Bioorganic & Medicinal Chemistry 17 (2009) 1339–1344

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Determination of 1-aryl-4-propylpiperazine pK_a values: The substituent on aryl modulates basicity

Enza Lacivita *, Marcello Leopoldo, Paola De Giorgio, Francesco Berardi, Roberto Perrone

Università degli Studi di Bari, Dipartimento Farmaco-Chimico, via Orabona, 4, 70125 Bari, Italy

ARTICLE INFO

Article history:
Received 1 August 2008
Revised 13 November 2008
Accepted 8 December 2008
Available online 24 December 2008

Keywords:
1-Arylpiperazine
Basicity
pK_a
Potentiometric titration

ABSTRACT

In order to design a potential drug, it is important to know its pK_a because the protonation state of the molecule will be critical for ligand–receptor interaction and for the pharmacokinetic of the molecule. pK_a values of a series of 1-(substitutedphenyl)-4-propylpiperazines were measured to study how the presence of a substituent on the phenyl ring modulates the basicity of N-4 nitrogen. pK_a values indicated that the position of the substituent was crucial. In general, the introduction of the substituent in *ortho*-position of the phenyl ring increased the basicity of the molecule. This effect appeared to be related to steric and conformational effects and not to the electronic properties of the substituent. On the other hand, *meta*- and *para*-substituted derivatives showed a slight decrease of pK_a that was qualitatively consistent with the electronic properties of the substituent.

1. Introduction

It is been estimated that 60–70% of drugs are ionizable molecules and, thus, one of the most important physicochemical properties of a drug molecule is the acid dissociation constant (pK_a). Several key physicochemical properties that regulates absorption and distribution processes, such as lipophilicity and solubility, are pK_a dependent.[1] The ionization state is a key parameter not only in ADME profiling, but also when the drug interacts with the biological target because the interaction occurs in aqueous environment at physiological pH. Moreover, pK_a can be important in determining the rate and the site of drug metabolism.[2] Finally, in drug formulation the ionization constant is important for choosing the correct excipients and counterions.[3] Therefore, pK_a should be accurately evaluated during drug optimization.

Ionization constant can be measured by several methods, including potentiometric and UV–vis spectrophotometric titrations which are frequently used. These methods require a lot of skills for both monitoring the ionization state of the molecule while changing pH and interpreting the experimental data taking full account of chemical equilibria theory, sample solubility, and overlapping pK_a values.[1] This explains the paucity of experimental data on the ionization constant of drug-like molecules. Several pK_a prediction models have been proposed, based on different descriptors, including atomic charges, topological distances, chemical reactivity models, and group philicity. However, the predicted values sometimes could be much off and can only be used as estimates.[4]

1-Arylpiperazines represent one of the most versatile template for obtaining molecules acting at several receptor system such as serotoninergic, adrenergic, and dopaminergic receptors.[5–7] A number of studies have described the synthesis, structure-activity relationships, and pharmacological evaluation of various classes of drugs with an arylpiperazine moiety in their structure. These research efforts have led to the identification of several arylpiperazine derivatives that reached late stage clinical trials for the treatment of depression, psychosis, and anxiety (Fig. 1). The specificity of arylpiperazine-based compounds for a given receptor system and the pharmacokinetic properties can be modulated through: (i) appropriate choice of the substituent on the phenyl ring linked to the piperazine; (ii) optimization of the intermediate alkyl chain length; (iii) selection of the different terminal fragment. During the last decade, our research group have studied several classes of 1-arylpiperazine derivatives with specificity for serotonin 5-HT_{1A} and 5-HT_7 receptors and for dopamine D_3 and D_4 receptors.[8–11] Although 1-arylpiperazine-based compounds have been studied in depth for their pharmacological properties, only a few papers dealt with ionization constant. Caccia et al. determined the ionization constants of several unsubstituted 1-arylpiperazines which may be formed during the in vivo biotransformation of central nervous system acting drugs.[12] Van Steen et al. determined the pK_a values of a series of N-4 alkylsubstituted heterobicyclic phenylpiperazines in order to evaluate if there was a correlation between the ionization state of the ligands and their affinities at 5-HT_{1A} receptors.[13] Bojarski et al. studied the effect of N-alkylation on the basicity of several cyclic amines, including some arylpiperazines, that are common fragments of several adrenergic, serotoninergic and dopaminergic ligands.[14]

* Corresponding author. Tel.: +39 080 5442750; fax: +39 080 5442231.
E-mail address: lacivita@farmchim.uniba.it (E. Lacivita).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.12.015

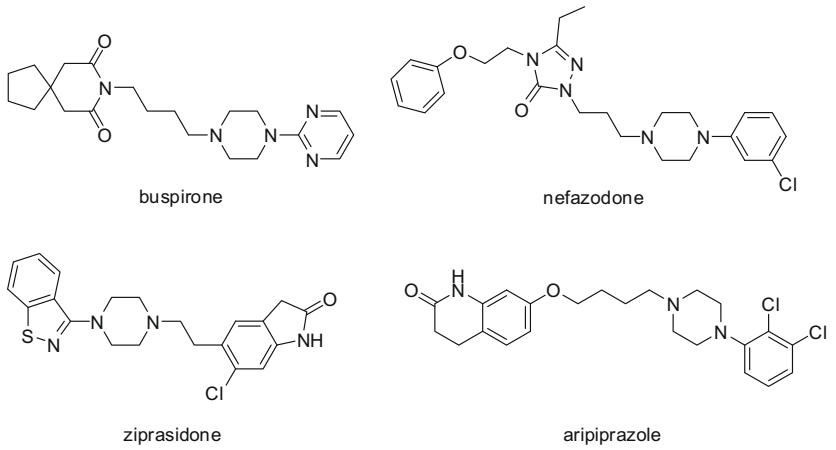


Figure 1. Structures of arylpiperazines entered in clinical trials.

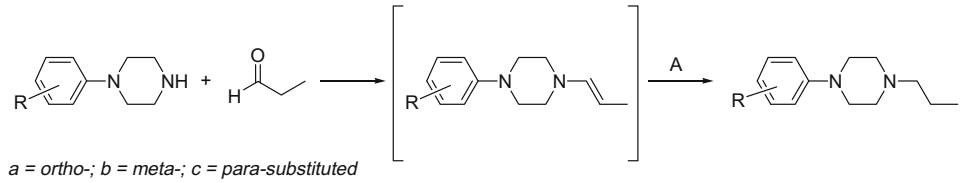
Recently, we have determined by using a potentiometric method the physicochemical properties of a series of dopamine D_3 ligands with *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamide structure. During that study, we observed that the ionization constant of the piperazine nitrogen linked to the butyl chain was influenced by the nature and the position of the substituent on the phenyl attached to the piperazine.[15] Starting from this observation, we prepared a series of 1-(substitutedphenyl)-4-propylpiperazines in order to study how the nature and position of the substituent on the phenyl ring can modulate the basicity of the *N*-propyl-substituted nitrogen. For this purpose, we selected substituents covering a wide range of electronic properties (CH_3, OCH_3, Cl, OH, CF_3, CN, NO_2, $CONH_2$). The *N*-propyl-1-arylpiperazine scaffold was chosen because a large number of biologically active 1-arylpiperazine derivatives bears an alkyl chain of variable length on the nitrogen. Finally, to the best of our knowledge, this aspect has not been documented in the literature.

2. Chemistry

The target compounds **9**, **10a–c**–**16a–c** were synthesized by reductive alkylation of the corresponding 1-arylpiperazines as depicted in Scheme 1. Propionaldehyde reacted with the appropriate 1-arylpiperazine to give the intermediate enamine which was reduced with $NaBH_4$ to give the target compound. The carboxamide derivatives **17a–c** were prepared by acidic hydrolysis of the corresponding cyano derivatives **16a–c**. The 1-arylpiperazines were obtained from commercial sources or were prepared according to the literature as detailed in Experimental Section.

3. Results and discussion

The experimental and the calculated pK_a values of compounds **10a–c**–**17a–c** are reported in Table 1. To evaluate if variations in pK_a values were related to Hammet σ-parameters of the substituents, the unsubstituted 4-phenyl-1-propylpiperazine **9** was prepared. pK_a data of *ortho*- (**10a**–**17a**) and *meta*-substituted (**10b**–**17b**) derivatives appeared to be not in correlation with the electronic properties of the substituents. The introduction of a substituent in *ortho*-position determined an increase of pK_a value of the N-4 piperazine nitrogen with exception of derivatives **15a** (R = NO_2) and **16a** (R = CN), that showed pK_a values close to that of the unsubstituted **9**. Moreover, the basicity-increasing effect was not related to the electronic properties of the substituent. In fact, **10a** (R = CH_3) and **14a** (R = CF_3) showed similar pK_a values despite the very different inductive effect of the substituents. On the other hand, the introduction of OCH_3 or OH (compounds **11a** and **12a**, respectively), which share nearly the same inductive effect, determined different variation in pK_a value (pK_a = 8.29 and 8.05, respectively). These data suggested that other factors, such as steric or conformational effects, are able to modulate the basicity of the N-4 nitrogen of the piperazine in a larger extent than electronic

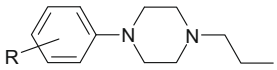


1R= H
2a-c: R = CH_3
3a-c: R = OCH_3
4a-c: R = OH
5a-c: R = Cl
6a-c: R = CF_3
7a-c: R = NO_2
8a-c: R = CN

9: R= H
10a-c: R = CH_3
11a-c: R = OCH_3
12a-c: R = OH
13a-c: R = Cl
14a-c: R = CF_3
15a-c: R = NO_2
B → **16a-c:** R = CN
B → **17a-c:** R = $CONH_2$

Scheme 1. Reagents and conditions: (A) $NaBH_4$, rt; (B) H_2SO_4, 70 °C.

Table 1
Calculated and experimental pK_a values of compounds **9**, **10a–c–17a–c**



Cpd	R	Calculated pK_a[a]	Experimental pK_a	ΔpK_a
9	H	7.14	7.96 ± 0.02	—
10a	2-CH_3	7.15	8.39 ± 0.03	+0.43
10b	3-CH_3	7.23	7.83 ± 0.01	−0.13
10c	4-CH_3	7.19	7.92 ± 0.02	−0.04
11a	2-OCH_3	7.16	8.29 ± 0.03	+0.33
11b	3-OCH_3	7.16	7.86 ± 0.03	−0.10
11c	4-OCH_3	7.16	7.88 ± 0.02	−0.08
12a	2-OH	7.08	8.05 ± 0.03	+0.09
12b	3-OH	7.14	7.82 ± 0.06	−0.14
12c	4-OH	7.23	7.92 ± 0.05	−0.04
13a	2-Cl	7.03	8.07 ± 0.02	+0.11
13b	3-Cl	7.06	7.83 ± 0.01	−0.13
13c	4-Cl	7.08	7.83 ± 0.02	−0.13
14a	2-CF_3	7.03	8.80 ± 0.01	+0.84
14b	3-CF_3	7.08	7.81 ± 0.01	−0.15
14c	4-CF_3	7.00	7.84 ± 0.01	−0.12
15a	2-NO_2	6.96	7.94 ± 0.01	−0.02
15b	3-NO_2	6.97	7.70 ± 0.04	−0.26
15c	4-NO_2	6.91	7.59 ± 0.03	−0.37
16a	2−CN	6.99	7.92 ± 0.01	−0.04
16b	3-CN	7.00	7.67 ± 0.06	−0.29
16c	4-CN	7.03	7.78 ± 0.01	−0.18
17a	2-$CONH_2$	7.07	8.14 ± 0.03	+0.18
17b	3-$CONH_2$	7.07	7.76 ± 0.04	−0.20
17c	4-$CONH_2$	7.04	7.84 ± 0.02	−0.12

[a] Calculated with ACD/Labs 7.0 (Advanced Chemistry Development, Inc., Toronto ON, Canada).

effects. The introduction of the substituent in *meta*-position of the phenyl ring linked to the piperazine had an overall basicity-lowering effect. Also in this case, this effect seemed to be not related to the electronic properties of the substituent. In fact, all compounds showed very similar pK_a values, being **16b** (R = CN) the less basic compound among the *meta*-substituted derivatives. The introduction of the substituent in *para* position determined a slight decrease of the pK_a value as compared to that of **9**. This effect was qualitatively consistent with the Hammet σ-parameters of the substituents. In particular, derivative **15c** (R = NO_2) showed the more marked decrease in pK_a as compared to that of **9** (ΔpK_a = −0.37) due to strong inductive and mesomeric effect of nitro group in *para*-position.

Taken together, these data clearly indicated that the electronic properties of the substituent on the phenyl ring are not the only feature that modulates the basicity of the N-4 nitrogen of the piperazine. It is interesting to note that the trend observed in the variation of pK_a in this serie of compounds paralleled the findings reported on the basicity of substituted anilines.[16–18] Those studies evidenced good correlation between pK_a and electronic properties of the substituent only among *para*-substituted anilines, whereas no correlation was found among the *ortho*-substituted isomers. Such results were rationalized considering that the introduction of a substituent in *ortho*-position increases the hydrophobicity in the region adjacent to the nitrogen atom, hindering the translational motion of water molecules. As a result, the space in which the substituent and the nitrogen are enclosed becomes inaccessible for water molecules. This creates the so-called 'hindrance effect' and leads to the formation of a layer of more structurized water around the species. Hydrophobic hydration decreases medium polarity in the vicinity of the molecule and changes the prototropic properties of the medium in the local microenvironment of the molecule. This translates into a decrease of the pK_a for the aniline nitrogen which depends on the thermodynamic of the solvation process and not on the electronic properties of the substituent. Considering that *ortho*-substituted phenylpiperazines **10a–17a** are structurally related to *ortho*-substituted anilines, it can be speculated that the 'hindrance effect' modulates the electron withdrawing effect of the 'anilinic' nitrogen of the piperazine which transmitted via σ-pathways to the N-4 nitrogen, thus determining a basicity increase. Moreover, a conformational effect of the *ortho*-substituent reflecting in pK_a variation cannot be ruled out. From a survey of crystal structures in Cambridge Structural Database it emerges that *N*-phenylpiperazines adopt different conformations depending on the presence of a substituent on the phenyl ring. In particular, unsubstituted derivatives can adopt a π-conjugated arrangement with the N-lone pair axis of the 'anilinic' nitrogen almost perpendicular to the adjacent aryl p-orbitals. The introduction of an *ortho*-substituent on the phenyl ring twists the N-lone pair axis by 45–75° from the plane of π-system, causing the uncoupling of π-conjugation. This twisting effect is less pronounced when the substituent is in *meta*-position, whereas it is nearly absent for *para*-substituted derivatives.

All the above considerations might explain the significant difference between experimental and calculated pK_a values of compounds **9**, **10a–c–17a–c**, because currently available pK_a prediction tools operate considering only the electronic properties of a substituent and its topological distance from reaction centre.

4. Conclusion

A series of 1-(substitutedphenyl)-4-propylpiperazines was prepared in order to study the effect of the substituent on the phenyl ring on the basicity of the alkylated nitrogen. The pK_a values indicated that the position of the substituent was crucial for the modulation of this physicochemical parameter. In general, the introduction of the substituent in *ortho*-position of the phenyl ring increased the basicity of the molecule. This effect appeared to be related to steric and conformational effects caused by the introduction of the substituent and not to its electronic properties. On the other hand, the presence of a substituent in *meta*- and *para*-position determined a slight decrease of pK_a that was qualitatively consistent with the electronic properties of the substituent. Clearly, the increasing-basicity effect of the *ortho*-substituent may reflect on lipophilicity and solubility of this class of compounds. Finally, calculated pK_a values were significantly different from the experimental ones. This aspect is not surprising because computational pK_a prediction tools are not yet sufficiently sophisticated to be of general practical value in pharmaceutical industry. However, this situation is expected to change as more experimental data become available and continued effort are spent to refine existing tools.

5. Experimental

5.1. Chemistry

Column chromatography was performed with 1:30 Merck silica gel 60A (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on free bases. All chemical shift

values are reported in ppm (δ). Recording of mass spectra was done on an HP6890-5973 MSD gas chromatograph/mass spectrometer; only significant *m/z* peaks, with their percentage of relative intensity in parentheses, are reported. The purity of new compounds that were essential to the conclusions drawn in the text was determined by HPLC on a Perkin–Elmer series 200 LC instrument using a Phenomenex Gemini RP-18 column, (250 × 4.6 mm, 5 μm particle size) and equipped with a Perkin–Elmer 785A UV–vis detector setting λ = 254 nm. Compounds were eluted with $CH_3OH/H_2O/Et_3N$, 4:1:0.01, v/v at a flow rate of 0.8 mL/min. A standard procedure was used to transform final compounds into their hydrochloride salts. The following compounds were synthesized according to published procedures: 1-(2-trifluoromethylphenyl)piperazine (**6a**),[19] 1-(3-nitrophenyl)piperazine (**7b**),[20] 1-(3-cyanophenyl)piperazine (**8b**).[21]

5.2. General procedure for the preparation of compounds 9, 10a,c–16a,c

To a solution of the appropriate 1-arylpiperazine (3.0 mmol) in MeOH (20 mL) propionaldehyde (3.6 mmol) was added dropwise and the mixture was stirred at rt for 1 h. After cooling at 0 °C, $NaBH_4$ (4.5 mmol) was added in small portions. The mixture was warmed at room temperature, stirred for 2 h and, then, quenched with H_2O. MeOH was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were collected, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude residue was purified by chromatography ($CHCl_3$/AcOEt, 1:1 as eluent) to give pure compounds as oils in 40–50% yield.

5.3. 1-Phenyl-4-propylpiperazine (9)

GC–MS *m/z* 205 (M^++1, 11), 204 (M^+, 74), 175 (100), 132 (26). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.49–1.60 (m, 2H), 2.33–2.39 (m, 2H), 2.61 (app. t, 4H), 3.21 (app. t, 4H), 6.81–6.88 (m, 1H), 6.91–6.95 (m, 2H), 7.24–7.30 (m, 2H). Anal. ($C_{13}H_{20}N_2$·2HCl·0.5H_2O) C, H, N.

5.4. 1-(2-Methylphenyl)-4-propylpiperazine (10a)

GC–MS *m/z* 219 (M^++1, 9), 218 (M^+, 63), 189 (100), 118 (43).^1H NMR: δ 0.94 (t, 3H, *J* = 7.4 Hz), 1.51–1.61 (m, 2H), 2.30 (s, 3H), 2.39 (app t, 2H), 2.62 (br s, 4H), 2.96 (app. t, 4H), 6.95–7.05 (m, 2H), 7.14–7.19 (m, 2H). Anal. ($C_{13}H_{20}N_2$·2HCl) C, H, N.

5.5. 1-(3-Methylphenyl)-4-propylpiperazine (10b)

GC–MS *m/z* 219 (M^++1, 14), 218 (M^+, 88), 189 (100), 70 (44).^1H NMR: δ 0.94 (t, 3H, *J* = 7.4 Hz), 1.49–1.62 (m, 2H), 2.32 (s, 3H), 2.33–2.38 (m, 2H), 2.60 (app. t, 4H), 3.20 (app. t, 4H), 6.67–6.76 (m, 3H), 7.12–7.18 (m, 1H). Anal. ($C_{13}H_{20}N_2$·2HCl) C, H, N.

5.6. 1-(4-Methylphenyl)-4-propylpiperazine (10c)

GC–MS *m/z* 219 (M^++1, 15), 218 (M^+, 90), 189 (100), 70 (44).^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.48–1.61 (m, 2H), 2.30 (s, 3H), 2.36 (app t, 2H), 2.61 (app t, 4H), 3.16 (app. t, 4H), 6.85 (d, 2H, *J* = 8.5 Hz), 7.07 (d, 2H, *J* = 8.8 Hz). Anal. ($C_{13}H_{20}N_2$·2HCl) C, H, N.

5.7. 1-(2-Methoxyphenyl)-4-propylpiperazine (11a)

GC–MS *m/z* 235 (M^++1, 14), 234 (M^+, 83), 205 (100), 190 (36), 177 (23). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.50–1.62 (m, 2H), 2.38 (app t, 2H), 2.66 (br s, 4H), 3.11 (br s, 4H), 3.86 (s, 3H), 6.84–7.02 (m, 4H). Anal. ($C_{14}H_{22}N_2O$·2HCl) C, H, N.

5.8. 1-(3-Methoxyphenyl)-4-propylpiperazine (11b)

GC–MS *m/z* 235 (M^++1, 15), 234 (M^+, 92), 205 (100), 162 (28). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.49–1.62 (m, 2H), 2.36 (app t, 2H), 2.60 (app t, 4H), 3.21 (app t, 4H), 3.79 (s, 3H), 6.39–6.43 (m, 1H), 6.46–6.47 (m, 1H), 6.55 (dd, 1H, *J* = 2.2, 8.2 Hz), 7.16 (t, 1H, *J* = 8.2 Hz). Anal. ($C_{14}H_{22}N_2O$·2HCl) C, H, N.

5.9. 1-(4-Methoxyphenyl)-4-propylpiperazine (11c)

GC–MS *m/z* 235 (M^++1, 17), 234 (M^+, 100), 205 (78), 135 (25). ^1H NMR: δ 0.92 (t, 3H, *J* = 7.4 Hz), 1.51–1.68 (m, 2H), 2.33–2.38 (m, 2H), 2.61 (app t, 4H), 3.11 (app t, 4H), 3.76 (s, 3H), 6.81–6.85 (m, 2H), 6.88–6.92 (m, 2H). Anal. ($C_{14}H_{22}N_2O$·2HCl) C, H, N.

5.10. 2-(4-Propyl-1-piperazinyl)phenol (12a)

GC–MS *m/z* 221 (M^++1, 11), 220 (M^+, 70), 191 (94), 148 (64), 134 (100), 120 (99). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.50–1.62 (m, 2H), 2.39 (app. t, 2H), 2.64 (br s, 4H), 2.92 (app. t, 4H), 5.0 (br s, 1H, D_2O exchanged), 6.85 (dt, 1H, *J* = 1.4, 7.7 Hz), 6.94 (dd, 1H, *J* = 1.4, 7.9 Hz), 7.04–7.10 (m, 1H), 7.18 (dd, 1H, *J* = 1.4, 7.7 Hz). Anal. ($C_{13}H_{20}N_2O$·2HCl·H_2O) C, H, N.

5.11. 3-(4-Propyl-1-piperazinyl)phenol (12b)

GC–MS *m/z* 221 (M^++1, 14), 220 (M^+, 88), 191 (100), 148 (28). ^1H NMR: δ 0.92 (t, 3H, *J* = 7.4 Hz), 1.50–1.63 (m, 2H), 2.35–2.40 (m, 2H), 2.61 (app. t, 4H), 3.19 (app. t, 4H), 4.85 (br s, 1H, D_2O exchanged), 6.28–6.31 (m, 1H), 6.36–6.38 (m, 1H), 6.47–6.50 (m, 1H), 7.09 (t, 1H, *J* = 8.0 Hz). Anal. ($C_{13}H_{20}N_2O$·2HCl·0.3H_2O) C, H, N.

5.12. 4-(4-Propyl-1-piperazinyl)phenol (12c)

GC–MS *m/z* 221 (M^++1, 16), 220 (M^+, 100), 191 (89), 120 (31). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.52–1.62 (m, 3H, 1H D_2O exchanged), 2.40 (app. t, 2H), 2.65 (br s, 4H), 3.12 (app. t, 4H), 6.74–6.80 (m, 2H), 6.82–6.91 (m, 2H). Anal. ($C_{13}H_{20}N_2O$) C, H, N.

5.13. 1-(2-Chlorophenyl)-4-propylpiperazine (13a)

GC–MS *m/z* 240 (M^++2, 11), 238 (M^+, 33), 211 (33), 209 (100), 166 (16). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.49–1.62 (m, 2H), 2.35–2.41 (m, 2H), 2.64 (br s, 4H), 3.09 (app. t, 4H), 6.93–6.99 (m, 1H), 7.05 (dd, 1H, *J* = 1.7, 8.0 Hz), 7.19 (m, 1H), 7.35 (dd, 1H, *J* = 1.7, 8.0 Hz). Anal. ($C_{13}H_{19}ClN_2$·HCl) C, H, N.

5.14. 1-(3-Chlorophenyl)-4-propylpiperazine (13b)

GC–MS *m/z* 240 (M^++2, 16), 238 (M^+, 49), 211 (33), 209 (100), 166 (21). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.48–1.61 (m, 2H), 2.32–2.38 (m, 2H), 2.58 (app. t, 4H), 3.20 (app. t, 4H), 6.76–6.81 (m, 2H), 6.86–6.88 (m, 1H), 7.15 (t, 1H, *J* = 8.0 Hz). Anal. ($C_{13}H_{19}ClN_2$·HCl) C, H, N.

5.15. 1-(4-Chlorophenyl)-4-propylpiperazine (13c)

GC–MS *m/z* 240 (M^++2, 18), 238 (M^+, 54), 211 (34), 209 (100), 166 (21). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.48–1.61 (m, 2H), 2.33–2.38 (m, 2H), 2.59 (app. t, 4H), 3.17 (app. t, 4H), 6.81–6.86 (m, 2H), 7.17–7.19 (m, 2H). Anal. ($C_{13}H_{19}ClN_2$·2HCl) C, H, N.

5.16. 1-(2-Trifluoromethylphenyl)-4-propylpiperazine (14a)

GC–MS *m/z* 273 (M^++1, 6), 272 (M^+, 20), 243 (100), 172 (12), 70 (16). ^1H NMR: δ 0.93 (t, 3H, *J* = 7.4 Hz), 1.53–1.60 (m, 2H), 2.39 (app

t, 2H), 2.61 (br s, 4H), 2.98 (app. t, 4H), 7.21 (t, 1H, J = 7.7 Hz), 7.38 (d, 1H, J = 7.9 Hz), 7.48–7.53 (m, 1H), 7.61 (dd, 1H, J = 1.1, 7.4 Hz). Anal. ($C_{14}H_{19}F_3N_2$·HCl) C, H, N.

5.17. 1-(3-Trifluoromethylphenyl)-4-propylpiperazine (14b)

GC–MS m/z 273 (M^++1, 6), 272 (M^+, 36), 243 (100), 200 (23), 172 (22). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.51–1.61 (m, 2H), 2.33–2.38 (m, 2H), 2.59 (app t, 4H), 3.29 (app. t, 4H), 6.91–6.93 (m, 1H), 7.10–7.12 (m, 2H), 7.27–7.29 (m, 1H). Anal. ($C_{14}H_{19}F_3N_2$·HCl·0.5H_2O) C, H, N.

5.18. 1-(4-Trifluoromethylphenyl)-4-propylpiperazine (14c)

GC–MS m/z 273 (M^++1, 6), 272 (M^+, 36), 243 (100), 200 (22), 172 (20). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.52–1.61 (m, 2H), 2.35 (app t, 2H), 2.59 (br s, 4H), 3.29 (app. t, 4H), 6.92 (d, 2H, J = 9.0 Hz), 7.46 (d, 2H, J = 9.0 Hz). Anal. ($C_{14}H_{19}F_3N_2$·HCl·0.5H_2O) C, H, N.

5.19. 1-(2-Nitrophenyl)-4-propylpiperazine (15a)

GC–MS m/z 250 (M^++1, 6), 249 (M^+, 31), 220 (87), 202 (57), 131 (100), 119 (72). ^{1}H NMR: δ 0.92 (t, 3H, J = 7.4 Hz), 1.47–1.59 (m, 2H), 2.34–2.39 (m, 2H), 2.60 (app t, 4H), 3.08 (app. t, 4H), 6.99–7.08 (m, 1H), 7.14 (dd, 1H, J = 1.1, 8.3 Hz) 7.43–7.49 (m, 1H) 7.74 (dd, 1H, J = 1.4, 8.1 Hz). Anal. ($C_{13}H_{19}N_3O_2$·HCl) C, H, N.

5.20. 1-(3-Nitrophenyl)-4-propylpiperazine (15b)

GC–MS m/z 250 (M^++1, 5), 249 (M^+, 29), 220 (100), 177 (14). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.49–1.62 (m, 2H), 2.34–2.39 (m, 2H), 2.61 (app t, 4H), 3.29 (app. t, 4H), 7.18 (dd, 1H, J = 2.4, 8.4 Hz), 7.36 (t, 1H, J = 8.0 Hz), 7.64 (dd, 1H, J = 2.2, 8.0 Hz) 7.70–7.72 (m, 1H). Anal. ($C_{13}H_{19}N_3O_2$·HCl·0.5H_2O) C, H, N.

5.21. 1-(4-Nitrophenyl)-4-propylpiperazine (15c)

GC–MS m/z 250 (M^++1, 5), 249 (M^+, 29), 220 (100), 177 (15). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.48–1.60 (m, 2H), 2.33–2.38 (m, 2H), 2.57 (app t, 4H), 3.43 (app. t, 4H), 6.78–6.84 (m, 2H), 8.08–8.14 (m, 2H). Anal. ($C_{13}H_{19}N_3O_2$·HCl·0.3H_2O) C, H, N.

5.22. 1-(2-Cyanophenyl)-4-propylpiperazine (16a)

GC–MS m/z 230 (M^++1, 4), 229 (M^+, 24), 200 (100), 157 (41). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.48–1.61 (m, 2H), 2.36–2.41 (m, 2H), 2.66 (app t, 4H), 3.26 (app. t, 4H), 6.96–7.02 (m, 2H), 7.44–7.50 (m, 1H), 7.54–7.57 (m, 1H). Anal. ($C_{14}H_{19}N_3$·HCl·0.4H_2O) C, H, N.

5.23. 1-(3-Cyanophenyl)-4-propylpiperazine (16b)

GC–MS m/z 230 (M^++1, 6), 229 (M^+, 33), 200 (100), 157 (20). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.48–1.61 (m, 2H), 2.32–2.38 (m, 2H), 2.59 (app t, 4H), 3.23 (app. t, 4H), 7.06–7.11 (m, 3H), 7.44–7.27–7.33 (m, 1H). Anal. ($C_{14}H_{19}N_3$·HCl·0.4H_2O) C, H, N.

5.24. 1-(4-Cyanophenyl)-4-propylpiperazine (16c)

GC–MS m/z 230 (M^++1, 6), 229 (M^+, 35), 200 (100), 157 (18). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.48–1.68 (m, 2H), 2.35 (app t, 2H), 2.57 (app t, 4H), 3.33 (app. t, 4H), 6.82–6.87 (m, 2H), 7.46–7.51 (m, 2H). Anal. ($C_{14}H_{19}N_3$·HCl·0.4H_2O) C, H, N.

5.25. General procedure for the preparation of compounds 17a–c

Compounds **16a–c** (2 mmol) were slowly added to cooled concd H_2SO_4 (10 mL). The mixture was stirred at 60 °C for 3 h, then, after cooling, was poured on ice. The aqueous solution was basified with concd NH_4OH and extracted with AcOEt (3 × 20 mL). The organic phases were collected, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by chromatography ($CHCl_3$/MeOH, 19:1 as eluent) to give pure compounds as white semisolids in 50% yield.

5.26. 1-(2-Carboxamidophenyl)-4-propylpiperazine (17a)

GC–MS m/z 248 (M^++1, 2), 247 (M^+, 4), 162 (100), 158 (68), 132 (68), 98 (39). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.48–1.60 (m, 2H), 2.34–2.39 (m, 2H), 2.62 (br s, 4H), 3.05 (app. t, 4H), 5.88 (br s, 1H, D_2O exchanged), 7.18–7.24 (m, 2H), 7.43–7.48 (m, 1H), 8.14–8.17 (m, 1H) 9.55 (br s, 1H, D_2O exchanged). Anal. ($C_{14}H_{21}N_3O$·HCl·0.5H_2O) C, H, N.

5.27. 1-(3-Carboxamidophenyl)-4-propylpiperazine (17b)

GC–MS m/z 248 (M^++1, 8), 247 (M^+, 46), 218 (100), 175 (17). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.49–1.61 (m, 2H), 2.33–2.38 (m, 2H), 2.60 (app t, 4H), 3.27 (app. t, 4H), 5.64 (br s, 1H, D_2O exchanged), 6.05 (br s, 1H, D_2O exchanged), 7.04–7.08 (m, 1H), 7.14–7.16 (m, 1H), 7.30 (t, 1H, J = 7.9 Hz), 7.43–7.44 (m, 1H). Anal. ($C_{14}H_{21}N_3O$·2HCl) C, H, N.

5.28. 1-(4-Carboxamidophenyl)-4-propylpiperazine (17c)

GC–MS m/z 248 (M^++1, 8), 247 (M^+, 47), 218 (100), 175 (18), 132 (20). ^{1}H NMR: δ 0.93 (t, 3H, J = 7.4 Hz), 1.49–1.61 (m, 2H), 2.33–2.38 (m, 2H), 2.58 (app t, 4H), 3.30 (app. t, 4H), 5.65 (br s, 2H, D_2O exchanged), 6.87–6.91 (m, 2H), 7.69–7.74 (m, 2H). Anal. ($C_{14}H_{21}N_3O$·HCl·0.5H_2O) C, H, N.

5.29. Determination of p*K*a by the pH-metric technique

pK_a values were determined by potentiometric titration using the Sirius GLpKa (Sirius Analytical Instruments Ltd., Forest Row, East Sussex, UK). All experiments were carried out at 25 ± 0.5 °C under a slow argon flow to avoid CO_2 absorption at high pHs. Details about instrument and procedures can be found elsewhere.[22–24] To measure pK_a values, a right amount of each compounds was dissolved in ionic strength adjusted water (0.15 M KCl) to achieve final sample concentration in the range 5×10^{-4}–1×10^{-4} M. The low aqueous solubility of the investigated compounds required pK_a measurements to be performed in the presence of methanol as cosolvent. Three separate 20 mL-semiaqueous solutions, in 15–35% w/w of MeOH (50–30% w/w of MeOH for compound **14a**), were initially acidified with 0.5 M HCl to pH 3.0. The solutions were then titrated with 0.5 M KOH to pH 11. The pH change per titrant addition was limited to 0.2 pH units and pH value in each point was collected when the pH-drift was lower than 0.002 pH per minute. Check of the pH-electrode with measurement of voltage in Orion pH 7.00 buffer and electrode calibration (Four-PlusTM parameters) by a blank titration were everyday performed. The initial estimates of the p$_s K_a$ values, which are the apparent ionization constants in the mixed solvent, were obtained by *Bjerrum* plots. These values were then refined by a weighted non linear least-squares procedure (Refinement Pro 1.0 software) to create a multiset, where the refined values were extrapolated to zero co-solvent concentration using the Yasuda–Shedlovsky equation.[25]

Acknowledgement

The authors wish to thank Dr. Concetta Pacifico and Dr. Antonio Carrieri (Universitá degli Studi di Bari) for supporting in Cambridge Structural Database search.

References and notes

1. Avdeef, A. *Curr. Top. Med. Chem.* **2001**, *1*, 277.
2. Upthagrove, A. L.; Nelson, W. L. *Drug Metab. Dispos.* **2001**, *29*, 1377.
3. Stahl, P. H. In *The Pratice of Medicinal Chemistry*; Vermuth, C., Ed.; Academic Press: London, 2003; p 601.
4. Morgenthaler, M.; Schweizer, E.; Hoffmann-Röder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Müller, K. *ChemMedChem* **2007**, *2*, 1100.
5. Lopez-Rodriguez, M. L.; Ayala, D.; Benhamù, B.; Morbillo, M. J.; Viso, A. *Curr. Med. Chem.* **2002**, *9*, 443.
6. Rosini, M.; Bolognesi, M. L.; Giardinà, D.; Minarini, A.; Tumiatti, V.; Melchiorre, C. *Curr. Top. Med. Chem.* **2007**, *7*, 147.
7. Soskić, V.; Joksimović, J. *Curr. Med. Chem.* **1998**, *5*, 493.
8. Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Lacivita, E.; Tortorella, V.; Leonardi, A.; Poggesi, E.; Testa, R. *J. Med. Chem.* **2001**, *44*, 4431.
9. Leopoldo, M.; Lacivita, E.; Contino, M.; Colabufo, N. A.; Berardi, F.; Perrone, R. *J. Med. Chem.* **2007**, *50*, 4214.
10. Leopoldo, M.; Lacivita, E.; De Giorgio, P.; Colabufo, N. A.; Niso, M.; Berardi, F.; Perrone, R. *J. Med. Chem.* **2006**, *49*, 358.
11. Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Tortorella, V. *J. Med. Chem.* **2000**, *43*, 270.
12. Caccia, S.; Fong, M. H.; Urso, R. *J. Pharm. Pharmacol.* **1985**, *37*, 567.
13. Van Steen, B. J.; van Wijngaarden, I.; Tulp, M. T.; Soudijn, W. *J. Med. Chem.* **1993**, *36*, 2751.
14. Bojarski, A. J.; Mokroz, M. J.; Paluchowska, M. H. *Pharmazie* **1995**, *50*, 569.
15. Leopoldo, M.; Lacivita, E.; De Giorgio, P.; Contino, M.; Berardi, F.; Perrone, R. *Bioorg. Med. Chem.* **2009**, *17*, 758.
16. Pankratov, A. N.; Uchaeva, I. M.; Doronin, S. Y.; Chernova, R. K. *J. Struct. Chem.* **2001**, *42*, 739.
17. Adam, K. R. *J. Phys. Chem. A* **2002**, *106*, 11963.
18. Graña, A. M.; Hermida-Ramón, J. M.; Mosquera, R. A. *Chem. Phys. Lett.* **2005**, *412*, 106.
19. Elworthy, T. R.; Ford, A. P. D. W.; Bantle, G. W.; Morgans, D. J.; Ozer, R. S.; Palmer, W. S.; Repke, D. B.; Romero, M.; Sandoval, L.; Sjogren, E. B.; Talamas, F. X.; Vazquez, A.; Wu, H.; Arredondo, N. F.; Blue, D. R.; De Sousa, A.; Gross, L. M.; Kava, M. S.; Lesnick, J. D.; Vimont, R. L.; Williams, T. J.; Zhu, Q.; Pfister, J. R.; Clarke, D. E. *J. Med. Chem.* **1997**, *40*, 2674–2687.
20. Becker, O. M.; Dhanoa, D. S.; Marantz, Y.; Chen, D.; Shacham, S.; Cheruku, S.; Heifetz, A.; Mohanty, P.; Fichman, M.; Sharadendu, A.; Nudelman, R.; Kauffman, M.; Noiman, S. *J. Med. Chem.* **2006**, *49*, 3116.
21. Koyama, M.; Kikuchi, C.; Ushiroda, O.; Ando, T.; Nagaso, H.; Fuji, K.; Okuno, M.; Hiranuma, T. PTC Appl. WO 9800400, 1998; *Chem. Abstr.* **1998**, *128*, 114961.
22. Avdeef, A. *Quant. Struct.-Act. Relat.* **1992**, *11*, 510.
23. Avdeef, A. *J. Pharm. Sci.* **1993**, *82*, 183.
24. Avdeef, A.; Box, K. J.; Comer, J. E.; Gilges, M.; Hadley, M.; Hibbert, C.; Patterson, W.; Tam, K. Y. *J. Pharm. Biomed. Anal.* **1999**, *20*, 631.
25. Takács-Novák, K.; Box, K. J.; Avdeef, A. *Int. J. Pharm.* **1997**, *151*, 235.