

Benzenesulfonamides with benzimidazole moieties as inhibitors of carbonic anhydrases I, II, VII, XII and XIII

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Abstract

A series of benzenesulfonamide derivatives, bearing benzimidazole moieties, were designed and synthesized as inhibitors of carbonic anhydrases (CAs). Their binding affinities to recombinant human CA isozymes I, II, VII, XII and XIII were determined by the thermal shift assay. A group of compounds containing a benzimidazole substituent in the *para* position of the benzenesulfonamide ring was found to exhibit higher binding potency toward tested CAs than *meta*-substituted benzenesulfonamides. Some of these compounds exhibited nanomolar affinities and selectivity toward the CA isozymes tested.

Keywords

Benzenesulfonamide, carbonic anhydrase, *N*-alkylated benzimidazoles, thermal shift assay, ThermoFluor[®]

History

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Introduction

Carbonic anhydrases (CAs) are widely investigated enzymes due to their involvement in various physiological and pathological processes^{1–4}. There are a total of 15 CA isoforms found in humans. However, three of them have no catalytic activity (CA VIII, X and XI). The 12 active isoforms (CA I–IV, VA, VB, VI, VII, IX, XII and XIV) are metalloenzymes, which participate in the regulation of acid–base balance and ion transport in many tissues and organs. The enhanced activity of CAs is often related to various diseases. The isoforms CA IX and CA XII were intensively investigated enzymes during the last decade due to their overexpression in various human cancers^{5–9}. Much work has been done to design selective CA IX and CA XII inhibitors. Some compounds such as glycosyl coumarins, *N*- β -glycosyl sulfamides and ureido-substituted sulfamates were found to be low nanomolar CA IX and XII inhibitors, while showing weak inhibition toward cytosolic isoforms CA I and CA II^{10–12}. Numerous inhibitors of various CA isoforms were reviewed by Alterio et al.¹. The design of CA inhibitors as drugs has been impeded by the large number of isoforms, their diffusion among many tissues/organs and the lack of isozyme-selective inhibitors among existing sulfonamides¹³. As the differences between the active sites of the 12 catalytically active human CAs are very subtle, CAs have proved to be a challenging drug target for the development of isozyme-specific inhibitors^{2,14}.

Primary sulfonamides present an important class of compounds which have been studied as drugs and drug candidates against diseases such as glaucoma, inflammation, edema,

epilepsy, dandruff, cancer and as antiviral compounds^{15,16}. The majority of clinically used CA inhibitors are also primary sulfonamides¹. To date, CA inhibitors such as acetazolamide (AZM), ethoxzolamide (EZA) and methazolamide are widely used as systemic antiglaucoma drugs¹⁷; sultiame and zonisamide as antiepileptic drugs¹⁸; and chlorthalidone, indapamide and furosemide as diuretics¹⁹ (Figure 1). The largest numbers of new CA inhibitors are benzenesulfonamides^{1,14}.

Benzenesulfonamides bind to the active site of CAs in their deprotonated form, with the nitrogen atom of the sulfonamide moiety coordinated to the Zn(II) ion. Depending on the nature of the substituent, additional interactions with hydrophobic and hydrophilic amino acids in the CA active center can be assigned. Therefore, a variety of substitutions on the benzenesulfonamide head group can be used for the development of novel inhibitors.

Recently, we have investigated the interaction of several CA isozymes with pyrimidine derivatives such as [(2-pyrimidinylthio)acetyl]benzenesulfonamides²⁰, 4-[*N*-(substituted 4-pyrimidinyl)amino]benzenesulfonamides²¹ and thiaziazole derivatives²². We have also reported on the synthesis of 2-substituted benzimidazoles *N*-alkylated with 2-chloro-5-bromoacetylbenzenesulfonamide (Figure 2, compounds **4(a–j)**) and their binding to four CA isozymes, namely CA I, CA II, CA VII and CA XIII²³. We extended these earlier investigations to new sulfonamides containing a benzimidazole group (compounds of series **1–3**).

Here we present the binding data of **4(a–j)** to the catalytic domain of CA XII, an anticancer drug target. The *N*-alkylated benzimidazoles **4(a–j)** exhibited dissociation constants in the range of 0.3–10 μ M against tested CAs. Furthermore, we compared series **4** to compounds of series **1–3**, which are 4-[(2-substituted-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide **1(a–j)**, 2-chloro-4-[(2-substituted-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide **2(a–j)** and 3-[(2-substituted-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide **3(a–j)**. Their binding was

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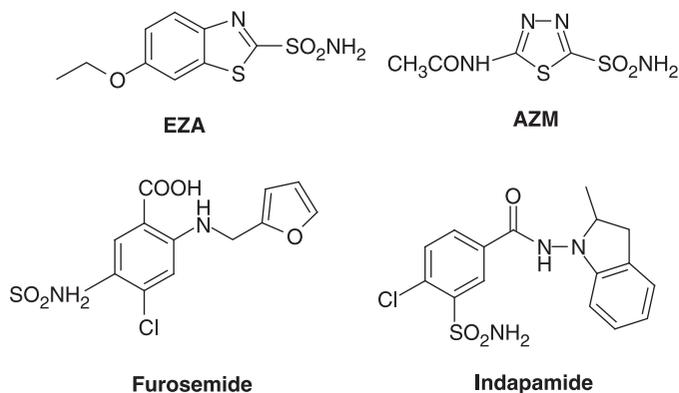
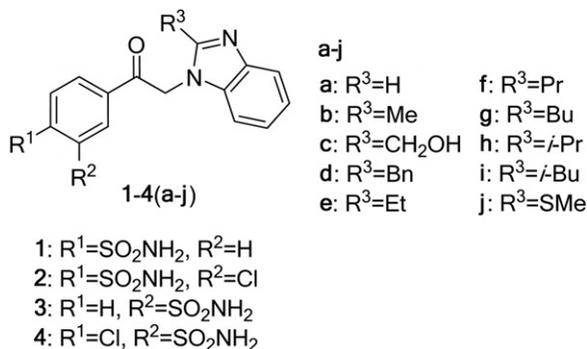


Figure 1. Chemical structures of some clinically used sulfonamides.

Figure 2. The structure of *N*-alkylated benzimidazole derivatives.

then assayed with five CA isozymes CA I, CA II, CA VII, CA XII and CA XIII. The compounds **2(a–j)** were found to be the most potent CA inhibitors exhibiting nanomolar affinities toward CA I, II, VII and XIII. Detailed structure–activity correlation could be drawn by comparing the affinities and selectivity of these compounds.

Materials and methods

Syntheses

Synthesis of 4-(bromoacetyl)benzenesulfonamide (**1**) and 3-(bromoacetyl)benzenesulfonamide (**3**) was achieved from commercially available 1-(4-aminophenyl)ethanone and 1-(3-aminophenyl)ethanone, respectively, as described by Fujikura et al.²⁴. Synthesis of 4-(bromoacetyl)-2-chlorobenzenesulfonamide (**2**) was accomplished from 1-(4-amino-3-chlorophenyl)ethanone as described by Fujikura et al.²⁴. 1-(4-Amino-3-chlorophenyl)ethanone was synthesized by the chlorination of 1-(3-aminophenyl)-*N*-ethanone with *N*-chlorosuccinimide in acetonitrile^{25,26}. 2-Substituted benzimidazoles (**b–i**) were prepared from 1,2-benzenediamine and the appropriate carboxylic acids according to Phillips' procedure²⁷. Benzimidazole (**a**) and 2-methylthiobenzimidazole (**j**) are commercially available and were used without further purification. All ingredients were purchased from Sigma-Aldrich (Milwaukee, WI) and Alfa Aesar GmbH (Karlsruhe, Germany).

The melting points of the compounds were determined in open capillaries on a Thermo Scientific 9100 Series apparatus (Rochford, UK) without further correction. IR spectra were obtained on a Perkin-Elmer (Upplands Vasby, Sweden) FT-IR spectrophotometer Spectrum BX II in KBr. 1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer (Palo Alto, CA) (300 and 75 MHz, respectively) in DMSO- d_6 using residual DMSO signals (2.52 and 40.21 ppm for 1H and ^{13}C NMR

spectra, respectively) as the internal standard. Thin-layer chromatography was performed with silica gel 60 F254 aluminum plates (Merck, Darmstadt, Germany) and visualized with UV light. High-resolution mass spectra (HRMS) were recorded on a Dual-ESI Q-TOF 6520 mass spectrometer (Agilent Technologies, Santa Clara, CA). The purities of target compounds were analyzed using an HPLC system with UV detection.

General procedure for the synthesis of 1–3(a–j)

A mixture of the corresponding compounds **1–3** (0.360 mmol), appropriate 2-substituted benzimidazole (**a–j**) (0.540 mmol) and sodium acetate (33.9 mg, 0.414 mmol) in tetrahydrofuran (2 ml) were stirred at r.t. for 24 h. The reaction mixture was poured into water. The precipitate was filtered out and then washed with water and diethyl ether to yield **1–3(a–j)**.

4-(1H-benzimidazol-1-ylacetyl)benzenesulfonamide (1a). Yield 60%, m.p. 242–244 °C. IR (ν , cm^{-1}): 3358 (NH₂), 1701 (CO). 1H NMR δ ppm: 6.11 (2H, s, CH₂CO), 7.24–7.27 (2H, m, C_{5',6'}-H), 7.57–7.60 (1H, m, C_{7'}-H), 7.67 (2H, s, NH₂), 7.70–7.73 (1H, m, C_{4'}-H), 8.07 (2H, d, $J=8.4$ Hz, C_{2,6'}-H), 8.24 (1H, s, C_{2'}-H), 8.31 (2H, d, $J=8.1$ Hz, C_{3,5'}-H). ^{13}C NMR δ ppm: 51.82, 111.38, 119.97, 122.32, 123.14, 126.80, 129.66, 135.31, 137.46, 143.60, 145.57, 149.09, 193.67. HRMS Calcd for C₁₅H₁₃N₃O₃S ([M + H]⁺): 316.0750, Found: 316.0746.

4-(1H-benzimidazol-1-ylacetyl)-2-chlorobenzenesulfonamide (2a). Yield 72%, m.p. 234–236 °C. IR (ν , cm^{-1}): 3379 (NH₂), 1705 (CO). 1H NMR δ ppm: 6.10 (2H, s, CH₂CO), 7.23–7.26 (2H, m, C_{5',6'}-H), 7.57–7.60 (1H, m, C_{7'}-H), 7.70–7.72 (1H, m, C_{4'}-H), 7.93 (2H, s, NH₂), 8.19–8.23 (3H, m, C_{2',5,6'}-H), 8.33 (1H, s, C_{3'}-H). ^{13}C NMR δ ppm: 51.88, 111.37, 120.05, 122.27, 123.09, 127.72, 130.18, 131.75 (2C), 135.33, 138.83, 143.77, 145.50, 145.67, 192.98. HRMS Calcd for C₁₅H₁₂ClN₃O₃S ([M + H]⁺): 350.0361, Found: 350.0363.

3-(1H-benzimidazol-1-ylacetyl)benzenesulfonamide (3a). Yield 83%, m.p. 247–249 °C. IR (ν , cm^{-1}): 3270 (NH₂), 1698 (CO). 1H NMR δ ppm: 6.13 (2H, s, CH₂CO), 7.22–7.28 (2H, m, C_{5',6'}-H), 7.57–7.61 (1H, m, C_{7'}-H), 7.64 (2H, s, NH₂), 7.69–7.75 (1H, m, C_{4'}-H), 7.88 (1H, t, $J=7.8$ Hz, C_{5'}-H), 8.19 (1H, d, $J=7.8$ Hz, C_{4'}-H), 8.22 (1H, s, C_{2'}-H), 8.40 (1H, d, $J=7.8$ Hz, C_{6'}-H), 8.50 (1H, s, C_{2'}-H). ^{13}C NMR δ ppm: 15.71, 111.38, 120.04, 122.23, 123.08, 125.63, 130.60, 131.18, 132.26, 135.41, 135.74, 143.81, 145.60, 145.69, 193.48. HRMS Calcd for C₁₅H₁₃N₃O₃S ([M + H]⁺): 316.0750, Found: 316.0752.

4-[(2-Methyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1b). Yield 63%, m.p. 146–148 °C. IR (ν , cm^{-1}): 3336 (NH₂), 1698 (CO). 1H NMR δ ppm: 2.45 (3H, s, CH₃), 6.06 (2H, s, CH₂CO), 7.14–7.21 (2H, m, C_{5',6'}-H), 7.46–7.49 (1H, m, C_{7'}-H), 7.57–7.60 (1H, m, C_{4'}-H), 7.66 (2H, s, NH₂), 8.07 (2H, d, $J=8.4$ Hz, C_{2,6'}-H), 8.32 (2H, d, $J=8.1$ Hz, C_{3,5'}-H). ^{13}C NMR δ ppm: 14.00, 50.84, 110.60, 118.80, 122.06, 122.23, 126.74, 129.79, 136.53, 137.47, 142.88, 149.21, 153.31, 193.81. HRMS Calcd for C₁₆H₁₅N₃O₃S ([M + H]⁺): 330.0907, Found: 330.0909.

2-Chloro-4-[(2-methyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2b). Yield 74%, m.p. 204–206 °C. IR (ν , cm^{-1}): 3385, 3332 (NH₂), 1707 (CO). 1H NMR δ ppm: 2.45 (3H, s, CH₃), 6.09 (2H, s, CH₂CO), 7.14–7.20 (2H, m, C_{5',6'}-H), 7.47–7.50 (1H, m, C_{7'}-H), 7.57–7.61 (1H, m, C_{4'}-H), 7.94 (2H, s, NH₂), 8.20 (2H, s, C_{5,6'}-H), 8.37 (1H, s, C_{3'}-H). ^{13}C NMR δ ppm: 13.97, 51.01, 110.75, 118.71, 122.19, 122.32, 127.80, 130.09, 131.71, 132.06, 136.41, 138.72, 142.59, 145.78, 153.29, 193.02. HRMS Calcd for C₁₆H₁₄ClN₃O₃S ([M + H]⁺): 364.0517, Found: 364.0517.

3-[(2-Methyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3b). Yield 62%, m.p. 295–297 °C. IR (ν , cm^{-1}): 3280 (NH₂), 1699 (CO). 1H NMR δ ppm: 2.46 (3H, s, CH₃), 6.09

(2H, s, CH₂CO), 7.14–7.21 (2H, m, C_{5',6'}-H), 7.47–7.51 (1H, m, C₇-H), 7.57–7.60 (1H, m, C_{4'}-H), 7.63 (2H, s, NH₂), 7.89 (1H, t, *J* = 7.8 Hz, C₅-H), 8.20 (1H, d, *J* = 8.1 Hz, C₄-H), 8.44 (1H, d, *J* = 7.8 Hz, C₆-H), 8.51 (1H, s, C₂-H). ¹³C NMR δ ppm: 14.03, 50.78, 110.62, 118.83, 122.03, 122.21, 125.70, 130.57, 131.31, 132.51, 135.66, 136.58, 142.96, 145.66, 153.33, 193.59. HRMS Calcd for C₁₆H₁₅N₃O₃S ([M + H]⁺): 330.0907, Found: 330.0903.

4-[(2-(Hydroxymethyl)-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1c). Yield 57%, m.p. 200–202 °C. IR (ν, cm⁻¹): 3375, 3314 (NH₂, OH), 1702 (CO). ¹H NMR δ ppm: 4.70 (2H, s, CH₂OH), 5.64 (2H, br s, OH), 6.09 (2H, s, CH₂CO), 7.20–7.26 (2H, m, C_{5',6'}-H), 7.53–7.56 (1H, m, C₇-H), 7.64–7.68 (3H, m, C₄-H, NH₂), 8.06 (2H, d, *J* = 8.4 Hz, C_{2,6}-H), 8.31 (2H, d, *J* = 8.4 Hz, C_{3,5}-H). ¹³C NMR δ ppm: 51.04, 57.59, 111.02, 119.59, 122.31, 123.01, 126.76, 129.72, 137.00, 137.54, 142.33, 149.04, 154.76, 193.49. HRMS Calcd for C₁₆H₁₅N₃O₄S ([M + H]⁺): 346.0856, Found: 346.0859.

2-Chloro-4-[(2-(hydroxymethyl)-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2c). Yield 52%, m.p. 195–197 °C. IR (ν, cm⁻¹): 3370, 3267 (NH₂, OH), 1707 (CO). ¹H NMR δ ppm: 4.69 (2H, d, *J* = 4.8 Hz, CH₂OH), 5.60 (1H, br s, OH), 6.08 (2H, s, CH₂CO), 7.20–7.25 (2H, m, C_{5',6'}-H), 7.50–7.57 (1H, m, C₇-H), 7.60–7.65 (1H, m, C₄-H), 7.92 (2H, s, NH₂), 8.19 (2H, s, C_{5,6}-H), 8.33 (1H, s, C₃-H). ¹³C NMR δ ppm: 51.11, 57.54, 110.97, 119.66, 122.27, 122.33, 127.74, 130.15, 131.05, 131.81, 137.01, 138.88, 142.50, 145.63, 154.71, 192.80. HRMS Calcd for C₁₆H₁₄ClN₃O₄S ([M + H]⁺): 380.0466, Found: 380.0463.

3-[(2-(Hydroxymethyl)-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3c). Yield 60%, m.p. 220–222 °C. IR (ν, cm⁻¹): 3364, 3272 (NH₂, OH), 1703 (CO). ¹H NMR δ ppm: 4.69 (2H, d, *J* = 3.3 Hz, CH₂OH), 5.63 (1H, br s, OH), 6.12 (2H, s, CH₂CO), 7.21–7.24 (2H, m, C_{5',6'}-H), 7.54–7.57 (1H, m, C₇-H), 7.62–7.67 (3H, m, C₄-H, NH₂), 7.87 (1H, t, *J* = 7.8 Hz, C₅-H), 8.18 (1H, d, *J* = 7.5 Hz, C₄-H), 8.40 (1H, d, *J* = 7.8 Hz, C₆-H), 8.49 (1H, s, C₂-H). ¹³C NMR δ ppm: 50.98, 57.64, 111.02, 119.63, 122.26, 122.97, 125.65, 130.59, 131.16, 132.33, 135.74, 137.07, 142.45, 145.63, 154.75, 193.20. HRMS Calcd for C₁₆H₁₅N₃O₄S ([M + H]⁺): 346.0856, Found: 346.0857.

4-[(2-Benzyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1d). Yield 62%, m.p. 241–243 °C. IR (ν, cm⁻¹): 3284 (NH₂), 1703 (CO). ¹H NMR δ ppm: 4.26 (2H, s, CH₂Ph), 6.06 (2H, s, CH₂CO), 7.15–7.28 (7H, m, C_{5',6'}-H, Ph-H), 7.44–7.47 (1H, m, C₇-H), 7.62–7.65 (1H, m, C₄-H), 7.68 (2H, s, NH₂), 8.04 (2H, d, *J* = 8.4 Hz, C_{2,6}-H), 8.22 (2H, d, *J* = 8.7 Hz, C_{3,5}-H). ¹³C NMR δ ppm: 33.42, 50.83, 111.03, 119.17, 122.28, 122.59, 126.63, 127.19, 129.06, 129.60 (2C), 129.70, 136.60, 137.36, 142.80, 149.03, 154.83, 193.15. HRMS Calcd for C₂₂H₁₉N₃O₃S ([M + H]⁺): 406.1220, Found: 406.1217.

4-[(2-Benzyl-1H-benzimidazol-1-yl)acetyl]-2-chlorobenzenesulfonamide (2d). Yield 71%, m.p. 210–212 °C. IR (ν, cm⁻¹): 3382, 3294 (NH₂), 1705 (CO). ¹H NMR δ ppm: 4.27 (2H, s, CH₂Ph), 6.07 (2H, s, CH₂CO), 7.19–7.26 (7H, m, C_{5',6'}-H, Ph-H), 7.45–7.49 (1H, m, C₇-H), 7.61–7.64 (1H, m, C₄-H), 7.93 (2H, s, NH₂), 8.07 (1H, d, *J* = 8.1 Hz, C₅-H), 8.18 (1H, d, *J* = 8.1 Hz, C₆-H), 8.25 (1H, s, C₃-H). ¹³C NMR δ ppm: 33.40, 50.91, 111.01, 119.21, 122.25, 122.56, 127.15, 127.66, 129.03, 129.62, 129.98, 131.59, 131.87, 136.61, 137.41, 138.67, 142.91, 145.63, 154.77, 192.46. HRMS Calcd for C₂₂H₁₈ClN₃O₃S ([M + H]⁺): 440.0830, Found: 440.0836.

3-[(2-Benzyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3d). Yield 88%, m.p. 146–148 °C. IR (ν, cm⁻¹): 3340 (NH₂), 1702 (CO). ¹H NMR δ ppm: 4.27 (2H, s, CH₂Ph), 6.08 (2H, s, CH₂CO), 7.17–7.36 (7H, m, C_{5',6'}-H, Ph-H), 7.45–7.52 (1H, m, C₇-H), 7.64–7.69 (1H, m, C₄-H, NH₂), 7.85 (1H, t, *J* = 7.8 Hz, C₅-H), 8.18 (1H, d, *J* = 7.8 Hz, C₄-H), 8.34 (1H, d,

J = 7.8 Hz, C₆-H), 8.41 (1H, s, C₂-H). ¹³C NMR δ ppm: 33.45, 50.76, 111.01, 119.22, 122.14, 122.22, 125.67, 127.18, 129.06, 129.63, 130.43, 131.16, 132.37, 135.63, 136.67, 137.44, 142.98, 145.55, 154.87, 193.01. HRMS Calcd for C₂₂H₁₉N₃O₃S ([M + H]⁺): 406.1220, Found: 406.1223.

4-[(2-Ethyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1e). Yield 71%, m.p. 236–238 °C. IR (ν, cm⁻¹): 3334 (NH₂), 1699 (CO). ¹H NMR δ ppm: 1.31 (3H, t, *J* = 7.5 Hz, CH₃), 2.79 (2H, q, *J* = 7.5 Hz, CH₂), 6.07 (2H, s, CH₂CO), 7.17–7.20 (2H, m, C_{5',6'}-H), 7.47–7.50 (1H, m, C₇-H), 7.61–7.64 (1H, m, C₄-H), 7.67 (2H, s, NH₂), 8.07 (2H, d, *J* = 8.1 Hz, C_{2,6}-H), 8.33 (2H, d, *J* = 8.1 Hz, C_{3,5}-H). ¹³C NMR δ ppm: 12.16, 20.50, 50.63, 110.70, 118.91, 122.12, 122.37, 126.73, 129.82, 136.49, 137.44, 142.62, 149.21, 157.44, 193.89. HRMS Calcd for C₁₇H₁₇N₃O₃S ([M + H]⁺): 344.1063, Found: 344.1062.

2-Chloro-4-[(2-ethyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2e). Yield 66%, m.p. 236–238 °C. IR (ν, cm⁻¹): 3333 (NH₂), 1706 (CO). ¹H NMR δ ppm: 1.30 (3H, t, *J* = 7.5 Hz, CH₃), 2.78 (2H, q, *J* = 7.5 Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.14–7.21 (2H, m, C_{5',6'}-H), 7.47–7.50 (1H, m, C₇-H), 7.60–7.63 (1H, m, C₄-H), 7.93 (2H, s, NH₂), 8.17–8.23 (2H, m, C_{5,6}-H), 8.38 (1H, s, C₃-H). ¹³C NMR δ ppm: 12.19, 20.46, 50.76, 110.76, 118.90, 122.14, 122.36, 127.79, 130.06, 131.71, 132.10, 136.44, 138.70, 142.59, 145.78, 157.41, 193.16. HRMS Calcd for C₁₇H₁₆ClN₃O₃S ([M + H]⁺): 378.0674, Found: 378.0670.

3-[(2-Ethyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3e). Yield 66%, m.p. 292–294 °C. IR (ν, cm⁻¹): 3288 (NH₂), 1699 (CO). ¹H NMR δ ppm: 1.31 (3H, t, *J* = 7.5 Hz, CH₃), 2.79 (2H, q, *J* = 7.5 Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.14–7.21 (2H, m, C_{5',6'}-H), 7.47–7.50 (1H, m, C₇-H), 7.62–7.69 (1H, m, NH₂, C₄-H), 7.89 (1H, t, *J* = 7.8 Hz, C₅-H), 8.20 (1H, d, *J* = 8.1 Hz, C₄-H), 8.45 (1H, d, *J* = 7.8 Hz, C₆-H), 8.51 (1H, s, C₂-H). ¹³C NMR δ ppm: 12.20, 20.53, 50.53, 110.65, 119.01, 121.99, 122.27, 125.71, 130.56, 131.31, 132.54, 135.66, 136.60, 142.92, 145.66, 157.46, 193.72. HRMS Calcd for C₁₇H₁₇N₃O₃S ([M + H]⁺): 344.1063, Found: 344.1065.

4-[(2-Propyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1f). Yield 65%, m.p. 245–247 °C. IR (ν, cm⁻¹): 3300 (NH₂), 1700 (CO). ¹H NMR δ ppm: 0.97 (3H, t, *J* = 7.5 Hz, CH₃), 1.78 (2H, sextet, *J* = 7.5 Hz, CH₂), 2.75 (2H, t, *J* = 7.2 Hz, CH₂), 6.08 (2H, s, CH₂CO), 7.14–7.22 (2H, m, C_{5',6'}-H), 7.45–7.48 (1H, m, C₇-H), 7.60–7.63 (1H, m, C₄-H), 7.69 (2H, s, NH₂), 8.07 (2H, d, *J* = 8.4 Hz, C_{2,6}-H), 8.33 (2H, d, *J* = 8.7 Hz, C_{3,5}-H). ¹³C NMR δ ppm: 14.47, 20.95, 28.84, 50.66, 110.75, 118.88, 122.12, 122.32, 126.71, 129.85, 136.38, 137.38, 142.66, 149.19, 156.32, 193.85. HRMS Calcd for C₁₈H₁₉N₃O₃S ([M + H]⁺): 358.1220, Found: 358.1222.

2-Chloro-4-[(2-propyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2f). Yield 71%, m.p. 240–242 °C. IR (ν, cm⁻¹): 3354 (NH₂), 1702 (CO). ¹H NMR δ ppm: 0.97 (3H, t, *J* = 7.5 Hz, CH₃), 1.78 (2H, sextet, *J* = 7.5 Hz, CH₂), 2.74 (2H, t, *J* = 7.5 Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.13–7.20 (2H, m, C_{5',6'}-H), 7.45–7.48 (1H, m, C₇-H), 7.59–7.62 (1H, m, C₄-H), 7.93 (2H, s, NH₂), 8.20 (2H, s, C_{5,6}-H), 8.39 (1H, s, C₃-H). ¹³C NMR δ ppm: 14.49, 21.00, 28.87, 50.78, 110.74, 118.96, 122.05, 122.24, 127.79, 130.05, 131.71, 132.13, 136.41, 138.69, 142.90, 145.78, 156.31, 193.14. HRMS Calcd for C₁₈H₁₈ClN₃O₃S ([M + H]⁺): 392.0830, Found: 392.0827.

3-[(2-Propyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3f). Yield 87%, m.p. 275–277 °C. IR (ν, cm⁻¹): 3314 (NH₂), 1701 (CO). ¹H NMR δ ppm: 0.97 (3H, t, *J* = 7.5 Hz, CH₃), 1.79 (2H, sextet, *J* = 7.5 Hz, CH₂), 2.75 (2H, t, *J* = 7.5 Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.13–7.21 (2H, m, C_{5',6'}-H), 7.46–7.49 (1H, m, C₇-H), 7.60–7.62 (3H, m, C₄-H, NH₂), 7.89 (1H, t, *J* = 7.8 Hz, C₅-H), 8.20 (1H, d, *J* = 8.1 Hz, C₄-H), 8.45 (1H, d,

$J = 7.8$ Hz, C₆-H), 8.50 (1H, s, C₂-H). ¹³C NMR δ ppm: 14.50, 20.98, 28.91, 50.58, 110.69, 118.99, 122.00, 122.22, 125.71, 130.56, 131.30, 132.58, 135.64, 136.50, 142.97, 145.65, 156.35, 193.66. HRMS Calcd for C₁₈H₁₉N₃O₃S ([M + H]⁺): 358.1220, Found: 358.1219.

4-[(2-Butyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1g). Yield 67%, m.p. 260–262 °C. IR (ν , cm⁻¹): 3299 (NH₂), 1699 (CO). ¹H NMR δ ppm: 0.90 (3H, t, $J = 7.5$ Hz, CH₃), 1.39 (2H, sextet, $J = 7.5$ Hz, CH₂), 1.75 (2H, quintet, $J = 7.5$ Hz, CH₂), 2.77 (2H, t, $J = 7.5$ Hz, CH₂), 6.08 (2H, s, CH₂CO), 7.14–7.21 (2H, m, C_{5',6'}-H), 7.45–7.48 (1H, m, C_{7'}-H), 7.60–7.63 (1H, m, C_{4'}-H), 7.69 (2H, s, NH₂), 8.07 (2H, d, $J = 8.4$ Hz, C_{2,6}-H), 8.34 (2H, d, $J = 8.4$ Hz, C_{3,5}-H). ¹³C NMR δ ppm: 14.48, 22.50, 26.63, 29.65, 50.66, 110.70, 118.93, 122.06, 122.26, 126.73, 129.84, 136.45, 137.42, 142.82, 149.20, 156.46, 193.89. HRMS Calcd for C₁₉H₂₁N₃O₃S ([M + H]⁺): 372.1376, Found: 372.1376.

4-[(2-Butyl-1H-benzimidazol-1-yl)acetyl]-2-chlorobenzenesulfonamide (2g). Yield 75%, m.p. 201–203 °C. IR (ν , cm⁻¹): 3333 (NH₂), 1708 (CO). ¹H NMR δ ppm: 0.90 (3H, t, $J = 7.2$ Hz, CH₃), 1.38 (2H, sextet, $J = 7.5$ Hz, CH₂), 1.74 (2H, quintet, $J = 7.5$ Hz, CH₂), 2.76 (2H, t, $J = 7.5$ Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.13–7.20 (2H, m, C_{5',6'}-H), 7.45–7.48 (1H, m, C_{7'}-H), 7.59–7.61 (1H, m, C_{4'}-H), 7.94 (2H, s, NH₂), 8.20 (2H, s, C_{5,6}-H), 8.39 (1H, s, C₃-H). ¹³C NMR δ ppm: 14.47, 22.50, 26.60, 29.66, 50.78, 110.74, 118.93, 122.06, 122.24, 127.77, 130.06, 131.71, 132.12, 136.42, 138.69, 142.85, 145.78, 156.44, 193.15. HRMS Calcd. for C₁₉H₂₀ClN₃O₃S ([M + H]⁺): 406.0987, Found: 406.0981.

3-[(2-Butyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3g). Yield 61%, m.p. 214–216 °C. IR (ν , cm⁻¹): 3322 (NH₂), 1702 (CO). ¹H NMR δ ppm: 0.90 (3H, t, $J = 7.2$ Hz, CH₃), 1.39 (2H, sextet, $J = 7.2$ Hz, CH₂), 1.75 (2H, quintet, $J = 7.5$ Hz, CH₂), 2.77 (2H, t, $J = 7.5$ Hz, CH₂), 6.09 (2H, s, CH₂CO), 7.13–7.20 (2H, m, C_{5',6'}-H), 7.45–7.48 (1H, m, C_{7'}-H), 7.60–7.69 (3H, m, C_{4'}-H, NH₂), 7.89 (1H, t, $J = 7.8$ Hz, C₅-H), 8.20 (1H, d, $J = 7.8$ Hz, C₄-H), 8.45 (1H, d, $J = 8.1$ Hz, C₆-H), 8.50 (1H, s, C₂-H). ¹³C NMR δ ppm: 14.47, 22.50, 26.64, 29.65, 50.57, 110.67, 118.98, 121.99, 122.20, 125.70, 130.56, 131.30, 132.56, 135.66, 136.52, 142.99, 145.65, 156.48, 193.67. HRMS Calcd for C₁₉H₂₁N₃O₃S ([M + H]⁺): 372.1376, Found: 372.1377.

4-[(2-Isopropyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1h). Yield 65%, m.p. 258–260 °C. IR (ν , cm⁻¹): 3341 (NH₂), 1706 (CO). ¹H NMR δ ppm: 1.30 (6H, d, $J = 6.6$ Hz, (CH₃)₂), 3.18 (1H, septet, $J = 6.6$ Hz, CH), 6.09 (2H, s, CH₂CO), 7.13–7.22 (2H, m, C_{5',6'}-H), 7.44–7.47 (1H, m, C_{4'}-H), 7.61–7.64 (1H, m, C_{7'}-H), 7.66 (2H, s, NH₂), 8.07 (2H, d, $J = 8.4$ Hz, C_{2,6}-H), 8.34 (2H, d, $J = 8.4$ Hz, C_{3,5}-H). ¹³C NMR δ ppm: 22.41, 26.20, 50.57, 110.82, 119.04, 122.12, 122.32, 126.72, 129.85, 136.28, 137.44, 142.71, 149.23, 161.05, 194.03. HRMS Calcd for C₁₈H₁₉N₃O₃S ([M + H]⁺): 358.1220, Found: 358.1218.

2-Chloro-4-[(2-isopropyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2h). Yield 57%, m.p. 149–151 °C. IR (ν , cm⁻¹): 3375, 3278 (NH₂), 1708 (CO). ¹H NMR δ ppm: 1.29 (6H, d, $J = 6.3$ Hz, (CH₃)₂), 3.16–3.24 (1H, m, CH), 6.13 (2H, s, CH₂CO), 7.10–7.26 (2H, m, C_{5',6'}-H), 7.48–7.50 (1H, m, C_{7'}-H), 7.61–7.63 (1H, m, C_{4'}-H), 7.93 (2H, s, NH₂), 8.20 (2H, s, C_{5,6}-H), 8.40 (1H, s, C₃-H). ¹³C NMR δ ppm: 22.39, 26.11, 50.78, 111.00, 118.88, 122.33, 122.48, 127.81, 130.04, 131.71, 132.19, 136.08, 138.64, 142.25, 145.81, 161.03, 193.22. HRMS Calcd for C₁₈H₁₈ClN₃O₃S ([M + H]⁺): 392.0830, Found: 392.0827.

3-[(2-Isopropyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3h). Yield 51%, m.p. 267–269 °C. IR (ν , cm⁻¹): 3319 (NH₂), 1706 (CO). ¹H NMR δ ppm: 1.30 (6H, d, $J = 6.6$ Hz, (CH₃)₂), 3.19 (1H, septet, $J = 6.9$ Hz, CH), 6.12 (2H, s, CH₂CO),

7.14–7.22 (2H, m, C_{5',6'}-H), 7.46–7.49 (1H, m, C_{7'}-H), 7.62–7.63 (3H, m, C_{4'}-H, NH₂), 7.89 (1H, t, $J = 7.8$ Hz, C₅-H), 8.20 (1H, d, $J = 7.8$ Hz, C₄-H), 8.47 (1H, d, $J = 7.8$ Hz, C₆-H), 8.51 (1H, s, C₂-H). ¹³C NMR δ ppm: 22.44, 26.19, 50.53, 110.87, 119.02, 122.13, 122.33, 125.72, 130.55, 131.32, 132.63, 135.61, 136.27, 142.67, 145.64, 161.07, 193.80. HRMS Calcd for C₁₈H₁₉N₃O₃S ([M + H]⁺): 358.1220, Found: 358.1221.

4-[(2-Isobutyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (1i). Yield 66%, m.p. 234–236 °C. IR (ν , cm⁻¹): 3333 (NH₂), 1703 (CO). ¹H NMR δ ppm: 0.96 (6H, d, $J = 6.6$ Hz, (CH₃)₂), 2.12–2.25 (1H, m, CH), 2.68 (2H, d, $J = 6.9$ Hz, CH₂), 6.07 (2H, s, CH₂CO), 7.13–7.22 (2H, m, C_{5',6'}-H), 7.43–7.46 (1H, m, C_{4'}-H), 7.61–7.64 (1H, m, C_{7'}-H), 7.66 (2H, s, NH₂), 8.07 (2H, d, $J = 8.4$ Hz, C_{2,6}-H), 8.33 (2H, d, $J = 8.4$ Hz, C_{3,5}-H). ¹³C NMR δ ppm: 23.08, 27.67, 35.76, 50.73, 110.82, 118.92, 122.12, 122.29, 126.72, 129.84, 136.34, 137.44, 142.77, 149.22, 155.71, 193.77. HRMS Calcd for C₁₉H₂₁N₃O₃S ([M + H]⁺): 372.1376, Found: 372.1377.

2-Chloro-4-[(2-isobutyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (2i). Yield 74%, m.p. 234–236 °C. IR (ν , cm⁻¹): 3378 (NH₂), 1706 (CO). ¹H NMR δ ppm: 0.95 (6H, d, $J = 6.3$ Hz, (CH₃)₂), 2.12–2.24 (1H, m, CH), 2.67 (2H, d, $J = 6.9$ Hz, CH₂), 6.10 (2H, s, CH₂CO), 7.13–7.21 (2H, m, C_{5',6'}-H), 7.45–7.47 (1H, m, C_{7'}-H), 7.60–7.62 (1H, m, C_{4'}-H), 7.93 (2H, s, NH₂), 8.20 (2H, s, C_{5,6}-H), 8.39 (1H, s, C₃-H). ¹³C NMR δ ppm: 23.07, 27.67, 35.66, 50.87, 110.88, 118.86, 122.17, 122.30, 127.79, 130.03, 131.69, 132.16, 136.24, 138.64, 142.67, 145.78, 155.69, 193.00. HRMS Calcd for C₁₉H₂₀ClN₃O₃S ([M + H]⁺): 406.0987, Found: 406.0989.

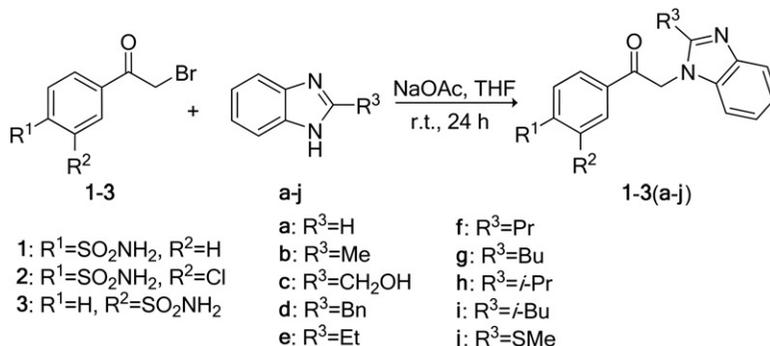
3-[(2-Isobutyl-1H-benzimidazol-1-yl)acetyl]benzenesulfonamide (3i). Yield 68%, m.p. 216–218 °C. IR (ν , cm⁻¹): 3312 (NH₂), 1703 (CO). ¹H NMR δ ppm: 0.96 (6H, d, $J = 6.6$ Hz, (CH₃)₂), 2.19 (1H, septet, $J = 6.9$ Hz, CH), 2.68 (2H, d, $J = 7.2$ Hz, CH₂), 6.10 (2H, s, CH₂CO), 7.13–7.21 (2H, m, C_{5',6'}-H), 7.45–7.47 (1H, m, C_{7'}-H), 7.63–7.69 (3H, m, C_{4'}-H, NH₂), 7.89 (1H, t, $J = 7.8$ Hz, C₅-H), 8.20 (1H, d, $J = 7.5$ Hz, C₄-H), 8.46 (1H, d, $J = 7.8$ Hz, C₆-H), 8.50 (1H, s, C₂-H). ¹³C NMR δ ppm: 23.11, 27.65, 35.77, 50.65, 110.79, 118.98, 122.06, 122.23, 125.72, 130.56, 131.30, 132.62, 135.63, 136.40, 142.96, 145.65, 155.75, 193.56. HRMS Calcd for C₁₉H₂₁N₃O₃S ([M + H]⁺): 372.1376, Found: 372.1375.

4-[[2-(Methylsulfanyl)-1H-benzimidazol-1-yl]acetyl]benzenesulfonamide (1j). Yield 62%, m.p. 213–215 °C. IR (ν , cm⁻¹): 3319 (NH₂), 1702 (CO). ¹H NMR δ ppm: 2.72 (3H, s, CH₃), 5.98 (2H, s, CH₂CO), 7.15–7.23 (2H, m, C_{5',6'}-H), 7.50–7.52 (1H, m, C_{4'}-H), 7.61–7.63 (1H, m, C_{7'}-H), 7.68 (2H, s, NH₂), 8.07 (2H, d, $J = 8.1$ Hz, C_{2,6}-H), 8.34 (2H, d, $J = 8.1$ Hz, C_{3,5}-H). ¹³C NMR δ ppm: 15.20, 50.96, 110.32, 118.26, 122.37(2C), 126.82, 129.82, 137.18, 173.72, 143.58, 149.29, 153.70, 193.05. HRMS Calcd for C₁₆H₁₅N₃O₃S₂ ([M + H]⁺): 362.0628, Found: 362.0628.

2-Chloro-4-[[2-(methylthio)-1H-benzimidazol-1-yl]acetyl]benzenesulfonamide (2j). Yield 53%, m.p. 195–197 °C. IR (ν , cm⁻¹): 3362, 3256 (NH₂), 1707 (CO). ¹H NMR δ ppm: 2.72 (3H, s, CH₃), 6.01 (2H, s, CH₂CO), 7.14–7.23 (2H, m, C_{5',6'}-H), 7.48–7.51 (1H, m, C_{7'}-H), 7.60–7.63 (1H, m, C_{4'}-H), 7.92 (2H, s, NH₂), 8.21 (2H, s, C_{5,6}-H), 8.39 (1H, s, C₃-H). ¹³C NMR δ ppm: 15.22, 51.09, 110.34, 118.26, 122.34, 122.38, 127.80, 130.17, 131.86, 132.04, 137.64, 138.43, 143.56, 145.91, 153.72, 192.37. HRMS Calcd for C₁₆H₁₄ClN₃O₃S₂ ([M + H]⁺): 396.0238, Found: 396.0241.

3-[[2-(Methylthio)-1H-benzimidazol-1-yl]acetyl]benzenesulfonamide (3j). Yield 54%, m.p. 242–244 °C. IR (ν , cm⁻¹): 3288 (NH₂), 1706 (CO). ¹H NMR δ ppm: 2.72 (3H, s, CH₃), 6.99 (2H, s, CH₂CO), 7.15–7.23 (2H, m, C_{5',6'}-H), 7.50–7.41 (1H, m, C_{7'}-H), 7.60–7.63 (3H, m, C_{4'}-H, NH₂), 7.88 (1H, t, $J = 7.5$ Hz, C₅-H), 8.19 (1H, d, $J = 7.5$ Hz, C₄-H), 8.45 (1H, d, $J = 7.8$ Hz,

Scheme 1. Synthesis of compounds 1–3(a–j).



C₆-H), 8.50 (1H, s, C₂-H). ¹³C NMR δ ppm: 15.24, 50.90, 110.36, 118.28, 122.28, 122.37, 125.69, 130.69, 131.43, 132.47, 135.45, 137.76, 143.62, 145.72, 153.65, 192.83. HRMS Calcd for C₁₆H₁₅N₃O₃S₂ ([M + H]⁺): 362.0628, Found: 362.0627.

Protein preparation

Expression and purification of CA I, II, VII, XII and XIII has been previously described: CA I by Baranauskiene et al.²⁸, CA II by Cimperman et al.²⁹, CA VII and XIII by Sudzius et al.²¹ and CA XII by Jogaite et al.³⁰.

Determination of compound binding to CAs by thermal shift assay

The thermal shift assay (TSA) measurements were performed in a Corbett Rotor-Gene 6000 (QIAGEN Rotor-Gene Q, Sydney, Australia) instrument using the blue channel (excitation 365 ± 20, detection 460 ± 15 nm). Samples contained 10 μM protein, 0–200 μM compound, 50 μM solvatochromic dye ANS (8-anilino-1-naphthalene sulfonate) and 50 mM phosphate buffer containing 100 mM NaCl at pH 7.0, with the final DMSO concentration at 2%. The applied heating rate was 1 °C/min. Data analysis was performed as previously described³¹.

Results

A series of benzenesulfonamides with a benzimidazole moiety **1–4(a–j)** were designed as CA inhibitors (Figure 2). The procedures for *N*-alkylation of 2-substituted benzimidazoles (**a–j**) with 5-(bromoacetyl)-2-chlorobenzenesulfonamide (**4**) have been described previously²³. *N*-substituted benzimidazole derivatives **1–3(a–j)** were prepared by *N*-alkylation of benzimidazoles **a–j** with 4-(bromoacetyl)benzene-sulfonamide (**1**), 4-(bromoacetyl)-2-chlorobenzenesulfonamide (**2**) and 3-(bromoacetyl)benzenesulfonamide (**3**) (Scheme 1).

The dissociation constants for compounds **1–4(a–j)** binding to five CA isozymes CA I, II, VII, XII and XIII are listed in Table 1. AZM and EZA were used as controls. The binding of these compounds was determined by the TSA. Figures 3 and 4 show representative binding data obtained by TSA. The data in Table 1 and Figure 5 show that the position of the sulfonamide group on the benzene ring significantly influences the binding strength to the various CA isoforms. In most cases, the *para*-substituted benzenesulfonamide (compounds **1–2(a–j)**) had higher binding affinity than its *meta*-substituted benzenesulfonamide equivalent (compounds **3–4(a–j)**) (Figure 3). All **1–2(a–j)** derivatives were nanomolar inhibitors of CA I, II, VII and XIII (dissociation constants (*K*_ds) in the range of 1.6–286 nM). The binding of **1–2(a–j)** to CA XII was weaker (*K*_d ranged from 62.5 nM to 8.3 μM). Most compounds of the **3–4(a–j)** series were micromolar inhibitors of all investigated CA isoforms (*K*_ds in the range of 0.1–100 μM). Compounds bearing a chlorine atom in the *ortho*

Table 1. Dissociation constants for compounds binding to five human recombinant CA isoforms, determined by TSA (37 °C, pH 7.0).

Inhibitors	R ³	Dissociation constants <i>K</i> _d (nM) to CA isoforms				
		CA I	CA II	CA VII	CA XII	CA XIII
1a		33.0	4.0	20.0	909.0	83.0
2a	H	12.0	8.3	4.0	833.0	19.0
3a		4350	4340	6670	12 500	2380
4a		11 100	1560	1000	2080	670.0
1b		10.0	6.3	28.0	769.0	76.9
2b	Me	5.88	10.5	11.0	200.0	23.2
3b		9090	7690	16 670	16 670	2000
4b		10 000	2000	2500	6250	400.0
1c		8.33	4.17	20.0	1050	62.5
2c	CH ₂ OH	10.0	14.3	5.0	1000	66.7
3c		5000	4000	5710	12 500	2000
4c		7140	1000	400.0	5560	333.0
1d		143.0	100.0	20.0	8 330	286.0
2d	C ₆ H ₅ CH ₂	8.33	2.86	8.33	167.0	6.67
3d		2500	1667	12 500	5000	1110
4d		8330	1560	3330	769.0	1000
1e		8.33	5.0	20.0	556.0	50.0
2e	Et	4.76	6.25	7.69	66.7	11.5
3e		4760	7690	25 000	14 300	3330
4e		7140	556.0	1670	1820	400.0
1f		7.14	5.56	17.2	667.0	28.6
2f	Pr	2.86	3.57	4.17	62.5	5.56
3f		1000	6670	25 000	11 100	1000
4f		10 000	833.0	2500	1430	1430
1g		11.1	4.17	20.0	1000	50.0
2g	Bu	3.33	2.70	3.33	112.0	2.50
3g		667.0	2860	25 000	11 100	667.0
4g		10 000	454.0	3 330	2860	400.0
1h		4.0	5.56	20.0	476.0	33.3
2h	i-Pr	5.0	14.3	5.0	333.0	40.0
3h		2500	16 700	100 000	20 000	5000
4h		4540	667.0	2000	588.0	370.0
1i		8.33	5.56	5.56	833.0	33.3
2i	i-Bu	6.67	14.3	5.0	500.0	40.0
3i		125.0	5000	25 000	8330	1430
4i		3330	384.0	1000	2000	2860
1j		3.57	4.0	18.2	2000	38.4
2j	SCH ₃	1.67	3.33	4.54	66.7	9.09
3j		1670	3450	25 000	5000	833.0
4j		5000	1000	1670	1430	1110
Indapamide		10 000	300.0	300.0	1400	100.0
EZA		14	0.71	0.71	36.0	13.0
AZM		1400	17.0	17.0	133.0	50.0

Average standard deviation for TSA data was below 25%.

position of the benzenesulfonamide ring were in most cases more effective CA inhibitors than compounds without a chlorine substitution in this position: (**2(a–j)** versus **1(a–j)** and **4(a–j)** versus **3(a–j)**). For example, compound **2g** exhibited 25 times greater affinity than **1g** to CA XIII whereas compound **4h** was 34

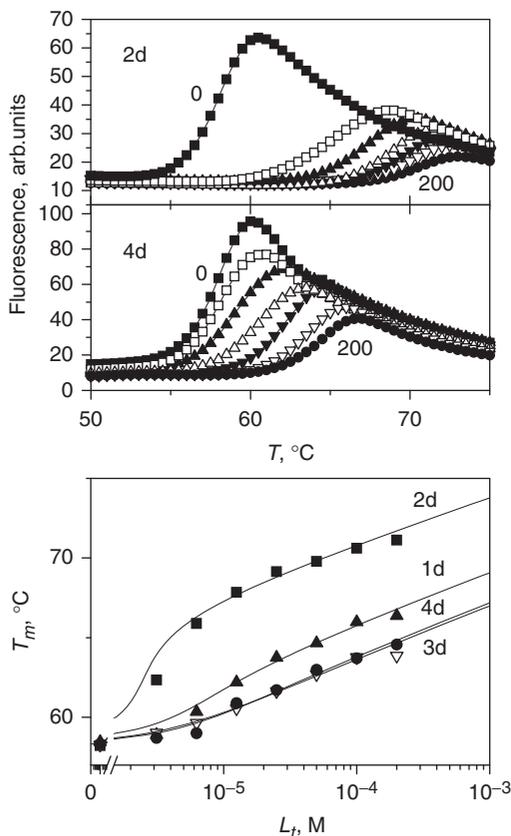


Figure 3. TSA data of **1–4d** binding to CA XIII. The top panels compare denaturation curves observed by fluorescence at 0–200 μM added compound concentrations. The bottom panel shows the dependence of the protein melting temperatures T_m on compound concentrations.

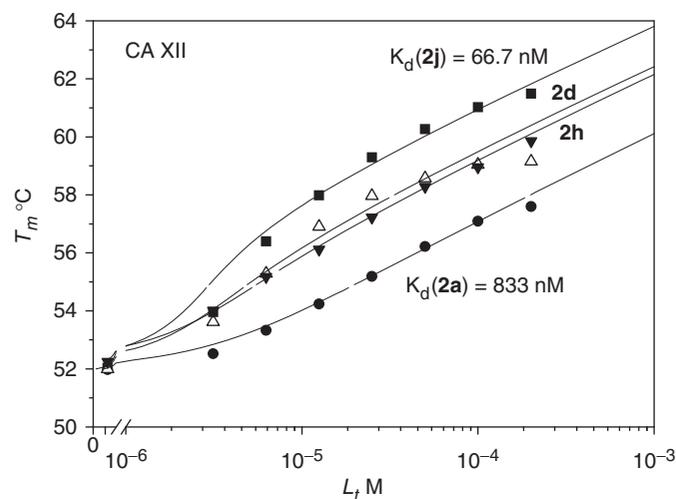


Figure 4. TSA data of **2a**, **2d**, **2h** and **2j** binding to CA XII.

times more potent than **3h** for CA VII. In contrast, CA I bound **4(a–j)** weaker than **3(a–j)**. The highest difference was observed between compounds **4i** and **3i**, with the latter being 27 times stronger CA I inhibitor than **4i**.

The binding of most benzenesulfonamides of types **1–4(a–j)** to CA I, II, VII, XII and XIII did not strongly depend on the substituent (R_3) on the benzimidazole ring. By comparing the series of compounds bearing methyl **1–4b**, hydroxymethyl **1–4c**, ethyl **1–4e**, propyl **1–4f** and butyl **1–4g** substituents, we showed that the tail length did not affect the binding affinity by more than two times to any of the tested CAs. For example, the K_{dS} for **1b**, **c**

and **e–j** for CA I varied only in the narrow nanomolar range (from 3.5 to 11.1 nM). The K_{dS} for CA I were higher for compounds **1a** and **1d**, 33 and 143 nM, respectively. CA II was best inhibited ($K_d = 4–100$ nM), while CA XII ($K_d = 0.48–8.33$ μM) least inhibited. Compounds bearing *iso*-propyl **1h** and *S*-methyl **1j** substitution on benzimidazole ring were good inhibitors of CA I and CA II (K_{dS} were about 4–5 nM), whereas *iso*-butyl-substituted compound **1i** potently bound CA VII ($K_d = 5.56$ nM). Benzyl-substituted **1d** had the weakest binding affinity, especially to CA XII ($K_d = 8.33$ μM), but it was selective for CA VII ($K_d = 20$ nM).

N-alkylated benzimidazoles (**2(a–j)**) were the best inhibitors of CA I, II, VII and XIII exhibiting affinities in the nanomolar range. These compounds were weaker CA XII inhibitors by about one order of magnitude. Compounds with methyl, ethyl, propyl and butyl substituents had a high affinity for CA I (K_d ranged from 2.9 to 5.9 nM), CAII (K_d from 10.5 to 2.70 nM), CA VII (K_d from 11.1 to 3.33 nM) and CA XIII (K_d from 23.3 to 2.50 nM). Benzimidazoles that contained a branched chain aliphatic tail (compounds **2h**, **2i**) had four to eight times reduced affinity for CA II (K_{dS} were 14.3 nM for both compounds), CA XII (K_{dS} were 333 and 500 nM, respectively) and CA XIII (K_{dS} were 40 nM for both compounds), as compared to compounds **2f** and **2g** which contained an unbranched tail. Benzimidazole with an *S*-methyl substituent, **2j**, bound to CA isoforms similarly as the propyl-substituted compound, **2f**.

3(a–j) *N*-benzimidazoles were the weakest CA inhibitors (K_{dS} in the range of 0.67–100 μM). Only three of them (**3g**, **i**, **j**) had a higher affinity for CA I and XIII, than for the other tested CAs. For example, **3i** binds to CA I 40 times stronger than CA II, 200 times stronger than CA VII, 66 times stronger than CA XII and 10 times stronger than CA XIII.

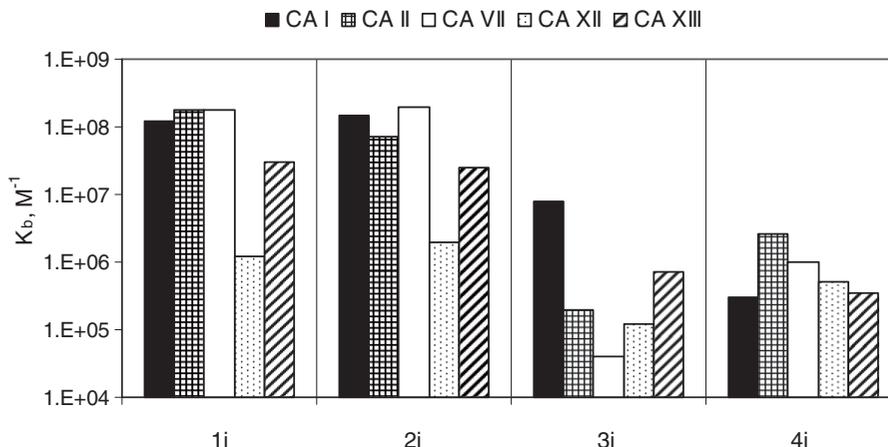
4(a–j) *N*-substituted benzimidazoles (K_{dS} in the range of 0.33–11.1 μM) were slightly better inhibitors than the **3(a–j)** compounds. They behaved as weak inhibitors against CA I (K_{dS} are in the micromolar range 3.3–11 μM). Six derivatives, namely **4a–c**, **e**, **g** and **h**, were better inhibitors of CA XIII, with K_{dS} in the range of 0.33–0.67 μM . Compound **4b**, which is structurally the most similar to the clinically used indapamide, was slightly less potent. The binding affinity of **4b** and indapamide to CA I was similar, but **4b** bound about seven times weaker to CA II and CA VII and four times weaker to CA XII and CA XIII than indapamide.

Discussion

Indapamide, which is a clinically used diuretic, may act as an inhibitor of various CA isozymes present in the kidneys and blood vessels, thus lowering the blood pressure in patients with hypertension and type-2 diabetes³². It has been reported previously by Supuran's group that the inhibition constants (K_i s) of indapamide for several CAs were: 51 900 nM for hCA I, 2520 nM for hCA II, 0.23 nM for hCA VII, 10 nM for CA XII and 13 nM for murine CA XIII¹⁹. The TSA data confirmed that indapamide was a weak inhibitor of the isoform CA I ($K_d = 10\,000$ nM). However, the K_d values toward other CAs differed from K_i values (K_{dS} for CA II and VII were 300 nM, CA XII = 1400 nM and CA XIII = 100 nM). These discrepancies could be observed due to the different techniques utilized for compound binding and inhibition measurements. Usually, the TSA and other biophysical binding techniques, such as isothermal titration calorimetry, yield dissociation constants that are close to the inhibition constants determined by the stop-flow carbon dioxide hydration assay^{28,33}.

Numerous indapamide analogues were designed in search of new, more potent and selective CA inhibitors. A diminished

Figure 5. The binding constants of **1i**, **2i**, **3i** and **4i** to CA I, II, VII, XII and XIII.



affinity of derivatives of both series **3(a–j)** and **4(a–j)**, which are most structurally related to indapamide when compared with the corresponding inhibitors **1(a–j)** and **2(a–j)**, towards isozyme I, II, VII, XII and XIII was observed. This is thought to occur due to the benzimidazole group in the *meta* position in relation to the benzenesulfonamide moiety, which is present in the first series of compounds. This could lead to steric hindrance when binding to the Zn ion within the enzyme active site.

It was interesting to notice that the chlorine atom in the *ortho* position of the sulfonamide group of the compounds **2(a–j)** and **4(a–j)** increased the affinity to all CAs, with the exception of CA I. All compounds of the series **4(a–j)** with a chlorine substitution in the *ortho* position possessed a lower binding potency with CA I than compounds **3(a–j)** without a chlorine substitution. Compound **3i**, bearing a bulky *iso*-butyl substituent in the benzimidazole moiety, behaved as a selective CA I inhibitor, having more than 10 times higher affinity against CA I isozyme as compared to other CAs.

Conclusions

Benzenesulfonamides bearing benzimidazole substituents were synthesized and assayed as inhibitors of five CA isozymes, CA I, II, VII, XII and XIII. 4-[(2-Substituted-1H-benzimidazol-1-yl)acetyl]benzenesulfonamides **1(a–j)** were more than two orders of magnitude stronger CA inhibitors than 3-[(2-substituted-1H-benzimidazol-1-yl)acetyl]benzenesulfonamides **3(a–j)**. Compounds of the series **1(a–j)** and **2(a–j)** were nanomolar inhibitors of CA I, II, VII and XIII (K_d s in the range of 1.6–286 nM).

Declaration of interest

The authors report no conflicts of interest.

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