Small-Molecule Inhibitors of Actin Dynamics and Cell Motility

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Abstract: Cell motility is a central feature of a range of normal and pathological processes, including embryonic development, tissue repair, immune cell function, angiogenesis, and cancer metastasis. The dynamics of the actin cytoskeleton power cell migration. A large number of proteins are known or suspected to play roles in regulating actin dynamics. While there are now many available small molecules that target the actin cytoskeleton directly, there is a paucity of specific inhibitors of actin-binding proteins and other immediate regulators of actin dynamics and cell movement. This makes the field of exceptional interest as a meeting place between the goals of chemical biology and the needs of cell biology. Furthermore, while regulators of the cell cycle have been recognized for some time as targets for anti-cancer drug development, controlling actin dynamics and cell motility as a therapeutic approach has received scant attention in comparison until recently. This review deals with small-molecule inhibitors of actin dynamics as they relate to cell shape change and motility, from compounds targeting actin directly to those targeting proteins involved in the fundamental control of the actin cytoskeleton.

Key Words: cell motility, cytoskeleton, actin dynamics, regulation, small molecule, chemical, inhibitors.

INTRODUCTION

Active cell shape change and movement are basic life processes in all multicellular animals and many unicellular organisms. Embryonic development, wound healing and tissue repair, immune system function and inflammation, angiogenesis, and cancer cell invasion and metastasis are some of the normal and pathological processes that involve cell migration. Morphological differentiation of different cell types also involves membrane protrusion and cell shape changes driven by cytoskeletal polymer dynamics that are similar to those occurring during cell migration. While there are numerous distinct mechanisms and structures that facilitate movement of different cells and subcellular systems, this review focuses specifically on pharmacological probes and inhibitors of animal cell crawling. The literature touching upon actin dynamics and cell motility is vast, and in the interest of space, some of the references in the more active areas are to excellent reviews. Furthermore, this review will focus on inhibitors of the central intracellular "engine" of animal cell motility and its immediate upstream components. The emphasis will therefore be on the inside of the cell, even though extracellular variables, such as cell adhesion, extracellular matrix composition and structure, and activity of matrix metalloproteinases, are also important determinants of cell migration in the organism.

Anti-cancer drug development strategies have traditionally focused on direct inhibition of cancer cell growth. However, other rate-limiting processes in the progression of cancers, particularly solid tumors, are also promising targets for intervention. Among these, angiogenesis has become by far the most intensely investigated alternative control point for anti-cancer drug development [1-12]. Many angiogenesis inhibitors, such as fumagillin and its derivatives [13], selectively block the growth of capillary endothelial cells, a requisite step in angiogenesis. However, cell shape change and migration are also involved in the recruitment of endothelial cells from neighboring capillaries leading to vascularization of solid tumors [4, 14, 15]. Endothelial cell motility and related aspects of the neo-vascularization process, such as cell adhesion and extracellular matrix remodeling, are promising targets for anti-angiogenesis drug development [2, 4]. Furthermore, tumor cell invasion and metastasis, later points in cancer progression, also inherently involve cell motility [16-18]. Therapeutic agents that potently inhibit invasion and metastasis could be effective in restraining new tumor formation when earlier therapy or surgery has failed or could increase successful containment of solid tumors in combination therapy with other agents. For all of these reasons, proteins involved in cell motility represent attractive targets for development of new chemotherapies [18, 19].

A number of inhibitors of microtubule dynamics and function, such as taxol and colchicine, are used in cancer chemotherapy [20-22]; although no actin-targeted inhibitors are currently used in the clinic, both filament systems are considered valid anti-cancer targets [21]. The therapeutic effect of the microtubule inhibitors arises from their ability to inhibit mitosis in dividing cells, a microtubule-dependent process. There are inherent side effects associated with this strategy, however, as cancer cells are not the only dividing cells in the body. In addition, microtubule inhibitors can

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affect other microtubule-dependent processes, such as neuronal function. Actin-targeted inhibitors in therapy would likely also have major side effects inherent in their mode of action, because many normal cells even in the adult must crawl to function and because actin is involved in a range of other processes as well, such as cytokinesis (the contractile ring is an actin- and myosin-based structure), endocytosis, and exocytosis. Therefore, in a drug development strategy geared toward inhibiting cell migration, specific upstream components of pathways to actin dynamics involved in cell motility are clearly more theoretically attractive targets, especially those regulatory proteins that may play a larger role in diseased cells over normal ones. A similar realization in the area of anti-proliferative drug development has led to greater interest in signaling molecules as targets rather than the basic machinery of cell division.

In addition to their potential as therapeutic compounds, inhibitors of cell motility are invaluable research probes for understanding the process of cell movement and its roles in the biology of the organism. Despite great advances in the last decade, there is still much missing from a comprehensive understanding of how different proteins involved in cell motility are integrated at the level of signal transduction, on the one hand, and the structural and biophysical changes associated with cytoskeletal dynamics, on the other. Further complicating this understanding, there appear to be multiple types of cell motility, perhaps even multiple ways for a given cell to move. Progress in the cell motility field would benefit enormously from the availability of more chemical probes specific to the many proteins involved. In fact, new inhibitor discovery is one of the rate-limiting factors in research on cytoskeletal regulation and cell motility and as such is one of the big opportunities in chemical biology.

ACTIN DYNAMICS AND CELL MOTILITY

The modern conception of how animal cells crawl dates from the work of Abercrombie and colleagues three decades ago [23-27]. The central engine of cell movement, as well as cell shape change in general, is the cytoskeleton. The key component of the cytoskeleton involved in animal cell locomotion is actin [28-39]. Microtubules play important supporting roles in cell migration through direct and indirect interactions with the actin cytoskeleton that regulate a number of processes such as establishment and maintenance of cell polarity, a precondition for directional locomotion [40-45]. Since microtubule inhibitors have helped to implicate microtubules in animal cell crawling and illuminate potential interactions between microtubules and the actin cytoskeleton, some of them will be discussed later in this context. In other types of motility, such as ciliary and flagellar movement, microtubules play a central and better-understood role. Intermediate filaments, the third and most heterogeneous filament system, are not considered to be a central active component of cell motility but are structurally dynamic, interact with the other cytoskeletal systems and play critical roles in signal transduction and in determining the mechanical properties of cells and tissues [46, 47]. They possess unique mechanical strength and

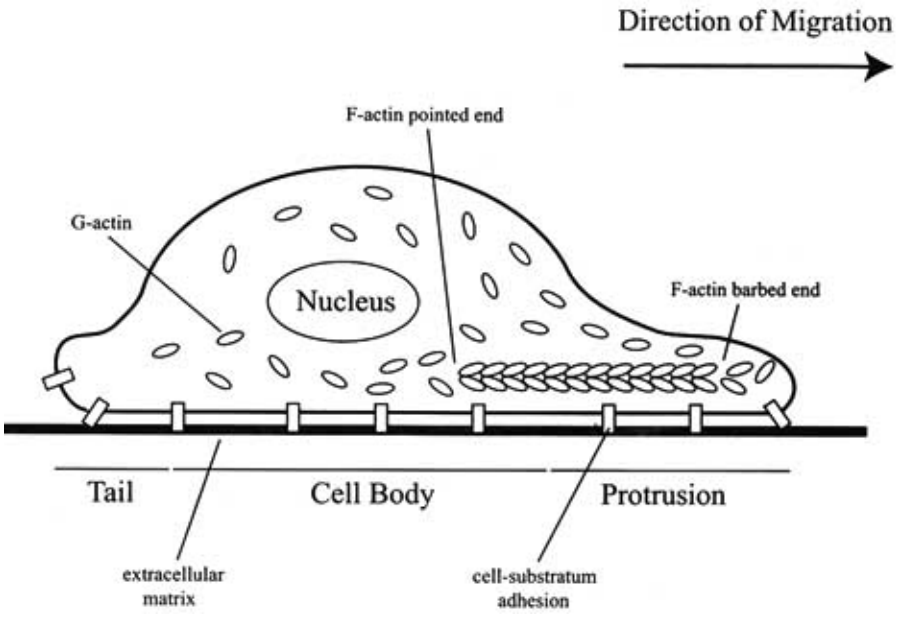


Fig. (1). A migrating animal cell, showing a lamellipodial membrane protrusion, polarity and orientation of actin filaments (F-actin), addition of actin monomer (G-actin) at the barbed end, dissociation of G-actin at the pointed end, and cell-substratum adhesion sites (the linkage to the actin cytoskeleton is not explicitly shown). Nucleation of new actin filaments, actin polymerization at the barbed end, filament crosslinking, network disassembly, actin depolymerization at the pointed end, cell-substratum adhesion, cell body contraction, and tail retraction occur concurrently in different parts of the cell and are controlled by proteins discussed in the text.

resistance to breakage, consistent with an expected role in maintaining cell and tissue integrity [48].

Animal cell crawling occurs by membrane protrusion, which itself results from regulated dynamics of the actin polymer system in the cell (Fig. (**1**)). The concept of actin dynamics generally implies changes in lengths of actin filaments due to polymerization and depolymerization. However, this concept also should rightly include changes that affect the organization and gel properties of actin networks, such as modulation of the density and geometry of filament crosslinking and rearrangement or remodeling of the actin cytoskeleton. All of these processes involve actin, are dynamic and regulated by a wide range of proteins, and only together do they make cell shape change and motility possible.

The cellular actin network is especially rich just below the plasma membrane (the cell's cortex), and localized changes in this cortical actin lattice are thought to drive membrane protrusion and animal cell crawling. Membrane protrusion by itself does not result in movement, although it does generally appear required for movement. Many adherent cells extend sheet-like protrusions called lamellipodia and related finger-like protrusions called filopodia even when not translocating from one point to another. During migration, however, the cell first becomes polarized along the axis in which the cell is to move; subsequent protrusive activity is then localized to the side of the cell that is in the direction of movement (the leading edge) and not randomly distributed around the cell.

The proponderance of evidence suggests that membrane protrusion results from actin polymerization and crosslinking at the leading edge. The actin filament (F-actin) is a polar polymer of variable length, with a fast-growing end called the barbed end (based on its appearance when myosin head fragments are bound to the filament) and a slow-growing end called the pointed end. Actin polymerization is the incorporation of monomeric actin (G-actin) into the F-actin polymer, each added G-actin molecule forming a subunit or protomer of the filament. In an activated cell, actin polymerization occurs from and requires free barbed ends, which are preferentially oriented in the direction of protrusion at the leading-edge membrane. However, in the cell's resting state, the ends of actin filaments are bound by specific capping proteins that prevent addition of actin monomers [31, 35]. Therefore, the process of new polymerization can be initiated only in a limited number of ways. One general route is through the regulated *de novo* nucleation of new actin filaments, particularly by the actin-related protein (Arp) 2/3 complex, a multimer of 7 proteins that stabilizes dimeric or trimeric actin nuclei to provide new free barbed ends while remaining bound to the pointed end [35, 37, 49]. Recently, evidence has emerged that formins, one of a broad and diverse group of proteins that contain proline-rich domains involved in protein-protein interactions, can also nucleate new filaments by a distinct mechanism independent of the Arp2/3 complex [50-53]. The second general route to new polymerization is elongation from existing actin filaments, and when starting from the resting state where filament barbed ends are capped, this can only proceed following dissociation of barbed-end capping proteins or breakage of the non-covalent associations between filament subunits, regulated processes known as barbed-end uncapping and filament severing, respectively [30, 31, 35].

During elongation of an actin filament, G-actin, in a complex with Mg^{2+}-adenosine triphosphate (ATP) and the G-actin-binding protein profilin [54], associates with the barbed end of the filament, with dissociation of profilin from the complex. Actin-bound ATP is hydrolyzed to adenosine diphosphate (ADP) by actin's intrinsic ATPase activity, followed by slow release of the resulting phosphate, steps which tend to destabilize the filament. The critical concentration (or concentration of G-actin above which there is net polymerization and below which there is net depolymerization) gives an indication of the thermodynamics of polymerization. The barbed end of an actin filament, where ATP-bound actin subunits predominate, has a higher affinity and lower critical concentration for ATP-G-actin than the pointed end, where the subunits are in the ADP-bound state. At concentrations of ATP-G-actin between the critical concentrations of the two ends and in the absence of filament-end capping, actin monomer tends to add at the barbed end and dissociate at the pointed end, a phenomenon known as treadmilling [55]. However, if the barbed end of a filament is capped by barbed-end capping proteins, such as gelsolin [56], Cap G [57, 58], or capping protein (Cap Z) [59, 60], and the pointed end is capped by the Arp2/3 complex, an actin filament will neither grow nor shrink unless the ends are first uncapped or the filament is severed.

Proteins that bind actin and control its dynamics *in vivo* fall into two broad groups: those that bind F-actin [61], such as capping proteins and other F-actin-binding proteins discussed later, and those that bind G-actin, like profilin [54] and thymosin β4 [62]. Profilin and thymosin β4 bind competitively to G-actin and can exchange rapidly [63]. Profilin increases the rate of nucleotide exchange [64], facilitating the exchange of ADP for ATP on the monomer, which promotes polymerization from the barbed end only (and not from the pointed end); in binding G-actin, profilin also helps prevent spontaneous nucleation [65, 66]. Thymosin β4 sequesters G-actin, inhibits spontaneous nucleation and polymerization [62], and reduces the rate of nucleotide exchange [63]. These proteins therefore work oppositely to control the concentration of polymerization-competent, ATP-bound G-actin. Furthermore, profilin, along with thymosin β4, helps ensure that nucleation of new filaments only occurs where the Arp2/3 complex is present. At the same time, profilin may help to improve specificity of G-actin for the barbed end of filaments, adding to the already higher intrinsic affinity for monomer of the barbed end over the pointed end. In addition, certain proline-rich proteins, such as Ena/VASP proteins, help to deliver profilin-ATP-G-actin to sites of polymerization and thereby promote actin filament assembly and membrane protrusion [67].

The actin filaments that arise from polymerization at the membrane are crosslinked in lamellipodia or bundled in filopodia by different actin-binding proteins to yield organelle-excluding F-actin gels of suitable strength to

support protrusion [68-71]. The Arp2/3 complex binds to the sides of existing filament so that new filaments are nucleated at a ~70º angle from existing ones *in vitro* and *in vivo*; this appears to initiate the formation of branched actin networks in lamellipodia [72-75]. There is other evidence, however, that Arp2/3 complex instead may bind barbed ends competitively with capping proteins and then promote polymerization and barbed-end branching [76]. In either case, formation of sufficiently strong actin gels appears to require the additional binding of specialized actin-crosslinking proteins like filamin A [77, 78]. It is not understood precisely how actin dynamics and membrane protrusion are coupled mechanically, and there are a number of physical models to describe the phenomenon [79-83].

As actin continues to polymerize and become crosslinked at the leading edge during membrane protrusion, a fixed point on an actin filament appears to move rearward away from the leading edge and toward the cell body, a dimly understood process that is not simply the result of actin treadmilling (continuous actin assembly at the leading edge and disassembly behind it) and may involve myosin motor proteins [30, 84-87]. Actin polymerization and crosslinking at the membrane occurs as network contraction, actin depolymerization, network disassembly, and recycling of components take place behind the protrusive structure. This disassembly process is facilitated by proteins that sever actin filaments and/or accelerate depolymerization from the pointed ends of filaments, such as proteins of the actin-depolymerizing factor (ADF)/cofilin [88-91] and gelsolin families [31, 92].

In order for a cell to translocate from one place to another, directional membrane protrusion must occur against a substratum for traction, requiring that a given point on the membrane transiently and reversibly pinned to an external support, such as the extracellular matrix. These transient attachments to the matrix during movement are mediated primarily by integrin proteins at sites called focal complexes [45], related to the more stable focal adhesions at the termini of stress fibers in stationary adherent cells [93, 94]. Integrins are transmembrane receptors for a number of matrix proteins, and their function is critical for cell motility [95]. These receptors are linked intracellularly to the cortical actin network, allowing both for "passive" tethering to and "active" pulling on the substratum, when contractile force is generated in the network. An array of structural proteins, such as talin, vinculin, α-actinin (an actin-bundling protein), and filamin A, have been shown to interact with the integrin complex, as have many regulatory proteins, including focal adhesion kinase, integrin-linked kinase, and phosphatidylinositol-3-kinase, and many of these different types of proteins have been shown to be involved in motility [93]. Adhesion to the matrix during cell migration is a cyclical process of attachment at a forward point (the leading edge) and detachment later at a more rearward point as the cell moves along, concomitant with formation of new forward attachments and repetition of the cycle. At the same time, the body of the cell has to contract, bringing the cell body forward. Finally, as the cell moves forward, it has to bring the lagging edge along. Therefore, most types of animal cell locomotion require directional membrane protrusion, cycles of adhesion and detachment, cell body contraction, and tail retraction.

A large and ever-expanding number of proteins are known to play a role in the regulation of actin dynamics and cell movement. Many extracellular molecules are known to initiate signal transduction cascades leading to motility in different cell types, including growth factors implicated in tumor invasion [18]. These signals include, among others, lysophosphatidic acid (LPA), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factors, transforming growth factors, insulin-like growth factors, keratinocyte growth factor, hepatocyte growth factor, vascular endothelial growth factor, and *N*-formyl peptides. These molecules bind to their cognate transmembrane receptors, leading to activation of a range of intracellular signaling pathways. Furthermore, if a growth factor or other motility stimulus is present in a gradient, formation of signaling complexes at the membrane, stimulation of actin polymerization, and protrusive activity will tend to polarize to the side of the cell that is nearest the stimulus source. In such a case, the cell will migrate up the gradient of stimulus toward the source, a process known as chemotaxis. It should be noted, however, that many cell types will exhibit crawling behavior by default even in the absence of a chemoattractant, although generally in a random fashion with frequent changes of direction.

Among known signaling proteins involved in motility, the Ras-related small guanosine triphosphatases (GTPases) of the Rho family are of particular importance, since activation of different members of this family lead to distinct modular changes in the state of the actin cytoskeleton [96-99]. These small GTPases are membrane-associated proteins that serve as molecular switches. They are activated through signal-induced exchange of bound guanosine diphosphate (GDP) in the resting state for GTP (a switch to a transient, GTP-bound "on" state), which is then hydrolyzed back to GDP by these enzymes' intrinsic GTPase activity (essentially, an internal "off" fuse). Exchange rates and hydrolysis rates are modulated by associated proteins known as guanine nucleotide exchange factors and GTPase-activating proteins, respectively. Rho proteins, the prototype members of this protein family, are associated with formation of stress fibers, stable focal adhesions, and contractile actin-myosin (actomyosin) bundles. Signaling through Rac proteins, a second multi-protein subset of the Rho family, is most correlated with formation of lamellipodia, membrane ruffles, transient focal complexes, and cell crawling behavior. Activation of Cdc42, another member of the Rho family, results in filopodial extension and is also linked to the control of cell polarity. The Rho-family proteins are involved in a range of other processes, including the regulation of cadherin-mediated cell-cell adhesion [100, 101], microtubule dynamics [40, 102], cell cycle progression and cell growth [103-105], and oncogenic transformation [106].

Phosphorylated inositol lipids or polyphosphoinositides (PPIs), particularly phosphatidylinositol-4,5-bisphosphate (PIP_2) and phosphatidylinositol-3,4,5-trisphosphate (PIP_3) in the membrane [107-110], are known to play critical roles in signaling to actin assembly and crosslinking. These

functions are related to the intact PPIs in the membrane and are distinct from the more familiar functions of the products of hydrolysis of PIP_2 by phospholipase C. The precise roles of the intact PPIs, and the extent to which their cellular functions are distinct or overlapping, are still open questions [109, 110]. PPIs have been shown to be involved in a number of Cdc42- [111-113] and Rac-dependent pathways [111, 113-115]. In fact, there is good evidence for a pathway from PPIs and activated Cdc42 to induction of Arp2/3-mediated nucleation of new actin filaments; this pathway involves neural Wiskott-Aldrich syndrome protein (N-WASP), which, despite its name, is a ubiquitously expressed protein that cooperatively binds Cdc42 and PIP_2 and then associates with the Arp2/3 complex and actin monomer, resulting in signal-controlled nucleation of new filaments at the membrane [116-121]. Intracellular Ca^{2+} also regulates actin dynamics and tends to have opposite effects to those of PPIs [107].

In addition to serving a mechanical requirement for cell movement, transmembrane cell adhesion receptors are important signal-transducing molecules. Cell adhesion receptors, such as clustered integrin molecules bound to extracellular matrix ligands, can activate intracellular signaling pathways and elicit cytoskeletal changes [93, 95, 122-124]. Signaling through cell adhesion receptors can also occur in the "inside-out" direction, with intracellular molecules regulating cell adhesion to extracellular molecules [93, 122, 123]. The composition and structure of the extracellular matrix and its remodeling by matrix metalloproteinases are also centrally relevant to cell migration *in vivo* [125-127]. The regulatory networks and interplay of the cytoskeleton, cell adhesion, and the extracellular matrix therefore constitute a complex and dynamic system, both being affected by and itself affecting a range of intra- and extracellular factors. There are both biochemical and mechanical levels of interaction, tied together to coordinate and carry out cell shape changes and movement.

There are different biochemical/signaling components that operate (i) in solution in the cytoplasm, as in the hydrophilic second-messenger Ca^{2+}, (ii) at the membrane, as in the inositol lipids and membrane-associated regulatory proteins, and (iii) in the solid-like gel phase, as in the signaling from extracellular matrix through integrins to the cyoskeleton. In the last type of system, part of the signaling process can be viewed as classically biochemical with induced conformational changes, modulation of protein-protein binding affinities, and associations and dissociations of components of otherwise insoluble hyper-complexes. However, there is also a higher-order mechanical aspect to such a system, where the transmembrane coupling between extracellular gels (the matrix) and intracellular gels (the cytoskeleton) provides a conduit for force transmission. The integration of these different levels of interaction is critical to cell movement, an inherently biochemical and mechanical process. No complete mechanism or theory that integrates the roles of all the components of cell motility presently exists. Much of the problem in sorting out the precise contribution of individual proteins to movement lies with the dearth of cell-permeant agents to selectively inhibit each protein involved in cell motility.

Dominant-negative mutant proteins, constitutively active mutant proteins, and certain toxic bacterial exonenzymes [128] are the main tools presently used to study the cellular function of actin-regulatory proteins, especially the Rho-family proteins. Fragments of the N-WASP protein are also used to control Arp2/3 complex activation; the so-called VCA domain fragment of N-WASP functions as a constitutively active form of N-WASP, while the CA fragment can be used as a dominant-negative to titrate Arp2/3 complex, preventing nucleation of new filaments [121]. However, none of these protein-based inhibitors are cell-permeant and so require microinjection of the protein or transfection of expression constructs into the cells of interest.

Ironically, while there is a lack of specific, small-molecule inhibitors for most of the unique proteins that regulate cytoskeletal dynamics, the cytoskeleton itself is the target of a large and growing number of natural products. Perhaps by virtue of its central importance in a range of cellular processes, it appears that natural selection has resulted in many toxins against the cytoskeleton. These molecules have become indispensable tools to understanding the function of the cytoskeleton. In fact, a history of scientific progress on the cytoskeleton would also be a history of the discovery and exploitation of pharmacological inhibitors of the cytoskeleton. More recently, progress has been made in the discovery of new chemical probes for proteins involved in the regulation of the actin cytoskeleton.

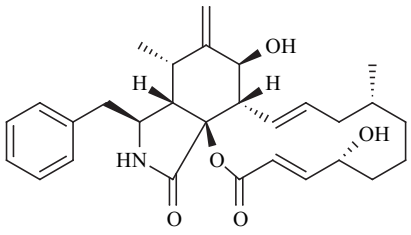
INHIBITORS THAT TARGET ACTIN DIRECTLY

Inhibitors that target actin have dramatic effects on cell shape, cell migration, cell division, endocytosis, exocytosis, and other actin-based processes in virtually all animal cells by affecting the function and organization of the actin cytoskeleton [129, 130]. In addition to their importance in present and future studies of actin function, they have played irreplaceable historical roles in helping to establish the basic biochemical properties of actin, on the one hand, and the importance of actin dynamics in cell motility and other cellular processes, on the other. Inhibitors that directly target actin can be classified into two broad categories: (i) those that primarily disrupt actin filament assembly by a variety of mechanisms and effectively destabilize filament, and (ii) those that stabilize filaments and induce actin polymerization. The former category is richer in terms of number of compounds known and apparent breadth of molecular mechanisms of action and will be dealt with first.

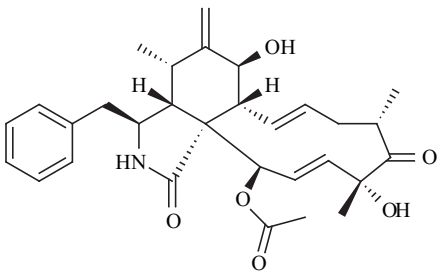
While some agents that destabilize F-actin do so by severing of filaments, compounds that simply bind monomer and prevent polymerization can also lead to the apparent disassembly of existing filaments. This can be viewed as a shift in the equilibrium between monomer and filament toward monomer and also as a consequence of the intrinsic dynamics of actin, with net loss of filamentous mass because normal depolymerization is not being offset by polymerization in the presence of such an inhibitor. In contrast, compounds that preferentially bind filamentous actin and stabilize the assembled polymer favor net

polymerization by shifting the equilibrium between monomer and filaments toward filaments.

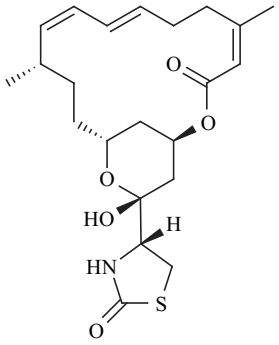
Cytochalasins are among the best-known actin-targeted small molecules and have been extensively reviewed elsewhere [129]. These fungal metabolites bind and block the barbed end of actin filaments, like barbed-end capping proteins, thereby inhibiting polymerization and depolymerization at that end. Cytochalasins B and D (Fig. (**2**)) are the most commonly used members of this group of molecules. Of these two, cytochalasin D is preferable in studies where inhibition of actin dynamics is desired because of greater selectivity for actin (cytochalasin B is known to also inhibit monosaccharide transport) and lower K_i for inhibition of dynamics at the barbed end. Cytochalasin D has been shown to also bind and sequester actin monomers and perhaps dimers [131-133]. Cytochalasin D and to a lesser extent cytochalasin B also accelerate the ATPase activity of actin [134, 135]. In addition, there are reports that cytochalasin B can shorten or sever filaments and in this respect resemble actin filament-severing proteins [136, 137].



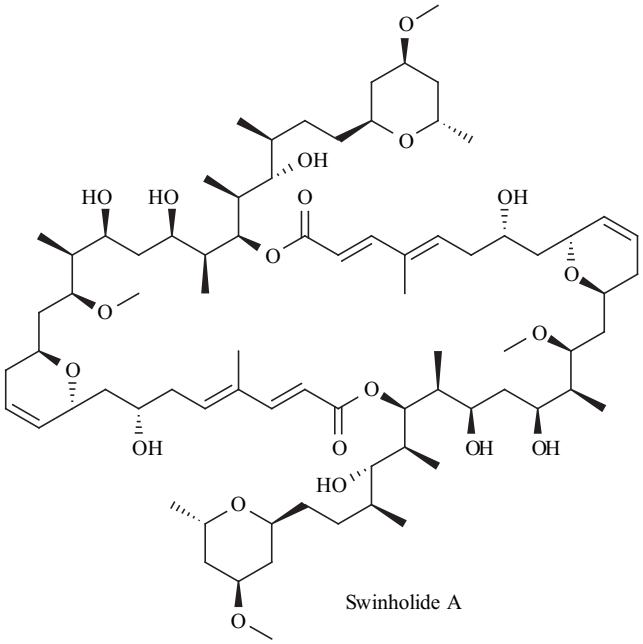
Cytochalasin B



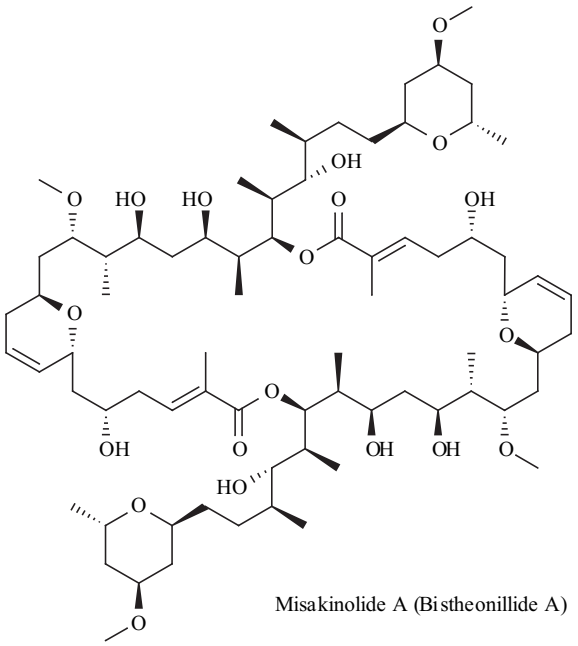
Cytochalasin D



Latrunculin A



Swinholide A



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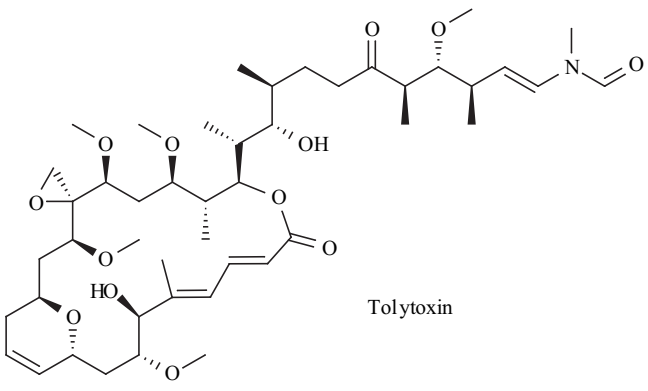

Tolytoxin

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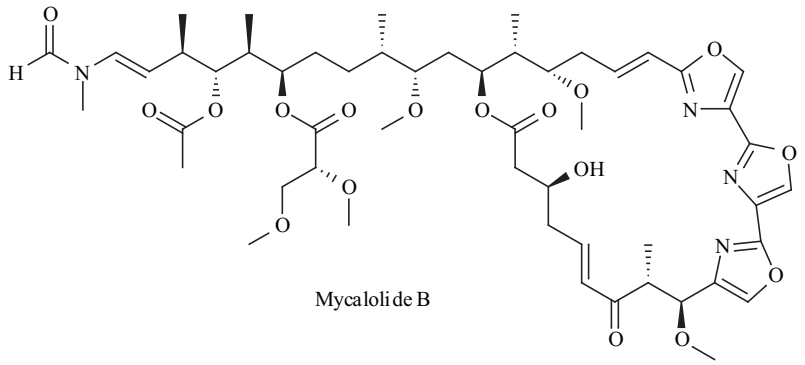
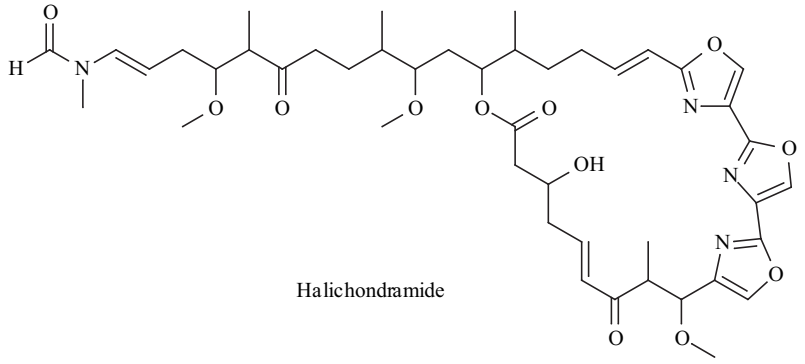
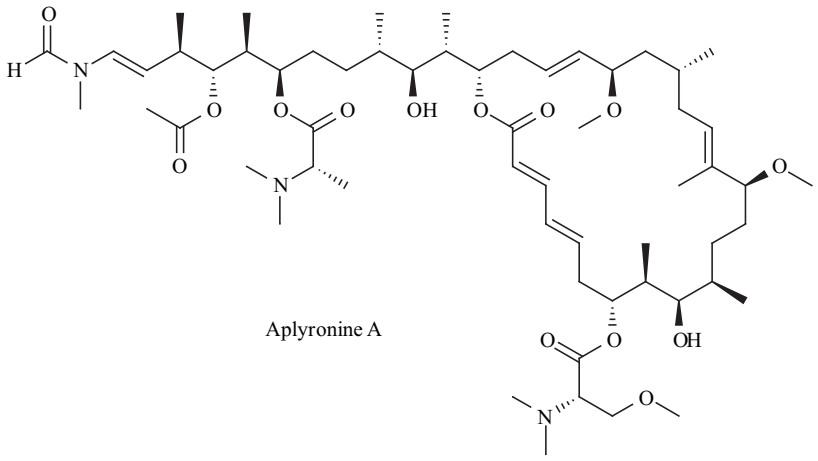


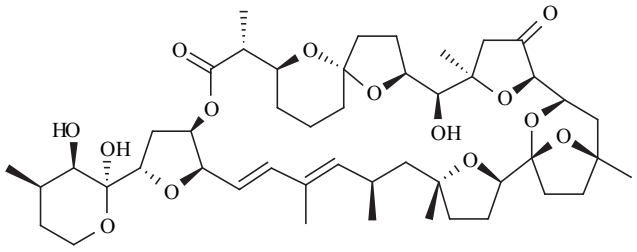
Mycalolide B



Halichondramide



Aplyronine A



Pectenotoxin 2

Fig. (2). Structures of compounds that destabilize actin filaments.

A growing number of new inhibitors that also prevent filament elongation or destabilize actin filaments are becoming available, including a number of macrolides derived from marine organisms [130], some of which are described below. Like the cytochalasins, many of these molecules resemble known actin-binding proteins in their mechanisms. The potential for competitive interactions with actin between endogenous proteins and exogenous small molecules, however, often complicates inference of clear-cut *in vivo* mechanisms for these compounds based on *in vitro* studies with purified actin. In addition, many of these compounds, including the cytochalasins, appear to have multiple effects on actin, complicating interpretation of results. Nevertheless, compounds that inhibit actin polymerization or destabilize actin filaments *in vitro* tend to have consistent effects *in vivo* when the structure of the actin cytoskeleton in treated cells is examined.

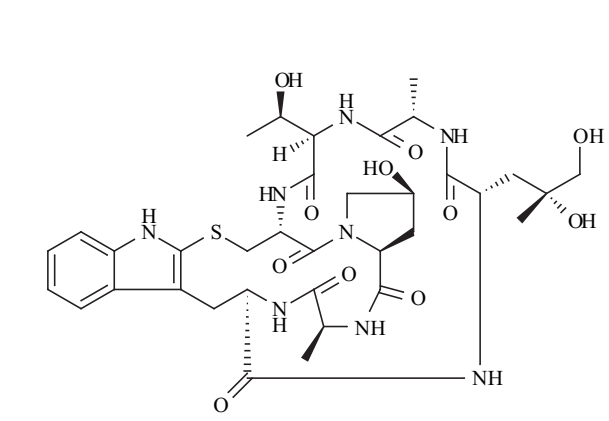
Latrunculins are thiazolidinone-containing macrolides first isolated from a Red Sea sponge [138]. They have advantages over cytochalasins in studies of actin function in that they are generally more potent and appear to have a simpler and more definable mode of action. Latrunculin A (Fig. (**2**)), the most potent member of this family, inhibits actin polymerization, binding G-actin in a 1:1 complex [139], and also inhibits nucleotide exchange in the monomer [140]. Unlike the cytochalasins, which bind the barbed end of filaments, latrunculin A appears to only associate with the actin monomer [140]. This mechanism is reminiscent of that displayed by the G-actin-sequestering protein thymosin β4. Latrunculins induce changes in cell shape and actin filament organization in cultured mammalian cells [141-143] in a manner distinct from those caused by cytochalasin D [142]. Latrunculin A disrupts the actin cytoskeleton in yeast as well, and certain mutations of the actin gene that make yeast resistant to latrunculin A map near the nucleotide-binding cleft of actin; the purified mutant actin is unable to bind latrunculin A and so polymerizes *in vitro* even in the presence of the compound [140]. A high-resolution structure of the actin/latrunculin A complex suggests that the inhibitor causes a conformational change in G-actin that seems to both lock the bound nucleotide in its binding site and alter the conformation of the interface between actin molecules, disfavoring subunit interactions and polymerization [144].

Swinholide A (Fig. (**2**)), first isolated from another Red Sea sponge, is a dimeric dilactone macrolide [145-147] that binds dimers of G-actin with high affinity and has F-actin-severing activity [148, 149]. Misakinolide A (also known as bistheonillide A; Fig. (**2**)), isolated from an Okinawan marine sponge [150], is very similar in structure to swinholide A but, surprisingly, has no filament severing activity [149, 151]. Instead, it caps the barbed end of filaments, although it does also bind actin dimers with an affinity equivalent to that of swinholide A [151]. Misakinolide A increases the rate of nucleotide exchange in G-actin, while swinholide A decreases it [149]. Scytophycins like tolytoxin (Fig. (**2**)) are macrolides resembling a monomeric unit of swinholide A that inhibit actin polymerization *in vitro* by an unknown mechanism [152].

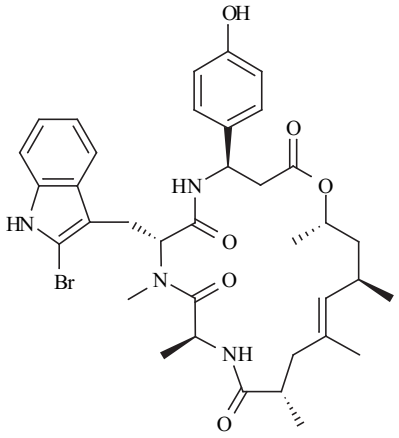
Mycalolide B (Fig. (**2**)), another macrolide isolated from a marine sponge [153], inhibits polymerization and induces rapid depolymerization of F-actin *in vitro*, apparently by severing F-actin and binding G-actin in a 1:1 complex [154-156]. The activity of mycalolide B is irreversible [149, 156] and appears due to covalent modification of actin by the compound [156]. Halichondramide (Fig. (**2**)) and dihydrohalichondramide, which are structurally related to mycalolide B, appear to possess barbed-end capping and F-actin severing activity [130]. Aplyronine A (Fig. (**2**)), although not possessing the same tris-oxazole ring system, has a similar side-chain structure to mycalolide B and appears to have a mode of action similar to mycalolide B [155]. Pectenotoxin 2 (Fig. (**2**)) appears to sequester actin monomer with no severing or capping activity [130]. Pectenotoxin 6, a derivative of pectenotoxin 2, has been shown to induce depolymerization of F-actin in neuroblastoma cells without resulting in cell detachment or apoptosis [157].

Compounds that stabilize actin filaments and promote actin polymerization do not resemble any known actin-binding proteins in their activity. The best known is phalloidin (Fig. (**3**)), a so-called phallotoxin from the deadly mushroom *Amanita phalloides*, is a bicyclic heptapeptide that binds and stabilizes actin filaments, shifting the equilibrium between G- and F-actin toward F-actin and lowering the critical concentration for polymerization by an order of magnitude [129]. It is not cell-permeant, however, which limits its usefulness as an inhibitor in most studies. However, fluorophore-conjugates of phalloidin have long been used to fluorescently stain and visualize F-actin in fixed and permeabilized cells [158].

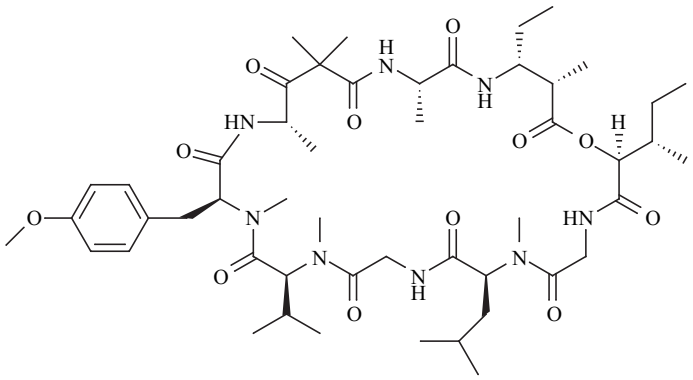
Jasplakinolide (jaspamide; Fig. (**3**)), a cyclodepsipeptide isolated from a marine sponge [159, 160], induces actin polymerization, binds F-actin competitively with phalloidin, and stabilizes actin filaments [161]. However, unlike phalloidin, jasplakinolide readily crosses the cell membrane, not requiring permeabilization of cells with detergent or microinjection into cells for use. This makes it a useful reagent for cellular studies where F-actin stabilization and increased actin polymerization are the desired manipulations. The F-actin-stabilizing effect of jasplakinolide has been directly observed *in vivo* [162, 163]. However, treatment of certain cells with jasplakinolide can lead to an apparently paradoxical disruption of the actin cytoskeleton, an *in vivo* effect that has been attributed to increased *de novo* nucleation resulting in disordered and amorphous F-actin masses with insufficient remaining G-actin for remodeling of organized actin structures such as stress fibers [164]. Jasplakinolide has been shown to inhibit lamellipodial protusion and migration of fibroblasts, as well as the actin-based motility of the intracellular pathogen *Listeria monocytogenes*; in fibroblasts, inhibition of F-actin disassembly is observed with short-term treatment, while increase in net actin polymer mass only appears with longer treatment [163]. Other compounds, such as dolastatin 11 [165], hectochlorin [166], and doliculide [167], have been recently found to induce assembly of F-actin (structures in Fig. (**3**)). Dolastatin 11 and hectochlorin, unlike jasplakinolide, are not competitive with phalloidin for binding to F-actin.



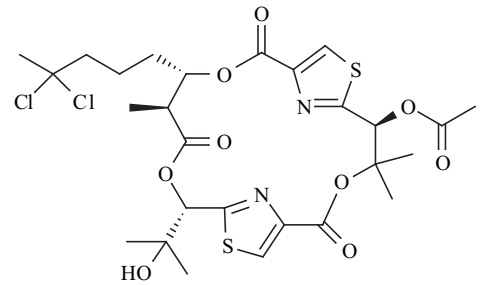
Phalloidin



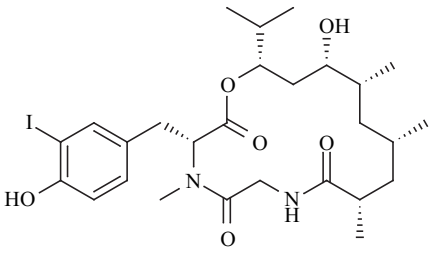
Jasplakinolide (Jaspamide)



Dolastatin 11



Hectochlorin



Doliculide

Fig. (3). Structures of compounds that stabilize actin filaments.

TUBULIN- AND MICROTUBULE-TARGETED INHIBITORS

Microtubles play important though still elusive roles in cell motility and in interactions with and possible regulation of actin dynamics and cell polarity [40-45]. These unexpected relationships have been suggested in large part through the use of microtubule inhibitors. Moreover, many of the basic properties of microtubules and tubulin, the heterodimer from which microtubules are assembled, were discovered using small-molecule inhibitors of tubulin- and microtubule-function, which are more thoroughly reviewed elsewhere [20]. Only a limited number of microtubule inhibitors will be discussed here with an emphasis on their effects on animal cell shape change and migration. As with the actin-targeted inhibitors, microtubule inhibitors can be superficially classified into: (i) those that inhibit polymerization and destabilize microtubules and (ii) those that promote polymerization and stabilize microtubules.

Since microtubules are highly dynamic structures with stochastic transitions between growing and shrinking phases [168, 169], compounds that bind tubulin heterodimer and inhibit polymerization also have the net effect of rapidly disassembling existing microtubules. On the other hand, drugs that stabilize microtubules also promote new microtubule assembly, sometimes leading to formation of unusual microtubule structures in cultured cells, as is the case with taxol [170].

Colchicine and colcemid (Fig. (**4**)) are well-known plant alkaloids that inhibit polymerization of tubulin and disrupt microtubules [20]. In fact, radioactively labeled colchicine was used to first discover tubulin in an early example of a small-molecule-based approach to the discovery of biological function [171-173]. Colchicine has been shown to inhibit migration of a wide range of cell types [174-180], as has colcemid [181-183]. In random migration of alveolar macrophages, colchicine reduces the proportion of properly

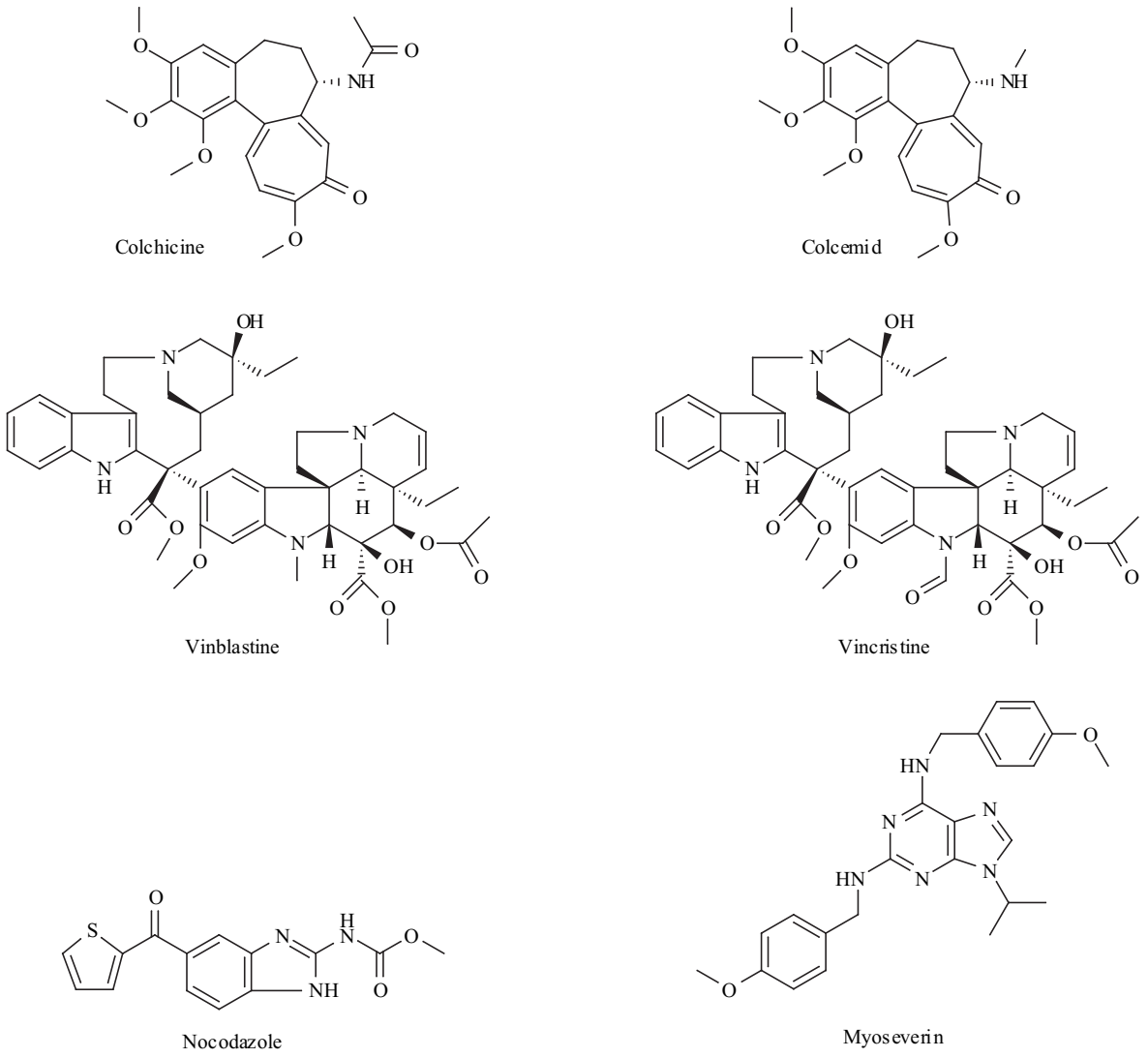


Fig. (4). Structures of compounds that destabilize microtubules.

polarized cells with a single lamellipodium (monopolar cells), with an increase in the number of bipolar cells (which do not migrate), but barely affects migration rates and persistence of the remaining subpopulation of monopolar cells [184]. These results imply that overall migration in the population of cells is inhibited by colchicine as a secondary consequence of deficient cell polarization and not from a direct effect on the basic machinery of motility.

The *Vinca* alkaloids, vinblastine and vincristine (Fig. (**4**)), are indole alkaloids found in periwinkle extracts; they are more potent microtubule-destabilizing agents than colchicine [20]. These natural products bind tubulin at a site distinct from the colchicine-binding site and prevent polymerization of the tubulin heterodimer into microtubules [20]. The *Vinca* alkaloids have been shown to inhibit cell migration [176, 185, 186]. Nocodazole (Fig. (**4**)) is a synthetic benzimidazole that destabilizes microtubules with a level of potency similar to that of the *Vinca* alkaloids; although structurally unrelated, nocodazole binds tubulin competitively with colchicine [20]. Nocodazole, like the other microtubule-disrupting drugs, inhibits animal cell motility [187-189].

Treatment of melanoma cells with nocodazole or the microtubule-stabilizing drug taxol, which will be discussed later, specifically inhibits cell body translocation and tail retraction during migration, implying that microtubule dynamics are critical for these processes [190]. Furthermore, initiation of new microtubule growth in fibroblasts by removal of nocodazole from treated cells leads to activation of Rac1, actin polymerization, and formation of lamellipodia, as does very short-term treatment with taxol [191]. Treatment of fibroblasts with colchicines has been shown to lead to activation of Rho A [192]. These and other results have been proposed to represent a positive feedback relationship between microtubule and actin dynamics during cell migration [42]. In this feedback model, polarized actin assembly and retrograde flow of F-actin reinforces and is itself reinforced by a gradient of microtubule dynamics. Net microtubule growth at the leading edge helps further induce actin polymerization and lamellipodial protrusion through Rac1 activation. Microtubule shortening in the cell body activates RhoA, which results in increased adhesion and formation of contractile actomyosin bundles/stress fibers. At the same time, the actin polymerization at the leading edge further promotes microtubule assembly there, while actin

depolymerization in the cell body and tail helps accelerate microtubule disassembly. Therefore, each gradient of dynamics would perpetuate the other, leading to persistent cell polarization and movement.

Microtubule-destabilizing agents can also have the effect of actually stimulating random locomotion in some cell types, but in a manner that is morphologically distinct from that occurring in normal polarization and migration [193, 194]. Treatment of initially spherical (but not already motile) polymorphonuclear leukocytes with colchicine, vinblastine or nocodazole leads to apparent polarization and cell locomotion but without normal membrane ruffling [193]. Similar results are found with treatment of carcinosarcoma cells, where instead of formation of normal ruffles and spikes, there is blebbing at the leading edge [194]. These studies also highlight putative interactions between the microtubule and actin cytoskeletons, since the cell shape changes induced in both of these systems by the microtubule-destabilizing agents can be suppressed by cytochalasin B [193, 194], suggesting that the effect ultimately depends on the function of the actin cytoskeleton.

Myoseverin (Fig. (**4**)) was identified from a library of synthetic 2,6,9-trisubstituted purines in a screen for compounds that affect morphological differentiation of muscle cells and found to induce fission of multinucleated myotubes into mononucleated fragments [195]. Myoseverin and related derivatives were then shown to inhibit microtubule assembly *in vitro* [195-197]. This is a successful example of a chemical genetic approach where a whole-cell screen for compounds that affect the cell shape and morphology has led to the discovery of a class of new microtubule inhibitors. Moreover, these efforts provide new evidence for a possible role of microtubules in muscle cell differentation, since myoseverin inhibits and reverses myogenic differentiation.

Taxol (paclitaxel; Fig. (**5**)), a natural product from the Pacific Yew, *Taxus brevifolia*, stabilizes microtubules *in*

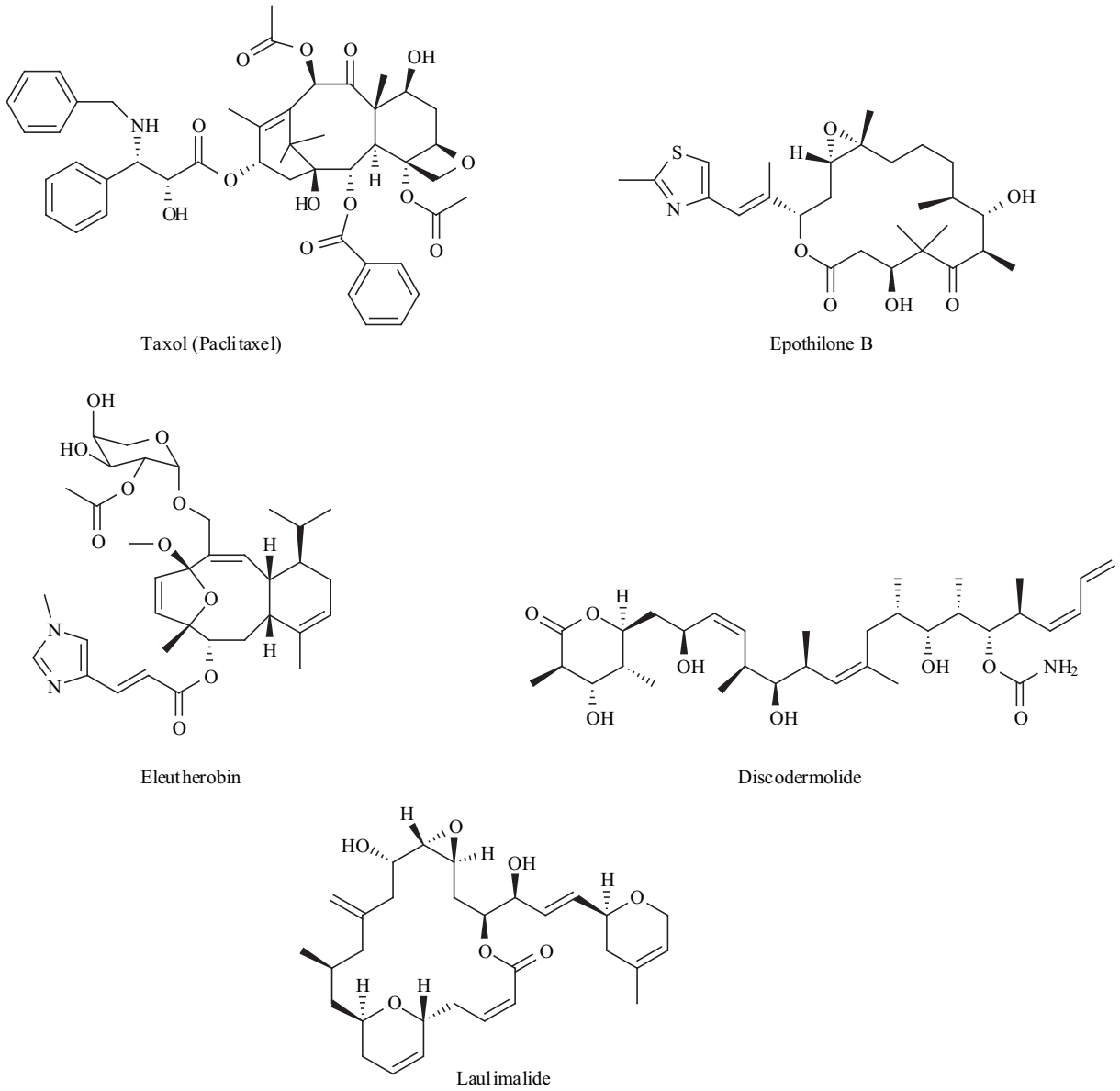


Fig. (5). Structures of compounds that stabilize microtubules.

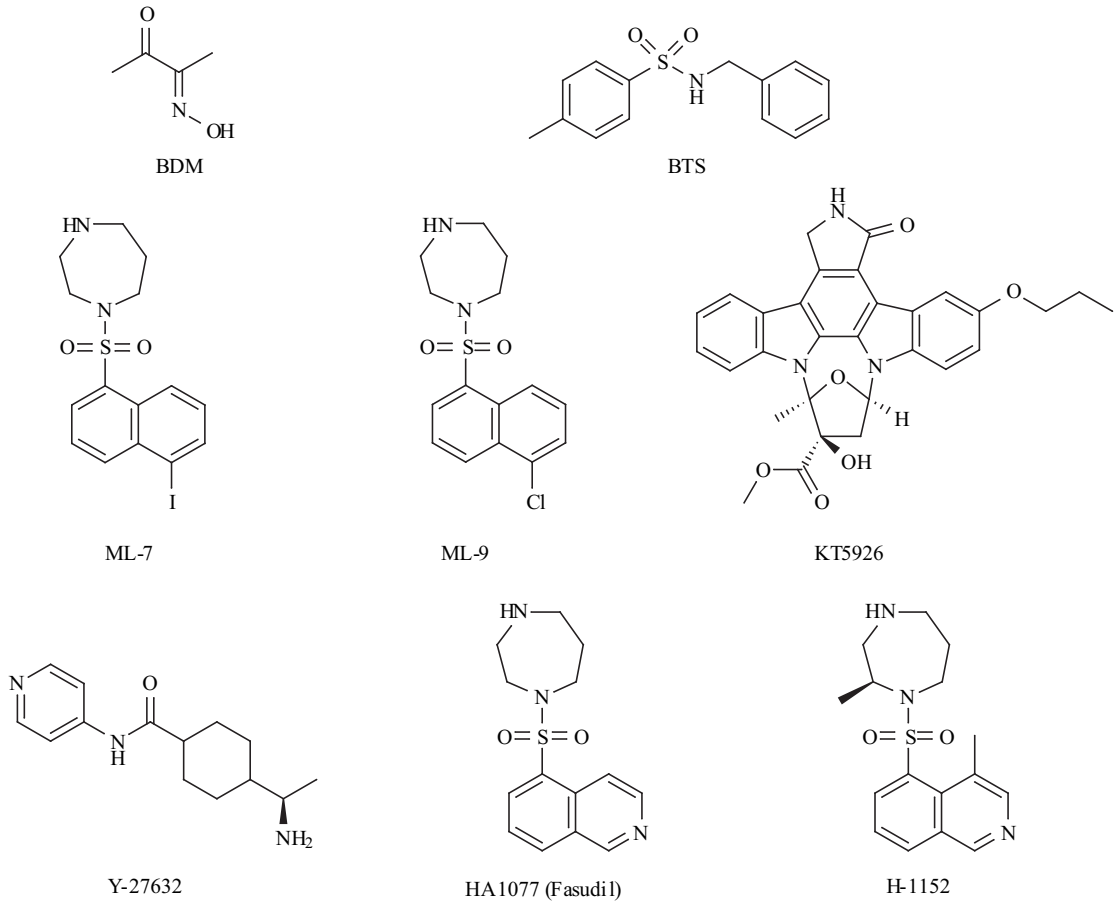
vitro [198] and in cultured cells [199] and induces assembly of new microtubules [200], leading to a reorganization of the microtubule network with extensive parallel arrays or bundles of microtubules forming in treated cells [170]. Taxol causes assembly of microtubules that have a smaller diameter than normal microtubules with only 12 protofilaments instead of the normal 13 [201] and results in unusual end-to-end joining of microtubules [202]. Taxol inhibits cell migration in different cell types [178, 194, 199, 203]. Inhibition of migration by taxol in fibroblasts is not accompanied by blockage of the formation of lamellipodia and filopodia [199]. These results demonstrate not only a "post-protrusion" role for microtubule disassembly in fibroblasts but also that membrane protrusion, while necessary, is not sufficient for cell crawling.

Epothilones [204], eleutherobin [205], discodermilide [206], and laulimalide [207] are other microtubule-stabilizing agents (structures in Fig. (**5**)). Epothilones, eleutherobin, and discodermolide bind microtubules competitively with taxol, and a common pharmacophore for these microtubule-stabilizing compounds has been proposed [208]. Laulimalide, on the other hand, binds at a site distinct from the taxol-binding site [209].

INHIBITORS OF ACTIN-BINDING PROTEINS

Most of the cellular functions of F-actin depend not only on actin itself but also on F-actin-binding proteins, a large, functionally and evolutionarily diverse group of proteins [61]. There are currently very few small-molecule ligands for actin-binding proteins, and identifying new specific inhibitors to modulate the function of each of these proteins is a real need in the cytoskeleton and cell motility fields. In fact, the only actin-binding protein for which directly binding inhibitors are known is myosin.

Proteins of the myosin superfamily are ATP-hydrolyzing motors responsible for actin filament contractility in both



cyclo(L-Lys-D-Phe-D-Pro-D-Phe-L-Phe-D-Pro-L-Gln)$_2$

187-1

Rhodamine B-Gln-Arg-Leu-Phe-Gln-Val-Lys-Gly-Arg-Arg

PBP10

Fig. (6). Structures of inhibitors that target proteins which modulate the function of the actin cytoskeleton. Note that 187-1 and PBP10 are not traditional small molecules but rather larger peptide-based structures.

muscle and non-muscle cells [210]. Non-muscle myosin II bundles F-actin into antiparallel arrays and generates tension in stress fibers and other contractile actomyosin structures through ATP-dependent, barbed-end-directed motion along F-actin. Although not required for the protrusive component of cell movement, non-muscle myosin II and other non-muscle myosins play still poorly understood roles in cell motility, possibly in cell body contraction, retrograde flow of F-actin, and/or tail retraction [30, 84-87, 211, 212].

2,3-Butanedione-2-monoxime (BDM; Fig. (**6**)) is a very weak inhibitor (with IC_{50} values in the mM range and questionable specificity) of the ATPase activity of skeletal muscle myosin II [213-215] and non-muscle myosin II and myosin V [216], although another report suggests that BDM does not inhibit non-muscle myosin II directly [217]. BDM also appears to affect other processes related to muscular function, including Ca^{2+} release from intracellular stores [213, 218], whole-cell Ca^{2+} conductance [219], and K^+ conductance [220]. BDM inhibits post-mitotic cell spreading [216] and migration of some non-muscle cell types [221-224]. BDM treatment has also been found to disorganize microtubules as well as the actin cytoskeleton in yeast, providing further evidence for interactions between the two filament systems [225]. Another synthetic small molecule, *N*-benzyl-*p*-toluene sulphonamide (BTS; Fig. (**6**)), was a recently identified from a screen for inhibitors of myosin ATPase activity and found to be a specific inhibitor of skeletal muscle myosin II [217]. In addition, a number of inhibitors of proteins upstream of myosin and other actin-binding proteins are known, as discussed in the following section.

INHIBITORS OF UPSTREAM SIGNALING MOLECULES

There are a considerable number of signaling proteins whose inhibition in certain cells and in response to certain stimuli affects cell motility quantitatively (positively or negatively affecting the rate of migration) or qualitatively (for instance, affecting leading-edge morphology without influencing rate of migration). This is especially true of components of various growth factor signaling pathways, such as the EGF pathway, that are tied to cell motility and tumor invasion [18]. Many familiar signaling proteins play important roles in a range of cell types and in diverse cellular processes within each cell. Furthermore, inhibition of a given signaling protein may have an effect on a cellular process in one cell and not another; similarly, such inhibition may affect movement of a single cell type following one stimulation but not another. It is not possible in the space of this review to consider inhibitors of every signaling protein whose activity may play a role in cell movement. In addition, the lack of good selectivity of some known inhibitors between members of a protein family or different protein isoforms can complicate matters.

The inhibitors discussed here are those that to date appear fairly selective for a single target, unless otherwise noted, and that affect pathways most closely tied to fundamental control of the actin cytoskeleton in cell motility. Inhibitors of other relevant systems are reviewed elsewhere, such as EGF receptor [226-229] and cell cycle inhibitors [230], some of which also inhibit motility. There are also a number of recent reviews on inhibitors of extracellular components of cell migration, such as antagonists of integrins [231-235] and synthetic and endogenous inhibitors of matrix metalloproteinases [236-239], and these active areas of research are too vast to describe here.

Myosin light chain kinase (MLCK) is an upstream regulator of myosin II in both muscle and non-muscle cells [240]. Ca^{2+}/calmodulin-mediated activation of MLCK results in phosphorylation of the regulatory light chain of myosin II, resulting in increased assembly and contraction of actomyosin-based structures, such as stress fibers in non-muscle cells. A number of inhibitors of MLCK are known, but a problem with most of these inhibitors is the lack of good selectivity between different protein kinases and sometimes even between protein kinases and inositol lipid kinases. Since kinases comprise such a vast group of proteins, such issues may be especially acute with kinase inhibitors, exacerbated by the fact that most kinase inhibitors target the ATP-binding site.

Among the more selective MLCK inhibitors presently available are the synthetic naphthalenesulfonamides ML-7 (Fig. (**6**)) [241] and ML-9 (Fig. (**6**)) [241, 242], both of which bind MLCK competitively with ATP. These compounds have been shown to inhibit cell migration in a number of different cell types [243-247]. KT5926 (Fig. (**6**)) is a synthetic derivative of the broad-spectrum kinase inhibitor K252a, a microbial natural product [248]; KT5926 was first reported as a potent ATP-competitive inhibitor of MLCK [249]. A number of other kinases tested, such as protein kinase C, were shown to be inhibited with considerably higher K_i, suggesting reasonable selectivity [249]. KT5926 was subsequently found to inhibit Ca^{2+}/calmodulin-dependent protein kinase II with slightly greater potency than MLCK [250]. This demonstrates a potential peril of the pharmacological and chemical genetic approaches; discovery of a high-affinity inhibitory interaction does not preclude the possibility that other high-affinity interactions exist, especially with functionally and structurally related proteins.

The small GTPase RhoA also regulates myosin II in smooth muscle and non-muscle cells [251, 252]. GTP-bound RhoA activates Rho-associated kinases (Rho-kinases), such as p160ROCK, resulting in inhibitory phosphorylation of myosin light chain phosphatase [253], which results in increased levels of phosphorylation of the regulatory light chain of myosin II [251, 252]. As with activation of MLCK, this favors formation and contraction of stress fibers and similar actomyosin contractile structures in non-muscle cells.

Y-27632 (Fig. (**6**)) is a synthetic pyridine derivative that inhibits the Rho-kinases $p160^{ROCK}$ (ROCK-1) and ROCK-II [254]. In cultured cells, it inhibits formation of stress fibers, as expected for an inhibitor of a critical downstream effector of Rho [254]. Y-27632 inhibits motility in a number of systems, including hepatoma cells [255, 256], prostate cancer cells [257], breast cancer and fibrosarcoma cells [258], and chemotaxis of vascular smooth muscle cells induced by

PDGF and LPA [245, 246]. HA1077 (fasudil; Fig. (**6**)), a synthetic isoquinolinesulfonamide that inhibits Rho-kinase activity with rather poor selectivity, has been shown to inhibit neutrophil migration [259]. Recently, another isoquinolinesulfonamide, H-1152 (Fig. (**6**)), has been developed that appears to be a more potent and selective Rho-kinase inhibitor with low-nM K_i and much weaker inhibitory effect on other serine/threonine protein kinases [260].

N-WASP links activated Cdc42 and PIP_2 at the membrane to Arp2/3-induced *de novo* nucleation of new actin filaments and formation of filopodia [116, 118-121]. A related protein, WAVE2, appears to mediate Rac1-dependent, Arp2/3-induced actin polymerization and formation of lamellipodia, although the Rac1/WAVE2 interaction does not appear direct, as in the case of Cdc42/N-WASP, but rather through an adaptor protein, the insulin receptor substrate IRSp53 [261]. In addition, Rac1 and the adapter protein Nck have recently been shown to activate actin polymerization through WAVE1 by apparent release of *trans*-inhibitory interactions [262]. Of the known components of these effector/nucleation complexes, only N-

WASP has a known exogenous inhibitory ligand associated with it. This inhibitor (187-1; (Fig. (**6**)), a synthetic 14-residue cyclodimeric peptide and therefore larger than the typical small molecule, was identified in a screen for compounds that inhibit PIP_2-induced actin polymerization in cell-free frog egg extracts [263]. The target of this inhibitory cyclic peptide was identified as N-WASP. Its effects are consistent with a mechanism by which it allosterically stabilizes the auto-inhibited state of the protein, and it may prove a useful mechanistic probe of the biochemistry of N-WASP *in vitro*.

Oligopeptides known to specifically bind PPIs constitute another class of molecules that, although again larger than a typical small molecule, has provided useful reagents to probe components of actin assembly pathways. These synthetic peptides are based on the sequence of gelsolin, whose uncapping from the barbed end of filaments is stimulated by PPIs. Peptides corresponding to segment 1 of gelsolin (residues 135–142) [264] or segment 2 of gelsolin (residues 150–169) [265] were found to bind PPIs and compete with full-length gelsolin for binding PPIs. Similar sequences are present in other PPI-binding proteins. These peptides are polyvalent cations with several hydrophobic residues, but binding to PPIs is not exclusively electrostatic, since it is much stronger than binding of PPIs to a number of other peptides of greater positive charge [265]. They appear to sequester and titrate endogenous PPIs, inhibiting PPI-dependent pathways in detergent-permeabilized or microinjected cells [113-115], as well as in cell-free extracts [112]. They block actin assembly, cell shape change, and cell motility in different cell types [113-115]. Recently, a membrane-permeant fluorescent derivative of a PPI-binding peptide has been described (PBP10; Fig. (**6**)) [266]. PBP10 corresponds to gelsolin residues 160-169 with a rhodamine B moiety linked to its *N*-terminus. It has been shown to inhibit actin polymerization and cell motility [266]. This cell-permeant peptide derivative therefore expands the usefulness of this class of inhibitor in cellular studies of PPI-dependent signaling.

INHIBITORS OF CELL MOTILITY WITH UNIDENTIFIED TARGETS

The activities of the inhibitors discussed up to this point are summarized in Table 1. These are inhibitors whose targets are known. However, a number of inhibitors of cell motility with as-yet unidentified cellular targets have been discovered using different whole-cell screening systems, three of which, migrastatin [267-271], motuporamine C [272-274], and UIC-1005 [275], are shown in Fig. (**7**). Various cell-based assays can be used to screen for new inhibitors of cell motility, including scrape-wound closure assays in multiwell tissue culture plates [267, 275] and systems using filter chambers (like Boyden and Transwell chambers) [17, 273, 276], often coated with Matrigel, which is used as a model for the basement membrane in tumor cell invasion assays [277]. These assays can be done in parallel, high-throughput formats. Migrastatin (Fig. (**7**)), a macrolide produced by at least two *Streptomyces* spp. [267, 271], was first discovered using human esophageal cancer cells in a wound closure assay, followed by additional analysis in a filter chamber assay (Chemotaxicell) [267]. Motuporamines, such as motuporamine C (Fig. (**7**)), are macrocyclic alkaloids with spermidine-like tails that were first isolated from the marine sponge *Xestospongia exigua* on the basis of

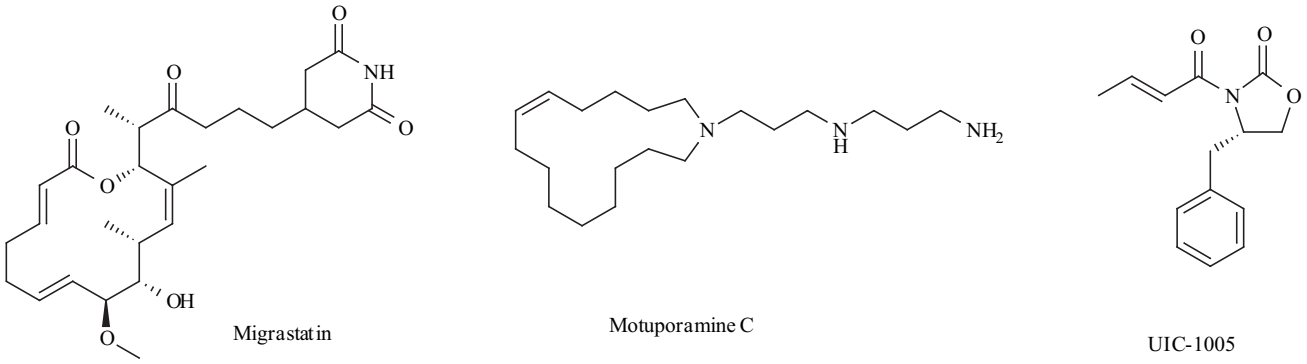


Fig. (7). Structures of three inhibitors of cell motility whose cellular targets are not yet known that have been discovered using cell migration assays.

Table 1. Activities of the Inhibitors Discussed in this Review. The Precise Modes of Action for Different Compounds with the same Activity may Vary. See main text for discussion and references

Compound	Activity
Cytochalasin B	F-Actin Destabilizer (Poor Selectivity)
Cytochalasin D	F-Actin Destabilizer
Latrunculin A	F-Actin Destabilizer
Swinholide A	F-Actin Destabilizer
Misakinolide A (Bistheonellide A)	F-Actin Destabilizer
Tolytoxin	F-Actin Destabilizer
Mycalolide B	F-Actin Destabilizer
Aplyronine A	F-Actin Destabilizer
Halichondramide	F-Actin Destabilizer
Pectenotoxin 2	F-Actin Destabilizer
Phalloidin	F-Actin Stabilizer
Jasplakinolide (Jaspamide)	F-Actin Stabilizer
Dolastatin 11	F-Actin Stabilizer
Hectochlorin	F-Actin Stabilizer
Doliculide	F-Actin Stabilizer
Colchicine	Microtubule Destabilizer
Colcemid	Microtubule Destabilizer
Vinblastine	Microtubule Destabilizer
Vincristine	Microtubule Destabilizer
Nocodazole	Microtubule Destabilizer
Myoseverin	Microtubule Destabilizer
Taxol (Paclitaxel)	Microtubule Stabilizer
Epothilone B	Microtubule Stabilizer
Eleutherobin	Microtubule Stabilizer
Discodermilide	Microtubule Stabilizer
Laulimalide	Microtubule Stabilizer
BDM	Myosin Inhibitor (Low Affinity)
BTS	Myosin Inhibitor
ML-7	Myosin Light Chain Kinase Inhibitor
ML-9	Myosin Light Chain Kinase Inhibitor
KT5926	Myosin Light Chain Kinase Inhibitor (Poor Selectivity)
Y-27632	Rho-Associated Kinase (Rho-Kinase) Inhibitor
HA1077 (Fasudil)	Rho-Kinase Inhibitor (Poor Selectivity)
H-1152	Rho-Kinase Inhibitor
187-1	N-WASP Inhibitor
PBP10	Polyphosphoinositide Sequesterer

their cytotoxic effects [272]. It was then later re-discovered in an independent screen of *Xestospongia* extracts using human breast carcinoma, prostate carcinoma, and glioma cells in an assay combining aspects of the Boyden chamber assay and a Matrigel "outgrowth" assay, followed by additional analysis in a wound closure assay [273]. UIC-1005 (Fig. (**7**)), a synthetic oxazolidinone derivative, was discovered using canine kidney epithelial cells in a wound closure assay [275]. Identification of the cellular targets of these compounds and elucidation of their modes of action in molecular detail may lead to advances in our understanding of cell migration. Whole-cell approaches to screening for inhibitors of cell migration complement more defined *in vitro* assays, such as the assay for PIP_2-induced actin polymerization in cell-free extracts used to discover the N-WASP inhibitor 187-1 [263]. Chemical genetic screens for compounds that affect actin dynamics and cell motility hold great promise for the discovery of new tools for cell biology and new therapeutic leads.

SUMMARY

Table 1 gives an indication of both the richness of molecules now known to target the cytoskeleton directly and the scarcity of molecules targeting actin-binding proteins and immediate regulators of actin dynamics. This situation is bound to change in the near future, as libraries of synthetic molecules and natural products are screened in relevant *in vitro* and cellular assays, yielding new specific inhibitors of actin-binding proteins and upstream components of actin-regulatory pathways. These efforts will engage researchers in natural products chemistry, combinatorial chemistry, medicinal chemistry, pharmacology, biochemistry, and cell biology, the confluence of which defines chemical biology. They will ultimately bear fruit in a more complete understanding of the mechanisms of cell motility and the ability to control cell movement therapeutically.

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