Bioorganic & Medicinal Chemistry Letters 28 (2018) 222–229

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 1-(4-((3-(4-methylpiperazin-1-yl)propyl)amino)benzyl)-5-(trifluoromethyl)pyridin-2(1*H*)-one, an orally active multi-target agent for the treatment of diabetic nephropathy

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ARTICLE INFO

Article history:
Received 13 April 2017
Revised 28 June 2017
Accepted 1 July 2017
Available online 4 July 2017

Keywords:
Anti-fibrosis
Anti-inflammatory
Anti-Diabetic Nephropathy
Multi-target
Pyridone derivatives

ABSTRACT

Oxidative stress, inflammation and fibrosis can cause irreversible damage on cell structure and function of kidney and are key pathological factors in Diabetic Nephropathy (DN). Therefore, multi-target agents are urgently need for the clinical treatment of DN. Using Pirfenidone as a lead compound and based on the previous research, two novel series (5-trifluoromethyl)-2(1*H*)-pyridone analogs were designed and synthesized. SAR of (5-trifluoromethyl)-2(1*H*)-pyridone derivatives containing nitrogen heterocyclic ring have been established for in vitro potency. In addition, compound 8, a novel agent that act on multiple targets of anti-DN with IC_{50} of 90 μM in NIH3T3 cell lines, $t_{1/2}$ of 4.89 ± 1.33 h in male rats and LD_{50} > 2000 mg/kg in mice, has been advanced to preclinical studies as an oral treatment for DN.

Diabetic Nephropathy (DN) is considered as one of the major complications of diabetes mellitus, which is the leading cause of end-stage renal disease (ESRD) and has become a serious threat to human health. There are 285 million people in 2010 and 347 million people in 2013 with diabetes mellitus all over the world,[1] and WHO projects that diabetics will be the seventh-leading cause of death by 2030.[2] Roughly one-third of the diabetic population will develop diabetic nephropathy,[3] which is a heavy social and economic burden.[4]

The occurrence and development of the DN is a complex process with the pathogenic nature of kidney fibrosis. Abundant evidence suggests that genetic predisposition,[5] hyperglycemia,[6–8] hyperlipidemia,[9] and microcirculatory disturbance are the major factors contributing to the initiation and progression of DN.[10] Although DN is associated with high morbidity and mortality and the prevalence of this disease is continuously increasing worldwide, there is currently no effective treatment strategy available other than symptom control. In recent years, numerous studies have shown that the development of oxidative stress,[11] inflammatory and renal fibrosis is the key to the occurrence and development of DN.[12,13] Therefore, compounds that can bring resistance to oxidative stress, inflammation, and fibrosis have been the focus of drug development studies on DN.

Reactive Oxygen Species (ROS) play an important role in DN which indicates that the direct scavenge of ROS is a potential strategy for drug discovery of anti-DN agents. Superoxide dismutase mimetic Tempol,[14] a nonspecific NOX inhibitors with IC_{50} of 0.9 μM, reduced the peroxide production in glomeruli. At the same time, due to increased myeloperoxidase, this drug failed to reduce

Abbreviations: ACR, albumin-to-creatinine ratio; AUC_{0-t}, area under the curve; BuOH, *n*-butanol; BUN, blood urea nitrogen; C_{max}, peak concentration; C^{13}-NMR, C^{13}-nuclear magnetic resonance; CCr, creatinine clearance rate; CCR2, CCchemkin receptor 2; $CDCl_3$, Deuterotrichloromethane; DBM, diabetes model; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; DN, diabetic nephropathy; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; ERSD, end stage renal disease; GC-MS, Gas Chromatograph-Mass Spectrometer-computer; GLP, Good laboratory practice of drug; GSI, glomerular sclerosis index; HMBC, heteronuclear multiple bond correlation; HPLC, high performance liquid chromatography; IR, infrared radiation; LC-MS, liquid chromatography-mass spectrum; LPS, lipopolysaccharide; MTT, methylthiazolyl diphenyl tetrazolium bromide; NIH3T3, mouse embryonic fibroblast cell line; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PD, pharmacodynamics; PDGF, platelet-derived growth factor; PFD, pirfenidone; PK, pharmacokinetics; ROS, reactive oxygen species; SAR, structure activity relationships; SCr, serum creatinine; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factors-α; UUO, unilateral ureteral occlusion.

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https://doi.org/10.1016/j.bmcl.2017.07.001
0960-894X/

albuminuria which is a result of the damage to the GBM. VAS-2870, a NOX specific depressor, inhibits ROS generation with an IC_{50} value of 10.6 mM in cell-free systems.[15] However, it failed in animal experiments assessing the pharmaceutical profile. Next, inflammation also plays a key role in the development and progression of DN. TLK-19705,[16] a novel CCR2 antagonist, has been tested in a group of db/db mice with dosages of 30 mg/kg/day and 10 mg/kg/day for 8 weeks by Okamoto. M' team. And the results show that the urinary ACR has improved in the animal experiment. Finally, renal fibrosis is the final common pathway of DN. IN-1130 showed efficacy in suppressing fibronectin in UUO rat kidneys by inhibiting Smad2 phosphorylation.[17] GW788388 can diminish renal fibrosis by inhibiting the proliferation of fibrosis cell.[18,19] HOE77, an inhibitor of the formation of collagen in the late stage of liver fibrosis, can cause cataracts in phase II clinical trial, and its investigation has been terminated.[20,21] The above many compounds effect in a link or target of fibrosis, which rarely passed clinical research, suggesting that single target inhibitor is not the ideal research direction of anti-DN drug discovery.

Pirfenidone (PFD), a multi-target drug, has been marketed mainly for idiopathic pulmonary fibrosis since 2008.[22] And a phaseⅢclinical trial of PFD for DN has been completed with 77 patients at San Digo Medical Center of California University.[23] Clinical effect of PFD is not satisfactory because its 5-methyl can be easily metabolized.[24] In addition, PFD showed a certain acute toxicity in animal experiments.[25] With PFD as the lead compound, our research group has carried on a series of transformation. The previous research results have been reviewed by Yi-Min Liu.[26] First, we gain Fluorofenidone (AKF-PD) by introducing fluorine in the meta position of the phenyl group of PFD.[27] AKF-PD, a "me-better" drug, show higher inhibitory activity and more alleviated fibrosis symptoms than PFD.[28] As with PDF, AKF-PD is prone to inactivation due to the metabolism of methyl groups on pyridone ring.[29] In order to prevent the metabolism of the 5-methyl group, our research group turn methyl into aromatic ring,[30] trifluoromethyl and methyl cyclization.[31,32] which greatly increase the activity of the compound. Among them, the activity of compound ZHC-102 with an IC_{50} of 290uM was obviously improved. In our early work, we found that the introduction of methylene into pyridine ring and phenyl group can improve the activity of the target compound. For the purpose of finding the target compounds with excellent activity and further exploring the structure-activity relationship, our group has developed a new series of compounds by converting the phenyl group at the 1-position of the pyridone ring to the benzyl group.[31] ZHC-159, a benzyl series compound, shows excellent activity with IC_{50} of 80uM in NIH3T3 cell lines, but it also has high toxicity.

The purpose of this study is to develop an anti-DN drug. Although the activity of the compound has been improved by previous studies, the drugability of these compounds is not sufficient because of poor water-solubility and high toxicity. Experiments revealed that the drugability of compounds could be improved by adding the larger group to 1-N of pyridone. Herein, our research group developed two novel series of (5-trifluoromethyl)-2(1*H*)-pyridone derivatives containing nitrogen heterocyclic ring to improve its medical properties (see Fig. 1).

The mouse fibroblast cell line (NIH3T3 cells), a well-recognized anti-fibrosis drugs in vitro screening model,[33] was used to test the potency of the target compounds. Here we detected NIH3T3 cell proliferation by MTT assay with AKF-PD as the positive control to test the potency of the anti-fibrosis compounds and discuss the SAR on anti-fibrotic activity. Compound 8 was finally identified in the research by conducting a series of research of SAR and pharmacokinetic experiments (see Table 1).

An efficient synthesis of a wide range of (5-trifluoromethyl)-2(1*H*)-pyridone derivative was reported earlier with our effort to discover NIH3T3 inhibitor. Based on this, PFD was extended by introducing versatile nitrogen heterocyclic ring side chains. The link chain between the aromatic amino group and the heterocycle is 2 to 3 carbon atoms in length. Representative nitrogen

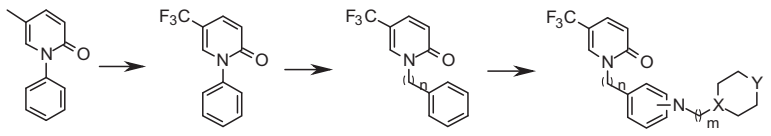

Fig. 1. The map of design route of the target compounds.

Table 1
The anti-DN compounds in preclinical or clinical research.