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Design, synthesis, and mechanism of action of 2-(3-hydroxy-5-methoxyphenyl)-6-pyrrolidinylquinolin-4-one as a potent anticancer lead

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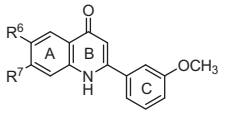
ABSTRACT

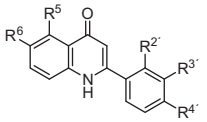
New 6- (or 6,7-) substituted 2-(hydroxyl substituted phenyl)quinolin-4-one derivatives were synthesized and screened for antiproliferative effects against cancer cell lines. Structure–activity relationship correlations were established and the most promising compound 2-(3-hydroxy-5-methoxyphenyl)-6-pyrrolidin-1-ylquinolin-4-one (**6h**) exhibited strong inhibitory activity against various human cancer cell lines, particularly non-small cell lung cancer NCI-H522. Additional studies suggested a mechanism of action resembling that of the antimitotic drug vincristine. The presence of a C-ring OH group in **6h** will allow this compound to be converted readily to a water soluble and physicochemically stable hydrophilic prodrug. Compound **6h** is proposed as a new anticancer lead compound.

In prior studies, we synthesized 2-phenylquinolin-4-one derivatives (2PQs)[1–17] and identified various compounds, for example, **2PQ-1**, **2PQ-2**, **2PQ-3**, and **2PQ-4**(Chart 1), with potent anticancer activity.[3] These potential antitumor agents, however, suffer from high toxicity and poor hydrophilicity, which has limited their further development. To overcome such drawbacks of 2PQs, we introduced a hydroxy group into the 2PQ skeleton to improve water solubility. Moreover, this type of 2PQ could be further converted to a water soluble prodrug. Accordingly, we synthesized a series of 2PQs with a hydroxy group on the A-ring (**2PQ-5**∼**10**, Chart 2), and among them, 5-hydroxy-6-methoxy derivatives (**2PQ-5**, and **2PQ-6**) exhibited good anticancer activity with IC_{50} values ranging from 0.03 to 0.11 μM against HL-60, HCT116, Hep3B and NCI-H460 cell lines.[5] Subsequently, **2PQ-6** was converted to a water soluble disodium monophosphate prodrug (**2PQ-6P**), which exhibited significant tumor growth suppression, without appreciably affecting normal biological function.[5] Based on this finding, we have now



2PQ-1: R^6, R^7 = -OCH_2O-
2PQ-2: R^6 = pyrrolidinyl, R^7 = H
2PQ-3: R^6 = morpholino, R^7 = H
2PQ-4: R^6 = dimethylamino, R^7 = H

Chart 1. Structures of compounds **2PQ-1**∼**4**.



2PQ-5: R^5 = OH, R^6 = OCH_3, $R^{2'}$ = F, $R^{3'}$ = $R^{4'}$ = H
2PQ-6: R^5 = OH, R^6 = OCH_3, $R^{3'}$ = F, $R^{2'}$ = $R^{4'}$ = H
2PQ-7: R^5 = OH, R^6 = OCH_3, $R^{4'}$ = F, $R^{2'}$ = $R^{3'}$ = H
2PQ-8: R^5 = R^6 = OH, $R^{2'}$ = F, $R^{3'}$ = $R^{4'}$ = H
2PQ-9: R^5 = R^6 = OH, $R^{3'}$ = F, $R^{2'}$ = $R^{4'}$ = H
2PQ-10: R^5 = R^6 = OH, $R^{4'}$ = F, $R^{2'}$ = $R^{3'}$ = H
2PQ-6P: R^5 = OPO_3Na_2, R^6=OCH_3, $R^{3'}$ = F, $R^{2'}$ = $R^{4'}$ = H

Chart 2. Structures of compounds **2PQ-5**∼**10** and **2PQ-6P**.

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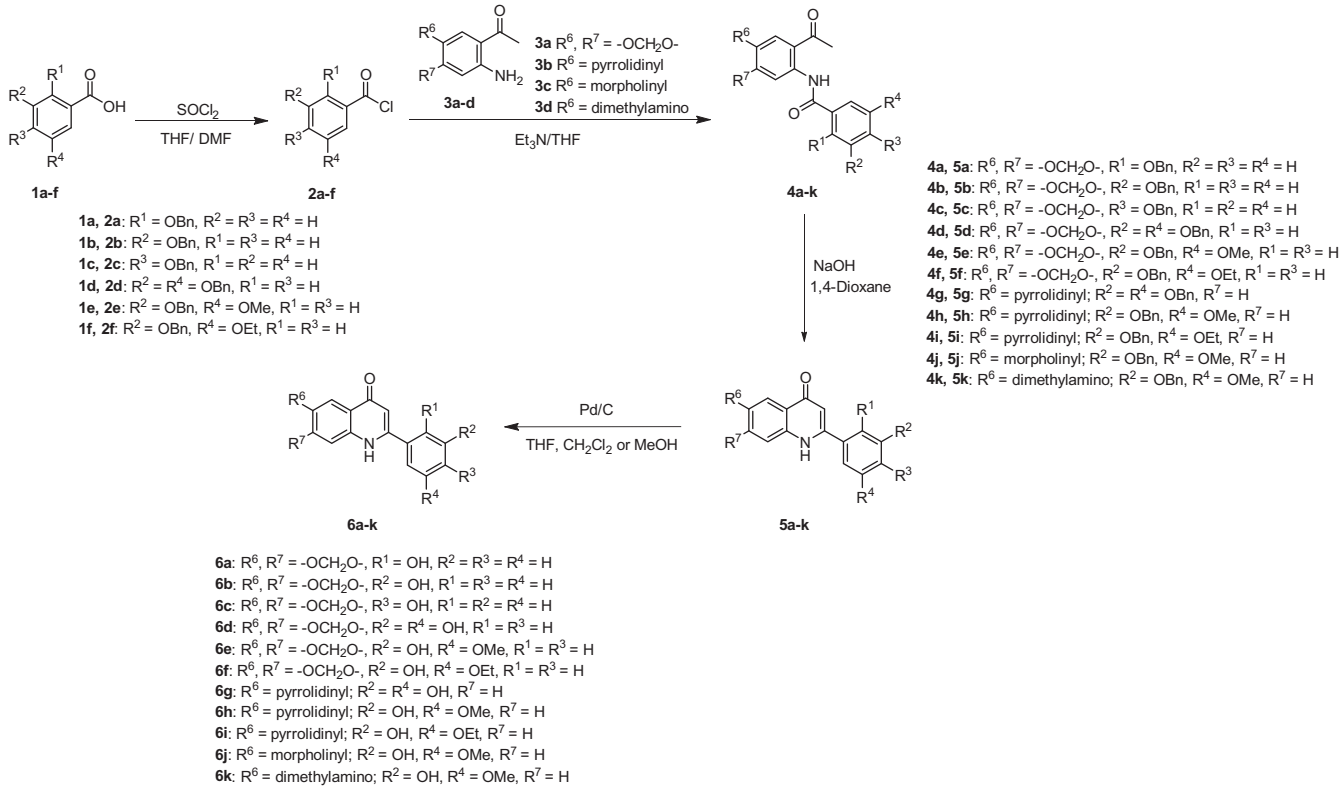
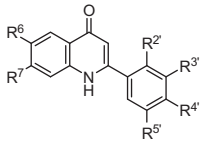
designed a new series of 2PQs with a hydroxy group on the C-ring (**6a**–**k**). Again, this hydroxy moiety should provide a 'synthetic handle' for potential useful prodrug design. The benefits of this design are to decrease toxicity, increase hydrophility, and facilitate



Scheme 1. Synthesis of compounds **6a**–**k**.

Table 1
In vitro cytotoxic activity of 6- (or 6,7-) substituted 2-(hydroxyl substituted phenyl)quinolin-4-one derivatives (**6a**–**k**)



Compounds **6a-k**

Compds							IC_{50}[a,b] (μM)			
	R^6	R^7	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	HL-60	Hep3B	NCI-H460	Detroit 551
2PQ-1	OCH_2O		H	OMe	H	H	0.08	0.08	0.20	4.77
6a	OCH_2O		OH	H	H	H	7.62	8.04	19.13	48.85
6b	OCH_2O		H	OH	H	H	0.25	1.05	17.00	21.80
6c	OCH_2O		H	H	OH	H	3.26	22.33	>100	40.98
6d	OCH_2O		H	OH	H	OH	8.71	>25	>25	ND[c]
6e	OCH_2O		H	OH	H	OMe	0.50	>50	>50	>50
6f	OCH_2O		H	OH	H	OEt	0.50	25	>25	ND[c]
6g	Pyrrolidinyl	H	H	OH	H	OH	0.34	1.58	13.89	>25
6h	Pyrrolidinyl	H	H	OH	H	OMe	0.10	0.50	2.12	>50
6i	Pyrrolidinyl	H	H	OH	H	OEt	0.02	0.07	0.59	13.23
6j	Morpholinyl	H	H	OH	H	OMe	3.90	6.03	>100	>100
6k	Dimethylamino	H	H	OH	H	OMe	0.22	6.15	>50	>50

[a] Data are presented as IC_{50} (μM) for each cell line; the concentration of compound that caused a 50% proliferation-inhibitory effect after 48 h incubation.
[b] Cell lines include human promyelocytic leukemia (HL-60), human hepatoma (Hep3B), human lung cancer (H460), and embryonic skin fibroblast (Detroit551) cell lines.
[c] ND = not determined.

Table 2
Inhibition of in vitro tumor cell growth by compound **6h**[a]

Cell line[e]	log GI_{50}[b]	log TGI[c]	log LC_{50}[d]
SR	<−8.00	>−4.00	>−4.00
NCI-H522	<−8.00	<−8.00	−5.41
Colo205	−7.27	−6.66	—[f]
SF-295	−6.96	−4.95	>−4.00
M14	−7.52	−4.80	>−4.00
MDA-MB-435	−8.00	−7.53	—[f]
SK-MEL-5	−7.61	−7.18	−6.31
OVCAR-3	−7.63	−7.05	−5.19
NCI/ADR-RES	−7.21	−6.29	>−4.00
SK-OV-3	−7.63	−4.60	>−4.00
RXF 393	−7.39	−4.62	>−4.00
DU-145	−7.51	−6.59	>−4.00
MDA-MB-468	−6.58	−5.70	>−4.00

[a] Data obtained from NCI's in vitro disease-oriented human tumor cells screen.
[b] Log concentrations that reduced cell growth to 50% of level at start of experiment.
[c] Log concentrations that caused total growth inhibition.
[d] Log concentrations that a given compound required to kill 50% of a test population.
[e] SR, leukemia; NCI-H522, non-small cell lung cancer; Colo205, colon cancer; SF-295, CNS cancer; M14, MDA-MB-435, SK-MEL-5, melanoma, M14; OVCAR-3, NCI/ADR-RES, SK-OV-3, ovarian cancer; RXF 393, renal cancer; DU-145, prostate cancer; MDA-MB-468, breast cancer cell lines.
[f] '—' no data.

possible hydrophilic prodrug derivatization. This report describes the chemical synthesis, antitumor activity screening in vitro, and mechanism of action of the new series of 6- (or 6,7-) substituted 2-(hydroxyl substituted phenyl)quinolin-4-ones (**6a**–**k**).

The synthesis of target compounds **6a**–**k** is illustrated in Scheme 1. As shown, variously substituted benzoic acids (**1a**–**f**) were chlorinated with thionyl chloride to afford compounds **2a**–**f**.[18] Without purification, **2a**–**f** were reacted with *o*-aminoacetophenones (**3a**–**d**)[2,3] to give the desired amides (**4a**–**k**),[19] which were then cyclized in the presence of basic dioxane solution to yield **5a**–**k**.[20] Catalytic hydrogenolysis of **5a**–**k** with palladium on active charcoal gave the final compounds, 6- (or 6,7-) substituted 2-(hydroxyphenyl)quinolin-4-ones (**6a**–**k**).[21] The spectroscopic data (^{1}H, ^{13}C NMr and HR-ESIMS) were consistent with the proposed structures.

The newly synthesized compounds were screened for antiproliferative activity against HL-60 leukemia, Hep3B hepatoma, NCI-H460 non-small cell lung cancer, and Detroit 551 human skin fibroblast cells.[22,23] The results are given in Table 1. Among the three mono-hydroxy derivatives (**6a**–**c**), **6b** with *meta*-hydroxy substitution was the most potent (Table1), but showed only moderate cytotoxicity compared with **2PQ-1**. Compound **6d** with an additional hydroxy group at the C-ring 5-position exhibited only weak activity. Based on the structures of active compounds **2PQ-1**∼**4**, we also synthesized compounds with a 3-hydroxy-5-alkoxyphenyl C-ring (**6e**–**f**) and changed the A-ring substitution from 6,7-methylenedioxy to 6-pyrrolidinyl (**6g**–**i**), 6-morpholinyl (**6j**), and 6-dimethylamino (**6k**). These seven compounds (**6e**–**k**) showed the highest cytotoxicity against the HL-60 cancer cell line. With hydroxy and methoxy groups on the C-ring, the rank order of HL-60 inhibitory activity was 6-pyrrolidinyl (**6h**) > 6-dimethylamino (**6k**) > 6,7-methylenedioxy (**6e**) > 6-morpholinyl (**6j**). A change from 5′-methoxy (**6h**) to 5′-ethoxy (**6i**) significantly increased anti-proliferative activity, but replacement with 5′-hydroxy (**6g**) decreased activity significantly, as also seen with **6e** versus **6d**. While **6i** was more potent than **6h**, unfortunately, **6i** also exhibited unsatisfactory toxicity against the Detroit 551 cell line. Therefore, among compounds **6a**–**k**, we selected**6h** as the lead compound for further investigation, and submitted it to NCI for anticancer evaluation.

The resulting activity profile fingerprint (Table 2 and Supplementary data) of **6h** against the NCI-60 human cancer cell line panel indicated significant inhibitory activity against a variety of cancer cell lines. Compound **6h** was particularly active against SR leukemia (log GI_{50} <−8.00), MDA-MB-435 melanoma (log GI_{50} <−8.00), and NCI-H522 non-small cell lung cancer (log TGI <−8.00) cell lines. We also evaluated the potency of **6h** in an anti-proliferation assay with NCI-H522 cells. MTT assay results showed that **6h** effectively inhibited proliferation of NCI-H522 cells with an IC_{50} value of 42.3 ± 1.2 nM at 48 h-incubation. The activity profile fingerprint of **6h** was further analyzed by COMPARE correlation at the GI_{50} level. As shown in Table 3, the fingerprint of **6h** correlated most closely with that of antimitotic *Vinca* alkaloids, such as vincristine and vinblastine. To support this supposition, we examined whether **6h** has an effect on tubulin assembly. In an in vitro tubulin polymerization assay, **6h** inhibited tubulin polymerization in a concentration-dependent manner, which was similar to the effects caused by vincristine and vinblastine (Fig. 1A).[24] Results from an in vivo tubulin assembly assay showed that **6h** inhibited α-tubulin and β-tubulin accumulation concentration-dependently in the cytoskeletal fraction, the same effect as vincristine and vinblastine, whereas paclitaxel caused tubulin polymerization (Fig. 1B).[25] Thus, the preliminary results indicated that the mechanism of action of **6h** resembles that of vincristine and vinblastine (Fig. 2).

In summary, 6- (or 6,7-) substituted 2-(hydroxyl substituted phenyl)quinolin-4-one derivatives were designed, synthesized, and evaluated for in vitro antitumor activity. Preliminary SAR correlations of the new analogs were established. The most promising compound **6h** demonstrated low toxicity against a normal cell line and significant inhibition against several cancer cell lines. In addition, the results of a COMPARE analysis suggested that **6h** might function as an antimitotic agent, such as *Vinca* alkaloids. Therefore, we believe that **6h** is a promising lead compound that deserves further optimization and derivatization as a hydrophilic prodrug.

Table 3
Results of COMPARE correlations at GI_{50} level for compound **6h**

Rank	Compound (NCI number)	r[a]
1	Vincristine sulfate (NSC 67574)	0.538
2	Maytansine (NSC 153858)	0.497
3	Vinblastine sulfate (NSC 49842)	0.489
4	Rhizoxin (NSC 332598)	0.476
5	DON (NSC 7365)	0.452
6	Didemnin B (NSC 325319)	0.450
7	AT-125 (NSC 163501)	0.442
8	*S*-Trityl-L-cysteine (NSC 83265)	0.421
9	Tiazofurin (NSC 286193)	0.402
10	Trimetrexate (NSC 352122)	0.401

[a] r = correlation coefficient.

Acknowledgments

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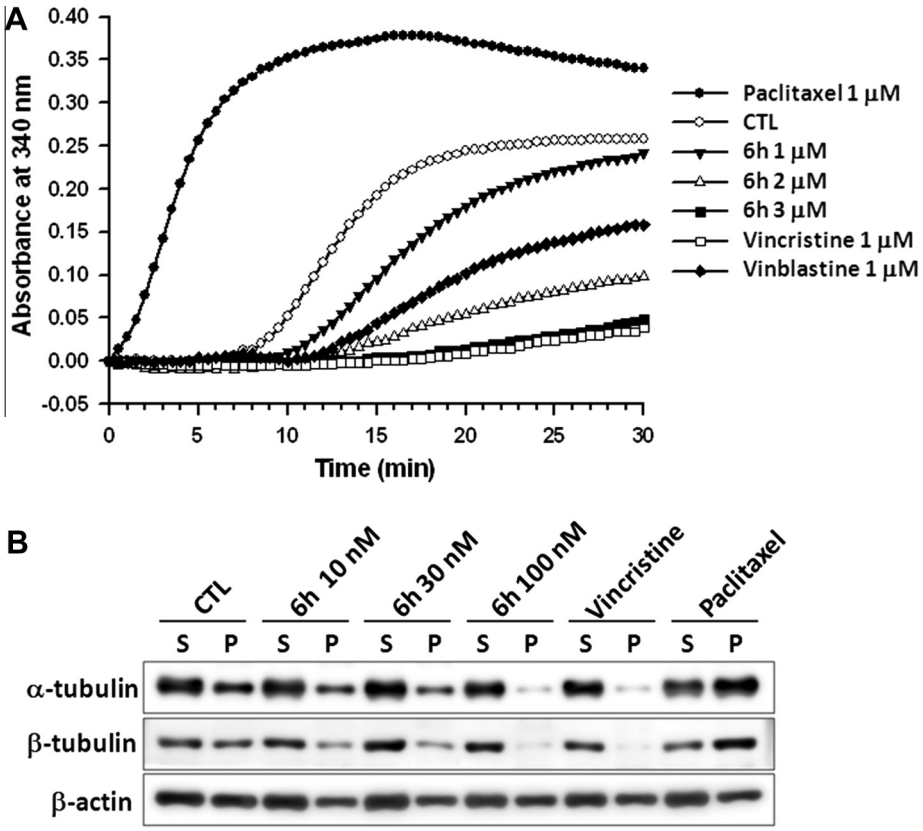

Figure 1. Action mechanism of **6h** for anti-proliferation of NCI-H522 non-small cell lung cancer cells. (A) Compound **6h**, vincristine, vinblastine, and paclitaxel were incubated with pure tubulin proteins in GPEM buffer. Alternation of tubulin assembly was recorded at absorbance 340 nm. (B) Cells were treated with vehicle (DMSO, as control), **6h** (10, 30, and 100 nM), 100 nM vincristine, and 100 nM paclitaxel for 24 h. Cytosolic (S, soluble) and cytoskeletal (P, polymerized tubulin) fractions were separated and followed by Western blot analysis for detection of α-tubulin, β-tubulin, and β-actin protein expression.

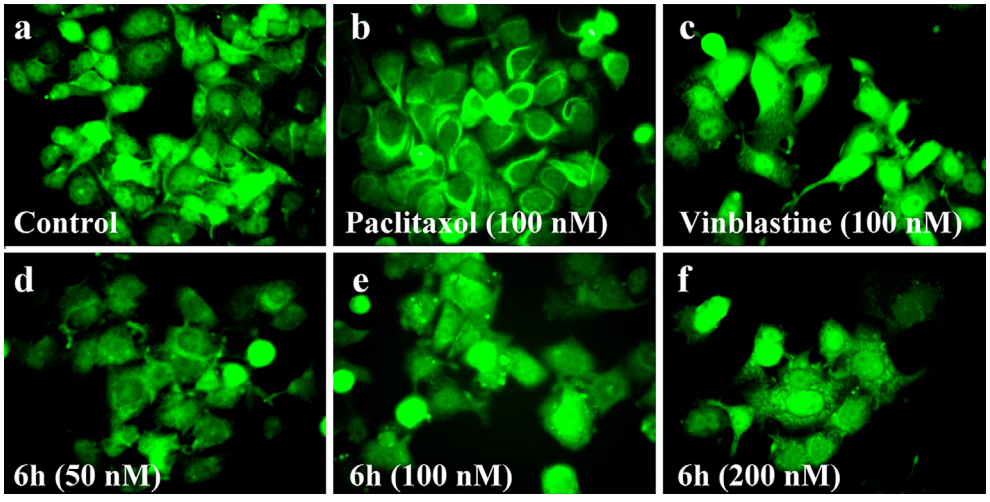

Figure 2. Immunostaining of non-small cell lung cancer NCI-H522 with α-tubulin-FITC. (a) Control; (b) 24 h treatment with paclitaxel at 100 nM; (c) 24 h treatment with vinblastine at 100 nM; (d–f) 24 h treatment with **6h** at 50, 100, 200 nM.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.083.

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18. *Preparation of arylcarbonyl chlorides (**2a–f**):* Arylcarboxylic acids (**1a–f**) were suspended in dry THF at room temperature. Thionyl chloride and DMF were added dropwise. The reaction mixtures were stirred for 12 h at room temperature and then evaporated to dryness. The residues were used directly in the next step without purification.
19. *Preparation of benzamides (**4a–k**):* Into solutions of **1a–f** in dry THF were added triethylamine and *o*-aminoacetophenones (**3a–d**).[2,3] The mixtures were stirred at room temperature for 12 h and evaporated. The residue was purified by column chromatography eluting with a mixture of *n*-hexane and EtOAc (1/1) to afford pure carboxamides (**4a–k**).
20. *Preparation of 2-(benzyloxy substituted phenyl)quinolin-4-ones (**5a–k**):* A mixture of carboxamide (**4a–k**) and NaOH was suspended in 1,4-dioxane. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the mixture was evaporated and then the residue was added to 10% NH_4Cl solution. The precipitate was collected, washed with water and acetone, and then purified by silica gel column chromatography to obtain 2-(benzyloxy substituted phenyl)quinolin-4-ones (**5a–k**).
21. *Preparation of 2-(hydroxyl substituted phenyl)quinolin-4-ones (**6a–k**):* A suspension of **5a–k** and palladium (10 wt.% on activated carbon) in MeOH or CH_2Cl_2 was stirred at room temperature under hydrogen gas atmosphere for 24 h. The precipitate was collected and dissolved in 10% NaOH solution and then filtered. The filtrate was acidified with dil aq HCl and the resulting precipitate was collected and washed with acetone and water to yield 2-(hydroxyl substituted phenyl)quinolin-4-ones (**6a–k**).
22. *Cell culture:* Human leukemia HL-60 and non-small-cell-lung cancer H460 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Human hepatocellular carcinoma cell line Hep3B was obtained from America Type Culture Collection (Manassas, VA, USA). Hep3B cells were cultured in DMEM/F12 medium supplemented with 10% FBS and penicillin (100 U/mL)/streptomycin (100 μg/mL) and maintained in a humidified incubator containing 5% CO_2. Normal skin Detroit 551 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Logarithmically growing cancer cells were used for all experiments.
23. *Cell viability assay:* The cell viability was detected by 3(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were cultured in 96-well plates at 37 °C and incubated with complete medium containing the vehicle (DMSO) or compounds for indicated times and concentrations. After treatment, cells were incubated with MTT solution (1 mg/mL in 1× PBS) at 37 °C for 2 h. The absorbance of the samples was read at 570 nm and corrected for inference at 630 nm.
24. *In vitro microtubule polymerization:* In vitro assay of microtubule polymerization was detected with the tubulin polymerization assay (Cat.# BK011P, Cytoskeleton Inc, Denver, CO, USA) Briefly, pure porcine brain tubulin proteins (300 μg) in 100 μL of GPEM buffer (80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, 5% glycerol) were incubated with test compounds at 37 °C. The alternation of tubulin polymerization was measured at absorbance 340 nm every 30 s for 30 min (Synergy 2; BioTek, Winooski, VT, USA).
25. *In vivo tubulin assembly assay:* Cells were lysed in hypotonic buffer (1 mM $MgCl_2$, 2 mM EGTA, 0.5% NP-40, 2 mM PMSF, 200 Units/mL aprotinin, 5 mM amino caproic acid, 1 mM benzamidine, and 20 mM Tris–HCl, pH 6.8) for 5 min at 37 °C followed by centrifugation, 15,000 *g* for 10 min at 25 °C. The supernatant contained cytosolic tubulin. The pellets of polymerized tubulin were resuspended in hypotonic buffer and sonication. Both fractions were subjected to Western blot analysis for detection of tubulin contents.