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Synthesis and structure–activity relationship studies in peripheral benzodiazepine receptor ligands related to alpidem

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Abstract—The exploration of the structure–affinity relationships concerning a new class of peripheral benzodiazepine receptor (PBR) ligands related to alpidem has been pursued in order to evaluate the consistency of the structure–affinity relationships among different classes (and subclasses) of PBR ligands. The target amide derivatives were prepared following a previously published procedure based on the condensation of pyrrolo[3,4-*b*]quinoline derivatives **11a**,**b** with glyoxylic acid mono-hydrate and the subsequent amidation of the acids obtained via mixed anhydride. On the other hand, the preparation of compound **9g** lacking the pharmacophoric (δ1) carbonyl group involved: (a) the double sequential attack of the dimethylmethyleneammonium salt obtained from bis(dimethylamino)methane and acetyl chloride to pyrrolo[3,4-*b*]quinoline derivative **11b**, (b) the quaternization of the obtained allylamine derivative **13** with methyl iodide, and (c) the palladium-catalyzed allylation of *N*-methyl-*p*-anisidine by quaternary allylammonium cation **14**. The structure–affinity relationship trends observed in this subclass of tricyclic alpidem-related PBR ligands find correlations in other classes (or subclasses) of PBR ligands. This result supports the initial pharmacophoric hypothesis and suggests a common mode of interaction at the PBR binding site.

1. Introduction

The peripheral benzodiazepine receptor (PBR), so called because it was originally discovered in the periphery by means of tritium-labeled diazepam,[1] is a 169-amino acid protein (18 kDa) with five trans-membrane domains localized on the mitochondrial outer membrane, although nonmitochondrial (nuclear or microsomal) localizations in some cells have been suggested. Photolabeling studies indicated that this receptor is functionally linked to the voltage-dependent anion channel (VDAC) and to the adenine nucleotide translocase (ANT) and might be implicated in the regulation of the opening of the mitochondrial permeability transition pore (MPTP). Tissue distribution analysis revealed an ubiquitous expression of PBR with particularly high densities in steroidogenic tissues such as adrenal gland, but also in kidney, heart, testis, and at a lower level in the brain parenchyma, ependyma, choroid plexus, and olfactory neurons. Furthermore, PBR is overexpressed in a variety of tumors and the expression appears to be related to the tumor malignancy degree.[2] Augmented concentrations of PBR were observed in lesioned brain areas in a variety of neuropathologies such as multiple sclerosis, Alzheimer's disease, and Huntington's disease.[3]

The exact physiological function of PBR is not yet fully understood, but a wide range of pharmacological activities, such as anticonvulsant, anxiolytic, immunomodulating, and cardiovascular, has been related to its activation.[4] In particular, this receptor appears to be involved in steroidogenesis, the regulation of which represents a potential clinical application of PBR ligands.[5]

Keywords: PBR; Peripheral benzodiazepine receptor; Palladium-catalyzed allylation; X-ray crystallography.
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The isoquinolinecarboxamide PK11195 (**1**, Fig. 1) is the most widely used pharmacological tool for the study of the expression and the function of PBR. Compound **1** labeled with positron emitter carbon-11 was used in the imaging of brain tumors, multiple sclerosis, cerebral infarction, and abnormalities of calcium channels in heart diseases by means of positron emission tomography (PET).[6]

The imidazopyridine alpidem binds with nanomolar affinity both CBR and PBR and is capable of stimulating pregnenolone formation from the mitochondria of C6-2B glioma cells.[7]

In recent years, a number of alpidem-related PBR ligands have been described. Some examples include indol-3-acetamides **2**,[7] benzofuran-3-acetamides **3**,[7] imidazo[1,2-*a*]pyridineacetamides **4**,[8] pyrazolo[1,5-*a*]pyrimidineacetamides **5**,[9] and indol-3-glyoxylamides **6**.[10] Another PBR ligand which may be included in the alpidem-related compounds is pyridazino[4,5-*b*]indole-1-acetamide SSR180575 (**7**). This compound shows high affinity and selectivity for the rat and human PBR, and increased pregnenolone accumulation in the brain and sciatic nerve suggests that its neuroprotective effects could be mediated via PBR stimulation of neurosteroid biosynthesis.[11]

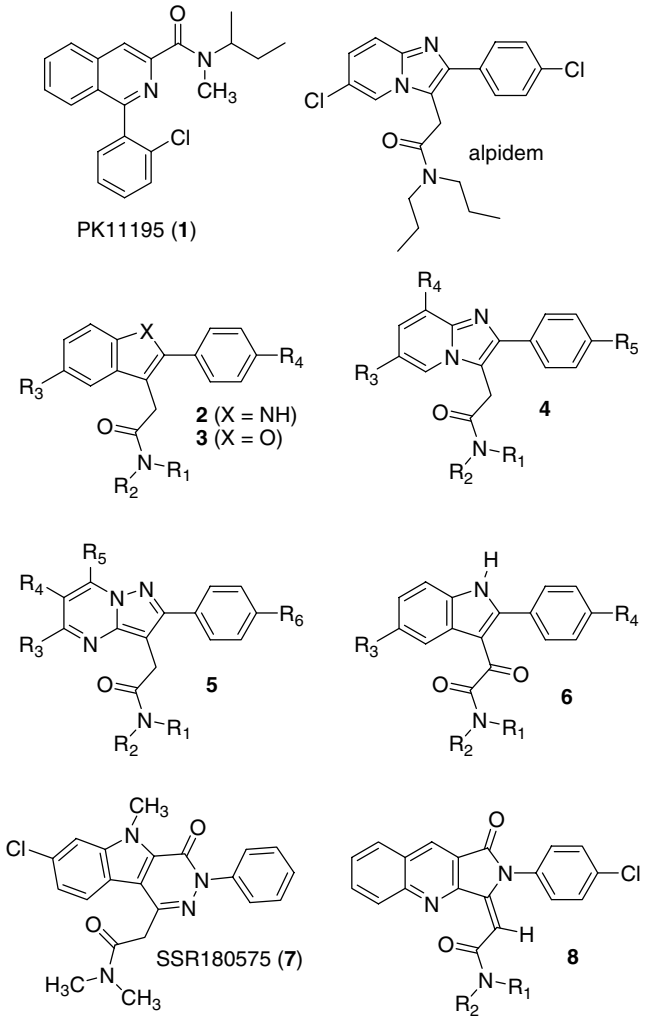


Figure 1. Structures of some PBR ligands.

The design and synthesis of PBR ligands led to development of potent compounds both in the class of alpidem-related substances (**8**)[12] and in the class of PK11195 derivatives[13] as well as to the development of theoretical models for the interaction of PBR with the best known ligands.[14] Some 2-quinolinecarboxamide derivatives related to PK1195 were ^{11}C-labeled and their preliminary evaluation suggested that these compounds are promising PET tracers[15] for the in vivo PET monitoring of neurodegenerative processes.[16] Moreover, the structure–affinity relationship (SAFIR) data obtained with PK11195-related compounds were used in the design of both the first PBR ligand bearing a carborane cage potentially useful in boron neutron capture therapy (BNCT)[17] and one of the most potent PET radiotracers for the in vivo imaging of the PBR so far described.[18]

The structural analysis of PBR ligands **1**–**8** suggests that they may share the common four component pharmacophore (δ1, LA, PAR, FRA, see Ref. 12) in which a single H-bonding acceptor moiety (δ1, usually a suitably oriented amide carbonyl group) plays a key role in the interaction with PBR. However, alpidem and compounds **1**–**6** show in their structure a bicyclic planar aromatic region (PAR), which is instead tricyclic in SSR180575[11] and in compounds **8**.[12]

Within the large program focused on the medicinal chemistry of PBR ligands, compounds **8** were subjected to structural modification in the three of the four postulated pharmacophoric groups (δ1, LA, and FRA) to give compounds **9** in order to evaluate the consistency of the structure–affinity relationship trends among different classes (and subclasses) of PBR ligands (Fig. 2).

In this paper, we describe the synthesis, the preliminary pharmacological evaluation, and the structure–affinity relationship analysis of a short series of PBR ligands **9** based on the tricyclic system pyrrolo[3,4-*b*]quinoline.

2. Results

2.1. Chemistry

The previously described target amide derivatives **8a**–**e** were re-prepared following the reported procedure based on the condensation of pyrrolo[3,4-*b*]quinoline derivative **11b** with glyoxylic acid mono-hydrate and

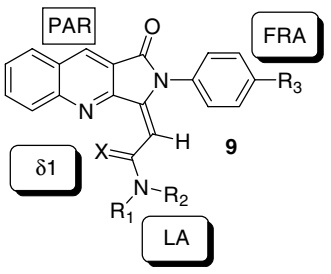


Figure 2. PBR ligands **9**. The pharmacophoric groups subjected to modification are highlighted.

the subsequent transformation of the ylideneacetic acid **12b** via mixed anhydride (Scheme 1).[12]

The newly designed compounds **9a**–**f** were synthesized by means of the same chemistry as shown in Scheme 1. Moreover, tertiary amide **8a** was obtained by N-methylation of the corresponding secondary amide **9b** with methyl iodide in the presence of sodium hydride as the base. This result demonstrates the feasibility of the labeling of **8a** with [^{11}C]CH_3I.

The preparation of compound **9g** lacking the postulated pharmacophoric (δ1) carbonyl group was based on the results of the studies performed in our laboratories on the chemistry of the pyrrolo[3,4-*b*]quinoline system. In fact, the access to target **9g** was envisioned when it was found that the dimethylmethyleneammonium salt[19] obtained from bis(dimethylamino)methane and acetyl chloride performed a double sequential attack (with intermediate elimination) to pyrrolo[3,4-*b*]quinoline derivative **11b** to give compound **13** (Scheme 2). Thus, dimethylamine derivative **13** was quaternized with methyl iodide to obtain quaternary allylammonium cation **14**. This compound was demonstrated to behave as an allylating agent under Tsuji-Trost conditions and reacted with *N*-methyl-*p*-anisidine to give allylamine derivative **9g**, the structure of which was confirmed by crystallography (Fig. 3).

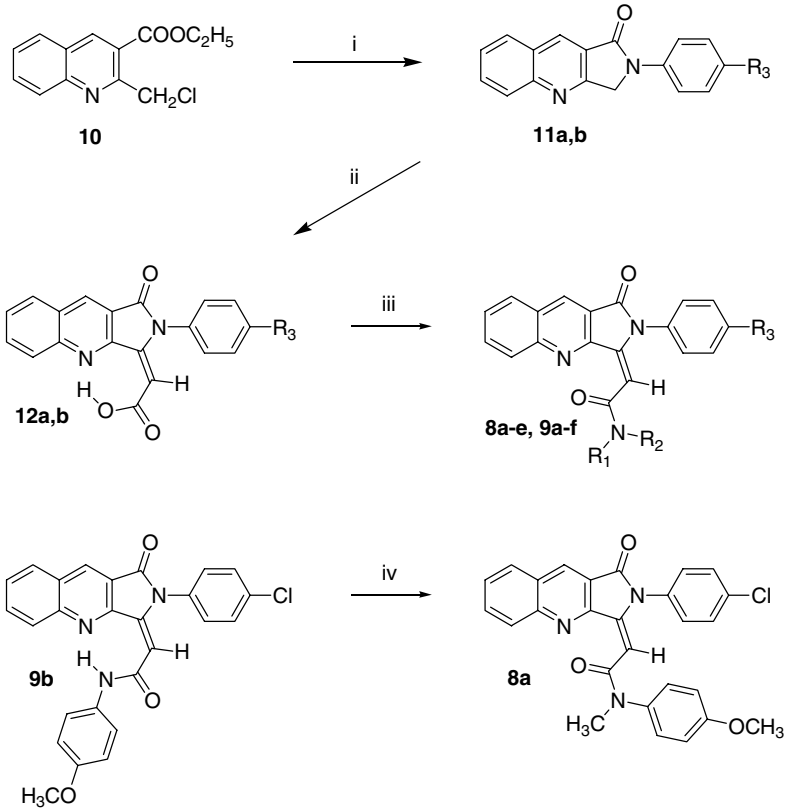
In the setting-up of the palladium-catalyzed allylation reaction, compounds **15** and **16** were isolated from the reaction mixture obtained in the absence of *N*-methyl-*p*-anisidine and with increased amounts (with respect to the catalytic ones) of palladium acetate and triphenylphosphine (Scheme 3).

The structure of intermediate allylpalladium complex **15** was characterized by crystallography, which demonstrated the existence of two conformational polymorphs of this compound (Fig. 4).

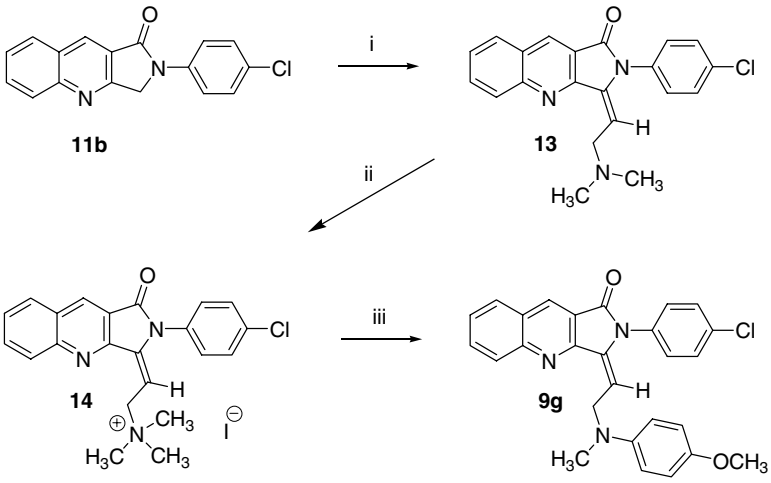
2.2. Binding studies

Compounds **8a**–**e** and **9a**–**g** were tested for their potential activity in inhibiting the specific binding of [^{3}H]**1** to rat cortical membrane in comparison with reference compound **1** and the results of the binding studies are shown in Table 1. Almost all the compounds tested show PBR affinities in the nanomolar range with a modulation suitable for structure affinity relationship analysis. Amides **8a**,**b** show IC_{50} values very similar to those previously reported, while benzyl derivatives **8c**,**d** and dipropylamide **8e** are significantly more potent than formerly reported.[12] We have no specific explanation for this discrepancy, but the final results fit better with the known SAFIR trends.[9,13,17a]

Comparison of the results obtained with compounds **8a** and **9b** confirms the previously observed difference in affinity between secondary and tertiary amides.[13] In fact, secondary amide **9b** shows a significantly lower PBR affinity when compared to its *N*-methylated counterpart **8a**. It



Scheme 1. Reagents: (i) $R_3C_6H_4NH_2$, C_2H_5OH; (ii) $CHOCOOH{\cdot}H_2O$, $(CH_3CO)_2O$, CH_3COOH; (iii) *i*-C_4H_9OCOCl, TEA, R_1NHR_2, CH_2Cl_2; (iv) CH_3I, NaH, DMF.



Scheme 2. Reagents: (i) $(CH_3)_2HCH_2N(CH_3)_2$, CH_3COCl, K_2CO_3, DMF; (ii) CH_3I, $C_2H_5OC_2H_5$, C_2H_5OH; (iii) $CH_3NH–C_6H_4OCH_3$, PPh_3, $Pd(CH_3COO)_2$, DMF.

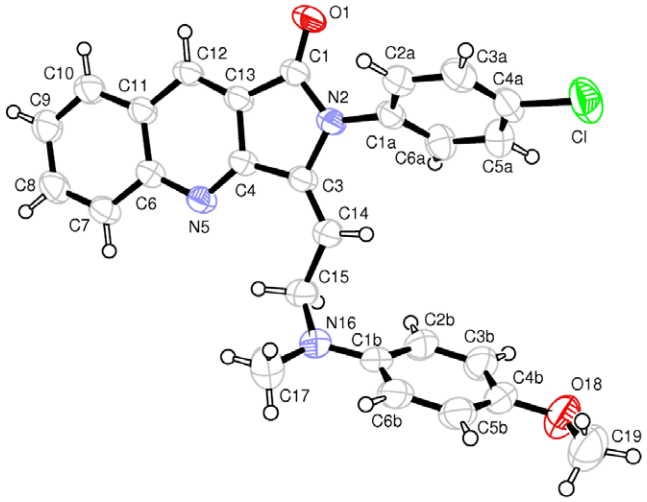


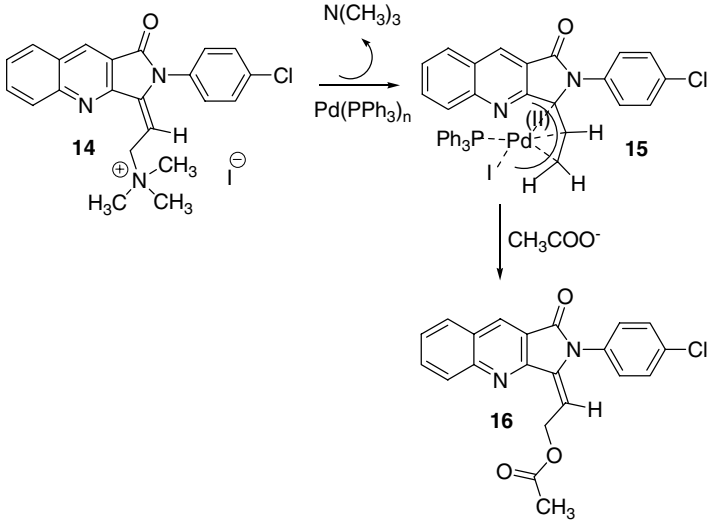
Figure 3. Crystallographic structure of **9g**. Ellipsoids enclose 50% probability.

is noteworthy that secondary amide **9b** shows the same IC_{50} value as its positional isomer **9c**, which is a tertiary amide with a hydrophilic substituent on the amide phenyl group. The comparison of the most potent compounds **8a**,**b** with **9a**,**c** demonstrates the importance of a lipophilic substituent in *para*-position of the amide phenyl.

The replacement of the amide phenyl group of compound **9a** with the benzyl of **8c** appears to be well tolerated by PBR, whereas the same substitution with a propargyl moiety is not accepted equally well (compare **9d** vs **9a**).

The removal of the lipophilic chlorine atom in the pendant phenyl ring of the most active compounds **8a**,**b** leads to a decrease in PBR affinity of about one order of magnitude (compounds **9e**,**f**).

The transformation of the amide carbonyl of **8a** into the methylene group of **9g** produces a dramatic decrease (2600 times) in the receptor affinity. This result can be



Scheme 3. Formation of the intermediate allylpalladium complex **15** and reaction with acetate anion to give **16**.

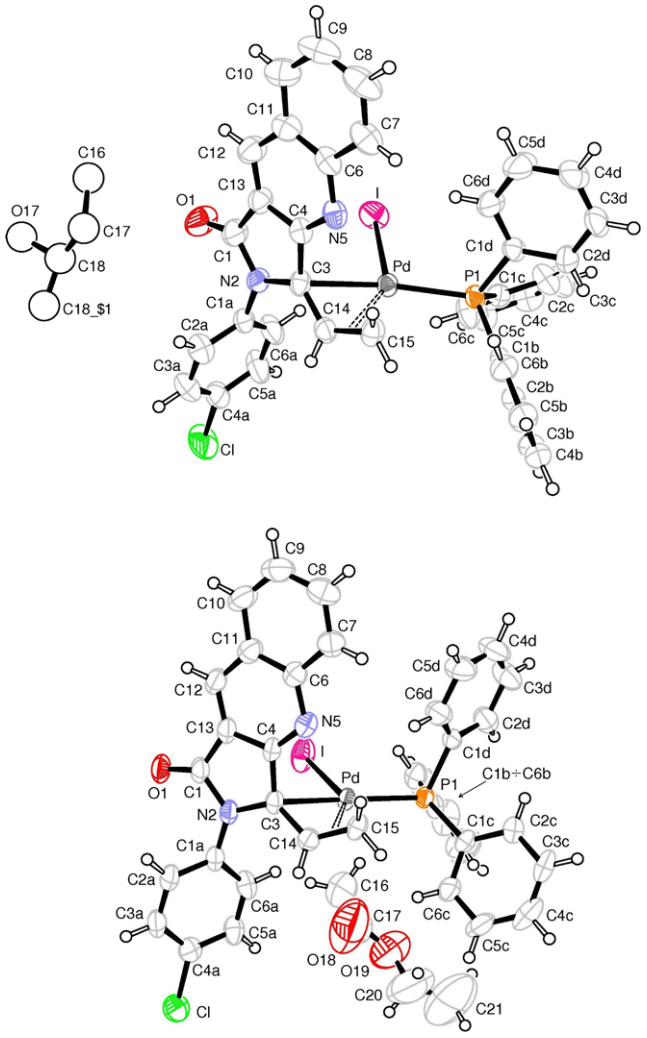


Figure 4. Crystallographic structures of the two polymorphic forms of allylpalladium complex **15** ethyl acetate solvate. Ellipsoids enclose 50% probability. Top: polymorph 1; **15**·0.5 EtOAc; symmetry op. \$1 = 3-*x*,-*y*,-*z*; EtOAc moiety: spheres have arbitrary dimensions and hydrogen atoms are omitted for clarity. Bottom: polymorph 2; **15**·EtOAc.

considered the first direct evidence of the key role played by the amide carbonyl in the interaction of these tricyclic compounds with PBR.

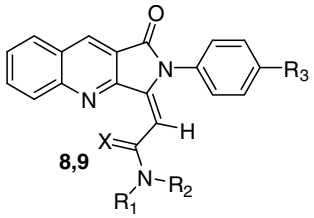
In conclusion, the structure–affinity relationship trends observed in this subclass of tricyclic alpidem-related PBR ligands find correlations in other classes (or subclasses) of PBR ligands (see Refs. 8–10,13,14,17a). This result supports the initial pharmacophoric hypothesis (Fig. 5) and suggests a common mode of interaction at PBR binding site.[20]

3. Experimental

3.1. Chemistry

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Microanalyses were carried out by means of a Perkin-Elmer Series II CHNS/O Analyzer 2400. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F_{254} were used for TLC. ^{1}H NMR spectra were recorded with a Bruker AC 200 spectrometer in the indicated solvents (TMS as internal standard); the values of the chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. Mass spectra were recorded on either a Varian Saturn 3 spectrometer or a ThermoFinnigan LCQ-Deca.

Table 1. PBR binding affinities of compounds **8a**–**e** and **9a**–**g**



Compound	R_1	R_2	R_3	X	IC_{50} (nM) ± SEM[a]
8a	4-$(CH_3O)C_6H_4$	CH_3	Cl	=O	0.90 ± 0.08
8b	4-ClC_6H_4	CH_3	Cl	=O	0.62 ± 0.05
8c	$CH_2C_6H_5$	CH_3	Cl	=O	26 ± 3.1
8d	$CH_2C_6H_5$	C_2H_5	Cl	=O	15 ± 1.3
8e	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	Cl	=O	21 ± 2.1
9a	C_6H_5	CH_3	Cl	=O	44 ± 3.9
9b	4-$(CH_3O)C_6H_4$	H	Cl	=O	76 ± 8.6
9c	4-$(OH)C_6H_4$	CH_3	Cl	=O	74 ± 8.0
9d	$CH_2C{\equiv}CH$	CH_3	Cl	=O	320 ± 29
9e	4-$(CH_3O)C_6H_4$	CH_3	H	=O	12 ± 1.4
9f	4-ClC_6H_4	CH_3	H	=O	10 ± 1.2
9g	4-$(CH_3O)C_6H_4$	CH_3	Cl	$=H_2$	2360 ± 274
1					2.1 ± 0.2

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half the maximum inhibition of [^{3}H]**1** (final concentration 1 nM) specific binding to rat cortical membranes.

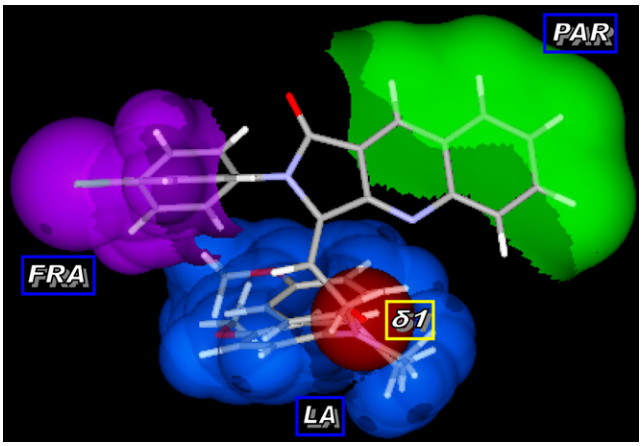


Figure 5. Superposition of **8a** crystallographic structure with a low energy conformer of **9g**. This result suggests that **9g** is able to populate the solid state conformation of **8a**, which is a very rigid and highly potent PBR ligand. Thus, the difference in PBR affinity between **8a** and **9g** is related to the key role played by the amide carbonyl in the interaction with PBR.

3.2. General procedure for the synthesis of compounds 11a,b

A mixture of **10** (2.2 g, 8.8 mmol) in 15 mL of ethanol with aniline (or 4-chloroaniline in the case of **11b**) (27 mmol) was refluxed for 52 h. The reaction mixture was cooled to room temperature, and the precipitate was collected by filtration, washed with ethanol and petroleum ether, and dried under reduced pressure to obtain **11a**,**b**.

3.3. 2,3-Dihydro-2-phenyl-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (11a)

The title compound was obtained as white crystals (yield 72%, mp 240–241 °C). ^{1}H NMR ($CDCl_3$): 5.02 (s, 2H), 7.21 (t, J = 7.3, 1H), 7.41–7.49 (m, 2H), 7.62 (t, J = 7.0, 1H), 7.80–7.92 (m, 3H), 8.00 (d, J = 8.1, 1H), 8.16 (d, J = 8.5, 1H), 8.67 (s, 1H). MS(ESI): m/z 261 ($M+H^+$).

3.4. 2-(4-Chlorophenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]-quinolin-1-one (11b)

The title compound was obtained as white crystals (yield 85%, mp 268–270 °C, lit.[12] 268–270 °C). ^{1}H NMR ($CDCl_3$): 5.03 (s, 2H), 7.41–7.46 (m, 2H), 7.66 (t, J = 7.3, 1H), 7.83–7.92 (m, 3H), 8.04 (d, J = 8.3, 1H), 8.19 (d, J = 8.5, 1H), 8.71 (s, 1H).

3.5. General procedure for the synthesis of compounds 12a,b

A mixture of the appropriate pyrroloquinoline derivative **11a**,**b** (1.0 mmol) in glacial acetic acid (5 mL) and acetic anhydride (5 mL) with glyoxylic acid monohydrate (0.30 g, 3.26 mmol) was heated at 110 °C for 5 h. Then 0.3 g of glyoxylic acid monohydrate was added, and the mixture was stirred at 110 °C for one additional hour. Afterwards, the cooled reaction mixture was poured into ice-water and allowed to stand at room temperature overnight. The precipitate was extracted with chloroform and the organic layer was extracted with diluted NaOH. The organic layer was discarded, the aqueous phase was acidified (pH 4–5) with 3 N HCl and extracted with chloroform. The combined extracts were dried over sodium sulfate and concentrated under reduced pressure to give the corresponding acids **12a**,**b**.

3.6. (*E*)-(1,2-Dihydro-1-oxo-2-phenyl-3*H*-pyrrolo[3,4- *b*]-quinolin-3-ylidene)acetic acid (12a)

The title compound was obtained as a light-brown solid (yield 50%, mp 298–300 °C). ^{1}H NMR ($CDCl_3$): 5.89 (s, 1H), 7.33–7.63 (m, 5H), 7.84 (t, J = 7.3, 1H), 8.04 (m, 1H), 8.17 (d, J = 7.9, 1H), 8.30 (d, J = 8.4, 1H), 8.95 (s, 1H), 16.22 (br s, 1H). MS(ESI): m/z 317 ($M+H^+$).

3.7. (*E*)-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]acetic acid (12b)

The title compound was obtained as an off-white solid (yield 55%, mp 271–272 °C, lit.[12] 272–273 °C). ^{1}H NMR ($CDCl_3$): 5.89 (s, 1H), 7.27–7.35 (m, 2H), 7.54–7.61 (m, 2H), 7.86 (t, J = 8.0, 1H), 8.03–8.11 (m, 1H), 8.19 (d, J = 8.4, 1H), 8.32 (d, J = 8.5, 1H), 8.96 (s, 1H), 16.13 (s, 1H).

3.8. General procedure for the synthesis of compounds 9a–f

A mixture of the appropriate carboxylic acid (**12a**,**b**, 0.40 mmol) in dichloromethane (10 mL) and TEA (0.19 mL, 1.4 mmol) was cooled at −10 °C, and isobutyl chloroformate (0.057 mL, 0.44 mmol) was added with stirring under argon for 30 min. Subsequently, the suitable amine (0.44 mmol) was added to the solution and the reaction mixture was allowed to reach room temperature and stirred for 1 h (for compound **9d** the reaction time was 17 h). The reaction mixture was then diluted with dichloromethane, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Crude amides **9a**–**f** were purified by flash-chromatography with the appropriate eluent.

3.9. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]-*N*- methyl-*N*-phenylacetamide (9a)

The title compound was purified by flash-chromatography with dichloromethane/ethyl acetate (95:5) as the eluent to give **9a** as a white crystalline solid (yield 64%, mp 138–140 °C). ^{1}H NMR ($CDCl_3$): 3.59 (s, 3H), 5.58 (s, 1H), 6.98 (m, 2H), 7.10–7.17 (m, 5H), 7.42 (m, 2H), 7.68 (t, J = 7.6, 1H), 7.87–8.03 (m, 2H), 8.33 (d, J = 8.4, 1H), 8.62 (s, 1H). MS(ESI): m/z 440 ($M+H^+$). Anal. Calcd for ($C_{26}H_{18}ClN_3O_2 \cdot 0.25\ CH_3COOC_2H_5$): C, 70.20; H, 4.36; N, 9.10. Found: C, 70.45; H, 4.32; N, 8.93.

3.10. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]-*N*-(4-methoxyphenyl)-acetamide (9b)

The title compound was purified by flash-chromatography with dichloromethane/ethyl acetate (9:1) as the eluent to give **9b** as a yellow solid (yield 90%). An analytical sample recrystallized from *n*-hexane–ethyl acetate melted at 272–274 °C. ^{1}H NMR ($CDCl_3$): 3.84 (s, 3H), 5.88 (s, 1H), 6.99 (m, 2H), 7.32 (m, 2H), 7.56 (m, 2H), 7.73–7.84 (m, 3H), 8.02 (t, J = 7.7, 1H), 8.15 (d, J = 8.0, 1H), 8.31 (d, J = 8.3, 1H), 8.90 (s, 1H), 13.45 (s, 1H). MS(ESI, negative ions): m/z 454 ($M-H^+$). Anal. Calcd for ($C_{26}H_{18}ClN_3O_3 \cdot 0.25\ CH_3COOC_2H_5$): C, 67.85; H, 4.22; N, 8.79. Found: C, 68.19; H, 3.90; N, 8.78.

3.11. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]-*N*-(4-hydroxyphenyl)-*N*-methylacetamide (9c)

The title compound was purified by flash-chromatography with chloroform/ethyl acetate (6:4) as the eluent to give **9c** as a white solid (yield 60%). An analytical sample recrystallized from dichloromethane melted at 205–207 °C. The ^{1}H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^{1}H NMR ($CDCl_3$): 3.36 (s), 3.48

(s), 5.51 (s), 5.88 (s), 6.56 (m), 6.81–6.94 (m), 7.32–7.41 (m), 7.52 (m), 7.65 (t, $J = 7.4$), 7.78–7.90 (m), 7.98 (d, $J = 8.1$), 8.15 (d, $J = 8.1$), 8.27 (d, $J = 8.4$), 8.60 (s), 8.71 (s). MS(ESI, negative ions): *m/z* 454 ($M-H^+$). Anal. Calcd for ($C_{26}H_{18}ClN_3O_3$): C, 68.50; H, 3.98; N, 9.22. Found: C, 68.33; H, 3.85; N, 9.10.

3.12. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene)-*N*-methyl-*N*-(prop-2-ynyl)acetamide (9d)

Crude amide **9d** was purified by flash-chromatography with chloroform/ethyl acetate (6:4) as the eluent to obtain a colorless glassy solid (yield 54%). The ^1H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^1H NMR ($CDCl_3$): 2.12 (t, $J = 2.3$), 2.32 (t, $J = 2.5$), 3.11 (s), 3.27 (s), 4.19 (d, $J = 2.4$), 4.53 (d, $J = 2.4$), 5.71 (s), 5.73 (s), 7.36–7.66 (m), 7.82 (m), 7.98 (d, $J = 8.1$), 8.10 (d, $J = 8.5$), 8.21 (d, $J = 8.6$), 8.66 (s). MS(ESI): *m/z* 402 ($M+H^+$). Anal. Calcd for ($C_{23}H_{16}ClN_3O_2 \cdot 0.2\ CHCl_3$): C, 65.45; H, 3.84; N, 9.87. Found: C, 65.28; H, 3.91; N, 9.87.

3.13. (*E*)-2-(1,2-Dihydro-1-oxo-2-phenyl-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene)-*N*-(4-methoxyphenyl)-*N*-methylacetamide (9e)

Crude amide **9e** was purified by flash-chromatography with dichloromethane/ethyl acetate (9:1) as the eluent to obtain a pale-yellow solid (yield 63%). An analytical sample recrystallized from petroleum ether–ethyl acetate melted at 193–195 °C. The ^1H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^1H NMR ($CDCl_3$): 3.41 (s), 3.54 (s), 3.71 (s), 3.87 (s), 5.60 (s), 5.92 (s), 6.66 (m), 6.98–7.09 (m), 7.39–7.71 (m), 7.90 (t, $J = 7.9$), 8.01 (d, $J = 7.8$), 8.25 (d, $J = 8.4$), 8.32 (d, $J = 8.4$), 8.63 (s), 8.75 (s). MS(ESI): *m/z* 436 ($M+H^+$). Anal. Calcd for ($C_{27}H_{21}N_3O_3$): C, 74.47; H, 4.86; N, 9.65. Found: C, 74.42; H, 4.55; N, 9.52.

3.14. (*E*)-*N*-(4-Chlorophenyl)-2-(1,2-dihydro-1-oxo-2-phenyl-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene)-*N*-methylacetamide (9f)

Crude amide **9f** was purified by flash-chromatography with dichloromethane/ethyl acetate (9:1) as the eluent to obtain a colorless crystalline solid (yield 50%). An analytical sample recrystallized from petroleum ether–ethyl acetate melted at 191–193 °C. The ^1H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^1H NMR ($CDCl_3$): 3.41 (s), 3.56 (s), 5.58 (s), 5.88 (s), 7.00–7.12 (m), 7.33–7.70 (m), 7.89 (t, $J = 7.5$), 8.00 (d, $J = 8.0$), 8.15 (d, $J = 7.9$), 8.28 (d, $J = 8.3$), 8.62 (s), 8.72 (s). MS(ESI): *m/z* 440 ($M+H^+$). Anal. Calcd for ($C_{26}H_{18}ClN_3O_2$): C, 70.99; H, 4.12; N, 9.55. Found: C, 71.05; H, 3.92; N, 9.50.

3.15. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]-*N*-(4-methoxyphenyl)-*N*-methylacetamide (8a)

To a solution of **9b** (0.030 g, 0.066 mmol) in 3 mL of dry DMF and methyl iodide (0.50 mL, 8.0 mmol) cooled at 0–5 °C was added NaH (1.6 mg, 0.067 mmol). The reaction mixture was stirred at room temperature for 30 min and then poured into ice-water. The precipitate was extracted with dichloromethane and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash-chromatography with dichloromethane/ethyl acetate (95:5) as the eluent gave **8a** as a pale yellow oil which crystallized on standing (0.022 g, yield 71%). Recrystallization from diethyl ether gave an analytical sample of **8a** mp at 158–160 °C (lit.[12] 159–160 °C). The ^1H NMR spectrum of this amide shows the presence of two different rotamers in equilibrium. For the sake of simplification the integral intensities have not been given. ^1H NMR ($CDCl_3$): 3.41 (s), 3.55 (s), 3.72 (s), 3.88 (s), 5.60 (s), 5.92 (s), 6.64–6.69 (m), 6.99–7.04 (m), 7.39–7.47 (m), 7.55–7.72 (m), 7.88–8.04 (m), 8.22–8.35 (m), 8.64 (s), 8.74 (s).

3.16. (*E*)-2-(4-Chlorophenyl)-2,3-dihydro-3-[2-(dimethylamino)ethylidene]-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (13)

To a solution of bis(dimethylamino)methane (0.51 mL, 3.7 mmol) in 5 mL of dry DMF cooled at −5 °C acetyl chloride (0.46 mL, 6.5 mmol) was slowly added and the resulting mixture was stirred at room temperature under argon for 10 min. The reaction mixture was added with potassium carbonate (1.2 g, 8.8 mmol) and of pyrroloquinoline derivative **11a** (0.50 g, 1.7 mmol), and the resulting mixture was heated at 60 °C for 2.5 h and poured into ice-water. The precipitate was extracted with dichloromethane and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash-chromatography with ethyl acetate/triethylamine (8:2) as eluent gave **13** as a pale yellow oil (0.51 g, yield 82%). ^1H NMR ($CDCl_3$): 2.36 (s, 6 H), 4.14 (d, $J = 7.0$, 2H), 5.57 (t, $J = 7.4$, 1H), 7.33 (m, 2H), 7.51 (m, 2H), 7.64 (t, $J = 7.3$, 1H), 7.85 (m, 1H), 8.01 (d, $J = 9.0$, 1H), 8.25 (d, $J = 8.5$, 1H), 8.71 (s, 1H).

3.17. (*E*)-2-(4-Chlorophenyl)-2,3-dihydro-3-[2-(trimethylammonium)ethylidene]-1*H*-pyrrolo[3,4-*b*]quinolin-1-one iodide (14)

To a solution of **13** (0.18 g, 0.49 mmol) in 5 mL of absolute ethanol and 5 mL of dry diethyl ether was added methyl iodide (0.034 mL, 0.55 mmol) and the resulting mixture was stirred at room temperature for 48 h. The precipitate was collected by filtration, washed with diethyl ether, and dried under reduced pressure to give **14** as a white solid (0.175 g, yield 70%, mp 219–222 °C). ^1H NMR (DMSO-d_6): 3.14 (s, 9H), 5.08 (d, $J = 8.7$, 2H), 5.36 (t, $J = 8.7$, 1H), 7.63 (m, 4H), 7.80 (t, $J = 7.5$, 1H), 8.01 (t, $J = 7.4$, 1H), 8.29 (d, $J = 8.1$, 1H), 8.43 (d, $J = 8.4$, 1H), 9.06 (s, 1H). MS(ESI): *m/z* 378 (M^+).

3.18. (*E*)-2-(4-Chlorophenyl)-2,3-dihydro-3-[2-[*N*-(4-methoxyphenyl)methylamino]ethylidene]-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (9g)

A mixture of **14** (0.090 g, 0.18 mmol) in 9 mL of dry DMF with triphenylphosphine (0.023 g, 0.088 mmol) was stirred at room temperature under a stream of argon for 10 min, and then treated with palladium acetate (0.010 g, 0.044 mmol) and *N*-methyl-*p*-anisidine (0.024 g, 0.175 mmol). The reaction mixture was stirred at room temperature for 20 h, poured into ice-water, and extracted with diethyl ether. The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash-chromatography with dichloromethane/ethyl acetate (95:5) as the eluent gave pure **9g** as a pale yellow oil which crystallized on standing. Recrystallization from diethyl ether–dichloromethane gave yellow prisms suitable for crystallographic studies (0.060 g, yield 73%, mp 160–163 °C). ^{1}H NMR ($CDCl_3$): 2.93 (s, 3 H), 3.72 (s, 3H), 5.10 (d, J = 6.9, 2H), 5.54 (t, J = 6.9, 1H), 6.81 (m, 4H), 7.27 (m, 2H), 7.46 (m, 2H), 7.67 (t, J = 7.5, 1H), 7.89 (t, J = 7.7, 1H), 8.04 (d, J = 8.0, 1H), 8.28 (d, J = 8.5, 1H), 8.74 (s, 1H). MS(E-SI): m/z 456 ($M+H^+$). Anal. Calcd for ($C_{27}H_{22}ClN_3O_2$): C, 71.13; H, 4.86; N, 9.22. Found: C, 70.89; H, 4.78; N, 9.37.

3.19. Palladium complex (15)

To a solution of **14** (0.075 g, 0.15 mmol) in 8 mL of anhydrous DMF with triphenylphosphine (0.040 g, 0.15 mmol) was added palladium acetate (0.017 g, 0.075 mmol) and the resulting mixture was stirred at room temperature for 20 h. The reaction mixture was then poured into ice-water and extracted with dichloromethane; the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue obtained was purified by flash-chromatography with dichloromethane/ethyl acetate (95:5) as eluent to give 0.020 g of **15** as a yellow solid which crystallized from diethyl ether–dichloromethane–ethyl acetate in two different polymorphic forms ethyl acetate solvate. ^{1}H NMR ($CDCl_3$): 3.41 (d, J = 8.5, 1H), 5.02 (d, J = 12.8, 1H), 5.39 (dd, J = 8.9, 12.6, 1H), 7.26–7.69 (m, 18H), 7.81–7.90 (m, 3H), 8.01–8.13 (m, 2H), 8.80 (s, 1H).

3.20. (*E*)-2-[2-(4-Chlorophenyl)-1,2-dihydro-1-oxo-3*H*-pyrrolo[3,4-*b*]quinolin-3-ylidene]ethyl acetate (16)

Compound **16** was obtained as a white solid (0.010 g, mp 171–173 °C) from the purification of the reaction mixture of **15** by flash-chromatography (more polar fractions). ^{1}H NMR ($CDCl_3$): 2.07 (s, 3H), 5.50 (t, J = 6.9, 1H), 5.80 (d, J = 6.9, 2H), 7.34 (m, 2H), 7.49–7.68 (m, 3H), 7.85 (m, 1H), 8.00 (d, J = 8.3, 1H), 8.25 (d, J = 8.5, 1H), 8.68 (s, 1H). MS(ESI): m/z 401 ($M+Na^+$).

3.21. X-ray crystallography

Single crystals of **9g** and the two polymorphic forms ethyl acetate solvate of **15** were submitted to X-ray data collection on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71069 Å). The $\omega/2\Theta$ scan technique was used. The structures were solved by direct methods and the refinements were carried out by full-matrix anisotropic least-squares of F^2 against all reflections. The hydrogen atoms were located on Fourier difference maps or placed in calculated positions and refined as riding atoms on their heavy atoms with isotropic temperature factor. Atomic scattering factors including f' and f'' were taken from Ref. 21. Structure solution was carried out by SHELXS-97.[22] Structure refinement and molecular graphics were performed by SHELX-97[21] and the WinGX package,[23] respectively.

CCDC 646033 (**9g**), 644752 (**15**-Polymorph 1), and 644753 (**15**-Polymorph 2) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

3.22. In vitro binding assays

Male Sprague–Dawley CD rats (Charles River Italia, Calco, CO, Italy) with body masses of 200–250 g were used. Rats were acclimatized to the new housing conditions for at least 1 week. They were housed six per cage under an artificial 12-h-light, 12-h-dark cycle at a constant temperature of 22 ± 2 °C and a relative humidity of 65%. They had free access to water and standard laboratory food at all times. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Rats were sacrificed by decapitation and their brains were rapidly dissected into the various areas which were stored at −80 °C until the day of the assay. The binding assays were performed as described in the lit.[8] The cerebral cortex was subsequently thawed and then homogenized in 50 volumes of ice-cold Dulbecco's phosphate-buffered saline (PBS), pH 7.4, at 4 °C with a Polytron PT 10 disrupter (setting 5 for 20 s). The homogenate was centrifuged at 40,000g and 4 °C for 30 min and the resulting pellet was resuspended in the same volume of fresh buffer and recentrifuged. The new pellet was resuspended in 10 volumes of the incubation buffer (PBS) and used for the binding assay.

[^{3}H]PK11195 binding was measured in a final volume of 500 μL, consisting of 50 μL of membrane suspension (0.15–0.20 mg of protein), 50 μL of [^{3}H]PK11195 (sa 85.5 Ci/mmol, New England Nuclear; final assay concentration of 1 nM), 5 μL of drug solution or solvent, 395 μL of PBS. The binding reaction was performed at 25 °C for 90 min and began with the addition of membranes. The incubation was terminated by rapid filtration through glass-fiber filters (Whatman GF/B) which had been presoaked with 0.3% polyethyleneimine and placed in a cell harvester filtration manifold (Brandel).

The filters were washed five times with 4 mL of ice-cold PBS, after which filter-bound radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 10 μM of unlabeled PK11195 (Sigma). Specific binding was determined by subtracting the nonspecific from the total binding and was about 80% of the total binding. The concentration of the test compounds that inhibited [^{3}H]ligand binding by 50% (IC_{50}) was determined by means of Jandel Sigmaplot[24] program with 6–10 concentrations of the displacers, each performed in triplicate.

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