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# Carbonic anhydrase inhibitors: *N*-(*p*-sulfamoylphenyl)-α-D-glycopyranosylamines as topically acting antiglaucoma agents in hypertensive rabbits

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**Abstract**—A series of *N*-(*p*-sulfamoylphenyl)-α-D-glycopyranosylamines was prepared by reaction of sulfanilamide with different monosaccharides in the presence of ammonium chloride. The new compounds were investigated for inhibition of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1), involved in aqueous humor secretion within the mammalian eye. Isozymes CA I and CA II were strongly inhibited by some of these compounds, which showed inhibition constants in the range of 510–1200 nM against CA I and 10–25 nM against CA II, similarly to clinically used sulfonamides, such as acetazolamide, methazolamide, dichlorophenamide, dorzolamide and brinzolamide. The presence of sugar moieties in these molecules induced an enhanced water solubility as compared to other sulfonamides. In hypertensive rabbits (a widely used animal model of glaucoma), two of the new compounds showed strong and long-lasting intraocular pressure (IOP) lowering, being more effective than dorzolamide and brinzolamide, the two clinically used, topically acting antiglaucoma sulfonamides with CA inhibitory properties.


## 1. Introduction

Although the treatment of glaucoma with inhibitors of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1) is very effective in reducing elevated intraocular pressure (IOP), the systemic administration of drugs such as acetazolamide **AZA**, methazolamide **MZA** or dichlorophenamide DCP leads to unpleasant side effects due to inhibition of the enzyme present in other tissues (kidneys, red cells, stomach, etc.) than the eye.[1–4] The possibility of the topical administration of the classical drugs from this class mentioned above (acetazolamide, methazolamide or dichlorophenamide) was extensively investigated by several researchers, but negative results have been constantly obtained, and for more than 40 years it was considered that CA inhibitors could only be given sistemically.[1] Important advances in this field have been then achieved by the Merck group, which discovered the first clinically used, topically effective antiglaucoma sulfonamide, dorzolamide DZA.[5] The approach for arriving to this compound (and in fact also to brinzolamide BRZ, the second such clinically used pharmacological agent)[6] is known as the 'ring approach', as it involved the exploration of a wide range of ring systems to which sulfamoyl moieties were incorporated.[2–4] We have explored on the other hand an alternative approach for the design of topically acting antiglaucoma sulfonamides, which consists in attaching tails that will induce the desired physico-chemical properties (such as for example water solubility, good penetrability through the cornea, etc.) to scaffolds of aromatic/heterocyclic sulfonamides also incorporating derivatizable moieties of the amino/hydroxy type.[2–4,7–10]

Many tails have been used for the design of topically acting sulfonamide CA inhibitor antiglaucoma agents,

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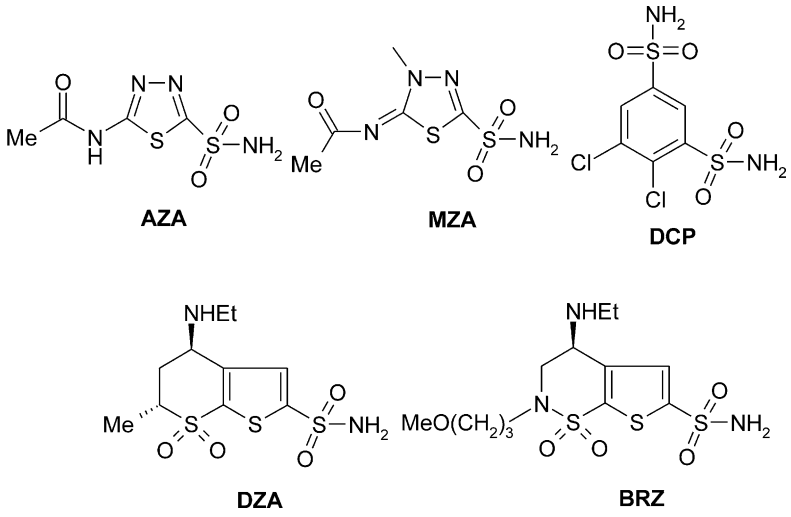



such as for example pyridine-carboxamido-, quinoline-sulfonylamido-, polyaminopolycarboxamido-, perfluoroalkyl/arylcarboxamido/sulfonamido, aminoacyl- or oligopeptidyl among others,[2–4,7–10] but no sugar moieties have been incorporated up to now in such compounds. Due to the highly hydrophilic character of such sugar moieties, one may expect a good water solubility for sulfonamides incorporating them, a feature desirable for topically acting drugs to be administered directly into the eye. Thus, we explored the possibility of synthesizing sugar-containing sulfonamides, prepared a series of such derivatives possessing α-glycosylamino tails, and studied their in vitro CA inhibitory properties against isozymes hCA I and hCA II. Some of the most effective CA inhibitors obtained were then investigated in vivo, in rabbits with high intraocular pressure (IOP), in order to detect compounds useful as topically acting antiglaucoma drugs.

## 2. Chemistry

Previous studies have shown that *N*-*p*-sulfamoylphenylglucosylamine **2a** and *N*-*p*-sulfamoylphenylmannosylamine **2c** possessed the same antibacterial properties as the lead compound, sulfanilamide, and were considerably less toxic and better tolerated.[11] Still, such compounds have never been investigated for their CA inhibitory properties. We decided to prepare a series of *N*-(*p*-sulfamoylphenyl)-α-D-glycopyranosylamines and test them for their interaction with the most abundant and physiologically relevant CA isozymes,[1–4] that is, hCA I and hCA II (Scheme 1).

The different glycosylamines **2** were prepared by a method previously described by Bognar and Nanasi.[12] Direct condensation of monosaccharides, such as glucose **1a**, galactose **1b**, mannose **1c**, ribose **1d**, arabinose **1e**, xylose **1f**, rhamnose **1g** and fucose **1h**, with sulfanilamide in 95% ethanol, using ammonium chloride as catalyst, led to the *N*-*p*-sulfamoylphenyl glycosylamine **2a–h** in good yields. All compounds were extensively characterized.[13]

## 3. CA inhibition

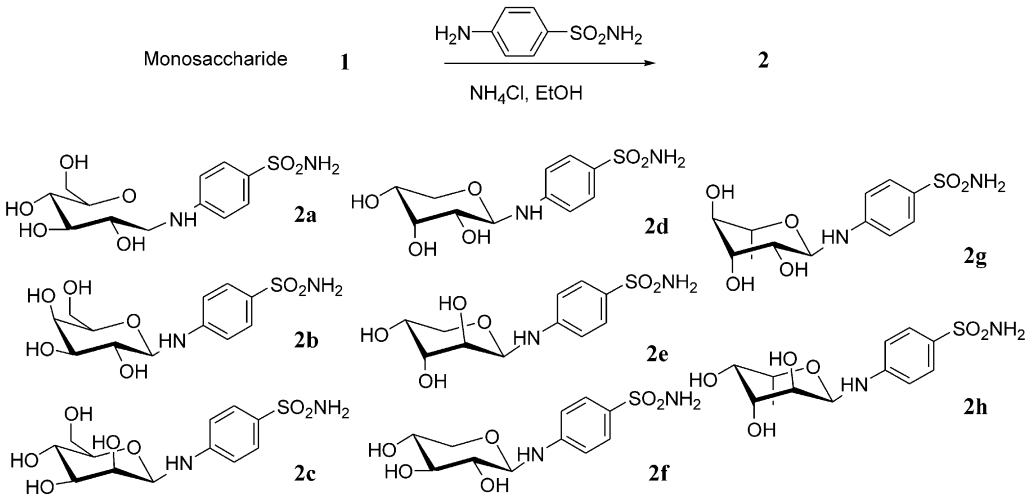
Inhibition data against isozymes hCA I and hCA II with the compounds **2a–h** reported here and standard CA inhibitors, such as acetazolamide, methazolamide, dichlorophenamide, dorzolamide, brinzolamide and sulfanilamide, are shown in Table 1.[16]

As seen from data of Table 1, the glycosylsulfanilamides **2a–h** reported here show a very compact behavior from the point of view of their CA I and II inhibitory properties, with all eight derivatives possessing inhibition constants in a limited range of values, i.e., of 510–1200 nM against hCA I, and of 10–25 nM against hCA II. Thus, in contrast to sulfanilamide, these sugar derivatives act as much more efficient inhibitors against both isozymes. Indeed, the parent compound, sulfanilamide, has a $K_i$ value of 28 μM against hCA I and of 300 nM against hCA II, being a weak inhibitor. The sugar derivatives **2a–h** are at least an order of magnitude better CA II inhibitors as compared to sulfanilamide, but the

**Table 1.** Inhibition data for derivatives **2a–h** reported in the present paper and standard sulfonamide CA inhibitors

| No. | Inhibitor | $K_i$[a] (nM) | |
|---|---|---|---|
| | | hCA I[b] | hCA II[b] |
| | Acetazolamide | 900 | 12 |
| | Methazolamide | 780 | 14 |
| | Dichlorophenamide | 1200 | 38 |
| | Dorzolamide | > 50,000 | 9 |
| | Brinzolamide | — | 3 |
| | Sulfanilamide | 28,000 | 300 |
| **2a** | | 1200 | 23 |
| **2b** | | 1000 | 25 |
| **2c** | | 930 | 18 |
| **2d** | | 840 | 16 |
| **2e** | | 630 | 12 |
| **2f** | | 750 | 15 |
| **2g** | | 510 | 10 |
| **2h** | | 680 | 14 |

[a] Mean from three different assays. Errors were in the range of 5–10% of the reported values.
[b] Human (recombinant) isozymes.




**Scheme 1.**

nature of the sugar moiety, although fine tuning potency, seems to be less important for the structure–activity relationship, as all derivatives showed rather similar potency. One must also mention that all the sugar derivatives used for the preparation of the new compounds belonged to the same series, which may explain these data. Derivatives **2a–h** showed the same potency against hCA II as the clinically used sulfonamides acetazolamide, methazolamide, dichlorophenamide and dorzolamide. Only brinzolamide behaved as a much stronger CA II inhibitor. The potency against CA I of the sugar derivatives **2a–g** was again comparable to that of acetazolamide, methazolamide and dichlorophenamide, whereas dorzolamide was a much weaker inhibitor of this isozyme (Table 1).

## 4. Topical antiglaucoma activity

Two of the best in vitro CA inhibitors investigated here, **2e** and **2g** have been formulated as 2% water solutions and administered topically to hypertensive rabbits, a widely used animal model of glaucoma.[2–6] The water solubility of these derivatives was indeed very good (data not shown) due to the presence of the hydrophilic sugar moieties in their molecule. Thus, it was easy to formulate them as eye-drops at neutral pH values, in contrast to dorzolamide and brinzolamide which are either not soluble enough at neutral pH and must be used as a suspension (brinzolamide)[6] or must be solubilized as hydrochloride salt (dorzolamide)[5] and as a consequence, the pH of the solution is rather acidic (around 5.5). Both these properties lead to undesired side effects of these two antiglaucoma drugs such as blurred vision, eye redening and irritation, and so on.[2–6] In the case of the sugar derivatives **2e** and **2g**, the presence of the glycosyl tail in their molecule highly enhanced water solubility at neutral pH, which constitutes a very desirable pharmacological feature for topically acting antiglaucoma drugs.

The variation of intraocular pressure (IOP) of hypertensive albino rabbits treated topically with one drop (50 μL) of 2% solution/suspension of inhibitors is shown in Figure 1.[17,18] The two standard drugs, dorzolamide DZA and brinzolamide BRZ were also included in our experiments. It may be seen that both DZA and BRZ show very similar patterns of IOP reduction, the two drugs being efficient lowering agents (curves 1 and 2 in Fig. 1), with a maximal effect (around 6.5–7 mm Hg) achieved at 1 h post-administration, and a return to basal IOP values after 3 h. The two sugar sulfanilamides investigated here were, on the other hand, much more effective IOP lowering agents as compared to the two clinically used drugs. Thus, the arabinose derivative **2e** was again maximally active after 1 h post-administration, producing an IOP lowering of around 10 mm Hg. A potent IOP lowering of 6–8 mm Hg was then maintained for the next 2–3 h (curve 3 in Fig. 1), and pressure returned to basal values after 5 h (data not shown). The best IOP lowering agent was the rhamnose derivative **2g** (curve 4 in Fig. 1). In this case, the maximal IOP lowering (of around 16 mm Hg) has been achieved after

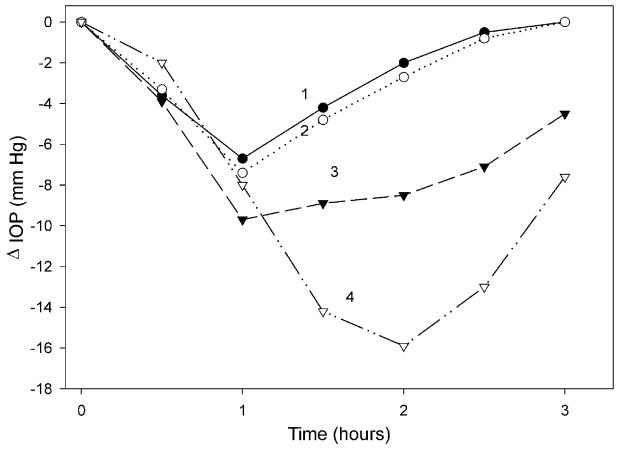



**Figure 1.** Effect of topically administered sulfonamide CA inhibitors (2% water solutions/suspensions) on the IOP of hypertensive albino rabbits (initial IOP of 33±3 mm Hg). Curve 1: dorzolamide **DZA** (hydrochloride salt, pH 5.5); curve 2: brinzolamide **BRZ** (2% suspension); curve 3: compound **2e** (solution, pH 7.0); curve 4: compound **2g** (solution, pH 7.0). Errors were in the range of 3–5% of the reported values.

2 h post-administration, and this very potent effect was maintained for the next 5–6 h when pressure returned to the basal values. Thus, the two derivatives investigated by us showed much more effective IOP lowering as compared to DZA and BRZ: both the magnitude of the effect as well as its duration of action were very much augmented, which constitute very desirable properties for topically acting antiglaucoma drugs. No ocular discomfort or eye irritation of the experimental animals have been observed after administration of these sugar-sulfonamide derivatives.

## 5. Conclusions

A small series of glycosyl-sulfanilamide derivatives was prepared and assayed as CA inhibitors. All compounds showed good CA II and CA I inhibitory properties, and two of them were very effective topical antiglaucoma agents in hypertensive rabbits. Indeed, the arabinosyl- and rhamnosyl-sulfanilamide derivatives produced maximal IOP lowering in the range of 10–16 mm Hg after administration directly into the eye, and the duration of action of this effect was much longer as compared to that of the standard drugs dorzolamide and brinzolamide. Furthermore, these sugar derivatives are water soluble at neutral pH, in contrast to the two clinically used drugs mentioned above, which may lead to highly reduced side effects after their topical administration within the eye.

## References and notes


1. Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595.
2. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146.
3. Supuran, C. T.; Scozzafava, A. *Curr. Med. Chem. Imm., Endoc., Metab. Agents* **2001**, *1*, 61.
4. Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Pat.* **2000**, *10*, 575.
5. Sugrue, M. F. *Progr. Ret. Eye Res.* **2000**, *19*, 87.
6. Silver, L. H. *Surv. Ophthalmol.* **2000**, *44* (Suppl 2), 147.
7. (a) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4884. (b) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4542.
8. (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Mincione, G.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 575. (b) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Supuran, C. T. *J. Med. Chem.* **2002**, *45*, 1466.
9. Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 551.
10. Supuran, C. T.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G. *Eur. J. Med. Chem.* **1999**, *34*, 799.
11. (a) Lehr, H.; Bloch, H.; Erlenmeyer, H. *Helv. Chim. Acta* **1945**, *28*, 1415. (b) Schering, A. G. French Patent, 824,726, 1939. *Chem. Abstr.* **1940**, *34*, 5857.
12. Bognar, R.; Nanasi, P. *J. Chem. Soc* **1953**, 1703.
13. Preparation of *N*-(*p*-sulfamoylphenyl)-glycosylamines:[12,14] 1 equiv of monosaccharide **1a**–**h**, 1 equiv of sulfanilamide and 0.06 equiv of ammonium chloride were refluxed in 95% ethanol until total dissolution of the reactants. The different glycosylamines **2a**–**h** crystallized on boiling the solution or on cooling. After filtration, the product was recrystallized from aqueous ethanol.

***N*-(*p*-sulfamoylphenyl)-α-D-glucopyranosylamine 2a**: mp 198–201 °C (lit.[14] 207 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.55 (d, 2H, $J$ = 8.5 Hz), 7 (s, 2H), 6.95 (d, 1H, $J$ = 7.7 Hz), 6.8 (d, 2H, $J$ = 8.5 Hz), 5.05 (d, 1H, $J$ = 4.5 Hz), 4.95 (d, 2H, $J$ = 5.1 Hz), 4.5 (m, 2H), 3.7 (m, 1H), 3.5 (s, 2H), 3.3 (m, 3H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 151.0, 132.5, 127.9, 113.3, 84.8, 78.3, 73.9, 73.1, 71.1, 62.1; IR (KBr) 3401, 2916, 1600, 1522, 1327, 1147, 1082 cm$^{-1}$; MS ESI$^+$ $m/z$ 357 (M + Na)$^+$. ESI$^-$ $m/z$ 333 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-D-galactopyranosylamine 2b**: mp 139–142 °C (lit.[12] 174–175 °C, 144 °C[3]); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.5 (d, 2H, $J$ = 8.7 Hz), 7 (s, 2H), 6.9 (d, 1H, $J$ = 7.8 Hz), 6.8 (d, 2H, $J$ = 8.7 Hz), 4.8 (m, 2H), 4.6 (s, 1H), 4.45 (m, 6H), 3.8 (s, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 151.1, 132.4, 128.2, 113.2, 85.3, 76.5, 75.1, 70.8, 69.2, 61.3; IR (KBr) 3355, 2895, 1601, 1526, 1315, 1153, 1093 cm$^{-1}$; MS ESI$^+$ $m/z$ 357 (M + Na)$^+$. ESI$^-$ $m/z$ 333 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-D-mannopyranosylamine 2c**: mp 191–192 °C (lit.[12] 194 °C, 188–190 °C,[2] 201 °C[3]); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.5 (d, 2H, $J$ = 8.5 Hz), 7 (s, 2H), 6.9 (d, 2H, $J$ = 8.5 Hz), 6.5 (d, 1H, $J$ = 9.2 Hz), 4.9 (d, 1H, $J$ = 5.2 Hz), 4.8 (m, 3H), 4.4 (t, 2H, $J$ = 5.4 Hz), 3.75 (m, 1H), 3.65 (m, 1H), 3.45 (m, 2H), 3.2 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 150.2, 132.9, 127.8, 113.6, 81.7, 78.7, 75.1, 71.6, 67.8, 62.1; IR (KBr) 3349, 2903, 1602, 1518, 1334, 1155, 1107, 1061 cm$^{-1}$; MS ESI$^+$ $m/z$ 357 (M + Na)$^+$. ESI$^-$ $m/z$ 333 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-D-ribopyranosylamine 2d**: mp 180–182 °C (lit.[14,15] 177–179 °C, 182 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.55 (d, 2H, $J$ = 8.4 Hz), 7 (s, 2H), 6.85 (d, 2H, $J$ = 8.4 Hz), 6.65 (d, 1H, $J$ = 8.5 Hz), 5.2 (d, 1H, $J$ = 4.4 Hz), 5 (d, 1H, $J$ = 4.7 Hz), 4.9 (m, 2H), 3.8 (s, 1H), 3.6 (m, 3H), 3.4 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 150.1, 132.9, 128.2, 113.4, 81.7, 71.4, 71.1, 68.1, 64.3; IR (KBr) 3350, 2906, 1605, 1521, 1321, 1153, 1101 cm$^{-1}$; MS ESI$^+$ $m/z$ 327 (M + Na)$^+$. ESI$^-$ $m/z$ 303 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-D-arabinopyranosylamine 2e**: mp 193–195 °C (lit.[14,15] 191 °C, 182–189 °C, 200 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.55 (d, 2H, $J$ = 8.5 Hz), 7 (s, 2H), 6.9 (d, 1H, $J$ = 7.9 Hz), 6.8 (d, 2H, $J$ = 8.5 Hz), 4.9 (m, 2H), 4.6 (d, 1H, $J$ = 3.5 Hz), 4.4 (t, 1H, $J$ = 7.6 Hz), 3.7 (m, 2H), 3.65 (m, 2H), 3.45 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 152.7, 132.5, 127.9, 113.3, 85.3, 74.3, 71.1, 68.9, 66.9; IR (KBr) 3338, 2983, 1599, 1534, 1307, 1152, 1062 cm$^{-1}$; MS ESI$^+$ $m/z$ 327 (M + Na)$^+$. ESI$^-$ $m/z$ 303 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-5D-xylopyranosylamine 2f**: mp 165–167 °C (lit.[14,15] 168–169 °C, 168 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.55 (d, 2H, $J$ = 8.7 Hz), 7 (s, 2H), 6.95 (d, 1H, $J$ = 7.9 Hz), 6.75 (d, 2H, $J$ = 8.7 Hz), 5.6 (s, 1H), 5 (d, 2H, $J$ = 7.2 Hz), 4.4 (t, 2H, $J$ = 7.9 Hz), 3.7 (m, 1H), 3.4 (m, 1H), 3.3 (m, 1H), 3.2 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 150.9, 132.7, 127.9, 113.4, 85.6, 78.4, 73.6, 70.6, 67.2; IR (KBr) 3331, 2908, 1602, 1529, 1309, 1157, 1097 cm$^{-1}$; MS ESI$^+$ $m/z$ 327 (M + Na)$^+$. ESI$^-$ $m/z$ 303 (M − H)$^-$.

***N*-(*p*-sulfamoylphenyl)-α-L-rhamnosylamine 2g**: mp 188–190 °C (lit.[14] 204 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 7.5 (d, 2H, $J$ = 8.6 Hz), 7 (s, 2H), 6.85 (d, 2H, $J$ = 8.6 Hz), 6.4 (d, 1H, $J$ = 9.3 Hz), 4.9 (d, 1H, $J$ = 5.1 Hz), 4.8 (m, 3H), 3.7 (s, 1H), 3.4 (m, 1H), 3.3 (m, 1H), 3.2 (m, 1H), 1.1 (d, 3H, $J$ = 5.9 Hz); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) δ 152.7, 132.9, 128.2, 113.6, 81.4, 74.8, 73.3, 72.8, 71.7, 18.8;

IR (KBr) 3381, 2913, 1603, 1518, 1311, 1141, 1074 $cm^{-1}$; MS $ESI^+$ $m/z$ 341 $(M+Na)^+$. $ESI^-$ $m/z$ 317 $(M-H)^-$.

***N*-(*p*-sulfamoylphenyl)-α-D-fucosylamine 2h**: mp 192–193 °C; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 7.55 (d, 2H, $J=7.6$ Hz), 7 (s, 2H), 6.9 (d, 1H, $J=8$ Hz), 6.8 (d, 2H, $J=7.6$ Hz), 4.8 (m, 2H), 4.5 (d, 1H, $J=4.3$ Hz), 4.4 (t, 1H, $J=8.1$ Hz), 3.7 (m, 1H), 3.4 (m, 3H), 1.1 (d, 3H, $J=6.4$ Hz); $^{13}C$ NMR (DMSO-$d_6$, 400 MHz) δ 151.1, 132.3, 127.9, 113, 85, 75.2, 72.1, 71.2, 70.5, 17.6; IR (KBr) 3341, 2891, 1602, 1530, 1305, 1145, 1078 $cm^{-1}$; MS $ESI^+$ $m/z$ 341 $(M+Na)^+$. $ESI^-$ $m/z$ 317 $(M-H)^-$.

14. Ojala, C. R.; Ostman, J. M.; Ojala, W. H.; Hanson, S. E. *Carbohydr. Res.* **2001**, *331*, 319.
15. (a) Ellis, G. P. *J. Chem. Soc.* **1966**, 572. (b) Ellis, G. P.; Honeyman, J. *Adv. Carbohydr. Chem.* **1955**, *10*, 95.
16. A stopped flow variant of the Pocker and Stone spectrophotometric method (Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668) has been employed, using an SX.18MV-R Applied Photophysics stopped flow instrument.
17. Adult male New Zealand albino rabbits weighing 3–3.5 kg were used in the experiments (three animals were used for each inhibitor studied). The experimental procedures conform to the Association for Research in Vision and Ophthalmology Resolution on the use of animals. The rabbits were kept in individual cages with food and water provided ad libitum. The animals were maintained on a 12 h/12 h light/dark cycle in a temperature controlled room, at 20–24 °C.
18. IOP was measured using a Tono-Pen XL digital instrument (Medtronic Solan, USA). The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration verifier. All IOP measurements were done by the same investigator with the same tonometer. One drop of 0.2% oxybuprocaine hydrochloride (novesine, Sandoz) diluted 1:1 with saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured three times at each time interval, and the means reported. IOP was measured first immediately before drug administration, then at 30 min after the instillation of the pharmacological agent, and then each 30 min for a period of 4–6 h. For all IOP experiments drug was administered to only one eye, leaving the contralateral eye as an untreated control. The ocular hypotensive activity is expressed as the average difference in IOP between the treated and control eye, in this way minimizing the diurnal, seasonal and interindividual variations commonly observed in the rabbit.