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Review Article

# Reshaping cytoskeleton: different acts of modulatory compounds

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**ABSTRACT**

The eukaryotic cytoskeleton is composed of filamentous structures, namely microfilaments, microtubules and intermediate filaments. The cytoskeleton is an essential component of cells due to its role in various cellular functions, such as intracellular transport, organelle positioning, chromosome segregation and cytokinesis. Abnormalities in cytoskeleton, as well as associated proteins and regulatory pathways, have been shown to contribute to disease pathomechanisms including cancer and neurodegenerative diseases. Therefore, cytoskeleton is an important therapeutic target and many compounds have been identified or developed to modulate the cytoskeleton. In this review, we focused on cytoskeleton modulatory compounds and summarized their mechanisms of action.

**Keywords:** Microtubule, actin, cytoskeleton modulatory compounds

## INTRODUCTION

The cytoskeleton is an intracellular network maintaining the internal organization of cells and also providing specific cell morphology and mechanical support. It plays a role in various cellular processes such as cell movement, division, contraction in muscle cells and axonal transport in neurons. It is made of different types of filaments, namely microfilaments, microtubules, and intermediate filaments. These filaments interact with each other with the help of associated proteins to establish and maintain the cytoskeletal network. Recent studies showed that defects in the cytoskeleton are involved in pathomechanisms of several diseases such as cancer, neurodegenerative disease, myopathies, cardiovascular disease, liver cirrhosis and pulmonary fibrosis. For this reason, many compounds have been investigated so far for therapeutic purposes. Additionally, such compounds are powerful tools in cell biology to understand the structural properties of filaments as well as functions of cytoskeleton related proteins. Therefore, a broad spectrum of cytoskeleton modulatory compounds has been extensively studied in in vitro and in vivo model systems as well as in clinical trials. Several synthetic and natural compounds obtained from minerals, microorganisms, and animals, have been studied mostly for altering microfilament and microtubule structures. These compounds generally alter the cytoskeleton by directly binding to monomer/filament or associated-proteins, targeting post-translational modifications and regulatory signaling pathways. In this review, we summarized different acts of cytoskeleton modulatory compounds considering their mechanisms of action.

### A. Microtubules and modulatory compounds

Microtubules are hollow cylindrical polymers and the thickest filaments (25 nm in diameter) of cytoskeleton. It is composed of two globular protein subunits, namely alpha (α) and beta (β) tubulin. The α and β tubulin proteins form a heterodimer, which is added sequentially in a certain direction to form protofilaments. Laterally interacting 13 protofilaments establish microtubule

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structure. The position of α and β tubulin proteins within a filament gives a polar feature to microtubules and forms plus and minus ends. The polymerization of tubulin heterodimers primarily occurs at plus ends. Minus ends are generally attached to the "microtubule organization center" (MTOC), a region where microtubules are formed. Microtubules are dynamic structures that shorten with depolymerization and elongate with polymerization. This dynamic behavior is called dynamic instability and GTP hydrolysis plays an important role in the regulation of this behavior (Lodish et al., 2003;Bora, Koyunoğlu, Sunguroğlu, & Yurter, 2019) (Figure 1). The dynamic structure of microtubules is regulated by microtubule-binding proteins such as microtubule-associated proteins (MAPs), microtubule plus-end-tracking proteins (+TIPs) and also post-translational modifications of tubulin subunits. Microtubules are involved in diverse cellular processes in eukaryotic cells such as cell movement, division, polarization, intracellular transport of macromolecules (protein, RNA, vesicle, etc) and organelle positions.

Microtubules are known as druggable targets and pharmacological alteration of microtubule structure has long been utilized for cancer treatment to arrest cell cycle by interfering mitotic spindle formation and also promote apoptotic cell death. Besides, dysregulations in microtubule dynamics contribute to the pathomechanisms of several neurodegenerative diseases (Bora, Sucularlı, Hensel, Claus, & Yurter, 2019). Reduced stability of neuronal microtubules has been reported in Alzheimer's disease, Parkinson's disease and Amyotrophic lateral sclerosis (Dubey, Ratnakaran, & Koushika, 2015), whereas microtubules are hyperstabilized in Hereditary spastic paraplegia (Hazan J.,1999; Evans K. J., 2005). Therefore, microtubules could also be promising targets for neurodegenerative diseases as well as for nervous system injuries. Microtubule

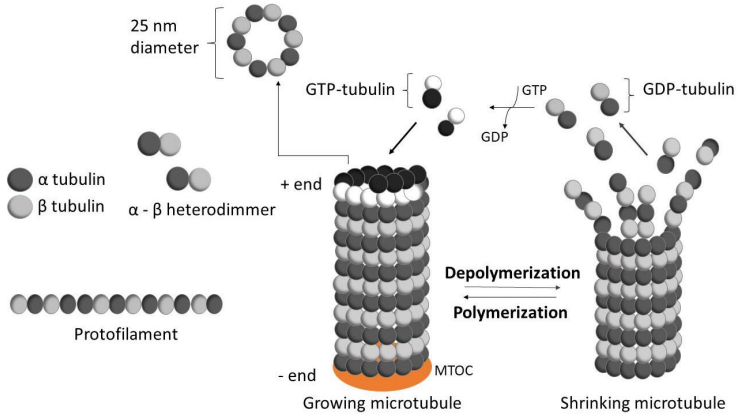


**Figure 1.** Microtubule structure and organization.

**Table 1. Microtubule stabilizers, destabilizers and its binding sites.**

| Compounds | Binding sites | | | Compounds | Binding sites | | |
|---|---|---|---|---|---|---|---|
| **Stabilizers** | **Between tubulin dimer** | **Within tubulin dimer** | **Microtubule luminal packet** | **Destabilizers** | **Between tubulin dimer** | **Within tubulin dimer** | **Microtubule luminal packet** |
| Paclitaxel* | | | + | Maytansine* | + | | |
| Epothilones** | | | + | Rotenone* | | + | |
| Discodermolide*** | | | + | Noscapine* | | + | |
| Docetaxel | | | + | Vinorelbine | | + | |
| Dictyostatin*** | | + | | Colchicine* | | + | |
| Zampanolide*** | | | + | Halichondrin B*** | + | | |
| Rhizoxin**** | + | | | Eribulin | | | + |
| Eleutherobin*** | | | + | Nocodazole | | + | |
| Laulimalide*** | | + | | Combrestatins A4* | + | | |
| Peloruside A*** | | + | | Tivantinib | | + | |
| Cyclostreptin** | | | + | Dolastatins-10*** | + | | |
| | | | | Pironetin** | + | | |

Natural compounds originally derived from * plants, **bacteria, ***marine species and **** fungi

modifying compounds having different mechanisms of action are summarized below.

**(i). Direct binding to tubulin or microtubule**

Several compounds alter microtubule structure by directly binding to either tubulins or microtubule filament (Table 1). These compounds are collectively called as microtubule targeting agents (MTAs), tubulin-binding agents (TBA) or anti-mitotic drugs. (Figure 2A) Tubulin-binding agents can interact with α and β tubulin proteins from six different binding sites. Mostly microtubule stabilizers bind to the taxane and/or laulimalide/peloruside sites in β tubulin, whereas microtubule depolymerizers bind to pironetin, colchicine, maytansine and vinca sites (Steinmetz & Prota, 2018). Microtubule stabilizers support tubulin polymerization and increase microtubule density, whereas destabilizers initiate microtubule depolymerization, disassembly and cause a reduction in microtubule density. Colchicine which is a natural compound derived from the *Colchicum autumnale,* is the first tubulin-targeting compound, in fact, it played a role in the discovery and characterization of tubulin proteins. It destabilizes microtubules by binding itself to β tubulin at the colchicine binding site. Colchicine binding leads to conformational changes of tubulin heterodimer, which can not further polymerize into microtubule thereby disrupting microtubule function (Niel & Scherrmann, 2006). Colchicine is an FDA approved drug for prophylaxis, gout and related inflammatory diseases such as Familial mediterranean fever (Zemer D,1986;Dalbeth, Lauterio, & Wolfe, 2014). Additionally, recent studies have provided evidence that colchicine increases the expression of a heat shock protein thereby it may be repurposed for Amyotrophic lateral sclerosis with a different mechanism (clinical trial ID: NCT03693781). Conversely, epothilone D (EpoD), a member of epothilone family isolated from the myxobacterium *Sorangium cellulosum*, is a strong promoter of tubulin polymerization *in vitro* that binds to the luminal surface of β-tubulin and inhibits microtubule depolymerization. In this way, it inhibits spindle formation and induces mitotic arrest (Lee & Swain, 2008). It has significant antitumor activity in patients with breast cancer (Overmoyer et al., 2005), but failed to show any activity in patients with non–small cell lung cancer (Yee et al., 2005). Additionally, EpoD has been investigated in Alzheimer's disease and it has been reported that EpoD treatment both increased axonal microtubule density and reduced axonal dystrophy. Although promising preclinical studies, phase I trial of EpoD in Alzheimer's disease was discontinued because of the severe toxic side-effect (Brunden et al., 2011).

**(ii). Modulation of tubulin post-translational modifications**

It is also possible to modify microtubules by altering post-translational modifications (PTMs) of tubulin proteins. PTMs occur at amino and carboxyl ends of α and β tubulins and form tubulin code regulating microtubule functions. Most of the PTMs occur after the formation of the microtubule structure, therefore,

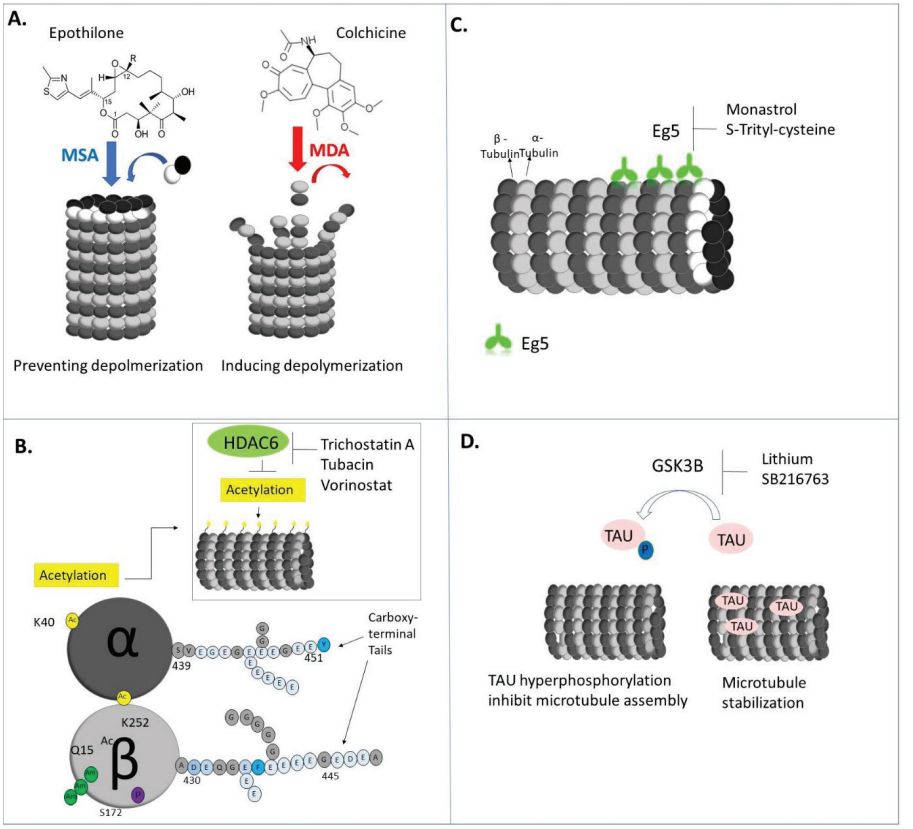


**Figure 2.** Mechanisms of action of microtubule modulatory compounds. (A) Compounds that can directly bind to tubulin or filament. MSA: microtubule-stabilizing agent, MDA: microtubule-destabilizing agent (B) Modulation of tubulin acetylation by HDAC inhibitors. (C) Eg5 inhibition by Monastrol and S-Trityl-cysteine (D) Modulation of signaling pathways through GSK3B kinase inhibitors. Ac: Acetylation, P: Phosphorylation

stable microtubules have more modifications than the dynamic ones. PTMs are enzymatically catalyzed, thereby it is possible to alter some of the modifications by inhibiting/activating tubulin-modifying enzymes. One of the most studied PTM is acetylation, which takes place at the 40$^{th}$ lysine (K40) of α tubulin within the microtubule lumen (Janke & Kneussel, 2010). Acetylation is catalyzed by tubulin acetyltransferase (TAT1), whereas deacetylation is catalyzed by sirtuin 2 (SIRT2) and histone deacetylase 6 (HDAC6) enzymes. It has been reported that inhibition of HDAC6 by inhibitors such as tubacin, trichostatin A and vorinostat leads to an increase in tubulin acetylation and enhancing cell motility (Palazzo, Ackerman, & Gundersen, 2003) (Figure 2B). Additionally, in an *in vitro* ALS model, HDAC inhibitors restore axonal transport defects, suggesting that increasing microtubule acetylation could have therapeutic potential (Guo et al., 2017). Acetylated microtubules are considered to be stable and long-lived, however, microtubule stabilization is not promoted by tubulin acetylation, therefore other acetylation related mechanisms should be involved.

**(iii). Modulation of microtubule-related proteins**

Several proteins play a role in regulating microtubule structure, therefore it is possible to modify it by inhibiting the activities of such proteins. For instance, kinesins are motor proteins that function in anterograde transport of membrane-bound organelles (Chen & Hancock, 2015). A member of the kinesin-5 family Eg5 is a plus-end kinesin and a key protein for spindle pole separation in many organisms, including humans. Monastrol and also S-Trityl-cysteine can selectively inhibit Eg5 protein, which causes mitotic arrest and apoptotic cell death due to defective spindle pole migration and the formation of radially aligned microtubules (Peterson & Mitchison, 2002)(Figure 2C). Several compounds were also identified to inhibit dyneins, another class of motor proteins, such as Ciliobrevin D and EHNA hydrochloride. These compounds affect the ATPase activity of dynein, therefore, are useful to address cellular functions of dynein proteins (Roossien, Miller, & Gallo, 2015).

**(iv). Alteration of signaling pathways**

The pharmacological alteration of signaling pathways is another approach to modify microtubule structure. Glycogen synthase kinase-3 beta (GSK-3β) is the major kinase that controlling microtubule structure and dynamics by differently regulating multiple types of microtubule-associated proteins such as MAP1B, MAP2C, TAU, CRMP2 and APC (Xu, Ge, Liu, & Gong, 2015), (Trivedi, Marsh, Goold, Wood-Kaczmar, & Gordon-Weeks, 2005), (Sánchez, Pérez, & Avila, 2000). High GSK3β activity induces phosphorylation of CRMP2 and APC thus inhibiting their microtubule polymerization/stabilization activities (Zumbrunn J., 2001), (Yoshimura T., 2005) However, loss of GSK3β activity results in dephosphorylation of MAP1B at growth cone that leads to decrease in growth cone motility necessary for axonal growth (Trivedi et al., 2005). Inhibition of GSK3β by compounds like lithium (LiCl) diminished TAU phosphorylation that leads to enhance microtubule polymerization and stabilization in Alzheimer's disease models (Trivedi et al., 2005; Lei, Ayton, Bush, & Adlard, 2011; Engel et al., 2006) Another compound, SB216763, also reduced TAU phosphorylation levels in the neuronal cultures, the hippocampus of postnatal rats, which correlated with its neuroprotective potential (Selenica et al., 2007) (Figure 2D).

**B. Microfilaments and modulatory compounds**

Microfilaments are the thinnest filaments (6 nm in diameter) of the cytoskeleton, which are composed of highly conserved globular actin proteins (G-actin). To form filamentous actin (F actin), three G-actin proteins come together using ATP and subsequent binding of G-actin to the plus end leads to the formation of a helix having two chains. Similar to microtubules, F actin shows polarity and plus end of the filament-barbed end-grows faster than the minus-pointed- end. Microfilaments are dynamic structures and both polymerization and depolymerization of actin subunits are regulated by several actin-binding proteins (Figure 3).

Actin filaments play an important role in the maintenance of cellular morphology as well as cell migration, cytokinesis, vesicle and organelle transport, and endocytosis. Therefore, modifying the actin cytoskeleton is a useful approach to understand the molecular mechanisms of actin-related biological processes. Besides, actin dysregulations are involved in the pathomechanisms of several diseases and targeting actin could have therapeutic potential for diseases such as cancer. There are several chemically diverse compounds, which are widely used as tools in cell biology but not in the clinic because of their cardiotoxic side effects (Bryce, Hardeman, Gun-

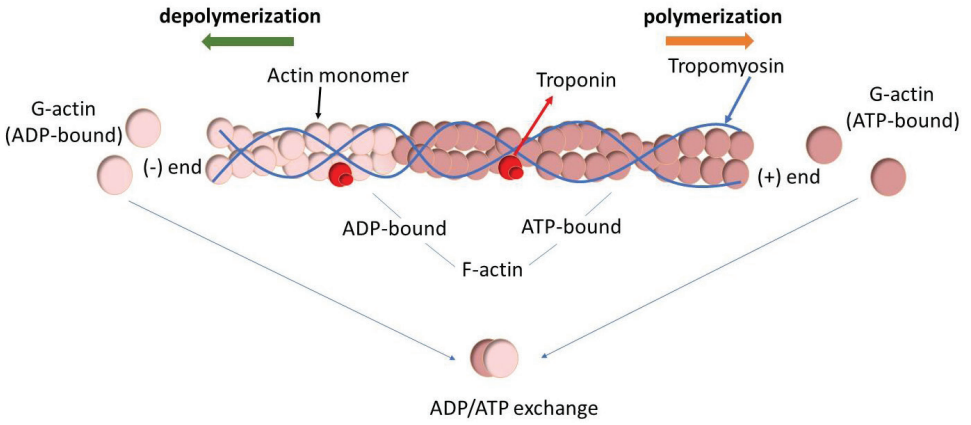


**Figure 3.** Actin filament structure and organization.

ning, & Lock, 2019). According to their targets, actin modifying compounds are grouped into three, as summarized below.

**(i). Direct binding to G-actin or F-actin**

Some compounds alter filament polymerization by binding to either G-actin monomer or F actin. These small actin-binding compounds are roughly divided as stabilizers and destabilizers depending on their effect on the actin cytoskeleton (Table 2). Stabilizers prevent depolymerization of F- actin and protecting it from proteolytic cleavage, while destabilizers inhibit filament formation thereby promote the depolymerization of filaments with a different mechanism (Coluccio & Tilney, 1984). One of the stabilizers is phalloidin, which is derived from *Amanita phalloides* (death cap mushroom), binds to actin filaments more tightly than monomers and prevents depolymerization (Figure 4A). There are fluorescent deriva-

**Table 2. Well-known actin modifying compounds and its binding sites.**

| Direct binding to G-actin or F- actin | Binding site | | Compounds that modulate actin regulatory proteins | Target |
|---|---|---|---|---|
| **Stabilizers** | **G- actin** | **F-actin** | Wiskostatin, 187-1 | N-WASP |
| **Cucurbitacin*** | | + | CK-0944636,CK0993548 CK-666 | Arp2/3 complex |
| **Jasplakinolide**** | | + | Blebbistatin | Myosin II |
| **Chondramide**** | | + | Migrastatin** | Fascin |
| **Dolastatin 11***** | | + | TR-100, ATM1001, ATM3507 | Tropomyosin |
| **Phalloidin******** | | + | Doxazosin | Talin |
| **Destabilizers** | | | SMIFH2 | Formins |
| **Cytochalasins******** | + | + | 6-B345TTQ | Paxilin |
| **Swinholide***** | | + | Cucurbitacin E and I* | Cofilin |
| **Staurosporine**** | | + | | |
| **Chaetoglobosins******** | | + | | |
| **Latrunculins***** | + | | | |

Natural compounds originally derived from * plants, **bacteria, ***marine species and **** fungi

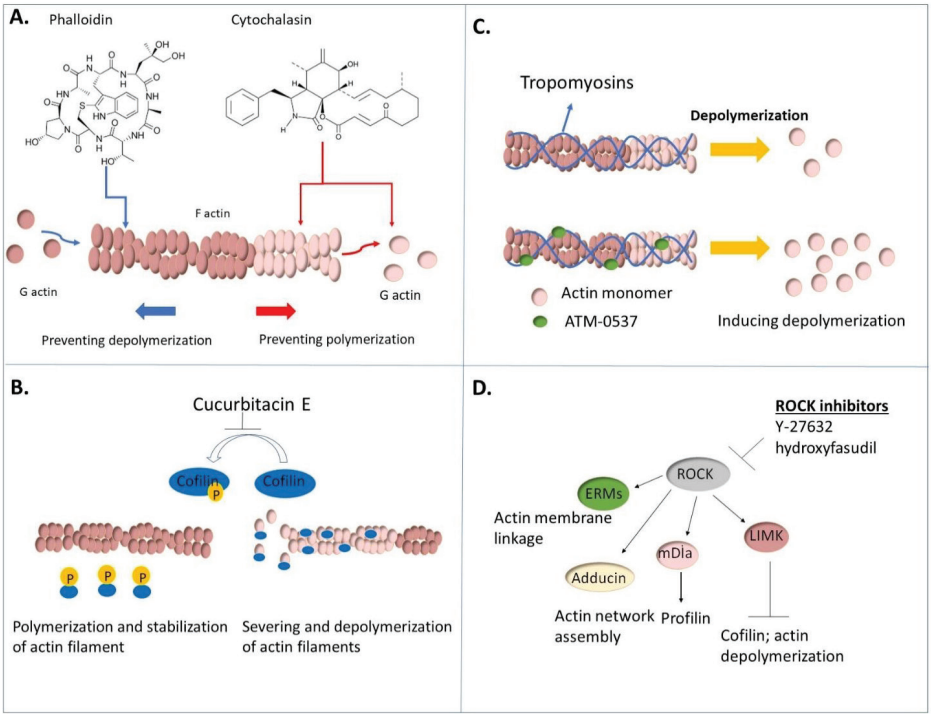


**Figure 4.** Mechanisms of action of actin modulatory compounds. (A) Compounds that can directly bind to G or F actin. (B) Inhibition of post-translational modification of an actin-binding protein, cofilin by compounds. (C) Inhibition of actin-binding protein, tropomyosin (D) Modulation of ROCK signaling pathway and downstream effectors through inhibitors.

tives of phalloidin, which are useful tools to visualize actin filaments in fixed tissues and also cultured cells (Gandalovičová et al., 2017). Cytochalasins, which are fungal toxins, are the most studied actin targeting agents that destabilize filaments by binding to the barbed end and also G-actin monomer thereby inhibiting F actin formation and polymerization (Figure 4A). Cytochalasins inhibit cytokinesis, cell motility and induce apoptosis. Currently, more than 60 different cytochalasins have been identified and some of them have been investigated as chemotherapeutics (Brown & Spudich, 1979).

**(ii). Modulation of actin-regulatory proteins**

Actin dynamics can be modified chemically via inhibiting/altering activities of regulatory proteins (Table 2). For instance, N-WASP, a protein regulating actin polymerization, binds to Cdc42 and undergoes a conformational change, which is required for the activation of the Arp2/3 complex. Arp2/3 functions in the formation of new actin filaments as a branch from the existing ones. There are two compounds, namely 187-1 and wiskostatin, which inhibit conformational changes of N-WASP and prevent the activation of the Arp2/3 complex (Peterson & Mitchison, 2002; Zigmond, 2000; Carlier, 2001). Similarly, tropomyosin, a coiled-coil F-actin binding protein, is a key to the stability of actin filaments. Tropomyosin inhibition by using ATM3507, induce depolymerization of actin filament (Figure 4C). Inhibition of tropomyosins or myosins have been used to reduce the motility of tumor cells by either affecting actin or actomyosin contractility (Currier et al., 2017).

In addition, post-translational modifications of actin regulatory proteins have an impact on actin dynamics. Cofilin is an F-actin binding protein, which plays a role in depolymerization and severing of actin filaments. Phosphorylation of cofilin on serine 3 by LIM kinases inhibits its depolymerizing activity. Cucurbitacins E and I, which are derived from traditional Chinese medicinal plants -pumpkins and gourds- have been shown to inhibit phosphorylation of cofilin and increased its severing and depolymerizing activities (Figure 4B) (Currie et al .,2017; Nakashima et al., 2010).

**(iii). Alteration of signaling pathways**

Actin filaments are regulated by signaling pathways. Among all, one of the key pathway regulating actin dynamics is Rho-associated kinases (ROCKs) which are serin/threonine kinases that phosphorylate various actin-regulating proteins like profilin, myosin light chain phosphatase (MLC) and LIM kinase (Amin et al., 2013). Therefore it involves various cellular processes in both neuronal and non-neuronal cells such as neurite elongation, stress fiber formation, cytokinesis, cell migration, and apoptosis. Small molecules, namely hydroxyfasudil and Y-27632 are useful tools for cell biology to inhibit ROCK for investigating alterations of both actin dynamics and actin related processes in physiological and pathological conditions (Figure 4D) (Brown & Spudich, 1981)(Schofield, Steels, & Bernard, 2012).

**C. Intermediate filaments and modulatory compounds**

Intermediate filaments (IFs), are strong and flexible polymers (10 nm in diameter), which provide mechanical strength to cells. Unlike microtubules and microfilaments, IFs are composed of different subunits. More than 70 proteins have been shown to form a filament structure in different cells. For filament assembly, two proteins interact with each other to form coiled-coil homo/heterodimers. Dimers associate with another one in an antiparallel fashion and form tetramers. End to end interaction of tetramers form protofilaments and laterally interacting eight protofilaments form the final intermediate filament structure. In contrast to microtubules and microfilaments, IFs are non-polar and generally exist in polymerized form. These structural properties make IFs generally not suitable as drug targets. Nevertheless, it has been identified that some compounds, such as withaferin A, which is derived from the Solanaceae family of plants, including *Withania somnifera* and *Acnistus arborescens*, and simvastatin target vimentin, a type III intermediate filament expressed in mesenchymal cells. Since vimentin is also expressed in cancer cells in epithelial-mesenchymal transition, therapeutic potential has been investigated for cancer. (Grin et al., 2012)(Trogden et al., 2018).

## CONCLUSION

The cytoskeleton is a dynamic network and involves almost all cellular processes. Structure and organization of cytoskeleton are still being investigated in a physiological and pathological context. Therefore, targeting cytoskeleton is a powerful approach not only for experimental purposes but also for the treatment of several diseases such as cancer and neurodegeneration. Currently, only microtubule targeted compounds are used in the clinic even though there is still a need for the development of new compounds because of their toxicity, and side effects, as well as the resistance of cancer cells. Increasing knowledge about the structure and organization of cytoskeletal elements is required to develop better modulatory drugs and can overcome the aforementioned limitations.

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