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Original article

# Natural biflavones as novel inhibitors of cathepsin B and K

G.-Z. Zeng[a,b], X.-L. Pan[a,b], N.-H. Tan[a,*], J. Xiong[a], Y.-M. Zhang[a]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*

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**Abstract**

Cathepsin B and K, two important members in lysosomal proteases, involve in many serious human diseases, such as tumor and osteoporosis. In order to find their novel inhibitors, we performed the inhibition assays of cathepsin B and cathepsin K in vitro, randomly screened compounds from plants, and found six biflavones, named AMF1-5 and HIF, can potently inhibit cathepsin B and cathepsin K, especially AMF4 and HIF with $IC_{50}$ of 0.62 and 0.58 μM against cathepsin B. They are novel inhibitors for cathepsin B and K. Inhibition and flexible docking studies indicated that these biflavones are reversible inhibitors of cathepsin B, and their binding patterns and interaction modes with cathepsin B made them more specific to cathepsin B endopeptidase.


*Keywords:* Biflavones; Inhibitors; Cathepsin B; Cathepsin K; Docking

## 1. Introduction

Cathepsins, also known as lysosomal proteases, are the main members of the papain-like protease family, which are implicated in many pathological situations and have been studied intensely as potential drug targets [1].

Cathepsin B (CatB, EC 3.4.22.1) and Cathepsin K (CatK, EC 3.4.22.38), two important members of cathepsins, are 30 and 27 kDa bilobal proteins, respectively. The active site and substrate-binding cleft of CatB are located at the interface between the two lobes with Cys29 on the left lobe and His199 on the right lobe, while for CatK are Cys25 and His162 residues. At optimal pH, a Cys29-$S^-$/His199-$ImH^+$ (for CatK is Cys25-$S^-$/His162-$ImH^+$) ion pair is formed by the side chains of Cys29 (Cys25) and His199 (His162), substrate peptide bond cleavage is mediated by the nucleophilic attack by $S^-$ from Cys29 (Cys25) on the carbonyl carbon atom, and followed by proton donation from His199 (His162) [2–4]. CatB is a unique member in cathepsins for its dual roles as both endopeptidase and exopeptidase (dipeptidyl carboxypeptidase). The occluding loop (Ser104–Pro126) of CatB is responsible for the latter activity. Because His110 and His111, two important residues, face the prime side of the substrate-binding cleft to stabilize the terminal carboxylate of a peptide, this arrangement accounts for its dipeptidyl carboxypeptidase activity [2–5]. There is a pH-dependent transition for CatB from exo- to endo-peptidase. Below pH 5.5, CatB mainly acts as an exopeptidase for its proteolytic degrading activity in lysosomal. Above pH 5.5, a mixture of endo- and exo-proteolytic CatB activities presents with a slightly greater percent activity as an endopeptidase. CatB is active in neutral condition, and it has maximum endopeptidase activity around pH 7.4. In tumor invasion and metastasis, CatB was found outside the lysosomal compartment; it acts mainly as endopeptidase and plays an important role in degrading extracellular matrix (ECM) and basement membrane [6–8]. Moreover, increased expression, activity and secretion of cathepsin B have been observed in more than one type of tumors, such as breast, gastric, lung, prostate carcinomas and so on [9]. So inhibitors specifically targeted endopeptidase of CatB will be potentially more therapeutic. CatK, expressed in bone-

*Abbreviations:* Abz-, *ortho*-aminobenzoic acid; AMC, [7-amino-4-methyl] coumarin; CA030, EtO-(2*S*,3*S*)-*t*Eps-Ile-Pro-OH; DMSO, dimethyl sulfoxide; Dnp, 2,4-dinitrophenyl; E-64, 1-[[(L-trans-epoxysuccinyl)-L-leucyl] amino]-4-guanidinobutane; EDTA, ethylenediaminetetraacetic acid; Leupeptin, acetyl-leucyl-leucyl-arginal; Z-, carbobenzyloxy-.

* Corresponding author.
*E-mail address:* nhtan@mail.kib.ac.cn (N.-H. Tan).

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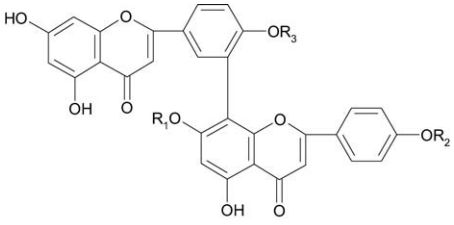
resorbing osteoclasts selectively and abundantly, can degrade type I collagen, which is the predominant component of the extracellular matrix of bone. Thus, CatK plays a pivotal role in osteoclast-mediated bone resorption and is a potential target in osteoporosis, and some of CatK inhibitors efficiently block bone resorption in vivo [4,10].

Many chemotypes have been described as inhibitors of cathepsins, such as cystatins, leupeptin, E-64, CA030. Most of inhibitors are peptide-based molecules. Though with potent inhibition, they generally possess poor pharmacokinetic properties [11]. Therefore, searching for new inhibitors of cathepsins, especially selective reversible nonpeptidic inhibitors, should be given increased emphasis [3]. For this purpose, we performed the inhibition assays of CatB and CatK in vitro and found six biflavones can inhibit CatB and CatK, which are a novel class of cathepsin inhibitors from plants. This paper describes the inhibition of these six nonpeptidic compounds against CatB and CatK.

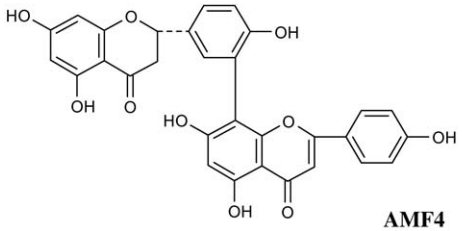
## 2. Chemistry

Natural biflavone compounds amentoflavone (AMF1), 4‴-methylamentoflavone (podocarpusflavone A, AMF2), and 7″,4‴-dimethylamentoflavone (AMF3) were isolated and identified from acetone extracts of leaves and branches of *Taxodium mucronatum* (Taxodiaceae) by chromatographic and spectral methods [12]; 2,3-dihydroamentoflavone (AMF4), 4′-methylamentoflavone (bilobetin, AMF5), and hinokiflavone (HIF) were isolated and identified from methanol extracts of leaves of *Cycas guizhouensis* (Cycadaceae) by chromatographic and spectral methods [13] (Fig. 1). Their purities are > 95%. Both *Taxodium mucronatum* and *Cycas guizhouensis* were collected from Kunming Botany Garden (Kunming Institute of Botany, Chinese Academy of Sciences, China). Detailed purifications and identifications of these six biflavones were described before by us [12,13].

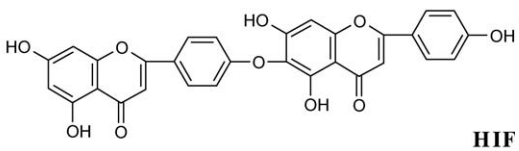


**AMF1:** $R_1$=$R_2$=$R_3$=H **AMF2:** $R_1$=$R_3$=H, $R_2$=$CH_3$

**AMF3:** $R_1$=$R_2$=$CH_3$, $R_3$=H **AMF5:** $R_1$=$R_2$=H, $R_3$=$CH_3$



**AMF4**



**HIF**

Fig. 1. Chemical structures of AFM1, 2, 3, 4, 5 and HIF.

## 3. Pharmacology

Natural compounds AMF1-5 and HIF were evaluated for their ability to inhibit CatB and CatK by fluorescence assays. Moreover, the specificities of six biflavones against CatB endopeptidase were assayed with different substrates and different assay buffers (Table 1). Enzyme inhibition data were expressed as $IC_{50}$ values (50% inhibitory concentration), and $IC_{50}$ values of each inhibitor were calculated by dose-response curves with four concentrations (dilution ratio = 1/2) and the highest tested concentration is 2.5 μg/ml. Results are expressed as mean $IC_{50}$ values ± standard deviation. Leupeptin and E-64 were used as reference compounds.

## 4. Results and discussion

By inhibition assays of CatB and CatK, we found six biflavones can potently inhibit cathepsin B and cathepsin K and with some degree of selectivity for CatB (Table 1). In kinetic studies of the hydrolysis conversion of Z-FR-AMC into AMC, inhibition of AMF4 and HIF against CatB activity was found to be time-dependent: Concentrations of AMC were less than the standard level in the initial time, but as time went on, they were equal to the standard level. Their inhibition were similar to leupeptin recognized as a reversible inhibitor, but did not like E-64, an irreversible inhibitor (Fig. 2). The reversible inhibition of other four biflavones, AMF1-3 and AMF5, against CatB were similar to that of AMF4 and HIF in our experiments. It demonstrated that these biflavones were reversible inhibitors of CatB.

The occluding loop of CatB is the major structural determinant of the exopeptidase specificity and relatively lower endopeptidase activity [14]. We have used one endopeptidase substrate Z-FR-AMC and two carboxyldipeptidase substrates Abz-GIVRAK(Dnp) and Abz-FRF($4NO_2$)A in our experiment to determine the specificity of these inhibitors against CatB, and

Table 1
Inhibitory activities of inhibitors against CatB and CatK

| Inhibitor | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|
| | Cathepsin B | | | | Cathepsin K |
| | Z-FR-AMC | | Abz-GIVRAK(Dnp) | Abz-FRF($4NO_2$)A | Z-GPR-AMC |
| | pH 5.5 | pH 7.4 | | | |
| AMF1 | 1.17 ± 0.25 | 0.49 ± 0.15 | 49.63 ± 4.99 | 41.74 ± 9.13 | 1.88 ± 0.38 |
| AMF2 | 1.03 ± 0.32 | 0.98 ± 0.23 | > 90.50 | 75.19 ± 0.76 | 2.51 ± 0.28 |
| AMF3 | 0.67 ± 0.16 | 0.52 ± 0.12 | > 88.26 | 80.84 ± 1.22 | 1.57 ± 0.41 |
| AMF4 | 0.62 ± 0.13 | 0.23 ± 0.03 | 8.54 ± 2.63 | 11.10 ± 5.37 | 1.39 ± 0.15 |
| AMF5 | 0.81 ± 0.11 | 0.68 ± 0.22 | 76.78 ± 5.19 | > 92.51 | 1.55 ± 0.25 |
| HIF | 0.58 ± 0.21 | 0.52 ± 0.08 | 36.65 ± 12.84 | 32.90 ± 14.38 | 1.54 ± 0.37 |
| LP | 0.051 ± 0.014 | 0.021 ± 0.005 | 0.130 ± 0.011 | 0.160 ± 0.009 | 0.039 ± 0.013 |
| E-64 | 0.014 ± 0.001 | 0.031 ± 0.007 | 0.016 ± 0.002 | 0.022 ± 0.006 | 0.003 ± 0.001 |

Averages were calculated from at least three independent experimental data. "> μM" meant that 50% inhibitory concentration of the compound is beyond the sample stock solution in our compound library.

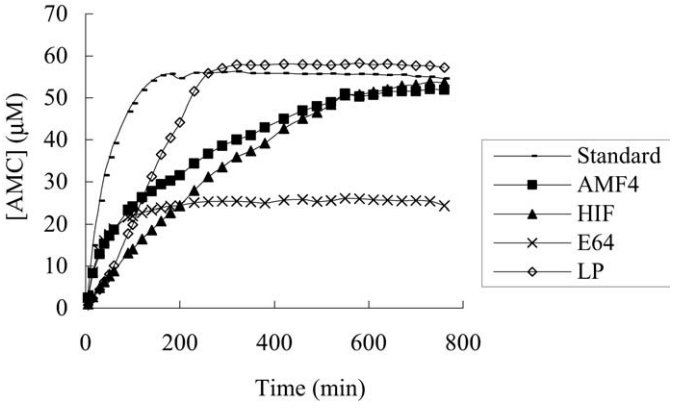



Fig. 2. Time-dependent inhibition and reversibility of biflavones. Relationship between [AMC] and reaction time was monitored (Z-FR-AMC hydrolyzed by CatB with buffer pH 5.5): Standard (without inhibitor), LP (0.13 μM), E-64 (0.016 μM), AMF4 (0.6 μM) and HIF (1.2 μM), respectively ($K_m$ is 0.504 mM for Z-FR-AMC in the reaction system).

found that inhibitory activities of six biflavones against CatB endopeptidase were about 14–132 times higher than their exopeptidase ones, which for leupeptin was only three times, and for E-64 was almost the same (Table 1).

To investigate the pH preference of CatB against the peptide Z-FR-AMC, we measured $k_{cat}/K_m$ values under pseudo first-order condition. The pH dependency is characterized by a gradual increase in activity when pH is raised from 3.0 to 7.4, and the $k_{cat}/K_m$ value at pH 7.4 is much higher than that at pH 5.5, which meant that, in weak neutral condition, CatB can hydrolyze the endopeptidase substrate Z-FR-AMC more efficiently, and it is with greater endopeptidase activity than exopeptidase one. Though Z-FR-AMC is not a suitable substrate to study the occluding loop function, the pH-dependent profile also showed that there is a better affinity between CatB and Z-FR-AMC at pH 7.4 than pH 5.5 (Fig. 3). We tested the inhibitory capacities of six compounds against CatB with Z-FR-AMC in acidic (pH 5.5) and weak neutral (pH 7.4) conditions, $IC_{50}$ results also indicated that under the weak neutral condition, the inhibitory activities of six biflavones are slightly more potent than those under acidic condition. While $IC_{50}$ for E-64 is contrary, $IC_{50}$ at pH 7.4 is twice as many as it at pH 5.5 (Table 1). These results correlated with that from different substrates. All results

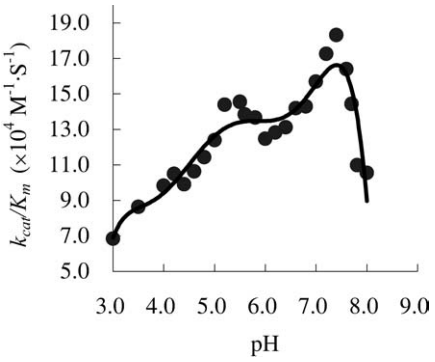



Fig. 3. The pH dependence of CatB activity with the substrate Z-FR-AMC.

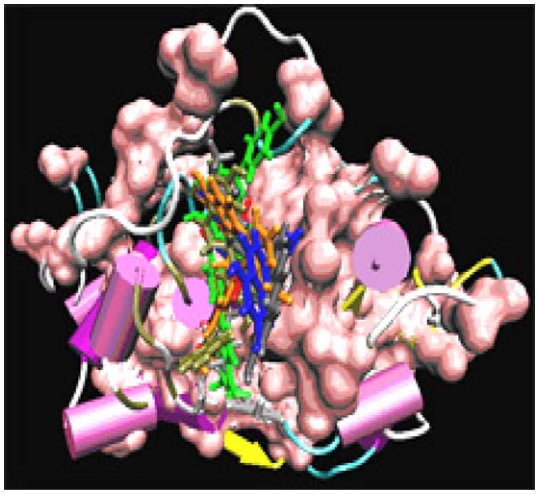


Fig. 4. Docking results of the complexes between AMF1-5, HIF and CA030 ligands and human CatB. The binding conformations of CA030, AMF1, AMF2, AMF3, AMF4, AMF5, and HIF are displayed in stick model representation with different colors for each compound: CA030 in red, AMF1 in blue, AMF2 in gray, AMF3 in orange, AMF4 in tan, AMF5 in silver, and HIF in green. The hydrophobic surface is displayed in a schematic representation in pink. This was prepared using VMD.

indicated that these biflavone inhibitors were more specific to CatB endopeptidase activity.

The binding conformations (Fig. 4) and binding energies (Table 2) of six biflavones with CatB were performed and predicted by molecular docking (Fig. 5). Six biflavones potently inhibited CatB with the order

Table 2
Docking energies (kJ/mol) of six biflavones

| Ligand | Total_score | G_score | PMF_score | D_score | Chem_score |
|---|---|---|---|---|---|
| AMF1 | –49.83 | –169.22 | –38.65 | –133.96 | –32.47 |
| AMF2 | –50.11 | –154.28 | –31.21 | –149.67 | –30.74 |
| AMF3 | –61.18 | –145.69 | –31.88 | –145.78 | –23.40 |
| AMF4 | –58.54 | –158.67 | –0.29 | –143.31 | –24.30 |
| AMF5 | –57.88 | –148.54 | –16.22 | –145.97 | –24.50 |
| HIF | –63.14 | –144.35 | –17.67 | –151.20 | –28.90 |

Docking scoring values for complexes of the human cathepsin B receptor with optimal structure of six biflavones ligands. G_score, PMF_score, D_score and Chem_score are based on hydrogen-bonding interaction, statistical ligand–receptor atom-pair interaction potentials, electrostatic and hydrophobic contributions to binding energy, a diverse training set of 82 receptor-ligand complexes, respectively. Lower scores indicate more favorable binding.

HIF > AMF4 > AMF3 > AMF5 > AMF2 > AMF1 (Table 1), which correlated well with the calculation results (Table 2). Results indicated that there is a good correlation between the binding energies and the experimental inhibitory potency $pIC_{50}$ ($-\log IC_{50}$) with the $r^2$ (correlation coefficient) value of 0.903 (Fig. 6). For AMF1-3 and AMF5, more methoxy groups the molecule contained, more potent inhibitory activity it had. It mainly attributed that methoxy group is electron donor which can increase the electron density, HOMO energy and hydrophobic group. Moreover, AMF5 and AMF2, which have one $OCH_3$ substitute except in different positions, have different inhibitory activities for the results of steric and electric effects which we proposed previously [15]. However the structures of HIF and AMF4 are different from the others. HIF, in which 4′ and 6″ substitutes were connected by an oxygen atom, was the most potent inhibitor in these biflavones, because it occupied almost the entire active site of CatB (Fig. 4), and formed many hydrophobic contacts with the active site of CatB (Fig. 5). AMF4, saturated at 2,3 positions, is more flexible, which made it interact with CatB active site cleft well with more potent activity. By flexible docking study, we found that six biflavones acted on the active site cleft of CatB just like CA030 did (Fig. 4), but their binding patterns and interaction modes were different from CA030 analogs that mainly interact with the residues His110, His111 and Cys29 [15]. Six biflavones interacted little with the occluding loop, which might be responsible for their specific inhibitory activities against CatB endopeptidase. AMF4 is exceptional in six biflavones, for its A-ring could interact with the occluding loop residues (His111, Gly121, and Cys119) (Fig. 5), which made it more potent on CatB exopeptidase activity (Table 1).

In conclusion, we found six biflavones potently inhibit activities of CatB and CatK by random screening. They are novel natural inhibitors for cathepsins and with some degree of selectivity for the inhibition of CatB over CatK. They are reversible inhibitors for CatB and could inhibit CatB endopeptidase activity specifically. The inhibitory specificities against CatB endopeptidase made them may be more potential in pathological state like tumor progression. Among these biflavones, AMF4 and HIF are two inhibitors with the best activities against CatB both for endopeptidase and exopeptidase. But their binding patterns and interaction modes differ from CA030, their endopeptidase inhibitory activities are 14–60 times higher than their exopeptidase ones. Docking results also supported these experimental results. Our findings could benefit the development of new strategies for design and screen of natural inhibitors of CatB.

## 5. Experimental protocols

### 5.1. Materials

Human liver cathepsin B and human recombinant cathepsin K were from Calbiochem (Cat# 219364, 219461, Darmstadt, Germany). The fluorogenic substrate Z-FR-AMC and Z-GPR-AMC were from Bachem (Cat# I-1160, I-1150, King of Prussia, Pennsylvania, USA). The fluorescent cleavage product AMC and cysteine protease inhibitors E-64 and Leupeptin (LP) were from Sigma (Cat# A9891, E3132, L9783, St. Louis, MO, USA). The fluoregenic substrates Abz-GIVRAK(Dnp) and Abz-FRF($4NO_2$)A were generously given by Dr. Luiz Juliano (Department of Biophysics, Universidade Federal de Sao Paulo, Brazil). All other reagents used were commercially available with analytical grade. Substrate hydrolysis was monitored in a cytofluor II fluorescent plate reader (PerSeptive Biosystems).

### 5.2. Inhibition assays of CatB

Assay of CatB endopeptidase activity was referenced by what Barrett [16] and Melo et al. [17] did, which was determined spectrofluorometrically using the fluorogenic substrate Z-FR-AMC. Two different buffers were used. One buffer (pH 5.5) contained 60 mM sodium acetate, 15 mM L-cysteine and 5 mM EDTA. The other buffer (pH 7.4) contained 60 mM sodium phosphate, 15 mM L-cysteine and 5 mM EDTA. Test compounds were dissolved in DMSO and diluted with buffers. The compound solution was mixed with diluted enzyme solution in a well of 96-well black microplate (Costar) and incubated for 5–10 min at 25 °C. The reaction was initiated by adding 75 μM fluorescence substrate solution to the well. After incubation for 45 min at 25 °C, in which produced fluorescence is sensitive enough to be detected, AMC released from Z-FR-AMC was monitored on the Cytofluor II fluorescent plate reader at 460/40 nm after excitation at 360/40 nm. The final concentration of CatB in the assay mixture was 3.73 nM. Assay of CatB exopeptidase activity was referenced by the method Cotrin used [18] with some modifications: The buffer

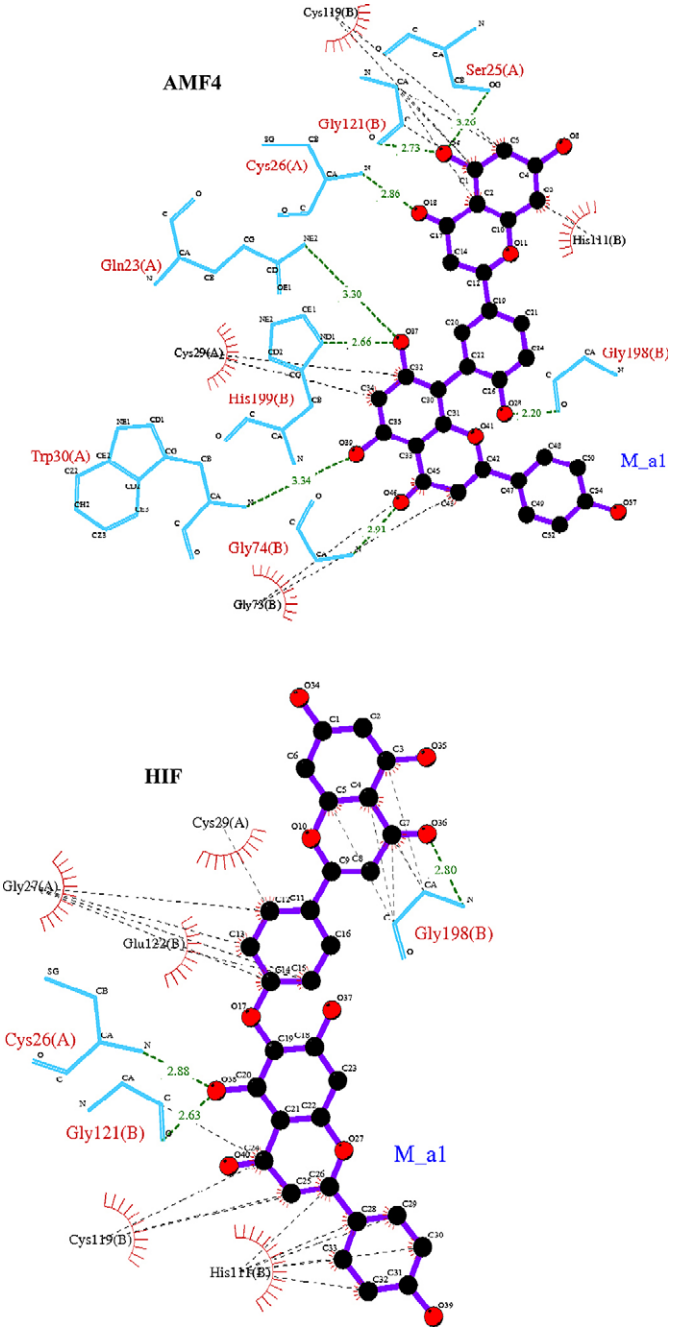



Fig. 5. Binding modes of the optional conformation poses of AMF4 and HIF ligands with the active sites of CatB receptor were drawn using the Ligplot program. Broken lines in green represent hydrogen bonds and spiked residues form hydrophobic contacts with the inhibitors (connected by broken lines in black).

(pH 5.0) contained 60 mM sodium acetate, 10 mM L-cysteine and 2.5 mM EDTA, and the hydrolysis reaction was initiated by adding 30 μM fluorescence substrate (Abz-GIVRAK(Dnp) or Abz-FRF(4$NO_2$)A) solution. After incubated for 45 min at 25 °C, produced fluorescence was monitored on the Cytofluor II fluorescent plate reader at 460/40 nm after excitation at 360/40 nm.

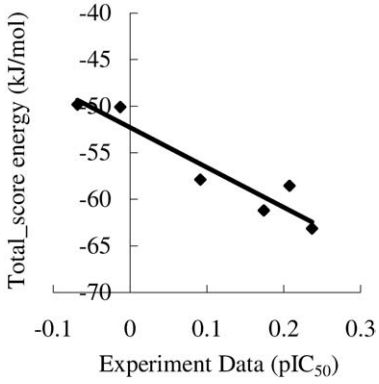



Fig. 6. Plots of experimental data versus Total_score energy for six biflavone inhibitors.

### 5.3. Kinetics of six biflavones against CatB

Experimental procedure was the same as assay of CatB except that increasing AMC hydrolyzed from Z-FR-AMC was monitored at 20–30 min interval in 700 min. The buffer (pH 5.5) contained 60 mM sodium acetate, 15 mM L-cysteine and 5 mM EDTA. Furthermore, by measuring initial rates at various substrate concentrations, kinetic parameter $K_m$ was determined based on the Michaelis–Menten equations.

### 5.4. pH dependence of CatB activity with the substrate Z-FR-AMC

The pH profile of CatB activity was obtained by measuring $k_{cat}/K_m$ values on the relationship $v = [E][S]k_{cat}/K_m$, at $[S] << K_m.k_{cat}/K_m$ values were determined by measuring initial rates at various substrate concentrations, when the enzyme final concentration was 3.73 nM. The reaction buffers were 60 mM sodium acetate (pH 3.0–5.8), 60 mM sodium phosphate (pH 5.8–8.0). Both contained 15 mM L-cysteine and 5 mM EDTA. Buffer pH ranged from 3.0 to 8.0 and was adjusted with 1 M NaOH or HCl.

### 5.5. Inhibition assay of CatK

Compound inhibitory activity against CatK was measured as the method Aibe et al. [19] and Barrett [16] used before with some modifications. Test compounds were diluted with the buffer (pH 5.0, 100 mM sodium acetate, 20 mM L-cysteine, and 2 mM EDTA), and were mixed with diluted enzyme solution for 5–10 min at 25 °C, then the 75 μM substrate Z-GPR-AMC was added to start the reaction. The final concentration of CatK in the assay mixture was 4.63 nM. After incubation for 120 min at 37 °C, produced fluorescence was monitored on the Cytofluor II fluorescent plate reader at 460/40 nm after excitation at 360/40 nm.

### 5.6. Flexible docking

The method is the same as our previous research [15] with some modifications. The 3D structures of six biflavones were

constructed by using molecular modeling software package Sybyl6.9 [20]. Partial atomic charges were calculated by Gasteiger–Huckel method, and energy minimizations were performed using the Tripos force field with a distance dependent dielectric and the powell conjugate gradient algorithm (convergence criterion of 0.001 kcal/mol Å). The X-ray crystal structure of CatB complexed with CA030 (pdb entry: 1csb) was retrieved from the Protein Data Bank (PDB) [21,22]. The active site was defined as all the amino acid residues enclosed within 6.5 Å radius sphere centered by the bound ligand, CA030. The docking procedure was repeated 100 times for each inhibitor and subsequent the Cscore program of Sybyl was employed to score each conformation. The chosen docked conformation for each inhibitor was selected from the docking results on the basis of their FlexX_score (Total_score).

## Acknowledgments

We thank Dr. Luiz Juliano (Department of Biophysics, Universidade Federal de Sao Paulo, Brazil) for generously giving us substrates Abz-GIVRAK(Dnp) and Abz-FRF(4$NO_2$)A of Cathepsin B and his expert suggestion for our experiment. The present work was supported by the Foundation of Chinese Academy Sciences (West Light Program, KSCX1-09-03-1 and KSCX-SW-11) and the National Natural Science Foundation of China (30572258).

## References

[1] B. Turk, D. Turk, V. Turk, Biochim. Biophys. Acta 1477 (2000) 98–111.
[2] J.S. Mort, D.J. Buttle, Int. J. Biochem. Cell Biol. 29 (1997) 715–720.
[3] G.Z. Zeng, N.H. Tan, R.R. Jia, X.L. Pan, Acta Bot. Yunnan 27 (2005) 337–354.
[4] X.L. Pan, N.H. Tan, G.Z. Zeng, H.J. Han, H.Q. Huang, Bioorg. Med. Chem. 14 (2006) 2771–2778.
[5] M.E. McGrath, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 181–204.
[6] B.E. Linebaugh, M. Sameni, N.A. Day, B.F. Sloane, D. Keppler, Eur. J. Biochem. 264 (1999) 100–109.
[7] L. Polgar, C. Csoma, J. Biol. Chem. 262 (1987) 14448–14453.
[8] B.E. Cathers, C. Barrett, J.T. Palmer, R.M. Rydzewski, Bioorg. Chem. 30 (2002) 264–275.
[9] J.E. Koblonski, M. Ahram, B.F. Sloane, Clin. Chim. Acta 291 (2000) 113–135.
[10] V. Turk, B. Turk, G. Guancar, D. Turk, J. Kos, Advan. Enzyme Regul. 42 (2002) 285–303.
[11] J.-P. Falgueyret, R.M. Oballa, O. Okamoto, G. Wesolowski, Y. Aubin, R.M. Rydzewski, P. Prasit, D. Riendeau, S.B. Rodan, M.D. Percival, J. Med. Chem. 44 (2001) 94–104.
[12] Y.M. Zhang, N.H. Tan, H.Q. Huang, R.R. Jia, G.Z. Zeng, C.J. Ji, Acta Bot. Yunnan 27 (2005) 107–110.
[13] J. Xiong, N.H. Tan, K. Gu, Chinese J. Org. Chem. 25 (Suppl.) (2005) 461.
[14] D.K. Nagler, A.C. Storer, F.C.V. Portaro, E. Carmona, L. Juliano, R. Menard, Biochemistry 36 (1997) 12608–12615.
[15] X.L. Pan, N.H. Tan, G.Z. Zeng, Y.M. Zhang, R.R. Jia, Bioorg. Med. Chem. 13 (2005) 5819–5825.
[16] A.J. Barrett, Biochem. J. 187 (1980) 909–912.
[17] R.L. Melo, L.C. Alves, E.D. Nery, L. Juliano, M.A. Juliano, Anal. Biochem. 293 (2001) 71–77.
[18] S.S. Cotrin, L. Puzer, W.A.S. Judice, L. Juliano, A.K. Carmona, M.A. Juliano, Anal. Biochem. 335 (2004) 244–252.
[19] K. Aibe, H. Yazawa, K. Abe, K. Teramura, M. Kumegama, H. Kawashima, K. Honda, Biol. Pharm. Bull. 19 (1996) 1026–1031.
[20] Tripos Inc, SYBYL Molecular Modeling Software, Sybyl Version 6.9, St. Louis; MO, 2001.
[21] D. Turk, M. Podobnik, T. Popovic, N. Katunuma, W. Bode, R. Huber, V. Turk, Biochemistry 34 (1995) 4791–4797.
[22] A. Yamamoto, K. Tomoo, K.I. Matsugi, T. Hara, I. Yasuko, M. Murata, K. Kitamura, T. Ishida, Biochim. Biophys. Acta 1597 (2002) 244–251.