

RESEARCH ARTICLE

Synthesis and characterization of novel dioxoacridine sulfonamide derivatives as new carbonic anhydrase inhibitors

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Abstract

Novel dioxoacridine sulfonamide compounds were synthesized from reaction of cyclic 1,3-diketones, sulfanilamide (4-amino benzene sulfonamide) and aromatic aldehydes. The structures of these compounds were confirmed by using spectral analysis (IR, H-NMR, ¹³C-NMR, and mass). Human carbonic anhydrase isoenzymes (hCA I and hCA II) were purified from erythrocyte cells by affinity chromatography. The inhibitory effects of sulfanilamide, acetazolamide (AAZ), and newly synthesized sulfonamides on hydratase and esterase activities of these isoenzymes have been studied *in vitro*. The IC₅₀ values of compounds for esterase activity are 0.71–0.11 μM for hCA I and 0.45–0.12 μM for hCA II, respectively. The K_i values of these inhibitors were determined as 0.38–0.008 μM for hCA I and 0.19–0.001 μM for hCA II, respectively.

Keywords: Dioxoacridine sulfonamide, carbonic anhydrase, inhibition, hydratase activity, esterase activity

Introduction

Carbonic anhydrases (CAs) (E.C. 4.2.1.1) are catalytic reversible hydration of carbon dioxide in a two-step reaction to yield bicarbonate and proton^{1–5}. Sixteen different CA isoenzymes were described in higher vertebrates, including humans⁶. The isoenzymes relevant to the human eye are hCA I, hCA II and hCA IV. The isoenzymes of hCA I and hCA II are cytosolic, whereas hCA IV is membrane-bound^{7–9}. The CA inhibitors, which reduce aqueous production with a corresponding decrease in intraocular pressure (IOP), have been used as ocular hypotensive agents for the treatment of glaucoma^{10,11}. The disease is the second leading cause of blindness worldwide^{12,13}. The risk factors for glaucoma disease include age, race, ocular hypertension, severe myopia and a family history of glaucoma. Here, the strongest risk factors are age, race and ocular hypertension. An elevated IOP was formerly synonymous with glaucoma⁷.

Sulfonamides, which are powerful CA inhibitors, are now widely used as the drug for the treatment or prevention of a variety of diseases^{4,5,14–16}. They are the best known inhibitors of CA enzyme in the treatment of glaucoma in

clinical medicine^{4,5,15}. These inhibitors are very effective in the treatment by reducing elevated IOP. Acetazolamide (AAZ) was the first compound used as the inhibitor of this potent hCA II. Then, it was suggested that reducing aqueous humor secretion might provide an effective means of lowering IOP to treat the disease¹⁷. Afterwards some systemic sulfonamide drugs were mainly used clinically for a long time as anti-glaucoma agents¹⁷. The orally administered drugs had affected various CA isozymes present in other tissues and led to an entire range of side effects. To decrease systemic side effects of oral CA inhibitors, dorzolamide (DZA) and brinzolamide (BRZ) ophthalmic suspension approved for the treatment of glaucoma have been used as the topical CA inhibitors^{7,11}. Each drug may be an effective anti-glaucoma agent, but these drugs tend to pose tolerability problems in many patients because of local side effects¹⁸. Thus, it is required to develop new compounds and new drugs.

In this study, novel dioxoacridine derivatives have been synthesized and characterized by IR, ¹H and ¹³C-NMR data and satisfactory mass spectral analyses, respectively. The synthesis of new CA inhibitors by using sulfanilamide and their effects on human

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CA isoenzymes (hCA I, hCA II) purified from human erythrocytes are reported. Further, the potential use of these compounds as new inhibitors of hCA I and hCA II isoenzymes in the treatment of glaucoma are investigated.

Material and methods

Materials

The chemicals used in the synthesis of dioxoacridine sulfonamide derivatives were provided from Merck and Aldrich Chemical Company and Sepharose 4B for affinity column and electrophoresis reagents were obtained from Sigma Chem. Co. All chemicals and solvents used for the synthesis were spectroscopic reagent grade. Melting points were measured on a Bibby Stuart Scientific apparatus. Fourier Transform Infrared (FT-IR) spectra were recorded from Bruker Optics, Andrtex 70 FT-IR spectrometer using ATR diamond crystal. The ^1H -NMR, and ^{13}C -NMR spectra were obtained with a Bruker DPX-400 FT-NMR instrument in CDCl_3 and $\text{DMSO}-d_6$ as solvent with trimethylsilane as the internal reference, at 400 and 100 MHz, respectively. Chemical shifts are expressed in δ units (ppm). The mass analyses were performed on Waters 2695 Alliance Micromass ZQ instrument LC/MS.

General procedure for preparation of dioxoacridine sulfonamide derivatives (4–12).

A mixture of a 5,5-dimethylcyclohexane-1,3-dione **1** (280 mg, 2 mmol), sulfanilamide **2** (172 mg, 1 mmol), 4-cyanobenzaldehyde **3** (131 mg, 1 mmol), and DBSA (420 mg, 10 mol%) in H_2O (40 mL) was stirred at refluxing for 4 h (6 h for cyclohexane-1,3-dione derivatives; 10 h for 4,4-dimethylcyclohexane-1,3-dione derivatives). The progress of the reaction was monitored by TLC. Once the reaction is completed, the mixture was cooled to room temperature and solid filtered off and washed with H_2O . The acridine-1,3-dione sulfonamide products were purified and recrystallized from the following solvent mixture for each compound (76%–91%).

4-(9-(4-Cyanophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (4)

As yellow crystals, (471 mg, 87%), mp 215°C (ethanol- H_2O). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 0.84 (s, 6H, $2 \times \text{CH}_3$), 0.96 (s, 6H, $2 \times \text{CH}_3$), 1.76–1.81 (m, 2H, $-\text{CH}_2$), 1.99–2.23 (m, 6H, $-\text{CH}_2$), 3.75 (s, 3H, $-\text{OCH}_3$), 5.05 (s, 2H, SO_2NH_2), 5.29 (s, 1H, $-\text{CH}$), 7.41 (d, 2H, $J=8.72$ Hz Ar-H), 7.44 (m, 4H, Ar-H), 8.20 (d, 2H, $J=8.38$ Hz Ar-H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 27.23, 28.96, 32.36, 33.96, 40.53, 50.04, 112.61, 113.89, 120.93, 122.58, 127.38, 130.40, 131.59, 134.18, 143.56, 150.72, 161.14, 197.03. IR (cm^{-1}): 3298 and 3172 w (NH_2), 3052 w (Ar-H), 2960 w (C-H), 2229 (CN), 1641 s (C=O), 1628 and 1575 m (C=C), 1361, 1220, 1170, 1145, 1019; MS(CI) m/z 530.90 ($\text{M} + 1$).

4-(9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (5)

As yellow crystals, (0.481 mg, 90%), mp 227°C (ethanol- H_2O). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 0.85 (s, 6H, $2 \times \text{CH}_3$), 0.98 (s, 6H, $2 \times \text{CH}_3$), 1.75–1.81 (m, 2H, $-\text{CH}_2$), 1.99–2.23 (m, 6H, $-\text{CH}_2$), 3.78 (s, 3H, $-\text{OCH}_3$), 5.06 (s, 2H, SO_2NH_2), 5.24 (s, 1H, $-\text{CH}$), 6.82 (d, 2H, $J=8.65$ Hz Ar-H), 7.34 (d, 2H, $J=8.65$ Hz Ar-H), 7.44 (d, 2H, $J=8.40$ Hz Ar-H), 8.16 (d, 2H, $J=8.40$ Hz Ar-H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 26.87, 28.99, 31.68, 32.64, 42.05, 51.10, 55.13, 113.29, 115.23, 123.05, 128.47, 130.88, 131.35, 138.15, 146.57, 157.61, 157.89, 195.68. IR (cm^{-1}): 3303 and 3222 w (NH_2), 3093 and 3071 w (Ar-H), 2997 w (C-H), 1633 s (C=O), 1626 and 1570 m (C=C), 1386, 1221, 1166, 1136, 1032; MS(CI) m/z 535.10 ($\text{M} + 1$), 428.00 ($\text{M}-\text{PhOCH}_3$).

4-(3,3,6,6-tetramethyl-1,8-dioxo-9-(pyridin-2-yl)-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (6)

As yellow crystals, (0.429 mg, 85%), mp 170°C (ethanol- H_2O). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 1.01 (s, 6H, $2 \times \text{CH}_3$), 1.11 (s, 6H, $2 \times \text{CH}_3$), 2.14–2.28 (m, 4H, $-\text{CH}_2$), 2.43–2.58 (m, 4H, $-\text{CH}_2$), 4.94 (s, 1H, $-\text{CH}$), 5.35 (s, 2H, SO_2NH_2), 7.19 (qxd, 2H, $J=2.29$ Hz, $J=1.44$ Hz Ar-H), 7.53–7.64 (m, 4H, Ar-H), 8.27 (dxq, 1H, $J=7.41$ Hz, $J=1.39$ Hz Ar-H), 8.39 (dxq, 1H, $J=4.80$ Hz, $J=0.64$ Hz Ar-H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 27.15, 29.30, 32.30, 34.45, 40.86, 50.75, 114.33, 121.40, 122.10, 124.96, 130.95, 131.77, 135.71, 145.02, 148.89, 161.72, 163.37, 196.98. IR (cm^{-1}): 3300 w (NH_2), 3065 w (Ar-H), 2960 w (C-H), 1652 s (C=O), 1621 and 1570 m (C=C), 1360, 1223, 1196, 1165, 1001; MS(CI) m/z 506.78 ($\text{M} + 1$).

4-(9-(2,4-dimethoxyphenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (7)

As yellow crystals, 396 mg, 78%, mp 243°C (chloroform). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 1.60–1.91 (m, 6H, $3 \times \text{CH}_2$), 2.19–2.46 (m, 6H, $3 \times \text{CH}_2$), 3.72 (s, 3H, $-\text{OCH}_3$), 3.80 (s, 3H, $-\text{OCH}_3$), 5.18 (s, 1H, $-\text{CH}$), 6.44 (dxq, 1H, $J=8.53$ Hz, $J=2.60$ Hz Ar-H), 6.50 (d, 1H, $J=2.54$ Hz Ar-H), 7.24 (d, 1H, $J=8.57$ Hz Ar-H), 7.62 (s, 2H, Ar-H), 7.69 (s, 2H, SO_2NH_2), 8.06 (d, 2H, $J=8.50$ Hz Ar-H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 21.40, 27.94, 31.96, 36.51, 55.73, 55.91, 99.03, 103.14, 116.90, 122.08, 124.54, 130.66, 131.13, 133.46, 144.80, 156.09, 159.48, 161.29, 196.08. IR (cm^{-1}): 3310 w (NH_2), 3171 and 3031 w (Ar-H), 2971 w (C-H), 1641 s (C=O), 1608 and 1568 m (C=C), 1362, 1234, 1089, 1042; MS(CI) m/z 509.65 ($\text{M} + 1$).

4-(9-(4-methoxyphenyl)-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl)benzene sulfonamide (8)

As yellow crystals, 0.397 mg, 83%, mp 181°C (ethanol- H_2O). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 1.54–1.93 (m, 6H, $3 \times \text{CH}_2$), 1.96–2.30 (m, 6H, $3 \times \text{CH}_2$), 3.52 (s, 3H,

–OCH₃), 5.11 (s, 1H, –CH), 6.64 (d, 2H, *J* = 8.80 Hz Ar-H), 6.77 (s, 2H, SO₂NH₂), 7.13 (d, 2H, *J* = 8.51 Hz Ar-H), 7.30 (d, 2H, *J* = 8.51 Hz Ar-H), 7.96 (d, 2H, *J* = 8.80 Hz Ar-H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 21.36, 27.95, 31.23, 36.48, 55.48, 113.70, 116.54, 122.16, 126.53, 130.69, 131.02, 138.29, 144.53, 150.63, 156.52, 196.43. IR (cm⁻¹): 3312 w (NH₂), 3125 and 3061 w (Ar-H), 2940 w (C-H), 1638 s (C=O), 1595 and 1539 m (C=C), 1385, 1131, 1084, 1011; MS(CI) *m/z* 479.22 (*M* + 1).

4-(9-(4-Cyanophenyl)-2,2,7,7-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (9).

As yellow crystals, 471 mg, 89%, mp 299°C (ethanol-H₂O). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.82 (s, 6H, 2 × CH₃), 0.93 (s, 6H, 2 × CH₃), 1.63–1.72 (m, 2H, –CH₂), 1.78–1.92 (m, 2H, –CH₂), 1.99–2.10 (m, 2H, –CH₂), 2.28–2.47 (m, 2H, –CH₂), 5.11 (s, 1H, –CH), 6.80 (s, 2H, SO₂NH₂), 7.33–7.46 (m, 6H, Ar-H), 8.01 (d, 2H, *J* = 8.30 Hz Ar-H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 24.08, 24.16, 24.35, 32.57, 33.84, 39.91, 112.08, 114.50, 120.54, 122.46, 127.45, 130.27, 131.65, 133.71, 144.10, 151.03, 160.23, 204.60. IR (cm⁻¹): 3356 and 3280 w (NH₂), 3076 w (Ar-H), 2957 w (C-H), 2222 (CN), 1643 s (C=O), 1619 and 1566 (C=C), 1349, 1220, 1198, 1160, 1010; MS(CI) *m/z* 530.93 (*M* + 1).

4-(9-(4-Hydroxyphenyl)-2,2,7,7-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (10)

As yellow crystals, 442 mg, 85%, mp 280°C (decompoze) (ethanol-H₂O). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 0.91 (s, 6H, 2 × CH₃), 1.00 (s, 6H, 2 × CH₃), 1.60–1.71 (m, 4H, –CH₂), 1.88–1.92 (m, 2H, –CH₂), 2.28–2.33 (m, 2H, –CH₂), 4.98 (s, 1H, –CH), 7.61 (d, 2H, *J* = 8.45 Hz Ar-H), 7.05 (d, 2H, *J* = 8.45 Hz Ar-H), 7.55 (d, 2H, *J* = 7.62 Hz Ar-H), 8.02 (d, 2H, *J* = 7.62 Hz Ar-H), 8.25 (s, 1H, Ar-OH), 9.02 (s, 2H, SO₂NH₂); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 24.12, 24.23, 24.38, 32.69, 33.81, 39.93, 114.56, 115.38, 122.49, 129.06, 130.50, 131.23, 139.51, 144.17, 154.91, 160.31, 204.56. IR (cm⁻¹): 3353 and 3278 w (NH₂), 3066 and 3029 w (Ar-H), 2971 w (C-H), 1631 s (C=O), 1610 and 1586 m (C=C), 1362, 1222, 1161, 1145, 1016; MS(CI) *m/z* 521.83 (*M* + 1).

4-(9-(3,4-Dimethoxyphenyl)-2,2,7,7-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (11)

As yellow crystals, 429 mg, 76%, mp 190°C (ethanol-H₂O). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.04 (s, 6H, 2 × CH₃), 1.11 (s, 6H, 2 × CH₃), 1.67–1.72 (m, 4H, 2 × CH₂), 1.85–2.03 (m, 2H, –CH₂), 2.15–2.28 (m, 2H, –CH₂), 3.86 (s, 3H, –OCH₃), 3.92 (s, 3H, –OCH₃), 5.01 (s, 2H, SO₂NH₂), 5.29 (s, 1H, –CH), 6.78 (m, 2H, Ar-H), 7.11 (s, 1H, Ar-H), 7.47 (d, 2H, *J* = 8.20 Hz, Ar-H), 8.14 (d, 2H, *J* = 7.50 Hz, Ar-H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 24.17, 24.20, 24.33, 32.98, 33.73, 39.88, 55.23, 55.29, 112.55, 114.30, 114.65, 122.41, 122.49, 130.53, 131.24, 137.62, 144.26, 156.49, 157.20, 160.72, 204.61. IR (cm⁻¹): 3306 and 3239 w (NH₂),

3096 w (Ar-H), 2963 w (C-H), 1631 s (C=O), 1579 and 1510 (C=C), 1362, 1263, 1227, 1159, 1027; MS(CI) *m/z* 565.88 (*M* + 1).

4-(9-(2,4-Dichlorophenyl)-2,2,7,7-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridine-10(9H)-yl) benzenesulfonamide (12)

As yellow crystals, 521 mg, 91%, mp 270°C (decompoze) (ethanol-H₂O). ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 0.81 (s, 6H, 2 × CH₃), 0.97 (s, 6H, 2 × CH₃), 1.52–1.58 (m, 2H, –CH₂), 1.60–1.70 (m, 2H, –CH₂), 1.84–1.95 (m, 2H, –CH₂), 2.19–2.23 (m, 2H, –CH₂), 5.20 (s, 1H, –CH), 7.30 (dxd, 1H, *J* = 6.00 Hz, *J* = 2.31 Hz, Ar-H), 7.38 (d, 1H, *J* = 2.77 Hz, Ar-H), 7.51 (d, 1H, *J* = 8.50 Hz, Ar-H), 7.61 (s, 2H, SO₂NH₂), 7.71 (m, 1H, Ar-H), 7.86 (m, 1H, Ar-H), 8.02 (d, 2H, *J* = 7.83 Hz, Ar-H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 24.13, 24.20, 24.30, 33.01, 33.69, 39.90, 114.88, 122.45, 127.04, 130.32, 130.44, 131.18, 131.27, 134.56, 137.71, 140.83, 144.20, 161.04, 204.51. IR (cm⁻¹): 3384 and 3319 w (NH₂), 3088 w (Ar-H), 2963 w (C-H), 1642 s (C=O), 1620 and 1573 (C=C), 1332, 1221, 1198, 1160, 1013; MS(CI) *m/z* 573.75 (*M* + 1).

Purification of carbonic anhydrase I and II from human erythrocytes

Erythrocytes were purified from human blood. The blood samples were centrifuged at 1500 rpm for 20 min and plasma was removed. Later, red cells were washed with NaCl (0.9%), and the erythrocytes were hemolyzed with 1.5 volumes of ice-cold water. Cell membranes were removed by centrifugation at 4°C, 20,000 rpm for 30 min. The pH of hemolysate was adjusted to 8.7 with solid Tris. The hemolysate was applied to affinity column with a structure of Sepharose-4B-L-tyrosine-*p*-aminobenzenesulfonamide and equilibrated with 25 mM Tris-HCl/0.1 M Na₂SO₄ (pH 8.7). The affinity gel was washed with solution of 25 mM Tris-HCl/22 mM Na₂SO₄ (pH 8.7). The hCA-I and hCA-II isozymes were diluted with the solution of 1 M NaCl/25 mM Na₂HPO₄ (pH 6.3) and 0.1 M NaCH₃COO/0.5 M NaClO₄ (pH 5.6), respectively¹⁹. For protein content estimation, Bradford method was used with bovine serum albumin as a standard^{20,21}. SDS polyacrylamide gel electrophoresis was performed after the purification of the enzyme (see Figure 1)²².

Determination of hydratase and esterase activities of hCA I and hCA II

The CO₂-hydratase activity of the enzyme was determined at 0°C in a veronal buffer (pH = 8.15) with pH-state method as indicator and saturated carbon dioxide solution as substrate in a final volume of 4.2 mL. The time (in seconds) taken for the solution to change from pH 8.15 to pH 6.5 was measured by pH meter. The enzyme unit (EU) is the enzyme amount that reduces the non-enzymatic reaction time by 50%. Activity of an enzyme unit was calculated by using the equation (*t*₀ – *t*_c/*t*_c) where *t*₀ and *t*_c are times for pH change of the non-enzymatic and enzymatic reactions, respectively^{23–25}. Esterase activities of hCA I

and hCA II isoenzymes, eluted from affinity column, were determined by hydrolysis of *p*-nitrophenylacetate. The change of absorbance was determined at 348 nm after 3 min²⁶.

Determination of IC_{50} and K_i values of compounds

The study consists of two parts. In the first part, IC_{50} values have been obtained as *in vitro*. The IC_{50} values of inhibitors (**4–12**) were assayed by the hydrolysis of *p*-nitrophenyl acetate on the esterase activities of CA isoenzymes in the presence of various inhibitor concentrations. The absorbance was determined at 348 nm after 3 min²⁷. Regression analysis graphs were drawn by plotting inhibitor concentrations versus enzyme activity.

To determine K_i value as well as the inhibition type, three different inhibitor concentrations giving 30%, 50% and 70% inhibition were selected. At each of these inhibitor concentrations, enzyme activity was measured in the presence of various substrate concentrations (0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM and 0.7 mM) and the data were linearized with Lineweaver–Burke plot for V_{max} and the K_i determination. Enzyme activity was also measured in the presence of the same substrate concentrations but in the absence of any inhibitor to determine the V_{max}^{26} .

Result and discussion

The general synthetic method shown in Scheme 1 is employed to prepare dioxoacridine sulfonamide derivatives (**4–12**). All spectral data are in agreement with the assigned structures.

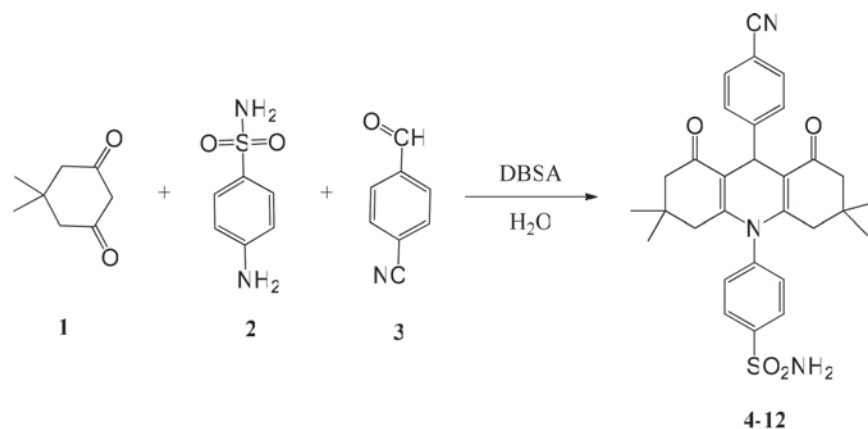
The synthesis of dioxoacridine sulfonamide compounds were realized in water in a single process through two successive reactions (Aldol condensation and Michael addition) and using a phase transfer catalyst–Bronsted acid as *p*-dodesylbenzenesulphonic acid (DBSA). In recent years, using DBSA as a combine catalyst (phase transfer catalyst–Bronsted acid) has been a popular application in organic chemistry^{28–30}. Sulfonamide compounds were prepared by one pot reaction in processing high yields and simple work-up procedure.

The infrared (IR) spectra of all the dioxoacridine sulfonamide compounds showed sharp peaks for the carbonyl groups in region between 1650 and 1631 cm^{-1} ³¹. The compounds **4**, **9** exhibited peaks that belong to CN group 2229 and 2222 cm^{-1} , respectively³². Besides, in the IR spectra of the compounds, aliphatic C–H stretching bands at 2997–2940 cm^{-1} and aromatic C–H stretching bands at 3171–3029 cm^{-1} were observed. The NH_2 vibrations of dioxoacridine sulfonamide compounds were observed in the region between 3384 and 3172 cm^{-1} ³³. The 1H -NMR spectra of compounds **4–6** showed singlet peaks that belong to protons of the methyl groups in position 3 and 6 between 0.84 and 1.11 ppm. The compounds **9–12** showed singlet peaks that belong to protons of the methyl groups in position 2 and 7 between 0.81 and 1.11 ppm³⁰. The CH_2 group protons of the cyclohexene rings of the compounds **4–12** showed multiple peaks in the 1.54–2.58 ppm range³⁰. Signals for the methoxy group protons for compounds **5**, **7**, **8**, **11** were shown in the range of 3.52–3.92 ppm. The signals for the CH protons at 4.98–5.35 ppm and signals for the aromatic protons in the range of 6.44–8.39 ppm were observed. Signals of pyridine ring protons for the compound **6** showed in the range of 7.01–8.39 ppm. Hydroxyl group proton of compound **10** was observed as broad signal at 8.25 ppm. The broad singlet peaks between 4.94 and 9.02 ppm were assigned to sulfonamide ($-SO_2NH_2$) groups protons of all the compounds **4–12** (Scheme 2).

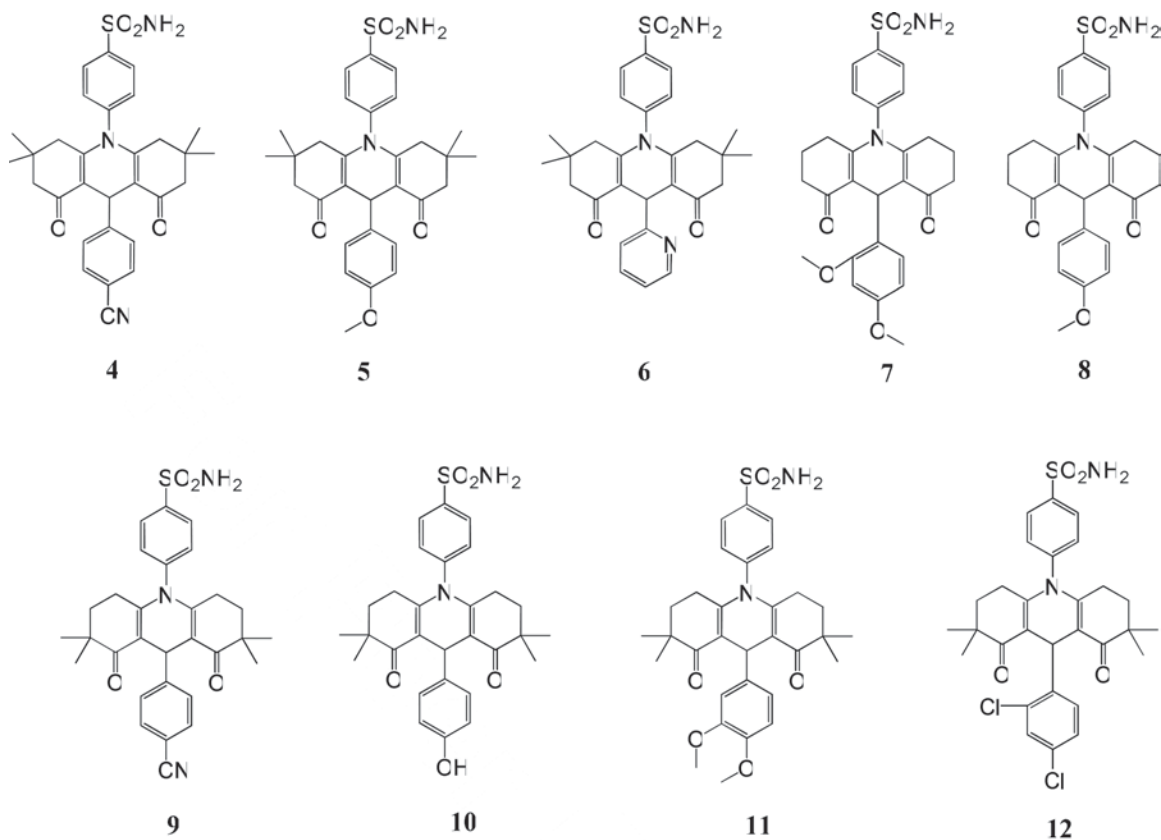
The concentration required inhibiting hCA I and hCA II activities of the purified proteins by 50% (IC_{50}) and inhibition equilibrium constant (K_i) was determined for each compound. Sulfanilamide and AAZ were used as control compounds to compare inhibition potential of newly synthesized compounds **4–12**.

According to *in vitro* studies, any inhibition effects of **4–12** compounds were not observed on hydratase activity of hCA I and hCA II isoenzymes. The IC_{50} and K_i values obtained from esterase activities of these compounds were shown in Table 1.

All of the new inhibitor compounds are more effective than control compounds (sulfanilamide and AAZ) for



Scheme 1. Synthesis of acridine-1,3-dion sulfonamides (**4–12**).



Scheme 2 . New CA inhibitors (4–12).

 Table 1. The K_i values obtained from *in vitro* inhibition experiments.

Inhibitor	K_i Values (μM)	
	hCA I	hCA II
AAZ	3.1–3.0	2.2–2.1
Sulfanilamide	4.6–4.4	3.9–3.8
4	0.38–0.66	0.19–0.31
5	0.08–0.07	0.18–0.12
6	0.05–0.06	0.12–0.14
7	0.01–0.02	0.001–0.002
8	0.008–0.009	0.006–0.008
9	0.13–0.14	0.09–0.10
10	0.04–0.05	0.11–0.14
11	0.19–0.22	0.11–0.13
12	0.21–0.23	0.03–0.04

hCA I and hCA II. Especially compounds **5**, **6**, **7**, **8** and **10** have shown remarkable inhibition on hCA I and hCA II isoenzymes.

The K_i values of all the novel compounds are lower than control compounds for hCA I and hCA II.

Compounds **5**, **7**, **8** and **11** contain methoxy group, while compound **10** contain hydroxyl group. These compounds are the most effective inhibitors in the newly synthesized compounds. It is thought that these groups in abovementioned compounds increase interactions of inhibitor-zinc metal and inhibitor-histidine residues in the active site of hCA I and hCA II isoenzymes³⁴.

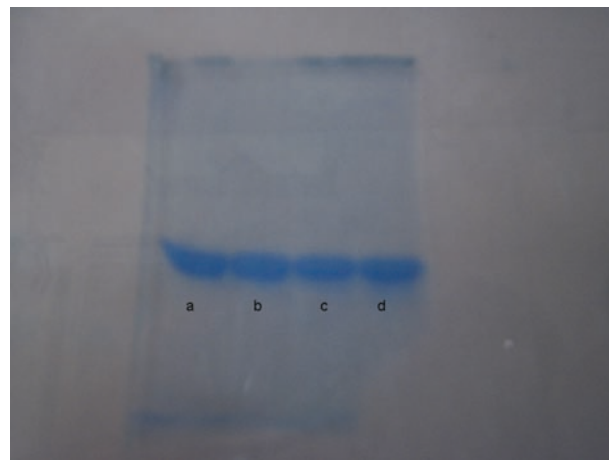


Figure 1. SDS-PAGE analysis of CA isoenzymes: (a) Standard hCA I, (b) isolated hCA I, (c) standard hCA II, (d) isolated hCA II.

In summary, these new compounds have potential inhibitory effects; so these compounds can be candidates for *in vivo* studies of glaucoma treatment.

Conclusion

A series of dioxoacridine sulfonamide containing nitrile, methoxy, halogen, and hydroxyl groups were synthesized. Synthesized compounds have inhibitory potential

on human erythrocyte hCA I and hCA II isoenzymes. So these compounds can be candidates for *in vivo* studies of glaucoma treatment.

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Declaration of interest

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