Monitor

Monitor provides an insight into the latest developments in the pharmaceutical and biotechnology industries. ***Chemistry*** examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while ***Profiles*** provides commentaries on promising lines of research, new molecular targets and technologies. ***Biology*** reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. ***Business*** reports on the latest patents and collaborations, and ***People*** provides information on the most recent personnel changes within the drug discovery industry.

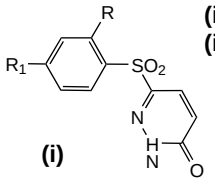
Chemistry

Molecules

The polyol pathway and diabetic complications: aldose reductase inhibitors

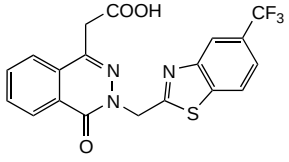
The elevated blood glucose levels present during diabetes are responsible for the development of microvascular conditions such as retinopathy, nephropathy and neuropathy. There are a number of hypotheses concerning how hyperglycemia causes diabetic complications [1], although the increased flux of glucose through the polyol pathway has received particular attention. This metabolic pathway comprises two enzymes: aldose reductase (ALR2), which catalyzes the reduction of glucose to sorbitol in the presence of NADPH; and sorbitol dehydrogenase (SDH), which converts sorbitol to fructose in the presence of NAD^+. There are two main hypotheses that link the activation of the polyol pathway to the onset of diabetic complications; the osmotic hypothesis, which describes an osmotic effect caused by sorbitol accumulation; and, more recently, the pseudohypoxia hypothesis, which attributes a pivotal role in diabetic complications to the imbalance of NADH and NAD^+ caused by the activation of SDH [2].

Studies on the *in vivo* inhibition of ALR2 for the prevention of diabetic complications have yielded inconsistent results. Several negative trials have been reported, but a positive effect on diabetic neuropathy of a potent aldose reductase inhibitor (ARI) has been confirmed in humans [3]. It is possible that the ARIs tested to date in Phase III studies have showed disappointing results because of the low dosages employed. In fact, dose selection was based on the sorbitol end-point (ED90), and in a patient efficacy trial the ARIs that were studied were not able to suppress nerve fructose content [2]. However, the therapeutic effect was obtained in experimental models (diabetic rats) with higher doses of ARIs that were able to suppress not only sorbitol but also nerve fructose levels by ≥ 90% [2]. Clinical Phase III trials were performed only on compounds of two chemical classes, the weakly acidic hydantoins [pK_a >8; e.g. sorbinil (Pfizer; http://www.pfizer.com)] and carboxylic acids [pK_a 3–4; e.g. zopolrestat (Pfizer)]. Owing to their acidity, carboxylic acids are less potent *in vivo* than hydantoins, but hydantoins are known to cause skin rashes, hypersensitivity or liver toxicity. Thus, there is a need for highly active non-carboxylic, non-hydantoin ARIs. Recently, high-throughput screening yielded compound **ia**, with a pK_a of 7.1, which was active *in vitro* (IC_{50} of 0.6 μM) and *in vivo* (normalization of elevated nerve sorbitol levels by 95% and fructose levels by 74% at doses of 50 mg/kg). Phenyl substitution led to compound **ib,** which had activity *in vitro* (IC_{50} of 0.19 μM) and *in vivo* (ED90 normalization of elevated nerve sorbitol and fructose levels was 5 mg/kg and 20 mg/kg, respectively) [2].



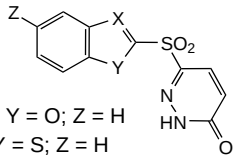
(i)

(ia) R = R_1 = H
(ib) R = R_1 = Cl



(ii) Zopolrestat

(iii)



(iiia) X = CH; Y = O; Z = H
(iiib) X = N; Y = S; Z = H
(iiic) X = CH; Y = S; Z = H
(iiid) X = N; Y = CH; Z = H
(iiie) X = CH; Y = O; Z = Cl

From previous structure–activity relationship (SAR) experience with zopolrestat (**ii**) [4], a series of analogs (**iii**)**,** containing a benzofuran (**iiia**), benzothiazole (**iiib**), benzothiophene (**iiic**) and indole (**iiid**) group were synthesized. Based on the results of *in vitro* assays, the analog incorporating the benzofuran moiety (**iiia**) was selected but it did not have the desired activity *in vivo*. To increase lipophilicity, a chlorine atom was introduced at the 5-position.

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Moreover, previous research has shown that whenever benzofuran has been employed in medicinal chemistry projects, substitution at the 3-position has played a remarkable role in enhancing *in vivo* potency. Therefore, it was decided to incorporate a methyl group at that position. Research indicated compound **iiie** to be the most potent ARI yet described (IC_{50} of 0.84 nM), possessing an ED90 for normalization of fructose in the chronic test (performed on streptozotocin-diabetic rats [2,4]) lower than that of zopolrestat (13-times) and sorbinil (3-times). Furthermore, this compound possesses optimal pharmacokinetic and physicochemical properties for potential once-a-day dosing in the clinic [2].

1 Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813–820

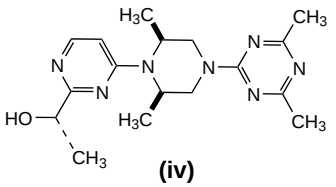
2 Mylari, B.L. *et al.* (2003) A highly selective, non-hydantoin, non-carboxylic acid inhibitor of aldose reductase with potent oral activity in diabetic rat models: 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2-H-pyridazin-3-one. *J. Med. Chem.* 46, 2283–2286

3 Hohman, T.C. *et al.* (1997) Nerve function and biochemistry in diabetic patients treated with ARI-509, a novel aldose reductase inhibitor. *Diabetologia* 40, A552

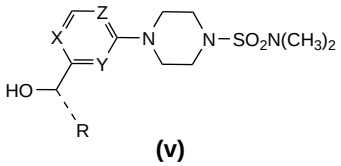
4 Mylari, B.L. *et al.* (1991) Novel, potent aldose reductase inhibitors: 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (zopolrestat) and congeners. *J. Med. Chem.* 34, 108–122

The polyol pathway and diabetic complications: sorbitol dehydrogenase inhibitors

Under diabetic conditions, excess flux through sorbitol dehydrogenase (SDH) creates an imbalance in the cytoplasmic NAD^+:NADH ratio. Inhibition of SDH can restore the altered cytoplasmic redox state and can influence the functional changes observed in experimental diabetes. *In vivo* experiments with a sorbitol dehydrogenase inhibitor (SDI) showed a marked increase of sorbitol in the sciatic nerve of normal and diabetic rats. However, in apparent contrast to expectations based on long-standing osmotic hypotheses, no adverse effects in nerves of normal rats were observed [5]. Furthermore, confounding results were reported in chronically diabetic rats [6]. At present, there are few SDIs available, and to clarify the role of SDH in the development of diabetic complications it is important to have access to potent and long-acting compounds. Recent research revealed



(iv)

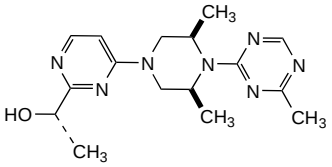


(v)

(va) X = Y = N; Z = CH; R = H
(vb) X = N; Y = Z = CH; R = H
(vc) X = Y = Z = N; R = H
(vd) X = Y = Z = N; R = CH_3

compound **iv** to be a potent SDI *in vitro* (IC_{50} of 0.005 μM) and *in vivo* (ED90 for normalizing elevated fructose levels of 0.05 mg/kg in the sciatic nerve of chronically diabetic rats) [6]. To find other chemotypes active as SDIs, two new templates, 2-hydroxymethylpyridine and 2-hydroxymethyltriazine, were considered [7].

Replacing the nitrogen at position 3 (compound **va**) with CH yielded the pyridine derivative **vb**. Despite the importance of the N1 of the pyrimidine moiety and the oxygen of the 2 hydroxymethyl moiety in liganding the catalytic zinc atom of SDH, **vb** is markedly less active than **va**. This reduction in activity is probably because of the protonation of **vb** at physiological pH (**vb** pK_a 9.6; **va** pK_a 6.1). Thus, the high pK_a value of **vb** favors protonation rather than zinc coordination at physiological pH. Substitution of CH at positions 3 and 5 of compound **vb** with nitrogen yielded the triazine derivative **vc**. Compound **vc** (pK_a 3.4) was less basic than the pyrimidine derivative **va** and more active than the pyridine derivative **vb**. Compound **vd** was active *in vitro* (IC_{50} of 0.2 μM) and *in vivo* (normalizing the elevated fructose levels in the diabetic rat sciatic nerve by 50% at an oral dose of 10 mg/kg). The potentiating effect of the (*R*)-2-hydroxyethyl moiety with respect to the hydroxymethyl group that was observed in the pyrimidine and pyridine series of compounds is also shared by this new template. Thus, on the basis of the positive effect of piperazine-triazine side-chains on SDI activity seen with compound **iv,** compound **vi** was synthesized and was found to be potent *in vitro* (IC_{50} of 0.059 μM) and *in vivo* (orally), giving rise to near normalization of sciatic nerve fructose in acutely (at doses of 5 mg/kg) and chronically (at doses of 10 mg/kg) diabetic rats [7]. This is the first example of a non-pyrimidine SDI that is active *in vitro* and *in vivo* [7].



(vi)

5 Geisen, K. *et al.* (1994) Sorbitol-accumulating pyrimidine derivatives. *Arzneim.- Forsch.* 44, 1032–1043

6 Mylari, B.L. *et al.* (2002) A sorbitol dehydrogenase inhibitor of exceptional *in vivo* potency with a long duration of action: 1-(*R*)-[4-[4-(4,6-dimethyl[1,3,5]triazin-2-yl)-2*R*,6*S*-dimethylpiperazin-1-yl]pyrimidin-2-yl]ethanol. *J. Med. Chem.* 45, 4398–4401

7 Mylari, B.L. *et al.* (2003) Design and synthesis of a novel family of triazine-based inhibitors of sorbitol dehydrogenase with oral activity: 1-[4-[3*R*,5*S*-dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl]-(*R*) ethanol. *Bioorg. Med. Chem.* 11, 4179–4188

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Inhibitors of TNF-α converting enzyme

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine and a key mediator in the inflammatory processes involved in rheumatoid arthritis (RA). The clinical success of biological therapeutics such as infliximab [Remicade® (Centocor; http://www.centocor.com)], a monoclonal TNF-α antibody, and entanercept [Enbrel® (Amgen; http://www.amgen.com)], a soluble TNF-α receptor, have validated the effectiveness of neutralizing TNF-α in treating RA. The success of these therapeutics has also strengthened the desire for an orally administered small molecule that is able to lower endogenous TNF-α levels. The active, soluble form of TNF-α is formed by proteolytic cleavage from its membrane-bound pro-form, catalyzed by a matrix metalloprotease (MMP) TNF-α converting enzyme (TACE). Broad spectrum MMP inhibitors have been found to cause undesirable side-effects in clinical trials, although they are useful starting points for the discovery of more selective inhibitors.

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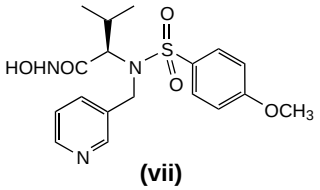
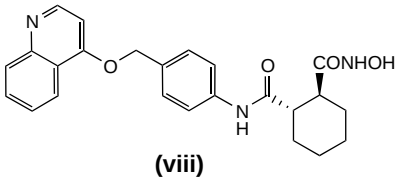
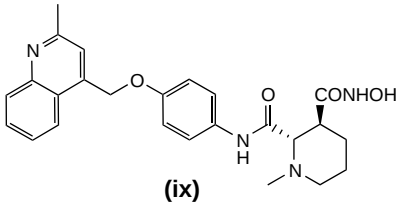
A group from Bristol–Myers Squibb (http://www.bms.com) used the Ciba–Geigy (http://www.ciba.com) sulfonamide CGS27023A (**vii**) and its cocrystal structure bound to MMP-3 to rationally design a series of cyclic succinate-based hydroxamic acid inhibitors of TACE, as exemplified by compound **viii** [8]. This compound shows good potency (IC_{50} of 8 nM) and high selectivity, possessing a K_i >2 μM for MMP-1, MMP-2, MMP-9 and all members of the related metalloprotease family. However, compound **viii** is an ineffective inhibitor (IC_{50} of >50 μM) of TNF-α release in a whole blood assay (WBA). This result was thought to be a consequence of the lipophilicity of the cyclohexyl ring giving rise to high plasma-protein binding. A nitrogen atom was introduced into the cyclohexyl ring to generate a new series of 3,4-piperidine carboxamide derivatives [9]. The introduction of a basic nitrogen atom



(vii)



(viii)



(ix)

greatly improved activity in the WBA but compounds often showed poor permeability in the Caco-2 assay. Walking the nitrogen around the ring eventually led to compound **ix**, which possesses an IC_{50} of 6.2 nM for TACE, maintains selectivity over related MMPs and has an IC_{50} of 20 nM in the WBA. The molecule demonstrated reasonable Caco-2 permeability and an oral bioavailability of 43% in dog.

8 Xue, C-B. *et al.* (2003) Rational design, synthesis and structure–activity relationships of a cyclic succinate series of TNF-α converting enzyme inhibitors. Part 1: Lead identification. *Biorg. Med. Chem. Lett.* 13, 4293–4297

9 Xue, C-B. *et al.* (2003) Rational design, synthesis and structure–activity relationships of a cyclic succinate series of TNF-α converting enzyme inhibitors. Part 2: Lead optimization. *Biorg. Med. Chem. Lett.* 13, 4299–4304

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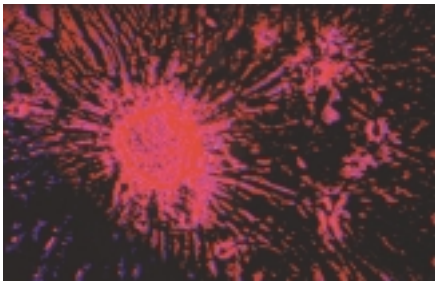
Biology

Cancer

New therapeutic potential for NSAIDs



Non- steroidal anti-inflammatory drugs (NSAIDs) inhibit the enzymatic activity of cyclooxygenase (COX), a protein catalyzing a key step in the synthesis of prostaglandins and often involved in the development and progression of colorectal cancer. Two isoforms have been identified so far. COX-1 is widely expressed and constitutively active, whereas COX-2 was found to be involved in inflammatory processes. Several Rho GTPases control COX-2 expression via regulation of transcription factor activity.

Rho GTPase deregulation has been reported for several human tumours, including breast, colon, pancreas, and head and neck squamous carcinomas. In a recent study [1], Benitah *et al.* analyzed the effects of two NSAIDs (Sulindac and NS-398) on COX-2 expression.

Constitutively active (QL) RhoA, Rac1 and cdc42 were shown to induce COX-2 expression in transformed NIH3T3, HT29 and MDCK cells. Among the various effectors of Rho GTPases, ROCK kinases were identified as mediators of COX-2 expression via NF-κB, which has a putative binding site in the COX-2 promotor region.

Treatment of RhoA-, Rac1- and cdc42-transformed cells with either Sulindac or NS-398 resulted in inhibited growth and proliferation and led to complete loss of COX-2 expression. NSAIDs were shown to inhibit NF-κB activation, as well as translocation to the nucleus. The capacity of NS-398 to inhibit growth *in vivo* was determined in mice transfected with MDCK-RhoAQL cells. Upon treatment with NSAIDs, a decrease in size of RhoAQL induced tumours was observed, compared to untreated mice.

NSAIDs were shown to limit Rho induced signaling by inhibiting activity of NF-κB. The results suggest that treatment of Rho disregulated tumours with specific NF-κB- inhibitors or NSAIDs like sulindac and NS-398 might offer a potential new avenue for antitumour therapy.

1 Aznar Benitah, S. *et al.* (2003) ROCK and nuclear factor- kappaB- dependent activation of cyclooxygenase-2 by Rho GTPases: effects on tumor growth and therapeutic consequences. *Mol. Biol. Cell* 14, 3041–3054

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VCP: a new role at the end of mitosis

VCP/p97/Cdc48 is a ubiquitous ATPase related to N-ethylmaleimide sensitive factor (NSF) and proteasomal ATPases. It is involved in different cellular activities, ranging from homotypic membrane function, ER-associated degradation, apoptosis, neurodegeneration and cancer

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