Carbonic anhydrase I, II, IV and IX inhibition with a series of 7-amino-3,4-dihydroquinolin-2(1H)-one derivatives

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ABSTRACT
A series of new derivatives was prepared by derivatisation of the 7-amino moiety present in 7-amino-3,4-dihydroquinolin-2(1H)-one, a compound investigated earlier as CAI. The derivatisation was achieved by: i) reaction with arylsulfonyl isocyanates/aryl isocyanates; (ii) reaction with fluorescein isothiocyanate; (iii) condensation with substituted benzoic acids in the presence of carbodimides; (iv) reaction with 2,4,6-trimethyl-pyrylium tetrafluoroborate; (v) reaction with methylsulfonyl chloride and (vi) reaction with maleic anhydride. The new compounds were assayed as inhibitors of four carbonic anhydrases (CA, EC 4.2.1.1) human (h) isoforms of pharmacologic relevance, the cytosolic hCA I and II, the membrane-anchored hCA IV and the transmembrane, tumour-associated hCA IX. hCA IX was the most inhibited isoform (Ki ranging between 243.6 and 2785.6 nm) whereas hCA IV was not inhibited by these compounds. Most derivatives were weak hCA I and II inhibitors, with few of them showing Ki < 10 μM. Considering that the inhibition mechanism with these lactams is not yet elucidated, exploring a range of such derivatives with various substitution patterns may be useful to identify leads showing isofrom selectivity or the desired pharmacologic action.

Introduction
CO₂, bicarbonate and protons are essential molecules/ions in important physiologic processes in the three life kingdoms (Bacteria, Archaea and Eukarya), and for this reason, relatively high amounts of the enzymes carbonic anhydrases (CAs, EC 4.2.1.1) are present in different tissues/cell compartments of most investigated organisms.1–11. The α-CAs are present in vertebrates, protozoa, algae and cytoplasm of green plants and in some Bacteria14–19, the β-CAs are predominantly found in Bacteria, algae and chloroplasts of both mono- as well as dicotyledons, but also in many fungi and some Archaea1–11. The γ-CAs were found in plants, Archaea and Bacteria1–11, whereas the δ-, ε- and θ-CAs seem to be present only in marine diatoms.11 The η-CA class has been discovered in protozoa such as those belonging to the genus Plasmodium20. In many organisms, these enzymes are involved in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (e.g. gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumourigenicity and many other physiologic or pathologic processes (thoroughly studied in vertebrates)1–11,21–26 whereas in algae, plants and some bacteria they play an important role in photosynthesis and other biosynthetic reactions.8,11. In diatoms δ- and ε-CAs play a crucial role in carbon dioxide fixation.11 Many such enzymes from vertebrates, fungi and bacteria are well-known drug targets, with inhibitors and activators possessing various pharmacologic applications.23–42.

Sulfonamides are the most important class of CA inhibitors CaIs4–12, with several compounds in clinical use for many years, as diuretics1,26,28, antiglaucoma agents1,27,33, antiepileptics30–34 and more recently as anticancer agents1,12. Although a large number of isofrom-selective sulfonamide CAs were reported ultimate, mostly by using the tail approach for their synthesis16–23,26, a large variety of other chemotypes were investigated for their interaction with these enzymes, which led to the development of a large number of non-classic CAs, belonging to various classes14,33. Here, we report a new series of such derivatives which incorporate the 7-amino-3,4-dihydroquinolin-2(1H)-one scaffold43.

Materials and methods
Chemistry
Anhydrous solvents and all reagents were purchased from Sigma-Aldrich (Milan, Italy). All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (1H-NMR, 13C-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d₆. 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; q, quadruplet; dd, double of doublet. The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D₂O.

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General procedure for the preparation of compounds 2–20

A solution of 7-amino-3,4-dihydroquinolin-2(1H)-one (1) in dry dimethylformamide (3–5 ml) was treated with a stoichiometric amount of appropriate isocyanates/isothiocyanate. The mixture was stirred at room temperature until the consumption of starting materials (TLC monitoring). The reaction was quenched with a 1.0 M aqueous solution of HCl to give a precipitate that was washed with diethyl ether (3 × 5 ml), filtered and dried under vacuum (compounds 2–19) or extracted with ethyl acetate (3 × 15 ml), the combined organic layers were washed with H2O (3 × 15 ml), dried over Na2SO4, filtered, and concentrated (compound 20) to afford the title compounds 2–20.

N-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)carbamoyl] benzenesulfonylamine (3)

Beige solid, yield 89%; m.p.: 272–273 °C; silica gel TLC Rf = 0.16 (MeOH/DCM 10% v/v); δH (400 MHz, DMSO-d6) 2.42 (2H, d, J 6.8), 2.81 (2H, d, J 6.8), 6.85 (1H, dd, J 2.0, 8.4), 7.01 (1H, d, J 2.0), 7.06 (1H, d, J 8.4), 7.67 (2H, t, J 8.0), 7.73 (1H, t, J 8.0), 8.00 (2H, d, J 8.0), 8.91 (1H, s, exchange with D2O, NH), 10.03 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.1, 31.5, 106.9, 113.4, 117.2 (d, J3-C = 23), 119.4, 128.8, 131.6 (d, J3-C = 10), 137.6 (d, J3-C = 17), 137.8, 139.5, 150.1, 165.6 (d, 1J-C = 250), 171.1; δH (376 MHz, DMSO-d6) –105.1 (1F, s); m/z (ESI negative) 362 (M – H)–.

1-(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)-3-(p-tolyl)urea (8)

White solid, yield 88%; m.p.: 276–277 °C; silica gel TLC Rf = 0.48 (MeOH/DCM 10% v/v); δH (400 MHz, DMSO-d6) 2.27 (3H, s, 2.46 (2H, t, J 7.6), 2.83 (2H, t, J 7.6), 7.00 (1H, dd, J 2.0, 8.4), 7.09 (4H, m), 7.35 (2H, d, J 8.4), 8.48 (1H, s, exchange with D2O, NH), 8.60 (1H, s, exchange with D2O, NH), 10.07 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 21.2, 25.1, 31.6, 106.0, 112.6, 117.7, 119.1, 126.7, 128.7, 129.7, 139.5, 139.6, 140.6, 153.3, 171.2; m/z (ESI positive) 282.0 [M + H]+.

1-(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)-3-(p-tolyl)urea (9)

White solid, yield 90%; m.p.: > 300 °C; silica gel TLC Rf = 0.47 (MeOH/DCM 10% v/v); δH (400 MHz, DMSO-d6) 2.27 (3H, s, 2.46 (2H, t, J 6.8), 2.83 (2H, t, J 6.8), 6.97 (1H, t, J 7.2), 7.07 (3H, m, 7.18 (2H, m), 7.89 (2H, m), 1H exchange with D2O, NH), 9.01 (1H exchange with D2O, NH), 10.11 (1H exchange with D2O, NH); δC (100 MHz, DMSO-d6) 18.8, 25.1, 31.7, 105.9, 112.5, 117.7, 121.6, 123.4, 127.1, 128.1, 128.8, 131.1, 138.4, 139.5, 139.8, 153.4, 171.2; m/z (ESI positive) 296.0 [M + H]+.

1-(4-Chlorophenyl)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)urea (10)

White solid, yield 97%; m.p.: 249–250 °C; silica gel TLC Rf = 0.55 (Ethyl acetate/1-hexane 80% v/v); δH (400 MHz, DMSO-d6) 2.46 (2H, t, J 7.6), 2.83 (2H, t, J 7.6), 7.00 (1H, dd, J 2.0, 8.4), 7.08 (2H, m), 7.35 (2H, d, J 9.2), 7.50 (2H, d, J 9.2), 8.08 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.2, 31.7, 106.2, 112.8, 118.0, 120.5, 126.1, 128.8, 129.5, 139.5, 139.6, 139.7, 153.3, 171.2; m/z (ESI positive) 316.0 [M + H]+.

1-(2-Chlorophenyl)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)urea (11)

White solid, yield 83%; m.p.: > 300 °C; silica gel TLC Rf = 0.50 (Ethyl acetate 100% v/v); δH (400 MHz, DMSO-d6) 2.46 (2H, t, J 7.2), 2.84 (2H, t, J 7.2), 7.08 (4H, m), 7.73 (1H, t, J 8.0), 7.49 (1H, d, J 8.0), 8.20 (1H, d, J 8.0), 8.30 (1H, s, exchange with D2O, NH), 9.41 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.1, 31.5, 107.0, 113.5, 119.5, 128.8, 130.1, 130.4, 137.8, 139.2, 139.6, 139.8, 150.1, 171.1; m/z (ESI negative) 378.0 [M – H]–.
1-(4-Fluorophenyl)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)urea (12)

White solid, yield 98%; m.p.: 257–258 °C; silica gel TLC Rf = 0.59 (Ethyl acetate 100% v/v); δH (400 MHz, DMSO-d6) 2.45 (2H, t, J 7.8), 2.83 (2H, t, J 7.8), 7.00 (1H, dd, J 2.0, 8.8) 7.08 (2H, m), 7.14 (2H, m), 7.48 (2H, m), 8.62 (1H, s, exchange with D2O, NH), 8.64 (1H, s, exchange with D2O, NH), 10.08 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.2, 31.6, 112.6, 116.1 (d, 3J_1C–C_2 22), 117.9, 120.7 (d, 3J_1C–C_8 8), 128.8, 137.0 (q, 3J_1C–C_2 2), 139.5, 139.6, 153.4, 158.5 (d, 3J_1C–C 237), 171.2; δF (376 MHz, DMSO-d6) –121.5 (1 F, s); m/z (ESI positive) 300.0 [M + H]+.

1-(4-Fluoro-3-methylphenyl)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)urea (13)

White solid, yield 89%; m.p.: >300 °C; silica gel TLC Rf = 0.47 (MeOH/DCM 10% v/v); δH (400 MHz, DMSO-d6) 2.22 (3H, d, J 1.5), 2.45 (2H, t, J 7.6), 2.82 (2H, t, J 7.6), 7.00 (1H, dd, J 2.0, 8.10), 7.07 (3H, m), 7.27 (1H, m), 7.38 (1H, m), 8.55 (1H, exchange with D2O, NH), 8.64 (1H, s, exchange with D2O, NH), 10.07 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 15.3 (d, 3J_1C–C_3 3), 25.2, 31.7, 106.2, 112.8, 115.8 (d, 3J_1C–C_23), 117.9, 118.2 (d, 3J_1C–C_8 8), 122.1 (d, 3J_1C–C_10 4), 125.1 (d, 3J_1C–C_18 4), 128.8, 136.6 (d, 3J_1C–C 3), 139.5, 139.7, 153.5, 157.0 (d, 3J_1C–C_236), 171.3; δF (376 MHz, DMSO-d6) –125.9 (1F, s); m/z (ESI positive) 314.0 [M + H]+.

1-(2,4-Difluorophenyl)-3-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)urea (14)

White solid, yield 95%; m.p.: 240–241 °C; silica gel TLC Rf = 0.42 (MeOH/DCM 10% v/v); δH (400 MHz, DMSO-d6) 2.26 (2H, t, J 7.8), 2.83 (2H, t, J 7.8), 7.07 (4H, m), 7.34 (1H, m), 8.13 (1H, m), 8.47 (1H, s, exchange with D2O, NH), 9.03 (1H, s, exchange with D2O, NH), 10.11 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.2, 31.7, 104.7 (t, 3J_1C–C_4 4), 106.0, 111.9 (d, 3J_1C–C_2 4), 112.6, 118.1, 122.7, (dd, 3J_1C–C_3 3, 9.0), 125.1 (dd, 3J_1C–C_10 4, 10.0), 128.9, 139.4, 139.6, 153.1 (dd, 3J_1C–C_12 2, 244.0), 153.2, 157.7 (d, 3J_1C–C_12 0, 240.0), 171.3; δF (376 MHz, DMSO-d6) –124.3 (1F, d, J 3.0), –118.2 (1F, d, J 3.0); m/z (ESI positive) 318.0 [M + H]+.

1-(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)-3-(perfluorophenyl)urea (15)

White solid, yield 88%; m.p.: 297–298 °C; silica gel TLC Rf = 0.8 (Ethyl acetate 100% v/v); δH (400 MHz, DMSO-d6) 2.45 (2H, d, J 7.2), 2.83 (2H, t, J 7.2), 7.00 (1H, dd, J 2.0, 8.0), 7.09 (2H, m), 8.41 (1H, s, exchange with D2O, NH), 9.07 (1H, s, exchange with D2O, NH), 10.10 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.2, 31.6, 106.5, 113.1, 115.0 (m, 3J_1C–C_15 15), 118.5, 128.8, 138.1 (m, 3J_1C–C_245 245), 139.1, 139.3 (m, 3J_1C–C_245 245), 139.6, 143.9 (m, 3J_1C–C_245 245), 152.8, 171.3; δF (376 MHz, DMSO-d6) –164.3 (1F, t, J 22), –159.9 (2F, t, J 23), –146.4 (2F, d, J 20); m/z (ESI negative) 370.0 [M – H]+.

1-(2-Oxo-1,2,3,4-tetrahydroquinolin-7-yl)-3-(4-(trifluoromethyl)phenyl)urea (16)

White solid, yield 72%; m.p.: 284–285 °C; silica gel TLC Rf = 0.55 (Ethyl acetate 100% v/v); δH (400 MHz, DMSO-d6) 2.46 (2H, t, J 7.6), 2.84 (2H, t, J 7.6), 7.02 (1H, dd, J 2.0, 8.0), 7.10 (2H, d, J 8.0), 7.67 (4H, m), 8.79 (1H, s, exchange with D2O, NH), 9.01 (1H, s, exchange with D2O, NH), 10.09 (1H, s, exchange with D2O, NH); δC (100 MHz, DMSO-d6) 25.1, 31.6, 106.3, 112.9, 118.3, 117.2, 122.6 (q, 3J_1C–C_3 32), 125.4 (q, 3J_1C–C_270 270), 126.9 (q, 3J_1C–C_4 4), 128.8, 139.1, 139.5, 144.3 (q, 3J_1C–C_1 1), 153.0, 171.1; δF (376 MHz, DMSO-d6) –60.1 (3F, s); m/z (ESI positive) 350.0 [M + H]+.
A solution of 1 (1 mmol) was treated with maleic anhydride (1.05 mmol) in dry DMF then heated up to 150°C. The reaction continued until the consumption of starting materials, quenched with 1 M aqueous HCl solution to obtain a precipitate which was washed with EtO\textsubscript{2} (3 × 5 ml) and dried under vacuum to afford desired product.

White solid, yield 15%, m.p.: 281–282°C; silica gel TLC R\textsubscript{f} = 0.23 (Ethyl acetate/n-hexane 50% v/v/v); \( \delta_{\text{H}} (400 \text{ MHz}, \text{DMSO-\text{d}_6}) 2.49 (2\text{H}, d, J 7.8), 2.89 (2\text{H}, t, J 7.8), 7.12 (1\text{H}, d, J 7.6), 7.23 (3\text{H}, m), 7.41 (2\text{H}, m), 7.65 (2\text{H}, m), 8.13 (1\text{H}, m), 10.18 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 10.47 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 12.04 (1\text{H}, s, exchange with D\textsubscript{2}O, OH); \( \delta_{\text{C}} (100 \text{ MHz}, \text{DMSO-\text{d}_6}) 25.3, 31.5, 105.4 (t, J\textsubscript{C,F} 26), 108.9, 112.9 (dd, J\textsubscript{C,F} 4, 21), 115.6, 118.5 (d, J\textsubscript{C,F} 19), 120.5, 125.0 (dd, J\textsubscript{C,F} 4, 14), 126.0 (d, J\textsubscript{C,F} 1), 128.7, 130.0 (d, J\textsubscript{C,F} 2), 132.6 (d, J\textsubscript{C,F} 5, 10), 134.8 (d, J\textsubscript{C,F} 3), 137.9, 139.5, 158.7 (d, J\textsubscript{C,F} 12), 159.1, 161.1 (dd, J\textsubscript{C,F} 3, 12), 163.6 (d, J\textsubscript{C,F} 12), 167.1, 171.2; \( m/z \) (ESI negative) 393.0 [M – H]\textsuperscript{+}.

(Z)-4-oxo-4-((2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)amino)but-2-enic acid (23)

A solution of compound 1 (1 mmol) was treated with maleic anhydride (1.05 mmol) in dry DMF then heated up to 150°C. The reaction continued until the consumption of starting materials, quenched with 1 M aqueous HCl solution to obtain a precipitate which was washed with EtO\textsubscript{2} (3 × 5 ml) and dried under vacuum to obtain desired product.

White solid, yield 30%; m.p. > 300°C; \( \delta_{\text{H}} (400 \text{ MHz}, \text{DMSO-\text{d}_6}) 2.46 (2\text{H}, t, J 7.6), 2.86 (2\text{H}, t, J 7.6), 6.67 (1\text{H}, d, J 15.3), 7.16 (2\text{H}, m), 7.23 (1\text{H}, dd, J 1.8, 8.0), 7.34 (1\text{H}, d, J 1.8), 10.20 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 10.51 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 13.03 (1\text{H}, s, exchange with D\textsubscript{2}O, OH); \( \delta_{\text{C}} (100 \text{ MHz}, \text{DMSO-\text{d}_6}) 25.2, 31.4, 107.3, 113.9, 120.2, 128.8, 131.4, 138.1, 138.4, 139.5, 162.3, 167.1, 171.1; m/z (ESI positive) 261.0 [M + H]\textsuperscript{+}.

N-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)methanesulfonamide (24)

A solution of 24 (0.4 mmol) was treated with iodomethane (0.4 mmol) in dry DMF (3 ml) at 0°C, followed by addition of K\textsubscript{2}CO\textsubscript{3} (0.4 mmol) then warmed up to rt. The reaction continued until the consumption of starting materials and quenched with Et\textsubscript{3}N (1 M) to obtain a residue which was filtered, washed with Et\textsubscript{2}O (3 × 5 ml) and dried under vacuum to afford desired compound.

White solid, yield 57%; m.p.: 236–237°C; silica gel TLC R\textsubscript{f} = 0.37 (MeOH/DCM 10% v/v); \( \delta_{\text{H}} (400 \text{ MHz}, \text{DMSO-\text{d}_6}) 2.46 (2\text{H}, t, J 7.6), 2.85 (2\text{H}, t, J 7.6), 2.98 (3\text{H}, s), 6.79 (1\text{H}, dd, J 2.4, 8.0), 6.84 (1\text{H}, d, J 2.4), 7.14 (1\text{H}, d, J 2.4), 9.66 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 10.13 (1\text{H}, s, exchange with D\textsubscript{2}O, NH); \( \delta_{\text{C}} (100 \text{ MHz}, \text{DMSO-\text{d}_6}) 25.1, 31.4, 39.9, 108.0, 114.5, 120.2, 129.2, 138.2, 139.9, 171.1; m/z (ESI negative) 239.0 [M – H]\textsuperscript{+}.

N-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-7-yl) methanesulfonamide (25)

A solution of 24 (0.4 mmol) was treated with iodomethane (0.4 mmol) in dry DMSO (3 ml) at 0°C, followed by addition of Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated to obtain a residue which was purified by silica gel column chromatography eluting with ethyl acetate/n-hexane 50% v/v to afford desired compound.

White solid, yield 20%; m.p.: 220–221°C; silica gel TLC R\textsubscript{f} = 0.18 (Ethyl acetate/n-hexane 50% v/v); \( \delta_{\text{H}} (400 \text{ MHz}, \text{DMSO-\text{d}_6}) 2.15 (3\text{H}, s), 2.31 (3\text{H}, s), 2.48 (2\text{H}, t, J 7.6), 2.87 (2\text{H}, t, J 7.6), 6.87 (2\text{H}, m), 6.98 (1\text{H}, m), 7.13 (3\text{H}, m), 7.23 (1\text{H}, d, J 2.0, 8.0), 7.34 (1\text{H}, td, J 2.0, 7.8), 7.42 (1\text{H}, d, J 2.0), 7.81 (1\text{H}, dd, J 2.0, 8.0), 9.15 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 10.16 (1\text{H}, s, exchange with D\textsubscript{2}O, NH), 10.32 (1\text{H}, s, exchange with D\textsubscript{2}O, NH); \( \delta_{\text{C}} (100 \text{ MHz}, \text{DMSO-\text{d}_6}) 14.5, 21.2, 25.3, 31.5, 108.9, 115.1, 115.5, 117.9, 118.8, 120.0, 120.8, 126.2, 126.8, 128.5, 130.0, 133.2, 138.6, 138.7, 139.3, 140.1, 147.1, 168.8, 171.2; m/z (ESI negative) 384.0 [M – H]\textsuperscript{+}.
working at the absorbance maximum of 557 nm, following the initial rates of the CA-catalysed CO$_2$ hydration reaction for 10–100 s. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.01 nm) were prepared in distilled-deionised water with 5% DMSO and dilutions up to 0.1 nm were done thereafter with the assay buffer. Enzyme and inhibitor were incubated for 6 h$^{48}$. The inhibition constant ($K_i$) was obtained by considering the classical Michaelis–Menten equation which has been fitted by using non-linear least squares with PRISM 3 (La Jolla, CA). All CA isozymes used in the experiments were purified, recombinant proteins obtained as reported earlier by our group$^{50–59}$.

**Results and discussion**

**Chemistry**

In a previous report from this group$^{43}$, we showed that 7-amino-3,4-dihydroquinolin-2(1H)-one (1) (Scheme 1) possesses interesting CA inhibitory properties against many human isoforms such as hCA VII, IX, XII and XIV, some of which are important drug targets for various applications of the CAIs. The lactam 1 was investigated as a CAI due to its structural similarity with the coumarins, a class of CAIs reported by this group$^{45–48}$. Indeed, unlike other classes of such pharmacological agents, the coumarins act as prodrug inhibitors, being hydrolysed by the CA esterase activity to substituted 2-hydroxy-cinnamic acids, which thereafter bind at the entrance of the active site cavity, far away from the catalytic Zn(II) ion with which most CAIs interact$^{13,45}$. That region is the most variable among the 15 human CAs, and this explains why coumarins and their derivatives are among the most isomselective CAIs reported so far$^{13,45–48}$. In fact, a large number of substitution patterns at the coumarin ring, isosteric replacements or various other modifications were done on this chemotype, leading to a large number of CAIs possessing interesting properties$^{13,45–48}$. Thus, the rationale of this work was to derivatise the 7-amino moiety of the lead 1, by reacting it with a variety of agents used earlier for the design of sulfonamide or dithiocarbamate CAIs (Scheme 1)$^{13–16,22–25,35–37,60,61}$.

As shown in Scheme 1, a multitude of derivatisations of the amino moiety of compound 1 were achieved, such as: (i) reaction with arylsulfonyl isocyanates (leading to arylsulfonylureido derivatives 2–6); (ii) reaction with isocyanates, leading to ureas 7–19; (iii) reaction with fluoresceine isothiocyanate, leading to the fluorescent thiourea 20; (iv) condensation with substituted benzoic acids in the presence of carbodiimides, leading to the amides 21 and 22; (v) reaction with 2,4,6-trimethyl-pyrylium tetrafluoroborate, leading to the pyridinium salt 26; (vi) reaction with methylsulfonyl chloride leading to the secondary sulfonamide 24, which was subsequently methylated with methyl iodide, leading to the 1-N methyl derivative 25, and (vii) reaction with maleic anhydride leading to the monoamide 23 (Scheme 1). All these compounds were thoroughly characterised by physicochemical procedures which confirmed their structures (see “Materials and methods” for details).

**CA inhibition**

Compounds 2–26 were assayed for their CA inhibitory activity by a stopped-flow, CO$_2$ hydrase method$^{44}$ against four isoforms of

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**Scheme 1.** Synthesis of compounds 2–26.
pharmacologic relevance, the cytosolic human (h) hCA I and II, the membrane-anchored hCA IV and the transmembrane, tumour-associated hCA IX (Table 1). The following structure-activity relationship can be observed from the inhibition data of Table 1:

i. hCA I was poorly inhibited by most derivatives 2–26, with only seven of them showing $K_I$ in the micromolar range (i.e. 3.20–8.75 μm), the remaining ones having $K_I > 10 \mu$m (Table 1). The more effective inhibitors were 2–4, 6, 17 and 18, which incorporate arylsulfonylureido and ureido moieties. The other substitution patterns led to compounds with much weaker hCA I inhibitory activity.

ii. hCA II, the dominant cytosolic isoform was generally also poorly inhibited by these derivatives ($K_I > 10 \mu$m) except the arylsulfonylureido ones 2–6 ($K_I$ of 4.43–7.46 μm) the ureas 15–18 ($K_I$ of 4.97–7.88 μm) and the thiourea 20 ($K_I$ of 3.37 μm), which was the best hCA II inhibitor in the series.

iii. hCA IV was the least sensitive isofom to these compounds with only one of them (17, $K_I$ of 3.80 μm) having an activity <10 μm (Table 1). It is rather difficult to explain this result considering that the inhibition mechanism with these lactams is not yet elucidated.

iv. The tumour-associated hCA IX was the most inhibited isoform among the four investigated ones, with $K_I$ ranging between 243.6 and 2758.6 nm (Table 1). Only four derivatives (8, 10, 16 and 21) had $K_I > 10 \mu$m, whereas the best hCA IX inhibitors were 15 and 24 with $K_I$ of 243.6–292.2 nm. These compounds rather different as the first one is a urea incorporating a pentafluorophenyl moiety, whereas the second one has the secondary sulfonamide functionality. It should be noted that small variations in the structures of such derivatives (as the N1-methylation of 24 leading to 25) or the reduction of the number of fluorine atoms on the phenyl ring, as in 14, led to a rather important reduction of the $K_I$ inhibitory power compared to 24 and 15, respectively. Generally, all other arylsulfonylureas/ureas 2–19 (except the two compounds mentioned above as weak inhibitors and 15 which is one of the best) showed a similar behaviour of medium potency hCA IX inhibitors with $K_I$ of 1.05–2.48 μm.

v. All the derivatives reported here showed much weaker CA inhibitory activity compared to the clinically used sulfonamide acetazolamide AAZ (Table 1).

**Conclusions**

A series of derivatives was prepared by derivatisation of the 7-amino moiety of 7-amino-3,4-dihydroquinolin-2(1H)-one, a compound investigated earlier as CAI. The derivatisation was achieved by: (i) reaction with arylsulfonyl isocyanates (ii) reaction with aryl isocyanates; (iii) reaction with fluoresceine isoiodoocyanate; (iv) condensation with substituted benzoic acids in the presence of carbodiimides; (v) reaction with 2,4,6-trimethyl-pyrylum tetrafluoroborate; (vi) reaction with methylsulfonyl chloride and (vii) reaction with maleic anhydride. The new compounds were assayed as inhibitors of four CA human isoforms of pharmacologic relevance, the cytosolic hCA I and II, the membrane-anchored hCA IV and the transmembrane, tumour-associated hCA IX. hCA IX was the most inhibited isofom ($K_I$ ranging between 243.6 and 2658.3 nm) whereas hCA IV was not inhibited by these compounds. Most derivatives were weak hCA I and II inhibitors, with few of them showing $K_I < 10 \mu$m. Considering that the inhibition mechanism with these lactams is not yet elucidated, exploring a large range of derivatives with various substitution patterns may be useful to identify leads showing isoform selectivity.

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**Disclosure statement**

One author (CTS) declares conflict of interest, being author of several patents in the field of CA inhibitors/activators. The other authors do not declare conflict of interest.

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