Check for updates

EurJOC
European Journal of Organic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

Title: 2-Sulfonylpyrimidines target the kinesin HSET via cysteine alkylation

Authors: Herbert Waldmann, Tim Förster, Erchang Shang, Kenshiro Shimizu, Emiko Sanada, Beate Schölermann, Mylene Hübecker, Gernot Hahne, Maria Pascual Lopez Alberca, Petra Janning, Nobumoto Watanabe, Sonja Sievers, Fabrizio Giordanetto, Takeshi Shimizu, Slava Ziegler, and Hiroyuki Osada

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201900586

Link to VoR: http://dx.doi.org/10.1002/ejoc.201900586

Supported by ACES Asian Chemical Editorial Society

WILEY-VCH

10.1002/ejoc.201900586

2-Sulfonylpyrimidines target the kinesin HSET via cysteine alkylation

Dr. Tim Förster[a,b], Dr. Erchang Shang[a], Kenshiro Shimizu[c], Emiko Sanada[c,e], Beate Schölermann[a], Mylene Huebecker[a,d], Dr. Gernot Hahne[a], Dr. Maria Pascual López-Alberca[a,e], Dr. Petra Janning[a], Prof. Dr. Nobumoto Watanabe[e], Dr. Sonja Sievers[a], Dr. Fabrizio Giordanetto[f,g], Dr. Takeshi Shimizu[c], Dr. Slava Ziegler[a], Prof. Dr. Hiroyuki Osada[c,e], Prof. Dr. Dr. h.c. Herbert Waldmann*[a,b]

[a] *Max Planck Institute of Molecular Physiology, Department of Chemical Biology, Otto-Hahn-Str. 11, 44227 Dortmund, Germany*

[b] *Technical University of Dortmund, Faculty of Chemistry and Chemical Biology, Otto-Hahn-Str. 6, 44227 Dortmund, Germany*

[c] *Chemical Biology Research Group, RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

[d] *Current address: University of Oxford, Department of Pharmacology, Oxford OX1 3QT, UK*

[e] *RIKEN-Max Planck Joint Research Division for Systems Chemical Biology, Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

[f] *Taros Chemicals GmbH & Co. KG, Emil-Figge-Str. 76, 44227 Dortmund*

[g] *Current address: D. E. Shaw Research, 120 West, 45th Street, New York, NY 10036, USA*

* corresponding author: Herbert Waldmann, email: Herbert.Waldmann@mpi-dortmund.mpg.de; URL: https://www.mpi-dortmund.mpg.de/research-groups/waldmann

Accepted Manuscript

This article is protected by copyright. All rights reserved.

Email addresses:

Tim Förster: tim.foerster@mpi-dortmund.mpg.de

Erchang Shang: shangech@163.com

Kenshiro Shimizu: kenshirou.shimizu.bs@gmail.com

Emiko Sanada: sanadae@riken.jp

Beate Schölermann: beate.schoelermann@mpi-dortmund.mpg.de

Mylene Hübecker: mylene.huebecker@pharm.ox.ac.uk

Gernot Hahne: gernot.hahne@web.de

Maria Pascual Lopez Alberca: mpascualla@gmail.com

Petra Janning: petra.janning@mpi-dortmund.mpg.de

Nobumoto Watanabe: nwatanab@riken.jp

Sonja Sievers: sonja.sievers@mpi-dortmund.mpg.de

Fabrizio Giordanetto: fabrizio.giordanetto@deshawresearch.com

Takeshi Shimizu: tshimizu@riken.jp

Slava Ziegler: slava.ziegler@mpi-dortmund.mpg.de

Hiroyuki Osada: cb-secretary@ml.riken.jp

Herbert Waldmann: herbert.waldmann@mpi-dortmund.mpg.de

Keywords: inhibitors, Protein modifications, Biological activity, Antitumor agents

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

Abstract

Supernumerary centrosomes are a source of aneuploidy and cells have adopted different mechanisms to avoid multipolar mitoses. The kinesin HSET is required for pseudo-bipolar mitoses in cancer cells with amplified centrosomes and suppression of HSET activity is regarded a potential anti-cancer approach. We report the identification of 2-sulfonylpyrimidine inhibitors of HSET enzymatic activity. HSET inhibition results in establishment of multipolar mitoses and simultaneous inhibition of the kinesin Eg5 restored bipolar spindle formation. Correlation of structure to activity revealed that the 2-sulfonylpyrimidinyl group is required for the activity of the compound class and that 2-sulfonylpyrimidines covalently modify HSET. In addition, these electrophiles react with glutathione, thereby causing oxidative stress. This general reactivity needs to be taken into account if 2-sulfonylpyrimidines will be employed in the development of biologically active small molecules.

Introduction

Covalent inhibition of protein function and activity has proven to be a viable option in drug discovery, e.g., in the development of novel anti-infectives and anti-cancer drugs.[1] In addition, nature employs covalent targeting by endogenous covalent ligands to regulate proteins, e.g., IKKβ and PPARδ,[2] and various natural products covalently modulate their targets.[3] Effective and safe covalent inhibitors must have tuned reactivity to avoid unspecific toxicity resulting from promiscuous reactions or metabolic inactivation[4], which poses particular challenges for their development. For structure-based design of covalent inhibitors, in general a reversible inhibitor is equipped with an electrophilic moiety (‘warhead’) that targets an amino acid, which is proximal to the binding site. Most frequently, cysteine is targeted, followed by lysine and other nucleophilic amino acids.[5]

This article is protected by copyright. All rights reserved.

Accepted Manuscript

Modulation of mitosis has been considered as a promising anti-cancer approach. However, the use of anti-mitotic drugs is limited by severe side effects mostly due to simultaneous targeting of non-cancerous cells. Thus, strategies that exploit differences between tumor cells and normal cells are in demand. Supernumerary centrosomes have been described for several cancers including breast, pancreas and prostate tumors.[6] Cancer cells with amplified centrosomes employ strategies to suppress multipolar mitosis, e.g., extra copies of chromosomes are extruded, inactivated, segregated or clustered to facilitate pseudo-bipolar mitosis.[7] Suppression of multipolar mitoses may offer novel opportunities for anti-cancer research, since small molecules interfering with this process may preferentially target cancer cells.[6a] The kinesin HSET (also termed KIFC1 or KNSL2) is important for cancer cells but dispensable in normal cells.[6a] HSET is a member of the kinesin-14 subfamily of minus-end directed kinesins that function as spindle pole organizing components.[8] HSET is involved in cross-linking and bundling of microtubules.[9] In normal cells, HSET is required for bipolar spindle assembly under acentrosomal conditions, while it is not essential for spindle assembly in the presence of functionally dominant centrosomes.[9b] In cancer cells, HSET is critical for clustering extra centrosomes and ensures pseudo-bipolar cell division.[6a] In addition, in tumor cells with correct centrosome number acentrosomal microtubule polymerization generates acentrosomal poles and requires HSET for proper clustering and focusing of these acentrosomal microtubule organizing centers for bipolar spindle assembly.[10] Therefore, HSET is an emerging target in anti-cancer drug discovery and recently the first inhibitors of HSET were identified.[11]

Here we report the discovery of 2-sulfonylpyrimidines as covalent HSET inhibitors that impair progression through mitosis due to the formation of multipolar mitotic spindles. Due to their electrophilic nature 2-sulfonylpyrimidines also react with glutathione (GSH) and cause oxidative stress. This general reactivity needs to be taken into account in the use of 2-sulfonylpyrimidines for bioactive compound development.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

Results

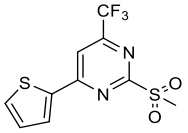
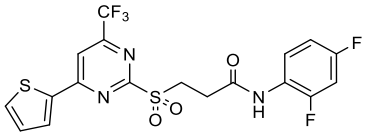
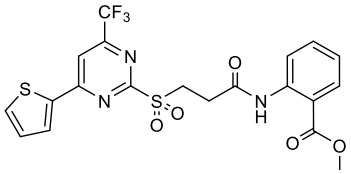
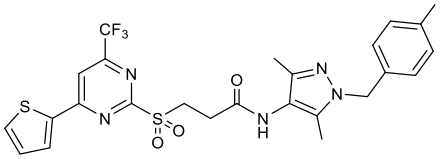
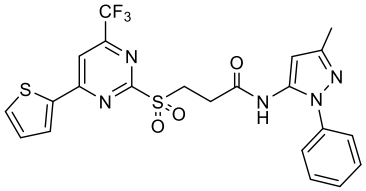
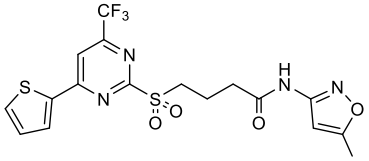
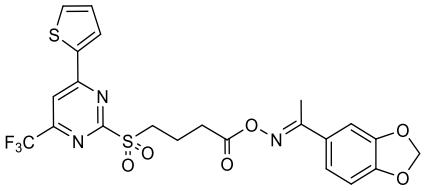
To identify inhibitors of HSET, a collection of 148,000 compounds was screened for inhibition of HSET's ATPase activity in the presence of microtubules by means of the ADP-Glo assay.[12] Hit compounds were assessed for inhibition of luciferase and for influence on microtubules to reveal false positives. This led to the identification of 2-sulfonylpyrimidines as a prominent class of HSET inhibitors (Table 1).

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

Table 1 **Hits identified in screening for HSET inhibition**. The activity of HSET was determined in the presence of microtubules using the ADP-Glo system. Data are mean values (n≥2) ± SD.

Entry	No.	Structure	IC_{50} ± s.d. [µM]
1	**1**		3.4 ± 0.9
2	**2**		3.8 ± 1.1
3	**3**		3.9 ± 1.1
4	**4**		5.8 ±4.4
5	**5**		6.6 ± 3.8
6	**6**		14.8 ± 9.9
7	**7**		16.5 ± 4.5

Accepted Manuscript

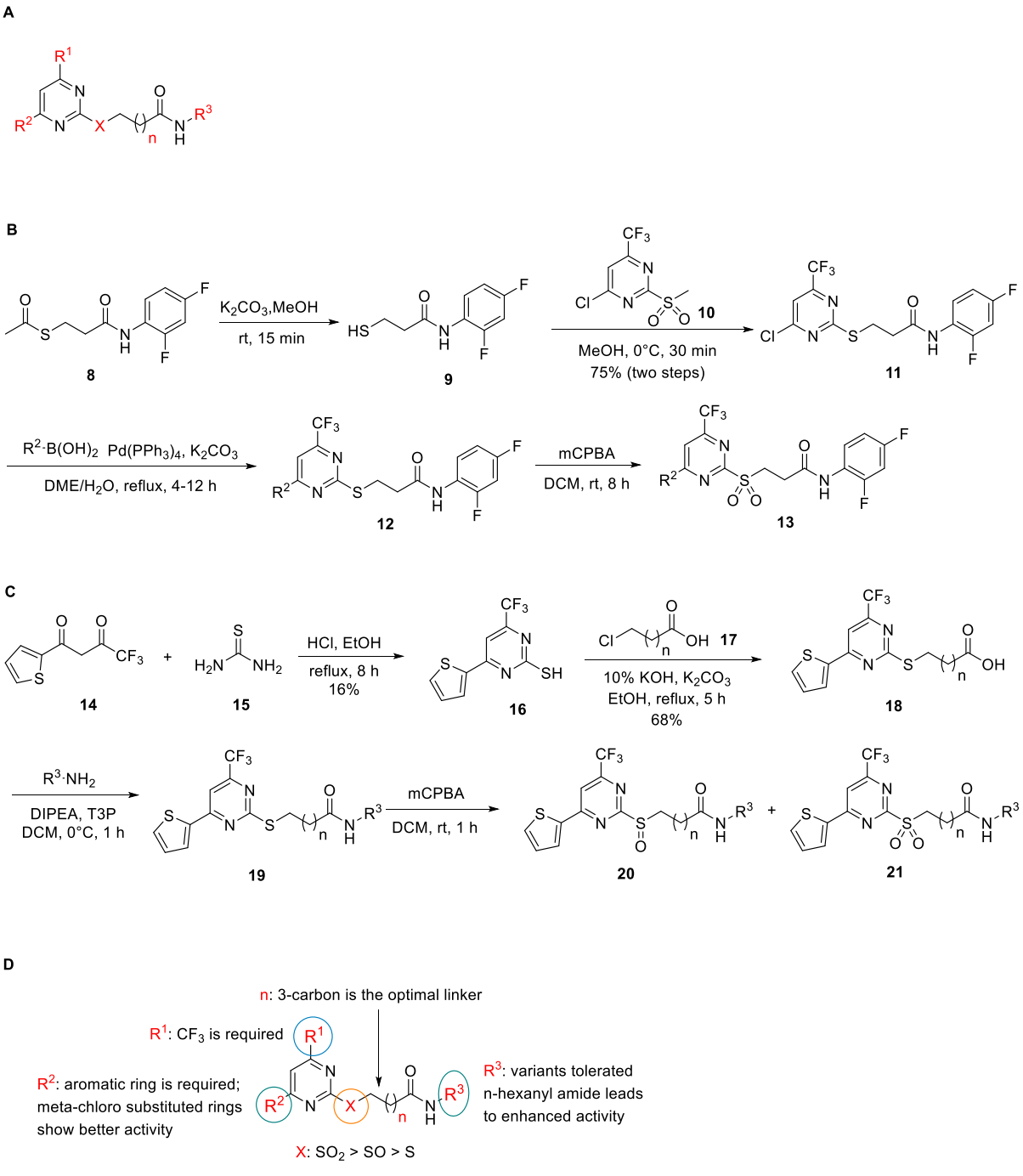
This article is protected by copyright. All rights reserved.

We designed and synthesized analogues based on the general structure depicted in Scheme 1A to establish a structure-activity relationship (SAR) and explored (i) the role of the trifluoromethyl group (R^1), (ii) replacement of the thiophene (R^2), (iii) the importance of sulfone-, sulfoxide- and thioether substituents (X), (iv) the length of the carbon linker between pyrimidine and amide (n), and (v) variation of amides (R^3). Scheme 1 shows two general strategies for the synthesis of the compounds with different R^2 group and different amides respectively (Scheme 1B-1C). The syntheses of further analogues were achieved with minor modifications of these synthetic routes (see the Experimental section for details). To investigate different R^2 substituents, aryl chloride **11** was synthesized by means of an efficient one-pot reaction. Deprotection of thioacetate **8** with potassium carbonate led to a solution of thiol **9**, which was added dropwise to a solution of pyrimidine **10** in methanol at 0°C to give the chloride **11**. With this compound in hand, different R^2 groups were introduced by Suzuki-Miyaura coupling. The resulting thioethers **12** were further oxidized with *meta*-chloroperoxybenzoic acid (mCPBA) to the target sulfones **13** (Scheme 1B). Different amides were introduced while retaining the trifluoromethyl- and thiophene groups. As shown in Scheme 1C, the condensation of diketone **14** and thiourea **15** led to pyrimidine **16**, which reacted with 3-chloropropionic acid **17** to yield acid **18**. Formation of amides **19** followed by the mCPBA mediated oxidation afforded mixtures of sulfoxides **20** and sulfones **21**, which were separated and subjected to biochemical investigation.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586



Scheme 1. General structure of the 2-sulfonylpyrimidines. (A) (B-C) Synthetic routes for analogues with different substituents at R^2 position (B) and analogues with different amides (C). (D) Structure-activity relationship for 2-sulfonylpyrimidines.

This article is protected by copyright. All rights reserved.

Accepted Manuscript

All compounds were tested for inhibition of HSET in a manual assay, which resulted in higher IC_{50} values for compound **2** as compared to the automated HTS. The trifluoromethyl- (R^1) as well as the pyrimidine ring are required for inhibition of HSET motor domain ATPase activity (**22 - 25**, see Table 2, entries 9 - 12). Replacement of the sulfone with sulfoxide or thioether reduced the activity of the compound (**19a, 20a**, see Table 2, entries 2 and 3). Variation of the carbon linker revealed that the three-carbon linker without side chain is most favorable (**21l - 21p**, see Table 2, entries 4 - 8).

Compared to compound **2**, replacement of the 2-thiophene with 2-furan or phenyl group retained the activity (**13d, 13e,** see Table 3, entries 5 and 6). However, replacement of the 2-thiophene with 3-thiophene or 3-methoxyphenyl resulted in a twofold increase of activity (**13a, 13f**, see Table 3, entries 2 and 7). Introduction of a chlorine at the *meta*-position of the aromatic ring led to three- to fivefold enhanced potency (**13g;** see also **13c** as comparable thiophene derivative; Table 3, entries 4 and 8), while analogues with small mono-substituents slightly increased the activity (**13h - 13l,** see Table 3, entries 9 - 13). Moreover, 3,5-di-trifluoromethyl substitution clearly increased the potency (**13o,** see Table 3, entry 16), while introduction of several substituents or bulkier derivatives led to decreased activity (**13n, 13p, 13r, 13s,** see Table 3, entries 15, 17, 19, 20). However, the potency of compound **13q** is comparable to the parent compound (see Table 3, entry 18). Thus, a 3-chlorophenyl or 3,5-ditrifluoromethylphenyl is most favourable as R^2.

Analogues with different amides (R^3) were also prepared based on the synthetic route shown in Scheme 1C. As shown in Table 4, 2,4-dichlorophenyl- (**21a**, see Table 4, entry 2), 3,5-difluorophenyl- (**21b**, see Table 4, entry 3) and phenethyl amides (**21j**, see Table 4, entry 11) led to nearly threefold enhanced HSET inhibition, while derivatives based on different amides (**21c - 21e, 21h - 21i**, see Table 4, entries 4 - 6, 9 - 10) retained or displayed only slightly improved potency. Moreover, some heterocyclic amides had reduced activity (**21f, 21k**, see Table 4, entries 7 and 12). Pleasingly, the replacement of the aromatic amide with

This article is protected by copyright. All rights reserved.

Accepted Manuscript

10.1002/ejoc.201900586

an n-hexanyl amide resulted in a fourfold increase in activity to an IC_{50} of 5.5 µM (**21g**, see Table 4, entry 8).

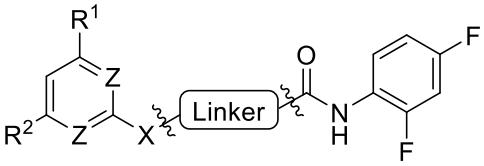
A summary of the SAR study is shown in Scheme 1D: a sulfone and an electron withdrawing group (trifluoromethyl) in the pyrimidine ring are essential for activity, while the thiophene (R^2), linker and amide (R^3) tolerate changes.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

Table 2: **Structure-activity relationship studies of 2-sulfonylpyrimidines.** IC_{50} values for inhibition of HSET activity by compounds with variation of the pyrimidine ring, the trifluoromethyl (R^1), thiophene (R^2) and sulfonyl groups (X) as well as the linker length were determined by means of the ADP-Glo™ Kinase Assay (n=2-3).

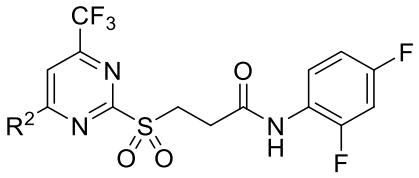


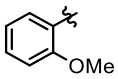
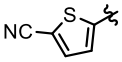
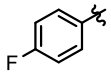
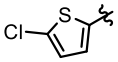
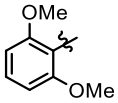
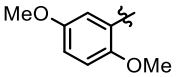
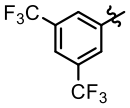
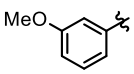
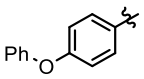
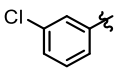
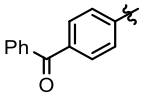
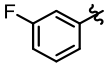
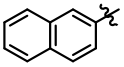
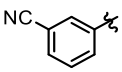
Entry	No.	R^1	R^2	X	Z	Linker	IC_{50} ± s.d. [µM]
1	**2**	CF_3		SO_2	N		19.6 ± 6.2
2	**19a**	CF_3		S	N		> 50
3	**20a**	CF_3		SO	N		> 50
4	**21l**	CF_3		SO_2	N		> 30
5	**21m**	CF_3		SO_2	N		5.9 ± 2.8
6	**21n**	CF_3		SO_2	N		> 30
7	**21o**	CF_3		SO_2	N		10.4 ± 3.0
8	**21p**	CF_3		SO_2	N		13.9 ± 0.5
9	**22**	CH_3		SO_2	N		> 50
10	**23**			SO_2	N		> 50
11	**24**	H	H	SO_2	N		> 50
12	**25**	H	H	SO_2	CH		> 50

Accepted Manuscript

This article is protected by copyright. All rights reserved.

Table 3: **Structure-activity relationship of 2-sulfonylpyrimidines with different substitutents at R^2.** Determination of IC_{50} values for inhibition of HSET activity was performed by means of ADP-Glo assay (n=2-3).



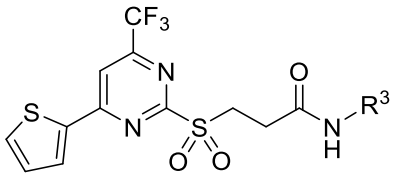
Entry	No.	R^2	IC_{50} ± s.d. [µM]	Entry	No.	R^2	IC_{50} ± s.d. [µM]
1	**2**		19.6 ± 6.1	11	**13j**		9.6 ± 0.7
2	**13a**		10.2 ± 2.5	12	**13k**		11.0 ± 5.2
3	**13b**		21.5 ± 5.1	13	**13l**		8.2 ± 0.5
4	**13c**		5.9 ± 1.3	14	**13m**		26.4 ± 1.6
5	**13d**		18.8 ± 3.1	15	**13n**		>50
6	**13e**		22.2 ± 6.2	16	**13o**		5.1 ± 1.7
7	**13f**		9.7 ± 0.3	17	**13p**		>50
8	**13g**		3.8 ± 0.1	18	**13q**		12.2 ± 5.4
9	**13h**		8.2 ± 1.8	19	**13r**		>50
10	**13i**		9.4 ± 1.8	20	**13s**		>50

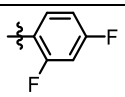
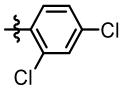
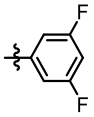
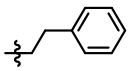
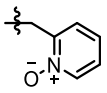
Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

Table 4: **Structure-activity relationship of 2-sulfonylpyrimidines with substitutions at R^3.** Determination of IC_{50} values for inhibition of HSET activity was performed by means of ADP-Glo™ Assay (n=2-3).



Entry	No.	R^3	IC_{50} ± s.d. [µM]	Entry	No.	R^3	IC_{50} ± s.d. [µM]
1	**2**		19.6 ± 6.1	7	**21f**		>30
2	**21a**		8.3 ± 1.3	8	**21g**		5.5 ± 1.4
3	**21b**		7.0 ± 0.8	9	**21h**		18.0 ± 6.8
4	**21c**		10.3 ± 0.9	10	**21i**		16.6 ± 4.4
5	**21d**		14.3 ± 6.6	11	**21j**		7.9 ± 1.1
6	**21e**		14.9 ± 5.8	12	**21k**		>30

Impairment of HSET function by small molecules should cause defective mitosis in cells with formation of additional centrosomes due to failure in centrosome clustering. Thus, the influence of the compound collection on mitosis, particularly focusing on mitotic spindle morphology, was assessed in the human cervix cancer cell line HeLa and breast carcinoma BT-549 cells. HeLa cell populations contain a small number of cells with amplified centrosomes [6a, 11b], while BT-549 is widely used to study HSET activity since 45% of the

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

cells display centrosome amplifications.[6a] Several compounds reduced the cell number or were cytotoxic (see Figure S1and S2). Interestingly, only compound **5** showed specific cellular activity and induced the accumulation of mitotic cells with multipolar mitotic spindles (Figure 1A and 1B, Figure S3A-S3B). This resulted in a 12-fold increased amount of mitotic cells compared to vehicle-treated cells (Figure 1C and 1D). In BT-549 breast carcinoma cells, treatment with **5** led to a threefold increase in the amount of mitotic cells (Figure S3C-S3D). This phenotype is prominent for small molecules that target microtubules.[13] However, compound **5** did not affect the *in vitro* tubulin polymerization (Figure 1E) and thus, most likely does not target tubulin/microtubules.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

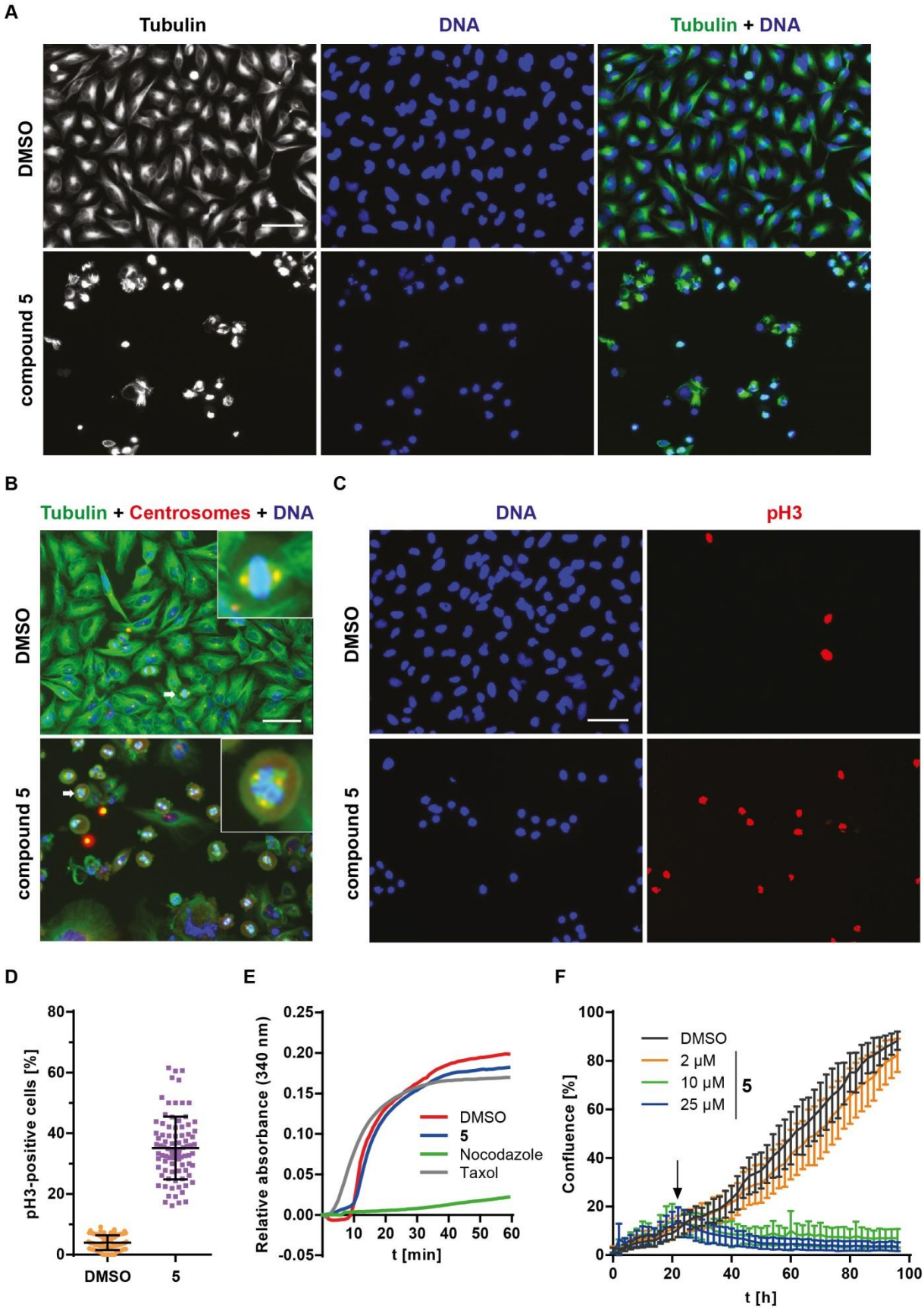


Figure 1. **Influence of compound 5 on mitosis in HeLa cells**. (A) HeLa cells were treated with **5** (25 µM) or DMSO as a control for 24 h prior to staining with an anti-α-tubulin antibody

This article is protected by copyright. All rights reserved.

Accepted Manuscript

(green) and DAPI (blue). Scale bar: 50 µm. (B) Compound **5** causes the formation of multipolar mitotic spindles. HeLa cells were treated were treated with **5** (25 µM) or DMSO as a control for 24 h prior to staining with an anti-α-tubulin antibody (green), anti-pericentrin antibody (red) and DAPI (blue). Scale bar: 50 µm. Arrows indicate inlets. (C and D) Compound **5** leads to mitotic arrest. HeLa cells were treated with **5** (25 µM) or DMSO as a control for 24 h prior to staining with an anti-phospho-histone H3 (pH3) antibody (red) or DAPI (blue). Images (C) and image-based quantification (D) of mitotic cells are shown. Scale bar: 50 µm. Data are percentage values of mitotic cells of 70 (DMSO) and 87 (compound **5**) images (n≥3 biological replicates) ± s.d. (E) Influence of **5** on *in vitro* tubulin polymerization. 50 µM compound **5** and 1 µM of the control substances were mixed with 10 µM tubulin followed by application of 500 µM GTP to start the reaction. Absorption at 340 nm was measured for 60 min and normalized to the value at t=0 for each probe. (F) Cell growth in the presence of compound **5**. HeLa cells were treated with 2, 10 and 25 µM of **5** and confluence was quantified as a measure of viability/cell growth for 72 h using real-time live-cell microscopy. Confluence was normalized to the value at the time of seeding for each probe. Arrow indicates time point of compound addition. Data is representative of three biological replicates.

The minus-end directed activity of HSET is antagonistic to the plus-end directed activity of the kinesin Eg5.[9b] Whereas Eg5 inhibition results in the formation of monoastral mitotic spindles, simultaneous inhibition of Eg5 and HSET can rescue the monoaster phenotype.[9b, 11b, 14] To assess the ability of compound **5** to revert the monopolar mitotic spindle phenotype, HeLa cells were co-treated with the Eg5 inhibitor S-trityl-L-cysteine (STLC) [15] in the presence or absence of 25 µM of **5** and the mitotic spindle architecture was analyzed (Figure 2). While single treatment with STLC or compound **5** led to the formation of monopolar and multipolar mitotic spindles respectively, mitotic spindles resembled a bipolar morphology

This article is protected by copyright. All rights reserved.

Accepted Manuscript

when both inhibitors were present (Figure 2). This finding is in line with HSET inhibition by derivative **5** in cells.

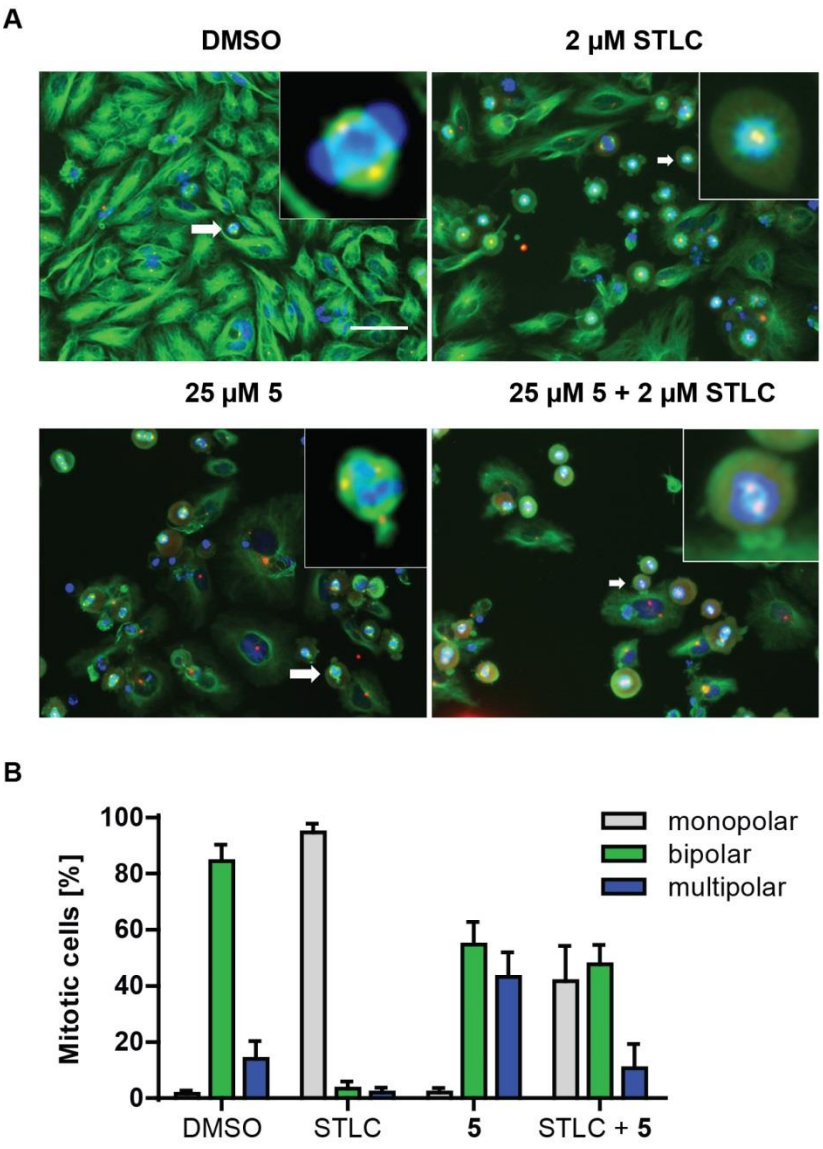


Figure 2. **Compound 5 rescues Eg5 inhibition.** HeLa cells were treated with 25 µM compound **5** with or without 2 µM STLC for 24 h. Cells were fixed and permeabilized prior to staining of DNA, centrosomes and microtubules using DAPI (blue), a pericentrin antibody (red) and an alpha-tubulin antibody (green). Representative images (A) and quantification (B) are shown. (B) Data are mean values ± s.d. of four individual experiments (n=4; 37-139 cells per condition). Scale bar: 50 µm. Arrows indicate inlets.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

Sulfones are good leaving groups when linked to electron-withdrawing aromatic rings.[16] Recently, 2-sulfonylpyrimidines were reported as mild alkylating agents, which react with highly nucleophilic cysteines.[17] Thus, we assumed a covalent binding mode for compound **5** as well. To this end, we monitored formation of covalent adducts of compound **5** and a peptide containing nucleophilic amino acids in an *in vitro* peptide trapping assay using mass spectrometry.[18] Upon incubation of **5** with the peptide Ac-YACAKASAHA, a new peak (m/z 1262.27) was detected in addition to the peptide peak (m/z 1034.45), thus proving the formation of peptide-compound conjugate. The mass increase (Δ m/z 227.82, measured) corresponds to the transfer of the 4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine moiety to the test peptide (Δ m/z 228.00, theoretical) (Figure 3A and B). To explore the putative covalent binding of compound **5** to HSET, nano-HPLC-MS/MS measurements upon incubation of HSET with 50 µM **5,** followed by tryptic digestion of the protein and a database search were performed. We considered the proposed nucleophilic aromatic substitution (S_NAr) for 2-sulfonylpyrimidines[17], elimination of substituted sulfinic acid and a transfer of 4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine to nucleophilic amino acids (Figure 3A). Oxidation of methionine, carbamindomethylation of cysteine and 4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine addition to cysteine, serine, lysine, or histidine, respectively, were defined as variable modifications. The modification of two prominent cysteine residues (Cys 485 and Cys 663) with 4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine according to the reaction proposed by Bauer et al.[17] were clearly identified (localization probability of 1 in both cases, PEP 1.1E-19 for Cys 485 and 2.1E-30 for Cys 663). Two further modifications (His 370 and Cys 405) were assumed but the localization and/or identification probabilities were worse (His 370: localization probability 0.999972, PEP 0.007; Cys 405: localization probability 0.864, PEP 1.21E-09). The MS/MS spectrum of the tryptic peptide KGQGGE**C**EIR modified at position Cys 485 is shown in Figure 3C (for the remaining MS/MS spectra see Figure S4) The modified residues were mapped near the protein surface, i.e., not the active site (Figure 3D). Thus, their alkylation is most likely due to favorable environment, i.e.,

This article is protected by copyright. All rights reserved.

Accepted Manuscript

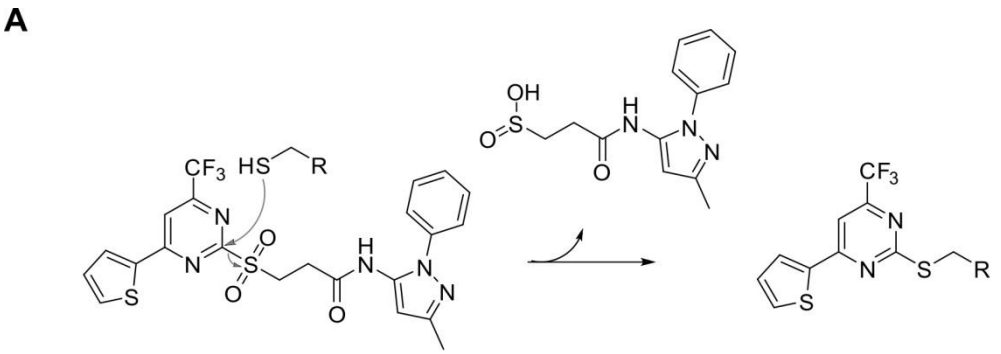
10.1002/ejoc.201900586

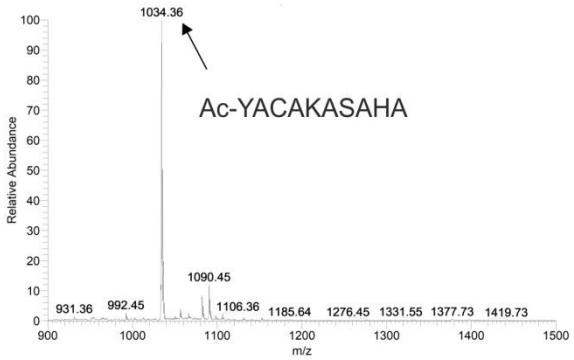
accessibility and nucleophilicity. These results confirm **5** as an alkylator and the covalent modification of HSET as the likely cause for HSET inhibition by **5**.

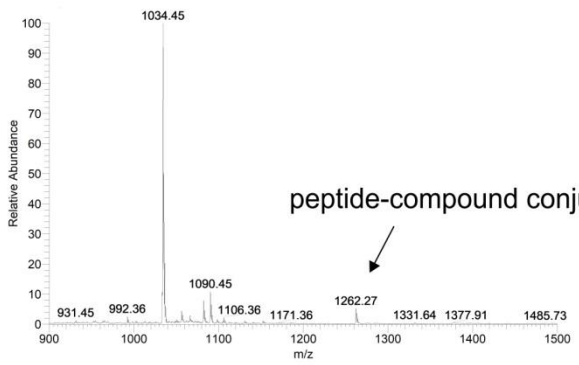
Accepted Manuscript

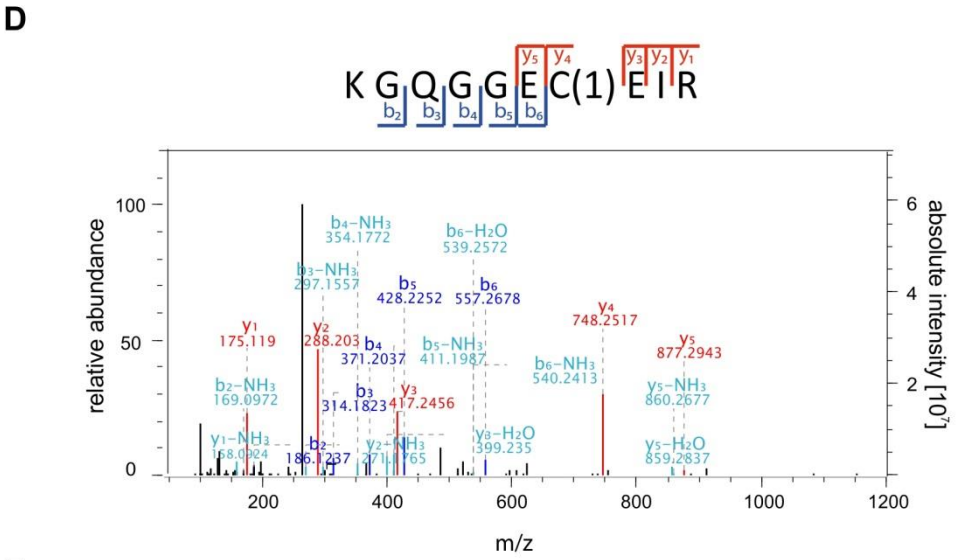
This article is protected by copyright. All rights reserved.

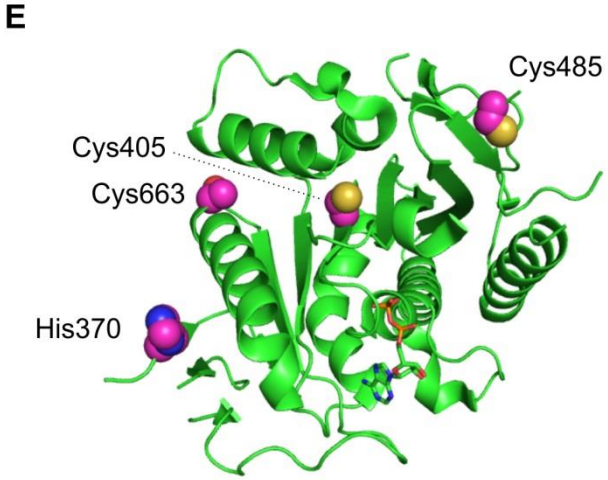
10.1002/ejoc.201900586











Accepted Manuscript

This article is protected by copyright. All rights reserved.

Figure 3**. Compound 5 alkylates HSET**. (A) Proposed mechanism of protein alkylation by **5**. (B and C) The model peptide Ac-YACAKASAHA was incubated with **5** (50 µM) and was then subjected to mass spectrometry. MS spectra for the peptide (B) and peptide + **5** (C) are shown. (D) Formation of compound-HSET conjugate. HSET was incubated with **5** (50 µM) followed by MS/MS analysis. The spectrum for the alkylated peptide ^{479}KGQGGECEIR^{488} is shown. The localization probability resulting from MaxQuant search is mentioned in brackets behind the modified residue. (E) Mapping of the alkylated residues in the structure of HSET. PDB: 5WDH.

The reactivity of sulfonyl pyrimidines may also be directed towards other cellular thiols than cysteines such as glutathione (GSH). Since sulfonyl-containing small molecules have been shown to react with GSH,[19] we analyzed the stability of compound **5** in the presence of 5 mM GSH, which corresponds to the estimated thiol concentration in cells.[20] Compound **5** was added at a concentration of 25 µM, resulting in a 200-fold excess of GSH. Mass spectrometry analysis of an aqueous solution of compound **5** revealed one peak. In contrast, three peaks were detected upon incubation of **5** in the presence of 5 mM GSH (Figure 4A). Mass analysis confirmed the nucleophilic substitution of the sulfonyl part of **5** and the formation of the 4-(thiophen-2-yl)-6-(trifluoromethyl)pyrimidine-2-yl-GSH conjugate (Figure 4A). Quantification of the unreacted compound mass peak revealed partial scavenging (51.7 ± 16.7 %) of compound **5** by thiols (Figure 4B). However, in the presence of GSH a large amount of unreacted compound remained, which indicates slow substitution of the sulfonyl by GSH. Thus, a sufficient amount of intact compound is likely to reach its target protein in cells under physiological conditions.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

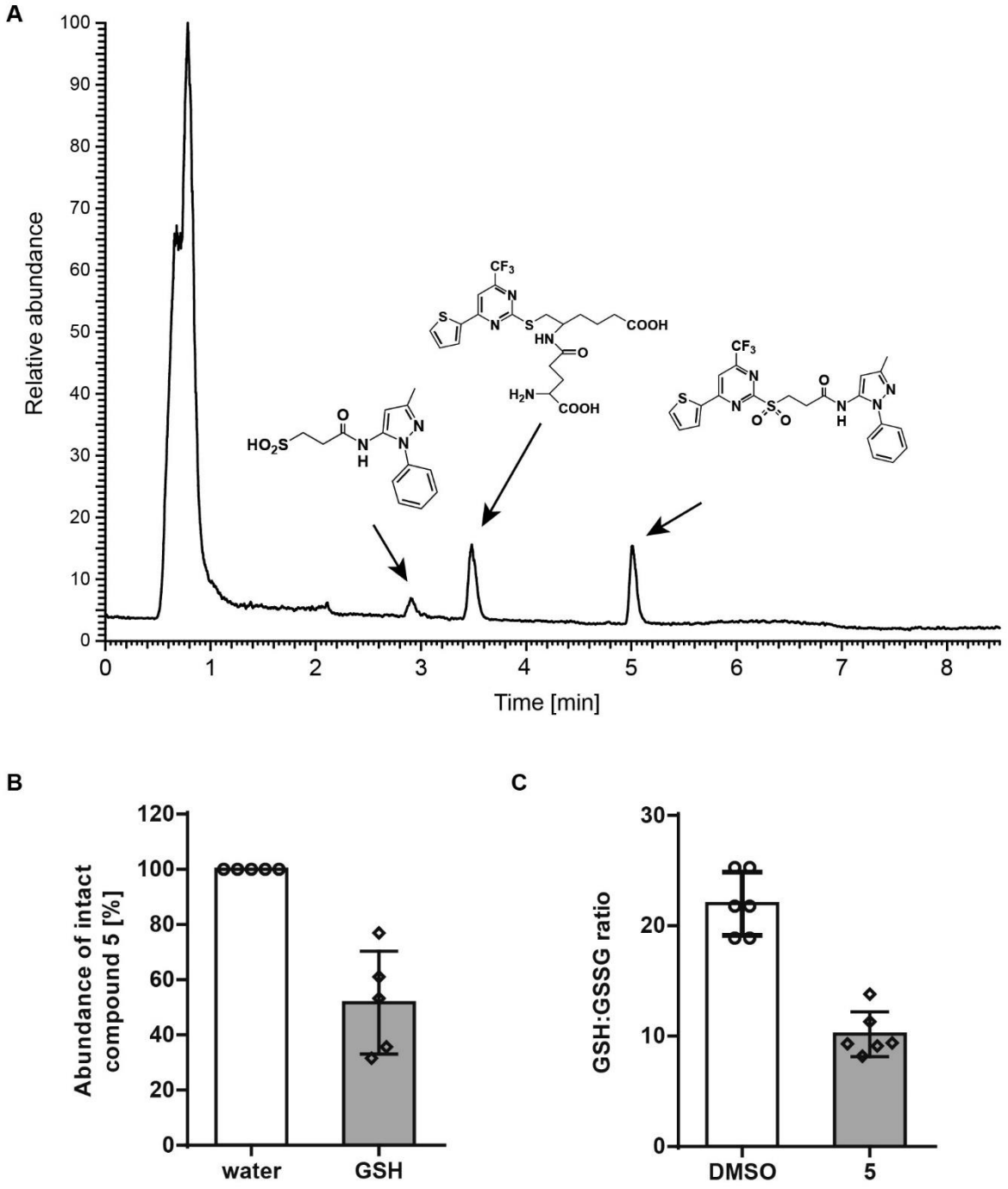


Figure 4. Compound 5 conjugates to GSH. (A und B) 5 mM GSH was incubated with **5** (25 µM) for 24 h at 37 °C prior to ESI-MS analysis. Representative spectra are shown. (B) Upon GSH treatment spectra were quantified for intact compound abundance and related to pure compound in H_2O. Data are mean values (n=5) ± SD. (C) Influence of **5** on cellular GSH/GSSG levels. HeLa cells were incubated with the compound (10 µM) or DMSO as a

This article is protected by copyright. All rights reserved.

Accepted Manuscript

control for 24 h prior to determination of GSH and GSSG levels. Data are mean values (n=6) ± SD.

GSH is the most abundant scavenger of reactive oxygen species and its depletion may cause oxidative stress and eventually cell death. Conjugation of reactive compounds to GSH in cells reduces cellular GSH levels. The ratio of the levels of GSH and its oxidized form GSSG is a measure of oxidative stress [21]. To assess a possible influence of compound **5** on the GSH/GSSG ratio, U2OS cells were treated with **5** for 24h before determining the levels of each GSH derivative. Although **5** did not change the level of reactive oxygen species in HeLa cells, it reduced the GSH/GSSG ratio as compared to cells, which were treated with DMSO (10.8 for **5** vs. 22 for the control, see Figure 4C), which indicates induction of oxidative stress by **5**.

Discussion

The kinesin HSET is essential only in cells with amplified centrosomes[6a] and selective inhibition of HSET opens up an opportunity to selectively target malignant cells with overduplicated centrosomes, while sparing healthy cells. HSET is overexpressed in a broad range of cancer types, including lung, breast, colon and cervical tumors,[22] which all exhibit centrosome amplifications.[6b] HSET has been proposed as biomarker for breast tumors, since HSET overexpression correlates with cancer development and aggressiveness as well as accelerated proliferation.[22] Similar observations have been made in ovarian cancer, where HSET overexpression is associated with poor prognosis in high-grade serous tumors.[23] To date, only a few small-molecule inhibitors of HSET have been reported.[11, 24] Here, we identified 2-sulfonylpyrimidines as a prominent class of hit compounds in a screen for HSET inhibition. A compound collection based on the 2-sulfonylpyrimidine scaffold was prepared to explore the structure-activity relationship. Introducing substituents like furan or phenyl instead of thiophene at R^2 retained the activity. However, replacement of the 2-

This article is protected by copyright. All rights reserved.

Accepted Manuscript

thiophene with 3-thiophene or 3-methoxyphenyl resulted in a twofold increase of activity. Introduction of a chlorine at the *meta*-position of the aromatic ring led to three- to fivefold enhanced potency, whereas bulkier groups were not tolerated. A three carbon linker was shown to be most advantageous and n-hexanyl amide was most appropriate as R^3 substituent. The 2-sulfonyl group as well as the 4-trifluoromethyl substituent in the pyrimidine ring are essential for HSET inhibition.

The sulfonyl-pyrimidine derivative **5** bearing a thiophene and 3-methyl-1-phenyl-1*H*-pyrazole moieties increased the amount of mitotic cells and provoked multipolar spindle formation in HeLa and BT-549 cells. The compound prevented the formation of monopolar mitotic spindles induced by Eg5 inhibition which is in line with inhibition of HSET and the opposing activity of HSET and Eg5 on the spindle.[9b] *In vitro* peptide trapping identified the substance as covalent binder of at least two cysteine residues in HSET and suggested the binding of the 4-trifluoromethyl-pyrimidine scaffold to cysteines. Reactivity towards cysteines is related also to reactivity to other cellular thiols, e.g., GSH. Derivative **5** is only partially GSH-reactive as a substantial amount of the compound remained unreacted upon 24 h of incubation in thiol-containing solution. Thus, the compound is likely to reach its target in the intracellular environment. However, compound **5** reduced the GSH/GSSG levels in cells, which is indicative of oxidative stress and of an additional mode-of-action along with HSET inhibition. Oxidative stress has been linked to mitotic arrest. [25] Therefore, reactive compounds that target GSH may also lead to mitotic arrest. However, rescue of Eg5-induced spindle monopolarity was observed for compound **5**, thus suggesting a more complex regulation of mitotic arrest than only through oxidative stress. Search for known bioactivity of the 3-methyl-1-phenyl-1H-pyrazole moiety revealed a link to kinesin inhibition [26] (see Figure S5A), whereas a different 3-methyl-1-phenyl-1H-pyrazole -bearing compound was toxic to L12102 and K562 cells[27] (see Figure S5B). In addition, various compounds bearing the 1-phenyl-1H-pyrazole moiety were among the hits from the initial screening for HSET modulators, which inhibited the HSET activity >50% at 50 µM. Thus, 3-methyl-1-phenyl-1H-pyrazole in compound **5** may guide the compound to mitotic kinesins, e.g. HSET, whereas

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

the 2-sulfonylpyrimidine part may mediate the reactivity to proteins and GSH. Sulfonyl group-bearing compounds have been described as small-molecule modulators of biological processes[5] including substituted 2-sulfonylpyrimidines.[17, 19, 28] Sulfones are good leaving groups when linked to electron withdrawing aromatic rings[19] and for various compounds conjugation to GSH has been confirmed.[17, 19, 29] Thus, this general reactivity needs to be taken into account if 2-sulfonylpyrimidines will be employed in the development of biologically active small molecules.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

Experimental Section

General Techniques

All solvents were reagent grade. Acetone, acetonitrile (MeCN), benzene, chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), dimethyl sulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc) methanol (MeOH), tetrahydrofuran (THF) *N*,*N*-dimethylformamide (DMF), pyridine and toluene were purchased from Sigma-Aldrich, FUJIFILM Wako Pure Chemical Corporation, and Tokyo Chemical Industry Co., Ltd. Compounds **1 - 7** were purchased from ChemDiv. Reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck precoated silica gel plates with F-254 indicator. E. Merck 0.5 mm precoated silica gel was used for preparative thin layer chromatography (PLC). Merck 60 (40-63 μm) silica gel and Kanto chemical silica gel 60 N (spherical, neutral) (40-50 μm) was used for flash chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX400 (400 MHz), Bruker DRX500 (500 MHz), INOVA500 (500 MHz), JEOL JNM-ECA-500 (500 MHz), and INOVA600 (600 MHz) spectrometer using $CDCl_3$ or CD_3OD as solvents. Data are reported in the following order: chemical shifts (δ) were reported in parts per million (ppm) with the solvent resonance as internal standard ($CDCl_3$: δ = 7.26 ppm for ^{1}H, δ = 77.16 ppm for ^{13}C; CD_3OD: δ = 3.31 ppm for ^{1}H, δ = 49.00 ppm for ^{13}C); multiplicities are indicated as following: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants (*J*) are given in Hertz (Hz). High resolution mass spectra were recorded on a LTQ Orbitrap mass spectrometer coupled to a Accela HPLC-System (HPLC column: Hypersyl GOLD, 50 mm x 1 mm, particle size 1.9 μm, ionization method: electron spray ionization) and Waters IMS Vion QTof system coupled to a Waters UPLC H-class (column: Waters UPLC BEH C18, 50 mm x 2.1 mm, particle size 1.7 μm, ionization method: electron spray ionization).

This article is protected by copyright. All rights reserved.

Accepted Manuscript

Subcloning, Expression and purification of HSET

pGEX-F/A-HSETΔ228 containing the kinesin motor domain of human HSET and corresponding to amino acids 229-673 as well as EG5_pQE80 containing the motor domain of Eg5 were kind gifts of T. U. Mayer, University of Konstanz, Germany. HSETΔ228 was subcloned by the Dortmund Protein Facility into pOPIN-CHis or pOPIN-NHis-EGFP plasmid by amplifying HSETΔ228 cDNA using 5'-CAAAGGAGATATACCATGGAGCTTCAGAAAAAACAGGTGGAATTGC-3'/ 5'-GATGGTGATGGTGATGTTTCTTCCTGTTGGCCTGAGCAGTAC-3' or 5'-GAAGTTCTGTTTCAGGGTCCCGAGCTTCAGAAAAAACAGGTGGAATTGC-3'/5'-TAAACTGGTCTAGAAAGCTTTACTTCCTGTTGGCCTGAGCAGTAC-3' respectively followed by sequence and ligation independent cloning[30] to obtain HSETΔ228 with a C-terminal His_8 tag (HSETΔ228-His) or HSETΔ228 with N-terminal His_8-EGFP tag (His-EGFP-HSETΔ228). pOPIN-HSETΔ228-His and EG5_pQE80 were transformed in *E. coli* strain BL21 DE3 Codon Plus RIL. Cells were grown overnight in auto-induction medium (TB medium supplemented with 0.01 % lactose and 2 mM $MgSO_4$). Cells were diluted 1:100 and were incubated for 4 h at 37 °C and 200 rpm prior to reduction of the temperature to 25 °C and further incubation for 20 h. Cells were harvested and lysed in buffer containing 50 mM Hepes, 300 mM NaCl, 20 mM imidazol, 1 mM TCEP, 0.5 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and 40 U/ml DNase using a cell disruptor. After centrifugation at 75,600 rcf at 10°C for 30 min the supernatant was loaded on a Ni-HisTrap FF crude column. After washing with 50 mM Hepes (pH 8.0), 300 mM NaCl, 30mM imidazol and 1 mM TCEP proteins were eluted with 50 mM Hepes (pH 8.0), 300 mM NaCl, 500 mM imidazol and 1 mM TCEP. Eluted proteins were further purified by size exclusion chromatography using HiLoad 16/60 Superdex 75.

Determination of kinesin activity using ADP-Glo™

This article is protected by copyright. All rights reserved.

Accepted Manuscript

In vitro HSET inhibition by sulfonylpyrimidines was measured by ADP generation using ADP-Glo™ assay (Promega) in presence of premade microtubules. Premade microtubules (Cytoskeleton) were resuspended in 15 mM PIPES pH 7.0, 1 mM $MgCl_2$ and 4 µM taxol and incubated at room temperature for 15 min until further use. Master mix containing 1 µM purified HSET and 2 µM premade microtubules was prepared in 40 mM Tris pH 7.5, 20 mM $MgCl_2$ and 0.1 mg/ml BSA. Compounds existed in stock solutions of 10 mM and were pre-diluted in DMSO. 0.5 µl of the compound pre-dilution was added to 50 µl master mix and shortly vortexed to mix. 3 µl were added to a 384 well plate (3673, Corning) in quadruplicates. ATP was added to each sample containing well to generate a final concentration of 400 µM.

All steps were performed at room temperature. The samples were incubated for 60 min to allow the ATPase reaction, followed by addition of 5 µl Glo reagent to each well. After further 40 min incubation to deplete remaining ATP and convert ADP to ATP, 10 µl detection reagent were applied to all wells to carry out the luciferase reaction. Luminescence was determined using a microplate reader (Infinite M200, Tecan). Settings: Shaking Linear Duration 5 s, Shaking Linear Amplitude 1.5 mm, Waiting time 5 s, Mode Luminescence, Attenuation NONE, Integration Time 1000 ms, Settle Time 5 ms.

Cell culture

BT-549 and HeLa cells were obtained from DSMZ, U2OS cells were purchased from CLS GmbH. All cells were cultured in Dulbecco's modified Eagle's medium with L-glutamine (DMEM, PAN Biotech) supplemented with 10% fetal bovine serum (FBS, Life Technologies), sodium pyruvate, non-essential amino acids, 100 µg/ml streptomycin and 100 U/ml penicillin (PAN Biotech) at 37°C and 5% CO_2 in humidified atmosphere.

Immunofluorescence

This article is protected by copyright. All rights reserved.

Accepted Manuscript

5.000 cells / well were seeded in a black 96 well plate with transparent bottom and incubated for 24 h at 37°C and 5 % CO_2 in humidified atmosphere. Cells were treated with compounds for additional 24 h. Fixation and permeabilization were carried out using 3.7 % formaldehyde and 0.1 % (v/v) Triton X-100 for 30 min prior to blocking by 2 % BSA (w/v) in TBS-T for 1 hour. Immunocytochemical staining with FITC-labeled anti-α-tubulin- (Sigma-Aldrich) and pericentrin-antibodies (Abcam) was performed overnight at 4 °C, followed by application of Alexa-594 coupled antibody (Thermo Scientific) for 1 hour at room temperature. Images were subsequently acquired at an automated microscope (Axiovert M200, Zeiss).

Peptide trapping

Selected compounds were evaluated for covalent binding to HSET and Ac-YACAKASAHA peptide by *in vitro* peptide trapping approach using ESI-MS technique. For binding studies with the Ac-YACAKASAHA peptide, 50 µM compound was added to 0.4 mM peptide in 75 µl Tris (25 mM, pH 7.4) in nitrogen atmosphere and incubated for 2 h at room temperature. The sample was centrifuged for 10 min at 5000 g at 4°C. The supernatant was 1:10 diluted in 95 % (v/v) H_2O/5 % (v/v) acetonitrile prior to measurement. Samples were subjected to flow-injection analysis using an Agilent 1100 HPLC system coupled to an LTQ-XL electrospray ionization mass spectrometer (Thermo Scientific). The flow rate was set to 300 µL/min; the eluent composition was water/acetonitrile 1/1 acidified with 0.1% formic acid; a mass range from 700 to 2000 m/z was recorded; and a source fragmentation of 20 V was used.

57 µg purified HSET in 25 mM Tris pH 7.4 were mixed with 50 µM compound or 0.5 % DMSO as vehicle control, followed by 30 min incubation at room temperature. The samples were concentrated by centrifugation using Amicon® Ultra 0.5ml filters (Merck Millipore) and digested using 1.5 µg trypsin overnight. Trypsinized samples were stage-tip purified [31] and subsequently measured using UltiMate™ 3000 RSLCnano-System coupled to a Q

This article is protected by copyright. All rights reserved.

Accepted Manuscript

Exactive™ Hybrid Quadrupole Orbitrap nano-HPLC/MS/MS equipped with a nano-spray source (all Thermo Scientific). Briefly, the lyophilized tryptic peptides were dissolved in 20 µl 0.1 % TFA and 3 µl of these samples were injected and enriched onto a C18 PepMap 100 column (5 µm, 100 Å, 300 µm ID * 5 mm, Dionex, Germany) using 0.1 % TFA and a flow rate of 30 µl/min for 5 min. Subsequently, the peptides were separated on a C18 PepMap 100 column (3 µm, 100 Å, 75 µm ID * 50 cm) using a linear gradient starting with 95% solvent A/5 % solvent B and increasing to 30.0% solvent B in 90 min, further increase to 60% solvent B in 5 min and to 95% solvent B in further 5 min, washing of the column with 95% solvent B for 5 min and re-equilibration of the system. The flow rate was set to 300 nl/min (solvent A: water containing 0.1 % formic acid, solvent B: acetonitril containing 0.1 % formic acid). The nano-HPLC was online coupled to the mass spectrometer using a standard coated Pico Tip emitter (ID 20 µm, Tip-ID 10 µM, New Objective, Woburn, MA, USA). Mass range of m/z 300 to 1650 was acquired with a resolution of 70000 for full scan, followed by up to ten high energy collision dissociation (HCD) MS/MS scans of the most intense at least doubly charged ions with a resolution of 17500.

Protein identification and relative quantification were performed using MaxQuant [1] v.1.4.1.2, including the Andromeda search algorithm and searching a contaminant database implemented in MaxQuant together with the sequence of the recombinant HSET protein. Briefly, a MS/MS ion search was performed for full enzymatic trypsin cleavages allowing two miscleavages. For protein modifications oxidation of methionine, carbamindomethylation of cysteine and formal $C_9N_2SF_3H_3$-addition to cysteine, serine, lysine, or histidine, respectively were chosen as variable modifications. The mass accuracy was set to 20 ppm for the first and 6 ppm for the second search. The false discovery rates for peptide and protein identification were set to 0.01.

GSH reactivity in solution

This article is protected by copyright. All rights reserved.

Accepted Manuscript

25 µM compound was dissolved in 5 mM glutathione (GSH) and incubated in humidified atmosphere at 37°C. Samples were measured for compound abundance, product formation with GSH and leftover fragments of the compound after 24h incubation in GSH. Samples were measured using an UltiMate™ 3000 RSLCnano-System (Dionex) coupled to an Orbitrap Velos Pro ion trap mass spectrometer (Thermo Scientific).

GSH/GSSG in cells

U2OS cells (2.000 cells per well) were seeded into white 384-well plates and incubated at 37°C and 5 % CO_2 overnight. Compounds were added using the ECHO 520 acoustic dispenser (Labcyte Inc., USA) and cells were incubated for 1.5 h at 37°C and 5 % CO_2. The glutathione content was determined using the GSH/GSSG-Glo Assay kit (Promega, USA) according to the manufacturer's instruction. Luminescence was detected using the Envision multilabel plate reader (PerkinElmer, USA). Data was normalized to the luminescence values of lysates of cells that were treated with DMSO.

Real-time live cell imaging and analysis

3.000 cells / well were seeded in a transparent 96 well plate and incubated for 24 h at 37°C and 5 % CO_2 in humidified atmosphere. Compounds were added to the cells and the plate was transferred to an IncuCyte ZOOM device (Essen Bioscience), where live-cell images were acquired every 60 min for 72 h. Cell growth was quantified by applying a respective analysis mask to differentiate between cells and background. Growth curves were normalized to the respective confluency at t=0.

Tubulin Polymerization Assay

To analyze their influence on *in vitro* polymerization of tubulin, 10 µM tubulin was mixed with 50 µM compound, 0.8 M monosodium glutamate and 0.88 mM $MgCl_2$. Upon 20 min

This article is protected by copyright. All rights reserved.

Accepted Manuscript

incubation, the samples were transferred to a 96-well plate and incubated for further 20 min on ice. 0.5 mM GTP was added to start the reaction. Absorption at 340 nm was measured each 30 sec for 60 min using a microplate reader (Infinite M200, Tecan). Curves were normalized to the respective t=0 value.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Dr. Toshihiko Nogawa, Christiane Heitbrink and Tanja Bojarzyn for HRMS analyses as well as Sascha Gentz for the synthesis of the model peptide. Moreover, the authors are thankful to the Compound Management and Screening Center (COMAS) for the high-throughput screening and Dr. Alexandra Friese for the HSET structure analysis. The authors also thank Prof. Dr. Thomas Mayer and Dr. Martin Möckel (University of Konstanz, Germany) for providing the HSET construct and for discussion on HSET activity assays and phenotypic analysis.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

References

[1] a) T. A. Baillie, *Angew Chem Int Ed Engl* **2016**, *55*, 13408-13421; b) J. Singh, R. C. Petter, T. A. Baillie, A. Whitty, *Nat Rev Drug Discov* **2011**, *10*, 307-317.

[2] a) A. Rossi, P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, M. G. Santoro, *Nature* **2000**, *403*, 103-108; b) T. Itoh, L. Fairall, K. Amin, Y. Inaba, A. Szanto, B. L. Balint, L. Nagy, K. Yamamoto, J. W. Schwabe, *Nat Struct Mol Biol* **2008**, *15*, 924-931.

[3] C. Drahl, B. F. Cravatt, E. J. Sorensen, *Angew Chem Int Ed Engl* **2005**, *44*, 5788-5809.

[4] R. Lagoutte, R. Patouret, N. Winssinger, *Curr Opin Chem Biol* **2017**, *39*, 54-63.

[5] M. Gehringer, S. A. Laufer, *J. Med. Chem.* **2019**, doi:10.1021/acs.jmedchem.1028b01153

[6] a) M. Kwon, S. A. Godinho, N. S. Chandhok, N. J. Ganem, A. Azioune, M. Thery, D. Pellman, *Genes & development* **2008**, *22*, 2189-2203; b) E. A. Nigg, *Nature reviews. Cancer* **2002**, *2*, 815-825.

[7] S. A. Godinho, M. Kwon, D. Pellman, *Cancer metastasis reviews* **2009**, *28*, 85-98.

[8] K. J. Verhey, J. W. Hammond, *Nature reviews. Molecular cell biology* **2009**, *10*, 765-777.

[9] a) S. Cai, L. N. Weaver, S. C. Ems-McClung, C. E. Walczak, *Molecular biology of the cell* **2009**, *20*, 1348-1359; b) V. Mountain, C. Simerly, L. Howard, A. Ando, G. Schatten, D. A. Compton, *The Journal of cell biology* **1999**, *147*, 351-366.

[10] J. Kleylein-Sohn, B. Pollinger, M. Ohmer, F. Hofmann, E. A. Nigg, B. A. Hemmings, M. Wartmann, *Journal of cell science* **2012**, *125*, 5391-5402.

[11] a) C. A. Watts, F. M. Richards, A. Bender, P. J. Bond, O. Korb, O. Kern, M. Riddick, P. Owen, R. M. Myers, J. Raff, F. Gergely, D. I. Jodrell, S. V. Ley, *Chemistry & biology* **2013**, *20*, 1399-1410; b) J. Wu, K. Mikule, W. Wang, N. Su, P. Petteruti, F. Gharahdaghi, E. Code, X. Zhu, K. Jacques, Z. Lai, B. Yang, M. L. Lamb, C. Chuaqui, N. Keen, H. Chen, *ACS chemical biology* **2013**, *8*, 2201-2208; c) B. Yang, M. L. Lamb, T. Zhang, E. J. Hennessy, G. Grewal, L. Sha, M. Zambrowski, M. H. Block, J. E. Dowling, N. Su, J. Wu, T. Deegan, K. Mikule, W. Wang, R. Kaspera, C. Chuaqui, H. Chen, *Journal of medicinal chemistry* **2014**, *57*, 9958-9970.

[12] H. Zegzouti, M. Zdanovskaia, K. Hsiao, S. A. Goueli, *Assay and drug development technologies* **2009**, *7*, 560-572.

[13] K. M. Comess, S. M. McLoughlin, J. A. Oyer, P. L. Richardson, H. Stockmann, A. Vasudevan, S. E. Warder, *Journal of medicinal chemistry* **2018**, *61*, 8504-8535.

[14] T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. L. Schreiber, T. J. Mitchison, *Science* **1999**, *286*, 971-974.

[15] S. DeBonis, D. A. Skoufias, L. Lebeau, R. Lopez, G. Robin, R. L. Margolis, R. H. Wade, F. Kozielski, *Molecular cancer therapeutics* **2004**, *3*, 1079-1090.

[16] a) W. M. Nolte, J. P. Fortin, B. D. Stevens, G. E. Aspnes, D. A. Griffith, L. R. Hoth, R. B. Ruggeri, A. M. Mathiowetz, C. Limberakis, D. Hepworth, P. A. Carpino, *Nature chemical biology* **2014**, *10*, 629-631; b) L. Li, X. Jiang, S. Q. Huang, Z. X. Ying, Z. L. Zhang, C. J. Pan, S. S. Li, X. D. Wang, Z. Y. Zhang, *Acs Medicinal Chemistry Letters* **2017**, *8*, 407-412.

[17] M. R. Bauer, A. C. Joerger, A. R. Fersht, *Proc Natl Acad Sci U S A* **2016**, *113*, E5271-5280.

[18] C. Wei, L. S. Chupak, T. Philip, B. M. Johnson, R. Gentles, D. M. Drexler, *Journal of biomolecular screening* **2014**, *19*, 297-307.

[19] L. Li, X. Jiang, S. Huang, Z. Ying, Z. Zhang, C. Pan, S. Li, X. Wang, Z. Zhang, *ACS Medicinal Chemistry Letters* **2017**, 407-412.

[20] V. I. Lushchak, *Journal of amino acids* **2012**, *2012*, 736837.

[21] J. B. Owen, D. A. Butterfield, *Protein Misfolding and Cellular Stress in Disease and Aging: Concepts and Protocols* **2010**, *648*, 269-277.

[22] S. Pawar, S. Donthamsetty, V. Pannu, P. Rida, A. Ogden, N. Bowen, R. Osan, G. Cantuaria, R. Aneja, *Journal of ovarian research* **2014**, *7*, 53.

[23] K. Mittal, H. Choi da, S. Klimov, S. Pawar, R. Kaur, A. K. Mitra, M. V. Gupta, R. Sams, G. Cantuaria, P. C. Rida, R. Aneja, *Journal of ovarian research* **2016**, *9*, 17.

This article is protected by copyright. All rights reserved.

Accepted Manuscript

[24] W. Zhang, L. Zhai, Y. Wang, R. J. Boohaker, W. Lu, V. V. Gupta, I. Padmalayam, R. J. Bostwick, E. L. White, L. J. Ross, J. Maddry, S. Ananthan, C. E. Augelli-Szafran, M. J. Suto, B. Xu, R. Li, Y. Li, *The Biochemical journal* **2016**, *473*, 1027-1035.

[25] G. F. Wang, Q. Dong, Y. Bai, J. Yuan, Q. Xu, C. Cao, X. Liu, *Free Radic Biol Med* **2017**, *103*, 177-187.

[26] X. Qian, J. Wang, G. Bergnes, B. P. Morgan, D. J. Morgans, Jr., M. Sarpong, D. Dhanak, S. D. Knight*, Vol. WO2007056078A2*, Cytokinetics, Inc., USA; Smithkline Beecham Corp. . **2007**, p. 142 pp.

[27] D. Raffa, M. C. Edler, G. Daidone, B. Maggio, M. Merikech, S. Plescia, D. Schillaci, R. Bai, E. Hamel, *Eur J Med Chem* **2004**, *39*, 299-304.

[28] a) D. Y. Zhai, P. Godoi, E. Sergienko, R. Dahl, X. Chan, B. Brown, J. Rascon, A. Hurder, Y. Su, T. D. Y. Chung, C. F. Jin, P. Diaz, J. C. Reed, *Journal of biomolecular screening* **2012**, *17*, 350-360; b) K. W. Sloop, F. S. Willard, M. B. Brenner, J. Ficorilli, K. Valasek, A. D. Showalter, T. B. Farb, J. X. C. Cao, A. L. Cox, M. D. Michael, S. M. G. Sanfeliciano, M. J. Tebbe, M. J. Coghlan, *Diabetes* **2010**, *59*, 3099-3107; c) R. Anthouard, V. J. DiRita, *Mbio* **2013**, *4*, doi:10.1128/mBio.00403-00413

[29] a) J. A. Pfefferkorn, J. H. Lou, M. L. Minich, K. J. Filipski, M. Y. He, R. Zhou, S. Ahmed, J. Benbow, A. G. Perez, M. H. Tu, J. Litchfield, R. Sharma, K. Metzler, F. Bourbonais, C. Huang, D. A. Beebe, P. J. Oates, *Bioorganic & Medicinal Chemistry Letters* **2009**, *19*, 3247-3252; b) J. Litchfield, R. Sharma, K. Atkinson, K. J. Filipski, S. W. Wright, J. A. Pfefferkorn, B. J. Tan, R. E. Kosa, B. Stevens, M. H. Tu, A. S. Kalgutkar, *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 6262-6267.

[30] M. Z. Li, S. J. Elledge, *Methods Mol Biol* **2012**, *852*, 51-59.

[31] J. Rappsilber, M. Mann, Y. Ishihama, *Nat Protoc* **2007**, *2*, 1896-1906.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ejoc.201900586

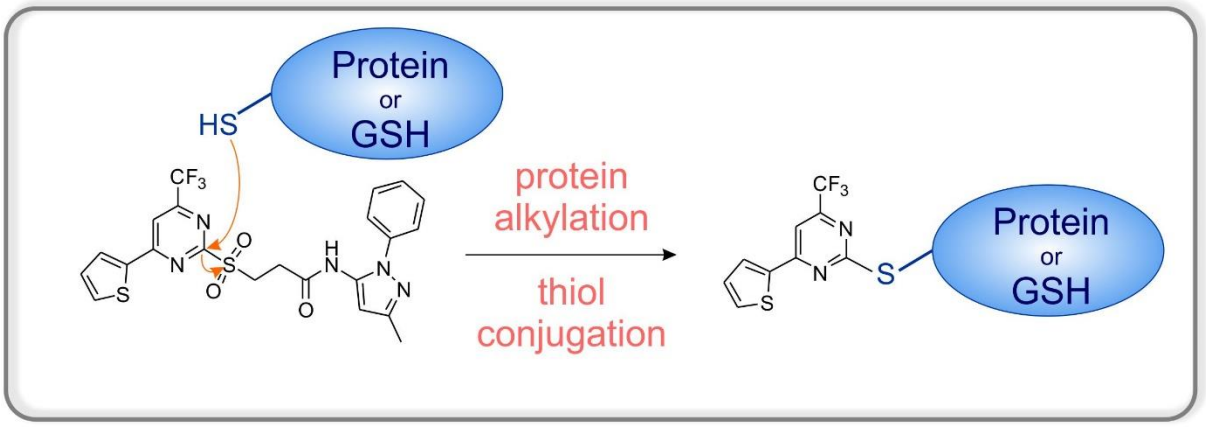
Table of Contents entry

2-Sulfonylpyrimidines were identified to inhibit the enzymatic activity of the kinesin HSET by covalently targeting cysteine residues and to induce multipolar mitoses and oxidative stress, thus the emphasizing of the pleiotropic effects of these alkylating agents in cells.

Key Topic

Sulphonyl-base alkylating agents, thiol reactivity

Graphical element



Accepted Manuscript

This article is protected by copyright. All rights reserved.