Tetrahedron Letters 54 (2013) 6682–6686

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis of novel dansyl-labeled Celecoxib derivatives

Andreas Lill [a], Klaus Scholich [b], Holger Stark [a,c,*]

[a] Johann Wolfgang Goethe University, Institute of Pharmaceutical Chemistry, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
[b] Johann Wolfgang Goethe University, Institute of Clinical Pharmacology, Theodor-Stern-Kai 7, 60596 Frankfurt am Main, Germany
[c] Heinrich-Heine-Universität Düsseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Universitätsstraße 1, 40225 Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 12 July 2013
Revised 5 September 2013
Accepted 10 September 2013
Available online 19 September 2013

Keywords:
Fluorescence labeling
Celecoxib
Dansyl
COX
NSAR
Linker

ABSTRACT

Four novel dansyl-labeled derivatives of Celecoxib, a cyclooxygenase-2 (COX-2) selective inhibitor, were designed and synthesized. To realize the fluorophore-linker-approach divergent and convergent synthetic strategies were applied. Therefore Celecoxib p-benzoic acid, **8**, was synthesized in a new and convenient way. The yield and the synthetic route to Celecoxib, **1**, its pyrazolylic acid, **7**, and its pyrazolylic methyl ester, **6**, were improved. Through a convenient synthesis 1,11-diamino-3,6,9-trioxundecane, **19**, was obtained in high yield and purity and used as a linker for the dansyl moiety.

Introduction

Visualization of molecules can help to understand the interactions and molecular pathways of pharmacologically active substances. Labeled substances are tools to identify unknown binding partners and to clarify the details of the mechanism of action. They can be used as a diagnostic or therapeutic marker to design assay systems for distinct binding partners as well, for example an enzyme or receptor associated with a disease. Aside from radiolabels or imaging agents for complex optical photon techniques, the introduction of a fluorescence moiety is the easiest method to visualize a molecule.[1,2]

It is well known that cyclooxygenase 2 (COX-2), the inducible form of two isoenzymes that catalyze the conversion of arachidonic acid to prostaglandin PGH_2, is overexpressed in inflamed and tumerous tissue, distinct from most normal tissue. Through the produced prostaglandins, COX-2 is a major contributor in cancer progression and might also be important in some neurological diseases.[3–6]

Celecoxib, **1**, a nonsteroidal anti-inflammatory drug (NSAID), is a selective COX-2 inhibitor. COX-2-dependent and independent anti-carcinogenic effects are observed independent of its concentration, but they are far from being understood.[7–9] Visualization of COX-2 and/or Celecoxib is one way to get better insights in the molecular actions involving Celecoxib.[6,10]

The visualization of COX-2 by molecular imagining has been shown by Marnett et al.[11] The unselective COX inhibitor indomethacin has been derivatized to a diagnostic and therapeutic fluorescent marker for COX-2.[12–14] Other groups have studied radiolabeled Celecoxib derivatives that are suitable for molecular imaging—for example introduction of ^{123}I and fluorination.[11,15,16] Recently Marnett et al. described Celecoxib derivatives that are linked to a fluorophore.[12,13] Here we report the synthesis of novel fluorescent labeled Celecoxib derivatives with a different design approach. The final compounds were tested for their COX-2 inhibitory activity and absorptions and emission spectra in different solvents were recorded.

Results

Modeling

For the design of fluorescence-labeled Celecoxib derivatives a linker approach was chosen to maintain the pharmacophore and introduce the labeling element (Fig. 1).

Known structure–activity-relationship (SAR) studies,[17] X-ray structure of COX-2 with bond ligands[18] and molecular docking-experiments on Celecoxib and its derivatives[19,20] revealed that the sulfonamide moiety is essential for biological activities. Whereas the trifluoromethyl group at the heterocycle points toward the entry tunnel of the active site and should not alter the affinity. The SAR showed that a pyrazole-3-carboxamide is still active on COX-2.[17] Derivatization of the tolyl group could serve as an

* Corresponding author. Tel.: +49 211 81 10478; fax: +49 211 81 13359.
E-mail address: stark@hhu.de (H. Stark).

0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.09.025

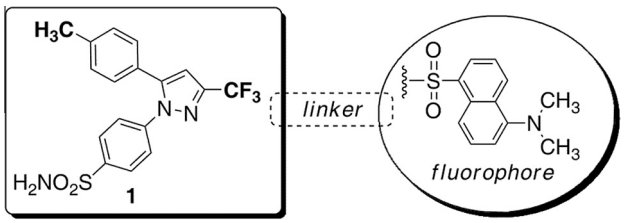


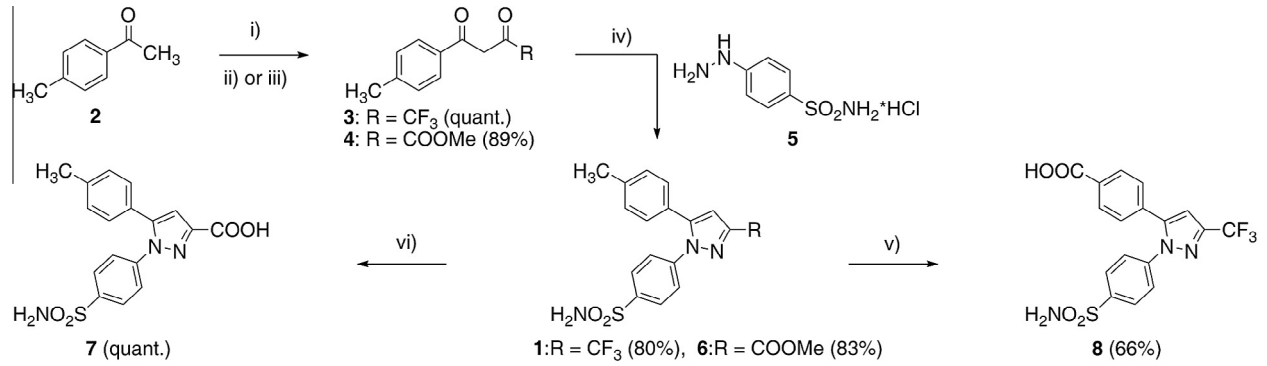
Figure 1. Linker approach for fluorescence-labeled derivatives with marked sites for derivatization (bold), Celecoxib, **1** (4-(5-*p*-tolyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide).

additional approach because it is unpredictable in which way fluorescence-labeled Celecoxib analogues may bind in the binding pocket.[18]

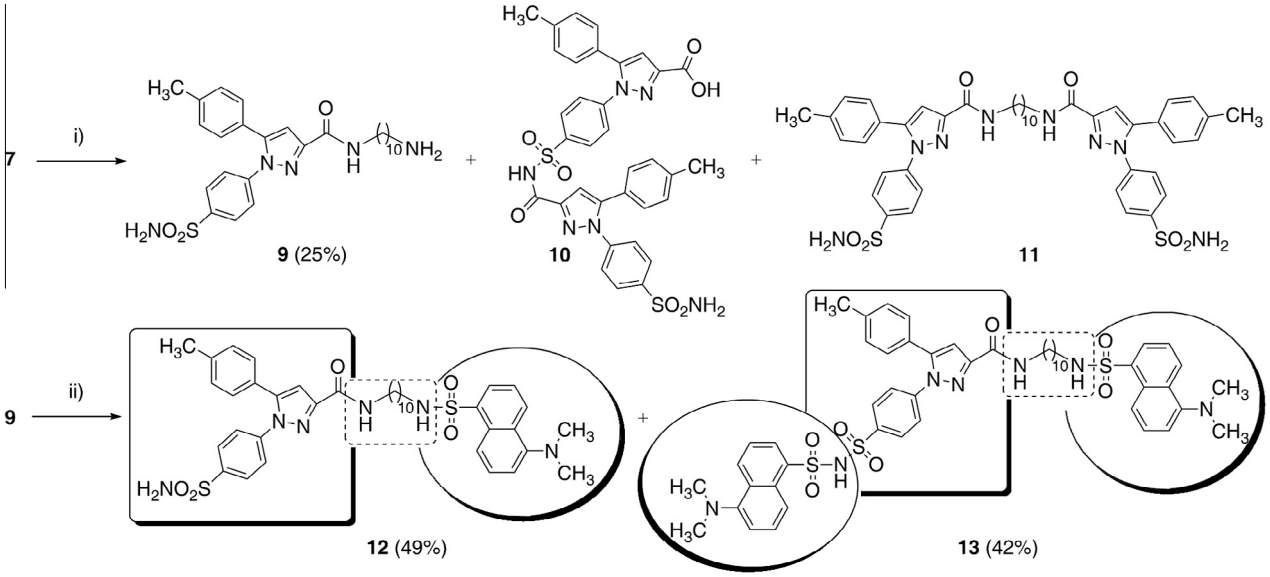
The physicochemical properties (e.g., lipophilicity) of the fluorescence-labeled Celecoxib derivative should be at best in a comparable range to that of Celecoxib, to avoid great changes in the pharmacological behavior, for example cell permeability. Molecular docking-experiments pointed out that the linker should have a length of about 14 atoms to allow the fluorescence moiety to stay out of the binding pocket. A tetraethylene glycol and an alkyl linker were chosen. Despite their different lipophilicity the alkyl linker was selected for simplicity reasons and it can serve as a negative control as well. 5-(Dimethylamino)naphthalene-1-sulfonamide (Dansyl) was used as a fluorophore because it is a small fluorophore with similar physicochemical properties compared to Celecoxib. In addition to that the spectral properties of dansyl conjugates are sensitive to their immediate environment which could be helpful in whole cell studies.[21–23]

Chemistry

Starting from 4-methylacetophenon, **2**, the Celecoxib acid derivatives **7** and **8** were synthesized in good overall yields (**7**:53%, **8**:74%). In both cases the corresponding 1,3-diketones **3** and **4** were obtained by Claisen condensation (Scheme 1) using sodium hydride[24] in THF since sodium methylate[17] resulted in poorer yields (30–40% lower) and longer reaction times. For the ester **4** ultrasound aided synthesis was applied. The diketones were reacted with 4-hydrazinyl benzenesulfonamide hydro chloride to give Celecoxib, **1**, and the methyl ester **6**, which were both separated from their corresponding 3,5-regioisomers by column chromatography. For the pyrazol substituted Celecoxib



Scheme 1. Synthesis of linkable Celecoxib derivatives **7** and **8**: (i) NaH, THF, 30 min, 0 °C; (ii) $F_3CCOOEt$, THF, 5 h, rt; (iii) $(COOMe)_2$, THF, 16 h, 45 °C, ultrasound; (iv) EtOH, 20 h (**1**)/3h (**4**), reflux; (v) $KMnO_4$, 2M NaOH, H_2O, 6 h, reflux; (vi) 1.1 M NaOH, THF, 20 h, reflux.



Scheme 2. Linear synthesis of dansyl-labeled Celecoxib derivatives **11** and analogues: (i) HOBt·H_2O, EDC, DMF, 1 h, rt, then 1,10-diaminodecane, **14**, DMF, 22 h, rt; (ii) DsCl, NEt_3, dioxane, 24 h, rt.