

Design, synthesis and preliminary pharmacological evaluation of rigid analogues of the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP)

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Dedicated to Prof. Vincenzo Tortorella on the occasion of his “Fuori Ruolo” status

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Abstract

Some frozen analogues of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) and of 1-(3-pyridyl)piperazine have been synthesized and tested on rat cerebral cortex by means of binding studies. Among the synthesized substances, only compound **2c** was found to displace [³H]-cytisine from the nicotinic binding sites on rat cerebral cortex. Some possible explanations for the inactivity of the other compounds are given.

Keywords: Nicotinic receptors ligands, tetrahydropyrazino[1,2-a]indoles, hexahydropyrazino[1,2-a]indoles, tetrahydropyrido[4',3':4,5]pyrrolo[1,2-a]pyrazines

Introduction

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels which modulate synaptic transmission. They are formed by five subunits arranged to form a pore, through which cations cross the cellular membrane. To date, 17 different subunits have been identified in vertebrate species, and according to the subunit composition, location, and sensitivity to α -bungarotoxin (α BTX), they can be classified into different groups: 1) muscle-type receptors, found at the skeletal neuromuscular junction and in the electric organs of *Torpedo* fishes, formed by four different subunits (α 1, β 1, γ , δ or α 1, β 1, ϵ , δ); 2) α BTX-insensitive neuronal receptors, found in the peripheral and central nervous systems, formed by different combinations of α 2-6 and β 2-4 subunits; 3) α BTX-sensitive neuronal receptors, found in the CNS, containing α 7-10 subunits. According to a recent classification, the subtypes formed by the most recently identified subunits (α 9 and α 10), which are expressed primarily in sensory epithelia, form a separate group.¹

There is great interest in nAChRs, since they seem to be involved in several physiological functions, such as synaptic transmission, modulation of presynaptic transmitter release, cognitive processes and control of movement in normal subjects, as well as in several pathological processes. In fact, dysfunction of nAChR has been linked to a number of human diseases such as depression, schizophrenia, Alzheimer's and Parkinson's diseases, Tourette's syndrome.² Moreover, some genetic forms of epilepsy (ADNFLE) and congenital myasthenic syndrome are associated to mutations in the gene coding for nAChR subunits.³⁻⁵ Other therapeutically important applications of nicotinic ligands are the treatment of nicotine addiction and the management of pain.⁶

For a long time the nicotinic receptor extracted from *Torpedo* fishes has been studied using electron microscopy to obtain valuable information about the structure and functioning of the receptor,⁷⁻¹⁰ but an important step toward the understanding of nAChR structure has come from the resolution of X-ray crystallography of the molluscan acetylcholine-binding protein (AChBP)¹¹ which has been used to model the extracellular domain of the nicotinic receptor where the agonist binding site is located.¹²⁻¹⁴ A three-dimensional model of the binding site is helpful to the design of new ligands; so far, nicotinic ligands have been designed using qualitative pharmacophoric models and 3D-QSAR analysis.¹⁵⁻²⁰ The nicotinic pharmacophore is formed by two groups: an H-bond acceptor atom, usually a pyridyl nitrogen or a carbonyl oxygen, and a positive nitrogen, which can be protonated or quaternarized; the proposed distance between these two groups ranges from 4.5 Å¹⁵ to 5.5 Å.^{16,21}

As a part of our research in the field of nicotinic ligands, we have recently reported the synthesis and pharmacological evaluation of a series of analogues of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP)^{22,23} (general formula A, Figure 1),

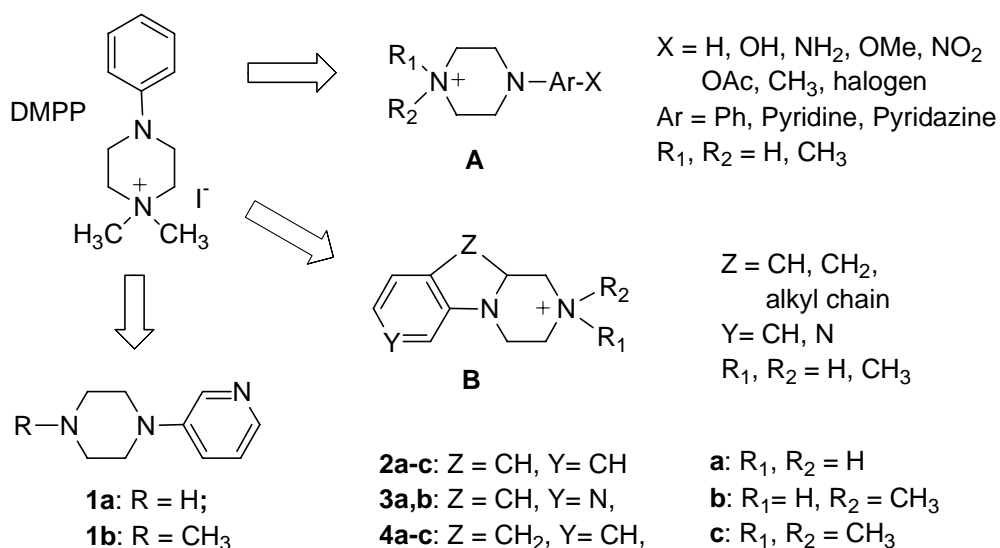


Figure 1

A well-known nicotinic agonist which, lacking the H-bond acceptor group, does not fit the proposed nicotinic pharmacophore. DMPP is reported to bind to the central nicotinic receptor with K_i values ranging from 57 nM²⁴ to 250 nM,²² it has been shown²³ that the introduction of

substituents on the phenyl ring greatly improved affinity but in general, derivatives with a permanent positive charge showed higher potency than their tertiary amino analogues. However, by introducing an H-bond forming group (NH₂, F, OCOMe, NO₂) in position 3 on the phenyl ring, or by replacing the phenyl ring with N-containing heterocycles, compounds were synthesized endowed with good affinity for the nicotinic receptor also as uncharged amines.

Since the possibility to cross the blood brain barrier is a crucial feature for drugs in the treatment of CNS pathologies, we decided to focus our attention on the uncharged 1-(3-pyridyl)piperazines **1a** and **1b** (Figure 1), endowed with good affinity (K_i 90 nM). In these molecules, as well as in DMPP, the rotation around the arylpiperazine bond is free; this observation prompted us to evaluate the effect on affinity, and eventually selectivity, of reducing the conformational freedom of this part of the molecule. Therefore, the compounds of general formula **B** (Figure 1) were designed, in which the two cycles (aromatic and piperazine rings) are connected through a suitable spacer (*Z*) that fixes their relative orientation.

This modification of the structure of DMPP and analogues could also help to find the bioactive conformation of this class of molecules. In fact, the conformational analysis of these molecules using different computational methods ends up with different low-energy conformations. As pointed out by Dijkstra,²⁵ the quantum mechanical semiempirical program AM1 yields a conformation of DMPP similar to that found in the crystal structure,²⁶ with the dihedral angle (C₂-C₁-N-lp, Figure 2) at 120°.

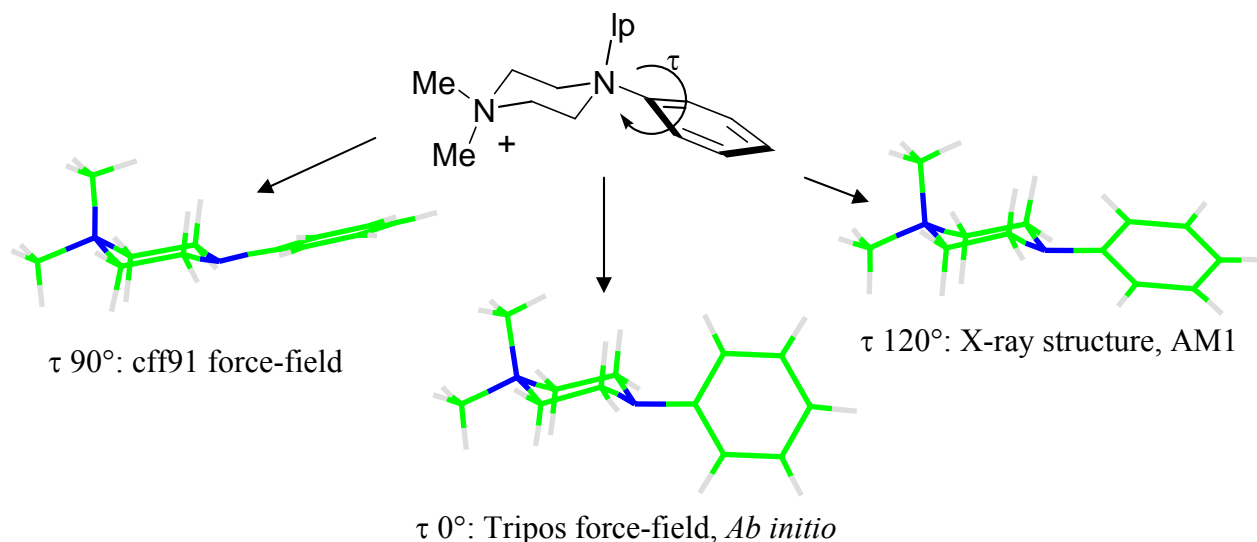


Figure 2. Low-energy conformation of DMPP, calculated with different methods. Hydrogen atoms are shown in gray, carbon atoms in green, nitrogen atoms in blue.

This minimum energy conformation has been explained by the presence of two opposite factors: the possibility of conjugation between the nitrogen lone pair and the π orbitals, and the steric hindrance between the aromatic ring and the methylene groups of the piperazine ring.^{25,26} Other computational methods produce different results: *ab initio* (HF) gives a conformation in which the two rings, phenyl and piperazine, are orthogonal (τ 0°), while molecular mechanics

calculations give different results according to the force-field used: a conformation with τ 90° (cff91 force-field within the Accelrys program Discover) and a conformation with τ 0° (Tripos force-field).

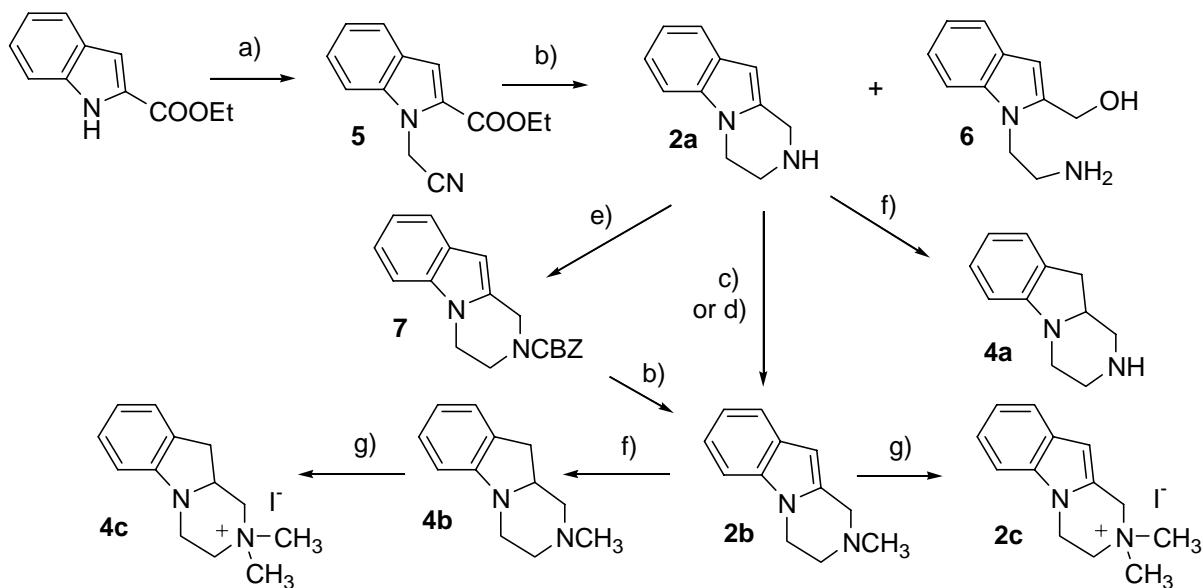
Compounds of general formula **B**, with Z being only a one-carbon unit, should mimic the 90° conformation, while the orthogonal conformation could be approached with Z being a longer saturated alkyl chain.

We decided to start with Z being only a one-carbon unit, synthesizing 1,2,3,4-tetrahydropyrazino[1,2-a]indoles (**2a-c**, Y = CH, Z = CH), their aza-analogues 1,2,3,4-tetrahydropyrido[4',3':4,5]pyrrolo[1,2-a]pyrazine (**3a,b**, Y = N, Z = CH), and their hydrogenated derivatives 1,2,3,4,10,10a-hexahydropyrazino[1,2-a]indoles (**4a-c**, Y = CH, Z = CH₂). Both secondary and tertiary amines (R = H, CH₃) and, when possible, also the corresponding methiodides were synthesized. In fact, although as mentioned before, compounds carrying a permanent positive charge are not suitable to be developed as drugs, if endowed with good affinity they can be useful as pharmacological tools to study the geometry of ligand interaction and to characterize nAChRs subtypes.

Results and Discussion

Chemistry

The synthesis of the 1,2,3,4-tetrahydropyrazino[1,2-a]indoles **2** started from the commercially-available ethyl indole-2-carboxylate which was reacted with chloroacetonitrile obtaining compound **5** (Scheme 1).²⁷



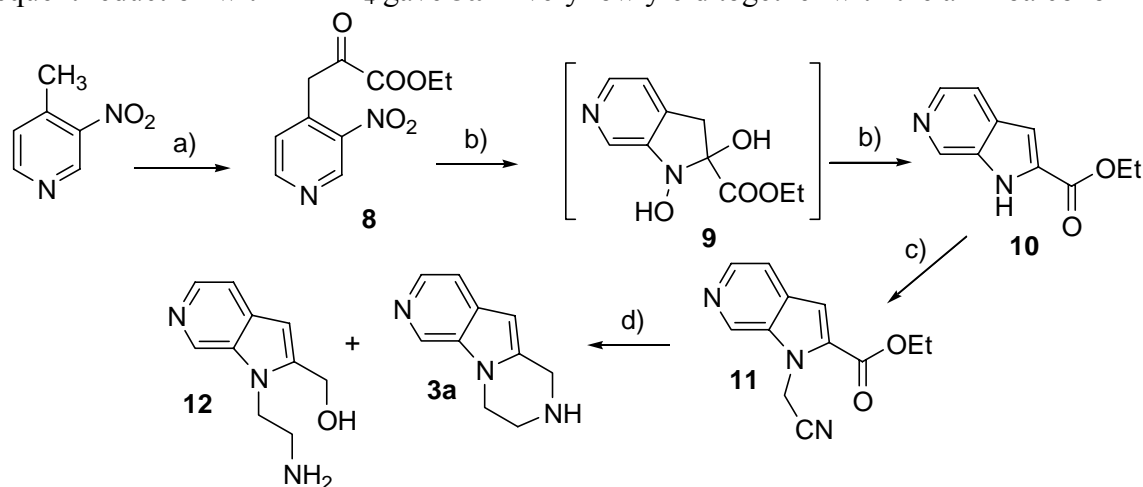
a) t-BuOK, ClCH₂CN; b) LiAlH₄; c) (CH₂O)_n, H₂/Pd/C; d) MeI (1 eq), DMF; e) ClCOOCH₂Ph; f) NaBH₃CN, MeOH; g) MeI, Et₂O.

Scheme 1

Treatment of **5** with LiAlH_4 resulted in the reduction of both the cyano and the ester functions with cyclization, giving **2a**²⁸ in moderate yield (24%) and in mixture with the aminoalcohol **6** (20%), which were separated by chromatography.

Methylation of **2a** was first attempted by reacting the secondary amine with formaldehyde and formic acid, but only decomposition of the starting material was observed. Treatment with paraformaldehyde in a *Parr* apparatus under hydrogen pressure and in the presence of Pd/C, according to Abreo,²¹ gave the expected product **2b**²⁸ in very low yield (10%), while MeI in DMF gave slightly better results (31% yield). Finally, treatment of **2a** with benzyl chloroformate gave **7**, which was then reduced with LiAlH_4 affording **2b** with good yield. Compounds **2a** and **2b** were successfully reduced with NaBH_3CN in acetic acid²⁹ obtaining the corresponding 1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles **4a**³⁰ and **4b**. Methiodides **2c** and **4c** were then obtained by treatment with MeI in ether.

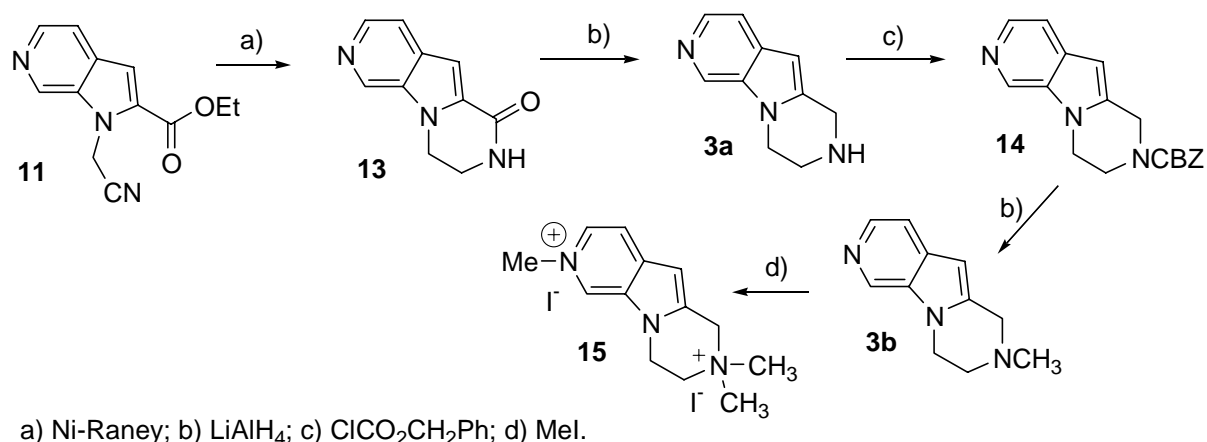
For the synthesis of 1,2,3,4-tetrahydropyrido[4',3':4,5]pyrrolo[1,2-*a*]pyrazines **3**, an analogous synthetic pathway was first attempted (Scheme 2). Compound **10** was prepared according to Fisher:³¹ the commercially available 4-methyl-3-nitropyridine was treated with diethyl oxalate and sodium ethoxide obtaining ethyl 3-(3-nitropyridin-4-yl)-2-oxo-propionate (**8**), which was hydrogenated in a *Parr* apparatus to **10**. Through this pathway, compound **10** was usually obtained in good yield, but sometimes, when scaling up the reaction, hydrogenation stopped at the hydroxylamine derivative **9**, which was isolated and further hydrogenated obtaining **10**. The azaindole derivative **10** was then alkylated obtaining the nitrile **11**, but subsequent reduction with LiAlH_4 gave **3a** in very low yield together with the aminoalcohol **12**.



a) diethyl oxalate, EtONa; b) $\text{H}_2/\text{Pd/C}$; c) $t\text{-BuOK}$, ClCH_2CN ; d) LiAlH_4

Scheme 2

Therefore, we decided to reduce **11** in two steps (scheme 3): the reaction with Raney/Ni gave the lactam **13** which was reduced with LiAlH_4 to **3a**, however without substantial improvement in the yields. To obtain the tertiary amine **3b**, **3a** was treated with benzyl chloroformate to give **14** which was then reduced with LiAlH_4 obtaining **3b**. Subsequent methylation with MeI failed to give the desired methiodide **3c**, and **15** was obtained as the only product.



Scheme 3

Biological evaluation

Compounds **2-4** were tested *in vitro* on rat brain homogenates to evaluate their affinity for the central nicotinic receptors, according to a previously reported experimental protocol.²³ [³H]-Cytisine was used as radioligand; this compound is reported to label the $\alpha 4\beta 2$ subtype, which is believed to represent up to 90% of the high affinity agonist binding site in the brain.^{32 33}

Among the frozen analogues of DMPP and of 3-pyridylpiperazine (compounds **2a-c**, **4a-c**, **3a** and **3b**), only methiodide **2c** shows affinity for the central nicotinic receptor with K_i 2.02 μ M (confidence limits 1.24-3.29 μ M); the other compounds do not displace [³H]-cytisine from rat cerebral cortex up to a 100 μ M concentration.

As far as the piperazino-indoles are concerned, the lack of affinity of secondary bases (**2a** and **4a**) and tertiary bases (**2b** and **4b**) is not surprising, since also 1-phenyl-4-methylpiperazine (the tertiary base of DMPP) is devoid of affinity.²³ On the contrary, it seems that the freezing of the phenylpiperazinium moiety into a tricyclic structure is detrimental for activity, since the methiodide **4c** does not interact with the receptor, and the affinity of compound **2c** is 8-fold lower than that of DMPP. The lack of affinity of the aza compounds **3a** and **3b** is also unexpected, since the parent compounds **1a** and **1b** show K_i values in the nanomolar range.

Some explanations regarding the inactivity of compounds **3-4** are however possible. The conformation of DMPP and of **1(a,b)**, which have been constrained, respectively, into the hexahydropyrazino[1,2-a]indole **4c** and the tetrahydropyrido[4',3':4,5]pyrrolo[1,2-a]pyrazines **3(a,b)**, may not be the right one. In this way, in fact, the molecules have been frozen into a pseudoplanar conformation, with a value of the dihedral angle τ of 90° (Figure 2), which may not be the bioactive conformation. In this respect, the synthesis of substances (general formula **B**, Figure 1) in which the central five-membered ring has been replaced by a larger ring that allows a more orthogonal disposition between the aromatic and the piperazine rings will help to clarify this point.

On the other hand, a limiting factor in the interaction of these molecules with the receptor may be the space available at the binding site. In fact, **2c** (K_i 2.02 μ M) and **4c** (K_i > 100 μ M), differing for the double/single bond within the indole ring, show a difference in their volume of 4.58 Å³. In this respect, it must be noticed that also 1,1,3-trimethyl-4-phenylpiperazinium iodide

(the 3-methyl analogue of DMPP)²³ is completely devoid of affinity. In addition, the reduction of the indole double bond, while reducing the surface of the aromatic part, induces a bending in the molecule (Figure 3) which may not be compatible with the space available at the binding site.

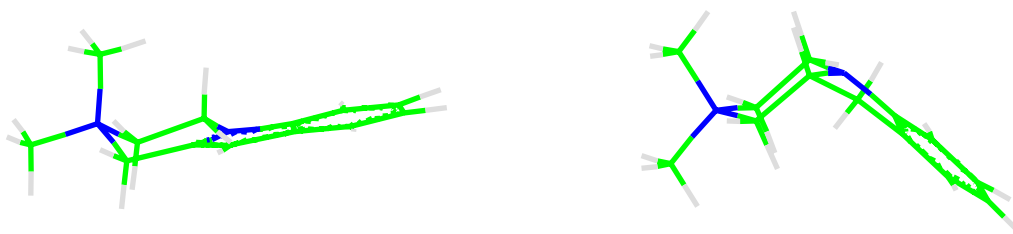


Figure 3. Minimized conformations of compound **2c** (left) and **4c** (right). Hydrogen atoms are shown in gray, carbon atoms in green, nitrogen atoms in blue.

A further consideration can be made regarding compounds **3a** and **3b**. One reason for their lack of affinity could be the wrong orientation of the pyridyl nitrogen in the binding site; in fact, in 1,2,3,4-tetrahydropyrido[4',3':4,5]pyrrolo[1,2-*a*]pyrazines **B** the pyridyl ring has been constrained into one of the two possible “planar” conformations. In this respect, the synthesis of the isomeric 6,7,8,9-tetrahydropyrido[2',3':4,5]pyrrolo[1,2-*a*]pyrazines **C** (Figure 4), which is on the way, may help to clarify this point.

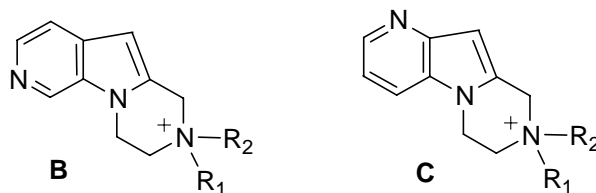


Figure 4

Conclusions

Some frozen analogues of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) and of 1-(3-pyridyl)piperazine (**1a** and **1b**) have been synthesized and tested on rat cerebral cortex by means of binding studies. Compound **2c** shows an 8-fold lower affinity for the nicotinic receptor than the lead compound DMPP; the other compounds do not interact with the nicotinic receptor. The decrease or lack of affinity of these compounds suggests that the structures in which the lead compounds have been frozen do not represent their bioactive conformations, or their volume is not compatible with the space available within the interaction site. The synthesis of other frozen analogues may help to clarify the bioactive conformation of aryl piperazine.

Experimental section

General Procedures. All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 681 spectrophotometer in a Nujol mull for solids and neat for liquids. Unless otherwise stated, NMR spectra were recorded on a Gemini 200 spectrometer. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063-0.200 mm, Merck) or flash chromatography (Kieselgel 40, 0.040-0.063 mm, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within $\pm 0.4\%$ of the theoretical values.

Ethyl 1-cyanomethyl-1H-indole-2-carboxylate (5).²⁷ To a solution of ethyl indole-2-carboxylate (1.2 g, 6.34 mmol) in anhydrous DMF (15 mL) potassium tert-butoxide (1.06 g, 9.4 mmol) was added at room temperature. After 40 min, chloroacetonitrile (0.8 mL, 12.6 mmol) was added dropwise and the solution heated at 65 °C for 30 min and left stirring at room temperature for 20 h. Water (20 mL) was then added and all the solvents distilled to give a solid residue that was treated with water and extracted with CH₂Cl₂. Drying (Na₂SO₄) and removal of the solvent gave 1 of .41 g the title compound (white solid, 97% yield). Mp 98-99 °C. ¹H-NMR (CDCl₃, δ): 1.44 (t, 3H, $J = 7.2$ Hz, CH₃CH₂O); 4.43 (q, 2H, $J = 7.2$ Hz, CH₃CH₂O); 5.62 (s, 2H, CH₂CN); 7.23-7.31 (m, 1H), 7.41-7.48 (m, 3H), 7.73 (d, 1H, $J = 8.1$ Hz) (aromatic protons) ppm. ¹³C-NMR (CDCl₃) δ : 14.43 (q), 32.46 (t), 61.32 (t), 109.67 (d), 112.67 (d), 115.07 (s), 122.05 (d), 123.23 (d), 126.42 (d), 126.42 (s), 126.67 (s), 138.71 (s), 161.87 (s) ppm.

1,2,3,4-Tetrahydropyrazino[1,2-*a*]indole (2a).²⁸ LiAlH₄ (2.41 g, 63.4 mmol) was suspended in 15 mL of dry DME and a solution of **5** (4.4 g, 19.3 mmol) in 20 mL of dry DME was added dropwise at room temperature. The mixture was heated at 50 °C for 7 h. After cooling, the excess of hydride was destroyed with ice and the solvent evaporated. The residue was dissolved in AcOEt and extracted with acidic water, the aqueous phase alkalized and extracted with AcOEt. The organic phase was dried and evaporated and the residue separated by flash chromatography (CHCl₃/petroleum ether/Et₂O/EtOH/NH₃ 360/900/360/180/9.9 as first eluent and CH₂Cl₂/petroleum ether/EtOH/NH₃ 340/60/65/8 as second eluent): 0.79 g of **2a** (low melting solid, 23.8% yield) and 0.75 g of [**1-(2-aminoethyl)-1H-indol-2-yl**]-methanol (**6**). (oil, 20.4 % yield).

6. ¹H-NMR (CDCl₃, δ): 2.97 (bs, 2H, OH/NH); 3.14 (t, 2H, $J = 5.1$ Hz, CH₂NH₂); 4.26 (t, 2H, $J = 5.1$ Hz, NCH₂CH₂NH₂); 4.73 (s, 2H, CH₂OH); 6.50 (s, 1H), 7.09-7.31 (m, 3H), 7.63 (d, 1H, $J = 7.3$ Hz) (aromatic protons) ppm. ¹³C-NMR (CDCl₃) δ : 39.60 (t), 45.63 (t), 55.83 (t), 101.62 (d), 109.34 (d), 119.89 (d), 121.16 (d), 121.76 (d), 127.90 (s), 136.37 (s), 140.55 (s) ppm. IR (neat) ν 3295, 3360 (NH₂) cm⁻¹. Anal. Calcd for C₁₁H₁₄N₂O: C, 69.45; H, 7.42; N, 14.73. Found: C, 69.70; H, 7.53; N, 14.56.

2a. ¹H-NMR (CDCl₃, δ): 1.82 (bs, 1H, NH); 3.36 (t, 2H, $J = 5.7$ Hz, CH₂CH₂NH); 4.02 (t, 2H, $J = 5.7$ Hz, CH₂CH₂NH); 4.23 (s, 2H, CCH₂NH); 6.21 (s, 1H), 7.08-7.31 (m, 3H), 7.58 (d, 1H, $J = 7.3$ Hz) (aromatic protons) ppm. ¹³C-NMR (CDCl₃) δ : 42.50 (t), 43.56 (t), 44.23 (t), 96.13 (d), 108.51 (d), 119.93 (d), 119.98 (d), 120.57 (d), 127.90 (s), 134.55 (s), 136.24 (s) ppm.

2a oxalate salt. Mp 199-200 °C. ¹H-NMR (D₂O, δ): 3.74 (t, 2H, *J* = 6.0 Hz, CH₂CH₂NH); 4.30 (t, 2H, *J* = 6.0 Hz, CH₂CH₂NH); 4.55 (s, 2H, CCH₂NH); 6.52 (s, 1H), 7.19 (dd, 1H, *J* = 7.3 Hz and 7.0 Hz), 7.29 (dd, 1H, *J* = 8.1 Hz and 7.0 Hz), 7.44 (d, 1H, *J* = 8.1 Hz), 7.64 (d, 1H, *J* = 7.3 Hz) (aromatic protons) ppm. ¹³C-NMR (D₂O, δ): 38.50 (t), 41.33 (t), 41.33 (t), 97.12 (d), 109.36 (d), 120.39 (d), 120.56 (d), 121.96 (d), 127.26 (s), 127.26 (s), 136.11 (s), 165.06 (s) ppm.

2-Methyl-1,2,3,4-tetrahydro-pyrazino[1,2-*a*]indole (2b).²⁸ *Method A:* a mixture of **2a** (0.1 g, 0.58 mmol), (CH₂O)_n (0.15 g, 5.14 mmol) and Pd/C 10% (0.02 g) in absolute ethanol (20 mL) was hydrogenated at 40 psi at room temperature for 2 days. The catalyst was filtered off and the solvent removed to give a residue that was purified by column chromatography (CHCl₃/petroleum ether/Et₂O/EtOH/NH₃ 360/900/360/180/9.9) obtaining 0.01 g of **2a** and 0.01 g of **2b** (9% yield).

Method B: a solution of **2a** (0.06 g, 0.35 mmol) and CH₃I (0.06 mL, 1.05 mmol) in anhydrous DMF (10 mL) was stirred at room temperature in the dark for 2 days. The solvent and the excess of reagent were distilled off and the residue treated with water and extracted with CH₂Cl₂. Drying (Na₂SO₄) and removal of the solvent gave 0.02 g (31% yield) of the title compound.

Method C: **2a** (0.15 g, 0.87 mmol) was dissolved in 4.4 mL of a 4:1 mixture of Na₂CO₃ 2M/1,4-dioxane and the solution kept at 0 °C. A solution of ClCOOBz (0.3 mL, 2.13 mmol) in 1.8 mL of 1,4-dioxane and a solution of NaOH 2M (1.1 mL, 2.2 mmol) were then added dropwise simultaneously. After 3.5 h of stirring, the organic solvent was distilled off and the mixture extracted with CH₂Cl₂. Drying (Na₂SO₄) and removal of the solvent gave 0.26 g of **benzyl 3,4-Dihydro-1H-pyrazino[1,2-*a*]indole-2-carboxylate (7)** (white solid, 97% yield). Mp 78-79 °C. ¹H-NMR (CDCl₃, δ): 4.02-4.12 (m, 4H, NCH₂CH₂N); 4.92 (s, 2H, CCH₂N); 5.22 (s, 2H, OCH₂Bz); 6.31 (s, 1H), 7.10-7.40 (m, 8H), 7.59 (d, 1H, *J* = 7.3 Hz) (aromatic protons) ppm. Compound **7** (0.26 g, 1.01 mmol) was dissolved in 4 mL of anhydrous DME and the solution added dropwise to a suspension of LiAlH₄ (0.24 g, 6.43 mmol) in 2 mL of anhydrous DME at -18 °C. The mixture was allowed to reach room temperature and after 1.5 h the excess of hydride was destroyed with ice. DME was evaporated, the residue dissolved in HCl 0.1 N and washed with CH₂Cl₂. The aqueous phase was then alkalized with NaOH and extracted with CH₂Cl₂. Drying (Na₂SO₄) and removal of the solvent gave a solid that was purified by flash chromatography (CHCl₃/petroleum ether/Et₂O/EtOH/NH₃ 360/900/360/180/9.9) to give 0.16 g of **2b** (white crystals, 85% yield). Mp 123-125 °C (lit.²⁸ 130-133). ¹H-NMR (CDCl₃, δ): 2.54 (s, 3H, NCH₃); 2.94 (t, 2H, *J* = 5.7 Hz, NCH₂CH₂NCH₃); 3.81 (s, 2H, CCH₂N); 4.15 (t, *J* = 5.7 Hz, NCH₂CH₂NCH₃); 6.26 (s, 1H), 7.12-7.33 (m, 3H), 7.61 (d, 1H, *J* = 7.0 Hz) (aromatic protons) ppm. ¹³C-NMR (CDCl₃, δ): 41.91 (t), 45.98 (q), 52.58 (t), 53.61 (t), 96.41 (d), 108.67 (d), 119.81 (d), 120.08 (d), 120.57 (d), 128.35 (s), 134.37 (s), 136.01 (s) ppm.

2b oxalate salt. Mp 167-169 °C. ¹H-NMR (D₂O, δ): 2.96 (s, 3H, NCH₃); 3.32-3.49 (m, 1H, NCH₂CH₂N); 3.76-3.82 (m, 1H, NCH₂CH₂N); 3.98-4.32 (m, 2H, NCH₂CH₂N); 4.17 (d, 1H, *J* = 15.2 Hz, CCH₂NHCH₃); 4.61 (d, 1H, *J* = 15.2 Hz, CCH₂NHCH₃); 6.42 (s, 1H), 7.11-7.37 (m, 3H), 7.60 (d, 1H, *J* = 7.3 Hz) (aromatic protons) ppm. ¹³C-NMR (D₂O, δ): 38.27 (t), 41.82 (q), 50.53 (t), 50.84 (t), 99.67, 109.49 (d), 120.50 (d), 120.67 (d), 122.18 (d), 126.79 (s), 127.30 (s), 136.07 (s), 164.93 (s) ppm.

2-Methyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indole (4b). NaCNBH₃ (0.19 g, 2.96 mmol) was added to a solution of **2b** (0.11 g, 0.59 mmol) in 3 mL of CH₃COOH at 0 °C and the mixture

was allowed to reach room temperature. After 3.5 h the reaction was quenched with ice. NaOH 10% (30 mL) was added and the mixture extracted with CH₂Cl₂. The organic phase was then washed with water, anhydri-fied and evaporated to give an oily residue. Purification by flash chromatography (CHCl₃/petroleum ether/Et₂O/EtOH/NH₃ 360/900/360/180/9.9) yielded 0.11 g (yellow oil, 99%) of the title compound. ¹H-NMR (CDCl₃, δ): 2.08 (t, 1H, *J* = 10.6 Hz); 2.17 (td, 1H, *J* = 11.7 Hz and 3.3 Hz); 2.33 (s, 3H, NCH₃); 2.58 (dd, 1H, *J* = 15.0 Hz and 8.1 Hz); 2.73-2.86 (m, 2H); 2.95-3.14 (m, 2H); 3.54-3.70 (m, 2H); 6.45 (d, 1H, *J* = 8.1 Hz), 6.62-6.69 (m, 1H), 7.04-7.11 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (CDCl₃, δ): 32.94 (t), 44.11 (t), 46.51 (q), 53.50 (t), 58.98 (t), 62.41 (d), 106.24 (d), 117.69 (d), 124.85 (d), 127.45 (d), 129.31 (s), 150.58 (s) ppm. Anal. Calcd for C₁₂H₁₆N₂: C, 76.55; H, 8.57; N, 14.88. Found C, 76.35; H, 8.48; N, 14.58.

4b oxalate salt. Mp 97-99 °C. ¹H-NMR (D₂O, δ): 2.56-3.41 (m, 7H); 2.76 (s, 3H, NCH₃); 3.74-3.90 (m, 2H); 6.67 (d, 1H, *J* = 7.7 Hz), 6.77-6.85 (m, 1H), 7.10-7.19 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (D₂O, δ): 31.55 (t), 41.07 (t), 43.28 (q), 50.73 (t), 54.08 (t), 58.85 (d), 108.60 (d), 120.27 (d), 125.51 (d), 127.75 (d), 128.90 (s), 147.56 (s), 164.04 (s) ppm.

1,2,3,4,10,10a-Hexahydropyrazino[1,2-*a*]indole (4a).³⁰ Following the procedure used for **4b**, starting from 0.27 g (1.57 mmol) of **2a** and 0.49 g (7.85 mmol) of NaCNBH₃, the desired product was obtained in 73% yield (0.2 g) as an oil. ¹H-NMR (CDCl₃, δ): 1.83 (bs, 1H, NH); 2.56 (dd, 1H, *J* = 15.0 Hz and 9.5 Hz); 2.73-3.13 (m, 6H); 3.33-3.62 (m, 2H); 6.45 (d, 1H, *J* = 8.1 Hz), 6.66 (m, 1H), 7.05-7.12 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (CDCl₃, δ): 32.95 (t), 44.68 (t), 45.66 (t), 50.25 (t), 64.09 (d), 105.84 (d), 117.73 (d), 124.72 (d), 127.38 (d), 128.91 (s), 151.07 (s) ppm.

4a oxalate salt. Mp 169-173 °C (with decomposition). ¹H-NMR (D₂O, δ): 2.56 (dd, 1H, *J* = 15.7 Hz and 5.9 Hz); 2.80-3.28 (m, 6H); 3.61-3.80 (m, 2H); 6.62 (d, 1H, *J* = 8.1 Hz), 6.74 (m, 1H), 7.04-7.14 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (DMSO, δ): 31.97, 40.91, 41.31, 45.30, 58.97, 106.83, 118.39, 124.76, 127.38, 127.98, 149.51, 164.57 ppm.

2,2-Dimethyl-1,2,3,4-tetrahydropyrazino[1,2-*a*]indol-2-ium iodide (2c). To a solution of **2b** (0.07 g, 0.27 mmol) in 5 mL of Et₂O MeI (0.2 mL, 3.21 mmol) was added and the solution stirred for 48 h in the dark. The mixture was then filtered and the solid residue dried to give 0.08 g of the title compound in 91% yield (white solid). Mp >250 °C. ¹H-NMR (DMSO, δ): 3.26 (s, 6H, N(CH₃)₂); 4.09 (t, 2H, *J* = 5.5 Hz, CH₂N); 4.46 (t, 2H, *J* = 5.5 Hz, CH₂N); 4.92 (s, 2H, CH₂N); 6.53 (s, 1H), 7.08-7.27 (m, 2H), 7.49-7.61 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (DMSO, δ): 37.40 (t), 50.50 (q), 57.73 (t), 58.87 (t), 100.72 (d), 110.08 (d), 120.38 (d), 120.49 (d), 121.95 (d), 126.72 (s), 127.48 (s), 136.33 (s) ppm. Anal. Calcd. for C₁₃H₁₇N₂I: C, 47.58; H, 5.22; N, 8.54. Found: C, 47.21; H, 5.27; N, 8.23.

2,2-Dimethyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indol-2-ium iodide (4c). Following the procedure used for **2c**, starting from 0.07 g (0.37 mmol) of **4b** and 0.2 mL (3.21 mmol) of MeI, the desired product was obtained in 49% yield (0.06 g) as a white solid. Mp 184-186 °C with decomposition. ¹H-NMR (D₂O, δ): 2.67 (dd, 1H, *J* = 16.1 Hz and 2.9 Hz, CH); 3.08-3.47 (m, 11H); 3.08 (s, 3H, CH₃N); 3.23 (s, 3H, CH₃N); 3.63 (td, 1H, *J* = 12.1 Hz and 3.3 Hz, CH); 3.85-3.93 (m, 1H, CH); 4.14-4.24 (m, 1H, CH); 6.76 (d, 1H, *J* = 7.7 Hz), 6.88 (dd, 1H, *J* = 7.7 Hz and 7.3 Hz), 7.17-7.26 (m, 2H) (aromatic protons) ppm. ¹³C-NMR (D₂O, δ): 31.52 (t), 38.13 (t), 46.70 (q), 55.19 (d), 56.61 (q), 58.12 (t), 61.16 (t), 108.83 (d), 120.34 (d), 125.69 (d), 127.88 (d),

128.72 (s), 147.40 (s) ppm. Anal. Calcd. for $C_{13}H_{19}N_2I$: C, 47.29; H, 5.80; N, 8.48. Found: C, 46.97; H, 5.70; N, 8.51.

Ethyl 3-(3-nitropyridin-4-yl)-2-oxo-propanoate (8).³¹ Diethyl oxalate (2.25 mL, 16.57 mmol) was added dropwise to a stirred solution of NaOEt (16.05 mmol, from 0.37 g of Na) in 20 mL of anhydrous EtOH; then, 4-methyl-3-nitro-pyridine (2 g, 14.48 mmol) dissolved in 30 mL of anhydrous toluene was added causing the formation of a deep red color. The solution was stirred at room temperature for 2.5 h, then the solvents were evaporated, the residue dissolved in water and treated with AcOH to form a red precipitate that was extracted with EtOAc. Drying and evaporation of the solvent gave a mixture that was purified by flash chromatography (toluene/EtOAc, 8/2 as first eluent and 5/5 as second eluent) to give 1.45 g of **8** (red solid, yield 42%). Mp 117-119 °C. ¹H-NMR ($CDCl_3$, δ) (as 2:1 mixture of keto and enol ester tautomers): 1.42 (t, 6H, $J = 7.1$ Hz, CH_2CH_3); 4.36-4.49 (m, 4H, $J = 7.1$ Hz, CH_2CH_3); 4.59 (bs, 1H, OH of enol form); 4.61 (s, 2H, CH_2CO); 6.99 (s, 1H, $CH=C$); 7.32 (d, 1H, $J = 5.1$ Hz), 8.83 (d, 1H, $J = 5.1$ Hz), 9.38 (s, 1H) (aromatic protons of keto form); 8.23 (d, 1H, $J = 5.5$ Hz), 8.78 (d, 1H, $J = 5.5$ Hz), 9.15 (s, 1H) (aromatic protons of enol form) ppm.

Ethyl 1,2-dihydroxy-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (9). A suspension of **8** (1.82 g, 7.65 mmol) in 120 mL of CH_2Cl_2 was hydrogenated at 30 psi at room temperature with Pd/C 10% (0.23 g) for 48 h. The solid was filtered off and washed several times with boiling EtOH. Removal of the solvents gave 1.31 g of the title compound in 76% yield (pale brown crystals). Mp 138-140 °C (with decomposition). ¹H-NMR (DMSO, δ): 1.17 (t, 3H, $J = 7.1$ Hz, CH_2CH_3); 3.00 (d, 1H, $J = 16.7$ Hz, benzylic proton); 3.34 (d, 1H, $J = 16.7$ Hz, benzylic proton); 4.13 (q, 2H, $J = 7.1$ Hz, CH_2CH_3); 6.69 (s, 1H, OH); 7.15 (d, 1H, $J = 4.8$ Hz), 7.95 (s, 1H), 8.08 (d, 1H, $J = 4.8$ Hz) (aromatic protons); 9.43 (s, 1H, OH) ppm. ¹³C-NMR (DMSO, δ): 14.37 (q), 39.67 (t), 61.66 (t), 97.02 (s), 120.13 (d), 132.26 (d), 134.90 (s), 142.93 (d), 147.81 (s), 170.11 (s) ppm. MS: 224 (M^+). IR (nujol) ν 3239 (OH), 1740 (C=O) cm^{-1} . Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.82; H, 5.45; N, 12.78.

Ethyl 1H-pyrrolo[2,3-c]pyridine-2-carboxylate (10).³¹ *Method A:* a suspension of **8** (1.45 g, 6.09 mmol) in 200 mL of CH_2Cl_2 was hydrogenated at 35 psi at room temperature with Pd/C 10% (0.18 g) for 72 h. The solid was filtered off and washed several times with CH_2Cl_2 /EtOH 1/1. Removal of the solvents gave 0.97 g of the title compound in 84% yield.

Method B: a suspension of **9** (0.68 g, 3.04 mmol) in 40 mL of abs. EtOH was hydrogenated at 110 psi at room temperature with Pd/C 10% (0.3 g) for 36 h. The solid was filtered off and washed several times with CH_2Cl_2 /EtOH 1/1. Removal of the solvents gave 0.54 g of the title compound in 93.5% yield (white crystals). Mp 207-208 °C. ¹H-NMR ($CDCl_3$, δ): 1.45 (t, 3H, $J = 7.2$ Hz, CH_2CH_3); 4.47 (q, 2H, $J = 7.2$ Hz, CH_2CH_3); 7.22 (s, 1H), 7.61 (d, 1H, $J = 5.5$ Hz), 8.35 (d, 1H, $J = 5.5$ Hz), 8.97 (s, 1H) (aromatic protons) ppm. ¹³C-NMR (DMSO, δ): 14.24 (q), 61.04 (t), 106.22 (d), 116.01 (d), 130.47 (s), 130.62 (s), 133.91 (s), 136.37 (d), 138.30 (d), 160.86 (s) ppm.

Ethyl 1-cyanomethyl-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (11). To a solution of **10** (0.15 g, 0.79 mmol) in anhydrous DMF (4 mL) potassium tert-butoxide (0.13 g, 1.18 mmol) was added at room temperature. After 40 min, chloroacetonitrile (0.07 mL, 1.18 mmol) was added dropwise and the solution heated at 65 °C for 30 min and left stirring at room temperature for 20 h. Water (20 mL) was then added and all the solvents distilled off to give a solid residue that

was treated with water and extracted with CH_2Cl_2 . Drying (Na_2SO_4) and removal of the solvent gave 0.18 g of the title compound (yellow solid, 84% yield). Mp 78-79 °C. $^1\text{H-NMR}$ (CDCl_3 , δ): 1.45 (t, 3H, $J = 7.1$ Hz, CH_2CH_3); 4.47 (q, 2H, $J = 7.1$ Hz, CH_2CH_3); 5.69 (s, 2H, CH_2CN); 7.37 (s, 1H), 7.61 (d, 1H, $J = 5.5$ Hz), 8.44 (d, 1H, $J = 5.5$ Hz), 8.98 (s, 1H) (aromatic protons) ppm. $^{13}\text{C-NMR-APT}$ (CDCl_3 , δ): 14.24, 32.76, 61.93, 110.93, 114.54, 116.65, 129.54, 131.02, 133.60, 134.86, 140.58, 161.15 ppm. Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 62.95; H, 5.03; N, 18.11.

3,4-Dihydropyrido[4',3':4,5]pyrrolo[1,2-a]pyrazin-1(2H)-one (13). Compound **11** (0.33 g, 1.44 mmol), dissolved in 30 mL of THF/ CH_3OH 1/1 was hydrogenated at 105 psi at room temperature with Ni-Raney (50% in H_2O , 2 g) for 72 h. The solid was filtered off and washed with CH_3OH , the solvents were evaporated and the residue separated by column chromatography (CH_2Cl_2 /petroleum ether/ EtOH/NH_3 340/60/65/8) to give 0.04 g of unreacted **11** and 0.1 g of **13** (yellow solid, 37% yield). Mp 248 °C (with decomposition). $^1\text{H-NMR}$ (DMSO, δ): 3.65-3.70 (m, 2H, $\text{NCH}_2\text{CH}_2\text{NH}$); 4.38-4.44 (m, 2H, $\text{NCH}_2\text{CH}_2\text{NH}$); 7.06 (s, 1H), 7.63 (d, 1H, $J = 5.5$ Hz), 8.19 (d, 1H, $J = 5.5$ Hz) (aromatic protons); 8.38 (bs, 1H, NH); 8.98 (s, 1H, aromatic proton) ppm. $^{13}\text{C-NMR}$ (DMSO, δ): 39.40 (t), 40.36 (t), 102.69 (d), 115.76 (d), 130.33 (s), 132.41 (s), 132.83 (s), 134.88 (d), 138.53 (d), 159.47 (s) ppm. Anal. Calcd. for $\text{C}_{10}\text{H}_9\text{N}_3\text{O}$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.42; H, 5.01; N, 22.28.

1,2,3,4-Tetrahydropyrido[4',3':4,5]pyrrolo[1,2-a]pyrazine (3a). *Method A:* a solution of **13** (0.31 g, 1.66 mmol) in 15 mL of anhydrous DME was added dropwise to a cooled (0 °C) suspension of LiAlH_4 (0.12 g, 3.16 mmol) in 10 mL of the same solvent and the mixture was heated at 80 °C for 3 h. The excess of hydride was quenched with ice and the solvent was distilled off. Extraction of the residue with CH_2Cl_2 / MeOH 1:1 gave 0.42 g of mixture that was separated by flash chromatography (CH_2Cl_2 / MeOH 9/1 as first eluent, CH_2Cl_2 /petroleum ether/ EtOH/NH_3 340/60/65/8 as second eluent) to give 0.1 g of unreacted **13** and 0.06 g of **3a** (pale red solid, 8% yield). Mp 144-145 °C (with decomposition). $^1\text{H-NMR}$ (CDCl_3 , δ): 2.01 (bs, 1H, NH); 3.36 (t, 2H, $J = 5.7$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}$); 4.09 (t, 2H, $J = 5.7$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}$); 4.23 (s, 2H, CCH_2NH); 6.18 (s, 1H), 7.41 (d, 1H, $J = 5.5$ Hz), 8.22 (d, 1H, $J = 5.5$ Hz), 8.65 (s, 1H) (aromatic protons) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , δ): 42.83 (t), 43.29 (t), 44.27 (t), 95.86 (d), 114.49 (d), 131.62 (d), 132.75 (s), 133.44 (s), 138.61 (s), 139.27 (d) ppm. Anal. Calcd. for $\text{C}_{10}\text{H}_{11}\text{N}_3$: C, 69.34; H, 6.40; N, 24.26. Found: C, 69.09; H, 6.49; N, 24.38.

3a oxalate salt. Mp 82-84 °C (with decomposition). $^1\text{H-NMR}$ (D_2O , δ): 3.92 (t, 2H, $J = 5.9$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}$); 4.67 (t, 2H, $J = 5.9$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}$); 4.84 (s, 2H, CCH_2NH); 6.91 (s, 1H), 8.04 (d, 1H, $J = 6.6$ Hz), 8.19 (d, 1H, $J = 6.6$ Hz), 9.03 (s, 1H) (aromatic protons) ppm.

Method B: LiAlH_4 (0.54 g, 14.21 mmol) was suspended in 25 mL of dry DME and a solution of **11** (0.98 g, 4.28 mmol) in 20 mL of dry DME was added dropwise at room temperature. The mixture was heated at 40 °C for 2 h, after cooling the excess of hydride was destroyed with ice and the solvent evaporated. The solid residue was extracted with a mixture of CH_2Cl_2 / EtOH 1/1. Evaporation of the solvents and separation by flash chromatography (CH_2Cl_2 /petroleum ether/ EtOH/NH_3 340/60/65/8) gave 0.1 g of [1-(2-aminoethyl)-1H-pyrrolo[2,3-c]pyridin-2-yl]-methanol (**12**) (oil, 12% yield) and 0.06 g of **3a** (10% yield).

$^1\text{H-NMR}$ (**12**) (CDCl_3 , δ): 3.21 (t, 2H, $J = 5.0$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}_2$); 4.35 (t, 2H, $J = 5.0$ Hz, $\text{NCH}_2\text{CH}_2\text{NH}_2$); 4.76 (s, 2H, CH_2OH); 6.51 (s, 1H), 7.51 (d, 1H, $J = 5.5$ Hz), 8.25 (d, 1H, $J =$

5.5 Hz), 8.70 (s, 1H) (aromatic protons) ppm. Anal. Calcd. for $C_{10}H_{13}N_3O$: C, 62.81; H, 6.85; N, 21.97. Found: C, 62.98; H, 6.67; N, 22.12.

Benzyl 3,4-dihydropyrido[4',3':4,5]pyrrolo[1,2-*a*]pyrazine-2(1*H*)-carboxylate (14). **3a** (0.04 g, 0.23 mmol) was dissolved in 1.2 mL of a 4:1 mixture of Na_2CO_3 2M/1,4-dioxane and the solution kept at 0 °C. A solution of ClCOOBz (0.04 mL, 0.28 mmol) in 0.4 mL of 1,4-dioxane and a solution of NaOH 2M (0.15 mL, 0.30 mmol) were then added dropwise simultaneously. After 1.5 h stirring, the organic solvent was distilled off and the mixture treated with water and extracted with CH_2Cl_2 . Drying (Na_2SO_4) and removal of the solvent gave 0.09 g of crude **14** (oil) that was used in the next reaction without further purification. 1H -NMR ($CDCl_3$, δ): 4.00-4.20 (m, 4H, NCH_2CH_2N); 4.94 (s, 2H, CCH_2N); 5.21 (s, 2H, OCH_2Bz); 6.29 (s, 1H), 7.28-7.46 (m, 6H), 8.23 (d, 1H, $J = 5.5$ Hz), 8.65 (s, 1H) (aromatic protons) ppm.

2-Methyl-1,2,3,4-tetrahydropyrido[4',3':4,5]pyrrolo[1,2-*a*]pyrazine (3b). Compound **14** (0.09 g, 0.29 mmol) was dissolved in 1.5 mL of anhydrous DME and the solution added dropwise to a suspension of $LiAlH_4$ (0.07 g, 1.86 mmol) in 1 mL of anhydrous DME at 0 °C. After 30 min, the mixture was allowed to reach room temperature and the excess of hydride was destroyed with ice. DME was evaporated, the residue dissolved in HCl 0.1 N and washed with CH_2Cl_2 . The aqueous phase was then alkalinized with NaOH and extracted with CH_2Cl_2 . Drying (Na_2SO_4) and removal of the solvent gave a solid that was purified by column chromatography ($CH_2Cl_2/MeOH$ 9/1) to give 0.03 g of **3b** (pale brown solid, 55% yield). Mp 121-122 °C. 1H -NMR ($CDCl_3$, δ): 2.53 (s, 3H, NCH_3); 2.96 (t, 2H, $J = 5.7$ Hz, $NCH_2CH_2NCH_3$); 3.82 (s, 2H, CCH_2N); 4.23 (t, $J = 5.7$ Hz, $NCH_2CH_2NCH_3$); 6.21 (s, 1H), 7.44 (d, 1H, $J = 5.5$ Hz), 8.23 (d, 1H, $J = 5.5$ Hz), 8.69 (s, 1H) (aromatic protons) ppm. ^{13}C -NMR ($CDCl_3$, δ): 42.21 (t), 45.91 (q), 52.12 (t), 53.39 (t), 96.09 (d), 114.57 (d), 131.73 (d), 133.15 (s), 138.43 (s), 139.12 (d) ppm. Anal. Calcd. for $C_{11}H_{13}N_3$: C, 70.56; H, 7.00; N, 22.44. Found C, 70.76; H, 7.13; N, 22.29.

3b oxalate salt. Mp 168-170 °C (with decomposition). ^{13}C -NMR-APT (D_2O , δ): 41.99, 45.03, 52.29, 52.91, 104.03, 119.72, 127.81, 131.38, 133.93, 140.37, 142.78, 167.14 ppm.

2,2,7-Trimethyl-1,2,3,4-tetrahydropyrido[4',3':4,5]pyrrolo[1,2-*a*]pyrazine-2,7-diium diiodide (15). MeI (0.005 mL, 0.09 mmol) was added to a solution of **3b** (0.015 g, 0.08 mmol) in 0.5 mL of anhydrous DMF and the mixture was kept under stirring at room temperature in the dark for 60 h. Distillation of the solvent gave the title compound as a brown solid (45% yield). Mp 218-220 °C. 1H -NMR (D_2O , δ): 3.38 (s, 6H, $N(CH_3)_2$); 4.14-4.20 (m, 2H, CH_2N); 4.36 (s, 3H, CH_3N); 4.75-4.80 (m, 2H, NCH_2); 5.08 (s, 2H, CH_2N); 6.93 (s, 1H), 8.02 (d, 1H, $J = 6.6$ Hz), 8.18 (d, 1H, $J = 6.6$ Hz), 9.15 (s, 1H) (aromatic protons) ppm. Anal. Calcd. for $C_{13}H_{19}N_3I_2$: C, 33.14; H, 4.06; N, 8.92. Found: C, 32.96; H, 4.18; N, 9.05.

Molecular modeling

For quantummechanical calculations, the compounds were sketched using Spartan '04 (PC Spartan '04 Windows, V. 1.0.0, Wavefunction, Inc., 18401 Von Karman Ave., Suite 370, Irvine, CA 92612, USA), and minimized to convergence using the quantum mechanical semiempirical program AM1 and the *ab initio* RHF procedure (basis set 6-31G*) within Spartan '04. The volumes of compounds **2c** and **4c** (226.35 \AA^3 and 230.93 \AA^3 , respectively) were calculated for the conformations optimized with the *ab initio* method. Molecular mechanics calculations were performed on a SGI R8000 workstation using the Accelrys programs InsightII and Discover (Version 2000), using the cff91 force-field for minimization, and Sybyl (Version 6.7). The

crystal structure of DMPP was retrieved from the Cambridge Structural Database (structure code DMPIPZ).

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