Exploring synthetically accessible alternatives to $P2Y_{12}$ antagonists using shape and electrostatics

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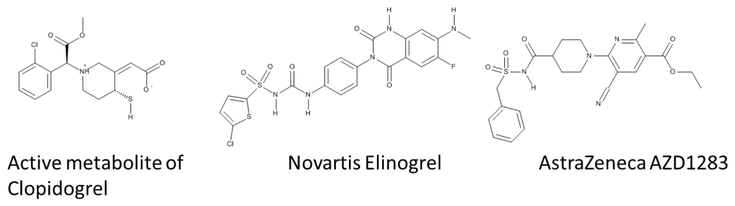
Abstract

Clopidogrel is a member of the thienopyridine class of ADP-induced platelet aggregation inhibitors. The mechanism of action requires oxidative activation, resulting in opening of the thiophene ring to generate the active antithrombotic agent which is an irreversible antagonist of the ADP receptor $P2Y_{12}$ (a GPCR). The mechanism of inactivation probably involves formation of a disulfide bond between the thiol group of the active metabolite and a cysteine residue in the receptor. Since this compound acts through an irreversible mechanism by which it is covalently bound, both slow onset and effect duration are an issue which can lead to problems, particularly for patients requiring bypass surgery.

We have used Cresset's field-based technology to explore potential binding interactions of recently developed antagonists. By applying our electrostatics to probe these examples in the context of new protein structures, we reveal some less intuitive interaction patterns. These insights relate all the current antagonists and suggest binding modes for Clopidogrel and Elinogrel.

Challenge

Although, faster acting variants have been developed, true reversible inhibitors are ideally still required. First generation examples closely resemble ADP (eg Cangrelor and Ticagrelor) and are thus likely to present selectivity / off-target issues. Development is still ongoing (eg. AstraZeneca with AZD128 and Novartis with Elinogrel) to deliver new reversible, non-substrate-like drug candidates against this target.



Active metabolite of Clopidogrel | Novartis Elinogrel | AstraZeneca AZD1283

Figure 1: Chemical structures of three $P2Y_{12}$ antagonists.

X-ray data and binding modes

Recent crystallographic data (Figure 2) revealing the actual binding positions of both the ADP–like and synthetic inhibitors of $P2Y_{12}$ show rather unusual modes of interaction for these inhibitors - however no data is yet publically available for Clopidogrel itself.

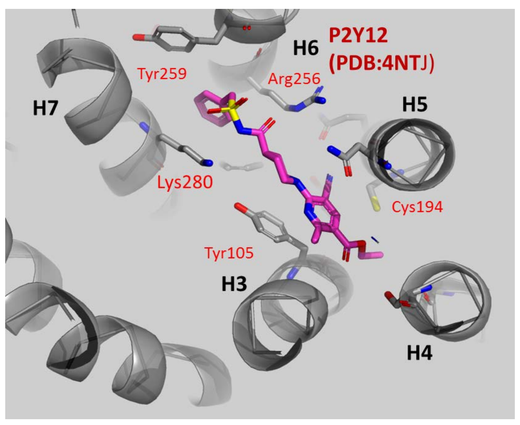


Figure 2: X-ray structure of AZ1283 in $P2Y_{12}$ (PDB:4NTJ).
The X-ray structure of AstraZeneca compound AZ1283shows H6 displacement outward to accommodate benzyl-acyl-sulfonamide moiety. Key interactions other than polar acid (bioisostere) to basic residues are two key π face interactions with Tyr105 and Tyr259.

Interpretation of ligand-protein interactions using electrostatics

The electrostatic interactions are key to understanding the ligand binding events. All use positive ligand field to complement negative protein field. The cyano-pyridine ester is particularly π deficient.

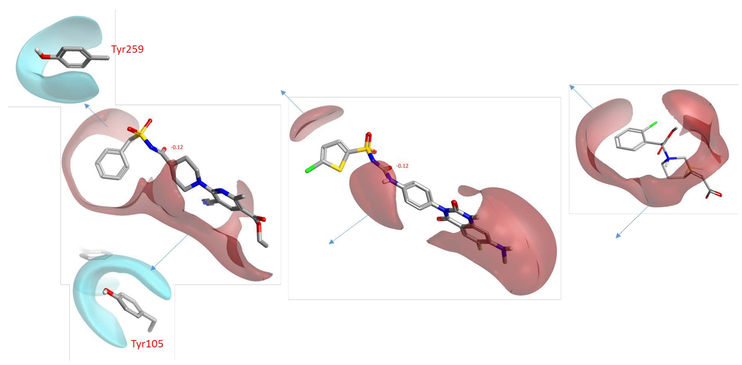


Figure 3: Antagonist positive electrostatics and protein residue interactions.

The Novartis compound Elinogrel switches chemotype, and thus geometry, to access an edge to face arrangement. Clopidogrel itself uses a π cation interaction to Y105.

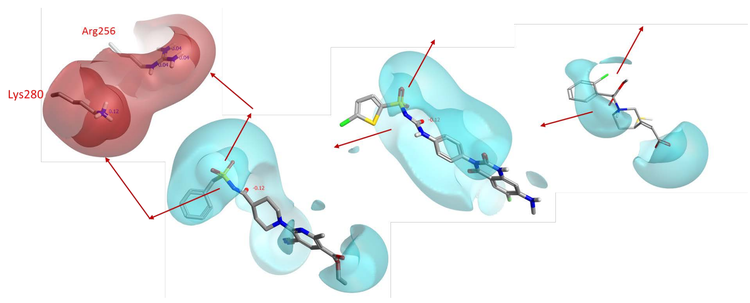


Figure 4: Antagonist negative electrostatics and protein residue interactions.

Acyl-sulfonamide and sulfonyl ureas are well known acid bioisosteres which make good interactions with Arg and Lys residues at the upper reaches of the binding pocket.

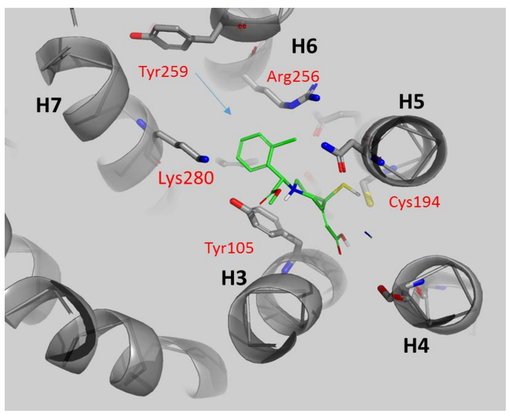


Figure 3: Binding hypothesis for Clopidogrel in $P2Y_{12}$
Showing a binding hypothesis for Clopidogrel (active metabolite) derived via considering the electrostatic fields of each of these inhibitors in the protein context. A candidate cysteine Cys194 covalent attachment point from H5 is in close proximity. Also maintains π electrostatic interactions with a putative receptor conformation with inward movement of H6. Evidence for H6 movement already has precedence – see PDB: 4PY0, 4PXZ.

Spark for useful chemical ideas

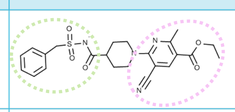
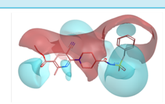
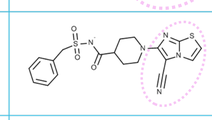
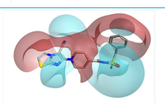
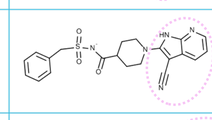
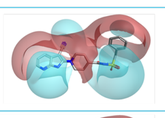
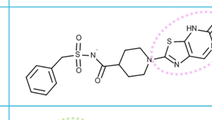
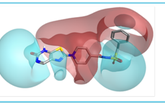
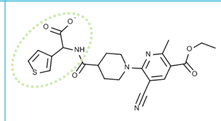
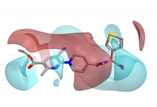
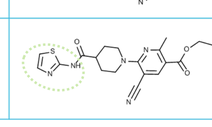
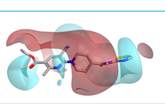
Searching for bioisosteric replacements is a valuable part of a medicinal chemist's toolbox. A bioisosteric core replacement can solve an ADMET or IP issue and move development into a new lead series, while bioisosteric replacements for leaf groups enable fine tuning of molecular properties without affecting the fundamental activity. Cresset's Spark application has proven popular for finding bioisosteric replacements for molecular cores.

In Spark, databases of molecular fragments are searched for replacements for a selected portion of the starting molecule. Fragments with the correct geometry are transformed into viable products that are energy minimized and scored for similarity to the starting point using shape and electrostatics. Recently, a new module was launched that enabled the processing of molecules into reagent databases. These databases can be derived from chemical catalogues or in-house reagents, enabling rapid searching of synthetically accessible chemistry space for novel replacements for R groups. In this case we wanted to illustrate how Spark can be utilized to find suitable synthetically accessible alternatives for the decoration of AZD1283.

Fragment search methodology

Spark requires a 3D conformation from which to generate 3D bioisosteric replacements and a ligand bound X-ray structure (PDB:4NTJ) is an excellent starting point. Searches were applied using this antagonist conformation as a template and the selections highlighted below. The left hand acyl-sulfonamide portion of the molecule was disconnected via the N atom and reconnected using reagent databases containing amines. The cyano-pyridine was disconnected via the secondary amine and thus aryl and heteroaryl halides were the choice of reagents with synthetic reconnection potential. The results are presented in Table 1.

Table 1 Spark fragment replacement results

Entry	Reagent Database	Spark output 2D structures	3D structures and fields	Similarity 2D / 3D fs
1	Reference			1.0 / 1.0
2	Zinc_16 Aryl halide			0.50 / 0.86
3	Zinc_16 Aryl halide			0.51 / 0.82
4	Zinc_16 Aryl halide			0.48 / 0.81
5	Zinc_16 AmineN			0.37 / 0.86
6	Zinc_16 AmineN			0.40 / 0.83

Discussion

The initial field analysis made clear that the ligands required specific electrostatic properties in order to satisfy the interactions. Entry 2 in the table shows a suitably electron deficient heteroaryl replacement (ie one which may closely stack face-on-face with Tyr105) that was found using Spark. Entry 5 is an interesting replacement of the acyl-sulfonamide sporting an α-(3-thienyl) amino acid as the electron deficient heteroaryl. Thienyl groups are clearly suitable replacements to engage Tyr259 in an edge to face arrangement evidenced by Elinogrel.

Summary

Spark can be used as a bioisosteric replacement method, which leverages the unique electrostatic force field and similarity algorithm provided by Cresset to provide insight and pragmatic solutions for medicinal chemists.

References

(1) New $P2Y_{12}$ inhibitors, M. Cataneo, *Circulation.* 2010;**121**:171-179**.** (2) Structure and stereochemistry of the active metabolite of clopidogrel, Pereillo et al, *Drug metabolism and disposition* **30,** (11), 1288-1295, 2002.