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Synthesis and Pharmacological Evaluation of New 5-aryl-1*H*-imidazoles Derivatives

Imidazole is an important nucleus that can be found in nature and is present in many biomolecules. This aromatic heterocycle with the formula $C_3H_4N_2$, exists in two tautomeric forms, because the proton can be located on N1 or N3 nitrogen. Moreover, imidazole's insertion is a key synthetic strategy in drug discovery which has found broad applications in medicinal chemistry. Compounds including an imidazole are used as new drugs for the treatment of infectious diseases, cancer, metabolic, cardiovascular, and Central Nervous System disorders as well as analgesic and inflammatory conditions (1).

Figure 1. Tautomeric forms of the imidazole

In the context of a project aiming at the development of new inhibitors of the Na^+/K^+ ATPase pump as anticancer agents, we became interested in the synthesis of a new family of compounds comprising a 5-aryl-1*H*-imidazole motif included in a macrocycle. The design of these new imidazole derivatives was based on Structure-Activity Relationship (SAR) studies on BIIA, an imidazoisoquinoline which is a drug acting against the Na^+/K^+ ATPase (Figure 2).

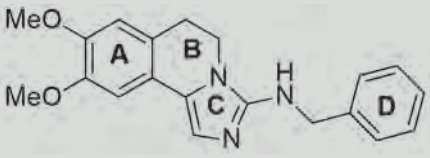


Figure 2. Structure of BIIA

It was shown that cycle B is not essential for the activity, cycle D was retained in this study, while cycles A and C are important. Accordingly, we decided to maintain these two latter cycles and, to obtain an original molecule, introduce a macrocycle of variable length connecting C2 and ortho-aryl positions (Figure 3).

Formation of a macrocycle will moreover allow increasing the conformational flexibility of the 5-aryl-1*H*-imidazole pharmacophore. A similar strategy has been used on several classes of pharmaceutical targets and has already made significant contributions to drug discovery (2).

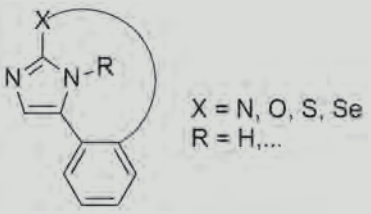
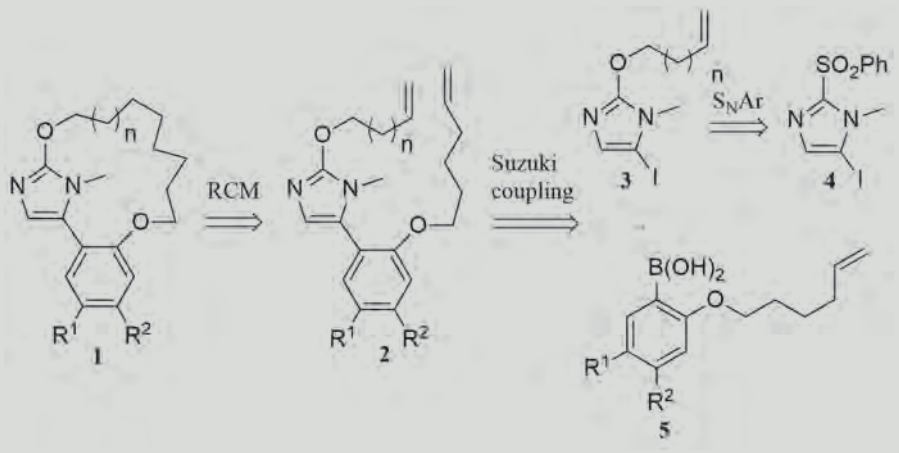
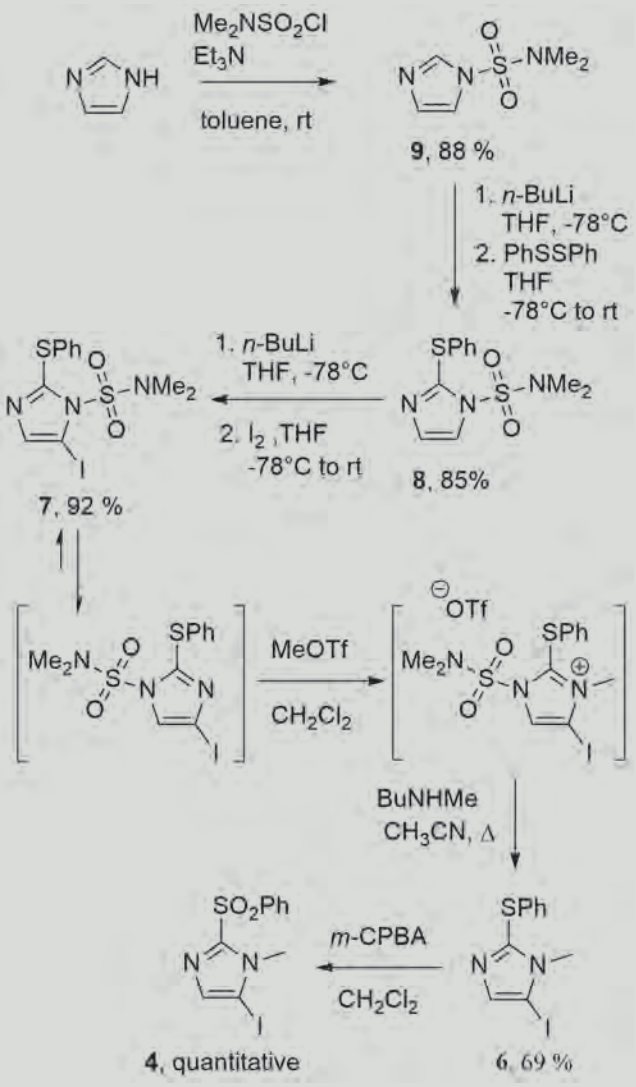


Figure 3. Schaffold of our original molecule



Scheme 1. Retro-synthetic pathway to obtain macrocycles 1



Scheme 2. Synthesis of key intermediate 4

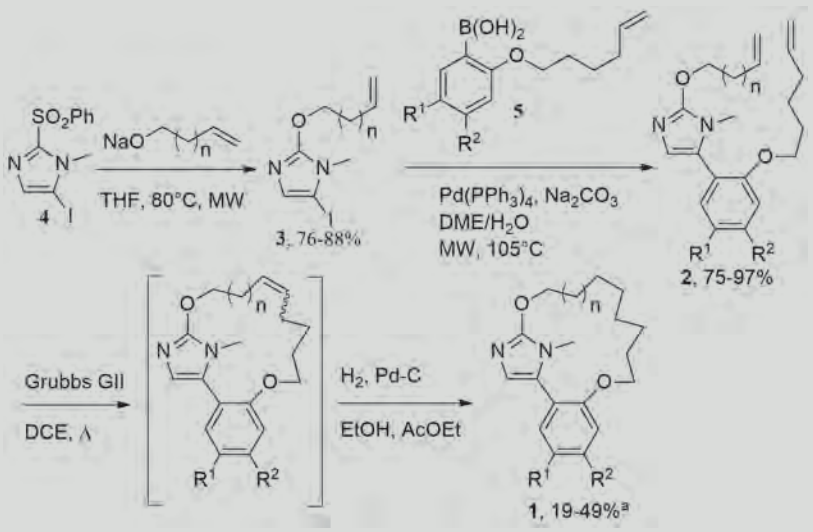
The envisioned synthesis of macrocycles **1** is based on three keys steps: a ring closure metathesis (RCM), a Suzuki coupling and an aromatic nucleophilic substitution (Scheme 1).

The first challenge was the synthesis of the common intermediate, the 1,2,5-trisubstituted imidazole **4**. Our lab has developed a regioselective synthesis of compound **4** in five steps from the (NH)-imidazole (Scheme 2) (3). The first step is protection of imidazole with an ortho-directing group, the dimethylsulfamoyl group. Different substitutions allow then introducing substituents at position C2 and C5 of the imidazole. The next step is the regioselective alkylation/deprotection of **7** and finally oxidation of the sulfide to sulfone. We obtained **4** in 47% overall yield.

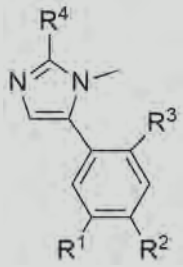
Having the common intermediate in hand, one can introduce the desired functional groups on the imidazole. The first variation, the arm in position 2, is added via an aromatic nucleophilic substitution (Scheme 3). The aryl part with the other alkyl chain is then placed via a Suzuki cross-coupling between imidazole **3** and boronic acids **5**. Finally, the ring closure metathesis and hydrogenation of the resulting double bond lead to macrocycles **1** (4).

It is interesting to mention that a detailed NMR and HPLC study revealed that some of our macrocycles **1** are chiral (5). Indeed, macrocycles with 15- and 16-membered rings (n = 1,2) are planar chiral molecules. The cyclophane-type structure of the macrocycle prevents the alicyclic ring from flipping from one side of the plane of the imidazole ring to the other (rope skipping motion). In contrast, their superior analogues, 17- and 18-membered rings (n = 3,4) isomerize rapidly at room temperature.

Unfortunately, biological assays showed that compounds **1a** and **2a** (n = 1, R^1, R^2 = H) have only low activity against Na^+/K^+ ATPase. We decided nonetheless to evaluate the *in vivo* grown inhibitory effect of **1a** (n = 1, R^1, R^2 = H) using a MTT colorimetric assay which is a rapid, sensitive quantification of proliferation and viability of cells. These *in vitro* growth inhibitory effects were determined on various cancer cell lines that we analyzed based on their display of sensitivity or resistance to pro-apoptotic stimuli. Four cancer cell lines displaying sensitivity to pro-apoptotic stimuli were analyzed: the mouse B16F10 melanoma, the human prostate



Scheme 3. Synthesis of macrocycles 1



Compound	R^4	R^3	R^2	R^1	IC_{50} APO S	IC_{50} APO R
(+/-)-1a§	$O(CH_2)_8O$		H	H	50 ± 1	68 ± 7
(+)-1a§	$O(CH_2)_8O$		H	H	43 ± 3	67 ± 3
(-)-1a§	$O(CH_2)_8O$		H	H	54 ± 6	71 ± 8
1b§	$O(CH_2)_{10}O$		H	H	29 ± 3	38 ± 4
2a§	$O(CH_2)_2CH=CH_2$	$O(CH_2)_4CH=CH_2$	H	H	13 ± 3	22 ± 6
2b	$O(CH_2)_4CH=CH_2$	$O(CH_2)_4CH=CH_2$	H	H	6 ± 1	7 ± 1
2c§	$O(CH_2)_4CH=CH_2$	OCH_3	H	H	11 ± 7	8 ± 2
2d*	OC_4H_9	OCH_3	H	H	59 ± 8	88 ± 4
2e*	$O(CH_2)_3Ph$	OCH_3	H	H	10 ± 6	3 ± 1
2f§	$O(CH_2)_2OCH_2CH=CH_2$	$O(CH_2)_4CH=CH_2$	H	H	31 ± 3	55 ± 7
2i*	$OCH_2(CF_2)_4CF_3$	OCH_3	H	H	56 ± 7	77 ± 5
2j§	$O(CH_2)_4CH=CH_2$	$O(CH_2)_4CH=CH_2$	naphthyl		33 ± 4	37 ± 9
2k§	$O(CH_2)_2CH=CH_2$	$O(CH_2)_4CH=CH_2$	OCH_3	H	38 ± 3	62 ± 7
2l§	$O(CH_2)_2CH=CH_2$	$O(CH_2)_4CH=CH_2$	F	H	56 ± 6	> 89 ± 6
2m	$O(CH_2)_4CH=CH_2$	OCH_3	H	*i*-Pr	4 ± 1	9 ± 3
2n	$O(CH_2)_4CH=CH_2$	OCH_3	H	Cl	37 ± 6	29 ± 1
6	$O(CH_2)_4CH=CH_2$	/	/	/	82 ± 12	>100

Table 1. IC_{50} growth inhibitory concentrations (in µM) of compounds determined by colorimetric MTT assay (APO S: sensitive against apoptotic stimuli; APO R: resistant against apoptotic stimuli.). § B16F10 cells were not assayed. * U373 cells were not assayed

PC-3 the breast MCF-7 and the colon LoVo carcinoma cell lines. In the same manner, four human cancer cell lines that displayed various levels of resistance to pro-apoptotic stimuli were also analyzed: the human U373 and T98G glioma, the SKMEL-28 melanoma and the A549 non-small cell lung carcinoma (NSCLC). These *in vitro* growth inhibitory effects were determined in each cancer cell line by calculating the concentration that decreased the growth of this cancer cell line by 50% (IC_{50}) after 72 h of culture in the presence of the drug of interest. Interestingly, these assays revealed that macrocycle **1a** displays significant *in vivo* growth inhibitory activity in the various cancer cell lines analyzed regardless of cells' levels of resistance to pro-apoptotic stimuli. With this attractive result, we decided to synthesize and test a series of macrocycles (**1**), "open compounds" (**2**) and intermediates (in total more than 60 compounds). Some representative results are reported in Table 1 (6).

Some conclusions can be drawn from observed inhibitory activities. First, the non-cyclized ("open") compounds (**2**) are systematically more active than their respective macrocyclic structure (**1**). In the case of enantiopure macrocycles (see **(+)-1a** and **(-)-1a**), one can see that the chirality has no effect on the activity. The nature of substituent in position 2 of the imidazole (R^4) appears to have a marked impact on the growth inhibition activity in cancer cell line models. Indeed, variations in the chain length (see **2a,b**), inclusion of fluorine (**2i**) or oxygen (**2f**) atoms induce large difference in IC_{50}. The terminal unsaturation is important for activity (compare **2a** and **d**), with the best results being obtained with a terminal double bond or a phenyl group (**2b** and **e**). The nature of the R^3 chain does not significantly influence the biological activity (compare for instance **2b** and **2c**). The aryl group is found to be crucial for the biological activity since compound **6** which misses this motif shows no or a very low growth inhibition activity. Concerning the substitution pattern, the obtained IC_{50} for compounds **2i-m** indicate that substitution of the aryl group (R^1, R^2) leads to a decrease of inhibition activity (except for R^1 = *i*-Pr, **2l**, which has no significant effect on the activity). Interestingly, a computer-assisted phase-contrast microscopy (quantitative videomicroscopy) study, using the two A549 and U373 cancer cell lineages, showed that our imidazoles are in fact cytostatic but do not display cytotoxic effects (Figure 4). Indeed, while the global growth of each of these two melanoma cell populations was decreased by approximately 50% between 48h and 72h of culture, no cell death occurred.

Compound **2b** was also analyzed by the *National Cancer Institute* (NCI). These results revealed any correlation between its growth inhibition profile with the NCI database compound profiles, suggesting that the mechanisms of action of **2b** as a potential anticancer agent should be distinct from the one of the > 763,000 compounds present in the NCI database, at least in terms of *in vitro* cancer cell growth inhibition.

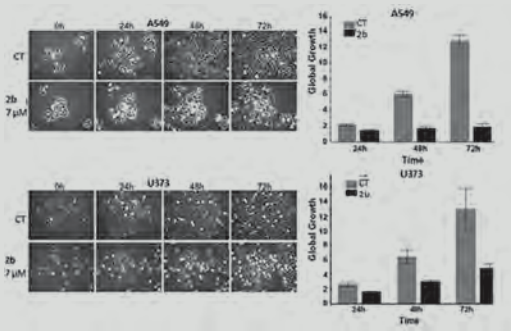


Figure 4. Quantitative video microscopy pictures of control test and test in the presence of compound 2b (left) and global growth (right). CT: control test

In conclusion, we have synthesized about sixty 5-aryl-1*H*-imidazoles, among which several compounds display single digit µM 50% growth inhibitory concentration against various cancer models, including apoptosis-resistant ones. Interestingly, the mechanism by which these compounds (at least **2b**) exert their anti-cancer cytostatic activities does not correlate with any compound from the NCI database. These compounds could represent a new way to combat apoptosis-resistant cancer types.

Acknowledgment

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