Review

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Modulation of dynamin function by small molecules

https://doi.org/10.1515/hsz-2018-0257
Received May 18, 2018; accepted July 17, 2018; previously published online July 24, 2018

Abstract: Dynamins are essential as membrane remodelers in various cellular processes, like receptor-mediated endocytosis, synaptic vesicle recycling and spermatogenesis. Moreover, dynamin is involved in the internalization of numerous viruses and in the motility of several cancer cell lines. As tools for dissecting the underlying mechanisms of these important biological processes and as potential future therapeutics, small molecules have been developed in the last two decades that modulate the functions of dynamin. In this review we give an overview of the compound classes that are currently in use and describe how they affect dynamin function.

Keywords: dynamin; endocytosis; GTPase activity; inhibitor; small molecules.

Introduction

Dynamins constitute a family of multi-domain high molecular weight GTPases best known for their multiple roles in membrane shaping processes. Most notably, dynamins are key players in receptor mediated endocytosis and synaptic vesicle recycling (Ferguson and De Camilli, 2012; Antonny et al., 2016). Moreover, dynamins are involved in the uptake of viral and bacterial pathogens (Harper et al., 2013) and are implicated in the motility of metastatic cancer cells (Meng, 2017).

Classical dynamins are the eponymous founding members of the dynamin family. They share five distinct domains (Figure 1): an N-terminal GTPase (G) domain that binds and hydrolyzes GTP, a helical stalk that mediates oligomer formation, a three-helix bundle called bundle signaling element (BSE) acting as flexible linker between the G domain and the stalk, a pleckstrin homology (PH) domain that binds acidic membrane phospholipids, and a C-terminal proline-rich domain (PRD) that mediates protein-protein interactions with src-homology 3 (SH3) domain containing proteins. Dynamins display low nucleotide affinity (reviewed in Sever et al., 2000) and high basal GTPase activity that can be stimulated up to 200-fold by helical self-assembly on suitable lipid templates (Stowell et al., 1999). Mammals express three classical dynamins. Dynamin 1 is expressed predominantly in neurons and brain tissue and mediates synaptic vesicle recycling (Nakata et al., 1991; Ferguson et al., 2007). Dynamin 2 is ubiquitously expressed and is involved in receptor-mediated endocytosis (Cook et al., 1994). Dynamin 3 is expressed in testes, lung, and in the postsynapse and has been implicated in post-synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid) receptor cycling (Lu et al., 2007) and megakaryocytogenesis (Reems et al., 2008; Wang et al., 2011). In this review, we use the term 'dynamin' to designate the classical mammalian dynamin isoforms.

Dynamins are able to form helical supramolecular assemblies around lipid tubules, e.g. the necks of nascent vesicles at the plasma membrane. The smallest building blocks of the helical assemblies are dynamin tetramers, which are thought to assemble the helix without energy consumption (Reubold et al., 2015). Within the helical filament, G domains from neighboring rungs are arranged such that their nucleotide binding sites are located opposite to each other (Chappie et al., 2011). Upon nucleotide binding the opposing G domains dimerize and thus stimulate their GTPase activity. Hydrolysis of the bound GTP changes the position of the G domain relative to the BSE and provides the mechanical energy necessary to constrict the helix and eventually to sever the membrane (Chappie et al., 2010, 2011). The mechanism of membrane fission is still unclear and two principal models, the two-state model and the constrictase/ratchet model, are being discussed (Antonny et al., 2016). According to the two-state model, assembly of the dynamin helix and GTP hydrolysis constrict the membrane tubule to enable the formation

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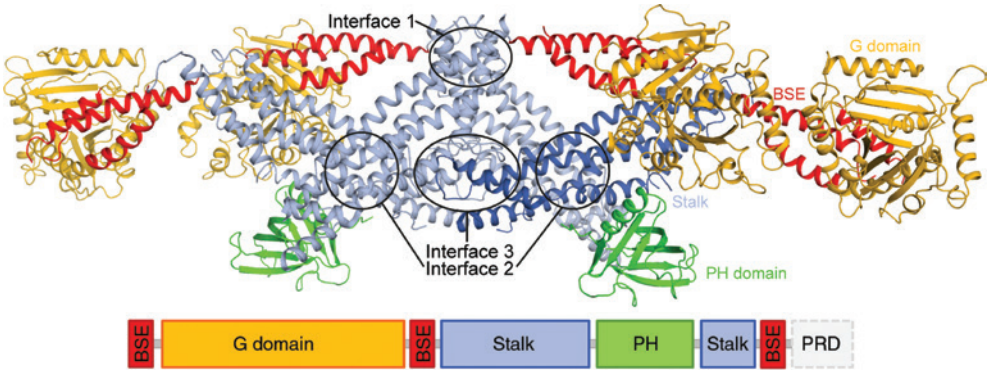

Figure 1: Crystal structure of the dynamin tetramer.
The three-dimensional structure of the tetramer of dynamin in isoform 3 (PDB code 5A3F, Reubold et al., 2015) is shown in cartoon representation. The individual domains of the monomers are colored according to the color code of the domain structure shown beneath the cartoon. The stalk of the rightmost monomer is depicted in dark blue for clarity. The interfaces that drive dimerization (interface 2) and tetramerization (interfaces 1 and 3) are marked. BSE, Bundle signaling element; G domain, GTPase domain; PH, pleckstrin homology domain; PRD, proline rich domain.

of hemi-fission intermediates. The subsequent release of inorganic phosphate loosens the helical scaffold and disassembly of the dynamin helix leads to complete fission of the membrane. In the constrictase/ratchet model the G domains act as molecular motors that trigger sliding of the helical turns that lead to constriction and twisting of the dynamin helix, which finally lead to fission of the membrane.

Besides its well-characterized role in endocytosis, dynamin has been implicated in a range of actin-based processes (Orth et al., 2002; Krueger et al., 2003; Gu et al., 2010; Destaing et al., 2013; Yamada et al., 2013; Zhang et al., 2014). Evidence for the involvement of dynamin in actin remodeling is derived from the fact that dynamin directly binds actin-regulatory proteins like cortactin (McNiven et al., 2000; Schafer et al., 2002), murine actin binding protein 1 (Kessels et al., 2001), and profilin (Witke et al., 1998), or actin itself (Gu et al., 2010). The true role of dynamin in the dynamics of the actin cytoskeleton is still unknown.

In order to provide the means to elucidate the mechanisms of these fundamental processes, numerous small-molecule modulators of dynamin function have been developed and employed in the last decade (see Table 1 and references therein). Most of the described compounds act as inhibitors. Different classes of compounds target different sites in dynamin and thereby impede the function of dynamin through different mechanisms. Substances that bind to the GTPase domain of dynamin and compete with GTP binding decrease the GTPase activity of dynamin required for vesicle scission. Agents that bind to the PH domain prevent the recruitment of dynamin to the membrane. Other points of action may lie in stimulation or inhibition of the assembly of the dynamin helix. This review provides an overview of the currently available assortment of small molecule modulators of dynamin function, describes examples of applications, and emphasize caveats on the use of some of the inhibitors.

Compounds targeting the PH domain

Alkyl ammonium salts

Long-chain alkyl ammonium salts were the first class of compounds to be described as specific dynamin inhibitors (Hill et al., 2004). Starting with long-chain primary amines, which displayed moderate inhibitory effects on lipid stimulated GTPase activity, the authors of this study found that the corresponding trimethyl ammonium salts myristyl trimethyl ammonium bromide (MiTMAB) and octadecyl trimethyl ammonium bromide (OcTMAB) showed improved inhibitory action with IC_{50} values of 5.79 and 3.15 μM, respectively (Figure 2A). Please note that in the literature the designation ‘MiTMABs’ is often used for the whole class of substances. In a follow-up study it was shown that these compounds disturb the interaction of dynamin 1 with lipids, thereby abolishing efficient stimulation of its GTPase activity (Quan et al., 2007). The inhibitory action was also confirmed in cell-based assays, including transferrin and EGF uptake that require functional dynamin 2, and synaptic vesicle endocytosis, which is mediated by dynamin 1. MiTMABs were also shown to significantly diminish proliferation and viability of a diverse panel of cancer cell lines (Joshi et al., 2010).

Table 1: Most potent representatives of the compound classes described in this review.

Compound	Class	Domain	IC_{50} *in vitro* (Dyn1)	IC_{50} RME	References
MiTMAB	AAS	PH	3.1 ± 0.2 μM	20.9 ± 3.2 μM[a]	Quan et al., 2007 Daniel et al., 2012
OcTMAB	AAS	PH	1.9 ± 0.24 μM	16.0 ± 4.2 μM[a]	Quan et al., 2007
Alendronate	BP	PH	n.d.	n.d.	Masaike et al., 2010
BisT-23	Bistyrphostins	Unknown	1.7 ± 0.2 μM	n.d.	Hill et al., 2005
Dynasore	HPH	Unknown	n.d.	~15 μM[b]	Macia et al., 2006 Harper et al., 2011
Dyngo-4a	HPH	Unknown	0.38 ± 0.05 μM	5.7 ± 1.0 μM[c]	McCluskey et al., 2013
Dynole-34-2	Dynoles	Unknown	1.3 ± 0.3 μM	5.0 ± 0.9 μM[c]	Hill et al., 2009
Fluvoxamine	PD	PH	14.7 ± 1.6 μM	n.d.	Daniel et al., 2015
Iminodyn-23	Iminodyns	Unknown	0.26 ± 0.08 μM	74.6 ± 8.8 μM[c]	Hill et al., 2010
Melophlin A	Tetramic acids	Unknown	n.d.	n.d.	Knoth et al., 2009
Naphthaladyn-29	Naphthaladyns	GTPase	18.5 ± 1.7 μM	66 μM[c]	Abdel-Hamid et al., 2015
Sertraline	PD	PH	7.3 ± 1.0 μM	n.d.	Otomo et al., 2008
Trifluoperazine	PD	PH	2.6 ± 0.70 μM	2.5 μM[c]	Daniel et al., 2015
Pthaladyn-23	Pthaladyns	GTPase	17.4 ± 5.8 μM	n.a.	Odell et al., 2010
Pyrimidyn-7	Pyrimidyns	GTPase/PH	1.1 ± 0.05 μM	12.1 ± 2.1 μM[a]	McGeachie et al., 2013
Quinodyn-49	Quinodyns	GTPase	10.6 ± 1.6 μM	200 ± 47 μM[c]	MacGregor et al., 2014a
Rhodadyn-D10	Rhodadyns	GTPase	4.5 ± 0.8 μM	5.9 ± 1.0 μM[c]	Robertson et al., 2012
RTIL-13	RTIL	PH	2.3 ± 0.3 μM	n.d.	Zhang et al., 2008

AAS, Alkyl ammonium salts; BP, bisphosphonates; n.a., not active; n.d., not determined; HPH, hydroxyphenyl hydrazones; PD, psychotropic drugs; RME, receptor-mediated endocytosis; RTIL, room-temperature ionic liquids.
[a]Uptake of epithelial growth factor in COS7 cells.
[b]Uptake of transferrin in HeLa cells.
[c]Uptake of transferrin in U2OS cells.

Further analysis revealed that the proliferation arrest was caused by a defective scission of the cleavage furrow in cytokinesis, confirming the previous observation that dynamin 2 is not involved in the constriction step but in the scission step of cytokinesis (Liu et al., 2008). Although non-tumorigenic fibroblasts also experienced a cytokinesis related growth arrest, their viability was much less compromised than that of the tumor cells.

Bisphosphonates

Bisphosphonates (BPs) are pyrophosphate analogs, some of which are being used as prescription drugs directed against loss of bone mass in diverse medical conditions (Maraka and Kennel, 2015). BPs can be sub-classified based on the presence of nitrogen into two subclasses, which are thought to act via different mechanisms of action (Reszka and Rodan, 2003). Non-nitrogen containing bisphosphonates (NN-BPs), like etidronate (Figure 2B), are supposed to be metabolized to non-hydrolyzable ATP analogs that subsequently induce apoptosis in osteoclasts (Halasy-Nagy et al., 2001). Nitrogen-containing bisphosphonates (N-BPs), like alendronate (Figure 2C) or zolendronate; are thought to inhibit farnesyl diphosphate synthase, which leads to osteoclast inhibition through interference with geranylgeranylation of small GTPases (Fisher et al., 1999). Some years ago, Masaike and colleagues reported dynamin 2 as a novel target for BP drugs (Masaike et al., 2010). They used alendronate as an affinity bait to isolate dynamin 2 from lysates of RAW264.7 cells, a virally transformed murine leukocyte cell line, and showed that the compound inhibited lipid stimulated GTPase activity. Furthermore, three different BPs inhibited cellular entry of simian virus 40 and adenovirus as well as uptake of transferrin. Based on titration experiments with acidic phospholipids the authors concluded that BPs bind to the PH domain thus preventing membrane recruitment of dynamin. Contradictory to this conclusion is their observation by electron microscopy that dynamin 2 was still able to decorate lipid tubules in the presence of alendronate, although tubule scission by addition of GTP was prevented (Masaike et al., 2010). Despite some unresolved issues concerning the proposed mechanism the results of the study warrant further evaluation of BPs as potential antivirals, especially given their established good safety profile and decade-long usage as osteoporosis drugs.

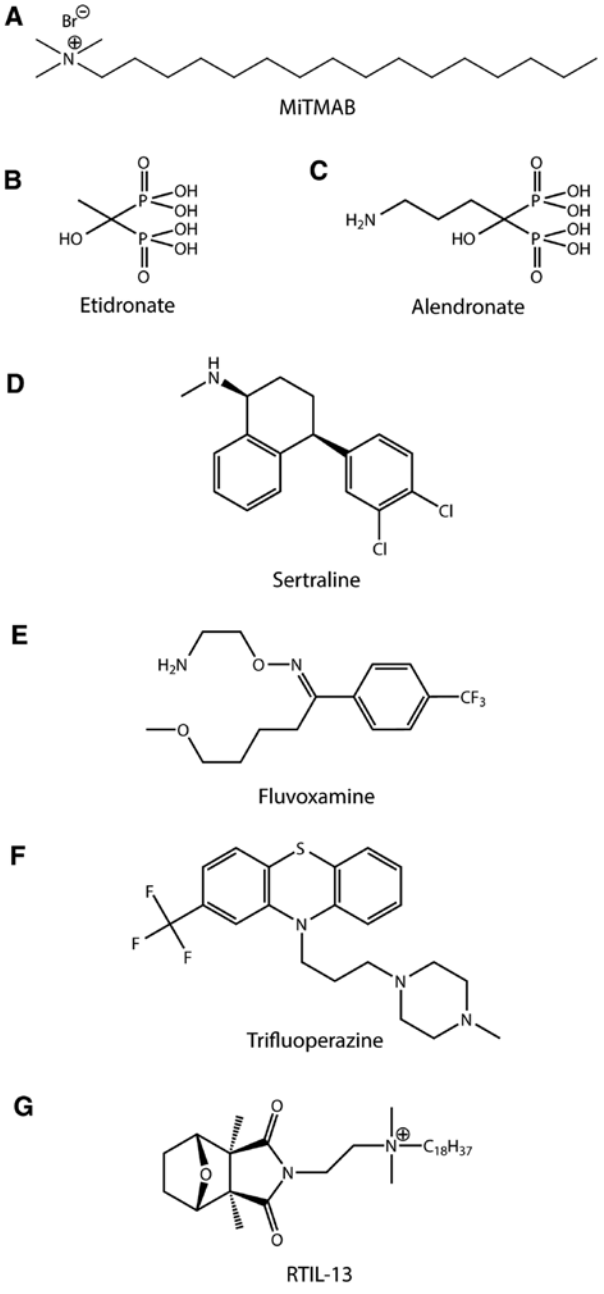

Figure 2: Structural formulae of modulators that target the PH domain.

Psychotropic drugs

In 2008, Otomo and co-workers published a study about the effects of a panel of antidepressants and selective serotonine reuptake inhibitors (SSRIs) on the GTPase activity of dynamin 1 (Otomo et al., 2008). To this end the authors used full-length dynamin 1 stimulated with lipid tubules composed of phosphatidyl serine for their assays. They found that the SSRIs sertraline (Figure 2D) and fluvoxamine (Figure 2E) displayed stronger inhibitory effects than the reference compound MiTMAB with IC_{50} values of 7.3 and 14.7 μM, respectively. An inhibitory effect of sertraline on transferrin internalization in HeLa and neuronal cells was found in a later study (Takahashi et al., 2010). Daniel and co-workers analyzed the structure-activity relationship of both clinically used phenothiazine-derived antipsychotic drugs (APDs) and non-clinically used phenothiazines in the context of dynamin inhibition (Daniel et al., 2015). The two compounds yielding the highest inhibition of the lipid-stimulated GTPase activity of dynamin 1 and dynamin 2 as well as of CME were the drug trifluoperazine (Figure 2F) and the non-APD compound 10-(3-piperazine-1-yl-propyl)-2-trifluoromethyl-10H-phenothiazine with IC_{50} values of 2.6 and 2.2 μM, respectively. Further analysis suggested that sertraline and phenothiazines, with the exception of chlorpromazine, target membrane recruitment of dynamin through competition with phoshpholipids.

Room-temperature ionic liquids (RTILs)

In an attempt to develop novel room-temperature ionic liquids (RTILs) to be used for the solubilization of the PH domain of dynamin, Zhang and colleagues synthesized a group of RTILs based on the scaffold of norcantharidine (Zhang et al., 2008). A common feature of the compounds is the presence of an alkyl ammonium group at the nitrogen atom of the maleimide moiety that contains one long chain alkyl group. The most potent compound, RTIL-13 (Figure 2G) displayed inhibitory activities towards the lipid-stimulated GTPase activity of dynamin 1 with an IC_{50} value of 2.3 μM. Based on the structural similarity to the MiTMABs (see above) the authors suggested a similar mechanism of action. The RTILs are supposed to bind to the PH domain of dynamin, which would competitively inhibit membrane binding. However, this hypothesis has not been experimentally verified to date.

Modulators targeting the GTPase domain

Naphthaladyns

The naphthaladyns are 1,8-naphthalimide derivatives that have been identified by combining virtual screening of a fragment library with structure-based drug design (Abdel-Hamid et al., 2015). Previously, 1,8-naphthalimide derivatives had been identified as clathrin

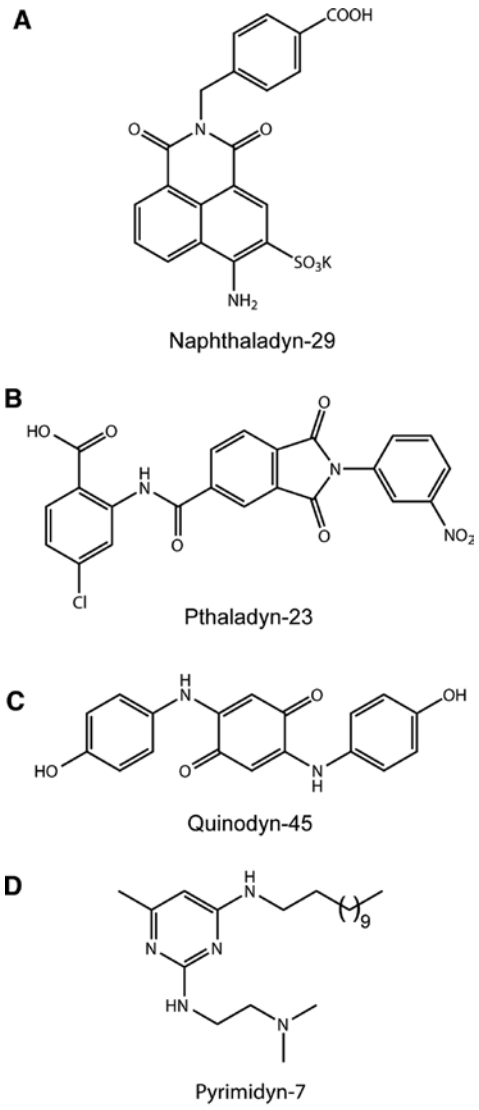

Figure 3: Structural formulae of modulators that target the G domain.

inhibitors (MacGregor et al., 2014b). To act on dynamin, the basic 1,8-naphthalimide scaffold was substituted with a sulfonic acid moiety and an amino group at the naphthyl ring, and with variable groups at the imide nitrogen. Naphthaladyn-29 (Figure 3A) displays an IC_{50} of 18.5 μM in the inhibition of lipid stimulated GTPase activity and an IC_{50} of 66 μM for inhibition of transferrin internalization.

Pthaladyns

The first class of dynamin inhibitors that has been developed by virtual screening is that of the pthaladyns (Odell et al., 2010). The authors of this study used the crystal structure of the GTPase domain of dynamin A from *Dictyostelium discoideum* to generate a homology model of the GTPase domain of human dynamin 1. Using the homology model as target, the authors screened a library containing 80 000 compounds and identified a substituted phthalimide derivative as lead compound. Optimization of the lead yielded the compound pthaladyn-23 (Figure 3B) that effectively inhibited the GTPase activity of dynamin 1 as well as the dynamin 1-mediated vesicle formation on isolated rat synaptosomes with IC_{50} values of 17.4 μM and 12.9 μM, respectively. Pthaladyn-23 showed competitive behavior for magnesium-GTP, which validated the basic concept of identifying compounds that occupy the GTP-binding site. However, in a later publication pthaladyn-23 has been reported to lack efficacy in the synaptic-vesicle endocytosis (SVE) assay with an IC_{50} value of 743 μM (Daniel et al., 2012).

Quinodyns

A further class of dynamin inhibitors that has been identified by virtual screening is that of the quinodyns (MacGregor et al., 2014a). The authors used the structure of a minimal dynamin construct containing the GTPase domain and the BSE (Chappie et al., 2011) as a target for docking. The most potent hit resulting from these trials, the substituted p-benzoquinone quinodyn-45 (Figure 3C), inhibited the phosphatidyl serine-stimulated GTPase activity of dynamin 1 with an IC_{50} of 11.1 μM and transferrin uptake by CME with an IC_{50} of 36 μM, respectively. According to a kinetic analysis, the quinodyns act through competition with magnesium-GTP.

Compounds targeting both the GTPase domain and the PH domain

Pyrimidyns

The pyrimidyns contain a pyrimidine ring that is substituted with a medium chain alkyl amino group and a dimethyl amino ethyl group (McGeachie et al., 2013). The most potent inhibitor, pyrimidyn 7 (Figure 3D), inhibits the lipid stimulated GTPase activity of dynamin 1 with an IC_{50} of 1.1 μM and CME of the transferrin receptor with an IC_{50} of 12.1 μM. The pyrimidyns are the only class of dynamin inhibitors so far that target two different dynamin domains at once, namely the GTPase domain and the PH domain (Odell et al., 2017). Analysis

of the binding behavior of pyrimidyn 7 revealed that the compound is competitive for GTP and phospholipid in binding to the GTPase domain and the PH domain, respectively. Consequently, pyrimidyn 7 inhibits both basal and stimulated GTPase activity and is able to displace GFP-tagged dynamin from the plasma membrane (McGeachie et al., 2013).

Pyrimidyn 7 has been used recently to assess the uptake mechanism of vesicles into brain lymphatic endothelial cells (BLECs) (van Lessen et al., 2017). The authors of this study found that incubation with pyrimidyn 7 almost completely abolished cargo uptake into BLECs and suggested that internalization of glycoproteins and polysaccharides is largely dynamin dependent in these cells.

Compounds with unknown target domain

Bistyrphostins

Tyrphostins (tyrosine phosphorylation inhibitors) had initially been described as EGF receptor kinase inhibitors almost 30 years ago (Yaish et al., 1988). In 2005, Hill and colleagues reported the synthesis of a series of dimerized tyrphostins (called Bis-Tyrphostins or BisT), the most potent of which, BisT-22 and BisT-23 (Figure 4A), inhibited phosphatidylserine stimulated dynamin 1 with IC_{50} values of 1.7 μM (Hill et al., 2005). BisT-22 has been described before as a potent inhibitor of EGF receptor kinase under the name Tyrphostin AG 537 (Levitzki and Gilon, 1991; Gazit et al., 1996). Recently, BisT-22 has been shown to also inhibit RNA editing ligase 1 of *Trypanosoma brucei* (Zimmermann et al., 2016).

Odell and colleagues attempted to map the BisT binding site on dynamin with the aid of asymmetric BisT derivatives, which carried a photoactivatable substituent on one of the phenyl rings instead of the hydroxyl groups (Odell et al., 2009). This approach proved to be inconclusive as no BisT-conjugated peptides could be detected by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). As possible explanations, the authors suggested that either the phenyl ring carrying the photoactivatable group was not able to bind to dynamin due to the absence of the second catechol moiety or the respective peptides may not have been recovered in the mass spectrometry (MS) experiment.

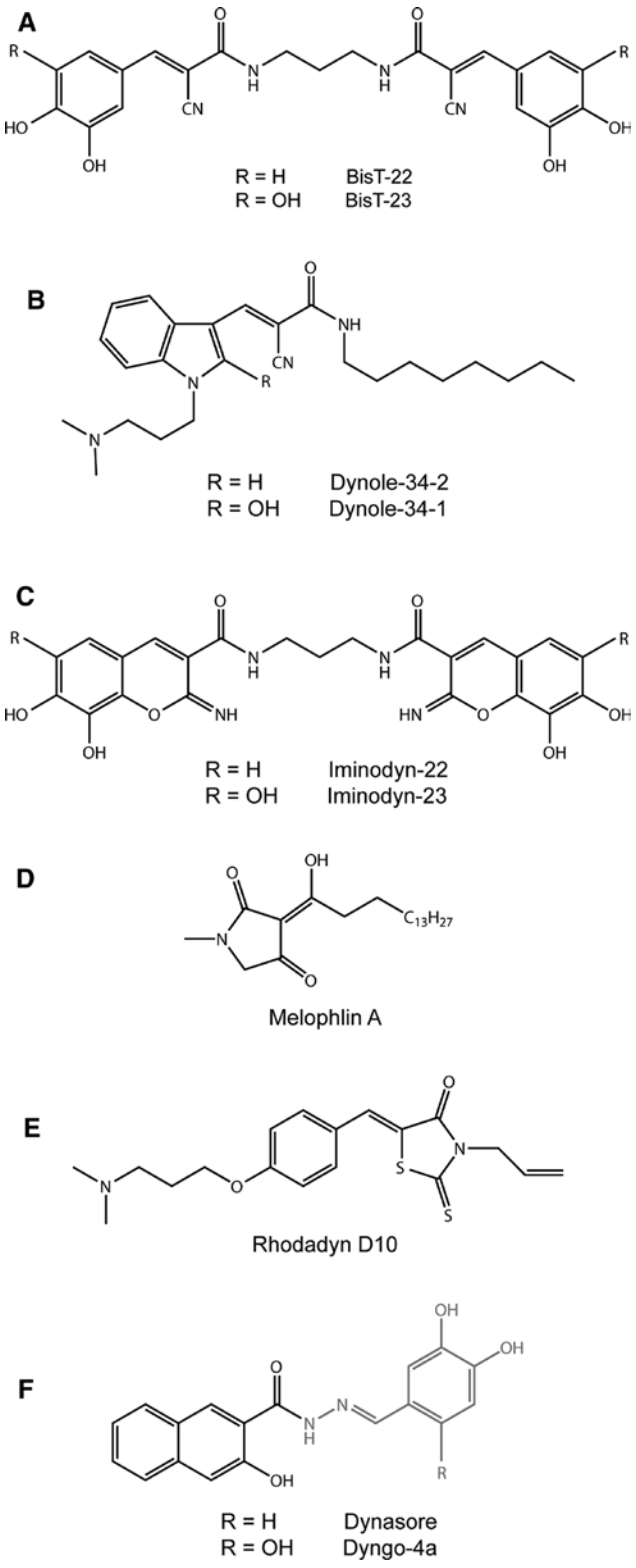

Figure 4: Structural formulae of modulators whose target domain is currently unknown.
The hydroxyphenyl hydrazone moiety of dynasore and dyngo is shown in red in panel (F).

A publication by Gu and co-workers reported that BisT-23 induced the formation of ring-like higher order oligomers of dynamin in the absence of lipid templates (Gu et al., 2014). Rather than inhibiting the GTPase activity of dynamin, as reported earlier (Hill et al., 2005), BisT-23 induced oligomerization stimulated a 6-fold increase of the basal GTPase activity. This increase is hard to reconcile with a closed dynamin ring, as here the GTPase domains would be aligned laterally without the possibility to form catalytically competent *trans* interactions. Furthermore, formation of a closed ring would require an unlikely distortion of the main oligomerization interface, which has been shown to inherently drive the assembly into helical oligomers (Reubold et al., 2015). A more likely scenario is that BisT compounds prompt the formation of lock-washer-like assemblies of dynamin, where the open ends of the ring overlap in a few monomers such that their GTPase domains are arranged in catalytically activating positions. The capacity of BisT-22 and BisT-23 to stimulate oligomerization may stem from stabilization of the oligomerization interface, thus shifting the equilibrium away from the tetramer towards higher order oligomers.

Schiffer et al. showed that BisT-23 is able to improve glomerular filtration in a range of both transient and chronic kidney disease models leading to proteinuria (Schiffer et al., 2015). Common to all proteinuric phenotypes is the effacement of so-called actin-rich foot processes in the podocytes that normally form the glomerular filtration barrier (Trimarchi, 2015). The described rescue effect is supposed to be mediated by BisT-23-induced dynamin oligomerization which in turn modulates the actin cytoskeleton such that foot processes are stabilized and filtration function is restored. A foot process inducing effect of BisT-23 has also been observed by Ono et al. (2017).

The newly discovered oligomerization inducing property of BisT-23 was also exploited in a study about the involvement of dynamin in exocytotic fusion pore expansion (FPE) (Jackson et al., 2015). The authors incubated chromaffin cells with BisT-23, which they renamed to ryngo 1-23 in this context, and measured the release of catecholamines as amperometric spikes. In cells incubated with ryngo 1-23 the number of release events did not change compared to control cells, but the spike charge significantly increased, which corresponds to an increase in the amount of transmitter released per fusion event. Incubation with either dynole 34-2 or dyngo-4a led to a reduction of both the number and intensity of individual releases. The authors hypothesized that stimulation of GTPase activity, ring formation, and actin polymerization by ryngo 1-23 would slow FPE and thus stabilize the fusion pore.

Dynoles

A class of dynamin inhibitors termed dynoles was developed by Hill and co-workers based on an indole scaffold (Hill et al., 2009). The most potent compound, dynole 34-2 (Figure 4B), inhibited the lipid stimulated GTPase activity of dynamin 1 and transferrin uptake in U2OS cells with IC_{50} values of 1.3 and 5.0 μM, respectively. Dynole 34-2, as well as some other dynole variants, were shown to induce multinucleation and to inhibit cytokinesis by arresting the scission step in a diverse panel of human cancer cell lines with subsequent induction of apoptosis, similar to the effects seen for MiTMABs (Joshi et al., 2010). As observed for MiTMABs, nontumorigenic fibroblasts were much less susceptible to the cytotoxic action of the dynoles. Development of a series of second-generation dynoles yielded two compounds with sub-micromolar potency against the GTPase activity of dynamin 1 while displaying greatly reduced cell toxicity (Gordon et al., 2013). Neither the binding site on dynamin nor the mechanism of action of the dynoles has been elucidated. The dynoles of the first series were shown to be uncompetitive for GTP, which is compatible with the proposed binding to an allosteric site (Hill et al., 2009). However, the fact that dynoles decrease the GTPase activity does not necessarily mean that the allosteric site is located in the GTPase domain as suggested by Hill and co-workers. This restriction is valid for all proposed allosteric inhibitors uncompetitive for GTP.

Interestingly, dynole 34-1 has also been recognized as a promising lead compound in a screening study to find substances targeting cancer specific tropomyosin isoforms (Stehn et al., 2013; Currier et al., 2017). In this context the compound was renamed to TR100 and molecular modelling suggested that the compound binds to the C-terminal end of dimers of the tropomyosin Tpm3.1 (Stehn et al., 2013). Binding of TR100 to Tpm3.1 is supposed to prevent the decoration of actin filaments with oligomeric tropomyosin leading to an increased actin depolymerization. The compound exerted marked cytotoxicity towards diverse cancer cell lines but none against liver and heart tissue. TR100 and a novel derivative showed synergistic effects when combined with anti-microtubule agents leading to an arrest in the G2/M phase of the cell cycle (Currier et al., 2017). Since G2/M arrest has been described previously as an effect of dynole 34-2 on cancer cells mediated by inhibition of dynamin 2 (Chircop et al., 2011), it may well be that

the cytotoxicity of dynole 34-1/TR100 exerted via dynamin may have contributed to the effects seen in the tropomyosin study.

Iminodyns

Based on the previously published BisT pharmacophore, Hill and colleagues chose the structurally similar iminochromene scaffold as a replacement for the tyrphostin moiety (Hill et al., 2010). The iminochromenes contain a second aromatic ring that encompasses the position of the nitrile carbon atom in BisT. The nitrile nitrogen of BisT is replaced by an imino group that assumes a similar but not identical position in the iminochromenes. The best inhibitors iminodyns 17, 22, and 23 (Figure 4C) displayed IC_{50} values of 260–450 nM against the lipid-stimulated GTPase activity of dynamin 1. However, cell-based activity against RME (dynamin 2) or SVE (dynamin 1) was much lower with IC_{50} values of 10.7 and 99.5 μM, respectively.

Nevertheless, iminodyn-22 has been used as a tool to elucidate the role of dynamin in several different processes (Ayala-Nunez et al., 2016; Hyndman et al., 2016; Marie-Anais et al., 2016; Muranen et al., 2017). Interestingly, iminodyn-22 was able to restore the actin cytoskeleton in cultured podocytes derived from patients with nephrotic syndrome in a similar manner as BisT-23 (see above) (Muller-Deile et al., 2016).

Melophlin

The naturally occurring tetramic acid derivative melophlin A (Figure 4D) has been found to reverse the phenotype of Ras transformed NIH3T3 cells (Knoth et al., 2009). Through an affinity-based mass spectrometry approach, dynamin was identified as a target of melophlin A. The authors of this study suggested that dynamin inhibition obviates the effect of the Ras transformation downstream of Ras *via* an arrest of endocytic trafficking of MAP kinase kinase, which in turn leads to incomplete activation of ERK1/2. The direct interaction of dynamin and melophlin A was verified by surface plasmon resonance yielding a dissociation constant (K_d) of 0.4 μM for dynamin 1 and 13.8 μM for dynamin 2. Interestingly, also dynamin-related protein 1 was able to bind melophlin A with a K_d of 0.6 μM. Melophlin A did neither inhibit the basal GTPase activity of full-length dynamin 2 nor its Grb2-stimulated GTPase activity, but it reduced endocytic uptake of transferrin by 70% at a concentration of 20 μM.

Rhodadyns

Starting from a selection of substituted rhodanines, Robertson and colleagues developed the rhodadyn series of dynamin inhibitors. Rhodadyn-D10 (Figure 4E), the most potent compound of the rhodadyns, inhibited lipid-stimulated GTPase activity of dynamin 1 with an IC_{50} value of 4.5 μM, as well as receptor-mediated endocytosis with an IC_{50} value of 5.9 μM (Robertson et al., 2012). Several other rhodadyns, like for instance rhodadyn-C8, display a comparable inhibitory action on the lipid stimulated GTPase activity, but the inhibitory potential against receptor-mediated endocytosis is lost. The observed differences in inhibitory action may be explained with differences in cell permeability or in elimination velocity by efflux mechanisms. The inhibitory mechanism is unknown.

Hydroxyphenyl hydrazones

The hydroxyphenyl hydrazone dynasore (Figure 4F) was identified in a high-throughput screen containing ~16 000 compounds directed against the GTPase activity of full-length human dynamin 1 stimulated by addition of the SH3-domain containing protein Grb2 (Macia et al., 2006). Dynasore inhibited the GTPase activity of dynamin assembled under low salt conditions as well as transferrin uptake with IC_{50} values of ~15 μM.

To improve the efficacy of dynasore, Lee and colleagues published a series of derivatives with variations in the substitution pattern of the aromatic rings, yielding two compounds with 3-fold reduced IC_{50} values in the inhibition of dynamin self-assembled via low salt exposure (Lee et al., 2010). From their observations, the authors concluded that the hydroxyl group in position 3 of the naphthol ring is dispensable. In a later study, McCluskey and co-workers reported another series of dynasore derivatives based on the observation that the structure of dynasore is similar to that of the BisT compounds (see above) (McCluskey et al., 2013). The new dynasore derivatives generally displayed strongly decreased cytotoxicity compared to the parent compound. By transferring the hydroxyl substitution patterns of the BisT series onto the phenyl ring of dynasore, they obtained several compounds with markedly improved inhibitory activity. The compound dyngo-4a (Figure 4F) displayed a 32-fold higher inhibitory potency towards the assembly stimulated GTPase activity of dynamin 1 (0.38 μM vs. 12.4 μM) and a seven-fold higher potency in CME (5.7 μM vs. 34.7 μM) compared to dynasore. During their work, the authors noticed a peculiar sensitivity of dynasore towards the detergent Tween-80,

which was included in their GTPase assay buffer. Addition of 0.06% Tween-80 (458 μM) to the assay buffer caused a 40-fold increase of the IC_{50} value for dynasore against the GTPase activity of dynamin 1. Most members of the dyngo series showed very low or no Tween sensitivity at all. The authors suggested that either removal of the hydroxyl group at position 4′ or addition of a 5′-hydroxyl group greatly reduces detergent sensitivity.

The authors of the original dynasore study (Macia et al., 2006) assumed that dynasore targets the GTPase domain and inhibits the GTP hydrolysis reaction in a non-competitive manner, as it not only inhibited assembly stimulated dynamin, but also the basal activity of a GST-fusion of the isolated GTPase domain of human dynamin 2 *in vitro* using purified protein. However, both the binding site and its mechanism of action remain obscure to date. Recently, a novel highly sensitive GTPase assay has been described that relies on the detection of the hydrolysis product GDP via specific antibodies (Mohanakrishnan et al., 2017). The authors found that neither dynasore nor dyngo-4a influenced the basal GTPase activity of unassembled dynamin 1, whereas the GTPase activity stimulated by assembly on lipid nanotubes was inhibited by dynasore or dyngo-4a with moderate IC_{50} values. These observations strongly argue against the GTPase domain as the domain targeted by dynasore/dyngo.

Dynasore and dyngo-4a have been used in numerous studies to inhibit endocytosis dependent processes in various contexts (Hardwick et al., 2017; Hegarty et al., 2017; Reventun et al., 2017; Yuan et al., 2018). However, the specificity of the dynasore and dyngo compounds has been questioned by a study from Pietro De Camilli and co-workers, who found that the compounds inhibited fluid-phase endocytosis and peripheral membrane ruffling in murine dynamin 1-3 triple knockout (TKO) fibroblasts (Park et al., 2013). Recently, Basagiannis and co-workers showed that dynasore inhibited the signaling of vascular endothelial growth factor receptor 2 independently of endocytosis (Basagiannis et al., 2017). Furthermore, dynasore and dyngo-4a were shown to prevent activation of mTORC1 independently of dynamin in dynamin TKO cells via the inhibition of the Ras-like GTPase RagA (Persaud et al., 2018).

A possible explanation for the unspecific effect of Dyngo-4a is provided by inspection of its chemical structure. Dynasore and dyngo-4a are hydroxyphenyl hydrazones (Figure 4F), which belong to a diverse group of substances called pan-assay interference compounds (PAINS). These have been found to frequently generate false positive results in chemical screening assays (Baell and Holloway, 2010; Baell and Walters, 2014). Hydroxyphenyl hydrazones are prone to covalently modify their targets, which may interfere with the function of the targets in an unpredictable manner. Therefore, strong off-target effects of dynasore and dyngo may blur their actual inhibitory action on dynamin. As dynasore and dyngo-4a have been frequently used as tools to mechanistically elucidate subcellular processes, caution in the interpretation of such results and use of an alternative inhibitor may be advisable.

Conclusions

The numerous compound classes that have been described as modulators of dynamin function show a strong emphasis on inhibition. The compounds have been validated using standard assays to probe the clathrin-mediated uptake of fluorescently labeled transferrin in a cell-based setup or to quantify the inhibition of lipid-stimulated GTPase activity of isolated dynamin. However, when interpreting the results of the assays to uncover the mode of action of the modulators, it is imperative to consider that there may be multiple possible points of attack which all may affect the readout of the assays in similar ways. This is in particular important for GTPase assays. Lipid stimulated GTPase activity for instance may be compromised by direct competitive inhibition of the G domain or by blocking the membrane binding site on the PH domain to prevent membrane recruitment. With the current lack of structural information about modulator binding, it might help to assay the GTPase activity of dynamin under different assembly conditions to pinpoint likely modes of action for individual modulator classes. However, for rational improvement of already existing modulator classes, the generation of high resolution co-crystal structures is still highly desirable.

Conflict of interest statement: The authors declare no conflict of interests.

References

Abdel-Hamid, M.K., Macgregor, K.A., Odell, L.R., Chau, N., Mariana, A., Whiting, A., Robinson, P.J., and McCluskey, A. (2015). 1,8-Naphthalimide derivatives: new leads against dynamin I GTPase activity. Org. Biomol. Chem. *13*, 8016–8028.

Antonny, B., Burd, C., De Camilli, P., Chen, E., Daumke, O., Faelber, K., Ford, M., Frolov, V.A., Frost, A., Hinshaw, J.E., et al. (2016). Membrane fission by dynamin: what we know and what we need to know. EMBO J. *35*, 2270–2284.

Ayala-Nunez, N.V., Hoornweg, T.E., van de Pol, D.P., Sjollema, K.A., Flipse, J., van der Schaar, H.M., and Smit, J.M. (2016). How antibodies alter the cell entry pathway of dengue virus particles in macrophages. Sci. Rep. *6*, 28768.

Baell, J.B. and Holloway, G.A. (2010). New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J. Med. Chem. *53*, 2719–2740.

Baell, J. and Walters, M.A. (2014). Chemistry: chemical con artists foil drug discovery. Nature *513*, 481–483.

Basagiannis, D., Zografou, S., Galanopoulou, K., and Christoforidis, S. (2017). Dynasore impairs VEGFR2 signalling in an endocytosis-independent manner. Sci. Rep. *7*, 45035.

Chappie, J.S., Acharya, S., Leonard, M., Schmid, S.L., and Dyda, F. (2010). G domain dimerization controls dynamin's assembly-stimulated GTPase activity. Nature *465*, 435–440.

Chappie, J.S., Mears, J.A., Fang, S., Leonard, M., Schmid, S.L., Milligan, R.A., Hinshaw, J.E., and Dyda, F. (2011). A pseudoatomic model of the dynamin polymer identifies a hydrolysis-dependent powerstroke. Cell *147*, 209–222.

Chircop, M., Perera, S., Mariana, A., Lau, H., Ma, M.P., Gilbert, J., Jones, N.C., Gordon, C.P., Young, K.A., Morokoff, A., et al. (2011). Inhibition of dynamin by dynole 34-2 induces cell death following cytokinesis failure in cancer cells. Mol. Cancer. Ther. *10*, 1553–1562.

Cook, T.A., Urrutia, R., and McNiven, M.A. (1994). Identification of dynamin 2, an isoform ubiquitously expressed in rat tissues. Proc. Natl. Acad. Sci. USA *91*, 644–648.

Currier, M.A., Stehn, J.R., Swain, A., Chen, D., Hook, J., Eiffe, E., Heaton, A., Brown, D., Nartker, B.A., Eaves, D.W., et al. (2017). Identification of cancer-targeted tropomyosin inhibitors and their synergy with microtubule drugs. Mol. Cancer. Ther. *16*, 1555–1565.

Daniel, J.A., Malladi, C.S., Kettle, E., McCluskey, A., and Robinson, P.J. (2012). Analysis of synaptic vesicle endocytosis in synaptosomes by high-content screening. Nat. Protoc. *7*, 1439–1455.

Daniel, J.A., Chau, N., Abdel-Hamid, M.K., Hu, L., von Kleist, L., Whiting, A., Krishnan, S., Maamary, P., Joseph, S.R., Simpson, F., et al. (2015). Phenothiazine-derived antipsychotic drugs inhibit dynamin and clathrin-mediated endocytosis. Traffic *16*, 635–654.

Destaing, O., Ferguson, S.M., Grichine, A., Oddou, C., De Camilli, P., Albiges-Rizo, C., and Baron, R. (2013). Essential function of dynamin in the invasive properties and actin architecture of v-Src induced podosomes/invadosomes. PLoS One *8*, e77956.

Ferguson, S.M. and De Camilli, P. (2012). Dynamin, a membrane-remodelling GTPase. Nat. Rev. Mol. Cell Biol. *13*, 75–88.

Ferguson, S.M., Brasnjo, G., Hayashi, M., Wolfel, M., Collesi, C., Giovedi, S., Raimondi, A., Gong, L.W., Ariel, P., Paradise, S., et al. (2007). A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. Science *316*, 570–574.

Fisher, J.E., Rogers, M.J., Halasy, J.M., Luckman, S.P., Hughes, D.E., Masarachia, P.J., Wesolowski, G., Russell, R.G., Rodan, G.A., and Reszka, A.A. (1999). Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation *in vitro*. Proc. Natl. Acad. Sci. USA *96*, 133–138.

Gazit, A., Chen, J., App, H., McMahon, G., Hirth, P., Chen, I., and Levitzki, A. (1996). Tyrphostins IV – highly potent inhibitors of EGF receptor kinase. Structure-activity relationship study of 4-anilidoquinazolines. Bioorg. Med. Chem. *4*, 1203–1207.

Gordon, C.P., Venn-Brown, B., Robertson, M.J., Young, K.A., Chau, N., Mariana, A., Whiting, A., Chircop, M., Robinson, P.J., and McCluskey, A. (2013). Development of second-generation indole-based dynamin GTPase inhibitors. J. Med. Chem. *56*, 46–59.

Gu, C., Yaddanapudi, S., Weins, A., Osborn, T., Reiser, J., Pollak, M., Hartwig, J., and Sever, S. (2010). Direct dynamin-actin interactions regulate the actin cytoskeleton. EMBO J. *29*, 3593–3606.

Gu, C., Chang, J., Shchedrina, V.A., Pham, V.A., Hartwig, J.H., Suphamungmee, W., Lehman, W., Hyman, B.T., Bacskai, B.J., and Sever, S. (2014). Regulation of dynamin oligomerization in cells: the role of dynamin–actin interactions and its GTPase activity. Traffic *15*, 819–838.

Halasy-Nagy, J.M., Rodan, G.A., and Reszka, A.A. (2001). Inhibition of bone resorption by alendronate and risedronate does not require osteoclast apoptosis. Bone *29*, 553–559.

Hardwick, J.C., Clason, T.A., Tompkins, J.D., Girard, B.M., Baran, C.N., Merriam, L.A., May, V., and Parsons, R.L. (2017). Recruitment of endosomal signaling mediates the forskolin modulation of guinea pig cardiac neuron excitability. Am. J. Physiol. Cell. Physiol. *313*, C219–C227.

Harper, C.B., Martin, S., Nguyen, T.H., Daniels, S.J., Lavidis, N.A., Popoff, M.R., Hadzic, G., Mariana, A., Chau, N., McCluskey, A., et al. (2011). Dynamin inhibition blocks botulinum neurotoxin type A endocytosis in neurons and delays botulism. J. Biol. Chem. *286*, 35966–35976.

Harper, C.B., Popoff, M.R., McCluskey, A., Robinson, P.J., and Meunier, F.A. (2013). Targeting membrane trafficking in infection prophylaxis: dynamin inhibitors. Trends Cell Biol. *23*, 90–101.

Hegarty, S.V., Sullivan, A.M., and O'Keeffe, G.W. (2017). Endocytosis contributes to BMP2-induced Smad signalling and neuronal growth. Neurosci. Lett. *643*, 32–37.

Hill, T.A., Odell, L.R., Quan, A., Abagyan, R., Ferguson, G., Robinson, P.J., and McCluskey, A. (2004). Long chain amines and long chain ammonium salts as novel inhibitors of dynamin GTPase activity. Bioorg. Med. Chem. Lett. *14*, 3275–3278.

Hill, T., Odell, L.R., Edwards, J.K., Graham, M.E., McGeachie, A.B., Rusak, J., Quan, A., Abagyan, R., Scott, J.L., Robinson, P.J., et al. (2005). Small molecule inhibitors of dynamin I GTPase activity: development of dimeric tyrphostins. J. Med. Chem. *48*, 7781–7788.

Hill, T.A., Gordon, C.P., McGeachie, A.B., Venn-Brown, B., Odell, L.R., Chau, N., Quan, A., Mariana, A., Sakoff, J.A., Chircop, M., et al. (2009). Inhibition of dynamin mediated endocytosis by the dynoles – synthesis and functional activity of a family of indoles. J. Med. Chem. *52*, 3762–3773.

Hill, T.A., Mariana, A., Gordon, C.P., Odell, L.R., Robertson, M.J., McGeachie, A.B., Chau, N., Daniel, J.A., Gorgani, N.N., Robinson, P.J., et al. (2010). Iminochromene inhibitors of dynamins I and II GTPase activity and endocytosis. J. Med. Chem. *53*, 4094–4102.

Hyndman, K.A., Arguello, A.M., Morsing, S.K., and Pollock, J.S. (2016). Dynamin-2 is a novel NOS1beta interacting protein and negative regulator in the collecting duct. Am. J. Physiol. Regul. Integr. Comp. Physiol. *310*, R570–R577.

Jackson, J., Papadopulos, A., Meunier, F.A., McCluskey, A., Robinson, P.J., and Keating, D.J. (2015). Small molecules demonstrate the role of dynamin as a bi-directional regulator of the

exocytosis fusion pore and vesicle release. Mol. Psychiatry *20*, 810–819.

Joshi, S., Perera, S., Gilbert, J., Smith, C.M., Mariana, A., Gordon, C.P., Sakoff, J.A., McCluskey, A., Robinson, P.J., Braithwaite, A.W., et al. (2010). The dynamin inhibitors MiTMAB and OcTMAB induce cytokinesis failure and inhibit cell proliferation in human cancer cells. Mol. Cancer Ther. *9*, 1995–2006.

Kessels, M.M., Engqvist-Goldstein, A.E., Drubin, D.G., and Qualmann, B. (2001). Mammalian Abp1, a signal-responsive F-actin-binding protein, links the actin cytoskeleton to endocytosis via the GTPase dynamin. J. Cell Biol. *153*, 351–366.

Knoth, T., Warburg, K., Katzka, C., Rai, A., Wolf, A., Brockmeyer, A., Janning, P., Reubold, T.F., Eschenburg, S., Manstein, D.J., et al. (2009). The Ras pathway modulator melophlin A targets dynamins. Angew. Chem. Int. Ed. *48*, 7240–7245.

Krueger, E.W., Orth, J.D., Cao, H., and McNiven, M.A. (2003). A dynamin-cortactin-Arp2/3 complex mediates actin reorganization in growth factor-stimulated cells. Mol. Biol. Cell *14*, 1085–1096.

Lee, S., Jung, K.Y., Park, J., Cho, J.H., Kim, Y.C., and Chang, S. (2010). Synthesis of potent chemical inhibitors of dynamin GTPase. Bioorg. Med. Chem. Lett. *20*, 4858–4864.

Levitzki, A. and Gilon, C. (1991). Tyrphostins as molecular tools and potential antiproliferative drugs. Trends Pharmacol. Sci. *12*, 171–174.

Liu, Y.W., Surka, M.C., Schroeter, T., Lukiyanchuk, V., and Schmid, S.L. (2008). Isoform and splice-variant specific functions of dynamin-2 revealed by analysis of conditional knock-out cells. Mol. Biol. Cell *19*, 5347–5359.

Lu, J., Helton, T.D., Blanpied, T.A., Racz, B., Newpher, T.M., Weinberg, R.J., and Ehlers, M.D. (2007). Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. Neuron *55*, 874–889.

MacGregor, K.A., Abdel-Hamid, M.K., Odell, L.R., Chau, N., Whiting, A., Robinson, P.J., and McCluskey, A. (2014a). Development of quinone analogues as dynamin GTPase inhibitors. Eur. J. Med. Chem. *85*, 191–206.

MacGregor, K.A., Robertson, M.J., Young, K.A., von Kleist, L., Stahlschmidt, W., Whiting, A., Chau, N., Robinson, P.J., Haucke, V., and McCluskey, A. (2014b). Development of 1,8-naphthalimides as clathrin inhibitors. J. Med. Chem. *57*, 131–143.

Macia, E., Ehrlich, M., Massol, R., Boucrot, E., Brunner, C., and Kirchhausen, T. (2006). Dynasore, a cell-permeable inhibitor of dynamin. Dev. Cell *10*, 839–850.

Maraka, S. and Kennel, K.A. (2015). Bisphosphonates for the prevention and treatment of osteoporosis. Br. Med. J. *351*, h3783.

Marie-Anais, F., Mazzolini, J., Herit, F., and Niedergang, F. (2016). Dynamin–actin cross talk contributes to phagosome formation and closure. Traffic *17*, 487–499.

Masaike, Y., Takagi, T., Hirota, M., Yamada, J., Ishihara, S., Yung, T.M., Inoue, T., Sawa, C., Sagara, H., Sakamoto, S., et al. (2010). Identification of dynamin-2-mediated endocytosis as a new target of osteoporosis drugs, bisphosphonates. Mol. Pharmacol. *77*, 262–269.

McCluskey, A., Daniel, J.A., Hadzic, G., Chau, N., Clayton, E.L., Mariana, A., Whiting, A., Gorgani, N.N., Lloyd, J., Quan, A., et al. (2013). Building a better dynasore: the dyngo compounds potently inhibit dynamin and endocytosis. Traffic *14*, 1272–1289.

McGeachie, A.B., Odell, L.R., Quan, A., Daniel, J.A., Chau, N., Hill, T.A., Gorgani, N.N., Keating, D.J., Cousin, M.A., van Dam, E.M., et al. (2013). Pyrimidyn compounds: dual-action small molecule pyrimidine-based dynamin inhibitors. ACS Chem. Biol. *8*, 1507–1518.

McNiven, M.A., Kim, L., Krueger, E.W., Orth, J.D., Cao, H., and Wong, T.W. (2000). Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape. J. Cell Biol. *151*, 187–198.

Meng, J. (2017). Distinct functions of dynamin isoforms in tumorigenesis and their potential as therapeutic targets in cancer. Oncotarget *8*, 41701–41716.

Mohanakrishnan, A., Tran, T.V.M., Kumar, M., Chen, H., Posner, B.A., and Schmid, S.L. (2017). A highly-sensitive high throughput assay for dynamin's basal GTPase activity. PLoS One *12*, e0185639.

Muller-Deile, J., Teng, B., Schenk, H., Haller, H., Reiser, J., Sever, S., and Schiffer, M. (2016). Drugs targeting dynamin can restore cytoskeleton and focal contact alterations of urinary podocytes derived from patients with nephrotic syndrome. Ann. Transl. Med. *4*, 439.

Muranen, T., Iwanicki, M.P., Curry, N.L., Hwang, J., DuBois, C.D., Coloff, J.L., Hitchcock, D.S., Clish, C.B., Brugge, J.S., and Kalaany, N.Y. (2017). Starved epithelial cells uptake extracellular matrix for survival. Nat. Commun. *8*, 13989.

Nakata, T., Iwamoto, A., Noda, Y., Takemura, R., Yoshikura, H., and Hirokawa, N. (1991). Predominant and developmentally regulated expression of dynamin in neurons. Neuron *7*, 461–469.

Odell, L.R., Chau, N., Mariana, A., Graham, M.E., Robinson, P.J., and McCluskey, A. (2009). Azido and diazarinyl analogues of bis-tyrphostin as asymmetrical inhibitors of dynamin GTPase. ChemMedChem *4*, 1182–1188.

Odell, L.R., Howan, D., Gordon, C.P., Robertson, M.J., Chau, N., Mariana, A., Whiting, A.E., Abagyan, R., Daniel, J.A., Gorgani, N.N., et al. (2010). The pthaladyns: GTP competitive inhibitors of dynamin I and II GTPase derived from virtual screening. J. Med. Chem. *53*, 5267–5280.

Odell, L.R., Abdel-Hamid, M.K., Hill, T.A., Chau, N., Young, K.A., Deane, F.M., Sakoff, J.A., Andersson, S., Daniel, J.A., Robinson, P.J., et al. (2017). Pyrimidine-based inhibitors of dynamin I GTPase activity: competitive inhibition at the pleckstrin homology domain. J. Med. Chem. *60*, 349–361.

Ono, S., Kume, S., Yasuda-Yamahara, M., Yamahara, K., Takeda, N., Chin-Kanasaki, M., Araki, H., Sekine, O., Yokoi, H., Mukoyama, M., et al. (2017). O-linked β-N-acetylglucosamine modification of proteins is essential for foot process maturation and survival in podocytes. Nephrol. Dial. Transplant. *32*, 1477–1487.

Orth, J.D., Krueger, E.W., Cao, H., and McNiven, M.A. (2002). The large GTPase dynamin regulates actin comet formation and movement in living cells. Proc. Natl. Acad. Sci. USA *99*, 167–172.

Otomo, M., Takahashi, K., Miyoshi, H., Osada, K., Nakashima, H., and Yamaguchi, N. (2008). Some selective serotonin reuptake inhibitors inhibit dynamin I guanosine triphosphatase (GTPase). Biol. Pharm. Bull. *31*, 1489–1495.

Park, R.J., Shen, H., Liu, L., Liu, X., Ferguson, S.M., and De Camilli, P. (2013). Dynamin triple knockout cells reveal off target effects of commonly used dynamin inhibitors. J. Cell. Sci. *126*, 5305–5312.

Persaud, A., Cormerais, Y., Pouyssegur, J., and Rotin, D. (2018). Dynamin inhibitors block activation of mTORC1 by amino acids independently of dynamin. J. Cell Sci. *131*, jcs211755.

Quan, A., McGeachie, A.B., Keating, D.J., van Dam, E.M., Rusak, J., Chau, N., Malladi, C.S., Chen, C., McCluskey, A., Cousin, M.A., et al. (2007). Myristyl trimethyl ammonium bromide and octadecyl trimethyl ammonium bromide are surface-active small molecule dynamin inhibitors that block endocytosis mediated by dynamin I or dynamin II. Mol. Pharmacol. *72*, 1425–1439.

Reems, J.A., Wang, W., Tsubata, K., Abdurrahman, N., Sundell, B., Tijssen, M.R., van der Schoot, E., Di Summa, F., Patel-Hett, S., Italiano, Jr., J., et al. (2008). Dynamin 3 participates in the growth and development of megakaryocytes. Exp. Hematol. *36*, 1714–1727.

Reszka, A.A. and Rodan, G.A. (2003). Mechanism of action of bisphosphonates. Curr. Osteoporos. Rep. *1*, 45–52.

Reubold, T.F., Faelber, K., Plattner, N., Posor, Y., Ketel, K., Curth, U., Schlegel, J., Anand, R., Manstein, D.J., Noe, F., et al. (2015). Crystal structure of the dynamin tetramer. Nature *525*, 404–408.

Reventun, P., Alique, M., Cuadrado, I., Marquez, S., Toro, R., Zaragoza, C., and Saura, M. (2017). iNOS-derived nitric oxide induces integrin-linked kinase endocytic lysosome-mediated degradation in the vascular endothelium. Arterioscler. Thromb. Vasc. Biol. *37*, 1272–1281.

Robertson, M.J., Hadzic, G., Ambrus, J., Pome, D.Y., Hyde, E., Whiting, A., Mariana, A., von Kleist, L., Chau, N., Haucke, V., et al. (2012). The rhodadyns, a new class of small molecule inhibitors of dynamin GTPase activity. ACS Med. Chem. Lett. *3*, 352–356.

Schafer, D.A., Weed, S.A., Binns, D., Karginov, A.V., Parsons, J.T., and Cooper, J.A. (2002). Dynamin2 and cortactin regulate actin assembly and filament organization. Curr. Biol. *12*, 1852–1857.

Schiffer, M., Teng, B., Gu, C., Shchedrina, V.A., Kasaikina, M., Pham, V.A., Hanke, N., Rong, S., Gueler, F., Schroder, P., et al. (2015). Pharmacological targeting of actin-dependent dynamin oligomerization ameliorates chronic kidney disease in diverse animal models. Nat. Med. *21*, 601–609.

Sever, S., Damke, H., and Schmid, S.L. (2000). Garrotes, springs, ratchets, and whips: putting dynamin models to the test. Traffic *1*, 385–392.

Stehn, J.R., Haass, N.K., Bonello, T., Desouza, M., Kottyan, G., Treutlein, H., Zeng, J., Nascimento, P.R., Sequeira, V.B., Butler, T.L., et al. (2013). A novel class of anticancer compounds targets the actin cytoskeleton in tumor cells. Cancer Res. *73*, 5169–5182.

Stowell, M.H., Marks, B., Wigge, P., and McMahon, H.T. (1999). Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring. Nat. Cell Biol. *1*, 27–32.

Takahashi, K., Miyoshi, H., Otomo, M., Osada, K., Yamaguchi, N., and Nakashima, H. (2010). Suppression of dynamin GTPase activity by sertraline leads to inhibition of dynamin-dependent endocytosis. Biochem. Biophys. Res. Commun. *391*, 382–387.

Trimarchi, H. (2015). Podocyturia: What is in a name? J. Transl. Int. Med. *3*, 51–56.

van Lessen, M., Shibata-Germanos, S., van Impel, A., Hawkins, T.A., Rihel, J., and Schulte-Merker, S. (2017). Intracellular uptake of macromolecules by brain lymphatic endothelial cells during zebrafish embryonic development. eLife *6*, e25932.

Wang, W., Gilligan, D.M., Sun, S., Wu, X., and Reems, J.A. (2011). Distinct functional effects for dynamin 3 during megakaryocytopoiesis. Stem Cells Dev. *20*, 2139–2151.

Witke, W., Podtelejnikov, A.V., Di Nardo, A., Sutherland, J.D., Gurniak, C.B., Dotti, C., and Mann, M. (1998). In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. EMBO J. *17*, 967–976.

Yaish, P., Gazit, A., Gilon, C., and Levitzki, A. (1988). Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. Science *242*, 933–935.

Yamada, H., Abe, T., Satoh, A., Okazaki, N., Tago, S., Kobayashi, K., Yoshida, Y., Oda, Y., Watanabe, M., Tomizawa, K., et al. (2013). Stabilization of actin bundles by a dynamin 1/cortactin ring complex is necessary for growth cone filopodia. J. Neurosci. *33*, 4514–4526.

Yuan, M., Yan, J., Xun, J., Chen, C., Zhang, Y., Wang, M., Chu, W., Song, Z., Hu, Y., Zhang, S., et al. (2018). Enhanced human enterovirus 71 infection by endocytosis inhibitors reveals multiple entry pathways by enterovirus causing hand-foot-and-mouth diseases. Virol. J. *15*, 1-017-0913-3.

Zhang, J., Lawrance, G.A., Chau, N., Robinson, P.J., and McCluskey, A. (2008). From Spanish fly to room-temperature ionic liquids (RTILs): synthesis, thermal stability and inhibition of dynamin 1 GTPase by a novel class of RTILs. N. J. Chem. *32*, 28–36.

Zhang, Y., Wang, Q.C., Han, J., Cao, R., Cui, X.S., Kim, N.H., Rui, R., and Sun, S.C. (2014). Involvement of dynamin 2 in actin-based polar-body extrusion during porcine oocyte maturation. Mol. Reprod. Dev. *81*, 725–734.

Zimmermann, S., Hall, L., Riley, S., Sorensen, J., Amaro, R.E., and Schnaufer, A. (2016). A novel high-throughput activity assay for the *Trypanosoma brucei* editosome enzyme REL1 and other RNA ligases. Nucleic Acids Res. *44*, e24.