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Solid-phase Synthesis of α-substituted 3-Bisarylthio *N*-Hydroxy Propionamides as Specific MMP Inhibitors

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Abstract—A novel series of potent and specific MMP-2,3,9,13 inhibitors has been obtained by modulation on solid phase by α and aryl substitutions on 3-arylthio-*N*-hydroxy-propionamides starting from itaconic acid.

Introduction

Matrix metalloproteinases (MMPs) are a class of zinc dependent proteolytic enzymes involved in the degradation and regeneration of the extracellular matrix.[1] Upregulation of specific MMPs has been associated with various pathologies including arthritis (MMP-1,3,13),[2] and metastatic cancer (MMP-2,9),[3] and therefore inhibition of these enzymes has been recognised as a valuable therapeutic approach. Among the compounds in clinical development we can distinguish those active on a large spectrum of MMPs (CGS 27023A[4] and Ro32-3555[Trocade][5] for arthrosis; BB-2516[Marimastat],[6] AG3340[Prinomastat][7] and BMS-275291[8] for cancer), and those more recently studied selective for MMP-13 (RS-130830[9] for arthrosis), or for MMP-2,9 (ABT-518[10] for cancer). The optimal degree of selectivity of MMP inhibitors as drugs is still debatable but access to specific inhibitors would help to understand the relationship between the various enzymes and different pathologies. For this purpose, we recently described a series of bisarylether tetrahedral analogues of the substrate transition-state, and for example **1**[11] is a very potent and specific inhibitor of MMP-2,3,9,13. Therefore the synthesis of the corresponding bisaryl thioether **2** by a methodology applicable to solid phase was envisaged to allow an extensive investigation of the P_1/P_2 mimic part of the molecule (Scheme 1).

Results and Discussion

Chemical synthesis

A retrosynthetic analysis, using classical modifications, could link **2** to α-substituted 4-hydroxybutyrate **4**. Then the correlation of **4** to itaconic acid **5**, based on a Michael addition of thiophenolate, could give a direct access to this new class of compounds. This approach is attractive for a rapid SAR study, since P_1 and P'_1 substitutions (respectively X and Y), could be exemplified independently from the common precursor **4**. Moreover, this type of synthon **4** could also be adapted to solid phase synthesis by anchoring the ester function on a hydroxamate resin[12] (Scheme 2).

Investigation on the P'_1 side. By a Michael addition of 4-bromophenyl-thiolate on the mono *t*-butyl ester of itaconic acid **5a**, followed by reduction of the carboxylic acid, the synthon **4** could be obtained in fairly good yield and in large scale from inexpensive starting material. In order to validate the chemical sequence, a SAR

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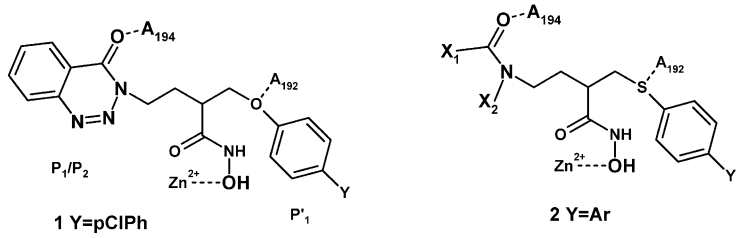
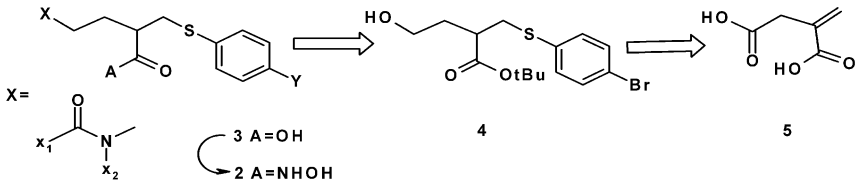
investigation on the P'_1 side by aryl substitution was carried out. Previous work in our laboratory had shown that a phenyltriazino group was well accepted at P_1[11] and therefore it was used to start this study. The substitution of the hydroxyl function of **4** was carried out under Mitsunobu reaction conditions with benzotriazine-4-one in very good yield to give **7**. As previously described,[13] a bisaryl substituant at P'_1 improved the selectivity index versus MMP-1, since crystallographic data showed a narrow S'_1 pocket, more shallow in MMP-1 than in the other MMPs (replacement of Arg by Leu). A series of aryl and heteroaryl were envisaged in order to select a bisaryl moiety for the solid phase procedure. These P'_1 terminal modifications were performed by substitution of the 4-bromophenyl on **7** by a Stille reaction[14] with various arylstannanes to give bis arylderivatives and then hydrolysis of carboxylic acids **3–1** to **3–5**. As an alternative, **7** could be transformed into a stannyl derivative and then arylated with 5-bromopyrimidine to gave the corresponding 4-phenylpyrimidine **3–6** as an example. The carboxylic acids **3–1** to **3–6** were then converted into their corresponding hydroxamic acids **2–1** to **2–6** in reasonable yield by coupling with allylhydroxamate and subsequent deprotection (Scheme 3 and Table 1).

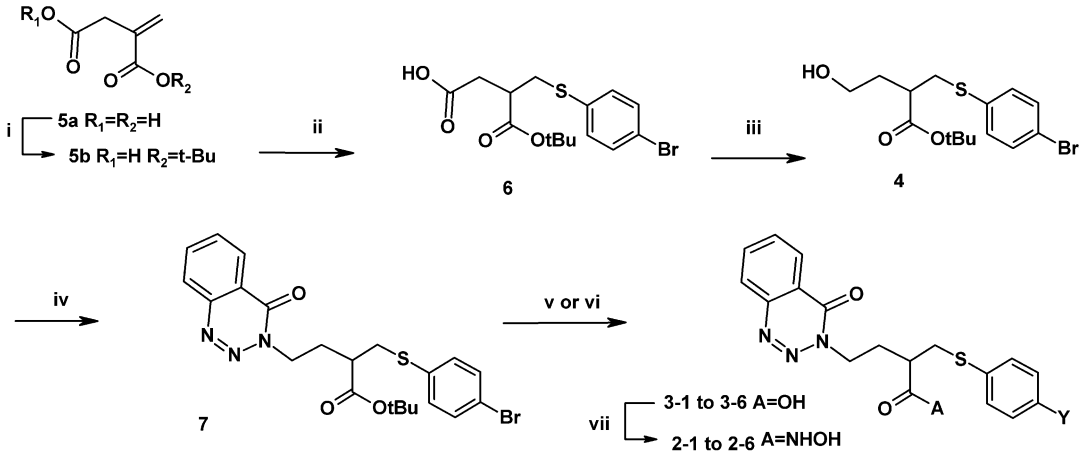
Investigation on the P_1/P_2 sides. Based on the studies of the active site specificity of MMP-2,9,[15] a preference for much lower hydrophobic residues was observed at the P_1/P_2 sites than at P'_1 (57/29% compared to 100%, respectively). We decided to take advantage of this to randomly modify the P_1/P_2 side, to combine potency, selectivity and improved pharmacokinetic values. For this purpose the solid phase methodology described by Floyd[12a] to generate hydroxamic acid from its corresponding carboxylic analogue was applied. The use of *O*-hydoxylamine Wang-resin allowed the last three steps of the synthesis described above to be by passed. In order to cover a larger substituent diversity, we considered either acyclic or cyclic variations of X_1 and X_2 in formula 2.



Scheme 1.



Scheme 2. Retrosynthesis.



Scheme 3. Synthesis of compounds **2-1** to **2-6** and **3-1** to **3-6.** (i) (a) MeOH, amberlyst-15, rt 80 h, (97%); (b) isobutene, H_2SO_4, CH_2Cl_2, rt 12 h (67.5%); (c) LiOH, H_2O/dioxane, 0 °C, 1.5 h, rt, 2 h (75%); (ii) MeONa, MeOH, 4-BrPhSH, rt, 12 h (**6**, 80%); (iii) (a) $ClCO_2iPr$, NMM, DME, 0 °C, +12 h, rt; (c) $NaBH_4$, H_2O/DME, −10 °C, 1 h, (**4**, 74%); (iv) PPh_3, DIAD, benzotriazin-4-one, THF, 0 °C–rt (**7**, 88%); (v) (a) $YSnBu_3$, $Pd(PPh_3)_4$, LiCl, toluene, Δ, 12 h; (b) TFA, CH_2Cl_2, 0 °C–rt, 18 h (**3-1** to **3-5**, %, Table 1); (vi) (a) $(Bu_3Sn)_2$, $Pd_2(OAc)_2(P(OPhCH_3)_3)_2$, toluene, 80 °C, 12 h, (66%); (b) YBr, $Pd_2(OAc)_2(P(OPhCH_3)_3)_2$, NMP, 80 °C, 18 h; (c) TFA, CH_2Cl_2, 0 °C–rt, 18 h (**3-6**, **%**, Table 1); (vii) (a) $H_2NOAllyl$, HOBT, EDC, NEt_3, CH_2Cl_2, rt; 12 h; (b) Bu_3SnH, $PdCl_2(PPh_3)_2$, AcOH, CH_2Cl_2, rt, 2 h (**2-1** to **2-6**, %, Table 1).

Table 1. Structures and yields of compounds **3-1** to **3-6** and **2-1** to **2-6**

Y	Ph	pCl-Ph	3-thienyl	3-Pyridyl	2-Pyrazinyl	5-Pyrimidinyl
Compound	**1-3**	**3-2**	**3-3**	**3-4**	**3-5**	**3-6**
iv or v%	60	47	71	39	59	56
Compound	**2-1**	**2-2**	**2-3**	**2-4**	**2-5**	**2-6**
vi %	40	35	48	47	16	43

To have access to a variety of cyclic amides or imides (X), we tried using Mitsunobu methodology on solid phase. Unfortunately, γ-hydroxyl hydroxamate lactamized very rapidly during this reaction condition on solid phase, and an alternative sequential approach had to be considered. The synthon **4** was first acylated, to allow the introduction of the 4-chlorophenyl moiety by a Stille reaction, then transformed to its γ-hydroxy acid and fixed on the Wang resin to give the adduct **8**. The various Mitsunobu reactions were then carried out, and acidic treatment released the corresponding carboxylic acids **3–8** to **3–19.** They were directly engaged as **3–7** on the hydroxamate resin to form the adducts **9–7** to **9–19** which were converted into the products **2–7** to **2–19** (Scheme 4 and Table 3).

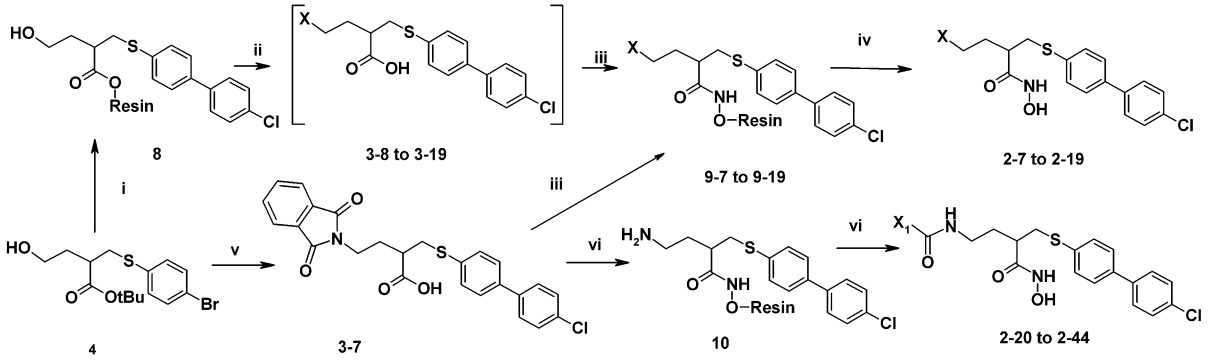
For the acyclic series ($X_2 = H$), a terminal primary amine was necessary to achieve *N* acylation. This precursor **10** was readily obtained from **4** by introduction of a phthalimido group, hydrolysis of the ester to give **3–7** and then treatment by hydrazine after the attachment to the resin. Then **8** was coupled with a series of carboxylic acids, and the final hydroxamates **2–20** to **2–44** were released by acidic treatment (Scheme 4 and Table 4).

Biological evaluation

The inhibitory activities of these compounds were examined against a panel of MMPs and the results were compared to the various clinical references. Since none of the carboxylic acids **3–1** to **3–7** displayed significant activities (IC_{50} in the μM range; data not shown), carboxylic acids were no longer evaluated. In the hydroxamate series **2–1** to **2–6**, all the compounds with various aryl or heteroaryl at P'_1 side showed very good selectivity versus MMP-1 as was the case in the ether series,[11] but the 4-chloro analogue **2–2** exhibited significantly improved inhibition of MMP-2,3,9,13 compared with the non substituted biphenyl analogue **2–1**. In our attempt to replace the chlorophenyl terminal in **2–2** by a heteroaryl, the 3-thiophenyl **2–3** maintained inhibitory activities for all these MMPs in the same range as **2–1**. However, its replacement by either a 3-pyridinyl (**2–4**), or a 5-pyrimidinyl (**2–6**), increased their IC_{50} values by one or two orders of magnitude. The replacement by a 2-pirazyl (**2–5**) was the most deleterious, with a loss of activity for all MMPs, illustrating the lipophilic discriminations of the interactions at P'_1 (Table 2).

Therefore, the 4-chlorobiphenyl moiety at P'_1 was maintained in order to enhance the interactions at the P_1 side. In the first selection, all attempts to replace this triazino group at P_1 by cyclic amide or imide functions (compounds **2–7** to **2–19** in Table 3) decreased affinities by factors of 10 to 50, with the exception of **2–9,** with a bulky tricyclic substituent, which showed a more marked decrease in these affinities. In conclusion, all these compounds remained potent inhibitors of the gelatinases (MMP-2,9) with IC_{50} values in the nM range. Selectivity indices for the other MMPs remained the same, except for compound **2–14** which had a slightly better selectivity versus MMP–13 (Table 3).

In the second selection, the random replacement of the P_1-triazine moiety of **2–2** by an aryl/heteroaryl mono or bicyclic amide significantly reduced the affinities for all the MMPs (compounds **2–20** to **2–44** in Table 4). However, three compounds (**2–22**, **2–28** and **2–34**) retained significant activities for MMP-2,9,13. Interestingly, there was a 10-fold difference in activity on these



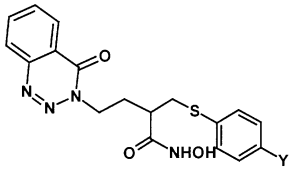
Scheme 4. Synthesis of compounds **2-7** to **2-44**. (i) (a) Ac_2O, pyridine, CH_2Cl_2, rt, 18 h, (92%); (b) 4-ClPhSnBu$_3$, Pd(PPh$_3$)$_4$, LiCl, toluene, 80 °C, 12 h, (76%); (c) TFA,CH_2Cl_2, rt, 48 h, (91.5%); (d) LiOH, H_2O/THF, 0 °C; (e) HATU, DIEA, (*o*-Cl-trityl)-resin; (ii) (a) PPh_3, DIAD, XH, THF/CH_2Cl_2, rt; (b) 5% TFA, CH_2Cl_2, 0 °C–rt, 18 h (**3-8** to **3-19**); (iii) HATU, DIEA, hyroxylamine-resin; (iv) 5% TFA, CH_2Cl_2 (**2-7** to **2-19**); (v) (a) PPh_3, DIAD, PhtNH, 0 °C—rt, 12 h, (99%); (b) 4-ClPhSnBu$_3$, Pd(PPh$_3$)$_4$, LiCl, toluene, 80 °C, 18 h, (62%); (c) TFA, CH_2Cl_2, 0 °C–rt, 18 h, (**3-7**, 100%); (vi) (a) HATU, DIEA, hyroxylamine-resin; (b) NH_2NH_2, MeOH; (vii) (a) X_1CO_2H, HATU, DIEA; (b) 5% TFA, CH_2Cl_2 (**2-20** to **2-44**).

enzymes between the 2- and 3-pyridinylamide, compounds **2–22** and **2–23**, respectively. When the two heteroatoms were present as in the pyrimidyl derivative **2–24,** the activities were only partially recovered. The introduction of a phenyl ring close to the nitrogen of the pyridinyl ring, resulting in the 2-(1-azanaphtyl) **2–34** compound, maintained the activities, but when the phenyl was in the other side, resulting in the 1-(2-azanaphtyl) **2–35** compound, the activities were significantly decreased. The synthesis of a new series of compounds would be planned to investigate the active site interaction at the S_2-S_3 region of this heteroarylnitrogen atom (Table 4).

Pharmacological evaluation

Selected compounds with $IC_{50} < 100$ nM on MMP-13 were evaluated in an in vitro model of MMP-dependent cartilage loss. Except compound **2–29,** almost all the acyclicamides **2–20** to **2–44** showed IC_{50} values over 1 μM for the inhibition of the degradation of proteoglycan. However, several cyclic derivatives, among them compounds **2–11**, **2–12** and **2–15**, showed significant activity ($IC_{50} < 0.5$ μM), but relatively lower than the references compounds. Moreover, these compounds are on the racemate form, and therefore the synthesis of the active isomer have been planned for continued study (Table 5).

Table 2. Structures and biological evaluation of compounds **2-1** to **2-6** and references



No.	Y	IC_{50} (nM) MMP 1	2	3	9	13
BB-2516		1.5	1.8	25	1.6	3.4
CGS27023A		96	15	14	10	12
Ro32-3555		(K_i) 7	154	527	58	3.4
AG3340		48	0.5	1.1	0.2	1.5
2-1		$>10^5$	1.2	61	1.3	76
2-2		$>10^4$	0.06	10	0.5	1.2
2-3		$>10^4$	1.3	66	4.7	13
2-4		$>10^3$	11	234	32	92
2-5		$>10^4$	357	966	375	$>10^3$
2-6		$>10^3$	6	164	24	20

As a preliminary evaluation for an oncological application, compounds were tested on the experimental B16F10 melanoma metastasis model in mice.[16] In this experiment, compound **2–2** at 100 and 200 mg/kg ip, showed a significant reduction of tumour burden (45 and 55%, respectively) and a marked reduction of the size of metastases (50 and 100% reduction of number of the metastases with diameter over 1 mm, respectively). Some in vitro pharmacokinetic parameters were also evaluated using human hepatic microsomes to predict metabolic stability and first pass metabolism (MF%) and using Caco2 cell line monolayers permeability to predict absorption (A%). Compound **2–2** exhibited a modest metabolic stability (MF = 20%), comparable to most of the reference compounds,[5,7] but was well absorbed (A = 92%) and is considered as a good candidate for continued study.

Conclusion

A synthetic access was developed from itaconic acid to 2-substituted-3-bisarylthiopropionic acid hydroxamates, a new class of inhibitors of MMPs. Key synthon **4** allowed independent optimisation of P and P′ sites adaptable to solid phase, leading to very potent inhibitors of MMP-2, 9, 13 and selective versus MMP-1. The activities of the selected compounds were validated in vitro in a cartilage degradation assay, and in vivo in a metastasis model.

Experimental

Chemistry general techniques

All reactions were carried out under nitrogen or argon atmosphere under anhydrous conditions unless otherwise noted.

Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous material unless otherwise stated.

All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated. All reactions were monitored by thin layer chromatography carried out on 0.2 mm Merck silicagel plates using UV light and ethanol phosphomolybdic acid or *p*-anisaldehyde and heat as developing agent. Preparative flash chromatography separations were carried out on Kiesegel 60 (0.04–0.063 mm) Merck silicagel.

Reverse-phase HPLC analysis was performed on an Agilent 1100 instrument using a Xtera Waters column with detection at 210 nM using a $H_2O/CH_3CN + 0.1\%$ TFA gradient over 15 min.

NMR spectra were recorded on a Brucker PPX 200 or 300 instrument as indicated, calibrated using TMS as an internal reference.

The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; b, broad.

IR spectra were recorded on a Brucker Vector 22 spectrophometer.

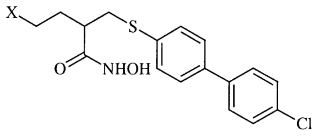
Electrospray mass spectra were recorded in a positive mode on a Finnigan TSQ 7000 spectrophometer, by infusion at 15 μL/min of a 0.1 mg/mL sample solution in a mixture of CH_3CN/H_2O (3/1:v/v).

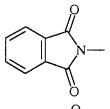
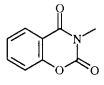
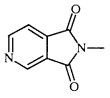
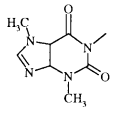
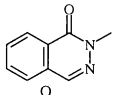
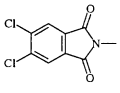
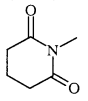
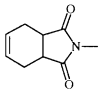
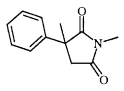
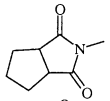
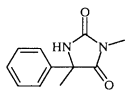
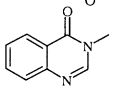
Preparation of tert-butyl-2-carboxymethyl acrylate (5b). Step 1: 2-methoxycarbonylmethyl acrylic acid. A heterogeneous mixture of itaconic acid (200 g, 1.54 mol) and Amberlyst 15 H^+ resin (240 g, washed to neutral, rinsed with methanol and dried overnight at 40 °C) in methanol (4 L) was shaken (120–140 r/min) on an oscillated plate during 3 days at room temperature. The resin was filtered off, and the mixture was evaporated to dryness. The residue (214 g, 97%) was used for the next step without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.5 (m, 1H; O*H*), 6.2–5.8 (2s, 2H, $CHCH_2$), 3.65 (s, 3H, CO_2CH_3), 3.4 (s, 2H, CH_2CO_2Me). IR: V_{max} 2750–2548, 1732, 1691 cm^{-1}.

Step 2: tert-butyl-2-methoxy carbonyl methyl acrylate. To a mixture of the compound obtained in step 1 (214 g) in CH_2Cl_2 (3 L) concentrated H_2SO_4 (15 mL) was added dropwise at 0 °C. Then a stream of isobutene was bubbled on the mixture at 0 °C during 15 min. The flask was sealed hermetically and the mixture was warmed to room temperature overnight. After cooling the flask was opened and the reaction mixture was carefully concentrated to 1 L, washed with diluted NaOH (1 N), and brine. The organic phase was dried over $MgSO_4$, filtered and the solvent removed in vacuo to give the title compound (200.8 g, 67.5%). The crude product was used without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 6.2–5.5 (2s, 2H, $CHCH_2$), 3.7 (s, 3H, CO_2CH_3), 3.3 (s, 2H, CH_2CO_2H), 1.4 (s, 9H, *tBu*). IR: V_{max} 1745, 1714, 1641 cm^{-1}.

Step 3: Preparation of tert-butyl-2-carboxymethyl acrylate (**5b**). To a mixture of the compound obtained in step 2 (200.8 g, ~1 mol) in dioxane (600 mL) was added an aqueous solution (600 mL) of LiOH, H_2O (62 g, 1.5 mol) at 0 °C. The reaction mixture was stirred 1 h and 30 min at 0 °C and then 2 h at room temperature. The residual diester was extracted with ether and the aqueous phase was acidified to pH 5 with 6 N HCl at 0 °C. The aqueous phase was extracted several times with ether and the combined organic extracts were washed with brine. The organic phase was dried over $MgSO_4$, filtrated and the solvent removed in vacuo to give the title compound (140.55 g, 75.3%) which was used without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.5 (m, 1H; O*H*), 6.3–5.7 (2s, 2H,

Table 3. Structures and biological Evaluation of compounds **2-7** to **2-19**



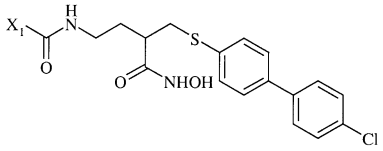
No.	X	IC_{50} (nM) MMP					No.	X	IC_{50} (nM) MMP				
		1	2	3	9	13			1	2	3	9	13
2–7		> 10^3	2.8	135	4.5	8.7	**2-14**		> 10^4	7	1100	8	332
2-8		> 10^3	3	638	12	84	**2-15**		> 10^3	5	182	7	53
2-9		> 10^3	63	708	42	353	**2-16**		> 10^3	2	134	6	31
2-10		311	2	132	2	16	**2-17**		> 10^3	2	78	4	23
2-11		> 10^3	3	335	4	36	**2-18**		> 10^3	1	190	2	55
2-12		> 10^3	5	104	6	25	**2-19**		> 10^3	1	225	3	23
2-13		> 10^3	2	652	2	66							

CHCH_2), 3.4 (s, 2H, CH_2CO$_2$H), 1.4 (s, 9H, *tBu*). IR: V_{max} 2500–2400, 1714, 1641 cm^{-1}. Anal. ($C_9H_{14}O_4$,0.14H_2O): C,H calcd: 57.29; 7.58. Found: 57.14; 7.51.

Preparation of tert-butyl-2-carboxy methyl-3-(4-bromophenylthio) propionate (6). To a mixture of NaOMe (13.8 g, 0.255 mol) in methanol (300 mL) was added a solution of 4-bromothiophenol (36.2 g, 0.19 mol) in methanol (400 mL). Then to this mixture was added dropwise a solution of compound prepared in step 3 (32 g, 0.17 mol) in methanol (150 mL).

The reaction mixture was stirred overnight at room temperature, then concentrated in vacuo. The residue was diluted with water and washed several times with ether. The aqueous phase was cooled at 0 °C, acidified

Table 4. Structures and biological evaluation of compounds **2-20** to **2-44**



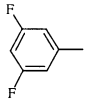
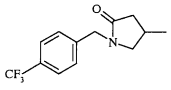
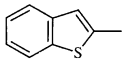
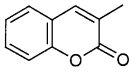
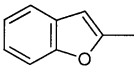
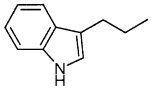
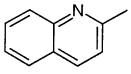
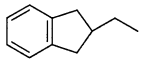
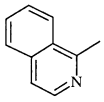
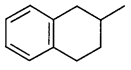
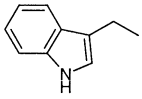
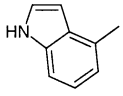
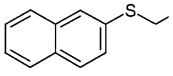
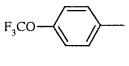
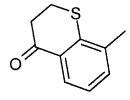
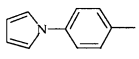
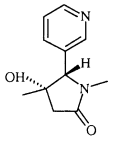
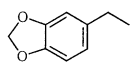
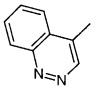
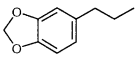
No.	X	IC_{50} (nM) MMP					No.	X	IC_{50} (nM) MMP				
		1	2	3	9	13			1	2	3	9	13
2-20		647	11	126	28	36	**2-33**		$>10^3$	12	76	10	22
2-21		$>10^3$	94	5258	284	314	**2-34**		328	3	25	3	7
2-22		$>10^3$	2	99	8	12	**2-35**		$>10^4$	47	179	68	45
2-23		$>10^3$	27	407	66	94	**2-36**		$>10^3$	57	352	208	324
2-24		436	19	173	17	115	**2-37**		$>10^4$	22	424	79	123
2-25		795	25	173	32	52	**2-38**		$>10^3$	29	865	52	134
2-26		$>10^3$	21	161	12	37	**2-39**		$>10^4$	61	1311	229	826
2-27		$>10^4$	25	141	36	234	**2-40**		$>10^3$	23	164	55	92
2-28		$>10^3$	5	35	6	9	**2-41**		$>10^4$	1715	$>10^4$	957	3580
2-29		545	24	34	5	60	**2-42**		$>10^3$	20	384	193	337
2-30		$>10^3$	102	520	187	293	**2-43**		$>10^3$	17	274	60	109
2-31		$>10^3$	226	5250	643	787	**2-44**		$>10^3$	18	238	35	91
2-32		$>10^3$	3	144	24	16							

Table 5. Inhibition of the proteoglycan degradation

No.	IC_{50} (μM)	No.	IC_{50} (μM)	No.	IC_{50} (μM)	No.	IC_{50} (μM)
BB-2516	0.016	**2-6**	>10	**2-15**	0.33	**2-29**	0.4
CGS27023A	0.022	**2-7**	0.9	**2-20**	2.2	**2-32**	1.9
AG3340	0.0015	**2-8**	0.8	**2-22**	1.3	**2-33**	>10
2-1	1.7	**2-10**	>10	**2-23**	0.64	**2-34**	5
2-2	1.1	**2-11**	0.32	**2-25**	>10	**2-35**	5
2-3	>10	**2-12**	0.32	**2-26**	>10	**2-40**	>10
2-4	>10	**2-13**	>10	**2-28**	1.5	**2-44**	3

with 4 N HCl and extracted several times with ether. The organic phase was dried over $MgSO_4$ washed with brine and concentrated in vacuo. Flash chromatography (silica gel, 20% ethyl acetate in petroleum ether) of the residue gave compound **6**. (48 g, 80.2%). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.4 (s, 1H; O*H*), 7.6–7.4 (2d, 4H, *Ph*), 3.2 (m, 2H, CH_2S), 2.8 (m, 1H, C*H*CO), 2.5 (m, 2H, CH_2CO$_2$H), 1.4 (s, 9H, *tBu*). IR: V_{max} 2665–2575, 1716 cm^{-1}. Anal. ($C_{15}H_{19}BrO_4S$): C, H, S calcd: 48.01; 5.10; 8.54. Found: 48.43; 5.21; 8.83.

Preparation of *tert*-butyl-2-(2-hydroxy-ethyl)-3-(4-bromophenylthio) propionate (4). To a mixture of compound **6** (48 g, 0.128 mol) in DME (200 mL) at 0 °C was added sequentially *N*-methylmorpholine (18 mL, 0.16 mol) and isobutylchloroformiate (22 mL, 0,16 mol). The reaction mixture was stirred overnight at room temperature and the precipitate removed by filtration. To the filtrate was added at −10 °C a solution of $NaBH_4$ (7.26 g, 0.19 mol) in water (150 mL). After 1 h, the reaction mixture was diluted with water (200 mL), acidified with 1 N HCl (100 mL) and extracted with ethyl acetate. The organic phase was washed with brine, dried over $NaSO_4$ and concentrated in vacuo. Flash chromatography (silica gel, gradient of ethyl acetate in petroleum ether) gave compound **4** (34 g, 74%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.4–7.25 (2d, 4H, *Ph*), 3.7 (q, 2H, OCH_2), 3.25–3.0 (2dd, 2H, CH_2S), 2.65 (q, 1H, C*H*CO), 1.9 (m, 2H, OCH$_2$CH_2), 1.6 (t, 1H, O*H*),1.45 (s, 9H, *tBu*). IR: V_{max} 3427, 1726 cm^{-1}. (M+) ($C_{15}H_{19}O_4S^{79}Br$): calcd: 360.0395. Found: 360.0378.($C_{15}H_{19}O_4S^{81}Br$): calcd: 362.0375. Found: 362.0362.

Preparation of tert-butyl-2-[2-(4-oxo-4H-benzo[d][,2,3]-triazin-3-ylethyl)]-3-(4-bromo-phenylthio)propionate (7). To a mixture of triphenylphosphine (37 g, 0.14 mol) and DIAD (28.6 g, 0.14 mol) in anhydrous THF (400 mL) at 0 °C was added a mixture of compound **4** (34 g, 0.094 mol) and benzotriazine (14 g, 0.095 mol) in anhydrous THF (400 mL).After 1 h and 30 min, the reaction mixture was concentrated in vacuo, diluted with diisopropylether and the precipitate was removed by filtration. The filtrate was concentrated in vacuo and compound **7** (40.6 g, 88%) was obtained by flash chromatography of the residue (silica gel, gradient of ethyl acetate in petroleum ether). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.35–8.15 (2d, 2H, *o-Phtriazine*), 7.95–7.8 (2t, 2H, *m-Phtriazine*), 7.35–7.15 (2d, 4H, *Ph*), 4.5 (t, 2H, NCH_2), 3.25–3.0 (2dd, 2H, CH_2S), 2.55 (q, 1H, C*H*CO), 2.3 (m, 2H, NCH$_2$CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1718, 1686 cm^{-1}. Anal. ($C_{22}H_{24}BrN_3O_3S$): C,H,N,Br,S calcd: 53.88; 4.93; 8.57,6.54,16.29. Found: 54.03; 4.92; 8.58,6.29,15.85.

Preparation of *N*-hydroxy-2-[2-(4-oxo-4H-benzo[d][,2,3]-triazin-3-ylethyl)]-3-(biphenyl-4-thio)-propionamide (2.1). Step 1: Preparation of *tert*-butyl-2-[2-(4-oxo-4H-benzo[d][,2,3]triazin-3-ylethyl)]-3- biphenyl thio propionate. To a mixture of compound **7** (3.7 g, 8.16 mmol) in toluene (80 mL) was added sequentially phenyl tri-*n*-butyltin (2.66 mL, 12.2 mmol), tetrakis(triphenyl-phosphine)-palladium(O) (0.47 g, 0.4 mmol) and lithium chloride (1.04 g, 24.5 mmol) at room temperature. Then the reaction mixture was heated under reflux for 12 h and concentrated in vacuo. The residue was diluted in heptane and extracted several times with acetonitrile. The organic phases were concentrated in vacuo and the residue purified by flash chromatography (silica gel, 20% ethyl acetate in petroleum ether) to give the title compound (2.56 g, 63%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.3–8.1 (2d, 2H, *o-Phtriazine*), 7.9–7.75 (2t, 2H, *m-Phtriazine*), 7.6–7.3 (m, 9H, *PhPh*), 4.5 (t, 2H, NCH_2), 3.3–3.05 (2dd, 2H, CH_2S), 2.65 (m, 1H, C*H*CO), 2.3 (m, 2H, NCH$_2$CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1720, 1688 cm^{-1}.

Step 2: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(biphenyl-4-thio) propionic acid (**3**–**1**). To a solution of compound obtained in step 1 (2.5 g, 5.15 mmol) in CH_2Cl_2 was added dropwise trifluoroacetic acid (8 mL) at room temperature. After 18 h, the reaction mixture was concentrated in vacuo and the residue was crystallised in hexane and diisopropylether to give **3.1** (2.1 g, 95%) which was used for the next step without further purification.

Step 3: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-biphenyl thio-propionamide. To a solution of compound **3.1** (2.1 g, 4.87 mmol) in CH_2Cl_2 (100 mL) was added successively EDC (1.1 g, 5.84 mmol), NEt_3 (2 mL, 14.6 mmol) and HOBT (0.8 g, 5.84 mmol) at room temperature. After 12 h, the mixture was dissolved in ethyl acetate. The organic phase was washed with diluted HCl (0.1 N) and brine and dried over $MgSO_4$. The solvent was removed in vacuo and the crude residue (2.1 g, ∼87%) was used for next step.

Step 4: Preparation of **2.1.** To a mixture of compound obtained in step 3 (2.1 g, 4.4 mmol) in CH_2Cl_2 was added bis(triphenyl phosphine) palladium (II) chloride (0.124 g, 0.176 mmol), acetic acid (0.6 mL, 11 mmol) and tri-*n*-butyltin hydride (2.3 mL, 8.75 mmol) at room tem-

perature. After 1 h, the reaction mixture was concentrated in vacuo, and the residue was crystallised twice in ethyl acetate and in acetonitrile/H_2O to give by filtration **2.1** (0.38 g, 49%) as an orange solid. IR: V_{max} 3500–3150, 1684, 1631 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.6–9 (2s, 2H, O*H*, N*H*), 8.3 (2d, 2H, *o-Phtriazine*), 8.15–7.8 (2t, 2H, *m-Phtriazine*), 7.65–7.4 (m, 9H, *PhPh*), 4.4 (t, 2H, NCH_2), 3.2 (d, 2H, CH_2S), 2.65 (m, 1H, C*H*CO), 2.15 (m, 2H, NCH$_2$CH_2). Anal. ($C_{24}H_{22}N_4O_3S$,0.65H_2O): C,H,N calcd: 62.85; 5.12; 12.22. Found: 62.60; 4.99; 11.90.

Preparation of *N*-hydroxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4′-chlorobiphenyl-4-thio) propionionamide (2–2). Step 1: Preparation of *tert*-butyl-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4′-chlorobiphenyl-4-thio) propionate. This compound was prepared by the same procedure as for 3-1 step 1 using 4-chlorophenyl tri-*n*-butyl stannane. Yield (2 g, 47%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.4–8.15 (2d, 2H, *oPhtriazine*), 7.9–7.75 (2t, 2H, *m-Phtriazine*), 7.4 (m, 8H, *PhPh*), 4.5 (t, 2H, NCH_2), 3.3–3.05 (2dd, 2H, CH_2S), 2.65 (m, 1H, C*H*CO), 2.35 (m, 2H, NCH$_2$CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1723, 1684 cm^{-1}. ($M+Na^+$) ($C_{28}H_{28}ClN_3O_3S$): calcd: 544.1438. Found: 544.1462.

Step 2: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4′-chlorobiphenyl-4-thio) propionic acid (**3–2**). This compound was prepared by the same procedure as for 3-1 step 2 using the compound obtained in step 1 as the starting material. Yield (2.1 g, 87%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.3–8.15 (2d, 2H, *o-Phtriazine*), 7.9–7.7 (2t, 2H, *m-Phtriazine*), 7.6–7.25 (m, 8H, *PhPh*), 4.6 (t, 2H, NCH_2), 3.4–3.1 (2dd, 2H, CH_2S), 2.7 (m, 1H, C*H*CO), 2.4 (m, 2H, NCH$_2$CH_2). IR: V_{max} 1735, 1649 cm^{-1}. ($M+Na^+$) ($C_{24}H_{20}ClN_3O_3S$): calcd: 488.0812. Found: 488.0841.

Step 3: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4′-chlorobiphenyl-4-thio) propionamide. This compound was prepared by the same procedure as for **2-1** step 3 using **3-2** as the starting material. Yield (1.7 g, 79%). ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) 11.15 (m, 1H, N*H*), 8.25 (dd, 2H, *o-Phtriazine*), 8.2–7.9 (m, 2H, *m-Phtriazine*), 7.8–7.5 (m, 6H, *PhPh*), 7.45 (d, 2H, *PhPh*),5.9 (m, 1H, C*H*CH$_2$), 5.3 (m, 2H, CHCH_2), 4.35 (t, 2H, NCH_2), 4.3 (d, 2H, OCH_2), 3.25 (m, 2H, CH_2S), 2.35 (m, 1H, C*H*CO), 2.15 (m, 2H, NCH$_2$CH_2). IR: V_{max} 3217, 1681, 1657 cm^{-1}. Anal. ($C_{27}H_{25}ClN_4O_3S$): C, H, N calcd: 62.24, 4.84, 10.75. Found: 62.19, 4.86, 10.92.

Step 4: Preparation of **2-2**: This compound was prepared by the same procedure as for **2-1** step 4 using the compound obtained in step 3 as the starting material. Yield (1.1 g, 50.6%). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.6–8.9 (2s, 2H, O*H*, N*H*), 8.25 (2d, 2H, *o-Phtriazine*), 8.2–7.85 (2t, 2H, *m-Phtriazine*), 7.7–7.35 (m, 8H, *PhPh*), 4.4 (t, 2H, NCH_2), 3.25 (m, 2H, CH_2S), 2.45 (m, 1H, C*H*CO), 2.15 (m, 2H, NCH$_2$CH_2). IR: V_{max} 3236, 1687, 1634 cm^{-1}. Anal. ($C_{24}H_{21}ClN_4O_3S$; 0.4 H_2O): C, H, N calcd: 58.99, 4.46, 11.47. Found: 59.03, 4.49, 11.12.

Preparation of *N*-hydroxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-thien-3-ylphenylthio) propionionamide (2-3). Step 1: Preparation of *tert*-butyl-2-[2-(4-oxo-4H-benzo[d][,2,3]triazin-3-ylethyl)]-3-(4-thien-3-ylphenylthio) propionate. This compound was prepared by the same procedure as for **3-1** step 1 using thien-3-yl tri-*n*-butyl stannane. Yield (2.85 g, 71%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.3–8.1 (2d, 2H, *o-Phtriazine*), 7.9–7.75 (2t, 2H, *m-Phtriazine*), 7.5–7.3 (m, 7H, *ThyPh*), 4.5 (t, 2H, NCH_2), 3.3–3.05 (2dd, 2H, CH_2S), 2.6 (m, 1H, C*H*CO), 2.3 (m, 2H, NCH$_2$CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1725, 1685 cm^{-1}. ($M+Na^+$) ($C_{26}H_{27}N_3O_3S_2$): calcd: 516.1392. Found: 516.1415.

Step 2: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-thien-3-ylphenyl thio) propionic acid (**3-3**). This compound was prepared by the same procedure as for **3-1** step 2 using the compound obtained in step 1 (2.13 g, 4.3 mmol) as the starting material. Yield (1.75 g, 93%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 12.5 (s, 1H, CO2*H*), 8.3–8.1 (2d, 2H, *o-Phtriazine*), 8.05–7.9 (2t, 2H, *m-Phtriazine*), 7.85–7.65 (m, 2H, 2, 5-*Thy*), 7.55–7.5 (m, 3H, *ThyPh*) 7.3 (d, 2H, Thyo-*Ph*), 4.4 (t, 2H, NCH_2), 3.4–3.05 (2dd, 2H, CH_2S), 2.6 (m, 1H, C*H*CO), 2.2 (m, 2H, NCH$_2$CH_2). IR: V_{max} 3219, 1788, 1663 cm^{-1}. MH^+(438).

Step 3: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4-thien-3-ylphenylthio) propionamide. This compound was prepared by the same procedure as for **2-1** step 3 using 3-3 as the starting material (1 g, 2.3 mmol). Yield (0.97 g, 86.5%). ^1H NMR (300 MHz, DMSO-d_6): δ (ppm) 11.2 (m, 1H, N*H*), 8.3–8.2 (dd, 2H, *o-Phtriazine*), 8.14–7.95 (m, 2H, *m-Phtriazine*), 7.85–7.65 (m, 4H, *2, 5-Thyo-Ph*), 7.55 (dd, 1H, *4-Thy*), 7.35 (d, 2H, *m-Ph*), 6.05–5.8 (m, 1H, C*H*CH$_2$), 5.4–5.2 (m, 2H, CHCH_2), 4.4 (t, 2H, NCH_2), 4.3 (d, 2H, OCH_2), 3.2 (m, 2H, CH_2S), 2.35 (m, 1H, C*H*CO), 2.15 (m, 2H, NCH$_2$CH_2). IR: V_{max} 3215, 1682, 1655 cm^{-1}. ($M+Na^+$) ($C_{25}H_{24}N_4O_3S_2$): calcd: 515.1188. Found: 515.1221.

Step 4: Preparation of **2-3.** This compound was prepared by the same procedure as for **2-1** step 4 using the compound obtained in step 3 (0.95 g, 1.93 mmol) as the starting material. Yield (0.52 g, 60%). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.6–8.9 (2s, 2H, O*H*, N*H*), 8.25 (2d, 2H, *o-Phtriazine*), 8.1–7.9 (2t, 2H, *m-Phtriazine*), 7.85–7.66 (m, 4H, *2, 5-Thyo-Ph*), 7.3 (d, 2H, *m Ph*), −4.35 (t, 2H, NCH_2), 3.15 (m, 2H, CH_2S), 2.4 (m, 1H, C*H*CO), 2.15 (m, 2H, NCH$_2$CH_2). IR: V_{max} 3210, 1681, 1627 cm^{-1}. Anal. ($C_{22}H_{20}N_4O_3S_2$;0.25H_2O): C, H, N calcd: 57.70, 4.48, 12.25. Found: 57.62, 4.48, 11.65.

Preparation of *N*-hydroxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-pyridin-3-ylphenylthio) propionionamide (2-4). Step 1: Preparation of *tert*-butyl-2-(2-benzotriazoethyl)-3-(4-pyridin-3-ylphenylthio) propionate. This compound was prepared by the same procedure as for **3-1** step 1 using compound **7** (5 g, 10.2 mmol) and pyridin-3-yl tri-*n*-butyl stannane. (7.5 g, 20.4 mmol). Yield (1.8 g; 39%). ^1H NMR (200 MHz,

$CDCl_3$): δ (ppm) 8.8 (s, 1H, *2-Pyr*), 8.55 (dd, 1H, *6-Pyr*), 8.3–8.1 (2dd, 2H, *o-Phtriazine*), 8.0–7.7 (m, 3H, *4-Pyr, m-Phtriazine*), 7.4 (m, 5H, *5-Pyr, Ph*), 4.55 (t, 2H, NCH_2), 3.3–3.08 (2dd, 2H, CH_2S), 2.65 (m, 1H, CHCO), 2.35 (m, 2H, NCH_2CH_2), 1.55 (s, 9H, *tBu*). IR: V_{max} 1727, 1686 cm^{-1}. Anal. ($C_{27}H_{28}N_4O_3S$): C, H, N,S calcd: 66.37, 5.78, 11.47,6.56. Found: 65.9, 5.84, 11.46, 6.53.

Step 2: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][2,3]-triazin-3-ylethyl)]-3-(4-pyridin-3-ylphenyl thio) propionic acid (**3-4**).

This compound was prepared by the same procedure as for **3-1** step 2 using the compound obtained in step 1 (1.7 g, 4.3 mmol) as the starting material. Yield (1.8 g, 82%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 13 (s, 1H, CO2*H*), 9.05 (s, 1H, *2-Pyr*), 8.75 (d, 1H, *6-Pyr*), 8.5 (d, 1H, *4-Pyr*), 8.25–8.15 (2dd, 2H, *o-Phtriazine*), 8.1–7.8 (m, 3H*, 5-Pyr, m-Phtriazine*), 7.65–7.45 (m, 4H, *Ph*), 4.45 (t, 2H, NCH_2), 3.3 (m, 2H, CH_2S), 2.6 (m, 1H, CHCO), 2.25 (m, 2H, NCH_2CH_2). Anal. ($C_{23}H_{20}N_4O_3S$; CF_3CO_2H): C, H, N calcd: 54.94, 3.87, 10.09. Found: 54.86, 3.95, 10.09. IR: V_{max} 3300–2500, 1742, 1721, 1674 cm^{-1}. MH^+ (433).

Step 3: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4-pyridin-3-ylphenylthio) propionamide. This compound was prepared by the same procedure as for **2-1** step 3 using **3-4** as the starting material (1.4 g, 2.55 mmol). Yield (1.18 g, 95%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 9.5 (m, 1H, N*H*), 8.6 (m, 2H, 2, *6-Pyr*), 8.2–8.05 (2dd, 2H, *o-Phtriazine*), 7.9–7.55 (m, 3H, *4-Pyr*, *m-Phtriazine*), 7.4–7.1 (m, 5H*, 5-Pyr, Ph*), 6.2–5.9 (m, 1H, $CHCH_2$), 5.4–5.3 (m, 2H, $CHCH_2$), 4.5 (m, 4H, NCH_2, OCH_2), 3.35–2.9 (2dd, 2H, CH_2S), 2.5–2.0 (m, 3H, CHCO, NCH_2CH_2). Anal. ($C_{26}H_{25}N_5O_3S$;$0.5H_2O$): C, H, N calcd: 62.90, 5.24, 14.10. Found: 62.97, 5.29, 13.96. IR: V_{max} 3600–2500, 1680, 1657 cm^{-1}.

Step 4: Preparation of **2-4**. This compound was prepared by the same procedure as for **2-1** step 4 using the compound obtained in step 3 (0.95 g, 1.93 mmol) as the starting material. Yield (0.52 g, 60%). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.6–9 (2s, 2H, O*H*, N*H*), 8.85–8.55 (d, 2H, 2,*6-Pyr*), 8.3–8.15 (m, 2H, *o-Phtriazine*), 8.1–7.85 (m, 3H, *4-Pyr*, *m-Phtriazine*), 7.65–7.5 (m, 5H, *5-Pyr, Ph*), 4.38 (t, 2H, NCH_2), 3.2 (m, 2H, CH_2S), 2.45 (m, 3H, CHCO), 2.15 (m, 1H, NCH_2CH_2). IR: V_{max} 3251, 1681, 1641 cm^{-1}. Anal. ($C_{23}H_{21}N_5O_3S$): C, H, N calcd: 61.73, 4.73, 15.65. Found: 61.25, 4.82, 15.08. MH^+ (448).

Preparation of *N*-hydroxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-pyrazin-2-ylphenylthio) propionionamide (2-5). Step 1: Preparation of *tert*-butyl-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4-pyrazin-2-ylphenylthio) propionate. This compound was prepared by the same procedure as for **3-1** step 1 using compound **7** (5 g, 10.2 mmol) and pyrazin-2-yl tri-*n*-butyl stannane. (5.5 g, 11.2 mmol). Yield (3.25 g, 59%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 9.0 (s, 1H, *3-Pyr*), 8.6 (m, 1H, *5-Pyr*), 8.3–8.1 (2dd, 2H, *o-Phtriazine*), 7.9–7.75 (m, 4H, m-*Ph*, *m-Phtriazine*), 7.45 (m, 2H, *o-Ph*), 4.55 (t, 2H, NCH_2), 3.35–3.1 (2dd, 2H, CH_2S), 2.65 (m, 1H, CHCO), 2.35 (m, 2H, NCH_2CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1727, 1686 cm^{-1}. ($M + Na^+$) ($C_{26}H_{27}N_5O_3S$): calcd: 512.1732. Found: 512.1742.

Step 2: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-pyrazin-2-ylphenyl thio) propionic acid (**3-5**).

This compound was prepared by the same procedure as for **3-1** step 2 using the compound obtained in step 1 (2.15 g, 4.35 mmol) as the starting material. Yield (1.75 g, 93%). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.7 (s, 1H, CO2*H*), 9.2 (d, 1H, *3-Pyr*), 8.7–8.6 (m, 2H, *5,6-Pyr)*, 8.18–8.12 (2dd, 2H, *o-Phtriazine*), 8.1–7.8 (m, 4H, *m-Ph, m-Phtriazine*), 7.4 (m, 2H, *o-Ph*), 4.45 (t, 2H, NCH_2), 3.3 (2dd, 2H, CH_2S), 2.55 (q, 1H, CHCO), 2.2 (q, 2H, NCH_2CH_2). Anal. ($C_{22}H_{19}N_5O_3S$; $0.4H_2O$): C,H,N calcd: 59.91, 4.49, 15.88. Found: 59.90, 4.52, 15.17. IR: V_{max} 3300–2500, 1676 cm^{-1}. MH^+ (434).

Step 3: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][2,3]triazin-3-ylethyl)]-3-(4-pyrazin-2-ylphenylthio) propionamide.

This compound was prepared by the same procedure as for **2-1** step 3 using **3-4** as the starting material (2.1 g, 4.8 mmol). Yield (1.84 g, 78.4%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 11.2 (m, 1H, N*H*), 9.2 (d, 1H, 2-*Pyr*), 8.7–8.6 (dd, 2H, *4*, *5-Pyr*), 8.25 (2d, 2H, *o-Phtriazine*), 8.1 (d, 2H, *m-Ph*), 8.15–7.85 (2d, 2H, *m-Phtriazine*), 7.45 (d, 2H, *o-Ph*) 5.95 (m, 1H, $CHCH_2$), 5.3 (m, 2H, $CHCH_2$), 4.4 (m, 2H, NCH_2), 4.3 (d, 2H, OCH_2), 3.3 (2dd, 2H, CH_2S), 2.4 (m, 1H, CHCO), 2.15 (m, 2H, NCH_2CH_2). IR: V_{max} 3346, 1693–1682, 1635 cm^{-1}. Anal. ($C_{25}H_{24}N_6O_3S$): C, H, N,S calcd: 61.46, 4.95, 17.2, 6.56. Found: 61.25, 4.93 17.23, 6.5.

Step 4: Preparation of **2-5**. This compound was prepared by the same procedure as for **2-1** step 4 using the compound obtained above in step 3 (1.8 g, 3.7 mmol) as the starting material. Yield (0.4 g, 24%). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.6–9 (2s, 2H, O*H*, N*H*), 9.2 (s, 1H, *3-Pyr*), 8.7-8.6 (m, 2H, *5*, *6-Pyr*), 8.3-7.9 (m, 4H, *o, m-Phtriazine*), 8.05 (dd, 2H, *m-Ph*), 7.45 (dd, 2H*, o-Ph*), 4.4 (t, 2H, NCH_2), 3.25 (m, 2H, CH_2S), 2.50 (m, 3H, CHCO), 2.15 (m, 2H, NCH_2CH_2). IR: V_{max} 3192–3071, 1681, 1625 cm^{-1}. Anal. ($C_{22}H_{20}N_6O_3S$): C, H, N calcd 58.92, 4.49, 18.74. Found: 58.72, 4.52, 18.28. MH^+ (448).

Preparation of *N*-Hydroxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-pyrimidin-5-ylphenylthio) propionionamide (2-6). Step 1: Preparation of 4-[2-*tert*-butyloxy-carbonyl-4-(4-oxo-4H-benzo[*d*][,2,3] triazin-3-yl)-butylthio] phenyltri-*n*-butyl stananne.

To a mixture of compound **7** (4.26 g, 8.7 mmol) in toluene (100 mL) was added sequentially bis(tri-*n*-butyltin) (5 g, 8.7 mmol) and bis(tritoluyloxyphosphine)-palladium diacetate (0.38 g, 0.05 mmol) at room tem-

perature. Then the reaction mixture was heated under reflux for 12 h and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, gradient ethyl acetate in petroleum ether) to give the title compound (1.65 g, 27%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.35–8.15 (2dd, 2H, *o-Phtriazine*), 7.95–7.8 (2t, 2H, *m-Phtriazine*), 7.3 (m, 4H, *Ph*), 4.5 (t, 2H, NCH_2), 3.25–3.05 (2dd, 2H, CH_2S), 2.65 (q, 1H, *CH*CO), 2.25 (q, 2H, NCH_2CH_2), 1.65–1.45 (s + m, 15H, *tBu*, *3CH*$_2$), 1.3 (m, 9H, *3CH*$_3$), 1.1–0.8 (m, 12H, *6 CH*$_2$). IR: V_{max} 1728, 1689 cm^{-1}.

Step 2: Preparation of *tert*-butyl-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4-pyrimidin-5-ylphenylthio) propionate. To a mixture of the compound obtained in step 1 above (1.65 g, 2.35 mmol) in *N*-methylmorpholine (25 mL) was added sequentially 5-bromopyrimidine (0.75 g, 4.7 mmol), tetrakis (triphenylphosphine) palladium (O) (0.11 g, 0.12 mmol) and lithium chloride (0.3 g, 7.5 mmol) at room temperature. Then the reaction mixture was heated under reflux for 18 h and cooled at room temperature. Then, this mixture was treated with a saturated solution of KF (1.4 mL) and stirred for 15 min. After filtration, the filtrate was diluted with diethylether and washed by diluted HCl (1 N) and brine. The organic phase was concentrated in vacuo and the residue was purified by flash chromatography (silica gel, 20% ethyl acetate in CH_2Cl_2) to give the title compound (0.65 g, 56%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 9.2 (s, 1H, 2-*Pyr*), 8.9 (s, 2H, *4-5Pyr*), 8.35–8.1 (2d, 2H, *o-Phtriazine*), 7.9–7.75 (2t, 2H, *m-Phtriazine*), 7.45 (m, 4H, *Ph*), 4.55 (t, 2H, NCH_2), 3.35–3.1 (2dd, 2H, CH_2S), 2.65 (q, 1H, *CH*CO), 2.3 (m, 2H, NCH_2CH_2), 1.5 (s, 9H, *tBu*). IR: V_{max} 1726, 1685 cm^{-1}. Anal. ($C_{26}H_{27}N_5O_3S$): C, H, N,S calcd: 63.78, 5.56, 14.3, 6.55. Found: 63.47, 5.61 13.86, 6.3.

Step 3: Preparation of 2-[2-(4-oxo-4H-benzo[*d*][,2,3]-triazin-3-ylethyl)]-3-(4-pyrimidin-5-ylphenylthio) propionic acid (**3-6**).

This compound was prepared by the same procedure as for **3-1** step 2 using the compound obtained in step 2 above (0.61 g, 1.25 mmol) as the starting material. Yield (0.415 g, 77.5%). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.5 (m, 1H, CO2*H*), 9.2 (d, 1H, *2-Pyr*), 9.1 (s, 2H, *5, 6-Pyr*), 8.22–8.15 (2dd, 2H, *o-Phtriazine*), 8.05–7.85 (2td, 2H, *m-Phtriazine*), 7.65–7.4 (2d, 4H, *o*, *m-Ph*), 4.45 (t, 2H, NCH_2), 3.4–3.15 (m, 2H, CH_2S), 2.6 (q, 1H, *CH*CO), 2.2 (m, 2H, NCH_2CH_2) Anal. ($C_{22}H_{19}N_5O_3S$): C, H, N calcd: 60.96, 4.42, 16.16. Found: 60.74, 4.59, 15.71. IR: V_{max} 3300–2500, 1674 cm^{-1}. MH$^+$(433).

Step 4: Preparation of *N*-allyloxy-2-[2-(4-oxo-4H-benzo[*d*][,2,3]triazin-3-ylethyl)]-3-(4-pyrimidin-5-ylphenylthio) propio-namide.

This compound was prepared by the same procedure as for **2-1** step 3 using **3-6** as the starting materia (0.415 g, 0.96 mmol). Yield (0.4 g, 85.5%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 11. (s, 1H, N*H*), 9.2 (s, 1H, 2-*Pyr*), 9.1 (s, 2H, *4,6-Pyr*), 8.2 (m, 2H, *o-Phtriazine*), 8.05–7.9 (2td, 2H, *m-Phtriazine*), 7.75 (d, 2H, *o-Ph*), 7.5 (d, 2H, *m-Ph*) 6-5.95 (m, 1H, *CH*CH_2), 5.4–5.2 (m, 2H, CHCH_2), 4.4 (t, 2H, NCH_2), 4.25 (d, 2H, OCH_2), 3.2 (m, 2H, CH_2S), 2.45 (m, 1H, *CH*CO), 2.15 (m, 2H, NCH_2CH_2). IR: V_{max} 3176, 1680 cm^{-1}. Anal. ($C_{25}H_{24}N_6O_3S$): C, H, N cal. 61.46, 4.95, 17.20. Found: 60.64, 4.89, 16.72.

Step 5: Preparation of **2-6**. This compound was prepared by the same procedure as for **2-1** step 4 using the compound obtained in step 1 above (1.8 g, 3.7 mmol) as the starting material. Yield (0.4 g, 24%). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 10.7–8.9 (2s, 2H, O*H*, N*H*), 9.2 (s, 1H, *2-Pyr*), 9.15 (s, 2H, *3, 5-Pyr*), 8.3–8.1(2d, 2H, *o-Phtriazine*), 8.1–7.8 (2t, 2H, *m-Phtriazine*) 7.75 (d, 2H, *m-Ph*), 7.45 (d, 2H, *o-Ph*), 4.4 (t, 2H, NCH_2), 3.2 (m, 2H, CH_2S), 2.45 (m, 1H, *CH*CO), 2.15 (m, 2H, NCH_2CH_2). IR: V_{max} 3257, 1683, 1640 cm^{-1}. Anal. ($C_{22}H_{20}N_6O_3S$): C, H, N calcd: 58.92, 4.49, 18.74. Found: 58.21, 4.55, 17.45. MH$^+$(449).

Preparation of *N*-hydroxy-2-(2-X-ethyl)-3-(4′-chlorobiphenylthio) propionate (2-7 to 2-19). A: Preparation of 2-(2-phthalimidoethyl)-3-(4′-chlorobiphenylthio) propionionamide propionic acid (3-7). Step 1: Preparation of *tert*-butyl-2-(2-phthalimidoethyl)-3-(4-bromophenylthio) propionate.

To a mixture of triphenylphosphine (25.5 g, 0.097 mol) and DIAD (19.58 g, 0.097 mol) in anhydrous THF (250 mL) at 0 °C was added compound **4** (23.4 g, 0.065 mol) in anhydrous THF (150 mL) and then phthalimide (14.3 g, 0.097 mol). The reaction mixture was stirred 12 h at room temperature and concentrated in vacuo. The residue was diluted with diisopropylether and the precipitate was removed by filtration. The filtrate was concentrated in vacuo and the title compound (29.7 g, 98%) was obtained by flash chromatography of the residue (silica gel, gradient of ethyl acetate in petroleum ether). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.8–7.7 (m, 4H, *Pht*), 7.35–7.2 (dd, 4H, *Ph*), 3.7 (t, 2H, NCH_2), 3.1 (2dd, 2H, CH_2S), 2.45 (m, 1H, *CH*CO), 2.05 (m, 2H, NCH_2CH_2), 1.45 (s, 9H, *tBu*). Anal. ($C_{23}H_{24Br}NO_4S$): C, H, N, S calcd: 56.33, 4.93, 2.86, 6.54. Found: 57.51, 5.01, 3.33, 6.42.

Step 2: Preparation of *tert*-butyl-2-(2-phthalimidoethyl)-3-(4′-chlorobiphenylthio) propionate. To a mixture of compound obtained in step 1 above (29.4 g, 0.064 mol) in toluene (80 mL) was added sequentially 4-chlorophenyl tri-*n*-butyltin (52.48 g, 0.128 mmol), tetrakis (triphenylphosphine) palladium(O) (3.7 g, 3.2 mmol) and lithium chloride (8.15 g, 0.128 mol) at room temperature. Then the reaction mixture was heated under reflux for 12 h. Then, this mixture was treated with a saturated solution of KF and stirred for 15 min. After filtration, the filtrate was washed with brine and dried over $MgSO_4$. The organic phase was concentrated in vacuo. The residue was diluted in heptane and extracted several times with acetonitrile. The organic phases were concentrated in vacuo and the residue purified by flash chromatography (silica gel, 10% ethyl acetate in petroleum ether) to give the title compound (20.7 g, 62%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.9–7.6 (m, 4H, *Pht*),

7.45 (m, 8H, *PhPh*), 3.75 (t, 2H, NCH_2), 3.3–3.05 (2dd, 2H, CH_2S), 2.55 (m, 1H, C*H*CO), 2.1 (q, 2H, NCH$_2$CH_2), 1.5 (s, 9H, *tBu*). Anal. ($C_{29}H_{28}ClNO_4S$): C, H, N, S calcd: 66.72, 5.41, 2.68, 6.14. Found: 66.67, 5.46, 2.74, 6.01. IR: V_{max} 1773, 1717 cm^{-1}.

Step 3: Preparation of (**3-7**). To a solution of compound obtained in step 2 above (20.71 g, 39.6 mmol) in CH_2Cl_2(300 mL) was added dropwise trifluoroacetic acid (61 mL) at 0 °C. The reaction mixture was stirred 6 h at room temperature and concentrated in vacuo. The residue was crystallised in hexane and diisopropylether to give **3–7** (18.5 g, quant). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 12.5 (m, 1H, CO2*H*), 7.8 (m, 4H, *3Pht*), 7.65–7.35 (2dd, 8H, *PhPh*), 3.65 (t, 2H, NCH_2), 3.2 (m, 2H, CH_2S), 2.5 (m, 1H, C*H*CO), 2.35 (m, 2H, NCH$_2$CH_2). Anal. ($C_{25}H_{20}ClNO_4S$): C, H, N, S calcd: 64.44, 4.33, 3.01, 6.88. Found: 64.12, 4.40, 3.09, 6.91. IR: V_{max} 3274, 1764, 1734, 1696 cm^{-1}. MH$^+$(466).

B: Preparation of 2-(2-X-ethyl)-3-(4′-chlorobiphenylthio) propionic acid (3-8 to **3-19**). B-1: Preparation of the carboxylic resin **8**. Step 1: Preparation of tert-butyl-2-(2-acetoxyethyl)-3-(4-bromophenylthio) propionate.

To a mixture of *tert*-butyl-2-(2-hydroxy-ethyl)-3-(4-bromophenylthio) propionate (4) (27.85 g, 0.077 mol) in CH_2Cl_2 (300 mL) was added sequentially pyridine (12.5 mL, 0.154 mol), and acetic anhydride (8.8 mL, 0.0925 mol) at room temperature. Then the reaction mixture was stirred 5 days and concentrated in vacuo. The residue was diluted with ethyl acetate washed by diluted HCl (1 N), saturated $NaHCO_3$ and brine. The organic phase was concentrated in vacuo and the residue was purified by flash chromatography (silica gel, 5% ethyl acetate in heptane) to give the title compound (28.7 g, 92%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.4–7.2 (2d, 4H, *Ph*), 4.1 (t, 2H, OCH_2), 3.2–2.95 (2dd, 2H, CH_2S), 2.6 (m, 1H, C*H*CO), 2.05 (s, 3H, COCH_3), 1.5 (m, 2H, OCH$_2$ CH_2), 1.45 (s, 9H, *tBu*). IR: V_{max} 1736, 1234, 1150 cm^{-1}.

Step 2: Preparation of *tert*-butyl-2-(2-acetoxyethyl)-3-(4′-chlorobiphenylthio) propionate.

To a mixture of compound obtained in step 1 above (28.5 g, 0.07 mol) in toluene (80 mL) was added sequentially 4-chlorophenyl tri-*n*-butyltin (49 g, 0.122 mol), tetrakis (triphenylphosphine) palladium(O) (4.1 g, 3.5 mmol) and lithium chloride (9.1 g, 0.23 mol) at room temperature. Then the reaction mixture was heated under reflux for 12 h. Then, this mixture was treated with a saturated solution of KF and stirred for 15 min. After filtration, the filtrate was washed with brine and dried over $MgSO_4$. The organic phase was concentrated in vacuo. The residue was diluted in heptane and extracted several times with acetonitrile. The organic phases were concentrated in vacuo and the residue purified by flash chromatography (silica gel, 5% ethyl acetate in petroleum ether) to give the title compound (23.2 g, 76%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.5–7.3 (m, 8H, *PhPh*), 4.1 (t, 2H, OCH_2CH), 3.25–3.0 (2dd, 2H, CH_2S), 2.6 (m, 1H, C*H*CO), 2.0(m, 2H, OCH$_2$CH_2), 2.0 (s, 3H, COCH_3) 1.5 (s, 9H, tBu). IR: V_{max} 1737, 1236–1150 cm^{-1}.

Step 3: Preparation of 2-(2-acetoxyethyl)-3-(4′-chlorobiphenylthio) propionic acid.

To a solution of compound obtained in step 2 above (23.2 g, 0.053 mol) in CH_2Cl_2 (100 mL) was added dropwise trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred 48 h at room temperature, concentrated in vacuo and the residue purified by flash chromatography (silica gel, 5% ethyl acetate, 1% acetic acid in CH_2Cl_2) to give the title compound (18.5 g, 89%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 12.5 (m, 1H, CO2*H*), 7.5 (m, 8H, *PhPh*), 4.15 (t, 2H, OCH_2CH), 3.3 (dd, 1H, CH_2S), 3.05 (dd, 1H, CH_2S), 2.75 (m, 1H, C*H*CO), 2.1(t, 2H, OCH$_2$CH_2), 1.95 (s, 3H, COCH_3). Anal. ($C_{19}H_{19}ClO_4S$): C, H, S, Cl calcd: 60.23, 5.05, 8.46, 9.36. Found: 60.28, 5.19, 8.78, 9.08. IR: V_{max} 3400, 3000, 1726, 1696 cm^{-1}.

Step 4: Preparation of 2-(2-hydroxyethyl)-(4′-chlorobiphenylthio) propionic acid.

To a solution of 2-(2-acetoxyethyl)-3-(4′-chlorobiphenylthio) propionic acid (7.2 g, 19 mmol) in THF (150 mL) was added a solution of LiOH, H_2O (2.4 g, 57 mmol) in water (40 mL). After 3 h stirring at room temperature, the reaction mixture was concentrated in vacuo and acidified with HCl 0.1 N. The alcohol was extracted with a mixture of ethylacetate/diethylether and the solution rapidly concentrated in vacuo (in order to avoid formation of the lactone) to give 2-(2-hydroxyethyl)-3-(4′-chlorobiphenylthio) propionic acid as a white wax (6 g, 93%). The instable γ-hydroxy acid was immediately coupled to the resin.

Step 5: Preparation of the resin **8**. 2-Chlorotritylchloride resin (Senn Chemicals, 100–200 mesh, 1.4 mmol/g) (20.35 g, 28.5 mmol) was pre-swollen in CH_2Cl_2 for 1 h in a semi-automated Labortec solid-phase synthesizer (SP4000). After filtration, a solution of the γ-hydroxy acid (6 g, 17.8 mmol) in dichloromethane (250 mL) was introduced in the reactor. *N*,*N*-diisopropylethylamine (8.3 mL, 47.5 mmol) was added and the suspension shaken for 16 h at room temperature. 10 mL methanol were added for capping. After 30 min, the resin was filtered and rinsed alternatively three times with DMF and isopropyl alcohol, three times with DCM, twice with Et_2O and dried in vacuo (24.1 g).

B-2: Preparation of 2-(2-X-ethyl)-3-(4′-chlorobiphenylthio) propionic acid (3-8 to **3-19**). Step 1: The ester resin (16 g) was evenly distributed in 15 reactors of a robotic Zymark solid-phase synthesizer (1.1 g per reactor, 0.79 mmol theor). The resins were washed three times with CH_2Cl_2, filtered and suspended in 3 mL CH_2Cl_2. 15 different imides pre-weighted (2.37 mmol) in individual vials were dissolved in 15 mL of a solution of triphenylphosphine (622 mg, 2.37 mmol) in THF/CH_2Cl_2 50/50. The solutions were introduced via

cannula to the 15 reactors and diisopropyl azodicarboxylate was manually added in four portions (479 mg, 2.37 mmol) at 5 min time intervals. The suspension was mixed by intermittent nitrogen bubbling for 2 h. After filtration, a second coupling was performed. The resins were automatically washed alternatively three times with DMF and isopropyl alcohol, three times with CH_2Cl_2.

Step 2: Preparation of **3-8** to **3-19**. The reactors were transferred to a semi-automated cleavage station and treated 30 min with 20 mL of 5% trifluoroacetic acid/CH_2Cl_2 solution. The filtrates were collected in vials and concentrated in vacuo in a Christ B-RVC parallel evaporator to give the carboxylic acids **3-8** to **3-19** which were used for next step without further purification.

C: Preparation of 2-7 to **2-19**. Step 1: Fmoc-hydroxylamine-o-chlorotrityl resin (Novabiochem, 200–400 mesh, 0.76 mmol/g, 15 g) was distributed evenly in 15 reactors of the Zymark solid-phase synthesizer[17] (1 g per reactor, 0.76 mmol). After three washings with CH_2Cl_2, 20 mL of a 20% piperidine/DMF solution was dispensed in each reactor to cleave the Fmoc protecting group. The resin was mixed by intermittent nitrogen bubbling for 20 min, filtered, and a second cleavage was performed for 20 min. The resin was washed three times with DMF, three times with CH_2Cl_2 and was suspended in 10 mL of a diisopropylethylamine/DMF solution (270 μL, 1.52 mmol).

The compounds **3-7** to **3-19** (0.76 mmol theor) prepared above were introduced in 13 individual vials, dissolved in a solution of HATU (289 mg per reactor, 0.76 mmol) in 7 mL DMF. The 13 solutions were introduced via cannula to the 13 reactors and the suspensions mixed by nitrogen bubbling for 10 h. After filtration, the resin was washed alternatively three times with DMF and isopropyl alcohol, three times with CH_2Cl_2.

Step 2: preparation of **2-7** to **2-19**: The reactors were transferred to a cleavage station and treated for 30 min with 20 mL of 5% trifluoroacetic acid/CH_2Cl_2 solution. The filtrates were collected in vials and concentrated in vacuo in a Christ B-RVC parallel evaporator. After crystallisation in acetonitrile and filtration, the precipitates were lyophilised in dioxane. Yields and analytical data of compounds **2-7** to **2-19** were summarized in Table 6.

Preparation of *N*-hydroxy-2-(2-X_1-aminocarbonylethyl)-3-(4′-chloro-biphenylthio) propionates 2-20 to 2-44. A: Preparation of resin 10. Step 1: Fmoc-hydroxylamine-*o*-chlorotrityl resin (Novabiochem, 200–400 mesh, 0.71 mmol/g, 25 g, 17.75 mmol) was pre-swollen in CH_2Cl_2 for 1 h on a semi-automated Labortec solid-phase synthesizer. After 2 washings with CH_2Cl_2 and DMF, the Fmoc protecting group was cleaved with a solution of 20% piperidine in DMF (2×20 min). The resin was rinsed three times with DMF, three times with CH_2Cl_2. A solution of **3-7** (12.4 g, 26.62 mmol) and HATU (10.2 g, 26.62 mmol) in 250 mL DMF was added to the resin, followed by *N*,*N*-diisopropylethylamine (7.75 mL, 44.38 mmol). The suspension was shaken for 16 h, filtered and washed alternatively three times with DMF and isopropyl alcohol, three times with CH_2Cl_2.

Step 2: The resin obtained in step 1, was treated with a 10% solution of hydrazine hydrate in methanol for 2 h. After filtration, a second cleavage was performed for 16 h. The resin was washed five times with DMF, alternatively three times with DMF and isopropyl alcohol, and three times with CH_2Cl_2.

B: Preparation of 2-20 to **2-44**. Step 1: The resin **10** was introduced in the mixing chamber of the robotic Zymark synthesizer, evenly distributed in the 25 reactors (0.71 mmol per reactor) and washed three times with CH_2Cl_2. Then 25 different acids pre-weighted in 25 vials (2.13 mmol) were dissolved in 7 mL of HATU/DMF solutions (810 mg per reactor, 2.13 mmol). After addition of 10 mL of a *N*,*N*-diisopropylethylamine/DMF solution (366 mg per reactor, 2.84 mmol) to the resins, the acid solutions were introduced via cannula to their corresponding reactor. The suspensions were mixed by nitrogen bubbling for 10 h, filtered and rinsed alternatively three times with DMF and isopropyl alcohol, three times with CH_2Cl_2.

Step 2: The reactors were transfered to a semi-automated cleavage station and treated for 30 min with 15 mL of 5% trifluoroacetic acid/CH_2Cl_2 solution. The filtrates were collected in vials and concentrated in vacuo in a Christ B-RVC parallel evaporator. After crystallisation in diisopropylether and filtration, the precipitates were lyophilised in dioxane. Yields and analytical data of compounds **2-20** to **2-44** were summarized in Table 7.

Enzyme assays

Human pro-MMPs were dissolved in Novex developing buffer (Cat. No LC2671) at the following concentrations: MMP-1 (Calbiotech) at 1.25 μg/ml; MMP-2 and MMP-9 (Boehringer) at 300 and 200 mU/mL respectively; MMP-3 (AbCys) at 1 μg/mL and MMP-13 (Pr G. Murphy, Univ. East Anglia) at 2 μg/mL. Pro-enzymes were activated by 2 mM *p*-aminophenilmercuric acetate

Table 6. Yields and analytical data of compounds **2-7** to **2-19**

	2-7	2-8	2-9	2-10	2-11	2-12	2-13	2-14	2-15	2-16	2-17	2-18	2-19
Yield %	68	15	9	24	16	18	3	7	6	16	14	19	23
MH^+	481	482	531	549	485	473	480	497	512	480	447	523	524
Purity %	95	91	87	90	79	93	94	92	83	88	97	88	88

Table 7. Yields and analytical data of compounds **2-20** to **2-44**

	2-20	2-21	2-22	2-23	2-24	2-25	2-26	2-27	2-28	2-29	2-30	2-31	2-32	2-33	2-34	2-35	2-36	2-37	2-38	2-39	2-40	2-41	2-42	2-43	2-44
Yield %	90	52	37	23	14	56	22	68	45	64	81	53	93	20	18	35	79	53	92	41	29	55	79	44	39
MH^+	473	491	456	456	457	539	518	513	527	499	620	511	523	495	506	506	508	522	509	509	494	565	539	553	507
Purity %	82	89	91	84	95	76	85	81	88	96	73	91	70	92	84	93	84	78	82	98	94	94	77	43:55	84

(APMA, Sigma) at 37 °C for 30 min (MMP-1, -2, -9) or 1 h (MMP-3,-13). Activation was stopped by transferring the samples to ice. Inhibitors were dissolved in dimethyl sulfoxide (DMSO) at 10^{-2} M, then serially diluted (1/10) in developing buffer at concentrations from 10^{-4} to 10^{-13} M. Fluorogenic substrates were purchased from Bachem. Substrate for MMP-3 was (7-methoxycoumarine-4-yl)-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2.[18] Substrate for MMP-1, -2, -9, -13 was Dnp-Pro-Cha-Gly-Cys(ME)-His-Ala-Lys (Nma)-NH_2.[19] They were dissolved in DMSO at 10^{-2} and 2×10^{-3} M, respectively, then diluted to 2×10^{-4} M in water. Assays were performed in 96-well plates by adding to each well 70 μL of developing buffer, 10 μL of inhibitor (or buffer for the control) and 10 μL of enzyme (or buffer for the blank). Each point was run in duplicate and each inhibitor was assayed at least twice. After a 30 min preincubation at 37 °C, 10 μL of substrate was added and the plates incubated for 6 h at 37 °C. Reading was then performed by a Spectrofluor Plus fluorimeter (Tecan), set at excitation and emission wavelengths of 340 and 440 nm, respectively. Substrate degradation in the presence of inhibitor at a given concentration was calculated as % fluorescence of control wells. IC_{50} of each product on each enzyme was calculated by EXCEL software using three points in the central linear range of fluorescence inhibition.

Assay of cartilage degradation

The protocol was adapted from that previously published.[20] Fragments (1–2 mg) of articular cartilage were isolated from the knees of one rabbit and labeled with 3.7 MBq (100 μCi) of $^{35}SO_4^-$ (Amersham) in 25 mL of Dulbecco's minimal essential medium (DMEM)/Ham's F12 media supplemented with 10% fetal calf serum (FCS) and 1% of a stock solution of 10^4 IU/mL penicillin and 10 mg/mL streptomycin (PS) (Life technologies). After 3 days, the unincorporated radioactivity was removed by six media changes over 24 h using DMEM/Ham's F12 supplemented with 0.1% bovine serum albumin and 1% PS. Fragments were then incubated in 25 mL of this media supplemented with 10 ng/mL of mouse IL-1 β (Sigma) for 24 h. After one final wash, each fragment was transferred to 96-well plates into 0.25 mL of media. In the treatment groups, media was supplemented with 5×10^{-4} M APMA, plus MMP inhibitors at 10^{-7} or 10^{-6} M. In the control group, media was supplemented with APMA and vehicle (DMSO); in the basal group, with vehicle alone. Each group was made of eight fragments. After a further 24 h, the fragments were collected and digested in 0.5 mL of 0.6 mg/mL papain, 1 mM ethylene diamino tetraacetic acid, 0.25 mg/mL dithiothreitol 20 mM sodium phosphate (Sigma), pH 6.8 at 56 °C for 16 h. Radioactivity in the culture media and in the tissue digest was measured by liquid scintillation using a β-counter (Beckman). Proteoglycan degradation in each fragment was expressed as the percentage of released radioactivity. The effect of MMP inhibitors was calculated from the mean values of proteoglycan degradation in each group by the formula: % inhibition = (control-treatment)/(control-basal)×100. IC_{50} of each product on proteoglycan degradation was calculated by a SAS-based software using at least three concentration points in the linear range of % inhibition.

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