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The discovery and optimization of hexahydro-2*H*-pyrano[3,2-*c*]quinolines (HHPQs) as potent and selective inhibitors of the mitotic kinesin-5

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ABSTRACT

Here we describe the discovery and optimization of hexahydro-2*H*-pyrano[3,2-*c*]quinolines (HHPQs) as potent and selective inhibitors of the mitotic kinesin-5 originally found during a high-throughput screening (HTS) campaign sampling our in-house compound collection. The compounds optimized subsequently and characterized herein were potently inhibiting the ATPase activity of Kinesin-5 and also exhibited consistent cellular activity, in that cells arrested in mitosis and apoptosis induction could be observed. X-ray crystallographic data demonstrated that these inhibitors bind in an allosteric pocket of Kinesin-5 distant from the nucleotide and microtubule binding sites. The selected clinical candidate **EMD 534085** caused strong growth inhibition in human tumor xenograft models using Colo 205 colon carcinoma cells at doses below 30 mg/kg administered twice weekly without showing severe toxicity as determined by loss of body weight.

The process of mitosis is a highly attractive point of therapeutic intervention in cancer therapy and a variety of antimitotic drugs are successfully being used in the clinic.[1] Especially microtubule targeting inhibitors are highly efficacious; however, these drugs are associated with a variety of side effects,[2] including peripheral neuropathies.[3] Moreover, the clinical efficacy of antimitotic drugs has been hampered due to the development of drug resistance. Therefore, it is of great interest to identify novel antimitotic therapeutics with alternate mechanisms of actions to either eliminate or reduce neurotoxicities as well circumvent drug resistance. Several potential new targets (e.g., mitotic kinases, kinesin ATPases) have been identified and are currently in preclinical and clinical development by many pharmaceutical companies.[4]

The mitotic kinesin-5 (KSP, KIF11, Eg5) belongs to this group of novel antimitotic targets. Kinesins are ATP-dependent motor proteins which transport cargoes along the microtubule or participate in chromosome or spindle movements.[5] Mitotic kinesins function during mitosis only. The mitotic kinesin-5 is essential for proper mitotic spindle formation.[6] Kinesin-5 interacts with the spindle and is thought to stabilize the bipolar architecture of the spindle apparatus.[7] The first identified inhibitor against Kinesin-5 was Monastrol, discovered by the groups of Stuart Schreiber and Tim Mitchison.[8] Analysis of Monastrol's mode of action has shown that the activity to hydrolyze ATP is absolutely required for its proper function.[9] The typical phenotype caused by kinesin-5 inhibition such as M phase arrest with characteristic monoastral spindles in cancer cells was nicely shown applying **EMD 534085** and other kinesin-5 inhibitors.[9c]

Here, we describe the discovery and optimization of hexahydro-2*H*-pyrano[3,2-*c*]quinolines (HHPQs) as potent and selective inhibitors of the mitotic kinesin-5 originally identified in the high-throughput screening (HTS). We were able to further optimize our initially discovered inhibitors to high potency and selectivity in line with consistent cellular mechanism of action. Protein X-ray structures of kinesin-5 in complex with ADP and several HHPQs demonstrate that these inhibitors bind in an allosteric pocket of Kinesin-5 distant from the nucleotide and microtubule binding sites.

Small molecule inhibitors of Kinesin-5 were found in a HTS of our in-house compound collection. An in vitro ATPase assay in the presence of microtubules measuring the compound's ability

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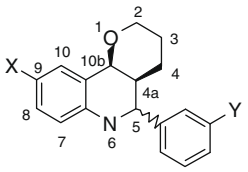


1	X=Y=H	IC50 > 10 μM
2	X=*tert.*-Bu, Y=H	IC50 = 300 nM
3	X=*tert.*-Bu, Y=F	IC50 = 870 nM
4	X=*tert.*-Bu, Y=Cl	IC50 > 10 μM

Figure 1. HHPQs as kinesin-5 inhibitors.

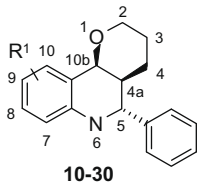
to prevent the hydrolysis of ATP to ADP was established, providing a simple and robust biochemical assay to determine the potency of enzyme inhibition.[10]

In an initial HTS screen around 1000 HHPQs out of 232,000 compounds were tested, of which 40 confirmed hits were identified. A R-group analysis showed clearly that the 9-position of the HHPQs plays an important role for the activity as indicated for compound **2** with a *tert*-butyl group (Fig. 1, IC_{50} = 300 nM) compared to the non-substituted compound **1** (IC_{50} >10 μM). As well, steric restrictions in the phenyl substituent of the molecule were identified; the potency dropped dramatically by exchanging a fluoro by a chloro substituent (compounds **3** and **4**).

The HHPQs were readily assembled by modification of the Povarov reaction[11] as shown in Scheme 1. The inverse electron-demand aza Diels–Alder reaction worked well with a wide range of anilines and aromatic aldehydes with dihydropyran in a one-pot reaction using 1 equiv of TFA at room temperature. The product mixture contained 2 diastereomers mainly in favor of the *trans*-product. Many publications described this reaction by variation of the Brønsted[12] or Lewis[13] acid catalyst and solvent.[14] Others induce the reaction photochemically[15] or describe solid-supported[16] or stereoselective approaches[17] to the compound class. In our case the two diastereomers could be easily separated by chromatography or crystallization. Since we could show that the *trans*-isomer is more stable and potent than the *cis*-isomer (easily forms compound **9**, also isolated in various yields as a side product during the reaction) the SAR is described for the racemic *trans*-isomer. For example, *trans*-isomer **31** (Table 2) expressing a kinesin-5 activity of IC_{50} = 40 nM is five times more potent than its corresponding *cis*-isomer, as well as *trans*-isomer **44**, which is 3 times more potent than its *cis*-isomer.

Looking at the SAR shown in Table 1 one can easily see that a spherical hydrophobic substituent at the C-9 position gives the most potent compounds in accordance to the protein X-ray structure of kinesin-5 in complex with **EMD 534085** (Fig. 2),[18] which can also be compared to co-crystal structures of several other inhibitors described in the literature.[19] The aromatic portion in the western part in addition with a non-lipophilic *tert*-butyl substituent at C-9 (compound **15**, Table 1) fills-up perfectly the hydrophobic pocket of the allosteric binding site of the kinesin-5 protein. The potency dropped when the steric bulkiness was reduced from *tert*-butyl to isopropyl (**14**) to ethyl (**12**) to methyl (**11**) and chlorine (**18**) or—in opposite—was increased (e.g., *n*-Pr, **13**). The CF_3 derivative appeared to be equally potent (**16**). In agreement with structural information obtained by protein X-ray crystallography, all attempts to introduce more polar substituents, like nitriles (**19**, **20**), amines (**23**), alcohols (**24**) or even acid functions (**22**) let to less active or inactive compounds. All efforts to vary the position of the hydrophobic group in the western part or to introduce additional substituents also reduced the potency dramatically. Only fluorine in 7-position was accepted and in-

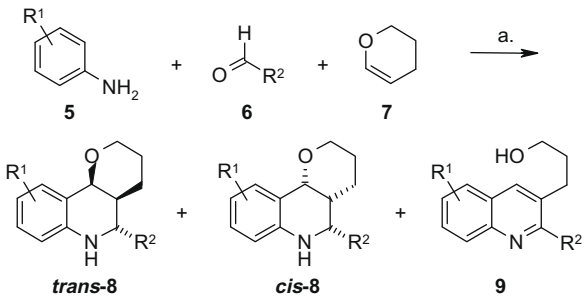
Table 1
Western part SAR



10-30

Entry	R^1	Kinesin-5 IC_{50} (nM)	Proliferation in HCT116 IC_{50} (nM)
10	H	>10,000	n.d.[a]
11	9-Me	420	840
12	9-Et	130	370
13	9-*n*-Pr	360	1800
14	9-*i*-Pr	130	170
15	9-*tert*-Bu	120	250
16	9-CF_3	110	280
17	9-SF_5	910	3400
18	9-Cl	720	1400
19	9-CN	>10,000	n.d.[a]
20	9-CH_2CN	500	580
21	9-OCF_3	3700	>10,000
22	9-COOH	>10,000	n.d.[a]
23	9-NMe_2	5300	8600
24	9-$(CH_2)_2OH$	10,000	>10,000
25	8-*tert*-Bu	>10,000	n.d.[a]
26	7-F,9-CF_3	90	130
27	7-F,8-Cl	540	1600
28	8,9-Di-Me	1250	1500
29	7,9-Di-Me	>10,000	n.d.[a]
30	8,10-Di-Me	>10,000	n.d.[a]

[a] Not determined.



5 **6** **7**

***trans*-8** ***cis*-8** **9**

Scheme 1. Synthesis of basic HHPQs. Reagents and conditions: (a) trifluoroacetic acid (1 equiv), acetonitrile, 0–5 °C→rt, 4 h.

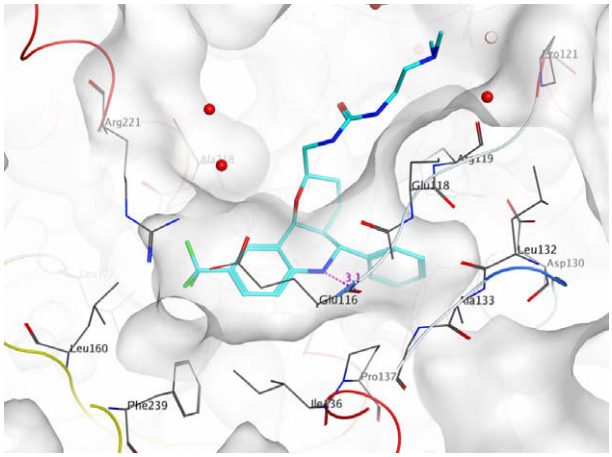


Figure 2. X-ray structure of **EMD 534085** in the allosteric binding pocket of kinesin-5.