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Original article

Evaluation of synthetic acridones and 4-quinolinones as potent inhibitors of cathepsins L and V

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ABSTRACT

Cathepsins, also known as lysosomal cysteine peptidases, are members of the papain-like peptidase family, involved in different physiological processes. In addition, cathepsins are implicated in many pathological conditions. This report describes the synthesis and evaluation of a series of *N*-arylanthranilic acids, acridones, and 4-quinolinones as inhibitors of cathepsins V and L. The kinetics revealed that compounds of the classes of acridones are reversible competitive inhibitors of the target enzyme with affinities in the low micromolar range. They represent promising lead candidates for the discovery of novel competitive cathepsin inhibitors with enhanced selectivity and potency. On the other hand, 4-quinolinones were noncompetitive inhibitors and *N*-arylanthranilic acids were uncompetitive inhibitors.

1. Introduction

Natural Products are a very important source of therapeutic drugs in medicine due to their ability to modulate many biochemical pathways [1]. The first products isolated from natural sources in the 19th century were alkaloids. Since then, hundreds of bioactive compounds have been isolated from plants, animals or microorganisms with different architectures, presenting a very high chemical diversity. This includes terpenes, coumarins, flavonoids, acetogenins, and many others. However, we have few examples of "natural drugs" which are formulated for ready availability as medicine [2]. In the majority of cases "natural drugs" must be chemically modified in order to improve their pharmacological profile [3].

Alkaloids are one of the most important classes of natural products due to their ability to modulate many pharmacological events. 4-Quinolinones are widely used as antibacterial and antimycobacterial drugs to treat various infectious diseases [4,5], and potent chemotherapeutic agents of first choice for the treatment of a broad range of bacterial infections, and are used as templates in the development of various inhibitors [6,7]. The toxicity of quinolinones is low and comparable to that of other commonly used antimicrobial agents; so they can be considered relatively well-tolerated [8]. Besides the antibacterial activity, several reports have shown the anti-parasitic activity of 4-quinolinones [9–13].

N-Arylanthranilic acids are an interesting class of compounds which show antibacterial [14–17] and anti-inflammatory [16] properties. They are also employed as precursor of biologically important compounds such as acridone alkaloids [17–25]. Santos et al. reported the evaluation of 11 acridones, isolated from *Swinglea glutinosa*, toward *Plasmodium falciparum*, *Leishmania donovani* and *Trypanosoma brucei* [25].

Cathepsins, also known as lysosomal cysteine peptidases, are members of the papain-like peptidase family, involved in different physiological processes. In addition, cathepsins are implicated in many pathological conditions [26].

Cathepsin V (catV) has been associated with the MHC class II complex in humans [27] and the enzyme has been considered as a potential diagnostic marker for colon tumors [28]. This enzyme also is expressed in colorectal and breast carcinomas but not in normal colon or mammary tissue [29]. In addition, cathepsin V together with the cathepsins L, K and S, have been implicated in atherosclerosis and are considered potential drug targets [30–33].

On the other hand, cathepsin L (catL) has been associated in processes of keratinocyte differentiation, heart functions and reproduction [34]. Studies on cathepsin expression revealed increased expression of cathepsins B and L in melanomas and

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carcinomas of breast, lung, colon and prostate [35]. Recently, it has been demonstrated that non-metastatic melanoma cells are converted into metastatic cells by overexpression of catL [36]. A potent inhibitor of catL, cystatin C, blocks the mobility and invasion of melanoma cells [37,38].

Though these enzymes have been intensively studied as valuable targets for drug discovery and development [34,39], few natural product-based inhibitors have been described in literature [30,31,40–42]. Recently, we reported synthetic flavones and natural acridones as potent inhibitors of catV, with IC_{50} values varying in the range of 0.8–4.1 μM and 1.2–5.2 μM, respectively [43,44].

This report describes the synthesis and inhibitory evaluation of a series of *N*-arylanthranilic acids, acridones, and 4-quinolinones. Most of the compounds are novel and their inhibitory potency against cathepsins V and L was determined.

2. Results and discussion

2.1. Synthesis of 4-quinolinones

Several synthetic methods have been developed for the preparation of 2-substituted-4-quinolones from different starting materials, and among them, acylated 2-aminoacetophenones and enamines are the most common [45,46]. These methodologies involve thermal cyclization usually under extremely harsh conditions [47]. In this work, we report the synthesis of a library of 4-quinolinones under microwave (MW) irradiation [48,49].

After a systematic study, looking for alternatives for the cyclization of 2-aminoacetophenones (**1**), we found that MW irradiation in the presence of *t*-BuOK in THF, for 10–20 min at 100–120 °C and 180 W is the best condition. We have prepared a series of 2-substituted-4-quinolinones in good yields (Table 1), excepting for quinolinone **2e** (entry 5) due to formation of the 2-quinolinone isomer as the major product.

2.2. Synthesis of N-arylanthranilic acids and aridones

The synthesis of *N*-arylanthranilic acids can be usually achieved by Ullmann condensation [50] between a 2-halobenzoic acid and an aryl amine [51,52] or an anthranilic acid and an aryl halide [53,54]. One of the major drawbacks of these methodologies is the need of heating during long reaction time [55]. Recently, we reported the synthesis of a series of novel *N*-arylanthranilic acids **5**, with good to excellent yields, employing MW as heat source to promote the Ullmann coupling between aryl bromides **3** and anthranilic acids **4** possessing electron donating or withdrawing groups [56].

To complete the synthesis of acridones **6**, a series of experiments, employing different reagents and conditions, was performed and it was observed that intramolecular Friedel–Crafts acylation was best performed employing $POCl_3$ as acylating reagent [57,58]. In general, the acridones were obtained in good to excellent yields, however, *N*-arylanthranilic acids bearing strong electron-withdrawing group at C3 position (aryl bromide **3b**) furnished no product (Table 2, entries 2, 9, 11, 14 and 15). Attempts to obtain the desired compounds employing previously reported methodologies such as PPA [59], PPE [60], TFAA [61,62] and H_2SO_4 [23] as acylating reagents also failed, which we attributed to the rightly deactivating character of nitro substituent, making the Friedel–Crafts acylation unfavorable.

2.3. Biochemical evaluation of synthetic compounds

The design of the compounds presented in this work is based on the results of previous catV inhibition studies on using natural acridone alkaloids [44]. First we evaluated the potential intrinsic autofluorescences of the compounds which could lead to false positives. None of the compounds exhibited intrinsic fluorescence. In the first inhibition screen assay, we used an initial inhibitor concentration of 25 μM. As most of compounds showed 100% inhibition of catV and L, they were subsequently diluted to 12.5, 2.5 and 0.25 μM and screened against cathepsins L and V. In order to explore the specificity–activity relationship (SAR) of cathepsins V and L inhibitors, we determined IC_{50} values for both cathepsins of ten 2-aminoacetophenones, ten 2-substituted-4-quinolones, seventeen *N*-arylanthranilic acids, and ten acridones, by making rate measurements for at least seven inhibitor concentration, as shown in Tables 3–5, respectively. The tested compounds showed low selectivity toward the enzymes studied.

Compounds **1a**–**j** did not show significant inhibition at a concentration of 25 μM, although quinolinones **2a**–**j** (excepting for **2e** which was not evaluated due to the low yield obtained during its preparation) presented values of IC_{50} varying in the range of 1.6–20 μM and 4.7–20 μM for the catL and catV, respectively. In this series, the most potent catL inhibitor was **2b** with an IC_{50} of 1.6 μM and the most potent catV inhibitors were **2a** and **2c**, both with IC_{50} values of 4.7 μM. In this small series with substitutions at the benzyl group, compound **2a** showed an IC_{50} value of 2.6 μM for the catL and 4.7 μM for catV. This represents a good basis for further alterations of the substituent at the ring position and to improve the potency of this class. Small variation of the size of the alkyl chain in benzyl group (position 4) already affected directly the inhibitory potency of this class.

Compounds **5a**–**q** showed significant inhibition with IC_{50} values varying in the range of 2–50 μM and 1–50 μM for the catL and catV, respectively. Although these inhibitors were sensitive for both cathepsins, the most potent *N*-arylanthranilic acid was **5i** with an IC_{50} value of 1.0 μM for catV. In general, *N*-arylanthranilic acid exhibited a slight selectivity for catV as can be seen for the selectivity ratio (S) >1. Despite the limited amount of structure–activity relationship (SAR) data, it is possible to note the following preferences: a) when R^1 is a nitro group, the compound is five times more potent than a methoxy group (see compounds **5b** and **5c**); b) addition of a benzyl group at R^1 in **5e** increases the potency twenty-fold when compared with compounds **5f**–**h**; c) addition of a methoxy group at R^3 decreases the potency when comparing compounds **5b** and **5k** (10-fold difference) and **5c** and **5l** (2-fold difference); and d) the presence of a methoxy group at R^4 and R^5 in **5n** increased the potency when compared with compound **5k**.

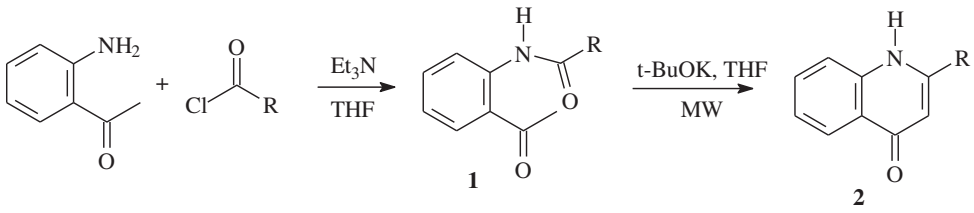
Acridones **6a**–**q** showed IC_{50} values varying in the range of 0.5–19.3 μM and 1.7–18 μM for the catL and catV, respectively. Compound **6g** was the most potent inhibitor for catL, with an IC_{50} of 0.5. The structural difference between compounds **6f**–**h** is the methoxy group, benzyl group and the hydrogen at the R^5 position.

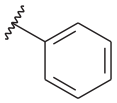
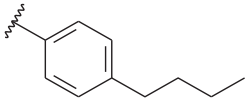
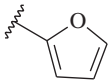
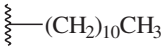
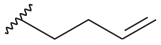
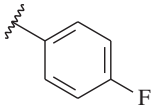
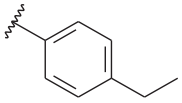
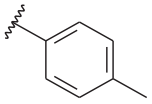
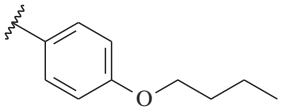
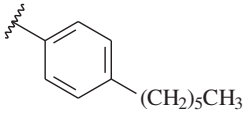
2.4. Mechanism of inhibition

Some 4-quinolinones (**2b**), *N*-arylanthranilic acids (**5i** and **5j**) and acridones (**6a** and **6g**) with low IC_{50} values were selected to determinate the type of inhibition of cathepsins L and V. Interestingly, selected compounds from *N*-arylanthranilic acids showed uncompetitive inhibition of catL and catV (Fig. 1), from acridones exhibited competitive inhibition (Fig. 2), whereas a 4-quinolinone showed noncompetitive inhibition (Fig. 3).

Uncompetitive inhibitors require the prior formation of the ES complex and bind exclusively to the ES complex. On the other hand, competitive inhibitors binds to the free enzyme and compete with the substrate with a mutually exclusive binding mode [63,64]. Noncompetitive inhibitors display binding affinity for free enzyme (E) and ES complex. These inhibitors do not compete with the substrate for binding to free enzyme, they bind at a site distinct from the active site.

Table 1
Synthesis of 2-substituted-4-quinolinones.



Entry	R	Acetophenone yield (%)[a]	Quinolinone yield (%)[a]
1		**1a**, 90	**2a**, 60
2		**1b**, 91	**2b**, 67
3		**1c**, 72	**2c**, 73
4		**1d**, 74	**2d**, 55
5		**1e**, 75	**2e**, 20
6		**1f**, 83	**2f**, 72
7		**1g**, 93	**2g**, 64
8		**1h**, 84	**2h**, 68
9		**1i**, 85	**2i**, 65
10		**1j**, 79	**2j**, 63

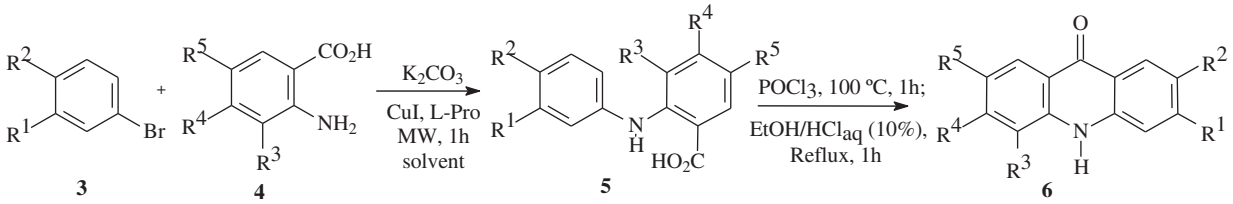
[a] Isolated yields after chromatographic purification.

The K_i values of the competitive and uncompetitive inhibitors were obtained using Dixon plot analysis, which consist in plotting the reciprocal of the initial velocity ($1/v_0$) *versus* a series of inhibitor concentrations at constant substrate concentration, where the lines converge above the *x*-axis, indicating the inhibition constant-K_i. The K_i values for inhibitors **5i**, **5j**, **6a** and **6g** are reported in Table 6.

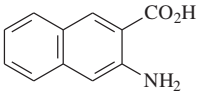
3. Conclusions

In this work we described the synthesis of 2-substituted-4-quinolinones employing microwave irradiation, instead of conventional heating methods, which significantly improved the reaction times and yields. Newly synthesized acridones and 4-quinolinones were shown to be potent inhibitors of cathepsins L and V. The kinetics revealed that compounds of the classes of acridones are reversible competitive inhibitors of the target enzyme with affinities in the low micromolar range. They represent promising lead candidates for the discovery of novel competitive cathepsin inhibitors with enhanced selectivity and potency. Interestingly, some *N*-arylanthranilic acid and 4-quinolinones showed uncompetitive and 4-quinolinones noncompetitive inhibition mechanisms, respectively.

Table 2
Synthesis of *N*-arylanthranilic acids and acridones.



3 **4** **5** **6**

Entry	Aryl bromide	Anthranilic acid	*N*-Arylanthranilic acid, yield (%)[a,b]	Acridone, yield (%)[b]
1	**3a**: R^1 = BnO; R^2 = H	**4a**: R^3 = R^4 = R^5 = H	**5a**, 78	**6a**, 82
2	**3b**: R^1 = NO_2; R^2 = BnO	**4a**	**5b**, 83	–
3	**3c**: R^1 = MeO; R^2 = BnO	**4a**	**5c**, 85	**6c**, 85
4	**3d**: R^1 = H; R^2 = MeO	**4a**	**5d**, 77	**6d**, 89
5	**3a**	**4b**: R^3 = R^4 = H; R^5 = NO_2	**5e**, 82	**6e**, 90
6	**3d**	**4b**	**5f**, 76	**6f**, 87
7	**3e**: R^1 = H; R^2 = BnO	**4b**	**5g**, 69	**6g**, 78
8	**3f**: R^1; R^2 = H	**4b**	**5h**, 82	**6h**, 82
9	**3b**	**4c** 	**5i**, 54	–
10	**3c**	**4c**	**5j**, 81	–
11	**3b**	**4d**: R^3 = MeO; R^4 = R^5 = H	**5k**, 92	–
12	**3c**	**4d**	**5l**, 70	–
13	**3f**	**4d**	**5m**, 54	**6m**, 99
14	**3b**	**4e**: R^3 = R^4 = R^5 = MeO	**5n**, 83	–
15	**3b**	**4f**: R^3 = H; R^4 = R^5 = F	**5o**, 54	–
16	**3c**	**4f**	**5p**, 38	**6p**, 93
17	**3e**	**4f**	**5q**, 65	**6q**, 95

[a] All reactions were performed in anhydrous *iso*-amyl alcohol as solvent at 140 °C, except for anthranilic acid **4b** which was performed in DMF/H_2O 90% at 160 °C [56].
[b] Isolated yield after purification.

4. Material and methods

4.1. Synthesis

Unless otherwise noted, 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 or Mass Spectrometer QuatroLC-Micromass. Elemental analyses were performed on a Fisons EA 1108 CHNS–O. Analytical thin-layer chromatography was performed on a 0.25 μm film of silica gel containing fluorescent indicator UV_{254} 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. Melting points were performed in Microquimica MQAPF – 301. Reactions were irradiated in a focused microwave oven CEM Discover.

4.1.1. General procedure for the preparation of acetophenone derivatives **1** [49]

To a mixture of triethylamine (1 mmol), anhydrous dichloromethane (2 mL) and 2′-aminoacetophenone (1 mmol), acyl chloride (1 mmol) was added in drop wise under ice bath. The solution was stirred for 2 h at room temperature. The mixture was extracted with dichloromethane (3 × 10 mL), and the combined organic layers washed with water (3 × 10 mL), then dried with Na_2SO_4. After removal of the solvent the product was purified by column chromatography (Hex/Acet 80:20).

4.1.1.1. *N*-(2-Acetylphenyl)benzamide (**1a**) [65].

Yield 90%. Mp 99 °C (dec.). IR (ν_{max}, KBr): 701, 764, 963, 1028, 1247, 1260, 1299, 1316, 1449, 1538, 1587, 1608, 1647, 1673, 2331, 2358, 3217 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H); 7.16 (ddd, 1H, *J* 1.2, 7.2, 7.9 Hz); 7.47–7.67 (m, 4H); 7.95 (dd, 1H, *J* 1.4, 1.6 Hz); 8.05–8.10 (m, 2H); 8.98 (dd, 1H, *J* 1.2, 1.4 Hz), 12.69 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.5, 120.9, 122.5, 127.5, 128.8, 130.4, 131.8, 131.9, 155.4, 167.9, 203.2. MS (*m*/*z*): 239 (M^+), 224, 200, 196, 106, 105 (100), 78, 77, 51.

4.1.1.2. *N*-(2-Acetylphenyl)-4-butylbenzamide (**1b**).

Yield 91%. Mp 120 °C (dec.). IR (ν_{max}, KBr): 758, 850, 897, 1246, 1651, 1682, 1785, 2859, 2930, 2957, 3223, 3245 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.93 (t, 3H, *J* 7.2 Hz); 1.36 (sex, 2H, *J* 7.2 Hz); 1.63 (qui, 2H, *J* 7.2 Hz); 2.64–2.72 (m, 5H); 7.13 (ddd, 1H, *J* 1.2, 1.3, 1.5 Hz); 7.26–7.34 (m, 2H); 7.60 (ddd, 1H, *J* 1.3, 1.5, 1.6 Hz); 7.92–8.07 (m, 3H); 8.98 (dd, 1H, *J* 1.2 Hz); 12.65 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.9, 22.3, 28.5, 33.2, 35.5, 120.8, 122.3, 127.5, 128.8, 130.6, 131.8, 135.3, 141.5, 147.4, 164.5, 216.1.

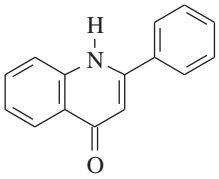
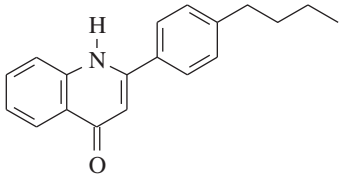
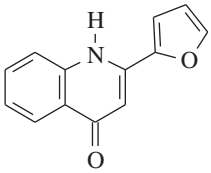
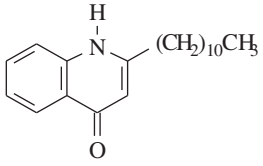
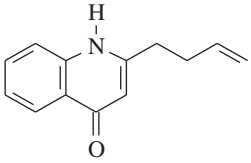
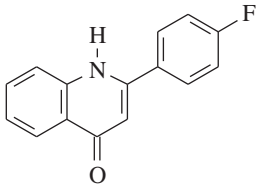
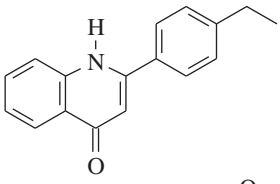
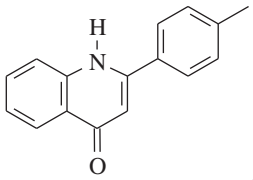
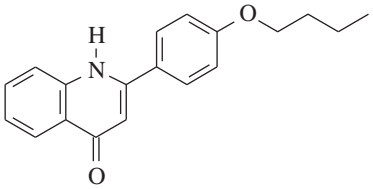
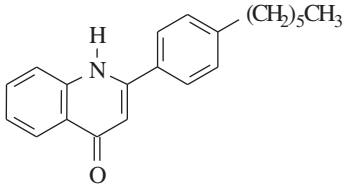
4.1.1.3. *N*-(2-Acetylphenyl)furan-2-carboxamide (**1c**) [66].

Yield 72%. Mp 105 °C (dec.). IR (ν_{max}, KBr): 750, 786, 883, 959, 1010, 1167, 1248, 1315, 1452, 1525, 1582, 1607, 1651, 1677, 2336, 2359, 3113, 3148 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H); 6.56 (dd, 1H, *J* 1.8, 1.9 Hz); 7.16 (ddd, 1H, *J* 0.8, 1.2, 1.6 Hz); 7.27 (dd, 1H, *J* 0.8, 1.2 Hz); 7.56–7.65 (m, 2H); 7.95 (dd, 1H, *J* 1.4, 1.6 Hz); 8.90 (dd, 1H, *J* 1.0, 1.2 Hz); 12.69 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.5, 107.2, 109.8, 120.9, 122.5, 127.5, 128.8, 131.9, 140.0, 141.4, 147.9, 167.9, 203.2. MS (*m*/*z*): 229 (M^+), 214, 186, 158, 146, 116, 95 (100), 90, 67, 51.

4.1.1.4. *N*-(2-Acetylphenyl)undecanamide (**1d**).

Yield 74%. Mp 115 °C (dec.). IR (ν_{max}, KBr): 704, 735, 1164, 1246, 1265, 1451, 1523, 1583, 1606, 1654, 1694, 1775, 2305, 2358, 2854, 2926, 2954, 2984 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.88 (t, 3H, *J* 6.5 Hz); 1.26–1.82 (m, 16H);

Table 3
Structures of 2-substituted-4-quinolinones and values of IC_{50} on cathepsin L and cathepsin V.

	Structure	CatL IC_{50} (μM)[a]	CatV IC_{50} (μM)[a]	S[b]		Structure	IC_{50} CatL (μM)[a]	IC_{50} CatV (μM)[a]	S[b]
2a		2.6 ± 0.1	4.7 ± 0.1	0.6	**2b**		1.6 ± 0.2	5.2 ± 0.1	0.3
2c		2.6 ± 0.1	4.7 ± 0.1	0.6	**2d**		2.9 ± 0.3	9.9 ± 0.2	0.3
2e		ND	ND	–	**2f**		20 ± 0.8	20 ± 0.5	1.0
2g		16 ± 1.3	16 ± 1.5	1.0	**2h**		15 ± 1.2	5.0 ± 0.9	3.0
2j		8.6 ± 0.7	15 ± 1.1	0.6	**2i**		9.0 ± 0.8	8.5 ± 0.7	1.0

ND = not determined.
[a] The values represent means of three individual experiments ± SD.
[b] Selectivity (S) = IC_{50} cathepsin L/IC_{50} cathepsin V.

2.43 (t, 2H, *J* 7.2 Hz); 2.67 (s, 3H); 7.10 (ddd, 1H, *J* 0.9, 1.1, 1.2 Hz); 7.55 (ddd, 1H, *J* 1.6, 1.2, 1.4 Hz); 7.98 (dd, 1H, *J* 1.5, 1.6 Hz); 8.78 (dd, 1H, *J* 0.9, 1.1 Hz); 11.71 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 14.3, 21.8, 25.2, 26.6, 28.3, 29.6, 30.1, 30.2, 30.3, 32.7, 38.9, 121.3, 122.3, 131.2, 136.0, 137.5, 139.4, 171.2, 203.8. MS (*m*/*z*): 225, 211, 199, 183, 169, 155, 135, 127, 109, 95, 85, 71, 57 (100).

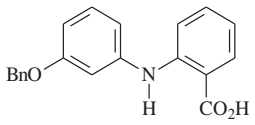
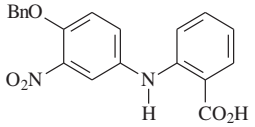
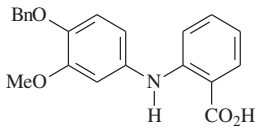
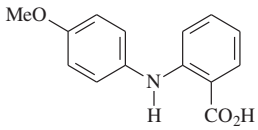
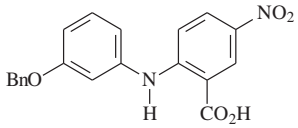
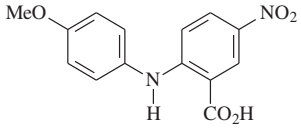
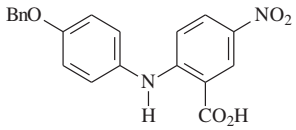
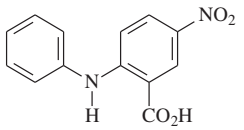
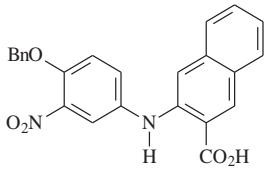
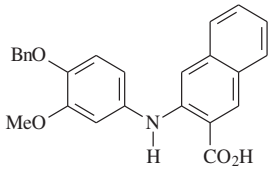
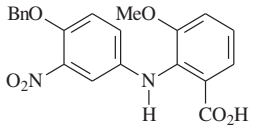
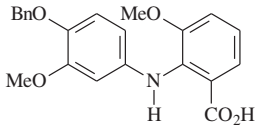
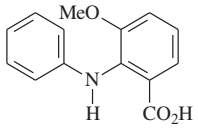
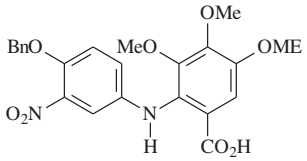
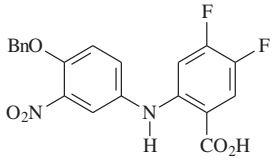
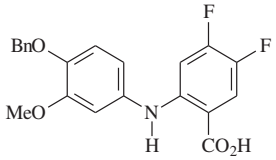
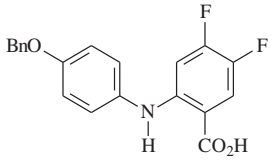
*4.1.1.5. N-(2-Acetylphenyl)pent-4-enamide (**1e**).* Yield 75%. Mp 110 °C (dec.). IR ($\nu_{max,}$ KBr): 517, 605, 735, 757, 916, 960, 1163, 1247, 1311, 1359, 1451, 1521, 1584, 1652, 1696, 2915, 2978, 3001, 3078 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 2.47–2.56 (m, 4H); 2.66 (s, 3H); 4.97–5.15 (m, 2H); 5.77–5.97 (m, 2H); 7.10 (ddd, 1H, *J* 1.4, 1.2, 0.8 Hz), 7.54 (ddd, 1H, *J* 1.8, 1.6, 1.4 Hz); 7.88 (dd, 1H, *J* 1.6, 1.8 Hz); 8.75 (dd, 1H, *J* 0.9, 1.2 Hz); 11.72 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 27.3, 29.2, 37.8, 115.5, 120.7, 122.2, 123.8, 131.6, 135.1, 136.6, 173.1, 200.7. MS (*m*/*z*): 217 (M^+), 202, 189, 174, 160, 135, 120 (100), 116, 92, 84, 65, 55.

*4.1.1.6. N-(2-Acetylphenyl)-4-fluorobenzamide (**1f**).* Yield 83%. Mp 105 °C (dec.). IR ($\nu_{max,}$ KBr): 459, 520, 576, 607, 628, 678, 754, 850, 960, 1008, 1097, 1232, 1319, 1450, 1506, 1539, 1593, 1652, 1672, 3076, 3166, 3184, 3199 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 2.71 (s, 1H); 7.17–7.29 (m, 3H); 7.61 (ddd, 1 H, *J* 1.6, 1.2, 1.7 Hz); 8.01–8.08 (m, 3H); 8.78 (dd, 1H, *J* 0.8, 1.2 Hz), 12.71 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 28.7, 116.4, 116.8, 121.3, 123.9, 130.6, 130.8, 133.1, 134.3, 135.9, 137.5, 161.9, 163.7, 165.9, 204.8. MS (*m*/*z*): 257 (M^+), 242, 214, 123 (100), 95, 91, 75, 51.

*4.1.1.7. N-(2-Acetylphenyl)-4-ethylbenzamide (**1g**).* Yield 93%. Mp 100 °C (dec.). IR ($\nu_{max,}$ KBr): 526, 603, 688, 763, 854, 960, 1120, 1188, 1245, 1315, 1357, 1448, 1510, 1535, 1608, 1645, 1670, 2871, 2931, 2960, 2972, 3217 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 1.26 (t, 3H, *J* 7.5 Hz); 2.65–2.77 (m, 5H); 7.11 (ddd, 1H, *J* 1.2, 0.8, 1.1 Hz); 7.33 (dt, 2H, *J* 0.6, 1.9 Hz); 7.58 (ddd, 1H, *J* 0.6, 1.2, 0.8 Hz); 7.90–8.01 (m, 3H); 8.51 (dd, 1H, *J* 0.9, 1.1 Hz); 12.65 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) *δ*: 15.2, 28.8, 29.8, 120.6, 120.7, 122.2, 123.8, 126.6, 127.5, 128.2, 129.2, 131.7, 135.3, 136.7, 141.5, 166.0, 202.5. MS (*m*/*z*): 267 (M^+), 252, 224, 207, 177, 134, 133 (100), 106, 105, 77, 51.

*4.1.1.8. N-(2-Acetylphenyl)-4-methylbenzamide (**1h**).* Yield 84%. Mp 95 °C (dec.). IR ($\nu_{max,}$ KBr): 474, 522, 607, 628, 648, 757, 833, 962, 1020, 1118, 1168, 1193, 1247, 1315, 1363, 1454, 1508, 1643, 1674, 2921, 2970, 3207 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) *δ*: 2.43 (s, 3H); 2.72 (s,

Table 4
Structures of *N*-arylanthranilic acids and values of IC_{50} on cathepsin L and cathepsin V.

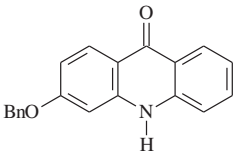
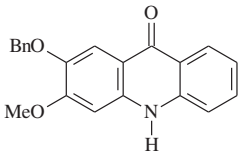
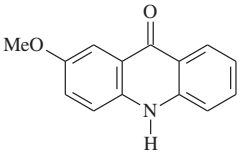
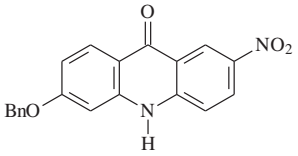
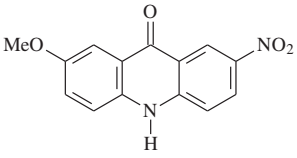
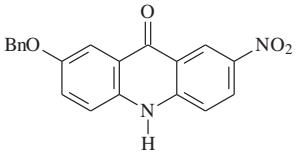
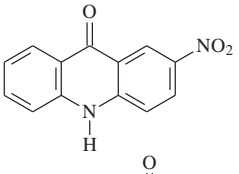
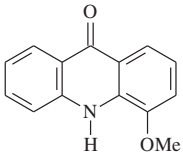
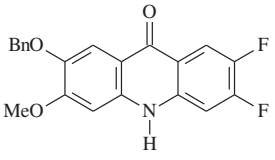
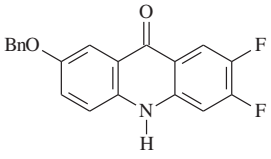
	Structure	CatL IC_{50} (μM)[a]	CatV IC_{50} (μM)[a]	S[b]		Structure	IC_{50} CatL (μM)[a]	IC_{50} CatV (μM)[a]	S[b]
5a		7.2 ± 0.3	2.1 ± 0.2	3.4	**5b**		5.7 ± 0.1	6.8 ± 0.2	0.8
5c		26 ± 0.3	25 ± 0.3	1.0	**5d**		>50	>50	–
5e		2.5 ± 0.05	1.5 ± 0.04	1.7	**5f**		45 ± 0.1	15 ± 0.5	3.0
5g		>50	>50	–	**5h**		43 ± 0.2	10 ± 0.8	4.3
5i		2.9 ± 0.3	1.0 ± 0.1	2.9	**5j**		2.1 ± 0.005	1.8 ± 0.01	1.2
5k		>50	>50	–	**5l**		>50	>50	–
5m		>50	>50	–	**5n**		18.2 ± 0.1	12.3 ± 1.3	1.5
5o		7.2 ± 0.2	3.5 ± 0. 1	2.0	**5p**		11 ± 0.3	9.0 ± 0.4	1.2
5q		3.2 ± 0.4	6.9 ± 0.1	0.5					

[a] The values represent means of three individual experiments ± SD.
[b] Selectivity (S) = IC_{50} cathepsin L/IC_{50} cathepsin V.

3H); 7.15 (ddd, 1H, *J* 1.9, 1.6, 1.2 Hz); 7.32 (d, 2H, *J* 7.8 Hz); 7.62 (ddd, 1H, *J* 0.7, 0.9, 1.2 Hz) 7.94–7.98 (m, 3H); 8.98 (dd, 1H, *J* 0.7, 7.8 Hz); 12.66 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.3, 28.8, 121.6, 123.7, 127.8, 128.8, 130.8, 131.3, 136.9, 137.4, 140.2, 141.6, 164.0, 203.7. MS (*m*/*z*): 253 (M^+), 235, 210, 119 (100), 106, 91, 77, 65, 41.

*4.1.1.9. N-(2-Acetylphenyl)-4-butoxybenzamide (**1i**).* Yield 85%. Mp 125 °C (dec.). IR (ν_{max}, KBr): 487, 522, 609, 682, 763, 840, 980, 1002, 1037, 1124, 1178, 1247, 1315, 1359, 1448, 1508, 1533, 1583, 1606, 1643, 1676, 2867, 2941, 2952, 3249, 3271, 3288 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 0.98 (t, 3H, *J* 7.3 Hz), 1.50 (sex, 2H, *J* 7.3 Hz); 1.79

Table 5
Structures of acridones and values of IC_{50} on cathepsin L and cathepsin V.

	Structure	CatL IC_{50} (μM)[a]	CatV IC_{50} (μM)[a]	S[b]		Structure	IC_{50} CatL (μM)[a]	IC_{50} CatV (μM)[a]	S[b]
6a		3.9 ± 0.1	3.6 ± 0.1	1.1	**6c**		1.1 ± 0.02	1.7 ± 0.09	0.6
6d		ND[c]	ND	–	**6e**		13 ± 0.1	8.2 ± 0.2	1.6
6f		1.6 ± 0.02	2.0 ± 0.02	0.8	**6g**		0.5 ± 0.01	1.7 ± 0.08	0.3
6h		6.8 ± 0.08	7.2 ± 0.08	0.9	**6m**		ND	ND	–
6p		19.3 ± 0.1	18 ± 0.2	1.1	**6q**		1.5 ± 0.1	4.9 ± 0.3	0.3

[a] The values represent means of three individual experiments ± SD.
[b] Selectivity (S) = IC_{50} cathepsin L/IC_{50} cathepsin V.
[c] ND = not determined.

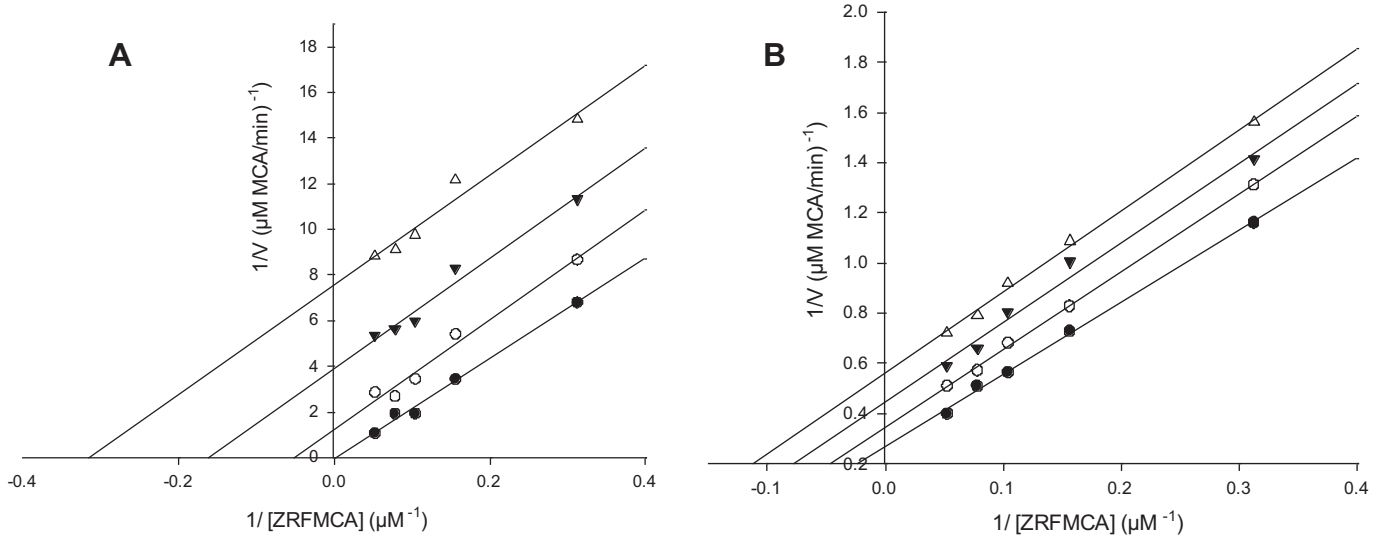


Fig. 1. Uncompetitive inhibition profile of compound **5i** (A) against catV and **5j** (B) against catL. Kinetic measurements were conducted in the presence of increasing concentrations of inhibitors. Panel A: 5 μM (△), 3 μM (▼) and 1 μM (○). Panel B: 7 μM (△), 5 μM (▼) and 3 μM (○). The absence of inhibitor is depicted by ● in panels A and B.

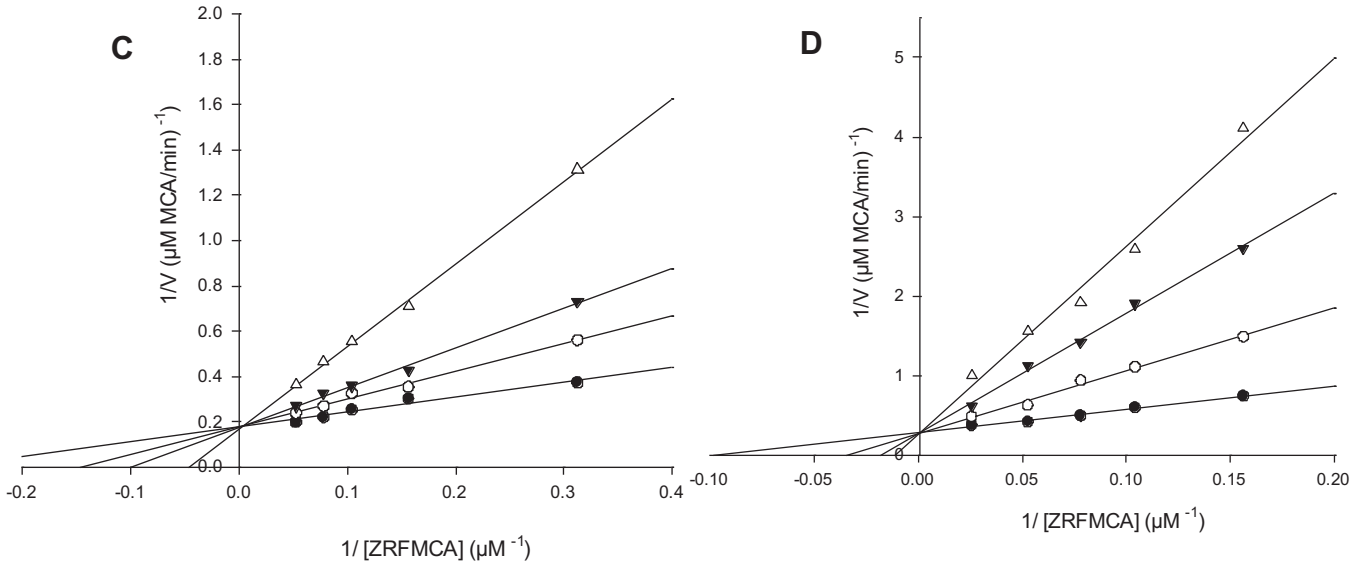


Fig. 2. Competitive inhibition profile of compound **6a** (C) against catL and **6g** (D) against catV. Kinetic measurements were conducted in the presence of increasing concentrations of inhibitors. Panel C: 5 µM (△), 3 µM (▼) and 1 µM (○). Panel D: 5 µM (△), 3 µM (▼) and 1 µM (○). The absence of inhibitor is depicted by ● in panels A and B.

(qui, 2H, *J* 7.3 Hz); 2.70 (s, 3H); 4.02 (t, 2H, *J* 6.5 Hz); 6.98 (dt, 2H, *J* 2.1, 2.9 Hz); 7.12 (ddd, 1H, *J* 1.4, 0.9, 1.2 Hz), 7.59 (ddd, 1H, *J* 1.6, 1.2, 1.4 Hz); 7.93 (dd, 1H, *J* 1.6, 1.4 Hz); 8.02 (dt, 2H, *J* 2.9, 2.1 Hz); 8.96 (dd, 1H, *J* 1.2, 0.9 Hz); 12.61 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.9, 22.3, 28.5, 33.2, 61.0, 120.8, 122.3, 127.5, 128.8, 130.6, 131.8, 135.3, 141.5, 147.4, 164.5, 216.1. MS (*m*/*z*): 311(M^+), 281, 268, 207, 177, 149, 137, 121(100), 107, 94, 91, 65, 55.

*4.1.1.10. N-(2-Acetylphenyl)-4-hexylbenzamide (**1j**).* Yield 79%. Mp 130 °C (dec.). IR (ν_{max}, KBr): 530, 609, 690, 757, 850, 896, 954, 1018, 1118, 1164, 1247, 1315, 1359, 1452, 1508, 1537, 1585, 1639, 1679, 2848, 2916, 2927, 2948 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.89 (t, 3H, *J* 7.3 Hz); 1.28–1.40 (m, 6H); 1.64 (qui, 2H, *J* 7.8 Hz); 2.68 (t, 2H, *J* 7.8 Hz); 2.71 (s, 1H); 7.15 (ddd, 1H, *J* 1.2, 1.3, 6.0 Hz); 7.31–7.34 (m, 2H); 7.61 (ddd, 1H, *J* 1.2, 1.5, 5.9 Hz); 7.94–8.00 (m, 3H); 8.98 (dd, 1H, *J* 1.2, 7.3 Hz); 12.66 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.0, 22.5, 28.6, 28.8, 31.1, 31.6, 35.8, 120.7, 121.8, 122.3, 127.4, 128.4, 128.8, 130.1, 131.7, 132.1, 135.3, 141.5, 147.4, 166.1, 203.1. MS (*m*/*z*): 323 (M^+), 305, 280, 248, 234, 222, 210, 189 (100), 180, 162, 145, 131, 118, 103, 91, 77, 65, 40.

*4.1.1.11. N-(2-Acetylphenyl)-4-fluoro-3-(trifluoromethyl)benzamide (**1k**).* Yield 84%. Mp 120 °C (dec.). IR (ν_{max}, KBr): 613, 763, 844, 910, 966, 1056, 1124, 1161, 1245, 1326, 1363, 1452, 1500, 1544, 1593, 1610, 1650, 1679, 3124, 3157 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H); 7.19 (ddd, 1H, *J* 0.7, 1.2, 6.0 Hz); 7.34 (t, 1H, *J* 9.3 Hz); 7.63 (ddd, 1H, *J* 1.2, 1.5, 5.9 Hz); 7.97 (dd, 1H, *J* 1.5, 6.0 Hz); 8.22–8.26 (m, 1H); 8.36 (dd, 1H, *J* 2.0, 4.5 Hz); 8.89 (dd, 1H, *J* 2.0, 7.5 Hz); 12.84 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 28.4, 115.9, 119.3, 121.0, 121.8, 122.3, 123.7, 124.9, 125.4, 127.6, 128.1, 130.5, 131.0, 136.1, 138.3, 144.2, 158.6, 161.0, 164.3, 203.6. MS (*m*/*z*): 325 (M^+), 310, 306, 282, 279, 264, 244, 234, 207, 191 (100), 170, 163, 143, 134, 106, 92, 75, 65, 43, 40.

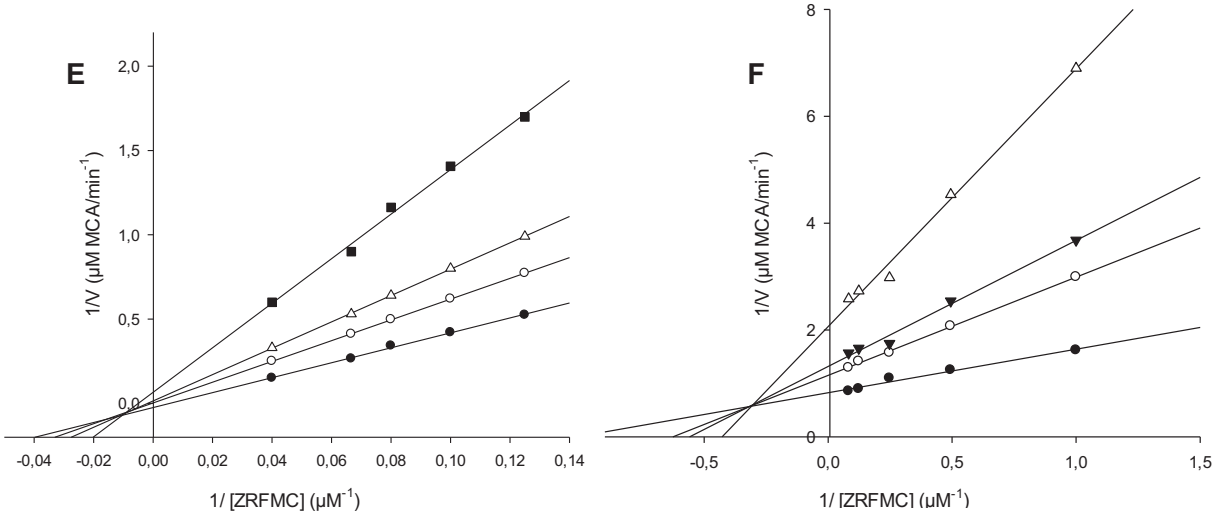


Fig. 3. Noncompetitive inhibition profile of compound **2b** (E) against catV and **2b** (F), against catL. Kinetic measurements were conducted in the presence of increasing concentrations of inhibitors. Panel E: 8.5 µM (△), 7.5 µM (▼) and 2.5 µM (○). Panel F: 7 µM (△), 5 µM (▼) and 3 µM (○). The absence of inhibitor is depicted by ● in panels A and B.

Table 6
K_i values of compounds **5i**, **5j**, **6a** and **6g**.

Compound	K_i (μM)	
	Cathepsin L	Cathepsin V
2b	1.5 noncomp	0.8 noncomp
5i	1.9 uncomp	0.8 uncomp
5j	1.9 uncomp	1.1 uncomp
6a	0.9 comp	1.3 comp
6g	0.2 comp	0.4 comp

*4.1.2. General procedure for the preparation of 4-quinolinones derivatives **2***

A 10 mL vial containing a magnetic stirring bar was charged with amides **1a**–**j** (0.3 mmol), *t*-BuOK (168.3 mg, 1.5 mmol) and THF (1.5 mL). The vial was sealed and the resulting suspension was heated at 100–120 °C, and 180 W for 10–20 min. The reaction mixture was cooled and poured in water (8 mL) and a solution of HCl 1 mol L^{-1} was added to adjusted pH 5–6. The solution was concentrated under reduced pressure until copious solid appeared. The solid was collected and washed successively with water and a cold mixture of acetone and dichloromethane (1:1 ratio) to give the pure product.

*4.1.2.1. 2-Phenyl-quinolin-4(1H)-one (**2a**) [67].* Yield 60%. Mp 255 °C (dec.). IR (ν_{max}, KBr): 690, 755, 772, 799, 839, 1474, 1505, 1547, 1583, 1595, 1610, 1636, 2329, 2357, 2969, 3067, 3090 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 6.54 (s, 1H); 7.32–7.73 (m, 5H); 7.98 (dd, 2H, *J* 1.4, 1.3 Hz); 8.25 (d, 1H, *J* 8.2 Hz); 8.83 (d, 1H, *J* 8.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 105.6, 118.9, 121.3, 125.0, 125.7, 129.4, 129.5, 131.8, 139.6, 158.0, 176.9. MS (*m*/*z*): 221 (M^+), 204, 193, 189, 161, 135, 119, 95, 91, 67, 55 (100).

*4.1.2.2. 2-(4-Butylphenyl)quinolin-4(1H)-one (**2b**).* Yield 67%. Mp ≥300 °C. IR (ν_{max}, KBr): 752, 829, 1257, 1319, 1400, 1440, 1500, 1539, 1573, 1595, 1631, 2858, 2927, 2952, 3074, 3147, 3257 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 0.88 (t, 3H, *J* 7.2 Hz); 1.31 (sex, 2H, *J* 7.4 Hz); 1.58 (qui, 2H, *J* 7.7 Hz); 2.64 (t, 2H, *J* 7.7 Hz); 6.49 (s, 1H); 7.31–7.67 (m, 7H); 8.18 (d, 1H, *J* 8.8 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 14.2, 23.3, 33.7, 34.6, 105.2, 119.7, 125.4, 126.0, 128.5, 130.4, 139.7, 147.8, 157.9, 175.4. Anal. Calc. for $C_{19}H_{19}NO$: C 82.31; H 6.86; N 5.05. Found: C 81.98; H 6.51; N 4.92.

*4.1.2.3. 2-(Furan-2-yl)quinolin-4(1H)-one (**2c**) [68].* Yield 73%. Mp 248 °C (dec.). IR (ν_{max}, KBr): 747, 762, 831, 839, 1465, 1501, 1569, 1647, 1699, 2336, 2359, 2860, 2916 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ: 7.27 (s, 1H); 7.58 (dd, 1H, *J* 1.8, 1.9 Hz); 8.11 (ddd, 1H, *J* 1.5, 1.3, 1.2 Hz); 8.25 (d, 1H, *J* 0.5 Hz); 8.45 (dt, 1H, *J* 1.7, 1.5 Hz); 8.57 (dd, 1H, *J* 0.8, 0.7 Hz); 8.81–8.89 (m, 2H), 12.46 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 103.8, 111.8, 116.8, 122.5, 124.6, 126.1, 131.9, 139.0, 140.9, 145.6, 154.8, 177.7. MS (*m*/*z*): 207 (M^+), 177, 162, 149, 135 (100), 120, 107, 98, 82, 69, 55.

*4.1.2.4. 2-Undecylquinolin-4(1H)-one (**2d**) [69].* Yield 55%. Mp 155 °C (dec.). IR (ν_{max}, KBr): 756, 765, 1246, 1356, 1365, 1441, 1489, 1552, 1593, 1657, 1713, 2331, 2358, 2850, 2870, 2920, 2954 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 0.86 (t, 3H, *J* 6.5 Hz); 1.22–1.52 (m, 16H); 1.71 (qui, 2H, *J* 7.8 Hz); 2.69 (t, 2H, *J* 7.9 Hz); 6.19 (s, 1H); 7.35–7.41 (m, 1H); 7.66–7.70 (m, 2H); 8.11 (d, 1H, *J* 8.0 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 22.7, 25.6, 26.9, 28.7, 28.8, 29.4, 29.6, 29.7, 31.7, 34.9, 107.6, 118.13, 123.8, 124.5, 124.7, 131.6, 139.3, 156.7, 179.3. MS (*m*/*z*): 274 (M^+), 177, 162, 135, 121, 120, 106, 92, 69, 55.

*4.1.2.5. 2-(But-3-enyl)quinolin-4(1H)-one (**2e**).* Yield 20%. Mp 185 °C (dec.). IR (ν_{max}, KBr): 455, 520, 667, 753, 908, 1387, 1430, 1502, 1550, 1597, 1639, 1658, 2846, 2880, 2949, 3001 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 1.54 (t, 2H, *J* 1.7 Hz); 1.94 (t, 2H, *J* 1.7 Hz); 5.06–5.23 (m, 2H); 5.86–6.06 (m, 1H); 6.72 (s, 1H); 7.18 (ddd, 1H, *J* 1.4, 1.2, 0.6 Hz), 7.63 (dt, 1H, *J* 1.6, 1.4 Hz); 7.97 (t, 1H, *J* 1.6 Hz); 8.85 (dd, 1H, *J* 1.2, 0.6 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 30.7, 34.1, 104.5, 111.7, 114.7, 118.6, 125.5, 129.5, 133.1, 136.1, 138.4, 146.6, 153.1, 177.9. Anal. Calc. for $C_{13}H_{13}NO$: C 78.39; H 6.53; N 7.03. Found: C 78.74; H 6.40; N 6.85. MS (*m*/*z*): 199 (M^+), 184 (100), 166, 154, 140, 128, 115, 92, 89, 77, 51.

*4.1.2.6. 2-(4-Fluorophenyl)quinolin-4(1H)-one (**2f**) [70].* Yield 72%. Mp ≥300°. IR (ν_{max}, KBr): 519, 592, 749, 830, 1162, 1240, 1400, 1514, 1592, 1606, 1635 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ: 6.89 (s, 1H); 7.41–7.53 (m, 4H), 7.81 (ddd, 1H, *J* 1.2, 1.0, 0.8 Hz); 8.00–8.07 (m, 1H); 8.15–8.23 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 104.4, 105.9, 114.6, 115.8, 116.2, 119.6, 123.9, 124.9, 130.5, 130.7, 132.6, 140.5, 150.9, 161.2, 166.2, 191.3.

*4.1.2.7. 2-(4-Ethylphenyl)quinolin-4(1H)-one (**2g**).* Yield 64%. Mp ≥300°. IR (ν_{max}, KBr): 532, 838, 1110, 1240, 1382, 1423, 1487, 1517, 1596, 1641, 2831, 2937, 2964, 3028 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 1.32 (t, 3H, *J* 7.7 Hz); 2.81 (q, 2 H, *J* 7.7 Hz); 7.36 (s, 1H); 7.56 (dt, 2H, *J* 1.9, 0.6 Hz); 7.82 (q, 1H, *J* 1.2 Hz); 7.93 (t, 2H, *J* 1.9 Hz); 8.08 (dt, 1H, *J* 1.6, 1.4 Hz); 8.20 (dd, 1H, *J* 0.8, 0.6 Hz); 8.46 (dd, 1H, *J* 0.8, 0.6 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 15.2, 28.0, 105.9, 120.0, 120.2, 123.3, 126.9, 128.6, 128.7, 129.2, 133.9, 140.1, 148.4, 154.1, 170.4. Anal. Calc. for $C_{17}H_{15}NO$: C 81.93; H 6.02; N 5.62. Found: C 82.05; H 5.93; N 5.74.

*4.1.2.8. 2-p-Tolylquinolin-4(1H)-one (**2h**) [47].* Yield 68%. Mp ≥300°. IR (ν_{max}, KBr): 482, 514, 536, 567, 671, 756, 815, 873, 958, 1024, 1141, 1186, 1245, 1315, 1357, 1440, 1471, 1510, 1542, 1595, 1635, 1652, 1701, 2914, 2964, 3066, 3087, 3116 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 2.46 (s, 3H); 6.78 (s, 1H); 7.42 (d, 2H, *J* 8.0 Hz); 7.52 (ddd, 1H, *J* 1.6, 1.7, 4.7 Hz), 7.73 (dd, 1H, *J* 1.7, 8.0 Hz); 7.80–7.90 (m, 2H); 8.31 (dd, 1H, *J* 0.8, 6.3 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 22.7, 103.7, 119.6, 123.7, 125.4, 125.6, 126.7, 127.9, 128.6, 132.8, 140.3, 141.5, 153.6, 176.4.

*4.1.2.9. 2-(4-Butoxyphenyl)quinolin-4(1H)-one (**2i**).* Yield 65%. Mp ≥300°. IR (ν_{max}, KBr): 540, 838, 1006, 1188, 1236, 1271, 1429, 1494, 1515, 1598, 1645, 2871, 2937, 2970, 3365 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 1.01 (t, 3H, *J* 7.2 Hz); 1.55 (sex, 2H, *J* 7.2 Hz); 1.82 (qui, 2H, *J* 5.9 Hz); 4.13 (t, 2H, *J* 6.2 Hz); 7.22 (dt, 2H, *J* 2.9, 1.9 Hz); 7.31 (s, 1H); 7.79 (q, 1H, *J* 1.2 Hz); 7.96 (dt, 2H, *J* 2.2, 1.9 Hz), 8.05 (ddd, 1H, *J* 1.7, 1.5, 1.2 Hz); 8.15 (dd, 1H, *J* 1.5, 1.4 Hz); 8.43 (dd, 1H, *J* 1.0, 0.9 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 12.7, 19.5, 31.2, 67.1, 104.3, 115.0, 118.0, 121.1, 123.2, 124.9, 125.9, 132.6, 140.0, 154.9, 160.8, 176.7. Anal. Calc. for $C_{19}H_{19}NO_2$: C 77.81; H 6.48; N 4.77. Found: C 77.68; H 6.15; N 5.09.

*4.1.2.10. 2-(4-Hexylphenyl)quinolin-4(1H)-one (**2j**).* Yield 63%. Mp ≥300°. IR (ν_{max}, KBr): 526, 584, 649, 756, 815, 829, 865, 1022, 1143, 1257, 1319, 1440, 1500, 1541, 1595, 1633, 2854, 2927, 2956, 3006 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 0.91 (t, 3H, *J* 6.9 Hz); 1.28–1.46 (m, 6H); 1.70 (qui, 2H, *J* 8.1 Hz); 2.77 (t, 2H, *J* 7.3 Hz); 7.37 (s, 1H); 7.53 (d, 2H, *J* 8.5 Hz); 7.81 (dt, 1H, *J* 1.4, 1.2 Hz), 7.93 (ddd, 2H, *J* 2.0, 1.9, 1.8 Hz); 8.08 (ddd, 1H, *J* 1.6, 1.5, 1.4 Hz); 8.20 (dd, 1H, *J* 0.8, 0.6 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 14.2, 22.2, 28.4, 31.1, 31.7, 37.3, 104.8, 118.1, 122.9, 125.7, 125.8, 125.9, 126.5, 127.3, 127.8, 132.6, 140.1, 142.6, 153.8, 177.2. Anal. Calc. for $C_{21}H_{23}NO$: C 82.62; H 7.54; N 4.59. Found: C 82.34; H 7.24; N 4.27.

*4.1.2.11. 2-(4-Fluoro-3-(trifluoromethyl)phenyl)quinolin-4(1H)-one (**2k**).* Yield 54%. Mp ≥300°. IR (ν_{max}, KBr): 757, 833, 1139, 1232, 1247, 1504, 1602, 1633 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ: 6.57 (s,

1H); 6.99 (d, 1H, *J* 8.6 Hz); 7.42 (ddd, 1H, *J* 0.8, 1.3, 4.7 Hz); 7.71–7.81 (m, 3H); 8.24 (dd, 1H, *J* 0.8, 6.8 Hz); 8.32 (d, 1H, *J* 2.5 Hz). ^{13}C NMR (50 MHz, CD_3OD) δ: 104.3, 112.9, 114.6, 119.4, 120.4, 120.8, 123.1, 125.7, 125.9, 126.6, 126.8, 127.7, 131.4, 132.7, 133.2, 139.6, 147.0, 156.1, 161.1, 176.2. Anal. Calc. for $C_{16}H_9NOF_4$: C 62.54; H 2.93; N 4.56. Found: C 62.31; H 3.01; N 4.33.

4.1.3. General procedure for the preparation of acridone alkaloids ***6***

A mixture of *N*-arylanthranilic acid [56] **5** (0.16 mmol) and $POCl_3$ (1.0 mL) was refluxed for 2 h under dry nitrogen atmosphere. $POCl_3$ excess was removed under reduced pressure and an 8:1 solution of ethanol: 10% chloridric acid (5.0 mL) was added to the crude product and the mixture was refluxed during 1 h. After this time, the reaction mixture was allowed to cool, water (5.0 mL) was added and the resulting precipitated was removed by filtration, washed with water and dried in a dissector to give **6** as yellow solid.

*4.1.3.1. 3-(Benzyloxy)acridin-9(10H)-one (**6a**).* Yield 82%. Mp 327 °C (dec.); ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 11.98 (s, 1H), 8.25 (d, 2H, *J* 8.0 Hz), 7.81 (t, 1H, *J* 8.0 Hz), 7.67 (d, 1H, *J* 8.0 Hz), 7.43 (m, 6H), 7.14 (dd, 1H, *J* 10.0, 2.0 Hz), 7.05 (d, 1H, *J* 2.0 Hz), 5.20 (s, 2H). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 167.4, 1662.2, 143.6, 140.0, 136.6, 134.3, 129.0, 127.6, 126.1, 125.6, 124.0, 120.2, 118.1, 114.5, 110.0, 97.8, 71.3. Anal. Calc. for $C_{20}H_{15}NO_2$: C 79.72; H 5.02; N 4.65. Found: C 79.63; H 4.91; 4.54.

*4.1.3.2. 2-(Benzyloxy)-3-methoxyacridin-9(10H)-one (**6c**).* Yield 85%. Mp 297 °C (dec.); IR (ν_{max}, KBr): 3146, 1633, 1591, 1562, 1501, 1276, 1128, 746 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 3.98 (s, 3H); 5.08 (s, 2H); 6.90 (s, 1H); 7.26–7.68 (m, 10H); 8.10 (d, 1H, *J* 8.0 Hz). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 56.7; 70.9; 97.1; 102.0; 109.8; 114.8; 117.8; 123.8; 124.9; 127.6 (2C); 128.6; 128.7 (2C); 134.7; 134.8; 138.3; 138.9; 148.0; 159.7; 165.3. Anal. Calc. for $C_{21}H_{17}NO_3$: C 76.12; H 5.17; N 4.23. Found: C 75.91; H 5.15; N 4.55.

*4.1.3.3. 2-Methoxyacridin-9(10H)-one (**6d**).* Yield 89%. Mp 278 °C (dec.); IR (ν_{max}, KBr): 2983, 1683, 1591, 1533, 1475, 1364, 1233, 1160, 1033, 757 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 3.84 (s, 3H); 7.27–7.74 (m, 7H); 8.17 (d, 1H, *J* 8 Hz). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 55.6; 100.4; 115,0; 116.0; 118,2; 119.9; 124.1; 125.2; 130.2; 135.4; 136.0; 138.8; 156.7; 167.8. Anal. Calc. for $C_{20}H_{17}NO_3$: C 74.65; H 4.92; N. 6.22. Found: C 74.57; H 4.15; N 7.13.

*4.1.3.4. 6-(Benzyloxy)-2-nitroacridin-9(10H)-one (**6e**).* Yield 90%. Mp 300 °C (dec.); IR (ν_{max}, KBr): 3188, 2912, 2852, 1638, 1603, 1570, 1507, 1471, 1322, 1182, 1122, 817, 552 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 7.19 (d, 1H, *J* 2.0 Hz); 7.26–7.68 (m, 7H); 7.87 (d, 1H, *J* 10 Hz); 8.45 (d, 1H, *J* 8.0 Hz); 8.57 (dd, 1H, *J* 8.0, 2.0 Hz); 9.37 (d, 1H, *J* 2.0 Hz). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 71.9; 99.1; 103.1; 115.2; 119.1; 120.6; 122.8; 127.5; 127.8 (2C); 129.1 (2C); 129.2; 129.3; 134.2; 142.6; 143.6; 144.8; 167.8; 171.4. Anal. Calc. For $C_{20}H_{14}N_2O_4$: C 69.36; H 4.07; N 8.09. Found: C 69.45; H 4.28; N 8.27.

*4.1.3.5. 2-Methoxy-7-nitroacridin-9(10H)-one (**6f**).* Yield 87%. Mp >350 °C; IR (ν_{max}, KBr): 3097, 1639, 1607, 1574, 1494, 1332, 1155, 826, 749 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 4.04 (s, 3H); 7.81–7.95 (m, 3H); 8.03 (d, 1H, *J* 10 Hz); 8.66 (d, 1H, *J* 8.0 Hz); 9.54 (s, 1H). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 56.2; 102.0; 115.9; 118.6; 120.7; 120.8; 122.9; 128.9; 132.6; 137.9; 141.5; 144.0; 158.7; 171.9. Anal. Calc. For $C_{14}H_{10}N_2O_4$: C 62.22; H 3.73; N 10.37. Found: C 62.73; H 3.92; N 10.56.

*4.1.3.6. 2-(Benzyloxy)-7-nitroacridin-9(10H)-one (**6g**).* Yield 78%. Mp 295 °C (dec.); IR (ν_{max}, KBr): 3269, 3152, 1612, 1604, 1569, 1329, 1181, 748 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 5.34 (s, 2H); 7.25–7.48 (m, 8H); 7.94 (d, 1H, *J* 10 Hz); 8.51 (d, 1H, *J* 10.0 Hz); 8.66 (dd, 1H, *J* 10.0, 2.0 Hz); 9.47 (d, 1H, *J* 2.0 Hz). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 72.1; 99.2; 111.9; 114.19; 120.2; 121.2; 122.8; 127.3; 127.8 (2C); 129,1 (2C); 129.2; 129.6; 134.2; 142.7; 143.9; 145.2; 168.2; 170.9. Anal. Calc. For $C_{20}H_{14}N_2O_4$: C 69.36; H 4.07; N 8.09. Found: C 69.27; H 4.10; N 8.49.

*4.1.3.7. 2-Nitroacridin-9(10H)-one (**6h**).* Yield 82%. Mp 295 °C (dec.); spectral data were according to those previously reported [71].

*4.1.3.8. 4-Methoxyacridin-9(10H)-one (**6m**).* Yield 99%. Mp 295 °C; IR (ν_{max}, KBr): 1603, 1331, 1156 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 4.05 (s, 3H); 7.15–7.47 (m, 4H); 7.84–7.95 (m, 3H); 8.36 (d, 1H, *J* 8 Hz). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 56.4; 113.2; 115.8; 116.3; 116.5; 118.5; 124.9; 125.5; 132.2; 136.6; 139.6; 147.4; 171.7. Anal. Calc. For $C_{14}H_{11}NO_2$: C 74.65; H 4.92; N 6.22. Found: C 74.57; H 5.02; N 6.54.

*4.1.3.9. 2-(Benzyloxy)-6,7-difluoro-3-methoxyacridin-9(10H)-one (**6p**).* Yield 93%. Mp 294 °C (dec.). IR (ν_{max}, KBr): 3266, 3186, 1649, 1288, 1200, 1093, 873, 807, 748, 698 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 4.06 (s, 3H); 5.19 (s, 2H); 7.07 (s, 1H); 7.36–7.69 (m, 8H); 7.95–8.04 (m, 1H). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 56.9; 71.57; 97.44; 102.1; 102.9; 105.7 (d, *J* 22 Hz); 101.1; 110.7 (d, *J* 22 Hz); 111.8; 127.8 (2C); 128.5 (2C); 134.4; 136.4; 139.8; 149.0; 159.9; 164.7. Anal. Calc. For $C_{21}H_{15}F_2NO_3$: C 68.66; H 4.12; N 3.81. Found: C 68.59; H 4.18; N 3.92.

*4.1.3.10. 7-(Benzyloxy)-2,3-difluoroacridin-9(10H)-one (**6q**).* Yield 93%. Mp 294 °C (dec.). IR (ν_{max}, KBr): 3100, 2919, 1647, 1599, 1490, 1382, 1289, 1097, 784, 564 cm^{-1}. ^{1}H NMR (200 MHz; $CDCl_3$/TFA 10%) δ: 5.26 (s, 2H); 7.40–7.46 (m, 6H); 7.73–7.91 (m, 4H); 8.33–8.38 (m, 1H). ^{13}C NMR (50 MHz; $CDCl_3$/TFA 10%) δ: 71.5; 101.6; 106.5; 101.7 (d, *J* 22 Hz); 112.6; 116.2; 120.7; 127.9 (2C); 128.0; 129.0 (2C); 132.6; 135.0; 137.6; 137.7; 157.7; 166.7. Anal. Calc. For $C_{21}H_{15}F_2NO_3$: C 68.66; H 4.12; N 3.81. Found: C 68.59; H 4.18; N 3.92.

4.2. Kinetic measurements

Recombinant human cathepsins V and L were produced using the *Pichia pastoris* expression system as previously described [72]. The molar concentrations of the enzymes were determined by active site titration using E-64 following the conditions previously described [73].

All commercially available reagents were purchased from Aldrich Chemical Co and Sigma. 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 cathepsins V and L at initial concentration of 25 μM. Inhibition measurements were carried out in triplicate in 96-well black plates as previously described [43,44].

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 inhibitor, and 1 μL of catV (32 nM) or catL (20 nM). The enzymes were activated with DTE at 27 °C for 5 min. Z-Phe-Arg-MCA was added to start the reaction, and the fluorescence of released 4-methyl-7-coumaryl-amide 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. The percentage of inhibition was calculated according to the equation: % 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. The selectivity ratio of the

inhibitors (catL/catV) was determinate according to the equation: Selectivity (S) = IC_{50} catL/IC_{50} catV.

Values of IC_{50} were determined by making rate measurements for at least seven inhibitor concentrations (inhibition range: 20–85%) from the collected data by nonlinear regression.

To determine the mechanism of inhibition and K_i values, compounds **2b**, **2d**, **5i**, **5j**, **6a** and **6g** were tested under the same experimental conditions for three different inhibitor concentrations (variable depending on IC_{50}) and seven concentrations of Z-Phe-Arg-MCA (0.8–39 μM) and were determined using the Lineweaver–Burk plot analysis. All kinetic parameters were analyzed using the SigmaPlot enzyme kinetics module. The values represent means of at least three individual experiments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2012.04.002. These data include MOL files and InChiKeys of the most important compounds described in this article.

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