), 7.72 (s, 2 H), 7.53−7.49 (m, 3 H), 7.05 (s, 1 H), 7.82 (d, 1 H, J = 1.5 Hz), 3.97 (s, 3 H), 3.96 (s, 6 H). MS (ESI) m/z 362.1 $(M + H)^+$. Anal. ($C_{19}H_{17}NO_5$) C, H, N.

*(4-Phenylpiperidin-1-yl)(3,4,5-trimethoxyphenyl)methanone (**9i**).* To a mixture of 4-phenylpiperidine **11** (5 mmol), EDCI (6 mmol), HOBt (5.5 mmol), and NMM (6 mmol) in CH_2Cl_2 (50 mL) was added 3,4,5-trimethoxybenzoic acid (5.3 mmol) and stirring continued at RT for overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, satd $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **9i**. (57.9%); mp 141−142 °C. ^{1}H NMR ($CDCl_3$) δ 7.35−7.21(m, 5 H), 6.66 (s, 2 H), 4.84 (br, 1 H), 3.95 (br, 1 H), 3.88 (s, 6 H), 3.86 (s, 3 H), 3.20−2.87 (br, 2 H), 2.85−2.74 (tt, 1 H, J = 3.6 Hz, J = 15.6 Hz) 1.92 (br, 2 H), 1.70 (br, 2 H). MS (ESI) m/z 378.1 $(M + Na)^+$. Anal. ($C_{21}H_{25}NO_4$) C, H, N.

*(Z)-(2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone Oxime (**13a**).* To a suspension of **1** (210 mg, 0.59 mmol) in 10 mL of ethanol was added an aqueous solution (2 mL) of hydroxylamine hydrochloride (127 mg, 1.83 mmol). Then 2 mL of 1N NaOH was added dropwise to the reaction mixture and the mixture was stirred at 55 °C for 3 h. After completion of the reaction, the residue was absorbed on silica gel and purified by column chromatography to give compounds **13a** (85 mg) and **13b** (50 mg); mp 150−152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.95 (s, 1 H), 8.35 (s, 1 H), 7.91−7.89 (m, 2 H), 7.50−7.44 (br, 3 H), 6.85 (s, 2 H), 3.73 (s, 6 H), 3.70 (s, 3 H). MS (ESI) m/z 393.1 $(M + Na)^+$; 368.9 $(M - H)^-$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

*(E)-(2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone oxime (**13b**).* Melting point: 176−177 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.49 (s, 1 H), 7.92−7.89 (m, 2 H), 7.64 (s, 1 H), 7.51−7.49 (m, 3 H), 7.34 (s, 1 H), 6.75 (s, 2 H), 3.75 (s, 6 H), 3.72 (s, 3 H). MS (ESI) m/z 393.1 $(M + Na)^+$; 368.9 $(M - H)^-$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

*(Z)-(2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone O-Methyl Oxime (**13c**).* To a suspension of **1** (110 mg, 0.59 mmol) in 10 mL of pyridine was added *O*-methylhydroxylamine hydrochloride (52 mg, 0.63 mmol) and the mixture was stirred at 60 °C for overnight. The reaction was quenched with 1 N HCl solution, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to give pure compounds **13c** (41 mg) and **13d** (33 mg); mp 116−117 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (s, 1 H), 7.96−7.94 (m, 2 H), 7.45−7.44 (m, 3 H), 6.94 (s, 2 H), 4.13 (s, 3 H), 3.91 (s, 6 H), 3.88 (s, 3 H). MS (ESI) m/z 407.2 $(M + Na)^+$. Anal. ($C_{20}H_{20}N_2O_4$) C, H, N.

*(E)-(2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone O-Methyl Oxime (**13d**).* Melting point: 91−92 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00−7.98 (m, 2 H), 7.44−7.43 (m, 3 H), 7.28 (s, 1 H), 6.70 (s, 2 H), 4.08 (s, 3 H), 3.91 (s, 6 H), 3.85 (s, 3 H). MS (ESI) m/z 407.0 $(M + Na)^+$. Anal. ($C_{20}H_{20}N_2O_4$) C, H, N.

*2-Phenyl-N-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (**13e**).* To a solution of **13a** (21 mg, 0.06 mmol) in 5 mL of CH_2Cl_2 was added *p*-toluenesulfonyl chloride (23 mg, 0.12 mmol) and NaH (5 mg, 60% i n light mineral oil). Then the reaction mixture was stirred for 20 min. After completion of the reaction, the residue was absorbed on silica gel and purified by Al_2O_3 column chromatography to give compound **13e** (15 mg); mp 157−158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.19 (s, 1 H), 8.02−7.99 (m, 2 H), 7.52−7.50 (m, 3 H), 7.07 (s, 2 H), 3.92 (s, 6 H), 3.85 (s, 3 H). MS (ESI) m/z 371.1 $(M + H)^+$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

*3,4,5-Trimethoxy-N-(2-phenylthiazol-4-yl)benzamide (**13f**).* To a solution of **13b** (26 mg, 0.07 mmol) in 5 mL of CH_2Cl_2 was added *p*-toluenesulfonyl chloride (27 mg, 0.14 mmol) and NaH (5 mg, 60% in light mineral oil). Then the reaction mixture was stirred for 20 min. After completion of the reaction, the residue was absorbed on silica gel and purified by Al_2O_3 column chromatography to give compound **13f** (15 mg); mp 154−156 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.94−7.91 (m, 2 H), 7.83 (s, 1 H), 7.48−7.46 (m, 3 H), 7.18 (s, 2 H), 3.97 (s, 6 H), 3.94 (s, 3 H). MS (ESI) m/z 393.1 $(M + Na)^+$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

*(Z)-4-(Hydrazono(3,4,5-trimethoxyphenyl)methyl)-2-phenylthiazole (**14a**).* To a mixture of **1** (230 mg, 0.65 mmol) in 3 mL of CH_2Cl_2 and 3 mL of ethanol was added hydrazine hydrate (2 mL). Then the mixture was refluxed for overnight. After completion of the reaction, the residue was absorbed on silica gel and purified by column chromatography to give compounds **14a** (80 mg) and **14b** (56 mg); mp 117−119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01−7.98 (m, 2 H), 7.49−7.46 (m, 5 H), 7.33 (s, 1 H), 6.82 (s, 2 H), 3.87 (s, 3 H), 3.85 (s, 6 H). MS (ESI) m/z 370.1 $(M + H)^+$. Anal. ($C_{19}H_{19}N_3O_3S$) C, H, N.

*(E)-4-(Hydrazono(3,4,5-trimethoxyphenyl)methyl)-2-phenylthiazole (**14b**).* Melting point: 65−66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04−8.01 (m, 2 H), 7.44−7.40 (m, 3 H), 6.95 (s, 1 H), 6.65 (s, 2 H), 5.62 (s, 2 H), 3.93 (s, 3 H), 3.87 (s, 6 H). MS (ESI) m/z 370.1 $(M + H)^+$. Anal. ($C_{19}H_{19}N_3O_3S$) C, H, N.

*(Z)-3-(2-Phenylthiazol-4-yl)-3-(3,4,5-trimethoxyphenyl)acrylonitrile (**15a**).* To a solution of 0.4 mL of 2.5 N *n*-BuLi in hexane and 10 mL of THF was added dropwise a solution of 177 mg (1 mmol) of diethyl cyanomethylphosphonate in 5 mL of THF at 0 °C under Ar_2. The ice bath was removed, and the mixture was stirred at 25 °C for 40 min. A solution of 200 mg (0.56 mmol) of **1** in 10 mL of THF was added dropwise at 0 °C, and the mixture was stirred for 1 h at RT. The reaction mixture was treated with saturated NH_4C1 solution. After a conventional workup, column chromatography (silica gel, petroleum ether/ethyl acetate) gave compounds **15a** (83 mg) and **15b** (76 mg); mp:192−193 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01−7.99 (m, 2 H), 7.44−7.40 (m, 3 H), 7.21 (s, 1 H), 6.74 (s, 2 H), 6.67 (s, 1 H), 3.93 (s, 3 H), 3.89 (s, 6 H). MS (ESI) m/z 401.1 $(M + Na)^+$. Anal. ($C_{21}H_{18}N_2O_3S$) C, H, N.

*(E)-3-(2-Phenylthiazol-4-yl)-3-(3,4,5-trimethoxyphenyl)acrylonitrile (**15b**).* Melting point: 111−114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07−8.05 (m, 2 H), 7.49−7.46 (m, 4 H), 6.66 (s, 2 H), 5.64 (s, 1 H), 3.91 (s, 3 H), 3.86 (s, 6 H). MS (ESI) m/z 401.1 $(M + Na)^+$. Anal. ($C_{21}H_{18}N_2O_3S$) C, H, N.

*N-((2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methylene)cyanamide (**16**).* First, 100 mg of **1** (0.28 mmol, 1 equiv) was dissolved in 10 mL of methylene chloride. Then titanium tetrachloride in methylene chloride (1.0 N, 0.7 mL, 2.5 equiv) was added dropwise at 0 °C and stirred for 30 min. Bis-trimethylsilylcarbodiimide (2.4 equiv) in 2 mL of methylene chloride was added and the reaction stirred overnight protected from air and moisture. The reaction was treated with ice−water mixture followed by extraction with methylene chloride. The organic phase was dried over magnesium sulfate, filtered through Celite, and concentrated to give the crude acetophenone cyanoimines, which were purified by flash column as isomers (35 mg) with a ratio of 3:7. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (br, 0.3 H), 8.63 (s, 0.7 H), 8.09−8.07 (m, 1.4 H), 7.99 (br, 0.6 H), 7.58−7.56 (br, 3 H), 7.26 (s, 1.4 H), 7.18 (s, 0.6 H), 3.84, 3.83 (s, s, 6 H), 3.82 (s, 3 H). MS (ESI) m/z 402.1 $(M + Na)^+$. Anal. ($C_{20}H_{17}N_3O_3S$) C, H, N.

*(2-Phenylthiazol-4-yl)(3,4,5-trimethoxyphenyl)methanol (**17**).* At 0 °C, to a solution of 104 mg of **12b** (0.55 mmol, 1 equiv) in 6 mL of

THF was added 3,4,5-trimethoxyphenylmagnesium bromide (0.5 N in THF, 2.9 mL). The mixtures were stirred for 30 min until aldehyde disappeared on TLC plates. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **17**; mp 49−51 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95−7.92 (m, 2 H), 7.44−7.43 (m, 4 H), 6.97 (s, 1 H), 6.76 (s, 2 H), 5.93 (d, 1 H, J = 3.6 Hz), 3.86 (s, 9 H). MS (ESI) m/z 402.1 $(M + Na)^+$. Anal. ($C_{19}H_{19}NO_4S$) C, H, N.

*4-(2-Methyl-1-(3,4,5-trimethoxyphenyl)prop-1-enyl)-2-phenylthiazole (**18**).* At −78 °C, to a solution of 223 mg of isopropyl triphenylphosphonium iodide (0.52 mmol) in 5 mL of THF was added dropwise 0.4 mL of 1.6 N *n*-BuLi in hexane under Ar_2 protection. And the mixture was stirred at 0 °C for 40 min. A solution of 140 mg (0.39 mmol) of **1** in 5 mL of THF was added dropwise at 0 °C. The mixture was stirred for 1 h at RT. The reaction mixture was treated with saturated NH_4C1 solution. After a conventional workup, column chromatography (silica gel, petroleum ether/ethyl acetate) gave compound **18** (86 mg, 57.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98−7.97 (m, 2 H), 7.45−7.40 (m, 3 H), 6.77 (s, 1 H), 6.48 (s, 2 H), 3.86 (s, 3 H), 3.82 (s, 6 H), 2.15 (s, 3 H), 1.81 (s, 3 H). MS (ESI) m/z 404.1 $(M + Na)^+$. Anal. ($C_{22}H_{23}NO_3S$) C, H, N.

*2-Phenyl-4-(3,4,5-trimethoxyphenyl)thiazole (**19**).* Bromine (160 mg, 1 mmol) was added dropwise to a stirred solution of 1-(3,4,5-trimethoxyphenyl)ethanone (210 mg, 1 mmol) in ethanol (30 mL), and the solution was stirred at 0 °C for 1 h and then poured into water to form a precipitate. This was recrystallized from ethanol to give bromoacetophenone (70%) and used directly for the next step. A mixture of bromoacetophenone (288 mg, 1 mmol) and benzothioamide (137 mg, 1 mmol) in ethanol was refluxed for 1 h. The reaction mixture was concentrated in vacuo and purified with a flash column to give **19** (167 mg, 51.1%); mp: 95−96 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05−8.03 (m, 2 H), 7.48−7.44 (m, 3 H), 7.41 (s, 1 H), 7.22 (s, 2 H), 3.97 (s, 6 H), 3.89 (s, 3 H). MS (ESI) m/z 350.1 $(M + Na)^+$. Anal. ($C_{18}H_{17}NO_3S$) C, H, N.

*(Z)-2-Phenyl-4-(3,4,5-trimethoxystyryl)thiazole (**20**).* Triphenylphosphine (3.41 g, 13 mmol) was added to a solution of 5-(bromomethyl)-1,2,3-trimethoxybenzene (2.61 g, 10 mmol) in dry THF (30 mL). The mixture was refluxed with stirring for 6 h. The resulting white solid was filtered and washed with ether/hexane to afford the product 3,4,5-trimethoxybenzyltriphenylphosphonium bromide in 96.4% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77−7.73, 7.65−7.61 (m, 15 H), 6.44 (d, 2 H, J = 1.5 Hz), 5.37 (d, 2 H, J = 14 Hz), 3.76 (s, 3 H), 3.51 (d, 6 H). MS (ESI) m/z 443.1 $(M - Br]^+$. At −78 °C, *n*-BuLi (0.42 mL, 2.5 N in hexane) was added to a solution of 3,4,5-trimethoxybenzyltriphenylphosphonium bromide (500 mg, 0.96 mmol) in 10 mL of THF. After stirring at RT for 2 h, aldehyde **12b** (109 mg, 0.58 mmol) in 3 mL of THF was charged and stirred for 30 min. The reaction mixture was treated with saturated NH_4C1 solution. After a conventional workup, column chromatography (silica gel, petroleum ether/ethyl acetate) gave compounds **20**(*Z*) (57 mg) and **21**(*E*) (99 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90−7.89 (m, 2 H), 7.42−7.40 (m, 3 H), 7.07 (s, 1 H), 6.71 (s, 2 H), 6.66 (s, 1 H), 3.87 (s, 6 H), 3.75 (s, 3 H). MS (ESI) m/z 376.1 $(M + Na)^+$. Anal. ($C_{20}H_{19}NO_3S$) C, H, N.

*(E)-2-Phenyl-4-(3,4,5-trimethoxystyryl)thiazole (**21**).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03−8.01 (m, 2 H), 7.52 (d, 1 H, J = 16 Hz), 7.47−7.44 (m, 3 H), 7.16 (s, 1 H), 7.05 (d, 1 H, J = 16 Hz), 6.79 (s, 2 H), 3.92 (s, 6 H), 3.88 (s, 3 H). MS (ESI) m/z 354.1 $(M + H)^+$. Anal. ($C_{20}H_{19}NO_3S$) C, H, N.

*S-Biphenyl-3-yl-O-Ethyl-Carbonodithioate (**22**).* To a solution of 1 equiv of biphenyl-3-amine (1 g, 5.92 mmol) in water (7.3 mL) at 0 °C was added concentrated hydrochloric acid (1 mL). A cold solution of 1.1 equiv of sodium nitrite (450 mg, 6.5 mmol) in water (3 mL) was added slowly and stirred for 15 min. The cold diazonium solution was added slowly to a solution of 1.3 equiv of potassium ethyl xanthate (1.16 g, 1.3 mmol) in water (1.3 mL) at 45 °C. The reaction mixture was stirred for an additional 30 min at 45 °C and then cooled to RT. The reaction mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with 1 N NaOH solution (100 mL), water (3 × 50 mL), brine (50 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting crude xanthate **22** was used directly in the next step without further purification. MS (ESI) m/z 275.0 $(M + H)^+$.

*Biphenyl-3-yl(3,4,5-trimethoxyphenyl)sulfane (**23**).* To a solution of **22** (1.1 g, crude compound) in ethanol (8 mL) was added potassium hydroxide (2.1 g, 12 mL) and heated to reflux for overnight. The solution was cooled to RT, and the ethanol was evaporated under reduced pressure. The residue was dissolved in water and washed with diethyl ether (10 mL). The aqueous layer was acidified with 2 N HCl and extracted with diethyl ether (3 × 50 mL). The organic extracts were washed with water (50 mL), brine (50 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure to afford 0.85 g (77.3%) of crude biphenyl-3-thiol product (overall, 3 steps). Into a round-bottomed flask, stirred magnetically, were placed 0.1 g (1.04 mmol) of sodium *tert*-butoxide and 83 mg of copper iodide (0.43 mmol). After the reaction vessel was sealed, 0.13 g (0.71 mmol) of 4-methoxybenzenethiol and 0.19 g (0.65 mmol) of 5-iodo-1,2,3-trimethoxybenzene in 3.0 mL of toluene were injected through the septum. The reaction mixture was heated for overnight at 110 °C. Purification was performed by flash chromatography and colorless oil **23** was obtained (40% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54−7.52 (m, 3 H), 7.44−7.41 (m, 3 H), 7.37−7.33 (m, 2 H), 7.23 (s, br, 1 H), 6.69 (s, 2 H), 3.86 (s, 3 H), 3.80 (s, 6 H). MS (ESI) m/z 353.2 $(M + H)^+$. Anal. ($C_{21}H_{20}O_3S$) C, H.

*3-(3,4,5-Trimethoxyphenylsulfonyl)biphenyl (**24**).* To a solution of 60 mg (0.17 mmol) of compound **23** and 5 mL of dichloromethane was added very slowly 2 equiv of *m*-CPBA over 3 h. Sulfoxide formation was monitored by thin-layer chromatography. Purification was performed with a flash chromatographic column, and an amorphous powder of **24** was obtained (73% yield); mp 99−101 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (br, 1 H), 7.89 (d, 1 H), 7.78 (d, 1 H), 7.59−7.56 (m, 3 H), 7.49−7.39 (m, 3 H), 7.19 (s, 2 H), 3.89 (s, 6 H), 3.87 (s, 3 H). MS (ESI) m/z 385.0 $(M + Na)^+$. Anal. ($C_{21}H_{20}O_5S$) C, H.

*3-(3,4,5-Trimethoxyphenylsulfinyl)biphenyl (**25**).* At 0 °C, to a solution of 500 mg (1.42 mmol) of compound **23** and 5 mL of dichloromethane was added very slowly 1 equiv of *m*-CPBA over 3 h. Sulfoxide formation was monitored by thin-layer chromatography. Purification was performed with a flash chromatographic column, and an amorphous powder of **25** was obtained (87% yield); mp 108−109 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (br, 1 H), 7.71 (d, 2 H), 7.62−7.60 (m, 3 H), 7.58−7.40 (m, 4 H), 6.94 (s, 2 H), 3.79 (s, 3 H), 3.74 (s, 6 H). MS (ESI) m/z 369.1 $(M + H)^+$. Anal. ($C_{21}H_{20}O_4S$) C, H.

*N-(3,4,5-Trimethoxyphenyl)biphenyl-3-sulfinamide (**26**).* A mixture of 65 mg of biphenyl-3-sulfonyl chloride (0.25 mmol), 44 mg of 3,4,5-trimethoxyaniline (0.24 mmol), and 0.3 mmol of triethylamine in 5 mL of DMF was stirred overnight. The reaction mixture was treated with water and extracted with ethyl acetate. After a conventional workup, column chromatography (silica gel, petroleum ether/ethyl acetate) gave 88 mg of **26** (91.7%); mp 48−50 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (t, 1 H, J = 1.8 Hz), 7.81−7.74 (m, 2 H), 7.57−7.40 (m, 6 H), 6.33 (s, 2 H), 3.86 (s, 3 H), 3.80 (s, 6 H). MS (ESI) m/z 422.1 $(M + Na)^+$. Anal. ($C_{21}H_{21}NO_5S$) C, H, N.

*(2-(4-Hydroxyphenyl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone (**29**).* At 0 °C, to a solution of **28** (0.2 mmol) in 5 mL of CH_2Cl_2 was added a solution of tetrabutylammonium fluoride in THF (1 N, 0.6 mmol) and stirred at RT for around 14 h until reaction was finished by TLC monitor; 67.0% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (s, 1 H), 8.51 (s, 1 H), 7.85 (d, 2 H, J = 8.50 Hz), 7.62 (s, 2 H), 6.91 (d,

2 H, J = 8.5 Hz), 3.86 (s, 6 H), 3.79 (s, 3 H). MS (ESI) m/z 394.1 $(M + Na)^+$, 369.9 $(M - H)^-$. Anal. ($C_{19}H_{17}FNO_5S$) C, H, N.

*(2-(1H-Indol-2-yl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone (**32**) Was Synthesized from **31** Using the Same Method As Used for **5**.* Yield 45.8%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.26 (s, 1 H), 8.11 (s, 1 H), 7.66 (d, 1 H, J = 8.0 Hz), 7.46 (s, 2 H), 7.42 (d, 1 H, J = 8.0 Hz), 7.29 (t, 1 H, J = 7.5 Hz), 7.16 (t, 1 H, J = 7.5 Hz), 7.10 (s, 1 H), 3.97 (s, 3 H), 3.93 (s, 6 H). MS (ESI) m/z 417.1 $(M + Na)^+$, 392.9 $(M - H)^-$. Anal. ($C_{21}H_{18}N_2O_4S$) C, H, N.

*(2-(1H-Indol-5-yl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone (**36**).* To a solution of *n*-BuLi (1.6 M, 1.7 mL) in 8 mL of THF was added a solution of 3,4,5-trimethoxybromobenzene (2.47 mmol) in 3 mL of THF under −78 °C. The mixture was allowed to stir for 2 h, and a solution of Weinreb amide **35** (1.24 mmol) in 3 mL of THF was charged. The temperature was allowed to increase at RT and stirred overnight. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was refluxed in 1 N NaOH in 5 mL of ethanol solution to obtain the deprotected compound **36** and purified by column chromatography to obtain pure compound as a light-yellow solid (36.3%); mp 162−164 °C. ^{1}H NMR (300M, $CDCl_3$) δ 8.36 (br, s, 1 H), 8.31 (s, 1 H), 8.21 (s, 1 H), 7.92, 7.89 (dd, 1 H, J = 1.8, 2.7 Hz), 7.46 (d, 1 H), 7.62 (s, 2 H, J = 8.7 Hz), 7.29 (t, 1 H, J = 2.7 Hz), 6.64 (br, 1 H), 3.97 (s, 6 H), 3.97 (s, 3 H). MS (ESI) m/z 417.1 $(M + Na)^+$, 392.9 $(M - H)^-$. Anal. ($C_{21}H_{18}N_2O_4S$) C, H, N.

*tert-Butyl 4-(4-(3,4,5-Trimethoxybenzoyl)thiazol-2-yl)benzylcarbamate (**38**).* A mixture of **37** (2.5 mmol), $CBrCl_3$ (3.2 mmol), and DBU (5.0 mmol) in CH_2Cl_2 (20 mL) was stirred overnight. The reaction mixture was absorbed on silica gel and purified by column chromatography to yield an intermediate thiazole Weinreb amide. To a solution of (3,4,5-trimethoxyphenyl)magnesium bromide (0.5 M, 5.5 mL) in THF was added a solution of the intermediate thiazole Weinreb amide (1.83 mmol) in 10 mL of THF under 0 °C and stirred for 30 min. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound as a light-yellow solid (32.3%); mp 123−124 °C. ^{1}H NMR (300M, $CDCl_3$) δ 8.27 (s, 1 H), 7.98 (d, 2 H, J = 8.1 Hz), 7.78 (s, 2 H), 7.39 (d, 2 H, J = 8.1 Hz), 4.93 (br, 1 H), 4.37 (br, d, 2 H), 3.96 (s, 3 H), 3.95 (s, 6 H), 1.47 (s, 9 H). MS (ESI) m/z 507.1 $(M + Na)^+$. Anal. ($C_{25}H_{28}N_2O_6S$) C, H, N.

*(2-(4-(Aminomethyl)phenyl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)-methanone Hydrochloride (**39**).* At 0 °C, to a solution of **38** (200 mg) in 10 mL of CH_2Cl_2 was added a solution of HCl in 1,4-dioxane (4 N, 2 mL) and stirred at RT for 4 h. The precipitate (**39**) was filtered and washed with diethyl ether. Yield 81.3%; mp 200−203 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (s, 1 H), 8.38 (br, 3 H), 8.10 (d, 2 H, J = 8.4 Hz), 7.66 (d, 2 H, J = 8.4 Hz), 7.62 (s, 2 H), 4.11 (s, 2 H), 3.87 (s, 6 H), 3.80 (s, 3 H). MS (ESI) m/z 385.1 $(M + H)^+$. Anal. ($C_{20}H_{20}N_2O_4S\cdot$HCl) C, H, N, Cl.

*(2-(4-((Methylamino)methyl)phenyl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone Hydrochloride (**40**).* At 0 °C, to a solution of **41** (60 mg) in 5 mL of CH_2Cl_2 was added a solution of HCl in 1,4-dioxane (4 N, 2 mL) and stirred at RT for overnight. The precipitate (**40**) was filtered and washed with diethyl ether. Yield 81.3%; mp 197−200 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.0 (s, 1 H), 8.29 (s, 1 H), 8.05 (d, 2 H, J = 6.0 Hz), 7.74 (s, 2 H), 7.72 (d, 2 H, J = 6.0 Hz), 4.15 (s, 2 H), 3.99 (s, 3 H), 3.96 (s, 6 H), 2.61 (s, 3 H). MS (ESI) m/z 399.1 $(M + H)^+$.; Anal. ($C_{21}H_{22}N_2O_4S\cdot$HCl$\cdot H_2O$) C, H, N.

*(2-(4-((Dimethylamino)methyl)phenyl)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone Hydrochloride (**42**).* To a solution of **39** (53 mg, 0.14 mmol) in 5 mL of CH_2Cl_2 was added formaldehyde solution (37% in H_2O, 340 mg, 4.2 mmol) and sodium cyanoborohydride (34 mg, 0.55 mmol), the reaction mixture was absorbed on silica gel, and free base was purified after flash column (41 mg, 70.9%). At 0 °C, to a solution of free base (41 mg) in 5 mL of CH_2Cl_2 was added a solution of HCl in 1, 4-dioxane (4 N, 2 mL) and stirred at RT for overnight. The precipitate (**42**) was filtered and washed with diethyl ether. Yield 71.3%; mp 94−96 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.0 (s, 1 H), 8.34 (s, 1 H), 8.13 (d, 2 H, J = 7.0 Hz), 7.82 (d, 2 H, J = 7.5 Hz), 7.75 (s, 2 H), 4.24 (s, 2 H), 3.99 (s, 3 H), 3.97 (s, 6 H), 2.83 (s, 6 H). MS (ESI) m/z 413.1 $(M + H)^+$. Anal. ($C_{22}H_{24}N_2O_4S\cdot$HCl) C, H, N.

General Procedure for the Synthesis of (2-(Arylamino)-thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanones (45a−c). At −78 °C, to a solution of 5-bromo-1,2,3-trimethoxybenzene (1.235 g, 5.0 mmol) in 30 mL of THF was charged *n*-BuLi in hexane (2.5 N, 2.4 mL, 6 mmol) under Ar_2 protection and stirred for 10 min. Weinreb amide **44a−c** (1 mmol) in 10 mL of THF was added to the lithium reagent and allowed to stir at RT for 2 h. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **45a−c**.

*(2-(Phenylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone (**45a**).* Yield 33.3%; mp 149−151 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.4 (s, 1 H), 7.85 (s, 1 H), 7.68 (d, 2 H, J = 8.0 Hz), 7.31 (t, 2 H, J = 8.0 Hz), 6.98 (t, 1 H, J = 8.0 Hz), 3.83 (s, 6 H), 3.78 (s, 3 H). MS (ESI) m/z 393.1 $(M + H)^+$, 368.9 $(M - H)^-$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

*(2-(p-Tolylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone (**45b**).* Yield 40.6%; mp 139−140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 1 H), 7.47 (s, 2 H), 7.30 (br, 1 H), 7.27 (d, 2 H, J = 8.5 Hz), 7.17 (d, 2 H, J = 8.5 Hz), 3.93 (s, 3 H). 3.90 (s, 6 H), 2.34 (s, 3 H). MS (ESI) m/z 385.1 $(M + H)^+$, 382.9 $(M - H)^-$. Anal. ($C_{20}H_{20}N_2O_4S$) C, H, N.

*(2-(p-Fluorophenylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)-methanone (**45c**).* Yield 39.6%; mp 129−130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (br, 1 H), 7.49 (s, 1 H), 7.45 (s, 2 H), 7.40−7.37 (q, 2 H, J = 4.5 Hz), 7.08−7.04 (t, 2 H, J = 8.0 Hz), 3.93 (s, 3 H), 3.89 (s, 6H). MS (ESI) m/z 389.3 $(M + H)^+$, 386.9 $(M - H)^-$. Anal. ($C_{19}H_{17}FN_2O_4S$) C, H, N.

General Procedure for the Synthesis of Hydrochloride Salts (46a−c). At 0 °C, to a solution of compound **45a−c** (0.1 mmol) in 5 mL of CH_2Cl_2 was added a solution of HCl in 1,4-dioxane (4 N, 2 mL) and stirred at RT for overnight. The precipitates **46a−c** were collected and washed with diethyl ether.

*(2-(Phenylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone Hydrochloride Salt (**46a**).* Yield 91.6%; mp 94−96 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.9 (br, 1 H), 7.49−7.46 (m, 2 H), 7.42−7.40 (m, 2 H), 7.37−7.34 (m, br, 2 H), 7.11 (s, 2 H), 3.94 (s, 3 H), 3.92 (s, 6 H), 3.57 (br, H_2O). MS (ESI) m/z 389.1 $(M + H)^+$. Anal. ($C_{19}H_{18}N_2O_4S\cdot$HCl$\cdot H_2O$) C, H, N.

*(2-(p-Tolylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)methanone Hydrochloride Salt (**46b**).* Yield 39.6%; mp 115−118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30−7.25 (m, br, 6 H), 7.12 (s, 2 H), 3.94 (s, 3 H), 3.92 (s, 6 H), 2.38 (s, 3 H). MS (ESI) m/z 389.1 $(M + H)^+$. Anal. ($C_{20}H_{20}N_2O_4S\cdot$2HCl) C, H, N.

*(2-(p-Fluorophenylamino)thiazol-4-yl)(3,4,5-trimethoxyphenyl)-methanone Hydrochloride Salt (**46c**).* Yield 89.3%; mp 102−104 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.55 (s, 1 H), 7.85 (s, 1 H), 7.72−7.69 (q, 2 H, J = 4.5 Hz), 7.50 (s, 2 H), 7.18−7.15 (t, 2 H, J = 8.5 Hz), 4.30 (br, H_2O), 3.82 (s, 6H), 3.78 (s, 3 H). MS (ESI) m/z 389.3 $(M + H)^+$. Anal. ($C_{19}H_{17}FN_2O_4S\cdot$1.5HCl$\cdot$0.5$H_2O$) C, H, N.

Cell Culture and Cytotoxicity Assay of Prostate Cancer and Melanoma. All cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), while cell culture supplies were purchased from Cellgro Mediatech (Herndon, VA, USA). We examined the antiproliferative activity of our antitubulin compounds

in four human prostate cancer cell lines (LNCaP, DU 145, PC-3, and PPC-1) and three melanoma cell lines (A375, B16−F1 and WM-164). Human ovarian cell line OVCAR-8 and its resistant cell line that overexpresses P-gp (NCI/ADR-RES) were used as MDR models. Both ovarian cell lines were obtained from National Cancer Institutes (NCI). All cell lines were tested and authenticated by either ATCC or NCI. All prostate cancer and ovarian cancer cell lines were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum (FBS). Melanoma cells were cultured in DMEM, supplemented with 5% FBS, 1% antibiotic/antimycotic mixture (Sigma-Aldrich, Inc., St. Louis, MO, USA), and bovine insulin (5 μg/mL; Sigma-Aldrich). The cytotoxic potential of the antitubulin compounds was evaluated using the sulforhodamine B (SRB) assay after 96 h of treatment.

Aqueous Solubility. The solubility of drugs was determined by a Multiscreen solubility filter plate (Millipore Corporate, Billerica, MA) coupled with LC-MS/MS. Briefly, 198 μL of phosphate buffered saline (PBS) buffer (pH 7.4) was loaded into 96-well plate, and 2 μL of 10 mM test compounds (in DMSO) was dispensed and mixed with gentle shaking (200−300 rpm) for 1.5 h at RT (N = 3). The plate was centrifuged at 800g for 5 min, and the filtrate was used to determine its concentration and solubility of test compound by LC-MS/MS as described below. Concentrations of tested compounds were determined with individual calibration curve with satisfied linearity (r > 0.995).

Pharmacokinetic Study. Female Sprague−Dawley rats (n = 3 or 4; 254 ± 4 g) were purchased from Harlan Inc. (Indianapolis, IN). Rat thoracic jugular vein catheters were purchased from Braintree Scientific Inc. (Braintree, MA). On arrival at the animal facility, the animals were acclimated for 3 days in a temperature-controlled room (20−22 °C) with a 12 h light/dark cycle before any treatment. Compound **1** was administered intravenously (IV) into the jugular vein catheters at a dose of 2.5 mg/kg (in DMSO/PEG300, 2/8), whereas **46a** and **46c** were dosed at 5 mg/kg (in DMSO/PEG300, 1/9). An equal volume of heparinized saline was injected to replace the removed blood, and blood samples (250 μL) were collected via the jugular vein catheters at 10, 20, 30 min, and 1, 2, 4, 8, 12, 24 h. Compounds **1**, **46a**, and **46c** were given (PO) by oral gavage at 10 mg/kg (in Tween80/DMSO/H_2O, 2/1/7). All blood samples (250 μL) after oral administration were collected via the jugular vein catheters at 30, 60, 90, 120, 150, 180, 210, 240 min, and 8, 12, 24 h. Heparinized syringes and vials were prepared prior to blood collection. Plasma samples were prepared by centrifuging the blood samples at 8000g for 5 min. All plasma samples were stored immediately at −80 °C until analyzed.

Analytes were extracted from 100 μL of plasma with 200 μL of acetonitrile containing 200 nM of the internal standard ((3,5-dimethoxyphenyl)(2-phenyl-1*H*-imidazol-4-yl)methanone). The samples were thoroughly mixed, centrifuged, and the organic extract was transferred to autosampler for LC-MS/MS analysis. Multiple reaction monitoring (MRM) mode, scanning m/z 356 → 188 (compound **1**), m/z 371 → 203 (compound **46a**), m/z 389 → 221 (compound **46c**), and m/z 309 → 171 (the internal standard), was used to obtain the most sensitive signals. The pharmacokinetic parameters were determined using noncompartmental analysis (WinNonlin, Pharsight Corporation, Mountain View, CA)

Analytical Method. Sample solution (10 μL) was injected into an Agilent series HPLC system (Agilent 1100 Series Agilent 1100 Chemstation, Agilent Technology Co, Ltd.). All analytes were separated on a narrow-bore C18 column (Alltech Alltima HP, 2.1 mm × 100 mm, 3 μm, Fisher, Fair Lawn, NJ). Two gradient modes were used. Gradient mode was used to achieve the separation of analytes using mixtures of mobile phase A [ACN/H_2O (5%/95%, v/v) containing 0.1% formic acid] and mobile phase B [ACN/H_2O (95%/5%, v/v) containing 0.1% formic acid] at a flow rate of 300 μL/min. Mobile phase A was used at 15% from 0 to 1 min, followed by a linearly programmed gradient to 100% of mobile phase B within 6 min, 100% of mobile phase B was maintained for 0.5 min before a quick ramp to 15% mobile phase A. Mobile phase A was continued for another 12 min toward the end of analysis.

A triple-quadruple mass spectrometer, API Qtrap 4000 (Applied Biosystems/MDS SCIEX, Concord, Ontario, Canada), operating with a TurboIonSpray source was used. The spraying needle voltage was set at 5 kV for positive mode. Curtain gas was set at 10; gas 1 and gas 2 were set 50. Collision-assisted dissociation (CAD) gas at medium and the source heater probe temperature at 500 °C. Data acquisition and quantitative processing were accomplished using Analyst software, Ver. 1.4.1 (Applied Biosystems).

In Vitro Tubulin Polymerization Assay. Bovine brain tubulin (0.4 mg, >97% pure) (Cytoskeleton, Denver, CO) was mixed with 5 μM of the test compounds and incubated in 100 μL of general tubulin buffer (80 mM PIPES, 2.0 mM $MgCl_2$, 0.5 mM EGTA, and 1 mM GTP) at pH 6.9. The absorbance of wavelength at 340 nm was monitored every 1 min for 20 min by the SYNERGY 4 Microplate Reader (Bio-Tek Instruments, Winooski, VT). The spectrophotometer was set at 37 °C for tubulin polymerization.

■ ASSOCIATED CONTENT

Supporting Information. 2D NOESY NMR and 1D NOE NMR to identify *syn*/*anti*-isomers of hydrazides **14a**−**14b** and acrylonitriles **15a**−**15b**, synthesis, mass, and NMR of intermediates **1**−**4**, **8a**−**e**, **10e**−**g**, **12a**, **12b**, **27**, **30**, **31**, **34**, **35**, **37**, **38**, **41**, **43a**−**c**, **44a**−**c**, and quantum chemical shifts calcula tions. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

ABC, ATP binding cassette; Boc_2O, *tert*-butyl dicarbonate; CA-4, combretastatin A-4; DMSO, dimethyl sulfoxide; MDR, multidrug resistance; MRM, Multiple reaction monitoring; NMM, *N*-methylmorpholine; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; PDC, pyridinium dichromate; P-gp, P-glycoprotein; phenylamino-thiazole, PAT; pharmacokinetic, PK; RT, Room temperature; SAR, Structure−activity relationship; SMART, 4-substituted methoxybenzoyl-aryl-thiazole; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; THF, tetrahydrofuran; TMP, 3,4,5-trimethoxyphenyl

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