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Enzyme inhibition potency enhancement by active site metal chelating and hydrogen bonding induced conformation-restricted cyclopropanecarbonyl derivatives

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Abstract—Two cyclopropanecarbonyl derivatives were independently found to be 15 and 14 times more potent than the corresponding isopropylcarbonyl analogues as inhibitors of 4-hydroxyphenylpyruvate dioxygenase and dihydroorotate dehydrogenase, respectively. A thorough examination of the co-crystal structures of available enzyme inhibitor complexes and the conformation of X-ray crystal structures of several synthesized cyclopropanecarbonyl derivatives revealed that this enhancement by one order of magnitude of inhibition potency exhibited by cyclopropanecarbonyl derivatives in both enzymes is probably caused by respective metal chelating and hydrogen bonding interactions at the ligand–receptor binding site. These specific interactions subsequently cause the cyclopropyl group of the molecules to adopt a fixed bisected conformation, which is unavailable for isopropylcarbonyl derivatives.

4-Hydroxyphenylpyruvate dioxygenase (HPPD)[1] is a non-heme Fe-dependent enzyme involved in the catabolism of tyrosine in most organisms. It catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate. The inhibition of HPPD activity may provide an effective drug therapy in treating fatal tyrosinaemia type I.[2] During our ongoing efforts to develop potent HPPD inhibitors as potential therapeutic agents, an interesting observation was made: the inhibition activity of 2-cyclopropylcarbonylcyclohexane-1,3-dione **4** toward HPPD was 15 times more potent than that of the corresponding 2-isopropyl derivative **3** (Table 1).[3] Similar observations were also made of a non-related enzyme, dihydroorotate dehydrogenase (DHODH).[4] DHODH is a key enzyme which catalyzes the fourth step of the synthesis of pyrimidine bases necessary for cell proliferation. Molecules with DHODH inhibition activity may have the potential to serve as an immunosuppressive drug for the treatment of rheumatoid arthritis.[5] Previous studies[6] have demonstrated that cyclopropanecarbonyl derivative **11** was 14 times more potent than the corresponding isopropylcarbonyl derivative **8** in inhibiting rat DHODH (Table 2). Although the role of the cyclopropyl ring remains unclear, it cannot be accounted for only by lipophilicity. These findings prompted us to investigate the possible biological role of cyclopropanecarbonyl functionality, which is present in various bioactive molecules. Here we report the X-ray crystal structures of several cyclopropanecarbonyl-containing compounds, whose carbonyl oxygen atoms are either metal-chelated or intramolecularly hydrogen-bonded. The observed bisected cyclopropane conformation relative to the adjacent carbonyl group in these molecules, together with the recently published co-crystal complex structure of factor Xa[7] with an inhibitor containing a constrained cyclopropylglycine core, supports the hypothesis that these cyclopropanecarbonyl compounds **4** and **11** may behave as novel ligand–receptor interaction induced conformation-restricted enzyme inhibitors.

2-(2-Nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC, Fig. 1),[8] a triketone derivative, is a US Food and Drug Administration approved drug for the treatment of tyrosinaemia type I by inhibition of the activity of HPPD. The molecular mode of action of this triketone-type HPPD inhibition has recently been characterized to be caused by a tight chelation of the enzyme-bound metal iron with enol tautomer of the 1,3-diketone moiety of the inhibitor.[9] We assert that the chelation of the active site iron ion with the carbonyl

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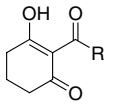
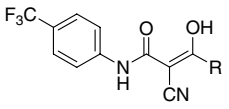
Table 1. SAR studies of pig HPPD inhibition[3]



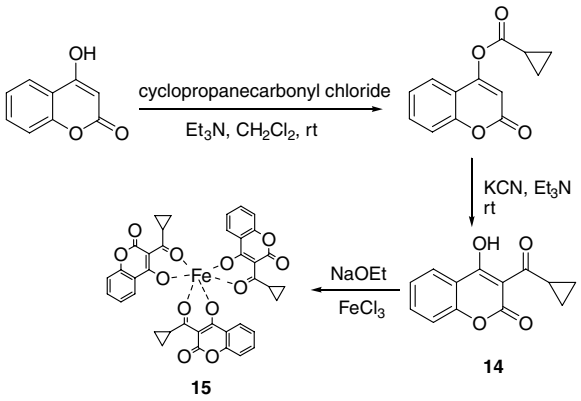
Compound	R	IC_{50} (μM)
1	CH_3	11.2
2	CH_2CH_3	17.8
3		93.3
4		6.0
5		21.0
6		33.1
7		364.5

Table 2. SAR studies of rat DHODH inhibition[6]



Compound	R	IC_{50} (nM)
8		295
9		251
10		23
11		21
12		282
13		31,600

oxygen atom in **4** may induce the cyclopropyl group to adopt the bisected conformation, making **4** an induced conformation-restricted analogue. Conformation-restricted analogs have long been used to improve the ligand–receptor binding affinity and to elucidate the receptor binding site topography.[10] Although the best way to verify this hypothesis is to examine the three-dimensional structure of HPPD and the cyclopropanecarbonyl-containing inhibitor complex, this desired co-crystal structure is presently unavailable. Alternatively, a triketone derivative **14** was selective as a model compound to explore the most stable conformation of a cyclopropane group given that the adjacent carbonyl oxygen atom is metal-chelated. Scheme 1 depicts the preparation of **14** in two steps by esterification of 4-hydroxycoumarin with cyclopropanecarbonyl chloride, followed by a KCN-catalyzed isomerization reaction.[11] The inhibition result indicated that **14** is a much less potent HPPD inhibitor with IC_{50} value of 322 μM. This observation suggested that the left-hand side moiety of **4** also plays an important role on HPPD inhibition, other than the capability of chelation with the active site iron. The cyclopropanecarbonyl derivative **14** was further treated with sodium ethoxide and $FeCl_3$ to yield iron-chelated trimer **15**.[12] X-ray crystal structure of iron complex **15** indeed indicated that cyclopropane is bisected to the iron-chelated carbonyl group (Fig. 2). Therefore, complex **15** can be regarded as a conformationally restricted molecule because of the fixed cyclopropane conformation. Assuming that **4** adopts the similar bisected conformation when chelated with iron at the HPPD active site, compound **4** may bind more tightly than does conformationally flexible isopropyl derivative **3**, presumably because of the conformationally restricted cyclopropane structure induced by the iron oxygen chelation. This specific iron chelation increases the electrophilic character of the carbonyl car-



Scheme 1. Preparation of compounds **14** and **15**.

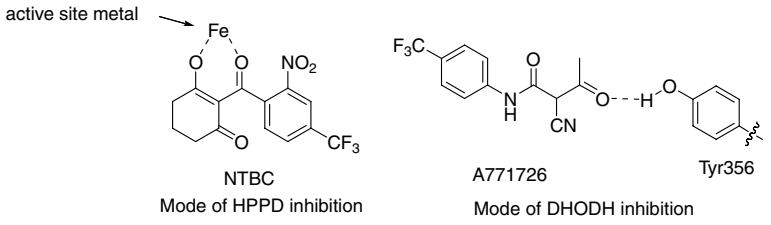


Figure 1. Structures of HPPD inhibitor NTBC and DHODH inhibitor A771726, and their modes of inhibition.

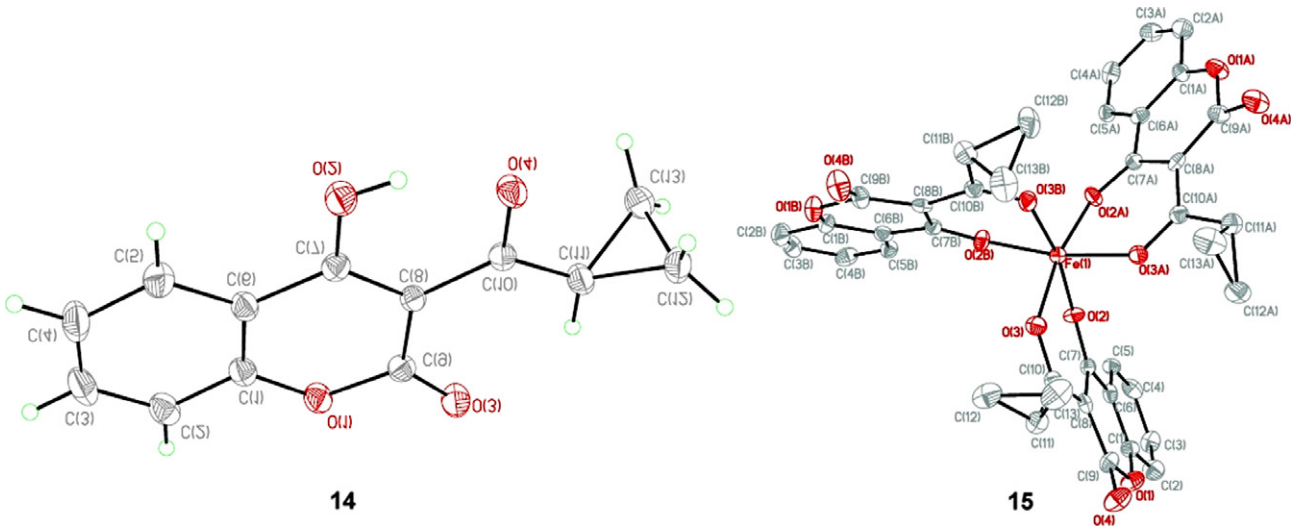


Figure 2. X-ray crystal structures of compounds **14** and **15**.

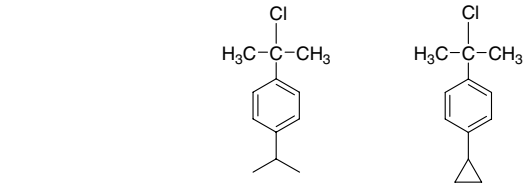


Figure 3. Relative solvolysis rate of *p*-isopropyl and *p*-cyclopropyl *tert*-cumyl chlorides in aqueous acetone.[13]

bon in **4**, subsequently causing the cyclopropane group to adopt the rigid bisected conformation relative to the carbonyl group. The resulting conformation-restricted cyclopropanecarbonyl analogues then exhibit an enzyme inhibition potency that exceeds by more than one order of magnitude that of the corresponding conformationally flexible isopropylcarbonyl compounds. A similar magnitude of rate enhancement on *p*-cyclopropyl *t*-cumyl chloride solvolysis in aqueous acetone relative to the corresponding *p*-isopropyl derivative has also been reported three decades ago. Brown[13] has demonstrated that a cyclopropyl substituent in the *para*-position increases the solvolysis rate of a tertiary benzylic chloride up to nine times, comparing with the corresponding isopropyl substituent (Fig. 3). This close to one order of magnitude rate enhancement has been attributed to the fact that the developing cationic center can simultaneously interact with two 'bent bonds' of the bisected cyclopropyl ring via the benzene p orbitals. This observation suggested that both cyclopropane ring and benzene ring are conformation-restricted during solvolysis. We speculate that sharing a similar enhancement property on two non-related events (enzyme inhibition potency and solvolysis rate) by cyclopropyl-containing derivatives relative to their corresponding isopropyl ones is unlikely to be a coincidence, but a common phenomenon. If this hypothesis held true, then this one order of magnitude inhibition potency enhancement phenomenon should be observed in other enzymatic systems as well.

With reference to DHODH inhibition, DHODH is not a metal-dependent enzyme, and compound **11** should have a different mode of action than that of **4**. Examining the co-crystal structure of human DHODH in complex with the inhibitor A771726 (an antiproliferative agent, Fig. 1) determined by Clardy[14] clearly reveals a hydrogen bonding between the acetyl oxygen in A771726 with the hydroxyl group of Tyr 356 in DHODH. If **11** and A771726 are assumed to share the same DHODH binding site, then this specific hydrogen bonding may increase the electrophilic character of the carbonyl carbon in **11**, subsequently causing the cyclopropane group to adopt a bisected conformation. Again, the triketone **14** was selective as a model compound to explore the most stable conformation of a cyclopropane group given that the adjacent carbonyl oxygen atom is hydrogen bonded. The X-ray crystal structures of **14**[12] were determined, as shown in Fig. 2. The cyclopropane moiety indeed adopts the expected bisected conformation which is induced by the intramolecular hydrogen bond. Thus, it is reasonable to assume

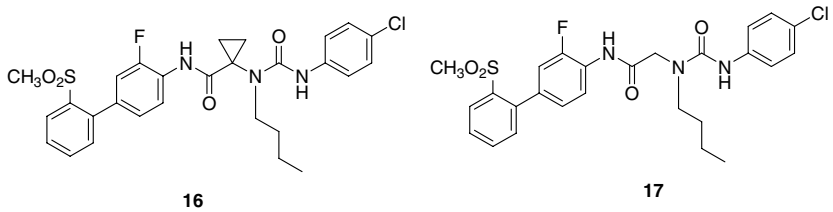


Figure 4. Structures of FXa inhibitor **16** and its glycine homolog **17**.

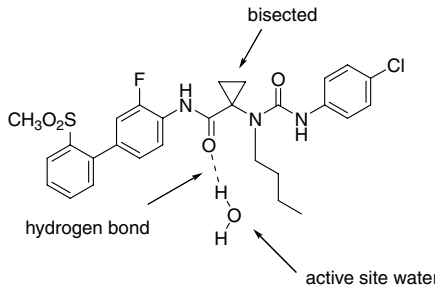


Figure 5. Mode of inhibition based on X-ray crystal structure of **16** in the active site of FXa.[7]

that cyclopropanecarbonyl derivative **11** was 14 times more potent than the corresponding isopropylcarbonyl derivative **8** in rat DHODH inhibition is because of the hydrogen bonding of the cyclopropanecarbonyl oxygen of **11** to the hydroxyl group of Tyr 356 in DHODH active site. This specific hydrogen bonding induces the cyclopropyl group in **11** to adopt the fixed bisected conformation, causing **11** to function as a conformation-restricted DHODH inhibitor. Therefore, the enzyme DHODH can be considered as the second example which exhibited the unique inhibition potency enhancement property by a cyclopropanecarbonyl-containing derivative.

This proposed hydrogen bonding-induced conformation-restricted enzyme inhibition potency enhancement has gained further support by the recent design of a factor Xa (FXa) inhibitor. FXa is a key protease at the central junction of coagulation pathways involved in the formation of blood clots. Kohrt[7] and co-workers have reported a potent FXa inhibitor **16** with a constrained cyclopropylglycine core to reduce the potential for bond rotation (Fig. 4). Based on the published co-crystal structure of FXa in complex with **16**, the cyclopropyl group indeed adopted the expected bisected conformation relative to the glycine carbonyl group. Similar to the DHODH inhibition, this rigid conformation was strengthened by a strong hydrogen bonding between the carbonyl group of cyclopropylglycine and water in the enzyme active site (Fig. 5). Furthermore, compound **16** was also found to be 7-fold more potent than its glycine homolog **17**, presumably due to the decreased entropy of **16** after binding with FXa. We speculate that compound **16** will be one order of magnitude more potent than the corresponding isopropyl derivative on FXa inhibition, although these data are currently unavailable.

In summary, the potency of cyclopropanecarbonyl derivatives in HPPD and DHODH inhibitions is one order of magnitude greater than that of corresponding isopropyl derivatives is probably associated with the respective metal chelating and hydrogen bonding interactions at the ligand–receptor binding site. These interactions increase the electrophilic character of the carbonyl carbon atom, causing the adjacent cyclopropane group to adopt a fixed bisected conformation. Therefore, these two cyclopropanecarbonyl compounds can be regarded as induced conformation-restricted enzyme inhibitors. Considering the fact that approximately one-third of the proteins contain metal ions and hydrogen bonding is one of the most frequently encountered interactions involved in the ligand–receptor complexes, this induced conformational restriction strategy can potentially be exploited in designing various other potent enzyme inhibitors.

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