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Review Article

Chymase inhibitors and their therapeutic potential

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CONTENTS

Introduction

Chymase (EC 3.4.21.39) is synthesized and stored in mast cells localized mainly in the heart, blood vessels and skin. Mast cells secrete chymase with other biologically active substances in response to immunological stimuli. Reports on the *in vitro* activities of chymase have indicated that it degrades IgG and extracellular matrices (such as type IV collagen, fibronectin and vitronectin), produces active-type collagenase from procollagenase and promotes the conversion of macrophages into foam cells by degrading apolipoprotein B-100 (1-3). However, few reports have discussed the physiological and pharmacological importance of chymase. Recently, attention has been focused on the localized production of angiotensin II (Ang II) by human chymase in the cardiovascular system (4-6): chymase levels have been found to increase in balloon-injured blood vessels of dogs and in the hearts of a hamster model of cardiomyopathy (7, 8), while increased chymase-dependent Ang II formation has been observed in human atherosclerotic aortae (9). In addition, several reports have suggested that chymase is involved not only in Ang II formation but also in tissue remodeling in the cardiovascular system (10, 11), and that chymase is one of the endothelin-processing enzymes (12, 13). It is also known that transforming growth factor β1 (TGF-β1) is produced through the proteolytic action of the co-localized chymase (14).

Independent of this, the localization of chymase in mast cells has prompted the study of its roles in allergic and inflammatory diseases. Chymase has been reported to be involved in the process of histamine release (15) and to provoke infiltration of inflammatory cells when injected (16). With particular reference to skin inflammation, involvement of chymase in the production of soluble-type stem cell factor (SCF) and of interleukin-1β (IL-1β) has been suggested (17-20), while more recently the relationship between genetic polymorphism of mast cell chymase and atopic eczema has been discussed (21-23). Chymase inhibitors, therefore, are now considered a potential new type of antiinflammatory and antiallergic agent.

Chymase is thus thought to play important roles in several biological reactions. With the recent discovery of potent chymase inhibitors featuring specificity and metabolic stability, their potential clinical application has widened. The present review focuses on chymase inhibitors and their therapeutic potential in chymase-induced diseases.

Peptidic chymase inhibitors

Peptidic serine protease inhibitors generally consist of a peptidic backbone (*e.g.*, P_3–P_1) recognized by the protease and a functional substituent to activate the P_1 carbonyl group toward nucleophilic addition by the active site Ser-195 hydroxy group of the protease. Chymase inhibitors have a similar structure. The peptidic inhibitors are shown in Figure 1. Compound **1** (chymostatin) is a well-known and naturally occurring inhibitor of chymotrypsin-like serine protease (24) and is generally used as a positive control for chymase. Peptidic inhibitors, including compound **1**, have mainly been developed around mechanism-based concepts, that is, by replacing the scissile amide bond of a protease substrate with a carbonyl group to form a hemiketal that resembles a tetrahedral intermediate. A number of functional groups have been used to activate the P_1 carbonyl group of peptidyl ketones toward nucleophilic addition by the active site Ser-195 hydroxy group of chymase, including the trifluoromethyl, difluoromethylene **2** (25, 26), ester **3** (27, 28) and amide groups (29). The boronic acid inhibitor **4** is capable of forming a tetrahedral adduct with the Ser-195 (15). These inhibitors are reversible; irreversible inhibitors are described below. The diphenyl phosphonate ester **5** is attacked by the Ser-195 to form a phosphonate ester and an enzyme through elimination of one phenol; another

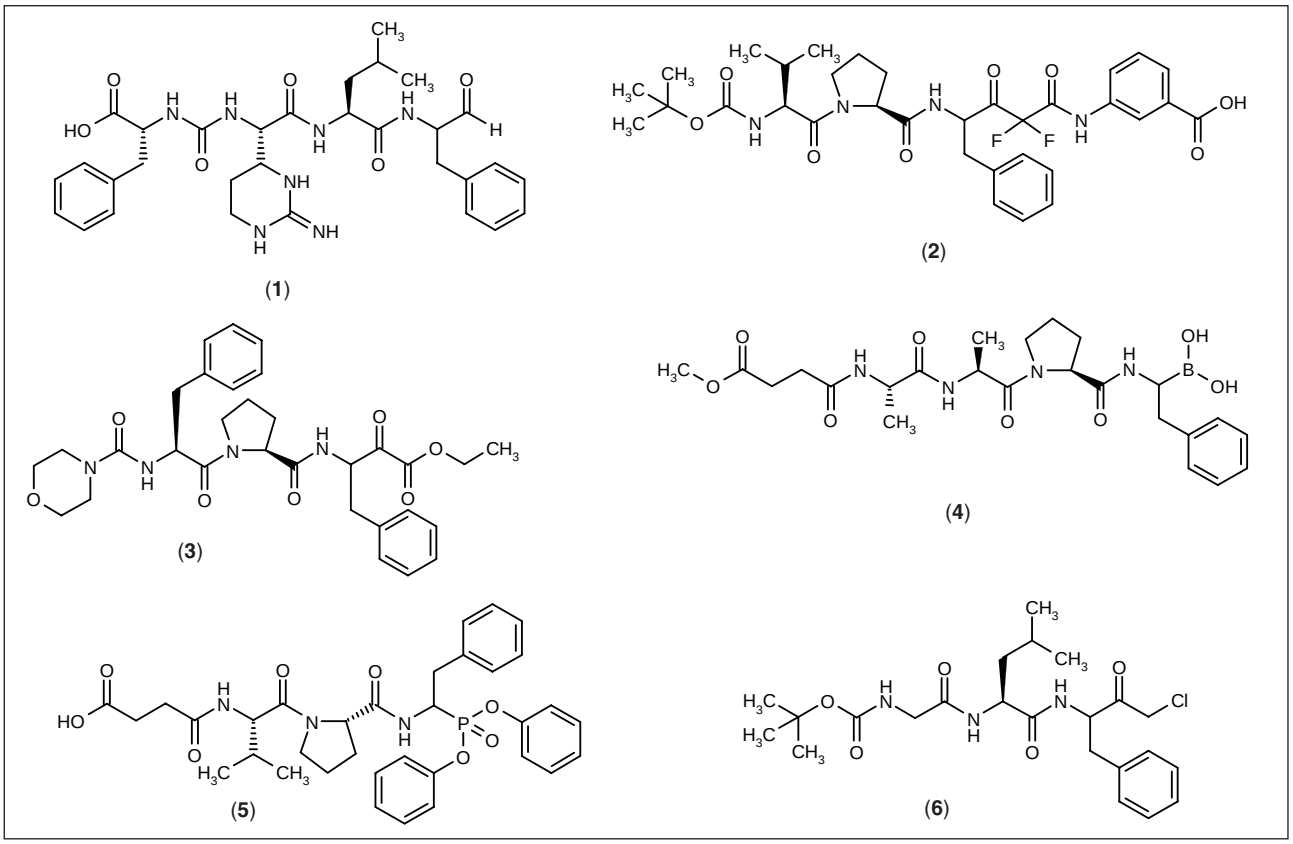

Fig. 1. Peptidic chymase inhibitors.

phenol ester is then hydrolyzed to generate a stable phosphonated enzyme (30). The chloromethyl ketone derivative **6**, a well studied inhibitor, interacts with the Ser-195 to give a tetrahedral hemiketal and then alkylates the active site His-57 residue to generate an alkylated enzyme (27, 31). Although these peptidic inhibitors have highly potent chymase-inhibitory activity, their therapeutic use is still limited because of their peptidic nature.

Nonpeptidic chymase inhibitors

Recently, several nonpeptidic chymase inhibitors have been described, including orally active ones. Nonpeptidic chymase inhibitors are shown in Figure 2. The saccharin **7** and isocoumarin **8** inhibit chymase by acylation of the active site Ser-195 of the enzyme followed by a ring-opening reaction (32, 33). The sulfonylfluoride **9** also inhibits human and rat chymases (RMCP II) irreversibly by sulfonylation of the Ser-195 (27). It is not yet certain whether the imidazolidine-2,4-dione derivative **10** and the quinazoline-2,4-dione derivative **11** (SUN-C8257) act by trapping the Ser-195 hydroxy group of chymase, but the 4-carbonyl of the imidazolidine and quinazoline ring and the sulfonyl groups are thought to interact with the oxyanion hole and the His-57 side chain, respectively (34, 35). The anhydride derivative **12** inhibits chymotrypsin-like proteases, including recombinant human chymase, and has been shown by kinetic studies of chymotrypsin to be a mechanism-based inactivator (36, 37); compound **12** presumably acylates the Ser-195. The β-lactam analogues including the cephalosporin derivative **13** are reported to be human chymase inhibitors in which the Ser-195 hydroxy group attacks the β-lactam carbonyl group of 1-oxocepham, leading to cleavage of the β-lactam ring and generation of an acylated enzyme (38-41). The inhibitory mechanism of compound **14** against chymase is assumed to involve nucleophilic substitution of the Ser-195 hydroxy group at the β-olefinic carbon atom activated by the two carbonyl groups, leading to the generation of serine hydroxy O-alkenyl enzyme while the phenyl group docks into the S_1 pocket (42). Recently, peptidomimetic design has also been successfully used to modify the P_3–P_2 dipeptide portion of a peptidic chymase inhibitor into the isosteric and isoelectronic fragment, which is represented by 5-amino-2-phenylpyrimidin-6-one. In the relevant research, several novel potent, selective and orally active nonpeptidic chymase inhibitors, typified by **15** (Y-40613), **16** (Y-40079) and **17** (Y-40018), were discovered (43-45). Most recently, **18** (NK-3201), which incorporates a similar framework, was reported as a potent and orally active chymase inhibitor (46).

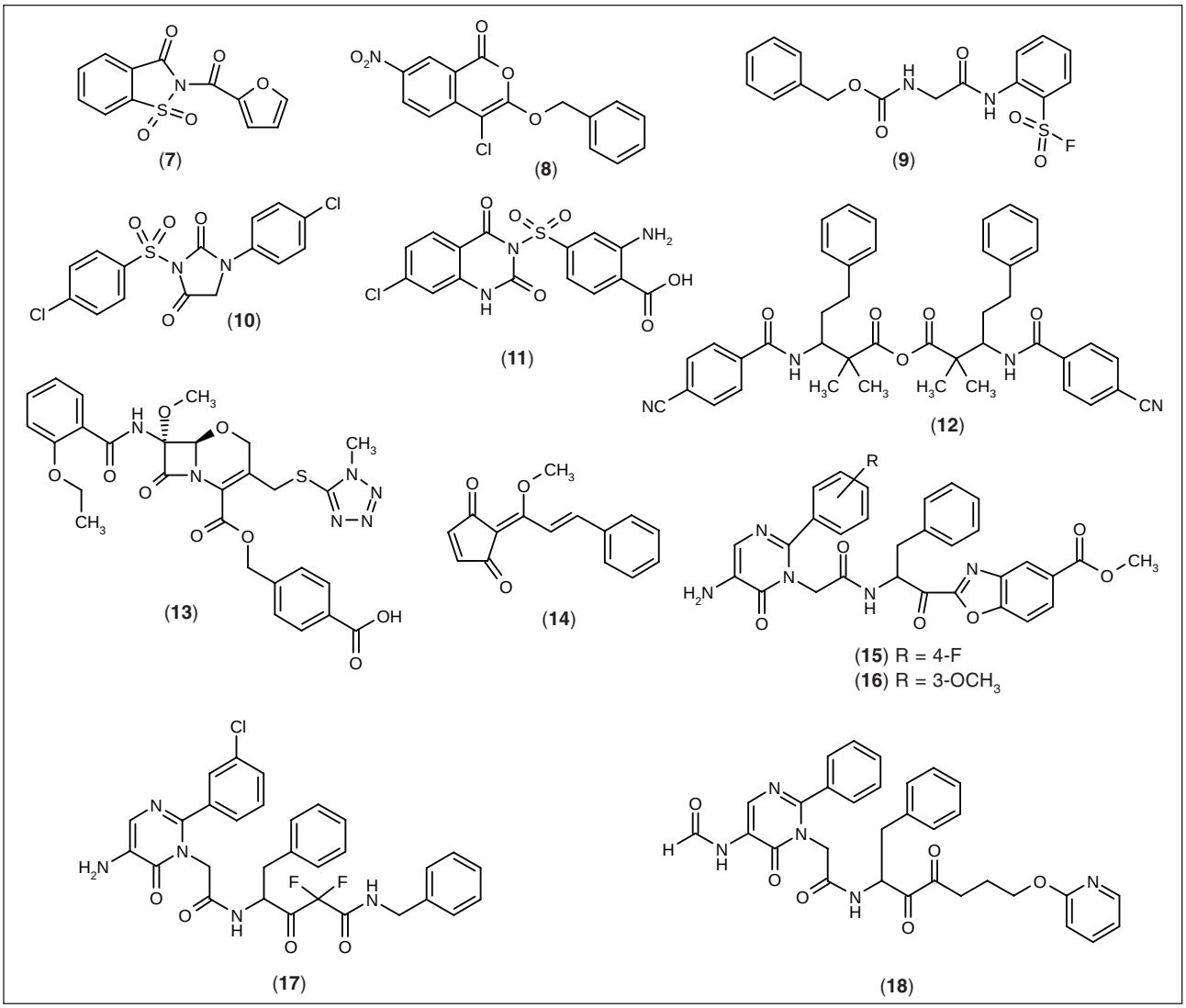

Fig. 2. Nonpeptidic chymase inhibitors.

Therapeutic potential of chymase inhibitors

The discovery of potent, specific and orally active chymase inhibitors has presented new opportunities to explore the role of chymase under both physiological and pathophysiological conditions. At the same time, recent advances in the understanding of the role of chymase in diverse states have widened the potential clinical application of chymase inhibitors. The therapeutic potential of specific chymase inhibitors as novel drugs is summarized below.

Restenosis after bypass graft or PTCA

The pivotal roles of locally produced Ang II in vascular proliferation are established. Chymase is believed to be a major enzyme in the production of Ang II in the vascular bed in some species, including humans. Chymase inhibitors may have the ability to suppress locally produced Ang II without affecting systemic blood pressure.

Takai *et al.* have reported the effect of chymase on proliferation of grafted veins in dogs (46-48). By 28 days after grafting, a significant vascular proliferation was observed in the grafted veins and chymase activity was also increased. Infiltration of the peptidic chymase inhibitor **5** (10 μM) into blood vessels extirpated during surgery significantly decreased chymase activity in the grafted veins and also reduced the intimal area of the grafted veins at 28 days. Oral administration of **18** (1 mg/kg/day) from 5 days before to the day of removal of the grafted veins suppressed the proliferation of the grafted veins and the increased chymase activity. Inhibition of chymase may, therefore, be useful in preventing vascular disease such as proliferation in grafted vessels.

Tissue adhesion

Adhesion formation is a major source of postoperative morbidity and mortality in many medical fields. Yao *et al.* reported that adhesion formation in mast cell-deficient W/W(V) mice 1 week after cecal scraping was significantly less severe than that in normal control mice (49). Recently, Okamoto *et al.* reported the preventive effect of the specific peptidic chymase inhibitor **5** on adhesion formation in a hamster experimental model (50). Hamsters underwent resection of the right uterine body and then **5** (10 μM) or placebo was injected into the abdomen. Two weeks after surgery, the scores for adhesion formation in the chymase inhibitor-treated group were significantly lower than in the placebo-treated group. These results suggest that chymase may be important in the development of adhesion formation.

Angiogenesis-related diseases

It has been reported that Ang II regulates angiogenesis in several experimental models. Muramatsu *et al.* investigated the angiogenic effect of chymase on angiogenesis using a hamster sponge implant model (51, 52). Exogenous administration of Ang II or Ang I directly into the sponges enhanced angiogenesis as determined from hemoglobin content in the sponge granuloma tissues. Daily injection of basic fibroblast growth factor (bFGF) into the implanted sponges also induced angiogenesis, which was suppressed by treatment with the specific inhibitor **1** (10 nmol/site/day) or TCV-116 (5 mg/kg/day p.o.), an antagonist of Ang II type 1 receptor. Chymase activity in the sponge granulomas increased in parallel with the rise in hemoglobin content induced by bFGF. These results suggest that chymase may induce angiogenesis *in vivo* partly through the local generation of Ang II. Moreover, since angiogenesis occurs under certain pathological conditions such as cancer, diabetic retinopathy and rheumatoid arthritis, chymase inhibitors figure as possible therapeutic agents for these disorders.

Atopic dermatitis

When purified rat chymase (RMCP-1) is intradermally injected into mice, a scratching reflex is observed (53), suggesting that chymase inhibitors may be effective against atopic dermatitis. Imada *et al.* reported that chymase inhibitors suppress the production of pruritus and IgE which are the two main symptoms of the disease (54, 55).

The specific chymase inhibitor **15** (3, 10 and 30 mg/kg p.o.) showed dose-dependent suppression of the scratching response in a recently established pruritus model using 2,4-dinitrofluorobenzene (DNFB)-induced passively sensitized BALB/c mice. It was of interest that **15** showed enhanced inhibition of the scratching response in combination with cyproheptadine, a histamine/serotonine antagonist, and prednisolone. These results suggest that chymase contributes to the development of pruritus by a mechanism or mechanisms different from those of conventional drugs.

Participation of chymase in the production of IgE was also examined *in vitro*. Although purified chymase increased the production of IgE from human and mouse B cells in the presence of IL-4, it was significantly suppressed in the presence of **15**. This effect was also confirmed *in vivo* with a Brown Norway (BN) rat model. Although increased serum IgE level was observed in BN rats injected in the back with mercury chloride, oral administration of **15** (3, 10 and 30 mg/kg/day) suppressed the response dose-dependently. These findings suggest that specific chymase inhibitors like **15** offer therapeutic potential and a novel approach to atopic dermatitis.

Tomimori *et al.* have recently reported that topical applications of DNFB to the ears of mice result in accumulation of mast cells and that administration of **11** (10 and 50 mg/kg i.p.) inhibited the increase in dermal mast cells (56). Since chymase is thought to control local mast cell numbers by processing of SCF associated with the surrounding cells including keratinocytes, chymase inhibitors may be effective in allergic skin disorders.

Conclusions

The discovery of a number of chymase inhibitors including orally active ones as described in the present review, has presented new opportunities to explore the pathophysiological role of chymase and has led to an increase in the last few years in the number of studies reporting their therapeutic potential in chymase-induced diseases. They have demonstrated their efficacy as therapeutic agents in restenosis following bypass graft or PTCA, tissue adhesion, angiogenesis-related diseases and atopic dermatitis. With energetic progress being made in the development of novel agents featuring potent chymase-inhibitory activity, specificity and metabolic stability, their clinical evaluation can be expected in the not too distant future.

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