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Contribution of indazolinone tautomers to kinase activity

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ABSTRACT

The design and synthesis of indazolinone containing kinase inhibitors are reported. Regioisomers that showed profound potency variation in previously-reported isoindolinone and aminoindazole systems were surprisingly found to have similar potencies in the case of the indazolinone chemical series. An interpretation using differential hinge hydrogen bonding and tautomeric equilibrium of indazolinone ring system is supported by quantum mechanics calculations. The equipotent inhibition of a representative kinase (KDR) by regioisomeric indazolinones **4** and **5** is clear evidence that in case of the indazolinone hinge, both tautomers are equally favored, and should be considered in design of inhibitors.

Analysis of the many available crystal structures of kinase/inhibitor complexes reveals conserved interactions that are important for the recognition of small heterocyclic molecules by the ATP binding site of this class of enzymes. Prominent among these are hydrogen bond interactions that inhibitors make with the backbone of the amino acid stretch that connects the kinase N- and C-terminal domains, the so-called 'hinge' loop. These hydrogen bonds position the inhibitors in an orientation that allow them to make multiple favorable contacts with the side chains of hydrophobic residues that normally comprise the environment of the adenine ring of ATP in the pocket. Given the importance of the hinge hydrogen bonds and the associated hydrophobic interactions to obtain binding affinity for the ATP pocket, one strategy to find new kinase inhibitors is based on the design of molecular scaffolds targeting these interactions.

Inspired by the hinge interaction in Staurosporine (**1**), Curtin et al. reported the elegant design and synthesis of isoindolinone ureas (**2**) as inhibitors of KDR (Fig. 1).[1] An overlay model of KDR and CDK2 with bound **2** indicated that the lactam N–H and C=O make interactions with the hinge carbonyl Glu915 and amide Cys917 respectively. Subsequently, Dai et al. reported 3-amino indazole containing biarylureas (**3**) wherein the 3-amino group and the N-2 nitrogen participate in hinge interactions, a maneuver which also resulted in potent KDR inhibitors.[2] In this communication, we report the design and synthesis of indazolinone inhibitors **4** and **5**.

The design strategy of this work relied on the potential ability of the indazolinone core to tautomerize between keto and hydroxy forms, as shown in Figure 2. It was anticipated that this tautomerization would allow the indazolinone hinge-binder **6** to make similar hydrogen bond interactions with the backbone hinge similar to either or both the isoindolinone core present in **2** and aminoindazole core in **3**. With no literature information on indazolinone tautomer preference as a guide, computer modeling studies were carried out on several test molecular systems.

Relative tautomer energies of structurally-simplified regioisomeric phenyl-substituted indazolinone structures were calculated and are given in Table 1.[3] Structures **7** and **8** match the core units of compounds **4** and **5**, respectively. Both **7** and **8** give, at most, a 1.7 kcal/more energy difference between the keto and hydroxy tautomers in various environments. Strikingly, there was a reversal of tautomer preference under conditions of low or high dielectric, with the keto form being preferred at high dielectric. Regardless of the exact environment, the calculations suggest that the keto and hydroxy tautomers are close enough in energy that under the varied conditions of dissolution in aqueous solution or being bound within the active-site, compounds **7** and **8** will have both tautomeric forms present to some extent.

The synthesis of **4** involved cyclization of the product of the Suzuki cross coupling reaction **10** with hydrazine hydrate to afford the indazolinone intermediate **11** (Scheme 1). Urea formation using meta trifluoromethyl phenylisocyanate afforded **4**. Similar synthetic elaboration of **12** afforded the regioisomer **5**.

Models of the binding modes of **4** and **5** in complex with KDR are shown in Figure 3a–b.[4] The previously reported model of isoindolinone **2** was based on Staurosporine and satisfactorily

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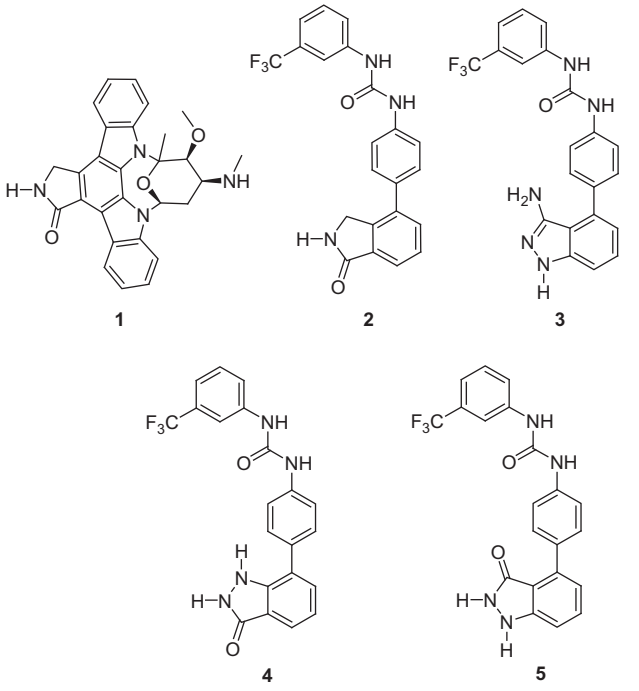


Figure 1. Staurosporine, representative biaryl urea inhibitors and target compounds from the present study.

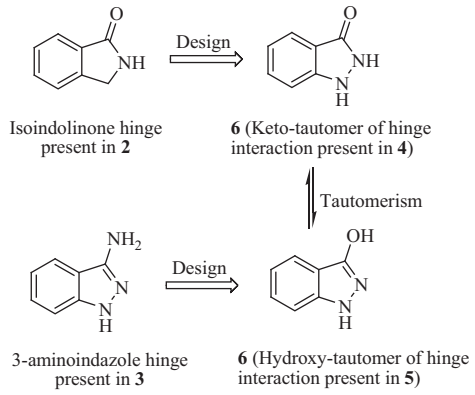
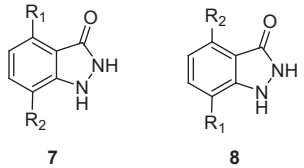
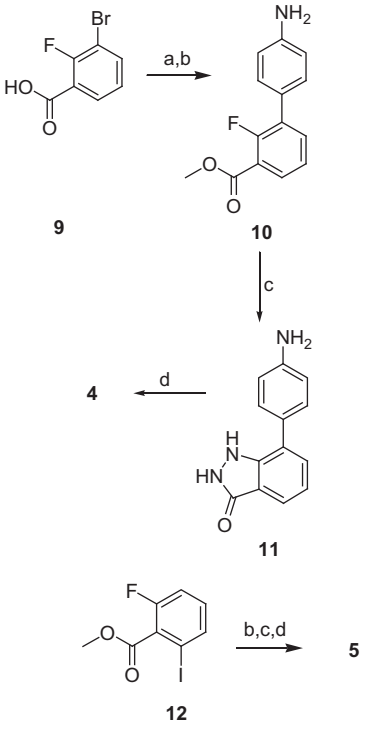


Figure 2. Design strategy – structural similarity of the indazolinone hinge 6, to the isoindolinone and 3-aminoindazole hinge.

Table 1
Relative energies of tautomers in gas and aqueous phase, calculated at HF/6-31G* level of quantum mechanics.[3] ε = dielectric constant, ΔE = energy difference for the tautomeric equilibrium, kcal/mole



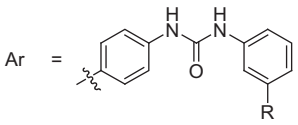
R_1	R_2	ε	ΔE	Preferred tautomer
H	Ph	1	−0.49	**7**, Hydroxy
H	Ph	80	+1.67	**7**, Keto
Ph	H	1	−0.90	**8**, Hydroxy
Ph	H	80	+0.66	**8**, Keto



Scheme 1. Reagents and conditions: (a) CH_3I, Cs_2CO_3, DMF, 3 h, 67%; (b) 4-anilinophenyl boronic acid, $PdCl_2dppf$, Na_2CO_3, DME:CH_3CH_2OH:H_2O, μwave, 160 °C, 20 min, 65%; (c) N_2H_4, pTsOH, 130 °C, 20 min, 25–40%; (d) 3-CF_3phenylisocyanate, DMF, 4 h, 70–75%.

correlated with the SAR reported in the study.[1] This model was used as a template in modeling compound **4** which possesses the same regiochemical substitution pattern as **2** (Fig. 3a). As mentioned previously, in this model, the inhibitor carbonyl accepts a H-bond from Cys917 and the 2-NH donates a H-bond to Glu915. In an analogous exercise, the previously reported model of aminoindazole **3** was based on a thienopyrimidine hinge,[5] and satisfactorily correlated with the SAR.[2] This model was used as a template in modeling compound **5** which possesses the same regiochemical substitution pattern as **3**. (Fig. 3b) In this model, the inhibitor hydroxy group donates a H-bond to the backbone carbonyl of Glu915 and the 5-membered ring nitrogen accepts a H-bond from the backbone NH of Cys917. Both models of **4** and **5** gave satisfactory structural fit within the active site. The models are consistent with compound **4** bound in the keto-tautomeric form and compound **5** bound in the hydroxy-tautomeric form.

Compounds **4** and **5** were evaluated for their KDR kinase activity (Table 2)



As depicted in Table 2, both **2** and **3** were potent inhibitors of KDR, whereas the regioisomeric biarylureas **13** and **14** were devoid of KDR activity. In contrast, both regioisomers of **4** and **5** were determined to be potent inhibitors of KDR with IC_{50} values of 0.04 μM and 0.01 μM, respectively.

The observation of this high level of potency for both 4 and 5 is striking given that the biaryl urea moiety is attached at diametrically opposite 4- and 7-positions on the benzo ring of the indazolinone ring system. In fact, the two compounds overlay within the active site with a similar orientation (schematized in Fig. 4). We

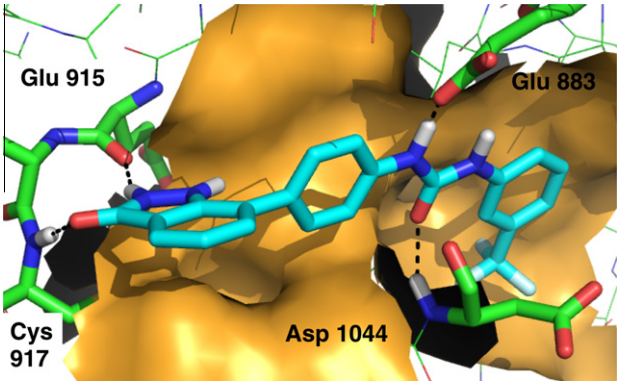


Figure 3a. Compound **4** bound to ATP-binding site of KDR kinase in the DFG-out conformation.

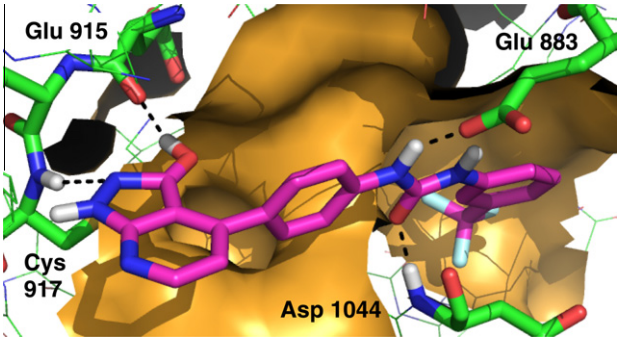
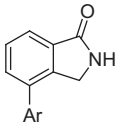
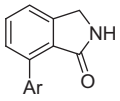
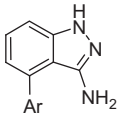
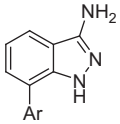
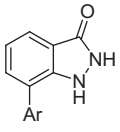
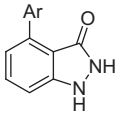


Figure 3b. Compound **5** bound to ATP-binding site of KDR kinase in the DFG-out conformation.

Table 2
KDR IC_{50} (1 mM ATP) of **4**, **5** and comparator analogs

Compound	Structure	R	KDR IC_{50} (μM)
2		CF_3	0.007
13		CH_3	>1000
3		CF_3	0.009
14		CH_3	>1000
4		CF_3	0.04
5		CF_3	0.01

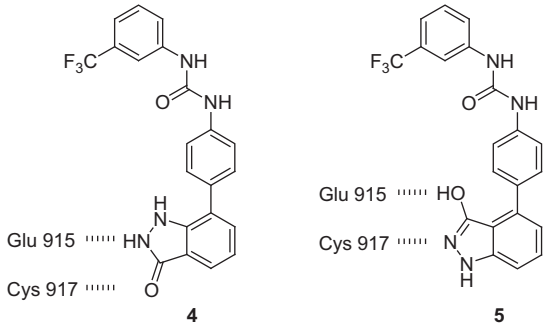


Figure 4. Bioactive hinge interactions of **4** and **5**.

postulate that this overlay is feasible because of the potential for tautomerism of the indazolidinone ring system and the different hydrogen bonding arrangements with the hinge (Fig. 4). In sharp contrast to the indazolinone systems reported here, the isoindolines (2, 13) and aminoindazoles (3, 14) are expected to be overwhelmingly in one tautomeric form and activity is observed for only one regioisomer in each case. Additional SAR on the similar kinase inhibition profile on close analogs of **4** and **5** are representative of a larger analoging effort (data not shown here).

In this Letter, we have reported on a new class of kinase inhibitors based on an indazolinone ring system.[6] We believe that two tautomers of this ring system exist, that permit two different chemical series to be generated, here represented by compounds **4** and **5**. The concept of tautomerism in kinase hinge interactions has been discussed previously, as have the challenges of differences between tautomerization states and conformation states in binding free-energy calculations.[7] This study demonstrates that in case of the indazolinone hinge, there exists the possibility of multiple tautomers that provide additional options in analog design. Therefore, both tautomers should be considered in design of kinase inhibitors.[8] For heterocycles that can exist in tautomeric forms with reasonable abundance, kinase inhibitor design can exploit unique features of each tautomer to discover novel hinge-binding units.

Acknowledgements

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3. Structures were geometry optimized at the HF/6-31G* level (gas phase) using Gaussian 98 software (Gaussian, Inc., Pittsburgh, PA). The gas phase energy difference between tautomeric structures, ΔE(g), was calculated at the MP2/6-31G* level using the geometry obtained at the HF/6-31G* level. The solution energy ΔE(s) = ΔE(g) + E(solv), where the solvation energy E(solv) was calculated at the B3LYP/6-31G* level using the same geometry and the conductor-like polarizability continuum model (CPCM). The estimated error in these energy differences was +/− 0.5 kcal/mole at ε = 1 and +/− 1.0 at ε = 80.
4. Previous modeling indicated that diaryl urea compounds such as **4** and **5** bind to a DFG-out form of KDR kinase [Ref.[2] and[3]]. DFG-out protein from entry 1YWN (PDB database, BMCL, **2005**, *15*, 2203) was selected and models of isoindolinone inhibitor **2**[1] and aminoindazole inhibitor **3**[2] were overlaid using a least-squared superposition of the alpha-carbon protein coordinates. Models of compounds **10** and **11** were built from structures 2 and 3, respectively (Insight II software,

Accelrys, San Diego, California). Energy minimization to eliminate close contacts provided the final structures illustrated in Figure 4.

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