European Journal of Medicinal Chemistry 138 (2017) 328–342

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Leucine rich repeat kinase 2 (LRRK2) inhibitors based on indolinone scaffold: Potential pro-neurogenic agents

Irene G. Salado [a, 1], Josefa Zaldivar-Diez [a, 1], Victor Sebastian [a], Lingling Li [b], Larissa Geiger [a], Silvia González [a], Nuria E. Campillo [a], Carmen Gil [a], Aixa V. Morales [b], Daniel I. Perez [a, **], Ana Martinez [a, *]

[a] Department of Chemical and Physical Biology, Centro de Investigaciones Biológicas-CSIC, Madrid, Spain
[b] Department of Molecular, Cellular and Developmental Neurobiology, Instituto Cajal-CSIC, Madrid, Spain

ARTICLE INFO

Article history:
Received 4 May 2017
Received in revised form
27 June 2017
Accepted 28 June 2017
Available online 29 June 2017

Keywords:
LRRK2 inhibitors
Protein kinase inhibitors
Indolinone
Adult neurogenesis
Neural stem cell
Regenerative medicine

ABSTRACT

Leucine-rich repeat kinase 2 (LRRK2) is one of the most pursued targets for Parkinson's disease (PD) therapy. Moreover, it has recently described its role in regulating Wnt signaling and thus, it may be involved in adult neurogenesis. This new hypothesis could give rise to double disease-modifying agents firstly by the benefits of inhibiting LRRK2 and secondly by promoting adult neurogenesis. Herein we report, the design, synthesis, biological evaluation, SAR and potential binding mode of indoline-like LRRK2 inhibitors and their preliminary neurogenic effect in neural precursor cells isolated from adult mice ventricular-subventricular zone. These results open new therapeutic horizons for the use of LRRK2 inhibitors as neuroregenerative agents. Moreover, the indolinone derivatives here prepared, inhibitors of the kinase activity of LRRK2, may be considered as pharmacological probes to study the potential neuroregeneration of the damaged brain.

1. Introduction

The search of effective treatments for neurodegenerative diseases is one of the urgent clinical and social needs today given their widespread and devastating nature. As most neurodegenerative disorders are age-dependent, due to longer life expectancy the prevalence of these diseases, including Alzheimer's and Parkinson's disease among others, increases daily. Common characteristics of neurodegenerative diseases include the progressive loss of neurons in specific regions of the nervous system underlying the subsequent decline in cognitive or motor function in patients. Given their largely unknown etiology and the lack of effective treatments there is a critical need to better understand the underlying disease pathophysiology and discover effective disease-modifying targets for their treatment.

Genetic studies carried on several families in Asia, the United States, and Europe led to discover in 2004 that mutations in the gene *LRRK2* encoding leucine-rich repeat kinase 2 (LRRK2) as a major genetic risk factor for both familial and sporadic Parkinson's disease (PD) [1]. Up to date among the disease-linked genetic polymorphisms (15% of the total PD population) LRRK2 mutations account for 4% of the cases and 1% of the sporadic ones [2]. Although these incidences are quite low and it remains unclear how mutations in *LRRK2* gene cause PD-related neurodegeneration, LRRK2 has raised as one of the most pursued targets for PD and its inhibition has been proposed to be beneficial for preventing neurodegeneration. Big efforts are being done at the moment both from academia and the pharmaceutical industry with the goal of developing selective and brain-permeable LRRK2 inhibitors as neuroprotective agents for PD [3,4].

LRRK2 is an unusually large (2527 amino acids) protein that is classified as a member of the Ras-like GTPase (ROCO) superfamily. At least 6 independent domains have been established including a kinase domain, a GTPase domain and several protein-protein interacting regions [5]. The physiological role of LRRK2 is poorly understood and many of its substrates remain unclear, however, great success was achieved by the identification of a subset of Rab GTPases as key LRRK2 substrates [6]. The downstream signals in

* Corresponding author.
** Corresponding author.
E-mail addresses: dperez@cib.csic.es (D.I. Perez), ana.martinez@csic.es (A. Martinez).
[1] Equally contributed to this work.

http://dx.doi.org/10.1016/j.ejmech.2017.06.060
0223-5234/

which LRRK2 is involved are not well characterized yet, however LRRK2 can be related to other pathological pathways outside the direct PD pathology. LRRK2 can be related to tau pathology, inflammatory response, oxidative stress, mitochondrial and synaptic dysfunction, deficiencies in the autophagy-lysosomal system [7] and its implication in adult neurogenesis through the Wnt signaling pathway [8].

Among these connections we considered as the most interesting one, the hypothesis that the pharmacological inhibition of LRRK2 may induce adult neurogenesis. There is evidence suggesting that neurogenesis is impaired in many neurodegenerative diseases, therapeutic approaches that stimulate neurogenesis may have potential to stimulate repair and even recovery, thereby providing innovative, disease-modifying treatments [9,10]. Therefore, LRRK2 inhibitors may have a dual therapeutic role: the first one due to the direct beneficial effect of LRRK2 inhibition and the second one as enhancing adult neurogenesis.

Based on these collective observations pointing to the critical role of LRRK2 in CNS function and connections to proneurogenic pathways like Wnt signaling [8], we initiated a medicinal chemistry program with the aim of finding new selective LRRK2 inhibitors with physicochemical properties compatible with crossing the blood brain barrier (BBB). Furthermore, we have used them as pharmacological probes of adult neurogenesis activators using neural stem cells isolated from the neurogenic niche of the adult mouse ventricular-subventricular zone [11]. In this communication we present a class of outstanding novel LRRK2 inhibitors that show a preliminary effect in increasing the proliferation of neural stem cells from the subventricular zone of adult mice and can therefore be considered as chemical probes to study the neuroregeneration potential of the damaged brain.

2. Results and discussion

2.1. Design of new compounds

Biology-oriented synthesis [12] employs chemical privileged scaffolds from natural sources for the development of focused libraries because natural products are particularly important as source of inspiration for new compounds [13]. A literature survey identified several isatin (1*H*-indole-2,3-dione) derivatives as, a natural scaffold present in different Chinese traditional medicines, with potent biological activity on different protein kinases (Fig. 1) [14]. Moreover, some of them such as sunitinib are FDA approved for human therapy while semaxanib is currently in clinical trials (Fig. 1) [15].

For the design of new LRRK2 inhibitors, we decided to keep the indolinone ring as common and privileged scaffold and to modify the nature of the atom directly joined to the oxindole ring introducing a nitrogen atom instead of the carbon atom present in the compounds of Fig. 1. Furthermore, the influence of various substituents on the aryl group was explored obtaining different compounds, with aromatic and aliphatic nature. In addition, the linker between the above mentioned substituent and the oxoindole heterocycle was modified. Imines and hydrazones were used for this purpose. Finally, alkylation of the heterocyclic nitrogen atom and substitution in different positions of the indolinone framework gave additional diversity to the final compounds (Fig. 2).

2.2. Chemistry

Preparation of the first family of these compounds was carried out using classical conditions to conduct the imine formation reaction between the isatin core and the corresponding aromatic amine under reflux in toluene and with 4-toluensulfonic acid as catalyst [16]. Several imino indolinone derivatives have been previously described. However, due to long reaction times and low yields, new synthetic reaction conditions were looked for. Microwave assisted organic synthesis in the absence of solvent and montmorillonite (MMT-K10) as surface catalyst were the best option found [17], although here an optimized work-up was used (described in the experimental part). MMT-K10 is a mixture of silicates in laminar arrangement recently used as inorganic base in organic synthesis. The reactions catalysed by MMT-K10 are normally performed under mild conditions to obtain high yield and selectivity. MMT-K10 was not only used as a base but also as a solid surface where the reaction took place [18]. For imine formation it is essential to remove the water from the reaction medium and normally 4-toluenesulfonic acid was used. In our case, the use of microwave assisted organic synthesis accelerates the dehydration of the reaction using a clay support MMT-K10. Thus, lower reaction times (10 min) and an increase in general yield was achieved except

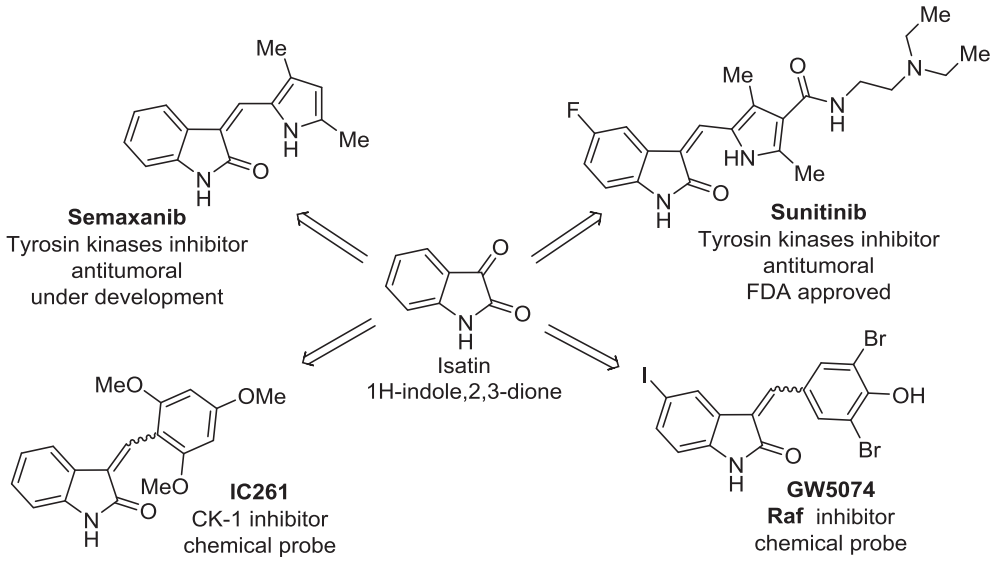

Fig. 1. Structure of known protein kinase inhibitors with indolinone scaffold.