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SPECIFIC INHIBITORS OF PUROMYCIN-SENSITIVE AMINOPEPTIDASE WITH A 3-(HALOGENATED PHENYL)-2,4(1*H*,3*H*)-QUINAZOLINEDIONE SKELETON

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Abstract – Specific puromycin-sensitive aminopeptidase (PSA) inhibitors with a 3-(halogenated phenyl)-2,4(1*H*,3*H*)-quinazolinedione skeleton were prepared and their structure–activity relationships were investigated. The nature (F, Cl or Br), number and position(s) of the halogen atom(s) introduced into the 3-phenyl group were concluded to be critical determinants of the inhibitory activity.

INTRODUCTION

Puromycin-sensitive aminopeptidase (PSA: EC 3.4.11.14) is a ubiquitous, 100-kDa, Zn^{2+} metallopeptidase with a substrate specificity similar to that of aminopeptidase N (APN), and is present at high concentrations in the brain (especially striatum, hippocampus and cerebellum).[1-7] Although PSA was initially purified as a candidate enkephalinase by Hersh and McKelvy in 1981,[8,9] its localization (predominantly in the cytoplasm) and its broad distribution in tissues argue against such a function.[6,10-12] Instead, PSA has been implicated in many physiological processes, including regulation of the cell cycle and onset of apoptosis,[6,13] antigen processing in the class I MHC pathway,[14-16] reproductive function,[17,18] and regulation of neuropeptide levels.[19,20]

PSA was recently identified as a major peptidase digesting neuronal TAU protein and showing protective activity against TAU-induced neurodegeneration in Alzheimer's disease and other tauopathies.[21-26] It was also demonstrated that PSA is a major peptidase responsible for the degradation of polyglutamine repeats, implicating this enzyme in the pathogenesis of polyQ diseases, including Huntington's disease.[27] PSA is also involved in digestion of polyglutamine sequences released by proteasomes and removal of neurotoxic polyglutamine-expanded Htt exon-1, ataxin-3, mutant synuclein and superoxide dismutase 1 via the autophagy system.[28] These reports suggest that PSA might represent a novel degradation

Dedicated to Professor Ei-ichi Negishi (Purdue University) on the occasion of his 77th birthday

mechanism targeting aggregate-prone neurotoxic protein substrates, including mutated Htt. Nevertheless, the physiological role(s)/function(s) of PSA have remained unclear because of the low substrate specificity of the enzyme and the lack of specific inhibitors.[5-7,12]

Puromycin (**1**) is an inhibitor of PSA that is effective at a low concentration,[8] but it is not a specific inhibitor of PSA. This is because the amino acid sequences recognized by **1**, i.e., the catalytic site for hydrolysis and the substrate-binding site, are similar among various neutral alanine-aminopeptidases.[29] On the other hand, we have reported potent non-peptide, small-molecular PSA-specific inhibitors with a homo-phthalimide or a quinazolinedione skeleton derived from thalidomide (**2**), including *N*-(2,6-diethylphenyl)homophthalimide (PIQ-22, **3**), 3-(2,6-diethylphenyl)-2,4(1*H*,3*H*)-quinazolinedione (PAQ-22, **4**) and 1-methyl-3-(2,6-diethylphenyl)-2,4(1*H*,3*H*)-quinazolinedione (MPAQ-22, **5**), ANTAQ (**6**) and DAMPAQ-22 (**7**) (Figure 1).[28,30-40] They are all potent PSA-specific non-competitive inhibitors with IC50 values of 3–8 μM. The potencies of these inhibitors are comparable to those of bestatin and actinonin (competitive inhibitors).[30-39] By employing these PSA inhibitors, we identified possible roles of the enzyme in cell mobility/invasion/apoptosis.[32-34,37,41] These inhibitors also showed dose-dependent cell invasion-inhibitory activity in a Matrigel assay using mouse melanoma B16F10/L5 cells, together with low cell toxicity.[38]

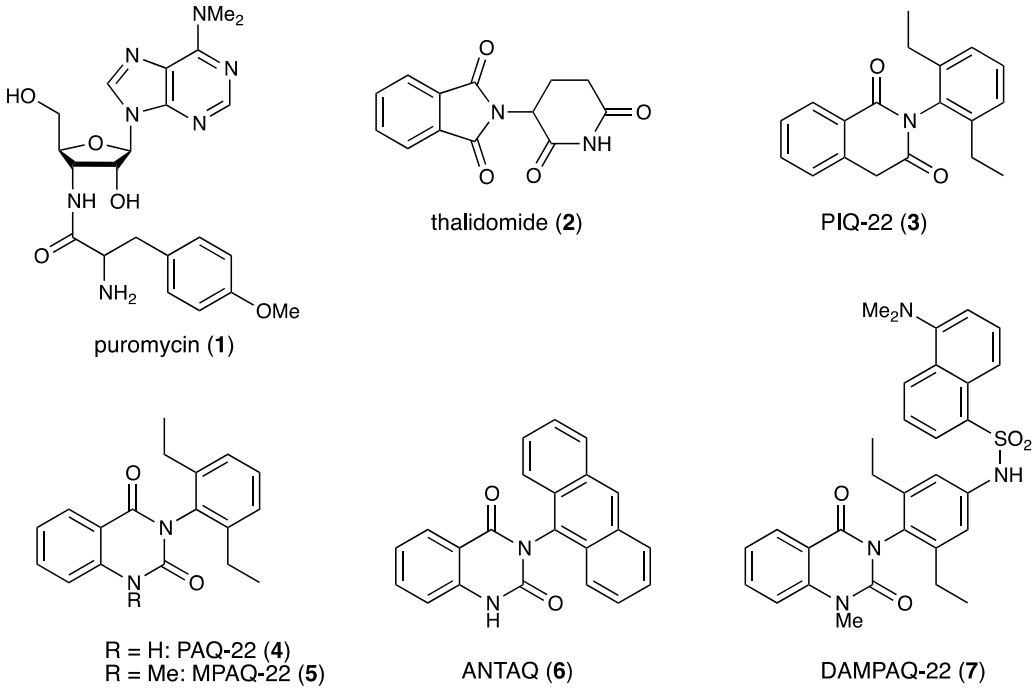


Figure 1. Structures of puromycin (**1**), thalidomide (**2**), PSA-specific inhibitors (PIQ- 22: **3**, PAQ-22: **4**, MPAQ-22: **5** derived from thalidomide, and the designed fluorescent bioprobes (ANTAQ: **6**, DAMPAQ-22: **7**).

On the other hand, a derivative of quinazolinedione named mdivi-1 (mitochondrial division inhibitor-1, *vide infra*, Scheme 2) has been reported as a selective inhibitor of mitochondrial fission-related Drp1 (dynamin-related protein 1).[42-44] Mdivi-1 inhibits GTPase activity by blocking the self-assembly of Drp1

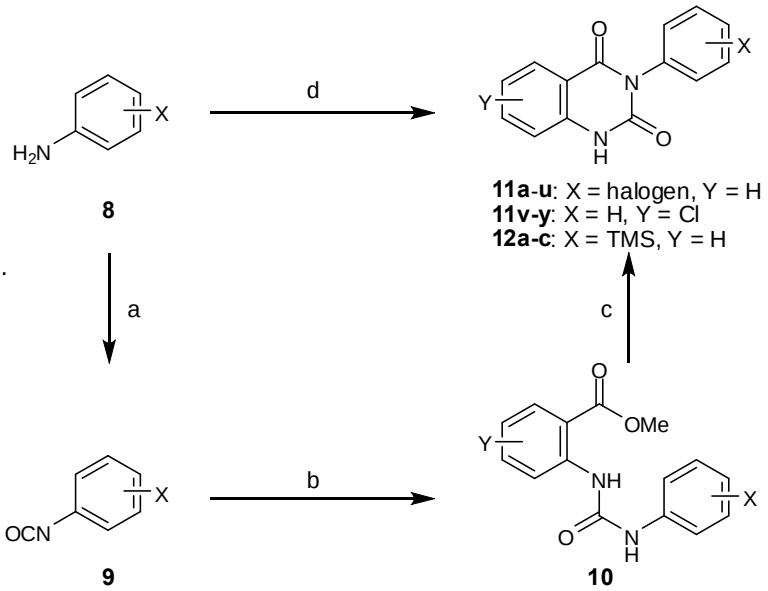
in vitro and causes rapid, reversible and dose-dependent formation of netlike mitochondria in wild-type cells; this may be significant, because mitochondrial dysfunction is known to be a key event in the pathogenesis of Huntington's disease, and mutant huntingtin has been reported to increase GTPase activity and to trigger mitochondrial fragmentation,[45] suggesting possible nerve cell-protecting activity of mdivi-1 in Huntington's disease. Therefore, both PSA and Drp1 might play a role in the pathophysiology of Huntington's disease. In addition, mdivi-1 possesses a quite similar structure to 3-phenyl chlorinated derivatives of the above-mentioned quinazolinediones, including PAQ-22 (**4**). This prompted us to examine the PSA-inhibitory activity of mdivi-1 and halogenated derivatives of quinazolinedione.

In this article, we describe studies on the structure-activity relationship of 3-(fluorophenyl, chlorophenyl, bromophenyl or silicon-substituted phenyl)-2,4(1*H*,3*H*)-quinazolinedione, as well as an examination of the PSA-inhibitory activity of mdivi-1, with the aim of structural optimization of PSA inhibitors to achieve potent and specific inhibitory activity.

RESULTS AND DISCUSSION

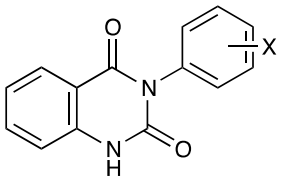
We first introduced a halogen atom(s) into the 3-phenyl group of 3-phenyl-2,4(1*H*,3*H*)-quinazolinedione to obtain **11a-u**. Also, 5-, 6-, 7- and 8-chloro-3-phenyl-2,4(1*H*,3*H*)-quinazolinediones (**11v-y**) were prepared. The synthetic method is summarized in Scheme 1.[38,46] Compounds **11a-y** were prepared by condensation of halophenyl isocyanate or phenyl isocyanate (**9**) with methyl anthranilate to give urea (**10**), followed by cyclization of the resulting urea under basic conditions in one pot (Scheme 1).[46] Halophenyl isocyanate (**9**) was prepared from the corresponding haloaniline (**8**), triphosgene and triethylamine in toluene. 3-((Trimethylsilyl)phenyl)quinazoline-2,4(1*H*,3*H*)-dione (**12a-c**) was prepared by condensation and cyclization of methyl 2-isocyanatobenzoate and the corresponding trimethylsilylaniline (**8**) (Scheme 1).

Scheme 1. Reagents and conditions: (a) triphosgene, triethylamine, toluene, reflux, 2 h. (b) methyl anthranilate, toluene, reflux 2 h. (c) 2 M NaOH, EtOH, 80 °C, 30 min, then acidified with 2 M HCl. (d) methyl 2-isocyanatobenzoate, triethylamine, MeCN, 80 °C, 4 h.



Inhibition of PSA by these compounds was assessed by measuring 7-amino-4-methylcoumarin (AMC) liberated from L-methylcoumarylamide (Ala-AMC) using intact human acute lymphoblastic leukemia MOLT-4 cells.[33,36,37,47] In order to examine the specificity of PSA-inhibitory activity, inhibition of another aminopeptidase, APN, by the compounds was also assessed by measuring AMC liberated from Ala-AMC with human promyelocytic leukemia HL-60 cells. All experiments were performed at least in duplicate, and the IC_{50} values obtained are given in Tables 1 and 2.

Table 1. Aminopeptidase (PSA and APN)-Inhibitory Activity (IC_{50} values) of 3-Phenylquinazoline-2,4(1*H*,3*H*)-dione Derivatives **11a-11u**



11a-u

compd	X	PSA IC_{50} (μM)	APN IC_{50} (μM)
4: PAQ-22	2,6-diethyl	3.8	>100
11a	*o*-F	>100	>100
11b	*m*-F	>100	>100
11c	*p*-F	>100	>100
11d	2,6-F	>100	>100
11e	3,5-F	>100	>100
11f	2,4,6-F	>100	>100
11g	2,3,4,5,6-F	>100	>100
11h	*o*-Cl	67.1	>100
11i	*m*-Cl	46.6	>100
11j	*p*-Cl	>100	>100
11k	2,6-Cl	17	>100
11l	3,5-Cl	>100	>100
11m	2,4,6-Cl	6.5	>100
11n	2,3,4,5,6-Cl	2.4	>100
11o	*o*-Br	46.9	>100
11p	*m*-Br	28.9	>100
11q	*p*-Br	>100	>100
11r	2,6-Br	8.3	>100
11s	3,5-Br	>100	>100
11t	2,4,6-Br	5.3	>100
11u	2,3,4,5,6-Br	0.82	>100

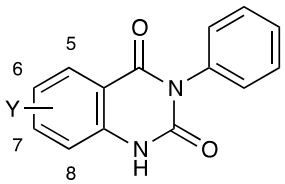
As shown in Table 1, all of the fluoro-substituted compounds (**11a**—**11g**) were inactive (the IC_{50} values are higher than 100 μM, though slight PSA inhibition was observed at this concentration; data not shown). Concerning chloro derivatives **11h**—**11n**, all of the compounds, except the *para*- (**11j**) and 3,5-disubstituted (**11l**) compounds, showed moderate PSA-inhibitory activity with IC_{50} values of 2.4—67.1 μM. Among the active chlorinated derivatives, the activity decreased in the order of

2,3,4,5,6-pentasubstituted (**11n**) > 2,4,6-trisubstituted (**11m**) > 2,6-disubstituted (**11k**) > *meta*-substituted (**11i**) > *ortho*-substituted (**11h**). This tendency is just the same as for the brominated derivatives **11o**—**11u**, and the *para*-bromo (**11q**) and 3,5-dibromo (**11s**) compounds are inactive, as in the case of the corresponding chlorinated derivatives, **11j** and **11l**, respectively. Like PAQ-22 (**4**), none of the compounds listed in Table 1 showed apparent APN-inhibitory activity.[38] Pentahalogenated derivatives, **11n** and **11u**, are more potent PSA-selective inhibitors than PAQ-22 (**4**). Among the active bromo and chloro derivatives, the bromo derivative is a more potent PSA inhibitor than the corresponding chloro derivative, i.e., **11o** >**11h**, **11p** >**11i**, **11r** >**11k**, **11t** >**11m**, and **11u** >**11n**. It seems quite difficult to interpret these structure-activity relationships at this stage. Among the mono-substituted derivatives, **11h**—**11j** and **11o**—**11q**, *meta*-substitution seems to be best for PSA-inhibitory activity, while *ortho*-substitution seems moderately effective, and *para*-substitution seems ineffective. However, among disubstituted derivatives, i.e., **11k**, **11l**, **11o** and **11q**, the *meta*-substituted (3,5-disubstitution) compounds are inactive, whereas *para*-substitution (2,6-disubstitution) seems to be effective.

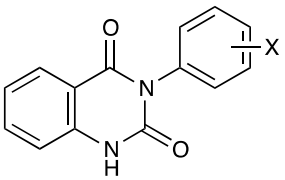
As for the effects of a substituent on the aromatic ring of the quinazolinedione moiety (**11v**—**11y**), only **11y** showed slight PSA-inhibitory activity (Table 2). This result is consistent with our previously reported structural development studies of PAQ-22 (**4**), in which we established the importance of the 8-position on the aromatic ring of the quinazolinedione moiety for PSA-inhibitory activity.[38]

Next, 3-(trimethylsilyl-substituted phenyl)-2,4(1*H*,3*H*)-quinazolinediones **12a**—**12c** were investigated to check the influence of the steric factor on PSA-inhibitory activity (Table 2). Among compounds **12a**—**12c**, only *ortho*-substituted derivative **12a** showed relatively potent PSA-inhibitory activity, while among the mono-halogenated derivatives, *meta*-substituted ones are more potent than the corresponding *ortho*-derivatives.

Table 2. Aminopeptidase-Inhibitory Activity of 3-Phenylquinazoline-2,4(1*H*,3*H*)-dione Derivatives **11v-11y** and **12a-12c**



11v-11y



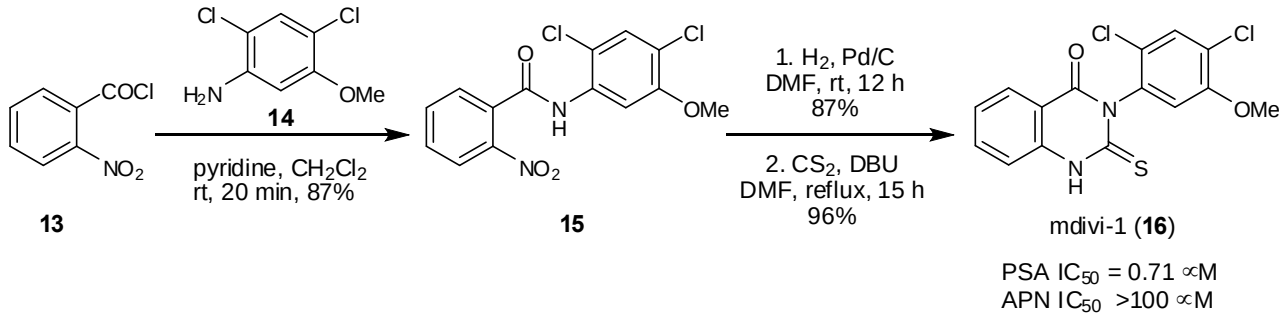
12a-12c

compd	Y	PSA IC_{50} (μM)	APN IC_{50} (μM)
PAQ-00	H	>100	>100
11v	5-Cl	>100	>100
11w	6-Cl	>100	>100
11x	7-Cl	>100	>100
11y	8-Cl	92.9	>100

compd	X	PSA IC_{50} (μM)	APN IC_{50} (μM)
12a	*o*-TMS	5.9	>100
12b	*m*-TMS	>100	>100
12c	*p*-TMS	>100	>100

Finally, we investigated whether mitochondrial division inhibitor (mdivi)-1 inhibits PSA.

Mdivi-1 was prepared as shown in Scheme 2, by treatment of the amide compound (**15**) with CS_2 and DBU in DMF. Amido compound (**15**) was prepared from 2-nitrobenzoyl chloride (**13**) and 2,4-dichloro-5-methoxyaniline (**14**). The aminopeptidase-inhibitory activities of mdivi-1 were evaluated. These results are summarized in terms of IC_{50} values in Scheme 2. Inhibitory activity of mdivi-1 towards PSA was more potent than that of PAQ-22 (**4**), but mdivi-1 showed no inhibitory activity toward APN. Therefore, even though mdivi-1 is a selective Drp1 inhibitor (*vide infra*), it should be noted that a part of its biological activities may be elicited by inhibition of PSA.



Scheme 2. Preparation and Aminopeptidase (PSA and APN)-Inhibitory Activity (IC_{50} values) of mdivi-1 (**16**)

In conclusion, specific inhibitors of PSA with 3-(halogenated phenyl)- and 3-(trimethylsilyl-substituted phenyl)-2,4(1*H*,3*H*)-quinazolinedione structures were prepared and their structure–activity relationships were investigated. Pentachlorinated (**11n)** and pentabrominated (**11u)** derivatives were discovered to be potent PSA-specific inhibitors among the prepared compounds. Compounds **12a** (a silicon-containing derivative) and mdivi-1 were also rather potent PSA-selective inhibitors. These results indicate generality of the quinazolinedione skeleton as a platform for specific inhibitors of PSA.

EXPERIMENTAL

Abbreviations. CS_2, carbon disulfide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOH, ethanol; EtOAc, ethyl acetate; Et_3N, triethylamine; Hex, n-hexane; MeOH, methanol.

General Comments. Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and results were within ±0.3% of the theoretical values. NMR spectra were recorded on a JEOL JNM-□-500 (500 MHz) spectrometer. Unless otherwise noted, samples were dissolved in $CDCl_3$. Chemical shifts are expressed in δ (ppm) values, and coupling constants are expressed in hertz (Hz). NMR spectra were referenced to tetramethylsilane as an

internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, quint = quintet, m = multiplet, and brs = broad singlet. Mass spectra were recorded on a JEOL spectrometer.

Materials. Unless otherwise noted, materials were purchased from Tokyo Kasei Co., Aldrich Inc., and other commercial suppliers and were used after appropriate purification (distillation or recrystallization).

General Procedure for the Synthesis of 3-Substituted 2,4(1*H*,3*H*)-Quinazolinediones from Amines

To a mixture of amine (**8**) (1.0 mmol) and Et_3N (2.0 mmol) in toluene (10 mL) was added triphosgene (0.40 mmol), and the resulting solution was heated at reflux until the starting amine disappeared (for *ca*. 2 h). Next, the appropriate methyl anthranilate (1.0 mmol) was added, and the resulting mixture was stirred at reflux for 2 h. The solvent was removed under reduced pressure, and EtOH (2 mL) and 2 N NaOH solution (1 mL) were added to the residue. The reaction mixture was stirred at 80 °C for 30 min. This solution was cooled, diluted with water, acidified with 2 N HCl (*ca*. 2 mL), and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel column chromatography (eluent: EtOAc/hexane or $CHCl_3$/MeOH) gave the 3-substituted 2,4(1*H*,3*H*)-quinazolinedione (**11a**-**y**).

3-(2-Fluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11a) According to the general procedure, **11a** was obtained in 70% yield as pale yellow solid after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.36 (br s, 1H), 8.14 (d, 1H, *J* = 8.0 Hz), 7.65 (dd, 1H, *J* = 7.6, 7.9 Hz), 7.47 (m, 1H), 7.35-7.24 (m, 4H), 7.16 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 257 $(M+H)^+$; Anal. Calcd for $C_{14}H_9FN_2O_2$: C, 65.62; H, 3.54; N, 10.93. Found: C, 65.62; H, 3.64; N, 10.90.

3-(3-Fluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11b) According to the general procedure, **11b** was obtained in 67% yield as white solid after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.41 (br s, 1H), 8.17 (d, 1H, *J* = 8.0 Hz), 7.66 (dd, 1H, *J* = 7.3, 8.0 Hz), 7.49 (m, 1H), 7.30-7.03 (m, 5H); FAB-MS m/z: 257 $(M+H)^+$.

3-(4-Fluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11c) According to the general procedure, **11c** was obtained in 59% yield as white plates after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.36 (br s, 1H), 8.17 (d, 1H, *J* = 8.0 Hz), 7.66 (dd, 1H, *J* = 7.4, 7.9 Hz), 7.29-7.20 (m, 5H), 7.03 (d, 1H, *J* = 8.6 Hz); FAB-MS m/z: 257 $(M+H)^+$; Anal. Calcd for $C_{14}H_9FN_2O_2$: C, 65.62; H, 3.54; N, 10.93. Found: C, 65.52; H, 3.68; N, 11.02.

3-(2,6-Difluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11d) According to the general procedure, **11d**

was obtained in 44% yield as pale yellow solid after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.35 (br s, 1H), 8.18 (d, 1H, *J* = 7.9 Hz), 7.67 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.45 (m, 1H), 7.29 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.10-7.05 (m, 3H); FAB-MS m/z: 275 $(M+H)^+$; Anal. Calcd for $C_{14}H_8F_2N_2O_2$: C, 61.32; H, 2.94; N, 10.22. Found: C, 61.12; H, 3.17; N, 10.06.

3-(3,5-Difluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11e) According to the general procedure, **11e** was obtained in 43% yield as white crystals after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.45 (br s, 1H), 8.13 (d, 1H, *J* = 7.9 Hz), 7.66 (dd, 1H, *J* = 7.3, 8.5 Hz), 7.26 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.16 (d, 1H, *J* = 8.5 Hz), 6.96-6.87 (m, 3H); FAB-MS m/z: 275 $(M+H)^+$; Anal. Calcd for $C_{14}H_8F_2N_2O_2$: C, 61.32; H, 2.94; N, 10.22. Found: C, 61.15; H, 3.07; N, 10.17.

3-(2,4,6-Trifluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11f) According to the general procedure, **11f** was obtained in 73% yield as yellow powder after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.18 (d, 1H, *J* = 8.0 Hz), 8.01 (br s, 1H), 7.69 (dd, 1H, *J* = 8.6, 7.4 Hz), 7.30 (dd, 1H, *J* = 8.0, 7.4 Hz), 7.06 (d 1H, *J* = 8.6 Hz), 6.86 (t, 2H, *J* = 7.9, 8.6 Hz); FAB-MS m/z: 293 $(M+H)^+$; Anal. Calcd for $C_{14}H_7F_3N_2O_2$: C, 57.54; H, 2.41; N, 9.57. Found: C, 57.42; H, 2.64; N, 9.51.

3-(2,3,4,5,6-Pentafluorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11g) According to the general procedure, **11g** was obtained in 88% yield as colorless plates after recrystallization from $CHCl_3$. mp 273-274 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.43 (br s, 1H), 8.18 (d, 1H, *J* = 7.9 Hz), 7.70 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.32 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.10 (d, 1H, *J* = 8.5 Hz); FAB-MS m/z: 329 $(M+H)^+$; Anal. Calcd for $C_{14}H_5F_5N_2O_2$: C, 51.23; H, 1.54; N, 8.54. Found: C, 51.17; H, 1.79; N, 8.54.

3-(2-Chlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11h) According to the general procedure, **11h** was obtained in 51% yield as white powder after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.82 (br s, 1H), 8.18 (d, 1H, *J* = 8.0 Hz), 7.65 (dd, 1H, *J* = 7.3, 8.0 Hz), 7.60 (m, 1H), 7.45 (m, 2H), 7.37 (m, 1H), 7.28 (m, 1H, *J* = 8.0 Hz), 7.04 (d, 1H, *J* = 8.0 Hz); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2$: C, 61.66; H, 3.33; N, 10.27. Found: C, 61.65; H, 3.58; N, 10.22.

3-(3-Chlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11i) According to the general procedure, **11i** was obtained in 69% yield as pale yellow powder after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.32 (br s, 1H), 8.17 (d, 1H, *J* = 7.3 Hz), 7.66 (dd, 1H, *J* = 6.7, 8.5 Hz), 7.46 (m, 2H), 7.33 (s, 1H), 7.30-7.20 (m, 2H), 7.04 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2 \cdot 0.2H_2O$: C, 60.86; H, 3.43; N, 10.14. Found: C, 60.98; H, 3.39; N, 10.21.

3-(4-Chlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11j) According to the general procedure, **11j** was obtained in 74% yield as white crystals after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.17 (d, 1H, *J* = 7.3 Hz), 8.05 (br s, 1H), 7.66 (m, 1H), 7.50 (d, 2H, *J* = 8.6 Hz), 7.29-7.23 (m, 3H), 7.04 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2 \cdot 0.2H_2O$: C, 60.86; H, 3.43; N, 10.14. Found: C, 61.12; H, 3.46; N, 10.15.

3-(2,6-Dichlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11k) According to the general procedure, **11d 11k** was obtained in 57% yield as white powder after recrystallization from $CHCl_3$. mp >300 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.82 (br s, 1H), 8.23 (d, 1H, *J* = 7.9 Hz), 7.68 (dd, 1H, *J* = 7.3, 8.5 Hz), 7.50 (d, 2H, *J* = 8.5 Hz), 7.38 (dd, 1H, *J* = 7.3, 8.5 Hz), 7.30 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.07 (d, 1H, *J* = 8.5 Hz); FAB-MS m/z: 307 $(M+H)^+$, 309 $(M+H)^+$; Anal. Calcd for $C_{14}H_8Cl_2N_2O_2$: C, 54.75; H, 2.63; N, 9.12. Found: C, 54.70; H, 2.75; N, 9.00.

3-(3,5-Dichlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11l) According to the general procedure, **11l** was obtained in 68% yield as white crystals after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.17 (br s, 1H), 8.16 (d, 1H, *J* = 7.4 Hz), 7.68 (dd, 1H, *J* = 8.0, 7.4 Hz), 7.47 (s, 1H), 7.30 (dd, 1H, *J* = 8.0, 7.4 Hz), 7.24 (s, 1H), 7.23 (s, 1H), 7.05 (d, 1H, *J* = 8.0 Hz); FAB-MS m/z: 307 $(M+H)^+$, 309 $(M+H)^+$; Anal. Calcd for $C_{14}H_8Cl_2N_2O_2$: C, 54.75; H, 2.63; N, 9.12. Found: C, 54.73; H, 2.72; N, 9.15.

3-(2,4,6-Trichlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11m) According to the general procedure, **11m** was obtained in 68% yield as colorless cubes after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.65 (br s, 1H), 8.19 (d, 1H, *J* = 7.9 Hz), 7.69 (dd, 1H, *J* = 7.9 Hz), 7.52 (s, 2H), 7.31 (dd, 1H, *J* = 7.9, 7.3 Hz), 7.07 (d 1H, *J* = 8.5 Hz); FAB-MS m/z: 341 $(M+H)^+$, 343 $(M+H)^+$; Anal. Calcd for $C_{14}H_7Cl_3N_2O_2$: C, 49.23; H, 2.07; N, 8.20. Found: C, 49.09; H, 2.20; N, 8.18.

3-(2,3,4,5,6-Pentachlorophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11n) According to the general procedure, **11n** was obtained in 37% yield as white crystals after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.84 (br s, 1H), 8.19 (d, 1H, *J* = 7.9 Hz), 7.71 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.32 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.09 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 409 $(M+H)^+$, 411 $(M+H)^+$, 413 $(M+H)^+$; Anal. Calcd for $C_{14}H_5Cl_5N_2O_2$: C, 40.97; H, 1.23; N, 6.82. Found: C, 41.17; H, 1.39; N, 6.83.

3-(2-Bromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11o) According to the general procedure, **11o** was obtained in 94% yield as white needles after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 8.76 (br s, 1H), 8.18 (d, 1H, *J* = 8.0 Hz), 7.77 (d, 1H, *J* = 8.0 Hz), 7.65 (dd, 1H, *J* = 7.4, 7.9 Hz), 7.50

(dd, 1H, *J* = 7.9, 8.0 Hz), 7.37 (m, 2H), 7.28 (m, 1H), 7.04 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 317 $(M+H)^+$, 319 $(M+H)^+$; Anal. Calcd for $C_{14}H_9BrN_2O_2 \cdot 1/3CHCl_3$: C, 48.27; H, 2.64; N, 7.86. Found: C, 48.20; H, 2.75; N, 7.79.

3-(3-Bromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11p) According to the general procedure, **11p** was obtained in 49% yield as colorless cubes after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.86 (br s, 1H), 8.16 (d, 1H, *J* = 8.0 Hz), 7.66-7.60 (m, 2H), 7.49 (s, 1H), 7.41 (dd, 1H, *J* = 7.9, 8.6 Hz), 7.27 (m, 2H), 7.02 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 317 $(M+H)^+$, 319 $(M+H)^+$; Anal. Calcd for $C_{14}H_9BrN_2O_2$: C, 53.02; H, 2.86; N, 8.83. Found: C, 52.97; H, 2.89; N, 8.87.

3-(4-Bromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11q) According to the general procedure, **11q** was obtained in 83% yield as pale yellow needles after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.18 (br s, 1H), 8.17 (d, 1H, *J* = 7.9 Hz), 7.66 (m, 1H), 7.65 (d, 1H, *J* = 8.6 Hz), 7.28 (m, 1H), 7.37 (m, 1H), 7.18 (d, 1H, *J* = 8.6 Hz), 7.03 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 317 $(M+H)^+$, 319 $(M+H)^+$; Anal. Calcd for $C_{14}H_9BrN_2O_2$: C, 53.02; H, 2.86; N, 8.83. Found: C, 52.81; H, 2.88; N, 8.68.

3-(2,6-Dibromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11r) According to the general procedure, **11r** was obtained in 49% yield as white plates after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.21 (d, 1H, *J* = 7.9 Hz), 8.07 (br s, 1H), 7.71-7.67 (m, 3H), 7.32-7.21 (m, 2H), 7.07 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 395 $(M+H)^+$, 397 $(M+H)^+$, 399 $(M+H)^+$; Anal. Calcd for $C_{14}H_8Br_2N_2O_2$: C, 42.46; H, 2.04; N, 7.07. Found: C, 42.31; H, 2.21; N, 7.04.

3-(3,5-Dibromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11s) According to the general procedure, **11s** was obtained in 81% yield as white solid after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.29 (br s, 1H), 8.16 (d, 1H, *J* = 7.9 Hz), 7.77 (s, 1H), 7.67 (dd, 1H, *J* = 7.3, 8.5 Hz), 7.43 (s, 2H), 7.29 (dd, 1H, *J* = 7.3, 7.9 Hz), 7.05 (d, 1H, *J* = 8.5 Hz); FAB-MS m/z: 395 $(M+H)^+$, 397 $(M+H)^+$, 399 $(M+H)^+$; Anal. Calcd for $C_{14}H_8Br_2N_2O_2 \cdot 0.5H_2O$: C, 41.51; H, 2.24; N, 6.92. Found: C, 41.66; H, 2.18; N, 6.91.

3-(2,4,6-Tribromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11t) According to the general procedure, **11t** was obtained in 53% yield as white cubes after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.80 (br s, 1H), 8.19 (d, 1H, *J* = 7.9 Hz), 7.87 (s, 2H), 7.68 (t, 1H, *J* = 7.3, 7.9 Hz), 7.30 (t, 1H, *J* = 7.3, 7.9 Hz), 7.08 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 475 $(M+H)^+$, 477 $(M+H)^+$; Anal. Calcd for $C_{14}H_7Br_3N_2O_2$: C, 35.41; H, 1.49; N, 5.90. Found: C, 35.36; H, 1.58; N, 5.89.

3-(2,3,4,5,6-Pentabromophenyl)-2,4(1*H*,3*H*)-quinazolinedione (11u) According to the general procedure, **11u** was obtained in 50% yield as white needles after recrystallization from $CHCl_3$/MeOH.; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.97 (br s, 1H), 8.19 (d, 1H, *J* = 8.0 Hz), 7.70 (dd, 1H, *J* = 7.3, 8.5 Hz), 7.32 (dd, 1H, *J* = 7.3, 8.0 Hz), 7.09 (d, 1H, *J* = 8.5 Hz); FAB-MS m/z: 630 $(M+H)^+$, 632 $(M+H)^+$, 634 $(M+H)^+$, 636 $(M+H)^+$; Anal. Calcd for $C_{14}H_5Br_5N_2O_2 \cdot 0.5MeOH$: C, 26.84; H, 1.12; N, 4.32. Found: C, 26.84; H, 1.16; N, 4.34.

5-Chloro-3-phenyl-2,4(1*H*,3*H*)-quinazolinedione (11v) According to the general procedure, **11v** was obtained in 28% yield as white solid after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.52 (br s, 1H), 7.42 (d, 2H, *J* = 7.3 Hz), 7.33-7.30 (m, 3H), 7.21 (t, 1H, *J* = 7.9 Hz), 7.06 (t, 1H, *J* = 7.3 Hz), 6.99 (d, 1H, *J* = 7.9 Hz); FAB-MS m/z: 273 $(M+H)^+$.

6-Chloro-3-phenyl-2,4(1*H*,3*H*)-quinazolinedione (11w) According to the general procedure, **11w** was obtained in 73% yield as white powder after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.62 (br s, 1H), 8.07 (s, 1H), 7.51-7.44 (m, 4H), 7.26 (m, 2H), 6.98 (d, 1H, *J* = 8.5 Hz); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2 \cdot 0.2H_2O$: C, 60.86; H, 3.43; N, 10.14. Found: C, 61.04; H, 3.52; N, 10.20.

7-Chloro-3-phenyl-2,4(1*H*,3*H*)-quinazolinedione (11x) According to the general procedure, **11x** was obtained in 36% yield as colorless crystalline needles after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.17 (br s, 1H), 8.10 (d, 1H, *J* = 8.5 Hz), 7.54 (t, 2H, *J* = 7.3, 7.9 Hz), 7.47 (m, 1H), 7.29 (d, 2H, *J* = 7.3 Hz), 7.23 (d, 1H, *J* = 8.5 Hz), 7.05 (s, 1H); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2$: C, 61.66; H, 3.33; N, 10.27. Found: C, 61.49; H, 3.46; N, 10.19.

8-Chloro-3-phenyl-2,4(1*H*,3*H*)-quinazolinedione (11y) According to the general procedure, **11y** was obtained in 62% yield as colorless crystalline needles after recrystallization from $CHCl_3$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.73 (br s, 1H), 8.09 (d, 1H, *J* = 8.0 Hz), 7.67 (d, 1H, *J* = 7.9 Hz), 7.53-7.44 (m, 3H), 7.29 (d, 2H, *J* = 8.0 Hz), 7.19 (dd, 1H, *J* = 7.9, 8.0 Hz); FAB-MS m/z: 273 $(M+H)^+$; Anal. Calcd for $C_{14}H_9ClN_2O_2$: C, 61.66; H, 3.33; N, 10.27. Found: C, 61.63; H, 3.41; N, 10.22.

General procedure for TMS compounds 12a-12c

Trimethylsilylaniline was prepared according to the reported method.[48] To a solution of trimethylsilylaniline in acetonitrile were added methyl 2-isocyanatobenzoate (1.05 eq) and triethylamine (1.0 eq) at rt, and the mixture was stirred at 80 °C for 4 h. The reaction mixture was evaporated. The

residue was washed with hexane and, if necessary, recrystallized from $CHCl_3$ to give **12a-12c** (54.8 -91.2%).

3-(2-(Trimethylsilyl)phenyl)quinazoline-2,4(1*H*,3*H*)-dione (12a) According to the general procedure, **12a** was obtained in 55% yield as white solid; mp 272-273 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.23 (s, 1H), 8.16 (d, 1H, *J* = 7.9 Hz), 7.71 (d, 1H, *J* = 7.3 Hz), 7.61-7.47 (m, 3H), 7.26-7.23 (m, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 6.97 (s, 1H), 0.16 (9H, s); ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 163.47, 152.55, 140.42, 139.55, 139.23, 136.38, 135.77, 131.03, 129.51, 129.04, 128.91, 123.96, 115.87, 115.42, 0.00; HRMS (FAB): calcd for $C_{17}H_{19}N_2O_2Si$ 311.1216, found 311.1217 $(M+H)^+$.

3-(3-(Trimethylsilyl)phenyl)quinazoline-2,4(1*H*,3*H*)-dione (12b) According to the general procedure, **12b** was obtained in 90% yield as white solid; mp >300 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 10.04 (s, 1H), 8.14 (d, 1H, *J* = 7.9 Hz), 7.64 (d, 1H, *J* = 7.3 Hz), 7.56-7.52 (m, 1H), 7.50-7.47 (m, 1H), 7.42 (s, 1H), 7.31-7.29 (m, 1H), 7.26-7.20 (m, 1H), 6.80 (d, 1H, *J* = 7.9 Hz), 0.28 (9H, s); ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 162.60, 151.78, 142.24, 138.77, 135.30, 134.35, 133.80, 133.13, 128.85, 128.80, 128.63, 123.43, 115.20, 114.86, 1.17; HRMS (FAB): calcd for $C_{17}H_{19}N_2O_2Si$ 311.1216, found 311.1219 $(M+H)^+$.

3-(4-(Trimethylsilyl)phenyl)quinazoline-2,4(1*H*,3*H*)-dione (12c) According to the general procedure, **12c** was obtained in 91% yield as white solid; mp >300 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.82 (s, 1H), 8.14 (d, 1H, *J* = 7.9 Hz), 7.69 (d, 2H, *J* = 7.3 Hz), 7.54-7.51 (m, 1H), 7.30 (d, 2H, *J* = 7.9 Hz), 7.26-7.20 (m, 1H), 6.92 (d, 1H, *J* = 7.9 Hz), 0.31 (9H, s).

***N*-(2,4-Dichloro-5-methoxyphenyl)-2-nitrobenzamide (15)**

To a solution of 2,4-dichloro-5-methoxyaniline (**14**) (384 mg, 2.00 mmol) and pyridine (0.1 mL) in CH_2Cl_2 (2 mL) was added 2-nitrobenzoyl chloride (**13)** (371 mg, 2.0 mmol). The mixture was stirred for 20 min at rt, and a pale yellow solid was precipitated. The precipitate was collected by filtration and washed with AcOEt to afford **15** (595 mg, 87%) as pale yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.28 (s, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 7.88 (brs, 1H), 7.78 (m, 1H), 7.69 (m, 2H), 7.42 (s, 1H), 3.98 (s, 3H).

3-(2,4-Dichloro-5-methoxyphenyl)-2,3-dihydro-2-thioxoquinazolin-4(1*H*)-one (mdivi-1) (16)

341 mg (1.0 mmol) of **15** was dissolved in DMF (5 mL) and hydrogenated (1 bar H_2) over 10 % palladium on charcoal. The mixture was filtered through a pad of Celite. The filtrate was diluted with AcOEt and washed with water and brine. The organic layer was dried over $MgSO_4$ and concentrated to

afford 2-amino-*N*-(2,4-dichloro-5-methoxyphenyl)benzamide (272 mg, 87%) as pale yellow solid.
^{1}H-NMR (500 MHz, $CDCl_3$) δ□: 8.34 (m, 2H), 7.52 (d, 1H), 7.40 (s, 1H), 7.29 (m, 1H), 6.75 (m, 2H), 5.59 (brs, 2H), 3.96 (s, 3H).

To a solution of 2-amino-*N*-(2,4-dichloro-5-methoxyphenyl)benzamide (80.6 mg, 260 mmol) and CS_2 (300 mL, 5.00 mmol) in DMF (2 mL) was added DBU (38.7 mL, 258 mmol). The mixture was stirred for 15.5 h at rt, then diluted with AcOEt. The organic layer was washed with 2 N HCl aq., water and brine, dried over anhydrous magnesium sulfate and concentrated to afford **16** (87.7 mg, 96%). The product was obtained as pale yellow solid from $CHCl_3$/MeOH.
^{1}H-NMR (500 MHz, $CDCl_3$) δ : 8.16 (d, 1H, J = 7.95 Hz), 7.72 (m, 1H), 7.58 (s, 1H), 7.35 (m, 1H), 7.24 (d, 1H, J = 8.55 Hz), 6.89 (s, 1H), 3.90 (s, 3H); MS (FAB) *m/z* 353, 355, 357 $(M+H)^+$.

Cells MOLT-4 cells or HL-60 cells were maintained in RPMI1640 medium supplemented with 10% v/v fetal bovine serum at 37 °C under an atmosphere of 5% CO_2 in air.

Assay of Enzyme Activities PSA and APN activities were evaluated in the usual way, by measuring 7-amino-4-methylcoumarin (AMC) liberated from *L*-alanine 4-methylcoumaryl-7-amide (Ala-MCA). Cell suspension: Cells were collected by centrifugation (2000 rpm, 5 min, 4ºC) and suspended in phosphate-buffered saline (PBS) at 2 × 10^6 cells/mL. Briefly, to Tris-HCl buffer (pH = 7.4, 395 μL/well) were added cell suspension (50 μL/well) and a test inhibitor (various concentrations, 5 μL/well) or DMSO, and the resulting suspension was pre-incubated at 37 °C for exactly 10 min. Then, Ala-MCA (1 mM in Tris-HCl buffer, 50 μL/well) was added. The suspension was further incubated at 37 °C for exactly 30 min, and AcONa-AcOH buffer (1 M, pH 4.0, 1.5 mL/well) was added. The amounts of liberated AMC were measured in terms of fluorescence intensity (excitation at 355 nm, emission at 460 nm). The assay was performed at least in duplicate, and the mean value was taken.

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