European Journal of Medicinal Chemistry 44 (2009) 1230–1239

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Natural polyprenylated benzophenones inhibiting cysteine and serine proteases

Felipe T. Martins [a,*], Diego M. Assis [a], Marcelo H. dos Santos [a], I. Camps [a], Márcia P. Veloso [a], Maria A. Juliano [b], Lira C. Alves [a], Antônio C. Doriguetto [a]

[a] Department of Exact Science, Federal University of Alfenas, Rua Gabriel Monteiro da Silva 714, Alfenas, MG 37130-000, Brazil
[b] Department of Biophysics, Federal University of São Paulo, Rua Três de Maio 100, CP 369, São Paulo, SP 04044-020, Brazil

ARTICLE INFO

Article history:
Received 3 May 2008
Received in revised form 7 August 2008
Accepted 4 September 2008
Available online 24 September 2008

Keywords:
Benzophenones
Proteases
Guttiferone A
Cathepsin G
SAR
Flexible docking

ABSTRACT

We have investigated the in vitro inhibition of papain, trypsin, and cathepsins B and G by five benzophenone-type compounds, three natural ones isolated from *Garcinia brasiliensis* and two synthetic derivatives. The activities of pentaprenylated trihydroxy-substituted benzophenone guttiferone A (**1**) on all assayed enzymes were approximately 2–69 folds higher than that manifested by mono-hydroxylated tetraprenylated and triprenylated compounds epiclusianone (**2**) and garciniaphenone (**3**), respectively, the other natural benzophenones that also inhibited significantly the four enzymes. Differently, the synthetic derivatives 2,2′,4-trihydroxybenzophenone (**4**) and diphenylmethanone (**5**) have inhibited weakly the studied proteases. Furthermore, compound **1** has bonded preferentially to cathepsin G, once its IC_{50} value (2.7 ± 0.1 μM) on such peptidase is quite similar to that of the classical inhibitor of serine proteases, chymostatin (2.1 ± 0.1 μM). Interesting structure–activity relationships (SARs) were confirmed by flexible docking simulations, likewise the potential usefulness of natural compound **1** as antitumoral drug is strengthened by our results concerning the antiproteolytic activity.

1. Introduction

Proteases or peptidases are enzymes that catalyze reactions on peptide chains, hydrolyzing them into short fragments by splitting the peptide bond between amino acid residues placed either within the ribbon, in this case they are known as endopeptidases, or at the polymeric backbone end (exopeptidases). These enzymes can be further joined according to the reactant groups that are present in the catalytic site, as the serine (EC 3.4.21), cysteine (EC 3.4.22), aspartic acid (EC 3.4.23), metalo (EC 3.4.24) and threonine (EC 3.4.25) proteases. Papain (EC 3.4.22.2), a plant cysteine protease isolated from *Carica papaya* latex, cleaves preferentially peptide chains on either Arg and Lys residues or hydrophobic Phe ones [1]. The cathepsins, a group of lysosomal enzymes, are proteases presenting several members. Two of them, cathepsin B (CatB, EC 3.4.22.1) and cathepsin G (CatG, EC 3.4.21.20), have been identified in human tissues, where they can be isolated [2]. CatB, a papain-like protease, is barely detected in the extracellular matrix, at least in nonpathological tissues, due to its low stability in neutral–alkaline pH values [3,4]. Concerning the neoplastic events, CatB is secreted out of the cells and it presents essentially an endopeptidase-functioned proteolytic activity crucial to tissue rupture around the tumor spreading area and metastasis [5–7]. Moreover, high expression, activity and secretion of CatB have been observed in several types of tumors, such as breast, gastric, lung and prostate ones [8]. The CatG is the major serine protease in the azurophilic granules of polymorphonuclear leukocytes [9,10] and is involved in the degradation of foreign bodies and injured tissues enclosed by phagosomal vesicles during inflammatory responses [11]. In opposition to CatB, CatG is commonly released towards the extracellular matrix. Its proteolytic activity is finely controlled by serum proteinase inhibitors, which can fail quantitatively or qualitatively in down-regulation due to genetic or acquired deficiencies resulting in a disturbed digestion of extracellular proteins that could contribute to the development of connective tissue diseases, such as emphysema, rheumatoid arthritis and perionditis [12]. Furthermore, recent evidences have suggested that this lysosomal protease presents alternative physiologic roles, as for instance platelet activation [13], monocyte and neutrophil chemotaxis [14], natural killer cytotoxicity increasing [15], proteolytic processing of interleukin-8 [16], complement C3 [17] and factor V [18], as well as other pathological ones, such as dissemination of tumor aggregates from

Abbreviations: CC, column chromatography; TLC, thin layer chromatography; MAP, mitogen-activated protein; XRD, X-ray diffraction; HPLC, High Performance Liquid Chromatography; Z-FR-AMC, carbobenzyloxy-Phe-Arg-(7-amino-4-methylcoumarin); EDTA, ethylenediaminetetraacetic acid; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; LP, leupeptin; E-64, 1-[[(L-*trans*-epoxysuccinyl)-L-leucyl]amino]-4-guanidinobutane; SBTI, soybean trypsin inhibitor; Abz-, *ortho*-aminobenzoic acid; -EDDnp, *N*-(ethylenediamine)-2,4-dinitrophenyl amide; IC_{50}, inhibitor concentration to decrease 50% enzymatic activity; VDW, van der Waals.

* Corresponding author. Tel. +55 35 33991261. fax: +55 35 32991262
E-mail address: felipetmartins@yahoo.com.br (F.T. Martins).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.09.018

primary tumor sites after to be secreted out by infiltrated neutrophils [19].

Trypsin (E.C. 3.4.21.4) has specificity for peptides containing Arg and Lys residues and its catalytic triad is composed of the amino acids serine, histidine and aspartate, in the same way that occurs in similar serine proteases [20]. Each amino acid of the triad has a specific role in the peptide bond cleavage of the substrates, wherein the carboxylate group of the aspartic acid is hydrogen bonded to the amine nitrogen atom of the histidine residue, contributing to increase the electronegativity of the imine nitrogen within the same heterocyclic side chain. In this way, the free electron pair of this last imidazolyl nitrogen atom is devoted to accept the hydrogen from the serine hydroxyl group, powering, therefore, the nucleophilic attack by this serine residue on the carbonyl carbon atom of the peptide bond that is properly oriented into the enzymatic active site. Cysteine proteases have a similar catalytic mechanism with a nucleophilic cysteine thiol group. In Fig. 1, a simplified nucleophilic attack scheme of serine and cysteine proteases is illustrated. After the peptide carbonyl group to be nucleophilically attacked, in a following step, the amide bond is cleaved due to an electronic rearrangement and the acyl-protease intermediate is generated. To release the free enzyme, a water molecule, in place of the N-terminal end of the cleaved peptide, attacks the carbonyl carbon keeping bonded the substrate–enzyme complex, resulting in the formation of a binding between the water oxygen atom and the peptide carbon one. Here, it should be highlighted that the imine nitrogen of the histidine residue plays an important role by accepting a proton from water. At last, the bond formed in the initial step between the serine and the carbonyl carbon is broken and the C-terminus of the substrate leaves then. We recommend consulting the literature for an overall approach on such hydrolase groups [20].

Our research group has dealt with synthetic and natural benzophenone derivatives regarding structural elucidation and screening for pharmacological properties on various biological systems. From the *Garcinia brasiliensis* fruits and seeds, three benzophenones possessing a polyprenylated bicyclo[3.3.1]-nonane-2,4,9-trione core were isolated through repeated column chromatography (CC), repeated preparative thin layer chromatography (TLC) and recrystallizations in methanol solutions: guttiferone A (**1**), an anti-HIV compound that was initially isolated in 1992 [21]; epiclusianone (**2**), a vasoactive substance identified in 1998 [22] that also presents effects against HIV infection [23,24]; and garciniaphenone (**3**), a first example of natural benzophenone presenting just three prenyl moieties that has been discovered for the first time in this plant by us. A trihydroxylated benzophenone named systematically (2,4-dihydroxyphenyl)(2-hydroxyphenyl)methanone, or simply 2,2′,4-trihydroxybenzophenone (**4**), was previously synthesized in our laboratories by aromatic electrophilic substitution [25]. All these benzophenone derivatives were spectroscopically featured and their structures have been accurately determined via single crystal X-ray diffraction techniques [22,26,27], likewise the anti-inflammatory and antioxidant properties were recently described for such natural products [25]. Furthermore, the polyisoprenylated benzophenone derivatives of the bicycle[3.3.1]-nonanetrione moiety are implicated in the inhibition of DNA topoisomerases and telomerase and also act as regulators within mitogen-activated protein (MAP) kinase signal transduction pathways, decreasing mitosis rate in cancerous and tumoral tissues, once MAP kinases are active enzymes during mitosis process [28]. Due to their merged effects on different pharmacological targets for antineoplastic therapy, the natural benzophenones in query can be useful as antitumoral compounds (Scheme 1).

As above mentioned, proteases are potentially involved in many diseases, including tumor growth and spreading and HIV maturation due to interaction with HIV-1 gp120 protein V3 loop [29]. Therefore, these enzymes are molecularly searched for more effective drugs that could be employed in the therapeutic. However, a notable challenge involved in the design and development of protease inhibitors has consisted to add properties such as high enzymatic inhibition rates and suitable bioavailability in a single molecule [30]. Several potent peptide inhibitors are available, whereas these compounds fail to satisfy the pharmacokinetic requirements in view of high hydrophilicity and hydrolysable moieties present in their structures. Thereby, small non-peptide molecules are currently preferred to survey novel antiproteolytic drugs. In this work, we have reported the in vitro inhibition of model enzymes of cysteine and serine proteases, papain and trypsin, respectively, CatB and CatG by four benzophenone derivatives presenting lipophilic character appropriate to cross biological membranes, which would make possible the use of most active assayed benzophenones as pharmaceutical ingredients. In addition, the results obtained with flexible docking simulations have shown interesting structure–activity relationships and can be useful in the rationalizing of the anti-HIV effect presented by these compounds.

2. Chemistry

The natural polyprenylated benzophenones **1** and **3** were isolated from the ethanolic and hexanic extracts of *G. brasiliensis* dried and powdered seeds (700 g) and fruits (1000 g), respectively, whereas compound **2** was obtained from both prior extracts. Chromatographic efforts on thin layer plates and filled columns have been employed in order to achieve the isolation of these compounds. For purification, several recrystallizations in methanol solution were performed to give 0.51, 2.80 and 0.65 g of **1**–**3**, respectively, which were characterized by spectroscopic methods (UV, IR, MS, 1D and 2D spectra of ^{1}H and ^{13}C NMR) and their intra- and inter-molecular geometries in the solid state were entirely determined and

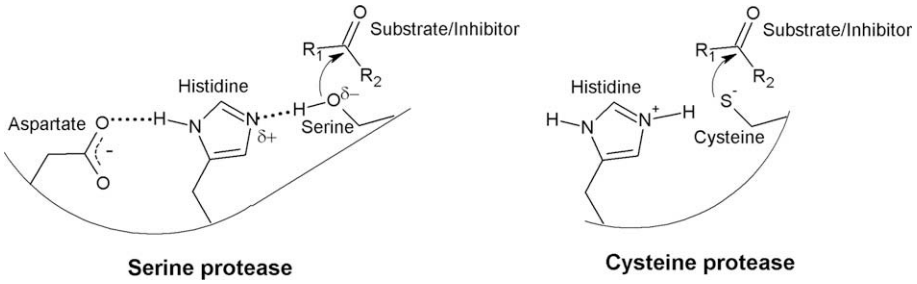

Fig. 1. A general view of the nucleophilic attack mode whereby serine and cysteine proteases either catalyze the hydrolysis of peptide backbones or bond irreversibly to some inhibitors. The side chains are shown for the amino acid residues which are well conserved into the enzymatic active site of these two protease classes. The arrows indicate the nucleophilic attack by the oxygen and sulfur atoms from serine and cysteine residues, respectively, on the electronically deficient carbonyl carbon of the peptide substrates and of various covalently bonded inhibitors.