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Short communication

Design, synthesis and characterization of N_9/N_7-substituted 6-aminopurines as VEGF-R and EGF-R inhibitors

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Abstract

In this study we report on the design, synthesis and biological characterization of novel N_9 or N_7 arylethanone-substituted 6-aminopurines and 6-methoxypurines, respectively, as EGF-R and VEGF-R inhibitors. The compounds were initially profiled in a panel of 24 cancer-relevant protein kinases. Dependent on the regio-substitution of the purine core we found inhibition activity for EGF-R and VEGF-R with IC_{50} values in the μM range. The two novel N_9/N_7 2-(6-amino-purine)-1-(1*H*-indole-3-yl)ethanone derivatives were characterized in an enhanced panel of 78 kinases showing the N_9 derivative to also inhibit MNK1 and IRR while the N_7 isomer was found to be specific for VEGF-R2.

Keywords: VEGF-R; EGF-R; Protein kinase inhibitors; N_7/N_9-Substituted 6-aminopurines

1. Introduction

Small molecule inhibitors of the receptor tyrosine kinases VEGF-R (vascular endothelial growth factor receptor) [1] and EGF-R (epidermal growth factor receptor) [2,3] are in particular of pharmaceutical interest because these protein kinases (PKs) are considered to be validated drug targets in angiogenesis and cancer [4]. In recent publications we presented compounds combining the purine system from the original cosubstrate ATP and phenyl moieties in order to explore possible interactions with the different regions of the ATP binding site in several disease-related protein kinases [5,6].

In this communication we report on the design, synthesis and results of our SAR investigation directed at the N_9/N_7-regiospecific substitution of the 6-aminopurine scaffold (I and II, Scheme 1) as VEGF-R/EGF-R inhibitors and the biological characterization of the compounds in a panel of 24 cancer-relevant PKs. Furthermore, two novel compounds have been tested in a specificity profile over 78 PKs.

2. Results and discussions

We designed the scaffolds I and II on the basis of the modelled binding mode of compound **1** (belonging to scaffold I) in the ATP pocket of VEGF-R2 (Fig. 1). Herein, the pose of **1** involves H-bond interactions of the 6-aminopurine moiety addressing the hinge-region carbonyl of Cys917. In contrast to the situation in ATP where N_1 is accepting an H-bond from the protein, in this binding mode an H-bond is accepted by N_7 of the adenine scaffold (Fig. 1). The N_9 indolylethanone substitution is directed towards the hydrophobic pocket I (HPI) where the indole moiety forms π–π stacking interactions to the Phe1045 residue of the DFG motif [7]. However, in particular this interaction towards HPI was considered to determine activity and selectivity of the inhibitor for VEGF-R2/3 [8]. Furthermore, in this binding mode of **1** the hydrophobic region II (HRII) is not yet addressed and, therefore, leaving chemical space for optimization. To prove the

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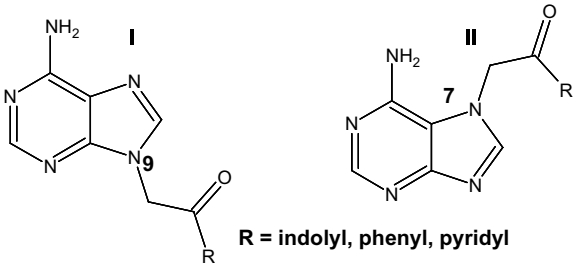

Scheme 1. N_9/N_7 regioselective-substituted 6-aminopurine scaffolds I and II.

concept of the inhibitor design as a lead structure the N_9/N_7-regiospecific-substituted 6-aminopurines **1**, **2** and related analogues were synthesized and evaluated for biological activity.

3. Chemistry

The synthesis for both I and II starts from 6-chloro-9*H*-purine. Due to the tautomeric situation in the purine nucleus, modifications of the purine scaffold in this study by base catalyzed S_N reaction with 2-bromo-1-arylethanone derivatives leads mainly to N_9-substituted purines with only 5–10% of N_7-substituted purines. Vice versa, major regioselective N_7-substitution was conveniently afforded by use of the auxiliary cobaloxime [9] $CH_3Co(DH)_2OH_2$ whereas N_9-substitution occurred only in 5–12% (Fig. 2) [10].

Accordingly, in this study the reaction performed well for 2-bromo-1*H*-indol-3-ylethanone and 2-bromo-1-phenylethanone to yield compounds **1**–**4** (Fig. 3). Unexpectedly, the cobaloxime strategy was not successful for 2-bromo-1-pyridylethanones **5** and **6** and hence only the N_9-substituted derivatives were obtained. Due to the additional pyridine nitrogen the cobaloxime–purine complex was probably not stable using these reaction conditions. The N_9/N_7-regiospecific-substituted isomers were isolated by flash chromatography and subsequently heated with concentrated ammonium hydroxide solution in a Berghof B25 pressure reactor to yield the corresponding 6-aminopurine derivatives (I/II, Fig. 3). In light of the aminolysis of 6-chloropurine derivatives, catalytic amounts of Cu(I)I decreased the yields and side products occurred [11]. The reaction of adequate N_9/N_7-precursors in a methanolic solution of ammonia gave compounds **7** and **8** [12] as major products besides minor amounts of **3** and **4** [13–16].

Compound **9** was obtained under basic conditions (K_2CO_3) by the reaction of 6-chloropurine and 2-bromo-1*H*-indol-3-ylethanone in non-distilled DMF which contains dimethylamine. Hence, in a one-pot reaction both the alkylation of N_9 according to the general scheme as well as the nucleophilic substitution of purine-C_6 by dimethylamine occurs.

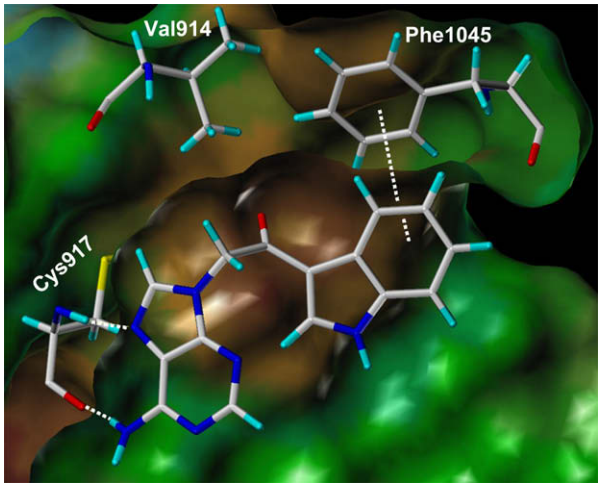

Fig. 1. Modelled binding mode of **1** in the crystallographically determined ATP binding pocket of VEGF-R2 (PDB 1Y6A [7]). Compound **1** forms key H-bonds to Cys917 (hinge-region) and π–π stacking interactions to Phe1045 in the hydrophobic pocket I; Val914 (gatekeeper).

4. Biological evaluation

These nine compounds have been evaluated for their potency against 24 cancer-relevant protein kinases belonging to different families of the kinome (Table 1) [17]. Consistent with the modelling hypothesis, **1** inhibited VEGF-R2 ($IC_{50} = 81$ μM) and slightly more potently blocked the epidermal growth factor receptor (EGF-R, $IC_{50} = 35$ μM; a modelled binding mode of **1** in EGF-R [18] comparable to VEGF-R2 is available in the Supporting information). Concerning SAR, the involvement of the amino-function of **1** in ligand–protein interactions to these PKs is supported by complete loss of the biological activity of aminomethylated derivative **9** in the panel. Inhibition in the μM range for VEGF-R2 was also found for **2**, **6** and **7** whereas EGF-R is inhibited by **2**, **3**, **5** and **6**. Compounds **1** and **2** inhibited VEGF-R2 but not the highly homologous VEGF-R3. In contrast, compound **7** shows an IC_{50} of 25 μM for VEGF-R2 and 45 μM for VEGF-R3 with specificity over the other kinases of this panel. In light of the modelled binding mode of **1** (Fig. 1) this result was unexpected since the amino-function in **1** (H-bond donor) is replaced in **7** by a methoxy group (H-bond acceptor). Furthermore, the corresponding N_7 regioisomer **8** was not inhibiting any of the PKs. However, the inhibition of **7** indicates an alternative binding mode in VEGF-R for this compound which was not predicted by our modelling studies. Interestingly, dependent on the regio-substitution of the 1*H*-indol-3-ylethanone moiety, compound **1** (N_9) inhibited EGF-R more potently than VEGF-R2 whereas the converse situation is true for **2** (N_7, VEGF-R2 over EGF-R).

However, in order to reveal their potential as multikinase inhibitors [19] **1** and **2** were additionally characterized in an enhanced specificity panel of 78 PKs at a concentration of 10 μM [20] (data available in the Supporting information). Herein, **1** inhibited MNK1 [21] (MAP kinase signal-integrating kinase, 73 ± 3% inhibition at 10 μM) and IRR [22] (insulin-receptor related receptor, 51 ± 1% inhibition at