Supporting Information

Discovery of 4-Aryl-2-benzoyl-imidazoles As Tubulin Polymerization Inhibitor with Potent Antiproliferative Properties

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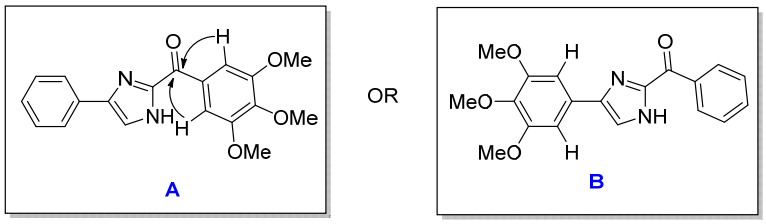
Contents of Supplementary Information:

Structure elucidation of **12a**



In order to confirm the structure of compound **12a**, we performed two-dimensional ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) NMR measurement. If the structure of **12a** is A as shown in the above figure, then the two protons on the trimethoxyphenyl (TMP) moiety would have long coupling to the carbonyl carbon in HMBC spectrum. In contrast, if the structure of **12a** is B as shown in the above, then there will no such long range coupling. As shown in the NMR spectrum below, there is a clearly strong correlation peak indicating the HMBC coupling between the two protons on TMP ring (δ 8.28 ppm) and the carbonyl carbon (δ 179.41 ppm). This confirms that the structure of compound **12a** is A.

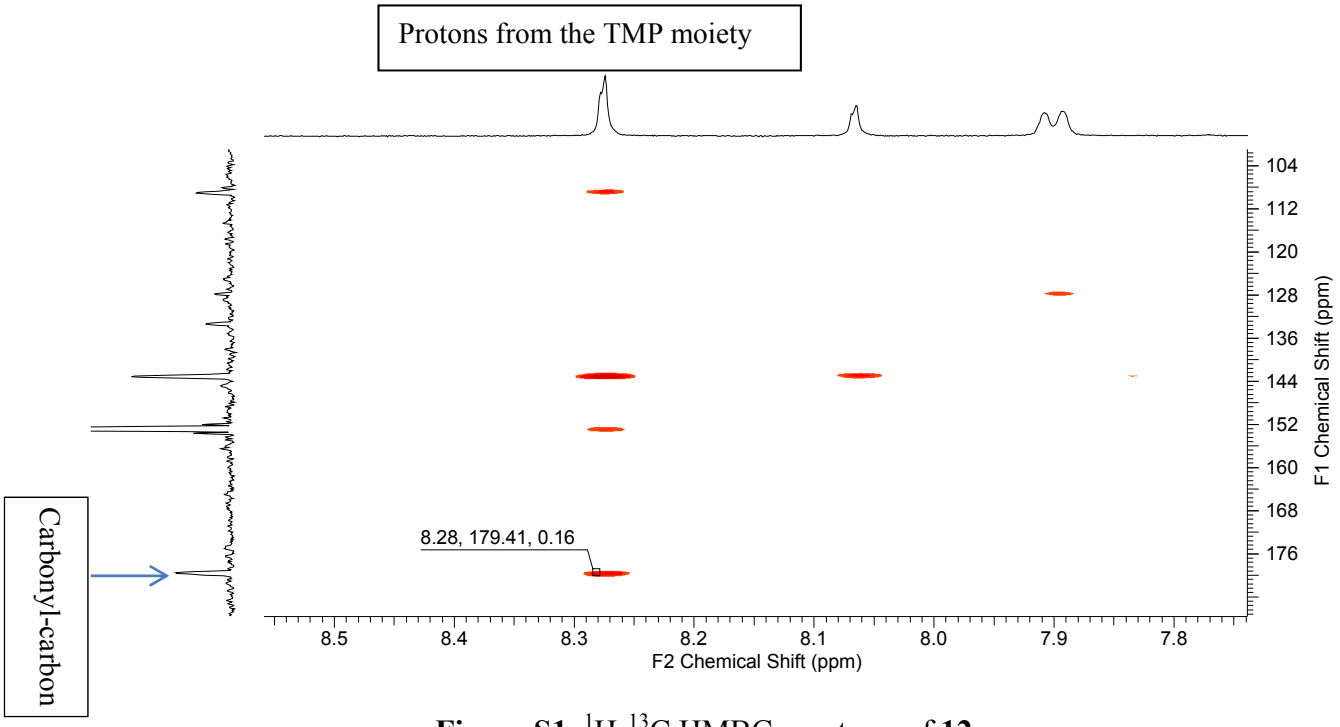


Figure S1. ^{1}H-^{13}C HMBC spectrum of **12a**

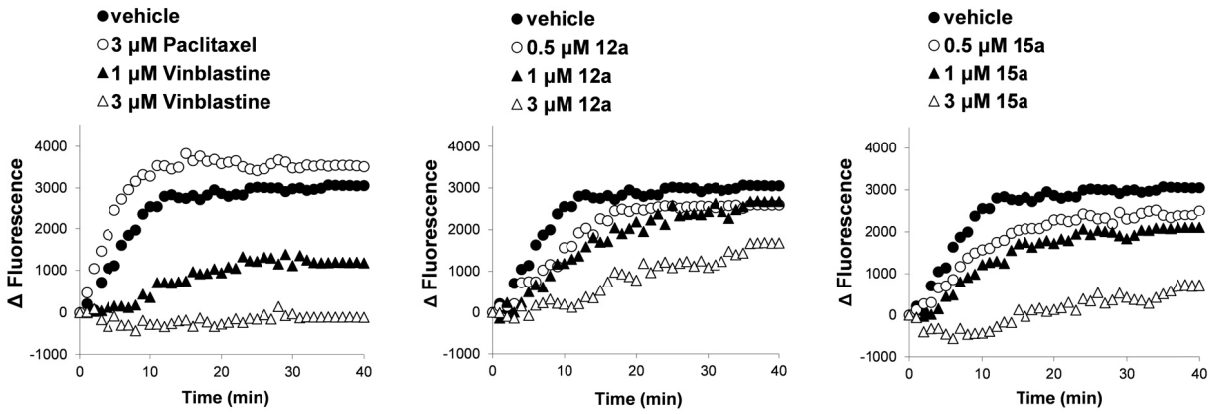


Figure S2. Effect of RABI compounds on tubulin polymerization *in vitro*

Experimental section for Figure S2.

The drug effects on tubulin polymerization were determined using a fluorescence-enhanced tubulin polymerization assay kit (Cytoskeleton, cat# BK011P). Porcine brain tubulin (0.56 mg, >99% pure) was mixed with test compounds and incubated in 50 μL of tubulin buffer (80 mM PIPES, 2.0 mM MgCl2, 0.5 mM EGTA, 1 mM GTP, and 10 μM fluorescent reporter) with 10% glycerol at 37^{o}C. The assay was monitored every 1 min for 40 min by the SYNERGY 4 Microplate Reader (Bio-Tek Instruments, Winooski, VT) at the excitation wavelength 340 to 360 nm and emission 410 to 460 in kinetic mode. Polymerization is measured by fluorescence enhancement.

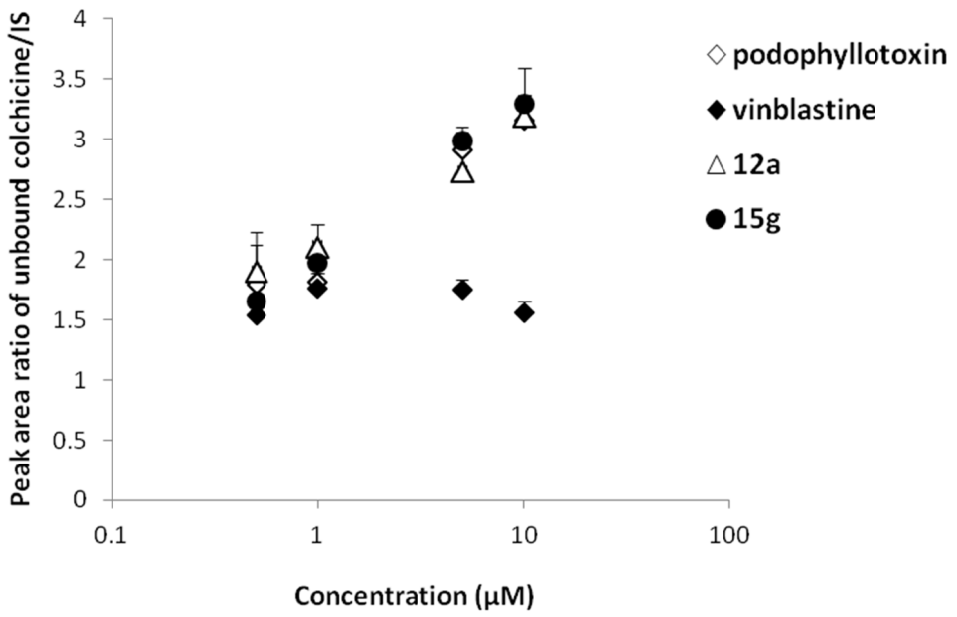


Figure S3. Competitive mass spectrometry binding showed RABI compounds competitively bound to the colchicine-binding site on tubulin. Podophyllotoxin is a known ligand binding to the colchicine site in tubulin and serves as a positive control. Vinblastine binds to a different site in tubulin and serves as a negative control.

Experimental Section for Figure S3.

Competitive Mass Spectrometry Binding Assay

The binding site of 12a and 15g in tubulin protein was evaluated by the competitive mass spectrometry binding assay as previously described.[1] Briefly, colchicine (1.2 μM) was incubated with bovine brain tubulin (1.0 mg/mL) in the incubation buffer [80 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), 2.0 mM magnesium chloride ($MgCl_2$), 0.5 mM ethylene glycol tetra acetic acid (EGTA), pH 6.9] at 37°C for 1 h. Different concentrations (0.5–10 μM) of podophyllotoxin (positive control), 12a, 15g, and vinblastine (negative control) were used to

compete with the binding of colchicine to tubulin. After 1 h incubation, the amount of unbound colchicine was obtained from the filtrate by ultrafiltration (Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-30 membrane, Millipore, Billerica, MA). The amount of unbound colchicine in the presence or absence of any competitor was measured. Each experiment was performed in triplicate.

Analysis method

An high performance liquid chromatography (HPLC) system (Model 1100 Series Chemstation, Agilent Technology Co, Santa Clara, CA), C12 column (Synergi™, 50 x 2 mm, phenomenex , Torrance, CA) with C12 guard column (SecurityGuard™, phenomenex , Torrance, CA) and triple-quadruple mass spectrometer (API Qtrap4000^{TM}, Applied Biosystems/MDS SCIEX, Concord, Ontario, Canada) with a Turbo Ion Spray source were used to develop this method. Gradient mode was used to achieve the separation of analytes using mixtures of mobile phase A [5%/95% (v/v) acetonitrile/H_2O containing 0.1% formic acid] and mobile phase B [95%/5% (v/v) acetonitrile/H_2O containing 0.1% formic acid] at a flow rate of 250 μL/min. Mobile phase A was used at 90% from 0 to 1 min followed by a linearly programmed gradient to 100% of mobile phase B within 3 min; 100% of mobile phase B was maintained for 1 min before a quick ramp to 90% mobile phase A. Mobile phase A was continued for another 6min toward the end of analysis. Multiple reaction monitoring mode, scanning *m*/*z* 400 → 310 (colchicine) and *m*/*z* 260.2 → 183.1 (internal standard, propranolol), was used to obtain the most sensitive signals for these tubulin ligands. The spraying needle voltage was set at 5000 V for positive mode, collision-assisted dissociation gas at medium, and the source heater probe temperature at 500°C. Collision energies were set at 40 and 25 V, respectively. Gas 1 and 2 were set at 50. Unit resolution was

selected for Q1 and Q3. The dwell time was set 300 ms. Data acquisition and quantitative processing were accomplished using Analyst^{TM} software, Ver. 1.4.2 (Applied Biosystems).

References:

1. Li, C. M.; Lu, Y.; Ahn, S.; Narayanan, R.; Miller, D. D.; Dalton, J. T., Competitive mass spectrometry binding assay for characterization of three binding sites of tubulin. *Journal of mass spectrometry : JMS* **2010,** *45* (10), 1160-6.