European Journal of Medicinal Chemistry 125 (2017) 1225–1234

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Imidazopyridine-fused [1,3]-diazepinones part 2: Structure-activity relationships and antiproliferative activity against melanoma cells

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ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form
7 November 2016
Accepted 10 November 2016
Available online 11 November 2016

Keywords:
Imidazo[1,2-*a*]pyridine
Polyfused heterocycles
Diazepinones
Antitumor activity
Melanoma

ABSTRACT

We recently described a pyrido-imidazodiazepinone derivative which could be a promising hit compound for the development of new drugs acting against melanoma cells. In this study, a series of 28 novel pyrido-imidazodiazepinones were synthesized and screened for their *in vitro* cytotoxic activities against the melanoma MDA-MB-435 cell line. Among the derivatives, seven of them showed 50% growth inhibitory activity at 1 μM concentration, and high selectivity against the melanoma cell line MDA-MB-435.

1. Introduction

Malignant melanoma is the most aggressive and life-threatening skin cancer. It represents about 5% of skin cancers, and over the last 50 years, it has increased by ~10% per year in industrialized countries [1]. On the local stage (stage I/II), surgical resection provides more than 90% survival at 5 years. However, when the diagnosis is delayed, melanoma metastasis spreads rapidly into the liver, bones, lungs, etc. (stage IV), and survival falls down dramatically, with a median survival of less than one year [2,3]. Until recently, the therapeutic options in stage IV melanoma were very limited, mostly centred on dacarbazine, a chemotherapeutic alkylating agent, used as monotherapy, and in various combinations. Recently, new therapies acting against metastatic melanoma have emerged and offer promising results, with a median survival improved from 6 to 10 months. Among these new therapies, small compounds inhibiting the RAF/MEK pathway (vemurafenib and dabrafenib, two BRAF inhibitors and trametinib a MEK inhibitor) have greatly improved median overall survival of mutated BRAF metastatic melanoma patients [3,4]. Immunotherapy based on immune checkpoint inhibitors (like ipilimumab, nivolumab, pembrolizumab) and on the first oncolytic virus T-VEC authorized by the U.S. Food and Drug Administration, also offers encouraging results [5]. However, despite these new therapies, the rapid emergence of drug resistance to initially responsive cancers has led to only marginal patient benefit [6]. The primary strategy to overcome single line treatment resistance is the use of combination therapies [7]. For example, BRAF inhibitor drugs could be combining with a MEK inhibitor. Several other drug associations are under study [8]. Another strategy consists in the development of new drugs acting against melanoma.

Recently, we have identified pyrido-imidazodiazepinone derivatives, which demonstrate cytotoxic activity on several cell lines. Such series appear particularly active towards the MDA-MB-435 melanoma cell line [9]. Moreover, no significant cytotoxic activity on healthy fibroblasts has been detected, even at 100 μM. Among the tested compounds, the bromo derivative JMV5038 (Fig. 1) showed the most promising activity against the melanoma MDA-MB-435 cell line, with a growth inhibition of 50% (GI_{50}) of 1.3 μM. Further investigations revealed that JMV5038 induced cell cycle arrest in S phase and increased apoptosis in subG1 phase. Microscopy observations of treated cells indicated a change in the cell morphology, concomitant with an increase of the membrane permeability and disruption of the actin network. Evaluation of

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http://dx.doi.org/10.1016/j.ejmech.2016.11.023
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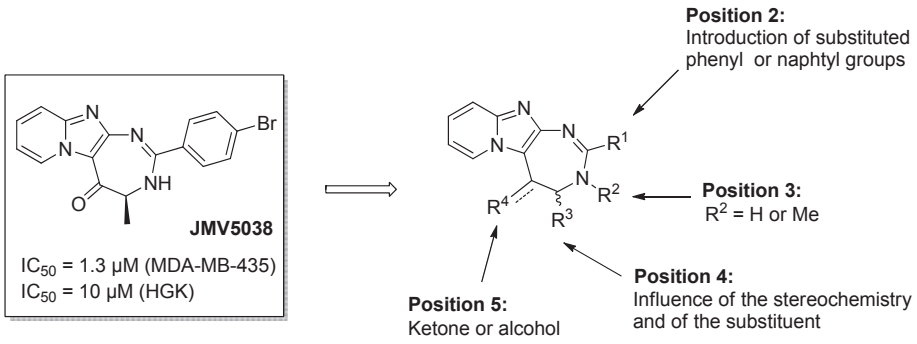


Fig. 1. Structure of JMV5038 and studied modifications.

JMV5038 on a panel of representative kinases suggested this compound could act as an HGK (Hepatocyte progenitor kinase-like kinase) inhibitor, with an IC_{50} of 10 μM [9].

We report herein a structure-activity relationship (SAR) study on the pyrido-imidazodiazepinone series, based on modifications of positions 2, 3, 4 and 5 of the diazepine core (Fig. 2). All compounds were evaluated for their cytotoxic potential on the MDA-MB-435 cell line. HGK inhibitory activities of representative diazepinone derivatives were also examined to evaluate their possible mechanism of action.

2. Results and discussion

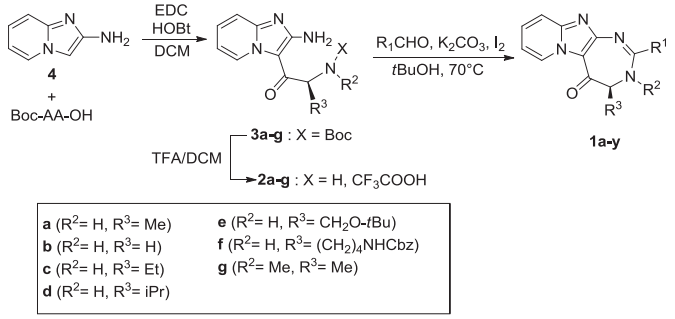
2.1. Chemistry

Pyrido-imidazodiazepinones **1a-1ab** were synthesized starting from 2-amino-imidazo[1,2-*a*]pyridine **4**, by selective *C*-3 acylation of the IP nucleus (Scheme 1), using 7 different *N*-Boc amino-acids (Boc-Ala-OH, Boc-Gly-OH, Boc-Abu-OH, Boc-Val-OH, Boc-Ser(*t*Bu)-OH, Boc-Lys(Cbz)-OH, Boc-*N*-Me-Ala-OH), according to our previously reported methodology [10–12]. *C*-3 acylated compounds **3a-g** were isolated in 50%–97% yield after chromatography on alumina gel. Only traces of the corresponding *N*-acylated derivatives were detected in the crude mixture by LC-MS analysis. After Boc removal using a mixture of trifluoroacetic acid/dichloromethane (50/50 v/v), the resulting 2-amino-3-acyl-imidazo[1,2-*a*] pyridines **2a-g** were isolated as trifluoroacetate salts, and used without further purification. In the case of compound **2e**, only Boc removal was obtained without the loss of the *tert*-butyl group. Indeed, as no scavenger was used during the Boc removal step, the temporary free hydroxyl group immediately trapped the *tert*-butyl radical formed in the acidic conditions. Such selective deprotection was previously reported by several groups [13,14].

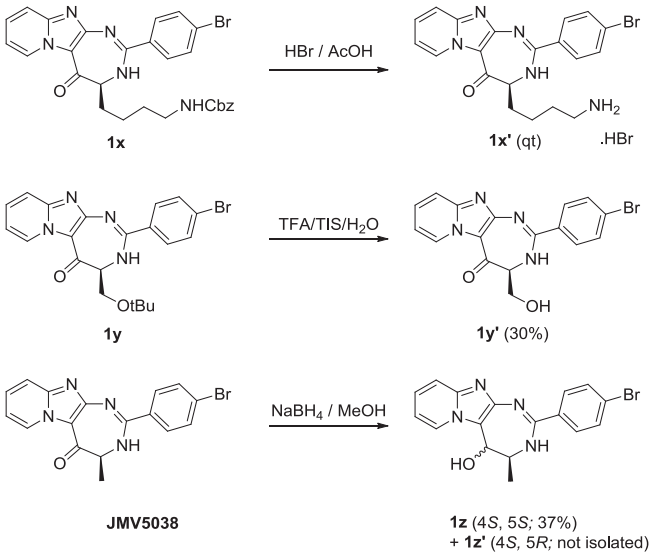
Diamines **3a-g** were then successively reacted with a set of aldehydes in *tert*-butanol with potassium carbonate and iodine at 70 °C overnight. Pyrido-imidazodiazepinones **1a-1y** were finally isolated in 12%–73% yield, after purification by chromatography on alumina gel.

The benzyloxycarbonyl protected derivative **1x** was treated by a solution of 33% hydrobromic acid in acetic acid to yield the corresponding hydrobromic acid salt derivative **1x'** in quantitative yield (Scheme 2). The hydroxymethyl derivative **1y'** was obtained after *tert*-butyl removal of **1y**, using acidic conditions in the presence of radical scavengers [trifluoroacetic acid/triisopropylsilane/water (95/2.5/2.5 v/v/v)]. Finally, the 5-hydroxy derivative **1z** was obtained, after reduction of JMV5038, using sodium borohydride in methanol (Scheme 2).

The reduction led to the formation of the two diastereomers in a 70/30 ratio (determined by integration of the two corresponding



Scheme 1. Synthesis of compounds **1a-y**. Reagents: Boc-AA-OH = Boc-Ala-OH, Boc-Gly-OH, Boc-Abu-OH, Boc-Val-OH, Boc-Ser(*t*Bu)-OH, Boc-Lys(Cbz)-OH or Boc-*N*-Me-Ala-OH. R^1, R^2 and R^3 substituents are referenced in Table 2.



Scheme 2. Synthesis of compounds **1x'-z'.**

peaks by HPLC at 214 nm). Purification of the mixture by chromatography allowed isolation of the major diastereomer **1z** as a pure compound. Whereas, a $^1H-^1H$ NOESY analysis was not informative to determine the absolute configuration of C4, ^1H NMR analysis of **1z** showed a coupling constant of 1.1 Hz for H-5, which indicated that the dihedral angle between the H4–C4–C5–H5 bonds, is close to 80°, regarding to the Karplus curve [15]. Nonetheless, the seven-membered ring system is not a planar ring and could exist as several conformers, which complicated the stereochemistry determination. For each stereomer (4*S*, 5*S*) or (4*S*, 5*R*),