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Novel anellated pyrazoloquinolin-3-ones: synthesis and in vitro BZR activity

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Abstract—A series of pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one derivatives **6**, **7a**–**c**, **8a**,**b**, **9a**,**b** and **10**–**12** were synthesized as modified pyrazoloquinolinone analogs (PQs) and evaluated for their ability to inhibit radioligand to central and peripheral benzodiazepine receptors (BZRs) and their effect on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Multistep synthesis starting from 5-nitroindole, via the Gould–Jacobs reaction to the quinoline nucleus, yielded key intermediates 9-chloro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylates. The reaction of the latter with methyl-hydrazine and various phenyl-hydrazines furnished the final compounds. In order to confirm the expected tetracyclic 2-substituted-2*H*-pyrazolopyrroloquinolin-3-one structure, IR spectrophotometric, mono-^{1}H and ^{13}C and bi-dimensional spectrometric and HRMS analyses were carried out: all compounds were found to be 2-substituted 3-keto tautomers; compound **6** only differed because it turned out to be 1-methyl-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-olo. The results of this work are consistent with those previously reported for PQs: **7**–**9** show high potency in displacing specific [^{3}H]flunitrazepam from its receptor site; no compound was active in inhibiting the binding of [^{3}H]PK 11195. They all act as antagonists at central BZR.

1. Introduction

Since 1982,[1] 2-aryl-pyrazoloquinolin-3-ones (PQs) have been known for their high affinity for central benzodiazepine receptors (BZRs) and later papers reported specific SARs and QSARs, indicating the structural requirements (lipophilic, electronic and steric) which cause changes in activity from state of an inverse agonist to an antagonist or agonist.[2,3] These compounds belong to a large number of BZ-binding site ligands, both classical benzodiazepine and non-benzodiazepine types, that is, polyheterocyclic structures synthesized and studied for their ability to interact with BZRs and all aimed at discovering more selective drugs. Extensive SARs and QSARs on the classes of compounds were useful in identifying pharmacophore models of the BZ-binding site, important tools for rational drug design, in the lack of sufficient structural insights into various receptor subtypes.[4]

The proposed agonist/antagonist pharmacophore model, based on 2-aryl-pyrazoloquinolinones, involves the interaction sites between the receptor and ligand necessary for activity,[5] whereas SAR and QSAR findings indicate the importance of various substitutions on the pyrazolo[4,3-*c*]quinolin-3-one skeleton which determine ligand affinities for various BZR subtypes.[3,5–8] As part of our screening research activity on pyrroloquinoline compounds,[9] we were interested in the synthesis of pyrazolopyrroloquinolin-3-ones and, in the light of specific literature data, we designed some derivatives conforming to the guidelines dictated by the postulated pharmacophore model. With the aim of obtaining ligands with high affinity for BZRs, we chose the angular pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one tetracycle, because it may be considered as pyrazolo[4,3-*c*]quinolin-3-one modified by a fused pyrrole ring at 8 and 9

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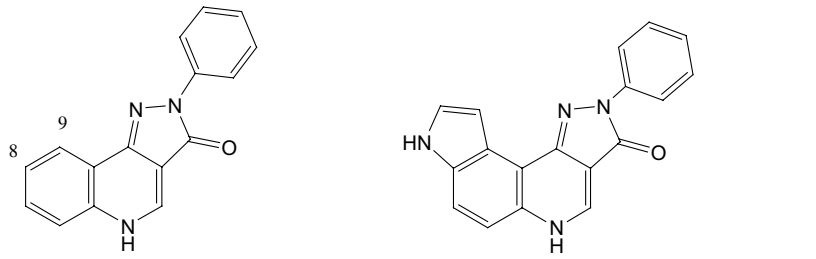


2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-3-one 2-Phenyl-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one

Figure 1.

positions (Fig. 1). These positions appeared to be the most suitable for substitution, particularly 9.[5] Moreover, the pyrrole ring may be subjected to further modifications aimed at optimizing the pharmacodynamic and pharmacokinetic properties of possible active agents. Our hypothesis was that the pyrrole moiety establishes connections with lipophilic region L_{Di} of the model pharmacophore/receptor and thus directs the type of activity (agonist or antagonist) (Fig. 2).[6] However, the data yielded by these tests would also be useful for more information on the complex nature of interactions at receptor lipophilic regions.[7,8]

This paper reports multistep synthesis, consisting of classical methods yielding the final tetracyclic compounds, results from affinity assays for central and peripheral BZRs, and activity on the $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ subtype receptor.

2. Results and discussion

2.1. Chemistry

The studied compounds (**6**, **7a**,**b**,**c**, **8a**,**b**, **9a**,**b**, **10**–**12**) were obtained starting from commercial 5-nitro-indole by classical and known methods, as shown in Scheme 1. For the synthesis of alkylated compounds **1b**,**c**, a previously described[10] modified alkylating procedure was adopted, which uses acetone and KOH at room temperature, with higher yields when compared with methods like NaH or other bases, alkyl iodide in dimethylformamide at higher temperature than room temperature. After catalytic reduction of **1b**,**c** with H_2 at atmospheric pressure to afford amino-derivatives **2b**,**c**, the next condensation reaction with diethyl ethoxymethylenemalonate yielded enamine derivatives **3b**,**c**, the structure of which was confirmed by the correctly positioned and coupled ^{1}H NMR signals of =*CH* and *NH* protons (about δ 8.45 and 10.86 in DMSO-d_6, $J = 14.0$, and about δ 7.63 and 10.07 in $CDCl_3$, $J = 10.1$ Hz, respectively). Both **3b**,**c** were submitted to thermal cyclization in boiling diphenyl ether, yielding pyrroloquinoline derivatives **4b**,**c** in the quinolinone form as the IR data showed (about 1619 cm^{-1} for *C*=*O*, ^{1}H NMR (about 12.30 δ for quinoline *NH*) and ^{13}C NMR (about 166 δ for C-9). Compounds **4a**,[11] **b**,**c** were transformed into 9-chloro derivatives by $POCl_3$ at boiling temperature, thus providing intermediate precursors **5a**,**b**,**c** for all 11 new final compounds. They furnished tetracyclic compounds **6**, **7a**,**b**,**c**, **8a**,**b**, **9a**,**b**, **10**, **11**, and **12** by reaction

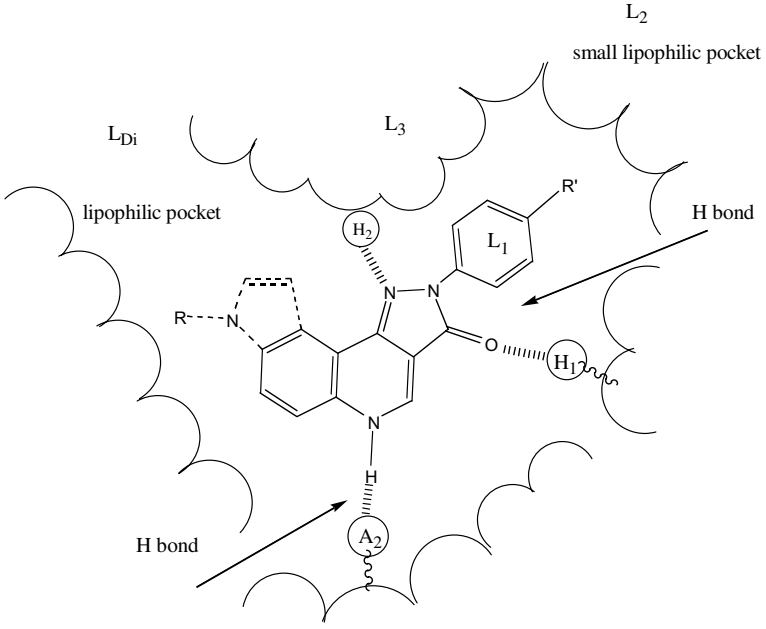
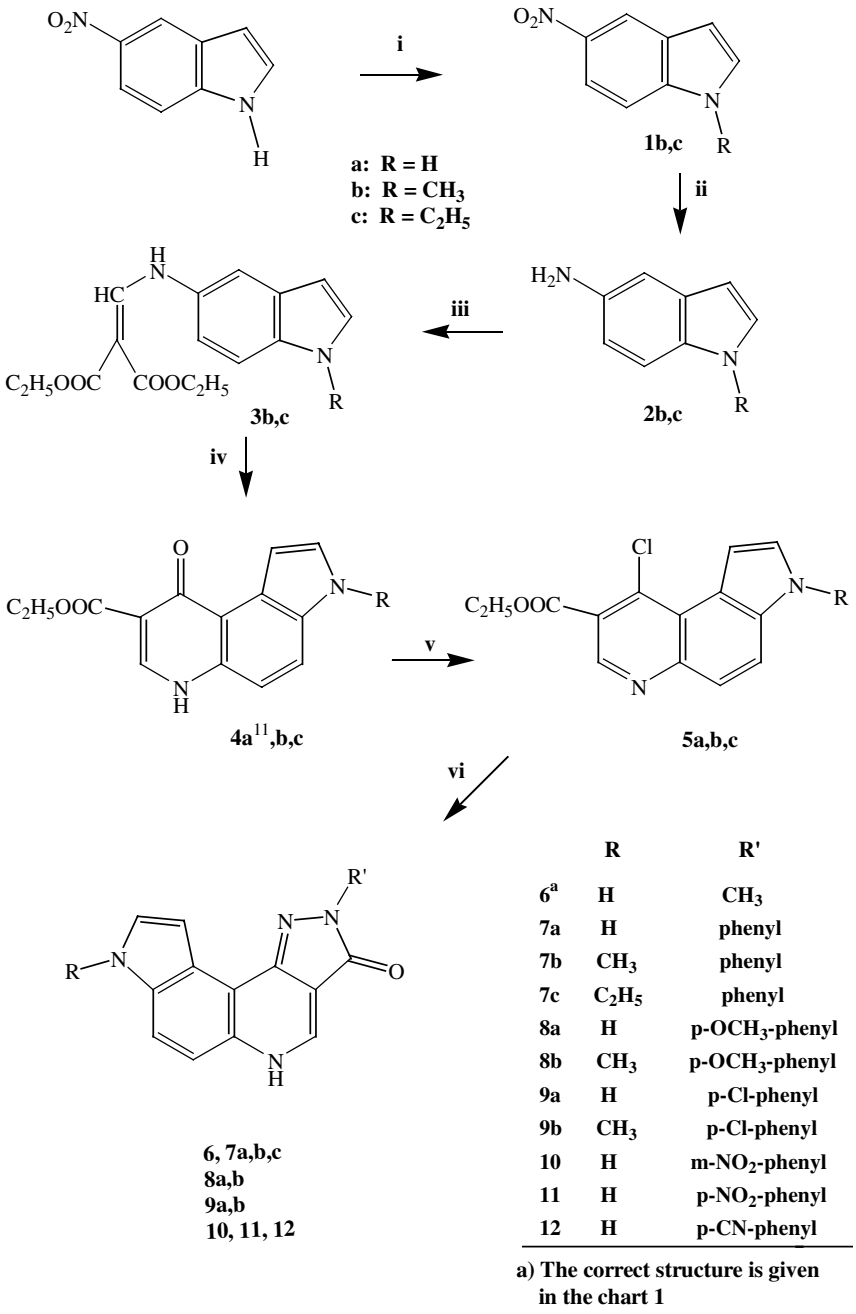


Figure 2. Proposed pharmacophore model for pyrazolo[4,3-*c*]quinolone lead compound[6] in which a pyrrole ring was designed by new synthesized tetracyclic pyrazolopyrroloquinolin-3-ones.

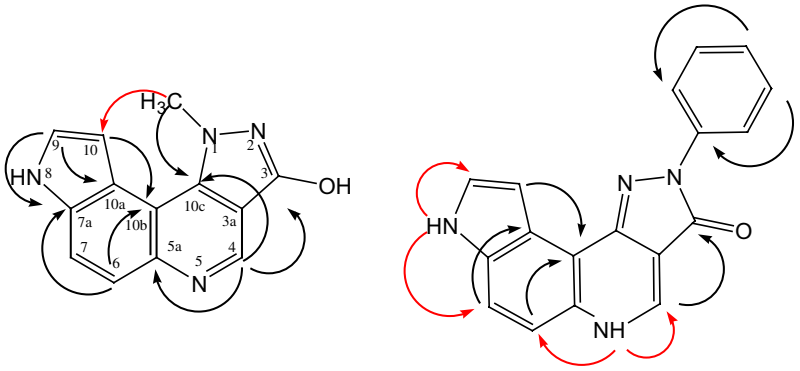


	R	R'
6[a]	H	CH_3
7a	H	phenyl
7b	CH_3	phenyl
7c	C_2H_5	phenyl
8a	H	p-OCH_3-phenyl
8b	CH_3	p-OCH_3-phenyl
9a	H	p-Cl-phenyl
9b	CH_3	p-Cl-phenyl
10	H	m-NO_2-phenyl
11	H	p-NO_2-phenyl
12	H	p-CN-phenyl

a) The correct structure is given in the chart 1

Scheme 1. Reagents and conditions. (i) CH_3I or C_2H_5I, acetone, KOH, reflux; (ii) H_2, Pd/C 5%, ethanol, 40 °C; (iii) diethyl ethoxymethylenemalonate, absolute ethanol, reflux; (iv) diphenyl ether, 250 °C; (v) $POCl_3$, 70 °C; (vi) hydrazine derivative, xylene, triethylamine, reflux.

with various purchased hydrazines in boiling xylene. The previously reported method, consisting of a nucleophylic substitution of the chlorine atom with the hydrazine derivate followed by cyclization,[1] again showed itself to be useful and profitable for the aims proposed. Spectroscopic, mass and elemental analyses, obtained for all new compounds, revealed the expected and designed 2H-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one structure (except for compound **6**, for which the HRMS spectrum gave a positive ion $[M + H]^+$ at *m/z* 239.10, suggesting a molecular formula of $C_{13}H_{10}N_4O$, calcd 239.09329 for $C_{13}H_{10}N_4O + H$). Spectral data indicated a 3-enol configuration instead of the 3-carbonyl one, and the methyl group in the 1 position thus 1-methyl-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-olo (Chart 1). The evidence for this structure followed from observations of the lack in the ^1H NMR spectra of the quinoline *NH* proton signal (doublet at $\delta > 12$), which caused the appearance of a singlet at about 8.7 δ in place of the doublet ($J = 6.4$ Hz) for *HC-4*, and the presence of a broadened signal at 11.4 δ, due to the *OH* proton. In addition, the C-4 signal and *C*═*O* peak did not appear in the ^{13}C NMR and IR spectra, whereas the corresponding peaks for C–OH and *OH* did.



1-Methyl-8-hydro-1*H*-pyrazolo [4,3-c]pyrrolo[3,2-f]quinolin-3-olo 6

2-Phenyl-5,8-dihydro-2*H*-pyrazolo[4,3-c]pyrrolo [3,2-f]quinolin-3-one 7a

Chart 1. Diagnostic HMBC (black arrows) and NOESY (red arrows) correlations for compound **6** and **7a**.

In order to confirm the assigned structures of these synthesized compounds, 2D-NMR experiments (HMQC, HMBC, COSY, NOESY) were performed: the chemical shifts of protonated carbons were obtained from an HMQC experiment. Complete elucidation of the structure was achieved by HMBC and NOESY experiments. Diagnostic long-range correlations were observed from the proton signal at δ 8.92 (HC-4) and carbons at δ 143.6 (C-10c), 143.3 (C-5a), 143.2 (C-3) and 109.2 (C-3a). The assignment of position 5a was confirmed by the HMBC correlation between the doublet at δ 7.82 and the carbon at δ 143.3 (C-5a). Further correlations from this proton signal (δ 7.82) determined the chemical shift of position 10a as δ 118.7. Assignment of position 7a was deduced from the HMBC correlations observed from proton signals at δ 7.82 (HC-7) and 7.56 (HC-9) and the carbon at δ 134.1 (C-7a). Long-range correlations were also observed between the signal at δ 7.23 and carbon at δ 110.5 (C-10b), and between the singlet at δ 4.16 and the carbon signal at δ 143.6 (C-10c). The NOESY experiment showed a strong correlation between the methyl group at δ 4.16 and the signal at δ 7.23, confirming that this group is linked in position 1. Thus, the structure of compound **6** was 1-methyl-8-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-olo (Chart 1).

For compound **7a**, taken as a sample among all the pyrazolopyrroloquinolinones, the HRMS spectrum gave a positive ion $[M + H]^+$ at *m/z* 301.10, indicating a molecular formula of $C_{18}H_{12}N_4O$ (calcd 301.10894 for $C_{18}H_{12}N_4O + H$); the ^1H NMR spectrum presented two signals, due to the amine protons at δ 11.76 (HC-8) and 12.86 (HC-5). In addition, the doublets of doublets were observed at δ 7.41 and 7.64 (1H each, $J = 2.65$ and 0.9 Hz) ascribable to positions HC-10 and HC-9, respectively; the *ortho*-coupled doublets at δ 7.48 and 7.76 (1H each, $J = 9.26$ Hz; HC-6 and HC-7, respectively), and the doublet at δ 8.67 (1H, $J = 6.4$ Hz, HC-4). Three doublets of doublets at δ 8.37 (2H, $J = 8.23$, 1.10 Hz), 7.46 (2H, $J = 8.23$, 9.23 Hz) and 7.16 (9.23, 1.10 Hz) were assigned to the phenyl moiety. Structure elucidation was achieved by DQF-COSY, NOESY, HMQC and HMBC experiments. In the DQF-COSY spectrum, the HC-4 signal (δ 8.67) showed coupling with the amine proton HC-5 (δ 12.86 Hz), whereas the latter (HC-5) showed correlations with the signals at δ 8.67 (HC-4) and 7.48 (HC-6). In addition, NOESY cross-peaks between the proton at δ 11.76 and the signals at δ 7.76 (HC-7) and 7.63 (HC-9) were also observed. The chemical shifts of protonated carbons were obtained by HMQC experiments. Structure elucidation was completed by HMBC correlations. Diagnostic HMBC correlations were observed between the singlet at δ 8.67 and the carbon signals at δ 144.5 (C-10c), 130.5 (C-5a), 106.3 (C3a) and 161.7 (C-3). Further long-range correlations were observed between the signal at 7.63 (HC-9) and the carbons at δ 133.3 (C-7a) and 121.4 (C-10a), and between the signal at 7.41 (HC-10) and the carbon at δ 111.5 (C-10b). The chemical shifts of the phenyl moiety were also deduced from HMBC correlations between the doublet of doublets at δ 7.16 (HC-4′) and the carbon signal at 119.2 (C-2′ and 6′) and between the signal at δ 8.37 (HC-2′ and 6′) and carbons at δ 140.9 (C-1′), 119.2 (C-2′ and 6′) and 124.4 (C-4′). Thus, the structure of compound **7a** was 2-phenyl-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]]quinolin-3-one (Chart 1).

2.2. Biological activity

To evaluate the possible interaction of pyrazolopyrroloquinolin-3-ones derivatives with the central BZRs, we studied the capability of these compounds to displace [^{3}H]flunitrazepam binding in comparison with the effect of diazepam. All the tested compounds inhibited the binding of [^{3}H]flunitrazepam. 1-Methyl derivative **6** showed low affinity, confirming the importance for the activity of aryl substitutions in the position 2 for these structures.The most potent compounds, in decreasing order, were **7c** (IC_{50} 1.7 nM), **8b** (IC_{50} 2.7 nM), **9a** (IC_{50} 3.5 nM), **7a** (IC_{50} 7.51 nM), **8a** (IC_{50} 8 nM). In comparison, diazepam inhibited [^{3}H]flunitrazepam binding with an IC_{50} of 11.5 nM (Table 1). To test whether these compounds affected peripheral benzodiazepine binding, compounds **6**–**12** were studied on [^{3}H]PK11195 binding. None of them were able to change this parameter. As reported in Table 1, the compounds result to have selective affinity for central BZRs.

Table 1. In vitro binding affinities[a] of pyrazolopyrroloquinolin-3-ones **6–12**

Compounds	IC_{50} (nM)	
	[^{3}H]Flunitrazepam binding	[^{3}H]PK 11195 binding
6	4450 ± 30	—
7a	7.51 ± 0.5	—
7b	27 ± 1	>10,000
7c	1.7 ± 0.1	>10,000
8a	8 ± 0.5	—
8b	2.7 ± 0.15	>10,000
9a	3.5 ± 0.2	>10,000
9b	70.1 ± 5	—
10	1011 ± 85	>10,000
11	7230 ± 60	>10,000
12	184 ± 15	>10,000
Diazepam	11.5 ± 0.7	>10,000
PK 11195		1.99 ± 0.1

[a] Each value is the mean ± SEM of three determinations. Missing data represent lack of affinity for the peripheral benzodiazepine receptor.

The affinity profile at the central BZR for **7–12** is consistent with substitutions on the 2-phenyl ring reported for pyrazoloquinolin-3-ones. Small groups with +M effect (see **7** and **9**) are well tolerated, although they do not confer much more affinity than the unsubstituted ring. Electron-withdrawing groups with −M effect (see **10** and **11**) are deleterious.

As regards the added pyrrole moiety, a general increase in affinity was noted for 8N-alkylated derivatives, particularly for 8N-ethylated **7c** (IC_{50} 1.7 nM). Although further investigations is needed, our results are consistent with the previous data suggesting that positive hydrophobic interactions occur in the lipophilic pocket L_{Di} (Fig. 2).

In order to clarify the action of these compounds on the $GABA_A$ receptor, the effects of **7a**, **8a**, **9a**, **8b**, and **7c** (0.001–10 μmol) were tested in *Xenopus laevis* oocytes expressing recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. Inward Cl^- currents, induced by application of an EC_{10} concentration of $GABA_A$, were not significantly affected by **7a**, **8a**, **9a** (Figs. 3–5) or **7c** (Fig. 7). However, **8b** (Fig. 6) at the highest concentration (10 μmol) significantly potentates by 41.8 ± 8.3% the $GABA_A$ receptor function ($p < 0.05$ Kruskal–Wallis ANOVA). By comparison, diazepam 1 μmol potentates this function with greater efficacy (∼+85%).

In addition, **7c** (0.001–10 μmol) inhibited the potentation of the $GABA_A$ receptor function induced by 1 μmol diazepam in a concentration-dependent fashion. Moreover, the enhancement of this function by diazepam was also completely blocked by 1 and 10 μmol **7c** (Fig. 8). Our results indicate that these compounds act at the $GABA_A$ receptor as benzodiazepine receptor antagonists.

3. Conclusions

Some new tetracycle pyrrolopyrazoloquinolin-3-ones were synthesized as pyrazoloquinolin-3-one analogs

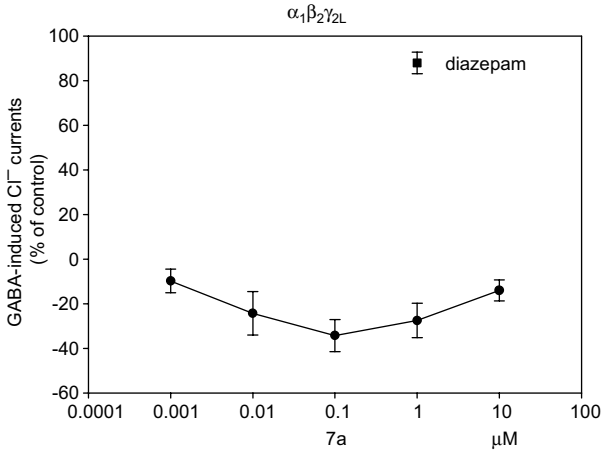


Figure 3. Lack of effect of **7a** (0.001–10 μmol) on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Compound **7a** was tested against an EC_{10} concentration of GABA. Data are means ± SEM of four oocytes.

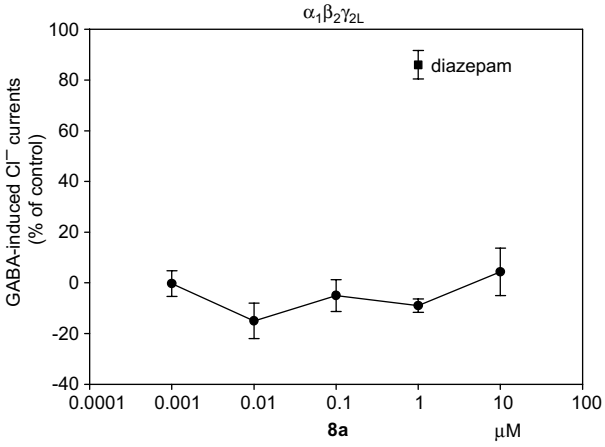


Figure 4. Lack of effect of **8a** (0.001–10 μmol) on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Compound **8a** was tested against an EC_{10} concentration of GABA. Data are means ± SEM of three oocytes.

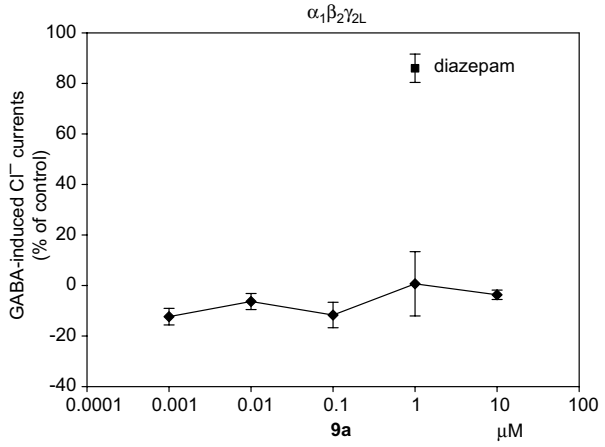


Figure 5. Lack of effect of **9a** (0.001–10 μmol) on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Compound **9a** was tested against an EC_{10} concentration of GABA. Data are means ± SEM of three oocytes.

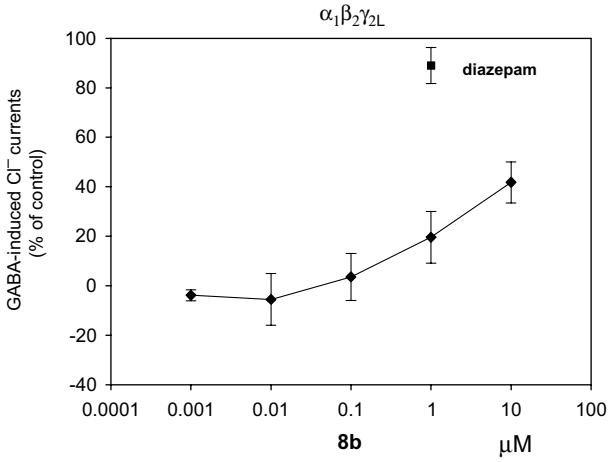


Figure 6. Concentration–response curve of **8b** (0.001–10 μmol) on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Compound **8b** (10 μmol) significantly enhanced an EC_{10} concentration of GABA. Data are means ± SEM of five oocytes.

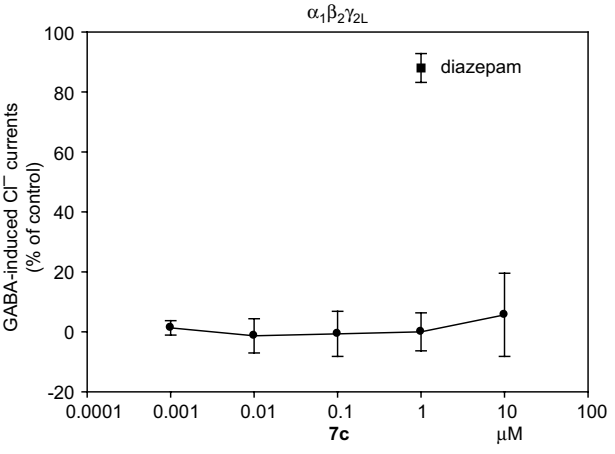


Figure 7. Lack of effect of **7c** (0.001–10 μmol) on $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus laevis* oocytes. Compound **7c** was tested against an EC_{10} concentration of GABA. Data are means ± SEM of three oocytes.

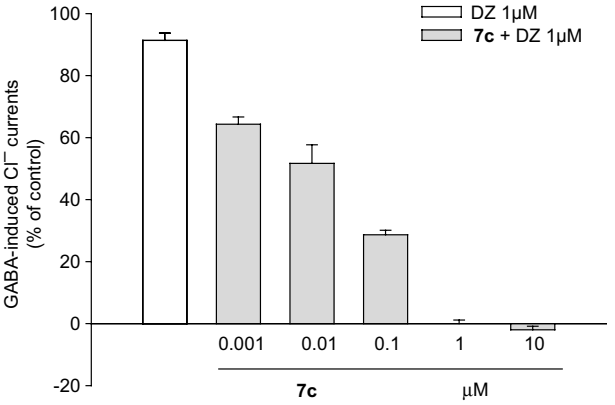


Figure 8. Compound **7c** (0.001–10 μmol) inhibited the enhancement of $GABA_A$ receptor function induced by diazepam (1 mol). $GABA_A$ $\alpha_1\beta_2\gamma_{2L}$ receptors were expressed in *Xenopus laevis* oocytes. Compound **7c** alone or in the presence of Diazepam (1 μmol) was tested against an EC_{10} concentration of GABA. Data are means ± SEM of three oocytes.

and, like their parent compounds, they showed high selective affinity for central BZRs acting as antagonists. Their affinity profiles and intrinsic activity fit previously reported PQs. Indeed, the higher affinity of compounds with an unsubstituted phenyl ring in position two (Table 1) confirms literature data on SARs and QSARs: the need for a not too heavily encumbered 2-phenyl ring and the slight importance of the electronic nature of its substitutions. Moreover, the most potent compounds were those with alkylated pyrrole 8-N, indicating that they fit the lipophilic pocket L_{Di} of the proposed pharmacophore/receptor model by establishing hydrophobic interactions and that this BZR pocket is large enough to receive the fourth fused pyrrole ring, even when N-ethylated, as in compound **7c**. Therefore, we believe that our work provides a further contribution to knowledge on pharmacophore/receptor topology and on specific SARs.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1760 FTIR spectrometer as potassium bromide pressed disks; values are expressed in cm^{-1}. UV–vis spectra were recorded on a Perkin–Elmer Lambda UV–vis spectrometer. ^1H NMR, ^{13}C NMR, HMBC, HMQC, COSY and NOESY spectra were recorded on a Bruker AMX spectrometer at 300.13 MHz for ^1H and 75.04 for ^{13}C, using the indicated solvents; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as internal reference. Coupling constant values are given in Hertz. In the case of multiplets, the chemical shift quoted was measured from the approximate centre. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, using a Perkin–Elmer Elemental Analyzer Model 240B; results fell in the range ±0.4% of theoretical values. High-resolution mass spectra were obtained with a Mat 112 Varian Mat Bremen (70 eV) mass spectrometer and Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was carried out on Merck silica gel (250–400 mesh ASTM); reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates. Solutions were concentrated in a rotary evaporator under reduced pressure. Starting 5-nitro-indole and all hydrazine derivatives employed in the synthesis in Scheme 1 were purchased from Aldrich and Acros Organics.

4.1.1. General procedure for synthesis of 1-alkyl-5-nitro-indoles (1b,c). A solution of 1g (6.2 mmol) of 5-nitro-indole in 20 mL of acetone, cooled by immersion in a water/ice bath, was added to 1.75 g (31.0 mmol) of powdered KOH. On vigorous stirring, alkyl iodide (12.4 mmol) was added and the mixture was stirred for

15 min at room temperature. After the addition of 90 mL of toluene, a precipitate of inorganic salts had formed, which was filtered off. The organic mixture was treated with saturated NaCl solution (20 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was re-crystallized from aqueous ethanol.

4.1.1.1. 1-Methyl-5-nitro-indole (1b). Yield 89%; mp 161 °C; R_f 0.52 (toluene/*n*-hexane 1:1); ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H, CH_3), 6.77 (d, 1H, $J_{3,2}$ = 3.1 Hz, HC-3), 7.62 (d, 1H, $J_{2,3}$ = 3.1 Hz, HC-2), 7.67 (d, 1H, $J_{7,6}$ = 9.0 Hz, HC-7), 8.06 (dd, 1H, $J_{6,7}$ = 9.0 and $J_{6,4}$ = 2.1 Hz, HC-6), 8.60 (d, 1H, $J_{4,6}$ = 2.1 Hz, HC-4). Anal. Calcd for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90. Found: C, 61.51; H, 4.65; N, 15.55.

4.1.1.2. 1-Ethyl-5-nitro-indoles (1c). Yield 80%; mp 94 °C; R_f 0.51 (toluene/*n*-hexane 1:1); IR (KBr) 1066, 2983, 3098 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.55 (t, 3H, J = 7.1 Hz, CH_3), 4.37 (q, 2H, J = 7.1 Hz, CH_2), 6.70 (d, 1H, $J_{3,2}$ = 3.2 Hz, HC-3), 7.65 (m, 2H, HC-7 and HC-2), 8.10 (dd, 1H, $J_{6,7}$ = 9 and $J_{6,3}$ = 2.1 Hz, HC-6), 8.62 (d, 1H, $J_{4,6}$ = 2.1 Hz, HC-4); ^{13}C NMR (DMSO-d_6) δ 15.6 (CH_3), 41.23, (CH_2), 103.94 (C-2), 110.57 (C-7), 116.60, (C-6), 117.89 (C-3), 127.68 (C-3°), 132.31, (C-4), 138.64 (C-5), 140.98 C-7a). Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.42; H, 5.57; N, 14.51.

4.1.2. General procedure for synthesis of 1-alkyl-5-amino-indoles (2b,c). A solution of 1-alkyl-5-nitro-indole, prepared as above, in absolute ethanol was slowly added to a palladium 5% on carbon ethanol suspension saturated with hydrogen and the mixture was hydrogenated at an atmospheric pressure of 40 °C until the starting material had disappeared, according to TLC analysis (eluent ethyl acetate/*n*-hexane 1:3). After 3–4 h, the mixture was filtered and the filtrate evaporated to dryness. The almost pure residue was used in the next reaction without purification.

4.1.2.1. 1-Methyl-5-amino-indole (2b). Yield 96%; mp 87 °C; R_f 0.44 (ethyl acetate/*n*-hexane 1:3); ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 1H, HC-3), 4.66 (bs, 2H, NH_2), 6.45 (d, 1H, $J_{3,2}$ = 2.4 Hz, HC-3), 6.82 (dd, 1H, $J_{6,4}$ = 1.9 and $J_{6,7}$ = 8.4 Hz, HC-6), 6.96 (d, 1H, $J_{4,6}$ = 1.9 Hz, HC-4), 7.32 (m, 2H, HC-7 and HC-2). Anal. Calcd for $C_9H_{10}N_2$: C, 73.94; H, 6.89; N, 19.16. Found: C, 73.64; H, 7.12; N, 18.89.

4.1.2.2. 1-Ethyl-5-amino-indole (2c). Yield 94%; semisolid product; R_f 0.22 (ethyl acetate/*n*-hexane 1:3); IR (KBr) 3346–3418, 2974, 2932, 1154 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.55 (t, 3H, J = 7.3, –CH_3), 4.30 (q, 2H, J = 7.3, –CH_2), 4.70 (bs, 2H, NH_2), 6.38 (d, 1H, $J_{2,3}$ = 3.1 and $J_{2,4}$ = 0.7, HC-3), 6.80 (dd, 1H, $J_{6,7}$ = 8.6 and $J_{6,4}$ = 2.3, HC-6), 6.96 (d, 1H, $J_{4,6}$ = 2.2, HC-4), 7.38 (m, 2H, HC-2 and HC-7); ^{13}C NMR (DMSO-d_6) δ 15.20 (–CH_3), 39.89 (CH_2), 98.57 (C-2), 103.46 (C-3), 109.37 (C-7a), 111.48 (C-6), 127.04 (C-3a), 128.86 (C-4), 129.28 (C-5), 140.82 (C-7). Anal. Calcd for $C_{10}H_{12}N_2$: C, 79.97; H, 7.35; N, 17.48. Found: C, 79.63; H, 7.08; N, 17.21.

4.1.3. General procedure for synthesis of 2-[(1-alkyl-1*H*-indol-5ylamino)-methylene]-malonic acid diethyl esters (3b,c). Equimolar amounts of 5-amino-indoles **2b**,**c** (11–12 mmol) and diethyl ethoxymethylenemalonate were mixed together and heated slowly to 130 °C in an oil bath. After about 3 h (TLC) at this temperature, the reaction product ethanol was evaporated under reduced pressure. Cooling to room temperature converted the semisolid crude malonate into a compact solid.

4.1.3.1. 2-[(1-Methyl-1*H*-indol-5ylamino)-methylene]-malonic acid diethyl ester (3b). Yield 96% (methanol); mp 66 °C; R_f 0.85 (ethyl acetate/*n*-hexane 7:4); ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 6H, J = 7.1 Hz, 2× CH_3), 3.90 (s, 3H, N–CH_3), 4.12 (2q, 4H, J = 7.1 Hz, 2× CH_2), 6.44 (d, 1H, $J_{2,3}$ = 2.9 Hz, HC-3), 7.17 (dd, 1H, $J_{6,4}$ = 2.1 and $J_{6,7}$ = 8.7 Hz, HC-6), 7.25 (d, 1H, $J_{2,3}$ = 2.9 Hz, HC-2), 7.38 (d, 1H, $J_{7,6}$ = 8.7 Hz, HC-7), 7.52 (d, 1H, $J_{4,6}$ = 1.9 Hz, HC-4), 8.45 (d, 1H, J = 14.0 Hz, *HC*=), 10.86 (d, 1H, J = 14.1 Hz, NH). Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.67; H, 6.28; N, 8.65.

4.1.3.2. 2-[(1-Ethyl-1*H*-indol-5ylamino)-methylene]-malonic acid diethyl ester (3c). Yield 95%; semisolid product; R_f 0.77 (ethyl acetate/*n*-hexane 7:4); IR (KBr) 2939–2983, 1714–1731, 1023.5 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.79 (m, 9H, 3× CH_3), 3.73 (m, 6H, 3× CH_2), 6.01 (d, 1H, $J_{3,2}$ = 3.3 Hz, HC-3), 6.71 (dd, 1H, $J_{6,7}$ = 8.7 and $J_{6,4}$ = 2.3 Hz, HC-6), 6.98 (d, 1H, $J_{2,3}$ = 3.2 Hz, HC-2), 7.05 (d, 1H, $J_{7,6}$ = 8.7 Hz, HC-7), 7.07 (d, 1H, $J_{4,6}$ = 2.2 Hz, HC-4), 8.03 (d, 1H, J = 14.0 Hz, *HC*=), 10.47 (d, 1H, J = 14.0 Hz, NH). Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.14; H, 6.95; N, 8.35.

4.1.4. General procedure for synthesis of 3-alkyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl esters (4b,c). The cyclization reaction was performed by adding portions of enamine malonate derivatives **3b**,**c** (2–3 mmol) to boiling phenyl ether, heating to reflux for 15 min to produce the tricyclic pyrroloquinoline 8-ethyl esters, cooling to room temperature and filtering. The collected precipitate was washed with diethylether and dried and the resulting product re-crystallized from 70% ethanol.

4.1.4.1. 3-Methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl ester (4b). Yield 69%; mp 268-269 °C; R_f 0.71 (ethyl acetate/*n*-hexane 9:1); ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H, J = 7 Hz, CH_3), 3.96 (s, 3H, N–CH_3), 4.24 (q, 2H, J = 7 Hz, O–CH_2), 7.38 (d, 1H, $J_{4,5}$ = 8.7 Hz, HC-4), 7.28 (d, 1H, $J_{1,2}$ = 2.5 Hz, HC-1), 7.60 (d, 1H, $J_{2,1}$ = 2.7 Hz, HC-2), 7.56 (d, 1H, $J_{5,4}$ = 8.7 Hz, HC-5), 8.45 (s, 1H, HC-7), 12.24 (bs, 1 H, NH). Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.42; H, 5.52; N, 10.09.

4.1.4.2. 3-Ethyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]-quinoline-8-carboxylic acid ethyl ester (4c). Yield 63.5%; mp 262 °C; R_f 0.75 (ethyl acetate/*n*-hexane 9:1); IR (KBr) 3104, 2978, 1703, 1620 cm^{-1}; ^1H NMR(DMSO) δ 1.29 (t, 3H, J = 7.4 Hz, O–CH_2–CH_3), 1.38 (t, 3H, J = 7.2 Hz, N–CH_2–CH_3), 4.22 (q, 2H, J = 7.4 Hz, O–CH_2), 4.31 (q, 2H, J = 7.2 Hz, N–CH_2), 7.36 (d, 1H, $J_{4,5}$ = 9.0 Hz, HC-4), 7.56 (s, 2H, HC-2 e HC-3), 7.90 (d, 1H, $J_{5,4}$ = 9.0 Hz, HC-5), 8.44 (d, 1H, J = 6.9 Hz, HC-7), 12.23 (d, 1H, J = 6.5 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 13.61 (–CH_3), 15.05 (–CH_3), 39.83 (N–CH_2), 58.69 (O–CH_2), 102.91 (C-1), 111.05 (C-8), 115.07 (C-5), 117.80 (C-9a), 128.39 (C-2), 129.29 (C-3a), 130.04 (C-9b), 131.05 (C-4), 135.95 (C-5a), 141.16 (C-7), 164.64 (C=O). Anal. Calcd for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.33; H, 5.82; N, 9.69.

4.1.5. General procedure for synthesis of 9-chloro-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl esters (5a,b,c). Pyrroloquinoline derivatives of **4a**,[11] **b**,**c** (3–4 mmol) were added to an amount corresponding to five times their weight of $POCl_3$, and the mixture was refluxed until the starting material had disappeared at TLC analysis (eluent ethyl acetate/*n*-hexane 8:2). The suspension was then cooled first to room temperature and then to 0 °C in a water/ice bath. Then, with stirring, it was carefully made alkaline with NaOH 28% aqueous solution and the resulting precipitate was collected, washed many times with water, and dried. The raw material was purified by silica gel flash chromatography (ethyl acetate/*n*-hexane 7:3).

4.1.5.1. 9-Chloro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl ester (5a). Yield 94%; mp 202–203 °C; R_f 0.84 (ethyl acetate/*n*-hexane 8:2); IR (KBr) 3195, 2990, 2870, 1699.4, 727.1 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.40 (t, 3H, J = 7 Hz, CH_3), 4.45 (q, 2H, J = 7 Hz, CH_2), 7.67 (m, 2H, HC-1 and HC-2), 7.82 (d, 1H, $J_{5,4}$ = 8.9 Hz, HC-5), 8.09 (d, 1H, $J_{4,5}$ = 8.9 Hz, HC-4), 8.98 (s, 1H, HC-7), 12.28 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 14.92 (CH_3), 62.58 (CH_2), 106.01 (C-1), 120.09 (C-2), 120.63 (C-8), 121.41 (C-9$_b$), 123.72 (C-5), 124.30 (C-9$_a$), 126.13 (C-4), 134.42 (C-3$_a$), 139.83 (C-9), 146.01 (C-7), 148.24 (C-5$_a$), 165.59 (C–O). Anal. Calcd for $C_{14}H_{11}N_2O_2Cl$: C, 61.21; H, 4.04; N, 10.20; Cl, 12.91. Found: C, 59.93; H, 4.08; N, 10.07; Cl, 13.15.

4.1.5.2. 3-Methyl-9-chloro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl ester (5b). Yield 80%; mp 180–181 °C; R_f 0.86 (ethyl acetate/*n*-hexane 8:2); IR (KBr) 3084, 2982, 1720, 1044 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (t, 3H, J = 7.2 Hz, CH_2–CH_3), 3.97 (s, 3H, –CH_3), 4.44 (q, 2H, J = 7.2 Hz, CH_2–CH_3), 7.21 (d, 1H, $J_{1,2}$ = 3.2 Hz, HC-2), 7.69 (dd, 1H, $J_{1,2}$ = 3.2 and $J_{1,4}$ = 0.7 Hz, HC-1), 7.78 (dd, 1H, $J_{4,5}$ = 9.0 and $J_{4,1}$ = 0.7 Hz, HC-4), 7.85 (d, 1H, $J_{5,4}$ = 9.0 Hz, HC-5), 8.99 (s, 1H, HC-7); ^{13}C NMR ($CDCl_3$) δ 13.82 (–CH_3), 33.08 (N–CH_3), 61.47 (O–CH_2), 105.87 (C-1), 116.62 (C-2), 120.98 (C-8), 123.02 (C-9c), 123.10 (C-9b), 123.19 (C-3a), 127.77 (C-5), 133.64 (C-4), 140.96 (C-9), 145.61 (C-7), 147.15 (C-5a), 164.8 (C=O). Anal. Calcd for $C_{15}H_{13}N_2O_2Cl$: C, 62.40; H, 4.54; N, 9.90, Cl, 12.28. Found: C, 62.31; H, 4.74; N, 9.72, Cl, 12.46.

4.1.5.3. 3-Ethyl-9-chloro-3*H*-pyrrolo[3,2-*f*]quinoline-8-carboxylic acid ethyl ester (5c). Yield 78%; mp 107–108 °C; R_f 0.92 (ethyl acetate/*n*-hexane 8:2); IR (KBr) 3102, 1726, 2927, 2966 913 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (t, 3H, J = 7.1 Hz, N–CH_2–CH_3), 1.56 (t, 3H, J = 7.0 Hz, O–CH_2–CH_3), 4.36 (q, 2H, J = 7.2 Hz, O–CH_2–CH_3), 4.51 (q, 2H, J = 7.0 Hz, N–CH_2–CH_3), 7.36 (d, 1H, $J_{1,2}$ = 2.9 Hz, HC-1), 7.79 (d, 1H, $J_{2,1}$ = 3.0 Hz, HC-2), 7.88 (d, 1H, $J_{4,5}$ = 9.1 Hz, HC-4), 7.92 (d, 1H, $J_{4,5}$ = 9.3 Hz, HC-5), 9.06 (s, 1H, HC-7); ^{13}C NMR ($CDCl_3$) δ 13.84 (O–CH_2–CH_3), 15.53 (N–CH_2–CH_3), 41.13 (O–CH_2–CH_3), 61.44 (N–CH_2–CH_3), 106.19 (C-1), 116.55 (C-2), 121.04 (C-8), 123.22 (C-3a and C-9b), 125.86 (C-5), 132.74 (C-4), 141.48 (C-9), 145.75 (C-7), 147.36 (C-5a), 164.89 (C=O). Anal. Calcd for $C_{16}H_{15}N_2O_2Cl$: C, 63.47; H, 4.99; N, 9.25; Cl, 11.71: found: C, 63.22; H, 4.68; N, 9.02; Cl, 11.96.

4.1.6. General procedure for synthesis of 2-substituted pyrazolopyrroloquinoline-3-ones (6, 7a,b,c, 8a,b, 9a,b, 10, 11, 12). 9-Chloro-pyrroloquinoline derivative (200–300 mg, 1–1.5 mmol) was dispersed in 20 mL of xylene and the suspension was heated to refluxing until complete dissolution. Then a slight excess of hydrazine compound, together with an equimolar amount of triethylamine with respect to the hydrazine, was added and the reaction mixture was refluxed for the required time (10–25 h), until the disappearance of the starting product, monitored by TLC analysis (ethyl acetate). In the meantime, a precipitate had formed which, at the end of the reaction, was collected, washed many times with hexane, dried under vacuum, and finally recrystallized with absolute ethanol.

4.1.6.1. 1-Methyl-8-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo-[3,2-*f*]quinolin-3-olo (6). Yield 80% by further flash chromatography; mp 310 °C; R_f 0.38 (ethyl acetate/methanol 1:1); IR (KBr) 3387.4 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.18 (s, 1H, CH_3), 7.25 (s, 1H, HC-10), 7.59 (t, 1H, $J_{9,10}$ = 2.6 Hz, HC-9), 7.81 (d, 2H, $J_{6,7}$ = 8.8 Hz, HC-6), 7.86 (d, 2H, $J_{7,6}$ = 8.8 Hz, HC-7), 8.90 (bs, 1H, HC-4), 11.4 (s, 1H, OH), 11.82 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6) δ 40.5 (CH_3), 105.32 (C-10), 110.5 (C-10b and 3a), 115.89 (C-7), 119.42 (C-10a), 124.63 (C-6), 125.08 (C-9), 134.05 (C-7a and C-3), 142.2 (C-4), 143.59 (C-10c); HRMS (*m*/*z*) 239.10 (M + 1). Anal. Calcd for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.47; H, 4.28; N, 23.45.

More analytical data on HMQC, HMBC, COSY and NOESY aimed at elucidating the exact structure of **6** are given and discussed in Section 2.

4.1.6.2. 2-Phenyl-5,8-dihydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (7a). Yield 91%; mp 326 °C; R_f 0.86 (ethyl acetate/methanol 4:6); IR (KBr) 3373, 1620, 1596, and 1554 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.17 (t, 1H, $J_{4',3'\text{ and }4',5'}$ = 7.3 Hz, HC-4′), 7.41 (dd, 1 H, $J_{10,9}$ = 2.63 and 0.9 Hz, HC-10), 7.45 (d, 2H, $J_{3',2'\text{ and }5',6'}$ = 8.26 Hz, HC-3′ and HC-5′), 7.48 (d, 1H,

$J_{7,6}$ = 9.3 Hz, HC-7), 7.64 (t, 1H, $J_{9,10}$ = 2.63 and 0.9 Hz, HC-9), 7.78 (d, 1H, $J_{6,7}$ = 9.3 Hz, HC-6), 8.37 (d, 2H, $J_{2',3' \text{ and } 6',5'}$ = 8.3 Hz, HC-2′and HC-6′), 8.69 (d, 1H, $J_{4,3}$ = 6.4 Hz, HC-4), 11.75 (s, 1H, indole NH), 12.84 (s, 1 H, quinoline NH); ^{13}C NMR (DMSO-d_6) δ 104.67 (C-10), 106.84 (C-3a), 112.11 (C-10b), 113.53 (C-6), 115.88 (C-7), 119.31 (C-2′ and C-6′), 121.66 (C-10a), 124.51 (C-4′), 127.60 (C-9), 129.54 (C-3′ and C-5′), 131.04 (C-5a), 133.76 (C-7a), 137.42 (C-4), 141.42 (C-1′), 144.92 (C-10c), 162.52 (C-3); HRMS (*m*/*z*) 301.10 (M + 1). Anal. Calcd for $C_{18}H_{12}N_4O$: C, 71.99; H, 4.03; N, 18.66. Found: C, 71.75; H, 3.81; N, 18.39.

More analytical data on HMQC, HMBC, COSY and NOESY aimed at elucidating the exact structure of **7a** are given and discussed in Section 2.

4.1.6.3. 2-Phenyl-8-methyl-5-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (7b). Yield 62%, mp > 300 °C; R_f 0.45 (ethyl acetate); IR (KBr) 3227, 3086, 2933, 1599 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 3H, CH_3), 7.19 (t, 1H, $J_{4',3'}$ and $_{4',5'}$ = 7.5 Hz, HC-4′), 7.28 (d, 1H, $J_{10,9}$ = 2.3 Hz, HC-10), 7.46 (t, 2H, $J_{3',2'} = J_{3',5',4'} = J_{5',6'}$ = 7.5 Hz, HC-3′ and HC-5′), 7.54 (d, 1H, $J_{6,7}$ = 9.0 Hz, HC-7), 7.63 (d, 1H, $J_{9,10}$ = 2.3 Hz, HC-9), 7.86 (d, 1H, $J_{7,6}$ = 9.0 Hz, HC-6), 8.37 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 7.6 Hz, HC-2′ and HC-6′), 8.69 (d, 1H, $J_{4,5}$ = 6.5 Hz, HC-4), 12.87 (d, 1H, $J_{5,4}$ = 6.5 Hz, quinoline HN); ^{13}C NMR (DMSO-d_6) δ 103.34 (C-3a), 106.38 (C-10), 111.62 (C-6), 112.95 (C-10b), 113.68 (C-7), 118.79 (C-2′ and C-6′), 121.44 (C-10a), 124.04 (C-4′), 129.07 (C-3′ and C-5′), 130.60 (C-9), 131.39 (C-5a), 133.72 (C-7a), 137.03 (C-4), 140.88 (C-1′), 144.26 (C-10c), 162.02 (C-3); HRMS (*m*/*z*) 315.23 (M + 1). Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.70; H, 4.49; N, 17.82. Found: C, 72.53; H, 4.18; N, 17.42.

4.1.6.4. 2-Phenyl-8-ethyl-5-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (7c). Yield 51%; mp >300 °C; R_f 0.26 (ethyl acetate); IR (KBr) 3069, 2872, 2932, 1626.2 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.43 (t, 3H, J = 7.2 Hz, CH_3), 4.36 (q, 2H, J = 7.2 Hz, CH_2), 7.17 (t, 1H, $J_{4',3' \text{ and } 4',5'}$ = 7.2 Hz, HC-4′), 7.41 (d, 1H, $J_{10,9}$ = 2.8 Hz, HC-10), 7.47 (t, 2H, HC-3′and HC-5′), 7.53 (d, 1H, $J_{7,6}$ = 8.4 Hz, HC-7), 7.69 (d, 1H, $J_{9,10}$ = 2.8 Hz, HC-9), 7.90 (d, 1H, $J_{6,7}$ = 8.4 Hz, HC-6), 8.37 (d, 2H, HC-2′and HC-6′), 8.69 (d, 1H, $J_{4,NH}$ = 7.1 Hz, HC-4); 12.86 (d, 1H, $J_{NH,4}$ = 7.1 Hz, NH); ^{13}C NMR (DMSO-d_6) δ 15.70 (CH_3), 40.68 (CH_2), 103.13 (C-3a), 105.89 (C-10), 111.28 (C-6), 112.49 (C-10b), 113.21 (C-7), 118.41 (C-2′ and C-6′), 121.13 (C-10a), 123.64 (C-4′), 128.58 (C-3′ and C-5′), 129.28 (C-9), 130.07 (C-5a), 132.28 (C-7a), 136.55 (C-4), 140.33 (C-1′), 143.82 (C-10c), 161.52 (C-3); HRMS (*m*/*z*) 329.28 (M + 1). Anal. Calcd for $C_{20}H_{14}N_4O_2$: C, 73.15; H, 4.91; N, 17.01. Found: C, 72.93; H, 4.81; N, 16.70.

4.1.6.5. 2-(4-Methoxy-phenyl)-5,8-dihydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (8a). Yield 75%; mp 356 °C (dec.); R_f 0.41 (ethyl acetate/methanol 8:2); IR (KBr) 3339, 3260, 2974, 1622, 1596 and 1554 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H, CH_3), 7.03 (d, 2H, $J_{2',3' \text{ and } 5',6'}$ = 9.1 Hz, HC-3′and HC-5′), 7.39 (s, 1H, HC-10), 7.47 (d, 1H, J_{7-6} = 9.0 Hz, HC-7), 7.61 (s, 1H, HC-9), 7.74 (d, J_{6-7} = 9.0 Hz, HC-6), 8.23 (d, 2H, $J_{2',3' \text{ and } 5,6'}$ = 9.1 Hz, HC-2′and HC-6′), 8.64 (s, 1H, HC-4), 11.71 (s, 1H, indole NH), 12.82 (s, 1H, quinoline NH); ^{13}C NMR (DMSO-d_6) δ 56.10 (CH_3), 104.66 (C-3a), 106.09 (C-10), 112.13 (C-10b), 113.41 (C-6), 114.69 (C-3′, C-5′), 115.72 (C-7), 120.89 (C-2′, C-6′ and C-10a), 127.49 (C-9), 130.60 (C-5a), 133.71 (C-7a), 134.99 (C-1′), 137.14 (C-4), 144.65 (C-10c), 156.53 (C-4′), 162.01 (C-3); HRMS (*m*/*z*) 331.12 (M + 1). Anal. Calcd for $C_{19}H_{14}N_4O_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 68.91; H, 4.05; N, 16.83.

4.1.6.6. 2-(4-Methoxy-phenyl)-8-methyl-5-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (8b). Yield 45%; mp > 300 °C; R_f 0.19 (ethyl acetate); IR (KBr) 3339, 3260, 2974, 1626 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H, N–CH_3), 3.95 (s, 3H, O–CH_3), 7.04 (d, 2H, HC-3′ and HC-5′), 7.38 (d, 1H, $J_{10,9}$ = 2.8 Hz, HC-10), 7.54 (d, 1H, HC-7), 7.62 (d, 1H, $J_{9,10}$ = 2.8 Hz, HC-9), 7.84 (d, 1H, HC-6), 8.25 (d, 1H, HC-2′ and HC-6′), 8.67 (d, 1H, HC-4), 12.83 (d, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 32.50 (N–CH_3), 54.73 (–OCH_3), 106.09 (C-3a), 106.38 (C-10), 112.13 (C-6), 113.41 (C-10b), 114.69 (C-3′ and C-5′), 115.72 (C-7), 120.89 (C-2′, C-6′ and C-10a), 127.49 (C-9), 130.60 (C-5a), 133.72 (C-7a), 134.99 (C-1′), 137.14 (C-4), 144.65 (C-1′), 156.53 (C-10c), 162.01 (C-3); HRMS (*m*/*z*) 345.16 (M + 1). Anal. Calcd for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.49; H, 4.30; N, 16.03.

4.1.6.7. 2-(4-Chloro-phenyl)-5,8-dihydro-2*H*-pyrazolo-[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (9a). Yield 78%; mp > 300 °C; R_f 0.36 (ethyl acetate); IR (KBr) 3274, 2979, 1605, 745 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.41 (m, 1H, HC-10), 7.49 (d, 1H, $J_{6,7}$ = 8.6 Hz, HC-6), 7.52 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.6 Hz, HC-3′ and HC-5′), 7.65 (t, 1H, $J_{9,8} = J_{9,10}$ = 7.6 Hz, HC-9), 7.79 (d, 1H, $J_{7,6}$ = 8.6 Hz, HC-7), 8.43 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.6 Hz, HC-2′ and HC-6′), 8.72 (d, 1H, $J_{4,5}$ = 6.7 Hz, HC-4), 11.76 (sa, 1H, indole NH), 12.93 (d, 1H, $J_{5,4}$ = 7.2 quinoline NH); ^{13}C NMR (DMSO-d_6) δ 105.27 (C-10), 107.20 (C-3a), 112.61 (C-10b), 114.14 (C-6), 116.63 (C-7), 121.22 (C-2′ and C-6′), 122.40 (C-10a), 128.27 (C-9), 130.09 (C-3′ and C-5′), 131.73 (C-5a), 134.39 (C-7a and C-4′), 137.02 (C-4), 140.85 (C-1′), 145.82 (C-10c), 163.17 (C-3); HR MS (*m*/*z*) 336.78 (M + 1). Anal. Calcd for $C_{18}H_{11}N_4OCl$: C, 64.58; H, 3.31; N, 16.74; Cl, 10.59. Found: C, 64.27; H, 3.10; N, 16.54; Cl, 10.73.

4.1.6.8. 2-(4-Chloro-phenyl)-8-methyl-5-hydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (9b). Yield 58%; mp > 300 °C; R_f 0.43 (ethyl acetate); IR (KBr) 3252, 3088, 1618, 801 cm^{-1}; ^{1}H NMR (DMSO-*d*6) δ 3.97 (s, 3H, CH_3), 7.39 (d, 1H, $J_{10,9}$ = 2.8 Hz, HC-10), 7.51 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.6 Hz, HC-3′ and HC-5′), 7.54 (d, 1H, $J_{6,7}$ = 9.0 Hz, HC-6), 7.64 (d, 1H, $J_{9,10}$ = 2.8 Hz, HC-9), 7.87 (d, 1H, $J_{7,6}$ = 9.0 Hz, HC-7), 8.44 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.6 Hz, HC-2′ and HC-6′), 8.73 (s, 1H, HC-4), 12.94 (s, 1H, NH); ^{13}C NMR

(DMSO-d_6) δ 33.30 (CH_3), 103.37 (C-10), 106.13 (C-3a), 111.52 (C-10b), 112.99 (C-6), 113.89 (C-7), 120.18 (C-10), 121.47 (C-10a), 127.77 (C-9), 128.98 (C-2′ and C-6′), 130.65 (C-5a), 131.49 (C-7a and C-4′), 131.49 (C-3′ and C-5′), 137.27 (C-4), 139.64 (C-1′), 144.59 (C-10c), 161.98 (C-3); HRMS (*m*/*z*) 349.53 (M + 1). Anal. Calcd for $C_{19}H_{13}N_4OCl$: C, 65.43; H, 3.76; N, 16.06; Cl, 10.16. Found: C, 65.19; H, 3.53; N, 15.88; Cl, 10.45.

4.1.6.9. 2-(3-Nitro-phenyl)-5,8-dihydro-2*H*-pyrazolo-[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (10). Yield 80%; mp 300 °C (dec.); R_f 0.81 (ethyl acetate/*n*-hexane 7:3); IR (KBr) 3349.3, 3245, 1639, 1600.8 and 1521.4 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.45 (s, 1H, HC-10), 7.55 (d, 1H, $J_{6,7}$ = 8.9 Hz, HC-6), 7.72 (d, 1H, $J_{9,10}$ = 2.5 Hz, HC-9), 7.80 (m, 2H, HC-7 and HC-5′), 8.04 (d, 1H, $J_{6',5'}$ = 8.2 Hz, HC-6′), 8.82 (m, 2H, HC-4 and HC-4′), 9.33 (s, 1H, HC-2′), 11.84 (s, 1H, indole NH), 13.07 (d, 1H, J = 6.4 Hz, quinoline NH); ^{13}C NMR (DMSO-d_6) δ 104.61 (C-10), 106.28 (C-3a), 111.88 (C-10b), 112.88 (C-10b), 113.60 (C-6), 116.33 (C-7), 118.74 (C-6′), 121.69 (C-10a), 124.80 (C-5′), 127.92 (C-9), 131.18 (C-2′), 131.30 (C-5a), 133.88 (C-7a), 138.04 (C-4), 142.04 (C-1′), 145.93 (C-10c), 149.02 (C-3′), 163.09 (C-3); HRMS (*m*/*z*) 346.09 (M + 1). Anal. Calcd for $C_{18}H_{11}N_5O_3$: C, 62.61; H, 3.21; N, 20.28. Found: C, 62.38; H, 3.02; N, 20.47.

4.1.6.10. 2-(4-Nitro-phenyl)-5,8-dihydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin- 3-one (11). Yield 98%; mp 300 °C (dec.); R_f 0.62 (ethyl acetate/methanol 7:3); IR (KBr) 3314, 1592, 1322, 850 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.44 (s, 1H, HC-10), 7.52 (d, 1H, $J_{6,7}$ = 8.7 Hz, HC-6), 7.67 (m, 1H, HC-9), 7.81 (d, 2H, $J_{7,6}$ = 8.7 Hz, HC-7), 8.35 (d, 2H, $J_{2',3'\text{and}5',6'}$ = 9.3 Hz, HC-2′ and HC-6′), 8.64 (d, 2H, $J_{2',3'\text{and}5',6'}$ = 9.3 Hz, HC-3′ and HC-5′), 8.78 (d, 1H, $J_{4,5}$ = 6.6 Hz, HC-4), 11.81 (s, 1H, indole NH), 13.05 (s, 1H, quinoline NH); ^{13}C NMR (DMSO-d_6) δ 104.71 (C-10), 106.00 (C-3a), 111.79 (C-10b), 113.61 (C-6), 116.51 (C-7), 118.57 (C-2′ and C-6′), 121.75 (C-10a), 125.86 (C-3′ and C-5′), 127.98 (C-9), 131.44 (C-5a), 133.92 (C-7a), 138.28 (C-4), 143.18 (C-1′), 146.37 (C-4′), 146.71 (C-10c), 163.66 (C-3); HRMS (*m*/*z*) 346.08 (M + 1). Anal. Calcd for $C_{18}H_{11}N_5O_3$: C, 62.61; H, 3.21; N, 20.28. Found: 62.43; H, 2.98; N, 20.56.

4.1.6.11. 2-(4-Cyano-phenyl)-5,8-dihydro-2*H*-pyrazolo[4,3-*c*]pyrrolo[3,2-*f*]quinolin-3-one (12). Yield 70%; mp 225–228 °C; R_f 0.88 (ethyl acetate); IR (KBr) 3317, 2969, 2224, 1612 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.43 (m, 1H, HC-10), 7.52 (d, 1H, $J_{6,7}$ = 9.0 Hz, HC-6), 7.66 (t, 1H, $J_{9,10}$ = 2.3 and $J_{9,8}$ = 2.9 Hz, HC-9), 7.81 (d, 1H, $J_{7,6}$ = 9.0 Hz, HC-7), 7.92 (d, 2H, $J_{3',2'} = J_{5'6'}$ = 8.6 Hz, HC-3′ and HC-5′), 8.60 (d, 2H, $J_{2',3'} = J_{6'5'}$ = 8.6 Hz, HC-2′ and HC-6′), 8.76 (d, 1H, $J_{4,5}$ = 6.2 Hz, HC-4), 11.79 (s, 1H, indole NH), 13.01 (d, 1H, $J_{5,4}$ = 6.2 Hz, quinoline NH); ^{13}C NMR (DMSO-d_6) δ 104.19 (C-10), 105.44 (C-3a), 111.37 (C-10b), 113.12 (C-6), 115.86 (C-7), 118.42 (C–CN), 119.66 (C-7_a), 121.23 (C-4′), 127.40 (C-9), 130.83 (C-2′ and C-6′ and C-5a), 133.39 (C-7a), 133.57 (C-3′ and C-5′), 137.57 (C-4), 144.18 (C-1′), 145.75 (C-10_c), 162.85 (C-3); HRMS (*m*/*z*) 326.11 (M + 1). Anal. Calcd for $C_{19}H_{11}N_5O$: C, 70.15; H, 3.41; N, 21.53. Found: C, 69.83; H, 3.12; N, 21.71.

4.2. Biological activity

4.2.1. [^{3}H]Flunitrazepam binding. Male Sprague–Dawley rats weighing 200–225 g were killed by decapitation and their brains were rapidly removed. The fresh brain cortical tissue was suspended in 50 vol of Tris–HCl 50 mmol, pH 7.4, homogenized with a polytron PT 10 for 30 s, then centrifuged twice at 48,000*g* for 10 min. The resulting pellet was resuspended in 50 vol of the same buffer and used for the assay.

As previously described,[12] the [^{3}H] flunitrazepam binding was determined in a final volume of 1 mL consisting of 400 μL of membranes (about 200–400 μg of protein), 100 μL of [^{3}H]flunitrazepam (specific activity 70–100 Ci/mmol), 5 μL of drug or solvent and Tris–HCl 50 mmol, pH 7.4. Stock drug solutions (20 mmol) were dissolved in dimethyl sulfoxide (DMSO). Non-specific binding was determined in the presence of diazepam 5 μM.

4.2.2. Expression of human GABA of cDNA subunits. A mixture of plasmids subcloned into pCDM8 vector and encoding the α1, β2, γ2L receptor subunits (total of 1.5 ng of cDNA in 30 nL in a 1α:1β:1γ ratio) was injected into the animal pole of oocytes as described,[13] using a microdispenser (Drummolond Scientific, Broomwall, PA). The injected oocytes were stored in incubation media (sterile Modified Barth Solution (MBS) supplemented with 10 mg/L streptomycin, 10,000 units/L penicillin, 50 mg/L gentamycin, 90 mg/L theophylline, and 220 mg/L pyruvate) and incubated at 15 °C.

4.2.3. Electrophysiology. Electrophysiological measurements were made in oocytes 1–4 days after injection. As previously described,[14] oocytes were placed in a rectangular chamber (approximately 100 μL vol) and perfused (1.7 mL/min) with MBS, via a roller pump (Cole-Parmer Instruments Co., Chicago, IL) through 18-gauge polyethylene tubing (Clay Adams Co., Parsippany, NJ). They were impaled in the animal pole with two glass electrodes (0.5–10 MΩ) filled with 3 M KCl and clamped at −70 mV using a Warner Instruments OC725C (Hamden, CT) oocyte clamp. Currents were continuously plotted using a chart recorder (Cole-Parmer Instrument Co). GABA (Sigma, St. Louis, MO) was dissolved in MBS containing (in mmol): NaCl 88, KCl 1, HEPES 10, $MgSO_4$ 0.82, $NaHCO_3$ 2.4, $CaCl_2$ 0.91, and $Ca(NO_3)_2$, 0.33 adjusted to pH 7.5 and applied for 30 s.

Oocytes expressing $GABA_A$ recombinant receptors were perfused with drugs for 60 s either in the absence of GABA or in the presence of an EC_{10} concentration of GABA (i.e., concentration of GABA producing peak currents equal 10% of the maximal current obtained by application of 1 mmol GABA). All compounds **6**, **7a**,**b**,**c**, **8a**,**b**, **9a**,**b**, **10**–**12** as well as diazepam were first dissolved in DMSO at a 100 mmol stock solution, and

then diluted in MBS to a final DMSO concentration not exceeding 0.1%. For each experiment, control responses were determined before and 10 min after drug application.

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