Bioorganic & Medicinal Chemistry 26 (2018) 4624–4634

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Antiprotozoal and cysteine proteases inhibitory activity of dipeptidyl enoates

Santiago Royo[a], Tanja Schirmeister[b], Marcel Kaiser[c,d], Sascha Jung[b], Santiago Rodríguez[a], José Manuel Bautista[e], Florenci V. González[a,*]

[a] Departament de Química Inorgànica i Orgànica, Universitat Jaume I, Avda. Sos Baynat s/n 12080 Castelló, Spain
[b] Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55099 Mainz, Germany
[c] Swiss Tropical and Public Health Institute, Socinstrasse 57, 4051 Basel, Switzerland
[d] University of Basel, Petersplatz 1, 4003 Basel, Switzerland
[e] Department of Biochemistry and Molecular Biology and Research Institute Hospital 12 de Octubre, Faculty of Veterinary Sciences, Complutense University of Madrid, 28040 Madrid, Spain

ARTICLE INFO

Keywords:
Inhibitors
Malaria
Sleeping sickness
Chagas disease
Cysteine proteases

ABSTRACT

A family of dipeptidyl enoates has been prepared and tested against the parasitic cysteine proteases rhodesain, cruzain and falcipain-2 related to sleeping sickness, Chagas disease and malaria, respectively. They have also been tested against human cathepsins B and L1 for selectivity. Dipeptidyl enoates resulted to be irreversible inhibitors of these enzymes. Some of the members of the family are very potent inhibitors of parasitic cysteine proteases displaying k_{2nd} ($M^{-1}s^{-1}$) values of seven orders of magnitude. *In vivo* antiprotozoal testing was also performed. Inhibitors exhibited IC_{50} values in the micromolar range against *Plasmodium falciparum, Trypanosoma brucei, Trypanosoma cruzi* and even more promising lower values against *Leishmania donovanii*.

1. Introduction

Malaria, sleeping sickness and Chagas disease are among the most important tropical diseases, and the last two are considered neglected.[1,2] Human African trypanosomiasis (HAT) or sleeping sickness, caused by the protozoan *Trypanosoma brucei*, is fatal when untreated, and current treatments are ineffective and have side effects. The cysteine protease rhodesain, being essential for the development of *T. brucei*, has been identified as an interesting target for the search of new drugs against this disease. Chagas disease is caused by the protozoan *Trypanosoma cruzi.* Approximately 7–8 million people are infected by *T. cruzi* in Central and South America, with over 100 million people at risk of infection.[1] It has recently emerged in North America and Europe as well. Benznidazole and nifurtimox are drugs currently available but both have variable efficacy and side effects.[3] The cysteine protease cruzain has been identified as a target for the search of new drugs against this disease.[4] Malaria is the most widespread and severe tropical infectious disease; in humans, it is caused by several species of the *Plasmodium* genus, with *Plasmodium falciparum* being the most dangerous and most prevalent. The cysteine protease falcipain-2 has been recognized as a potential drug target.

All three above mentioned parasitic cysteine proteases rhodesain, cruzain and falcipain-2 belong to the papain superfamily. The alignment of these three proteases with their homologous cathepsin B and L shows striking similarities at three main functional regions (see Supplementary Material)[5] despite of some other structural differences allowing the potential design of inhibitors selectivity as antiparasitic compounds.[6]

Michael acceptors are among the most interesting inhibitors of cysteine proteases. For example, K1777 a dipeptidyl vinyl sulfone is a potent irreversible inhibitor.[7]

We previously reported dipeptidyl enoates as efficient inhibitors against rhodesain,[8] and we now report a structure–activity study of dipeptidyl enoates as irreversible inhibitors against the parasitic cysteine proteases falcipain-2, cruzain and rhodesain as compared to their activity against human cathepsins B and L. As it is reported herein *in vitro* and antiprotozoal activity of these inhibitors which contain an enoate moiety at the carboxyl terminus depended very much on the residues within the peptidic framework and the protecting group of the amino terminus.

* Corresponding author.
E-mail address: fgonzale@uji.es (F.V. González).

https://doi.org/10.1016/j.bmc.2018.07.015
Received 10 May 2018; Received in revised form 2 July 2018; Accepted 7 July 2018
Available online 10 July 2018
0968-0896/ © 2018 Elsevier Ltd. All rights reserved.

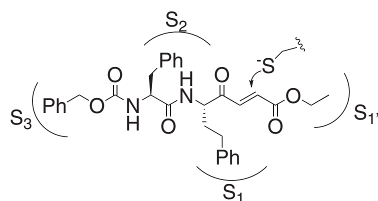


Fig. 1. Schematic representation of binding of the dipeptidyl enoate inhibitors into the active site and the binding pockets.

2. Results and discussion

2.1. Structure design of inhibitors

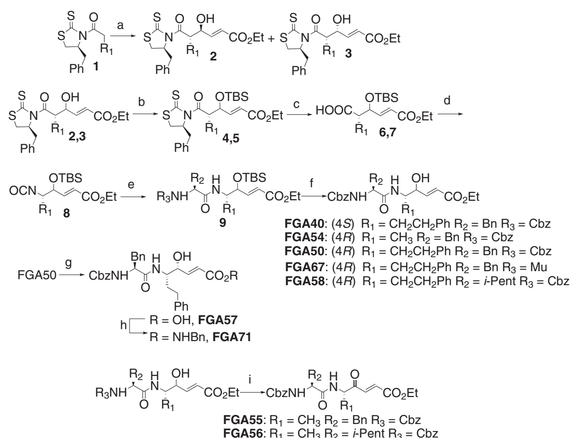
The structure of the inhibitors is a modified dipeptide having a carbon–carbon double bond conjugated with an ester at the carboxyl-terminus of the dipeptide and a protecting group at the amino-terminus (Fig. 1). The conjugated double bond at the carboxyl-terminus is the warhead to be attacked by the thiolate of the cysteine. Analogues with different substituens were prepared in order to optimize the interactions at the sites S_1, S_2, S_3 and S_1'.

2.2. Synthesis of inhibitors

The designed inhibitors were initially prepared through a synthetic route resulting from a combination of an asymmetric Evans aldol reaction, followed by protection/deprotection steps and then a Curtius reaction to afford the corresponding isocyanate which upon coupling with corresponding *N*-protected aminoacid yielded the hydroxylic enoates FGA40, FGA54, FGA50, FGA67 and FGA58 (Scheme 1). Since the aldol reaction affords a mixture of separable isomers, epimeric inhibitors differing in the configuration of carbon-4 could also be prepared. Then, the oxidation of FGA54 afforded the ketones FGA55 and FGA56 (Scheme 1).

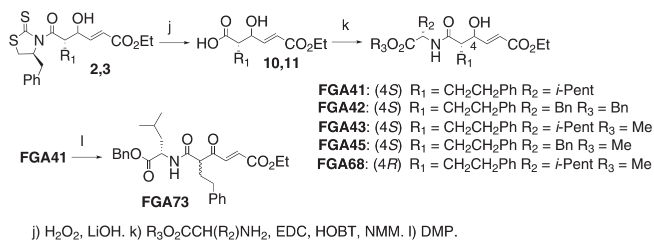
Analogues displaying a reverse sequence were also prepared by coupling the carboxylic acids resulting from chiral auxiliary removal with an aminoester (FGA41, 42, 43, 45, 68) (Scheme 2). Chemical derivatizations of these compounds afforded further members of the family. FGA41 was oxidized to give the corresponding ketone FGA73, and the ester FGA50 was hydrolized into carboxylic acid FGA57 which was subsequently derivatized into amide FGA71 (Scheme 2).

In order to prepare more dipeptidyl enoates, the synthetic strategy was changed. A straightforward approach was applied by preparation of phosphonates derived from the corresponding diprotected dipeptide.



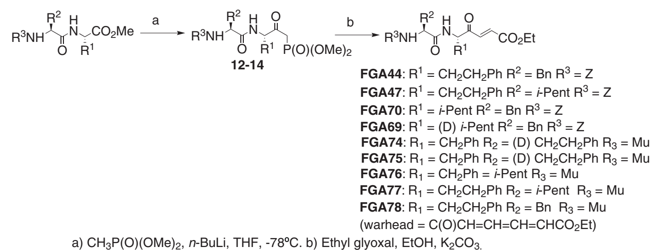
a) 1. Ethyl fumaraldehyde, $MgBr_2$-Et_2O, TMSCl, AcOEt. 2. HCl. b) TBSOTf, 2,6-lutidine. c) H_2O_2, LiOH. d) DPPA, Et_3N, Tol. e) $R_3NHCH(R_2)CO_2H$, DMAP. f) TBAF. g) KOH, EtOH. h) $BnNH_2$, EDC, HOBT, NMM. i) DMP.

Scheme 1. Preparation of inhibitors through an Evans aldol/Curtius sequence.



j) H_2O_2, LiOH. k) $R_3O_2CCH(R_2)NH_2$, EDC, HOBT, NMM. l) DMP.

Scheme 2. Preparation of inhibitors displaying a reversed sequence.



a) $CH_3P(O)(OMe)_2$, *n*-BuLi, THF, -78ºC. b) Ethyl glyoxal, EtOH, K_2CO_3.

Scheme 3. Preparation of inhibitors through phosphonates.

Then, Horner-Emmons reactions between dipeptidyl phosphonate and ethyl glyoxalate afforded the corresponding inhibitors in good yield (Scheme 3).

2.3. Inhibitory activity against cysteine proteases

In the alcohol series, *in vitro* testing against cysteine proteases denote inhibitors to display a time-dependent inhibition with IC_{50} values in the micromolar range (Table 1). The most active inhibitor of this series resulted to be FGA67 displaying a morpholinyl carbonyl protecting group instead of the benzyloxy carbonyl which is part of the other compounds. Inhibitor FGA67 gave IC_{50} values between 1 and 4 µM. Interestingly, the diastereomeric inhibitors FGA50 and FGA40 differing only in the configuration of one stereocenter showed different activities. FGA50, displaying S configuration at C-4, was more active than FGA40, with *R* configured C-4 atom.

For all assayed cysteine proteases, FGA54 with an l-alanine residue at the *P*1 site showed similar inhibitory (slightly lower) activity than its l-homophenylalanine counterpart FGA50.

Inhibitor FGA57 having a carboxylic acid was slightly less active than the ethyl ester FGA50, and the benzylamide FGA71 was not active.

FGA41, FGA42, FGA43 and FGA45 consisting of a reverse amide bond as compared to the rest of inhibitors were not active which demonstrates the importance of the correctly oriented amide bond for these dipeptidic inhibitors.

Inhibitors with a ketone group resulted to be more potent against the tested cysteine proteases than the counterparts with hydroxyl group (Table 2). The inhibitors FGA44, FGA55 and FGA75 containing a l-phenylalanine residue at P-2 site were more active than FGA47, FGA56 and FGA77 having an l-leucine residue at this position. For the *P*-1 site l-homophenylalanine (FGA44, FGA47, FGA74, FGA75 and FGA77) or l-leucine (FGA69 and FGA76) gave better results than l-alanine (FGA55 and FGA56). Inhibitors FGA69 and FGA 70 differing in the configuration of the leucine residue gave similar results with the l-leucine derivative being slightly more active. A similar result was observed when comparing inhibitors FGA74 and FGA75.

Inhibitors having a morpholine carbonyl group were less potent than the ones with a benzyloxy carbonyl group. For example, FGA44 displayed higher IC_{50} values than FGA75, and the kinetic constants ratio k_{2nd} was found to be higher against rhodesain; similar results were observed when comparing FGA70 with FGA76 or FGA47 with FGA77.

Interestingly, compound FGA78 having a dienoate warhead is a reversible inhibitor as opposite to the ones having an enoate moiety

Download English Version:

https://daneshyari.com/en/article/8954995

Download Persian Version:

https://daneshyari.com/article/8954995

Daneshyari.com