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New prostaglandin derivative for glaucoma treatment

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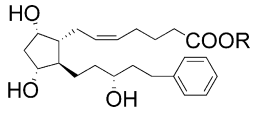
ABSTRACT

A hydrogen sulphide-releasing derivative of latanoprost acid (ACS 67) was synthesized and tested in vivo to evaluate its activity on reduction of intraocular pressure and tolerability. Glutathione (GSH) and cGMP content were also measured in the aqueous humour. The increased reduction of intraocular pressure, with a marked increase of GSH and cGMP and the related potential neuroprotective properties, make this compound interesting for the treatment of glaucoma. This is the first time that an application of a hydrogen sulphide-releasing molecule is reported for the treatment of ocular diseases.

Glaucoma is a group of diseases of the optic nerve involving progressive degeneration of retinal ganglion cells resulting in a pattern of irreversible loss of vision.[1] Intraocular pressure (IOP) represents a significant risk factor for developing glaucoma and can be, at least partially, controlled by administering drugs which either reduce the production of aqueous humour within the eye or increase the fluid drainage.[2]

The use of prostaglandins for IOP reduction has been discussed for the first time by Camras in 1981 when it was shown that $PGF_{2\alpha}$ effectively reduces IOP in monkeys.[3] Since then there has been an increasing interest in the research of new prostaglandin derivatives to be used as drugs for glaucoma treatment.[4,5] The first approach in modifying $PGF_{2\alpha}$ included esterification of the carboxylic acid end of the molecule to improve bioavailability and reduce the side effects.[6] An extensive series of structure–activity studies led to the synthesis of latanoprost (Xalatan® eye drop, Pfizer, Fig. 1) the most prescribed prostaglandin derivative for treatment of glaucoma.[7]



R = H latanoprost acid
R = CH-$(CH_3)_2$ latanoprost

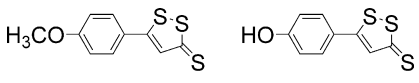
Figure 1. Structures of latanoprost acid and latanoprost.

In recent years the physiological role of hydrogen sulphide (H_2S), a compound that belongs to, along with nitric oxide (NO) and carbon monoxide (CO), the family of labile biological mediators called gasotransmitters, has been elucidated.[8,9] Several effects are reported on biological targets, such as cytoprotection through the increase of the intracellular glutathione (GSH) content, smooth muscle relaxation and vasodilatatory effects through K_{ATP} or Cl^- channels activation.[9]

Our research group has recently discovered that the class of compounds known as dithiolethiones has the ability to release H_2S in a controlled and long lasting way.[10] We had previously applied the concept of preparing H_2S-releasing hybrids in other pharmacological fields such as inflammation, with a hybrid of diclofenac,[10–12] male erectile dysfunction, with a hybrid of sildenafil,[13] cardiovascular, with a hybrid of aspirin.[14]

It has also been reported that anethole dithiolethione (**ADT**) is effective in in vitro lens cell protection against radiation injury. Anethole dithiolethione (Sulfarlem®)[15] is a commercial drug used as a hepatoprotective agent and for the treatment of drug- and radiation-induced xerostomia, and 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (**ADTOH**, Fig. 2) is its main metabolite which could be released from the former by in vivo demethylation.[16]

The working idea was to prepare a H_2S-releasing derivative of latanoprost acid, potentially able to maintain or even increase



ADT **ADTOH**

Figure 2. Structures of ADT and ADTOH.

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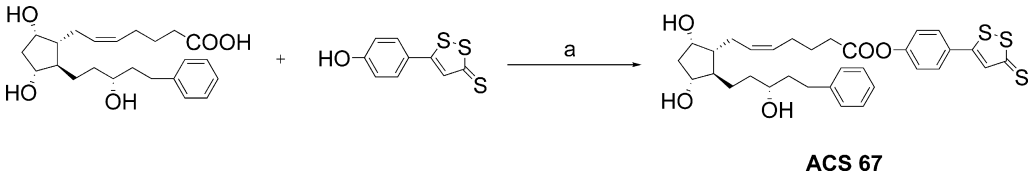

Scheme 1. Reagents and conditions: (a) EDAC, DMAP, anhydrous THF, N_2, rt, 3 h, 65%.

the anti-IOP activity and possessing significant neuroprotective properties through the increase of anti-oxidant substances, such as GSH. Thus a new compound (**ACS 67**) was designed by replacing the isopropyl moiety of latanoprost with a phenyldithiolethione group.

The route used for the synthesis of **ACS 67** is reported in Scheme 1 and required firstly the preparation of the **ADTOH** intermediate[10] which was then esterified with latanoprost acid in the presence of EDAC and DMAP.[17]

The effects of **ACS 67** versus latanoprost on IOP in glaucomatous pigmented rabbit over a four-hour time course are reported in Figure 3. The rabbits were made glaucomatous by injecting carbomer into anterior chamber and, after stabilization, the IOP was measured after 30, 60, 120 and 240 min.[18]

The AUC (mm Hg h) was 75.2 ± 3.3 for **ACS 67** and 108.8 ± 1.0 for latanoprost with a 30.8% reduction in IOP and the difference between the two groups was statistically significant already after 30 min.

A similar experiment was conducted in albino rabbits (Fig. 4) since it is reported that latanoprost failed to produce hypotensive effects in albino rabbit and cat eyes. These differences may be attributed to the different selectivity for prostaglandin receptors and differences in prostaglandin receptor subtypes distribution in the eye of these species.[19] The increased IOP response in wild rabbits might be due to an increased affinity or an increased number of prostaglandin receptors.

The AUC (mm Hg h) was 106.1 ± 4.2 for **ACS 67** and 119.0 ± 5.0 for latanoprost and there was a statistical difference at fourth hour.

To exclude the fact that this response could only have been a transient phenomenon, the effects of **ACS 67** versus latanoprost on IOP in glaucomatous pigmented rabbits were determined after repeated administrations in a five-day time course,[18] and the results reported in Figure 5.

The AUC (mm Hg h) was 2088.0 ± 31.0 for **ACS 67** and 2658.0 ± 59.1 for latanoprost with a 27% reduction versus latanoprost ($p < 0.001$).

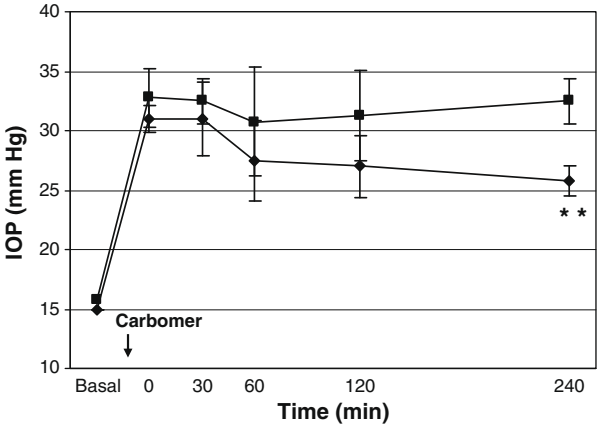

Figure 4. Effect on IOP in carbomer-induced glaucoma in New Zealand albino rabbits after topical treatment with one drop (50 μl) of 0.005% **ACS 67** (-♦-) versus 0.005% latanoprost (-■-). $^{**}p < 0.01$ versus latanoprost.

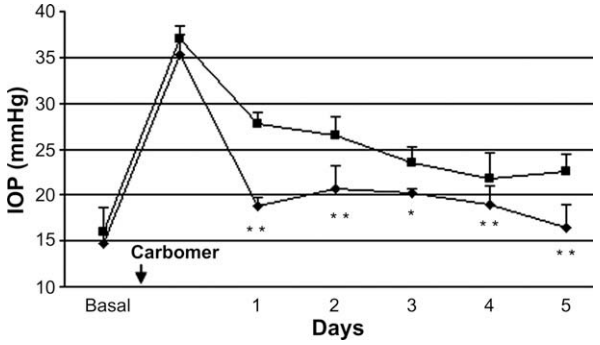

Figure 5. Effect on IOP in carbomer-induced glaucoma in pigmented rabbits after repeated topical treatment with 0.005% **ACS 67** (-♦-) versus 0.005% latanoprost (-■-). $^{*}p < 0.05$; $^{**}p < 0.01$ versus latanoprost.

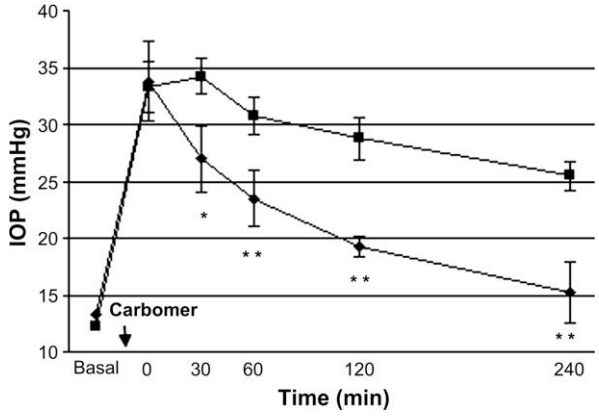

Figure 3. Effect on IOP in carbomer-induced glaucoma in pigmented rabbits after topical treatment with one drop (50 μl) of 0.005% **ACS 67** (-♦-) versus 0.005% latanoprost (-■-). $^{*}p < 0.05$; $^{**}p < 0.01$ versus latanoprost.

We also measured the IOP after single administration (Table 1) and after 5-day repeated administration (Table 2) of ADTOH, and of the combination ADTOH + latanoprost at the concentration of 0.005% in albino and pigmented rabbit. The data show the contribution of the sulfurated moiety to IOP reduction in pigmented rabbits.

The GSH content in the aqueous humour of normotensive rabbits at 3 different dose levels (Table 3) after single administration and in glaucomatous pigmented rabbits after 5-day repeated administration (Table 4) was determined.[20]

ACS 67 significantly increases reduced glutathione levels in normotensive pigmented rabbits after single administration in a dose related manner, moreover **ACS 67** also significantly increases, versus latanoprost, GSH levels in glaucomatous pigmented rabbits after repeated 5-day administration (Table 4).

We also measured the GSH levels in normotensive pigmented rabbits (basal 36.3 ± 2.9) after single administration of ADTOH (58.3 ± 3.7), latanoprost (38.7 ± 3.5) and the combination

Table 1
IOP (mm Hg) in albino and pigmented rabbits after single administration of ADTOH and latanoprost $n = 4$ (±sd)

Strain	Treatment	Basal	Carbomer	Time (min)				
				30	60	120	180	240
Albino rabbit	ADTOH	12.5 (±2.9)	31.0[#] (±4.5)	29.5 (±1.9)	28.0 (±2.3)	26.2 (±2.2)	25.0 (±1.4)	25.2 (±1.5)
	ADTOH + latanoprost	13.2 (±2.5)	32.2[#] (±3.9)	30.5 (±3.4)	29.2 (±4.1)	27.7 (±4.0)	26.2 (±4.8)	27.2 (±4.6)
Pigmented rabbit	ADTOH	11.2 (±1.9)	32.5[#] (±6.0)	30.0 (±5.2)	29.0 (±4.2)	26.0 (±3.3)	24.5 (±2.1)	23.5 (±2.4)
	ADTOH + latanoprost	12.5 (±1.3)	32.3[#] (±3.1)	29.7 (±3.3)	23.5[*] (±0.6)	19.2[*] (±2.2)	16.5[**] (±1.9)	16.2[**] (±2.1)

[#] $p < 0.01$ carbomer versus basal.
[*] $p < 0.05$ ADTOH versus ADTOH + latanoprost.
[**] $p < 0.01$ ADTOH versus ADTOH + latanoprost.

Table 2
IOP (mm Hg) in pigmented rabbits after repeated 5-day administration of ADTOH and latanoprost $n = 4$ (±sd)

Strain	Treatment	Basal	Carbomer	Time (days)				
				1	2	3	4	5
Pigmented rabbit	ADTOH	11.2 (±1.9)	32.5[#] (±6.0)	26.7 (±3.4)	26.0 (±1.6)	23.0 (±2.6)	22.2 (±1.3)	22.0 (±1.4)
	ADTOH + latanoprost	12.5 (±1.3)	32.5[#] (±3.1)	19.2[*] (±2.2)	19.2[**] (±2.2)	16.2[**] (±1.3)	17.0[**] (±1.4)	16.2[**] (±1.3)

[#] $p < 0.01$ carbomer versus basal.
[*] $p < 0.05$ ADTOH versus ADTOH + latanoprost.
[**] $p < 0.01$ ADTOH versus ADTOH + latanoprost.

Table 3
GSH levels in normotensive pigmented rabbits after single administration

GSH (pmol/ml)	
Basal	38.68 ± 2.15
ACS 67 0.005%	60.04 ± 11.03
ACS 67 0.008%	69.83 ± 6.63
ACS 67 0.01%	82.89 ± 5.69

Table 4
GSH levels in glaucomatous pigmented rabbits after repeated 5-day administration

GSH (pmol/ml)	
Vehicle/carbomer	20.92 ± 8.49
ACS 67 0.005%	65.27 ± 10.97
Latanoprost 0.005%	36.44 ± 4.76

ADTOH + latanosprost (61.2 ± 1.6) and in glaucomatous pigmented rabbits (basal 36.3 ± 2.9) after 5-day repeated administration of ADTOH (60.4 ± 3.1), latanoprost (33.7 ± 3.0), ADTOH + latanoprost (63.9 ± 4.9) at the concentration of 0.005% .

These data support the evidence that the increase of GSH is due to the sulfurated moiety of ACS 67.

The cGMP content in the aqueous humour of glaucomatous pigmented rabbits after 5-day repeated administration of latanoprost and **ACS 67** is reported (Table 5).[21] Moreover the cGMP levels after repeated 5-day administration in glaucomatous pigmented rabbits (basal 73.7 ± 2.8) of ADTOH (121.6 ± 17.5); latanoprost (69.9 ± 14.7); ADTOH + latanoprost (123.3 ± 13.6) at the concentration of 0.005% were also measured.

ACS 67 significantly increases the cGMP levels in glaucomatous pigmented rabbits after 5-day repeated administration and the data with the combination support the evidence that the increase of cGMP is due to the sulfurated moiety of ACS 67.

Table 5
Cyclic GMP levels in glaucomatous pigmented rabbits after repeated 5-day administration of latanoprost or compound **ACS 67** at 0.005% concentration

cGMP (fmol/ml)	
Vehicle/carbomer	72.00 ± 3.76
ACS 67 0.005%	118.16 ± 9.20
Latanoprost 0.005%	71.82 ± 4.34

Table 6
Tolerability of **ACS 67** in Draize test

Draize score	
Vehicle	0/5
ACS 67 0.005%	0/5
ACS 67 0.008%	0/5
ACS 67 0.01%	0/5

Finally the tolerability was measured in New Zealand albino rabbits at three different concentrations of **ACS 67** versus placebo according to the Draize test[22] and the results are reported in Table 6.

This study shows that **ACS 67**, the new H_2S-donating $PGF_{2\alpha}$ derivative, was significantly more potent than latanoprost in reducing IOP after either single or repeated topical administration to glaucomatous pigmented rabbits. The compound was also very well tolerated in Draize test.

Furthermore the compound was found to be more potent than latanoprost in reducing IOP also in the albino glaucomatous rabbits particularly in consideration that the dose of **ACS 67** is 28% lower on a molar basis. This finding is of relevance since it is known that in these latter animals latanoprost is only very poorly effective.[19,23] Thus both PG-dependent and independent effects in reducing IOP can be identified for **ACS 67**. The PG-dependent effects could be mediated by cAMP content similar in latanoprost and **ACS 67** groups (unpublished data). The PG-independent effects are likely to be due to the contribution of cGMP and sulfurated moiety via its ability to release H_2S.

Recently, H_2S was found to possess relevant vasorelaxant effects in mice with depletion of cystathionine-γ-lyase.[24] Furthermore H_2S was found to be neuroprotective through the GSH formation.[8]

The increased anti-IOP response was accompanied by a marked increase in GSH levels in aqueous humour of **ACS 67** treated rabbits as compared to the related values in latanoprost treated animals. The observed GSH increase suggests a potential neuroprotective effect for **ACS 67**.

This is the first time that an application of a hydrogen sulphide-releasing molecule is reported for the treatment of ocular diseases.

References and notes

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17. *Synthesis of (5Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl] cyclopentyl]-5-heptenoic acid 4-(3H-1,2-dithiole-3-thione-5-yl)-phenyl ester* (**ACS 67**): 5-(4-Hydroxyphenyl)-3*H*-1,2-dithiol-3-thione, prepared as previously described[10] (91 mg, 0.40 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) were added to a solution of (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoic acid (latanoprost acid, 84 mg, 0.20 mmol) in 2 ml of anhydrous tetrahydrofuran (THF), stirring under nitrogen at 0 °C. After few minutes 1-(3-dimethylaminoisopropyl)-3-ethyl-carbodiimide hydrochloride (EDAC, 57 mg, 0.30 mmol) was added and the reaction was stirred at room temperature for 3 h. After evaporation of THF, the residue was dissolved in dichloromethane and washed with cold water. The organic solution was dried on anhydrous sodium sulphate, evaporated to dryness and the crude solid was purified by column chromatography on silica gel (ethyl acetate/cyclohexane, 70:30) and then washed with ethyl ether/ethyl acetate (70:30). The obtained red-coloured product (78 mg, yield 65%) has melting point (Büchi) 94–95 °C.
HRMS (Apex II ICR-FTMS Bruker Daltonics; ESI) *m/z* calcd for $[C_{32}H_{38}S_3O_5+Na]^+$: 621.17736; found: 621.17694.
^{1}H NMR (Varian Mercury 300VX; $CDCl_3$): δ 7.65 (d, 2H); 7.40 (s, 1H); 7.30–7.15 (m, 7H); 5.60–5.40 (m, 2H); 4.20 (dd, 1H); 3.95 (dd, 1H) 3.70–3.60 (m, 1H); 2.80–2.60 (m, 4H); 2.40–2.20 (m, 4H); 1.95–1.30 (m, 12H).
18. High IOP levels were obtained injecting carbomer 0.25% (Siccafluid Farmila THEA Pharmaceutical) 0.1 ml bilaterally into anterior chamber in pre-anesthetized rabbits with 1 ml iv of pentobarbital sodium (Pentothal Abbott, Campoverde di Aprilia, LT). The IOP increase was tested, every 30 min during the first 3 h, and at least three times a day until stabilization. As soon as the IOP was stabilized on high levels, the drugs in study were administered by instillation, once for single dose experiment and every day for 5 days for the repeated dose experiment, and IOP was measured, using a Tono-Pen tonometer (Solan Ophtalmic Products Jacksonville, USA). Aqueous humour (both posterior and anterior chamber fluids) was withdrawn before inducing ocular hypertension, when IOP was increased and after each treatment with the drugs in study and used for GSH and cGMP level determinations.
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20. Determination of GSH levels in aqueous humour: aqueous humour was centrifuged at 4 °C at 100.000*g* for 30 min and a fixed volume of supernatant was diluted with phosphate-EDTA buffer to a final volume of 5 ml. For GSH evaluation, 200 μl of 40 mM N-ethylmaleimide was added to 0.5 ml of the supernatant, incubated for 30 min and diluted with 0.1 N NaOH to a final volume of 5 ml. Phosphate-EDTA buffer and 0.1 N NaOH (1.8 and 0.1 ml, respectively) and o-phthaldialdehyde solution (1 mg/ml, 0.1 ml) were added to both mixtures. The samples were incubated at room temperature for 15 min and then the fluorescence evaluated fluorometrically at 420 nm emission and 350 nm excitation. Samples will be calibrated using a standard curve of GSH.
21. Determination of cyclic GMP (cGMP) levels in aqueous humour. The concentration of cGMP was determined by means a radioimmunoassay kit using [^{121}I] labelled cGMP (Amersham, Bucks, UK). Five hundred μl of 10% trichloroacetic acid (TCA) were added to the samples which were then centrifuged and TCA extracted with 0.5 M tri-*n*-octylamine dissolved in 1,1,2-trichloro-1,2,2-trifluoroethane. The samples were then acetylated in acetic anhydrase and the amount of cGMP measured in aqueous phase. The values were expressed as fmoles of cGMP per ml.
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