Bioorganic Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis, characterization and pharmacological evaluation of certain enzymatically cleavable NSAIDs amide prodrugs

Tilal Elsaman [a,*], Omar A.A. Aldeeb [b], Tarek Aboul-Fadl [c], Elnazeer I. Hamedelneil [d]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, Omdurman Islamic University, P.O. Box 2587, Khartoum, Sudan
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[c] Department of Medicinal Chemistry, College of Pharmacy, Assiut University, Assiut 71526, Egypt
[d] Department of Pharmaceutics, College of Pharmacy, Omdurman Islamic University, P.O. Box 2587, Khartoum, Sudan

ARTICLE INFO

Article history:
Received 4 July 2016
Accepted 20 December 2016
Available online xxxx

Keywords:
NSAIDs
Prodrugs
Amino acid ester
Ulcerogenicity

ABSTRACT

The presence of free carboxylic acid group in majority of non-steroidal anti-inflammatory drug (NSAIDs) is responsible from GI irritation. Coupling of the appropriate NSAIDs (diclofenac, naproxen, dexibuprofen and meclofenamic acid) **1**–**4**, respectively with the appropriate amino acid ester **5** using dicyclohexylcarbodiimide afforded prodrugs **6**–**13**. The structures of the prodrugs were verified based on spectral data. Chemical hydrolysis studies performed in three different non enzymatic buffer solutions at pH 1.2, 5.5 and 7.4, as well as in 80% human plasma and 10% rat liver homogenate using HPLC indicate no conversion of prodrugs to their respective NSAID in the studied buffers, while they underwent a reasonable plasma and rat liver homogenate hydrolysis. Furthermore, ulcerogenicity of prodrugs **9** and **12** were studied and results revealed no gastro-ulcerogenic effects.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs for treatment of pain and various inflammatory conditions [1,2]. NSAIDs exert their anti-inflammatory effect through inhibition of cyclooxygenase an enzyme that catalyzes the transformation of arachidonic acid to prostaglandins (PGs) and thromboxanes [3]. Enormous literature has been published regarding their gastrointestinal (GI) side effects, which ranging from stomach irritation to ulceration and bleeding [4]. The properties of NSAIDs that contribute to ulcerogenesis can be divided into two categories topical irritancy (local mechanism), which is associated with those NSAIDs with a carboxylic acid residue (Diclofenac, Ibuprofen, Naproxen Fenamic acids) and the suppression of PG synthase activity (systemic mechanism) [5,6]. The design and synthesis of prodrugs for NSAIDs have been given much attention, especially in the last decade after documentation of association between the use of COX-2 selective inhibitors and increased incidence of stroke and myocardial infarction [7]. One approach that has been used to decrease NSAID induced GI toxicity without adversely affecting their anti-inflammatory activity is to mask the carboxylic acid group [8]. A major requisite for these prodrugs is that, they must be readily hydrolyzed, enzymatically or chemically after oral absorption to quantitatively release the parent drug [9]. In addition, the pro-moiety should be non-toxic and readily excreted. The salient features of the usefulness of conjugation of amino acids with drugs are reported [9–11].

In light of the above statements, the fewer attempts that have been made to develop amide prodrugs of NSAIDs utilizing amino acids esters and in continuation of our research to develop safe NSAIDs using amino acids esters as promoiety [1,12–14], a series of diclofenac **1**, S-naproxen **2**, dexibuprofen **3** and meclofenamic acid **4** amides of certain amino acids esters were synthesized, characterized and assessed in terms of pharmacology, chemical and enzymatic stability.

2. Results and discussion

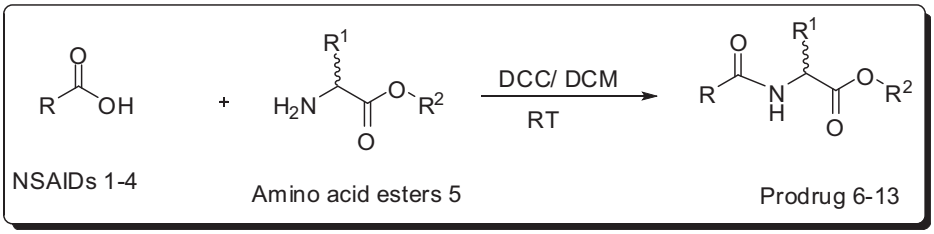
2.1. Chemistry

The target derivatives were obtained by coupling of the appropriate NSAID **1**–**4** with the appropriate amino acid ester **5** using dicyclohexylcarbdiimide (DCC) in dicholoromethane (DCM) at room temperature (Scheme 1, Table 1).

The IR spectra of the prodrugs **6**–**13** exhibited, in each case, a band in the region $\bar{\upsilon}$1754–1627 cm^{-1} due to the carbonyl absorptions of amide and ester groups, whereas the absorption band of

* Corresponding author.
E-mail address: Tilochem@yahoo.com (T. Elsaman).

http://dx.doi.org/10.1016/j.bioorg.2016.12.005
0045-2068/



Scheme 1. Synthesis of the target amide derivatives **6**–**13**.

Table 1
Structures, M.P., yield and Clog P of compounds **6**–**13**.

Prodrug	NSAID	Amino acid ester (5)	M.P. (°C)	Yield%	Clog P
6	1	d-valine methyl ester	90–92	58	5.47
7	1	l-leucine ethyl ester	72–74	65	6.03
8	2	Octyl glycinate	57–59	42	6.02
9	3	l-phenyl alanine ethyl ester	44–46	64	5.44
10	3	(S)-benzyl-l-cysteine ethyl ester	52–54	65	6.28
11	3	l-leucine ethyl ester	58–61	73	5.48
12	4	l-valine ethyl ester	76–78	45	6.92
13	4	l-leucine methyl ester	64–66	67	6.92

NH amide function appeared in the region $\bar{\upsilon}$ 3421–3299 cm^{-1}, but it was obscured by strong and sharp NH secondary amine absorption at $\bar{\upsilon}$ 3421–3292 cm^{-1} in case of **6**, **7**, **12** and **13**. The ^{1}H NMR spectra of compounds **6**–**13** revealed the presence of D_2O exchangeable NH signals in the regions δ 6.1–6.6 due to amidic NH. Moreover, **6**, **7**, **12** and **13** displayed additional signal at δ 9.10–9.20 ppm due to secondary amine of Ar—NH—Ar in addition to the characteristic signals of aromatic moieties in each case around δ 7.07–7.45 ppm. Furthermore, the amino acid protons were distinguished in each molecule. Prodrugs **6** and **7** showed signal referring to the benzylic methylene group at δ 3.58 ppm, which appeared as singlet. Prodrug **8** displayed the characteristic signals of naproxen moiety at δ1.40–1.64 ppm (d, —CH3), δ 3.76–3.80 ppm (q, —CH), δ 3.86–3.94 ppm (s, —OCH_3), 4.51–4.53 (CH—NH) and the naphthyl moiety protons at δ 7.14. Prodrugs **9**–**11** displayed the characteristic ibuprofen protons signals, which were shown at δ 0.82–0.91 (d, $(CH_3)_2$—CH—), δ 1.22–1.63 (d, —$CHCH_3$), 1.52–1.86 (m, $CH_3)_2$—CH—), 2.37–2.46 (s, Ar—CH_2—), 3.44–3.6 (q, $ArCHCH_3$) and δ 7.15–7.23 ppm (ArH). Prodrugs **12** and **13** ^{1}H NMR spectra showed a singlet at δ 2.42 integrating for three protons was assigned to Ar—CH_3 of meclofenamic acid.

^{13}C NMR spectra of **6**–**13** exhibited the characteristic ^{13}C aromatic carbons signals in each case at δ 114.71–145.69 ppm, in addition to the distinguished signals of each amino acids esters carbons. diclofenac prodrugs 7 and 8 displayed signal at δ 38.92 ppm assigning for benzylic ($ArCH_2$—). Moreover, prodrugs **12** and**13** displayed a signal at displayed signal at δ 20.64 ppm assigned for the one methyl carbon attached to the aromatic ring. These prodrugs displayed two signals at δ 168–169 ppm and δ 170–171 ppm assigning for the amidic and ester carbonyl, respectively.

The synthesized prodrugs provide, in each case molecular ion peaks corresponding to their molecular weights in their mass spectra.

2.2. Biological investigations

2.2.1. Estimation of the lipophilicity of the synthesized prodrugs

Lipophilicity is a physicochemical property of principal importance in drug discovery and development. It affects three phases of drug activity its pharmaceutical, pharmacokinetic and pharmacodynamic action [15,16]. Lipophilicity of the synthesized compounds expressed in the term of their Clog P values is shown in Table 1. Computation of the log P was based on the fragment method developed by Leo contained in a PC software package since many studies of the most commonly used methods confirm the superiority of the fragmental methods over the atom-based approaches [17]. The lipophilicity of the synthesized NSAIDs prodrugs is significantly enhanced, when compared to the parent drugs, this may enhance the bioavailability of this prodrugs (see Fig. 1).

2.2.2. Chemical stability of the synthesized prodrugs

It has been reported that the essential pre-requisite for success in the use of NSAIDs prodrugs is that the masked compounds should be acid-stable to prevent the direct contact effects with the gastric mucosa as well as the local inhibition of the prostaglandins [18]. Therefore, hydrolytic stabilities were studied in buffer solutions of pH of microclimate of stomach l.2, 5.5 (non enzymatic SGF), and isotonic phosphate buffer of pH 7.4 (physiological pH) at 37 °C. They were selected to mimic the appropriate clinical range. Furthermore, the chemical stability studies were carried out in aqueous buffer in order to indicate whether the prodrugs hydrolyze in aqueous medium and to what extent or not. Moreover, *in vitro* hydrolysis of compounds in simulated fluids will help to predict the amount of drug that would be available at the site of action [19]. Accordingly, chemical stability was studied for the

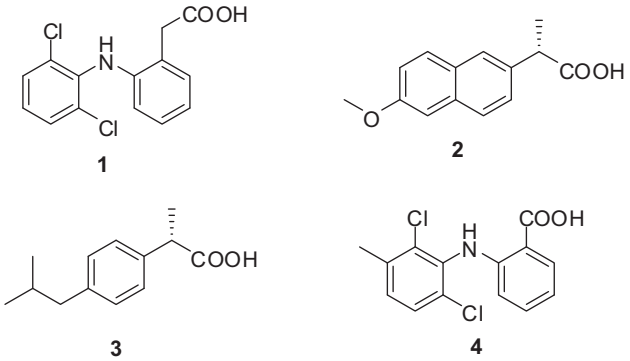


Fig. 1. Structure of NSAIDs 1–4.