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Original article

Synthesis and evaluation of vinyl sulfones as caspase-3 inhibitors. A structure–activity study

Ana S. Newton [1], Paulo M.C. Glória [1], Lídia M. Gonçalves, Daniel J.V.A. dos Santos, Rui Moreira, Rita C. Guedes, Maria M.M. Santos*

Medicinal Chemistry, iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-019 Lisboa, Portugal

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ABSTRACT

The first structure–activity relationship study of vinyl sulfones as caspase-3 inhibitors is reported. A series of 12 vinyl sulfones was synthesized and evaluated for two downstream caspases (caspases-3 and -7). Dipeptidyl derivatives were significantly superior to their counterparts containing only Asp at P_1, as caspase-3 inhibitors. Fmoc-Val-Asp-*trans*-CH=CH–SO_2Me was the most potent inhibitor of caspase-3 in the series, with a IC_{50} of 29 μM and a second-order rate constant of inactivation, k_{inact}/K_i, of 1.5 M^{-1} s^{-1}. Computational studies suggest that the second amino acid occupies position S_3 of the enzyme. In addition, Fmoc-Val-Asp-*trans*-CH=CH–SO_2Ph was inactive for caspase-7 for the tested concentrations.

1. Introduction

Caspases are a family of cysteine endoproteases that are involved in cytokine maturation and apoptosis [1–3]. Excessive neuronal apoptosis leads to a variety of diseases such as stroke, Alzheimer's disease, Huntington's disease and Parkinson's disease [4,5]. In consequence, caspases are recognized as novel therapeutic targets for central nervous diseases in which cell death occurs mainly by an apoptosis mechanism.

One type of irreversible cysteine proteases inhibitors that has received special attention in the last few years are the ones based on Michael acceptor scaffolds. This class of inhibitors includes vinyl sulfones, which have been developed as highly potent inhibitors of many clan CA cysteine proteases including papain, cathepsins B, L, S, and K, calpains, and cruzain [6]. In contrast, there are almost no reports of vinyl sulfones as inhibitors of cysteine proteases from the clan CD, to which caspases belong to (e.g. VSB-C11, Fig. 1) [7]. The lack of adequate activity–structure relationships regarding caspase inhibition by vinyl sulfones has precluded the optimization of this scaffold.

One of the most striking features of caspases is their stringency for Asp at the P_1 residue. For example, the DEVD and LETD sequences are optimal sequences for caspase-3 and caspase-8, respectively [1]. However, a truncated sequence (e.g. AD or VD) is generally enough to obtain selective and potent inhibitors of caspases by coupling a reactive functionality to these recognition moieties (e.g. activated ketones) [8,9]. With this in mind, vinyl sulfones **3a–d** and **6a–h** (Scheme 1) containing Asp at P_1 were designed to bind in the S_1 and S_2 subsites of the enzyme.

2. Results and discussion

2.1. Chemistry

Vinyl sulfones **3a–d** derivatized with N-protected aspartic acid were prepared by Horner–Wadsworth–Emmons condensation reaction. Aldehydes **1a–b** were obtained from the appropriate *N*-Cbz and *N*-Fmoc aspartic acids using Weinreb chemistry [10]. Acid deprotection with TFA afforded compounds **3a–d** with yields of 32–56% from the correspondent aldehydes (Scheme 1). To improve the potency of the inhibitors, we decided to extend the recognition structure by incorporating a second amino acid. The side chains at the R^2 position were chosen based upon literature precedent, which suggest that Ala and Val are preferred at the S_2 subsite. In order to compare the effect on caspase-3 inhibition, a third amino acid, Ile, was also used. The first approach to obtain the dipeptidyl vinyl sulfones evolved the deprotection of the nitrogen atom of the vinyl sulfones **2**, in order to couple the second

* Corresponding author. Tel.: + 351 21 794 6400; fax: + 351 21 794 6470.
E-mail address: mariasantos@ff.ul.pt (M.M.M. Santos).
[1] Ana S. Newton and Paulo M.C. Glória contributed equally to this work.

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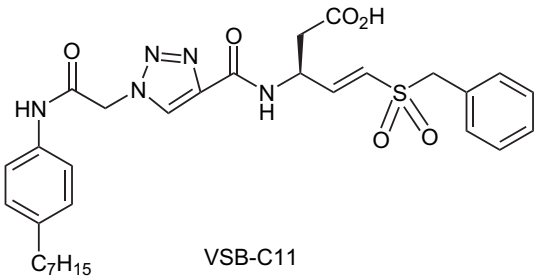


Fig. 1. Vinyl sulfone caspase inhibitor.

amino acid. However, deprotection of the Cbz group, in the presence of H_2/Pd, did not result. Instead, the double bond of the Michael acceptor was reduced.

Deprotection of the Fmoc group using several different basic conditions (piperidine, triethylamine, DBU) was also not successful leading to yields of 4% of unprotected product. We then decided to use as starting material the dipeptidyl aldehydes **4a**–**d** synthesized from the correspondent dipeptide acids [11] using Weinreb chemistry [10]. Deprotection of vinyl sulfones **5a**–**h** with TFA, afforded vinyl sulfones **6a**–**h** with yields of 34–75% from the correspondent aldehydes (Scheme 1).

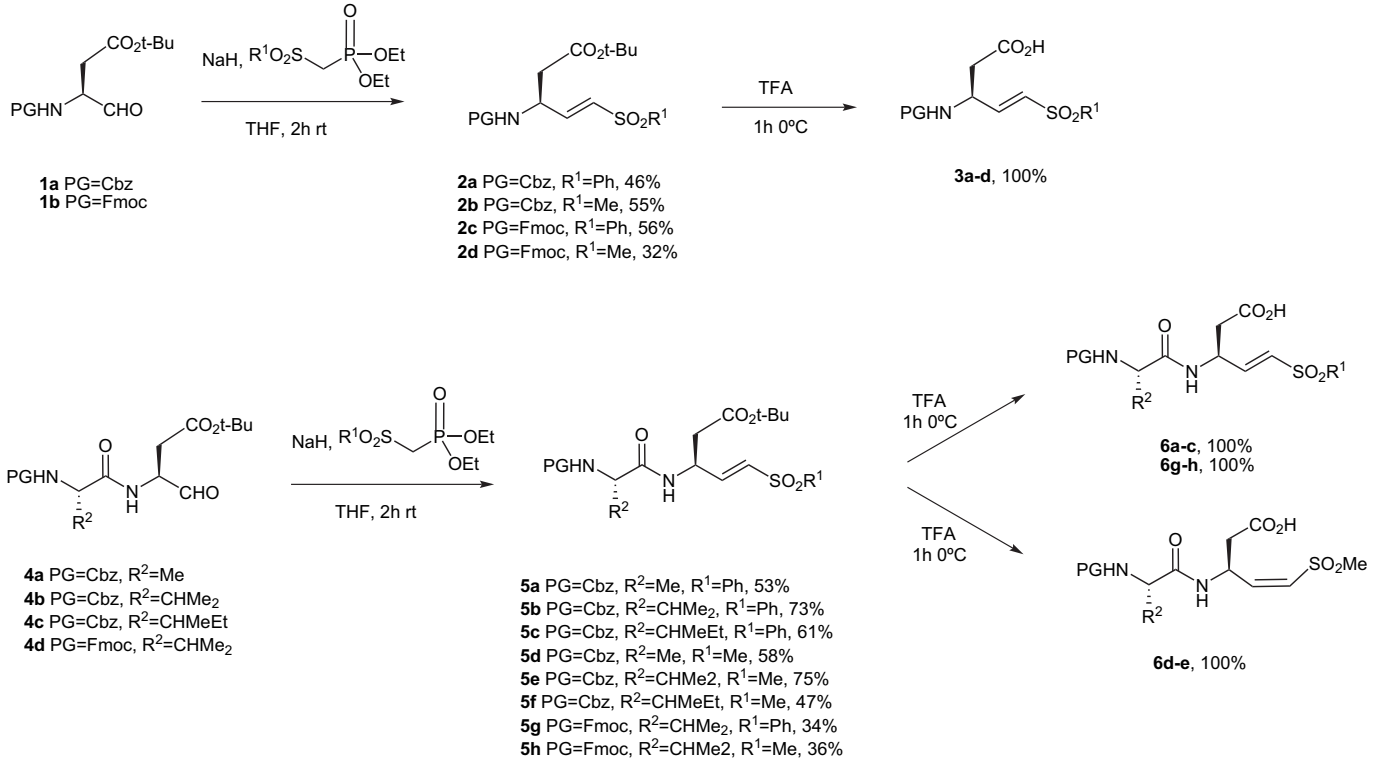
All of the proposed structures were established by NMR (^{1}H, ^{13}C, COSY and HMQC), IR, and MS. The stereochemistry around the double bond was established using the corresponding ^{1}H NMR coupling constant. A double doublet (J = 4.0, 15.0 Hz) and a doublet (J = 15.0 Hz) or double doublet (J = 1.5, 15.0 Hz) at δ 6.5–7.0 ppm are observed for the vinyl sulfones **3a**–**d**, **6a**–**c** and **6f**–**h** confirming the presence of *E* isomer. Surprisingly, deprotection with TFA of *N*-Cbz methyl vinyl sulfones **5e**–**f** led to isomerization of the double bond, as vinyl sulfones **6e**–**f** show a singlet for 2 protons at δ 6.8 ppm. Interestingly, deprotection of the Cbz-Ala and Cbz-Val methyl vinyl sulfones **5d**–**e** led only to the formation of the *Z* isomer, while Cbz-Ile methyl vinyl sulfone **5f** led to a mixture of *Z*/*E* isomers in a 1:3 ratio.

2.2. Biological activity

The vinyl sulfones synthesized were examined for their ability to inhibit the activity of recombinant human effector caspases-3 and -7. The IC_{50} values were determined for compounds **3a**–**d** and **6a**–**h** using a fluorimetric assay. The tetrapeptidyl aldehyde Ac-DEVD-CHO was used as positive control. All vinyl sulfones displayed IC_{50} values in the μM range against caspase-3 (Table 1). These values are in the same range of caspase-7 inhibition observed for the vinyl sulfone VSB-C11 [7]. In contrast, compounds **3** and **6** showed to be inactive against caspase-7, thus indicating that our vinyl sulfones are selective for caspase-3.

Inspection of the data in Table 1, allows the following observations to be made:

1. For inhibitors containing only the P_1 residue (i.e. Asp), the presence of a more bulky protective group, such as Fmoc seems to improve the inhibitory activity against caspase-3 (e.g. Fmoc vinyl sulfones **3c** and **3d** versus their Cbz-protected counterparts **3a** and **3b**). The same trend was observed for the dipeptidyl vinyl sulfones (vinyl sulfones **6g**–**h** versus compounds **6a**–**f**).
2. The presence of a P_2 amino acid residue, i.e. vinyl sulfones **6a**–**h**, improves, but not dramatically, the inhibitory activity against caspase-3, when compared to the compounds in which the P_2 residue is lacking, i.e. **3a**–**d**. For example, the inhibitory activity of the *N*-Cbz-Ile-Asp vinyl sulfones **6c** and **6f** are *ca* 2- and 4-fold higher than that of its truncated counterpart, the *N*-Cbz-Asp vinyl sulfone **3a** and **3b**, respectively, while the



Scheme 1. Synthesis of vinyl sulfones **3a**–**d** and **6a**–**h**.