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Synthesis and stability evaluation of novel peptidomimetic Caspase-1 inhibitors for topical application

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ABSTRACT

During our search for topically-active Caspase-1 inhibitors, we identified a novel class of potent inhibitors based on a 1,3,5-trisubstituted uracil motif equipped with an L-aspartate semi-aldehyde derived warhead. In the literature, the majority of Caspase-1 inhibitors possessing the same warhead have been designed and evaluated for oral administration as the ethyl acetal pro-drug form. For our topical program, the pro-drug acetal form was not fully hydrolysed in the skin and was unstable in many of our standard topical excipients, therefore, we were obliged to focus on the actual hemiacetal drug form of the molecule during our drug discovery program. Our work focuses on both the synthesis and achiral and chiral stability of the final drug molecules in topical excipients.

1. Introduction

Caspases are a family of protease enzymes called Cysteine Aspartic Proteinases. There are 12 known Caspases in humans. Caspases 1, 4, 5, 11 and 12 are all inflammatory caspases and 2, 3, 6, 7, 8, 9 and 10 are all associated with apoptosis [1]. All caspase substrates are cleaved from the C-terminus of an aspartic acid residue C-terminal in the P1 region of the binding site. Interleukin-1β converting enzyme (ICE), also known as Caspase-1, is the principal enzyme responsible for cleavage and activation of pro-Interleukin-1β (pro-IL-1β) to its active form IL-1β, which in turn is involved in the pathogenesis of several inflammatory disorders including acne vulgaris [2]. The cleavage of pro-IL-1β to IL-1β occurs through deprotonation of a cysteine residue via a neighbouring histidine. Cytokine IL-1β plays an important role in local and systemic inflammation and has been reported to target numerous cells involved in inflammatory acne [3]. In a recent study, we reported a comparison of lesional versus non-lesional biopsies of patients with inflammatory acne and through transcriptomic and proteomic studies, we showed a strong induction of IL-1β mRNA and IL-1β protein in lesional biopsies [4]. Consequently, the treatment of inflammatory acne with a topical agent targeting Caspase-1 presented an exciting and novel opportunity for the treatment of moderate to severe acne.

Medicinal chemists have developed potent inhibitors of the catalytic site principally by using masked aldehydes [5]. These form a reversible covalent linkage with the cysteine residue to block the enzyme from functioning. In 1992, Thornberry et al., inspired from the actual sequence of Caspase I substrate pro-IL-1β, discovered a tetrapeptide motif, Ac-YVAD-CHO with nanomolar potency against Caspase I. The C-terminus of the aspartic acid residue was modified to an aldehyde resulting in reversible covalent inhibition of the enzyme through a hemithioacetal linkage (Fig. 1).

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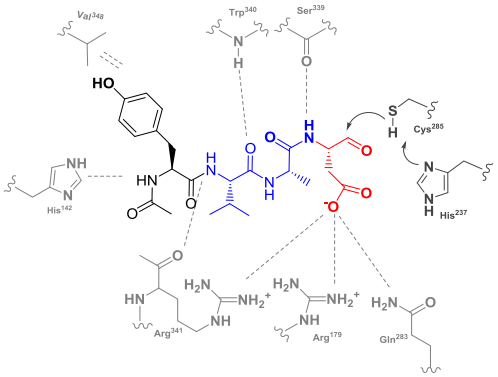


Fig. 1. Binding mode of Ac-YVAD-CHO in Caspase I (Black, P4 pocket; blue P3/P2 region; red, P1 warhead region).

To overcome the poor ADMET of the early peptidic leads, potent peptidomimetic inhibitors of Capsase-1 [6], Pralnacasan (VX740) and Belnacasan (VX765) were developed and have been progressed to clinical studies for the treatment of rheumatoid arthritis and treatment-resistant epilepsy. Inspired by their approach, we set about designing our own cyclic peptidomimetic inhibitors based on scaffolds while preserving the 3 point H-bonding network and the aspartaldehyde warhead (Fig. 2) [7].

2. Results and discussion

We developed several synthetic strategies to deliver the piperidin-2,6-dione and pyrrolidin-2,5-dione based inhibitors that we have already detailed in a recent communication [8], focusing principally on: a) P1 warhead exploration; and b) probing the P4 region both exemplified in Schemes 1 and 2, respectively.

For topical medicinal chemistry, activity is always an important driver but the solution stability of our actives is another major consideration as the API is not formulated in solid form but in wet form such as creams, lotions or gels [9]. The topical excipient selected for screening purposes was a 96% w/w aqueous ethanol. Solutions at 0.01% (w/w) were monitored by HPLC during 3 months. Ideally, we would expect our developable candidate to have >90% of its original peak area remaining after 3 months at 40 °C. This can be extrapolated to a shelf-life over >1 yr in the final topical formulation at room temperature [10].

Early on in our investigations, we established that the ethyl acetal pro-drug form (*eg.*, **8**) of our final compounds was actually less stable than the hemiacetal drug form (*eg.*, **1** and **2**) in all of our topical excipients. Moreover, the pro-drug was also not cleaved sufficiently quickly enough to the drug form in our in vitro and

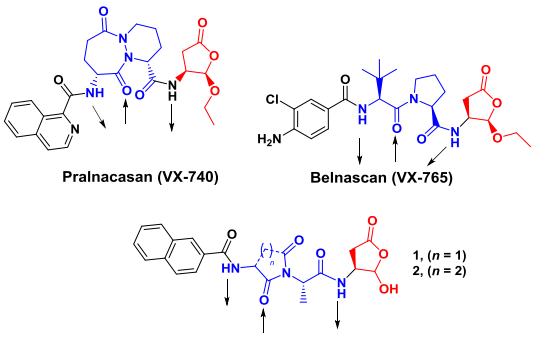
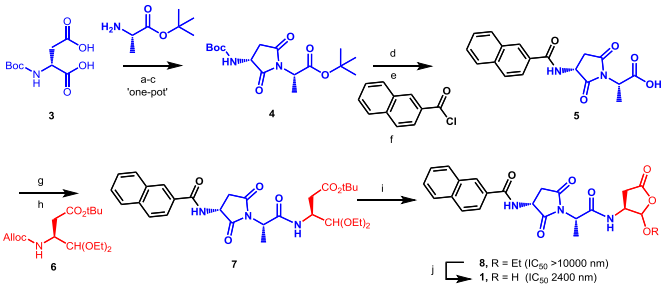
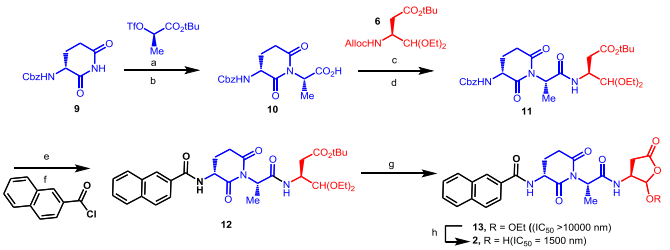


Fig. 2. Our design rationale for a new classes of imide-based peptidomimetic Caspase-1 inhibitors.



Scheme 1. A representative route designed for P1 exploration and used to deliver *eg.*, **1**. Reagents and conditions: (a) EDCI, DCM, 16 h, r.t.; (b) H-Ala-OtBu.HCl, DIPEA, 2 h, r.t.; (c) EDCI, HOBt (1.5 eq.), 16 h, r.t., 58%; (d) 4 M HCl in dioxane, EtOAc, 5 °C, 16 h, quant.; (e) 2-naphthoyl chloride, DIPEA, THF, 0 °C-r.t., 4 h, 59%; (f) TFA-DCM (1:3), r.t., 6 h, quant., (g) $Pd(PPh_3)_4$, DMBA, DCM, r.t., 15 min; (h) **5** pre-activated EDCI, HOBt, DCM, DMF, 0 °C, 15 min, amine from step (g) added, r.t., 16 h, 72%; (i) TFA, DCM, r.t., 59%; (j) 2N HCl (aq), MeCN, 0 °C-r.t., 32%.



Scheme 2. A representative route designed for P4 exploration and used to deliver *eg.*, **2**. Reagents and conditions: (a) K_2CO_3, MeCN, r.t., 53%; (b) TFA, 0 °C-r.t., quant.; (c) $Pd(PPh_3)_4$, DMBA, DCM, r.t., 15 min; (d) **10** pre-activated EDCI, HOBt, DCM, DMF, 0 °C, 15 min, amine from step (c) added, r.t., 16 h, 62%; (e) H_2, $Pd(OH)_2$-C, EtOAc, r.t., 78%; (f) TEA, DCM or 43%; (g) TFA, DCM, r.t., 75%; (h) 2N HCl (aq), MeCN, 0 °C-r.t., 52%.

in vivo assays. Therefore, we targeted the preparation of the drug form of our final compounds for evaluation in our testing cascade.

Both pyrrolidin-2,5-dione and piperin-2,6-dione scaffolds **1** and **2** provided our first lead compounds with moderate activity 2400 nM and 1500 nM, respectively, against Caspase-1 (Schemes 1 and 2). However, the stability studies were very disappointing with all of our early designs. Both drug (**1**, **2**) and pro-drug (**8**) did not achieve an acceptable level of stability (>90%) at 40 °C after just 30 d and only **2** had acceptable stability with just 9% degradation at 23 °C after 30 days (Fig. 2). It is also interesting to note that the drug form **1** was more stable at 40 °C than the pro-drug form **8** with 70% versus 58% of the original peak remaining, respectively (Fig. 3).

By HRMS, we managed to identify the principal degradation products of our lead piperidin-2,6-dione series being due to ring-opening and loss of the warhead, not through hydrolysis of the amide bond but through tautomerisation followed by alcoholysis of

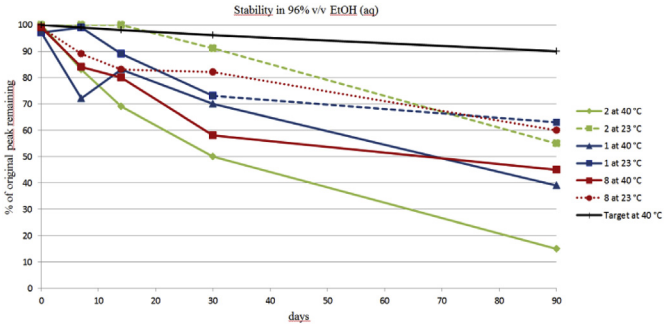


Fig. 3. Comparative stability evaluation of **1**, **2** and **8**.