Potent Antitubulin Tumor Cell Cytotoxins Based on 3-Aroyl Indazoles

Jian-Xin Duan,* Xiaohong Cai, Fanying Meng, Leslie Lan, Charles Hart, and Mark Matteucci*

Threshold Pharmaceuticals, 1300 Seaport Boulevard, Redwood City, California 94063

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A series of 3-aroyl indazoles was synthesized. Modification of the C-7 position resulted in a significant structure−activity relationship (SAR) with acetylene modifications conferring unusual potency in a tumor cell cytotoxicity assay. The most potent compounds exceeded the activity of combretastatin A4 (CA-4), showing single digit nM IC_{50} values against all cell lines tested including those with known efflux resistance pumps. The inhibition of *in vitro* tubulin polymerization was comparable to CA-4, consistent with tubulin being the target for these compounds. Competition binding experiments employing [^{3}H]colchicine and purified tubulins demonstrated that the compound specifically binds to the colchicine site.

Introduction

Tubulin-containing structures such as microtubules are important for diverse cellular functions, including chromosome segregation during cell division, intracellular transport, cell motility, and cell shape.[1] Combretastatin A4 (CA-4[a])[2] is a low-molecular weight natural product that binds to the colchicine site of tubulin and inhibits tubulin polymerization. A water soluble phosphate prodrug of combretastatin A4 (CA-4P)[3] is currently in clinical trials for the treatment of solid tumors. The activity and structural simplicity of CA-4 have stimulated enormous efforts to develop new analogues that mimic CA-4 activity and improve on its pharmacological properties. One analogue series has utilized diphenyl ketones[4] that were shown to be tubulin inhibitors with only mild cytotoxicity. Liou[5] et al. have extended their initial observation and reported that aroylindoles were potent tubulin inhibitors and highly cytotoxic to tumor cells in culture.

We report here that 3-(3,4,5-trimethoxylbenzoyl)indazoles are highly cytotoxic agents to tumor cells in cell culture and inhibitors of tubulin polymerization *in vitro*. The interest in the indazole series was driven by the postulate that the N-2 nitrogen on the indazole and the oxygen on the carbonyl group could form a chelate with a metal such as magnesium or a hydrogen-bonded complex with water as shown below in Figure 1. Such a preordered structure could conformationally mimic the cis configuration of CA-4.

Synthesis. The core structure was readily synthesized based on an adoption of the literature method[6,7] as shown below in Scheme 1. **1** was coupled to trimethylsilylacetylene using palladium catalyst followed by desilylation to give compound **2**.[6] Subsequent coupling with 4-iodo-3-nitroanisole gave compound **3**, which was readily reduced by iron powder to afford **4**. The core **5** was prepared from **4** following the reported procedure.[7] Iodination of **5** was highly regioselective and gave compound **6** in very good yield (85%) (Scheme 2). This iodo derivative was a key synthon for further elaboration. Coupling reaction of **6** with various terminal alkynes gave **7** in good yields (Scheme 3). Careful hydrogenation of **10** and desilylation resulted in compounds **12** and **13** (Scheme 4). Compound **15** was synthesized as a test of the effect of C-7 modification on the previously published compound **14** (BPROL075) of the indole series (Scheme 5).[5]

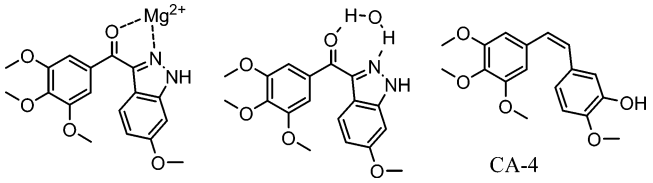
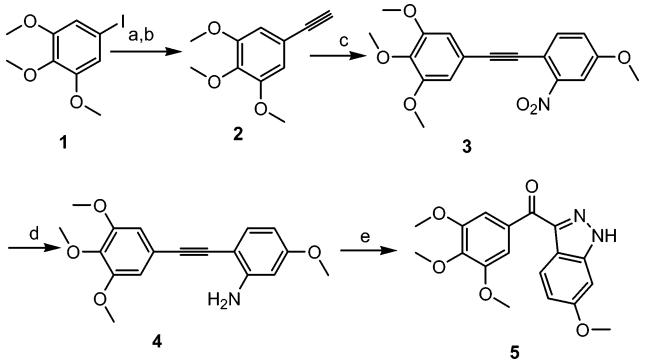


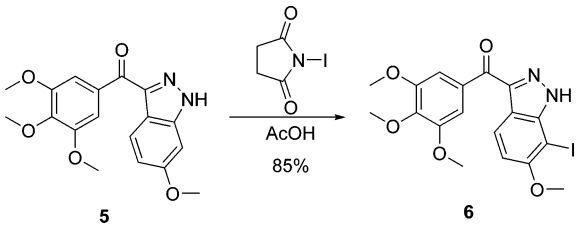
Figure 1. Potential metal-chelated and hydrogen-bonded indazole structures.

Scheme 1[a]



[a] (a) $PdCl_2(PPh_3)_2$, CuI/TEA (89%), trimethylsilylacetylene. (b) NBu_4F/THF (82%). (c) $PdCl_2(PPh_3)_2$, CuI/TEA, 4-iodo-3-nitroanisole (90%). (d) Fe, HCl/EtOH (54%). (e) $NaNO_2$, HCl (80%).

Scheme 2



Biological Evaluation. All of the reported compounds were evaluated for cytotoxicity toward H460 cells, a human non-small lung cancer cell line. CA-4 and **14** were included as

* To whom correspondence should be addressed. Phone: 650-474-8231. Fax: 650-474-0408. E-mail: jduan@thresholdpharm.com (J.D.). Phone: 650-474-8225. Fax: 650-474-0408. E-mail: mmatteucci@thresholdpharm.com (M. M).

[a] Abbreviations: CA-4, combretastatin A4; IC_{50}, the half maximal inhibitory concentration; MDR, multidrug drug resistance; MRP, multiple resistant protein; GTP, guanosine triphosphate; TLC, thin layer chromatography; HRMS, high resolution mass spectra; HPLC, high pressure liquid chromatography.

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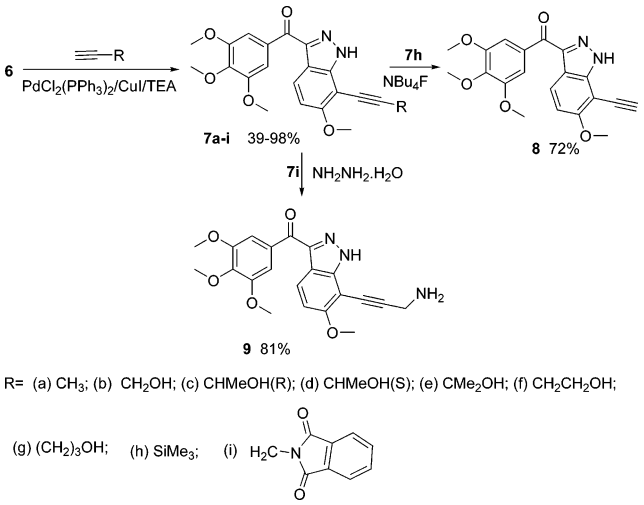
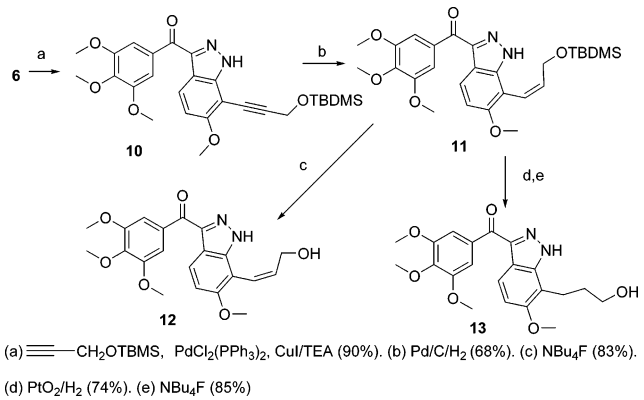
Scheme 3



Scheme 4[a]

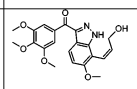
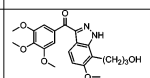
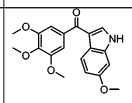
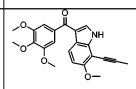
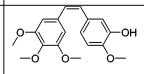


[a] (a) ≡EnDashCH$_2$OTBMS, PdCl$_2$(PPh$_3$)$_2$, CuI/TEA (90%). (b) Pd/C/H$_2$ (68%). (c) NBu$_4$F (83%). (d) PtO$_2$/H$_2$ (74%). (e) NBu$_4$F (85%).

controls. The IC_{50} values for the inhibition of proliferation are shown in Table 1.

The core indazole compound **5** had an IC_{50} of 64 nM which is approximately 4 times less active than the corresponding indole analogue **14** (IC_{50} = 16 nM). When acetylenes were introduced onto the C-7 position of **5**, the resulting compounds showed dramatically improved cytotoxic activities. The activity of the simple acetylene derivative **8** was increased by 20 times (IC_{50} = 3 nM for **8** vs 64 nM for **5**), while the propyne derivative **7a** proved to be the most potent compound being 60 times more potent (IC_{50} = 1 nM for 7a) than **5**. Alcohols were well tolerated with the linear substituted alcohols demonstrating significant potency (IC_{50} values for **7b**, **7f**, and **7g** are 8, 14, and 8 nM, respectively). The tertiary alcohol was found to be much less active (IC_{50} for **7e** is 195 nM). Amino substitution was less tolerated, with compound **9** being about 15 times less active than the corresponding alcohol analogue **7b**. The *cis*-allyl alcohol derivative had comparable activity to the propargyl alcohol **7b**, while the completely hydrogenated propanol **13** was much less active (IC_{50} = 8 nM for **7b** vs IC_{50} = 13 nM for **12** vs IC_{50} = 283 nM for **13**). The intriguing activity imparted by the propyne modification in the indazole series did not transfer to the indole series. The propyne analogue of **14** was inactive at concentrations up to 1000 nM.

Table 1. IC_{50} Values (nM) of **7**−**9**, **12**−**14**, and **16**[a]

Compd		IC_{50}(nM)	Compd	Structure	IC_{50}(nM)
7a	CH_3	1	**12**		13
7b	CH_2OH	8			
7c	CHMeOH(R)	29	**13**		283
7d	CHMeOH(S)	14			
7e	CMe_2OH	195	**14**		16
7f	CH_2CH_2OH	14			
7g	$CH_2(CH_2)_2OH$	8	**16**		>1000
8	H	3			
9	CH_2NH_2	247	**CA-4**		10

[a] All assays performed in triplicate; cells were treated with compound for 3 days.

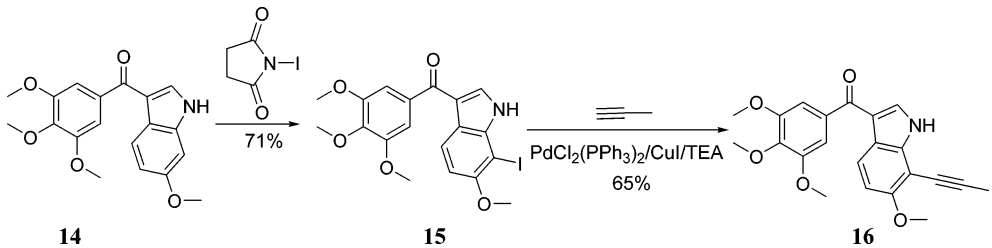
Table 2. IC_{50} Values (nM) of **7a**, **7b**, and **8** against HT29, PC-3, and HeLa Cell Lines

tumor cell line	tumor type	**7a**	**7b**	**8**	CA-4
HT29	colon	3.0	1.0	1.7	>1000
PC3	prostate	1.1	1.0	3.0	3.2
Hela	cervical	0.9	0.8	2.3	2.7

Three of most potent compounds **7a**, **7b**, and **8** were tested in three other human cancer cell lines: PC-3 human prostate cancer cells, HT29 human colorectal cancer cells, and HeLa human cervical cancer cells. As shown in Table 2, all three compounds exhibited similar potent cytotoxic effects against these lines. The CA-4 control demonstrated similar low nanomolar potency against PC-3 and HeLa cells. CA-4 was virtually inactive against HT29 cells consistent with published data demonstrating HT29 resistance to CA-4.[8]

Tubulin Inhibitors Overcome Drug Resistance. The lead compounds **7a**, **7b**, and **8** were further evaluated against cell lines possessing the well characterized drug resistance efflux pumps MDR-1 (P-glycoprotein, ABCB1) and MRP-1 (AB-

Scheme 5



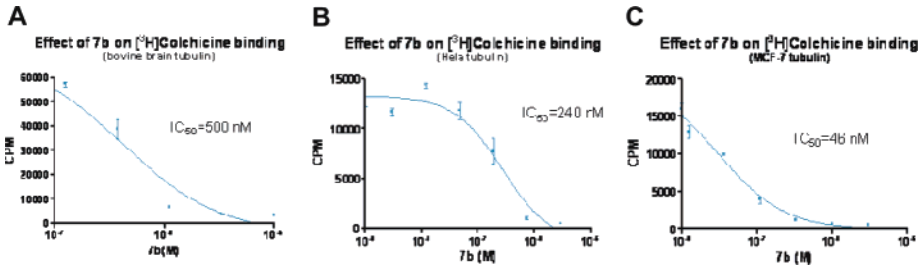


Figure 2. Inhibition of colchicine binding by **7b**. Purified tubulin from bovine brain (A), Hela cells (B) and MCF-7 cells (C), was preincubated with **7b** for 10 min at 37 °C. [^{3}H]Coclchicine was added into the reaction. The samples were spotted on DE81 filters, and bound and unbound [^{3}H]colchicine were separated by washing three times. The bound [^{3}H]colchicine was measured using a scintillation counter.

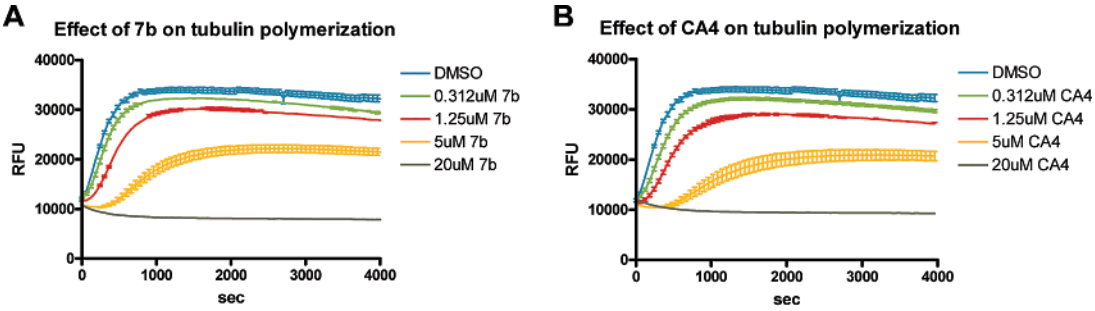


Figure 3. **7b** (A) and CA4 (B) inhibited tubulin polymerization in a concentration-dependent manner.

Table 3. IC_{50} Values (nM) of **7a**, **7b**, and **8** against Drug Resistant Cell Lines

cell line	Messa	Messa/DX5	HCT-15	ACHN	NCI-H69	H69 AR
type	sensitive	resistant	resistant	resistant	sensitive	resistant
origin	gastric	gastric	colon	renal	small cell lung	small cell lung
resistance mechanism	none	MDR-1	MDR-1	MDR-1	none	MRP-1
CA4	1.6	1.4	2.5	1.6	1.7	2.5
7a	1.6	1.9	1.9	1.6	1.6	2.5
7b	1.7	1.8	1.9	1.6	1.6	2.6
8	1.9	3.9		1.6	2.5	2.2
paclitaxel	1.6	>1000	79.4	25	1.9	2.5
daunorubicin	20	>400	>400	63	100	>1000
colchicine	6.3	63	100	39.8		
vinblastine	1.6	8.9	79.4	25	1.6	1.6

CC1).[9] These results along with data enabling a comparison to some efflux-sensitive chemotherapeutics are shown in Table 3.MESSA/DX5 was derived from the parent MESSA gastric cancer cell line and is highly resistant to daunorubicin, paclitaxel, and colchicine due to overexpression of the MDR-1 pump.[10] HCT-15 and ACHN cells also have high expression levels of MDR-1 and display the multiple drug resistance phenotype.[11] H69AR was derived from NCI-H69 and is highly resistant to daunorubicin due to overexpression of the MRP-1 pump.[12] As shown in Table 3, all three compounds exhibited potent antiproliferative activities in all of the drug resistant cell lines examined, with the IC_{50} values ranging from 1.6 nM to 3.9 nM. These results demonstrate that **7a**, **7b**, and **8** are not substrates for the MDR-1 and MRP-1 pumps.

7b Specifically Binds to Colchicine Binding Site. The ability of lead compound **7b** to compete with [^{3}H]colchicine binding to purified tubulins, including bovine brain tubulin, HeLa cell tubulin, and MCF-7 tubulin, was evaluated. As shown in Figure 2A, **7b** inhibited 50% of the colchicine binding to bovine brain tubulin at 500 nM. Similarly, **7b** was able to compete with [^{3}H]-colchicine binding to purified tubulin from Hela cells and MCF-7 cells. As shown in Figure 2B and 2C, **7b** inhibited colchicine binding to Hela tubulin and MCF-7 tubulin with IC_{50} values of 46 nM and 240 nM, respectively. The different inhibitory potency to tubulins derived from various sources might be related to differential expression of tubulin isoforms (unpublished data).

7b Inhibits Tubulin Polymerization *in Vitro*. The effects of **7b** on microtubule formation were assessed using an *in vitro* tubulin polymerization assay,[13] in which microtubule formation was monitored by the increase in fluorescent intensity of the reaction mixture. The addition of **7b** inhibited tubulin polymerization in a concentration-dependent manner (Figure 3) with an IC_{50} of 6 μM. The control compound, CA-4, displayed a similar inhibition effect in this assay. The concentrations required to inhibit tubulin polymerization (IC_{50}: 6 μM) are much higher than those required for cytotoxicity, a phenomenon well documented in the literature.[14] These results, along with the structural similarity to known tubulin polymerization inhibitors, indicate that the indazole ketone's biological activity is due to interaction with tubulin.

A highly potent class of cytotoxic compounds has been identified based on novel acetylene substitutions of an indazole core. Three compounds, **7a**, **7b**, and **8**, have emerged as lead compounds. These compounds have been shown to be nearly equally potent against a panel of cancer cell lines and are not substrates of two common drug efflux pumps. On the basis of these excellent properties, these compounds are worthy of further *in vitro* and *in vivo* evaluation.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer (400 and 75 MHz, respectively) using $CDCl_3$, CD_3-OD, or DMSO-d_6 as solvents with TMS as an internal standard.

High-resolution mass spectra analysis was performed on a GCT-MS Micromass UK mass spectrometer. Column chromatography was performed with silica gel (230−400 mesh). All chemicals and solvents were purchased from Sigma-Aldrich and Fisher Scientific.

1-Trimethylsilyl-2-(3,4,5-trimethoxyphenyl)acetylene. A 50-mL two-necked round-bottomed flask equipped with a septum, a stir-bar, and a water condenser topped with a nitrogen inlet was charged with a mixture of compound **1** (588 mg, 2.0 mmol), $PdCl_2(PPh_3)_2$ (70 mg, 0.1 mol), CuI (19 mg, 0.1 mol), and triethylamine (TEA, 15 mL). Trimethylsilylacetylene (0.47 mL, 3.4 mmol) was added to it at room temperature (rt). After 30 min the solution was heated to 50 °C under nitrogen. After complete consumption of starting material (monitored by thin layer chromatography (TLC)) the mixture was cooled to rt and filtered, and the solid was washed with dichloromethane (DCM, 10 mL). The filtrate was concentrated under reduced pressure to give a crude product, which subjected to flash chromatography purification on silica gel to give the pure product (470 mg, 89%). ^{1}H NMR (DMSO-d_6) δ 6.73 (s, 2H), 3.78 (s, 6H), 3.66 (s, 3H), 0.22 (s, 9H).

3,4,5-Trimethoxyethynylbenzene (**2**).[6] Compound **2** was synthesized in 82% yield based on the literature method reported for the compound.[6] ^{1}H NMR (DMSO-d_6) δ 6.78 (s, 2H), 4.13 (*s*, 1H), 3.77 (s, 6H), 3.66 (s, 3H).

1,2,3-Trimethoxy-5-(2-(4-methoxy-2-nitrophenyl)ethynyl)benzene (**3**). To a solution containing compound **2** (240 mg, 1.25 mmol) and 4-iodo-3-nitroanisole (345 mg, 1.24 mmol) were added $PdCl_2(PPh_3)_2$ (44 mg, 5.0 mol %) and CuI (12 mg, 5.0 mol %) in TEA (15 mL). The mixture was stirred at 55 °C for 3 h, cooled, and filtered. The filtrate was concentrated under reduced pressure. Chromatography of the residue on silica gel gave compound **3** (380 mg, 90%). ^{1}H NMR (DMSO-d_6) δ 8.06 (d, J = 2.4 Hz, 1H), 7.82 (dd, J = 2.0, 8.8 Hz, 1H), 7.42 (d, J = 8.8 Hz), 6.89 (*s*, 2H), 3.97 (s, 3H), 3.81 (s, 6H), 3.7 (s, 3H).

5-Methoxy-2-(2-(3,4,5-trimethoxyphenyl)ethynyl)amine (**4**). Compound **3** (140 mg, 0.41 mmol) was suspended in EtOH (95%, 15 mL) and stirred at 80 °C for 30 min. To this mixture were added concentrated HCl (0.017 mL) and iron powder (230 mg, 8.3 mmol). The reaction mixture was refluxed for 2 h, cooled, and filtered. The filtrate was concentrated under reduced pressure. Chromatography of the residue on silica gel gave compound **4** (69 mg, 54%). ^{1}H NMR (DMSO-d_6) δ 7.11 (d, J = 11.6 Hz, 1H), 6.88 (s, 2H), 6.28 (d, J = 2.8 Hz, 1H), 6.13 (dd, J = 2.8, 11.6 Hz, 1H), 5.52 (br, s, NH_2), 3.80 (s, 6H), 3.69 (s, 3H), 3.67 (s, 3H).

(6-Methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**5**). To a solution containing compound 4 (170 mg, 0.54 mmol) in water/acetone (V/V = 1:2 10 mL) was added dropwise 10% HCl (3 mL). The resulting mixture was cooled down to −10 °C. A solution of $NaNO_2$ (56 mg, 0.81 mmol) in water (1 mL) was added to the reaction mixture and stirred for 30 min at −10 to −5 °C. After water (100 mL) was added, the reaction mixture was warmed to rt, stirred for 30 min, and extracted with AcOEt (2 × 15 mL). The organic phase was washed with 10% $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatography of the residue on silica gel afforded compound **5** (147 mg, 80%). ^{1}H NMR (DMSO-d_6) δ 13.8 (s, NH), 8.12 (d, J = 8.8 Hz, 1H), 7.70 (s, 2H), 7.06 (s, 1H), 6.98 (d, J = 8.4 Hz 1H), 3.86 (s, 9H), 3.78 (s, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 55.6, 56.3, 61.0, 91.3, 108.5, 115.7, 118.5, 123.4, 133.1, 142.4, 142.5, 143.2, 152.9, 160.1, 188.2. HRMS Calcd for $C_{18}H_{18}N_2O_5$: 342.1216. Found 342.1214.

(7-Iodo-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**6**). To a solution of *N*-iodosuccinimide (45 mg, 0.2 mmol, 1.0 equiv) in AcOH (1 mL) was added a solution of **5** (68 mg, 0.2 mmol, 1.0 equiv) in AcOH (2 mL) at rt. The mixture was stirred at rt for 2 h, diluted with 8 mL water and extracted with EtOAc (10 mL × 3). Combined organic layers were neutralized to pH 7 with 10% $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was crystallized from MeOH to yield compound **6** (80 mg, 85%). ^{1}H NMR ($CDCl_3$) δ 10.2 (br, s, NH), 8.36 (d, J = 8.8 Hz, 1H), 7.75 (s, 2H), 7.03 (d, J = 8.8 Hz, 1H), 4.04 (s, 3H), 3.97 (s, 3H), 3.96 (s, 6H). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 55.9, 57.0, 60.5, 62.1, 108.2, 109.7, 119.0, 123.4, 132.2, 142.1, 143.8, 144.4, 152.4, 157.9, 187.4. HRMS Calcd for $C_{18}H_{17}IN_2O_5$: 468.0182. Found 468. 0187.

(6-Methoxy-7-(prop-1-ynyl)-1*H*-indazol-3-yl)(3,4,5-tri-methoxyphenyl)methanone (**7a**)**.** A mixture of compound **6c** (46 mg, 0.1 mmol), $PdCl_2(PPh_3)_2$ (7 mg, 0.1 mmol), and CuI (2 mg, 0.1 mmol) in Et_3N (6 mL) was thrice degassed and exchanged with propyne. Propyne contained in a balloon was kept in contact with the reaction mixture. The system was stirred at 45 °C overnight and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified with flash chromatography on silica gel to yield compound **7a** (22 mg, 58%). ^{1}H NMR ($CDCl_3$) δ 11.3 (br, s, NH), 8.27 (d, J = 8.8 Hz, 1H), 7.71 (s, 2H), 7.01 (d, J = 9.2 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.87 (s, 6H), 2.12 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 4.8, 56.1, 56.9, 60.8, 70.8, 93.6, 95.6, 108.4, 110.0, 118.3, 123.2, 132.4, 142.4, 143.0, 144.2, 152.6, 159.9, 187.1. HRMS Calcd for $C_{21}H_{20}N_2O_5$: 380.1372. Found 380.1377.

General Procedure for Preparation of Compounds 7b−i from Compound 6. A mixture of compound **6** (468 mg, 1 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol), and CuI (10 mg, 0.05 mmol) in Et_3N (70 mL) was thrice degassed and exchanged with argon followed by addition of alkynes at rt. The reaction mixture was stirred at 55 °C for 5−10 h (monitored by TLC) and filtered. The filtrate was concentrated under reduced pressure, and the residue was separated employing flash chromatography on silica gel to yield compounds **7b-i**.

(7-(3-Hydroxyprop-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**7b**)**.** Compound **7** was obtained as white solid in 39% yield. ^{1}H NMR (DMSO-d_6) δ 14.03 (s, NH), 8.21 (d, J = 9.2 Hz, 1H), 7.69 (s, 2H), 7.24 (d, J = 9.2 Hz, 1H), 5.35 (br, s, OH), 4.43 (s, 2H), 3.95 (s, 3H), 3.86 (s, 6H), 3.79 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 49.9, 56.0, 56.6, 60.1, 75.5, 92.5, 98.9, 108.1, 110.2, 118.0, 123.2, 132.2, 141.8, 142.6, 142.9, 152.4, 159.5, 186.1. HRMS Calcd for $C_{21}H_{20}N_2O_6$: 396.1321. Found 396.1319.

***R*-(7-(3-Hydroxybut-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (7c).** Compound **7c** was obtained as a light yellow solid in 52% yield. ^{1}H NMR (DMSO-d_6) δ 13.97 (s, NH), 8.20 (d, J = 9.2 Hz, 1H), 7.71 (s, 2H), 7.23 (d, J = 9.2 Hz, 1H), 5.43 (d, J = 5.2 Hz, OH), 4.72 (m, 1H), 3.94 (s, 3H), 3.87 (s, 6H), 3.79 (s, 3H), 1.48 (d, J = 6.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) 25.2, 56.7, 57.4, 57.9, 60.9, 74.8, 93.3, 103.1, 108.9, 111.1, 118.8, 123.8, 133.0, 142.6, 143.2, 143.7, 153.1, 160.0, 186.9. HRMS Calcd for $C_{22}H_{22}N_2O_6$: 410.1478. Found 420.1481.

S-(7-(3-Hydroxybut-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**7d**). Compound **7d** was obtained as a light yellow solid in 55% yield. ^{1}H NMR (DMSO-d_6) δ 14.0 (br, s, NH), 8.20 (d, J = 8.8 Hz, 1H), 7.71 (s, 2H), 7.24 (d, J = 9.2 Hz, 1H), 5.44 (br, *s*, OH), 4.71 (m, 1H), 3.94 (s, 3H), 3.87 (s, 6H), 3.79 (s, 3H), 1.48 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$ + CD_3OD) 23.8, 55.9, 56.7, 58.4, 60.6, 75.1, 92.6, 100.1, 108.3, 109.8, 118.4, 123.6, 132.4, 142.1, 142.9, 143.5, 152.5, 159.5, 187.5. HRMS Calcd for $C_{22}H_{22}N_2O_6$: 410.1478. Found 420.1480.

(7-(3-Hydroxy-3-methylbut-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (7e). Compound **7e** was obtained as a light yellow solid in 61% yield. ^{1}H NMR ($CDCl_3$) δ 12.3 (s, NH), 8.24 (d, J = 9.2 Hz, 1H), 7.75 (s, 2H), 6.87 (d, J = 9.2 Hz, 1H), 5.02 (br, *s*, OH), 3.90 (s, 3H), 3.89 (s, 3H), 3.84 (s, 6H), 1.79 (s, 6H). ^{13}C NMR ($CDCl_3$ + CD_3OD) 30.9, 55.9, 56.8, 60.6, 65.5, 73.5, 92.6, 102.8, 108.3, 110.0, 118.4, 123.6, 132.4, 142.1, 142.8, 143.6, 152.5, 159.4, 187.3. HRMS Calcd for $C_{23}H_{24}N_2O_6$: 424.1634. Found 424.1636.

(7-(4-Hydroxybut-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (7f). Compound **7f** was obtained as a light yellow solid in 85% yield. ^{1}H NMR (DMSO-d_6) δ 14.0 (s, NH), 8.17 (d, J = 8.8 Hz, 1H), 7.70 (s, 2H), 7.22 (d, J = 9.2 Hz, 1H), 4.94 (t, J = 5.6 Hz, HO), 3.93 (s, 3H), 3.87 (s, 6H), 3.79 (s, 3H), 3.69 (m, 2H), 2.69 (t, J = 6.8 Hz, 1H). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 23.7, 55.7, 56.6, 60.2, 60.4, 72.7, 93.4, 96.6, 108.2, 109.7, 118.3, 122.9, 132.4, 141.9, 142.9, 143.3, 152.3, 159.6, 187.4. HRMS Calcd for $C_{22}H_{22}N_2O_6$: 410.1478. Found 420.1482.

(7-(5-Hydroxypent-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**7g**). Compound **7g** was obtained as a light yellow solid in 71% yield. ^{1}H NMR (DMSO-d_6) δ 14.0 (s, NH), 8.16 (d, J = 8.8 Hz, 1H), 7.70 (s, 2H), 7.21 (d, J = 8.8 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 6H), 3.79 (s, 3H), 3.57 (t, J = 6.4 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 1.79 (m, 2H). ^{13}C NMR ($CDCl_3$+CD_3OD) δ 16.6, 30.8, 56.1, 56.9, 60.8, 61.0, 72.4, 93.7, 99.1, 108.4, 110.0, 118.4, 123.1, 132.6, 142.3, 143.3, 143.7, 152.6, 159.6, 187.5. HRMS Calcd for $C_{23}H_{24}N_2O_6$: 424.1634. Found 424.1636.

(6-Methoxy-7-trimethylsilylethynyl-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (7h). Crude **7h** (0.43 g, 98%) was obtained and used for the preparation of compound 8 without further purification.

2-(3-(6-Methoxy-3-(3,4,5-trimethoxybenzoyl)-1*H*-indazole-7-yl)prop-2-ynyl)isoindoline-1,3-dione (7i). Compound **6** (117 mg 1 equiv) and *N*-2-propynylphthalimide (93 mg 2 equiv) were reacted to yield compound **7i** (70 mg, 59%). ^{1}H NMR (DMSO-d_6) δ 14.06 (s, NH), 8.21 (d, J = 9.2 Hz, 1H), 7.96 (m, 2H), 7.89 (m, 2H), 7.67 (s, 2H), 7.23 (d, J = 8.8 Hz, 1H), 4.76 (s, 2H), 3.94 (s, 3H), 3.84 (s, 6H), 3.77 (s, 3H).

(7-Ethynyl-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (8). To a solution containing crude compound **7h** (430 mg), water (0.5 mL), and THF (9.5 mL) was added tetrabutylammonium fluoride solution in THF (1 M, 3.0 mL) at 0 °C. The mixture was stirred from 0 °C to rt 4 h. After the solvent was removed under reduced pressure, DCM (10 mL) was added. The organic phase was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **8** (260 mg, 72%). ^{1}H NMR (DMSO-d_6) δ 14.07 (s, NH), 8.23 (d, J = 8.8 Hz, 1H), 7.67 (s, 2H), 7.25 (d, J = 8.8 Hz, 1H), 4.72 (s, 1H), 3.96 (s, 3H), 3.86 (s, 6H), 3.79 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 56.3, 57.0, 60.9, 75.3, 86.6, 91.5, 108.5, 109.9, 118.4, 125.0, 132.3, 142.6, 143.0, 144.6, 152.8, 161.2, 187.0. HRMS Calcd for $C_{20}H_{18}N_2O_5$: 366.1216. Found 366.1219.

(7-(3-Aminoprop-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (**9**). N_2H_4•H_2O (0.2 mL) was added to a solution of compound **7i** (100 mg) in a mixed solvent (MeOH (10 mL)/THF (3 mL)) at rt. The mixture was refluxed for 3 h. After removal of solvent under vacuum, the residue was purified by flash chromatography on silica gel to yield compound **9** (67 mg, 81%). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.23 (d, J = 9.2 Hz, 1H), 7.67 (s, 2H), 6.98 (d, J = 9.2 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 6H), 3.87 (s, 3H), 3.77 (s, 2H), 3.4 (br, s, NH_2). ^{13}C NMR ($CDCl_3$+CD_3OD) δ 31.8, 56.0, 56.8, 60.7, 75.1, 92.7, 97.4, 108.4, 109.6, 118.5, 123.8, 132.6, 142.2, 143.3, 143.7, 152.6, 159.5, 187.5. HRMS Calcd for $C_{21}H_{21}N_3O_5$: 395.1481. Found 395.1479.

(7-(3-*tert*-Butyldimethylsilanyloxy-prop-1-ynyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (10). The same reaction procedures were carried out as for the compounds **7b**−**i**. Compound **6** (468 mg 1 equiv) and *tert*-butyldimethyl(prop-2-ynyloxy)silane (425 mg 2.5 equiv) were used as starting materials to yield compound **10** (460 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 10.6 (br, s, NH), 8.35 (d, J = 9.2 Hz, 1H), 7.72 (s, 2H), 7.07 (d, J = 9.2 Hz, 1H), 4.71 (s, 2H), 4.02 (s, 3H), 3.96 (s, 6H), 3.95 (s, 3H), 0.97 (s, 9H), 0.22 (s, 6H).

(*Z*)-(7-(3-*tert*-Butyldimethylsilanyloxy-prop-1-enyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (11). A 20 mg amount of 10% Pd/C was added to a solution of 180 mg of compound **10** in AcOEt (25 mL) under a nitrogen atmosphere, and the mixture was then purged with hydrogen thrice, stirred under hydrogen at rt overnight, and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified with flash chromatography on silica gel to yield compound **11** (123 mg, 68%). ^{1}H NMR ($CDCl_3$) δ 12.5 (s, NH), 8.43 (d, J = 8.8 Hz, 1H), 7.93 (s, 2H), 7.09 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 11.2 Hz, 1H), 6.09 (m, 1H), 4.23 (d, J = 8.8 Hz, 2H), 3.98 (s, 3H), 3.97 (s, 6H), 3.96 (s, 3H), 1.03 (s, 9H), 0.20 (s, 6H).

(*Z*)-(7-(3-Hydroxyprop-1-enyl)-6-methoxy-1*H*-indazol-3-yl)-(3,4,5-trimethoxyphenyl)methanone (12). The same reaction procedures were carried out as for compound **8**. Compound **11** (128 mg 1 equiv) and tetrabutylammonium fluoride solution in THF (1 M, 0.75 mL) were used as starting material to yield compound **12** (83, mg 83%). ^{1}H NMR ($CDCl_3$) δ 12.75 (s, NH), 8.24 (d, J = 8.8 Hz, 1H), 7.61 (s, 2H), 7.04 (d, J = 8.8 Hz, 1H), 8.67 (d, J = 11.2 Hz, 1H), 6.10 (m, 1H), 4.06 (d, J = 7.6 Hz, 2H), 3.90 (s, 3H), 3.88 (s, 9H), 3.36 (br, s, HO). ^{13}C NMR ($CDCl_3$) δ 56.4, 56.8, 59.1, 60.9, 106.1, 108.4, 109.9, 118.9, 123.6, 126.1, 129.2, 132.8, 140.6, 142.5, 143.8, 152.8, 157.0, 187.5. HRMS Calcd for $C_{21}H_{22}N_2O_6$: 398.1478. Found 398.1482.

(7-(3-Hydroxypropyl)-6-methoxy-1*H*-indazol-3-yl)(3,4,5-trimethoxyphenyl)methanone (13). A 7 mg amount of PtO_2 was added to a solution of 69 mg of compound **11** in AcOEt (12 mL), purged with hydrogen thrice, stirred under hydrogen at rt overnight, and filtered. The filtrate was concentrated under reduced pressure, and the residue was then treated with a solution of THF (95%, 4 mL) and tetrabutylammonium fluoride solution (1 M in THF, 0.3 mL) at 0 °C for 4 h. After the solvent was removed under reduced pressure, DCM (7 mL) was added. The organic phase was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **13** (34 mg, 85%). ^{1}H NMR ($CDCl_3$) δ 11.77 (br, s, NH), 8.25 (d, J = 8.8 Hz, 1H), 7.70 (s, 2H), 7.09 (d, J = 8.8 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93 (s, 6H), 3.63 (m, 2H), 3.11 (t, J = 6.4 Hz, 2H), 1.94 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 20.0, 31.1, 56.3, 56.8, 60.87, 60.97, 108.4, 109.6, 100.6, 118.6, 121.4, 132.9, 142.4, 142.9, 144.0, 152.8, 156.3, 197.5. HRMS Calcd for $C_{21}H_{24}N_2O_6$: 400.1634. Found 400.1631.

(7-Iodo-6-Methoxy-1*H*-indol-3-yl)(3,4,5-trimethoxyphenyl)-methanone (15). Starting material **14** was synthesized based on the literature method,[5] and **15** was prepared in a similar way as compound **6**, except the starting material was **14** and was obtained as light yellow solid at a 71% yield. ^{1}H NMR ($CDCl_3$) δ 8.79 (br, s, NH), 8.26 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 2.8 Hz, 1H), 7.10 (s, 2H), 6.93 (d, J = 8.4 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.88 (s, 6H).

(6-Methoxy-7-(prop-1-ynyl)-1*H*-indol-3-yl)(3,4,5-trimethoxyphenyl)methanone (16). The same reaction procedures were carried out as for compound **7a**, and compound **16** was obtained as light yellow sold in 65% yield. ^{1}H NMR ($CDCl_3$) δ 8.89 (br, s, NH), 8.25 (d, J = 8.8 Hz, 1H), 7.67 (d, J = 2.4 Hz, 1H), 7.11 (s, 2H), 6.98 (d, J = 8.8 Hz, 1H), 3.99 (s, 3H), 3.94 (s, 3H), 3.90 (s, 6H), 2.22 (s, 3H).

***In Vitro* Proliferation Assay.** Exponentially growing cells were seeded at a density ranging from 4000 to 6000 cells per well in 96-well plates and incubated at 37 °C in 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of compounds. The next day, the cell population for each cell line at the time of drug addition (T_0) was measured using the AlamarBlue assay. Compounds were solubilized in 100% DMSO at 200 times the desired final test concentration. At the time of drug addition, compounds were further diluted to 4 times the desired final concentration with complete medium. Following drug addition, the plates were incubated for 72 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. At the end of incubation, the viable cells were quantified using AlamarBlue. Growth inhibition of 50% (IC_{50}) was calculated using Prism software at each of the drug concentrations.

Tubulin Binding. 7b at different concentrations was incubated with purified tubulin (bovine brain, MCF-7 cells, or Hela cells) for 10 min at 37 °C. [^{3}H]Colchicine was then added to the samples and incubated for an additional 30 min at 37 °C. To separate the bound from unbound [^{3}H]colchicine, the samples were spotted onto DE81 filter and washed three times to remove unbound [^{3}H]-colchicine. The bound colchicine on the filter was measured using a scintillation counter.

***In Vitro* Tubulin Polymerization Assay.** *In vitro* tubulin polymerization assays were conducted with reagents as described by the manufacturer (Cytoskeleton Inc.). In brief, **7b** was incubated with purified bovine tubulin and buffer containing 10% glycerol

and 1 mM GTP at 37 °C, and the effect of **7b** on tubulin polymerization was monitored kinetically using a fluorescent plate reader.

Supporting Information Available: Characterization (^{1}H NMR, ^{13}C NMR, and HRMS data) of all the new compounds and the purities of all the new compounds from normal-phase HPLC and reverse-phase HPLC analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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