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# Molecular basis for the enantioselective binding of a novel class of cytochrome bc1 complex inhibitors

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**Abstract**

The recently solved co-crystal structures of mitochondrial cytochrome bc1 complex with inhibitors have provided an important structural framework for the elucidation of modes of binding of various bc1 complex inhibitors. *N*-Phenyl triazolones, a novel class of bc1 complex ubiquinol oxidation ($Q_o$)-site inhibitors, were found to exhibit atropisomerism; in few cases, the atropisomers were resolved and shown to express different biological activities. However, the underlying mechanism for such differential binding of the enantiomers to bc1 complex is unknown. Here molecular docking is used to examine the binding modes of the *N*-phenyl triazolones fungicides. Our docking studies allow the molecular basis for the enantioselective binding of atropisomeric triazolones to be elucidated. Furthermore, the mode of binding of azoxystrobin has also been clarified.


*Keywords:* Fungicide; Strobilurin; Molecular docking; Atropisomeric; Scoring

## 1. Introduction

Mitochondrial cytochrome bc1 complex (ubiquinol–cytochrome *c* oxidoreductase) is a very fruitful target for the discovery of fungicides useful in controlling crop diseases [1–4]. Two distinct ubiquinone (or ubiquinol) binding sites are known: ubiquinol oxidation site ($Q_o$ site) and ubiquinol reduction site ($Q_i$ site) [5]. Several $Q_o$-site inhibitors (e.g., **2–5** in Scheme 1) and one $Q_i$-site inhibitor (**6**) of the bc1 complex have been commercialized [1–4].

Most of the commercialized $Q_o$-site inhibitors of the cytochrome bc1 complex could be considered as analogues of the naturally occurring $Q_o$-site inhibitor strobilurins and oudemansins [3]; famoxadone represents a structurally distinct $Q_o$-site inhibitor [2]. Lead optimization programs at several companies have resulted in a large number of strobilurin analogues and structure–activity relationships (SARs) were elucidated for those analogue series. The methoxyacrylate head group as in strobilurin A is normally considered as the pharmacophore (or toxiphore) for the inhibition of cytochrome bc1 complex. However, as shown in Scheme 1, the methoxyacrylate can be replaced by isosteric groups as in kresoxim-methyl and metominostrobin without losing biological activity [3]. Scheme 2 lists several additional variations of this pharmacophore. Early SAR study also established the requirement of the E configuration of the central double bond for biological activity since the Z isomer is inactive [6]. Recently, X-ray crystallographic studies on cytochrome bc1 complexed with various inhibitors have been reported [7–11].

*N*-Phenyl triazolones (as exemplified by compound **12** in Scheme 3) are one novel class of strobilurin analogues [12]. The triazolone ring could be considered as a tied-back version of the methoxyacrylate pharmacophore. Like the biphenyls, such *N*-phenyl-triazolone strobilurin analogs are twisted with large inter-ring torsion angles [13]. In principle, they could exhibit atropisomerism. Indeed, when the phenyl ring is substituted at the ortho positions (positions 2 and 6), the torsion barrier around the inter-ring C–N bond is large enough to allow atropisomers to be stable even at room temperature [13]. The atropisomers of compound **12** have been resolved and their bioactivities were evaluated [12]. One enantiomer is found to be much more active than the other. However, the underlying mechanism for such enantioselective binding is unknown.

Here molecular docking is used to elucidate the mode of binding of compound **12** in the hope of uncovering the underlying mechanism of differential binding of the enantiomers.

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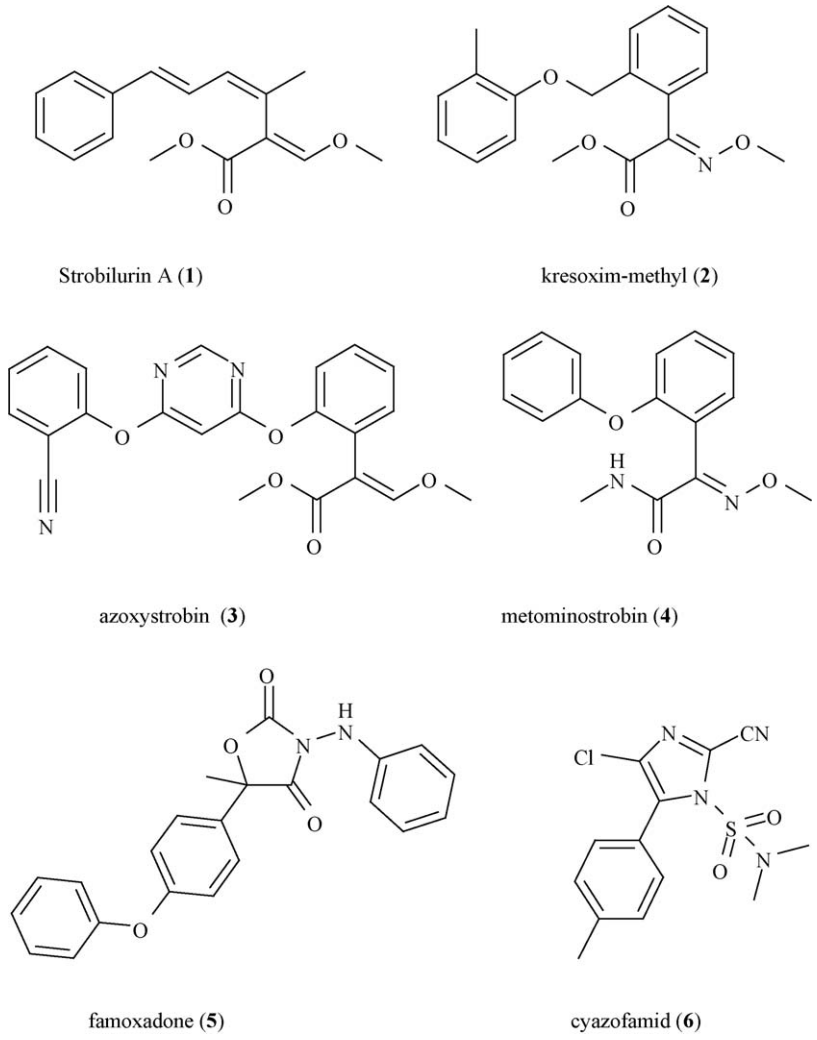



Scheme 1.

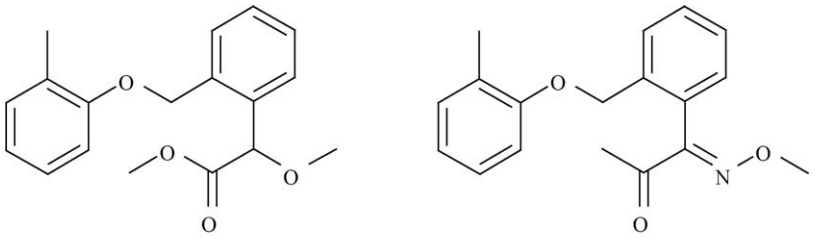
## 2. Theoretical procedures

Molecular docking was performed using QXP program [14], a part of the Flo+ software [15]. The MCDOCK and MCDOCK+ procedures were used. For each inhibitor, an initial 100 Monte Carlo steps were used to place the inhibitor inside the binding pocket; then 10,000 repeated cycles of Monte Carlo (MC) followed by energy minimization and additional scoring were used to yield an ensemble of 25 top scoring inhibitor poses. Simulations were repeated several times using different starting inhibitor conformations to assess convergence. The protein binding pocket surface mesh was generated using Flo+ with a previously described procedure [16]. The co-crystal structure of bc1 with bound azoxystrobin (**3**) was used [8c]. To assess the reliability of the docking software, the bound azoxystrobin was taken out and flexibly redocked into the binding site. Owning to the low resolution of the X-ray crystal structure, there were some uncertainties concerning the details of the bound conformation of azoxystrobin. For instance, the conformation of the methoxyacrylate head group could be either s-*cis* or s-*trans*. Previous SAR data were then used in conjunction with the docking experiments to elucidate the binding mode of azoxystrobin. Once the performance of the docking software is established, we proceed to examine the binding of compound **12**. A pdb file containing the docked compound **12** in the binding pocket is available upon request from the author.

## 3. Results and discussions

### 3.1. Mode of binding of azoxystrobin

For conjugated unsaturated compounds as shown in Scheme 4, there are two possible conformation, s-*cis* and s-*trans*. The energy difference between them is dependent of the steric size of the X group. For acrolein (where X = H), the sterically less crowded s-*trans* conformation is favored over the c-*cis* conformation by 1.7 kcal/mol and the interconversion energy barrier is about 5.0–6.4 kcal/mol [17]. In the cases of acrylic acid (X = OH) and methyl acrylate ($X = OCH_3$), the energy differences between the s-*cis* and s-*trans* conformations are only 0.17 kcal/mol and 0.31 kcal/mol, respectively [17]. Even though the energy difference between the s-*cis* and s-*trans* conformation of azoxystrobin has not been determined



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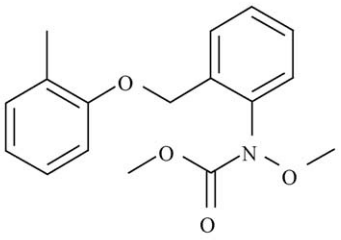

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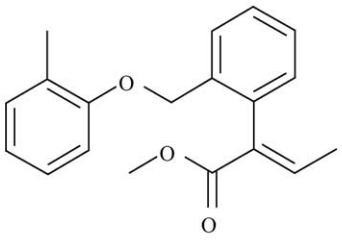

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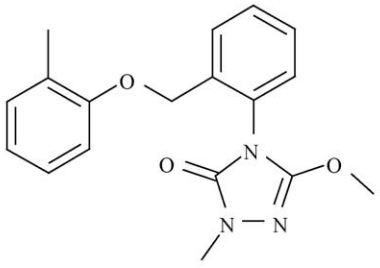

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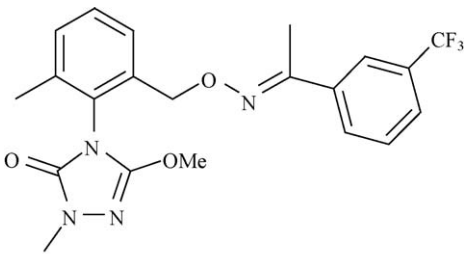
Scheme 2.

experimentally, it is very reasonable to assume that the energy difference is rather small and both conformations are equally plausible.

The X-ray co-crystal structure of bc1 with azoxystrobin was determined at 2.7 Å resolution [8c]. Since the cyctochrome b structure has been solved several times under different conditions (with and without inhibitors), the structure of the protein itself is accurate. However, at such resolution, there is uncertainty regarding the bound conformation of the small molecular weight inhibitor. In fact, in the bc1/azoxystrobin structure, the methoxyacrylate is refined as s-*cis* while in the analogous bc1/myxothiazol, an s-*trans* conformation was assumed. To address this issue, the bound azoxystrobin was taken out of the active and re-docked into the binding site. During the docking process, the protein is kept at the X-ray structure and the ligand is flexibly docked. The top 25 scoring structures were examined in detail and Fig. 1 showed the overlap of top 25 scoring docked poses of azoxystrobin in the $Q_o$ site.



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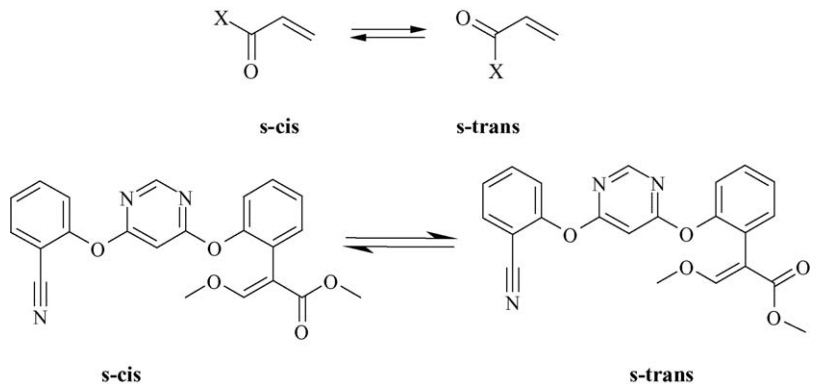
Scheme 3.

Overall, the docking poses are quite consistent. The pyrimidine and the phenyl rings are essentially at the same locations in the binding pocket. The position of cyanophenoxy shows considerable variations, which are consistent with the active site topology. The methoxyacrylate moiety is s-*trans* in all of the 25 poses as opposed to the s-*cis*; the docked poses also indicate that the ester function could be in either Z or E configuration. This is likely an artifact of the simulation since it is known experimentally that the Z configuration ($CH_3$ is trans to the acrylate C=O bond) is generally favored in carboxylic esters [18]. Fig. 2 displays the overlap of the docked poses with the ester in the Z configuration and the refined structure (white) by Esser et al. [8c]. All of the top scoring docked poses agree with the refined structure by Esser et al. [8c] with respect to the




Scheme 4. Conformation of conjugated unsaturated compounds.

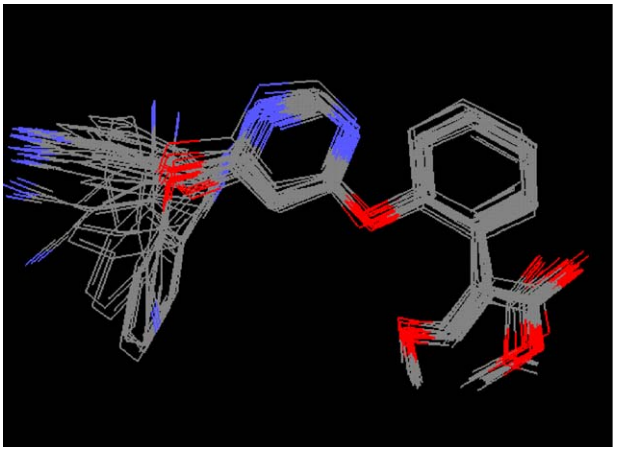


Fig. 1. The overlap of 25 top scoring poses of azoxystrobin in the bc1 $Q_o$ site.

overall alignment of the ligand within the binding site. The differences lie in the exact conformations at the two termini of the ligand. In view of the previously published extensive SAR data, it is highly plausible that the s-*trans* conformation as predicted by the docking study is indeed the 'true' bound conformation of azoxystrobin. Previous SAR studies indicated that there is a hydrogen bond between the ester moiety and the protein. In principle, either one of the two ester oxygen atoms could be involved in such a hydrogen bonding interaction. However, since the ketone analogs remain active, the hydrogen bond could only be between the carbonyl oxygen and the protein. In agreement with this argument, replacing the carbonyl (C═O) by either C═S or C═$CH_2$ has resulted in loss of activity [3]. Only the s-*trans* conformation in the active site allows for such a hydrogen bond (between ester carbonyl oxygen and the amide N–H of Pro270). Similarly, the high activity of compounds such as **11** and **12**, which are tied-back version of the normal methoxyacrylate pharmacophore, provides definitive support for the s-*trans* conformation of the methoxyacrylate.

### 3.2. Mode of binding of N-phenyl triazolone

Now, with a better understanding of how azoxystrobin binds in the binding pocket, we proceed to examine how *N*-phenyl triazolones such as **11** and **12** may bind in the active site. Compounds such as **12** exist as atropisomers due to the large rotational barrier around the inter-ring C–N bond [13]. The enantiomers of **12** (Scheme 5) have been resolved and shown to behave differently biologically [12].

Following the same docking procedure, compound **12** was docked into the $Q_o$ binding site. The top 25 scoring poses were displayed in Fig. 3 in two perspectives. The binding site pocket was also displayed as color-coded surface mesh, which provides an excellent way to visualize the fitness of a ligand within a binding pocket. The yellow, red, and blue meshes refer to hydrophobic, hydrogen bonding donor, and hydrogen bonding acceptor regions of the binding site, respectively. Ideally, for effective binding interactions, the ligand atoms should be on or near the yellow mesh and penetrating the mesh when there is strong hydrogen bonding interactions present.

As demonstrated clearly in Fig. 3, the inhibitor fits snugly in the binding site. The docked poses are highly consistent among themselves. The *N*-phenyl triazolone portion of the inhibitor is essentially in the same location in all poses. The binding position of the trifluoromethylphenyl moiety displays some scattering and alternative ring orientations; this part of binding pocket is bulky. The mode of binding of **12** is very similar to

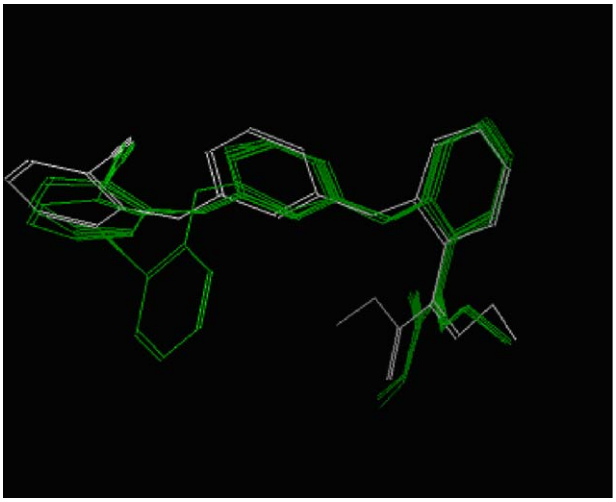
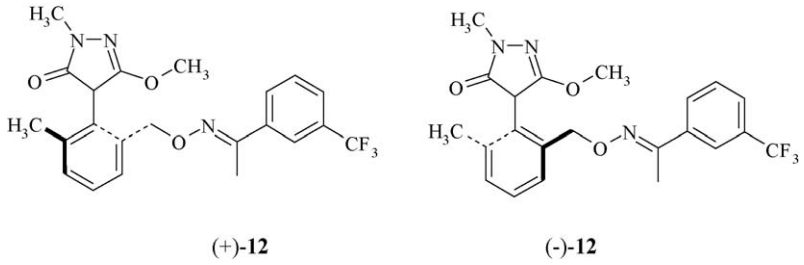


Fig. 2. The docked poses (green) and the bound structure (white) as proposed by Esser et al. [8c].



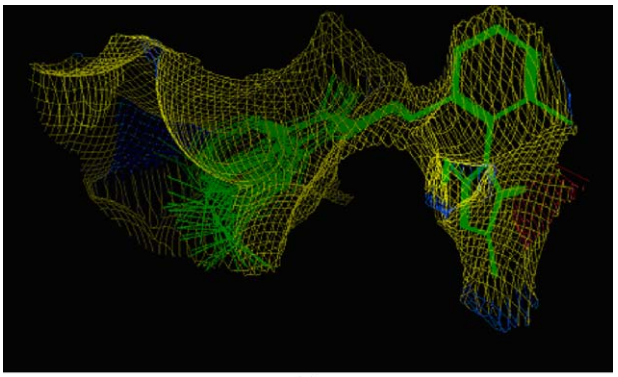
(+)-**12** (-)-**12**

Scheme 5.

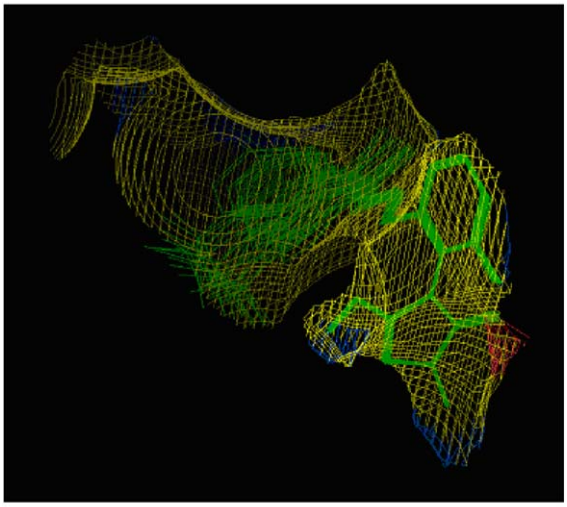
that of azoxystrobin, again consistent with the notion that the triazolone pharmacophore of **12** is a tied-back version of the normal methoxyacrylate pharmacophore of strobilurins.

The binding mode of **12** in the binding site also provides a straightforward explanation as to why only the (+)-**12** enantiomer is biologically active. Binding of the (−)-**12** enantiomer would require the flip of the triazolone ring along the inter-ring C–N bond in the binding pocket, switching positions of C=O with $–OCH_3$, and the N–$CH_3$ with the $sp^2$ ring N atom. Since in the binding site (Fig. 3), the $sp^2$ ring N is already on the surface mesh, switching the $sp^2$ ring N by N–$CH_3$ will result in significant clash between the methyl group and protein atoms. Thus, the (−)-**12** enantiomer cannot fit well in the binding pocket.

Xia and co-workers proposed previously that aromatic–aromatic (Ar–Ar) interactions play a critical role in the binding of famoxadone and azoxystrobin to the bc1 complex [8]. Several aromatic amino acids such as Tyr-131, Tyr-278, Tyr-273, and Phe-274 were found to be in close contact with the bound famoxadone [8a]. Interestingly, Tyr-131, Tyr-278, and Phe-274 are also in close contact with (+)-**12** in the binding pocket as revealed by our docking calculations. Thus, the proposed Ar–Ar interactions may also contribute significantly to the binding affinity of *N*-phenyl triazolones.



(a)



(b)

Fig. 3. Binding of compound (+)-**12** in the $Q_o$ site in two (a and b) perspectives.

## 4. Conclusions

The binding mode of azoxystrobin in the $O_o$ site of mitochondrial bc1 complex was examined using a molecular docking procedure. Based on previous extensive SAR, X-ray crystallographic studies, and the present molecular docking study, a consistent binding mode for azoxystrobin is presented. In this binding mode, azoxystrobin forms a hydrogen bond with the amide N–H of Pro270 through the ester carbonyl oxygen atom. The methoxyacrylate is in the s-*trans* form instead of the s-*cis* as proposed by Esser et al. previously. Subsequently, the binding mode of *N*-phenyl triazolone inhibitors with a conformationally constrained pharmacophore is investigated. According to our docking study, the binding site topology is consistent with only (+)-**12**, the biologically active enatiomer; the other atropisomer does not fit well. Our study also demonstrated the usefulness of molecular docking in elucidating the mode of binding of bc1 inhibitors. Similar approaches could be used to examine the binding modes of other novel inhibitors.

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