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Spiro[1,2,4-benzotriazine-3(4*H*),4′-(1′-substituted)piperidines] and related compounds as ligands for sigma receptors

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Abstract

As analogues of some conformationally restricted spiropiperidine derivatives which are endowed with high affinity for σ_1 receptor, a set of 16 spiro[1,2,4-benzotriazine-3(4*H*),4′-(1′-substituted)piperidines] and congeneric compounds was prepared and tested for affinity to σ_1 receptor subtype. All *N*-arylalkyl substituted derivatives exhibited high affinity for the relevant receptor, with K_i in the low nanomolar range. Affinity for σ_2 subtype (assayed only for a few representative compounds) was from one to three order of magnitude lower. Spiro[1,2,4-benzotriazine-3(4*H*),4′-(1′-benzyl)piperidine] (**2**), with a ratio $K_i\sigma_2/K_i\sigma_1 = 7000$ should represent the most selective σ_1 ligand so far described.

Keywords: 3,4-Dihydro-1,2,4-benzotriazine derivatives; Spiro compounds; Sigma receptors ligands

1. Introduction

Sigma receptors occur in at least two classes of binding sites, namely σ_1 and σ_2, which are widely distributed in CNS and in several peripheral tissues and are, also, expressed in some types of tumoral cells [1–3].

The specific functional roles of the two receptor subtypes are being progressively defined, acquiring clinical relevance. Therefore, the design of selective sigma receptor ligands is the subject of a large number of studies due to their possible involvement in the therapy of various pathologies concerning the CNS (mental and motor disorders), as well as liver, spleen, kidney, immune system, sterol biosynthesis and in cancer diagnostics and chemotherapy [4–11]. The achievement of atypical antipsycotics which are devoid of the extrapyramidal side effects associated with classical neuroleptics, is pursued particularly [4,5].

A number of structurally unrelated compounds are able to bind on sigma receptors, even if only few bind with high affinity and selectivity to sigma receptor subtypes [12–16].

Some conformationally restricted spiropiperidine derivatives, exemplified by compound **A** and **B**, deserve a particular interest; the former is endowed with combined high affinity for σ and 5-HT$_2$ receptor [17], while the latter is characterized with high selectivity towards σ_1 receptor subtype [14].

Pursuing our researches on dihydrobenzotriazine derivatives [18–22], we are herewith describing a set of spiro[1,2,4-benzotriazine-3(4*H*),4′/3′-(1′-substituted)polymethyleneimines] (**C**), whose molecular shape and size are similar to those of **A** and **B**. These compounds have been assayed for affinity to σ_1 receptor subtype; for a few representative compounds the selectivity versus σ_2 subtype has been also investigated. In order to investigate the space requirement for the nitrogen binding site and support the supposed existence of a bulk tolerating region [12,13] in the relevant receptor, a few congeners which contain a bicyclic (**D**) or an open chain basic moiety (**E**) were additionally prepared and tested.

A still more suitable compound for the above purpose would have been the bulky spiro[1,2,4-benzotriazine-3(4*H*),4′-(1′-benzyl-2′,2′,6′,6′-tetramethyl)piperidine] (**F**), but its preparation failed and an unexpected benzotriazole derivative was instead formed.

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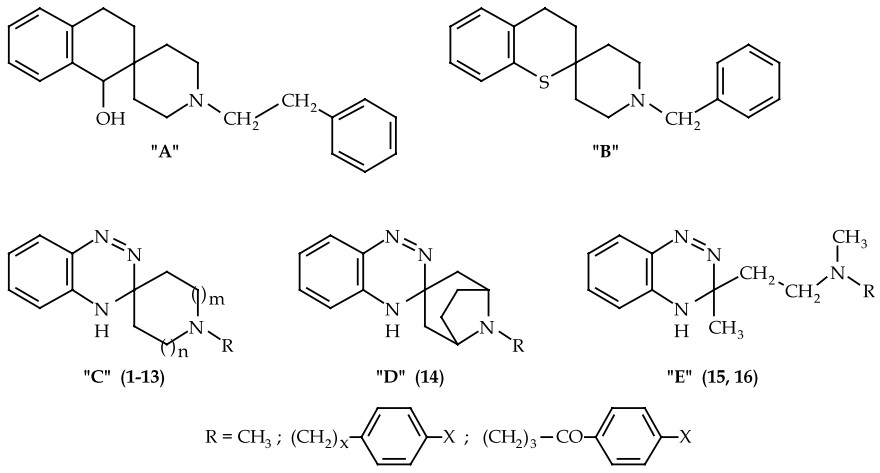
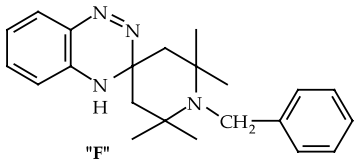




2. Chemistry

Compounds of structure **C**, **D**, **E** (**1**–**16**; see Tables 1a and 1b) were obtained through the air oxidation of 2-aminophenylhydrazones of N-substituted piperidones or analogous cyclic or open chain aminoketones (Scheme 1), which at their turn were commonly obtained by catalytic hydrogenation of the corresponding 2-nitrophenylhydrazones (**17**–**32**; see Tables 2a and 2b).

In the case of *N*-[3-(4-*R*-benzoyl)propyl]-4-piperidones,which possess two ketone groups, using a 1/1 ratio of 2-nitro-phenylhydrazine/ketone, only the piperidone carbonyl group was involved in the reaction [21].

Usually in the reduction of *N*-benzylpiperidone-2-nitrophenyl-hydrazones, no significant hydrogenolytic debenzylation was observed, which however does occur in a large measure in the case of 1-(*N*-benzyl-*N*-methyl)aminobutan-3-one. In such a case the nitro group was reduced by means of zinc and acetic acid.

Some of the required N-substituted cyclic aminoketones were commercially available; all the remaining N-substituted piperidones were prepared by adapting the method described in two patents [23,24] for the synthesis of *N*-[3-(4-*R*-benzoyl)propyl]-4-piperidones. Thus 4-piperidone ethylene ketal (1,4-dioxo-8-azaspiro[4,5]decane) was reacted with the suitable arylalkylhalide, followed by acid hydrolysis of ketal (Scheme 2).

N-Benzyltropinone was obtained condensing 1,3-aceton-dicarboxylic acid with benzylamine and succinic aldehyde, through the method described by Schöpf and Lehmann [25] for similar compounds, with minor modifications. Succinic aldehyde was generated in situ from 2,5-dimethoxytetrahydrofuran [26] (Scheme 3).

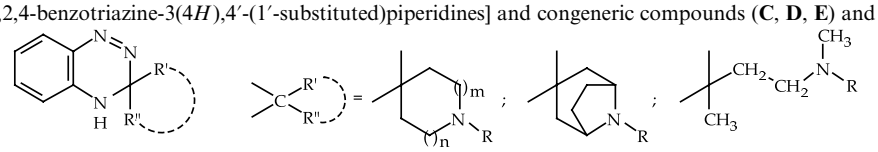
Finally 1-(*N*-benzyl/phenethyl-*N*-methyl)-aminobutan-3-ones were formed by reacting methylvinylketone with *N*-benzyl/-phenethyl-*N*-methylamines (Scheme 3).

In an attempt to prepare compound **F**, 1-benzyl-2,2,6,6-tetra-methylpiperidin-4-one (obtained as described by Guareschi [27]) was reacted with 2-nitrophenylhydrazine. However, instead of the expected 2-nitrophenylhydrazone a colourless compound of formula $C_{13}H_{14}N_4O$ was isolated. The ^{1}H NMR ($CDCl_3$) do not exhibit signals due to methyl groups, but shows a singlet at δ 4.0 due to the benzyl methylene, a multiplet at δ 7.5–6.9 for aromatic protons and signals for three exchangeable protons at about δ 9.0. Such a spectrum suggests that this compound was formed in a reaction between 2-nitrophenylhydrazine and benzylamine, which could be generated from *N*-benzyltetramethylpiperidone through a β-elimination reaction (Scheme 4).

In fact, the same compound was formed in high yield when benzylamine was reacted with 2-nitrophenylhydrazine or with 1-hydroxybenzotriazole, which could arise from the latter by intramolecular loss of water. The UV spectrum, with maxima at 273, 281 and 313 nm, differs from that of 1-hydroxybenzotriazole, but closely resembles that of 3-methylbenzotriazole-1-oxide [28–30], suggesting that compound $C_{13}H_{14}N_4O$ could be a salt between benzylamine and the N-oxide tautomer of 1-hydroxybenzotriazole.

Structures of the final compounds **C**, **D**, **E** were supported by elemental analyses, UV, IR and ^{1}H NMR spectra, which were fully consistent with them. Since the ^{1}H NMR spactra do not exhibit any unusual feature, only a few of them are reported in Section 3.

Table 1a
Characteristics of spirol[1,2,4-benzotriazine-3(4*H*),4′-(1′-substituted)piperidines] and congeneric compounds (**C**, **D**, **E**) and results of binding assays



C **D** **E**

Compound	Structure	m	n	R	Formula*	M. p. °C	Yield %	Ki** (nM) σ_1	Ki** (nM) σ_2
1[a]	**C**	1	1	CH_3	$C_{12}H_{16}N_4$	141-142	67	3410	
2[a]	**C**	1	1	$CH_2C_6H_5$	$C_{18}H_{20}N_4$	178-179	63	2.8 0.6[c]	4220[d]
3	**C**	1	1	$CH_2C_6H_4F(4)$	$C_{18}H_{19}FN_4$	144-146	41	2.8	
4	**C**	1	1	$CH_2C_6H_4Cl(4)$	$C_{18}H_{19}ClN_4$	140-142	39	1.25	346
5	**C**	1	1	$CH_2C_6H_4CH_3(4)$	$C_{19}H_{22}N_4$+HCl	198-200	52	2.5	
6	**C**	1	1	$CH_2C_6H_4OCH_3(4)$	$C_{19}H_{22}N_4O$	155-157	27	4.0	
7[a]	**C**	1	1	$CH_2CH_2C_6H_5$	$C_{19}H_{22}N_4$	125-126	54	4.0[c]	
8	**C**	1	1	$(CH_2)_4C_6H_5$	$C_{21}H_{26}N_4$+HCl+0,25H_2O	192-193	79	3.8	43
9	**C**	1	1	$(CH_2)_5C_6H_5$	$C_{22}H_{28}N_4$+0,25H_2O	oil	26		
					$C_{22}H_{28}N_4$+HCl	181-182	16	0.94	52
10	**C**	1	1	$(CH_2)_3COC_6H_5$	$C_{21}H_{24}N_4O$+HCl+0,25H_2O	209-210	56	14	277
11[a]	**C**	1	1	$(CH_2)_3COC_6H_4F(4)$	$C_{21}H_{23}FN_4O$	135-136	50	12[c]	
12	**C**	0	2	$CH_2C_6H_5$	$C_{18}H_{20}N_4$	oil	15	10.6	
					$C_{18}H_{20}N_4$+HCl	206-207	3		
13	**C**	0	1	$CH_2C_6H_5$	$C_{17}H_{18}N_4$+H_2O	107-109	25	47	
14	**D**	-	-	$CH_2C_6H_5$	$C_{20}H_{22}N_4$	112-114	14	2.6	
15	**E**	-	-	$CH_2C_6H_5$	$C_{18}H_{22}N_4$	oil	8.9		
					$C_{18}H_{22}N_4$+HCl	[b]	5.9	35	
16	**E**	-	-	CH_2 $CH_2C_6H_5$	$C_{19}H_{24}N_4$	oil	9.5		
					$C_{19}H_{24}N_4$+HCl	[b]	3.0	37.5	
haloperidol								6.0	59

* All compounds were analyzed for C, H, N and results were within ±0.4% of calculated values.

** Mean of duplicate experiments: each value differed from the mean by less than 10% (CEREP data unless otherwise stated).

[a] Known [21].

[b] Hydrochlorides of compounds **15** and **16**, in spite of satisfactory elemental analyses, melt in a wide range of temperature with progressive decomposition.

[c] For this compound the K_i value was determined at MDS-Panlabs and the corresponding K_i for reference haloperidol was 2.0 nM.

[d] For this compound the K_i value was determined at MDS-Panlabs and the corresponding K_i for reference ifenprodil was 1.4 nM.

Table 1b
Analytical results of compounds of Table 1a

Comp.	Formula	C%		H%		N%	
		Found	Calc.	Found	Calc.	Found	Calc.
1	$C_{12}H_{16}N_4$	66.65	66.64	7.49	7.46	26.00	25.91
2	$C_{18}H_{20}N_4$	73.75	73.94	6.89	6.90	19.20	19.16
3	$C_{18}H_{19}FN_4$	69.97	69.66	6.05	6.17	18.00	18.05
4	$C_{18}H_{19}ClN_4$	66.48	66.15	6.03	5.86	17.26	17.14
5	$C_{19}H_{22}N_4+HCl$	66.58	66.56	7.01	6.76	16.38	16.34
6	$C_{19}H_{22}N_4O$	70.86	70.78	6.98	6.88	17.15	17.38
7	$C_{19}H_{22}N_4$	74.61	74.47	7.24	7.24	18.21	18.29
8	$C_{21}H_{26}N_4+HCl+0.25H_2O$	67.05	67.18	7.46	7.38	14.62	14.92
9	$C_{22}H_{28}N_4+0.25H_2O$	75.42	74.85	8.62	8.14	15.45	15.88
	$C_{22}H_{28}N_4+HCl$	68.72	68.64	7.89	7.59	14.49	14.55
10	$C_{21}H_{24}N_4O+HCl+0.25H_2O$	64.54	64.77	6.50	6.60	14.28	14.39
11	$C_{21}H_{23}FN_4O$	68.62	68.83	6.28	6.33	15.20	15.29
12	$C_{18}H_{20}N_4+HCl$	65.81	65.74	6.45	6.44	17.23	17.04
13	$C_{17}H_{18}N_4+H_2O$	68.91	68.90	6.83	6.80	19.09	19.90
14	$C_{20}H_{22}N_4$	75.60	75.44	7.26	6.96	17.59	17.60
15	$C_{18}H_{22}N_4$	72.15	73.43	7.44	7.53	19.20	19.03
	$C_{18}H_{22}N_4+HCl$	65.09	65.34	6.95	7.01	17.21	16.93
16	$C_{19}H_{24}N_4+HCl$	65.85	66.17	7.32	7.31	16.35	16.24

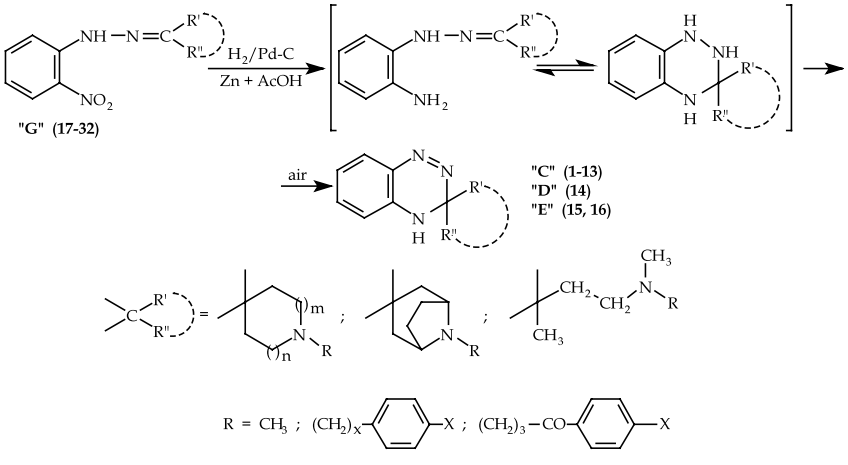
In the oxidative cyclization of 2-aminophenylhydrazone of *N*-benzyl-3-piperidone and *N*-benzyl-3-pyrrolidinone a chiral carbon is generated and compounds **12** and **13** are racemates. No attempts were made so far to separate the corresponding enantiomers.

On the other hand, in the cyclization of *N*-benzyltropanone-2-aminophenylhydrazone two geometric isomers can be produced; however the isolated dihydrobenzotriazine derivative **14**, behaves as a unitary compound in different TLC systems. ^1H and ^{13}C NMR spectra also indicate that the compound is unitary; infact they do not exhibit any splitted signal, as it should be expected in the case of a mixture of isomers (see Section 3).

3. Experimental

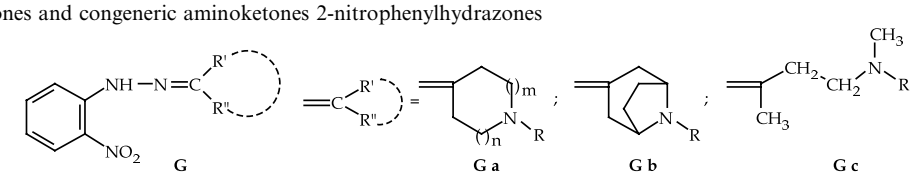
3.1. Chemistry

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected. The elemental analyses were performed with CE EA 1110 CHNS-O instrument and the results obtained for the indicated elements were within ±0.4% of the calculated values. UV and IR spectra were recorded, respectively, on Perkin–Elmer mod. 550 S and Paragon 1000PC spectrophotometers; ^1H NMR spectra were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ as solvents, with TMS as internal standard.



Scheme 1.

Table 2a
N-substituted piperidones and congeneric aminoketones 2-nitrophenylhydrazones



G Ga Gb Gc

Compound	Structure	m	n	R	Formula*	M. p. °C	Yield %
17[a]	**Ga**	1	1	CH_3	$C_{12}H_{16}N_4O_2$	81-82	75
18[a]	**Ga**	1	1	$CH_2C_6H_5$	$C_{18}H_{20}N_4O_2$	123-124	94
19	**Ga**	1	1	$CH_2C_6H_4F(4)$	$C_{18}H_{19}FN_4O_2+0.5H_2O$	118-120	77
20	**Ga**	1	1	$CH_2C_6H_4Cl(4)$	$C_{18}H_{19}ClN_4O_2$	124-125	81
21	**Ga**	1	1	$CH_2C_6H_4CH_3(4)$	$C_{19}H_{22}N_4O_2$	86-88	69
22	**Ga**	1	1	$CH_2C_6H_4OCH_3(4)$	$C_{19}H_{22}N_4O_3$	87-88	81
23[a]	**Ga**	1	1	$CH_2CH_2C_6H_5$	$C_{19}H_{22}N_4O_2$	70-71	87
24	**Ga**	1	1	$(CH_2)_4C_6H_5$	$C_{21}H_{26}N_4O_2$	82-84	80
25	**Ga**	1	1	$(CH_2)_5C_6H_5$	$C_{22}H_{28}N_4O_2$	oil	82
26	**Ga**	1	1	$(CH_2)_3COC_6H_5$	$C_{21}H_{24}N_4O_3$	96-97	81
27[a]	**Ga**	1	1	$(CH_2)_3COC_6H_4F(4)$	$C_{21}H_{23}FN_4O_3$	122-123	73
28	**Ga**	0	2	$CH_2C_6H_5$	$C_{18}H_{20}N_4O_2$	93-94.5	62
29	**Ga**	0	1	$CH_2C_6H_5$	$C_{17}H_{18}N_4O_2$	85-86	86
30	**Gb**	-	-	$CH_2C_6H_5$	$C_{20}H_{22}N_4O_2$	89-92	75
31	**Gc**	-	-	$CH_2C_6H_5$	$C_{18}H_{22}N_4O_2$	oil	88
32	**Gc**	-	-	$CH_2\ CH_2C_6H_5$	$C_{19}H_{24}N_4O_2$	oil	60

* All compounds were analyzed for C, H, N and results were within ±0.4% of calculated values.
[a] Known [21].

3.1.1. Basic ketones

N-Methyl-, *N*-benzyl-, *N*-phenethyl-4-piperidones, *N*-benzyl-3-piperidone and *N*-benzyl-3-pyrrolidinone were commercially available.

3.1.2. N-substituted-4-piperidones

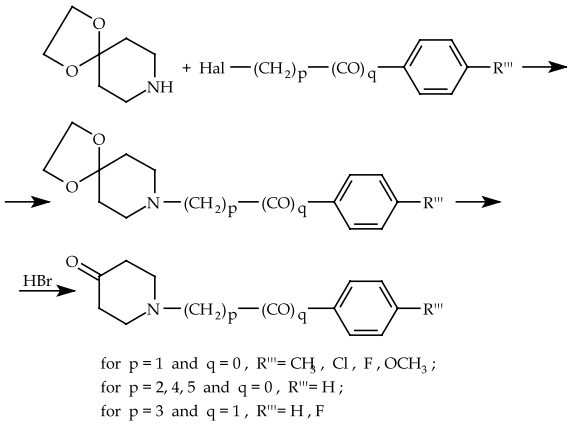
The suitable arylalkylhalide or ω-halobutyrophenone (6–14 mmol) and 4-piperidone ethylene ketal (12–28 mmol) were dissolved in toluene and the solution was refluxed for 8 h. After cooling the 4-piperidone ethylene ketal hydrochloride was filtered and the *N*-substituted-4-piperidoneketal was extracted with 0.5 N HCl. The acid solution was washed with ether, basified and again extracted with ether. Any residual 4-piperidone ethylene ketal was removed by chromatography on neutral alumina (1:10) using dry ether as eluent. The obtained ketals were hydrolized by refluxing (3–8 h) with 48% HBr (1 ml for each g). The solution was basified with 6 N NaOH, followed by extraction with ether; the crude N-substituted piperidones were finally chromatographed on alumina (1:10) eluting with dry ether.

3.1.3. N-Benzyl-nor-tropinone (N-benzyl-1αH,5αH-tropan-3-one)

a) 1,5-Dimethoxytetrahydrofuran (6 g, 45 mmol) was treated with 20 ml of 0.025 N HCl for 20 h at about

Table 2b
Analytical results of compounds of Table 2a

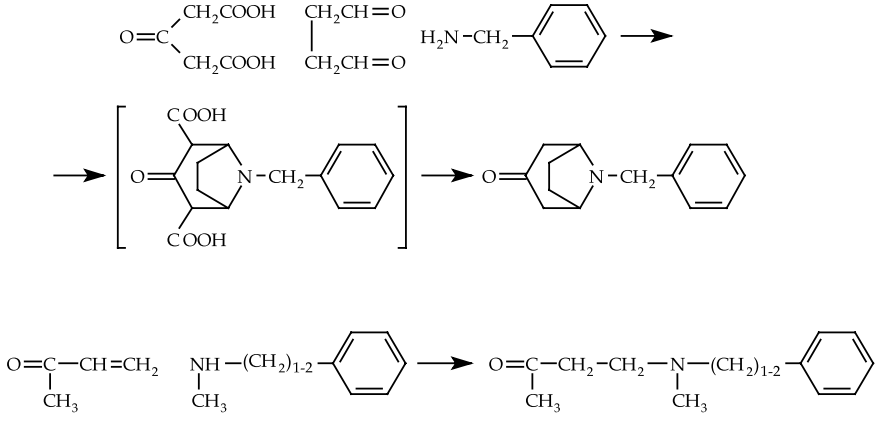
Comp.	Formula	C%		H%		N%	
		Found	Calc.	Found	Calc.	Found	Calc.
17	$C_{12}H_{16}N_4O_2$	57.91	58.05	6.42	6.50	22.43	22.57
18	$C_{18}H_{20}N_4O_2$	66.52	66.65	6.21	6.22	17.16	17.27
19	$C_{18}H_{19}FN_4O_2+0.5H_2O$	61.75	61.52	5.46	5.74	15.78	15.95
20	$C_{18}H_{19}ClN_4O_2$	60.50	60.25	5.18	5.34	15.58	15.61
21	$C_{19}H_{22}N_4O_2$	67.50	67.44	6.60	6.55	16.45	16.56
22	$C_{19}H_{22}N_4O_3$	64.47	64.39	6.37	6.26	15.70	15.81
23	$C_{19}H_{22}N_4O_2$	67.41	67.43	6.55	6.55	16.46	16.56
24	$C_{21}H_{26}N_4O_2$	68.75	68.83	7.12	7.15	15.11	15.29
25	$C_{22}H_{28}N_4O_2$	69.25	69.45	7.57	7.42	14.88	14.72
26	$C_{21}H_{24}N_4O_3$	66.63	66.30	6.44	6.36	14.54	14.73
27	$C_{21}H_{23}FN_4O_3$	63.16	63.30	5.83	5.82	14.04	14.06
28	$C_{18}H_{20}N_4O_2$	66.81	66.65	6.04	6.21	17.48	17.27
29	$C_{17}H_{18}N_4O_2$	65.60	65.79	6.03	5.85	18.25	18.05
30	$C_{20}H_{22}N_4O_2$	68.31	68.55	6.52	6.33	15.97	15.99
31	$C_{18}H_{22}N_4O_2$	65.98	66.24	7.00	6.79	17.40	17.17
32	$C_{19}H_{24}N_4O_2$	65.00	67.04	7.08	7.11	17.40	16.46



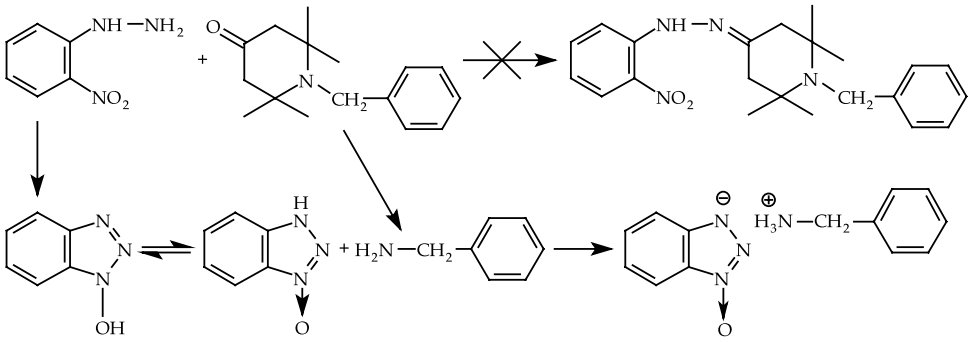
Scheme 2.

4 °C. The pH value was then corrected up to 6 by adding a few drops of saturated solution of $NaHCO_3$ and the solution diluted with water to 30 ml.

b) To a solution of sodium dihydrogenphosphate monohydrate (4.6 g, 34 mmol) in 500 ml of water, 1,3-acetonedicarboxylic acid (12 g, 82 mmol), NaOH (6,58 g, 164 mmol) and benzylamine (9.4 g, 88 mmol) were added in the order, under vigorous stirring. The solution of succinic aldehyde prepared as under (a) was added and pH was adjusted to 7 by adding about 18 ml of 1 N HCl. The mixture was maintained, under stirring, at room temperature (r.t.) for a week. The yellowish precipitate was filtered, dissolved in CH_2Cl_2 and the solution was shaken with water and then dried with Na_2SO_4.



Scheme 3.



Scheme 4.

After removing the solvent, the oily residue (4.9 g) was distilled twice (100–140 °C, at 0.01 torr) obtaining 1.3 g (13.3% yield) of the title ketone.

3.1.4. 1-(N-Benzyl/phenethyl-N-methyl)-amino-butan 3-one

To a solution of 15 mmol of *N*-benzyl(or phenethyl)-*N*-methylamine in 2 ml of dry dioxane, a solution of 1.37 ml (1.16 g, 16.5 mmol) of freshly distilled methyl vinyl ketone in 2 ml of dry dioxane was added slowly and the mixture is maintained at 70–80 °C for 45–60 min. After cooling the mixture was partitioned between ether and 1 N HCl (15 ml); the ether solution was further extracted with acidulated water that was joined to the acid solution. The acid solution was extracted with ether, basified with 6 N NaOH solution and again extracted with ether. After removing the solvent, the oily amino-ketone was obtained in 95% yield. In the case of 1-(*N*-phenethyl-*N*-methyl)amino-butan-3-one, a small amount of the starting secondary amine was removed by chromathography on neutral alumina (1:10) eluting with dry ether. Final yield of pure amino-ketone was 84.7%.

3.1.5. N-Benzyl-2,2,6,6-tetramethyl-piperidin-4-one

The title compound was prepared as indicated by Guareschi [27] with some modification.

To a solution of benzylamine (5 g, 4.7 mmol) in 5 ml of water, phorone (6.6 g, 4.7 mmol) was added and the mixture was stirred at r.t. for 48 h. At the end 0.5 N HCl was added till the solution was clearly acidic; unreacted phorone was filtered and then the solution was extracted with ether. The acid solution was basified with 6 N KOH solution and extracted with ether. After removing the solvent 9.23 g of pale yellow oil were obtained. This oil was chromatographed (in three portion) on silica (1:20) eluting with dry ether. The title compound is mainly present in the middle fractions, while it is associated respectively with phorone in the former fractions and with benzylamine and its open chain adducts with phorone in the tail fractions. Repeating the chromatography on the middle fractions a practically unitary compound is obtained (6.9 g, 59.8% yield), which, however on standing exhibits again three spots in the TLC, suggesting that a β-elimination reaction takes place progressively.

*3.1.6. 2-Nitrophenylhydrazones (**G**: **17**–**32**)*

The suitable N-substituted cyclic or open chain amino-ketone and 2-nitrophenylhydrazine (7–12 mmol each) were dissolved in EtOH (10–20 ml) and the solution was heated under reflux for 2–3 h. The solvent was removed under reduced pressure and the residue chromatographed on alumina (1:10) eluting with dry ether (**20**–**22**, **24**–**26**, **30**, **31**) or CH_2Cl_2 (**19**, **28**, **29**). Most compounds are then crystallized from dry ether or dry ether–pentane (**30**). In some cases red or orange coloured oils are obtained, that crystallize only when treated with a few drops of ether and after standing a long time in freezer; successively, after washing with cold ether, a product of good purity is obtained (**24**–**26**, **29**). The 2-nitrophenylhydrazone **32** still after repeated chromatography on alumina (1:20), eluting with ether or CH_2Cl_2, exhibit at TLC traces of compounds which increased on standing, suggesting that it is not stable.

^{1}H NMR ($CDCl_3$) of compound **19**: δ 10.82 (s, 1H, NH of phenylhydrazone, collapses after D_2O exchange), 8.22–8.08 (pseudo-dd, 1H arom.), 7.92–6.78 (pseudo-dd, 1H arom.), 7.60–7.42 (m, 1H arom.), 7.40–7.22 (m, 2H arom. p-substitution), 7.12–6.94 (m, 2H arom. p-substitution), 6.86–6.70 (m, 1H, arom.) 3.55 (s, 2H, CH_2 of benzyl group), 2.80–2.40 (m, 8H, piperidine ring).

*3.1.7. Spiro[1,2,4-benzotriazine-3(4H),4′-(1′-substitited)piperidines] and congeneric compounds (**C**: **1**–**13**, **D**: **14**, **E**: **15**, **16**)*

a) Most of the foregoing 2-nitrophenylhydrazones of N-substituted amino-ketones (5–10 mmol) were dissolved in EtOH and hydrogenated at atmospheric pressure in the presence of 10% Pd on charcoal (0.1 g for each g of nitro-compound). When the hydrogen absorption terminated, the catalyst was removed, the stoichiometric volume of 1 N ethanolic HCl was added (with the exception

of compounds **9** and **13**) and air was insufflated into the solution for a time variable from a few hours to 1 day. The formation of the dihydrobenzotriazine was followed up through the appearance and progressive increase of the absorption maximum in the range of 420–450 nm. When the latter maximum did not increase any more, the solvent was removed under reduced pressure and the residue (hydrochloride or free base) was worked up differently depending on the single compound. Crude hydrochlorides of compounds **5**, **8** and **10** were crystallized from absolute EtOH. From crude hydrochlorides of compounds **3** and **6** the corresponding bases were obtained by treatment with 6 N NaOH solution and extraction with ether. The free base **3** was crystallized from dry ether, while compound **6** required a triple chromatography on neutral alumina (1:20) eluting with dry ether, followed by ether plus 0.5% of MeOH; finally compound **6** was crystallized from dry ether. The crude free base **13** was crystallized from EtOH, while the base **9** was purified through a triple chromatography on neutral alumina (1:20) eluting with dry ether containing increasing amount of MeOH. From the third chromatography, eluting with dry ether plus 0.5% MeOH, an oily compound was obtained, which exhibit the expected UV and ^{1}H NMR spectra, but whose carbon content still differed for +0.57% from the calculated value. Finally the base was converted into the hydrochloride which was crystallized from absolute EtOH.

^{1}H NMR ($CDCl_3$) of compound **3**: δ 7.88–7.74 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 7.40–7.14 (m, 1H arom. dihydrobenzotriazine ring+2H arom. p-substitution), 7.10–6.94 (m, 2H arom. p-substitition), 6.94–6.80 (m, 1H arom. dihydrobenzo-triazine ring), 6.70–6.56 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 3.72 (s, 1H, NH of dihydrobenzotriazine ring, collapses after D_2O exchange), 3.57 (s, 2H, CH_2 of benzyl group), 2.96–2.74 (m, 2H, piperidine ring), 2.60–2.36 (m, 2H, piperidine ring), 2.36–2.14 (m, 2H, piperidine ring), 1.88–1.64 (m, 2H, piperidine ring).

^{1}H NMR ($CDCl_3$) of compound **13**: δ 7.82–7.70 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 7.40–7.12 (m, 5H arom. benzyl group+1H arom. dihydrobenzotriazine ring), 6.90–6.76 (m, 1H arom. dihydrobenzotriazine ring), 6.66–6.54 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 4.45 (s, 1H, NH of dihydro-benzotriazine ring, collapses after D_2O exchange), 3.68 (s, 2H, CH_2 of benzyl group), 3.10–2.82 (m, 3H, pyrrolidine ring), 2.80–2.62 (m, 1H, pyrrolidine ring), 2.62–2.46 (m, 1H, pyrrolidine ring), 2.12–1.92 (m, 1H, pyrrolidine ring).

5)nce in preliminary experiments some hydrogenolytic debenzylation or chlorine elimination was observed, the reduction of 2-nitrophenylhydrazones **20**, **28**, **30**, **31** was effected with the following method, which was applied also to the 2-nitrophenylhydrazone **32** even if lacking any chlorine or benzyl group. 2-Nitrophenylhydrazones (5–8 mmol) were dissolved or suspended in EtOH (50–80 mmol) and treated with 50–80 mmol of AcOH diluted with the same volume of water. Then, under stirring and a stream of nitrogen, zinc powder (30–48 mmol) was added in small portion. The mixture was maintained at about 80 °C for 30 min. After cooling the unreacted zinc was removed by filtration and through the almost colourless solution air was bubbled for several (6–10) hours. The solvent was removed under reduced pressure and the residue was dissolved in water, basified with 6 N NaOH solution and extracted thoroughly with ether or CH_2Cl_2. After a first extraction it may be convenient to filter the insoluble zinc salts to avoid the formation of a stable emulsion. After the elimination of the solvent, the residue was crystallized from dry ether (**4**) or chromatographed on neutral alumina (1:20) eluting with dry ether (**12**, **14**, **15**, **16**). The oily compound **12** was converted into the hydrochloride, which was crystallized from absolute EtOH. The oily compound **14** crystallized when treated with drops of dry ether. From the oily compounds **15** and **16** consistent amounts of impurities were removed by distillation in vacuo (up to 150 °C at 0.01 torr) and residues were again chromatographed on alumina (1:30) eluting with dry ether. The obtained brownish oils were finally converted into the hydrochlorides (very hygroscopic), which were washed several times with dry ether.

^{1}H NMR ($CDCl_3$) of compound **14**: δ 7.80–7.68 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 7.50–7.10 (m, 5H arom. benzyl group+1H arom. dihydrobenzotriazine ring), 6.90–6.74 (m, 1H arom. dihydrobenzotriazine ring), 6.60–6.48 (pseudo-dd, 1H arom. dihydrobenzotriazine ring), 3.88 (s, 1H, NH of dihydro-benzotriazine ring, collapses after D_2O exchange), 3.62 (s, 2H, CH_2 of benzyl group), 3.45–3.25 (pseudo-s centered at 3.33, 2H, 2 CH of tropane ring), 2.40–2.00 (m, 6H, tropane ring), 2.00–1.80 (m, 2H, tropane ring).

^{13}C NMR and DEPT ($CDCl_3$) of compound **14**: δ 140.37 (C arom.), 136.26 (C arom.), 133.37 (CH arom.), 132.62 (C arom.), 129.68 (CH arom.), 129.02 (2 CH arom. benzyl group), 128.75 (2 CH arom. benzyl group), 127.40 (CH arom.), 119.07 (CH arom.), 115.37 (CH arom.), 70.81 (C tropane ring), 58.84 (2 CH tropane ring), 56.69 (CH_2 benzyl

group), 43.34 (2 CH_2 tropane ring), 27.33 (2 CH_2 tropane ring).

c) When the 2-nitro-phenylhydrazone of the 1-(*N*-benzyl-*N*-methyl-amino)butan-3-one (**31**) was reduced catalitically, as under point (a), a consistent *N*-debenzylation occurred. After the air oxidation and the usual working up, a brown oil was obtained, which on standing partially crystallized. By washing with dry ether, yellow crystals melting at 116–117 °C were obtained, corresponding to compound **E** with R = H.

Anal. Found: C, 64.60; H, 7.91; N, 27.51. Calc. for $C_{11}H_{16}N_4$: C, 64.68; H, 7.89; N, 27.43%.

^{1}H NMR ($CDCl_3$): δ 7.85–7.70 (pseudo-dd, 1H arom.), 7.30–7.10 (m, 1H arom.), 6.90–6.70 (m, 1H arom.), 6.70–6.55 (pseudo-dd, 1H arom.), 6.08 (s, 1H, NH of dihydrobenzotriazine ring, collapses after D_2O exchange), 3.00–2.70 (m, 2H, $-CH_2-CH_2-NH-CH_3$), 2.50–2.30 (m, with 1 superimposed s at 2.39, 1H of $-CH_2-CH_2-NH-CH_3$ + 3H of $-CH_2-CH_2-NH-CH_3$), 2.30–2.15 (m, 1H of $-CH_2-CH_2-NH-CH_3$), 1.20 (s, 3H, CH_3 in **3** of dihydrobenzotriazine ring), 1.00 (s, 1H, $-CH_2-CH_2-NH-CH_3$, collapses after D_2O exchange).

3.1.8. Salt between 1-hydroxybenzotriazole and benzylamine

a) To a solution of *N*-benzyl-2,2,6,6-tetramethylpiperidin-4-one (1.07 g, 4.3 mmol) in EtOH, 2-nitrophenylhydrazine (0.67 g, 4.4 mmol) was added and the solution was refluxed for 3 h. The solvent was removed under reduced pressure and the residue was treated with dry ether, which extracted 0.66 g of an untractable red oil, leaving a colourless solid. After crystallization from CH_2Cl_2 0.52 g (50% yield) of flat white crystals, melting at 124–126 °C were obtained.

b) The same compound was obtained in 74% yield when the EtOH solution of equimolar amounts of benzylamine and 2-nitro-phenylhydrazine were heated for 3 h and treated as above.

c) The same product was obtained in 69% yield when the EtOH solution of equimolar amounts of benzylamine and 1-hydroxy-benzotriazole were heated for 3 h and treated as usual.

Anal. Found: C, 64.29; H, 5.60; N, 23.25. Calc. for $C_{13}H_{14}N_4O$: C, 64.45; H, 5.82; N, 23.12%.

^{1}H NMR ($CDCl_3$): δ 9.35–9.00 (s, 3H, NH_3^+, collapses after D_2O exchange), 7.50–7.30 (m, 1H arom.), 7.30–6.90 (m, 8H arom.), 4.00 (s, 2H, CH_2 of benzyl group).

UV (EtOH): λmax (log ε) 273 (3.4); 281 (3.5); 313 (4.38).

3.2. Biological evaluation

3.2.1. Receptor binding assays

Binding assays were performed at the Receptology Department of CEREP, Celle L'Evescault, France, or at MDS-Panlabs, Bothell, WA, USA.

Compounds **1**–**16** were tested for displacement of specific radioligands from σ_1 (guinea pig brain membranes) [31], σ_2 (rat brain) [31,32], serotonin 5-HT_{2A} (rat brain) [33] and dopamine D_{2L} (human recombinant expressed by CHO cells) [34,35] receptors.

Incubation conditions were as follows:

σ_1 (CEREP): [^{3}H](+)pentazocine 2 nM (K_D = 3.4 nM), 150 min at 22 °C (non-specific binding was estimated in the presence of 10 μM haloperidol);alternatively (Panlabs): [^{3}H](+)pentazocine 1 nM (K_D = 4.3 nM), 30 min at 25 °C (non-specific binding was estimated in the presence of 10 μM (+)3-PPP);

σ_2 (CEREP): [^{3}H]DTG 5 nM (+300 nM (+)pentazocine) (K_D = 32 nM), 120 min at 22° (non specific binding in presence of 10 μM haloperidol);alternatively (Panlabs): [^{3}H]ifenprodil 3 nM (K_D = 4.8 nM), 60 min at 37 °C (non-specific binding in presence of 10 μM ifenprodil);

5-HT_{2A} (Panlabs): [^{3}H]ketanserin 0.5 nM (K_D = 0.82 nM), 40 min at 25 °C (non-specific binding in the presence of 1 μM ketanserin);

D_{2L} (Panlabs): [^{3}H]spiperone 2 nM (K_D = 0.08 nM), 120 min at 25 °C (non-specific binding in presence of 10 μM haloperidol).

Following incubation, the membranes were rapidly filtered under vacuum through glass fibre filters (Filtermat). Filters were washed three times with ice-cold buffer and bound radioactivity was measured with a scintillation counter (LKB Betaplate), using (CEREP) a solid scintillant (MeltiLex B/HS, Wallac) or (Panlabs) a liquid scintillation fluid (Packard).

Compounds were assayed, either as hydrochlorides or as free bases, at four or five different concentrations in the range 10 μM–1 nM; each concentration was run in duplicate. The free bases were dissolved in DMSO at 1–10 mM conc. and then diluted with water to 100 μM conc.

3.2.2. Acetylcholinesterase inhibition assay

Compound **2** was tested as acetylcholinesterase inhibitor by the method of Ellmann et al. [36]. Human recombinant acetyl-cholinesterase (Sigma, C-1682) is used.

Test compound and/or vehicle is incubated with acetylthiocholine iodide and 5,5-dithio-bis-2-nitrobenzoic acid in sodium phosphate buffer (pH 7.4) at 25 °C. The reaction is initiated by addition of 2 ng acetylcholinesterase and the thiocholine generated reacts con-

tinuously with dithio-bis(nitrobenzoic)acid to produce a yellow anion (5-thio-2-nitro-benzoic acid) proportional to enzymatic activity which is determined after 20 min by spectrophotometry at 405 nm. Physostigmine is used as reference compound with $IC_{50} = 0.12$ nM.

4. Results and discussion

Results of binding assays to sigma receptors are collected in Table 1a. With the only exception of the *N*-methylderivative **1**, all compounds exhibited good affinity for σ_1 receptors subtype, indicating that an arylalkyl substituent on the basic nitrogen is necessary to have good affinity for the relevant subtype.

Commonly the spiro[1,2,4-benzotriazine-3(4*H*),4′-(1′-substituted)-piperidines (**2**–**11**) were more active than the remaining compounds. The lengthening of the aliphatic chain, between the piperidine nitrogen and the aryl moiety, from one to four methylenes does not change significantly the affinity, which is however improved when five methylenes are present (**9**).

Similarly, the presence of para substituent on the *N*-benzyl residue produces only small variations of the K_i value, which, anyhow, is lowest for the chloro derivative (1.25 nM) and highest for the methoxy one (4 nM).

The comparison of the butyrophenone derivatives **10** and **11** with the phenylbutyl derivative **8** indicates that the carbonyl group is not useful for affinity, as it was also observed for the 1-(arylalkyl)-quinolizidine derived ligands for σ_1 receptors [37]. When the joining position of dihydro-benzotriazine ring to piperidine is changed from C-4 to C-3, as in compounds **2** and **12**, a decrease of affinity is observed; however the real effect of this structural modification cannot be correctly evaluated without the separation of enantiomers composing the racemate **12**. Research for this purpose will be undertaken nextly.

Similar considerations may be done for compound **13**, which also is a racemate; anyhow comparing the two racemate **12** and **13** it is evident that the smaller pyrrolidine ring derivative is less qualified to fit σ_1 receptor subtype.

On the other hand it is interesting to observe how the *N*-benzyl-piperidine derivative **2** and the more cumbersome *N*-benzyl-tropane derivative **14** exhibited the same K_i value, suggesting that some bulkiness around the basic nitrogen is tolerated by the receptor, provided that the benzyl residue is maintained in the proper position. This result supports further the claimed existence of a bulk tolerating region in the σ_1 receptor [12,13].

The formal cleavage of piperidine ring of compounds **2** and **7** afforded compounds **15** and **16** respectively, whose affinity for σ_1 subtype was about one order of magnitude lower. Thus the decrease of activity with the increased mobility of the basic arylalkyl chain, indirectly supports the importance of a 4-spiro-substituted piperidine ring to allow the correct fitting of the *N*-arylalkyl substituent on the receptor.

Concerning the σ_2 receptor subtype, only five representative compounds were assayed for affinity, which resulted from moderate to very poor, with K_i in the range 43–4220 nM. Therefore, the dihydrobenzotriazine derivatives seem endowed with a rather high degree of selectivity for σ_1 versus σ_2 subtype. The ratio $K_i\sigma_2/K_i\sigma_1$ varies from 11, for compound **8**, to 7033 for compound **2**. The last value is many times higher than the ratio observed for spipethiane (832 [14]) and for the (+)-enantiomer of 3,3-dimethyl-1-[3-(5-methoxy-1,2,3,4-tetrahydronaphtalene-1-yl)-*n*-propyl]piperidine (1340 [15]). Thus, even with some caution for the somewhat different binding conditions, compound **2** should be considered, so far, the most selective ligand for σ_1 versus σ_2 subtype.

The same compound exhibited also a good selectivity versus 5-HT_2 ($K_i = 1470$ nM; ratio K_i5-$HT_2/K_i\sigma_1 = 2450$) and D_2 receptor subtypes (only 14% inhibition of [^{3}H]-spiperone binding at 10 μM conc.).

Such a poor affinity to D_2 receptors agrees with the only moderate affinity observed in the past [21] for the 4-fluorobutyrophenone derivative **11** ($K_1 = 164$ nM), in spite of its strict structural similarity to haloperidol, which is a well known potent ligand for both σ_1 and D_2 receptor subtype.

To further define the pharmacological profile of compound **2**, the possible inhibition of acethylcholinesterase was also investigated. In fact several *N*-benzylpiperid-4-yl-alkyl derivatives of bicyclic systems as 1-indanone [38], phthalimide [39] and benzisoxazole [40] are endowed with AChE inhibitory activity, which become outstanding when suitable substituents are introduced on the benzene ring of the bicyclic system, as in the case of donepezil (E 2020), recently launched as drug for the treatment of Alzheimer disease.

In spite of some structural similarity, compounds **2** completely failed to inhibit the acetylthiocholine hydrolysis by means of the human erythrocytes enzyme at concentrations up to 10 μM.

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