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Research Article

Synthesis of MMP inhibitor radiotracers [^{11}C]methyl-CGS 27023A and its analogs, new potential PET breast cancer imaging agents

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Summary

[^{11}C]Methyl-CGS 27023A (**1a**) and its analogs [^{11}C]methyl-2-picolyl-CGS 27023A (**1b**), [^{11}C]methyl-benzyl-CGS 27023A (**1c**), [^{11}C]methyl-2-nitro-CGS 27023A (**1d**), [^{11}C]methyl-3-nitro-CGS 27023A (**1e**), and [^{11}C]methyl-4-nitro-CGS 27023A (**1f**), novel radiolabeled matrix metalloproteinase (MMP) inhibitors, have been synthesized for evaluation as new potential positron

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emission tomography (PET) breast cancer imaging agents. The appropriate precursors for radiolabeling were obtained in four to five steps from starting material amino acid D-valine with moderate to excellent chemical yields. Precursors were labeled by [^{11}C]methyl triflate through ^{11}C–O-methylation method at the aminohydroxyl position under basic conditions and isolated by solid-phase extraction (SPE) purification to produce pure target compounds in 40–60% radiochemical yields (decay corrected to end of bombardment), in 20–25 min synthesis time. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: matrix metalloproteinase inhibitor; breast cancer; radiotracer; carbon-11; positron emission tomography; [^{11}C]methyl-CGS 27023A

1. Introduction

Breast cancer is the most commonly diagnosed cancer of women and the second leading cause of cancer deaths among women. Experiments in animal models of breast cancer have shown that matrix metalloproteinase (MMP) inhibitors can significantly reduce the growth rate of both primary and secondary tumors and can block the process of metastasis.[1] MMPs are a family of zinc-containing enzymes responsible for the breakdown of connective tissue proteins. The ability of these enzymes, present in high concentrations in many tumor types, to modify the extracellular matrix (ECM) allows tumor cells to establish metastases and undergo rapid growth.[2] The overexpression of MMPs in breast tumors provides a target for positron emission tomography (PET) imaging of breast cancer.[3] Radiolabeled MMP inhibitor analogs labeled with positron emitting radionuclides carbon-11 or fluorine-18 may enable non-invasive monitoring of breast cancer MMP levels and breast cancer response to MMP inhibitor therapy using PET imaging techniques.

There has been a great interest in the design and development of MMP inhibitors (MMPIs) as therapeutic agents.[4] The development of specific bioavailable inhibitors of the MMPs would aid in delineating the role of these enzymes in normal and disease states.[5,6] Several MMP inhibitors have been entering human trials such as Batimastat (BB-94, British Biotech),[7] Marimastat (BB2516, British Biotech),[8] CGS 27023A (Novartis),[9] CT-1746 (Celltech Therapeutics)[10] and AG-3340 (Agouron).[11] Among these MMP inhibitors, CGS 27023A possesses the combination of favorable pharmacokinetics and nanomolar IC_{50} potencies for several MMP subtypes,[6,9] and aminohydroxyl and/or

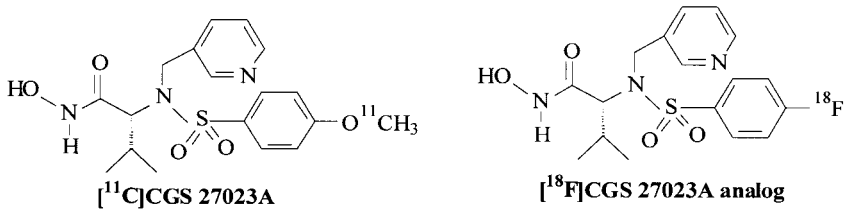


Figure 1. Structure of [^{11}C]CGS 27023A and [^{18}F]CGS 27023A analog

O-methyl positions amenable to labeling with carbon-11. These same properties are often beneficial in a diagnostic radiotracer.

A number of carbon-11 and fluorine-18 labeled CGS 27023A analogs have been developed in this laboratory.[12] [^{11}C]CGS 27023A and [^{18}F]CGS 27023A analog (Figure 1) are our initial target radiotracers, however, the low radiochemical yields, long reaction time and complicated purification methods in their radiochemical syntheses have prevented the reliable routine production and automation of these tracers. In order to explore novel possible radiolabeled analogs of CGS 27023A as alternative candidate agents, we have synthesized [^{11}C]methyl-CGS 27023A (**1a**) and its analogs [^{11}C]methyl-2-picolyl-CGS 27023A (**1b**), [^{11}C]methyl-benzyl-CGS 27023A (**1c**), [^{11}C]methyl-2-nitro-CGS 27023A (**1d**), [^{11}C]methyl-3-nitro-CGS 27023A (**1e**), and [^{11}C]methyl-4-nitro-CGS 27023A (**1f**).

Results and discussion

The requirement for a molecule to be an effective inhibitor of the MMP class of enzymes is a functional group (e.g. carboxylic acid, hydroxamic acid, and sulfhydryl, etc.) capable of chelating the active-site zinc (II) ion (this will be referred to as zinc binding group or ZBG), at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains which undergo effective van der Waals interactions with the enzyme subsites.[4] It is now apparent that this requirement can be satisfied by a variety of different structural classes of MMP inhibitors which have been discovered by a number of methods including structure-based design[13–29] and combinatorial chemistry.[30,31] The mechanism-based inhibition of MMPs by MMPIs and the reaction mechanism for proteolysis by MMPs has been rationalized by the structure-based information of MMPI-MMP

complex.[26] Therefore, we have assumed that the structural modification of CGS 27023A to methylated CGS 27023A (**1a**) and its analogs (**1b–f**), is not likely to cause a major change in their inhibitory properties, because the ZBG of O-methylated hydroxamic acid has the same function as the ZBG of hydroxamic acid to coordinate with zinc at the active site. The evidence to support this assumption is provided by the biological assay of compounds **1a–f** in comparison with CGS 27023A (**6a**).

CGS 27023A is a potent MMP inhibitor for several MMP subtypes such as MMP-3 (stromelysin) and MMP-1 (collagenase). In order to examine effects of modified compounds methyl-CGS 27023A and its analogs (**1a–f**) on MMP activity, we performed a fibril degradation assay[32] using fluorogenic substrates specific to MMP-1. Two hundred nanomolar of individual modified compound was incubated at room temperature for 2 h with *p*-aminophenylmercuric acetate (APMA) activated 100 ng of human MMP-1 and 12.5 μM MMP-1 specific fluorogenic substrates in a total 100 μl reaction mixture consisting of 500 mM Tris–HCl (pH 7.6), 1.5 M NaCl, 50 mM $CaCl_2$ and 2 mM sodium azide. The substrate was intensively labeled with fluorophores and digestion of substrate by MMP-1 would liberate fluorophores from a quenching effect of nearby fluorophores. Fluorescent intensity was measured by FluoroMax-2 spectrofluorometer (Instruments S.A., Inc.). The absorption and emission wavelengths were set to 382 and 441 nm, respectively. To examine the statistical significance of the inhibition effects, a student's *t*-test was conducted and *p*-values <0.05 were considered statistically significant. Distilled water without any addition of testing compound was used as a negative control. The results show that all these modified compounds **1a–f** exhibit strong inhibitory effectiveness on MMP-1 ($p<0.01$) at a level similar to CGS 27023A (**6a**) (Figure 2), although their inhibition effect levels are slightly varied.

New MMP inhibitor radiotracers **1a–f** were synthesized as shown in Schemes 1 and 2.

The commercially available starting material amino acid D-valine (**2**) was converted into its ester (**3**). The ester (**3**) was coupled with various sulfonyl chlorides to give the corresponding sulfonylamino esters (**4**) and (**7**). The esters (**4**) and (**7**) were reacted with different picolyl chlorides or benzyl bromide to provide the key intermediates (**5**) and (**8**). Hydroxamic acids were synthesized using two methods (**A** and **B**).[9,13,33] In Method **A**, esters (**5**) were reacted directly with hydroxylamine to produce hydroxamic acids CGS 27023A (**6a**), 2-picolyl-CGS 27023A

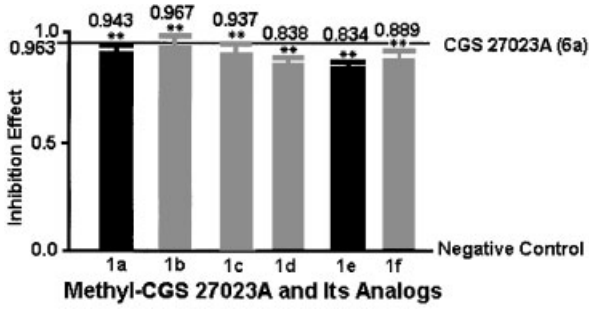
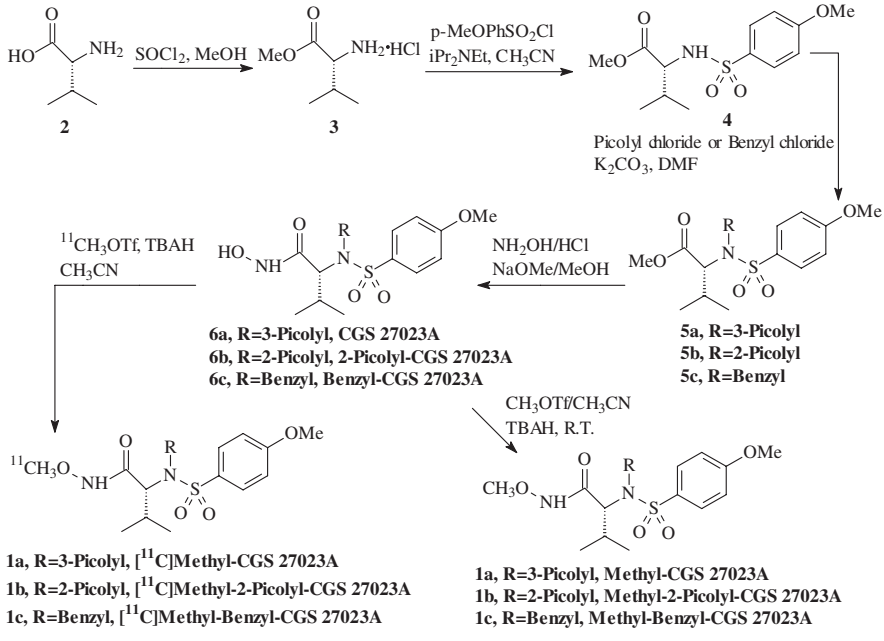


Figure 2. Relative inhibition effects of modified compounds on MMP-1 activity determined by a fibril degradation assay. The inhibition levels are normalized with a negative control, which the distilled water was used to replace tested inhibiting substance CGS 27023A or any modified compound. Inhibition effect = 1-relative activity; relative activity = tested inhibiting substance/negative control; we assumed that relative activity for negative control = 1, then inhibition effect of negative control = 0. The bar on each column indicates standard deviation in three experiments. The asterisk indicates statistically significant difference ($p < 0.01$) from the negative control

(**6b**), and benzyl-CGS 27023A (**6c**). In Method **B**, we adapted a synthetic method under acidic condition, because the nitro compounds (**8**) are unstable under the condition of strong base sodium methoxide (NaOMe); in this method, the conversion of esters (**8**) to hydroxamic acid includes the hydrolysis of esters (**8**) into acids followed by coupling with O-*tert*-butylhydroxylamine hydrochloride in the presence of *N*-[(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotrizole (HOBT) and *N*-methylmorphorine (NMM) to give *t*-butylated hydroxamic acids (**9**), and the deprotection of **9** by hydrogen chloride gas in dichloroethane (DCE) to give hydroxamic acids 2-nitro-CGS 27023A (**10d**), 3-nitro-CGS 27023A (**10e**), and 4-nitro-CGS 27023A (**10f**). The overall chemical yields of hydroxamic acids from D-valine were moderate to excellent.

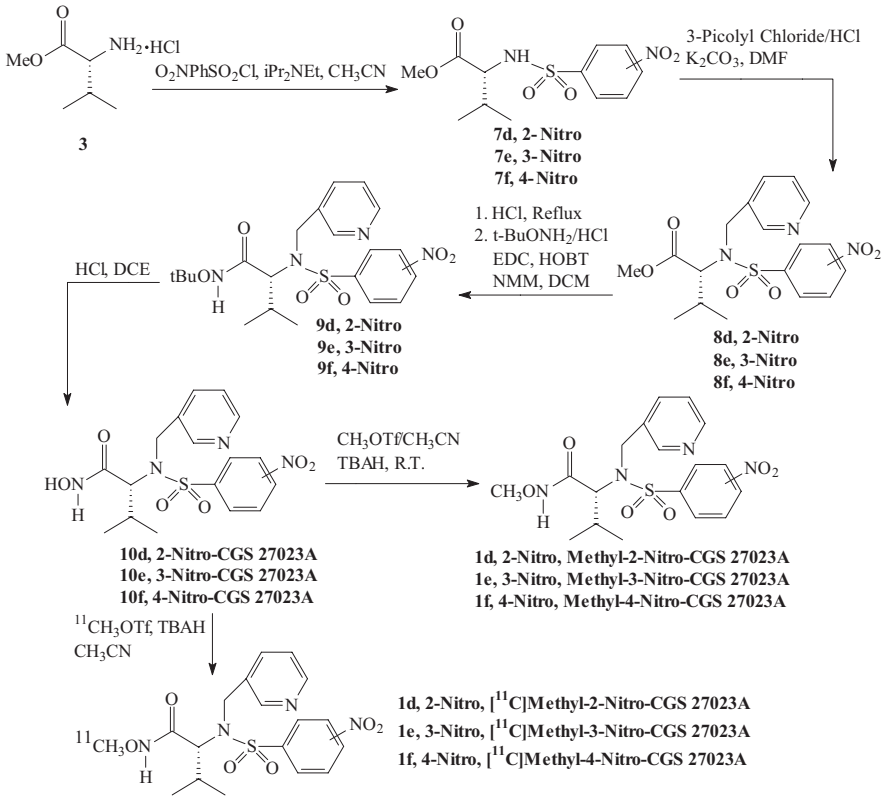
The hydroxamic acids (**6a–c, 10d–f**) were stirred with methyl triflate at room temperature under basic condition to give the standard samples unlabeled **1a–f**. The final products were characterized by analytical data mp, ^{1}HNMR and MS. The alkylation of the hydroxylamine on the nitrogen position is a potential competing reaction, however, the evidence provided by the ^{1}HNMR data (there are δ 3.63–3.72 for $\underline{CH_3}ONH$, and there are not δ ~2–3 for $HON\underline{CH_3}$) of methylated CGS 27023A analogs shows that the compounds are methylated on the oxygen position of the hydroxylamine. These results are consistent with



Scheme 1. Synthesis of [$^{11/12}$C]Methyl-CGS 27023A, [$^{11/12}$C]Methyl-2-Picolyl-CGS 27023A and [$^{11/12}$C]Methyl-Benzyl-CGS 27023A

the theoretical explanation that the deprotonization at the hydroxyl position of the hydroxylamine is easier than at the amine position since the acidity of HO– of the hydroxylamine is greater than the acidity of HN– of the hydroxylamine, and the methylation of the hydroxylamine will prefer to occur at the oxygen position rather than at the nitrogen position. The existence of strong base in methylation reaction of the hydroxylamine may epimerise amides, fortunately the epimerization did not occur during the reaction, which was monitored and confirmed by analytical HPLC method,[34] although tetrabutylammonium hydroxide in acetonitrile is quite forcing.

The stereochemistry of the analogues prepared is important. The starting stereochemistry is defined by the starting material D-valine. Based on the organic reaction mechanism and stereochemistry theory, the racemization of a chiral compound is due to the conversion to its non-chiral analog. In the synthetic approaches shown in Schemes 1 and 2, there is not any formation of non-chiral intermediates, and the chiral center is not destroyed by any synthetic step. Therefore, we can assume that stereochemistry is conserved throughout the synthetic sequences and no racemization occurred during the various steps.



Scheme 2. Synthesis of [$^{11/12}$C]Methyl-2-Nitro-CGS 27023A, [$^{11/12}$C]Methyl-3-Nitro-CGS 27023A and [$^{11/12}$C]Methyl-4-Nitro-CGS 27023A

The hydroxamic acid precursors CGS 27023A (**6a**) and its analogs (**6b–c, 10d–f**) were labeled by [^{11}C]methyl triflate[35] through ^{11}C-O-methylation method[36–38] at aminohydroxyl position under basic conditions and isolated by solid-phase extraction (SPE) purification[36] to produce pure target compounds in 40–60% radiochemical yields (based on $^{11}CO_2$, decay corrected to end of bombardment), in 20–25 min synthesis time. The large polarity difference between the hydroxamic acid precursor and the labeled methylated product permitted the use of SPE technique for purification of radioligands from radiolabeling reaction mixture. The reaction mixture was diluted with $NaHCO_3$ and loaded onto C-18 cartridge by gas pressure. The cartridge column was washed with water to remove unreacted acid precursor and reaction solvent, then final labeled product was eluted with ethanol. Chemical purity, radiochemical purity, and specific

radioactivity were determined by analytical HPLC methods. The chemical purity of precursors **6a–c**, **10d–f**, and standard samples **1a–f** was >95%. The radiochemical purity of target radiotracers **1a–f** was >99%. The chemical purities of target radiotracers **1a–f** were ~95% (**1a**), ~98% (**1b**), ~98% (**1c**), ~95% (**1d**), ~92% (**1e**), and ~93% (**1f**). The average ($n = 5-10$) specific activity of target radiotracers **1a–f** was 0.6–0.8 Ci/μmol at end-of-synthesis (EOS).

Conclusion

We have developed synthetic procedures that provide MMP inhibitor radiotracers **1a** and its analogs **1b–f**. Preliminary findings of biological assay indicate the analogs synthesized have strong inhibitory effectiveness on MMP-1 in comparison with CGS 27023A. The results warrant further evaluation of these radiotracers as new potential PET breast cancer imaging agents *in vivo*.

Experimental

All commercial reagents and solvents were used without further purification unless otherwise specified. The [^{11}C]methyl triflate was made according to a literature procedure[16] by the metathetical reaction of [^{11}C]methyl bromide over a hot column of silver triflate supported on porous graphite beads. One silver triflate column was used for 20–40 [^{11}C]methyl triflate runs before replacement. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal standard TMS (δ 0.0). The low resolution mass spectra were obtained using a Hewlett–Packard Engine mass spectrometer, and the high resolution mass measurements were obtained using a ThermoFinnigan MAT95 mass spectrometer, at Purdue University Campus-wide Mass Spectrometry Center. Chromatographic solvent proportions are expressed on a volume: volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates (5 × 10 cm). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60

(230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 250 mm; 3:1:3 CH_3CN: MeOH: 20 mM, pH 6.7 $KHPO_4^-$ mobile phase, flow rate 1.5 mL/min, and UV (185 nm) and γ-ray (NaI) flow detectors. Semi-prep C-18 silica guard cartridge column 1 × 1 cm was obtained from E. S. Industries, Berlin, NJ, Part number 300121-C18-BD 10 μM. Sterile vented Millex-GS 0.22 μM vented filter unit was obtained from Millipore Corporation, Bedford, MA.

D-valine methyl ester hydrochloride (3)

To a solution of D-valine (**2**) (3.58 g, 30.56 mmol) in MeOH (75 ml) was added thionyl chloride (10 g, 84 mmol) in an ice bath. After the solution was stirred at room temperature (RT) overnight, the solvent was removed under vacuum to give a white solid **3** (4.94 g, 96%). The crude product was used for the next step reaction without further purification.

N-[(4-methoxyphenyl)sulfonyl]-D-valine methyl ester (4)

To a solution of compound **3** (3.26 g, 19.44 mmol) and *p*-$MeOPhSO_2Cl$ (4.61 g, 22.36 mmol) in CH_3CN (100 ml) was added iPr_2NEt (5.0 ml) dropwise in an ice bath. After the resulting solution was stirred at RT for 2 days, the solvent was evaporated. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1/3 EtOAc/Hexane) to give a solid **4** (5.10 g, 83%). TLC R_f 0.45 (1/3 EtOAc/Hexane), mp 91–92°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.86–0.88 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.93–0.96 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.98–2.03 (m, 1H, $(CH_3)_2\underline{CH}$), 3.47 (s, 3H, $CO_2\underline{CH_3}$), 3.69–3.74 (dd, $J = 5.1$, 10.3 Hz, 1H, CHN), 3.86 (s, 3H, $PhO\underline{CH_3}$), 5.12–5.15 (d, $J = 10.3$ Hz, 1H, NH), 6.94–6.97 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.74–7.77 (d, $J = 8.8$ Hz, 2H, H–Ph).

Methyl 2(R)-[[(4-methoxyphenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoate (5a)

A mixture of compound **4** (2.04 g, 6.48 mmol), 3-picolyl chloride/HCl salt (1.27 g, 7.77 mmol) and K_2CO_3 (5.36 g, 38.88 mmol) in dry DMF

(50 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (75 ml × 3). The combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (1/3 EtOAc/Hexane) to give the product **5a** (2.37 g, 93%). TLC R_f 0.50 (EtOAc), mp 63–65°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.76–0.78 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.81–0.84 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.89–1.93 (m, 1H, $(CH_3)_2\underline{CH}$), 3.44 (s, 3H, $CO_2\underline{CH_3}$), 3.86 (s, 3H, $PhO\underline{CH_3}$), 4.14–4.18 (d, $J = 11$ Hz, 1H, CHN), 4.63–4.64 (d, $J = 2.2$ Hz, 2H, $N\underline{CH_2}Py$), 6.90–6.93 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.23–7.27 (m, 1H, H–Py), 7.67–7.70 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.87–7.89 (d, $J = 8.1$ Hz, 1H, H–Py), 8.49–8.54 (m, 2H, H–Py).

Methyl 2(R)-[[(4-methoxyphenyl)sulfonyl](2-picolyl)amino]-3-methyl butanoate (5b)

A mixture of compound **4** (0.58 g, 1.77 mmol), 2-picolyl chloride/HCl salt (0.58 g, 3.54 mmol) and K_2CO_3 (1.46 g, 10.74 mmol) in dry DMF (25 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (75 ml × 3). The combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (1/3 EtOAc/Hexane) to give a thick oil **5b** (0.75 g, 93%), it was converted to be a solid in the freezer. TLC R_f 0.35 (1/2 EtOAc/Hexane), mp 61–62°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.77–0.80 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.84–0.86 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.04–2.11 (m, 1H, $(CH_3)_2\underline{CH}$), 3.44 (s, 3H, CO_2CH_3), 3.86 (s, 3H, $PhO\underline{CH_3}$), 4.11–4.14 (d, $J = 10.3$ Hz, 1H, CHN), 4.69–4.75 (d, $J = 16.9$ Hz, 1H, $N\underline{CH_a}Py$), 4.83–4.88 (d, $J = 17.2$ Hz, 1H, $N\underline{CH_b}Py$), 6.91–6.94 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.10–7.16 (m, 1H, H–Py), 7.65–7.67 (m, 2H, H–Py), 7.73–7.76 (d, $J = 8.8$ Hz, 2H, H–Ph), 8.45–8.46 (d, $J = 4.4$ Hz, 1H, H–Py).

Methyl 2(R)-[[(4-methoxyphenyl)sulfonyl]benzylamino]-3-methylbutanoate (5c)

A mixture of compound **4** (1.08 g, 3.42 mmol), benzyl bromide (0.70 g, 4.11 mmol) and K_2CO_3 (2.85 g, 20.8 mmol) in dry DMF (25 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (100 ml × 3). The

combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (1/4 EtOAc/Hexane) to give a solid **5c** (1.35 g, 97%). TLC R_f 0.65 (1/4 EtOAc/Hexane), mp 65–66°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.76–0.78 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.79–0.81 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.86–1.98 (m, 1H, $(CH_3)_2\underline{CH}$), 3.40 (s, 3H, $CO_2\underline{CH_3}$), 3.86 (s, 3H, $PhO\underline{CH_3}$), 4.13–4.17 (d, 1H, CHN), 4.49–4.54 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_a}Ph$), 4.64–4.70 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_b}Ph$), 6.89–6.92 (d, $J = 8.8$ Hz, 2H, H–PhOMe), 7.23–7.30 (m, 3H, H–Ph), 7.36–7.41 (m, 2H, H–Ph), 7.68–7.71 (d, $J = 8.8$ Hz, 2H, H–PhOMe).

N-hydroxy-2(R)-[[(4-methoxyphenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (CGS 27023A, 6a)

Ester **5a** (0.73 g, 1.94 mmol) was dissolved in anhydrous MeOH (6.0 ml). To this solution was added hydroxylamine hydrochloride (0.65 g, 9.35 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (0.34 g, 14.78 mmol) dissolved in MeOH (5 ml). After the resulting reaction mixture was stirred at RT for 2 days, the solvent was removed under vacuum. The residue was diluted with EtOAc (75 ml), washed with brine, and dried over Na_2SO_4. The solution was evaporated to give the crude product, which was purified by flash chromatography (50/1 EtOAc/MeOH) to give a white solid **6a** (0.34 g, 46%). TLC R_f 0.35 (1/10 MeOH/EtOAc), mp 107–108°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.62–0.64 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.10–2.20 (m, 1H, $(CH_3)_2\underline{CH}$), 3.82 (s, 3H, $PhO\underline{CH_3}$), 3.83–3.86 (d, $J = 10.4$ Hz, 1H, CHN), 4.55–4.61 (d, $J = 15.1$ Hz, 1H, $N\underline{CH_a}Py$), 4.66–4.71 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_b}Py$), 6.84–6.87 (d, $J = 7.8$ Hz, 2H, H–Ph), 7.19–7.25 (m, 1H, H–Py), 7.55–7.58 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.76–7.78 (d, $J = 7.4$ Hz, 1H, H–Py), 8.38–8.39 (d, $J = 3.7$ Hz, 1H, H–Py), 8.49 (s, 1H, H–Py). MS (DCI, CH_4) m/z 394 $(M+H)^+$. CGS 27023A hydrochloride, mp 168–170°C.

N-hydroxy-2(R)-[[(4-methoxyphenyl)sulfonyl](2-picolyl)amino]-3-methylbutanamide (2-picolyl-CGS 27023A, 6b)

Ester **5b** (0.49 g, 1.25 mmol) was dissolved in anhydrous MeOH (5.0 ml). To this solution was added hydroxylamine hydrochloride (0.52 g, 7.50 mmol), followed by the addition of sodium methoxide, freshly

prepared from sodium (0.20 g, 8.79 mmol) dissolved in MeOH (5 ml). After the resulting reaction mixture was stirred at RT for 2 days, the solvent was removed under vacuum. The residue was diluted with EtOAc (75 ml), washed with brine, and dried over Na_2SO_4. The solution was evaporated to give the crude product, which was purified by flash chromatography (100/1 EtOAc/MeOH) to give a white solid **6b** (0.15 g, 30%). TLC R_f 0.44 (1/20 MeOH/EtOAc), mp 103–104°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.68–0.70 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.78–0.81 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.96–2.02 (m, 1H, $(CH_3)_2\underline{CH}$), 3.84 (s, 3H, $PhO\underline{CH_3}$), 3.85–3.89 (d, $J = 10.1$ Hz, 1H, CHN), 4.68–4.74 (d, $J = 16.9$ Hz, 1H, $N\underline{CH_a}Py$), 4.82–4.87 (d, $J = 16.9$ Hz, 1H, $N\underline{CH_b}Py$), 6.88–6.91 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.10–7.12 (m, 1H, H–Py), 7.65–7.67 (m, 2H, H–Py), 7.73–7.76 (d, $J = 8.8$ Hz, 2H, H–Ph), 8.45–8.47 (d, $J = 4.4$ Hz, 1H, H–Py).

N-hydroxy-2(R)-[[(4-methoxyphenyl)sulfonyl]benzylamino]-3-methylbutanamide (benzyl-CGS 27023A, 6c)

Ester **5c** (0.53 g, 1.41 mmol) was dissolved in anhydrous MeOH (5.0 ml). To this solution was added hydroxylamine hydrochloride (0.63 g, 9.13 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (0.24 g, 10.46 mmol) dissolved in MeOH (5 ml). After the resulting reaction mixture was stirred at RT for 2 days, the solvent was removed under vacuum. The residue was diluted with EtOAc (75 ml), washed with brine, and dried over Na_2SO_4. The solution was evaporated to give the crude product, which was purified by flash chromatography (EtOAc) to give a white solid **6c** (0.13 g, 24%). TLC R_f 0.45 (1/50 MeOH/EtOAc), mp 110–112°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.47–0.49 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.80–0.82 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.12–2.20 (m, 1H, $(CH_3)_2\underline{CH}$), 3.69–3.72 (d, $J = 10.3$ Hz, 1H. CHN), 3.85 (s, 3H, $PhO\underline{CH_3}$), 4.45–4.50 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_a}Ph$), 4.63–4.68 (d, $J = 15.5$ Hz, 1H, $N\underline{CH_b}Ph$), 6.88–6.90 (d, $J = 8.2$ Hz, 2H, $\underline{H–Ph}OMe$), 7.26–7.32 (m, 3H, H–Ph), 7.35–7.38 (m, 2H, H–Ph), 7.59–7.62 (d, $J = 8.8$ Hz, 2H, $\underline{H–Ph}OMe$).

N-[(2-nitrophenyl)sulfonyl]-D-valine methyl ester (7d)

To a solution of compound **3** (1.29 g, 7.71 mmol) and 2-nitrophenyl sulfonyl chloride (2.20 g, 9.93 mmol) in CH_3CN (75 ml) was added

iPr_2NEt (5.0 ml) dropwise in an ice bath. After the resulting solution was stirred at RT for 2 days, the solvent was evaporated. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1/1 EtOAc/Hexane) to produce a yellow solid **7d** (2.10 g, 86%). TLC R_f 0.48 (1/10, MeOH/Hexane), mp 81–82°C. ^1H NMR (300 MHz, $CDCl_3$): 0.86–0.88 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.93–0.95 (d, $J = 6.6$ Hz. 3H, $\underline{CH_3}CH$), 2.00–2.10 (m, 1H, $(CH_3)_2\underline{CH}$), 3.52 (s, 3H, CO_2CH_3), 3.70–3.75 (dd, $J = 5.0$, 10.0 Hz, 1H, CHN), 5.13–5.15 (d, $J = 10.0$ Hz, 1H, NH), 7.40–7.48 (m, 2H), 7.57–7.58 (m, 1H), 8.43 (s, 1H).

N-[(3-nitrophenyl)sulfonyl]-D-valine methyl ester (7e)

To a solution of compound **3** (1.23 g, 7.31 mmol) and 3-nitrophenyl sulfonyl chloride (2.43 g, 10.97 mmol) in CH_3CN (75 ml) was added iPr_2NEt (5.0 ml) dropwise in an ice bath. After the resulting solution was stirred at RT for 2 days, the solvent was evaporated. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1/1 EtOAc/Hexane) to produce a yellow solid **7e** (2.17 g, 94%). TLC R_f 0.52 (1/2, EtOAc/Hexane), mp 86–88°C. ^1H NMR (300 MHz, $CDCl_3$): 0.84–0.86 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.90–0.99 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.02–2.12 (m, 1H, $(CH_3)_2\underline{CH}$), 3.52 (s, 3H, CO_2CH_3), 3.81–3.86 (dd, $J = 4.8$, 10.1 Hz, 1H, CHN), 5.28–5.32 (d, $J = 10.2$ Hz, 1H, NH), 7.56–7.62 (t, $J = 8.1$ Hz, 1H), 7.72–7.74 (d, $J = 7.4$ Hz, 1H), 8.33–8.37 (dd, $J = 1.8, 8.1$ Hz, 1H), 8.42 (s, 1H).

N-[(4-Nitrophenyl)sulfonyl]-D-valine methyl ester (7f)

To a solution of compound **3** (1.39 g, 8.27 mmol) and 4-nitrophenyl sulfonyl chloride (2.75 g, 12.41 mmol) in CH_3CN (75 ml) was added iPr_2NEt (5.0 ml) dropwise in an ice bath. After the resulting solution was stirred at RT for 2 days, the solvent was evaporated. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1/2 EtOAc/Hexane) to give a yellow solid **7f** (2.58 g, 98%). TLC R_f 0.45 (1/3 EtOAc/Hexane), mp 95–97°C. ^1H NMR (300 MHz, $CDCl_3$): 0.87–0.89 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.97–0.99 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.07–2.14 (m, 1H, $(CH_3)_2\underline{CH}$),

3.52 (s, 3H, CO_2CH_3), 3.82–3.87 (dd, $J = 4.8$, 10.1 Hz, 1H, CHN), 5.28–5.31 (d, $J = 10.3$ Hz, 1H, NH), 8.02–8.05 (d, $J = 8.8$ Hz, 2H, H-Ar), 8.34–8.36 (d, $J = 8.8$ Hz, 2H, H-Ar).

Methyl 2(R)-[[(2-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoate (8d)

A mixture of compound **7d** (2.18 g, 6.90 mmol), 3-picolyl chloride/HCl salt (2.26 g, 13.80 mmol) and K_2CO_3 (5.70 g, 41.40 mmol) in dry DMF (30 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (100 ml × 3). The combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (2/1 EtOAc/Hexane) to give a yellow solid **8d** (2.45 g, 87%). TLC R_f 0.55 (EtOAc), mp 91–93°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.88–0.90 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.93–0.95 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.11–2.18 (m, 1H, $(CH_3)_2\underline{CH}$), 3.58 (s, 3H, CO_2CH_3), 4.29–4.33 (d, $J = 10.3$ Hz, 1H, CHN), 4.60–4.65 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_a}Py$), 4.91–4.96 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_b}Py$), 7.09–7.11 (m, 1H), 7.47–7.50 (m, 1H), 7.60–7.62 (m, 2H), 7.68–7.71 (d, $J = 8.1$ Hz, 1H), 7.78–7.81 (d, $J = 7.4$ Hz, 1H), 8.42–8.43 (d, $J = 4.4$ Hz, 1H), 8.56 (s, 1H).

Methyl 2(R)-[[(3-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoate (8e)

A mixture of compound **7e** (1.22 g, 3.88 mmol), 3-picolyl chloride/HCl salt (1.27 g, 7.75 mmol) and K_2CO_3 (2.97 g, 21.52 mmol) in dry DMF (25 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (100 ml × 3). The combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (2/1 EtOAc/Hexane) to give a solid **8e** (1.35 g, 77%). TLC R_f 0.54 (2/1, EtOAc/Hexane), mp 94–95°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.91–0.93 (d, $J = 6.6$ Hz, 6H, $(\underline{CH_3})_2CH$), 2.05–2.10 (m, 1H, $(CH_3)_2\underline{CH}$), 3.57 (s, 3H, CO_2CH_3), 4.34–4.37 (d, $J = 10.3$ Hz, 1H, CHN), 4.52–4.57 (d, $J = 15.4$ Hz, 1H, $N\underline{CH_a}Py$), 4.65–4.71 (d, $J = 16.2$ Hz, 1H, $N\underline{CH_b}Py$), 7.16–7.20 (m, 1H), 7.56–7.62 (t, $J = 8.1$ Hz, 1H), 7.72–7.74 (d, $J = 7.4$ Hz, 1H), 7.91–7.94 (d, $J = 8.1$ Hz, 1H), 8.33–8.36 (dd, $J = 1.5$, 8.1 Hz, 1H), 8.43–8.46 (m, 3H).

Methyl 2(R)-[[(4-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoate (8f)

A mixture of compound **7f** (0.56 g, 1.76 mmol), 3-picolyl chloride/HCl salt (0.43 g, 2.64 mmol) and K_2CO_3 (1.46 g, 10.56 mmol) in dry DMF (25 ml) was stirred at RT under nitrogen overnight. The reaction mixture was diluted with H_2O (75 ml) and extracted with Et_2O (100 ml × 3). The combined organic phase was dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash chromatography (1/1 EtOAc/Hexane) to give a solid **8f** (0.69 g, 82%). TLC R_f 0.55 (1/2, EtOAc/Hexane), mp 97–99°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.86–0.88 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.88–0.90 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.00–2.08 (m, 1H, $(CH_3)_2\underline{CH}$), 3.52 (s, 3H, CO_2CH_3), 4.25–4.29 (d, $J = 11.0$ Hz, 1H, CHN), 4.54–4.60 (d, $J = 6.2$ Hz, 1H, $N\underline{CH_a}Py$), 4.67–4.72 (d, $J = 6.2$ Hz, 1H, $N\underline{CH_b}Py$), 7.17–7.22 (dd, $J = 5.1$, 8.2 Hz, 1H, H–Py), 7.73–7.76 (d, $J = 8.1$ Hz, 1H, H–Py), 7.81–7.84 (d, $J = 8.8$ Hz, 2H, H-Ar), 8.23–8.26 (d, $J = 8.8$ Hz, 2H, H-Ar), 8.50 (bs, 2H, H–Py).

N-(tert-butyloxy)-2(R)-[[(2-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (9d)

A solution of ester **8d** (0.79 g, 2.03 mmol) in concentrated HCl (5.0 ml) was refluxed for 8 h. The solvent was removed by vacuum and the residue was dissolved in CH_2Cl_2 (10 ml). Into this solution were added EDC (0.43 g, 2.23 mmol), HOBT (0.36 g, 2.67 mmol) and NMM (1.0 ml). After this solution was stirred at RT for 1 h, $tBuONH_2$/HCl (0.38 g, 3.00 mmol) was added. The resulting reaction solution was stirred at RT for 1 day. The solvent was removed by evaporation. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. Evaporation gave the crude product, which was purified by flash chromatography (EtOAc) to give a white solid **9d** (0.78 g, 83%). TLC R_f 0.45 (EtOAc), mp 70–71°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.56–0.59 (d, $J = 5.9$ Hz, 3H, $\underline{CH_3}CH$), 0.87–0.89 (d, $J = 5.9$ Hz, 3H, $\underline{CH_3}CH$), 1.26 (s, 9H, tBu), 2.10–2.13 (m, 1H, $(CH_3)_2\underline{CH}$), 3.70–3.74 (d, $J = 11.0$ Hz, 1H, CHN), 6.62 (bs, 2H, $N\underline{CH_2}Py$), 6.86–6.89 (d, $J = 8.8$ Hz, 1H), 7.16–7.20 (m, 2H), 7.54–7.57 (d, $J = 8.1$ Hz, 1H), 7.73–7.75 (d, $J = 7.6$ Hz, 1H), 8.37 (s, 1H), 8.46–8.47 (d, $J = 3.0$ Hz, 1H), 8.55 (s, 1H).

N-(tert-butyloxy)-2(R)-[[(3-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (9e)

A solution of ester **8e** (0.48 g, 1.23 mmol) in concentrated HCl (5.0 ml) was refluxed for 8 h. The solvent was removed by vacuum and the residue was dissolved in CH_2Cl_2 (10 ml). Into this solution were added EDC (0.26 g, 1.35 mmol), HOBT (0.20 g, 1.48 mmol) and NMM (1.0 ml). After this solution was stirred at RT for 1 h, $tBuNH_2$/HCl (0.20 g, 1.60 mmol) was added. The resulting reaction solution was stirred at RT for 1 day. The solvent was removed by evaporation. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. Evaporation gave the crude product, which was purified by flash chromatography (EtOAc) to give a white solid **9e** (0.48 g, 84%). TLC R_f 0.56 (EtOAc), mp 77–79°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.72–0.74 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.96–0.98 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.38 (s, 9H, tBu), 2.20–2.31 (m, 1H, $(CH_3)_2\underline{CH}$), 3.85–3.89 (d, $J = 11.1$ Hz, 1H, CHN), 4.69 (bs, 2H, $N\underline{CH_2}Py$), 7.16–7.18 (m, 1H), 7.55–7.68 (t, $J = 8.2$ Hz, 1H), 7.72–7.77 (t, $J = 8.1$ Hz, 1H), 8.30–8.32 (m, 2H), 8.37 (s, 1H), 8.44–8.46 (m, 1H), 8.50 (s, 1H).

N-(tert-butyloxy)-2(R)-[[(4-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (9f)

A solution of ester **8f** (0.58 g, 1.44 mmol) in concentrated HCl (5 ml) was refluxed for 8 h. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 (10 ml). Into this solution were added EDC (0.30 g, 1.58 mmol), HOBT (0.25 g, 1.87 mmol) and NMM (1.0 ml). After this solution was stirred at RT for 1 h, $tBuONH_2$/HCl (0.36 g, 2.88 mmol) was added. The resulting reaction solution was stirred at RT for 1 day. The solvent was removed by evaporation. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. Evaporation gave the crude product, which was purified by flash chromatography (EtOAc) to give a white solid product **9f** (0.65 g, 94%). TLC R_f 0.42 (1/1 EtOAc/Hexane), mp 88–89°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.73–0.75 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.93–0.95 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 1.26 (s, 9H, tBu), 2.27–2.33 (m, 1H, $(CH_3)_2\underline{CH}$), 3.86–3.90 (d, $J = 11.0$ Hz, 1H, CHN), 4.73 (s, 2H, $N\underline{CH_2}Py$), 7.12–7.19 (m, 1H, H–Py), 7.70–7.72 (d, $J = 8.1$ Hz, 2H, H-Ar), 8.15–8.18 (d, $J = 8.1$ Hz, 2H, H-Ar), 8.47–8.48 (d, $J = 3.6$ Hz, 1H, H–Py), 8.58 (s, 1H, H–Py), 8.91 (s, 1H, H–Py).

N-hydroxy-2(R)-[[(2-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (2-nitro-CGS 27023A, 10d)

The protected hydroxamic acid **9d** (0.60 g, 1.29 mmol) was dissolved in 1,2-dichloroethane (DCE) (20 ml) and EtOH (0.06 g, 1.29 mmol) in a flask. HCl gas was bubbled through the flask for 0.5 h. The reaction flask was sealed and stirred at RT for 3 days. The solvent was reduced to 1/3 volume by evaporation and diluted with ether. The mixture was filtered, and the solid was collected, and dried under vacuum to provide a white solid hydrochloride salt of **10d** (0.51 g, 89%). TLC R_f 0.35 (1/10, MeOH/EtOAc), mp 150–151°C. It was converted to a free base **10d** by Et_3N. ^1H NMR (300 MHz, $CDCl_3$): 0.82–0.84 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.92–0.94 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.20–2.36 (m, 1H, $(CH_3)_2\underline{CH}$), 3.92–3.96 (d, $J = 11.0$ Hz, 1H, CHN), 4.73–4.79 (d, $J = 15.0$ Hz, 1H, $N\underline{CH_a}Py$), 4.92–4.99 (d, $J = 15.0$ Hz, 1H, $N\underline{CH_b}Py$), 7.02–7.10 (m, 1H), 7.39–7.48 (m, 1H), 7.52–7.62 (m, 3H), 7.65–7.67 (bs, 1H), 8.31–8.32 (bs, 1H), 8.51 (s, 1H). HRMS calcd for $C_{17}H_{20}N_4O_6S$ 408.1104, found 408.1099.

N-hydroxy-2(R)-[[(3-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (3-nitro-CGS 27023A, 10e)

The protected hydroxamic acid **9e** (0.39 g, 0.85 mmol) was dissolved in 1,2-dichloroethane (DCE) (20 ml) and EtOH (0.04 g, 0.85 mmol) in a flask. HCl gas was bubbled through the flask for 0.5 h. The reaction flask was sealed and stirred at RT for 3 days. The solvent was reduced to 1/3 volume by evaporation and diluted with ether. The mixture was filtered, and the solid was collected, and dried under vacuum to provide a white solid hydrochloride salt of **10e** (0.35 g, 97%). TLC R_f 0.45 (1/10, MeOH/EtOAc), mp 154–156°C. It was converted to a free base **10e** by Et_3N. ^1H NMR (300 MHz, $CDCl_3$): 0.77–0.79 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.82–0.84 (d, $J = 6.6$ Hz, $\underline{CH_3}CH$), 2.22–2.33 (m, 1H, $(CH_3)_2\underline{CH}$), 3.85–3.88 (d, $J = 11.0$ Hz, 1H, CHN), 4.73 (bs, 2H, $N\underline{CH_2}Py$), 7.17–7.20 (m, 1H), 7.50–7.55 (m, 1H), 7.77–7.79 (m, 1H), 7.84–7.86 (m, 1H), 8.22–8.23 (m, 1H), 8.34–8.47 (m, 3H). HRMS calcd for $C_{17}H_{20}N_4O_6S$ 408.1104, found 408.1102.

N-hydroxy-2(R)-[[(4-nitrophenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide (4-Nitro-CGS 27023A, 10f)

The protected hydroxamic acid **9f** (0.61 g, 1.30 mmol) was dissolved in 1,2-dichloroethane (DCE) (30 ml) and EtOH (0.06 g, 1.30 mmol) in a

flask. HCl gas was bubbled through the flask for 0.5 h. The reaction flask was sealed and stirred at RT for 3 days. The solvent was reduced to 1/3 volume by evaporation and diluted with ether. The mixture was filtered, and the solid was collected, and dried under vacuum to provide a white solid hydrochloride salt of **10f** (0.57 g, 99%). TLC R_f 0.36 (1/10 MeOH/EtOAc), mp 151–153°C. It was converted to a free base **10f** by Et_3N. ^1H NMR (300 MHz, $CDCl_3$): 0.56–0.58 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.79–0.81 (d, $J = 6.6$ Hz Hz, 3H, $\underline{CH_3}CH$), 1.94–2.02 (m, 1H, $(CH_3)_2\underline{CH}$), 3.75–3.77 (d, $J = 10.3$ Hz, 1H, CHN), 4.57–4.62 (d, $J = 16.2$ Hz, 1H, $N\underline{CH_a}Py$), 4.72–4.77 (d, $J = 16.2$ Hz, 1H, $N\underline{CH_b}Py$), 7.13–7.27 (m, 1H, H–Py), 7.71–7.33 (d, $J = 8.1$ Hz, 2H, H–Ph), 8.15–8.18 (d, $J = 8.8$ Hz, 2H, H–Ph), 8.46–8.47 (d, $J = 3.6$ Hz, 1H, H–Py), 8.59 (s, 1H, H–Py), 8.90 (s, 1H, H–Py). HRMS calcd for $C_{17}H_{20}N_4O_6S$ 408.1104, found 408.1098.

Typical experimental procedure for the synthesis of the standard samples unlabeled methyl-CGS 27023A (1a), methyl-2-picolyl-CGS 27023A (1b), methyl-benzyl-CGS 27023A (1c), methyl-2-nitro-CGS 27023A (1d), methyl-3-nitro-CGS 27023A (1e), and methyl-4-nitro-CGS 27023A (1f)

Hydroxamic acid precursor **6–10e**, or **10f** (0.1–0.2 mmol) was dissolved in CH_3CN (5–10 ml). To this solution was added tetrabutylammonium hydroxide (TBAH) (0.3–1.0 ml, 1 M solution in methanol) and methyl triflate (0.4–0.6 mmol). The mixture was stirred at RT under nitrogen overnight. The solvent was evaporated. The residue was diluted with EtOAc, washed with brine and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (EtOAc) to give the solid unlabeled target compounds **1a-f**. The yields of **1a–f** were 60–85%. ^1H NMR (300 MHz, $CDCl_3$): **1a**, 0.60–0.62 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.86–0.88 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.15–2.22 (m, 1H, $(CH_3)_2\underline{CH}$), 3.65 (s, 3H, $\underline{CH_3}ONH$), 3.74–3.77 (d, $J = 10.3$ Hz, 1H, CHN), 3.84 (s, 3H, $PhO\underline{CH_3}$), 4.57–4.62 (d, $J = 16.2$ Hz, 1H, $N\underline{CH_a}Py$), 4.72–4.77 (d, $J = 15.5$ Hz, 1H, $N\underline{CH_b}Py$), 6.84–6.87 (d, 2H, $J = 9.8$ Hz, H–Ph), 7.17–7.21 (m, 1H, H–Py), 7.57–7.60 (d, $J = 9.6$ Hz, 2H, H–Ph), 7.77–7.80 (d, $J = 9.1$ Hz, 1H, H–Py), 8.46–8.48 (d, $J = 5.2$ Hz, 1H, H–Py), 8.61 (s, 1H, H–Py). HRMS calcd for $C_{19}H_{26}N_3O_5S\ (M+H)^+$ 408.1593, found 408.1572. **1b**. 0.57–0.60 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 0.84–0.86 (d, $J = 6.6$ Hz, 3H, $\underline{CH_3}CH$), 2.10–2.20 (m, 1H, $(CH_3)_2\underline{CH}$), 3.63 (s, 3H, $\underline{CH_3}ONH$), 3.77–3.80 (d, $J = 11.0$ Hz, 1H, CHN), 3.85 (s, 3H, $PhO\underline{CH_3}$), 4.55–4.60

(d, $J = 16.2$ Hz, 1H, NCH$_a$Py), 4.70–4.75 (d, $J = 16.2$ Hz, 1H, NCH$_b$Py), 6.84–6.87 (d, $J = 8.8$ Hz, 2H, H–Ph), 7.12–7.17 (m, 1H, H–Py), 7.63–7.67 (m, 2H, H–Py), 7.73–7.76 (d, $J = 8.6$ Hz, 2H, H–Ph), 8.45–8.47 (d, $J = 4.4$ Hz, 1H, H–Py). HRMS calcd for $C_{19}H_{26}N_3O_5S$ $(M+H)^+$ 408.1593, found 408.1104. **1c**, 0.58–0.60 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 0.87–0.89 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 2.10–2.20 (m, 1H, (CH$_3$)$_2$CH), 3.63 (s, 3H, CH$_3$ONH), 3.74–3.77 (d, $J = 11.0$ Hz, 1H, CHN), 3.84 (s, 3H, PhOCH$_3$), 4.58–4.63 (d, $J = 15.4$ Hz, 1H, NCH$_a$Ph), 4.64–4.68 (d, $J = 15.6$ Hz, 1H, NCH$_b$Ph), 6.87–6.90 (d, $J = 8.8$ Hz, 2H, H–PhOMe), 7.22–7.27 (m, 3H, H–Ph), 7.36–7.41 (m, 2H, H–Ph), 7.68–7.71 (d, $J = 8.7$ Hz, 2H, H–PhOMe). HRMS calcd for $C_{20}H_{27}N_2O_5S$ $(M+H)^+$ 407.1641, found 407.1621. **1d**, 0.87–0.89 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 0.96–0.98 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 2.25–2.30 (m, 1H, (CH$_3$)$_2$CH), 3.69 (s, 3H, CH$_3$ONH), 3.88–3.92 (d, $J = 10.3$ Hz, 1H, CHN), 4.76–4.81 (d, $J = 15.4$ Hz, 1H, NCH$_a$Py), 4.96–5.01 (d, $J = 15.4$ Hz, 1H. NCH$_b$Py), 7.05–7.09 (m, 1H), 7.40–7.46 (m, 2H), 7.58–7.78 (m, 1H), 7.65–7.68 (d, $J = 7.4$ Hz, 1H), 8.39–8.41 (d, $J = 3.7$ Hz, 1H), 8.56 (s, 1H), 8.93 (s, 1H). HRMS calcd for $C_{18}H_{23}N_4O_6S$ $(M+H)^+$ 423.1338, found 423.1320. **1e**, 0.76–0.78 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 0.95–0.97 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 2.28–2.32 (m, 1H, (CH$_3$)$_2$CH), 3.72 (s, 3H, CH$_3$ONH), 3.89–3.92 (d, $J = 10.1$ Hz, 1H, CHN), 4.73 (s, 2H, NCH$_2$Py), 7.16–7.18 (m, 1H), 7.52–7.58 (t, $J = 8.1$ Hz, 1H), 7.71–7.74 (d, $J = 7.3$ Hz, 1H), 7.81–7.83 (d, $J = 7.3$ Hz, 1H), 8.29–8.32 (d, $J = 8.8$ Hz, 1H), 8.36 (s, 1H), 8.44 (s, 1H), 8.53 (s, 1H). HRMS calcd for $C_{18}H_{23}N_4O_6S$ $(M+H)^+$ 423.1338, found 423.1326. **1f**, 0.72–0.74 (d, $J = 6.0$ Hz, 3H, CH$_3$CH), 0.93–0.95 (d, $J = 6.6$ Hz, 3H, CH$_3$CH), 1.98–2.10 (m, 1H, (CH$_3$)$_2$CH), 3.72 (s, 3H, CH$_3$ONH), 3.85–3.89 (d, $J = 11.1$ Hz, 1H, CHN), 4.73–4.75 (d, $J = 3.7$ Hz, 2H, NCH$_2$Py), 7.14–7.18 (m, 1H, H–Py), 7.71–7.73 (d, $J = 8.1$ Hz, 2H, H–Ph), 8.15–8.18 (d, $J = 8.8$ Hz, 2H, H–Ph), 8.46–8.48 (d, $J = 3.7$ Hz, 1H, H–Py), 8.59 (s, 1H, H–Py), 8.90 (s, 1H, H–Py). HRMS calcd for $C_{18}H_{22}N_4O_6S$ 422.1260, found 422.1246.

Typical experimental procedure for the radiosynthesis of [^{11}C]methyl-CGS 27023A (1a), [^{11}C]methyl-2-picolyl-CGS 27023A (1b), [^{11}C]methyl-benzyl-CGS 27023A (1c), [^{11}C]methyl-2-nitro-CGS 27023A (1d), [^{11}C]methyl-3-nitro-CGS 27023A (1e), and [^{11}C]methyl-4-nitro-CGS 27023A (1f).

Hydroxamic acid precursor **6a–c**, **10d**, **10e**, or **10f** (0.6–1.0 mg) was dissolved in CH_3CN (300 µl). To this solution was added tetrabutyl-

ammonium hydroxide (TBAH) (2–3 μl, 1 M solution in methanol). The mixture was transferred to a small volume, three-neck reaction tube. [^{11}C]methyl triflate was passed into the air-cooled reaction tube at −15 to −20°C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until activity reached a maximum (~3 min), then the reaction tube was heated at 70–80°C for 3 min. The contents of the reaction tube were diluted with 0.1 M $NaHCO_3$ (1 ml). This solution was passed onto a C-18 cartridge by gas pressure. The cartridge was washed with 2 × 3 ml H_2O, and the aqueous washing was discarded. The product was eluted from the column with 2 × 3 ml EtOH, then passed onto a rotary evaporator. The solvent was removed by evaporation under high vacuum. The labeled product **1a–f** was formulated with 50 mM NaH_2PO_4, whose volume was dependent upon the use of the labeled product **1a–f** in tissue biodistribution studies (~6 ml, 3 × 2 ml) or in micro-PET imaging studies (1–3 ml) of the breast cancer athymic mice, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis time was ~20 min. The decay corrected yield, from $^{11}CO_2$, was 40–60%, and the radiochemical purity was >99% by analytical HPLC. Retention times in the analytical HPLC system were: RT**6a** = 2.90 min, RT**6b** = 3.18 min, RT**6c** = 6.13 min, RT **10d** = 2.99 min, RT**10e** = 3.21 min, RT**10f** = 3.20 min; RT**1a** = 3.72 min, RT**1b** = 4.40 min, RT**1c** = 9.56 min, RT**1d** = 3.54 min, RT**1e** = 4.24 min, RT**1f** = 4.06 min.

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References

1. Brown PD, *Breast Cancer Res Treat* 1998; **52**: 125–136.

2. Greenwald RA, Zucker S, Golub LM, (ed.). Inhibition of matrix metalloproteinases, therapeutic applications. *Ann NY Acad Sci* 1999; **878**: 1–761.
3. Hahnel E, Harvey JM, Joyce R, Robbins PD, Sterrett GF, Hahnel R. *Int J Cancer* 1993; **55**: 771–774.
4. Whittaker M, Floyd CD, Brown P, Gearing AJH. *Chem Rev* 1999; **99**: 2735–2776.
5. Hagmann WK, Lark MW, Becker JW. *Annual Report in Medical Chemistry* Bristol JA (ed.), vol 31. Academic Press: New York, 1996; 231–240.
6. Summers JB, Davidsen SK. *Annual Report in Medical Chemistry*. Bristol JA. (ed.), vol 33. Academic Press: New York, 1998; 131–140.
7. Rasmussen HS, McCann PP. *Pharmacol Ther* 1997; **75**: 69–75.
8. Steward WP. *Cancer Chemother Pharmacol* 1999; **43** (Suppl): S56–S60.
9. MacPherson LJ, Bayburt EK, Capparelli MP, *et al. J Med Chem* 1997; **40**: 2525–2532.
10. Afzal S, Lalani el-N, Foulkes WD, *et al. Lab Invest* 1996; **74**(2): 406–421.
11. Price A, Shi Q, Morris D, *et al. Clin Cancer Res* 1999; **5**(4): 845–854.
12. Zheng Q-H., Hutchins GD, Mock BH, Winkle WL *J. Label Compd Radiopharm* 2001; **44 (**Suppl 1**)**: S104-S106.
13. Natchus MG, Bookland RG, De B, *et al. J Med Chem* 2000; **43**: 4948–4963.
14. Babine RE, Bender SL. *Chem Rev* 1997; **97**: 1359–1472.
15. Tamura Y, Watanabe F, Nakatani T, *et al. J Med Chem* 1998; **41**: 640–649.
16. Pikul S, Dunham KLM, Almstead NG, *et al. J Med Chem* 1998; **41**: 3568–3571.
17. Pikul S, Dunham KLM, Almstead NG, *et al. J Med Chem* 1999; **42**: 87–94.
18. Kiyama R, Tamura Y, Watanabe F, Tsuzuki H, Ohtani M, Yodo M. *J Med Chem* 1999; **42**: 1273–1278.
19. Almstead NG, Bradley RS, Pikul S, *et al. J Med Chem* 1999; **42**: 4547–4562.
20. Cheng MY, De B., Almstead NG, et al. *J Med Chem* 1999; **42**: 5426–5436.
21. Cheng MY, De B, Pikul S, et al. *J Med Chem* 2000; **43**: 369–380.
22. Robinson RP, Laird ER, Blake JF, *et al. J Med Chem* 2000; **43**: 2293–2296.
23. Scozzafava A, Supuran CT. *J Med Chem* 2000; **43**: 3677–3687.
24. Natchus MG, Bookland RG, De B, *et al. J Med Chem* 2000; **43**: 4948–4963.
25. Brown S, Bernardo MM, Li ZH, *et al. J Am Chem Soc* 2000; **122**: 6799–6780.
26. Natchus MG, Bookland RG, Laufersweiler MJ, *et al. J Med Chem* 2001; **44**: 1060–1071.
27. Pikul S, Ohler NE, Ciszewski G, *et al. J Med Chem* 2001; **44**: 2499–2502.

28. Hanessian S, MacKay DB, Moitessier N. *J Med Chem* 2001; **44**: 3074–3082.
29. Schroder J, Henke A, Wenzel H, *et al*. *J Med Chem* 2001; **44**: 3231–3243.
30. Shuttleworth S. *Advances in Drug Discovery Techniques*, Harvey AL (ed.), Wiley: New York, 1998; p. 115.
31. Whittaker M. *Curr Opin Chem Biol* 1998; **2**: 386–396.
32. Nagase H, Fields CG, Fields GB. *J Biol Chem* 1994; **269**(33): 20952–20957.
33. Jeng AY, Chou M, Parker DT. *Bioorg Med Chem Lett* 1998; **8**: 897–902.
34. Zheng Q-H, Mulholland GK. *Syn Commun* 2000; **30**: 333–340.
35. Mock BH, Mulholland GK, Vavrek MT. *Nucl Med Biol* 1999; **26**(4): 467–471.
36. Zheng Q-H, Mulholland GK. *Nucl Med Biol* 1996; **23**(8): 981–986.
37. Zheng Q-H, Mulholland G.K. *J Label Compd Radiopharm* 1997; **40** (Suppl 1): 551–553.
38. Zheng Q-H, Winkle W, Carlson K, Mulholland GK. *J Label Compd Radiopharm* 1997; **40** (Suppl 1): 548–550.

