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# IMIDAZOPYRIDINE-FUSED [1,3]-DIAZEPINONES PART 2: STRUCTURE-ACTIVITY RELATIONSHIPS AND ANTIPROLIFERATIVE ACTIVITY AGAINST MELANOMA CELLS

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## 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  $\mu$ M 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  $\mu$ M. Among the tested compounds, the bromo derivative JMV5038 (Figure 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  $\mu$ M. 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 JMV5038 on a panel of representative kinases suggested this compound could act as an HGK (Hepatocyte progenitor kinase-like kinase) inhibitor, with an IC<sub>50</sub> 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 (Figure 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.

< Insert Figure 1 >

## 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% to 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% to 73% yield, after purification by chromatography on alumina gel.

< Insert Scheme 1 >

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).

### < Insert Scheme 2 >

The reduction led to the formation of the two diastereomers in a 70/30 ratio (determined by integration of the two corresponding 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 <sup>1</sup>H-<sup>1</sup>H NOESY analysis was not informative to determine the absolute configuration of C4, <sup>1</sup>H 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*), two puckered modes of the diazepine ring have been recognized, based on molecular dynamic simulations, using MM+ force field implemented in Hyperchem 6.0. They were defined based on the position of the N3 atom that could be above (N3-up) or below (N3-down) the median plan of the diazepine ring (Table 1).

### < Insert Table 1 >

Whatever the isomer considered, the "N3-up" conformer was more stable. Considering the "N3-up" conformers, the H4-C4-C5-H5 dihedral angles were determined at 76.7° and 61.7° for the (4*S*, 5*S*)-**1z** and (4*S*, 5*R*)-**1z'** compounds, respectively. Similar angles were measured on the "N3-down" conformers. Based on this result and according to the Karplus curve, only the (4*S*, 5*S*)-**1z** isomer was compatible with the NMR data, *i.e.* <sup>3</sup>*J* = 1.1 Hz.

## 2.2. Structure Activity Relationships

The cytotoxicity of diazepine derivatives **1a-1z** was measured towards the MDA-MB-435 melanoma cell line, using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide] assay. Growth inhibitions 50 (GI<sub>50</sub>) are reported in Table 2.

### < Insert Table 2 >

We started our SAR investigations by focusing our attention on the position 2 of the diazepine core. As the initial hit possesses a bromine atom in the *para* position of the phenyl ring, 4-fluoro, 4-chloro and 4-iodo analogues were investigated. Replacement of the bromine by a fluorine atom (compound **1c**) led to the total loss of cytotoxic activity. The 4-chloro derivative **1d** showed a weaker activity compared to its 4-bromo analog (GI<sub>50</sub> = 5 μM), whereas the 4-iodo derivative **1g** kept the initial activity (GI<sub>50</sub> = 1.35 μM). From these results, it seemed that the cytotoxic activity could be positively correlated to the halogen atom size or inversely correlated with the electronegativity of the halogen.

Then, modification of the halogen position was investigated. For bromine series, the *meta* derivative (Entry 3, product **1a**) was inactive. Some cytotoxic activity was retained with the *ortho* isomer (Entry 4, product **1b**: GI<sub>50</sub> = 8.3 μM compared to 1.3 for compound **1**). The same correlation was observed in the chlorine series (Entries 6-8). The *para* chloro derivative **1d** showed the best activity (GI<sub>50</sub> = 5 μM), followed by the *ortho* derivative **1f** (GI<sub>50</sub> = 6.5 μM), and by the *meta* analog **1e** (GI<sub>50</sub> = 10 μM). We could also observe in the chlorine series, that variation of the halogen position had a weaker impact on the biological activity than in the bromine series, suggesting that steric hindrance had more influence than electronic effects. Introduction of a supplementary withdrawing trifluoromethyl group on the 4-chloro derivative (compound **1i**) led to a strong decrease of the cytotoxicity (GI<sub>50</sub> = 30 μM), confirming that a substitution in the *meta* position was not favourable. An additional chlorine atom in the *ortho* position (Entry 10, compound **1h**) led to a similar activity to JMV5038 (GI<sub>50</sub> = 1.15 μM).

Other substituents than halogen atoms were then investigated. Introduction of a 2-methyl group (**1j**), a 3,4-dimethoxy (**1k**), an ethyl ester (**1l**), an amide (**1m** and **1n**) or a strong electro-withdrawing substituent like a cyano or a trifluoromethyl group (**1o** and **1p** respectively) on the 2-phenyl ring, led to the total loss of the cytotoxic activity. When

the 4-bromophenyl group was replaced by either a 1-naphthyl (**1q**) or a 2-naphthyl (**1r**) group, the initial activity was recovered ( $GI_{50} = 1 \mu\text{M}$ ). These results showed that an electronegative atom was not required for the cytotoxic activity, but that the later might depend on the presence of a bulky group in position 2 of the diazepine core. This could suggest that the 4-bromophenyl or the 1- or 2-naphthyl groups could occupy a large hydrophobic pocket on the target. Surprisingly, the biphenyl derivative, compound **1s**, was weakly active ( $GI_{50} = 40 \mu\text{M}$ ), probably due to an unsuitable geometry of the biphenyl substituent.

Study of the position 4 of the diazepine ring was then carried out, keeping constant the 4-bromophenyl group at R1 (Fig. 1). Replacing the 4-methyl group by a hydrogen atom led to the loss of activity (compound **1u**). We then studied the introduction of various alkyl chains. We have previously reported that the (*S*) configuration of the 4-methyl group was necessary for the cytotoxic activity (by comparison of compound JMV5038 and compound **1**, Table 1),[9] so all further modifications kept constant the (*S*) configuration. Whereas a short elongation of the chain preserved the activity (ethyl derivative **1w**), a branching chain was detrimental (isopropyl derivative **1v**). Introduction of a hydroxyl group (hydroxymethyl derivative **1y'**) did not modify the activity, as well as a bulky *tert*-butyloxymethyl chain (compound **1y**). A free or a protected butylamine chain in this position led also to the loss of cytotoxic activity (compound **1x**). These results could suggest that the position 4 of the diazepine ring did not tolerate long alkyl chains or aromatic moieties and could tolerate bulky groups, but not too closed to the diazepine ring (isopropyl derivative **1v** was inactive whereas *tert*-butyloxymethyl compound **1y'** was active). However, as the activities of compounds **1y** and **1y'** were very similar, it could also suggest that the *tert*-butyl ether derivative **1y** was deprotected *in cellulo*, leading to the bioactive free hydroxyl compound **1y'**. Further studies will be done to evaluate this hypothesis.

Finally, methylation of the diazepine N-3 or reduction of the carbonyl in position 5 of compound **1** led to a loss of activity (compounds **1t** and **1z** respectively). These results suggested that the NH and the carbonyl of the diazepine ring might be involved in the formation of hydrogen bonds within the target. The loss of activity could be also explained by a structural change of the diazepine ring geometry.

### 2.3. Antiproliferative activities on a cancer cell line panel

Among the most active compounds on the MDA-MB-435 cell line, four diazepine derivatives (compounds **1d**, **1h**, **1r** and **1y**) were selected and evaluated 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 (NCI evaluation). Compounds were tested at a single 10  $\mu$ M concentration, using the sulforhodamine B assay (SRB). The SRB test provides a direct cell density index, and the amount of dye incorporated by the cells varies concomitantly with the increase (or the decrease) of the total protein biomass. The cell growth was evaluated spectrophotometrically after 48 h exposure and reported as the percent of untreated control cells. Values were compared to the JMV5038 activity (Figure 2 and SI) [9]. Considering the mean of cell growth, compounds **1h** and **1y** provided similar results to those obtained with JMV5038, with a mean of cell growth around 50%. The 4-chloro analogue **1d** was less active (mean of cell growth for the 60 cell lines = 86%), while the 2-naphtyl derivative **1r** was the most potent (mean = 42%). Among the 60 cell lines tested, the MDA-MB-435 cell line was the most sensitive to compounds **1d**, **1h**, **1r** and **1y**, as previously observed with JMV5038. Compound **1d** led to 67% of cell growth inhibition at a 10  $\mu$ M concentration, and in the case of the three most active compounds **1h**, **1r** and **1y**, a total inhibition of cell growth was obtained at a 10  $\mu$ M concentration. Once again, these results demonstrate a singular selectivity against this melanoma cell line.

### < Insert Figure 2 >

#### 2.4. Activity against HGK

In our previous study, we have reported that JMV5038 is a moderate inhibitor of HGK ( $IC_{50}$  = 10  $\mu$ M). HGK, also called MAP4K4, is a serine threonine kinase, broadly expressed in human tumour cells, which activates the c-Jun N-terminal kinase (JNK) pathway, playing a crucial role in stress responses, cell proliferation, apoptosis, and oncogenesis [16–19]. We have tested 6 analogues of JMV5038 for their HGK inhibitor activity. Compounds **1h** and **1y**, which possess a cytotoxic activity comparable to JMV5038 on MDA-MB-435 cell line ( $GI_{50}$  ~ 1  $\mu$ M), two moderate cytotoxic compounds **1s** and **1v** ( $GI_{50}$  between 10  $\mu$ M and 50  $\mu$ M), and two non-cytotoxic compounds **1** and **1n** ( $GI_{50}$  > 100  $\mu$ M), were selected to determine the possible correlation between the cytotoxic activity and the HGK inhibition potency. Results are reported in Table 3.

### < Insert Table 3 >

While compound **1y** displayed a better inhibition potency of HGK than JMV5038 ( $IC_{50} = 5.6 \mu M$ ), compound **1h**, which exhibit a similar cytotoxic activity on the melanoma cell line MDA-MB-435 to both JMV5038 and compound **1y** ( $IC_{50} \sim 1 \mu M$ ), did not have any activity on the kinase inhibition. For compound **1v**, the weak cytotoxicity seems to be correlated with the weak HGK inhibition. However, compound **1s**, which displayed similar cytotoxic activity to compound **1v**, was totally inactive against HGK. These results indicated that no strict correlation exists between the cytotoxicity on the MDA-MB-435 cell line and the HGK inhibition potency of these compounds. So it seems that the main activity of our compounds is not based on HGK inhibition and that the mechanism of action of pyrido-imidazodiazepinone derivatives still remains to be elucidated.

### 3. Conclusion

Based on a first hit (JMV5038), which showed significant cytotoxic activity against several cancer cell lines, including the melanoma cell line MDA-MB-435, we have hypothesized that this compound could act as a HGK inhibitor, a kinase involved in metastasis. A SAR study was then carried out. Modification of position 2, 3, 4 and 5 of the diazepine core was successively investigated. In position 2, a 4-iodophenyl group, a 2,4-dichlorophenyl, a 1-naphtyl, and a 2-naphtyl group without significant loss of the cytotoxic activity successfully replaced the 4-bromophenyl substituent. All other modifications on this position yielded analogues with a decrease or a total abolishment of the activity. Concerning the position 4, several modifications were tolerated, like short elongation of the alkyl chain, and introduction of bulky groups such as a *tert*-butyloxymethyl group. Moreover, a NH in position 3 and a carbonyl in position 5 were necessary for the activity. A screening against the NCI 60 cell lines panel showed a particular, intriguing specificity against the MDA-MB-435 cell line of this series of compounds.

Moreover, we didn't observe any straightforward correlation between the cytotoxic activity and the HGK inhibition of these derivatives, and the possibility that these derivatives interact with a HGK-like enzyme present in melanoma still remains an attractive hypothesis. Further biological studies are on going to elucidate the

mechanism of action of these compounds, with the goal to develop new anti-melanoma specific drugs.

## 4. Experimental

### 4.1 General

Commercially available reagents and solvents were used without further purification. Reactions were monitored by an analytical Waters Alliance 2690 HPLC instrument, equipped with a photodiode array and an analytical Chromolith Speed Rod RP-C18 185 Pm column (50x4.6 mm, 5 mm), at a flow rate of 3.0 mL/min, and gradients of 100/0 to 0/100 eluents A/B for 5 min (eluent A = H<sub>2</sub>O/0.1% TFA and B = CH<sub>3</sub>CN/0.1% TFA). Detection was performed at 214 nm. Retention times (Tr) are reported in min. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker spectrometers (300 or 400 MHz) at room temperature in deuterated solvents. Chemical shifts (δ) are expressed in parts per million (ppm), relative to the resonance of CDCl<sub>3</sub> = 7.26 ppm for <sup>1</sup>H (77.16 ppm for <sup>13</sup>C), CD<sub>3</sub>OD = 3.31 ppm for <sup>1</sup>H (49.00 ppm for <sup>13</sup>C) or DMSO *d*<sub>6</sub> = 2.50 ppm for <sup>1</sup>H (39.52 ppm for <sup>13</sup>C). The following abbreviations were used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Analytical thin-layer chromatography (TLC) was performed using 60 F254 aluminium-backed silica gel plates coated with a 0.2 mm thickness of neutral aluminium oxide. The LC/MS system consisted of a Waters Alliance 2695 HPLC, coupled to a Micromass (Manchester, UK) ZQ spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using a Merck Chromolith Speed rod C18, 25x4.6 mm reversed-phase column. A flow rate of 3mL/min and a gradient of (0–100)% B over 3 min (or over 15 min) were used. Eluent A: water/0.1% HCO<sub>2</sub>H; eluent B: acetonitrile/0.1% HCO<sub>2</sub>H. Retention times (RT) are given in minutes. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 100 to 1000 m/z in 0.1 s intervals; 10 scans were summed up to get the final spectrum. High-resolution mass spectrometry analyses were performed with a Waters Synapt G2-S time-of-flight mass spectrometer fitted with an electrospray ionisation source. All measurements were performed in the positive ion mode. Melting points (Mp) are uncorrected and were recorded on a capillary melting point apparatus.

Compounds **3a**, **3d-f**, **1**, **1j**, **1k** and JMV5038 were synthesized according to the previous reported procedures [9–12] and their physical characteristics were in agreement with the published data.

#### 4.2 Synthesis of compounds **3**

To a suspension of trifluoro-*N*-imidazo[1,2-*a*]pyridin-2-ylacetamide (0.5 g, 2.18 mmol) in 5 N aqueous sodium hydroxide (9 mL), was added THF (0.5 mL). The solution was stirred at 40°C for 2h. The solution was extracted with dichloromethane (3 x 20 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (20 mL) and either Boc-Gly-OH, Boc-Abu-OH, Boc-Ser(*t*Bu)-OH or Boc-*N*-Me-Ala-OH (2.4 mmol, 1.1 equiv), HOBt (325 mg, 2.4 mmol, 1.1 equiv), EDCI (460 mg, 2.4 mmol, 1.1 equiv), or triethylamine (334  $\mu$ L, 2.4 mmol, 1.1 equiv), were added at 0°C. The solution was stirred at room temperature for 1 night. The solution was then washed with a saturated NaHCO<sub>3</sub> solution (2 x 50 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was concentrated *in vacuo*. The residue was purified by chromatography (Al<sub>2</sub>O<sub>3</sub>, DCM/EtOH 99/1 v/v) to yield compounds **3**.

##### 4.2.1 2-Boc-amino-1-(2-aminoimidazo[1,2-*a*]pyridin-3-yl)-ethan-1-one (**3b**)

White solid (480 mg, yield 76%); mp: 183-184°C; <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 300 MHz):  $\delta$  ppm 1.40 (s, 9H), 4.14 (d, 2H, *J* = 5.9 Hz), 6.45 (bs, 2H), 6.98 (ddd, 1H, *J* = 8.0 Hz, 5.7 Hz, 1.1 Hz), 7.35 (d, 1H, *J* = 8.7 Hz), 7.49 (d, 1H, *J* = 8.7 Hz, 8.0 Hz), 9.49 (d, 1H, *J* = 5.7 Hz); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 75 MHz):  $\delta$  ppm 28.2, 47.5, 77.9, 106.6, 112.5, 113.6, 128.3, 130.0, 146.7, 156.1, 157.8, 182.1; HPLC, Tr = 1.03 min; MS (ESI<sup>+</sup>): *m/z* 291.3 [M+H]<sup>+</sup>; HRMS calcd for C<sub>14</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> 291.1457, found 291.1458.

##### 4.2.2 (2*S*)-2-Boc-amino-1-(2-aminoimidazo[1,2-*a*]pyridin-3-yl)-butan-1-one (**3c**)

White solid (347 mg, yield 50%); mp: 145-146°C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +24 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  ppm 0.91 (t, 3H, *J* = 7.4 Hz), 1.40 (s, 9H), 1.60-1.74 (m, 1H), 1.82-1.93 (m, 1H), 4.84 (m, 1H), 5.53 (d, 1H, *J* = 8.8 Hz), 6.00 (bs, 2H), 6.85 (td, 1H, *J* = 6.7 Hz, 1.5 Hz), 7.33 (d, 1H, *J* = 8.5 Hz), 7.39 (dd, 1H, *J* = 8.5 Hz, 6.7 Hz), 9.63 (d, 1H, *J* = 6.7 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  ppm 10.3, 26.0, 55.8, 80.4, 108.3, 113.2 (2C),

114.3, 129.4, 130.7, 147.7, 156.9, 158.8, 179.0, 185.8; HPLC, Tr = 1.23 min; MS (ESI<sup>+</sup>): m/z 319.0 [M+H]<sup>+</sup>; HRMS calcd for C<sub>16</sub>H<sub>23</sub>N<sub>4</sub>O<sub>3</sub> 319.1770, found 319.1775.

#### 4.2.3 (2S)-2-Boc-N-methyl-amino-1-(2-aminoimidazo[1,2-a]pyridin-3-yl)-propan-1-one (**3g**)

Beige Gum (409 mg, yield 59%);  $[\alpha]_D^{20} = +18$  (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 1.37 (d, 3H, J = 6.6 Hz), 1.46 (s, 9H), 2.79 (s, 3H), 4.15 (q, 1H, J = 6.6 Hz), 6.33 (bs, 2H), 6.90 (t, 1H, J = 6.9 Hz), 7.35 (d, 1H, J = 8.4 Hz), 7.45 (dd, 1H, J = 8.4, 6.9 Hz), 9.72 (bs, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 13.7, 28.3, 53.5, 60.9, 81.1, 113.4, 113.5, 129.7, 131.1, 146.4, 156.9, 157.2, 172.4, 183.7; HPLC, Tr = 1.30 min; MS (ESI<sup>+</sup>): m/z 319.0 [M+H]<sup>+</sup>; HRMS calcd for C<sub>16</sub>H<sub>23</sub>N<sub>4</sub>O<sub>3</sub> 319.1770, found 319.1769.

### 4.3 General procedure for the synthesis of diazepinones **1a-1y**

A solution of compound **3a-g** (0.33 mmol) in a 50/50 v/v mixture of trifluoroacetic acid and dichloromethane (5 mL) was stirred at room temperature for 1 h. After completion of the reaction (HPLC analysis), the solution was concentrated to dryness and co-evaporated several times with acetonitrile to completely remove TFA. Then were added, *tert*-butanol (8 mL), K<sub>2</sub>CO<sub>3</sub> (182 mg, 4 equiv.), iodine (251 mg, 3 equiv.), and the appropriate aldehyde (1 equiv.). The mixture was stirred under heating at 70°C for one night. *Tert*-butanol was then removed under reduced pressure and 30 mL of dichloromethane were added. The organic phase was successively washed with a 10% m/v sodium thiosulfate aqueous solution (2 x 30 mL) and then with a saturated NaHCO<sub>3</sub> aqueous solution (2 x 30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on alumina, eluted by DCM/EtOH 99/1 v/v.

#### 4.3.1 (4S)-4-methyl-2-(3-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo[4,5-d][1,3]diazepin-5-one (**1a**)

Yellow solid (72 mg, yield 59%); mp: 154-156°C;  $[\alpha]_D^{20} = -22$  (c 0.3, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz): δ ppm 1.64 (d, 3H, J = 7.0 Hz), 4.34 (q, 1H, J = 7.0 Hz), 7.46 (t, 1H, J = 6.8 Hz), 7.56 (t, 1H, J = 8.4 Hz), 7.84 (d, 1H, J = 8.9 Hz), 7.94 (m, 2H), 8.02 (d, 1H, J = 8.4 Hz), 8.25 (t, 1H, J = 1.7 Hz), 9.67 (d, 1H, J = 6.8 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD,

75 MHz):  $\delta$  ppm 14.5, 59.5, 113.7, 115.1, 117.9, 123.8, 130.3, 130.4, 131.8, 134.4, 134.6, 135.2, 138.0, 144.1, 151.0, 162.8, 182.1; HPLC, Tr = 1.42 min; MS (ESI<sup>+</sup>): m/z 369.1 [M+H]<sup>+</sup>, 371.2 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>OBr 369.0351, found 369.0350.

#### 4.3.2 (4S)-4-Methyl-2-(2-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1b**)

Yellow solid (16 mg, yield 13%); mp: 69-70°C;  $[\alpha]_{\text{D}}^{20} = +48$  (c 0.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  ppm 1.56 (d, 3H, *J* = 6.9 Hz), 4.10 (q, 1H, *J* = 7.0 Hz), 7.17 (td, 1H, *J* = 6.6, 2.0 Hz), 7.45 (m, 2H), 7.60 (dd, 1H, *J* = 7.4, 2.0 Hz), 7.69 (m, 3H), 8.07 (s, 1H), 9.55 (d, 1H, *J* = 6.6 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz):  $\delta$  ppm 15.7, 60.0, 114.2, 115.3, 116.8, 123.5, 128.7, 129.5, 132.2, 132.5, 132.9, 133.2, 134.2, 134.4, 139.1, 147.8, 183.9; HPLC, Tr = 0.91 min; MS (ESI<sup>+</sup>): m/z 369.1 [M+H]<sup>+</sup>, 371.1 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>OBr 369.0351, found 369.0349.

#### 4.3.3 (4S)-4-Methyl-2-(4-fluorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1c**)

Yellow solid (66 mg, yield 65%); mp: 136-137°C;  $[\alpha]_{\text{D}}^{20} = -9$  (c 0.5, MeOH); <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 600 MHz):  $\delta$  ppm 1.36 (d, 3H, *J* = 6.9 Hz), 3.99 (q, 1H, *J* = 6.9 Hz), 7.17 (td, 1H, *J* = 6.7 Hz, 1.8 Hz), 7.36 (t, 2H, *J* = 8.5 Hz), 7.65 (m, 2H), 8.06 (m, 2H), 9.46 (d, 1H, *J* = 6.9 Hz); <sup>19</sup>F NMR (DMSO *d*<sub>6</sub>, 400 MHz):  $\delta$  ppm -109.11; <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 100 MHz):  $\delta$  ppm 15.5, 56.6, 108.0, 113.6, 115.1, 115.3, 115.8, 127.7, 130.4, 131.6, 131.7, 142.2, 146.0, 164.2 (d, *J* = 247 Hz), 182.9; HPLC, Tr = 0.90 min; MS (ESI<sup>+</sup>): m/z 308.9 [M+H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>FN<sub>4</sub>O 309.1152, found 309.1155.

#### 4.3.4 (4S)-4-Methyl-2-(4-chlorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1d**)

Yellow solid (44 mg, yield 41%); mp: 126-127°C;  $[\alpha]_{\text{D}}^{20} = -37$  (c 0.5, MeOH); <sup>1</sup>H NMR (DMSO *d*<sub>6</sub>, 600 MHz):  $\delta$  ppm 1.36 (d, 3H, *J* = 6.9 Hz), 4.00 (q, 1H, *J* = 6.9 Hz), 7.18 (td, 1H, *J* = 6.6 Hz, 1.7 Hz), 7.60 (d, 2H, *J* = 8.5 Hz), 7.66 (m, 2H), 8.02 (d, 2H, *J* = 8.5 Hz), 9.46 (d, 1H, *J* = 6.6 Hz); <sup>13</sup>C NMR (DMSO *d*<sub>6</sub>, 100 MHz):  $\delta$  ppm 15.7, 56.7, 112.3, 113.7, 115.8, 127.7, 128.4, 130.5, 130.9, 134.1, 134.2, 136.3, 142.2, 146.0, 158.8,

182.8; HPLC, Tr = 0.98 min; MS (ESI<sup>+</sup>): m/z 324.9 [M+H]<sup>+</sup>, 326.8 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>ClN<sub>4</sub>O 325.0856, found 325.0858.

#### 4.3.5 (4S)-4-Methyl-2-(3-chlorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1e**)

Yellow solid (35 mg, yield 33%); mp: 179-180°C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +19 (c 0.3, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  ppm) 1.55 (d, 3H, *J* = 7.0 Hz), 4.26 (q, 1H, *J* = 7.0 Hz), 7.09 (t, 1H, *J* = 6.8 Hz), 7.28-7.38 (m, 2H), 7.52 (m, 2H), 7.94 (m, 3H), 9.54 (d, 1H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  ppm 16.6, 59.9, 112.4, 114.7, 115.5, 127.7, 128.7, 129.5, 130.1, 131.4, 132.0, 134.8, 136.4, 145.1, 153.9, 156.3, 182.7; HPLC, Tr = 0.96 min; MS (ESI<sup>+</sup>): m/z 325.2 [M+H]<sup>+</sup>, 327.2 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>ClN<sub>4</sub>O 325.0856, found 325.0857.

#### 4.3.6 (4S)-4-Methyl-2-(2-chlorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1f**)

Yellow solid (35 mg, yield 33%); mp: 100-101°C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -20 (c 0.7, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  ppm) 1.61 (s, 3H, *J* = 7.2 Hz), 4.21 (q, 1H, *J* = 7.2 Hz), 6.88 (d, 1H, *J* = 8.8 Hz), 6.96 (t, 1H, *J* = 6.9 Hz), 7.27-7.36 (m, 4H), 7.72 (m, 1H), 9.47 (d, 1H, *J* = 6.9 Hz) (NH missing); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  ppm 16.6, 61.7, 112.4, 114.2, 115.5, 127.4, 128.7, 130.5, 130.7, 132.4, 133.0, 133.8, 134.3, 145.7, 153.3, 155.1, 182.3; HPLC, Tr = 0.88 min; MS (ESI<sup>+</sup>): m/z 325.2 [M+H]<sup>+</sup>, 327.3 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>ClN<sub>4</sub>O 325.0856, found 325.0859.

#### 4.3.7 (4S)-4-Methyl-2-(4-iodophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1g**)

Yellow solid (37 mg, yield 27%); mp: 103-104°C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +13 (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  ppm 1.46 (d, 3H, *J* = 7.0 Hz), 4.06 (q, 1H, *J* = 7.0 Hz), 7.15 (ddd, 1H, *J* = 6.8, 6.0, 2.4 Hz), 7.65 (m, 2H), 7.76 (d, 2H, *J* = 8.5 Hz), 7.88 (d, 2H, *J* = 8.5 Hz), 9.53 (d, 1H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz):  $\delta$  ppm 15.8, 59.2, 99.9, 114.7, 115.2, 116.6, 129.6, 132.2, 132.3, 136.3, 138.6, 138.9, 147.9, 160.2, 184.8; HPLC, Tr = 1.05 min; MS (ESI<sup>+</sup>): m/z 417.0 [M+H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>OI 417.0212, found 417.0215.

4.3.8 (4S)-4-Methyl-2-(2,4-dichlorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1h**)

Yellow solid (46 mg, yield 39%); mp: 112-114°C;  $[\alpha]_{\text{D}}^{20} = +18$  (c 0.6, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz): δ ppm 1.54 (d, 3H, *J* = 7.0 Hz), 4.10 (q, 1H, *J* = 7.0 Hz), 7.18 (td, 1H, *J* = 6.8, 1.2 Hz), 7.47 (dd, 1H, *J* = 8.4, 1.9 Hz), 7.65 (m, 4H), 9.56 (d, 1H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz): δ ppm 15.8, 58.5, 114.7, 115.4, 116.8, 128.5, 129.5, 130.8, 132.2, 133.8, 135.6, 135.7, 138.1, 144.8, 145.1, 147.8, 183.8; HPLC, Tr = 1.01 min; MS (ESI<sup>+</sup>): *m/z* 359.0 [M+H]<sup>+</sup>, 361.0 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>OCl<sub>2</sub> 359.0466, found 359.0465.

4.3.9 (4S)-4-Methyl-2-(3-trifluoromethyl-4-chlorophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]-imidazo-[4,5-d][1,3]diazepin-5-one (**1i**)

Yellow solid (37 mg, yield 29%); mp: 118-119°C;  $[\alpha]_{\text{D}}^{20} = -16$  (c 0.5, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz): δ ppm 1.48 (d, 3H, *J* = 7.0 Hz), 4.09 (q, 1H, *J* = 7.0 Hz), 7.15 (t, 1H, *J* = 7.0 Hz), 7.65 (m, 2H), 7.75 (d, 1H, *J* = 8.5 Hz), 8.22 (d, 1H, *J* = 8.2 Hz), 8.45 (d, 1H, *J* = 1.8 Hz), 9.53 (d, 1H, *J* = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 16.7, 61.1, 112.5, 114.6, 115.3, 120.7, 124.3, 128.5, 128.6, 128.8, 131.1, 131.9, 133.6, 133.9, 135.9, 145.4, 154.2, 182.7; NMR <sup>19</sup>F (MeOD, 300 MHz) : δ -63.96; HPLC, Tr = 1.25 min; MS (ESI<sup>+</sup>): *m/z* 393.1 [M+H]<sup>+</sup>; HRMS calcd for C<sub>18</sub>H<sub>13</sub>ClF<sub>3</sub>N<sub>4</sub>O 393.0730, found 393.0735.

4.3.10 (4S)-4-Methyl-2-(4-ethoxycarbonylphenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1l**)

Yellow solid (48 mg, yield 40%); mp: 160-165°C (dec);  $[\alpha]_{\text{D}}^{20} = -6$  (c 0.1, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 1.40 (t, 3H, *J* = 7.1 Hz), 1.63 (d, 3H, *J* = 6.8 Hz), 4.22 (q, 1H, *J* = 6.8 Hz), 4.38 (q, 2H, *J* = 7.1 Hz), 7.03 (td, 1H, *J* = 6.9, 1.1 Hz), 7.29 (m, 1H), 7.45 (dd, 1H, *J* = 8.5, 7.3 Hz), 8.07 (m, 4H), 9.52 (d, 1H, *J* = 6.9 Hz) (NH missing); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 14.4, 16.6, 60.6, 61.5, 112.6, 114.1, 115.7, 128.6, 129.1, 129.9, 130.6, 130.7, 133.3, 138.9, 145.9, 154.2, 165.9, 182.5; HPLC, Tr = 1.03 min; MS (ESI<sup>+</sup>): *m/z* 363.1 [M+H]<sup>+</sup>; HRMS calcd for C<sub>20</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> 363.1457, found 363.1458.

4.3.11 (4S)-4-Methyl-2-(4-methylbenzamide)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1m**)

Yellow solid (14 mg, yield 12%); mp: 136-137°C;  $[\alpha]_{\text{D}}^{20} = -58$  (c 0.3, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  ppm 1.54 (d, 3H,  $J = 7.0$  Hz), 2.96 (s, 3H), 4.19 (q, 1H,  $J = 7.0$  Hz), 7.26 (td, 1H,  $J = 6.7, 1.6$  Hz), 7.74 (m, 2H), 7.97 (d, 2H,  $J = 8.4$  Hz), 8.12 (d, 2H,  $J = 8.4$  Hz), 9.60 (d, 1H,  $J = 6.7$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75 MHz):  $\delta$  ppm 15.5, 30.7, 59.4, 116.0, 116.3, 117.6, 128.5, 129.8, 131.1, 133.1, 134.6, 138.1, 139.2, 151.6, 156.3, 166.5, 184.0; HPLC, Tr = 0.79 min; MS (ESI<sup>+</sup>): m/z 348.1 [M+H]<sup>+</sup>; HRMS calcd for  $\text{C}_{19}\text{H}_{18}\text{N}_5\text{O}_2$  348.1460, found 348.1461.

4.3.12 (4S)-4-Methyl-2-(4-acetamidophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1n**)

Yellow solid (14 mg, yield 12%); mp: 146-150°C (dec);  $[\alpha]_{\text{D}}^{20} = +21$  (c 0.2, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  ppm 1.68 (d, 3H,  $J = 7.0$  Hz), 2.20 (s, 3H), 4.42 (q, 1H,  $J = 7.0$  Hz), 7.36 (td, 1H,  $J = 6.7$  Hz, 1.4 Hz), 7.79-7.84 (m, 2H), 7.90 (d, 2H,  $J = 8.7$  Hz), 7.99 (d, 2H,  $J = 8.7$  Hz), 9.60 (d, 1H,  $J = 6.7$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta$  ppm 14.2, 24.1, 59.9, 113.6, 116.8, 117.0, 120.4, 124.5, 129.9, 133.3, 133.8, 146.5, 146.7, 151.2, 163.3, 172.2, 181.7; HPLC, Tr = 0.84 min; MS (ESI<sup>+</sup>): m/z 348.2 [M+H]<sup>+</sup>; HRMS calcd for  $\text{C}_{19}\text{H}_{18}\text{N}_5\text{O}_2$  348.1460, found 348.1459.

4.3.13 (4S)-4-Methyl-2-(4-cyanophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1o**)

Yellow solid (35 mg, yield 34%); mp: 174-175°C (dec);  $[\alpha]_{\text{D}}^{20} = -17$  (c 0.5, MeOH); two conformers detected by NMR in DMSO.  $^1\text{H}$  NMR ( $\text{DMSO } d_6$ , 300 MHz):  $\delta$  ppm 1.32 (d, 3H,  $J = 7.1$  Hz), 4.03 (m, 1H), 7.19 (td, 1H,  $J = 6.8, 1.9$  Hz), 7.66 (m, 2H), 8.02 (d, 2H,  $J = 8.5$  Hz), 8.20 (d, 2H,  $J = 8.5$  Hz), 8.76 (d, 1H,  $J = 5.7$  Hz), 9.48 (d, 1H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{DMSO } d_6$ , 75 MHz):  $\delta$  ppm 15.4, 56.5, 112.9, 113.8, 113.9, 116.1, 118.4, 127.8, 129.6, 129.8, 130.4, 132.2, 132.3, 140.0, 146.3, 152.7, 157.4, 183.0; HPLC, Tr = 0.90 min; MS (ESI<sup>+</sup>): m/z 316.1 [M+H]<sup>+</sup>; HRMS calcd for  $\text{C}_{18}\text{H}_{14}\text{N}_5\text{O}$  316.1198, found 316.1200.

4.3.14 (4S)-4-Methyl-2-(4-trifluoromethylphenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1p**)

Yellow solid (15 mg, yield 13%); mp: 140-141°C;  $[\alpha]_D^{20} = +5$  (c 0.5, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  ppm 1.48 (d, 3H,  $J = 7.0$  Hz), 4.10 (q, 1H,  $J = 7.0$  Hz), 7.15 (m, 1H), 7.65 (m, 2H), 7.81 (d, 2H,  $J = 8.2$  Hz), 7.89 (s, 1H), 8.20 (d, 2H,  $J = 8.2$  Hz), 9.53 (d, 1H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75 MHz):  $\delta$  ppm 15.9, 59.3, 114.2, 115.3, 116.7, 126.4, 126.5, 129.6, 131.2, 132.3, 134.1, 134.5, 140.5, 147.9, 159.3, 184.6; HPLC, Tr = 1.09 min; MS (ESI<sup>+</sup>): m/z 359.0 [M+H]<sup>+</sup>; HRMS calcd for  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_3$  359.1120, found 359.1118.

4.3.15 (4S)-4-Methyl-2-(1-naphthyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1q**)

Yellow solid (67 mg, yield 60%); mp: 137-139°C;  $[\alpha]_D^{20} = +25$  (c 0.4,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  ppm 1.70 (d, 3H,  $J = 6.8$  Hz), 4.33 (q, 1H,  $J = 6.8$  Hz), 4.67 (bs, 1H), 6.70 (d, 1H,  $J = 8.8$  Hz), 6.99 (td, 1H,  $J = 7.0, 1.1$  Hz), 7.28 (m, 1H), 7.45 (m, 3H), 7.79 (ddd, 2H,  $J = 8.1, 7.0, 1.6$  Hz), 7.88 (d, 1H,  $J = 8.2$  Hz), 8.1 (d, 1H,  $J = 8.2$  Hz), 9.49 (d, 1H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  ppm 16.8, 62.3, 111.9, 114.0, 115.1, 124.8, 125.0, 126.4, 127.4, 128.5, 129.2, 129.8, 130.6, 131.5, 131.8, 132.2, 133.8, 145.4, 152.7, 155.5, 182.3; HPLC, Tr = 0.97 min; MS (ESI<sup>+</sup>): m/z 341.21 [M+H]<sup>+</sup>; HRMS calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_4\text{O}$  341.1402, found 341.1405.

4.3.16 (4S)-4-Methyl-2-(2-naphthyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1r**)

Yellow solid (70 mg, yield 62%); mp: 146-147°C;  $[\alpha]_D^{20} = -67$  (c 0.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  ppm 1.62 (d, 3H,  $J = 6.9$  Hz), 4.00 (q, 1H,  $J = 6.9$  Hz), 6.90 (t, 1H,  $J = 6.9$  Hz), 7.08 (bs, 1H), 7.22 (d, 1H,  $J = 7.6$  Hz), 7.42 (m, 2H), 7.76 (m, 3H), 8.02 (dd, 1H,  $J = 8.6, 1.7$  Hz), 8.43 (s, 1H), 9.44 (d, 1H,  $J = 6.9$  Hz) (1H missing);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  ppm 16.6, 60.7, 112.5, 113.7, 115.6, 125.9, 126.8, 127.6, 127.8, 128.5 (2C), 128.9, 129.8, 130.2, 132.0, 132.7, 134.9, 145.9, 154.9, 156.5, 182.8; HPLC, Tr = min; MS (ESI<sup>+</sup>): m/z [M+H]<sup>+</sup>, 385.5 [M+2H]<sup>+</sup>; HRMS calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_4\text{O}$  341.1402, found 341.1404.

4.3.17 (4S)-4-Methyl-2-(4-Biphenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1s**)

Yellow solid (70 mg, yield 58%); mp: 109-110°C;  $[\alpha]_{\text{D}}^{20} = -65$  (c 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ ppm 1.70 (bs, 3H), 4.13 (q, 1H, *J* = 6.7 Hz), 6.29 (bs, 1H), 6.91 (t, 1H, *J* = 6.0 Hz), 7.21 (m, 1H), 7.33-7.44 (m, 4H), 7.52 (d, 2H, *J* = 7.5 Hz), 7.61 (d, 2H, *J* = 8.3 Hz), 8.07 (d, 2H, *J* = 7.8 Hz), 9.48 (d, 1H, *J* = 6.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ ppm 16.7, 63.5, 113.5, 114.9, 127.2, 127.3, 128.0, 128.3, 128.9, 129.8, 129.9, 130.0, 134.0, 140.0, 143.4, 145.4, 153.0, 153.9, 182.7; HPLC, Tr = 1.16 min; MS (ESI<sup>+</sup>): *m/z* 367.3 [M+H]<sup>+</sup>; HRMS calcd for C<sub>23</sub>H<sub>19</sub>N<sub>4</sub>O 367.1559, found 367.1559.

#### 4.3.18 (4S)-3,4-Dimethyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1t**)

Yellow solid (41 mg, yield 32 %); mp: 94-96°C;  $[\alpha]_{\text{D}}^{20} = +153$  (c 0.3, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 1.32 (d, 3H, *J* = 7.3 Hz), 3.05 (s, 3H), 3.92 (q, 1H, *J* = 7.3 Hz), 6.98 (ddd, 1H, *J* = 8.1, 7.0, 1.2 Hz), 7.47 (ddd, 1H, *J* = 8.9, 8.1, 1.2 Hz), 7.54 (d, 2H, *J* = 8.5 Hz), 7.67 (m, 3H), 9.47 (d, 1H, *J* = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 13.9, 42.2, 65.1, 113.0, 113.9, 116.9, 126.3, 128.4, 130.3, 131.9, 132.2, 135.3, 146.8, 157.4, 160.4, 183.4; HPLC, Tr = 1.04 min; MS (ESI<sup>+</sup>): *m/z* 383.0 [M+H]<sup>+</sup>, 385.0 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>18</sub>H<sub>16</sub>BrN<sub>4</sub>O 383.0507, found 383.0507.

#### 4.3.19 2-(4-Bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1u**)

Yellow solid (71 mg, yield 61%); mp: 186-188°C (dec); <sup>1</sup>H NMR (DMSO d<sub>6</sub>, 400 MHz): δ ppm 4.04 (s, 2H), 7.17 (dd, 1H, *J* = 6.5 Hz, 2.2 Hz), 7.65 (m, 2H), 7.74 (m, 3H), 7.94 (d, 2H, *J* = 8.4 Hz), 8.05 (bs, 1H), 9.40 (d, 1H, *J* = 6.5 Hz); <sup>13</sup>C NMR (DMSO d<sub>6</sub>, 100 MHz): δ ppm 54.9, 113.8, 115.8, 125.3, 127.8, 129.6, 130.0, 130.5, 130.8, 131.4, 134.2, 145.9, 157.7, 180.9; HPLC, Tr = 0.94 min; MS (ESI<sup>+</sup>): *m/z* 355.2 [M+H]<sup>+</sup>, 357.1 [M+2+H]<sup>+</sup>; HRMS calcd for C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>OBr 355.0194, found 355.0192.

#### 4.3.20 (4S)-4-Isopropyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1v**)

Yellow solid (96 mg, yield 73%); mp: 131-132°C;  $[\alpha]_{\text{D}}^{20} = +37$  (c 0.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 0.94 (d, 3H, *J* = 5.5 Hz), 1.12 (d, 3H, *J* = 5.5 Hz), 1.24 (m, 1H), 3.74 (d, 1H, *J* = 9.3 Hz), 6.99 (dd, 1H, *J* = 6.8 Hz, 7.9 Hz), 7.45 (m, 4H), 7.90 (d, 2H, *J* = 8.2 Hz), 9.52 (d, 1H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 19.7, 28.8,

31.3, 69.4, 113.1, 113.7, 116.0, 126.5, 127.3, 128.4, 129.8, 130.3, 130.6, 131.8, 134.4, 146.5, 182.1; HPLC, Tr = 1.13 min; MS (ESI<sup>+</sup>): m/z 397.1 [M+H]<sup>+</sup>, 399.1 [M+2+H]<sup>+</sup>; HRMS calcd for C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>OBr 397.0664, found 397.0664.

#### 4.3.21 (4S)-4-Ethyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1w**)

Yellow solid (32 mg, yield 25%); mp: 104-105°C; [α]<sub>D</sub><sup>20</sup> = +67 (c 0.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 1.00 (t, 3H, J = 7.4 Hz), 1.78 (m, 1H), 4.06 (dd, 1H, J = 8.8, 6.2 Hz), 7.05 (t, 1H, J = 7.1 Hz), 7.37 (d, 2H, J = 8.5 Hz), 7.50 (m, 2H), 7.85 (d, 2H, J = 8.5 Hz), 9.50 (d, 1H, J = 7.1 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 10.9, 24.0, 65.6, 112.3, 115.0, 115.3, 127.5, 128.8, 131.2, 131.6, 131.9, 132.9, 144.6, 144.7, 153.2, 182.4; HPLC, Tr = 1.09 min; MS (ESI<sup>+</sup>): m/z 383.5 [M+H]<sup>+</sup>, 385.5 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>OBr 383.0507, found 383.0507.

#### 4.3.22 (4S)-4-Benzoyloxycarbonylaminobutyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1x**)

Yellow solid (66 mg, yield 36%); mp: 176-177°C; [α]<sub>D</sub><sup>20</sup> = -13.9 (c 0.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ ppm 0.82 (m, 2H), 1.23 (m, 2H), 1.99 (m, 3H), 3.22 (d, 2H, J = 5.0 Hz), 3.91 (dd, 1H, J = 5.6 Hz, 6.9 Hz), 4.94 (t, 1H, J = 5.8 Hz), 5.03 (s, 2H), 6.97 (t, 1H, J = 7.0 Hz), 7.30 (m, 6H), 7.40 (dd, 1H, J = 7.0 Hz, 8.8 Hz), 7.50 (d, 2H, J = 8.5 Hz), 7.83 (d, 2H, J = 8.5 Hz), 9.47 (d, 1H, J = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ ppm 23.4, 29.8, 29.9, 40.9, 66.8, 66.9, 113.9, 126.6, 126.7, 128.1, 128.2, 128.5, 128.6, 129.8, 130.4, 130.7, 132.0, 134.3, 135.3, 136.7, 153.4, 156.6, 156.7, 183.2; HPLC, Tr = 1.39 min; MS (ESI<sup>+</sup>): m/z 560.0 [M+H]<sup>+</sup>, 562.1 [M+2+H]<sup>+</sup>; HRMS calcd for C<sub>28</sub>H<sub>27</sub>N<sub>5</sub>O<sub>3</sub>Br 560.1297, found 560.1300.

#### 4.3.23 (4S)-4-Tert-butyloxymethyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo-[4,5-d][1,3]diazepin-5-one (**1y**)

Yellow solid (80 mg, yield 55%); mp: 118-119°C; [α]<sub>D</sub><sup>20</sup> = -29 (c 0.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ ppm 1.23 (s, 9H), 3.70 (t, 1H, J = 9.9 Hz), 3.85 (dd, 1H, J = 9.9 Hz, 4.2 Hz), 4.18 (m, 1H), 4.66 (m, 1H), 7.02 (t, 1H, J = 7.0 Hz), 7.51 (dd, 1H, J = 8.1 Hz, 7.0 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.68 (d, 1H, J = 8.1 Hz), 8.01 (d, 2H, J = 8.8 Hz), 9.56 (d, 1H, J = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ ppm 29.9, 59.4, 61.5, 74.6,

114.7, 117.1, 127.1, 128.2, 128.8, 129.9, 130.6, 130.8, 132.1, 135.2, 147.5, 158.7, 181.7; HPLC, Tr = 1.26 min; MS (ESI<sup>+</sup>): m/z 441.0 [M+H]<sup>+</sup>, 443.0 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>Br 441.0926, found 441.0923.

#### 4.4 Synthesis of (4S)-4-aminobutyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo[4,5-d][1,3]diazepin-5-one (**1x'**)

A solution of compound **1x** (21 mg, 0.037 mmol) in a mixture of HBr 33% in acetic acid (5 mL) was stirred at room temperature for 30 min. After completion of the reaction (monitoring by HPLC), the solvent was removed under reduced pressure. Water (5 mL) was added and the solution was treated with a 28% aqueous ammonia solution and extracted with dichloromethane (3 x 20 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was evaporated *in vacuo*. The crude was then washed with diethyl ether (2 x 10 mL) to afford compound **1x'** as a red powder (11 mg, yield 67%). Mp: 91-92°C; [α]<sub>D</sub><sup>20</sup> = -18 (c 1.0, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz): δ ppm 1.60 (m, 4H), 1.79 (m, 2H), 2.76 (dd, 2H, J = 6.4 Hz, 6.4 Hz), 3.98 (dd, 1H, J = 6.6 Hz), 7.18 (td, 1H, J = 6.8 Hz, 2.2 Hz), 7.69 (m, 4H), 7.97 (d, 2H, J = 8.6 Hz), 9.56 (d, 1H, J = 6.87 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz): δ ppm 23.5, 28.1, 32.1, 40.1, 58.0, 115.7, 119.5, 129.5, 130.4, 131.0, 131.3, 132.2, 132.8, 133.5, 135.9, 144.0, 168.4, 186.3; HPLC, Tr = 0.94 min; MS (ESI<sup>+</sup>): m/z 426.1 [M+H]<sup>+</sup>, 428.1 [M+2H]<sup>+</sup>; HRMS calcd for C<sub>20</sub>H<sub>21</sub>N<sub>5</sub>OBr 426.0929, found 426.0927.

#### 4.5 Synthesis of (4S)-4-hydroxymethyl-2-(4-bromophenyl)-3,4-dihydro-5H-pyrido[1',2':1,2]imidazo[4,5-d][1,3]diazepin-5-one (**1y'**)

A solution of compound **1y** (75 mg, 0.17 mmol) in a 95/2.5/2.5 v/v/v mixture of trifluoroacetic acid, triisopropylsilane and water (10 mL) was stirred at room temperature for 3 h. After completion of the reaction (HPLC analysis), the medium was evaporated to dryness. A saturated NaHCO<sub>3</sub> aqueous solution (10 mL) was added and then extracted with dichloromethane (3 x 10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on alumina, eluted first with DCM/EtOH 98/2 v/v and then by DCM/EtOH 95/5 v/v, to afford compound **1y'** as a white powder (20 mg, yield 30.5%).

Mp: 158-160°C (dec);  $[\alpha]_{\text{D}}^{20} = +167$  (c 0.1, DMSO);  $^1\text{H NMR}$  (DMSO  $d_6$ , 300 MHz):  $\delta$  ppm 3.53 (m, 1H), 3.98 (m, 1H), 5.12 (m, 1H), 7.18 (m, 1H), 7.66 (m, 2H), 7.75 (d, 2H,  $J = 8.1$  Hz), 7.96 (d, 2H,  $J = 8.1$  Hz), 8.72 (bs, 1H), 9.45 (d, 1H,  $J = 6.6$  Hz);  $^{13}\text{C NMR}$  (DMSO  $d_6$ , 75 MHz):  $\delta$  ppm 58.9, 59.2, 113.8, 115.9, 125.2, 127.9, 130.6, 131.2, 131.3, 131.6, 146.3, 151.6, 156.8, 161.4, 181.3; HPLC, Tr = 0.94 min; MS (ESI<sup>+</sup>): m/z 385.0 [M+H]<sup>+</sup>, 387.0 [M+2+H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>Br 385.0300, found 385.0294.

#### 4.6 Synthesis of (4S,5S)-4-methyl-2-(4-bromophenyl)-4,5-dihydro-3H-pyrido[1',2':1,2]imidazo[4,5-d][1,3]diazepin-5-ol (**1z**)

A solution of compound JMV5038 (50 mg, 0.13 mmol) in MeOH (2 mL) was cooled to 0°C. Then NaBH<sub>4</sub> (3 equiv., 15 mg, 0.39 mmol) was added and the mixture was stirred at 0°C for 40 min (monitoring by TLC). After reaction completion, a saturated NaHCO<sub>3</sub> aqueous solution (5 mL) was added to quench the reaction. The solvent was then removed and the resulting residue was dissolved in ethyl acetate (20 mL). The solution was washed with a saturated NaHCO<sub>3</sub> aqueous solution. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed under reduced pressure. The residue was purified by chromatography on alumina, eluted first by DCM and then by DCM/EtOH 98/2 v/v to afford compound **1z** as a white powder (18 mg, yield 37%). Mp: 121-122°C;  $[\alpha]_{\text{D}}^{20} = -43$  (c 0.4, MeOH);  $^1\text{H NMR}$  (CD<sub>3</sub>OD, 300 MHz):  $\delta$  ppm 1.60 (d, 3H,  $J = 6.9$  Hz), 3.67 (m, 1H), 5.26 (d, 1H,  $J = 1.0$  Hz), 6.95 (td, 1H,  $J = 6.9$  Hz, 1.1 Hz), 7.32 (ddd, 1H,  $J = 9.1$  Hz, 6.9 Hz, 1.2 Hz), 7.47 (dd, 1H,  $J = 9.1$  Hz, 1.1 Hz), 7.59 (d, 2H,  $J = 8.6$  Hz), 7.81 (d, 2H,  $J = 8.6$  Hz), 8.26 (d, 1H,  $J = 6.9$  Hz);  $^{13}\text{C NMR}$  (CD<sub>3</sub>OD, 75 MHz):  $\delta$  ppm 17.22, 54.6, 69.4, 113.4, 114.4, 116.5, 125.1, 126.4, 126.5, 131.7, 132.4, 136.9, 143.7, 149.0, 159.8; HPLC, Tr = 0.85 min; MS (ESI<sup>+</sup>): m/z 371.1 [M+H]<sup>+</sup>, 373.0 [M+2+H]<sup>+</sup>; HRMS calcd for C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>OBr 371.0507, found 371.0509.

#### 4.7 Biological evaluation

##### 4.7.1 MTT assay

*Cell culture conditions:* human melanoma (MDA-MB-435 from ATCC, American Type Culture Collection, Manassas, VA) cells were cultured in a 1:1 mixture of Ham's F12 and Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal

calf serum (FCS), 50  $\mu\text{g}\cdot\text{mL}^{-1}$  gentamicin, 100 IU penicillin, and 100  $\mu\text{g}\cdot\text{mL}^{-1}$  streptomycin. The cell line was allowed to grow at 37°C in an atmosphere containing humidified air with 5%  $\text{CO}_2$ .

*Cytotoxicity evaluation:* cells were seeded into 96-well plates at  $10^4$  cells per well in 100  $\mu\text{L}$  culture medium and allowed to grow for 24 h. All compounds were freshly dissolved in media at a concentration of 100  $\mu\text{M}$ . Then cells were incubated for 48 h with compounds in a 0.01-100  $\mu\text{M}$  concentration range. Then, a MTT assay was performed to evaluate the toxicity. Briefly, cells were incubated for 4 h with 0.5  $\text{mg}\cdot\text{mL}^{-1}$  MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in media. The MTT media solution was then removed and the precipitated crystals were dissolved in EtOH/DMSO (1/1 v/v). The solution absorbance was evaluated at 540 nm.

#### 4.7.2 SRB assay

Primary anticancer assay was performed on a panel of sixty human tumour cell lines derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, Maryland, USA [20]. Tested compounds were added to the cell culture at a single concentration (10  $\mu\text{M}$ ) and the cultures were incubated for 48h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of treated cells when compared to untreated control cells. The percentage growth was evaluated spectrophotometrically *versus* controls not treated with test agents.

#### 4.7.3 HGK assay

Kinase selectivity was evaluated on HGK at CEREP (Paris, France) [21]. The effects of compounds **1y**, **1h**, **1v**, **1s**, **1n** and **1** on HGK kinase activity were determined by measuring the phosphorylation of the substrate phospho-Ulight-FLGFTYVAP using a human recombinant enzyme. The LANCE detection method was used. All compounds were tested at concentrations of 1.0, 5.0, 10.0, 20.0, 50.0 and 100.0  $\mu\text{M}$ . Staurosporine was used as a reference compound. Data are expressed as a percent of control specific activity (observed specific activity/control specific activity x 100) obtained in the presence of the test compounds. The  $\text{IC}_{50}$  value is the concentration causing a half-maximal inhibition of control specific activity.

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## Supplementary data

Supplementary data related to this article can be found at

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- [21] Cerep website, <http://www.cerep.fr>.

## List of captions

**Fig 1.** Structure of JMV5038 and studied modifications

**Scheme 1. 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<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> substituents are referenced in Table 2

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

**Table 1.** Analysis of **1z** conformers by molecular dynamic simulation

**Table 2.** Structure and GI<sub>50</sub> of compounds on the MDA-MB-435 cell line

**Fig. 2.** Evaluation of cell growth at a single-dose (10 μM) of compounds **JMV5038, 1d, 1h, 1y** and **1r**, according to the NCI-60 cancer cell lines screening (sulforhodamine B test). For all data, see SI

**Table 3.** HGK IC<sub>50</sub> (μM) and GI<sub>50</sub> on the MDA-MB-435 cell line of selected compounds.

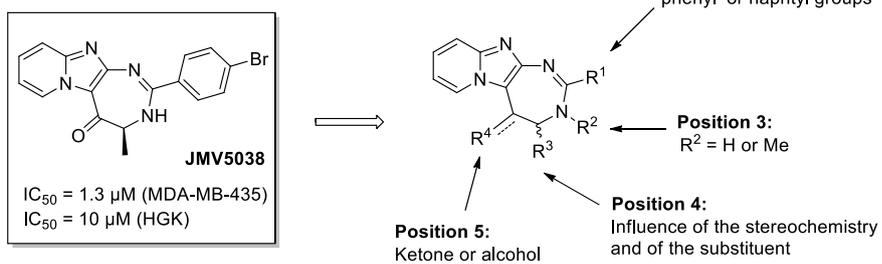
## Highlights

28 novel pyrido-imidazodiazepinone derivatives were synthesized

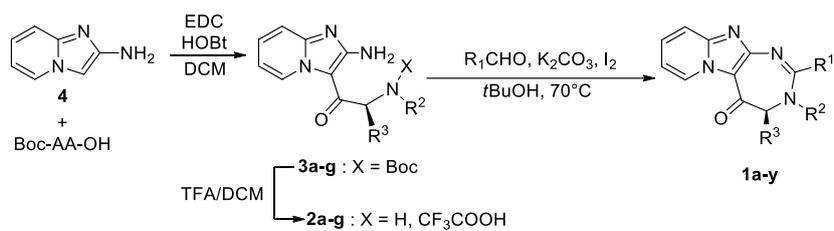
SAR studies of position 2, 3, 4 and 5 of the diazepine core were performed

Their cytotoxicities were evaluated against the melanoma MDA-MB-435 cell line

Several compounds showed a specificity against the melanoma MDA-MB-435 cell line

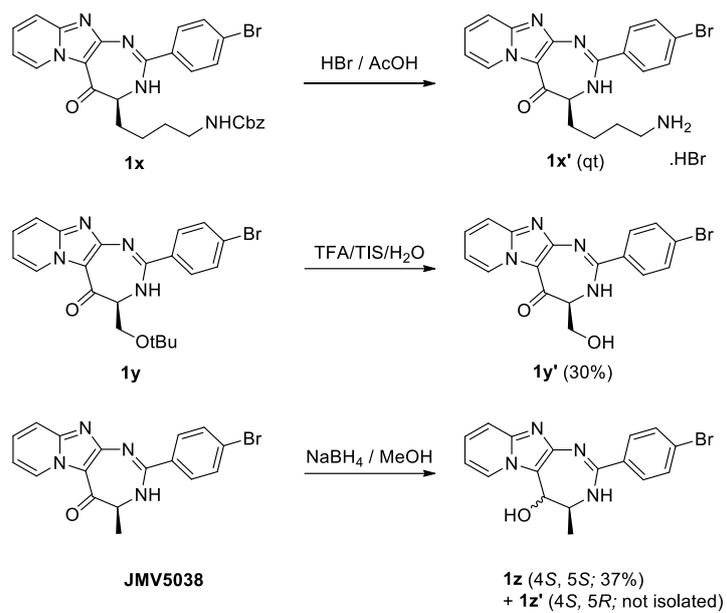


**Fig. 1.** Structure of JM5038 and studied modifications



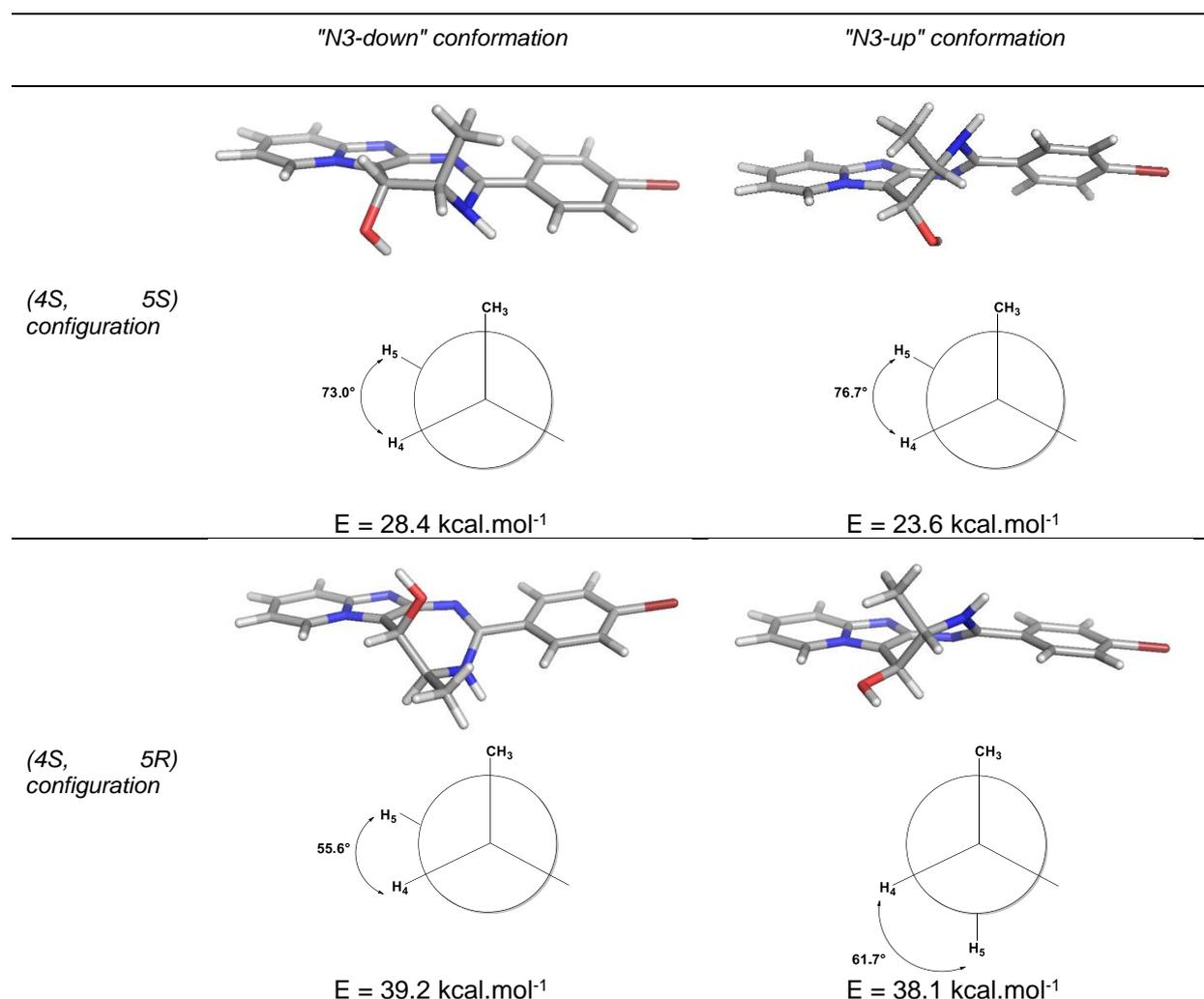
<b>a</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = \text{Me}$ )	<b>e</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = \text{CH}_2\text{O-}t\text{Bu}$ )
<b>b</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = \text{H}$ )	<b>f</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = (\text{CH}_2)_4\text{NHCbz}$ )
<b>c</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = \text{Et}$ )	<b>g</b> ( $\text{R}^2 = \text{Me}, \text{R}^3 = \text{Me}$ )
<b>d</b> ( $\text{R}^2 = \text{H}, \text{R}^3 = \text{iPr}$ )	

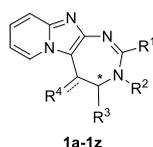
**Scheme 1.** Synthesis of compounds **1a-y**. Reagents: Boc-AA-OH = Boc-Ala-OH, Boc-Gly-OH, Boc-Abu-OH, Boc-Val-OH, Boc-Ser(*t*Bu)-OH, Boc-Lys(Cbz)-OH or Boc-*N*-Me-Ala-OH.  $\text{R}^1$ ,  $\text{R}^2$  and  $\text{R}^3$  substituents are referenced in Table 2



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

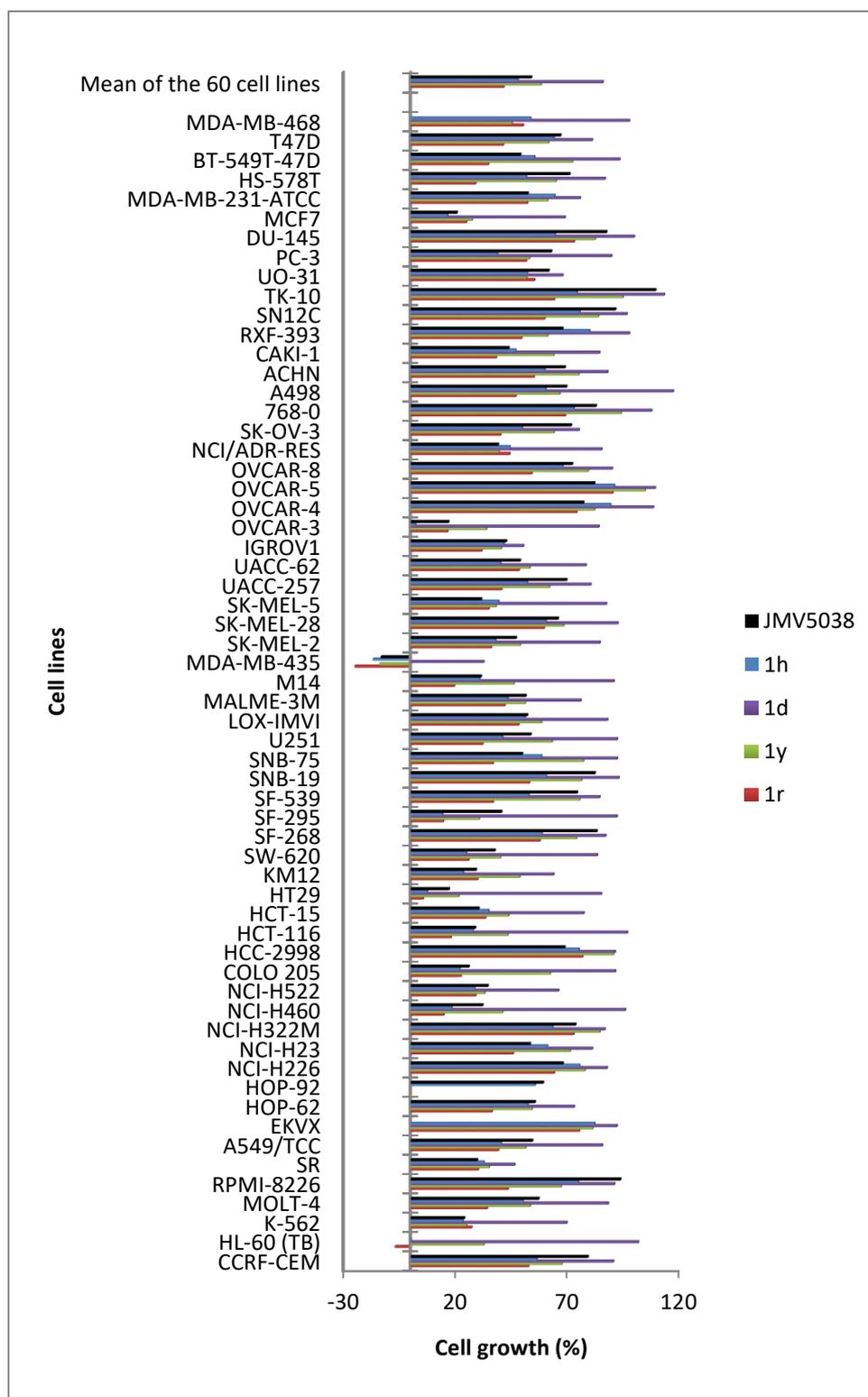
**Table 1.** Analysis of **1z** conformers by molecular dynamic simulation



**Table 2.** Structure and GI<sub>50</sub> of compounds on the MDA-MB-435 cell line

Entry	Compounds	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	GI <sub>50</sub> (μM) <sup>a</sup>
1	<b>JMV5038</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	1.3 ± 0.53
2	<b>1</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(R) Me	=O	> 100
3	<b>1a</b>	3-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
4	<b>1b</b>	2-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	8.35 ± 0.6
5	<b>1c</b>	4-F-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
6	<b>1d</b>	4-Cl-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	5.0 ± 0.5
7	<b>1e</b>	3-Cl-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	10 ± 3
8	<b>1f</b>	2-Cl-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	6.5 ± 0.5
9	<b>1g</b>	4-I-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	1.35 ± 0.3
10	<b>1h</b>	2,4-diCl-C <sub>6</sub> H <sub>3</sub>	H	(S) Me	=O	1.15 ± 0.22
11	<b>1i</b>	4-Cl,3-CF <sub>3</sub> -C <sub>6</sub> H <sub>3</sub>	H	(S) Me	=O	> 10
12	<b>1j</b>	2-Me-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
13	<b>1k</b>	3,4-diOMe-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
14	<b>1l</b>	4-CO <sub>2</sub> Et-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
15	<b>1m</b>	4-CONHMe-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
16	<b>1n</b>	4-NHCOMe-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
17	<b>1o</b>	4-CN-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
18	<b>1p</b>	4-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	> 10
19	<b>1q</b>	1-naphtyl	H	(S) Me	=O	1.4 ± 0.8
20	<b>1r</b>	2-naphtyl	H	(S) Me	=O	1.4 ± 0.45
21	<b>1s</b>	4-C <sub>6</sub> H <sub>4</sub> -C <sub>6</sub> H <sub>4</sub>	H	(S) Me	=O	40 ± 8
22	<b>1t</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	Me	(S) Me	=O	> 10
23	<b>1u</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	H	=O	> 10
24	<b>1v</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) iPr	=O	50 ± 11
25	<b>1w</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) Et	=O	1.0 ± 0.34
26	<b>1x</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) (CH <sub>2</sub> ) <sub>4</sub> NHCbz	=O	> 10
27	<b>1y</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) CH <sub>2</sub> O <sup>t</sup> Bu	=O	0.95 ± 0.31
28	<b>1x'</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) (CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	=O	> 10
29	<b>1y'</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) CH <sub>2</sub> OH	=O	1.0 ± 0.19
30	<b>1z</b>	4-Br-C <sub>6</sub> H <sub>4</sub>	H	(S) Me	OH	> 10

<sup>a</sup> Values are mean of three independent experiments.



**Fig. 2.** Evaluation of cell growth at a single-dose (10  $\mu$ M) of compounds **JMV5038**, **1d**, **1h**, **1y** and **1r**, according to the NCI-60 cancer cell lines screening (sulforhodamine B test). For all data, see SI.

**Table 3.** HGK IC<sub>50</sub> (μM) and GI<sub>50</sub> on the MDA-MB-435 cell line of selected compounds.

<b>Compound</b>	<b>MDA-MB-435 GI<sub>50</sub> (μM)</b>	<b>HGK IC<sub>50</sub> (μM)<sup>a</sup></b>
<b>JMV5038</b> [9]	1.3 ± 0.53	10
<b>1y</b>	0.95 ± 0.31	5.6
<b>1h</b>	1.15 ± 0.22	> 100
<b>1v</b>	50 ± 11	44
<b>1s</b>	40 ± 8	> 100
<b>1n</b>	> 100	> 100
<b>1</b>	> 100	> 100

<sup>a</sup> The results are expressed as the mean of two independent separate experiments.