European Journal of Medicinal Chemistry 218 (2021) 113258

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Bioinspired imidazo[1,2-*a*:4,5-*c*']dipyridines with dual antiproliferative and anti-migrative properties in human cancer cells: The SAR investigation

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ARTICLE INFO

Article history:
Received 10 November 2020
Received in revised form
28 January 2021
Accepted 30 January 2021
Available online 13 February 2021

Keywords:
Imidazo[1,2-*a*:4,5-*c*']dipyridines
Antiproliferative activity
Anti-migration activity
Harmine analogues

ABSTRACT

Herein, we report the design, synthesis and evaluation of novel bioinspired imidazo[1,2-*a*:4,5*c*']dipyridines. The structural optimization identified four anti-proliferative compounds. Compounds **11**, **18**, **19** and **20** exhibited excellent anticancer activities *in vitro* with IC_{50} of 0.4–5 μM against three human cancer cell lines (MDA-MB-468, MDA-MB-435s and MDA-MB-231). These four compounds induced apoptosis in MDA-MB-231 cells in a dose-dependent manner, targeting different apoptotic proteins expression: **11** increased the expression of pro-apoptotic Bax protein while **18**–**20** reduced the level of anti-apoptotic Bcl-2 protein. Compounds **18** and **19** also reduced MDA-MB-231 cells proliferation as measured by Ki-67 staining.

Furthermore, compounds were also tested for the ability to inhibit cell migration in the highly aggressive human MDA-MB-435s cell line. Six compounds of this series (**8, 15, 18, 22, 23, 24**) inhibited cell migration by 41–50% while four compounds (**20**, **25**, **27**, **30**) inhibited the migration by 53–62% in wound-healing experiments. Interestingly, compound **20** presented both antiproliferative and anti-migration activities and might be a promising anti-metastatic agent for cancer treatment.

1. Introduction

Breast cancer is the most commonly occurring cancer in women worldwide and the second most common cancer overall. From the World Health Organization, about 2.1 million new patients are diagnosed with breast cancer each year and about 627,000 died worldwide from the disease in 2018 [1]. According to the American Cancer Society, the 5-year survival rate for a localized breast cancer has improved significantly to 99%, while this rate drops to 26% if the cancer is diagnosed at a distant metastasis stage [2]. Metastasis development is a complex cascade that involves a first and necessary step of invasion, with the acquisition of the ability by malignant cells to degrade and migrate through extracellular matrices to distant tissues [3]. Inhibition of cancer cells migrative abilities constitutes a highly interesting approach. Indeed the biggest hurdle in controlling breast cancer associated mortality is the lack of effective antimetastatic therapy. Thus, anti-invasive drugs are urgently requested. They would be used in combination with other anticancer agents, not only for the treatment of localized tumors, but also in patients presenting with metastases at diagnosis.

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https://doi.org/10.1016/j.ejmech.2021.113258
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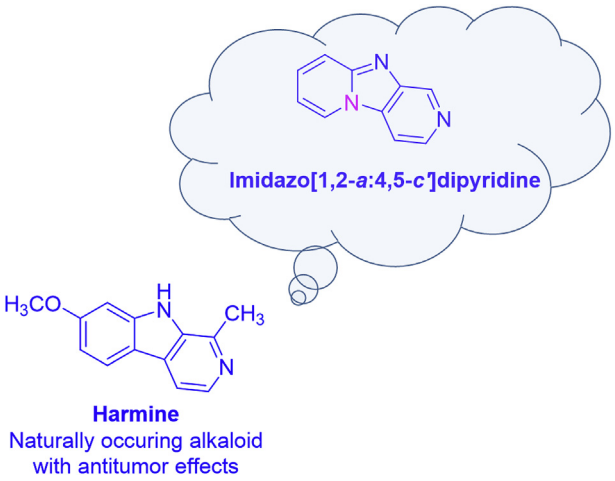


Fig. 1. Chemical structures of harmine, as representative β-carboline derivative, and designed imidazo[1,2-*a*:4,5-*c*']dipyridine core.

Indeed, a growing body of evidence indicates that the burden put on the primary tumor during radiotherapy or antiproliferative treatment may promote further metastatic dissemination and shorten survival time [4].

Natural product-oriented synthetic derivatives have played an important role in drug discovery [5], and the majority of chemotherapeutic agents currently used in clinical settings are derived from natural product scaffolds such as paclitaxel and vinblastine. In recent years, our laboratories have described an efficient one-pot sequence for the preparation of imidazo[1,2-*a*:4,5-*c*']dipyridines [6], analogues of the β-carboline scaffold present in many naturally occurring alkaloids (Fig. 1) [7]. β-Carbolines alkaloids such as harmine and its derivatives display several pharmacological activities [8], particularly antitumor effects. It is commonly reported that both natural and synthetic β-carboline alkaloids elicit their anticancer activities through multiple mechanisms such as DNA intercalation [9], topoisomerase I and II inhibition [10] or kinase inhibition (CDK, PLKs, IKK, DYRK1A) [11].

Imidazo[1,2-*a*:4,5-*c*']dipyridine scaffold was scarcely studied in the literature, and to the best of our knowledge only one publication mentioned an antimalarial activity for such tricyclic system [12]. However, imidazo[1,2-*a*:5,4-*b*']dipyridines were lately presented as anticancer agents inhibiting human prostate cancer cell DU-145 proliferation with an IC_{50} of 1.6 μM for the most active compound [13].

Regarding the similarity between β-carboline and imidazo[1,2-*a*:4,5-*c*']dipyridine, we got interested in investigating the potential anticancer activity of our compounds, drawing on all the relevant literature. Indeed the β-carboline analogues were hugely studied from decades in the field of oncology [14] and the structure-activity relationships of this scaffold against cancer cells was described in 2007 by Cao et al. [8a]. Six sites of modulation were characterized (Fig. 2).

Several harmine derivatives were developed on this model and studied in order to promote antiproliferative properties. Despite a high selectivity for cancer cells and an IC_{50} close to micromolar concentrations, these derivatives are generally difficult to develop as lead compounds for pre-clinical studies because of their poor solubility [15].

Imidazo[1,2-*a*:4,5-*c*']dipyridine structure present a supplementary nitrogen atom compared to harmine (Fig. 1). This difference seems tenuous enough to conserve antitumoral properties, but could critically affect the pharmacokinetic (specially improving the aqueous solubility) and the reactivity of the scaffold to extend the scope of the functionalization. Based on the SAR of the β-carboline scaffold (Fig. 2), we proposed in a first approach to explore positions 1, 2, 3, 7 and 10. Nevertheless, conversely to β-carboline, we rapidly noticed that position 2 of imidazo[1,2-*a*:4,5-*c*']dipyridines could not be studied. Indeed, N-2 was less nucleophilic than N-10 in our series of compounds, and the benzylation did not occurred on the 2 position but on the 10 position. Finally, we explored positions 1, 3, 7 and 10 (Fig. 3). As antiproliferative activities seemed to be conserved with the preliminary screened 10-substituted compounds (**8** and **10**, Table 1) we concluded that the presence but not the position of this benzyl group is determinant. Moreover, we have never been confronted to solubility limitation for our new imidazo [1,2-*a*:4,5-*c*']dipyridines.

In the present study, we explored the antiproliferative and antimigrative activities of this new series of compounds against breast cancer cells as part of our on-going research, and attempted to further elucidate the mechanisms of action, providing a basis for future development of these compounds as human breast cancer therapy.

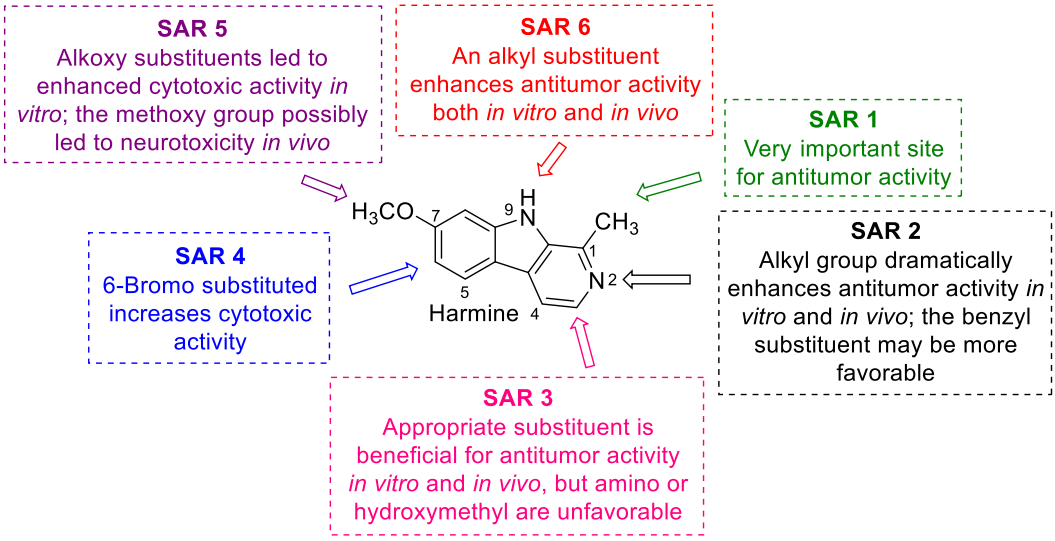


Fig. 2. The structure-activity relationships of β-carboline derivatives against tumor cells by Cao et al. [8a].

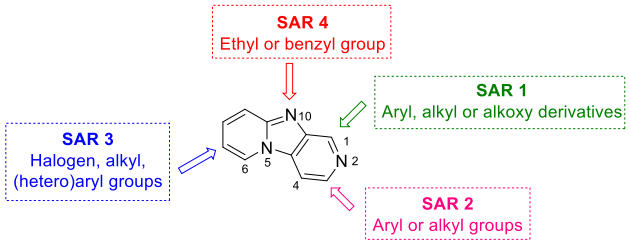


Fig. 3. Proposed SAR explorations around imidazo[1,2-*a*:4,5-*c*']dipyridine core.

2. Results and discussion

2.1. Chemistry

The starting materials for the preparation of the attempted imidazo[1,2-*a*:4,5-*c*']dipyridines **6a-p** are the 3-alkynyl-2-cyano-imidazo[1,2-*a*]pyridines **5a-f**.

The synthetic route to **5a-f** is presented in Scheme 1 [6]. Esters **1a-b** were transformed into amides **2a-b** using aqueous solution of ammonia (30%) at room temperature overnight. Amides **2a-b** were subjected to phosphorus oxychloride at reflux for 4 h to generate nitriles **3a-b**. Iodination at 3 position of **3a** was performed as previously described [6a], when **3b** was iodinated in the presence of NIS overnight at reflux of acetonitrile. The Sonogashira coupling reaction with various alkynes was then applied to **4 a-b** in the presence of Pd_2dba_3 and CuI in dioxane at room temperature for 3 h.

Scheme 2 displayed the preparation of the tricyclic **6a-h** from **5a-d**, by a Grignard reagent addition sequence to the nitrile group, followed by a 6-*endo-dig* cyclization on the triple bond at room temperature for 30 min to 2 h [6]. In order to introduce an ester function in position 3 of the scaffold, an alternative protocol was achieved to avoid the reaction between ester and Grignard reagent. The starting material **5b** with a THP protected hydroxyl group was first obtained and cyclized to **6b** in the same conditions than described above. After removal of the protecting group in HCl 6M at room temperature for 1 h, the alcohol was oxidized to carboxylic acid and esterified to **6i** in non-optimized poor yield**.**

To extend the scope of the pharmacomodulation at position 1, a new alternative protocol was developed using sodium methoxide as nucleophile, in refluxing methanol overnight, in order to obtain the 1-methoxy substituted compounds **6j-l**.

The same synthetic route was then applied to the 6-brominated starting materials **5e-f** leading to compounds **6m-o** *via* the Grignard procedure and compound **6p** after sodium methoxide reaction (Scheme 3).

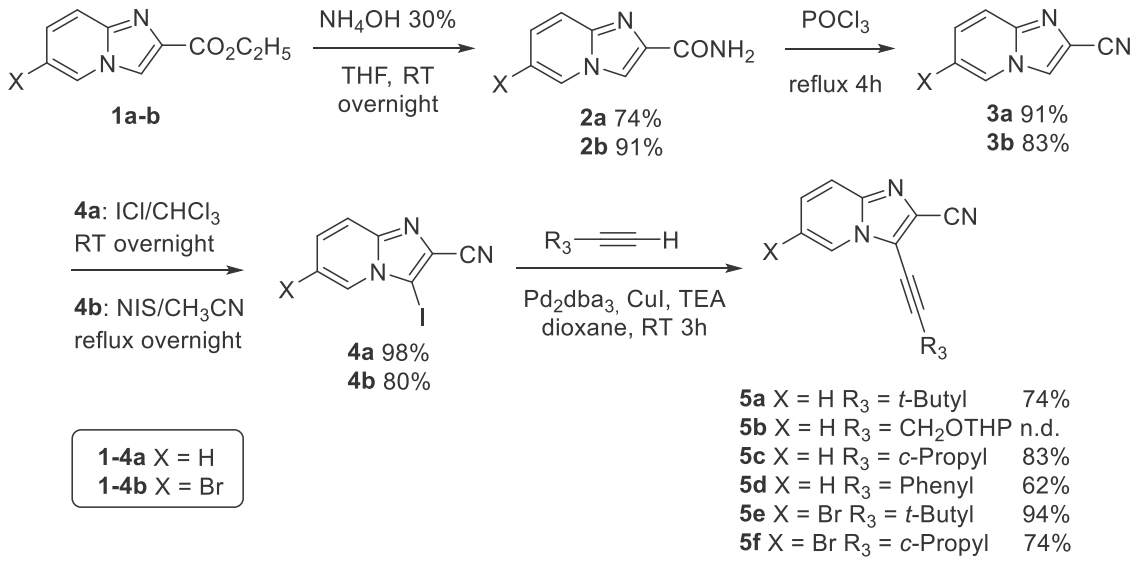
Finally, the 6-brominated imidazo[1,2-*a*:4,5-*c*']dipyridines **6o-p** were involved in a Suzuki-Miyaura metal-catalyzed coupling reaction leading to 7-methyl or (hetero)aryl-1-phenyl-3-*tertio*butylimidazo[1,2-*a*:4,5-*c*']dipyridines **7a-d** or 1-methoxy-7-pyridin-4-yl-3-*tertio*butyl analogue **7e** (Scheme 4).

All these compounds were converted into quaternary ammonium salts **8**–**30**, after reaction with benzyl bromide or ethyl iodide in acetonitrile at 80 °C for 24–48 h (Scheme 5, Table 1). The structure of the salt **8** was confirmed by X-ray crystal structure analysis (Fig. 4). This transformation not only aimed to improve the solubility [16] but also to introduce substituent on the 10-position, which has already been shown to be beneficial for antiproliferative activity against cancer cell lines in the β-carboline series (Fig. 2) [17]. During this salt formation, compound **6j** underwent a demethylation of the methoxy group leading to the 1-hydroxy derivative **22**. To avoid this demethylation, the reaction time with benzyl bromide was limited to 24 h leading to compound **18**. Interestingly, with a pyridine at the C7 position (**7c** and **7e**) the salt did not form selectively on N10 like for the other compounds but on pyridine only (**30**) or on both pyridine and N10 (**29**), respectively.

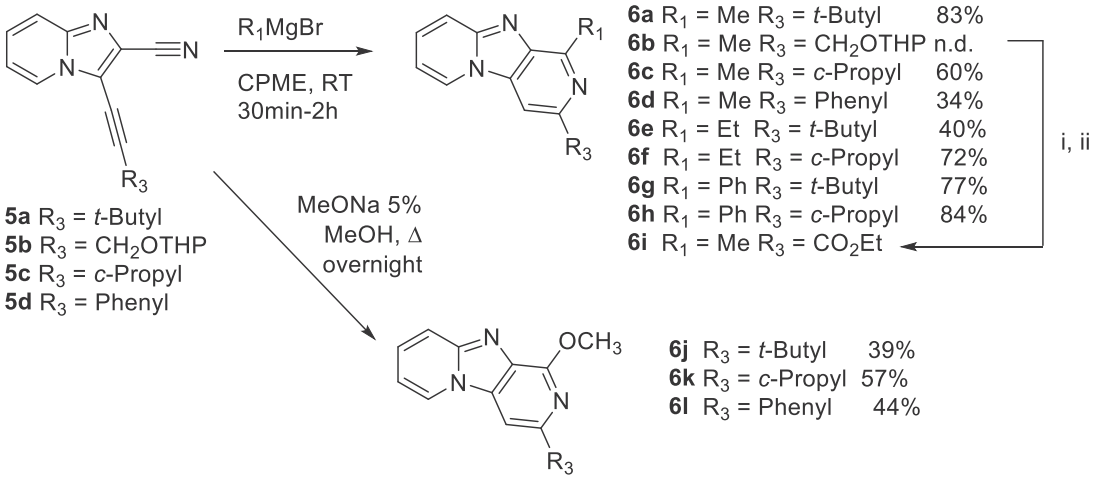
Table 1
Compounds **8**–**30** and their anti-proliferative (cell culture over five days) and cytotoxic (over 24 h) activities (n = 3 independent experiments).

Cpd	R_1	R_3	R_7	R_{10}	Antiproliferative activity (IC_{50}, μM)				Cytotox. (IC_{50}, μM)
					MDA-MB-468	MDA-MB-435s	MDA-MB-231	4T1	MDA-MB-435s
8	Me	*t*-Bu	H	Bn	18.4 ± 6.1	16.4 ± 3.1	n.d.	n.d.	74.4 ± 28.0
9	Me	CO_2Et	H	Bn	>100	>100	n.d.	n.d.	>100
10	Me	c-Pr	H	Bn	30.7 ± 11.4	43.9 ± 14.1	n.d.	n.d.	>100
11	Me	Ph	H	Bn	1.2 ± 0.9	22.4 ± 1.5	2.8 ± 0.2	>100	>100
12	Me	*t*-Bu	H	Et	99.4 ± 15.0	90.5 ± 0.5	>100	n.d.	>100
13	Et	*t*-Bu	H	Bn	8.7 ± 1.7	4.8 ± 0.3	10.7 ± 2.6	n.d.	35.4 ± 14.6
14	Et	c-Pr	H	Et	>100	28.3 ± 4.7	>100	n.d.	>100
15	Ph	*t*-Bu	H	Bn	13.3 ± 2.3	6.4 ± 1.0	11.9 ± 2.3	19.7 ± 5.2	21.3 ± 8.6
16	Ph	c-Pr	H	Bn	31.1 ± 16.1	13.1 ± 6.5	32.1 ± 4.5	n.d.	48.6 ± 2.3
17	Ph	c-Pr	H	Et	>100	47.6 ± 20.4	>100	n.d.	>100
18	OMe	*t*-Bu	H	Bn	0.4 ± 0.4	1.1 ± 0.1	1.7 ± 0.3	0.5 ± 0.3	11.9 ± 1.7
19	OMe	c-Pr	H	Bn	3.6 ± 1.2	1.3 ± 0.1	3.1 ± 0.4	1.5 ± 0.4	16.3 ± 3.3
20	OMe	Ph	H	Bn	4.7 ± 0.2	5.0 ± 3.5	3.0 ± 0.4	6.0 ± 1.3	16.9 ± 2.4
21	OMe	c-Pr	H	Et	37.7 ± 5.4	6.3 ± 3.0	24.8 ± 3.7	n.d.	>100
22	OH	*t*-Bu	H	Bn	>100	33.4 ± 1.2	>100	n.d.	>100
23	Me	*t*-Bu	Br	Bn	11.0 ± 9.2	10.7 ± 0.5	36.0 ± 1.1	n.d.	59.6 ± 14.8
24	Et	c-Pr	Br	Bn	47.1 ± 4.7	62.2 ± 25.8	55.7 ± 12.1	n.d.	>100
25	Ph	*t*-Bu	Br	Bn	14.1 ± 11.0	5.2 ± 0.4	27.6 ± 4.9	31.8 ± 8.3	29.1 ± 1.7
26	Ph	*t*-Bu	Me	Bn	n.d.	n.d.	>100	>100	>100
27	Ph	*t*-Bu	Ph	Bn	n.d.	n.d.	17.3 ± 5.7	25.3 ± 2.9	37.2 ± 5.0
28	Ph	*t*-Bu	Thien-2-yl	Bn	n.d.	n.d.	62.9 ± 0.4	27.7 ± 3.1	30.8 ± 6.5
29	OMe	*t*-Bu	1-benzyl pyridinium-4-yl	Bn	n.d.	n.d.	14.3 ± 2.0	18.1 ± 0,7	98.7 ± 7.9
30	Ph	*t*-Bu	1-benzyl pyridinium-4-yl	H	n.d.	n.d.	>100	>100	>100
Harmine					n.d.	17,1 ± 3,7	n.d.	n.d.	>100

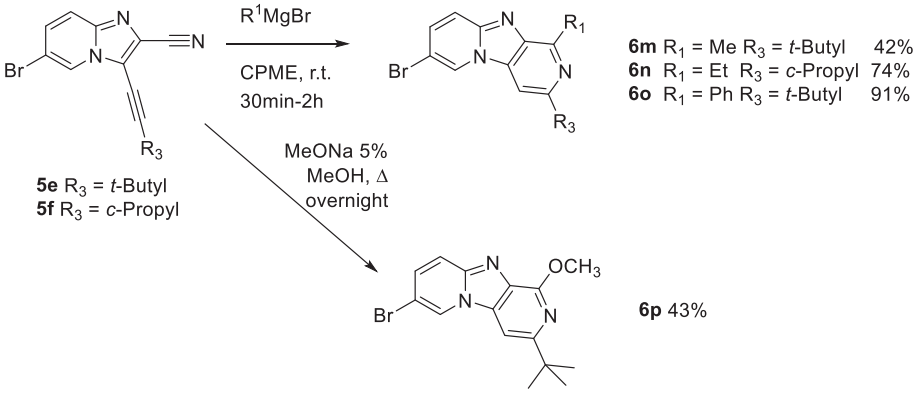
n.d. stands for not determinated.



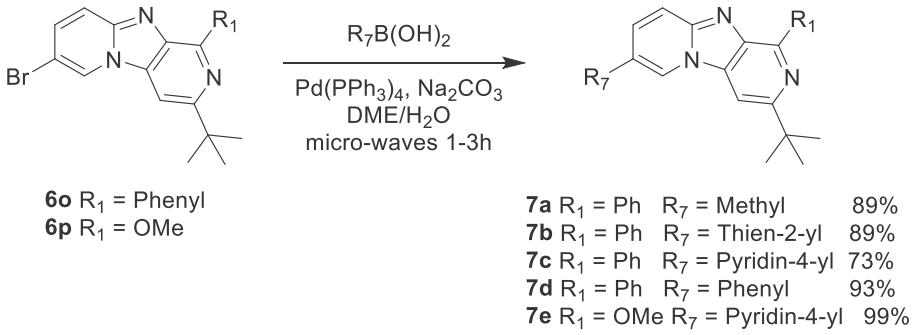
Scheme 1. Synthetic routes to the compounds **5a-f** (THP = tetrahydropyrane, *c*-propyl = cyclopropyl, n.d. = not determined).



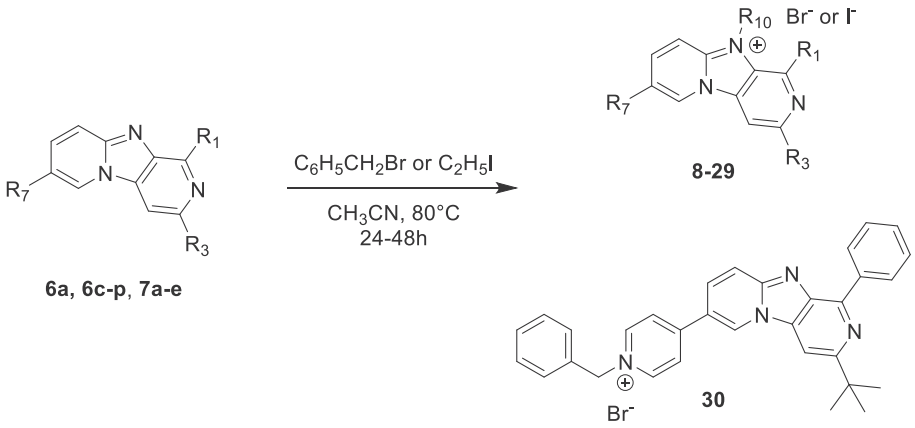
Scheme 2. Synthetic routes to the compounds **6a-l**. Reactions conditions for **6i**: i: HCl 6M, CH_2Cl_2, r.t., 1 h, 93%; ii: a) $KMnO_4$, acetone, 40 °C, overnight; b) EtOH, H_2SO_4, reflux, 4 h, 7%.



Scheme 3. Synthetic route to the brominated compounds **6m-p**.



Scheme 4. Synthetic route to the 7-substituted imidazo[1,2-*a*:4,5-*c*']dipyridines **7a-e**.



Scheme 5. Formation of quaternary ammonium salts **8**–**30**.

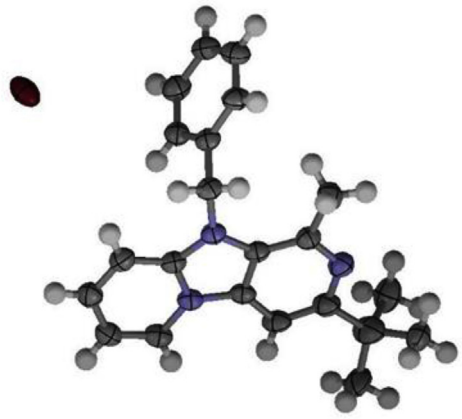


Fig. 4. ORTEP diagram for **8** [18].

2.2. Biological evaluation

2.2.1. In vitro antiproliferative activity against MDA-MB-468, MDA-MB-435, MDA-MB-231 human cancer cells

Determination of *in vitro* growth inhibitory activity was preliminary performed on three highly aggressive human cancer cell lines (MDA-MB-468, MDA-MB-435s, MDA-MB-231) using a colorimetric MTT assay. The best compounds were then tested against 4T1 murine mammary carcinoma *in vitro* to anticipate the evaluation of our compounds in the experimental mouse model. Cells were cultured during five days in presence (test) or absence (control) of compounds **8**–**30** or harmine, at concentrations from 10 nM to 100 μM. For each compound, the concentration required to reduce the global growth by 50% (IC_{50}) was determined on each cell line (Table 1). Dimethylsulfoxyde (DMSO) was used in the control condition.

Compounds **8**–**10** from our initial screening, were preliminary tested for their cancer cell growth inhibitory activity on MDA-MB-468 and MDA-MB-435s cells (Table 1). Compound **8** exhibited the best activity with IC_{50} of 18.4 and 16.4 μM, respectively, compare to **9** (completely inactive) and **10** (30.7 and 43.9 μM). These first encouraging results gave a trend for the C3 position: *t*-butyl > *c*-propyl ≫ CO_2Et. All the next newly synthetized compounds **11**–**25** were then tested on the three human cancer cell lines. To complete the study focusing on the C3 position, the phenyl analogue **11** (R_1 = Methyl and R_3 = Phenyl) was prepared and exhibited the best antiproliferative activity against MDA-MB-468. The superiority of the phenyl group at the C3 position was not recovered with compound **20** (R_1 = OMe, R_3 = Phenyl) compare to analogues **18** (R_1 = OMe, R_3 = *t*-Butyl) and **19** (R_1 = OMe, R_3 = *c*-Propyl). In the methoxylated series, the best compound **18** present a *tertio*-butyl in position 3.

Changing the benzyl group (compound **8**) to an ethyl group (compound **12**) at the N-10 position resulted in a complete loss of activity. This observation (benzyl ≫ Ethyl) was confirmed for compounds **17** and **21**, compare to **16** and **19** respectively, and for compound **14**, regardless of the nature of substituents in other positions.

We then explored the C1 position with compounds **13** (R_1 = Ethyl) and **15** (R_1 = Phenyl) compare to **8** (R_1 = Methyl). These three compounds with a *t*-butyl group at the C3 position gave

similar results, with a slightly higher activity of the ethyl group. We then moved to 1-methoxy substituted compounds **18**–**21**. Besides compound **21** (R_{10} = Ethyl), they all exhibited strong antiproliferative effect with IC_{50} from 0.4 to 6.0 μM regardless of the cancer cell line, whereas harmine presented an IC_{50} of 17.1 μM against MDA-MB-435s cells. Compound **18** (R_3 = *t*-Butyl) proved to be the most active derivative with the lowest IC_{50} on the three cell lines. On the opposite, introduction of a hydroxyl group at the C1 position led to a loss of activity (compound **22**).

Finally, we introduced structural modifications at the C7 position. A bromine atom was well tolerated in this position if we compare the 7-brominated compounds **23** (R_1 = Methyl) and **25** (R_1 = Phenyl) to the corresponding analogues **8** (R_1 = Methyl) and **15** (R_1 = Phenyl) with almost no effect on the IC_{50} values. The following compounds **26**–**30** were then tested only on MDA-MB-231 and 4T1 murine mammary carcinoma. Introduction of a methyl or thienyl moiety drastically affected the antiproliferative activity (compounds **26** and **28**) while a phenyl or benzylpyridinium group seems to be tolerated in this position (IC_{50} around 15 μM respectively for **27** and **29** on MDA-MB-231). We could again confirm the critical importance of the benzyl substituent in position 10, with the inactive compound **30**.

From the *in vitro* growth inhibitory activity evaluation, **18** appeared as the most promising antiproliferative agent against the three highly aggressive human cancer cell lines tested (MDA-MB-468, MDA-MB-435s, MDA-MB-231) as well as against 4T1 murine mammary carcinoma cells. As in the SAR investigation of the β-carboline series (Fig. 2), we can observe that the nature of the substituent in position 1 deeply influences the antiproliferative efficacy when position 3 is less decisive.

We also measured the cytotoxic activity of these compounds by assessing cell survival after one-day treatment to exclude any compound with acute toxic effect that could induce high toxicity *in vivo*. Among the best compounds, **11, 18**–**20** were chosen for further evaluation because of their low IC_{50} for antiproliferative activity and higher IC_{50} for cytotoxicity test.

2.2.2. Effect on apoptosis in MDA-MB-231 cells

A double staining flow cytometric assay using annexin-V fluorescein isothiocyanate (FITC) and propidium iodide (PI) was carried out to evaluate the potential of compounds **11** and **18**–**20** to induce apoptosis in MDA-MB-231 cancer cells. MDA-MB-231 cells were treated with compounds **11** and **18**–**20** for 48 h at two different concentrations, 3 and 30 μM corresponding to about 1xIC_{50} and 10xIC_{50}, respectively (Fig. 5). Dimethylsulfoxyde (DMSO) 0.1%, used as a vehicle for these compounds, was used in the control condition. The different parts of the graph represented the percentage of living, apoptotic, and necrotic cell populations. Compared to the control group with a total apoptosis rate of 2.38%, almost no effect was observed at 3 μM with apoptosis of 3.30, 3.34, 3.50 and 4.11% for compounds **11** and **18**–**20**, respectively. Nevertheless, at 30 μM the total apoptosis rate strongly increased for compounds **11**, **18**–**20** to 7.78, 6.72, 12.5 and 6.03% respectively. Late apoptotic and necrotic cell population also dramatically increased with 30 μM of **11** (from 2.94% in control group to 13.4%) and **18**–**20** (from 2.94% to around 30%).

To further investigate the induction of apoptosis by our compounds, we examined the expression of pro-apoptotic proteins Bax and Bak, anti-apoptotic Bcl-2 and Bcl-XL, and the cleavage state of PARP in MDA-MB-231 cells in response to compounds **11**, **18**–**20** at the single dose of 30 μM. MDA-MB-231 cells were treated with or without (control condition with 0.1% DMSO) the tested compounds for 48 h and then lysed and analyzed by western blotting. β-Actin expression was used as an internal control (Fig. 6). It was revealed that the relative levels of pro-apoptotic Bax expression was increased in the presence of **11**, while **18**–**20** reduced the levels of anti-apoptotic Bcl-2 expression. Furthermore, the four tested compounds resulted in more significant cleavage of PARP than the control group. Taken together, these results confirm that **11**, **18**–**20** induced apoptosis in MDA-MB-231 cells. Nevertheless, a different signaling pathway seems to be impacted by **11** compare to **18**–**20**.

2.2.3. Effect on MDA-MB-231 cell proliferation using Ki-67 staining

In addition to the induction of apoptosis by compounds **11**, **18**–**20**, the reduction of cell number could be attributed to a reduced cell proliferation. Cell proliferation was evaluated using Ki-67 staining in MDA-MB-231 cells treated with 30 μM of compounds **11**, **18**–**20** for 48 h. A decrease in nuclear Ki-67 staining indicates a blockage in cell cycle and a decrease in cell proliferation. All compounds induced a decrease in nuclear Ki-67 expression with compounds **18** and **19** being the most effective (Fig. 7).

Altogether, compounds **11**, **18**–**20** lead to cancer cell growth inhibition with slightly different mechanisms. Compounds **18, 19** and **20** are the most potent to induce apoptosis and compounds **18** and **19** also reduced cell proliferation.

2.2.4. Effects on MDA-MB-435s cells migration in vitro

Cell invasion and migration are critical processes in tumor metastasis. The inhibitory effect of compounds **8**–**30** and harmine on migration of the highly metastatic human cancer cell lines MDA-MB-435s was investigated using time-lapse videomicroscopy of *in vitro* wound healing. These experiments were conducted at 1 μM, concentration for which no cytotoxicity at 24 h can be observed (Fig. 8).

In this assay, six compounds inhibited the migration of MDA-MB-435s cells by 41–50% (compounds **8**, **15**, **18**, **22**–**24**) while four compounds inhibited the migration by 53–62% (**20**, **25**, **27**, **30**). From these ten compounds, eight present a *tertio*-butyl group in position 3 and a structural diversity in position 1, which seems to be less decisive for the anti-migrative activity than for antiproliferative activity.

Three closely related compounds **25**, **27**, and **30** deeply inhibited MDA-MB-435s cell migration at 1 μM by 62, 53 and 62%, respectively. Their structures present a phenyl group in position 1 and a *tertio*-butyl group in position 3. Diversity occurs in position 7 with either a bromine atom for compound **25**, a phenyl group for **27** and a benzylpyridinium for **30**. As the 10-benzyl part is absent in compound **30**, this substituent did not appear to be essential for the anti-migration activity. Interestingly, compound **20** presented dual antiproliferative and anti-migration activity with an average IC_{50} of 3.2 μM for the inhibition of the MDA-MB-231, MDA-MB-468 and MDA-MB-435s cell proliferation, and 43% inhibition in the MDA-MB-435s migration assay at 1 μM (Fig. 6). Conversely, harmine had almost no effect on migration of MDA-MB-435s cells (MDA-MB-435s cells migration of 93%).

3. Conclusions

In conclusion, a series of bioinspired imidazo[1,2-*a*:4,5-*c*']dipyridines, diversely substituted on positions 1, 3, 7 and 10, were synthetized and tested for their *in vitro* antitumor activity against three strains of highly aggressive human cancer cell lines (MDA-MB-468, MDA-MB-435s and MDA-MB-231). Among them, compounds **18**–**20** exhibited excellent cancer inhibitory activity *in vitro* with IC_{50} of 0.4–5 μM against the three cell lines, higher than harmine activity against MDA-MB-435s. Compound **11** was also selected for further exploration (IC_{50} of 1.2 and 2.8 μM against MDA-MB-468 and 231 respectively).

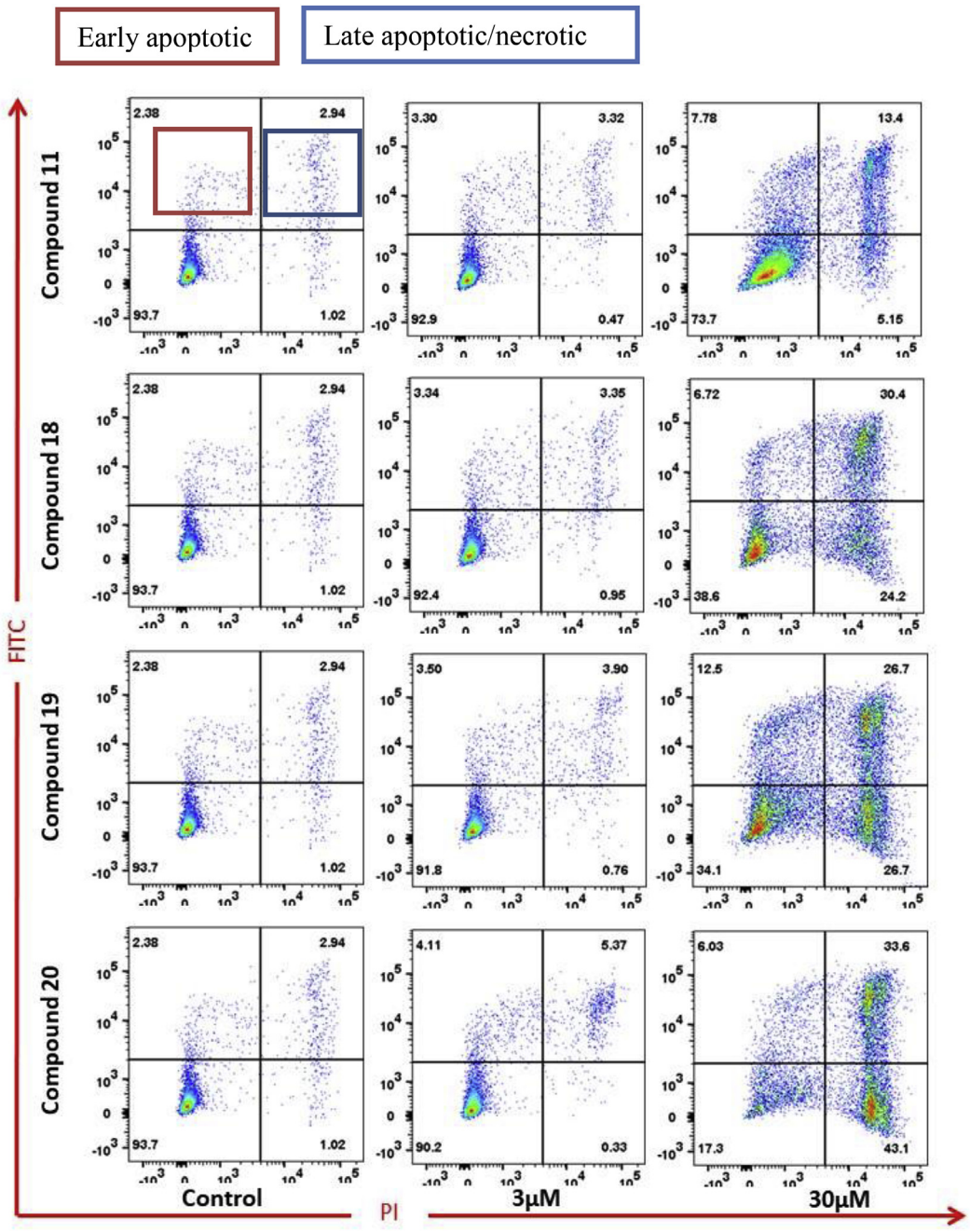


Fig. 5. Annexin V/PI staining for detection of apoptosis of MDA-MB-231 cells treated with compounds **11**, **18**–**20** at 3 and 30 μM for 48 h (Representative of n = 3 independent experiments).

SAR analysis showed that the 10-benzyl group played a critical role in the antitumor activity, as well as the 1-methoxy group that deeply improved the antiproliferative effect. On the opposite, the presence of an ester group on the 3 position or the substitution of the 7 position seems detrimental.

Flow cytometry assay showed that compounds **11** and **18**–**20** induce apoptosis in MDA-MB-231 cells in a dose-dependent manner, compounds **18**–**20** being more potent than **11**. Moreover, a different signaling pathway seems to be impacted by **11** compare to 1-methoxylated **18**–**20**: **11** increased the expression of pro-apoptotic Bax while **18**–**20** reduced the level of anti-apoptotic Bcl-2 expression. The effect of these compounds on MDA-MB-231 cell proliferation using Ki-67 staining revealed that **18** and **19** also reduced cell proliferation.

Furthermore, wound-healing experiments showed that this series of compounds present also very interesting anti-migration activity (conversely to harmine that proved to be inactive) with six compounds inhibiting MDA-MB-435s cell migration by 41–50% while four compounds (**20**, **25**, **27**, **30**) inhibited the migration by 53–62%. SAR analysis showed that the nature of the substitution in position 1 and the presence of a 10-benzyl group are less discriminant for anti-migration activity. The 7 position appeared to be important to explore further.

Interestingly, compound **20** may constitute a promising drug candidate with dual antiproliferative and anti-migration activity on aggressive human breast cancer cell lines.

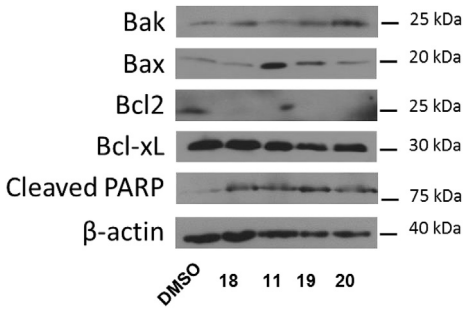


Fig. 6. Immunoblot analyses of levels of pro-apoptotic proteins Bax and Bak, anti-apoptotic Bcl-2 and Bcl-XL, and the cleavage state of PARP in MDA-MB-231 cells in response to compounds **11**, **18**–**20** treatment at 30 μM for 48 h (Representative of n = 3 independent experiments).

4. Experimental section

4.1. General methods of chemistry

All reagents were used directly as obtained commercially. Harmine was purchased from Sigma-Aldrich (St Louis, MO, USA). Thin-layer chromatographies (TLC) were performed using Merck® silica gel 60F_{254} plates. Column chromatographies were performed using Merck Geduran® Si 60 (40–63 μm) silica. Melting points were determined on a capillary apparatus (Stuart, Staffordshire, United Kingdom) and are uncorrected. Microwave heating was performed using CEM® Explorer SP 12 S class apparatus (maw power 300 W). NMR experiments were performed at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Bruker-Avance 300 MHz spectrometer. Assignment of carbons noted C* may be interchanged. Mass spectra were determined on a Hewlett Packard 5988A spectrometer or on a Shimadzu QP 2010 spectrometer by direct inlet at 70 eV. Compounds **1a**, **2a**, **3a**, **4a**, **5a**, **5c**, **5d**, **6a**, **6c**, **6d**, **6f** and **6h** were prepared according to literature [6].

4.1.1. Ethyl 6-bromoimidazo[1,2-a]pyridine-2-carboxylate (*1b*)

To a solution of 2-amino-5-bromopyridine (8.5 g, 49 mmol, 1 eq) in DME (300 mL) was added dropwise ethyl bromopyruvate (11 mL, 73.7 mmol, 1.5 eq). After stirring for 5 h, the solid was filtered and washed with DME. The solid was then transfered in a second flask and heated in refluxing ethanol (500 mL) overnight. The ethanol was evaporated to dryness and the resulting solid was partitioned between water and CH_2Cl_2 (300 mL/300 mL). The aqueous phase was alkalinized with a saturated solution of Na_2CO_3 and extracted with twice 300 mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated to yield 11.5 g (42,7 mmol, 87%) of crude product, as a beige solid. **1b** was used directly for the next step without further purification.

m.p.: 122–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.31 (d, ^4J = 1.0 Hz, 1H, H-5), 8.14 (s, 1H, H-3), 7.61 (d, ^3J = 9.6 Hz, 1H, H-8), 7.32 (dd, ^3J = 9.6 Hz, ^4J = 1.7 Hz, 1H, H-7), 4.46 (q, ^3J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 1.44 (t, ^3J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.8 (CO), 143.7 (C-8a), 137.5 (C-2), 130.2 (C-7), 126.3 (C-5), 119.8 (C-3), 116.9 (C-8), 109.1 (C-6), 61.6 ($O\underline{C}H_2CH_3$), 14.5 ($OCH_2\underline{C}H_3$). HRMS (ESI): *m/z* calc. for $C_{10}H_9^{79}BrN_2O_2$ $[M+I]^+$: 268.99202, found: 268.99118; *m/z* calc. for $C_{10}H_9^{81}BrN_2O_2$ $[M+I]^+$: 270.98997, found: 270.98902.

4.1.2. 6-Bromoimidazo[1,2-a]pyridine-2-carboxamide (*2b*)

1b (11.5 g, 42.7 mmol) was stirred at room temperature overnight in a mixture of 65 mL of THF and 350 mL of an aqueous solution of ammonia (30%). Solvents were then evaporated to dryness. The obtained solid was washed with water and CH_2Cl_2 to afford 9.35 g of a beige solid (38.9 mmol, 91%). **2b** was used directly for the next step without further purification.

m.p.: 253–257 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.94 (d, ^4J = 1.0 Hz, 1H, H-5), 8.29 (s, 1H, H-3), 7.76 (bs, 1H, NH), 7.58 (d, ^3J = 9.6 Hz, 1H, H-8), 7.46–7.42 (m, 2H, H-7 & NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.8 (CO), 142.3 (C-8a*), 140.5 (C-2*), 129.1 (C-7), 127.7 (C-5), 118.4 (C-3), 115.1 (C-8), 106.8 (C-6). HRMS (ESI): *m/z* calc. for $C_8H_6^{79}BrN_3O$ $[M+I]^+$: 239.97670, found: 239.97592; *m/z*

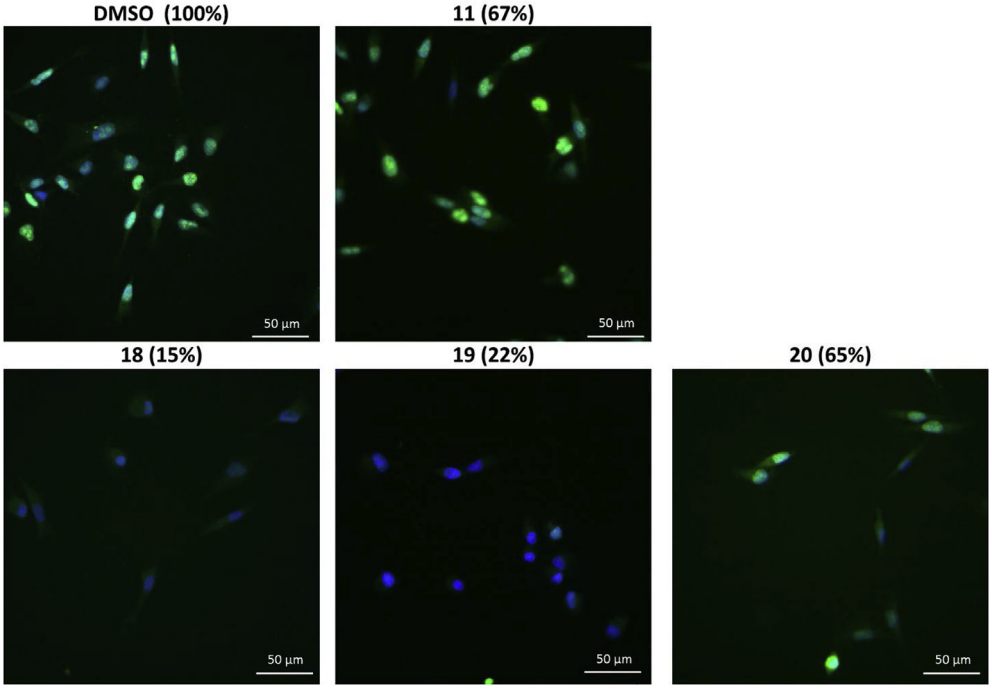


Fig. 7. Ki-67 staining for detection of cell proliferation of MDA-MB-231 cells treated with compounds **11**, **18**–**20** at 30 μM for 48 h. Total nuclei are stained with Dapi. % of Ki-67 positive cells is indicated (Representative of n = 3 independent experiments).

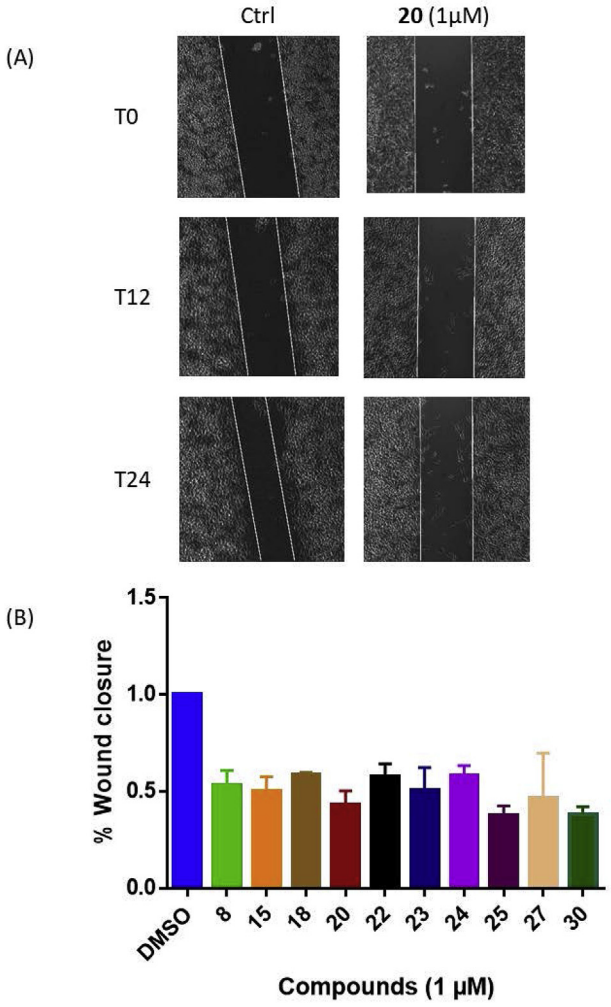


Fig. 8. (A) Effect of compound **20** on MDA-MB-435s cell migration in a wound healing assay using time-lapse video microscopy. Representative photographs taken at 0 h (T0), 12 h (T12) and 24 h (T24) in the control condition (DMSO) or under the treatment with compound **20** (1 μM). (B) Effect of the most active compounds on MDA-MB-435 cells migration for 24 h in wound healing assay. Results are presented as means ± SEM of three independent experiments.

calc. for $C_8H_6^{81}BrN_3O$ $[M+I]^+$: 241.97465, found: 241.97374.

*4.1.3. 6-Bromoimidazo[1,2-a]pyridine-2-carbonitrile (**3b**)*

2b (8.47 g, 35 mmol) was charged in a 250 mL round bottom flask, followed by $POCl_3$ (66 mL, 705 mmol, 20 eq). The reaction mixture was heated at reflux for 4 h. $POCl_3$ was then directly evaporated from the flask, using vacuum pump equipped with ice-cooled trap. The resulting solid was then suspended in a mixture of water and CH_2Cl_2 (200 mL/200 mL), followed by alkalization with aqueous saturated solution of Na_2CO_3. The aqueous phase was then extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to yield 6.5 g (29 mmol, 83%) of crude product, as a brown solid. **3b** was used directly for the next step without further purification.

m.p.: 223–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.97 (dd, ^4J = 1.8 Hz, ^5J = 0.9 Hz, 1H, H-5), 8.69 (s, 1H, H-3), 7.66 (d, ^3J = 9.7 Hz, 1H, H-8), 7.57 (dd, ^3J = 9.7 Hz, ^4J = 1.9 Hz, 1H, H-7). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 143.3 (C-8a), 130.9 (C-7), 127.7 (C-5), 121.5 (C-3), 118.4 (C-8), 116.5 (C-2), 115.0 (CN), 108.3 (C-6). HRMS (ESI): *m/z* calc. for $C_8H_4^{79}BrN_3$ $[M+I]^+$: 221.96614, found: 221.96547; *m/z* calc. for $C_8H_4^{81}BrN_3$ $[M+I]^+$: 223.96409, found: 223.96336.

*4.1.4. 6-Bromo-3-iodoimidazo[1,2-a]pyridine-2-carbonitrile (**4b**)*

To a solution of **3b** (8.4 g, 37.8 mmol) in CH_3CN (170 mL) was added NIS (11.1 g, 49.2 mmol, 1.3 eq) in one portion. The reaction mixture was heated at reflux overnight. The CH_3CN was then evaporated to dryness and the solid was washed several times with water, petroleum ether and diethyl ether. The crude mixture was purified by flash chromatography on alumina (CH_2Cl_2) to afford 10.5 g (30 mmol, 80%) of **4b** as a white solid.

m.p.: 275–279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.56 (dd, ^4J = 1.7 Hz, ^5J = 1.0 Hz, 1H, H-5), 7.67 (dd, ^3J = 9.6 Hz, ^5J = 0.9 Hz, 1H, H-8), 7.61 (dd, ^3J = 9.6 Hz, ^4J = 1.7 Hz, 1H, H-7). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 145.8 (C-8a), 131.6 (C-7), 127.8 (C-5), 124.0 (C-2), 118.9 (C-8), 115.0 (CN), 110.0 (C-6), 78.3 (C-3). HRMS (ESI): *m/z* calc. for $C_8H_3^{79}BrIN_3$ $[M+I]^+$: 347.86278, found: 347.86179; *m/z* calc. for $C_8H_3^{81}BrIN_3$ $[M+I]^+$: 349.86073, found: 349.85975.

*4.1.5. General method for Sonogashira cross-coupling (**5b**, **5e**, **5f**)*

4 (5 mmol), Pd_2dba_3 (0.25 mmol, 5 mol%) and CuI (0.5 mmol, 10 mol%) were introduced in a sealed tube. Vacuum/refilling with argon cycles were repeated thrice and dioxane (5.5 mL), Et_3N (5.5 mL) and finally alkyne (1.2 eq) were added. The reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then filtered on celite® with CH_2Cl_2. Organic phase was washed with a saturated solution of NH_4Cl, dried over $MgSO_4$ and evaporated. The crude mixture was purified by flash chromatography with silica or alumina with appropriate eluent.

*4.1.6. 3-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl) imidazo[1,2-a]pyridine-2-carbonitrile (**5b**)*

Compound **5b** was obtained after purification as an inseparable mixture (80/20) with de-iodinated side-product. It was used like this for the cyclization.

*4.1.7. 6-Bromo-3-(3,3-dimethylbut-1-yn-1-yl)imidazo[1,2-a] pyridine-2-carbonitrile (**5e**)*

Silica, PE/Et_2O 80/20 → 50/50, yellow solid, m.p.: 137–141 °C, 3.26 g. Yield: 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.26 (dd, ^4J = 1.8 Hz, ^5J = 0.9 Hz, 1H, H-5), 7.53 (dd, ^3J = 9.6 Hz, ^5J = 0.8 Hz, 1H, H-8), 7.42 (dd, ^3J = 9.6 Hz, ^4J = 1.8 Hz, 1H, H-7), 1.42 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 143.1 (C-8a), 131.6 (C-7), 125.4 (C-5), 121.1 (C-2), 119.3 (C-8), 114.7 (C-3*), 113.9 (C-6*), 110.1 (CN), 63.5 (-C≡C-*t*-Bu), 30.7 (CH_3 *t*-Bu), 29.0 (Cq *t*-Bu). One carbon is missing. HRMS (ESI): *m/z* calc. for $C_{14}H_{12}^{79}BrN_3$ $[M+I]^+$: 302.02874, found: 302.02784; *m/z* calc. for $C_{14}H_{12}^{81}BrN_3$ $[M+I]^+$: 304.02669, found: 304.02566.

*4.1.8. 6-Bromo-3-(cyclopropylethynyl)imidazo[1,2-a]pyridine-2-carbonitrile (**5f**)*

Alumina, CH_2Cl_2, yellow solid, m.p.: 161–165 °C, 1.05 g. Yield: 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.33 (dd, ^4J = 1.8 Hz, ^5J = 0.9 Hz, 1H, H-5), 7.52 (dd, ^3J = 9.6 Hz, ^5J = 0.9 Hz, 1H, H-8), 7.41 (dd, ^3J = 9.6 Hz, ^4J = 1.8 Hz, 1H, H-7), 1.72–1.58 (m, 1H, CH c-Pr), 1.13–0.95 (m, 4H 2 CH_2 c-Pr). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 143.0 (C-8a), 131.6 (C-7), 125.5 (C-5), 121.3 (C-2), 119.3 (C-8), 113.9 (C-3), 110.1 (CN*), 110.1 (C-6*), 59.6 (-C≡C-c-Pr), 9.9 (CH_2 c-Pr), 0.7 (CH c-Pr). *One carbon is missing.* HRMS (ESI): *m/z* calc. for $C_{13}H_8^{79}BrN_3$ $[M+I]^+$: 285.99744, found: 285.99663; *m/z* calc. for $C_{13}H_8^{81}BrN_3$ $[M+I]^+$: 287.99539, found: 287.99444.

*4.1.9. General method for cyclization with Grignard reagent (compounds **6e**, **6g**, **6i**, **6m**, **6n** and **6o**)*

To a solution of 3-alkynylimidazo[1,2-*a*]pyridine-2-carbonitriles (*e.g.* 1 mmol) in CPME (10 mL) were added dropwise Grignard reagent (2 eq) at room temperature. After completion of the reaction (followed by TLC, *ca.* 30min-2h), 10 mL of NH_4Cl aqueous saturated solution were added. After 30 min of hydrolysis the phases are

separated and aqueous phase was extracted with thrice 15 mL of EtOAc. The crude mixture was purified by flash chromatography on silica gel (petroleum ether/diethyl ether mixture).

*4.1.10. 3-(tert-Butyl)-1-ethylimidazo[1,2-a:4,5-c']dipyridine (**6e**)*

PE/Et_2O: 50/50, yellow solid, m.p.: 121–125 °C, 45 mg. Yield: 40%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.41 (d, ^3J = 6.9 Hz, 1H, H-6), 7.73 (d, ^3J = 9.4 Hz, 1H, H-9), 7.60 (s, 1H, H-4), 7.42 (ddd, ^3J = 9.3 Hz, 6.6 Hz, ^4J = 1.1 Hz, 1H, H-8), 6.84 (td, ^3J = 6.7 Hz, ^4J = 0.9 Hz, 1H, H-7), 3.39 (q, ^3J = 7.5 Hz, 2H, $C\underline{H}_2CH_3$), 1.51–1.46 (m, 12H, *t*-Bu & $CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.0 (C-3), 155.7 (C-1), 148.2 (C-9a), 137.1 (C-10a), 133.4 (C-4a), 129.9 (C-8), 125.6 (C-6), 119.0 (C-9), 110.9 (C-7), 97.6 (C-4), 37.8 (Cq *t*-Bu), 30.8 (CH_3, *t*-Bu), 27.2 ($\underline{C}H_2CH_3$), 13.0 ($CH_2\underline{C}H_3$). HRMS (ESI): *m/z* calc. for $C_{16}H_{19}N_3$ $[M+I]^+$: 254.16517, found: 254.16426.

*4.1.11. 3-(tert-Butyl)-1-phenylimidazo[1,2-a:4,5-c']dipyridine (**6g**)*

PE/Et_2O: 50/50, yellow solid, m.p.: 128–132 °C, 105 mg. Yield: 77%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.93–8.91 (m, 2H, Ph-2,6), 8.42 (d, ^3J = 6.9 Hz, 1H, H-6), 7.76 (d, ^3J = 9.4 Hz, 1H, H-9), 7.72 (s, 1H, H-4), 7.60–7.55 (m, 2H, Ph-3,5), 7.47–7.39 (m, 2H, Ph-4 & H-8), 6.83 (td, ^3J = 6.8 Hz, ^4J = 0.7 Hz, 1H, H-7), 1.56 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.8 (C-3), 148.7 (C-9a), 147.7 (C-1), 138.8 (Ph-1), 137.0 (C-10a*), 135.2 (C-4a*), 130.2 (C-8), 129.5 (Ph-2,6), 128.9 (Ph-4), 128.5 (Ph-3,5), 125.5 (C-6), 119.1 (C-9), 111.0 (C-7), 98.8 (C-4), 38.0 (Cq *t*-Bu), 30.9 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{20}H_{19}N_3$ $[M+I]^+$: 302.16517, found: 302.16415.

*4.1.12. Ethyl 1-methylimidazo[1,2-a:4,5-c']dipyridine-3-carboxylate (**6i**)*

Note: The sequence leading to compound **6i** is an un-optimized sequence which was performed only once.

Cyclization was performed on **5b** (981 mg: mixture with de-iodinated side-product, 80/20) following general procedure. 529 mg of 1-methyl-3-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl) imidazo[1,2-*a*:4,5-*c*']dipyridine (**6b**) were obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.41 (d, ^3J = 6.9 Hz, 1H), 7.82 (s, 1H), 7.72 (d, ^3J = 9.4 Hz, 1H), 7.45 (ddd, ^3J = 9.4, 6.6 Hz, ^4J = 1.2 Hz, 1H), 6.88 (td, ^3J = 6.9 Hz, ^4J = 0.9 Hz, 1H), 5.09 (d, ^2J = 13.3 Hz, 1H), 4.86–4.81 (m, 2H), 3.98–3.91 (m, 1H), 3.60–3.54 (m, 1H), 3.00 (s, 3H), 1.94–1.55 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 151.8, 148.5, 148.3, 138.7, 133.1, 130.3, 125.7, 118.7, 111.2, 101.1, 98.6, 70.2, 62.5, 30.6, 25.4, 20.3, 19.6.

Deprotection of the THP was performed by adding 10 mL of HCl (6M) to a solution of **6b** (529 mg, 1.78 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was stirred at room temperature for 1 h. The aqueous phase was alkalinized with Na_2CO_3 aqueous saturated solution and extracted with CH_2Cl_2 (twice 40 mL). The organic phase was dried over $MgSO_4$ and then evaporated to give 141 mg of a yellow solid. The aqueous phase was cooled in an ice-bath for 2 h. After filtration, 214 mg more of yellow solid was obtained. All the solids were combined to yield 355 mg of the free alcohol (1-methylimidazo[1,2-*a*:4,5-*c*']dipyridin-3-yl)methanol (1.66 mmol, 93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.84 (d, ^3J = 7.0 Hz, 1H), 8.08 (s, 1H), 7.70–7.62 (m, H), 7.08–7.03 (m, 1H), 4.92 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 152.9, 151.8, 150.3, 138.8, 135.2, 133.4, 128.2, 118.4, 113.3, 102.5, 65.7, 19.7.

218 mg (1.02 mmol) of (1-methylimidazo[1,2-*a*:4,5-*c*']dipyridin-3-yl)methanol was dissolved in 22 mL of acetone and 403 mg (2.55 mmol, 2.5 eq) of $KMnO_4$ were added in one portion. The reaction mixture was heated at 40 °C overnight. The mixture was then filtrated, the solid washed with water and the filtrate was evaporated to dryness. The obtained residue was directly used for the next step and dissolved in 10 mL of EtOH. 250 µL of H_2SO_4 were added and the reaction mixture was heated to reflux for 4 h. The solvent was evaporated to dryness and the residue was partitioned between 10 mL of a saturated aqueous solution of Na_2CO_3 and 10 mL of $CHCl_3$. The aqueous solution was extracted two more times with 10 mL of $CHCl_3$. The combined organic phases were dried over $MgSO_4$ and evaporated. The crude mixture was purified on alumina column with a mixture CH_2Cl_2/AcOEt (9/1) as eluent. 17 mg (0.07 mmol, 7%) of **6i** were obtained. Due to the small amount obtained, **6i** was entirely engaged in the salt formation and only the proton NMR was recorded. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.63 (s, 1H), 8.53 (d, ^3J = 6.9 Hz, 1H), 7.81 (d, ^3J = 9.4 Hz, 1H), 7.58 (ddd, ^3J = 9.3, 6.7 Hz, ^4J = 1.2 Hz, 1H), 7.01 (td, ^3J = 6.8 Hz, ^4J = 0.9 Hz, 1H), 4.53 (q, ^3J = 7.1 Hz, 2H), 3.08 (s, 3H), 1.48 (t, ^3J = 7.1 Hz, 3H).

*4.1.13. 7-Bromo-3-(tert-butyl)-1-methylimidazo[1,2-a:4,5-c'] dipyridine (**6m**)*

PE/Et_2O: 10/90, yellow solid, m.p.: 226–230 °C, 90 mg. Yield: 42%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.54 (s, 1H, H-6), 7.62 (d, ^3J = 9.8 Hz, 1H, H-9), 7.57 (s, 1H, H-4), 7.44 (d, ^3J = 9.8 Hz, 1H, H-8), 2.97 (s, 3H, CH_3), 1.46 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.8 (C-3), 151.8 (C-1), 146.4 (C-9a), 137.9 (C-10a), 133.3 (C-8), 132.9 (C-4a), 125.8 (C-6), 119.7 (C-9), 105.3 (C-7), 97.8 (C-4), 37.7 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu), 20.7 (Me). HRMS (ESI): *m/z* calc. for $C_{15}H_{16}^{79}BrN_3$ $[M+I]^+$: 318.06004, found: 318.05912; *m/z* calc. for $C_{15}H_{16}^{81}BrN_3$ $[M+I]^+$: 320.05799, found: 320.05698.

*4.1.14. 7-Bromo-3-cyclopropyl-1-ethylimidazo[1,2-a:4,5-c'] dipyridine (**6n**)*

CH_2Cl_2/EtOAc: 70/30 → 60/40, tan solid, m.p.: 121–125 °C, 164 mg. Yield: 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.46 (dd, ^4J = 1.5 Hz, ^5J = 0.6 Hz, 1H, H-6), 7.60 (dd, ^3J = 9.6 Hz, ^5J = 0.6 Hz, 1H, H-9), 7.43 (dd, ^3J = 9.6 Hz, ^4J = 1.8 Hz, 1H, H-8), 7.36 (s, 1H, H-4), 3.30 (q, ^3J = 7.5 Hz, 2H, $C\underline{H}_2CH_3$), 2.25–2.16 (m, 1H, CH c-Pr), 1.43 (t, ^3J = 7.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.11–0.99 (m, 4H, 2 CH_2 c-Pr). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.2 (C-1), 153.9 (C-3), 146.1 (C-9a), 137.6 (C-10a), 133.3 (C-8), 125.7 (C-6), 119.8 (C-9), 105.2 (C-7), 99.2 (C-4), 27.4 ($\underline{C}H_2CH_3$), 17.9 (CH c-Pr), 13.1 ($CH_2\underline{C}H_3$), 9.8 (CH_2 c-Pr). One carbon is missing. HRMS (ESI): *m/z* calc. for $C_{15}H_{14}^{79}BrN_3$ $[M+I]^+$: 316.04439, found: 316.04351; *m/z* calc. for $C_{15}H_{14}^{81}BrN_3$ $[M+I]^+$: 318.04234, found: 318.04135.

*4.1.15. 7-Bromo-3-(tert-butyl)-1-phenylimidazo[1,2-a:4,5-c'] dipyridine (**6o**)*

PE/Et_2O: 10/90, yellow solid, m.p.: 216–220 °C, 230 mg. Yield: 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.85–8.83 (m, 2H, Ph-2,6), 8.63 (s, 1H, H-6), 7.76 (d, ^3J = 9.7 Hz, 1H, H-9), 7.71 (s, 1H, H-4), 7.60–7.43 (m, 4H, H-8 & Ph-3,4,5), 1.55 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.7 (C-3), 148.3 (C-1), 146.8 (C-9a), 138.5 (Ph-1), 136.9 (C-4a*), 134.9 (C-10a*), 133.7 (C-8), 129.6 (Ph-2,6), 129.2 (Ph-4), 128.5 (Ph-3,5), 125.7 (C-6), 119.9 (C-9), 105.4 (C-7), 98.7 (C-4), 38.1 (Cq *t*-Bu), 30.8 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{20}H_{18}^{79}BrN_3$ $[M+I]^+$: 380.07569, found: 380.07470; *m/z* calc. for $C_{20}H_{18}^{81}BrN_3$ $[M+I]^+$: 382.07364, found: 382.07253.

*4.1.16. General method for cyclization with sodium methoxide (compounds **6j-l** and **6p**)*

3-Alkynylimidazo[1,2-*a*]pyridine-2-carbonitriles (*e.g.* 1 mmol) were added in one portion to a 0.4 M solution of MeONa in MeOH (12 mL). The reaction mixture was refluxed overnight. Methanol was then evaporated and crude solid was partitioned between 25 mL of water and 25 mL of CH_2Cl_2. Aqueous phase was extracted several time with 20 mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and evaporated to dryness. The crude mixture was purified by flash chromatography on silica gel with appropriate eluent.

*4.1.17. 3-(tert-Butyl)-1-methoxyimidazo[1,2-a:4,5-c']dipyridine (**6j**)*

PE/Et_2O: 10/90, beige solid, m.p.: 80–84 °C, 45 mg. Yield: 39%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.40 (d, $^3J = 6.5$ Hz, 1H, H-6), 7.81 (d, $^3J = 9.2$ Hz, 1H, H-9), 7.44 (dd, $^3J = 8.8$ Hz, $^4J = 6.8$ Hz, 1H, H-8), 7.34 (s, 1H, H-4), 6.92 (t, $^3J = 6.4$ Hz, 1H, H-7), 4.19 (s, 3H, OCH_3), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.2 (C-3), 154.7 (C-1), 147.2 (C-9a), 134.8 (C-4a*), 129.9 (C-8), 126.3 (C-10a*), 125.4 (C-6), 118.8 (C-9), 111.9 (C-7), 94.4 (C-4), 53.6 (OCH_3), 37.8 (Cq *t*-Bu), 30.4 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{15}H_{17}N_3O$ $[M+I]^+$: 256.14444, found: 256.14351.

*4.1.18. 3-Cyclopropyl-1-methoxyimidazo[1,2-a:4,5-c']dipyridine (**6k**)*

PE/Et_2O: 10/90, yellow solid, m.p.: 188–192 °C, 88 mg. Yield: 57%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.26 (dt, $^3J = 6.9$ Hz, $^4J = 1.0$ Hz, 1H, H-6), 7.69 (d, $^3J = 9.3$ Hz, 1H, H-9), 7.36 (ddd, $^3J = 9.3$ Hz, 6.6 Hz, $^4J = 1.2$ Hz, 1H, H-8), 7.23 (s, 1H, H-4), 6.83 (td, $^3J = 6.8$ Hz, $^4J = 1.0$ Hz, 1H, H-7), 4.11 (s, 3H, OCH_3), 2.12–2.04 (m, 1H, CH c-Pr), 1.14–1.09 (m, 2H, 2 $C\underline{H}_aH_b$ c-Pr), 0.97–0.91 (m, 2H, 2 $CH_a\underline{H}_b$ c-Pr). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9 (C-1), 151.5 (C-3), 147.3 (C-9a), 135.0 (C-10a*), 129.2 (C-8), 127.5 (C-4a*), 125.3 (C-6), 119.1 (C-9), 111.4 (C-7), 96.6 (C-4), 53.4 (OCH_3), 17.5 (CH c-Pr), 9.3 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{14}H_{13}N_3O$ $[M+I]^+$: 240.11314, found: 240.11230.

*4.1.19. 1-Methoxy-3-phenylimidazo[1,2-a:4,5-c']dipyridine (**6l**)*

PE/EtOAc: 50/50 → 30/70, beige solid, m.p.: 248–252 °C, 100 mg. Yield: 44%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.37 (d, $^3J = 6.9$ Hz, 1H, H-6), 8.15–8.12 (m, 2H, Ph-2,6), 7.80 (s, 1H, H-4), 7.72 (d, $^3J = 9.4$ Hz, 1H, H-9), 7.50–7.35 (m, 4H, H-8 & Ph-3,4,5), 6.89 (t, $^3J = 6.8$ Hz, 1H, H-7), 4.30 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9 (C-1), 148.3 (C-9a), 146.0 (C-3), 139.6 (Ph-1), 135.4 (C-4a), 129.6 (C-8*), 129.4 (C-10a), 128.8 (Ph-3,5), 128.3 (Ph-4*), 126.7 (Ph-2,6), 125.4 (C-6), 119.3 (C-9), 111.7 (C-7), 96.3 (C-4), 53.8 (OCH_3). HRMS (ESI): *m/z* calc. for $C_{17}H_{13}N_3O$ $[M+I]^+$: 276.11314, found: 276.11235.

*4.1.20. 7-Bromo-3-(tert-butyl)-1-methoxyimidazo[1,2-a:4,5-c'] dipyridine (**6p**)*

CH_2Cl_2, beige solid, m.p.: 207–211 °C, 143 mg. Yield: 43%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.51 (d, $^4J = 1.5$ Hz, 1H, H-6), 7.66 (d, $^3J = 9.7$ Hz, 1H, H-9), 7.44 (dd, $^3J = 9.7$ Hz, $^4J = 1.8$ Hz, 1H, H-8), 7.30 (s, 1H, H-4), 4.19 (s, 3H, OCH_3), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.7 (C-3), 155.1 (C-1), 145.7 (C-9a), 134.7 (C-10a), 132.7 (C-8), 127.6 (C-4a), 125.5 (C-6), 119.8 (C-9), 105.9 (C-7), 94.2 (C-4), 53.6 (OCH_3), 37.8 (Cq *t*-Bu), 30.4 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{15}H_{16}^{79}BrN_3O$ $[M+I]^+$: 334.05495, found: 334.05389; *m/z* calc. for $C_{15}H_{16}^{81}BrN_3O$ $[M+I]^+$: 336.05290, found: 336.05169.

*4.1.21. General method for Suzuki-Miyaura cross-coupling (compounds **7a-e**)*

7-Bromo-imidazo[1,2-*a*:4,5-*c*']dipyridines (**6n-o**) (*e.g.* 100 mg), $Pd(PPh_3)_4$ (10 mol%), Na_2CO_3 (2 eq) and boronic acid (1.1 eq) were introduced in a microwave reaction vial. DME (1 mL) and water (500 μL) were then added. The mixture was heated using microwave oven until completion (*ca.* 1–3 h). The crude mixture was partitioned between 20 mL of water and 20 mL of CH_2Cl_2. Aqueous phase was then extracted with twice 20 mL of CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and evaporated to dryness. The crude mixture was purified by flash chromatography on silica gel with appropriate eluent.

*4.1.22. 3-(tert-Butyl)-7-methyl-1-phenylimidazo[1,2-a:4,5-c'] dipyridine (**7a**)*

CH_2Cl_2, pale yellow solid, m.p.: 190–194 °C, 74 mg. Yield: 89%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.91–8.88 (m, 2H, Ph-2,6), 8.23 (s, 1H, H-6), 7.71–7.68 (m, 2H, H-4 & H-9), 7.59–7.54 (m, 2H, Ph-3,5), 7.46–7.40 (m, 1H, Ph-4), 7.31 (dd, $^3J = 9.5$ Hz, $^4J = 1.7$ Hz, 1H, H-8), 2.41 (s, 3H, Me), 1.55 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.6 (C-3), 148.1 (C-9a), 147.7 (C-1), 138.9 (Ph-1), 137.2 (C-10a*), 135.1 (C-4a*), 133.7 (C-8), 129.5 (Ph-2,6), 128.9 (Ph-4), 128.5 (Ph-3,5), 122.8 (C-6), 120.7 (C-7), 118.5 (C-9), 98.8 (C-4), 38.0 (Cq *t*-Bu), 30.9 (CH_3 *t*-Bu), 18.3 (Me). HRMS (ESI): *m/z* calc. for $C_{21}H_{21}N_3$ $[M+I]^+$: 316.18082, found: 316.17996.

*4.1.23. 3-(tert-Butyl)-1-phenyl-7-(thien-2-yl)imidazo[1,2-a:4,5-c'] dipyridine (**7b**)*

CH_2Cl_2, pale yellow solid, m.p.: 229–233 °C, 90 mg. Yield: 89%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.91–8.88 (m, 2H, Ph-2,6), 8.60 (s, 1H, H-6), 7.80 (dd, $^3J = 9.6$ Hz, $^5J = 0.8$ Hz, 1H, H-9), 7.75 (s, 1H, H-4), 7.71 (dd, $^3J = 9.6$ Hz, $^4J = 1.8$ Hz, 1H, H-8), 7.60–7.55 (m, 2H, Ph-3,5), 7.47–7.42 (m, 1H, Ph-4), 7.37–7.35 (m, 2H, thienyl-3,5), 7.15 (dd, $^3J = 4.9$ Hz, 3.8 Hz, 1H, thienyl-4), 1.57 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.3 (C-3), 148.0 (C-1), 147.8 (C-9a), 139.4 (thienyl-2), 138.7 (Ph-1), 137.3 (C-4a), 135.5 (C-10a), 130.4 (C-8), 129.5 (Ph-2,6), 129.0 (Ph-4), 128.5 (Ph-3,5 & thienyl-4), 125.6 (thienyl-5*), 124.4 (thienyl-3*), 121.1 (C-6), 119.6 (C-7), 119.1 (C-9), 98.9 (C-4), 38.1 (Cq *t*-Bu), 30.9 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{24}H_{21}N_3S$ $[M+I]^+$: 384.15289, found: 384.15177.

*4.1.24. 3-(tert-Butyl)-1-phenyl-7-(pyridin-4-yl)imidazo[1,2-a:4,5-c']dipyridine (**7c**)*

CH_2Cl_2 → CH_2Cl_2/MeOH 99/1, pale yellow solid, m.p.: 254–258 °C, 72 mg. Yield: 73%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.89–8.86 (m, 2H, Ph-2,6), 8.72 (bs, 2H, pyr-2,6), 8.67 (s, 1H, H-6), 7.82 (d, $^3J = 9.6$ Hz, 1H, H-9), 7.77 (s, 1H, H-4), 7.67 (dd, $^3J = 9.6$ Hz, $^4J = 1.5$ Hz, 1H, H-8), 7.56–7.51 (m, 4H, Ph-3,5 & pyr-3,5), 7.45–7.40 (m, 1H, Ph-4), 1.56 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.5 (C-3), 150.7 (pyr-2,6), 148.1 (C-1*), 147.9 (C-9a*), 144.1 (pyr-4), 138.5 (Ph-1), 137.3 (C-4a), 135.5 (C-10a), 129.5 (C-8), 129.5 (Ph-2,6), 129.1 (Ph-4), 128.4 (Ph-3,5), 123.5 (C-6), 122.5 (C-7), 121.2 (pyr-3,5), 119.5 (C-9), 98.9 (C-4), 38.1 (Cq *t*-Bu), 30.8 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{25}H_{22}N_4$ $[M+I]^+$: 379.19172, found: 379.19058.

*4.1.25. 3-(tert-Butyl)-1,7-diphenylimidazo[1,2-a:4,5-c']dipyridine (**7d**)*

CH_2Cl_2, orange solid, m.p.: 267–271 °C, 92 mg. Yield: 93%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.93–8.89 (m, 2H, 1-Ph-2,6), 8.60 (m, 1H, H-6), 7.86 (dd, $^3J = 9.6$ Hz, $^5J = 0.8$ Hz, 1H, H-9), 7.78 (s, 1H, H-4), 7.75 (dd, $^3J = 9.6$ Hz, $^4J = 1.8$ Hz, 1H, H-8), 7.68–7.67 (m, 2H, 7-Ph-2,6), 7.60–7.41 (m, 6H, 1-Ph-3,4,5 & 7-Ph-3,4,5), 1.56 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.1 (C-3), 148.1 (C-1*), 148.0 (C-9a*), 138.8 (1-Ph-1), 137.4 (7-Ph-1), 136.9 (C-10a), 135.6 (C-4a), 131.3 (C-8), 129.6 (1-Ph-2,6), 129.4 (1-Ph-3,5*), 129.0 (1-Ph-4*), 128.5 (7-Ph-3,5*), 128.3 (7-Ph-4*), 127.0 (7-Ph-2,6), 125.6 (C-7), 122.5 (C-6), 119.1 (C-9), 98.9 (C-4), 38.1 (Cq *t*-Bu), 30.1 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{26}H_{23}N_3$ $[M+I]^+$: 378.19647, found: 378.19548.

*4.1.26. 3-(tert-Butyl)-1-methoxy-7-(pyridin-4-yl)imidazo[1,2-a:4,5-c']dipyridine (**7e**)*

CH_2Cl_2/MeOH 99/1 → CH_2Cl_2/MeOH 96/4, brown solid, m.p.: 190–194 °C, 106 mg. Yield: 99%. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.75–8.73 (m, 2H, pyr-2,6), 8.62 (dd, $^4J = 1.5$ Hz, $^5J = 0.9$ Hz, 1H, H-6), 7.81 (dd, $^3J = 9.6$ Hz, $^5J = 0.7$ Hz, 1H, H-9), 7.65 (dd, $^3J = 9.6$ Hz, $^4J = 1.7$ Hz, 1H, H-8), 7.59–7.56 (m, 2H, pyr-3,5), 7.39 (s, 1H, H-4), 4.19 (s, 3H, OCH_3), 1.45 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.4 (C-3), 155.2 (C-1), 150.7 (pyr-2,6), 147.0 (C-9a), 144.5 (pyr-4),

135.4 (C-4a*), 128.4 (C-10a*), 128.3 (C-8), 123.4 (C-6), 122.8 (C-7), 121.3 (pyr-3,5), 119.7 (C-9), 94.3 (C-4), 53.5 (OCH_3), 37.9 (Cq *t*-Bu), 30.5 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{20}H_{20}N_4O$ $[M+I]^+$: 333.17099, found: 333.16997.

*4.1.27. General method for salt formation (compounds **8**–**30**)*

To a solution of **6a**, **6b-p** or **7a-e** (*e.g.* 0.5 mmol) in CH_3CN (5 mL) was added benzyl bromide or ethyl iodide (8 eq for **6a**, **6b-i**, **6m-o** and **7a-d** or 1.5 eq for **6j-l**, **6p** and **7e**). The tube was sealed and the reaction heated to 80 °C for *ca.* 24–72 h. After cooling in an ice bath the solid was filtered and washed with EtOAc.

*4.1.28. 10-Benzyl-3-(tert-butyl)-1-methylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**8**)*

Beige solid, m.p.: 138–142 °C, 156 mg. Yield: 69%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.84 (d, $^3J = 6.6$ Hz, 1H, H-6), 8.70 (s, 1H, H-4), 8.50–8.42 (m, 2H, H-8 & H-9), 7.82 (m, 1H, H-7), 7.40–7.36 (m, 3H, Ph-3,4,5), 7.25–7.23 (m, 2H, Ph-2,6), 6.18 (s, 2H, $C\underline{H}_2$Ph), 2.76 (s, 3H, CH_3), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.3 (C-3), 144.0 (C-1*), 143.6 (C-9a*), 141.0 (C-8), 135.7 (Ph-1), 133.9 (C-4a), 130.3 (C-6), 129.1 (Ph-3,5), 128.0 (Ph-4), 125.7 (Ph-2,6), 125.5 (C-10a), 117.0 (C-7), 111.2 (C-9), 102.0 (C-4), 48.2 ($C\underline{H}_2$Ph), 37.7 (Cq *t*-Bu), 30.2 (CH_3 *t*-Bu), 22.4 (Me). HRMS (ESI): *m/z* calc. for $C_{22}H_{24}N_3$ $[M]^+$: 330.19647, found: 330.19572.

*4.1.29. 10-Benzyl-3-(ethoxycarbonyl)-1-methylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**9**)*

Beige solid, m.p.: 256–260 °C, 5 mg. Yield: 15%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.73 (d, $^3J = 6.9$ Hz, 1H, H-6), 9.28 (s, 1H, H-4), 8.52 (ddd, $^3J = 9.3$ Hz, 7.2 Hz, $^4J = 0.9$ Hz, 1H, H-8), 8.37 (d, $^3J = 9.3$ Hz, 1H, H-9), 7.87 (t, $^3J = 6.9$ Hz, 1H, H-7), 7.44–7.37 (m, 3H, Ph-3,4,5), 7.26–7.23 (m, 2H, Ph-2,6), 6.24 (s, 2H, $C\underline{H}_2$Ph), 4.55 (q, $^3J = 7.1$ Hz, 2H, $OC\underline{H}_2CH_3$), 2.99 (s, 3H, CH_3), 1.49 (t, $^3J = 7.1$ Hz, 3H, $OCH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, CD_3OD) δ: 165.3 (C-3), 147.8 (C-1), 146.6 (C-9a), 143.53 (C-4a), 143.47 (C-8), 135.9 (C-10a), 135.2 (Ph-1), 131.7 (C-6), 130.6 (Ph-3,5), 129.7 (Ph-4), 126.7 (Ph-2,6), 119.4 (C-7), 112.6 (C-9), 110.4 (C-4), 63.5 ($O\underline{C}H_2CH_3$), 50.3 ($\underline{C}H_2$Ph), 22.4 (Me), 14.6 ($OCH_2\underline{C}H_3$). CO is missing. HRMS (ESI): *m/z* calc. for $C_{21}H_{20}N_3O_2$ $[M]^+$: 346.15500, found: 346.15400.

*4.1.30. 10-Benzyl-3-cyclopropyl-1-methylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**10**)*

Brown solid, m.p.: 158–162 °C, 7 mg. Yield: 39%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.54 (d, $^3J = 6.8$ Hz, 1H, H-6), 8.49–8.43 (m, 2H, H-8 & H-4), 8.31 (d, $^3J = 9.3$ Hz, 1H, H-9), 7.81 (td, $^3J = 7.0$ Hz, $^4J = 0.8$ Hz, 1H, H-7), 7.44–7.36 (m, 3H, Ph-3,4,5), 7.25–7.22 (m, 2H, Ph-2,6), 6.17 (s, 2H, $C\underline{H}_2$Ph), 2.90 (s, 3H, $C\underline{H}_3$), 2.47–2.39 (m, 1H, C$\underline{H}$ c-Pr), 1.26–1.20 (m, 4H, 2 $C\underline{H}_2$ c-Pr). ^{13}C NMR (75 MHz, CD_3OD) δ: 158.6 (C-3), 146.9 (C-1*), 146.0 (C-9a*), 143.7 (C-8), 137.2 (Ph-1), 135.7 (C-4a), 131.5 (C-6), 130.6 (Ph-3,5), 129.7 (Ph-4), 127.9 (C-10a), 126.8 (Ph-2,6), 119.2 (C-7), 112.5 (C-9), 105.1 (C-4), 50.3 ($\underline{C}H_2$Ph), 20.6 (Me), 16.8 (CH c-Pr), 11.6 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{21}H_{20}N_3$ $[M]^+$: 314.16517, found: 314.16443.

*4.1.31. 10-Benzyl-1-methyl-3-phenylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**11**)*

Brown solid, m.p: 238–242 °C, 35 mg. Yield: 30%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.85 (d, $^3J = 6.9$ Hz, 1H, H-6), 9.37 (s, 1H, H-4), 8.54–8.47 (m, 2H, H-8 & H-9), 8.26 (d, $^3J = 7.2$ Hz, 2H, Ph-2,6), 7.91 (td, $^3J = 6.9$ Hz, $^4J = 0.9$ Hz, 1H, H-7), 7.61–7.47 (m, 3H, Ph-3,4,5), 7.42–7.26 (m, 5H, CH_2Ph), 6.23 (s, 2H, $C\underline{H}_2$Ph); 2.84 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 150.4 (C-3), 144.8 (C-1), 144.2 (C-9a), 141.5 (C-8), 137.5 (3-Ph-1), 135.7 (CH_2Ph-1*), 134.7 (C-4a*), 130.4 (C-6), 129.5 (3-Ph-4), 129.1 (CH_2Ph-3,5*), 129.0 (3-Ph-3,5*), 128.1 (CH_2Ph-4), 126.8 (C-10a), 126.7 (3-Ph-2,6), 125.7 (CH_2Ph-2,6), 117.4 (C-7), 111.4 (C-9), 103.2 (C-4), 48.3 ($C\underline{H}_2$Ph), 22.4 (Me). HRMS (ESI): *m/z* calc. for $C_{24}H_{20}N_3$ $[M]^+$: 350.16517, found: 350.16432.

*4.1.32. 3-(tert-Butyl)-10-ethyl-1-methylimidazo[1,2-a:4,5-c'] dipyridin-10-ium iodide (**12**)*

Brown solid, m.p: 244–248 °C, 52 mg. Yield: 63%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.72 (d, $^3J = 6.9$ Hz, 1H, H-6), 8.61 (s, 1H, H-4), 8.50–8.40 (m, 2H, H-9 & H-8), 7.76 (td, $^3J = 6.7$ Hz, $^4J = 1.2$ Hz, 1H, H-7), 4.88 (q, $^3J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 3.08 (s, 3H, Me), 1.49 (t, $^3J = 7.2$ Hz, 3H, $CH_2C\underline{H}_3$), 1.44 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.9 (C-3), 143.6 (C-1*), 142.8 (C-9a*), 140.3 (C-8), 133.4 (C-4a), 129.9 (C-6), 125.2 (C-10a), 116.6 (C-7), 111.4 (C-9), 101.8 (C-4), 40.8 ($C\underline{H}_2CH_3$), 37.6 (Cq *t*-Bu), 30.2 (CH_3 *t*-Bu), 22.7 (Me), 15.2 ($CH_2C\underline{H}_3$). HRMS (ESI): *m/z* calc. for $C_{17}H_{22}N_3$ $[M]^+$: 268.18082, found: 268.17990.

*4.1.33. 10-Benzyl-3-(tert-butyl)-1-ethylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**13**)*

Beige solid, m.p: 158–162 °C, 30 mg. Yield: 40%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.68 (d, $^3J = 6.8$ Hz, 1H, H-6), 8.52 (s, 1H, H-4), 8.42 (ddd, $^3J = 9.3$ Hz, 6.9 Hz, $^4J = 0.9$ Hz, 1H, H-8), 8.29 (d, $^3J = 9.3$ Hz, 1H, H-9), 7.78 (t, $^3J = 6.9$ Hz, 1H, H-7), 7.43–7.21 (m, 5H, Ph), 6.18 (s, 2H, $C\underline{H}_2$Ph), 3.23 (q, $^3J = 7.4$ Hz, 2H, $C\underline{H}_2CH_3$), 1.52 (s, 9H, *t*-Bu), 1.30 (t, $^3J = 7.4$ Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, CD_3OD) δ: 165.8 (C-3), 150.1 (C-1), 145.5 (C-9a), 142.2 (C-8), 136.0 (Ph-1*), 135.4 (C-4a*), 131.1 (C-6), 130.5 (Ph-3,5), 129.5 (Ph-4), 126.7 (Ph-2,6), 126.5 (C-10a), 118.5 (C-7), 112.3 (C-9), 102.5 (C-4), 50.4 ($\underline{C}H_2$Ph), 39.2 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu), 28.7 ($\underline{C}H_2CH_3$), 12.6 ($CH_2\underline{C}H_3$). HRMS (ESI): *m/z* calc. for $C_{23}H_{26}N_3$ $[M]^+$: 344.21212, found: 344.21126.

*4.1.34. 3-Cyclopropyl-1,10-diethylimidazo[1,2-a:4,5-c']dipyridin-10-ium iodide (**14**)*

Pale yellow solid, m.p.: 256–260 °C, 30 mg. Yield: 15%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.52 (d, $^3J = 6.9$ Hz, 1H, H-6), 8.47–8.38 (m, 3H, H-4, H-8 & H-9), 7.75 (td, $^3J = 6.6$ Hz, $^4J = 1.5$ Hz, 1H, H-7), 4.83 (q, $^3J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 3.32 (q, $^3J = 7.5$ Hz, 2H, $C\underline{H}_2CH_3$), 2.35–2.27 (m, 1H, CH c-Pr), 1.47 (t, $^3J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 1.38 (t, $^3J = 7.3$ Hz, 3H, $CH_2C\underline{H}_3$), 1.13–1.02 (m, 4H, 2 CH_2 c-Pr). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 156.2 (C-3), 148.7 (C-1), 142.7 (C-9a), 140.2 (C-8), 133.5 (C-4a), 129.6 (C-6), 124.5 (C-10a), 116.7 (C-7), 111.4 (C-9), 102.7 (C-4), 41.1 ($C\underline{H}_2CH_3$), 27.1 ($C\underline{H}_2CH_3$), 17.2 (CH c-Pr), 15.0 ($CH_2C\underline{H}_3$), 12.2 ($CH_2C\underline{H}_3$), 10.2 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{17}H_{20}N_3$ $[M]^+$: 266.16517, found: 266.16450.

*4.1.35. 10-Benzyl-3-(tert-butyl)-1-phenylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**15**)*

Yellow solid, m.p: 208–212 °C, 54 mg. Yield: 34%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.94 (d, $^3J = 6.6$ Hz, 1H, H-6), 8.92 (s, 1H, H-4), 8.49 (t, $^3J = 8.7$ Hz, 1H, H-8), 8.42 (d, $^3J = 9.0$ Hz, 1H, H-9), 7.88 (t, $^3J = 6.6$ Hz, 1H, H-7), 7.52–7.41 (m, 5H, 1-Ph), 7.20–7.13 (m, 3H, CH_2Ph-3,4,5), 6.56 (d, $^3J = 7.2$ Hz, 2H, CH_2Ph-2,6), 5.64 (s, 2H, $C\underline{H}_2$Ph), 1.46 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.3 (C-3), 145.2 (C-1), 144.3 (C-9a), 141.5 (C-8), 136.8 (1-Ph-1), 134.9 (C-4a), 134.0 (CH_2Ph-1), 130.6 (C-6), 129.4 (1-Ph-4), 129.2 (1-Ph-2,6), 128.5 (1-Ph-3,5*), 128.3 (CH_2Ph-3,5*), 127.8 (CH_2Ph-4), 125.8 (CH_2Ph-2,6), 124.5 (C-10a), 117.3 (C-7), 111.5 (C-9), 103.3 (C-4), 48.5 ($\underline{C}H_2$Ph), 37.9 (Cq *t*-Bu), 30.2 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{27}H_{26}N_3$ $[M]^+$: 392.21212, found: 392.21099.

*4.1.36. 10-Benzyl-3-cyclopropyl-1-phenylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**16**)*

Beige solid, m.p.: 252–256 °C, 133 mg. Yield: 55%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.57 (d, $^3J = 6.8$ Hz, 1H, H-6), 8.46 (s, 1H, H-4), 8.41 (ddd, $^3J = 9.1$ Hz, 7.1 Hz, $^4J = 1.1$ Hz, 1H, H-8), 8.18 (d, $^3J = 9.3$ Hz, 1H, H-9), 7.79 (td, $^3J = 7.0$ Hz, $^4J = 0.9$ Hz, 1H, H-7), 7.56–7.49 (m, 1H,

Ph-4), 7.44–7.40 (m, 4H, Ph-2,3,5,6), 7.25–7.14 (m, 3H, Bn-3,4,5), 6.69–6.67 (m, 2H, Bn-2,6), 5.60 (s, 2H, $C\underline{H}_2Ph$), 2.48–2.39 (m, 1H, CH c-Pr), 1.16–1.14 (m, 4H, CH_2 c-Pr). ^{13}C NMR (75 MHz, CD_3OD) δ: 160.1 (C-3), 148.4 (C-1), 145.9 (C-9a), 142.5 (C-8), 138.0 (1-Ph-1), 136.2 (C-4a), 135.0 (CH_2Ph-1), 131.2 (C-6), 130.9 (1-Ph-4), 130.4 (1-Ph-3,5*), 130.0 (1-Ph-2,6*), 129.5 (CH_2Ph-3,5*), 129.2 (CH_2Ph-4), 126.8 (CH_2Ph-2,6), 126.2 (C-10a), 118.6 (C-7), 112.7 (C-9), 104.7 (C-4), 50.2 ($C\underline{H}_2Ph$), 18.3 (CH c-Pr), 11.3 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{26}H_{22}N_3$ $[M]^+$: 376.18082, found: 376.18001.

4.1.37. 3-Cyclopropyl-10-ethyl-1-phenylimidazo[1,2-a:4,5-c'] dipyridin-10-ium iodide (**17**)

Pale yellow solid, m.p.: 237–241 °C, 103 mg. Yield: 44%. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.62 (d, $^3J = 6.9$ Hz, 1H, H-6), 8.63 (s, 1H, H-4), 8.48–8.43 (m, 2H, H-8 & H-9), 7.81 (td, $^3J = 6.6$ Hz, $^4J = 1.5$ Hz, 1H, H-7), 7.68–7.60 (m, 5H, Ph), 4.29 (q, $^3J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 2.45–2.35 (m, 1H, CH c-Pr), 1.16–1.02 (m, 4H, 2 CH_2 c-Pr), 0.95 (t, $^3J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 156.8 (C-3), 145.9 (C-1), 143.4 (C-9a), 140.8 (C-8), 136.9 (Ph-1), 134.4 (C-4a), 129.8 (C-6*), 129.7 (Ph-4*), 129.1 (Ph-2,6*), 128.5 (Ph-3,5*), 124.4 (C-10a), 117.0 (C-7), 111.7 (C-9), 103.9 (C-4), 40.7 ($C\underline{H}_2CH_3$), 17.3 (CH c-Pr), 13.5 ($CH_2C\underline{H}_3$), 10.5 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{21}H_{20}N_3$ $[M]^+$: 314.16517, found: 314.16421.

4.1.38. 10-Benzyl-3-(tert-butyl)-1-methoxyimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**18**)

White solid, m.p: 188–192 °C, 38 mg. Yield: 50%. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.71 (d, $^3J = 6.6$ Hz, 1H, H-6), 8.49–8.38 (m, 2H, H-8 & H-9), 8.34 (s, 1H, H-4), 7.79 (t, $^3J = 6.6$ Hz, 1H, H-7), 7.43–7.33 (m, 5H, Ph), 6.03 (s, 2H, $C\underline{H}_2Ph$), 4.11 (s, 3H, OCH_3), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 161.9 (C-3); 149.8 (C-1), 142.8 (C-9a), 140.0 (C-8), 135.3 (C-4a*), 135.1 (Ph-1*), 130.1 (C-6), 128.8 (Ph-3,5), 128.2 (Ph-4), 127.2 (Ph-2,6), 117.0 (C-7), 115.2 (C-10a), 111.5 (C-9), 98.0 (C-4), 54.0 (OCH_3), 48.9 ($C\underline{H}_2Ph$), 37.8 (Cq *t*-Bu), 29.9 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{22}H_{24}N_3O$ $[M]^+$: 346.19139, found: 346.19042.

4.1.39. 10-Benzyl-3-cyclopropyl-1-methoxyimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**19**)

Off-white solid, m.p: 218–222 °C, 88 mg. Yield: 43%. ^1H NMR (300 MHz, $CDCl_3$) δ: 10.08 (d, $^3J = 6.8$ Hz, 1H, H-6), 8.53 (s, 1H, H-4), 8.35 (d, $^3J = 9.3$ Hz, 1H, H-9), 8.14 (m, 1H, H-8), 7.54 (t, $^3J = 6.9$ Hz, H-7), 7.37–7.29 (m, 5H, Ph), 6.21 (s, 2H, $C\underline{H}_2Ph$), 4.14 (s, 3H, OCH_3), 2.34–2.26 (m, 1H, CH c-Pr), 1.17–1.01 (m, 4H, 2 CH_2 c-Pr). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.1 (C-3), 151.0 (C-1), 141.9 (C-9a), 139.5 (C-8), 134.6 (C-4a*), 134.2 (Ph-1*), 131.2 (C-6), 129.5 (Ph-3,5), 129.1 (Ph-4), 127.5 (Ph-2,6), 117.7 (C-7), 115.0 (C-10a), 112.5 (C-9), 100.3 (C-4), 54.4 (OCH_3), 50.5 ($C\underline{H}_2Ph$), 17.8 (CH c-Pr), 10.8 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{21}H_{20}N_3O$ $[M]^+$: 330.16009, found: 330.15921.

4.1.40. 10-Benzyl-1-methoxy-3-phenylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**20**)

Off-white solid, m.p: 190–194 °C, 48 mg. Yield: 49%. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.71 (d, $^3J = 6.6$ Hz, 1H, H-6), 9.06 (s, 1H, H-4), 8.53–8.42 (m, 2H, H-8 & H-9), 8.26 (d, $^3J = 7.5$ Hz, 2H, 3-Ph-2,6), 7.86 (t, $^3J = 6.6$ Hz, 1H, H-7), 7.60–7.31 (m, 8H, 3-Ph-3,4,5 & CH_2Ph), 6.08 (s, 2H, $C\underline{H}_2Ph$), 4.23 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 150.6 (C-1), 148.6 (C-3), 143.0 (C-9a), 140.4 (C-8), 137.1 (C-4a), 135.7 (3-Ph-1), 135.2 (CH_2Ph-1), 130.2 (C-6), 129.6 (3-Ph-4), 129.0 (3-Ph-3,5*), 128.8 (CH_2Ph-3,5*), 128.2 (CH_2Ph-4), 127.2 (CH_2Ph-2,6), 126.5 (3-Ph-2,6), 117.4 (C-7), 116.4 (C-10a), 111.7 (C-9), 99.2 (C-4), 54.4 (OCH_3), 49.0 ($C\underline{H}_2Ph$). HRMS (ESI): *m/z* calc. for $C_{24}H_{20}N_3O$ $[M]^+$: 366.16009, found: 366.15899.

4.1.41. 3-Cyclopropyl-10-ethyl-1-methoxyimidazo[1,2-a:4,5-c'] dipyridin-10-ium iodide (**21**)

Yellow solid, m.p.: >260 C, 69 mg. Yield: 47%. ^1H NMR (300 MHz, DMSO-d_6) δ: 9.46 (d, $^3J = 6.8$ Hz, 1H, H-6), 8.45 (d, $^3J = 9.3$ Hz, 1H, H-9), 8.36 (ddd, $^3J = 9.0$ Hz, 6.9 Hz, $^4J = 1.2$ Hz, 1H, H-8), 8.17 (s, 1H, H-4), 7.73 (td, $^3J = 6.8$ Hz, $^4J = 1.0$ Hz, 1H, H-7), 4.77 (q, $^3J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 4.13 (s, 3H, OCH_3), 2.28–2.20 (m, 1H, CH c-Pr), 1.45 (t, $^3J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 1.08–1.06 (m, 4H, 2 CH_2 c-Pr). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 155.1 (C-3), 150.8 (C-1), 142.0 (C-9a), 139.2 (C-8), 134.8 (C-4a), 129.4 (C-6), 116.7 (C-7), 114.9 (C-10a), 111.6 (C-9), 99.0 (C-4), 54.2 (OCH_3), 41.5 ($C\underline{H}_2CH_3$), 17.1 (CH c-Pr), 15.1 ($CH_2C\underline{H}_3$), 9.9 (CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{16}H_{18}N_3O$ $[M]^+$: 268.14444, found: 268.14368.

4.1.42. 10-Benzyl-3-(tert-butyl)-1-hydroxyimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**22**)

Yellow solid, m.p.: >260 °C, 31 mg. Yield: 80%. ^1H NMR (300 MHz, CD_3OD) δ: 9.31 (dt, $^3J = 6.9$ Hz, $^4J = {}^5J = 0.9$ Hz, 1H, H-6), 8.24–8.22 (m, 2H, H-8 & H-9), 7.69 (m, 1H, H-7), 7.51–7.48 (m, 2H, Ph-2,6), 7.36–7.33 (m, 4H, Ph-3,4,5 & H-4), 6.20 (s, 2H, $C\underline{H}_2Ph$), 1.48 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, CD_3OD) δ: 156.9 (C-3*), 156.8 (C-1*), 143.4 (C-9a), 139.3 (C-8), 136.1 (Ph-1), 134.9 (C-4a), 130.4 (C-6), 130.2 (Ph-3,5), 129.8 (Ph-4), 128.8 (Ph-2,6), 120.2 (C-10a), 118.9 (C-7), 112.7 (C-9), 89.6 (C-4), 49.8 ($C\underline{H}_2Ph$), 36.8 (Cq *t*-Bu), 29.3 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{21}H_{22}N_3O$ $[M]^+$: 332.17574, found: 332.17473.

4.1.43. 10-Benzyl-7-bromo-3-(tert-butyl)-1-methylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**23**)

Pale yellow solid, m.p.: 220–224 °C, 145 mg. Yield: 99%. ^1H NMR (300 MHz, DMSO-d_6) δ: 10.23 (d, $^4J = 1.2$ Hz, 1H, H-6), 8.70 (s, 1H, H-4), 8.66 (dd, $^3J = 9.7$ Hz, $^4J = 1.7$ Hz, 1H, H-8), 8.49 (d, $^3J = 9.8$ Hz, 1H, H-9), 7.41–7.31 (m, 3H, Ph-3,4,5), 7.24–7.21 (m, 2H, Ph-2,6), 6.19 (s, 2H, $C\underline{H}_2Ph$), 2.74 (s, 3H, Me), 1.45 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.6 (C-3), 143.9 (C-1), 143.3 (C-9a), 142.9 (C-8), 135.6 (C-4a), 133.6 (Ph-1), 130.6 (C-6), 129.1 (Ph-3,5), 128.1 (Ph-4), 125.7 (Ph-2,6), 125.5 (C-10a), 112.5 (C-9), 110.7 (C-7), 102.2 (C-4), 48.4 ($C\underline{H}_2Ph$), 37.7 (Cq *t*-Bu), 30.1 (CH_3 *t*-Bu), 22.4 ($\underline{Me}$). HRMS (ESI): *m/z* calc. for $C_{22}H_{23}^{79}BrN_3$ $[M]^+$: 408.10699, found: 408.10601; *m/z* calc. for $C_{22}H_{23}^{81}BrN_3$ $[M]^+$: 410.10494, found: 410.10392.

4.1.44. 10-Benzyl-7-bromo-3-cyclopropyl-1-ethylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**24**)

Pale yellow solid, m.p.: 232–236 °C, 31 mg. Yield: 20%. ^1H NMR (300 MHz, DMSO-d_6) δ: 10.09 (s, 1H, H-6), 8.65 (dd, $^3J = 9.7$ Hz, $^4J = 1.2$ Hz, 1H, H-8), 8.51–8.47 (m, 2H, H-4 & H-9), 7.36–7.34 (m, 3H, Ph-3,4,5), 7.22–7.19 (m, 2H, Ph-2,6), 6.16 (s, 2H, $C\underline{H}_2Ph$), 3.01 (q, $^3J = 7.3$ Hz, 2H, $C\underline{H}_2CH_3$), 2.34–2.24 (m, 1H, CH c-Pr), 1.11–1.02 (m, 7H, $2CH_2$ c-Pr & $CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 157.1 (C-3), 149.0 (C-1), 143.3 (C-8), 142.8 (C-9a), 135.2 (Ph-1), 133.7 (C-4a), 130.3 (C-6), 129.0 (Ph-3,5), 128.1 (Ph-4), 125.7 (Ph-2,6), 124.7 (C-10a), 112.5 (C-9), 110.6 (C-7), 102.8 (C-4), 48.7 ($C\underline{H}_2Ph$), 26.8 ($C\underline{H}_2CH_3$), 17.3 (CH c-Pr), 12.1 ($CH_2C\underline{H}_3$), 10.4 (2 CH_2 c-Pr). HRMS (ESI): *m/z* calc. for $C_{22}H_{21}^{79}BrN_3$ $[M]^+$: 406.09134, found: 406.09033; *m/z* calc. for $C_{22}H_{21}^{81}BrN_3$ $[M]^+$: 408.08929, found: 408.08815.

4.1.45. 10-Benzyl-7-bromo-3-(tert-butyl)-1-phenylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**25**)

Yellow solid, m.p.: 156–160 °C, 112 mg. Yield: 33%. ^1H NMR (300 MHz, $CDCl_3$) δ: 10.67 (d, $^4J = 0.9$ Hz, 1H, H-6), 9.05 (s, 1H, H-4), 8.50 (d, $^3J = 9.7$ Hz, 1H, H-9), 8.15 (dd, $^3J = 9.6$ Hz, $^4J = 1.1$ Hz, 1H, H-8), 7.57–7.41 (m, 5H, 1-Ph), 7.18–7.10 (m, 3H, CH_2Ph-3,4,5), 6.75–6.72 (m, 2H, CH_2Ph-2,6), 5.92 (s, 2H, $C\underline{H}_2Ph$), 1.48 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.3 (C-3), 146.3 (C-1), 143.2 (C-8), 142.5 (C-9a), 136.7 (1-Ph-1), 133.9 (CH_2Ph-1*), 133.4 (C-4a*), 130.7 (C-6), 130.1 (1-Ph-4), 129.7 (1-Ph-2,6), 129.2 (1-Ph-3,5*), 128.8

(CH_2Ph-3,5*), 128.7 (CH_2Ph-4), 126.4 (CH_2Ph-2,6), 124.7 (C10a), 114.6 (C-9), 112.1 (C-7), 104.1 (C-4), 50.4 ($\underline{C}H_2$Ph), 38.8 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{27}H_{25}^{79}BrN_3$ $[M]^+$: 470.12264, found: 470.12126; *m/z* calc. for $C_{27}H_{25}^{81}BrN_3$ $[M]^+$: 472.12059, found: 472.11915.

*4.1.46. 10-Benzyl-3-(tert-butyl)-7-methyl-1-phenylimidazo[1,2-a:4,5-c']dipyridin-10-ium bromide (**26**)*

White solid, m.p.: 237–241 °C, 57 mg. Yield: 53%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.56 (s, 1H, H-6), 8.62 (s, 1H, H-4), 8.30 (dd, ^3J = 9.4 Hz, ^4J = 1.5 Hz, 1H, H-8), 8.10 (d, ^3J = 9.4 Hz, 1H, H-9), 7.57–7.40 (m, 5H, 1-Ph), 7.25–7.13 (m, 3H, CH_2Ph-3,4,5), 6.69–6.66 (m, 2H, CH_2Ph-2,6), 5.61 (s, 2H, $C\underline{H}_2$Ph), 2.65 (s, 3H, Me), 1.53 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, CD_3OD) δ: 166.1 (C-3), 147.6 (C-1), 144.9 (C-8), 144.8 (C-9a), 138.5 (1-Ph-1), 135.9 (C-4a), 135.1 (CH_2Ph-1), 130.8 (1-Ph-4), 130.6 (1-Ph-2,6), 130.0 (1-Ph-3,5*), 129.8 (C-7), 129.5 (CH_2Ph-3,5*), 129.2 (CH_2Ph-4), 129.0 (C-6), 126.9 (CH_2Ph-2,6), 126.2 (C-10a), 111.9 (C-9), 103.5 (C-4), 50.1 ($\underline{C}H_2$Ph), 39.2 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu), 17.9 (Me). HRMS (ESI): *m/z* calc. for $C_{28}H_{28}N_3$ $[M]^+$: 406.22777, found: 406.22663.

*4.1.47. 10-Benzyl-3-(tert-butyl)-1,7-diphenylimidazo[1,2-a:4,5-c'] dipyridin-10-ium bromide (**27**)*

White solid, m.p.: 244–248 °C, 103 mg. Yield: 97%. ^{1}H NMR (300 MHz, CD_3OD) δ: 9.99 (s, 1H, H-6), 8.86 (s, 1H, H-4), 8.74 (dd, ^3J = 9.6 Hz, ^4J = 1.7 Hz, 1H, H-8), 8.27 (d, ^3J = 9.6 Hz, 1H, H-9), 7.97–7.94 (m, 2H, 7-Ph-2,6), 7.72–7.39 (m, 8H, 7-Ph-3,4,5 & 1-Ph), 7.30–7.12 (m, 3H, CH_2Ph-3,4,5), 6.74–6.72 (m, 2H, CH_2Ph-2,6), 5.67 (s, 2H, $C\underline{H}_2$Ph), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, CD_3OD) δ: 166.4 (C-3), 147.7 (C-1), 145.1 (C-9a), 142.1 (C-8), 138.4 (1-Ph-1), 136.5 (C-4a), 135.5 (7-Ph-1), 135.1 (CH_2Ph-1), 133.1 (C-7), 130.8 (7-Ph-4), 130.7 (1-Ph-3,4,5), 130.6 (7-Ph-3,5), 130.0 (CH_2Ph-3,5*), 129.6 (1-Ph-2,6*), 129.3 (CH_2Ph-4), 128.6 (7-Ph-2,6), 128.3 (C-6), 126.9 (CH_2Ph-2,6), 126.4 (C-10a), 112.6 (C-9), 104.0 (C-4), 50.3 ($\underline{C}H_2$Ph), 39.3 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{33}H_{30}N_3$ $[M]^+$: 468.24342, found: 468.24191.

*4.1.48. 10-Benzyl-3-(tert-butyl)-1-phenyl-7-(thien-2-yl)imidazo [1,2-a:4,5-c']dipyridin-10-ium bromide (**28**)*

Off-white solid, m.p.: 251–255 °C, 119 mg. Yield: 90%. ^{1}H NMR (300 MHz, CD_3OD) δ: 10.0 (q, ^4J = 1.8 Hz, ^5J = 0.9 Hz, 1H, H-6), 8.87 (s, 1H, H-4), 8.70 (dd, ^3J = 9.6 Hz, ^4J = 1.8 Hz, 1H, H-8), 8.23 (dd, ^3J = 9.6 Hz, ^5J = 0.7 Hz, 1H, H-9), 7.85 (dd, ^3J = 3.7 Hz, ^4J = 1.1 Hz, 1H, thienyl-3), 7.71 (dd, ^3J = 5.1 Hz, ^4J = 1.1 Hz, 1H, thienyl-5), 7.59–7.41 (m, 5H, 1-Ph), 7.29 (dd, ^3J = 5.1 Hz, 3.7 Hz, 1H, thienyl-4), 7.24–7.15 (m, 3H, CH_2Ph-3,4,5), 6.72–6.70 (m, 2H, CH_2Ph-2,6), 5.65 (s, 2H, $C\underline{H}_2$Ph), 1.55 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, CD_3OD) δ: 166.4 (C-3), 147.7 (C-1), 144.8 (C-9a), 140.9 (C-8), 138.4 (1-Ph-1), 137.6 (thienyl-2), 136.4 (C-4a), 135.0 (CH_2Ph-1), 130.8 (1-Ph-4), 130.6 (1-Ph-2,6), 130.03 (1-Ph-3,5*), 130.00 (thienyl-4), 129.6 (CH_2Ph-3,5*), 129.34 (CH_2Ph-4*), 129.32 (thienyl-5*), 128.2 (thienyl-3), 127.4 (C-7), 126.9 (CH_2Ph-2,6), 126.4 (C-6), 126.3 (C-10a), 112.9 (C-9), 104.0 (C-4), 50.3 ($\underline{C}H_2$Ph), 39.3 (Cq *t*-Bu), 30.7 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{31}H_{28}N_3S$ $[M]^+$: 474.20039, found: 474.19820.

*4.1.49. 10-Benzyl-7-(1-benzylpyridin-1-ium-4-yl)-3-(tert-butyl)-1-methoxyimidazo[1,2-a:4,5-c'] dipyridin-10-ium dibromide (**29**)*

Red solid, m.p.: 208–212 °C, 65 mg. Yield: 32%. ^{1}H NMR (300 MHz, CD_3OD) δ: 10.38 (s, 1H, H-6), 9.28 (d, ^3J = 6.7 Hz, 2H, pyr-2,6), 8.89 (dd, ^3J = 9.7 Hz, ^4J = 1.5 Hz, 1H, H-8), 8.79 (d, ^3J = 6.7 Hz, 2H, pyr-3,5), 8.52 (d, ^3J = 9.7 Hz, 1H, H-9), 8.45 (s, 1H, H-4), 7.67–7.29 (m, 10H, Ph), 6.16 (s, 2H, 10-$C\underline{H}_2$Ph), 5.95 (s, 2H, pyr-$C\underline{H}_2$Ph), 4.24 (s, 3H, OCH_3), 1.52 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, CD_3OD) δ: 165.8 (C-3), 152.3 (C-1*), 152.0 (pyr-4*), 146.5 (pyr-2,6), 144.4 (C-9a), 139.2 (C-8), 137.1 (C-4a), 135.9 (10-CH_2Ph-1), 134.6 (pyr-CH_2Ph-1), 132.3 (C-6), 131.1 (10-CH_2Ph-4*), 130.8 (10-CH_2Ph-3,5*), 130.32 (pyr-CH_2Ph-3,5*), 130.26 (10-CH_2Ph-2,6*), 129.8 (pyr-CH_2Ph-4*), 128.4 (pyr-CH_2Ph-2,6*), 127.4 (pyr-3,5), 125.9 (C-7), 117.3 (C-10a), 113.5 (C-9), 99.2 (C-4), 65.4 (pyr-$\underline{C}H_2$Ph), 54.9 (OCH_3), 51.3 (10-$\underline{C}H_2$Ph), 39.4 (Cq *t*-Bu), 30.6 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{34}H_{34}N_4O$ $[M]^{2+}$: 257.13608, found: 257.13525.

*4.1.50. 1-Benzyl-4-(3-(tert-butyl)-1-phenylimidazo[1,2-a:4,5-c'] dipyridin-7-yl)pyridin-1-ium bromide (**30**)*

Orange solid, m.p.: 324–328 °C, 103 mg. Yield: 97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.09 (s, 1H, H-6), 9.34 (d, ^3J = 7.0 Hz, 2H, pyr-2,6), 8.92–8.84 (m, 2H, 1-Ph-2,6), 8.69 (d, ^3J = 7.0 Hz, 2H, pyr-3,5), 8.53 (s, 1H, H-4), 8.21 (dd, ^3J = 9.9 Hz, ^4J = 1.8 Hz, 1H, H-8), 7.94 (d, ^3J = 9.7 Hz, 1H, H-9), 7.64–7.61 (m, *2*H, CH_2Ph-2,6), 7.56–7.40 (m, 6H, 1-Ph-3,4,5 & CH_2Ph-3,4,5), 5.88 (s, 2H, $C\underline{H}_2$Ph), 1.54 (s, 9H, *t*-Bu). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 159.7 (C-3), 151.8 (pyr-4), 147.6 (C-9a), 145.7 (C-1), 144.6 (pyr-2,6), 137.8 (1-Ph-1), 136.7 (C-4a), 136.2 (C-10a), 134.6 (CH_2Ph-1), 130.2 (C-6), 129.4 (C-8), 129.3 (1-Ph-4*), 129.2 (1-Ph-3,5*), 129.1 (CH_2Ph-4*), 128.8 (1-Ph-2,6), 128.6 (CH_2Ph-2,6), 128.3 (CH_2Ph-3,5*), 124.1 (pyr-3,5), 118.2 (C-7), 117.7 (C-9), 101.7 (C-4), 62.4 ($\underline{C}H_2$Ph), 37.7 (Cq *t*-Bu), 30.6 (CH_3 *t*-Bu). HRMS (ESI): *m/z* calc. for $C_{32}H_{29}N_4$ $[M]^+$: 469.23867, found: 469.23716.

4.2. Cell culture

Human cancer MDA-MB-435s, MDA-MB-231, MDA-MB-468 cells and murine mammary cancer 4T1 lines were obtained from the American Type Culture Collection (LGC Promochem, France). MDA-MB-435s, MDA-MB-231 and MDA-MB-468 cell lines were cultivated in plastic culture flasks in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS). 4T1 cells were grown in RPMI supplemented with 10% FBS. All cells were cultured at 37 °C under a humidified atmosphere containing 5% CO_2.

Test compounds were dissolved in DMSO at a stock solution of 100 mM and further diluted to 100 μM, 10 μM, 1 μM, 0.1 μM and 0.01 μM in culture medium with a final concentration of DMSO of 0.1% including the control condition.

4.3. Cell viability and toxicity assay

Cell viability and toxicity were evaluated *in vitro* using the MTT assay. This assay is based on that the yellow tetrazolium MTT can be reduced by living cells to colored formazan dye. The absorbance of the dye was measured and is proportional to the cell viability. Cells were seeded in 24 well plates at a density of 5×10^3 - 30×10^3 cells/well in 500 μL of medium containing FBS and allowed to attach overnight at 37 °C and 5% CO_2. The growth medium in each well was then replaced with culture medium containing compounds to be tested at 5 different concentrations and incubated for 1 day for toxicity or for 5 days for viability assay. After incubation, the medium was replaced by MTT reagent (5 mg/mL prepared in DMEM+5%FCS) and incubated for 30–40 min in the incubator at 37 °C, 5% CO_2. The formazan crystals were dissolved in DMSO and the absorbance was measured using a microplate spectrophotometer (Epoch, BioTek Instruments Inc, USA) at 570 nm. The obtained data were normalized to the DMSO treated cells and IC_{50}, corresponding to the concentration of the test compound giving 50% inhibition of growth, were calculated by using nonlinear regression with variable slope using graph GraphPad Prism™ v.5 (GraphPad Software, Incorporation, California, USA).

4.4. Apoptosis assay

Apoptosis was measured using BD Biosciences - FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, France) according to manufacturer recommendation. Briefly cells treated with 30 μM of compounds for 48 h were detached using accutase and stained with Annexin V and propidium iodide. Samples were analyzed on a flow cytometer Canto, BD Biosciences.

4.5. Western blotting

Pro-apoptotic and anti-apoptotic proteins were detected by standard western blot protocol. Cell lysate were loaded on polyacrylamide gel and then transferred on PVDF membranes. Primary antibodies against Bak, Bax, Bcl-2, Bcl-xL were from cell signaling (Ozyme, France) and against cleaved PARP were from Abcam, France.

4.6. Ki-67 staining

Cells were seeded in Labteck glass chambers and incubated with 30 μM of compounds for 48 h. Then cells were fixed in 4% paraformaldehyde and permeabilized using 0.3% Triton-X-100. After saturation in 3% BSA, Ki-67 were stained using primary antibody from BD Bioscience (France) and secondary AlexaFluor488 antibody (Invitrogen, France). Slides were mounted with Vectashield Vibrance with Dapi (Eurobio, France) and pictures were taken with Nikon Eclipse (Nikon Corp., Tokyo, Japan) equipped with NIS Elements software.

4.7. Wound healing assay

Migration studies were performed using 2-well culture inserts (ibidi, Regensburg, Germany) which were transferred into 24-well culture plate to form a well-defined gap without scratching the cell monolayer. Cells were cultivated in 70 μL of growth medium in a density of 40×10^3 per chamber of the culture insert and incubated overnight. After removal of inserts using a sterile forceps, different subtoxic concentrations of each test compound in 500 μL culture medium were added to each well plate. Then cells were monitored and photographed every 15 min for 24 h for wound closure using Nikon Eclipse (Nikon Corp., Tokyo, Japan) equipped with NIS Elements software. The open area was analyzed with Tscratch software (CSElab, Zurich, Switzerland).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We gratefully acknowledge the generous support provided by the Ligue Nationale Contre le Cancer – Interrégion Grand-Ouest. Abdulrahim Alzain research was supported by a grant from the Ministry of Higher Education and University of Gezira, Sudan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113258.

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