Available online at www.sciencedirect.com
SCIENCE DIRECT®
IL FARMACO 59 (2004) 849–856
IL FARMACO
http://france.elsevier.com/direct/FARMAC/

Original article

Synthesis, central and peripheral benzodiazepine receptor affinity of pyrazole and pyrazole-containing polycyclic derivatives

Francesco Campagna [a,*], Fausta Palluotto [a], Angelo Carotti [a], Elisabetta Maciocco [b]

[a] *Dipartimento Farmacochimico, Università di Bari, via Orabona 4, 70126 Bari, Italy*
[b] *Dipartimento di Biologia Sperimentale, Sezione di Neuropsicofarmacologia, Istituto Nazionale di Neuroscienze, Università degli Studi di Cagliari, Italy*

Received 15 March 2004; accepted 14 May 2004

Available online 15 September 2004

Abstract

A series of new pyrazole-condensed 6,5,5 tricyclic compounds were synthesized and tested to evaluate their binding affinities at both central (CBR) and peripheral (PBR) benzodiazepine receptors. Some 1-aryl-5-phenylpyrazole derivatives were also prepared and tested for comparison with their corresponding rigid tricyclic analogs. Among the newly synthesized 1-aryl-1,4-dihydro-indeno[1,2-c]pyrazoles bearing both an ethoxycarbonyl group at position 3 and a carbonyl function at the position 4, compound **4b** emerged as a new potent (IC_{50} = 26.4 nM) and selective CBR ligand. The 4-oxo-1-aryl-1,4-dihydro-indeno[1,2-c]pyrazole diethylamide derivative **14a** was instead identified as a relatively potent (IC_{50} = 124 nM) but highly selective PBR ligand.

Keywords: 1-Aryl-1,4-dihydro-indeno[1,2-c]pyrazoles; Central benzodiazepine receptor; Peripheral benzodiazepine receptor; Binding assay; Structure–activity relationships

1. Introduction

Benzodiazepines bind two main classes of receptors, the central-type benzodiazepine receptor (CBR) and the peripheral-type benzodiazepine receptor (PBR).

CBR, which is present exclusively in the central nervous system (CNS), has been identified as part of $GABA_A$-receptor/Cl^- ionophore supramolecular complex [1,2], a pentameric protein, formed by different combinations of 21 distinct subunits (6α, 4β, 4γ, 1ϵ, 1δ, 3ρ 1ϖ, and 1π), 16 of which have been found in the mammalian CNS [3–5]. Allosteric modulation of $GABA_A$ by CBR ligands involves three distinct events: ligand binding to recognition site, transduction of the signal to the GABA effector site, modification in GABA-gated conductance.

Agonists (GABA-positive ligands), increasing the frequency of Cl^- channel opening, induce sedative/hypnotic, muscle relaxant, anticonvulsant and anxiolytic activities. Inverse agonists (GABA-negative ligands) decrease channel open frequency and display (pro)convulsant and anxiogenic activities. Antagonists do not exhibit, per se, any relevant biological effect but antagonize the action of agonists and inverse agonists [6].

Benzodiazepines, β-carbolines, imidazopyridines, pyrazoloquinolines, and imidazoquinoxalines represent major families of compounds with high affinity for CBR [7,8]. Among them, flunitrazepam **I** (Chart 1) and pyrazoloquinolines, such as CGS 9896 **II**, exhibit high affinity for CBR but not for PBR, while benzodiazepine **III** (diazepam) and imidazopyridine **IV** (alpidem) bind with high affinity to both CBR and PBR.

Several pharmacophore models have been proposed for CBR, and among them, the one developed by Cook et al. [7] for agonists, antagonists and inverse agonists received much consensus. It consists of four essential features: a lipophilic region (L_1), two hydrogen-bond donor sites (HB_1 and HB_2) and a hydrogen-bond acceptor site (A_2). Lipophilic regions named L_2, L_3 and LDi have been identified as three additional, but not essential, binding regions. A schematic representation of this pharmacophore model is reported in Fig. 1.

PBR is ubiquitously expressed throughout the body especially in steroid producing tissues, such as adrenal, testis, placenta and brain. It is localised mainly in the mitochondria

* Corresponding author. Fax: +39-080-544-2724.
E-mail address: Campagna@farmchim.uniba.it (F. Campagna).

0014-827X/$ - see front matter © 2004 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2004.05.008

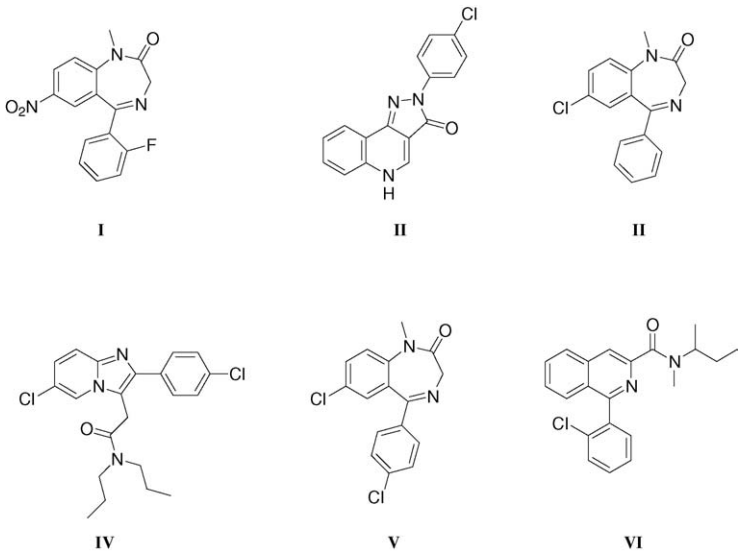


Chart 1.

and its role in several biological functions, such as the regulation of cell proliferation, mitochondrial oxidative phosphorylation, steroidogenesis, immune and inflammatory responses, has been recognized [9–16].

Many compounds have been discovered which bind to PBR with varying affinities. Among them, Ro 5-4864 **V** (Chart 1) and PK 11195 **VI** are noteworthy, due to their high PBR affinity and PBR/CBR selectivity. The pharmacophore models recently developed for PBR ligands converge in identifying four relevant features: three hydrophobic moieties (L_1, L_3 and L_4) and a hydrogen-bond donor group (HB_2) as shown in Fig. 2 [17–19].

In recent years, we have extensively investigated new central and peripheral benzodiazepine receptors ligands built on the pyridazino[4,3-b]indole or indeno[1,2-c]pyridazine nuclei (Fig. 3). The highest CBR affinity and selectivity was

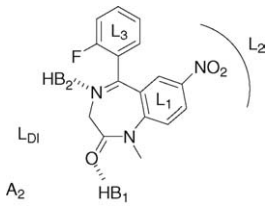


Fig. 1. Schematic representation of CBR pharmacophore model proposed by Cook [7]. The molecular structure of flunitrazepam is sketched to help interpretation.

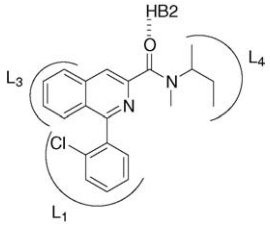
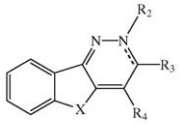


Fig. 2. Schematic representation of PBR pharmacophore model as proposed by different groups [16–18]. The molecular structure of PK11195 is sketched to help interpretation.



X= CH_2, CO, NH, NR
R_2 = H, Aryl, Alkyl
R_3 = Phenyl, Arylalkoxy, =O
R_4= H, $COOC_2H_5$, CON $(C_2H_5)_2$

Fig. 3. Pyridazino[4,3-b]indoles and indeno[1,2-c]pyridazines.

found for 2-aryl-2,5-dihydropyridazino[4,3-b]indol-3(3*H*)-ones, which possess structural features fitting the pharmacophore requirements. Interestingly, these compounds showed in vivo a full spectrum of intrinsic activities spanning from full agonism to inverse agonism [20–22].

2-Aryl-pyridazino[4,3-b]indole and 2-aryl-indeno[1,2-c]pyridazine derivatives bearing an ethoxycarbonyl or a carboxamido group at C-4 did not show any affinity for CBR, whereas in contrast they showed a selective, but weak, binding to PBR. Within that research program, relatively good PBR binding affinities had been found for compounds belonging to the 3-arylmethyloxy-pyridazinoindole series [23].

As a continuation of our ongoing research in this field, we synthesized new pyrazole-condensed 6,5,5 tricyclic compounds (**3a–e, 4a–e, 8b, 9d, 10d, 13a, 14a,b**, and **18a–c**) and measured their binding affinity at both CBR and PBR (Table 2). 1-Aryl-5-phenylpyrazole derivatives **21a–e**, were also prepared and tested for comparison with their corresponding rigid tricyclic analogs.

2. Chemistry

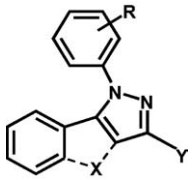
Scheme 1 outlines the synthetic pathway leading to ethyl 1-aryl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylates **3a–e** and **4a–e**. In short, ethyl (3-hydroxy-1*H*-inden-2-yl)(oxo)-acetate **2** was prepared by reacting the 1-indanone **1** with

Table 1
Physical and spectroscopic data of newly synthesized compounds (**3b–e, 4a–d, 7b, 8b, 9d, 10d, 12a–b, 13a, 14a–b, 16, 17a–c, 18a–c, 21c**)

Compound	m.p. (°C) (crystallization solvent)	IR ν_{max} (cm^{-1})	^{1}H-NMR, δ (ppm), J (Hz)
3b	215–216 (ethanol)	1730, 1500	1.71 (t, 3H, CH_3, J = 7.1); 4.10 (s, 2H, CH_2); 4.73 (q, 2H, CH_2, J = 7.1); 7.50–7.60 (m, 2H, Arom); 7.65–7.70 (m, 1H, Arom); 7.75–7.85 (m, 3H, Arom); 7.90–8.00 (m, 2H, Arom)
3c	141–142 (ethanol)	1725, 1595	1.45 (t, 3H, CH_3, J = 7.1); 3.84 (s, 2H, CH_2); 4.48 (q, 2H, CH_2, J = 7.1); 7.25–7.35 (m, 2H, Arom): 7.40–7.60 (m, 4H, Arom); 7.65–7.70 (m, 1H, Arom); 7.80–7.85 (m, 1H, Arom)
3d	131–133 (ethanol)	1735, 1535, 1520	1.45 (t, 3H, CH_3, J = 7.1); 3.85 (s, 2H, CH_2), 4.45 (q, 2H, CH_2, J = 7.1); 6.95–7.00 (m, 1H, Arom), 7.15–7.35 (m, 3H, Arom); 7.40–7.65 (m, 4H, Arom)
3e	201–202 (ethanol)	1725, 1500	1.45 (t, 3H, CH_3, J = 7.1); 3.83 (s, 2H, CH_2); 4.47 (q, 2H, CH_2, J = 7.1); 7.25–7.40 (m, 2H, Arom); 7.40–7.50 (m, 1H, Arom); 7.50–7.60 (m, 1H, Arom); 7.60–7.80 (m, 4H, Arom)
4a	207–209 (chloroform/hexane)	1740, 1720, 1610, 1520, 1480	1.46 (t, 3H, CH_3, J = 7.1); 4.48 (q, 2H, CH_2, J = 7.1); 7.10-7.40 (m, 4H, Arom); 7.50-7.75 (m, 5H, Arom)
4b	197–200 °C (ethanol)	1740, 1720, 1600	1.44 (t, 3H, CH_3, J = 7.2); 4.47 (q, 2H, CH_2, J = 7.2); 7.10–7.15 (m, 1H, Arom); 7.30–7.40 (m, 2H, Arom); 7.50–7.60 (m, 2H, Arom); 7.60–7.70 (m, 3H, Arom)
4c	175–180 °C (ethanol)	1735, 1715, 1600, 1590	1.47 (t, 3H, CH_3, J = 7.1); 4.50 (q, 2H, CH_2, J = 7.1); 7.15–7.20 (m, 1H, Arom); 7.35–7.40 (m, 2H, Arom); 7.50–7.55 (m, 2H, Arom); 7.60–7.70 (m, 2H, Arom); 7.75–7.80 (m, 1H, Arom)
4d	162–164 °C (ethanol)	1730, 1715, 1525	1.45 (t, 3H, CH_3, J = 7.1); 4.50 (q, 2H, CH_2, J = 7.1); 6.65–6.75 (m, 1H, Arom); 7.25–7.35 (m, 3H, Arom); 7.45–7.70 (m, 4H, Arom)
7b	193–197 °C (ethanol)	3250, 1705, 1650	6.34 (s, 1H, NH); 6.70–6.80 (m, 2H, Arom); 7.20–7.30 (m, 2H, Arom); 7.60–7.70 (m, 2H, Arom); 7.70–7.90 (m, 2H, Arom); 7.91(s, 1H, CH); 9.90–10.20 (br, 1H, NH)
8b	220–221 °C (ethanol)	1715, 1610, 1520	7.10–7.20 (m, 1H, Arom); 7.30–7.35 (m, 2H, Arom); 7.50–7.65(m, 5H, Arom); 7.65 (s, 1H, CH)
9d	129–130 °C (diethyl ether)	1775, 1725, 1610	1.05 (t, 3H, CH_3, J = 7.1); 1.20–1.30 (m, 2H, CH_2); 1.41 (d, 3H, CH_3, J = 6.3); 5.10–5.30 (m, 1H, CH); 6.60–6.80 (m, 1H, Arom); 7.20–7.35 (m, 2H, Arom); 7.45–7.60 (m, 2H, Arom); 7.60–7.70 (m, 3H, Arom)
10d	124–125 °C (diethyl ether)	1735, 1720, 1615	1.24 (d, 6H, $2CH_3$, J = 6.8); 1.55–1.60 (m, 1H, CH); 4.21 (d, 2H, CH_2, J = 6.8); 7.20–7.35 (m, 3H, Arom); 7.45–7.60 (m, 2H, Arom); 7.60–7.70 (m, 3H, Arom)
12a	236–238 °C (dioxane)	3420, 1725, 1600	7.09–7.18 (m, 1H, Arom); 7.35–7.50 (m, 2H, Arom); 7.50–7.70 (m, 4H, Arom); 7.70–7.80 (m, 2H, Arom)
12b	299–300 °C dec (chloroform)	3500–2500 br, 1725, 1600	7.17 (d, 1H, Arom, J = 7.0); 7.40–7.50 (m, 2H, Arom); 7.57 (d, 2H, Arom, J = 6.70); 7.73 (d, 1H, Arom, J = 8.6); 7.83 (d, 2H, Arom, J = 8.6)
13a	124–126 °C (ethanol)	1730, 1630	1.20–1.40 (m, 6H, $2CH_3$); 3.60 (q, 2H, CH_2, J = 7.1); 3.85 (s, 2H, CH_2); 3.95 (q, 2H, CH_2, J = 7.1); 7.25–7.35 (m, 3H, Arom); 7.40–7.60 (m, 4H, Arom); 7.70–7.80 (m, 2H, Arom).
14a	113–115 °C (chloroform/hexane)	1715, 1645	1.22 (t, 3H, CH_3, J = 7.1); 1.25 (t, 3H, CH_3, J = 7.1); 3.55 (q, 2H, CH_2, J = 7.1), 3.61 (q, 2H, CH_2, J = 7.1); 7.20-7.40 (m, 4H, Arom); 7.50-7.80 (m, 5H, Arom)
14b	145–146 °C (ethyl acetate)	1715, 1630	1.20 (t, 3H, CH_3, J = 7.1); 1.27 (t, 3H, 3H, CH_3, J = 7.1); 3.52 (q, 2H, CH_2, J = 7.1); 3.59 (q, 2H, CH_2, J = 7.1); 7.15–7.20 (m, 1H, Arom); 7.30–7.35 (m, 2H, Arom); 7.52–7.60 (m, 2H, Arom); 7.60–7.70 (m, 3H, Arom)
16	132–133 °C (ethanol)	1690, 1640, 1620, 1600	1.50 (t, 3H, CH_3, J = 7.0); 4.56 (q, 2H, CH_2, J = 7.0); 7.20–7.35 (m, 1H, Arom); 7.48 (d, 1H, Arom, H, J = 8.4); 7.55–7.655 (m, 1H, Arom); 7.84 (d, 1H, Arom, J = 8.6); 11.83 (s, 1H, OH)
17a	129–131 °C (ethanol)	3210, 1725, 1700, 1615	0.76 (t, 3H, CH_3, J = 7.1); 4.00 (q, 2H, CH_2, J = 7.1); 5.31 (s, 1H, NH); 7.00–7.15 (m, 2H, Arom); 7.20–7.40 (m, 4H, Arom); 7.60–7.80 (m, 2H, Arom), 12.53 (s, 1H, OH)
17b	135–137 °C (ethanol)	3450, 1730, 1685	0.78 (t, 3H, CH_3, J = 7.1); 4.00 (q, 2H, CH_2, J = 7.1); 5.28 (s, 1H, NH); 7.10–7.50 (m, 6H, Arom); 7.60–7.80 (m, 2H, Arom), 12.53 (s, 1H, OH)
17c	164–166 °C (ethanol)	3600–3200, 1716, 1700, 1615	0.75 (t, 3H, CH_3, J = 7.1); 4.01 (q, 2H, CH_2, J = 7.1); 5.27 (s, 1H, NH); 7.10–7.70 (m, 8H, Arom); 12.53 (s, 1H, OH)
18a	193–194 °C (ethanol)	1730, 1595	1.50 (t, 3H, CH_3, J = 7.1); 4.54 (q, 2H, CH_2, J = 7.1); 7.30–8.0 (m, 9H, Arom)
18b	203–206 °C (ethanol)	1730, 1500	1.49 (t, 3H, CH_3, J = 7.1); 4.53 (q, 2H, CH_2, J = 7.1); 7.3–7.9 (m, 8H, Arom)
18c	153–154 °C (ethanol)	1730, 1595	1.50 (t, 3H, CH_3, J = 7.1); 4.56 (q, 2H, CH_2, J = 7.1); 7.25–7.55 (m, 4H, Arom); 7.65–7.75 (m, 1H, Arom); 7.80–7.90 (m, 2H, Arom); 7.95–8.00 (m, 1H, Arom)
21c	71–73 °C (ethanol)	1710, 1590	1.43 (t, 3H, CH_3, J = 7.1); 4.46 (q, 2H, CH_2, J = 7.1); 7.00–7.50 (m, 10H, Arom)

^{1}H-NMR spectra were recorded in $CDCl_3$ (**3b–e, 4a–d, 7b, 8b, 9d, 10d, 13a, 14a–b, 16, 17a–c, 18a–c, 21c**) or in DMSO-d6 (**12a,b**).

Table 2
Binding data of pyrazoles and pyrazole-containing polycyclic derivatives



Compound	X	Y	R	CBR [a]	PBR [b]
3a	CH2	COOEt	H	11±0.5%	45±4%
3b	CH_2	COOEt	4–Cl	0%	40±3%
3c	CH_2	COOEt	3–Cl	4±0.3%	31±3%
3d	CH_2	COOEt	2–Cl	33±2% [c]	26±1.3%
3e	CH_2	COOEt	4–Br	0%	6%±0.4
4a	CO	COOEt	H	137±10	750±60
4b	CO	COOEt	4–Cl	26.4±1.5	10±1%
4c	CO	COOEt	3–Cl	51.5±2.5	671±45
4d	CO	COOEt	2–Cl	670±50	12±1.1%
4e	CO	COOEt	4–Br	141±12	25±0.8%
8b	CO	H	4–Cl	9±0.5%	7±0.5%
9d	CO	COO–sBut	2–Cl	30±1.5% [c]	435±35%
10d	CO	COO–iBut	2–Cl	29±2 [c]	23±2%
13a	CH_2	$CONEt_2$	H	0%	552±37
14a	CO	$CONEt_2$	H	0%	124±10
14b	CO	$CONEt_2$	4–Cl	0%	460±25
18a	O	COOEt	H	38±2.5%	5±0.3%
18b	O	COOEt	4–Cl	0%	17±1.4%
18c	O	COOEt	3–Cl	22±1.2%	4±0.3%
21a	–	COOEt	H	581±50	2±0.1%
21b	–	COOEt	4–Cl	0%	3±0.2%
21c	–	COOEt	3–Cl	5±0.3%	8±0.5%
21e	–	COOEt	4–Br	0%	2±0.2%
Diazepam				11.5±1.1	
PK 11195					3.3±0.5

Each value is the mean ± SEM of three determinations.

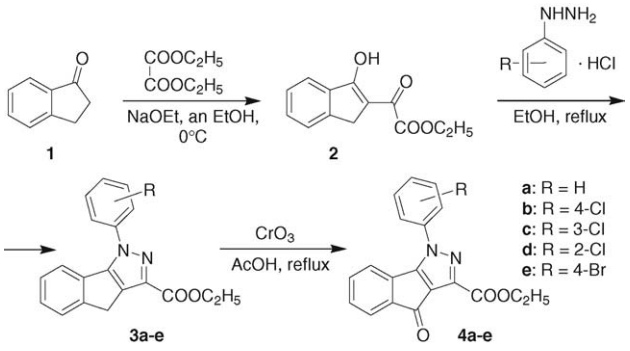
[a] Inhibition of [^{3}H] flunitrazepam binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.

[b] Inhibition of [^{3}H] PK 11195 binding expressed as IC_{50} (nM) or as percentage of inhibition at 1 μM concentration.

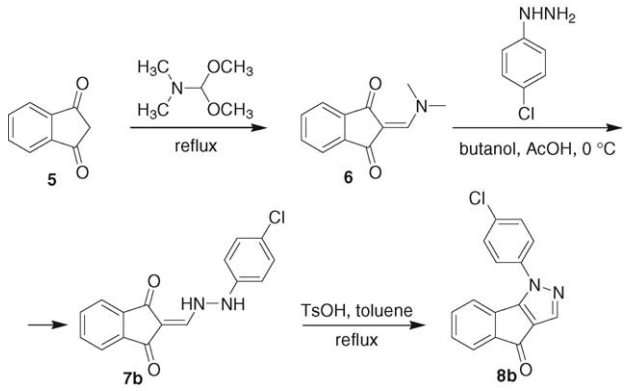
[c] Percentage of inhibition at 10 μM concentration.

diethyl oxalate in the presence of sodium ethoxide according to reported methods [24,25].

Compound **2** was subsequently condensed with the appropriate arylhydrazine hydrochloride to afford the ethyl 1-aryl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylates **3a–e**, which were oxidized by chromic anhydride in refluxing acetic acid to give the corresponding 4-oxo derivatives **4a–e**. The already known compounds **3a** [26] and **4e** [27] were obtained through the reported procedure [26] and through the new method described herein, respectively.



Scheme 1.



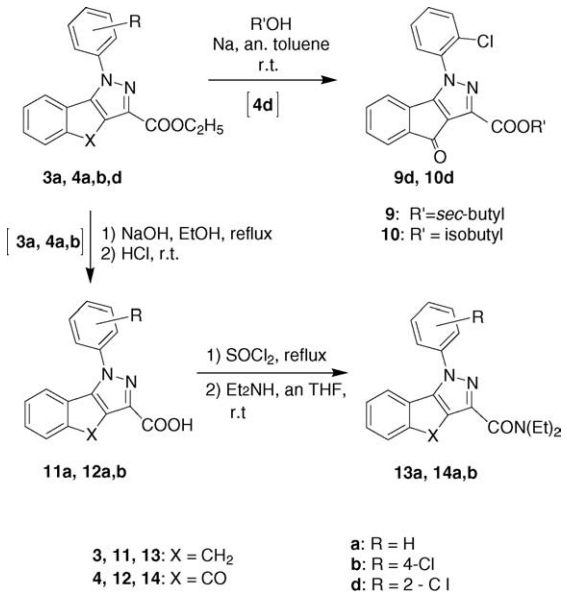
Scheme 2.

The synthesis of 1-(4-chlorophenyl)-indeno[1,2-c]-pyrazol-4(1*H*)-one **8b** was accomplished according to the method reported in Ref. [28] for the preparation of the 4-phenyl derivative (Scheme 2).

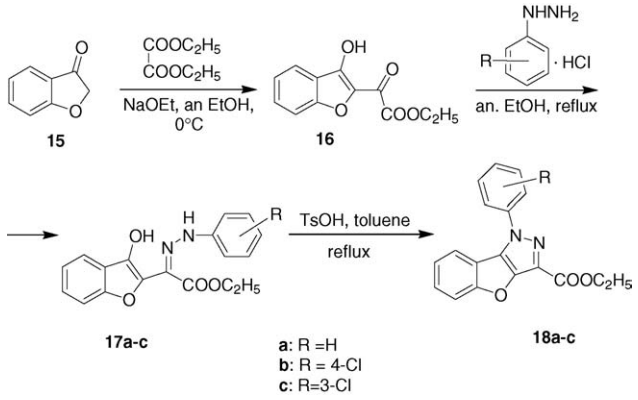
Sec and isobutyl esters **9d** and **10d** were prepared by transesterification of the ethyl ester **4d** with sodium sec- and isobutoxide, respectively, in anhydrous toluene (Scheme 3).

Diethylcarboxamides **13a** and **14a,b** were prepared through a preliminary hydrolysis of esters **3a** and **4a,b** to carboxylic acids **11a** [26] and **12a,b**, respectively, followed by a transformation to the corresponding acid chlorides and, finally, a reaction with diethylamine (Scheme 3).

Ethyl 1-aryl-1*H*-[1]benzofuro[3,2-c]pyrazol-3-carboxylates **18a–c** were prepared as outlined in Scheme 4. Ethyl (3-hydroxy-1-benzofuran-2-yl) (oxo) acetate **16**, obtained from 1-benzofuran-3-(2H)-one **15** and diethyl oxalate, was condensed with the appropriate arylhydrazine hydrochloride



Scheme 3.



Scheme 4.

to afford the arylhydrazones **17a–c**, which furnished the ethyl 1-aryl-1*H*-[1]benzofuro[3,2-c]pyrazole-3-carboxylates **18a–c**, by refluxing in toluene in the presence of *p*-toluenesulfonic acid.

Ethyl 1,5-diaryl-1*H*-pyrazole-3-carboxylates **21a–c,e** were synthesized (Scheme 5) by condensation of the corresponding arylhydrazine hydrochloride with the ethyl (3Z)-4-hydroxy-2-oxo-4-phenylbut-3-enoate **20**, prepared from acetophenone and diethyloxalate according to a reported method [29]. Compounds **21a,b,e** have been already prepared by others through a different route [30,31].

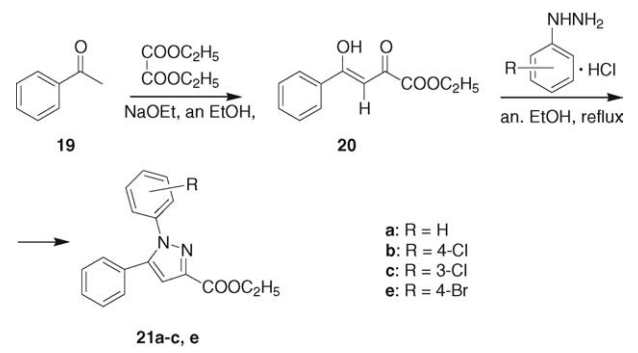
Physical and spectroscopic data of the newly synthesized compounds are reported in Table 1.

3. Binding studies

All the synthesized compounds were evaluated for their *in vitro* binding affinity at 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 [32,33]. Binding data, reported in Table 2, are expressed as IC_{50} (nM) or as percentage of inhibition of specific radioligand binding at 1 μM compound concentration.

4. Results and discussion

From the data shown in Table 2 it appears that only the indeno[1,2-c]pyrazole derivatives, bearing both the ethoxycarbonyl group at position 3 and the carbonyl function at the position 4, bind with a good affinity to the CBR. In fact, indanones **4a–e** are more active than the corresponding indeno **3a–e** and benzofuropyrazoles analogs **18a–c**, both lacking the carbonyl group at position 4.



Scheme 5.

Interestingly, also compound **8b**, in which the ethoxycarbonyl group at position 3 is missing, was also inactive. The role played by the above chemical groups in the receptor binding is noteworthy. As a matter of fact, when a methylene group (compounds **3a–e**) or an oxygen atom (compounds **18a–c**) are present at position 4 or when position 3 is unsubstituted (i.e., compound **8b**), the receptor affinity dramatically decreases, and this may indicate that two hydrogen bonds, likely involving the carbonyl oxygens at the 3 and 4 positions and the HB_1 and HB_2 donor sites on the receptor, may be formed.

Another important observation can be made by analyzing the different effect arising form the introduction of a chloro substituent in the N-1 phenyl ring. For ligands **4**, the introduction of a Cl substituent at the *para* and *meta* positions (but not at the *ortho* position) determined a significant improvement of the binding affinity whereas for the other two classes of ligands the affinity remained unchanged or even diminished (compare **3b** vs. **3a, 14b** vs. **14a** and **18b** vs. **18a**). Taking these results into account, we could hypothesize that the N-1 aryl ring probably interacts with L_1 lipophilic region of CBR.

Bulky substituents in position 3 do not favour CBR binding since the replacement of the ethoxycarbonyl substituent with 3-sec(*iso*)-butyloxycarbonyl-(compounds **9d, 10d**) or with 3-diethylcarboxamido-(compounds **14a,b**) groups resulted in a marked drop of the CBR affinity. Conversely, an increase of affinity was observed at the PBR. In particular, a carboxamide function similar to that present in PK11195, showed a preferential binding to PBR. A comparison of the binding data of amides **14a,b** with those of esters **4a,b** suggests that *N,N*-disubstituted amidic groups might engage a better interaction with the lipophilic region L_4 of PBR.

The presence of a carbonyl function at position 4 results less critical for binding to PBR than CBR. However, it must say that a significantly higher affinity was shown by the indanone derivatives **4a,c** and **14a** (IC_{50}, 124 nM) in comparison with the corresponding indeno derivatives **3a,c** and **13a** (IC_{50}, 552 nM).

Unlike the 6,5,5 rigid tricyclic analogs, pyrazole-1,5-diphenyl derivatives **21a–e** did not display any CBR and/or PBR affinity.

5. Conclusions

Among the newly synthesized 1-aryl-1,4-dihydro-indeno[1,2-c]pyrazoles, compound **4b** emerged as a new potent (IC_{50} = 26.4 nM) and selective CBR ligand whereas the 4-oxo-1-aryl-1,4-dihydro-indeno[1,2-c]pyrazole diethylamide derivative **14a** was identified as a less potent (IC_{50} = 124 nM) but highly selective PBR ligand.

The key importance of the ethoxycarbonyl substituent in position 3 and of a carbonyl group in position 4 for a high CBR affinity and of a *N,N*-diethylamidic group in position 3 and, to a lesser extent, of a 4-carbonyl group for an acceptably high PBR affinity was clearly pointed out.

As far as the pharmacophore for CBR ligands is concerned, our findings support the key role of a lipophilic interaction at the L1 region and of hydrogen bond formation at the HB_1, HB_2 donor sites. The good CBR activity of compound **4b** would deserve in vivo pharmacological studies, whereas the switching of the selectivity toward PBR triggered by the replacement of the COOEt with the $CON(Et)_2$ group (compare **4a** vs. **14a** and **4b** vs. **14b**) call for further structural modifications of the *N,N*-alkylamidic groups aimed at an optimization of the lipophilic interaction at the L4 region of PBR. We are presently working along these two research lines.

6. Experimental

6.1. Chemistry

Melting points were taken on a Gallenkamp MFB 595010 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. ^{1}H 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 widely spread on the base line and thus very difficult to detect. Reagents and solvents were purchased from Sigma (Aldrich).

*6.1.1. Ethyl 1-aryl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylates **3b–e***

Arylhydrazine hydrochloride (6 mmol) was added to a solution of ethyl (3-hydroxy-1*H*-inden-2-yl)(oxo)acetate 2 [23–25] (1.16 g, 5 mmol) in anhydrous ethanol (33 ml). The reaction mixture was refluxed for 1 h 30 min. After cooling, the precipitate was filtered, washed with ethanol and recrystallized from ethanol affording: **3b** 1.34 g, 77% yield; **3c** 1.28 g, 75% yield; **3d** 1.45 g, 85% yield; **3e** 1.10 g, 58% yield).

*6.1.2. Ethyl 1-aryl-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylates **4a–e***

A solution of CrO_3 in acetic acid (10%, 4 ml) was added drop wise to a solution of ethyl 1-aryl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate **3a–e** (0.5 mmol) in acetic acid (12.5 ml) and the mixture refluxed for 48 h. After cooling, the reaction mixture was poured on ice and extracted with chloroform. The extracts were dried over anhydrous sodium sulfate and evaporated to dryness giving a oil residue, which was purified by chromatography (**4a**: eluent mixture, ethyl acetate/petroleum ether 3:7, Rf = 0.32, 0.034 g, 21% yield; **4b**: eluent mixture, ethyl acetate/petroleum ether 3:7, Rf = 0.3, 0.035 g, 20% yield; **4c**: eluent mixture, ethyl acetate/petroleum ether 3:7, Rf = 0.37, 0.035 g, 20% yield; **4d**: eluent mixture, ethyl acetate/petroleum ether 4:6, Rf = 0.42; 0.035 g, 20% yield; **4e**: eluent mixture ethyl acetate/petroleum ether 3:7, Rf = 0.43, 0.040 g, 22% yield).

*6.1.3. 2-{[2-(4-Chlorophenyl)hydrazono]methylene}-1H-indene-1,3(2H)-dione **7b***

4-Chlorophenylhydrazine (0.596 g, 4.2 mmol) in 1-butanol (4 ml) was slowly added with stirring to a solution of 2-[(dimethylamino)methylene]-1*H*-indene-1,3(2*H*)-dione **6** [28] (0.804 g, 4 mmol) in 1-butanol (16 ml) and acetic acid (0.6 ml). The resulting solution was stirred at 0 °C for 1 h. The precipitate was filtered and recrystallized from ethanol to furnish compound **7b** (0.597 g, 50% yield).

*6.1.4. 1-(4-Chlorophenyl)-indeno[1,2-c]pyrazol-4(1H)-one **8b***

A suspension of 2-{[2-(4-chlorophenyl)hydrazono]-methylene}-1*H*-indene-1,3(2H)-dione **7b** (0.343 g, 1.15 mmol) and *p*-toluensulfonic acid (0.006 g) in anhydrous toluene (17 ml) was refluxed in a Dean–Stark apparatus for 3 h, cooled, washed with 1 N sodium hydroxide and water, dried over sodium sulfate and evaporated under reduced pressure. The residue was crystallized from ethanol (0.273 g, 85% yield).

*6.1.5. Sec-butyl 1-(2-chlorophenyl)-4-oxo-1,4-dihydro-indeno[1,2-c]pyrazole-3-carboxylate **9d** and isobutyl 1-(2-chlorophenyl)-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate **10d***

The solution of sodium butoxide in anhydrous toluene—prepared from anhydrous toluene (17 ml), sodium (0.008 g) and isobutyl alcohol (0.77 ml) or sec-butyl alcohol (0.77 ml)—was added drop wise to a suspension of ethyl 1-(2-chlorophenyl)-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate **4d** (0.088 g, 0.25 mmol) in anhydrous toluene (0.84 ml). The mixture was stirred at room temperature for 2–3 h, then was diluted with chloroform and washed with cold water. The organic phase was evaporated to dryness, dried over anhydrous sodium sulfate and the residue purified by chromatography, using ethyl acetate/petroleum ether 4:6 as the eluent mixture (**9d**: 0.029 g, 30% yield, Rf = 0.53; **10d**: 0.073 g, 77% yield, Rf = 0.53).

*6.1.6. 1-Aryl-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylic acids **12a,b***

A solution of NaOH (0.618 g, 15.5 mmol) in ethanol (77 ml) was added to a suspension of ethyl 1-aryl-4-oxo-

1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylate **4a,b** (1.55 mmol). The reaction mixture was refluxed for 2 h. The solvent was evaporated under reduced pressure and the residue suspended in water. After acidification with concentrated HCl, the precipitate was filtered, washed with water and recrystallized from ethanol (**12a**: 0.313 g, 70% yield; **12b**: 0.452 g, 90% yield).

6.1.7. N,N-*diethyl-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide* ***13a***

The mixture of 1-phenyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylic acid **11a** (0.150 g, 0.543 mmol) and thionyl chloride (7.1 ml, 97 mmol) was refluxed for 7 h. The unreacted thionyl chloride was evaporated and to the residue, suspended in anhydrous THF (10 ml), the diethylamine (0.021 ml, 0.4 mmol) was added. The mixture was stirred at room temperature for 2–3 h. After washing with diluted HCl, the organic phase was evaporated to dryness and the residue purified by crystallization from ethanol to give **13a** (0.130 g, 72% yield).

6.1.8. N,N-*diethyl-1-aryl-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide* ***14a,b***

The mixture of 1-aryl-4-oxo-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxylic acid **12a,b** (0.54 mmol) and thionyl chloride (7.1 ml, 97 mmol) was refluxed for 7 h. The unreacted thionyl chloride was evaporated and to residue suspended in anhydrous THF (10 ml) the diethylamine (0.032 ml, 0.6 mmol) was added. The mixture was stirred at room temperature for 2–3 h. After washing with dil. HCl, the organic phase was evaporated to dryness, and the residue purified by chromatography to give **14a** (eluent mixture, ethyl acetate/petroleum ether 6:4, Rf = 0.59, 0.047 g, 25% yield) or **14b** (eluent mixture, ethyl acetate/petroleum ether 9:1 Rf = 0.83, 0.051 g, 25% yield).

6.1.9. Ethyl (3-hydroxy-1-benzofuran-2-yl) (oxo) acetate ***16***

A solution of sodium ethoxide (4 mmol) in anhydrous ethanol (2.4 ml) was added drop wise to a mixture, cooled at –12 °C, of **15** (0.536 g, 4 mmol) and ethyl oxalate (0.54 ml, 4 mmol). The mixture was stirred at 0 °C for 75 min; then poured on ice/concentrated H_2SO_4 (10/0.2 ml). The orange solid was filtered, washed with water, dried and crystallized from ethanol (0.237 g, 25% yield).

6.1.10. Ethyl (2Z)-(3-hydroxy-1-benzofuran-2-yl)(arylhydrazono) acetate ***17a–c***

Arylhydrazine hydrochloride (2 mmol) was added to a solution of ethyl (3-hydroxy-1-benzofuran-2-yl) (oxo) acetate **16** (0.351 g, 1.5 mmol) in anhydrous ethanol (7 ml). The mixture was refluxed for 1 h 30 min. Upon cooling to 0 °C, the resulting precipitate was collected by filtration and crystallized from ethanol (**17a**: 0.097 g, 20% yield, **17b**: 0.081 g, 15% yield; **17c**: 0.169 g, 32% yield).

6.1.11. Ethyl 1-aryl-1H-[1]benzofuro[3,2-c]pyrazole-3-carboxylate ***18a–c***

To a solution of ethyl (2Z)-(3-hydroxy-1-benzofuran-2-yl)(arylhydrazono) acetate **17a–c** (0.80 mmol) in toluene (5 ml), *p*-toluenesulfonic acid (catalytic amount) was added. The solution was refluxed for 3 h. After cooling to 0 °C, the resulting precipitate was filtered, washed with toluene and crystallized from ethanol (**18a**, 0.134 g, 55% yield; **18b**, 0.136 g, 50% yield; **18c**, 0.040 g, 32% yield).

6.1.12. Ethyl 1,5-diaryl-1H-pyrazole-3-carboxylate ***21a–c,e***

Arylhydrazine hydrochloride (0.9 mmol) was added to a solution of ethyl (3Z)-4-hydroxy-2-oxo-4-phenylbut-3-enoate **20** [29]. in anhydrous ethanol (5 ml). The mixture was refluxed for 1 h. After cooling at 0 °C, the resulting precipitate was filtered and crystallized from ethanol (**21a**: 0.081 g, 40% yield; **21b**: 0.091 g, 40% yield, **21c**: 0.096 g, 42% yield, **21e**: 0.114 g, 50% yield).

References

[1] I.B. Kerr, J. Ong, GABA agonists and antagonists, Med. Res. Rev. 12 (1992) 593–636.

[2] R. Squires (Ed.), GABA and Benzodiazepine Receptors, 1–2, CRC Press, USA, Boca Raton, FL, 1988.

[3] T.M. De Lorey, R.W. Olsen, γ-Aminobutyric $acid_A$ receptor structure and function, J. Biol. Chem. 262 (1992) 6747–6750.

[4] G.B. Smith, W.R. Olsen, Functional domains of $GABA_A$ receptors, Trends Pharmacol. Sci. 16 (1995) 162–168.

[5] E.A. Bernard, $GABA_A$ receptor and anxiety, G. Biggio, E. Sanna, E. Costa (Eds.), From Neurobiology to Treatment, Raven Press, New York, 1995, pp. 1–16.

[6] J. Tulinsky, L.B. Gammill, The chemistry and pharmacology at GABA-A and GABA-B ligands, Curr. Med. Chem. 3 (1994) 226–2523.

[7] X. He, C. Zhang, J.M. Cook, Model of the BzR binding site: correlation of data from site-directed mutagenesis and the pharmacophore/receptor model, Med. Chem. Res. 10 (5) (2001) 269–308.

[8] A. Carotti, C. Altomare, L. Savini, L. Chiasserini, C. Pellerano, M.P. Mascia, E. Maciocco, F. Busonero, M. Mameli, G. Biggio, E. Sanna, High affinity central benzodiaepine receptor ligands. Part 3: insights into the pharmacophore and pattern recognition study of intrinsic activities of pyrazolo[4,3-c]quinolin-3-ones, Bioorg. Med. Chem. 11 (2003) 5259–5272.

[9] C. Braestrup, R.F. Squires, Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H] diazepam binding, Proc. Natl. Acad. Sci. SSA 74 (1977) 3805–3809.

[10] A.B. Basile, P. Skolnick, Subcellular localization of "peripheral type" binding sites for benzodiazepines in rat brain, J. Neurochem. 46 (1986) 305–308.

[11] G.B. O'Beirne, D.C. Williams, The subcellular location in rat kidney of the peripheral benzodiazepine acceptor, Eur. J. Biochem. 175 (1988) 413–421.

[12] A.G. Mukhin, V. Papadopoulos, E. Costa, K.E. Krueger, Mitochondrial benzodiazepine receptors regulate steroid biosynthesis, Proc. Natl. Acad. Sci. 86 (1989) 9813–9816.

[13] V. Papadopoulos, A.G. Mukhin, E. Costa, K.E. Krueger, The peripheral-type benzodiazepine receptor is functionally linked to leydig cell steroidogenesis, J. Biol. Chem. 265 (1990) 3772–3779.

[14] V. Papadopoulos, Structure and function of the peripheral-type benzodiazepine receptor in steroidogenic cells, PSEBM 217 (1998) 130–142.

[15] A. Beurdeley-Thomas, L. Miccoli, S. Oudard, B. Dutrillaux, M.F. Poupon, The peripheral benzodiazepine receptors: a review, J. Neuro-Oncol. 46 (2000) 45–56.

[16] P. Casellas, S. Galiegue, A.S. Basile, Peripheral benzodiazepine receptors and mitochondrial function, Neurochem. Int. 40 (2002) 475–486.

[17] G. Campiani, V. Nacci, I. Fiorini, M.P. De Filippis, A. Garofalo, S.M. Ciani, G. Greco, E. Novellino, D.C. Williams, D.M. Zisterer, M.J. Woods, C. Mihai, C. Manzoni, T. Mennini, Synthesis, biological activity, and SARs of pyrrolobenzoxazepine derivatives, a new class of specific "peripheral-type" benzodiazepine receptor ligands, J. Med. Chem. 39 (1996) 3435–3450.

[18] M. Anzini, A. Cappelli, S. Vomero, M. Seeber, M.C. Menziani, T. Langer, B. Hagen, C. Manzoni, J.J. Bourguignon, Mapping and fitting the peripheral benzodiazepine receptor binding site by carboxamide derivatives. Comparison of different approaches to quantitative ligand–receptor interaction modeling, J. Med. Chem. 44 (2001) 1134–1150.

[19] N. Cinone, H.D. Höltje, A. Carotti, Development of a unique 3D interaction model of endogenous and synthetic peripheral benzodiazepine receptor ligands, J. Computer-Aided Mol. Design 14 (2000) 753–768.

[20] F. Campagna, A. Carotti, G. Casini, F. Palluotto, G. Genchi, G.B. De Sarro, 2-aryl-2,5-dihydropyridazino[4,3-b]indol-3(3H)-ones: novel rigid planar benzodiazepine receptor ligands, Bioorg. Med. Chem. 1 (1993) 437–446.

[21] F. Palluotto, A. Carotti, G. Casini, F. Campagna, G. Genchi, M. Rizzo, G.B. De Sarro, Structure–activity relationships of 2-aryl-2,5-dihydropyridazino[4,3-b]indol-3(3H)-ones at the benzodiazepine receptor, Bioorg. Med. Chem. 4 (1996) 2091–2104.

[22] F. Campagna, F. Palluotto, A. Carotti, G. Casini, G. Genchi, Synthesis and structure–affinity relationships at the central benzodiazepine receptor of pyridazino[4,3-b]indoles and indeno [1,2-c]pyridazines, Bioorg. Med. Chem. 7 (1999) 1533–1538.

[23] F. Campagna, F. Palluotto, M.P. Mascia, E. Maciocco, C. Marra, A. Carotti, A. Carrieri, Synthesis an biological evaluation of pyridazino[4,3-b]indoles and indeno[1,2-c]pyridazines as new ligands of central and peripheral benzodiazepine receptors, Il Farmaco 58 (2003) 129–140.

[24] H. Leuchs, G. Kowlski, Uber den α-hydrindon-β-oxalester, Berichte 58B (1925) 2288–2293.

[25] J.M. Mussinu, S. Ruiu, A.C. Mulè, A. Pau, M.A.M. Carai, G. Loriga, G. Murineddu, G.A. Pinna, Tricyclic pyrazoles. Part 1: synthesis and biological evaluation of novel 1,4-dihydroindeno[1,2-c]pyrazol-based ligands for CB1 and CB2 cannabinoid receptors, Bioorg. Med. Chem. 11 (2003) 251–263.

[26] R.W. Hamilton, The antiarrhythmic and antiinflammatory activity of tricyclic pyrazoles, J. Heterocyclic Chem 13 (3) (1976) 545–553.

[27] A.S. Shawali, M.S.T. Mekki, M.A. Abdallah, I.M. Abbas, M. Ikhlass, A one step synthesis of 1,3-disubstituted indeno[1,2-c]pyrazol-4(H)-ones, Arch. Pharmacol. Res. 13 (4) (1990) 382–384.

[28] P. Schenone, L. Mosti, G. Menozzi, Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles.1.Synthesis of 1,5-disubstituted 4-acylpyrazoles, J. Heterocyclic Chem 19 (1982) 1355–1361.

[29] L. Brecker, M. Pogorevc, H. Griengl, W. Steiner, T. Kappe, D.W. Ribbons, Synthesis of 2,4-diketo acids and their aqueous solution structures, New J. Chem. 23 (4) (1999) 437–446.

[30] M.A.P. Martins, R.A. Freitag, A. da Rosa, A.F.C. Florex, N. Zanatta, H.G. Bonacorso, Haloacetylated enol ethers. 11[16]. Synthesis of 1-methyl-and 1-phenyl pyrazole-3(5)-ethyl esters. A one-pot procedure, J. Heterocyclic Chem. 36 (1) (1999) 217–220.

[31] S.A. Khattab, S.A. Shawai, A.M. Farag, Structure of the diazonium coupling product of γ-phenyl-Δβ,γ-butenolide, J. Chem. Eng. Data 22 (1) (1977) 104–110.

[32] L. Savini, P. Massarelli, C. Nencini, C. Pellerano, G. Biggio, E. Maciocco, G. Tuligi, A. Carrieri, N. Cinone, A. Carotti, High affinity central benzodiazepine receptor ligands: synthesis and structure–activity relationship studies of a new series of pyrazolo[4,3-c]quinoline-3-ones, Bioorg. Med. Chem. 6 (1998) 389–399.

[33] M. Serra, M. Littera, M.G. Pisu, M. Muggironi, R.H. Purdy, G. Biggio, Steroidogenesis in rat brain induced by short- and long-term administration of carbamazepine, Neuropharmacology 39 (2000) 2448–2456.