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Preliminary communication

Mannich bases of 3*H*-pyrrolo[3,2-*f*]quinoline having vasorelaxing activity

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Abstract

Mannich bases obtained by aminoalkylation of 3*H*-pyrrolo[3,2-*f*]quinoline were designed and prepared as potential vasorelaxing agents. Compounds **Ia**–**Va** were characterised by IR, ^{1}H-NMR, mass spectral data and elemental analysis; **IIb**,**c**–**Vb**,**c** were also confirmed by ^{1}H-NMR spectra of reaction mixtures. To estimate their vascular activity, prototypes 1-(*N*,*N*-dimethylaminomethyl)- (**Ia**) and 1-(4-phenyl-piperazin-1-ylmethyl)- (**IVa**) 3*H*-pyrrolo[3,2-*f*]quinoline derivatives were studied in rat-tail arteries. In tissues precontracted with 0.5 μM 5-hydroxytryptamine (5-HT), 3 μM phenylephrine or 80 mM KCl, **Ia** and **IVa** showed endothelium-independent relaxing action. In a preliminary study on the cellular mechanisms of **Ia**, the influence of propranolol, a β-receptor antagonist, and ketanserin, a 5-HT$_{2A}$-receptor antagonist, was checked. In the presence of phenylephrine, the vasorelaxing effect of **Ia** was not affected by these inhibitors. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: 3*H*-pyrrolo[3,2-*f*]quinoline derivatives; Mannich bases; vasorelaxation; 5-HT receptors

1. Introduction

Drugs are available for the treatment of diseases such as hypertension, intermittent claudication and Raynaud's disease, but the possibility of new therapeutic tools is still a goal of medical research. Vascular tone depends on the simultaneous influences of constricting and dilating stimuli on vascular smooth muscle. 5-Hydroxytryptamine (5-HT) plays an important modulatory function. In human vessels, 5-HT interacts with endothelial receptors to stimulate nitric oxide synthase and to evoke vascular relaxation, or, with other 5-HT receptors, to elicit contraction in smooth muscle [1]. The vascular effect of 5-HT depends on the extent of endothelial damage and the presence of various types of 5-HT receptors [2,3].

The aim of this research was to synthesise compounds with vasorelaxing activity interacting with the 5-HT system. Data from literature showed that Mannich bases of benzazoles have a relaxing effect on smooth muscle [4]. Compounds with indole moiety, present in 5-HT and related drugs, are well-known to influence resistance in human vessels. Also, the indole alkaloid gramine (*N*,*N*-dimethyl-3-aminomethylindole) interacts with the 5-HT system. Recently, halogen-gramine derivatives demonstrated a vasorelaxing action in isolated rat aorta [5]. Heterocycles with aminoethyl or piperazine side-chains in the C-3 substituent of the indole were generally useful for interaction with 5-HT receptors [6,7]. All these observations were the basis for our project: Mannich synthesis was used to prepare a new series of dialkyl-aminomethyl derivatives of 3*H*-pyrrolo[3,2-*f*]quinoline, a tricyclic nucleus containing the indole moiety. The aminomethylation reaction of pyrroloquinoline, obtained from its 9-chloro derivative by reductive elimination, was accomplished, employing formaldehyde and five secondary amines in a ratio of 1:1:1 and following a known procedure for the preparation of analogous indole derivatives [8]. The structure of Mannich bases was established on the basis of elemental analysis, IR, ^{1}H-NMR and mass spectral data.

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Preliminary studies on vascular activity focused on 1-(*N*,*N*-dimethyl-aminomethyl)- and 1-(4-phenyl-piperazin-1-ylmethyl)-3*H*-pyrrolo[3,2-*f*]quinoline derivatives **Ia** and **IVa**, used as prototypes. These compounds were tested on rat artery tissues precontracted by 5-HT, phenylephrine or KCl, and had an appreciable endothelium-independent vasorelaxing action. Experiments were also carried out to investigate the role of β-receptors and 5-HT_{2A} receptors in the action of **Ia**.

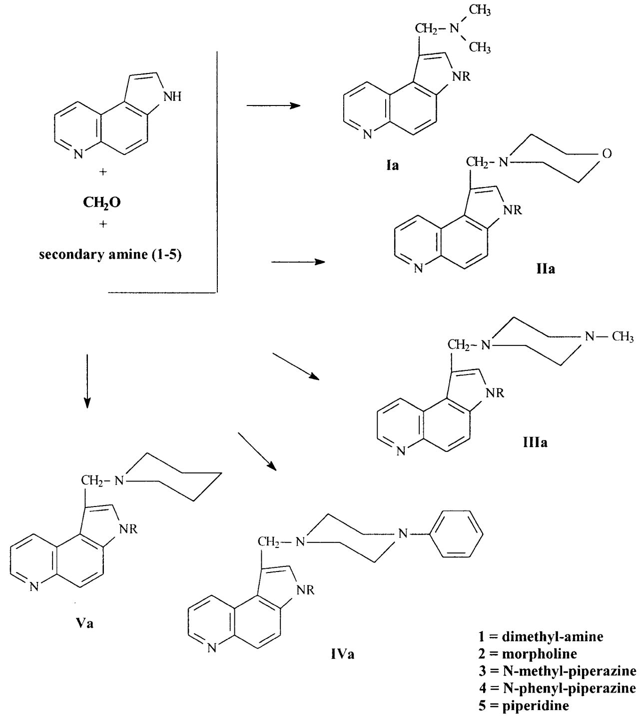

Fig. 1. Synthetic route to 3*H*-pyrrolo[3,2-*f*]quinoline Mannich bases **I**–**Va**, R = H.

Table 1
Chemical shifts of methylene groups of compounds **I**–**V**

Compound	a	b	c
	=C–CH_2–N	=C–CH_2–N –N–CH_2–N	=C–CH_2–N –N–CH_2–OH
I	3.86[a]	–	–
II	3.91[a]	3.78[b] 4.82	3.81 (s, CH_2)[c] 5.62 (d, CH_2) 6.61 (t, OH)
III	3.87[b]	3.82[b] 4.92	3.82[b] 5.75
IV	3.93[b] 3.87[c]	3.88[c] 5.18	3.87[b] 5.75
V	3.81[b]	3.80[b] 4.92	3.79 (s, CH_2)[c] 5.67 (d, CH_2) 6.57 (t, OH)

[a] Deutero-methanol.
[b] Deutero-chloroform.
[c] Deutero-dimethylsulphoxide.

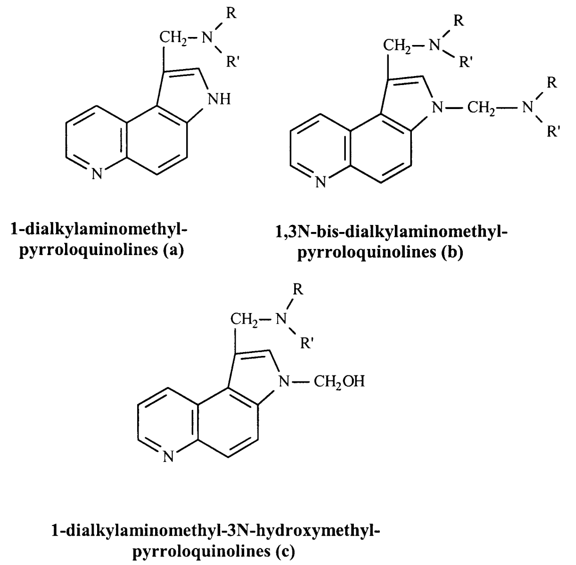

Fig. 2. Products (**a**, **b**, **c**) identified from aminoalkylation reaction of 3*H*-pyrrolo[3,2-*f*]quinoline, carried out as described in Section 6.

2. Chemistry

Mannich bases were prepared as described in Fig. 1. Pyrroloquinoline was obtained from the 9-chloro derivative by reductive elimination [9], performed with H_2 in the presence of 5% Pd/C, in a 90% yield. The dialkyl-aminomethylation reaction was carried out following the classical method, which employs formaldehyde, secondary amine, and the reactive compound in a 1:1:1 ratio. In all cases, except for the dimethylaminomethyl derivative, the formation of three compounds was confirmed by TLC analysis and ^{1}H-NMR spectral data (Table 1): the desired 1-Mannich base (**a**), 1,3-bis-Mannich base (**b**), and 1-Mannich base-3-hydroxymethyl derivative (**c**) (Fig. 2).

3. Pharmacology

1-(*N*,*N*-Dimethyl-aminomethyl)- and 1-(4-phenyl-piperazin-1-ylmethyl)-3*H*-pyrrolo[3,2-*f*]quinoline derivatives **Ia** and **IVa** were tested on rat-tail artery as prototypes of the synthesised Mannich bases. More precisely, the compounds were studied in varying conditions of precontracture (5-HT, phenylephrine or KCl), with or without endothelium. Further experiments were carried out with **Ia** to evaluate the cellular mechanism of the vasorelaxing action, using propranolol and ketanserin, well-known antagonists selective respectively for β and 5-HT_{2A} receptors.

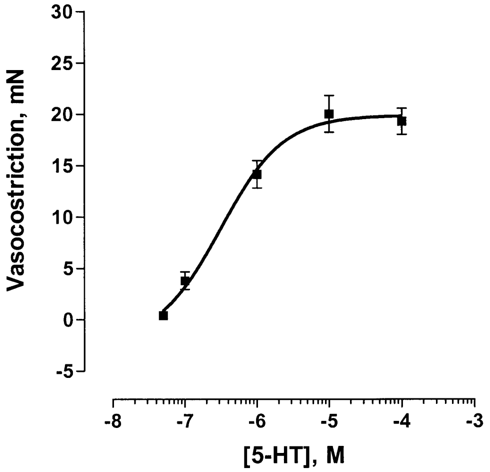

Fig. 3. Concentration–effect curve of 5-HT on rat-tail artery. Values are mean ± S.E.M. of eight experiments. S.E.M.s not indicated fall within respective symbols.

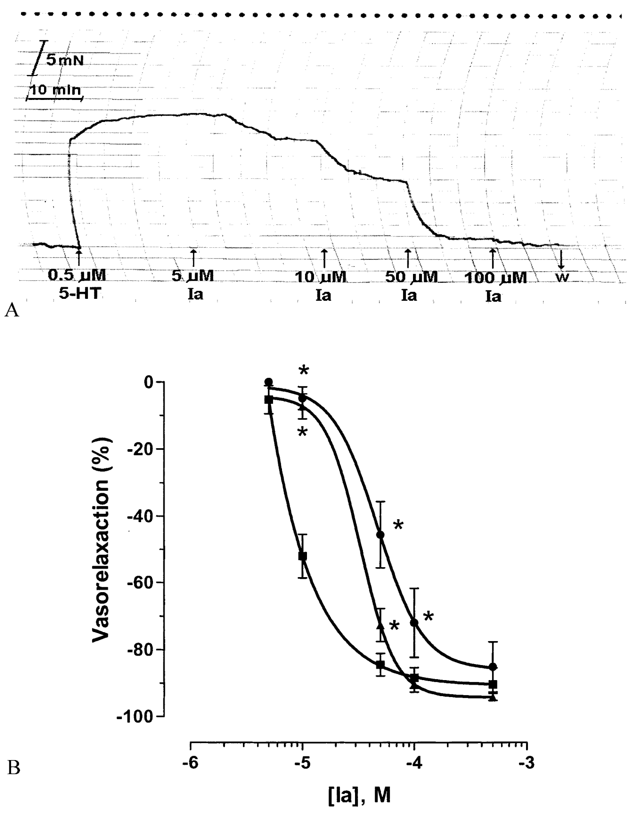

Fig. 4. Example of original tracing (A) obtained by pyrrolo[3,2-*f*]quinoline **Ia** on rat-tail artery precontracted with 0.5 µM 5-HT, and concentration–response curves (B) of **Ia** in rat-tail artery precontracted with 0.5 µM 5-HT (■), 3 µM PE (▲) and 80 mM KCl (●). Values are mean ± S.E.M. of 6–8 experiments. * $P < 0.05$ vs. **Ia** in tissues precontracted with 5-HT.

4. Results

4.1. Chemical characteristics

In comparison with the behaviour of indole in the Mannich reaction [10], pyrrolo[3,2-*f*]quinoline showed greater reactivity of the indole moiety, certainly due to the fused pyridine ring: the 3-H atom becomes more acid and higher stability of 3-substituted derivatives also follows. Similar results were obtained when different reagent ratios and reaction conditions were adopted, in order to obtain better yields of the 1-aminomethyl derivative. Obviously, reaction performance was partly conditioned by which secondary amine was used in the Mannich reaction, as indicated by the different but constant amounts in which the three compounds **a**, **b** and **c** formed in each reaction. The mutual ratios were noted by means of integral values in ^{1}H-NMR spectra of raw reaction material (data not reported). Among labelled **b** and **c** compounds, only **IVb** was isolated.

4.2. Pharmacological characteristics

4.2.1. Activity of *Ia* and *IVa*

1-(*N*,*N*-Dimethylaminomethyl)-3*H*-pyrrolo[3,2-*f*]quinoline (**Ia**) and 1-(4-phenyl-piperazin-1-ylmethyl)-3*H*-pyrrolo[3,2-*f*]quinoline (**IVa**) were tested in a range of concentrations from 10 nM to 0.5 mM, and maintained in an organ bath solution for 15 min at each concentration. In this condition, the compounds were unable to modify the resting tension of rat-tail artery (data not reported).

4.2.2. Effect of *Ia* in the presence of 5-HT, phenylephrine or KCl

In order to obtain the EC$_{50}$ value, we tested 5-HT from 50 nM to 100 µM on rat-tail arterial rings (Fig. 3). The pD$_2$ was 6.5 ± 0.2 (CE$_{50}$ $\sim$ 0.3 µM). With precontracture by 0.5 µM 5-HT, a concentration slightly higher than that of the EC$_{50}$, **Ia** evoked concentration-related relaxation (Fig. 4). The pD$_2$ was 5.0 ± 0.1. α_1-Adrenoceptor agonist phenylephrine, tested from 0.1 to 100 µM, evoked concentration-related contraction in arterial rings (Fig. 5). The pD$_2$ value was 5.4 ± 0.1 (CE$_{50}$ $\sim$ 3.6 µM). With precontracture by 3 µM phenylephrine (EC$_{50}$), **Ia** caused concentration-related relaxation (Fig. 4). The pD$_2$ was 4.5 ± 0.1. Increased isometric tension in artery rings can be obtained by direct depolarisation of tissue with KCl (80 mM). In this condition too, **Ia** evoked concentration-related relaxation (Fig. 4). The pD$_2$ was 4.3 ± 0.1. The potency of **Ia** was higher in tissues precontracted with 5-HT than with phenylephrine or KCl.

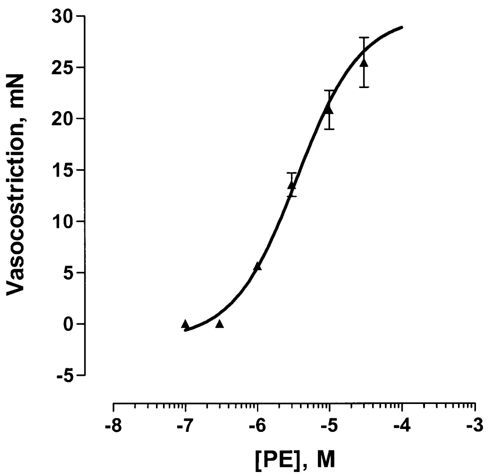

Fig. 5. Concentration–effect curve of phenylephrine (PE) in rat-tail artery. Values are mean ± S.E.M. of seven experiments. SEMs not indicated fall within respective symbols.

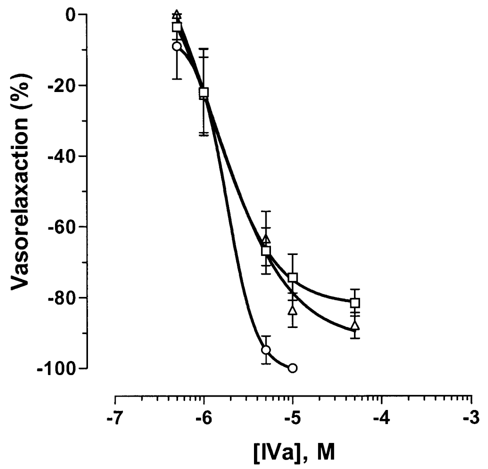

Fig. 6. Comparison among concentration–response curves of pyrrolo[3,2-*f*]quinoline **IVa** in rat-tail artery precontracted with 0.5 μM 5-HT (□), 3 μM PE (△) and 80 mM KCl (○). Values are mean ± S.E.M. of 6–8 experiments. SEMs not indicated fall within respective symbols.

4.2.3. *Effect of* ***IVa*** *in the presence of 5-HT, phenylephrine or KCl*

In the presence of 0.5 μM 5-HT, 3 μM phenylephrine or 80 mM KCl, **IVa** evoked concentration-related relaxation. The pD_2 were 5.9 ± 0.3, 5.9 ± 0.5 and 5.6 ± 0.2, respectively. The concentration-dependent relaxation curves obtained with **IVa**, in the various conditions of precontraction, are compared in Fig. 6. Derivative **IVa** showed comparable potency in vasorelaxing arterial tissues precontracted with 5-HT, phenylephrine, and KCl.

4.2.4. *Studies on vasorelaxing mechanisms*

All vascular preparations were subjected to a starting procedure to determine the viability of endothelium: they were first contracted with 10 μM phenylephrine and then, at the plateau of contraction, 30 μM acetylcholine was added. In this condition, acetylcholine was able to evoke vasorelaxation only if the endothelium was not damaged [11,12]. The vasorelaxing action of **Ia** and **IVa** was studied in rings with uninjured endothelium as well as in damaged endothelium; the curve-contraction effects did not change in the presence or absence of endothelium (data not shown).

Preliminary studies on cellular mechanisms using **Ia** were also carried out, since this compound showed selectivity in the 5-HT system. Derivative **Ia** in arterial rings precontracted by 3 μM phenylephrine evoked vasodilatation. In order to evaluate the role of β-adrenergic receptors, **Ia** was tested in the presence of 1 μM propranolol, a known blocker of β-adrenoceptor, which also has $5\text{-HT}_{1A}/5\text{-HT}_{1B}$ receptor antagonist activity [13,14]. Propranolol in itself did not modify the effect of phenylephrine used to precontract arterial rings. In this condition, the concentration-dependent relaxation curve of **Ia** was not modified (Fig. 7).

The role of 5-HT_{2A} receptor was also examined, using ketanserin, a well-known 5-HT_{2A} antagonist [15,16]. Alone ketanserin (50 nM) did not influence precontracture by phenylephrine, and did not change the relaxation induced by derivative **Ia** (Fig. 7).

5. Discussion

Compounds **Ia** and **IVa** evoked concentration-related relaxation in rat-tail artery precontracted with various active agents such as 5-HT, phenylephrine and potassium chloride. Concentration–response curves were obtained in a range from 0.1 to 500 μM. The vasorelaxing effects were endothelium-independent. Comparing the

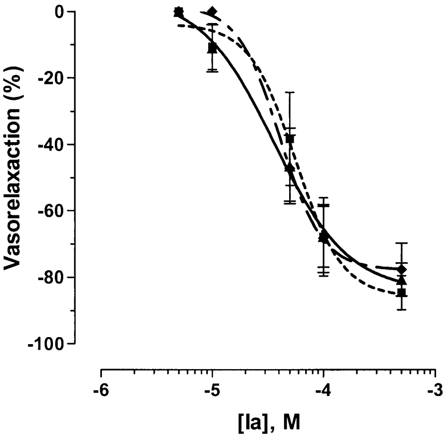

Fig. 7. Vasorelaxing effects induced by pyrrolo[3,2-*f*]quinoline **Ia** in rings precontracted with 3 μM PE, in the absence (control, ▲) and presence of 1 μM propranolol (◆) or 50 nM ketanserin (■). Values are mean ± S.E.M. of 6–8 experiments. SEMs not indicated fall within respective symbols.

relaxing effect induced by **Ia** with that of **IVa**, it was evident that **IVa** had a higher potency but less selectivity on the 5-HT system. These data indicate that the 1-(*N*,*N*-dimethylaminomethyl) group in 3*H*-pyrrolo-[3,2-*f*]quinoline **Ia** is important for selectivity on 5-HT receptors, whereas the 1-(4-phenyl-piperazin-1-yl-methyl) group eventually is useful for increasing potency. The other synthesised compounds, **IIa**, **IIIa** and **Va**, will be investigated pharmacologically in the near future.

Drugs with relaxing activity on vascular vessels independent of endothelium are of great value, because the endothelial layer is often damaged in cardiovascular diseases [17]. In these pathological conditions, physiological modulators such as acetylcholine and ATP lose their relaxing action on arterial tone, so that endothelium-independent relaxing agents are useful.

As regards the mechanism adopted by derivatives inducing relaxation, some preliminary experiments on **Ia** were carried out, which was the more selective on the 5-HT system. β-Adrenoceptors are expressed in vascular tissues regulating resistance [18–20]. Pretreatment with 1 μM propranolol, a well-known β-antagonist, did not change the vasorelaxing action of **Ia**; this experimental observation excludes the direct activation of **Ia** on β-adrenoceptors. It was also shown that propranolol is an antagonist of 5-HT$_{1A}$/5-HT$_{1B}$ receptors [13,14], so that the involvement of 5-HT$_{1A}$/5-HT$_{1B}$ receptors in vasorelaxing action by **Ia** is not probable, partly because this subtype of receptor is poorly represented in rat-tail artery [21]. The effect of ketanserin, a well-known antagonist of 5-HT$_{2A}$ receptors [15,16], with slight α-antagonist activity was also studied here [22,23]. Ketanserin did not change the vasorelaxing action of **Ia**, indicating that the compound does not act through 5-HT$_{2A}$ receptors. This is an intricate point: as it was not possible to carry out experiments with ketanserin with 5-HT precontracture since 5-HT is largely inhibited by ketanserin, we worked with vessels precontracted by phenylephrine. However, therefore we probably lost the component of **Ia** on 5-HT$_{2A}$: compound **Ia** cannot inhibit 5-HT receptors if they are not preactivated! Obviously, more research is necessary to clarify the cellular mechanisms by which **Ia** induces relaxation of precontracted rat-tail artery. The only consideration that, at this moment, may be made on dimethylaminomethylpyrrolo[3,2-*f*]quinoline **Ia** is based on its different potency in the various conditions of precontracture studied here. Its potency in reducing vascular tone is greater in rat-tail rings precontracted by 5-HT, less with phenylephrine, and the least by KCl. Future binding studies on the 5-HT receptors potentially involved may clarify this important point.

In conclusion, the most interesting observation is that pyrrolo[3,2-*f*]quinoline derivatives **Ia** and **IVa** have concentration-dependent relaxing effects, which are endothelium-independent. Data also show a slight selectivity of **Ia** on the 5-HT system and exclude the involvement of β-receptors. These derivatives may represent a source of endothelium-independent vasorelaxing agents.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Gallenkamp MFB-595-010M Melting Point Apparatus, and are uncorrected.

^{1}H-NMR spectra were obtained on a Varian Gemini 200MHz spectrometer using indicated solvents and TMS as internal reference. ^{1}H-NMR signals are reported in parts per million (ppm), and are characterised as singlets (s), doublets (d), triplets (t), quartets (q), or multiplets (m). In the case of multiplets, the quoted chemical shift corresponds to the multiplet centre. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Coupling constants are expressed in Hertz (Hz).

Mass spectra were obtained on a Varian Mat 112 mass spectrometer.

Elemental analyses were performed using Perkin–Elmer elemental analysis apparatus, model 240B; results for C, H, and N were within ± 0.4% of theoretical values.

IR spectra were recorded in cm^{-1} on a Perkin–Elmer 1760FTIR spectrophotometer as potassium bromide pressed disks.

Thin-layer chromatography (TLC) was performed using Merck silica gel on F254 polystyrene-backed plates.

Column flash chromatography was performed with Merck silica gel (250–400 mesh ASTM).

40% Dimethylamine was purchased from Fluka, all other secondary amines from Aldrich, and 37% formaldehyde from Acros.

6.1.1. 3H-Pyrrolo[3,2-f]quinoline

A solution of 9-chloro-pyrroloquinoline [9] (2.39 mmol) in 200 ml methanol was slowly added to 5% palladium in a charcoal–methanol suspension saturated with hydrogen, and the mixture was hydrogenated at atmospheric pressure at 40 °C until the starting material disappeared, according to TLC analysis (eluent ethyl acetate). After 2 h, the mixture was filtered and the filtrate evaporated to dryness. The residue was used in the Mannich reaction without purification. Yield 90%, pinkish-white solid; m.p. 181–82 °C (aceton) [24]; r.f. 0.41 (eluent ethyl acetate–*n*-hexane 7:3); ^{1}H-NMR (DMSO-d_6), δ: 7.43 (m, 1H, HC-1), 7.78 (t, 1H, $J_{2,3,1} = 2.74$ Hz, HC-2), 8.10–8.03 (m, 2H, HC-5, HC-8), 8.29

(d, 1H, $J_{4,5} = 9.52$ Hz, HC-4), 9.09 (dd, 1H, $J_{7,8} = 5.38$ Hz, $J_{7,9} = 1.40$ Hz, HC-7); 9.52 (d, 1H, $J_{9,8} = 7.70$ Hz, HC-9), 12.43 (bs, 1H, indolic NH).

6.1.2. General procedure for preparation of 1-alkyl-aminomethyl-pyrroloquinolines (Mannich bases) ***I–V***

An equimolar mixture of 37% formaldehyde and a secondary amine, made acid with glacial CH_3COOH, was previously prepared in a vessel at room temperature. After about 30 min, powdered pyrroloquinoline (1–3 mmol) was added, and the suspension was vigorously stirred for some time. It was then heated to 40 °C and maintained at that temperature until the end of the reaction, as indicated by TLC analysis (about 24 h). Next, the dark reaction mixture was poured into a cold 10% KOH water solution (6 ml) and kept at 0 °C under stirring for several hours. A semisolid precipitate formed and, if it became crystalline by standing at 0 °C for a further 24 h, it was filtered out, washed with water and dried; otherwise, it was extracted using a suitable solvent.

Purification by recrystallisation or flash chromatography was needed in order to obtain pure products.

*6.1.2.1. 1-(N,N-Dimethylaminomethyl)-3H-pyrrolo[3,2-f]quinoline (**Ia**).* Yield 50%, pale pink crystals; m.p. 192 °C dec. (methanol); r.f. 0.15 (eluent ethyl acetate–methanol 8:2); IR (KBr) 3186 (NH) cm^{-1}; ms (70 eV), *m/z* (relative abundance): 226 (M^+, 15%), 182 (226–44, 100%), 58 (226–168, 14%); ^{1}H-NMR (deutero-methanol), δ: 2.33 (s, 6H, 2 CH_3); 3.85 (s, 2H, –CH_2–N); 7.34 (s, 1H, CH-2); 7.54 (dd, 1H, $J = 8.5$ and 4.4 Hz, HC-8); 7.70 (d, 1H, $J = 9.0$ Hz, HC-4); 7.83 (d, 1H, $J = 9.0$ Hz, HC-5); 8.67 (dd, 1H, $J = 4.4$ and 1.6 Hz, HC-9); 8.00 (dd, 1H, $J = 8.5$ and 1.6 Hz, HC-7), 11.54 (bs, 1H, NH DMSO-d_6). Anal. calc. for $C_{14}H_{15}N_3$: C, 74.64; H, 6.71; N, 18.65. Found: C, 74.31; H, 6.93; N, 18.33%.

*6.1.2.2. 1-Morpholin-4-ylmethyl-3H-pyrrolo[3,2-f]quinoline (**IIa**).* This compound was prepared according to the general procedure but, as it did not solidify, it was extracted with ethyl acetate and extracts were evaporated to dryness. As the resulting crude product contained three compounds, **IIa–c** (^{1}H-NMR), having similar r.f. at TLC analysis, **IIa** was isolated by fractionated recrystallisation with absolute ethanol. Yield 20%; m.p. 160–63 °C dec.; r.f. 0.71 (eluent methanol); IR (KBr) 3125 (NH) cm^{-1}; ms (70 eV) *m/z* (relative abundance) 267 (M^+, 4%), 182 (267–86, 100%), 100 (267–167, 13%), 86 (267–182, 13%); ^{1}H-NMR (deutero-methanol) δ: 2.57 (t, 4H, $J = 4.7$ Hz, CH_2–O–CH_2), 3.67 (t, 4H, $J = 4,7$ Hz, morpholinic CH_2–N–CH_2) 3.91 (s, 2H, CH_2), 7.35 (s, 1H, HC-2), 7.54 (dd, 1H, $J = 8.4$ and 4.5 Hz, HC-8), 7.70 (d, 1H, $J = 9.1$ Hz, HC-4), 7.83 (d, 1H, $J = 9.1$ Hz, HC−5), 8.67(dd, 1H, $J = 4.5$ and 1.8 Hz, HC−9), 9.18 (dd, 1H, $J = 8.4$ and 1.8 Hz, HC−7), 11.57 (bs, 1H, NH DMSO-d_6). Anal. calc. for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.60; H, 6.58; N, 15.51%.

*6.1.2.3. 1-(4-Methyl-piperazin-1-ylmethyl)-3H-pyrrolo-[3,2-f]quinoline (**IIIa**).* This compound was prepared according to the general procedure but, as it did not solidify, it was extracted with chloroform and extracts evaporated to dryness. As the residue was composed of three compounds, **IIIa–c**, **IIIa** was separated by fractionated recrystallisation with absolute ethanol. Yield 35%; m.p. 235–236 °C (pale yellowish); r.f. 0.17 (eluent methanol); IR (KBr) 3136 (NH) cm^{-1}; ms (70 eV) *m/z* (relative abundance) 281 (M +, 30%), 182 (281–99, 85%), 112 (281–113, 9%), 99 (281–182, 43%), 56 (100%); ^{1}H-NMR (DMSO-d_6) δ: 2.12 (s, 3H, –CH_3), 2.29 (bm, 4H, 3′- and 5′-CH_2), 2.50 (bm, 4H, 2′ and 6′-CH_2, overlapped with DMSO signal), 3.78 (s, 2H, CH_2–), 7.39 (d, 1H, $J = 2.2$ Hz, HC-2), 7.47 (dd, 1H, $J = 8.5$ and 4.2 Hz, HC-8), 7.66 (d, 1H, $J = 9.0$ Hz, HC-4), 7.81 (d, 1H, $J = 9.0$ Hz, HC-5), 8.72 (dd, 1H, $J = 4.2$ and 1.5 Hz, HC-9), 8.96 (dd, 1H, $J = 8.5$ and 1.5 Hz, HC-7), 11.54 (bs, 1H, NH). Anal. Calc. for $C_{17}H_{20}N_4$: C, 72.3; H, 7.19; N, 19.98. Found: C, 72.55; H, 7.40; N, 19.70%.

*6.1.2.4. 1-(4-Phenyl-piperazin-1-ylmethyl)-3H-pyrrolo-[3,2-f]quinoline (**IVa–c**).* Prepared according to the general procedure, a white precipitate formed from the reaction mixture after 12–24 h at 0 °C. It was filtered out and dried. As it was composed of three compounds (TLC, ^{1}H-NMR), it was chromatographed by flash chromatography (eluent ethyl acetate–*n*-hexane 7:3), affording pure **IVa** and **IVb**.

IVa: yield 42%; m.p. 246–249 °C dec.; r.f. 0.25 (eluent ethyl acetate–*n*-hexane 7:3); IR (KBr) 3146 (NH) cm^{-1}; ms (70 eV) *m/z* (relative abundance) 343 (M^+, 45%), 182 (343–162, 66%), 162 (343–182, 52%), 121 (100%), 77 (343–266, 31%), 56 (96%); ^{1}H-NMR ($CDCl_3$) δ: 2.73 (bt, 4H, 3′ and 5′ –CH_2–), 3.20 (bt, 4H, 2′ and 6′ –CH_2–), 3.93 (s, 2H, –CH_2–N), 6.84 (t, 1H, $J = 7.2$ Hz, phenyl), 6.92 (d, 2H, $J = 7.8$ Hz, phenyl), 7.26 (t, 2H, $J = 7.2$ Hz, phenyl), 7.44 (d, 1H, $J = 2.2$, HC-2), 7.5 (dd, 1H, $J = 8.3$ and 4.2 Hz, HC-8), 7.75 (d, 1H, $J = 9.1$ Hz, HC-4), 7.87 (d, 1H, $J = 9.0$ Hz, HC-5), 8.81 (dd, 1H, $J = 2.7$ and 4.3 Hz, HC-9), 9.03 (bs, 1H, NH), 9.15 (dd, 1H, $J = 8.3$ and 2.7 Hz, HC-7), 11.6 (s, 1H, NH DMSO). Anal. Calc. for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36. Found: C, 76.91; H, 6.30; N, 16.52%.

IVb: Yield 30%; m.p. 220–23 °C dec.; r.f. 0.18 (eluent ethyl acetate–*n*-hexane 7:3); IR (KBr) 1594 and 1502 (phenyl) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.66 (bm, 8H, bis-piperazinic 3′ and 5′ –CH_2–), 3.11 (bm, 8H, bis-piperazinic 2′ and 6′ –CH_2–), 3.88 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.74 (m, 2H, bis-phenylic), 6.89 (m, 4H, bis-phenylic) 7.17 (m, 4H, bis-phenylic), 7.53 (m, 2H, HC-8 and HC-2), 7.74 (d, 1H, $J_{4,5} = 9.1$ Hz, HC-4), 8.15 (d, 1H, $J_{5,4} = 9.1$ Hz, HC-5), 8.74 (dd, 1H, $J = 1.4$ and 4.4 Hz, HC-7), 9.04 (dd, 1H, $J = 1.0$ and 8.5 Hz, HC-9). Anal. Calc. for $C_{33}H_{36}N_6$: C, 76.71; H, 7.02; N, 16.27. Found: C, 76.48; H, 7.13; N, 16.44%.

*6.1.2.5. 1-Piperidin-1-ylmethyl-3H-pyrrolo[3,2-f]quinoline (**Va**).* Following the general procedure, a solid beige precipitate separated from the reaction mixture, was filtered out, washed with water, and dried. As TLC analysis indicated the presence of several compounds having similar r.f., its isolation by means of fractionated crystallisation with various solvents was necessary. Yield 15%; m.p. 189.193 °C.; r.f. 0.45 (eluent methanol); IR (KBr) 3146 (NH) cm^{-1}; ms (70 eV) m/z (relative abundance) 266 (M^+, 9%), 182 (266–84, 100%), 98 (266–169, 30%), 84 (266–182, 39%), 56 (18%). ^1H-NMR ($CDCl_3$) δ: 1.0 (bm, 6H, piperidinic 3′, 4′ and 5′ –CH_2–), 2.51 (bm, 4H, piperidinic 2′ and 6′ –CH_2–), 3.81 (s, 2H, CH_2–N), 7.23 (d, 1H, $J = 2.1$ Hz, HC-2), 7.46 (dd, 1H, $J = 8.3$ and 4.3 Hz, HC-8), 7.74 (d, 1H, $J = 9.1$ Hz, HC-4), 7.87 (d, 1H, $J = 9.1$ Hz, HC-5), 8.82 (dd, 1H, $J = 4.3$ and 1.7 Hz, HC-9), 9.14 (dd, 1H, $J = 8.3$ and 1.7 Hz, HC-7), 11.81 (s, 1H, NH, DMSO). Anal. Calc. for $C_{17}H_{19}N_3$: C, 76.95; H, 7.22; N, 15.84. Found: C, 7716; H, 7.35; N, 15.59%.

6.2. Pharmacological methods

Male rats (Wistar, 250–350 g) were anaesthetised with inhalation of methoxyfluoran and then sacrificed by cervical dislocation. The proximal 2 cm of the tail artery were carefully removed and cleaned of adhering tissue. Arterial rings (3 mm long) were suspended between two tungsten wires to measure isometric tension and placed in a tissue bath containing 15 ml of Krebs–Ringer solution at 37 °C, with the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, D-glucose 11.1, bubbled with 95% O_2 and 5% CO_2 ($P_{O_2} = 345 \pm 8$ mmHg), pH 7.2. Tissues were stretched to a basal tension of 20 mN, this amount of resting tension having been shown to maximise the contractile response to phenylephrine, a standard vasoconstricting agent. The tail artery was preserved for a maximum period of 5 days in Krebs–Ringer solution at 4 °C [12]. The contractility of vascular tissue was recorded by means of a high-sensitivity transducer connected to a chart recorder (Ugo Basile, type DYO and Unirecord System, Model 7050, Comerio (VA), Italy). Vessel segments were allowed to equilibrate for 60 min before viability was assessed with a standard start procedure, consisting in constriction by 10 μM phenylephrine and, when contraction reached a plateau, relaxation by 50 μM acetylcholine; this procedure are also useful to check the condition of endothelium [22].

In experiments carried out on depolarised tissue by 80 mM KCl, the Krebs–Ringer solution was modified in order to counterbalance the increased concentration of KCl by an equal reduction of NaCl. Pharmacological experiments with untreated and treated tissues were performed on the same preparation.

6.2.1. Drugs

5-HT, L-phenylephrine hydrochloride and acetylcholine chloride were dissolved in saline. Ketanserin tartrate and propranolol hydrochloride were prepared in dimethylsulphoxide and ethanol, respectively. **Ia** and **IVa** were dissolved in dimethylsulphoxide. The concentration of organic solvents added never exceeded 0.2% V/V of the organ bath; in this condition, the solvents did not modify the contractility of vascular tissue.

6.2.2. Data analysis and statistics

All values presented are means ± S.E.M. for 7–8 experiments. Changes in tension are expressed in mN or as percentages of the maximum contraction obtained. For statistical comparisons between treatment and control data, student's two-tail paired t-test was used; the difference between mean group data was significant at $P < 0.05$.

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