SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW BENZOXAZOLES AS pH SENSORS

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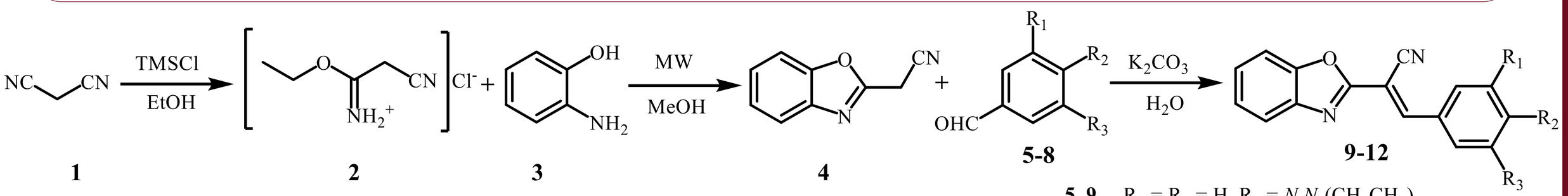
Benzoxazole has been incorporated as an essential pharmacophore and substructure in the structure of various medicinally important compounds, offering a range of biological and pharmacological activities such as, anticancer, antiviral, antibacterial, antimicrobial and others. [1,2] Herein we present the synthesis, biological evaluation and spectroscopic characterization of acrylonitrile derived benzoxazoles prepared by aldol condensation from benzaldehyde and 2-cyanomethylbenzoxazole in water. [3] The structures of newly prepared compounds were confirmed by means of ^{1}H and ^{13}C NMR spectroscopy.



Scheme 1. Synthesis of novel acrylonitrile derivatives

Antiproliferative activity *in vitro* was evaluated on several human cancer cells (Table 1). All compounds showed strong to moderate activity. Compounds **9** and **11** showed strong and selective activity towards **CAPAN-1** cell (IC_{50} 1.1. and 0.7 μM). Obtained results revealed that a larger number of OH groups improves the activity. The antibacterial activity *in vitro* was tested on a Gram-positive and Gram-negative bacterial strains but the compounds did not show significant activity (Table 2).

Additionally, UV/Vis spectroscopic characterization in several organic solvents with different polarity and pH spectroscopic titrations were performed to determine possible application of chosen compounds as pH sensors in solutions followed by determination of p*K*a values experimentally as well as computationally. [4] Studies compounds have proven to have pH sensing spectroscopic properties which would allowed them the potential use as sensitive and selective optical sensors.

Table 1. Antiproliferative activity *in vitro* of acrylonitrile derivatives

cpd	Cytotoxicity Adherent Cells (IC_{50})				Cytotoxicity Suspension Cells (IC_{50})				Cytotoxicity Normal Cells (IC_{50})
	Capan-1	HCT-116	LN229	NCI-H460	DND-41	HL-60	K562	Z138	PBMC
9	**1.1**	5.3	4.1	4.0	**1.6**	**1.8**	**1.1**	2.6	>50
10	24.7	>50	>50	25.9	39.2	34.6	>50	35.0	>50
11	**0.7**	4.7	20.1	18.5	4.8	4.6	31.5	22.0	>50
12	**1.2**	5.2	9.2	5.0	3.9	4.6	14.8	5.4	>50

Table 2. Antibacterial activity *in vitro* of acrylonitrile derivatives

cpd	*S.aureus* ATCC 29213	*E.faecalis* ATCC 29212	*E.coli* ATCC 25922	*E.coli* efflux del.	*P.aeruginosa* ATCC 27853	*A.baumannii* ATCC 17978
9	>64	>64	>64	>64	>64	>64
10	>64	>64	>64	>64	>64	>64
11	32	>64	>64	>64	>64	>64
12	>64	>64	>64	>64	>64	>64

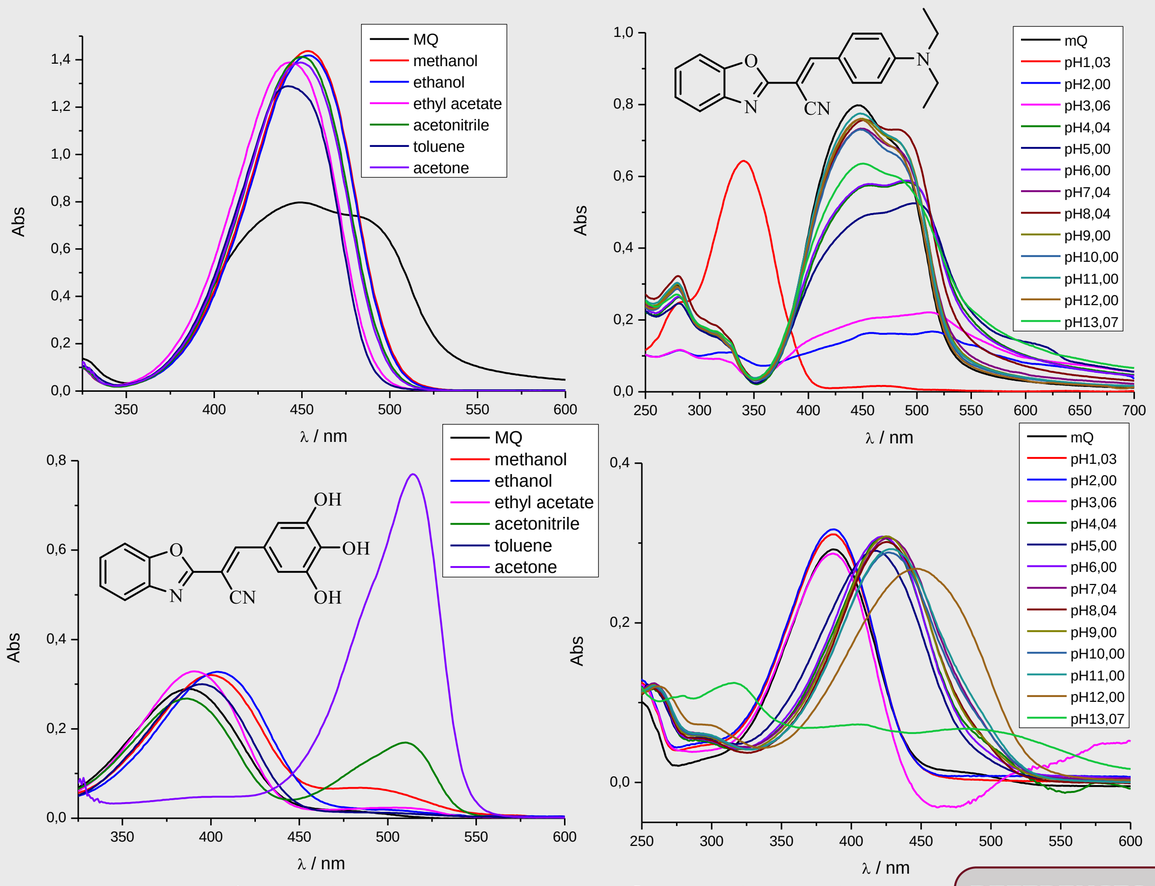


Figure 1. UV/Vis spectra and UV/Vis pH titrations of compounds **9** and **12**

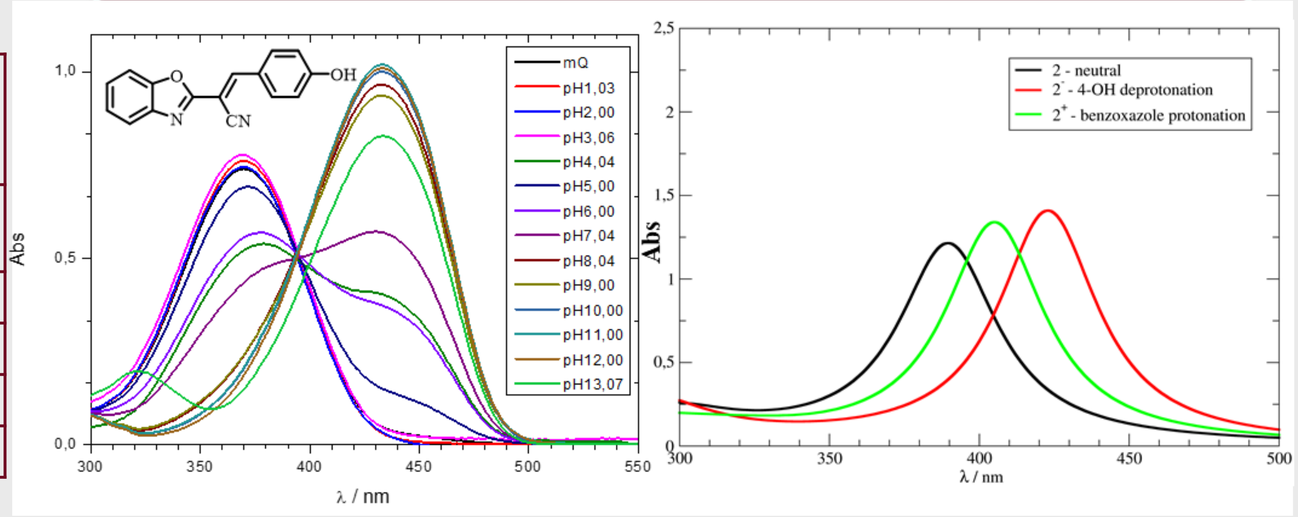


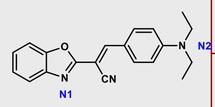
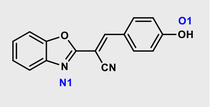
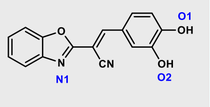
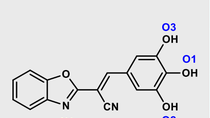
Figure 2. a) UV/Vis spectra of **10** ($2x10^{-5}$ $moldm^{-3}$) at different pH values
b) Calculated absorbtion spectra of neutral and protonated **10**

As shown in Fig.2, the predicted band at 389 nm confirms the dominance of the neutral form 2 in the range up to pH = 7.02 and corresponds to the measured absorption maximum at 368 nm, while the calculated band at 423 nm corresponds to the measured absorption maximum at 443 nm and confirms the deprotonated form 2^{-}. On the other hand, the band that would correspond to the species protonated on benzoxazole 2+ does not exist in the experimental spectrum, which confirms the very low basicity of that fragment.of p*K*a = –0.5, making this process invisible under the experimental conditions.

CONCLUSION:

- **The agreement between the calculated and experimental data is in principle good and acceptable, although in some cases there is a noticeable deviation in the p*K*a values.**
- **The tested systems represent good optical sensors for changing pH conditions in an aqueous solution, especially for low (pH ≈ 2–4) and high pH conditions (pH ≈ 12–13).**

Table 3. Calculated aqueous solution p*K*a values

System	Protonation Reaction	pK_a(calc)	pK_a(exp)
	**N2 → N2⁺**	1.4	2.98
	N1 → N1⁺	–2.4	–
	**O1⁻ → O1**	8.3	7.02
	N1 → N1⁺	–0.5	–
	**O2⁻ → O2**	12.9	12.36
	O1⁻ → O1	3.6	–
	N1 → N1⁺	–0.6	–
	**O3⁻ → O3**	13.1	–
	O2⁻ → O2	12.5	12.31
	O1⁻ → O1	2.8	–
	N1 → N1⁺	–0.4	–

REFERENCES:
[1] X. K. Wong, K. Y. Yeong, *ChemMedChem* 16 (2021) 3237 - 3262; [2] A. Abdullahi, K. Y. Yeong, *Med. Chem. Res.* 33 (2024) 406 - 438; [3] C. Youssef, et all. *J. Heterocycl. Chem.* 48 (2011) 1126-1131; [4] A. Beč, R. Vianello, M. Hranjec, *J. Mol. Liq.* 386 (2023) 122493.

This work was funded by the Croatian Science Foundation (IP-2020-02-8090).