Solution Phase Synthesis of a Combinatorial Library of Chalcones and Flavones as Potent Cathepsin V Inhibitors

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Cathepsin V is a papain-like cysteine protease. It is involved in the control of human T cells (responsible for cell immunity), and presents the largest elastolytic activity among the proteolytic enzymes. Therefore, cathepsin V is a potential molecular target for the treatment of atherosclerosis. In the present work, natural flavonoids were screened against cathepsin V, and two flavones were identified as potent inhibitors of cathepsin V. On the basis of this result, a combinatorial library of chalcones and flavones was prepared, in solution phase employing a scavenger reagent, and fully evaluated.

Introduction

Coronary arterial disease is one of the major causes of human death. In this context, atherosclerosis, which is characterized by a thickening and loss of elasticity of the arterial wall, is the leading cause of cardiac illness. Cathepsin V is a papain-like cysteine protease, involved in the control of human T cells (responsible for cell immunity), and presents the largest elastolytic activity among proteolytic enzymes.[1,2] It is predominantly expressed in the thymus, testicles, and epithelial corneal. Although cathepsin V can be considered a potential molecular target for the treatment of atherosclerosis, few inhibitors are described in the literature.[2,3]

The benzopyranone-ring system, found in flavonoids, is a molecular scaffold of considerable interest, and some reports describe this class of compounds as inhibitors of cathepsin acitvity. Kenyon and co-workers reported the activity of chalcone derivatives toward the enzyme cathepsin B.[4,5] Patil et al. isolated a new dimeric dihydrochalcone and a prenylated flavone from *Artocarpus altilis* (Moraceae) which are potent inhibitors of cathepsin K, with IC_{50} values of 98 and 840 nM, respectively.[6] Pan et al.[7] and Zeng et al.[8] described biflavones isolated from *Taxodium mucronatum* (Taxodiacea) and *Cycas guizhouensis* (Cycadaceae) with activity against cathepsin B and K. Bauvois et al. examined the effects of synthetic flavones on a number of metalloproteinases, γ-glutamyl transpeptidase as well as serine proteases such as cathepsin G.[9]

Medicinal chemistry efforts have focused on diversifying the benzopyranone scaffold and utilizing combinatorial chemistry approaches to construct small benzopyranone libraries.[10] In the literature are described several flavonoid libraries synthesized in solution phase, such as, for example, chalcones[11–13] and flavones.[14,15]

Solid-phase organic synthesis (SPOS) has long been an integral component of the synthetic repertoire employed by combinatorial chemists.[16] In SPOS, solid support-bound substrates are elaborated synthetically by using an excess of reagents to drive reactions to completion. Desired products can then be isolated easily by simple filtration and removed from the support material. Previous reviews have discussed the importance of combinatorial libraries of flavonoids prepared by solid phase synthesis.[17–19] Nicolaou et al. reported a solid-phase synthesis of a 2,2-dimethylbenzopyran motif employing a cycloloading strategy that relies on the use of a polystyrene-based selenenyl bromide resin.[20] Harikrishnan and Showalter prepared a small library of isoflavone analogues employing a solid-supported diisopropylsilyloxy traceless linker.[21] Cheng et al. prepared a small library of chalcones via parallel solid phase synthesis.[22] A different strategy was employed by Yao et al. for the preparation of a flavone library.[23] A functionalized flavone was prepared in solution phase by classical Claisen condensation, mixed acid nitration, and oxidative cyclization reaction with iodine-dimethyl sulfoxide (DMSO). Then, the flavone was employed as scaffold for the synthesis of a combinatorial library in solid phase. Arai et al. developed an efficient one-pot synthesis by Michael aldol reaction of chromone and flavonoid derivatives bearing heterocyclic units.[24] The 2,3-heterocyclic-substituted chromones were obtained in one step. Moreover, the use of substituted benzaldehydes and subsequent addition of heterocyclic aldehydes gave 3-pyridyl-substituted flavones. They have also examined these one-pot reactions in the solid phase by attaching the acetophenone derivative to (4-methoxyphenyl)-diisopropylsilylpropyl polystyrene beads.

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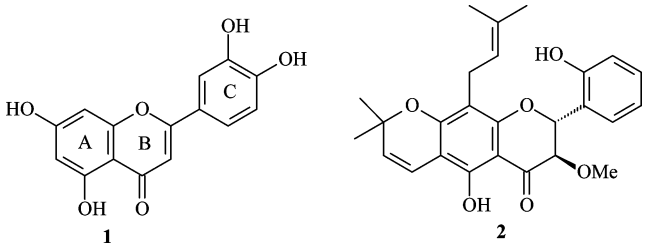


Figure 1. Natural flavonoids evaluated against cathepsin V.

SPOS has many advantages but also many limiting drawbacks: it can be difficult to adapt conventional solution phase chemistry to a solid-phase format, some solid-phase reactions are significantly slower than they are when run in solution, and the progress of solid-phase reactions can be difficult to monitor.[11] An alternative is the use of polymer-supported reagents, catalysts, and cleanup agents (scavengers) which facilitate reactions of substrates in solution. For example, a solid-supported reagent can be used to drive to completion a coupling reaction between two reactive substrates, one of which is present in excess. A support-bound scavenger can then be used to remove the excess reagent, yielding pure product. Or a support-bound capture reagent can be used to remove the coupling product directly, which is then cleaved from the support.[25] Bhat et al. employed a piperazinyl Merrifield resin in the synthesis of a library of flavones.[26] Huang et al. described a solid-phase synthesis of flavones employing a Lewis acid-mediated polystyrene-supported selenium induced intramolecular cyclization of chalcones.[27] After the oxidative cleavage of selenium resin, the flavones were obtained in good overall yields and high purity.

In the present work, we describe the use of tosyl hydrazide scavenger resin in the preparation of a combinatorial library of chalcone and flavone derivatives, identified as potent inhibitors of cathepsin V.

Results and Discussion

In an ongoing program toward the development of specific, potent inhibitors of cathepsin V enzyme, several natural products were screened. The percentage of inhibition was calculated according to the equation:

$$\% \text{ inhibition} = 100 \times (1 - V_i/V_0)$$

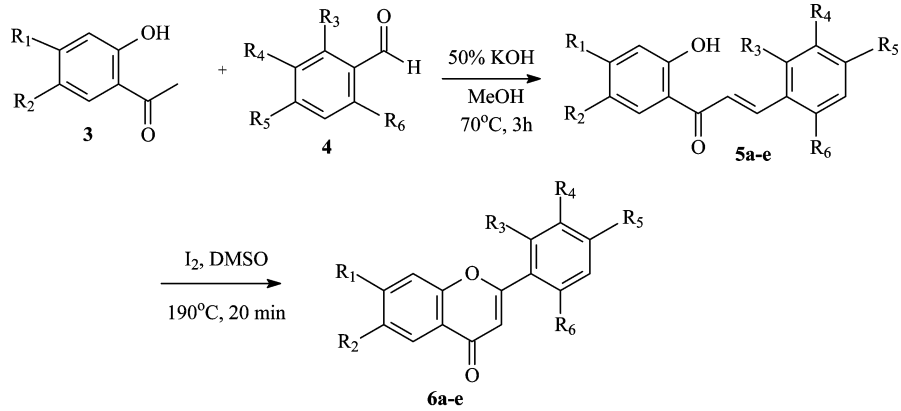
where V_i and V_0 are initial velocities (enzyme activities) determined in the presence and in the absence of inhibitor, respectively.

Among some other active compounds, a series of flavonoids showed interesting activity at initial concentration of 25 μM. The most potent compounds were flavones **1** and **2** (Figure 1), isolated from *Vitex polygama* (Verbenaceae)[28] and *Lonchocarpus subglaucescens* (Leguminoseae),[29] respectively, with $IC_{50} = 2.5$ and 13.5 μM. The IC_{50} values correspond to the concentration of compound required for 50% inhibition of cathepsin V, and were determined from collected data by non-linear regression analysis.

On the basis of these results, a small library of flavonoids was prepared in solution phase employing the Claisen−Schmidt condensation,[30] followed by oxidative cyclization catalyzed by I_2 (Scheme 1).[31] The library components were obtained in moderate to good yields and were characterized by ^{1}H NMR and MS. The compounds then were submitted to biochemical evaluation against cathepsin V enzyme (Table 1).

In this small series we have already identified flavone (**6d**) with a higher inhibition activity than the natural products **1**

Scheme 1. Synthesis of Flavonoid Derivatives



R_1	R_2	R_3	R_4	R_5	R_6	product	yield (%)[a]	
							5	**6**
MeO	H	MeO	H	MeO	H	**a**	32	80
F	H	MeO	H	H	Cl	**b**	50[b]	62
OBn	H	H	MeO	H	H	**c**	71[c]	82
H	Br	MeO	H	MeO	H	**d**	70	75
F	H	F	H	H	Cl	**e**	14	50

[a] After chromatographic purification. [b] Chalcone **5b** was obtained as a byproduct in the synthesis of chalcone **5e**, via a nucleophilic substitution of a fluoride by a methoxy group. [c] Chalcone **5c** was prepared using 50% NaOH in dioxane at 50 °C for 24 h.

Table 1. Inhibitory Activity of a Series of Flavonoids against Cathepsin V

compounds	inhibition (%) at 25 μM	$IC_{50}(\mu M)$[a]
5a	36	>50
5b	14	>50
5c	64	>50
6a	nd[b]	nd
6b	18	>50
6c	nd	nd
6d	100	1.2 ± 0.1
6e	19	>50

[a] The values represent the mean values of three individual experiments ± SD. [b] nd = not determinate because compound showed fluorescence superior of the control.

and **2**. To evaluate a larger number of flavonoids and establish some structure−activity relationships, a flavone library was prepared through parallel synthesis using the polystyrene-tosyl hydrazide (PS-Ts-$NHNH_2$) (**7**) as a carbonyl scavenging resin.[32] Before carrying out the synthesis of the library some tests were realized to optimize the reaction conditions using the resin. The best results were obtained using 0.075 mmol of 2-hydroxy-4-methoxyacetophenone, 0.1 mmol of resin **7**, and 0.1 mmol of 2,4-dimethoxybenzaldehyde, at room temperature for 2 h. The mixture was analyzed by gas chromatography (GC), and the presence of the aldehydes was not observed. Following the reaction conditions previously tested, a library of chalcones and flavones was prepared (Scheme 2) using 4 acetophenones (**3**) (Figure 2) and 6 aldehydes (**4**) as building blocks (Figure 3). The diversity of the library was planned based on the structure of natural products **1** and **2**, which are highly oxygenated, and also on the commercial availability of reagents.

The reactions were carried out in 24 reaction vessels of a Büchi Syncore synthesizer. Initially, for the Claisen−Schmidt condensation, to a solution containing the acetophenone **3** and the aldehyde **4** in methanol was added an aqueous solution of 50% KOH. After 3 h at 75 °C, an aqueous solution of 10% HCl was added to neutralize the mixture. Excess of aldehyde was removed from the reaction mixture by the use of resin scavenger **7** in dimethylformamide (DMF). Oxidative cyclization was carried out in DMSO at 150 °C for 1 h using iodine as catalyst.

Thirteen chalcones **5** were purified by preparative TLC and evaluated against cathepsin V enzyme (Table 2). As observed before, the chalcones showed no activity. The flavones were purified by SPE or preparative TLC. Analyses

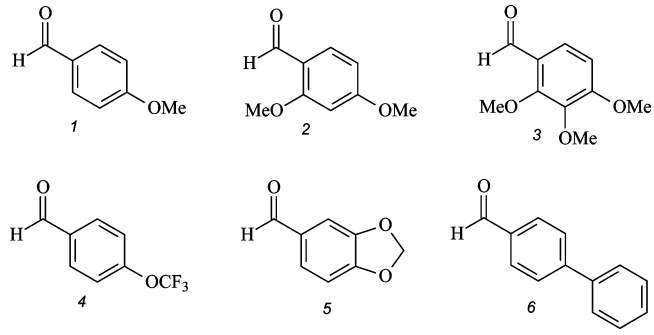


Figure 3. Aldehydes **4** employed in the synthesis of the combinatorial library.

Table 2. Inhibitory Activity of a Series of Chalcones **5** against Cathepsin V

chalcone	R_1	R_2	R_3	R_4	R_5	R_6	inhibition (%)[a]
5{*1,1*}	BnO	H	H	H	H	MeO	21
5{*1,3*}	BnO	H	H	MeO	MeO	MeO	35
5{*1,4*}	BnO	H	H	H	H	CF_3O	22
5{*2,1*}	MeO	H	MeO	H	H	MeO	24
5{*2,2*}	MeO	H	MeO	MeO	H	MeO	22
5{*2,3*}	MeO	H	MeO	MeO	MeO	MeO	1
5{*2,6*}	MeO	H	MeO	H	H	C_6H_5	57
5{*3,1*}	H	F	H	H	H	MeO	53
5{*3,4*}	H	F	H	H	H	CF_3O	26
5{*3,6*}	H	F	H	H	H	C_6H_5	48
5{*4,6*}	H	MeO	H	H	H	C_6H_5	7

[a] All compounds were tested at 25 μM and showed IC_{50} > 50 μM.

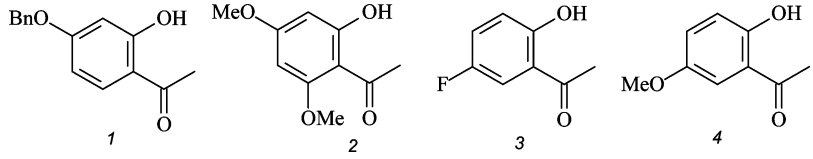


Figure 2. Acetophenones **3** employed in the synthesis of the combinatorial library.

Scheme 2. Synthetic Route for Preparation the Combinatorial Library

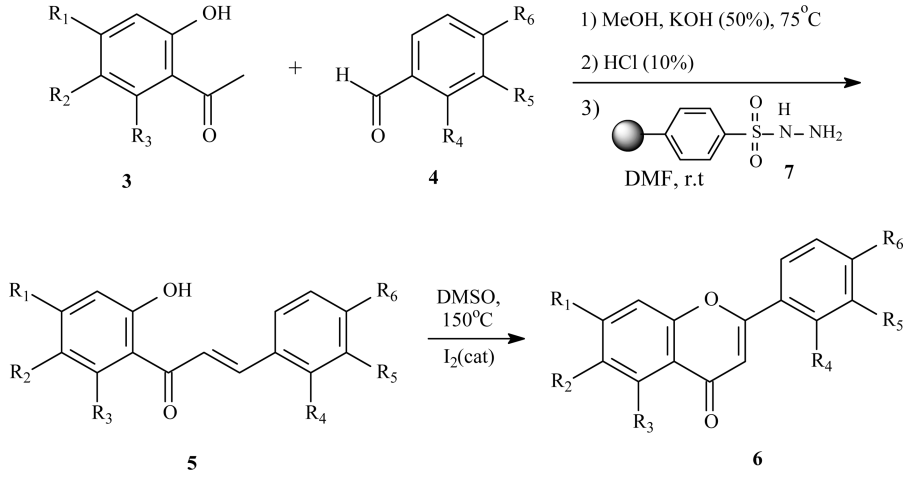


Table 3. Synthesis and Evaluation of the Flavone Combinatorial Library

flavones	R_1	R_2	R_3	R_4	R_5	R_6	yield (%)	purity (%)	inhibition (%) at 25 μM	IC_{50}(μM)[a]
6{*1,1*}	BnO	H	H	H	H	MeO	68	>95[c]	100	nd[d]
6{*1,2*}	BnO	H	H	MeO	H	MeO	46	>95	nd	nd
6{*1,3*}	BnO	H	H	MeO	MeO	MeO	38	>95	52	nd
6{*1,4*}	BnO	H	H	H	H	CF_3O	60	>95	56	2.7 ± 0.3
6{*1,5*}	BnO	H	H	H	O−CH_2-O		42	>95	100	1.5 ± 0.2
6{*1,6*}	BnO	H	H	H	H	C_6H_5	29	>80	85	11.1 ± 1.0
6{*2,1*}	MeO	H	MeO	H	H	MeO	30	>95	100	nd
6{*2,2*}	MeO	H	MeO	MeO	H	MeO	13	>95	56	19.0 ± 1.8
6{*2,3*}	MeO	H	MeO	MeO	MeO	MeO	25	>95	6	>50
6{*2,4*}	MeO	H	MeO	H	H	CF_3O	68	>95	nd	nd
6{*2,5*}	MeO	H	MeO	H	O−CH_2-O		35	>95	51	25.1 ± 2.2
6{*2,6*}	MeO	H	MeO	H	H	C_6H_5	50	>90	26	>50
6{*3,1*}	H	F	H	H	H	MeO	52	>95	100	0.8 ± 0.05
6{*3,2*}	H	F	H	MeO	H	MeO	68	>90	100	1.6 ± 0.2
6{*3,3*}	H	F	H	MeO	MeO	MeO	25	>95	84	4.1 ± 0.4
6{*3,4*}	H	F	H	H	H	CF_3O	*b*			
6{*3,5*}	H	F	H	H	O−CH_2-O		*b*			
6{*3,6*}	H	F	H	H	H	C_6H_5	66	>95	74	2.9 ± 0.3
6{*4,1*}	H	MeO	H	H	H	MeO	43	>95	100	12.0 ± 0.9
6{*4,2*}	H	MeO	H	MeO	H	MeO	24	>95	30	>50
6{*4,3*}	H	MeO	H	MeO	MeO	MeO	24	>95	35	>50
6{*4,4*}	H	MeO	H	H	H	CF_3O	31	>90	nd	nd
6{*4,5*}	H	MeO	H	H	O−CH_2-O		*b*			
6{*4,6*}	H	MeO	H	H	H	C_6H_5	33	>90	65	19.2 ± 1.8

[a] The values represent means of at three individual experiments ± SD. [b] Product not obtained. [c] Analysis by HPLC at three different wavelengths (254, 280, and 320 nm). [d] nd = not determinate because the solution showed fluorescence superior of the control.

by HPLC in three different wavelengths (254, 280, and 320 nm) showed that 21 flavones **6** were prepared in low to moderate yield (13−68%) and good to high purity (80−95%). All compounds were characterized by ^{1}H and ^{13}C NMR, and the spectroscopic data were compared with those reported in the literature.[33−57] The library components were submitted to biochemical evaluation (Table 3).

The first aspect of the validation of these results was the analysis of false positives. Depending on the assay design and detection method used for the screening assay, various artifacts can occur that lead to wrong results. Before performing the screening, all compounds were analyzed on a fluorimeter using the test conditions without the addition of the enzyme. Flavones **6a**, **6c**, **6**{*1,2*}, **6**{*2,4*}, and **6**{*4,4*} showed intrinsic fluorescence; thus, it was not possible to verify their potency as inhibitors. Compounds that inhibited more than 50% were selected for determination of IC_{50} values. When diluted, flavones **6**{*1,1*}, **6**{*1,3*}, and **6**{*2,1*} showed fluorescence above the control, suggesting that at lower concentrations these compounds may cause a conformational change of the protein, and it was not possible to observe the fluorescence quenching, thus interfering with the response of the test.

Most of the flavones showed significant inhibition with IC_{50} values varying in the range of 0.8−25.1 μM. Primary studies on the structure−activity relationships (SAR) of the flavonoids suggest the importance of the ring C. Interestingly the chalcones exhibited lower inhibition in a unique concentration demonstrating that cyclization to form the C ring is relevant to interaction between enzyme site and inhibitor. We also observed that among the most potent compounds, flavones **6d**, **6**{*3,1*}, and **6**{*3,2*} (IC_{50} = 1.2, 0.8, and 1.6 μM, respectively) possess one halogen (bromine or fluorine) as substituent in the A ring and methoxy groups in the C ring.

In conclusion, despite the lack of a detailed experimental enzyme−inhibitor complex, from the natural products screening, we have been able to identify synthetic flavones with increased potency. These results indicate the potential of this class as promising inhibitors of cathepsin V. To further investigate these results and thus generate useful data for the computer aided molecular design, modeling studies are being performed through docking protocol.

3. Experimental Section

All commercially available reagents were purchased from Aldrich Chemical Co. Reagents and solvents were purified when necessary according to the usual procedures described in the literature. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-200 (200 and 50 MHz respectively). The IR spectra refer to films and were measured on a Bomem M102 spectrometer. Mass Spectra were recorded on a Shimadzu GCMS-QP5000. Analytical thin-layer chromatography was performed on a 0.25 μm film of silica gel containing fluorescent indicator UV254 supported on an aluminum sheet (Sigma-Aldrich). Flash column chromatography was performed using silica gel (Kieselgel 60, 230−400 mesh, E. Merck). Gas chromatography was performed in a Shimadzu GC-17A with H_2 as carrier and using a DB-5 column. Solid-phase extraction (SPE) was carried out using silica cartridges (Phenomenex Strata Silica SL-1, 500 mg, 6 mL). HPLC analyses were performed in Shimadzu equipment with a LC-20AT pump, SIL-10A injector, SPD-20A detector, and in a Phenomenex column Luna C18(2) 5 μm (150 × 4.6 mm). The combinatorial library was prepared in a Syncore Büchi synthesizer.

General Procedure for the Synthesis of Chalcones 5a−e. To a solution of acetophenone **3** and aldehyde **4** in methanol was added an aqueous solution of KOH 50% (Table 5). The mixture was stirred at 70 °C for 3 h, and

Table 4. Preparation of Chalcones **5a**−**e**

acetophenone (mg/mmol)	aldehyde (mg/mmol)	KOH 50% (mL)	MeOH (mL)	product (mg)	yield (%)
100/0.60	150/0.90	0.90	4.2	**5a** (60)	32
100/0.65	133/0.84	0.84	4.5	**5b** (99)	50
				5e (27)	14
107/0.44	63/0.46	10[a]	10[b]	**5c** (113)	71
70/0.32	70/0.42	0.42	2.1	**5d** (81)	70

[a] 50% NaOH was used as base. [b] 1,4-Dioxane was employed as solvent.

Table 5. Preparation of Flavones **6a**−**e**

chalcone (mg/mmol)	I_2(mg)	DMSO (mL)	product (mg)	yield (%)
5a (30/0.09)	1.0	1.3	**6a** (24)	80
5b (15/0.05)	1.0	0.7	**6b** (9)	62
5c (50/0.14)	1.0	1.7	**6c** (41)	82
5d (60/0.16)	2.8	2.0	**6d** (43)	75
5e (22/0.08)	1.0	1.0	**6e** (11)	50

then an aqueous solution of HCl 10% was added. The organic layer was extracted with ethyl acetate, dried with Na_2SO_4, and concentrated under vacuum. The crude product was purified by flash column chromatography in silica gel or by recrystallization.

2′-Hydroxy-2,4,4′-trimethoxychalcone (5a).[33] mp: 153−155 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.84 (s, 3H); 3.85 (s, 3H); 3.91 (m, 3H); 6.42−6.50 (m, 3H); 6.53 (dd, J = 8.5, 2.3 Hz, 1H); 7.56 (d, J = 8.5 Hz, 1H); 7.60 (d, J = 15.5 Hz, 1H); 7.75−7.89 (m, 1H); 8.12 (d, J = 15.5 Hz, 1H); 13.69 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.7; 98.5; 101.0; 105.4; 107.4; 114.4; 117.1; 118.4; 131.1; 140.2; 160.5; 163.2; 165.8; 166.5; 192.5. MS *m*/*z*: 314 (M^+), 299, 283, 257, 240, 211, 191, 177, 164, 151 (100), 149, 121, 108, 91, 77, 51. IR (ν_{max}, KBr): 2935, 1627, 1608, 1558, 1506, 1216, 1153, 1020, 835 cm^{-1}.

2-Chloro-4′-fluoro-2′-hydroxy-6-methoxychalcone (5b). mp: 133−135 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.98 (s, 3H); 6.57−6.78 (m, 2H); 6.90 (d, J = 8.1 Hz, 1H); 7.10 (dd, J = 8.1, 1.2 Hz, 1H); 7.28 (t, J = 8.1, 1H); 7.88 (dd, J = 8.8, 6.4 Hz, 1H); 8.03 (d, J = 15.8 Hz, 1H); 8.26 (d, J = 15.8 Hz, 1H); 13.26 (d, J = 1.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 56.0; 105.0 (d, J = 23.4 Hz); 107.0 (d, J = 22.5 Hz); 109.7; 117,2 (d, J = 2.0 Hz); 121.8; 122.6; 125.4; 131.1; 132.1 (d, J = 11.5 Hz); 137.1; 137.8; 160.2; 166.1; 167.3 (d, J = 254.8 Hz); 193.6. MS *m*/*z*: 308 (M^+ +2), 306 (M^+), 271, 256, 199, 165, 139, 125, 111, 103, 89, 83 (100), 63, 57. IR (ν_{max}, KBr): 1635, 1598, 1560, 1469, 1351, 1268, 1205, 1043, 836 cm^{-1}.

4′-Benzyloxy-2′-hydroxy-3-methoxychalcone (5c).[34] mp 131−132 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.86 (s, 3H); 5.11 (s, 2H); 6.52−6.62 (m, 2H); 6.97 (ddd, J = 8.0, 2.5, 1.2 Hz, 1H); 7.13−7.19 (m, 1H); 7.20−7.28 (m, 1H); 7.29−7.36 (m, 1H); 7.37−7.48 (m, 5H); 7.55 (d, J = 15.5 Hz, 1H); 7.85 (d, J =15 Hz, 1H); 7.78−7.93 (m, 1H); 13.39 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.4; 70.2; 102.2; 108.2; 113.6; 114.3; 116.4; 120.7; 121.2; 127.5; 128.3; 128.7; 130.0; 131.3; 135.9; 136.2; 144.4; 160.0; 165.3; 166.6; 191.8. MS *m*/*z*: 360 (M^+), 235, 134, 91 (100), 51. IR (ν_{max}, KBr): 2923, 1639, 1619, 1573, 1251, 1132, 1037, 850, 736 cm^{-1}.

5′-Bromo-2′-hydroxy-2,4-dimethoxychalcone (5d). mp 116−118 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.87 (s, 3H); 3.93 (s, 3H); 6.48 (d, J = 2.4 Hz, 1H); 6.56 (dd, J = 8.8, 2.4 Hz, 1H); 6.91 (d, J = 8.8 Hz, 1H); 7.53 (dd, J = 8.8, 2.4 Hz, 1H); 7.57 (d, J = 15.4 Hz, 1H); 7.61 (d, J = 8.8 Hz, 1H); 7.99 (d, J = 2.4 Hz, 1H); 8.19 (d, J = 15.4 Hz, 1H); 13.0 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.7; 98.5; 105.8; 110.2; 116.7; 117.3; 120.5; 121.6; 131.6; 131.8; 138.3; 142.3; 160.9; 162.4; 163.8; 193.2. MS *m*/*z*: 364 (M^+ + 1), 362 (M^+ − 1), 333, 331, 225, 201, 199, 164, 149 (100), 138, 121, 105, 91, 77, 63, 51. IR (ν_{max}, KBr): 2925, 1850, 1751, 1616, 1558, 1506, 1419, 1267, 1162, 1027, 819 cm^{-1}.

2-Chloro-4′,6-difluoro-2′-hydroxychalcone (5e). mp 126−128 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.55−6.82 (m, 2H); 7.01−7.18 (m, 1H); 7.20−7.42 (m, 2H); 7.75−7.97 (m, 2H); 8.12 (d, J = 15.8 Hz, 1H); 13.08 (d, J = 1.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 105.1 (d, J = 23.4 Hz); 107.3 (d, J = 22.6 Hz); 114.9 (d, J = 23.4 Hz); 117.0 (d, J = 2.0 Hz); 121.8 (d, J = 13.6 Hz); 126.2 (d, J = 3.7 Hz); 126.6 (d, J = 14.4 Hz); 131.2 (d, J = 10.6 Hz); 132.2 (d, J = 11.9 Hz); 135.3 (d, J = 2.0 Hz); 136.8 (d, J = 5.3 Hz); 162.2 (d, J = 254.8 Hz); 165.6 (d, J = 54.0 Hz); 168.2 (d, J = 187.2 Hz); 192.7. MS *m*/*z*: 296 (M^++2), 294 (M^+), 277, 259, 201, 183, 165, 139, 120, 110, 99, 83, 57 (100). IR (ν_{max}, KBr): 1652, 1602, 1506, 1457, 1419, 1353, 1270, 1214, 921, 850, 775 cm^{-1}.

General Procedure for the Synthesis of Flavones 6a−e. To a solution of chalcone **5a**−**e** in DMSO was added iodine (Table 4). The mixture was stirred for 20 min at 190 °C, and then water was added. The organic layer was extracted with ethyl acetate, washed with an aqueous solution of 0.1 M sodium thiosulfate and brine, then dried with Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash column chromatography in silica gel or by recrystallization.

2′,4′,7-Trimethoxyflavone (6a).[35] mp 133−134 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (s, 3H); 3.91 (s, 6H); 6.55 (d, J = 2.3 Hz, 1H); 6.62 (dd, J = 8.7, 2.3 Hz, 1H); 6.90 (dd, J = 2.4, 0.4 Hz, 1H); 6.95 (dd, J = 8.7, 2.4 Hz, 1H); 7.06 (s, 1H); 7.86 (d, J = 8.7 Hz, 1H); 8.12 (dd, J = 8.7, 0.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.6; 55.7; 98.9; 100.3; 105.2; 111.2; 113.7; 113.9; 117.7; 126.9; 130.3; 158.1; 159.5; 160.4; 163.1; 163.9; 178.4. MS *m*/*z*: 312 (M^+), 162, 151 (100), 142, 119, 91, 65. IR (ν_{max}, KBr): 2923, 1623, 1440, 1263, 1162, 1027, 833 cm^{-1}.

2′-Chloro-7′-fluoro-6′-methoxyflavone (6b). mp 167−169 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.82 (s, 3H); 6.40 (s, 1H); 6.93 (dd, J = 8.4, 0.8 Hz, 1H); 7.07−7.22 (m, 3H);

7.41 (t, J = 8.4, 1H); 8.22−8.34 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.2; 104.9 (d, J = 25.1 Hz); 109.6; 113.8 (d, J = 22.6 Hz); 115.0; 120.9 (d, J = 1.8 Hz); 121.5; 121.9; 128.2 (d, J = 10.5 Hz); 132.1; 134.6; 158.0 (d, J = 13.4 Hz); 158.5; 160.4; 165.6 (d, J = 253.0 Hz); 177.2. MS *m/z*: 306 (M^+ +2), 304 (M^+), 269, 254, 165, 165, 139 (100), 110, 82. IR (ν_{max}, KBr): 2925, 2836, 1623, 1562, 1506, 1475, 1353, 1299, 1268, 1143, 1052, 1027, 896, 846, 792 cm^{-1}.

7-Benzyloxy-3′-methoxyflavone (6c).[34] mp 155−157 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.85 (s, 3H); 5.18 (s, 2H); 6.74 (s, 1H); 7.02−7.06 (m, 2H); 7.06−7.11 (m, 1H); 7.34−7.53 (m, 8H); 8.13 (ddd, J = 8.8, 1.1, 1.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 70.5; 101.5; 107.8; 111.7; 114.9; 117.0; 118.1; 118.6; 127.1; 127.5; 128.4; 128.8; 130.1; 133.2; 135.7; 157.9; 160.0; 162.8; 163.3; 177.8. MS *m/z*: 358 (M^+), 139, 102, 91 (100), 63. IR (ν_{max}, KBr): 2921, 1639, 1602, 1440, 1238, 1033, 854, 696 cm^{-1}.

6-Bromo-2′,4′-dimethoxyflavone (6d).[36] mp 185−188 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.92 (s, 3H); 6.54 (d, J = 2.4 Hz, 1H); 6.62 (dd, J = 8.8, 2.4 Hz, 1H); 7.15 (s, 1H); 7.39 (dd, J = 8.8, 0.4 Hz, 1H); 7.72 (dd, J = 8.8, 2.4 Hz, 1H); 7.86 (d, J = 8.8 Hz, 1H); 8.23 (dd, J = 2.4, 0.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.7; 98.9; 105.4; 111.2; 113.1; 118.1; 119.8; 125.1; 128.2; 130.4; 136.2; 155.1; 159.8; 161.0; 163.5; 177.4. MS *m/z*: 362 (M^+ +1), 360 (M^+ −1), 201, 199, 162, 147, 119, 91, 63 (100). IR (ν_{max}, KBr): 1623, 1562, 1475, 1353, 1299, 1268, 1143, 1052, 846, 792 cm^{-1}.

2′-Chloro-6′,7-difluoroflavone (6e). mp 167−168 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.50 (s, 1H); 7.00−7.25 (m, 3H); 7.29−7.55 (m, 2H); 8.20−8.35 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 104.9 (d, J = 25.2 Hz); 114.5 (d, J = 46.2 Hz); 114.9 (d, J = 42.6 Hz); 115.0 (d, J = 42.6 Hz); 120.7 (d, J = 2.8 Hz); 120,9 (d, J = 13.5 Hz); 122.2; 125.9 (d, J = 3.5 Hz); 128.3 (d, J = 10.6 Hz); 132.5 (d, J = 9.4 Hz); 134.5 (d, J = 3.0 Hz); 157.5 (d, J = 9.4 Hz); 160.4 (d, J = 253.3 Hz); 165.8 (d, J = 253.9 Hz); 176.8. MS *m/z*: 294 (M^+ +2), 292 (M^+), 266, 264, 201, 181, 154, 138, 110 (100), 100, 82, 63, 57. IR (ν_{max}, KBr): 2925, 2854, 1660, 1625, 1446, 1371, 1257, 1147, 1033, 854, 788 cm^{-1}.

Synthesis of the Combinatorial Library of Flavones 6. In 24 reaction vessels of a Büchi Syncore synthesizer was placed a solution of acetophenone **3**{*1-4*} (0.075 mmol) and aldehyde **4**{*1-6*} (0.10 mmol) (Figure 2) in methanol (2.0 mL). Then it was added an aqueous solution of KOH 50% (150 μL). The mixture was stirred at 75 °C for 3 h, and then an aqueous solution of HCl 10% (750 μL) was added. The solvent was evaporated, then ethyl acetate (3 mL) was added and the organic layer was transferred to another vessel. This procedure was repeated three times and then concentrated under vacuum. Then DMF (1 mL) was added followed by PS-Ts-$NHNH_2$ (**7**) (50 mg, 0.1 mmol). The mixture was stirred for 2 h at room temperature, then it was washed with methylene chloride (3 mL), and the organic layer was transferred to another vessel. This procedure was repeated three times; then the solvent was evaporated furnishing chalcones **5**. One third was separated for further characterization and bioassays.

To the previously prepared chalcones **5** was added a 1 mg/mL solution of iodine in DMSO (1 mL). The mixture was stirred for 1 h at 150 °C, and then an aqueous solution of 0.1 M sodium thiosulfate (0.1 mL) was added. The mixture was concentrated under vacuum, and the crude product was purified by SPE using silica gel (500 mg) and methylene chloride: ethyl acetate (8:2) or preparative TLC using hexane: ethyl acetate (8:2) as eluent. The flavones **6** were analyzed by HPLC (see Table 3), characterized by ^{1}H and ^{13}C NMR, and evaluated against cathepsin V enzyme.

4′-Benzyloxy-2′-hydroxy-4-methoxychalcone (5{*1,1*}).[37] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.86 (s, 3H); 5.11 (s, 2H); 6.47−6.62 (m, 2H); 6.94 (d, J = 8.6 Hz, 2H); 7.30−7.53 (m, 6H); 7.61 (d, J = 8.6 Hz, 2H); 7.82 (d, J = 2.8 Hz, 1H); 7.88 (d, J = 8.7 Hz, 1H); 13.52 (s, 1H).

4′-Benzyloxi-2′-hydroxy-2,4,6-trimethoxychalcone (5{*1,3*}).[38] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.90 (s, 3H); 3.92 (s, 3H); 3.97 (s, 3H); 5,11 (s, 2H); 6.50−6.62 (m, 2H); 6.73 (d, J = 8.9 Hz, 1H); 7.31−7.52 (m, 6H); 7.63 (d, J = 15.6 Hz, 1H); 7.78−7.90 (m, 1H); 8.05 (d, J = 15.6 Hz, 1H); 13.56 (s, 1H).

4′-Benzyloxy-2′-hydroxy-4-trifluoromethoxychalcone (5{*1,4*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.12 (s, 2H); 6.52−6.64 (s, 2H); 7.25−7.32 (m, 1H); 7.35−7.47 (m, 5H); 7.54 (J = 15.5 Hz, 1H); 7.63−7.75 (m, 2H); 7.84 (d, J = 9.5 Hz, 1H); 7.86 (J = 15.5 Hz, 1H); 13.32 (s, 1H).

2′-Hydroxy-4,4′,6′-trimethoxychalcone (5{*2,1*}):[39] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.84 (s, 3H); 3.85 (s, 3H); 3.92 (s, 3H); 5.97 (d, J = 2.4, 1H); 6.11 (d, J = 2.4 Hz, 1H); 6.87−7.02 (m, 2H); 7.51−7.63 (m, 2H); 7.80 (s, 2H); 14.40 (s, 1H).

2′-Hydroxy-2,4,4′,6′-tetramethoxychalcone (5{*2,2*}).[40] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.83 (s, 3H); 3.86 (s, 3H); 3.90 (s, 3H); 3.91 (s, 3H); 5.96 (dd, J = 2.4, 0.2 Hz, 1H); 6.10 (d, J = 2.4 Hz, 1H); 6.47 (dd, J = 2.4, 0.3 Hz, 1H); 6.53 (ddd, J = 8.5, 2.3, 0.3 Hz, 1H); 7.55 (d, J = 8.5 Hz, 1H); 7.90 (d, J = 15.6 Hz, 1H); 8.11 (d, J = 15.6 Hz, 1H); 14.54 (s, 1H).

2′-Hydroxy-2,3,4,4′,6′-pentamethoxychalcone (5{*2,3*}).[41] ^{1}H NMR(200 MHz, $CDCl_3$) δ: 3.84 (s, 3H); 3.89 (s, 3H); 3.91 (s, 3H); 3.95 (s, 3H); 5.96 (d, J = 2.4, 1H); 6.11 (d, J = 2.4 Hz, 1H); 6.72 (d, J = 8.8 Hz, 1H); 7.35 (d, J = 8.8 Hz, 1H); 7.90 (d, J = 15.8 Hz, 1H); 8.02 (d, J = 15.8 Hz, 1H); 14.44 (s, 1H).

4-Phenyl-2′-hydroxy-4′,6′-dimethoxychalcone (5{*2,6*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.85 (s, 3H); 3.94 (s, 3H); 5.98 (d, J = 2.3 Hz, 1H); 6.12 (d, J = 2.3 Hz, 1H); 7.35−7.55 (m, 3H); 7.57−7.75 (m, 6H); 15.65 (d, J = 15.7 Hz, 1H); 7.96 (d, J = 15.4 Hz, 1H); 14.32 (s, 1H).

5′-Fluoro-2′-hydroxy-4-methoxychalcone (5{*3,1*}).[42] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (s, 3H); 6.91−7.06 (m, 3H); 7.17−7.32 (m, 1H); 7.42 (d, J = 15.4 Hz, 1H); 7.52−7.2 (m, 3H); 7.93 (d, J = 15.4 Hz, 1H); 12.65 (s, 1H).

5′-Fluoro-2′-hydroxy-2,4-dimethoxychalcone (5{*3,2*}). mp 106−107 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.87 (s, 3H); 3.93 (s, 3H); 6.48 (d, J = 2.2 Hz, 1H); 6.55 (dd, J = 8.5, 2.2 Hz, 1H); 6.97 (dd, J = 9.0, 4.8 Hz, 1H); 7.14−7.32 (m, 1H); 7.52−7.67 (m, 3H); 8.18 (d, J = 15.6 Hz, 1H); 12.81 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.6;

98.5; 105.7; 114.5 (d, $J = 23.1$ Hz); 116.7; 117.5; 119.6 (d, $J = 7.0$ Hz); 119.9 (d, $J = 6.2$ Hz); 123.2 (d, $J = 23.5$ Hz); 131.8; 142.2; 154.8 (d, $J = 237.5$ Hz); 159.6 (d, $J = 1.6$ Hz); 160.9; 163.7; 193 (d, $J = 2.8$ Hz). MS *m/z*: 302 (M^+), 287, 271, 245, 199, 164, 149 (100), 147, 133, 121, 110, 83, 77, 57, 51. IR (ν_{max}, KBr): 2952, 2917, 2848, 1635, 1558, 1506, 1471, 1457, 1290, 1180, 1024, 823, 808 cm^{-1}.

5′-Fluoro-2′-hydroxy-2,3,4-trimethoxychalcone (5{*3,3*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.90 (s, 3H); 3.93 (s, 3H); 3.99 (s, 3H); 6.74 (d, $J = 8.8$ Hz, 1H); 6.99 (dd, $J = 9.1$, 4.6 Hz, 1H); 7.14−7.33 (m, 1H); 7.40 (d, $J = 8.8$, 1H); 7.57 (dd, $J = 9.1$, 3.1 Hz, 1H); 7.60 (d, $J = 15.6$ Hz, 1H); 8.12 (d, $J = 15.6$ Hz, 1H); 12.69 (s, 1H).

5′-Fluoro-2′-hydroxy-3,4-methylenedioxychalcone (5{*3,5*}).[43] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.06 (s, 2H); 6.82−6.92 (m, 1H); 6.99 (dd $J = 9.1$, 4.7 Hz, 1H); 7.12−7.23 (m, 3H); 7.37 (d, $J = 15.3$, 1H); 7.56 (dd, $J = 9.1$, 3.0 Hz, 1H); 7.87 (d, $J = 15.3$ Hz, 1H); 12.60 (s, 1H).

4-Phenyl-5′-fluoro-2′-hydroxychalcone (5{*3,6*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.01 (dd, $J = 9.1$, 4.7 Hz, 1H); 7.30−7.50 (m, 4H); 7.51−7.83 (m, 8H); 8.00 (d, $J = 15.5$ Hz, 1H); 12.56 (s, 1H).

4-Phenyl-2′-hydroxy-5′-methoxychalcone (5{*4,6*}).[44] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.86 (s, 3H); 6.99 (d, $J = 9.1$ Hz, 1H); 7.16 (dd, $J = 9.1$, 3.0 Hz, 1H); 7.32−7.55 (m, 5H); 7.56−7.87 (m, 6H); 7.98 (d, $J = 15.5$ Hz, 1H); 12.40 (s, 1H).

7-Benzyloxy-4′-methoxyflavone (6{*1,1*}).[45] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (s, 3H); 5.18 (s, 2H); 6.69 (s, 1H); 6.96−7.10 (m, 4H); 7.32−7.55 (m, 5H); 7.77−7.92 (m, 2H); 8.10−8.18 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 70.5; 101.5; 106.1; 114.4; 114.7; 117.9; 124.1; 127.1; 127.4; 127.8; 128.3; 128.7; 135.8; 157.8; 162.3; 163.1; 163.2; 177.8.

7-Benzyloxy-2′,4′-dimethoxyflavone (6{*1,2*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.92 (s, 3H); 5.17 (s, 2H); 6.56 (d, $J = 2.3$ Hz, 1H); 6.62 (dd, $J = 8.7$, 2.3 Hz, 1H); 6.99 (d, $J = 2.3$ Hz, 1H); 7.04 (dd, $J = 8.7$, 2.3 Hz, 1H); 7.05 (s, 1H); 7.33−7.54 (m, 5H); 7.84 (d, $J = 8.7$ Hz, 1H); 8.14 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.6; 55.7; 70.5; 99.9; 101.4; 105.3; 111.3; 113.8; 114.5; 117.9; 127.1; 127.5; 128.4; 128.8; 130.3; 135.9; 158.0; 159.6; 161.6; 163.0; 163.1, 178.4.

7-Benzyloxy-2′,4′,6′-trimethoxyflavone (6{*1,3*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.91 (s, 3H); 3.94 (s, 6H); 5.18 (s, 2H); 6.79 (d, $J = 8.9$ Hz, 1H); 6.91 (s, 1H); 6.98 (d, $J = 2.4$ Hz, 1H); 7.06 (dd, $J = 8.9$, 2.4 Hz, 1H); 7.33−7.46 (m, 5H); 7.51 (d, $J = 8.9$ Hz, 1H); 8.15 (d, $J = 8.9$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 56.1; 61.0; 61.2; 70.5; 101.5; 107.4; 111.1; 114.6; 118.0; 119.2; 124.0; 127.2; 127.5; 128.4; 128.8; 135.8; 142.9; 153.0; 156.1; 158.0; 161.1, 163.1, 178.1.

7-Benzyloxy-4′-trifluoromethoxyflavone (6{*1,4*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.20 (s, 2H); 6.74 (s, 1H); 7.01−7.13 (m, 2H); 7.31−7.55 (m, 7H); 7.87−8.01 (m, 2H); 8.14 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 70.7; 101.6; 108.0; 115.1; 118.0; 120.4 (d, $J = 258.6$ Hz); 121.1 (q, $J = 1.0$ Hz); 127.3; 127.5; 127.9; 128.5; 130.4; 135.7; 151.4 (q, $J = 2.0$ Hz); 157.9; 161.7; 163.4, 177.6.

7-Benzyloxy-4′-piperonylflavone (6{*1,5*}).[46] 1H NMR (200 MHz, $CDCl_3$) δ: 5.18 (s, 2H); 6.07 (s, 2H); 6.64 (s, 1H); 6.92 (dd, $J = 8.2$, 0.3 Hz, 1H); 7.01 (d, $J = 2.4$ Hz, 1H); 7.05 (dd, $J = 8.7$, 2.4 Hz, 1H); 7.33 (dd, $J = 1.8$, 0.3 Hz, 1H); 7.35−7.47 (m, 5H); 7.49 (d, $J = 1.8$ Hz, 1H); 8.13 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 70.5; 101.5; 101.9; 106.2; 106.6; 108.7; 114.8; 118.0; 121.3; 125.8; 127.1; 127.5; 128.4; 128.7; 135.8; 148.4; 150.5; 157.8; 162.2; 163.2; 177.7.

7-Benzyloxy-4′-phenylflavone (6{*1,6*}).[47] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.20 (s, 2H); 6.82 (s, 1H); 7.02−7.13 (m, 2H); 7.34−7.55 (m, 8H); 7.60−7.69 (m, 2H); 7.70−7.79 (m, 2H); 7.93−8.04 (m, 2H); 8.15−8.21 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 70.6; 101.5; 107.4; 114.9; 118.0; 126.7; 127.1; 127.2; 127.5; 127.6; 128.1; 128.4; 128.8; 129.0; 130.5; 135.7; 139.8; 144.3; 157.9; 162.9; 163.3; 177.9.

4′,5,7-Trimethoxyflavone (6{*2,1*}).[50] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (s, 3H); 3.91 (s, 3H); 3.95 (s, 3H); 6.37 (d, $J = 2.3$, 1H); 6.56 (d, $J = 2.3$ Hz, 1H); 6.59 (s, 1H); 6.95−7.05 (m, 2H); 7.77−7.87 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.7; 56.4; 92.9; 96.2; 107.8; 109.3; 114.5; 124.0; 127.7; 159.9; 160.7; 161.0; 162.1; 164.4; 177.6.

5,7-Dimethoxy-2′,4′-dimethoxyflavone (6{*2,2*}).[49] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88 (s, 3H); 3.89 (s, 3H); 3.91 (s, 3H); 3.95 (s, 3H); 6.36 (d, $J = 2.2$, 1H); 6.52 (d, $J = 2.2$ Hz, 1H); 6.54 (d, $J = 2.3$ Hz, 1H); 6.61 (dd, $J = 8.7$ and 2.3 Hz, 1H); 6.98 (s, 1H); 7.84 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.3; 55.6; 55.7; 56.4; 92.7; 95.8; 98.8; 105.1; 112.8; 113.4; 114.3; 130.0; 158.1; 159.5; 160.0; 160.8; 162.9; 163.7; 178.2.

2′,3′,4′,5,7-Pentamethoxyflavone (6{*2,3*}).[50] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.90 (s, 3H); 3.93 (s, 6H); 3.96 (s, 3H); 6.36 (d, $J = 2.3$ Hz, 1H); 6.51 (d, $J = 2.3$ Hz, 1H); 6.77 (d, $J = 8.9$ Hz, 1H); 6.82 (s, 1H); 7.49 (d, $J = 8.9$ Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.7; 56.1; 56.4; 61.0; 61.1; 92.8; 95.9; 107.3; 109.3; 112.7; 118.9; 123.8; 142.8; 152.9; 155.9; 158.7; 160.0; 161.0; 163.9; 177.9.

5,7-Dimethoxy-4′-trifluoromethoxyflavone (6{*2,4*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.92 (s, 3H); 3.96 (s, 3H); 6.39 (d, $J = 2.2$ Hz, 1H); 6.56 (d, $J = 2.2$ Hz, 1H); 6.69 (s, 1H); 7.28−7.42 (m, 2H); 7.85−8.02 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.8; 56.4; 92.8; 96.3; 109.3; 102.3 (q, $J = 253.2$ Hz); 121.1; 127.7; 130.0; 132.1; 151.3 (q, $J = 2.0$ Hz); 159.4; 159.8; 161.0; 164.3; 177.5.

5,7-Dimethoxy-4′-piperonylflavone (6{*2,5*}).[51] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.91 (s, 3H); 3.96 (s, 3H); 6.06 (s, 2H); 6.38 (d, $J = 1.1$ Hz, 1H); 6.55 (d, $J = 1.1$ Hz, 1H); 6.56 (s, 1H); 6.91 (d, $J = 4.2$ Hz, 1H); 7.31 (d, $J = 1.0$ Hz, 1H); 7.44 (dd, $J = 4.2$, 1.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.8; 56.5; 92.8; 96.2; 101,8; 106.1; 108.1; 108.7; 121.0; 125.6; 148.4; 150.3; 159.8; 160.4; 161.0; 162.0; 164.0; 177.6.

4′-Phenyl-5,7-dimethoxyflavone (6{*2,6*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.93 (s, 3H); 3.96 (s, 3H); 6.38 (d, $J = 2.3$ Hz, 1H); 6.59 (d, $J = 2.3$ Hz, 1H); 6.72 (s, 1H); 7.35−7.54 (m, 3H); 7.57−7.82 (m, 4H); 7.87−8.05 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.8; 56.5; 92.9; 96.3; 109.0; 109.4; 126.4; 127.2; 127.6; 128.1; 129.0; 130.4; 139.9; 144.0; 160.0; 160.5; 161.0; 164.2; 177.7.

6-Fluoro-4′-methoxyflavone (6{*3,1*}).[52] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.90 (s, 3H); 6.74 (d, $J = 0.6$ Hz, 1H);

6.98−7.08 (m, 2H); 7.40 (ddd, J = 9.1, 7.6, 3.1 Hz, 1H); 7.58 (ddd, J = 9.1, 4.2, 0.5 Hz, 1H); 7.81−7.93 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 105.5; 110.6 (d, J = 23.9 Hz); 114.5; 119.9 (d, J = 7.8 Hz); 121.6 (d, J = 25.5 Hz); 123.8; 125.1 (d, J = 5.8 Hz); 128.0; 152.4 (d, J = 1.6 Hz); 159.6 (d, J = 246.6 Hz); 162.6; 163.7; 177.5 (d, J = 2.5 Hz).

6-Fluoro-2′,4′-dimethoxyflavone (6{*3,2*}). mp: 162−164 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.92 (s, 3H); 6.55 (d, J = 2.4 Hz, 1H); 6.63 (dd, J = 8.8, 2.4 Hz, 1H); 7.13 (d, J = 0.5 Hz, 1H); 7.37 (ddd, J = 9.1, 7.6, 3.1 Hz, 1H); 7.51 (ddd, J = 9.1, 4.3, 0.5 Hz, 1H); 7.85 (ddd, J = 8.3, 3.1, 0.5 Hz, 1H); 7.87 (d, J = 8.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.6; 98.9; 105.4; 110.4 (d, J = 23.4 Hz); 110.5; 113.3; 119.8 (d, J = 7.8 Hz); 121.3 (d, J = 25.4 Hz); 124.9 (d, J = 7.4 Hz); 130.7; 152.5 (d, J = 2.0 Hz); 159.3 (d, J = 244.2 Hz); 159.7; 161.1; 163.4; 178.0 (d, J = 2.0 Hz). MS m/z: 300 (M^+), 269, 214, 161, 139 (100), 119, 91, 65. IV (ν_{max}, KBr): 1627, 1606, 1558, 1436, 1353, 1274, 1255, 1216, 1020, 811 cm^{-1}.

6-Fluoro-2′,3′,4′-trimethoxyflavone (6{*3,3*}).[53] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.92 (s, 3H); 3.95 (s, 3H); 3.96 (s, 3H); 6.81 (d, J = 9.0 Hz, 1H); 7.00 (s, 1H); 7.39 (ddd, J = 9.1, 7.6, 3.1 Hz, 1H); 7.52 (dd, J = 9.1, 4.3 Hz, 1H); 7.55 (d, J = 9.0 Hz, 1H); 7.87 (dd, J = 8.2, 3.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 56.2; 61.0; 61.2; 107.5; 110.5; 110.6 (d, J = 24.0 Hz); 118.9; 120.0 (d, J = 8.0 Hz); 121.6 (d, J = 26.0 Hz); 124.1; 125.1; 143.0; 152.7; 153.2; 156.5; 159.5; (d, J = 246.2 Hz); 161.

4′-Phenyl-6-fluoroflavone (6{*3,6*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.86 (s,1H); 7.35−7.55 (m, 4H); 7.56−7.72 (m, 3H); 7.72−7.82 (m, 2H); 7.88 (dd, J = 8.1, 3.0 Hz, 1H); 7.95−8.03 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 106.7; 110.7 (d, J = 23.5 Hz); 120.1 (d, J = 8.2 Hz); 121.9 (d, J = 25.6 Hz); 125.2 (d, J = 7.4 Hz); 126.8; 127.1; 127.7; 128.3; 129.0; 130.3; 139.7; 144.6; 152.4 (d, J = 1.6 Hz); 159.6 (d, J = 247.0 Hz); 163.4; 177.5 (d, J = 2.1 Hz).

4′,6-Dimethoxyflavone (6{*4,1*}).[54] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.91 (s, 3H); 3.93 (s, 3H); 6.57 (d, J = 2.3 Hz, 1H); 6.64 (dd, J = 8.7, 2.3 Hz, 1H); 7.13 (s, 1H); 7.26 (dd, J = 9.0, 3.1 Hz, 1H); 7.45 (d, J = 9.0 Hz, 1H); 7.60 (d, J = 3.1 Hz, 1H); 7.89 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.9; 105.0; 105.6; 114.5; 119.4; 123.5; 124.2; 124.6; 128.0; 151.0; 157.0; 162.4; 163.2; 178.2.

2′,4′,6-Trimethoxyflavone (6{*4,2*}).[55] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H); 3.91 (s, 3H); 3.93 (s, 3H); 6.57 (d, J = 2.3 Hz, 1H); 6.64 (dd, J = 8.7, 2.3 Hz, 1H); 7.13 (s, 1H); 7.26 (dd, J = 9.0, 3.1 Hz, 1H); 7.45 (d, J = 9.0 Hz, 1H); 7.60 (d, J = 3.1 Hz, 1H); 7.89 (d, J = 8.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5; 55.7; 56.0; 99.0; 104.8; 105.3; 110.6; 113.8; 119.3; 123.4; 124.4; 130.4; 151.3; 156.7; 159.6; 160.7; 163.2; 178.8.

2′,3′,4′,6-Tetramethoxyflavone (6{*4,3*}).[56] ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.92 (s, 3H); 3.94 (s, 3H); 3.96 (s, 3H); 6.80 (d, J = 9.0 Hz, 1H); 6.98 (s, 1H); 7.27 (dd, J = 9.1, 3.1 Hz, 1H); 7.45 (d, J = 9.1 Hz, 1H); 7.54 (d, J = 9.0 Hz, 1H); 7.61 (d, J = 3.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 56.0; 56.2; 61.0; 61.2; 104.9; 107.4; 110.5; 119.3; 119.4; 123.6; 124.1; 124.5; 142.9; 151.3; 153.1; 156.2; 156.9; 161.3; 178.6.

6-Methoxy-4′-trifluoromethoxyflavone (6{*4,4*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.92 (s, 3H); 6.80 (s, 1H); 7.31 (dd, J = 9.2, 3.1 Hz, 1H); 7.34−7.43 (m, 2H); 7.52 (d, J = 9.2 Hz, 1H); 7.61 (d, J = 3.1 Hz, 1H); 7.92−8.05 (m, 2H).

4′-Phenyl-6-methoxyflavone (6{*4,6*}). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.92 (s, 3H); 6.88 (s, 1H); 7.30 (dd, J = 9.1, 3.1 Hz, 1H); 7.40−7.44 (m, 2H); 7.45−7.57 (m, 2H); 7.62 (d, J = 3.1 Hz, 1H); 7.63−7.70 (m, 2H), 7.72−7.82 (m, 2H); 7.97−8.07 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 56.0; 105.0; 106.8; 119.5; 123.8; 124.7; 126.7; 127.1; 127.7; 128.2; 129.0; 130.7; 139.8; 144.3; 151.1; 157.1; 163.0; 178.3.

Inhibitory Activity Assay. Recombinant human cathepsin V was expressed and purified as described previously.[57] The molar concentration of this enzyme was determined by active site titration with E-64.

Kinetic measurements were carried out in a Molecular Devices Spectra MAX GEMINI XS. Stock solutions of the compounds were prepared at a concentration of 1 mM in DMSO, and the inhibitors were screened against the cathepsin V at initial concentration of 25 μM. The protease inhibitor activity was carried out in triplicate in 96-well black plate with the method previously described by Barret et al.[58] The reaction mixture contained 192 μL of a sodium acetate buffer (100 mM, 5 mM EDTA, 5 mM DTE, pH 5.5), 2 μL of 1 mM Z-Phe-Arg-MCA, 5 μL of sample, and 1 μL of cathepsin V (32 nM). The enzyme was activated during 5 min with DTE at 27 °C, after the solution was incubated during 5 min with the sample. The substrate was added to start the reaction, and the fluorescence of 4-methyl-7-coumaryl-amide (release MCA) was measured at Ex 355 nm and Em 460 nm. Control assays were performed without inhibitor (negative control) and in the presence of the E-64 (positive control), an irreversible inhibitor for cysteine peptidase. Values of IC_{50} were determined by making rate measurements for at least seven inhibitor concentrations. Kinetics parameters were determined from collected data employing the SigmaPlot enzyme kinetics module.

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