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Original article

Discovery of water-soluble semicarbazide-containing sulfonamide derivatives possessing favorable anti-glaucoma effect *in vivo* and drug-like properties

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ABSTRACT

In order to obtain topical and non-irritating anti-glaucoma drugs, novel semicarbazide-containing sulfonamide derivatives were designed and synthetized by sugar tail method in this study. The hydrophilic monosaccharides were expected to form interaction with the hydrophilic site of hCA II meanwhile the linker semicarbazides are used to further enhance water solubility, and more importantly, regulate the pH values of the target compounds in aqueous solution. First, all target compounds were synthesized and evaluated for their CA inhibitory activities. The results showed our target compounds demonstrated comparable activity to the positive control drug acetazolamide. The best derivative **11d** exhibits an IC_{50} value of 14 nM for hCA II and 2086-fold selectivity over CA I. Subsequently, physicochemical properties study showed that the target compounds displayed very good water solubility (up to 3 %) and neutral pH value in solutions. Meanwhile, the artificial membrane permeability assay was performed to verify that the target compound could also pass through the membrane structure despite their strong water solubility. In the glaucomatous rabbit eye model, the applied topically representative compounds showed strongly lowered intraocular pressure (IOP), as 1 % or 2 % water solutions. Subsequent drug-like evaluation showed our target compounds possessed low hemolysis effect and low cytotoxicity toward human corneal epithelial cell line. Also, it was not found that these target compounds had significant inhibition of hERG and CYP. In addition, these novel analogs also displayed good liver microsomal metabolic stability and plasma stability. Finally, docking studies provided the rational binding modes of representative compounds in complex with hCA II. Taken together, these results suggested that compound **11d** may be a promising hCA II inhibitor deserving further development.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous zinc metal enzymes, which play pivotal roles in a variety of physiological activities by catalyzing the reversible conversion of carbon dioxide to bicarbonate and proton ($CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$) (Supuran, 2008,2016,2020,2021a; Sonmez et al., 2014, Capasso and Supuran, 2015, Kumar et al., 2021, Nocentini et al., 2021, Nocentini and Supuran, 2018, Nocentini and Supuran, 2019). Carbonic anhydrase inhibitors (CAIs) were widely employed for the treatment of multiple diseases such as glaucoma, cancer, edema and altitude sickness in clinical practice (Mishra et al., 2020). Among the CAs, the CA II, located in the epithelial cells of the ciliary body, is a major target for developing antiglaucoma therapies (Alterio et al., 2012).

At present, there have been two generations of carbonic anhydrase inhibitors used in the clinical treatment of glaucoma. Acetazolamide (AAZ), methazolamide (MZA), and dichlorophenamide (DCP) are first-generation CAIs used as systemic drugs for the management of this disease (Fig. 1). Their main barrier, however, is the lack of isoenzyme selectivity due to the presence of 15 CA isoforms, which are widely distributed in many human tissues and organs. Thus, the systemically used CAIs lead to several undesired adverse effects, due to the inhibition of off-targets (Nixon, 2009, Supuran, et al., 2019, Nocentini and Supuran, 2019).

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Topical CAIs dorzolamide (DZA) and brinzolamide (BRZ) represent second-generation inhibitors. Compared to first-generation drugs, topical CAIs have fewer adverse effects but are also less potent. Moreover, their short duration of action requires 2–3 times daily dosing, decreasing persistence and adherence. In addition, in order to be prepared in topical drop form, dorzolamine must be prepared as a hydrochloride, which will cause eye irritation (Supuran, 2017,2018,2019,2021a,b; Berrino and Supuran, 2019; Bua and Supuran, 2019; Nocentini and Supuran, 2019; Angeli and Supuran, 2019; Supuran, et al., 2003; Supuran and Scozzafava, 2002; Casini, et al., 2000). In recent years, several hydrophilic tails with different chemical scaffolds had been used to design topical used CA inhibitors. The sugar tail, in particular, allows the target compound to be prepared into a highly concentrated aqueous solution without the need to prepare a hydrochloride (Hou et al., 2020).

Given the advantage of the sugar tail, novel carbohydrate-containing sulfonamide derivatives with semicarbazides as linker were designed and synthesized in this study. The approach is shown in Fig. 2. The hydrophilic monosaccharides were expected to form interaction with the hydrophilic site of hCA II. As we know, the sulfanilamide group is acidic. Therefore, the semicarbazides was chosen as the linker because its multiple alkaline amino groups can not only improve the water solubility, but also balance the acidity of sulfonamide so that the target compounds possess neutral pH in water. The target compounds resulting from this design are expected to be developed into topical and non-irritating anti-glaucoma drugs. More specifically, target compounds **11a**–**11h** (containing glucose, galactose, mannose, glucosamine, xylose, arabinose, rhamnose and ribose) were designed and synthesized. Then, their pharmacological activities, metabolic stability and primary safety assessment were conducted.

2. Experimental

2.1. Chemistry

All chemicals were purchased from commercial sources. All the ^{1}H NMR and ^{13}C NMR spectra were tested by employing a Bruker 600 MHz spectrometer, with tetramethyl silane as an internal reference. Column chromatography was performed using the 200–300 mesh silica gel (Qingdao Haiyang Chemical, China). Reactions were monitored by TLC analysis. ESI-MS and HRMS were performed using Agilent ESI-QTOF instrument.

2.1.1. Synthesis of target compound *11a*

The derivative **5a** was synthesized using previously described route (Guo et al., 2022). To a solution of 4-sulfamoylbenzoic acid **6** (1.00 g, 4.97 mmol) in $SOCl_2$ (12.5 mL), the DMF (0.02 mL) was added. The reaction mixture was refluxed for 12 h. Then 4-sulfonamidobenzoyl chloride **7** (1.05 g) was obtained by evaporation with a yield of 96 %, and the yellow crude products were directly used for the next reaction without purification. Then, to a solution of 4-sulfonamidobenzoyl chloride **7** (1.04 g, 4.77 mmol) in dry acetone (10.0 mL), sodium azide (0.62 g, 9.44 mmol) was added at 0 °C. The reaction mixture was stirred for 2 h. The white solid was filtered and washed with water to obtain intermediate **8** (0.97 g) in 90 % yield. To a solution of intermediate **8** (0.97 g, 4.29 mmol) in dry toluene (10.0 mL) was refluxed for 1 h. To the hot solution of intermediate **9** in toluene, the **5a** (2.62 g, 4.29 mmol) was then added and the mixture was further refluxed for 4 h. The solid was filtered off while hot and washed with ethanol and petroleum ether to obtain intermediate **10a** (1.74 g) in 51 % yield. To a solution of intermediate **10a** (1.74 g, 2.15 mmol) in MeOH (20 mL), the NaOMe in MeOH solution (1.0 mol/L, 10 mL) was added. The reaction mixture was stirred for 10 min and then was neutralized by using Dowex H^{+} resin until pH = 7. The mixture was filtered and evaporated. The filtrate was purified by a silica gel column chromatography (CH_2Cl_2: MeOH, 7:1) to obtain white solid **11a** (0.84 g) with a yield of 99 %. The compounds **11b**–**11h** were synthesized according to the above synthetic method.

*2.1.1.1. 4-(1-β-D-glucopyranosyl) semicarbazide) benzenesulfonamide (**11a**).* m.p. 154.2–156.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.93 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.17 (s, 2H), 6.83 (d, J = 9.1 Hz, 1H), 6.41 (t, J = 9.0 Hz, 1H), 5.01 (s, 2H), 4.68 (t, J = 9.1 Hz, 1H), 4.37 (s, 2H), 3.65 (dd, J = 11.7, 1.5 Hz, 1H), 3.43 (dd, J = 11.9, 5.3 Hz, 1H), 3.22 (t, J = 8.8 Hz, 1H), 3.15–3.11 (m, 1H), 3.10–3.06 (m, 1H), 2.99 (t, J = 9.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.71, 143.55, 137.03, 127.29, 117.61, 81.17, 78.76, 78.02, 73.36, 70.45, 61.38. ESI-MS (m/z): 415.1 [M + Na] $^{+}$; HRMS (ESI): Calcd for [M + Na] $^{+}$ $C_{13}H_{20}N_4NaO_8S$: 415.1012, Found 415.1037.

*2.1.1.2. 4-(1-β-D-galactopyranosyl) semicarbazide) benzenesulfonamide (**11b**).* m.p. 151.6–153.8 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.17 (s, 2H), 6.79 (d, J = 9.2 Hz, 1H), 6.21 (t, J = 9.0 Hz, 1H), 4.86–4.62 (m, 2H), 4.52 (d, J = 8.9 Hz, 3H), 3.69 (d, J = 2.8 Hz, 1H), 3.50 (dd, J = 10.7, 6.1 Hz, 1H), 3.43 (dd, J = 10.6, 6.0 Hz, 1H), 3.39 (t, J = 6.3 Hz, 1H), 3.37 (dd, J = 9.4, 3.1 Hz, 1H), 3.32 (t, J = 9.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.76, 143.58, 136.99, 127.30, 117.56, 81.65, 76.92, 74.65, 70.55, 68.77, 60.93. ESI-MS (m/z): 415.1 [M + Na] $^{+}$; HRMS (ESI): Calcd for [M + Na] $^{+}$ $C_{13}H_{20}N_4NaO_8S$: 415.0992, Found 415.0996.

*2.1.1.3. 4-(1-β-D-mannopyranosyl) semicarbazide) benzenesulfonamide (**11c**).* m.p. 160.3–162.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (d, J = 7.5 Hz, 1H), 7.66 (d, J = 7.4 Hz, 2H), 7.55 (d, J = 7.7 Hz, 2H), 7.32 (s, 2H), 7.04 (s, 1H), 6.20 (t, J = 9.0 Hz, 1H), 5.46 (d, J = 2.5 Hz, 1H), 5.30 (d, J = 2.7 Hz, 1H), 5.06–5.01 (m, 2H), 4.88 (d, J = 7.6 Hz, 1H), 3.87 (d, J = 5.3 Hz, 1H), 3.69 (d, J = 11.4 Hz, 1H), 3.63 (d, J = 5.4 Hz, 2H), 3.57 (dd, J = 11.5, 3.7 Hz, 1H), 3.52–3.41 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.89, 133.83, 129.81, 129.13, 127.78, 91.44, 74.90,

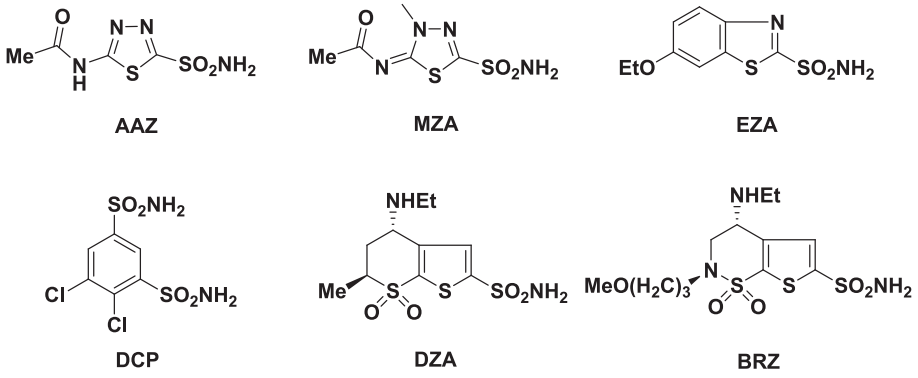


Fig. 1. The chemical structures of clinically used antiglaucoma CAIs.

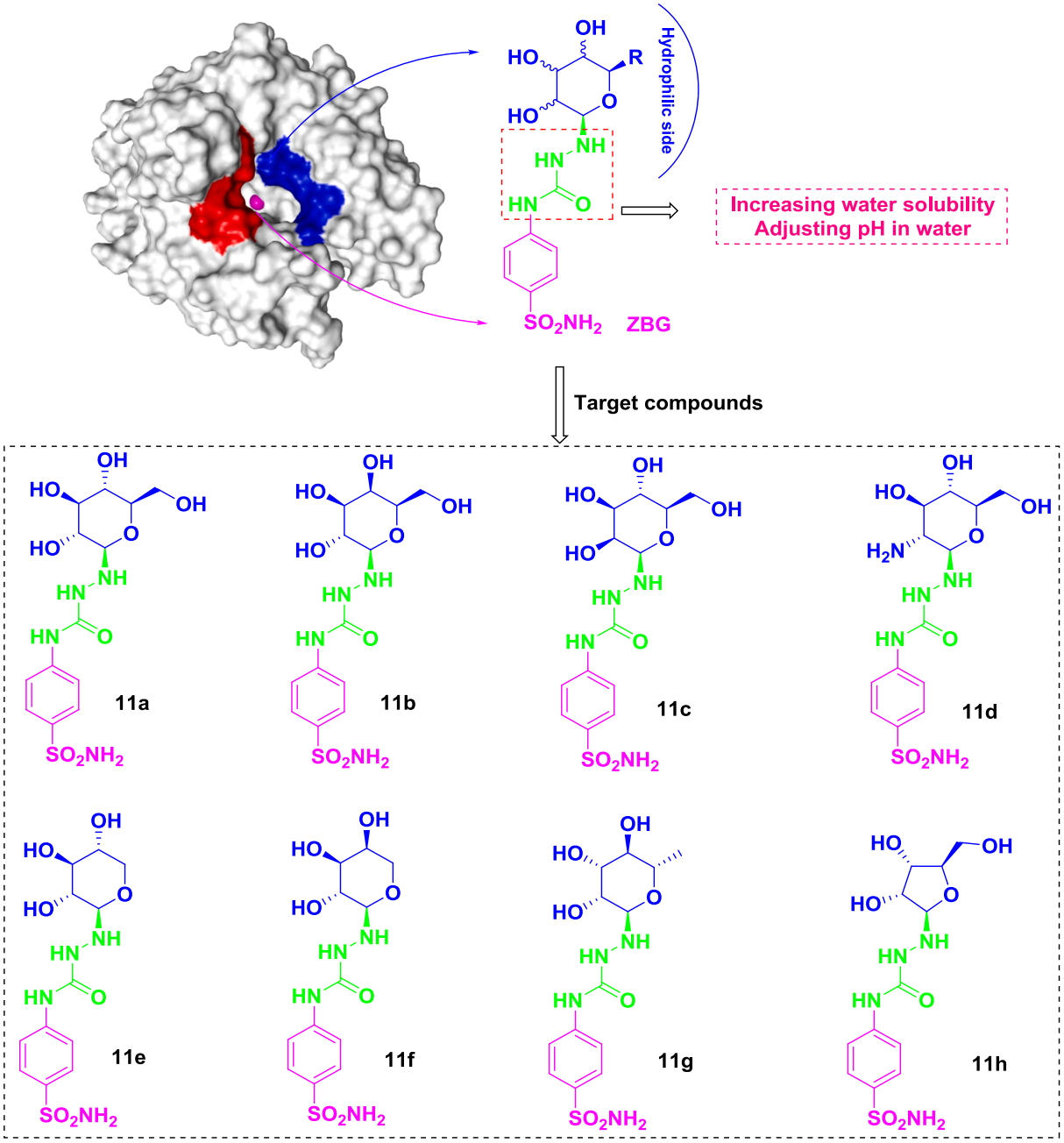


Fig. 2. Design strategies for the target compounds.

73.66, 68.98, 67.93, 61.57. ESI-MS (*m/z*): 415.1 [M + Na] $^{+}$; HRMS (ESI): Calcd for [M + Na] $^{+}$ $C_{13}H_{20}N_4NaO_8S$: 415.0893, Found 415.0877.

*2.1.1.4. 4-(1-β-D-glucosaminogly) semicarbazide) benzenesulfonamide (**11d**).* m.p. 159.4–161.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.46 (s, 1H), 7.71 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 9.4 Hz, 1H), 7.19 (s, 2H), 6.21 (t, J = 9.0 Hz, 1H), 6.04–5.43 (m, 2H), 5.22 (d, J = 12.9 Hz, 1H), 4.93 (t, J = 9.5 Hz, 1H), 4.62 (d, J = 7.9 Hz, 1H), 3.66 (d, J = 11.7 Hz, 1H), 3.46 (d, J = 8.4 Hz, 1H), 3.41–3.38 (m, 1H), 3.16 (d, J = 6.0 Hz, 3H), 2.77 (t, J = 9.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.63, 143.50, 137.16, 127.24, 117.74, 79.15, 79.07, 74.92, 70.32, 61.09, 56.01, 55.39. ESI-MS (*m/z*): 414.1 [M + Na] $^{+}$; HRMS (ESI): Calcd for [M + Na] $^{+}$ $C_{13}H_{21}N_5NaO_7S$: 414.1064, Found 414.1078.

*2.1.1.5. 4-(1-β-D-xylopyranosyl) semicarbazide) benzenesulfonamide (**11e**).* m.p. 150.6–152.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.17 (s, 2H), 6.86 (d, J = 9.0 Hz, 1H), 6.21 (t, J = 9.0 Hz, 1H), 4.74 (s, 1H), 4.62 (t, J = 8.9 Hz, 1H), 4.47 (s, 2H), 3.66 (dd, J = 11.2, 5.2 Hz, 1H), 3.29 (ddd, J = 10.1, 8.8, 5.3 Hz, 1H), 3.18 (t, J = 7.8 Hz, 1H), 3.07 (t, J = 10.7 Hz, 1H), 3.01 (t, J = 8.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.76, 143.54, 137.05, 127.28, 117.62, 81.95, 77.80, 73.07, 70.13, 67.47. ESI-MS (*m/z*): 385.1 [M + Na] $^{+}$; HRMS (ESI): Calcd for [M + Na] $^{+}$ $C_{12}H_{18}N_4NaO_7S$: 385.0781, Found 385.0792.

*2.1.1.6. 4-(1-β-L-arabinopyranosyl) semicarbazide) benzenesulfonamide (**11f**).* m.p. 150.8–152.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.17 (s, 2H), 6.97 (d, J = 9.1 Hz, 1H), 6.30 (t, J = 9.0 Hz, 1H), 4.75 (dd, J = 8.9, 6.7 Hz, 1H), 4.25 (s, 3H), 3.71 (d, J = 2.1 Hz, 1H), 3.62 (dd, J = 11.8, 4.7 Hz,

1H), 3.51 (dd, J = 7.5, 3.2 Hz, 1H), 3.45–3.38 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.57, 143.71, 136.93, 127.27, 117.55, 80.72, 73.10, 70.58, 67.30, 65.35. ESI-MS (m/z): 385.1 [M + Na] $^+$; HRMS (ESI): Calcd for [M + Na] $^+$ $C_{12}H_{18}N_4NaO_7S$: 385.0775, Found 385.0783.

*2.1.1.7. 4-(1-β-L-rhamnosyl) semicarbazide) benzenesulfonamide (**11g**).* m.p. 161.3–164.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.02 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 8.3 Hz, 2H), 7.49 (s, 2H), 6.86 (d, J = 9.6 Hz, 1H), 6.22 (t, J = 9.0 Hz, 1H), 5.22 (d, J = 8.6 Hz, 1H), 5.04 (d, J = 4.5 Hz, 1H), 4.80 (d, J = 5.3 Hz, 1H), 4.73 (d, J = 5.9 Hz, 1H), 3.71 (d, J = 3.3 Hz, 1H), 3.37 (ddd, J = 9.0, 5.9, 3.3 Hz, 1H), 3.24–3.20 (m, 1H), 3.17 (dd, J = 10.9, 4.6 Hz, 1H), 1.16 (d, J = 5.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.21, 146.99, 137.06, 128.69, 126.05, 78.89, 74.31, 73.97, 72.11, 71.04, 18.41.ESI-MS (m/z): 399.1 [M + Na] $^+$; HRMS (ESI): Calcd for [M + Na] $^+$ $C_{13}H_{20}N_4NaO_7S$: 399.0948, Found 399.0960.

*2.1.1.8. 4-(1-β-D-ribofuranosyl) semicarbazide) benzenesulfonamide (**11h**).* m.p. 156.8–158.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.15 (s, 1H), 7.93 (s, 4H), 7.50 (s, 2H), 6.96 (d, J = 9.4 Hz, 1H), 6.18 (t, J = 9.0 Hz, 1H), 5.63 (s, 1H), 5.33 (dd, J = 8.1, 4.1 Hz, 1H), 5.21 (d, J = 6.9 Hz, 1H), 4.92 (d, J = 5.8 Hz, 1H), 3.92 (s, 1H), 3.64–3.61 (m, 1H), 3.59–3.54 (m, 2H), 3.36 (q, J = 9.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.76, 147.18, 137.51, 128.09, 126.43, 77.34, 71.72, 67.48, 67.03, 60.96.ESI-MS (m/z): 385.1 [M + Na] $^+$; HRMS (ESI): Calcd for [M + Na] $^+$ $C_{12}H_{18}N_4NaO_7S$: 385.0782, Found 385.0760.

2.2. Biological assays

2.2.1. CA inhibition

The esterase assay has been utilized for assessing the inhibitory activities of new synthetic molecules against carbonic anhydrases, as reported before (Hou et al., 2017). The carbonic anhydrases were purchased from Novus Biologicals USA and the purity of these enzymes is >95 %. The detailed procedures were showed in the Supplementary Material.

2.2.2. Cytotoxic activities study

Cell viability assay were achieved referring to the previous method (Hou et al., 2017), and the procedures were noted in the Supplementary Material.

2.2.3. Artificial membrane permeability assay

A 1.8 % lecithin solution (w/v) was prepared in dodecane and the mixture was ultrasonically treated to ensure complete dissolution. Then, lecithin/dodecane mixture (6 μL) was pipeted into each acceptor plate well (top compartment). After the artificial membrane was applied (within 10 min), 300 μL of PBS (1X PBS, pH 7.4) solution was added to each well of the acceptor plate. 300 μL of drug-containing solutions were added to each well of the donor plate (bottom compartment) in triplicate. The acceptor plate was slowly and carefully placed into the donor plate, making sure the underside of the membrane is in contact with the drug-containing solutions in all wells. Replace the plate cover and incubate at 25℃ at 60 RPM for 16 h. After incubation, aliquots of 50 μL from each well of acceptor and donor plate are transferred into a 96-well plate. 450 μL of methanol was added into each well. Samples were centrifuged at 3220 g for 20 min. The concentration of the compound was determined by LC/MS/MS.

2.2.4. Preliminary safety assessment

Hemolytic assays, Inhibition Evaluation on CYP and hERG K^+ Channel were performed as reported (Xu et al., 2021), and the detailed experimental method were described in the Supplementary Material.

2.2.5. Metabolic stability assays

The metabolic stability of new synthetic molecules in liver microsomes and plasma was tested, as reported previously (Xu et al., 2021).

2.2.6. Molecular docking study

The crystallographic structure of CA II complex (PDB code: 6UFC) were used to perform the molecular docking studies. Firstly, the protein structure was obtained by removing waters and ligands. Then, target compounds were docked into the active pocket of CA II. Finally, the interaction models between protein and target compounds were analyzed.

3. Results

3.1. Chemistry

The preparation of representative compound **11a** in this study was depicted in Schemes 1–3. In Scheme 1, commonly-available D-glucose **1a** was used as the starting material. Firstly, the intermediate **2a** was prepared *via* reacting D-glucose with benzoyl chloride in the presence of pyridine. Then, **2a** reacted with HBr-AcOH to generate the benzoyl glycosyl bromide **3a**. Thereafter, derivative **4a** was prepared *via* nucleophilic substitution by benzoyl glycosyl bromide **3a** with NH_2NHBoc. Next, the removal of the Boc group of **4a** leads to intermediate **5a**.

Subsequently, 4-sulfamoylbenzoic acid **6** reacted with dichlorosulfoxide to afford 4-sulfamoylbenzoyl chloride **7** in 96 % yield. The aroyl chloride **6** was then treated with sodium azide at 0 °C to yield the desired aroyl azide **8**, followed by Curtius rearrangement to produce the key intermediate 4-isocyanatobenzenesulfonamide **9**.

In Scheme 3, the key intermediate **10a** was synthesized by reacting **9** with derivative **5a** (Sonmez et al., 2014). Finally, the benzyl group of compound **10a** was removed to produce the target compound **11a**. Other compounds **11b–11h** were also synthesized by using this method.

3.2. Biological activity

3.2.1. Carbonic anhydrase isoforms inhibition assay

All the newly prepared compounds **11a–11h** were assessed for their CA inhibitory activities toward three isoforms hCA I, II, and IX by the use of the esterase assay, utilizing acetazolamide (AAZ) as reference CAI. The CA inhibition data were displayed in Table 1.

3.2.2. Water solubility and the pH value test.

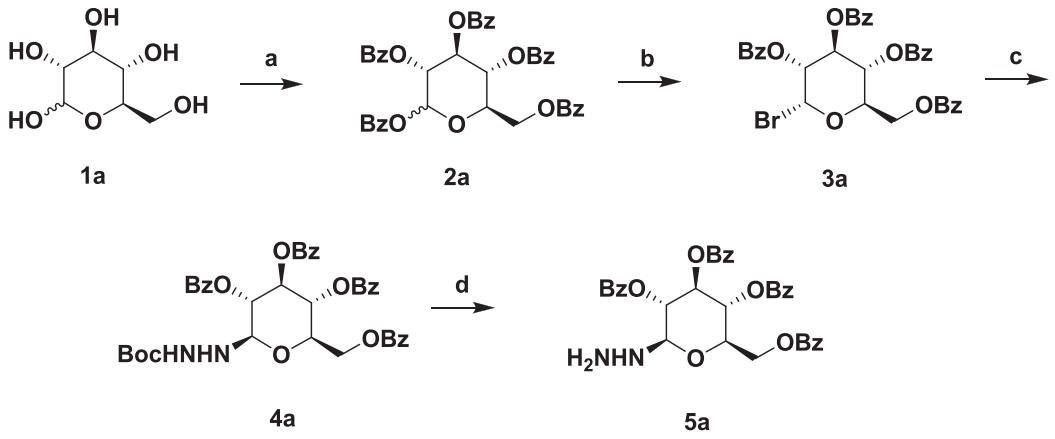
The new generation of anti-glaucoma drugs must be in the form of topical drops in order to reduce systemic side effects, which requires the target compound to have a high water solubility. With this in mind, we firstly determined the solubility of the target compounds in water (Fig. 3). Anti-glaucoma drugs with low eye irritation are more advantageous, which re-quires that the pH values of the new compounds in water were near neutral. Therefore, we also tested the pH value of the target compound in aqueous solution (Fig. 3).

3.2.3. Membrane permeability test

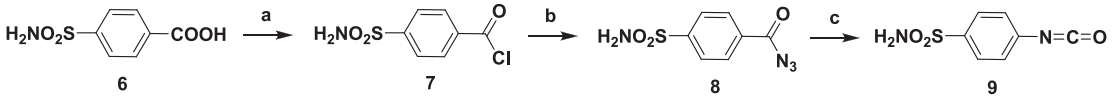
The complexity of eye structure makes it difficult to develop local eye drops. Our sugars are highly water-soluble and can be easily prepared as eye drops, which has the advantage of avoiding systemic side effects. However, the ability of highly water-soluble compounds to penetrate the cornea is critical to their ability to reach the target tissue, the ciliary body. To investigate whether the target compounds could cross the cornea to reach the target tissue, the artificial membrane permeability assay was performed in this study (Table 2).

3.2.4. In vitro cytotoxicity against mammalian cells

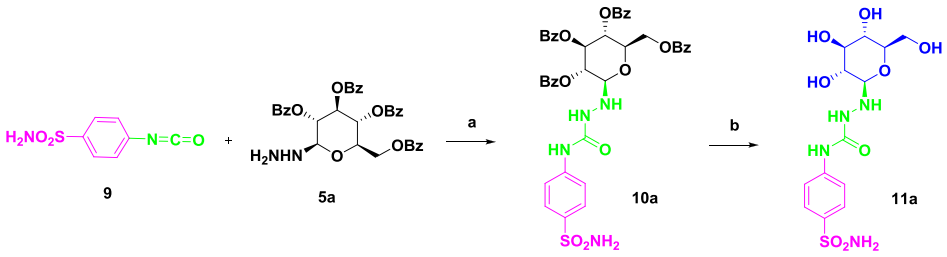
In this study, we tested the inhibitory activity of the compounds against carbonic anhydrase II and IX. Carbonic anhydrase II is associated with glaucoma, while carbonic anhydrase IX is a drug target for tumors.



Scheme 1. Preparation of the derivative **5a**.



Scheme 2. Preparation of key intermediate **9**.



Scheme 3. Synthesis of representative compound **11a**.

Table 1
Inhibition data of three CA isoforms with the target compounds by the esterase assay.

Compounds	IC_{50} (μM) [a]		Selectivity Ratio (SR)		
	hCA I	hCA II	hCA IX	hCA I/hCA II	hCA IX/ hCA II
p-Sulfamoylbenzoic acid	120	15	24	8	1.6
11a	17.0	0.015	0.051	1133	3.4
11b	21.2	0.019	0.064	1116	3.4
11c	22.4	0.015	0.045	1493	3.0
11d	29.2	0.014	0.067	2086	4.8
11e	33.4	0.052	0.088	642	1.7
11f	19.4	0.045	0.092	431	2.0
11 g	32.0	0.058	0.128	551	2.2
11 h	30.8	0.047	0.132	655	2.8
AAZ	0.3	0.013	0.030	23	2.3

[a] Errors in the range of 5–10% of the reported values, from three different determinations.

Therefore, we evaluated the compound's inhibitory activity on both human corneal epithelial cell lines (HCEC) and HT-29 cells (CA IX high expression) before conducting *in vivo* activity test. The evaluation of the inhibitory activity on corneal cells was conducted to determine the safety of the compounds as anti-glaucoma agents. Meanwhile, testing the activity on HT-29 cells can also indicate whether our target compounds have the potential to be used as anti-tumor agents. The results of this experiment will help us in deciding the next steps for our research. The data were described in Table 3.

3.2.5. *Evaluation of antiglaucoma activity in vivo*

Considering the excellent physicochemical properties, membrane permeability and cytotoxicity of the target compounds, the results of subsequent *in vivo* experiments are of utmost concern to us, which can also further prove whether the target compounds can pass through the cornea. In this study, brinzolamide (1 %) was chosen as positive drug and the most potent compounds **11a** and **11d** were chosen as representative compounds. The results were shown in Fig. 4.

3.2.6. *Assessment of hemolysis*

Topical drops could reduce systemic side effects, but a small amount of the drug still enters the circulatory system. Therefore, it is still necessary to carry out a series of drug-like evaluation. It is worth noting that hemolysis rate test is a necessary method to evaluate the safety of preferred compounds in preclinical studies. Therefore, the hemolysis rate experiment was conducted to explore the safety of the most potent compounds **11a**, **11c** and **11d** on rabbit erythrocyte. The results were shown in Table 4.

3.2.7. *Effect of on CYP enzyme inhibitory activity*

Cytochrome P450s (CYP) play an important role in the metabolism of many drugs. Drug metabolism through the cytochrome P450 system easily cause drug interactions, resulting in drug toxicity, reduced pharmacological action and adverse drug reactions. Therefore, the inhibitory effect of the most compound **11d** on human P450s such as 1A2, 2C9, 2C19, 2D6, and 3A4 was evaluated to investigate the potential

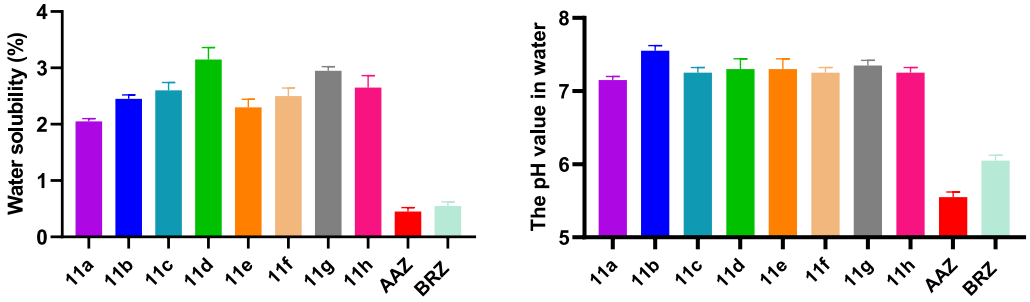


Fig. 3. The water solubility and the pH value test.

Table 2
The effective permeability coefficients (-Log Pe) of test compounds.

Compound	-Log Pe	Compound	-Log Pe	Compound	-Log Pe
Methotrexate	7.9	Testosterone	4.4	**11d**	5.1

Table 3
Inhibitory activity against mammalian cells.

Compound	**IC_{50} (μM)**	**Compound**	**IC_{50} (μM)**
	HCEC		**HT-29**
11a	>100	**11a**	92
11b	>100	**11b**	>100
11c	>100	**11c**	82
11d	>100	**11d**	74
11e	>100	**11e**	>100
11f	>100	**11f**	90
11 g	>100	**11 g**	86
11 h	>100	**11 h**	81
AAZ	88	AAZ	>100

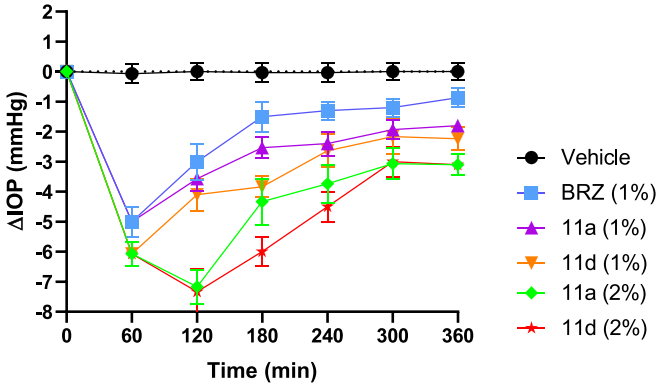


Fig. 4. The evaluation of antiglaucoma activity *in vivo*.

Table 4
The hemolysis ratio of the target compounds.

Compound	**Hemolysis ratio**				
	4 μg/mL	8 μg/mL	16 μg/mL	32 μg/mL	64 μg/mL
11a	0	0	0	0	0
11c	0	0	0	3.15	5.05
11d	0	0	0	5.24	6.36

Table 5
Inhibitory effects of compound **11d** on CYP450 enzymes.

Compound	**IC_{50} (μM)**				
	CYP1A2	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
11d	>50	>50	>50	>50	>50
Reference drugs	1.18[a]	0.617[b]	0.235[c]	1.34[d]	0.070[e]

[a] Phenacetin. [b] Diclofenac. [c] Mephenytoin. [d] Dextromethorphan. [e] Midazolam were selected as the reference drugs.

risk of drug interaction (Table 5).

3.2.8. *Inhibition evaluation on hERG channel*

Inhibition of the hERG channel will result in serious cardiotoxicity which is one of the main causes of drug failure. Early evaluation of hERG toxicity is important for drug development. Thinking of this, the representative compounds **11a**, **11c** and **11d** were further evaluated for inhibition of the hERG channel (Table 6).

3.2.9. *In vitro liver microsomal stability assay*

Liver microsomal stability is a key pharmacokinetic parameter in drug development. Therefore, the metabolic stability of target compounds in liver microsomes were also tested. Diclofenac and propafenone were used as control with poor or moderate metabolic stability. The results were shown in Table 7.

3.2.10. *Stability of compound **11d** in plasma*

The *in vitro* metabolic stability of the target compounds is an important component to be considered in the evaluation of drug-like properties. Therefore, the *in vitro* stability in rat plasma of the representative compound was also conducted and was shown in Table 8.

3.2.11. *Molecular docking study*

To obtain the binding interactions of target compounds with hCA II (PDB code: 6UFC), molecular docking studies were performed in this study. The results were shown in Fig. 5.

4. Discussion

4.1. *Carbonic anhydrase isoforms inhibition assay*

The following should be noted regarding CA inhibitory data of Table 1: (i) Target compounds **11a**–**11h** were poor hCA I inhibitors, with IC_{50} values in the micromolar range, as mentioned, between 17.0 and 33.4 μM. Notable is, these compounds showed weaker inhibitory

Table 6
The potassium channel Herg test of representative compounds.

Compound	**IC_{50} (μM)**	**Compound**	**IC_{50} (μM)**
11a	>100	**11c**	>100
11d	88.32	**Cisapride**	0.018

Table 7
Metabolic stabilities of compounds **11a**, **11c**, **11d** in mouse liver microsomes.

Compound	$T_{1/2}$ (min)	CL (mL/min/kg)
11a	1432	3.83
11c	1239	4.43
11d	1467	3.74
Diclofenac	40.0	137.4
Propafenone	2.3	2433.0

Table 8
Plasma stability of compound **11d**.

Time Point (min)	0	10	30	60	120
Remaining (%)	100	138	98	100	105
$T_{1/2}$ (min)	>2240				

activity than positive drug AAZ (IC_{50} = 0.3 μM). The low inhibition of these compounds containing sugars against the CA I may possess a positive feature, since hCA I was considered an important off-target when it comes to the study of CAIs class of antiglaucoma drugs. (ii) The parent p-sulfamoylbenzoic acid was ineffective hCA II inhibitor. Intriguingly, the introduction of hydrophilic sugar moieties into the parent p-sulfamoylbenzoic acid leads to a clear increase of the hCA II inhibitory activity (IC_{50}: 14–58 nM). The inhibitory activity of compounds **11a**–**11h** on hCA II was comparable to AAZ (IC_{50} = 13 nM). The slightly reduced inhibitory activity demonstrated by the compounds **11e–11f** may be due to a decrease in the number of hydroxyl groups on the sugar. (iii) Target compounds **11a**–**11h** exhibited good inhibitory potency against the isoform hCA IX (IC_{50} values between 45 and 132 nM). Among them, compound **11c** exhibited the strongest inhibitory activity against hCA IX (IC_{50} = 45 nM). (iv) Subsequently, the selectivity ratios for CAII and CAIX over CAI were investigated. The results showed that the target compounds possessed significant I/II inhibitory selectivity, with selectivity ratios spanning between 431 and 2086. All the compounds showed SR higher than the reference AAZ (SR = 23).

4.2. Water solubility and the pH value test

As expected, all compounds showed good water solubility (2 %–3%), which ensures that our compounds are easy to prepare into eye drops. The results showed that the pH values of the target compounds were in the range of 7–7.5, which means that our compounds may be low irritation to eyes.

4.3. Membrane permeability test

The compounds can be categorized into high and low permeability. Generally speaking, compounds which have a -Log Pe < 6 are classified as high permeability and compounds with a -Log Pe > 6 are classified as low permeability. We used methotrexate and testosterone as positive controls. The compound **11d** showed high permeability with a -Log Pe value of 5.1. In view of the membrane permeability, compound **11d** was chosen for further study.

4.4. In vitro cytotoxicity against mammalian cells

The data (Table 3.) suggested all the target compounds showed lower cytotoxicity toward human corneal epithelial cell line (IC_{50} > 100 μM), compared with AAZ (IC_{50} = 88 μM). Accordingly, these target compounds are safe with low side effects on normal cells than clinically used AAZ. Although most target compounds showed enhanced antitumor activity compared to the positive control AAZ, the inhibitory activity against HT-29 cells remains at the micromolar level. Therefore, we believed that our target compounds were better suited for development as anti-glaucoma drugs.

4.5. Evaluation of antiglaucoma activity in vivo

At 1 % concentration, BRZ and compound **11a** showed the same maximal lowing intraocular pressure effect (around 5 mm Hg) at 1 h post-administration. Compound **11d**, has a maximum intraocular pressure lowering effect of 6 mmHg at 2 h post-administration. It is interesting to note the more potent IOP lowering of compounds **11a** and **11d** was then maintained for the next 3–6 h compared to BRZ. Our compounds have the advantages over brinzolamide that they can be configured as a 2 % aqueous solutions. Therefore, we tested the ability of the compound to reduce IOP at 2 % concentration. As shown in Fig. 4, compounds **11a** and **11d** were both maximally active after 2 h post-administration, producing an IOP lowering of around 7 mm Hg and lasted for up to 6 h. Overall, our target compound demonstrated better *in vivo* anti-glaucoma activity compared to the positive control brinzolamide. Moreover, the good results of *in vivo* experiments can also prove that the target compound can successfully pass through the cornea to the target tissue ciliary body.

4.6. Assessment of hemolysis

From Table 4, it can be concluded that compound **11a** showed no activity toward the rabbit erythrocyte. Meanwhile, compound **11c** and **11d** showed only weak hemolytic effects at high concentrations (>32

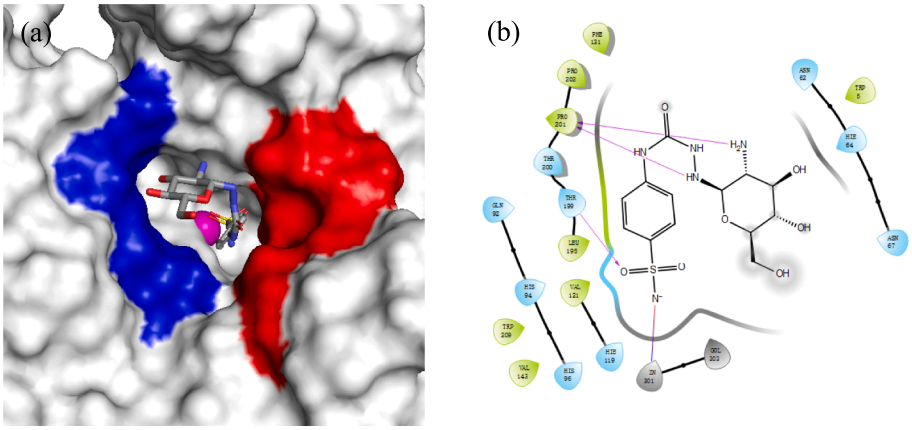


Fig. 5. The 3D docking pose displaying interaction of compound **11d** (a) in the binding site of hCA II (PDB ID: 6UFC). Highlighted hydrophobic (red) and hydrophilic (blue) residues. (b) The 2D docking pose of compounds **11d**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

μg/mL). In general, the target compounds showed some promising results.

4.7. Effect of on CYP enzyme inhibitory activity

To our delight, compound **11d** showed almost no inhibitory activity against all tested CYP isoforms with IC_{50} values over 50 μM, suggesting that compound **11d** might not cause drug interactions (Table 5).

4.8. Inhibition evaluation on hERG channel

The results displayed in Table 6 demonstrated that target compounds **11a** and **11c** had no inhibitory action on hERG ($IC_{50} > 100$ μM) while compound **11d** showed very weak inhibitory activity ($IC_{50} = 88.32$ μM). The results showed that our compounds may not be cardiotoxic.

4.9. In vitro liver microsomal stability assay

Liver microsomal stability is a key pharmacokinetic parameter in drug development. The results illustrated our target compounds displayed considerably longer half-lives of 1239–1467 min (Table 7).

4.10. Stability of compound *11d* in plasma

The results showed that compound **11d** contained desirable plasma stability, with a half-life value longer than 2240 min (Table 8).

4.11. Molecular docking study

The obtained results indicated that the deprotonated sulfonamide moiety of **11d** could anchor to the Zn (II) ion and form hydrogen bonding with the OH group of Thr199 residue. Meanwhile, the semicarbazides linker of **11d** could form hydrogen binding with Pro201. More specifically, the sugar groups of the compounds could form with hydrogen bond with Pro201 in CA II (Fig. 5).

5. Conclusion

In the present study, we designed and synthesized water-soluble semicarbazides-containing sulfonamide derivatives by sugar tail method. Notably, these target compounds exhibited interesting inhibition potency against hCA II, with IC_{50} values in nanomolar range from 14 to 58 nM. As a highlight, target compounds displayed very good water solubility (up to 3 %) and neutral pH value in solutions. The artificial membrane permeability assay was performed to verify that the target compound could also pass through the membrane structure despite their strong water solubility. More intriguingly, representative compounds showed no significant hemolysis or cytotoxicity to human corneal epithelial cell line. Also, representative compounds possessed low CYP and hERG inhibition. In addition, these target compounds demonstrated optimal profiles on good metabolic stability and plasma stability. Finally, molecular docking indicated a positive correlation between the obtained values of docking studies and experimental data. Hence, compound **11d** represents a promising lead compound for the discovery of more effective hCA II inhibitors as topical and non-irritating anti-glaucoma drugs.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2024.101969.

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