European Journal of Medicinal Chemistry 75 (2014) 382–390

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Imidazopyridine-fused [1,3]-diazepinones: Synthesis and antiproliferative activity

Audrey Gallud [a,1], Ophélie Vaillant [a,1], Ludovic T. Maillard [a], Dominique P. Arama [a], Joëlle Dubois [b], Marie Maynadier [a], Vincent Lisowski [a], Marcel Garcia [a], Jean Martinez [a], Nicolas Masurier [a,*]

[a] Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, Universités Montpellier I et II, UFR des Sciences Pharmaceutiques et Biologiques, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France
[b] Institut de Chimie des Substances Naturelles, UPR 2301 CNRS, Centre de Recherche de Gif, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form
17 January 2014
Accepted 18 January 2014
Available online 30 January 2014

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

ABSTRACT

A series of 15 pyrido-imidazo-1,3-diazepin-5-ones and pyrido-1,3-diazepine-2,5-diones were synthesized and their anticancer activities were evaluated. Among tested compounds on a cell lines panel, compound **6a** presents the best growth inhibition activity on 21 cell lines with a cytotoxic effect on MDA-MB-435 melanoma cells. This compound led to deep cell morphological changes and revealed to be an inhibitor of the Hepatocyte progenitor kinase-like kinase (HGK), which is known to be implicated in the migration, adhesion and invasion of various tumor cells.

1. Introduction

Cancer is a notably complex, widespread and lethal disease accounting for 7.6 million deaths (around 13% of all deaths) in 2008, that is projected to continue rising, with an estimated 13.1 million deaths in 2030 [1]. Several lines of evidence support the view that chemotherapy has become one of the most significant treatment modalities in cancer management. Classical chemotherapy using small molecules or bioactive natural products is still the mainstay of chemotherapy, whereby the major cellular targets are DNA, tubulin [2,3], along with various kinases [4]. However, the absence of selectivity and acute toxicity of many antitumor agents beside the development of cellular drug resistance have been the major drawback in their usage, prompting the search for new more selective, efficient, and safe antitumor agents [5].

Azepine core is considered as a privileged structure to access to active compounds displaying a wide range of pharmacological activities. In particular, polyfused azepine derivatives led to the discovery of compounds with anticancer potency. Pyrrolo[1,2-*c*][1,3]benzodiazepines (Fig. 1) revealed to be cytotoxic for Jurkat and neuronal cells, with induction of DNA cleavage [6]. Hymenialdisine, a marine natural product, which also has a pyrrolo-azepine core, is a potent inhibitor of cyclin dependent kinases (CDK) [7]. Paullone derivatives, which are based on the indolo[3,2-*d*][1]benzazepinone system, are CDK inhibitors with very potent antitumor activity [8,9]. Kenpaullone, alsterpaullone and their analogs were also reported to be inhibitors of glycogen synthase kinase-3 (GSK3) [10–17] and of Hepatocyte progenitor kinase-like kinase (HGK) [18]. Moreover, some structural isomers of paullones, based on the indolo[2,3-*d*][2]benzazepinone framework, were reported to inhibit tubulin polymerization [19].

Recently, we reported an efficient approach to access polyfused diazepinone derivatives, based on the imidazo[1,2-*a*]pyridine (IP) nucleus, an aza analog of indole [20,21]. In continuation to this study and since some polyfused IP derivatives have been described to possess antitumor activities [22,23], we decided to evaluate the pharmacological potency of IP-fused diazepinones. We reported herein the synthesis of some pyrido-imidazo [1,3]diazepin-5-ones

* Corresponding author. Tel.: +33 4 11 75 96 42; fax: +33 4 11 75 96 41.
E-mail address: nicolas.masurier@univ-montp1.fr (N. Masurier).
[1] These authors contributed equally to the work.

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http://dx.doi.org/10.1016/j.ejmech.2014.01.044

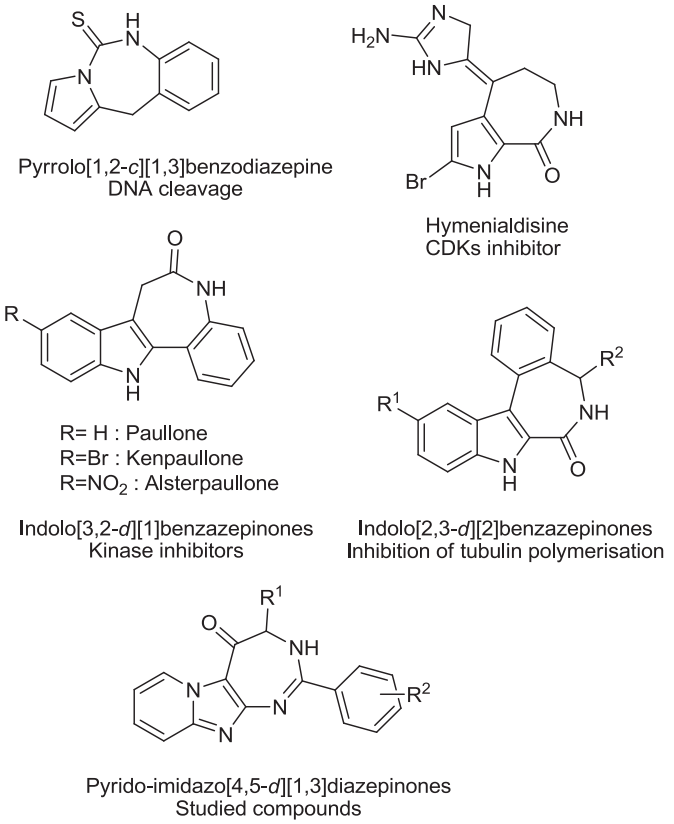
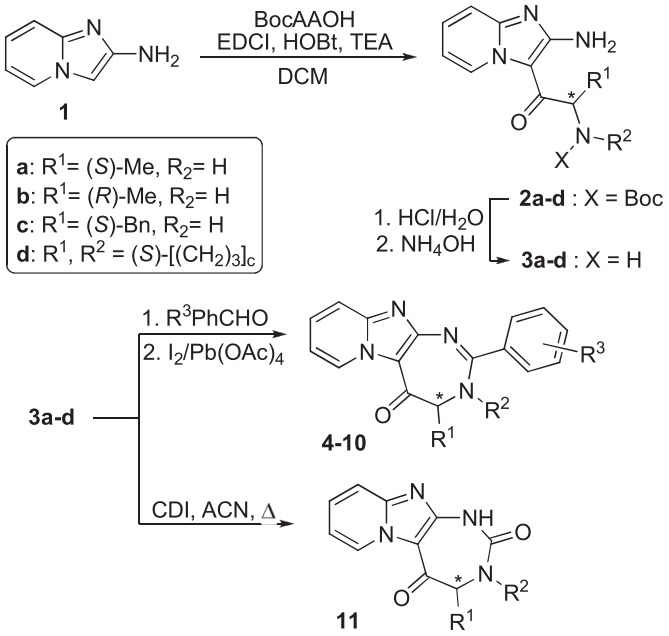


Fig. 1. Representative examples of azepine derivatives with antitumor potency.



Scheme 1. Synthesis of pyrido-imidazodiazepinones **4**–**10** and pyrido-imidazodiazepinediones **11**. Abbreviations used: BocAAOH = Boc-Ala-OH, Boc-DAla-OH, Boc-Phe-OH or Boc-Pro-OH; EDCI: *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride; HOBt: hydroxybenzotriazole; TEA: triethylamine; CDI: 1,1′-carbonyldiimidazole; ACN: acetonitrile. R^1, R^2 and R^3 substituents are referenced in Table 1.

and pyrido-imidazo [1,3]diazepine-2,5-diones derivatives and their evaluation for growth inhibitory activities on cancer cell lines.

2. Results and discussion

2.1. Chemistry

Pyrido-imidazodiazepine derivatives **4**–**11** were synthesized according to our previous published methodologies [20,21]. Briefly, 2-amino-imidazo[1,2-*a*]pyridine **1** [24,25] was selectively acylated at *C*-3 position, using four different *N*-Boc protected amino acids: Boc-Ala-OH, Boc-D-Ala-OH, Boc-Phe-OH or Boc-Pro-OH. IP is known to be an electron-rich aromatic ring that could lead to electrophilic aromatic substitutions at *C*-3 [26,27]. Therefore, a simple activation at room temperature of a *N*-Boc protected amino-acid by EDCI/HOBt in dichloromethane (DCM) led to the *C*-3 acylated IP derivatives **2a**–**d** in 62–88% isolated yields (Scheme 1). In accordance with previous results, N-addition side-products were only detected as traces by LC–MS [20,21]. After Boc removal by HCl treatment, the resulting hydrochloride salts were neutralized with aqueous ammonia and 2-amino-3-acyl-imidazo[1,2-*a*]pyridines **3a**–**d** were extracted with chloroform and used in the next step without further purification. Diamines **3** were then successively reacted with a set of benzaldehydes in chloroform at reflux overnight. The aminal intermediates were then oxidized without isolation, using a mixture of lead tetraacetate and iodine, to lead to pyrido-imidazodiazepinones **4**–**10** in 23–71% isolated yields (Scheme 1, Table 1). On the other hand, intramolecular carbonylation of diamines **3** using carbonyldiimidazole (CDI) led to pyrido-imidazodiazepinediones **11** in 78–99% isolated yields.

2.2. Antiproliferative activities on cancer cell lines panel

2.2.1. Sulforhodamine B (SRB) assay

Diazepine derivatives **4**–**11** were evaluated by the NCI towards a panel of sixty cancer cell lines corresponding to nine different cancer types, *i.e.*, leukemia, melanoma, as well as lung, colon, central nervous system, ovarian, renal, prostate and breast cancers. The compounds were tested at a single concentration (10 μM) and incubated for 48 h on the cell cultures. Cellular densities were determined by Sulforhodamine B (SRB) assay, which is based on the cellular protein content. The SRB test is a direct index of cell density since the amount of dye incorporated by the cells varies concomitantly with the increase (or the decrease) of the total protein biomass [28]. The cell growth was evaluated spectrophotometrically after 48 h exposure and reported as the percent of untreated control cells (Table 1 and SI). Pyrido-imidazodiazepinediones (**11a**, **11c** and **11d**), as well as pyrido-imidazodiazepinones (**4d**, **5c**, **5d**, **8c** and **10c**) appeared inactive whatever the cell line considered. **5a** showed a moderate activity on 4 cell lines (growth < 50%). The best results were obtained with the bromo analog **6a** which inhibited cell growth (growth < 50%) in 21 cell lines. **6a** was particularly active on MDA-MB-435 and MDA-MB-468 cells with a cytotoxic effect at 10 μM (0% growth). Interestingly, its enantiomer **6b** was inactive on all cell lines suggesting a very specific mechanism of action.

Compound **6a** was selected for an advanced assay on the NCI-60 cancer cell lines panel and was tested in a five-dose testing mode, to determine IC_{50} values. IC_{50} for 40 cell lines were lower than 10 μM (Fig. 2). The best activities were obtained on glioblastoma SNB-75 cells (IC_{50} = 0.7 μM), non-small-cell lung cancer HOP-92 cells (IC_{50} = 1.1 μM) and melanoma MDA-MB-435 cells (IC_{50} = 1.3 μM).

2.2.2. MTT assay

To confirm these first results, dose–response and time-course experiments were carried out on the most active compound **6a**