Molecular Basis of Avanafil's Superior Isoform Selectivity Toward Phosphodiesterase 5A1

Phosphodiesterase 5A1 (PDE5) is a key target to treat cardiovascular diseases and erectile dysfunction. In this report, based on the crystal structure of the PDE5-avanafil complex, the underlyng mechanism of the isoform selectivity is revealed and a new concept of structure-based drug design is proposed.

cAMP and cGMP are second messengers that play a critical role in signal transduction pathways that regulate many cellular processes. The levels of cAMP and cGMP are tightly controlled through their synthesis by nucleotidyl cyclases and hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). The human genome encodes 21 PDE genes that are divided into 11 families, PDE1–11, based on sequence similarity, substrate specificity and regulatory properties. Alternative splicing of mRNA or multiple promoters and transcription-starting sites further generates more than 100 PDE isoforms that vary in tissue and subcellular distribution.

PDEs have been considered main therapeutic targets for a long time; numerous PDE inhibitors have been developed to treat various diseases. For example, PDE3 inhibitor milrinone and PDE4 inhibitor roflumilast are widely used to treat heart failure and particular inflammatory lung diseases, respectively.[1,2] The presence of unwanted side effects resulting from the inability to target individual PDE isoforms is, however, the major limiting factor to success. For instance, the three first-generation PDE5 inhibitors (sildenafil, vardenafil, tadalafil) to treat erectile dysfunction, benign prostatic hyperplasia and pulmonary arterial hypertension might cross-inhibit PDE1, PDE6 and PDE11 and result in visual disturbance, hearing loss and dyspepsia.[3-5] The exceptional specificity showed by FDA-proved second-generation PDE5 inhibitor avanafil is hence remarkable.[6]

To understand the detailed mechanism of avanafil's superior isoform selectivity, a research team led by Nei-Li Chan (National Taiwan University) solved the crystal structure of PDE5 in a complex with avanafil by X-ray crystallography.[7] The X-ray diffraction data were collected at **TLS 15A1** and **TLS 13C1** of NSRRC. The final model contains the entire

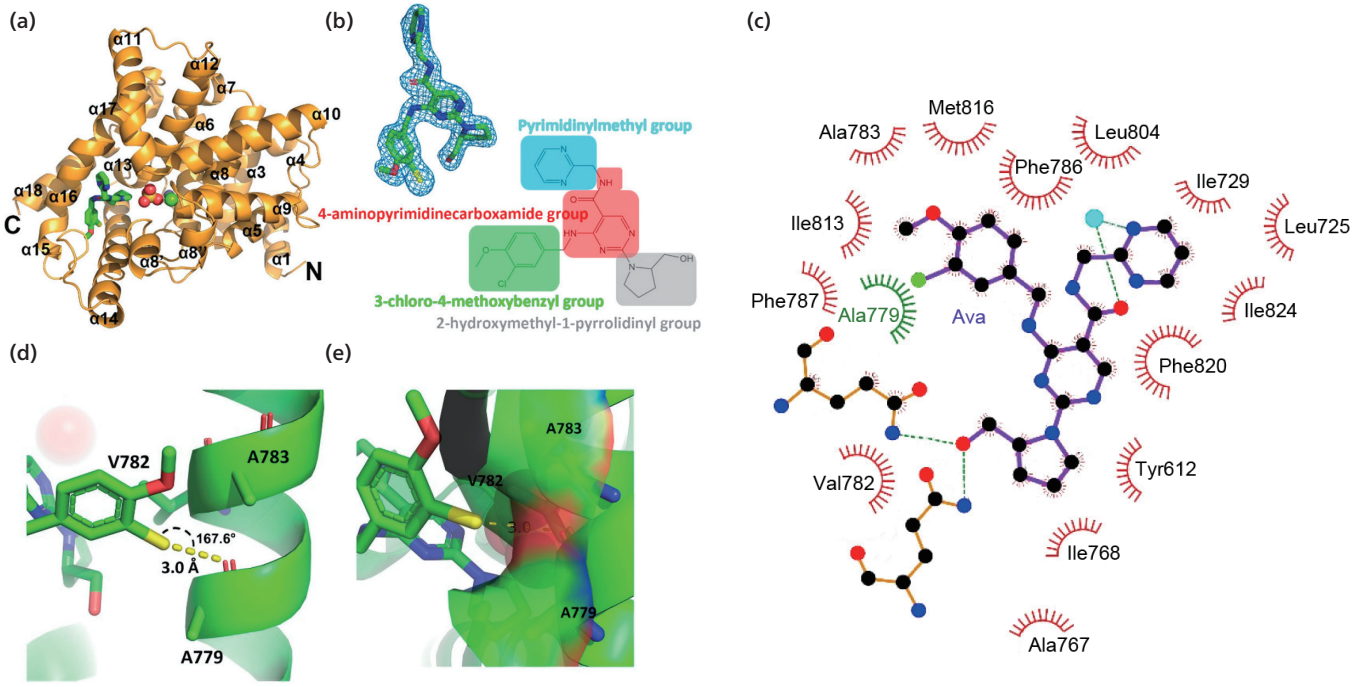

Fig. 1: (a) Overall structure of the PDE5–avanafil complex. The avanafil is represented as green sticks. Mg^{2+} and Zn^{2+} ions are shown as green and purple spheres; sulfur and oxygen atoms of SO_4^{2-} are shown as brown and red spheres. (b) Chemical structure of avanafil and $2F_o - F_c$ electron density (contoured at 1.0 σ) for the bound avanafil. (c) Schematic diagram of interactions between avanafil and PDE5. (d) The carbonyl oxygen of A779 from helix α14 forms a halogen bond with the chlorine atom of the 3-chloro-4-methoxybenzene ring of avanafil. (e) The side chains of A779, V782 and A783 enclose a hole with the carbonyl oxygen of A779 at the bottom to accommodate the chlorine atom of avanafil. [Reproduced from Ref. 7]

PDE5 catalytic domain, one avanafil, one Zn^{2+}, one Mg^{2+} and one sulfate (**Fig. 1(a)**). A distinct feature of avanafil is the employment of a halogen-substituted benzyl moiety, the 3-chloro-4-methoxybenzene ring, to mediate target binding (**Fig. 1(b)**). The interactions between PDE5 and avanafil are mainly through noncovalent interactions of multiple types and a unique, avanafil-specific, halogen bond (**Fig. 1(c)**). The hydrogen bonds are shown as green dashed lines, and residues making van der Waals interactions with avanafil are shown in red arcs with spokes. A779, of which the carbonyl oxygen forms a halogen bond with avanafil, is shown as a green arc with spokes. A water molecule (cyan) bridges the interaction between avanafil and α16. The chlorine atom of this aryl halide moiety is located atypically near (3.0 Å) the carbonyl oxygen of A779 (**Fig. 1(d)**), indicating the formation of a halogen bond between Cl and O. A key feature of this halogen bond formed between avanafil and PDE5 is that A779, the halogen-bond acceptor, belongs to helix α14 (**Fig. 1(d)**). This finding illustrates that particular main-chain carbonyl-oxygen atoms from α-helices can participate in protein–ligand interactions through acting as a halogen-bond acceptor. Furthermore, this chlorine atom makes van der Waals interactions with side chains of nearby residues (A779, V782 and A783) that form a binding pocket (**Fig. 1(e)**). This finding demonstrates that the accessibility of such a carbonyl oxygen depends on the width of the hole entrance that is determined by the sizes and shapes of the surrounding side chains.

In summary, the crystal structure of the PDE5–avanafil complex was determined at resolution 1.9 Å. Analysis of the protein–drug interactions reveals the structure–activity relation of avanafil and provides a molecular basis of its superior isoform selectivity. Moreover, a halogen bond was observed between the aryl halide moiety of avanafil and a backbone carbonyl oxygen from an α-helix flanking the drug-binding site, which illustrates the feasibility of exploiting the α-helix backbone in structure-based drug development. (Reported by Chia-Liang Lin)

This report features the work of Nei-Li Chan and his colleagues published in J. Med. Chem. ***63****, 8485 (2020).*

TLS 15A1 Biopharmaceuticals Protein Crystallography
TLS 13C1 SW60 – Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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