European Journal of Medicinal Chemistry xxx (2013) 1–8

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and biological evaluation of phosphorylated flavonoids as potent and selective inhibitors of cholesterol esterase

Yingling Wei [a], Ai-Yun Peng [a,*], Bo Wang [a], Lin Ma [a], Guoping Peng [b], Yidan Du [a], Jingming Tang [a]

[a] School of Chemistry & Chemical Engineering, Sun Yat-sen University, 135 Xingangxi Lu, Guangzhou 510275, China
[b] College of Bioscience and Biotechnology, Hunan Agricultural University, Furong District, Changsha 410428, China

ARTICLE INFO

Article history:
Received 17 September 2012
Received in revised form
9 March 2013
Accepted 13 March 2013
Available online xxx

Keywords:
Cholesterol esterase
Acetylcholinesterase
Inhibitor
Phosphorylated flavonoids
Organophosphate
Selectivity

ABSTRACT

A series of phosphorylated flavonoids were synthesized and investigated in vitro as inhibitors of pancreatic cholesterol esterase (CEase) and acetylcholinesterase (AChE). The results showed that most of the synthesized compounds exhibited nanomolar potency against CEase, much better than the parent flavonoids. Furthermore, these phosphorylated flavonoids demonstrated good to high selectivity for CEase over AChE, which only showed micromolar potency inhibition of AChE. The most selective and potent inhibitor of CEase (**3e**) had IC_{50} value of 0.72 nM and 11800-fold selectivity for CEase over AChE. The structure–activity relationships revealed that the free hydroxyl group at position 5 and phosphate group at position 7 of the phosphorylated flavonoids are favorable to the inhibition of CEase. The inhibition mechanism and kinetic characterization studies indicated that they are irreversible competitive inhibitors of CEase.

1. Introduction

Pancreatic cholesterol esterase (CEase; EC 3.1.1.13) is an important serine hydrolase that plays significant roles in the absorption of dietary cholesterol. Inhibition of CEase has attracted much attention in the last decades as a potential approach to treat hypocholesterolemia and atherosclerosis by limiting the bioavailability of dietary cholesterol [1]. Several classes of potent CEase inhibitors have been developed [2], including 6-chloro-2-pyrones [3], thieno [1,3]-oxazin-4-ones [1,4], carbamates [5], aryl phosphates and phosphonates [6], chloroisocoumarins [7], phosphaisocoumarins [8], and thiazolidinediones [9] (Fig. 1). However, most of these inhibitors are not highly selective and they could also inhibit other serine hydrolases, such as acetylcholinesterase (AChE; EC 3.1.1.7), butyrylcholinesterase (BChE, EC 3.1.1.8), *Pseudomonas* species lipase (PSL, EC 3.1.1.3), chymotrpsin (CT, EC 3.4.21.1) and trypsin (EC 3.4.21.4) [1,3,5d,5f,5h]. One main reason for the poor selectivity is that all serine hydrolases as well as serine proteases share the similar catalytic triad of Ser-His-Asp (Glu) and mechanism of acylation–deacylation [10]. Therefore, these enzymes usually can be inhibited by the same class of compounds and it is challenging to improve the selectivity when developing inhibitors of them.

Nevertheless, some selective inhibitors of certain serine hydrolase have been designed and developed. For example, acetylcholinesterase (AChE) is one of the most studied serine hydrolases as a viable target to treat Alzheimer's disease (AD) and many high selective inhibitors of AChE with nanomolar to picomolar potency have been identified, three of which have been used clinically to treat AD [11]. In contrast, only a few selective inhibitors of CEase were reported. In 1999, Deck's group [3a] found that 6-chloro-3-(1-ethyl-2-cyclohexyl)-2-pyranone could effectively inhibit CEase with a *Ki* value of 25 nM and its selectivity for CEase over chymotrypsin was greater than 1000-fold. Recently, Gütschow and Pietsch developed two CEase inhibitors with micromolar potency and medium selectivity for CEase over AChE; one is a 1,3-oxazin-4-one [1], and the other is a thiazolidinedione [9]. Apparently, it is still desirable to develop novel inhibitors of CEase with high potency and high selectivity.

Flavonoids, including flavones, flavonols, isoflavones and flavanones, are a large class of polyphenolic compounds widely distributed in herbs and foods of plant origin, and they exhibit diversified biological activities, such as antioxidant, antiviral, anticancer and enzyme inhibition [12]. Recently, we prepared five fully phosphorylated flavones and found that they displayed excellent

* Corresponding author. Tel.: +86 020 84110918; fax: +86 020 84112245.
E-mail address: cespay@mail.sysu.edu.cn (A.-Y. Peng).

0223-5234/$ – see front matter Crown Copyright © 2013 Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.03.025

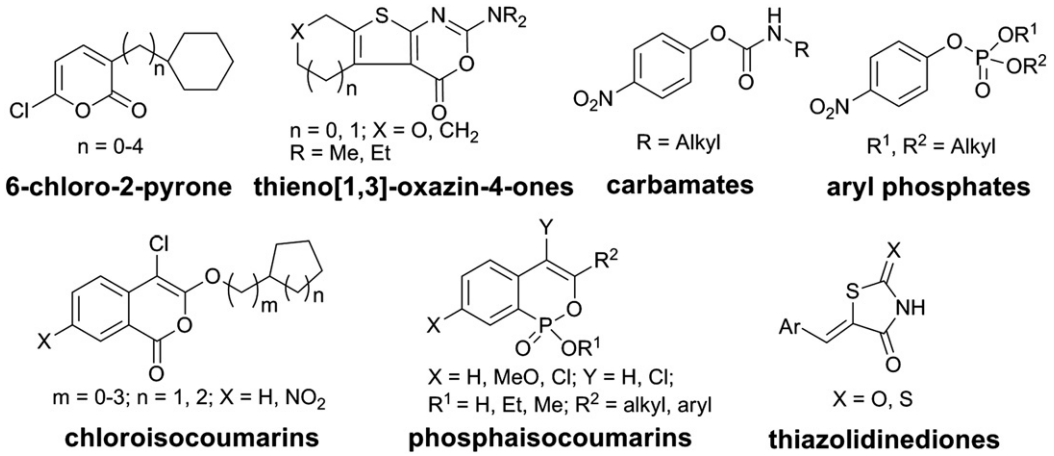


Fig. 1. Structures of some reported inhibitors of CEase.

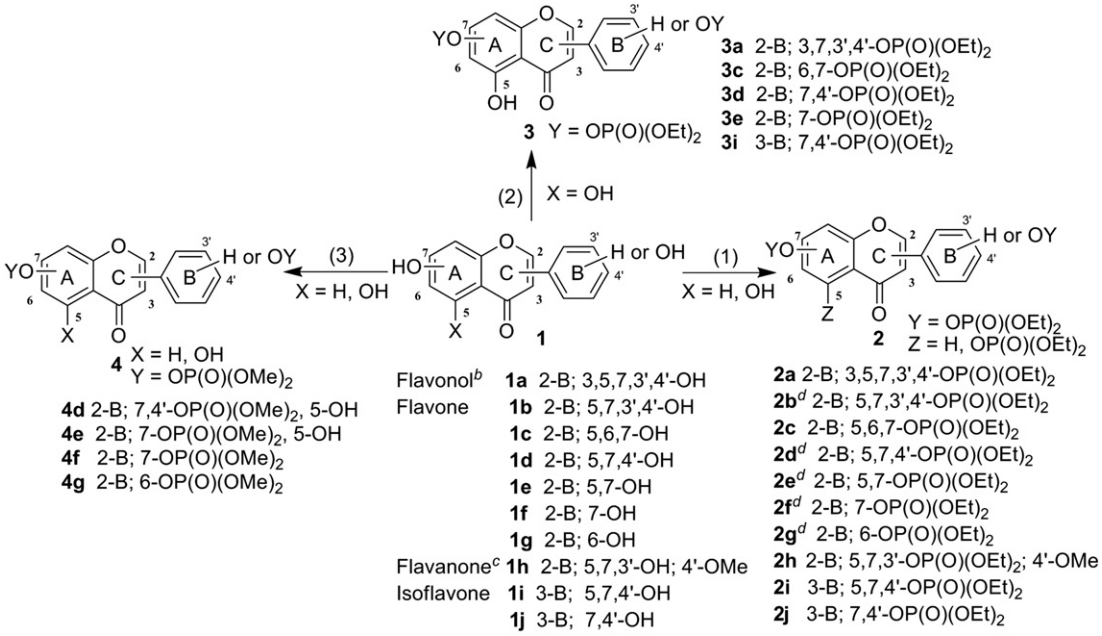
CEase inhibitory activities with IC_{50} values in the nanomolar range [13]. This work indicated that we may develop inhibitors of CEase with better activities by investigating more kinds of phosphorylated flavonoids. Furthermore, aryl phosphates, especially those with good leaving groups, such as diethyl 4-nitrophenyl phosphate (paraoxon), are well-known irreversible inhibitors of many serine hydrolases and proteases and widely used as insecticides [6a,14]. Taking into account that many phosphate insecticides are toxic and the neurotoxicity of them are mainly associated with their AChE inhibition [14], we think it necessary to examine whether these flavonoid phosphate derivatives can selectively inhibit CEase over AChE so as to preliminarily know about their application prospect.

In this paper, we presented a series of fully and partially phosphorylated flavonoids as inhibitors of CEase with low nanomolar potency and high selectivity for porcine CEase over AChE from Electrophorus electricus. The highest selectivity reached to over 10000-fold. Besides, we examined the inhibition mechanism and kinetics, the results indicated that they are irreversible competitive inhibitors of CEase.

2. Results and discussion

2.1. Chemistry

The synthesis of the phosphorylated flavonoids **2**–**4** was outlined in Scheme 1. According to our previous procedure [13], the fully phosphorylated flavonoids **2a**–**2j** could be obtained by the treatment of **1a**–**1j** with $ClP(O)(OEt)_2$, Et_3N and 4-dimethylamiopryidine (DMAP) in THF at room temperature for 24 h. To accelerate the reaction process, we carried out these reactions under reflux conditions, and found that all the reactions completed within 3 h (condition (1), Scheme 1). During these



[a] Reagents and conditions: (1) DMAP, Et_3N, $ClP(O)(OEt)_2$, reflux, 3 h; (2) $HP(O)(OEt)_2$, Et_3N, CCl_4, room temperature, 24 h. (3) $HP(O)(OMe)_2$, Et_3N, CCl_4, room temperature, 24 h. [b] 2-B means the B ring is linked to position 2, and 3-B means the B ring is linked to position 3. [c] For flavanones **1h** and **2h**, the bond between position 2 and 3 is single bond instead of double bond. [d] These compounds have been reported in Ref. 13.

Scheme 1. General synthetic procedure for the preparation of compounds **2**, **3** and **4**.[a].

Please cite this article in press as: Y. Wei, et al., Synthesis and biological evaluation of phosphorylated flavonoids as potent and selective inhibitors of cholesterol esterase, European Journal of Medicinal Chemistry (2013), http://dx.doi.org/10.1016/j.ejmech.2013.03.025