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Novel aminopeptidase N inhibitors derived from 1,3,4-thiadiazole scaffold

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ABSTRACT

The aminopeptidase N (APN/CD13), overexpressed in tumor cells, plays a critical role in angiogenesis. In this study, we report the synthesis and in vitro enzyme inhibition assay of 1,3,4-thiadiazole scaffold compounds. These new compounds have potent inhibitory activities toward APN with IC_{50} values in the micromol rang.

1. Introduction

Aminopeptidase N (APN), also known as CD13, is a widespread ectopeptidase which preferentially releases neutral and basic amino acids from the N-terminal end of peptides.[1] In the intestinal brush border, APN is involved in the terminal degradation of small peptides. In synaptic membranes, the enzyme inactivates neuropeptides (endorphins and enkephalins).[2] APN was also shown to be the major receptor for the TGEV and HCV229E, and *Bacillus thuringensis* Cry1A toxin.[3–5] Many experimental results suggest that APN is involved in the down-regulation of several biological active molecules, such as fMLP, IL-8, angiotensin III, and major histocompatibility complex (MHC) class II molecules.[6–11] APN plays an essential role in the entry of HIV into host cells.[12] Furthermore, tumor cells which overexpress APN (such as melanoma cells, acute lymphocytic leukemic cells, and urological cancer cells) are highly motile and capable of migration through extracellular matrix.[13,14] On the other hand, angiogenesis emanating from microvascular endothelial cells plays a central role in tumor growth and metastasis.[15] APN antagonists (CD13 antibodies or bestatin) significantly block induced-retinal neovascularization in mice and in chorioallantoic membrane angiogenesis in vitro.[16,17] All these findings make this enzyme an interesting target for possible therapeutic applications, which require the development of potent and selective inhibitors. To date, several inhibitors of APN, including Bestatin, Amastatin, and Actinonin, have been developed and some of them are currently investigated for clinical uses.[18] APN inhibition by Bestatin leads to a loss of motility. Likewise, CD13 antibodies inhibit cell growth and cell motility.[19–21]

Inhibitors containing zinc-binding groups (ZBGs) should be designed to inhibit the activity of APN. This class of inhibitors can interact with the zinc ions of zinc-dependent metalloenzymes and sequentially inhibit the metastatic spread of tumors and block the processes of tumor neovascularization.[22] There are two hydrophobic domains beside the catalytic activity center of APN, called pockets S_1 and S_1', respectively. APN exhibits a broad specificity for peptides with a N-terminal neutral or basic amino acid such as alanine, arginine or leucine. Thiadiazole derivatives and cinnamic acid have been known to have broad biological activities, especially anti-tumor properties.[23,24] According to the 'combination principles', if we link thiadiazole with alanine and cinnamic acid, the resulting 1,3,4-thiadiazole scaffold compounds should be capable of inhibiting the enzymatic activity of APN.

2. Chemistry

In order to study the SAR of these novel peptidomimetic compounds, different 1,3,4-thiadiazole derivatives were designed and synthesized via the route outlined in Scheme 1. The readily available carboxylic acid **4a–g** was condensed with *N*-aminothiourea **5** in presence of Phosphorus oxychloride, yielding 5-substituted-1,3,4-thiadiazol-2-amine **6a–g**.[25] The coupling of D-alanine **7** with compound **8a–c** led to amide **9a–c**.[26,27] Finally, the intermediate **6a–g** and **9a–c** were reacted using DCC to give target compounds **1a–g**, **2a–g**, and **3a–g**.

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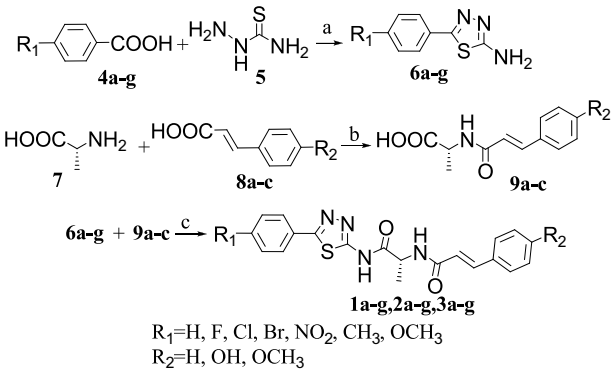


Scheme 1. Reagents and conditions: (a) $POCl_3$, 110 °C; (b and c) *N*-hydroxysuccinimide, DCC, rt, by WenFang Xu.

3. Results and discussion

All the inhibition results are summarized in Table 1. The inhibition results showed that most of the target compounds display excellent potency toward APN with IC_{50} values lying in micromolar level. Comparing **2a–g**, we could confirm that hydroxyl group was positively related with the inhibitory activities. This could be due to hydroxyl group forming hydrogen bond with the enzyme. The more active compounds are **2d** and **2e**, which have been shown to recognize efficiently the S_1 and S_1' subsites of APN. To further understand the interactions of **2d** with APN, the preferred pharmacophore docking studies were carried out via the FlexX flexible-Dock program. The interaction of **2d** with active site of *Escherichia coli* APN (PDB:2DQM)[28] is shown in Figs. 1 and 2.[29] The carbonyl group of alanine can interact with the zinc ion with the distance of 2.85 Å and form hydrogen bond with Tyr-383. In addition, the hydroxyl and thiadiazole form hydrogen bond with Gln-821 and Ala-262, respectively. The hydrophobic parts of phenyl rings are in contact with nonpolar surface areas of APN. In addition, all of target compounds, except compound **3e**, show inactivity toward MMP-2 which is a zinc-dependent endopeptidase associated with the tumorigenic process.

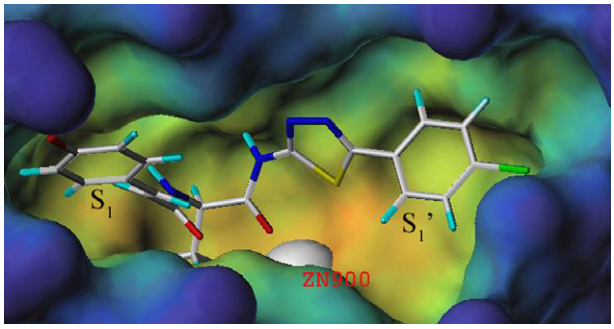
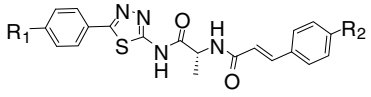


Figure 1. The FlexX docking of compound **2d** with active-site in *Escherichia coli* APN (PDB:2DQM) by WenFang Xu.

Although the computed information partially supported our assumption, the exact binding model of the thiadiazole derivatives with APN should be obtained from further X-ray crystal studies.

Table 1
In vitro enzyme assay (APN and MMP-2) results for compounds 1-3 and Bestatin



Compound	R_1	R_2	IC_{50}[a]/μM	
			APN	MMP-2
1a	—H	-H	215.6 ± 12.4	na[b]
1b	—F	—H	183.4 ± 8.7	na
1c	—Cl	—H	152.6 ± 11.6	na
1d	—Br	—H	114.9 ± 13.7	na
1e	$-NO_2$	—H	84.7 ± 9.0	na
1f	$-CH_3$	—H	206.2 ± 15.7	na
1g	$-OCH_3$	—H	31.4 ± 18.0	na
2a	—H	—OH	123.9 ± 9.7	na
2b	—F	—OH	115.4 ± 12.6	na
2c	—Cl	—OH	104.9 ± 17.9	na
2d	—Br	—OH	35.3 ± 0.9	na
2e	$-NO_2$	—OH	54.1 ± 8.4	na
2f	$-CH_3$	—OH	126.8 ± 12.9	na
2g	$-OCH_3$	—OH	68.8 ± 7.9	na
3a	—H	$-OCH_3$	131.1 ± 14.4	na
3b	—F	$-OCH_3$	90.7 ± 20.8	na
3c	—Cl	$-OCH_3$	134.6 ± 15.2	na
3d	—Br	$-OCH_3$	108.2 ± 11.9	na
3e	$-NO_2$	$-OCH_3$	44.6 ± 10.7	297.3 ± 28.5
3f	$-CH_3$	$-OCH_3$	141.1 ± 12.8	na
3g	$-OCH_3$	$-OCH_3$	78.5 ± 0.6	na
	Bestatin		8.5 ± 0.6	9.5 ± 0.5

[a] Values are means of three experiments, standard deviation is given.
[b] not activity.

4. Conclusion

In conclusion, we have developed a new series of potent APN inhibitors. Most of compounds have potent inhibitory activities toward APN and could be used as lead compounds for the development of low molecular-weight peptidomimetic APN inhibitors. Because the critical point in designing selective inhibitors is to optimize the recognition of APN.

5. Experimental

5.1. Enzyme inhibition assay (in vitro)

The target compounds were evaluated for inhibitory activity toward APN and MMP-2. The IC_{50} values against APN were determined using L-Leu-p-nitroanilide as substrate and microsomal aminopeptidase from Porcine Kidney Microsomes (Sigma) as the enzyme in 50 mM PBS, pH 7.2, at 37 °C.[30] MMP assay was performed according to the literature.[31] The gelatinase(MMP-2), substance, and inhibitor were dissolved in sodium borate (pH 8.5, 50 mmol/L) and incubated for 30 min at 37 °C, and then 0.03% TNBS was added and incubated for another 20 min, the resulting solution was detected under 450 nm wavelength to gain absorption.

5.2. Chemistry: general procedures

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All the solvents except DMF have been distilled before use. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light. Column chromatography was performed on silica gel (200–300 mesh). ESI-MS was determined on a Aglient-1100 series LC/MSD trap spectrometer. IR was recorded on a FTIR-8400 spectrometer. Melting point was determined on a electrothermal melting point apparatus and is uncorrected. ^{1}H NMR spectra was obtained on a brucker-400. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane (TMS) as internal standard. Signifi-

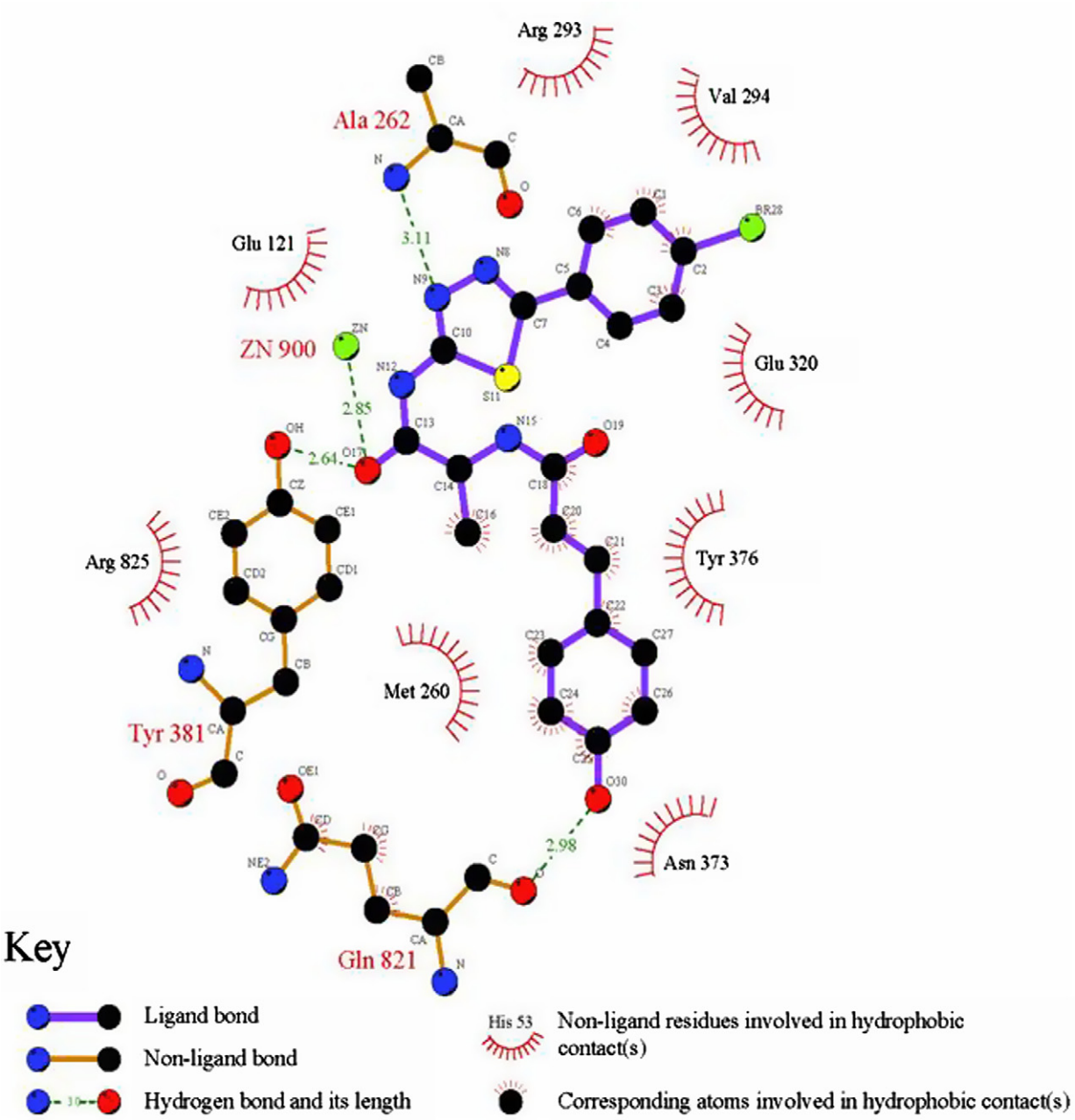


Figure 2. Diagram (LIGPLOT) of the hydrogen bonds and hydrophobic interactions of the compound **2d** with active-site residues in *Escherichia coli* APN (PDB:2DQM) by WenFang Xu.

cant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) number of protons.

5.2.1. General procedure for preparing 5-substituted-1,3,4-thiadiazol-2-amine. Synthesis of 5-phenyl-1,3,4-thiadiazol-2-amino (6a)

A stirring mixture of benzoic acid (50 mmol), *N*-aminothiourea (50 mmol) and $POCl_3$ (13 ml) was heated at 75 °C for 0.5 h. After cooling down to room temperature, water was added. The reaction mixture was refluxed for 4 h. After cooling, the mixture was basified to pH 8 by the dropwise addition of 50% NaOH solution under stirring. The precipitate was filtered and recystallized from ethanol to yield 7.0 g (79.1%) of the title compounds as white solid; mp: 224–226 °C; IR (KBr, σ/cm^{-1}): 3276, 3114 (υ_{NH}), 1634 ($\upsilon_{C=N}$), 691 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ 7.40 (s, 2H), 7.43–7.49 (dd, J_1 = 6.38 Hz, J_2 = 14.00 Hz, 2H), 7.74–7.76 (d, J = 6.94 Hz, 2H), ESI-MS: m/z [M+H]$^+$ 178.3.

The following compounds were prepared according to the general procedure as described for the preparation of compound **6a** starting from compounds **4a**–**g** by coupling with compound **5**.

5.2.2. 5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amino (6b)

White solid, yield: 82.5%; mp: 232–234 °C; IR (KBr, σ/cm^{-1}): 3341, 3246 (υ_{NH}), 1061 (υ_{C-F}), 687(υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ *7.29–7.33* (t, J = 8.77 Hz, 2H), 7.41 (s, 2H), 7.79–7.83 (dd, J_1 = 5.46 Hz, J_2 = 8.59 Hz, 2H), ESI-MS: m/z [M+H]$^+$ 196.4.

5.2.3. 5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amino (6c)

White solid, yield: 77.4%; mp: 214–216 °C; IR (KBr, σ/cm^{-1}): 3269, 3098 (υ_{NH}), 1636 ($\upsilon_{C=N}$), 694 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ

7.47 (s, 2H), 7.52–7.54 (d, J = 6.55 Hz, 2H), 7.76–7.78 (d, J = 6.24 Hz, 2H), ESI-MS: m/z $[M+H]^+$ 212.6.

5.2.4. 5-(4-Bromophenyl)-1,3,4-thiadiazol-2-amino (6d)

White solid, yield: 84.1%; mp: 222–224 °C; IR (KBr, σ/cm^{-1}):3286, 3090 (υ_{NH}), 1638 ($\upsilon_{C=N}$), 690 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ 7.47 (s, 2H), 7.65–7.72 (dd, J_1 = 8.48 Hz, J_2 = 16.97 Hz, 4H), ESI-MS: m/z $[M+H]^+$ 256.2.

5.2.5. 5-(4-Nitrorophenyl)-1,3,4-thiadiazol-2-amino (6e)

Yellow solid, yield: 80.6%; mp: 250–252 °C; IR (KBr, σ/cm^{-1}): 3398, 3288 (υ_{NH}), 1630 ($\upsilon_{C=N}$), 691 (υ_{C-S}), 1506 (υ_{NO_2}); ^{1}H NMR (DMSO-d_6): δ 7.74 (s, 2H), 8.00–8.01 (d, J = 2.20 Hz, 2H), 8.28–8.29 (d, J = 2.28 Hz, 2H); ESI-MS: m/z $[M+H]^+$ 223.3.

5.2.6. 5-(4-Methylphenyl)-1,3,4-thiadiazol-2-amino (6f)

White solid, yield: 90.2%; mp: 210–211 °C; IR (KBr, σ/cm^{-1}):3288, 3100 (υ_{NH}), 1635 ($\upsilon_{C=N}$), 690 (υ_{C-S}), 2952 (υ_{CH}); ^{1}H NMR (DMSO-d_6): δ 2.33(s, 3H), 7.25–7.27 (d, J = 8.00 Hz, 2H), 7.36 (s, 2H), 7.63–7.65 (d, J = 8.12 Hz, 2H); ESI-MS: m/z $[M+H]^+$ 192.2.

5.2.7. 5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-amino (6g)

White solid, yield: 92.3%; mp: 192–194 °C; IR (KBr, σ/cm^{-1}): 3286, 3107 (υ_{NH}), 1628 ($\upsilon_{C=N}$), 1254 (υ_{Ar-O-C}), 2960 (υ_{CH}); ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 2H), 7.02 (s, 2H), 7.27 (s, 2H), 7.68 (s, 2H), ESI-MS: m/z $[M+H]^+$ 208.2.

5.2.8. General procedure for preparing *N*-acyl-D-alanine. Synthesis of *N*-cinnamoyl-D-alanine (9a)

N,*N*-Dicyclohexylcarbodiimide (32 mmol) was added to a cooled solution of cinnamic acid (30 mmol) and *N*-hydroxysuccinimide (32 mmol) in freshly distillation dioxane (46 ml). The reaction mixture was stirred for 5 h at room temperature. The insoluble material was filtered off and washed with cold dioxane. The filtrate was added to a solution of D-alanine (34 mmol) and $NaHCO_3$ (34 mmol) in H_2O (48 ml) and the reaction mixture stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residual was dissolved in water and the insoluble material was filtered off. The aqueous layer was extracted with EtOAc (3× 30 ml). The resulting aqueous layer was acidified with concentrated HCl (pH 2) and allowed to cool. The precipitate formed was filtered, recrystallized from methanol and dried to afford the target compound as white solid 3.7 g, yield: 56.6%. mp: 154–156 °C; IR (KBr, σ/cm^{-1}): 3323 (υ_{NH}), 1656 ($\upsilon_{C=O}$), 2943 (υ_{OH}), 1724 ($\upsilon_{C=O}$). ^{1}H NMR (DMSO-d_6): δ *1.35–1.37* (d, J = 7.28 Hz, 3H), 4.35–4.42(m, 1H), 6.73–6.77 (d, J = 15.84 Hz, 1H), 7.37–7.50 (m, 4H), 7.58–7.59 (d, J = 7.28 Hz, 2H), 8.42–8.44 (d, J = 7.32 Hz, 1H), 12.62 (s, 1H). ESI-MS: m/z $[M+H]^+$ 220.2.

5.2.9. *N*-(*p*-Coumaroyl)-D-alanine (9b)

The title compound was prepared starting from *p*-coumaric acid as described for compound **9a**. Yield: 62.6%; white solid; mp: 188–190 °C. IR (KBr, σ/cm^{-1}): 3325 (υ_{NH}), 1647 ($\upsilon_{C=O}$), 3099 (υ_{OH}), 1724 ($\upsilon_{C=O}$). ^{1}H NMR (DMSO-d_6): δ *1.33–1.34* (d, J = 7.28 Hz, 3H), 4.31–4.39(m, 1H), 6.49–6.53 (d, J = 15.76 Hz, 1H), 6.81–6.83 (d, J = 8.55 Hz, 2H), 7.34–7.43 (m, 3H), 8.26–8.28 (d, J = 7.36 Hz, 1H), 9.62–9.64 (d, J = 7.45 Hz, 1H), 12.59 (s, 1H). ESI-MS: m/z $[M+H]^+$ 236.3.

5.2.10. *N*-(*p*-Methoxycinnamoyl)-D-alanine (9c)

The title compound was prepared starting from *p*-methoxycinnamic acid as described for compound **9a**. Yield: 68.2%; white solid; mp: 162–164 °C. IR (KBr, σ/cm^{-1}): 3375 (υ_{NH}), 1650 ($\upsilon_{C=O}$), 3018 (υ_{OH}), 1717 ($\upsilon_{C=O}$). ^{1}H NMR (DMSO-d_6): δ *1.33–1.35* (d, J = 7.28 Hz, 3H), 3.79 (s, 3H), 4.33–4.40 (m, 1H), 6.57–6.1 (d, J = 15.76 Hz, 1H), 6.97–7.00 (d, J = 8.72 Hz, 2H), 7.39–7.43 (d, J = 15.80 Hz, 1H), 7.51–7.54 (d, J = 8.76 Hz, 2H), 8.31–8.33 (d, J = 7.40 Hz, 1H), 12.57 (s, 1H). ESI-MS: m/z $[M+H]^+$ 250.2.

5.2.11. General procedure for preparing propanamide. Synthesis of (2*R*)-*N*-[5-phenyl-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1a)

N,*N*-Dicyclohexylcarbodiimide (5.7 mmol) was added to a cooled solution of *N*-cinnamoyl-D-alanine (**9a**) (5.6 mmol) and *N*-hydroxysuccinimide (5.6 mmol) in freshly distillation dioxane (30 ml). The reaction mixture was stirred overnight at room temperature. The insoluble material was filtered off and washed with cold dioxane. 5-Phenyl-1,3,4- thiadiazol-2-amino (**6a**) (5.5 mmol) was added to the filtrate and the reaction mixture was stirred for 48 h at room temperature. The solvent was removed under reduced pressure. The residual was dissolved in EtOAc and the insoluble material was filtered off. The filtrate was washed successively with saturated Na_2CO_3 solution (3× 20 ml), water (1× 20 ml), 0.1 M HCl (3× 20 ml) and water (1× 20 ml). The organic layer evaporated in vacuo, the residual was recrystallized from methanol/acetonitrile (5:1) and dried to afford the target compound as white solid 0.34 g, yield: 16.3%. mp: 209–210 °C. IR (KBr, σ/cm^{-1}): 3417, 3262 (υ_{NH}), 1684,1654 ($\upsilon_{C=O}$), 690 (υ_{C-S}), 1620 ($\upsilon_{C=N}$). ^{1}H NMR (DMSO-d_6) δ: 1.42–1.43 (d, J = 7.16 Hz, 3H), 4.64–4.71(m, 1H), 6.74–6.78 (d, J = 15.96 Hz, 1H), 7.40–7.48 (m, 4H), 7.53–7.60 (m, 5H), 7.94–7.96 (m, 2H), 8.61–8.63 (d, J = 6.48 Hz, 1H), 12.86 (s, 1H). ESI-MS: m/z $[M+H]^+$ 379.2.

The following compounds were prepared according to the general procedure as described for the preparation of compound **1a** starting from compounds **9a**–**c** by coupling with compounds **6a**–**g**.

5.2.12. (2*R*)-*N*-[5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1b)

White solid, yield: 28.3%; mp: 249–250 °C; IR (KBr, σ/cm^{-1}): 3402, 3261 (υ_{NH}), 1701, 1658 ($\upsilon_{C=O}$), 686 (υ_{C-S}), 1623 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.40–1.42* (d, J = 7.08 Hz, 3H), 4.63–4.66 (t, J = 6.86 Hz, 1H), 6.72-6.76 (d, J = 15.88 Hz, 1H), 7.36–7.47 (m, 6 H), 7.57–7.59 (d, J = 7.24 Hz, 2H), 7.99–8.02 (dd, J_1 = 5.56 Hz, J_2 = 8.24 Hz, 2H), 8.64–8.66 (d, J = 6.40 Hz, 1H), 12.90 (s, 1H); ESI-MS: m/z $[M+H]^+$ 397.1.

5.2.13. (2*R*)-*N*-[5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]- propanamide (1c)

White solid, yield: 39.4%; mp: 207–208 °C; IR (KBr, σ/cm^{-1}): 3437, 3155 (υ_{NH}), 1701, 1659 ($\upsilon_{C=O}$), 686 (υ_{C-S}), 1620 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.41–1.43* (d, J = 7.20 Hz, 3H), 4.64–4.71 (m, 1H), 6.73–6.77 (d, J = 15.92 Hz, 1H), 7.39–7.48 (m, 4H), 7.58–7.61 (m, 4H), 7.96–7.98 (dd, J_1 = 1.92 Hz, J_2 = 6.60 Hz, 2H), 8.62–8.63 (d, J = 6.48 Hz, 1H), 12.91 (s, 1H); ESI-MS: m/z $[M+H]^+$ 413.1.

5.2.14. (2*R*)-*N*-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1d)

White solid, yield: 14.9%; mp: 158–60 °C; IR (KBr, σ/cm^{-1}): 3301, 3166 (υ_{NH}), 1698, 1659 ($\upsilon_{C=O}$), 688 (υ_{C-S}), 1622 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.40–1.42* (d, J = 7.12 Hz, 3H), 4.64–4.67 (t, J = 6.86 Hz, 1H), 6.72–6.76 (d, J = 15.80 Hz, 1H), 7.47–7.51 (d, J = 16.64 Hz, 1H), 7.58–7.72 (m, 5H), 7.73–7.75 (d, J = 8.48 Hz, 2H), 7.89–7.91 (d, J = 8.48 Hz, 2H), 8.66–8.67 (d, J = 6.40 Hz, 1H), 12.95 (s, 1H); ESI-MS: m/z $[M+H]^+$458.0.

5.2.15. (2*R*)-*N*-[5-(4-Nitrorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1e)

Yellow solid, yield: 50.2%; mp: 265–267 °C; IR (KBr, σ/cm^{-1}): 3422, 3320 (υ_{NH}), 1700, 1659 ($\upsilon_{C=O}$), 688 (υ_{C-S}), 1617 ($\upsilon_{C=N}$), 1522 (υas_{NO_2}), 1333 (υs_{NO_2}); ^{1}H NMR (DMSO-d_6): δ 1.39–1.41 (d, J = 6.86 Hz, 3H), 4.59–4.63 (t, J = 6.78 Hz, 1H), 6.78–6.82 (d,

J = 15.82 Hz, 1H), 7.39–7.46 (dd, J_1 = 10.37 Hz, J_2 = 16.32 Hz, 4H), 7.58–7.60 (d, J = 7.12 Hz, 2H), 8.18–8.20 (d, J = 8.30 Hz, 2H), 8.33–8.35 (d, J = 8.41 Hz, 2H), 8.48 (s, 1H), 12.96 (s, 1H); ESI-MS: m/z $[M+H]^+$ 424.1.

5.2.16. (2*R*)-*N*-[5-(4-Methylphenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1f)

White solid, yield: 29.3%; mp: 210–212 °C; IR (KBr, σ/cm^{-1}): 3427, 3310 (υ_{NH}), 1703, 1660 ($\upsilon_{C=O}$), 691 (υ_{C-S}), 1621 ($\upsilon_{C=N}$); ^{1}H NMR ($CDCl_3$): δ *1.66–1.68* (d, J = 7.40 Hz, 3H), 2.42 (s, 3H), 4.92–5.00 (m, 1H), 6.39–6.43 (d, J = 15.72 Hz, 1H), 7.05–7.26 (m, 4H), 7.35–7.38 (t, J = 6.32 Hz, 2H), 7.53–7.56 (d, J = 15.72 Hz, 1H), 7.86–7.88 (d, J = 8.08 Hz, 2H), 8.65–8.67 (d, J = 7.04 Hz, 1H), 13.33 (s, 1H); ESI-MS: m/z $[M+H]^+$ 393.3.

5.2.17. (2*R*)-*N*-[5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl]-2-[(cinnamoyl)amino]-propanamide (1g)

White solid, yield: 35.2%; mp: 176–178 °C; IR (KBr, σ/cm^{-1}): 3437, 3209 (υ_{NH}), 1670, 1654 ($\upsilon_{C=O}$), 1627 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.40–1.42* (d, J = 7.12 Hz, 3H), 3.83 (s, 3H), 4.64–4.67 (t, J = 6.90 Hz, 1H), 6.72–6.76 (d, J = 15.88 Hz, 1H), 7.08–7.10 (d, J = 8.72 Hz, 2H), 7.39–7.47 (m, 4H), 7.57–7.59 (d, J = 7.08 Hz, 2H), 7.87–7.89 (d, J = 8.72 Hz, 2H), 8.59–8.61 (d, J = 6.36 Hz, 1H), 12.77 (s, 1H); ESI-MS: m/z $[M+H]^+$ 409.1.

5.2.18. (2*R*)-*N*-[5-Phenyl-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2a)

White solid, yield: 24.9%; mp: 215-217 °C; IR (KBr, σ/cm^{-1}): 3389, 3327 (υ_{NH}), 1700, 1651 ($\upsilon_{C=O}$), 690 (υ_{C-S}), 1628 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.39–1.41* (d, J = 7.04 Hz, 3H), 4.64 (s, 1H), 6.50–6.54 (d, J = 15.64 Hz, 1H), 6.80–6.82 (d, J = 8.48 Hz, 2H), 7.33–7.37 (d, J = 15.78 Hz, 1H), 7.40–7.42 (d, J = 8.46 Hz, 2H), 7.53–7.54 (d, J = 2.08 Hz, 3H), 7.93–7.94 (d, J = 3.40 Hz, 2H), 8.46–8.48 (d, J = 6.24 Hz, 1H), 9.87 (s, 1H), 12.83 (s, 1H); ESI-MS: m/z $[M+H]^+$ 395.0.

5.2.19. (2*R*)-*N*-[5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2b)

White solid, yield: 23.3%; mp: 220–223 °C; IR (KBr, σ/cm^{-1}): 3262, 3159 (υ_{NH}), 1695, 1655 ($\upsilon_{C=O}$); ^{1}H NMR (DMSO-d_6): δ *1.38–1.40* (d, J = 7.12 Hz, 3H), 4.61–4.64 (t, J = 6.86 Hz, 1H), 6.50–6.54 (d, J = 15.76 Hz, 1H), 6.79–6.82 (d, J = 8.48 Hz, 2H), 7.32–7.42 (m, 5H), 7.99–8.02 (dd, J_1 = 5.40 Hz, J_2 = 8.64 Hz, 2H), 8.50–8.52 (d, J = 6.36 Hz, 1H), 9.92 (s, 1H), 12.88 (s, 1H); ESI-MS: m/z $[M+H]^+$ 413.2.

5.2.20. (2*R*)-*N*-[5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2c)

White solid, yield: 42.8%; mp: 265–266 °C; IR (KBr, σ/cm^{-1}): 3388, 3165 (υ_{NH}), 1685, 1655 ($\upsilon_{C=O}$), 697 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ *1.40–1.41* (d, J = 7.16 Hz, 3H), 4.62–4.68 (m, 1H), 6.51–6.54 (d, J = 15.80 Hz, 1H), 6.80–6.82 (d, J = 8.48 Hz, 2H), 7.34–7.38 (d, J = 15.76 Hz, 1H), 7.40–7.67 (m, 4H), 7.96–7.98 (t, J = *4.30* Hz, 2H), 8.47–8.28 (d, J = 6.48 Hz, 1H), 9.87 (s, 1H), 12.88 (s, 1H); ESI-MS: m/z $[M+H]^+$ 429.1.

5.2.21. (2*R*)-*N*-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2d)

White solid, yield: 32.7%; mp: 242–244 °C; IR (KBr, σ/cm^{-1}): 3341, 3203 (υ_{NH}), 1706, 1653 ($\upsilon_{C=O}$); ^{1}H NMR (DMSO-d_6): δ *1.38–1.40* (d, J = 7.08 Hz, 3H), 4.61–4.64 (t, J = 6.86 Hz, 1H), 6.49–6.53 (d, J = 15.76 Hz, 1H), 6.79–6.81 (d, J = 8.40 Hz, 2H), 7.32–7.36 (d, J = 15.76 Hz, 1H), 7.40–7.42 (d, J = 8.36 Hz, 2H), 7.73–7.75 (d, J = 8.44 Hz, 2H), 7.89–7.91 (d, J = 8.40 Hz, 2H), 8.50–8.52 (d, J = 6.28 Hz, 1H), 9.92 (s, 1H), 12.92 (s, 1H); ESI-MS: m/z $[M+H]^+$474.3.

5.2.22. (2*R*)-*N*-[5-(4-Nitrorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2e)

Yellow solid, yield: 23.1%; mp: 225–227 °C; IR (KBr, σ/cm^{-1}): 3429, 3327 (υ_{NH}), 1696, 1654 ($\upsilon_{C=O}$), 690 (υ_{C-S}), 1621 ($\upsilon_{C=N}$), 1517 (υas_{NO_2}), 1345 (υs_{NO_2}); ^{1}H NMR (DMSO-d_6): δ *1.40–1.42* (d, J = 7.12 Hz, 3H), 4.63–4.66 (t, J = 6.88 Hz, 1H), 6.50–6.53 (d, J = 15.80 Hz, 1H), 6.80–6.82 (d, J = 8.44 Hz, 2H), 7.33–7.37 (d, J = 15.76 Hz, 1H), 7.40–7.42 (d, J = 8.44 Hz, 2H), 8.23–8.25 (d, J = 8.76 Hz, 2H), 8.35–8.38 (d, J = 8.79 Hz, 2H), 8.50–8.51 (d, J = 6.18 Hz, 1H), 9.89 (s, 1H), 13.04 (s, 1H); ESI-MS: m/z $[M+H]^+$ 440.2.

5.2.23. (2*R*)-*N*-[5-(4-Methylphenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2f)

White solid, yield: 35.8%; mp: 195–197 °C; IR (KBr, σ/cm^{-1}): 3333, 3253 (υ_{NH}), 1710, 1655 ($\upsilon_{C=O}$), 690 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ *1.39–1.41* (d, J = 7.08 Hz, 3H), 2.37 (s, 3H), 4.62–4.65 (t, J = 6.88 Hz, 1H), 6.51–6.55 (d, J = 15.76 Hz, 1H), 6.80–6.82 (d, J = 8.40 Hz, 2H), 7.33–7.37 (t, J = 7.70 Hz, 3H), 7.41–7.43 (d, J = 8.44 Hz, 2H), 7.82–7.84 (d, J = 7.96 Hz, 2H), 8.50–8.52 (d, J = 6.36 Hz, 1H), 9.93 (s, 1H), 12.84 (s, 1H); ESI-MS: m/z $[M+H]^+$ 409.1.

5.2.24. (2*R*)-*N*-[5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-coumaroyl)amino]-propanamide (2g)

White solid, yield: 42.1%; mp: 215–216 °C; IR (KBr, σ/cm^{-1}): 3408, 3272 (υ_{NH}), 1695, 1655 ($\upsilon_{C=O}$); ^{1}H NMR (DMSO-d_6): δ *1.38–1.40* (d, J = 7.16 Hz, 3H), 3.83 (s, 3H), 4.62 (s, 1H), 6.50–6.54 (d, J = 15.92 Hz, 1H), 6.79–6.81 (d, J = 8.48 Hz, 2H), 7.07–7.10 (d, J = 8.72 Hz, 2H), 7.32–7.36 (d, J = 15.76 Hz, 1H), 7.40–7.42 (d, J = 8.52 Hz, 2H), 7.86–7.89 (d, J = 8.68 Hz, 2H), 8.47 (s, 1H), 9.88 (s, 1H), 12.75 (s, 1H); ESI-MS: m/z $[M+H]^+$ 425.1

5.2.25. (2*R*)-*N*-[5-Phenyl-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3a)

White solid, yield: 23.5%; mp: 145–147 °C; IR (KBr, σ/cm^{-1}): 3405, 3286 (υ_{NH}), 1700, 1650 ($\upsilon_{C=O}$), 691 (υ_{C-S}), 1620 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.39–1.41* (d, J = 6.96 Hz, 3H), 3.79 (s, 3H), 4.63–4.65 (d, J = 6.92 Hz, 1H), 6.57–6.61 (d, J = 15.72 Hz, 1H), 6.98–7.00 (d, J = 8.16 Hz, 2H), 7.37–7.41 (d, J = 16.32 Hz, 1H), 7.54 (s, 5H), 7.93–7.94 (d, J = 4.48 Hz, 2H), 8.85–8.55 (d, J = 6.08 Hz, 1H), 12.88 (s, 1H); ESI-MS: m/z $[M+H]^+$ 409.1.

5.2.26. (2*R*)-*N*-[5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3b)

White solid, yield: 68.4%; mp: 183–185 °C; IR (KBr, σ/cm^{-1}): 3405,3277 (υ_{NH}), 1691,1653 ($\upsilon_{C=O}$), 1621 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.39–1.41* (d, J = 7.12 Hz, 3H), 3.79 (s, 3H), 4.62–4.65 (t, J = 6.86 Hz, 1H), 6.57–6.61 (d, J = 15.84 Hz, 1H), 6.98–7.01 (d, J = 8.64 Hz, 2H), 7.36–7.41 (dd, J_1 = 9.36 Hz, J_2 = 14.84 Hz, 3H), 7.52–7.54 (d, J = 8.64 Hz, 2H), 7.99–8.02 (dd, J_1 = 5.40 Hz, J_2 = 8.64 Hz, 2H), 8.54–8.86 (d, J = 6.40 Hz, 1H), 12.89 (s, 1H); ESI-MS: m/z $[M+H]^+$ 427.1.

5.2.27. (2*R*)-*N*-[5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3c)

White solid, yield: 35.8%; mp: 125–128 °C; IR (KBr, σ/cm^{-1}): 3405, 3333 (υ_{NH}), 1700, 1655 ($\upsilon_{C=O}$), 1627 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.39–1.41* (d, J = 7.12 Hz, 3H), 3.79 (s, 3H), 4.62–4.66 (t, J = 6.84 Hz, 1H), 6.57–6.61 (d, J = 15.80 Hz, 1H), 6.98–7.01 (d, J = 8.52 Hz, 2H), 7.37–7.42 (d, J = 15.76 Hz, 1H), 7.52–7.62 (m, 4H), 7.96–7.98 (d, J = 8.40 Hz, 2H), 8.55–8.57 (d, J = 6.28 Hz, 1H), 12.93 (s, 1H); ESI-MS: m/z $[M+H]^+$ 443.2.

5.2.28. (2*R*)-*N*-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3d)

White solid, yield: 45.2%; mp: 134–135 °C; IR (KBr, σ/cm^{-1}): 3405, 3285 (υ_{NH}), 1698, 1652 ($\upsilon_{C=O}$), 1620 ($\upsilon_{C=N}$); ^{1}H NMR

(DMSO-d_6): δ *1.39–1.41* (d, *J* = 7.12 Hz, 3H), 3.79 (s, 3H), 4.62–4.65 (t, *J* = 6.88 Hz, 1H), 6.57–6.61 (d, *J* = 15.80 Hz, 1H), 6.98–7.00 (d, *J* = 8.64 Hz, 2H), 7.37–7.41 (d, *J* = 15.76 Hz, 1H), 7.52–7.54 (d, *J* = 8.64 Hz, 2H), 7.73–7.75 (d, *J* = 8.48 Hz, 2H), 7.89–7.91 (d, *J* = 8.48 Hz, 2H), 8.54–8.56 (d, *J* = 6.40 Hz, 1H), 12.93 (s, 1H); ESI-MS: *m*/*z* $[M+H]^+$ 488.1.

5.2.29. (2*R*)-*N*-[5-(4-Nitrorophenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3e)

Yellow solid, yield: 51.3%; mp: 265–266 °C; IR (KBr, σ/cm^{-1}): 3425, 3281 (υ_{NH}), 1694, 1655 ($\upsilon_{C=O}$), 690 (υ_{C-S}), 1517 (υas_{NO_2}), 1345 (υs_{NO_2}); ^{1}H NMR (DMSO-d_6): δ 1.40–1.42 (d, *J* = 7.08 Hz, 3H), 3.80 (s, 3H), 4.63–4.67 (t, *J* = 6.86 Hz, 1H), 6.57–6.61 (d, *J* = 15.76 Hz, 1H), 6.98–7.01 (d, *J* = 8.60 Hz, 2H), 7.38–7.42 (d, *J* = 15.72 Hz, 1H), 7.52–7.54 (d, *J* = 8.60 Hz, 2H), 8.23–8.25 (d, *J* = 8.76 Hz, 2H), 8.36–8.38 (d, *J* = 8.76 Hz, 2H), 8.54–8.56 (d, *J* = 6.04 Hz, 1H), 13.05 (s, 1H); ESI-MS: *m*/*z* $[M+H]^+$454.3.

5.2.30. (2*R*)-*N*-[5-(4-Methylphenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3f)

White solid, yield: 32.8%; mp: 188–190 °C; IR (KBr, σ/cm^{-1}): 3420, 3270 (υ_{NH}), 1700, 1655 ($\upsilon_{C=O}$), 1622 ($\upsilon_{C=N}$); ^{1}H NMR (DMSO-d_6): δ *1.38–1.40* (d, *J* = 7.08 Hz, 3H), 2.37 (s, 3H), 3.79 (s, 3H), 4.63 (s, 1H), 6.57–6.61 (d, *J* = 15.80 Hz, 1H), 6.98–7.00 (d, *J* = 8.48 Hz, 2H), 7.34–7.35 (d, *J* = 7.96 Hz, 2H), 7.37–7.41 (d, *J* = 15.84 Hz, 1H), 7.52–7.54 (d, *J* = 8.52 Hz, 2H), 7.82–7.84 (d, *J* = 7.88 Hz, 2H), 8.53–8.55 (d, *J* = 6.32 Hz, 1H), 12.84 (s, 1H); ESI-MS: *m*/*z* $[M+H]^+$ 424.1.

5.2.31. (2*R*)-*N*-[5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl]-2-[(*p*-methoxycinnamoyl)amino]-propanamide (3g)

White solid, yield: 60.4%; mp: 144–145 °C; IR (KBr, σ/cm^{-1}): 3407, 3280 (υ_{NH}), 1695, 1653 ($\upsilon_{C=O}$), 690 (υ_{C-S}); ^{1}H NMR (DMSO-d_6): δ *1.38–1.40* (d, *J* = 7.00 Hz, 3H), 3.79–3.83 (d, *J* = 14.28 Hz, 6 H), 4.61–4.65 (t, *J* = 6.80 Hz, 1H), 6.57–6.61 (d, *J* = 15.76 Hz, 1H), 6.98–7.00 (d, *J* = 8.52 Hz, 2H), 7.07–7.09 (d, *J* = 8.68 Hz, 2H), 7.37–7.41 (d, *J* = 15.76 Hz, 1H), 7.52–7.54 (d, *J* = 8.48 Hz, 2H), 7.87–7.89 (d, *J* = 8.60 Hz, 2H), 8.53–8.54 (d, *J* = 6.24 Hz, 1H), 12.79 (s, 1H); ESI-MS: *m*/*z* $[M+H]^+$ 439.1.

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References and notes

1. Riemann, D.; Kehlen, A.; Langner, J. *Immunol. Today* **1999**, *20*, 83.
2. Hooper, N. M. *FEBS Lett.* **1994**, *354*, 1.
3. Delmas, B.; Gelfi, J.; L'Haridon, R.; Vogel, L. K.; Sjostrom, H.; Noren, O.; Laude, H. *Nature* **1992**, *357*, 417.
4. Nakanishi, K.; Yaoi, K.; Nagino, Y.; Hara, H.; Kitami, M.; Atsumi, S.; Miura, N.; Sato, R. *FEBS Lett.* **2002**, *519*, 215.
5. Yeager, C. L.; Ashmun, R. A.; Williams, R. K.; Cardellichio, C. B.; Shapiro, L. H.; Look, A. T.; Holmes, K. V. *Nature* **1992**, *357*, 420.
6. Hattori, A.; Tsujimoto, M. *Biol. Pharm. Bull.* **2004**, *27*, 777.
7. Irazusta, J.; Valdivia, A.; Fernandez, D.; Agirregoitia, E.; Ochoa, C.; Casis, L. *J. Androl.* **2004**, *25*, 733.
8. Kanayama, N.; Kajiwara, Y.; Goto, J.; el Maradny, E.; Maehara, K.; Andou, K.; Terao, T. *J. Leukocyte Biol.* **1995**, *57*, 129.
9. Larsen, S. L.; Pedersen, L. O.; Buus, S.; Stryhn, A. *J. Exp. Med.* **1996**, *184*, 183.
10. Mitsui, T.; Nomura, S.; Itakura, A.; Mizutani, S. *Biol. Pharm. Bull.* **2004**, *27*, 768.
11. Shipp, M. A.; Look, A. T. *Blood* **1993**, *82*, 1052.
12. Pulido-Cejudo, G.; Conway, B.; Proulx, P.; Brown, R.; Izaguirre, C. A. *Antiviral Res.* **1997**, *36*, 167.
13. Fujii, H.; Nakajima, M.; Saiki, I.; Yoneda, J.; Azuma, I.; Tsuruo, T. *Clin. Exp. Metastasis* **1995**, *13*, 337.
14. Ishii, K.; Usui, S.; Sugimura, Y.; Yoshida, S.; Hioki, T.; Tatematsu, M.; Yamamoto, H.; Hirano, K. *Int. J. Cancer* **2001**, *92*, 49.
15. Tomanek, R. J.; Schatteman, G. C. *Anat. Rec.* **2000**, *261*, 126.
16. Bhagwat, S. V.; Lahdenranta, J.; Giordano, R.; Arap, W.; Pasqualini, R.; Shapiro, L. H. *Blood* **2001**, *97*, 652.
17. Hashida, H.; Takabayashi, A.; Kanai, M.; Adachi, M.; Kondo, K.; Kohno, N.; Yamaoka, Y.; Miyake, M. *Gastroenterology* **2002**, *122*, 376.
18. Sato, Y. *Biol. Pharm. Bull.* **2004**, *27*, 5.
19. Antczak, C.; De Meester, I.; Bauvois, B. *Bioessays* **2001**, *23*, 251.
20. Lohn, M.; Mueller, C.; Langner, J. *Leuk. Lymphoma* **2002**, *43*, 407.
21. Saiki, I.; Fujii, H.; Yoneda, J.; Abe, F.; Nakajima, M.; Tsuruo, T.; Azuma, I. *Int. J. Cancer* **1993**, *54*, 137.
22. Becker, J. W.; Marcy, A. I.; Rokosz, L. L.; Axel, M. G.; Burbaum, J. J.; Fitzgerald, P. M. D.; Cameron, P. M.; Esser, C. K.; Hagmann, W. K.; Hermes, J. D.; Springer, J. P. *Protein Sci.* **1995**, *4*, 1966.
23. Rzeski, W.; Matysiak, J.; Kandefer-Szerszeń, M. *Bioorg. Med. Chem.* **2007**, *15*, 3201.
24. Akao, Y.; Maruyama, H.; Matsumoto, K.; Ohguchi, K.; Nishizawa, K.; Sakamoto, T.; Araki, Y.; Mishima, S.; Nozawa, Y. *Biol. Pharm. Bull.* **2003**, *26*, 1057.
25. Foroumadi, A.; Daneshtalab, M.; Shafiee, A. *Arzneimittel-Forschung* **1999**, *49*, 1035.
26. Levy, M.; Palmer, A. H. *J. Biol. Chem.* **1942**, *146*, 493.
27. Song, K. S.; Ishikawa, Y.; Kobayashi, S.; Sankawa, U.; Ebizuka, Y. *Phytochemistry* **1992**, *31*, 823.
28. Kim, H.; Burley, S. K.; Lipscomb, W. N. *J. Mol. Biol.* **1993**, *230*, 722.
29. Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. *Protein Eng.* **1995**, *8*, 127.
30. Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*, 3549.
31. Baragi, V. M.; Shaw, B. J.; Renkiewicz, R. R.; Kuipers, P. J.; Welgus, H. G.; Mathrubutham, M.; Cohen, J. R.; Rao, S. K. *Matrix Biol.* **2000**, *19*, 267.