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Original article

Structure-based optimization of oxadiazole-based GSK-3 inhibitors

Fabio Lo Monte [a,*], Thomas Kramer [a], Jiamin Gu [a], Martin Brodrecht [a], Johannes Pilakowski [a], Ana Fuertes [b], Juan Manuel Dominguez [b], Batya Plotkin [c], Hagit Eldar-Finkelman [c], Boris Schmidt [a,*]

[a] Clemens Schöpf – Institute of Organic Chemistry and Biochemistry, Technische Universität Darmstadt, Petersenstrasse 22, 64287 Darmstadt, Germany
[b] Noscira S.A., Drug Discovery, Tres Cantos 28760 Madrid, Spain
[c] Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel

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ABSTRACT

Inhibition of glycogen synthase kinase-3 (GSK-3) induces neuroprotective effects, e.g. decreases β-amyloid production and reduces tau hyperphosphorylation, which are both associated with Alzheimer's disease (AD). The two isoforms of GSK-3 in mammalians are GSK-3α and β, which share 98% homology in their catalytic domains. We investigated GSK-3 inhibitors based on 2 different scaffolds in order to elucidate the demands of the ATP-binding pocket [1]. Particularly, the oxadiazole scaffold provided potent and selective GSK-3 inhibitors. For example, the most potent inhibitor of the present series, the acetamide **26d**, is characterized by an IC_{50} of 2 nM for GSK-3α and 17 nM for GSK-3β. In addition, the benzodioxane **8g** showed up to 27-fold selectivity for GSK-3α over GSK-3β, with an IC_{50} of 35 nM for GSK-3α. Two GSK-3 inhibitors were further profiled for efficacy and toxicity in the wild-type (wt) zebrafish embryo assay to evaluate simultaneously permeability and safety.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and characterized by the presence of abnormal filamentous protein inclusions in nerve cells of the brain [2]. The neuropathological hallmarks of AD were first reported by Alois Alzheimer and date back to 1907 [3,4]. These inclusions are formed by extracellular amyloid deposits and intracellular microtubule-associated protein tau [5]. Early onset forms of familial Alzheimer's disease (FAD) have been linked to mutations in the amyloid precursor protein (APP), presenilin-1 (PS-1) and presenilin-2 (PS-2). These mutations adversely affect APP processing and result in the increased production of the 40-42 amino acid long β-amyloid (Aβ) peptides, which are the major component of amyloid deposits. Several risk factors have been associated with sporadic Alzheimer's disease (SAD). The most prevalent is aging and the presence of specific ApoE isoforms, which have been implicated in Aβ clearance. The activation of β-secretase may be involved in Aβ generation, which in combination with a deficiency in Aβ clearance will result in the accumulation of Aβ aggregates [2,6]. Partially phosphorylated tau in the normal adult brain features sequences that support association with tubulin, which entails the stabilization of microtubules. The pathological hyperphosphorylation of tau causes destabilization of microtubules, which in turn interferes with tubulin binding. The misfolding of hyperphosphorylated tau leads to the formation of insoluble neurofibrillary tangles (NFTs) and intraneuronal aggregates of paired helical filaments (PHFs) [7,8]. GSK-3 was shown to phosphorylate tau both *in vitro* and *in vivo* on multiple sites [7]. Several studies demonstrate that inhibition of GSK-3 induces decreased Aβ production and a reduction in tau hyperphosphorylation [9,10]. GSK-3 was identified in the late 1970s and is a constitutively active, ubiquitously expressed serine/threonine kinase, which participates in a number of physiological processes [2,11]. Two related isoforms of GSK-3 exist in mammals, GSK-3α and β, which share 98% homology in their catalytic domains and have similar biochemical properties [7,12]. The isoforms differ significantly outside of their catalytic domains at their N-terminal regions [13]. Furthermore, an alternative splice variant of GSK-3β: GSK-3β2, has been reported for rodents and humans [14,15]. The crystal structure of GSK-3β was determined in 2001 [16,17]. GSK-3 is highly enriched in the brain and several publications indicate that the GSK-3β isoform is a key kinase required for abnormal hyperphosphorylation of tau [18–20]. Lithium chloride was the first GSK-3 inhibitor to be discovered. However there are several other biological targets for lithium cations, which impose limits on the therapeutic window. Considering the homology of GSK-3α and β within the ATP-binding pocket it appears difficult to identify an

* Corresponding authors. Tel.: +49 6151 164531; fax: +49 6151 163278.
E-mail addresses: Fabio.Lo-Monte@gmx.de (F. Lo Monte), schmidt_boris@t-online.de (B. Schmidt).

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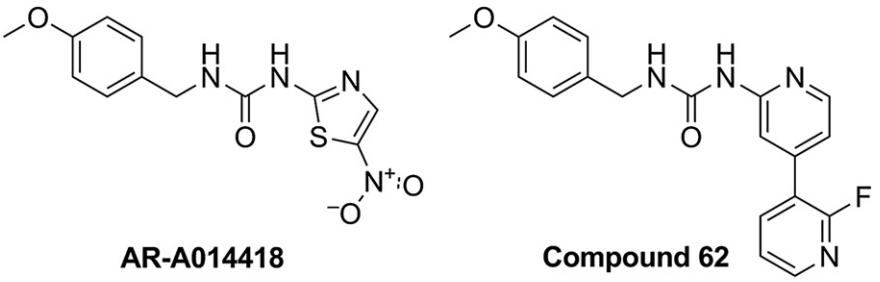
inhibitor that differentiates the two isoforms. All GSK-3 inhibitors developed until now are able to inhibit the two isoforms with almost similar potency, except compound **Λ-OS1**, which showed up to 7-fold selectivity for GSK-3α and compound **15b**, which showed up to 92-fold selectivity for GSK-3α [21–24]. A plethora of GSK-3 inhibitors has been described and most of the effects were observed *in vitro* and cellular studies (Fig. 1) [5,25,26]. These studies and the ongoing patent filing indicate that GSK-3 is a potential drug target not just for the treatment of AD. Several research groups and pharmaceutical companies are interested in the discovery of novel GSK-3 inhibitors with good selectivity and bioavailability despite of the Wnt-pathway associated risks, which may result in adverse cell proliferation. In the present study, the 1,3,4-oxadiazole-moiety served as a scaffold for a variety of GSK-3 inhibitors. This particular heterocycle was chosen because it has favorable properties over 1,2,4-oxadiazoles and other five-membered heterocycles [27]. The inhibition potencies of these compounds were compared to those of the previously reported 1,3,4-oxadiazoles and substituted ureas in the attempt to identify additional heterocycles and substituents that enhance GSK-3 inhibition [1,28–30]. The resulting interactions with the ATP-binding pocket of GSK-3α and β were also investigated to generate a hypothesis for isoform discrimination. We employed the wild-type zebrafish embryo in order to validate the utility of these compounds *in vivo*.

2. Chemistry

The esterfication of the carboxylic acids **1a**, **b** afforded compounds **2a**, **b** which were converted to the hydrazides **3a**, **b**. The hydrazide **3c** was commercially available. Reaction of the hydrazides **3a**–**c** with carbon disulfide (CS_2) afforded the oxadiazoles **4a**–**c** (Scheme 1) [28,31,32].



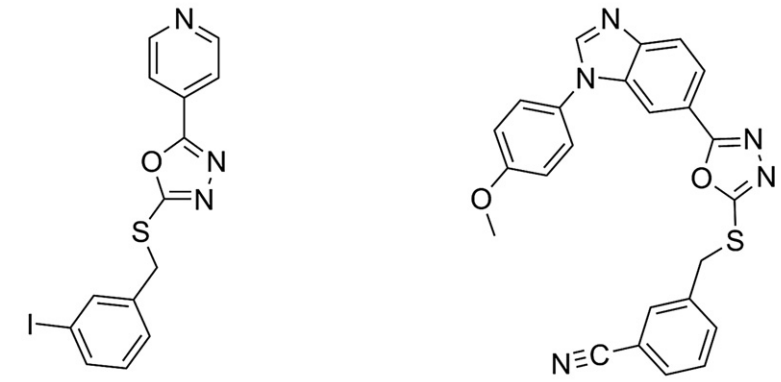
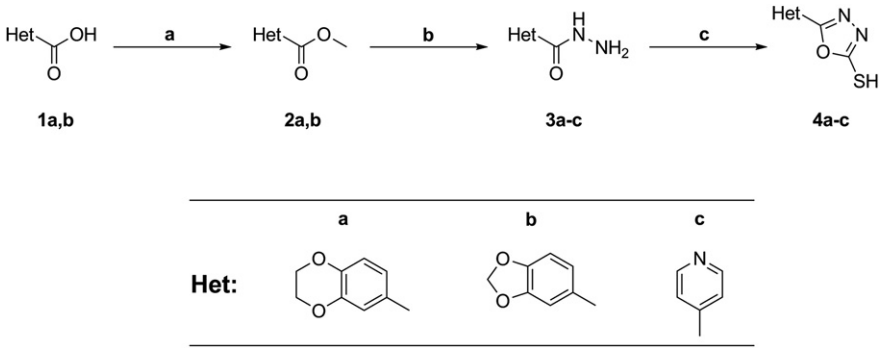


Fig. 1. Structures of previously reported GSK-3 inhibitors.



Scheme 1. Reagents and conditions: (a) MeOH, $SOCl_2$, 0 °C–50 °C, 83–89%; (b) $NH_2NH_2{\bullet}H_2O$, EtOH, reflux, 67–75%; (c) CS_2, Et_3N, EtOH, reflux, 79–89%.