CH···O and CH···N Hydrogen Bonds in Ligand Design: A Novel Quinazolin-4-ylthiazol-2-ylamine Protein Kinase Inhibitor

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Aryl CH hydrogen bonds play an important role in the binding of several analogues of a pyrazol-3-ylquinazolin-4-ylamine inhibitor of glycogen synthase kinase 3 (GSK3). Understanding the importance of these CH···O and CH···N hydrogen bonds allowed the design of a novel quinazolin-4-ylthiazol-2-ylamine inhibitor of GSK3 with a structurally confirmed CH···O hydrogen bond to the protein.

Introduction

The role of traditional hydrogen bonds (XH···X, where X is O or N) is routinely taken into account in studies of structure−activity relationships (SAR) and ligand design. On the other hand, CH···X hydrogen bonds have generally not been considered in these situations. Given the large body of evidence supporting the existence of these hydrogen bonds,[1−3] including their occurrence within proteins[4,5] and at protein interfaces,[6] it seems likely that they are relevant to protein−ligand interactions and inhibitor design. In fact, two recent studies suggest that CH···X hydrogen bonds do make important contributions to protein−ligand binding.[7,8] However, there have so far been no cases where CH···X hydrogen bonds played a key role in inhibitor SAR or ligand design. In our work designing inhibitors of glycogen synthase kinase 3 (GSK3) we believe we have come across such a case.

Discussion

Given the numerous pharmacological possibilities for a GSK3 inhibitor[9,10] and our recent determination of the structure of the enzyme,[11] we set out to discover inhibitors of this important enzyme. An early lead in this effort was **1**, a potent inhibitor with a K_i of 24 nM. The determination of a ligand-bound structure of GSK3 with **1** revealed that the aminopyrazole moiety forms three key hydrogen bonds with the hinge strand in the ATP binding site (Figure 1). With this information, **2**−**6** were synthesized to develop a structure−activity relationship around the lead (Table 1). As expected, removing a key hydrogen bond donor by changing the pyrazole to the isoxazole of **2** led to a dramatic loss of activity. The more surprising results came from **3** and **4**. Both compounds were expected to maintain a high level of potency because all key interactions with the protein were conserved from **1**. Nevertheless, **3** was 100-fold less potent than the lead compound, and **4** was 1000-fold less potent. More surprising SAR was revealed when **5** and **6** were titrated against the enzyme. These are the des-methyl versions of **1** and **4**, respectively. Removing the methyl group from **1** leads to **5**, with a moderate 4-fold loss in potency, while the corresponding change

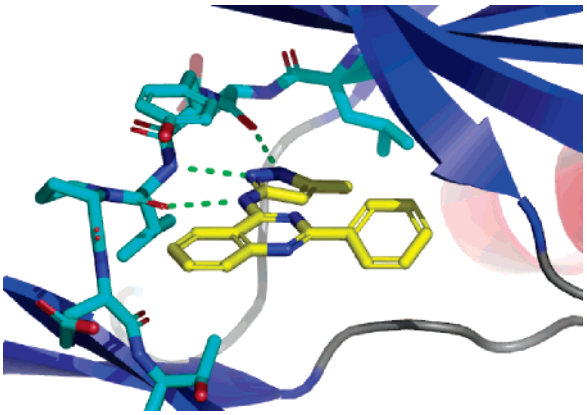
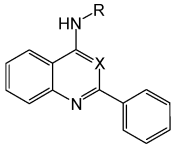


Figure 1. Crystal structure of **1** bound to the ATP binding site of GSK3. The pyrazole and quinazoline ring systems are nearly coplanar, and the three hydrogen bonds to the hinge (cyan tubes) are highlighted.

Table 1. Inhibition Constants of **1**−**6**



	R	X	GSK3 K_i(μM)
1		N	0.024
2		N	>2.0
3		C	2.5
4		N	23.0
5		N	0.11
6		N	0.25

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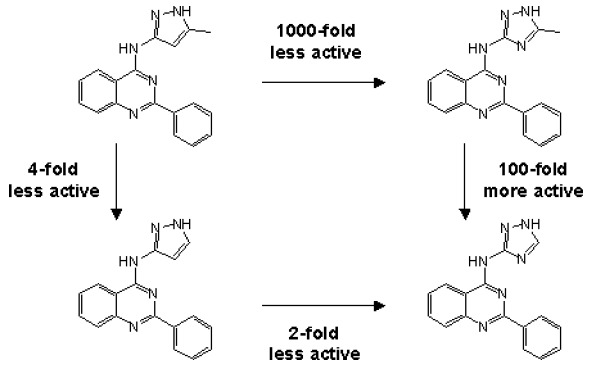

Figure 2. Illustration of unexpected SAR. Parallel arrows represent analogous structural transformations, which had been expected to yield analogous changes in potency.

in **4** leads to **6**, with a nearly 100-fold gain in potency. Figure 2 highlights several of these unexpected points of SAR.

The meaning of these results was unclear until the potential role of CH···X hydrogen bonds was considered (where X can be O or N). These moderately polar interactions are well-known from studies of small-molecule crystal structures[1,2] and have more recently been recognized in proteins[5] and at protein−protein interfaces.[6] Also, a recent analysis of protein kinase crystal structures demonstrates that CH···O hydrogen bonds are also important in protein−ligand binding interactions.[7] This analysis finds the most compelling evidence for heteroaromatic CH groups (those adjacent to a heteroatom in an aromatic ring) as hydrogen bond donors, but numerous studies have shown the broad hydrogen-bonding potential of aromatic CH groups.[12,13] Although the donor acidity in these aromatic CH interactions is far less than that in traditional hydrogen bonds, it seems that the reduced desolvation penalty upon binding leads to inhibitors with comparable potency. We believe that the SAR of **1**−**6** can only be explained in light of these aryl CH···X hydrogen bonds, not only between the ligand and the protein but also in stabilizing the appropriate inhibitor conformation through intramolecular hydrogen bonds.

Relative to **1**, the activities of **2** and **5** are easily explained by traditional protein−ligand interactions. In the first case, replacement of a hydrogen bond with an acceptor−acceptor pair leads to a sharp drop in potency, while in the second the loss of hydrophobic interactions leads to a moderate loss of activity. The loss of activity in **3** is less easily explained unless one considers that the intramolecular hydrogen bond between the pyrazole CH and quinazoline nitrogen stabilizes the planar binding conformation. Replacing this donor−acceptor pair with the donor−donor pair in **3** changes the preferred conformation to that shown in Figure 3a. This conformation presents the protein with only one of three hydrogen-bonding groups, and the equivalent coplanar structure in Figure 3b, while capable of forming the appropriate hydrogen bonds, is 1.9 kcal/mol less favored. It is this preference for an unproductive binding conformation that leads to the poor activity of this compound. All intramolecular energies and conformations are derived from Hartree−Fock ab initio calculations performed with the 6-31G** basis set in Gaussian 98.[14] For conformations described as preferred and coplanar

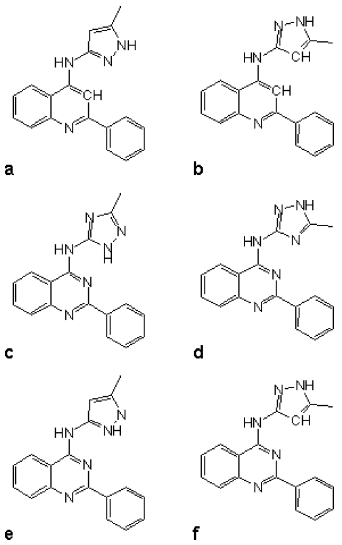

Figure 3. Illustrations of various tautomers and conformations available to **3** (a, b), **4** (c, d), and **1** (e, f). The relative energies and their effect on binding are described in the text and in Figure 5.

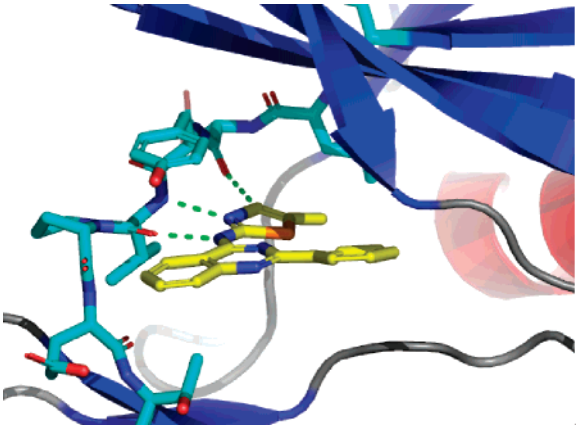

Figure 4. Crystal structure of **7** in the ATP site of GSK3, revealing the coplanar arrangement of the quinazoline and thiazole, two traditional hydrogen bonds to the hinge (cyan tubes), and a CH···O hydrogen bond to the hinge.

both dihedral angles linking the two aromatic ring systems, when optimized, have absolute values of less than 0.2°.

The relative activity of **4** is explained by a similar conformational preference. Because of an intramolecular NH···N hydrogen bond, the conformation depicted in Figure 3c is favored over that in Figure 3d by 10.1 kcal/mol. While the former conformation presents two of the three requisite hydrogen-bonding groups, the methyl group sterically prohibits the formation of the hydrogen bonds. Because of this steric constraint, **4** binds in the expected conformation shown in Figure 3d (unpublished data). Although its tautomeric form cannot be determined crystallographically, no tautomer can make the requisite intra- and intermolecular hydrogen bonds necessary for tight binding. With this knowledge, Figure 3c shows that if the methyl group is removed from this strongly preferred conformation, **6** has not only removed the sterically disfavored methyl group but also presented a third hydrogen-bonding CH group. It is due to the combination of intramolecular and protein−ligand hydrogen bonds that the triazole of **6** binds so much

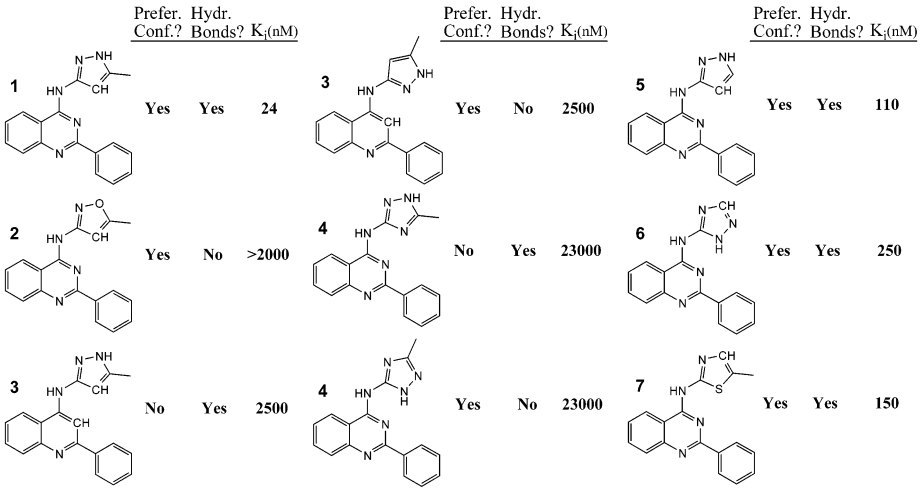

Figure 5. Tautomers and conformations of **1**−**7**, along with an assessment of whether the tautomer or conformer shown is energetically preferred and capable of forming three hydrogen bonds to the hinge region of the ATP site of GSK3. Both requirements must be met for potent (<1 μM) GSK3 inhibition.

better than the triazole of **4** and nearly equivalently to the pyrazole of **5**. In addition, the fact that **5** and **6** have such similar activity suggests that the exchange of a traditional NH···O hydrogen bond to the protein for an unconventional CH···O hydrogen bond has a very modest effect on ligand binding.

A final concern with this hypothetical requirement for intramolecular stabilization of a conformation capable of making a triplet of hydrogen bonds to the protein is that one would expect **1** to actually prefer the conformation in Figure 3e. In this conformation, the alternative pyrazole tautomer of **1** donates a traditional NH···N hydrogen bond to the pyrimidine nitrogen. Yet this conformation, which would appear to be more stable, could not form the necessary hydrogen bonds with the protein. However, ab initio calculations reveal that this conformation is not more stable. The tautomer in Figure 3e is sufficiently disfavored that even the intramolecular hydrogen bond still leaves this structure 0.4 kcal/mol higher in energy than the binding orientation depicted in Figure 3f. So it seems that all of these results support the necessity of intramolecular stabilization of the hydrogen-bonding conformer.

As an additional test of this conclusion, further calculations were performed in an attempt to find a new compound with an appropriate combination of the necessary conformational preferences and hydrogen-bonding functionality. Compound **7**, the aminothiazole analogue of **1**, shows a striking 9 kcal/mol preference for the planar conformation shown in Figure 4. This conformation is also capable of making the requisite triplet of hydrogen bonds, including that donated by the thiazole CH group. Since this compound met all proposed requirements for binding to GSK3, it was synthesized and tested for activity against the enzyme. As expected, the compound is quite potent with a K_i of 150 nM. The crystal structure of this ligand bound to GSK3, shown in Figure 4, shows that it binds in the expected conformation forming a CH···O hydrogen bond to the protein hinge. This is actually an exceptional case in that the binding conformational preference is not maintained through an internal hydrogen bond. Rather, the ab initio calculations reveal that this conformation is stabilized through a polar interaction between the partial positive charge (+0.35) of the thiazole sulfur and the partial negative charge (−0.65) of the pyrimidine nitrogen. Although this seems to be an unusual interaction, similar geometric preferences have been reported previously.[15] Despite its different means of stabilizing the binding conformation, this compound does provide further support for the importance of this conformational preference for potency, as provided by internal hydrogen bonds in **1**, **5**, and **6**. It also provides another example of a CH donor replacing an NH donor for a key protein−ligand hydrogen bond.

This collection of results makes a very consistent argument for the importance of aryl CH···X hydrogen bonds in protein−ligand binding. Figure 5 summarizes these results for each compound, listing whether the depicted conformation is favored, the protein−ligand hydrogen-bonding potential of the conformation, and the K_i against GSK3. For the relatively potent compounds, only the binding conformation is depicted. For the less potent compounds, both planar conformations are depicted to show that neither conformation possesses the necessary binding elements. This summary clearly shows that all compounds with a favored conformation capable forming three hydrogen bonds to the protein are potent inhibitors. At the same time compounds that cannot form the hydrogen bonds in their favored conformation are poor inhibitors. This finding is not surprising because internal energy and protein−ligand hydrogen bonding are known to be important predictors of ligand activity. What is surprising is how many of these vital interactions are mediated by CH···N and CH···O hydrogen bonds. Every compound in this set with a K_i under 1 μM depends on at least one such hydrogen bond for its activity. To our knowledge, this is the first example of a ligand series in which CH···X hydrogen bonds play such a widespread and important role in binding and where CH···X hydrogen bonds were deliberately used in ligand design. As such, this study reaffirms the importance of these interactions in protein−ligand binding and inhibitor design.

Experimental Section

Synthesis of (5-Methyl-1*H*-pyrazol-3-yl)-(2-phenylquinazolin-4-yl)amine (1). 4-Chloro-2-phenylquinazoline (248 mg,

1.03 mmol) and 5-methyl-2*H*-pyrazol-3-ylamine (100 mg, 1.03 mmol) were heated in DMF (8 mL) at 120 °C under nitrogen for 16 h. The reaction mixture was allowed to cool, then diluted with EtOAc (100 mL) and washed with water (2 × 50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a yellow oil, which was purified by silica gel column chromatography using 1:1 petrol/EtOAc as eluent to give the title compound as a white solid (106 mg, 34%).

Synthesis of (5-Methylisoxazol-3-yl)-(2-phenyl-quinazolin-4-yl)amine (2). 4-Chloro-2-phenylquinazoline (200 mg, 0.83 mmol) and 5-methylisoxazol-3-ylamine (98 mg, 0.83 mmol) were heated in THF (8 mL) at 65 °C under nitrogen for 16 h. The reaction mixture was allowed to cool, and a pale-yellow precipitate formed, which was removed by filtration to give the desired compound (76 mg, 31%).

Synthesis of (5-Methyl-2*H*-pyrazol-3-yl)-(2-phenyl-quinolin-4-yl)amine (3). 4-Chloro-2-phenylquinoline (530 mg, 2.2 mmol) was suspended in diphenyl ether (5 mL) and 3-amino-5-methylpyrazole (430 mg, 4.4 mmol). The mixture was stirred at 200 °C for 24 h. The cooled mixture was treated with petroleum ether (20 mL), and the resulting precipitate was filtered. This crude product was then further purified by flash chromatography (5−10% MeOH/DCM). The product was heated in ethanol to remove trace impurities. The product, a white solid (77 mg, 0.25 mmol), was isolated by filtration.

Synthesis of (5-Methyl-1*H*-[1,2,4]triazol-3-yl)-(2-phenylquinazolin-4-yl)amine (4). To a solution of 4-chloro-2-phenylquinazoline (0.16 mmol) in DMF (1 mL) was added 5-methyl-1*H*-[1,2,4]triazol-3-ylamine (0.32 mmol). The mixture was heated in DMF at 100−110 °C for 16 h and then poured into water (2 mL). The precipitate was collected by filtration and purified by HPLC to afford 109 mg (36% yield) of a pale-yellow solid.

Synthesis of (2-Phenylquinazolin-4-yl)-(1*H*-pyrazol-3-yl)amine (5). 4-Chloro-2-phenylquinazoline (200 mg, 0.83 mmol) and 1*H*-pyrazol-3-ylamine (70 mg, 0.83 mmol) were heated in THF (8 mL) at 65 °C under nitrogen for 14 h. The reaction mixture was allowed to cool, and a precipitate formed, which was removed by filtration to give the title compound (130 mg, 54%).

Synthesis of (2-Phenylquinazolin-4-yl)-(2*H*-[1,2,4]triazol-3-yl)amine (6). 4-Chloro-2-phenylquinazoline (481 mg, 2 mmol) and 2-amino-1,3,5-triazole (336 mg, 4 mmol) in dioxane (15 mL) were heated at 110 °C for 48 h and then cooled and filtered to give a colorless solid (420 mg, 73%). Recrystallization from ethanol gave the product as colorless crystals.

Synthesis of (5-Methylthiazol-2-yl)-(2-phenylquinazolin-4-yl)amine (7). 4-Chloro-2-phenylquinazoline (481 mg, 2 mmol) and 2-amino-5-methylthiazole (456 mg, 4 mmol) in dioxane (15 mL) were stirred at 110 °C for 3 days and then cooled and filtered. The collected solid was washed with THF to give a sticky yellow solid. Chromatography on silica gel eluting with 10% methanol/dichloromethane gave an off-white solid. Trituration with hot ethanol gave the product as a colorless solid (190 mg, 30%).

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Supporting Information Available: Compound characterization data, summary of crystallography information, and representative optimized structures from ab initio calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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