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New triazolothiadiazole and triazolothiadiazine derivatives as kinesin Eg5 and HIV inhibitors: synthesis, QSAR and modeling studies

Abstract: A new series of fused 1,2,4-triazoles, namely 6-aryl-3-(furan-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **3a**–**h** and **4a**–**f** as well as 6-aryl-3-(furan-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **5a**–**h**, were synthesized by the condensation of 4-amino-5-(furan-2-yl)-4*H*-1,2,4-triazole-3-thiol (**2**) with substituted aromatic acids and phenacyl bromides, respectively. The structures of the newly synthesized compounds were established using spectroscopic analysis, while that of **3e** was confirmed independently by a single-crystal X-ray structure determination. The compounds were evaluated for their antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells using an MTT assay. In a docking study, **4b** interacted with several amino acids in the reverse transcriptase (RT) binding site of HIV-1. Some new analogues were selected for evaluation of their Eg5 inhibitory activity using an *in vitro* malachite green ATPase assay. The QSAR of these new analogues was studied as well.

Keywords: anti-HIV activity; kinesin Eg5 inhibitors; molecular modeling study; NNRTIs; triazolothiadiazines; triazolothiadiazoles.

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1 Introduction

Uncontrolled proliferation in cancer cells is dependent on mitosis – a highly regulated process in cell division. The mitotic spindle, therefore, is a validated target in cancer chemotherapy [1]. The multicomponent mitotic spindle is responsible for accurate segregation of duplicated chromosomes into their daughter cells in eukaryotes during cell division [2]. The spindle formation is essentially driven by microtubule-associated proteins and molecular motors, cytoplasmic dynein, and kinesins from various subfamilies [3].

Small molecules of natural as well as synthetic origin that target the microtubule cytoskeleton have been proven invaluable for investigating the mechanisms of cell division. Well-known antiproliferative agents, such as vinca alkaloids, colcimid, paclitaxel, and epothilones, that modulate the microtubule polymerization, interfere with the polymerization–depolymerization process by altering microtubule dynamics resulting in extended mitotic arrest and cell death [4]. Microtubule disrupters have been proven effective chemotherapeutics. However, the toxicity of microtubule disrupters imposes limitations to their use as anticancer agents [5], as microtubules are essential for many cellular functions.

More recently, new mitotic targets such as kinesins have attracted great interest worldwide as potential candidates for new generation antiproliferative drugs [6, 7]. There are roughly 14 classes of kinesin motors with the functions ranging from vesicular transport to cell division. Most prominent of these is the kinesin spindle protein (KSP), also known as Eg5, essential for the formation and separation of bipolar spindles during human cell division [8]. Eg5 is a homotetrameric motor formed by the antiparallel arrangement of two dimers [9] and has the ability to crosslink antiparallel microtubules emanating from the two centrosomes at G2/M. Through its plus-end-directed microtubule-based motor activity, Eg5 is capable of generating outward forces that contribute to the separation of two centrosomes, a process that is essential for

the successful establishment of a bipolar spindle during mitosis [3].

Anticancer drug screening and chemical biological approaches have identified a range of structurally diverse KSP inhibitors that have been found to cause cell cycle arrest, differentiation and/or apoptosis of tumor cells. Over the past decade, the first generation of KSP inhibitors, including monastrol [10], *(S)*-trityl-L-cysteine (STLC) [11], *N*-(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (K858) [12], [(*5R,11aS*)-2-benzyl-5-(3-hydroxyphenyl)-6*H*-1,2,3,5,11,11a-hexahydroimidazo[1,5-*b*]-β-carboline-1,3-dione (Trans-24) [13], Ispinesib [14], and MK-0731 [15], have been reported.

On the other hand, HIV-1 reverse transcriptase (HIV-1 RT) that catalyzes several steps in the replication of HIV has been identified as an attractive target for the development of anti-AIDS drugs. The rapid emergence of drug resistance has increased the need for more diverse anti-HIV agents, with either new structures or mechanisms of action [16, 17]. Consequently, there is a need to develop novel, selective, potent, safe and inexpensive antiviral agents that are also effective against mutant strains of HIV.

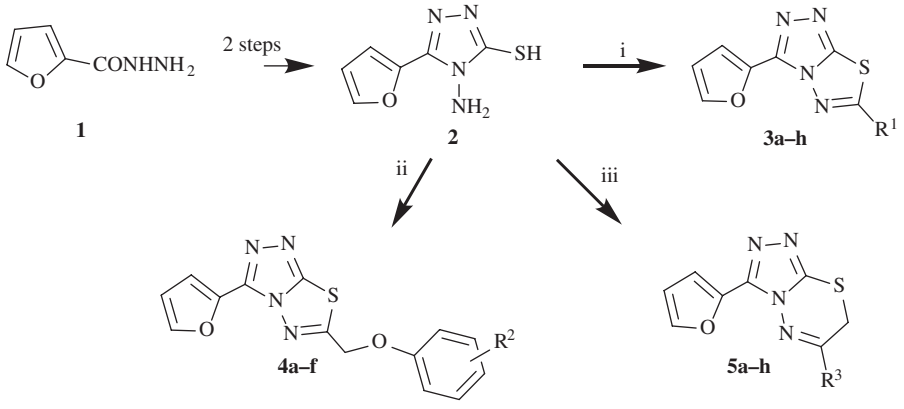
In continuation of our ongoing studies on triazolothiadiazoles and triazolothiadiazines [18, 19], we report here the synthesis of new analogues of these compounds with evaluation of their anti-HIV and kinesin Eg5 inhibitory activity.

2 Results and discussion

2.1 Chemistry

In the present work, 4-amino-5-(furan-2-yl)-4*H*-[1,2,4] triazole-3-thiol (**2**), prepared previously from the available hydrazide **1** in two steps following a reported procedure [20], has been selected as a key intermediate for the synthesis of the target compounds. Treatment of **2** with substituted benzoic acids in the presence of phosphoryl chloride furnished the triazolo-thiadiazole derivatives **3a**–**h** in 71–79 % yield, while similar treatment with substituted phenoxyacetic acids afforded the analogues **4a**–**f** in 70–80 % yield (Scheme 1).

The structures of **3a**–**h** and **4a**–**f** were confirmed by the ^{1}H NMR spectra, which exhibited additional signals for the protons of the newly introduced aromatic ring. The methylene protons in compounds **4a**–**f** appeared as singlets in the region $\delta = 5.68$–5.53 ppm. The aromatic



Entry	R^1	Entry	R^2	Entry	R^3
3a	2-F,4-Cl-C_6H_3	**4a**	2-OH	**5a**	4-$OCH_3C_6H_4$
3b	3-Cl-4-F-C_6H_3	**4b**	4-OH	**5b**	4-Cl-C_6H_4
3c	3-furanyl	**4c**	2-CH_3	**5c**	4-F-C_6H_4
3d	2-CH_3-3-furanyl	**4d**	4-CH_3	**5d**	4-CH_3-C_6H_4
3e	4-F-$C_6H_4CH_2$	**4e**	4-OCH_3	**5e**	biphen-4-yl
3f	2-Cl-4,5-F_2-C_6H_2	**4f**	4-F	**5f**	naphthalen-l-yl
3g	2-pyrrolyl			**5g**	4-NO_2-C_6H_4
3h	4-$OC_2H_5C_6H_4CH_2$			**5h**	3,4-Cl_2-C_6H_3

Scheme 1 Reagents and conditions: (i) $ArCO_2H$, $POCl_3$, reflux, 6–7 h; (ii) $ArOCH_2CO_2H$, $POCl_3$, reflux, 6–7 h; (iii) $PhC(=O)CH_2Br$, EtOH, reflux, 6–7 h.

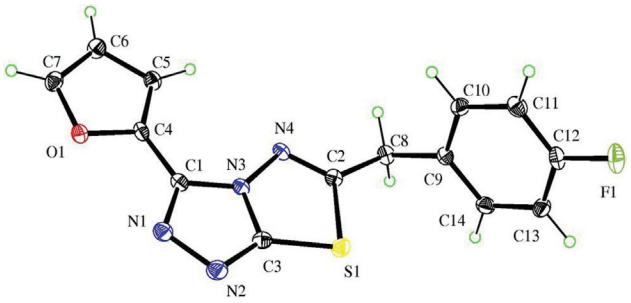


Fig. 1 Molecular structure of 6-(4-fluorobenzyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole (**3e**) in the crystal. Displacement ellipsoids are drawn at the 50 % probability level, H atoms as spheres with arbitrary radius.

protons of the phenoxy and furan rings have been fully assigned (cf. Experimental Section). In the ^{13}C NMR spectra of **3a**–**h** and **4a**–**f**, C-3 atoms of the triazolothiadiazole ring resonated in the range $\delta = 154.5$–145.8 ppm, while the C-6 signals were observed at $\delta = 172.7$–169.4 ppm. The resonances at $\delta = 163.7$–155.6 ppm were assigned to C-8 of the fused nucleus. The structure of **3e** was independently confirmed by a single-crystal X-ray diffraction analysis (Fig. 1 and Experimental Section).

Treatment of **2** with various substituted phenacyl bromides afforded 6-aryl-3-(furan-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **5a**–**h** in 73–81 % yield. The structural build-up of these compounds was assigned from the ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectra of **5a**–**h**, the signals at $\delta = 4.63$–4.43 ppm were assigned to the CH_2 protons of the thiadiazine moiety. In the ^{13}C NMR spectra, the resonances at $\delta = 23.1$–23.6 ppm were assigned to the methylene carbon atoms of the thiadiazine ring. Compound **5h** was selected for further NMR studies, since its gradient HMBC spectrum [21] revealed three couplings of CH_2 protons ($\delta_H = 4.46$ ppm): a ^{3}J coupling to C-8 of the triazolothiazdiazine ring at $\delta_C = 154.8$ ppm, a $^{2}J_{C,H}$ coupling with C-6 of the same ring at $\delta_C = 162.9$ ppm as well as ^{3}J coupling with C-1′ of the aromatic ring at $\delta_C = 133.9$ ppm. Additionally, a $^{3}J_{C,H}$ coupling between 3-H of the furan moiety at $\delta_H = 7.22$ ppm and C-3 of the triazole ring at $\delta_C = 112.8$ ppm was observed (Fig. 2).

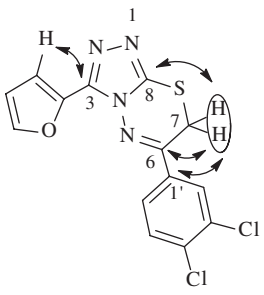


Fig. 2 $J_{C,H}$ correlations in the HMBC NMR spectrum of **5h**.

2.2 Bioactivities

2.2.1 *In vitro* anti-kinesin Eg5 activity

Antimitotic agents that have been used so far in cancer treatment, such as taxanes and vinca alkaloids, perturb the tubulin polymerization/depolymerization, cause mitotic arrest and subsequent cell death [22]. However, these drugs produce serious side effects because microtubules also have essential intracellular functions in non-dividing cells [5]. A new alternative approach to prevent mitotic-spindle formation is the inhibition of proteins, such as the mitotic motors (kinesins) that interact with microtubules and cause mitotic arrest [3].

Monastrol (MA) has been reported as one of the antimitotic agents that arrest the cells in mitosis by specifically inhibiting Eg5 [10], a member of the kinesin 5 family. Like many enzyme inhibitors, monastrol might be substrate-competitive, inhibiting the ATP hydrolysis cycle of Eg5 by directly competing with ATP [3, 10] or microtubule binding. Alternatively, monastrol might inhibit the motor domain allosterically, either by inhibiting ATP hydrolysis or by uncoupling partner head interactions to inhibit motor but not ATPase activity [23].

Compounds **3a**, **3c**, **3f**, **4a**, **4d**, **5b**, and **5g** were screened for their inhibition of Eg5 activity using an *in vitro* malachite green ATPase assay (enzyme-coupled assay) [24]. Compound **4a** matched the selection criteria of a selective inhibitor of Eg5 in this assay in comparison to monastrol. It showed an ATPase inhibition value of 40 % at 100 μM concentration (Fig. 3). From the data, it appears that a hydroxyl group at position 2 of the aromatic ring is important for inhibition.

2.2.2 *In vitro* anti-HIV activity

Compounds **3a**–**h**, **4a**–**f** and **5a**–**h** were evaluated for their *in vitro* anti-HIV-1 (strain III_B) and anti-HIV-2 (strain ROD) activity and monitored by the inhibition of the

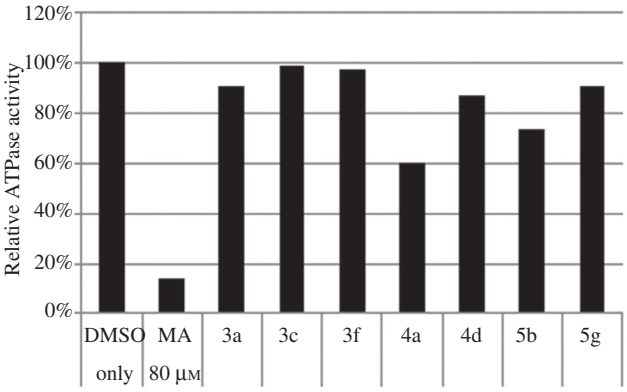


Fig. 3 *In vitro* ATPase activity of mitotic kinesin Eg5 of some triazolothiadiazine derivatives.

virus-induced cytopathic effect in the (MT-4) cells, based on an MTT assay [25]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [26] and azidothymidine (DDN/AZT) [27] are included for comparison. Compounds **3e**, **4b** and **5f** were found to be the only compounds from the series inhibiting HIV-1 and 2 replication in cell cultures with EC_{50} of > 1.51, > 0.89 and > 0.67 μM, respectively, but no selectivity was witnessed (SI < 1). However, implantation of halogen (e.g.,: F, Cl) or hydroxyl groups in 2 and 4 positions of the phenyl ring considerably increased the anti-HIV activity, in comparison to the effectiveness of other functional groups. This inhibitory activity can be shown also with the triazolo[3,4-*b*][1,3,4]thiadiazine-substituted naphthalene moiety (e.g., **5f**).

2.2.3 Molecular modeling analysis

The molecular modeling studies of **3a**, **4a** and **5f** were performed as HIV-1 reverse transcriptase (HIV-1 RT) inhibitors using the software package SYBYL 6.5 (TRIPOS Assoc., St Louis, MO, USA) running on a Silicon Graphics Octane 10 000 workstation. Conformational energies were calculated through the molecular mechanics TRIPOS force field [28]. Full geometry optimizations were realized with the semiempirical molecular orbital method AM1.

Table 1 *In vitro* anti-HIV-1[a] and HIV-2[b] activity of triazolothiadizole and triazolothiadiazine derivatives **3a–h**, **4a–f** and **5a–h**.

Entry	EC_{50} (μg mL^{-1})[c]	EC_{50} (μg mL^{-1})[c]	CC_{50} (μg mL^{-1})[d]	SI[e] (III_B)	SI[e] (ROD)
3a	>47.53	>47.53	47.53	<1	<1
3b	>78.03	>78.03	78.03	<1	<1
3c	>67.53	>67.53	67.53	<1	<1
3d	>37.05	>37.05	37.05	<1	<1
3e	>1.51	>1.51	1.51	<1	<1
3f	>66.25	>66.25	66.25	<1	<1
3g	>59.15	>59.15	59.15	<1	<1
3h	>21.68	>21.68	21.68	<1	<1
4a	>11.75	>11.75	11.75	<1	<1
4b	>0.89	>0.89	0.89	<1	<1
4c	>79.48	>79.48	79.48	<1	<1
4d	>88.50	>88.50	88.50	<1	<1
4e	>30.98	>30.98	30.98	<1	<1
4f	>89.10	>89.10	89.10	<1	<1
5a	>1.29	>1.29	1.29	<1	<1
5b	>71.28	>71.28	71.28	<1	<1
5c	>31.45	>31.45	31.45	<1	<1
5d	>11.56	>11.56	11.56	<1	<1
5e	>5.13	>5.13	5.13	<1	<1
5f	>0.67	>0.67	0.67	<1	<1
5g	>83.20	>83.20	83.20	<1	<1
5h	>50.38	>50.38	50.38	<1	<1
Nevirapine	0.050	>4.00	>4.00	>80	<1
AZT	0.0022	0.00094	>25	>11 363	>26 596

[a]Anti-HIV-1 activity measured on strain III_B. [b]Anti-HIV-2 activity measured with strain ROD. [c]Compound concentration required to achieve 50 % protection of MT-4 cells from HIV-1 and 2-induced cytopathogenic effect. [d]Compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %. [e]Selectivity index (CC_{50}/EC_{50}).

Table 2 Binding energies, *Ki*, and inhibition constant values of **3a–g**, **4a–f** and **5a–g** at $T = 298.5$ K.

Compd.	Binding energy[a]	*Ki* (μM)	Intermolecular energy[a]	Internal energy[a]	Torsional energy[a]	Unbound external energy[a]
3a	−7.07	6.58	−7.69	−0.53	0.6	−0.55
3b	−7.24	4.89	−7.53	−0.28	0.3	−0.27
3c	−6.90	8.73	−7.2	−0.15	0.3	−0.16
3d	−6.84	9.63	−7.11	−0.27	0.3	−0.24
3e	−6.95	8.05	−7.41	−0.51	0.6	−0.37
3f	−6.92	8.52	−7.18	−0.4	0.3	−0.37
3g	−6.25	26.23	−6.57	−0.24	0.3	−0.26
4a	−8.61	0.4892	−8.5	−1.19	0.89	−0.19
4b	−9.60	4.81	−8.13	−0.39	0.89	−0.37
4c	−7.47	3.36	−7.9	−0.49	0.6	−0.33
4d	−7.29	4.56	−7.81	−0.41	0.6	−0.34
4e	−6.97	7.79	−7.7	−0.55	0.89	−0.38
4f	−6.98	7.65	−7.47	−0.39	0.6	−0.28
5a	−7.38	3.9	−7.69	−0.41	0.6	−0.39
5b	−7.52	3.07	−7.81	−0.37	0.3	−0.35
5c	−7.19	5.35	−7.48	−0.34	0.3	−0.33
5d	−7.41	3.67	−7.7	−0.37	0.3	−0.35
5e	−8.85	0.3276	−9.43	−0.68	0.6	−0.67
5f	−9.83	0.0618	−10.43	−0.9	0.6	−0.9
5g	−7.14	5.85	−7.72	−0.42	0.6	−0.41

[a]The energy values are in kcal mol^{-1}.

The prospective ligands were ranked according to the highest binding energy of the best conformers. Thus, the binding energy score for the **3a–g**, **4a–f**, and **5a–g** series ranged from −6.25 to −9.83 kcal mol^{-1} (Table 2), indicating selectivity and potency profiles of these derivatives to bind the active site of HIV-RT pocket, especially those carrying hydroxyaryl residues (e.g., **4b**). Compound **4b** has been selected to show its binding to the enzyme pocket. As shown in Fig. 4, the phenyl ring of **4b** fitted into an arene-rich subpocket surrounded by the aromatic side chains of Tyr179, Tyr186, and Trp227 residues and apparently developing π-π stacking interactions with the three residues. The triazolothiadiazole backbone is located in the middle of the binding pocket, anchoring the oxygen atom of the furan ring in a favorable position for hydrogen bonding with the Lys102 of the reverse transcriptase (RT) enzyme, in addition to the same bonding between the amino group of Lys101 with the hydroxyl group of the

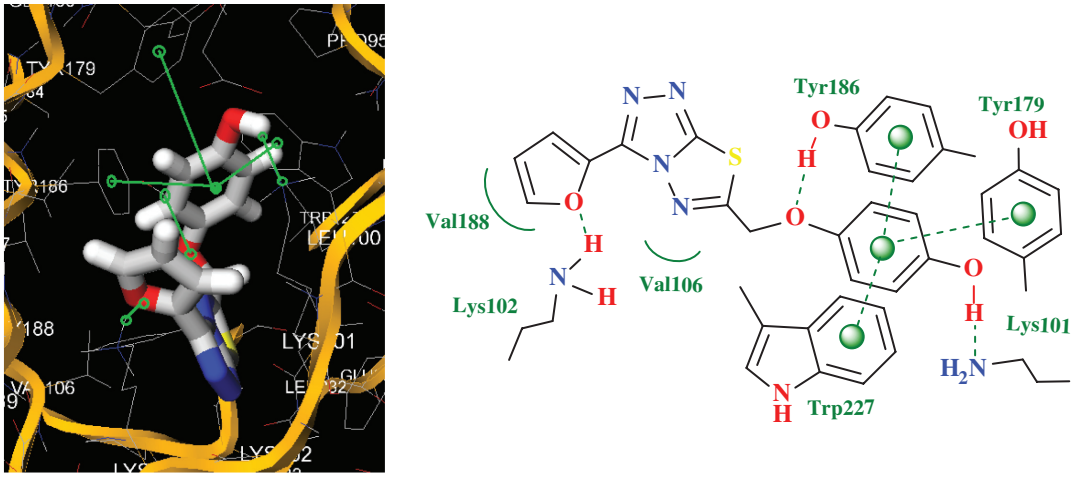


Fig. 4 Compound **4b** shows three hydrogen bonds: Lys102 with the oxygen of the furan ring, Lys101 with the OH group of the phenyl group and Tyr186 with the oxygen atom of the benzyl group. In addition, hydrophobic interactions between of the phenyl moiety of **4b** and Tyr179, Tyr186 as well as Trp227 of reverse transcriptase (RT) enzyme residues were observed.

benzyl moiety. Overall, the combination of hydrophobic interactions and π stacking appears to govern the binding of **4b** with HIV RT (binding energy –9.83 kcal mol^{-1}).

2.2.4 Quantitative structure-activity relationship

The binding scores for **3a**, **4b**, and **5f** were congruent with their anti-HIV activity. A good correlation between the predicted binding free energies and the experimentally observed inhibitory activities (EC_{50}) (Tables 2 and 3) suggests that the identified binding conformations of these inhibitors are reliable. The results of docking studies provide an insight into the pharmacophoric structural requirements for the HIV-1 RT inhibitory activity of this class of molecules.

For an understanding of the quantitative structure-activity relationships, a statistical analysis using a genetic function approximation (GFA) method [29] was made. First, a study table was belted and presented in Table 2. Second, a correlation matrix by using Friedman LOF measure [30] (Table 3) was derived, and finally regression parameters were obtained. Regarding this method, various statistical measures can be adapted to calculate the fitness of a GFA model during the evolution process.

Table 3 shows the GFA analysis which gives a summary of the input parameters used for the calculation. Also, it reports whether the GFA algorithm converged in a specified number of generations. The Friedman's lack-of-fit (LOF) score in Table 3 evaluates the QSAR model. The lower the LOF, the less likely it is that the GFA model will fit the data. The significant regression is given by the *F* test, the higher the value, the better is the model. Using this method, compounds **4a**–**f** were selected for studying the predicted equation for EC_{50} calculation (Table 4).

Table 3 Validation table of the genetic function approximation.

Friedman LOF	4.95×10^{-4}
R^2	1.00
Adjusted R^2	1.00
Cross validated R^2	0.999983
Significant regression	yes
Significance-of-regression *F* value	2.229275×10^7
Critical SOR *F* value (95 %)	225.749641
Replicate points	0
Computed experimental error	0.00
Lack-of-fit points	1
Min expt. error for non-significant LOF (95 %)	0.004866

Table 4 Calculated and predicted EC_{50} of **4a**–**f**.

Compd.	EC_{50} calcd.	EC_{50} predicted	Residuals
4a	11.750000	11.745702	0.004298
4b	0.890000	0.893131	–0.003131
4c	79.480000	79.484749	–0.004749
4d	88.500000	88.494036	0.005964
4e	30.980000	30.980246	–0.000246
4f	89.100000	89.102137	–0.002137

$$EC_{50} = 221.046349884 \times X5 + 10629.588732628 \times X9 + 2508.141746599 \times X15 - 752.566106747 \times X34 - 22049.514189079$$

where:
X5: H: AlogP
X9: L: Balaban index JX
X15: R: Kappa-3
X34: AK: Chi (3): path (valence modified).

3 Conclusion

In conclusion, we report the *in vitro* inhibition of kinesin Eg5 *via* the ATPase activity assay as well as the anti-HIV activity. Compound **4b**, as the only compound of the triazolothiadiazine series, showed moderate inhibition of ATPase activity (40 %) at 100 μM. In addition, compounds **3e**, and **5f** inhibited the replication of HIV-1 and HIV-2 in cell cultures, but the compounds need to be further pursued for toxicity and pharmacokinetics.

4 Experimental

4.1 General

Melting points were measured on a Stuart melting point apparatus (SMP3) in open capillaries and are uncorrected. IR spectra were recorded on an FTS 3000 MX, Bio-Rad Merlin (Excalibur model) spectrophotometer. NMR spectra were recorded either on a 300- or 400-MHz spectrometer (Avance III; Bruker, Reinstetten, Germany), using the residual solvent signal for calibration of the spectrum. Heteronuclear assignments were verified by HMBC spectroscopy experiments. The R_f values were determined by employing Merck 60 F_{254} precoated silica gel plates; the eluent was $CHCl_3$-MeOH (9:1). Product spots were visualized under UV light at 254 and 365 nm. The elemental

analysis was performed on a Leco CHNS-932 Elemental Analyzer (Leco, St. Joseph, MI, USA).

4.2 General procedure for the preparation of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles 3a–h and 4a–f

A mixture of **2** (182 mg, 1.00 mmol) and a substituted aromatic acid (1.10 mmol) in $POCl_3$ (5 mL) was heated under reflux for 6–7 h. The reaction mixture was slowly poured into crushed ice with stirring and neutralized with solid sodium bicarbonate. The solid product obtained was filtered, washed with cold water, and recrystallized from ethanol to give the desired products.

6-(4-Chloro-2-fluorophenyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3a) From 4-chloro-2-fluorobenzoic acid (191 mg). Yield: 228 mg (71%), light-yellow solid, m.p.: 257–258 °C, $R_f = 0.73$. – IR (neat): $\nu = 3066$ (Ar-H), 1606 (C=N), 1574, 1514 (2 × C=C), 1204 (C-F), 1024 (C-Cl) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.05$ (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.90 (d, 1H, $J_{3,F} = 7.5$ Hz, 3-$H_{arom.}$), 7.62 (d, 1H, $J_{5,6} = 8.4$ Hz, 6-$H_{arom.}$), 7.42 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 7.36 (d, 1H, $J_{5,6arom.} = 8.4$ Hz, 5-$H_{arom.}$), 6.82 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.2$ Hz, 4-H_{furan}) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 170.9$ (C-6), 163.7 (C-8), 157.4 (d, $J_{C2,F} = 248$ Hz, C-2$_{arom.}$-F), 154.9 (C-2$_{furan}$), 149.7 (C-3), 141.9 (C-5$_{furan}$), 137.8 (d, $J_{4,F} = 20$ Hz, C-4$_{arom.}$), 134.6 (d, $J_{6,F} = 22$ Hz, C-6$_{arom.}$), 130.6 (C-5$_{arom.}$), 127.5 (d, $J_{C1,F} = 120$ Hz, C-1$_{arom.}$), 117.2 (d, $J_{C3,F} = 121$ Hz, C-3$_{arom.}$), 112.5 (C-4$_{furan}$), 108.7 (C-3$_{furan}$) ppm. – $C_{13}H_6ClFN_4OS$ (320.73): calcd. C 48.68, H 1.89, N 17.47; found C 48.51, H 1.74, N 17.59.

6-(3-Chloro-4-fluorophenyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3b) From 3-chloro-4-fluorobenzoic acid (191 mg). Yield: 234 mg (73%), off-white solid, m.p.: 263–264 °C, $R_f = 0.75$. – IR (neat): $\nu = 3062$ (Ar-H), 1625 (C=N), 1597, 1525 (2 × C=C), 1221 (C-F), 1012 (C-Cl) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.37$ (d, 1H, $J_{H2,F} = 6.6$ Hz, 2-$H_{arom.}$), 8.04 (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.73–7.67 (m, 1H, 6-$H_{arom.}$ + 5-$H_{arom.}$), 7.49 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 6.82 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.2$ Hz, 4-H_{furan}) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 169.9$ (C-6), 163.7 (C-8), 156.8 (d, $J_{C4,F} = 250$ Hz, C-4$_{arom.}$-F), 153.9 (C-2$_{furan}$), 149.8 (C-3), 141.8 (C-5$_{furan}$), 133.5 (C-1$_{arom.}$), 130.7 (d, $J_{C2',F} = 22$ Hz, C-2$_{arom.}$), 130.5 (d, $J_{C6,F} = 22$ Hz, C-6$_{arom.}$), 127.5 (d, $J_{C3,F} = 123$ Hz, C-3$_{arom.}$), 117.0 (d, $J_{C5,F} = 120$ Hz, C-5$_{arom.}$), 112.8 (C-4$_{furan}$), 107.9 (C-3$_{furan}$) ppm. – $C_{13}H_6ClFN_4OS$ (320.73): calcd. C 48.68, H 1.89, N, 17.47; found C 48.76, H 1.76, N 17.56.

3-(Furan-2-yl)-6-(furan-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3c) From furan-3-carboxylic acid (123 mg).Yield: 186 mg (72%), light-brown solid, m.p.: 177–178 °C, $R_f = 0.72$. – IR (neat): $\nu = 3021$ (Ar-H), 1621 (C=N), 1591, 1518 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.03$ (d, 1H, $J_{4,5furan} = 1.5$ Hz, H^5_{furan}), 8.00 (s, 1H, H^2_{furan}), 7.36 (m, 2H, 3-H_{furan} + 5-H_{furan}), 7.17 (d 1H, $J_{4,5furan} = 1.1$ Hz, 4-H_{furan}), 6.80 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 172.7$ (C-6), 160.2 (C-8), 153.6 (C-2$_{furan}$), 146.5 (C-3), 145.8 (C-5$_{furan}$), 140.5 (C-5$_{furan}$), 139.6 (C-2$_{furan}$), 135.1 (C-3$_{furan}$), 112.6 (C-4$_{furan}$), 112.2 (C-5$_{furan}$), 108.7 (C-3$_{furan}$) ppm. – $C_{11}H_6N_4O_2S$ (258.26): calcd. C 51.16, H 2.34, N 21.69; found C 50.97, H 2.15, N 21.57.

3-(Furan-2-yl)-6-(2-methylfuran-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3d) From 2-methylfuran-3-carboxylic acid (139 mg). Yield: 214 mg (78%), light-yellow solid, m.p.: 190–191 °C, $R_f = 0.71$. – IR (neat): $\nu = 3096$ (Ar-H), 1620 (C=N), 1592, 1521 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.80 (d, 1H, $J_{4',5'furan} = 2.0$ Hz, 5′-H_{furan}), 7.28 (d, 1H, $J_{3,4furan} = 3.3$ Hz, 3-H_{furan}), 7.02 (d, 1H, $J_{4',5'furan} = 2.0$ Hz, 4′-H_{furan}), 6.80 (dd, 1H, $J_{3,4furan} = 3.3$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 2.14 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 171.5$ (C-6), 161.3 (C-8), 155.0 (C-2$_{furan}$), 153.5 (C-2a$_{furan}$), 145.8 (C-5a$_{furan}$ + C-3), 140.5 (C-5$_{furan}$), 139.5 (C-3a$_{furan}$), 112.2 (C-4$_{furan}$), 112.0 (C-4a$_{furan}$), 110.3 (C-3$_{furan}$), 14.3 (CH_3) ppm. – $C_{12}H_8N_4O_2S$ (272.28): calcd. C 52.93, H, 2.96, N 20.58; found C 52.81, H 2.88, N 20.41.

6-(4-Fluorobenzyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3e) From 2-(4-(fluorophenyl)acetic acid (154 mg). Yield: 228 mg (76%), light-brown solid, m.p.: 150–151 °C, $R_f = 0.68$. – IR (neat): $\nu = 3064$ (Ar-H), 1621 (C=N), 1592, 1521 (2 × C=C), 1138 (C-F) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.01$ (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.52–7.47 (m, 2H, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.25–7.19 (m, 3H, 3-$H_{arom.}$ + 6-$H_{arom.}$ + 3-H_{furan}), 6.78 (dd, 1H, $J_{3,4furan} = 3.3$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 4.51 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 171.4$ (C-6), 163.7 (C-8), 157.5 (d, $J_{C4',F} = 249$ Hz, C-4$_{arom.}$-F), 154.5 (C-2$_{furan}$), 145.8 (C-3), 140.6 (C-5$_{furan}$), 132.0 (C-1$_{arom.}$), 131.8 (d, $J_{C2,4,F} = 21$ Hz, C-2$_{arom.}$ + C-6$_{arom.}$), 116.2 ($J_{C2,4,F} = 118$ Hz, C-3$_{arom.}$ + C-5$_{arom.}$), 112.6 (C-4$_{furan}$), 111.9 (C-3$_{furan}$), 36.7 (CH_2) ppm. – $C_{14}H_9FN_4OS$ (300.31): calcd. C 55.99, H 3.02,N 18.66; found C 55.84, H 2.92, N 18.50.

6-(2-Chloro-4,5-difluorophenyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3f) From 2-chloro-4,5-difluorobenzoic acid (211 mg). Yield: 254 mg (75%), light-yellow solid, m.p.: 226–227 °C, $R_f = 0.64$. – IR (neat): $\nu = 3058$ (Ar-H), 1622 (C=N), 1591, 1515 (2 × C=C), 1182 (C-F),

1020 (C-Cl) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.36$ (m, 1H, 4-$H_{arom.}$), 8.11 (m, 1H, 6-$H_{arom.}$), 8.04 (d, $J_{4,5furan} = 1.0$ Hz, 5-H_{furan}), 7.43 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 6.81 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.8$ Hz, 4-H_{furan}) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 170.9$ (C-6), 163.6 (C-8), 158.2 (2 × d, $J_{C4,F4} = 249$ Hz, $J_{C4',F5} = 118$ Hz, C-$4_{arom.}$-F), 156.8 (C-2_{furan}), 151.3 (C-3), 147.7 (2 × d, $J_{C5,F5} = 247$ Hz, $J_{C5,F4} = 119$ Hz, C-$5_{arom.}$-F),134.5 (C$^1_{arom.}$), 129.1 (d, $J_{C2,F4} = 25$ Hz, C-$2_{arom.}$), 121.0 (m, C-$3_{arom.}$ + C-$6_{arom.}$), 112.1 (C-4_{furan}), 110.6 (C-3_{furan}) ppm. – $C_{13}H_5ClF_2N_4OS$ (338.32): calcd. C 46.10, H 1.49, N 16.54; found C 45.97, H 1.32, N 16.59.

3-(Furan-2-yl)-6-(1H-pyrrol-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3g) From 1*H*-pyrrol-2-carboxylic acid (122 mg). Yield: 203 mg (79 %), black solid; m.p.: 223–224 °C, $R_f = 0.66$. – IR (neat): $\nu = 3119$ (N-H), 3054 (Ar-H), 1621 (C=N), 1581, 1518 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 12.55$ (s, 1H, NH), 8.02 (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.22 (m, 2H, 3-H_{furan} + 5-$H_{pyrrole}$), 7.02–6.94 (m, 2H, 4-H_{furan} + 3-$H_{pyrrole}$), 6.79 (dd, 1H, $J_{3,4pyrrole} = 3.6$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-$H_{pyrrole}$) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 169.4$ (C-6), 159.6 (C-8), 154.5 (C-2_{furan} + C-3), 138.7 (C-5_{furan}), 127.8 (C-$2_{pyrrole}$), 122.5 (C-$5_{pyrrole}$), 115.3 (C-4_{furan} + C-$4_{pyrrole}$), 107.1 (C-3_{furan} + C-3_{pyrrol}) ppm. – $C_{11}H_7FN_5OS$ (257.27): calcd. C 51.35, H 2.74, N 27.22; found C 51.23, H 2.58, N 27.39.

6-(4-Ethoxybenzyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (3h) From 2-(4-(ethoxyphenyl) acetic acid (198 mg). Yield: 273 mg (77 %), light-brown solid, m.p.: 158–159 °C, $R_f = 0.70$. – IR (neat): $\nu = 3071$ (Ar-H), 1611 (C=N), 1585, 1527 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.01$ (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.34 (d, 2H, $J_{2,3} = J_{6,5} = 8.7$ Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.22 (d, 1H, $J_{3,4furan} = 3.3$ Hz, 3-H_{furan}), 6.93 (d, 2H, 3-$H_{arom.}$ + 5-$H_{arom.}$), 6.78 (dd, 1H, $J_{3,4furan} = 3.3$ Hz, $J_{4,5furan} = 1.2$ Hz, 4-H_{furan}), 4.42 (s, 2H, CH_2), 4.01 (q, 2H, $J = 6.9$ Hz, CH_2), 1.31 (t, 3H, $J = 6.9$ Hz, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 172.4$ (C-8), 158.5 (C-6),154.5 (C-2_{furan} + *C*-OEt), 145.8 (C-3), 140.6 (C-5_{furan}), 131.0 (C-$2_{arom.}$ + C-$6_{arom.}$), 127.4 (C-$1_{arom.}$), 115.3 (C-$3_{arom.}$ + C-$5_{arom.}$), 112.6 (C-4_{furan}), 111.9 (C-3_{furan}), 63.5 (OCH_2), 36.8 (CH_2), 15.1 (CH_3) ppm. – $C_{17}H_{14}N_4O_3S$ (354.38): calcd. C 57.62, H 3.98, N 15.81; found C 57.81, H 3.79, N 15.62.

2-((3-(Furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)methoxy)phenol (4a) From 2-(2-hydroxyphenoxy)acetic acid (185 mg). Yield: 232 mg (70 %), off-white solid, m.p.: 180–181 °C, $R_f = 0.74$. – IR (neat): $\nu = 3147$ (OH), 3035 (Ar-H), 1618 (C=N), 1551, 1508 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (br s, 1H, 5-H_{furan}), 7.49–7.40 (m, 4H, $H_{arom.}$), 7.22 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 6.79 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.8$ Hz, 4-H_{furan}), 5.68 (s, 2H, CH_2), 5.48 (s, 1H, OH). – ^{13}C NMR ([D_6]DMSO): $\delta = 167.2$)C-6), 161.2 (C-8), 154.9 (C-2_{furan}), 149.7 (C-$1_{arom.}$), 145.4 (C-OH), 144.8 (C-3), 141.9 (C-5_{furan}), 128.3, 127.5 (C-$4_{arom.}$ + C-$5_{arom.}$), 117.2 (C-$6_{arom.}$ + C-$3_{arom.}$), 112.1 (C-4_{furan}), 109.8 (C-3_{furan}), 65.5 (CH_2) ppm. – $C_{14}H_{10}N_4O_3S$ (314.32): calcd. C 53.50, H 3.21, N 17.82; found C 53.37, H 3.07, 17.69.

4-((3-(Furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)methoxy)phenol (4b) From 2-(4-hydroxyphenoxy)acetic acid (185 mg). Yield: 232 (74 %), off-white solid, m.p.: 174–175 °C, $R_f = 0.75$. – IR (neat): $\nu = 3115$ (OH), 3029 (Ar-H), 1622(C=N), 1546, 1498 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (br s, 1H, 5-H_{furan}), 7.46–7.42 (m, 4H, $H_{arom.}$), 7.20 (d, 1H, $J_{3,4furan} = 3.3$ Hz, 3-H_{furan}), 6.78 (dd, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 5.62 (s, 2H, CH_2), 5.44 (s, 1H, OH) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 166.1$) C-6 + C-8), 154.8 (C-2_{furan}), 149.7 (C-$1_{arom.}$), 145.4 (C-OH), 144.9 (C-3), 142.9 (C-5_{furan}), 129.7, 129.2 ($C_{arom.}$), 112.1 (C-4_{furan}), 109.8 (C-3_{furan}), 65.3 (CH_2) ppm. – $C_{14}H_{10}N_4O_3S$ (314.32): calcd. C 53.50, H 3.21, N 17.82; found C 53.59, H 3.11, N 17.74.

3-(Furan-2-yl)-6-(2-tolyloxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4c) From 2-(tolyloxy)acetic acid (183 mg). Yield: 234 mg (75 %), off-white solid, m.p.: 186–187 °C, $R_f = 0.76$. – IR (neat): $\nu = 3041$ (Ar-H), 1605 (C=N), 1589, 1516 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.32 (br s, 1H, 3-H_{furan}), 7.23–7.17 (m, 2H, 3-$H_{arom.}$ + 4-$H_{arom.}$), 7.12 (d, 1H, $J = 7.8$ Hz, 6-$H_{arom.}$), 6.94 (t, 1H, $J = 7.8$ Hz, 5-$H_{arom.}$), 6.79 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 5.61 (s, 2H, CH_2), 2.25 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 169.6$ (C-6 + C-8), 155.6 (C-$1_{arom.}$), 154.1 (C-2_{furan}), 145.9 (C-3), 140.5 (C-5_{furan}), 131.3, 126.6, 122.3 ($C_{arom.}$), 112.7 (C-4_{furan}), 112.6 (C-$6_{arom.}$), 111.9 (C-3_{furan}), 65.4 (CH_2), 16.4 (CH_3) ppm. – $C_{15}H_{12}N_4O_2S$ (312.35): calcd. C 57.68, H 3.87, N 17.94; found C 57.52, H 3.99, N 17.81.

3-(Furan-2-yl)-6-(4-tolyloxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4d) From 2-(tolyloxy)acetic acid (183 mg). Yield: 228 mg (73 %), off-white solid, m.p.: 191–192 °C, $R_f = 0.75$. – IR (neat): $\nu = 3039$ (Ar-H), 1611 (C=N), 1588, 1508 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.22 (d, 1H, $J_{3,4furan} = 3.7$ Hz, 3-H_{furan}), 7.15 (d, 2H, $J = 8.4$ Hz, 3-$H_{arom.}$ + 5-$H_{arom.}$), 7.02 (d, 2H, $J = 7.8$ Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 6.79 (dd, 1H, $J_{3,4furan} = 3.7$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 5.56 (s, 2H, CH_2), 2.24 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 169.3$ (C-6 + C-8), 155.4 (C-$1_{arom.}$), 154.1 (C-2_{furan}), 145.9 (C-3), 140.4 (C-5_{furan}), 131.5, 130.5 (C-$3_{arom.}$ + C-$5_{arom.}$), 115.4 (C-$2_{arom.}$ + C-$6_{arom.}$), 112.6 (C-4_{furan}), 111.9 (C-3_{furan}), 65.4 (CH_2), 20.6 (CH_3) ppm. – $C_{15}H_{12}N_4O_2S$ (312.35): calcd. C 57.68, H 3.87, N 17.94; found C 57.60, H 3.72, N 17.85.

3-(Furan-2-yl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4e) From 2-(4-methoxyphenoxy)acetic acid (200 mg). Yield: 253 mg (77 %), off-white solid, m.p.: 148–149 °C, $R_f = 0.72$. – IR (neat): $\nu = 3026$ (Ar-H), 1620 (C=N), 1592, 1505 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (d, 1H, $J_{4,5furan} = 1.2$ Hz, 5-H_{furan}), 7.22 (d, 1H, $J_{3,4furan} = 3.3$ Hz, 3-H_{furan}), 7.06 (d, 2H, $J = 7.5$ Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 6.90 (d, 2H, $J = 7.5$ Hz, 3-$H_{arom.}$ + 5-$H_{arom.}$), 6.78 (dd, 1H, $J_{3,4furan} = 3.3$ Hz, $J_{4,5furan} = 1.2$ Hz, 4-H_{furan}), 5.53 (s, 2H, CH_2), 3.70 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 169.4$ (C-6 + C-8), 154.9 (C-2_{furan}), 154.1 (C-OMe), 151.4 (C-$1_{arom.}$), 145.9 (C-3), 140.4 (C-5_{furan}), 116.7 (C-$2_{arom.}$ + C-$6_{arom.}$), 115.2 (C-$3_{arom.}$ + C-$5_{arom.}$), 112.6 (C-4_{furan}), 111.9 (C-3_{furan}), 66.0 (CH_2), 55.8 (OMe) ppm. – $C_{15}H_{12}N_4O_3S$ (328.35): calcd. C 54.87, H 3.68, N 17.06; found C 54.71, H 3.48, N 16.92.

6-((4-Fluorophenoxy)methyl)-3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4f) From 2-(4-fluorophenoxy)acetic acid (187 mg). Yield: 253 mg (80 %), light-brown solid, m.p.: 164–165 °C, $R_f = 0.77$. – IR (neat): $\nu = 3019$ (Ar-H), 1625 (C=N), 1552, 1503 (2 × C=C), 1203 (C-F) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.02$ (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.22–7.13 (m, 5H, $H_{arom.}$ + 3-H_{furan}), 6.78 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 5.59 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 168.8$ (C-6), 159.4 (C-8), 155.3 (d, $J_{C4,F} = 249$ Hz, C-$4_{arom.}$-F), 154.2 (C-$1_{arom.}$), 153.9 (C-2_{furan}), 145.9 (C-3), 140.4 (C-5_{furan}), 117.2 (d, $J_{C3,5,F} = 118$ Hz, C-$3_{arom.}$ + C-$5_{arom.}$), 116.6 (d, $J_{C2,6,F} = 20$ Hz, C-$2_{arom.}$ + C-$6_{arom.}$), 112.6 (C-4_{furan}), 111.9 (C-3_{furan}), 65.9 (CH_2) ppm. – $C_{14}H_9FN_4O_2S$ (316.31): calcd. C 53.16, H 2.87, N 17.71; found C 53.22, H 2.70, N 17.56.

4.3 General procedure for the synthesis of 1,2,4-triazolo[3,4-b][1,3,4]thiadiazines 5a–h

A mixture of **2** (182 mg, 1.00 mmol) and a substituted phenacyl bromide (1.20 mmol) was heated under reflux in abs. ethanol (10 mL) for 6–7 h. The reaction mass was poured into crushed ice and neutralized with $NaHCO_3$. The solid product obtained was filtered, washed with water, dried, and recrystallized from EtOH to afford the desired products.

3-(Furan-2-yl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a) From 4-methoxyphenacyl bromide (275 mg). Yield:253 mg (81 %), yellow solid, m.p.: 214–215 °C, $R_f = 0.62$. – IR (neat): $\nu = 3015$ (Ar-H), 2912, 2857 (2 × C-H), 1604 (C=N), 1556, 1510 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.05$ (d, 2H, $J = 8.7$ Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.99 (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.24 (d, 1H, $J_{3,4furan} = 2.7$ Hz, 3-H_{furan}), 7.14 (d, 2H, $J = 8.7$ Hz, 3-$H_{arom.}$ + 5-$H_{arom.}$), 6.77 (dd, 1H, $J_{3,4furan} = 2.7$ Hz, $J_{4,5furan} = 1.5$ Hz, 4-H_{furan}), 4.43 (s, 2H, CH_2), 3.86 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 162.9$ (C-6 + C-OMe), 156.2 (C-8 + C-2_{furan}), 145.7 (C-3), 142.4 (C-5_{furan}), 130.0 (C-$2_{arom.}$ + C-$6_{arom.}$), 125.7 (C-$1_{arom.}$), 115.1 (C-$3_{arom.}$ + C-$5_{arom.}$), 113.6 (C-4_{furan}), 112.6 (C-3_{furan}), 56.1 (OCH_3), 23.1 (CH_2) ppm. – $C_{15}H_{12}N_4O_2S$ (312.35): calcd. C 57.68, H 3.87, N 17.94; found C 57.53, H 3.66, N 17.85.

6-(4-Chlorophenyl)-3-(furan-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5b) From 4-chlorophenacyl bromide (280 mg). Yield: 260 mg (79 %), light-yellow solid, m.p.: 231–232 °C, $R_f = 0.66$. – IR (neat): $\nu = 3037$ (Ar-H), 2910, 2824 (2 × C-H), 1591 (C=N), 1562, 1515 (2 × C=C), 1076 (C-Cl) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.70$ (br s, 2H, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.99 (d, 1H, $J_{4,5furan} = 1.8$ Hz, 5-H_{furan}), 7.67 (br s, 2H, 3-$H_{arom.}$ + 5-$H_{arom.}$), 7.22 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 6.77 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.8$ Hz, 4-H_{furan}), 4.45 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 163.1$ (C-6), 155.6 (C-8 + C-2_{furan}), 145.8 (C-3), 142.2 (C-5_{furan}), 137.4 (C-$4_{arom.}$-Cl), 132.6 (C-$1_{arom.}$), 129.9 (C-$3_{arom.}$ + C-$5_{arom.}$), 129.7 (C-$2_{arom.}$ + C-$6_{arom.}$), 113.6 (C-4_{furan}), 112.6 (C-3_{furan}), 23.3 (CH_2) ppm. – $C_{14}H_9ClN_4OS$ (316.77): calcd. C 53.08, H 2.86, N 17.69; found C 52.96, H 2.74, N 17.56.

6-(4-Fluorophenyl)-3-(furan-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5c) From 4-fluorophenacyl bromide (260 mg). Yield: 231 mg (77 %), colorless solid, m.p.: 248–249 °C, $R_f = 0.69$. – IR (neat): $\nu = 3049$ (Ar-H), 2912, 2843 (2 × C-H), 1599 (C=N), 1556, 1511 (2 × C=C), 1223 (C-F) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 8.14$ (m, 2H, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.99 (d, 1H, $J_{4,5furan} = 1.8$ Hz, 5-H_{furan}), 7.46 (m, 2H, 3-$H_{arom.}$ + 5-$H_{arom.}$), 7.23 (d, 1H, $J_{3,4furan} = 3.6$ Hz, 3-H_{furan}), 6.77 (dd, 1H, $J_{3,4furan} = 3.6$ Hz, $J_{4,5furan} = 1.8$ Hz, 4-H_{furan}), 4.46 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): $\delta = 166.5$ (d, $J_{C4,F} = 249$ Hz, C4$_{arom.}$-F), 163.2 (C-6), 155.7 (C-8 + C-2_{furan}), 145.7 (C-3), 142.2 (C-5_{furan}), 130.7 (C-$1_{arom.}$), 130.3 (d, $J_{C2,6,F} = 18$ Hz, C-$2_{arom.}$ + C-$6_{arom.}$), 116.9 (d, $J_{C3,5,F} = 123$ Hz, C-$3_{arom.}$ + C-$5_{arom.}$), 113.6 (C-4_{furan}), 112.9 (C-3_{furan}), 23.4 (CH_2) ppm. – $C_{14}H_9FN_4OS$ (300.31): calcd. C 55.99, H 3.02, N 18.66; found C 55.84, H 2.90, N 18.51.

3-(Furan-2-yl)-6-(p-tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5d) From 4-methylphenacyl bromide (255 mg). Yield: 219 mg (74 %), light-orange solid, m.p.: 190–191 °C, $R_f = 0.69$. – IR (neat): $\nu = 3016$ (Ar-H), 2917, 2837 (2 × C-H), 1606 (C=N), 1584, 1509 (2 × C=C) cm^{-1}. – ^1H NMR ([D_6]DMSO): $\delta = 7.99$ (d, 1H, $J_{4,5furan} = 1.5$ Hz, 5-H_{furan}), 7.96 (d,

2H, J = 8.1 Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 7.40 (d, 2H, J = 8.1 3-Hz, $H_{arom.}$ + 5-$H_{arom.}$), 7.23 (d, 1H, $J_{3,4furan}$ = 3.3 Hz, 3-H_{furan}), 6.77 (dd, 1H, $J_{3,4furan}$ = 3.3 Hz, $J_{4,5furan}$ = 1.5 Hz, 4-H_{furan}), 4.43 (s, 2H, CH_2), 2.40 (s, 3H, CH_3) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 164.2 (C-6), 156.5 (C-8 + C-2$_{furan}$), 146.0 (C-3), 142.9 (C-5$_{furan}$), 140.9 (C-4$_{arom.}$-CH_3), 130.9 (C-1$_{arom.}$), 130.2 (C-2$_{arom.}$ + C-6$_{arom.}$), 128.1 (C-3$_{arom.}$ + C-5$_{arom.}$), 113.5 (C-4$_{furan}$), 112.7 (C-3$_{furan}$), 23.3 (CH_2), 21.5 (CH_3) ppm. – $C_{15}H_{12}N_4OS$ (296.35): calcd. C 60.79, H 4.08, N 18.91; found C 60.62, H 4.18, N 18.74.

6-([1,1′-Biphenyl]-4-yl)-3-(furan-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5e) From 4-phenylphenacyl bromide (330 mg). Yield: 276 mg (77 %), yellow solid, m.p.: 228–229 °C, R_f = 0.64. – IR (neat): ν = 3034 (Ar-H), 2915, 2851 (2 × C-H), 1605 (C=N), 1551, 1517 (2 × C=C) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 8.16 (d, 2H, J = 8.4 Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 8.01 (d, 1H, $J_{4,5furan}$ = 1.5 Hz, 5-H_{furan}), 7.92 (d, 2H, J = 8.4 Hz, 3-$H_{arom.}$ + 5-$H_{arom.}$), 7.79 (d, 2H, J = 7.2 Hz, 3′-$H_{arom.}$ + 5′-$H_{arom.}$), 7.53 (d, 2H, J = 7.2 Hz, 2′-$H_{arom.}$ + 6′-$H_{arom.}$), 7.43 (t, 1H, J = 7.2 Hz, 4-$H_{arom.}$), 7.23 (d, 1H, $J_{3,4furan}$ = 3.6 Hz, 3-H_{furan}), 6.79 (dd, 1H, $J_{3,4furan}$ = 3.3 Hz, $J_{4,5furan}$ = 1.5 Hz, 4-H_{furan}), 4.50 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 163.6 (C-6), 156.2 (C-8 + C-2$_{furan}$), 145.8 (C-3), 144.1 (C-4$_{arom.}$), 142.3 (C-5$_{furan}$), 140.9 (C-1$_{arom.}$), 129.6 (C-4$_{arom.}$), 128.8, 128.7 (C-2$_{arom.}$ + C-6$_{arom.}$), 127.8, 127.4 (C-3$_{arom.}$ + C-5$_{arom.}$), 113.8 (C-4$_{furan}$), 112.5 (C-3$_{furan}$), 23.3 (CH_2) ppm. – $C_{20}H_{14}N_4OS$ (358.42): calcd. C 67.02, H 3.94, N 15.63; found C 66.91, H 3.82, N 15.64.

3-(Furan-2-yl)-6-(naphthalen-1-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5f) From 2-bromo-2′-acetonaphthone (299 mg). Yield: 242 mg (73 %), yellow solid, m.p.: 219–220 °C, R_f = 0.68. – IR (neat): ν = 3056 (Ar-H), 2915, 2842 (2 × C-H), 1605 (C=N), 1596, 1512 (2 × C=C) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 8.67 (br s, 1H, 2-$H_{arom.}$), 8.19 (m, 1H, 4-$H_{arom.}$), 8.18 (m, 2H, $H_{arom.}$), 8.03 (m, 2H, $H_{arom.}$ + 5-H_{furan}), 7.67 (m, 2H, $H_{arom.}$), 7.30 (d, 1H, $J_{3,4furan}$ = 3.3 Hz, 3-H_{furan}), 6.80 (dd, 1H, $J_{3,4furan}$ = 3.3 Hz, $J_{4,5furan}$ = 1.8 Hz, 4-H_{furan}), 4.60 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 162.8 (C-6), 156.8 (C-8 + C-2$_{furan}$), 145.9 (C-3), 142.4 (C-5$_{furan}$), 134.8 (C-8a$_{arom.}$), 132.9 (C-4a$_{arom.}$), 129.9 (C-1$_{arom.}$), 129.5 (C-4$_{arom.}$), 129.4, 128.9, 128.2, 127.7, 123.6 ($C_{arom.}$), 113.5 (C-4$_{furan}$), 112.7 (C-3$_{furan}$), 23.2 (CH_2) ppm. – $C_{18}H_{12}N_4OS$ (332.38): calcd. C 65.04, H 3.64, N 16.86; found C 64.98, H 3.75, N 16.69.

3-(Furan-2-yl)-6-(4-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5g) From 4-nitrophenacyl bromide (293 mg). Yield: 245 mg (75 %), yellow solid, m.p.: 270–271 °C, R_f = 0.67. – IR (neat): ν = 3031 (Ar-H), 2931, 2856 (2 × C-H), 1599 (C=N), 1574, 1513 (2 × C=C), 1469, 1344 (2 × NO_2) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 8.42 (d, 2H, J = 8.7 Hz, 3-$H_{arom.}$ + 5-$H_{arom.}$), 8.29 (d, 2H, J = 8.7 Hz, 2-$H_{arom.}$ + 6-$H_{arom.}$), 8.01 (d, 1H, $J_{4,5furan}$ = 1.5 Hz, 5-H_{furan}), 7.24 (d, 1H, $J_{3,4furan}$ = 3.3 Hz, 3-H_{furan}), 6.77 (dd, 1H, $J_{3,4furan}$ = 3.3 Hz, $J_{4,5furan}$ = 1.5 Hz, 4-H_{furan}), 4.53 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 163.1 (C-6), 154.9 (C-8 + C-2$_{furan}$), 149.7 (C-4$_{arom.}$-NO_2 + C-3), 142.2 (C-5$_{furan}$), 140.6 (C-1$_{arom.}$), 129.5 (C-2$_{arom.}$ + C-6$_{arom.}$), 124.6 (C-3$_{arom.}$ + C-5$_{arom.}$), 113.9 (C-4$_{furan}$), 113.0 (C-3$_{furan}$), 23.5 (CH_2) ppm. – $C_{14}H_9N_5O_3S$ (327.32): calcd. C 51.37, H 2.77, N 21.40, found C 51.26, H 2.58, N 21.47.

6-(3,4-Dichlorophenyl)-3-(furan-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5h) From 2,4-dichlorophenacyl bromide (320 mg). Yield: 267 mg (76 %), off-white solid, m.p.: 261–262 °C, R_f = 0.72. – IR (neat): ν = 3020 (Ar-H), 2908, 2851 (2 × C-H), 1685 (C=N), 1548, 1515 (2 × C=C), 1017 (C-Cl) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 8.29 (d, 1H, $J_{4,5furan}$ = 1.5 Hz, 5-H_{furan}), 8.04 (br s, 2H, 5-$H_{arom.}$ + 6-$H_{arom.}$), 7.90 (s, 1H, 2-$H_{arom.}$), 7.22 (d, 1H, $J_{3,4furan}$ = 3.6 Hz, 3-H_{furan}), 6.78 (dd, 1H, $J_{3,4furan}$ = 3.6 Hz, $J_{4,5furan}$ = 1.5 Hz, 4-H_{furan}), 4.46 (s, 2H, CH_2) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 162.9 (C-6), 154.8 (C-8), 149.7 (C-2$_{furan}$), 142.7 (C-5$_{furan}$), 134.7 (C-4$_{arom.}$-Cl), 133.9 (C-3$_{arom.}$-Cl + C4$_{arom.}$-C1), 130.1 (C-2$_{arom.}$), 129.5 (C-5$_{arom.}$), 128.3 (C-2$_{arom.}$), 112.8 (C-3$_{furan}$), 113.7 (C-4$_{furan}$), 23.6 (CH_2) ppm. – $C_{14}H_8Cl_2N_4OS$ (351.21): calcd. C 47.88, H 2.30, N 15.95; found C 47.65, H 2.36, N 15.84.

4.4 Crystal structure determination of 3e

Data were collected on a Bruker APEXII Kappa CCD single-crystal diffractometer equipped with a graphite monochromator. The structure was solved by Direct Methods and refined by full-matrix least-squares procedures on $F2$ with the program SHELXS-97 [31] and TITAN [32]. Hydrogen atoms were placed in idealized positions, and their displacement parameters were refined isotropically with U_{eq} = 1.2–1.5 U_{eq}(C), while other nonhydrogen atoms were

Table 5 Hydrogen bond geometry of **3e** (Å and deg)[a].

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
C(8)–H(8B)···O(1)^{ii}	0.99	2.59	3.386(2)	137.7
C(14)–H(14)···N(1)^{ii}	0.95	2.67	3.385(2)	132.1
C(6)–H(6)···F(1)^{iii}	0.95	2.61	3.140(2)	115.2
C(8)–H(8B)···O(1)^{iv}	0.99	2.54	3.410(2)	146.5
C(11)–H(11)···N(2)^{v}	0.95	2.51	3.382(2)	151.8
C(7)–H(7)···F(1)^{vi}	0.95	2.63	3.223(2)	120.8

[a]Symmetry codes: (ii) x, y+1, z; (iii) –x+1/2, –y+1/2, z–1/2; (iv) –x+1, –y, –z+1; (v) x–1/2, y+1/2, –z+3/2; (vi) x, –y, z–1/2.

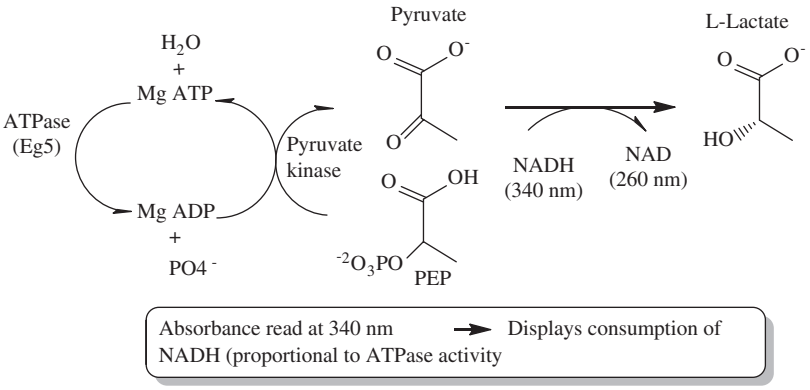


Fig. 5 ATPase activity screening assay.

refined with anisotropic displacement parameters. Table 5 shows the hydrogen bond geometry of **3e**.

Crystal structure data $C_{14}H_9FN_4OS$, $M_r = 300.31$, light-brown plates, crystals dimension = 0.47 × 0.45 × 0.19 mm^3, orthorhombic, space group *Pbca*, $a = 13.699(3)$ $b = 9.328(2)$ $c = 20.497(4)$ Å, $Z = 8$, $V = 2619.3(10)$ Å^3. $D_{calcd.} = 1.52$ mg cm^{-3}, $\mu(MoK_\alpha) = 0.3$ mm^{-1}, $F(000) = 1232$ *e*, $T = 90(2)$ K. MoK_α radiation, $\lambda = 0.71073$ Å, 28565 collected refls. (*hkl* –17/14, ±11, ±25), 2639 unique refls. ($R_{int} = 0.0685$); 190 refined parameters. *R*1/*wR*2 = 0.0464/0.0969 (all data), GOF = 1.022, $\Delta\rho_{fin}$ (max/min) = 0.23/–0.31 *e* Å^{-3}.

CCDC 990263 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via*www.ccdc.cs.ac.uk/data_request/cif.

4.5 Anti-kinesin Eg5 assay

The ATPase activity of the Eg5 motor domain was measured using the malachite green assay as described earlier [24]. The reactions were performed in a buffer (80 mM Pipes, pH = 6.8; 1 mM EGTA, 1 mM $MgCl_2$, 0.1 mgmL^{-1} BSA, 1 mM taxol) supplemented with Eg5 (48 nM) fusion protein and microtubules (200 nM). Ten minutes after compound addition, reactions were started by the addition of ATP (50 mM) and incubated at r.t. for 7 min. The reactions were stopped by adding perchloric acid (444 mM, Fluka), and the color reaction was started by adding the developer solution [1 M HCl (Sigma), 33 mM malachite green (Sigma), 775 mM ammonium molybdate tetrahydrate (Sigma)]. After 20 min, the absorbance at 610 nm was measured using a plate reader (Victor2, Perkin-Elmer). The IC_{50} values were determined in three independent experiments for each compound. Fig. 5 shows the ATPase activity screening assay.

4.6 *In vitro* anti-HIV assay

Evaluation of the antiviral activity of compounds **3a–h**, **4a–f** and **5a–h** against the HIV-1 strain (III_B) and the HIV-2 strain (ROD) in MT-4 cells was performed using an MTT assay as described previously [25]. In brief, stock solutions (10 times final concentration) of test compounds were added in 25-µL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control, HIV-, and mock-infected cell samples, were included for each sample. HIV-1(III_B) [33] or HIV-2 (ROD) [34] stock (50 µL) at 100–300 $CCID_{50}$ (50 % cell culture infectious dose) or culture medium was added to either of the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells [35] were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells per mL, and 50-µL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of the mock- and HIV-infected cells was examined spectrophotometrically.

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References

[1] O. Rath, F. Kozielski, *Nat. Rev. Cancer* **2012**, *12*, 527–539.

[2] J. Cahu, A. Olichon, C. Hentrich, H. Schek, J. Drinjakovic, C. Zhang, A. Doherty-Kirby, G. Lajoie, T. Surrey, *PLoS One* **2008,** *3*, e3936.

[3] D. J. Sharp, G. C. Rogers, J. M. Scholey, *Nature* **2000**, *407*, 41–47.

[4] J. Zhou, P. Giannakakou. *Curr. Med. Chem.: Anti-Cancer Agents* **2005**, *5*, 65–71.

[5] D. Rodriguez, C. Ramesh, L. H. Henson, L. Wilmeth, S. Kadavakollu, R. Hirsch, J. Montoya, P. R. Howell, D. Alexander, D. L. Johnson, J. B. Arterburn, C. B. Shuste, *Bioorg. Med. Chem.* **2011**, *19*, 5446–5453.

[6] D. Huszar, M. E. Theoclitou, J. Skolnik, R. Herbst, *Cancer Metastasis Rev*. **2009**, *28*, 197–208.

[7] S. D. Knight, C. A. Parrish, *Curr. Top. Med. Chem.* **2008**, *8*, 888–904.

[8] C. J. Lawrence, R. K. Dawe, K. R. Christie, D. W. Cleveland, S. C. Dawson, S. A. Endow, L. S. Goldstein, H. V. Goodson, N. Hirokawa, J. Howard, R. L. Malmberg, J. R. McIntosh, H. Miki, T. J. Mitchison, Y. Okada, A. S. Reddy, W. M. Saxton, M. Schliwa, J. M. Scholey, R. D. Vale, C. E. Walczak, L. Wordeman, *J. Cell. Biol.* **2004**, *167*, 19–22.

[9] A. S. Kashina, R. J. Baskin, D. G. Cole, K. P. Wedaman, W. M. Saxton, J. M. Scholey, *Nature* **1996**, *379*, 270–272.

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

[11] S. DeBonis, D. Skoufias, G. Robin, L. Lebeau, R. Lopez, R. Margolis, R. H. Wade, F. Kozielski, *Mol. Cancer. Ther.* **2004**, *3*, 1079–1090.

[12] R. Nakai, S. Lida, T. Takahashi, T. Tsujita, S. Okamoto, C. Takada, K. Akasaka, S. Ichikawa, H. Ishida, S. Akinaga, C. Murakata, S. Honda, M. Nitta, H. Saya, Y. Yamashita, *Cancer Res*. **2009**, *69*, 3901–3909.

[13] N. Sunder-Plassmann, V. Sarli, M. Gartner, M. Utz, J. Seiler, S. Huemmer, T. U. Mayer, T. Surrey, A. Giannis, *Bioorg. Med. Chem.* **2005**, *13*, 6094–6111.

[14] H. Carol, R. Lock, P. J. Houghton, C. L. Morton, E. A. Kolb, R. Gorlick, C. P. Reynolds, J. M. Maris, S. T. Keir, C. A. Billups, M. A. Smith, *Pediatr. Blood Cancer* **2009**, *53*, 1255–1263.

[15] K. Holen, R. DiPaola, G. Liu, A. R. Tan, G. Wilding, K. Hsu, N. Agarwal, C. Chen, L. L. Xue, E. Rosenberg, M. Stein, *Invest. New Drugs* **2012**, *30*, 1088–1095.

[16] E. De Clercq, *Med. Res. Rev.* **1996**, *16*, 125–157.

[17] A. M. Vandamme, K. Van Laethem, E. De Clercq, *Drugs* **1999**, *57*, 337–361.

[18] M. H. Khan, T. Akhtar, K. A. Yasin, N. A. Al-Masoudi, P. G. Jones, S. Hameed, *Z. Naturforsch.* **2010**, *65b*, 178–184.

[19] Y. A. Al-Soud, N. A. Al-Masoudi, R. Loddo, P. La Colla, *Arch. Pharm. (Weinheim, Ger.)* **2008**, *341*, 365–369.

[20] M. Koparır, A. Cansız, A. Demirdağ, *Molecules* **2004**, *9*, 204–212.

[21] W. Willker, D. Leibfritz, R. Kerssebaum, W. Bermel, *Magn. Reson. Chem.* **1993**, *31*, 287–292.

[22] K. N. Bhalla, *Oncogene* **2003**, *22*, 9075–9086.

[23] Z. Maliga, T. M. Kapoor, T. J. Mitchison, *Chem. Biol.* **2002**, *9*, 989–996.

[24] R. D. Henkel, J. L. VandeBerg, R. A. Walsh, *Anal. Biochem.* **1988**, *169*, 312–318.

[25] C. Pannecouque, D. Daelemans, E. De Clercq, *Nat. Protoc.* **2008**, *3*, 427–434.

[26] K. D. Hargrave, J. R. Proudfoot, K. G. Grozinger, E. Cullen, S. R. Kapadia, U. R. Patel, V. U. Fuchs, S. C. Mauldin, J. Vitous, *J. Med. Chem.***1991**, *34*, 2231–2241.

[27] H. Mitsuya, K. J. Weinhold, P. A. Furman, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096–7100.

[28] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **1999**, *28*, 235–242.

[29] D. Rogers, A. J. Hopfinger, *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 854–866.

[30] J. H. Friedman, *Technical Report No. 102*, 2nd ed., Laboratory for Computational Statistics, Stanford University, Stanford, **1988**.

[31] G. M. Sheldrick, *Acta Crystallogr.* **2008**, *A64*, 112–122.

[32] K. A. Hunter, J. Simpson, TITAN2000, University of Otago, Otago, New Zealand, **1999**.

[33] M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **1984**, *224*, 497–500.

[34] F. Barré-Sinoussi, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, *Science* **1983**, *220*, 868–871.

[35] I. Miyoshi, H. Taguchi, I. Kubonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, T. Akagi. *Gann Monogr. Cancer Res,* **1982**, *28*, 219–228.