HHS Public Access

Author manuscript

J Chem Inf Model. Author manuscript; available in PMC 2020 June 11.

Published in final edited form as:

J Chem Inf Model. 2009 December ; 49(12): 2801–2812. doi:10.1021/ci900289x.

Comparison of Nine Programs Predicting pK_a Values of Pharmaceutical Substances

Chenzhong Liao, **Marc C. Nicklaus***

Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, DHHS, NCI-Frederick, 376 Boyles St., Frederick, Maryland 21702

Abstract

Knowledge of the possible ionization states of a pharmaceutical substance, embodied in the pK_a values (logarithm of the acid dissociation constant), is vital for understanding many properties essential to drug development. We compare nine commercially available or free programs for predicting ionization constants. Eight of these programs are based on empirical methods: ACD/pK_a DB 12.0, ADME Boxes 4.9, ADMET Predictor 3.0, Epik 1.6, Marvin 5.1.4, Pallas pKalc Net 2.0, Pipeline Pilot 5.0, and SPARC 4.2; one program is based on a quantum chemical method: Jaguar 7.5. We compared their performances by applying them to 197 pharmaceutical substances with 261 carefully determined and highly reliable experimental pK_a values from a literature source. The programs ADME Boxes 4.9, ACD/pK_a DB 12.0, and SPARC 4.2 ranked as the top three with mean absolute deviations of 0.389, 0.478, and 0.651 and r^2 values of 0.944, 0.908, and 0.894, respectively. ACD/pK_a DB 12.0 predicted all sites, whereas ADME Boxes 4.9 and SPARC 4.2 failed to predict 5 and 18 sites, respectively. The performance of the quantum chemical-based program Jaguar 7.5 was not as expected, with a mean absolute deviation of 1.283 and an r^2 value of 0.579, indicating the potential for further development of this type of approach to pK_a prediction.

INTRODUCTION

Most pharmaceutical substances will be protonated or deprotonated in aqueous solution; for example, data from the 1999 World Drug Index suggest that 63% of the 51600 listed drugs are ionizable, of which 15% are acids, 67% bases, and 18% ampholytes.[1] The ionization ability is quantified by a parameter, the (logarithm of the) acid ionization constant (pK_a), which is also called the protonation constant, equilibrium constant, or (acid) dissociation constant in the literature. Along with the partition coefficient, solubility, and reaction rate, pK_a is the most important physicochemical property of a substance to be formulated into a useful medicine. As a function of its intrinsic pK_a value(s) and the pH value of the solution, the extent of ionization of a drug controls its solubility, dissolution rate, and consequently

*Corresponding author phone: +1 301 846 5903; fax: +1 301 846 6033; mn1@helix.nih.gov.

Note Added after ASAP Publication. This paper was published ASAP on December 4, 2009 with an incorrect version of Figure 1. The corrected version was published ASAP on December 9, 2009.

Supporting Information **Available:** The data set used in this study and calculation results. This material is available free of charge via the Internet at http://pubs.acs.org.

has great impact on gastrointestinal uptake into the bloodstream, distribution, cell permeability, drug–receptor binding, reaction kinetics, metabolism, elimination, etc.[2,3] In the preformulation stage, knowledge of ionization constants is useful when trying to form a salt in order to obtain biopharmaceutical properties and solid-state characteristics that may be lacking in the free form of the compound.

pK_a values can be either measured or calculated. There are a number of methods to use for the experimental determination of pK_a values and closely related quantities such as pH values.[4–6] These methods have been used extensively in drug discovery and various development stages in the pharmaceutical industry. The highly accurate measurement methods include conductance methods (reliable to ±0.0001 pK units or better) and electrochemical cells without liquid junction potentials (reliable to ±0.001 pK units or better), both of which, unfortunately, have been rarely used to measure ionization data for pharmaceutically relevant organic acids and bases. For this class of substances, most pK_a measurements are based on relationships between the measured solution pH and a measured physicochemical quantity such as added titrant concentration, solubility, etc., which limits the expected accuracy. Measurement of pK_a values has become easier and more convenient over recent years. Nevertheless, in early drug discovery, measuring millions of compounds in large screening libraries is costly and simply not practical, and outright impossible for virtual libraries, which makes in silico prediction of pK_a values vital in modern drug discovery.

At present, standard methods for pK_a prediction for pharmaceutical substances can be classified into two major groups: empirical methods and quantum chemical methods. On the basis of the detailed approach used, the empirical methods can be further divided into three groups: (1) linear free-energy relationships (LFER), methods utilizing the empirical relations of Hammett and Taft, (2) quantitative structure–property relationships (QSPR), methods correlating calculated structural descriptors with pK_a values, and (3) database lookup, i.e., methods searching of similar structures in a predetermined database of molecules with known measured pK_a values.[7] One of the strengths of the empirical methods is their high speed, useful when processing large databases of drug-like molecules. The alternative to empirical methods, quantum chemical methods, are supposed to have higher accuracy because they are based on, or closer to, first principles when calculating quantum mechanical descriptors. However, these methods are much more time-consuming than empirical methods.

While a significant number of recent publications can be found reporting on new and better programs and methods for pK_a prediction (see refs 8–16 and 17–21 for additional approaches using empirical or quantum chemical methods, respectively), albeit mostly for specific classes of compounds, much less prior work exists comparing such approaches. Melun et al. used the REGDIA regression diagnostics algorithm in S-Plus to examine the accuracy of pK_a predictions of four programs: ACD/pK_a, Marvin, PALLAS, and SPARC.[22] Three different validation data sets were taken from literature, including 64 pK_a values for different drugs. We therefore felt there was the need to not only compare a larger number of available programs but also use more pK_a values for pharmaceutical substances in order to cover as much as possible of the drug-like chemical space. In this article, we use eight

empirical programs and one quantum chemical program (Table 1) to predict 261 carefully determined pK_a values of 197 pharmaceutical substances in order to compare the predictive power of the nine programs.

While we have tried to bring together as many of the existing programs that have some usage, we do not claim that we have tested each and every code that predicts pK_a values. One program, for example, that we are aware of but which was not included is MoKa,[13] marketed by Molecular Discovery, Ltd.[35] Unfortunately, a mutually satisfactory agreement to gain access to the program for the purpose of this study could not be reached with the company.

DATA SET

Significant numbers of experimental pK_a data on aqueous ionization chemicals have been collected over the decades. Some of them have found their way into literature compilations[36–40] which, however, are not focused on pharmaceutical substances, although they do include some such compounds. In 2007, a book titled *Profiles of Drug Substances, Excipients, and Related Methodology: Critical Compilation of pK_a Values for Pharmaceutical Substances* was published.[41] The author of this book systematically collected nearly 3500 reported pK_a values for drugs and related compounds from the pertinent primary and secondary literature and then, using the IUPAC classification and guidelines given below, assessed the reliability of these reported pK_a values. On the basis of the aspects of a pK_a measurement, such as the experimental method, mathematical definition selected to calculate the value from the raw data, and degree to which technical refinements have been applied, the IUPAC established in the 1960s the criteria for its compilations of dissociation constants for weak organic acids and bases: *very reliable* (VR; pK_a error < ±0.005), *reliable* (R; pK_a error ±0.005 to ±0.02), *approximate* (A; pK_a error ±0.02 to ±0.04), and *uncertain* (U; pK_a, error > ±0.04).[36,37] The author of this book set the cutoff for *uncertain* to > ±0.06 pK_a unit. On the basis of this modified IUPAC criteria, about 74% of the collected pK_a, values in this book were found to be of *uncertain* quality, whereas only 0.1% qualified as *very reliable* and 0.33% qualified as *reliable*, which left ~25% being classified as *approximate*. The compilation has two sections: Appendix A comprises pK_a values for which the measurements were sufficiently well described for the data to be assessed for reliability, and Appendix B comprises those pK_a values for which little reliability data could be assessed, which were mostly from the secondary literature.

For this project, the pK_a values used to compare the abilities of the nine programs to predict pK_a values for the nine programs were chosen according to the following criteria: (1) Only pK_a values that came from Appendix A of the above-mentioned reference were chosen. (2) The data qualities had to be VR, R, or A. (3) The solvent contained only water except some possible inorganic ions. (4) The temperature at which the measurement was taken was in the range of 25 ± 2 °C (except for compound **134**, measured at 20 °C, which was included because otherwise we would have lost the only thiol in the final set). These quite rigorous criteria finally left us with 261 pK_a values measured for 197 compounds whose structures are shown in Figure 1. The molecular weight and pK_a value distributions and numbers of acidic and basic sites of these compounds are shown in Figure 2 and Table 2, respectively,

both of which demonstrate that, as far as chemical species, number, and value distribution are concerned, this data set can be considered high-quality for the purpose of this comparison. A cautionary note is warranted for compound **99**, which may be capable of forming aggregates[42,43] at high concentration because this compound likely has low solubility in water as a monomer. This might then affect the measured pK_a values. Because firm evidence exists neither for nor against aggregation, however, we decided to keep it in our compound set (hoping that the authors of the original paper were aware of this issue and performed the necessary procedures to rule it out or prevent it).

It is worthwhile to point out that although this data set has dozens of multiprotic molecules, not all of the acidic and basic sites of every multiprotic molecule were predicted in this comparison. The main reason is that the book does not note every pK_a value of every multiprotic molecule or that the data quality of some of these sites is below A.

It should also be pointed out and kept in mind by the reader, that these programs tend to be trained with as much of the data available in the literature as possible. Testing these programs using exclusively information from the literature, as we unavoidably had to do for this study, therefore entails the risk that the programs may effectively "look up" known pK_a values rather than predict them. It would therefore not be a wrong strategy for the serious user to test any of these programs themselves using unpublished or private pK_a data if possible.

COMPUTATIONAL METHODS

All eight programs based on empirical methods were executed one compound at a time or in batch mode. Some programs such as ACD/pK_a, DB, ADME Boxes, Epik, Marvin, and SPARC can calculate different kinds of pK_a values. For this study, only the pK_a types that were designated as predicting experimental pK_a values were calculated. ACD/pK_a DB 12.00, ADME Boxes 4.9, ADMET Predictor 3.0, Marvin 5.1.4, Pallas pKalc Net 2.0, and Pipeline Pilot 5.0 were run using the default options in their respective graphical user interfaces on a Windows XP computer. SPARC 4.2 was run via a web-based interface (IE 7.0) on a Windows XP machine. Epik 1.6 was run via command line on a Linux system.

pK_a values are in direct proportion to $\Delta G°$, the free energy change for transition from the protonated state to the deprotonated state. A small calculation error of $\Delta G°$ (on the order of a few kcal/mol) can therefore lead to a significant prediction error for pK_a for programs that are based on quantum–chemical methods. To correct for this deficiency that affects the quantum chemistry-based program Jaguar, this program employs two additional empirical parameters, scaling and additive factors.

pK_a predictions of Jaguar 7.5 consist of a series of calculations on the protonated and deprotonated forms of the target molecule, followed by the aforementioned empirical correction. Because the calculated results partly depend on the conformation of the target molecule, first a conformational search was performed with MacroModel.[44] As recoimnended by Schrödinger, the lowest-energy conformers found were used for further pK_a calculations. Jaguar calculates microscopic (atomic) pK_a values but not macroscopic

(experimental) pK_a values. If two or more microscopic pK_a values lie within one pK_a unit of each other, the macroscopic pK_a values can markedly differ from the corresponding microscopic values. Therefore, for such a multiprotic molecule, in order to obtain the macroscopic pK_a values, Schrödinger suggests in the Jaguar manual to run 2^n states (n being the number of close pK_a values in a multiprotic molecule) and then to assemble the titration curve. Given that each QM computation takes already orders of magnitude longer than the corresponding empirical calculation, this would have heavily increased the amount of calculation necessary for the project while creating hard-to-assess additional sources of potential error. We therefore decided to drop the calculation of such protonation sites. We set the cutoff here for two experimental pK_a values in a multiprotic molecule being too close to each other if their difference was less than or equal to 2.5 pK_a units. This led to a reduction of the number of calculated sites from 261 to 204. For some kinds of acidic or basic sites, Jaguar 7.5 does not have parameters for an aqueous solution, which led to another 11 sites being dropped to yield the final number of 193 sites included in the calculations. For those multiprotic molecules whose experimental pK_a values are well separated (by >2.5 pK_a units), when calculating the lowest pK_a value, the sites with higher pK_a values were in the protonated states; when calculating the middle pK_a value, the sites with higher and lower pK_a values were in the protonated and deprotonated states, respectively; and when calculating the highest pK_a value, the sites with lower pK_a values were in the deprotonated states.

Among the 197 compounds, some molecules, for example, compounds **79**, **128**, and **164**, have two equivalent sites for protonation or deprotonation. In this situation, the need for a statistical correction factor arises from the increased entropy of the appropriate species. A correction of +0.60 ($\log_{10}2^2$) or −0.60 was added by hand to the result obtained from running the pK_a prediction module on the basis of whether the calculated molecule has two equivalent acidic sites or basic sites because Jaguar 7.5 does not automatically recognize equivalent sites.

RESULTS AND DISCUSSION

Overview.

Execution Speeds.—Program execution was very fast for all programs based on empirical methods except SPARC 4.2. For example, ADMET Predictor 3.0 and Pipeline Pilot 5.0 finished the calculation of predicting the pK_a values of these 197 pharmaceutical substances in less than 1 s on a Windows computer. Epik 1.6 took 119 s on one CPU (AMD 64, dual core FX-61) of our Linux cluster. It is therefore possible to predict pK_a values of millions of compounds in a tractable time by using these seven programs. The calculation speed of SPARC 4.2, because of its use of the PMO theory, was much slower than that of the other seven programs. For example, submission of compound **74**, which has five protonation sites, resulted in the following message: “This will result in ~5120 calculations and may take as long as 51.87 minutes”. In fact, the server did not produce any result for this compound but an error message: “The request has exceeded the allowable time limit Tag”. Yet for simpler compounds with one or two protonation sites, the calculation time was acceptable.

One-by-one submission and longer execution times make SPARC 4.2 not suitable for predicting pK_a values of large number of compounds.

The pK_a prediction of Jaguar 7.5 was very time-consuming, and it was strongly dependent on the size and flexibility of the molecule. For examples, on the above-mentioned AMD 64, dual core FX-61 CPU, it took about 4, 23, 96, 202, 1568, and 3831 min, respectively, to predict pK_a values of the carboxylic acid sites in compounds **3** and **148**, the barbituric acid site in compound **36**, the pyridine site in compound **145**, the carboxylic acid site in compound **104**, and the tertiary amine site in compound **116**. The molecular weights of these six compounds in the same order are 46.03, 123.11, 226.28, 282.22, 573.67, and 592.69, respectively. After more than three days of computation, Jaguar 7.5 failed for an unknown reason (but presumably due to resource exhaustion) in the prediction of the primary amine site of compound **99**, which is the largest and most flexible molecule in the test set with a molecular weight of 744.05. With such slow calculation speeds, we can conclude that Jaguar 7.5 is not a practical solution to handle many compounds, especially when they are large, flexible, and/or multiprotic with close pK_a values.

Prediction Results.—The prediction results for the 261 pK_a protonation sites are shown in Table S1 of the Supporting Information and Figure 3 and summarized in Table 3 and Figure 4. Only ACD/pK_a DB 12.0, ADMET Predictor 3.0, and Marvin 5.1.4 predicted all 261 protonation sites; ADME Boxes 4.9, Epik 1.6, Pallas pKalc Net 2.0, Pipeline Pilot 5.0 and SPARC 4.2 failed for 5, 4, 2, 9, and 18 sites, respectively. Jaguar 7.5 failed for 11 sites with the error message “No pK_a functional group with parameters for water could be identified”.

When based on mean absolute deviation (MAD), the rank order of this comparison is ADME Boxes 4.9, ACD/pK_a DB 12.0, SPARC 4.2, ADMET Predictor 3.0, Pipeline Pilot 5.0, Pallas pKalc Net 2.0, Marvin 5.1.4, Epik 1.6, and Jaguar 7.5. In terms of r^2, the situation changes a little, but the top three are still ADME Boxes 4.9, ACD/pK_a DB 12.0, and SPARC 4.2. It might be argued that this criteria is actually unfair for the three programs that predicted all protonation sites.

When predicting pK_a values as part of the drug discovery process, researchers may typically be interested mostly in the protonation state at physiological pH, i.e., 7.4. Table 4 shows the performance of these nine programs, specifically for those 116 sites whose measured pK_a values are in the range of 5.4–9.4. In general, on the basis of the mean absolute deviation, most programs performed worse in this range than for the full set of sites, except Jaguar 7.5 and Pallas pKalc Net 2.0. The top three performers in terms of MAD were ADME Boxes 4.9, ACD/pK_a DB 12.00, and Pallas pKalc Net 2.0; and ADME Boxes 4.9, ACD/pK_a DB 12.00, and SPARC 4.2 in terms of r^2.

About thirty of the compounds were consistently predicted poorly, i.e., more than six of the programs predicted them with an deviation of at least 0.5 log units or even failed to predict one or more sites of them altogether: **6**, **9**, **52**, **53**, **60**, **62**, **67**–**69**, **74**–**76**, **79**, **85**, **99**, **102**, **110**, **119**, **121**, **128**, **129**, **134**, **144**, **155**, **156**, **174**, **175**, **178**, and **188**–**190**. It is not clear

whether this points to a general weakness in the understanding and/or algorithms in the field for these molecules or if this may indicate potential problems with the experimental results.

ACD/pK_a DB 12.0.

AC D/pK_a DB 12.0 ranks second in this comparison with an MAD of 0.478 and an r^2 of 0.908. A strong point of ACD/pK_a DB was that it calculated all 261 protonation sites. It produced 64 (24.52%) and 185 (70.88%) predicted values with accuracies of ±0.1 and ±0.5 log unit, respectively. This makes it a well-built and robust program for the prediction of ionization states of pharmaceutical substances. Nevertheless, there are still 10 sites (3.83%) whose predicted accuracies were more than 2.0. The two largest deviations are 7.07 and −4.05 log units, which occurred on the general C substituted amide site of compound **6** and the substituted aniline site of compound **93**, respectively. For the tertiary amine sites of the three tetracycline antibiotics (compounds **74**–**76**), the prediction errors are significant: all of them are around +3.50 log units. The other very poorly predicted sites include the primary amine sites of three penicilloic acids (compounds **67**–**69**), the phenol site of ebifuramin (compound **102**), and the benzodiazepine site of compound **156**.

ADME Boxes 4.9.

This program ranks first if one only focuses on the MAD (0.389) or r^2 (0.944) values. However, it failed to predict five protonation sites: one enol site of compound **23** (another enol site was successfully predicted), one acid site of compound **60** (which actually is an inorganic acid), the substituted aniline site of compound **93**, and the two heterocycle acid sites of compounds **118** and **119**. Apart from these cases, ADME Box 4.9 predicted normal organic compounds very well: it produced 95 (37.11%) and 197 (76.95%) predicted values with accuracies of ±0.1 and ±0.5 log unit, respectively. Each of these two values is at the top of its list for the nine programs. There are seven sites (2.73%) whose prediction accuracies were worse than 2.0 log units, which also represents the top spot among the nine compared programs. The largest deviation was −3.5, which occurred for one of the two acid sites of carbonic acid (compound **62**). The other very poorly predicted six sites include the three phenol sites of the three tetracycline antibiotic compounds **74** – **76**, which all were predicted higher than experimentally measured, one tertiary amine site of the antipsychotic compound **85**, the phenol site of compound **102**, and one carboxylic acid site of compound **128**.

ADMET Predictor 3.0.

This program ranks fourth with a MAD of 0.659 and an r^2 of 0.837. It also is one of the three programs that did not fail to predict even one site. It gave 40 (15.33%) and 159 (60.92%) predicted values with accuracies of ±0.1 and ±0.5 log unit, respectively. There were 18 sites (6.90%) whose predicted accuracies were more than 2.0 log units. The calculated largest deviation is −7.86, which occurred for compound **60**. Besides this site, there are 17 additional rather poorly predicted sites: both of the two acid sites of carbonic acid, compound **62**; the three primary amine sites of the three penicilloic acids (compounds **67**–**69**); the three enol sites, and the three tertiary amine sites of compounds **74**–**76** (whereas the three phenol sites of these three compounds were predicted quite well, with absolute deviations between 0.24 and 0.79); the substituted aniline site of compound **93**; the thiol site

of compound **134**; the pyridine site of compound **145**; the primary amine sites of compounds **155** and the **175**; and the heterocycle acid site of compound **190**.

Epik 1.6.

The r^2 and MAD values of this program were 0.802 and 0.893, respectively, which represents the sixth and last rank, respectively, among the eight programs utilizing empirical methods. The prediction of two protonation sites, the acid site of the inorganic acid compound **60**, and the thiol site of compound **134** were not completed by this program. The enol sites of compounds **75** and **102** were tautomerized into carbonyl groups, which are not protonation sites (it is interesting to note that the enol sites of compounds **74** and **76** were not tautomerized in the pK_a prediction process by the program, although the structures of the compounds **74**–**76** are almost identical to each other). All in all, Epik predicted 257 sites and failed for four sites. It produced 30 (11.67%) and 117 (45.52%) predicted values with accuracies of ±0.1 and ±0.5 log unit, respectively. There were 23 sites (8.95%) whose predicted accuracies were off by more than 2.0 log units. The largest deviation was 6.26, which occurred for the CH acid site of compound **157**. The other very poorly predicted protonation sites comprised both of the two acid sites of carbonic acid, compound **62**; the three primary amine sites of the three penicilloic acids (compounds **67**–**69**); the three guanidine sites of compounds **70**–**72**; the two tertiary amine sites of compounds **74** and **76**; the phenol sites of compounds **81, 144**, and **176**; the tertiary amine site of compound **88**; the substituted aniline site of compound **93**; the primary amine sites of compounds **9** and **99**; the secondary amine site of compound **103**; the carboxylic acid site of compound **109**; the imine site of compound **151**; the heterocycle site of compound **156**; and the heterocycle acid site of compound **190**.

Jaguar 7.5.

Even though based on a more sophisticated fundamental approach than the other eight programs, which are based on empirical methods, Jaguar 7.5 did not pull ahead of any one of them on the basis of our analysis. Only 193 protonation sites were predicted. One reason is the lack of parameters for some less frequently occurring sites, the other is the closeness of the pK_a values for some sites in a multiprotic molecule. The r^2 value for Jaguar 7.5 was 0.579, 0.178 lower than the worst-performing one of the other eight programs. Likewise, its MAD was 1.283, 0.390 higher than the next-ranked program. Jaguar 7.5 produced 24 (12.44%) and 62 (32.12%) predicted values with accuracies of ±0.1 and ±0.5 log units, respectively. The 24 sites whose predicted values were very close to the best literature values include the barbituric acid site of compound **37**, the carboxylic acid site of compound **77**, the phenol site of compound **81**, the secondary amine sites of compounds **87**, **105**, and **131**, the tertiary amine site of compound **114**, the heterocycle site and the primary amine site of compound **118**, one carboxylic acid site of compound **130**, and several others. One strong point of Jaguar 7.5 over the other eight programs is that it can distinguish diastereomers. For example, compounds **152** and **153** are diastereomers of each other. The experimental and predicted pK_a values for the two primary amine sites of compound **152** are 9.05 and 9.0, respectively, and for compound **153**, they are 9.19 and 9.3, respectively. Nevertheless, this version of Jaguar would not appear to be an ideal tool for predicting pK_a values of

pharmaceutical substances. There were 40 sites (20.73%) whose predicted accuracies were off by more than 2.0 log units. Frequently, Jaguar 7.5 worked incorrectly when there were one or more charged groups in the molecule, even though this molecule is not multiprotic. For example, for the three antibiotic compounds **15**, **18**, and **20**, which are monoprotic, the predictions for the three carboxylic acid sites were acceptable: The calculated deviations were 0.4, 0.7, and 0.9 respectively. However, the deviations for the other three antibiotic compounds **16**, **17**, **19** were 4.5, 5.7, and 6.3, which are clearly not acceptable. Each of these three compounds has an $-NH_2$ group, which we set as protonated and then maintained positively charged for the prediction of the pK_a value of the carboxylic acid site. When calculating the pK_a value of the second acid group of carbonic acid (compound **62**), the first carboxylic acid was deprotonated. This led to the pK_a value being predicted as 1.6, which is even lower than the first calculated one and then brings the deviation to −8.8. The compounds **89**–**92** are acids with quaternary ammonium groups; however, they are monoprotic, not diprotic. The positive quaternary ammonium groups induced big deviations for every carboxylic acid site: 3.3, 4.2, 5.7, and 4.4, respectively. In fact, among the 40 very poorly predicted sites (>2.0 log units), 29 were calculated when a charge existed in the molecule. Schrödinger's explanation in the Jaguar manual acknowledges this shortcoming: "When the ionziable groups are close together in the molecule, the calculated pK_a may not be as accurate because the two groups could interact in ways that the existing parameterization cannot handle."

Marvin 5.1.4.

With no sites with failed prediction attempts, the r^2 and MAD values for the program were 0.763 and 0.872, respectively, which makes this program rank seventh. It produced 39 (14.94%) and 130 (49.81%) predicted values with accuracies of ±0.1 and ±0.5 log units, respectively. Marvin calculated barbituric acid sites relatively poorly. The average deviation for the 29 barbituric acid sites was 1.254; two deviations were more than 2.0 log units. There were 22 sites (8.43%) whose predicted accuracies were more than 2.0 log units. The worst five predicted deviations were −9.88, 6.93, −5.88, 5.34, and −4.08, which happened on the substituted aniline site of compound **93**, the primary amine site of compound **26**, the pyridine site of compound **159**, the carboxylic acid site of compound **26**, and the tertiary amine site of compound **10**, respectively. The other very poorly estimated sites include the four guanidine sites of the four biguanidine compounds **70**–**73**, the primary amine site of compound **9**, one enol site of compound **23**, the phenol sites of compounds **75** and **102**, one tertiary amine site of compound **85**, the tertiary amine site of compound **115**, the pyridine site of compound **149**, the benzodiazepine site of compound **156**, the enol site of compound **166**, the imide site of compound **179**, and the heterocycle site of compound **190**.

Pallas pKalc Net 2.0.

This program failed to predict two sites: the acid site of compound **60** and the enol site of compound **166**. With this, it garnered an r^2 value of 0.803 and an MAD value 0.787, which helped it rank fifth and sixth, respectively, among the nine programs. It produced 48 (18.53%) and 153 (59.07%) predicted values with accuracies of ±0.1 and ±0.5 log units, respectively. There were 26 sites (10.04%) whose calculated deviations were more than 2.0

log units, among which the worst three predicted deviations occurred on the enol sites of the antibiotic compounds **74**–**76**. The other 23 very poorly predicted protonation sites included the alcohol sites of compound **5**, the general C substituted amide site and the heterocycle site of compound **6**, the primary amine site of compound **9**, one acid site of compound **62**, the tertiary amine sites of compounds **65** and **66**, the guanidine site of compound **70**, the phenol sites of compound **81**, **102**, and **176**, the substituted aniline site of compound **93**, the secondary amine sites of compounds **103**, **123**, and **188**, one carboxylic acid site of compound **128**, the thiol site of compound **134**, the pyridine site of compound **145**, the quinolin-2-one site of compound **151**, the pyridine sites of compounds **174** and **175**, the imide site of compound **179**, and the heterocycle site of compound **190**.

Pipeline Pilot 5.0.

With nine failed sites, the r^2 and MAD values of this program were 0.757 and 0.769, respectively, which made it rank eighth and fifth in these two categories. The failed nine sites were the carboxylic acid site of compound **3**, the alcohol sites of compound **5**, the two sites of compound **6**, the heterocycle sites of compounds **27**, **118**, **119**, and **190**, and the quinolin-2-one site of compound **151**. Pipeline Pilot 5.0 produced 77 (29.62%) and 151 (57.85%) predicted values with accuracies of ±0.1 and ±0.5 log units, respectively. The quality of the predictions of Pipeline Pilot for the pK_a values of the barbituric acids varied strongly. Some compounds, such as **36** and **56**, were predicted well, with deviations close or equal to zero, whereas other barbituric acids were predicted very poorly. For example, the deviations for compounds **30** and **52** were −4.62 and −4.96, respectively. The predicted values of the two enol sites of compound **23** were very close to the experimental values; unfortunately, Pipeline Pilot assigned them to the wrong sites, which led to the two largest deviations, 7.43 and −7.22. Besides these four sites, there were 20 sites whose predicted accuracies were off by more than 2.0 log units. These were the carboxylic acid sites of compounds **9**, **79** and **149**, the barbituric acid sites of compounds **53** and **54**, the tertiary amine sites of compounds **61, 74**, and **183**, the two acid sites of compound **62**, the primary amine site of compound **99**, the phenol sites of compound **102**, **106**–**108**, one tertiary amine site of compound **120**, the thiol site of compound **134**, the pyridine site of compound **145**, the heterocycle site of compound **156**, and the sulfonamide site of compound **185**.

SPARC 4.2.

This program gave up on the prediction of 18 protonation sites, mostly because it exceeded its time limit. It ranks third with an r^2 value of 0.894 and an MAD value of 0.651 in this comparison. This program produced 30 (12.35%) and 126 (51.85%) predicted values with accuracies of ±0.1 and ±0.5 log units, respectively. These two values are actually the second worst ones in each category among the eight empirical methods; nevertheless, this program did not produce too exorbitantly bad deviations. Only 12 deviations (4.94%) were larger than 2.0 log units. The worst two were −3.11 and 3.10, which happened for one acid site of compound **62** and the benzodiazepine site of compound **156**. This would seem to confirm that the PMO method is effective in preventing big deviations when it is used to predict pK_a values of pharmaceutical substances, albeit at the cost of prolonging calculation times. The other 10 poorly predicted sites involve the primary amine sites of compounds **9** and **99**, the

guanidine site of compound **82**, one tertiary amine site of compound **85**, one carboxylic acid site of compound **128**, the phenol site of compound **129**, the tertiary amine sites of compounds **139** and **140**, the enol site of compound **166**, and the heterocycle site of compound **190**.

CONCLUSION

Predicting pK_a values of pharmaceutical substances is both challenging and important in drug development and thus is an intriguing task in computational chemistry. We have compared nine currently available programs, including eight based on empirical methods and one based on a quantum chemical approach as to their ability to accurately predict 261 carefully experimentally measured pK_a values of 197 pharmaceutical compounds. It had been suggested that an approach based on a quantum chemical method would have led to better predictions, but our study did not bear this out. On the contrary, the only program in this comparison based on a higher level of theory than empirical methods ranked last in essentially all respects, indicating that the more recently introduced quantum chemical approach for predicting pK_a values has not yet reached the maturity level of the empirical methods.

Among the eight programs that are based on empirical methods, we found several that performed very well with this test set, with two predicting all or nearly all protonation sites, and doing so with an r^2 value of better than 0.9 and a mean absolute deviation of less than half a log unit. While extrapolation to any possible compound of interest in drug development is obviously risky, we believe that the best pK_a predicting programs currently available are useful tools in the arsenal of the drug developer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We would like to acknowledge Advanced Chemistry Development, Inc., Pharma Algorithms, Inc., ChemAxon, Ltd., and CompuDrug International, Inc. for giving us access to their programs.

REFERENCES AND NOTES

(1). Comer J; Tam K In Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical, and Computational Strategies; Testa B, Waterbeemd H van de, Folkers G, Guy R, Eds.; Wiley-VCH: Weinheim; New York, 2001; pp 275–304.

(2). Comer JEA In Comprehensive Medicinal Chemistry II. Testa B, Waterbeemd H van de, Eds.; Elsevier: Oxford, UK, 2007; Vol. 5, pp 357–398.

(3). Mitra R; Shyam R; Mitra I; Miteva MA; Alexov E Calculating the protonation states of proteins and small molecules: Implications to ligand-receptor interactions. Curr. Comput-Aided Drug Des. 2008, 4, 169–179.

(4). Prue JE Ionic Equilibria. Pergamon Press: Oxford, New York, 1966.

(5). Poole SK; Patel S; Dehring K; Workman H; Poole CF Determination of acid dissociation constants by capillary electrophoresis. J. Chromatogr., A 2004, 1037, 445–454. [PubMed: 15214681]

(6). Meloun M; Bordovska S; Galla L The thermodynamic dissociation constants of four non-steroidal anti-inflammatory drugs by the least-squares nonlinear regression of multiwavelength spectrophotometric pH-titration data. J. Phann. Biomed. Anal 2007, 45, 552–564.

(7). Fraczkiewicz R In Comprehensive Medicinal Chemistry II. Testa B, Waterbeemd H van de, Eds.; Elsevier: Oxford, UK, 2007; Vol. 5, pp 603–626.

(8). Sipila J; Nurmi H; Kaukonen AM; Hirvonen J; Taskinen J; Yli-Kauhaluoma J A modification of the Hammett equation for predicting ionisation constants of *p*-vinyl phenols. Eur. J. Pharm. Sci 2005, 25, 417–425. [PubMed: 15979536]

(9). Kaminski GA Accurate prediction of absolute acidity constants in water with a polarizable force field: Substituted phenols, methanol, and imidazole. J. Phys. Chem. B 2005, 109, 5884–5890. [PubMed: 16851640]

(10). Zhang J; Kleinoder T; Gasteiger J Prediction of pK_a values for aliphatic carboxylic acids and alcohols with empirical atomic charge descriptors. J. Chem. Inf. Model. 2006, 46, 2256–2266. [PubMed: 17125168]

(11). Brown TN; Mora-Diez N Computational determination of aqueous pK_a values of protonated benzimidazoles (part 1). J. Phys. Chem. B 2006, 110, 9210–9219.

(12). Brown TN; Mora-Diez N Computational determination of aqueous pK_a values of protonated benzimidazoles (Part 2). J. Phys. Chem. B 2006, 110, 20546–20554. [PubMed: 17034242]

(13). Milletti F; Storchi L; Sforna G; Cruciani G New and original pK_a prediction method using grid molecular interaction fields. J. Chem. Inf. Model. 2007, 47, 2172–2181. [PubMed: 17910431]

(14). Meloun M; Bordovská S; Syrový T A novel computational strategy for the pK_a estimation of drugs by non-linear regression of multiwavelength spectrophotometric pH-titration data exhibiting small spectral changes. J. Phys. Org. Chem 2007, 20, 690–701.

(15). Jelfs S; Ertl P; Selzer P Estimation of pK_a for druglike compounds using semiempirical and information-based descriptors. J. Chem. Inf. Model. 2007, 47, 450–459. [PubMed: 17381168]

(16). Jover J; Bosque R; Sales J Neural network-based QSPR study for predicting pK_a of phenols in different solvents. QSAR & Combin. Sci. 2007, 26, 385–397.

(17). Caballero NA; Melendez FJ; Munoz-Caro C; Nino A Theoretical prediction of relative and absolute pK_a values of aminopyridines. Biophys. Chem 2006, 124, 155–160. [PubMed: 16844281]

(18). Lu H; Chen X; Zhan CG First-principles calculation of pK_a for cocaine, nicotine, neurotransmitters, and anilines in aqueous solution. J. Phys. Chem. B 2007, 111, 10599–10605. [PubMed: 17691837]

(19). Bryantsev VS; Diallo MS; Goddard WA pK_a calculations of aliphatic amines, diamines, and aminoamides via density functional theory with a Poisson–Boltzmann continuum solvent model. J. Phys. Chem. A 2007, 111, 4422–4430. [PubMed: 17469810]

(20). Trummal A; Rummel A; Lippmaa E; Burk P; Koppel IA IEF-PCM calculations of absolute pK_a for substituted phenols in dimethyl sulfoxide and acetonitrile solutions. J. Phys. Chem. A 2009,113, 6206–6212. [PubMed: 19413308]

(21). Verdolino V; Cammi R; Munk BH; Schlegel HB Calculation of pK_a values of nucleobases and the guanine oxidation products guanidinohydantoin and spiroiminodihydantoin using density functional theory and a polarizable continuum model. J. Phys. Chem. B 2008, 112, 16860–16873. [PubMed: 19049279]

(22). Meloun M; Bordovska S Benchmarking and validating algorithms that estimate pK_a values of drugs based on their molecular structures. Anal. Bioanal. Chem 2007, 389, 1267–1281. [PubMed: 17676314]

(23). Predict Ionization Constant, Acid–Base Dissociation Constant, pKa, Experimental pKa. http://www.acdlabs.com/products/phys_chem_lab/pka/ (accessed September 17, 2009).

(24). Physicochemical and ADMET Laboratory. http://pharma-algorithms.com/ionization.htm (accessed September 17, 2009).

(25). Simulations Plus. http://www.simulations-plus.com (accessed September 17, 2009).

(26). Epik, Schrödinger, LLC: New York, 2008.

(27). Shelley JC; Cholleti A; Frye LL; Greenwood JR; Timlin MR; Uchimaya M Epik: A software program for pK_a prediction and protonation state generation for drug-like molecules. J. Comput. Aided Mol. Des 2007, 21, 681–691. [PubMed: 17899391]

(28). Jaguar, version 7.5; Schrödinger, LLC: New York, 2008.

(29). Klicić JJ; Friesner RA; Liu S-Y; Guida WC Accurate prediction of acidity constants in aqueous solution via density functional theory and self-consistent reaction field methods. J. Phys. Chem. A 2002, 106, 1327–1335.

(30). p*K*a Plugin ionization equilibrium partial charge distribution. http://www.chemaxon.com/product/pka.html (accessed September 17, 2009).

(31). CompuDrug, Latest Upgrades. http://www.compudrug.com (accessed September 17, 2009).

(32). Pipeline Pilot data analysis and reporting platform. http://accelrys.com/products/scitegic/ (accessed September 17, 2009).

(33). Sparc On-Line Calculator. http://ibmlc2.chem.uga.edu/sparc/ (accessed September 17, 2009).

(34). Lee PH; Ayyampalayam SN; Carreira LA; Shalaeva M; Bhattachar S; Coselmon R; Poole S; Gifford E; Lombardo F In silico prediction of ionization constants of drugs. Mol. Pharmacol 2007, 4, 498–512.

(35). MoKa–p*K*a Modeling. http://www.moldiscovery.com/soft_moka.php (accessed September 30, 2009).

(36). Dissociation Constants of Organic Acids in Aqueous Solution Kortüm G, Ed.; International Union of Pure and Applied Chemistry, Commission on Electrochemical Data; Butterworths: London, 1961.

(37). Perrin DD In Dissociation Constants of Organic Bases in Aqueous Solution. Butterworths: London, 1965.

(38). Perrin DD; Dempsey B; Serjeant EP In pK_a Prediction for Organic Acids and Bases. Chapman and Hall: London, New York, 1981.

(39). Ionization Constants of Organic Acids in Aqueous Solution Serjeant EP; Dempsey B, Eds.; International Union of Pure and Applied Chemistry, Commission on Electrochemical Data; Pergamon Press: Oxford, New York, 1979.

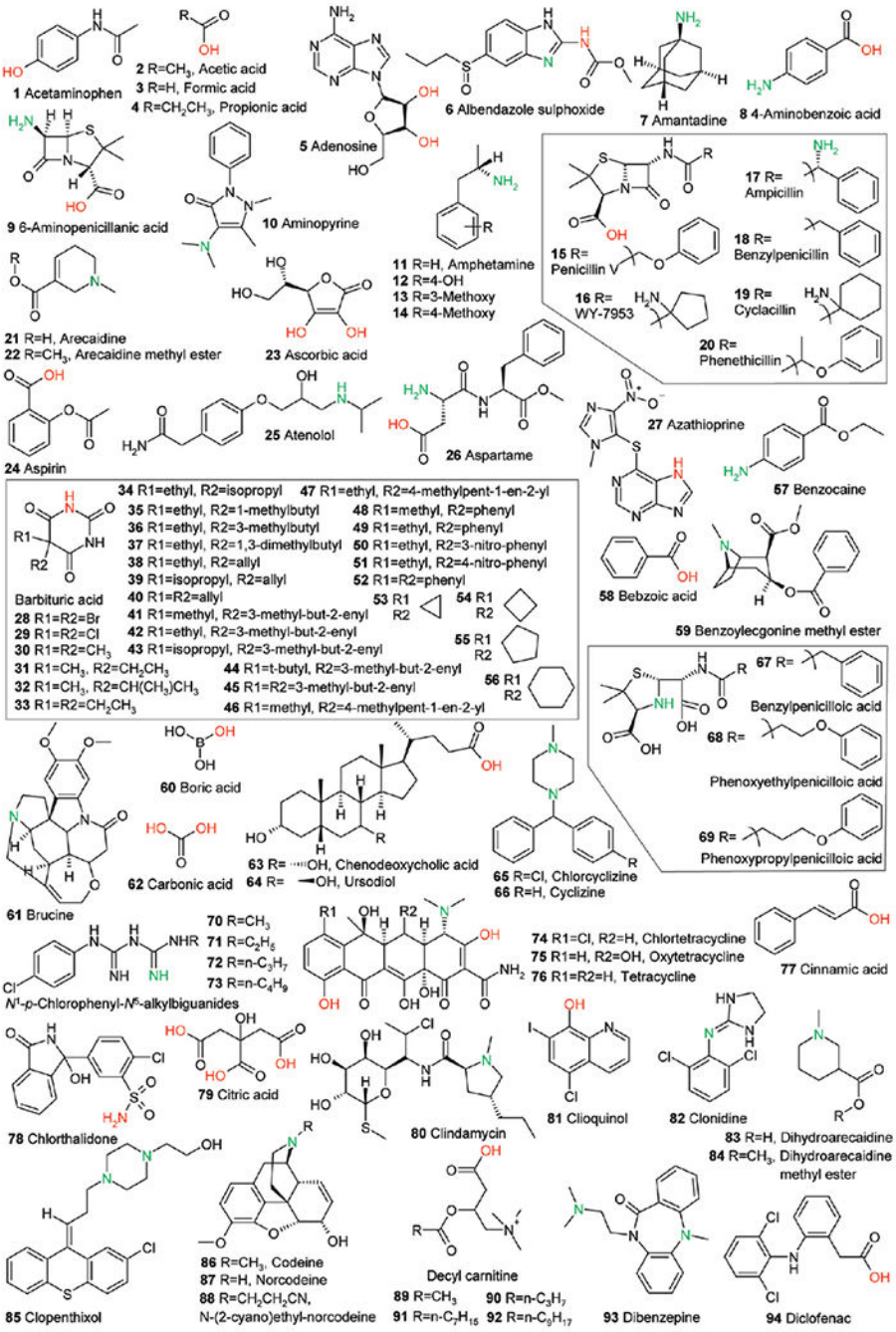
(40). Perrin DD Dissociation Constants of Weak Bases in Aqueous Solution. Butterworths: London, 1972.

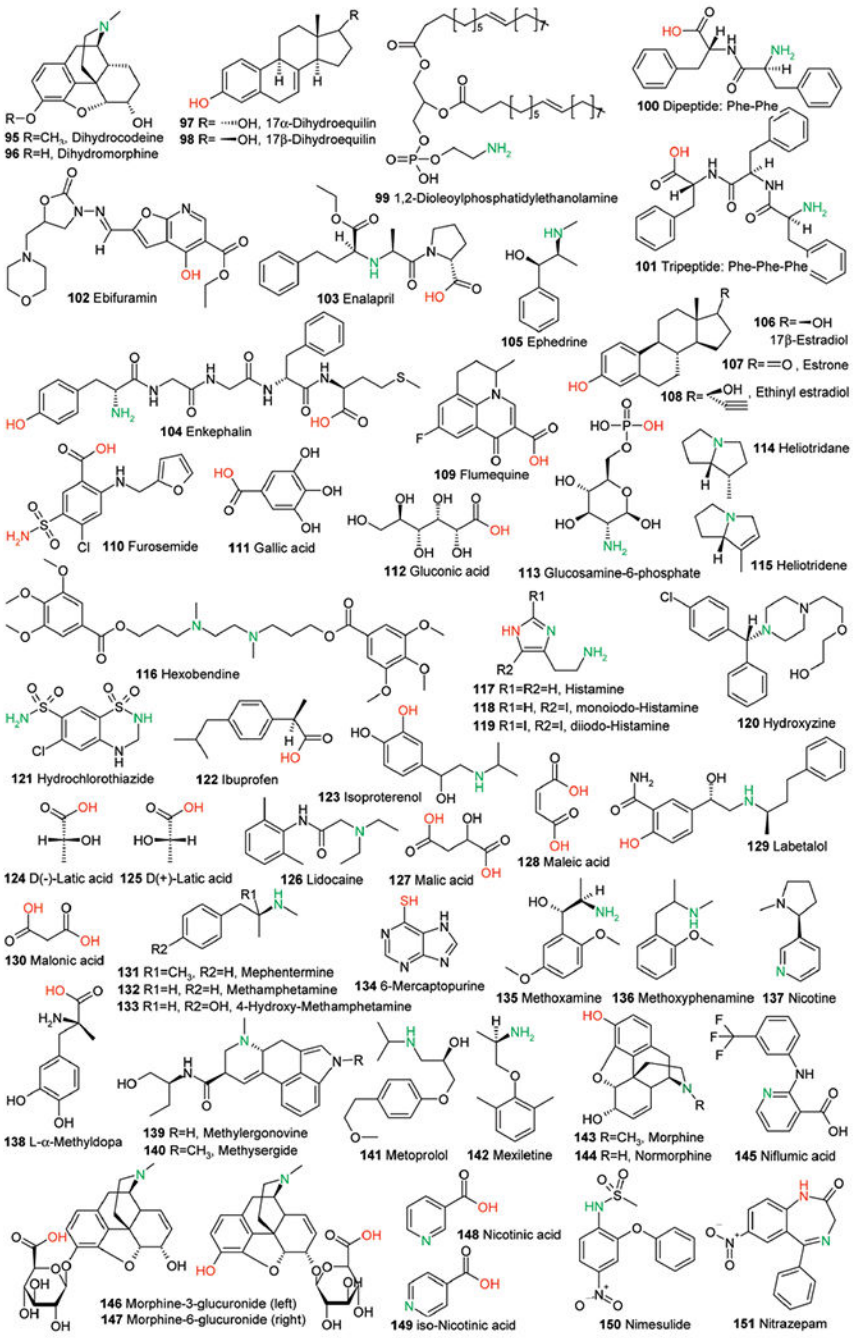
(41). Prankerd RJ In Profiles of Drug Substances, Excipients, and Related Methodology. Elsevier Academic Press: San Diego, CA, 2007; Vol. 33.

(42). Frenkel YV; Clark AD Jr.; Das K; Wang YH; Lewi PJ; Janssen PA; Arnold E Concentration and pH dependent aggregation of hydrophobic drug molecules and relevance to oral bioavailability. J. Med. Chem 2005, 48, 1974–1983. [PubMed: 15771441]

(43). Coan KE; Maltby DA; Burlingame AL; Shoichet BK Promiscuous aggregate-based inhibitors promote enzyme unfolding. J. Med. Chem 2009, 52, 2067–2075. [PubMed: 19281222]

(44). Macromodel, version 9.6; Schrödinger, LLC: New York, 2008.





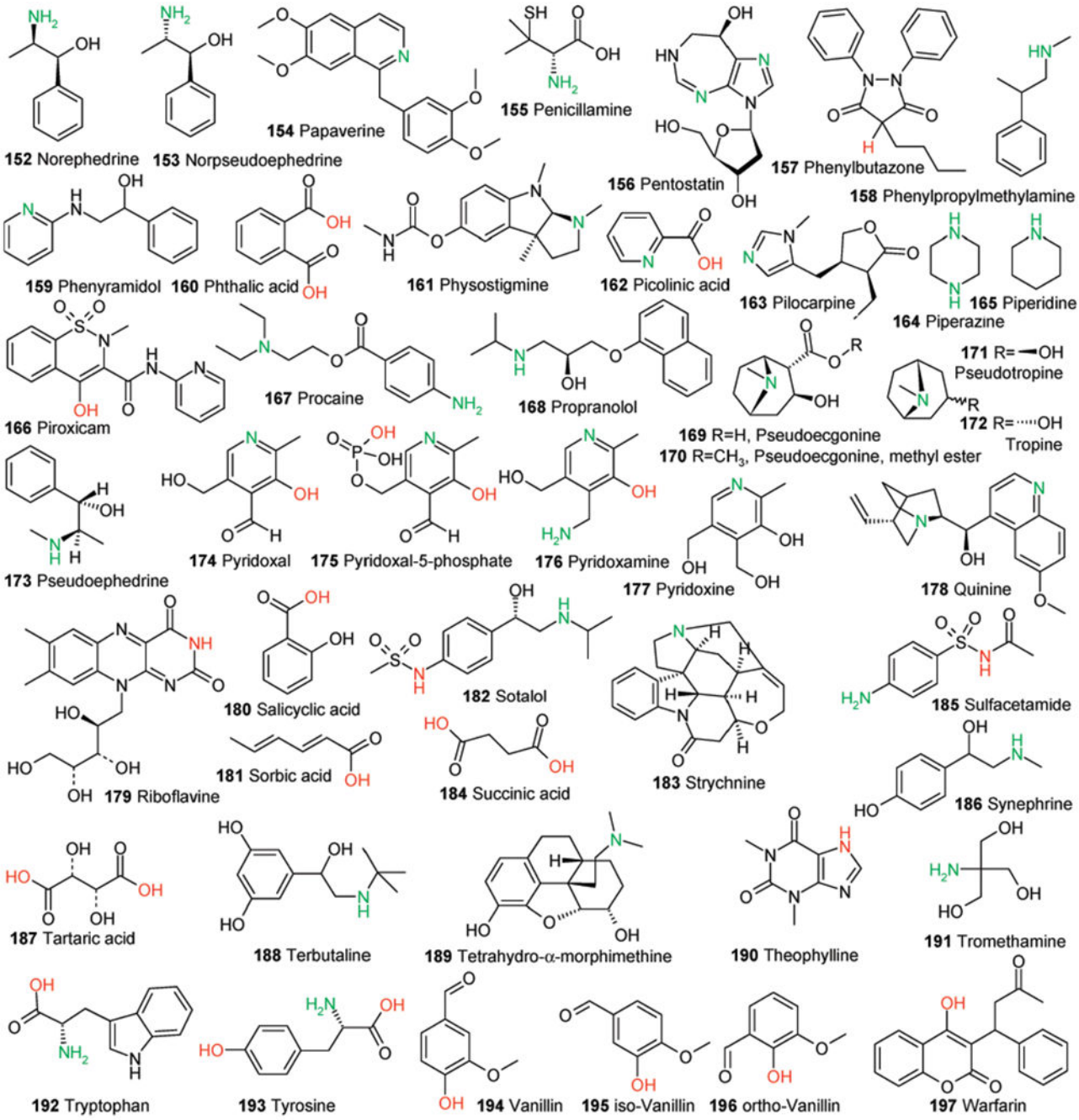


Figure 1.

Part 3 of 3. Structure set used for this study. All structures were extracted from ref 41. Calculated acidic and basic atoms are indicated in red and green, respectively. Structures of compounds **23**, **74**, **140**, and **154** in the original reference are wrong and are shown here in their corrected form.

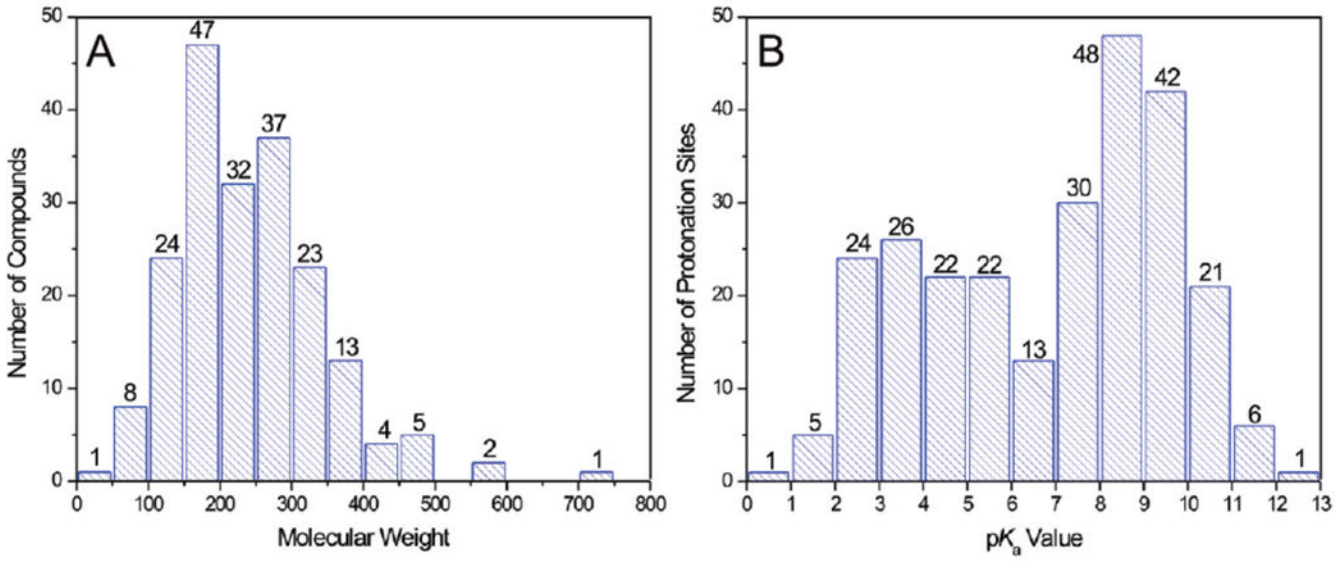


Figure 2.

Distribution of (A) molecular weights of the calculated 197 compounds and (B) experimental pK_a values of the 261 protonation sites used in the study.

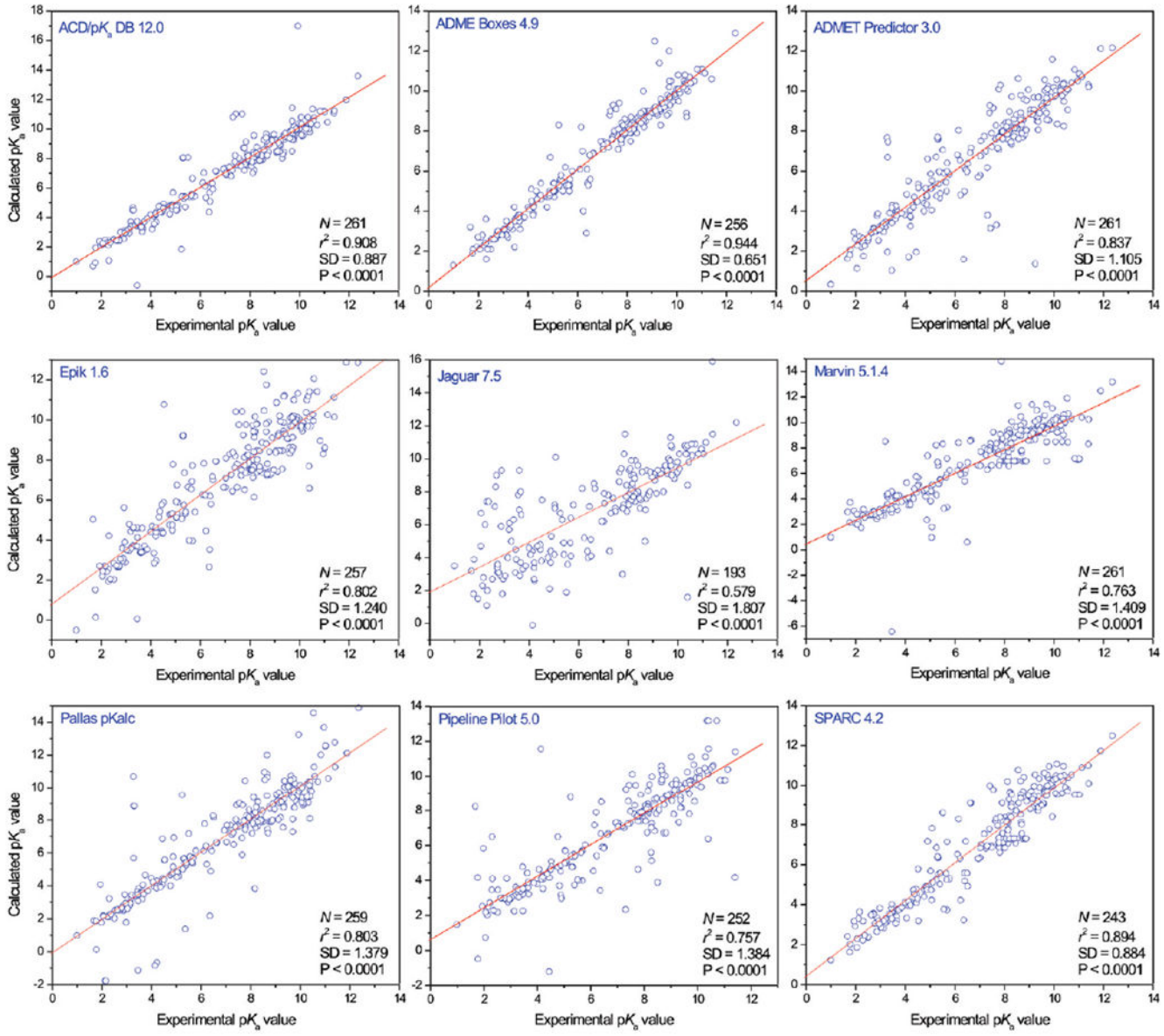


Figure 3.

Predicted pK_a values calculated by nine different programs versus experimental pK_a values for 261 protonation sites.

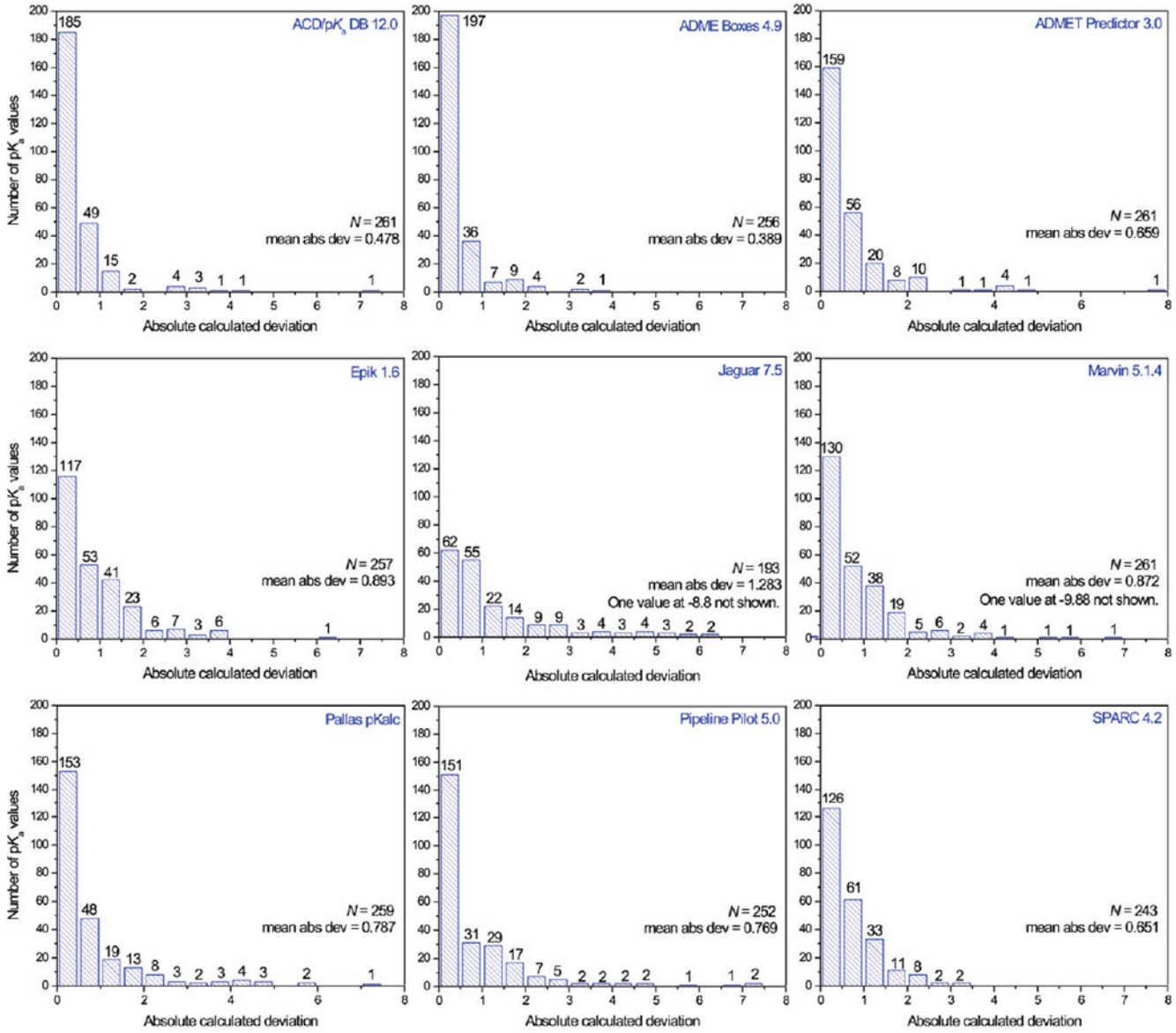


Figure 4.

Summary of the pK_a value predictions of 261 protonation sites. The predictions are binned by prediction accuracy with a resolution of 0.5 log unit.

Table 1.

Programs Used For This Study

program	version	company	method
ACD/pK_a DB[23]	12.00	Advanced Chemistry Development, Inc.	LFER
ADME Boxes[24]	4.9	Pharma Algorithms, Inc.	QSPR
ADMET Predictor[25]	3.0	Simulations Plus, Inc.	QSPR
Epik[26,27]	1.6	Schrödinger, LLC.	LFER
Jaguar[28,29]	7.5	Schrödinger, LLC.	quantum–chemical method (DFT) with empirical correction
Marvin[30]	5.1.4	ChemAxon Ltd.	QSPR
Pallas pKalc[31]	Net 2.0	CompuDrug International, Inc.	LFER
Pipeline Pilot[32]	5.0	SciTegic, Inc.	QSPR
SPARC[33,34]	4.2	University of Georgia/U.S. Environmental Protection Agency	blend of LFER and perturbed molecular orbital (PMO) method

J Chem Inf Model. Author manuscript; available in PMC 2020 June 11.

Table 2.

Number of Different Acidic and Basic Sites Used for This Comparison

acids		bases	
alcohol	1	primary amines	25
enols	7	secondary amines	24
phenols	24	tertiary amines	42
carboxylic acids (not conjugated)	42	anilines	5
carboxylic acids (conjugated)	17	heterocycles	17
thiol	1	amidine	1
sulfonamides	7	benzodiazepine	1
imide	1	guanidines	5
barbituric acids	29		
heterocycles	4		
NH acids	2		
CH acid	1		
phosphoric acids	2		
others	3		
∑	141	∑	120

Table 3.

Performance of the Nine pK_a Prediction Programs Using a Set of 261 Protonation Sites, Sorted Alphabetically by Program Name

program	number handled	predicted excellently (AD[a] ≤ 0.1)	predicted well (0.1 < AD ≤ 0.5)	predicted poorly (1.0 < AD ≤ 2.0)	predicted awfully (AD > 2.0)	r^2	MAD[b]
ACD/pK_a DB	261	64	121	17	10	0.908	0.478
ADME Boxes	256	95	102	16	7	0.944	0.389
ADMET Predictor	261	40	119	28	18	0.837	0.659
Epik	257	30	87	64	23	0.802	0.893
Jaguar	193	24	38	36	40	0.579	1.283
Marvin	261	39	91	57	22	0.763	0.872
Pallas pKalc	259	48	105	32	26	0.803	0.787
Pipeline Pilot	252	77	74	46	24	0.757	0.769
SPARC	243	30	96	45	12	0.894	0.651

[a] AD: absolute deviation.

[b] MAD: mean absolute deviation.

Table 4.

Performance for pK_a Range 5.4–9.4; Programs Sorted Alphabetically

program	number handled	predicted excellently (AD[a] ≤ 0.1)	predicted well (0.1 < AD ≤ 0.5)	predicted poorly (1.0 < AD ≤ 2.0)	predicted awfully (AD > 2.0)	$r^2_{5.4–9.4}$	MAD[b]
ACD/pK_a DB	116	25	51	9	4	0.574	0.504
ADME Boxes	115	33	60	8	5	0.682	0.452
ADMET Predictor	116	14	53	15	8	0.350	0.744
Epik	114	9	29	39	9	0.403	0.979
Jaguar	84	10	16	20	9	0.422	1.044
Marvin	116	10	35	35	8	0.374	0.961
Pallas pKalc	115	21	45	19	6	0.469	0.680
Pipeline Pilot	114	35	30	28	10	0.422	0.762
SPARC	108	7	30	27	10	0.479	0.876

[a] AD: absolute deviation.

[b] MAD: mean absolute deviation.