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Review Article

Fluoro-Sulfate-Based Covalent Inhibitors: Design Principles and Therapeutic Potential

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ABSTRACT

Covalent inhibitors first recognize a biological target and can then form a chemical bond with it. Fluorosulfate groups have become of interest in medicinal chemistry because, under suitable conditions, they can take part in sulfur (VI) fluoride exchange (SuFEx) reactions with nucleophilic groups in proteins. In this process, sulfur is the reactive centre and fluoride may leave as the new bond forms. The reaction depends on the chemistry of the compound as well as the way it sits in the target protein. This review looks at the structure and proposed action of fluorosulfate-based inhibitors, including target binding, positioning of the reactive group, nucleophilic attack, fluoride displacement and formation of a covalent complex. It also discusses the main design considerations, possible benefits, limitations, therapeutic areas and questions that still need further study.

INTRODUCTION

Many medicines work by attaching to a biological target through interactions such as hydrogen bonds, ionic attractions, hydrophobic contacts and van der Waals forces. These interactions are usually reversible, so a drug may detach from its target as its concentration falls. Covalent inhibitors work differently. After the molecule has bound to the target, a reactive group may form a chemical bond with an amino-acid residue in the protein. When this happens, the target can remain affected for longer than it would with reversible binding alone.

A targeted covalent inhibitor generally has two important parts: a scaffold that helps it recognize the target and a reactive group called a warhead. The scaffold supports binding, while the warhead can react with a suitably placed nucleophilic residue. In this way, the molecule's fit within the protein helps guide the chemical reaction instead of depending only on a highly reactive group. [5–8]

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Medicinal chemists use several types of covalent warheads, including acrylamides, chloroacetamides, aldehydes, epoxides and sulfur (VI)-based groups. Fluorosulfates are of interest because they can participate in sulfur (VI) fluoride exchange chemistry in appropriate conditions. Their common structural pattern is R–O–SO_2–F. The sulfur atom is electrophilic, and the bond to fluorine can be broken during a nucleophilic substitution reaction. The surrounding protein environment can affect whether this reaction takes place, so the position of the group within the binding site is important. [1–4]

2. Chemical Structure and Mechanism

In a fluorosulfate group, sulfur is in the +6-oxidation state and is attached to oxygen-containing groups and fluorine. The electronic environment makes the sulfur centre susceptible to attack by a nucleophile, while fluoride can leave during substitution. How readily the group reacts depends on the rest of the molecule and the environment around it. If the molecule binds to a protein in the right position, the fluorosulfate group may be brought close to a suitable residue. [1–4]

2.1 Steps in covalent inhibition

Step 1—Target binding: The inhibitor first associates with the target through reversible interactions. These may include hydrogen bonding, hydrophobic contacts and ionic interactions within the binding pocket. [5,7]

Inhibitor + target ⇌ target–inhibitor complex

Step 2—Positioning the reactive group: Once the inhibitor is bound, the fluorosulfate group must be close enough to a nucleophilic residue and point in a suitable direction. If the geometry is unfavourable, a reaction may not occur even when the group has suitable chemical reactivity. [3,7,8]

Step 3—Availability of a nucleophile: The amino-acid side chain must be able to attack the reactive sulfur centre. Tyrosine, lysine, serine, threonine and cysteine may be considered in suitable protein environments. Their reactivity depends on factors such as local pKa, hydrogen bonding, electrical effects, solvent exposure and orientation.

Step 4—Nucleophilic attack: The nucleophilic atom on the amino-acid side chain attacks the electrophilic sulfur (VI) centre. This begins the substitution process. [1–4]

Step 5—Fluoride leaves: During the reaction, fluoride can be displaced from sulfur. A new covalent bond can then connect the protein residue to the sulfur-containing part of the inhibitor. [1,2]

Step 6—Covalent complex forms: The inhibitor and protein are now joined by a covalent bond. If this modification prevents the protein from working normally, inhibition may last longer than with a reversible inhibitor. [5,6]

Target–Nu + inhibitor → Target–Nu–inhibitor

Step 7—Effect may continue: The target can remain affected after the amount of free inhibitor in the body has decreased. The time needed for activity to return depends on whether the modified protein is repaired, broken down or replaced by newly made protein. [5,6]

3. Nucleophilic Residues and Design Principles

Different amino-acid residues have different chemical groups: tyrosine has a phenolic hydroxyl group, lysine has an ε-amino group, serine and threonine have hydroxyl groups, cysteine has a thiol group, and histidine has an imidazole nitrogen. These groups do not react equally in every protein. Their accessibility and the conditions inside the binding site can change their reactivity. Cysteine can be a strong nucleophile, but reactions with unintended proteins still need to be avoided. [3,4]

3.1 Target affinity and orientation

The part of the inhibitor that recognizes the target should bind well enough to bring the fluorosulfate group into a useful position. Strong binding can help, but it does not guarantee that a covalent bond will form. The reactive group must also be oriented correctly and able to reach the intended residue. A compound may therefore bind to a protein yet show limited covalent activity if its geometry is not suitable. [5,7,8]

3.2 Reactivity and stability

The aim is to make an inhibitor reactive enough to modify its intended target, but not so reactive that it readily reacts with unrelated proteins. Excessive reactivity can lead to off-target effects, lower selectivity

and possible safety problems. If the group is too unreactive, covalent bond formation may be slow and inhibition may be weak. The compound must also remain chemically stable during preparation, formulation and exposure to biological conditions. [5,8,14,15,25]

3.3 Linker, steric and electronic effects

The linker connects the target-binding scaffold to the fluorosulfate group. Its length and flexibility affect how far the reactive group can reach and how it is oriented. A more rigid structure may help hold the group in place. Nearby bulky groups can shield the sulfur centre and reduce unwanted reactions, but too much shielding may stop the intended residue from reaching it. Other groups in the molecule can change the electronics of the warhead and therefore its reactivity. These features need to be considered together during compound design. [7,14,20,23]

4. Proximity, Structure–Activity Relationship and Selectivity

In proximity-driven covalent inhibition, the inhibitor first binds to the target and brings its reactive group close to a particular nucleophilic residue. This closeness makes a productive reaction more likely. The sequence can be summarized as target binding, correct positioning, nucleophilic attack and covalent modification. [3,8,10,14,23]

Structure–activity relationship studies help researchers understand how changes to a molecule affect its activity. For fluorosulfate-based inhibitors, useful features to examine include the position of the fluorosulfate group, linker length, molecular flexibility, steric hindrance, electronic properties, polarity, hydrophobicity and overall size. These features can affect binding, access to the residue, cell permeability and pharmacokinetic behaviour. The aim is to find a balance between binding strength and selective covalent reaction. [5,7,8,10,14,20,23]

Selectivity remains an important challenge. A fluorosulfate group may react with more than one type of nucleophilic residue, so poorly controlled reactivity can modify proteins other than the intended target. Researchers can try to improve selectivity by strengthening target recognition, placing the warhead correctly, adjusting its reactivity, controlling steric access and taking advantage of features unique to the target's binding pocket. Limiting exposure of the reactive group to unrelated molecules may also help. [5,6,9,12,16]

Fluorosulfates are not the only option for covalent drug design. Acrylamides, chloroacetamides, aldehydes and epoxides have different chemical properties and may be more suitable for particular targets. The choice of warhead should consider target binding, selectivity, drug behaviour in the body and safety—not just how reactive the group is.

5. Advantages, Limitations and Pharmacological Considerations

5.1 Potential advantages

A covalent bond may keep an inhibitor attached to its target even after the free-drug concentration has fallen. This can extend target engagement and may contribute to strong inhibition. Covalent approaches can also help researchers investigate residues or binding pockets that are difficult to address with reversible compounds. Fluorosulfate groups can be added to a range of molecular scaffolds, giving medicinal chemists another option to explore. These potential advantages must still be confirmed through appropriate experiments. [3,5,7,8]

5.2 Limitations and challenges

One concern is modification of proteins other than the intended target, which may reduce selectivity and create safety risks. The compound must be stable enough to reach its target, yet retain the ability to react when correctly positioned. Metabolism can reduce the amount of active compound available. It is also difficult to predict which protein residues will react because their behaviour depends on the local structure and chemical environment. Since covalent changes may last for a long time, unintended protein modification must be examined carefully. Fluorosulfate-based therapeutic chemistry is still developing and requires more evidence before its clinical value can be established. [5,6]

5.3 Two-step inhibition and kinetic terms

A simplified model for covalent inhibition is E + I ⇌ EI → E–I. E represents the target protein, I the inhibitor, EI the reversible complex and E–I the covalent complex. First, the inhibitor binds; then, if the arrangement and chemistry are suitable, a covalent bond forms. In fluorosulfate-based inhibitors, the sulfur (VI) centre is electrophilic and fluoride can act as the leaving group. [1–4,7]

K_i is used to describe affinity in the reversible binding step. A lower K_i usually means stronger binding under the model being used, but it does not tell us how quickly the covalent bond forms. $k_{ina}ct$ describes the rate of the covalent step after the reversible complex has formed. The ratio $k_{ina}ct/K_i$ combines information about binding and covalent reaction efficiency. A higher ratio generally suggests more efficient modification under the relevant conditions, but it should be considered alongside selectivity, assay conditions, target turnover and exposure in cells. [5,7]

The duration of the effect also depends on how quickly the target protein is replaced, whether the cell can recover the affected pathway, and how the drug is absorbed and distributed. A covalent bond can prolong inhibition, but it does not automatically mean that the drug's overall action will be longer in every situation.

6. Therapeutic Potential and Future Perspectives

6.1 Cancer and infectious diseases

Cancer is an important field for covalent drug research because some cancer-related proteins contain residues that can be targeted chemically. Covalent strategies have shown that selected proteins can be inhibited through targeted modification. Fluorosulfate chemistry may offer a way to explore certain cancer-associated enzymes and signalling proteins, but each proposed target needs direct experimental support. [5,7,13,18,21–23]

Covalent inhibitors may also be studied against enzymes from viruses, bacteria and parasites. Differences between pathogen proteins and human proteins could help researchers find selective targets. However, each compound must be tested to establish whether it acts on the pathogen protein without causing unacceptable effects on human proteins. [18,19,21,23]

6.2 Inflammatory and neurological disorders

Proteins involved in inflammatory pathways may also be considered for covalent inhibition. A selective inhibitor could potentially produce a sustained effect on a disease-related pathway, although this depends on the target and the compound's off-target activity. Work on neurological disorders faces an additional challenge: a candidate must have suitable properties to cross the blood–brain barrier and reach its target in the central nervous system. [13,17,20]

6.3 Future research

Further research should explain more clearly how the structure of a fluorosulfate compound, the local protein environment and reaction speed affect one another. Better ways to predict residue reactivity could help researchers design more selective inhibitors. Structural studies can reveal how the reactive group sits in the binding pocket and whether its orientation favours covalent bond formation. Scientists also need to improve stability and pharmacokinetic properties so that compounds can reach their targets without reacting too readily elsewhere. Combining medicinal chemistry, structural biology, chemical biology and computational methods may help address these questions. The main task is to balance potency with selectivity, stability and safety.

CONCLUSION

Fluorosulfate-based covalent inhibitors are an emerging approach in medicinal chemistry. They combine recognition of a target through non-covalent interactions with the possibility of forming a covalent bond to a suitably positioned nucleophilic residue. The proposed process involves target binding, positioning of the fluorosulfate group, attack at sulfur (VI), fluoride displacement and formation of a covalent protein–inhibitor complex.

This approach may extend target engagement and provide new ways to investigate residues that are difficult to target with reversible inhibitors. However, successful development depends on careful control of binding, warhead orientation, reactivity, selectivity, metabolic

stability and safety. Further research is needed to show which targets are best suited to fluorosulfate chemistry and whether these compounds can offer clear therapeutic benefits.

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