European Journal of Medicinal Chemistry 126 (2017) 550–560

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Optimization of substituted imidazobenzodiazepines as novel asthma treatments

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ARTICLE INFO

Article history:
Received 1 October 2016
Received in revised form
19 November 2016
Accepted 21 November 2016
Available online 24 November 2016

Keywords:
XHE-III-74
Deuterated compounds
Asthma
$GABA_A$ receptor
Airway hyperresponsiveness
Airway smooth muscle

ABSTRACT

We describe the synthesis of analogs of XHE-III-74, a selective α4β3γ2 $GABA_AR$ ligand, shown to relax airway smooth muscle *ex vivo* and reduce airway hyperresponsiveness in a murine asthma model. To improve properties of this compound as an asthma therapeutic, a series of analogs with a deuterated methoxy group in place of methoxy group at C-8 position was evaluated for isotope effects in preclinical assays; including microsomal stability, cytotoxicity, and sensorimotor impairment. The deuterated compounds were equally or more metabolically stable than the corresponding non-deuterated analogs and increased sensorimotor impairment was observed for some deuterated compounds. Thioesters were more cytotoxic in comparison to other carboxylic acid derivatives of this compound series. The most promising compound **16** identified from the *in vitro* screens also strongly inhibited smooth muscle constriction in *ex vivo* guinea pig tracheal rings. Smooth muscle relaxation, determined by reduction of airway hyperresponsiveness with a murine ovalbumin sensitized and challenged model, showed that **16** was efficacious at low methacholine concentrations. However, this effect was limited due to suboptimal pharmacokinetics of **16**. Based on these findings, further analogs of XHE-III-74 will be investigated to improve *in vivo* metabolic stability while retaining the efficacy at lung tissues involved in asthma pathology.

1. Introduction

Asthma is a major healthcare challenge, effecting an estimated 300 million people globally [1]. Over $56 billion in asthma-related healthcare expenses occur in the United States annually [2]. Moreover, asthma accounts for the majority of missed school/work days, doctor and emergency room visits, and patient hospitalizations in young persons [1–3]. Consequently, asthma continues to be a significant healthcare burden in terms of morbidity, productivity, and medical costs. Beta 2-adrenergic agonists and inhaled corticosteroids (ICs) are the most often prescribed treatments for the acute and chronic management of asthma. Both agents present efficacy, compliance, and adverse side effect concerns [4–8]. Hence, there is an unmet need for asthma therapies with novel mechanisms of action to better control diseases with decreased adverse side effects.

The $GABA_A$ receptor ($GABA_AR$) is a ligand-gated chloride ion channel best known for its role in central nervous system (CNS) inhibitory neurotransmission. $GABA_ARs$ are heteropentameric receptors mainly comprised of combinations of 19 different subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, θ, ρ_{1-3}). Classical $GABA_AR$ consist of two α, two β and one "tertiary" subunit (γ, δ, ε, θ, or π) [9,10]. The receptor subunits have been identified in airway smooth muscle, airway epithelium, and inflammatory cells, and their ligand-mediated activation has been shown to reduce immune response measures and reduce airway hyperresponsiveness (*ex vivo* and *in vivo*)

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http://dx.doi.org/10.1016/j.ejmech.2016.11.045
0223-5234/

[11–16]. In these studies, GABA dose-dependently reduced IL-12 and IL-6 production in LPS stimulated macrophages [15]. GABA and muscimol also inhibited anti-CD3 and antigen specific T cell proliferation [17]. Honokiol, a $GABA_AR$ agonist, reduced cardinal features of the asthma-like phenotype including inflammation (reduced airway eosinophilia), mucous cell metaplasia, collagen deposition, and airway hyperresponsiveness in an acute and chronic ovalbumin-induced murine asthma models [18]. However, nonselective $GABA_AR$ activation is associated with unwanted CNS effects [19] and increased mucous production [16,20,21]. To preclude these side effects in the treatment of asthma, subtype-selective $GABA_AR$ ligands were identified, especially those exhibiting preferential efficacy at $\alpha4/\alpha5\beta3\gamma2$ $GABA_AR$ [22–25]. In these studies, $\alpha4\beta3\gamma2$ $GABA_AR$ selective compounds CMD-45 and XHE-III-74 relaxed pre-contracted airway smooth muscle. In addition, XHE-III-74 reduced airway resistance in a murine house dust mite model of asthma. The ethyl ester of XHE-III-74 reduced airway hyperresponsiveness in a murine ovalbumin sensitized and challenged (S/C) asthma model, whereas XHE-III-74A, the corresponding carboxylic acid, significantly reduced airway eosinophilia [26] and reduced IL-2 production following PMA/PHA activation of Jurkat cells. Furthermore, XHE-III-74A exhibited no CNS effects because its negative charge precluded brain penetration.

Our previous studies showed that the C-8 methoxy group of XHE-II-74 (herein indicated as C-7) is essential for $\alpha4\beta3\gamma2$ $GABA_AR$ selectivity [27].To improve the metabolic stability of this group, we herein describe the synthesis of compounds bearing a deuterated form ($-OCD_3$) because the C-D bond is stronger than the C–H bond. We further report the synthesis and characterization of a series XHE-III-74 analogs additionally bearing different ester, thioester, and amide functionalities at the C-3 position to identify compounds with high metabolic stability, absence of CNS effects, low toxicity, and efficacy in a murine asthma model.

2. Results

In agreement with the earlier route of Fryer and Gu [27] [28]the synthesis of XHE-III-74 (**7**) started with the synthesis of 5-methoxyanthranilic acid **2** (Scheme 1) from 5-methoxy-2-nitrobenzoic acid **1** by hydrogenation.

The aniline **2** was converted into isotoic anhydride **3** with triphosgene. Anhydride **3** was heated with L-proline in DMSO to generate the corresponding benzodiazepine **4**. This compound was converted into the imidazobenzodiazepine, XHE-III-74 ethyl ester **5.** The introduction of the OCD_3 group was achieved using the demethylation-alkylation sequence as depicted in Scheme 2.

As shown in Scheme 2, ester **5** was demethylated at C-8 using aluminum chloride and ethanethiol in methylene chloride at room temperature to furnish the corresponding phenol **6**. The phenol was re-alkylated with deuterated iodomethane (CD_3I) using Cs_2CO_3 in methylene chloride at room temperature in excellent yield producing the OCD_3 analog **5a**.

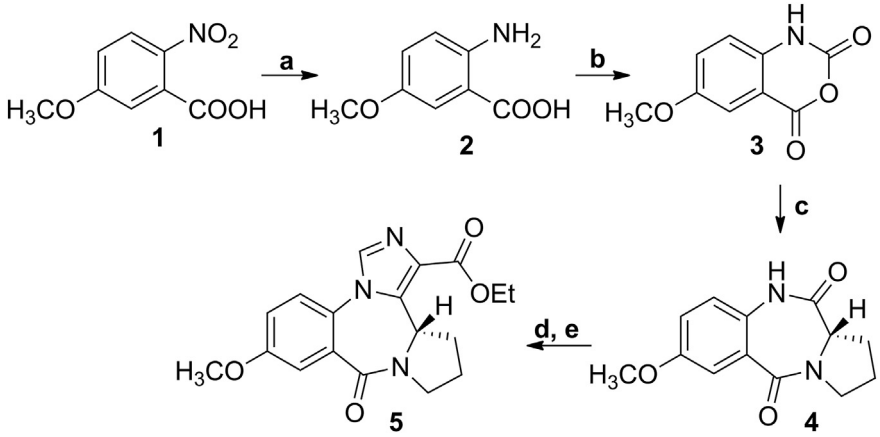
The esters **5**, **5a** and **6** were converted into the corresponding *tert*-butyl analogs **7** (XHE-III-74), **7a** and **8**, respectively, via *trans*-esterification with lithium in the presence of *tert*-butanol in THF at 50 °C (Scheme 3).

For generation of the other ester and amide analogs of XHE-III-74 (**7**), a three step protocol was used (Scheme 4).

The ethyl esters **5** and **5a** were saponified to give the corresponding acids **9** and **9a.** The subsequent reactions with thionyl chloride in methylene chloride yielded the corresponding acid chlorides, which in turn were converted to esters, thioesters, and amides (**11, 11a-19,** and **19a**). The methyl esters **10** and **10a** were formed in the presence of NaOMe in methanol.

Initially, human and mouse liver microsomal stability was investigated to identify metabolically labile compounds and deuterated compounds that are more stable than their non-deuterated counterparts. The results are summarized in Table 1.

The parent compound XHE-III-74 (**7**) was metabolized rapidly by mouse liver microsomes with 16.2% remaining after 1 h (Table 1, Entry 4). The corresponding half-life was less than 24 min. However, **7** was stable in the presence of human liver microsomes, similar to the majority of compounds investigated. Less than 80% of the parent was observed after 1 h for compounds **9**, **11**, **11a**, **12a,** and **19** (Table 1, Entries 7, 11, 12, 14 and 27). For **9** and **19**, stability of the deuterated analog (**9a** and **19a)** is significantly increased (Table 1, Entries 7, 8, 27, and 28). A smaller number of compounds were stable in the presence of mouse liver microsomes for 1 h. The most stable compounds (as judged by less than 20% loss at 1 h) were **5**, **5a**, **6, 9a**, **10a**, **16**, **16a**, **17a, 19**, and **19a** (Table 1, Entries 1, 2, 3, 8, 10, 21, 22, 24, 27, and 28). All compounds that exhibited good stability in mouse microsomes were also stable in the presence of human liver microsomes. Importantly, different metabolic rates for deuterated and non-deuterated compounds in the presence of mouse liver microsomes were observed for **9**, **10, 11,** and **17** and their corresponding deuterated analogs (Table 1, Entries 7–12, 23, 24). Further characterization of these compounds included the determination of their cytotoxicity using three different cell lines; HEK293 kidney cells, HepG2 liver cells, and BEAS2B lung epithelial cells (Table 2).



Scheme 1. Synthesis of XHE-III-74 EE. a. H_2, Pd/C, EtOAc, rt, 8 h, 97%; b. triphosgene, HCl/H_2O, rt, 4 h, 89%; c. L-proline, DMSO, 160 °C, 2 h, 96%; d. *t*-BuOK, $(EtO)_2POCl$, THF, −20 °C to rt, 4 h; e. *t*-BuOK, $CNCH_2CO_2Et$, −20 °C to rt, 8 h, 60%.