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Solubility of Mono-Aminoacridines

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Solubilities of mono-aminoacridines (AAs) in a variety of solvents were studied computationally using COSMO-RS method and selectively confirmed by experiment (liquid chromatography and gravimetry). The results confirm the reliability of COSMO-RS for ranking solvents on the basis of dissolving a specific AA, but not ranking the solubilities of different AAs in the same solvent. Pyrrolidine and some pyrrolidine-based mixtures were predicted to deliver outstandingly high solubilities of AAs (10–30% by mass, depending on the compound). Hydrogen bonding was found to have a considerable role in the solubility of AAs, which can act as hydrogen bond donors and acceptors. The obtained data and conclusions can facilitate solvent selection for purification of AAs, sample preparation for MALDI-MS if an AA is used as a matrix, and can be helpful for solubility-related applications involving other compounds.

Introduction

Solubility of compounds in different media plays a crucial role in the fields of chemistry and medicine, as well as chemical, pharmaceutical, and food industries. For example, the selection of a proper solvent is important for acquiring synthesis products with high purity. The habitual solubility measurement techniques are high performance liquid chromatography (HPLC)[1–4] and gravimetry.[1,5,6] For solubility calculations, the conductor-like screening model for real solvents (COSMO-RS)[7–13] and the non-random two liquids approach (NRTL)[14,15] are the current leaders. Reliable computational screening of solvents is often preferable to extensive experimental investigations as it reduces the efforts and cost along with saving resources and the environment.

The dissolution of a crystalline solid can be viewed as a two-step process: first, fusion of the crystal, and second, mixing the obtained liquid with the solvent. Mixing of two liquids, despite being a complex process, can be modeled acceptably well by modern computational methods. The bottleneck of solubility predictions in the case of solids is estimating the Gibbs free energy of fusion of the solid. It is much harder to predict, and therefore some methods (including COSMO-RS) use the experimental fusion Gibbs free energy values whenever available.[16] This parameter can be estimated from an experimental solubility value and experimental or computed Gibbs free energy of mixing in the same solvent. Therefore, having experimental solubility values in just a few solvents can advance solubility predictions for a diverse set of media.

In this work, we explore solubility of five possible mono-aminoacridine (AA) isomers (Figure 1). This study complements our previous works[17,18] on the use of AA isomers as matrix compounds in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Solubility is important for MALDI-MS process, where obtaining a homogenous solution of sample and matrix compound is needed for producing high-quality mass spectra.[19–21] The most used of the isomers, 9-AA, also finds applications as an intracellular pH indicator,[22] topical antiseptic,[23] fluorescent dye,[24] and drug precursor.[25–27] The other AAs have been used less widely,[17,18] but the amino-acridine moiety itself is part of several dyes, such as acridine yellow and acridine orange. To the best of our knowledge, there is no work dedicated to the solubility of the AA isomers or closely related compounds.

In the present work, solubilities of AAs in a wide range of solvents were predicted by the COSMO-RS approach and solubilities in a few representative solvents were measured by gravimetry and HPLC techniques. The solid-liquid equilibrium method (SLE)[9] with different reference solvents was used for most calculations. The results obtained in this work can help in the selection of suitable solvent/solvent mixtures for purification processes, synthesis of novel compounds, and/or MALDI-MS measurements.

Results and discussion

Experimental results and evaluation of the computational approach

The solvents used in the experiments – methanol (MeOH)/isopropanol (*i*PrOH), acetonitrile (MeCN), and dichloromethane (DCM)/chloroform ($CHCl_3$) – were selected as model solvents for protic polar, aprotic polar and non-polar solvents, respectively. In addition, one solubility value in pyrrolidine was obtained for an additional validation of computational predictions. Table 1

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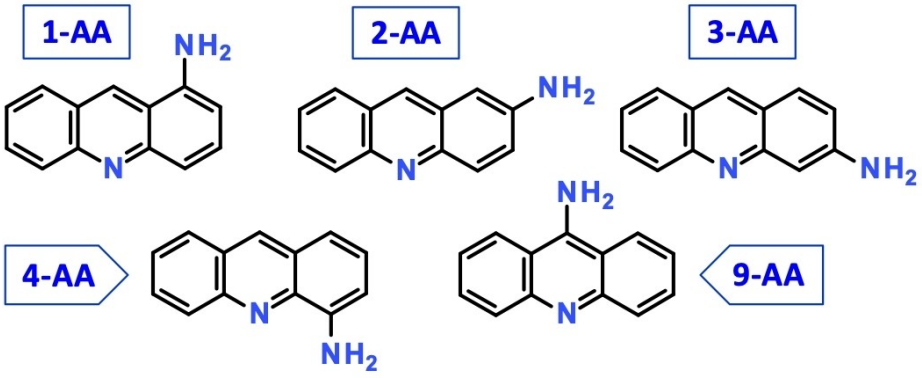


Figure 1. Mono-aminoacridines studied in this work.

Table 1. Experimental and predicted solubilities (in mass fractions) of mono-aminoacridines in different solvents. Experimental averages are given with standard uncertainties (see Experimental Section).

Solute	Solvent	Experimental solubility			Calculated solubility			
		HPLC	Gravimetry	Average	Ref: H_2O	Ref: MeOH/*i*PrOH[b]	Ref: MeCN	Ref: DCM/$CHCl_3$[c]
1-AA	H_2O			$1.17 \cdot 10^{-5}$ [a]		$5.0 \cdot 10^{-5}$ [b]	$9.9 \cdot 10^{-5}$	$2.6 \cdot 10^{-5}$
	*i*PrOH	0.033	0.033	0.033 ± 0.008	0.0080		0.064	0.017
	MeCN	0.028	0.027	0.027 ± 0.007	0.0030	0.013[b]		0.0065
	DCM	0.0087	0.0056	0.0072 ± 0.0024	0.0032	0.014[b]	0.032	
2-AA	H_2O			$9.74 \cdot 10^{-6}$ [a]		$1.4 \cdot 10^{-5}$	$2.1 \cdot 10^{-5}$	$1.8 \cdot 10^{-5}$
	MeOH	0.029	0.029	0.029 ± 0.007	0.021		0.043	0.038
	MeCN	0.0069	0.0077	0.0073 ± 0.0025	0.0035	0.0049		0.0064
	DCM	0.0073	0.0076	0.0074 ± 0.0025	0.0040	0.0056	0.0085	
3-AA	H_2O			$2.92 \cdot 10^{-5}$ [a]		$4.4 \cdot 10^{-5}$	$5.3 \cdot 10^{-5}$	$5.8 \cdot 10^{-5}$
	MeOH	0.091	0.092	0.091 ± 0.022	0.064		0.11	0.12
	MeCN	0.013	0.014	0.013 ± 0.003	0.0069	0.010		0.014
	DCM	0.014	0.016	0.015 ± 0.004	0.0072	0.011	0.014	
4-AA	H_2O			$1.36 \cdot 10^{-5}$ [a]		$1.6 \cdot 10^{-5}$	$2.5 \cdot 10^{-5}$	$3.6 \cdot 10^{-5}$ [c]
	MeOH	0.062	0.058	0.059 ± 0.014	0.054		0.099	0.14[c]
	MeCN	0.12	0.13	0.12 ± 0.03	0.063	0.067		0.18[c]
	$CHCl_3$	0.21	0.22	0.21 ± 0.05	0.094	0.10	0.16	
9-AA	H_2O			$1.17 \cdot 10^{-5}$ [a]		$4.3 \cdot 10^{-5}$	$3.3 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
	MeOH	0.092	0.085	0.089 ± 0.021	0.028		0.070	0.049
	MeCN	0.0039	0.0048	0.0043 ± 0.0015	0.0016	0.0058		0.0030
	DCM	0.0010	0.0010	0.0010 ± 0.0003	0.00053	0.0020	0.0014	
	Pyrrolidine	0.21	0.21	0.21 ± 0.05	0.31	0.43	0.41	0.37

[a] Taken from ref. [28], at 24 °C. Water density at 1 atm and 24 °C is 997.30 g $L^{[29]}$. [b] *i*PrOH was used as reference for 1-AA [c] $CHCl_3$ was used as reference for 4-AA

and Figure 2 show the experimental solubilities of AAs measured by gravimetry and HPLC techniques, average experimental values, and values predicted by COSMO-RS method using different reference solvents. The computations indicate that low contents of water in the solvents have a minimal effect on the predicted solubilities (see Table S6 in the SI), therefore, calculated solubilities in pure solvents are reported here.

The trends observed for computationally predicted solubilities are, in most cases, qualitatively consistent with the experimental counterparts for each AA. The exceptions are predictions of 1AA with protic solvents (*i*PrOH or water) as reference: the predicted solubilities in DCM are marginally higher than in MeCN, while in reality, the solubility in MeCN is about three times higher than in DCM. Overall, COSMO-RS is a reliable method for qualitative solubility evaluation when the solubility order of the same solute in a series of solvents is needed. However, the accuracy of the predicted absolute solubility values depends strongly on the reference solvent used for approximation of Gibbs free energy of fusion. For all solubility values except one (9-AA in pyrrolidine), using water as the reference solvent led to negative bias ranging from −9 to −89% and averaging −51%. These observations could mean that either (1) the used literature values in water are consistently underestimated, (2) most values determined in this work are overestimated or (3) there is computational bias in COSMO-RS if water is used as reference solvent. The exception - solubility of 9-AA in pyrrolidine with water as reference – is overestimated by about 50%. The reason is not certain, but potential causes for such difference are possible experimental error and issues with computing van der Waals and/or hydro-

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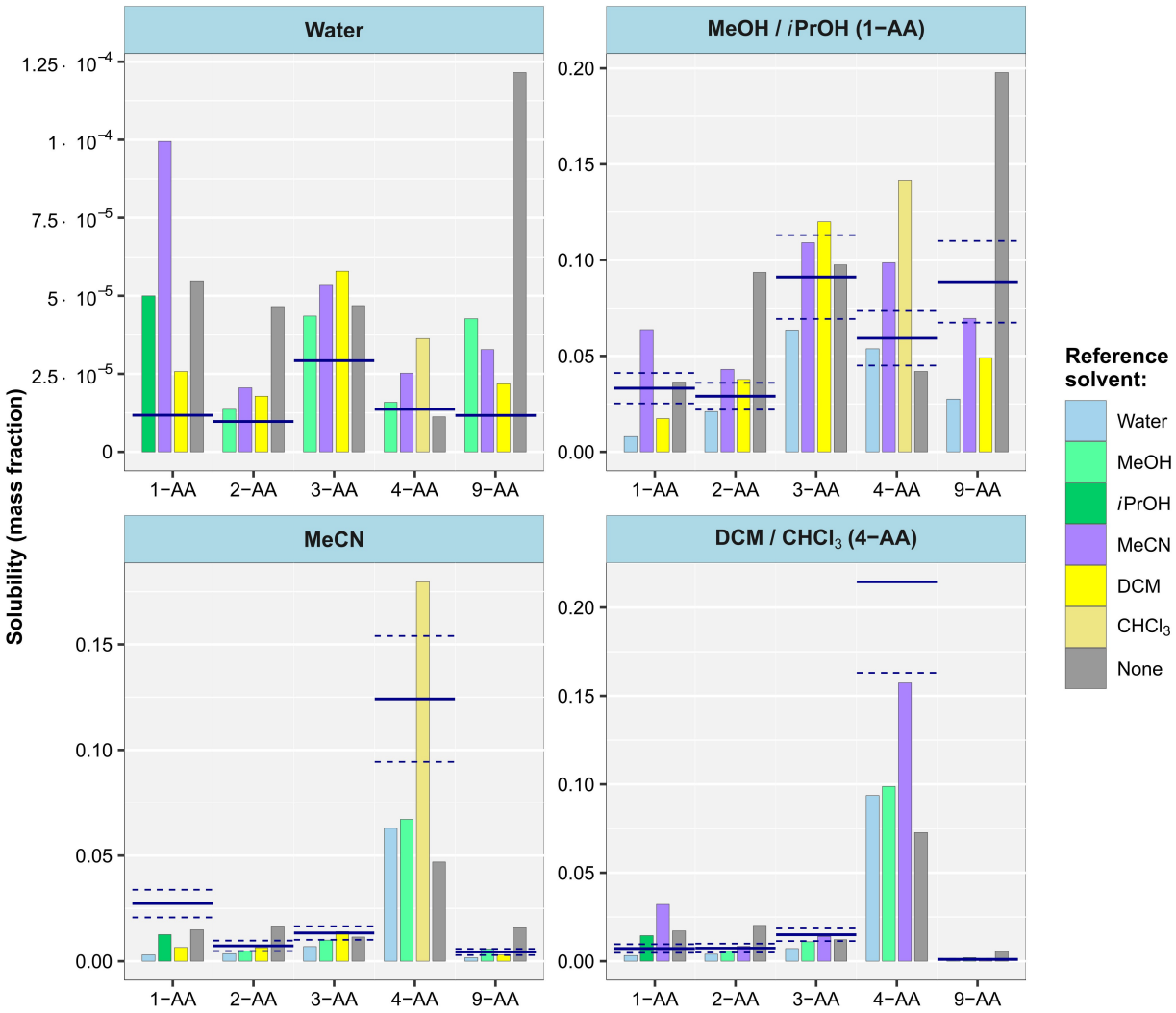


Figure 2. Experimental and the corresponding predicted solubilities of AAs. The facets correspond to the target solvents. Solid lines = experimental solubilities, dashed lines = standard uncertainty limits, bars = predicted solubilities (SLE approach with different reference solvents). A different layout of this figure (grouped by AAs rather than solvents) is available in the SI (Figure S2).

gen bond interaction energies of pyrrolidine with 9-AA. Secondary and tertiary amines were known to be problematic compounds for COSMO-RS, although advances have been made to mitigate the issue.[8,30]

Using MeOH or *i*PrOH as reference tends to cause moderate negative bias for predictions in MeCN but seemingly random (in direction and magnitude) deviations for predictions in DCM. Use of MeCN and DCM/$CHCl_3$ as references yields similarly inconsistent discrepancies. Overall, water as a reference solvent yielded the best results, with the deviations from experimental values being the lowest and most consistent in character.

For 1-AA, 3-AA and 4-AA, using no reference solvents (in which case the free energy of fusion will be estimated by COSMOtherm software using QSPR model) produces results of similar quality to the ones obtained with reference solubility data. However, in case of 9-AA and to lesser extent 2AA, using no reference solvent leads to significant overestimation of solubility. It is possible that the structure of these two compounds leads to effective specific interactions (e.g hydrogen bonding) in the crystals, which the software understandably fails to predict from single-molecule data.

On the other hand, the orders of solubilities of different AAs in one specific solvent were not satisfactorily reproduced by calculations: only a couple of target solvent/reference solvent combinations yielded the correct solute ranking. Therefore, COSMO-RS solubility predictions are reliable for qualitatively screening solvents for a specific solute, but the lack of an accurate Gibbs free energy of fusion leads to inaccurate results or incorrect ranking when different solutes are considered.

Solubility prediction in other pure solvents and solvent mixtures

Solubilities of AAs in a range of pure solvents and solvent mixtures were predicted using COSMO-RS method to find media providing the highest solubilities for each AA. A diverse set of solvents was included: polar protic (water, methanol,

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ethanol, *n*-propanol, isopropanol, *n*-butanol), polar aprotic (dimethyl sulfoxide, diethyl sulfoxide, tetramethylene sulfoxide, dimethyl formamide, methyl acetate, acetone, acetonitrile, and nitromethane), medium polarity (ethyl acetate, *n*-propyl acetate, isopropyl acetate, *n*-butyl acetate, pyridine, diethylamine, pyrrolidine, dioxane, aniline, tetrahydrofuran, and diethyl ether), and low-polarity solvents (toluene, tetrachloromethane, chloroform, dichloromethane, hexane, and cyclohexane). This solvent set contains HB donor solvents, HB acceptor solvents, and solvents without HB ability. The predicted solubilities of AAs in these solvents using water as a reference solvent are shown in Figure 3. Water was selected as the reference as it yielded the most consistent biases across different solvents (see above). The predicted data for all target and reference solvents in the tabulated form are presented in Table S5 in the SI. The predicted solubilities show that pyrrolidine is an outstandingly effective solvent. In addition, sulfoxide solvents are among the best, while non-polar solvents and water are the least effective ones.

The solubility of 9-AA in pyrrolidine was determined experimentally to validate the prediction claiming this solvent as exceptionally effective. Although the obtained mass fraction of 0.21 is about 50% lower than the corresponding prediction, the computations correctly identified pyrrolidine as considerably more effective than the other studied solvents for 9-AA.

Considering the C1–C4 alcohols, increasing the number of carbon atoms (and consecutively decreasing polarity) tends to lower the solubility of AAs. The same was observed in acetates – growth of the carbon chain led to decreasing solubilities of AAs. Among the solvents examined experimentally (Table 1), methanol is the most effective solvent for all but 4-AA, which dissolves best in chloroform. Using computational results we aimed to identify the solvents promising higher solubility than methanol (or chloroform in the case of 4-AA). Figure 3 shows these solvents to be pyrrolidine, sulfoxides, and dimethylformamide. For 9-AA, pyridine is also noteworthy. For 4-AA, there are many solvents potentially better than chloroform, mainly medium to high polarity ones including diethylamine, acetone, and tetrahydrofuran.

Another important factor in solvent selection is safety. Figure S3 in the SI relates the predicted solubilities of each AA to solvent safety ranking[32] (recommended, problematic, hazardous, and highly hazardous). Considering different factors important in solvent selection (such as solubility, volatility, availability, and safety) it appears that ethanol for all (except for 4-AA) and acetone for 4-AA are the best solvents. The solvents providing higher solubilities than these are mostly hazardous or problematic in some other way.

In addition to pure solvents, some pyrrolidine-based mixtures were computationally screened to further increase solubility. Tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), and tetramethylene sulfoxide (TMSO) were selected as co-solvents. Results presented in Figure 4 suggest that for all aminoacridines except 9-AA the solubilities can be slightly improved by adding DMSO or TMSO. The addition of THF did

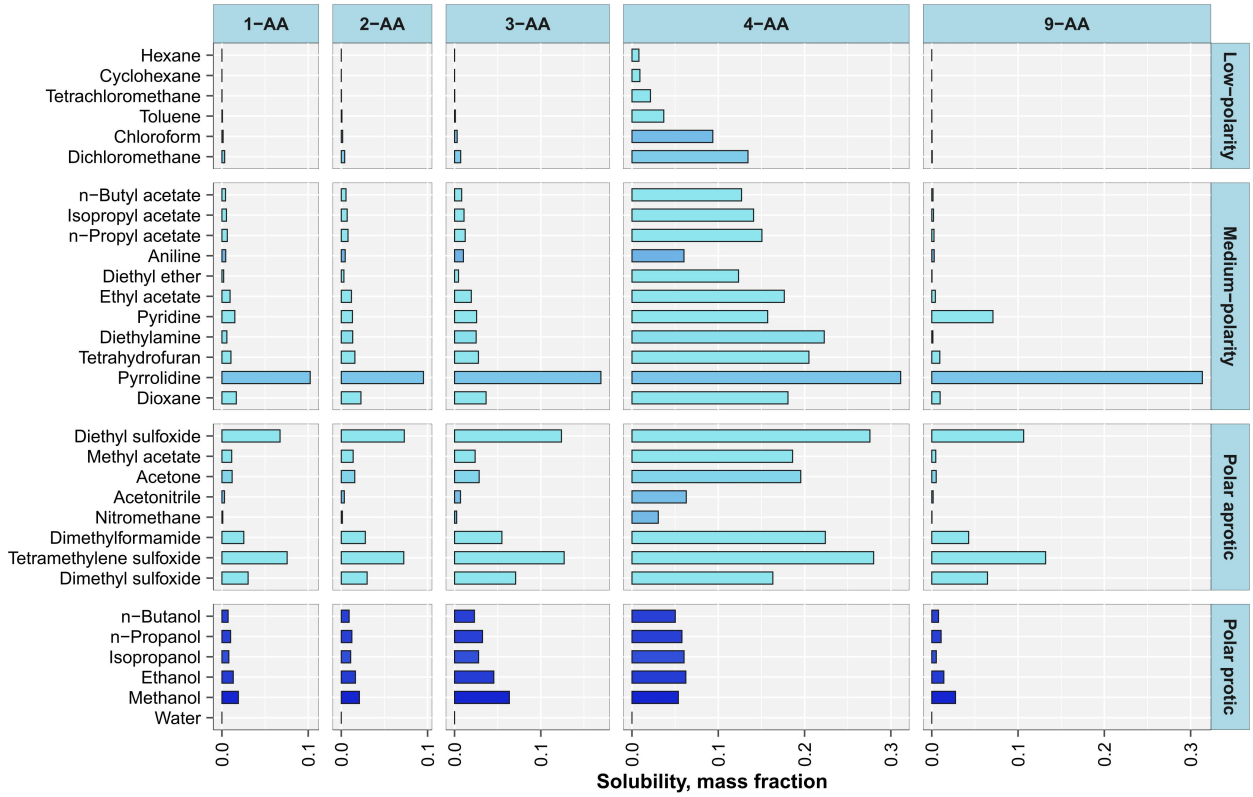


Figure 3. Solubilities of AAs predicted by COSMO-RS method with water as reference solvent. The solvents within groups are ordered by lipophilicity. Colour of the bar corresponds to Kamlet-Taft α parameter of the solvent (darker tone = better HB donating ability; data from ref. [31]).

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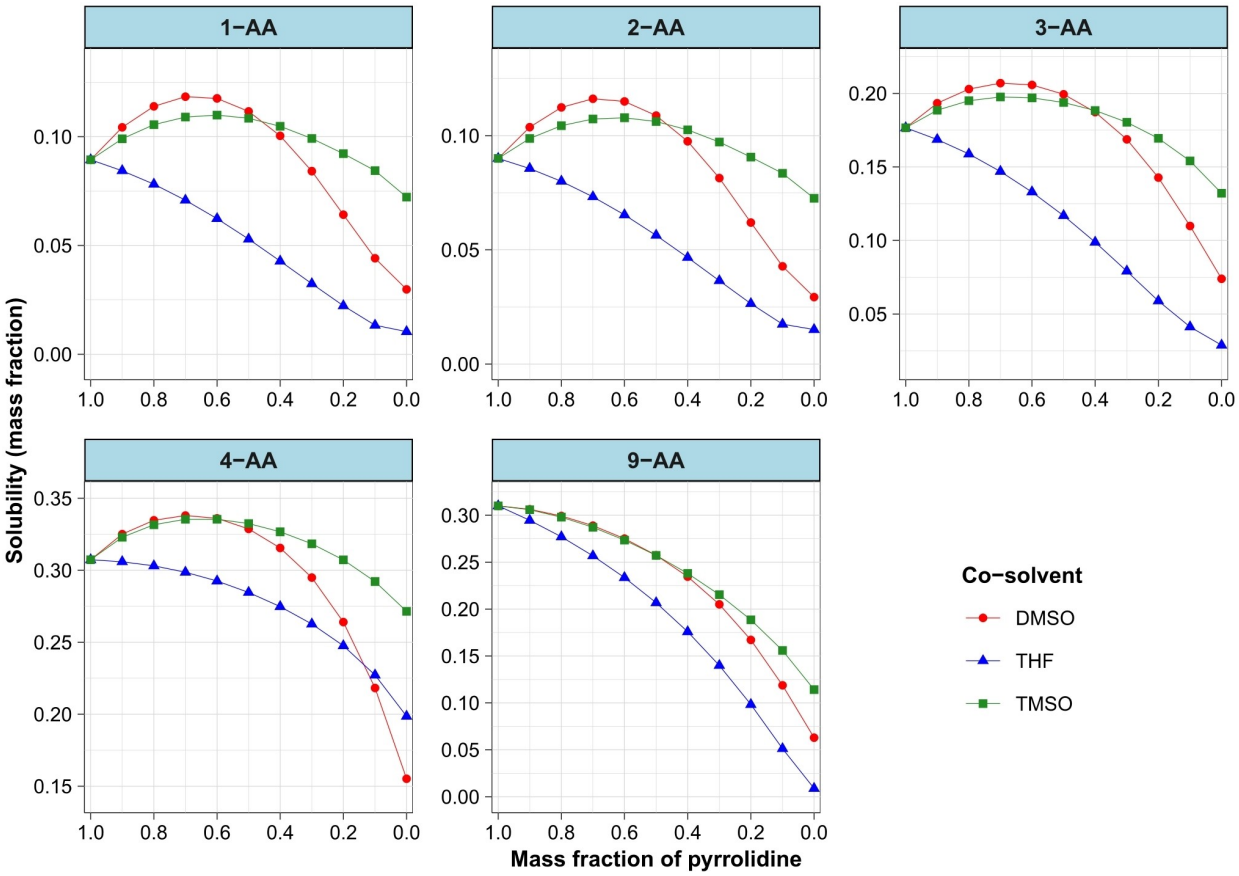


Figure 4. Computational solubilities of aminoacridines in pyrrolidine-containing mixtures (calculated using fast iterative method with water as reference solvent).

not produce solubility maxima; 9-AA showed no solubility maxima in any of the studied mixed solvents.

Driving forces behind solubility

In acetonitrile the experimental solubility trend 4-AA > 1AA > 3AA > 2-AA ≈ 9-AA was observed. This order is almost the exact reverse of the order of melting points (104,[33] 165–170,[34] 170,[35] 218,[36] 236–238[37] °C, respectively), suggesting that in MeCN (very weakly acidic and weakly basic solvent) the free energy of fusion may be the decisive factor in comparison to solvation energies. However, consistency between melting points and solubilities does not hold in all solvents: the experimental solubility orders in MeOH (3-AA ≈ 9-AA ≈ 4-AA > 2-AA, 1AA could not be measured), in DCM (3-AA > 2-AA ≈ 1-AA > 9-AA, 4-AA could not be measured) and in water (3-AA > 4-AA > 1-AA ≈ 9-AA > 2-AA, data from ref. [28]) do not match the orders of either melting points, lipophilicities, or acid-base properties (see Table S7 in SI), leading to the conclusion that in most solvents the relative solubilities of AAs are determined by the interplay of a number of factors none of which is dominating.

The structure of 4-AA suggests the presence of intramolecular hydrogen bonding, which would explain its low melting point due to reduced intermolecular forces in crystal structure, and high solubility (especially in low- and medium-polarity solvents) compared to the other AA isomers. Atom in molecule (AIM) and natural bond orbital (NBO) analyses were performed in order to verify the existence of intramolecular hydrogen bonding in 4-AA. Figure 5 and Table 2 show the molecular graph and the results of AIM analysis for 4-AA, respectively. The bond critical point (BCP, orange point) between H_{24} and N_{22}, and also ring critical point (RCP, yellow point), confirm the existence of intramolecular hydrogen bonding in 4-AA. The values in Table 2 show that this bond is of

Table 2. Selected bond lengths, angle and the corresponding parameters obtained from AIM analysis for 4-AA.

Parameter	Description	Value[a]
R N_{22}–H_{24}	Bond length	2.27
R N_{23}–H_{24}	Bond length	1.01
A N_{22}–H_{24}–N_{23}	Angle	104.18
ϱ^{BCP}	Electron density	0.0175
$\nabla^2\varrho^{BCP}$	Laplacian of electron density	0.0785
V^{BCP}	Potential energy density	-0.01255
G^{BCP}	Kinetic energy	0.01608
H^{BCP}	Energy density	0.003531
E_{HB}[b]	HB energy	1.73

[a] R: Angstrom, A: degree, AIM parameters: atomic unit and E_{HB}: kcal/mol. [b] $E_{HB} = 0.277\ |V^{BCP}| - 0.45$ equation[38] was used for HB energy calculation.

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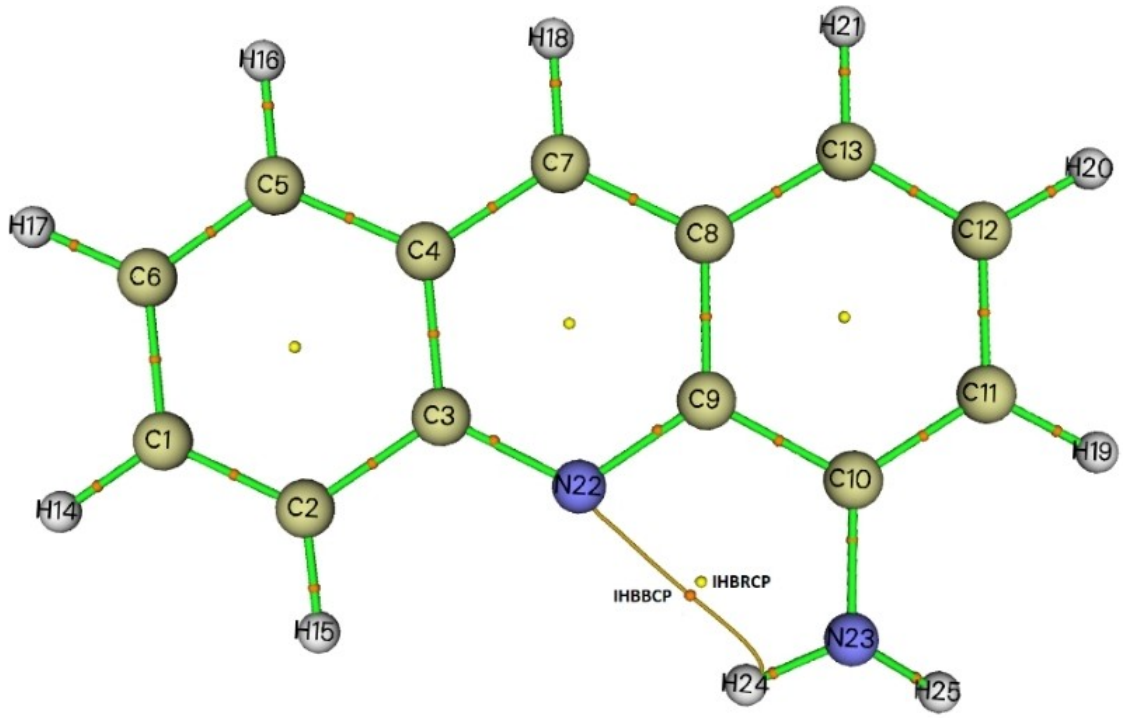


Figure 5. AIM molecular graph for 4-AA obtained at the B3LYP/6–311++G(d,p) level of theory. BCPs and RCPs are shown as orange and yellow circles, respectively. The corresponding BCP and RCP for intramolecular hydrogen bonding are marked as IHBBCP and IHBRCP, respectively.

weak type. On the other hand, the complimentary NBO analysis shows stabilization of lone pair orbital of ring nitrogen, N_{22}, through its interaction with antibonding orbital of N_{23}-H_{24} sigma bond, LP (N_{22})→σ* (N_{23}–H_{24}) that supports that AIM results.

In order to better understand the interactions responsible for the observed solubility trends, and to express solubility-promoting solvent properties via easily available quantitative parameters, the computationally predicted data were correlated with five Abraham solvent descriptors (values from ref. [39], available for 23 solvents out of the studied 31). Multilinear regression method was used as described by equation 1:

$$lnx = \sum_{i=1}^{n} D_i \cdot c_i + c_0 \quad (1)$$

where x is the predicted solubility (in mole fraction), D_i is the value of the descriptor i of the specific solvent, c_i is the respective regression coefficient, and c_0 is the intercept.

The results are shown in Figure 6. The relevance of each solvent property for solubility was expressed via the p value of the corresponding descriptor in regression. For four out of five aminoacridines, the most significant interaction is hydrogen bonding between solute (as a donor) and solvent (as an acceptor), represented by Abraham parameter a. The second most important interaction is cavity formation (parameter v), closely followed by dipolarity/polarizability interactions (parameter s). The importance of HB between solute as an acceptor and solvent as a donor (parameter b) is in agreement with the basicity order of AAs: 9AA > 3-AA > 1-AA > 2AA > 4-AA. As might be expected, the pattern of importance of the solvent proper-

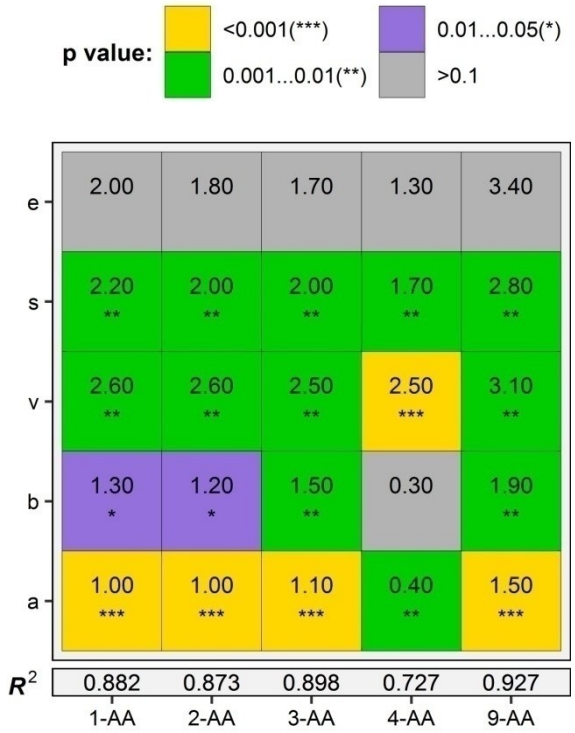


Figure 6. Statistical significance of Abraham solvent parameters in the multilinear equations connecting them to predicted solubilities. Lower p value indicates higher significance; numbers in cells are equation coefficients. Parameters describe the solvation energy dependence on solute's HB donicity (a), HB acceptivity (b), molecular volume (v), dipolarity/polarizability (s), and excess molar refraction (e). R^2 is the correlation coefficient of the equation.

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ties to the solubility of 4-AA differs from the remaining aminoacridine isomers.

Comparison of components of solvation energies computed by COSMO-RS confirms that different strengths of HB interactions in solutions are clearly correlated with differences in solvation energies of AAs, while van der Waals interactions are both weaker and more similar for different AAs. Thus, the rule of thumb for solubility improvement of aminoacridines is searching for a solvent with higher HB acceptor ability, and higher Abraham volume and dipolarity coefficients.

Conclusions

Solubilities of five mono-aminoacridine isomers were predicted by COSMO-RS method and measured experimentally in several representative solvents. The available data indicate that in most cases, COSMO-RS provides an accurate ranking of solvents for a given solute. Hydrogen bonding between solute as donor and solvent as acceptor was identified as the main factor accounting for solubility differences between solvents. In addition, lower melting point and higher solubility of 4-AA were explained by intramolecular hydrogen bonding that was confirmed by computations. Suitable solvents for each AA were proposed based on solubility, volatility, and safety issues. Although this work focuses specifically on aminoacridines, the presented approaches and found relationships can be useful for predicting or rationalizing solubility of many other compounds in different solvents.

Experimental section

Synthesis and characterization

Chemicals used in this work, their origin and purity are reported in the SI (Table S1). For the preparation of 4-AA, the procedure presented in Shimo *et al.*[40] was followed. For the preparation of 1-AA, the same methodology was used with the following modifications: 1-nitroacridine was separated from 3-nitroacridine with column chromatography (eluent: toluene/ethyl acetate/triethylamine 100 : 5 : 1) and then reduced on 10 % Pd/C in MeOH using H_2. 3-AA was synthesized based on the method presented in the ref. [41], except the obtained crude 3-acetamido-6-aminoacridine was used directly for the next step, without purification. Also, a more extensive purification of the mixture was performed, including multiple extractions and three column chromatographic separations to obtain the pure 3-AA. The purities of the AAs were determined with the quantitative NMR method (qNMR, described in detail in the SI). The ^{1}H and ^{13}C NMR spectra of all five AAs have been reported in our previous publication.[18]

Experimental limitations

Only very low amounts of some of the AAs were available due to experimental difficulties in synthesis. As this led to small volumes of saturated solutions, conventional experimental procedures had to be altered. Filtration of saturated solution was not always feasible and centrifugation was less helpful than expected. In most cases only one saturated solution was prepared and analyzed with two different methods in lieu of replicate measurements. All these limitations were taken into account when estimating the systematic error (bias) of our methodology, and lack of replicate measurements was compensated by conservative uncertainty estimate. We consider the obtained accuracy adequate for the intended purpose of confirmation of computation-based conclusions and solvent rankings.

Preparation of saturated solutions

The water content of the main solvents used in the solubility experiments was determined with the Karl Fischer titration[42] using the Mettler Toledo DL32 Karl Fischer Coulometer device. For each solvent at least seven replicate measurements were made and the injected solvent mass in one experiment was around 0.5–1.0 g. The water contents in the solvents together with their standard deviations were as follows: (41 ± 3) ppm in dichloromethane, (165 ± 3) ppm in methanol, (50 ± 2) ppm in acetonitrile, (277 ± 3) ppm in chloroform, and (76 ± 4) ppm in isopropanol.

Based on the preliminary solubility calculations, 10–100 mg of AA and 0.3–1.5 ml of solvent were combined in a 2 ml vial with a PTFE-lined screw cap. The mixture was vigorously stirred for 10–20 seconds on a vortex and thereafter visually checked to confirm that some AA remained undissolved. The suspension was allowed to settle overnight at room temperature.

In several exploratory experiments with AAs, isatin and acridine yellow the initial solutions with excess solid were stirred using a magnetic stirrer for 20–72 hours, followed by standing for 2–24 hours, and centrifuged/filtrated if very fine suspension was formed. We observed no systematic difference between results obtained by longer equilibration and the shorter procedure described above. Solution of 9-AA in pyrrolidine, notably more viscous solvent than others in this work, was equilibrated for ca 70 hours to ensure saturation. Please see the SI for more discussion of equilibration time.

Solutions of 2-AA and 3-AA showed discolouration and in some cases precipitation of dark green solid (presumably oxidation product) when exposed to air. Speed of degradation depended on the solvent (fastest in acetonitrile, slowest in methanol) and the extent of exposure to air and elevated temperature. To impede degradation, the solutions of 2-AA and 3-AA in acetonitrile were prepared using nitrogen-aerated solvent and vials were filled with N_2 before closing.

Two other problems were encountered: 1) a suspiciously high amount of 1-AA dissolved in methanol was detected, forming a dark red solution; and 2) the saturated solution of 4-AA in dichloromethane solidified overnight. In the case of these solute-solvent pairs, doubts arose as to whether only solubility processes were taking place. For this reason, in these two cases the solvents were replaced with isopropanol and chloroform, respectively, where such phenomena did not occur.

The concentration of the solution was then determined by gravimetric and chromatographic methods. All experiments were conducted at ambient temperature (20.8–24.5 °C, median 22.6 °C).

Solubility measurement methods

For gravimetric method the analytical balance Sartorius GENIUS (resolution of 0.01 mg) was used. Saturated solution was carefully drawn using a plastic syringe with a needle or an automatic pipette, avoiding drawing in the crystals of the undissolved AA. The collected solution was weighed ($m_{solution}$) and left to dry at room

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temperature, sometimes aided by nitrogen flow, until constant mass (m_{AA}). Solutions of 2-AA and 3-AA in acetonitrile were dried under nitrogen flow to minimize oxidation. When measuring solubility of 9-AA in pyrrolidine the solid residue was redissolved in methanol because of slow drying, presumably due to unusually stable solvates. Depending on the solvent, the complete evaporation of the solvent took 0.5–3 days. Solubility (in mass fraction) was calculated according to equation 2:

$$w = \frac{m_{AA}}{m_{solution}} \quad (2)$$

The HPLC-related experimental details for each AA are summarized in the SI (Table S3). Saturated solutions of AAs were either filtered (0.45 μm PVDF syringe filter, Whatman), centrifuged, diluted (typically 20- to 50-fold, 2–4 replicates), or combination of the above. When filtration was used, only the last portion of the solution passing the filter was used for analysis to minimize the effect of adsorption. To improve accuracy, saturated solutions in DCM were diluted with less volatile MeOH, MeCN, or DMSO, and the measurement results corrected by the ratio of solvent densities. HPLC analysis was carried out using Agilent 1200 series HPLC instrument with UV-Vis detection, using a reverse-phase column (Agilent Zorbax Eclipse XDB-C18, 4.6×250 mm, 5 μm particle size) and eluent composed of methanol and acidic buffer solution. The exact composition of the eluent and detection wavelength was optimized for each analyte. A calibration graph including 3 to 6 data points was constructed for every AA using MeOH or DCM as a solvent.

The results (in mass fraction) were calculated according to equation 3:

$$w_{sat} = \frac{(A_{dil} \cdot a + b) \cdot m_{dil} \cdot \rho_{cal}}{m_{sat} \cdot \rho_{dil}} \quad (3)$$

where A_{dil} is the peak area obtained from the diluted solution, a and b are the parameters of the calibration graph, m_{dil} is the mass of diluted solution, m_{sat} is the mass of saturated solution taken for dilution, ρ_{cal} and ρ_{dil} are the densities of the calibration solution and the diluted saturated solution, respectively. The densities were assumed to be equal to the density of the solvent/diluent.

Estimation of measurement uncertainty

The relative combined standard uncertainty $u_{c,rel}$ (see the course in ref. [43] for uncertainty-related terminology) was found according to the within-lab validation approach as formalized by Nordtest[44] according to equation 4:

$$u_{c,rel} = \sqrt{u_{rand}^2 + u_{syst}^2} \quad (4)$$

where u_{rand} accounts for the random and u_{syst} for systematic effects.

The relative systematic component of uncertainty (u_{syst}) was estimated via biases of solubility determinations of several heterocyclic compounds for which solubility values from literature were available. The results and the corresponding literature values are reported and illustrated in the SI (Table S4, Figure S1). The experimental limitations applicable to AAs were reproduced in these experiments.

The results indicate that for solubilities above mole fraction of ca 0.005 the bias is mostly positive, and in most cases does not exceed 20%. This is to be expected, as the results of both HPLC and gravimetry are more likely to be overestimated than underestimated due to solvent evaporation and accidentally picking up solid particles along with saturated solution. The remarkable outlier is 2-methylimidazole, showing almost two-fold difference between our results and literature value[45] (0.043 vs 0.022, mole fraction). The experiment was done in replicate to confirm that this discrepancy is not caused by random effects. Assuming that the literature value is accurate, a possible explanation is the presence of some specific solubility-enhancing impurity since chemicals of similar overall purity but different origin were used.

An average absolute bias is 29% including all results listed in Table S4 and 17% excluding 2-methylimidazole. We use an average of 23% as the estimate of the systematic uncertainty component (u_{syst}).

The relative random uncertainty component (u_{rand}) was found as a pooled standard deviation of chromatographic and gravimetric results for all saturated solutions successfully analyzed in this work. The relative bias in case of low solubilities is remarkably higher than for higher ones. Therefore u_{rand} was computed separately for solubilities below and above 1% (w/w). For lower range it amounted to 25%, for higher range to 4%. The bias was not estimated separately for different ranges due to insufficient amount of available data.

The combined relative uncertainty $u_{c,rel}$ is thus 24% for solubilities above 1% (w/w) and 34% for lower solubilities.

Computational methods

The molecular geometries were optimized at DFT BP86/TZVP level of theory using Turbomole software.[46] Frequency calculations were performed at the same level of theory to confirm that the structure corresponds to local minimum (evidenced by lack of imaginary frequencies in the vibrational spectra). After that, single point energy calculations were carried out at BP86/def2-TZVPD level of theory with *Fine* cavity parameter. The results of DFT calculations were used for solubility predictions using COSMOtherm software.[47] The recommended approach, solid-liquid equilibrium (SLE), was used in combination with Gibbs free energies of fusion derived from available experimental solubilities. The theory behind the calculation is described in the SI. Somewhat less accurate but faster iterative approach with free energy of fusion derived from experimental solubilities was used for mixture screening (Figure 4).

For AIM analysis of 4-AA, B3LYP/6-311++G(d,p) level of theory was used for geometry optimization, frequency calculation, population analysis, and wfn input file creation. For this aim, Gaussian 16[48] was used. Then, the corresponding file was applied for AIM analysis using Multiwfn software.[49]

Supporting information

Experimental details and comprehensive computational data are provided in the Supporting Information: the used chemicals, purity determination with qNMR method, discussion of equilibration time, details of HPLC measurements and uncertainty estimation, all predicted solubility values, additional figures, incl. illustration of solvent safety ranking, study of the effect of water contamination on solubility, melting points, lipophilicities, and acid-base properties of AAs.

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The authors have cited additional references within the Supporting Information.[50–63]

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: COSMO-RS · Hydrogen Bonding · Mono-Aminoacridines · Solubility · Solvent Effect

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