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Fragment screening of inhibitors for MIF tautomerase reveals a cryptic surface binding site

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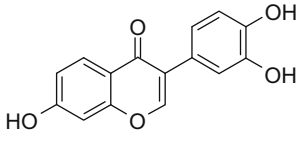
ABSTRACT

In the course of a fragment screening campaign by in silico docking followed by X-ray crystallography, a novel binding site for migration inhibitory factor (MIF) inhibitors was demonstrated. The site is formed by rotation of the side-chain of Tyr-36 to reveal a surface binding site in MIF that is hydrophobic and surrounded by aromatic side-chain residues. The crystal structures of two small inhibitors that bind to this site and of a quinolinone inhibitor, that spans the canonical deep pocket near Pro-1 and the new surface binding site, have been solved. These results suggest new opportunities for structure-based design of MIF inhibitors.

Since fragment-based approaches have become a common practice for discovering drug leads,[1] we applied fragment-based screening to identify potential novel inhibitors for the tautomerase activity of migration inhibitory factor (MIF). With an unusual enzymatic activity of catalyzing the tautomerization of dopachrome to hydroxyindole, MIF is an important cytokine involved in various biological functions.[2] An inhibitor bound to the active site of MIF tautomerase could be useful in preventing its cytokine signaling function.[3] We report here fragment-based screening by X-ray crystallography of two dozen compounds selected from a docking study. The results revealed the formation of a cryptic pocket near the intended binding cavity at the active site.

At the time we initiated the screening campaign, all reported MIF inhibitors were shown to bury within a deep pocket near Pro-1, at the bottom of which is Asn-97 to which they form hydrogen-bonds.[4,5] More recently, we[6] and Winner[7] have shown the potential for covalent bond formation to Pro-1, in the same binding site. Crichlow et al.[8] reported an inhibitor that does not reach Asn-97 and adopts a binding mode wherein the phenolic hydroxyl points out, toward the aqueous phase. But this compound also binds in the canonical site near Pro-1. NAPQI forms an acetaminophen dimer that binds to MIF at the mouth of the active site,[9] with a slight shift in the side-chain position of Tyr-36 (relative to its position in the crystal structure 1GCZ)[5] to form an H-bond to one of the amides of the inhibitor; the other amide points into the canonical pocket.

In light of these results, we focused attention on the Pro-1/Asn-97 site for fragment screening. Prior to the fragment screening campaign, the structure of a small molecule inhibitor **1** (IC_{50} of 38 nM, reported by Orita et al.),[5] bound to MIF was solved during development of the crystallization protocols.[10] In contrast to the proposed binding mode in the Orita's publication, we found that the orientation of this compound is 'flipped.' The carbonyl oxygen is hydrogen bonded to the backbone NH of Ile-64 (Fig. 1) and not to the phenolic OH of Tyr-95. Thus, the carbonyl groups of both molecules **1** and **2** point in the same direction (Fig. 1, right). Compound **1** also retains the hydrogen-bond to the side-chain of Asn-97 by rotation of the hydroxy-chromenone ring system. Steric constraints around the opening of the pocket may account for part of this rotation.



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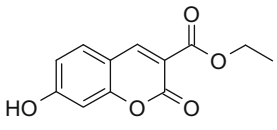

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This work established an in-house platform suitable for a fragment screening approach,[11] Our interest was in constrained heterocycles, similar in structure to the hydroxy-coumarin ligand **2** that would bind in the same, buried pocket. A small fragment

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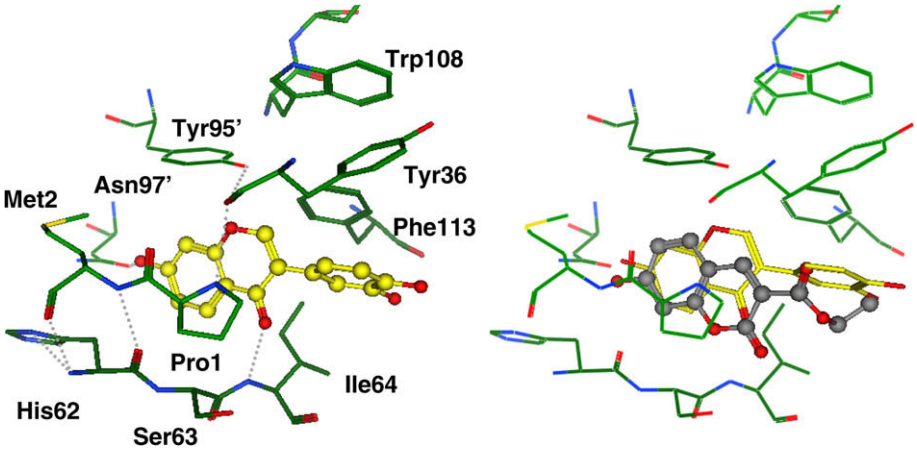
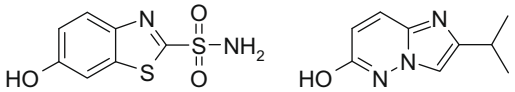


Figure 1. Crystal structure of **1** complex with MIF at 1.94 Å (left) and superposed on ligand **2** (shown in gray ball & stick) from Orita's 1GCZ (right).

library focusing on [5,5], [5,6], and [6,6]-bicyclic ring structures was constructed and used for virtual screening by GLIDE docking[12] with H-bonding constraints to either Ile-64 or Asn-97.

Compounds from the virtual screen were selected for soaking experiments with crystals of MIF on the basis of their size and potential for follow-up chemistry. Of the 200 compounds identified by GLIDE docking, 40 compounds were chosen for crystal soaking studies. Of these, 23 were available to be tested: three were insoluble, 15 were not observed in the electron density, and five structures were solved. Three of the solved structures, of compounds **3**–**5,** will be reported here.



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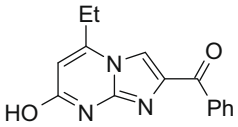


5

Among the five solved structures, three bound to the coumarin site, as expected. One example is shown in Figure 2. As in known coumarin inhibitors, such as those from Orita et al.,[5] the phenolic oxygen of **3** forms a hydrogen-bond with the side-chain carbonyl of Asn-97; the benzothiophene ring bridges between the hydroxyl of Tyr-95 and the backbone nitrogen of Ile-64. A polar interaction from the sulfonamide to Ser-63 side-chain hydroxyl is also observed. Pro-1 and Lys-32 are displaced by approximately 2 Å from the positions observed in an in-house *apo* structure and the structures reported by Orita (1GCZ) and Lubetsky (1LJT). It is not clear if this is the result of the bulk and interactions of the sulfonamide or due to the precise positioning of the benzothiophene ring between Tyr-95 and Ile-64. The data do suggest some flexibility within the canonical binding pocket and, especially, of Pro-1.

The most interesting finding from the fragment screening was that two of the compounds (**4** and **5**) bind to a previously unidentified surface site. The inhibitor **4** is found at the mouth of the coumarin pocket, trapped between Phe-113 and Tyr-36. These compounds do not enter the active site pocket at all. Rather, the pocket in structure **4** contains glycerol from the cryo-protectant, through which a hydrogen-bond network is made with a crystallographic water (Fig. 3, left).

The novel site is exposed through displacement of Tyr-36 by the inhibitor, which leads to formation of a hydrophobic site at the 'rim' of the protein. Figure 4 illustrates the rotation of the Tyr-36 side-chain by comparing the structures of 1GCZ and the complex with **4**. This hydrophobic rim site is dominated by aromatic residues (Tyr-36, Tyr-95, Phe-113, and Trp-108) and is distinct from the surface site reported for 3DJI (Fig. 3, right).

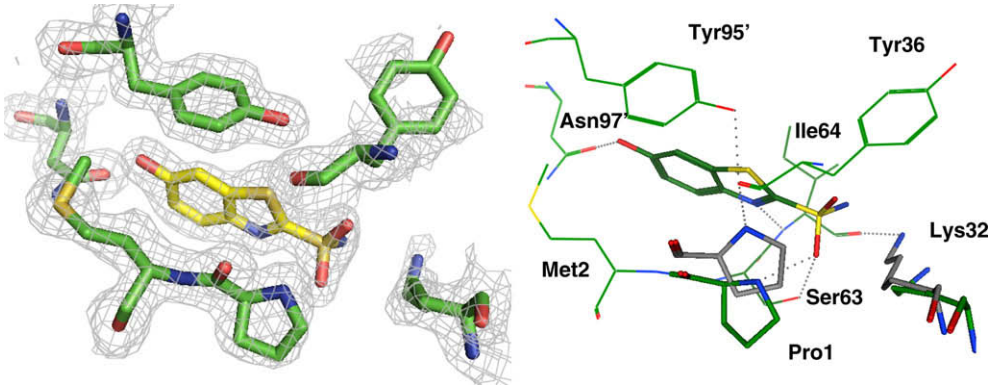


Figure 2. Structure of **3** bound to MIF and refined at 1.90 Å (left) with $2F_o-F_c$ electron density contoured at 1σ. Displacement of Pro-1 and Lys-32 in the **3** structure (green) from that of the *apo* structure (gray) solved at 1.70 Å (right).

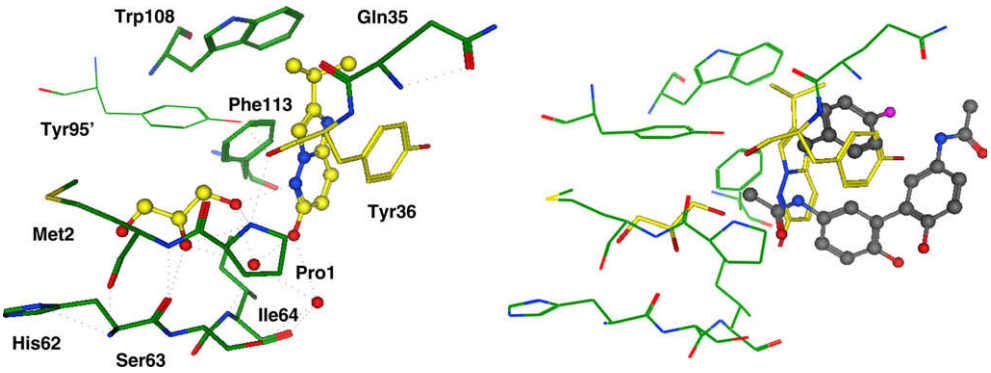


Figure 3. Crystal structure of **4** (shown as ball&stick) in MIF at 1.80 Å (left) and superposed with 3DJI (right; **4** and its Tyr-36 in yellow; 1DJI ligand and its Tyr-36 in gray).

The rotation of Tyr-36 is less dramatic in the structure with **5** and the canonical pocket is filled with solvent water (Fig. 5, left), as the crystal was cryo-protected with sucrose. Figure 5 (right) illustrates the range of Tyr-36 positions revealed by these structures, in comparison to the 1GCZ structure. Tyr-36 of *apo* MIF is in the same position as observed in 1GCZ. On the other hand, the displacement of Tyr-36 induced by inhibitors **4** and **5** is almost identical to the observed conformation of Tyr-36 in the *apo* structure of Pro-1 to Gly-1 mutant (1P1G),[7] which is devoid of both catalytic and biological functions.

It is conceivable that both **4** and **5** were inhibitors too weak to bind to the coumarin pocket by displacing either glycerol or solvent. The observed binding to the cryptic hydrophobic pocket, formed at the surface site by rotation of Tyr-36, could be merely an artifact under the crystallographic conditions. It also raised the question, if the formation of the cryptic site could have limited

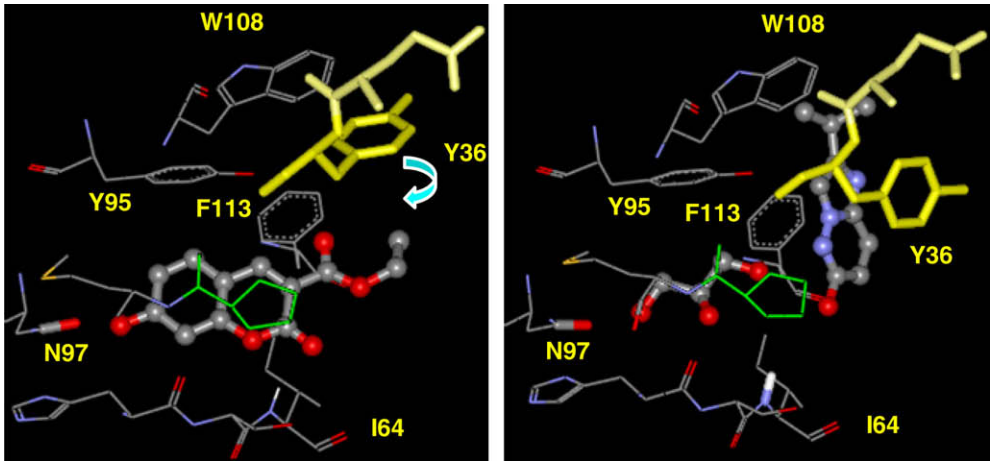


Figure 4. Position of Tyr-36 in 1GCZ (left) and in MIF complex with **4** (right).

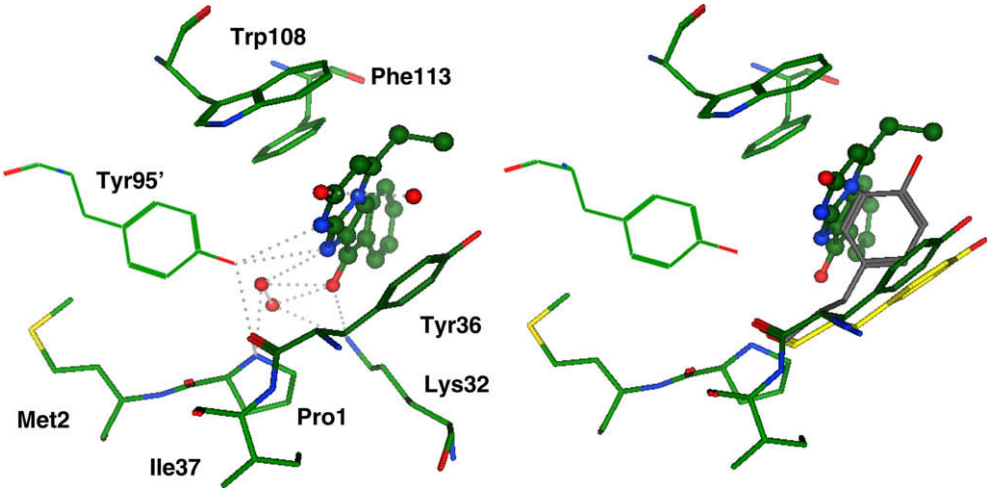
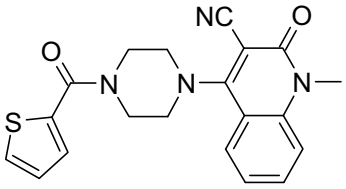


Figure 5. Crystal structure of **5** in MIF (1.86 Å, left) and superposed to show the positions of Tyr-36 in 1GCZ (gray), **4** (yellow), and **5** (green).



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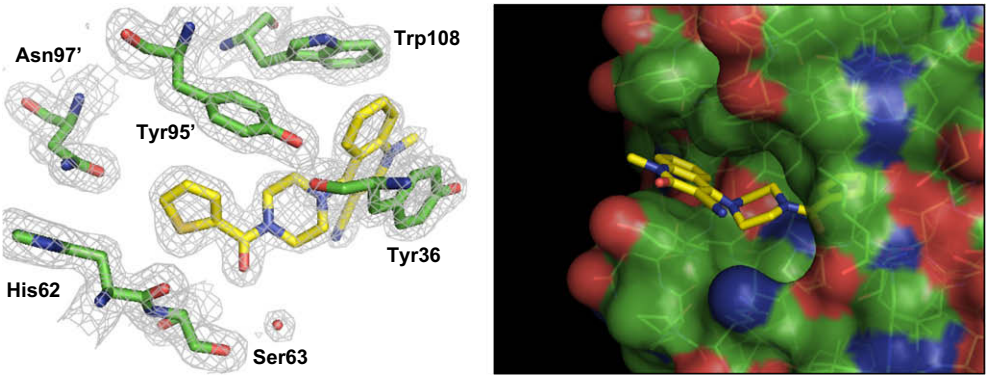

Figure 6. Crystal structure of **6** with MIF at 1.86Å with $2F_o-F_c$ electron density contoured at 1σ (left) and in surface representation (right).

the access to the coumarin pocket as a result of Tyr-36 rotation. Thus, compounds that span both the coumarin and cryptic surface sites may be particularly interesting and useful inhibitors. Such inhibitors that simultaneously occupy the two sites would show that the newly recognized surface pocket is genuine. We found one such example in the literature: the inhibitor **6**, shown in Figure 6.[13]

The piperazine of this inhibitor forms a bridge between the canonical 'coumarin' pocket and the newly identified, cryptic 'hydrophobic rim' binding site, into which the quinolinone ring binds. The inhibitor thiophene does not extend deep enough into the canonical pocket to form any interactions with Asn-97, but the piperizine amide oxygen does hydrogen-bond to Ile-64. The position of Tyr-36 is very similar to that observed with compound **4** and is somewhat displaced further away from the position it adopts in the *apo* structure. Thus, Tyr-36 adopts a position parallel to that of the quinolinone ring.

Contrary to our initial expectations, fragment-based screening revealed the formation of a cryptic surface binding pocket at the MIF tautomerase substrate binding site. Identification of this new binding site not only offers opportunities for structure-based virtual screening, but also opens up possibilities for novel inhibitor design. In addition, it may be possible to investigate the effect of the rotation of Tyr-36 on biological activity using discrete inhibitors to probe the different sites.

Acknowledgments

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