Synthesis and biological activity of novel amidino-substituted imidazo[4,5-*b*]pyridines

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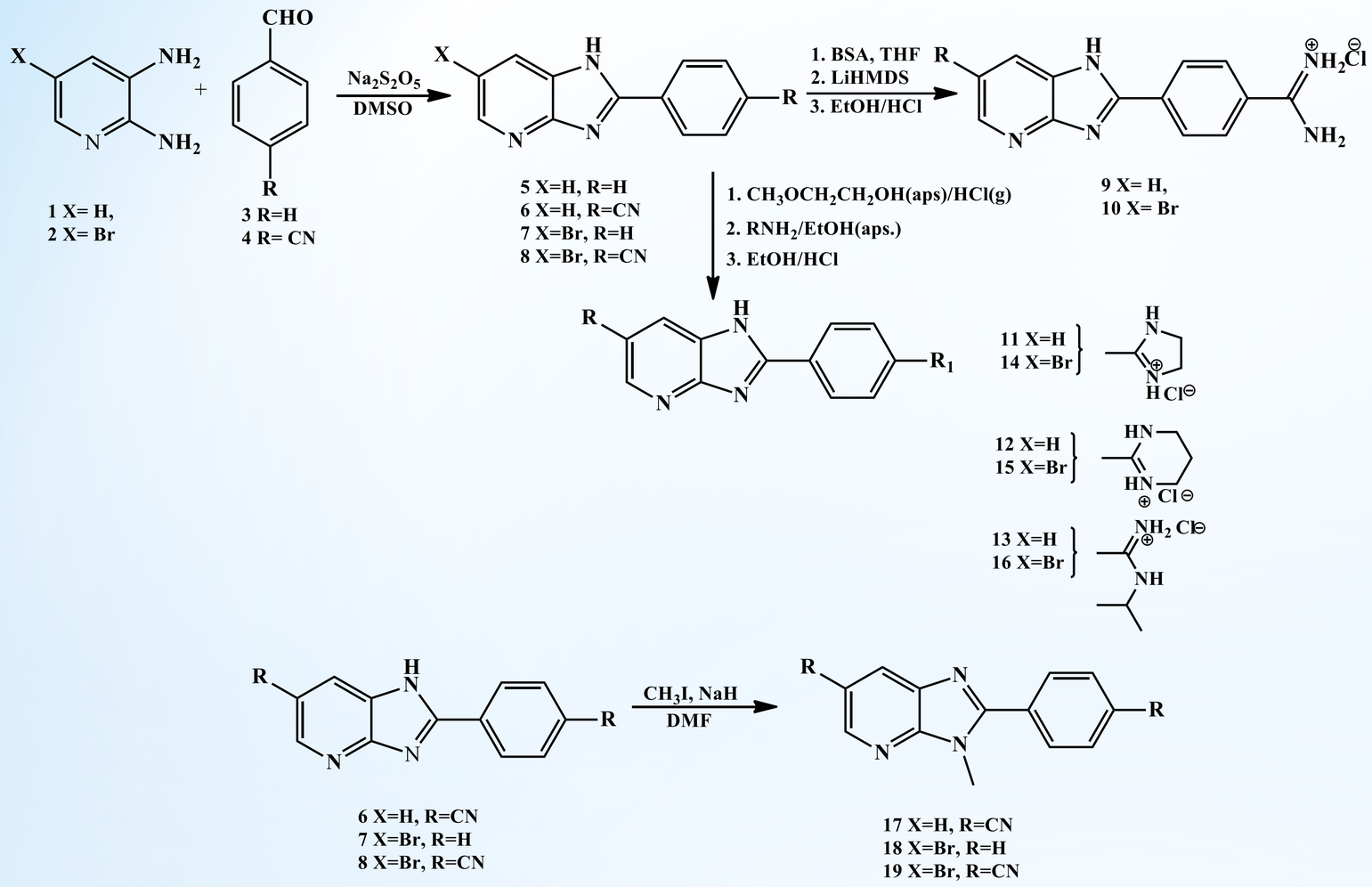
Imidazo-pyridines are important scaffold in organic and medicinal chemistry due to their promising biological activity. [1] Some of the derivatives have already found application in medicine, while many others are currently in clinical testing. [2]



Scheme 1. Synthesis of novel derivatives

Main precursors for the synthesis of targeted derivatives were prepared by DMSO-mediated cyclization.[3] Unsubstituded amidines were prepared using lithium hexamethyldisilazane, while other were obtained by Pinner reaction. The structures of newly prepared compounds were confirmed by means of ^{1}H and ^{13}C NMR spectroscopy as well as MS spectrometry.

Antibacterial activity of the prepared compounds was evaluated against four different bacterial strains. Gram positive bacterial strains included *S. aureus* and *S. pneumoniae*, while the panel of Gram-negative bacteria included two different strains of *E. coli*. Tested compounds showed no antibacterial activity against tested strains.

Table 1. Antibacterial activity *in vitro*

Cpd	*S. aureus* ATCC 29212	*E. coli* ATCC 29213	*E. coli* efflux del	*S. Pneumoniae* ATCC 49619
5	>64	>64	>64	>64
6	>64	>64	>64	>64
7	>64	>64	>64	>64
8	>64	>64	>64	>64
9	>64	>64	>64	>64
10	>64	>64	>64	>64
11	>64	>64	>64	>64
12	>64	>64	>64	>64
13	>64	>64	>64	>64
14	>64	>64	**32**	>64
15	>64	>64	>64	>64
16	>64	>64	>64	>64
17	>64	>64	>64	>64
18	>64	>64	>64	>64
19	>64	>64	>64	>64
Ampicillin	1	1	2	<0.125
Ceftazidime	16	0.25	0.25	0.25
Ciprofloxacin	0.25	<0.125	<0.125	0.25
Meropenem	<0.125	<0.125	<0.125	<0.125

Table 2. Antiproliferative activity *in vitro* IC_{50}

Cpd	Conc. unit	LN-229 glioblastoma	Capan-1 pancreatic adenocarcinoma	HCT-116 colorectal carcinoma	NCI-H460 lung carcinoma	DND-41 acute lymphoblastic leukemia	HL-60 acute myeloid leukemia	K-562 chronic myeloid leukemia	Z-138 non-Hodgkin lymphoma
5	µM	>100	**95.9**	>100	>100	>100	**63.3**	>100	>100
11	µM	**81.7**	**48.9**	>100	**69.8**	**48.8**	>100	>100	**52.8**
14	µM	**8.0**	**9.4**	**13.6**	**13.0**	**10.8**	**9.5**	**49.7**	**8.5**
15	µM	**73.5**	>100	>100	>100	**17.0**	>100	>100	**29.1**
16	µM	**52.4**	**52.7**	**77.2**	>100	**11.9**	**91.4**	**53.6**	**12.1**
Docetaxel	nM	4.1	3.8	2.5	3.4	2.5	2.2	8.5	2.3
Staurosporine	nM	66.8	51.9	70.1	44.8	54.8	58.6	37.4	48.4

All prepared compounds were evaluated for their antiproliferative activity *in vitro* on several human cancer cells. Compounds **8**, **14**, **15**, **16** and **19** showed marked antiproliferative activity towards tested cell lines while compounds **9**, **12**, **13** and **17** had no significant effect on proliferation of the cells on all tested cell lines. No selectivity toward any cell line was observed, except for compounds **5**, **6** and **18**, which were selective towards HeLa cells (GI_{50} between 20 µM and 60 µM). Also, PC3 cells are in general less sensitive towards tested compounds. On the other hand, compound **10** showed significant antiproliferative activity on SW 620 cells (GI_{50} in submicromolar range) while other compounds have GI_{50} values in micromolar range.

Table 3. Antiproliferative activity *in vitro* GI_{50}

Cpd	PC3 colon carcinoma	HeLa prostate carcinoma	SW 620 cervical carcinoma
5	> 100	**13.6±3.4**	> 100
6	> 100	**28.9±7.2**	> 100
8	**3.2±0.7**	**1.8±0.02**	**2.0±0.2**
9	> 100	> 100	> 100
10	≥ 100	**1.3±0.26**	**0.4±0.1**
11	≥ 100	**11.1±3.5**	**12.1±3.7**
12	> 100	> 100	> 100
13	> 100	> 100	> 100
14	**1.5±0.5**	**4.3±2.6**	**0.7±0.3**
15	**8.6±0.7**	**7.3±0.4**	**3.5±0.2**
16	**13.3±2.6**	**13.3±4.3**	**7.4±1.1**
17	> 100	≥ 100	> 100
18	> 100	**48.0±0.2**	> 100
19	**28.8±15.0**	**26.1±0.01**	**63.8±7.4**

Table 4. Cytotoxicity *in vitro* CC_{50}

Cpd	Conc. (µM)	HEL 299	Huh7	MDCK
5	100	45.6	>100	58.4
6	100	>100	>100	>100
7	100	>100	>100	>100
8	100	>100	>100	>100
9	100	>100	>100	>100
10	100	>100	>100	>100
11	100	>100	30.1	>100
12	100	>100	>100	>100
13	100	62.1	>100	>100
14	100	9.3	<0.8	33.9
15	100	>100	50.3	>100
16	100	>100	1.5	42.3
17	100	>100	>100	>100
18	100	>100	>100	>100
19	100	>100	>100	>100
Remdesivir	10	>10	>10	-
Ribavirin	250	>250	8.9	67
Zanamivir	100	-	-	>100
Zanamivir	100	-	-	>100
BVDU	100	-	-	>100

Table 5. Antiviral activity *in vitro* EC_{50}

Cpd	HCoV 229E HEL 299	HCoV OC43 HEL 299	HCoV NL63 Huh7	Influenza H1N1 MDCK	Influenza H3N2 MDCK	Influenza B MDCK	RSV A Long HEL 299	HSV-1 KOS HSV-1	YFV 17D Huh7	Zika Virus mr766 Huh7	Sindbis Huh7
7	>100	>100	>100	>100	>100	>100	**21**	>100	>100	>100	>100
8	>100	>100	>100	**72.35**	**35.85**	**56.7**	>100	>100	>100	>100	>100
10	>100	>100	>100	**71.35**	**79.2**	**93.95**	**73.7**	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100	**58**	>100	>100	>100	>100
19	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Remdesivir	0.06	0.06	0.03	-	-	-	0.03	-	6.2	0.7	>10
Ribavirin	82.6	170.1	>250	10.5	4	2.8	10.8	-	>250	>250	148.1
Zanamivir	-	-	-	0.13	16.8	0.05	-	-	-	-	-
Zanamivir	-	-	-	4.4	0.05	>100	-	-	-	-	-
BVDU	-	-	-	-	-	-	-	0.05	-	-	-

Compounds **8** and **10** showed specific activity against Influenza virus.

Antiviral activity of the prepared compounds was evaluated against eleven different viral strains including two strain of Human coronavirus, three strains of Influenza, Respiratory syncytial virus, Herpes simplex virus, Yellow fever virus, Zika virus and Sindbis virus.

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