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RESEARCH ARTICLE

Synthesis and carbonic anhydrase I, II, IV and XII inhibitory properties of N-protected amino acid – sulfonamide conjugates

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Abstract

N-protected amino acids (Gly, Ala and Phe protected with Boc and Z groups) were reacted with sulfonamide derivatives, leading to the corresponding N-protected amino acid–sulfonamide conjugates. The carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity of the new compounds was assessed against four human (h) isoforms, hCA I, hCA II, hCA IV and hCA XII. Among them, hCA II, IV and XII are antiglaucoma drug targets, being involved in aqueous humor secretion within the eye. Low nanomolar inhibition was measured against all four isoforms with the 20 reported sulfonamides, but no selective inhibitory profiles, except for some CA XII-selective derivatives, were observed. hCA I, II and XII were generally better inhibited by sulfonamides incorporating longer scaffolds and Gly/Ala, whereas the best hCA IV inhibitors were homosulfanilamide derivatives, incorporating Phe moieties. The amino acid–sulfonamide conjugates show good water solubility and effective hCA II, IV and XII inhibition, and may be considered as interesting candidates for antiglaucoma studies.

Keywords

Carbonic anhydrase, glaucoma, inhibitor, N-protected amino acid, sulfonamide

History

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Introduction

There are six different genetic families encoding for the metalloprotein carbonic anhydrase (CA, EC 4.2.1.1), a superfamily of metalloenzymes acting as catalysts for the interconversion between CO_2 and bicarbonate. These are the α-, β-, γ-, δ-, ζ- and η-CA classess[1–11]. The catalytic/inhibition mechanisms for the physiologic and other catalyzed by these enzymes are well understood processes[12–21]. The discovery of new classes of CA inhibitors (CAIs), possessing different inhibition mechanisms compared with the classical inhibitors of the sulfonamide/anion type[1–3,5], has also seen important developments ultimately[13]. At least five different CA inhibition mechanisms were reported so far: (i) the zinc binders are the inhibitors which coordinate to the catalytically crucial Zn(II) ion from the enzyme active site. The metal ion may be in a tetrahedral or trigonal bipyramidal geometries, with the sulfonamides and their isosteres (sulfamides, sulfamates, etc.), most anions, dithiocarbamates and their isosteres, carboxylates and hydroxamates binding in this way[1–3,5,13]; (ii) the inhibitors that anchor to the zinc-coordinated water molecule/hydroxide ion, represented by the phenols, some carboxylates, the polyamines, 2-thioxocoumarins, and sulfocoumarins[1–3,13,14]; (iii) the inhibitors which occlude the entrance to the active site cavity (coumarins and their isosteres), this binding site coinciding with that where CA activators bind[13–20]; (iv) the compounds which bind out of the active site cavity (a carboxylic acid derivative was seen to inhibit CA in this manner)[21], and (v) compounds for which the inhibition mechanism is not known, among which the secondary/tertiary sulfonamides as well as imatinib/nilotinib are the most investigated examples[13,22–26].

The sulfonamides however remain the main class of CAIs with many clinically used drugs as antiglaucoma agents[27–29], diuretics[30], antiobesity drugs[31–33], antiepilpetics[34], and more recently agents for the management of hypoxic tumors[35–38], neuropathic pain[39] and ischaemia[40,41].

Continuing our interest in designing biologically active compounds incorporating N-protected amino acid moieties (the coumarine[19] conjugates as CAIs as well as quinine conjugates were recently reported[42]), we focused on the synthesis of amino acid–sulfonamide conjugates by using the N-(protected α-aminoacyl)benzotriazole methodology[43] and explore the enzyme inhibitory activities of such compounds against physiologically important isoforms, such as the cytosolic human (h) hCAI and II, as well as the membrane-asssociated/transmembrane hCA IV and XII[1–5].

Materials and methods

Chemistry

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich (Milan, Italy), Acros (Milan, Italy) and Merck (Florence, Italy). All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried

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glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; dd, double of doubles. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. All microwave-assisted reactions were carried out in a microwave oven system manufactured by GEM (CEM Corporation, Matthews, NC, USA) under a nitrogen atmosphere. The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stir bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation. Melting points (mp) were measured in open capillary tubes and are uncorrected, using a Gallenkamp MPD350.BM3.5 apparatus. N-protected amino acids[19,43–45]; benzyl (2-1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)carbamate (**I**), *tert*-butyl (2-1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)carbamate (**II**), (*S*)-benzyl (1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)carbamate (**III**), (*S*)-*tert*-butyl (1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (**IV**) and sulfonamide derivatives[46]; 2-(4-sulfamoylphenoxy)ethanaminium 2,2,2-trifluoroacetate, 3-(4-sulfamoylphenoxy)propan-1-aminium chloride, 4-(4-sulfamoylphenoxy)butan-1-aminium 2,2,2-trifluoroacetate used as starting materials were prepared according to literature procedure. Compounds **3**[47], **4**[48], **5**[48] and **10**[47] were found in the literature but their synthesis methods need harsh reaction conditions and cumbersome work-up procedures. Thus, these compounds were synthesized by a new, one-step, easy synthetic method, using N-(protected α-aminoacyl)benzotriazoles in this work.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 1–3

A mixture of equivalent amounts of the appropriate N-protected aminoacylbenzotriazole and 3-aminobenzenesulfonamide was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((3-sulfamoylphenyl)amino)ethyl)carbamate, ***1***

White solid (83%); silica gel TLC $R_f = 0.16$ (MeOH/CH_2Cl_2 5% *v/v*); mp 182–183 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.32 (s, 1H, N*H*), 8.21 (s, 1H, Ar-*H*), 7.79 (d,1H, Ar-*H*, $J = 4.0$ Hz), 7.62 (t, 1H, N*H*, $J = 8.0$ Hz), 7.56 (s, 2H, NH_2), 7.42–7.36 (m, 7H, Ar-*H*), 5.10 (s, 2H, OCH_2Ph), 3.88 (d, 2H, CH_2NH, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.3 (*C*OCH$_2$NH), 157.6 (*C*OOCH$_2$Ph), 145.6, 140.1, 137.9, 130.4, 129.3, 128.8, 128.7, 122.9, 121.3, 117.1, 117.4 (Ar-*C*), 66.5 (OCH$_2$Ph), 45.1 (*C*H$_2$NH). HRMS *m/z* for $C_{16}H_{17}N_3O_5S$ $[M+H]^+$ calcd. 364.1, found 364.0; $[M+NH_4]^+$ calcd. 381.1, found 381.1; $[M+Na]^+$ calcd. 386.1, found 386.0; $[M-H]^-$ calcd. 362.1, found 362.1; $[M+Cl]^-$ calcd. 398.1, found 398.0.

(S)-benzyl (1-oxo-1-((3-sulfamoylphenyl)amino)propan-2-yl) carbamate, ***2***

White solid (76%); silica gel TLC $R_f = 0.20$ (MeCOOEt/Hexane 50% v/v); mp 178–179 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.32 (s, 1H, N*H*), 8.24 (s, 1H, Ar-*H*), 7.79 (d,1H, Ar-*H*, $J = 4.0$ Hz), 7.68 (d, 1H, N*H*, $J = 8.0$ Hz), 7.57 (s, 2H, NH_2), 7.52–7.36 (m, 7H, Ar-*H*), 5.08 (s, 2H, OCH_2Ph), 4.23 (d, 1H, C*H*NH, $J = 8.0$ Hz), 1.35 (d, 3H, CH_3, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.9 (*C*OCH$_2$NH), 156.7 (*C*OOCH$_2$Ph), 145.5, 140.2, 137.9, 130.3, 129.2, 128.7, 128.6, 123.0, 121.2, 117.2 (Ar-*C*), 66.4 (OCH$_2$Ph), 51.8 (*C*HNH), 18.7 (*C*H$_3$). HRMS *m/z* for $C_{17}H_{19}N_3O_5S$ $[M+H]^+$ calcd. 378.1, found 378.0; $[M+NH_4]^+$ calcd. 395.1, found 395.1; $[M+Na]^+$ calcd. 400.1, found 400.0; $[M-H]^-$ calcd. 376.1, found 376.0; $[M+Cl]^-$ calcd. 412.1, found 412.1.

(S)-benzyl (1-oxo-3-phenyl-1-((3-sulfamoylphenyl)amino) propan-2-yl)carbamate, ***3***

White solid (86%); silica gel TLC $R_f = 0.29$ (MeOH/CH_2Cl_2 5% v/v); mp 139–140 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.46 (s, 1H, N*H*), 8.21 (s, 1H, Ar-*H*), 7.80–7.77 (m, 2H, Ar-*H* + N*H*), 7.56 (s, 2H, NH_2), 7.40–7.25 (m, 12H, Ar-*H*), 5.01 (s, 2H, OCH_2Ph), 4.48–4.43 (m, 1H, C*H*NH), 3.11–3.07 (m, 1H, CH_2), 2.93–2.87 (m, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 171.9 (*C*OCH$_2$NH), 156.9 (*C*OOCH$_2$Ph), 145.5, 140.1, 138.6, 137.8, 130.4, 130.1, 129.2, 129.0, 128.7, 128.5, 127.3, 123.1, 121.4, 117.3 (Ar-*C*), 66.3 (OCH$_2$Ph), 57.9 (*C*HNH), 38.2 (*C*H$_2$CH). HRMS *m/z* for $C_{23}H_{23}N_3O_5S$ $[M+H]^+$ calcd. 454.1, found 454.1; $[M+NH_4]^+$ calcd. 471.1, found 471.1; $[M+Na]^+$ calcd. 476.1, found 476.1; $[M-H]^-$ calcd. 452.1, found 452.2; $[M+Cl]^-$ calcd. 488.1, found 488.2.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 4–6

A mixture of equivalent amounts of the appropriate N-protected aminoacylbenzotriazole and 4-aminobenzenesulfonamide was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)carbamate, ***4***

White solid (84%); silica gel TLC $R_f = 0.38$ (MeOH/CH_2Cl_2 5% v/v); mp 186–187 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.34 (s, 1H, N*H*), 7.82–7.78 (m, 4H, Ar-*H*), 7.61 (t, 1H, N*H*, J = 8.0 Hz), 7.42–7.37 (m, 5H, Ar-*H*), 7.27 (s, 2H, NH_2), 5.10 (s, 2H, OCH_2Ph), 3.88 (d, 2H, CH_2NH, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.4 (*C*OCH$_2$NH), 157.5 (*C*OOCH$_2$Ph), 142.7, 139.3, 137.9, 129.2, 128.7, 128.6, 127.6, 119.5 (Ar-*C*), 66.4 (OCH$_2$Ph), 45.1 (*C*H$_2$NH). HRMS *m/z* for $C_{16}H_{17}N_3O_5S$ $[M+H]^+$ calcd. 364.1, found 364.0; $[M+Na]^+$ calcd. 386.1, found 386.0; $[M-H]^-$ calcd. 362.1, found 362.1; $[M+Cl]^-$ calcd. 398.1, found 398.0.

(S)-benzyl (1-oxo-1-((4-sulfamoylphenyl)amino)propan-2-yl) carbamate, ***5***

White solid (77%); silica gel TLC $R_f = 0.25$ (MeCOOEt/Hexane 50% v/v); mp 240–241 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.36 (s, 1H, N*H*), 7.81 (brs, 4H, Ar-*H*), 7.70 (d, 1H, N*H*, J = 8.0 Hz), 7.41–7.40 (m, 5H, Ar-*H*), 7.29 (s, 2H, NH_2), 5.08 (s, 2H, OCH_2Ph), 4.26 (t, 1H, C*H*NH, $J = 8.0$ Hz), 1.35 (d, 3H, CH_3, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 173.1 (*C*OCH$_2$NH), 156.8 (*C*OOCH$_2$Ph), 152.8, 142.8, 139.4, 137.9, 129.3, 128.7, 128.6, 127.6, 119.7, 113.4 (Ar-*C*), 66.4 (OCH$_2$Ph), 51.8 (*C*HNH), 18.8 (*C*H$_3$). HRMS *m/z* for $C_{17}H_{19}N_3O_5S$ $[M+H]^+$ calcd. 378.1, found 378.0; $[M+Na]^+$ calcd. 400.1, found 400.0; $[M-H]^-$ calcd. 376.1, found 376.1; $[M+Cl]^-$ calcd. 412.1, found 412.1.

(S)-benzyl (1-oxo-4-phenyl-1-((3-sulfamoylphenyl)amino) propan-2-yl)carbamate, ***6***

White solid (87%); silica gel TLC $R_f = 0.32$ (MeOH/CH_2Cl_2 5% v/v); mp 216–217 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 10.47 (s, 1H, N*H*), 7.84–7.78 (m, 6H, Ar-*H* + N*H*), 7.38–7.23 (m, 14H, Ar-*H* + NH_2), 5.02 (s, 2H, OCH_2Ph), 4.52–4.47 (m, 1H, C*H*NH), 3.12–3.07 (m, 1H, CH_2), 2.95–2.89 (m, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.0 (*C*OCH$_2$NH), 156.9 (*C*OOCH$_2$Ph), 142.6, 139.5, 138.5, 137.8, 130.1, 129.2, 129.0, 128.7, 128.5, 127.6, 127.3, 119.8 (Ar-*C*), 66.3 (O*C*H$_2$Ph), 57.9 (*C*HNH), 38.3 (*C*H$_2$CH). HRMS *m/z* for $C_{23}H_{23}N_3O_5S$ $[M+H]^+$ calcd. 454.1, found 454.1; $[M+Na]^+$ calcd. 476.1, found 476.1; $[M-H]^-$ calcd. 452.1, found 452.2; $[M+Cl]^-$ calcd. 488.1, found 488.2.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 7–9

A mixture of equivalent amounts of the appropriate N-protected aminoacyl-benzotriazole and (4-sulfamoylphenyl)methanaminium chloride was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((4-sulfamoylbenzyl)amino)ethyl)carbamate, ***7***

White solid (95%); silica gel TLC $R_f = 0.20$ (MeOH/CH_2Cl_2 5% v/v); mp 182–183 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 8.51 (t, 1H, N*H*, $J = 4.0$ Hz), 7.89 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.54 (t, 1H, N*H*, $J = 8.0$ Hz), 7.47–7.35 (m, 10H, Ar-*H* + NH_2), 5.08 (s, 2H, OCH_2Ph), 4.38 (d, 2H, CH_2Ph, $J = 4.0$ Hz), 3.71 (d,2H, CH$_2$NH, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.2 (*C*OCH$_2$NH), 157.4 (*C*OOCH$_2$Ph), 144.4, 143.5, 137.9, 129.2, 128.7, 128.6, 128.4, 126.5 (Ar-*C*), 66.4 (O*C*H$_2$Ph), 44.5 (*C*H$_2$Ph), 42.6 (*C*H$_2$NH). HRMS *m/z* for $C_{17}H_{19}N_3O_5S$ $[M+H]^+$ calcd. 378.1, found 378.0; $[M+NH_4]^+$ calcd. 395.1, found 395.1; $[M+Na]^+$ calcd. 400.1, found 400.0; $[M+Cl]^-$ calcd. 412.1, found 412.1; $[M+HCOO]^-$ calcd. 422.1, found 422.1

(S)-benzyl (1-oxo-1-((4-sulfamoylbenzyl)amino)propan-2-yl) carbamate, ***8***

White solid (93%); silica gel TLC $R_f = 0.36$ (MeOH/CH_2Cl_2 5% v/v); mp 181–182 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 8.55 (t, 1H, N*H*, $J = 8.0$ Hz), 7.80 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.46–7.35 (m, 10H, Ar-*H* + N*H* + NH_2), 5.07 (s, 2H, CH_2Ph), 4.38 (d, 2H, CH_2Ph, $J = 8.0$ Hz), 4.15–4.10 (m, 1H, C*H*), 1.28 (d, 3H, CH_3, $J = 4.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 173.7 (*C*OCH$_2$NH), 156.7 (*C*OOCH$_2$Ph), 144.6, 143.5, 137.9, 129.3, 128.8, 128.7, 128.2, 126.6 (Ar-*C*), 66.4 (O*C*H$_2$Ph), 51.2 (*C*H$_2$Ph), 42.6 (*C*H), 19.2 (*C*H$_3$). HRMS *m/z* for $C_{18}H_{21}N_3O_5S$ $[M+H]^+$ calcd. 392.1, found 392.1; $[M+NH_4]^+$ calcd. 409.1, found 409.2; $[M+Na]^+$ calcd. 414.1, found 414.1; $[M+Cl]^-$ calcd. 426.1, found 426.1; $[M+HCOO]^-$ calcd. 436.1, found 436.1.

(S)-benzyl (1-oxo-3-phenyl-1-((4-sulfamoylbenzyl)amino) propan-2-yl)carbamate, 9

White solid (94%); silica gel TLC $R_f = 0.29$ (MeOH/CH_2Cl_2 5% v/v); mp 230–231 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 8.68 (t, 1H, N*H*, $J = 8.0$ Hz), 7.77 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.64 (d,1H, N*H*, $J = 8.0$ Hz), 7.36–7.30 (m, 14H, Ar-*H* + NH_2), 5.01 (s, 2H, OCH_2Ph), 4.39–4.30 (m, 2H, CH_2Ph + C*H*), 3.07–3.03 (m, 1H, CH_2CH), 2.88–2.82 (m, 1H, CH_2CH). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.5 (*C*OCH$_2$NH), 156.8 (*C*OOCH$_2$Ph), 144.3, 143.6, 138.9, 137.9, 130.2, 129.2, 129.0, 128.6, 128.4, 128.3, 127.2, 126.5 (Ar-*C*), 66.2 (O*C*H$_2$Ph), 57.3 (*C*H$_2$Ph), 42.7 (*C*H), 38.4 (*C*H$_3$). HRMS *m/z* for $C_{24}H_{25}N_3O_5S$ $[M+H]^+$ calcd. 368.2, found 468.1; $[M+Na]^+$ calcd. 490.1, found 460.1; $[M+Cl]^-$ calcd. 502.1, found 502.2.1; $[M+HCOO]^-$ calcd. 512.1, found 512.2.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 10–13

A mixture of equivalent amounts of the appropriate N-protected aminoacylbenzotriazole and 4-(2-aminoethyl)benzenesulfonamide was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)carbamate, ***10***

Beige solid (95%); silica gel TLC $R_f = 0.58$ (MeOH/CH_2Cl_2 5% v/v); mp 161–162 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.99 (t, 1H, N*H*, $J = 4.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.45–7.40 (m, 8H, Ar-*H* + N*H*), 7.33 (s, 2H, NH_2), 5.08 (s, 2H, OCH_2Ph), 3.61 (d, 2H, CH_2Ph, $J = 8.0$ Hz), 3.33 (t, 2H, CH$_2$CH_2NH, $J = 8.0$ Hz), 2.83 (t, 2H, CH_2CH$_2$NH, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.0 (*C*OCH$_2$NH), 157.4 (*C*OOCH$_2$Ph), 144.6, 143.0, 138.0, 130.0, 129.3, 128.7, 128.6, 126.6 (Ar-*C*), 66.4 (O*C*H$_2$Ph), 44.5 (*C*H$_2$NH), 41.1 (*C*H$_2$CH$_2$Ph), 35.7 (CH$_2$*C*H$_2$Ph). HRMS *m/z* for $C_{18}H_{21}N_3O_5S$ $[M+H]^+$ calcd. 392.1, found 392.1; $[M+NH_4]^+$ calcd. 409.2, found 409.1; $[M+Na]^+$ calcd. 414.1, found 414.0; $[M+Cl]^-$ calcd. 426.1, found 426.1; $[M+HCOO]^-$ calcd. 436.1, found 436.1.

tert-Butyl (2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl) carbamate, ***11***

White solid (89%); silica gel TLC $R_f = 0.60$ (MeOH/CH_2Cl_2 30% v/v); mp 201–202 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.89 (t, 1H, N*H*, $J = 4.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.43 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.32 (s, 2H, NH_2), 6.94 (t, 1H, N*H*, $J = 4.0$ Hz), 3.52 (d, 2H, CH_2NH, $J = 4.0$ Hz), 3.34 (t, 2H, CH$_2$CH_2N, $J = 8.0$ Hz), 2.82 (t, 2H, NCH$_2$CH_2, $J = 8.0$ Hz), 1.42 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.2 (*C*OCH$_2$NH), 156.7 (*C*OOCH$_2$Ph), 144.6, 143.0, 130.0, 126.6 (Ar-*C*), 79.0 ((CH$_3$)$_3$*C*O, 44.2 (*C*H$_2$NH), 40.9 (NH*C*H$_2$CH$_2$), 35.7 (NHCH$_2$*C*H$_2$), 29.1 ((*C*H$_3$)$_3$C). HRMS *m/z* for $C_{15}H_{23}N_3O_5S$ $[M+Na]^+$ calcd. 380.1, found 380.1; $[M+Cl]^-$ calcd. 392.1, found 392.1; $[M+HCOO]^-$ calcd. 402.1, found 402.1.

(S)-benzyl (1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl) carbamate, ***12***

Yellow solid (92%); silica gel TLC $R_f = 0.46$ (EtOAc/Hexane 30% v/v); mp 130–131 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.99 (t, 1H, N*H*, $J = 8.0$ Hz), 7.93 (d, 1H, N*H*, $J = 4.0$ Hz), 7.81–7.78 (m, 2H, Ar-*H*), 7.46–7.35 (m, 9H, Ar-*H* + NH_2), 5.08 (s, 2H, OCH_2Ph), 4.02 (t, 2H, CH_2CH$_2$Ph, $J = 8.0$ Hz), 3.39–3.31 (m, 1H, C*H*CH$_3$), 2.82 (t, 2H, PhCH_2CH$_2$, $J = 8.0$ Hz), 1.20 (d, 3H, CH$_3$, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 173.4 (*C*OCH$_2$NH), 155.7 (*C*OOCH$_2$Ph), 144.6, 143.0, 138.0, 130.1, 130.0, 129.3, 128.7, 126.6, 126.0, 115.9 (Ar-*C*), 66.3 (O*C*H$_2$Ph), 51.1 (*C*HNH), 42.9 (N*C*H$_2$CH$_2$), 35.7 (CH$_2$*C*H$_2$Ph), 19.2 (*C*H$_3$). HRMS *m/z* for $C_{19}H_{23}N_3O_5S$ $[M+H]^+$ calcd. 406.1, found 406.1; $[M+Na]^+$ calcd. 428.1, found 428.1; $[M+Cl]^-$ calcd. 440.1, found 440.1; $[M+HCOO]^-$ calcd. 450.1, found 450.2.

(S)-benzyl (1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino) propan-2-yl)carbamate, ***13***

Beige solid (97%); silica gel TLC $R_f = 0.66$ (MeOH/CH_2Cl_2 30% v/v); mp 195–196 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 8.15 (t, 1H, N*H*, $J = 8.0$ Hz), 7.77 (d, 2H, Ar*H*, $J = 4.0$ Hz), 7.75 (d, 1H, N*H*, $J = 4.0$ Hz), 7.37–7.24 (m, 14H, Ar-*H* + NH_2), 4.99 (s, 2H, OCH_2Ph), 4.23–4.22 (m, 1H, NHC*H*CH$_2$Ph), 3.36 (t, 2H, PhCH$_2$CH_2, $J = 8.0$ Hz), 3.54 (t, 2H, PhCH_2CH$_2$, $J = 8.0$ Hz), 2.85–2.76 (m, 3H, CH_2), 2.97–2.93 (m, 1H, CHCH_2), 2.81–2.78 (m, 3H, CHCH_2 + PhCH_2CH$_2$) . ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.3 (*C*OCH$_2$NH), 156.7 (*C*OOCH$_2$Ph), 144.6, 143.0, 138.0, 139.0, 138.0, 130.1, 129.2, 129.0, 128.6, 128.4, 127.2, 126.6 (Ar-*C*), 66.2 (OCH$_2$Ph), 57.2 (*C*HNH), 40.7 (*C*H$_2$CH$_2$Ph), 38.5 (NCH*C*H$_2$), 35.7 (CH$_2$*C*H$_2$Ph). HRMS *m/z* for $C_{25}H_{27}N_3O_5S$ $[M + H]^+$ calcd. 482.2, found 482.2; $[M + Na]^+$ calcd. 504.2, found 504.1; $[M + Cl]^-$ calcd. 516.1, found 516.2; $[M + HCOO]^-$ calcd. 526.2, found 526.3.

Benzyl (2-oxo-2-((2-(4-sulfamoylphenoxy)ethyl)amino)ethyl) carbamate, ***14***

A mixture of benzyl (2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-oxoethyl)carbamate (0.12 g; 0.39 mmol), 2-(4-sulfamoylphenoxy)ethanaminium 2,2,2-trifluoroacetate (0.13 g; 0.39 mmol) and Et_3N (0.06 mL; 0.43 mmol) was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

White solid (0.14 g, 93%); silica gel TLC $R_f = 0.75$ (MeOH/CH_2Cl_2 5% v/v); mp 183–184 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.99 (t, 1H, N*H*, $J = 4.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.45–7.40 (m, 8H, Ar-*H* + N*H*), 7.33 (s, 2H, NH_2), 5.08 (s, 2H, OCH_2Ph), 3.61 (d, 2H, CH_2Ph, $J = 8.0$ Hz), 3.33 (t, 2H, CH$_2$CH_2NH, $J = 8.0$ Hz), 2.83 (t, 2H, CH_2CH$_2$NH, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.0 (*C*OCH$_2$NH), 157.4 (*C*OOCH$_2$Ph), 144.6, 143.0, 138.0, 130.0, 129.3, 128.7, 128.6, 126.6 (Ar-*C*), 66.4 (OCH$_2$Ph), 44.5 (*C*H$_2$NH), 41.1 (*C*H$_2$CH$_2$Ph), 35.7 (CH$_2$*C*H$_2$Ph). HRMS *m/z* for $C_{18}H_{21}N_3O_6S$ $[M + Na]^+$ calcd. 430.1, found 430.0.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 15 and 16

A mixture of equivalent amounts of the appropriate N-protected aminoacylbenzotriazole, 3-(4-sulfamoylphenoxy)propan-1-aminium chloride and Et_3N was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((3-(4-sulfamoylphenoxy)propyl)amino)ethyl)-carbamate, ***15***

White solid (91%); silica gel TLC $R_f = 0.5$ (MeOH/CH_2Cl_2 5% v/v); mp 125–126 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.97 (t, 1H, N*H*, $J = 4.0$ Hz), 7.77 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.46–7.35 (m, 6H, Ar-*H* + N*H*), 7.22 (s, 2H, NH_2), 7.10 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 5.06 (s, 2H, OCH_2Ph), 4.09 (t, 2H, CH$_2$CH_2OPh, $J = 8.0$ Hz), 3.80 (d, 2H, CH_2NH, $J = 4.0$ Hz), 3.27 (t, 2H, CH$_2$CH_2NH, $J = 8.0$ Hz), 1.91 (t, 2H, CH$_2$CH_2CH$_2$, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.0 (*C*OCH$_2$NH), 161.9 (OC$_6$H$_4$), 157.4 (*C*OOCH$_2$Ph), 137.9, 137.0, 129.3, 128.7, 128.6, 128.5, 115.4 (Ar-*C*), 66.6 (OCH$_2$Ph), 66.4 (CH$_2$*C*H$_2$O), 44.5 (*C*H$_2$NH), 36.9 (NH*C*H$_2$CH$_2$), 29.6 (CH$_2$*C*H$_2$*C*H$_2$). HRMS *m/z* for $C_{19}H_{23}N_3O_6S$ $[M + Na]^+$ calcd. 444.1, found 444.1; $[M + Cl]^-$ calcd. 456.1, found 456.1; $[M + HCOO]^-$ calcd. 466.1, found 466.1.

(S)-benzyl (1-oxo-3-phenyl-1-((3-(4-sulfamoylphenoxy)propyl) amino)propan-2-yl)carbamate, ***16***

White solid (89%); silica gel TLC $R_f = 0.45$ (MeOH/CH_2Cl_2 5% v/v); mp 183–184 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 8.11 (t, 1H, N*H*, $J = 4.0$ Hz), 7.77 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.55 (d, 1H, N*H*, $J = 8.0$ Hz), 7.39–7.28 (m, 10H, Ar-$_H$), 7.23 (s, 2H, NH_2), 7.08 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 4.99 (s, 2H, OCH_2Ph), 4.22 (m, 1H, C*H*CH$_2$), 4.01 (t, 2H, CH$_2$CH_2O, $J = 8.0$ Hz), 3.24 (m, 2H, CH$_2$CH_2NH), 3.01–2.96 (m, 1H, CHCH_2), 2.81–2.78 (m, 1H, CHCH_2), 1.86 (m, 2H, CH$_2$CH_2CH$_2$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 172.2 (*C*OCH$_2$NH), 161.8 (OC$_6$H$_4$), 156.7 (*C*OOCH$_2$Ph), 138.9, 137.9, 137.0, 130.0, 129.2, 128.9, 128.6, 128.5, 128.0, 127.1, 115.3 (Ar-*C*), 66.6 (OCH$_2$Ph), 66.1 (*C*HCH$_2$), 57.2 (CH$_2$*C*H$_2$O), 38.6 (NH*C*H$_2$CH$_2$), 36.3 (CH*C*H$_2$), 29.5 (CH$_2$*C*H$_2$CH$_2$). HRMS *m/z* for $C_{26}H_{29}N_3O_6S$ $[M + H]^+$ calcd. 512.2, found 512.1; $[M + Na]^+$ calcd. 534.2, found 534.1.

General procedure for the synthesis of amino acid–sulfonamide conjugates, 17–20

A mixture of equivalent amounts of the appropriate N-protected aminoacylbenzotriazole, 4-(4-sulfamoylphenoxy)butan-1-aminium 2,2,2-trifluoroacetate and Et_3N was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction, all volatiles were removed by rotavapour and the obtained crude product was crystallized from methanol.

Benzyl (2-oxo-2-((4-(4-sulfamoylphenoxy)butyl)amino)ethyl) carbamate, ***17***

White solid (94%); silica gel TLC $R_f = 0.55$ (MeOH/CH_2Cl_2 5% v/v); mp 135–136 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.97 (t, 1H, N*H*, $J = 4.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.45 (t, 1H, N*H*, $J = 4.0$ Hz), 7.40–7.36 (m, 5H, Ar-*H*), 7.23 (s, 2H, NH_2), 7.11 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 5.07 (s, 2H, OCH_2Ph), 4.08 (t, 2H, CH$_2$CH_2OPh, $J = 8.0$ Hz), 3.63 (d, 2H, CH_2NH, $J = 4.0$ Hz), 3.16 (q, 2H, CH$_2$CH_2NH, $J = 8.0$ Hz), 1.78–1.74 (m, 2H, CH_2CH$_2$O), 1.60–1.57 (m, 2H, NCH_2CH$_2$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.9 (*C*OCH$_2$NH), 162.0 (OC$_6$H$_4$), 157.4 (*C*OOCH$_2$Ph), 138.0, 137.0, 129.3, 128.7, 128.6, 128.5, 115.4 (Ar-*C*), 68.6 (OCH$_2$Ph), 66.4 (CH$_2$*C*H$_2$O), 44.5 (*C*H$_2$NH), 39.1 (CH$_2$*C*H$_2$NH), 26.9 (*C*H$_2$CH$_2$O), 26.6 (NCH$_2$*C*H$_2$). HRMS *m/z* for $C_{20}H_{25}N_3O_6S$ $[M + Na]^+$ calcd. 458.1, found 458.1; $[M + Cl]^-$ calcd. 470.1, found 470.1; $[M + HCOO]^-$ calcd. 480.1, found 480.1.

tert-butyl (2-oxo-2-((4-(4-sulfamoylphenoxy)butyl)amino)ethyl)-carbamate, ***18***

White solid (88%); silica gel TLC $R_f = 0.29$ (EtOAc/Hexane 30% v/v); mp 75–76 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) 7.80 (t, 1H, N*H*, $J = 4.0$ Hz), 7.77 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.22 (s, 2H, NH_2), 7.10 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 6.93 (t, 1H, N*H*, $J = 8.0$ Hz), 4.08 (t, 2H, CH$_2$CH_2OPh, $J = 8.0$ Hz), 3.53 (d, 2H, CH_2NH, $J = 8.0$ Hz), 3.16 (q, 2H, CH$_2$CH_2NH, $J = 8.0$ Hz), 1.78–1.74 (m, 2H, CH_2CH$_2$O), 1.60–1.56 (m, 2H, NCH_2CH$_2$), 1.42 (s, 9H, (CH_3)$_3$). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 170.0 (*C*OCH$_2$NH), 161.9 (OC$_6$H$_4$), 156.8 (*C*OOCH$_2$Ph), 137.0, 128.6, 115.3 (Ar-*C*), 78.9 (OC(CH$_3$)$_3$), 68.7 (CH$_2$*C*H$_2$O), 52.2 (*C*H$_2$NH), 44.2 (CH$_2$CH$_2$NH), 38.9 (*C*H$_2$CH$_2$O), 29.1 (NCH$_2$*C*H$_2$), 26.8 (*C*H$_3$). HRMS *m/z* for $C_{17}H_{27}N_3O_6S$ $[M^+]$ calcd. 401.2, found 401.2; $[M + Na]^+$ calcd. 424.2, found 424.1; $[M + HCOO]^-$ calcd. 446.2, found 446.1.

(S)-benzyl (1-oxo-1-((4-(4-sulfamoylphenoxy)butyl)amino) propan-2-yl)carbamate, ***19***

Beige solid (93%); silica gel TLC $R_f = 0.45$ (EtOAc/Hexane 30% v/v); mp 76–77 °C; ^{1}H NMR (DMSO-d$_6$, 400 MHz) 8.29 (t, 1H, N*H*, $J = 8.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.44–7.35 (m, 6H, Ar-*H* + N*H*), 7.22 (s, 2H, NH_2), 7.10 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 5.05 (s, 2H, OCH_2Ph), 4.09–3.99 (m, 3H, $CH_2CH_2OPh + CH$), 3.16–3.12 (m, 2H, CH_2CH_2NH), 1.77–1.74 (m, 2H, CH_2CH_2O), 1.60–1.56 (m, 2H, NCH_2CH_2), 1.23 (d, 3H, CH_3, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d$_6$, 400 MHz) δ 173.2 ($COCH_2NH$), 161.9 (OC_6H_4), 156.5 ($COOCH_2Ph$), 138.0, 137.0, 129.2, 128.7, 128.6, 128.5, 115.3 (Ar-*C*), 68.6 (OCH_2Ph), 66.2 (CH_2CH_2O), 51.1 (*C*HNH), 39.0 (CH_2CH_2NH), 26.8 (CH_2CH_2O), 26.5 (NCH_2CH_2), 19.3 (CH_3). HRMS *m/z* for $C_{21}H_{27}N_3O_6S$ $[M + H]^+$ calcd. 450.2 Found 450.1; $[M + Na]^+$ calcd. 472.2, found 472.1.

(S)-benzyl (1-oxo-3-phenyl-1-((4-(4-sulfamoylphenoxy)butyl) amino)propan-2-yl)carbamate, ***20***

White solid (97%); silica gel TLC $R_f = 0.77$ (MeOH/CH_2Cl_2 30% v/v); mp 197–198 °C; ^{1}H NMR (DMSO-d$_6$, 400 MHz) 8.04 (t, 1H, N*H*, $J = 4.0$ Hz), 7.78 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 7.52(d, 1H, N*H*, $J = 8.0$ Hz), 7.37–7.28 (m, 10H, Ar-*H*), 7.23 (s, 2H, NH_2), 7.11 (d, 2H, Ar-*H*, $J = 8.0$ Hz), 4.99 (s, 2H, OCH_2Ph), 4.27–4.21 (m, 1H, $CHCH_2$), 4.06 (t, 2H, CH_2CH_2O), 3.19–3.12 (m, 2H, CH_2CH_2N), 3.01–2.97 (m, 1H, $CHCH_2Ph$), 2.84–2.78 (m, 1H, $CHCH_2Ph$), 1.71 (t, 2H, CH_2CH_2O, $J = 8.0$ Hz), 1.55 (t, 2H, NCH_2CH_2, $J = 8.0$ Hz). ^{13}C NMR (DMSO-d$_6$, 400 MHz) δ 172.1 ($COCH_2NH$), 162.0 (OC_6H_4), 156.7 ($COOCH_2Ph$), 139.0, 138.7, 138.0, 137.0, 130.1, 129.0, 128.6, 128.4, 127.2, 115.4 (Ar-*C*), 68.6 (OCH_2Ph), 66.1 (*C*HNH), 57.2 (CH_2CH_2O), 39.1 (CH_2CH_2NH), 26.8 (CH_2CH_2O), 26.5 (NCH_2CH_2). HRMS *m/z* for $C_{27}H_{31}N_3O_6S$ $[M + H]^+$ calcd. 526.2 Found 526.1; $[M + Na]^+$ calcd. 548.2, found 548.1.

CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO2 hydration activity by using method of Khalifah[49]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM HEPES (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7–17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5%–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-square methods using PRISM (www.graphpad.com), and non-linear least squares methods, values representing the mean of at least three different determinations, as described earlier by us[50–56].

Results and discussion

New N-protected amino acid–sulfanamide conjugates (**1**–**20**) were synthesized by the treatment of the appropriate sulfonamide derivatives, incorporating primary amine moieties, with N-protected aminoacylbenzotriazoles under microwave heating at 70 °C for 30 min, with good yields of 83%–97%. The synthetic pathway of sulfonamides **1**–**20** is summarized in Scheme 1.

We have included in the study amino-sulfonamides having the NH_2 group directly linked to the benzene ring on which the sulfonamide zinc-binding group is found (for example, metanilamide and sulfanilamide) as well as derivatives having various linkers between the benzenesulfonamide fragment and the primary amine group. These were of the alkylene type (methylene, ethylene) or longer ones, of the -$(CH_2)_n$O- type, with n varying between 2 and 4 (Scheme 1). The amino acids chosen for derivatization were Gly, Ala and Phe, in order to investigate whether this part of the inhibitor scaffold influences the CA inhibitory properties. Furthermore, the amino group from the amino acid reagent was protected with the benzyloxycarbonyl (Z) or tert-butyloxycarbonyl (Boc) protecting groups, as shown in Scheme 1.

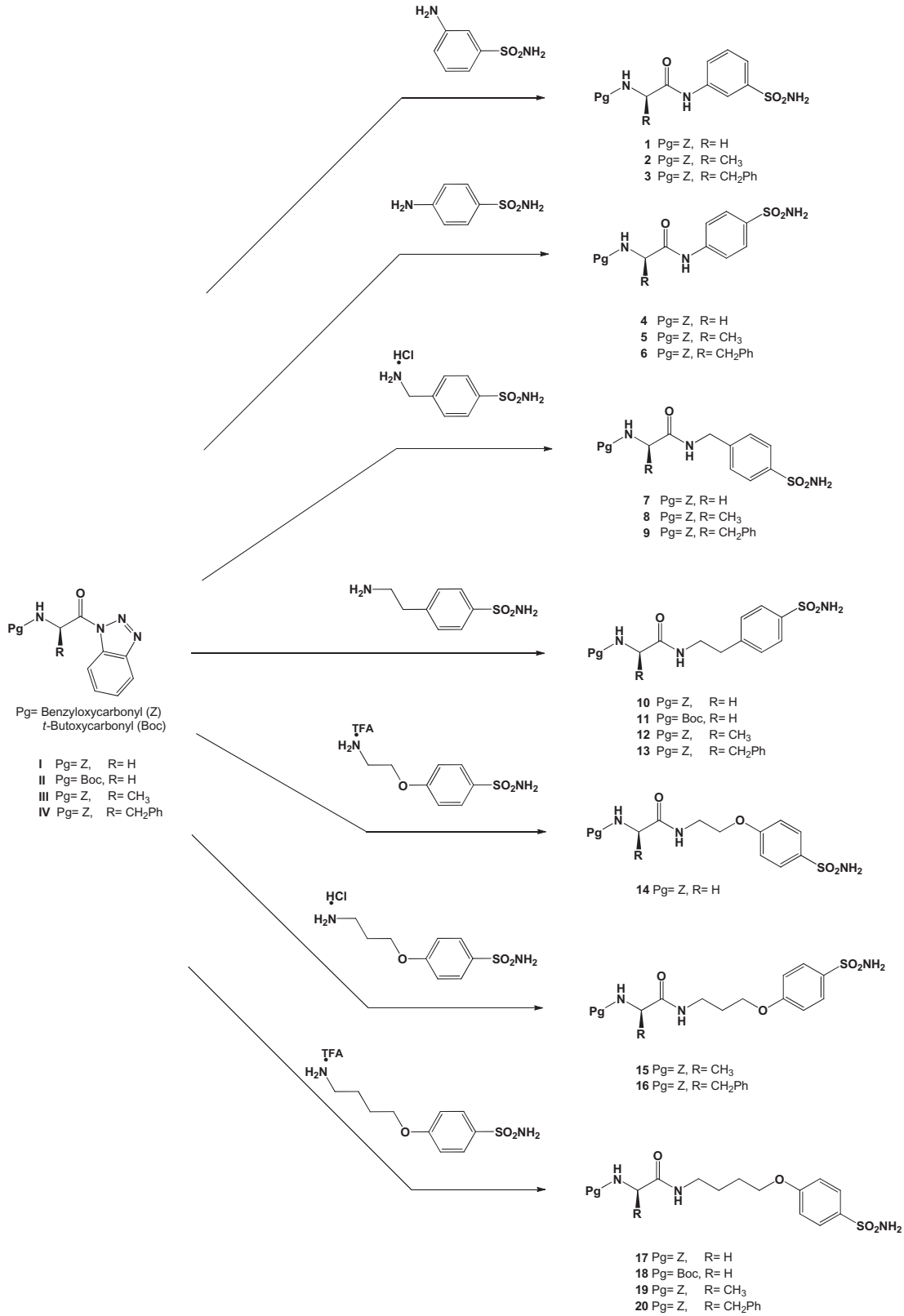
The structures of N-protected amino acid–sulfonamide conjugates (**1**–**20**) were elucidated by ^{1}H NMR, ^{13}C NMR and mass spectrometric analyses. All spectral data were in agreement with the proposed structures. The characteristic *NH* resonances of the sulfonamide part of the conjugates **1**–**6** and **2**–**20** were observed between 10.32–10.47 and 7.80–8.68 ppm, respectively. Other amino group resonances for the sulfonamide conjugates **1**–**20** were observed between 6.93–8.24 ppm. Carbonyl resonances of the amide carbonyl and carbamate carbonyl were observed around 170 and 156 ppm, respectively. The characteristic NH_2 resonances for sulfonamide functional group were observed between 7.22–7.57 ppm in the ^{1}H NMR spectrum. All other aliphatic and aromatic protons and carbons were observed in the expected regions (see Materials and Methods).

The hCA I, II, IV and XII enzyme inhibitory activity of the N-protected amino acid–sulfonamide conjugates **1**–**20** are shown in Table 1. We have chosen these isforms as three of them (CA II, IV and XII) are antiglaucoma drug targets[1,27–29] whereas CA I, due to its diffuse distribution in the blood and gastrointestinal tract is one of the main off-targets for such pharmacologic agents[57].

The following structure–activity relationship (SAR) can be observed from the inhibition data of Table 1.

hCA I was potently inhibited by sulfonamides **11**, **14**, **15**, **17**–**19** (inhibition constants ranging between 5.6 and 24.5 nM), moderately inhibited by **6**, **7**, **9**, **10**, **12**, **13** and **16** (K_Is in the range of 45.9–102.4) and poorly inhibited by the remaining derivatives, which showed K_Is in the range of 351.1–1946 nM (Table 1). Thus, best inhibition for this isoform was associated with compounds incorporating a long linker between the amino and benzenesulfonamide moieties (**14**, **15**, **17**–**19**), whereas the nature of the protecting group (Pg) seem to be less important for activity (compare the activity of **17**–**19**). The Gly and Ala derivatives were generally much better hCA I inhibitors compared with the corresponding Phe derivatives. Acetazolamide (**AZA**, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide), a clinically used compound, was a medium potency CAI for this isoform, with an inhibition constant of 250 nM.

Only three derivatives showed highly effective hCA II inhibitory activity, comparable to that of **AZA**, that is, **17**–**19** (K_Is in the range of 8.6–11.9 nM). Again they incorporate the longest scaffold, being Gly or Ala derivatives with Z- or Boc-protecting groups. A number of other compounds (**1**, **4**–**6**, and **8**–**15**) were medium potency hCA II inhibitors, with K_Is in the range of 24.5–97.2 nM (Table 1). They incorporate all the sulfonamide scaffolds employed in this study and this reveals that in this case the nature of the linker and that of the amino acid moiety/protecting groups are the most important factors influencing the CA II inhibitory power. The remaining derivatives (**2**, **3**, **7** and **20**) were the least effective inhibitors, with K_Is in the range of 139–694 nM.



Scheme 1. Synthesis pathways of the new sulfonamide conjugates of N-protected amino acids.

hCA IV, a membrane-anchored isoform[1], was the least sensitive CA among the investigated ones to inhibition by these compounds. A group of derivatives, **1**, **4**–**6**, **9**, **13** and **17** showed inhibitory activity in the same range or better than **AZA**, with K_Is in the range of 27.9–93.5 nM (K_I of **AZA** is of 74 nM). Interestingly, the most potent CA IV inhibitor, **6**, was a Phe derivative (also incorporating Z as protecting group and the homosufanilamide scaffold). Thus, apart 17 which has an elongated molecule, all the best inhibitors of this isoform had a more compact scaffold, being derivatives of metanilamide or homosufanilamide. The remaining derivatives were either inactive (**2** and **20** had K_Is > 10 μM) or were poorly active CAIs, with K_Is in the range of 174.9–1453 nM (Table 1).

hCA XII, a transmembrane isoforms with an out of the cell active site[1] was more sensible to inhibition by the reported sulfonamides, which with few exceptions (**8**, **9**, **11** and **18**) had

Table 1. hCA I, II, IV and XII inhibition data with amino acid–sulfonamide conjugates **1**–**20**, by a stopped-flow CO_2 hydrase assay[49].

	K_I (nM)*			
Compound	hCA I	hCA II	hCA IV	hCA XII
1	851.7	52.3	81.2	89.8
2	1946	224.1	>10 000	73.1
3	1915	451.7	174.9	9.5
4	391.6	31.9	89.6	61.2
5	359.2	42.9	72.3	95.7
6	60.9	26.6	27.9	72.1
7	51.4	139.0	545.0	90.8
8	351.1	97.2	365.2	109.0
9	95.3	62.2	93.5	101.8
10	93.9	70.6	362.0	9.7
11	5.6	35.9	383.0	951.2
12	95.4	69.1	1453	34.7
13	102.4	28.4	76.7	10.3
14	15.0	29.1	692.1	99.1
15	24.5	50.3	1358	83.9
16	45.9	24.5	401.5	76.0
17	17.1	8.6	43.5	91.2
18	9.3	8.7	838.9	803.1
19	22.1	11.9	825.1	96.3
20	595.0	694.0	>10 000	85.7
AZA	250	12	74	5.7

Acetazolamide (**AZA**) was used as a standard inhibitor for all CAs investigated here.

*Mean from 3 different assays, errors in the range of ±5%–10% of the reported values (data not shown).

K_Is < 100 nM. The least active compounds, mentioned above, were the two homosulfanilamide derivatives **8** and **9** incorporating Ala and Phe moieties, and had similar K_Is of 101.8–109.0 nM, and two Boc-protected Gly derivatives incorporating longer scaffolds, **11** and **18**, which showed K_Is of 803.1–951.2 nM. The most effective hCA XII inhibitors (similar to **AZA**) were **3**, **10** and **13**, with K_Is in the range of 9.5–10.3 nM. They incorporate either metanilamide or 4-aminoethyl-benzenesulfonamide scaffolds, and Gly or Phe moieties. The remaining sulfonamides showed a rather flat SAR, with K_Is of 34.7–96.3 nM, proving that a lot of scaffolds and substitution patterns lead to effective (but not highly potent) hCA XII inhibitors.

Some of the new compounds showed rather good selectivity levels for inhibiting hCA XII over the other isoforms (for example, **3**, **10**, **12** and **13**) but generally no highly isoform-selective inhibition profiles were detected for these derivatives (Table 1).

Conclusions

A series of N-protected amino acids (Gly, Ala and Phe protected with Z or Boc groups) were reacted with sulfonamide derivatives, leading to the corresponding N-protected amino acid–sulfonamide conjugates in high yields (85%–98%). The carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity of the new sulfonamides was assessed against human (h) isoforms involved in serious pathologies (glaucoma, cancer, etc.), such as hCA I, hCA II, hCA IV and hCA XII. Among these four isoforms, hCA II, IV and XII are antiglaucoma drug targets, as all of them are involved in aqueous humor secretion within the eye. Low nanomolar inhibition was observed against all these CAs with the 20 prepared sulfonamides, but no selective inhibitory profiles were observed, except for few CA XII-selective derivatives. hCA I, II and XII were generally better inhibited by sulfonamides incorporating longer scaffolds and Gly/Ala, whereas the best hCA IV inhibitors were homosulfanilamide derivatives, incorporating Phe moieties. As the amino acid–sulfonamide conjugates show good water solubility and effective hCA II, IV and XII inhibition, they may be considered as interesting candidates for antiglaucoma studies.

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Declaration of interest

The authors declare no conflict of interest.

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