CHAPTER ELEVEN

# Type-2 Diabetes and Associated Comorbidities as an Inflammatory Syndrome

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## Contents



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## 1. INTRODUCTION

Acute inflammation represents a protective response to injury or infection which normally resolves after the threat has been eliminated. Incomplete resolution or repeated attempts to neutralize nonexisting threats leads to chronic inflammation. Persistent low-level inflammation is involved in the etiology of many chronic human diseases, including type-2 diabetes (T2D), cardiovascular disease, and chronic kidney disease.[1] While certain cellular pathways are common to many of these, specific mechanisms are particularly important to each pathology. For this review, inflammation is defined broadly and includes the activity of immune cells and processes occurring in parenchymal cells.

T2D is characterized by pancreatic deficiency, insulin resistance, and hyperglycemia. Prominent among the consequences of chronic hyperglycemia are microvascular complications that include diabetic nephropathy, retinopathy, and neuropathy. This chapter reviews recent evidence on the clinical and preclinical effects of a wide range of anti-inflammatory compounds in these conditions. Due to space constraints, we will not review the inflammatory aspects of atherosclerosis (a macrovascular complication associated with diabetes), which are nevertheless equally important.[2]

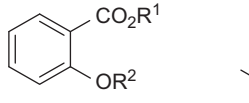
While the role of inflammatory/immune pathways in the etiology of type-1 diabetes (T1D, autoimmune attack on pancreatic islets that produce insulin) is distinct from T2D, the microvascular complications resulting from uncontrolled hyperglycemia are similar across both diseases. Consequently, clinical studies in diabetic microvascular diseases often enroll both types of patients, and preclinical hyperglycemia-driven models of them are just as often induced by insulin resistance (T2D) as by pancreatic destruction (T1D).

## 2. TYPE-2 DIABETES

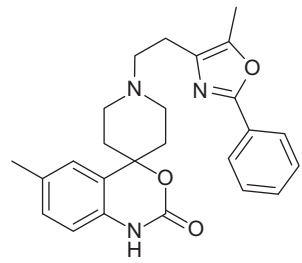
T2D is associated with multiple clinical markers of systemic inflammation (e.g., increased serum levels of acute-phase proteins and inflammatory cytokines).[3] Only a small number of the anti-inflammatory compounds described preclinically as potential antidiabetic agents have progressed into clinical trials. Due to the safety requirements for antidiabetic agents, nonspecific immunosuppressive drugs have generally not been given much clinical consideration. However, although beyond the scope of this review, we note the various anti-IL-1β biological agents currently in late-stage T2D clinical trials.[3]

## 2.1. Salicylates

Salicylic acid (**1**) and its derivatives possess anti-inflammatory activity due to their inhibition of cyclooxygenases and of NF-κB activation. Following promising pilot studies with high-dose aspirin (**2**), salsalate (**3**), a prodrug of salicylic acid, produced a highly significant reduction in blood glucose, hemoglobin-A1c (−0.49% vs. placebo, $p = 0.001$), and triglyceride levels when dosed to T2D patients for 14 weeks at doses up to 4 g/day.[4] A large Phase 3 trial (target > 560 patients) was initiated in 2008 and should complete soon.[5] While the mechanism of action for **3** is not entirely clear, the available clinical data are promising. Although it already has a long history of use in arthritic patients, its safety profile in T2D with respect to gastrointestinal bleeding and tinnitus will be critical to its therapeutic utility.




**1**: $R^1$ = H; $R^2$ = H
**2**: $R^1$ = H; $R^2$ = Ac
**3**: $R^1$ = (2-$CO_2$H)Ph; $R^2$ = H

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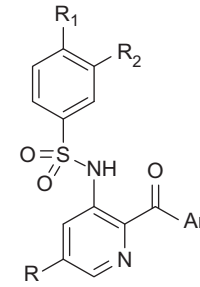


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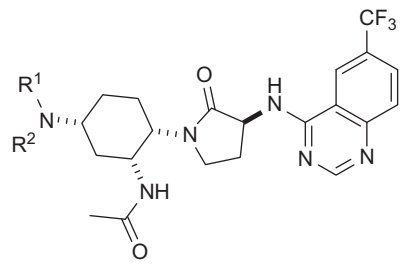



**6a**: $R^1$ = $C(CH_3)_3$, $R^2$ = H
**6b**: $R^1$ = $CH(CH_3)_2$, $R^2$ = $CH_3$

## 2.2. CCR2 antagonists

Much of the systemic inflammation in T2D appears to be driven by inflammation of adipose tissue (and perhaps also liver), a process recently linked to monocyte recruitment. Monocytes can differentiate into macrophages in these tissues, driving forward the vicious cycle of inflammation. C–C

chemokine receptor 2 (CCR2) and its main ligand MCP-1 (monocyte chemoattractant protein 1) regulate inflammatory macrophage recruitment to adipose tissue and liver.[6,7] CCR2 antagonist RS504393 (**4**) inhibits MCP-1-induced chemotaxis of murine spleen cells with an $IC_{50}$ of 0.8 μM. After 12 weeks of dosing in chow to *db/db* mice at 2 mg/kg/day, **4** ameliorated adipose tissue inflammation (MCP-1 expression and lipid hydroperoxide levels) and macrophage infiltration, concurrently with improved insulin sensitivity and hepatic steatosis.[8]

The orally active CCR2 antagonist CCX140-B potently ($IC_{50} = 8$ nM) inhibits CCR2-mediated chemotaxis of human monocytes while lacking effects on any other chemokine receptor. CCX140-B improved hyperglycemia and insulin sensitivity in a diet-induced-obese mouse model, by a mechanism involving reduction in adipose macrophage content and improved insulin sensitivity.[9,10] CCX140-B (10 mg/day; 28 days) was well tolerated and reduced plasma glucose and hemoglobin-A1c levels (0.23% reduction from baseline; $p = 0.045$ vs. placebo) in a 159-patient Phase 2 T2D trial.[11] Unlike other CCR2 antagonists described in the literature, CCX140-B did not alter circulating levels of monocytes or MCP-1, which would have been potentially detrimental to the desired therapeutic effect. CCX140-B was reported to belong to the class represented by formula **5**.[9,12] CCX140-B is undergoing further clinical study in DN (Section 3).

BMS-741672, an orally active CCR2 antagonist whose potency and structure have not been disclosed, but believed to be **6a** or **6b**,[13,14] was tested in T2D. The Phase 2 trial, completed in 2009, targeted 58 drug–naïve subjects, randomized to placebo or 50 mg BMS-741672 for 12 weeks. The primary end point was changes in hemoglobin-A1c from baseline.[15] Clinical results have not been disclosed, and there is no recent development activity for BMS-741672.

## 3. DIABETIC NEPHROPATHY

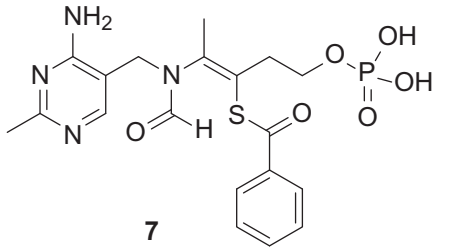
The first line of defense against microvascular diabetic complications is intensive control of hyperglycemia, dyslipidemia, and hypertension. However, this strategy is insufficient, as patients continue to develop these complications at a high rate.[16] Thus, therapies directed more proximally to the pathophysiology of these diabetic complications are needed. Pivotal diabetic nephropathy (DN) clinical trials generally aim to demonstrate clear benefits on number of renal failures (transplant or dialysis) or deaths, while Phase 2 studies often rely on clinical assessments such as estimated glomerular

filtration rate (eGFR) and proteinuria, which generally correlate with the desired long-term outcomes. Proteinuria (increased urinary excretion of protein, mostly albumin) reflects glomerular injury and also contributes directly to tubular and interstitial damage by activation of renal tubular cells, production of proinflammatory chemokines and cytokines, and interstitial recruitment of leukocytes, mainly monocytes/macrophages.[17] Even though no animal model completely recapitulates all pathological features of human DN, certain aspects (particularly proteinuria, leukocyte infiltration, glomerular hypertrophy, renal hyperfiltration, and selected histopathological features) can be modeled preclinically.

## 3.1. AGE inhibitors

Advanced glycation end-products (AGEs) are formed by nonenzymatic reaction of amino groups in proteins with breakdown products of glucose, such as pyruvate. This process is exacerbated by high glucose levels and oxidative stress. AGEs are recognized by various receptors (known as RAGE), the physiological role of which is unknown. The AGE–RAGE axis stimulates cellular proinflammatory responses that are involved in chronic injury in all major diabetic complications.[18] These effects are mediated by activation of the transcription factor NF-κB. Inhibition of AGE formation or stimulation of AGE cross-link breakage provides renal protection in experimental models of DN.

Benfotiamine (**7**), a vitamin B1 analogue, was shown to block renal AGE accumulation and urinary excretion in a diabetic rat model in which **7** was administered orally at 70 mg/kg/day for 24 weeks.[19] Pyridoxamine (**8**) is a derivative of vitamin B6 that inhibits AGE formation and advanced lipoxidation end-products during lipid peroxidation reactions. Following encouraging preclinical and pilot clinical results, a recent trial, in which 317 proteinuric DN patients were randomized to placebo or pyridoxamine (150 or 300 mg twice daily) for 1 year, failed to demonstrate any benefit, either on eGFR or proteinuria.[20] LR-90 (**9**) was shown in experimental DN models (e.g., Zucker diabetic fatty rat) to decrease AGE accumulation in kidney glomeruli and nitrotyrosine deposition in the renal cortex. *In vitro*, LR-90 was capable of trapping reactive carbonyl compounds and was a potent metal chelator, which is thought to be important for inhibition of AGE formation. LR-90 also blocked the development of DN, based on its lowering of serum creatinine levels and proteinuria.[21] There are no clinical reports for **9**.

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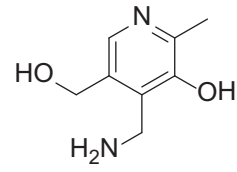


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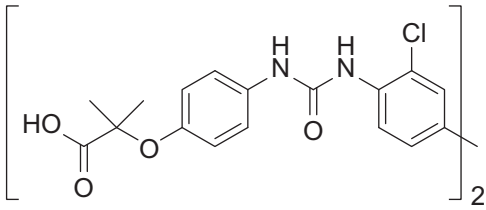


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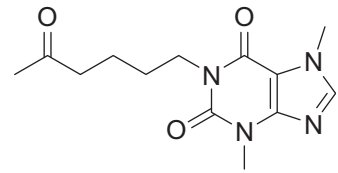


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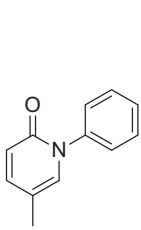
## 3.2. Inhibitors of TNF-α production

Pentoxifylline (**10**), a nonselective phosphodiesterase inhibitor, inhibits TNF-α synthesis and inflammation and is approved for the treatment of intermittent claudication in peripheral vascular disease. Over the years, pentoxifylline has been studied in a series of small clinical trials in DN, generally producing improvements in proteinuria and urinary markers of renal injury. For example, in one study involving 61 proteinuric T2D subjects, pentoxifylline (1.2 g/day) for 4 months reduced urinary albumin excretion from 900 to 791 mg/day ($p < 0.001$).[22,23] However, results have been less conclusive regarding eGFR, perhaps due to the small size and short duration of these clinical trials. Pentoxifylline continues to be studied clinically in DN in a randomized trial in which 169 DN subjects will be randomized to pentoxifylline (1200 mg/day) or placebo for 24 months. The primary outcome measure will be the difference in eGFR between the groups.[17]

## 3.3. Antifibrotic agents

Pirfenidone (**11**) is an orally active antifibrotic drug approved in Japan and Europe for idiopathic pulmonary fibrosis. While its molecular target is not known, pirfenidone decreases the production of the profibrotic cytokine TGF-β and scavenges radical oxygen species (ROS), among other activities, in cell culture and animal models of renal damage, such as the cyclosporine-induced chronic nephrotoxicity model. In a pilot Phase 2 trial involving 77

proteinuric DN subjects for 1 year, 1.2 g/day pirfenidone produced a small ($\Delta = 5.5$ ml/min/1.73 $m^2$) but statistically significant improvement in eGFR relative to placebo; the higher dose of compound (2.4 g/day) did not reach significance due, in part, to the high drop-out rate. There was no improvement in proteinuria.[24] There are no reports of further development in DN.

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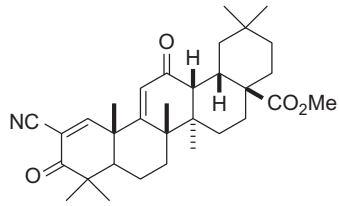


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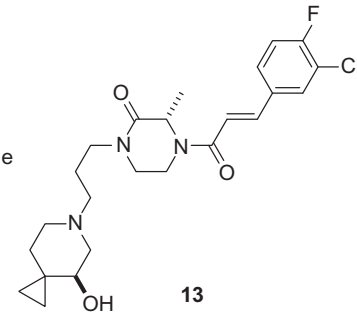


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## 3.4. Antioxidant compounds

The transcription factor NF-E2-related factor 2 (Nrf2) regulates expression of over 300 genes involved in modulating oxidative stress and inflammation. Its activity is suppressed in DN animal models, and kidney tissue from Nrf2-knockout mice shows impaired antioxidant activity, increased oxidative damage, and multiple histopathological changes. Bardoxolone methyl (**12**) is an oral activator of the Nrf2 pathway by preventing Nrf2 interaction with the negative regulator KEAP1 (kelch-like ECH-associated protein 1). **12** inhibits NF-κB activation *in vitro* and increases production of antioxidant molecules.[25] In a Phase 2 DN trial, 227 subjects were randomized to placebo or various doses (25–150 mg) of **12** for 1 year. At the higher doses, eGFR increased rapidly by about 5–10 ml/min/1.73 $m^2$, an effect that was sustained throughout the study.[26] Since eGFR is calculated from serum creatinine levels, some controversy exists as to whether this calculation accurately reflects true GFR or whether changes to creatinine metabolism and/or blood pressure might account for the lowering of creatinine levels.[25] Using a new formulation of **12**, a Phase 3 trial (20 mg/day) is ongoing, targeting 1600 T2D subjects with significantly impaired renal function (eGFR: 15–30 ml/min/1.73 $m^2$). The primary outcome assessment will be time-to-composite end point of renal failure or cardiovascular death.[27] A number of other putative Nrf2 activators have been shown to possess renoprotective effects in experimental models.[25]

### 3.5. CCR2 antagonists

The CCR2/MCP-1 axis is critical for recruitment of monocytes from blood into diabetic kidneys, where they differentiate into macrophages. Administration of **4** to diabetic *db/db* mice decreased proteinuria and mesangial expansion, and suppressed profibrotic and proinflammatory cytokine synthesis.[8] In uninephrectomized *db/db* mice, which suffer a more pronounced loss of renal function, dosing of CCR2 antagonist RO5234444 (**13**) at 100 mg/kg in chow for 8 weeks resulted in improved glomerulosclerosis, albuminuria, and glomerular filtration rate.[28]

CCX140-B (**5**) is currently in two Phase 2 DN trials.[29,30] The larger trial targets 135 DN patients with mild–moderate proteinuria, reduction of which will be a key study outcome. Placebo or CCX140-B (5 or 10 mg/day) will be administered for 12 weeks. Given the high selectivity of CCX140-B for human versus murine CCR2, preclinical data were generated with CCX417 (structure undisclosed), a close analogue that blocks mouse CCR2 with an $IC_{50}$ of 70 nM. Administration of CCX417 (30 mg/kg s.c.) to diabetic proteinuric *db/db* mice resulted in rapid (2 days) and sustained (2 weeks) reduction in proteinuria, as well as normalization of serum markers (creatinine, BUN) of renal function.[31]

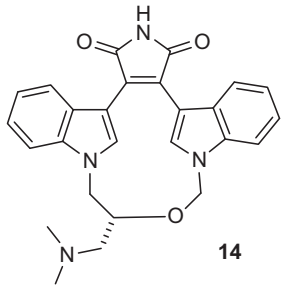
### 3.6. PKC-β inhibitors

Hyperglycemia-induced activation of protein kinase C beta (PKC-β) and downstream signaling pathways has been implicated in the vascular injury underlying diabetic microvascular complications. PKC-β is upregulated and activated in diabetic kidney, a process leading to cell growth, fibrosis, and tissue injury. Although perhaps not entirely an anti-inflammatory mechanism, PKC-β inhibition deserves mention here because at least some of the diabetic stimuli (e.g., AGE) that activate PKC-β are inflammatory in nature and because ruboxistaurin (**14**) is the only compound that has received wide attention in all three diabetic complications reviewed here. Ruboxistaurin is an orally bioavailable PKC-β inhibitor shown to ameliorate DN in various animal models. For example, administration of ruboxistaurin (10 mg/kg/day) to diabetic *db/db* mice for 16 weeks significantly ameliorated their proteinuria by $>50\%$, reduced the extent of glomerular mesangial expansion, and prevented the enhanced expression of extracellular matrix proteins and TGF-β.[32] In a Phase 2 trial, 123 T2D patients with persistent albuminuria were randomized to ruboxistaurin (32 mg/day) or placebo for 1 year. Drug treatment resulted in a 24% reduction in albuminuria ($p = 0.02$) and a stabilization

of eGFR.[33] Ruboxistaurin has been most extensively studied in DR (see Section 4).

### 3.7. Miscellaneous immunosuppressants

Strongly immunosuppressive drugs such as mycophenolate mofetil (**15**), a reversible inhibitor of IMPDH (inosine-5′-monophosphate dehydrogenase) that blocks lymphocyte proliferation, have demonstrated benefit on proteinuria, inflammatory cell infiltration, and fibrosis in experimental models of DN.[17,34] In spite of this, the clinical community has generally been reluctant to expose DN patients to strongly immunosuppressive agents, suggesting that clinical progress will be limited to agents that target specific pathways in the pathophysiology of DN.

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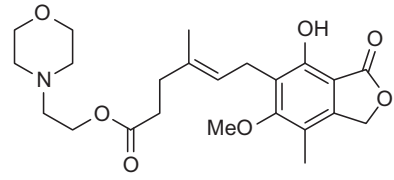


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## 4. DIABETIC RETINOPATHY

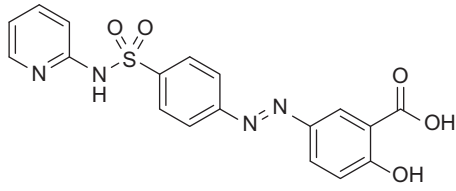
Diabetic retinopathy (DR) affects ~75% of diabetics and is a leading cause of adult blindness. The earliest stages of DR involve altered retinal blood flow and vascular inflammation, characterized by the release of inflammatory cytokines and chemokines, oxidative stress, and leukocyte accumulation. An initial nonproliferative stage, characterized by retinal hemorrhage and macular edema (ME), may be followed by a final proliferative stage characterized by the ischemia-induced formation of new blood vessels. In ME, leakage of blood and fluids into the retinal *macula* (which provides fine resolution of images) reduces vision quality. New blood vessels formed during proliferative DR are fragile, bleed easily, and may undergo fibrosis/contraction, leading to retinal detachment and other complications resulting in vision loss. Laser photocoagulation of bleeding blood vessels, which also destroys neural tissues, remains the main standard of care.

DR displays signs of chronic subclinical inflammation. In fact, topical and intravitreal application of corticosteroids such as dexamethasone and

triamcinolone, with their broad-spectrum anti-inflammatory effects, is a standard treatment option. The search for targeted anti-inflammatory treatments has gained attention in recent years, and several excellent reviews are available on this topic.[35–37]

## 4.1. Salicylates and NSAIDs

The benefit of aspirin on DR was first described 50 years ago.[38] Later prospective trials on its effects on the development of DN yielded contradictory results. However, these studies were conducted at doses deemed too low for anti-inflammatory effects.[35] Various salicylate-based drugs (salicylic acid, aspirin, sulfasalazine (**16**)), differing in their ability to inhibit prostaglandin synthesis by cyclooxygenases but sharing the ability to block activation of NF-κB, inhibited the development of retinal vascular pathology in diabetic rats and dogs.[39] In a small randomized trial, the NSAID sulindac (**17**) demonstrated a significant preventive effect on the development and progression of DR.[40] Various NSAIDs (nepafenac (**18**), bromfenac (**19**), diclofenac (**20**), and ketorolac (**21**)), either as topical or intravitreous formulations, are undergoing clinical evaluation in ME secondary to DR.[41–44] Ketorolac is also being studied in a Phase 2 trial in proliferative DR.[45]

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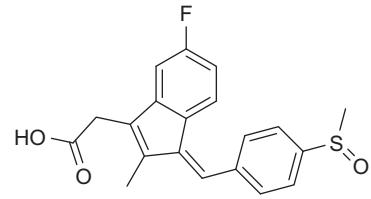


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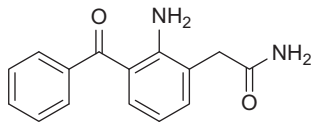


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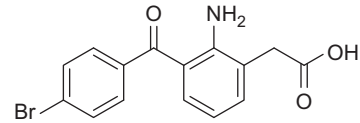


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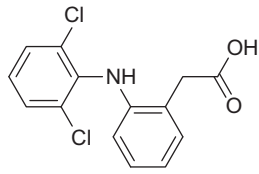


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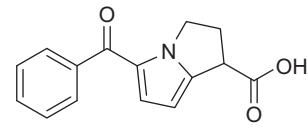


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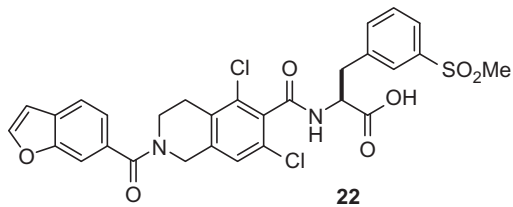
## 4.2. AGE inhibitors

AGE levels are greatly increased in diabetic vitreous and retinal vasculature. AGE receptors (RAGE) are expressed by numerous cells in the retina, including Müller (glia) cells, endothelial cells, and neurons. The AGE–RAGE system activates many downstream effects, including NF-κB activation, ROS formation, and leukocyte recruitment. The previously mentioned AGE inhibitor **9** administered in drinking water (50 mg/ml) to insulin-deficient diabetic rats for 32 weeks produced statistically significant benefits in the number of acellular capillaries and pericytes.[46]

## 4.3. Leukocyte adhesion inhibitors

Cell adhesion molecules are upregulated on endothelial cells in inflammatory settings and facilitate the adhesion and extravasation of circulating leukocytes. Leukocytes (neutrophils, monocytes/macrophages, T cells) accumulate in and around the abnormal vasculature in diabetic retina and choroid. These leukocytes disrupt vascular integrity and facilitate proliferative damage in DR. LFA-1 (lymphocyte function-associated antigen-1) is an integrin expressed on leukocytes which is important for leukocyte–endothelial cell interaction. SAR1118 (**22**) inhibits the binding of LFA-1 to ICAM-1 on Jurkat cells with an $IC_{50}$ of 2 nM. When delivered via eye drops, **22** reduced leukostasis and retinal vascular leakage in a diabetic rat model.[47]

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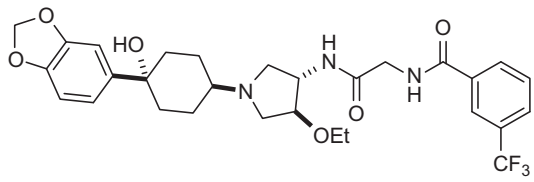
## 4.4. CCR2 antagonists

Certain common inflammatory pathways are involved in multiple vitreoretinal diseases, including DR, macular degeneration, and retinal detachment. For example, levels of IL-6, IL-8, and MCP-1 were highly correlated with each other in all vitreoretinal diseases evaluated.[48] MCP-1 levels in DR vitreous correlate with disease severity, which has been confirmed in a rat DR model.[49] It was recently shown that the CCR2/MCP-1 axis mediates recruitment of monocytes/macrophages and photoreceptor apoptosis in an experimental retinal detachment model.[50] INCB3344 (**23**) is a potent inhibitor of human and mouse MCP-1 binding ($IC_{50}$ = 7 and 10 nM for human

and mouse, respectively) and is also a functional inhibitor of CCR2-mediated chemotaxis (human monocyte $IC_{50} = 4$ nM). Intravitreal administration of **23** suppressed macrophage recruitment and choroidal neovascularization in a laser-induced mouse model of age-related macular vascularization.[51] To date, there have been no preclinical or clinical reports regarding evaluation of CCR2 antagonists in DR.

## 4.5. Miscellaneous anti-inflammatory agents

Among other anti-inflammatory drugs evaluated in preclinical DR models, minocycline (**24**), a tetracycline derivative with poorly defined anti-inflammatory effects (inhibition of microglia proliferation, activation, and apoptosis), was shown to inhibit neuronal cell death in the retinal ganglion cell layer of diabetic rats.[52] Long-term administration of minocycline also significantly inhibited the degeneration of retinal capillaries in diabetic mice.[53] An exploratory clinical trial is being conducted with **24** (100 mg b.i.d. for 24 months) in diabetic ME.[54]

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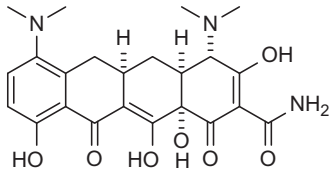


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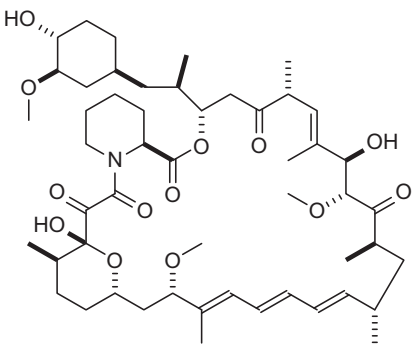



**25**

The immunosuppressive agent rapamycin (**25**), an inhibitor of mTOR (mammalian target of rapamycin), is currently in a placebo-controlled Phase 2 trial involving 120 subjects with diabetic ME secondary to DR.[55] The biological rationale behind this clinical trial has not been described in the literature; however, it appears to relate to the activation of PI3-kinase/AKT/mTOR (phosphatidylinositol 3-kinase/protein kinase B/mTOR)

signaling pathways activated by growth factors involved in angiogenesis and vascular (endothelial) cell proliferation and survival.[56]

A number of agents that are effective in animal models of DR may, at least in part, do so by interfering with proinflammatory processes. For example, aldose reductase inhibition by sorbinil (**26**) blocked 55% and 71% of the genes involved in TGF-β signaling and oxidative stress, respectively, which were upregulated in an experimental rat DR model.[57]

### 4.6. PKC-β inhibitors

Ruboxistaurin showed promising efficacy in several large clinical trials in DR. In a 3-year study involving 685 patients with moderate-to-severe DR, **14** markedly reduced the risk of sustained vision loss compared with placebo.[58] An NDA was filed in 2006 and the drug received priority review by the FDA. Following a regulatory request for additional clinical data, the compound no longer appears to be under active clinical development.[59]

## 5. DIABETIC NEUROPATHY

Diabetic neuropathy (DNeu) represents a series of progressive axonopathies (nerve death that starts with axon degeneration) impacting ~50% of diabetics. The longest axons, those innervating the feet, are generally affected first. Typical symptoms include pain, numbness, and weakness. DNeu increases the risk of lower-limb ulcerations and amputations. In the absence of data on compounds aimed at prevention of nerve damage, this review covers anti-inflammatory approaches that ameliorate the neuropathic pain associated with such damage.

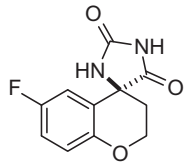
### 5.1. PKC-β inhibitors

Alterations in the microvasculature, similar to those noted in DN and DR, are also observed in DNeu.[60] However, unlike the efficacy documented in those other conditions, **14** only demonstrated efficacy on select end points (e.g., skin microvascular blood flow), while failing on others (e.g., sensory symptoms, neurological deficits, and nerve conduction studies) in a small 6-month Phase 2 DNeu trial.[61] Development of **14** in DNeu has been discontinued.[59]

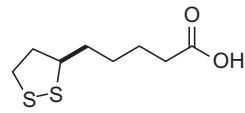
### 5.2. Antioxidant agents

Neurons are highly susceptible to glucose-mediated injury. Excess glucose leads to intracellular ROS generation, lactate accumulation, $NAD^+$ depletion, and activation of back-up pathways such as the polyol and hexose

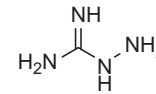
pathways, both of which lead to increased oxidative burden and neuronal damage.[60] Despite this, very few antioxidant strategies have been carefully explored in DNeu, with the possible exception of α-lipoic acid (**27**), a standard of diabetic care in Germany. According to a meta-analysis comprising 1258 patients, infusions of **27** (600 mg/day) ameliorated neuropathic symptoms after 3 weeks. In a randomized trial, 460 DNeu patients were assigned to oral **27** (600 mg/day) or placebo. **27** was well tolerated and, after 4 years, a significant difference in neuropathic deficits was noted relative to placebo.[62]




**26**




**27**




**28**

## 5.3. AGE inhibitors

In experimental diabetes models, RAGE expression is elevated in peripheral epidermal axons, sural axons, Schwann cells, and dorsal root ganglia (DRG) neurons, following the pattern of electrophysiological and structural abnormalities associated with neuropathy. The contribution of AGE to the development of DNeu has been studied preclinically using AGE inhibitors such as aminoguanidine (**28**) and benfotiamine and RAGE-knockout mice.[63]

## 5.4. CCR2 antagonists

CCR2 antagonists are worth noting because they may intervene at two distinct points in the pathology of DNeu, possibly slowing down peripheral nerve loss as well as ameliorating the resulting pain. Macrophages are recruited to areas of axon damage and DRG following damage to the afferent nerves. This process, which may accelerate nerve destruction and set in motion pain pathways, requires CCR2-mediated recruitment of circulating monocytes to the injury site.[64] CCR2 and its ligand MCP-1 are upregulated not only at the site of nerve injury but also distally in the DRG and spinal cord, where it regulates microglia–neuron communication.[65] AZ889 (undisclosed structure) is a potent antagonist of mouse CCR2, blocking mCCL2-induced calcium mobilization with an $IC_{50}$ of 1.3 nM in HEK293 cells, and is also a potent antagonist of rat CCR5, blocking MIP-1α-induced calcium mobilization with an $IC_{50}$ of 79 nM in CHO cells. Single intrathecal injections (1–30 nmol/rat) of AZ889 to rats who

had undergone sciatic nerve ligation (a model of neuropathic pain) resulted in a significant increase in their threshold to mechanical and thermal pain, suggesting that at least some of the antinociceptive effects of CCR2 antagonists are centrally (spinal cord) mediated.[66] Treatment (0.05–1 mg/kg; 7 days) with the orally active CCR2/CCR5 antagonist peptide RAP-103 (undisclosed structure) blocked mechanical allodynia and development of thermal hyperalgesia after partial ligation of the sciatic nerve in rats. RAP-103 also reduced spinal microglial activation and monocyte infiltration. RAP-103 was reported to be a potent antagonist of both CCR2- ($IC_{50} = 4.2$ pM) and CCR5- ($IC_{50} = 0.18$ pM) mediated monocyte chemotaxis.[67]

BMS-741672 (**6**) was tested in a Phase 2 DNeu trial.[68] The study, completed in 2009, targeted 50 diabetics with painful sensory-motor neuropathy secondary to diabetes. Subjects received either placebo or 100 mg BMS-741672 daily for 3 weeks. Results of this trial have not been disclosed.

## 6. CONCLUSIONS

Multiple anti-inflammatory compounds, with various degrees of selectivity for a well-defined pathway, continue to be tested clinically in T2D and associated microvascular complications. At this time, DN appears to have the largest number of active programs moving toward large-scale clinical trials.

## REFERENCES


(1) Manabe, I. *Circ. J.* **2011**, *75*, 2739.
(2) Libby, P.; Okamoto, Y.; Rocha, V.Z.; Folco, E. *Circ. J.* **2010**, *74*, 213.
(3) Donath, M.; Shoelson, S. *Nat. Rev. Immunol.* **2011**, *11*, 98.
(4) Goldfine, A.; Fonseca, V.; Jablonski, K.; Pyle, L.; Staten, M.; Shoelson, S. *Ann. Intern. Med.* **2010**, *152*, 346.
(5) http://clinicaltrials.gov/ct2/show/NCT00799643.
(6) Oh-da, Y.; Morinaga, H.; Talukdar, S.; Bae, E.; Olefsky, J. *Diabetes* **2012**, *61*, 346.
(7) Obstfeld, A.; Sugaru, E.; Thearle, M.; Francisco, A.; Gayet, C.; Ginsberg, H.; Ables, E.; Ferrante, A. *Diabetes* **2010**, *59*, 916.
(8) Kang, Y.; Lee, M.; Song, H.; Ko, G.; Kwon, O.; Lim, T.; Kim, S.; Han, S.; Han, K.; Lee, J.; Han, J.; Kim, H.; Cha, D. *Kidney Int.* **2010**, *78*, 883.
(9) Hanefeld, M.; Schell, E.; Gouni-Berthold, I.; Melichar, M.; Vesela, I.; Sullivan, T.; Miao, S.; Johnson, D.; Jaen, J.; Bekker, P.; Schall, T.J. *Diabetologia* **2011**, *54*(S1), S87.
(10) Jaen, J.; Sullivan, T.; Miao, Z.; Zhao, N.; Berahovich, R.; Ert, L.; Baumgart, T.; Krasinski, A.; Greenman, K.; Bekker, P.; Powers, J.; Schall, T. *Diabetologia* **2011**, *54* (S1), S365.
(11) Hanefeld, M.; Schell, E.; Gouni-Berthold, I.; Melichar, M.; Vesela, I.; Sullivan, T.; Miao, S.; Johnson, D.; Jaen, J.; Bekker, P.; Schall, T. *Diabetes* **2011**, *60*(S1), A85.

(12) Basak, A.; Charvat, T.; Chen, W.; Dairaghi, D.; Hansen, D.; Jin, J.; Krasinski, A.; Moore, J.; Pennell, A.; Punna, S.; Ungashe, S.; Wang, Q.; Wei, Z.; Wright, J.; Zeng, Y. Patent Application WO 008431-A2, **2008**.
(13) Carter, P.; Duncia, J.; Mudryk, B.; Randazzo, M.; Xiao, Z.; Yang, M.; Zhao, R. Patent Application WO 014360-A2, **2008**.
(14) Yang, M.; Cherney, R.; Eastgate, M.; Muslehiddinoglu, J.; Prasad, S.; Xiao, Z. Patent Application WO 014381-A2, **2008**.
(15) http://clinicaltrials.gov/ct2/show/NCT00699790.
(16) Gaede, P.; Vedel, P.; Larsen, N.; Jensen, G.V.; Parving, H.; Pedersen, O. *N. Engl. J. Med.* **2003**, *348*, 383.
(17) Navarro-Gonzalez, J.; Mora-Fernandez, C.; Muros-de-Fuente, M.; García-Pérez, J. *Nat. Rev. Immunol.* **2011**, *7*, 327.
(18) Bierhaus, A.; Nawroth, P. *Diabetologia* **2009**, *52*, 2251.
(19) Karachalias, N.; Babaei-Jadidi, R.; Rabbani, N.; Thornalley, P. *Diabetologia* **2010**, *53*, 1506.
(20) Lewis, E.; Greene, T.; Spitalewiz, S.; Blumenthal, S.; Berl, T.; Hunsicker, L.; Pohl, M.; Rohde, R.; Raz, I.; Yerushalmy, Y.; Yagil, Y.; Herskovits, T.; Atkins, R.; Reutens, A.; Packham, D.; Lewis, J. *J. Am. Soc. Nephrol.* **2012**, *23*, 131.
(21) Figarola, J.; Loera, S.; Weng, Y.; Shanmugam, N.; Natarajan, R.; Rahbar, S. *Diabetologia* **2008**, *51*, 882.
(22) McCormick, B.; Sydor, A.; Akbari, A.; Fergusson, D.; Doucette, S.; Knoll, G. *Am. J. Kidney Dis.* **2008**, *52*, 454.
(23) Badri, S.; Dashti-Khavidaki, S.; Lessan-Pezeshki, M.; Abdollahi, M. *J. Pharm. Pharm. Sci.* **2011**, *14*, 128.
(24) Sharma, K.; Ix, J.; Mathew, A.; Cho, M.; Pflueger, A.; Dunn, S.; Francos, B.; Sharma, S.; Falkner, B.; McGowan, T.; Donohue, M.; Ramachandrarao, S.; Xu, R.; Fervenza, F.; Kopp, J. *J. Am. Soc. Nephrol.* **2011**, *22*, 1144.
(25) Thomas, M.; Cooper, M. *Nat. Rev. Nephrol.* **2011**, *7*, 552.
(26) Pergola, P.; Raskin, P.; Toto, R.; Meyer, C.; Huff, J.; Grossman, E.; Krauth, M.; Ruiz, S.; Audhya, P.; Christ-Schmidt, H.; Wittes, J.; Warnock, D. *N. Engl. J. Med.* **2011**, *365*, 327.
(27) http://clinicaltrials.gov/ct2/show/NCT01351675.
(28) Sayyed, S.; Ryu, M.; Kulkarni, O.; Schmid, H.; Lichtnekert, J.; Gruner, S.; Green, L.; Mattei, P.; Hartmann, G.; Anders, H. *Kidney Int.* **2011**, *80*, 68.
(29) http://clinicaltrials.gov/ct2/show/NCT01440257.
(30) http://clinicaltrials.gov/ct2/show/NCT01447147.
(31) Sullivan, T.; Miao, Z.; Zhao, N.; Berahovich, R.; Powers, J.; Ertl, L.; Dang, T.; Zhao, R.; Liu, S.; Miao, S.; Seitz, L.; Bekker, P.; Schall, T.; Jaen, J. *J. Am. Soc. Nephrol.* **2011**, *22*(S1), 236A.
(32) Koya, D.; Haneda, M.; Nakagawa, H.; Isshiki, K.; Sato, H.; Maeda, S.; Sugimoto, T.; Yasuda, H.; Kashiwagi, A.; Ways, D.; King, G.; Kikkawa, R. *FASEB J.* **2000**, *14*, 439.
(33) Tuttle, K.; Bakris, G.; Toto, R.; McGill, J.; Hu, K.; Anderson, P. *Diabetes Care* **2005**, *28*, 2686.
(34) Rodríguez-Iturbe, B.; Quiroz, Y.; Shahkarami, A.; Li, Z.; Vaziri, N. *Kidney Int.* **2005**, *68*, 1041.
(35) Tang, J.; Kern, T. *Prog. Retin. Eye Res.* **2011**, *30*, 343.
(36) Zhang, W.; Liu, H.; Al-Shabrawey, M.; Caldwell, R.; Caldwell, R. *J. Cardiovasc. Dis. Res.* **2011**, *2*, 96.
(37) Noda, K.; Nakao, S.; Ishida, S.; Ishibashi, T. *J. Ophthalmol.* **2012**. http://dx.doi.org/10.1155/2012/279037 ePub Nov 2, 2011.
(38) Powell, E.; Field, R. *Lancet* **1964**, *2*, 17.
(39) Zheng, L.; Howell, S.; Hatala, D.; Huang, K.; Kern, T. *Diabetes* **2007**, *56*, 337.

(40) Hattori, Y.; Hashizume, K.; Nakajima, K.; Nishimura, Y.; Naka, M.; Miyanaga, K. *Curr. Med. Res. Opin.* **2007**, *23*, 1913.
(41) http://clinicaltrials.gov/ct2/show/NCT01331005.
(42) http://clinicaltrials.gov/ct2/show/NCT00758628.
(43) http://clinicaltrials.gov/ct2/show/NCT00619034.
(44) http://clinicaltrials.gov/ct2/show/NCT00900887.
(45) http://clinicaltrials.gov/ct2/show/NCT00907114.
(46) Bhatwadekar, A.; Glenn, J.; Figarola, J.; Scott, S.; Gardiner, T.; Rahbar, S.; Stitt, A. *Br. J. Ophthalmol.* **2008**, *92*, 545.
(47) Rao, V.; Prescott, E.; Shelke, N.; Trivedi, R.; Thomas, P.; Struble, C.; Gadek, T.; O'Neill, C.; Kompella, U. *Invest. Ophthalmol. Vis. Sci.* **2010**, *51*, 5198.
(48) Brucklacher, R.; Patel, K.; VanGuilder, H.; Bixler, G.; Barber, A.; Antonetti, D.; Lin, C.; LaNoue, K.; GarDNeur, T.; Bronson, S.; Freeman, W. *BMC Med. Genomics* **2008**, *1*, 26.
(49) Yoshimura, T.; Sonoda, K.; Sugahara, M.; Mochizuki, Y.; Enaida, H.; Oshima, Y.; Ueno, A.; Hata, Y.; Yoshida, H.; Ishibashi, T. *PLoS One* **2009**, *4*, e8158.
(50) Nakazawa, T.; Hisatomi, T.; Nakazawa, C.; Noda, K.; Maruyama, K.; She, H.; Matsubara, A.; Miyahara, S.; Nakao, S.; Yin, Y.; Benowitz, L.; Hafezi-Moghadam, A.; Miller, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2425.
(51) Xie, P.; Kamei, M.; Suzuki, M.; Matsumura, N.; Nishida, K.; Sakimoto, S.; Sakaguchi, H.; Nishida, K. *PLoS One* **2011**, *6*, e28933.
(52) Krady, J.; Basu, A.; Allen, C.; Xu, Y.; LaNoue, K.; GarDNeur, T.; Levison, S. *Diabetes* **2005**, *54*, 1559.
(53) Vincent, J.; Mohr, S. *Diabetes* **2007**, *56*, 224.
(54) http://clinicaltrials.gov/ct2/show/NCT01120899.
(55) http://clinicaltrials.gov/ct2/show/NCT00656643.
(56) Jacot, J.; Sherris, D. *J. Ophthalmol.* **2011**, *31*, 95S. http://dx.doi.org/10.1155/2011/589813 Article ID 589813.
(57) Gerhardinger, C.; Dagher, Z.; Sebastiani, P.; Park, Y.; Lorenzi, M. *Diabetes* **2009**, *58*, 1659.
(58) *Drugs R&D* **2007**, *8*, 193.
(59) Danis, R.; Sheetz, M. *Expert Opin. Pharmacother.* **2009**, *10*, 2913.
(60) Vincent, A.; Callaghan, B.; Smith, A.; Feldman, E. *Nat. Rev. Neurol.* **2011**, 7, 573.
(61) Casellini, C.; Barlow, P.; Rice, A.; Casey, M.; Simmons, K.; Pittenger, G.; Bastyr, E.; Wolka, A.; Vinik, A. *Diabetes Care* **2007**, *30*, 896.
(62) Ziegler, D. *Diabetes Care* **2009**, *32*(S2), S414.
(63) Cameron, N.; Gibson, T.; Nangle, M.; Cotter, M. *Ann. N.Y. Acad. Sci.* **2005**, *1043*, 784.
(64) Abbadie, C.; Lindia, J.; Cumiskey, A.; Peterson, L.; Mudgett, J.; Bayne, E.; DeMartino, J.; MacIntyre, D.; Forrest, M. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7947.
(65) Abbadie, C. *Trends Immunol.* **2005**, *26*, 529.
(66) Serrano, A.; Paré, M.; McIntosh, F.; Elmes, S.; Martino, G.; Jomphe, C.; Lessard, E.; Lembo, P.; Vaillancourt, F.; Perkins, M.; Cao, C. *Mol. Pain* **2010**, *6*, 90.
(67) Padi, S.; Shi, X.; Zhao, Y.; Ruff, M.; Baichoo, N.; Pert, C.; Zhang, J. *Pain* **2012**, *153*, 95.
(68) http://clinicaltrials.gov/ct2/show/NCT00683423.