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RESEARCH ARTICLE

A class of sulfonamides as carbonic anhydrase I and II inhibitors

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Abstract

Four groups of novel sulfonamide derivatives: (i) acetoxybenzamide, (ii) triacetoxybenzamide, (iii) hydroxybenzamide and (iv) trihydroxybenzamide, all having thiazole, pyrimidine, pyridine, isoxazole and thiadiazole moieties were prepared and their inhibitory effects were studied on two metalloenzymes, i.e. carbonic anhydrase isozymes (hCA I and II), purified from human erythrocyte cells by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography. These enzymes are present in almost all living organisms to catalyse the synthesis of bicarbonate ion (HCO_3^-) from carbon dioxide and water. The sulfonamide derivatives were found to be active against hCA I and II in the range of 2.62–136.54 and 5.74–210.58 nM, respectively.

Keywords

Enzyme inhibition, gallic acid, p-hydroxybenzoic acid

History

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Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc-containing metalloenzymes that catalyse the reversible reaction of carbon dioxide and water to produce bicarbonate (HCO_3^-) and proton (H^+)[1–5]. CAs play crucial pathophysiological roles including pH regulation, CO_2 homeostasis and biosynthetic reactions such as gluconeogenesis, respiration, transport of CO_2 and HCO_3^- among metabolizing tissues, lung electrolyte secretion, bone resorption, ureagenesis, lipogenesis, production of biological fluids, tumorigenicity, cell adhesion, proliferation, calcification, and in the growth and virulence of various fungal and bacterial pathogens[6–9].

CA appears to be almost ubiquitously expressed in living organisms. Thus far, six genetically distinct CA families are known, including the α-, β-, γ-, δ-, ζ- and η-class enzymes[10–13]. Currently, there are 16 known human CA isoforms, which differ in their cellular localization and rate of enzymatic activity[14]. The mammalian enzymes belong to the α-CA family and have different functions, kinetic parameters, inhibitory properties and cell and tissue localizations[14–18]. Humans encode 12 catalytically active α-CA isozymes, many of which have been studied both functionally and structurally. These CAs comprise CA I, II, III, IV, VA, VB, VI, VII, IX, XII, XIII and XIV[19]. CAs I, II, III, VII and XIII are cytosolic isozymes[20–23], CAs VA and VB are localized in mitochondria[24,25], CA VI is a unique secreted isozyme[25], CAs IX, XII and XIV are transmembrane proteins[26,27], and CAs IV and XV are glycosylphosphatidylinositol-anchored to the cell membrane[28].

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The CA isozymes have an increasing attention as important targets for designing inhibitors or activators for biomedical applications including treatments of epilepsy, glaucoma, idiopathic intracranial hypertension and altitude sickness[29–31]. Sulfonamides ($R\text{-}SO_2NH_2$) have found widespread applications in design of CA inhibitors as they are among the most important groups for zinc ion (Zn^{2+}) binding and most of the clinically used CA inhibitors contain sulfonamide moieties[32]. Since the first evidence of their CA inhibition, their physiological, pharmacological and kinetic properties were largely investigated[32–36]. Crystallographic studies of their adducts with several CA isozymes elucidate the key factors responsible for the binding of the sulfonamide moiety to the CA active site and provide a rationale for the unique tailored properties of this anchoring group[32,37,38]. Although binding of the sulfonamide derivatives was indicated to be driven by coordination of the deprotonated sulfonamide nitrogen to the catalytic zinc ion (Zn^{2+}), additional interactions with the hydrophilic and/or hydrophobic region of the active site may take place, depending on the nature of the substituent group[32,39–41]. These studies demonstrated that sulfonamide is an ideal ligand of the CA active site as it combines the negative charge of the deprotonated nitrogen with the zinc ion, and the presence of a proton on the coordinated nitrogen atom provides Thr199 atom with a strong H-bond[32].

Sulfonamides, which are the basis of several groups of drugs, were reported to have significant inhibitory activity against many CA classes. These studies are widely related with inhibition of mammalian isoforms[42,43]. It was reported that the discovery of isoform-selective CA inhibitors or at least organ-specific targeting inhibitors would represent one of the most important aims of the CA research, which has been very intense recently[44]. However, there are critical barriers need to be addressed on design of CAI as therapeutic agent such as high number of CA isoforms in human body, their rather diffuse localization in many tissues and organs,

and lack of isozyme selectivity of the currently available inhibitors of sulfonamide/sulfamates[45,46].

The natural phenolic acids, gallic (3,4,5-trihydroxybenzoic) acid and *p*-hydroxybenzoic acid, found in many plants and used as preservatives in food, possess antibacterial, anticarcinogenic, antioxidant, antifungal and antimutagenic activities, and cytotoxicity toward tumor cells[47–49]. Innocenti et al. showed that *p*-hydroxybenzoic acid had better inhibition properties on hCA I and hCA II compared with the natural products, polyphenols and phenolic acids[42]. Caffeic acid phenethyl ester which is also a polyphenolic compound, was derived from natural products. It possesses a range of biological activities[50] against such as hCA I, hCA II, hCA IX, hCA XII and comprises potent activities toward HIV-1. Wang et al. showed that designed inhibitors, mimicing caffeic acid phenethyl ester as bearing polyphenols at one side, sulphonamide moieties in the middle and various phenyl groups at the other side can be effective integrase inhibitors[51]. The study presented herein targets zinc ion (Zn^{2+}) instead of Mg^{2+} or Mn^{2+} and replaced catechol with gallic acid and *p*-hydroxyphenolic acid. In place of sulfonamide linker and phenyl group, sulfonamide drugs having thiazole, pyrimidine, pyridine, isoxazole and thiadiazole moieties were introduced. Although, it was believed that amine group of sulfonamide should be a primary agent acting as a CA inhibitor, recent studies indicated that secondary and even tertiary sulfonamides can act as affective CA inhibitors and they can be selective[52–55]. In this study, four groups of novel sulfonamide derivatives: (i) acetoxybenzamide, (ii) triacetoxybenzamide (iii) hydroxybenzamide and (iv) trihydroxybenzamide, all having thiazole, pyrimidine, pyridine, isoxazole and thiadiazole moieties are disclosed. In addition to their hydroxyl forms, we decided to investigate their acetylated derivatives since it is a general application in medicinal chemistry to esterify polar hydroxyl groups to convert them into prodrugs to promote physicochemical, biopharmaceutical and pharmacokinetic properties, membrane permeability and oral absorption[56–59]. In this study, we focus on inhibition of two metalloenzymes, CA isozymes (hCA I and II), purified from human erythrocyte cells by Sepharose-4B-L-tyrosine-sulphanilamide affinity chromatography.

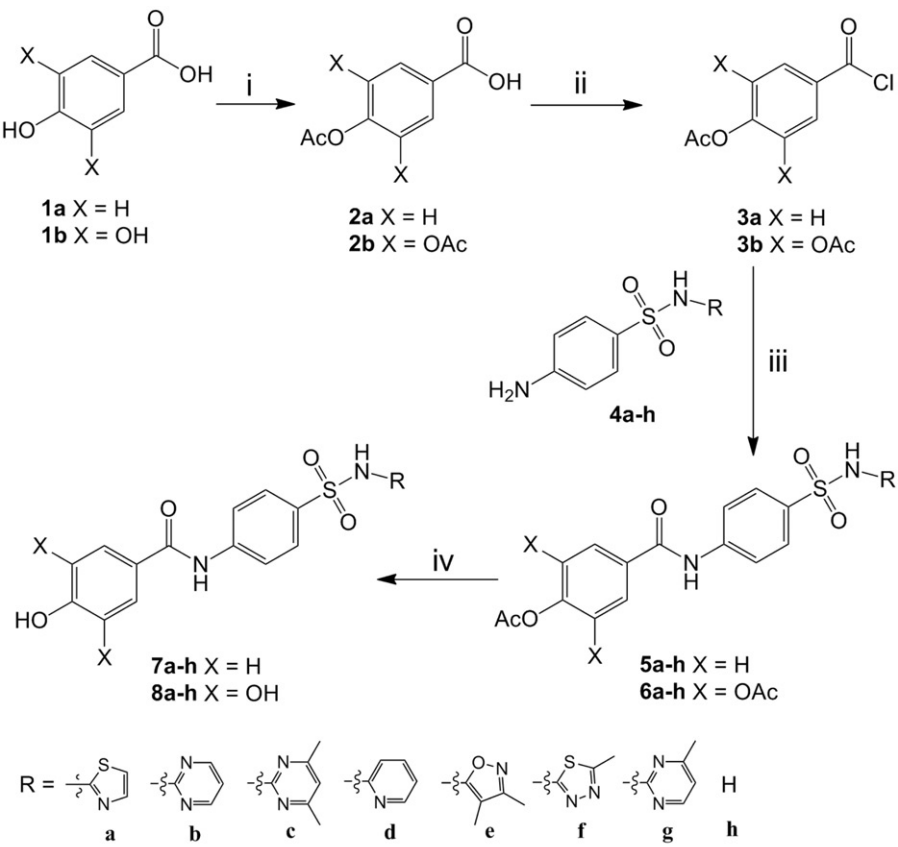
Methods

Syntheses of the sulfonamide derivatives were conducted starting from 4-hydroxy **1a** and 3,4,5-trihydroxy **1b** benzoic acids (Scheme 1), which are naturally occurring compounds in many plants and fruits[60–63]. Their reaction with acetic anhydride selectively produced 4-acetoxy **2a** and 3,4,5-triacetoxy **2b** benzoic acids, reactions of which with thionyl chloride yielded the benzoyl chlorides **3a**,**b**. Then, sulfonamides **4**, possessing thiazole, pyrimidine, pyridine, isoxazole and thiadiazole moieties, were reacted with the benzoyl chlorides **3a**,**b** to obtain first two classes of sulfonamide derivatives: (i) acetoxybenzamides **5a**–**h** and (ii) triacetoxybenzamides **6a**–**h**. Acid treatment of the first sulfonamide derivatives gave the second two classes of the derivatives **7a**–**h** and **8a**–**h**. Compounds **5g**[64], **6c**[65], **8a**[65], **8c**[66] and **8h**[51] are listed in literature but no CA inhibition activity was reported before.

Chemistry

All starting materials and the reagents were purchased from commercial suppliers. Progress of the reaction was monitored by TLC using silica gel-60 F254 plates with detection by short wave

Scheme 1. Synthesis of the compounds.



Reagents and conditions. (i) Acetic anhydride, H_2SO_4, rt, 1 h; (ii) $SOCl_2$, 80 °C, 5 h; (iii) sulfonamide, acetone, pyridine, 0 °C - rt, 20 h; (iv) THF, MeOH, HCl, 60 °C, 1 h.

UV fluorescence ($\lambda = 254$ nm) and staining with cerium sulphate. Silica gel flash chromatography was performed using silica gel 60 Å (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer at 25 °C. Chemical shifts for ^{1}H and ^{13}C NMR spectra obtained in DMSO-d_6 are reported in ppm relative to residual solvent proton ($\delta = 2.50$ ppm) and carbon ($\delta = 39.52$ ppm) signals, respectively. Multiplicity is indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet). Coupling constants are reported in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on BrukerMicroTOF-Q at positive electro spray ionization (ESI+) mode. ^{1}H and ^{13}C NMR, and HRMS spectra of compounds are provided in Supporting Information. Melting points were determined by Barnstead Electrothermal IA9200. Purities of compounds determined by C, H, N analysis by Thermo Finnigan Flash 1112 EA Series and also by HPLC-UV at 270 nm and >95%.

General procedure for acetylation of hydroxyl substituted benzoic acids 1a,b

To the stirred solution of benzoic acid **1** (20 mmol) in acetic anhydride (120 mmol), few drops of concentrated H_2SO_4 was added[67]. A fast temperature rise was observed and after all the solid was dissolved, the stirring was continued for an hour. Then, water (100 mL) was added and the solution was stirred for further 2.5 h to remove any excess acetic anhydride left. The solid precipitate was filtered, washed with water (2 × 50 mL) and dried on filter by air suction for 10 min and under vacuum for overnight.

p-Acetoxybenzoic acid (**2a**). White solid, yield 90%. ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 7.99 (d, $J = 8.6$ Hz, 2H), 7.26 (d, $J = 8.6$ Hz, 2H), 2.28 (s, 3H).

3,4,5-Triacetoxybenzoic acid (**2b**). White solid, yield 85%. ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 7.72 (s, 2H), 2.30 (s, 3H), 2.27 (s, 6H).

General procedure for preparation of acyl chlorides 3

A mixture of acetyl protected benzoic acid **2** (20 mmol) and thionyl chloride (300 mmol) was heated at 80 °C for 5 h. Excess of thionyl chloride was then evaporated under reduced pressure at 50 °C. To the residue was added acetone (20 mL) and the solution was used for the next step without further purification[51].

General procedure for preparation of *N*-(sulfonamide)-acetoxybenzamide (5 and 6)

To a mixture of sulfonamide **4** (20 mmol) and pyridine (20 mmol) in acetone (50 mL) was added dropwise acyl chloride **3** (20 mmol) in acetone (20 mL) with stirring at 0 °C, after which stirring was continued at room temperature for overnight[51]. Progress of the reaction was monitored by TLC, staining with ceric sulphate. The precipitated products were filtered. Otherwise, the solvent was evaporated under reduced pressure. The crude product was purified by re-crystallization from ethanol or flash column chromatography using ethyl acetate/hexane (1:1) mixture as an eluent.

N-(sulfathiazole)-*p*-acetoxybenzamide (5a)

Off white powder, yield 58%. m.p. 267–268 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.57 (s, 1H), 8.00 (d, $J = 8.7$ Hz, 2H), 7.93 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.31 (d, $J = 8.6$ Hz, 2H), 7.25 (d, $J = 4.6$ Hz, 1H), 6.82 (d, $J = 4.6$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.27, 169.16, 165.61, 153.57, 142.79, 137.21, 132.46, 129.79, 127.23, 124.83, 122.36, 120.18, 108.55, 21.33. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{16}N_3O_5S_2$ [M + H$^+$]: 418.0526, found 418.0526.

N-(sulfadiazine)-*p*-acetoxybenzamide (5b)

White powder, yield 55%. m.p. 269–270 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 11.71 (s, 1H), 10.61 (s, 1H), 8.51 (d, $J = 4.8$ Hz, 2H), 7.98 (m, 6H), 7.31 (d, $J = 8.7$ Hz, 2H), 7.05 (t, $J = 4.8$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.38, 165.74, 158.79, 157.37, 153.62, 143.55, 134.94, 132.41, 129.82, 129.14, 122.37, 119.95, 116.23, 21.32. HRMS (ESI$^+$): *m/z* calculated for $C_{19}H_{16}N_4O_5S_1Na_1$ [M + Na$^+$]: 435.0734, found 435.0738.

N-(sulfamethazine)-*p*-acetoxybenzamide (5c)

Off white powder, yield 62%. m.p. 245–246 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.58 (s, 1H), 8.00 (d, $J = 8.7$ Hz, 2H), 7.98 (d, $J = 8.9$ Hz, 2H), 7.94 (d, $J = 8.9$ Hz, 2H), 7.31 (d, $J = 8.6$ Hz, 2H), 6.77 (s, 1H), 2.31 (s, 3H), 2.26 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.37, 167.72, 165.66, 157.08, 153.29, 132.41, 130.72, 129.79, 129.54, 122.35, 119.60, 114.19, 112.26, 23.54, 21.31. HRMS (ESI$^+$): *m/z* calculated for $C_{21}H_{21}N_4O_5S_1$ [M + H$^+$]: 441.1227, found 441.1214.

N-(sulfapyridine)-*p*-acetoxybenzamide (5d)

White powder, yield 65%. m.p. 259–260 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.57 (s, 1H), 8.03 (d, $J = 3.6$ Hz, 1H), 7.99 (d, $J = 8.6$ Hz, 2H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.7$ Hz, 2H), 7.75–7.68 (m, 1H), 7.31 (d, $J = 8.6$ Hz, 2H), 7.15 (d, $J = 8.6$ Hz, 1H), 6.88 (t, $J = 5.9$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.94, 165.23, 153.15, 152.96, 142.55, 140.11, 136.07, 132.00, 129.38, 127.64, 126.63, 121.91, 119.75, 115.78, 113.53, 20.88. HRMS (ESI$^+$): *m/z* calculated for $C_{20}H_{18}N_3O_5S_1$ [M + H$^+$]: 412.0962, found 412.0963.

N-(sulfisoxazole)-*p*-acetoxybenzamide (5e)

Off white powder, yield 47%. m.p. 221–222 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.68 (s, 1H), 8.02 (d, $J = 8.7$ Hz, 2H), 8.00 (d, $J = 8.9$ Hz, 2H), 7.76 (d, $J = 8.9$ Hz, 2H), 7.32 (d, $J = 8.7$ Hz, 2H), 2.31 (s, 3H), 2.09 (s, 3H), 1.66 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.39, 165.81, 161.86, 155.94, 153.65, 143.95, 134.53, 132.34, 129.86, 128.20, 122.39, 120.42, 105.58, 21.33, 10.77, 6.33. HRMS (ESI$^+$): *m/z* calculated for $C_{20}H_{20}N_3O_6S_1$ [M + H$^+$]: 430.1067, found 430.1066.

N-(sulfamethizole)-*p*-acetoxybenzamide (5f)

White powder, yield 58%. m.p. 244.5–246 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.60 (s, 1H), 8.01 (d, $J = 8.7$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.78 (d, $J = 8.8$ Hz, 2H), 7.31 (d, $J = 8.7$ Hz, 2H), 2.47 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.37, 168.24, 165.65, 154.88, 153.60, 143.11, 136.74, 132.40, 129.80, 127.20, 122.36, 120.30, 21.32, 16.50. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{17}N_4O_5S_2$ [M + H$^+$]: 433.0635, found 433.0636.

N-(sulfamerazine)-*p*-acetoxybenzamide (5g)

Off white powder, yield 62%. m.p. 260–261 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 11.61 (s, 1H), 10.60 (s, 1H), 8.33 (d, $J = 5.1$ Hz, 1H), 8.00 (d, $J = 8.6$ Hz, 2H), 7.98 (d, $J = 9.0$ Hz, 2H), 7.95 (d, $J = 9.0$ Hz, 2H), 7.31 (d, $J = 8.6$ Hz, 2H), 6.91 (d, $J = 5.1$ Hz, 1H), 2.33 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.39, 168.80, 165.71, 158.10, 156.98, 153.61, 143.43, 135.12, 132.41, 129.81, 129.34, 122.36, 119.80, 115.28, 23.69, 21.30. HRMS (ESI$^+$): *m/z* calculated for $C_{19}H_{19}N_3O_8S_1$ [M + H$^+$]: 449.0887, found 449.0890.

N-(sulfanilamide)-*p*-acetoxybenzamide (5h)

White powder, yield 55%. m.p. 251–252 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.58 (s, 1H), 8.03 (d, $J = 8.6$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.82 (d, $J = 8.8$ Hz, 2H), 7.32 (d, $J = 8.6$ Hz, 2H), 7.28 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.38, 165.61, 153.58, 142.51, 139.20, 132.44, 129.79, 126.96, 122.36, 120.22, 21.32. HRMS (ESI$^+$): *m/z* calculated for $C_{15}H_{15}N_2O_5S_1$ [M + H$^+$]: 335.0696, found 335.0694.

N-(sulfathiazole)-3,4,5-triacetoxybenzamide (6a)

Yellowish powder, yield 50%. m.p. 177–178 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.63 (s, 1H), 7.90 (d, $J = 8.9$ Hz, 2H), 7.82 (s, 2H), 7.80 (d, $J = 8.8$ Hz, 2H), 7.24 (d, $J = 4.6$ Hz, 1H), 6.82 (d, $J = 4.6$ Hz, 1H), 2.34 (s, 3H), 2.33 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.77, 167.99, 166.94, 163.50, 143.14, 141.91, 137.55, 137.20, 132.46, 126.81, 124.59, 120.69, 119.95, 108.10, 20.31, 19.87. HRMS (ESI$^+$): *m/z* calculated for $C_{22}H_{20}N_3O_9S_2$ [M + H$^+$]: 534.0635, found 534.0641.

N-(sulfadiazine)-3,4,5-triacetoxybenzamide (6b)

Off white powder, yield 40%. m.p. 207–208 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.64 (s, 1H), 8.48 (d, $J = 4.8$ Hz, 2H), 7.95 (d, $J = 8.9$ Hz, 2H), 7.90 (d, $J = 8.9$ Hz, 2H), 7.79 (s, 2H), 7.02 (t, $J = 4.9$ Hz, 1H), 2.31 (s, 3H), 2.29 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.43, 167.37, 164.08, 158.79, 157.33, 143.58, 143.13, 138.03, 135.30, 132.84, 129.13, 121.15, 120.16, 116.23, 20.73, 20.29. HRMS (ESI$^+$): *m/z* calculated for $C_{23}H_{21}N_4O_9S_1$ [M + H$^+$]: 529.1024, found 529.1015.

N-(sulfamethazine)-3,4,5-triacetoxybenzamide (6c)

White powder, yield 48%. m.p. 236–238 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.64 (s, 1H), 7.99 (d, $J = 8.7$ Hz, 2H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.82 (s, 2H), 6.76 (s, 1H), 2.34 (s, 3H), 2.33 (s, 6H), 2.26 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 169.16, 168.43, 167.38, 164.01, 156.60, 143.58, 142.81, 139.52, 138.01, 135.80, 132.86, 129.55, 121.14, 119.80, 23.27, 21.45, 20.28. HRMS (ESI$^+$): *m/z* calculated for $C_{25}H_{25}N_4O_9S_1$ [M + H$^+$]: 557.1337, found 557.1336.

N-(sulfapyridine)-3,4,5-triacetoxybenzamide (6d)

White powder, yield 62%. m.p. 213–214 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.61 (s, 1H), 8.00 (s, 1H), 7.90–7.82 (m, 4H), 7.79 (s, 2H), 7.70 (t, $J = 8.0$ Hz, 1H), 7.13 (d, $J = 8.6$ Hz, 1H), 6.85 (t, $J = 6.3$ Hz, 1H), 2.32 (s, 3H), 2.30 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.44, 167.38, 164.01, 153.47, 143.89, 143.58, 142.53, 140.66, 138.00, 136.94, 132.89, 128.07, 121.13, 120.38, 116.14, 114.04, 20.73, 20.28. HRMS (ESI$^+$): *m/z* calculated for $C_{24}H_{22}N_3O_9S_1$ [M + H$^+$]: 528.1071, found 528.1068.

N-(sulfisoxazole)-3,4,5-triacetoxybenzamide (6e)

White powder, yield 54%. m.p. 147–148 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.72 (s, 1H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.82 (s, 2H), 7.75 (d, $J = 8.8$ Hz, 2H), 2.33 (s, 3H), 2.31 (s, 6H), 2.07 (s, 3H), 1.63 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.02, 166.96, 163.74, 161.43, 155.51, 143.17, 143.13, 137.65, 134.48, 132.38, 127.80, 120.79, 120.21, 105.14, 20.33, 19.89, 10.35, 5.90. HRMS (ESI$^+$): *m/z* calculated for $C_{24}H_{23}N_3O_{10}S_1Na$ [M + Na$^+$]: 568.0996, found 568.0997.

N-(sulfamethizole)-3,4,5-triacetoxybenzamide (6f)

Yellowish powder, yield 40%. m.p. 225–226 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.68 (s, 1H), 7.94 (d, $J = 8.7$ Hz, 2H), 7.84 (s, 2H), 7.80 (d, $J = 8.7$ Hz, 2H), 2.47 (s, 3H), 2.35 (s, 3H), 2.33 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 167.83 (d, $J = 22.4$ Hz), 167.75–167.27 (m), 166.84 (s), 163.46 (s), 154.39 (s), 143.06 (s), 142.19 (s), 137.49 (s), 136.56 (s), 132.30 (s), 126.69 (s), 120.63 (s), 120.01 (s), 20.21 (s), 19.77 (s), 15.97 (s). HRMS (ESI$^+$): *m/z* calculated for $C_{22}H_{20}N_4O_9S_2Na$ [M + Na$^+$]: 571.0564, found 571.0564.

N-(sulfamerazine)-3,4,5-triacetoxybenzamide (6g)

Yellowish powder, yield 42%. m.p. 234–236 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 10.66 (s, 1H), 8.33 (d, $J = 5.1$ Hz, 1H), 7.99 (d, $J = 8.9$ Hz, 2H), 7.93 (d, $J = 8.9$ Hz, 2H), 7.82 (s, 2H), 6.91 (d, $J = 5.1$ Hz, 1H), 2.34 (s, 3H), 2.33 (s, 6H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.26, 168.00, 166.95, 163.63, 157.53, 156.56, 143.17, 142.61, 137.61, 135.08, 132.45, 128.93, 120.75, 119.58, 114.85, 23.25, 20.31, 19.87. HRMS (ESI$^+$): *m/z* calculated for $C_{24}H_{23}N_4O_9S_1$ [M + H$^+$]: 543.1180, found 543.1180.

N-(sulfanilamide)-3,4,5-triacetoxybenzamide (6h)

White powder, yield 54%. m.p. 222–223 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 10.61 (s, 1H), 7.89 (d, $J = 8.8$ Hz, 2H), 7.81 (s, 2H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.26 (s, 2H), 2.32 (s, 3H), 2.30 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.02, 166.96, 163.53, 143.17, 141.69, 139.09, 137.57, 132.45, 126.57, 120.71, 120.02, 20.33, 19.88. HRMS (ESI$^+$): *m/z* calculated for $C_{19}H_{18}N_2O_9S_1Na$ [M + Na$^+$]: 473.0625, found 473.0625.

General procedure for *N*-(sulfonamide)-hydroxybenzamide (7 and 8)

N-(sulfonamide)-acetoxybenzamide **5** or **6** (1 g) was added to a solution of tetrahydrofuran (10 mL), methanol (10 mL) and concentrated hydrochloric acid (5 mL)[53]. The mixture stirred at 60 °C for one hour. The solvent was evaporated under reduced pressure and the crude product was purified by re-crystallization from ethanol or by flash column chromatography using ethyl acetate/hexane (1:1).

N-(sulfathiazole)-*p*-hydroxybenzamide (7a)

Off white powder, yield 48%. m.p. 293–294 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 12.68 (s, 1H), 10.28 (s, 1H), 10.16 (s, 1H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.86 (d, $J = 8.7$ Hz, 2H), 7.76 (d, $J = 8.8$ Hz, 2H), 7.24 (d, $J = 4.6$ Hz, 1H), 6.87 (d, $J = 8.7$ Hz, 2H), 6.81 (d, $J = 4.6$ Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.16, 165.85, 161.30, 143.19, 136.75, 130.35, 127.15, 125.33, 124.77, 120.02, 115.42, 108.48. HRMS (ESI$^+$): *m/z* calculated for $C_{16}H_{14}N_3O_4S_2$ [M + H$^+$]: 376.0420, found 376.0419.

N-(sulfadiazine)-*p*-hydroxybenzamide (7b)

White powder, yield 44%. m.p. 298–299 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.33 (s, 1H), 8.51 (d, $J = 4.9$ Hz, 2H), 7.94 (s, 4H), 7.85 (d, $J = 8.7$ Hz, 2H), 7.04 (t, $J = 4.9$ Hz, 1H), 6.87 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 165.96, 161.37, 158.77, 157.38, 143.96, 134.42, 130.39, 129.05, 125.24, 119.79, 116.21, 115.43. HRMS (ESI$^+$): *m/z* calculated for $C_{17}H_{15}N_4O_4S_1$ [M + H$^+$]: 371.0809, found 371.0817.

N-(sulfamethazine)-*p*-hydroxybenzamide (7c)

Off white powder, yield 48%. m.p. 162–163 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.31 (s, 1H), 10.17 (s, 1H), 7.95 (q, $J = 9.0$ Hz, 4H), 7.87 (d, $J = 8.7$ Hz, 2H), 6.88 (d, $J = 8.7$ Hz, 2H), 6.76 (s, 1H), 2.26 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 167.79, 165.90, 161.34, 156.67, 143.66, 134.84, 130.36, 129.46, 125.25, 119.45, 115.42, 114.01, 23.33. HRMS (ESI$^+$): *m/z* calculated for $C_{19}H_{19}N_4O_4S_1$ [M + H$^+$]: 399.1122, found 399.1123.

N-(sulfapyridine)-*p*-hydroxybenzamide (7d)

White powder, yield 50%. m.p. 282–283 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.29 (s, 1H), 8.03 (s, 1H), 7.91 (d, $J = 8.7$ Hz, 2H), 7.84 (two doublets, 4H), 7.71 (d, 1H), 7.14 (d, $J = 8.5$ Hz, 1H), 6.87 (3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 165.45, 160.93, 152.85, 143.94, 142.98, 140.02, 135.45, 129.91, 127.54, 124.76, 119.57, 115.42, 114.96, 113.43. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{16}N_3O_4S_1$ [M + H$^+$]: 370.0856, found 370.0856.

N-(sulfisoxazole)-*p*-hydroxybenzamide (7e)

Off white powder, yield 41%. m.p. 235–236 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.92 (s, 1H), 10.39 (s, 1H), 10.19 (s, 1H), 7.99 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.6$ Hz, 2H), 7.73 (d, $J = 8.8$ Hz, 2H), 6.88 (d, $J = 8.6$ Hz, 2H), 2.09 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 166.02, 161.84, 161.42, 156.01, 144.37, 134.05, 130.44, 128.12, 125.19, 120.25, 115.46, 105.52, 10.77, 6.32. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{17}N_3O_5S_1Na$ [M + Na$^+$]: 410.0781, found 410.0781.

N-(sulfamethizole)-*p*-hydroxybenzamide (7f)

Off white powder, yield 39%. m.p. 272–273 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.29 (s, 1H), 10.14 (s, 1H), 7.91 (d, $J = 8.9$ Hz, 2H), 7.84 (d, $J = 8.7$ Hz, 2H), 7.73 (d, $J = 8.8$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.20, 165.89, 161.34, 154.85, 143.54, 136.26, 130.38, 127.13, 125.27, 120.14, 115.43, 16.51. HRMS (ESI$^+$): *m/z* calculated for $C_{16}H_{15}N_4O_4S_2$ [M + H$^+$]: 391.0529, found 391.0530.

N-(sulfamerazine)-*p*-hydroxybenzamide (7g)

Off white powder, yield 50%. m.p. 271–272 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.36 (s, 1H), 8.33 (d, $J = 5.1$ Hz, 1H), 7.95 (s, 4H), 7.89–7.86 (m, 2H), 6.91 (d, $J = 5.2$ Hz, 1H), 6.90–6.87 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.65, 165.93, 161.40, 157.96, 157.00, 143.86, 134.56, 130.38, 129.22, 125.17, 119.65, 115.44, 115.35, 23.70. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{17}N_4O_4S_1$ [M + H$^+$]: 385.0965, found 385.0965.

N-(sulfanilamide)-*p*-hydroxybenzamide (7h)

White powder, yield 47%. m.p. 308–309 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.30 (s, 1H), 10.18 (s, 1H), 7.95 (d, $J = 8.9$ Hz, 2H), 7.89 (d, $J = 8.7$ Hz, 2H), 7.80 (d, $J = 8.8$ Hz, 2H), 7.26 (s, 2H), 6.89 (d, $J = 8.7$ Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 165.52, 160.93, 142.56, 138.34, 129.99, 126.54, 124.96, 119.73, 115.08. HRMS (ESI$^+$): *m/z* calculated for $C_{13}H_{13}N_2O_4S_1$ [M + H$^+$]: 293.0591, found 293.0594.

N-(sulfathiazole)-3,4,5-trihydroxybenzamide (8a)

Off white powder, yield 50%. m.p. 289–290 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.19 (s, 1H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.72 (d, $J = 8.8$ Hz, 2H), 7.22 (d, $J = 4.6$ Hz, 1H), 6.94 (s, 2H), 6.79 (d, $J = 4.6$ Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.07, 166.31, 145.96, 143.30, 137.59, 136.54, 127.09, 124.84, 124,87, 119.95, 108.50, 107.85. HRMS (ESI$^+$): *m/z* calculated for $C_{16}H_{14}N_3O_6S_2$ [M + H$^+$]: 408.0319, found 408.0315.

N-(sulfadiazine)-3,4,5-trihydroxybenzamide (8b)

Off white powder, yield 43%. m.p. 244–245 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.26 (s, 1H), 8.50 (d, $J = 4.9$ Hz, 2H), 7.96–7.89 (m, 4H), 7.04 (t, $J = 4.9$ Hz, 1H), 6.96 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 166.41, 158.77, 157.36, 145.97, 144.06, 137.67, 134.25, 128.98, 124.77, 119.72, 116.21, 107.89. HRMS (ESI$^+$): *m/z* calculated for $C_{17}H_{15}N_4O_6S_1$ [M + H$^+$]: 403.0707, found 403.0707.

N-(sulfamethazine)-3,4,5-trihydroxybenzamide (8c)

Off white powder, yield 35%. m.p. 270.5–271.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.23 (s, 1H), 7.96–7.89 (m, 4H), 6.96 (s, 2H), 6.75 (s, 1H), 2.25 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 167.47, 166.02, 156.29, 145.63, 143.43, 137.30, 134.30, 129.07, 124.45, 119.03, 113.68, 107.52, 22.99. HRMS (ESI$^+$): *m/z* calculated for $C_{19}H_{19}N_4O_6S_1$ [M + H$^+$]: 431.1020, found 431.1026.

N-(sulfapyridine)-3,4,5-trihydroxybenzamide (8d)

White powder, yield 48%. m.p. 268–269 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.21 (s, 1H), 8.04 (d, $J = 4.5$ Hz, 1H), 7.92–7.88 (m, 2H), 7.85–7.80 (m, 2H), 7.71 (ddd, $J = 8.9$, 7.3, 1.9 Hz, 1H), 7.15 (d, $J = 8.6$ Hz, 1H), 6.96 (s, 2H), 6.91–6.86 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 166.35, 153.26, 145.97, 143.53, 140.47, 137.63, 135.73, 129.22, 127.98, 124.83, 119.92, 116.40, 113.86, 107.83. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{16}N_3O_6S_1$ [M + H$^+$]: 402.0754, found 402.0754.

N-(sulfisoxazole)-3,4,5-triacetoxybenzamide (8e)

White powder, yield 57%. m.p. 252–253 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.93 (s, 1H), 10.32 (s, 1H), 7.98 (d, $J = 8.9$ Hz, 2H), 7.71 (d, $J = 8.9$ Hz, 2H), 6.99 (s, 2H), 2.09 (s, 3H), 1.66 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 166.45, 161.84, 156.01, 145.98, 144.48, 137.75, 133.90, 128.07, 124.68, 120.15, 107.85, 105.49, 10.77, 6.32. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{17}N_3O_7S_1Na$ [M + Na$^+$]: 442.0679, found 442.0675.

N-(sulfamethizole)-3,4,5-trihydroxybenzamide (8f)

Off white powder, yield 62%. m.p. 177–178 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.23 (s, 1H), 7.92 (d, $J = 8.9$ Hz, 2H), 7.73 (d, $J = 8.9$ Hz, 2H), 6.96 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 167.82, 165.98, 154.49, 145.57, 143.26, 137.27, 135.69, 126.71, 124.40, 119.69, 107.41, 16.13. HRMS (ESI$^+$): *m/z* calculated for $C_{16}H_{14}N_4O_6S_2Na$ [M + Na$^+$]: 445.0247, found 445.0249.

N-(sulfamerazine)-3,4,5-trihydroxybenzamide (8g)

White powder, yield 54%. m.p. 214–215 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8 ppm): 10.24 (s, 1H), 8.31 (d, $J = 5.1$ Hz, 1H), 7.92 (s, 4H), 6.97 (s, 2H), 6.89 (d, $J = 5.2$ Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 168.39, 166.09, 157.63, 156.66, 145.67, 143.63, 137.36, 134.09, 128.89, 124.46, 119.28, 114.99, 107.58, 23.40. HRMS (ESI$^+$): *m/z* calculated for $C_{18}H_{17}N_4O_6S_1$ [M + H$^+$]: 417.0863, found 417.0862.

Table 1. IC_{50}, K_i and the selectivity values of the compounds.

	IC_{50} (nM)		K_i (nM)				
Compounds	hCA I	hCA II	hCA I	hCA II	hCA II/hCA I	hCA I OH/Ac	hCA II OH/Ac
Sulfathiazole (4a)	77.01	77.01	136.54 ± 24.21	118.45 ± 14.90	0.87		
Sulfathiazole-*p*-acetoxybenzamide **(5a)**	17.83	15.78	12.71 ± 0.04	8.85 ± 0.03	0.70	2.20	5.84
Sulfathiazole-3,4,5-triacetoxybenzamide **(6a)**	22.35	10.66	12.21 ± 3.03	9.11 ± 1.34	0.75	2.16	7.11
Sulfathiazole-*p*-hydroxybenzamide **(7a)**	31.51	49.51	27.91 ± 1.38	51.69 ± 16.52	1.85		
Sulfathiazole-3,4,5-trihydroxybenzamide **(8a)**	36.47	43.31	26.33 ± 2.75	64.75 ± 15.30	2.46		
Sulfadiazine (4b)	77.01	86.63	46.82 ± 3.49	107.43 ± 62.80	2.29		
Sulfadiazine-*p*-acetoxybenzamide **(5b)**	7.59	40.98	6.17 ± 0.01	39.39 ± 0.03	6.38	4.44	1.74
Sulfadiazine-3,4,5-triacetoxybenzamide **(6b)**	16.12	9.91	11.82 ± 1.64	7.34 ± 0.74	0.62	5.31	19.99
Sulfadiazine-*p*-hydroxybenzamide **(7b)**	40.76	63.01	27.38 ± 1.09	68.52 ± 22.74	2.50		
Sulfadiazine-3,4,5-trihydroxybenzamide **(8b)**	49.51	63.01	62.74 ± 20.42	146.74 ± 93.16	2.34		
Sulfamethazine (4c)	36.47	115.51	22.98 ± 5.32	134.36 ± 47.61	5.85		
Sulfamethazine-*p*-acetoxybenzamide **(5c)**	9.07	47.69	7.01 ± 0.01	137.96 ± 0.64	19.68	1.34	0.12
Sulfamethazine-3,4,5-triacetoxybenzamide **(6c)**	12.38	8.77	12.43 ± 2.20	5.74 ± 1.17	0.46	2.19	7.80
Sulfamethazine-*p*-hydroxybenzamide **(7c)**	10.89	43.94	9.37 ± 0.03	16.84 ± 0.02	1.80		
Sulfamethazin-3,4,5-trihydroxybenzamide **(8c)**	27.72	49.51	27.18 ± 1.53	44.78 ± 5.57	1.65		
Sulfapyridine (4d)	34.65	20.38	23.79 ± 4.70	14.54 ± 1.12	0.61		
Sulfapyridine-*p*-acetoxybenzamide **(5d)**	11.59	53.35	7.49 ± 0.04	19.15 ± 0.03	2.56	0.35	1.97
Sulfapyridine-3,4,5-triacetoxybenzamide **(6d)**	18.24	10.19	15.69 ± 3.80	8.29 ± 2.33	0.53	0.44	4.39
Sulfapyridine-*p*-hydroxybenzamide **(7d)**	8.35	43.42	2.62 ± 0.05	37.69 ± 0.18	14.39		
Sulfapyridine-3,4,5-trihydroxybenzamide **(8d)**	9.25	40.65	6.89 ± 0.01	36.41 ± 0.13	5.28		
Sulfisoxazole (4e)	57.75	77.01	60.11 ± 6.11	210.58 ± 75.36	3.50		
Sulfisoxazole-*p*-acetoxybenzamide **(5e)**	7.38	40.43	4.82 ± 0.01	25.15 ± 0.13	5.22	1.53	3.08
Sulfisoxazole-3,4,5-triacetoxybenzamide **(6e)**	23.89	7.62	14.75 ± 0.74	10.24 ± 1.96	0.69	0.39	3.85
Sulfisoxazole-*p*-hydroxybenzamide **(7e)**	9.93	47.76	7.37 ± 0.04	77.45 ± 0.24	10.51		
Sulfisoxazole-3,4,5-trihydroxybenzamide **(8e)**	4.99	40.65	5.75 ± 0.02	39.41 ± 0.14	6.85		
Sulfamethizole (4f)	17.24	87.39	19.08 ± 0.07	31.39 ± 0.04	1.65		
Sulfamethizole-*p*-acetoxybenzamide **(5f)**	9.48	24.36	9.26 ± 0.06	29.85 ± 0.06	3.22	0.84	2.35
Sulfamethizole-3,4,5-triacetoxybenzamide **(6f)**	7.07	49.68	2.75 ± 0.05	22.61 ± 0.08	8.22	1.71	3.44
Sulfamethizole-*p*-hydroxybenzamide **(7f)**	9.54	34.48	7.81 ± 0.02	70.12 ± 0.44	8.98		
Sulfamethizole-3,4,5-trihydroxybenzamide **(8f)**	7.04	29.62	4.69 ± 0.04	77.86 ± 0.25	16.60		
Sulfamerazine (4g)	17.92	13.89	11.14 ± 0.01	26.56 ± 0.02	2.38		
Sulfamerazine-*p*-acetoxybenzamide **(5g)**	13.26	38.78	14.19 ± 0.01	64.82 ± 0.30	4.57	0.42	0.29
Sulfamerazine-3,4,5-triacetoxybenzamide **(6g)**	5.44	20.82	3.67 ± 0.02	17.37 ± 0.02	4.73	3.07	2.58
Sulfamerazine-*p*-hydroxybenzamide **(7g)**	14.07	21.07	6.01 ± 0.01	18.97 ± 0.01	3.16		
Sulfamerazine-3,4,5-trihydroxybenzamide **(8g)**	8.38	36.28	11.25 ± 0.04	44.76 ± 0.05	3.98		
Sulfanilamide (4h)	24.53	100.73	20.76 ± 0.04	120.22 ± 0.25	5.79		
Sulfanilamide-*p*-acetoxybenzamide **(5h)**	13.06	17.21	13.57 ± 0.06	33.98 ± 0.07	2.50	0.30	1.53
Sulfanilamide-3,4,5-triacetoxybenzamide **(6h)**	12.42	11.91	11.69 ± 0.01	14.92 ± 0.02	1.28	0.54	1.05
Sulfanilamide-*p*-hydroxybenzamide **(7h)**	7.01	53.31	4.06 ± 0.80	52.15 ± 4.56	12.84		
Sulfanilamide-3,4,5-trihydroxybenzamide **(8h)**	12.25	12.73	6.27 ± 0.01	15.73 ± 0.08	2.51		
***p*-Acetoxybenzoic acid (2a)**	53.31	31.51	39.11 ± 3.41	41.29 ± 29.92	1.06		
3,4,5-Triacetoxybenzamide acid (2b)	46.21	99.01	57.75 ± 7.71	104.61 ± 30.64	1.81		
***p*-Hydroxybenzoic acid (1a)**	46.21	57.75	35.69 ± 12.03	37.96 ± 1.48	1.06		
3,4,5-Trihydroxybenzoic acid (1b)	57.75	31.51	32.12 ± 5.85	38.16 ± 12.05	1.19		
AZA*	21.95	15.17	19.92 ± 0.16	9.76 ± 0.03	0.49		

*Acetazolamide (AZA) was used as a standard inhibitor for both CA isozymes.

N-(sulfanilamide)-3,4,5-trihydroxybenzamide (8h)

White powder, yield 40%. m.p. 307–308 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 10.22 (s, 1H), 7.93 (d, $J = 8.8$ Hz, 2H), 7.77 (d, $J = 8.8$ Hz, 2H), 7.25 (s, 2H), 6.99 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 166.00, 145.63, 142.69, 138.22, 137.27, 126.52, 124.53, 119.66, 107.48. HRMS (ESI$^+$): *m/z* calculated for $C_{13}H_{13}N_2O_6S_1$ [M + H$^+$]: 325.0489, found 325.0477.

Carbonic anhydrase inhibition

CA isozymes (hCA I and II) were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single purification step[2]. The column material, Sepharose-4B-L-tyrosine-sulfanilamide, was prepared according to a reported method[22,25]. Thus, pH of the solution was adjusted to 8.7, using solid Tris. Subsequently, the supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulfanilamide affinity column[68]. The proteins from the column were spectrophotometrically determined at 280 nm. For determination of the purity of the isozymes, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), having 10% and 3% acrylamide as an eluent and packing gel, respectively, with 0.1% SDS, was performed, through which a single band was observed for each isozyme.

CA isozyme activities were determined following to the methods described by Verpoorte et al.[69] and the methods reported previously. Absorbance change at 348 nm from *p*-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded by 3 min intervals at the room temperature (25 °C) using a spectrophotometer (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto, Japan). Quantity of the protein was measured spectrophotometrically at 595 nm during the purification steps according to the Bradford method. As reported previously, bovine serum

albumin (BSA) was used as a standard protein[14]. An activity (%)–[Sulfonamide] graph was depicted to determine the inhibition effect of each sulfonamide derivative. For K_i values, three different sulfonamide derivatives were tested. NPA was used as a substrate at five different concentrations and Lineweaver–Burk curves were drawn as described previousely[11].

Results and discussion

The isozymes CA I and CA II, examined in this study, have different activities. In mammals, CA II, which generally exists in red blood cells in lower concentrations, has approximately 10 times higher activity compared with CA I[30]. Cytosolic isozyme hCA I is ubiquitously expressed in body and available in high concentrations in blood and gastrointestinal tract. As it was demonstrated that this isozyme is involved in retinal and cerebral edema, its inhibition could be a valuable tool for fighting the condition. It is generally accepted that if K_i value of a tested compound is less than 50 μM ($K_i > 50$ μM), that compound is considered to be inactive against hCA I[55]. The results presented in Table 1 indicate that the new sulfonamide derivatives had effective inhibition profile against slow cytosolic isoform hCA I, and cytosolic dominant rapid isozymes hCA II. The cytosolic isozyme hCA I was inhibited by all the synthesized sulfonamide derivatives in nanomolar level, the K_i of which varied between 2.62 ± 0.05 and 136.54 ± 24.21 nM. On the other hand, acetazolamide (AZA), considered being a broad-specificity CA inhibitor owing to its widespread inhibition of CAs, showed K_i value of 19.92 ± 0.16 nM against hCA I. Among the inhibitors, sulfonamide derivative **7d** (*N*-(sulfapyridine)-*p*-hydroxybenzamide) was found to be the best hCA I inhibitor with K_i of 2.62 ± 0.05 nM. The inhibition effects of the sulfonamide derivatives (**6a–8h**) were found to be the same or lower than that of acetazolamide. Comparison of hydroxyl groups with their acetylated derivatives, in terms of hCA I inhibition, no clear trend was observed. Compound **5c** showed the best hCA I inhibition selectivity over hCA II inhibition.

The cytosolic hCA II is not only a very effective catalyst for interconversion between CO_2 and HCO_3^-, it also shows some catalytic versatility, participating in several other hydrolytic processes, which presumably involve nonphysiological substrates[32,70,71]. Against the physiologically dominant isoform hCA II, sulfonamide derivatives showed K_is varying from 5.74 ± 1.17 to 210.58 ± 75.36 nM (Table 1), among which the compound **6c**, (*N*-(sulfamethazine)-3,4,5-triacetoxybenzamide), was the best hCA II inhibitor (K_i: 5.74 ± 1.17 nM). Thus, the new sulfonamide derivatives (**6a–8h**) had high inhibition affinity toward hCA II. On the other hand, AZA, which may interact with the distinct hydrophobic and hydrophilic halves of the CA II active site, showed K_i of 9.76 ± 0.03 nM. Comparison of hydroxyl groups with their acetylated derivatives, in terms of hCA II inhibition, generally acetylated groups showed better hCA II inhibition activity. Compound **6c** showed the best hCA II inhibition selectivity over hCA I inhibition.

Conclusions

Four groups of sulfonamide derivatives, having thiazole, pyrimidine, pyridine, isoxazole and thiadiazole, groups, were synthesized and their inhibition activities toward hCA I and II isozymes purified from human erythrocyte cells by affinity chromatography were evaluated, which indicated that they are sufficiently active compared to clinically used drug AZA (acetazolamide). The most active inhibitors in the both series were found to be the compound **7d** (*N*-(sulfapyridine)-*p*-hydroxybenzamide), which had K_i value of 2.62 ± 0.05 nM for hCA I, and compound **6c** (*N*-(sulfamethazine)-3,4,5-triacetoxybenzamide) with K_i value of 5.74 ± 1.17 nM for hCA II. Although almost all the synthesized sulfonamides showed good inhibition activities compared with the used standard, AZA (acetazolamide), as a structure–activity relationship (SAR) there is no clear cut evidence on the individual effects of the introduced groups on the activities of the sulfonamides.

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Declaration of interest

The authors have declared no conflict of interest.

References

1. Gülçin İ, Beydemir Ş, Büyükokuroğlu ME. In vitro and in vivo effects of dantrolene on carbonic anhydrase enzyme activities. Biol Pharm Bull 2004;27:613–16.
2. Beydemir Ş, Gülçin İ. Effects of melatonin on carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes in vivo. J Enzyme Inhib Med Chem 2004;19:193–7.
3. ArasHisar Ş, Hisar O, Beydemir Ş, et al. Effect of vitamin E on carbonic anhydrase enzyme activity from rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vitro and in vivo. Acta Vet Hung 2004;52:413–22.
4. Topal M, Gülçin İ. Rosmarinic acid: a potent carbonic anhydrase isoenzymes inhibitor. Turk J Chem 2014;38:894–902.
5. Rahman TU, Khattrak KF, Liaqat W, Zaman K. Characterization of one novel flavone and four new source compounds from the bark of *Milletia ovalifolia* and in vitro inhibition of carbonic anhydrase-II by the novel flavonoid. Rec Nat Prod 2015;9:553–60.
6. Şentürk M, Gülçin İ, Daştan A, et al. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. Bioorg Med Chem 2009;17:3207–11.
7. Innocenti A, Gülçin İ, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Antioxidant polyphenols effectively inhibit mammalian isoforms I-XV. Bioorg Med Chem Lett 2010;20:5050–3.
8. ÖztürkSarikaya SB, Topal F, Şentürk M, et al. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. Bioorg Med Chem Lett 2011;21:4259–62.
9. Gülçin İ, Beydemir S. Phenolic compounds as antioxidants: carbonic anhydrase isoenzymes inhibitors. Mini Rev Med Chem 2013;13:408–30.
10. Zimmerman SA, Ferry JG, Supuran CT. Inhibition of the archaeal beta-class (Cab) and gamma-class (Cam) carbonic anhydrases. Curr Top Med Chem 2007;7:901–8.
11. Çetinkaya Y, Göçer H, Göksu S, Gülçin İ. Synthesis and carbonic anhydrase isoenzymes I and II inhibitory effects of novel benzylamine derivatives. J Enzyme Inhib Med Chem 2014;29:168–74.
12. Çetinkaya Y, Göçer H, Gülçin İ, Menzek A. Synthesis and carbonic anhydrase isoenzymes inhibitory effects of brominated diphenylmethanone and its derivatives. Arch Pharm (Weinheim) 2014;347:354–9.
13. Güney M, Coşkun A, Topal F, et al. Oxidation of cyanobenzocycloheptatrienes: synthesis, photooxygenation reaction and carbonic anhydrase isoenzymes inhibition properties of some new benzotropone derivatives. Bioorg Med Chem 2014;22:3537–43.
14. Boztaş M, Çetinkaya Y, Topal M, et al. Synthesis and carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties. J Med Chem 2015;58:640–50.
15. Hisar O, Beydemir Ş, Gülçin İ, et al. The effects of melatonin hormone on carbonic anhydrase enzyme activity in rainbow trout (*Oncorhynchus mykiss*) erythrocytes in vitro and in vivo. Turk J Vet Anim Sci 2005;29:841–5.

16. Çoban TA, Beydemir Ş, Gülçin İ, Ekinci D. Morphine inhibits erythrocyte carbonic anhydrase in vitro and in vivo. Biol Pharm Bull 2007;30:2257–61.
17. Çoban TA, Beydemir Ş, Gülçin İ, Ekinci D. The effect of ethanol on erythrocyte carbonic anhydrase isoenzymes activity: an in vitro and in vivo study. J Enzyme Inhib Med Chem 2008;23:266–70.
18. Çankaya M, Hernandez AM, Ciftci M, et al. An analysis of expression patterns of genes encoding proteins with catalytic activities. BMC Genomics 2007;8:232.
19. Tanpure RP, Ren B, Peat TS, et al. carbonic anhydrase inhibitors with dual-tail moieties to match the hydrophobic and hydrophilic halves of the carbonic anhydrase active site. J Med Chem 2015;58: 1494–501.
20. Montgomery JC, Venta PJ, Eddy RL, et al. Characterization of the human gene for a newly discovered carbonic anhydrase, CA VII, and its localization to chromosome 16. Genomics 1991;11:835–48.
21. Lehtonen J, Shen B, Vihinen M, et al. Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family. J Biol Chem 2004;279:2719–27.
22. Akbaba Y, Akıncıoğlu A, Göçer H, et al. Carbonic anhydrase inhibitory properties of novel sulfonamide derivatives of aminoindanes and aminotetralins. J Enzyme Inhib Med Chem 2014;29: 35–42.
23. Akbaba Y, Bastem E, Topal F, et al. Synthesis and carbonic anhydrase inhibitory effects of novel sulfamides derived from 1-aminoindanes and anilines. Arch Pharm (Weinheim) 2014;347: 950–7.
24. FujikawaAdachi K, Nishimori I, Taguchi T, Onishi S. Human mitochondrial carbonic anhydrase VB. cDNA cloning, mRNA expression, subcellular localization, and mapping to chromosome x. J Biol Chem 1999;274:21228–33.
25. Aksu K, Nar M, Tanç M, et al. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine. Bioorg Med Chem 2013;21:2925–31.
26. FujikawaAdachi K, Nishimori I, Taguchi T, Onishi S. Human carbonic anhydrase XIV (CA14): cDNA cloning, mRNA expression, and mapping to chromosome 1. Genomics 1999;61:74–81.
27. Ratto F, Witort E, Tatini F, et al. Plasmonic particles that hit hypoxic cells. Adv Funct Mater 2015;25:316–23.
28. Hilvo M, Salzano AM, Innocenti A, et al. Cloning, expression, post-translational modifications and inhibition studies on the latest mammalian carbonic anhydrase isoform, CA XV. J Med Chem 2009;52:646–54.
29. Göçer H, Akıncıoğlu A, Öztaşkın N, et al. Synthesis, antioxidant, and antiacetylcholinesterase activities of sulfonamide derivatives of dopamine-related compounds. Arch Pharm (Weinheim) 2013;346: 783–92.
30. Göçer H, Çetinkaya Y, Göksu S, et al. Carbonic anhydrase and acetylcholinesterase inhibitory effects of carbamates and sulfamoylcarbamates. J Enzyme Inhib Med Chem 2015;30:316–20.
31. Şentürk M, Gülçin İ, Beydemir Ş, et al. In vitro inhibition of human carbonic anhydrase I and II isozymes with natural phenolic compounds. Chem Biol Drug Des 2011;77:494–9.
32. Alterio V, Di Fiore A, D'Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem Rev 2012;112:4421–68.
33. Clare BW, Supuran CT. A perspective on quantitative structure–activity relationships and carbonic anhydrase inhibitors. Expert Opin Drug Metab Toxicol 2006;2:113–37.
34. Thiry A, Dogne' JM, Supuran CT, Masereel B. Anticonvulsant sulfonamides/sulfamates/sulfamides with carbonic anhydrase inhibitory activity: drug design and mechanism of action. Curr Pharm Des 2008;14:661–71.
35. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
36. Winum JY, Monter JL, Scozzafava A, Supuran CT. Functional, structural, and disease applications. In: Supuran CT, Winum JY, Wang B, eds. Drug design of zinc-enzyme inhibitors. Hoboken (NJ): Wiley; 2009:39.
37. Alterio V, Di Fiore A, D'Ambrosio K, et al. Functional, structural, and disease applications. In: Supuran CT, Winum JY, Wang B, eds. Drug design of zinc-enzyme inhibitors. Hoboken (NJ): Wiley; 2009:73.
38. Di Fiore A, Monti SM, Hilvo M, et al. Crystal structure of human carbonic anhydrase XIII and its complex with the inhibitor acetazolamide. Proteins 2008;74:164–75.
39. King RW, Burgen AS. Sulphonamide complexes of human carbonic anhydrases. Ultraviolet difference spectroscopy. Biochim Biophys Acta 1970;207:278–85.
40. Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. Med Res Rev 2003;23:146–89.
41. Abbate F, Supuran CT, Scozzafava A, et al. Nonaromatic sulfonamide group as an ideal anchor for potent human carbonic anhydrase inhibitors: role of hydrogen-bonding networks in ligand binding and drug design. J Med Chem 2002;45:3583–7.
42. Innocenti A, ÖztürkSarıkaya SB, Gülçin İ, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of mammalian isoforms I-XIV with a series of natural product polyphenols and phenolic acids. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine. Bioorg Med Chem 2010;18:2159–64.
43. Marini AM, Maresca A, Aggarwal M, et al. Tricyclic sulfonamides incorporating benzothiopyrano[4,3-c]pyrazole and pyridothiopyrano[4,3-c]pyrazole effectively inhibit α- and β-carbonic anhydrase: x-ray crystallography and solution investigations on 15 Isoforms. J Med Chem 2012;55:9619–29.
44. Ceruso M, Antel S, Vullo D, et al. Inhibition studies of new ureido-substituted sulfonamides incorporating a GABA moiety against human carbonic anhydrase isoforms I-XIV. Synthesis and carbonic anhydrase inhibitory properties of sulfamides structurally related to dopamine. Bioorg Med Chem 2014;22:6768–75.
45. Maresca A, Temperini C, Pochet L, et al. Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. J Med Chem 2010;53:335–44.
46. ÖztürkSarikaya SB, Gülçin İ, Supuran CT. Carbonic anhydrase inhibitors: inhibition of human erythrocyte isozymes I and II with a series of phenolic acids. Chem Biol Drug Des 2010;75:515–20.
47. Chen H-M, Wu Y-C, Chia Y-C, et al. Gallic acid, a major component of *Toona sinensis* leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. Cancer Lett 2009;286:161–71.
48. Hsu C-L, Lo W-H, Yen G-C. Gallic acid induces apoptosis in 3T3-L1 pre-adipocytes via a Fas- and mitochondrial-mediated pathway. J Agric Food Chem 2007;55:7359–65.
49. Manuja R, Sachdeva S, Jain A, Chaudhary J. A comprehensive review on biological activities of P-hydroxy benzoic acid and its derivatives. Int J Pharm Sci Rev Res 2013;22:109–15.
50. Gülçin İ, Scozzafava A, Supuran CT, et al. The effect of caffeic acid phenethyl ester (CAPE) on metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, lactoperoxidase, and carbonic anhydrase isoenzymes I, II, IX, and XII. J Enzyme Inhib Med Chem 2015. [Epub ahead of print]. DOI: 10.3109/14756366.2015.1094470.
51. Wang P, Liu C, Sanches T, et al. Design and synthesis of novel nitrogen-containing polyhydroxylated aromatics as HIV-1 integrase inhibitors from caffeic acid phenethyl ester. Bioorg Med Chem Lett 2009;19:4574–8.
52. Moeker J, Peat TS, Bornaghi LF, et al. Cyclic secondary sulfonamides: unusually good inhibitors of cancer-related carbonic anhydrase enzymes. J Med Chem 2014;57:3522–31.
53. Di Fiore A, Maresca A, Alterio V, et al. Carbonic anhydrase inhibitors: X-ray crystallographic studies for the binding of N-substituted benzenesulfonamides to human isoform II. Chem Commun 2011;47:11636–8.
54. Métayer B, Mingot A, Vullo D, et al. New superacid synthesized (fluorinated) tertiary benzenesulfonamides acting as selective hCA IX inhibitors: toward a new mode of carbonic anhydrase inhibition by sulfonamides. Chem Commun 2013;49:6015–17.
55. Yıldırım A, Atmaca U, Keskin A, et al. N-Acylsulfonamides strongly inhibit human carbonic anhydrase isoenzymes I and II. Bioorg Med Chem 2015;23:2598–605.
56. Ahmed M, Azam F, Gbaj A, et al. Ester prodrugs of ketoprofen: synthesis, *in vitro* stability, *in vivo* biological evaluation and *in silico* comparative docking studies against COX-1 and COX-2. Curr Drug Discov Technol 2016;13:41–57.
57. Carroux CJ, Rankin GM, Moeker J, et al. A prodrug approach toward cancer-related carbonic anhydrase inhibition. J Med Chem 2013;56:9623–34.
58. Müller CE. Prodrug approaches for enhancing the bioavailability of drugs with low solubility. Chem Biodivers 2009;6:2071–83.
59. Lv P-C, Elsayed MSA, Agama K, et al. Design, synthesis, and biological evaluation of potential prodrugs related to the

experimental anticancer agent indotecan (LMP400). J Med Chem 2016;59:4890–9.
60. Farhat MB, Landoulsi A, Chaouch-Hamada R, et al. Profiling of essential oils and polyphenolics of *Salvia argentea* and evaluation of its by-products antioxidant activity. Ind Crops Prod 2013;47: 106–12.
61. Smeriglio A, Mandalari G, Bisignano C, et al. Polyphenolic content and biological properties of Avola almond (*Prunus dulcis* Mill. D.A. Webb) skin and its industrial byproducts. Ind Crops Prod 2016;83: 283–93.
62. Kıvrak İ, Kıvrak Ş, Harmandar M, Çetintaş Y. Phenolic compounds of *Pinus brutia* Ten.: chemical investigation and quantitative analysis using an ultra-performance liquid chromatography tandem mass spectrometry with electrospray ionization source. Rec Nat Prod 2013;7:313–19.
63. Kalın P, Gülçin İ, Gören AC. Antioxidant activity and polyphenol content of *Cranberries* (*Vaccinium macrocarpon*). Rec Nat Prod 2015;9:496–502.
64. Patki VM, Shirsat MV. Chemotherapy of intestinal infections. I. Synthesis of N4-acyl derivatives of N1-substituted sulfanilamides. J Sci Ind Res 1959;18C:113–16.
65. Lin C, Lin X, Liu B, et al. Preparation of 3,4,5-trihydroxygallic acid amide containing benzene sulphonamide derivatives for treating osteoarthritis. Fam Zhuanli Shenqing 2014; CN 104193690 A 20141210.
66. Lin X, Zheng L, Liu Q, et al. In vitro effect of a synthesized sulfonamido-based gallate on articular chondrocyte metabolism. Bioorg Med Chem Lett 2014;24:2497–503.
67. Ye J, Abiman P, Crossley A, et al. Building block syntheses of gallic acid monomers and tris-(*o*-gallyl)-gallic acid dendrimers chemically attached to graphite powder: a comparative study of their uptake of Al(III) ions. Langmuir 2010;26:1776–85.
68. Nar M, Çetinkaya Y, Gülçin İ, Menzek A. (3,4-Dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. J Enzyme Inhib Med Chem 2013;28:402–6.
69. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967;242:4221–9.
70. Taslimi P, Gulcin İ, Ozgeris B, et al. The human carbonic anhydrase isoenzymes I and II (hCA I and II) inhibition effects of trimethoxyindane derivatives. J Enzyme Inhib Med Chem 2016; 31:152–7.
71. Scozzafava A, Kalın P, Supuran CT, et al. The impact of hydroquinone on acetylcholine esterase and certain human carbonic anhydrase isoenzymes (hCA I, II, IX, and XII). J Enzyme Inhib Med Chem 2015;30:941–6.

Supplementary material available online