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Protease Inhibitors: Synthesis of Bacterial Collagenase and Matrix Metalloproteinase Inhibitors Incorporating Arylsulfonylureido and 5-Dibenzo-suberenyl/suberyl Moieties

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Abstract—Novel matrix metalloproteinase (MMP)/bacterial collagenase inhibitors are reported, considering the sulfonylated amino acid hydroxamates as lead molecules. A series of compounds was prepared by reaction of arylsulfonyl isocyanates with *N*-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)- and *N*-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl) methyl glycocolate, respectively, followed by the conversion of the COOMe to the carboxylate/hydroxamate moieties. The corresponding derivatives with methylene and ethylene spacers between the polycyclic moiety and the amino acid functionality were also obtained by related synthetic strategies. These new compounds were assayed as inhibitors of MMP-1, MMP-2, MMP-8 and MMP-9, and of the collagenase isolated from *Clostridium histolyticum* (ChC). Some of the new derivatives reported here proved to be powerful inhibitors of the four MMPs mentioned above and of ChC, with activities in the low nanomolar range for some of the target enzymes, depending on the substitution pattern at the sulfonylureido moiety and on the length of the spacer through which the dibenzosuberenyl/suberyl group is connected with the rest of the molecule. Several of these inhibitors also showed selectivity for the deep pocket enzymes (MMP-2, MMP-8 and MMP-9) over the shallow pocket ones MMP-1 and ChC.

Introduction

Proteases, such as the matrix metalloproteinases (MMPs)[1,2] or the bacterial proteases (BPs)[3] have recently become interesting targets for the drug design, in the search of novel types of anticancer, anti-arthritis, antibacterial or other pharmacological agents useful in the management of inflammatory processes.[1–5] All these conditions are generally associated with enhanced activity of several zinc endopeptidases, of which the different MMPs actually known (more than 20 such enzymes were reported for the moment)[1–7] and the large number of BPs[3] isolated in many pathogenic bacterial species, are responsible for the efficient degradation of all components of the extracellular matrix (ECM). ECM turnover is involved in crucial physiological and physiopathological events, such as embryonic development, blastocyst implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption and remodeling (such as in the case of wound healing), bone remodeling, apoptosis, cancer invasion and metastasis, arthritis, atherosclerosis, aneurysm, breakdown of blood–brain barrier, periodontal disease, skin and corneal ulceration, gastric ulcer, or liver fibrosis in the case of the vertebrate enzymes mentioned above.[1–7] In bacteria, proteases are involved in critical processes such as colonization and evasion of host immune defenses, acquisition of nutrients for growth and proliferation, facilitation of dissemination, or tissue damage during infection.[3]

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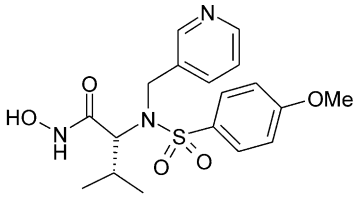
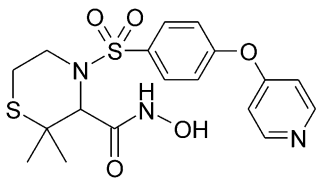
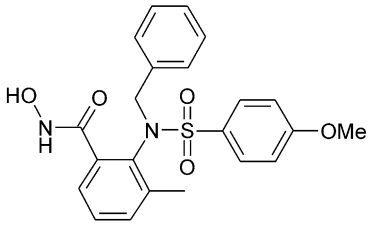


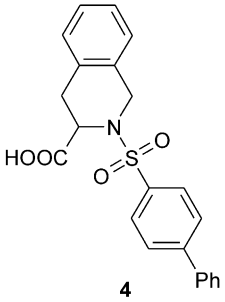
1: CGS-27023A K_I (MMP-3): 43 nM



2: AG-3340 K_I (MMP-2): 0.08 nM



3: K_I (MMP-9): 23 nM



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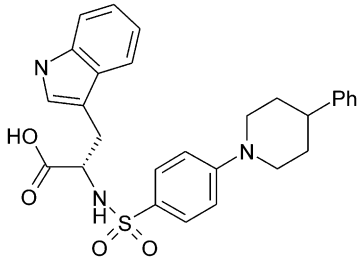
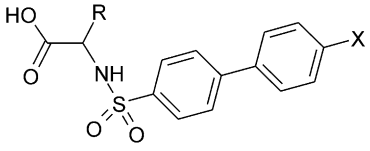
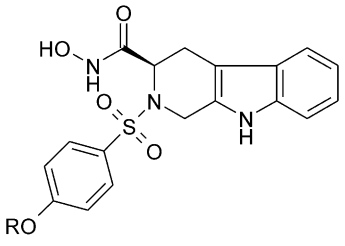


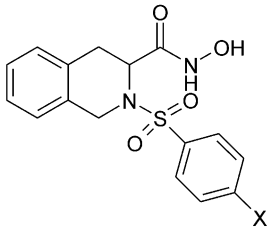
5: K_I (MMP-3): 19 nM



6: R = i-Pr; Bn; X H, Cl, Br
K_I (MMP-2): 2-30 nM



7: R = Me, Ph (K_I(MMP-8) = 3-7 nM)



8: X = H, PhO, MeO, etc
(K_I(MMP-8) = 2-20 nM)

After the report[8] that CGS-27023A **1** acts as a broad spectrum, potent inhibitor of some MMPs, a large number of sulfonylated amino acids and their hydroxamate derivatives have been investigated in the search of more potent and selective such agents.[9–15] All such derivatives target the zinc binding site in the catalytic domain of the enzyme, and incorporate a strong zinc-binding function of the hydroxamate (derivatives **1**–**3**) or carboxylate (derivatives **4**–**6**) type, as well as a scaffold that assures favorable interactions with the primed side of the protease active site (generally with the $S_{1'}$ and $S_{2'}$ sites).[9–15] The main problem with such protease inhibitors is their lack of selectivity towards the different MMPs, which are generally classified into two main types, depending on their $S_{1'}$ pocket: (i) the deep pocket enzymes (such as MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13), possessing a relatively big $S_{1'}$ pocket, and (ii) the shallower pocket enzymes (MMP-1, MMP-7 and MMP-11 among others), which possess a somehow smaller specificity $S_{1'}$ pocket due to its partial occlusion by bulkier amino acid residues, such as those in position 193 (MMP-8 numbering), which from Leu in MMP-8 becomes Arg in MMP-1, Tyr in MMP-7, and Gln in MMP-11.[2b,4] The $S_{2'}$ and $S_{3'}$ subsites are also important for the binding of inhibitors, as well as for the specificity of such inhibitors towards the different proteases. The $S_{2'}$ subsite is generally a solvent-exposed cleft with a preference for hydrophobic $P_{2'}$ residues, in both substrates and MMP inhibitors.[1–4] The $S_{3'}$ subsite on the other hand is a relatively ill-defined, solvent exposed region.[1–4]

Some progress has been made ultimately in the design of both bioavailable and more specific MMP/BP inhibitors.[9–16] A common feature for many of these compounds, such as the carboxylate/hydroxamate deri-

vatives **1**–**8**, is the presence of rather bulky scaffolds in their molecule, which by interaction with the protease subsites mentioned above, lead to selectivity towards some of the target enzymes for which these inhibitors have been designed.[9–16] Although no absolutely selective MMP/BP inhibitors are available up to now, this 'bulky scaffold' strategy seems to be one of the best approaches reported for the design of pharmacologically interesting such enzyme inhibitors. Indeed, a closer look to the X-ray crystal structure of some sulfonylated MMP inhibitors such as **1**[2] and **4**[13] bound within the catalytic domain of MMP-8, afford important information for the drug design of this type of pharmacological agents (Fig. 1). Thus, in such adducts, the hydroxamate/carboxylate moiety of the inhibitor is coordinated to the Zn(II) ion as mentioned above, being also hydrogen-bonded to the carboxylate of Glu 219, similarly to other types of MMP inhibitors (such as for example the succinyl hydroxamates, which are among the best studied inhibitors of these enzymes).[1–4] The importance of the sulfonamide moiety is stressed by the fact that one of the oxygens belonging to the SO_2 moiety of the inhibitor molecule participates in two hydrogen bonds with the main chain amide nitrogens of Leu 181 and Ala 182, whereas the sulfonyl substituent makes extensive hydrophobic contacts with the $S_{1'}$ site, as shown schematically in Fig. 1.[2,13] The pyridylmethyl moiety of **1**, as well as the tetrahydroisoquinolin moiety of **4** are fixed within the $S_{2'}$ pocket of the enzyme.[2,13] These multiple interactions may explain the unexpectedly high affinity of the sulfonylated hydroxamates/carboxylates for different enzymes belonging to this family, but also the capability of both the $S_{1'}$ and $S_{2'}$ subsites to accommodate rather bulky moieties. Unfortunately, no X-ray structures of adducts of MMPs with inhibitors possessing bulkier $P_{2'}$ moieties (such as for example **3**, **5**, **7**, or **8**) are available at this moment in order to allow a more detailed analysis for the influence of this moiety to the binding of inhibitors. Still, the fact that such compounds were reported to act as very potent MMP inhibitors proves that the bulky scaffold' strategy is worth being developed for the design of novel types of such protease inhibitors.

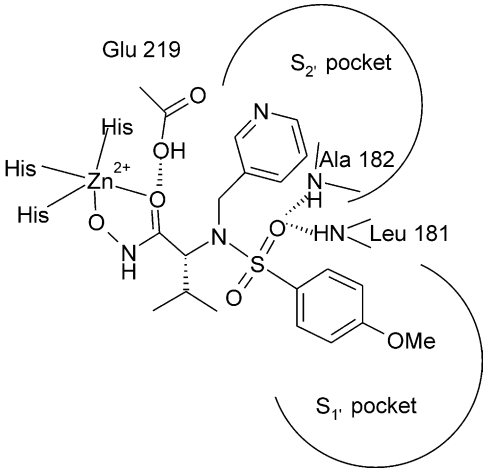


Figure 1. Key protein–ligand interactions for the binding of **1** to MMP-8.

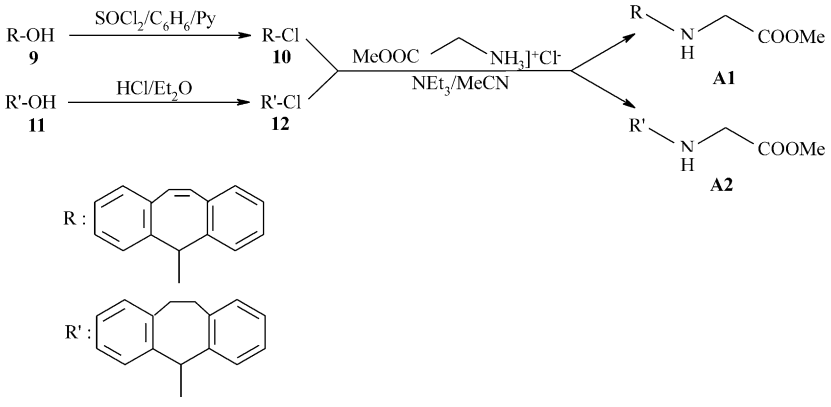
In this paper, we report the preparation of a series of MMP and *Clostridium histolyticum* collagenase (ChC, EC 3.4.24.3) inhibitors incorporating arylsulfonylureido-glycine hydroxamate, as well as bulky 5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl (= 5-dibenzosuberenyl) and 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl (= 5-dibenzosuberyl) moieties in their molecule. Some of the new compounds reported here, assayed for inhibition of purified collagenases (MMP-1 and MMP-8), gelatinases (MMP-2 and MMP-9) and ChC, showed affinities in the low nanomolar range for some of these enzymes, as well as a good degree of selectivity towards the two classes of proteases mentioned above (deep/shallow pocket enzymes).

Results

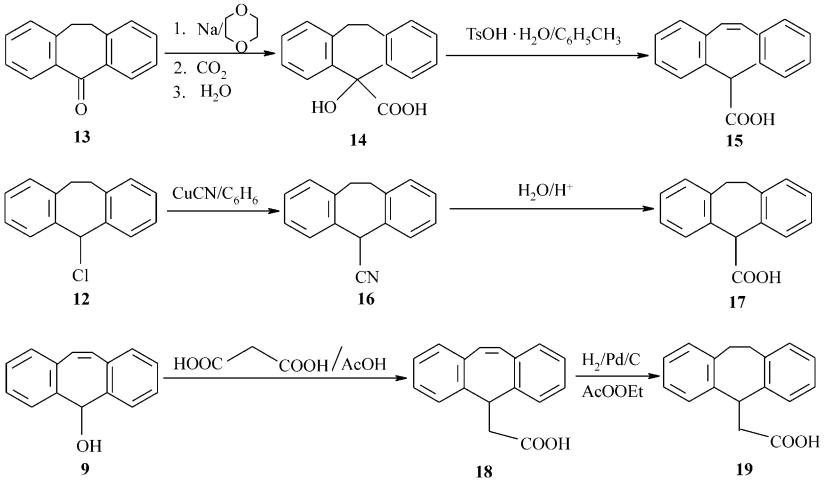
Synthesis

Classical synthetic procedures,[17–23] adapted and optimized in our laboratory, afforded the key intermediates [5 - dibenzosuberenyl/5 - dibenzosuberyl - (alkyl) - methyl glycocolates] **A1**, **A2**, **B1**, **B2**, **C1**, **C2** (Schemes 1–4).

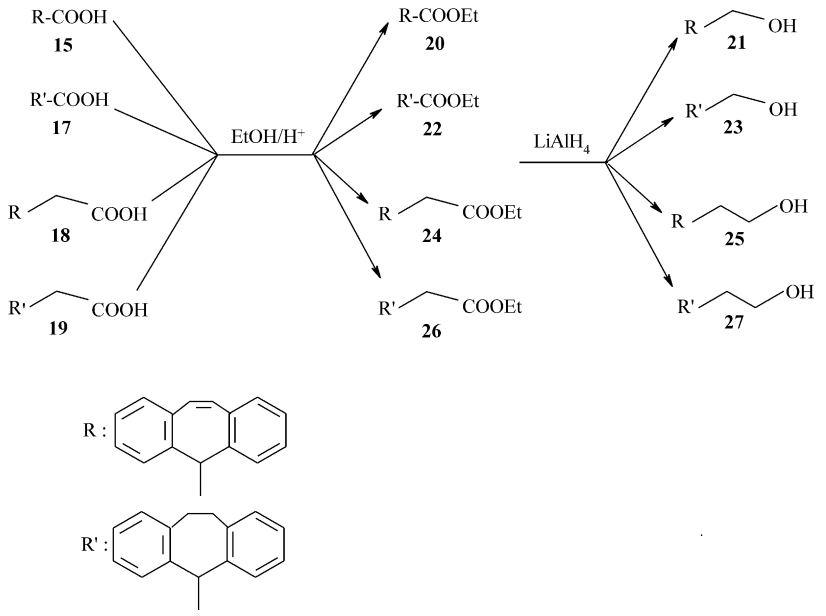
The alcohols **9** and **11** were transformed to the corresponding chloroderivatives **10** and **12** and then to the glycine derivatives **A1** and **A2** by routine synthetic procedures outlined in Scheme 1. Compounds shown in Scheme 2 were obtained by literature procedures, as



Scheme 1.



Scheme 2.

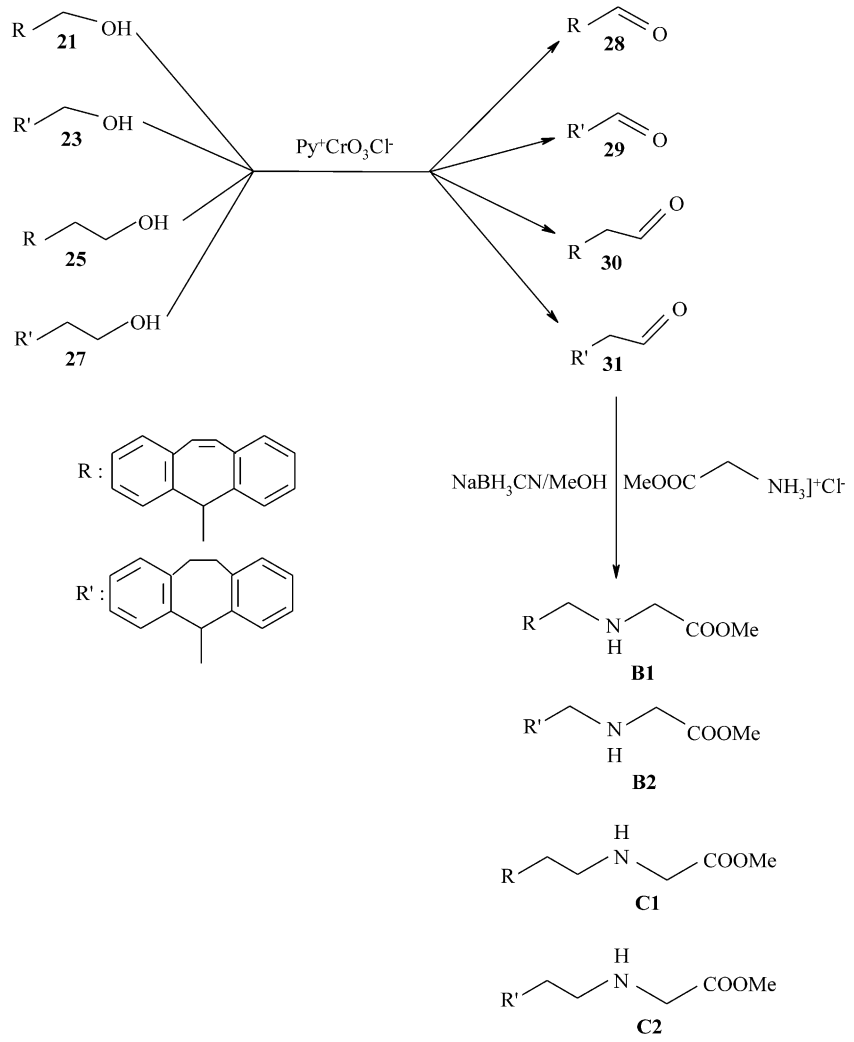


Scheme 3.

follows: 5-hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-carboxylic acid **14** was prepared[17] from 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one **13**; 5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-carboxylic acid **15** was generated[18] from the key intermediate **14**; 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-carbonitrile **16** and 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-carboxylic acid **17** were obtained[19] from 10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-yl-chloride **12**; 5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-acetic acid **18** and 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-acetic acid **19** were prepared by classical procedures adapted from ref 20, starting from 5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol **9**. Carboxylic acids **15**, **17**, **18** and **19** were converted into the corresponding ethyl esters **20**, **22**, **24**, **26** (Scheme 3) and these esters were reduced to the corresponding alcohols **21**, **23**, **25**, **27**, using nonexceptional synthetic procedures, as outlined in Scheme 3. Alcohols **21**, **23**, **25**, **27** were then selectively oxidized[21] to the corresponding aldehydes **28**–**31**, by treating them with pyridinium chlorochromate, as described in Scheme 4.

The key intermediates **A1**, **A2**, **B1**, **B2**, **C1**, **C2** were subsequently derivatized with arylsulfonyl isocyanates **32** to sulfonylureas **D1**–**D30**. These esters were then converted either to the corresponding carboxylic acids **E1**–**E30** (by acid hydrolysis of the methyl ester functionality) or to the hydroxamic acids **F1**–**F30** by reaction with hydroxylamine sodium methoxide, affording the new MMP/ChC inhibitors reported here (Scheme 5).[14–16]

The synthetic routes used for the preparation of the inhibitors reported here were generally related to those applied by MacPherson et al.,[8] Hanessian et al.,[10d] Almstead's and Natchus' groups,[10a,c] and our group,[14–16] for obtaining sulfonylated/sulfonylureido amino acid hydroxamates.



Scheme 4.

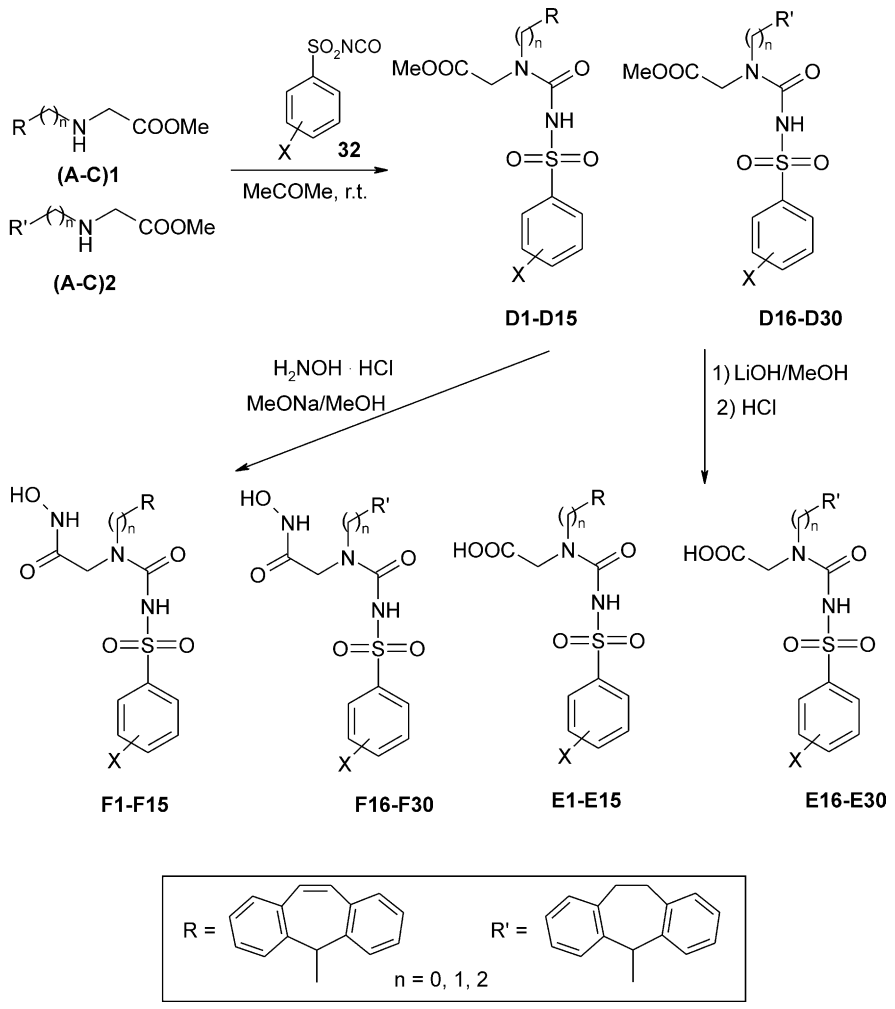
MMP and ChC inhibitory activity. Inhibition data against four MMP-s (MMP-1, MMP-2, MMP-8 and MMP-9) and type II ChC with the compounds **E1–E30** and **F1–F30** reported in the present paper are shown in Table 1.

Inhibition data against the above mentioned five proteases with some of the new compounds reported here and the analogous derivatives incorporating benzyl moieties at $P_{2'}$ instead of 5-dibenzosuberenyl/5-dibenzosuberyl-methyl are shown in Table 2. Such data are useful for validating the bulky scaffold strategy proposed in this paper.

Discussion

Chemistry

Sulfonylated amino acid hydroxamates and sulfonylated amino acids (carboxylates) were recently discovered to act as efficient MMP/ChC inhibitors.[8–16] The most active compounds from this class of non-peptide protease inhibitors possessed the following general structural features: (i) an arylsulfonyl group, occupying (but generally not filling) the specificity $S_{1'}$ pocket of the enzyme.[8–13] It has been proved that the SO_2 moiety of such an inhibitor is involved in several strong hydrogen bonds with amino acid residues from the active site cleft, which strongly stabilize the enzyme-inhibitor adduct (Fig. 1).[8–13] Recently, we have shown that this moiety may be successfully replaced by an arylsulfonylureido group;[14–16] (ii) a bulky hydrophobic moiety possibly placed in α to the hydroxamic/carboxylic acid group (which acts as the zinc-binding function), which is thought to play a role in increasing the selectivity/specificity towards some of the different MMPs/BPs.[8–13] It must be mentioned that some strong and selective MMP/BP inhibitors do not possess in their molecule this structural element; (iii) an isobutyl, pyridylmethyl or benzyl moiety substituting the amino nitrogen atom, which binds within the $S_{2'}$ solvent-exposed pocket of the enzyme. As mentioned earlier, it has recently been proved that this moiety may also be much bulkier, leading in this way to MMP inhibitors with increased selectivity.[9,10,19a] This was the reason why we decided to investigate compounds incorporating 5-dibenzosuberenyl/5-dibenzosuberyl- moieties in this position, together with their congeners containing a methylene or ethylene spacer between the amino acid functionality



Scheme 5.

and the polycyclic moiety (it must be mentioned that the simple 5-dibenzosuberenyl/5-dibenzosuberyl-derivatives of types **9**, **11**, **13**–**15** or **17**–**19**, do not possess MMP/BP inhibitory properties in the nano-micromolar range—data not shown). Furthermore, we did not use the simple sulfonylated amino acids as lead molecules in our drug design,[8–13] but the structurally related arylsulfonylureido compounds, which, as mentioned above, were recently shown to possess strong MMP inhibitory properties.[14–16]

The only commercially available compounds possessing the tricyclic scaffold of interest for us were the alcohols **9** and **11**, as well as dibenzosuberone **13**. Thus, all the syntheses were initiated starting from one of them. In order to obtain the two key intermediates without a spacer (**A1** and **A2**), the two alcohols mentioned above (**9** and **11**) were converted to the corresponding chloroderivatives **10** and **12** either with thionyl chloride or hydrochloric acid in diethyl ether. By reaction with methyl glycocolate, these chloroderivatives (**10** and **12**) afforded the first two key intermediates, **A1** and **A2**, respectively (Scheme 1). This synthetic strategy did not work on the other hand for obtaining the remaining four key intermediates, incorporating a methylene/ethylene spacer, of types **B1**, **B2**, **C1**, **C2**. Thus, alternative approaches were used for preparing these compounds, which involved the reductive condensation of 5-dibenzosuberenyl/5 - dibenzosuberyl - methyl/ethyl - carbaldehydes **28**–**31** with methyl glycocolate in the presence of sodium cyanoborohydride, when the intermediate imines were converted in situ to the secondary amines **B1**, **B2**, **C1**, **C2** (Scheme 4). Thus, the main problem was to prepare the four aldehydes mentioned above, **28**–**31**. For this reason, dibenzosuberone **13** was converted to the carboxylic acid **15** following literature procedures[18] outlined in Scheme 2. The saturated carboxylic acid **17** was on the other hand obtained by a different strategy, by coupling the chloroderivative **12** mentioned above with copper(I) cyanide, followed by acidic hydrolysis of the nitrile **16**. Condensation of the unsaturated alcohol **9** with malonic acid led on the other hand to 5-dibenzosuberenyl acetic acid **18**, which was catalytically (Pd on charcoal) hydrogenated to the corresponding 5-dibenzosuberyl derivative **19** (Scheme 2). The carboxylic acids **15**, **17**–**19** were then esterified with ethyl alcohol, and the corresponding esters **20**, **22**, **24**, **26**, reduced with lithium aluminum hydride to the alcohols **21**, **23**, **25** and **27**, respectively (Scheme 3). Oxidation of the alcohols **21**, **23**, **25** and **27** with pyridinium chlorochromate finally afforded the desired aldehydes **28**–**31** (Scheme 4). In the last steps, the esters (**A**,**B**,**C**)**1**,**2** were reacted with

Table 1. Inhibition data of several MMP-s and ChC with the compounds **E1–E30, F1–F30** and **1**

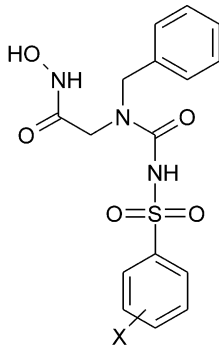
Compd	*n*	X	K_I[a] (nM)				
			MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
E1	0	H	nt	19±2	25±1.8	nt	125±7
E2	0	4-F	>250	15±1.7	26±3	29±2	140±11
E3	0	4-Cl	>250	17±3	29±2	35±2	125±12
E4	0	4-Me	nt	21±1.3	30±2	nt	133±9
E5	0	2-Me	nt	29±3	43±2.4	nt	150±13
E6	1	H	>250	13±0.8	20±1.4	24±2.1	35±3.4
E7	1	4-F	180±9	9±1	13±1.6	15±1.7	21±2
E8	1	4-Cl	230±15	12±1.7	14±2	18±1.1	25±1.9
E9	1	4-Me	>250	15±1.3	27±2.3	26±1.8	38±3.1
E10	1	2-Me	nt	24±1.7	nt	nt	40±3.9
E11	2	H	nt	18±0.8	24±1.9	30±2	86±7.6
E12	2	4-F	>250	13±1	20±2.1	21±1.5	33±3.4
E13	2	4-Cl	190±14	17±2	22±1.6	34±2.9	26±1.8
E14	2	4-Me	>250	24±2	31±1.8	nt	40±4
E15	2	2-Me	nt	25±1.6	37±2	nt	45±3
E16	0	H	nt	29±2	31±1.6	nt	nt
E17	0	4-F	>250	24±1.9	30±2	34±2.1	163±13
E18	0	4-Cl	>250	28±2.1	35±1.6	35±1.9	170±15
E19	0	4-Me	nt	33±2.9	nt	nt	165±14
E20	0	2-Me	nt	38±1.8	nt	nt	189±20
E21	1	H	nt	25±2.6	27±1.8	38±3	nt
E22	1	4-F	200±18	19±1.5	nt	nt	60±5
E23	1	4-Cl	nt	21±2	nt	35±2.6	nt
E24	1	4-Me	>250	25±3.1	nt	nt	42±5
E25	1	2-Me	nt	28±1.7	nt	40±3.7	43±2.5
E26	2	H	nt	27±2.4	28±3	30±2.5	nt
E27	2	4-F	>250	21±1.3	nt	30±1.9	50±4
E28	2	4-Cl	nt	24±1.8	25±2	37±3.3	nt
E29	2	4-Me	nt	26±2.3	33±1.6	nt	55±4.9
E30	2	2-Me	nt	nt	33±	nt	61±5
F1	0	H	25±2.4	9±0.6	14±1.2	12±0.9	16±0.9
F2	0	4-F	21±1.6	5±0.2	13±1.4	9±1.1	13±0.6
F3	0	4-Cl	24±0.7	7±0.3	15±1.2	11±0.8	14±0.4
F4	0	4-Me	32±2.6	15±1.1	16±1.8	14±0.9	18±1.1
F5	0	2-Me	36±2.5	15±0.8	18±2.1	18±2.5	21±1.3
F6	1	H	13±0.7	1.9±1.1	2.4±0.9	3.0±0.4	8±0.5
F7	1	4-F	11±1.0	1.5±0.6	2.0±0.1	2.3±0.3	6±0.4
F8	1	4-Cl	14±2.1	1.7±0.2	2.3±0.2	2.8±0.4	7±0.3
F9	1	4-Me	15±1.3	1.9±0.4	3.1±0.5	3.0±0.3	12±0.8
F10	1	2-Me	15±0.8	1.9±0.1	3.3±0.3	3.6±0.4	15±1
F11	2	H	nt	7±0.5	nt	nt	13±0.6
F12	2	4-F	17±2	3.4±0.9	nt	nt	10±0.8
F13	2	4-Cl	20±1.6	5±0.4	nt	nt	nt
F14	2	4-Me	24±1.6	8±0.7	nt	12±1.5	15±0.7
F15	2	2-Me	29±2.5	9±1.0	nt	13±1.5	nt
F16	0	H	nt	13±1.4	17±2	16±1.3	nt
F17	0	4-F	25±1.5	6±0.3	nt	14±1.6	18±2
F18	0	4-Cl	nt	7±0.6	19±1.6	15±1.4	21±1.5
F19	0	4-Me	nt	18±2	20±1.5	nt	nt
F20	0	2-Me	39±3.3	21±1.7	24±2.1	nt	28±3
F21	1	H	15±0.6	2.6±0.3	2.4±0.5	3.8±0.3	10±1
F22	1	4-F	13±1.2	2.2±0.4	2.7±0.6	2.9±0.3	7±0.5
F23	1	4-Cl	nt	2.7±0.3	nt	nt	9±0.6
F24	1	4-Me	18±	3.5±0.4	3.4±0.2	4.0±0.3	19±2
F25	1	2-Me	nt	3.9±0.5	3.3±0.3	nt	nt
F26	2	H	nt	9±1	nt	nt	15±0.9
F27	2	4-F	21±1.8	5±0.6	nt	nt	13±1
F28	2	4-Cl	24±2.5	7±0.3	9±0.5	nt	nt
F29	2	4-Me	30±2.8	11±1.4	nt	15±1	18±0.8
F30	2	2-Me	38±3	13±2	nt	nt	20±1.7
1		—	33±1.5	20±1.2	10±1	9±0.6	41±3.5

[a] K_Is values were obtained from Easson–Stedman[27] plots using a linear regression program, from at least three different assays. Standard errors were of 5–10%; nt, not tested due to the limited amount of inhibitor available.
[b] With the thioester substrate Ac-ProLeuGly-S-LeuLeuGlyOEt, spectrophotometrically.[25,26]
[c] With FALGPA as substrate, spectrophotometrically.[28]

arylsulfonyl isocyanates **32** leading to the arysulfonylureas **D1–D30**, which were either hydrolyzed with lithium hydroxide/methanol to the corresponding carboxylic acids **E1–E30**, or transformed to hydroxamic acids **F1–F30** by reaction with hydroxylamine hydrochloride and sodium methoxide in methanol (Scheme 5).

MMP and ChC inhibitory activity. Inhibition data of Table 1 show that the entire class of sulfonylureidoglycine hydroxamate/carboxylate derivatives incorporating 5-dibenzosuberenyl/5-dibenzosuberyl-moieties reported here, of types **E1–E30** and **F1–F30**, act as efficient MMP/ChC inhibitors, but important differences of activity were detected against the diverse enzymes, for the different substitution patterns of the sulfonylurea moiety, as well as for the length of the spacer between the polycyclic and amino acid part of these molecules. The following features should be noted regarding MMP/ChC inhibition data of Table 1: (i) the hydroxamates **F1–F30** were stronger inhibitors as compared to the corresponding carboxylates **E1–E30**. This is probably due to the enhanced Zn(II) coordinating properties of the CONHOH moiety (bidentate binding to Zn(II)) as compared to the COOH group (generally monodentate binding to the zinc ion) and was thoroughly documented by our previous work, as well as by contributions of other groups.[8–16] It must be noted anyhow that the differences between the hydroxamates and the corresponding carboxylates are generally not so high for this class of MMP/ChC inhibitors as compared to other inhibitors reported in the literature, and this is a rather positive aspect of derivatives reported here, since carboxylates generally possess a reduced toxicity as compared to the hydroxamates;[8–16] (ii) the compounds incorporating 5-dibenzosuberenyl- moieties were without exception more active than the corresponding derivatives incorporating 5-dibenzosuberyl-groups; (iii) the spacer connecting the polycyclic moiety to the amino acid part of the molecule of both carboxylate as well as hydroxamate protease inhibitors reported here was the most important parameter influencing biologic activity. Thus, the most active compounds were those containing a methylene spacer ($n=1$) between the two structural elements mentioned above, followed by the corresponding derivatives with an ethylene spacer ($n=2$), which in turn were more inhibitory than the compounds without a spacer ($n=0$). This is probably due to the steric hindrance within the $S_{2'}$ pocket of the enzyme, where presumably this moiety binds. Probably the best binding is assured when the bulky tricyclic moiety is connected to the amino acid part of the molecule by means of a CH_2 linker (or a CH_2–CH_2 one), whereas the absence of such a linker increases the steric bulk and destabilizes the enzyme-inhibitor adduct (it should be remembered that the $S_{2'}$ is a relatively shallow and solvent-exposed pocket);[1,4] (iv) the substitution pattern of the arylsulfonylureido part of the molecule also drastically influenced the MMP/ChC inhibitory properties of these derivatives. Inhibition power generally decreased in the following order for the X-Ar-SO_2NHCONH substituted congeners: 4-F > 4-Cl ≅ H > 4-Me > 2-Me-substituted derivatives; (v) the four MMPs and the bacterial collagenase investigated here

Table 2. Inhibition data of several MMP-s and ChC with some of the new compounds reported here (**F7–F10**) and the analogous derivatives containing benzyl moieties at P2′, of type **33a–33d**



33

Compd	X	K_I[a] (nM)				
		MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
33a	4-F	135	9	13	12	21
33b	4-Cl	143	10	15	15	15
33c	4-Me	170	19	20	21	16
33d	2-Me	162	18	25	24	17
F7	4-F	11	1.5	2.0	2.3	6
F8	4-Cl	14	1.7	2.3	2.8	7
F9	4-Me	15	1.9	3.1	3.0	12
F10	2-Me	15	1.9	3.3	3.6	15

[a]K_Is values were obtained from Easson–Stedman[27] plots using a linear regression program, from at least three different assays. Standard errors were of 5–10%. Data for **33a–33d** are from ref 15a.
[b]With the thioester substrate Ac-ProLeuGly-S-LeuLeuGlyOEt, spectrophotometrically.[25,26]
[c]With FALGPA as substrate, spectrophotometrically.[28]

possessed quite different affinities to this class of inhibitors. Thus, carboxylates **E1–E30** were rather ineffective MMP-1 inhibitors and medium potency ChC inhibitors, whereas they possessed nanomolar affinities against MMP-2, MMP-8 and MMP-9 (all these three MMPs are deep pocket enzymes). The order of affinity of such inhibitors for the five proteases investigated was MMP-2 > MMP-8 ≅ MMP-9 > ChC >> MMP-1. The same order of affinity was also shown by the hydroxamate inhibitors **F1–F30**, but the inhibition constants, K_Is, were much lower in this case. Thus, affinity for MMP-1 was in the range of 11–40 nM, that for MMP-2 in the range of 1.5–21 nM, for MMP-8 and MMP-9 in the range of 2–24 nM, whereas for ChC in the range of 6–28 nM. It may be seen that these inhibitors generally discriminate between the deep pocket and shallow pocket enzymes, with ChC showing a somehow intermediate behaviour.

In order to better evaluate the validity of the bulky scaffold strategy proposed here for obtaining MMP/BP inhibitors, we compared the inhibition data of some of the new compounds described here (such as, e.g., **F7–F10**) with the analogous derivatives incorporating a benzyl moiety instead of the 5-dibenzosuberenyl/5-dibenzosuberyl-methyl ones, of type **33a–33b**, previously reported by us[15a] (Table 2). Data of Table 2 unequivocally show the compounds incorporating bulkier $P_{2'}$ moieties, of type **F7–F10**, to act as much stronger MMP/BP inhibitors than the corresponding derivatives incorporating benzyl $P_{2'}$ groups, of types **33a–33d**. This applies to all five proteases investigated here and represents the required proof-of-concept of this bulky scaffold strategy.

Conclusion

We describe here a novel class of strong inhibitors of the zinc proteases MMP-1, MMP-2, MMP-8, MMP-9 and ChC (EC 3.4.24.3), a collagenase from *C. histolyticum*. The drug design has been done considering the 'bulky scaffold' strategy, which involved the introduction of bulky, hydrophobic 5-dibenzosuberenyl and 5-dibenzosuberyl moieties, in the molecule of arylsulfonylureido amino acid derivatives. Both carboxylate and hydroxamate inhibitors were obtained in this way, with different spacers (i.e., no spacer, methylene- and ethylene spacers, respectively) between the tricyclic and amino acid part of the molecule. The key intermediates incorporating the 5-dibenzosuberenyl and 5-dibenzosuberyl moieties, were obtained by nonexceptional but rather laborious synthetic procedures, and were then converted into arylsulfonylureido-glycine hydroxamates/carboxylates. These last compounds were assayed for their MMP and ChC inhibitory activity. Some of the new derivatives reported here proved to be powerful inhibitors of four MMPs with activities in the low nanomolar range for some of the target enzymes. Some of them also showed selectivity for the deep pocket enzymes (MMP-2, MMP-8 and MMP-9) over MMP-1 and ChC.

Experimental

General

Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400–4000 cm^{-1} Nicolet Avatar 360 FTIR spectrometer; NMR spectra: Varian Gemini 300BB apparatus (chemical shifts are expressed as δ values relative to Me_4Si as internal standard for proton spectra and to the solvent resonance for carbon spectra). Elemental analysis (±0.4% of the theoretical values, calculated for the proposed formulas for all the compounds reported here): Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with MeOH–$CHCl_3$ 1:4 v/v. Preparative HPLC was performed on a Dynamax-60A column (25 × 250 mm), with a Beckman EM-1760 instrument. The detection wavelength was 254 nm. 5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ol **9**, 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol **11**, 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one **13**, methyl glycocolate hydrochloride, arylsulfonyl isocyanates **32**, trietylamine, carbodiimides, hydroxylamine, sodium methoxide, 5,5′-dithiobis-(2-nitrobenzoic acid), FALGPA, buffers and other reagents used in the synthesis were commercially available compounds, from Sigma, Acros or Aldrich (Milan, Italy). The thioester MMP substrate, AcProLeuGly-S-LeuLeuGlyOEt was from Bachem. Acetonitrile, methanol, dioxane, ethyl acetate (E. Merck, Darmstadt, Germany) or other solvents used in the synthesis were double distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

Preparation of 5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl-chloride, 10. One gram (4.8 mmol) of 5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol **9** was dissolved in 20 mL of anhydrous benzene, under magnetical stirring, at room temperature. To the obtained solution were added dropwise a mixture of 3 mL of anhydrous benzene, 1.5 mL of thionyl chloride and one drop of pyridine. The reaction mixture was stirred for 10 min at room temperature, then another 10 min at 70 °C (TLC control). The solvent was evaporated in vacuo and the resulted crude product was washed with 30 mL petroleum ether and recrystallized from benzene. Yield was around 75%.

Preparation of 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-chloride, 12 (optimized from the literature procedure).[22] An amount of 3 g (14.2 mmol) of 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol **11** was dissolved in 40 mL of anhydrous diethylether, with energic (magnetic) stirring, at room temperature. HCl gas was then bubbled into the reaction mixture for 30 min, when **12** was obtained in good yield (TLC control). The solvent was evaporated in vacuo and the resulted crude product was recrystallized from petroleum ether. A white needle-shaped crystalline solid was obtained with an yield around 60%.

General procedure for the preparation of compounds 20, 22, 24, 26. An amount of 48 mmol of carboxylic acid **15**, **17**, **18**, or **19**, respectively, was treated under stirring at room temperature with anhydrous ethanol, working at a molar ratio of carboxylic acid–alcohol of 1:20, in the presence of one drop of 98% H_2SO_4 as catalyst. The obtained homogenous solution was then refluxed under anhydrous conditions for 10–24 h (reaction monitored by TLC). After all the acid was converted to the corresponding ester, the mixture was concentrated to half the volume by evaporating the solvent in vacuo, when an abundant white precipitate was obtained. Stirring of this crude product with 35 mL of water for 30 min, at room temperature, followed by vacuum filtration, washing with water until a pH of 5.5 was reached, and recrystallized from methanol, afforded the pure title compounds.[17] Overall yields were in the range of 70–80%.

General procedure for the preparation of compounds 21, 23, 25, 27

A solution was prepared by dissolving 43 mmol of ester obtained as described above (compound **20**, **22**, **24**, **26**, respectively) in 220 mL anhydrous diethylether. Separately, 52 mmol (a 20% excess relative to the stoichiometric amount) of $LiAlH_4$ were suspended in 60 mL anhydrous diethylether, under argon. The etheric solution of the ester was then slowly added to it, drop by drop, during a period of 2 h, under the argon atmosphere and strictly anhydrous conditions. After this time, the obtained reaction mixture was refluxed for 8–14 h (TLC control), then cooled to 0 °C, and quenched with 15% aqueous solution of H_2SO_4 added dropwise, when two distinct layers were obtained. The etheric layer was separated, and the aqueous one extracted twice with 75 mL of diethylether. The combined organic layers were washed with water till the neutral pH, and dried over anhydrous $CaCl_2$. The solvent was evaporated in vacuo yielding a white oil, which cristallyzed by standing. Recrystallization from hexane afforded the pure title compounds, as white crystals, with overall yields between 70 and 80%.[17,18]

General procedure for the preparation of compounds 28–31 (adapted from literature procedure)[21]

Pyridinium chlorochromate (4.5 mmol) was suspended in about 6 mL of methylene chloride, then the corresponding alcohol (**21**, **23**, **25**, **27**, respectively), dissolved in the same solvent, was rapidly added under good stirring, working at room temperature, and afforded to react for 2–3 h (TLC control). When the reaction was completed, the resulted dark mixture was diluted with 80 mL of anhydrous diethylether. The organic phase was decanted whereas the inorganic solid (the reduced reagent) was washed three times with anhydrous ether. The pooled organic fractions containing the ether solution of the aldehyde was evaporated in vacuum and the resulted crude product was used directly for the reductive coupling with methylglycocolate/sodium borohydride, as described later in the text.

General procedure for the preparation of compounds A1–A2

Chloroderivative (4.8 mmol) **10** and **12** respectively, and the stoichiometric amount of methyl glycocolate hydro-

chloride were suspended in 10 mL of anhydrous acetonitrile, with magnetical stirring. Triethylamine (9.6 mmol) was added and the obtained mixture was refluxed for 24–48 h, until a reasonable conversion was reached (TLC control). The solvent was evaporated in vacuum, and the obtained precipitate was extracted with a mixture of 15 mL CH_2Cl_2–10 mL water. The separated aqueous layer was reextracted twice with 10 mL of CH_2Cl_2. The combined organic layers (25 mL) were treated with 15 mL of saturated aqueous $NaHCO_3$ solution, then with water until a pH = 7 was reached. The organic layer was dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated in vacuo. In order to eliminate traces of the alcohol used as starting material, the resulted crude solid product was washed with 50–60 mL of anhydrous diethylether, then converted into the corresponding ester hydrochloride by bubbling dried gaseous HCl (generated in situ from concentrate H_2SO_4 and solid KCl). The obtained precipitate was separated by filtration under argon, washed with ether and retaken in the minimum volume of anhydrous MeOH. For generating the free amine, the methanolic solution was treated with solid $NaHCO_3$ and heated at reflux for 15–30 min. The inorganic precipitate was discarded and the solvent was evaporated in vacuum. The obtained crude solid was purified by column chromatography (adsorbent: silicagel—60 Å, 40–60 μm, Merck; eluent: CH_2Cl_2–MeOH 4:1 v/v). Recrystallization from absolute ethanol afforded the pure compounds **A1**, **A2**. Overall yields were in the range of 60–70%.

General procedure for the preparation of compounds B1, B2, C1, C2 (adapted from literature procedure)[23]

A molar ratio of aldehyde–methyl glycocolate hydrochloride–$NaBH_3CN$ of 1:3:0.6 was used in the reductive coupling. An amount of 4.27 mmol aldehyde was treated with 12.81 mM methyl glycocolate hydrochloride, then a solution of sodium methoxide required for the neutralization of the acid was added at room temperature to the reaction mixture, under magnetical stirring. A 5% excess relative to the stoichiometric amount of solid anhydrous Na_2SO_4 was also introduced in this reaction mixture, for absorbing the water resulted in reaction. 2.56 mmol of $NaBH_3CN$ were then added into the reaction mixture, and stirring was continued for 48 h at room temperature (TLC control). The solvent was evaporated in vacuo and 25 mL of water were added, then a quench was performed by treating the resulted mixture with concentrated HCl until the pH was brought to 1–1.5. Solid NaOH was then added till pH > 8.5 was reached. The resulted mixture was extracted with methylene chloride, for three times, each time with a volume of 25 mL. The combined organic layers were treated with 10 mL of aqueous NaCl solution, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated in vacuo. The resulted crude solid was purified following the procedure described above for obtaining the pure compounds **A1**, **A2**. Overall yields were in the range of 60–80%.

General procedure for the preparation of compounds D1–D30

An amount of 10 mmol of key intermediate **A1**, **A2**, **B1**, **B2**, and **C1**, **C2**, respectively, and the stoichiometric amount of arylsulfonyl isocyanate **32** were suspended in 50 mL of anhydrous acetone, and 150 μL (10 mmol) of triethylamine were added. The reaction mixture was stirred at room temperature for 2–6 h (TLC control). The solvent was evaporated in vacuo, and the crude product was taken up in ethyl acetate (15 mL), poured into a 5% solution of $NaHCO_3$ (25 mL), and extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and the solvent was removed in vacuo. The obtained crude products were recrystallized from ethanol. Yields were quantitative.

General procedure for the preparation of compounds E1–E30

An amount of 5 mmol of methyl ester **D1**–**D30** was dissolved in water–methanol–THF (1:1:1, v/v/v) and excess lithium hydroxide (200 mg) was added. The resulting mixture was stirred overnight at room temperature. The reaction was acidified with 1 N HCl and the mixture extracted with methylene chloride. The organic extracts were dried over sodium sulfate and then concentrated to an oil under reduced pressure. Preparative HPLC (Dynamax-60A column (25 × 250 mm); 90% acetonitrile/10% water; flow rate of 30 mL/min) afforded the pure carboxylic acids **E1**–**E30**.

General procedure for the preparation of compounds F1–F30

An amount of 5 mmol of ester **D1**–**D30** was dissolved in 20 mL of methanol. To this solution hydroxylamine hydrochloride (0.76 g, 11 mmol) was added, followed by the addition of sodium methoxide (15 mmol) freshly obtained from sodium (350 mg) dissolved in methanol (10 mL). The reaction mixture was stirred overnight at room temperature, and was then worked up by partitioning between dilute hydrochloric acid (pH = 3) and ethyl acetate. The aqueous phase was extracted with ethyl acetate, the combined organic layers dried (Na_2SO_4) and the solvent evaporated. The products were then purified by HPLC (Dynamax-60A column (25 × 250 mm); 90% acetonitrile/ 10% methanol; flow rate of 30 mL/min). Yields were in the range of 65–82%.

All the new compounds were characterized by IR, ^1H and ^{13}C NMR spectroscopy as well as elemental analysis. Representative data are provided below.

***N*-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-yl)methyl glycocolate A1.** White crystals, mp 64–66 °C; IR (nujol), cm^{-1}: 894; 972; 1070; 1243; 1560; 1673; 3200 br; ^1H NMR ($CDCl_3$, δ, ppm, *J*, Hz): 3.12 (s, 2H, N–CH_2); 3.67 (s, 3H, OMe); 4.87 (s, 1H, H-5); 6.95 (s, 2H, H-10-11); 7.23 (m, 8H, H-arom); ^{13}C NMR ($CDCl_3$, δ ppm): 172.29 (CO); 138.69 (C-q); 133.57 (C-q); 130.86 (CH-10-11); 130.54 (CH-arom); 130.46 (CH-arom); 130.22 (CH-arom); 130.07

(CH-arom); 68.88 (C-5); 51.57 (OMe); 48.30 (N–CH_2). Anal., found: C, 77.36; H, 6.16; N, 4.95. $C_{18}H_{17}NO_2$ requires: C, 77.42; H, 6.09; N, 5.02.

***N*-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methyl glycocolate A2.** White crystals, mp 85–87 °C; IR (nujol), cm^{-1}: 867; 954; 1082; 1255; 1560; 1674; 3200 br; ^1H NMR 2.88 (m, 4H, H-10-11, syst. A_2B_2); 3.28 (s, N–CH_2); 3.70 (s, 3H, OMe); 4.78 (s, 1H, H-5); 7.18 (m, 8H, H-1-4 and 6-9); ^{13}C NMR ($CDCl_3$, δ, ppm): 32.51 (C-10-11) 48.72 (N-CH_2); 51.77 (OMe); 68.93 (C-5); 125.97 (CH-arom); 127.81 (CH-arom); 129.92 (CH-arom); 130.55 (CH-arom); 139.22 (C-q); 140.55 (C-q); 173.24 (CO). Anal., found: C, 76.79; H, 6.71; N, 4.88. $C_{18}H_{19}NO_2$ requires: C, 76.87; H, 6.76; N, 4.98.

***N*-[(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-yl)methylen]methyl glycocolate B1.** Amorphous colorless solid; IR (nujol), cm^{-1}: 895; 966; 1059; 1248; 1560; 1671; 3200 br; ^1H NMR ($CDCl_3$, δ, ppm, *J*, Hz): 2.99 (d, 2H, H-51, 7.9); 3.30 (s, 2H, N–CH_2); 3.63 (s, 3H, OMe); 4.32 (t, 1H, H-5, 7.9); 6.88 (s, 2H, H-10-11); 7.22 (m, 8H, H-arom); ^{13}C NMR ($CDCl_3$, δ, ppm): 47.53($\underline{C}H_2NHCH_2$); 49.31 (N–CH_2); 51.87 (OMe); 54.04 (C-5); 126.98 (CH-arom); 129.89 (CH-arom); 128.99 (CH-arom); 130.21 (CH-arom); 130.72 (CH-10-11); 134.16 (C-q); 138.03 (C-q); 171.46 (CO). Anal., found: C, 77.75; H, 6.39; N, 4.81. $C_{19}H_{19}NO_2$ requires: C, 77.81; H, 6.48; N, 4.78.

***N*-[(10,11-Dihydro-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methylene]methyl glycocolate B2.** Amorphous colorless solid; IR (nujol), cm^{-1}: 872; 969; 1081; 1267; 1563; 1667; 3200 br; ^1H NMR ($CDCl_3$, δ, ppm, *J*, Hz): 3.03 and 3.35 (m, 4H, H-10-11, syst. A_2B_2); 3.41 (s, 2H, N–CH_2); 3.67(d, 2H, H-51, 7.9); 3.68 (s, 3H, OMe); 4.26 (t, 1H, H-5, 7.9); 7.17 (m, 8H, H-arom); ^{13}C NMR ($CDCl_3$, δ, ppm): 33.29 (C-10-11); 50.53 (CH_2–N); 50.53 (N–CH_2); 51.71 (OMe); 55.13 (C-5); 126.18 (CH-arom); 126.90 (CH-arom); 130.38 (CH-arom); 130.41 (CH-arom); 139.21 (C-q); 139.85 (C-q); 172.55 (CO). Anal., found: C, 77.21; H, 7.19; N, 4.69. $C_{19}H_{21}NO_2$ requires: C, 77.29; H, 7.12; N, 4.74.

***N*-[(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-yl)ethylene]methyl glycocolate C1.** Amorphous colorless solid; IR (nujol), cm^{-1}: 874; 975; 1049; 1241; 1574; 1668; 3200 br; ^1H NMR ($CDCl_3$, δ, ppm, *J*, Hz): 1.91 (q, 2H, CH_2-α to the tricyclic ring, 7.3); 2.26 (t, 2H, CH_2-β to the tricyclic ring, 7.3); 3.24 (s, 2H, N–CH_2); 3.65 (s, 3H, OMe); 4.09 (t, 1H, H-5, 7.3); 6.87 (s, 2H, H-10-11); 7.21 (m, 8H, H-arom); ^{13}C NMR ($CDCl_3$, δ, ppm): 30.11 (C-α to the tricyclic ring); 47.62 (C-β to the tricyclic ring); 50.55 (N–CH_2); 51.56 (OMe); 52.28 (C-5); 126.25 (CH-arom); 128.58 (CH-arom); 129.43 (CH-arom); 129.74 (CH-arom); 130.77 (CH-10-11); 133.94 (C-q); 140.73 (C-q); 172.76 (CO). Anal., found: C, 78.08; H, 6.79; N, 4.51; $C_{20}H_{21}NO_2$ requires: C, 78.17; H, 6.84; N, 4.56.

***N*-[(10,11-Dihydro-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)ethylene]methyl glycocolate C2.** Amorphous colorless solid; IR (nujol), cm^{-1}: 863; 975; 1078; 1267; 1565; 1671; 3200 br; ^1H NMR ($CDCl_3$, δ, ppm, *J*, Hz): 2.28 (q, 2H, CH_2-α to the tricyclic ring, 7.4); 2.56 (t, 2H, CH_2-β to the tricyclic ring, 7.4); 3.07 and 3.31 (m, 4H, H-10-11, syst. A_2B_2); 3.35 (s, 2H, N–CH_2); 3.70 (s, 3H, OMe); 4.74 (t, 1H, H-5, 7.4); 5.65 (bs, 1H, NH, deuterable); 7.16 (m, 8H, H-arom); ^{13}C NMR ($CDCl_3$, δ, ppm): 32.90 (C-α to the tricyclic ring); 33.03 (C-10-11); 47.74 (C-β to the tricyclic ring); 49.73 (N–CH_2); 51.78 (OMe); 51.82 (C-5); 125.98 (CH-arom); 126.51 (CH-arom); 129.77 (CH-arom); 130.09 (CH-arom); 139.24 (C-q); 141.05 (C-q); 171.81 (CO). Anal., found: C, 77.52; H, 7.62; N, 4.43. $C_{20}H_{23}NO_2$ requires: C, 77.67; H, 7.44; N, 4.53.

***N*-4-Fluorophenylsulfonylureido-*N*-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-glycine E2.** Colorless crystals, mp 136–137 °C; IR (KBr), cm^{-1}: 1153 (SO_2^{sym}), 1287 (amide III), 1379 (SO_2^{as}), 1584 (amide II), 1712 (amide I), 1755 (COOH); 3060 and 3300 (NH, OH); ^1H NMR (DMSO-d_6, δ, ppm, *J*, Hz): 3.62 (s, 2H, CH_2), 5.73 (s, 1H, H_{C5}), 7.17 (s, 2H, $H_{10,11}$), 7.55 (m, 8H, H_{Ph}), 7.69 (d, 2H, H_{ortho} of 4-FC_6H_4), 7.95 (d, $^3J_{HH}=8.1$, 2H, H_{meta} of 4-FC_6H_4); 8.12 (bs, 2H, NHCONH); 11.47 (bs, 1H, COOH); ^{13}C NMR (DMSO-d_6), δ, ppm: 45.39 (CH_2), 68.54 (CH), 128.73 (enlarged), 129.61 (C_q), 130.03 (Ph), 130.10 (C_{meta} of FC_6H_4), 130.26 (Ph), 130.45 (Ph), 130.58 (Ph), 130.85 (Ph), 132.63 (NHCONH), 133.40 (C_q), 135.13 (C_{ortho} of FC_6H_4), 148.92 (C_{ipso} of FC_6H_4), 149.82 (C_{para} of FC_6H_4), 170.31 (COOH). Anal. ($C_{24}H_{19}FN_2SO_5$) C, H, N.

***N*-4-Toluenesulfonylureido-*N*-[(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)methylen]glycine E9.** White crystals, mp 133–135 °C; IR (KBr), cm^{-1}: 1164 (SO_2^{sym}), 1288 (amide III), 1377 (SO_2^{as}), 1580 (amide II), 1715 (amide I), 1751 (COOH); 3060 and 3300 (NH, OH); ^1H NMR (DMSO-d_6), δ, ppm: 2.51 (s, 3H, C$H_3C_6H_4$), 2.93 (d, 2H, $^3J_{HH}=7.9$, CH–CH_2), 3.26 (s, 2H, CH_2COO), 4.23 (t, 1H, $^3J_{HH}=7.9$, H_{C5}), 6.87 (s, 2H, $H_{10,11}$), 7.30 (m, 8H, H_{Ph})7.68 (d, $^3J_{HH}=8.2$, 2H, H_{ortho} of $CH_3C_6H_4$), 7.99 (d, $^3J_{HH}=8.2$, 2H, H_{meta} of $CH_3C_6H_4$); 8.25 (bs, 2H, NHCONH); 11.73 (bs, 1H, COOH); ^{13}C NMR (DMSO-d_6), δ, ppm: 26.1 ($CH_3C_6H_4$), 45.61 (CH_2COO), 50.49 (CHCH_2), 55.10 (CH), 126.77, 128.49, 129.83, 130.11, 130.63 (all from Ph), 130.54 (C_{meta} of $CH_3C_6H_4$), 132.71 (NHCONH), 134.17 and 138.70 (C_q), 135.62 (C_{ortho} of $CH_3C_6H_4$), 145.80 (C_{ipso} of $CH_3C_6H_4$), 148.14 (C_{para} of $CH_3C_6H_4$), 175.91 (COOH). Anal. ($C_{26}H_{24}N_2SO_5$) C, H, N.

***N*-4-Chlorophenylsulfonylureido-*N*-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-glycine hydroxamate F3.** Colorless crystals, mp 157–158 °C; IR (KBr), cm^{-1}: 1162 (SO_2^{sym}), 1281 (amide III), 1385 (SO_2^{as}), 1574 (amide II), 1715 (amide I), 1757 (COOH); 3060 and 3300 (NH, OH); ^1H NMR (DMSO-d_6, δ, ppm, *J*, Hz): 3.60 (s, 2H, CH_2), 5.77 (s, 1H, H_{C5}), 7.19 (s, 2H, $H_{10,11}$), 7.55 (m, 8H, H_{Ph}), 7.66 (d, 2H, H_{ortho} of 4-ClC_6H_4), 7.94 (d, $^3J_{HH}=8.1$, 2H, H_{meta} of 4-ClC_6H_4); 8.15 (bs, 2H, NHCONH); 11.41 (bs, 1H, COOH); ^{13}C NMR (DMSO-d_6), δ, ppm: 45.26 (CH_2), 68.37 (CH), 128.79 (enlarged), 129.54 (C_q), 130.12 (Ph), 130.15 (C_{meta} of ClC_6H_4), 130.26 (Ph), 130.44 (Ph), 130.61 (Ph), 130.82 (Ph), 132.69 (NHCONH), 133.74 (C_q), 135.10 (C_{ortho} of ClC_6H_4), 149.14 (C_{ipso} of ClC_6H_4), 149.87 (C_{para} of ClC_6H_4), 170.20 (COOH). Anal. ($C_{24}H_{20}ClN_3SO_5$) C, H, N.

***N*-4-Toluenesulfonylureido-*N*-[(10,11-dihydro-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)ethylene] glycine hydroxamate F29.** Colorless crystals, mp 108–110 °C; IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1285 (amide III), 1381 (SO_2^{as}), 1575 (amide II), 1710 (amide I), 1754 (COOH); 3060 and 3300 (NH, OH); ^1H NMR (DMSO-d_6), δ, ppm: 2.25 (q, 2H, $^3J_{HH} = 7.3$, $CHCH_2CH_2$), 2.60 (t, 2H, $^3J_{HH} = 7.3$, $CHCH_2CH_2$), 2.66 (s, 3H, $CH_3C_6H_4$), 3.05 (m, 2H, H_{11A}, H_{11B}), 3.33 (m, 2H, H_{10A}, H_{10B}), 3.37 (s, 2H, CH_2COO), 4.10 (t, 1H, $^3J_{HH} = 7.4$, H_{C5}), 7.00–7.32 (m, 8H, H_{Ph}), 7.60 (d, $^3J_{HH} = 8.3$ Hz, 2H, H_{ortho} of $CH_3C_6H_4$), 7.95 (d, $^3J_{HH} = 8.3$ Hz, 2H, H_{meta} of $CH_3C_6H_4$); 8.26 (bs, 2H, NHCONH); 8.79 (bs, 1H, NHOH); 10.66 (brs, 1H, NHOH); ^{13}C NMR (DMSO-d_6), δ, ppm: 26.71 (s, $CH_3C_6H_4$), 33.12 ($C_{10}+C_{11}$), 47.65 (CH_2COO), 49.83 ($CHCH_2CH_2$), 51.78 (CH), 125.76, 126.54, 130.19, 130.34 (all from Ph), 130.53 (C_{meta} of $CH_3C_6H_4$), 132.86 (NHCONH), 135.62 (C_{ortho} of $CH_3C_6H_4$), 139.24 and 141.05 (C_q), 145.35 (C_{ipso} of $CH_3C_6H_4$), 148.97 (C_{para} of $CH_3C_6H_4$), 172.53 (s, CONHOH). Anal. ($C_{27}H_{29}N_3SO_5$) C, H, N.

Enzyme preparations and assay

Human purified MMP-s (MMP-1, MMP-2, MMP-8 and MMP-9) were purchased from Calbiochem (Inalco, Milano, Italy). They were activated[24] in the assay buffer by adding bovine trypsin (50 μL, 0.6 mg/mL) to the proenzyme, followed by incubation at 37 °C for 10 min. The trypsin was then inactivated with aprotinin (50 μL, 1.2 mg/mL). Initial rates for the hydrolysis of the thioester substrate AcProLeuGly-S-LeuLeuGlyOEt, coupled to the reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) were used for assessing the catalytic activity and inhibition of the four MMP-s mentioned above, by the spectrophotometric method of Powers and Kam,[25] and Johnson et al.[26] The change of absorbance ($\varepsilon = 19.800\ M^{-1}\ cm^{-1}$)[25] at 405 nm was monitored continuously at room temperature, using a Cary 3 spectrophotometer interfaced with a PC. A typical 100 μL reaction contained 50 mM MES, pH 6.0, 10 mM $CaCl_2$, 100 μM substrate, 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) and 5 nM MMP. For the K_I determinations, DMSO solutions of the inhibitor were included in the assay, resulting in a final concentration of 2% DMSO in the reaction mixture. In these conditions, K_I values varied from 5 to 10% in replicate experiments. K_Is were then determined by using Easson–Stedman[27] plots and a linear regression program.

C. histolyticum highly purified collagenase and its substrate, FALGPA (furanacryloyl-leucyl-glycyl-prolyl-alanine) were purchased from Sigma-Aldrich (Milano, Italy), and their concentrations were determined from the absorbance at 280 nm and the extinction coefficients furnished by the supplier. The activity of such preparations was in the range of 10 NIH units/mg solid. The potency of standard and newly obtained inhibitors was determined from the inhibition of the enzymatic (amidolytic) activity of the collagenase, at 25 °C, using FALGPA as substrate, by the method of van Wart and Steinbrink.[28] The substrate was reconstituted as 5 mM stock in 50 mM Tricine buffer, 0.4 M NaCl, 10 mM $CaCl_2$, pH 7.5. The rate of hydrolysis was determined from the change in absorbance at 324 nm using an extinction coefficient for FALGPA $\varepsilon_{305} = 24.700\ M^{-1}\ cm^{-1}$ in the above-mentioned reaction buffer.[28] Measurements were made using a Cary 3 spectrophotometer interfaced with a PC. Initial velocities were thus estimated using the direct linear plot-based procedure reported by van Wart and Steinbrink.[28] K_Is were then determined according to Easson–Stedman[23] plots and a linear regression program.

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References and Notes

1. (a) Woessner, J. F., Jr.; Nagase, H. *Matrix Metalloproteinases and TIMPS*; Oxford University Press: Oxford, 2000; p 1. (b) Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds. *Metalloproteinases as Targets for Anti-Inflammatory Drugs*. Birkhäuser: Basel, Boston, Berlin, 1999; p 1.
2. (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305. (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735. (c) Skotnicki, J. S.; Levin, J. I.; Zask, A.; Killar, L. M. Matrix Metalloproteinase Inhibitors; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Birkhäuser: Basel, Boston, Berlin, 1999; p 17 (d) Bottomley, K. M. K.; Johnson, W. H.; Walter, D. S. *J. Enzyme Inhib.* **1998**, *13*, 79. (e) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
3. (a) Supuran, C. T.; Scozzafava, A.; Mastrolorenzo, A. *Exp. Opin. Ther. Pat.* **2001**, *11*, 221. (b) Supuran, C. T.; Scozzafava, A.; Clare, B. W. *Med. Res. Rev.* **2002**, *22*, 329.
4. Supuran, C. T.; Scozzafava, A. Matrix Metalloproteinases (MMPs); Smith, H. J., Simons, C., Eds.; *Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development*; Taylor & Francis: London and New York, 2002; p 35.
5. Nagase, H.; Woessner, J. F., Jr. *J. Biol. Chem.* **1999**, *274*, 21491.
6. Giavazzi, R.; Taraboletti, G. *Crit. Rev. Oncol. Hematol.* **2001**, *37*, 53.
7. Casini, A.; Scozzafava, A.; Supuran, C. T. *Exp. Opin. Ther. Pat.* **2002**, *12*, 1307.
8. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. *J. Med. Chem.* **1997**, *40*, 2525.
9. (a) Matter, H.; Schwab, W.; Barbier, D.; Billen, G.; Haase, B.; Neises, B.; Schudok, M.; Thorwart, W.; Schreuder, H.; Brachvogel, V.; Lonze, P.; Weithmann, K. U. *J. Med. Chem.* **1999**, *42*, 1908. (b) Matter, H.; Schwab, W. *J. Med. Chem.* **1999**, *42*, 4506.
10. (a) Natchus, M. G.; Bookland, R. G.; Laufersweiler, M. J.; Pikul, S.; Almstead, N. G.; De, B.; Janusz, M. J.; Hsieh, L. C.; Gu, F.; Pokross, M. E.; Patel, V. S.; Garver, S. M.; Peng, S. X.; Branch, T. M.; King, S. L.; Baker, T. R.; Foltz, D. J.; Mieling, G. E. *J. Med. Chem.* **2001**, *44*, 1060. (b) Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. *J. Med. Chem.* **1999**, *42*, 4547. (c)

Cheng, M.; De, B.; Almstead, N. G.; Pikul, S.; Dowty, M. E.; Dietsch, C. R.; Dunaway, C. M.; Gu, F.; Hsieh, L. C.; Janusz, M. J.; Taiwo, Y. O.; Natchus, M. G. *J. Med. Chem.* **1999**, *42*, 5426. (d) Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1691.
11. (a) Kiyama, R.; Tamura, Y.; Watanabe, F.; Tsuzuki, H.; Ohtani, M.; Yodo, M. *J. Med. Chem.* **1999**, *42*, 1723. (b) O'Brien, P. M.; Ortwine, D. F.; Pavlovsky, A. G.; Picard, J. A.; Sliskovic, D. R.; Roth, B. D.; Dyer, R. D.; Johnson, L. L.; Man, C. F.; Hallak, H. *J. Med. Chem.* **2000**, *43*, 156.
12. (a) Levin, J. I.; Gu, Y.; Nelson, F. C.; Zask, A.; DiJoseph, J. F.; Sharr, M. A.; Sung, A.; Jin, G.; Cowling, R.; Chanda, P.; Cosmi, S.; Hsiao, C. L.; Edris, W.; Wilhelm, J.; Killar, L. M.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 239. (b) Levin, J. I.; Du, M. T.; DiJoseph, J. F.; Killar, L. M.; Sung, A.; Walter, T.; Sharr, M. A.; Roth, C. E.; Moy, F. J.; Powers, R.; Jin, G.; Cowling, R.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 235.
13. Gavuzzo, E.; Pochetti, G.; Mazza, F.; Gallina, C.; Gorini, B.; D'Alessio, S.; Pieper, M.; Tschesche, H.; Tucker, P. A. *J. Med. Chem.* **2000**, *43*, 3377.
14. (a) Clare, B. W.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2001**, *44*, 2253. (b) Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 1858. (c) Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 3677.
15. (a) Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **2000**, *35*, 299. (b) Supuran, C. T.; Scozzafava, A. *Eur. J. Pharm. Sci.* **2000**, *10*, 67. (c) Supuran, C. T.; Briganti, F.; Mincione, G.; Scozzafava, A. *J. Enzyme Inhib.* **2000**, *15*, 111.
16. (a) Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2000**, *8*, 637. (b) Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 499. (c) Scozzafava, A.; Supuran, C. T. *Eur. J. Pharm. Sci.* **2000**, *11*, 69.
17. Van der Stelt, C. US Patent 1967, 3.349.093.
18. Horning, D. E.; Muchowski, J. M. *Can. J. Chem.* **1968**, *46*, 3665.
19. Van der Stelt, C.; Haasjes, A.; Tersteege, H. M.; Nauta, W. T. *Recueil* **1965**, *84*, 1466.
20. Cope, A.; Fenton, S. *J. Am. Chem. Soc.* **1951**, *73*, 1673.
21. Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *31*, 2647.
22. Pless, J. *Helv. Chim. Acta* **1976**, *59*, 409.
23. Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *73*, 2897.
24. Nagase, H. *Biol. Chem.* **1997**, *378*, 151.
25. Powers, J. C.; Kam, C. M. *Meth. Enzymol.* **1995**, *248*, 3.
26. Johnson, L. L.; Bornemeier, D. A.; Janowicz, J. A.; Chen, J.; Pavlovsky, A. G.; Ortwine, D. F. *J. Biol. Chem.* **1999**, *274*, 24881.
27. Bieth, J. G. *Meth. Enzymol.* **1995**, *248*, 59.
28. Van Wart, H. E.; Steinbrink, D. R. *Anal. Biochem.* **1981**, *113*, 156.