Eur. J. Med. Chem. 35 (2000) 323–331

S0223523400001355/FLA

Original article

The low affinity PCP sites in the rat cerebellum not only bind TCP-like but also BTCP-like structures

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Received 14 June 1999; revised 7 October 1999; accepted 18 October 1999

Abstract – Congeners of the potent dopamine (DA) re-uptake inhibitor 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]piperidine (BTCP) are unexpectedly able to bind in the rat cerebellum, although this structure is devoid of dopaminergic nerve endings. In line with previous studies the hypothesis that they bind to low affinity PCP sites labelled with [^{3}H]TCP in the rat cerebellum, even though they do not bind to the high affinity PCP sites in the forebrain, was considered. Analogues of 1-[1-(2-thiophenyl)cyclohexyl]piperidine (TCP) and BTCP with a modified aromatic moiety and with O or S atoms substituted in the cyclohexyl ring were prepared and tested in competition experiments both in rat forebrain and cerebellum membranes labelled with [^{3}H]TCP, and in rat striatum membranes labelled with [^{3}H]BTCP. Results indicated that BTCP and congeners could bind to low affinity PCP sites labelled with [^{3}H]TCP in the rat cerebellum with a decrease of the selectivity for the DA transporter. On the contrary, some TCP analogues displayed a very high selectivity for these low affinity sites; they might be important pharmacological tools to elucidate the nature and function at yet unknown of these sites. © 2000 Éditions scientifiques et médicales Elsevier SAS

TCP / TCP analogues / BTCP / BTCP analogues / PCP receptor subsites / DA transporter / rat cerebellum

1. Introduction

1-[1-(2-Benzo[b]thiophenyl)cyclohexyl]piperidine (BTCP) is a phencyclidine (1-(1-phenylcyclohexyl) piperidine, PCP) analogue highly selective for the dopamine (DA) neuronal transporter (DAT) when compared to PCP, since it binds very poorly to the PCP receptor within the NMDA-gated Ca^{++} channel [1, 2]. Accordingly, it inhibits DA re-uptake [1, 3, 4], increases striatal levels of extracellular DA in rats [5] and evokes behaviour related to dopaminergic stimulation [2, 6–9]. It was thus tempting to hypothesize that positron-emitting radioligands derived from BTCP might enable the selective determination of the degree of DA fibre loss in Parkinson's disease. Unexpectedly the regional brain distribution of radioligands in rats revealed a strikingly similar accumulation of radioactivity in both the striatum and the cerebellum [10], although the latter is devoid of dopaminergic nerve endings. This binding pattern obviously precluded the use of such tracers for in vivo imaging and addressed the question of binding sites for BTCP and congeners in the cerebellum. We have recently shown that TCP analogues with O or S atoms substituted in the cyclohexyl ring and/or with a methyl-substituted heteroaromatic ring might yield selective ligands for low affinity sites labelled with [^{3}H]TCP in the rat cerebellum [11]. Taken together these results opened the hypothesis that BTCP and congeners could bind to these low affinity sites in the rat cerebellum even though they do not bind to the high affinity PCP sites in the forebrain [1, 2]. Since the nature and the role of the low affinity binding sites remains poorly documented we have decided to investigate this hypothesis by: (i) preparing TCP analogues with enlarged aromatic moieties to partly mimic bicyclic systems like in BTCP and with O or S atoms substituted in the cyclohexyl ring; (ii) preparing BTCP analogues with O or S atoms substituted in the cyclohexyl ring; (iii) using these new molecules, and some of those

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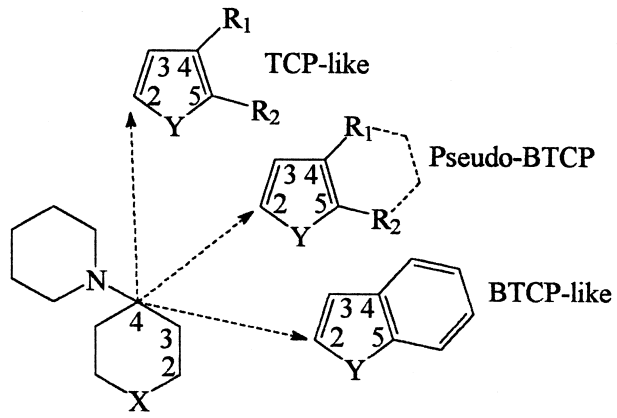
previously obtained, for competition experiments in rat forebrain or cerebellum membranes labelled with [^{3}H]TCP, and in rat striatal membranes labelled with [^{3}H]BTCP. For clarity, since[^{3}H]TCP labels three sites in the rat CNS [11], the different binding sites discussed here will be marked PCP_1 (high affinity binding sites in the forebrain, i.e. the original PCP receptor within the NMDA receptor associated ionic channel), PCP_2 (high affinity binding sites in the cerebellum, similar to PCP_1 but of lower affinity [11]), and finally PCP_3 ([^{3}H]TCP low affinity binding sites in the cerebellum). Finally this work was designed to determine how structural modifications of the aromatic moiety and/or hetero-substitutions in the cyclohexyl ring affect the molecular targeting to the different binding sites labelled by [^{3}H]TCP in the rat forebrain or cerebellum, and to the binding sites labelled by [^{3}H]BTCP in the rat striatum.

2. Chemistry

The newly synthesized compounds and some of those previously obtained are presented in *figure 1*. They resulted from various modifications on the TCP and BTCP models. Oxygen and sulfur atoms were substituted at the 4 position of the cyclohexyl ring and the aromatic moiety was chosen from (i) unsubstituted thiophenyl or furanyl rings to yield TCP-like structures; (ii) substituted thiophenyl or furanyl rings to partly mimic the steric bulk existing in BTCP to yield pseudo-BTCP structures; (iii) benzothiophenyl or benzofuranyl rings to yield BTCP-like structures.

Each of these compounds was obtained by means of a Bruylants reaction. Briefly, it consists of the replacement of a cyano group by an aryl or hetero-aryl group during the reaction between an α-aminonitrile and a Grignard reagent [12]. The suitable α-aminonitrile resulted from a Strecker-like synthesis in an organic medium as previously described [11, 13] (*figure 2*). Compounds **2** and **3** were previously prepared [11] from a Grignard reagent easily obtained from 2-bromo-thiophene and Mg turnings. In the present work, compounds **1** and **4**–**13** were prepared from Grignard reagents best obtained by a Mg/Li exchange reaction between $MgBr_2$ and a suitable hetero-aromatic Li derivative [14] (*figure 2*). The synthesis of non-commercial hetero-aromatic starting materials was required. 2,3-Dimethyl-furan was obtained in a three-step preparation: (i) reduction with $LiAlH_4$ of ethyl 2-methyl-3-furoate; (ii) reduction/chlorination with thionyl chloride of 3-hydroxymethyl-2-methyl-furan in a pH controlled medium; (iii) reduction with $LiAlH_4$ in diglyme of the 3-chloromethyl-2-methyl-furan intermediate (not isolated) [15–17]. 3-Ethyl-thiophene and 2,3-dimethyl-thiophene were respectively obtained from the commercially available 3-acetyl-thiophene and 3-methyl-2-thiophene-carboxaldehyde by means of a Wolff-Kishner reduction [18]. 3-Vinylthiophene resulted from the reduction of 3-acetylthiophene followed by a dehydration step [19]. The metallation reaction was done with n-butyl-lithium in the presence of TMEDA. Under the conditions used, the metallation occurred mainly at the α position from the heteroatom (S or O). Consequently the hetero-aromatic ring was linked by this same α position



Compound	*Aromatic*	*X*	*Y*	*R_1*	*R_2*
TCP	TCP-like	[a]-	S	H	H
1	TCP-like	O	O	H	H
2	TCP-like	O	S	H	H
3	TCP like	S	S	H	H
4	TCP like	O	O	H	CH_3
5	TCP like	O	S	CH_3	H
6	pseudo-BTCP	O	S	CH_2CH_3	H
7	pseudo-BTCP	S	S	CH_2CH_3	H
8	pseudo-BTCP	S	S	H	CH_2CH_3
9	pseudo-BTCP	O	S	$CHCH_2$	H
10	pseudo-BTCP	O	O	CH_3	CH_3
11	pseudo-BTCP	O	S	CH_3	CH_3
12	pseudo-BTCP	S	S	CH_3	CH_3
13	BTCP-like	O	O	-	-
14	BTCP-like	O	S	-	-
15	BTCP-like	S	S	-	-
16	BTCP-like	S	O	-	-
BTCP	BTCP-like	[a]-	S	-	-

[a] X = CH_2.

Figure 1. Structure of compounds.

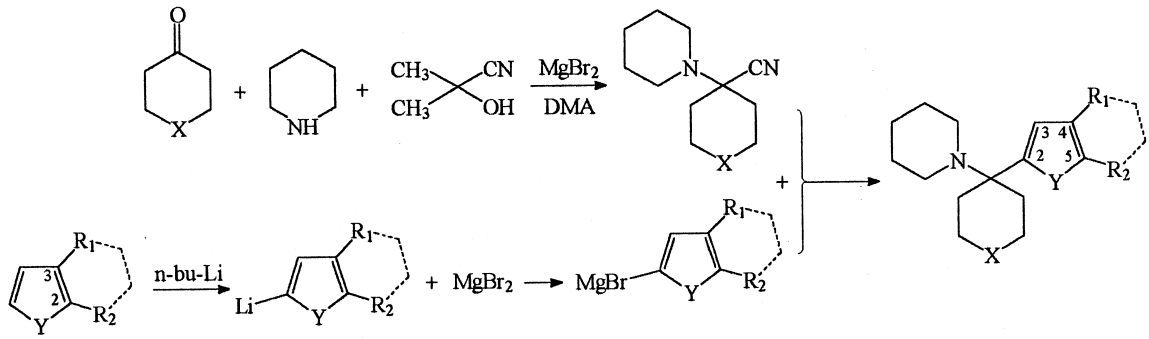


Figure 2. Synthetic scheme (X and Y are sulfur or oxygen atoms, see *figure 1*).

to the cyclohexyl ring in the final compound. However, in the case of 3-methyl-, 3-ethyl-, and 3-vinyl-thiophene the metallation was conducted without TMEDA. This modification allowed us to obtain a pure isomer which was a 2,4-disubstituted thiophenyl compound (**5**, **6**, **7** and **9**). This procedure was unsuccessful with 3-methyl-furan (obtained from methyl 3-methyl-2-furoate [20]) which gave, after completion of the reaction steps, and whatever the conditions used, a non-separable isomeric mixture of 2,3- and 2,4-disubstituted furanyl compounds (not shown). Structures of the new compounds were all checked by ^{13}C-NMR spectroscopy of the hydrochloride salts since in these series protonation induces a conformational trapping [11, 21, 22]. Purity was controlled by GC/MS and elemental analysis.

3. Pharmacology

The binding assays in rat forebrain and cerebellum membranes and the data analysis are described in the experimental protocols (section 6.2.). We have previously shown that a two-site model was more probable than a single-site model to describe the inhibitory effect of TCP and MK-801 in the rat cerebellum membranes labelled with [^{3}H]TCP [11]. The IC_{50}s obtained were consistent with those previously reported [23–25]. Thus, the same treatment was applied to BTCP and compounds belonging to the TCP- and BTCP-like or pseudo-BTCP series (*figure 1*). The results are summarized in *table I*. The affinities attributed to the population of high affinity sites, i.e. PCP_2-sites, were checked by additional competition experiments in rat cerebellum membranes labelled with [^{3}H]MK-801. Since MK-801 binds more than 1 000 times better to PCP_2- than to PCP_3-sites (see [11]: IC_{50} = 9.3 nM and 11 125 nM respectively,) the inhibition of [^{3}H]MK-801 and [^{3}H]TCP binding to PCP_2-sites should, respectively, appear in a similar range (*table II*). Finally, affinities for the DA transporter were measured by competition experiments in rat striatal membranes labelled with [^{3}H]BTCP (Experimental protocols, section 6.2.) and reported in *table III*.

4. Results and discussion

4.1. Inhibition of [^{3}H]TCP binding

In forebrain homogenates (PCP_1-sites), competition curves yielded Hill numbers close or equal to unity with the exception of **15** (*table I*) and therefore the single-site model was the most probable. On the contrary, in cerebellum homogenates, most Hill numbers were significantly lower than unity and the inhibition curves were best fitted to a two-site model (PCP_2- and PCP_3-sites) which produced a significant reduction in the sum of squares ($P < 0.05$, Student's *t*-test) and a Durbin-Watson coefficient between 1.5 and 2.5 (Experimental protocols, section 6.2.1.4.). Most compounds, including BTCP and BTCP-like compounds (**13**–**16**), were thus more or less able to interact with PCP_3-sites. However, the interaction of compounds **8**–**10** and **12** in the cerebellum (Hill numbers between 0.74 and 0.91) was better described by a single-site model, thus suggesting similar affinities for both sites. Interestingly compound **13**, a moderately effective ligand for PCP_3-sites (K_i = 372 nM), was very selective since it displayed a very low affinity (> 100 μM) for PCP_1- and PCP_2-sites. In this respect compound **6** was also found very selective for PCP_3-sites, mostly because it is a very high affinity ligand (K_i = 1.1 nM, see *table I*). It is noticeable that results presented in *table I* confirmed that [^{3}H]TCP labelled two sites in the cerebellum in about a 70/30 mean relative proportion (PCP_2/PCP_3 = 71.2 ± 5.6/27.8 ± 5.4).

Table I. Inhibition of [^{3}H]TCP binding in rat forebrain and cerebellum membranes. The mean of at least three independent determinations was analysed according to a single-site model in the forebrain and cerebellum (K_i[a], nM, Hill's number) or a two-site model in the cerebellum (SEM in brackets). When the two-site model was more probable, the proportion of PCP_2 and PCP_3 sites (%) and affinities (K_i[a], nM) are given.

Compound	Forebrain single-site model		Cerebellum single-site model		Cerebellum two-site model			
	K_i (PCP_1)	n_H	K_i[e]	n_H	K_i (PCP_2)	% (PCP_2)	K_i (PCP_3)	% (PCP_3)
TCP	7.7	1.00	188 (63)	0.56 (0.05)	56.5 (16)	72.6 (3.6)	3 700 (1 200)	29.8 (4.5)
1	5 100 (800)	0.85 (0.07)	10 300 (1 400)	0.63 (0.05)	51 000 (17 000)	70.6 (8.5)	19.5 (5.6)	29.3 (6.8)
2[c]	1 000 (120)	0.98 (0.05)	2 400 (480)	0.58 (0.12)	5 500 (1 600)	80.3 (6.5)	35.0 (18)	15.5 (5.7)
3[c]	59.3 (8.7)	1.05 (0.05)	178 (40)	0.68 (0.10)	560 (92)	75.2 (8.8)	8.5 (4.5)	25.2 (7.2)
4	15 000 (2 600)	0.89 (0.07)	10 100 (5 600)	0.63 (0.09)	14 400 (2 900)	77.0 (7.5)	266 (163)	23.0 (4.5)
5	1 700 (110)	0.95 (0.02)	3 600 (900)	0.48 (0.06)	11 600 (3 300)	65.3 (3.5)	183 (39)	33.5 (3.4)
6	880 (55)	0.88 (0.02)	1 800 (450)	0.58 (0.04)	2 500 (350)	76.8 (2.1)	1.1 (0.4)	19.4 (1.5)
7	49.6 (5.9)	1.01 (0.02)	270 (67)	0.45 (0.05)	62.2 (13.0)	56.4 (5.5)	12 000 (9 000)	43.1 (5.8)
8	315 (25)	0.98 (0.02)	265 (16.5)	0.89 (0.01)	–[b]	–	–[b]	–
9	1 700 (330)	0.85 (0.07)	4 700 (500)	0.79 (0.07)	–[b]	–	–[b]	–
10	12 000 (1 200)	1.01 (0.03)	15 000 (4 200)	0.74 (0.07)	–[b]	–	–[b]	–
11	13 600 (880)	1.03 (0.01)	4 400 (760)	0.63 (0.05)	6 400 (1 600)	80.5 (4.5)	15.0 (3.2)	22.4 (3.8)
12	1 200 (220)	0.93 (0.03)	1 500 (560)	0.91 (0.10)	–[b]	–	–[b]	–
13	> 100 μM	–	> 100 μM	–	> 100 μM	68.0 (3.9)	372 (167)	34.1 (5.0)
14[d]	41 000 (3 000)	0.83 (0.11)	10 500 (1 200)	0.45 (0.04)	40 000 (23 000)	63.2 (7.0)	301 (112)	35.1 (5.2)
15[d]	66 000 (6 000)	0.71 (0.05)	6 700 (990)	0.52 (0.07)	57 000 (22 000)	65.3 (3.8)	90 (47)	33.1 (6.3)
16[d]	22 700 (4 900)	0.90 (0.10)	6 600 (2 900)	0.64 (0.16)	25 000 (7 900)	65.0 (8.0)	59.0 (29.3)	33.4 (8.4)
BTCP	5 000	1.00	6 800 (2 800)	0.50 (0.07)	23 000 (2 800)	64.7 (9.5)	195 (85)	32.8 (7.1)

[a]Inhibition by unlabelled drugs of specific [^{3}H]TCP binding on specified sites; [b]a single-site model was more probable, suggesting similar affinities for both PCP_2- and PCP_3-sites; [c]from [26]; [d]from [11]; [e]IC_{50} values computed according to a single-site model.

4.2. Inhibition of [^{3}H]MK-801 binding

As expected, competition experiments in rat cerebellum membranes labelled with [^{3}H]MK-801 revealed that ligand interactions were better described by a single-site model (PCP_2-sites) (*table II*). Interestingly the K_i computed from competition experiments in cerebellum membranes labelled either by [^{3}H]MK-801 or [^{3}H]TCP (PCP_2-sites) were in the same range or even similar in several instances (*table II*). Taken together these results confirmed that: (i) MK-801 binds essentially one family of sites in the cerebellum; (ii) these sites are very likely similar to the 70% population, marked PCP_2-sites, bound by TCP itself.

4.3. Inhibition of [^{3}H]BTCP binding

Inhibition of [^{3}H]BTCP binding to rat striatal membranes (*table III*) revealed important structural features: (i) an oxygen atom introduced in the six-membered ring in place of a sulfur atom or a methylene group generally decreased affinities for the DAT (**1**, **2**, **4**–**6**, **9**–**11**, **13** and **14**) whatever the aromatic moiety was; (ii) as expected, BTCP-like compounds (**13**–**16**) interacted potently with the DAT, **15** being at least as efficient as BTCP; (iii) when comparing compounds differing only by substitutions on the thiophenyl or furanyl moiety the pseudo-BTCP com-

Table II. Inhibition by unlabelled drugs of specific [^{3}H]MK-801 (K_i nM, Hill's number) and [^{3}H]TCP (K_i nM from *table I*) binding to PCP_2 in rat cerebellum membranes (SEM in brackets).

Compound	PCP_2 ([^{3}H]MK801)		PCP_2 ([^{3}H]TCP)
	K_i	n_H	K_i
TCP	25.7 (4.8)	1.00 (0.10)	59 (17)
MK-801	5.6 (0.5)	0.97 (0.05)	9.3 (4.6)
1	7 300 (930)	0.96 (0.01)	53 000 (18 000)
2	2 900 (600)	1.00 (0.02)	5 800 (1 700)
3	–[b]	–[b]	586 (96)
4	9 600 (600)	0.98 (0.01)	15 000 (3 000)
5	2 400 (170)	0.98 (0.02)	12 100 (3 400)
6	1 170 (100)	0.98 (0.01)	2 600 (370)
7	59.1 (3.2)	0.99 (0.01)	65 (14)
8	300 (3.6)	1.01 (0.02)	–[b]
9	2 600 (260)	1.00 (0.01)	–[b]
10	28 000 (1 100)	0.98 (0.01)	–[b]
11	9 800 (600)	1.00 (0.01)	6 700 (1 700)
12	1 100 (130)	0.99 (0.01)	–[b]
13	> 100 μM	–	> 100 μM
14	40 000 (8 000)	0.97 (0.02)	42 000 (24 000)
15	> 100 μM	–	59 000 (23 000)
16	–[b]	–[b]	26 600 (8 200)
BTCP	10 400 (1 400)	1.04 (0.02)	24 000 (2 900)

[a]A single-site model was more probable for **8**, **9**, **10**, and **12** with respective Hill's numbers: 0.89 (0.01), 0.79 (0.07), 0.74 (0.07), and 0.91 (0.10) (*table I*); [b]not tested.

Table III. Inhibition of [^{3}H]BTCP binding to rat striatal membranes (IC_{50} nM, Hill's number) by the unlabelled drugs (SEM in brackets) and selectivity for DAT and PCP_3-sites with respect to other sites.

Compound	DA transporter		DA selectivity				PCP_3 selectivity	
	K_i	n_H	PCP_3[a]	PCP_2[b]	PCP_1[c]	DA[d]	PCP_2[e]	PCP_1[f]
TCP	1 250	0.82	0.34	22.1	162	2.96	65.5	480
1	58 000 (10 000)	0.93 (0.07)	3 000	1.13	11.3	0.0003	0.0004	0.004
2	7 200 (240)	0.89 (0.03)	206	1.31	7.2	0.006	0.006	0.035
3	1 400 (120)	1.00 (0.06)	165	2.5	23.6	0.006	0.015	0.14
4	9 100 (540)	0.89 (0.04)	34.2	0.63	0.61	0.029	0.018	0.018
5	940 (57)	0.91 (0.05)	5.14	0.08	0.55	0.19	0.016	0.11
6	1 800 (270)	0.93 (0.05)	1 636	0.72	2.04	0.0006	0.0004	0.001
7	403 (48.1)	1.06 (0.08)	0.03	6.50	8.12	29.8	192.9	241.9
8	295 (15)	1.01 (0.05)	–[g]	–[g]	0.93	–[g]	–[g]	–[g]
9	670 (75)	0.95 (0.04)	–[g]	–[g]	0.39	–[g]	–[g]	–[g]
10	1 500 (170)	1.07 (0.06)	–[g]	–[g]	0.13	–[g]	–[g]	–[g]
11	295 (5.0)	0.91 (0.01)	19.7	0.05	0.02	0.05	0.002	0.0009
12	31.2 (1.3)	0.94 (0.04)	–[g]	–[g]	0.026	–[g]	–[g]	–[g]
13	210 (15.4)	0.90 (0.02)	0.59	< 0.002	< 0.002	1.77	< 0.004	< 0.004
14	98.2 (18)	0.93 (0.02)	0.33	0.0025	0.0024	2.89	0.007	0.006
15	5.9 (0.6)	0.97 (0.05)	0.07	0.0001	0.0001	15.3	0.0016	0.001
16	13.7 (3.7)	1.0 (0.10)	0.23	0.0005	0.0006	4.3	0.002	0.003
BTCP	7.9 (0.2)	0.98 (0.01)	0.04	0.0003	0.002	24.7	0.008	0.04

[a]$K_i(DA)/K_i(PCP_3)$; [b]$K_i(DA)/K_i(PCP_2)$; [c]$K_i(DA)/K_i(PCP_1)$; [d]$K_i(PCP_3)/K_i(DA)$; [e]$K_i(PCP_3)/K_i(PCP_2)$; [f]$K_i(PCP_3)/K_i(PCP_1)$; [g]not determined, a single-site model was more probable (see *table I*).

pounds **10**–**12** were the most potent (**10** > **4** > **1**; **11** > **5** > **2**; **12** > **7** and **8** > **3**)

4.4. Binding selectivities

Finally, the binding selectivity of the molecules for: (i) the DAT with regard to the three PCP sites and (ii) the selectivity for the PCP_3-sites with regard to the DAT, PCP_1- and PCP_2-sites were examined (*table III*). Among the molecules tested, BTCP and **15** were the most selective ligands for the DAT. However, this selectivity was moderate because they bind only 26 and 15 times better to the DAT than to the PCP_3-sites, respectively. Interestingly, a progressive increase in the selectivity for the DAT is observed when passing from TCP-like to pseudo-BTCP molecules and finally to BTCP-like molecules. Suitable pharmacomodulations might thus yield, in the future, molecules devoid of affinity in the cerebellum. On the contrary, interesting selectivities for the PCP_3-sites were encountered in TCP-like compounds especially with molecules **1** and **6**. It is noticeable that both are built from a tetrahydropyranyl moiety which hypothetically might contribute, partly at least, to the observed selectivities.

5. Conclusion

Two lines of evidence can be drawn from the present work which confirms hypothesis and previous observations:

— BTCP and BTCP-like structures are able to bind in the rat cerebellum to sites marked here PCP_3-sites. Although the nature and function of these low affinity TCP binding sites in the cerebellum remain unknown they can probably play a role in the pharmacology of BTCP-like molecules in addition to their well known properties of DA re-uptake inhibition.

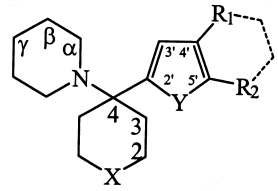
— importantly, a TCP-like and a pseudo-BTCP compound built from a tetrahydropyranyl six-membered ring displayed a high affinity and selectivity for low affinity PCP binding sites in the rat cerebellum. Such specific ligands might be valuable tools to elucidate the nature and function of these sites.

6. Experimental protocols

6.1. Chemistry

Melting points (uncorrected) were determined with a digital melting point apparatus (Electrothermal, IA9300).

Table IV. ^{13}C-NMR spectra of hydroclorides in $CDCl_3$ at 50.323 MHz (δ^a ppm from TMS).



Carbon	**1**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
2,6	64.3	64.3	64.2	64.1	25.3	25.2	64.1	64.3	64.2	25.2	64.3
3,5	30.8	30.6	33.3	33.2	33.5	33.4	33.2	30.6	33.1	33.3	30.8
4	65.6	65.6	67.1	67.0	69.3	69.4	67.0	65.7	67.1	69.2	66.0
α	47.3	46.9	46.7	46.6	47.1	47.0	46.8	46.9	46.5	46.9	46.7
β	22.7	22.5	22.7	23.4	22.9	23.3	22.6	22.6	22.6	22.8	22.6
γ	22.3	22.2	22.4	22.6	22.3	22.8	22.3	22.3	22.2	22.3	22.2
2′	146.3	143.7	134.4	134.2	134.5	131.7	135.3	142.4	*134.3*	*134.3*	148.1
3′	*114.1*	*115.1*	132.4	131.2	131.4	124.4	*128.1*	*117.2*	133.0	133.1	111.3
4′	*111.4*	*107.4*	138.9	145.7	145.8	130.4	141.3	*115.8*	*136.7*	*136.6*	126.9
5′	144.6	154.6	124.0	122.6	122.5	151.0	*124.7*	150.1	128.8	129.2	154.8
R_1	–	–	15.2	22.3 14.2	22.3 14.2	–	130.0 115.5	9.7	*13.6*	*13.5*	–
R_2	–	13.6	–	–	–	22.3 15.3	–	11.5	*13.1*	*13.0*	–

[a]Italicized chemical shifts may be exchanged.

Yields were not optimized. Elemental analysis was performed at the CNRS Microanalytical Section in Montpellier on the hydrochloride salts and were within ± 0.4% of theoretical values. ^{13}C-NMR spectra were obtained on a Bruker AC200 spectrometer at 50.32 MHz in 5 mm sample tubes in the FT mode. For some ^{13}C-signal assignments, a spin-echo sequence (Jmod) was used. Chemical shifts (δ) are reported in ppm downfield from TMS and collected in *table IV*. GC/MS analyses were performed on a Hewlett-Packard 5890 instrument equipped with a 9825B computer through a 25 m OV-1 capillary column. For NMR measurements and binding experiments, compounds were used as their hydrochloride salts precipitated by adding a dry HCl ethereal solution to a solution of base in ether. After filtration, the solid collected was dried under vacuum.

Synthesis of compounds **2** [26, 27], **3**, **14**, **15** and **16** [11, 26] have been previously described. A typical synthesis of new compounds is detailed in section 6.1.7., however their purification and properties are collected in *table V*.

6.1.1. Synthesis of α-aminonitriles

Acetone cyanohydrine (2.2 g, 25.8 mmol, 1 eq.) was added dropwise to a stirred mixture of tetrahydro-thiopyran-4-one (3 g, 25.8 mmol, 1 eq.), anhydrous $MgSO_4$ (9.3 g, 77.4 mmol, 3 eq.), dimethylacetamide (2.25 g, 25.8 mmol, 1 eq.), and piperidine (4.4 g, 51.6 mmol, 2 eq.). The pasty mixture was heated at 45 °C for 48 h, cooled to room temperature, poured onto ice and stirred for 30 min. The aqueous mixture obtained was then extracted with ether and the organic layer was washed with water until neutrality, dried over $MgSO_4$, filtered, and concentrated under vacuum to yield 5.3 g (98%) of 4-piperidin-1-yl-tetrahydro-thiopyran-4-carbonitrile as an orange oil pure enough for use in the next step. 4-Piperidin-1-yl-tetrahydro-pyran-4-carbonitrile was similarly obtained from tetrahydro-pyran-4-one (80.6%, F = 47 °C).

6.1.2. Synthesis of 3-hydroxymethyl-2-methyl-furan

Ethyl 2-methyl-furan-3-carboxylate (24.5 g, 159 mmol, 1 eq.) was added to an ethereal solution (1 M) of lithium aluminium hydride (4.8 g, 127 mmol, 0.8 eq.) and the mixture was refluxed for 2 h. The vessel was cooled in an ice bath and 5 mL of ice-cold water, then 5 mL of 15% NaOH, and 14.5 mL of water were successively added with caution. The white precipitate of LiOH was filtered and rinsed with ether. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to yield an oily compound distilled under reduced pressure to obtain 15.5 g (87%) of a colourless oil. GC/MS R_t =

Table V. Purification and properties of new compounds.

	Purification method	Solvent (v/v)	F_{base} (°C)	F_{HCl} (°C)	GC/MS (min, m/e)	Yield (%)
1	column chromatography (SiO_2)	petroleum ether/ether (60/40)	–	180.7–180.9	14.11, 235	42
4	column chromatography (SiO_2)	petroleum ether/ether (75/25)	76.6–77.0	230	14.49, 249	34.8
5	column chromatography (SiO_2)	petroleum ether/ether (90/10)	–	209.4–209.8	20.91, 285	22.7
6	column chromatography (SiO_2)	petroleum ether/ether (80/20)	oil	165.2–165.5	17.84, 279	34.6
7	column chromatography (SiO_2)	petroleum ether/ether (95/5)	oil	154.4–154.6	19.40, 295	56.1
8	crystallization	petroleum ether	69.5–69.7	164.8–165.1	21.63, 295	64.4
9	column chromatography (SiO_2)	petroleum ether/ether (70/30)	oil	177.8–178.0	19.02, 277	37.5
10	column chromatography (SiO_2)	petroleum ether/ether (70/30)	106.2–106.6	160	15.67, 263	63.9
11	column chromatography (SiO_2)	petroleum ether/ether (70/30)	97.7–98.0	170	19.33, 279	49.1
12	crystallization	ether	118.4–118.7	175	21.81, 295	67.8
13	column chromatography (Al_2O_3)	petroleum ether/ether (90/10)	111.2–111.5	209.6–210.2	19.01, 285	38.3

5.06 min, m/e = 112; ^{1}H-NMR 7.22 (d, 1H 5), 6.32 (d, 1H 4), 4.34 (s, 2H), 3.20 (s, 1H, OH), 2.22 (s, 3H); ^{13}C-NMR 148.9 (C2), 140.1 (C5), 118.6 (C3), 110.8 (C4), 55.9 (CH_2OH), 11.2 (CH_3).

6.1.3. Synthesis of 2,3-dimethyl-furan

Pyridine (11.3 mL, 140 mmol, 1 eq.), thionyl chloride (17.2 g, 140 mmol, 1 eq.), 3-hydroxymethyl-2-methyl-furan (15.5 g, 138 mmol, 1 eq.) in 26 mL of petroleum ether (30–40 °C), and 17 mL of anhydrous ether were successively added to a stirred mixture of 175 mL of petroleum ether (30–40 °C) and Na_2CO_3 (16.1 g, 152 mmol, 1.1 eq.) at –20 °C. The mixture obtained was stirred at 0 °C for 45 min, then at room temperature for 30 min and filtered. The filtrate, kept at 0 °C, was concentrated under vacuum to yield a brown oil. Lithium aluminium hydride (3.9 g, 105 mmol, 0.75 eq.) was added to a solution of the crude oil dissolved in diglyme (87 mL) at 0 °C. The mixture was stirred for 16 h at room temperature, then cooled at 0 °C, treated with 17.5 mL of water and distilled. The organic layer was washed with water, then with a copper (II) sulfate aqueous solution, and three times with water. The organic layer was then stirred for 10 min with coal, copper and potassium carbonate, filtered, and distilled to yield 8.2 g (61.9%) of 2,3-dimethylfurane as a colourless oil. ^{1}H-NMR 7.23 (d, 1H 5), 6.18 (d, 1H 4), 2.22 (s, 3H 2-Me), 1.97 (s, 3H 3-Me).

6.1.4. Synthesis of 3-ethyl-thiophene

3-Acetylthiophene (4.03 g, 32 mmol, 1 eq.) and hydrazine hydrate 88% (3.44 mL, 71 mmol, 2.5 eq.) was added to a solution of KOH (3.65 g, 65 mmol, 2 eq.) in ethylene glycol (24.3 mL) at 60 °C, heated for 1 h at 160–180 °C, and distilled at the same temperature. The distillate was decanted, the organic layer separated, and the aqueous layer extracted with ether. The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated under vacuum to yield 3.1 g (86%) of a pale yellow oil. ^{1}H-NMR 7.35 (s, 1H 5), 7.00 (s, 1H), 6.97 (s, 1H), 2.70 (q, 2H), 1.29 (t, 3H); ^{13}C-NMR 144.5 (C3), 127.8 (C4), 125.1 (C5), 119.1 (C2), 23.3 (CH_2), 14.6 (CH_3).

6.1.5. Synthesis of 3-vinyl-thiophene

A solution of 3-acetyl-thiophene (12 g, 95 mmol, 1 eq.) in 20 mL of ether was added dropwise to a solution of $LiAlH_4$ (5.2 g, 137 mmol, 1.45 eq.) in ether (40 mL). The mixture was stirred at room temperature for 4 h, then cooled to 0 °C, and 5 mL of water, 5 mL of NaOH 15%, and 15 mL of water were successively added with caution. The white precipitate of LiOH was filtered and washed with ether. The combined organic phases were dried over $MgSO_4$, concentrated in vacuum to yield 11.0 g (90.5%) of 1-thiophene-3-yl-ethanol as a yellow oil. A mixture of 1-thiophene-3-yl-ethanol (10 g, 78 mmol) and hydroquinone (5 mg) was distilled at 190 °C and the distillate decanted. The organic layer, dried over $MgSO_4$, was concentrated in vacuum to yield 2.8 g (33%) of a colourless oil. GC/MS R_t = 6.03 min, m/e = 128.

6.1.6. Synthesis of 2,3-dimethyl-thiophene

3-Methyl-thiophene-2-carboxaldehyde (25 g, 198 mmol, 1 eq.) and hydrazine hydrate 88% (21.7 mL, 500 mmol, 2.5 eq.) were added to a solution of KOH (28.05 g, 0.50 mol, 2.5 eq.) in 150 mL of ethylene glycol at 60 °C. The mixture was heated at 160 °C for 1 h, then distilled at 180 °C; the distillate was decanted, the aqueous phase was extracted with ether and the combined organic layers washed with water until neutrality, then dried over $MgSO_4$, concentrated under vacuum to yield a yellow oil. A distillation yielded 19.6 g (88%) of a colourless oil. ^{1}H-NMR 7.05 (d, 1H 5), 6.86 (d, 1H 4), 2.43 (s, 2-CH_3), 2.23 (d, 3-CH_3); ^{13}C-NMR 132.1 (C3), 131.7 (C2), 129.1 (C4), 119.7 (C5), 12.7 (3-CH_3), 12.1 (2-CH_3).

6.1.7. Typical synthesis of TCP and BTCP analogues: 1-[4-(2-furyl)-tetrahydro-pyran-4-yl]piperidine **1**

Firstly, a $MgBr_2$ solution was prepared from 1,2-dibromo-ethane (9.0 g, 48 mmol, 4 eq.) in ether (100 mL) and magnesium turnings (1.2 g, 48 mmol, 4 eq.) in a nitrogen atmosphere. Secondly, a solution of 2-furyl-lithium was prepared in a nitrogen atmosphere by the dropwise addition of a n-butyl-lithium solution 1.6 M in hexane (40 mL, 60 mmol, 5 eq.) to a mixture of furane (4.4 mL, 60 mmol, 5 eq.) and TMEDA (9.0 mL, 60 mmol, 5 eq.) in anhydrous ether (130 mL) at –20 °C (without TMEDA in the synthesis of **5**, **6**, **7** and **9**). The mixture was then stirred for 2 h at room temperature and added dropwise to the $MgBr_2$ solution in ether. The new mixture was stirred at room temperature for 0.5 h, then a solution of 4-piperidin-1-yl-tetrahydro-pyran-4-carbonitrile (2.3 g, 12 mmol, 1 eq.) in ether was added dropwise at room temperature. The mixture, refluxed for 16 h, cooled to room temperature, was treated as follows: the mixture was poured carefully onto an ice-cold saturated solution of NH_4Cl, stirred for 30 min, extracted with ether, the combined organic layers were extracted 3 times with 10% HCl and 20% NH_4OH was added to the acidic aqueous phase until neutrality. The aqueous phase was extracted with ether, the organic phase washed with water, dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product obtained was purified as described in *table V* to yield 1.18 g (42%) of **1** as a white solid.

6.2. Pharmacology

6.2.1. Binding assays

6.2.1.1. [^{3}H]TCP

[^{3}H]TCP (Amersham, 48 Ci/mmol) binding to the PCP_1-, PCP_2- and PCP_3-sites was measured as previously described [23]. Briefly, the rat (Wistar) brain (minus the cerebellum) or the cerebellum, stored at –80 °C, was homogenized with an Ultraturax (Ika Werke, maximum setting) in a 50 mM Tris/HCl, pH 7.7 buffer for 20 s at 4 °C. The homogenate was then centrifuged at 49 000 *g* for 20 min. The pellet was resuspended in the same buffer and the homogenization–centrifugation steps performed a second time. The final pellet was resuspended in 10 volumes of a 50 mM Tris/Hepes, pH 7.7 buffer and used without further purification.

The forebrain or cerebellum homogenate (0.5–0.8 mg protein/mL) was incubated with [^{3}H]TCP (1 nM or 2.5 nM, respectively) in a 5 mM Tris/Hepes, pH 7.7 buffer (0.5 mL or 1 mL, respectively) in the absence (total binding) or in the presence of the competing drug for 30 min at 25 °C. The incubation was terminated by filtration over GF/B (Whatman) glass fibre pre-soaked in 0.05% polyethyleneimine (Aldrich) with an MR24 Brandel cell harvester. The filters were rinsed three times with 5 mL of 50 mM NaCl, Tris HCl 10 mM, pH 7.7 buffer and the radioactivity retained was counted in 3.5 mL ACS (Amersham) with an Excel 1410 (LKB) liquid scintillation spectrophotometer. The non-specific binding was determined in parallel experiments in the presence of 100 μM unlabelled TCP.

6.2.1.2. [^{3}H]MK-801

[^{3}H]MK-801 (New England Nuclear, 20.3 Ci/mmol) binding to the PCP_1-, PCP_2- and PCP_3-sites was measured as described above for [^{3}H]TCP (see 6.2.1.1.) with incubation times fixed at 3 h instead of 30 min.

6.2.1.3. [^{3}H]BTCP

[^{3}H]BTCP binding to the DAT was measured according to a previously described method [4]. Rat striata stored at –80 °C were homogenized with an ultraturax in a 320 mM sucrose, 10 mM Tris/HCl (pH 7.4) buffer and centrifuged at 1 000 *g* for 10 min. The supernatant was then centrifuged at 49 000 *g* for 20 min. The resulting pellet (crude membrane preparation) was resuspended in the same buffer (1 mL per striatum). The homogenate (0.05–0.1 mg protein/mL) was incubated with [^{3}H]BTCP (0.2–0.5 nM) (C.E.A, 55 Ci/mmol) in the absence or in the presence of the competing drug in a 50 mM Na_2HPO_4 (pH 7.4) buffer in a volume of 2 mL for 90 min at 4 °C. The incubation was terminated by filtration over GF/B glass fibre filters (Whatman) pre-soaked in 0.5% polyethyleneimine (Aldrich) with an MR24 Brandel cell harvester (Beckman). The filters were rinsed three times with 5 mL of a 50 mM NaCl, Tris/HCl 10 mM (pH 7.7) buffer and the radioactivity retained counted in 3.5 mL ACS (Amersham) with an Excel 1410 (LKB) liquid scintillation spectrophotometer. The non-specific binding was determined in parallel incubations in the presence of 100 μM unlabelled BTCP.

6.2.1.4. Data analysis

In each experiment, values are the mean of three independent determinations. Each experiment was performed 3–5 times. The data from competition experiments were first analysed by the Hill's representation and then by a non-linear regression method (Marquardt-Levenberg algorithm) according to a single or two-site model using the Sigmaplot® 4 software (Jandel). The two-site interaction was represented by: $[LB] = (B_1 + B_2) - \{([I] \times B_1/(IC_{50_1} + [I])) + ([I] \times B_2/(IC_{50_2} + [I]))\}$, where [LB] was the % of radioligand specifically bound, [I] the competitor concentration, B_1 and B_2 the % of each binding site, IC_{50_1} and IC_{50_2} the concentrations of unla-

belled competitor that inhibited 50% of specific [^{3}H]TCP binding on specified sites. Two constraints were fixed: (i) IC_{50_1}, IC_{50_2}, B_1, $B_2 > 0$, (ii) $95\% < (B_1 + B_2) < 105\%$ because of the uncertainty on the total binding (10%). Experimental results were submitted to an ANOVA followed by a Durbin-Watson test. The two-site model was preferred when it produced a significant reduction in the sum of squares ($P < 0.05$, Student's *t*-test) and when the Durbin-Watson coefficient was closer to 2 than in the single-site model and was between 1.5 and 2.5. Finally K_i values were computed from the Cheng-Prusov equation: $K_i = IC_{50}/(1 + L/K_D)$.

Acknowledgements

The authors wish to thank D.R.E.T. for the financial support given to F. Espaze. Thanks are due to M. Michaud for her excellent technical assistance.

References

[1] Vignon J., Pinet V., Cerruti C., Kamenka J.M., Chicheportiche R., Eur. J. Pharmacol. 148 (1988) 427–436.

[2] Ilagouma A.T., Maurice T., Duterte-Boucher D., Coderc E., Vignon J., Costentin J., Kamenka J.M., Eur. J. Med. Chem. 28 (1993) 377–385.

[3] Cerruti C., Drian M.J., Kamenka J.M., Privat A., Brain Res. 55 (1991) 51–57.

[4] Maurice T., Vignon J., Kamenka J.M., Chicheportiche R., J. Neurochem. 56 (1991) 553–559.

[5] Martin-Fardon R., Arnaud M., Rousseau E., Kamenka J.M., Privat A., Vignon J., Neurosci. Lett. 211 (1996) 179–182.

[6] Koek W., Colpaert F.C., Woods J.H., Kamenka J.M., J. Pharmacol. Exp. Ther. 250 (1989) 1019–1027.

[7] Duterte-Boucher D., Kamenka J.M., Costentin J., Psychopharmacology 101 (1990) 344–353.

[8] French E.D., Lopez M., Peper S., Kamenka J.M., Roberts D.C.S., Behav. Pharmacol. 6 (1995) 223–228.

[9] Prinssen E.P.M., Kleven M.S., Vignon J., Kamenka J.M., Koek W., J. Pharmacol. Exp. Ther. 276 (1996) 904–911.

[10] Loustau-Then I., Ponchant M., Fuseau C., Kamenka J.M., Vignon J., Crouzel C., Nucl. Med. Biol. 24 (1997) 513–518.

[11] Hamon J., Espaze F., Vignon J., Kamenka J.M., Eur. J. Med. Chem. 34 (1999) 125–135.

[12] Bruylants P., Bull. Soc. Chim. Belg. 33 (1924) 467–478 and 35 (1926) 139–154.

[13] Geneste P., Kamenka J.M., Dessapt P., Bull. Soc. Chim. Fr. 3 & 4 II (1980) 186–191.

[14] Minato A., Tarrao K., Hayashi T., Susuki K., Kumada M., Tetrahedron Lett. 52 (1981) 5319–5322.

[15] Winberg H.E., Fawcett F.S., Mochel W.E., Theobald C.W., J. Am. Chem. Soc. 82 (1960) 1428–1435.

[16] Rice K.C., Dyer J.R., J. Heterocycl. Chem. 12 (1975) 1325–1326.

[17] Mann J., Holland H., Tetrahedron 43 (1987) 2533–2542.

[18] Mohamadi F., Spees M., Grindey J., J. Med. Chem. 35 (1992) 3012–3016.

[19] Yoshironi T., Milton L., Raymond N., J. Heterocycl. Chem. 18 (1981) 967–972.

[20] Rabjohn N. (Ed.), Organic Synthesis, Coll. Vol. IV, Wiley, London, 1963, p. 628.

[21] Geneste P., Kamenka J.M., Org. Magn. Res. 7 (1975) 579–584.

[22] Manoharan M., Eliel E.L., Kanan W., Tetrahedron Lett. 25 (1984) 3267–3268.

[23] Vignon J., Privat A., Chaudieu I., Thierry A., Kamenka J.M., Chicheportiche R., Brain Res. 378 (1986) 133–141.

[24] Ebert B., Wong E.H.F., Krogsgaard-Larsen P., Eur. J. Pharmacol. 208 (1991) 49–52.

[25] Berman F.W., Murray T.F., J. Biochem. Toxicol. 11 (1996) 217–226.

[26] Hamon J., Vignon J., Kamenka J.M., Eur. J. Med. Chem. 31 (1996) 489–495.

[27] Eiden F., Schmidt M., Buchborn H., Arch. Pharmacol. 320 (1987) 348–361.