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Scaffold hopping identifies 6,8-disubstituted purines as novel anaplastic lymphoma kinase inhibitors

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Abstract: Rearrangements of anaplastic lymphoma kinase (ALK) are associated with several cancer diseases. Due to resistance development against existing ALK-inhibitors, new, structurally unrelated inhibitors are required. By a scaffold hopping strategy, 6,8-disubstituted purines were designed as analogues of similar ALK-inhibiting thieno[3,2-*d*]pyrimidines. While the new title compounds indeed inhibited ALK and several ALK mutants in submicromolar concentrations, they retained poor water solubility.

Abbreviations

ALK: anaplastic lymphoma kinase; cMet, hepatocyte growth factor receptor; DIPEA, *N,N*-Diisopropylethylamine; DMF, *N,N*-Dimethylformamide; EML4, echinoderm microtubule-associated protein-like 4; FDA, Food and Drug Administration; gk, gatekeeper; µW, micro wave; NSCLC, non-small cell lung cancer; pdb, protein data bank; RT: room temperature; TEA, triethly amine; wt, wild type.

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Introduction

In developed countries, lung cancer is top on the cancer death rate list, with non-small cell lung cancer (NSCLC) accounting for the majority of cases [1]. As found with other cancer diseases, in NSCLC the hyperactivity of distinct protein kinases is often responsible for tissue hyperproliferation. In recent years, this finding has been translated into targeted therapies employing small molecules that inhibit oncogenic tumor-driving protein kinases [2]. The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is involved in brain development and is physiologically expressed during embryogenesis [3]. In 2007 it was shown that an inversion within chromosome 2p generates a fusion gene consisting of the *ALK* gene and parts of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene. This *EML4-ALK* fusion was found in 6.7% of the surveyed NSCLC patients [4]. Later it was shown that the *EML4-ALK* fusion occurs typically in patients with adenocarcinoma histology, female gender, and a non-smoking history [5]. Several oncogenic variants of *EML4-ALK* have been reported [6, 7], as well as oncogenic fusions of *ALK* with other partner genes [8].

The *EML4-ALK* fusion and other *ALK* translocations lead to constitutive protein kinase hyperactivity resulting in tissue proliferation, increased survival and eventually cancer development [9]. Consequently, ALK was suggested as a target for personalized therapy of *ALK*-positive NSCLC patients [4]. In 2011 the dual ALK/c-Met receptor tyrosine kinase inhibitor crizotinib (**1**) was approved by the FDA [10]. In the ALK-positive sub-group of patients, crizotinib is rather effective compared with the standard chemotherapy first line treatment. Unfortunately, most patients acquire resistance within the first year after start of treatment [11]. While in some cases resistance development is due to activation of other tumor drivers [12] or to

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amplification of the *ALK* fusion gene, the majority of resistances are the result of single point mutations of the *ALK* kinase domain [13], prominent examples being the gate-keeper mutation L1196M and the C1156Y mutation [14]. The second generation ALK inhibitors alectinib (**2**) [15, 16] and ceritinib (**3**) [11, 17] were approved recently. Both drugs show a protein kinase inhibition selectivity pattern different to crizotinib and also inhibit the gate-keeper mutation L1196M. The second generation ALK-inhibitors are used for the therapy of patients who have progressed on previous crizotinib treatment [10]. Because it can be predicted that also against these second generation inhibitors resistances will emerge, the development of further ALK inhibiting chemotypes is of high interest. In a recent patent application, thieno[3,2-*d*]pyrimidines like compound **4a** were disclosed as ALK inhibitors (IC_{50} **4a** on ALK wt ~ 1 µM) [18], which unfortunately display poor solubility (Fig. 1). Scaffold hopping is a strategy for the discovery of structurally novel bioactive compounds starting from known drug molecules by replacement of their core structure [19]. Predicted by the method of Wildman and Crippen [20], the purine ring system as an isolated element shows lower lipophilicity (clogP = 0.35) compared to the thieno[3,2-*d*]pyrimidine scaffold (clogP = 1.69) present in **4a**. In an attempt to develop ALK inhibitors based on novel chemical matter with improved solubility, we designed molecules of the series **5** by exchanging the thieno[3,2-*d*]pyrimidine core structure for a purine ring system (Fig. 2). The comparative pairwise solubility prediction of **4** and **5** by the method of Ali et al. [21] via the Swiss ADME interface (http://www.swissadme.ch) yielded distinctly better results for the latter congeners. Since docking analyses predicted a good fit of the designed molecules to the ATP binding pocket of the ALK target kinase, the purine analogues **5** were synthesized and tested for inhibition of ALK, of cancer-relevant ALK-mutants, and of related protein kinases.

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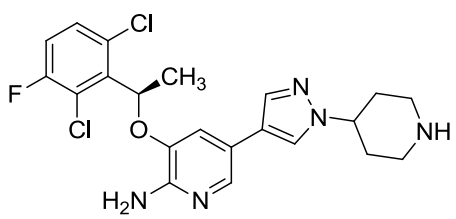

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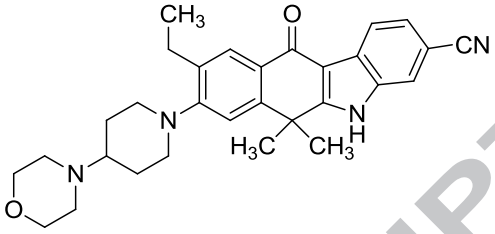

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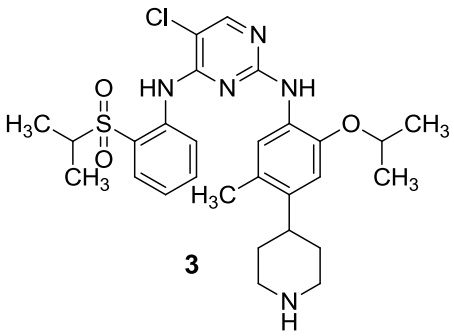

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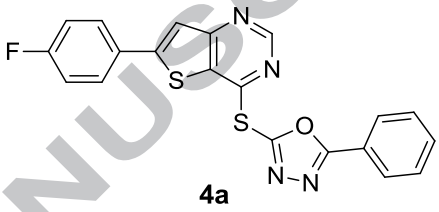

4a

Fig. 1 ALK-inhibitors: Crizotinib (**1**), Alectinib (**2**), Ceritinib (**3**), proprietary thieno[3,2-*d*]pyrimidine **4a**.

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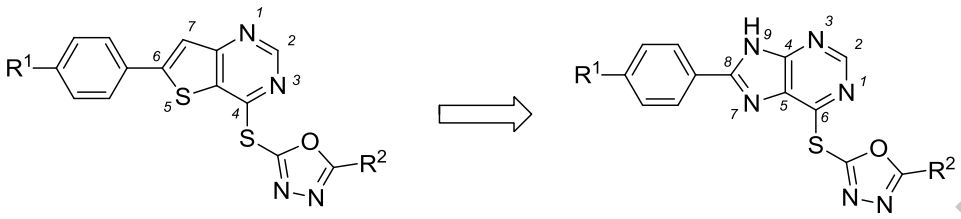

4 **5**

Fig. 2 Scaffold hopping: Exchange of the thieno[3,2-d]pyrimidine core structure in **4** [18] for a purine system leads to the title compounds **5**.

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Docking

To estimate the chances for successful scaffold hopping, we performed docking-experiments with **5** in the ATP binding site of ALK. The co-crystal structure of ALK wild type with bound ceritinib (PDB-code 4MKC)[11] was used as template for these studies. Ceritinib (**3**) forms two hydrogen bonds to Met1199 (gk+3) of the hinge region (Figure 3, A). Taking into account the binding mode of ceritinib, constraints were implemented to provoke the formation of two hydrogen bonds between the purine-scaffold of **5a** and residues of the ALK hinge region. The docking routine placed compound **5a** into a position in which the N(9)-H group acts as hydrogen bond donor for the carbonyl group of Met1199 (3 residues downstream of gatekeeper; gk+3) and in which the N(3)-atom of the ligand accepts a hydrogen bond from the NH-group of Met1199. While the 1,3,4-oxadiazole moiety is located in the center of the binding pocket, its phenyl substituent fills the back part of the pocket (Fig. 3, B). In contrast, the docking pose of compound **4** shows only one hydrogen bond between the N(3)-atom of the ligand and Met1199 (Fig. 3, C). According to the docking results, the scaffold hopping from thieno[3,2-*d*]pyrimidine to purine derivatives enables the formation of an additional hydrogen bond between the ligand and the protein kinase. Because docking of compound **5a** into the ATP binding site of the gatekeeper mutant ALK L1996M (PDB-code: 2YFX [22]) generated a similar docking pose (Figure 3, D), it was expected that both wild type and mutant form of ALK would be inhibited by **5a** and congeners. Considering the docking pose of **5a** in ALK, we expected that introduction of an additional amino group in 2-position of the purine core as displayed in congeners **5cc** and **5dd** would enable a third hydrogen bond between ligand and hinge area. This speculation was corroborated by the

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results of docking **5cc** into the ATP binding pocket of ALK (PDB: 4MKC). Hence, we also prepared these compounds in the hope to improve the ALK inhibitory activity. Furthermore, we synthesized the analogues **14a-c** to evaluate the contribution of the 8-aryl substituent of the title compounds for binding to and for inhibition of ALK.

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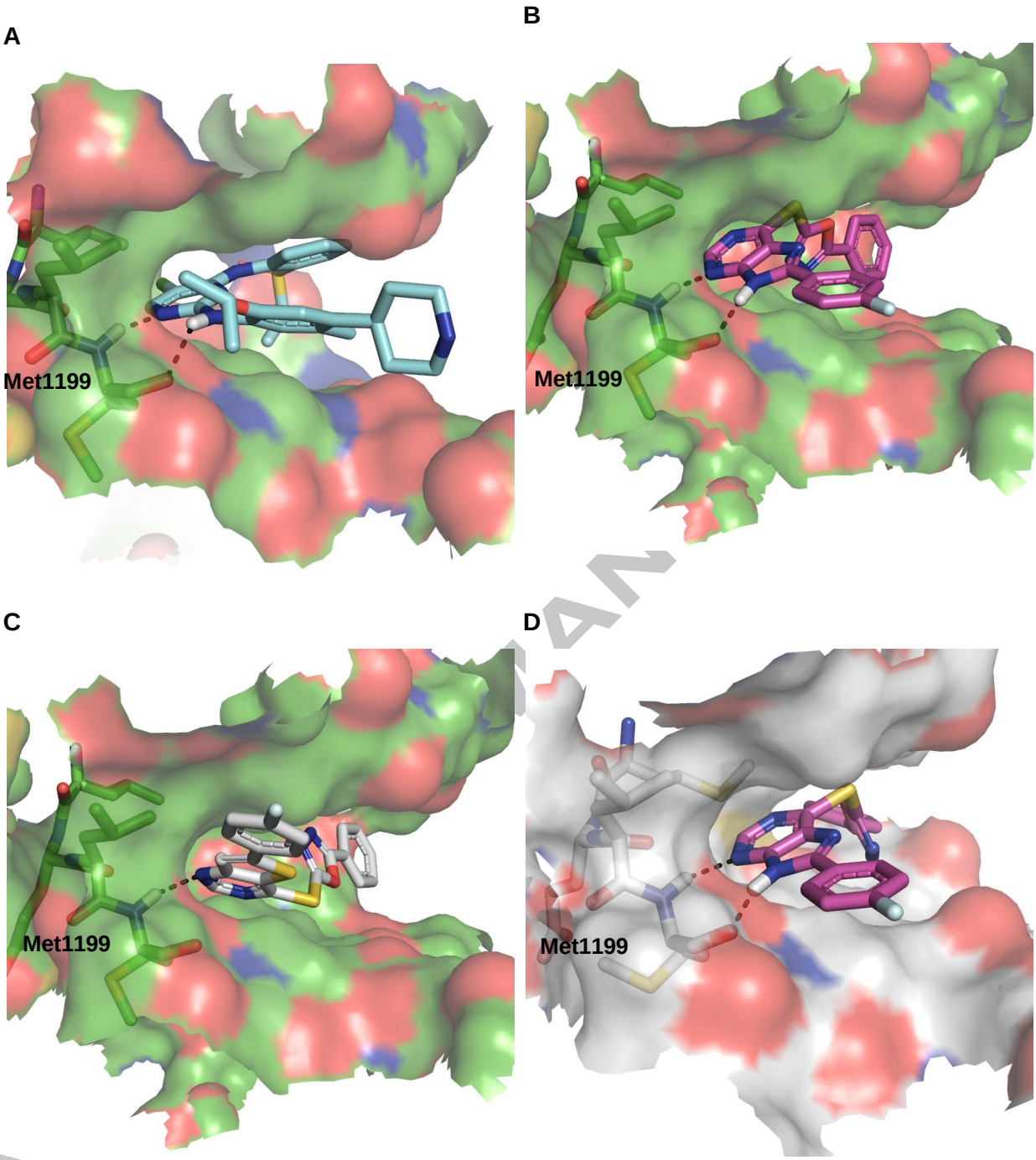

Fig. 3 Results of docking studies. 3A: co-crystal structure of Ceritinib (**3**, blue) and ALK wt (4MKC [11], green); 3B: docking-pose of **5a** (magenta) in the ATP binding site of ALK wt (4MKC, green); 3C: docking-pose of **4** (gray) in the ATP binding site of ALK wt (4MKC, green); 3D: docking-pose of **5a** (magenta) in the ATP binding site of ALK L1196M (2YFX [22], gray). Docking was carried out with GOLD [23] (refer to supporting information for details).

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Synthesis

The central step of the synthesis of the title compounds **5** consisted in a nucleophilic substitution reaction of the 8-aryl-6-chloropurines **8** with various 1,3,4-oxadiazole-2-thioles **9**. The starting materials **8** were synthesized according to the method developed by Legraverend et al.[24, 25] by ring closure reaction of 6-chloropyrimidine-4,5-diamine (**7**) with carboxylic acid chlorides **6** in $POCl_3$ in the presence of NH_4Cl. The 1,3,4-oxadiazole-2-thioles **9** were prepared by ring closure reaction of hydrazides with CS_2 by literature procedures [26-29]. The synthesis of the 2-aminopurine derivatives **5cc**, **5dd** started from 2-amino-8-bromo-1*H*-purin-6(9*H*)-one (**10**) which yielded 2-amino-8-(4-fluorophenyl)-1*H*-purin-6(9*H*)-one (**11**) by Suzuki reaction with 4-fluorophenylboronic acid in a microwave-assisted reaction. Treatment of **11** with $POCl_3$ catalyzed by *N,N*-dimethylaniline led to the 6-chloro derivative **12** [30-32]. Subsequently, the 1,3,4-oxadiazole-2-thioles **9** and the purine derivatives **8** or **12**, respectively, were reacted yielding the title compounds **5a**-**5dd**. Under conventional conditions, these nucleophilic substitution reactions required up to 40 h in refluxing propan-2-ol or butan-1-ol. Assisted by microwave irradiation, similar reactions in DMF needed only 20-30 min (scheme 1). In an analogous process, the commercially available 2-amino-6-chloropurine (**13**) reacted with 1,3,4-oxadiazole-2-thioles **9** furnishing the 8-unsubstituted congeners **14** (scheme 2).

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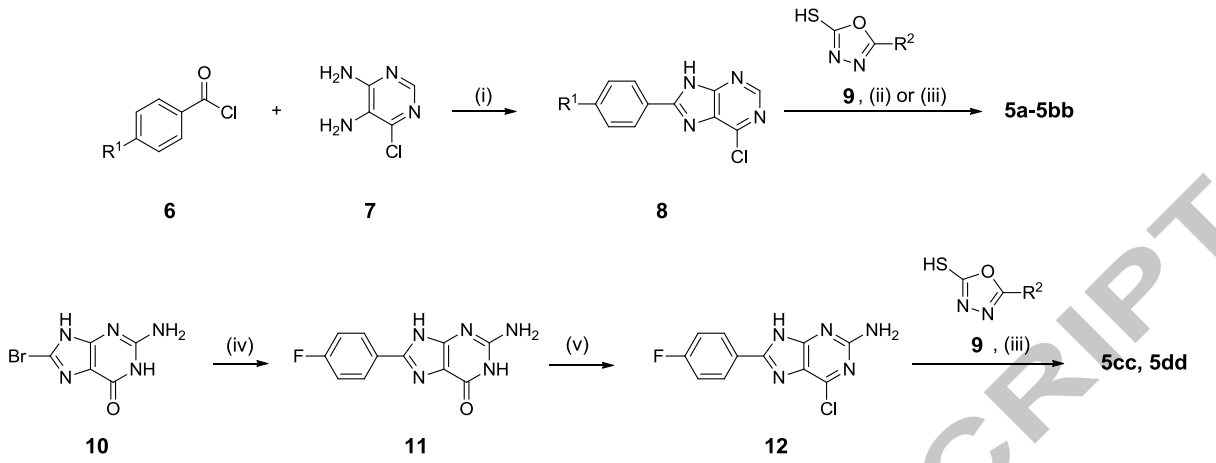

Scheme 1 Synthesis of compounds **5**: (i) $POCl_3$, NH_4Cl, 100 °C, 15 – 24 h, yield: 20 – 59%; (ii) TEA or DIPEA, propan-2-ol or butan-1-ol, reflux, 15-40 h, 18 – 65%; (iii) TEA or DIPEA, µW, DMF, 120 °C, 20 - 30 min, 10-66%; (iv) 4-fluorophenylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O 3:1, µW, 130 °C, 1 h, yield: 31%; (v) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 2 h, yield: 26%. For residues R^1 and R^2 refer to table 1.

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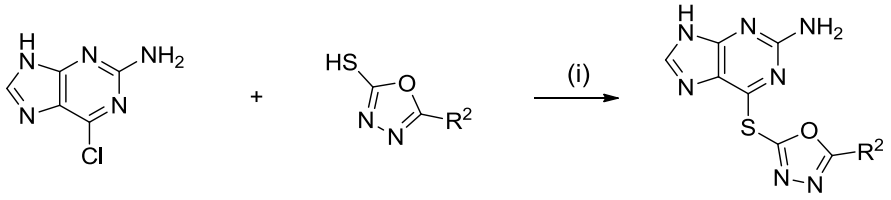

13 **9** **14a-c**

Scheme 2 Synthesis of compounds **14a-c**: (i) DMF, TEA, 120 °C, µW, 30 min, 61–74%. For residues R^2 refer to table 2.

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Results and discussion

The title compounds **5** and their 8-unsubstituted analogues **14** were evaluated for inhibitory activity against wild type ALK, ALK mutants, ALK fusion gene products and other tumor relevant protein kinases in a radiometric protein kinase assay. For means of comparison, crizotinib (**1**) was tested as a standard ALK inhibitor (Tables 1, 2, and 3; for complete data refer to tables S1 and S2 in the supporting information). The results revealed that the scaffold hopping was successful. Four of the new title compounds (**5b**, **5h**, **5l**, **5u**) displayed IC_{50}-values towards ALK wt in submicromolar concentrations, with compound **5l** exhibiting the strongest inhibition (IC_{50} = 457 nM). Structurally closely related compounds showed slightly decreased ALK inhibition in single digit micromolar concentrations. All new compounds were clearly inferior compared to crizotinib which inhibited ALK with an IC_{50} value of 21 nM in our assay. The test outcome allows a preliminary statement regarding structure activity relationships in the new family of ALK inhibitors. It is obvious from the results collected in Table 1 that substitution of the oxadiazole moiety by aliphatic instead of aromatic substituents R^2 (**5q**, **5r**, **5s**, **5aa**) leads to decrease of ALK wild type inhibition potency by two orders of magnitude. Similarly, substitution at this position by heteroaromatic residues, e.g. pyridin-4-yl (**5g**, **5m**, **5y**) or furan-2-yl (**5bb**) impaired ALK inhibitory potency. In contrast to our expectation and the results of our docking studies, the addition of a 2-amino group at the purine ring (**5cc**, **5dd**) failed to optimize the potency, but led to loss of activity. The analogues **14a-c** lacking the 8-aryl substitution were even less potent compared to **5cc** and **5dd** (Table 2). Most of the new compounds **5** and **14** maintained activity upon testing against the important gatekeeper mutant ALK L1196M, displaying an advantage over crizotinib which loses potency by one order of magnitude on the indicated mutant. While crizotinib is a well-

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known inhibitor of the cancer-relevant kinase c-Met [33, 34], the new compounds reported here showed, if any, only marginal inhibition of c-Met. A set of selected compounds from series **5** and **14** were also tested on various additional relevant ALK mutants and fusion proteins (refer to collected data in tables S1-S3 in the supporting information); some results for **5l** and **5u** are shown in Table 3. Crizotinib inhibited all ALK mutants with comparable potency, with the exception of the gatekeeper mutant L1196M. In contrast, **5l** performed less consistent, inhibiting two of the mutants (ALK C1156Y and ALK F1174L) in three- to fourfold higher concentrations. Compound **5u** turned out to be most robust with regard to ALK mutations, since all tested mutants were inhibited in concentrations between 0.8 and 1.4 µM.

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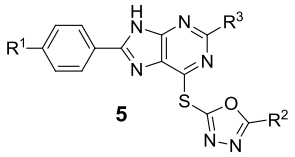
Table 1: Protein kinase inhibition by compounds **5** (IC_{50}, [µM]) [a]



5

entry	R^1	R^2	R^3	ALK wt	ALK L1196M	cMet
1	n.a.[b]	n.a.[b]	n.a.[b]	0.021	0.233	0.104
5a	F	Phenyl	H	6.01	4.86	33.6
5b	F	4-Cl-Phenyl	H	0.816	0.822	6.89
5c	F	4-CF_3-Phenyl	H	1.95	4.80	22.8
5d	F	4-OCH_3-Phenyl	H	4.98	4.94	> 100
5e	F	2-Cl-Phenyl	H	6.34	7.12	> 100
5f	F	2,4-di-Cl-Phenyl	H	5.18	3.58	> 100
5g	F	4-Pyridyl	H	> 100	> 100	> 100
5h	OCH_3	4-Cl-Phenyl	H	0.961	1.17	66.7
5i	OCH_3	4-CF_3-Phenyl	H	1.90	1.86	> 100
5j	OCH_3	4-OCH_3-Phenyl	H	4.81	4.95	> 100
5k	OCH_3	2-Cl-Phenyl	H	4.80	5.08	> 100
5l	OCH_3	2,4-di-Cl-Phenyl	H	0.457	0.875	> 100
5m	OCH_3	4-Pyridyl	H	9.64	9.40	> 100
5n	OCH_3	4-CH_3-Phenyl	H	2.08	1.95	33.3
5o	OCH_3	2-CH_3-Phenyl	H	2.06	1.81	> 100
5p	OCH_3	4-OH-Phenyl	H	2.99	3.04	> 100
5q	OCH_3	Benzyl	H	51.7	30.9	> 100
5r	OCH_3	*tert*-Butyl	H	31.4	41.0	75.5
5s	OCH_3	Morpholinomethyl	H	> 100	> 100	> 100
5t	H	4-Cl-Phenyl	H	5.95	7.00	> 100
5u	H	4-CF_3-Phenyl	H	0.857	0.963	> 100

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5v	H	4-OCH_3-Phenyl	H	4.29	3.12	> 100
5w	H	2-Cl-Phenyl	H	8.81	6.04	81.1
5x	H	2,4-di-Cl-Phenyl	H	1.60	1.93	> 100
5y	H	4-Pyridyl	H	26.6	51.1	> 100
5z	H	4-CH_3-Phenyl	H	10.5	7.16	> 100
5aa	H	Benzyl	H	75.5	54.6	> 100
5bb	H	2-Furyl	H	22.5	18.0	> 100
5cc	F	4-Cl-Phenyl	NH_2	13.5	12.1	21.7
5dd	F	2,4-di-Cl-Phenyl	NH_2	13.0	4.58	> 100

[a] Z'-factors [35] for high and low controls ≥0.54. [b] n.a. = not applicable

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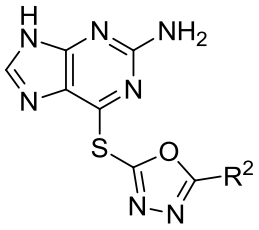
Table 2: Protein kinase inhibition by compounds **14** (IC_{50}, [µM]) [a]



entry	R^2	ALK wt	ALK L1196M	cMet
14a	4-Cl-Ph	21.1	13.1	> 100
14b	4-CF_3-Ph	21.4	16.0	> 100
14c	2-Cl-Ph	18.6	17.2	> 100

[a] Z'-factors[35] for high and low controls ≥0.54.

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Table 3: Comparison of ALK mutant selectivity profile of crizotinib with 8-aryl-6-(1,3,4-oxadiazol-2-ylthio)purines **5l** and **5u** (IC_{50}, [µM]) [a]

Protein kinase	**Crizotinib** (**1**)	**5l**	**5u**
ALK *wt*	0.021	0.457	0.857
ALK L1196M	0.233	0.875	0.963
ALK C1156Y	0.033	1.73	1.11
ALK F1174L	0.035	2.30	1.13
ALK F1174S	0.039	n.d.[b]	1.36
ALK R1275Q	0.021	0.332	0.977
NPM1- ALK	0.034	0.322	0.965
NPM1- ALK/F1174L	0.054	0.313	0.961

[a] Z'-factors[35] for high and low controls ≥0.54. [b] n.d.=not determined

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Despite the integration of the purine scaffold, the new compounds presented here still display a high degree of lipophilicity and a low sp^3/sp^2 ratio, both unfavorable features for water solubility of organic molecules. Indeed, none of four representative test compounds (**5b**, **5f**, **5cc**, **5dd**) which were subjected to thermodynamic solubility determination (aqueous phosphate buffer, pH 7.4) produced in the HPLC runs signals exceeding the limit of quantification (4 µM). Additional molecular modifications aiming at increasing water solubility are therefore essential for a further rational development process in this class of compounds.

Conclusion

Starting from thieno[3,2-*d*]pyrimidine derivatives **4**, 6,8-disubstituted purine derivatives **5** were developed as a class of new ALK inhibitors, demonstrating a vivid example for the efficiency of the scaffold hopping approach. Four of the new compounds exhibited ALK inhibitory activity in the submicromolar concentration range. Compound **5l** proved to be the most potent ALK inhibitor in the series (IC_{50} = 457 nM). Preliminary data regarding structure activity relationships in the series **5** show that aromatic substituents both at position 8 of the purine system and at position 2 of the 1,3,4-oxadiazole ring are important for kinase inhibition potency. In contrast to the approved ALK inhibitor crizotinib, **5** are able to maintain inhibition potency for of the gatekeeper mutant ALK L1196M. Docking studies suggest that **5** bind to the ATP binding pocket of ALK, where they are rendered in position by a pair of hydrogens bonds between the purine scaffold and Met1199 (gk+3) of the host protein. Inspired by the inhibitor poses produced through molecular docking, derivatives **5cc** and **5dd** were prepared which bear an additional amino group at

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position 2 of the purine ring as anchoring point for a third hydrogen bond to the hinge region of the kinase. This strategy was not successful, for both compounds showed decreased potency compared to the corresponding unsubstituted congeners **5b** and **5f**. The derivatives **5** presented here still display only poor water solubility. Additional molecular modifications are therefore ongoing to optimize these properties, as well as to investigate the cellular activities and the pharmacokinetic properties in the compound class.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the only version, at http://dx.doi.org/XXXXXXXXXXXXXXX

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Scaffold hopping identifies novel 6,8-disubstituted purines as anaplastic lymphoma kinase inhibitors

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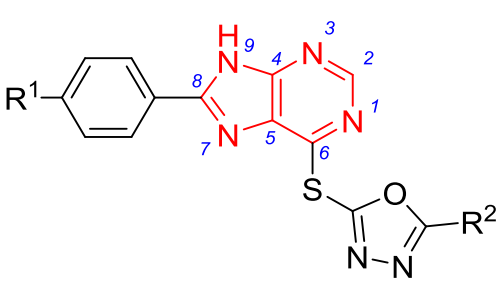

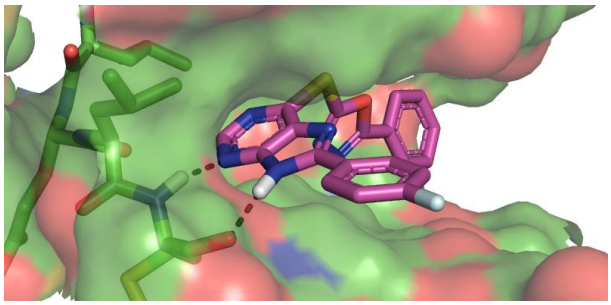

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