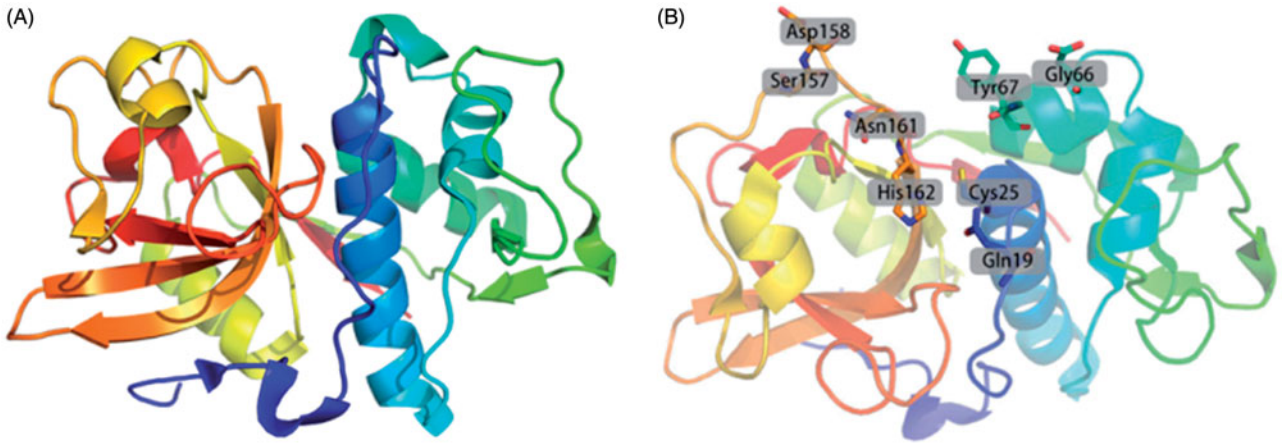

Figure 1. Ribbon drawing of human Cat K and the active sites of Cat K. (A) The overall ribbon structure of human Cat K. The structure is from Protein Data Bank (PDB ID: 5TDI). (B) The residues in active sites of human Cat K.

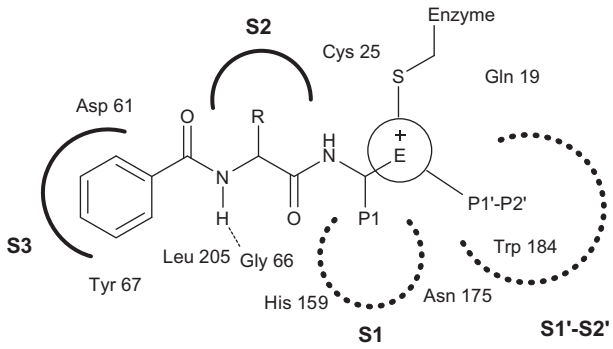

Figure 2. Key binding features of active sites in Cat K.

Cat K inhibitors based on ketone warhead

In 1997, Veber *et al.* reported a series of selective and reversible Cat K inhibitors based on a poorly electrophilic 1,3-bis(acylamino)-2-propanone scaffold[19]. Through modelling the interaction of active-sites and simplifying the structure of inhibitors, they developed an accessible symmetrical ketone **1** with a $K_{i,app}$ value of 22 nM against Cat K (Figure 3). It was interesting to note that **1** exhibited excellent selectivity over other members of cathepsin family ($K_{i,app}$ cathepsin L (Cat L), 0.34 μM; cathepsin B (Cat B), 1.3 μM; cathepsin S (Cat S), 0.89 μM)[20]. N-methyl analog of **1** examined effects of methylation, ketone **2**, was 4-fold less active than **1**. Whereas, in order to span the distance of both sides of its active site (picking up the Trp184 aromatic interaction) **3**, the chemical moiety of Cbz-Leu in **1** substituted by 4-phenoxyphenyl sulfonamide, showed 10-fold more active than their original peptide-based lead.

On the other hand, extension of aromatic moiety interacted with Try67, DesJarlais *et al.*[21] developed a variety of sulfonyl inhibitors, among which **4** with the biphenyl group replacing Cbz showed greater than 500-fold selectivity over Cat B, S, L ($K_{i,app}$ Cat K, 1.4 nM; Cat B, >10000 nM; Cat S, 910 nM; Cat L, >1000 nM) (Figure 3). The biphenyl group that best matched the conformation of prime side is more rigid and bulky than the benzyl carbamate. From the analysis of X-ray co-crystal structure, the biphenyl system in **4** occupied the S3 site rather than the substrate backbone binding site and formed an aromatic–aromatic interaction with Tyr67.

Marquis *et al.*[22] designed an azepanone-basediInhibitor of Cat K **5**, which possessed some special structures including a C-4 chiral center as S and an azepanone ring in a pseudo-boat conformation (Figure 3). The C-4 S stereochemistry was critical for potent inhibition that predicted the higher energy axial orientation bound within the active site of Cat K by molecular modelling. Compound **5**, which incorporated the replacement of the carbonylbenzyloxy group with the benzofuran-2-carboxyamide showed a potently reversible inhibitor of human Cat K with a $K_i = 0.16$ nM and a relatively acceptable selectivity against Cat B, S, L ($K_{i,app}$ Cat B, 500 nM; Cat S, 4 nM; Cat L, 2.2 nM). Comparison of the transport of cyclic and acyclic analogs, the results from pharmacokinetic analysis revealed inhibitor **5** with cyclic has good oral bioavailability in the rat of 42% with a $T_{1/2}$ of 30 min.

The ketone inhibitors of Cat K pioneered by GSK scientists have been taken a huge number of efforts to realise the desired inhibition and selectivity. The discovery of **6** embodying extremely potent inhibition with picomolar affinity, known as relacatib or SB-462795 (Developed by GSK), was considered as an important milestone (Figure 3)[23]. Compound **6** in a chair conformation has an axial methyl group at C-7 position, which contacts with the S1′ hydrophobic pocket, while the sulfonylpyridine interacts with the S2′ hydrophobic pocket. The interactions between compound **6** and Cat K are shown in Figure 4. Furthermore, conformational analysis revealed that the methyl group at C-4 increased the configurational stability. The 7-methyl substituted azepanone analog shows favorable pharmacokinetic characteristics, good oral bioavailability (89%), and an *in vivo* clearance rate of 19.5 ml/min/kg. However, in spite of those advantages, compound **6** exhibits a rather low or no selectivity over other off-target cathepsins ($K_{i,app}$ Cat K, 0.041 nM; Cat L, 0.068 nM; cathepsin V (Cat V), 0.053 nM; Cat B, 15 nM; Cat S, 1.6 nM)[24].

During the systemic research of odanacatib, Boyd *et al.* investigated that the replacement of nitrile with cyclic ketone warheads was based on the experience of ketones as reversible Cat K inhibitors[8,25]. Substitution of the benzofuran moiety of compound **5** with an odanacatib-like 4-methylsulfonylphenyl backbone, to provide compound **7**, conceivably allowed interactions on the prime side[26]. The biphenyl of the substrate intensively participates a ring-ring interaction between Tyr67 at the S3 pocket. In spite of this, compound **7** only furnished improved partial selectivity as well as more than 10-fold reduced inhibition.

Merck Frost also provided a series of ketone inhibitors of various cyclic aminoketone ring size and nitrogen substitution, such as **8**, **9** (Figure 3)[27]. The IC_{50} values against humanised rabbit Cat K and selectivity against human Cat L, B, and S exhibited no significant difference compared with inhibitor **7**[28]. Moreover, from the rat bile cannulation study, these inhibitors were rapidly cleared