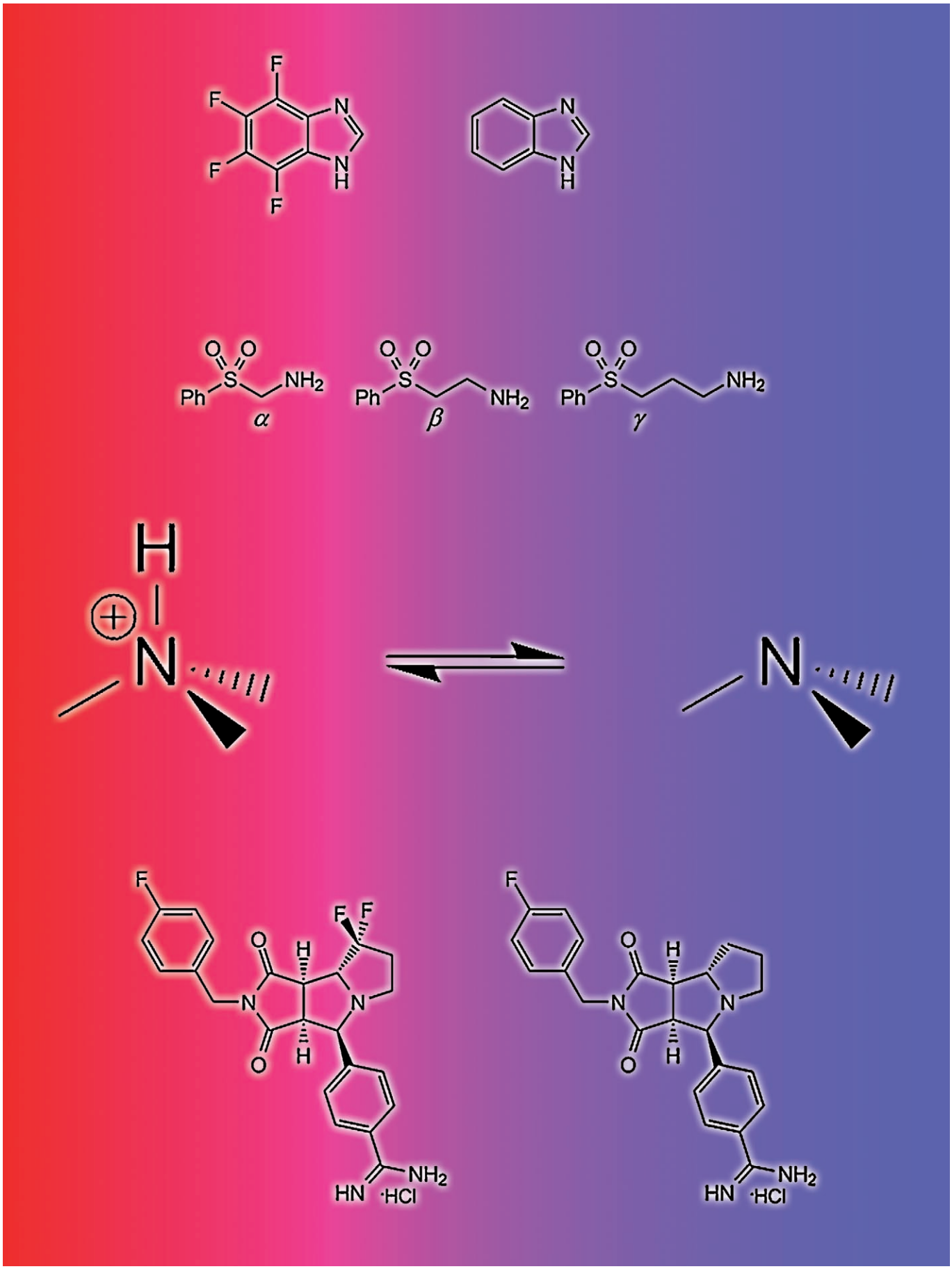

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Predicting and Tuning Physicochemical Properties in Lead Optimization: Amine Basicities

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This review describes simple and useful concepts for predicting and tuning the pK_a values of basic amine centers, a crucial step in the optimization of physical and ADME properties of many lead structures in drug-discovery research. The article starts with a case study of tricyclic thrombin inhibitors featuring a tertiary amine center with pK_a values that can be tuned over a wide range, from the usual value of around 10 to below 2 by (remote) neighboring functionalities commonly encountered in medicinal chemistry. Next, the changes in pK_a of acyclic and cyclic amines upon substitution by fluorine, oxygen, nitrogen, and sulfur functionalities, as well as carbonyl and carboxyl derivatives are systematically analyzed, leading to the derivation of simple rules for pK_a prediction. Electronic and stereoelectronic effects in cyclic amines are discussed, and the emerging computational methods for pK_a predictions are briefly surveyed. The rules for tuning amine basicities should not only be of interest in drug-discovery research, but also to the development of new crop-protection agents, new amine ligands for organometallic complexes, and in particular, to the growing field of amine-based organocatalysis.

1. Introduction

Modern drug-discovery chemistry is based on a multidimensional optimization (MDO) approach, in which the optimization of physicochemical properties, aspects of ADME (absorption, distribution, metabolism, and excretion), and safety of a lead compound are considered at an equal level together with potency and selectivity. Optimization of physicochemical properties such as pK_a and $\log D$ benefits from empirical knowledge available through both internal and publicly available databases.[1] In fact, with the establishment of high-throughput analytical methodologies, the amount of physicochemical data is growing rapidly, allowing hundreds of compounds to be measured rapidly and precisely each day.[2] The availability of large datasets covering a sufficiently broad structural diversity increases the chances that improved schemes for reasonably successful predictions of physicochemical properties can be developed. Both databases and chemoinformatics tools aim at enhancing the capacity of medicinal chemists to design compounds with desired physicochemical and pharmacological properties.

A large fraction of the drugs currently on the market or in development contain one or more basic nitrogen atoms. They contribute to essentially all molecular properties relevant to the MDO process. Whether or not basic nitrogen atoms are protonated in the biological environment may not only be critical for binding potency at the target, but also affects properties such as lipophilicity, membrane permeability,[3,4] amphiphilicity, and the potential liability for phospholipidosis,[5] as well as interference with the hERG (human ether-a-go-go-related gene) potassium ion channel and associated cardiovascular toxicity.[6–8] It is therefore important not only to know the pK_a value of an amine-containing compound, but even more so, to be able to modulate its basicity in a rational, structure-based fashion.

The incentive to survey possibilities for tuning amine basicity arose from our exploration of tricyclic inhibitors of thrombin, a

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serine protease from the blood coagulation cascade, to map the fluorophobicity/fluorophilicity of an enzyme active site.[2,9–12] In that investigation, we observed that the pK_a value of a tertiary non-aromatic amine could be shifted from values of around 10 to below 2 through the cumulative interaction effects of relatively remote functionalities (see Section 2). Simple rules for predictions of amine basicity were subsequently developed based on extensive database mining. These are summarized herein (see Section 3) along with a short overview of the current understanding of stereochemical and conformational factors that influence amine basicities (see Section 4). We are well aware that after seminal publications on predictions of the strength of organic bases,[13,14] numerous reports have dealt with this subject, particularly within the context of drug-discovery chemistry. Nevertheless, we feel that a survey such as this is long overdue and will be of great benefit to research groups involved in drug discovery as well as many other areas in which amine basicity plays a crucial role.

It should be noted that the experimental data collection for this review (both in the main text and the Supporting Information (SI)) has been possible through high-throughput photometric or routine potentiometric pK_a determinations,[2,15,16] which over the past years have replaced classical low-throughput titrimetric or electrochemical methods. The accuracy of pK_a determinations in the range of 3–11 is now typically $\Delta pK_a \pm 0.1$ for compounds with sufficient aqueous solubility and UV absorption (compared with $\Delta pK_a \pm 0.5$ for older methods prior to 1990). For these reasons, all pK_a values in this review are rounded to one digit after the decimal point. Nevertheless, it should be mentioned that uncertainties in comparing data might arise from systematic pK_a shifts due to different solvent systems, temperature, ionic strength, and other hidden experimental effects. Finally, this review contains several experimental pK_a values not previously published or re-measured for the sake of consistent comparison; these new or corrected pK_a values are marked with an asterisk (*) in the figures throughout this account for easy identification.

2. Tricyclic Thrombin Inhibitors

Our thrombin inhibitors are readily constructed using the 1,3-dipolar cycloaddition of azomethine ylides to maleimides as the key step to build the central tricyclic scaffold.[17] This strategy allows substituents to be introduced and modified in a versatile fashion for occupation of the selectivity pocket S1, the large distal hydrophobic D pocket, the tight proximal hydrophobic P pocket, and the region around the catalytic serine and the oxyanion hole. How these substituents alter the pK_a value of the tertiary amine center in the tricyclic skeleton of the targeted inhibitors and some of their precursors (the latter data are mostly in the Supporting Information), are analyzed in the following sections.

2.1. Variation of the S1 pocket vector

The investigation started with the unexpected finding that two significantly different pK_a values were measured for the tertiary amine center in (±)-**1** (Figure 1), depending on whether the photometric titration was conducted from pH 2 to pH 13 (pK_a 5.2) or in the reverse direction, from pH 13 to pH 2

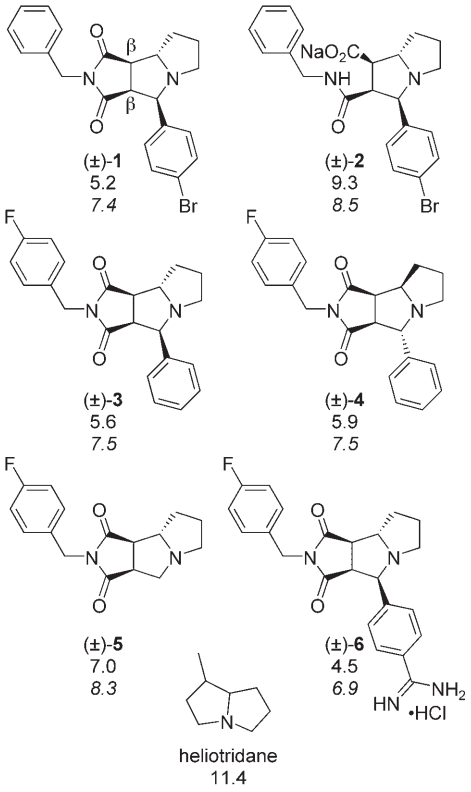


Figure 1. Experimental pK_a values for the tertiary amine center in precursors to tricyclic thrombin inhibitors, reflecting the basicity-lowering effects of β-carbonyl and α-aryl substituents. The numbers in italics below the experimental values indicate calculated[39] pK_a values discussed in Section 5.

(pK_a 9.3). Subsequent chemical analysis revealed that (±)-**1** (and a large number of related imides) are unstable in even mildly basic solution and that the succinimide moiety undergoes rapid and regioselective ring opening by formation of (±)-**2**.[2,18] The pK_a of the carboxylate group (4.0) in (±)-**2** could be determined by potentiometric titration.

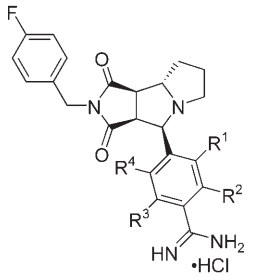
To shed light on the origin of the remarkably low pK_a value of (±)-**1** (5.2), compounds (±)-**3** to (±)-**5** were measured. The pK_a of (±)-**5** (7.0) is still three units smaller than the approximate value for a simple trialkylamine (~10.2) and some four units below the pK_a of heliotridane (11.4, (1*S*,7a*S*)-2,3,5,6,7,7a-hexahydro-1-methyl-1*H*-pyrrolizine),[19] which could be taken as the closest available parent compound in this series. A search in the MedChem database[1,2] revealed that a carbonyl group in the β-position can lower the pK_a value of an aliphatic amine by ~1.8 units. As there are two imide C=O groups in (±)-**5**, each in β-position to the tertiary amine center, the overall effect could naively be guessed as $\Delta pK_a = 2 \times \sim -1.8 = \sim -3.6$ units. Several remarks are appropriate at this point: First, the pK_a values observed for acyclic amines may represent conformational averages and therefore may not be transferable directly to cyclic amines, which often adopt one specific confor-

mation with fixed spatial relationships between the electron-withdrawing group and the amine function. This is particularly true for bi- and polycyclic amines. Second, in rigid (poly)cyclic systems, substantial direct through-space interactions may operate in addition to σ-transmission effects. Third, the transferability of pK_a decrements may be best for 6-membered piperidine units in which properly staggered synclinal and anti-periplanar arrangements of functional groups are closest to the situation in acyclic amines. However, for amines embedded in 5- or 4-membered rings this may not hold, because significant deviations from ideally staggered conformations are observed, and σ-transmission effects may be attenuated accordingly (see Section 4). Finally, σ-transmissions typically operate through all intervening σ-pathways between the electron-withdrawing function and amine group.[13] This is nicely illustrated, for example, by the shift from piperidine (pK_a 11.1) to 2-(2′-oxopropyl)piperidine (pK_a 9.5), with one σ-transmission pathway, and from 2,2,6,6,-tetramethylpiperidine (pK_a 11.1) to 4-oxo-2,2,6,6-tetramethylpiperidine (pK_a 7.9), with two σ-transmission pathways (see Figure 14a below). Such σ-transmitted electron-withdrawing effects in cyclic systems are discussed further in Section 3.

The decrease in pK_a from 7.0 (for (±)-**5**) into the range between 5 and 6 for the arylated tricycles (±)-**1**, (±)-**3**, and (±)-**4** reflects the σ-transmission effects of the aryl rings in α-position to the nitrogen center, in agreement with the MedChem database (ΔpK_a(α-phenyl) = −1.4). It is interesting to note that the pK_a-lowering effects are significantly different for the *endo*-phenyl ring in (±)-**3** (pK_a 5.6) and the *exo*-phenyl ring in (±)-**4** (pK_a 5.9) (*endo* and *exo* refer to the orientation of the aryl substituent with respect to the bicyclic perhydropyrrolo[3,4-*c*]pyrrole scaffold). The relatively high pK_a of (±)-**2** (9.3) may be explained by the decreased electron-withdrawing power of a carboxylate unit (σ(COOH) = −1.9; σ(C(=O)NH_2) = 1.68),[13] as well as, and probably most importantly, a direct (through-space) stabilization of the protonated ammonium center by the carboxylate anion.

The opposite effect can be observed upon introduction of a positive charge into the molecule by incorporating the amidinium unit into the benzyl group of (±)-**3**. From data in the MedChem database,[1] benzylamines with strong but neutral electron-withdrawing groups in the *para* position, such as cyano or nitro, produce an additional pK_a shift of ~−0.8 relative to the parent benzyl group. Hence, the substantial shift of −1.1 observed in going from (±)-**3** to (±)-**6** may reflect the additional destabilization of the protonated ammonium center by the presence of a positive charge.

The phenylamidinium ring for occupancy of the S1 pocket was subsequently fluorinated as shown for (±)-**7** to (±)-**11** (Figure 2), in order to decrease the basicity of the amidine residue and thus potentially enhance the bioavailability of these compounds.[2] Although this objective was reached (pK_{a2}((±)-**6**) 11.1; pK_{a2}((±)-**11**) 10.1), binding affinity unfortunately dropped dramatically (K_i((±)-**6**) = 0.057 μM; K_i((±)-**11**) = 9 μM). Fluorine substitution expectedly influences the pK_a value of the tertiary amine center as well; upon introduction of one or two F atoms, pK_{a1} decreases with one notable exception. The pK_{a1} value of compound (±)-**9** (4.2), with two F atoms *meta* to the amidinium group, is 0.5 units higher than that of compound (±)-**11** (3.7) with two F atoms in the *ortho* position. This increase in basicity is readily explained by the fact that in (±)-**9**, one of two F atoms must approach the nitrogen lone pair (the aryl ring is roughly perpendicular to the mean plane of the pyrrolidine ring to which it is attached), leading to both lone-pair repulsion and unfavorable dipolar alignment, whereas upon protonation, the C–F and N^+–H bonds can undergo favorable dipolar alignment and lone-pair repulsion is eliminated.[20] Similar trends were also observed in the corresponding series of nitrile precursors (Figure 1 SI).



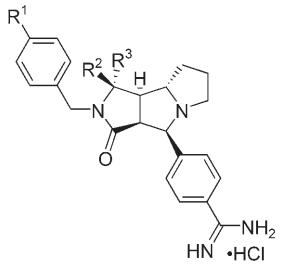
Compd	R^1	R^2	R^3	R^4	pK_{a1}	p$K_{a1\ calcd}$[39]	pK_{a2}
(±)-**6**	H	H	H	H	4.5	6.9	11.1
(±)-**7**	H	F	H	H	4.2	7.0	10.7
(±)-**8**	F	H	H	H	4.1	6.9	10.8
(±)-**9**	F	H	H	F	4.2	6.4	10.4
(±)-**10**	F	F	H	H	3.7	6.5	10.3
(±)-**11**	H	F	F	H	3.7	6.6	10.1

Figure 2. Effects of F substitution on the pK_a values of amine (pK_{a1}) and amidinium residues (pK_{a2}) in the tricyclic thrombin inhibitors (±)-**6** to (±)-**11**.

2.2. Variation of the D and P pocket vector

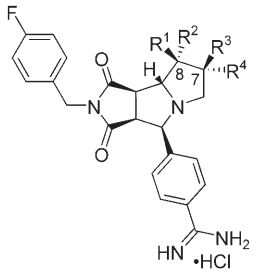
When the F substituent in the *para* position of the benzylic ring in (±)-**6**, which occupies the D pocket of the thrombin active site, is changed to Cl, Br, OMe, or OH, or the phenyl group exchanged for a 4-pyridyl ring, the pK_a value of the distant tertiary amine center is not affected and remains at 4.5 ± 0.1.[10] Upon changing from the tricyclic imide to the tricyclic lactam series, on the other hand, the pK_a values change substantially (Figure 3). Lactams (±)-**12** to (±)-**14** feature an isopropyl group to favorably fill the P pocket of thrombin, which results in improved binding to thrombin (K_i between 5 and 15 nM) and high selectivities over trypsin (~400–1600-fold).[9,10] Compared with imide (±)-**6** (Figure 2, pK_a 4.5) and the *para*-substituted benzyl analogues mentioned above, the pK_a values of the tertiary amine centers in (±)-**12** to (±)-**14** are raised by nearly two units to 6.2–6.5. This is clearly the result of removing one C=O group in the β-position.

Whereas the change from the isopropyl group in (±)-**12** to other hydrocarbon residues has expectedly only marginal effects (see compounds (±)-**15** to (±)-**18** in Figure 3), the introduction of fluoroalkyl residues (see (±)-**19** to (±)-**22** in Figure 3) to fill the P pocket can change the pK_a value signifi-



Compd	R^1	R^2	R^3	pK_a	p$K_{a\ calcd}$[39]
(±)-**12**	F	H	$CHMe_2$	6.5	7.7
(±)-**13**	Cl	H	$CHMe_2$	6.2	7.7
(±)-**14**	OMe	H	$CHMe_2$	6.3	7.7
(±)-**15**	F	H	CH_2CH_3	6.3	7.8
(±)-**16**	F	H	$CH{=}CH_2$	6.0	7.5
(±)-**17**	F	H	$CH_2CH_2CH_3$	6.2	7.8
(±)-**18**	F	H	$CH_2CH{=}CH_2$	6.2	7.7
(±)-**19**	F	H	CH_2F	6.0	7.3
(±)-**20**	F	H	CHF_2	5.6	6.9
(±)-**21**	F	H	CH_2CH_2F	6.1	7.6
(±)-**22**	F	H	CH_2CHF_2	6.0	7.4
(±)-**23**	F	OH	CF_3	5.5	6.1
(±)-**24**	F	OMe	CF_3	5.2	6.0
(±)-**25**	F	CF_3	OMe	4.9	6.0

Figure 3. Long-range effects on the pK_a value of the tertiary amine center in tricyclic thrombin inhibitors. Note: (±)-**12** measured in 0.1 M KNO_3 by potentiometric titration; (±)-**13** and (±)-**14** measured in 0.15 M SGA buffer (10% MeOH) by Sirius ProfilerSGA photometric titration;[16] the slightly different pK_a values for these three compounds can most likely be ascribed to the unequal solvent mixtures and ionic strengths.



Compd	R^1	R^2	R^3	R^4	pK_a	p$K_{a\ calcd}$[39]
(±)-**6**	H	H	H	H	4.5	6.9
(+)-**26**	OH	H	H	H	4.1	5.8
(+)-**27**	OMe	H	H	H	3.7	5.5
(+)-**28**	F	H	H	H	3.4	4.4
(+)-**29**	H	OH	H	H	4.2	5.8
(+)-**30**	H	OMe	H	H	4.2	5.5
(+)-**31**	H	F	H	H	3.3	4.4
(+)-**32**	F	F	H	H	<2	1.8
(+)-**33**	OMe	OMe	H	H	3.3	4.1
(+)-**34**	H	H	OH	H	3.9	5.8
(+)-**35**	H	H	OMe	H	3.6	5.5
(+)-**36**	H	H	F	H	3.3	4.5
(+)-**37**	H	H	H	OH	3.9	5.8
(+)-**38**	H	H	H	OMe	3.7	5.5
(+)-**39**	H	H	H	F	3.3	4.5
(±)-**40**	H	H	F	F	<2	2.1
(±)-**41**	H	H	OMe	OMe	3.2	4.2

Figure 4. Additional F, OH, or OMe substitution in the terminal pyrrolidine ring changes the protonated tertiary ammonium ion into a moderately strong acid.

cantly, despite the large topological distance of the F atoms from the tertiary amine center, which is five or six bonds away.[11] Thus, a CHF_2 substituent, as in (±)-**20** (F atoms in δ-position to the N atom), lowers the pK_a to a value of 5.6. In the presence of both OH/OMe and CF_3 substituents in γ-position to the N atom, as in (±)-**23** to (±)-**25**, measured pK_a values are as low as 4.9–5.5. This documents once more the substantial electron-withdrawing effects of fluorine and oxygen atoms and their dramatic effects on an amine center, even in rather remote locations.

2.3. Variation of substituents pointing towards the oxyanion hole

The introduction of F, OH, and OMe substituents at various positions of the terminal pyrrolidine ring ((+)-**26** to (±)-**41** in Figure 4) to probe their bioisosteric behavior in thrombin binding produced striking effects on the basicity of the adjacent tertiary amine center.[12] Inductive effects are transmitted over two σ-pathways, as substituents at positions 8 ((+)-**26** to (+)-**33**) and 7 ((+)-**34** to (±)-**41**) are both located in β- and γ-position to the amine center. With one substituent, the pK_a is decreased by amounts of −0.3 to −1.2 relative to the parent compound (±)-**6**. Remarkably, difluorination at either position produces essentially neutral amines with pK_a values below 2 ((+)-**32** and (±)-**40**). Several trends are noticeable: First, the interaction effects of fluorine are (expectedly) much stronger than those of OH or OMe, resulting in pK_a decrements that are larger by −0.3 to −0.9 units. Second, the ΔpK_a effects of OH and OMe are somewhat similar, differing between 0 and 0.4 units, depending on the position and relative configuration at the center of substitution. Third, the effects of two *gem* substituents (F or OMe) seem to be more or less additive. Finally, the pK_a decrements are essentially independent on the stereochemistry at C7, but show a marked configurational dependence for substituents at C8. Substituents at C8 are more strongly subjected to the anisotropic steric bulk of the tricyclic core, which could result in differential solvation, thus affecting the pK_a values.

The trends reported in Figures 3 and 4 are paralleled by the experimental pK_a values measured for a series of precursor compounds not containing the charged benzamidinium group. These pK_a data are collected in Figures 2 SI and 3 SI, respectively.

All these pK_a data for our tricyclic thrombin inhibitors, documenting significant long-range effects not only of F atoms, but also of OH and OMe substituents, led us to re-examine the pK_a decrements induced by electron-withdrawing groups as a function of topological distance to an amine in a systematic manner.

3. Trends for pK_a Prediction of Amines

3.1. Alkyl substituents

While gas-phase acidities of alkylammonium ions show an increase in pK_a with alkyl substitution ($NH_4^+ < RNH_3^+ < R_2NH_2^+ < R_3NH^+$), it is well known that the less-favorable solvation of a protonated tertiary ammonium ion lowers its pK_a in water, leading to the series NH_4^+ (pK_a 9.2) < $MeNH_3^+$ (10.6) < $Me_2NH_2^+$ (10.8) > Me_3NH^+ (9.8).[21] The pK_a values are relatively insensitive to alkyl chain length, except for methyl versus ethyl groups (or longer alkyl chains, Figure 5a). α-Branching in an aliphatic substituent tends to increase the basicity of only tertiary amines, but not primary or secondary amines. This may be interpreted by the steric bulk around the N atom leading to a decrease of the amine's pyramidal shape and thus an increase of the nitrogen lone-pair energy with a concomitant destabilization of the neutral amine. Single β-branching has little or no influence in all three types of aliphatic amines; however, double β-branching, such as in neopentylamine, decreases the pK_a (Figure 5b). This finding can be rationalized by a destabilizing effect of the neopentyl group in the protonated ammonium ion due to unfavorable solvation in staggered conformations and unavoidable 1,5-type steric interferences. The α- and β-branching effects on amine basicity are nicely corroborated by pK_a values recently measured for a short series of *N*-(4′-arylbutyl)-*N*,*N*-dimethylamines (Figure 5c), containing a *gem*-dimethyl group in α-, β-, or δ-position of the amine group.[22,23]

Aliphatic ring size seems to have little influence on amine basicity (Figure 5d). Exceptions are the 3-membered aziridine systems, which are substantially less basic.

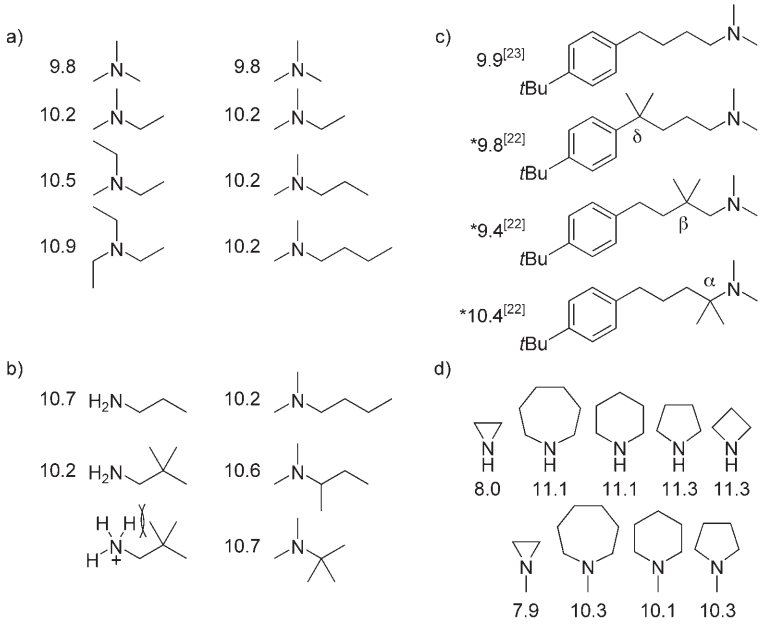


Figure 5. pK_a values of acyclic and cyclic aliphatic amines.

3.2. σ-Acceptor substitution: general remarks

As clearly demonstrated in the preceding analysis of the pK_a values of substituted tricyclic thrombin inhibitors, the incorporation of strong σ-acceptor substituents can modulate the basicity of an amine group dramatically. Fluorine, sulfones, various neutral O- or N-containing functional groups, and to a lesser extent nitriles, are σ-acceptors commonly encountered in medicinal chemistry. For a general treatment of σ-acceptor properties of C–X bonds in which X is a main-group element from groups IVa–VIIa in the periodic table, the reader is referred to a recent article by Alabugin and Zeidan[24] and to comprehensive tables of Hammett σ-parameters.[25]

3.3. Fluorine substitution in open-chain aliphatic amines

Fluorine substitution in the vicinity of an amine center not only lowers its basicity but also enhances metabolic stability, for example, against oxidation or dealkylation by cytochrome P450 enzymes, one of the major incentives for fluorine introduction in lead-optimization processes. In open-chain molecules, fluorination at the β-position to an amine center leads to a decrease in pK_a of ~1.7 units for each additional fluorine atom (Figure 6a). It appears that each additional fluorine contributes similarly to electron withdrawal from the amine and thus to a successively larger destabilization of the protonated ammonium form.

Increasing the number of carbon atoms between F acceptor and amine sites leads to an exponential attenuation of the pK_a shifts, being typically a factor of 0.5 between two homologs that differ by one σ-bond between the fluorine and amine function (Figure 6b). The additional sp^3 carbon atoms act as insu-

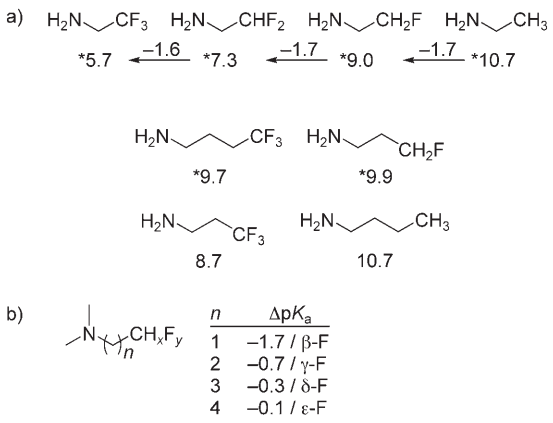


Figure 6. a) The additive effect of multiple fluorine atoms on amine basicity; the pK_a values for the fluorinated ethylamines were re-measured and confirm those given in the MedChem database,[1] except for difluoroethylamine (MedChem database: 7.5); experimental ΔpK_a values are indicated above the arrows. b) Dependence of average pK_a shifts (ΔpK_a) induced per fluorine substituent as a function of its topological distance from the basic center.

lators, leading to a nonlinear loss of the acceptor magnitude. The table in Figure 6b summarizes the approximate pK_a-lowering effects by one fluorine atom at a specified position derived from various singly and multiply F-substituted amines, assuming additivity of the observed F-induced pK_a decrements.[13] Based on these data, one can easily and accurately calculate the approximate pK_a value of a multiply fluorinated acyclic aliphatic amine. However, this additivity scheme may have its intrinsic limitations if many (strong) σ-acceptors are present which may attenuate the σ-transmissions of more remote acceptors and which may cooperatively stabilize conformations in which the basicity-lowering effects of certain groups are characteristically different (see Sections 3.4 and 4).

3.4. Fluorine substitution in cyclic aliphatic amines

As indicated in Section 2 and advocated by Perrin and co-workers[13,14] in cyclic systems the electron-withdrawing effect towards the σ-acceptor is transmitted via both σ-pathways connected to the nitrogen atom. The idea is outlined in Figure 7a.

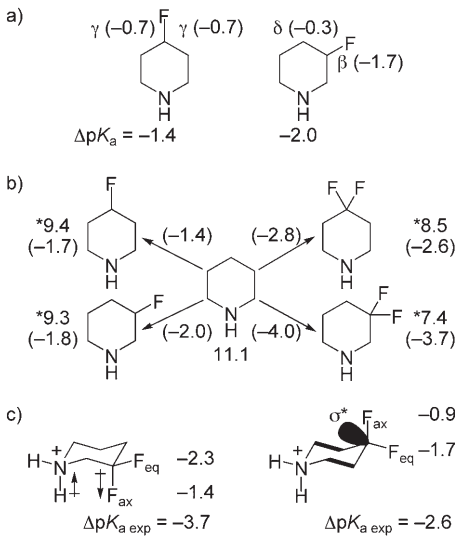


Figure 7. a) Consideration of the two available σ-transmission paths in predicting pK_a shifts for piperidine as a result of F introduction in the 4- or 3-position, taking the ΔpK_a values of Figure 6b. b) Experimental pK_a values of mono- and difluorinated piperidines, and ΔpK_a values (referenced to the parent piperidine) given in parentheses below; the estimated ΔpK_a values based on the pK_a shifts of Figure 6b are placed parenthetically above and below the arrows. c) ΔpK_a values for equatorial and axial F substituents in piperidine, as derived from the pK_a shifts given by Bols and co-workers for equatorial F substituents in the 3- and 4-positions of polyhydroxypiperidine derivatives,[26,33] and the experimental ΔpK_a values of 3,3- and 4,4-difluoropiperidine with possible rationales for the conformationally differentiated ΔpK_a effects.

Taking 4-fluoropiperidine as an example, the fluorine is located in γ-position to the amine when passing along both alkyl chains (Figure 7a). According to the table in Figure 6b, this results in a theoretical pK_a shift of −1.4 with respect to unsubstituted piperidine. In comparison with the decrement of ΔpK_a = −1.7, estimated from the pK_a value of a polyhydroxypiperidine derivative carrying an equatorial fluorine substituent in the 4-position,[26] this approximation is reasonable, albeit an underestimation. The actual measured pK_a value of 4-fluoropiperidine is 9.4 (Figure 7b), which is highly consistent with the somewhat larger pK_a decrement. By analogy, the pK_a shift in 3-fluoropiperidine can be calculated using the values for β- and δ-fluorination, resulting in an expected decrease of 2.0 pK_a units (Figure 7a): again, close to but below the decrement of 2.3, estimated from a pair of polyhydroxylated piperidines, in which one compound contains a fluorine substituent in a 3-equatorial position.[26] This pK_a decrement would have us expect a pK_a value for the 3-fluoropiperidine of 8.8; however, the actual measured pK_a is 9.3 (Figure 7b), with a concomitant ΔpK_a of −1.8. In a series of elegant NMR studies, Lankin, Snyder, and co-workers established that in various organic and water solutions, protonated 3-fluoropiperidine exists exclusively as a single conformer with the fluorine in axial orientation, whereas after deprotonation the substituent adopts the equatorial position.[27] In the axial orientation the dipole of the polar C–F bond is favorably aligned antiparallel to the dipole of the H–N^+ bond. This arrangement stabilizes the protonated state and explains why the experimental pK_a value of 3-fluoropiperidine is higher than expected. Thus, 3-fluoropiperidine presents an interesting case, in which the experimental pK_a shift (−1.8) would be expected to lie between that of an equatorial 3-F substituent (−2.3) and that of an axial 3-F substituent (−1.4, see Figure 7c).

Based on the experimental pK_a values of 4,4- and 3,3-difluoropiperidine (Figure 7b) and the pK_a decrements of 1.7 and 2.3 for equatorial fluorine substituents in the 4- and 3-positions, respectively, the pK_a decrements due to the second (axial) fluorine are calculated to be, respectively, 0.9 and 1.4. Thus, pK_a decrements for axial fluorine substituents are considerably smaller than for the equatorial counterparts and reflect the dramatic dependence of ΔpK_a values on the relative orientation of a fluorine substituent with respect to the amine function. Gratifyingly, these results are well corroborated by the pK_a shifts derived from experimental pK_a values in the compound series **42**, *cis*-**43**, *cis*-**44**, and *trans*-**44** (Figure 8a),[28] as well as for the ΔpK_a of −0.9 for an axial 4-fluoro substituent, as obtained from the pK_a values reported for **45** and its 4-fluoro derivative **46** (Figure 8b). The average ΔpK_a values for conformationally differentiated fluorine substituents in the 3- and 4-position of piperidine (−1.8 and −0.7, respectively) compare remarkably well with the corresponding respective ΔpK_a values obtained for β- and γ-fluorine substituents in acyclic aliphatic amines.

In this context, we report the pK_a values measured for the simple pair of epimeric 4-benzyl-3-fluoropiperidines, *cis*-**47** and *trans*-**47** (Figure 8c). The difference between their pK_a values (0.5) is only about half of what we might have expected (~0.9) from our previous discussions. Close inspection of their conformational behavior as neutral base and protonated salts in water by NMR spectroscopic analysis reveals that *cis*-**47** keeps the 3-fluorine substituent in axial position in both its neutral and protonated form (Figure 4a SI). By contrast, the epimeric

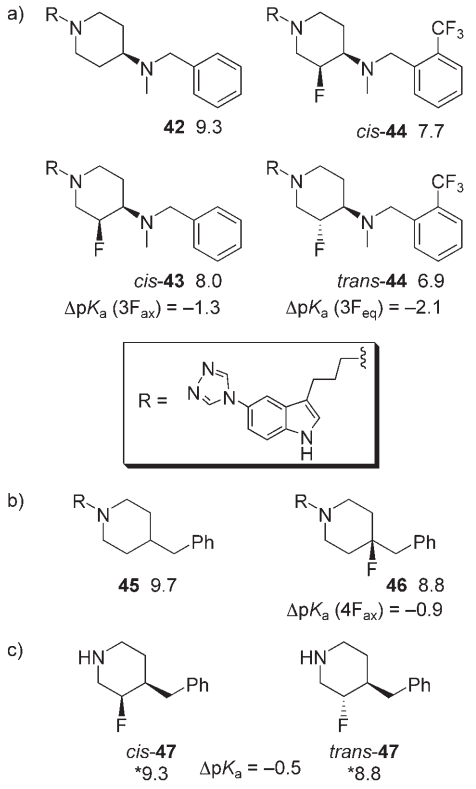


Figure 8. Confirmation of the unequal ΔpK_a effects of axial and equatorial fluorine substitution in a series of piperidine derivatives.[28]

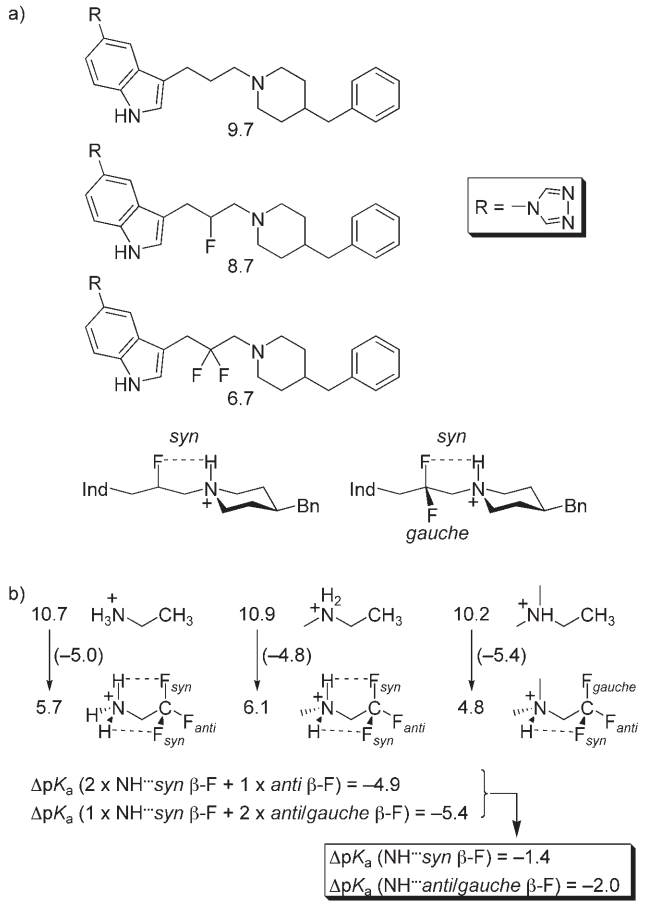


Figure 9. a) Unequal pK_a shifts observed for the first and second F atom introduced into the aliphatic linker attached to the N atom in piperidine derivatives[28] and possible rationalization in terms of the presence and absence of a 1,3-*syn* CF···$^+$NH interaction stabilizing the protonated ammonium ion. b) ΔpK_a values for the replacement of a β-CH_3 by a β-CF_3 group in primary, secondary, and tertiary amines and possible rationalization in terms of the presence and absence of 1,3-*syn* CF···$^+$NH interactions stabilizing the protonated ammonium ion. Assuming that a β-fluorine atom in an anti-periplanar or *gauche* conformation without *syn* interaction results in similar ΔpK_a effects, the corresponding pK_a shift parameters for 1,3-*syn* and *trans*/*gauche* arrangements can be estimated; they are in remarkably close agreement with the parameters obtained for equatorial and axial F substituents in piperidines (Figures 7 and 8).

trans-**47** adopts the expected diequatorial form only in its neutral state, but when protonated, exhibits the characteristics of two rapidly interconverting, roughly equally populated chair forms with the substituents in diequatorial and diaxial arrangements (Figure 4 b SI). Hence, *trans*-**47** does not exhibit the full pK_a decrement expected for an equatorial 3-fluoro substituent, as it acquires substantial 1,3-*syn* CF···$^+$NH stabilization in its partially populated diaxial conformer.

The fluorine-induced pK_a shifts identified for piperidines can be translated back into the acyclic situation. Take, for example, the case reported by van Niel et al.,[28] in which one and two fluorine atoms were introduced into the propyl linker unit between a piperidine and an indole unit (Figure 9 a). Introduction of the first fluorine atom results in a moderate pK_a shift by only −1 unit, whereas the second F atom produces a very pronounced additional shift of −2 units. The average pK_a decrement per fluorine is 1.5 units, in good agreement with the shift expected for a fluorine in β-position to an amine (Figure 6 b). However, the dramatic difference between the two pK_a shifts clearly points to a specific inequality of the two F atoms. Because the propyl chain in these molecules most likely adopts an all-*trans* conformation, the first F atom will assume a *gauche* conformation with respect to the piperidine N atom. The most favorable position will be the one in which the polar C–F bond is 1,3-*syn* to the N^+–H bond of the protonated piperidine, with concomitant stabilization of the protonated base, while the second F atom will have to take the other *gauche* position devoid of favorable 1,3-*syn* interactions with the ammonium group. This case not only nicely illustrates that the same principles operate similarly in both cyclic and acyclic systems, but also provides an important new piece of information: F atoms in β-position, either *trans* or *gauche* but not 1,3-*syn*, to a (protonated) amine group, produce similar (strong) pK_a shifts.

With this information in hand, the case of trifluoroethylamine and its *N*-methyl and *N*,*N*-dimethyl derivatives is illuminative. In all three cases, fluorine atoms can be assigned to *syn* and *anti*/*gauche* orientations with respect to the hydrogen atoms at the protonated amine (Figure 9 b). For primary and secondary amines, $\Delta pK_a \sim -4.9$ is observed when all hydrogens in β-position are replaced by fluorine. Two 1,3-*syn* CF···$^+$NH interactions are possible, while one fluorine in *anti* orientation does not interact with the amine hydrogens. For the tertiary amine, $\Delta pK_a = -5.4$ indicates a decreased stabilization of the

protonated state, as only one 1,3-*syn* interaction is possible, while two fluorines adopt non-interacting *anti* and *gauche* orientations. Taking these results together, the pK_a shift contribution of a single 1,3-*syn* CF···$^+$NH interaction can be calculated to be $\Delta pK_a = -1.4$, whereas the *anti*- or *gauche*-oriented fluorines contribute $\Delta pK_a = -2.0$, in good agreement with the basicity shift effects identified for β-fluorinated piperidines (Figure 7c).

3.5. Fluorine substitution in benzimidazoles

Although this review deals mainly with the effects of σ-acceptor substituents on the pK_a values of aliphatic amines, one example from our research should also be added to illustrate the predictability of the effects of such substituents on the basicity of aromatic amines. Specifically, we present fluorine effects on the pK_a values of benzimidazoles, common scaffolds in medicinal chemistry and used as a central platform in our inhibitors of the metalloprotease neprilysin (NEP).[29,30]

Figure 10 gives the pK_a values for the protonation (pK_{a1}) and deprotonation (pK_{a2}) of benzimidazole (**48a**) and its differently fluorinated derivatives **48b**–**j**. Interesting correlations between the pK_a values and the degree of fluorination at the benzene ring were observed. They are explicitly shown in Figures 5SI–10SI and are summarized in the following. Note that for nonsymmetrical cases such as **48b**, two different values for each pK_{a1} and pK_{a2} would have to be considered in principle if tautomerization were slow relative to pK_a measurements; however, we have no experimental evidence for "split" pK_a values.

Upon initial inspection of the data, increasing fluorination of the phenyl ring strongly affects both pK_a values, as expected,

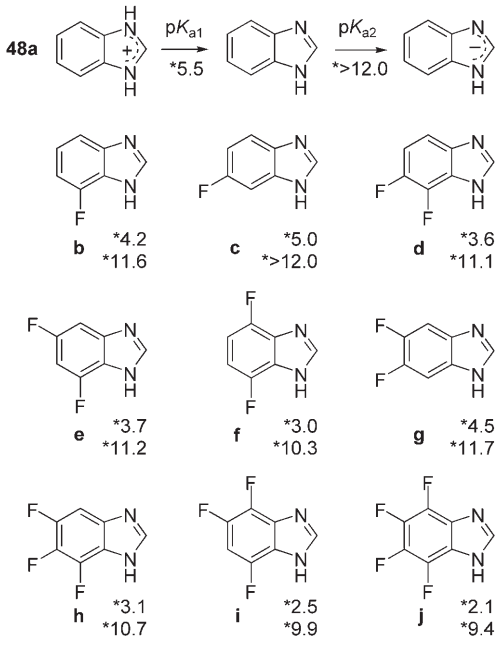


Figure 10. Experimental values for pK_{a1} (deprotonation of benzimidazolium) and pK_{a2} (deprotonation of benzimidazole) of benzimidazole **48a** and all its possible derivatives **48b**–**j**, having one to four F substituents in the benzene moiety.

which shift in the case of pK_{a1} from 5.5 (**48a**) to 2.1 (**48j**), and in the case of pK_{a2} from >12 (**48a**) to 9.4 (**48j**). At equal degrees of fluorination, remarkable variation in the pK_a values is observed which corroborates the importance of the position of the fluorine atoms. Introduction of fluorine *ortho* to an N atom in the benzimidazole system has a larger effect than *meta* substitution. Thus, *ortho* derivative **48b** features pK_{a1} 4.2, whereas for *meta* derivative **48c**, a value of 5.0 is measured. Similarly, in the difluorinated series, **48f** with two *ortho* fluorines features pK_{a1} 3.0, whereas the value for bis-*meta*-substituted **48g** is 1.5 units higher (4.5).

A more detailed analysis of the data shows remarkably consistent correlations. When comparing pairs of compounds that differ by only one additional fluorine at the *ortho* position to an imidazole nitrogen, a decrease in pK_{a1} of 1.2 ± 0.2 units is observed, independent of the number of fluorine atoms and their substitution pattern in the starting compound (Figure 5SI).

Similarly, introduction of an additional fluorine at the *meta* position decreases the pK_{a1} values by 0.5 ± 0.1 units for all pairs of compounds (Figure 6SI). Shifting a fluorine from *meta* to *ortho* leads to a decrease in pK_{a1} by 0.7 ± 0.1 for each series of mono- to tri-fluorinated benzimidazoles, again corroborating the weaker effect of *meta* fluorination (Figure 7SI). Interestingly, and quite remarkably, virtually identical patterns are observed for pK_{a2} values as a function of the F-substitution pattern (Figures 8SI–10SI).

3.6. Oxygen-containing functional groups

The oxygen in ethers and alcohols also acts as a strong σ-acceptor and is well suited, but remarkably underused, as a pK_a-modulating substituent (see also Figure 4). Figure 11a shows that the pK_a values of amino groups change incrementally as a function of the distance between N-donor and O-acceptor in open-chain aliphatic hydroxy, ether, and acetoxy derivatives, as previously demonstrated for fluorinated compounds. The ΔpK_a values in Figure 11a refer to *N*,*N*-dimethylbutylamine (pK_a 10.2) as the parent base. It is apparent from the data that hydroxy and methoxy groups have a nearly identical inductive effect. The influence of these acceptors drops considerably for substitution at or beyond the δ-position. In comparison, an acetoxy group causes distinctly larger decreases in pK_a, as it is more efficient in electron withdrawal due to the additional carbonyl group. The data nicely illustrate that the pK_a shifts by hydroxy or methoxy substituents at a given topological distance from the basic center are approximately equal to those of an acetoxy substituent at a distance one bond further away.

For cyclic ethers such as morpholine, similar effects can be identified. As the electron-withdrawing effect of the oxygen atom acts through both σ-pathways, as already pointed out in Section 3.4, morpholine can be viewed as a secondary amine with double methoxy substitution in β-position with an expected pK_a shift of -1.2 units through each σ-path (Figure 11b). Indeed, the experimental values for both morpholine and *N*-methylmorpholine are close to those expected.

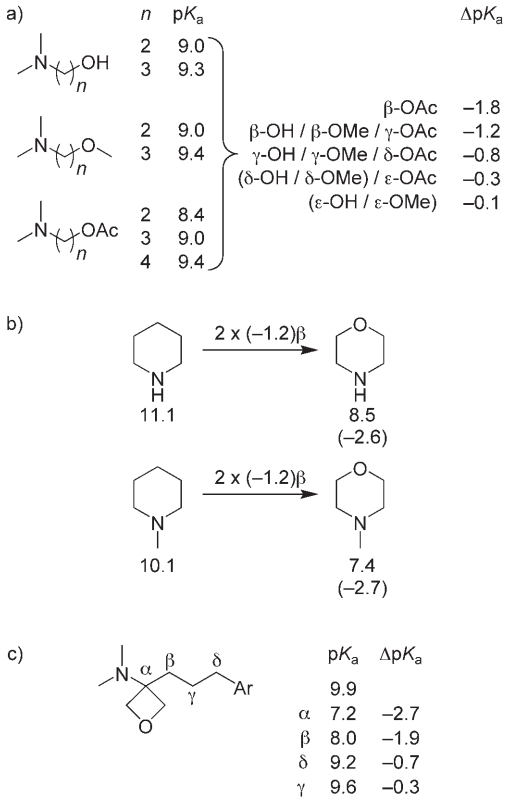


Figure 11. Effects of oxygen-containing functional groups on amine pK_a values. a) Distance dependence of ΔpK_a effects in open-chain alcohols, ethers, and acetates; ΔpK_a effects for OH and OMe groups in δ- and ε-positions are estimated by comparison with the corresponding value for the OAc group and assuming continued attenuation. b) The consideration of two σ-transmission pathways in morpholine derivatives provides a consistent rationalization (estimated value indicated above the arrow) of the experimental pK_a values (shown with ΔpK_a values in parentheses) below the structures. c) Experimental pK_a values in *N*-arylbutyl amines bearing an oxetane unit at different topological distances from the amine center.[23] The comparison of ΔpK_a values indicates a distinct deviation from the more typical exponential attenuation observed for other electron-withdrawing groups.

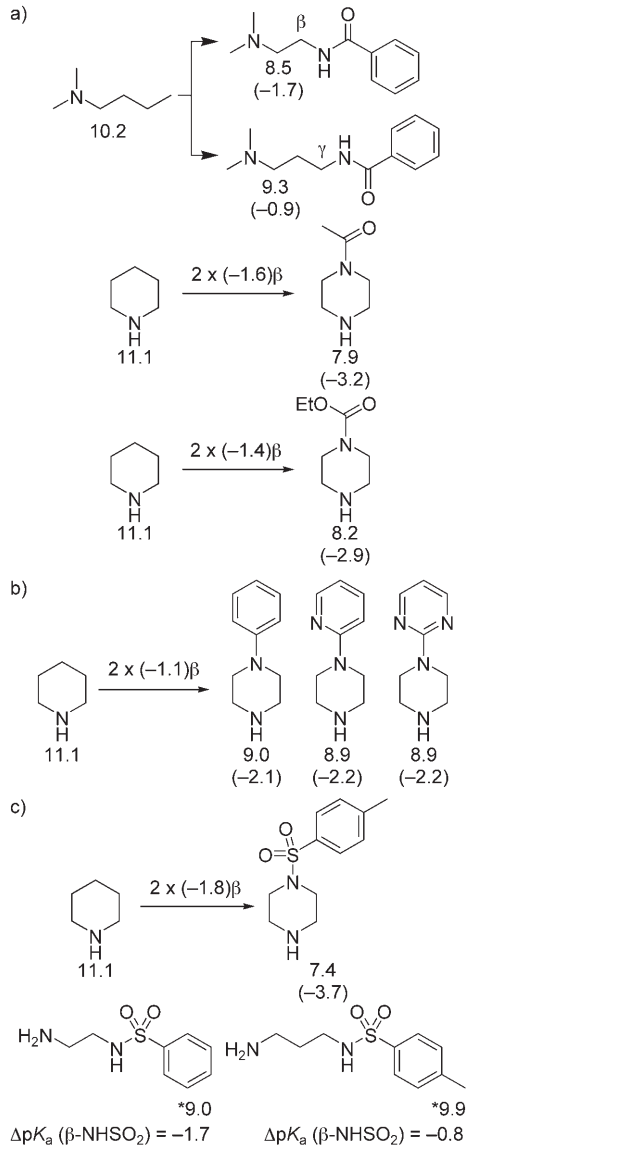


Figure 12. Modulation of amine basicity by a) amides and carbamates, b) *N*-aryl- and *N*-heteroarylamines, and c) sulfonamides. Experimental pK_a and ΔpK_a values given in parentheses below. Estimated ΔpK_a values (taking into account two σ-transmission pathways in the cyclic systems) are placed parenthetically above the arrows. The pK_a shifts in a) and c) are estimated with reference to *N*-*n*-butyl-*N*,*N*-dimethylamine (pK_a 10.2) and *n*-butylamine (pK_a 10.7), respectively.

Another useful class of ethers are oxetanes, as they not only modulate amine basicity, but can also favorably affect other essential physicochemical and pharmacological compound properties.[23] The compounds in Figure 11 c demonstrate the applicability of the rules for ΔpK_a of open-chain ethers and alcohols given in Figure 11 a. Closer inspection of these pK_a data reveals that the pK_a decrements as a function of topological distance between the oxetane and the amine group are not attenuated in a strictly exponential fashion, as may have been expected. This hints to specific conformation-dependent intramolecular interactions operating differently in various members of this series. They are the focus of ongoing research.[22]

3.7. Nitrogen-containing functional groups

Among the many N-containing functional groups, neutral amides, carbamates, sulfonamides, and *N*-arylamines play a considerable role in medicinal chemistry, and some of their pK_a-lowering effects are documented, albeit by only a comparatively small set of available data (Figure 12). We note again relatively consistent patterns for ΔpK_a shifts in going from the monoacylated ethylenediamine to the cyclic piperazine cases, taking into account the dual σ-transmission paths in the latter, as well as in going from the *N*-monoacylated ethylenediamine to the corresponding propylenediamine, allowing us in principle to estimate the pK_a shifts due to more distantly placed *N*-acylamino groups. We also note a slight reduction of ΔpK_a effects for such groups in β-position in going from *N*-acetyl, to *N*-benzoyl, and to *N*-ethoxycarbonyl, as might have been expected. Likewise, *N*-aryl tends to have distinct but considerably weaker effects that are similar to, but somewhat smaller than those of hydroxy, methoxy, or acetoxy groups. Of particular in-

terest is the case of *N*-tosylpiperazine, from which it is apparent that a sulfonamide function exhibits a very strong pK_a-lowering effect, more pronounced than that of an *N*-acyl unit and perhaps more comparable to that of a keto-carbonyl unit (see Section 3.9). Again, we note a consistent pattern in comparing this pK_a decrement with those derived for two acyclic monoarylsulfonylated diamines using *n*-butylamine (pK_a 10.7) as reference.

3.8. Sulfur-containing functional groups

The sulfone group is an important module in medicinal chemistry owing to its powerful electron-withdrawing character that affects many physicochemical parameters accordingly, and that is topped only by highly fluorinated or cyano substituents. Figure 13a shows how arylsulfones lower amine basicities in a saturated acyclic system, taking *n*-butylamine (pK_a 10.7) as reference.[31] The basicity-lowering effect of sulfones can again be viewed as an exponential function of the topological distance to the amine center. Similar effects are also found for secondary and tertiary amines. In addition, the example in Figure 13b affirms again that both σ-pathways have to be considered in cyclic amines. A β-sulfone group in an acyclic system would result in a pK_a shift of −2.9 units which is consistent with the experimental ΔpK_a value of −5.7 for the cyclic case.

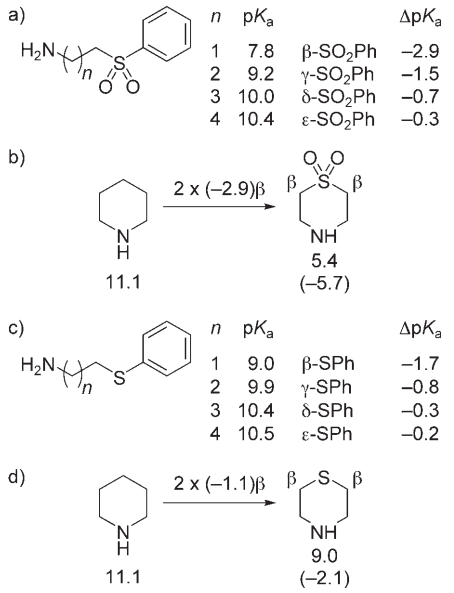


Figure 13. Modulation of amine basicity by a) aryl sulfones in acyclic amines, b) a sulfone unit in a cyclic system, c) aryl thioethers in acyclic amines,[31] and d) a thioether unit in a cyclic system. Experimental pK_a values are followed by ΔpK_a values given in parentheses below. Estimated ΔpK_a values (taking into account two σ-transmission pathways in the cyclic systems) are placed parenthetically above the arrows.

Phenylsulfoxides may exhibit similar pK_a-lowering effects as corresponding phenylsulfones (Figure 11 SI), although with only one reliable example currently available, no general statement can be made.

It is also interesting to look at the pK_a-lowering effect of the phenylthio group (Figure 13c). As expected, the ΔpK_a values are considerably lower than those of structurally analogous sulfones, but larger than for a methylthio group (β-(methylthio)ethylamine, pK_a 9.5 (ΔpK_a = −1.2)).[32] The latter pK_a decrement is consistent with the experimental value of thiomorpholine (Figure 13d, pK_a 9.0 (ΔpK_a = −2.1)), taking into account two σ-transmission paths in the cyclic system.[13]

3.9. Carbonyl and carboxyl groups

The electron-withdrawing effects of carbonyl and carboxyl derivatives on the basicity of neighboring amine groups are shown in Figure 14. The data for ketones depicted in Fig-

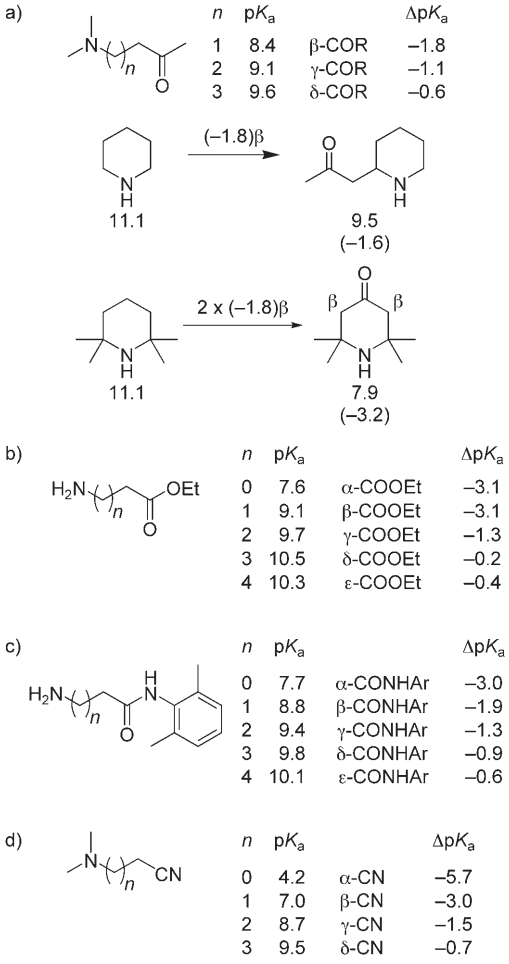


Figure 14. Modulation of amine basicity by carbonyl-containing functional groups in a)–c) and nitriles in d). The respective pK_a shifts are estimated with reference to *N*-*n*-butyl-*N*,*N*-dimethylamine (pK_a 10.2) and *n*-butylamine (pK_a 10.7). For the piperidine examples in a), experimental pK_a values are given with ΔpK_a in parentheses below; estimated ΔpK_a values (taking into account two σ-transmission pathways in the cyclic systems) are placed parenthetically above the arrows. The pK_a values for the two nitrile cases in d) with $n = 2$ and $n = 3$ are estimated from experimental pK_a values for the corresponding *N*,*N*-diethylamine derivatives (9.3 and 10.1, respectively) by taking into account a pK_a shift of −0.6 in going from *N*,*N*-diethyl- to *N*,*N*-dimethylamines.[31] The ΔpK_a values for nitriles represent averages from pK_a shifts observed for various amines.

ure 14a are referenced to *N*,*N*-dimethylbutylamine (pK_a 10.2). The amine pK_a decrement induced by a keto-carbonyl group in β-position appears to be in the range of 1.6–1.8 units, as judged by both acyclic and cyclic systems. This value range is similar to pK_a decrements observed for carboxyester and carboxamide groups in β-position to an amine (Figure 14b and c). However, there is some scatter in the currently available pK_a data as well as a suspected systematic shift towards lower pK_a values in the series of ω-aminocarboxyesters and amides, suggesting the need to reassess various experimental data prior to drawing further conclusions. Nevertheless, it appears that the carbonyl group accounts for most of the electron withdrawal in all these cases.

Finally, nitriles belong to the most powerful pK_a-lowering functional groups, as illustrated by the pK_a shifts in Figure 14d. These effects parallel those identified for the phenylsulfone group and are similar or somewhat larger than those of a CF_3 group at an equal topological distance from an amine unit (see Section 3.3).[31] Again, an exponential attenuation of the pK_a decrements as a function of topological distance between the cyano and amino function is evident.

Carboxylic acids represent a special class of basicity-lowering functional groups. When in close proximity to the amine, the influence on amine basicity is relatively small due to intramolecular stabilization of the protonated nitrogen atom by the carboxylate anion, present at physiological pH (Figure 15). This

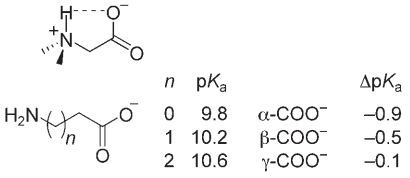


Figure 15. Intramolecular stabilization of the protonated ammonium center decreases the basicity-decreasing effect of adjacent carboxylates.

results in markedly lower ΔpK_a decrements relative to the other carbonyl and carboxyl derivatives shown in Figure 14.

4. Electronic and Stereoelectronic Considerations

The conformational dependence of functional-group-induced pK_a decrements is only at the beginning of being identified and understood, but as discussed in Section 3.4, it can be substantial for fluorine-substituted piperidine derivatives. In the systematic study of polyhydroxylated piperidines, Bols and coworkers identified distinct differences of pK_a shifts for axial and equatorial hydroxy groups.[26,33] Without exception, axial OH groups at the 3-position of the piperidine ring resulted in markedly decreased ΔpK_a values compared with equatorial OH groups. Interestingly, the difference in pK_a shifts of 1.0 ± 0.3 units, observed for many equatorial–axial pK_a couples, corresponds closely to the ΔpK_a differences observed for fluorine atoms in β-position to a cyclic or acyclic amine, as discussed in Section 3.4.

Different pK_a decrements were also diagnosed for hydroxy groups in the 4-position of a piperidine ring,[26] the effect of 4-OH_{eq} (−0.6) being more pronounced than that of 4-OH_{ax} (−0.2). This qualitatively parallels the findings for 4-fluorinated piperidines, as mentioned in Section 3.4. For the interpretation of the stronger pK_a-modulating effects of a polar C–X bond in the equatorial 4-position, either a better aligned C–X bond dipole or pronounced through-bond σ-conjugative transmission through properly aligned σ^*_{C-X} and intervening σ_{C-C} orbitals may be invoked (see Figure 7c).[26]

Here we include two examples with recently measured pK_a values that not only complement these findings, but also illustrate another important conformational aspect. The first is shown in Figure 16.

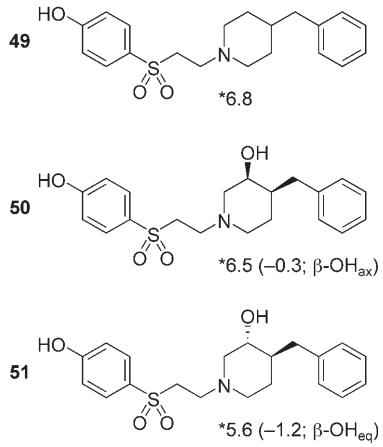


Figure 16. A 3-hydroxy group in piperidine derivatives exhibits conformationally dependent pK_a shifts similarly to fluorine (see Figures 7c and 8a). The pK_a-lowering effect of the axial OH group is much less pronounced than that of the equatorial OH group.

The parent compound **49**, a 4-benzyl piperidine with an arylsulfonylethyl substituent at the N atom, exhibits a pK_a value of 6.8, which corresponds nicely to expectation, taking into account the strong basicity-lowering effect of an arylsulfone group in β-position and the slight pK_a-reducing effects of the remote phenyl group (~ −0.2) operating through both cyclic σ-paths. Taking compound **49** as reference, the presence of an OH group in the 3-position of the piperidine in the *cis* (axial) derivative **50** results in only a small pK_a shift as anticipated. By contrast, the basicity-lowering effect is much more pronounced in the *trans* (equatorial) epimer **51**. Gratifyingly, the difference in pK_a shifts amounts to one pK_a unit, as observed for polyhydroxylated piperidines.[26]

The second example is given for a cyclic and an acyclic representative of a series of 3,3,3-trifluoro-2-hydroxypropylamines (Figure 17).[34–37] This functional group has been of interest in medicinal chemistry[35,36] and as a chiral auxiliary in asymmetric synthesis.[37] The measured pK_a values of the two hydroxylated amines indicate a pK_a shift of approximately −2.3 relative to the corresponding *N*-propylamines for the combined effects of the CF_3 and the OH group both in β-position to the amine center. Because the ΔpK_a effect due to a β-CF_3 group is about

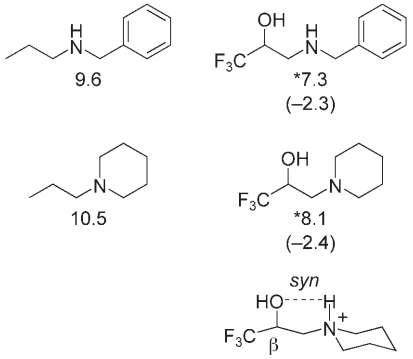


Figure 17. Two examples of compounds bearing a 3,3,3-trifluoro-2-hydroxypropylamine unit. The hydroxy group is forced by the bulky CF_3 group to take a *syn*-orientation to the $^+$N–H bond, thus stabilizing the protonated ammonium center.

−2.0, the additional pK_a shift due to the β-OH is only ~−0.3. However, this would be expected if the bulky CF_3 group is assumed to be *trans* to the amine center, thus forcing the hydroxy group into a *gauche* orientation, where it could stabilize the protonated form by similar favorable interactions as diagnosed for 3-hydroxypiperidine derivatives. Several X-ray crystal structures for different 3,3,3-trifluoro-2-hydroxypropylamines are available,[34–36] all of which show a conformational arrangement of the CF_3 and OH groups as depicted in Figure 17.

The first example (Figure 16) illustrates a case with good additivity of individual functional group effects operating essentially independently of each other. In the second example (Figure 17), the two pK_a-modulating functions cooperate in specific and mutually exclusive conformational orientations. In such a case, a simple additivity scheme may not hold if one or both functional groups exhibit a distinct conformational dependence of pK_a-lowering effects.

The conformational dependence of pK_a shifts has been recognized only in recent years, and only fragmentary data are available for most common functional groups. Much more experimental work is needed based on carefully designed model systems to clarify such important effects. All examples given above refer to systems in which the functional group (F or OH) is placed either in the 6-membered piperidine ring or in a simple aliphatic unit, both being able to adopt well-staggered conformations. Whether and to what extent the pK_a-lowering effects observed in 6-membered rings or acyclic systems can be transferred to cases in which the electron-withdrawing effects are transmitted through σ-paths with poorly staggered or even eclipsed arrangements, as in substituted 5- and 4-membered amines or in bicyclic ring systems, remains to be explored.

This is illustrated by a representative group of substituted pyrrolidine derivatives (Figure 18). The experimental pK_a values may be referenced to the unsubstituted pyrrolidine (pK_a 11.3) or *N*-benzylpyrrolidine (estimated pK_a ~8.9; for details of the calculations, see Figure 12 SI), which then results in the total pK_a decrements indicated in parentheses below each formula. The expected total downshifts, calculated by pK_a decrements derived from acyclic systems (or 6-membered model ring systems) and taking into account the dual σ-transmission paths, are given parenthetically in italics. The calculated total decrements must be reduced typically by a correctional factor of 0.6–0.8 in order to match the observed values. This may indicate that σ-transmission effects in these 5-membered ring systems are suboptimal compared with those in well-staggered acyclic or 6-membered ring systems.

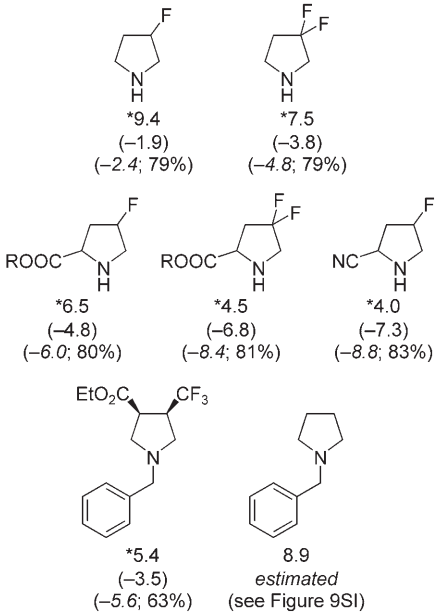


Figure 18. Experimental pK_a values of σ-acceptor-substituted pyrrolidine derivatives. The first values in parentheses give the actual ΔpK_a relative to unsubstituted pyrrolidine (11.3). The expected total downshifts are given in italics in a second set of parentheses; they must be multiplied by a correction factor of 0.6–0.8 to match the observed values. For details of the calculations, see Figure 12 SI.

Returning to the outset of tricyclic thrombin inhibitors of Figure 4, we note that the annulated succinimide unit positions each of the two imide carbonyl groups in both a β- and γ-position to the N atom of the central pyrrolidine. Hence, taking into account both σ-transmission paths for each carbonyl unit, and accounting for the various other substituents accordingly (see Figure 13 SI), the calculated total pK_a decrements again overestimate the electron-withdrawing effects relative to the corresponding values derived from the measured pK_a values of compounds (±)-**26** to (±)-**41** with reference to the pK_a value (11.4) of the unsubstituted hexahydropyrrolizine. Interestingly, a correction factor of about 0.6–0.8 is again required to match calculated and experimentally derived ΔpK_a values. As previously emphasized, a more detailed understanding of pK_a decrements in imperfectly staggered systems requires more experimental work with carefully designed model systems.

5. Comments on pK_a Prediction Methods and Developments of Computational Algorithms

We already referred to the outstanding publications by Perrin and co-workers, allowing us an easy and remarkably accurate

prediction of pK_a values.[13,14] Their approach is pragmatic and more concerned with experimentally derived linear free-energy relations for pK_a predictions than with attempts to derive pK_a values from first principles. In this way, the authors developed easy-to-use algorithms for certain classes of compounds which can be used as guiding rules for a large variety of molecules. More importantly, the relationship between molecular structure and pK_a-lowering effects remains transparent, which is a prerequisite for rational, structure-guided modulation of amine basicity.

Numerous other publications deal with the topic of computational pK_a predictions, but most of the algorithms are applicable only to limited classes of compounds for which the calculation method was parameterized. Unfortunately, the amount of useful and publicly available data is remarkably limited and increases at a relatively slow rate.[38] Furthermore, algorithms built on large fragment-based parameter sets applying multi-linear correlation or neural-network-type techniques tend to become black boxes that, even with reasonably accurate predictions within the substructural calibration domain, make it difficult to the user to back-translate calculated results into structural features.

Up to now, all prediction methods have been based on molecular topology only and do not take into account conformational or other 3D structural aspects. In view of the important and sometimes substantial yet still not sufficiently explored conformational effects, any such method has to be applied with due care.

An additional complication may arise from differences in solvation of the protonated versus unprotonated amine as well as the electron-withdrawing substituents, particularly in bicyclic or otherwise sterically congested amines. Such effects may operate in the tricyclic thrombin inhibitors as well as in some of the bicyclic polyhydroxylated piperidine analogues discussed above; they may account for significant pK_a shifts.

Owing to such inherent limitations, currently available computational pK_a prediction schemes are of limited practical value for general applications. Nevertheless, they can be helpful in specific cases to predict trends in series of related compounds.

An in-depth discussion of the merits of the various computational approaches towards pK_a prediction is clearly far beyond the scope of this review. However, the increasing number of recent reports mandates that some of this ongoing work is briefly introduced; this may help the reader to get a quick entry into this developing field.

Besides the experimentally derived pK_a values of tricyclic thrombin inhibitors discussed above, Figures 1–4 also provide estimated values calculated by the commercially available and relatively widely used ACD/pK_a prediction tool.[39] Although the general trends observed for the experimental pK_a values are reproduced for most inhibitors, the calculated pK_a values are systematically some 1–1.5 units too high. Furthermore, this tool does not account for the different pK_a values of epimeric compounds, such as (±)-**24** and (±)-**25** or (±)-**3** and (±)-**4**. More generally, we find reasonable predictions for relatively simple amines for which experimental data are available for calibration. However, for amine compounds with more complex structures or for which no data are yet available in the public domain, the method should be used with due care, as also evident in a recent publication of pK_a values of amines containing a sulfone group at different topological distances to the amine center.[31] It is hoped, however, that this method may be continuously improved as more measured pK_a values become available; it remains to be seen whether this predictive method can be extended to include conformational effects on pK_a as well.

Xing and Glen developed a simple but effective model for pK_a predictions.[40] Based on a compound set of 384 basic and 645 acidic aqueous pK_a values extracted from *Lange's Handbook of Chemistry*,[41] an ionization model was generated. Combined descriptors ranked by cross-validated partial least-squares analysis were mapped onto molecular tree constructs around the ionizable center. The derived model is based on the hypothesis that the ionization of a particular group is dependent on its sub-environments constituted by its neighboring atoms and bonds in topologically defined concentric shells. The idea is thus related to the concept of Perrin et al.[13] In a direct comparison with Perrin's approach on a set of 25 independent test compounds, the described model had slightly less predictive power. In a second phase, Xing and co-workers refined their algorithm for selected and well-represented subclasses of basic and acidic compounds, resulting in improved predictions, albeit at the cost of uniform applicability.[42]

Eckert and Klamt used an algorithm based on improved dielectric continuum solvation methods (DCSMs), also accounting for short-range electrostatics of polar solutes and ions as well as hydrogen bonding.[43] Compared with other algorithms, the model was calibrated with a relatively small dataset of 43 bases, excluding aliphatic amines. A fitted model was derived that was remarkably successful in predicting pK_a values of unsaturated heterocyclic bases, conjugated amines, and primary aliphatic amines. However, the method systematically failed for secondary and tertiary amines, for which separate ad hoc correction factors had to be introduced. In this way, the pK_a values of a test set of 58 more complex bioactive N-containing basic compounds could be predicted reasonably well, with some 85% of the predicted pK_a values lying within one unit of the experimental pK_a values. This method, which has been made commercially available,[43] is still computationally intense, as it requires the energy calculation of the base in its neutral and protonated states by relatively high-level density functional theory (DFT).

Seybold and co-workers performed a theoretical analysis on a limited set of 19 mono-substituted anilines (as well as 19 mono-substituted phenol derivatives) to test the usefulness of different partial atomic charges obtained from high-level ab initio self-consistent field (SCF) calculations for pK_a predictions.[44] Among various electron-density-partitioning schemes, they found partial atomic charges based on Bader's "atoms-in-molecules" scheme, Löwdin's orthogonal atomic orbital method, and natural population analysis to correlate best with experimental pK_a data. However, significant deviations between correlation-predicted and actual pK_a values were noted for several cases. We are not aware of an extension of this

analysis to other classes of organic bases, particularly to non-conjugated amines, to assess the potential of a more general applicability of this method.

A limited set of 15 small aliphatic amines, nine of which having one or more hydroxy groups in β-position to the basic center, was investigated by da Silva and Svendsen using high-level quantum-mechanical calculations of gas-phase basicities, complemented by polarizable continuum methods and Monte Carlo free-energy perturbations to estimate solvation energies.[45] Although this work pointed to the importance of intramolecular hydrogen bonding modulating basicities, the overall pK_a-predictive power of this computationally demanding scheme appears to be limited.

Zhang and co-workers investigated a set of 74 drugs containing a basic center with experimental aqueous pK_a values ranging between 6 and 11.[46] They applied both a multi-linear heuristic model and a nonlinear neural network algorithm based on radial basis functions incorporating a vast set of some 700 topological, geometrical, and quantum-mechanical descriptors. Based on a training set of 58 compounds (thus leaving only 16 compounds for testing) they concluded that the nonlinear neural-network-based method was overall more successful than the linear heuristic model. However, both methods showed a significant scatter of predicted versus experimental pK_a data, which still appears to limit the general applicability of either method.

Nagy et al. found good linear correlations between the pK_a of nitrogen bases and the minimum of the molecular electrostatic potential (MEP), estimated by either semi-empirical or small-basis-set ab initio SCF calculations.[47] This methodology provided reasonable prediction schemes for individual subsets of aliphatic amines, pyridines, and anilines. It was noted that a generalization over all three subsets was not possible, except with a significant sacrifice of pK_a predictability. Furthermore, each compound subset contained only a small number of representatives with limited structural diversity, making it difficult to assess the proposed method regarding its potential for more general applications even within only a given subclass. In a subsequent investigation of solvation effects, Nagy found that hydration at the basic center as well as conformational changes of neighboring groups can strongly impact on the calculated MEP and thus on predicted pK_a values.[48]

A similar finding was described by Manivet and co-workers in a recent theoretical investigation of the basicity of serotonin.[49] Based on high-level DFT calculations, complemented by estimates of solvation effects by a polarizable continuum model, they showed that computed pK_a values may differ substantially depending on the conformation of the aminoethyl side chain, thus on the relative position of the terminal amino/ammonium group relative to the 5-hydroxyindolyl unit.

Brown and Mora-Diez calculated the first pK_a of one di-substituted and 12 mono-substituted benzimidazoles using an approach similar to Eckert and Klamt (see above), also taking into account possible tautomers of the neutral base.[50] Best performance was noted with a standard, but high-level DFT approach. Whereas directly calculated pK_a values differed substantially from experimental data, correlated values gave acceptable agreement. However, it was noted that the application of their method to two mono-substituted benzimidazoles, not included in their original correlation, were moderately successful. Thus, the authors concluded that although their method gives overall good pK_a predictions for this important structural motif, the predictive power may still be insufficient for practical applications.

Finally, Chattaraj and co-workers introduced the concept of "group philicity"[51] to predict pK_a values for a set of 63 small-molecule compounds containing carboxylic and phosphoric acids, alcohols, and anilines.[52] While individual subclasses showed promising linear correlations, this was not the case for all compounds taken together. A polynomial second-order correlation resulted in a significantly improved correlation for all compounds. Whether this "group philicity"-based approach can also be extended to a diverse set of saturated amines and refined to a level of sufficient prediction accuracy to become a method for general practical applications remains to be seen.

In conclusion, most pK_a prediction methods proposed or available today were derived and validated on data of relatively small structural series. Therefore, any application of such methods to compounds with new topological features should be done with due care, and spot-check measurements are highly recommended in such cases. This holds true even for applications of more established tools such as ACD/pK_a, which is based on relatively large datasets.

6. Conclusions

This review provides useful insight for predicting and tuning amine basicities, one of the crucial factors determining molecular physicochemical properties of concern in lead optimization. Since the pioneering overviews by Perrin and co-workers some decades ago, many new data have become available for both relatively complex structures, such as typically encountered in medicinal chemistry, and series of simpler model compounds. This has provided the incentive for a review on the current state-of-affairs regarding structural factors influencing amine basicity along with new complementary experimental pK_a values. Computational pK_a prediction tools are not yet sufficiently sophisticated to be of general practical value in the pharmaceutical industry, but this situation is expected to change in the future, as more experimental data become available, and continued efforts are spent to refine existing tools. While this article primarily addresses scientists in drug-discovery chemistry, it should be equally useful to those in developing crop-protection agents. Furthermore, it should appeal to the broad community pursuing the development of amine ligands for organometallic complexes and, in particular, amine-based organocatalysis, for which fine-tuning of acid/base properties is of utmost importance.[53]

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Keywords: amines · basicity · database mining · medicinal chemistry · pK_a values

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