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Specific Inhibitor of Puromycin-Sensitive Aminopeptidase with a Homophthalimide Skeleton: Identification of the Target Molecule and a Structure–Activity Relationship Study

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Abstract—2-(2,6-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (**2**: PIQ-22) was found to be a potent and specific inhibitor of puromycin-sensitive aminopeptidase (PSA). Lineweaver–Burk plot analysis showed that PSA is inhibited by PIQ-22 in a non-competitive manner. Structure–activity relationship studies indicated that tautomerism of the imidobenzoylketone group in the cyclic imide moiety of the PIQ-22 skeleton is important for the inhibitory activity.

Introduction

Aminopeptidases as a class are widely distributed and play important roles in the turnover of proteins and peptides leading to uptake or re-use of amino acids in various tissues. Some aminopeptidases possess quite strict substrate-specificity and limited distribution, and their function has been shown to be related to physiological and pathophysiological phenomena such as modulation of the regulation of physiological events by peptide hormones, regulation of cell adhesion and mobility by degrading connective tissue barriers, and so on. Specific aminopeptidase inhibitors would be useful as bioprobes for investigation of the physiological/pathophysiological functions of these enzymes, and might also be clinically useful, e.g., as tumor metastasis inhibitors.

For example, the natural aminopeptidase inhibitor bestatin (**1**) (Fig. 1), which was first isolated from culture filtrate of *Streptomyces*,[1] has attracted considerable attention since its discovery due to its numerous biological activities, including as an immune response modifier, a potential analgesic, and an inhibitor of tumor metastasis.[2–4] Though bestatin is widely employed clinically as an anti-tumor agent with host-mediated actions, it has drawbacks for clinical application. These include low bioavailability, proteolytic lability, rapid biliary excretion, short duration of action, and so on, all of which are general features of peptide-type drugs. Therefore, development of non-peptide specific aminopeptidase inhibitors is desirable.

Recently, we have reported novel non-peptide small molecular inhibitors of aminopeptidase with a phthalimide/homophthalimide skeleton.[5–9] Some of them were structurally derived from thalidomide.[10] Among them, 2-(2,6-diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (**2**: PIQ-22) (Fig. 1) is a more potent aminopeptidase inhibitor than bestatin in our assay system (vide infra).[6–9] A potent inhibitory effect of PIQ-22 on tumor cell invasion has also been demonstrated.[11] Though the substrate selectivity of the aminopeptidase whose activity is potently inhibited by PIQ-22 indicated that the enzyme can be classified as a neutral aminopeptidase,[6–8] it has not been well characterized at the molecular level.[9,11]

In this article, we first describe the molecular characterization of the target aminopeptidase of PIQ-22. Structural development of PIQ-22 and the results of structure–activity relationship studies are also described.

Molecular characterization of the target aminopeptidase of PIQ-22

PIQ-22 (**2**) showed more potent aminopeptidase-inhibiting activity (IC_{50} value 0.12 μg/mL) than bestatin (**1**)

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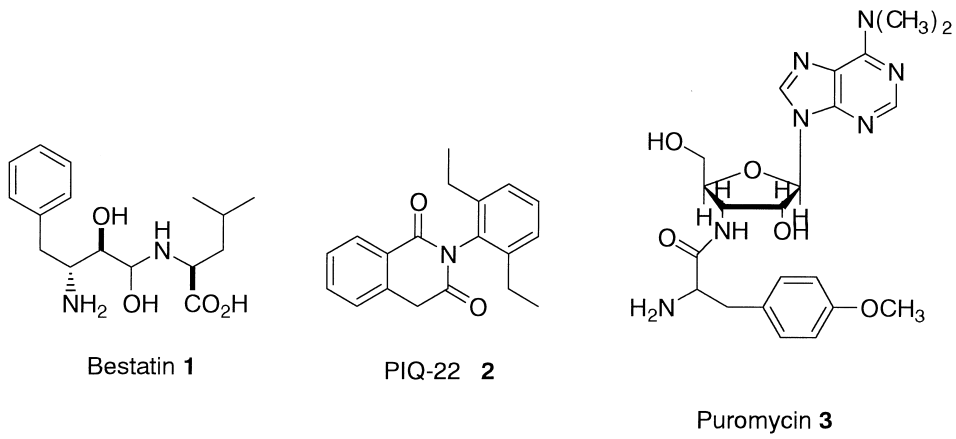


Figure 1. Structures of bestatin (**1**), PIQ-22 (**2**) and puromycin (**3**).

(IC_{50} value of 0.81 μg/mL) when assayed in a system using human acute lymphoblastic leukemia cells, MOLT-4, as a source of the enzyme, and L-alanine 7-amino-4-methylcoumaryl-7-amide (Ala-AMC) as a substrate.[6–9] Aminopeptidase expressed in another human lymphocytic cell line, Jurkat, was also sensitive to PIQ-22.[12] On the other hand, the PIQ-22-sensitivity of aminopeptidase(s) expressed in human monocytic cells, such as HL-60 and THP-1, was much lower (details will be published elsewhere).[12]

Because monocytic cells exclusively express aminopeptidase N (APN), which is identical to the myeloid differentiation antigen CD13,[13] and lymphocytic cells are reported to be APN/CD13-negative,[14] our result suggests that the target molecule of PIQ-22 is different from APN. In fact, PIQ-22 was inactive toward authentic APN (porcine, Sigma; data not shown).[11,12] Concerning lymphocytic cells, Wex et al. reported the presence of aminopeptidase with an isoelectric point of pH 5.4, which is different from that of APN/CD13 (isoelectric point: pH 3.5–4.8), though their enzyme has not been fully characterized.[15] Therefore, we conducted a molecular characterization of the PIQ-22-sensitive aminopeptidase expressed in MOLT-4 cells.

Cultured MOLT-4 cells were fractionated and the soluble fraction was separated successively by ammonium sulfate fractionation and Mono-Q high-performance liquid column chromatography (HPLC). Activity of PIQ-22-sensitive aminopeptidase was recovered as a single ammonium sulfate-precipitated fraction (50–60% ammonium sulfate), and eluted as a single peak on Mono-Q HPLC (Fig. 2). Analysis of each eluted fraction by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) showed that the 102 kDa band (p102) coincided with the aminopeptidase activity (Fig. 2). Direct activity staining of the electrophoresis gel[16] showed a single stained band at the location of p102, which strongly suggested that MOLT-4 cells express a unique PIQ-22-sensitive aminopeptidase of 102 kDa.

The active Mono-Q HPLC fractions were collected and further separated by gel filtration HPLC using a Superose 6 column under non-denaturing conditions (Fig. 3). The PIQ-22-sensitive aminopeptidase activity was eluted as a single peak with the estimated apparent molecular weight of 93.3 kDa, which accords reasonably well with the molecular weight of 102 kDa estimated by SDS–PAGE of the Mono-Q HPLC fractions (Fig. 2). In the non-denaturing Superose 6 HPLC, proteins with higher molecular weight and without aminopeptidase activity (possibly aggregated and/or inactivated proteins) were eluted with the retention time of 45–47 min (Fig. 3). Analysis of each Superose 6 HPLC fraction by SDS–PAGE again showed that the behavior of only the 102 kDa band (p102) is consistent with that of the aminopeptidase activity (Fig. 3). Therefore, we concluded that p102 is the target aminopeptidase of PIQ-22, and its amino acid sequence was analyzed.

The band of p102 on SDS–PAGE was blotted on a poly(vinylidene difluoride) (PVDF) membrane filter and analyzed with a gas-phase protein sequencer. The N-terminal amino acid sequence of PEKRPFERLPADVSPINY was obtained. A database homology search revealed that this sequence was identical with the reported sequence of human puromycin-sensitive aminopeptidase (PSA), and its calculated molecular weight, 99 kDa, is consistent with that of p102 estimated by gel filtration HPLC and SDS–PAGE, i.e., 93.3 and 102 kDa, respectively.[17,18] The result indicated that the PIQ-22-sensitive aminopeptidase existing in MOLT-4 cells is PSA. Moreover, it is suggested that the neutral aminopeptidase predominantly expressed in lymphoid CD13-negative cells is PSA, while that expressed in monocytic cells is APN/CD13.

Mode of PSA inhibition elicited by PIQ-22

PSA is a neutral aminopeptidase with a similar substrate specificity to that of APN, which was first found as a candidate aminopeptidase for degrading enkephalins, and is present in brain in much higher amounts than APN.[19–21] Recently, increased anxiety and impaired pain response in PSA gene-deficient mice obtained by a mouse gene-trap method was reported.[22] However, the physiological/pathophysiological role of PSA has not yet been fully clarified.

As mentioned before, PIQ-22 is inactive toward APN (porcine), though bestatin is a potent inhibitor of both

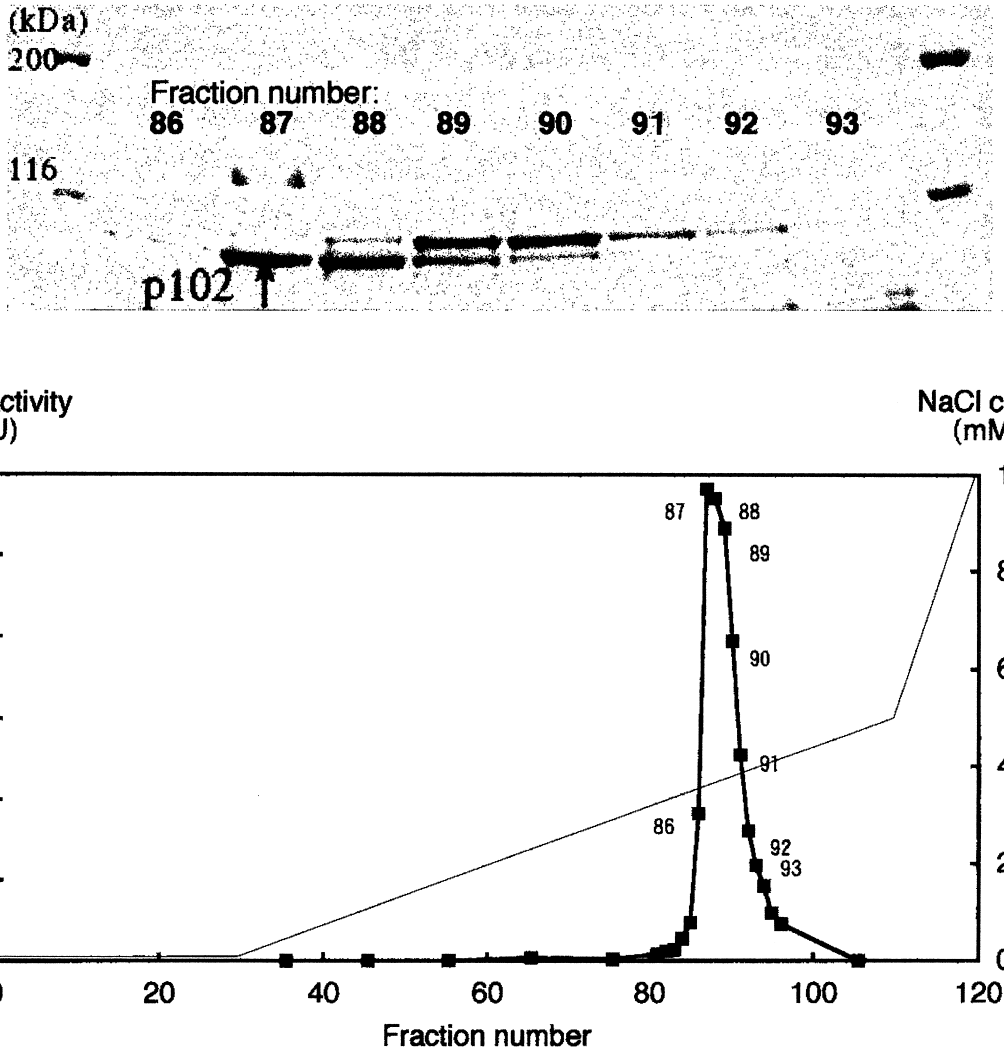


Figure 2. Mono-Q HPLC chromatogram and SDS–PAGE analysis of the fractions. The chromatogram was monitored by assay of PIQ-22-sensitive neutral aminopeptidase activity (AP activity: the left vertical scale) measured as described in the Experimental. The horizontal scale is the fraction number of the HPLC, and the concentration of NaCl in the eluent is shown on the right vertical scale. The SDS–PAGE gel was stained with CBB for fractions 86–93. The positions of molecular weight markers are also shown.

APN and PSA. PIQ-22 was also inactive towards other typical neutral aminopeptidases, leucine aminopeptidase (LAP, porcine, Sigma), dipeptidylpeptidase type IV (porcine, Sigma), trypsin (bovine, Sigma) and chymotrypsin (bovine, Sigma) (data not shown), suggesting that PIQ-22 is specific to PSA. Because PIQ-22 has been shown to be a potent inhibitor of tumor cell invasion,[11] PSA might be one of the target molecules of tumor cell invasion inhibitors.

PSA is reported to be approximately 100-fold more sensitive to puromycin (**3**) (Fig. 1) than APN, with an IC_{50} value of 0.5 μM.[19–21] In our assay system using MOLT-4 cells, puromycin inhibited the PIQ-22-sensitive aminopeptidase with the IC_{50} value of 0.4 μM, which is in good agreement with the reported value. Because there is no obvious structural relationship between PIQ-22 (**2**) and puromycin (**3**), the mode of inhibition elicited by these two inhibitors, as well as bestatin (**1**), was analyzed by Lineweaver–Burk plot analysis (Fig. 4). The analysis clearly indicated that PIQ-22 inhibits PSA in a non-competitive manner (Fig. 4(C): the two dose–response lines cross at the horizontal axis), while puromycin (Fig. 4(A)) and bestatin (Fig. 4(B)) inhibit PSA in a competitive manner (the two dose–response lines cross at the vertical axis in both cases).[9,11] The results suggest that PIQ-22 does not act as a substrate-mimic, but binds at a specific site of PSA which is not the substrate-binding site, while puromycin and bestatin act as substrate-mimics.

Structural development of PIQ-22: effects of substituents introduced into the *N*-phenyl moiety

The results mentioned above, as well as our previous studies on neutral aminopeptidase inhibitors with a phthalimide/homophthalimide skeleton,[6–9] suggest that the structure of PIQ-22 is critically recognized by the non-competitive site of PSA. We therefore modified the structure of PIQ-22 in various ways to obtain information concerning the non-competitive binding site of PSA (Table 1).

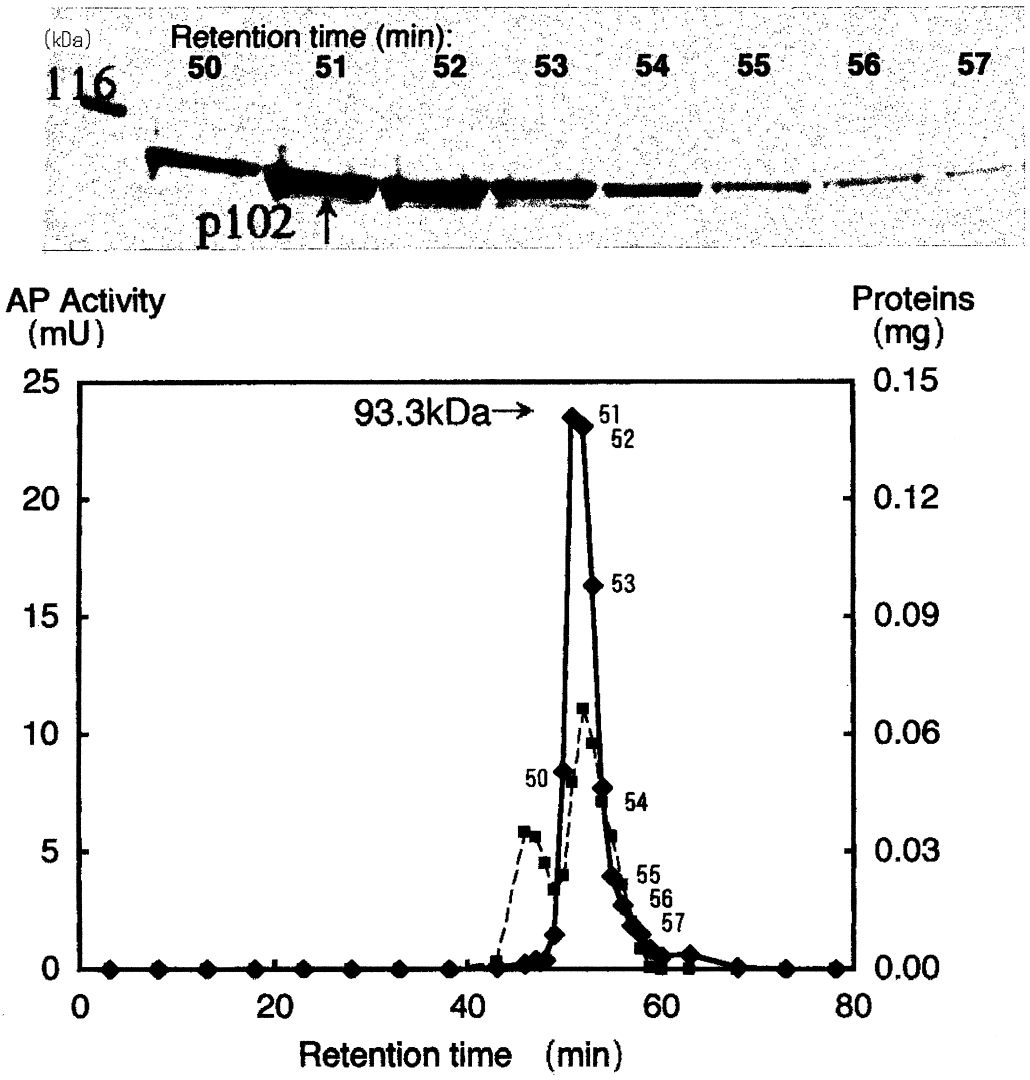


Figure 3. Superose 6 HPLC chromatogram and SDS–PAGE analysis of the fractions. The chromatogram was monitored by assay of PIQ-22-sensitive neutral aminopeptidase activity (AP activity: the left vertical scale, the solid line) measured as described in the Experimental and the amount of protein contained in each fraction (measured by the Lowry method: the right vertical scale, the broken line). The horizontal scale is the retention time (min). The molecular weight marker proteins were analyzed under the same conditions, and the apparent molecular weight of the aminopeptidase peak was estimated to be 93.3 kDa by the least-squares method. The SDS–PAGE gel was stained with CBB for fractions with retention times of 50–57 min.

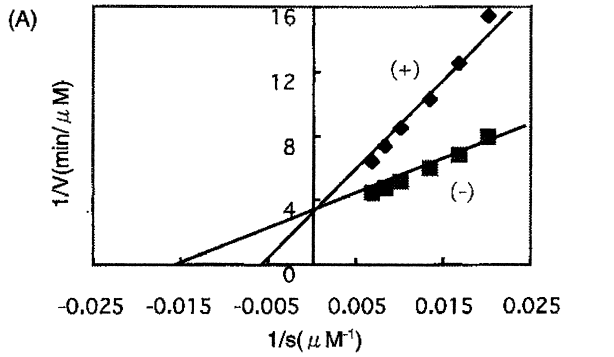
First, the effects of alkyl substituents on the *N*-phenyl moiety were examined (**2**, **4**–**27**). The corresponding non-alkylated derivative, **4**, is almost inactive. Comparison of the PSA-inhibiting activity of monoalkylated derivatives (**5**–**10**) roughly indicated that an ethyl substituent introduced at the *ortho*- or *meta*-position (**6** or **7**) is superior to a methyl (**5**) or isopropyl (**9**) substituent for potent activity. The substitution position should be *ortho* or *meta*, because the *para*-monoalkylated derivatives (**8** and **10**) are inactive.

The superiority of an ethyl group over other alkyl groups was also observed in dialkylated derivatives (**2**, **11**–**26**). The potency of PSA inhibition elicited by dimethylated regioisomers decreased in the order of: *m,m*′-(**16**) > *o,o*′-(**11**) > *o*(2),*m*(5) - (**12**) > *o,p* - (**13**) > *m,p* - (**15**) > *o*(2),*m*(3) - (**14**), while that of diethylated regioisomers decreased in the order of: *o,o*′-(**2**: PIQ-22) > *m,m*′-(**21**) > *o*(2),*m*(5)-(**17**) > *m,p*-(**20**) > *o,p*-(**18**) > *o*(2),*m*(3)-(**19**). PIQ-22 (**2**) is the most potent inhibitor among these compounds. The *N*-(α)-naphthyl derivative (**27**) was also potent.

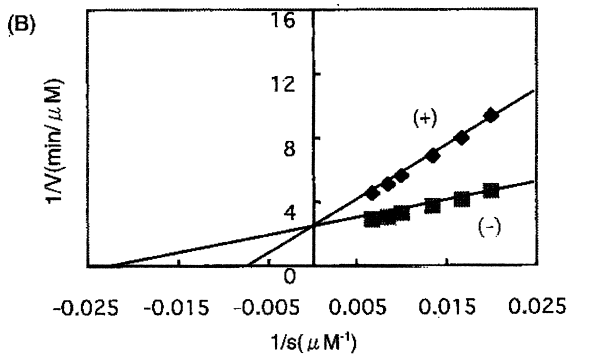
Introduction of a methoxy or methylthio group (**28**–**33**) also resulted in the appearance of potent activity. In this case, introduction at the *para*-position (**30**, **33**) was also effective.

Dimethoxy derivatives (**34**–**37**) were also potent PSA inhibitors, except the *m,p*-regioisomer (**36**). Introduction of two chlorine atoms at the two *ortho* positions (**38**) resulted in appearance of the activity, but introduction of two fluorine atoms at the corresponding positions (**39**) had no effect.

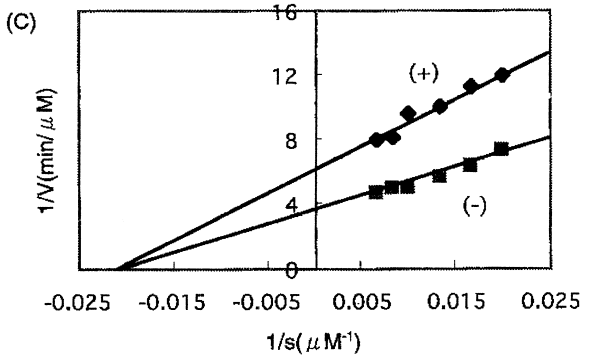
These results suggest that moderately bulky and hydrophobic circumstances around the nitrogen atom in the cyclic imide moiety and perpendicular orientation of the *N*-aryl group with respect to the plane of the homo-



(+); in the presence of puromycin, (-); in the absence of puromycin



(+); in the presence of bestatin, (-); in the absence of bestatin



(+); in the presence of PIQ-22, (-); in the absence of PIQ-22

Figure 4. Lineweaver–Burk plot analysis of inhibition of PSA by puromycin (A), bestatin (B) and PIQ-22 (C). ■, In the absence of an inhibitor; ◆, in the presence of an inhibitor. V, Rate of enzymatic hydrolysis of the substrate (Ala-AMC) for PSA (μM/min). Inhibitors were added at the concentration of 0.5 μM for puromycin (A) and PIQ-22 (C) and 2.5 μM for bestatin (B). The PSA activity was assessed as described in the Experimental.

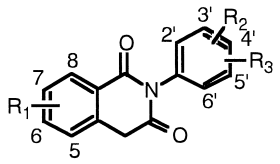
phthalimide moiety are critical factors for potent PSA-inhibiting activity.

Structural development of PIQ-22: effects of derivatization of the cyclic imide ring

As previously reported,[6–9,12] ring reduction of PIQ-22, i.e., *N*-2,6-diethylphenylphthalimide (**40**), resulted in disappearance of the activity (Fig. 5, Table 2). However, introduction of an electron-donating group such as an amino (**41**, **42**) or a hydroxyl (**43**, **44**) group into the phthalimide moiety of inactive **40** resulted in reappearance of the activity. On the other hand, introduction of an electron-withdrawing nitro group (**45**, **46**) had no effect.

The ring-expanded analogue of PIQ-22, i.e., **47**, was also inactive. Decarbonylation of PIQ-22, i.e., **48**, retained the activity, though the activity was weaker than that of PIQ-22. On the other hand, carbonylation of PIQ-22, i.e., **49**, resulted in complete disappearance of the activity. We have found that PIQ-22 is photolabile in a protic solvent in air, and a methanol solution of PIQ-22 exposed to daylight in air yielded the tricarbonyl analogue (**49**) quantitatively in a molecular oxygen-dependent manner (details will be published elsewhere). Briefly, a ^{1}H NMR spectroscopic study showed that PIQ-22 was light-dependently auto-oxidized in methanol to give the

Table 1. PSA-inhibitory activity (IC_{50} values) of phenyl-homophthalimide analogues



Compound	R_1	R_2	R_3	IC_{50} (μg/ml)
4	H	H	H	93.7
5	H	2′-Me	H	41.5
6	H	2′-Et	H	17.8
7	H	3′-Et	H	11.7
8	H	4′-Et	H	>100
9	H	2′-iPr	H	54.3
10	H	4′-iPr	H	>100
28	H	2′-OMe	H	6.2
29	H	3′-OMe	H	9.5
30	H	4′-OMe	H	7.8
31	H	2′-SMe	H	0.9
32	H	3′-SMe	H	8.0
33	H	4′-SMe	H	6.2
11	H	2′-Me	6′-Me	8.7
12	H	2′-Me	5′-Me	11.6
13	H	2′-Me	4′-Me	16.6
14	H	2′-Me	3′-Me	30.0
15	H	3′-Me	4′-Me	22.8
16	H	3′-Me	5′-Me	1.5
2: PIQ-22	H	2′-Et	6′-Et	0.12
17	H	2′-Et	5′-Et	4.3
18	H	2′-Et	4′-Et	19.5
19	H	2′-Et	3′-Et	47.0
20	H	3′-Et	4′-Et	7.0
21	H	3′-Et	5′-Et	2.5
22	H	2′-iPr	6′-iPr	3.5
23	H	2′-Me	6′-Et	0.43
24	H	2′-Me	6′-iPr	0.78
25	H	2′tBu	5′-tBu	>100
26	H	3′tBu	5′-tBu	7.3
27	H	2′,3′-fused-Ph		1.7
34	H	5′-OMe	5′-OMe	3.2
35	H	4′-OMe	4′-OMe	4.9
36	H	4′OMe	4′-OMe	>100
37	H	5′OMe	5′-OMe	5.3
38	H	6′-Cl	6′-Cl	44.1
39	H	6′-F	6′-F	>100
51	7-NH_2	6′-Et	6′-Et	8.2
52	7-NO_2	6′Et	6′-Et	28.0
1: Bestatin				0.81

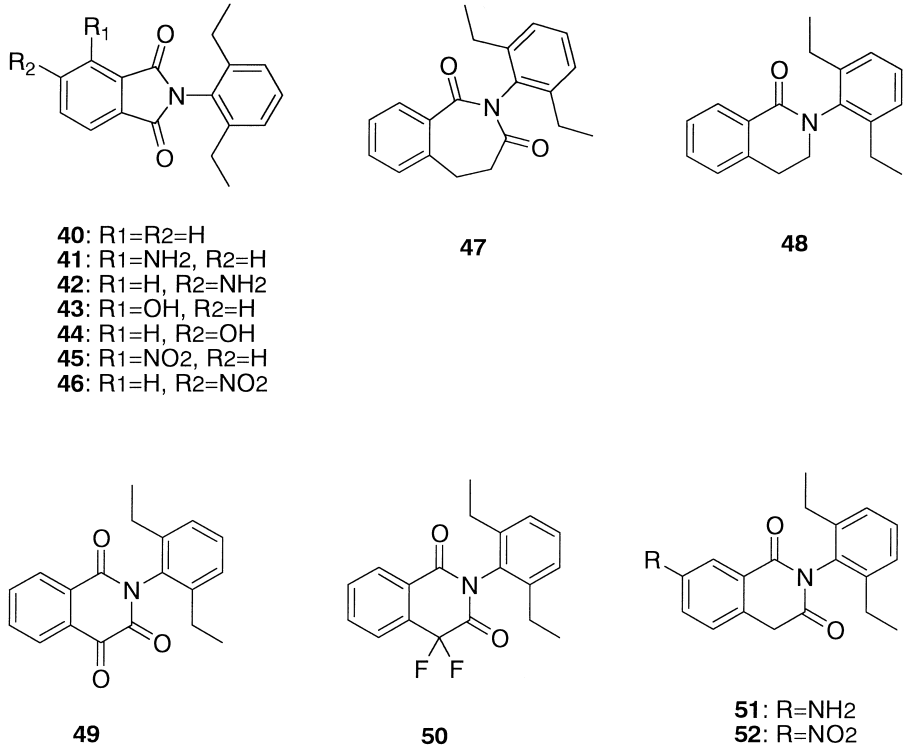


Figure 5. Structures of some derivatives of PIQ-22.

Table 2. PSA-inhibitory activity (IC_{50} values) of some compounds structurally related to PIQ-22

Compound	IC_{50} (μg/ml)
2	0.12
40	> 100
41	2.5
42	5.6
43	10.2
44	9.6
45	> 100
46	> 100
47	> 100
48	2.4
49	> 100
50	> 100
Bestatin	0.81

corresponding 4-methoxy hemiacetal analogue quantitatively, and this afforded the tricarbonyl analogue (**49**) after work up and recrystalization.[12] Therefore, we designed a non-oxidizable analogue, i.e., the difluorinated derivative (**50**). Compound **50** was prepared from **49** by reaction of the carbonyl group at the benzyl position with (diethylamino)sulfur trifluoride (DAST).[23] However, **50** was unexpectedly inactive.

Possible relevance of tautomerism of the cyclic imide moiety to the PSA-inhibiting activity

One possible interpretation of the results of our structure–activity relationship studies mentioned above is that moderate bulkiness/hydrophobicity around the cyclic imide nitrogen atom and the orientation of the *N*-aryl group are critical factors for potent PSA-inhibiting activity (vide supra). PIQ-22 is the most potent PSA inhibitor (more potent than bestatin) among the compounds described in this paper.

Comparison of the structures of active compounds with those of inactive/weakly active compounds, i.e., active amino/hydroxy-containing arylphthalimides (**41**–**44**) versus inactive arylphthalimides (**40** and nitrated analogues **45**, **46**), active PIQ-22 analogues (PIQ-22: **2** and its decarbonylated analogue **48**) versus inactive derivatives (tricarbonyl analogue **49**, difluorinated analogue **50** and ring-expanded **47**), led us to consider another possible interpretation (vide infra). The active compounds listed above possess a common feature, i.e., they can all be regarded as existing in a keto/enol tautomeric mixture (Fig. 6). Keto/enol tautomerism of homophthalic anhydride and trapping of the enol form by Diels–Alder reaction with a naphthoquinone derivative have been reported by Tamura et al.[24,25]

In contrast to the putative keto/enol tautomerism of the active compounds (**41**–**44**, PIQ-22, **48**), the inactive derivatives listed above (**40**, **45**–**47**, **49**, **50**) can be regarded as compounds which would not readily tautomerize to the corresponding enol forms. Among the *N*-arylphthalimide analogues, compounds **41**–**44** are expected to tautomerize to the corresponding enol forms more easily than **40**, **45** and **46** because of the electron-donating nature of the introduced amino or hydroxyl group. The tautomerization of the inactive ring-expanded analogue **48** to the corresponding enol form should be difficult because of ring torsion of the seven-membered ring. The inactive tricarbonyl analogue **49** and difluorinated analogue **50** cannot tautomerize because of the lack of

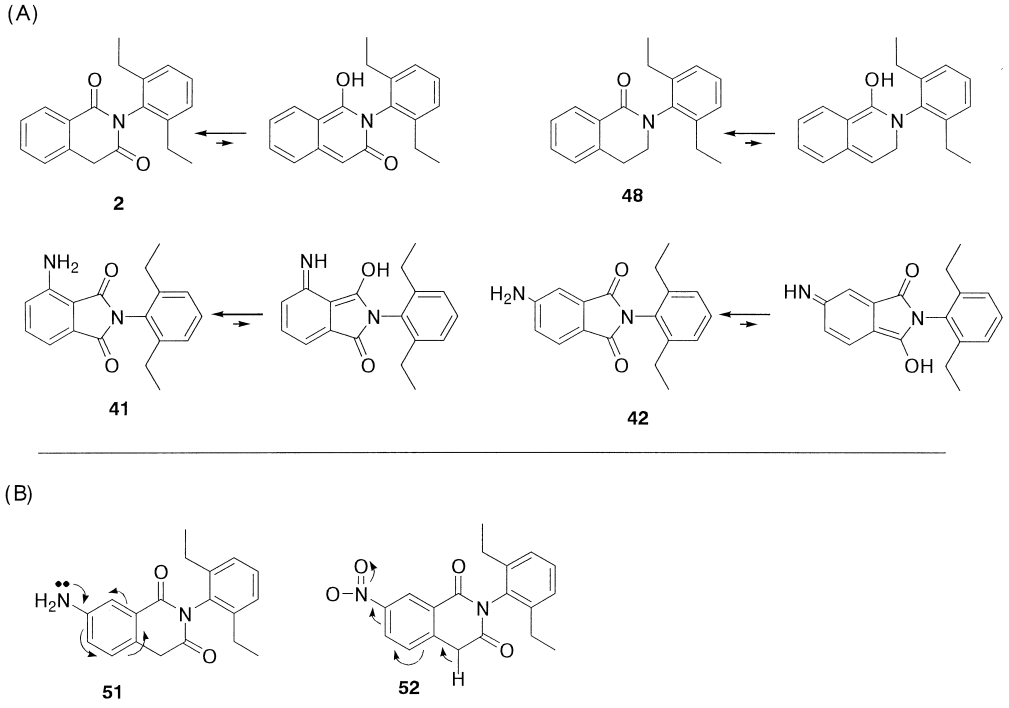


Figure 6. Possible relationship of tautomerism to aminopeptidase-inhibitory activity. (A) Compounds with high activity are considered to exist as tautomeric mixtures. (B) Compounds with low activity are considered unlikely to tautomerize readily.

active benzyl methylene hydrogens. Therefore, the enol forms might be the active forms of the compounds which directly bind to the non-competitive site of PSA.

If this were the case, introduction of a functional group with an electronic effect into the 7-position of the homophthalimide moiety of PIQ-22 should cause reduced potency of the PSA-inhibiting activity, because either an electron-donating or an electron-withdrawing group at the 7-position would make tautomerization of the imidobenzoylketone in the cyclic imide moiety unfavorable (Fig. 6(B)). In fact, introduction of an amino group (**51**) or a nitro group (**52**) at the 7-position of PIQ-22 decreased the potency of the activity, though the 7-amino derivative (**51**) was moderately active (Table 1).

Conclusion

First, PSA was identified as a PIQ-22-sensitive neutral aminopeptidase from MOLT-4 cells. This suggests that the so-far-unidentified aminopeptidase expressed in lymphocytic cells is PSA. Second, PIQ-22 (**2**) was established to be a potent, specific, non-peptide, small-molecular, non-competitive inhibitor of PSA. Because almost all of the potent protease inhibitors so far known are substrate mimics/peptide mimics, as in the case of bestatin (**1**), and the physiological/pathophysiological roles of PSA have not yet been well established, PIQ-22 should be a superior bioprobe for investigation of PSA. The potent inhibitory activity of PIQ-22 on tumor cell invasion[10–12] also suggests the usefulness of the compound as a lead compound for novel drugs, including antimetastatic agents.

Experimental

Assay of enzyme activity

Neutral aminopeptidase activity was evaluated in the usual assay by measuring 7-amino-4-methylcoumarin (AMC) liberated from L-alanine 4-methylcoumaryl-7-amide (Ala-AMC).[26,27] Briefly, Ala-AMC (1 mM) was dissolved in 50 mM Tris–HCl (pH 7.4) in the presence or absence of a test inhibitor (various concentrations). To the solution was added enzymatic activity (MOLT-4 cell extract, commercially available proteases, HPLC fractions, and so on), and the mixture was incubated at 37 °C for exactly 60 min. Then 6 volumes of 1 M AcONa–AcOH (pH 4.0) were added to stop the enzymatic reaction. The amounts of liberated AMC was measured in terms of fluorescence intensity (excitation at 380 nm, emission at 420 nm). The assay was performed in triplicate, and the mean value was taken. Though the values deviated from experiment to experiment, the results (order of potency) were basically reproducible, and a typical set of data is shown in the tables.

Identification of the neutral aminopeptidase expressed in MOLT-4

Cells. MOLT-4 cells were maintained in an RPMI1640 medium supplemented with 10% v/v fetal bovine serum at 37 °C under a 5% CO_2 atmosphere. Cells were collected by centrifugation (2000 rpm, 2 min, 4 °C), and homogenized in phosphate-buffered saline (PBS). The homogenate was ultracentrifuged at 100,000 *g* at 4 °C for 1 h, and the supernatant was separated. The supernatant was fractionated by successive addition of ammonium sulfate; the precipitate of each fraction was

collected by centrifugation (20,000 $g \times 3$ min at 4 °C). The aminopeptidase activity was recovered in the fraction of 50–60% saturated $(NH_4)_2SO_4$. The fraction was dialyzed and separated by HPLC equipped with a Mono-Q column by eluting with a linear gradient (Fig. 2) of NaCl (0–1 M) in 20 mM Tris–HCl (pH 8.0)/250 mM sucrose. The fractions showing aminopeptidase activity were collected, concentrated by ultrafiltration, and then further separated by HPLC with a Superose 6 column (eluted with 20 mM Tris–HCl (pH 8.0)/250 mM sucrose) (Fig. 3). The fractions showing aminopeptidase activity were collected, concentrated by ultrafiltration, and then separated by SDS–PAGE according to Laemmli.[28] The bands on the gel were blotted on a Millipore Immobilon PVDF membrane, and the membrane was stained with Coomassie Brilliant Blue (CBB). The band of p102 was cut out and the N-terminal amino acid sequence was analyzed with a gas phase amino acid sequencer (Shimadzu PPSQ-10). Homology search of the obtained sequence was performed by FASTA search (http://www.fasta.genome.ad.jp).

Chemicals. Cyclic imide derivatives were prepared as described previously.[5,6] Typically, phthalic/homophthalic anhydride was condensed with an appropriate amine. The syntheses of **4**, **11**, **15**, **17**, **22**–**27**, **32**, **33**, **37**, **41**, **43**, **45** and **47** were already reported.[29–32]

2-(2,6-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (PIQ-22: 2). Mp 108–110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.14 (6H, t, $J = 7.33$ Hz), 2.40 (4H, q, $J = 7.33$ Hz), 4.25 (2H, s), 7.24 (2H, d, $J = 7.83$ Hz), 7.36–7.40 (2H, m), 7.50 (1H, t, $J = 7.83$ Hz), 7.67 (1H, dt, $J = 7.83$, 1.43 Hz), 8.26 (1H, d, $J = 6.85$ Hz). Anal. calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.69; H, 6.43; N, 4.56.

2-(2-Methylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (5). Mp 107–108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.14 (3H, s), 4.24 (2H, s), 7.10 (1H, d, $J = 7.2$ Hz), 7.31–7.37 (4H, m), 7.49 (1H, t, $J = 8.0$ Hz), 7.66 (1H, dt, $J = 7.5$, 1.0 Hz), 8.26 (1H, dd, $J = 7.5$, 1.0 Hz). Anal. calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.47; H, 5.17; N, 5.28.

2-(2-Ethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (6). Mp 123–124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.16 (3H, t, $J = 7.63$ Hz), 2.45 (2H, q, $J = 7.63$ Hz), 4.24 (2H, s), 7.31–7.35 (1H, m), 7.37 (1H, d, $J = 7.63$ Hz), 7.41–7.42 (2H, m), 7.49 (1H, t, $J = 7.94$ Hz), 7.66 (1H, dt, $J = 7.63$, 1.22 Hz), 8.25 (1H, dd, $J = 7.94$, 0.92 Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.04; H, 5.70; N, 5.21.

2-(3-Ethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (7). Mp 76.5–78 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.17 (3H, t, $J = 8.0$ Hz), 2.72 (2H, q, $J = 8.0$ Hz), 4.23 (2H, s), 7.03 (2H, d, $J = 8.0$ Hz), 7.29 (1H, d, $J = 8.0$ Hz), 7.35 (1H, d, $J = 8.0$ Hz), 7.42 (1H, t, $J = 8.0$ Hz), 7.64 (1H, dt, $J = 7.5$, 1.2 Hz), 8.25 (1H, d, $J = 8.0$ Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.03; H, 5.74; N, 5.30.

2-(4-Ethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (8). Mp 139–140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.28 (3H, t, $J = 7.5$ Hz), 2.72 (2H, q, $J = 7.5$ Hz), 4.22 (2H, s), 7.12 (2H, d, $J = 8.0$ Hz), 7.35 (1H, d, $J = 7.6$ Hz), 7.48 (1H, d, $J = 8.0$ Hz), 7.64 (1H, dt, $J = 7.6$, 1.0 Hz), 8.25 (1H, dd, $J = 8.0$, 1.0 Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.94; H, 5.70; N, 5.26.

2-(2-Isopropylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (9). Mp 158–160 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.17 (6H, t, $J = 6.85$ Hz), 2.70 (1H, q, $J = 6.85$ Hz), 4.24 (2H, s), 7.05 (1H, d, $J = 8.32$ Hz), 7.26–7.33 (1H, m), 7.37 (1H, d, $J = 7.33$ Hz), 7.42–7.48 (1H, m), 7.50 (1H, d, $J = 7.83$ Hz), 7.66 (1H, dt, $J = 7.83$, 1.47 Hz), 8.26 (1H, dd, $J = 7.83$, 1.0 Hz). Anal. calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.23; H, 6.37; N, 4.84.

2-(4-Isopropylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (10). Mp 170–172 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.29 (6H, t, $J = 8.85$ Hz), 2.97 (1H, q, $J = 6.85$ Hz), 4.23 (2H, s), 7.10–7.13 (1H, m), 7.12 (2H, d, $J = 8.31$ Hz), 7.34–7.36 (1H, m), 7.36 (2H, d, $J = 8.31$ Hz), 7.47 (1H, t, $J = 7.34$ Hz), 7.64 (1H, dt, $J = 7.83$, 1.47 Hz), 8.25 (1H, d, $J = 7.34$ Hz). Anal. calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.33; H, 6.29; N, 4.75.

2-(2,5-Dimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (12). Mp 125–126 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.08 (3H, s), 2.35 (3H, s), 4.23 (2H, s), 6.92 (1H, s), 7.16 (1H, d, $J = 7.94$ Hz), 7.24 (1H, d, $J = 7.63$ Hz), 7.36 (1H, d, $J = 7.63$ Hz), 7.49 (1H, t, $J = 7.94$ Hz), 7.65 (1H, dt, $J = 7.63$, 1.22 Hz), 8.25 (1H, d, $J = 7.94$ Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.03; H, 5.71; N, 5.27.

2-(2,4-Dimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (13). Mp 171 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.09 (3H, s), 2.37 (3H, s), 4.23 (2H, s), 6.98 (1H, d, $J = 7.93$ Hz), 7.16 (1H, d, $J = 7.93$ Hz), 7.17 (1H, s), 7.36 (1H, d, $J = 7.63$ Hz), 7.48 (1H, t, $J = 7.93$ Hz), 7.65 (1H, dt, $J = 7.63$, 1.53 Hz), 8.25 (1H, dd, $J = 7.94$, 0.92 Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.97; H, 5.75; N, 5.25.

2-(2,3-Dimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (14). Mp 145–146 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.02 (3H, s), 2.35 (3H, s), 4.24 (2H, s), 6.95 (1H, d, $J = 7.02$ Hz), 7.20–7.24 (2H, m), 7.36 (1H, d, $J = 7.63$ Hz), 7.49 (1H, t, $J = 7.93$ Hz), 7.65 (1H, dt, $J = 7.63$, 1.22 Hz), 8.25 (1H, d, $J = 7.93$ Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.58; H, 5.69; N, 5.26.

2-(3,5-Dimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (16). Mp 129–130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.36 (6H, s), 4.21 (2H, s), 6.82 (2H, s), 7.07 (1H, s), 7.34 (1H, d, $J = 7.63$ Hz), 7.47 (1H, t, $J = 7.63$ Hz), 7.63 (1H, dt, $J = 7.63$, 1.22 Hz), 8.24 (1H, d, $J = 7.94$ Hz). Anal. calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.04; H, 5.73; N, 5.23.

2-(2,4-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (18). Mp 107–108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.15 (3H, t, J = 7.5 Hz), 1.28 (3H, t, J = 7.5 Hz), 2.41 (2H, q, J = 7.5 Hz), 2.70 (2H, q, J = 7.5 Hz), 4.23 (2H, s), 6.99 (1H, d, J = 8.0 Hz), 7.16 (1H, dd, J = 8.0, 1.5 Hz), 7.22 (1H, d, J = 1.5 Hz), 7.36 (1H, d, J = 7.8 Hz), 7.49 (1H, t, J = 7.5 Hz), 7.65 (1H, dt, J = 7.5, 1.0 Hz), 8.25 (1H, d, J = 7.8 Hz). Anal. calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.53; H, 6.53; N, 4.50.

2-(2,3-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (19). Mp 155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.03 (3H, t, J = 7.5 Hz), 1.29 (3H, t, J = 7.5 Hz), 2.46 (2H, q, J = 7.5 Hz), 2.74 (2H, q, J = 7.5 Hz), 4.24 (2H, s), 6.93 (1H, dd, J = 7.2, 1.5 Hz), 7.27 (1H, t, J = 7.2 Hz), 7.31 (1H, dd, J = 7.2, 1.5 Hz), 7.37 (1H, dd, J = 7.8, 1.0 Hz), 7.49 (1H, dd, J = 7.6, 1.0 Hz), 7.66 (1H, dd, J = 7.6, 1.0 Hz), 8.25 (1H, dd, J = 7.8, 1.0 Hz). Anal. calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.82; H, 6.47; N, 4.70.

2-(3,4-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (20). Mp 121–122 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.24 (3H, t, J = 7.5 Hz), 1.26 (3H, t, J = 7.5 Hz), 2.69 (2H, q, J = 7.5 Hz), 2.70 (2H, q, J = 7.5 Hz), 4.22 (2H, s), 6.98 (2H, d, J = 8.0 Hz), 7.30 (1H, t, J = 8.0 Hz), 7.35 (1H, d, J = 7.9 Hz), 7.49 (1H, dt, J = 7.6, 1.0 Hz), 7.63 (1H, dt, J = 7.6, 1.0 Hz), 8.24 (1H, dd, J = 7.9, 1.0 Hz). Anal. calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.77; H, 6.62; N, 4.76.

2-(3,5-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (21). Mp 130–131 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.26 (6H, t, J = 7.7 Hz), 2.67 (4H, q, J = 7.7 Hz), 4.22 (2H, s), 6.85 (2H, s), 7.12 (2H, s), 7.34 (1H, d, J = 7.6 Hz), 7.47 (1H, d, J = 7.6 Hz), 7.63 (1H, dt, J = 7.6, 1.0 Hz), 8.24 (1H, dd, J = 7.6, 1.0 Hz). Anal. calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.45; H, 6.72; N, 4.46.

2-(2-Methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (28). Mp 129–130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.78 (3H, s), 4.22 (2H, d, J = 11.3 Hz), 7.04–7.09 (2H, m), 7.17 (1H, dd, J = 7.63, 1.52 Hz), 7.30 (1H, d, J = 7.63 Hz), 7.43 (1H, dt, J = 8.24, 1.83 Hz), 7.48 (1H, t, J = 7.63 Hz), 7.65 (1H, dt, J = 7.83, 1.47 Hz), 8.26 (1H, dd, J = 7.83, 1.47 Hz). Anal. calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.97; H, 4.89; N, 5.19.

2-(3-Methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (29). Mp 169 °C. ^{p1}H NMR (500 MHz, $CDCl_3$) δ: 3.82 (3H, s), 4.23 (2H, d, J = 11.3 Hz), 6.75 (1H, t, J = 2.0 Hz), 6.80 (1H, dq, J = 8.0, 1.0 Hz), 7.00 (1H, ddd, J = 8.0, 2.5, 1.0 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.41 (1H, t, J = 8.0 Hz), 7.49 (1H, t, J = 7.5 Hz), 7.64 (1H, dt, J = 7.5, 1.0 Hz), 8.25 (1H, dd, J = 8.0, 1.0 Hz). Anal. calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.81; H, 4.94; N, 4.94.

2-(4-Methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (30). Mp 195 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.85 (3H, s), 4.22 (2H, d, J = 11.3 Hz), 7.00–7.03 (2H, m), 7.11–7.14 (2H, m), 7.35 (1H, d, J = 8.0 Hz), 7.48 (1H, dt, J = 8.0, 1.0 Hz), 7.64 (1H, dt, J = 8.0, 1.0 Hz), 8.25 (1H, dd, J = 8.0, 1.0 Hz). Anal. calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.83; H, 4.95; N, 4.92.

2-(2-Methylthiophenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (31). Mp 106–108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.41 (3H, s), 4.20 (1H, d, J = 2.5 Hz), 4.30 (1H, d, J = 2.5 Hz), 7.17 (1H, d, J = 7.83 Hz), 7.31 (1H, dt, J = 7.83, 2.0 Hz), 7.34 (1H, d, J = 7.34 Hz), 7.40–7.47 (1H, m), 7.48 (1H, t, J = 7.83 Hz), 7.65 (1H, dt, J = 7.83, 1.47 Hz), 8.26 (1H, dd, J = 7.83, 1.47 Hz). Anal. calcd for $C_{16}H_{13}NO_2S$: C, 67.82; H, 4.62; N, 4.94. Found: C, 67.86; H, 4.58; N, 4.70.

2-(2,5-Dimethoxylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (34). Mp 207–209 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.73 (3H, s), 3.77 (3H, s), 6.74 (1H, dd, J = 2.44, 0.98 Hz), 6.97 (2H, s), 7.35 (1H, d, J = 7.81 Hz), 7.47 (1H, dt, J = 7.81, 0.98 Hz), 7.63 (1H, dt, J = 7.81, 0.98 Hz), 8.25 (1H, dd, J = 7.81, 0.98 Hz). Anal. calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.35; H, 5.08; N, 4.61.

2-(2,4-Dimethoxylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (35). Mp 194–195 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.75 (3H, s), 3.84 (3H, s), 4.21 (2H, d, J = 7.81 Hz), 6.57–6.59 (2H, m), 7.06 (1H, dd, J = 9.28, 2.44 Hz), 7.34 (1H, d, J = 7.81 Hz), 7.46 (1H, t, J = 7.81 Hz), 7.62 (1H, dt, J = 7.81, 1.47 Hz), 8.24 (1H, d, J = 7.81 Hz). Anal. calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.22; H, 5.16; N, 4.54.

2-(3,4-Dimethoxylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (36). Mp 205–207 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.87 (3H, s), 3.92 (3H, s), 4.23 (2H, s), 6.70 (1H, d, J = 2.44 Hz), 6.78 (1H, dd, J = 8.30, 2.44 Hz), 6.98 (1H, d, J = 8.30 Hz), 7.35 (1H, d, J = 7.32 Hz), 7.49 (1H, t, J = 7.32 Hz), 7.65 (1H, dt, J = 7.81, 1.46 Hz), 8.26 (1H, dd, J = 7.81, 0.98 Hz). Anal. calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.58; H, 5.06; N, 4.62.

2-(2,6-Dichlorophenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (38). Bp 250 °C (2.0 mmHg). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.27 (2H, s), 7.34–7.39 (2H, m), 7.41–7.52 (3H, m), 7.68 (1H, dt, J = 7.32, 1.22 Hz), 8.27 (1H, d, J = 7.32 Hz). Anal. calcd for $C_{15}H_9Cl_2NO_2$: C, 58.85; H, 2.96; N, 4.58. Found: C, 58.57; H, 2.91; N, 4.48.

2-(2,6-Difluorophenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione (39). Mp 209–210 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.26 (2H, s), 7.06 (2H, t, J = 7.33 Hz), 7.37 (1H, d, J = 7.94 Hz), 7.40–7.46 (1H, m), 7.50 (1H, t, J = 7.33 Hz), 7.67 (1H, dt, J = 7.33, 0.92 Hz), 8.27 (1H, d, J = 7.94 Hz). Anal. calcd for $C_{15}H_9F_2NO_2$: C, 65.94; H, 3.32; N, 5.13. Found: C, 66.01; H, 3.24; N, 5.05.

2-(2,6-Diethylphenyl)-1*H*-isoindole-1,3-dione (40). Mp 154 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.16 (6H, t, J = 7.5 Hz), 2.47 (4H, q, J = 7.5 Hz), 7.32 (3H, m), 7.91 (4H, m). Anal. calcd for $C_{18}H_{17}NO_2$: C, 77.11; H, 6.05; N, 4.86. Found: C, 77.39; H, 6.13; N, 5.01.

2-(2,6-Diethylphenyl)-5-amino-1*H*-isoindole-1,3-dione (42). Mp 175 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.13 (6H, t, J = 7.5 Hz), 2.46 (4H, q, J = 7.5 Hz), 4.40 (2H, s), 6.90 (1H, dd, J = 8.0, 2.0 Hz), 7.13 (1H, d, J = 2.0 Hz), 7.22 (1H, d, J = 7.5 Hz), 7.36 (1H, t, J = 7.50 Hz), 7.72 (1H, d, J = 8.0 Hz). Anal. calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.59; H, 6.02; N, 9.23.

2-(2,6-Diethylphenyl)-5-hydroxy-1*H*-isoindole-1,3-dione (44). Mp 177–178 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.14 (6H, t, J = 7.5 Hz), 2.46 (4H, q, J = 7.5 Hz), 7.18 (1H, dd, J = 8.0, 2.8 Hz), 7.23 (1H, d, J = 8.0 Hz), 7.36 (1H, d, J = 2.0 Hz), 7.38 (1H, t, J = 8.0 Hz), 7.86 (1H, d, J = 8.0 Hz). Anal. calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.20; H, 5.79; N, 4.84.

2-(2,6-Diethylphenyl)-5-nitro-1*H*-isoindole-1,3-dione (46). Mp 134–135 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.14 (6H, t, J = 7.6 Hz), 2.44 (4H, q, J = 7.6 Hz), 7.26 (2H, d, J = 7.5 Hz), 7.43 (1H, t, J = 7.5 Hz), 8.17 (1H, d, J = 8.1 Hz), 8.70 (1H, dd, J = 8.1, 2.0 Hz), 8.80 (1H, d, J = 2.0 Hz). Anal. calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.76; H, 4.86; N, 8.42.

***N*-(2,6-Diethylphenyl)-3-(2-carboxyphenyl)propionimide (47).** 3-(2-Carboxyphenyl)propionic acid anhydride was condensed with *m*-diethylaniline in CH_2Cl_2. Then, to the mixture was added pivaloyl chloride and Et_3N, and the whole was stirred for 3 days. Mp 126–158 °C. FAB-Mass: 308 $(M+1)^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.07 (6H, t, J = 7.5 Hz), 2.40 (4H, q, J = 7.5 Hz), 2.76 (2H, t, J = 7.6 Hz), 3.50 (2H, t, J = 7.6 Hz), 7.04 (2H, d, J = 7.5 Hz), 7.16 (1H, t, J = 7.5 Hz), 7.39 (1H, t, J = 7.5 Hz), 7.49 (1H, d, J = 7.5 Hz), 7.58 (1H, d, J = 7.5 Hz), 8.11 (1H, d, J = 7.5 Hz).

2-(2,6-Diethylphenyl)-3,4-dihydroisoquinolin-1(2*H*)-one (48). PIQ-22 was treated with $NaBH_4$ to give 2-(2,6-diethylphenyl)isoquinolin-1(2*H*)-one (pale yellow oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.13 (6H, t, J = 7.5 Hz), 2.35–2.45 (4H, m), 6.59 (1H, d, J = 7.5 Hz), 6.93 (1H, d, J = 7.5 Hz), 7.24 (2H, d, J = 7.8 Hz), 7.37 (1H, t, J = 7.8 Hz), 7.53 (1H, dd, J = 7.5, 1.0 Hz), 7.60 (1H, d, J = 8.0 Hz), 7.70 (1H, dd, J = 7.5, 1.0 Hz), 8.59 (1H, dt, J = 8.0, 0.5 Hz)), which was further hydrogenated by H_2 gas catalyzed by 5% Pd/C to give **48**. Pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.23 (6H, t, J = 7.5 Hz), 2.69 (4H, s), 3.19 (2H, t, J = 6.5 Hz), 3.77 (2H, t, J = 6.5 Hz), 7.20 (2H, d, J = 7.5 Hz), 7.27 (1H, d, J = 7.5 Hz), 7.28 (1H, t, J = 7.5 Hz), 7.39 (1H, t, J = 7.5 Hz), 7.48 (1H, dt, J = 7.5, 1.0 Hz), 8.16 (1H, d, J = 7.5 Hz).

2-(2,6-Diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3,4-trione (49). Mp 163–165 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.14 (6H, t, J = 7.5 Hz), 2.41 (4H, q, J = 7.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 7.42 (1H, t, J = 7.5 Hz), 7.92 (1H, td, J = 7.5, 1.0 Hz), 7.97 (1H, td, J = 7.5, 1.0 Hz), 8.34 (1H, dd, J = 7.5, 1.0 Hz), 8.41 (1H, dd, J = 7.5, 1.0 Hz). Anal. calcd for $C_{19}H_{17}N_3$: C, 74.24; H, 5.59; N, 4.56. Found: C, 74.32; H, 5.84; N, 4.43.

2-(2,6-Diethylphenyl)-4,4-difluoro-1,2,3,4-tetrahydroisoquinoline-1,3-dione (50). Mp 112–123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.14 (6H, t, J = 7.5 Hz), 2.40 (4H, q, J = 7.5 Hz), 7.26 (2H, d, J = 7.5 Hz), 7.42 (1H, t, J = 7.5 Hz), 7.81 (1H, t, J = 7.5 Hz), 7.90 (1H, t, J = 7.5 Hz), 8.00 (1H, d, J = 7.5 Hz), 8.32 (1H, d, J = 7.5 Hz). Anal. calcd for $C_{19}H_{17}NO_2F_2$: C, 69.30; H, 5.17; N, 4.25. Found: C, 69.02; H, 5.11; N, 4.46.

2-(2,6-Diethylphenyl)-7-amino-1,2,3,4-tetrahydroisoquinoline-1,3-dione (51). Mp 153–155 °C. HRMS(EI) M^+: 308.151 (calcd for $C_{19}H_{20}N_2O_2$: 308.153). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.13 (6H, t, J = 7.3 Hz), 2.39 (4H, q, J = 7.3 Hz), 3.87 (2H, br s), 4.12 (2H, s), 6.97 (1H, dd, J = 8.2, 2.4 Hz), 7.15 (1H, d, J = 8.2 Hz), 7.23 (2H, d, J = 7.6 Hz), 7.36 (1H, t, J = 7.6 Hz), 7.51 (1H, d, J = 2.4 Hz).

2-(2,6-Diethylphenyl)-7-nitro-1,2,3,4-tetrahydroisoquinoline-1,3-dione (52). Mp 170–171 °C. HRMS(EI) M^+: 338.128 (calcd for $C_{19}H_{18}N_2O_4$: 338.127). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.15 (6H, t, J = 7.5 Hz), 2.39 (4H, q, J = 7.5 Hz), 4.35 (2H, s), 7.26 (2H, d, J = 7.5 Hz), 7.41 (1H, t, J = 7.5 Hz), 7.60 (1H, d, J = 8.5 Hz), 8.50 (1H, dd, J = 8.5, 2.5 Hz), 9.09 (1H, d, J = 2.5 Hz).

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