Tetrahedron 74 (2018) 4531–4537

Contents lists available at ScienceDirect

Tetrahedron

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

Discovery of 2,4-dimethoxypyridines as novel autophagy inhibitors

Lucas Robke [a, b], Tiago Rodrigues [c], Peter Schröder [a], Daniel J. Foley [a], Gonçalo J.L. Bernardes [c, d], Luca Laraia [a, e, **], Herbert Waldmann [a, b, *]

[a] Max Planck-Institute of Molecular Physiology, Otto-Hahn-Str. 11, 44227, Dortmund, Germany
[b] Department of Chemistry and Chemical Biology of the Technical University Dortmund, Otto-Hahn-Str. 6, 44227, Dortmund, Germany
[c] Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av Prof Egas Moniz, 1649-028, Lisboa, Portugal
[d] Department of Chemistry, University of Cambridge, Lensfield Road, CB2 1EW, Cambridge, UK
[e] Technical University of Denmark, Department of Chemistry, Kemitorvet, Building 207, DK-2800, Kgs. Lyngby, Denmark

ARTICLE INFO

Article history:
Received 6 June 2018
Received in revised form
4 July 2018
Accepted 9 July 2018
Available online 17 July 2018

Keywords:
Autophagy
Phenotypic screening
High-throughput screening
Natural products
Medicinal chemistry

ABSTRACT

Autophagy is a catabolic process, which mediates degradation of cellular components and has important roles in health and disease. Therefore, small molecule modulators of autophagy are in great demand. Herein, we describe a phenotypic high-content screen for autophagy inhibitors, which led to the discovery of a dimethoxypyridine-based class of autophagy inhibitors, which derive from previously reported, natural product-inspired MAP4K4 inhibitors. Comprehensive structure-activity relationship studies led to a potent compound, and biological validation experiments indicated that the mode of action was upstream or independent of mTOR.

1. Introduction

Macroautophagy (hereafter autophagy) is a highly conserved cellular process in eukaryotes, which mediates the degradation of cellular components within specialised subcellular compartments (autophagosomes) [1–3]. Autophagosomes derive from the phagophore, a double membrane structure that engulfs cellular components that are to be recycled. The autophagosomes subsequently fuse with lysosomes to form autophagolysosomes, in which degradation carried out by lysosomal enzymes takes place. This multistep process is tightly regulated by upstream signaling and is modulated by growth factors, the concentration of amino acids and the energy level of the cell. The purpose of this degradation is not only to compensate for a temporary lack of nutrients but also to eliminate dispensable, long-lived proteins, protein aggregates and organelles. Moreover, autophagy has been linked to microbiological infections and aging. Although primarily a protective pathway, autophagy can also be involved in cell death. Due to its involvement in these (patho)physiological processes, autophagy is an essential mechanism for the development, homeostasis and survival of cells.

Autophagy plays a crucial role in the degradation of protein aggregates, which cause several neurodegenerative diseases, including Alzheimer's, Huntington's and Parkinson's diseases [4–6]. Additionally, there is evidence that autophagy is involved in the prevention of cancer by degrading toxic metabolites in the cell [7,8]. However, it is also reported to promote the survival of cancer cells under conditions of nutrient deprivation [9–13]. Consequently, whereas an upregulation of autophagy might serve as a preventative strategy against cancer, autophagy inhibition is a potential approach for cancer therapy after its onset. Since many unanswered questions remain regarding the dual role of autophagy in physiology and pathophysiology, there is a strong interest in a deeper understanding of its mechanisms. Selective autophagy modulators are valuable tools to study this process at different stages of disease [14–17]. In this regard, phenotypic screening offers a useful starting point for the identification of new biologically active compounds by representing a disease relevant system [18–21].

Herein we report the identification and validation of dimethoxypyridine-containing autophagy inhibitors (DMPs)

* Corresponding author. Max-Planck-Institute of Molecular Physiology, Dortmund, Germany.
** Corresponding author. Technical University of Denmark, Department of Chemistry, Kemitorvet, Building 207, DK-2800, Kgs. Lyngby, Denmark.
E-mail addresses: luclar@kemi.dtu.dk (L. Laraia), herbert.waldmann@mpi-dortmund.mpg.de (H. Waldmann).

https://doi.org/10.1016/j.tet.2018.07.021
0040-4020/

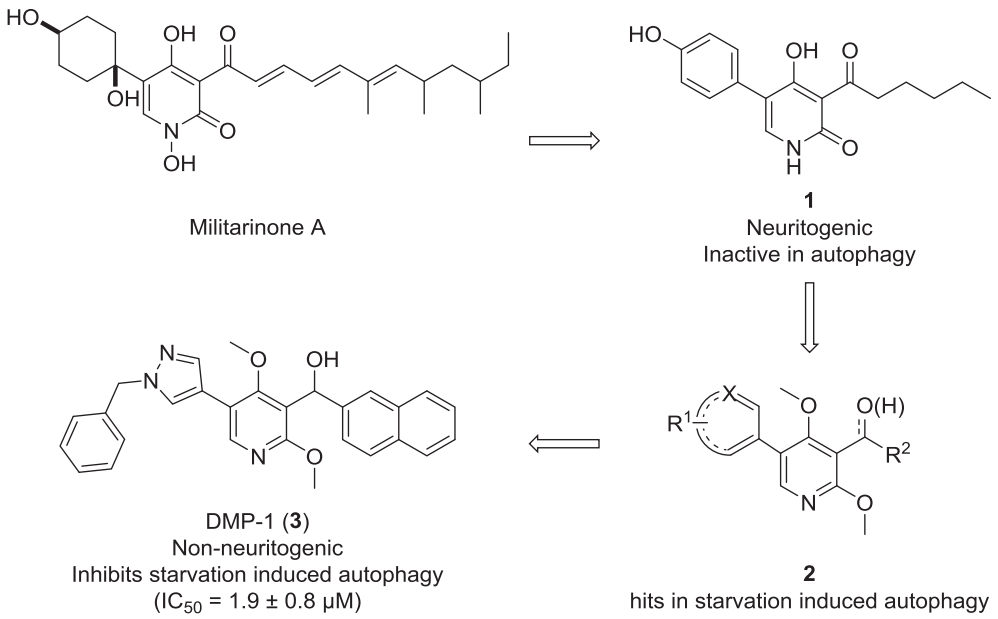


Fig. 1. Structural development of an autophagy inhibitor from a neuritogenic MAP-kinase inhibitor. Dimethoxypyridine **2** represents a structural precursor for the neuritogenic MAP-kinase inhibitor **1**. By varying the substitution pattern of **2** and maintaining the alcohol adjacent to the pyridine, autophagy inhibitors (including **3**) were identified.

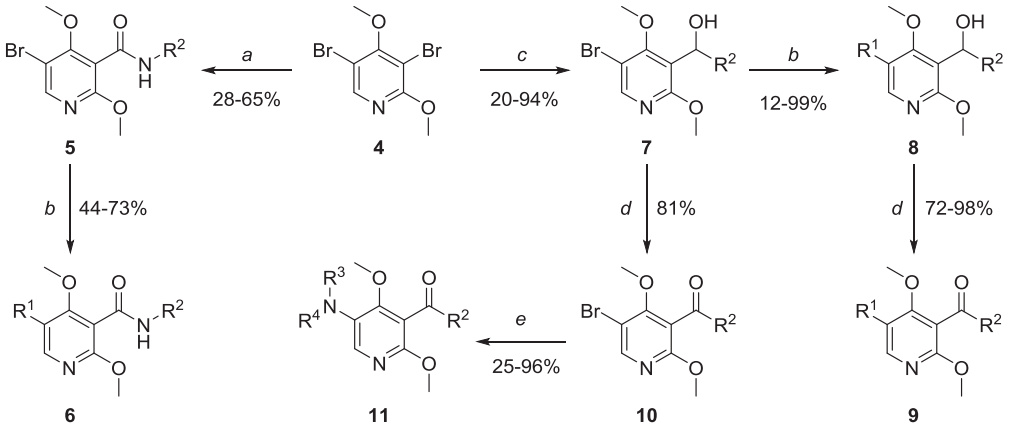
identified through a phenotypic screen. The DMPs were originally synthesised in the context of a biology-oriented synthesis (BIOS) [22,23] effort to synthesise pyridones based on the natural product Militarone, which were found to be MAPK inhibitors [24]. We describe a comprehensive SAR analysis, detailing the requirements for activity, in addition to biological validation experiments.

2. Results

2.1. Phenotypic screening for autophagy inhibitors

For monitoring and quantifying autophagy, MCF7 cells stably transfected with green fluorescent protein tagged light chain 3 (eGFP-LC3) were employed, as developed by Balgi *et al.* [25–27] Upon autophagy induction, the ubiquitin-like cytosolic protein LC3 is conjugated to phosphatidylethanolamine (PE) and consequently recruited to the autophagosomal membrane [28]. Whereas eGFP-LC3, and by association the fluorescence signal, is distributed throughout the cytoplasm without autophagy induction, eGFP-LC3 accumulates at autophagosomes under starvation conditions, which can be visualised as fluorescent puncta. The puncta are detected by automated fluorescence microscopy and quantified using automated image analysis [25]. Incubating the cells with Earle's balanced salt solution (EBSS) induces autophagy through amino acid starvation. Chloroquine (CQ), which is also included in the starvation medium, inhibits the fusion of autophagosomes with lysosomes, preserving eGFP fluorescence by inhibiting its degradation by the autolysosomes. This increases the dynamic range of the assay [29].

As part of our ongoing programme to identify autophagy inhibitors [30–32], we identified a 2,4-dimethoxypyridine (DMP) based compound class, which inhibited starvation-induced autophagy (Fig. 1). These compounds are synthetic precursors of a Militarinone-inspired, neuritogenic compound collection. The finding that synthetic precursors have different biological activity underlines the utility of the BIOS concept [33]. We sought to explore the SAR of this compound class and optimised them for autophagy inhibition, leading to a compound we termed DMP-1 (Fig. 1).



Scheme 1. General scheme for the synthesis of derivatives of DMP-1 and other analogues starting from precursor **4.** *a*) *s*-BuLi, THF, −78 °C, 1 h, then R^2NCO, −78 °C → rt, overnight; *b*) $R^1B(OH)_2$, $Pd(PPh_3)_4$, dppf, Na_2CO_3, PhMe/EtOH, Δ, 16 h; *c*) *s*-BuLi, THF, −78 °C, 1 h, then R^2CHO, −78 °C → rt, overnight; *d*) MnO_2, CH_2Cl_2, rt, 2 d *or* Dess–Martin periodinane, CH_2Cl_2, rt, 2 h; *e*) R^3R^4NH, *t*BuXPhos, Pd_2dba_3, NaO*t*Bu, PhMe, 80 °C, overnight. dppf = 1,1′-Bis(diphenylphosphino)ferrocene, THF = tetrahydrofuran, dba = dibenzylidineacetone.