SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL OXAZOLO[4,5-*b*]PYRIDINE DERIVATIVES

Jurja Vukovinski[1], Marijana Hranjec[1], Leentje Persoons[2], Dirk Daelemans[2], Maja Karnaš[3], Karolina Vrandečić[3]

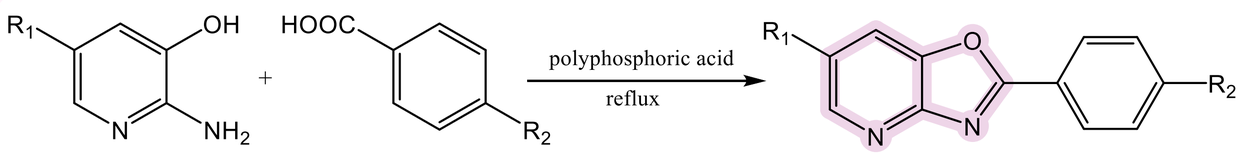
[1] Department of organic Chemistry, University of Zagreb Faculty of Chemical Engineering and Technology, Marulicev trg 19, Zagreb, Croatia, e-mail: jvukovins@fkit.unizg.hr; [2] KU Leuven, Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, Molecular Genetics and Therapeutics in Virology and Oncology Research Group, Leuven, Belgium;
[3] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

INTRODUCTION

Nitrogen-containing heterocycles represent an important class of organic compounds due to their diverse chemical and biological properties. Fused bicyclic heteroaromatic systems have attracted considerable attention as key structural motifs in biologically active molecules and as valuable scaffolds in the rational design of novel drugs.[1] Oxazolo[4,5-*b*]pyridines are fused heteroaromatic systems consisting of an oxazole ring fused to a pyridine ring. Their rigid, planar structure and unique electronic properties contribute to a broad range of versatile biological activities. Furthermore, their isosteric relationship to naturally occurring purine bases can facilitate interactions with important biological targets, including DNA, RNA, and proteins.[2,3]

SYNTHESIS

In this work, we prepared novel oxazolo[4,5-*b*]pyridine derivatives via an optimized cyclocondensation reaction of 2-amino-3-hydroxypyridine derivatives with selected benzoic acids in polyphosphoric acid (PPA). The synthesized compounds were structurally characterized by NMR spectroscopy and mass spectrometry, confirming the formation and structural identity of the target oxazolo[4,5-*b*]pyridine scaffolds.



1 R_1 = H
2 R_1 = Br

3 R_2 = H
4 R_2 = F
5 R_2 = Br
6 R_2 = NH_2
7 R_2 = $NHCH_3$

8 R_1 = H, R_2 = H
9 R_1 = Br, R_2 = H
10 R_1 = H, R_2 = F
11 R_1 = Br, R_2 = F
12 R_1 = H, R_2 = Br
13 R_1 = Br, R_2 = Br
14 R_1 = H, R_2 = NH_2
15 R_1 = Br, R_2 = NH_2
16 R_1 = H, R_2 = $NHCH_3$
17 R_1 = Br, R_2 = $NHCH_3$

Scheme 1. Synthesis of oxazolo[4,5-*b*]pyridine derivatives **8–17**.

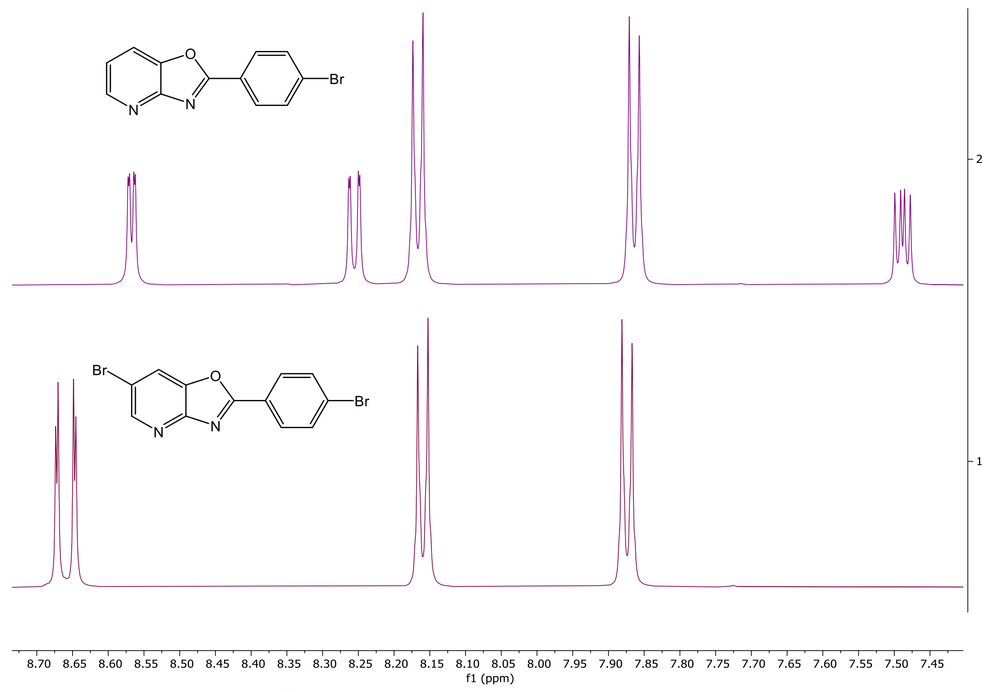


Figure 1. ^{1}H NMR spectra of derivatives **12** and **13**.

ANTIFUNGAL ACTIVITY *IN VITRO*

Antifungal assays was performed against agricultural phytopathogens (Table 1.). Although the majority of the evaluated oxazolo[4,5-*b*]pyridine derivatives exhibited a time-dependent decrease in antifungal efficacy, derivatives **10** and **12** demonstrated potent initial mycelial growth inhibition, whereas **15** remained the least effective overall. Ultimately, **16** emerged as the most promising candidate, maintaining superior and highly stable long-term antifungal activity against both *Botrytis cinerea* (54.18 %) and *Sclerotinia sclerotiorum* (40.18 %) after 72 hours.

Table 1. Antifungal activity *in vitro* of tested compounds.

Cpd	% Inhibition of mycelial growth			
	Sclerotinia sclerotiorum		*Botrytis cinerea*	
	48 h	72 h	48 h	72 h
8	32.79±5.00	25.67± 2.73	54.09±5.00	49.58±1.16
9	38.50±1.82	30.13±2.23	39.06±7.18	24.79±1.42
10	**51.81±4.22**	32.09±2.30	**55.29±2.78**	46.74±1.16
11	28.52±7.11	20.09±4.74	45.07±3.02	43.91±1.16
12	**52.76±2.85**	34.60±1.58	**57.69±1.96**	46.03±0.82
13	34.22±2.69	20.93±2.79	33.65±3.93	29.04±0.82
14	40.40±1.82	25.39±6.78	24.64±2.30	24.79±0.82
15	22.34±2.39	11.44±0.56	46.27±4.11	37.18±6.98
16	**42.30±2.85**	**40.18±0.91**	**58.89±5.00**	**54.18±3.73**
17	46.10±2.39	36.83±4.65	48.08±6.80	41.08±2.83
nc	0.00±00	0.00±00	0.00±00	0.00±00

*(nc = negative control)

ANTIPROLIFERATIVE ACTIVITY *IN VITRO*

All derivatives were tested for their antiproliferative activity *in vitro* on human cancer cells, showing overall weak activity (Table 2.).
Nocodazole and etoposide were used as standard drugs. Most of the tested compounds **8-10** and **12-14** did not exhibit any antiproliferative effect, with IC_{50} > 100 µM across all evaluated cancer cell lines. Among the few derivatives that displayed measurable activity, compound **15** showed moderate potency, against LN229 cell line (IC_{50} = 61.63 µM). Weak antiproliferative effects were also observed for compounds **11** (IC_{50} = 94.27 µM) and **17** (IC_{50} = 90.60 µM) against LN229 cell line, as well as for compound **16** against NCI-H460 (IC_{50} = 78.47 µM) and MOLT-4 (IC_{50} = 96.52 µM) cell lines.

Table 2. Antiproliferative activity *in vitro* of tested compounds.

Cpd	IC_{50} (µM)							
	Capan-1	HCT-116	LN229	NCI-H460	MOLT-4	HL-60	K562	Z138
8	>100	>100	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100	>100	>100
11	>100	>100	94.27±5.73	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100	>100	>100
15	>100	>100	**61.63±0.13**	>100	>100	>100	>100	>100
16	>100	>100	>100	78.47±29.18	96.52± 3.48	>100	>100	>100
17	>100	>100	90.60±2.64	>100	>100	>100	>100	>100
ETO	0.35±0.05	1.30±0.00	5.85±0.45	1.20±0.50	/	0.45±0.05	5.21±0.38	0.57±0.15
NOC	0.25±0.05	0.25±0.05	0.13±0.02	0.12±0.01	/	0.02±0.01	0.04±0.01	0.13±0.02

CONCLUSIONS

1. novel oxazolo[4,5-*b*]pyridine derivatives **8–17** were evaluated for their antifungal and antiproliferative activity *in vitro*
2. compound **16** exhibited the most pronounced and sustained antifungal activity
3. all derivatives displayed weak antiproliferative activity
4. compound **15** demonstrated weak but selective antiproliferative activity against LN229 cell line (IC_{50} = 61.63 µM).

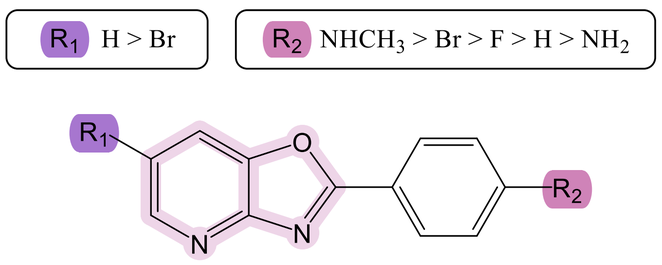


Figure 2. Structure-antiproliferative activity relationship of compounds **8**-**17**.

REFERENCES
1.I. Celik, M. Erol, G. Kuyucuklu, *New J. Chem.* 45 (2021) 11108–11118.
2.M. Monier, D. Abdel-Latif, A. El-Mekabaty, K. M. Elattar, *Synth. Comm.* 50 (2019) 1-32.
3.R. Sireesha, M. B. Tej, N. Poojith, R. Sreenivasulu, M. Musuluri, M. Subbarao, *Polycyclic Aromatic Compounds, 43* (2021) 915 - 932.

The Croatian Science Foundation funded this work (project **BenzHetPot IP-2024-05-7208**).