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New compounds resulting from structural and biochemical similarities between GBR 1278 and BTCP, two potent inhibitors of dopamine uptake

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GBR 12783 / BTCP / dopamine uptake inhibitors / molecular modelling

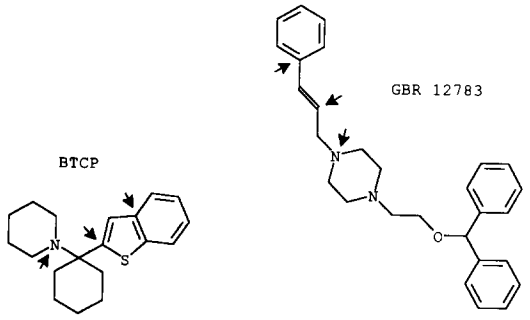
Introduction

N-[1-(2-Benzo[*b*]thiophenyl)cyclohexyl]piperidine (BTCP) and analogues bind to the dopamine (DA) uptake complex to inhibit the DA uptake process [1, 2] and as such act as indirect DA agonists. The biological properties of BTCP have often been compared to those of the well-known DA uptake inhibitor GBR 12783 (1-(2-diphenylmethoxy-ethyl)-4-(3-phenyl-2-propenyl)piperazine), one of the most active molecules among a numerous series of piperazinyl derivatives [3]. Both compounds compete for the same binding sites labelled with [^{3}H]-BTCP or [^{3}H]-GBR 12783, with a rather similar affinity [4–6].

A first sight they are very different with respect to their chemical structures (scheme 1); however, in an attempt to explain their biochemical similarities, we have tried to fit these molecules using ChemX software (Chemical Design) on a PC.

Fitting

GBR 12783 is able to adopt numerous conformations. In one of the lowest energy conformations we have obtained, the styryl part of the structure (fig 1) was apparently able to fit with the active conformation



Scheme 1. Structure of BTCP and GBR 12783. Arrows indicate the atoms used in the 3-point fitting.

of BTCP with the heteroaromatic ring axially positioned [2, 7]. Both molecules were independently minimized using the MM program in ChemX. Then, a rigid fit was made between BTCP (benzothiophenyl and piperidine moieties) and GBR 12783 (piperazinyl and phenylpropenyl moieties) using the fitting program of the software. A 3-point interaction (scheme 1) with identical weighting constants was applied: i) the 2 nitrogen atoms; ii) the cyclohexyl-substituted quaternary carbon in the 2-benzo[*b*]thiophenyl ring of BTCP and the unsaturated secondary β-carbon to the nitrogen atom in GBR 12783; iii) the quaternary bridged carbon from the sulphur atom in BTCP and the benzenic quaternary carbon atom in GBR 12783.

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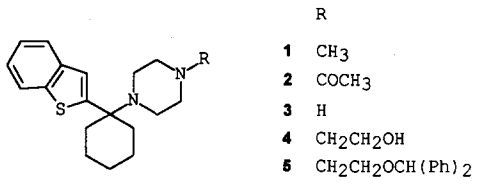
To test the apparently very good fit obtained, a flexible fitting was then applied to improve the closeness of fit by a MME optimization. After only 2 cycles, optimization ended, confirming the goodness of fit.



Scheme 2. BTCP-piperazinyl structures prepared.

In the model obtained, the phenyl-propenyl part of GBR 12783 resembled a desulfurized benzothiophenyl ring. Furthermore, the 4-methylene group in the piperidine ring of BTCP was in close correlation with the 4-piperazinyl nitrogen in the GBR 12783 molecule (distance < 0.25 Å) (fig 2).

Such a result prompted us to investigate a new series of BTCP-piperazinyl derivatives where the piperidine ring is replaced with a piperazinyl ring.

Synthesis

We decided to test the potential of the new series defined by molecular modelling. Thus, we prepared and tested *in vitro* 3 of the compounds presented in scheme 2, differing in the *N*-piperazinyl substitution: $-CH_3$, **1**, CH_2-CH_2OH, **4**, and finally the typical GBR 12783 substitution, **5**. Thus, we expected to define a general outline of BTCP-piperazinyl derivatives acceptable by the DA transporter.

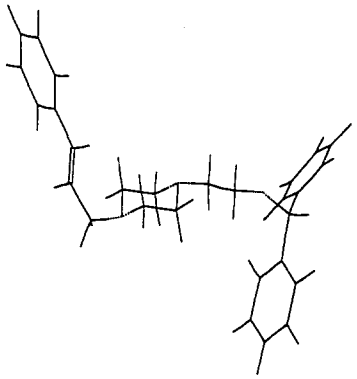


Fig 1. The GBR 12783 conformation used in the fitting.

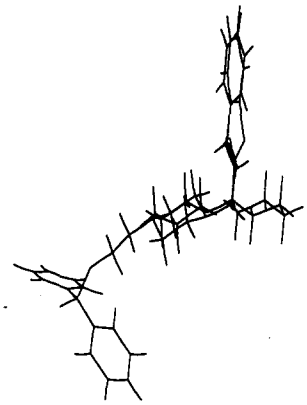
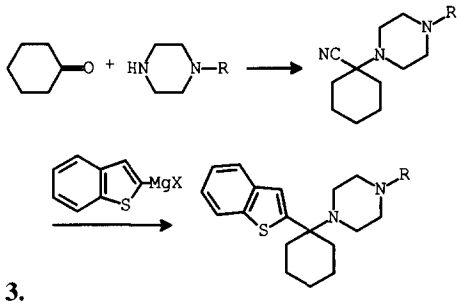


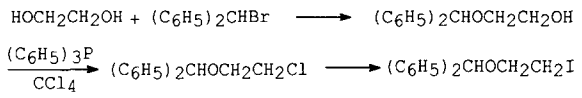
Fig 2. Superimposition of BTCP and GBR 12783 (the hard copy is from Alchemy^R software).

Compound **1** and the synthons for **4** and **5** were obtained by the Bruylants reaction [8, 9] on the corresponding α-aminonitrile according to scheme 3. α-Aminonitriles resulted from the previously described synthesis in an organic medium using dimethylacetamide (DMA) as solvent [10, 11]. It should be noticed that the 2-benzo[*b*]thiophenyl Grignard reagent was prepared, in this study, by either of 2 methods: i) from the 2-iodobenzo[*b*]thiophene [11]; ii) by reacting $MgBr_2$ with 2-lithio-benzo[*b*]thiophene [12]. The latter seemed better than the former when considering the cost of iodine and the low overall yield of the synthesis of 2-iodobenzo[*b*]thiophene.



Scheme 3.

Compound **3**, intermediate for the synthesis of **4** and **5**, was obtained more easily by the hydrolysis of the *N*-acetyl derivative **2**. Indeed, when using the unprotected piperazine in the α-aminonitrile synthesis, a great amount of the bis α-aminonitrile was produced. A reaction between **3** and 2-iodoethanol produced compound **4**. In the same way, compound **5** resulted from the reaction between **3** and 1-iodo-2-diphenylmethoxy-ethane obtained according to scheme 4.



Scheme 4.

After purification by chromatography, **1**, **4**, and **5** were subjected to a hydrochloration in ether to obtain water soluble molecules for the biochemical assays.

Results and discussion

Results of the binding experiments (table I) are interesting: compounds **1** (R = -CH_3) and **4**) (R = CH_2CH_2 OH) display high affinities and, on the contrary, compound **5** (R = 2-diphenylmethoxy-ethyl) displays a very low affinity for the transporter sites labelled with [^{3}H]-BTCP. Interestingly, **1** and **4** are equipotent and their affinities are in the same range as that of BTCP and GBR 12783. The very good results obtained with compounds **1** and **4** apparently validate the method used to generate new compounds from the structural similarities displayed by the 2 model structures. Surprisingly, **5**, expected to be the best compromise between BTCP and GBR 12783, was a very weak ligand.

To explain such a discrepancy different hypotheses could be drawn: i) a good fit was obtained using a non-significant conformational model for GBR 12783, too far from the active conformation; ii) DA uptake complex sites were unable to tolerate the simultaneous crowding caused by the cyclohexyl ring and the 2-diphenylmethoxy-ethyl substitution; iii) in the complex formed between the BTCP-piperazinyl ligand and the binding site, the 2-diphenylmethoxy-ethyl moiety does not occupy the space normally occupied by GBR 12783.

If the first hypothesis were true, none of the tested compounds would have a high affinity for the transporter sites. The second hypothesis might be true–nevertheless, it would be difficult to understand why 4 different molecules (BTCP, GBR 12783, **1**, **4**) would all have affinities in the same range for the DA transporter.

The last hypothesis is probably the most likely. Indeed, all the tested structures possess a symmetry axis through the 2 piperazinyl nitrogen atoms (4-piperidyl position in BTCP). A rotation of BTCP-piperazinyl molecules around this axis will shift the heteroaromatic ring upon the phenyl ring of the GBR 12783 molecule in the fitting model (fig 3). Such a rotation would fit the methyl **1** and the hydroxyl group **4** in close vicinity to their original position because they be on (or very near) the axis. On the contrary, the bulky diphenylmethyl group in **5** will move considerably from its original position. During the modelization, we have superimposed as best as possible a phenylpropenyl group and a benzothiophenyl group. Their surfaces are clearly different (fig 3); thus in the binding site, the 2-benzo[*b*]thiophenyl ring, which is bigger and bearing a bulky sulphur atom, may be shifted from the fitted model position for a better interaction with a flat lipophilic receptor zone. This will cause a rotation of the structure around the axis of the 2 nitrogen atoms and result in the diphenylmethyl group, causing steric interactions that do not exist with GBR 12783. If such a mechanism is true, it is possible to understand the differences in affinity observed for **1**, **4** and **5**. Furthermore, chemical substitutions at the phenyl rings of GBR 12783 decrease the affinity for the transporter most probably because of unfavoured steric interactions [3, 13]. Thus, the fitting of the diphenylmethyl moiety to the binding sites appears crucial for the GBR 12783 series as well as for the new BTCP-piperazinyl series.

From a structure obtained by molecular modelling we have prepared 2 of the best ligands yet obtained in the series. Work is in progress to enlarge the study of BTCP-piperazinyl derivatives.

Table I. Inhibition constants (nM) of the binding of [^{3}H]-BTCP ($K_{0.5}$) and Hill's number (n_H) on rat striatal membranes.

Compound	$K_{0.5}$	n_H	
BTCP	8.4 ± 0.2	0.98 ± 0.01	(n = 12)
GBR 12783	17.0 ± 2.5	0.97 ± 0.05	(n = 4)
1	11.5 ± 2.7	0.89 ± 0.01	(n = 4)
4	14.7 ± 2.1	0.96 ± 0.02	(n = 4)
5	1290 ± 540	1.26 ± 0.14	(n = 7)

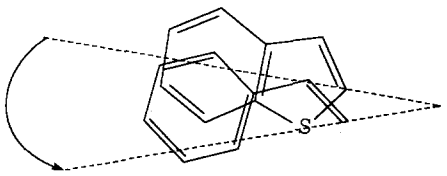


Fig 3. A rotation around the nitrogens axis may cause a shift of the aromatic moiety of BTCP upon the phenylpropenyl moiety of GBR 12783.

Experimental protocols

Binding experiments

[^{3}H]-BTCP (55 Ci/mmol) was obtained from the Service des Molécules Marquées (Saclay, France). The method used for the [^{3}H]-BTCP binding to the DA uptake complex was as described by Vignon *et al* [1]. Briefly, rat striata were dissected on ice and homogenized with an Ultra-turrax in 320 mM sucrose, Tris 10 mM pH 7.4 buffer and centrifuged at 1000 *g* for 10 min. The supernatant was then centrifuged at 49 000 *g* for 20 min. The resulting pellet (synaptosomal homogenate) 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) in the absence or the presence of the competing drug in a 50 mM Na_2HPO_4 pH 7.4 buffer in a vol of 0.5 ml for 90 min at 4°C. The incubation was terminated by filtration over GF/B (Whatman) glass fibre filters pre-soaked in 0.5% PEI (Aldrich) with a MR24 Brandel cell harvester. The filters were rinsed twice with 5 ml of 50 mM NaCl, Tris/HCl 10 mM, pH 7.7 and the radioactivity retained counted in 3.5 ml ACS (Amersham) with an Excel 1410 (LKB, France) liquid scintillation spectrophotometer. The non-specific binding was determined in the presence of 10 µM unlabeled BTCP.

Chemistry

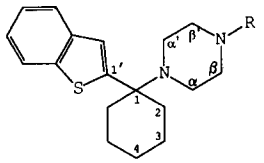
Melting points were determined with a Büchi–Tottoli apparatus and are uncorrected. Elemental analyses were performed at the CNRS Microanalytical Section in Montpellier and were within ±0.3% of theoretical values. GC/MS was performed on a Hewlett–Packard 5890 instrument equipped with a 9825B computer through a 25-m OV-1 capillary column. ^{13}C-NMR spectra (table II) were obtained on a Brucker WP 80 DS spectrometer at 20.147 MHz in 10-mm sample tubes in the FT mode. Chemical shifts are reported in (δ) ppm downfield from TMS. All compounds were isolated as their hydrosoluble HCl salts for use in the *in vitro* experiments. They were precipitated by bubbling a dry stream of HCl in an ethereal solution of the base. After filtration, solids collected were dried *in vacuo*.

Synthesis of 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]-4-methyl-piperazine ***1***

1-(1-cyano-cyclohexyl)-4-methyl-piperazine. To a mixture of cyclohexanone (16.7 g, 0.17 mol), anhydrous magnesium sulphate (60.2 g, 0.5 mol), dimethylacetamide (10 g, 0.11 mol), and 4-methyl-piperazine (20 g, 0.18 mol), acetone cyanohydrin (25.5 g, 0.3 mol) was added with stirring. The pasty mixture obtained was heated at 45°C for 48 h. After cooling to room temperature the mixture was poured onto ice and vigorously stirred for 30 min. The aqueous mixture was extracted with ether and the organic phase washed to neutrality with water. Evaporation under reduced pressure of the ethereal solution, dried on Na_2SO_4, yielded 25 g (60%) of an oily yellow residue pure enough for use in the next step. GC/MS: Rt = 8.4 min, *m/e* 207.

Grignard reagent and Bruylants reaction. During the first step, a $MgBr_2$ solution was prepared by a slow addition of 1,2-dibromoethane (14.1 g, 0.075 mol) in a minimum of an-

Table II. ^{13}C-NMR spectra of bases. Underlined chemical shifts could be exchanged (20.147 MHz, in $CDCl_3$, δ ppm from TMS).



Carbon	*R = CH$_3$*	*COCH$_3$*	*H*	*(CH$_2$)$_2$OH*	*(CH$_2$)$_2$O(Ph)$_2$*
1	60.4	60.3	60.7	60.1	60.2
2	35.2	35.2	35.0	35.2	35.2
3	22.3	22.1	22.2	22.1	22.2
4	25.9	25.8	25.8	25.8	25.9
5	22.3	22.1	22.2	22.1	22.2
6	35.2	35.2	35.0	35.2	35.2
α	45.1	45.8	44.6	45.0	45.1
α'	45.1	49.1	44.6	45.0	45.1
β	56.0	47.0	45.4	53.7	54.5
β'	56.0	42.1	45.4	53.7	54.5
1'	147.9	147.3	147.7	147.4	147.6
CH_3	45.8	20.9	–	–	–
CO	–	168.4	–	–	–
CH_2	–	–	–	59.4	57.7
CH_2	–	–	–	57.6	67.1
7C[a]	139.6 to 121.0	139.4 to 120.9	139.4 to 121.3	139.4 to 120.9	139.2 to 126.9

[a]Two quaternary and 5 secondary carbons from the benzothiophenyl group.

hydrous ether to Mg turnings (1.8 g, 0.075 mol) covered by 100 ml anhydrous ether. Simultaneously, a solution of 2-lithiobenzo[*b*]thiophene was prepared at –20°C in nitrogen atmosphere, by the careful addition of a 1.6 M solution of *n*-butyllithium in hexane (50 ml, 0.08 mol) to an ethereal solution of benzo[*b*]thiophene. The resulting solution was refluxed for 2 h, cooled and added under nitrogen to the $MgBr_2$ solution to yield the Grignard reagent. After stirring at room temperature for 30 min, the α-aminonitrile (5.5 g, 0.027 mol) in ether was added dropwise. The resulting mixture was stirred and refluxed for 16 h. After cooling at room temperature the resulting solution was poured onto an ice-cold saturated solution of NH_4Cl in water. The mixture was stirred for 30 min and treated as follows: decantation, extraction with 3 x 50 ml ether, washing of the pooled organic phases with 3 x 50 ml 10% HCl, neutralization of the aqueous phase with 20% NH_4OH, extraction with 3 x 50 ml ether, washing of the organic phases with water until neutrality. The organic layers, dried over Na_2SO_4, were evaporated under reduced pressure to yield an oily residue purified by chromatography on a column (aluminoxide Merck 2–3). A mixture of ether/petroleum ether (80:20) eluted 2.6 g (31%) of **1** as a white solid (mp: 96–97°C, mp (HCl): 173–174 and 210–212°C).

Synthesis of 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]-4-acetyl-piperazine ***2***

1(1-Cyano-cyclohexyl)-4-acetyl-piperazine. This synthon was obtained in a similar way as for the synthesis of **1** from cyclohexanone (10 g, 0.102 mol), $MgSO_4$ (61.4 g, 0.51 mol), DMA (8.89 g, 0.102 mol), 1-acetyl-piperazine (19.61 g, 0.153 mol), and acetone cyanohydrine (13.03, 0.153 mol). 11 g (45.8%) of a yellow oil was obtained.

Grignard reagent and Bruylants reaction. To a Grignard reagent prepared (under nitrogen) from 2-iodo-benzo[*b*]thiophene (5 g, 0.019 mol) and Mg turnings (0.5 g, 0.02 mol) in ether (100 ml), 1-(1-cyano-cyclohexyl)-4-acetyl-piperazine (2.3 g, 0.0098 mol) in 100 ml ether was added dropwise at room temperature. After the addition was completed, the solution was refluxed for 20 h, cooled at room temperature, and poured onto an ice-cold saturated solution of NH_4Cl in water. The procedure was the same as above with each time the last ether extraction replaced by an extraction with 50 ml dichloromethane. In the end, the oily residue obtained was purified by chromatography on a column to elute with ether 1.3 g (43%) of **2** as a white solid (mp: 154–156°C).

Synthesis of 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]-piperazine ***3***

Compound **2** (0.6 g, 0.0175 mol) was dissolved in ethanol 99% (11 ml) and heated at 50°C in a nitrogen atmosphere before addition of KOH pellets (1.5 g, 0.0267 mol). The solution was heated at 50–60°C and stirred for 7 h, then stirred at room temperature for an additional period of 12 h. The solution was diluted with water, the alcohol evaporated *in vacuo*, then the resulting aqueous solution extracted with methylene chloride. After drying (Na_2SO_4) the solvent was evaporated under reduced pressure to yield an oily residue crystallized in ether to give 0.19 g (36%) of a white solid (mp: 170°C dec).

Synthesis of 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]-4-(2-hydroxy-ethyl)-piperazine ***4***

A solution made from compound **3** (1.5 g, 0.005 mol) dissolved in acetonitrile, diisopropylethylamine (1.75 ml, 0.01 mol), and 1-iodoethanol (0.585 ml, 0.0075 mol) was stirred at room temperature for 60 h. After dilution with water, the solution was extracted with methylene chloride (3 x 50 ml). The organic phase obtained was washed with water until neutrality and dried (Na_2SO_4), then evaporated *in vacuo* to yield an oily residue purified by chromatography on silicagel (Lobar column, Merck). A mixture of methylene chloride and ethanol (90/10) yielded 0.4 g **4** (24%) as a white solid (mp: 131–133°C) immediately poured in an ethereal HCl solution to give the hydrochloride (mp: 158–160°C).

Synthesis of 1-[1-(2-benzo[b]thiophenyl)cyclohexyl]-4-(1-[2-diphenylmethoxy-ethyl]-piperazine ***5***

Preparation of 2-diphenylmethoxy-ethanol. A mixture of ethyleneglycol (5.4 g, 0.087 mol) and NaOH 50% (0.647 g, 0.0081 mol) was heated and stirred at 60°C for 30 min. Then, benzhydryl bromide (2 g, 0.0081 mol) was added and the solution was stirred at 60°C for 24 h. After cooling at room temperature, the solution was poured into ice-cold water, then extracted with 3 x 50 ml petroleum ether. The organic phase, washed with water until neutrality, dried (Na_2SO_4), and evaporated under reduced pressure, yielded an oily residue of 2-diphenylmethoxy-ethanol, crystallized in ether/petroleum ether to give 1.16 g (63%) of a solid identified by GC/MS.

Preparation of 1-chloro-2-diphenylmethoxy-ethane. To the former alcohol 1.5 g (0.0066 mol), dissolved in 10 ml acetonitrile, were added carbon tetrachloride (1.9 ml, 0.0197 mol) and, portionwise, triphenylphosphine (2.1 g, 0.008 mol). After completing the addition, the resulting mixture was stirred for 4 h at room temperature. The solvent was evaporated under reduced pressure, the residue triturated twice with petroleum ether, and then with ether, each operation being followed by filtration to eliminate triphenylphosphine oxide. The purification was achieved by a filtration on aluminoxide (Merck 2–3) in a mixture of petroleum ether/ether (60:40) yielding after evaporation of the solvents under reduced pressure 1.5 g (92%) of an oily residue identified by GC/MS.

Preparation of 1-iodo-2-diphenylmethoxy-ethane. NaI (0.51 g, 0.0034 mol) was added to a solution in 10 ml acetone of the chloride obtained above (0.7 g, 0.0028 mol). The suspension was stirred at room temperature for a 24-h period then at 60°C for an 11-d period with GC/MS controls. Finally, controls revealed 80% iodinated compound and 20% unreacted chlorinated compound. The cooled solution was diluted in water and extracted with petroleum ether. After workup of the organic phase an oily mixture (0.8 g) of the 2 halides was obtained in the above proportion.

Reaction between 1-iodo-2-diphenylmethoxy-ethane and ***3***. To a solution of **3** (0.4 g, 0.0013 mol), diisopropylethylamine (0.47 ml, 0.0026 mol) in acetonitrile (3 ml), 0.8 g of the above-obtained mixture of halides in acetonitrile (3 ml) was added. After stirring at room temperature for 2 h, the solution was poured onto ice-cold water. After extraction of the aqueous phase with methylene chloride and workup of the organic phase, the oily residue obtained was purified by chromatography on a column filled with aluminoxide (Merck 2–3). A mixture (80:20) of petroleum ether/ether eluted 0.4 g (60%) pure **5** (mp (HCl): 128–134°C).

Acknowledgments

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