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New Colchicine-Derived Triazoles and Their Influence on Cytotoxicity and Microtubule Morphology

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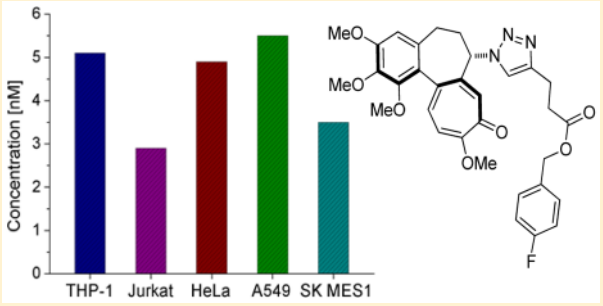
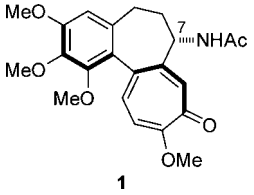
Supporting Information

ABSTRACT: A series of new colchicinoids with a variable triazole unit at C-7 was synthesized through Cu(I)-catalyzed 1,3-dipolar cycloaddition (*click*-chemistry) of a colchicine-derived azide with various alkynes and the cytotoxicity against THP-1 and Jurkat cancer cell lines was used for structural optimization. Three particularly active compounds ($IC_{50} \leq 5$ nM) were additionally investigated with respect to their efficacy against relevant solid tumor cell lines (HeLa, A549, and SK MES 1). Besides distorting the microtubule morphology by tubulin depolymerization, one compound also exhibited a pronounced centrosome declustering effect in triple negative breast cancer cells (MDA-MB-231) and nonsmall cell lung cancer cells (H1975).



KEYWORDS: *Colchicine, click chemistry, tubulin, antitumoral compounds, resistance*

Microtubules are highly dynamic polymers of α,β-tubulin heterodimers that play a key role in essential cellular processes such as accurate cell division, intracellular transport, and cell motility.[1] Therefore, the tubulin polymerization/depolymerization equilibrium represents an attractive target for the development of anticancer drugs.[2−5] Colchicine (**1**, Figure 1) is a long-known and powerful antimitotic agent extracted



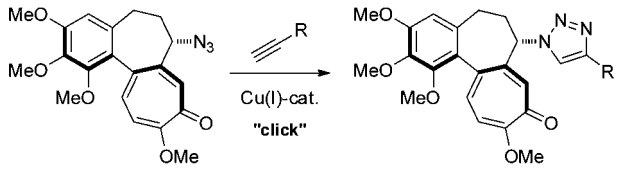
1

Figure 1. Natural product colchicine (**1**).

from *Colchicum autumnale* and clinically used mainly in the treatment of gout.[6] It acts by destabilizing microtubules via depolymerization leading to cell cycle arrest in the metaphase and, as a consequence, apoptotic cell death. While the use of colchicine (**1**) in cancer chemotherapy is hampered by its toxicity,[7] its remarkable biological activity motivates the search for new analogues with improved pharmacological properties.[8−14]

In this context, we recently developed a "click conjugation" approach allowing a fast and efficient variation of the C7-side chain at the colchicine core (Scheme 1).[15−17] The method is based on the microwave-assisted Cu-catalyzed 1,3-dipolar

Scheme 1. Synthesis of C7-Modified Colchicine Derivatives through Click-Chemistry[a]



2 **3**

[a]Reagents and conditions: $CuSO_4 \cdot 5H_2O$, (5 mol %), sodium ascorbate (10 mol %), H_2O/*tert*-BuOH (1:1), μW (300 W), 85 °C, 20−45 min. See Chart 1 for structures and isolated yields.

cycloaddition[18,19] of various alkynes to azide **2**, which is readily prepared from colchicine (**1**) in only four steps. Furthermore, it was shown that some of the resulting triazoles of type **3** exhibit promising levels of activity. We here report the continuation of this study, which has led to the identification of some new and highly active compounds.

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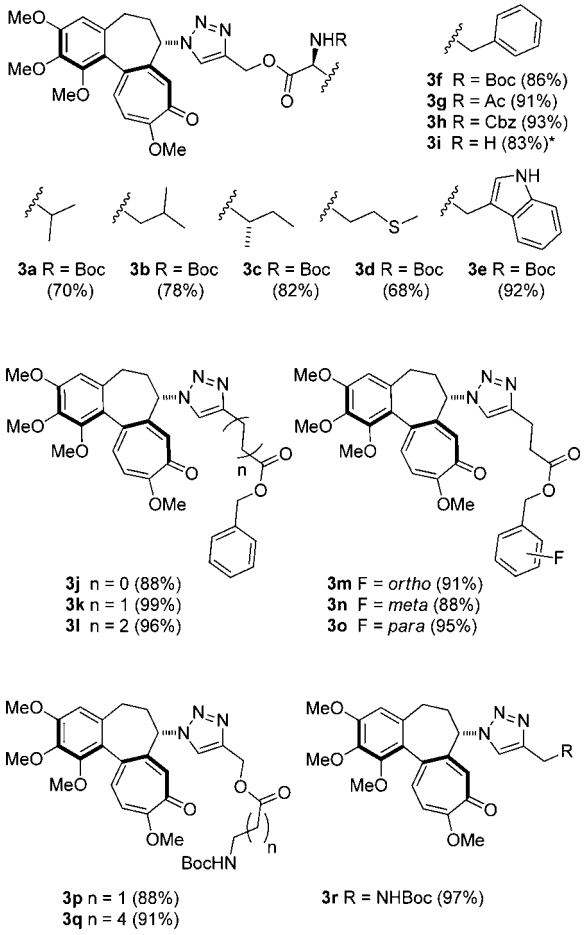
Following an improved protocol (see Supporting Information) the azide **2** was obtained from *N*-deacetyl-colchicin[15−17,20] through diazo transfer in 86% yield. The triazoles of type **3** were then prepared through "click chemistry" by microwave-induced heating of **2** and a terminal alkyne to 85 °C in the presence of 5 mol % of $CuSO_4 \cdot 5H_2O$ and 10 mol % of sodium ascorbate in a H_2O/*tert*-BuOH (1:1) solvent mixture. As a triazole of type **3** with an amino acid-derived side chain had shown significant activity in our previous study,[15−17] we focused on compounds derived from (*N*-protected) amino acid propargyl esters. The synthesized library of new colchicine conjugates (Chart 1), however, not only comprises compounds

Chart 1. New Colchicine-Derived Triazoles of Type 3 Synthesized According to Scheme 1[a]



[a]Yields of purified products are given in brackets. *3i was prepared from 3f by treatment with TFA.

derived from amino acids (**3a**−**3i**) but also compounds with an *O*-benzylated carboxylic acid substituent (**3j**−**3o**) or a *N*-Boc-protected aminoalkyl side chain (**3p**−**3r**), respectively.

The cytotoxicity (IC_{50}) of all compounds was determined by means of a bioluminescence cell viability assay[21,22] using THP-1 acute monocytic leukemia cells as well as Jurkat T cell lymphocytes. While all compounds were found to exhibit cytotoxic activity (Table 1), the phenylalanine derivative **3f** and the *β*-alanine ester **3p** stood out in the initial screening by inhibiting cell growth even at low nanomolar concentrations (IC_{50} < 10 nM). Interestingly, these compounds carry a lipophilic moiety (phenyl or *tert*-butyl) in a certain distance to

Table 1. Cytotoxic Activity of Colchicine-Derived Compounds against THP-1 and Jurkat Cells[a]

compd	IC_{50} [nM] THP-1	IC_{50} [nM] Jurkat
1	20.4 ± 2.7	13.5 ± 4.7
2	20.9 ± 0.7	
3a	8.1 ± 1.8	
3b	6.6 ± 5.5	
3c	17.3 ± 6.6	
3d	10.6 ± 9.3	
3e	7.3 ± 2.3	
3f	4.1 ± 1.7	15.3 ± 3.5
3g	25.5 ± 0.9	84.2 ± 5.9
3h	23.3 ± 4.8	21.4 ± 3.8
3i	24.0 ± 6.1	25.1 ± 2.9
3j	139.8 ± 34.6	
3k	31.0 ± 0.8	
3l	35.2 ± 12.8	16.7 ± 7.1
3m	12.7 ± 2.4	9.7 ± 3.1
3n	9.3 ± 0.2	4.9 ± 1.4
3o	5.1 ± 1.1	2.9 ± 1.6
3p	5.7 ± 2.2	5.0 ± 1.5
3q	5.4 ± 0.3	9.4 ± 4.0
3r	23.1 ± 10.1	

[a]Values represent cytotoxic activity (IC_{50}) after 24 h (THP-1) or 48 h (Jurkat) compound incubation. Data shown are mean values ± SD from at least 3 independent experiments with biological replicates ≥3, respectively.

the colchicine core (i.e., the main pharmacophore), as does also the benzylester **3k**. Shortening or lengthening the linking unit to the lipophilic group resulted in a decrease of activity[13] as illustrated by compounds **3i**−**3l** or **3p**−**3r** (Table 1). While phenylalanine derivatives **3g**−**3i** differing from **3f** only in the *N*-protecting group did not exhibit improved activities as compared to **3f**, the introduction of an additional fluorine substituent in the aryl moiety of **3k** led to an increase of the cytotoxic activity (compounds **3m**−**3o**). These results indicate a favorable hydrophobic interaction between the side chain and a lipophilic pocket of the target protein.

The three most active compounds, that is the phenylalanine derivative **3f**, the 4-fluorobenzyl ester **3o**, and the *β*-alanine ester **3p**, were further evaluated with respect to their cytotoxic activity against solid tumor cell lines, that is HeLa cervix carcinoma cells as well as A549 and chemotherapy resistant SK MES 1 lung cancer cells (Table 2). As a reference, paclitaxel, a marketed chemotherapeutic drug, was used as a strongly mictrotubule-stabilizing compound. Interestingly, compound **3o** proved to exhibit remarkable cytotoxic efficacy by inhibiting

Table 2. Cytotoxicity and Tubulin Polymerization Inhibition Activity of Compounds 3f, 3o, and 3p[a]

compd	IC_{50} [nM] HeLa	IC_{50} [nM] A549	IC_{50} [nM] SK MES1	IC_{50} [μM] tubulin
paclitaxel	18.9 ± 1.7	13.2 ± 3.1	13.5 ± 3.2	
1	21.8 ± 1.1	23.9 ± 4.6	18.6 ± 1.8	6.7 ± 0.05
3f	70.7 ± 3.1	73.7 ± 20.5	39.2 ± 14.2	3.2 ± 0.3
3o	4.9 ± 2.7	5.5 ± 1.7	3.5 ± 0.9	2.1 ± 0.6
3p	20.0 ± 2.2	25.4 ± 4.1	9.6 ± 1.6	2.44 ± 0.4

[a]Cytotoxic activity (IC_{50}) after 48 h (n = 3). Inhibition of tubulin polymerization (n = 2). Data shown are mean values ± SD from n independent experiments.

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cell growth already at concentrations below 6 nM clearly surpassing paclitaxel and colchicine (**1**). In addition, compounds **3f**, **3o**, and **3p** inhibited tubulin polymerization by 50% at concentrations of 2−3 μM in an *in vitro* assay. This demonstrates the strong cytotoxic activity of these compounds resulting from their microtubule-destabilizing activity, as expected.

In an additional set of experiments we explored the effect of colchicinoids **3f**, **3o**, and **3p** on the microtubule cytoskeleton morphology of MDA-MB-231 breast cancer cells by means of immune fluorescence microscopy. For this purpose, the cells were incubated with **3f**, **3o**, or **3p** at concentrations of 100 nM and microtubules, centrosomes, and DNA were visualized using fluorescence stains (Figure 2). Centrosomes are the major

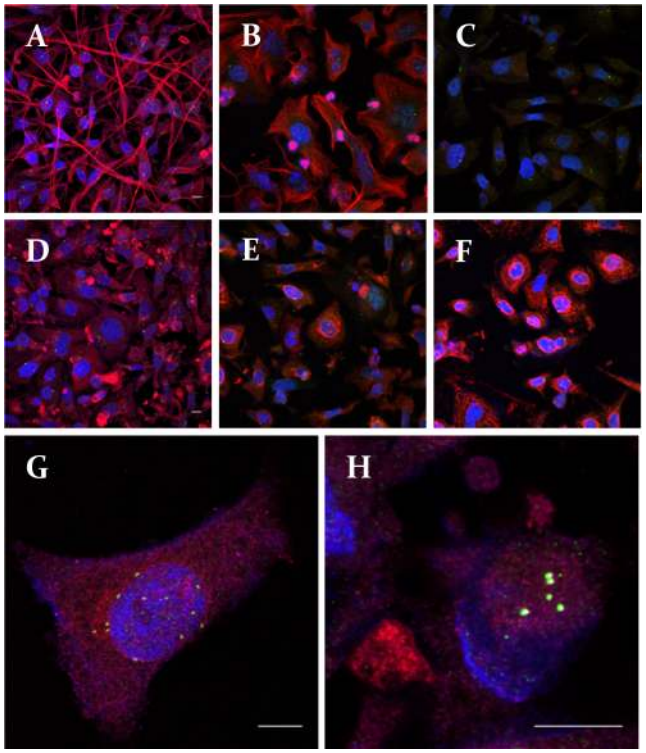

Figure 2. Microtubule morphology of MDA-MB-231 breast cancer cells after 24 h incubation with different compounds: (A) control (untreated cells); (B) paclitaxel (100 nM); (C) **1** (100 nM); (D) **3f** (100 nM); (E) **3o** (100 nM); (F) **3p** (100 nM). Centrosome declustering: (G) **3f** (100 nM) in MDA-MB-231 breast cancer cells; (H) **3f** (500 nM) in H1975 lung cancer cells. Microtubules (red) and centrosomes (green) were stained with antibodies, while DAPI (4,6-diamidino-2-phenylindole) was used to visualize DNA (blue). The white scale bars correspond to a distance of 10 μm.

microtubule organizing centers of animal cells.[1,23] While untreated cells (A) showed a typical microtubule network, incubation with 100 nM of paclitaxel as a microtubule-stabilizing agent resulted in the expected segregation of the cells (B). As a second control, colchicine completely collapsed the microtubule network (C). As Figure 2 clearly shows, compounds **3f**, **3o**, and **3p** also suppressed the formation of the microtubule network (at 100 nM); however, short microtubule fragments (tending to localize around the cell nucleus) were still visible (D−F).[13,14]

In contrast to normal cells, which contain a pair of centrosomes, cancer cells exhibit extra number of centrosomes, which contributes to cancer cell invasion.[24,25] The amplified centrosomes in cancer cells are clustered together so that cancer cells are able to avoid multipolar mitosis. Thus, preventing centrosome clustering has been recognized to be an attractive cancer target.[26,27] Interestingly, the phenylalanine derivative **3f** (as the only one of the investigated compounds) was found to exhibit a strong centrosome declustering effect on MDA-MB-231 breast cancer cells (G) and also on H1975 small cell lung cancer cells (H). Notably, H1975 cells have been identified to be a therapy resistant cancer against currently available tyrosine receptor kinase inhibitors.[28]

In conclusion, we have demonstrated that readily available colchicine-derived triazoles of type **3** may exhibit remarkable biological effects in dependence of the side chain structure. Besides pronounced cytotoxic activities the remarkable centrosome-declustering effect exhibited by compound **3f** may justify further investigation. Since tubulin heterodimers are core structural components also of centrosomes,[29] targeting their regulatory mechanisms by small molecules may offer interesting new options for therapeutic intervention in cancer chemotherapy.[30]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00418.

Experimental procedures, characterization of all compounds, and protocols for biological assays (PDF)

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Author Contributions

P.T. performed the synthesis, purification, and characterization of new colchicinoids. J.S. carried out the antiproliferation and tubulin polymerization assays. A.M. performed the immune fluorescence microscopic studies. N.T., J.G., and H.G.S. initiated and supervised the project. The manuscript was written, based on a first draft by P.T., through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Mol. Biol. Cell*, 6th ed.; Garland Publishing, 2014.

(2) Dumontet, C.; Jordan, M. A. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat. Rev. Drug Discovery* **2010**, *9*, 790−803.

(3) Seligmann, J.; Twelves, C. Tubulin: an example of targeted chemotherapy. *Future Med. Chem.* **2013**, *5*, 339−352.

DOI: 10.1021/acsmedchemlett.5b00418
ACS Med. Chem. Lett. 2016, 7, 188−191

(4) Nepali, K.; Ojha, R.; Sharma, S.; Bedi, P. M. S.; Dhar, K. L. Tubulin Inhibitors: A Patent Survey. *Recent Pat. Anti-Cancer Drug Discovery* **2014**, *9*, 176−220.
(5) Chandrasekaran, G.; Tatrai, P.; Gergely, F. Hitting the brakes: targeting microtubule motors in cancer. *Br. J. Cancer* **2015**, *113*, 693−698.
(6) Richette, P.; Bardin, T. Colchicine for the treatment of gout. *Expert Opin. Pharmacother.* **2010**, *11*, 2933−2938.
(7) Finkelstein, Y.; Aks, S. E.; Hutson, J. R.; Juurlink, D. N.; Nguyen, P.; Dubnov-Raz, G.; Pollak, U.; Koren, G.; Bentur, Y. Colchicine poisoning: the dark side of an ancient drug. *Clin. Toxicol.* **2010**, *48*, 407−414.
(8) Massarotti, A.; Theeramunkong, S.; Mesenzani, O.; Caldarelli, A.; Genazzani, A. A.; Tron, G. C. Identification of Novel Antitubulin Agents by Using a Virtual Screening Approach Based on a 7-Point Pharmacophore Model of the Tubulin Colchi-Site. Tron. *Chem. Biol. Drug Des.* **2011**, *78*, 913−922.
(9) Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quin, F.; Suffness, M.; Silverton, J. Colchicine and its analogues: Recent findings. *Med. Res. Rev.* **1988**, *8*, 77−94.
(10) Nicolaou, K. C.; Valiulin, R. A.; Pokorski, J. K.; Chang, V.; Chen, J. S. Bio-inspired synthesis and biological evaluation of a colchicine-related compound library. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3776−3780.
(11) Vilanova, C.; Díaz-Oltra, S.; Murga, J.; Falomir, E.; Carda, M.; Redondo-Horcajo, M.; Díaz, J. F.; Barasoain, I.; Marco, J. A. Design and Synthesis of Pironetin Analogue/Colchicine Hybrids and Study of Their Cytotoxic Activity and Mechanisms of Interaction with Tubulin. *J. Med. Chem.* **2014**, *57*, 10391−10403.
(12) Cosentino, L.; Redondo-Horcajo, M.; Zhao, Y.; Santos, A. R.; Chowdury, K. F.; Vinader, V.; Abdallah, Q. M. A.; Abdel-Rahman, H.; Fournier-Dit-Chabert, J.; Shnyder, S. D.; Loadman, P. M.; Fang, W.-S.; Díaz, J. F.; Barasoain, I.; Burns, P. A.; Pors, K. Synthesis and Biological Evaluation of Colchicine B-Ring Analogues Tethered with Halogenated Benzyl Moieties. *J. Med. Chem.* **2012**, *55*, 11062−11066.
(13) Zefirova, O. N.; Nurieva, E. V.; Shishov, D. V.; Baskin, I. I.; Fuchs, F.; Lemcke, H.; Schröder, F.; Weiss, D. G.; Zefirov, N. S.; Kuznetsov, S. A. Synthesis and SAR requirements of adamantane−colchicine conjugates with both microtubule depolymerizing and tubulin clustering activities. *Bioorg. Med. Chem.* **2011**, *19*, 5529−5538.
(14) Zefirova, O. N.; Lemcke, H.; Lantow, M.; Nurieva, E. V.; Wobith, B.; Onishchenko, G. E.; Hoenen, A.; Griffiths, G.; Zefirov, N. S.; Kuznetsov, S. A. Unusual Tubulin-Clustering Ability of Specifically C7-Modified Colchicine Analogues. *ChemBioChem* **2013**, *14*, 1444−1449.
(15) Nicolaus, N.; Zapke, J.; Riesterer, P.; Neudörfl, J.-M.; Prokop, A.; Oschkinat, H.; Schmalz, H.-G. Azides Derived from Colchicine and their Use in Library Synthesis: a Practical Entry to New Bioactive Derivatives of an Old Natural Drug. *ChemMedChem* **2010**, *5*, 661−665.
(16) Nicolaus, N.; Reball, J.; Sitnikov, N.; Velder, J.; Termath, A.; Fedorov, A. Y.; Schmalz, H.-G. A Convenient Entry to New C-7-Modified Colchicinoids through Azide Alkyne [3 + 2]-Cycloaddition: Application of Ring-Contractive Rearrangements. *Heterocycles* **2011**, *82*, 1585−1600.
(17) Kuznetsova, N. R.; Svirshchevskaya, E. V.; Sitnikov, N. S.; Abodo, L.; Sutorius, H.; Zapke, J.; Velder, J.; Thomopoulou, P.; Oschkinat, H.; Prokop, A.; Schmalz, H.-G.; Fedorov, A. Y.; Vodovozova, E. L. Lipophilic prodrugs of a triazole-containing colchicine analogue in liposomes: Biological effects on human tumor cells. *Russ. J. Bioorg. Chem.* **2013**, *39*, 543−552.
(18) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.
(19) Meldal, M.; Tornøe, C. W. Cu-Catalyzed Azide−Alkyne Cycloaddition. *Chem. Rev.* **2008**, *108*, 2952−3015.
(20) Bagnato, J. D.; Eilers, A. L.; Horton, R. A.; Grissom, C. B. Synthesis and Characterization of a Cobalamin−Colchicine Conjugate as a Novel Tumor-Targeted Cytotoxin. *J. Org. Chem.* **2004**, *69*, 8987−8996.
(21) Kangas, L.; Grönroos, M.; Nieminen, A. L. Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents in vitro. *Med. Biol.* **1984**, *62*, 338−343.
(22) Crouch, S. P. M.; Kozlowski, R.; Slater, K. J.; Fletcher, J. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Methods* **1993**, *160*, 81−88.
(23) Bornens, M. The Centrosome in Cells and Organisms. *Science* **2012**, *335*, 422−426.
(24) Godinho, S. A.; Picone, R.; Burute, M.; Dagher, R.; Su, Y.; Leung, C. T.; Polyak, K.; Brugge, J. S.; Théry, M.; Pellman, D. Oncogene-like induction of cellular invasion from centrosome amplification. *Nature* **2014**, *510*, 167−171.
(25) Ganem, N. J.; Godinho, S. A.; Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **2009**, *460*, 278−282.
(26) Ogden, A.; Rida, P. C. G.; Aneja, R. Let's huddle to prevent a muddle: centrosome declustering as an attractive anticancer strategy. *Cell Death Differ.* **2012**, *19*, 1255−1267.
(27) Pannu, V.; Rida, P. C. G.; Celik, B.; Turaga, R. C.; Ogden, A.; Cantuaria, G.; Gopalakrishnan, J.; Aneja, R. Centrosome-declustering drugs mediate a two-pronged attack on interphase and mitosis in supercentrosomal cancer cells. *Cell Death Dis.* **2014**, *5*, 1538−1548.
(28) Laurila, N.; Koivunen, J. EGFR inhibitor and chemotherapy combinations for acquired TKI resistance in EGFR-mutant NSCLC models. *Med. Oncol.* **2015**, *32*, 1−7.
(29) Avidor-Reiss, T.; Gopalakrishnan, J. Cell cycle regulation of the centrosome and cilium. *Drug Discovery Today: Dis. Mech.* **2013**, *10*, e119−e124.
(30) Kim, S.-K.; Cho, S.-M.; Kim, H.; Seok, H.; Kim, S.-O.; Kwon, T. K.; Chang, J.-S. The colchicine derivative CT20126 shows a novel microtubule-modulating activity with apoptosis. *Exp. Mol. Med.* **2013**, *45*, e19.

DOI: 10.1021/acsmedchemlett.5b00418
ACS Med. Chem. Lett. 2016, 7, 188−191