5-(Sulfamoyl)thien-2-yl 1,3-oxazole inhibitors of carbonic anhydrase II with hydrophilic periphery

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ABSTRACT
Hydrophilic derivatives of an earlier described series of carbonic anhydrase inhibitors have been designed, prepared and profiled against a panel of carbonic anhydrase isoforms, including the glaucoma-related hCA II. For all hydrophilic derivatives, computational prediction of intraocular permeability routes showed the predominance of conjunctival rather than corneal absorption. The potentially reactive primary or secondary amine periphery of these compounds makes them suitable candidates for bioconjugation to polymeric drug carriers. As was shown previously, the most active hCA II inhibitor is efficacious in alleviating intraocular pressure in normotensive rabbits with efficacy matching that of dorzolamide.

GRAPHICAL ABSTRACT

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Introduction
Glaucoma-related high intraocular pressure can be alleviated by the use of eye drops of prostaglandin analogues, beta blocking agents and carbonic anhydrase inhibitors (CAIs)\textsuperscript{1}. The recent approval of rho kinase inhibitors and NO donors significantly expands the range of treatment options\textsuperscript{2,3}. The clinically used topical CAIs for glaucoma treatment include dorzolamide (1) and brinzolamide (2), compounds that are (a) relatively lipophilic and (b) non-selective as inhibitors of a particular carbonic anhydrase isoform\textsuperscript{4}. Acetazolamide (3) and methazolamide (4) are also used as anti-glaucoma agents (Figure 1), but they are oral medications which frequently cause adverse drug reactions\textsuperscript{5}. Potent and selective inhibition of carbonic anhydrase II isoform (hCA II) is an important mechanism of action due to the critical importance of this enzyme in reduction of glaucoma-related intraocular pressure\textsuperscript{6}.

Topical ocular drugs are typically designed as rather lipophilic, because they absorb to the eye across the cornea\textsuperscript{7}. Lipophilicity leads to decreased water solubility and, thus, lowers the achievable drug concentration in the tear fluid. On the contrary, higher concentration in the tear fluid can be achieved with hydrophilic compounds. Such compounds may absorb into their ocular targets via conjunctiva and sclera that allow permeation of relatively hydrophilic compounds\textsuperscript{8}. Specifically designing hydrophilic compounds that can utilise this route will lower the loss of hydrophilic compounds to the bloodstream across conjunctiva\textsuperscript{8}. Anti-glaucoma CAIs exert their action in the ciliary body located next to the sclera, thereby making non-corneal absorption of highly potent, hydrophilic derivatives an interesting approach. Moreover, in comparison to the cornea, the conjunctiva has wider inter-cellular space for permeation of hydrophilic compounds\textsuperscript{8}.

Previously, we described a series of 5-(sulfamoyl)thien-2-yl 1,3-oxazoles \textsuperscript{5a–c} which displayed a remarkably potent inhibition...
profile towards human carbonic anhydrase (CA, EC 4.2.1.1) and, in particular, its hCA II isoform\(^{10}\) which is the primary target for intraocular pressure-reducing antiglaucoma drugs.\(^{6}\) Later on, a related – and similarly potent against hCA II – benzenesulfonylamide series (6a–c) showed high efficacy in vivo lowering ocular hypertension in rabbits. Furthermore, the high potency and the pronounced selectivity towards the CA isoform of this series was rationalised in rabbits. Furthermore, the high potency and the pronounced selectivity towards the CA isoform of this series was rationalised by X-ray crystallographic structure of complex of CAII inhibitors with the protein\(^{11}\). Considering that compounds 5a–c contain the primary sulphonamide group linked to a thioephene moiety, it makes them structurally closer to the clinically used drugs 1–4 all of which have a five-membered heterocyclic core as a primary sulphonamide-bearing scaffold. Thus, we selected carboxamides 5b–c as the prototype scaffold for the introduction of peripheral functional groups which would increase the resulting compounds’ hydrophilicity and also a reactive ‘handle’ for subsequent chemical conjugation to polymer nanoparticles. These notions resulted in the design of series 7 (Figure 2).

Eye drop treatment for glaucoma is notoriously hampered by the poor patient compliance and the progression of the disease and loss of vision\(^{12}\). Longer-acting intraocular drug delivery with polymeric systems could potentially solve this issue\(^{13}\). The electron-donor influence of the sulphonamide group on the electrophilicity of the ester functionality in 8 turned out to be of advantage in subsequent synthesis of the target compounds 7a–e. Indeed, on reaction requiring no additional activation, with 2.5-fold excess of mono-Boc-protected dibasic amines 9a–e at r.t. in MeOH, respective amides 10a–e were obtained and deprotected with TFA in 1,4-dioxane at 60°C and purified chromatographically to give the target compounds 7a–e (Scheme 1).

The inhibitory profile obtained for sulphonamides 7a–e in a stopped-flow kinetics assay against human CA I, II, IV and XII is shown in Table 1. In addition to hCA II, the other three isoforms were selected to preliminarily gauge the off-target profile of the compounds intended to inhibit the target isoform. Moreover, inhibition profile against hCA IV and XII was thought to be of significance as these isoforms are also involved in the secretion of the intraocular liquor\(^{17}\).

To our delight, all four inhibitors 6a–d preserved the potent inhibition profile against the target hCA II isoform (although their hCA II potency deteriorated somewhat compared to the less hydrophilic initial leads 5a–c) and a clearly better hCA II selectivity profile compared to acetazolamide (4) employed as a

**Results and discussion**

The key building block – ethyl 5- (4-sulamoylphenyl)oxazole-2-carboxylate (8) – was synthesised in several straightforward steps from \(\alpha\)-aminoacetoephene hydrochloride as described previously\(^{10,15}\). The electron-withdrawing influence of the sulphonamide group on the electrophilicity of the ester functionality in 8 turned out to be of advantage in subsequent synthesis of the target compounds 7a–e. Indeed, on reaction requiring no additional activation, with 2.5-fold excess of mono-Boc-protected dibasic amines 9a–e at r.t. in MeOH, respective amides 10a–e were obtained and deprotected with TFA in 1,4-dioxane at 60°C and purified chromatographically to give the target compounds 7a–e (Scheme 1).

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reference inhibitor. Interestingly, the replacement of the morpholine oxygen atom in 5c with hydrogen bond donating/accepting piperazine NH in compound 7a (a rather drastic change from the standpoint of potential molecular interactions which resulted in the change of the binding mode, *vide infra*) led to only a three-fold drop in hCA II potency. This clearly makes compound 7a stand out as the hydrophilic (and potentially ‘bioconjugatable’) follow-on to compound 5c. Of course, the ultimate efficacy profile of this inhibitor reducing the glaucoma-related intraocular pressure (IOP) would depend on a multitude

![Scheme 1. Synthesis of hydrophilic sulphonamides 7a-e investigated in this work.](image)

| Compound | Structure | $K_{i}$ (nM)$^a$ |
|----------|-----------|-----------------|
|          | $h$CA I   | $h$CA II | $h$CA IV | $h$CA XII |
| 7a       | ![Image](image) | 4.0    | 0.069   | 21.6     | 3.9     |
| 7b       | ![Image](image) | 56.8   | 0.92    | 23.7     | 8.9     |
| 7c       | ![Image](image) | 31.3   | 0.41    | 30.6     | 5.7     |
| 7d       | ![Image](image) | 72.9   | 3.9     | 5.2      | 9.3     |
| 7e       | ![Image](image) | 58.3   | 3.1     | 4.6      | 8.8     |
| 3b$^b$   | ![Image](image) | 250    | 12      | 75       | 5.7     |

$^a$Mean from three different assays by stopped flow technique (errors were in the range of ± 5–10% of the reported values).

$^b$Sulfonamide inhibitor acetazolamide (AAZ) used as a reference pan-CA inhibitor in stopped flow CO$_2$ hydrase assay.
of factors among which permeability characteristics (intrinsically linked to a favourable set of molecular parameters) will be of significance.

In order to visualise the binding of the prototype compound 5c in comparison to the hydrophilic lead derivative 7a and to possibly understand the origins of the essentially preserved hCA II potency in case of the latter, we performed the docking of both ligands into the active site hCA II. In the case of both prototype molecule 5c and the advanced hydrophilic lead compound 7a the thiophene sulphonamide moiety, predictably, acted as a zinc binding group displaying typical orientation which is well known from a wide range of crystallographic studies 18. Specifically, the sulphonamide moiety interacted with the catalytic Zn^{2+} ion as well as with Thr199. At the same time, the thiophene ring was oriented towards the hydrophobic pocket lined up with the residues Leu141, Val143, and Phe131. Furthermore, the 1,3-oxazole ring of the ligands was involved in interactions with Phe131 and formed a hydrogen bond with Gln92. Interestingly, we found the
The intraocular pressure (IOP) lowering effect of newly developed hydrophilic  hvaII inhibitor 7a was tested in normotensive New Zealand White rabbits. The results are shown as percentage changes in Figure 5. Compound 7a (1% eye drop) (tested twice consecutively) showed a clear IOP lowering effect which was comparable to the effect produced by 1 (dorzolamide, administered as 2% eye drops).

For compounds 7a–e, we have calculated a series of chemical descriptors (Table 2) from which critical ocular permeability parameters can be deduced. It is apparent, that all five compounds are distinctly hydrophilic.

morpholineamide moiety in the compound 5c was oriented towards the NH-groups of the Trp5 and Asn67. In contrast, the piperazine ring in compound 7a formed a salt bridge with Glu69. As it follows from this analysis, presumably, the ligand–protein interactions displayed by both morpholineamide moiety in 5a and piperazine amide substituent in 7a resulted in the favourable energy for the molecules’ binding within the active site of hvaII and thus leading the potent inhibitory action of the compounds against the CA isoform (Figure 3).

In order to test the robustness of the docking poses identified, we performed 120 ns molecular dynamics simulation of ligand 7a docked in the active site of hvaII in comparison with the clinically used (non-selective) hvaII inhibitor acetazolamide (3). The RMSD values of the protein backbone (blue), the ligand relative to hvaII (red) and the ligand relative to its original, pre-simulation docking pose (purple) were found to stabilise to fit the range of 1–3 Å (robust fit) within 23.36 ns for acetazolamide and within 77 ns for ligand 7a (Figure 4). The longer relaxation time observed for 7a has likely to do with the greater conformational flexibility of the piperazine carboxamide side chain which took longer to restore the network of critical hydrogen-bonding contacts. Overall, the molecular dynamics simulation demonstrated the robustness of the docking pose presented in Figure 3(B).

The Table 4. Formulas for estimating permeability properties of carbonic anhydrase inhibitors.

| Formula References | Reference |
|---------------------|-----------|
| Corneal permeability of rabbit (cm/s) | −3.885 − 0.183(HBtot) − 0.0792(HBd) | 19 |
| Corneal permeability of porcine (cm/s) | −4.6823 − 0.7670(logPSA) − 0.6121(logPSA) − 0.1346(HBd) − 3.0024(Halogen ratio) | 21 |
| Conjunctival permeability of porcine (cm/s) | −4.1594 − 0.6012(logPSA) − 0.6012(logPSA) − 0.1346(HBd) − 3.0024(Halogen ratio) | 21 |

LogPapp: logarithmic value of apparent permeability; HBtot: total amount of hydrogen bond formers; LogP: logarithmic value of partition coefficient; LogD7.4: logarithmic value of distribution coefficient at pH 7.4; LogPSA: logarithmic value of polar surface area; MW: molecular weight; LBb: hydrogen bond acceptors; HBd: hydrogen bond donors; HBtot: total amount of hydrogen bond formers; LogD8.0: logarithmic value of distribution coefficient at pH 7.4/8.0; PSA: polar surface area; LogPSA: logarithmic value of polar surface area.

Table 3. Calculated permeability (Papp) values of dorzolamide (1) and compounds 7a–e.

| Compound | Papp (cm/s) | % of 1 | Papp (cm/s) | % of 1 | Papp (cm/s) | % of 1 |
|----------|-------------|--------|-------------|--------|-------------|--------|
| 7a       | 7.79E−06    | 100    | 1.75E−07    | 100    | 1.86E−06    | 100    |
| 7b       | 9.68E−07    | 12     | 1.71E−07    | 96     | 1.83E−06    | 98     |
| 7c       | 3.27E−07    | 4      | 1.20E−07    | 69     | 1.47E−06    | 79     |
| 7d       | 3.76E−07    | 5      | 1.18E−07    | 67     | 1.45E−06    | 78     |
| 7e       | 2.68E−07    | 3      | 8.29E−08    | 48     | 1.17E−06    | 63     |
| 7a       | 1.68E−07    | 2      | 8.29E−08    | 48     | 1.17E−06    | 63     |

For compounds 7a–e, we have calculated a series of chemical descriptors (Table 2) from which critical ocular permeability parameters can be deduced. It is apparent, that all five compounds are distinctly hydrophilic.
The chemical descriptors presented in Table 2 allowed us to calculate the predicted corneal and conjunctival permeability values for compounds 7a–e in comparison with dorzolamide (1) (Table 3). These calculations are based on the earlier formulas by Kidron et al. and Ramsay et al. It is apparent that the conjunctival permeation route becomes a principal one for hydrophilic compounds 7a–e in comparison with more lipophilic dorzolamide (1) (Table 4).

In summary, we have described next-generation 5-(sulfamoyl)thien-2-yl 1,3-oxazole carbonic anhydrase inhibitors endowed with a primary or secondary amine periphery. The compounds were designed with a dual goal of increasing compounds’ hydrophilicity and provide a reactive ‘handle’ for potential conjugation to sustained-release nanoparticles. Increased hydrophilicity, while desirable for increased drug residence in the intraocular space could be generally viewed as an obstacle for corneal drug absorption. However, hydrophilic compounds may be efficiently absorbed via conjunctiva and thus have greater efficacy which may be expected if corneal absorption alone is considered. Out of the compounds described herein, the lead compound (7a) displayed a potent and selective inhibition of hCA II isoform, a glaucoma target and showed comparable efficacy as 1% eye drops in reducing the intraocular pressure in normotensive rabbit to that of clinically used 2% dorzolamide eye drops. This is despite the fact that the corneal permeability of these hydrophilic compounds was predicted to be significantly lower than that of dorzolamide. The data additionally support the concept of hydrophilic compounds permeating across the conjunctiva and sclera into the ciliary body.

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Disclosure statement

No potential conflict of interest was reported by all author(s) except CTS. C. T. Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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