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# A Solvent-free Strategy to Prepare Amorphous Salts of Folic Acid with Enhanced Solubility and Cell Permeability

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Eight new amorphous organic salts of folic acid (FA) were prepared by mechanochemistry. FA can prevent cardiovascular and neurological diseases. Mechanochemistry overcomes serious FA solubility issues avoiding the use of toxic solvents. Due to low FA solubility, therapeutic effects in supplements and drugs are not achieved. Current strategies to improve FA solubility include its derivatization by using complex synthetic procedures. Herein, a simple and green procedure, avoiding structural modifications, was designed using mechanochemistry. Biocompatible amine-derivative coformers were strategically combined with FA to obtain salts with good physicochemical properties. New 1:1 and 1:2 amorphous FA salts offer 10 to 10,000 times better aqueous solubility and 10 to 100 times better octanol-water partition coefficient values ($K_{octanol/water}$) than FA alone. $K_{octanol/water}$ is considered as a surrogate of cell permeability. No toxic effects in normal human primary dermal fibroblasts were detected for the prepared FA salts. Our findings suggest that 1:2 FA salts of choline hydroxide and derivatives could be good candidates for future pharmaceutical/nutraceutical applications.

## Introduction

Folic acid (FA), a synthetic compound from the naturally occurring folates, belongs to the Vitamin B family and is involved in important cellular pathways.[1] Deficiency of FA is associated with several diseases, including baby brain defects, cardiovascular problems, colon cancer and dementia (Alzheimer and Parkinson).[2] As a result, the U.S. Department of Health and Human Services recommends that FA is added to processed fruits, vegetables and grain products, as a nutrition fortifier, to ensure 400 μg intake for adults and an additional 200 μg for pregnant women.[3] However, the amount of folates introduced by a diet is still not enough to reach the expected concentration in the body due to their low stability and limited absorption in the gastrointestinal tract.[4] The use of nutraceutical products containing FA often does not satisfy the body requirements as just a limited amount of the administered dose is dissolved in the stomach and then absorbed.[5] This is mainly due to the very low solubility of FA, measured to be $<10$ mg/L in the pH range 1.0–8.0.[1a,6] Therefore, increasing the solubility of FA is crucial for drug delivery, absorption and transport in the human body.

Until now, few strategies have been reported to overcome the solubility and bioavailability limitations of FA. Trindade et al. reported the functionalization of FA terminal γ-carboxylic acid with the purpose of selectively deliver chemotherapeutics to cancer cells.[7] Later, Pagano *et al.*, described the successful preparation of inorganic-organic nanostructured hybrids to store and release FA, once in contact with biological fluids, promoting its dissolution in the stomach.[1a]

Another study from Beagan *et al.*, demonstrated the possibility of creating an FA co-polymer through its esterification with poly(2-hydroxyethylmethacrylate) in dimethylsulfoxide (DMSO), improving its solubility and its release, at low pH values, by a factor of 10 to 20 times.[8] More recently, a work by Crinivec *et al.* reported the successful encapsulation of FA in hydrogels with the purpose of incorporating FA in food supplements, demonstrating a 30% higher retention of FA in the human body.[9] However, all these strategies involved non-sustainable and complex synthetic processes as well as the need for chemical modifications of the FA structure.

Herein, we report a simple, green and straightforward method to prepare FA salts in 1:1 and 1:2 stoichiometry, which may offer the required improvement in the aqueous solubility profile and cell permeability. FA was mechanochemically combined with three types of biocompatible amines (Figure 1) to form salts:[10] i) quaternary ammonium derivatives, such as

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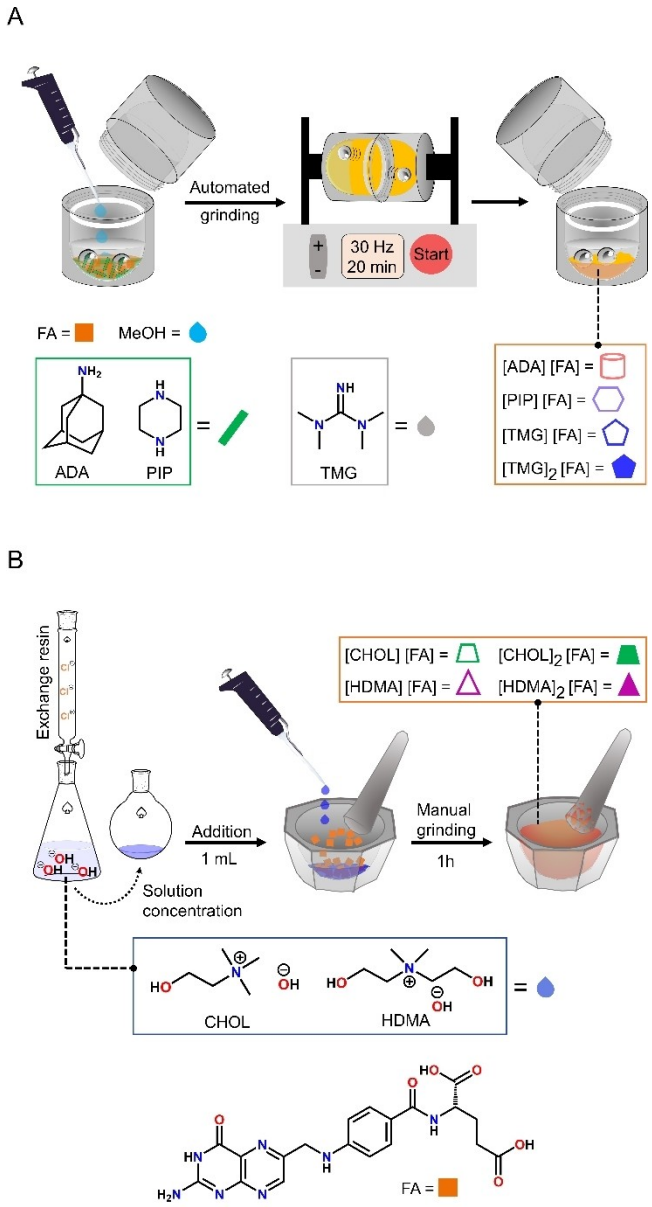


**Figure 1.** Schematic representation of the synthetic methods used to prepare both 1 : 1 and 1 : 2 FA salts: A – by automated ball mill grinding and B – by manual grinding. FA and coformers structures are also presented as symbols. Empty symbols depict 1 : 1 FA:coformer salts while full symbols depict 1 : 2 FA:coformer salts.

choline [CHOL] and bis(2-hydroxyethyl)dimethylammonium [HDMA] hydroxides; ii) primary amines, such as adamantylamine (ADA) and iii) secondary amines such as piperazine (PIP) and the organic superbase tetramethylguanidine (TMG). Most of these amines (ii and iii) are commercially available. However, quaternary ammonium salts, such as CHOL hydroxides and HDMA hydroxides are sold as stable chloride salts instead of the required hydroxide salts, these being too unstable on storage. Therefore, CHOL chloride and HDMA chloride were transformed into the corresponding hydroxy forms before considering the preparation of the [CHOL][FA] and [HDMA][FA] salts.

Conventional solution-based synthetic strategies are unsuitable to prepare the desired FA organic salts due to the low solubility of this vitamin in water and in organic solvents (*e.g.*, ethanol, methanol, acetonitrile). FA dissolves only in warm DMSO and after cooling it precipitates from this solvent. Liu *et al.* reported a synthetic strategy based on the FA derivatization with fluoropyrimidine derivatives to create a new chemotherapy with improved solubility and cytotoxicity towards human colorectal tumor cells.[11] For the derivatization synthesis, the authors considered several steps involving some "initial" protection of FA functional groups with 2-acetyloxyethylamine in order to increase its solubility in organic solvents and promote the desired derivatization reaction.[11] Here, an alternative and simple mechanochemical procedure for the preparation of FA amine-based salts is used, overcoming the serious low solubility issue typically encountered with traditional solution-based methods.

## Results and Discussion

We start by exemplifying how the challenges presented by the introduction of different amines in the preparation of FA salts, require the use of different mechanochemical procedures, such as ball mill grinding or the use of mortar and pestle. Ball mill grinding is suitable and most effective when transforming solid reagents with minimal or no liquid addition.[10] In this methodology, the reaction is mechanically activated by the collision of the ball bearings with the powder inside the milling jar while the jars are shaking at a specific frequency for a given time period. The energy generated from those collisions are then transferred to the solids, allowing a complete transformation of FA and the selected amines into a salt. In the presence of small quantities of solvent (liquid assisted grinding – LAG), the reaction can be accelerated, decreasing the time necessary to achieve a complete conversion of the starting materials into the desired products.[12] For solid amines like ADA and PIP (Figure 1 A), ball mill LAG is preferred as small quantities of solvent can be used (10–20 μL for 600 mg powder). For these particular amine coformers, salt formation was performed using 30 μL of methanol ($\eta = 0.05$) and milled at the maximum frequency of 30 Hz. These conditions help to achieve a faster kinetic process, 20 minutes to obtain the salt in quantitative yield.[13] On the other hand, for liquid amine coformers such as TMG, ball mill neat grinding conditions (absence of added solvent) is the best option as the liquid from the amine replaces the solvent normally used in LAG conditions.

The critical challenge comes from selecting a simple and effective procedure to transform the commercially available quaternary ammonium chlorides (CHOL chloride and HDMA chloride) into the required short-lived hydroxides. Pure CHOL and HDMA hydroxides can be obtained by an ionic exchange procedure (Figure 1 B) as reported by us in the mechanochemical preparation of pharmaceutical ionic liquids (*MechanoAPILs*).[14] This procedure allowed the preparation of a (1–2 mL) diluted but stable methanolic pure fraction containing the desired hydroxide salt. Due to the unstable nature of these

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hydroxide salts, further concentration by evaporation to obtain small fractions of around 200 μL or even a solid residue is not feasible. The use of a slurry-like mixture for ball mill grinding is totally unsuitable as the impact of the balls could be dissipated by the liquid. The use of a mortar and pestle, on the other hand, present just the right opportunity to resolve this challenge. The mortar being an open vessel, allows for the evaporation of methanol during grinding, while the pestle with its high surface area acting as shear forces is the best equipment for the mechanochemical treatment of mixtures similar to slurries. The manual use of mortar and pestle also allows for the repeated addition of small portions (200 μL) of the hydroxide salt solution to FA powder until the final amorphous FA salt is formed. The use of these quaternary ammonium hydroxides assures that the only side product from the acid-base reaction is water, becoming a green strategy for the preparation of non protic pharmaceutical salts. Overall, around 1 h is required for the complete stochiometric addition of the 1 mL hydroxide salt solution to the FA powder and formation of the final amorphous salt product.

The mechanochemical combination of FA with the selected bases highlights the advantages of using mechanochemistry in the preparation of pharmaceutical salts: 1) it is possible to successfully react the solid FA with either solid amines (ADA and PIP) under LAG conditions or liquid amines (TMG) under neat conditions (Figure 1A); 2) when using solutions containing unstable hydroxide ammonium salts, it is possible to perform the reaction with FA processing several small fractions of the solution freshly obtained after the ionic exchange process (Figure 1B) using mortar and pestle and; 3) in all acid-base reactions, a solid product is obtained in high yield (94 to 96% mol/mol).

Both 1:1 and 1:2 stoichiometric salts were characterized by $^{1}$H-NMR, $^{13}$C-NMR, X-ray diffraction and elemental analysis (Figures S6–S21 and S26, and experimental details). From the integration of the NMR peaks of $^{1}$H-NMR data it was possible to confirm the right stoichiometry obtained for each salt. Furthermore, by evaluating the $^{13}$C-NMR data, we observed that the signals of one or both carbon atoms from the two carboxylic groups of FA are shifted towards lower field (*e.g.* for [CHOL][FA], 175.2 ppm and 174.5 ppm) with respect to the pure FA (173.7 ppm and 173.9 ppm).[15] This observation supports the deprotonation event occurring in these groups upon acid-base reaction with the amines. For the 1:1 stoichiometry, the signals of only one carbon shifted (one deprotonation occurs), while for the 1:2 salts, the signals of both carbon shifted (two deprotonations occur).

All the prepared FA salts are amorphous, and revealed to be stable at room temperature over two months. The attained glass transition temperatures were in the range of 43 to 70 °C (Figure 2 and Figures S22–S25), supporting that all these salts become amorphous on milling.[16] This solid-state procedure should allow for the preparation of stable FA amorphous salts at room temperature for future pharmaceutical application.

Preliminary solubility studies and octanol-water partition coefficient measurements were performed for all prepared salts.

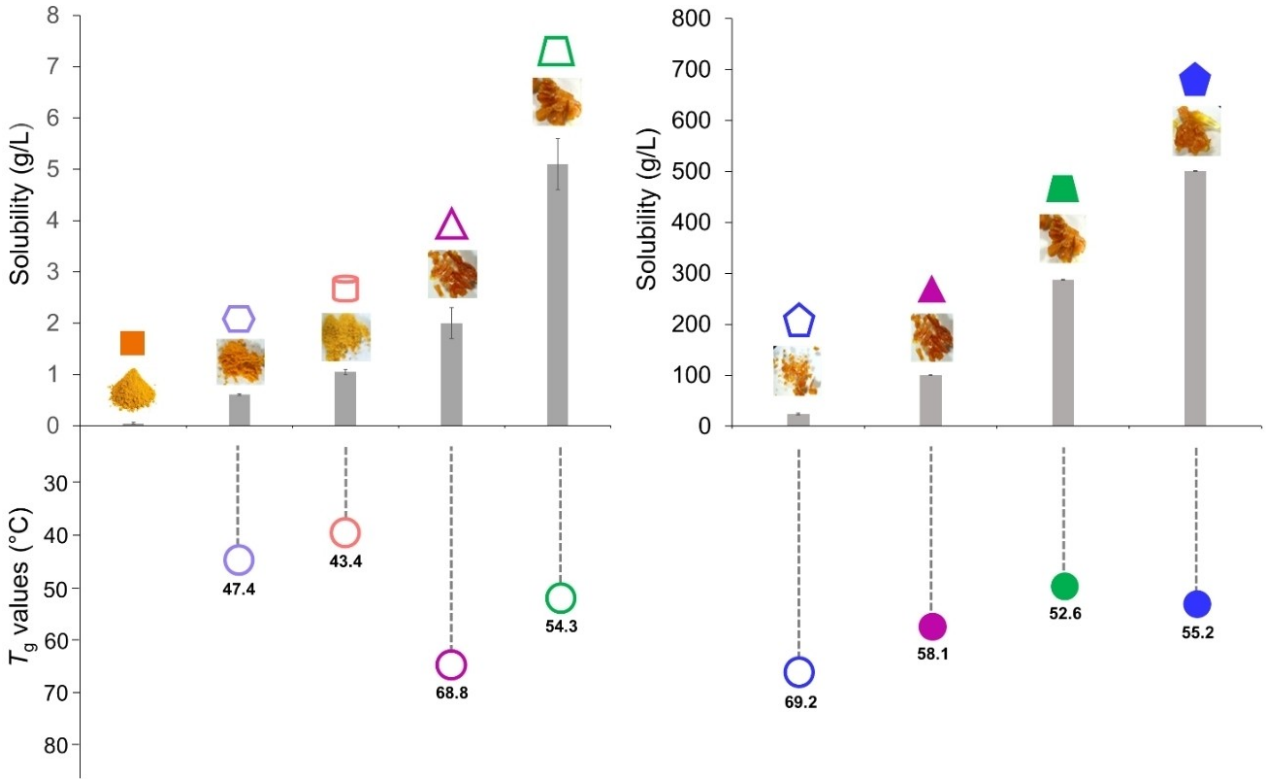


**Figure 2.** Representation of both solubility (in water at 37 °C) and glass transition temperature ($T_g$) values for FA 1:1 and 1:2 salts. Pictures showing the physical aspect of all amorphous salts are also presented alongside with their labelling scheme as defined in Figure 1. Empty symbols represent 1:1 FA salts while full symbols represent 2:1 FA salts.

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A remarkable improvement in FA solubility was observed for all the FA salts in comparison with pure FA, as can be observed in Figure 2. 1:2 FA:coformer salts have shown to performe extremely well, presenting very high aqueous solubility, increasing from the measured 50 mg/L for FA to 100 g/Lfor $[HDMA]_2[FA]$, 287 g/L for $[CHOL]_2[FA]$ and even 500 g/L for $[TMG]_2[FA]$ (Figure 2 and Table S1). More modest increase in solubility were obtained for the 1:1 FA:coformer salts, most of them having solubilities lower than 6 g/L except for TMG salt, exhibiting a solubility as high as 25 g/L. The higher solubility obtained for the 1:2 FA:coformer salts, with respect to the 1:1 salts, is somehow expected due to their highest polarity.[17] Furthermore, the use of counter-cations with higher hydrophilic character, such as TMG, CHOL and HDMA, also contribute to their solubility improvement. Both CHOL and HDMA possess hydroxyl groups in the alkyl chains, enhancing their polarity and hydrogen bonding capabilities. These coformers are therefore responsible for the significant increase in the aqueous solubility profile of their corresponding FA salts, when compared to salts containing protic coformers, such as [PIP][FA] and [ADA][FA].

It is known that amorphous materials exhibit, in general, higher solubility than crystalline materials due to the decrease of particle size to the nanoscale, significantly increasing the surface area.[18] However, their stability can be compromised, as these salts are metastable and can easily convert either to more stable forms (crystalline phases) or to pure crystalline API (salt disintegration).[19] The integrity of all salts in saturated aqueous solution at 37 °C was investigated by analyzing the powder X-ray diffraction data of the slurry residues obtained from the solubility experiments after completion (see supplementary information Figure S27). Here, the amorphous nature of the prepared salts was preserved after precipitation from the saturated solutions except for: [PIP][FA], [ADA][FA], [TMG][FA] and $[TMG]_2[FA]$ salts, that dissociated, leading to the detection of crystalline FA. In these salts, the counter-cations used are protic, in contrast to CHOL and HDMA, which enables the migration of the labile hydrogen atoms to FA, hence increasing the possibility of salt dissociation.

The hydrophilicity/lipophilicity of APIs is usually correlated to their capacity to cross cell membranes, a topic of high relevance to the pharmaceutical industry. The octanol-water partition coefficient ($K_{octanol/water}$) can often be correlated with biological effects and it is seen as a surrogate of the cell permeability evaluation. This coefficient is defined as the ratio of the equilibrium concentrations of the compound neutral species in octanol and water.[20] A study of the $K_{octanol/water}$ values was performed for the most promising FA salts such as [TMG][FA], $[TMG]_2[FA]$, [CHOL][FA], $[CHOL]_2[FA]$, [HDMA][FA] and $[HDMA]_2[FA]$. The relationship between these values and solubility at 37 °C are depicted in Figure 3. FA salts in 1:2 stoichiometry present the highest aqueous solubility and the lowest $K_{octanol/water}$, varying between 0.03 and 0.06. On the other hand, the salts in 1:1 stoichiometry, present comparatively low solubility and high $K_{octanol/water}$; the values being two times to six times larger than the ones for the corresponding 1:2 FA:coformer salts. It can be stated that the $K_{octanol/water}$ profile of the amorphous FA salts follow the opposite trend to their solubility, *i.e.*, higher $K_{octanol/water}$ means lower solubility. This observation is in agreement with results reported for other API salt systems.[21] However, when inspecting in more detail the behaviour for the three 1:2 FA:coformer salts, there is a direct correlation between the solubility and the $K_{octanol/water}$ values; the salt with the highest aqueous solubility, $[TMG]_2[FA]$, also presents comparatively the highest $K_{octanol/water}$ value.

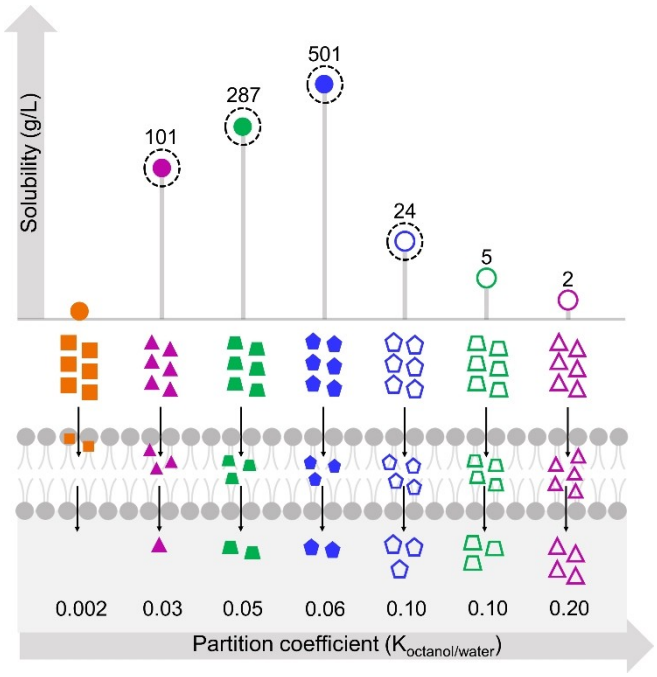


**Figure 3.** Schematic representation of the correlation between solubility and $K_{octanol/water}$ values (cell permeability) of FA and its most promising 1:1 and 1:2 salts candidates. The labelling schemes used for each salt composition are the same as depicted in Figure 1. Symbols: orange – FA; pink – HDMA; green – CHOL; blue – TMG. Empty symbols represent 1:1 FA salts while full symbols represent 2:1 FA salts. The four encircled symbols represent those FA salts most suitable as drug candidates, as they demonstrate the best balance between solubility and permeability (partition coefficient).

In a pharmaceutical perspective, a good drug needs to present an acceptable compromise between the aqueous solubility and cell permeability. From the prepared set of FA salts, the best candidates are [TMG][FA], $[TMG]_2[FA]$, $[CHOL]_2[FA]$ and $[HDMA]_2[FA]$, as highlighted in Figure 3. Although [CHOL][FA] and [TMG][FA] present similar $K_{octanol/water}$, [CHOL][FA] has not been considered a good candidate as its solubility is five times lower. Unfortunately, because [TMG][FA] and $[TMG]_2[FA]$ disintegrate in solution (Figure S27), they are not considered suitable candidates for drug formulation. Additionally, the biological activity of choline derivatives (CHOL and HDMA) has been proven to reduce the risk of brain pathologies. Therefore, $[CHOL]_2[FA]$ and $[HDMA]_2[FA]$ are the best salts, promoting a synergy between the therapeutic effect of FA and its coformers.[22]

Human primary dermal fibroblasts are a good surrogate model test system for human healthy cells. Therefore, they were selected to assess whether the amorphous formulations (presenting better solubility) showed in vitro cytotoxicity. As such,

these cells were incubated for 48 hours with a 50 μM concentration of each of these salts, FA and respective coformers, and the cell viability assessed. Figures 4 and S5, show that none of the FA salts or starting materials displayed any in vitro cytotoxicity at high concentrations (50 μM). The good cytotoxicity at such high concentration demonstrates the extremely benign nature of these FA based salts towards healthy cells, providing a good platform for future pharmaceutical applications.

## Conclusion

In conclusion, we have developed a viable strategy for improving FA aqueous solubility and $K_{octanol/water}$ through the synthesis of amorphous pharmaceutical salts, using a green synthetic strategy, based on mechanochemistry. This technique offers enormous benefits. The preparation of FA salts can now be performed using a solvent-free procedure. This overcomes previous problems encountered when attempting to prepare FA salts by solution-based methods, due to the very low FA solubility in most solvents. By using this strategy, FA does no longer requires to be chemically modified to increase its solubility. This avoids complex synthetic chemistry approaches in the production of FA derivatives intended to improve its solubility and bioavailability.

From the preliminary solubility tests conducted, all prepared amorphous salts dramatically improved FA aqueous solubility profile, revealing also the absence of toxicity to primary dermal fibroblasts. Furthermore, they also present a good stability even above room temperature over two months, an advantage for possible pharmaceutical formulations. In practice, the shelf-life stability of possible FA formulations containing these amorphous salts can be maintained even in places where the temperatures are above 30 °C.

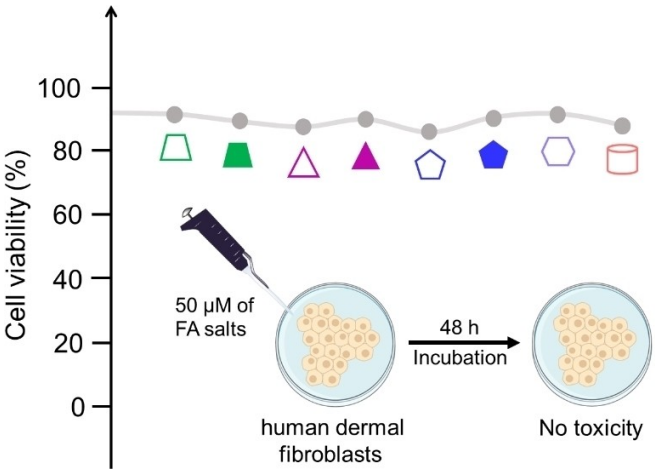


**Figure 4.** Schematic representation of the cytotoxicity of the most promising 1:1 and 1:2 FA salts to human primary dermal fibroblasts. The labelling schemes used for each salt composition are the same as depicted in Figure 1. Empty symbols represent 1:1 FA salts while full symbols represent 1:2 FA salts.

A pronounced improvement in the $K_{octanol/water}$, seen as a surrogate of the cell permeability, was observed for salts containing CHOL, HDMA and TMG as cations. The use of CHOL and HDMA presents two main advantages: i) help in the improvement of FA solubility properties and; ii) create a synergy between their therapeutic effect on reducing the risk of brain pathologies[22] and those of FA.

The aqueous solubility, absorption and permeability are three of the key properties for the successful delivery of a drug in the human body. The results presented herein demonstrate that the two selected amorphous FA salts ($[CHOL]_2[FA]$ and $[HDMA]_2[FA]$) are potential future candidates for being incorporated into pharmaceuticals and nutraceuticals. Considering the possibility of further development of formulations containing these FA salts, thorough and systematic solubility and cell permeability studies must be performed. The preliminary results herein reported demonstrate that an application of these FA salts will enables the reduction of the amount of FA in drug formulations and food supplements. This in turn can reduces the costs associated with the formulation process, presenting technological advantages for the pharmaceutical industry.

## Experimental Section

### Mechanochemical experiments

**[CHOL][FA]** – A concentrated solution of CHOL hydroxide in methanol (0.137 g; 1.133 mmol) was first prepared, through ionic exchange process described in supplementary information. This solution was then concentrated by evaporation (1–2 mL) and 200 μL aliquots were slowly added to the agate mortar, containing the FA (0.500 g; 1.133 mmol), and the mixture was manually ground during 1 h until the complete methanol evaporation. The obtained orange color product (0.602 g; yield 94.5 %) was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**$[CHOL]_2[FA]$** – A concentrated solution of 2 equivalent of CHOL hydroxide in methanol (0.274 g; 2.266 mmol) was first prepared, through the ionic exchange process described in supplementary information. This solution was then concentrated (1–2 mL) and 200 μL aliquots were slowly added to the agate mortar, containing the FA powder (0.500 g; 1.133 mmol), and the mixture was manually ground during 1 h until the complete methanol evaporation. The obtained strong orange color product (0.745 g; yield 96.3 %) was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**[HDMA][FA]** – A concentrated solution of HDMA hydroxide in methanol (0.171 g; 1.133 mmol) was first prepared, through the ionic exchange process described in supplementary information. This solution was then concentrated (1–2 mL) and 200 μL aliquots were slowly added to the agate mortar, containing the FA (0.500 g; 1.133 mmol), and the mixture was manually ground during 1 h until the complete methanol evaporation. The obtained strong orange color product (0.647 g; yield 96.4 %) was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**$[HDMA]_2[FA]$** – A concentrated solution of 2 equivalent of HDMA hydroxide in methanol (0.342 g; 2.266 mmol) was first prepared, through the ionic exchange process described in supplementary information. This solution was then concentrated (1–2 mL) and 200 μL aliquots were slowly added to the agate mortar, containing

the FA (0.500 g; 1.133 mmol), and the mixture was manually ground during 1 h until the complete methanol evaporation. The obtained strong orange color product (0.813 g; yield 96.6%) was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**[ADA][FA]** – FA (0.500 g; 1.13 mmol) and ADA (0.171 g; 1.133 mmol) were ground using an automated horizontal oscillatory ball mill grinder (for details, see supplementary information), during 20 min at 30 Hz, and in the presence of 30 μL of methanol ($\eta = 0.04$). The obtained yellowish color powder (0.633 g; yield 94.3%), was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**[PIP][FA]** – FA (0.500 g; 1.13 mmol) and PIP (0.098 g; 1.133 mmol) were ground using an automated horizontal oscillatory ball mill grinder (for details, see supplementary information), during 20 min at 30 Hz, and in the presence of 30 μL of methanol ($\eta = 0.05$). The obtained orange color powder (0.576 g; yield 96.4%), was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**[TMG][FA]** – FA (0.500 g; 1.13 mmol) and TMG (0.131 g; 1.133 mmol) were ground using an automated horizontal oscillatory ball mill grinder (for details, see supplementary information), during 20 min at 30 Hz. The obtained amorphous yellow color powder (0.612 g; yield 97.0%), was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

**[TMG]$_2$[FA]** – FA (0.500 g; 1.13 mmol) was ground with 2 equivalents of TMG (0.261 g; 2.266 mmol), using an automated horizontal oscillatory ball mill grinder (for details, see supplementary information), during 20 min at 30 Hz. The obtained amorphous yellow color powder (0.737 g; yield 96.8%), was dried under high vacuum and analyzed by solution NMR, elemental analysis, PXRD and DSC.

### Nuclear magnetic resonance (NMR) experiments

$^{1}$H and $^{13}$C NMR spectra were recorded on a Bruker Avance III 300/400 spectrometer, operating at 300/400 MHz and 75/100 MHz, respectively. Chemical shifts and coupling constants (*J*) are reported in ppm and Hz, respectively. Resonance and structural assignments were based on the analysis of coupling patterns, including the $^{13}$C-$^{1}$H coupling profiles, obtained in bidimensional heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments, performed with standard pulse programs. All NMR data are presented in Figures S6–S21.

### Powder X-ray diffraction analysis

Data was collected in a D8 Advance Bruker $\theta - 2\theta$ diffractometer, from 5°–37°, with a copper radiation source (Cu Kα, $\lambda = 1.5406$ Å) and a secondary monochromator, operated at 40 kV and 40 mA. The step width used was 0.2. Figures S26 and S27 show the results obtained for all FA salts before and after solubility studies, respectively.

### Elemental analysis

Elemental analyses were performed in a Fisons Instrument Mod EA-108, at *Laboratório de Análises (IST)*. All samples were weighted in a Sn capsule and heated until combustion. Two independent determinations for each compound were performed.

### Differential scanning calorimetry (DSC)

A differential scanning calorimeter (2920 MDSC system from TA Instruments Inc.) equipped with a cooling accessory was used. The instrument temperature scale was calibrated with five standards and the heat flow scale was calibrated with indium and tin.[23] Samples were accurately weighed (approximately 0.1 μg) in aluminium pans on a Mettler UMT2 ultra-micro balance under air. All the measurements were performed under dry high helium gas (Air Liquid N55) at a flow rate of 30 mL min$^{-1}$. The protocol used: i) cooling at - 100 °C; and ii) heating at 10 °C min$^{-1}$ until 140 °C, followed by an identical thermal cycle. DSC thermograms refer to the second thermal cycle, as in the first one the water content was removed. $T_g$ values were assigned to the onset point of the thermal events (Figures S22–S25).

### Solubility tests

Given the high aqueous solubility of the 1:2 FA;co-former salts, the solubility tests were performed using two methods:

Method 1, for [CHOL][FA], [HDMA][FA], [ADA][FA] and [PIP][FA]: aqueous saturated solutions of the compounds (1 or 2 mL) were prepared and kept stirring for 1 hour at 37 °C. The concentration of the supersaturated solutions was determined immediately after filtration by using UV-Vis spectroscopy (Lambda 35, Perkin Elmer) with respect to a previously established calibration curve:

$$Abs_{360nm} = 5542.6[salt]_{mol/L} + 0.0461 \ (R^2 = 0.995)$$

This calibration curve was determined by measuring the absorbance, at 360 nm, of different FA concentration solutions in a DMSO: water mixture (50:50) (Figure S3). This solvent mixture had to be used as it was impossible to obtain higher FA concentrations only in water.

Method 2, for [TMG][FA], [CHOL]$_2$[FA], [HDMA]$_2$[FA] and [TMG]$_2$[FA]: An exact amount of each compound (around 40 mg) was added to a vial. Vials were kept at 37 °C and aliquots of 5 μL of distilled water initially added to the solution until the solid was nearly dissolved. To achieve the complete dissolution of the compounds, 1–2 μL of distilled water was carefully added. For both methods, three independent solubility experiments were performed, and the results are presented as mean ± SD. Table S1 presents the results of all the solubility studies.

### Octanol-water partition coefficient measurements

The octanol-water partition coefficients ($K_{octanol/water}$) of selected FA amorphous organic salts ([CHOL][FA], [CHOL]$_2$[FA], [HDMA][FA], [HDMA]$_2$[FA] [TMG][FA] and [TMG]$_2$[FA]) were determined by the slow-stirring method, described in the OECD guidelines for testing the chemicals.[24] Before performing the partition experiments, water and 1-octanol were mutually saturated during 48 h by using the slow-stirring conditions. Solutions containing FA and its salts were prepared using water saturated in 1-octanol. Approximately 4 mL of each of these solutions were carefully added to test vials containing a magnetic stirrer bar and 4 mL of 1-octanol previously saturated with water. Test vials were stirred during 24 h and kept in an oil bath under a controlled temperature of 37 °C. Samples were taken from water-rich phase at chosen times (from 30 min to 24 h). Finally, the concentration of the compounds was determined by using UV-Vis spectroscopy, at $\lambda = 7$ 360 nm (Lambda 35, Perkin Elmer). Saturated solution of 1-octanol in water was used in the reference cell. The correlation between the $K_{octanol/water}$ and solubility is presented on Figure S4.

### Citotoxicity assays

Human primary dermal Fibroblasts (ATCC® PCS-201-010) were cultivated in 96-well plates at a concentration of 7500 cells per well and incubated for 24 h at 37 °C, 5% (v/v) CO2 and 99% (v/v) of relative humidity in Dulbecco Modified Eagle Medium (DMEM) culture medium. The medium was then replaced with fresh medium with the appropriate concentrations of the compounds and respective ions. All dilutions were made in culture medium. Cells were then incubated for another 48 h in the presence of FA, co-formers and respective FA:coformer salts (1:1 and 1:2 stoichiometries). Cell viability was then determined with CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, Promega, Madison, WI, USA) as previously described. Briefly, this is a colorimetric test that measures the formation of formazan, by viable cells, from a tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]. The absorbance of samples at 490 nm was measured in an Infinite M200 microplate reader (Tecan, Männedorf, Switzerland). Three independent experiments were performed, and the results are presented with mean ± SD (Figure S5).

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## Conflict of Interest

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:** Folic acid · amorphous salts · mechanochemistry · solubility & permeability · choline derivatives

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