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Synthesis and biological evaluation of pyridazino[4,3-b]indoles and indeno[1,2-c]pyridazines as new ligands of central and peripheral benzodiazepine receptors

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Abstract

A large number of pyridazino[4,3-b]indoles and indeno[1,2-c]pyridazines were synthesised and tested to evaluate their binding affinities at both central (CBR) and peripheral (PBR) benzodiazepine receptors. Relatively good PBR binding affinities were found for ligands belonging to the 3-arylmethyloxy-pyridazinoindole series, whereas only 2-aryl-indenopyridazines **7a**, **8a** and **10a** display a weak binding affinity for CBR. To find out the main structural determinants affecting PBR affinity, a molecular modelling study based on the comparative analysis of the three-dimensional properties of four properly selected derivatives **24a**, **3b**, **18a** and **10d**, with those of highly active and selective PBR ligands, taken as reference, was performed.

Keywords: Pyridazino[4,3-b]indoles; Indeno[1,2-c]pyridazines; Peripheral benzodiazepine receptor; Binding assay; Structure–activity relationships

1. Introduction

Benzodiazepines bind two main classes of receptors, namely the central (CBR) and the peripheral (PBR) benzodiazepine receptor. CBR is present exclusively in the central nervous system where benzodiazepines produce their pharmacological effects by modulating the action of GABA at $GABA_A$-receptor/Cl^- ionophore supramolecular complex [1,2], a pentameric protein, formed by different combinations of distinct subunits called α, β, δ, γ and ρ [3–5].

CBR ligands bind at allosteric modulatory sites (ω sites) enhancing (agonists) or reducing (inverse agonists) the GABA-induced Cl^- ion flux. The antagonists, exhibit, per se, no relevant biological effects but antagonise the action of agonists and inverse agonists [6].

Benzodiazepines, β-carbolines, imidazopyridines, pyrazoloquinolines, and imidazoquinoxalines represent the major families of compounds with high affinity for CBR. In addition to their anxiolitic properties, most of the drugs in clinical use, such as diazepam, possess anticonvulsant, sedative, and muscle relaxant effects [2].

In the central nervous system (CNS), diazepam also binds to PBR [7]. This receptorial protein is distinct from the CBR protein even if both modulate the activity of γ-aminobutyric acid in the brain. PBR, initially discovered in a wide range of peripheral tissues (kidney, liver and lung), has been found to occur in particularly high density in steroid-producing tissues, such as adrenal, testis, placenta and brain tissues and it is localised mainly in the mitochondria [8–10].

PBRs are involved in several functions such as cell proliferation, immune response modulation, regulation of mitochondrial oxidative phosphorylation and regulation of steroidogenesis [11–14].

Many compounds bind to PBR with varying affinities. Among them, isoquinoline carboxamides (PK11195), benzodiazepines (Ro 5-4864), imidazopyr-

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idines (*alpidem* and *zolpidem*), 3-indole-acetamides (FGIN-1-27), and pyrrolobenzoxazepines (NF 182) display outstanding binding affinities. From a medicinal chemistry point of view, the synthesis of new, PBR ligands should prove useful to determine some key structural features for high affinity and selectivity and to better characterise the pharmacological and therapeutic roles of PBR.

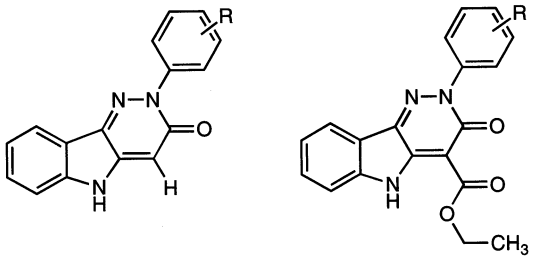
In the search for novel anxiolitic agents devoid of the undesirable side effects of classical benzodiazepines, we extensively explored a new class of CBR ligands, namely the 2-aryl-2,5-dihydropyridazino[4,3-b]indol-3(3H)ones (**I**, Plate 1), which showed a wide range of pharmacological activity spanning from agonist to inverse agonist [15–17].

According to the pharmacophore proposed by Cook [18], these compounds can efficiently interact with the lipophilic regions L1 and L2 as well as with HB1 and HB2 hydrogen-bond donors sites and finally with the A2 hydrogen-bond acceptor site (Fig. 1).

During our investigation of new classes of CBR ligands, we observed that, contrary to many derivatives of **I**, the esters **II** [15] (Plate 1), intermediates for the synthesis of **I**, showed no [^{3}H]flunitrazepam displacement from its CBR binding site up to a 20 μM concentration.

We hypothesised that the steric hindrance of the ethoxycarbonyl group at the S1 region of the CBR (Fig. 1), might probably be responsible for the lack of affinity of **II** and that, on the basis of the actual knowledge on the structural requirements for affinity at PBR, this moiety should not instead be detrimental for binding to this receptor. The esters **II**, in fact, may possess the structural features required for the interaction with PBR, that are three lipophilic regions (L1, L3 and L4), and a hydrogen-bond donor group (HB2), generally a carbonyl oxygen, as reported in several 3D QSAR and pharmacophore models [19,24,25] (Fig. 2).

In order to validate our hypothesis, we tested the ability of esters **3a** and **b** (Table 2) to displace the high affinity radioligand [^{3}H]PK11195 from its PBR binding site in rat cortex homogenate. The experimental binding data showed that **3b** bound with a relatively good affinity and a high selectivity to PBR ($IC_{50} = 610$ nM),



I **II**

Plate 1.

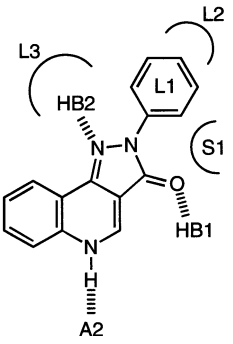


Fig. 1. Proposed pharmacophore model for CBR [18]. The main binding sites for pyrazoloquinoline CGS-8216 are indicated as HB1 and HB2 (HB donor sites), A2 (HB acceptor site), L1 and L2 (hydrophobic sites). L3 is another lipophilic region reached by the 5-phenyl ring of classical benzodiazepines. S1 represents a sterically inaccessible region. Binding to HB2 and A2 is not necessary for inverse agonist activity.

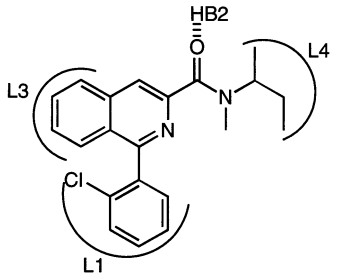
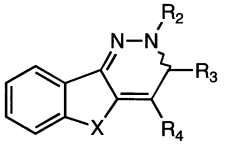


Fig. 2. Schematic representation of PBR pharmacophore model as proposed by different groups [19,24,25]. The molecular structure of PK1195 is sketched to help interpretation.

but, unexpectedly, no significant binding was found for **3a**.

In order to explain this difference and to gain further information on the molecular determinants for both high affinity and selectivity at PBR, within our classes of compounds, we synthesised a new series of pyridazino[4,3-b]indoles and indeno[1,2-c]pyridazines having the general formula **III** (Plate 2) and evaluated their binding affinities at both CBR and PBR.

The structural modifications were mainly made on the 2, 3, 4 and 5 positions, bearing in mind that two aromatic rings and an electron rich group are the most likely ligand anchoring points in the interaction model with PBR.



III

Plate 2.

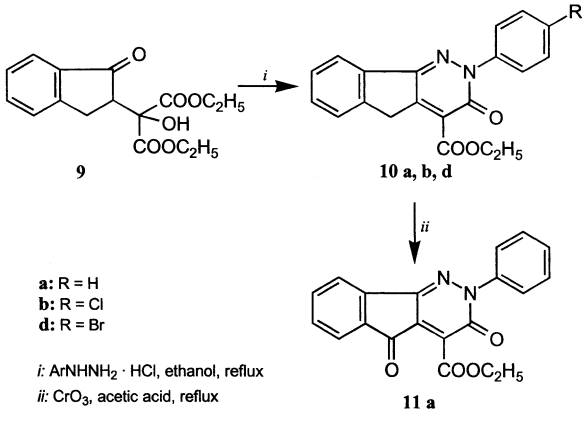
2. Chemistry

2-Aryl-2,5-dihydropyridazino[4,3-b]indol-3(3H)ones (**1**–**4**) were prepared according to methods previously reported by us [15,16]. Carboxamide derivatives **5b** and **6b** were synthesised by standard procedures starting from the corresponding parent carboxylic acid [15] as described in Section 5. Compound **8a** was obtained by oxidation of the indenopyridazine **7a** described in ref. [17]. Scheme 1 outlines the synthetic route to 2-aryl-indeno[1,2-c]pyridazine-4-carboxylic acid ethyl esters **10a**, **b**, **d** and **11a**.

In short, compounds **10a**, **b**, and **d** were synthesised refluxing the 2-hydroxy-2-(1-oxo-indan-2-yl)malonic acid diethyl ester (**9**), prepared according to the method reported in ref. [20], with the appropriate phenyl hydrazine hydrochloride in ethanol. The oxidation of **10a** by means of chromic anhydride in refluxing acetic acid gave the corresponding 3,5-dioxo derivative **11a**.

The new 3-aryl-pyridazino[4,3-b]indole-4-carboxylic acid ethyl esters **15a**, **e** and **18a** were synthesised as indicated in Scheme 2. In brief, 3-oxo-2-(3-oxo-1,3-dihydro-indol-2-ylidene)-3-aroyl-propionic acid ethyl esters **14a**, **e** were prepared by refluxing isatin **12** with phosphorus pentachloride in anhydrous benzene to give 2-chloro-3*H*-indol-3-one (**13**), which was then condensed with appropriate ethylaroylacetate sodium salt according to the method previously described by us [15,16]. The cyclization with hydrazine hydrate in anhydrous ethanol easily furnished the pyridazine derivatives **15a**, **e**. Treatment of **15a** with sodium hydride and ethyl iodide gave the N-5 ethyl derivative **18a**. Carboxamide **17e** was prepared starting from the carboxylic acid **16e** by standard methods as described in Section 5.

3-Arylmethyloxy derivatives **22a**, **b**, **f**, **23a**, **b**, **24a**, **b**, **f**, **25a** and **26a** were prepared starting from 3-hydroxy-*5H*-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**21**) [21], as outlined in Scheme 3. In short, treatment of the appropriate benzyl or halophenylmethylhalide with an equimolar mixture of **21** and sodium hydride in dry dimethylformamide (DMF) gave the 3-arylmethyloxy derivative **22a**, **b**, **f**, and **23a**, **b**, which were further ethylated at N-5 by means of sodium hydride and iodoethane to give **24a**, **b**, **f**. Finally, the diethylamide **26a** was prepared from the acid **25a** by standard methods. Physical and spectroscopic data of the newly synthesised compounds are reported in Table 1.

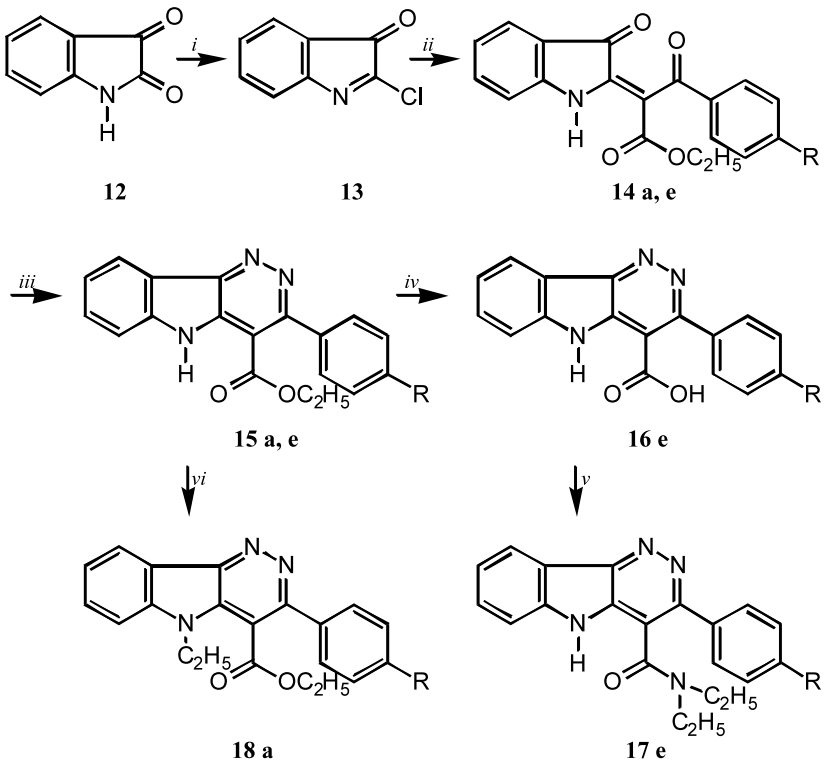


Scheme 1.

3. Binding and SAR studies

All the compounds were evaluated for their in vitro binding affinity at the CBR and PBR by means of a binding assay using [^{3}H]Flunitrazepam and [^{3}H]PK11195, respectively, as radioligands and cerebral cortices from male Sprague–Dawley rats as receptor source [22,23]. The binding data are expressed as IC_{50} or as percentage of inhibition of specific radioligand binding at 1 μM compound concentration (reported in square brackets in the tables). The measured binding affinities for CBR and PBR are reported in Tables 2–4 for condensed 2-arylpyridazine, condensed 3-arylpyridazine and 3-arylmethyloxy pyridazinoindole derivatives, respectively.

2-Aryl-pyridazino[4,3-b]indoles **1a**, **b**–**2a**, **c** and 2-aryl-indeno[1,2-c]pyridazines **7a** and **8a**, both devoid of carbonyl group at C-4, display an appreciable affinity for CBR. A lower CBR potency was found for indenopyridazines, as already observed by us [17], and no significant affinity for PBR was detected. On the contrary, ligands **3a**, **b**, **4a**, **10a**, **b**, **d**, **11a**, and **5b**, **6b**, containing an ethoxycarbonyl or a carboxamido group, respectively, bind sometimes weakly, but selectively at PBR. The highest potency was measured for **3b** (IC_{50} = 610 nM). These results confirm that in the series of condensed pyridazine derivatives bearing an aryl substituent in position 2, a bulky substituent at C-4 is not only well-tolerated but also necessary for binding to PBR. In addition to that, taking into account the relatively higher affinity of halogenated compounds **3b** and **10d** (IC_{50} = 610 and 730 nM, respectively), it seems reasonable to hypothesise that the halogen substituent at the N-2 phenyl ring could interact with the L1 lipophilic region, determining an appreciable improvement of the PBR affinity. The *p*-chloroderivative **10b** is the only exception to this trend. The replacement of the ethoxycarbonyl group of **3b** with carboxamide functions, even similar to those present in PK11195, results in a marked loss of PBR affinity in **5b** and **6b** (7 and 16%, respectively at 1 μM). These data suggest that our ligands may bind to a different less lipophilic, or less accessible, receptor region. Like the 2-aryl-pyridazino[4,3-b]indoles and 2-aryl-indeno[1,2-c]pyridazines, 3-aryl analogues **15a**, **e**, **17e** and **19**, although showing selective binding at PBR, do not possess a good PBR



i: PCl_5, anh. benzene, reflux; *ii:* R-C_6H_4-$COCH^-COOC_2H_5Na^+$, anh. dioxane, r.t.;
iii: $NH_2NH_2 \cdot H_2O$, ethanol, r.t.; *iv:* 1) NaOH, ethanol, reflux - 2) HCl, r.t.;
v: 1) $SOCl_2$, cat. DMF, reflux - 2) $(C_2H_5)_2NH$, anh. THF; *vi:* NaH, DMF, C_2H_5I, r.t.

Scheme 2.

affinity. A modest, but significant increase of the affinity of **15a** (31% [3H] PK11195 displacement at 1 μM), is observed by ethylation of its N-5 leading to **18a** (IC_{50} = 658 nM). An analogous substantial enhancement of the PBR affinity resulted from the N-5 alkylation of the 3-aryloxy derivatives **22a** and **22f** leading to **24a** and **24f**, respectively. The former is endowed with the highest binding potency observed for all the tested compounds (IC_{50} = 240 nM). This result might indicate that the N-5 alkyl group probably reaches a lipophilic pocket close to the receptor region accommodating the C-4 carbonyl group. The replacement of the ethoxycarbonyl group with a carboxamide function in the case of **24a**, as well as of 2-aryl-pyridazinoindole analogue **3b**, yields a marked drop of PBR affinity.

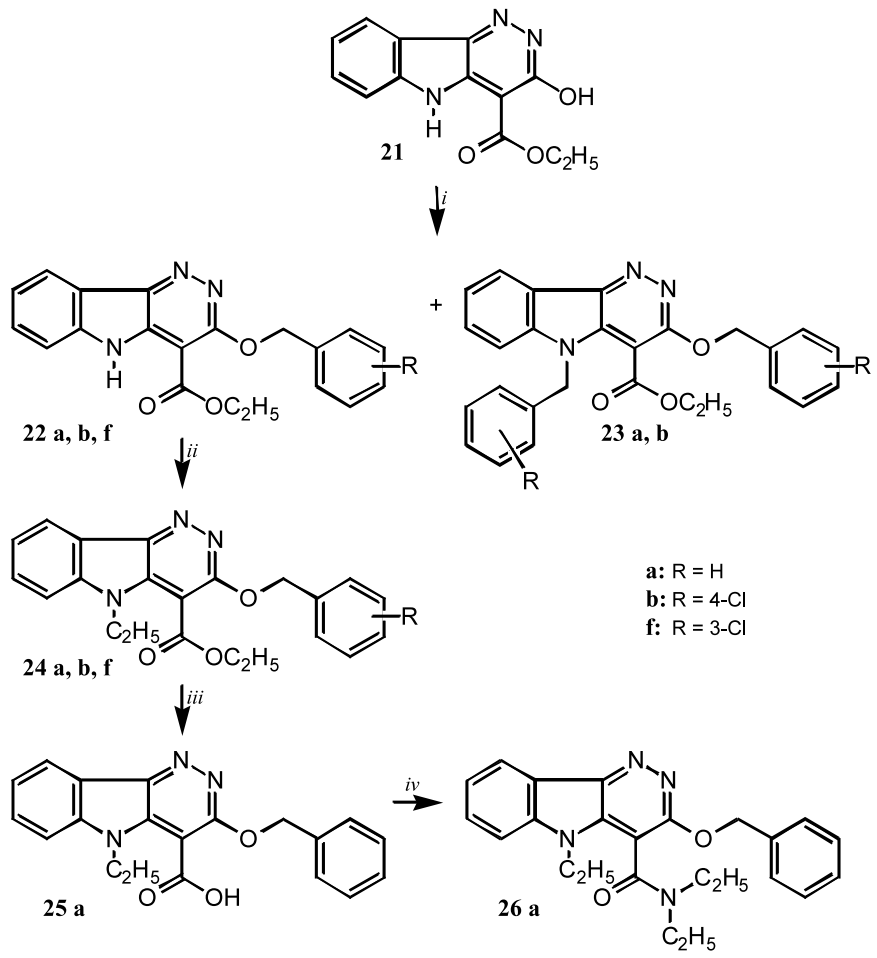
The introduction of a chloro substituent on the phenyl ring of the benzyloxy substituent determined, in the case of *para*-halophenylmethyloxy derivatives **22b**, **24b** and diversely from the 2-aryl-pyridazinoindole and 2-aryl indenopyridazine analogues **3b** and **10d**, a decrease of the activity compared with the corresponding unsubstituted parent compounds **22a** and **24a**. The PBR affinity was substantially conserved in the *meta*-halophenylmethyloxy derivatives **22f** and **24f**.

4. Molecular modelling studies

To find out the main structural determinants affecting the measured PBR binding affinities, a molecular modelling study based on the analysis of three-dimensional properties of four properly selected derivatives was performed.

The convergent pharmacophore models recently developed and referred by different groups [19,24,25] for PBR ligands were taken into account to interpret, in a qualitative manner, the structure–affinity relationships (SAFIR). These models are all characterised by four relevant features: three hydrophobic moieties (L1, L3 and L4) and a hydrogen-bond acceptor group (HB2) as schematically indicated in Fig. 2.

In the present study, three highly active and selective PBR ligands namely isoquinoline carboxamide PK11195, pyrrolo[2,1-d][1,5]benzoxazepin-7yl diethyl-



Scheme 3.

carbamate (**27**) and (2E) 1-oxo-1,2-dihydro-3*H*-pyrrolo[3,4-b]quinolin-3-ylidene acetamide (**28**) from ref. [19] (Plate 3) were chosen as molecular templates to compare their structural features with those shown by our selected molecules, that are **24a**, **3b**, **18a** and **10d**.

Relevant distances among the proposed pharmacophore features, namely L1–L3, L1–HB2 and L3–HB2, were measured for low energy conformers of our molecules and compared with those seen on the selected templates. The centroid characterising the important, but not essential hydrophobic region L4, was not taken into account due to its less precise spatial location. Since our ligands bind less tightly to PBR with respect to the templates, some significant differences in the intramolecular distances of the pharmacophoric points would have been expected.

The analysis of the distances reported in Table 5 suggests that the L1–L3 distance might be a crucial geometrical feature for an efficient binding process at least within our set of ligands. Indeed, unlikely compounds **3b**, **18a** and **10d**, ligand **24a** present a L1–L3 distance very close to those observed in the templates (5.98 versus 5.13–6.38 Å) and moreover, even the L1–HB2 distance is compatible with those measured in the templates (6.11 versus 4.88–6.59 Å). This may justify, at least in part, the higher affinity of **24a**. The difference of about two orders of magnitude of its IC_{50} compared with that of the templates could be ascribed to an unfavourable very low value of the HB2–L3 distance (3.68 versus 5.86–6.44 Å) and/or to a large 'excluded volume' from its tricyclic ring system that may impact a probably forbidden zone of the receptor, as can be seen in the molecular superposition onto PK11195 reported in Fig. 2.

It is worth noting that the low active ligand **18a** does not fit the geometrical features required for an efficient binding to PBR, especially the HB2–L3 distance.

The low activity of derivative **10d** could also arise from the presence of additional chemical groups (i.e. the carbonyl of the dihydropyridazinone moiety and the

Table 1
Physical and spectroscopic data of compounds **5**, **6**, **8**, **10**, **11**, **14**–**18**, **22**–**26**

Comp.	m.p. (°C) (crystallisation solvent)	IR ν_{max} (cm^{-1})	^1H NMR, δ (ppm), J (Hz) [a]
5b	283–285 (ethanol)	3075, 1655, 1630, 1620	1.17 (t, 3H, CH_3, $J = 7.1$); 1.32 (t, 3H, CH_3, $J = 7.1$), 3.44 (q, 2H, CH_2, $J = 7.1$); 3.64 (q, 2H, CH_2, $J = 7.1$); 7.10–7.20 (m, 2H, Arom); 7.35–7.50 (m, 3H, Arom); 7.55–7.65 (m, 2H, Arom); 7.84 (d, 1H, Arom, $J = 7.8$); 9.60 (s, 1H, NH)
6b	242–246 (ligroin)	1650, 1620, 1610	0.75 (t, 3H, CH_3, $J = 7.3$); 1.00 (t, 3H, CH_3, $J = 7.3$); 1.60–1.80 (m, 4H, $2CH_2$); 3.36 (t, 2H, CH_2, $J = 7.3$); 3.53 (t, 2H, CH_2, $J = 7.3$); 7.18 (t, 2H, Arom, $J = 7.4$); 7.40–7.50 (m, 3H, Arom); 7.55–7.65 (m, 2H, Arom); 7.91 (d, 1H, Arom, $J = 7.8$); 9.00 (s, 1H, NH)
8a	230–235 dec. (ethanol)	1720, 1655, 1625	7.35 (s, 1H, CH); 7.40–7.50 (m, 1H, Arom); 7.50–7.65 (m, 4H, Arom); 7.67 (dt, 1H, Arom, $J = 7.2$, 0.7); 7.85 (dt, 1H, Arom, $J = 7.4$. 0.7); 7.80–7.95 (m, 2H, Arom)
10a	149–150 (ethanol)	1710, 1670, 1625	1.35 (t, 3H, CH_3, $J = 7.3$); 4.15 (s, 2H, CH_2), 4.35 (q, 2H, CH_2, $J = 7.3$); 7.40–7.65 (m, 8H, Arom), 7.79 (d, 1H, $J = 7.5$)
10b	148–150 (ethanol)	1715, 1680	1.41 (t, 3H, CH_3, $J = 7.3$); 4.17 (s, 2H, CH_2); 4.45 (q, 2H, CH_2, $J = 7.3$); 7.40–7.55 (m, 5H, Arom); 7.60–7.70 (m, 2H, Arom), 7.85–7.95 (m, 1H, Arom)
10d	162–163 (ethanol)	1700, 1665	1.42 (t, 3H, CH_3, $J = 7.3$); 4.35 (q, 2H, CH_2, $J = 7.3$); 7.40–7.55 (m, 3H, Arom); 7.55–7.65 (m, 4H, Arom); dd (1H, Arom, $J = 6.6$, 1.7)
11a	155–157 (ethanol)	1740, 1665, 1630	1.33 (t, 3H, CH_3, $J = 7.1$); 4.41 (q, 2H, CH_2, $J = 7.1$); 7.45–7.70 (m, 6H; Arom); 7.85–7.95 (m, 3H, Arom)
14a	196–198 (ethanol)	3380, 1705, 1675, 1655, 1600	1.15 (t, 3H, CH_3, $J = 7.1$); 4.21 (q, 2H, CH_2, $J = 7.1$); 6.90–7.00 (m, 2H, Arom); 7.40–7.60 (m, 5H, Arom); 7.90–8.00 (m, 2H, Arom); 9.26 (s, 1H, NH)
14e	202–204 (ethanol)	3380, 1720, 1680, 1610, 1600	1.06 (t, 3H, CH_3, $J = 7.1$); 4.18 (q, 2H, CH_2, $J = 7.1$); 6.97 (t, 1H, Arom, $J = 7.4$); 7.30 (d, 1H, Arom, $J = 8.2$); 7.44 (d, 1H, Arom, $J = 7.4$); 7.59 (dt, 1H, Arom, $J = 7.7$, 1.0); 8.15–8.20 (m, 2H, Arom); 8.25–8.35 (m, 2H, Arom)
15a	210–212 (CH_3CN)	3200br, 1700, 1600	0.99 (t, 3H, CH_3, $J = 7.1$); 4.20 (q, 2H, CH_2, $J = 7.1$); 7.45–7.50 (m, 4H, Arom); 7.53 (d, 1H, Arom, $J = 7.8$); 7.55–7.70 (m, 3H, Arom); 8.57 (d, 1H, Arom, $J = 7.7$); 9.71 (s, 1H, NH)
15e	241–243 (chloroform/hexane)	3260 br, 1700, 1595	1.04 (t, 3H, CH_3, $J = 7.1$), 4.25 (q, 2H, CH_2, $J = 7.1$); 7.48 (t, 1H, Arom, $J = 7.2$); 7.58 (d, 1H, Arom, $J = 8.2$); 7.70 (dt, 1H, Arom, $J = 7.7$, 1.2); 7.75–7.85 (m,2H, Arom); 8.30–8.40 (m,2H, Arom); 8.59 (d, 1H, Arom, $J = 7.8$); 9.81 (s, 1H, NH)
16e	> 350	3070 br, 1630, 1585, 1570	7.46 (dt, 1H, Arom, $J = 7.3$, 1.0); 7.72 (dt, 1H, Arom, $J = 7.2$, 1.1); 7.80 (d, 1H, Arom, $J = 8.1$); 7.85–7.95 (m, 2H, Arom); 8.30–8.40 (m, 2H, Arom); 8.45 (d, 1H, Arom, $J = 7.7$); 12.14 (s, 1H, NH); 14.10 (s, 1H, COOH)
17e	314–316 (ethyl acetate)	3230, 1611, 1600	0.64 (t, 3H, CH_3, $J = 7.0$); 1.12 (t, 3H, CH_3, $J = 7.0$); 2.60–2.75 (m, 1H, CH), 2.85–3.00 (m, 1H, CH), 3.10–3.30 (m, 1H, CH), 3.85–3.95 (m, 1H, CH), 7.43 (t, 2H, Arom, $J = 7.7$); 7.61 (t, 1H, Arom, $J = 7.7$); 8.15 (d, 2H, Arom, $J = 8.6$); 8.35 (d, 2H, Arom, $J = 8.1$); 8.52 (d, 1H, Arom, $J = 7.9$); 9.38 (s, 1H, NH)
18a	169–170 (ethanol)	1720, 1575	1.04 (t, 3H, CH_3, $J = 7.1$); 1.42 (t, 3H, CH_3, $J = 7.1$); 4.20 (q, 2H, CH_2, $J = 7.1$), 4.38 (q, 2H, CH_2, $J = 7.1$); 7.45–7.55 (m, 5H, Arom); 7.65–7.75 (m, 3H, Arom); 8.63 (d, 1H, Arom, $J = 7.7$)
22a	194–196 (acetonitrile/water)	3360, 1670, 1640, 1605	1.31 (t, 3H, CH_3, $J = 7.1$); 4.35 (q, 2H, CH_2, $J = 7.1$); 5.42 (s, 2H, CH_2); 7.20–7.35 (m, 6H, Arom); 7.50–7.60 (m, 2H, Arom); 7.91 (d, 1H, Arom, $J = 7.7$); 11.50 (s, 1H, NH)
22b	184–186 (acetonitrile)	3520, 3340, 1660, 1630	1.31 (t, 3H, CH_3, $J = 7.0$); 4.35 (q, 2H, CH_2, $J = 7.0$); 5.40 (s, 2H, CH_2); 7.20–7.30 (m, 1H, Arom); 7.30–7.45 (m, 4H, Arom); 7.52 (d, 2H, Arom, $J = 4.0$); 7.91 (d, 1H, Arom, $J = 7.8$); 11.50 (s, 1H, NH)
22f	142–145 (acetonitrile/water)	3360, 1700, 1590	1.32 (t, 3H, CH_3, $J = 7.1$); 4.35 (q, 2H, CH_2, $J = 7.1$); 5.41 (s, 2H, CH_2); 7.20–7.30 (m, 2H, Arom); 7.30–7.40 (m, 3H, Arom); 7.50–7.55 (m, 2H, Arom); 7.92 (d, 1H, Arom, $J = 7.6$); 11.50 (s, 1H, NH)
23a	166–168 (acetonitrile/water)	1720, 1640, 1620	0.91 (t, 3H, CH_3, $J = 7.1$); 4.00 (q, 2H. CH_2, $J = 7.1$); 5.30 (s, 2H, CH_2); 5.42 (s, 2H, CH_2); 7.09 (dd, 2H, Arom, $J = 7.3$, 1.5); 7.20–7.40 (m, 10H, Arom); 7.54 (dt, 1H, Arom, $J = 7.7$, 1.0); 8.00 (d, 1H, Arom, $J = 7.4$)
23b	203–205 (acetonitrile)	1720, 1640, 1600	0.94 (t, 3H, CH_3, $J = 7.2$); 4.02 (q, 2H. CH_2, $J = 7.2$); 5.29 (s, 2H, CH_2); 5.41 (s, 2H, CH_2); 7.11 (d, 2H, Arom, $J = 8.5$); 7.25–7.45 (m, 8H, Arom); 7.55 (dt, 1H, Arom, $J = 7.7$, 1.2); 8.00 (d, 1H, Arom, $J = 7.7$)
24a	174–175 (acetonitrile/water)	1715, 1640, 1605	1.22 (t, 3H, CH_3, $J = 7.2$); 1.32 (t, 3H, CH_3, $J = 7.2$); 4.03 (q, 2H, CH_2, $J = 7.2$); 4.38 (q, 2H, CH_2, $J = 7.2$); 5.41 (s, 2H, CH_2); 7.25–7.40 (m, 6H, Arom); 7.50–7.65 (m, 2H, Arom); 7.95 (d, 1H, Arom, $J = 7.4$)
24b	181–183 (acetonitrile)	1700, 1635, 1605	1.22 (t, 3H, CH_3, $J = 7.1$); 1.32 (t, 3H, CH_3, $J = 7.1$); 4.01 (q, 2H, CH_2, $J = 7.1$); 4.37 (q, 2H, CH_2, $J = 7.1$); 5.40 (s, 2H, CH_2); 7.30–7.50 (m, 5H, Arom); 7.50–7.65 (m, 2H, Arom); 7.96 (d, 1H, Arom, $J = 7.7$)
24f	160–162 (acetonitrile/water)	3425, 1720, 1630, 1610	1.33 (t, 3H, CH_3, $J = 7.2$); 1.42 (t, 3H, CH_3, $J = 7.2$); 4.02 (q, 2H, CH_2, $J = 7.2$); 4.47 (q, 2H, CH_2, $J = 7.2$); 5.44 (s, 2H, CH_2); 7.15–7.30 (m, 4H, Arom); 7.35–7.40 (m, 1H, Arom); 7.44 (s, 1H, Arom); 7.51 (t, 1H, Arom, $J = 7.9$); 8.00 (d, 1H, Arom, $J = 7.5$)

Table 1 (*Continued*)

Comp.	m.p. (°C) (crystallisation solvent)	IR ν_{max} (cm^{-1})	^1H NMR, δ (ppm), J (Hz) [a]
25a	228–234 (dioxane)	3400, 1720	1.25 (t, 3H, CH_3, $J = 7.0$); 4.24 (q, 2H, CH_2, $J = 7.0$); 5.45 (s, 2H, CH_2); 7.25–7.40 (m, 6H, Arom); 7.50–7.65 (m, 2H, Arom); 7.97 (d, 1H, Arom, $J = 7.6$); 14.01 (br, 1H, COOH)
26a	Low melting point	1615, 1630	1.13 (t, 3H, CH_3, $J = 7.0$); 1.29 (t, 3H, CH_3, $J = 7.0$); 1.33 (t, 3H, CH_3, $J = 7.0$); 3.31 (dq, 2H, CH_2, $J = 7.1, 2.3$); 3.40–3.55 (m, 1H, CH); 3.70–3.85 (m, 1H, CH); 3.85–4.05 (m, 1H, CH); 4.05–4.20 (m, 1H, CH); 5.45 (d, 1H, H_A, CH2–Ph, AB system, $J_{AB} = 19.6$); 5.51(d, 1H, H_A, CH2-Ph, AB system, $J_{AB} = 19.6$); 7.10–7.40 (m, 5H, Arom); 7.40–7.55 (m, 3H, Arom); 8.00 (d, 1H, Arom, $J = 7.7$)

[a] ^1H NMR spectra were recorded in $CDCl_3$ (**5b**, **6b**, **10b**, **10d**, **14a**, **15a**, **15e**, **17e**, **18a**, **26a**) or in DMSO-d_6 (**8a**, **10a**, **11a**, **14e**, **16e**, **22a**, **22b**, **22f**, **23a**, **23b**, **24a**, **24b**, **24f**, **25a**).

benzene of the indole ring) which might generate some unfavourable steric and/or electrostatic interactions with the receptor (see Fig. 3).

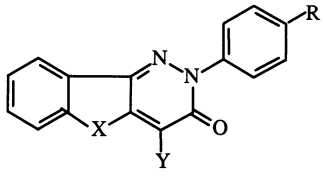
In summary, the present modelling study allowed us to understand the main reasons underlying the relatively low PBR affinity displayed by our ligands and suggested proper structural modifications for the synthesis of more active compounds. We are presently working on this and drastic molecular simplifications have been planned to confer to the new molecules the essential, and possibly minimum, structural requirements for a high PBR binding affinity.

5. Experimental

5.1. Chemistry

Melting points were taken on a Gallenkamp MFB 595 010 M apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 analyser for C, H, N; experimental results agreed to within ± 0.40% of the theoretical values. IR spectra were recorded using potassium bromide disks on a Perkin-Elmer 283 spectrophotometer, only the most significant and diagnostic absorption bands being reported. ^1H

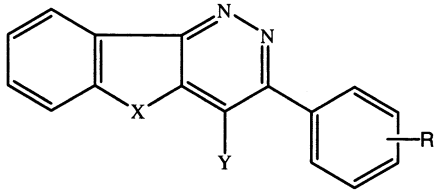
Table 2
Binding data of condensed 2-arylpyridazine derivatives



Comp.	X	Y	R	CBR [a]	PBR [b]
1a	NH	H	H	238	[0%]
1b	NH	H	4-Cl	31	[10%]
2a	N–CH_3	H	H	1560	[3%]
2c	N–CH_3	H	4-OCH_3	994	[0%]
3a	NH	$COOC_2H_5$	H	[0%]	[5%]
3b	NH	$COOC_2H_5$	4-Cl	[0%]	610
4a	N–CH_3	$COOC_2H_5$	H	N.D.	[16%]
5b	NH	$CON(C_2H_5)_2$	4-Cl	[0%]	[7%]
6b	NH	$CON(nC_3H_7)_2$	4-Cl	[0%]	[16%]
7a [c]	CH_2	H	H	2180	[11%]
8a	CO	H	H	[30%] 20 μM	[0%]
10a	CH_2	$COOC_2H_5$	H	[45%] 20 μM	[43%]
10b	CH_2	$COOC_2H_5$	4-Cl	[0%]	[30%]
10d	CH_2	$COOC_2H_5$	4-Br	[0%]	730
11a	CO	$COOC_2H_5$	H	[0%]	[18%]

[a] Inhibition of [3H] flunitrazepam binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.
[b] Inhibition of [3H] PK11195 binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.
[c] Reported in ref. [17].

Table 3
Binding data of condensed 3-arylpyridazine derivatives



Comp.	X	Y	R	CBR [a]	PBR [b]
15a	NH	$COOC_2H_5$	H	[0%]	[31%]
15e	NH	$COOC_2H_5$	NO_2	[0%]	[9%]
17e	NH	$CON(C_2H_5)_2$	NO_2	[0%]	[13%]
18a	$N–C_2H_5$	$COOC_2H_5$	H	[3%]	658
19 [c]	CO	$COOC_2H_5$	H	[0%]	2300

[a] Inhibition of [3H] flunitrazepam binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.
[b] Inhibition of [3H] PK11195 binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.
[c] Reported in ref. [26].

NMR spectra were recorded on a Bruker AM 300 WB 300 MHz or on a Varian 300 Mercury spectrometers. Chemical shifts are expressed in δ (ppm) and the coupling constants J in Hz. The following abbreviations were used: s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet. Exchange with deuterium oxide was used to identify OH and NH protons, which in some cases gave broad signals spread widely on the base line and these were very difficult to detect. Reagents and solvents were purchased from Sigma-Aldrich Chemie.

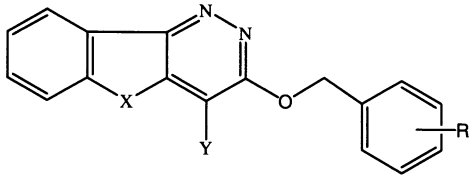
*5.1.1. 2-Chlorophenyl-3,5-dihydro-3-oxo-2H-pyridazino[4,3-b]indole-4-carboxylic acid dialkylamides (**5b**, **6b**)*

Catalytic amount of anhydrous DMF was added to a mixture of 2-chlorophenyl-3,5-dihydro-3-oxo-2*H*-pyridazino[4,3-b]indole-4-carboxylic acid, obtained from the ester **3b** as reported in ref. [15], (0.051 g, 0.15 mmol) and thionyl chloride (20.5 mmol, 1.5 ml). The resulting mixture was refluxed for 7 h. The unreacted thionyl chloride was evaporated and to the residue suspended in anhydrous THF (2 ml) the appropriate amine (0.55 mmol) was added. The mixture was stirred at room temperature (r.t.) for 2 h. In the case of **5b**, the residue, obtained after evaporation of the solvent in vacuo, was chromatographed on silica gel using ethyl acetate/petroleum ether 9:1 (**5b**, Rf = 0.6, 0.03 g, 52% yield). In the case of **6b**, the final suspension was filtered, the resulting solution evaporated in vacuo and the residue crystallised from ligroin **6b** (0.060 g, 57% yield).

*5.1.2. 2-Phenyl-2H-indeno[1,2-c]pyridazine-3,5-dione (**8a**)*

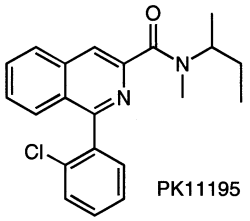
CrO_3 (0.140 g, 1 mmol) was added to a solution of 2-phenyl-2,5-dihydro-indeno[1,2-c]pyridazine-3-one (**7a**) [17] (0.260 g, 1 mmol) in acetic acid (4.6 ml) and the resulting mixture refluxed for 2 h. After cooling, the reaction mixture was poured on ice (40 ml) and the precipitate filtered, dried and chromatographed, using chloroform/ethyl acetate 9:1 as an eluant, to obtain **8a** (Rf = 0.67, 49% yield).

Table 4
Binding data of 3-arylmethyloxypyridazinoindole derivatives



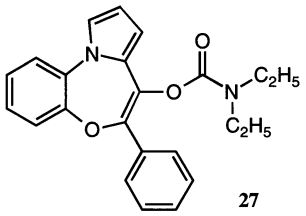
Comp.	X	Y	R	CBR [a]	PBR [b]
22a	NH	$COOC_2H_5$	H	[0%]	[27%]
22b	NH	$COOC_2H_5$	4-Cl	[0%]	[26%]
22f	NH	$COOC_2H_5$	3-Cl	[0%]	1470
23a	$N–CH_2C_6H_5$	$COOC_2H_5$	H	[0%]	1570
23b	$N–CH_2–C_6H_4–4Cl$	$COOC_2H_5$	4-Cl	[0%]	[18%]
24a	$N–C_2H_5$	$COOC_2H_5$	H	[0%]	240
24b	$N–C_2H_5$	$COOC_2H_5$	4-Cl	[0%]	[26%]
24f	$N–C_2H_5$	$COOC_2H_5$	3-Cl	[0%]	770
26	$N–C_2H_5$	$CON(C_2H_5)_2$	H	[0%]	[26%]

[a] Inhibition of [3H] flunitrazepam binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.
[b] Inhibition of [3H] PK11195 binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.



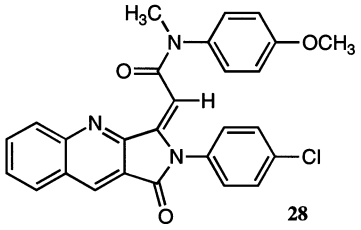
PK11195

N-(*sec*-butyl)-1-(2-chlorophenyl)-*N*-methylisoquinoline-3-carboxamide



27

6-phenyl-pyrrolo[2,1-d][1,5]benzossazepin-7-yl diethylcarbamate



28

(2E)-*N*-(4-methoxyphenyl)-*N*-methyl-2[1-oxo-2-(4-chlorophenyl)-1,2-dihydro-3*H*-pyrrolo[3,4-b] quinolin-3-ylidene

Plate 3.

Table 5
Intramolecular distances (Å) between pharmacophoric elements

Comp.	pIC_{50}	L1–L3	L1–HB2	HB2–L3
PK11195 (R) [a]	8.70	5.13	6.59	5.86
27 [a]	9.17	6.38	5.81	6.17
28 [a]	8.72	6.20	4.88	6.44
24a	6.62	5.98	6.11	3.68
3b	6.21	7.36	6.96	6.63
18a	6.18	4.29	4.54	3.66
10d	6.14	7.38	6.74	6.52

[a] Ref. [19].

*5.1.3. 3-Oxo-2-aryl-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxylic acid ethyl esters (**10a**, **b**, **d**)*

The appropriate arylhydrazine hydrochloride (1.2 mmol) was added to a solution of 2-hydroxy-2-(1-oxo-indan-2-yl)-malonic acid diethyl ester (**9**) [20] (0.307 g, 1 mmol) in ethanol (6 ml) and the resulting mixture refluxed 8–19 h (16 h for **10a**, 19 h for **10b**, 8 h for **10d**). The resulting precipitate was collected and chromatographed on silica gel using ethyl acetate/petroleum ether 3:7 as an eluant to give the esters **10a** (Rf = 0.50, 0.086 g, 26% yield), **10b** (Rf = 0.33, 0.260 g, 71% yield) and **10d** (Rf = 0.47, 0.296 g, 72% yield).

*5.1.4. 3,5-Dioxo-2-phenyl-3,5-dihydro-2H-indeno[1,2-c]pyridazine-4-carboxylic acid ethyl ester (**11a**)*

CrO_3 (0.07 g, 0.50 mmol) was added to a solution of 3-oxo-2-phenyl-3,5-dihydro-2*H*-indeno[1,2-c]pyridazine-4-carboxylic acid ethyl ester (**10a**) (0.166 g, 0.50 mmol) in acetic acid (2.3 ml) and the mixture refluxed for 2 h. After cooling, the reaction mixture was poured on ice (25 ml) and the resulting precipitate filtered, dried and chromatographed using chloroform/ethyl acetate 5:5 as an eluant to obtain **11a** (Rf = 0.82, 0.104 g, 60% yield).

*5.1.5. 3-Oxo-2-(3-oxo-1,3-dihydro-indol-2-yl)-3-aryl-propionic acid ethyl esters (**14a**, **e**)*

A mixture of 1*H*-indole-2,3-dione (**12**) (10.00 g, 68 mmol), phosphorus (V) chloride (15.00 g, 81.6 mmol) and anhydrous benzene (30 ml) was refluxed for 4 h. After cooling, the precipitate (imidoylchloride) was collected by filtration under dry nitrogen. After washing with petroleum ether, the solid was immediately dissolved in anhydrous dioxane (100 ml) and the equimolecular solution of ethylaroylacetate sodium salt in anhydrous dioxane (120 ml), was added dropwise. The reaction mixture was stirred at r.t. overnight. The precipitate sodium chloride was filtered off and the solution evaporated in vacuo. The residue was triturated

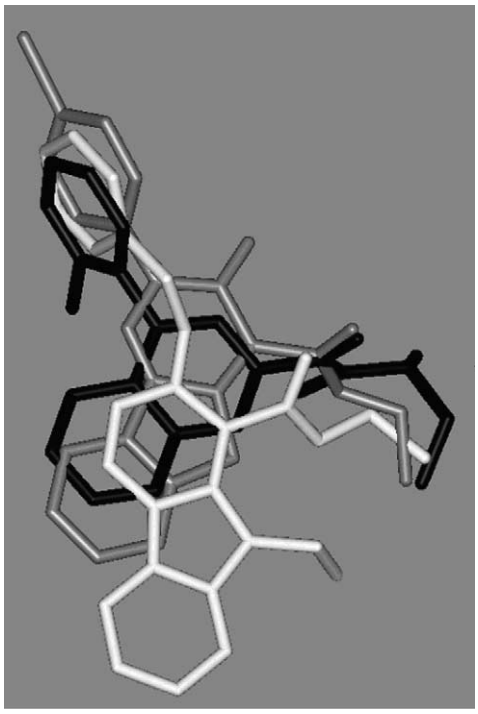


Fig. 3. Molecular superposition of PK11195 (black), **24a** (white) and **10d** (grey) generated as follows: the centroid of the benzene ring of isoquinoline, the centroid of the *ortho*-substituted phenyl ring and the carbonyl oxygen of PK11195 were fitted onto the centroid of the pyridazine ring, the centroid of the benzyloxy and the carbonyl oxygen of the ester **24a** and the centroid of the benzene ring of indole, the centroid of the 2-phenyl and the carbonyl oxygen of the ester for **10d**, respectively.

with diethyl ether to give a solid which was recrystallised from ethanol to afford **14a** (6.55 g, 30% yield), and **14e** (6.23 g, 25% yield).

*5.1.6. 3-Aryl-5H-pyridazino[4,3-b]indole-4-carboxylic acid ethyl esters (**15a, e**)*

Hydrazine hydrate (0.265 ml, 5.4 mmol) was added to a stirred solution of 3-oxo-2-(3-oxo-1,3-dihydro-indol-2-yl)-3-aryl-propionic acid ethyl ester (**14a** or **14e**) (5 mmol) in anhydrous ethanol (250 or 500 ml, respectively) and stirring was continued for 3 h at r.t. The solvent was evaporated in vacuo and the residue crystallised from methanol/water and then from acetonitrile to afford **15a** (0.400 g, 25% yield) or triturated with ethanol and then crystallised from chloroform/hexane to afford **15e** (0.905 g, 50% yield).

*5.1.7. 3-(4-Nitro-phenyl)-5H-pyridazino[4,3-b]indole-4-carboxylic acid (**16e**)*

A solution of NaOH (0.500 g, 14 mmol) in ethanol (47 ml) was added to a stirred suspension of 3-*p*-nitrophenyl-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**15e**) (0.346 g, 1 mmol) in ethanol (10 ml) and then refluxed for 3 h. The reaction mixture was cooled to r.t. and the solvent evaporated in vacuo. The residue was dissolved in water (40 ml), acidified with conc. HCl to pH 1 and stirred at r.t. for 30 min. The yellow precipitate was filtered, washed with water and dried to give **16e** (0.286 g, 90% yield).

*5.1.8. 3-(4-Nitro-phenyl)-5H-pyridazino[4,3-b]indole-4-carboxylic acid diethylamide (**17e**)*

A mixture of 3-(4-nitro-phenyl)-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid (**16e**) (0.159 g, 0.5 mmol) and thionyl chloride (4 ml) was refluxed for 6 h. The unreacted thionyl chloride was evaporated in vacuo and to the residue suspended in anhydrous THF (4 ml), diethylamine (0.192 ml) was added. The reaction mixture was stirred at r.t. for 24 h. The solid, obtained after evaporation of the solvent, was suspended in chloroform and filtered. The filtrate was evaporated in vacuo and the residue chromatographed on silica gel using ethyl acetate as an eluant to obtain **17e** (Rf = 0.70, 0.035 g, 18% yield).

*5.1.9. 3-Phenyl-5-ethyl-5H-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**18a**)*

Sodium hydride 95% (0.020 g, 0.80 mmol) was added to a stirred suspension of 3-phenyl-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**15a**) (0.162 g, 0.50 mmol) in dry DMF (3 ml) under nitrogen. After 1 h, iodoethane (0.064 ml, 0.80 mmol) was added and stirring was continued for 4 h. The mixture was then poured on ice (40 ml) and the precipitate was filtered, dried and chromatographed on silica gel using chloroform/ethyl acetate 8:2 as an eluant to give **18a** (Rf = 0.47, 0.040 g, 23% yield).

*5.1.10. 3-Arylmethyloxy-5H-pyridazino[4,3-b]indole-4-carboxylic acid ethyl esters (**22a, b, f** and **23a, b**)*

Sodium hydride 95% (0.038 g, 1.5 mmol) was added to a stirred suspension of 3-hydroxy-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**21**) [21] (0.384 g, 1.5 mmol) in dry DMF (13 ml) under nitrogen. After 30 min, benzyl bromide or 4-chlorobenzylchloride or 3-chlorobenzyl bromide (1.5 mmol) was added and stirring was continued for 3 h. The mixture was then poured on ice (150 ml) and the precipitate filtered, washed with water and dried. Chromatographic purification of the crude solid on silica gel using ethyl acetate/petroleum ether 5:5 as an eluant furnished **22a** and **23a** (**23a**: Rf = 0.76, 0.052 g, 8% yield; **22a**: Rf = 0.47, 0.224 g, 43% yield) or **22b** and **23b** (**23b**: Rf = 0.68, 0.076 g, 10% yield; **22b**: Rf = 0.40, 0.383 g, 67% yield). The crystallisation of the crude precipitate from acetonitrile/water furnished **22f** (0.241 g, 40% yield).

*5.1.11. 3-Arylmethyloxy-5-ethyl-5H-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**24a, b, f**)*

Sodium hydride 95% (0.009 g, 0.35 mmol) was added to a stirred suspension of 3-arylmethyloxy-5*H*-pyrida-

zino[4,3-b]indole-4-carboxylic acid ethyl ester (**22a**, **b**, **f**) (0.21 mmol) in dry DMF (1.5 ml) under nitrogen. After 40 min, iodoethane (0.028 ml, 0.35 mmol) was added and stirring was continued for 2 h (in the case of **22a**, **f**) or overnight (in the case of **22b**). The mixture was then poured on ice (20 ml). In the case of **22a**, **b**, the precipitate was filtered, washed with water, dried and chromatographed on silica gel using ethyl acetate/petroleum ether 5:5 as an eluant to furnish **24a** (Rf = 0.63, 0.049 g, 61% yield) or **24b** (Rf = 0.58, 0.058 g, 67% yield). In the case of **22f**, the mixture was extracted twice with 20 ml of chloroform and the organic phases washed with water, dried on anhydrous sodium sulphate and evaporated in vacuo. The obtained residue was chromatographed on silica gel using ethyl acetate/petroleum ether 5:5 as an eluant to give **24f** (Rf = 0.65, 0.022 g, 26% yield).

*5.1.12. 3-Benzyloxy-5-ethyl-5H-pyridazino[4,3-b]indole-4-carboxylic acid (**25a**)*

A solution of NaOH (0.100 g, 2.5 mmol) in ethanol (10 ml) was added with stirring to a suspension of 3-benzyloxy-5-ethyl-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid ethyl ester (**24a**) (0.094 g, 0.25 mmol) in ethanol (2 ml) and the resulting mixture refluxed for 2 h. After cooling, the precipitate was filtered, washed with ethanol and suspended in water (15 ml). The suspension was acidified with conc. HCl to pH 1 and then stirred at r.t. for 1 h. The solid **25a** was filtered, washed with water and dried (0.066 g, 76% yield).

*5.1.13. 3-Benzyloxy-5-ethyl-5H-pyridazino[4,3-b]indole-4-carboxylic acid diethylamide (**26a**)*

Catalytic amount of anhydrous DMF was added to a mixture of 3-benzyloxy-5-ethyl-5*H*-pyridazino[4,3-b]indole-4-carboxylic acid (**25a**) (0.030 g, 0.08 mmol) and thionyl chloride (1 ml). The resulting mixture was refluxed for 7 h. The unreacted thionyl chloride was evaporated and to the residue dissolved in anhydrous THF (1.55 ml), diethylamine (0.057 ml, 0.55 mmol) was added. The mixture was stirred at r.t. for 2 h. The residue, obtained after evaporation of the solvent in vacuo, was chromatographed on silica gel using ethyl acetate/petroleum ether 5:5 to obtain **26a** as an oil Rf = 0.26, 0.014 g, 44% yield).

5.2. Modelling

5.2.1. Methods

Molecular models of all the studied compounds were built using standard bond lengths and angles within the Catalyst software (ver. 4.6 Accelrys, San Diego, CA, USA).

Low energy conformers of reference ligands PK11195, **27** and **28** were chosen in order to comply with previously published pharmacophoric models of PBR.

Conformational analysis of reference ligands and compounds **3b**, **10d**, **18a**, **24a** was performed using the BEST SEARCH module implemented in Catalyst [27,28]. All the calculations were run on a SGI-O2 R10000 workstation.

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