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Entry into a new class of protein kinase inhibitors by pseudo ring design

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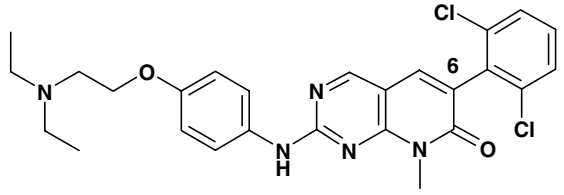
Abstract—A pyrimidin-4-yl-urea motif forming a pseudo ring by intramolecular hydrogen bonding has been designed to mimic the pyrido[2,3-*d*]pyrimidin-7-one core structure of a well-established class of protein kinase inhibitors. Potent inhibition of a number of protein kinases was obtained with the first prototype compound synthesized to probe the design concept.

Designing molecular mimics of established lead compounds is one of the major approaches in medicinal chemistry to generate new biologically active chemotypes. In such efforts, one aims at introducing the chemical functions conferring the desired biological activity in new, different, molecular frameworks. One thus expects to obtain molecules reaching the level of activity of the reference lead compound and presenting some additional benefit such as easier synthetic access, better patentability or different physico-chemical properties that might turn out advantageous in the drug development phase.

Pseudo six-membered rings resulting from the formation of an intramolecular hydrogen bond in a planar conjugated moiety of a molecule are considered to be particularly stable. They are often observed in crystal structures of organic molecules.[1] Replacing real rings by such pseudo rings is a new and non-conventional strategy to try to mimic established lead compounds. Recently, we have reported the discovery of new classes of potent VEGF-R and EGF-R tyrosine kinase inhibitors following this approach.[2,3] In this letter, we report another example of successful pseudo ring design applied to the search for new kinase inhibitor scaffolds.

Molecules based on a pyrido[2,3-*d*]pyrimidin-7-one core structure constitute one of the most prominent classes of protein kinase inhibitors.[4,5] In particular, attachment of an aryl moiety in position 6 of the ring system provides broadly active tyrosine kinase inhibitors exemplified by PD 166285.[6] Thus, our interest in identifying new tyrosine kinase inhibitor scaffolds led us to take PD 166285 (Fig. 1) as a template for the design of prototype mimics. Previous efforts in this direction are reported in the literature.[7] They consist of replacing one of the rings of the core pyrido[2,3-*d*]pyrimidin-7-one system by a similar



PD166285

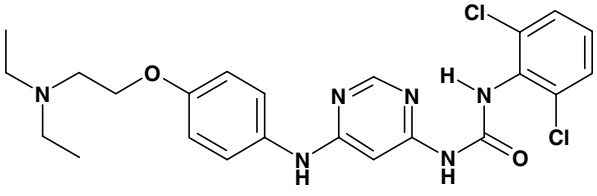


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Figure 1. Chemical structures of PD 166285 and prototype compound **1**.

Keywords: Kinase; Scaffold morphing; Pseudo ring.
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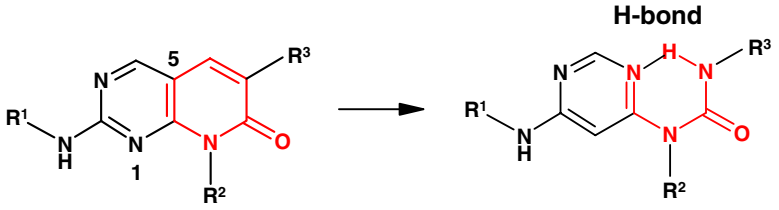


Figure 2. Pseudo ring design concept.

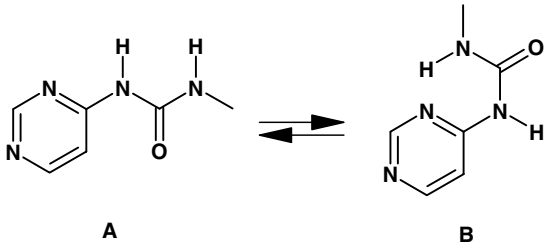


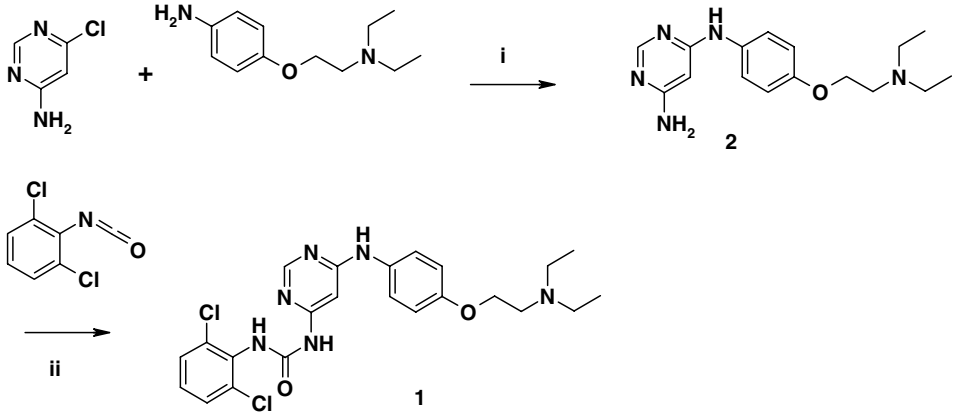
Figure 3. Model compound studied by ab initio calculations. Using the DFT-B3LYP/6-31G** method, the pseudo cyclic conformation B is predicted to be more stable than the extended conformation A.

heterocyclic moiety, in a traditional manner. We decided to follow a more risky but novel approach to achieve this objective. As illustrated in Figure 2, one of our design ideas was to move the nitrogen atom in position 1 of the pyrimidine ring of PD 166285 to position 5 and replacing the pyrimidone ring by a urea moiety able to form an intramolecular hydrogen bond with this nitrogen atom. The pseudo six-membered ring thus obtained looked to us as an excellent mimic of the pyrimidone ring and the synthesis of compound **1** as a first prototype for the designed pyrimidinyl urea scaffold was envisaged.

Before engaging in the synthesis of **1**, we sought some experimental and computational evidence that the pseudo cyclic conformation required to mimic the pyrido[2,3-*d*]pyrimidin-7-one bicyclic system was indeed stable and would not prohibit binding to a kinase active site because of a too high energy cost to pay for conformational adaptation. A search in the Cambridge Structural Database using a pyrimidin-4-yl-urea substructure query returned seven molecules with available crystal structure coordinates.[8] Encouragingly, we noticed that in all of them the pyrimidin-4-yl-urea motif adopts the pseudo cyclic conformation presenting an intramolecular hydrogen bond between the pyrimidine ring and the urea group. We then performed ab initio calculations on the model compound shown in Figure 3. The difference in energy between the extended and pseudo cyclic conformations of this model compound was computed in the gas phase and using a water solvation model. In both cases, the pseudo cyclic conformation was calculated to be more stable, strengthening our motivation to synthesize the envisaged prototype compound.[9]

The pyrimidinyl urea **1** was prepared in two steps from commercially available starting materials (Scheme 1). 6-Chloro-pyrimidin-4-ylamine and 4-(2-diethylamino-ethoxy)-phenylamine were condensed in *n*-butanol at 120 °C using 1 equiv of hydrochloric acid in dioxane. The intermediate **2** was further condensed with 1,3-dichloro-2-isocyanato-benzene in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) at room temperature.

Compound **1** was tested in biochemical assays measuring its ability to inhibit the catalytic activity of various recombinant protein kinases.[10] The experimental data are reported in Table 1. To our satisfaction, several tyrosine kinases such as c-Src, EGF-R, c-Abl, and Tie-2 were inhibited in the submicromolar range giving support to our design concept. Based on the known binding mode of pyrido[2,3-*d*]pyrimidin-7-one kinase inhibi-



Scheme 1. Synthesis of compound **1**. Reagents and conditions: (i) 4 N HCl in dioxane, *n*-butanol, 120 °C; (ii) solvent: *N*,*N*′-dimethyl-*N*,*N*′-propylene urea, rt, yield: 37%.

Table 1. IC_{50} values (μM) of compound **1** in enzymatic assays and nature of gate keeper residue

Kinase	IC_{50}[a]	Gate keeper
c-Src	0.066	Thr
EGF-R	0.38	Thr
c-Abl	0.25	Thr
FGFR-1	0.57	Val
c-Kit	0.93	Thr
KDR	0.96	Val
Tie-2	0.30	Ile
PDGFR-β	1.4	Thr
B-raf V599E	0.15	Thr
EphB4	0.43	Thr
P38	0.35	Thr
FLT3	>10	Phe
c-Met	>10	Leu
IGF1-R	>10	Met
JAK2	>10	Met
CDK2	>10	Phe
PKA	>10	Met
PKB	>10	Met
Axl	>10	Leu

[a] All IC_{50} values represent averages of at least three experimental determinations.

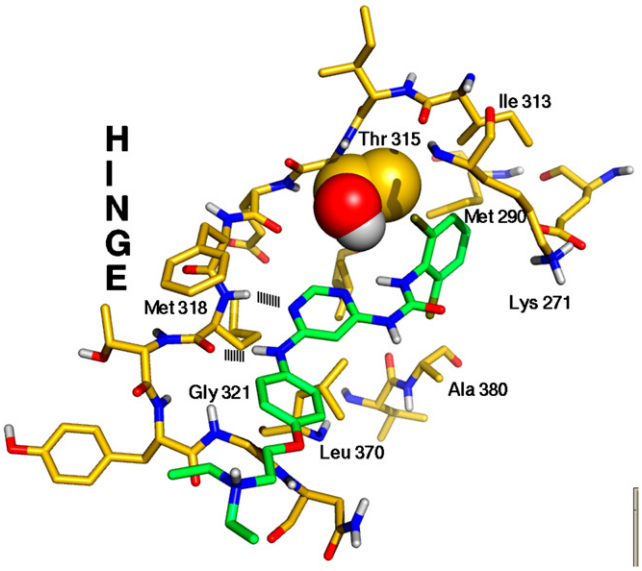


Figure 4. Model of compound **1** (green) docked in the ATP pocket of the c-Abl kinase (yellow). A CPK representation is given to the gate keeper residue, a threonine for this kinase. The canonical hydrogen bonds to the hinge segment are indicated by dashed lines.

tors[11] a model of **1** docked in the ATP pocket of one of these kinases (c-Abl) was constructed (Fig. 4).[12] In the model, the dichlorophenyl moiety of our pyrimidin-4-yl-urea mimic makes favorable van der Waals contacts with the hydrophobic back pocket of the ATP binding site, in particular with the threonine gate keeper residue.[13] The model suggests a very tight fit between the inhibitor and the enzyme in this region of the ATP pocket. As a consequence, an adverse steric clash between the compound and the gate keeper residue is expected if this is significantly larger than a threonine. In full agreement with this notion, none of the kinases of the Table 1 panel possessing a bulky gate keeper residue is inhibited by **1** at a concentration as high as 10 μM. This is the case for the FLT3, JAK2, and c-Met kinases, for instance, which present a phenylalanine, a methionine, and a leucine, respectively, at the gate keeper position. In contrast, all the kinases tested having a threonine or a residue of similar size (valine or isoleucine) at this position are inhibited by **1** in the micromolar or submicromolar range.

In conclusion, the results presented here give an additional illustration that employing a pseudo ring resulting from the formation of an intramolecular hydrogen bond is a valuable novel approach for mimicking a real ring in a lead compound. Prototype **1** has given us an entry in a new class of protein kinase inhibitors of potential interest for a variety of therapeutic targets.[14–16]

Acknowledgment

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the 6-31G** basis set and doing full geometry optimization. A preliminary systematic conformational analysis of the model compound by molecular mechanics varying all torsion angles identified the extended and pseudo cyclic conformations A and B as the low energy conformations of this system. In the subsequent quantum mechanical calculations, B was computed to be more stable than A: by 3.2 kcal/mol in the gas phase and by 0.5 kcal/mol applying the water solvation model available in Jaguar.

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