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Synthesis and pharmacological evaluation of Tic-hydantoin derivatives as selective σ_1 ligands. Part 2

Amaury Cazenave Gassiot,[†] Julie Charton,[‡] Sophie Girault-Mizzi, Pauline Gilleron,[§] Marie-Ange Debreu-Fontaine, Christian Sergheraert and Patricia Melnyk*

UMR CNRS 8525, Université de Lille II, Institut de Biologie et Institut Pasteur de Lille 1 rue du Professeur Calmette, B.P. 447, 59021 Lille cedex, France

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Abstract—Herein is described a new class of selective σ_1 ligands consisting of tetrahydroisoquinoline-hydantoin (Tic-hydantoin) derivatives. Compound **1a** has high affinity (IC_{50} = 16 nM) for σ_1 receptor and is selective in a large panel of therapeutic targets. This study presents structural changes on the side chain of the Tic-hydantoin core. Analogs of higher affinity could be identified ($IC_{50} \approx 2$–3 nM).

To date, multiple σ binding sites have been identified and σ receptors are classified into two distinct subtypes denoted as σ_1 and σ_2.[1] Recent evidence has indicated that σ_1 receptors may be involved in regulating a variety of neurotransmitters in the central nervous system, including cholinergic,[2,3] dopaminergic,[4] and glutamatergic systems.[5,6] Thus, there is a sustained interest for using selective σ_1 ligands, which stems from the possibility of developing new drug candidates particularly for the treatment of depression,[7] psychiatric disorders, memory deficits, or drug addiction.[8,9] σ_2 ligands may be developed for attenuating motor side effects associated with typical antipsychotic agents or for diagnossis and treatment of cancer.

In a profile screening, compound **1a** (Fig. 1) was identified as an interesting ligand of guinea pig σ_1 receptor with an IC_{50} of 16 nM. Binding of this compound at

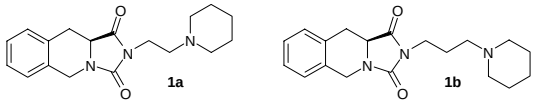

Figure 1. Compounds **1a** and **1b**.

σ_1 receptor could be explained by the association of an aromatic moiety and a nitrogen atom. That type of structure has previously proved to be a pharmacophoric element in the binding of a series of phenylalkylpiperidines and phenylalkylpiperazines to σ receptors.[10]

The tetrahydroisoquinoline-hydantoin (Tic-hydantoin) core was of particular interest on account of the specificity of the pharmacological profile obtained for compound **1a**.[11]

Two parallel studies were investigated to improve the affinity of the lead compound **1a** toward σ_1 receptor. The first study, described in the previous article, dealt with structural changes on the Tic-hydantoin core, while preserving the alkylpiperidine moiety of compound **1a**. In this letter, parallel work consisting in the study of the side chain of compound **1a** while preserving the Tic-hydantoin core is reported. Two series of compounds were designed (Fig. 2).

First, the piperidino group was replaced by differently substituted piperazino moieties, while conserving the

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* Corresponding author. Tel.: +33 3 20 87 12 19; fax: +33 3 20 87 12 33; e-mail: patricia.melnyk@ibl.fr
† Present address: School of Chemistry, University of Southampton—Highfield, Southampton, SO17 1BJ, UK.
‡ Present address: Laboratoire de Chimie Générale et Organique, UMR8525, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Professeur Laguesse, B.P. 83, 59006 Lille, France.
§ Present address: ICPAL—EA 2692—Université de Lille II, 3 rue du Professeur Laguesse, B.P. 83, 59006 Lille, France.

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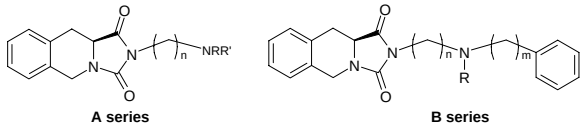


Figure 2. Target compounds.

two and three methylene linkers, which were found to be relevant in our parallel study (A series). Indeed, affinity of compounds **1a** and **1b** (Fig. 1) for σ_1 receptor (IC_{50} = 16 and 21.5 nM, respectively) and selectivity were equivalent.

Second, the structure was varied according to a model (Fig. 3) recently proposed to account for the binding of ligands at σ_1 receptor (B series).[10,12,13]

In this model, a nitrogen atom appears as a required pharmacophoric element between two hydrophobic aromatic regions: the first corresponding, restrictively, to a phenyl ring, whilst the second tolerates various structural features because it likely spills over a region of bulk tolerance (Fig. 3). The Tic-hydantoin core could play the role of this second hydrophobic group.

To obtain compounds **5a**–**j** of A series (Table 1), the starting material was commercial (*S*)-(−)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **2** (L-Tic-OH) whose secondary amine function was protected using Boc_2O before its transformation into methyl ester **4** (Scheme 1). The release of the amine function was realized by treatment with a TFA/CH_2Cl_2 1:1 mixture.

The urea was formed and cyclization was achieved as described in the previous article in a "one pot" reaction by the action of appropriate 3-chloropropyl- or 2-chloroethylisocyanate in a DIEA/CH_2Cl_2 mixture.[11,14] Compounds **1a**–**b** and **5a**–**j** were obtained by nucleophilic substitution of the chlorine atom.

For compounds **10a**–**r** of the B series (Table 2), the method described in Scheme 1 was first tried using appropriate chloroalkylisocyanates with the additional step of substitution of the chlorine atom by *N*-alkylmethylamine. This procedure was feasible when using primary amines (R = H), for instance *N*-benzylamine and 4-phenyl-1-butylamine, led to compounds **10a** and **10b**, respectively,

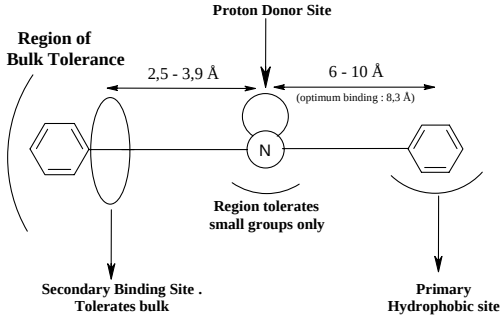
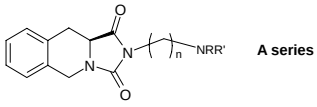
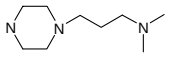
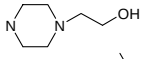
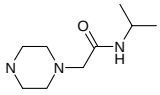
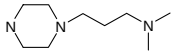
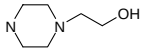
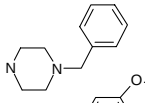
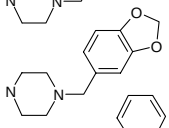
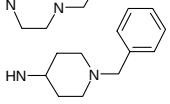
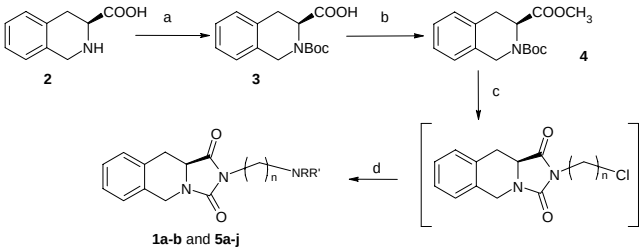


Figure 3. Pictorial representation of proposed features for σ_1 binding.[10]

Table 1. σ-binding assays on compounds **1a**–**b** and **5a**–**j**. Mean IC_{50} ± SD values for two to three independent experiments are shown



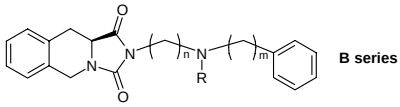
Compound	*n*	NRR′	IC_{50} σ_1 (nM)
Haloperidol	—	—	2.1 ± 0.3
1a	2		16 ± 3
5a	2		>100
5b	2		>1000
5c	2		>100
5d	2		>100
1b	3		21.5 ± 4.0
5e	3		>100
5f	3		>100
5g	3		>100
5h	3		32 ± 6
5i	3		23.8 ± 3.7
5j	3		82.3 ± 10.9



Scheme 1. Reagents and conditions: (a) Boc_2O 1.1 equiv, NaOH 1 M 1.1 equiv, dioxane, rt, 12 h, 98%; (b) (i) Cs_2CO_3 0.5 equiv, H_2O, MeOH, rt, 10 min, (ii) CH_3I 1.1 equiv, DMF, rt, 12 h, 90%; (c) (i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 equiv, CH_2Cl_2, rt, 15 min, (iii) 3-chloropropyl- or 2-chloroethylisocyanate 2.5 equiv, CH_2Cl_2, rt, 12 h; (d) HNRR′ 8 equiv, K_2CO_3 3 equiv, CH_3CN, reflux, several hours, 30–70%.

in medium overall yields (50 and 35%, respectively). But the first attempts using *N*-methylbenzylamine gave poor overall yields, respectively, 16 and 9% for $n = 2$ and 3

Table 2. σ-binding assays on compounds **1a** and **10a–o**



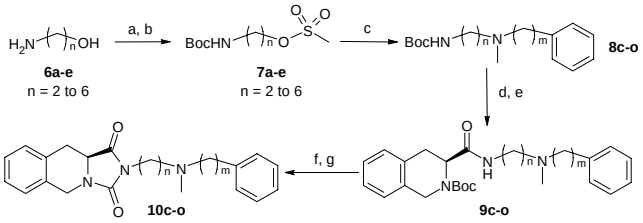
Compound	R	*n*	*m*	IC_{50} σ_1 (nM)	IC_{50} σ_2 (nM)[a]	Ratio σ_2/σ_1[a]
Haloperidol				2.1 ± 0.3	70 ± 20	33
1a	—	—	—	16 ± 3	>1000	>60
10a	H	3	1	9.6 ± 1.4	>1000	>100
10b	H	3	4	10.5 ± 2	nd	nd
10c	CH_3	2	1	4.5 ± 0.1	972 ± 41	216
10d	CH_3	2	2	12.6 ± 1.6	nd	nd
10e	CH_3	2	3	13.6 ± 1.9	nd	nd
10f	CH_3	2	4	3.2 ± 0.2	358 ± 60	112
10g	CH_3	2	5	2.9 ± 0.1	>1000	>340
10h	CH_3	3	1	3.9 ± 0.1	502 ± 98	129
10i	CH_3	3	2	2.1 ± 0.1	21 ± 11	10
10j	CH_3	4	1	4.2 ± 0.1	>100	>20
10k	CH_3	4	2	9.9 ± 0.1	nd	nd
10l	CH_3	5	1	5.0 ± 0.9	81 ± 16	16
10m	CH_3	5	2	11.7 ± 0.3	nd	nd
10n	CH_3	6	1	9.4 ± 1.8	nd	nd
10o	CH_3	6	2	29.5 ± 3.6	nd	nd
10p		3	1	>100	nd	nd
10q	—	—	1	2.4 ± 0.1	24 ± 11	10
10r	CH_3	3	0	>100	nd	nd

Mean IC_{50} ± SD values for two to three independent experiments are shown.
[a] nd, not determined.

(compounds **10c** and **10h**). Under the same conditions, compound **10d** was obtained from *N*-methylphenethylamine in poor yield (15%). *N*-methylphenylalkylamines showed indeed low reactivity, resulting in an increase in time of reaction and then decomposition of the compounds under the heating conditions. Thus, another strategy was to introduce diversity directly on the amine, before coupling with the Tic-hydantoin core. Protected amines **8c–o** were first synthesized in a three-step process starting from an appropriate alkylaminoalcohol (Scheme 2). Then, the deprotected amines were coupled with Boc-L-Tic-OH **3** using HOBt/EDCI activation. After deprotection of the secondary amino group, 1,1′-carbonyldiimidazole (CDI) was used to yield hydantoins **10c–o**. This procedure allows easy access to diversify hydantoin based products further.[15] Experimental procedure was optimized to allow rapid synthesis of a large set of hydantoins.

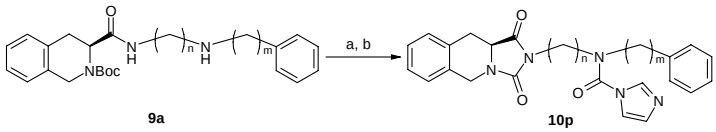
This last method could not be used to obtain secondary amine derivatives, such as compounds **10a** and **10b**. Indeed, when trying to obtain compound **10a** using the CDI method, the secondary nitrogen atom of the amino derivative **9a** reacts with CDI during the cyclization step to give the compound **10p** (Scheme 3). The use of one equivalent of CDI did not avoid this secondary reaction.

Finally, two more compounds were synthesized using this last method: **10q** with a more rigid scaffold (from 4-amino-1-benzylpiperidine) and **10r** with a less basic nitrogen atom (starting from *N*-(3-aminopropyl)-*N*-methylaniline) to verify the importance of the binding of this nitrogen with a proton donor site (Fig. 4).



Scheme 2. Reagents and conditions: (a) Boc_2O 1.1 equiv, CH_2Cl_2, rt, 12 h; (b) MsCl 1.3 equiv, TEA 2 equiv, CH_2Cl_2, 0 °C, 2 h; (c) phenylalkylmethylamine 3 equiv, K_2CO_3 4 equiv, CH_3CN, 40 °C, 24–72 h; (d) (i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 equiv, CH_2Cl_2, rt, 15 min; (e) compound **3** 1 equiv, HOBt 1.1 equiv, EDCI 1.1 equiv, CH_2Cl_2, rt, 12 h; (f) (i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 equiv, THF, rt, 15 min; (g) CDI 2 equiv, THF, reflux, 12 h.

All the compounds were assayed in binding assays on guinea pig cerebral cortex σ_1 receptor using haloperidol as reference compound. For compounds showing high σ_1 affinity, binding assays were also performed on rat σ_2 receptor.[16–19] The specific ligand binding to the receptors is defined as the difference between the total binding and the non-specific binding determined in the presence of an excess of unlabeled ligand. The biochemical results are presented as IC_{50} value, concentration causing a half-maximal inhibition of control-specific



Scheme 3. Reagents and conditions: (a) (i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 equiv, THF, rt, 15 min; (b) CDI 2 equiv, THF, reflux, 12 h, 30%.

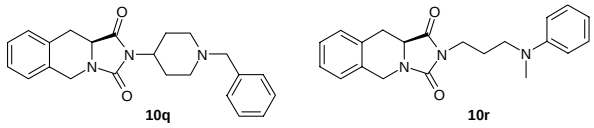


Figure 4. Compounds **10q** and **10r**.

binding[20] (Tables 1 and 2). Some compounds were tested for cytotoxicity upon a human diploid embryonic lung cell line (MRC-5 cells) using the colorimetric MTT assay (Table 3).[21]

With regard to A series (Table 1), introduction of benzylpiperazine templates results in retention of σ_1 affinity (compounds **5h** and **5i**). In contrast, the piperazines **5b–g**, which are not substituted by aromatic groups, lost σ_1 affinity. The 4-amino-1-benzylpiperidinyl compound **5j** showed moderate σ_1 affinity. In this series, a second hydrophobic group seems to be required for σ_1 affinity, which is consistent with the previously proposed model (Fig. 3). As stated in Introduction, it was first considered that the Tic core fitted in the left hydrophobic site of the proposed model due to the fact that this site was likely to tolerate steric bulk. However, it was recently suggested that the right hydrophobic, could tolerate steric bulk as well.[22] Taking that into account, it was suggested that two modes of binding may be possible: binding of the Tic core at the left hydrophobic site or at the right one. Whether the Tic core binds at the left or right region might be depending on the presence or not of a benzylic moiety.

Most active compounds of the A series were submitted to σ_2 binding test, but very low inhibition percentages were obtained at 10 μM, showing a good σ_2/σ_1 ratio.

With regard to B series, the σ_1 affinity was enhanced for a great majority of compounds (except compounds **10d**, **10e**, **10o**, **10p**, and **10r**) compared with the lead compound **1a**. This suggests that there is no minimal chain length requirement. Conversely, increasing the distance between the two aromatic groups (compound **10o**: *n*,*m* = 6,2) was detrimental to the σ_1 affinity.

Table 3. Cytotoxicity on MRC-5 cells and selectivity index

Compound	CC_{50} (μM)	IS
10b	31 ± 1	2952
10c	107 ± 4	23778
10e	136 ± 7	10000
10h	196 ± 11	50256
10k	106 ± 3	10707
10l	101 ± 20	20200

Mean CC_{50} ± SD values for two to three independent experiments are shown.

The compounds of B series were designed according to Glennon's model, which describes optimum length for the chain linking the nitrogen atom and the phenyl ring. Regardless of the lower σ_1 affinities of the compounds of B series compared to the affinities of the phenylpiperazines or phenylpiperidines described in Glennon's work,[10] the spacer length on both sides of the central nitrogen atom does not seem to significantly influence the affinity. For instance compounds **10c** (*n*,*m* = 2,1), **10f** (*n*,*m* = 2,4), **10g** (*n*,*m* = 2,5), and **10l** (*n*,*m* = 5,1) displayed similar affinity. Assuming that the Tic core binds at the left hydrophobic site would then lead to the conclusion that there is actually no direct correlation between the affinity and respective side chain length. Nevertheless, present results could also be interpreted in terms of different ways of binding. The decrease in affinity from **10c** (*n*,*m* = 2,1; IC_{50} = 4.5 nM) to **10d** and **10e** (*n*,*m* = 2,2 and 2,3; IC_{50} = 12.6 and 13.6, respectively) could be explained by the hypothesis that these compounds bind in one way which is disfavored by an increase in chain length. The fact that compounds **10f** and **10g** (*n*,*m* = 2,4 and 2,5; IC_{50} = 3.2 and 2.9 nM, respectively) showed similar affinity as **10c**, despite their even longer side chain, could then be explained by the existence of another (opposite) way of binding.

However, conclusions can hardly be drawn without further investigations.

The significant loss of affinity for compounds **10p** and **10r** confirmed the contribution of the proton-accepting nitrogen atom for σ_1 receptor binding, as previously described by Glennon. The secondary amino compound **10a** showed a slightly lower σ_1 affinity than its tertiary N-methylated analog **10h**. More constrained compound **10q** provided an increase in affinity but detriment to the selectivity σ_2/σ_1.

A preliminary study of the cytotoxicity upon MRC-5 cells of some compounds from the B series was investigated to evaluate the therapeutic potential of this series. As shown in Table 3, tested compounds present a low cytotoxicity with CC_{50} values superior to 100 μM for tertiary amino compounds. All these compounds provided a selectivity index (ratio CC_{50}/IC_{50} on σ_1 receptor) superior to 10,000. Replacement of the tertiary nitrogen by a secondary nitrogen (compound **10b**), in addition to be detrimental to the affinity, seems to increase the cytotoxicity.

By holding the Tic-hydantoin template constant, it has been possible to explore the nature of the side chain.

It led us to optimize the affinity of the lead compound **1a** by highlighting new selective σ_1 ligands with high affinity, among them being compounds **10f**, **10g**, and **10h**. This study was performed in parallel to the evaluation and optimization of the Tic-hydantoin core described in the previous article. Further investigations combining the results of these two studies should allow us to reach σ_1 ligands with higher affinity and better selectivity. Nevertheless, with such a high selectivity index, the described compounds could be considered as potential leads or candidates for therapy.

Acknowledgments

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16. σ_1 receptors were extracted from guinea-pig cerebral cortex according to Bowen et al.[17] Membranes were incubated with 2 nM [^{3}H](+)pentazocine in 5 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.5) for 150 min at 22 °C. Non-specific binding was determined under similar conditions but in the presence of 10 μM unlabeled haloperidol.[18]
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19. σ_2 receptors were extracted from rat cerebral cortex according to Bowen et al.[17] Membranes were incubated with 5 nM [^{3}H]DTG (+300 nM (+)pentazocine) in 5 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.5) for 120 min at 22 °C. Non-specific binding was determined under similar conditions but in the presence of 10 μM unlabeled haloperidol.
20. IC_{50} values from competitive inhibition experiments were determined using the Marquardt–Levenberg nonlinear curve fitting procedure of Xfit macro (Microsoft Excel).
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