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HIV-1 proteins join the family of LIM kinase partners. New roads open up for HIV-1 treatment

Fabrizio Manetti

Dipartimento Farmaco Chimico Tecnologico, Via Aldo Moro 2, I-53100 Siena, Italy

LIM kinases (LIMK) exert their functions by recruiting many macromolecular partners that could contribute to modulate LIMK activity in a positive or negative manner. In addition to proteins that interact with LIMK in human or animal cells and tissues, recent data show that LIMK activity is also influenced by HIV-1 viral proteins. These results suggest new strategies for the treatment of HIV-1 infection, based on the inhibition of LIMK-mediated cofilin inactivation and consequent actin depolymerization. Further efforts are however required to unravel the mechanism by which the virus interferes with LIMK activity and with the right balance of actin remodeling.

Introduction

The family of LIM kinases (LIMK) is populated by only two members (LIMK1 and LIMK2) discovered more than 15 years ago. Their structure is characterized by two zinc-binding domains (namely, the LIM domains; the name derives from Lin-11, Isl-1 and Mec-3 proteins in which LIM domains were identified for the first time) and a PDZ domain [whose name derives from the postsynaptic density protein-95 (PSD-95), the *Drosophila* disk large tumor suppressor (Dlg) and the zonula occludens-1 protein (ZO-1)] which are responsible for most of the direct interactions found between LIMK and their macromolecular partners. A proline/serine-rich sequence that contains phosphorylation sites (as an example, Ser310 and Ser323 are amino acids of LIMK1 that can be phosphorylated) bridges PDZ and the kinase domain at the C-terminus. LIMK1 does exist in three isoforms derived from alternative splicing, whereas only two variants of LIMK2 (a and b) have been discovered to date. Isoform 1 of LIMK1 and isoform a of LIMK2 share a high overall amino acid identity (about 54%). Alignment of their amino acid sequences, performed with ClustalW algorithm [1] at the Universal Protein Resource (UniProt) website (http://www.uniprot.org) shows 343 identical positions (conserved residues) and 181 similar positions. Single domains also share a significant sequence identity: 48% between LIM1 domains, 63% between LIM2 domains, 45% between PDZ domains and 71% between the kinase domains of isoform 1 of LIMK1 and LIMK2a.

LIMK share their primary function of regulating the actin cycle by controlling the phosphorylation of cofilin (Fig. 1). In particular, LIMK are involved in signaling pathways originating from the small GTPases of the Rho family (Rho, Rac, Cdc42) and their effectors (ROCK, PAK and MRCK) that activate LIMK by phosphorylation at specific threonine residues of the kinase domain (Thr508 for LIMK1 and Thr505 for LIMK2). Activated LIMK can directly phosphorylate and inactivate the three members of the cofilin family [cofilin-1 or non-muscle cofilin, cofilin-2 or muscle cofilin and destrin, which is also referred to as the actin depolymerizing factor (ADF)], thus hampering filamentous actin (F-actin) destabilization and its cleavage into shorter chains or globular actin (G-actin).

In addition to downstream effectors of the Rho family small GTPases, an ever-increasing number of positive regulators of LIMK activity is currently known. Among them, some macromolecular partners have been found to associate directly with LIMK in secondary, ternary or multicomponent complexes. Such proteins directly phosphorylate LIMK at specific amino acid positions, thus enhancing LIMK activity toward cofilin. As an example, co-immunoprecipitation experiments demonstrated a physical association between LIMK1 and the membrane type 1 matrix metalloproteinase (MT1-MMP) within the Golgi vesicles, facilitating localization of MT1-MMP at the plasma membrane [2]. In many cases, however, enhancement of LIMK activity is secondary to the induction of the signaling pathways that depend on Rho GTPases, as in the recent cases of leptin that was found to increase LIMK phosphorylation by

E-mail address: fabrizio.manetti@unisi.it.

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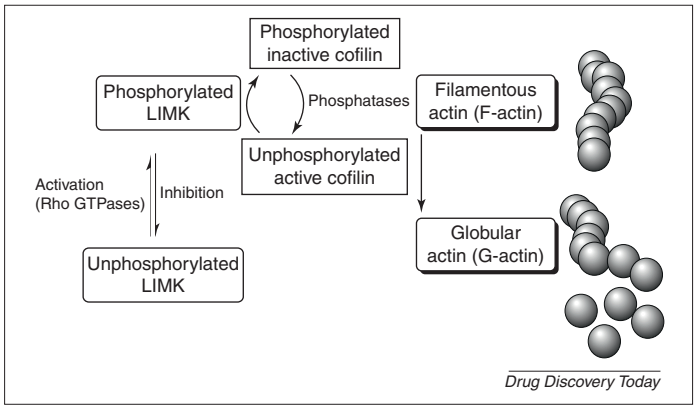

FIGURE 1

Modulation of LIMK activity is responsible for cofilin-mediated actin severing. Activity of LIMK is regulated by positive modulators (such as the Rho-family small GTPases Rho, Rac and Gdc42 that phosphorylate threonine residues of LIMK and activate it) or inhibitors that dephosphorylate LIMK or block their phosphorylation. Phosphorylated LIMK can in turn phosphorylate active cofilin substrates and inhibit their ability to cleave F-actin into G-actin. Phosphatases could reactivate cofilin and actin dynamics.

the RhoA/ROCK signal [3] or DGCR6L, a polypeptide that associates with the p21-activated kinase 4 (PAK4) to upregulate LIMK activity [4]. Other activators of LIMK previously discovered and described that can phosphorylate specific threonine and serine positions or activate LIMK by unknown or indirect mechanisms have been reviewed in a recent and exhaustive paper [5].

By contrast, macromolecular partners do exist that act as negative regulators of the kinase activity of LIMK, by downregulation of the Rho GTPase signaling pathways or by direct interaction with LIMK. Moreover, a biological outcome similar to that resulting from the inhibition of LIMK derives from the activity of cofilin activators that, at the same time, are not necessarily LIMK inhibitors (as in the case of the slingshot phosphatase SSH) [5].

In this context, the correct balance between phosphorylated and unphosphorylated LIMK and cofilin is responsible for the dynamic cycling of F-actin and G-actin and for dynamic remodeling of the actin cytoskeleton. Because actin dynamics influence many cellular functions (such as morphogenesis, motility, division, differentiation and apoptosis), dysregulation of LIMK activity is expected to be involved in pathological conditions and disorders. In fact, a survey of recent literature clearly shows that LIMK, mainly through their activity on cofilin, are involved in neurocognitive deficits (such as Smith-Lemli-Opitz syndrome [6], Alzheimer's disease [7], Parkinson's disease [8] and Williams' disease and its symptoms such as hyperacusis and hearing loss [9]), pulmonary artery hypertension [10], thoracic aortic dissection [11], intracranial aneurysm [12], testicular failure and male infertility [13], psoriasis [14], osteoarthritis [3], nephropathy [15], ocular hypertension and glaucoma [16]. Moreover, abnormal regulation of LIMK-dependent signaling pathways is often associated with cancer, cell invasion and metastasis. As examples, upregulation and mislocalization of LIMK is responsible for cytokinesis failure required for the process of tumor initiation [17]. In addition, LIMK activity is involved in the signaling pathways of tumor cells [18,19] and is necessary to modulate the function of other factors that drive cells to invasion [2,20–22] and metastasis [23,24]. Because LIMK are centrally positioned in the pathways leading to cytoskeleton dynamics and regulation, they could be considered as valuable targets for actin regulation. Fine modulation of LIMK activity could be a major challenge to inhibit tumor cell invasion and metastasis mediated by one or a combination of the upstream signaling factors. In a similar way, restoring the right level of phosphorylated and unphosphorylated LIMK could fruitfully contribute to the treatment of several human diseases associated with LIMK activity dysregulation.

Although LIMK show a significant structural similarity and share their major activity of cofilin phosphorylation, they have a different tissue-specific expression pattern and subcellular localization [25,26]. As an example, LIMK1 expression during mouse organogenesis is spatially restricted and cell-type specific [27]. However, because LIMK expression serves as a guide for multiple developmental processes, LIMK expression has a certain spatial and temporal dynamics. In fact, the level of LIMK and the fine tuning of their activity toward cofilin results from the combined action of several factors, including spatio-temporal conditions [28,29]. Moreover, even if LIMK show a common activity toward cofilin, they are also included in different signaling pathways that regulate distinct cellular functions. As an example, nucleo-cytoplasm shuttling of LIMK2 depends on activation of protein kinase C (PKC) which is unable to influence subcellular localization of LIMK1 because the LIMK1 structure does not possess phosphorylation sites for PKC [30]. Moreover, the polarity protein Par-3 activates LIMK2 but not LIMK1 by direct interaction [31].

Actin polymerization and depolymerization during HIV-1 infection

HIV-1 has evolved strategies to manage actin cycling and cytoskeletal remodeling that participate in virus entry into host cells, intracellular transport and viral progeny egress from cells.

HIV-1 binding and entry into host cells are mediated by the involvement of actin and coreceptors (such as CD4 and CXCR4) upon exposure of T lymphocytes to the viral envelope glycoprotein gp120. In fact, confocal immunofluorescence microscopy showed a surface staining pattern with a minimal overlap between CD4 and CXCR4 in the absence of gp120, whereas treatment with the envelope protein led to sequential aggregation of both receptors and their co-localization. These events are actin-dependent, because the overlap and co-localization of coreceptors were not found upon pretreatment with the mycotoxin cytochalasin D (CytoD), a macrocyclic alkaloid that specifically blocks actin polymerization. In agreement, HIV-1 entry and infection are strongly impaired by the inhibition of actin polymerization. In fact, a quantitative inhibition of viral growth was evident in cells pretreated with CytoD as a consequence of the blockage of post-CD4 binding events. These findings were reported for the first time in 1998 by Iyengar and co-workers [32] and have laid the foundations to discover many other elements required for HIV-1 binding and entry into host cells.

Today, the spatiotemporal complexity of HIV-1 infection of T cells is, at least in part, unraveled. Recently, Liu and co-workers reported a sequence of events involved in the binding of HIV-1 to lymphocyte membrane [33], based on the current literature reports. However, additional key components and their role in modulation of the actin cycle required for HIV-1 infection are now known (Fig. 2). The starting point that initiates the entire process is

the binding of viral gp120 to the host CD4 coreceptor. This interaction triggers several events that involve three actin-interacting proteins: filamin-A, exrin–radixin–moesin (ERM) proteins and cofilin. In particular, filamin should be considered as a structural support where cellular coreceptors CD4 and CXCR4 are anchored: interaction of CD4 and CXCR4 with filamin allows for coreceptor clustering on the cell membrane. Moreover, filamin also has a functional role resulting in the activation of the RhoA-dependent signaling pathway leading to cofilin phosphorylation and inactivation, with a consequent increase of actin polymerization [34]. Coreceptor clustering is also mediated by moesin, a protein of the ERM complex. Active (phosphorylated) moesin is responsible for the attachment of actin filaments to the plasma membrane required for clustering and direct interaction of host coreceptors CD4 and CXCR4 [35]. On the basis of these findings, filamin-A and ERM proteins (in particular moesin) are considered as facilitators of early steps of the infection, including receptor co-localization and clustering, membrane fusion and virus entry into host cells.

These evidences clearly show that ERM proteins and actin have a dual activity during HIV-1 infection. In fact, during the very early step of infection at the time of gp120 binding to CD4, moesin facilitates virus adhesion and entry by inducing actin redistribution and reorganization at the plasma membrane, actin-mediated CD4–CXCR4 interaction, membrane fusion and, finally, viral infection [36–39]. At the same time, because actin polymerization is required to enrich the concentration of CD4 and CXCR4 locally at the plasma membrane, cofilin undergoes a filamin-dependent Rho-mediated inactivation. In the next step, to enable post-entry events, conformational changes occurring on the structure of gp120 are responsible for its interaction with CXCR4, thus activating cofilin and leading to actin depolymerization (Fig. 2). Cofilin is dephosphorylated and re-activated to initiate actin depolymerization and remodeling necessary for HIV-1 intracellular migration and replication [40–42].

Paradoxically, moesin can also block viral replication before reverse transcription by disrupting stable microtubules in cultured cells [38]. In a similar way, although actin polymerization is required for virus entry, it constitutes a barrier for intracellular movement of HIV-1 and has to be reduced by cofilin activation [40]. Unfortunately, at the moment, there is no further experimental support to solve these paradoxes. In fact, although it is hypothesized that additional proteins are involved in these processes, their identity, spatiotemporal expression and the modulation of their activity are not known or fully understood yet. However, it was recently demonstrated that post-entry events, such as HIV-1 nuclear localization and integration, are promoted by a cofilin-mediated cytoskeletal reorganization dependent on the exposure of T cells to several chemokines (CCL19, CXCL9, CXCL10 and CCL20) that can synergize with the signaling pathways activated by the interactions between gp120, CD4 and CXCR4 [43,44].

LIMK are involved in the HIV-induced actin polymerization and depolymerization during HIV-1 infection

The temporal course of actin polymerization and depolymerization caused by the binding of the HIV-1 virus to T cells is also influenced by additional factors that belong to the virus and the host cells alike. In particular, actin polymerization triggered by gp120 at the earliest time of HIV-1 infection corresponds to a transient activation of LIMK1 [34,45], in turn regulating the CD4–CXCR4 direct interaction and clustering as well as viral entry. HIV-1-mediated LIMK activation is found to be a crucial pathway for the initiation of early stages of viral infection. In a recent study [45], to address the effects of LIMK on viral entry and infection, and to support further the hypothesis of a direct involvement of LIMK in actin polymerization required for HIV-1 entry, LIMK1 expression was suppressed in T cells by small interfering RNA (siRNA). As a result, a decrease of F-actin and an increase of CXCR4 internalization were found. Moreover, LIMK knockdown slightly decreased viral entry at high viral dosages but not at the low viral dosages. The latter result could be accounted for by the fact that a decreased actin activity associated to LIMK knockdown probably interferes with receptor clustering, thus affecting the entry of high concentrations of virus. LIMK knockdown also diminished HIV infection of human T cells, particularly viral DNA synthesis.

The very early step of gp120-induced actin polymerization is followed by cofilin activation and actin depolymerization which are required for viral nuclear migration [40]. To unravel the role of cofilin and LIMK1, a 16-amino acid peptide (S3), corresponding to the N-terminal portion of cofilin, was used as a competitor to inhibit cofilin phosphorylation at Ser3 by LIMK. The resulting activation of cofilin led to enhanced viral replication.

These data demonstrated that HIV-1 infection is dependent on the spatiotemporal dynamics and remodeling of actin that, in turn, result from modulation of the LIMK–cofilin pathway. In general, either actin polymerization or depolymerization could enhance HIV infection promoting viral entry and migration, respectively. Consequently, downstream effectors of chemokine signaling (such as cofilin and LIMK) emerge as druggable targets to be included in strategies that inhibit HIV infection. However, it is important to realize that targeting LIMK could result in enhanced or reduced HIV infection, depending on the extent and duration of LIMK inhibition.

Small molecule compounds able to modulate LIMK activity could be used to impair HIV-1 infection. As an example, if LIMK activity is required to phosphorylate and inactivate cofilin and, thus, to induce actin polymerization that promotes initial HIV-1 binding and fusion steps, inhibition of LIMK activity should result in a reduction of HIV-1 entry and infection. This hypothesis is in evident agreement with the decade-old knowledge that inhibition of actin polymerization strongly inhibits HIV entry [32]. However, as also noted by Vorster and co-workers, 'there is no specific LIMK inhibitor' [45], although at least two different classes of compounds that can impair LIMK activity are currently known. In fact, thiazolylurea and thiazolylamide derivatives [46], as well as pyrrolo-pyrimidines [16], have been described as inhibitors of LIMK activity (Fig. 3). Unfortunately, such compounds suffer from major limitations: several of them target multiple kinases, such as p38, ROCK and TNF-α, without significant selectivity; their mechanism of action toward LIMK (competitive or allosteric) is unknown; congeneric compounds (belonging to the same structural class) act toward LIMK and tubulin. Further efforts are required to identify their mechanism of action and to render their activity specific for LIMK.

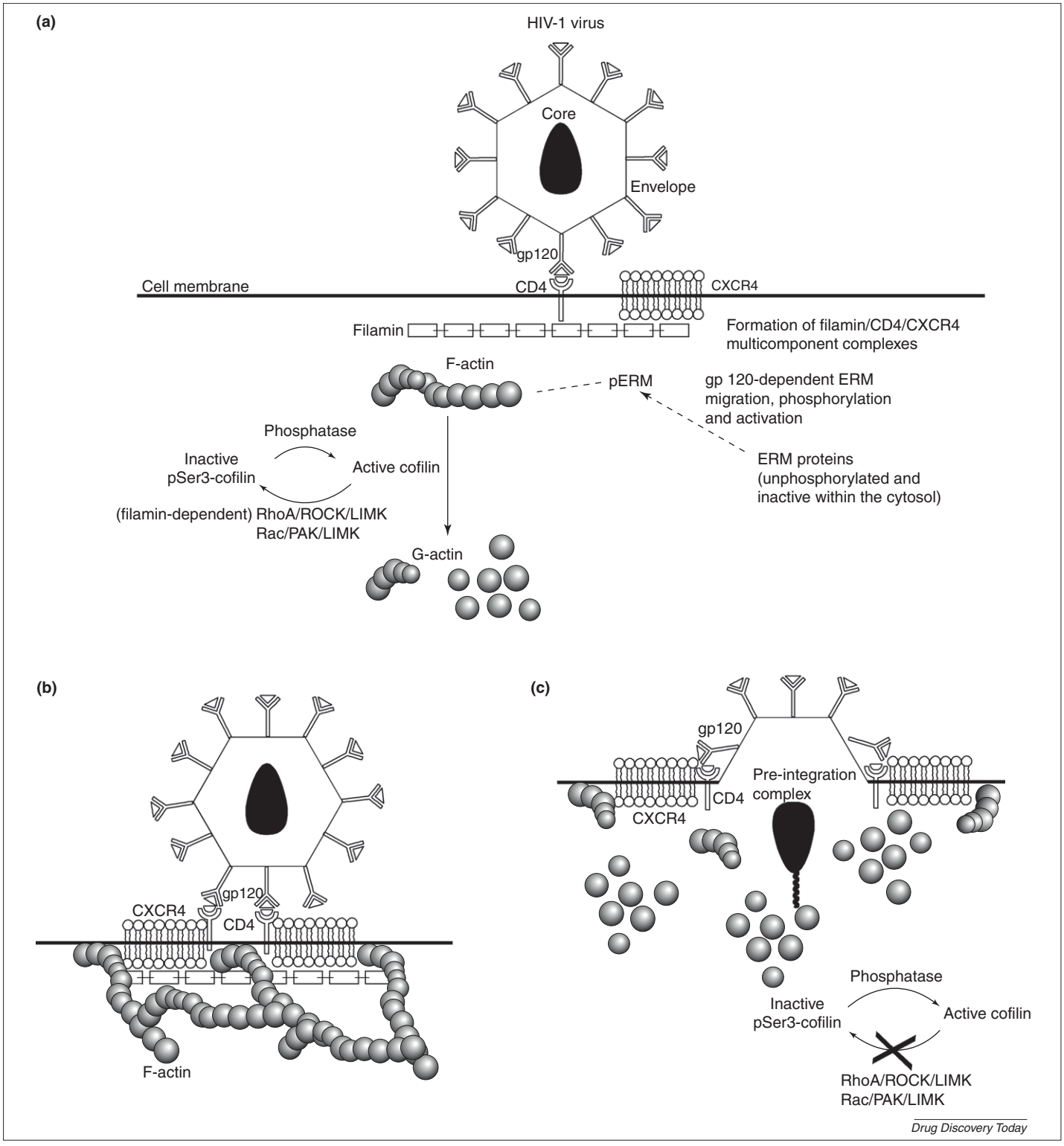

FIGURE 2

Schematic representation of the sequential steps describing HIV-1 attachment and penetration into host cells. **(a)** Binding of the viral envelope protein gp120 to host CD4 is the triggering event for co-localization and aggregations of CD4 and CXCR4 that is also mediated by formation of a filamin-A/CD4/CXCR4 multicomponent complex. At the same time, ERM proteins that are unphosphorylated and inactive within the cytosol, undergo a gp120-dependent migration toward the cell membrane, phosphorylation and activation. They serve to attach F-actin to the membrane and to enhance coreceptor clustering. By contrast, small GTPases of the Rho family (namely, RhoA and Rac) and their downstream effectors (ROCK and PAK, respectively) activate LIMK by phosphorylation, thus inactivating cofilin and leading to an increase of F-actin levels required for the early step of coreceptor clustering. The Rho-dependent signaling pathway is directly activated by filamin-A, whereas the Rac-dependent signal is also modulated (even if at a later stage) by the viral protein Nef. Involvement of LIMK gives the suggestion that inhibition of LIMK activity and actin remodeling could be a profitable tool to block the early stage of HIV-1 infection. **(b)** The structural role of filamin and ERM proteins leads to coreceptor enrichment and clustering on the cell membrane, further supported by attached F-actin. **(c)** Conformational changes affecting gp120 and leading to the exposure of gp41 toward the host cell membrane are responsible for viral particle fusion with the host cell surface,

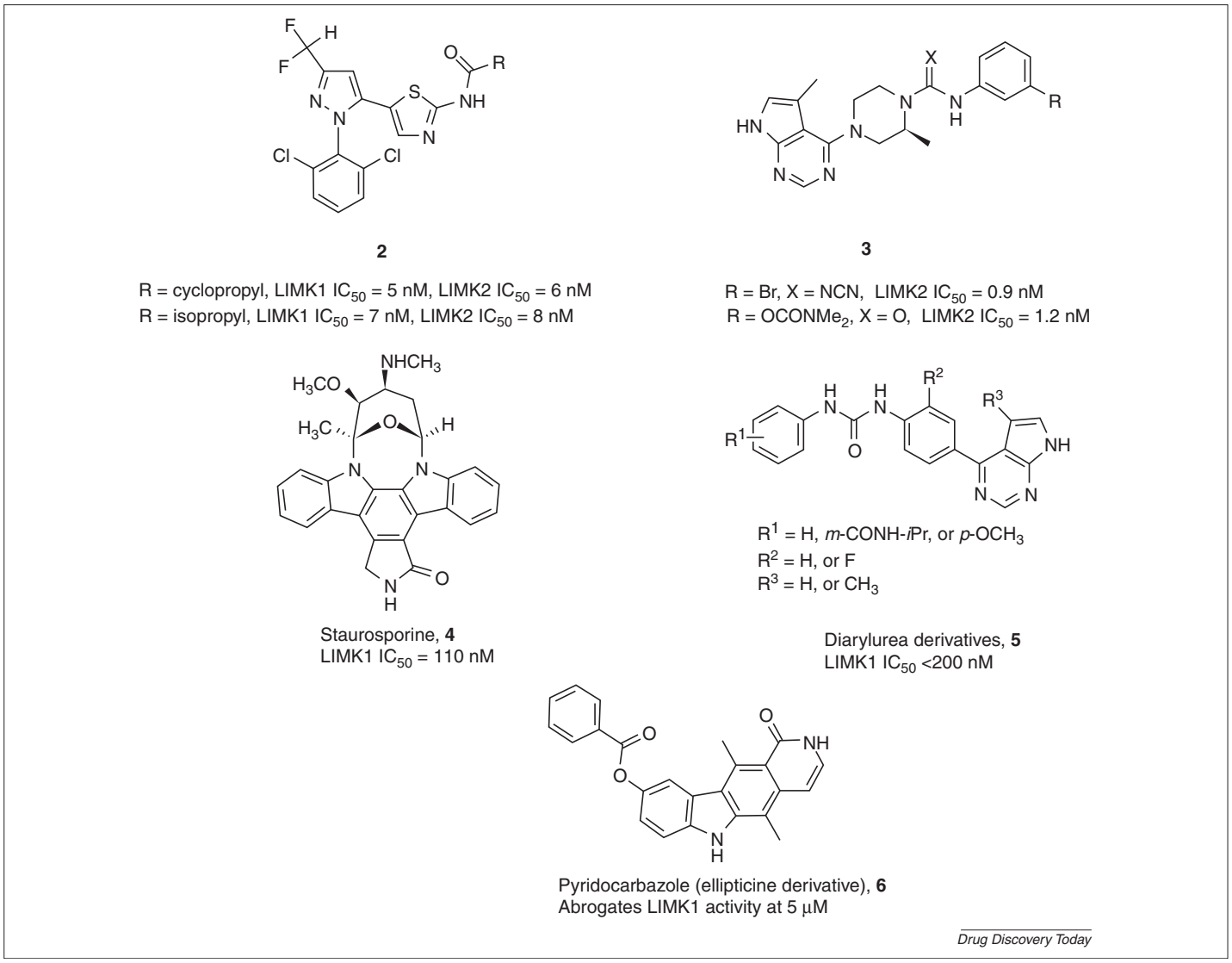

FIGURE 3

Schematic representation of the small molecule LIMK inhibitors currently available. None of the currently known inhibitors of LIMK activity is specific because they all target multiple kinases without selectivity or have tubulin as a target. Moreover, their binding site on LIMK and their mechanism of action (ATP-competitive or allosteric) are still unknown.

Transient actin polymerization induced by HIV-1 infection and LIMK1 activation is similar to the stromal-cell-derived factor (SDF)-1α-dependent Rac-mediated LIMK1 phosphorylation and activation found in Jurkat human leukemic T cells [47]. In both cases, LIMK1 activation by phosphorylation leads to cofilin phosphorylation and inactivation, with a consequent decrease or abrogation of actin cleavage and an increase of actin polymerization. Recently, early LIMK1 activation by HIV-1 was associated with a Rac-mediated increase of PAK phosphorylation and activation (Fig. 2). In particular, PAK1 and PAK2 were found to be phosphorylated at Thr423 and Thr402, respectively, upon HIV-1 treatment of T cells [45]. However, the PAK2-dependent phosphorylation of cofilin also requires a direct interaction of PAK2 with Nef [48], a crucial viral factor in AIDS pathogenesis, exclusively expressed by the lentiviruses HIV-1, HIV-2 and simian immunodeficiency virus (SIV). One of the major evidences of Nef expression is a marked inhibition of T-lymphocyte chemotaxis induced by SDF-1α and the consequent actin remodeling, similar to that found for gp120 [49].

LIMK could be recruited in a multicomponent complex involving PAK2 and viral Nef

Direct association of Nef with PAK2 was first discovered in 1996 [50]. Although the existence of a labile multiprotein complex was hypothesized, its full composition is still not determined. However, recent evidence strongly suggests 'the presence of another cofilin kinase in the Nef-associated protein complex' [48], in addition to Nef, PAK2, Rac1 and the guanine exchange factor VAV1 [51] (this complex is reminiscent of the Rac1–MOCA/PAK2/LIMK signaling pathway [52]). From an analysis of the

uncoating and release of the pre-integration complex. During this step, cofilin is reactivated to cleave F-actin into G-actin, thus allowing for remodeling required for post-entry events and nuclear import.

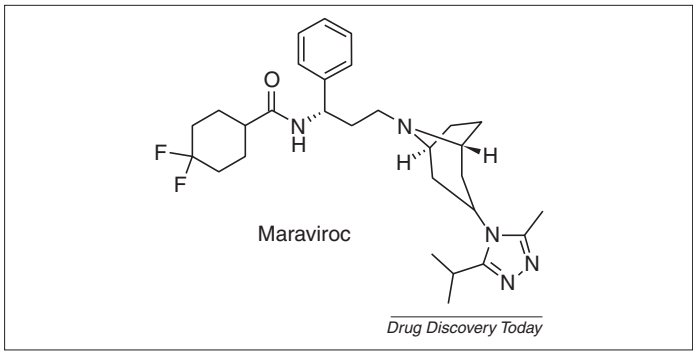

FIGURE 4

Representation of the chemical structure of maraviroc. Maraviroc, **1** is the first licensed chemokine coreceptor 5 (CCR5) antagonist in clinical practice.

currently available data, the hypothesis that LIMK could be one additional partner possibly belonging to the Nef–PAK2 multiprotein complex cannot be excluded. In fact, biological results reported by Stolp and co-workers [48] show that treatment of Nef-expressing cells with PAK2-specific RNAi was unable to restore fully cell motility impaired by Nef-PAK2-mediated cofilin inactivation. To explain this result, there could be an incomplete knockdown of PAK2 expression and/or the involvement of additional cofilin regulators. If other cofilin modulators are involved they are required to phosphorylate and inactivate cofilin, thus reducing cell motility, as found by Stolp and co-workers [48]. Moreover, the known ability of PAK to activate LIMK by phosphorylation upon direct interaction also suggests that LIMK and PAK2 could be partners in a multicomponent complex.

The fact that HIV-1 could drive CXCR4 and CD4 to activate LIMK suggests that LIMK has a crucial role in the viral infection process. In this context, LIMK regulation could result the major controller of the spatiotemporal cycling of actin that enables HIV infection. In fact, early and transient LIMK activation and actin depolymerization was associated with viral entry and DNA synthesis, whereas subsequent LIMK inactivation and actin depolymerization promotes intracellular migration of HIV-1.

The crucial role of LIMK in HIV-1 infection further supports the hypothesis that LIMK could be considered as valuable targets for drug-mediated actin regulation that, in this case, could result in the prevention of viral infection and contribute to combinatorial approaches in HIV therapy.

Cellular cofactors currently represent attractive new targets for HIV-1 chemotherapy, because targeting a cellular cofactor required for viral entry or replication should help to overcome viral resistance. Accordingly, in addition to the approved drug maraviroc [53] (**1**, Fig. 4) that blocks the chemokine (C–C motif) receptor 5 (CCR5), additional attempts are ongoing to block host cofactors, such as the aspartic-glutamic-alanine-aspartic (DEAD) box polypeptide 3 (DDX3) [54]. In this context, inhibition of LIMK activity and actin polymerization could open up a new strategy for impairing HIV-1 attachment and entry. Moreover, targeting a host protein as LIMK could also show the advantage of overcoming the emergence of resistant strains of HIV-1.

Design of new inhibitors of LIMK

Literature reports two classes of compounds found to be LIMK inhibitors (Fig. 3). The ability of such compounds to inhibit kinase activity of LIMK has been determined by incorporating radiolabeled phosphorous into the cofilin substrate (expressed as IC_{50} values). These compounds belong to the class of thiazolylureas and thiazolylamides described by Bristol-Myers Squibb (**2**) [46] and to the class of pyrrolo[2,3-*d*]pyrimidines from Lexicon Pharmaceuticals (**3**) [16] with activity spanning from subnanomolar to micromolar concentrations. Considering the wide range of activity (over four orders of magnitude or log units), a significantly different chemical structure, and homogeneity of biological data (they have been obtained following the same protocol in a cell-free *in vitro* assay), activity values have the optimal requisites to be used for *in silico* drug design purposes. Accordingly, to identify chemical features that are responsible for activity toward LIMK2 and to design novel inhibitors with improved activity, Sun and co-workers [55] recently reported a molecular modeling approach based on three-dimensional quantitative structure–activity relationship (3D QSAR) analysis and docking calculations for the pyrrolo-pyrimidine LIMK2 inhibitors [16]. The ligand-based approach based on Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA) led to validated statistical models that could be used to design new LIMK2 inhibitors with optimized activity and selectivity, although the authors did not apply them for these purposes. By contrast, the lack of structural information on the macromolecular target LIMK2 resulted in structure-based simulations that suffered from at least two major limitations. In particular, the NMR model of the second LIM domain of LIMK2, deposited in the Protein Data Bank with the entry code 1x6a, was chosen as the three-dimensional template structure for docking simulations. This appears as an arbitrarily and possibly inappropriate choice because 1x6a contains only a portion of the entire structure of LIMK2 whose three-dimensional arrangement could be very different from that of the same domain embedded in the overall structure of the kinase. In addition, there is no experimental evidence that pyrrolo-pyrimidine inhibitors target the second LIM domain of LIMK2, as supposed in that paper. By contrast, considering that pyrrolo-pyrimidine are known to be kinase inhibitors with an ATP-competitive mechanism [56], it is probable that such compounds could block LIMK2 by binding to its ATP-binding site and, thus, interfering with the ATP-based catalytic machinery.

In addition to these compounds, staurosporine (**4**; Fig. 3), known as a non-selective inhibitor of many kinases owing to its high affinity for the ATP-binding site, also shows significant inhibition of the kinase activity of LIMK1. The submicromolar activity of staurosporine toward LIMK1 (IC_{50} = 110 nM), measured on human recombinant Sf21 cells by a time-resolved fluorescence resonance energy transfer assay (http://www.cerep.fr/cerep/users/pages/catalog/affiche_condexp_test.asp?test=2934) based on LANCE® Ultra technology (http://las.perkinelmer.com/Catalog/CategoryPage.htm?CategoryID=LANCE+Ultra), also accounts for deposphorylation and activation of cofilin found by Yoder and co-workers on T cells treated with staurosporine [40]. The authors stated that staurosporine could act directly on the LIMK1-mediated cofilin phosphorylation pathway, but they described as unexpected the ability of staurosporine to decrease strongly the phosphorylation of cofilin by LIMK1 and, thus, to activate cofilin itself. However, the fact that staurosporine is well known as a multiple kinase inhibitor, combined with the ability of this

compound to inhibit directly LIMK1 as well, leads to the suggestion that cofilin activation found by Yoder *et al*. [40] was consequent to the inhibition of LIMK1 activity by staurosporine with a resulting increase of dephosphorylated cofilin levels.

Finally, three additional classes of compounds have been disclosed recently as LIMK1 inhibitors, although only a few details on their biological profile have been reported. In particular, aryl sulfonamides with a low nanomolar activity have been described [57], and only five diarylurea derivatives (**5**; Fig. 3) with $IC_{50} < 200$ nM have been disclosed by Feng and co-workers [58]. Moreover, among tetracyclic pyridocarbazoles belonging to the class of ellipticine derivatives, **6** (Fig. 3) can completely abrogate LIMK1 activity at a concentration of 5 μM [59].

Concluding remarks

LIMK are involved in many physiopathological conditions by the modulation of cofilin phosphorylation through signaling pathways where a plethora of additional macromolecular targets are also required. One of the last experimental findings shows that LIMK can associate with viral proteins and regulate entry and post-entry processes of HIV-1 infection. In particular, enhanced LIMK activity is necessary to freeze actin dynamics during the early steps of viral infection, whereas cytoskeleton remodeling and LIMK inactivation must occur for post-entry intracellular moving of HIV-1. Although many details of the spatiotemporal activation-inactivation process of LIMK and cofilin have been discovered and described, many more efforts are required to understand fully the relationships between LIMK-associated pathways and HIV-1 infection. As an example, there is a seemingly clear rationale that links the inhibition of LIMK activity with a reduction of HIV-infection. In fact, actin polymerization is required for HIV-1 recognition and entry into host cells. On this basis, blocking LIMK activity, thus enhancing actin cycling, should impair the early steps of HIV-1 infection. However, the hypothesis that LIMK inhibitors currently available could be useful tools to block or drastically reduce HIV-1 infection by interfering with the first stages should be supported by experimental evidence.

Moreover, the mechanism of action of currently known inhibitors of LIMK should be discovered. In fact, in particular the pyrrolo-pyrimidine derivatives [16] are likely to act as ATP-competitive agents by the occupation of the ATP-binding site on LIMK, whereas there is a lower probability that they could bind to the second LIM domain as recently reported [55].

Finally, in the attempt to shed further light on the mechanism of HIV-1 infection, the hypothesis that LIMK could be partner proteins involved in the multicomponent complex to which Nef and PAK2 belong should also be validated. In fact, recent works suggest that a not yet identified kinase could participate in this complex and that the multicomponent system can regulate cofilin phosphorylation and actin remodeling.

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