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Synthesis and Structure–Affinity Relationships at the Central Benzodiazepine Receptor of Pyridazino[4,3-*b*]indoles and Indeno[1,2-*c*]pyridazines

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Abstract—A series of 2-aryl-3-chloro-2*H*-pyridazino[4,3-*b*]indoles, 2-aryl-3-methoxy-2*H*-pyridazino[4,3-*b*]indoles, and 2-aryl-2,5-dihydroindeno[1,2-*c*]pyridazino-3(3*H*)-ones has been prepared and tested for their ability to inhibit the [^{3}H]flunitrazepam binding to the central benzodiazepine receptor. SAR are presented and discussed in comparison with existing pharmacophore models.

Introduction

Benzodiazepine receptor (BZR) ligands produce their pharmacological effects by modulating the action of GABA at $GABA_A$-receptor/Cl^- ionophore supramolecular complex.[1,2] Such a complex can be formed by different combinations of distinct subunits. A total of at least 13 subunits (α1 to α6, β1 to β3, γ1 to γ3 and δ)[3–5] have been identified by molecular cloning, and are thought to assemble into a pentameric structure to form a Cl^- channel. Most functional subtypes of $GABA_A$ receptors contain α,β and γ subunits, with different subtypes showing diverse sensitivity to different benzodiazepine receptor ligands.[6–8] BZR ligands bind to allosteric modulatory sites (ω sites) enhancing (agonists) or reducing (inverse agonists) the GABA-induced Cl^- ion flux. A third class of ligands, called antagonists, exhibit, per se, no relevant biological effects but antagonize the action of agonists and inverse agonists.[9] Despite the recent significant advances in the structural and functional studies of BZR,[4] and in the molecular pharmacology and physiopathology of anxiety disorders,[3] much remains to be achieved for the design and development of truly innovative drugs, lacking the numerous side effects of benzodiazepines.[10] From a medicinal chemistry view point, the recent years have seen the proposal and refinement of several pharmacophore models,[11–18] which may constitute a useful guide for the design of new potent ligands. An interesting contribution in this field came also from our recent 2-D and 3-D QSAR studies[19,20] of a new class of BZR ligands, namely the 2-aryl-2,5-dihydropyridazino[4,3-*b*]-indol-3-(3*H*)-ones (PIs), which are structurally related to the well known 2-aryl-2,5-dihydropyrazolo[4,3-*c*]-quinoline-3-(3*H*)ones (PQs).[21] As a continuation of our ongoing research on this topic we synthesized the 3-chloro (**2a–d**) and 3-methoxy (**3a–d**) pyridazino[4,3-*b*]-indoles and the indeno[1,2-*c*]pyridazines (**8**, **9a–d**) (Schemes 1 and 2) to better define the influence of both the indolic NH function, as a hydrogen bond donor group, and the carbonyl group, as hydrogen bond acceptor group, on the benzodiazepine receptor affinity of the new BZR ligands.

Results and Discussion

Chemistry

Scheme 1 outlines the synthetic route to 2-aryl-3-chloro-2*H*-pyridazino[4,3-*b*]indoles **2a–d** and 2-aryl-3-methoxy-2*H*-pyridazino[4,3-*b*]indoles **3a–d**. In short, the 2-aryl-3,5-dihydro-pyridazino[4,3-*b*]indolones **1a–d** were reacted with phosphorus oxychloride at 90–100°C to give the corresponding 3-chloro derivatives **2a–d**. The displacement of chloride ion with CH_3ONa in dry methanol gave 3-methoxy derivatives **3a–d**, which, by heating at 200°C, underwent the thermal rearrangement to the N-5 methyl isomers **4a–d**, previously reported through the alkylation of parent compounds **1a–d**.[20]

Key words: Benzodiazepine receptor; binding assay; structure–activity relationships; pyridazino[4,3-*b*]indoles; indeno[1,2-*c*]pyridazines.
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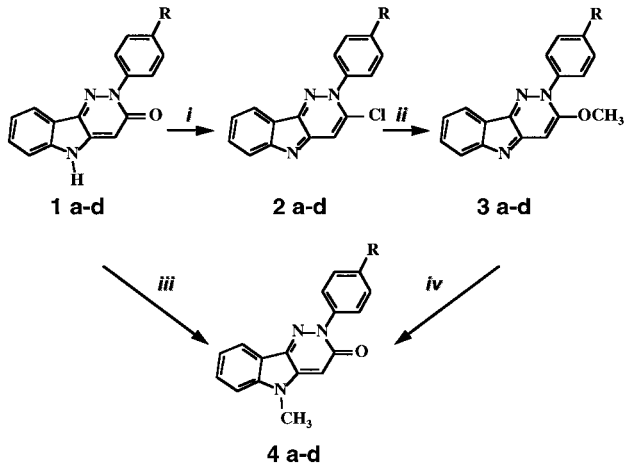
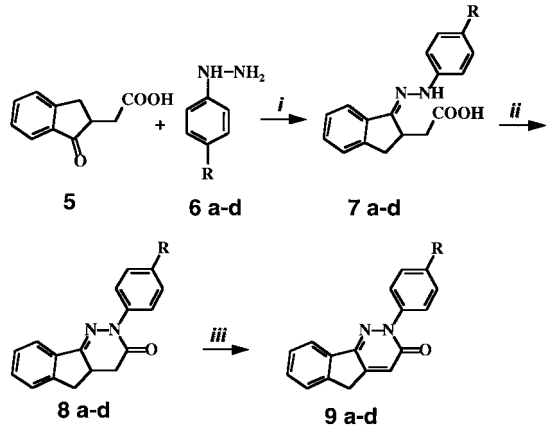


Scheme 1. (i) $POCl_3$, 100°C; (ii) CH_3ONa, anhydrous MeOH, reflux; (iii) NaH, anhydrous THF, MeI, rt; (iv) Δ, 200°C. **a**: R = H; **b**: R = Cl; **c**: R = Br; **d**: R = OCH_3.



Scheme 2. (i) Anhydrous MeOH, rt; (ii) DCC, anhydrous CH_3CN, rt; (iii) Br_2, CH_3COOH, 100°C. **a**: R = H; **b**: R = Cl; **c**: R = Br; **d**: R = OCH_3.

Compounds **2a–d** and **3a–d** show UV–Vis spectra indicative of an electronic structure more conjugated than parent compounds **1a–d** and N-5 methyl homologues **4a–d** (Table 1). The 2-aryl-indeno[1,2-*c*]pyridazinone derivatives **8a–d** and **9a–d** were synthesized as indicated in Scheme 2. In brief, 1-keto-2-indanylacetic acid **5**[22] was reacted at room temperature with the appropriate phenylhydrazine **6** to give the intermediate arylhydrazones **7a–d**, which were then easily cyclized to 2-aryl-2,4,4a,5-tetrahydroindeno[1,2-*c*]pyridazino-3(3*H*)-ones **8a–d** by treatment with dicyclohexylcarbodiimide in anhydrous acetonitrile. Compounds **8a,b**, and **d**, had spectroscopic and physicochemical properties consistent with those reported by Nakao and co-workers, who prepared the same compounds by a different procedure.[23] Finally 2-aryl-2,5-dihydroindeno[1,2-*c*]pyridazino-3(3*H*)-ones **9a–d** were obtained by oxidation of **8a–d** with bromine in acetic acid at 100°C. Physical and spectroscopic properties of compounds **2**, **3**, **7**, **8**, and **9** are listed in Table 2. Compounds **3** had melting points consistent with their complete thermal rearrangement to the corresponding N-5 methyl congeners **4**.

Table 1. [a]UV–Vis data (ethanol) of compounds **1**, **2**, **3** and **4**

Compd	λ_{max} (nm) (log ε)		
1a	218 (4.43)	272 (4.60)	332 (3.77)
1b	220 (4.52)	272 (4.67)	332 (3.92)
1c	220 (4.51)	272 (4.66)	332 (3.92)
1d	218 (4.52)	268 (4.62)	338 (3.86)
2a	222 (4.32)	278 (4.56)	426 (3.30)
2b	220 (4.42)	278 (4.56)	422 (3.35)
2c	222 (4.41)	278 (4.54)	426 (3.31)
2d	224 (4.40)	278 (4.54)	422 (3.31)
3a	216 (4.45)	286 (4.69)	430 (3.36)
3b	216 (4.47)	286 (4.63)	426 (3.33)
3c	216 (4.70)	286 (4.86)	428 (3.58)
3d	218 (4.35)	280 (4.52)	420 (3.28)
4a	220 (4.51)	276 (4.68)	334 (3.85)
4b	222 (4.51)	276 (4.64)	334 (3.91)
4c	222 (4.44)	274 (4.58)	334 (3.84)
4d	222 (4.50)	270 (4.59)	340 (3.85)

[a] The UV–Vis data of compounds **2** and **3** are reported together with those of previously described congeners **1** and **4**,[20] for comparison.

Binding studies

All the new ligands **2**, **3**, **8** and **9** were evaluated for their ability to displace [^{3}H]flunitrazepam from BZR (Table 3), by a previously described method.[19] Data from the present work revealed that compounds with electron rich substituents in the *para* position of the N-2 phenyl ring were, in all the series, more active than the corresponding unsubstituted congeners. Binding affinities of 3-chloro derivatives **2a–d** were higher than the corresponding 3-methoxy analogues **3a–d**. This may be ascribed, at least in part, to the formation of a hydrogen bond with a proton-accepting group, called H_1 in the BZR pharmacophore model proposed by Cook.[18]

To support this hypothesis, a molecular modelling study was undertaken. The results thereof showed that the lower BZR affinity of **3a–d** with respect to **2a–d** analogues might indeed result from the different location and orientation of the lone pairs of oxygen and chlorine atoms, possibly involved in a hydrogen bonding at the H_1 site. The subtle differences between the 3-D structures of **1a**, **2a**, and **3a** may be better perceived by a simple visual inspection of the molecular superposition realized via the fitting of three supposed receptor anchoring points that are the N-1 atom and the centroids C1 and C2 of the benzene of the benzofused moiety and of the 2-phenyl ring, respectively (Fig. 1). In particular, the overlay of the three ligands shows that the oxygen lone pairs of the methoxy group (**3a**, violet), unlike those of the oxygen of the carbonyl (**1a**, red) and those of chlorine (**2a**, green) do not point toward the H_1 region. Moreover the dramatic drop of activity observed for compounds **3a–d** can be, at least in part, attributed to the steric hindrance caused by the OCH_3 group at the S_1 unaccessible region, in accord with the same pharmacophore model. Thus, as in the case of **4a–d** previously reported by us,[20] both the steric hindrance and the removal of a possible hydrogen bond with a specific binding site on the receptor may be responsible for their poor affinity. The 5-deaza-analogues **8a–d** and **9a–d**, which possess topological features similar to **1a–d**,

Table 2. Physical and spectroscopic data of compounds **2**, **3**, **7**,[a] **8**, and **9**

Compd	mp (°C) (crystallization solvent)	IR ν_{max} (cm^{-1})	^1H NMR δ [ppm, *J* (Hz)][b]
2a	271–273 dec (ligroin)	1610, 1550	7.25–7.35 (m, 1H, Arom), 7.45–7.60 (m, 5H, Arom), 7.63 (s, 1H, Arom), 7.65–7.75 (m, 1H, Arom), 7.83 (d, 1H, Arom, *J* = 8.2), 8.19 (d, 1H, Arom, *J* = 7.7)
2b	280–285 dec (ligroin)	1610, 1550, 1545	7.25–7.35 (m, 1H, Arom), 7.40–7.50 (m, 2H, Arom), 7.50–7.60 (m, 2H, Arom), 7.61 (s, 1H, Arom), 7.65–7.75 (m, 1H, Arom), 7.82 (d, 1H, Arom, *J* = 8.1), 8.16 (d, 1H, Arom, *J* = 7.9)
2c	290–300 dec (ligroin)	1610, 1560	7.30–7.45 (m, 3H, Arom), 7.62 (s, 1H, Arom), 7.65–7.75 (m, 3H, Arom), 7.82 (d, 1H, Arom, *J* = 7.9), 8.17 (d, 1H, Arom, *J* = 7.7)
2d	305–310 dec (ligroin)	1600, 1560	3.90 (s, 3H, CH_3), 7.00–7.10 (m, 2H, Arom), 7.25–7.35 (m, 1H, Arom), 7.35–7.45 (m, 2H, Arom), 7.61 (s, 1H, Arom), 7.65–7.70 (m, 1H, Arom), 7.82 (d, 1H, Arom, *J* = 8.1), 8.18 (d, 1H, Arom, *J* = 7.4)
3a	217–219[c] (methanol)	1570	4.04 (s, 3H, CH_3), 7.10–7.20 (m, 1H, Arom), 7.22 (s, 1H, Arom), 7.50–7.70 (m, 7H, Arom), 8.03 (d, 1H, Arom, *J* = 8.0)
3b	273–274[c] (methanol)	1565	4.05 (s, 3H, CH_3), 7.15–7.25 (m, 2H, Arom), 7.55–7.65 (m, 2H, Arom), 7.65–7,75 (m, 4H, Arom), 8.03 (d, 1H, Arom, *J* = 7.7)
3c	272–274[c] (methanol)	1570	4.03 (s, 3H, CH_3), 7.10–7.25 (m, 2H, Arom), 7.50–7.65 (m, 4H, Arom), 7.75–7.85 (m, 2H, Arom), 8.02 (d, 1H, Arom, *J* = 7.5)
3d	220–222[c] (methanol)	1565	3.85 (s, 3H, CH_3), 4.03 (s, 3H, CH_3), 7.05–7.25 (m, 4H, Arom), 7.50–7.60 (m, 4H, Arom), 8.02 (d, 1H, Arom, *J* = 7.4)
7b	129–132 (methanol)	3310, 1715, 1605	2.00–2.15 (m, 1H, CH_2), 2.70–2.95 (m, 2H, CH_2), 3.30–3.40 (m, 1H, CH_2), 3.60–3.75 (m, 1H, CH), 7.15–7.35 (m, 7H, Arom), 7.55–7.65 (m, 1H, Arom), 9.50 (s, 1H, NH), 12.20 (br s, 1H, OH)
7c	143–144 (methanol)	3310, 1710, 1600	2.00–2.15 (m, 1H, CH_2), 2.70–2.90 (m, 2H, CH_2), 3.30–3.40 (m, 1H, CH_2), 3.60–3.75 (m, 1H, CH), 7.10–7.20 (m, 2H, Arom), 7.20–7.40 (m, 5H, Arom), 7.55–7.65 (m, 1H, Arom), 9.51 (s, 1H, NH), 12.15 (br s, 1H, OH)
7d	115–117 (methanol)	3300, 1715, 1610	1.95–2.15 (m, 1H, CH_2), 2.70–2.95 (m, 2H, CH_2), 3.30–3.40 (m, 1H, CH_2), 3.60–3.75 (m, 1H, CH), 3.68 (s, 3H, CH_3, overlapped to the previous multiplet), 6.80–6.90 (m, 2H, Arom), 7.10–7.20 (m, 2H, Arom), 7.20–7.35 (m, 3H, Arom), 7.50–7.60 (m, 1H, Arom), 9.16 (s, 1H, NH), 12.24 (br s, 1H, OH)
8a	143–144 (methanol)	1670, 1660	2.50 (t, 1H, H-4A, *J* = 16.1), 2.80 (dd, 1H, H-5A, *J* = 16.3 and 5.5), 3.08 (dd, 1H, H-4B, *J* = 16.1 and 6.9), 3.20–3.40 (m, 1H, H-4a), 3.47 (dd, 1H, H-5B, *J* = 16.3 and 8.5), 7.20–7.50 (m, 6H, Arom), 7.55–7.62 (m, 2H, Arom), 7.78 (d, 1H, Arom, *J* = 7.4)
8b	132–133 (ethanol)	1670	2.52 (t, 1H, H-4A, *J* = 16.1), 2.80 (dd, 1H, H-5A, *J* = 16.4 and 5.6), 3.07 (dd, 1H, H-4B, *J* = 16.1 and 6.8), 3.20–3.40 (m, 1H, H-4a), 3.47 (dd, 1H, H-5B, *J* = 16.4 and 8.5), 7.30–7.50 (m, 5H, Arom), 7.50–7.60 (m, 2H, Arom), 7.77 (d, 1H, Arom, *J* = 7.5)
8c	152–154 (ethanol)	1675	2.51 (t, 1H, H-4A, *J* = 16.0), 2.79 (dd, 1H, H-5A, *J* = 16.4 and 5.5), 3.07 (dd, 1H, H-4B, *J* = 16.0 and 6.8), 3.20–3.35 (m, 1H, H-4a), 3.47 (dd, 1H, H-5B, *J* = 16.4 and 8.5), 7.30–7.60 (m, 7H, Arom), 7.77 (d, 1H, Arom, *J* = 7.4)
8d	136–138 (ethanol)	1675, 1600	2.51 (t, 1H, H-4A, *J* = 16.0), 2.79 (dd, 1H, H-5A, *J* = 16.3 and 5.6), 3.06 (dd, 1H, H-4B, *J* = 16.0 and 6.9), 3.20–3.35 (m, 1H, H-4a), 3.46 (dd, 1H, H-5B, *J* = 16.3 and 8.5), 3.81 (s, 3H, CH_3), 6.90–7.00 (m, 2H, Arom), 7.25–7.50 (m, 5H, Arom), 7.77 (d, 1H, Arom, *J* = 7.6)
9a	165–167 (methanol)	1655, 1605	3.96 (s, 2H, CH_2), 7.05–7.10 (m, 1H, H-4), 7.35–7.55 (m, 6H, Arom), 7.60–7.70 (m, 2H, Arom), 7.85–7.95 (m, 1H, Arom)
9b	220–222 (methanol)	1670, 1620	4.05 (s, 2H, CH_2), 7.10–7.15 (m, 1H, H-4), 7.40–7.70 (m, 7H, Arom), 7.79 (d, 1H, Arom, *J* = 7.4)
9c	215–217 (methanol)	1670, 1620	4.06 (s, 2H, CH_2), 7.15–7.20 (m, 1H, H-4), 7.40–7.55 (m, 2H, Arom), 7.55–7.65 (m, 3H, Arom), 7.65–7.75 (m, 2H, Arom), 7.73 (d, 1H, Arom, *J* = 7.3)
9d	180–182 dec (methanol)	1650, 1600	3.81 (s, 3H, O-CH_3), 4.04 (s, 2H, CH_2), 7.00–7.10 (m, 2H, Arom), 7.10–7.15 (m, 1H, H-4), 7.40–7.55 (m, 4H, Arom), 7.55–7.65 (m, 1H, Arom), 7.75–7.85 (m, 1H, Arom)

[a] Physical and spectroscopic data of compound **7a** have been reported in our previous paper.[27]
[b] ^1H NMR spectra were recorded in $CDCl_3$ (**2**, **8**) or in DMSO-d_6 (**3**, **7**, **9**), the abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet(s); br, broad signal.
[c] Melting point refers to the corresponding thermally rearranged compound **4**.

but lack the NH indolic function, displayed an affinity for BZR six- to 10-fold lower than the corresponding ligands **1a**–**d** but comparable to that of **2a**–**d**. The latter, unlike compounds **8** and **9**, have topological features similar to the enol tautomers of **1a**–**d** and similarly to compounds **8** and **9** do not have a hydrogen bond donor group. Thus the observed drop of the activity can be substantially attributed to the removal of a conceivable HB donation from the NH group to a proton-accepting group in the receptor. Nonetheless, **2d**, which lacks a hydrogen bond donor group at 5-position conserves a moderate affinity (IC_{50} = 90.7 nM) for the BZR. Finally, the higher BZR affinity of **8a**–**d** with respect to **9a**–**d** may indicate that the topographic planarity or quasi-planarity of these molecules is not a crucial element for their BZR affinity, unlike that observed from Nakao et al. for a series of higher homologues.[23]

A preliminary analysis of the structure–intrinsic efficacy relationships was undertaken on the basis of the GABA ratio values reported in Table 3. The GABA ratio can be taken as a good estimate of agonist, antagonist and inverse agonist activity for a BZR ligand.[24,25] As reported in a previous study,[19] the experimental method used

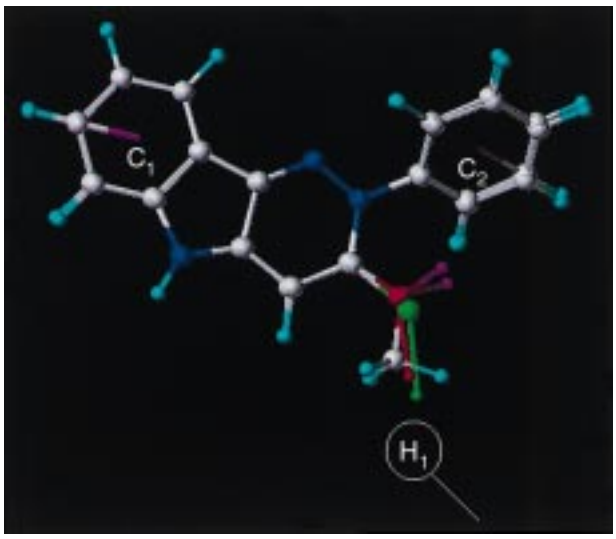


Figure 1. Fitted elements are C_1 and C_2 (aromatic centroids) and N_1. The oxygen and chlorine lone pairs, potentially involved in a HB at the H_1 site, are represented in red and in green for compounds **1a** and **2a**, respectively. The oxygen lone pairs of the methoxy group of compound **3a** (violet) are not correctly aligned to make HB at the H_1 site.

Table 3. Inhibition of [^{3}H]flunitrazepam binding and GABA-ratio[a]

Compd	R	IC_{50} nM (±SD)	GABA-ratio[b]
1a	H	238 ± 18	0.94
1b	Cl	31.1 ± 1	1.12
1c	Br	39.4 ± 2	1.13
1d	OCH_3	23.9 ± 1	1.02
2a	H	2490 ± 184	N.D.[d]
2b	Cl	253 ± 36	1.17
2c	Br	282 ± 58	1.01
2d	OCH_3	90.7 ± 8.7	0.88
3a	H	[37][c]	N.D.
3b	Cl	915 ± 48	N.D.
3c	Br	1180 ± 53	N.D.
3d	OCH_3	3520 ± 800	N.D.
4a	H	1560 ± 513	N.D.
4b	Cl	2485 ± 449	N.D.
4c	Br	[37][c]	N.D.
4d	OCH_3	994 ± 44	N.D.
8a	H	2670 ± 440	N.D.
8b	Cl	171 ± 5	1.47
8c	Br	180 ± 5	1.20
8d	OCH_3	180 ± 6	1.43
9a	H	2180 ± 170	N.D.
9b	Cl	407 ± 27	0.98
9c	Br	293 ± 31	1.04
9d	OCH_3	398 ± 16	1.02

[a] The binding affinities and GABA-ratios of the new ligands **2**, **3**, **8**, and **9** are reported together with those of already described cogeners **1** and **4** for comparison.
[b] GABA-ratio: IC_{50} (compound)/IC_{50} (compound + 20 μM-GABA).
[c] Percentage of inhibition of specific [^{3}H]flunitrazepam binding at 20 μM compound concentration.
[d] N.D.: not determined.

by us to measure the BZR binding led to IC_{50} values generally four to ten times higher than those reported in the literature for the same reference compounds. Moreover, in the case of agonists, our method led also to lower GABA-ratio values. Thus the GABA ratio values of the tetrahydroindenopyridazines **8b–d** (1.20–1.47) suggested a full agonist activity. Also for the *para* chloro derivative **2b** the GABA ratio indicated a similar agonist profile as observed for the corresponding parent compound **1b** and the pyrazoloquinoline analogue (PQ) **CGS 9896**.[26] The GABA-ratio values of compounds **2c** and 9**b–d** suggested a probable antagonist activity, unlike the corresponding congeners from the PQ and the PI series, for which different intrinsic activities have been previously reported.[19–21] Unexpectedly **2d** displayed an inverse agonist activity (GABA ratio 0.88) unlike the corresponding congeners of the PQ and PI series for which an antagonistic activity has been found.[19,26]

Conclusions

The PIs[20] have been recently shown to bind with high affinities to BZR. The relatively low affinity of the newly synthesized compounds **2**, **3**, **8**, and **9**, underlines the importance of the indolic NH function, as a hydrogen bond donor group, for a high binding potency at the central BZR of PI ligands **1**. Analogues **3**, which lack the HB with H1 site and might be subjected to some steric hindrance at the S1 region (see Cook model, Ref[18]), are essentially inactive. Furthermore it was confirmed that compounds with electron rich substituents in the *para* position of the N-2 phenyl ring were, in all the series, more active than the corresponding unsubstituted congeners. In vivo studies have been planned on compounds **2** and **3**, which upon metabolic transformation, could furnish the corresponding more active compounds **1**, as we have previously observed for the N-5 methylated congener **4b**, whose anticonvulsant activity was comparable to that of diazepam.[20]

Experimental

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 analyzer 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. ^{1}H NMR spectra were recorded on a Bruker AM 300 WB 300 MHz spectrometer. Chemical shifts are expressed in δ (ppm) and the coupling constants *J* in Hz. Exchange with deuterium oxide was used to identify OH and NH protons. UV–Vis spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. Chromatographic separations were carried out on silica gel columns (70–230 mesh, Merck). Phenylhydrazine free bases, not available commercially, were prepared from the corresponding phenylhydrazine hydrochlorides.

2-Aryl-3-chloro-2*H*-pyridazino[4,3-*b*]indoles (2a–d). A mixture of 2-aryl-2,5-dihydropyridazino[4,3-*b*]indol-3(3*H*)-one **1** (1 mmol) and phosphorus oxychloride

(5.9 mL) was heated with stirring at 90–100°C for 3 h. After cooling, petroleum ether (40 mL) was added dropwise to the stirred reaction mixture. The oily residue was separated from the ethereal solution by decantation and then basified with diluted NH_4OH. The resulting suspension was stirred for 10 min and then the orange precipitate filtered, washed with water and dried. Recrystallization from ligroin furnished **2a** (0.234 g, 84% yield), **2b** (0.223 g, 71% yield), **2c** (0.262 g, 73% yield), and **2d** (0.251 g, 81% yield).

2-Aryl-3-methoxy-2*H*-pyridazino[4,3-*b*]indoles (3a–d). A solution of sodium methoxide (0.015 g, 0.28 mmol) in dry methanol (0.8 mL) was added to a stirred suspension of **2** (0.21 mmol) in dry methanol (3 mL). The reaction mixture was refluxed under nitrogen for 2.5 h. After cooling, the solid was filtered and then recrystallized from acetonitrile/water to give **3a** (0.040 g, 68% yield), **3b** (0.056 g, 86% yield), **3c** (0.037 g, 50% yield), **3d** (0.049 g, 70% yield).

Thermal isomerization of 3a–d to 4a–d. Compound **3** was heated in a sealed glass tube at 200°C for 10 min and then cooled at room temperature. The solid was recrystallized from methanol to give **4** in quantitative yield.

2-Carbethoxymethylen-indan-1-one arylhydrazones (7b–d). A solution of 1-keto-2-indanylacetic acid **5**[22] (0.380 g, 2 mmol) and appropriate phenylhydrazine **6** (2.4 mmol) in anhydrous methanol (4 mL) was stirred at room temperature for 3 h. After removal of the solvent under reduced pressure, the crude residue was recrystallized from methanol to afford **7b** (0.284 g, 45% yield), **7c** (0.466 g, 65% yield), **7d** (0.186 g, 30% yield). Physical and spectroscopic data of compound **7a** have been reported in our previous paper.[27]

2-Aryl-2,4,4a,5-tetrahydroindeno[1,2-*c*]pyridazin-3(3*H*)-ones (8a–d). Dicyclohexylcarbodiimide (0.227 g, 1.1 mmol) was added to a stirred solution of the appropriate 2-carbethoxymethylen-indan-1-one arylhydrazone **6** (1 mmol) in dry acetonitrile (14 mL) at room temperature. Stirring was continued for 30 min. The precipitate of dicyclohexylurea was then filtered off and the filtrate evaporated in vacuo. The residue, recrystallized from methanol (**8a**, 0.200 g, 76% yield) or from ethanol (**8b**,**c**,**d**, 0.164 g, 55% yield, 0.202 g, 59% yield and 0.184 g, 63% yield, respectively), furnished a pure compound.

2-Aryl-2,5-dihydroindeno[1,2-*c*]pyridazin-3(3*H*)-ones (9a–d). A solution of 0.062 mL (0.192 g, 1.2 mmol) of bromine in acetic acid (2.5 mL) was added dropwise to a stirred solution of **8** (1 mmol) in acetic acid (8.3 mL) at 95–100°C and the heating was continued for 15 min. In the case of **9a**–**c**, the precipitate separated after cooling was filtered, washed with methanol and suspended in aqueous saturated sodium carbonate solution (10 mL). The suspension was stirred for 20 min and the solid was then filtered, washed with water, dried and recrystallized from methanol to give **9a** (0.156 g, 60% yield), **9b** (0.147 g, 50% yield) and **9c** (0.224 g, 66% yield), respectively. In the case of **9d** the dried solid was purified by chromatography on silica gel (ethyl acetate/petroleum ether, 8/2 as eluent) and then recrystallized from methanol to give **9d** (0.087 g, 30% yield).

Binding studies. The binding affinities data, reported in Table 3 were obtained by the method previously described.[19]

Molecular modelling studies. Molecular models of the heterocyclic compounds **1a**, **2a**, and **3a** were built on a Silicon Graphics Indigo 2 workstation from the fragments library of SYBYL 6.4 molecular modelling software (Tripos, St. Louis, MO, USA) and fully optimized by the AM1 Hamiltonian.[28] The minimum energy conformers selected upon a conformational search on the torsion angle of the N-2 phenyl ring, were aligned with the RIGIDFIT module of SYBYL by superimposing the three supposed anchoring points that are the N-1 atom and the centroids C1 and C2 of the benzofused moiety and of the 2-phenyl ring, respectively. The superimposed molecules are shown in Figure 1.

References

1. Kerr, I. B.; Ong, J. *Med. Res. Rev.* **1992**, *12*, 593.
2. *GABA and Benzodiazepine Receptors*; Squires R., Ed.; CRC: Boca Raton, 1988; Vols. 1–2.
3. Bernard, E. A. In *$GABA_A$ Receptor and Anxiety. From Neurobiology to Treatment*; Biggio, G., Sanna, E., Costa, E., Eds.; Raven: New York, 1995; pp 1–16.
4. Grigorenko, E. V. *J. Neurosci. Res.* **1995**, *41*, 567.
5. Smith, G. B.; Olsen, W. R. *Trends in Pharmac. Sci.* **1995**, *16*, 162.
6. De Lorey, T. M.; Olsen, R. W. *J. Biol. Chem.* **1992**, *262*, 6747.
7. Macdonald, R. L.; Olsen, R. W. *Annu. Rev. Neurosci.* **1994**, *17*, 569.
8. McKernan, R. M.; Whiting, P. J. *Trends Neurosci.* **1996**, *19*, 139.
9. Tulinsky, J.; Gammill, L. B. *Curr. Med. Chem.* **1994**, *3*, 226.
10. Gardner, C. R. *Progr. Neuro-Psychopharmacol. Biol. Psychiatry* **1992**, *16*, 755.
11. Borea, P. A.; Gilli, G.; Bertolasi, V.; Ferretti, V. *Mol. Pharmacol.* **1987**, *31*, 334.
12. Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Comput. Aid. Mol. Des.* **1987**, *1*, 1534.
13. Allen, M. S.; Tan, Y. C.; Trudell, M. L.; Schindler, L. R.; Martin, M. J.; Schulz, C.; Hagen, T. J.; Koehler, K. F.; Codding, P. W.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1990**, *33*, 2343.
14. Villar, H. O.; Davies, M. F.; Loew, G. H.; Maguire, P. A. *Life Sci.* **1991**, *48*, 593.
15. Diaz-Arauzo, H.; Koehler, K. F.; Hagen, T. J.; Cook, J. M. *Life Sci.* **1991**, *49*, 207.
16. Allen, M. S.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1992**, *35*, 368.
17. Schove, L. T.; Perez, J. J.; Loew, G. H. *Bioorg. Med. Chem.* **1994**, *2*, 1029.
18. Zhang, F.; Koehler, K. F.; Zhang, P.; Cook, J. M. *Drug Des. Dis.* **1995**, *12*, 193.
19. Campagna, F.; Carotti, A.; Casini, G.; Palluotto, F.; Genchi, G.; De Sarro, G. B. *Bioorg. Med. Chem.* **1993**, *1*, 437.
20. Palluotto, F.; Carotti, A.; Casini, G.; Campagna, F.; Genchi, G.; Rizzo, M.; De Sarro, G. B. *Bioorg. Med. Chem.* **1996**, *4*, 2091.
21. Savini, L.; Massarelli, P.; Nencini, C.; Pellerano, C.; Biggio,

G.; Maciocco, E.; Tuligi, G. A.; Carrieri, A.; Cinone, N.; Carotti, A. *Bioorg. Med. Chem.* **1998**, *6*, 389.
22. House, H. O.; Babad, H.; Toothill, R. B.; Noltes, A. W. *J. Org. Chem.* **1962**, *27*, 4141.
23. Nakao, T.; Kawakami, M.; Morita, K.; Morimoto, Y.; Takehara, S.; Tahara, T. *Yakugaku Zasshi* **1990**, *110*, 561.
24. Ehlert, F. J.; Roeske, W. R.; Braestrup, C.; Yamamura, S. H.; Yamamura, H. I. *Eur. J. Pharmacol.* **1981**, *70*, 593.
25. Braestrup, C.; Schmiechen, R.; Neff, G.; Neilsen, M.; Petersen, E. N. *Science* **1982**, *216*, 1241.
26. Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.
27. Palluotto, F.; Delle Monache, G.; Casini, G.; Campagna, F. *Heterocycles* **1997**, *45*, 1337.
28. Dewar, M. J. S.; Zoebish, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.