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Determination of Masked Basicity in Imidazo[1,5-*a*]pyridine Carboxylic Acids Using Esters

Abstract

Imidazo[1,5-*a*]pyridine carboxylic acids are useful building blocks for medical chemistry, but their synthesis scale-up and isolation depend strongly on pH. For zwitterion-prone representatives, a direct titration of acids does not always reveal the basicity of the heteroaromatic center, which is critical for their further use. Therefore, a series of acids, their corresponding esters, and hydrochlorides were analyzed by the potentiometric titration together with $^{1}H/^{13}C$ NMR, HPLC, LCMS, and HRMS. The acids showed apparent pK_a values of 5.13–6.11, while the esters exposed the basic-center pK_a values in the range of 2.75–4.64. NMR data indicate close electronic similarity within the acid/ester pairs, supporting the use of esters as models for acids with masked basicity.

Keywords: imidazo[1,5-*a*]pyridine; carboxylic acids; esters; potentiometric titration; pK_a; NMR spectroscopy; medicinal chemistry

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Визначення маскованої основності в імідазо[1,5-*a*]піридинкарбонових кислотах з використанням естерів

Анотація

Імідазо[1,5-*a*]піридинкарбонові кислоти є корисними будівельними блоками для медичної хімії, однак масштабування їх синтезу та виділення суттєво залежать від pH. Для сполук, схильних до утворення цвітер-йонних форм, пряме титрування кислот не завжди дозволяє визначити основність гетероароматичного центру, що є критично важливим для їх подальшого застосування. Тому серію кислот, відповідних естерів та їх гідрохлоридів було досліджено методом потенціометричного титрування в поєднанні з $^{1}H/^{13}C$ ЯМР-спектроскопією, HPLC, LCMS та HRMS. Для кислот було визначено значення pK_a в межах 5,13–6,11, тоді як естери демонстрували значення pK_a основного центру в діапазоні 2,75–4,64. Дані ЯМР свідчать про близьку електронну подібність у парах кислота/естер, що обґрунтовує використання естерів як моделей для кислот із замаскованою основністю.

Ключові слова: імідазо[1,5-*a*]піридин; карбонові кислоти; естери; потенціометричне титрування; pK_a; ЯМР-спектроскопія; медична хімія

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Supporting information: Potentiometric titration protocol and datasets, all ^{1}H and ^{13}C NMR spectra, HPLC, LCMS, and HRMS data for all samples analyzed.
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Introduction

Imidazo[1,5-*a*]pyridine is a versatile fused *aza*-heterocyclic scaffold that continues to attract attention because of its broad functional relevance in both materials-oriented and medicinal chemistry research [1]. Representative biologically active series based on this core include cannabinoid CB2 agonists [2], spleen tyrosine kinase inhibitors [3], mitogen-activated protein kinase (MEK) inhibitors [4], 5-HT4 receptor partial agonists [5], and the GCN2 activator HC-7366, which has entered clinical investigation [6] (**Figure 1**). At the same time, synthetic methods for the preparation and functionalization of imidazo[1,5-*a*]pyridines continue to expand [7, 8]. Regioisomeric carboxylic acids of this scaffold are especially attractive as building blocks for further derivatization, including amide-coupling-based medicinal chemistry programs.

However, synthetic accessibility alone is not sufficient for practical scale-up. In our case, the isolation and purification of imidazo[1,5-*a*]pyridine carboxylic acids proved strongly dependent on the products' ionization state. Therefore, determining the pH window for the pH-controlled precipitation is essential for the reproducible multigram-scale isolation. For such zwitterion-prone heteroaromatic acids, the protonation of the ring nitrogen and deprotonation of the carboxyl group may overlap under aqueous conditions, so their direct titration does not always reveal the basic center pK_a needed for the rational precipitation control. To address this problem, we analyzed the corresponding esters as model compounds. Since the ester analogs retain the same heteroaromatic framework while masking the exchangeable carboxyl proton, they can serve as practical probes for the basicity of the imidazo[1,5-*a*]pyridine core.

In this short communication, we report the potentiometric characterization of a set of parent regioisomeric imidazo[1,5-*a*]pyridine carboxylic acids (**Figure 2**) and the corresponding esters, supported by $^{1}H/^{13}C$ NMR, HPLC, LCMS, and HRMS. The main goal of the work was not synthetic novelty, but rather the determination of acid-base parameters relevant to the multigram-scale isolation and purification. It can provide broad perspectives on such chemotypes for use as building blocks in MedChem programs.

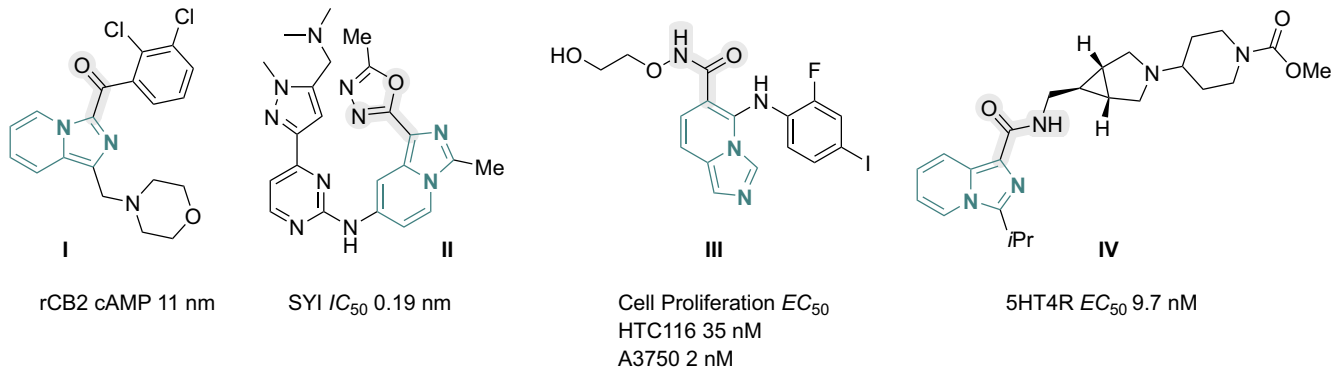


Figure 1. Imidazo[1,5-*a*]pyridines derivatives in medicinal chemistry

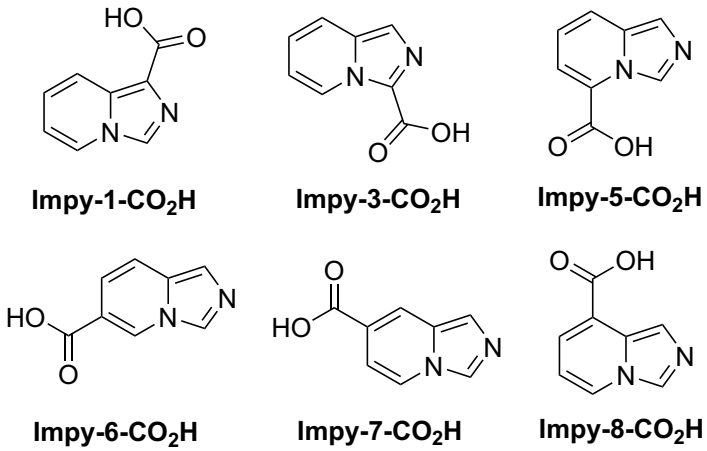


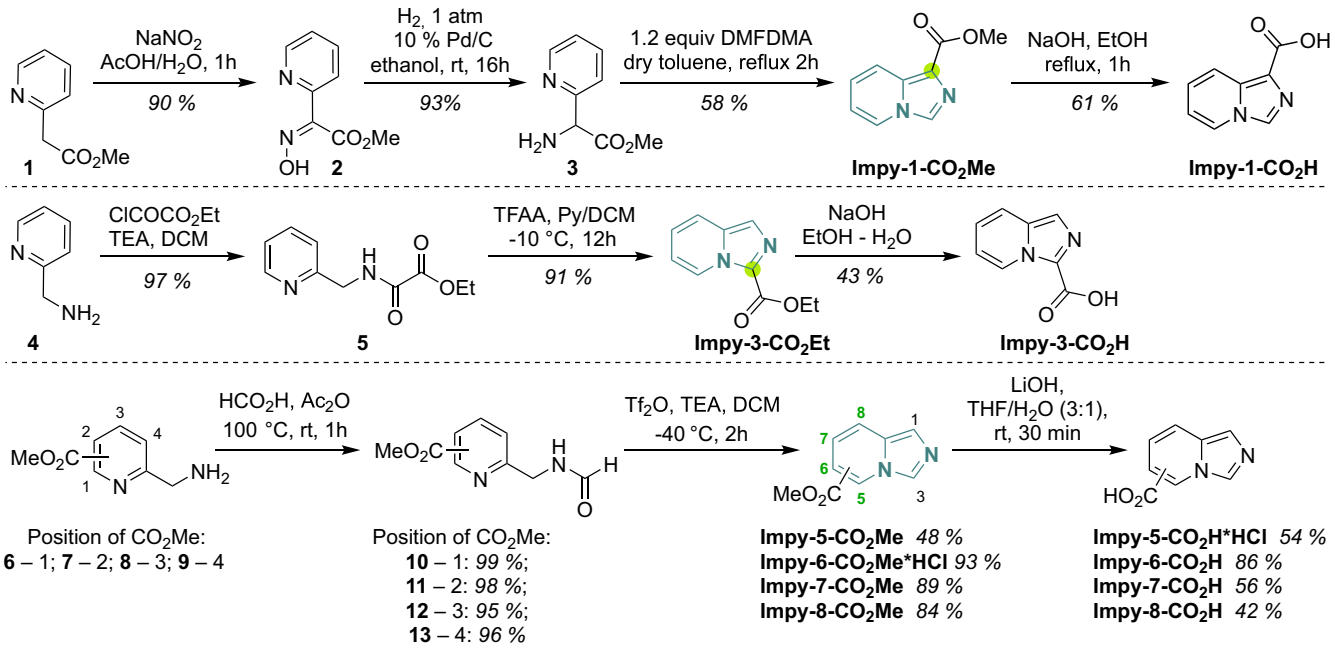
Figure 2. Structures of the imidazo[1,5-*a*]pyridine carboxylic acids studied

■ Materials and methods

The imidazo[1,5-*a*]pyridine carboxylic acids and esters studied were prepared by analogy to reported procedures [9, 10]. The structures of the compounds are shown in **Figure 2**. The acid **Impy-1-CO_2H** was prepared from the bulk methyl 2-(pyridin-2-yl)acetate (**1**) in 4 steps, following literature protocols [11]. Compound **1** was subjected to nitrosation with $NaNO_2$ in AcOH giving oxime **2**, which was hydrogenated at 1 atm over 10% Pd/C in methanol to afford **3**. The freshly prepared **3** was used in [5+1] heterocyclization with DMFDMA in toluene giving **Impy-1-CO_2Me**, which was subsequently hydrolyzed by NaOH in EtOH to the final **Impy-1-CO_2H.** The synthesis of **Impy-3-CO_2H** was initiated from the bulk 2-aminomethylpyridine (**4**) acylated with ethyl 2-chloro-2-oxoacetate to afford **5**, and cyclized in a TFAA-DCM-Py system giving **Impy-3-CO_2Et** in accordance with the published protocol [12]. The alkaline hydrolysis to the final **Impy-3-CO_2H** was performed in accordance with the *Gao and Wang* procedure [10]. The acids **Impy-5-CO_2H** to **Impy-8-CO_2H** were obtained by one general approach, starting from commercially available methyl picolinate **6**, nicotinates **7** and **9**, and isonicotinate **8**. All these amines were formylated by a mixed formic-acetic anhydride *in situ* generated from Ac_2O in HCO_2H in accordance with the patent procedure [13]. The following heterocyclization to methyl imidazo[1,5-*a*]pyridine carboxylates, **Impy-5-CO_2Me** to **Impy-8-CO_2Me**, was performed using the protocol recently developed with the Tf_2O-TEA-DCM system at -10 °C, giving the desired compounds in high preparative yields [14]. Finally, the alkaline hydrolysis by LiOH in THF at room temperature by analogy with [15] gave target acids **Impy-5-CO_2H** to **Impy-8-CO_2H** in good preparative yields. The reaction sequences and yields are shown in **Scheme 1**.

In the course of this work, it was found that the above-mentioned literature protocols yielded the compounds **Impy-6-CO_2Me** and **Impy-6-CO_2H** with rather high impurity levels, up to 10–15%. Therefore, these compounds were purified by treating their ethanol solutions with a saturated solution of dry HCl in dioxane [16], giving the corresponding analytically pure salts, **Impy-6-CO_2Me**·HCl and **Impy-6-CO_2H**·HCl, which were isolated and included in the analytical series. This observation provided an additional practical example of the importance of the pH/protonation-state control during the isolation.

The extent of literature characterization for these compounds is uneven. Surprisingly, among all acids, **Impy-1-CO_2H** to **Impy-8-CO_2H**, only **Impy-3-CO_2Et** [9] and **Impy-3-CO_2H** [10] were fully characterized. For the compound **Impy-5-CO_2H**, only ^{1}H NMR spectra in DMSO-d_6 were published [17]. The compounds **Impy-6-CO_2H**, **Impy-6-CO_2Me** [15], **Impy-7-CO_2Me** [13], **Impy-7-CO_2H** [18] were only mentioned in literature without characterization, and compounds **Impy-5-CO_2Me**, **Impy-8-CO_2Me** appeared to be new ones. Therefore, before proceeding with the study of their properties, we made their full characterization (see *SI file*).



Scheme 1. The synthesis of the imidazo[1,5-*a*]pyridine carboxylic acids and corresponding esters studied

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Potentiometric titrations were carried out in the aqueous medium using a TitroLine® 7000 automatic titrator (SI Analytics/Xylem). Carboxylic acids and hydrochlorides were titrated with a standardized aqueous NaOH, whereas neutral ester derivatives with pK_a values below 7 were titrated with a standardized aqueous HCl. The exact titration protocol, titrant concentrations, sample masses, pH values, and pointwise pK_a calculations are provided in the *SI file*. The pK_a values discussed below were estimated from the Henderson–Hasselbalch relationship using the buffer regions of the respective titration curves.

Potentiometric titrations were carried out in the aqueous medium using standardized 0.09910 N NaOH and 0.10967 N HCl; the exact titration protocol was provided in the *SI file*. The pK_a values discussed below were estimated from the Henderson–Hasselbalch relationship using the buffer regions of the respective titration curves. Since the aim of the present communication is practical pK_a determination rather than the exhaustive equilibrium modeling, only the constants directly relevant to the preparative problem are discussed in the main text.

All compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy, HPLC, LCMS, and HRMS. These methods were used to confirm the sample identity and purity before acid–base measurements. The complete analytical data, including full NMR spectra and results of chromatographic and high-resolution mass spectra for all samples, are provided in the *SI file*.

■ Results and discussion

A direct potentiometric titration of the carboxylic acids afforded apparent pK_a values in the range of 5.13–5.91 for the neutral acid series (**Table 1**). Thus, compounds **Impy-1-CO$_2$H**, **Impy-3-CO$_2$H**, **Impy-7-CO$_2$H**, and **Impy-8-CO$_2$H** showed pK_a values of 5.91, 5.64, 5.66, and 5.18, respectively, while **Impy-6-CO$_2$H·HCl** isolated as a hydrochloride showed an apparent pK_a of 5.13. Compound **Impy-5-CO$_2$H·HCl**, also available as the hydrochloride, displayed two transitions at pK_a 3.02 and 6.11, illustrating the presence of overlapping acid-base equilibria in this family. In preparative terms, these data are sufficient to describe the deprotonation behavior of the carboxyl-containing compounds, but they do not by themselves provide an unambiguous estimate of the heteroaromatic basicity.

The corresponding ester analogs solved this problem. Since they preserve the same imidazo[1,5-*a*]pyridine framework while removing the carboxylic acid proton, their potentiometric behavior is more informative with respect to the protonation of the ring nitrogen. The relevant basic-center pK_a values are listed in **Table 1**. Note that although **Impy-3-CO$_2$Et** is an ethyl rather than methyl ester, it was retained as the available ester analog of the 3-substituted acid; for the present determination, the essential structural requirement is masking of the carboxylic acid proton while preserving the imidazo[1,5-*a*]pyridine core. The isolated hydrochloride **Impy-6-CO$_2$Me·HCl** gave a closely related value of 4.47. Taken together, these results show that the basicity of the imidazo[1,5-*a*]pyridine nucleus depends markedly on the substitution pattern and, for several regioisomers, lies close enough to the carboxyl-related transition to complicate a direct interpretation of the titration curves of the acids. Representative overlayed titration curves are shown in **Figure 3**.

Table 1. Relevant acid-base constants of the imidazo[1,5-*a*]pyridine derivatives studied

Compound	Form	Relevant pK_a value(s)	Comment
Impy-1-CO$_2$H	acid	5.91	apparent pK_a of acid
Impy-3-CO$_2$H	acid	5.64	apparent pK_a of acid
Impy-5-CO$_2$H·HCl	acid hydrochloride	3.02; 6.11	two-step ionization
Impy-6-CO$_2$H·HCl	acid hydrochloride	5.13	apparent pK_a of acid-containing species
Impy-7-CO$_2$H	acid	5.66	apparent pK_a of acid
Impy-8-CO$_2$H	acid	5.18	apparent pK_a of acid
Impy-1-CO$_2$Me	methyl ester	2.83	basic-center pK_a model
Impy-3-CO$_2$Et	ethyl ester	2.95	basic-center pK_a model
Impy-5-CO$_2$Me	methyl ester	4.67	basic-center pK_a model
Impy-6-CO$_2$Me·HCl	methyl ester hydrochloride	4.47	protonated ester reference
Impy-7-CO$_2$Me	methyl ester	4.42	basic-center pK_a model
Impy-8-CO$_2$Me	methyl ester	4.71	basic-center pK_a model

Note: Values are rounded to two decimal places. Full titration worksheets, complete curves, and additional transitions observed outside the main discussion are given in the *SI file*

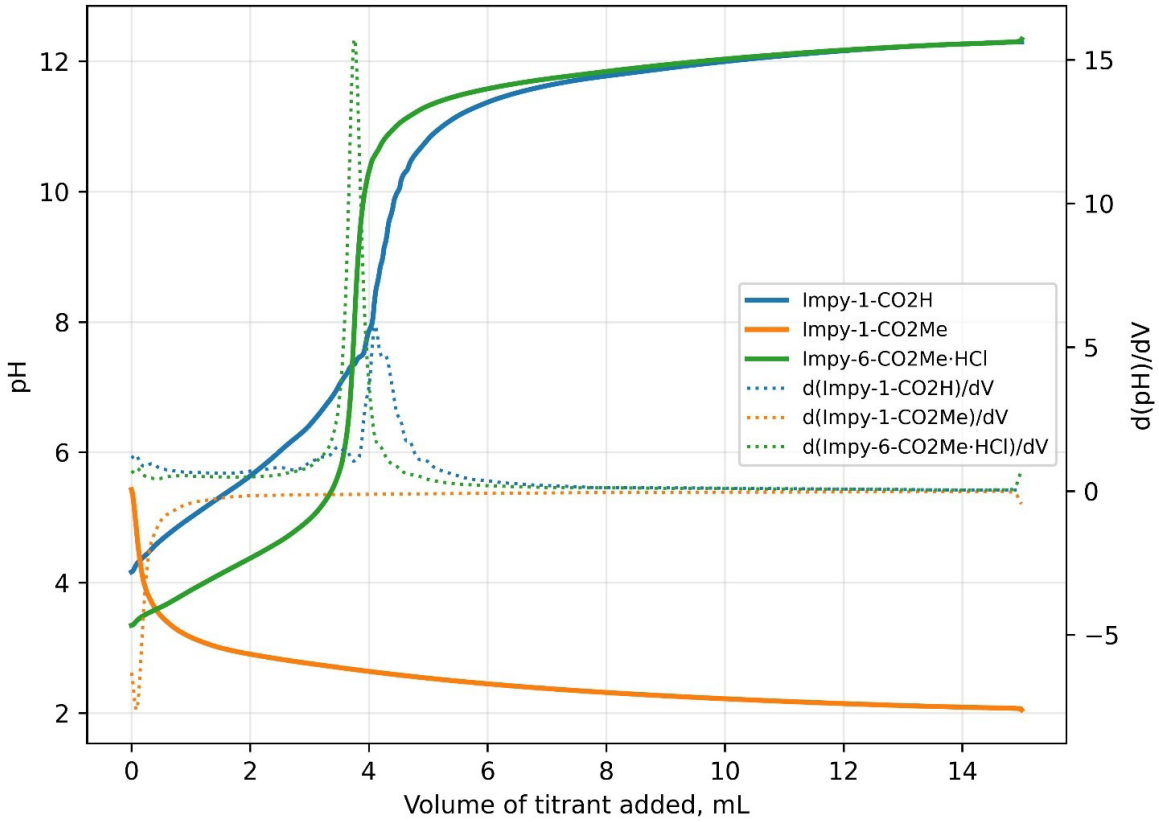

Figure 3. Overlayed potentiometric titration curves of **Impy-1-CO_2H**, **Impy-1-CO_2Me**, and **Impy-6-CO_2Me·HCl**; dotted lines show the corresponding differential curves

This interpretation is supported by the NMR data. For the matched acid/ester pairs, the aromatic regions of the ^{1}H NMR spectra are very similar. For example, the pair **Impy-1-CO_2H/Impy-1-CO_2Me** shows closely corresponding aromatic signals, and the same tendency is observed for the pairs **Impy-7-CO_2H/Impy-7-CO_2Me** and **Impy-8-CO_2H/Impy-8-CO_2Me**. The ^{13}C NMR data also show only modest changes in the heteroaromatic carbon resonances upon the conversion of CO_2H into CO_2R. Therefore, the esterification does not significantly alter the electronic structure of the imidazo[1,5-*a*]pyridine core, and the esters can be regarded as chemically reasonable models of the basicity of the corresponding acids.

An additional qualitative observation comes from hydrochlorides. In these samples, the aromatic signals are shifted downfield relative to the corresponding neutral compounds, which is consistent with the protonation in the heteroaromatic system. At the same time, the CO_2H signal is absent in the hydrochloride spectra, whereas it is observed for several neutral acids in DMSO-d_6. Thus, the spectroscopic data are complementary to the titration data: NMR does not replace the aqueous p*K*a determination, but it supports the protonation-state determinations and validates the use of esters as model compounds.

From a practical point of view, the most important conclusion is that the regioisomeric imidazo[1,5-*a*]pyridine carboxylic acids cannot be treated as a single analytical class. The 1- and 3-substituted systems have distinctly lower basic-center pK_a values in the corresponding ester series, whereas the 5- to 8-substituted series cluster around pK_a 4.3–4.6. As these values approach the apparent pK_a values of the acids themselves, the direct pH adjustment during work-up can easily lead to the partial co-precipitation, incomplete precipitation, or irreproducible isolation unless the underlying paired equilibria are taken into account. In this respect, the paired analysis of acids and esters provides not only a structural interpretation, but also a practical guide for scale-up.

■ Conclusions

A practical acid-base determination for a series of regioisomeric imidazo[1,5-*a*]pyridine carboxylic acids and corresponding esters has been obtained. A direct potentiometric titration of acids provided the apparent pK_a values relevant to

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the carboxyl-containing series, whereas the ester analogs revealed the protonation constants of the heteroaromatic basic center. The close similarity of the ^{1}H and ^{13}C NMR patterns within the acid/ester pairs shows that the esters are appropriate electronic models for this purpose. The combined titration and spectroscopic data explain why the direct pK_a determination in acids may be ambiguous and provide a practical basis for selecting pH windows for the precipitation and purification of these zwitterion-prone heteroaromatic building blocks.

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