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Review

Elucidating the functionality of kinesins: An overview of small molecule inhibitors

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ABSTRACT

Kinesin motor proteins are ubiquitously involved in multiple fundamental cellular processes, coordinating transport and mediating changes to cellular architecture. Thus, specific small molecule kinesin inhibitors can shed new light on the functions of kinesins and the dynamic roles in which they participate. Here we review the range of known inhibitors, their key characteristics, and specificity, and discuss their potential suitability for chemical genetics as starting points to further investigate complex kinesin-mediated processes.

Contents

Abbreviations: AMP-PCP, 5′-adenylyl (beta, gamma-methylene)diphosphonate; AMP-PNP, adenylyl-imidodiphosphate; AS, adociasulfate; CENP-E, centromere-associated protein-E; DPC-4, *deleted in pancreas cancer locus 4*; Hklp2, human kinesin-like protein 2; HSET, human spleen embryo testes; HT, high throughput; KLC, kinesin light chain; KRMP1, kinesin-related motor interacting with Pin1; KRPs, kinesin related proteins; MCAK, mitotic centromere-associated kinesin; MTs, microtubules; Ncd, nonclaret disjunctional; Paprotrain, passenger proteins transport inhibitor; PP1, protein phosphatase 1; PRC1, protein regulator of cytokinesis 1; SAR, structure–activity relationship; SPR, surface plasmon resonance; SQAGs, sulfoquinovosylacylglycerols; STLC, *S*-trityl L-cysteine; TPX2, targeting protein for Xklp2; Xklp2, *Xenopus* kinesin-like protein 2.

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1. Introduction

1.1. Inhibitors as tools to dissect complex molecular motor-driven processes

Chemical genetics refers to a research method whereby chemical compounds with known specificity for a given target are used to study its function in cells, or even entire organisms [1]. In recent years, compounds have been used intelligently either alone or in combination to gain "insights into complex biological processes": of particular interest are those which can be applied to dissecting mitotic processes [2–4]. Compared to siRNA studies, chemical compounds usually have a fast mode of action, are often reversible, and do not deplete the targeted proteins from the cell, thus providing high experimental temporal resolution [2]. Developing specific kinesin inhibitors may therefore enable us to more precisely examine the role of kinesin family members, and allow questions regarding the temporal need of a particular motor protein to be addressed, particularly throughout mitosis. Specific inhibitors may have additional value since mitosis and particularly MT-based dynamic processes may be valid chemotherapeutic targets. Their value is, however, inextricably linked to their target specificity: the less specific a compound is, the more complex the subsequent deconvolution and interpretation of the observed cellular effects become. This is an important limitation in their practical use.

1.2. The kinesin superfamily

Eukaryotic cells possess a plethora of molecular machines that coordinate intracellular transport along cytoskeletal filaments and are fundamental to create or maintain dynamic morphological changes in cellular architecture. They belong to three superfamilies: myosin, kinesin and dynein. All employ adenosine triphosphate (ATP) in producing a directed force along microtubule (MT) or actin tracks to perform their multiple roles. Kinesins form a superfamily of at least 650 distinct MT-dependent motor proteins, so far found only in eukaryotes [5]. This superfamily is divided into at least fourteen families (kinesin-1 to kinesin-14) by phylogenetic analysis of their characteristic motor domains of 330–440 residues in size [6]. Outside of the motor domain, kinesins are structurally divergent with very little sequence conservation. The motor domain is situated at either the N-terminal (N-type kinesins), internally (Kin I/M-type kinesins), or the C-terminal (C-type kinesins). N-terminal motors move to the plus-end of MTs (i.e. the direction where β-tubulin is pointing in each tubulin heterodimers on the MT lattice), whereas C-type kinesins travel to the MT minus end. M-type kinesins (kinesin-13) do not move but rather diffuse along MTs until they reach the end where they promote MT depolymerisation [7]. A typical N-terminal kinesin of the type primarily featured in this review comprises of the motor domain containing the nucleotide binding pocket and MT interacting regions, followed by a neck/neck linker region. This connects the motor domain to an internal α-helical region, thereby forming a coiled coil which is responsible for oligomerisation into dimers or even higher oligomers. Following the coiled-coil region is the C-terminal tail domain, where interactions with cargo either directly or indirectly occur, thereby affording kinesins their diverse functionalities in transport, mitosis and meiosis [5,8]

This review aims to inform researchers about known kinesin inhibitors. The rationale behind this is two-fold: (1) an increasing number of kinesin inhibitors are known in the patent literature, but have not yet reached the public domain; (2) while a number of excellent in-depth reviews on kinesin inhibitors are available, these primarily focus on Eg5 with a limited discussion on inhibitors of other kinesins [9–11]. The fundamental value of these novel inhibitors as tools to dissect and study of the mechanistic details of complex cellular processes has therefore been overlooked. Our focus is restricted to inhibitors that are specific to at least the kinesin superfamily: ATP-like compounds such as AMP-PNP and AMP-PCP were therefore excluded. All compounds described in this review are listed in Supplementary Table 1 along with the commercial supplier or reference describing the synthesis. We also provide an overview of the key characteristics and functions of the kinesins discussed in this review.

2. Kinesin-1 family: conventional kinesin inhibitors

Conventional kinesin, i.e. kinesin-1, was the first kinesin identified [12,13]. Three kinesin-1 motors are expressed in mammals: Kif5A, Kif5B and Kif5C. While Kif5B is found ubiquitously, Kif5A and Kif5C seem to be specifically expressed in neurons [14]. Kinesin-1 motors are plus-end directed, processive dimers with extensive involvement in intracellular transport. During axonal, dendritic and conventional cargo transport (reviewed in [15]), they can bind cargos directly to their tail domains, through association with partner proteins or *via* forming tetramers with kinesin light chains (KLCs). Consequently, kinesin-1 inhibitors may be of great use in understanding their multiple roles.

While numerous inhibitors of kinesin-1 have been identified, none are specific and thus have limited practical use. The natural product adociasulfate-2 (AS-2, Fig. 1, compound **1**), isolated from the marine sponge *Haliclona* was the first kinesin inhibitor identified [16]. AS-2 inhibited the MT-stimulated ATPase activity of the fungal kinesin T1-γ from *Thermomyces lanuginosus* with IC_{50} = 2.7 μM, and disrupted MT attachment during an *in vitro* motility assay. AS-2 is competitive with MTs, but does not compete with ATP and is proposed to interact at the conserved MT binding site [16–18]. While AS-2 does not inhibit ATPases such as rabbit kidney ATPase, EDTA-activated myosin II or pyruvate

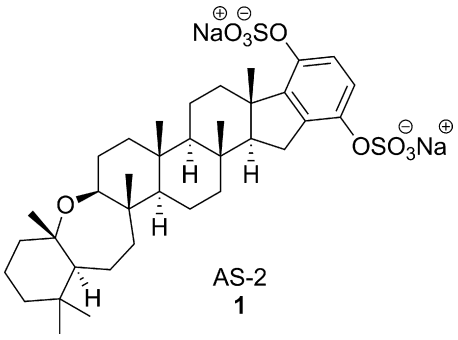

Fig. 1. Structure of adociasulfate-2.