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N-Sulfonyl-aminobiaryls as Antitubulin Agents and Inhibitors of Signal Transducer and Activator of Transcription 3 (STAT3) Signaling

Mei-Jung Lai,[‡,&] Hsueh-Yun Lee,[†,&] Hsun-Yueh Chuang, Li-Hsun Chang,[#] An-Chi Tsai,[¶] Mei-Chuan Chen,[§] Han-Lin Huang,[¶] Yi-Wen Wu,[¢] Che-Ming Teng,[#] Shiow-Lin Pan,[¶,¢] Yi-Min Liu,[†] Samir Mehndiratta,[†] Jing-Ping Liou[*,†,¥]

School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wuxing Street, Taipei 11031, Taiwan. Ph.D Program for the Clinical Drug Discovery from Botanical Herbs, College of Pharmacy, Taipei Medical University. Center for Translational Medicine, Taipei Medical University. School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan. The Ph.D program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University. School of Pharmacy, National Defense Medical Center, Taipei, Taiwan. Department of Pharmacology, College of Medicine, Taipei Medical University, Taipei, Taiwan.

[&] Contributed equally to this work.

[†] School of Pharmacy, College of Pharmacy, Taipei Medical University.

[§] Ph.D Program for the Clinical Drug Discovery from Botanical Herbs, College of Pharmacy, Taipei Medical University.

[‡] Center for Translational Medicine, Taipei Medical University.

[#] Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan.

[¶] The Ph.D Program for Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University.

[¥] School of Pharmacy, National Defense Medical Center, Taipei, Taiwan.

[¢] Department of Pharmacology, College of Medicine, Taipei Medical University, Taipei, Taiwan.

ABSTRACT. A series of *N*-sulfonyl-aminobiaryl derivatives have been examined as novel antitubulin agents. Compound **21** [N-(4'-cyano-3'-fluoro-biphenyl-2-yl)-4-methoxy-benzenesulfonamide] exhibits remarkable antiproliferative activity against four cancer cell lines (pancreatic AsPC-1, lung A549, liver Hep3B, and prostate PC-3) with a mean GI_{50} value of 57.5 nM. Additional assays reveal that **21** inhibits not only tubulin polymerization but also the phosphorylation of STAT3 inhibitory with an IC_{50} value of 0.2 μM. Four additional compounds (**8**, **10**, **19**, and **35**) are also able to inhibit this phosphorylation. This study describes novel *N*-sulfonyl-aminobiaryl (biaryl-benzenesulfonamides) as potent anticancer agents targeting both STAT3 and tubulin.

Introduction

Microtubules are assembled by the aggregation of α- and β-tubulin heterodimers in a head-to-tail manner to form hollow cylindrical filaments.[1] In the cell cycle, specifically the M-phase, microtubules assembled as a spindle act as guides that direct chromosomes into individual cells; they therefore are a crucial target for development of anticancer agents. Natural products such as paclitaxel (**1**), vinca alkaloids (**2**), and colchicine (**3**) were found to bind to microtubules at specific binding sites and thus display remarkable microtubule-targeting activity. These microtubule-targeting compounds are functionally categorized into microtubule-stabilizers (**1**) and microtubule-destabilizers (**2** and **3**).[2] In addition to their involvement in the proliferation process, microtubules are associated with several functions including cell migration and cell skeleton development. These alternative functions are probably correlated with post-translational modifications such as acetylation, polyglycylation, polyglutamylation, and tyrosination of tubulin.[3]

Signal transducers and activators of transcription 3 (STAT3) proteins are transcription factors normally resident in the cytoplasm. They are activated by phosphorylation by the JAK family of kinases and subsequently translocate into the nucleus where they regulate gene transcription.[4,5] This signaling pathway is transient in normal cells but in cancer cells, STAT3 is constitutively activated, triggering

proliferation and oncogenesis.[6-9] The poorly understood association of STAT3 protein with tubulin correlates promotion of cell migration[10], but is probably destroyed by microtubule targeting agents. For example, paclitaxel (**1**), a tubulin stabilizing agent, has been reported to be capable of triggering the inhibition of STAT3 expression.[11] Consequently, development of multifunctional bioactive molecules can support clinical indications in the treatment of diverse cancers.

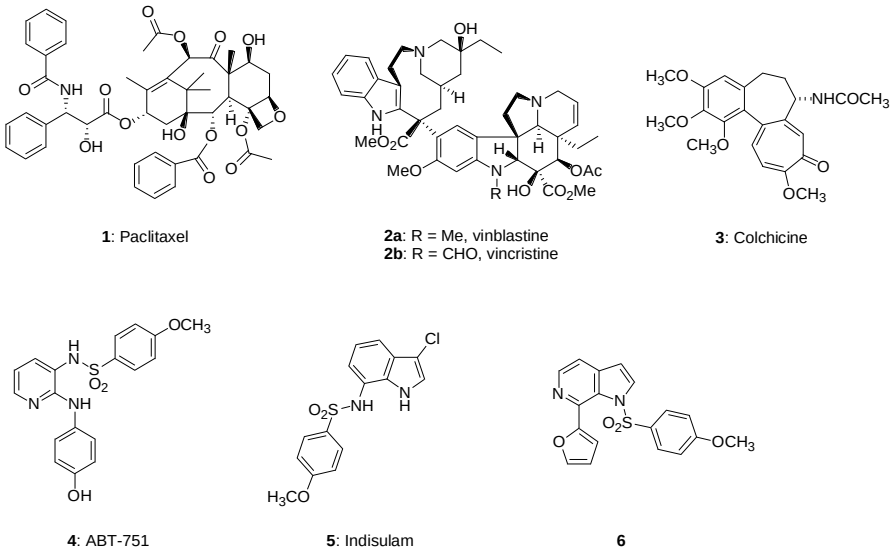
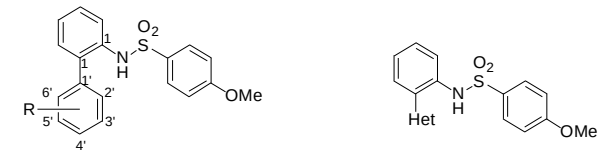


Figure 1. Natural microtubule-targeting molecules and synthetic benzensulfonamides.

The benzenesulfonamide moiety has been observed in numerous antitubulin agents such as ABT-751 (**4**) and indisulam (**5**), which are currently undergoing clinical trials.[12] Our earlier work on the modification of compound ABT-751 revealed that the benzenesulfonamide moiety is crucial for the compound's anticancer activity.[13-15] In addition, biaryl moieties have been frequently observed in the development of anti-tubulin agents.[15-17] Compound **6**, with both benzenesulfonamide and biaryl moieties, was found in our previous study to have remarkable antiproliferative activity.[15] Literature surveys indicate that the *N*-sulfonyl-aminobiaryl moiety has been comprehensively included in various heterocycles such as carbazole[18], phenanthridinones, and phenanthridines[19,20]; but little investigation of its biological potential has been reported., This study therefore, is aimed at synthesis of a series of *N*-sulfonyl-aminobiaryl derivatives (**7**-**26**) and assays of their activity against the growth of cancer cells. In

view of the known correlation of microtubule-targeting molecules with inhibition of the STAT3 protein, the synthetic compounds (**7**-**26**) have also been examined for their inhibitory activities in tubulin polymerization and STAT3 phosphorylation.



7: R = H
8: R = 4'-F
9: R = 4'-Cl
10: R = 4'-CN
11: R = 4'-OH
12: R = 4'-OMe
13: R = 4'-COOMe
14: R = 4'-COOH
15: R = 3'-F
16: R = 3'-Cl
17: R = 3'-CN
18: R = 3'-CF_3
19: R = 3',4'-diF
20: R = 3'-Cl-4'-F
21: R = 3'-F-4'-CN
22: R = 3',4'-diOMe
23: R = 3',4',5'-triOMe

24: Het = 4-pyridinyl
25: Het = 3-pyridinyl
26: Het = 2-furanyl

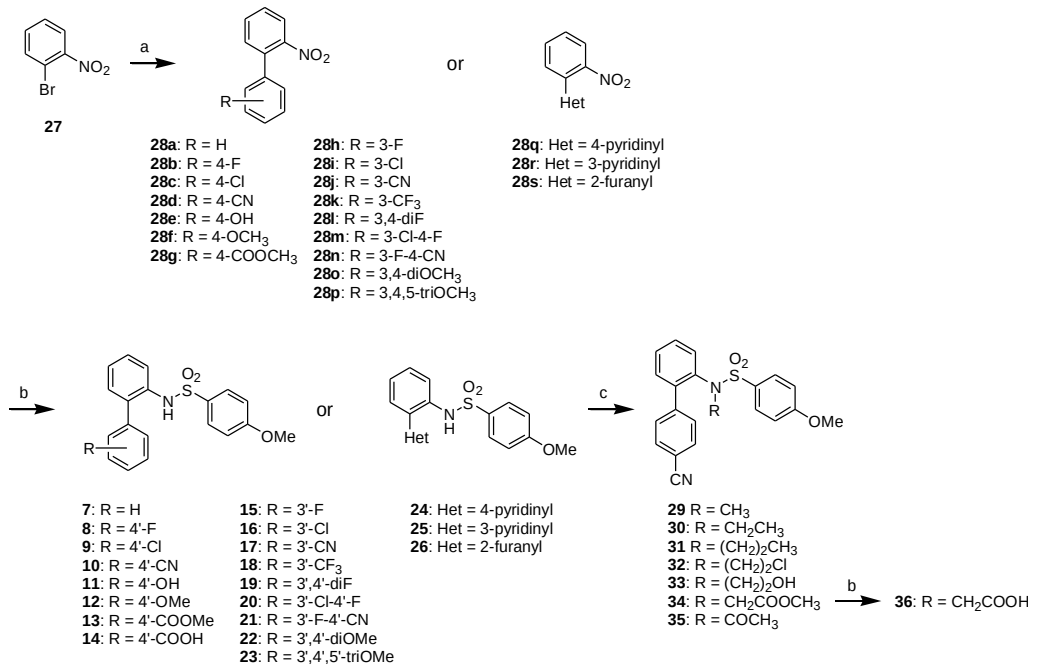
Figure 2. Synthetic *N*-sulfonyl-aminobiaryl compounds (**7**-**26**).

Results and Discussion

Chemistry

Scheme 1 shows the synthetic routes to *N*-sulfonyl-aminobiaryl compounds (**7**-**26** and **29**-**36**). 1-Bromo-2-nitrobenzene (**27**) was allowed to react with various phenylboronic acids under conditions of the Suzuki reaction, yielding the corresponding 2-nitrobiphenyls (**28a**-**s**). The nitro groups were reduced by iron powder in the presence of NH_4Cl and refluxing EtOH to afford the corresponding amines and this was followed by reaction with 4-methoxybenzenesulfonyl chloride to provide compounds **7**-**26**. To explore the effect of *N*-substitution on biological activity, compound **10** was reacted with various alkyl halides in the presence of K_2CO_3, yielding compounds **29**-**35**. The ester group of compound **34** was hydrolyzed by LiOH to afford the corresponding carboxylic acid **36**.

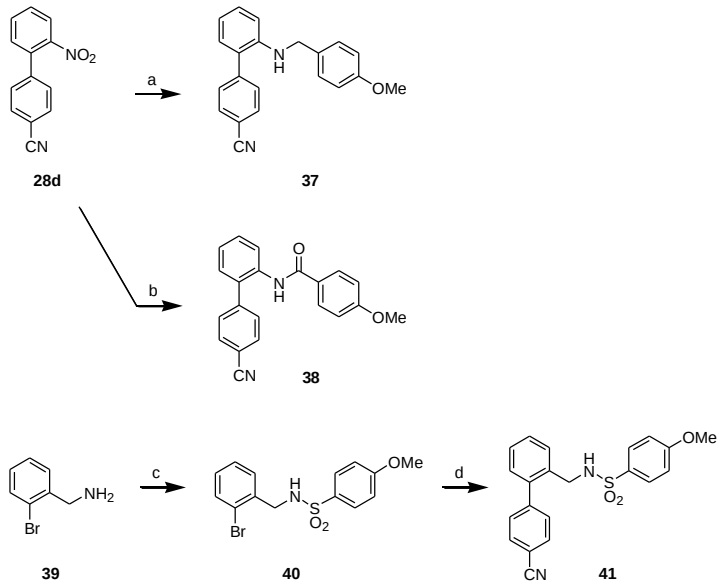
Scheme 1. Synthetic Approaches to Compounds **7**-**26** and **29**-**36**[a]



[a]Reagents and conditions: (a) substituted phenylboronic acid, $Pd(PPh_3)_4$, 2M $K_2CO_{3(aq)}$, EtOH, toluene, reflux; (b) i. iron powder, NH_4Cl, i-PrOH, H_2O; ii. 4-methoxybenzensulfonyl chloride, pyridine, 50 ^oC; (c) for **10**: K_2CO_3, alkyl halides, DMF or MeCN, rt or heating; (d) 1M $LiOH_{(aq)}$, 1,4-dioxane, 40 ^oC.

Scheme 2 describes the synthesis of compounds **37**-**38** and **41** which possess alternative linking patterns between the biaryl and the benzene ring. Compound **28d** was reduced by iron powder and NH_4Cl to afford the corresponding amine product, which was reacted with 4-methoxybenzyl bromide, or chloride, to give compound **37**. Alternatively, the resulting amine products were reacted with *p*-anisoyl chloride to yield the corresponding amide **38**. Benzyl derivative **41**, in which a carbon atom is inserted between the sulfonamide moiety and the biphenyl ring, was synthesized by the reaction of compound **39** with 4-methoxybenzenesulfonyl chloride, and subsequent Suzuki arylation with 4-cyanophenylboronic acid.

Scheme 2. Synthetic Approaches to Compounds **37**-**38** and **41**[a]



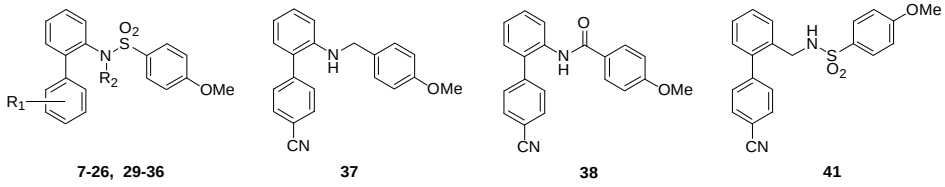
[a]Reagents and conditions: (a) i. Fe, NH_4Cl, H_2O, *i*-PrOH, reflux; ii. anisaldehyde, $NaBH_3CN$, acetic acid, MeOH; (b) i. Fe, NH_4Cl, H_2O, *i*-PrOH, reflux; ii. *p*-anisoyl chloride, pyridine or THF, rt; (c) 4-methoxybenzenesulfonyl chloride, pyridine, 90 ^oC; (d) 4-cyanophenylboronic acid, $Pd(PPh_3)_4$, 2M $K_2CO_{3(aq)}$, EtOH, toluene, reflux.

Biological Evaluation.

A. *In Vitro* Cell Growth Inhibitory Activity.

Table 1 summarizes the structure-activity relationships in the synthetic compounds, together with reference compound **4** on the inhibition of cancer cells. Specifically, the antiproliferative activity against four human cancer cell lines, pancreatic carcinoma AsPC-1, lung carcinoma A549, hepatocellular carcinoma Hep3B, and prostatic carcinoma PC-3 (Table 1) was examined.

Table 1. Antiproliferative activity of Tested Compounds and Reference Compound **4**.



compd	R_1	R_2	AsPC-1	A549	Hep3B	PC-3
			$GI_{50} \pm SD$ (μM)[a]			
7	H	H	0.88±0.07	0.60±0.09	0.36±0.02	0.44±0.01
8	4-F	H	0.30±0.05	0.36±0.01	0.34±0.01	0.25±0.01

9	4-Cl	H	0.63±0.24	0.60±0.05	0.42±0.05	0.59±0.08
10	4-CN	H	0.03±0.01	0.10±0.01	0.15±0.01	0.19±0.01
11	4-OH	H	4.77±0.71	1.21±0.12	2.31±0.01	1.60±0.02
12	4-OCH_3	H	2.23±0.82	>10	1.19±0.13	1.15±0.02
13	4-$COOCH_3$	H	>10	>10	>10	>10
14	4-COOH	H	>10	>10	>10	>10
15	3-F	H	0.79±0.15	0.54±0.44	0.31±0.02	0.52±0.04
16	3-Cl	H	2.16±1.68	1.13±0.03	0.65±0.02	0.60±0.03
17	3-CN	H	0.51±0.05	0.78±0.21	0.51±0.05	0.55±0.03
18	3-CF_3	H	1.79±0.20	3.02±0.13	2.96±0.03	3.43±0.07
19	3,4-F	H	0.28±0.05	0.30±0.02	0.15±0.03	0.19±0.02
20	3-Cl, 4-F	H	0.34±0.04	0.53±0.08	0.40±0.01	0.24±0.02
21	3-F, 4-CN	H	0.02±0.01	0.10±0.01	0.03±0.01	0.08±0.01
22	3,4-OCH_3	H	4.65±0.67	>10	2.19±0.17	2.17±0.29
23	3,4,5-OCH_3	H	>10	>10	>10	>10
24	4-pyridine	H	0.66±0.19	2.25±0.39	2.10±0.03	1.93±0.01
25	3-pyridine	H	1.84±0.39	1.00±0.26	1.70±0.42	1.91±0.01
26	2-furan	H	3.85±0.09	6.13±0.57	3.26±0.21	1.75±0.04
29	4-CN	Me	1.74±0.19	0.74±0.15	0.39±0.03	0.73±0.01
30	4-CN	Et	1.5±0.02	0.77±0.06	1.10±0.11	1.84±0.04
31	4-CN	Pr	5.21±0.93	1.14±0.07	1.41±0.17	1.88±0.05
32	4-CN	2-Cl-Et	4.79±0.04	1.50±0.23	1.55±0.06	6.52±0.07
33	4-CN	2-OH-Et	1.52±0.28	0.86±0.07	0.66±0.01	0.51±0.04
34	4-CN	-CH_2COOMe	>10	>10	>10	>10
35	4-CN	Ac	0.49±0.06	0.30±0.01	0.21±0.01	0.48±0.02
36	4-CN	-CH_2COOH	>10	>10	>10	>10
37	–	–	1.65±0.06	0.93±0.06	0.87±0.06	1.52±0.04
38	–	–	>10	>10	>10	>10
41	–	–	>10	>10	>10	>10
4	–	–	4.11±0.32	5.33±1.14	0.84±0.03	0.62±0.02

[a]SD: standard deviation, all experiments were independently performed at least three times.

Compound **7** with no substitution, exhibits activity that is slightly improved over that of compound **4**, suggesting that the biphenyl-containing sulfonamide provides a useful skeleton for the development

of bioactive molecules. Compound **7** inhibits the growth of AsPC-1, A549, Hep3B, and PC-3 cells with GI_{50} values of 0.88, 0.60, 0.36, and 0.44 μM, respectively. Replacement of a hydrogen atom with fluorine or chlorine at C3' or C4' position (**8**, **9**, and **15**) is tolerated but the C3'-Cl in compound **16** leads to a slight decrease in activity. Among mono-halogenated compounds (**8**, **9**, **15**, and **16**), compound **8** with a fluorine at C4' position showed about 2-fold improved activity over that of **7** for AsPC-1, A549, and PC-3 cells. Comparison of the mono-halogenated compounds indicated that the order of influence of halogen atoms on the antiproliferative activity is F > Cl; and a C4'-halogen is favored. The additional halogen atoms at the C3' position (**19** and **20**) have only minimal influence on the antiproliferative activity when compared to compound **8**. A variety of functional groups such as hydroxyl, methoxy, methoxycarbonyl, and aminocarbonyl groups lead to marked decrease of antiproliferative activity but the cyano group in compounds **10**, **17**, and **21** contributes to an improvement in the biological activity. Compound **10** possessing a C4'-CN is 2- to 10-fold more potent than compound **8** which has a C4'-F and inhibits the growth of AsPC-1, A549, Hep3B, and PC-3 cells with GI_{50} values of 0.03, 0.10, 0.15, and 0.19 μM, respectively. Moving the cyano group from C4' to C3', as in **17**, leads to a 3- to 17-fold decrease of antiproliferative activity as compared to its regioisomer **10**, and this is consistent with the observation that C4'-substitution is favored. The slight influence of an additional C3'-F of compound **21** was observed to lead to a 5-fold increase of inhibitory activity of Hep3B cells when compared to compound **10**. The replacement of phenyl ring with pyridine (**24** and **25**) or furan (**26**) resulted in a significant decrease of activity compared to **7**. The introduction of substituents at the nitrogen atom of the sulfonamide group (**29**-**36**) caused a remarkable decrease of activity when compared to **10**, and this appears to be independent of steric and electronic effects. Modification of the sulfonamide (**37**, **38**, and **41**) leads to decrease of potency, indicating that the sulfonamide moiety is crucial for antiproliferative activity.

B. Inhibition of Tubulin Polymerization.

Compound **21** was tested for tubulin polymerization inhibitory activity, using paclitaxel and vincristine as reference compounds (Figure 3), and was found to inhibit tubulin polymerization in a dose-dependent manner. As shown in Figure 3A, both compound **21** and vincristine destroy the tubulin assembly at the same concentration of 10 μM. The result of immunofluorescence staining revealed that compound **21** acts as a microtubule-destabilizing agent, similar to vincristine (Figure 3B). Generally, microtubule-destabilizing agents bind to β-tubulin either at vinca binding site or colchicine binding site.[21] Since compound **21** acts as a microtubule-destabilizing agent, we further identify the binding site of compound **21** using [^{3}H]-colchicine competitive binding assay. As shown in Figure 3C, both compound **21** and colchicine can inhibit the binding of [^{3}H]-colchicine to tubulin significantly, with IC_{50} values of 0.67 and 2.21 μM respectively. This result indicated that compound **21** could directly bind to tubulin at colchicine binding site.

C. Inhibition of STAT3 tyrosine phosphorylation.

Five compounds (**8**, **10**, **19**, **21**, and **35**) were examined for their ability to inhibit the phosphorylation of tyrosine residues of STAT3 in human non-small cell lung A549 cancer cells. The results are presented as IC_{50} values in Table 2. Compound **21**, which exhibits the most potent antiproliferative activity, inhibits the phosphorylation of STAT3 with an IC_{50} value of 0.2 μM. Removal of C3'-F (**10**) led to a 2-fold decrease of activity as compared with compound **21**. This phenomenon, that a C3'-F weakens the inhibitory activity of STAT3 phosphorylation is also observed in the comparison of **8** with **19**. Although compound **35** exhibits weaker antiproliferative activity, it displays slight better inhibitory activity of STAT3 phosphorylation than compound **10**. This result indicates that the C4'-CN plays a significant role in inhibition of the tyrosine phosphorylation of STAT3. We also conducted Western blot analysis to confirm the inhibitory activity of the synthetic compounds on STAT3 phosphorylation. As shown in Figure 4, compound **21**, at 0.1 μM, inhibits STAT3 constitutive activation, consistent with the results in Table 2. Compounds **35** and **10**, which exhibited slightly higher IC_{50} values in STAT3 tyrosine phosphorylation than **21**, suppressed STAT3 constitutive activation at the dose of 1 μM. Evidence has

shown that microtubule targeting agents may suppress STAT3 signaling[10,11], which may not exclude the fact that STAT3 inhibition may be the consequence of microtubule-destabilizing effect. However, in our case, compound **21** may need 3 μM to cause tubulin depolymerization and 0.67 μM to bind to colchicine binding site (Figure 3A and Figure 3C), whereas the IC_{50} value of STAT3 inhibition is only around 0.2 μM (Table. 2), which indicated that STAT3 inhibition effect of compound **21** may be stronger than tubulin binding effect. We also used STAT3 inhibitor S3I-201 to prove that whether STAT3 inhibition may cause G2/M arrest. As shown in Figure 5, STAT3 inhibition did not affect the percentage of G2/M cells. Therefore, we may conclude that the inhibition effect of compound **21** on STAT3 phosphorylation may be independent of the effect on microtubule destabilization.

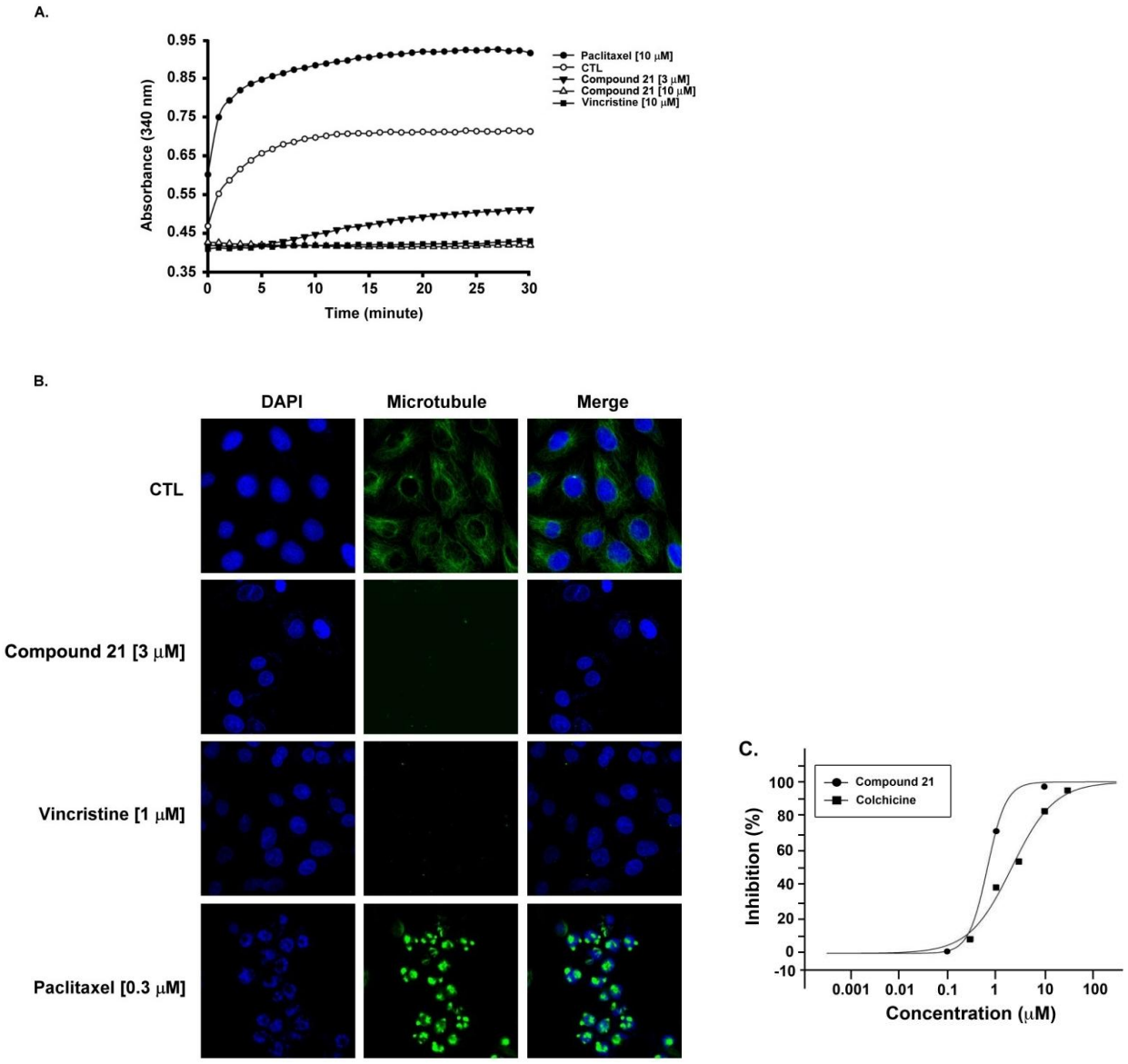


Figure 3. Compound **21** inhibits tubulin polymerization. (A) Tubulin in reaction buffer was incubated at 37 °C in the presence of control (DMSO), the indicated concentration of **21** (3 and 10 μM), 0.3 μM paclitaxel, and 1 μM vincristine. The microtubule assembly was measured by spectrophotometry. (B) A549 cells were incubated with DMSO (Control; CTL), 3 μM **21**, 1 μM paclitaxel, and 1 μM vincristine. The cellular microtubule network was analyzed by a confocal microscopy using monoclonal anti-β-tubulin antibody, FITC-conjugated anti-mouse antibody, and counterstaining with DAPI. (C) Tubulin in reaction buffer was incubated at 37 °C in the presence of compound **21** or colchicine followed by the addition of [^{3}H]-colchicine to compete with colchicine binding site.

Table 2. Inhibition of STAT3 Tyrosine Phosphorylation of Compounds **8**, **10**, **19**, **21**, and **35**.

compd	IC_{50} (μM ± SD)
8	4.02 ± 0.75
10	0.44 ± 0.04
19	2.75 ± 0.22

21	0.20 ± 0.03
35	0.32 ± 0.03

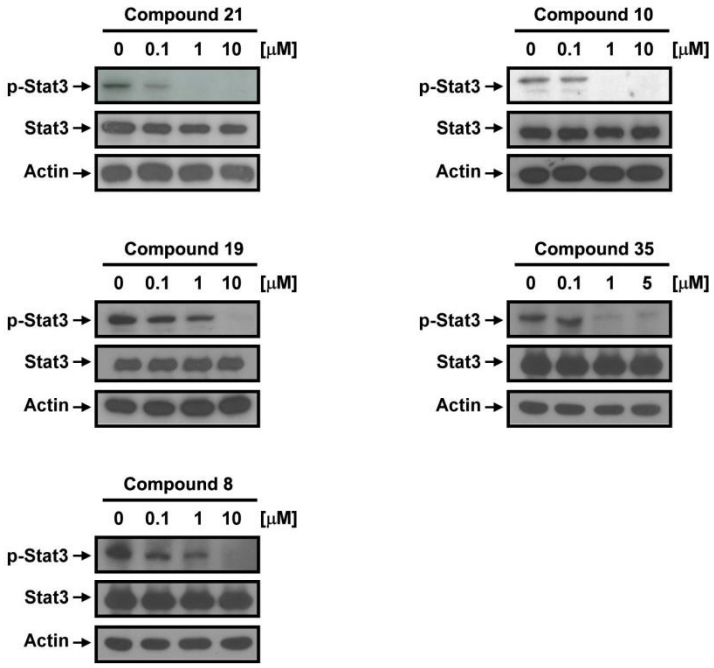


Figure 4. *N*-Sulfonyl-aminobiaryl analogues (**8**, **10**, **19**, **21**, and **35**) inhibit STAT3 tyrosine phosphorylation. A549 cells were treated with various concentrations of tested compounds for 24 hr. Then, whole cell lysates of A549 were subjected to Western blot analysis. Similar resultes were obtained in at least three independent experiments.

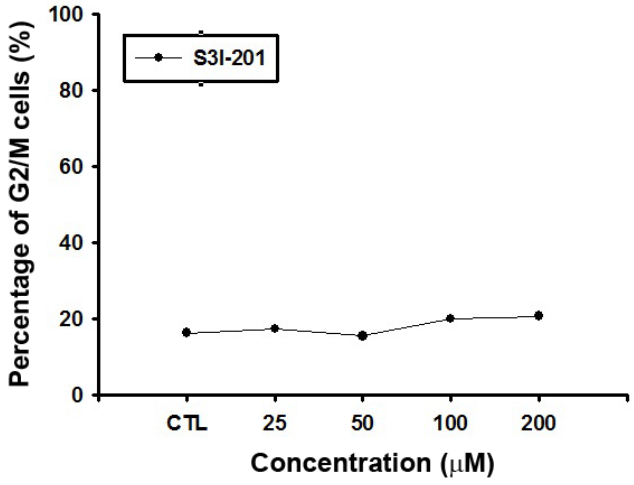


Figure 5. A549 cells were treated with S3I-201 (STAT3 inhibitor) for 24 h and then cell cycle was analyzed by flow cytometry.

Conclusion

This study describes novel *N*-sulfonyl-aminobiaryl derivatives acting as antitubulin agents with STAT3 inhibitory activity. A series of *N*-sulfonyl-aminobiaryl compounds has been synthesized and the relevant pharmacological assays have been conducted. Compound **21** exhibits remarkable antiproliferative activity against four cancer cell lines with a mean GI_{50} value of 57.5 nM. It interrupts tubulin assembly acting, like vincristine as a microtubule-destabilizing agent. Compound **21** reduces the intracellular level of phosphorylated STAT3 with an IC_{50} value of 0.2 μM. Compounds **35** and **10** are also able to inhibit the STAT3 activation in both IC_{50} value evaluation and Western blot analysis. Interestingly, the cyano substitution seems to be necessary for inhibition of STAT3 phosphorylation.

Experimental Section

(A) **Chemistry.** Nuclear magnetic resonance (^{1}H NMR) spectra were obtained with Bruker DRX-500 spectrometer (operating at 500 MHz), with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a JEOL (JMS-700) electron impact (EI) mass spectrometer. Elemental analyses were performed on a Heraeus varioIII-NCH. Purity of the final compounds were determined using an Agilent 1100 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) and were found to be ≥ 95%. Flash column chromatography was done using silica gel (Merck Kieselgel 60, no. 9385, 230-400 mesh ASTM). All reactions were carried out under an atmosphere of dry N_2.

General procedures for the synthesis of *N*-sulfonyl-2-aminobiaryl analogues (7-26): *N*-(4'-hydroxy-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (11).

A mixture of compound **27** (0.5 g, 2.48 mmol), $Pd(PPh_3)_4$ (0.2 g, 0.17 mmol), $K_2CO_{3\,(aq)}$ (2M, 3 mL), and toluene (9 mL) was stirred under N_2 at room temperature for 10 min. To this mixture was added a solution of 4-hydroxyphenylboronic acid (0.38 g, 2.72 mmol) in EtOH (9 mL) and then it was heated to reflux until the reaction was complete. The reaction mixture was filtrated through a pad of Celite and the

filtrate was extracted with H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was collected and purified by flash chromatography over silica gel (1:20 EtOAc/*n*-hexane) to yield the corresponding 2-nitrobiaryl product. To the resulting product (0.33 g, 1.52 mmol) was added NH_4Cl (0.16 g, 2.97 mmol), iron powder (0.25 g, 4.46 mmol), H_2O (3 mL), and i-PrOH (15 mL) and then the mixture was heated to reflux until the reaction was complete. The reaction mixture was cooled and filtrated through a pad of Celite. The filtrate was extracted by H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give the corresponding biphenyl-2-amine. A solution of the resulting product (0.23 g, 1.24 mmol), 4-methoxybenzenesulfonyl chloride (0.18 g, 0.89 mmol), and pyridine (2 mL) was stirred at 50 ℃ overnight. After the reaction completed, the mixture was purified by flash chromatography over silica gel (1:2 EtOAc/*n*-hexane) to afford compound **11** as a yellow solid (0.4 g, 91% three steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.56 (s, 1H), 6.76 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 7.07 (dd, *J* = 2.0, 7.5 Hz, 1H), 7.11 (td, *J* = 1.0, 7.5 Hz, 1H), 7.29 (dt, *J* = 1.5, 8.5 Hz, 1H), 7.54 (d, *J* = 7.0 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 355 (M^+); HRMS (EI) for $C_{19}H_{17}NO_4S$ (M^+) calcd 355.0878, found 355.0878.

***N*-(Biphenyl-2-yl)-4-methoxybenzenesulfonamide (7)**

The title compound was obtained in 89% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 100.0-102.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.57 (s, 1H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.88-6.90 (m, 2H), 7.09 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.14 (td, *J* = 1.0, 7.5 Hz, 1H), 7.30-7.36 (m, 4H), 7.51 (d, *J* = 7.0 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 339 (M^+); HRMS (EI) for $C_{19}H_{17}NO_3S$ (M^+) calcd 339.0929, found 339.0930.

***N*-(4'-Fluorobiphenyl-2-yl)-4-methoxybenzenesulfonamide (8)**

The title compound was obtained in 81% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 113.0-114.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.43 (s, 1H), 6.84-6.89 (m, 4H), 7.02-7.09 (m, 3H), 7.13-7.17 (m, 1H), 7.31-7.36 (m, 1H), 7.51-7.4 (m,

1H), 7.67 (s, 1H); MS (EI) *m*/*z* 357 (M^+); HRMS (EI) for $C_{19}H_{16}FNO_3S$ (M^+) calcd 357.0835, found 357.0832.

_N_-(4'-Chloro-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (**9**)

The title compound was obtained in 87% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 124.0-126.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.39 (s, 1H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 7.06 (d, *J* = 9.0 Hz, 1H), 7.14 (t, *J* = 7.0 Hz, 1H), 7.29-7.36 (m, 3H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 373 (M^+); HRMS (EI) for $C_{19}H_{16}ClNO_3S$ (M^+) calcd 373.0539, found 373.0542.

_N_-(4'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (**10**)

The title compound was obtained in 57% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 167.0-169.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.29 (s, 1H), 6.87 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.10 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.21 (td, *J* = 1.0, 7.5 Hz, 1H), 7.39 (td, *J* = 1.5 7.5 Hz, 1H), 7.52 (dd, *J* = 1.5, 7.0 Hz, 2H), 7.62-7.65 (m, 3H); MS (EI) *m*/*z* 364 (M^+); HRMS (EI) for $C_{20}H_{16}N_2O_3S$ (M^+) calcd 364.0882, found 364.0885.

4-Methoxy-_N_-(4'-methoxy-(1,1'-biphenyl)-2-yl)benzenesulfonamide (12)

The title compound was obtained in 29% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 102.1-104.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.83 (s, 3H), 3.84 (s, 3H), 6.58 (s, 1H), 6.80-6.89 (m, 6H), 7.06-7.12 (m, 2H), 7.27-7.31 (m, 1H), 7.52 (d, *J* = 7.0 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 369 (M^+); HRMS (EI) for $C_{20}H_{19}NO_4S$ (M^+) calcd 369.1035, found 369.1032.

Methyl 2'-(4-methoxyphenylsulfonamido)-(1,1'-biphenyl)-4-carboxylate (13)

The title compound was obtained in 43% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 136.0-138.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H),

3.96 (s, 3H), 6.44 (s, 1H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 7.10 (dd, *J* = 1.0, 7.5 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.0 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 2H); MS (EI) *m*/*z* 397 (M^+); HRMS (EI) for $C_{21}H_{19}NO_5S$ (M^+) calcd 397.0984, found 397.0982.

2'-(4-Methoxyphenylsulfonamido)-(1,1'-biphenyl)-4-carboxylic acid (14)

$LiOH_{(aq)}$ (1M, 1 mL, 1.0 mmol) was added to a solution of compound **13** (0.2 g, 0.5 mmol) in 1,4-dioxane (3 mL) and stirred at 40 ℃ overnight. The reaction was concentrated *in vacuo* and the resulting residue was acidified to pH value of 5~6, and then was extracted by H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was purified by flash chromatography (10:1 CH_2Cl_2/MeOH) to yield the desired compound **14** as a yellow solid (0.15 g, 78%): mp 197.0-200.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.75 (s, 3H), 6.77 (dd, *J* = 1.5, 7.0 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 7.06 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.14 (td, *J* = 1.0, 7.5 Hz, 1H), 7.21 (td, *J* = 1.5, 8.0 Hz, 1H), 7.29 (dd, *J* = 2.0, 7.5 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 2H); MS (EI) *m*/*z* 383 (M^+); HRMS (EI) for $C_{20}H_{17}NO_5S$ (M^+) calcd 383.0827, found 383.0828.

***N*-(3'-Fluoro-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (15)**

The title compound was obtained in 55% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 118.3-119.5 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.41 (d, *J* = 9.0 Hz, 1H), 6.46 (s, 1H), 6.74 (d, *J* = 7.5 Hz, 1H), 6.84 (dd, *J* = 2.0, 7.5 Hz, 2H), 7.05-7.10 (m, 2H), 7.16 (t, *J* = 7.0 Hz, 1H), 7.32-7.37 (m, 2H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.72 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 357 (M^+); HRMS (EI) for $C_{19}H_{16}FNO_3S$ (M^+) calcd 357.0835, found 357.0835.

***N*-(3'-Chloro-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (16)**

The title compound was obtained in 78% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 93.0-95.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.41

(s, 1H), 6.66 (s, 1H), 6.85-6.88 (m, 3H), 7.06 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.17 (td, *J* = 1.0, 7.5, Hz, 1H), 7.28-7.39 (m, 3H), 7.46 (d, *J* = 7.0 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 357 (M^+); HRMS (EI) for $C_{19}H_{16}ClNO_3S$ (M^+) calcd 373.0539, found 373.0540.

_N_-(3'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (17)

The title compound was obtained in 52% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 97.0-100.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.89 (s, 3H), 6.21 (s, 1H), 6.80-6.81 (m, 1H), 6.90-6.93 (m, 2H), 7.06 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.26-7.29 (m, 1H), 7.397.43 (m, 1H), 7.46-7.51 (m, 3H), 7.63 (dt, *J* = 1.0, 8.0 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 364 (M^+); HRMS (EI) for $C_{20}H_{16}N_2O_3S$ (M^+) calcd 364.0882, found 364.0885.

4-Methoxy-_N_-(3'-(trifluoromethyl)-(1,1'-biphenyl)-2-yl)benzenesulfonamide (18)

The title compound was obtained in 67% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 96.0-98.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.35 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 2H), 7.03 (s, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.48-7.53 (m, 3H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 407 (M^+); HRMS (EI) for $C_{20}H_{16}F_3NO_3S$ (M^+) calcd 407.0803, found 407.0801.

_N_-(3',4'-Difluoro-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (19)

The title compound was obtained in 66% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 93.0-95.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.33 (s, 1H), 6.49-6.53 (m, 1H), 6.67-6.70 (m, 1H), 6.87 (d, *J* = 8.5 Hz, 2H), 7.05 (dd, *J* = 1.0, 7.5 Hz, 1H), 7.12-7.18 (m, 2H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 375 (M^+); HRMS (EI) for $C_{19}H_{15}F_2NO_3S$ (M^+) calcd 375.0741, found 375.0743.

_N_-(3'-Chloro-4'-fluorobiphenyl-2-yl)-4-methoxybenzenesulfonamide (20)

The title compound was obtained in 78% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 117.0-119.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.32 (s, 1H), 6.72 (dd, *J* = 2.0, 7.0 Hz, 1H), 6.82-6.91 (m, 3H), 7.05 (d, *J* = 7.5 Hz, 1H), 7.11-7.20 (m, 2H), 7.36-7.40 (m, 1H), 7.47-7.50 (m, 2H), 7.72 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 391 (M^+); HRMS (EI) for $C_{19}H_{15}ClFNO_3S$ (M^+) calcd 391.0445, found 391.0443.

***N*-(4'-Cyano-3'-fluoro-[1,1'-biphenyl]-2-yl)-4-methoxybenzenesulfonamide (21)**

The title compound was obtained in 67% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 121.2-123.5 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.27 (s, 1H), 6.65 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 6.94 (dd, *J* = 1.0, 8.0 Hz, 1H), 7.09 (dd, *J* = 1.0, 7.5 Hz, 1H), 7.23-7.26 (m, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.59-7.63 (m, 2H); MS (EI) *m*/*z* 382 (M^+); HRMS (EI) for $C_{20}H_{15}FN_2O_3S$ (M^+) calcd 382.0787, found 382.0789.

***N*-(3',4'-Dimethoxy-(1,1'-biphenyl)-2-yl)-4-methoxybenzenesulfonamide (22)**

The title compound was obtained in 64% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 150.5-152.3 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.83 (s, 3H), 3.93 (s, 3H), 6.46 (d, *J* = 1.5 Hz, 1H), 6.51 (d, *J* = 8.0 Hz, 1H), 6.71 (s, 1H), 6.83-6.87 (m, 3H), 7.10-7.15 (m, 2H), 7.28-7.33 (m, 1H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 399 (M^+); HRMS (EI) for $C_{21}H_{21}NO_5S$ (M^+) calcd 399.1140, found 399.1142.

4-Methoxy-*N*-(3',4',5'-trimethoxybiphenyl-2-yl)benzenesulfonamide (23)

The title compound was obtained in 81% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 139.0-141.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (s, 6H), 3.83 (s, 3H), 3.90 (s, 3H), 6.19 (s, 2H), 6.74 (s, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 7.13-7.15 (m, 2H), 7.30-7.34 (m, 1H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.65 (d, *J* = 8.5 Hz, 1H); MS (EI) *m*/*z* 429 (M^+); HRMS (EI) for $C_{22}H_{23}NO_6S$ (M^+) calcd 429.1246, found 429.1248.

4-Methoxy-*N*-(2-(pyridin-4-yl)phenyl)benzenesulfonamide (24)

The title compound was obtained in 41% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 224.0-227.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.75 (s, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 6.99 (s, 2H), 7.13 (d, *J* = 7.5 Hz, 1H), 7.22-7.26 (m, 1H), 7.39 (t, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 1H); MS (EI) *m*/*z* 340 (M^+); HRMS (EI) for $C_{18}H_{16}N_2O_3S$ (M^+) calcd 340.0882, found 340.0880.

4-Methoxy-*N*-(2-(pyridin-3-yl)phenyl)benzenesulfonamide (25)

The title compound was obtained in 73% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: mp 180.0-183.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.36 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 2H), 7.11 (dd, *J* = 1.0, 7.5 Hz, 1H), 7.20 (t, *J* = 8.0 Hz, 1H), 7.28-7.30 (m, 2H), 7.37-7.41 (m, 1H), 7.54 (d, *J* = 9.0 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 1H), 8.16 (s, 1H), 8.62 (t, *J* = 3.0 Hz, 1H); MS (EI) *m*/*z* 340 (M^+); HRMS (EI) for $C_{18}H_{16}N_2O_3S$ (M^+) calcd 340.0882, found 340.0884.

***N*-(2-(Furan-2-yl)phenyl)-4-methoxybenzenesulfonamide (26)**

The title compound was obtained in 14% overall yield from compound **27** in a manner similar to that described for the preparation of **11**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (s, 3H), 6.34 (d, *J* = 3.5 Hz, 1H), 6.43 (dd, *J* = 1.5, 3.5 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 2H), 7.12 (td, *J* = 1.0, 7.5 Hz, 1H), 7.25-7.29 (m, 1H), 7.35 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.49 (dd, *J* = 2.0, 6.5 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.88 (s, 1H); MS (EI) *m*/*z* 329 (M^+); HRMS (EI) for $C_{17}H_{15}NO_4S$ (M^+) calcd 329.0722, found 329.0725.

***N*-(4'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxy-*N*-methylbenzenesulfonamide (29)**

K_2CO_3 (0.17 g, 1.23 mmol) was added to a solution of compound **10** (0.15 g, 0.41 mmol) in DMF (3 mL), and the mixture was stirred at room temperature for 10 min, and then MeI (0.05 mL, 0.82 mmol) was added. After stirring overnight, the reaction was extracted with H_2O (10 mL) and EtOAc (3 × 10 mL). The combined organic extract was dried over anhydrous $MgSO_4$ and evaporated to give a residue

which was purified by flash chromatography (1:1 EtOAc/*n*-hexane) to yield compound **29** as a white solid (0.15 g, 99%); mp 111.0-113.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.03 (s, 3H), 3.87 (s, 3H), 6.91-6.94 (m, 3H), 7.30-7.42 (m, 3H), 7.48-7.51 (m, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.68-7.71 (m, 2H); MS (EI) *m*/*z* 378 (M^+); HRMS (EI) for $C_{21}H_{18}N_2O_3S$ (M^+) calcd 378.1038, found 378.1040.

_N_-(4'-Cyano-(1,1'-biphenyl)-2-yl)-_N_-ethyl-4-methoxybenzenesulfonamide (30)

The title compound was obtained in 90% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: mp 121.0-120.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (t, *J* = 7.5 Hz, 3H), 3.25-3.40 (m, 2H), 3.88 (s, 3H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 2H), 7.31-7.43 (m, 3H), 7.59 (d, *J* = 9.0 Hz, 2H), 7.66-7.71 (m, 4H); MS (EI) *m*/*z* 392 (M^+); HRMS (EI) for $C_{22}H_{20}N_2O_3S$ (M^+) calcd 392.1195, found 392.1195.

_N_-(4'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxy-_N_-propylbenzenesulfonamide (31)

The title compound was obtained in 78% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: mp 139.0-141.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.60 (t, *J* = 7.5 Hz, 3H), 1.15-1.23 (m, 2H), 3.00-3.40 (m, 2H), 3.90 (s, 3H), 6.92 (dd, *J* = 1.0, 8.0 Hz, 1H), 6.96 (d, *J* = 9.0 Hz, 2H), 7.31-7.42 (m, 3H), 7.62 (d, *J* = 9.0 Hz, 2H), 7.67-7.72 (m, 4H); MS (EI) *m*/*z* 406 (M^+); HRMS (EI) for $C_{23}H_{22}N_2O_3S$ (M^+) calcd 406.1351, found 406.1349.

_N_-(2-Chloroethyl)-_N_-(4'-cyano-(1,1'-biphenyl)-2-yl)-4-methoxybenzene sulfonamide (32)

The title compound was obtained in 86% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: mp 150.0-152.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.00-3.40 (m, 2H), 3.50 (t, *J* = 7.5 Hz, 2H), 3.90 (s, 3H), 6.96-7.00 (m, 3H), 7.34-7.37 (m, 2H), 7.42-7.46 (m, 1H), 7.63-7.72 m, 7H); MS (EI) *m*/*z* 426 (M^+); HRMS (EI) for $C_{22}H_{19}ClN_2O_3S$ (M^+) calcd 426.0805, found 426.0802.

_N_-(4'-Cyano-(1,1'-biphenyl)-2-yl)-_N_-(2-hydroxyethyl)-4-methoxybenzenesulfonamide (33)

The title compound was obtained in 81% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: mp 130.0-133.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.98-3.17 (m, 4H), 3.54 (s, 3H), 4.23 (s, 2H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 9.0 Hz, 2H), 6.99-7.12 (m, 3H), 7.13 (d, *J* = 9.0 Hz, 2H), 7.36-7.42 (m, 4H); MS (EI) *m*/*z* 408 (M^+); HRMS (EI) for $C_{22}H_{20}N_2O_4S$ (M^+) calcd 408.1144, found 408.1144.

Methyl 2-(*N*-(4'-cyano-(1,1'-biphenyl)-2-yl)-4-methoxyphenylsulfonamido) acetate (34)

The title compound was obtained in 99% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.55 (s, 3H), 3.88 (s, 3H), 6.93 (d, *J* = 8.5 Hz, 2H), 7.22-7.26 (m, 1H), 7.29-7.33 (m, 2H), 7.38-7.42 (m, 1H), 7.60-7.70 (m, 6H); MS (EI) *m*/*z* 436 (M^+); HRMS (EI) for $C_{23}H_{20}N_2O_5S$ (M^+) calcd 436.1093, found 436.1094.

***N*-(4'-Cyano-(1,1'-biphenyl)-2-yl)-*N*-((4-methoxyphenyl)sulfonyl)acetamide (35)**

The title compound was obtained in 31% overall yield from compound **10** in a manner similar to that described for the preparation of **29**: mp 206.0-208.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.90 (s, 3H), 3.88 (s, 3H), 6.91(d, *J* = 9.0 Hz, 2H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.45-7.61 (m, 5H), 7.70-7.75 (m, 4H); MS (EI) *m*/*z* 406 (M^+); HRMS (EI) for $C_{22}H_{18}N_2O_4S$ (M^+) calcd 406.0987, found 406.0988.

2-(*N*-(4'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxyphenylsulfonamido)acetic acid (36)

The title compound was obtained in 99% overall yield from compound **34** in a manner similar to that described for the preparation of **14**: mp 175.0-178.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.88 (s, 3H), 6.91 (d, *J* = 9.0 Hz, 2H), 7.24-7.26 (m, 1H), 7.30-7.36 (m, 2H), 7.40-7.43 (m, 1H), 7.55-7.60 (m, 4H), 7.65 (d, *J* = 8.0 Hz, 2H); MS (EI) *m*/*z* 422 (M^+); HRMS (EI) for $C_{22}H_{18}N_2O_5S$ (M^+) calcd 422.0936, found 422.0938.

2'-((4-Methoxybenzyl)amino)-(1,1'-biphenyl)-4-carbonitrile (37)

A mixture of compound **28d** (0.4 g, 1.78 mmol), iron powder (0.3 g, 5.35 mmol), NH_4Cl (0.19 g, 3.57 mmol), H_2O (4 mL), and i-PrOH (18 mL) was heated to reflux for 3 h. Then the reaction was cooled and filtrated through a pad of Celite. The filtrate was concentrated and extracted with H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was purified by flash chromatography (1:2 EtOAc/*n*-hexane) to yield the corresponding amine product. Anisaldehyde (0.08 mL, 0.65 mmol), AcOH (0.1 mL), and MeOH (10 mL) were added to the resulting product (0.2 g, 1.03 mmol). After stirring for 10 min, sodium cyanoborohydride (0.08 g, 1.27 mmol) was added and stirred at room temperature overnight. The reaction was filtered and the filtrate was concentrated to afford compound **37** as a white solid (0.21 g, 100%): mp 159.0-161.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.79 (s, 3H), 4.24 (s, 2H), 6.72 (d, J = 8.5 Hz, 1H), 6.80 (t, J = 7.5 Hz, 1H), 6.84-6.88 (m, 2H), 7.06 (dd, J = 1.0, 8.0 Hz, 1H), 7.20-7.27 (m, 4H), 7.58 (dd, J = 1.5, 6.5 Hz, 2H), 7.71 (d, J = 1.5, 6.5 Hz, 2H); MS (EI) m/z 314 (M^+); HRMS (EI) for $C_{21}H_{18}N_2O_2$ (M^+) calcd 314.1419, found 314.1421.

***N*-(4'-Cyano-(1,1'-biphenyl)-2-yl)-4-methoxybenzamide (38)**

A mixture of compound **28d** (0.4 g, 1.78 mmol), iron powder (0.3 g, 5.35 mmol), NH_4Cl (0.19 g, 3.57 mmol), H_2O (4 mL), and i-PrOH (18 mL) was heated to reflux for 3 h. The reaction was cooled and filtrated through a pad of Celite. The filtrate was concentrated and extracted with H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was purified by flash chromatography (1:2 EtOAc/*n*-hexane) to yield the corresponding amine product. A mixture of the resulting product (0.2 g, 1.03 mmol), *p*-anisoyl chloride (0.14 mL, 1.03 mmol), and pyridine (3 mL) was stirred at 90 ℃ overnight. The mixture was concentrated *in vacuo*, and then the residue was purified by flash chromatography (1:2 EtOAc/*n*-hexane) to afford compound **38** as a white solid (0.22 g, 65%): mp 136.0-138.0 ^oC; ^1H NMR (500 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.89-6.93 (m, 2H), 7.24-7.29 (m, 2H), 7.45-7.49 (m, 1H), 7.57 (d, J = 8.5 Hz, 4H), 7.62 (s, 1H), 7.77 (d, J = 8.5 Hz, 2H), 8.31 (d, J = 8.0 Hz, 1H); MS (EI) m/z 328 (M^+); HRMS (EI) for $C_{21}H_{16}N_2O_2$ (M^+) calcd 328.1212, found 328.1212.

***N*-(2-Bromobenzyl)-4-methoxybenzenesulfonamide (40)**

A mixture of compound **39** (0.5 g, 2.69 mmol), 4-methoxybenzenesulfonyl chloride (0.4 g, 1.92 mmol), and pyridine (5 mL) was stirred at 90 ℃ overnight. The mixture was concentrated *in vacuo*, and then the residue was purified by flash chromatography (1:1 EtOAc/*n*-hexane) to afford compound **40** as a yellow solid (0.94 g, 99%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (s, 3H), 4.21 (s, 1H), 4.22 (s, 1H), 4.99 (s, 1H), 6.91 (d, *J* = 9.0 Hz, 2H), 7.10 (dt, *J* = 1.5, 8.0 Hz, 1H), 7.21 (dt, *J* = 1.0, 7.5 Hz, 1H), 7.26-7.31 (m, 3H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 2H), 8.60 (d, *J* = 4.0 Hz, 1H).

***N*-((4'-Cyano-(1,1'-biphenyl)-2-yl)methyl)-4-methoxybenzenesulfonamide (41)**

A mixture of compound **40** (0.2 g, 0.56 mmol), $Pd(PPh_3)_4$ (0.05 g, 0.17 mmol), 4-cyanophenylboronic acid (0.09 g, 0.62 mmol), 2M K_2CO_3 (0.7 mL), and in DMF (5 mL) was heated to reflux for 18 h. The reaction mixture was filtrated through a pad of Celite and the filtrate was extracted with H_2O (10 mL) and EtOAc (3 × 10 mL). The organic layer was purified by flash chromatography (1:3 EtOAc/*n*-hexane) to yield compound **41** as a light yellow solid (0.09 g, 42%): mp 163.0-166.0 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.90 (s, 3H), 4.00-4.02 (d, *J* = 6.0 Hz, 2H), 4.40-4.43 (m, 1H), 6.92 (d, *J* = 8.5 Hz, 2H), 7.16-7.19 (m, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.34-7.41 (m, 3H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 9.0 Hz, 2H); MS (EI) *m*/*z* 378 (M^+); HRMS (EI) for $C_{21}H_{18}N_2O_3S$ (M^+) calcd 378.1038, found 378.1040.

(B) Biology.

(a) Materials

Sulforhodamine B (SRB) (Sigma-Aldrich, 230162, St. Louis, MO), 4',6-diamidino-2-phenylindole (DAPI) was purchased from Roche Molecular Biochemicals (Cat. 10236276001, Mannheim, Germany). Antibodies to STAT3 (Cat. 610189) were purchased from BD Biosciences (San Jose, CA). The antibody to STAT3 (Tyr705) (Cat. 2236-1) was obtained from Epitomics (Burlingame, CA), and the antibody to actin (Cat. MAB1501) was purchased from Millipore (Temecula, CA). Stat3 inhibitor S3I-201 (Cat. 14336) was purchased from Cayman Chemical (Ann Arbor, MI).

(b) Cell culture

Human NSCLC cell line A549, human pancreatic adenocarcinoma cell line AsPC-1, human hepatoma cell line Hep3B and human prostate cancer cell line PC-3 were obtained from American Type Culture Collection (ATCC) (Manassas, VA). Cells were maintained in RPMI 1640 medium or DMEM with 10% fetal bovine serum (FBS) and penicillin (100 U/ml)/streptomycin (100 μg/mL)/amphotericin (0.25 μg/mL) at 37 °C in a humidified incubator with 5% CO_2.

(c) Sulforhodamine B (SRB) assay

All cells were seeded in 96-well culture plates at a density of 3-5 x 10^3 cells/well. After attachment, cells were fixed with 10% trichloroacetic acid (TCA) to provide a measurement of the cell population at the time of drug addition. The following day, cells were treated with vehicle (0.1% DMSO) or an increasing gradient concentration of the indicated compounds for 48 h, after which cells were fixed with 10% TCA and stained with 0.4% (w/v) SRB dissolved in 1% AcOH. The protein-bound dye was subsequently extracted with 10 mM trizma base to determine the absorbance at a wavelength of 515 nm. The inhibition rate on cell proliferation (GI_{50}) as a function of test drug concentration was calculated for each well as 100-[($A515_{\text{treated cells}}$ - $A515_{\text{time}}$)].

(d) *In vitro* tubulin polymerization assay

The effect of identified compounds on tubulin polymerization was determined kinetically using the CytoDYNAMIX Screen kit (BK006P, Cytoskeleton Inc., Denver, CO). Cold porcine tubulin protein (> 99% purity) was added to G-PEM buffer (80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, pH 6.9) containing 15% glycerol with or without the identified compounds. The sample mixture was dotted onto a prewarmed 96-well plate, which was immediately transferred to a 37 °C plate reader (SpectraMax Plus, Molecular Devices Inc., Sunnyvale, CA). The absorbance was read every minute for 30 min at 340 nm.

(e) Immunofluorescence confocal microscopy

A549 cells were seeded sparsely in eight-well chamber slides and treated with or without identified compounds for 24 h. Following treatment, cells were fixed with cold MeOH at -20 °C for 15 min, washed three times with PBS and blocked with 1% PBS plus 0.1% Triton X-100 for 30 min at 37 °C.

Microtubules were detected by incubation with a monoclonal anti-β-tubulin at 37 °C for 1 h. Then, the cells were washed with PBS and incubated with a FITC-conjugated anti-mouse IgG antibody. Nuclei were stained with DAPI, and microtubule distribution images were acquired with a Leica TCS SP2 Confocal Spectral Microscope.

(f) [^{3}H]-Colchicine competitive binding assay

Compound **21** was submitted to Eurofins Panlabs Pharma Services (Taiwan Ltd) for [^{3}H]-colchicine competitive binding assay. In briefly, tubulin was preincubated with compound 21 or colchicine in incubation buffer (10 mM Sodium Phosphate, pH 6.7, 10 mM $MgCl_2$, 1 mM EGTA, 0.1 mM GTP) for 1 h followed by the addition of [^{3}H]-colchicine. After 3 h of incubation at 37 °C, bound [^{3}H]-colchicine were analyzed by scintillation counter.

(**g) STAT3 kit**

The level of STAT3 phosphorylation in response to test compounds treatment was determined using the PathScan® Phospho-Stat3 (Try705) Sandwich ELISA kit (Cell Signaling Technology) according to the manufacturer's instructions. The absorbance was read within 30 min at 405 nm using a microplate ELISA reader.

(h) Western blot analysis

For Western blot analysis, cell lysates were prepared, and proteins were separated by 7.5-15% SDS-PAGE, transferred onto PVDF membrane, and then immunoblotted with specific antibodies. Proteins were visualized with an ECL detection system.[22]

(i) Flow Cytometry

Cells were treated with indicated concentration of S3I-201 for 24 h and then fixed in ethanol (75%, v/v) overnight at -20℃. After centrifugation, fixed cells were washed with ice-cold PBS once and incubated in 0.1M of phosphate–citric acid buffer (0.2 M $NaHPO_4$, 0.1 M citric acid, pH 7.8) for 30 min at room temperature, and then stained with propidium iodide staining buffer containing Triton X-100 (0.1%, v/v),

RNase A (100 mg/ml) and propidium iodide (80 mg/ml). Cell cycle distribution was performed using a FACScan flow cytometry with CellQuest software (Becton Dickinson, Mountain View, CA, USA).

(j) Statistical Analysis

Results are expressed as the mean ± SD for the indicated number of separate experiments. Means were assessed for significant differences using *t*-test and *P*-values < 0.05 were considered significant.

Author Information.

Corresponding Author

*Phone: 886-2-2736-1661 ext 6130. E-mail: jpl@tmu.edu.tw.

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Abbreviations Used

STAT3, Signal transducer and activator of transcription 3; LiOH, lithium hydroxide; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); DMF, dimethylformamide; $NaBH_3CN$, sodium cyanoborohydride.

Supporting Information Available: The HPLC results and ^{1}H-NMR spectrum of target compounds **7-26**, **29-38**, and **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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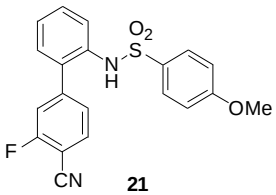
Graphic for manuscript



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AsPC-1 cells GI_{50} = 0.02 μM
Hep3B cells GI_{50} = 0.03 μM
STAT3 IC_{50} = 0.2 μM