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Title: Dynamin Functions and Ligands: Classical Mechanisms Behind

Running title: Dynamin Function and Ligands

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Title: Dynamin Functions and Ligands: Classical Mechanisms Behind

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List of non-standard abbreviations: 78

Aβ	Amyloid beta
ABP1	Actin binding protein 1
ATP	Adenosine triphosophate
AD	Alzheimer's disease
ADBE	Activity dependent bulk endocytosis
ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP-2	Adaptor protein-2
APP	Amyloid precursor protein
ATPase	Adenosine triphosphatase
BACE 1	Beta-site APP cleaving enzyme 1
BAR	Bin/Amphiphysin/Rvs
BDNF	Brain derived neurotrophic factor
BSE	Bundle signaling element
CaMK1	Calcium/ calmodulin-dependent protein kinase
CCP	Clathrin coated pit
CDK1	Cyclin dependent kinase 1
CKD	Chronic kidney disease
CME	Clathrin-mediated endocytosis
CMRI	Children's medical research institute

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CMT	Charcot-Marie-Tooth disease
CNM	Centronuclear myopathy
CypD	Cyclophylin D
DNM1	Dynamin 1
DNM2	Dynamin 2
DNM3	Dynamin 3
DNMBP	Dynamin binding protein
DLPs	Dynamin like proteins
DOA	Dominant optic atrophy
Drp	Dynamin related protein
Drp1	Dynamin related protein 1
DS	Down syndrome
DTNBP1	Dytsrobrevin-binding protein 1
DYRK1A	Dual specificity tyrosine-(Y)-phosphorylation regulated kinase 1A
FA	Focal adhesion
Fis1	Fission 1
GABA	Gamma-aminobutyric acid
GAP	GTPase activating protein
GDP	Guanosine diphosphate
GED	GTPase effector domain
GEF	Guanosine exchange factor
GTPase	Guanosine triphosphatase

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GTP	Guanidine triphosphate
HCC	Hepatocellular carcinoma
HD	Huntington's disease
HF	Heart failure
Htt	Huntington's protein
htt	Huntington's gene
IR	Ischemia reperfusion
LDL	Low density lipoprotein
LOAD	Late-onset of Alzheimer's disease
LRRK2	Leucine-rich repeat kinase 2
LV	Left ventricle
LVEFF	Left ventricle ejection fraction failure
LVHF	Left ventricle heart failure
MD	Middle Domain
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
mHtt	Mutant Huntington's protein
mRNA	Mitochondrial ribonucleic acid
MRP2	Myotubularin-related protein 2
MQC	Mitochondrial quality control
NMDA	N-methyl-D-Aspartate
OPA1	Mitochondrial dynamin like GTPase

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PCA	Prostate cancer
PD	Parkinson's disease
PHD	Pleckstrin homology domain
PKA	Protein kinase A
PRD	Proline rich domain
PI(3)P	Phosphatidylinositol-3-phosphate
PIP	Phosphatidylinositol phosphate
PIP_2	Phosphatidylinositol-4-5-bisphosphate
RCAN1	Regulator of calcineurin 1
RGC	Retinal ganglionic cell
RME	Receptor mediated endocytosis
RRP	Readily releasable pool
SH3D	SRC homology 3 domain
SUMO	Small ubiquitin-like modifier
UPS	Ubiquitin proteasomal system

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Title: Dynamin Functions and Ligands: Classical Mechanism Responsible

Abstract:

Dynamin is a GTPase that plays a vital role in clathrin-dependent endocytosis and other vesicular trafficking processes by acting as a pair of molecular scissors for newly formed vesicles originating from the plasma membrane. Dynamins and related proteins are important components for cleavage of clathrin-coated vesicles, phagosomes, mitochondria, etc. These proteins help in organelle division, viral resistance, and mitochondrial fusion/fission. Dysfunction and mutations in dynamin have been implicated in the pathophysiology of various disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Charcot-Marie-Tooth disease (CMT), Heart failure (HF), schizophrenia, epilepsy, cancer, Dominant optic atrophy (DOA), osteoporosis, Down’s syndrome (DS), etc. This review is an attempt to illustrate the dynamin related mechanisms involved in above mentioned disorders and to help medicinal chemists to design novel dynamin ligands, which could be useful in the treatment of dynamin-related disorders.

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1. Introduction:

Dynamins were originally discovered in the brain and identified as microtubule binding partners. Dynamin is a 100kDa protein macromolecule, belonging to the superfamily of GTPases, which plays a major role in synaptic vesicle transport. Members of dynamin family are found throughout the eukaryotic kingdom. Dynamin family includes, Dynamin 1, Dynamin 2 and Dynamin 3 are known as classical dynamins. Each of these dynamins has five different domains which are as follows: large N-terminal GTPase domain, middle domain (MD), pleckstrin homology domain (PH), GTPase effector domain (GED) and proline-rich domain (PRD), (Heymann and Hinshaw 2009)

Dynamins other than classical dynamins, come under the category of Dynamin-like proteins (DLPs), which lacks PH and PRD domains and assist in recruiting classical dynamins to cleave the vesicles. It has been observed that membrane fission involves the activities of dynamins and guanosine triphosphate (GTP). GTPase-activating proteins (GAPs) facilitate hydrolysis of GTP into GDP with the help of GTPases while guanine nucleotide exchange factors (GEFs) displace the generated GDP, thus favoring the next cycle of hydrolysis. The GTP-hydrolysis-dependent conformational change of GTPase dynamin assists in membrane fission, leading to generation of endocytic vesicles (Ferguson and De Camilli, 2012), (Praefcke and McMahon, 2004). Dynamin has been correlated to the pathophysiology of various disorders such as Alzheimer's, Parkinson's, Huntington's, CharcotMarie-Tooth disease, Heart failure, schizophrenia, epilepsy, cancer, optic atrophy, Down’s syndrome, osteoporosis etc.

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Domains of a dynamin

Dynamin and GTP together boost membrane fission by GTP hydrolysis and rapid displacement of dynamin from the membrane surface. The distinguishing architectural features which are common to all dynamins and are distinct from other GTPases include the 300 amino acid large GTPase domain or the globular G-domain along with the presence of two additional domains; the middle domain and the GTPase effector domain. The globular G-domain is composed of a central core that extends from a six-stranded-beta-sheet to an eight-stranded one by addition of 55 amino acids and this domain is necessary for guanine nucleotide binding, resulting in hydrolysis (Reubold *et al.*, 2005). Two more characteristic sequences of dynamin super-family are middle domain and a C-terminal GTPase effector domain which together constitute two distinct domains as stalk and bundle signaling elements (BSE). BSE consists of three helices located on the N and C terminus of GTPase domain and C-terminus of the GED. The BSE conveys nucleotide-dependent conformational changes from the GTPase domain to the stalk and control membrane activity in membrane fission. The stalk is a combination of MD and the amino terminal portion of GED. Depending on the information received from BSE, stalk mediates dimerization and tetramerization, and results in formation of rings and spirals (Wenger *et al.*, 2013). The MD and GED are linked by PH domain which is limited to classical dynamins, interacts directly with membrane bilayer (Ramachandran, 2011) (Klein *et al.*, 1998) (Faelber *et al.*, 2012). This domain is highly conserved and essential for dynamin functioning. It helps in binding to the negatively charged head group of phosphatidylinositol-4-5-biphosphate (PIP_2), a membrane lipid, plays role in clathrin-mediated endocytosis (CME). Mutations in the MD, R361S, R399A or in the GED, I690K, in human Dynamin 1, result in defective dimerization.

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If these mutations occur in the stalk domain, they yield abnormally stable dynamin polymers resistant to disassembly and disturb the process of GTP hydrolysis. The PRD has a binding site for SRC Homology 3 (SH3) domain, present in various dynamin-related proteins such as amphiphysin, endophilin, and syndapin. Thus, amphiphysin, endophilin, and syndapin serve as binding partners of classical dynamins. The PRD is involved in interaction with SH3 domain and thus multiple dynamins are engaged in making a network with protein carrying SH3 domains (Urrutia *et al.*, 1997) (Ford *et al.*, 2011).

Fig:1

Mechanics of fission: Assembly and polymerization

Purified dynamin always assembles as a spiral structure supporting the hypothesis that dynamin wraps around necks of nascent vesicles. Techniques, like gel filtration and electron microscopy, have found a large molecular weight helical structure (50nm wide and few NM long) that is in accordance with the proposed hypothesis (Hinshaw, 2000). When purified dynamins are added to negatively charged liposomes and supplemented with PIP2, they polymerize into helical polymers encircling membrane tubules with increased diameter. On the constriction of the helix, the radius of the neck could be reduced at a level, at which the membrane would fuse onto itself and break. Despite lipid membranes being highly flexible, attaining high curvatures requires a large force. Dynamin works against the force which is generated because of membrane elasticity and lipoidal nature. Lipid bilayers are auto-sealable, as the pore opens, it spontaneously closes, and only tension higher than 10^{-3}-10^{-2} N/m can rupture the membrane. These membranes are as resistant to stress as rubber. Obviously, the auto-sealable property of lipid bilayers makes them difficult to break, thus, seen from a membrane mechanics perspective, membrane fission is far from being a spontaneous process (Pelkmans and Helenius, 2002). Dynamin binds to PIP2

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through its PH domain and to negatively charged lipids through its positive residues. When a dynamin binds to the vesicle membrane, the PH domain orients towards the inner part of the helix and polymerization drives membrane flow inside the helix, due to which the membrane gets constrained (50nm to 20nm) by dynamin coat. Elasticity of membrane competes with the rigidity of dynamin coat. Kinetics of dynamin fission depends on bending rigidity, tension, and constriction torque (Morlot *et al.*, 2012).

During polymerization the force generated is responsible for the deformation of the membrane, which can be measured with optical tweezers using single membrane tubule. In vitro study indicates that dynamin is strong enough to curve the membrane and the late arrival of dynamin at curvature shows that it is recruited when the curvature starts forming. Amphiphysin and endophilin are supposed to recruit dynamin at the neck of clathrin coated pits (CCPs). The assemblage of dynamins at curvatures depends on various factors, such as negatively charged membranes, PIP2, the initial curvature of the membrane, pH level, and salt concentration (Schmid and Frolov, 2011).

Hydrolysis and conformational changes:

The GTPase domain of dynamin structurally similar to the ATPase domain of kinesin, is responsible for hydrolyzing GTP molecules through GTPase activity (Song *et al.*, 2004). A GTP binding motif known as switch-1 allows the GTPase domain to directly position itself in the most favorable hydrolytic conformation where positioning depends on interactions with other GTPase domains. Dynamin monomers do not work cooperatively meaning each monomer burns its GTP independently. The conformational change associated with GTP hydrolysis has been partially elucidated in the case of dynamin. GTP hydrolysis modulates the helical structure of dynamin

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and constriction can reduce the radius of the membrane in its helix which is opposed by the elasticity of the membrane (Roux *et al.*, 2006). Dynamin generates rotational force, during constriction, producing a conformational change which can be evaluated. Torque required for one turn of dynamin to constrict a membrane tube from a 10NM radius (radius of non-constricted dynamin) to a 5NM radius (constricted radius in the presence of GMP-PCP), by Canham-Helfrich theory, and values close to 500 pN.NM have been found (Lenz *et al.*, 2009). (Morlot and Roux, 2013).

Mechanism of membrane fission:

Mechanism of membrane fission by dynamins has always been a subject of debate and has been analyzed in living cells, broken cells and artificial lipid bilayers. For fission, pinches off, poppase and molecular switch model as three mechanisms have been described. In pinches off model, dynamin acts as a mechanoenzyme where it pinches the budding vesicle by hydrolyzing the bound GTP to GDP, whereas in poppase model, stretch-like a spring with the help of GTP hydrolysis. In molecular switch model, dynamin recruits other proteins which trigger the fission (Roux *et al.*, 2006). Dynamin spirals around the neck of the nascent vesicle and GTPase domain causes it to constrict by performing twisting or stretching action that promotes membrane fission, also termed as constriction mechanism. This mechanism is based on the capacity of dynamin to form a self- assembly as helical polymer around the membrane, followed by constriction upon GTP hydrolysis and finally leading to fission. It was suggested that the membrane could be broken by the rapid extension of the helix, tearing off the neck. The spring model relies on the speed of extension i.e. if the dynamin helix extends faster than membrane can flow, then the membrane ruptures else the membrane flows into the cylindrical volume of the helix and adjusts

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to the new conformation of the polymer without breaking. Thus, fission would occur if constriction were faster than the viscoelastic time of lipid membranes (Danino *et al.*, 2004). This whole process is additionally assisted by actin or myosin motors. Heterogeneous lipid distribution to both the sides of constriction increases line tension (Lee and De Camilli, 2002). The dynamin helix constriction has been shown by electron microscopy, biochemical, structural, and biophysical data. This constriction is necessary, but not sufficient for fission, and membrane elastic parameters have an opposite role in constriction (Roux *et al.*, 2010). Other partners such as actin, involved in many fission reactions, could help to control membrane tension. Dynamin has many partners that have a role in membrane remodeling. The future goal is to understand the combined effects of dynamin and its partners involved in fission via constriction (Lenz *et al.*, 2009), (Sweitzer and Hinshaw, 1998).

Dynamin and Endocytosis:

Endocytosis is characterized by internalization of molecules from cell surface to the internal cellular compartment. Vesicular trafficking can either be clathrin-mediated or clathrin-independent. Clathrin pathway is a well-established mechanism of internalization of pathogens, nutrients, various growth factors, neurotransmitters etc. Soluble clathrin from cytoplasm reaches the plasma membrane where it assembles as a lattice and coat the pits which, are finally pinched off from the plasma membrane with the help of dynamin. Clathrin binding adaptors such as adaptor protein-2 (AP-2) bind to cargo vesicles, help in forming a clathrin coat around the vesicles and mediates endocytosis. PIP_2 also facilitates vesicle formation and budding through epsin, a clathrin adaptor. The coated vesicles fuse with endosomes after endocytosis and the vesicles are either recycled or degraded by lysosomes (Kozlov, 1999),(Lenz *et al.*, 2009).

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Dynamin interacts with a number of SH3 domain containing proteins or dynamin binding partners, during the endocytic process through its C-terminal proline-rich domain (PRD). These dynamin-binding partners are intersectin, amphiphysin and endophilin (Sundborger and Hinshaw, 2014). Out of binding partners endophilin controls a fast-acting tubulovesicular endocytic pathway which is independent of AP2 and clathrin, and is inhibited by inhibitors of dynamin (Boucrot *et al.*, 2015). The existence of the clathrin independent pathway has been supported by uptake of Simian virus-40, interlukin-2 receptor-β etc. in living cells. It could be GTPase dependant or independent (Mayor and Pagano, 2007), (Takei *et al.*, 2005), (Sundborger and Hinshaw, 2014).

Figure: 2

Expression of dynamin

Transcriptional and translational mechanisms may control the expression of dynamin. The mammalian genome has three genes for dynamin and resultant proteins (dynamin 1, 2 and 3) have 80% homology. All three dynamins have different expression pattern. Neurons have high levels of dynamin 1 (DNM1), whereas dynamin 2 (DNM2) is expressed ubiquitously. Dynamin 3 (DNM3) is expressed in brain, testes and lungs. Dynamin and dynamin-related proteins perform a variety of cellular functions, apart from endocytosis (Van Der Bliek, 1999), (Cao *et al.*, 1998).

Binding partners or modifiers of dynamin

Binding partners of dynamins interact with the PRD domain of classical dynamins via SH3 domain. Actin-binding protein (ABP1) is an example of binding partner, which binds to human DNM2 via SH3 domain. BIN/amphiphysin/Rvs (BAR) domain containing proteins such as amphiphysin, endophilin, and syndapin also interact with PRD of classical dynamins via SH3

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domains and help in tubulation of lipids (Scaife and Margolis, 1997). Endophilin as dynamin binding partner binds the membrane bilayer via its N-terminal region and to both dynamin and synaptojanin (an inositol 5-phosphatase) via its C-terminal SH3 domain thus coordinates the function of these proteins in endocytosis. Amphiphysin directs dynamin towards clathrin-coated pits and there endophilin recruits dynamin to the curvature of the necks of nascent vesicles (Henley *et al.*, 1998), (Sundborger *et al.*, 2011). Dynamin modifiers, such as kinases, phosphatase, ubiquitin ligase, small ubiquitin like modifier (SUMO) ligases and proteases, moderate dynamin activity via complex protein-dynamin interactions. Reversible phosphorylation of human DRP1 at synaptic vesicles occurs via calcium/calmodulin-dependent protein kinase (CaMK1), cyclin dependent kinase1 (CDK1) and protein kinase A (PKA). Apart from phosphorylation, DRP1 has been shown to undergo sumoylation which increases mitochondrial fission (Mishra *et al.*, 2004).

Regulated activation and polymerization:

Purified dynamin always polymerizes/self-assembles into rings and helices, in solutions of suitable ionic strength (Carr and Hinshaw, 1997). Dynamin tubulates membrane bilayers and forms a continuous coat around them. Dynamin polymerization results from the parallel arrangement of dimers at a specific angle which decides the diameters of the rings. Stalk forms the core of the ring while Bundle signaling molecule (BSE) and G domain of dimer project towards adjacent rungs of dynamin helix. Dimerization of GTPase domain is critical for GTP hydrolysis which can be stimulated during polymerization. Fission could be a result of GTP hydrolysis and membrane destabilization as constricted rings disassemble (Hinshaw and Schmid, 1995).

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Dynamin, defective mitochondrial dynamics and neurodegenerative disorders

The role of abnormal mitochondrial dynamics in Alzheimer's, Huntington's, Parkinson's and various other disorders has been well-established. In mitochondrial fission and fusion, Dynamin-related protein 1 (Drp1), mitochondrial fission 1 protein (Fis1), and fusion proteins (Mfn1, Mfn2, and Opa1) are essential to provide ATP to neurons in order to maintain normal fission-fusion process. Drp1 is involved in several cellular functions like mitochondrial and peroxisomal fragmentation, sumoylation, phosphorylation, ubiquitination, and cell death. In neurodegenerative diseases, including AD, PD, HD, and Amyotrophic lateral sclerosis (ALA), mutant proteins interact with Drp1 and activates mitochondrial fission machinery. This activation leads to excessive mitochondrial fragmentation and impairs mitochondrial dynamics which finally causes mitochondrial dysfunction and neuronal damage (Reddy *et al.*, 2011).

Advancements in molecular biology and genetic analysis have revealed that mutations of human DNM1and DNM2 are very well linked to various disorders such as Alzheimer's, Parkinson's, Huntington's, Charcot-Marie-tooth disease (CMT), heart failure, schizophrenia, epilepsy, cancer, optic atrophy etc. CMT results from a defect in the PH domain of dynamin leading to defective lipid binding. Defects in MD and PH domains are very well linked to centronuclear myopathy (CNM).

Dynamin in Alzheimer's disease (AD):

Alzheimer's disease is the most prevalent age-related disorder characterized by neurodegeneration and cognitive decline. Synaptic dysfunction is one of the important events in the pathogenesis of AD (Kelly *et al.*, 2005). The causes include accumulation of amyloid-beta protein, phosphorylated tau and neurofibrillary tangles in the brain. DNM1 is involved in

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regulation of amyloid generation through modulation of BACE1. It reduces both secreted and intracellular amyloid beta levels in cell culture.

Amyloid beta (Aβ) and phosphorylated tau interact with dynamin-related protein 1 (Drp1), the mitochondrial fission protein, and cause excessive fragmentation of mitochondria, which leads to abnormal mitochondrial dynamics and synaptic degeneration in neurons, responsible for Alzheimer's disease (Kandimalla and Reddy, 2016).

Interaction of Aβ and Drp1 initiates mitochondrial fragmentation in AD neurons and abnormal interaction increases with disease progression (Manczak *et al.,* 2011). Amyloid-beta protein causes synaptic disturbances which result in neuronal death (Zhu *et al.,* 2012).

Down-regulation of dynamin2 (DNM2) have also been linked to beta-amyloid in hippocampal neurons. Dynamin binding protein (DNMBP) gene, located on chromosome 10 is associated with late-onset Alzheimer's disease (LOAD) (Kuwano *et al.,* 2006),(Aidaralieva *et al.,* 2008). The connection between DNM2 and LOAD is not clear, but a decreased expression of hippocampal DNM2 mRNA has been found in LOAD. DNM2 dysfunction affects metabolism and localization of the amyloid beta protein and amyloid precursor protein (APP). Real-time PCR analysis showed that amount of DNM2 mRNA was significantly lower in the temporal cortex of AD brains and peripheral blood of dementia patients as compared to that of the control. However, DNM1 and DNM3 were not significantly affected. Analysis of peripheral leukocyte in dementia patients showed that levels of DNM2 were significantly lower than those of the control. Hence, it was assumed that reduced levels of DNM2 mRNA caused dysfunction in DNM2 (Aidaralieva *et al.,* 2008), (Kamagata *et al.,* 2009). Research has been done to investigate the relationship between DNM2 dysfunction and amyloid beta production which is a key event in

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AD pathology. Neuroblastoma cells with dominant negative DNM2 resulted in amyloid beta protein (Ab) secretion and most of the amyloid precursor protein (APP) in these cells was localized to the plasma membrane. An accumulation of APP near plasma membrane shows DNM2 dysfunction, which is normally transported via endoplasmic reticulum and Golgi apparatus to the plasma membrane and finally taken up by endosomes via endocytosis for Ab generations (Carey *et al.*, 2005). Lipid raft is also a major source of Ab generation and plasma membrane of DNM2 negative neuronal cells have been found with an increase in the concentration of lipid raft. Increased amount of flotillin-1 a marker of lipid raft was found to be in the plasma membrane of DNM2 negative neuronal cells, confirming increased presence of lipid raft (Meister *et al.*, 2014). DNM1 knockout mice show reduced levels of secreted and intracellular levels of Aβ in cell cultures. A dramatic reduction in beta-site APP-Cleaving Enzyme 1 (BACE-1) cleavage products of APP has been found in DNM1 knockout mice. A decrease in Ab with DNM1 and BACE1 inhibitors does not show combined effect, which indicates that effects of DNM1 inhibition are mediated through the regulation of BACE1 (Sinjoanu *et al.*, 2008).

Figure-3:

Epilepsy:

An epileptic seizure is characterized by social, cognitive and psychological impairment. It results from abnormal neuronal discharge originating from the brain (Singh and Jadhav, 2014). Synaptic transmission is an important communication between pre-synaptic and post-synaptic neurons and depends on the synaptic vesicle formation, release and endocytosis. Abnormalities in synaptic transmission are responsible for various neurological disorders, including epilepsy.

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Up regulation of Dynamin 1 has been correlated in some epilepsy patients (Li *et al.*, 2015). Dynamin, synapsin and syndapins are involved in vesicle formation, neurotransmitter release, and recycling of neurotransmitter which binds to postsynaptic receptors i.e., Inhibitory γ-aminobutyric acid (GABA) receptors and to the excitatory glutamate receptors. Activation of glutamate receptor, further activates a variety of postsynaptic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), kainate and metabotropic receptors. The activation of the receptors triggers various signaling cascades and results in a vast array of effects, which can be modulated by numerous auxiliary regulatory subunits. Different neuropeptides, such as neuropeptide Y, brain-derived neurotrophic factor (BDNF), somatostatin, ghrelin, and galanin, also act as regulators of diverse synaptic functions (Casillas-Espinosa *et al.*, 2012). There is no direct evidence linking dynamins to epilepsy, but electrophysiological responses from neurons of mutant mice show defects in GABAergic neurotransmission, which are similar to dynamin-1 knockout mice. Missense mutations in dynamin1 have been found to cause epilepsy in the fitful mouse model (Boumil *et al.*, 2010). Perturbations of dynamin-1 function, can enhance proneness towards seizures. Inhibition of dynamin binding to syndapin with a peptide based inhibitor slows down the abnormal neuronal firing. Syndapins (Synaptic dynamin binding proteins) knockout mice show high impairment in the recruitment of dynamin to the nascent vesicle and suffer from seizures. (Koch *et al.*, 2011). Synapsin 1, 2, 3 or phosphoprotein interacts with synaptic vesicles and prevent them from being trafficked to presynaptic membrane. During action potential generation, they are phosphorylated and allow vesicles to release neurotransmitter. Mutations in synapsin-1 and inhibition of dynamin also alter the process and have been linked with abnormal neuronal discharge

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(Newton *et al.*, 2006). Thus, there is a need to search legends which inhibit abnormal neuronal discharge through endocytosis and at the same time allow normal neuronal activity to occur (Baldelli *et al.*, 2007)(Anggono *et al.*, 2006).

Huntington's disease (HD):

It is a genetic autosomal dominant neurodegenerative disease, caused by the expansion of a CAG repeat in the huntingtin (htt) gene and results in ataxia, chorea, dementia as major symptoms (Bates *et al.*, 2015). It was first described by George Huntington in 1872. Dysfunction of the Huntington protein (Htt) and disturbances in the mitochondrial electron transport chain are supposed to be the main causes of HD (Zeviani and Carelli, 2005). Mitochondrion is one of the organelles whose electron transport chain, calcium buffering capacity and morphology is severely affected in HD. In recent years, biochemical and genetic studies have shown that there is a link between Htt and clathrin mediated endocytosis (Harjes and Wanker, 2003), (Chakraborty *et al.*, 2014). The cell death process in HD is initiated in the mitochondria and Htt aggregates are found in their vicinity. Recently, mutant Huntington protein (mHtt) has been shown to affect the activity of dynamin related protein-1 (DRP1) via sumoylation and nitrosylation, resulting in excessive endocytic fission (Otera *et al.*, 2013). The increase in Drp1, Fis1, CypD and the decrease in Mfn1 and Mfn2 have been linked with abnormal mitochondrial dynamics in the cortex of HD patients. The presence of mutant Htt oligomers in HD neurons and in mitochondria may affect normal neuronal functions. In the affected brain regions of HD patients, mutant Htt in association with impaired mitochondrial dynamics, alters the axonal transport of mitochondria and results in decreased mitochondrial function and damaged neurons (Shirendeb *et al.*, 2011). Cortex and cerebellum parts of the brain of the HD patient show stage dependent increase in

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DRP1. DRP1 is a substrate for mitochondrial ubiquitin ligase and may affect the activity of the ubiquitin proteasomal systemm (UPS) and dysfunction of the cellular UPS has been found to be the main reason of increased mHtt aggregate formation, which is a result of reduced mitochondrial electron transport chain complex III. Recent studies have found that mutant Huntingtin (mHtt) interacts with Dynamin-related protein 1 (Drp1), and causes excessive fragmentation of mitochondria and is associated with impaired transmission(Reddy, 2014), (Shirendeb *et al.*, 2011).

Figure-4

Mitochondrial fission or division is controlled DRP1 and Translocation of DRP 1 to mitochondria is regulated by its GTPase activity, phosphorylation, SUMOylation, and nitrosylation. mHtt is now known to increase GTPase activity and nitrosylation, thereby increasing DRP1 affinity to mitochondria, and results in enhanced mitochondrial fission process.

Charcot-Marie-Tooth peripheral neuropathy (CMT) and centronuclear myopathy (CNM)

This monogenetic disease is characterized by impaired motor and sensory neuronal functions resulting in muscle weakness and foot ulcers leading to frequent infections. The disease is a result of mutations of genes associated with intracellular trafficking (Szigeti and Lupski, 2009), (Durieux *et al.*, 2010). Mutations in dynamin 2 result in Charcot-Marie-Tooth peripheral neuropathy. These mutations are mainly clustered in the N-terminal part of the PH domain of dynamin which is involved in interactions with phosphoinositides and resultes in dominant intermediate CMT type 2B (Sidiropoulos *et al.*, 2012). Interestingly, mutations in myotubularin-related protein-2 (MRP2) which is responsible for the metabolism of phosphatidylinositol bisphosphate and phosphatidylinositol 3 phosphate leads to different types of CMTs confirming

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role of synaptic transport in CMT. Clathrin-mediated endocytosis is necessary for proper myelination and defects in this function are caused by CMT mutants thus, CMT mutants are major contributors of pathology of CMT subtype 2B (Koutsopoulos *et al.*, 2011). Defects in clathrin-mediated endocytosis result in improper myelination in CMT-associated mutants. The dependency of myelination on DNM2 and clathrin-mediated endocytosis gives a hint that clathrin-mediated endocytosis could be a new target for CMT treatment. Mutations in DNM2 lead to dominant intermediate Charcot-Marie-Tooth neuropathy type B, and other mutations in DNM2 cause autosomal dominant centronuclear myopathy (Sidiropoulos *et al.*, 2012). DNM2 related CNM is slow progressive, rare, inherited disorder, accompanied by a facial, general muscle weakness, ptosis, and extraocular muscle palsy. Cognitive impairment has also been reported in some CNM patients. It was speculated that DNM2 mutants would cause CNM by interfering through centrosomes. Mutations in the C-terminal α-helix of PH domain appear to cause conformational changes in dynamin and affect GTP hydrolysis cycle. DNM2 mutations, affecting the MD and the PH domain have been identified in autosomal dominant centronuclear myopathy (CNM) (Kenniston and Lemmon, 2010),(Bitoun *et al.*, 2009). In a patient-based study DNM2 mutations were found to be the major cause of CNM but the molecular mechanisms that lead to neuropathy and myopathy need to be explored more (Echaniz-Laguna *et al.*, 2013).

Parkinson's disease (PD):

Parkinson's disease has been known to be associated with tremors, slow movement and cognitive difficulties. Amphiphysin and endophilin are two BAR proteins which bind to dynamin, where amphiphysin targets dynamin to clathrin-coated pits and endophilin directs dynamin to the necks of the nascent clathrin-coated pits. There is a link between Parkinson's disease gene, Parkin, and

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endocytic protein endophilin. Parkin performs degradation of DRP1 and mutation leads to accumulation of DRP1 for mitochondrial fragmentation. A hypothesis proposed that endophilin helps in recruiting Parkin at endocytic pathways to prevent or regulate the degradation of synaptic proteins. Mutations of Parkin, an E3 ubiquitin protein ligase, lead to autosomal juvenile-onset of Parkinson's disease. Endophilin binds to the Ubi domain of Parkin via SH3 domain and is said to get ubiquitinated,(Wang *et al.*, 2011) (Stafa *et al.*, 2014). Parkin levels significantly increase in the brain and fibroblasts of endophilin mutant mice (Cao *et al.*, 2014). The absence of endophilin or synaptojanin knockout results in a robust increase of Parkin in the brain. Endophilin-Parkin interactions may affect the synaptic vesicle transmission and might be involved in the pathogenesis of PD. Drp1, is a regulator of mitochondrial fission, is found to be reduced in wild-type DJ-1 cells and increased in mutant DJ-1 cells. DRP1 knockdown in these mutant DJ-1 cells restores the normal mitochondria morphology. DJ-1 is involved in the regulation of mitochondrial dynamics through modulation of DLP1/DRP expression. PD-associated DJ-1 mutations may cause PD by impairing mitochondrial dynamics and function through DRP (Hu *et al.*, 2012). Mutations in leucine-rich repeat kinase (LRRK2), and interactions of LRRK2 with endophilin, further interactions of endophilin with Parkin, are probable causes of autosomal familial Parkinson's disease. LRRK2 is involved in synaptic vesicle endocytosis and exocytosis, it has been linked with DNM1, DNM2, and DNM3. LRRK2 also interacts with dynamin-related proteins which are involved in mitochondrial fission and fusion (Smith *et al.*, 2005), (Cao *et al.*, 2014).

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Cancer:

Altered mitochondrial functions are also associated with cancer. Targeting mitochondria for restoration of normal functioning or insisting mitochondria-induced cell death are some important strategies for cancer treatment. Dynamin-related protein (Drp1) has been found to be up-regulated in certain types of cancers such as lung and breast cancer. A further role of Drp1 in cell migration and apoptosis in cancer cells has been linked recently, uncovering Drp1 mediated mitochondrial fission as an effective therapy for cancer(Frank *et al.*, 2001),(Qian *et al.*, 2013). Prostate cancer (PC) is the second most fatal cancer in men, although the mortality rate is reduced significantly in the recent era. Significantly increased expression of DNM2 has been found in advanced stages of progressive prostate cancer (PCA) as compared to the starting stage though the importance of expression is largely unknown. In some preclinical studies, suppression of DNM2 gene significantly reduces cell migration and tumor size, both in vitro and in vivo respectively. The conclusion is that over-expression of DNM2 is associated with neoplastic prostate epithelium and is a potential target for PCA. Dynamin 2 is essential for endocytosis of some proteins associated with cancer motility and invasiveness, such as integrin β-1 and focal adhesion (FA) kinase. Over expression of DNM2 in PCA and requirement of DNM2 in endocytosis of FA kinase and integrin, open the gate for a new therapy which can control the expression of DNM2 (Xu *et al.*, 2014). In hepato-cellular carcinoma (HCC), DNM3 has been a candidate tumor suppressor gene (Inokawa *et al.*, 2013).

Depletion of endogenous Dyn2 inhibited PDGFRα-stimulated phosphorylation of Akt, Erk1/2. Tyrosine-protein phosphatase, a non-receptor type-2 (SHP-2), interacts with Dyn2 and PDGFRα signaling. Dyn2 mediates PDGFRα-SHP-2-induced glioma tumor growth and invasion,

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suggesting that targeting the PDGFRα-SHP-2-Dyn2 pathway could give a new hope to patients with malignant glioblastomas (Feng *et al.*, 2012). Current treatments for glioblastomas include, nitrosoureas, cisplatin, irinotecan, gefitinib and erlotinib. Findings from the children's medical research institute (CMRI) correlate the role of dynamin2 in the treatment of glioblastoma. Since, Dynamin2 plays a role in the final stage of mitosis and cytokinesis, inhibitors of dynamin2 could help treat glioblastoma.

Dominant optic atrophy (DOA):

Optic nerve fibers are responsible for carrying image information from retina to brain. Defect in any fiber can impair vision due to disruption of impulses being sent to the brain. This abnormal condition is known as optic atrophy. The patient complains of having a blurred vision, trouble with peripheral and color vision. A gradual loss of visual activity is observed which often leads to blindness (Lenaers *et al.*, 2012). Optic atrophy is linked to optic atrophy gene1 (OPA1) which is a GTPase of dynamin family present in the inner mitochondrial membrane and is hypothesized to be involved in mitochondrial fission. 45% of existing dominated optic dystrophy cases are said to arise from mutation of OPA1(Delettre *et al.*, 2000). Mitochondrial network dynamics, fission, and fusion mediate mitochondrial quality (MQC). Proteolytic cleavage of OPA1 prevents mitochondrial fusion. OPA1L (long isoform) counteracts cytochrome C release and hence acts as an anti-apoptotic. An OPA1 mutant affects MQC rendering cells susceptible to stress, especially retinal ganglionic cells (RGCs) and risks to RGCs are very well-linked to glaucoma and DOA. OPA1 polymorphisms have been associated with certain forms of glaucoma(Alavi and Fuhrmann, 2013).

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Schizophrenia:

Schizophrenia is a devastating mental disorder characterized by a breakdown in thinking and poor emotional response. Neuropathological evidence suggests that dopaminergic, GABAergic and glutamatergic transmissions are involved in symptomatology of schizophrenia. Dystrobrevin-binding protein 1 (DTNBP1) or dysbindin gene located on chromosome 6p has been linked to the etiology of schizophrenia. It affects neurotransmission and is responsible for cognitive dysfunctions associated with schizophrenia. The expression level of dysbindin is found to be reduced in the hippocampus and prefrontal cortex of schizophrenia patients (Numakawa *et al.*, 2004). Dysbindin protein expression also affects the levels of dopamine and glutamate in the hippocampus. Studies support the role of CME in the pathophysiology of schizophrenia and bipolar disorders. Dysbindin is involved in processes closely related to CME and membrane trafficking (Chen *et al.*, 2008), (Schubert *et al.*, 2012). Dysbindin deficiency is responsible for 'dysbindin like defects' such as slow fusion kinetics, decreased neurotransmitter release and reduced small readily releasable pool (RRP). Finally, synaptic neurotransmission is affected in schizophrenia (Feng *et al.*, 2008),(Dickman and Davis, 2011). Further, antipsychotic drugs have been found to interact with clathrin-interacting proteins. Thus, involvement of dysbindin in membrane trafficking and interaction of antipsychotic medicines with clathrin protein, indicates a new approach to be explored in schizophrenia.

Heart failure:

Cardiac mitochondria serve as major source of energy and radicals, and are important for normal functioning of the heart. There has always been a link between mitochondrial number and structure, mitochondrial fusion and fission including mitophagy. Left ventricle ejection fraction

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failure or left ventricle heart failure (LVEFF/ LVHF), hypertension, idiopathic cardiomyopathies are various etiologies of heart failure (Palaniyandi *et al.*, 2010). Mitochondria are dynamic cell organelles which keep on dividing and fusing in order to maintain their number and integrity (Murray *et al.*, 2007). Abnormal mitochondrial morphologies have been linked to many cardiac diseases, strongly suggesting that mitochondrial fusion and fission is required for normal functioning of heart. Disruption of dynamin-related protein 1 (Drp 1) leads to mitochondrial elongation and inhibition of mitochondrial autophagy, inducing mitochondrial dysfunction which causes cardiac dysfunction (Ikeda *et al.*, 2015).

Drp1 activation during ischemia-reperfusion (IR) results in LV dysfunction implying that Drp1 inhibition is beneficial for heart activity (Sharp *et al.*, 2014). Cardiac myopathy is linked to a decreased level of OPA1. The protein OPA1 is a GTPase of dynamin family and is present in the inner mitochondrial membrane. It is expected to be involved in mitochondrial fission. Studies in rats having HF, found that reduced number of mitochondria and structural changes such as disorganized cristae/reduced cristae density.. This provides evidence that Drp1 induced fission could further be explored for HF (Chen *et al.*, 2009).

Osteoporosis:

Pyrophosphates have long been identified as the first choice of treatment for osteoporosis. These drugs are believed to inhibit prenylation and disrupt the signaling pathways downstream of prenylated small GTPase(Wark, 1996). Prenylation inhibitors are found to be antiviral agents and investigation shows that prenylation independent pathway can also suppress viral infections. Recently, it has been found that bisphosphonates target dynamin 2 by inhibiting their GTPase activity thereby, blocking the endocytosis of adenovirus. Thus, by inhibiting dynamin-mediated

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endocytosis, bisphosphonate class of drugs provides a new strategy for treatment of osteoporosis (Masaike *et al.*, 2010).

Down syndrome (DS):

Down syndrome or trisomy 21 is a condition in which a person is born with extra genetic material from chromosome 21 that causes, learning, language, and memory disabilities. Over-expression of regulator of calcineurin1 (RCAN1), an endogenous calcineurin inhibitor, affects calcineurin phosphatase signaling leading to Down syndrome (Kuruvilla *et al.*, 2004). Activity-dependent bulk endocytosis (ADBE) is one of the mechanisms by which synaptic vesicles reform. ADBE couples neuronal activity and calcineurin causes dephosphorylation of dynamin 1 so that it binds with syndapin. In simpler words, calcineurin modifies dynamin1 and stimulates ADBE. Disruption of the calcineurin-dynamin 1 interaction inhibits clathrin-mediated endocytosis. It has been proved that all downstream defects in brain function in DS are due to dysfunction of dynamin 1 (Lai *et al.*, 1999). Up-regulation of RCAN1 and DYRK1 result in dysfunction of dynamin 1 phosphorylation and give rise to defective ADBE (Clayton and Cousin, 2009), (Fuentes *et al.*, 2000).

Nephrosis:

The function of Bowman's capsule, in the kidney, is to filter blood by allowing small molecules such as salts, sugar, water etc. to pass through and retaining beneficial macromolecules such as proteins. Podocytes are visceral epithelial cells of Bowman's capsule that wrap around capillaries of glomerulus. Bis-T-23, a small molecule, promotes actin-dependent dynamin oligomerization and increases actin polymerization in injured podocytes, which results in improved renal health in diverse models of both transient kidney disease and CKD (Schiffer *et al.*, 2015). There has

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been a link between the mechanism which governs podocyte processes and neuronal synapse development. Dynamins act on actin filaments of cytoskeleton of podocytes and help in the development of podocyte network, after which, podocyte foot process levels of dynamin 1 and dynamin 2 are suppressed (Soda *et al.*, 2012). Disruption of this sequence of events causes nephrotic syndrome.

Ligands of dynamin

Dynasore

Inhibition of dynamin reversibly blocks synaptic vesicle endocytosis. Dynasore rapidly inhibits the GTPase activity of dynamin with high specificity without disturbing exocytosis. In the presence of dynasore, a stimulation of weak frequency can cause accumulation of vesicular proteins on the cell surface, even after stimulation is terminated. This shows that events of endocytosis rely on dynamin and that dynasore successfully inhibits these events. It has been further proved by ultrastructural analysis that dynasore causes a reduction in density of synaptic vesicles. Macia and colleagues proposed a dual role of dynamin in endocytosis i.e. one during detachment of the vesicle and second, at the time of invagination. They screened 16000 thousand compounds and came up with dynasore that interfered with the in vitro activity of DNM1, DNM2 and DRP1. Dynasore has the ability to block GTPase activity of dynamin. It non-competitively inhibits basal and stimulated rates of GTP hydrolysis without changing GTP binding. In cultured cells, it blocks clathrin mediated endocytosis completely. Dynasore acts as a potent inhibitor of endocytosis by rapidly blocking the formation of coated vesicles and is supported by half formed and O and U-shaped pit formation. It acts as a non-competitive inhibitor of GTPase activity of DNM1 and DNM2. Dynasore interferes with all functions of

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dynamin in endocytosis such as low density lipoprotein (LDL) and transferrin transport which happens through CME. Transferrin uptake was well blocked by pretreatment of cells with dynasore. It does not affect the functions which are independent of dynamin. The action of dynasore is very fast and it depends on diffusion of the molecule to coated pits in required concentration (Macia *et al.*, 2006),(McGeachie *et al.*, 2013),(Nankoe and Sever, 2006).

Other ligands:

Suho lee et al. have synthesized 2-naphthohydrazides, 2-naphthoamides and naphthoates which show potent inhibition of CME as compared to dynasore. Starting materials for these compounds are substituted 3-hydroxynaphthoic acids and are available commercially. A carboxylic acid group of these starting materials was converted to esters via Fischer esterification reaction. Resultant ester was substituted with hydrazine hydrate in ethanol to get hydrazide. Further derivatives were obtained by the reaction of hydrazide with various substituted aldehydes. For amide compounds, activated napthoic acid was reacted with various amine compounds in desired conditions. DD-6 (R1,R2,R3,R6= H,R4,R5=OH) and DD-11 (R1,R3=OH) compounds more potently inhibit membrane fission. The introduction of chlorine or dimethyl substitution on phenyl ring abolishes the inhibitory activity of dynasore. Hydroxyl group at the third and a methoxy group at the 4th position of naphthyl ring increase its activity. Physiologically and kinetically also these molecules are better than dynasore (Lee *et al.*, 2010).

Figure-5:

MecGregor, K.A. et al. have synthesized naphthalimides which inhibit the interaction between clathrin N-terminal domain and endocytic accessory proteins. One out of 17000 small molecules has been identified at ChemBioNet library. Further screening of various libraries resulted in 1, 8-

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naphthol imides as comparable inhibitors of clathrin. Refinement of 4-aminobenzyl moiety gives a more active compound with better IC50 values for clathrin inhibition. The author concludes that bulky molecule fails to follow Lipinski's rule of five and is synthetically difficult to prepare. 1,8-naphthyl anhydrides as starting materials are commercially available and were used to synthesize and screen 1,8- naphthalimides. These compounds were found to have modest clathrin inhibiting activity (Macgregor *et al.*, 2014).

Figure-6:

Mutations in leucine-rich repeat kinase (LRRK2) is a frequent cause of autosomal familial Parkinson's disease. LRRK2 is involved in synaptic vesicle endocytosis and exocytosis and has been linked with DNM1, DNM2, and DNM3. Kavanagh E.M. et al. have reported amino pyrimidines GNE-7915 as brain-penetrating and non-toxic LRRK2 inhibitors. Kim et al. have reported G-969 as an LRRK2 inhibitor with excellent potency (structure not disclosed). A number of compounds have been patented as LRRK2 inhibitors, but still, there is a need to explore their detailed mechanism of action (Kavanagh *et al.*, 2013).

Mcgechie A.B. et al. have reported pyrimidine compounds as potent lipid stimulated GTPase activity of both DNM1 and DNM2. They have reported pyrimidyn-7 as most potent compound in dynamin inhibitor category. These compounds directly compete with GTP and thus block endocytosis (McGeachie *et al.*, 2013). Robertson J.M. et al. have reported small molecules Rhodadyns as inhibitors of dynamin GTPase activity. From focused rhodadyn based libraries, 13 compounds were found to be very potent for inhibition of GTPase activity. These compounds block receptor-mediated endocytosis effectively and two compounds, C10 and D10, have very good IC50 values for receptor-mediated endocytosis (Robertson *et al.*, 2012). Wang, D. et al.

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have reported small molecule inhibitors of tyrosine-(Y)-phosphorylation regulated kinase 1A (DYRK1A) which gives a hope for treatment of DS. Compounds have been found to be potent in in vitro cell based assays. After performing structure based virtual screening, 6 novel molecules have been reported as potent DYRKA1 inhibitors. The mechanism can be further explored to see the clinical benefits of these compounds in DS (Wang *et al.*, 2012). Gordon, P.C has reported a second generation potent indole-based dynamin GTPase inhibitor. Compound no. 24 is found to be most active in the series (Gordon *et al.*, 2013). Odell, R.L. et al. have reported series of compounds as pthaladyns based on homology model for the GTP-binding domain of human dynamin 1. Pthaladyan-23 was found to be a potent inhibitor of dynamin1 mediated synaptic vesicle endocytosis in brain synaptosomes (Odell *et al.*, 2010). Hill, A.T. has reported amines and Dynoles for inhibition of dynamin-mediated endocytosis. Dynole 34-2 have been reported to be the most active inhibitor of RME and transferrin uptake (Hill *et al.*, 2004), (Hill *et al.*, 2005), (Hill *et al.*, 2009). Takahashi, K. reported Sertraline as an inhibitor of dynamin GTPase activity and dynamin-dependent endocytosis. Authors have supported their hypothesis by performing cell line assays where Sertraline suppresses dynamin1 as well as dynamin2. Sertraline affects endocytosis via dynamin2 (Takahashi *et al.*, 2010), (Yamada *et al.*, 2009)

Figure-7:

Conclusion and discussion: Dynamin family member carries out a large number of functions in cell biology, including scission of vesicles, mitochondrial fusion, and tubulation during cytokinesis etc. The presence of GED, a middle domain, and cooperative GTPase activities are essential for biological function. Deep molecular level understanding of dynamin interactions with their binding partners during vesicle biogenesis is still lacking. Though the role of dynamin

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has been linked with various disorders such as Alzheimer's, Parkinson's, Huntington's, Charcot-Marie-Tooth disease, heart failure, schizophrenia, epilepsy, cancer, optic atrophy, Down syndrome, osteoporosis but the establishment of the pathophysiological role is still a challenge as animal models are not easily available. Considerable progress has been made for understanding structural characterization of dynamins but still more details need to be explored. This review attempts to not only illustrate the mechanism and role of dynamin in above-mentioned diseases but also serves as a platform for a medicinal chemist to design novel dynamin ligands for various disorders.

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This article has not been copyedited and formatted. The final version may differ from this version.

References:

Aidaralieva NJ, Kamino K, Kimura R, Yamamoto M, Morihara T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Kida T, Okuda J-I, Uema T, Yamagata H, Miki T, Akatsu H, Kosaka K, and Takeda M (2008) Dynamin 2 gene is a novel susceptibility gene for late-onset Alzheimer disease in non-APOE-epsilon4 carriers. *J Hum Genet* **53**:296–302.

Alavi M V, and Fuhrmann N (2013) Dominant optic atrophy, OPA1, and mitochondrial quality control: understanding mitochondrial network dynamics. *Mol Neurodegener* **8**:32.

Anggono V, Smillie KJ, Graham ME, Valova VA, Cousin MA, and Robinson PJ (2006) Syndapin I is the phosphorylation-regulated dynamin I partner in synaptic vesicle endocytosis. *Nat Neurosci* **9**:752–60.

Baldelli P, Fassio A, Valtorta F, and Benfenati F (2007) Lack of synapsin I reduces the readily releasable pool of synaptic vesicles at central inhibitory synapses. *J Neurosci* **27**:13520–13531.

Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, Nance M, Ross C a., Scahill RI, Wetzel R, Wild EJ, and Tabrizi SJ (2015) Huntington disease. *Nat Rev Dis Prim* 15005.

Bitoun M, Durieux AC cile, Prudhon B, Bevilacqua JA, Herledan A, Sakanyan V, Urtizberea A, Cartier L, Romero NB, and Guicheney P (2009) Dynamin 2 mutations associated with human diseases impair clathrin-mediated receptor endocytosis. *Hum Mutat* **30**:1419–1427.

Boucrot E, Ferreira APA, Almeida-Souza L, Debard S, Vallis Y, Howard G, Bertot L, Sauvonnet N, and McMahon HT (2015) Endophilin marks and controls a clathrin-independent endocytic pathway. *Nature* **517**:460–5.

Boumil RM, Letts VA, Roberts MC, Lenz C, Mahaffey CL, Zhang Z, Moser T, and Frankel WN

Molecular Pharmacology Fast Forward. Published on November 22, 2016 as DOI: 10.1124/mol.116.105064
This article has not been copyedited and formatted. The final version may differ from this version.

(2010) A missense mutation in a highly conserved alternate exon of dynamin-1 causes epilepsy in fitful mice. *PLoS Genet* **6**.

Cao H, Garcia F, and Mcniven MA (1998) Differential Distribution of Dynamin Isoforms in Mammalian Cells. *Mol Biol Cell* **9**:2595–2609.

Cao M, Milosevic I, Giovedi S, and De Camilli P (2014) Upregulation of Parkin in endophilin mutant mice. *J Neurosci* **34**:16544–9.

Carey RM, Balcz BA, Lopez-Coviella I, and Slack BE (2005) Inhibition of dynamin-dependent endocytosis increases shedding of the amyloid precursor protein ectodomain and reduces generation of amyloid beta protein. *BMC Cell Biol* **6**:30.

Carr JF, and Hinshaw JE (1997) Dynamin assembles into spirals under physiological salt conditions upon the addition of GDP and □??-phosphate analogues. *J Biol Chem* **272**:28030–28035.

Casillas-Espinosa PM, Powell KL, and O'Brien TJ (2012) Regulators of synaptic transmission: roles in the pathogenesis and treatment of epilepsy. *Epilepsia* **53 Suppl 9**:41–58.

Chakraborty J, Rajamma U, and Mohanakumar KP (2014) A mitochondrial basis for Huntington's disease: Therapeutic prospects.

Chen L, Gong Q, Stice JP, and Knowlton AA (2009) Mitochondrial OPA1, apoptosis, and heart failure. *Cardiovasc Res* **84**:91–99.

Chen XW, Feng YQ, Hao CJ, Guo XL, He X, Zhou ZY, Guo N, Huang HP, Xiong W, Zheng H, Zuo PL, Zhang CX, Li W, and Zhou Z (2008) DTNBP1, a schizophrenia susceptibility gene, affects kinetics of transmitter release. *J Cell Biol* **181**:791–801.

Clayton EL, and Cousin MA (2009) The molecular physiology of activity-dependent bulk endocytosis of synaptic vesicles.

Molecular Pharmacology Fast Forward. Published on November 22, 2016 as DOI: 10.1124/mol.116.105064
This article has not been copyedited and formatted. The final version may differ from this version.

Danino D, Moon KH, and Hinshaw JE (2004) Rapid constriction of lipid bilayers by the mechanochemical enzyme dynamin. *J Struct Biol* **147**:259–267.

Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquellec L, Arnaud B, Ducommun B, Kaplan J, and Hamel CP (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* **26**:207–210.

Dickman DK, and Davis GW (2011) The Schizophrenia Susceptibility Gene Dysbindin Controls Synaptic Homeostasis. *Science (80-)* **326**:1127–1130.

Durieux A-C, Prudhon B, Guicheney P, and Bitoun M (2010) Dynamin 2 and human diseases. *J Mol Med (Berl)* **88**:339–350.

Echaniz-Laguna A, Biancalana V, Böhm J, Tranchant C, Mandel JL, and Laporte J (2013) Adult centronuclear myopathies: A hospital-based study. *Rev Neurol (Paris)* **169**:625–631.

Faelber K, Held M, Gao S, Posor Y, Haucke V, No?? F, and Daumke O (2012) Structural insights into dynamin-mediated membrane fission.

Feng H, Liu KW, Guo P, Zhang P, Cheng T, McNiven M a, Johnson GR, Hu B, and Cheng SY (2012) Dynamin 2 mediates PDGFRα-SHP-2-promoted glioblastoma growth and invasion. *Oncogene* **31**:2691–2702.

Feng YQ, Zhou ZY, He X, Wang H, Guo XL, Hao CJ, Guo Y, Zhen XC, and Li W (2008) Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia. *Schizophr Res* **106**:218–228.

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

Ford MGJ, Jenni S, and Nunnari J (2011) The crystal structure of dynamin. *Nature* **477**:561–566.

Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL, and Youle RJ (2001) The Role of Dynamin-Related Protein 1, a Mediator of Mitochondrial Fission, in Apoptosis. *Dev Cell* **1**:515–525.

Fuentes JJ, Genescà L, Kingsbury TJ, Cunningham KW, Pérez-Riba M, Estivill X, and de la Luna S (2000) DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. *Hum Mol Genet* **9**:1681–1690.

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

Harjes P, and Wanker EE (2003) The hunt for huntingtin function: Interaction partners tell many different stories.

Henley JR, Krueger EWA, Oswald BJ, and McNiven MA (1998) Dynamin-mediated internalization of caveolae. *J Cell Biol* **141**:85–99.

Hill TA, Gordon CP, McGeachie AB, Venn-Brown B, Odell LR, Chau N, Quan A, Mariana A, Sakoff JA, Chircop M, Robinson PJ, and McCluskey A (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 TA, Odell LR, Quan A, Abagyan R, Ferguson G, Robinson PJ, and McCluskey A (2004) Long chain amines and long chain ammonium salts as novel inhibitors of dynamin GTPase activity. *Bioorganic Med Chem Lett* **14**:3275–3278.

Hill T, Odell LR, Edwards JK, Graham ME, McGeachie AB, Rusak J, Quan A, Abagyan R, Scott JL, Robinson PJ, and McCluskey A (2005) Small molecule inhibitors of dynamin I GTPase activity: Development of dimeric tyrphostins. *J Med Chem* **48**:7781–7788.

Hinshaw JE (2000) Dynamin and its role in membrane fission. *Annu Rev Cell Dev Biol* **16**:483–519.

Hinshaw JE, and Schmid SL (1995) Dynamin self-assembles into rings suggesting a mechanism for coated vesicle budding.

Hu S, Xie G, Zhang DX, Davis C, Long W, Hu Y, Wang F, Kang X, Tan F, Ding L, and Wang Y (2012) Synthesis and biological evaluation of crown ether fused quinazoline analogues as potent EGFR inhibitors. *Bioorganic Med Chem Lett* **22**:6301–6305.

Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, Nomura M, Mihara K, Egashira K, Ohishi M, Abdellatif M, and Sadoshima J (2015) Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. *Circ Res* **116**:264–278.

Inokawa Y, Nomoto S, Hishida M, Hayashi M, Kanda M, Nishikawa Y, Takeda S, Fujiwara M, Koike M, Sugimoto H, Fujii T, Nakayama G, Yamada S, Tanaka C, Kobayashi D, and Kodera Y (2013) Dynamin 3: A new candidate tumor suppressor gene in hepatocellular carcinoma detected by triple combination array analysis. *Onco Targets Ther* **6**:1417–1424.

Kamagata E, Kudo T, Kimura R, Tanimukai H, Morihara T, Sadik MG, Kamino K, and Takeda M (2009) Decrease of dynamin 2 levels in late-onset Alzheimer's disease alters A?? metabolism. *Biochem Biophys Res Commun* **379**:691–695.

Kandimalla R, and Reddy PH (2016) Multiple faces of dynamin-related protein 1 and its role in Alzheimer's disease pathogenesis.

Kavanagh ME, Doddareddy MR, and Kassiou M (2013) The development of CNS-active LRRK2 inhibitors using property-directed optimisation.

Kelly BL, Vassar R, and Ferreira A (2005) ??-amyloid-induced dynamin 1 depletion in hippocampal neurons: A potential mechanism for early cognitive decline in Alzheimer

disease. *J Biol Chem* **280**:31746–31753.

Kenniston J a, and Lemmon M a (2010) Dynamin GTPase regulation is altered by PH domain mutations found in centronuclear myopathy patients. *EMBO J* **29**:3054–3067.

Klein DE, Lee A, Frank DW, Marks MS, and Lemmon MA (1998) The pleckstrin homology domains of dynamin isoforms require oligomerization for high affinity phosphoinositide binding. *J Biol Chem* **273**:27725–27733.

Koch D, Spiwoks-Becker I, Sabanov V, Sinning A, Dugladze T, Stellmacher A, Ahuja R, Grimm J, Schüler S, Müller A, Angenstein F, Ahmed T, Diesler A, Moser M, tom Dieck S, Spessert R, Boeckers TM, Fässler R, Hübner CA, Balschun D, Gloveli T, Kessels MM, and Qualmann B (2011) Proper synaptic vesicle formation and neuronal network activity critically rely on syndapin I. *EMBO J* **30**:4955–4969.

Koutsopoulos OS, Koch C, Tosch V, B??hm J, North KN, and Laporte J (2011) Mild functional differences of dynamin 2 mutations associated to centronuclear myopathy and charcot-marie-tooth peripheral neuropathy. *PLoS One* **6**.

Kozlov MM (1999) Dynamin: possible mechanism of “Pinchase” action. *Biophys J* **77**:604–16.

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G, Ye H, and Ginty DD (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. *Cell* **118**:243–255.

Kuwano R, Miyashita A, Arai H, Asada T, Imagawa M, Shoji M, Higuchi S, Urakami K, Kakita A, Takahashi H, Tsukie T, Toyabe S, Akazawa K, Kanazawa I, Ihara Y, Nunomura A, Chiba S, Takahashi S, Tomita N, Ito J, Hanyu H, Kimura H, Kitamura S, Shinotoh H, Iwamoto H, Takahashi M, Harigaya Y, Ikeda M, Amari M, Takahashi T, Nakano R, Nishizawa M, Suga M, Hasegawa M, Kawase Y, Honda K, Kumanishi T, Takeuchi Y, Ishikawa A, Morita M,

Yoshii F, Akatsu H, Kosaka K, Yamada M, Hamaguchi T, Masuzugawa S, Matsubara E, Kawarabayashi T, Takao T, Ota N, Sasaki K, Fujisawa Y, Nakata K, Wakutani Y, Nakashima K, Hayabara T, Ooya T, Takahashi M, Yamada T, Miyakawa T, Uyama E, Yuzuriha T, Nakagawa R, Yoshimoto S, and Serikawa K (2006) Dynamin-binding protein gene on chromosome 10q is associated with late-onset Alzheimer's disease. *Hum Mol Genet* **15**:2170–2182.

Lai MM, Hong JJ, Ruggiero AM, Burnett PE, Slepnev VI, De Camilli P, and Snyder SH (1999) The calcineurin-dynamin 1 complex as a calcium sensor for synaptic vesicle endocytosis. *J Biol Chem* **274**:25963–25966.

Lee E, and De Camilli P (2002) Dynamin at actin tails. *Proc Natl Acad Sci U S A* **99**:161–166.

Lee S, Jung KY, Park J, Cho JH, Kim YC, and Chang S (2010) Synthesis of potent chemical inhibitors of dynamin GTPase. *Bioorganic Med Chem Lett* **20**:4858–4864.

Lenaers G, Hamel CP, Delettre C, Amati-Bonneau P, Procaccio V, Bonneau D, Reynier P, and Milea D (2012) Dominant optic atrophy. *Orphanet J Rare Dis* **7**:46.

Lenz M, Morlot S, and Roux A (2009) Mechanical requirements for membrane fission: Common facts from various examples.

Li YY, Chen XN, Fan XX, Zhang YJ, Gu J, Fu XW, Wang ZH, Wang XF, and Xiao Z (2015) Upregulated dynamin 1 in an acute seizure model and in epileptic patients. *Synapse* **69**:67–77.

Macgregor KA, Robertson MJ, Young KA, Von Kleist L, Stahlschmidt W, Whiting A, Chau N, Robinson PJ, Haucke V, and McCluskey A (2014) 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.

Manczak M, Calkins MJ, and Reddy PH (2011) Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: Implications for neuronal damage. *Hum Mol Genet* **20**:2495–2509.

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

Mayor S, and Pagano RE (2007) Pathways of clathrin-independent endocytosis. *Nat Rev Mol Cell Biol* **8**:603–612.

McGeachie AB, Odell LR, Quan A, Daniel JA, Chau N, Hill TA, Gorgani NN, Keating DJ, Cousin MA, Van Dam EM, Mariana A, Whiting A, Perera S, Novelle A, Young KA, Deane FM, Gilbert J, Sakoff JA, Chircop M, McCluskey A, and Robinson PJ (2013) Pyrimidyn compounds: Dual-action small molecule pyrimidine-based dynamin inhibitors. *ACS Chem Biol* **8**:1507–1518.

Meister M, Zuk A, and Tikkanen R (2014) Role of dynamin and clathrin in the cellular trafficking of flotillins. *FEBS J* **281**:2956–2976.

Mishra RK, Jatiani SS, Kumar A, Simhadri VR, Hosur R V., and Mittal R (2004) Dynamin interacts with members of the sumoylation machinery. *J Biol Chem* **279**:31445–31454.

Morlot S, Galli V, Klein M, Chiaruttini N, Manzi J, Humbert F, Dinis L, Lenz M, Cappello G, and Roux A (2012) Membrane shape at the edge of the dynamin helix sets location and duration of the fission reaction. *Cell* **151**:619–629.

Morlot S, and Roux A (2013) Mechanics of dynamin-mediated membrane fission. *Annu Rev*

Biophys **42**:629–49.

Murray AJ, Edwards LM, and Clarke K (2007) Mitochondria and heart failure. *Curr Opin Clin Nutr Metab Care* **10**:704–11.

Nankoe SR, and Sever S (2006) Dynasore puts a new spin on dynamin: a surprising dual role during vesicle formation.

Newton a J, Kirchhausen T, and Murthy VN (2006) Inhibition of dynamin completely blocks compensatory synaptic vesicle endocytosis. *Proc Natl Acad Sci U S A* **103**:17955–17960.

Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N, Ozaki N, Taguchi T, Tatsumi M, Kamijima K, Straub RE, Weinberger DR, Kunugi H, and Hashimoto R (2004) Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. *Hum Mol Genet* **13**:2699–2708.

Odell LR, Howan D, Gordon CP, Robertson MJ, Chau N, Mariana A, Whiting AE, Abagyan R, Daniel JA, Gorgani NN, Robinson PJ, and McCluskey A (2010) The pthaladyns: GTP competitive inhibitors of dynamin I and II GTPase derived from virtual screening. *J Med Chem* **53**:5267–5280.

Otera H, Ishihara N, and Mihara K (2013) New insights into the function and regulation of mitochondrial fission. *Biochim Biophys Acta* **1833**:1256–68.

Palaniyandi SS, Qi X, Yogalingam G, Ferreira JCB, and Mochly-Rosen D (2010) Regulation of mitochondrial processes: A target for heart failure.

Pelkmans L, and Helenius A (2002) Endocytosis via caveolae. *Traffic* **3**:311–320.

Praefcke GJK, and McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* **5**:133–47.

Qian W, Wang J, and Van Houten B (2013) The role of dynamin-related protein 1 in cancer

growth: a promising therapeutic target? *Expert Opin Ther Targets* **17**:997–1001.

Ramachandran R (2011) Vesicle scission: Dynamin.

Reddy PH (2014) Increased mitochondrial fission and neuronal dysfunction in Huntington's disease: Implications for molecular inhibitors of excessive mitochondrial fission.

Reddy PH, Reddy TP, Manczak M, Calkins MJ, Shirendeb U, and Mao P (2011) Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases.

Reubold TF, Eschenburg S, Becker A, Leonard M, Schmid SL, Vallee RB, Kull FJ, and Manstein DJ (2005) Crystal structure of the GTPase domain of rat dynamin 1. *Proc Natl Acad Sci U S A* **102**:13093–13098.

Robertson MJ, Hadzic G, Ambrus J, Pom?? DY, Hyde E, Whiting A, Mariana A, Von Kleist L, Chau N, Haucke V, Robinson PJ, and McCluskey A (2012) The Rhodadyns, a new class of small molecule inhibitors of dynamin gtpase activity. *ACS Med Chem Lett* **3**:352–356.

Roux A, Koster G, Lenz M, Sorre B, Manneville J-B, Nassoy P, and Bassereau P (2010) Membrane curvature controls dynamin polymerization. *Proc Natl Acad Sci U S A* **107**:4141–4146.

Roux A, Uyhazi K, Frost A, and De Camilli P (2006) GTP-dependent twisting of dynamin implicates constriction and tension in membrane fission. *Nature* **441**:528–531.

Scaife RM, and Margolis RL (1997) The role of the PH domain and SH3 binding domains in dynamin function.

Schiffer M, Teng B, Gu C, Shchedrina V a, Kasaikina M, Pham V a, Hanke N, Rong S, Gueler F, Schroder P, Tossidou I, Park J-K, Staggs L, Haller H, Erschow S, Hilfiker-Kleiner D, Wei C, Chen C, Tardi N, Hakroush S, Selig MK, Vasilyev A, Merscher S, Reiser J, and Sever S (2015) Pharmacological targeting of actin-dependent dynamin oligomerization ameliorates

chronic kidney disease in diverse animal models. *Nat Med* **21**:601–9.

Schmid SL, and Frolov V a. (2011) Dynamin: Functional Design of a Membrane Fission Catalyst. *Annu Rev Cell Dev Biol* **27**:79–105.

Schubert KO, Föcking M, Prehn JHM, and Cotter DR (2012) Hypothesis review: are clathrin-mediated endocytosis and clathrin-dependent membrane and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder? *Mol Psychiatry* **17**:669–681.

Sharp WW, Fang YH, Han M, Zhang HJ, Hong Z, Banathy A, Morrow E, Ryan JJ, and Archer SL (2014) Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: Therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. *FASEB J* **28**:316–326.

Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, and Reddy PH (2011) Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: Implications for selective neuronal damage. *Hum Mol Genet* **20**:1438–1455.

Sidiropoulos PNM, Miehe M, Bock T, Tinelli E, Oertli CI, Kuner R, Meijer D, Wollscheid B, Niemann A, and Suter U (2012) Dynamin 2 mutations in Charcot-Marie-Tooth neuropathy highlight the importance of clathrin-mediated endocytosis in myelination. *Brain* **135**:1395–1411.

Singh M, and Jadhav HR (2014) Melatonin: Functions and ligands. *Drug Discov Today* **19**.

Sinjoanu RC, Kleinschmidt S, Bitner RS, Brioni JD, Moeller A, and Ferreira A (2008) The novel calpain inhibitor A-705253 potently inhibits oligomeric beta-amyloid-induced dynamin 1 and tau cleavage in hippocampal neurons. *Neurochem Int* **53**:79–88.

Molecular Pharmacology Fast Forward. Published on November 22, 2016 as DOI: 10.1124/mol.116.105064
This article has not been copyedited and formatted. The final version may differ from this version.

Smith WW, Pei Z, Jiang H, Moore DJ, Liang Y, West AB, Dawson VL, Dawson TM, and Ross C a (2005) Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci U S A* **102**:18676–18681.

Soda K, Balkin DM, Ferguson SM, Paradise S, Milosevic I, Giovedi S, Volpicelli-Daley L, Tian X, Wu Y, Ma H, Son SH, Zheng R, Moeckel G, Cremona O, Holzman LB, De Camilli P, and Ishibe S (2012) Role of dynamin, synaptojanin, and endophilin in podocyte foot processes. *J Clin Invest* **122**:4401–4411.

Song BD, Leonard M, and Schmid SL (2004) Dynamin GTPase domain mutants that differentially affect GTP binding, GTP hydrolysis, and clathrin-mediated endocytosis. *J Biol Chem* **279**:40431–40436.

Stafa K, Tsika E, Moser R, Musso A, Glauser L, Jones A, Biskup S, Xiong Y, Bandopadhyay R, Dawson VL, Dawson TM, and Moore DJ (2014) Functional interaction of Parkinson's disease-associated LRRK2 with members of the dynamin GTPase superfamily. *Hum Mol Genet* **23**:2055–2077.

Sundborger A, Soderblom C, Vorontsova O, Evergren E, Hinshaw JE, and Shupliakov O (2011) An endophilin-dynamin complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling. *J Cell Sci* **124**:133–143.

Sundborger AC, and Hinshaw JE (2014) Regulating dynamin dynamics during endocytosis. *F1000Prime Rep* **6**:85.

Sweitzer SM, and Hinshaw JE (1998) Dynamin undergoes a GTP-dependent conformational change causing vesiculation. *Cell* **93**:1021–1029.

Szigeti K, and Lupski JR (2009) Charcot-Marie-Tooth disease. *Eur J Hum Genet* **17**:703–10.

Takahashi K, Miyoshi H, Otomo M, Osada K, Yamaguchi N, and Nakashima H (2010)

Molecular Pharmacology Fast Forward. Published on November 22, 2016 as DOI: 10.1124/mol.116.105064
This article has not been copyedited and formatted. The final version may differ from this version.

Suppression of dynamin GTPase activity by sertraline leads to inhibition of dynamin-dependent endocytosis. *Biochem Biophys Res Commun* **391**:382–387.

Takei K, Yoshida Y, and Yamada H (2005) Regulatory mechanisms of dynamin-dependent endocytosis.

Urrutia R, Henley JR, Cook T, and McNiven M a (1997) The dynamins: redundant or distinct functions for an expanding family of related GTPases? *Proc Natl Acad Sci U S A* **94**:377–84.

Van Der Bliek AM (1999) Functional diversity in the dynamin family.

Wang D, Wang F, Tan Y, Dong L, Chen L, Zhu W, and Wang H (2012) Discovery of potent small molecule inhibitors of DYRK1A by structure-based virtual screening and bioassay. *Bioorg Med Chem Lett* **22**:168–71.

Wang H, Song P, Du L, Tian W, Yue W, Liu M, Li D, Wang B, Zhu Y, Cao C, Zhou J, and Chen Q (2011) Parkin ubiquitinates Drp1 for proteasome-dependent degradation: Implication of dysregulated mitochondrial dynamics in Parkinson disease. *J Biol Chem* **286**:11649–11658.

Wark JD (1996) Osteoporotic fractures: Background and prevention strategies, in *Maturitas* pp 193–207.

Wenger J, Klinglmayr E, Fröhlich C, Eibl C, Gimeno A, Hessenberger M, Puehringer S, Daumke O, and Goettig P (2013) Functional mapping of human dynamin-1-like GTPase domain based on x-ray structure analyses. *PLoS One* **8**:e71835.

Xu B, Teng LH, Silva SD da, Bijian K, Al Bashir S, Jie S, Dolph M, Alaoui-Jamali MA, and Bismar TA (2014) The significance of dynamin 2 expression for prostate cancer progression, prognostication, and therapeutic targeting. *Cancer Med* **3**:14–24.

Yamada H, Abe T, Li SA, Masuoka Y, Isoda M, Watanabe M, Nasu Y, Kumon H, Asai A, and

Takei K (2009) Dynasore, a dynamin inhibitor, suppresses lamellipodia formation and cancer cell invasion by destabilizing actin filaments. *Biochem Biophys Res Commun* **390**:1142–1148.

Zeviani M, and Carelli V (2005) Dominance in mitochondrial disorders.

Zhu L, Su M, Lucast L, Liu L, Netzer WJ, Gandy SE, and Cai D (2012) Dynamin 1 Regulates Amyloid Generation through Modulation of BACE-1. *PLoS One* **7**.

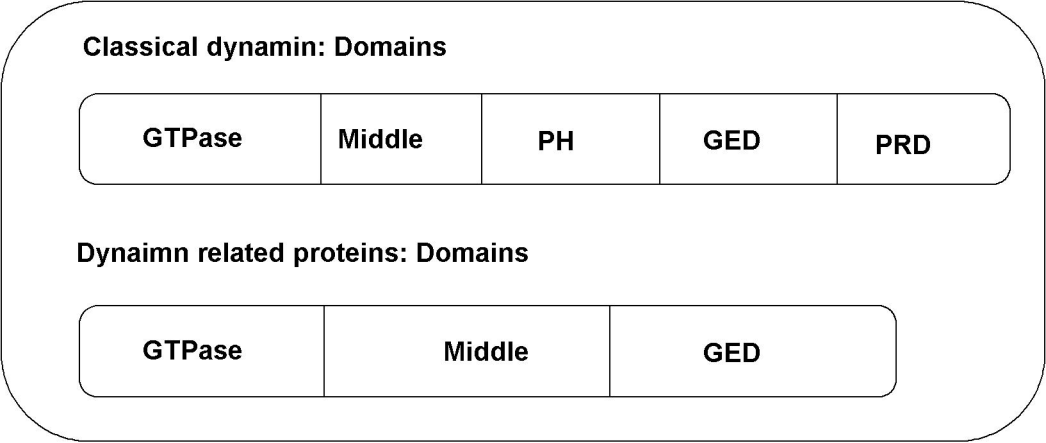
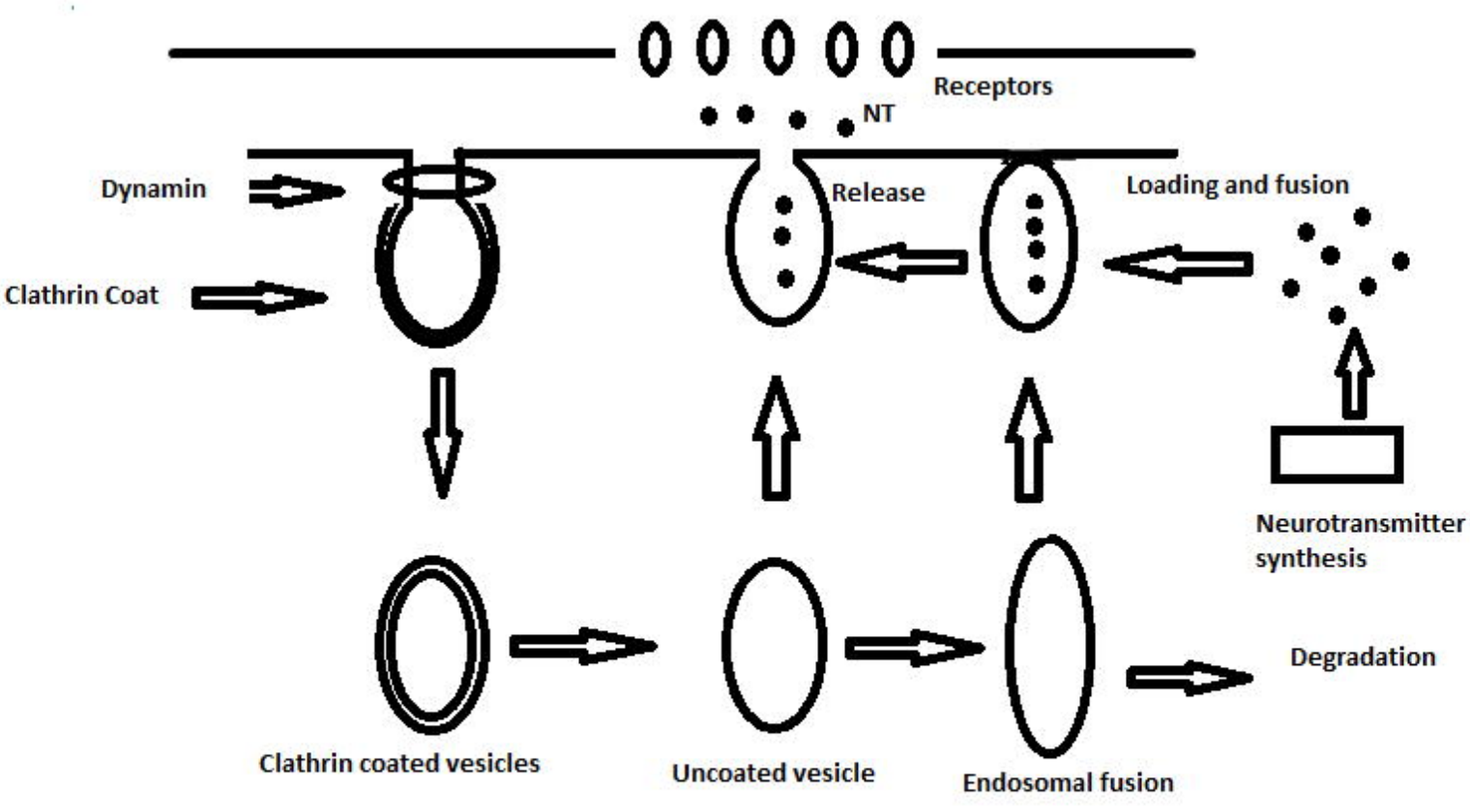

Classical dynamin: Domains
GTPase
Middle
PH
GED
PRD
Dynaimn related proteins: Domains
GTPase
Middle
GED

Figure 1


Receptors
NT
Dynamin
Clathrin Coat
Release
Loading and fusion
Neurotransmitter synthesis
Degradation
Clathrin coated vesicles
Uncoated vesicle
Endosomal fusion

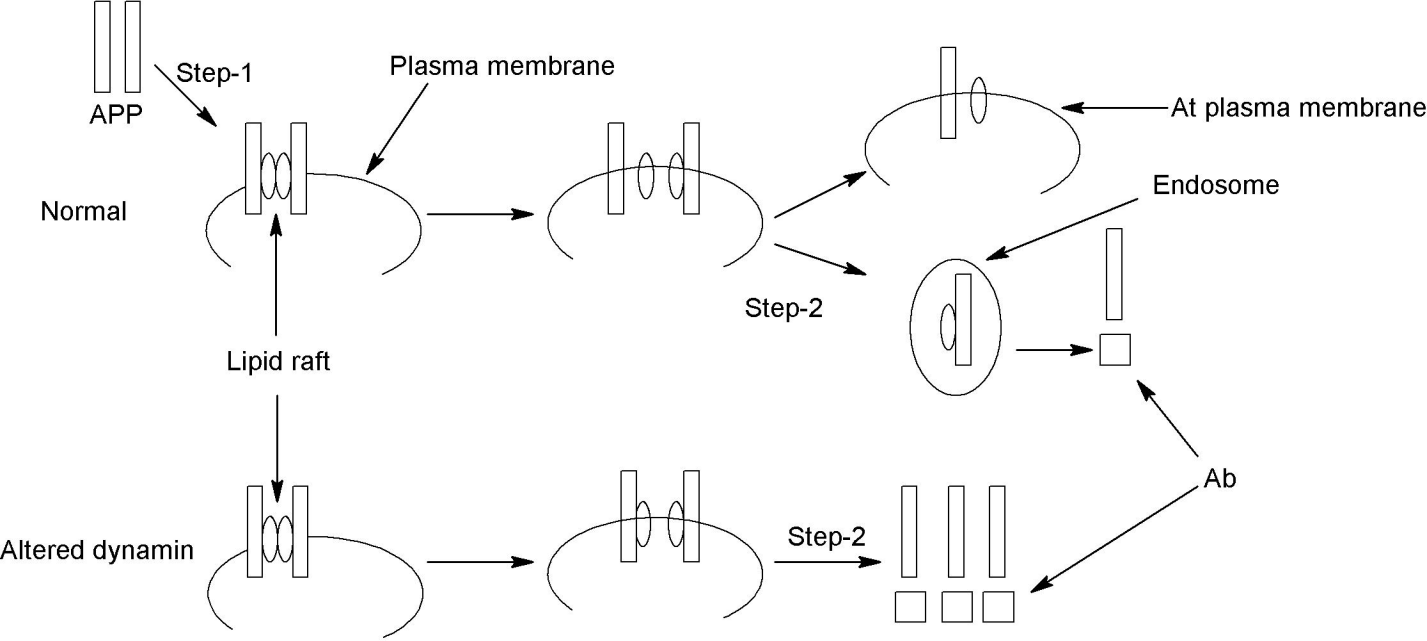

APP
Step-1
Plasma membrane
At plasma membrane
Normal
Endosome
Step-2
Lipid raft
Ab
Altered dynamin
Step-2

Figure 3

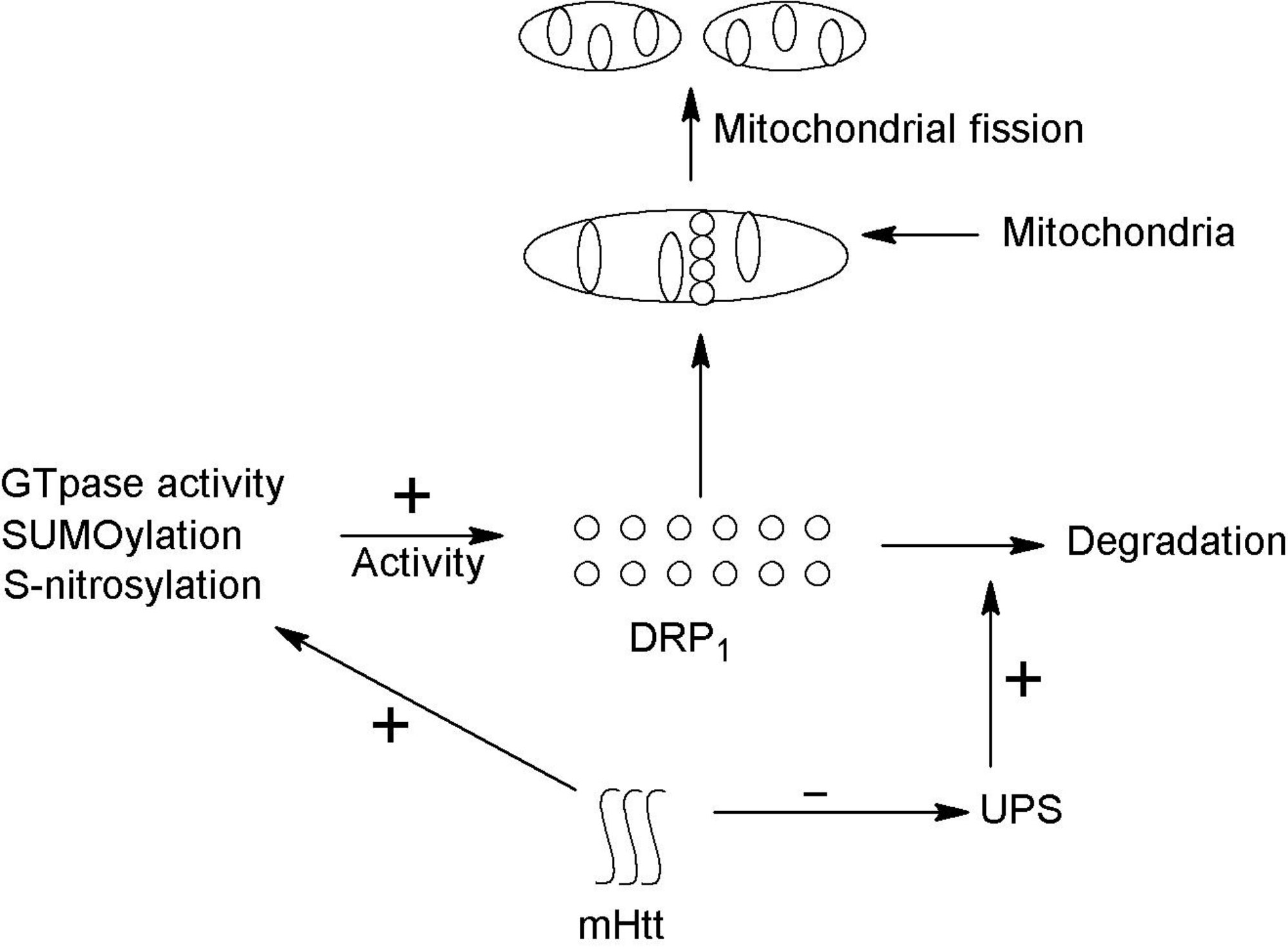

Mitochondrial fission
Mitochondria
GTpase activity
SUMOylation
S-nitrosylation
+
Activity
DRP1
Degradation
+
+
–
UPS
mHtt

Figure 4

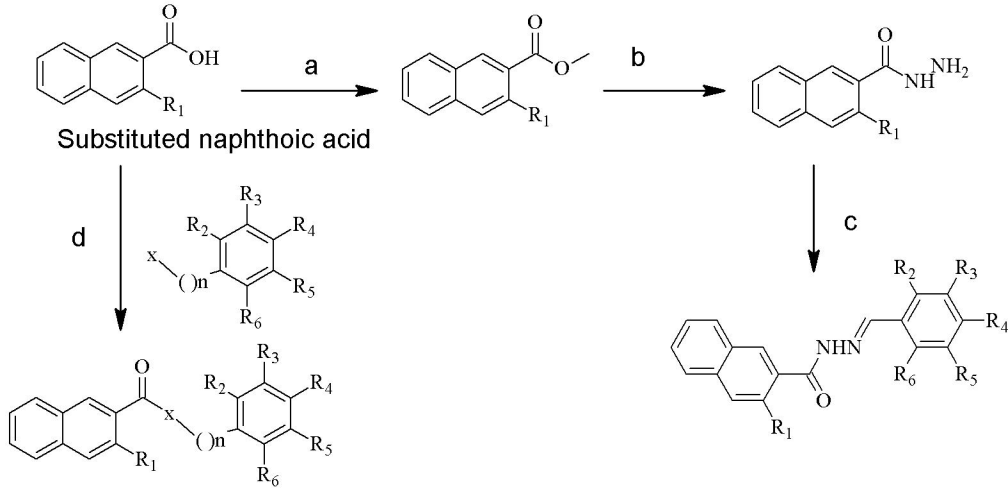


Figure 5

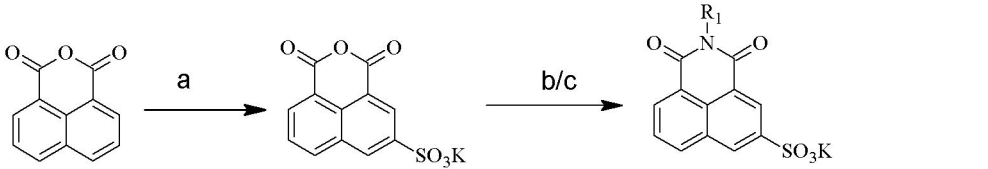
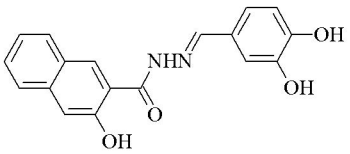
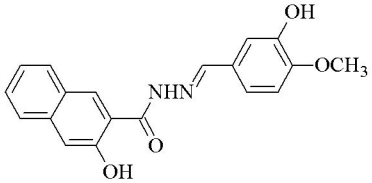

a
b/c
SO_3K
SO_3K
R_1
N
1,8-Napthtahlic anhyride
3-Sulfo-1,8-Napthtahlic anhyride
Substituted naphthylimides

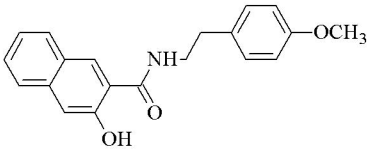
Figure 6



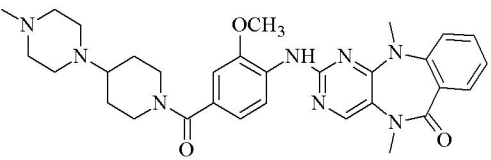
Dynasore



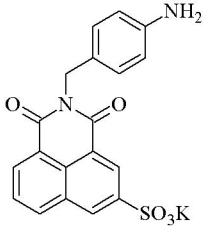
Napthohydrazide



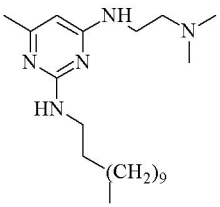
Napthoamide



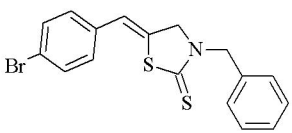
$LRRK_2IN_1$



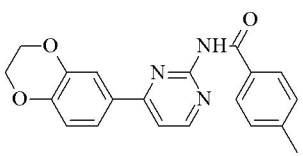
1,8 Nphthalimides



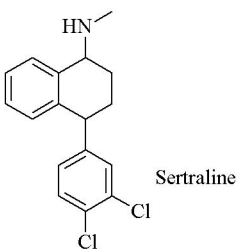
Pyrimdyn compound-6



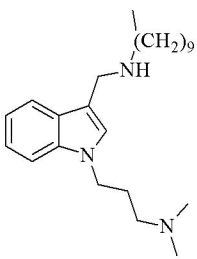
Rhodadyn A_1



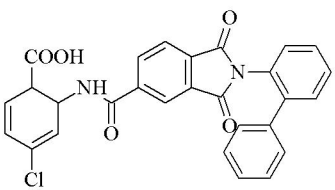
Compound-5, $DYRK_1A$ inhibitor



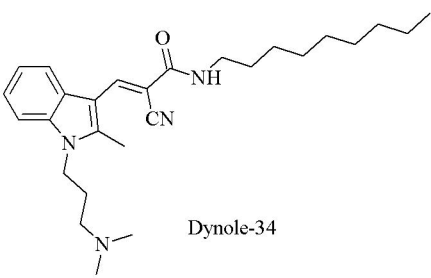
Sertraline



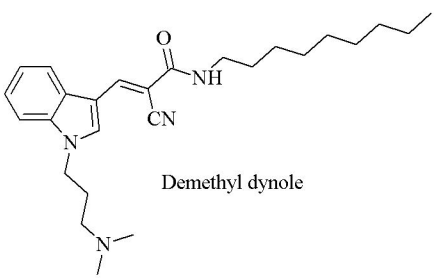
Indole-24



Phthaladyn-1



Dynole-34



Demethyl dynole

Figure 7