European Journal of Medicinal Chemistry 45 (2010) 256–263

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Sigma-1 ligands: Tic-hydantoin as a key pharmacophore

Marion Toussaint [a], Deborah Mousset [a], Catherine Foulon [b], Ulrich Jacquemard [a], Claude Vaccher [b], Patricia Melnyk [a,*]

[a] UMR CNRS 8161 - Université Lille Nord de France - Institut Pasteur de Lille 1 rue du Professeur Calmette, B.P. 447, 59021 Lille cedex, France
[b] EA4034, Faculté de Pharmacie de Lille, Université Lille Nord de France, 3 rue du Professeur Laguesse, 59006 Lille, France

ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form
10 August 2009
Accepted 1 October 2009
Available online 9 October 2009

Keywords:
Sigma receptor
Hydantoin
Tetrahydroisoquinoline

ABSTRACT

Sigma-1 receptors are involved in numerous pathological dysfunctions and the synthesis of selective ligands is of interest. We identified a fused tetrahydroisoquinoline-hydantoin (Tic-hydantoin) structure with high affinity and selectivity for these receptors. We report here our efforts towards the pharmacomodulation of this substructure, the synthesis of 9 analogs with stereochemistry inversion, opening of isoquinoline ring, removal of isoquinoline nitrogen, replacement of isoquinoline by pyridine, of Tic-hydantoin moiety by quinazolinedione heterocycle. All these analogs provided a loss in the affinity for the sigma-1 receptor. The present work underlines the real importance of the Tic-hydantoin moiety for the obtainment of high affinity ligands.

1. Introduction

Sigma receptors were first described by Martin et al. in 1976 [1]. They are now classified into two distinct subtypes denoted σ_1 and σ_2 [2]. These subtypes display a different tissue distribution and a distinct physiological and pharmacological profile in the central and peripheral nervous system. Based on the predicted 223 amino acid sequence, sigma-1 receptor shares no homology with any mammalian proteins. Homology analysis of the amino acid sequence has suggested that the sigma-1 receptor has two transmembrane segments, resulting in an extracellular loop of approximately 50 amino acids and an intracellular C-terminus of approximately 125 amino acids [3,4]. According to this model, the N-terminus is very short and also localised intracellularly. The ligand binding region together with the second hydrophobic region has been suggested to be important for the binding of ligands [5] but the exact binding site has still to be elucidated. σ_1 receptors were described to modulate the transmission of neurotransmitters such as norepinephrine, dopamine, serotonin, acetylcholine and glutamate as well as the activity of opiate receptors [6]. Consequently, they are associated with some functions or disorders such as nociception [7], cocaine addiction [8–10], mnesic disorders [11], depression [12], anxiety, epilepsy [13] and are implicated in neuroprotection [14,15]. Furthermore, σ_1 proteins are over expressed in tumor cells, which make them a possible target for cancer treatment [16].

Previous studies in our laboratory evidenced the affinity of compound **1** (Fig. 1) containing the tetrahydroisoquinoline-hydantoin structure towards sigma-1 receptors [17] and a chemical approach to this structure was developed [18]. This compound was first optimized modifying in parallel the Tic-hydantoin structure and the amino side chain to provide a first lead **2** (Fig. 1). The modification of the Tic-hydantoin part consisted in substitution of the aromatic nucleus, aromatic deletion, reduction of the size of the central quinoline ring, reduction of the hydantoin ring and formation of a thiohydantoin [17]. The modification of the amino side chain took advantage of Glennon and Ablorpeddey model [19,20]. The optimal chain was elected as $n = 3$ and $m = 1$, regarding several criteria such as sigma-1 affinity (IC_{50} guinea pig = 3.9 nM), selectivity towards sigma-2 receptor ($IC_{50} > 500$ nM), a limited number of free rotation bond and a very low cytotoxicity providing a high selectivity index (ratio CC_{50}/IC_{50}) greater than 50,000 [21].

In this paper, we report our continuing efforts towards the modulation of Tic-hydantoin core of the lead compound **2** ($n = 3$, $m = 1$) and complete our previous study. Obviously, we decided to evaluate different changes in the substructure (Fig. 2). We focused

Abbreviations: P_{HPLC}, Purity determined by HPLC; TLC, Thin-layer chromatography; t_R, HPLC retention time; CDI, 1,1′-Carbonyldiimidazole; tCDI, 1,1′-Thiocarbonyldiimidazole; EP, Petroleum ether; AcOEt, Ethyl acetate; Hex, *n*-Hexane; Cyh, Cyclohexane; DCM, Dichloromethane; CAN, Acetonitrile; THF, Tetrahydrofuran; IsoQ, Isoquinoline; Pyr, Pyridine; DIEA, Diisopropylethylamine; TFA, Trifluoroacetic acid; PTSA, Paratoluenesulfonic acid; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-Hydroxybenzotriazole; aro, Aromatic.

* Corresponding author. Tel.: +33 3 20 87 12 19; fax: +33 3 20 87 12 33.
E-mail address: patricia.melnyk@ibl.fr (P. Melnyk).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.10.004

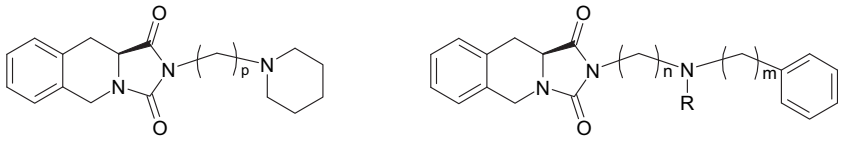


Fig. 1. Structure of compounds **1** and **2**.

our work on the importance of the stereochemistry, the synthesis of thiohydantoin, moderate modifications that were shown to be effective in our last work. To evaluate the importance of the constrained structure, we designed an open quinoline ring. The major changes were introduced with the deletion of the nitrogen atom, the deletion of the central ring with the dance of the nitrogen atom and the extension of the hydantoin ring.

2. Chemistry

N-methyl-*N*-benzyl-1,3-diamine **3** was synthesized according to our previously described procedure [21,22]. Slightly unstable and difficult to purify, it is conserved as its Boc protected form **4**. The synthesis of enantiomer compound **5** follows the same synthetic strategy as compound **2** and is described in Scheme 1. The starting material was commercial Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **6** (Boc-D-Tic-OH) whose secondary amine function was protected using Boc_2O. Amine **3** was preliminary deprotected by a TFA/CH_2Cl_2 1:1 treatment and followed by a large excess of DIEA (15 equiv), then coupled with acid **6** using HOBt/EDCI activation and an excess of DIEA to yield intermediate **7**. After deprotection of the secondary amino group, evaporation, and addition of THF and DIEA, 1,1′-carbonyldiimidazole (CDI) was added to yield hydantoin **5**. Both enantiomers are chemically and enantiomerically stable. The enantiomeric purity of both enantiomers was evaluated higher than 98% (chiral HPLC). The optical rotation of the two compounds are $[\alpha] = +132.0$ at 28 °C for compound **2** and $[\alpha] = -132.1$ at 28 °C for **5**. Preliminary study showed that those compounds were stable under acidic or neutral solution for more than 48 h.

Synthesis of thiohydantoin analog **8** was similar, starting from L-Tic-OH **9**. The cyclisation step was performed using thiocarbonyldiimidazole (Scheme 2). This thiohydantoin revealed a low chemical stability. Furthermore, HPLC experiments showed a complete racemisation of the compound. After preparative separation of both enantiomers, each compound showed a complete racemisation in ethanol after 30 min at 50 °C. Further details of the racemisation process will be published elsewhere.

The synthesis of analog **11** (Scheme 3) necessitates the preparation of N-Boc-N-methylphenylalanine **12** according to published method [23].

In order to obtain the analog compound **15** without the quinoline nitrogen, a Diels Alder cycloaddition of maleimide **13** and orthoquinodimethane was envisaged. Though modest, the higher yields were obtained according to the synthetic pathway described in Scheme 4. Maleimide **13** was synthesized from amine **3** and maleic anhydride in two steps. Intermediate orthoquinodimethane was obtained from sultine **14** easily prepared from α,α′-dichloro-*o*-xylene [24]. Pyridine analogs **16** and **17** were synthesized according to the same methodology starting from pyridine-2,3-dicarboxylic anhydride for **16** and pyridine-3,4-dicarboxylic anhydride for **17** (Scheme 5). The poor yields were due to the instability of the final compounds towards nucleophiles (methanol for instance) and the difficulty to take them out of silica.

Quinazolinedione analogs **23**–**25** were synthesized in two steps starting from isatoic anhydrides (Scheme 6). These latter compounds were either commercially available or synthesized according to Carter et al. [25] (compounds **18**, **19**).

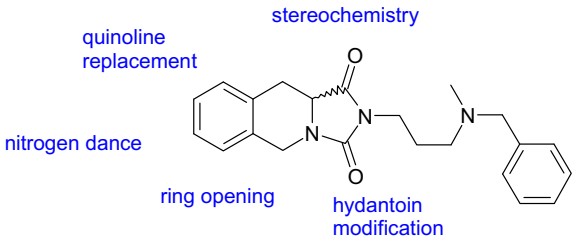


Fig. 2. Pharmacomodulations around the Tic-hydantoin core.

3. Biological results and discussion

All the compounds were evaluated in binding assays on human cerebral cortex σ_1 receptor using haloperidol as reference compound [26]. For compounds showing high σ_1 affinity, binding assays were also performed on rat σ_2 receptor [27]. 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 unlabelled ligand. The biochemical results are presented as IC_{50} value, concentration causing a half-maximal inhibition of control specific binding (Table 1) [28].

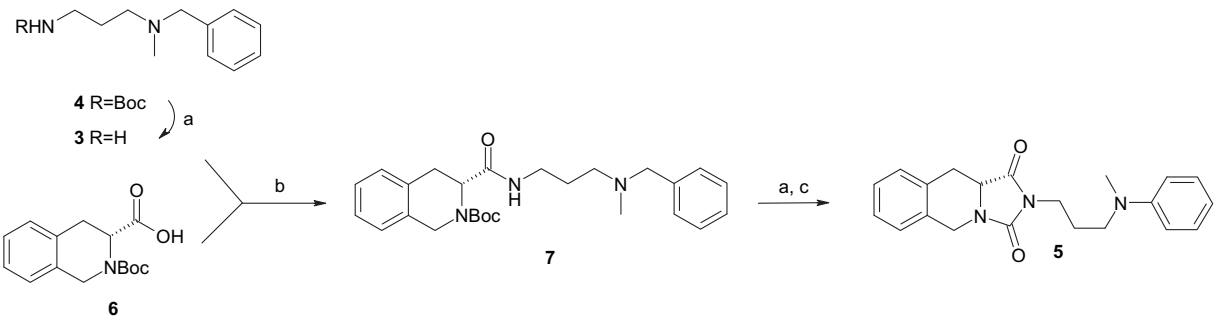
The lead compound **2** has a high affinity for σ receptors and good σ_1 versus σ_2 selectivity. The configuration of the asymmetrically substituted carbon seems to have an interesting influence on the affinity for σ_1 receptor resulting in a slight increase for the (*R*)-enantiomer **5**, while the selectivity versus σ_2 receptor is preserved.

Replacement of urea by thiourea in compound **8** increased the affinity, as we already described [17] but in this case the results have to take into account the chemical and enantiomeric unstability of the compound. Moreover, thiourea-containing compounds often show many adverse reactions, while in many cases the corresponding urea compounds do not cause similar toxicity [29].

Concerning the modifications of the Tic core, the opening of the isoquinoline ring in amine **11** causes a dramatic loss of affinity, highlightening the importance of a constrained structure whereas the elimination of the isoquinoline nitrogen in compound **15** results in a 6.5-fold decrease in affinity.

The replacement of the Tic core by a more polar pyridine nucleus core provides complete loss of σ_1 affinity for compounds **16** and **17**, which is consistent with Glennon's model.

As our lead compound **2** does not fit exactly with this model because of a too big distance between hydrophobic Tic substructure and central nitrogen atom, the replacement of Tic-hydantoin structure by quinazolinedione heterocycles should be of interest. In our case, non polar substituents were introduced on the aromatic ring providing in all examples an important decrease of affinity at least 10-fold.



Reagents: (a) i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 eq., THF, rt, 15 min; (b) HOBt 1.2 eq., HBTU 1.2 eq., CH_2Cl_2, rt, 12 h; (c) CDI 3 eq., THF, reflux, 12 h

Scheme 1. Synthesis of enantiomer **5**.

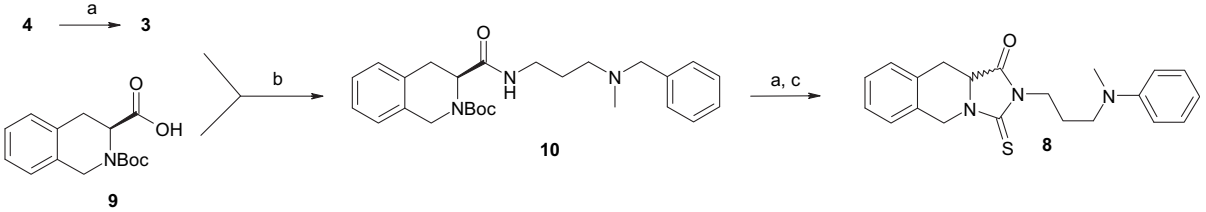
4. Conclusion

Sigma-1 receptors are associated with a lot of neuropsychiological disorders and the identification of potent ligands is of great interest. In the past, we identified a high affinity sigma-1 ligands related to the Tic-hydantoin structure and optimized the amino side chain. In this work, we described our efforts to modulate this substructure and the synthesis of about 9 new compounds. All of them showed a lower affinity or even no affinity for this receptor. Our work underlines the importance of the Tic-hydantoin core for affinity.

5. Materials and methods

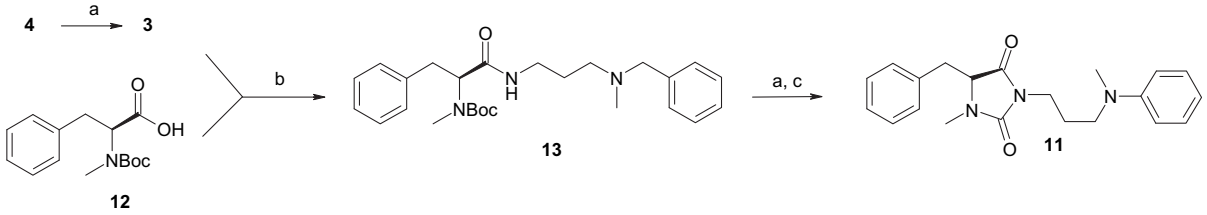
5.1. Chemistry

All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. Chromatography was undertaken using silica gel 60 (230–400 mesh ASTM) from Macherey-Nagel. Thin layer chromatography (TLC) was performed using silica gel from Merck, from which the compounds were extracted by the following solvent system: CH_2Cl_2/MeOH/NH_4OH, 80:20:1. NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. The attributions of the carbons are deduced after 2D experiments have been performed. Mass spectra were recorded on a MALDI-TOF Voyager-DE STR (Applied Biosystems, Palo Alto CA) with a trihydroxy-acetophenone matrix. The purity of final compounds was verified by two types of high pressure liquid chromatography (HPLC) columns: C18 Deltapak (C18N) and C4 Interchrom UP5WC4-25QS (C4). Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in Tampon B or MeOH and injected through a 50 μL loop. The following eluent systems were used: Tampon A (H_2O/TFA, 100:0.05) and Tampon B (CH_3CN/H_2O/TFA, 80:20:0.05). HPLC retention times (HPLC t_R) were obtained, at a flow rate of 1 mL/min, using the following conditions: for the 10 min method: a gradient run from 100% eluent A during 30 s, then to 100% eluent B over the next 8 min and for the 40 min method: a gradient run from 100% eluent A during 1 min, then to 100% eluent B over the next 30 min. Chiral chromatography was carried out on a Chiralpak AD (tris-2,5-dimethylphenylcarbamate, 250 × 4.6 mm I.D.; 10 μm; Daicel Chemical Industries, Baker, France) using a gradient Waters 600E metering pump model equipped with a Waters 996 photodiode array spectrophotometer (Waters, St Quentin-en-Yvelines, France). Chromatographic data were collected and processed on a computer running Millennium 2010. The sample loop was 20 μL (Rheodyne 7125 injector). The column eluate was monitored at 206 nm and 266 nm. For hydantoïns **2** and **5**, chiral separation was performed at 40 °C using an isocratic mobile phase of n-hexane/ethanol (40:60, v/v) at a flow rate of 1 mL/min. For thiohydantoïn **8**, chiral separation was performed at 20 °C using an isocratic mobile phase of n-hexane/2-propanol (90:10, v/v) at a flow rate of 1 mL/min. The peak of the solvent front was considered to be equal to the dead time (t_0) and was about 4.50 min. Compounds were chromatographed by dissolving them in ethanol to a concentration of 0.5 mM and passed through a 0.45 μm membrane filter prior to loading the column. The optical rotation of methanolic solutions (concentration 1 mg/mL) using Na D line (589 nm) was obtained using a polarimeter (Perkin Elmer Life and Analytical Systems, Boston, MA). The volume of the cell and the optical path were 1 mL and 10 cm, respectively. Measurements were performed at 28 °C. Reagents were obtained from Acros, Aldrich, Lancaster, Novabiochem and Avocado.



Reagents: (a) i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 eq., THF, rt, 15 min; (b) HOBt 1.2 eq., HBTU 1.2 eq., CH_2Cl_2, rt, 12 h; (c) tCDI 3 eq., THF, reflux, 12 h.

Scheme 2. Synthesis of thiohydantoin **8**.



Reagents: (a) i) TFA/CH_2Cl_2 1:1, rt, 30 min, (ii) DIEA 15 eq., THF, rt, 15 min; (b) HOBt 1.2 eq., HBTU 1.2 eq., CH_2Cl_2, rt, 12 h; (c) CDI 3 eq., THF, reflux, 24 h.

Scheme 3. Synthesis of compound **11**.

*5.1.1. (R)-3-[3-(Benzyl-methyl-amino)-propylcarbamoyl]-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester **7***

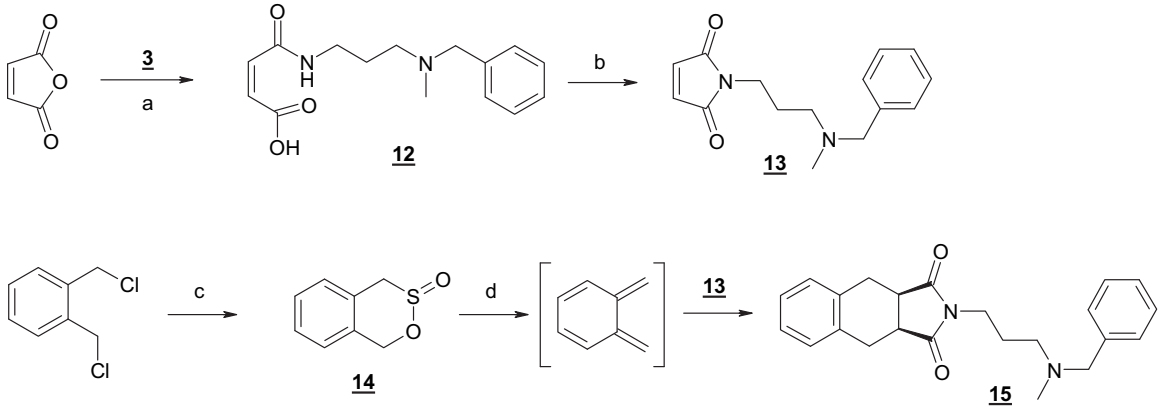
A solution of compound **4** (500 mg, 1.79 mmol) in DCM/TFA (10/10 mL), was stirred at room temperature during 30 min. The solvent was then evaporated to provide free amine **3**. A solution of Boc-D-1,2,3,4-tetrahydroisoquinoleine-3-carboxylic acid **6** (R) (Boc-D-TicOH; 496 mg, 1 equiv), HOBt (290 mg, 1.2 equiv), HBTU (814 mg, 1.2 equiv) in DCM (30 mL) was stirred at room temperature during 15 min. Then, a solution of free amine **3** and DIEA (4.7 mL, 15 equiv) in DCM (15 mL) was added and the reaction was stirred at room temperature overnight. The organic layer was washed with a solution of $NaHCO_3$ 5% (1×20 mL), brine (1×20 mL) and then dried over $MgSO_4$. The residue was purified by TLC (DCM/methanol, 9/1) to yield expected compound **7** as yellow oil (485 mg, 62% yield). TLC: R_f 0.6 (DCM/methanol, 9/1). HPLC (C18-10 min) P_{HPLC} 90%, t_R 5.3 min. ^{1}H NMR ($CDCl_3$): 1.4–1.5 (11H, m, $C(CH_3)_3$ and H_2), 1.9–2.0 (3H, m, NCH_3), 2.2–2.3 (2H, m, H_1), 2.9–3.0 (2H, m, H_3), 3.3–3.4 (4H, m, isoQ-H_4 and NCH_2Ph), 4.3–4.6 (3H, m, isoQ-H_1 and isoQ-H_3), 7.2–7.3 (9H, m, H_{aro}); ^{13}C NMR ($CDCl_3$) 25.9 (CH_2), 28.5 ($C\underline{(CH_3)_3}$), 31.8 (isoQ-C_4), 37.3 (C_1), 45.0 (isoQ-C_1), 50.5 (NCH_3), 54.9 ($\underline{C}_3$), 56.8 (isoQ-C_3), 62.1 ($N\underline{C}H_2Ph$), 126.3–126.9–127.6–128.6–129.4 (Caro); Formula $C_{26}H_{36}N_3O_3$, molecular weight 437.6, *m/z* 438.3 $[M+H]^+$.

*5.1.2. (R)-2-[3-(Benzyl(methyl)amino)propyl]-10,10a-dihydro-5H-imidazo[1,5-b]isoquinoline-1,3-dione **5***

A solution of compound **7** (528 mg, 1.2 mmol) in DCM/TFA (2/2 mL) was stirred at room temperature during 30 min. The solvent was evaporated *in vacuo* and the residue taken up in THF (20 mL). DIEA (3.15 mL, 15 equiv) was added. After stirring the mixture at room temperature for 10 min, CDI (588 mg, 3 equiv) was added and stirring was continued for 12 h at reflux. The solvent was evaporated *in vacuo* and the residue taken up in ethyl acetate (30 mL). The organic layer was washed with a solution of $NaHCO_3$ 5% (2×15 mL), brine (1×15 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by TLC (DCM/methanol/NH_4OH, 9/1/0.2) to yield expected compound **5** as yellow oil (379 mg, 87% yield). TLC: R_f 0.5 (DCM/methanol/NH_4OH, 9/1/0.2). HPLC (C18, 10 min) P_{HPLC} 99%, t_R 4.5 min; HPLC (C4, 40 min) P_{HPLC} 99%, t_R 14.4 min. ^{1}H NMR ($CDCl_3$): 1.89 (2H, quint, CH_2, $J = 7$ Hz), 2.21 (3H, s, NCH_3), 2.45 (2H, t, NCH_2, $J = 7$ Hz), 2.78 (1H, dd, H_{10}, $^2J = 16$ Hz, $J_{10-10a} = 12$ Hz), 3.25 (1H, dd, H_{10}, $^2J = 16$ Hz, $J_{10-10a} = 5$ Hz), 3.49 (2H, s, NCH_2Ph), 3.64 (2H, t, NCH_2, $J = 7$ Hz), 4.05 (1H, dd, H_{10a}, $J_{10a-10} = 12$ Hz, $J_{10a-10a} = 5$ Hz), 4.42 (1H, d, H_5, $^2J = 17$ Hz), 5.03 (1H, d, H_5, $^2J = 17$ Hz), 7.1–7.3 (m, 9H, H_{aro}); ^{13}C NMR ($CDCl_3$) 25.8 (CH_2), 30.9 (C_{10}), 37.1 (NCH_2), 41.4 (C_5), 41.8 (NCH_3), 54.3 (NCH_2), 54.3 (C_{10a}), 62.1 (CH_2Ph), 126.6–128.5–129.3 (C_{aro}); Formula $C_{22}H_{25}N_3O_2$, molecular weight 363.4, *m/z* 364.1 $[M+H]^+$; $[\alpha]_D^{28} = +132.0$ (MeOH, 1.0 mg/mL).

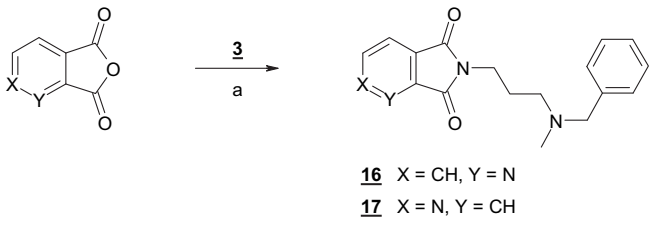
*5.1.3. (S)-3-[3-(Benzyl(methyl)amino)propylcarbamoyl]-3,4-dihydro-1H-isoquinoline-2-carboxylic acid tert-butyl ester ***10***

A solution of compound **4** (411 mg, 1.48 mmol) in DCM/TFA (10/10 mL) was stirred at room temperature during 30 min. The solvent was then evaporated to provide free amine **3**. A solution of Boc-L-1,2,3,4-tetrahydroisoquinoleine-3-carboxylic acid **9** (S)



Reagents: (a) DIEA (2 eq.), THF, rt, 12h; (b) (acetic anhydride/acetic acid) (2/1), 55°C, overnight; (c) rongalite (2 eq.), TBAB (0.2 eq.), DMF, rt overnight; (d) **13** (1.2 eq.), toluene, reflux, 24h.

Scheme 4. Synthesis of compound **15**.



Reagents: (a) PTSA (0.05 eq.), toluene, reflux, overnight.

Scheme 5. Synthesis of compounds **16** and **17**.

(Boc-L-TicOH; 411 mg, 1 equiv), HOBt (240 mg, 1.2 equiv), HBTU (673 mg, 1.2 equiv) in DCM (30 mL) was stirred at room temperature during 15 min. Then, a solution of free amine **3** and DIEA (3.9 mL, 15 equiv) in DCM (15 mL) was added and the reaction was stirred overnight at room temperature. The organic layer was washed with a solution of $NaHCO_3$ 5% (1×20 mL), brine (1×20 mL) and then dried over $MgSO_4$. The residue was purified by TLC (DCM/methanol, 9/1) to yield expected compound **10** as yellow oil (576 mg, 89% yield). TLC: R_f 0.6 (DCM/methanol, 9/1). HPLC (C18-10 min) P_{HPLC} 90%, t_R 5.3 min. ^{1}H NMR ($CDCl_3$): 1.4–1.5 (11H, m, $C(CH_3)_3$ and H_2), 1.9–2.0 (3H, m, NCH_3), 2.2–2.3 (2H, m, H_1), 2.9–3.0 (2H, m, H_3), 3.3–3.4 (4H, m, isoQ-H_4 and NCH_2Ph), 4.3–4.6 (3H, m, isoQ-H_1 and isoQ-H_3), 7.2–7.3 (9H, m, H_{aro}); ^{13}C NMR ($CDCl_3$) 25.9 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 31.8 (isoQ-C_4), 37.3 (C_1), 45.0 (isoQ-C_1), 50.5 (NCH_3), 54.9 (C_3), 56.8 (isoQ-C_3), 62.1 ($N\underline{C}H_2Ph$), 126.3–126.9–127.6–128.6–129.4 (C_{aro}); Formula $C_{26}H_{36}N_3O_3$, molecular weight 437.6, m/z 438.3 $[M+H]^+$.

5.1.4. 2-[3-(Benzyl(methyl)amino)propyl]-3-thioxo-2,3,10,10a-tetrahydro-5H-imidazo[1,5-b] isoquinoline-1-one *8*

A solution of compound **10** (366 mg, 0.84 mmol) in DCM/TFA (4/4 mL) was stirred at room temperature during 30 min. The solvent was evaporated *in vacuo* and the residue taken up in THF (20 mL). DIEA (2.18 mL, 15 equiv) was added. After stirring the mixture at room temperature for 10 min, tCDI (447 mg, 3 equiv) was added and stirring was continued overnight at reflux. The solvent was evaporated *in vacuo* and the residue taken up in ethyl acetate (30 mL). The organic layer was washed with a solution of $NaHCO_3$ 5% (2×15 mL), brine (1×15 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by TLC (DCM/methanol/NH_4OH, 9/1/0.1) to yield expected compound **8** as yellow oil (83 mg, 26% yield). TLC: R_f 0.4 (DCM/methanol, 95/5). HPLC (C18, 10 min) P_{HPLC} 90%, t_R 5.3 min; HPLC (C4, 40 min) P_{HPLC} 90%, t_R 19.4 min. ^{1}H NMR ($CDCl_3$): 1.94 (2H, quint, CH_2, $J = 7$ Hz), 2.19 (3H, s, NCH_3), 2.46 (2H, t, NCH_2, $J = 7$ Hz), 2.81 (1H, dd, H_{10}, $^2J = 15$ Hz, $J_{10-10a} = 12$ Hz), 3.26 (1H, dd, H_{10}, $^2J = 15$ Hz, $J_{10-10a} = 5$ Hz), 3.48 (2H, s, NCH_2Ph), 3.96 (2H, t, NCH_2, $J = 7$ Hz), 4.12 (1H, dd, H_{10a}, $J_{10a-10} = 12$ Hz, $J_{10a-10a} = 5$ Hz), 4.60 (1H, d, H_5, $^2J = 17$ Hz), 5.48 (1H, d, H_5, $^2J = 17$ Hz), 7.1–7.3 (m, 9H, H_{aro}); ^{13}C NMR ($CDCl_3$) 25.5 (CH_2), 30.9 (C_{10}), 40.2 (NCH_2), 42.2 (NCH_3); 46.1 (C5); 54.8 (NCH_2); 57.5 (C10a); 62.4 (CH_2Ph); 125.3–126.9–127.0–127.5–127.7–128.3–129.2–129.4 (C_{aro}); Formula $C_{22}H_{25}N_3OS$, molecular weight 379.5, m/z 380.2 $[M+H]^+$.

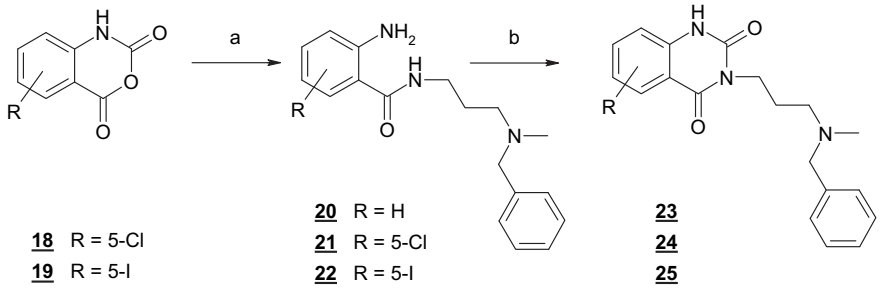
Table 1
Binding affinities for target compounds on σ_1 and σ_2 sites.

Compound	IC_{50} σ_1 (nM)	IC_{50} σ_2 (nM)	Ratio σ_2/σ_1
Haloperidol	23.0	230	10
2	8.7	650	75
5	13.7	1000	76
8	4.5	–	–
11	>200	–	–
15	57	–	–
16	>200	–	–
17	>200	–	–
23	116	–	–
24	>200	–	–
25	103	–	–

Mean IC_{50} values for 2–3 independent experiments are shown with less than 10% deviation

5.1.5. {1-[3-(Benzyl(methyl)amino)propylcarbamoyl]-2-phenylethyl}methylcarbamic acid tert-butyl ester *13*

A solution of compound **4** (114 mg, 0.41 mmol) in DCM/TFA (2/2 mL), was stirred at room temperature during 30 min. The solvent was then evaporated to provide free amine **3**. A solution of *N*-*tert*-butoxycarbonyl-*N*-methylphenylalanine **12** (S) (114 mg, 1 equiv), HOBt (66 mg, 1.2 equiv), HBTU (186 mg, 1.2 equiv) in DCM (8 mL) was stirred during 15 min. Then, a solution of free amine **3** and DIEA (1.1 mL, 15 equiv) in DCM (8 mL) was added and the reaction was stirred overnight at room temperature. The organic layer was washed with a solution of $NaHCO_3$ 5% (2×10 mL), brine (1×10 mL) and then dried over $MgSO_4$. The residue was purified by TLC (DCM/methanol, 95/5) to yield expected compound **13** as white powder (50 mg, 28% yield). TLC: R_f 0.3 (DCM/methanol, 95/5). HPLC (C18-10 min) P_{HPLC} 96%, t_R 2.5 min. ^{1}H NMR ($CDCl_3$): 1.30 (9H, s, $C(CH_3)_3$), 1.60 (2H, m, CH_2), 2.16 (3H, s, NCH_3), 2.35 (2H, t, NCH_2, $J = 6$ Hz), 2.68 (3H, s, $NBocCH_3$), 2.72 (2H, m, CH_2Ph), 3.22 (2H, m, $NHCH_2$), 4.43 (2H, s, NCH_2Ph), 4.67 (1H, m, CO–CH), 7.20–7.30 (m, 10H, H_{aro}); ^{13}C NMR ($CDCl_3$) 27.4 (CH_2), 29.0 ($C(CH_3)_3$), 31.7 ($\underline{C}H_2Ph$), 35.2 ($NHCH_2$), 39.8 (NCH_3), 54.5 (NCH_2), 62.2 (NCH_2Ph), 62.5 ($CO\underline{C}H$),



Reagents: (a) **3** (1.3 eq.), DIEA (5 eq.), DCM, rt, overnight; (b) DIEA (5 eq.), DCM, rt, 10 min, then CDI (3 eq.), THF, reflux, overnight.

Scheme 6. Synthesis of compounds **23**–**25**.

127.2–127.5–128.4–128.8–129.3–129.7 (C_{aro}); Formula $C_{26}H_{37}N_3O_3$, molecular weight 439.6, *m/z* 440.5 $[M+H]^+$.

5.1.6. (S)-5-Benzyl-3-[3-(benzyl(methyl)amino)propyl]-1-methylimidazolidine-2,4-dione ***11***

A solution of compound **13** (324 mg, 0.96 mmol) in DCM/TFA (4/4 mL) was stirred at room temperature during 30 min. The solvent was evaporated *in vacuo* and the residue taken up in THF (15 mL). DIEA (2.49 mL, 15 equiv) was added. After stirring the mixture at room temperature for 10 min, CDI (156 mg, 3 equiv) was added and stirring was continued overnight at reflux. The solvent was evaporated *in vacuo* and the residue taken up in ethyl acetate (40 mL). The organic layer was washed with a solution of $NaHCO_3$ 5% (20 mL), brine (20 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by TLC (AcOEt/methanol/NH_4OH, 9/1/0.05) to yield expected compound **11** as yellow oil (126 mg, 36% yield). TLC: R_f 0.4 (AcOEt/methanol, 96/4). HPLC (C18, 10 min) P_{HPLC} 90%, t_R 4.3 min; HPLC (C4, 40 min) P_{HPLC} 97%, t_R 21.8 min. ^{1}H NMR ($CDCl_3$): 1.50 (2H, quint, CH_2, $J=7$ Hz), 2.11 (3H, s, NCH_3), 2.24 (2H, t, NCH_2, $J=7$ Hz), 2.94 (3H, s, NCH_3), 3.15 (2H, m, CH_2Ph), 3.42 (m, 4H, NCH_2, NCH_2Ph), 4.07 (1H, t, COCH, $J=4$ Hz), 7.22 (m, 5H, H_{aro}); ^{13}C NMR ($CDCl_3$) 26.5 (CH_2), 29.8 (NCH_3), 35.1 (CH_2Ph), 37.1 (NCH_2), 41.8 (NCH_3), 54.5 (NCH_2), 62.2 (NCH_2Ph), 62.5 (CHCO), 127.2–127.5–128.4–128.8–129.3–129.7 (C_{aro}); Formula $C_{22}H_{27}N_3O_2$, molecular weight 365.4, *m/z* 366.3 $[M+H]^+$.

5.1.7. (Z)-3-[3-(Benzyl(methyl)amino)propylcarbamoyl]acrylic acid ***12***

A solution of amine **3** (373 mg, 0.96 mmol) and DIEA (0,22 mL, 2 equiv) in THF (2 mL) was stirred during 10 min. Maleic anhydride (113 mg, 1,2 equiv) was added and stirring was continued overnight at room temperature. The solvent was evaporated. The residue was purified by flash chromatography (Acetone/NH_4OH, 9/1) to yield expected compound **12** as yellow oil (228 mg, 86% yield). TLC: R_f 0.3 (Acetone/NH_4OH, 9/1). HPLC (C18, 10 min) P_{HPLC} 97%, t_R 2.9 min. ^{1}H NMR ($CDCl_3$): 1.86 (2H, quint, CH_2, $J=7$ Hz), 2.17 (3H, s, NCH_3), 3.35 (2H, t, NCH_2, $J=7$ Hz), 3.75 (2H, s, NCH_2Ph), 5.88 (2H, d, CH–CONH, $J=12$ Hz), 6.25 (2H, d, CH–COOH, $J=12$ Hz), 7.3–7.4 (5H, m, H_{aro}), 10.11 (1H, s, OH); ^{13}C NMR ($CDCl_3$) 27.2 (CH_2), 32.2 (NCH_3), 40.6 (NCH_2), 56.9 (NCH_2), 63.5 (NCH_2Ph), 130.9–131.3–132.4 (CH, C_{aro}), 138.2 (CH); Formula $C_{15}H_{20}N_2O_3$, molecular weight 276.3, *m/z* 277.1 $[M+H]^+$.

5.1.8. 1-[3-(Benzyl(methyl)amino)propyl]pyrrole-2,5-dione ***13***

A solution of acid **13** (218 mg, 0.79 mmol) in glacial acetic acid/acetic anhydride (2/1 mL) was stirred under nitrogen atmosphere at 50 °C for 24 h. The solvent was evaporated. The residue was purified by TLC (DCM/methanol, 95/5) to yield expected compound **13** as yellow oil (82 mg, 40% yield). TLC: Rf 0.3 (DCM/methanol, 95/5). HPLC (C18, 10 min) P_{HPLC} 95%, t_R 3.4 min. ^{1}H NMR ($CDCl_3$): 1.79 (2H, quint, CH_2, $J=7$ Hz), 2.16 (3H, s, NCH_3), 2.39 (2H, t, NCH_2, $J=7$ Hz), 3.46 (2H, s, NCH_2Ph), 3.58 (2H, t, NCH_2, $J=7$ Hz), 6.65 (2H, s, CH), 7.2–7.3 (5H, m, H_{aro}); ^{13}C NMR ($CDCl_3$) 26.4 (CH_2), 36.4 (NCH_2), 42.1 (NCH_3), 54.7 (NCH_2), 62.5 (NCH_2Ph), 127.2–128.4–129.3 (C_{aro}), 134,3 (CH); Formula $C_{15}H_{18}N_2O_2$, molecular weight 258.3, *m/z* 259.2 $[M+H]^+$.

5.1.9. 2-[3-(Benzyl(methyl)amino)propyl]-3a,4,9,9a-tetrahydrobenzo[f]isoindole-1,3-dione ***15***

To a solution of sultin **14** (100 mg, 0.59 mmol) in toluene (10 mL) was added maleimide **13** (183 mg, 1.2 equiv). Stirring was continued under nitrogen atmosphere at 50 °C for 24 h. The solvent was evaporated. The residue was purified by TLC (DCM/AcOEt/methanol, 6/4.5/0.5) to yield expected compound **15** as colorless oil (132 mg, 62% yield). TLC: R_f 0.5 (DCM/methanol, 95/5). HPLC (C18, 10 min) P_{HPLC} 98%, t_R 5.0 min; HPLC (C4, 40 min) P_{HPLC} 98%, t_R 11.2 min. ^{1}H NMR ($CDCl_3$): 1.33 (2H, quint, CH_2, $J=7$ Hz), 1.95 (3H, s, NCH_3), 1.96 (2H, t, NCH_2, $J=7$ Hz), 2.88 (2H, m, CH, $^2J=14$ Hz), 3.13 (2H, m, CH, $^2J=14$ Hz), 3.1–3.2 (2H, m, CHCO), 3.24 (2H, s, NCH_2Ph), 3.34 (2H, t, NCH_2, $J=7$ Hz), 7.1–7.3 (9H, m, H_{aro}); ^{13}C NMR ($CDCl_3$) 24.8 (CH_2), 29.6 (CH), 36.8 (NCH_2), 39.8 (CHCO), 41.4 (NCH_3), 54.0 (NCH_2), 61.2 (NCH_2Ph), 126.8–127.2–127.7–128.0–128.9 (C_{aro}); Formula $C_{23}H_{26}N_2O_2$, molecular weight 362.4, *m/z* 363.2 $[M+H]^+$.

5.1.10. 6-[3-(Benzyl(methyl)amino)propyl]pyrrolo[3,4-b]pyridine-5,7-dione ***16***

To a solution of amine **3** (135 mg, 0.76 mmol) in toluene (5 mL) in a Dean–Stark apparatus was added PTSA (9.5 mg, 0.05 equiv) and pyridine-2,3-dicarboxylic anhydride (113 mg, 1 equiv). Stirring was continued at 110 °C overnight. The organic layer was washed with a solution of $NaHCO_3$ 5% (10 mL), brine (10 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by TLC (AcOEt/Cyh, 9/1) to yield expected compound **16** as colorless oil (68 mg, 29% yield). TLC: R_f 0.5 (AcOEt/Cyh, 9/1). HPLC (C18, 10 min) P_{HPLC} 99%, t_R 3.3 min; HPLC (C4, 40 min) P_{HPLC} 99%, t_R 9.8 min. ^{1}H NMR ($CDCl_3$): 1.99 (2H, quint, CH_2, $J=7$ Hz), 2.27 (3H, s, NCH_3), 2.60 (2H, t, NCH_2, $J=7$ Hz), 3.59 (2H, s, NCH_2Ph), 3.83 (2H, t, NCH_2, $J=7$ Hz), 7.10–7.30 (9H, m, H_{aro}), 7.61 (1H, dd, Pyr-H_3, $J=7$ Hz, $J=5$ Hz), 8.15 (1H, dd, Pyr-H_4, $J=7$ Hz, $J=1$ Hz), 8.97 (1H, dd, Pyr-H_2, $J=5$ Hz, $J=1$ Hz); ^{13}C NMR ($CDCl_3$) 25.8 (CH_2), 36.6 (NCH_2), 41.5 (NCH_3), 54.4 (NCH_2), 61.9 (NCH_2Ph), 127.4 (Pyr-C_3), 128.6–128.8–129.6 (C_{aro}), 131.3 (Pyr-C_4), 155.3 (Pyr-C_2); Formula $C_{18}H_{19}N_3O_2$, molecular weight 309.3, *m/z* 310.1 $[M+H]^+$.

5.1.11. 2-[3-(Benzyl(methyl)amino)propyl]pyrrolo[3,4-c]pyridine-1,3-dione ***17***

To a solution of amine **3** (129 mg, 0.73 mmol) in toluene (5 mL) in a Dean–Stark apparatus was added PTSA (6.9 mg, 0.05 equiv) and pyridine-3,4-dicarboxylic anhydride (108 mg, 1 equiv). Stirring was continued at 110 °C overnight. The organic layer was washed with a solution of $NaHCO_3$ 5% (10 mL), brine (10 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by TLC (AcOEt/Cyh, 9/1) to yield expected compound **16** as colorless oil (7 mg, 3% yield). TLC: R_f 0.7 (DCM/methanol, 95/5). HPLC (C18, 10 min) P_{HPLC} 95%, t_R 3.3 min; HPLC (C4, 40 min) P_{HPLC} 96%, t_R 9.4 min. ^{1}H NMR ($CDCl_3$): 1.89 (2H, quint, CH_2, $J=7$ Hz), 2.16 (3H, s, NCH_3), 2.45 (2H, t, NCH_2, $J=7$ Hz), 3.46 (2H, s, NCH_2Ph), 3.79 (2H, t, NCH_2, $J=7$ Hz), 7.10–7.30 (9H, m, H_{aro}), 7.74 (1H, dd, Pyr-H_4, $J=5$ Hz, $J=1$ Hz), 9.06 (1H, d, Pyr-H_3, $J=5$ Hz), 9.13 (1H, d, Pyr-H_1, $J=1$ Hz); ^{13}C NMR ($CDCl_3$) 26.4 (CH_2), 37.1 (NCH_2), 42.2 (NCH_3), 54.9 (NCH_2), 62.6 (NCH_2Ph), 117.3 (Pyr-C_4), 127.3–128.5–129.3 (C_{aro}), 144.9 (Pyr-C_1), 155.9 (Pyr-C_3); Formula $C_{18}H_{19}N_3O_2$, molecular weight 309.3, *m/z* 310.1 $[M+H]^+$.

5.1.12. Opening of isatoic anhydrides, general protocol A

A solution of isatoic anhydride (0.83 mmol), amine **3** (1.3 equiv) and DIEA (5 equiv) in DCM (5 mL) was stirred overnight at room temperature under inert atmosphere. The solvent was evaporated. The residue was purified by flash chromatography (DCM/methanol, 98/2) to yield the desired compound.

5.1.13. 2-Amino-N-[3-(benzyl(methyl)amino)propyl]benzamide ***20***

White solid (84% yield). TLC: R_f 0.4 (DCM/methanol, 9/1). HPLC (C18, 10 min) P_{HPLC} 96%, t_R 3.5 min. ^{1}H NMR ($CDCl_3$): 1.79 (2H, quint, CH_2, $J=7$ Hz), 2.24 (3H, s, NCH_3), 2.57 (2H, t, NCH_2, $J=7$ Hz), 3.40–3.50 (4H, m, $NHCH_2$, NCH_2Ph), 5.62 (2H, large s, NH_2), 6.50 (1H, td, H_5, $J=8$ Hz, $J=1.5$ Hz), 6.66 (1H, dd, H_3, $J=8$ Hz,

$J = 1.5$ Hz), 7.12 (1H, dd, H_6, $J = 8$ Hz, $J = 1.5$ Hz), 7.17 (1H, td, H_2, $J = 8$ Hz, $J = 1.5$ Hz), 7.2–7.3 (5H, m, H_{aro}); ^{13}C NMR ($CDCl_3$) 27.6 (CH_2), 42.1 ($NHCH_2$), 44.0 (NCH_3), 59.3 (NCH_2), 65.5 (NCH_2Ph), 118.7 (C_5), 119.4 (C_3), 129.4 (C_{aro}), 129.5 (C_6), 130.6–131.5 (C_{aro}), 134.1 (C_2); Formula $C_{18}H_{23}N_3O$, molecular weight 297.4, *m/z* 298.2 $[M + H]^+$.

5.1.14. 2-Amino-N-[3-(benzyl(methyl)amino)propyl]-5-chlorobenzamide ***21***

Colorless oil (89% yield). TLC: R_f 0.5 (DCM/methanol, 9/1). HPLC (C18, 10 min) P_{HPLC} 98%, t_R 4.2 min. ^{1}H NMR ($CDCl_3$): 1.68 (2H, quint, CH_2, $J = 7$ Hz), 2.19 (3H, s, NCH_3), 2.46 (2H, t, NCH_2, $J = 7$ Hz), 3.35 (2H, q, $NHCH_2$, $J = 7$ Hz), 3.43 (2H, s, NCH_2Ph), 5.54 (2H, large s, NH_2), 6.50 (1H, d, H_3, $J = 8.5$ Hz), 7.00 (1H, dd, H_4, $J = 8.5$ Hz, $J = 2.5$ Hz), 7.08 (1H, d, H_6, $J = 2.5$ Hz), 7.10–7.20 (5H, m, H_{aro}), 7.98 (1H, large t, NH); ^{13}C NMR ($CDCl_3$) 23.9 (CH_2), 38.8 ($NHCH_2$), 40.8 (NCH_3), 55.2 (NCH_2), 61.7 (NCH_2Ph), 117.3 (C_3), 125.9 (C_6), 126.3–127.3–128.0 (C_{aro}), 130.6 (C_4); Formula $C_{18}H_{22}ClN_3O$, molecular weight 331.1–333.1, *m/z* 332.3–334.3 $[M + H]^+$.

5.1.15. 2-Amino-N-[3-(benzyl(methyl)amino)propyl]-5-iodobenzamide ***22***

Brown oil (58% yield). TLC: R_f 0.5 (DCM/methanol, 9/1). HPLC (TSK gel, 10 min) P_{HPLC} 91%, t_R 6.9 min. ^{1}H NMR (DMSO-d_6): 1.69 (2H, quint, CH_2, $J = 7$ Hz), 2.11 (3H, s, NCH_3), 2.34 (2H, t, NCH_2, $J = 7$ Hz), 3.20 (2H, q, $NHCH_2$, $J = 7$ Hz), 3.45 (2H, s, NCH_2Ph), 6.52 (2H, large s, NH_2), 6.54 (1H, d, H_3, $J = 8.5$ Hz), 7.20–7.29 (5H, m, H_{aro}), 7.37 (1H, dd, H_4, $J = 8.4$ Hz, $J = 2$ Hz), 7.69 (1H, d, H_6, $J = 2$ Hz), 8.31 (1H, large t, NH); ^{13}C NMR (DMSO-d_6) 27.1 (CH_2), 38.0 ($NHCH_2$), 42.2 (NCH_3), 55.1 (NCH_2), 62.1 (NCH_2Ph), 119.6 (C_3), 127.5–128.9–129.4 (C_{aro}), 136.5 (C_6), 140.2 (C_4); Formula $C_{18}H_{22}IN_3O$, molecular weight 423.3, *m/z* 424.1 $[M + H]^+$.

5.1.16. Formation of quinazolinediones, general protocol B

A solution of 2-amidobenzamide (0.90 mmol) and DIEA (5 equiv) in DCM (30 mL) was stirred for 10 min at room temperature under inert atmosphere. CDI (3 equiv) was added and the reaction mixture was heated overnight at reflux. The solvent was evaporated *in vacuo* and the residue taken up in ethyl acetate (40 mL). The organic layer was washed with a solution of $NaHCO_3$ 5% (20 mL), brine (20 mL) and then dried over $MgSO_4$. The solvent was evaporated. The residue was purified by flash chromatography (DCM/methanol, 95/5) to yield expected compound.

5.1.17. 3-[3-(Benzyl(methyl)amino)propyl]-1H-quinazoline-2,4-dione ***23***

White solid (99% yield). TLC: R_f 0.3 (DCM/methanol, 9/1). HPLC (C18, 10 min) P_{HPLC} 98%, t_R 4.0 min; HPLC (C4, 40 min) P_{HPLC} 99%, t_R 9.9 min. ^{1}H NMR ($CDCl_3$): 1.95 (2H, quint, CH_2, $J = 7$ Hz), 2.21 (3H, s, NCH_3), 2.54 (2H, t, NCH_2, $J = 7$ Hz), 3.51 (2H, s, NCH_2Ph), 4.18 (2H, t, NCH_2, $J = 7$ Hz), 7.03 (1H, d, H_8, $J = 8$ Hz), 7.22 (1H, d, H_6, $J = 8$ Hz), 7.20–7.30 (5H, m, H_{aro}), 7.54 (1H, d, H_7, $J = 8$ Hz), 8.13 (1H, d, H_5, $J = 8$ Hz); ^{13}C NMR ($CDCl_3$) 28.0 (CH_2), 42.1 (NCH_2), 44.5 (NCH_3), 57.4 (NCH_2), 64.6 (NCH_2Ph), 117.3 (C_8), 125.8 (C_6), 129.3–130.7–130.9 (C_{aro}), 131.5 (C_5), 137.4 (C_7); Formula $C_{19}H_{21}N_3O_2$, molecular weight 323.3, *m/z* 324.1 $[M + H]^+$.

5.1.18. 3-[3-(Benzyl(methyl)amino)propyl]-6-chloro-1H-quinazoline-2,4-dione ***24***

White solid (73% yield). TLC: R_f 0.5 (DCM/methanol, 9/1). HPLC (C18, 10 min) P_{HPLC} 98%, t_R 5.1 min; HPLC (C4, 40 min) P_{HPLC} 92%, t_R 10.5 min. ^{1}H NMR (DMSO-d_6): 1.69 (2H, quint, CH_2, $J = 7$ Hz), 2.01 (3H, s, NCH_3), 2.32 (2H, t, NCH_2, $J = 7$ Hz), 3.36 (2H, s, NCH_2Ph), 3.86 (2H, t, NCH_2, $J = 7$ Hz), 7.00–7.30 (6H, m, H_8, H_{aro}); 7.62 (1H, dd, H_7, $J = 8.5$ Hz, $J = 2.5$ Hz), 7.78 (1H, d, H_5, $J = 2.5$ Hz), 11.47 (1H, large s, NH); ^{13}C NMR (DMSO-d_6) 25.6 (CH_2), 39.4 (NCH_2), 42.7 (NCH_3), 55.1 (NCH_2), 62.1 (NCH_2Ph), 118.0 (C_8), 127.0 (C_5), 127.5–128.8–129.4 (C_{aro}), 135.5 (C_7); Formula $C_{19}H_{20}ClN_3O_2$, molecular weight 357.1–359.1, *m/z* 358.2–360.2 $[M + H]^+$.

5.1.19. 3-[3-(Benzyl(methyl)amino)propyl]-6-iodo-1H-quinazoline-2,4-dione ***25***

White solid (46% yield). TLC: R_f 0.5 (DCM/methanol, 9/1). HPLC (C18, 10 min) P_{HPLC} 95%, t_R 5.3 min; HPLC (C4, 40 min) P_{HPLC} 93%, t_R 10.6 min. ^{1}H NMR ($CDCl_3$): 2.08 (2H, quint, CH_2, $J = 7$ Hz), 2.36 (3H, s, NCH_3), 2.69 (2H, t, NCH_2, $J = 7$ Hz), 3.68 (2H, s, NCH_2Ph), 4.28 (2H, t, NCH_2, $J = 7$ Hz), 6.95 (1H, d, H_8, $J = 8.5$ Hz), 7.20–7.50 (5H, m, H_{aro}), 7.87 (1H, dd, H_7, $J = 8.5$ Hz, $J = 2$ Hz), 8.55 (1H, d, H_5, $J = 2$ Hz), 10.45 (1H, large s, NH); ^{13}C NMR ($CDCl_3$) 25.5 (CH_2), 39.7 (NCH_2), 42.0 (NCH_3), 54.9 (NCH_2), 62.0 (NCH_2Ph), 116.9 (C_8), 126.9–128.2–129.0 (C_{aro}), 136.9 (C_5), 143.4 (C_7); Formula $C_{19}H_{20}IN_3O_2$, molecular weight 449.2, *m/z* 450.1 $[M + H]^+$.

5.2. Binding assays to σ receptors

The σ_1 binding assays were performed by CEREP (Paris, France), according to Ganapathy et al. [26] for σ_1 binding and Bowen et al. [27] for σ_2 binding. In brief, the σ_1 binding assay was performed by incubating Jurkat cell membranes (10–20 mg protein per tube) with [^{3}H](+)-pentazocine (8 nM) and a range of concentrations of test compounds, at 22 °C for 2 h, in 5 mM Tris/HCl buffer, pH = 7.4. The σ_2 binding assay was performed by incubating rat cerebral cortex membranes (10–20 mg protein per tube) with [^{3}H](+)-DTG (5 nM), in the presence of (+)-pentazocine (300 nM) to saturate σ_1 sites, and a range of concentrations of test compounds, at 22 °C for 2 h, in 5 mM Tris/HCl buffer, pH = 7.4. The final assay volume was 0.5 mL. Non-specific binding was defined, in both assays, as that remaining in the presence of 10 μM haloperidol. The reaction was terminated by rapid filtration through Whatman GF/B filters, which were then washed with 5 × 1 mL ice-cold 0.9% NaCl (saline) solution and allowed to dry before bound radioactivity was measured using liquid scintillation counting. The protein concentration in the homogenates was determined using the method of Bradford [30].

Acknowledgments

We express our thanks to Gérard Montagne for NMR experiments, Marie-Ange Debreu-Fontaine for her contribution in organic synthesis and Hervé Drobecq for MS spectra. This work was supported by Université de Lille II and MILDt. M.T. was a recipient of fellowships from the Région Nord-Pas de Calais and CNRS.

References

[1] W.R. Martin, C.E. Eades, J.A. Thompsom, R.E. Huppler, The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197 (1976) 517–532.
[2] R. Quirion, W.D. Bowen, Y. Itzhak, J.L. Junien, J.M. Musacchio, R.B. Rothman, T.P. Su, S.W. Tam, D.P. Taylor, A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13 (1992) 85–86.
[3] E. Aydar, C.P. Palmer, V.A. Klyachko, M.B. Jackson, The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. Neuron 34 (2002) 399–410.
[4] G. Duncan, L. Wang, Focus on molecules: the sigma-1 receptor. Exp. Eye Res. 81 (2005) 121–122.
[5] H. Yamamoto, R. Miura, T. Yamamoto, K. Shinihara, M. Watanabe, S. Okuyama, A. Nakasato, T. Nukada, Amino acid residues in the transmembrane domain of the type 1 sigma receptor critical for ligand binding. FEBS Lett. 445 (1999) 19–22.

[6] D.L. DeHaven-Hudkins, L.C. Fleissner, Competitive interactions at [3H]1,3-di(2-tolyl)guanidine (DTG)-defined sigma recognition sites in guinea pig brain. Life Sci. 50 (1992) 65–70.
[7] D.H. Roh, H.W. Kim, S.Y. Yoon, H.S. Seo, Y.B. Kwon, K.W. Kim, H.J. Han, A.J. Beitz, J.H. Lee, Intrathecal administration of sigma-1 receptor agonists facilitates nociception: involvement of protein kinase C-dependant pathway. J. Neurosci. Res. 86 (2008) 3644–3654.
[8] T. Maurice, R. Martin-Fardon, P. Romieu, R.R. Matsumoto, Sigma(1) (sigma(1)) receptor antagonists represent a new strategy against cocaine addiction and toxicity. Neurosci. Biobehav. Rev. 26 (2002) 499–527.
[9] X. Guitart, X. Codony, X. Monroy, Sigma receptors: biology and therapeutic potential. Psychopharmacology 174 (2004) 301–319.
[10] P. Romieu, R. Martin-Fardon, W.B. Bowen, T. Maurice, Sigma-1 receptor-related neuroactive steroids modulate cocaine-induced reward. J. Neurosci. 23 (2003) 3572–3576.
[11] K. Matsuno, T. Senda, T. Kobayashi, S. Mita, Involvement of sigma 1 receptor in (+)-*N*-allylnormetazocine-stimulated hippocampal cholinergic functions in rats. Brain Res. 690 (1995) 200–206.
[12] H.C. Akunne, K.T. Zoski, S.Z. Whetzel, J.J. Cordon, R.M. Brandon, F. Roman, T.A. Pugsley, Neuropharmacological profile of a selective sigma ligand, igmesine: a potential antidepressant. Neuropsychopharmacology 41 (2001) 138–149.
[13] A. Meurs, R. Clinckers, G. Ebinger, Y. Michotte, I. Smolders, Sigma 1 receptor-mediated increase in hippocampal extracellular dopamine contributes to the mechanism of the anticonvulsant action of neuropeptide Y. Eur. J. Neurosci. 11 (2007) 3079–3092.
[14] T. Hayashi, T.P. Su, An update on the development of drugs for neuropsychiatric disorders: focusing on the sigma 1 ligand. Expert Opin. Ther. Targets 12 (2008) 45–58.
[15] C. Katnik, W.R. Guerrero, K.R. Pennypacker, Y. Herrera, J.J. Cuevas, Sigma-1 receptor activation prevents intracellular calcium dysregulation in cortical neurons during in vitro ischemia. Pharmacol. Exp. Ther. 319 (2006) 1355–1365.
[16] E. Aydar, P. Onganer, R. Perrett, M.B. Djamgoz, C.P. Palmer, The expression and functional characterization of sigma (sigma) 1 receptors in breast cancer cell lines. Cancer Lett. 242 (2006) 245–257.
[17] J. Charton, A. Cazenave Gassiot, S. Girault-Mizzi, P. Gilleron, M.-A. Debreu-Fontaine, C. Sergheraert, P. Melnyk, Synthesis and pharmacological evaluation of Tic-hydantoin derivatives as selective σ_1 ligands. Part 1. Bioorg. Med. Chem. Lett. 15 (2005) 4833–4837.
[18] J. Charton, A. Cazenave Gassiot, P. Melnyk, S. Girault-Mizzi, C. Sergheraert, Optimized Synthesis of tetrahydroisoquinoline-hydantoins. Tetrahedron Lett. 45 (2004) 7081–7085.
[19] R.A. Glennon, Pharmacophore identification for sigma-1 (σ_1) receptor binding: application of the "deconstruction–reconstruction–elaboration" approach. Mini-Rev. Medic. Chem. 5 (2005) 927–940.
[20] S.Y. Ablordeppey, J. Fischer, H. Law, R.A. Glennon, Probing the proposed phenyl-A region of the sigma-1 receptor. Bioorg. Med. Chem. 10 (2002) 2759–2765.
[21] A. Cazenave Gassiot, J. Charton, S. Girault-Mizzi, M.-A. Debreu-Fontaine, P. Melnyk, C. Sergheraert, Synthesis and pharmacological evaluation of Tic-hydantoin derivatives as selective σ_1. Part 2. Bioorg. Med. Chem. Lett. 15 (2005) 4828–4832.
[22] M. Toussaint, B. Delair, C. Foulon, N. Lempereur, C. Vaccher, T. Maurice, P. Melnyk, Tic hydantoin sigma-1 agonist: pharmacological characterization on cocaine-induced stimulant and appetitive effects. Eur. Neuropsychopharm. 19 (2009) 504–515.
[23] L. Rivas, L. Quintero, J.L. Fourrey, R. Benhida, A short synthesis of aspergillamide B. The marine natural product from *Aspergillus* sp. Tetrahedron Lett. 43 (2002) 7639–7642.
[24] M.D. Hoey, D.C. Dittmer, A convenient synthesis of 1,4-dihydro-2,3-benzoxathiin 3-oxide, a useful precursor of o-quinodimethane. J. Org. Chem. 56 (1991) 1947–1948.
[25] M.C. Carter, D.G. Alber, R.C. Baxter, S.K. Bithell, J. Budworth, A. Chubb, G. Stuart Cockerill, V.C.L. Dowdell, E.A. Henderson, S.J. Keegan, R.D. Kelsey, M.J. Lockyer, J.N. Stables, L.J. Wilson, K.L. Powell, 1,4-Benzodiazepines as inhibitors of respiratory syncytial virus. J. Med. Chem. 49 (2006) 2311–2319.
[26] M.E. Ganapathy, P.D. Prasad, W. Huang, P. Seth, F.H. Leibach, V.J. Ganapathy, Molecular and ligand-binding characterization of the sigma-receptor in the Jurkat human T lymphocyte cell line. Pharmacol. Exp. Ther. 289 (1999) 251–260.
[27] W.D. Bowen, B.R. de Costa, S.B. Hellewell, M. Walker, K.C. Rice, [^{3}H](+)-pentazocine: a potent and highly selective benzomorphan-based probe for sigma$_1$ receptors. Mol. Neuropharmacol. 3 (1993) 117–126.
[28] IC_{50} values from competitive inhibition experiments were determined using the Marquardt-Levenberg nonlinear curve fitting procedure of Xfit macro (Microsoft Excel).
[29] R.C.A. Onderwater, J.N.M. Commandeur, E.J. Groot, A. Sitters, W.M.P.B. Menge, N.P.E. Vermeulen, Cytotoxicity of a series of mono- and di-substituted thiourea in freshly isolated rat hepatocytes: a preliminary structure–toxicity relationship study. Toxicology 125 (1998) 117–129.
[30] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1976) 248–254.