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Synthesis and pharmacological evaluation of Tic-hydantoin derivatives as selective σ_1 ligands. Part 1

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Abstract—Herein is described a new class of selective σ_1 ligands consisting of tetrahydroisoquinoline-hydantoin (Tic-hydantoin) derivatives. Compound **3a** has high affinity (IC_{50} = 16 nM) for the σ_1 receptor and is selective in a large panel of therapeutic targets. This first study presents structural changes around the Tic-hydantoin core, leading to a Tic-hydantoin analogue with a higher σ_1 affinity ($IC_{50} \approx 1$ nM).

Di- and trisubstituted hydantoins have been widely used in biological screening, resulting in numerous pharmaceutical applications.[1–3] As part of our strategy towards the preparation and biological evaluation of hydantoin-containing heterocycles, the hydantoin ring (compounds **1**) was fused by its C-5 and N-1 positions to another ring to design a new series of more constrained derivatives **2** (Fig. 1). Indeed, the tetrahydroisoquinoline ring (Tic) was selected after screening different combinatorial libraries, frequently containing such molecular structures,[4,5] and a convenient and efficient method to prepare Tic-hydantoins was described.[6] Among several interesting compounds, pharmacological screening of the hydantoin **3a** (Fig. 1) was then performed to define potential targets.

Interestingly, following screening of a large range of receptors, compound **3a** was found to bind selectively to the guinea pig σ_1 receptor with an IC_{50} of 16 nM.[7]

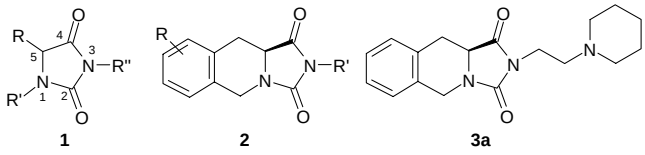

Figure 1. Di- and trisubstituted hydantoins **1**, more constrained Tic derivatives **2**, and compound **3a**.

σ receptors have been widely characterized in binding studies. They are distinct from any other known receptors and have been defined by their high affinity for various compounds including *N*-allyl-normetazocine, pentazocine, 1,3-di-*o*-tolylguanidine (DTG), (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine and haloperidol.[8] It is now widely accepted that there are at least two subtypes of σ receptors denoted as σ_1 and σ_2.[8] The σ_1 subtype is the best characterized and remains the only one to have been cloned from many sources, including guinea pig liver,[9] human placental choriocarcinoma cells,[10] human brain,[11] or rat brain.[12] It consists of a 228 amino acid protein with a molecular weight of ~25 kDa. Its amino acid sequence is shared with more than 90% similarity between the different species and presents no analogy with any known mammalian protein.[8] Recent evidence has indicated that σ_1 receptors may be involved in regulating a variety of neurotransmitters in the central nervous system, including cholinergic,[13,14] dopaminergic[15] and glutamatergic systems.[16,17] Thus, there is a sustained interest for developing

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selective σ_1 ligands, which stems from the possibility of developing new drug candidates particularly for the treatment of depression,[18] psychiatric disorders, memory deficits and drug addiction.[19,20] The σ_2 subtype has not been cloned yet but was found to be a 18–21 kDa protein.[21] σ_2 receptors have been implicated in motor control behaviour[22] and in the regulation of cell proliferation and viability.[23] Thus, σ_2 ligands may be developed to attenuate motor side effects associated with typical antipsychotic agents or for the diagnossis and treatment of cancer.

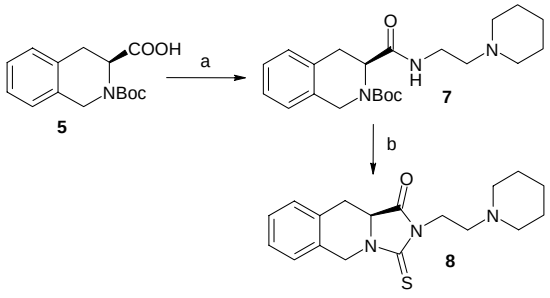
Although there are an increasing number of new selective σ ligands,[24–27] there is still a need to continue developing new structures. The interest in the Tic-hydantoin core is not only due to σ_1/σ_2 selectivity of compound **3a**, but especially the selectivity towards more than 35 other receptors. The specificity of this pharmacological profile prompted the evaluation of the relative importance of both parts of the compounds: the Tic-hydantoin group or the basic side chain. In this paper, we report on our efforts towards the modulation of the Tic-hydantoin core of the lead compound **3a**. Parallel work for the evaluation and optimization of the side chain will be described in the following article.

Compounds **3a**–**b** were synthesized, according to the procedure described in Scheme 1. The starting material was (*S*)-(−)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **4S** (L-Tic-OH);[6] due to reactivity and solubility issues, two protection steps were necessary before reaction with the appropriate isocyanate, the secondary amine function being protected using Boc_2O (compound **5**) before its transformation into methyl ester **6** with methyl iodide. The deprotection of the secondary amino group of compound **6** was performed using a TFA/CH_2Cl_2 1:1 mixture. The use of a chloroalkylisocyanate did not allow the cyclization in a methanolic 1 M NaOH solution, as previously described.[6] A 'one pot' reaction was applied, addition of appropriate isocyanate to a solution of intermediate L-Tic-OMe in dry CH_2Cl_2, in the presence of a large excess of DIEA (15 equiv), led directly to the formation of chloroalkylhydantoins. These latter intermediates were not isolated and, after evaporation to dryness, the substitution of the terminal chlorine atom was performed by treating the hydantoin with an excess of appropriate amine in refluxing acetonitrile, in the presence of K_2CO_3.

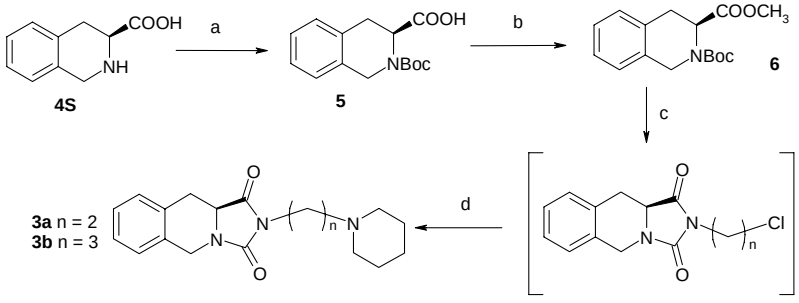
The importance of oxygen atoms to the hydantoin ring was tested, first by replacing the urea functionality by a thiourea and then by a partial or complete reduction of the hydantoin ring.

The procedure applied to obtain sulfur analogue **8** of compound **3a** was adapted from the one previously designed in the laboratory (Scheme 2).[28] Boc-protected L-Tic-OH **5** was coupled with 1-(2-aminoethyl)piperidine using HOBt/EDCI activation and DIEA as a base. This coupling method was retained due to the good solubility of by-products in the aqueous phase.[29] After deprotection of the secondary amino group using a TFA/CH_2Cl_2 mixture, the crude product was dissolved in THF and excess DIEA (4.5 equiv). 1,1′-Ihiocarbonyldiimidazole was added to yield the thiohydantoin **8**.[26]

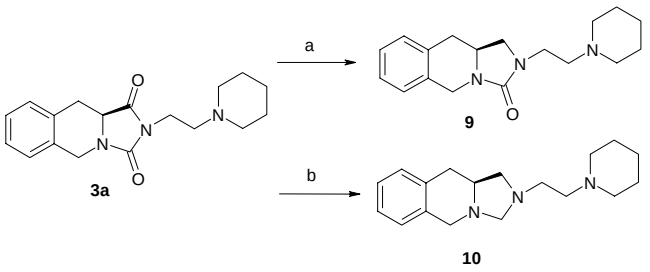
The reduction of hydantoin **3a** in urea **9** or in amine **10** was realized by using, respectively, the BH_3/THF complex or $LiAlH_4$, in refluxing THF (Scheme 3). In the second case, the expected amine was easily extracted from aluminium/lithium salts after a specific treatment with an aqueous solution of NaOH 15% and water according to a previously published procedure.[30,31]



Scheme 2. Reagents and conditions: (a) aminoethylpiperidine 1 equiv, HOBt 1.1 equiv, EDCI 1.1 equiv, DIEA 2 equiv, CH_2Cl_2, rt, 2 h, 90%; (b) (i) TFA/CH_2Cl_2 1:1, rt, 1 h, (ii) 1,1′-thiocarbonyldiimidazole 1.5 equiv, DIEA 4.5 equiv, THF, reflux, overnight, 60%.



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, 1 h, (ii) DIEA 15 equiv, CH_2Cl_2, rt, 15 min, (iii) appropriate 3-chloropropyl- or 2-chloroethylisocyanate 2.5 equiv, CH_2Cl_2, rt, 12 h; (d) piperidine 8 equiv, K_2CO_3 3 equiv, CH_3CN, reflux, 24 h, 50–65%.



Scheme 3. Reagents and conditions: (a) BH_3/THF 10 equiv, dry THF, reflux, 12 h, 45%; (b) $LiAlH_4$, 8 equiv, dry THF, reflux, 12 h, 28%.

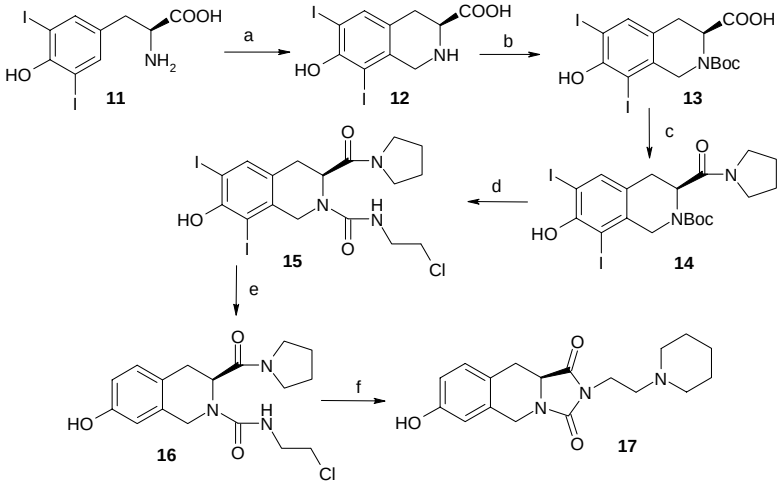
The next modifications were made around the Tic core by substituting or eliminating the aromatic group and modifying the size of the isoquinoline cycle. To reduce the hydrophobicity of the aromatic ring, the compound **17** substituted by a hydroxyl group in 7-position was synthesized, according to the synthetic sequence described in Scheme 4.

The Pictet–Spengler reaction has been applied as a key step to access the tetrahydroisoquinoline moiety starting from 3,5-diiodo-L-tyrosine **11**. Compound **12** was easily obtained[32] and transformed into the corresponding Boc derivative **13** by the action of Boc_2O. The protected precursor **13** was converted to the activated 1-hydroxy-1,2,3-benzotriazole (HOBt) ester and condensed with pyrrolidine to give the amide **14**. After deprotection of the secondary amino group, 2-chloroethylisocyanate was introduced to yield the urea **15**. This latter compound has been dehalogenated by catalytic hydrogenation with 10% Pd/C furnishing compound **16**, according to a described procedure.[32] The hydroxyl derivative **17** was obtained after cyclization and substitution steps in the presence, respectively, of aqueous NaOH 1 M and 1-ethylpiperidine.

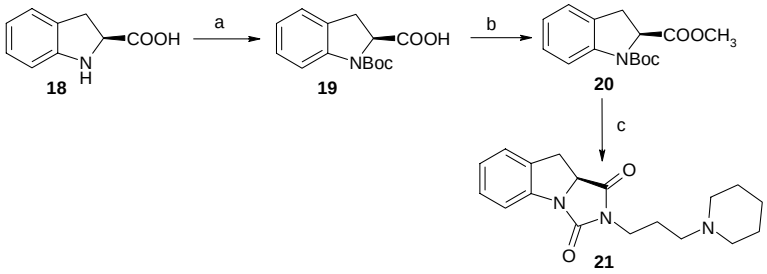
The importance of the Tic core was also evaluated, first by its replacement by an indoline core in compound **21**, which was synthesized, according to a procedure similar to that described in Scheme 1, starting from (*S*)-(−)-indoline-2-carboxylic acid **18** (Scheme 5).

Then compound **23**, in which the aromatic cycle of the Tic core is missing, was synthesized, according to a procedure similar to that described in Scheme 2, starting from *N*-Boc-pipecolinic acid **22** (Scheme 6).

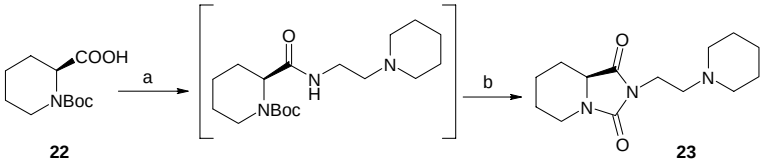
With the aim of evaluating the importance of the configuration of the centre of chirality, (*R*)-derivative **24** was obtained, according to the procedure described in



Scheme 4. Reagents and conditions: (a) formaldehyde 4 equiv, HCl 35%, 1,2-dimethoxyethane, 72 °C, 18 h, 45%; (b) Boc_2O 1.4 equiv, Et_3N 1.8 equiv, DMF, H_2O, rt, 4 h, 90%; (c) DCC 1 equiv, HOBt 1 equiv, pyrrolidine 1 equiv, CH_2Cl_2, rt, 12 h, 82%; (d) (i) TFA/CH_2Cl_2 1:1, rt, 1 h, (ii) DIEA 15 equiv, CH_2Cl_2, rt, 15 min, (iii) 2-chloroethylisocyanate 5 equiv, rt, 12 h; (e) H_2, Pd/C 10%, Et_3N 2 equiv, rt, 5 days, 28%; (f) aqueous NaOH 1 M 1.1 equiv, CH_2Cl_2, rt, 4 h, then piperidine 10 equiv, 1-ethylpiperidine 2 equiv, CH_2Cl_2, reflux, 12 h, 30%.



Scheme 5. 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, 1 h, (ii) DIEA 15 equiv, CH_2Cl_2, rt, 15 min, (iii) 3-chloropropylisocyanate 2.5 equiv, CH_2Cl_2, rt, 12 h, (iv) piperidine 8 equiv, K_2CO_3 3 equiv, CH_3CN, reflux, 12 h, 15%.



Scheme 6. Reagents and conditions: (a) aminoethylpiperidine 1 equiv, HOBt 1.1 equiv, EDCI 1.1 equiv, DIEA 2 equiv, CH_2Cl_2, rt, 2 h; (b) (i) TFA/CH_2Cl_2 1:1, rt, 1 h, (ii) 1,1′-carbonyldiimidazole 1.5 equiv, DIEA 4.5 equiv, THF, reflux, overnight, 65%.

Scheme 1 starting from (*R*)-(−)-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid **4R** (Fig. 2).

All the compounds were assayed in binding assays on guinea pig cerebral cortex σ_1 receptor using haloperidol as reference compound.[33,34] Most active compounds were tested on rat cerebral cortex σ_2 receptor.[34,36] The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The biochemical results are presented as IC_{50} value, concentration causing a half-maximal inhibition of control specific binding (Table 1).[37]

The lead compound **3a** has a high affinity for σ receptors and good σ_1 versus σ_2 selectivity. No significant difference in affinity and selectivity can be seen between compound **3a** with the two methylene side chain and its three methylene side-chain homologue **3b**. However, the influence of the side-chain length will be studied in the following article.

With regard to modifications of the Tic core, the reduction of hydantoin **3a** in urea **9** or in amine **10** is detrimental to the σ_1 affinity, resulting in a 3- and 5.5-fold decrease in affinity, respectively, while selectivity is conserved. In the first instance, this lower affinity seems to indicate the need for H bond acceptors. However, replacement of urea by thiourea in compound **8** increased the affinity, even though the sulfur atom is not an H bond acceptor. According to the latter observation, role of oxygen and sulfur atoms must be interpreted differently. Nevertheless, thiourea-containing compounds often show many adverse reactions, while in many cases the corresponding urea compounds do not cause similar toxicity.[38] The affinity of compound **8** for σ_2 receptor is higher, though the selectivity σ_2/σ_1 is preserved.

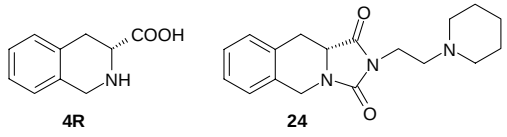


Figure 2. Compound **24** and its reagent **4R**.

Table 1. Binding assays on σ_1 and σ_2 receptors

Compound	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
3a	16 ± 3	>1000	>60
3b	21.5 ± 4.0	>1000	>45
8	0.9 ± 0.1	507 ± 80	563
9	54 ± 5	>1000	>15
10	77 ± 6	>1000	>10
17	>1000	nd	nd
21	66 ± 8	>1000	>15
23	>1000	nd	nd
24	6.6 ± 1.9	>1000	>150

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

[a] nd, not determined.

The replacement of the Tic core by an indoline core (compound **21**) produces a 3.5-fold decrease in σ_1 affinity. The absence of the aromatic ring or its substitution by a hydroxyl group in 7-position, in compounds **23** and **17**, respectively, causes a dramatic loss of affinity. It has been previously described that arylalkylamines are pharmacophores for σ_1 binding and a pharmacophore model has been proposed.[39] Loss of σ_1 affinity for compounds **17** and **23** is consistent with this model.

The configuration of the asymmetrically substituted carbon seems to have an interesting influence on the affinity for σ_1 receptor, resulting in a more than 2-fold increase for the (*R*)-enantiomer **24**, while the selectivity versus σ_2 receptor is preserved.

These first results highlight the Tic-hydantoin and Tic-thiohydantoin cores as new pharmacophoric moieties for σ_1 receptor ligands with high affinity. Our parallel and independent effort towards the evaluation and optimization of the side chain will be described in the following article.

Acknowledgments

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