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Original article

Pyrido-imidazodiazepinones as a new class of reversible inhibitors of human kallikrein 7

Dominique P. Arama [a, 1], Feryel Soualmia [b, 1], Vincent Lisowski [a], Jean-François Longevial [a], Elodie Bosc [b], Ludovic T. Maillard [a], Jean Martinez [a], Nicolas Masurier [a, *], Chahrazade El Amri [b, *]

[a] Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, Université de Montpellier, UFR des Sciences Pharmaceutiques et Biologiques, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France

[b] Sorbonne Universités, UPMC Univ Paris 06, UMR 8256, B2A, Biological Adaptation and Ageing, Integrated Cellular Ageing and Inflammation, Molecular & Functional Enzymology, 7 Quai St Bernard, F-75005 Paris, France

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ABSTRACT

The human tissue kallikrein-7 (KLK7) is a chymotryptic serine protease member of tissue kallikrein family. KLK7 is involved in skin homeostasis and inflammation. Excess of KLK7 activity is also associated with tumor metastasis processes, especially in ovarian carcinomas, prostatic and pancreatic cancers. Development of Kallikrein 7 inhibitors is thus of great interest in oncology but also for treating skin diseases. Most of the developed synthetic inhibitors present several drawbacks such as poor selectivity and unsuitable physico-chemical properties for *in vivo* use. Recently, we described a practical sequence for the synthesis of imidazopyridine-fused [1,3]-diazepines. Here, we report the identification of pyrido-imidazodiazepinone core as a new potential scaffold to develop selective and competitive inhibitors of kallikrein–related peptidase 7. Structure-activity relationships (SAR), inhibition mechanisms and selectivity as well as cytotoxicity against selected cancer cell lines were investigated.

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1. Introduction

Tumor invasion and dissemination are complex and multi-step processes involving several key cellular events. Among the many events leading to metastasis, loss of intercellular adhesion is one of the earliest phenomenon, which could lead to cells dissemination [1]. One of the most well characterized mechanisms by which tumor cells can lose adhesion is by up-regulating the expression of extracellular proteases, which are able to cleave one or more of these cellular adhesion proteins. Among these various families of proteases, the human tissue kallikrein serine proteases (KLKs) are known to be over-expressed in various cancers [2,3].

In this context, kallikrein 7 (KLK7) a chymotryptic enzyme [4,5], was reported to be implicated in several tumor metastasis processes, especially in ovarian carcinomas, pancreatic cancers, by the hydrolysis of several cadherin subtypes [6,7]. KLK7 facilitates metastasis *via* degradation of extracellular matrix components such as fibronectin, desmosomal proteins particularly desmogleins 1 and 2, thus reducing cell–cell adhesion. In prostatic cancers, KLK7 induces epithelial to mesenchymal transition-like changes in prostate carcinoma cells, implying a role in prostate cancer invasion and progression [8]. Furthermore, KLK7 like other kallikreins such as KLK5, KLK8, KLK14 and matriptase plays an important role in normal skin desquamation [9–11]. Such enzymes can hydrolyze extracellular proteins that are part of intercellular adhesion structures such as corneodesmosomes in the stratum corneum [12]. As a consequence, mutation in the *Spink5* gene of LEKTI, the physiological inhibitor of skin kallikreins, is responsible of the Netherton

Abbreviations: AMC, 7-amino-4-methylcoumarin; DCM, dichloromethane; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulfoxide; EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, hydroxybenzotriazole; IP, imidazo[1,2-*a*]pyridine; KLK5, kallikrein-related peptidase 5; KLK7, kallikrein-related peptidase 7; KLK8, kallikrein-related peptidase 8; KLK14, kallikrein-related peptidase 14; LEKTI, lymphoepithelial Kazal-type–related inhibitor; PDB, Protein Data Bank; TFA, trifluoroacetic acid; THF, tetrahydrofuran; XTT, 2,3-Bis-(2-Methoxy-4-Nitro-5-sulfophenyl)-2H-Tetrazolium-5-Carboxanilide.

* Corresponding authors.
E-mail addresses: nicolas.masurier@univ-montp1.fr (N. Masurier), chahrazade.el_amri@upmc.fr (C. El Amri).

[1] These authors contributed equally to the work.

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syndrome, a lethal recessive autosomal skin disorder [13]. Recently, in an organotypic human skin culture used as a model system of Netherton Syndrome, it has been shown that genetic inhibition of KLK5 and KLK7 ameliorates epidermal architecture demonstrating the relevance of these two enzymes as targets for treating this disease [14]. On the other hand, KLK7 transgenic mice exhibit a phenotype comparable to psoriasis with cutaneous hyperproliferation and immune cell infiltration [15]. Obese subjects often suffer from psoriatic lesions associated with high levels of adipokines such as chemerine. Beck-Sickinger and coll. have recently shown that KLK7 mediated pro-chemerine processing contributing thus to the development of these psoriatic lesions [16].

Consequently, the development of KLK7 inhibitors to control unopposed proteolytic cascades could represent a potential therapeutic approach to avoid or decrease metastasis associated with over-expression of KLK7 as well as for treating some skin disorders. Among the reported inhibitors of KLK7 [17], a few consist in peptide derivatives such as chloromethylketones [5], LEKTI-derived peptides [18] and sunflower trypsin inhibitor (SFTI-1) peptide derivatives [19]. More recently some heterocyclic compounds such as natural isocoumarins (as 8,8′-paepalantine) [20] isomannide derivatives [21,22] or a mercaptobenzimidazolyl-quinazoline derivative [23] have also shown significant activities against KLK7 (Fig. 1). All of them are reversible KLK7 inhibitors with moderate activity ($10 < IC_{50} < 200$ μM). Additionally, some acyl-1,2,4-triazoles were reported to be more potent inhibitors ($0.04 < IC_{50} < 3.5$ μM), with the ability to form transient covalent complexes (acyl-enzymes) with both KLK7 and KLK5 [24]. Since, most of the identified inhibitors suffer from low selectivity, moderate activity and unsuitable physico-chemical properties for *in vivo* use, the identification of new chemical scaffold for potent and reversible inhibition of KLK7 is thus needed.

A survey of the literature shows that some compounds bearing various heterocyclic pharmacophores can be found as KLK7 inhibitors. However, very few structure-activity relationships were carried out in these series and no co-crystallization of small molecules in the active site of KLK7 has been reported yet. So, crucial determinants for KLK7 specific targeting and inhibition still need to be addressed. All these points prompted us to screen some heterocyclic derivatives from our internal library to study their potential inhibiting activity against KLK7. We report herein the identification of the pyrido-imidazodiazepinone structure as a new potential scaffold to develop selective and competitive KLK7 inhibitors. Structure-activity relationship (SAR), inhibition mechanisms and selectivity, as well as biological evaluations against selected cancer cells were investigated.

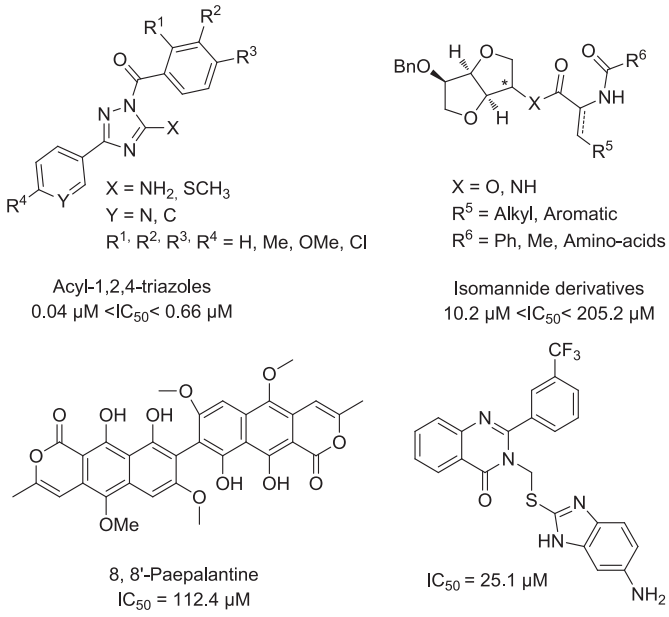

Fig. 1. Heterocyclic compounds reported as KLK7 inhibitors.

1.1. Screening of the diazepinone library and rationale for the synthesis of analogs

Due to the heterogeneity of the heterocyclic scaffolds used to obtain KLK7 inhibition, 65 heterocyclic derivatives from our internal library were chosen for an initial screening (data not shown), including imidazo[1,2-*a*]pyridine (IP) and 1,3-thiazole derivatives, due to our particular interest in these heterocyclic scaffolds [25–31].

Compounds were all tested at the single dose of 50 μM. Among them, a pyrido-imidazodiazepin-5-one showed an inhibition capability higher than 30% (compound **1a**, Table 1) ($IC_{50} = 57 \pm 1.0$ μM). The reversibility of the inhibition was evaluated by dilution of the reaction mixture after 15 and 60 min incubation of **1a** with KLK7 at pH8 and 37 °C. The enzymatic activity was immediately restored in agreement with no formation of a covalent bond between the protease and the inhibitor. Lineweaver–Burk double reciprocal plot was used to determine the mechanism of action of compound **1a**. Plots are consistent with a competitive inhibitor behavior with an inhibition constant K_i of 50.6 ± 0.8 μM (see Fig. S1, SI). It is worth noting that no noticeable inhibition was observed at 50 μM on other screened serine proteases (KLK5, KLK8 and KLK14), meaning that compound **1a** had a selective action on KLK7.

A SAR study was then initiated, starting from compound **1a**. A set of 30 new pyrido-imidazodiazepinones as well as 9 acyl-IP derivatives, which can be seen as "opened pyrido-imidazodiazepinones", were synthesized and their potency was evaluated against KLK7 and other serine proteases to check selectivity.

1.2. Rationale for the synthesis of optimized diazepinone analogs

Modifications at positions 2, 3, 4 and 5 of the diazepinone core were envisioned (Fig. 2). Synthesis of "opened-diazepinones" that possessed a higher flexibility was also investigated.

Pyrido-imidazodiazepinones **1a-ad** were synthesized starting from 2-amino-imidazo-[1,2-*a*]pyridine **5** by selective *C*-3 acylation of the IP nucleus (Scheme 1), using 7 different *N*-Boc amino-acids (Boc-Phe-OH, Boc-D-Phe-OH, Boc-Ala-OH, Boc-Ser(Bn)-OH, Boc-1-Nal-OH, Boc-Tyr(Bn)-OH, Boc-*N*-Me-Phe-OH) according to our previously reported methodology [25,32]. *C*-3 acylated compounds **4a-g** were isolated in 27%–88% yield after chromatography on alumina gel. Only traces of the corresponding *N*-acylated derivatives were detected by LC-MS analysis in the crude. After Boc removal in acidic media (by using concentrated hydrochloric acid or a mixture of trifluoroacetic acid/dichloromethane (50/50 v/v)), the resulting 2-amino-3-acyl-imidazo-[1,2-*a*]pyridines **3a-g** were isolated in basic medium, without further purification. Diamines **3a-g** were then successively reacted with a set of aldehydes in chloroform at reflux for one night. Intermediate aminals were then oxidized without isolation, using a mixture of lead tetraacetate and iodine, to lead to pyrido-imidazodiazepinones **1a-1w** that were purified by chromatography on alumina gel.

The pyrido-imidazodiazepine-2,5-dione **1aa** was synthesized by intracarbonylation of diamine **2a**, using 1,1′-carbonyldiimidazole in acetonitrile at 60 °C and was isolated in 78% yield [32]. "Opened derivatives" **2a-i** were synthesized by a coupling reaction between the diamine **3a** and a set of commercially available carboxylic acids.

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