Novel iminocoumarin derived imidazo[4,5-*b*]pyridines as potential pH sensors

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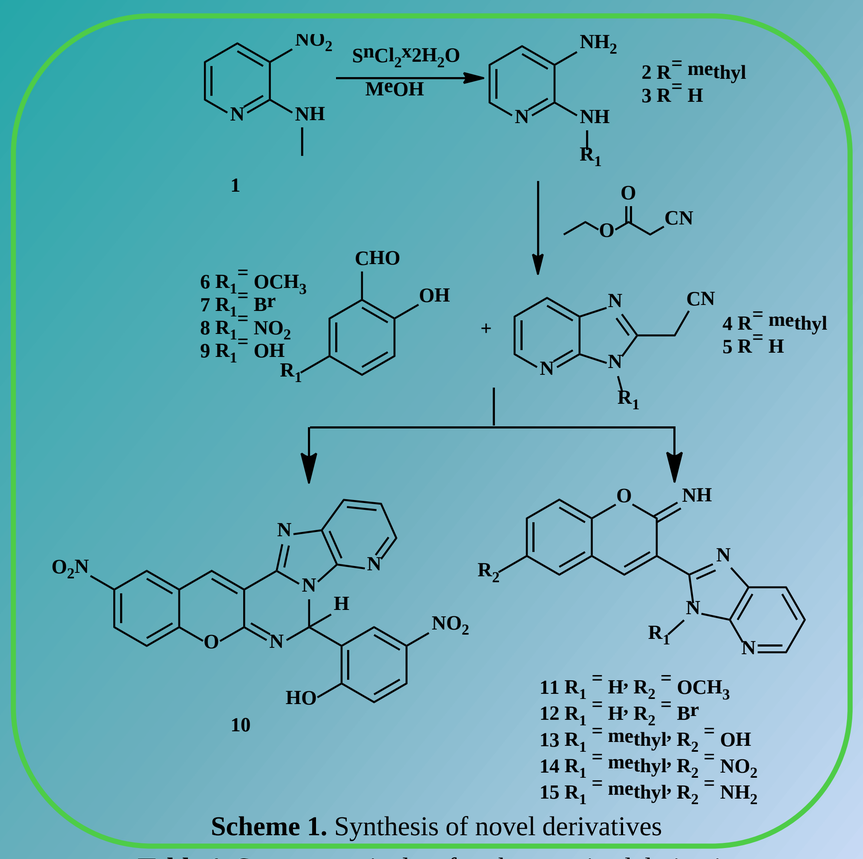
Iminocoumarin derivatives are known for their interesting spectroscopic properties as well as their application as fluorescent sensors. Monitoring pH range is crucial for studying many biological processes in live cell organelles. Conjugates of benzazoles and iminocoumarins are already being explored for pH sensing applications due to excellent spectroscopic response in correlation with pH value.[1,2]



Scheme 1. Synthesis of novel derivatives

Table 1. Spectroscopic data for characterized derivatives

	Toluene	$(C_2H_5)_2O$	Dioxane	EtAc	CH_2Cl_2	ACN	EtOH	MeOH	Water
$E_T(30)$	33.9	34.5	36.0	38.1	40.7	45.6	51.9	55.4	63.1
Compound 10		401			403	398	401	397	400
λ_{max}/nm	405 382 364	378 359 279	402 379 360	401 377 359	381 362 281	378 359 277	378 358 279	377 360 279	377 361 280
$\varepsilon\times 10^3$	7.6 12.5 11.6	8.1 13.3 12.3 10.3	7.3 28.0 12.1	7.1 12.6 11.8	7.7 12.8 12.1 10.6	10.5 13.6 12.5 9.1	7.2 12.3 12.0 10.2	8.7 13.2 12.4 10.1	8.1 13.2 13.1 9.9
λ_{emiss}/nm	453	464	459	462	467	469	448	439	448
Rel. Fluo. Int.	253	118	204	140	101	22	164	69	25
Compound 11		410	410	408	412	408			
λ_{max}/nm	415 392 359 340	389 372 355 288	387 353 337 279	388 371 354 338	390 374 357 341	385 352 335 278	388 353 377 280	385 352 336 280	384 350 335 280
$\varepsilon\times 10^3$	17.0 27.4 24.3 20.5	16.1 27.5 25.7 24.2 20.5	14.7 25.2 22.3 19.1 8.7	19.0 30.8 28.6 27.5 23.4	19.0 29.5 27.0 26.9 23.1	15.1 26.0 24.5 21.3 9.7	24.9 24.5 22.2 9.5	22.9 23.2 21.7 9.0	23.5 24.8 23.6 10.1
λ_{emiss}/nm	454	448 425	453 433	452 427	452 428	454	456	459	470
Rel. Fluo. Int.	225	431 307	340 243	356 249	302 204	302	186	152	143
Compound 12		398	397		402	376	378	374	
λ_{max}/nm	401 381 368 347	364 342 280	376 262 341 278	378 363 341	380 366 347 276	361 341 322 275	362 344 327 277	360 342 323 278	371 283
$\varepsilon\times 10^3$	13.7 24.5 23.9 17.7	14.0 26.1 20.6 8.6	13.6 25.2 24.8 17.5 7.6	25.2 25.4 17.7	14.8 26.2 25.4 19.3 8.9	26.6 26.7 19.8 12.3 9.4	24.8 25.2 19.6 13.5 8.2	25.8 26.0 20.4 14.1 8.8	16.7 7.7
λ_{emiss}/nm	455	450	456	456	455	456	455	456	461
Rel. Fluo. Int.	432	463	672	573	505	556	454	319	192
Compound 13	380	373	367	370	377	365	374	371	364
λ_{max}/nm	319	316	316	313	316	308	310	308	308
$\varepsilon\times 10^3$	7.8 13.6	9.0 14.2	9.4 13.7	9.5 15.4	8.3 14.3	8.7 13.8	7.2 15.2	7.1 15.1	7.9 15.0
λ_{emiss} /nm	470	457	460	463	467	463	478	480	494
Rel. Fluo. Int.	568	704	550	567	405	683	417	380	7.5
Compound 14	342	386	338	335	341	332	334	333	334
λ_{max}/nm		266	268		268	267	268	266	270
$\varepsilon\times 10^3$	13.9	14.4 19.4	15.2 18.4	15.3	16.4 22.8	13.9 19.3	14.7 19.6	17.3 23.8	14.3 19.9
λ_{emiss}/nm	463	459	467	467	467	480	474	478	0
Rel. Fluo. Int.	190	280	109	140	37	2	20	8	0
Compound 15	400	405	395	400	392	393	407	399	382
λ_{max}/nm	322	320 246	317	317	319 245	313 245	314 247	312 245	311 239
$\varepsilon\times 10^3$	5.0 15.6	4.8 17.2 21.1	4.9 16.3	5.3 18.8	5.8 16.6 20.3	4.3 16.0 20.9	3.9 18.1 19.2	3.9 1.8 19.0	5.2 17.3 19.0
λ_{emiss}/nm	558	540	547	557	547	567	599	592	0
Rel. Fluo. Int.	248	662	193	307	324	260	45	29	0

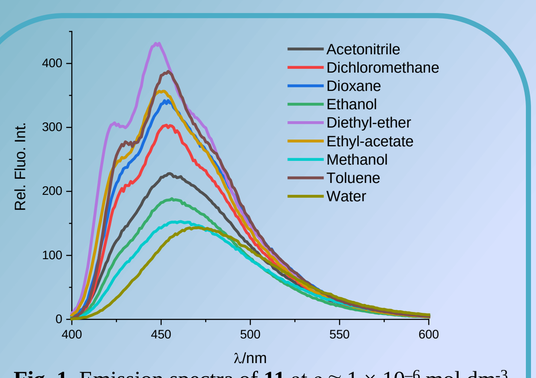


Fig. 1. Emission spectra of **11** at c ≈ 1 × 10^{-6} mol dm^{-3}

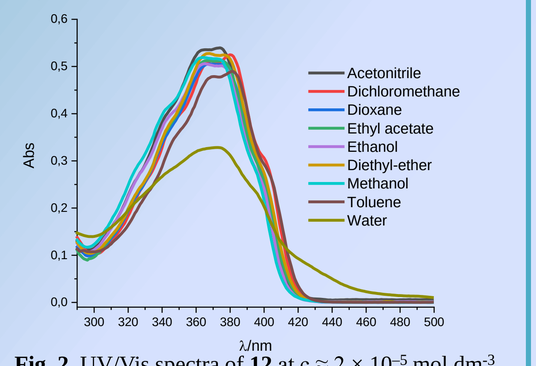


Fig. 2. UV/Vis spectra of **12** at c ≈ 2 × 10^{-5} mol dm^{-3}

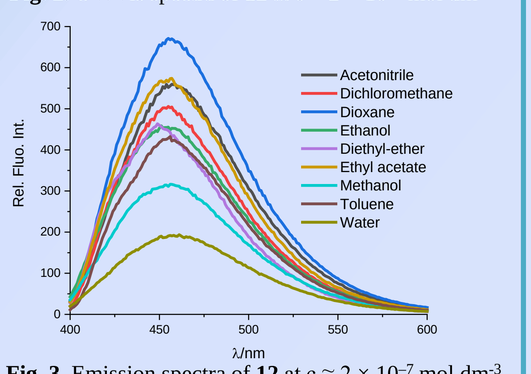


Fig. 3. Emission spectra of **12** at c ≈ 2 × 10^{-7} mol dm^{-3}

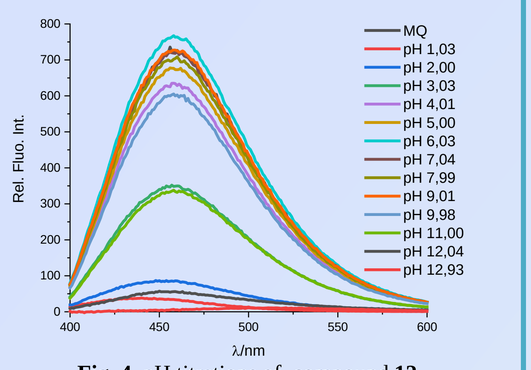


Fig. 4. pH titrations of compound **12**

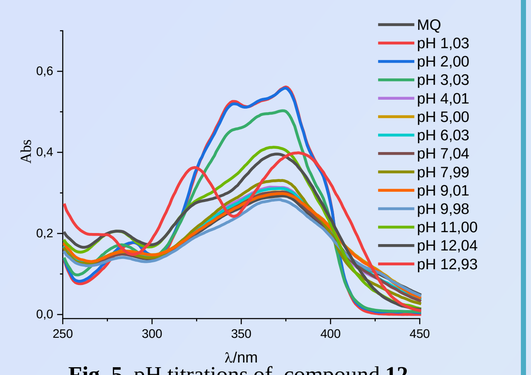


Fig. 5. pH titrations of compound **12**

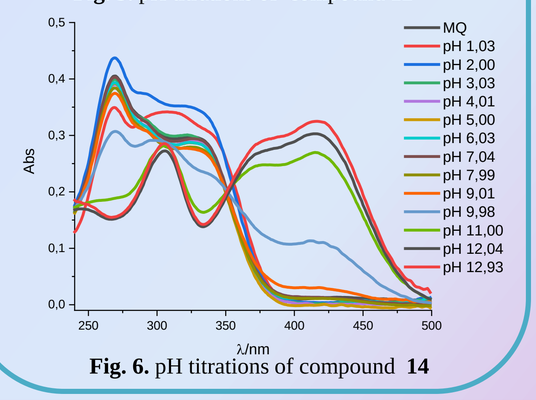
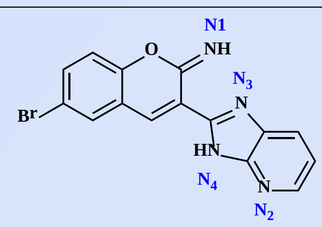
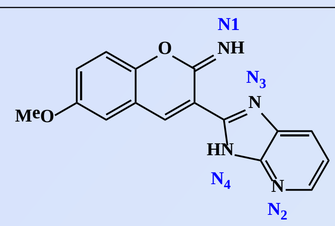
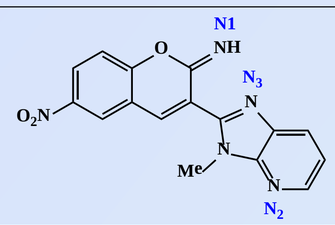
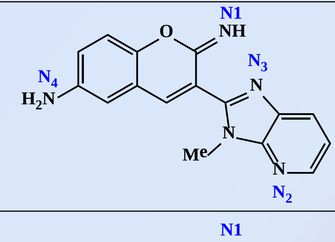
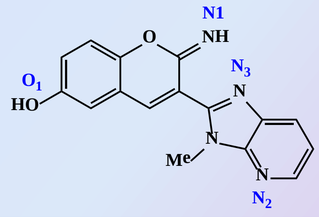
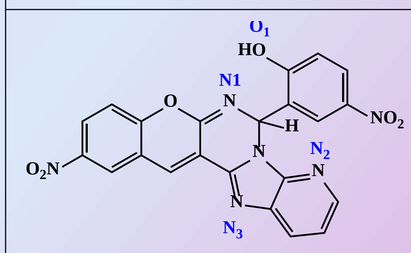


Fig. 6. pH titrations of compound **14**

The spectroscopic properties of chosen compounds were studied in several polar and non-polar solvents. Obtained absorption spectra revealed that we can observe the strong impact of the substituent placed at the iminocoumarin skeleton as well as the polarity of solvents and the pH value on the spectroscopic characteristics. Suchlike systems have proven to have excellent spectroscopic properties that allowed them the potential use as sensitive and selective optical sensors in a wide range of biological, environmental, and chemical processes.[3]

Table 2. Calculated aqueous solution pK_a values for each protonation state of investigated systems

System	Protonation Reaction	pK_a(calc)	pK_a(exp)
	**N1⁻ → N1**	21.3	
	N4⁻ → N4	19.7	
	N1 → N1⁺	10.9	
	N2 → N2⁺	3.8	3.33
	N3 → N3⁺	–7.4	
	**N1⁻ → N1**	24.8	
	N4⁻ → N4	20.1	
	N1 → N1⁺	12.2	12.39
	N2 → N2⁺	4.1	3.30
	N3 → N3⁺	–11.1	
	**N1⁻ → N1**	14.1	
	N1 → N1⁺	10.4	10.28
	N2 → N2⁺	3.4	
	N3 → N3⁺	–4.6	
	**N1⁻ → N1**	23.2	
	N1 → N1⁺	13.7	
	N2 → N2⁺	4.4	3.08
	N4 → N4⁺	–0.8	
	**N1⁻ → N1**	26.9	
	O1⁻ → O1	19.3	
	N1 → N1⁺	12.7	
	N2 → N2⁺	4.0	2.40
	N3 → N3⁺	–3.9	
	**O1⁻ → O1**	9.7	
	N3 → N3⁺	5.9	5.97
	N1 → N1⁺	1.2	
	N2 → N2⁺	–4.5	
	O1 → O1⁺	–26.9	

To determine the precise protonation state of studied systems under different pH conditions, we calculated the corresponding pKa values in water using the B3LYP/6–31+G(d) model and the implicit SMD solvation. The obtained results are found in very good agreement with experiments, and reveal that, under neutral conditions (pH = 7), most of the examined systems are present as cations monoprotonated at the *egzo*-imine, while, under considered pH range (pH ≈ 1–13), they exchange between neutral and diprotonated forms, which is responsible for alterations in UV/Vis responses following changes in pH conditions.

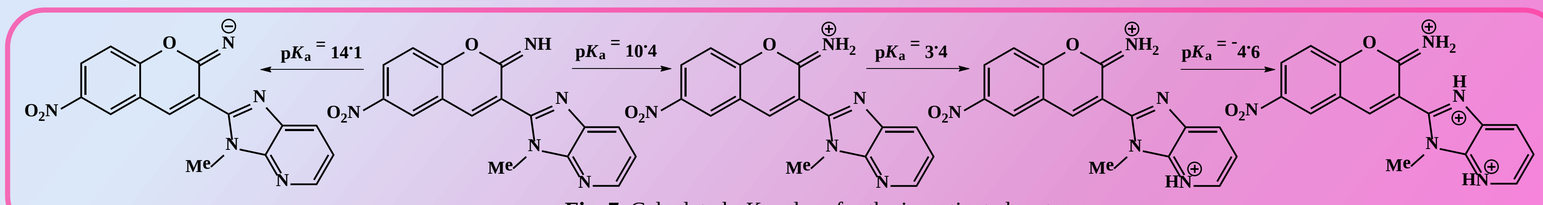


Fig. 7. Calculated pK_a values for the investigated systems

At pK_a values 3.4 and 10.4 molecule transits from monoprotonated to diprotonated and from monoprotonated to neutral form which is in excellent agreement with experimental pK_a values.

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[1] N. Perin, M. Cindrić, P. Vervaeke, S. Liekens, T. Mašek, K. Starčević and M. Hranjec, *Medicinal Chemistry* **2021**, *17*, 13-20.
[2] A. S. Vasylevska, A. A. Karasyov, S. M. Borisov, C. Krause, *Anal Bioanal Chem* **2007**, *387*, 2131–2141.
[3] I. Boček, K. Starčević, I. Novak Jovanović, R. Vianello and M. Hranjec, *J. Mol. Liq.* **2021**, *342*, 117527.