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Determination of Acidity Constant (pKa), Lipophilic Partition (LogP) and Distribution Coefficients (LogD) Values of Some 3-(2-Phenylethyl)-Tetrahydro-2H-1,3,5-Thiadiazine-2-Thione-5-Acetic Acid Derivatives

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Research Article

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ABSTRACT

Invasive fungal infections pose a significant threat to human health, emphasizing the urgent need for new and effective antifungal agents. 3,5-Disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives have shown promising antifungal activity. This study focused on characterizing the physicochemical properties, specifically the acidity constant (pKa), lipophilic partition coefficient (logP), and distribution coefficient (logD), of four 3-(2-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione-5-acetic acid derivatives (EP1, EP2, EP3, and EP4) with varying α-carbon substituents. pKa values were determined using two independent methods: parallel factor analysis (PARAFAC) of UV spectroscopic data and reversed-phase high-performance liquid chromatography (RP-HPLC). Lipophilicity was assessed by measuring logD values using the shake-flask method with n-octanol, and logP values were calculated based on the determined pKa and logD values. The pKa values determined by both PARAFAC and RP-HPLC showed a good correlation. The α-carbon substituent significantly influenced pKa, with electron-withdrawing substituents resulting in lower pKa values, consistent with inductive effects. The logD-pH profiles exhibited typical bell-shaped curves, with logP values at pH 5.5 ranging from -0.38 to 3.00. EP1, EP2, and EP4 displayed lipophilic characteristics, while EP3 showed higher hydrophilicity. This study provides the first reported pKa, logP, and logD values for these novel thiadiazine derivatives. The findings emphasize the influence of structural modifications on physicochemical properties, which are critical for drug absorption, distribution, and target interaction. These data provide a valuable basis for elucidation of the structure-activity relationship and formulation optimization of these antifungal agents.

Keywords: Acidity constant, Lipophilic partition, Distribution coefficients, PARAFAC, HPLC.

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Introduction

Invasive fungal infections present a serious and growing global health threat, causing an estimated 1.5 million deaths annually [1]. Despite medical progress in treating bacterial infections, the limited availability of effective antifungal drugs poses a significant challenge. Individuals with weakened immune systems, such as those undergoing chemotherapy, organ transplantation, or living with HIV/AIDS, are particularly susceptible to these infections, leading to severe consequences [2]. The emergence of antifungal resistance further complicates treatment efforts and highlights the urgent need for novel antifungal drug discovery [3].

The 3,5- disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione scaffold represents a promising source of novel therapeutic agents. Previous research has demonstrated the diverse biological activities of compounds containing this core structure, including antibacterial, antifungal, anti-inflammatory, antileishmanial, antitubercular, and anticancer properties [4-10]. Among these, 3-(2-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione-5-acetic acid derivatives, synthesized via a well-established two-step process, have emerged as particularly promising candidates.

The journey of a drug molecule from the laboratory bench to a patient's bedside is a complex one, influenced by a delicate interplay of factors. Among these, the molecule's inherent physicochemical properties stand out as critical determinants of its fate and effectiveness within the body. These properties, including its acidity constant (pKa), lipophilic partition coefficient (logP), and distribution coefficient (logD), govern how a drug interacts with biological systems, ultimately influencing its absorption, distribution, metabolism, excretion, and overall therapeutic efficacy.

A drug molecule's degree of ionization, directly related to its pKa value, significantly impacts its behavior and efficacy. The pKa dictates the ratio of ionized to non-ionized forms at a specific pH, influencing crucial factors like solubility, cell membrane permeability, binding to plasma proteins, and interactions with target molecules [11]. While not the sole determinant of efficacy, understanding how a drug's ionization changes with pH is essential for optimizing its delivery to fungal cells,

ensuring effective target engagement, and predicting its overall pharmacokinetic profile. Therefore, pKa is a critical consideration throughout the design and development of new antifungal agents.

Understanding the logP and logD values provides further insights into a drug's behavior. While logP, a measure of the molecule's affinity for a lipophilic environment, offers valuable information, it only paints a partial picture. In contrast, logD, which takes into account the ionization state of the molecule across a range of pH values, provides a more comprehensive view of its lipophilic character in biological systems [12]. This becomes particularly crucial when considering that most drugs exist as a mixture of ionized and non-ionized forms under physiological conditions.

PARAFAC has proven to be a valuable tool for pKa determination, with successful applications in analyzing UV [13-16], voltammetric [17, 18] and fluorescence [19] data.

To determine the pKa of these molecules, we employed two independent methods, including PARAFAC, which was applied to UV spectroscopic data and HPLC. PARAFAC, a powerful three-way analysis method (Figure 1), dissects the complex pH-dependent UV absorbance data, allowing us to discern the equilibrium between the acidic (HA) and basic (A^-) forms of the molecule and precisely pinpoint its pKa value (Figure 2) [20, 21].

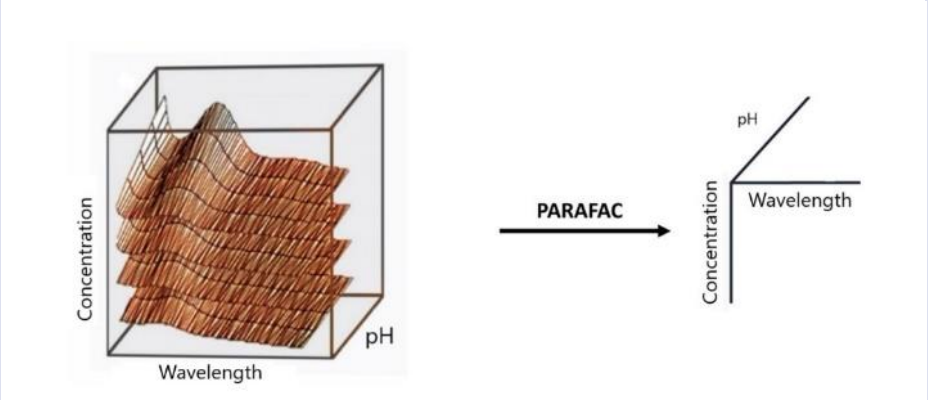

Figure 1. Datasets generated to determine pKa by PARAFAC three-way data analysis.

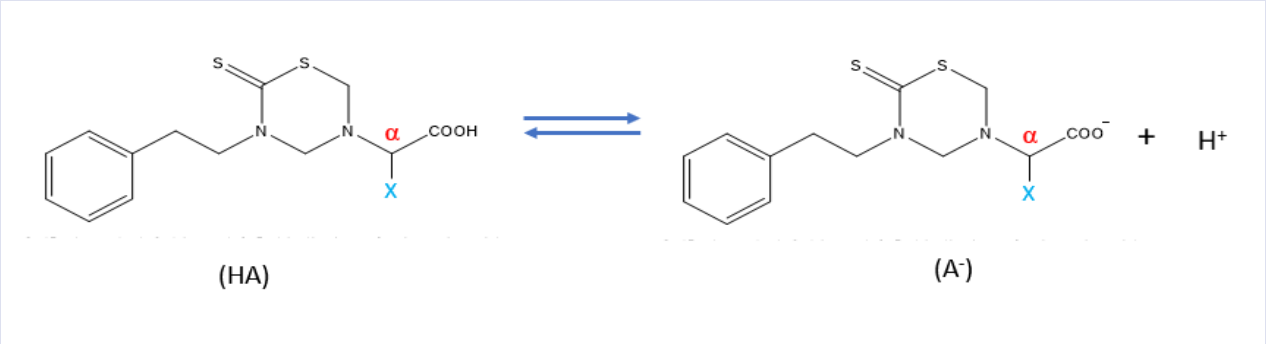

Figure 2. Acid dissociation equilibrium of EP0 molecule.

According to Equation 1, when the acidic form concentration [HA] and the base form concentration [A^-] are equal, the pH of the solution becomes equal to the pKa value of the relevant substance. Analytical signals can be decomposed with three-way analysis tools by arranging them as three-way data strings. This distinction helps us to observe the profiles of the three variants for all the components (HA and A^- concentrations) that contribute to the signals.

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad (1)$$

In additionally, in this study, the pKa values of some 3-(2-phenylethyl)-tetrahydro-2h-1,3,5-thiadiazine-2-thione-5-acetic acid derivatives were determined by the RP-HPLC method using acetonitrile (ACN)-water mobile phase. In RP- HPLC method, acidic form (HA) and basic form (A-) of ionizable substances have different retention times. In the RP-HPLC method, the retention times of both species are expressed by the capacity factor (k') (Equation 2).

$$k' = \frac{t_r - t_0}{t_0} \quad (2)$$

In the equation, k' is the capacity factor, t_r is the retention time of the substance, and t_0 is the dead time.

Determining the pH value of the mobile phase is very important in pKa determination studies with HPLC. Different buffers and organic solvents (acetonitrile, methanol, etc.) or mixtures of these in mobile phases with certain ratios can be used for pKa determination in the HPLC method. In this case, the pH value of the aqueous phase and the pH value of the mobile phase with an

organic modifier may differ. For this reason, different pH scales in HPLC studies can be used. Although there are many different pH scales, three of them are widely used in pH scaling. The first of these is the aqueous pH scale ($^{w}_{w}pH$); In this scale, the electrode system is calibrated with aqueous buffers and pH is measured in the aqueous buffers before adding organic modifiers. The second case is the pH scale relative to the solvent ($^{s}_{w}pH$); In this scaling, the electrode system is calibrated with aqueous buffers, but the pH measurement is made in the mobile phase obtained by mixing the aqueous buffers with the organic modifier. In this case, the pH value will differ from the standard aqueous pH scale. the third is the pH scale in the mobile phase ($^{s}_{s}pH$); The electrode system is calibrated with buffers prepared in the same mixed solvent used in the mobile phase and pH measurement is performed in the mobile phase. In this case, the pH value differs from the standard aqueous pH scale and also differs from the pH scale depending on the state of the solvent [22].

Determination of pKa using the k' and pH values of the mobile phase in liquid chromatography was first suggested by Horvat [23]. Later, further studies were performed on different compounds to measure the pKa values from the capacity factors [24, 25]. In this study, pKa values of EP1, EP2, EP3 and EP4 pharmaceuticals were determined with the help of k'-pH curves.

To unravel the lipophilic nature of these compounds, we employed UV-VIS spectroscopic methods in conjunction with the classic bottle-shaking method using n-octanol as the lipophilic phase, adhering to established OECD guidelines [26]. This approach allowed us to determine the partition coefficient (logP), a standard measure of lipophilicity [27], and the more understandable partition coefficient (logD), providing a comprehensive picture of the behavior of molecules in a two-phase system [12].

Lipophilicity is the affinity of a molecule for a lipophilic medium. It is measured by the dispersion behavior in a two-phase system, usually a liquid-liquid or solid-liquid system [27]. Passively absorbed active substances must cross biological membranes before entering the bloodstream. Due to the lipid structure of the membranes, the drug's passage rate directly depends on the lipophilic structure of the active substance. The logP observed in the water-n-octanol system of a molecule is adopted as the standard measure of lipophilicity (Equation 3). Most drugs in biological systems have an ionic nature. Therefore, evaluating the lipophilicity of the ionic forms of drugs is more important than the lipophilicity of the molecular form. The lipophilicity of the ionic structure of a drug is defined by its logD. The distribution coefficient is the ratio of the sum of the concentrations of all compound species in octanol to the sum of the concentrations of all compound species in water (Equation 4).

$$LogP = Log\frac{[sum\ of\ the\ concentrations\ of\ neutral\ species]_{octanol}}{[sum\ of\ the\ concentrations\ of\ neutral\ species]_{wather}} \quad (3)$$

$$LogD = Log\frac{[sum\ of\ the\ concentrations\ of\ all\ microspecies]_{octanol}}{[sum\ of\ the\ concentrations\ of\ all\ microspecies]_{wather}} \quad (4)$$

A certain balance of lipophilicity and hydrophilicity is required for a successful drug candidate substance. The solubility of a drug in water is directly proportional to its solubility in plasma and other aqueous biological fluids. Still, lipophilicity is the ability of the substance to penetrate biological membranes and is crucial for all drug compounds. The log D is a widely used coefficient to measure the lipophilicity of ionizable compounds. For non-ionizing compounds, lipophilicity is expressed by logP, and the logP value is equal to the logD value for these compounds' overall pH ranges. For ionizable compounds, logD considers both ionized and molecular forms. The logP values of acidic molecules can be calculated according to the Equation 5 depending on the pH, logD and pKa values [12].

$$logP_{(pH)} = logD + log[1 + 10^{(pH-pK_a)}] \quad (5)$$

By meticulously analyzing the lipophilic profiles of these compounds across a wide pH range and quantifying their relationship using quadratic functions, we aim to gain a deeper understanding of their physicochemical properties and potential implications for drug development. This comprehensive analysis will provide crucial information for predicting the absorption, distribution, and ultimately, the antifungal efficacy of these promising drug candidates.

Our study marks the first comprehensive determination of pKa, logP, and logD values for these novels 3-(2-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione-5-acetic acid derivatives. The insights gained from our research will not only contribute to the growing body of knowledge surrounding this intriguing class of compounds but also pave the way for the development of potent and effective antifungal agents to combat the rising tide of fungal infections.

Material and Method

Chemicals and Reagents

Stock solutions (5000 ppm) of EP1, EP2, EP3, and EP4 were prepared in methanol and stored at 4°C. These stock solutions were used for all pKa, logD, and logP determination experiments. A 20 mM phosphate buffer was used throughout the study, with pH adjustments made using 0.1 M sodium hydroxide and 0.1 M hydrochloric acid solutions as needed. The calibrations of the pH electrode were made using aqueous buffers.

All reagents, including acetonitrile, methanol, octanol, sodium chloride, sodium dihydrogen phosphate, hydrochloric acid, and sodium hydroxide (Sigma-Aldrich), were used as received. All solutions were prepared using Milli-Q water (Barnstead Nanopure™ system).

Synthesis of EP1, EP2, EP3 and EP4 compounds

Following the synthesis procedure outlined by Ertan et al., four 3-(2-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione-5-acetic acid derivatives (EP1, EP2, EP3, and EP4, Figure 3) were prepared starting from phenylethylamine [4].

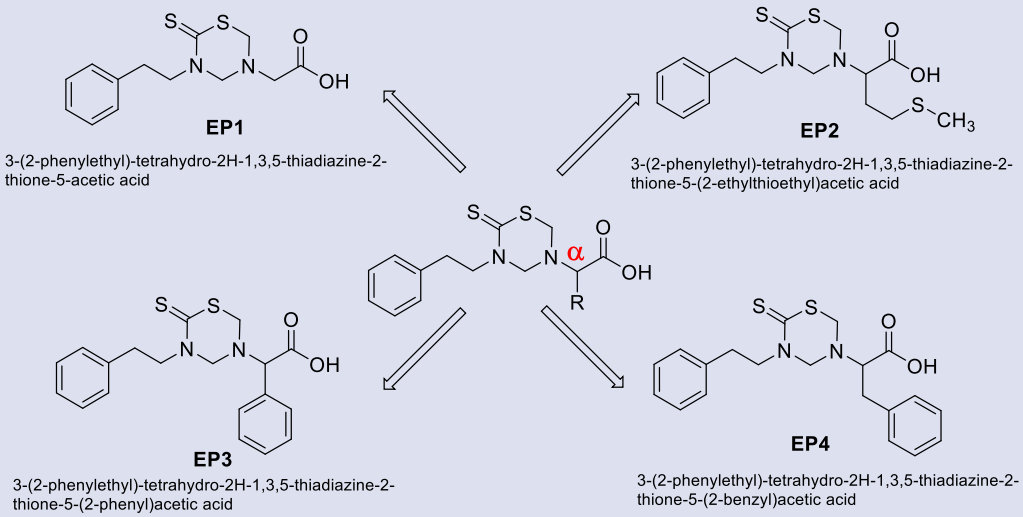

Figure 3. Structure and nomenclature of EP1, EP2, EP3 and EP4.

The synthesis involved reaction with carbon disulfide and potassium hydroxide to yield the potassium phenylethyl dithiocarbamate intermediate, followed by cyclocondensation with formaldehyde and the appropriate amino acids (Figure 4). This key step formed the thiadiazine ring and introduced the acetic acid moiety at position 5, a crucial structural element influencing the compounds' properties and potential antifungal activity. These compounds share a common core structure but differ in the substituent attached to the alpha (α) carbon of the acetic acid moiety, allowing for investigation into the impact of these subtle structural variations on their physicochemical properties and antifungal potential. This study aimed to comprehensively characterize these properties.

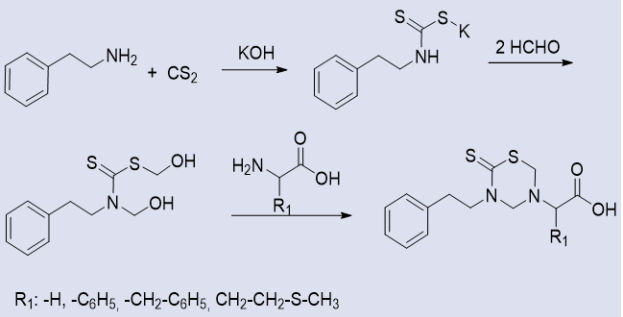

Figure 4. Synthesis reaction steps of 3-(2-phenylethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thion-5-acetic acid derivatives.

pKa Determination Using PARAFAC Analysis of UV Spectroscopic Data

UV spectra of the compounds were recorded for each compound at eleven pH levels (pH 2-12) using a Shimadzu UV-1280 UV/VIS spectrophotometer. Spectra were acquired in the range of 200-350 nm with a 0.1 nm interval and a scan speed of 100 nm/min. Buffer solutions were prepared and pH values were measured using a Thermo Fisher Orion Star 200 digital pH meter equipped with glass electrodes (uncertainty ±0.01 pH units).

UV spectra were collected using Shimadzu UVProbe 2.43 software and exported to Microsoft Excel. Three-way data arrays, consisting of pH, wavelength, and absorbance data, were constructed and processed in MATLAB R2023a using the N-way Toolbox [28].

The application of PARAFAC for spectrophotometric pKa determination involves a straightforward procedure focused on constructing and analyzing three-dimensional (3D) datasets.

I. UV absorbance spectra of analytes (EP1, EP2, EP3, EP4) were recorded at a concentration (15 ppm) at a selected pH level (n=3).

II. Spectral recordings were repeated for each pH level within the relevant pH range (pH 2-12) to capture the changes in absorbance associated with ionization.

III. The UV absorbance data matrices obtained at each pH level were combined to create a three-way data array with dimensions of absorbance x wavelength x pH.

This three-way data array serves as the input for PARAFAC analysis, enabling the deconvolution of the spectral data and accurately determining pKa values.

pKa Determination Using RP-HPLC

Acidity constants were also determined using an RP-HPLC (Agilent 1100) system equipped with a UV detector. Chromatographic separations were performed on an ACE-121-2546 C18 column (4.6 x 250 mm, 5 µm particle size).

The mobile phase consisted of 20 mM phosphate buffer (pH 2-12) (A) and acetonitrile (ACN) (B) in isocratic elution mode. In pKa determination studies using the HPLC method, the pH value of the mobile phase was adjusted after mixing ACN with buffer solution in appropriate proportions, and the measured pH ($_{w}^{s}pH$) values were used for pKa calculation. To ensure accurate and reliable measurements, buffer solutions were prepared using ultra-pure water and stored in capped bottles to prevent carbon dioxide intrusion.

The column temperature was maintained at 25°C, and the injection volume was 10 µL. UV detection was

performed at 283 nm. Data acquisition and processing were carried out using Agilent ChemStation for LC software (B.04.03-SP1). The column dead time (t_0) was 1.7 minutes, determined with methanol injection.

Capacity factors (k') were determined for each compound at various pH values (n=6). The relationship between k' and pH was analyzed using the Nonlinear Regression Shapes toolbox [29] in MATLAB. pKa values were determined from the second derivative of the resulting k'-pH curves.

To assess the robustness of retention time estimations, experiments were also conducted using different ACN:water ratios in the mobile phase (65:35, 70:30, and 75:25, v/v). For each pH value, three independent replicates were performed to ensure accurate determination of k' values.

Determination of Lipophilicity (logP and logD)

Lipophilicity was assessed by determining the logD using the classical shake-flask method with n-octanol, following OECD guidelines [26].

n-octanol and water were pre-saturated by shaking together for 24 hours. Aqueous phases were prepared using phosphate buffer, with pH adjustments (2.0-10.0) made using 0.1 M HCl or 0.1 M NaOH solutions. Ionic strength (μ) was maintained at 0.15 M using NaCl.

For each compound, 4 mL of the aqueous phase (at the desired pH) and 2 mL of n-octanol were combined and shaken for 1 hour. The samples were then centrifuged at 2000 g for 10 minutes to separate the phases.

Distribution coefficients (D) were determined using Equation 6:

$$D = \left(\frac{A_i - A_f}{A_f}\right) x \frac{V_w}{V_o} \qquad (6)$$

Where: A_i: UV absorbance of the aqueous phase before extraction, A_f: UV absorbance of the aqueous phase after extraction, V_w: Volume of the water-based layer, V_o: Volume of the n-octanol layer.

Each determination was performed in triplicate at each pH value. UV absorbance measurements were carried out using a Shimadzu UV-1280 spectrophotometer. All experiments were conducted at room temperature (22.3 ± 0.2°C). To account for spectral shifts due to pH, absorbance values were measured at 285 nm for pH 2-5 and at 290 nm for pH 5-10.

D values were calculated for each compound at pH 2-10 using Equation 6. LogD values were then plotted as a function of pH, and quadratic equations describing the relationship were derived using polynomial second-order regression in MATLAB. LogP values were calculated from the logD values using Equation 5.

Determination of pKa values is crucial to understand the behavior of drug candidates. In this study, pKa values of EP1, EP2, EP3 and EP4 with antifungal activity were determined using two independent methods (PARAFAC analysis of UV spectroscopic data and RP-HPLC).

Results and Discussion

Acidity Constant Determination via PARAFAC

The pH-dependent change in a three-way data set was analyzed to determine the pKa values of EP1, EP2, EP3, and EP4. UV absorbance spectra were recorded for each compound using the PARAFAC method. Spectra were obtained at eleven pH values (pH 2–12, ΔpH = 1) in the wavelength range of 200–340 nm and at a concentration of 15 ppm (Figure 5a–b). This process produced a series of 140 × 1 wavelength × absorbance data matrices for each of the eleven pH levels.

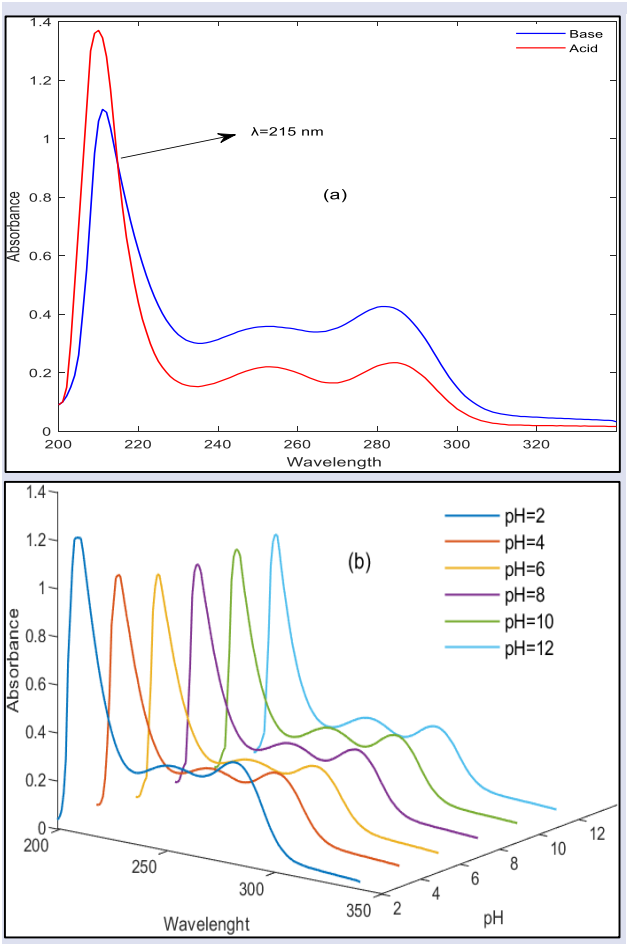

Figure 5. (a) UV spectrum (200-340 nm) of acidic and basic forms EP1 (15 ppm). (b) pH - absorbance - wavelength graph EP1 (15 ppm).

The eleven data matrices were then combined along the pH axis, resulting in a three-way data array (1 x 140 x 11 dimensions; absorbance x wavelength x pH) for analysis. This array was deconvoluted using the PARAFAC algorithm to differentiate the acidic and basic forms of the compounds. Both constrained (positive mode only) and unconstrained (positive and negative modes) PARAFAC models were evaluated. The unconstrained mode was selected for further analysis due to the negligible difference observed in the pH values at which the acidic and basic forms intersected between the two models.

Two-component PARAFAC modeling in the unconstrained mode, using CORCONDIA [30], yielded the optimal profiles for spectral, pH, and absorbance modes. The lowest CORCONDIA value obtained was 99.06% for all compounds using this two-component model. This result, coupled with the observation that the sum of the acidic and basic forms approximated unity, strongly suggests that the ionizable proton in these molecules is associated solely with the carboxylic acid group.

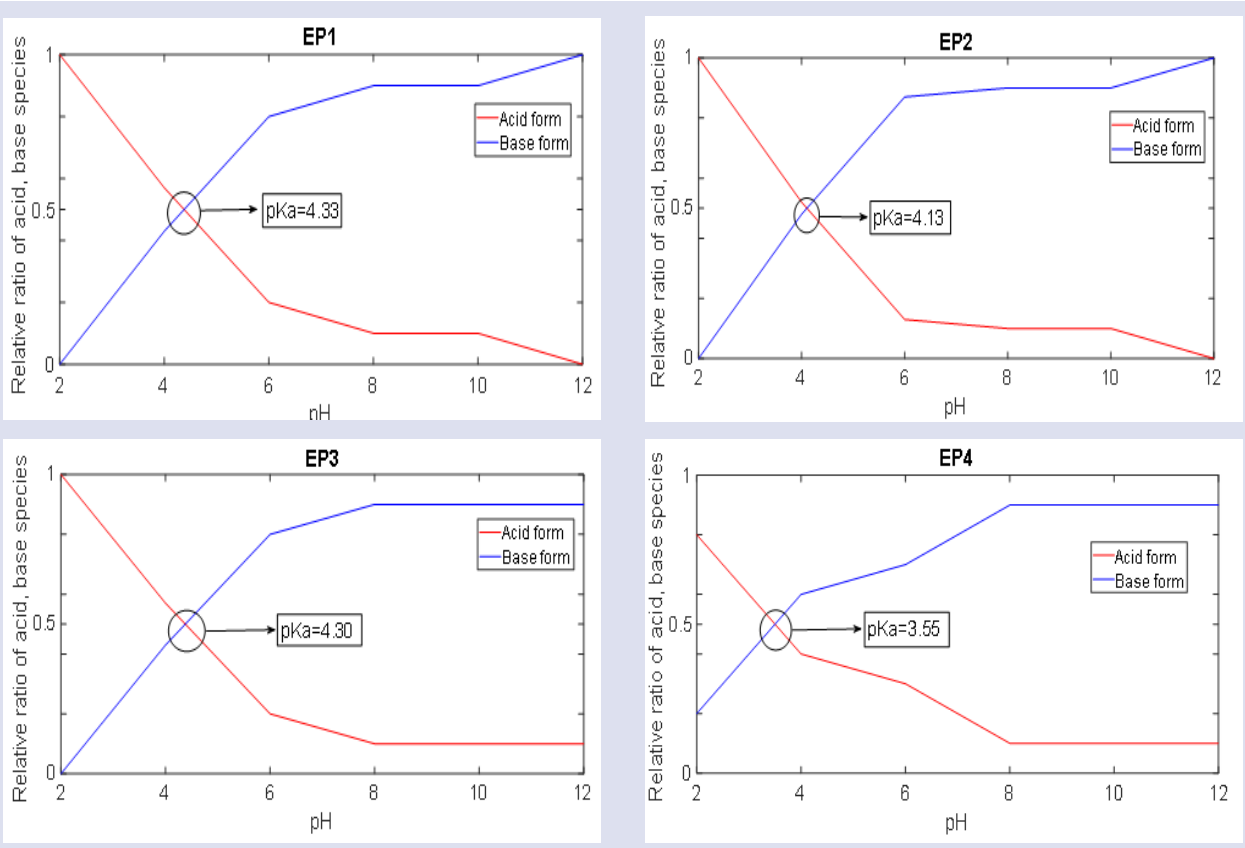

Figure 6. Estimated profiles for wavelength, pH, and concentration loadings of three-dimensional data in the PARAFAC deconvolution procedure.

Figure 6 displays the estimated profiles for absorbance, wavelength, and pH loadings resulting from the PARAFAC deconvolution. The red curves represent the relative amount of the compound in its acidic form, while the blue curves depict the relative amount in its basic form. The pKa value for each compound, representing the pH at which the acidic and basic forms are equal (intersection of the curves), is indicated on the graph (Equation 1). The pKa values determined using the PARAFAC method are presented in Table 1.

Isosbestic points in a UV absorption spectrum represent wavelengths where the absorbance of a molecule remains constant regardless of its ionization state (acid, base, or zwitterion). These points signify a direct relationship between the number of ionizable groups and the pKa values of the molecule. As the pKa value decreases, the corresponding isosbestic points generally shift to shorter wavelengths. The presence of a single isosbestic point at approximately 215 nm in the UV spectra of EP1, EP2, EP3, and EP4 suggests that each molecule possesses a single ionizable group with a relatively low pKa value.

Acidity Constant Determination via HPLC

The pKa values of EP1, EP2, EP3, and EP4 were also determined using RP-HPLC under various elution conditions. Retention factors were measured for each compound at different pH values and mobile phase compositions (ACN: buffer ratios of 65:35, 70:30, and 75:25 v/v).

Capacity factor (k') values were plotted as a function of pH, and the resulting curves were fitted using second-order nonlinear regression (Figure 7). pKa values were then determined from the second derivative of these fitted equations (Table 1). The RP-HPLC method yielded pKa values of 4.270 ± 0.121, 4.041 ± 0.280, 4.124 ± 0.319, and 3.332 ± 0.015 for EP1, EP2, EP3, and EP4, respectively. As shown in Figure 7, a strong correlation was observed between the experimental k' and the fitted curves across the entire pH range studied.

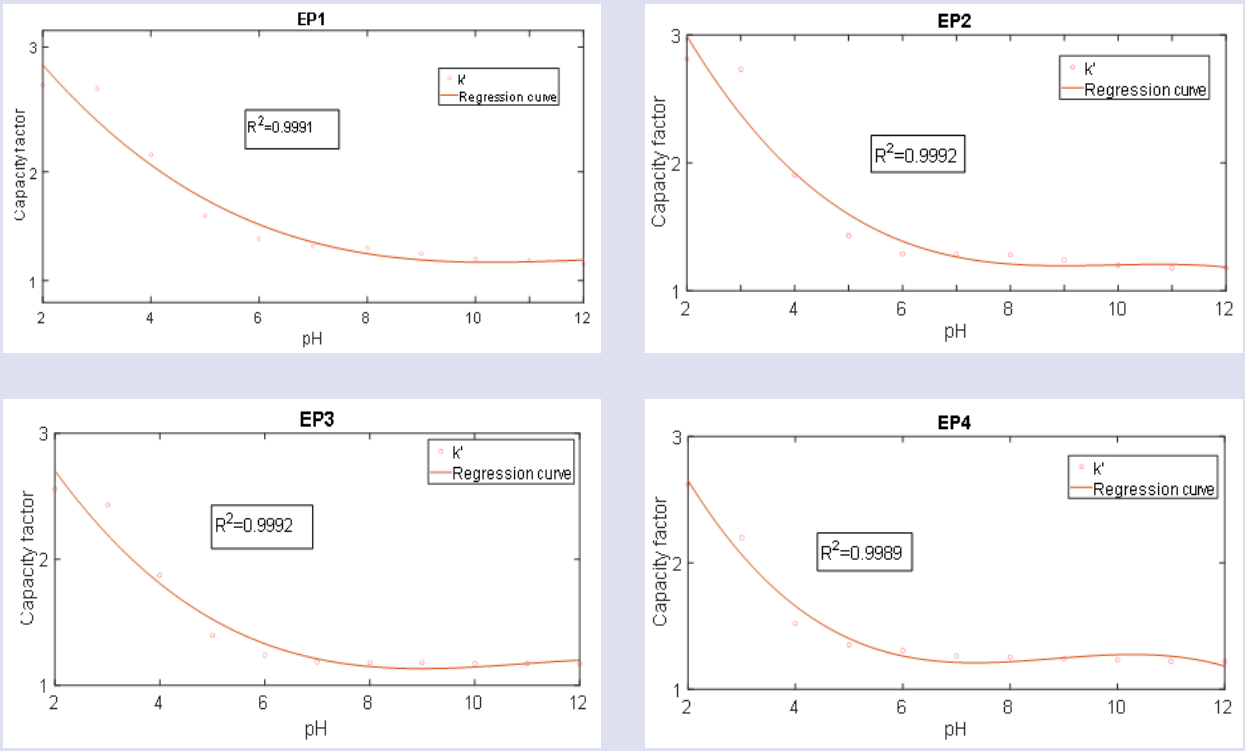

Figure 7. 2nd-degree nonlinear regression pH- k' curves of EP1, EP2, EP3 and EP4

Table 1. Experimental pKa Values for EP1, EP2, EP3 and EP4

	PARAFAC[a]	RP-HPLC[a,b]
EP1	4.332±0.016	4.270±0.121
EP2	4.137±0.030	4.041±0.280
EP3	4.305±0.029	4.124±0.319
EP4	3.510±0.022	3.332±0.015

[a] pKa ± standard error (SE)

[b] The average of the pKa values obtained in three different mobile phase compositions (ACN: buffer 65:35, 70:30, 75:25 v/v).

A thorough understanding of ionization mechanisms is crucial in pharmaceutical science, as a drug's pKa value significantly influences key properties such as solubility, absorption, distribution, excretion, and stability.

The molecules investigated in this study contain two tertiary nitrogen atoms within the thiadiazine ring, theoretically allowing for up to three distinct pKa values. However, only a single pKa value was detected using both PARAFAC and RP-HPLC methods. This discrepancy might be attributed to the high reactivity of tertiary amines. Literature suggests that pKa values associated with cyclic tertiary nitrogen atoms typically fall within the range of 9-11 [33]. It is plausible that buffer components, interacting with various species formed within this pH range, could suppress the ionization of these nitrogen atoms during the experimental procedures.

The pKa values of carboxylic acids are influenced by the inductive effects of substituents attached to the α-carbon. Electron-withdrawing groups (-I effect) decrease the pKa value, while electron-donating groups (+I effect) increase it. In this study, the experimentally determined pKa values were evaluated in light of the inductive effects exerted by the substituents on the α-carbon. Based on inductive effects alone, the following trends were expected:

The predicted pKa values for EP2, EP3, and EP4 were influenced by the electron-withdrawing nature of their respective α-carbon substituents. The strongly electron-withdrawing sulfur atom in the 2-methylthioethyl group of EP2 suggested a lower pKa than both EP1 and EP3. Similarly, the phenyl ring of EP3, being more electron-withdrawing than a hydrogen atom, was expected to result in a lower pKa than EP1. Finally, the benzyl group of EP4, exerting both inductive and resonance electron-withdrawing effects, was anticipated to yield the lowest pKa among all the compounds.

The experimentally observed pKa values were consistent with these predictions based on inductive effects, supporting the influence of substituent electronics on the acidity of these compounds.

In our previous study, the antifungal activity of EP1, EP2, EP3, and EP4 against C. albicans, C. parapsilosis, C. stellatoidea, and C. pseudotropicalis, minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were determined [4]. Comparing the pKa values determined in this study with the previously reported antifungal activities revealed a trend of lower average activity for compounds with pKa values between 4.0 and 4.3. Interestingly, EP2 and EP4, with pKa values outside of this range, exhibited approximately 30% higher average activity. While a statistically significant difference in activity was not observed, these preliminary

findings highlight the potential influence of pKa on antifungal efficacy and warrant further investigation. More comprehensive activity studies are needed to fully elucidate the relationship between pKa and antifungal activity for these compounds.

Distribution and Partition Coefficients

Following the OECD guidelines, as previously outlined, the distribution coefficients (D) of EP1, EP2, EP3, and EP4 were determined across a range of pH values (Table 2). The minimum logD value was observed at pH 6.0 for EP1 and EP2, and at pH 5.0 for EP3 and EP4.

The relationship between logD and pH was further analyzed by fitting the data to quadratic equations. The resulting equations and corresponding graphical representations are presented in Figure 8, demonstrating the pH-dependent lipophilicity profiles of each compound.

Table 2 logD experimental values for EP1, EP2, EP3 and EP4.

pH	EP1 [c]	EP2 [c]	EP3 [c]	EP4 [c]
2	2.250±0.010	0.758±0.782	-0.792±0.185	0.348±0.151
3	2.081±0.814	0.405±0.103	-1.213±0.936	0.247±0.253
4	1.913±0.665	0.057±0.574	-1.646±0.689	0.135±0.835
5	1.717±0.129	-0.292±0.823	-2.119±0.743	-0.001±0.929
6	1.706±0.050	-0.496±0.828	-2.094±0.247	-0.033±0.142
7	1.882±0.998	-0.261±0.470	-1.000±0.117	0.036±0.871
8	2.252±0.137	0.107±0.518	0.584±0.303	0.208±0.521
9	2.631±0.781	0.794±0.015	1.641±0.593	0.476±0.883
10	3.034±0.545	1.452±0.313	2.695±0.846	0.752±0.366

[c] logD±SE: Standard error

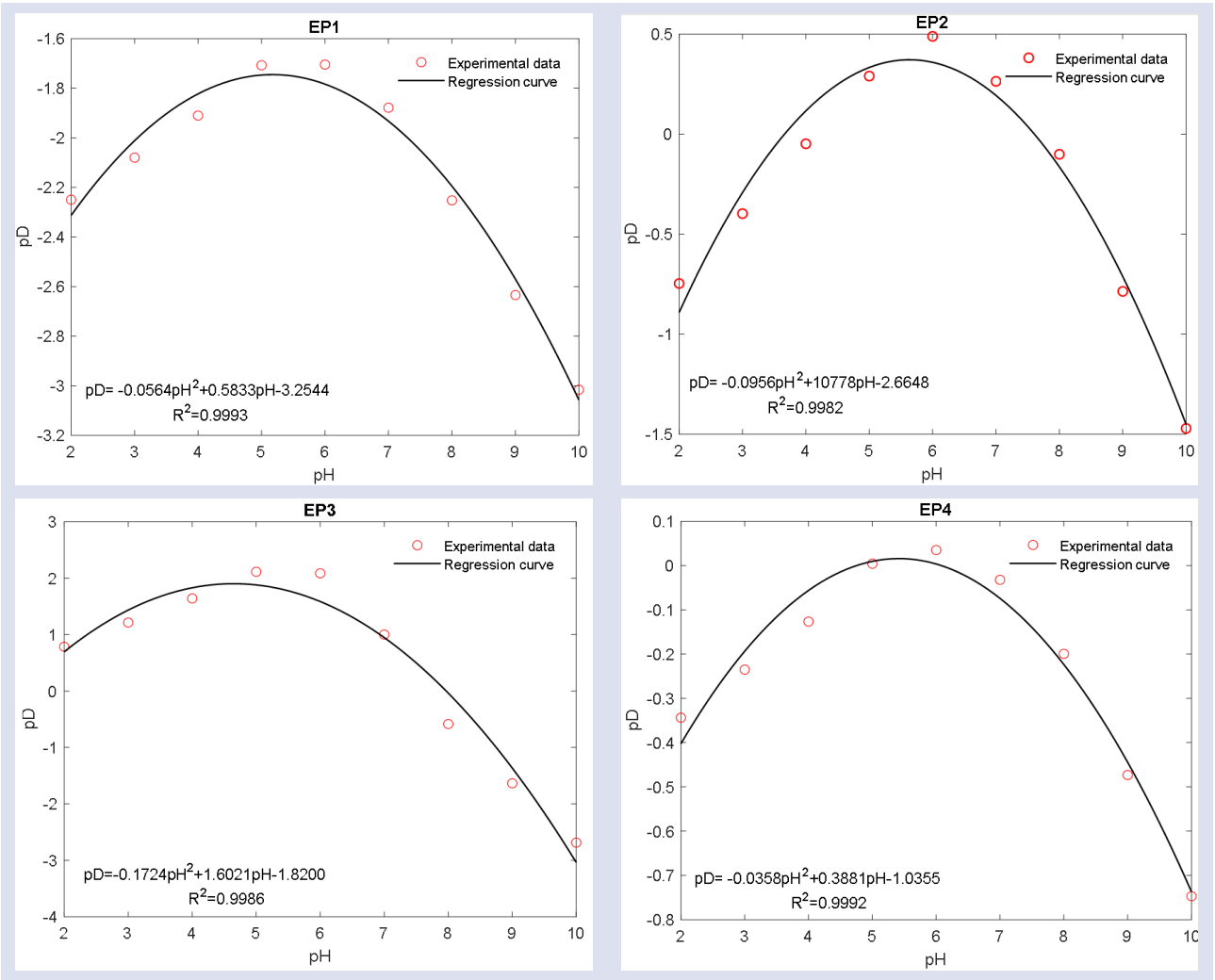

Figure 8. The logD - pH profiles and equations for EP1, EP2, EP3 and EP4.

To determine the logP values representing the neutral (molecular) forms of the compounds, the measured logD values were corrected for the relative proportions of ionized species at each pH using the pKa values [31, 32]. This correction is crucial because logD reflects the overall distribution of both ionized and non-ionized forms, while logP specifically describes the partitioning of the neutral form. The calculated logP values were as follows: EP1 (3.00), EP2 (1.10), EP3 (-0.38), and EP4 (2.19).

The efficacy of antifungal drugs and their ability to reach target tissues are closely related to the logD partition coefficient, an important parameter reflecting

both water and lipid solubility of the drug molecule. The curves in the logD-pH profiles in Figure 8 showed a conical parabolic shape, which is typical for each analyzed compound in all four cases. However, the shape of the EP3's curve is comparatively less sharp than the others. This means that the logD of EP3 is less pH dependent than other substances. When highly acidic or basic conditions are evaluated, it can be said that logD values are higher in extremely acidic conditions.

LogP is a key parameter for predicting the in vivo absorption of a drug. Compounds with logP values between 1 and 3 typically exhibit good absorption. In this study, EP1, EP2, and EP4, with logP values above 1, are predicted to have favorable absorption profiles. A logP value below 1 generally indicates a hydrophilic compound, while a value above 1 suggests lipophilicity. Thus, EP1, EP2, and EP4 can be classified as lipophilic, while EP3, with a logP below 1, is hydrophilic.

Conclusion

This study determined the pKa values of several 2-(5-phenethyl-6-thioxo-1,3,5-thiadiazinan-3-yl) acetic acid derivatives for the first time, employing both UV spectroscopy and RP-HPLC. The results strongly suggest that proton removal occurs primarily from the carboxyl group, as illustrated by the proposed protonation reaction in Figure 4.

Accurate pKa determination relies on meticulous experimental techniques. For both UV spectroscopy and RP-HPLC, using freshly prepared buffer solutions tailored to the specific pH range is crucial. In PARAFAC analysis, careful selection of the appropriate number of components is critical, as even a single component deviation can result in a pKa error of approximately ±25%. Furthermore, analyzing absorbance data within a wavelength range sensitive to pH-dependent changes enhances the sensitivity of PARAFAC analysis.

While both PARAFAC and RP-HPLC proved effective for pKa determination, the UV spectroscopy-assisted PARAFAC approach offered a significant time advantage over the longer elution times associated with RP-HPLC. These findings underscore the utility of both methods, particularly the PARAFAC approach, for accurate and efficient pKa determination.

The logD-pH profiles for all analyzed compounds exhibited a characteristic bell-shaped curve. The highest logD value for each compound was observed at a pH where approximately 50% of the molecules were in their ionized form, a point closely aligned with their respective pKa values. LogP values, calculated from the logD data, indicated that EP1, EP2, and EP4 possess favorable lipophilicity profiles for drug development. However, EP3, with a lower logP value, suggests higher hydrophilicity and potentially lower stability in acidic environments compared to the other compounds.

The accurate pKa values established in this work may be leveraged to systematically explore how modifications to the α-carbon substituent or other regions of the core scaffold impact both the physicochemical properties and the antifungal efficacy of these derivatives in future studies. These findings can then guide in vivo studies using relevant animal models to evaluate the pharmacokinetic behavior of these compounds.

The pKa and logP/logD data generated in this study provide valuable parameters that will help develop optimal pharmaceutical dosage forms. These formulations should aim to enhance solubility, stability, and targeted delivery to sites of fungal infection. For example, pro-drug strategies that leverage pH-dependent ionization could be beneficial for EP3 because of its relatively low lipophilicity. Such strategies could potentially improve the absorption, distribution, and overall pharmacokinetic profile of this compound.

Employing computational modeling techniques, researchers can further investigate the relationship between the physicochemical properties, 3D structures, and potential interactions of these compounds with their molecular targets within fungal cells. This approach could provide valuable insights into their mechanism of action and guide further structural optimization efforts.

By pursuing these research avenues, a more comprehensive understanding of this promising class of antifungal agents can be achieved, potentially leading to the development of new and effective treatments for invasive fungal infections.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Ethical Approval Statement

Ethical approval was not required for this study, as it involved only chemical synthesis, physicochemical characterization, and in silico analysis, with all procedures adhering to standard laboratory practices.

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