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Rigid *versus* flexible anilines or anilides confirm the bicyclic ring as the hydrophobic portion for optimal σ_2 receptor binding and provide novel tools for the development of future σ_2 receptor PET radiotracer

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Abstract

Despite their uncertain identification, σ_2 receptors are promising targets for the development of diagnostics and therapeutics for tumor diseases. Among the σ_2 ligands developed, the class of the flexible benzamides furnished an optimal pharmacophore for σ_2 receptor high affinity ligands. A recent investigation suggested that flexible benzamides bind σ_2 receptor in a bicyclic-like conformation due to an intramolecular H-bond, with 3,4-dihydroisoquinolinone derivatives reaching excellent σ_2 affinity and selectivity. Herein, the bicyclic-preferred conformation for σ_2 binding was confirmed through the development of 3,4-dihydroquinolin-(1*H*)2-one isomeric derivatives, 1,2,3,4-tetrahydroquinolines and the corresponding flexible anilides and anilines, all linked to the 6,7-dimethoxytetrahydroisoquinoline as basic moiety. 3,4-Dihydroisoquinolin-(1*H*)2-one (**10a**) and 1,2,3,4-tetrahydroisoquinoline (**11b**) emerged for the high σ_2 affinity combined to an excellent σ_2 *versus* σ_1 selectivity. In particular, compound **11b** with its low nanomolar σ_2 affinity and impressive 2807-fold σ_2 *versus* σ_1 selectivity largely exceeded the biological profile of the best 3,4-dihydroisoquinolin-(2*H*)1-one reference compounds (**1**). Because of the absence of a cytotoxic effect, the modest interaction with the P-gp, an appropriate lipophilicity and the presence of easily radiolabeling functions, **11b** deserves further investigation for the imaging of σ_2 receptors via PET in tumors.

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1. Introduction

Sigma (σ) receptors are intriguing targets for the therapy and diagnosis of cancer and neurodegenerative diseases. They were first discovered in the 1976, when they were misidentified as opioid receptors. First designated as 'σ-opioid' receptors,[1] they were later recognized as a distinct class of proteins, and in the early 1990s two distinct sigma receptor-types were identified, namely σ_1 and σ_2. The σ_1 subtype has been cloned and recently crystallized and several agents acting at the σ_1 receptors have been developed.[2] Its implication in pathologies such as anxiety, depression, Alzheimer's disease, juvenile lateral sclerosis has been shown, with role in neuroprotection and microglia modulation.[3-7] While considerable progress has been made over four decades, the mechanisms activated by σ_1 receptor are complex and deserve further characterization because of the importance that it is gaining as a target for the treatment of human diseases. Less is known about the σ_2 receptor which has never been cloned. Attempts of identification led to propose it as a histone protein first, and as a progesterone-receptor membrane component 1 (PGRMC1) complex later.[8-10] Recent literatures seem to agree about the identification of the σ_2 as the PGRMC1 as they refer to the protein as to PGRMC1/σ_2 protein.[11-13] However, a number of controversies are emerging pointing out a possible misidentification of the σ_2 as the PGRMC1.[14-16] Nevertheless, despite the absence of clear information about the σ_2 receptor structure, interest by the scientific research is increasing because of the important therapeutic or diagnostic potentials that range from the treatment and diagnosis of different cancers to the recently proposed involvement in neurodegenerative pathologies such as the Alzheimer's disease.[17,12,13] Development of novel σ_2 ligands is a crucial step to further clarify the binding site of the σ_2 and finally push towards the therapeutic and diagnostic exploitation of these receptors. Conformationally flexible benzamides have provided a successful pharmacophore for the development of high affinity σ_2 ligands.[18] While extending the investigation about these structures, we proposed the presence of an intramolecular H-bond in the binding mode of these molecules to the σ_2 binding site, mimicking a bicycle

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structure, which is also common to other σ_2 ligands such as the tetraline and the indole-based derivatives.[19-21] Support to this hypothesis came from freezing the H-bond into the corresponding isoquinolinone structures (Figure 1).[22,23] Therefore, if the binding of this molecule-types to the σ_2 receptors prefers the bicyclic-like conformation, then, the inverted amide, i.e. the corresponding 3,4-dihydroquinolinone derivatives would bind σ_2 receptors as well. In order to confirm this hypothesis and produce novel σ_2 high affinity and selectivity ligands, we produced 3,4-dihydroquinolin-(1*H*)2-one derivatives and the corresponding 1,2,3,4-tetrahydroquinoline that according to our hypothesis would bind σ_2 receptors equally well. Besides the 3,4-dihydroquinolin-(1*H*)2-one and 1,2,3,4-tetrahydroquinoline rings, also the corresponding 5-methoxy or 6-fluoro functionalized rings were investigated because the same groups led to the most promising σ_2 receptor ligands in the isoquinolinones series, providing convenient functions for Positron Emission Tomography (PET) tracers development (Compounds **1** and **2**, Figure 1). The corresponding flexible anilides and anilines were also produced to investigate the σ_2 receptor binding site and test how the absence of the H-bond-driven bicyclic structures influences the interaction with the σ_2 receptor. In view of a possible application of these agents for diagnostic purposes, we evaluated their cytotoxicity as well as their interaction with P-glycoprotein (P-gp), since we frequently found σ_2 receptor ligands bearing cyclic basic moieties such as 6,7-dimethoxytetrahydroisoquinoline, to modulate the P-gp efflux pump.[22,24,25]

2. Results and discussion

2.1. Chemistry

The synthesis of final compounds is described in Scheme 1 and 2. The already known 3-chloropropanamides **3a-c**[26] which were obtained by alkylation of the appropriate anilines were used to alkylate 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline to afford final compounds **4a-c**. Reduction of these amides with $LiAlH_4$ afforded final amines **5a-c**, which were then acetylated by acetyl chloride to provide final acetamides **6a-c**. Reduction of these latter with $LiAlH_4$ provided final

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ethylamines **7a-c** (Scheme 1). 5-Methoxy-3,4-dihydroquinolin-2(1*H*)-one (**8b**) and 6-fluoro-3,4-dihydroquinolin-2(1*H*)-one (**8c**) were obtained by chromatographic separation from the corresponding 3,4-dihydroisoquinolin-1(2*H*)-one derivatives according to the synthesis previously reported for the latter compounds,[23] whereas 3,4-dihydroquinolin-2(1*H*)-one (**8a**) was commercially available. Alkylation of 3,4-dihydroquinolin-2(1*H*)-one derivatives (**8a-c**) with 1-bromo-3-chloropropane using NaH as a base afforded 3-chloropropyl derivatives (**9a-c**) whose reaction with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline led to final compounds **10a-c** (Scheme 2). Reduction of **10a-c** with BH_3·DMS afforded final 1,2,3,4-tetrahydroquinoline **11a-c**. All of the final amine compounds were converted into their hydrochloride salts with gaseous HCl, in anhydrous diethyl ether except for compound **6b** which was crystallized as oxalate salt. Physical properties of the salts are listed in the Table of Physical Properties of Novel Compounds in the Supporting Information.

2.2. Biology

2.2.1. σ Receptors radioligand binding. Results from binding assays are expressed as inhibition constants (K_i values) in Table 1 and 2. As expected for most of the compounds carrying the 6,7-dimethoxytetrahydrisoquinoline as the basic moiety, the σ_1 receptor affinity was low (K_i values ranging from 144 nM to 4627 nM). Among the different series, the propanamides (**4a-c**) and the propanamines (**5a-c**) displayed higher σ_1 affinities in comparison with the other series such as the corresponding acetamides (**6a-c**), ethylamines (**7a-c**), 3,4-dihydroquinolin-2(1*H*)-ones (**10a-c**) and tetrahydroquinolines (**11a-c**) series. The presence of the Fluoro substituent on the benzene ring consistently determined in all the anilines - flexible and rigid - an increase in the σ_1 receptor affinity, with **5c**, **7c**, and **11c** displaying the highest affinities within their series (K_i values = 144 nM, 320 nM, 876 nM, respectively). As for the σ_2 affinity, the flexible anilides displayed worse σ_2 binding than corresponding anilines (compare **4a-c** with **5a-c** and **6a-c** with **7a-c**). This could reflect the partial conjugation of the N-atom lone pair with the benzene ring in the anilines, that determines a partial rigidification driving a bicyclic-like conformation like in the dihydroquinolines. This is not the case in the anilide derivatives (**4a-c** and **6a-c**) in which the delocalization of the N-atom lone

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pair is shared with the amide function. On the other hand and in agreement with these observations, when the amide is inserted in a rigid bicyclic structure, such as the 3,4-dihydroisoquinolin-(1*H*)2-one derivatives (**10a-c**), excellent σ_2 receptor affinity are displayed (K_i values from 7.11 nM to 34.2 nM). The functionalization on the benzene ring did not seem to drive any particular effect in the σ_2 binding, except for the propanamides series where the insertion of a 3-methoxy or 4-fluorine group determined a 5-fold increase in the σ_2 receptor affinity. The highest σ_2 receptor affinities were recorded for anilines **5a** (K_i = 3.58 nM) and **5c** (K_i = 2.70 nM), for 3,4-dihydroquinolinone **10a** (K_i = 7.11 nM) and tetrahydroquinoline **11b** (K_i = 1.42 nM). However, excellent selectivity values versus the σ_1 subtype were reached by the bicyclic derivatives **10a** (650-fold) and particularly for **11b** (2807-fold), whose affinity and selectivity values were superior to those displayed by reference 3,4-dihydroisoquinolin-(2*H*)1-one compounds **1** and **2**, and in line with more recently developed and most promising flexible benzamides.[18] Moreover, **10a** and **11b** showed an improved σ_2 receptor biological profile compared to reference flexible benzamide RHM-1 (σ_1 receptor K_i = 3078 nM; σ_2 receptor K_i = 10.3 nM)[27,28] and its fluoroethoxy analogue ISO-1 (σ_1 receptor K_i = 330 nM; σ_2 receptor K_i = 7.0 nM) which is currently recruiting for three different clinical trials for tumor imaging.[28-32] For compounds such as **7a**, **7c**, **11a** and **11c**, the K_i could not be determined because of a non-competitive binding with the σ_2 reference ligand di-1,2-bis(*o*-tolyl)guanidine (DTG) that led to high percentages of displacement of the radioligand (from 67 % to 80%) at very low concentrations (10^{-11} M). The same binding assay was repeated with cell membranes from MCF7 cells, where σ_2 receptors are overexpressed. However, the same results were recorded, indicating a likely allosteric binding of these compounds with the σ_2 receptors.

2.2.2. Calcein-AM assay. P-gp inhibiting activity of the newly synthesized compounds **4a-c**–**7a-c**, **10a-c**, **11a-c** and the reference compound 6,7-dimethoxy-2-{3-[4-methoxy-3,4-dihydro-2*H*-naphthalen-(1*E*)-ylidene]propyl}-1,2,3,4-tetrahydroisoquinoline (**MC18**)[33] was determined by fluorescence measurement using Calcein-AM probe in MDCK-MDR1 cell line as previously

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reported.[34] In comparison to recently developed P-gp modulators whose EC_{50} are in the low nanomolar range[34-37] all the compounds showed a moderate interaction with the efflux pump, with **4a** displaying the weakest interaction (EC_{50} = 9.51 μM) and **7b** the strongest (EC_{50} = 0.86 μM). Higher modulating P-gp activity was shown by the ethylamines (**7a-c**) and the bicyclic compounds **10a-c** and **11a-c** (EC_{50} ranging from 1.81 μM to 2.74 μM), without differences conferred by the substituent on the benzene ring or by the presence of the amide function.

2.2.3. Antiproliferative activity. The antiproliferative activity of all of the novel compounds was evaluated through the MTT assay in human breast adenocarcinoma MCF7 cells. These cells were previously deeply investigated and represent a model for the evaluation of the σ_2 mediated activity because of the constitutive overexpression of σ_2 receptors.[14,38] None of the compounds exerted antiproliferative activity (EC_{50} > 100 μM) in accordance with data from the isoquinolinone derivatives.[22,23] The absence of antiproliferative activity as an indication of a low toxicity of these compounds suggests future investigation of the highest affinity of these novel compounds as diagnostic tools for the imaging of σ_2 receptors in cancer pathologies.

3.1. Chemistry

Both column chromatography and flash column chromatography were performed with 60 Å pore size silica gel as the stationary phase (1:30 w/w, 63−200 μm particle size, from ICN, and 1:15 w/w, 15−40 μm particle size, from Merck, respectively). Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. ^{1}H NMR (499.801 MHz) and ^{13}C NMR (125.686 MHz) spectra were recorded on a 500-vnmrs500 Agilent spectrometer. Chemical shift (δ) in parts per million (ppm), and for ^{1}H NMR multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integration, and coupling constant(s) in hertz were reported. ^{13}C NMR spectra were performed on representative final compounds in their hydrochloride or oxalate forms. Recording of mass spectra was done on an Agilent 6890-5973 MSD gas chromatograph/mass spectrometer, or on an Agilent 1100 series LC-MSD trap system VL mass spectrometer; only significant *m*/*z* peaks,

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with their percentage of relative intensity in parentheses, are reported. Chemicals were from Aldrich, TCI and Alpha Aesar and were used without any further purification.

3.2. General procedure for the synthesis of *N*-phenyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-propanamides (4a-c).

A solution in CH_3CN (25 mL) of one among the intermediate propanamides **3a-c** (2.5 mmol) was added with K_2CO_3 (2.5 mmol, 0.34 g) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (2.5 mmol, 0.48 g). The resulting mixture was stirred under reflux overnight. The solvent was then removed under reduced pressure and the residue was taken up with water and extracted with ethyl acetate (3 × 20 mL). The collected organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude oil which was purified by column chromatography (CH_2Cl_2/CH_3OH, 95:5) to afford the title compounds.

3.2.1. *N*-phenyl-3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamide (4a). Was obtained as as yellow solid (0.51 g, 60% yield), mp = 135-137 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.63 (t, *J* = 5.8 Hz, 2H, $ArCH_2$), 2.78-2.98 (m, 6H, $COCH_2$, $N(CH_2)_2$), 3.72 (s, 2H, NCH_2Ar), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.85-7.38 (m, 7H, aromatic), 11.05 (broad s, 1H, D_2O exchanged); GC-MS *m/z* 339 (M^+ – 1, 0.5), 205 (20), 192 (100), 164 (30). Anal. ($C_{20}H_{24}N_2O_3 \cdot HCl \cdot 0.25H_2O$) C, H, N.

3.2.2. *N*-(3-methoxyphenyl)-3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamide (4b). Was obtained as yellow oil (0.62 g, 67% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.62 (t, *J* = 5.8 Hz, 2H, $ArCH_2$), 2.78-2.98 (m, 6H, $COCH_2$, $N(CH_2)_2$), 3.70 (s, 3H, OCH_3), 3.75 (s, 2H, NCH_2Ar), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.58-7.18 (m, 6H, aromatic), 11.18 (broad s, 1H, D_2O exchanged); ^{13}C NMR (125 MHz, CD_3OD) δ 27.30, 33.00, 53.04, 55.54, 55.62, 55.80, 56.10, 56.30, 108.31, 109.64, 110.21, 111.41, 116.46, 126.51, 126.83, 129.90, 139.50, 146.70, 148.20, 160.61, 173.70; GC-MS *m/z* 369 (M^+ – 1, 0.5), 205 (20), 192 (100), 164 (30). Anal. ($C_{21}H_{26}N_2O_4 \cdot HCl \cdot 0.75H_2O$) C, H, N.

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3.2.3. *N*-(4-fluorophenyl)-3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamide (4c). Was obtained as a white waxy solid (0.61 g, 68% yield), mp = 136-138 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.55-2.65 (m, 2H, $ArCH_2$), 2.85-2.98 (m, 6H, $COCH_2$, $N(CH_2)_2$), 3.65 (s, 3H, OCH_3), 3.83 (s, 2H, NCH_2Ar), 3.88 (s, 3H, OCH_3), 6.58 (s, 1H, aromatic), 6.65 (s, 1H, aromatic), 6.85-7.38 (m, 4H, aromatic), 11.05 (broad s, 1H, D_2O exchanged). Anal. ($C_{20}H_{23}N_2O_3$·HCl·0.25H_2O) C, H, N.

3.3. General procedure for the synthesis of *N*-phenyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamines (5a-c). A solution of one of the appropriate amides **4a-c** (2.0 mmol) in the same solvent (10 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (4.35 mmol, 0.16 g) in anhydrous THF (20 mL) kept under a stream of N_2 and cooled in an ice bath. After the addition, the mixture was refluxed for 5 h, cooled and the excess of $LiAlH_4$ was quenched with water. The aqueous layer was extracted with Et_2O (2 × 10 mL) and ethyl acetate (20 mL). The collected organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude oil as a dense brown oil which was either transformed into the corresponding hydrochloride salt or used for the next step without any further purification.

3.3.1. *N*-phenyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (5a). Was obtained as orange oil (0.38 g, 58% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.88-1.95 (m, 2H, $CH_2CH_2CH_2$), 2.64-2.68 (m, 2H, $ArCH_2$), 2.74 (t, 2H, J = 5.8 Hz, NCH_2), 2.86 (t, 2H, J = 5.8 Hz, NCH_2), 3.25 (t, 2H, J = 6.3 Hz, $NHCH_2$), 3.58 (s, 2H, NCH_2Ar), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.52-7.18 (m, 7H, aromatic); GC-MS *m/z* 326 (M^+, 8), 192 (100). Anal. ($C_{20}H_{26}N_2O_2$·2HCl·0.75H_2O) C, H, N.

3.3.2. *N*-(3-methoxyphenyl)-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (5b). Was obtained as brown oil (0.48 g, 68% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.7 (br s, 1H, NH), 1.70-1.95 (m, 2H, $CH_2CH_2CH_2$), 2.60-2.78 (m, 6H, 2 NCH_2 and $ArCH_2$), 3.22 (t, 2H, J = 10.7 Hz, $NHCH_2$), 3.58 (s, 2H, NCH_2Ar), 3.73 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.18-7.10 (m, 6H, aromatic); GC-MS *m/z* 356 (M^+, 8), 192 (100). Anal.

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($C_{21}H_{29}N_2O_3 \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

3.3.3. *N*-(4-fluorophenyl)-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (5c). Was obtained as brown oil (0.521 g, 75% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87-1.94 (m, 2H, $CH_2CH_2CH_2$), 2.64-2.76 (m, 4H, NCH_2 and $ArCH_2$), 2.85 (t, 2H, J = 5.8 Hz, NCH_2), 3.20 (t, 2H, J = 6.3 Hz, $NHCH_2$), 3.57 (s, 2H, NCH_2Ar), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.47-6.88 (m, 6H, aromatic); ^{13}C NMR (125 MHz, CD_3OD) δ 27.10, 27.35, 41.13, 56.11, 56.20, 56.31, 59.70, 63.40, 108.34, 111.41, 116.30, 118.90, 126.51, 126.90, 143.19, 146.72, 148.21, 155.70; GC-MS *m/z* 344 (M^+, 8), 192 (100); Anal. ($C_{20}H_{25}N_2O_2F \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

3.4. General procedure for the synthesis of *N*-phenyl-*N*-[(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-propan-3-yl]acetamides (6a-c). A solution of one of the appropriate amines **5a-c** (2.0 mmol) in CH_2Cl_2 (20 mL) and Et_3N (4.0 mmol, 0.5 mL) was added to a solution of CH_3COCl (2.0 mmol, 0.14 mL) in the same solvent (5 mL) cooled in an ice bath. After the addition, the mixture was stirred to room temperature overnight. H_2O was added to the mixture and extracted with CH_2Cl_2 (3 × 10 mL). The collected organic layers, were dried (Na_2SO_4) and concentrated under reduced pressure to provide a crude residue which was purified by column chromatography with CH_2Cl_2/MeOH (95:5) as eluent.

3.4.1. *N*-phenyl-*N*-[(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-propan-3-yl]acetamide (6a). Was obtained as brown oil (0.23 g, 32% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80-1.88 (m, 5H, $CH_2CH_2CH_2$, $COCH_3$), 2.50-2.54 (m, 2H, $ArCH_2$), 2.64-2.68 (m, 2H, NCH_2), 2.78 (t, 2H, J = 5.87 Hz, NCH_2), 3.50 (s, 2H, NCH_2Ar), 3.76-3.80 (m, 2H, $CONCH_2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.49 (s, 1H, aromatic), 6.58 (s, 1H, aromatic), 7.18-7.45 (m, 5H, aromatic). GC-MS *m/z*: 368 (M^+, 0.5), 192 (100). Anal. ($C_{22}H_{28}N_2O_3 \cdot HCl \cdot 1.25H_2O$) C, H, N.

3.4.2. *N*-(3-methoxyphenyl)-*N*-[(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-propan-3-yl]acetamide (6b). Was obtained as brown oil (0.25 g, 31% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80-1.90 (m, 5H, $CH_2CH_2CH_2$, $COCH_3$), 2.50-2.54 (m, 2H, $ArCH_2$), 2.67 (t, 2H, J = 5.87 Hz, NCH_2), 2.78 (t, 2H, J = 5.87 Hz, NCH_2), 3.51 (s, 2H, NCH_2Ar), 3.77 (t, 2H, J = 7.34 Hz,

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$CONCH_2$), 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.50 (s, 1H, aromatic), 6.58 (s, 1H, aromatic), 6.71-6.92 (m, 3H, aromatic), 7.32 (t, 1H, J = 8.32 Hz, aromatic). ^{13}C NMR (125 MHz, CD_3OD) δ 23.10, 24.41, 27.32, 40.18, 55.81, 56.13, 56.22, 56.31, 59.71, 63.45, 108.34, 111.43, 116.45, 119.80, 124.05, 125.42, 126.53, 126.92, 142.72, 146.70, 148.21, 160.83, 168.92; GC-MS *m/z*: 398 (M^+, 0.5), 192 (100). Anal. ($C_{25}H_{30}N_2O_4\cdot(COOH)_2\cdot0.75H_2O$) C, H, N.

3.4.3. *N*-(4-fluorophenyl)-*N*-[(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-propan-3-yl]acetamide (6c). Was obtained as brown oil (0.62 g, 80% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.92-2.00 (m, 5H, $CH_2CH_2CH_2$, $COCH_3$), 2.77-2.83 (m, 2H, $ArCH_2$), 2.90-2.94 (m, 2H, NCH_2), 2.98-3.02 (m, 2H, NCH_2), 3.74-3.77 (m, 2H, $CONCH_2$), 3.81 (s, 2H, NCH_2Ar), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.50 (s, 1H, aromatic), 6.60 (s, 1H, aromatic), 7.10-7.24 (m, 4H, aromatic). GC-MS *m/z*: 386 (M^+, 0.5), 192 (100). Anal. ($C_{22}H_{27}N_2O_3F\cdot HCl\cdot0.25H_2O$) C, H, N.

3.5. General procedure for the synthesis of *N*-phenyl-*N*-ethyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamines (7a-c). A solution of one of the appropriate acetamides **6a-c** (2 mmol) in anhydrous THF (10 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (4.2 mmol, 0.16 g) in the same solvent (10 mL) kept under a stream of N_2 and cooled in an ice bath. After the addition, the mixture was refluxed for 2 h, cooled and the excess of $LiAlH_4$ was quenched with water. The aqueous layer was extracted with Et_2O (2 × 10 mL) and ethyl acetate (20 mL). The collected organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude oil which was purified by column chromatography with CH_2Cl_2/MeOH (98:2) as eluent.

3.5.1. *N*-phenyl-*N*-ethyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (7a). Was obtained as yellow oil (0.21 g, 30 %); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16 (t, 3H, J = 6.85 Hz, CH_3CH_2), 1.90-1.95 (m, 2H, $CH_2CH_2CH_2$), 2.50-2.54 (m, 2H, $ArCH_2$), 2.72 (t, 2H, J = 5.87 Hz, NCH_2), 2.84-2.88 (m, 2H, NCH_2), 3.35-3.40 (m, 4H, $ArN(CH_2)_2$), 3.57 (s, 2H, NCH_2Ar), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.53 (s, 1H, aromatic), 6.62 (s, 1H, aromatic), 6.65 (t, 1H, J = 6.85

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Hz, aromatic), 6.72 (d, 2H, *J* = 8.80 Hz, aromatic), 7.20-7.23 (m, 2H, aromatic). GC-MS *m/z*: 354 (M^+, 20), 232 (90), 192 (100). Anal. ($C_{22}H_{30}N_2O_2 \cdot 2HCl \cdot 0.75H_2O$) C, H, N.

3.5.2. *N*-(3-methoxyphenyl)-*N*-ethyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (7b). Was obtained as orange oil (0.38 g, 50%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16 (t, 3H, *J* = 6.85 Hz, CH_3CH_2), 1.88-1.95 (m, 2H, $CH_2CH_2CH_2$), 2.54-2.60 (m, 2H, NCH_2), 2.72 (t, 2H, *J* = 5.87 Hz, $ArCH_2$), 2.84 (t, 2H, *J* = 5.87 Hz, NCH_2), 3.34-3.40 (m, 4H, $ArN(CH_2)_2$) 3.56 (s, 2H, NCH_2Ar), 3.78 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.23 (d, 1H, *J* = 8.31 Hz, aromatic), 6.27 (s, 1H, aromatic), 6.35 (d, 1H, *J* = 8.31 Hz, aromatic), 6.53 (s, 1H, aromatic), 6.62 (s, 1H, aromatic), 7.12 (t, 1H, *J* = 8.31 Hz, aromatic). ^{13}C NMR (125 MHz, CD_3OD) δ 9.32, 20.39, 24.42, 47.42,48.22, 50.17, 52.36, 52.46, 53.88, 55.05, 55.13, 109.39, 111.21, 113.64, 118.91, 122.76, 131.25, 131.36, 148.49, 149.33, 161.40, 161.45; GC-MS *m/z*: 384 (M^+, 15), 232 (90), 192 (100). Anal. ($C_{23}H_{32}N_2O_3 \cdot 2HCl \cdot 1.25H_2O$) C, H, N.

3.5.3. *N*-(4-fluorophenyl)-*N*-ethyl-3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanamine (7c). Was obtained as orange oil (0.22 g, 30 %); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (t, 3H, *J* = 6.85 Hz, CH_3CH_2), 1.84 (m, 2H, $CH_2CH_2CH_2$), 2.52-2.55 (m, 2H, NCH_2), 2.71 (t, 2H, *J* = 5.87 Hz, ArN), 2.84 (t, 2H, *J* = 5.87 Hz, NCH_2), 3.30-3.36 (m, 4H, $ArN(CH_2)_2$), 3.55 (s, 2H, NCH_2Ar), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.53 (s, 1H, aromatic), 6.61 (s, 1H, aromatic), 6.64-6.68 (m, 2H, aromatic), 6.89-6.94 (m, 2H, aromatic). GC-MS *m/z*: 372 (M^+, 10), 232 (80), 192 (100). Anal. ($C_{22}H_{29}N_2O_2F \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

3.6. General procedure for the synthesis of final 2-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-3,4-dihydroquinolin-2(1*H*)-one compounds (10a-c). To a solution of the appropriate alkylchloride derivative (2.0 mmol) in CH_3CN (20 mL), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (2.0 mmol, 0.46 g) and K_2CO_3 (3.0 mmol, 0.42 g) were added and the mixture was refluxed overnight. After cooling to room temperature, the solvent was evaporated under vacuum and the residue was treated with H_2O and extracted with CH_2Cl_2 (3 × 10 mL). The collected organic layers, were dried (Na_2SO_4) and concentrated under reduced pressure to

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provide a crude residue which was purified by column chromatography with CH_2Cl_2/MeOH (95:5) as eluent.

3.6.1. 2-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-3,4-dihydroquinolin-2(1*H*)-one (10a). Was obtained as waxy oil (0.55 g, 72%); ^{1}H NMR (300 MHz) δ 1.85-2.00 (m, 2H, $CH_2CH_2CH_2$), 2.55-2.90 [m, 10H, $Ar(CH_2)_2$, $COCH_2$, $N(CH_2)_2$], 3.55 (s, 2H, NCH_2Ar), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.02 (t, 2H, J = 7.44 Hz, $CONCH_2$), 6.50 (s, 1H, aromatic), 6.58 (s, 1H, aromatic), 6.62-7.20 (m, 4H, aromatic); ^{13}C NMR (125 MHz, CD_3OD) δ 24.40, 26.62, 27.16, 27.33, 40.41, 56.11, 56.33, 56.41, 59.73,63.40, 108.34, 111.42, 116.90, 124.83, 126.14, 126.56, 126.90, 127.83, 130.94, 138.92, 146.72, 148.22, 169.83; LC-MS (ESI^+) *m/z*: 403 $[M+Na]^+$. Anal. ($C_{23}H_{28}N_2O_3$·HCl) C, H, N.

3.6.2. 2-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-5-methoxy-3,4-dihydroquinolin-2(1*H*)-one (10b). Was obtained as yellow oil (0.57 g, 70%); ^{1}H NMR (300 MHz) δ 1.90-2.00 (m, 2H, $CH_2CH_2CH_2$), 2.55-2.95 [m, 10H, $Ar(CH_2)_2$, $COCH_2$, $N(CH_2)_2$], 3.58 (s, 2H, NCH_2Ar), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.02 (t, J = 7.42 Hz, $CONCH_2$), 6.51 (s, 1H, aromatic), 6.59 (s, 1H, aromatic), 6.61-6.62 (m, 1H, aromatic), 6.74 (d, 1H, J = 8.25 Hz, aromatic), 7.15 (t, 1H, J = 8.25 Hz, aromatic); GC-MS *m/z*: 410 (M^+, 10), 192 (100). Anal. ($C_{24}H_{30}N_2O_4$·HCl·1.6H_2O) C, H, N.

3.6.3. 2-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-6-fluoro-3,4-dihydroquinolin-2(1*H*)-one (10c). Was obtained as yellow oil (0.56 g, 70%); ^{1}H NMR (300 MHz) δ 1.95-2.05 (m, 2H, $CH_2CH_2CH_2$), 2.58-2.95 [m, 10H, $Ar(CH_2)_2$, $COCH_2$, $N(CH_2)_2$], 3.60 (s, 2H, NCH_2Ar), 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.01 (t, J = 7.42 Hz, $CONCH_2$), 6.49 (s, 1H, aromatic), 6.59 (s, 1H, aromatic), 6.85-7.05 (m, 3H, aromatic); GC-MS *m/z*: 398 (M^+, 0.5), 192 (100). Anal. ($C_{23}H_{27}N_2O_3F$·HCl·0.75H_2O) C, H, N.

3.7. General procedure for the synthesis of final 1-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl))-1,2,3,4-tetrahydroquinolines compounds (11a-c). A solution of one of the appropriate amides **10a-c** (2.0 mmol) in anhydrous THF (20 mL) was added with a solution of

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BH_3·DMS (10 mmol, 1 mL) kept under a stream of N_2 and cooled in an ice bath. After the addition, the mixture was refluxed for 3 h, cooled and added with MeOH (20 mL). HCl 3N was added and the mixture was refluxed for 1 h. After that the mixture was made basic with NaOH 3N and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The collected organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude oil as a dense brown oil which was purified by column chromatography (CH_2Cl_2/CH_3OH, 95:5) to afford the title compound.

3.7.1. 1-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl))-1,2,3,4-tetrahydroquinoline (11a). Was obtained as yellow oil (0.68 g, 90%); ^{1}H NMR (500 MHz) δ 1.90-2.00 [m, 4H, ($CH_2CH_2CH_2$)$_2$], 2.55-2.60 (m, 2H, $ArCH_2$), 2.70-2.90 [m, 6H, $ArCH_2$, N(CH_2)$_2$], 3.28-3.40 (m, 4H, ArN(CH_2)$_2$), 3.55 (s, 2H, NCH_2Ar), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.50-6.64 (m, 4H, aromatic), 6.92-7.06 (m, 2H, aromatic); ^{13}C NMR (125 MHz, CD_3OD) δ 16.20, 20.20, 24.31, 24.56, 48.59, 50.20, 52.48, 52.59, 54.30, 55.08, 55.17, 109.45, 111.27, 119.08, 122.08, 122.88, 127.45, 128.66, 130.85, 130.94, 136.31, 148.51, 149.35; GC-MS *m/z*: 366 (M^+, 20), 192 (100). Anal. ($C_{23}H_{30}N_2O_2$·2HCl·0.25H_2O) C, H, N.

3.7.2. 1-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-5-methoxy-1,2,3,4-tetrahydroquinoline (11b). Was obtained as yellow oil (0.45 g, 55%); ^{1}H NMR (500 MHz) δ 1.86-1.98 [m, 4H, ($CH_2CH_2CH_2$)$_2$], 2.60-2.70 [m, 4H, N(CH_2)$_2$], 2.75-2.95 [m, 4H, 2 $ArCH_2$], 3.20-3.25 (m, 2H, $ArNCH_2$), 3.33-3.38 (m, 2H, $ArNCH_2$), 3.60 (s, 2H, NCH_2Ar), 3.78 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.20-6.22 (m, 1H, aromatic), 6.33 (d, 1H, J = 8.25 Hz, aromatic), 6.51 (s, 1H, aromatic), 6.60 (s, 1H, aromatic), 6.99 (t, 1H, J = 8.32 Hz, aromatic); ^{13}C NMR (125 MHz, CD_3OD) δ 15.60, 18.94, 20.32, 24.55, 48.18, 50.21, 52.53, 52.62, 53.98, 55.05, 55.07, 55.16, 109.44, 111.24, 119.05, 119.24, 122.85, 127.56, 137.19, 148.53, 149.36, 158.23; GC-MS *m/z*: 396 (M^+, 22), 192 (100). Anal. ($C_{24}H_{32}N_2O_3$·2HCl·0.25H_2O) C, H, N.

3.7.3. 1-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-6-fluoro-1,2,3,4-tetrahydroquinoline (11c). Was obtained as yellow oil (0.53 g, 67%); ^{1}H NMR (500 MHz) δ 1.80-1.89 (m, 2H, $CH_2CH_2CH_2$), 1.90-1.96 (m, 2H, $CH_2CH_2CH_2$), 2.52-2.58 (m, 2H, $ArCH_2$), 2.68-2.76

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[m, 4H, $N(CH_2)_2$], 2.82-2.86 (m, 2H, $ArCH_2$), 3.22-3.26 (m, 2H, $ArNCH_2$), 3.28-3.34 (m, 2H, $ArNCH_2$), 3.56 (s, 2H, NCH_2Ar), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.51 (s, 1H, aromatic), 6.52-6.56 (m, 1H, aromatic), 6.60 (s, 1H, aromatic), 6.65-6.74 (m, 2H, aromatic); ^{13}C NMR (125 MHz, CD_3OD) δ 16.43, 20.28, 24.55, 24.84,48.67, 50.21, 52.57, 52.63, 53.78, 55.04, 55.13, 109.40, 111.24, 116.80, 119.03, 122.82, 123.00, 132.95, 133.27, 148.54, 149.39, 160.24, 162.22; GC-MS *m/z*: 384 (M^+, 20), 192 (100). Anal. ($C_{23}H_{29}N_2O_2F \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

3.8. Biology

3.8.1. Materials. [^{3}H]-DTG (29 Ci/mmol) and (+)-[^{3}H]-pentazocine (32 Ci/mmol) were purchased from PerkinElmer Life Sciences (Zavantem, Belgium). DTG was purchased from Tocris Cookson Ltd., U.K. (+)-Pentazocine was obtained from Sigma-Aldrich-RBI s.r.l. (Milan, Italy). Male Dunkin guinea-pigs and Wistar Hannover rats (250-300 g) were from Harlan, Italy. Cell culture reagents were purchased from EuroClone (Milan, Italy). Calcein-AM, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) were obtained from Sigma-Aldrich (Milan, Italy).

3.8.2. Competition Binding Assays. All the procedures for the binding assays were previously described. σ_1 And σ_2 receptor binding were carried out according to Berardi et al.[21] The specific radioligands and tissue sources were respectively: (a) σ_1 receptor, (+)-[^{3}H]-pentazocine, guinea-pig brain membranes without cerebellum; (b) σ_2 receptor, [^{3}H]-DTG in the presence of 1 μM (+)-pentazocine to mask σ_1 receptors, rat liver membranes. The following compounds were used to define the specific binding reported in parentheses: (a) (+)-pentazocine (73-87%), (b) DTG (85-96%). Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) were determined by using six to nine different concentrations of the drug studied in two or three experiments with samples in duplicate. Scatchard parameters (K_d and B_{max}) and apparent inhibition constants (K_i) values were determined by nonlinear curve fitting, using the Prism, version 3.0, GraphPad software (1998).[39]

3.8.3. Cell Culture. MDCK-MDR1 cells was a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Nederland. Human MCF7 breast adenocarcinoma cells was purchased from ICLC

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(Genoa, Italy). MCF7 and MDCK-MDR1 cells were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, in a humidified incubator at 37 °C with a 5 % CO_2 atmosphere.

3.8.4. Cell Viability. Determination of cell growth was performed using the MTT assay at 48 h.[40] On day 1, 25,000 cells/well were seeded into 96-well plates in a volume of 100 μl. On day 2, the various drugs concentration (0.1 μM-100 μM) were added. In all the experiments, the various drug-solvents (EtOH, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with drugs (48 h), MTT (0.5 mg/ml) was added to each well, and after 3-4 h incubation at 37 °C, the supernatant was removed. The formazan crystals were solubilized using 100 μl of DMSO/EtOH (1:1) and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences.

3.8.5. Calcein-AM experiment. These experiments were carried out as described by Pati et al. with minor modifications.[34] Calcein-AM is a profluorescent probe and is a P-gp substrate. In cells overexpressing P-gp, Calcein-AM is not able to permeate cell membrane whereas when the efflux pump is not present or is inhibited, the probe enters living cells and is converted to fluorescent Calcein by intracellular esterases. Calcein is not able to diffuse through the membrane since it is hydrophilic and is not a P-gp substrate; thus, Calcein accumulates in cells when the pump is blocked. Therefore, the fluorescent signal is directly correlated to the amount of P-gp inhibition. MDCK-MDR1 cell line (50,000 cells per well) was seeded into black CulturePlate 96/wells plate with 100 μL medium and allowed to become confluent overnight. 100 μL of test compounds were solubilized in culture medium and added to monolayers. 96/Wells plate was incubated at 37 °C for 30 min. Calcein-AM was added in 100 μL of Phosphate Buffered Saline (PBS) to yield a final concentration of 2.5 μM and plate was incubated for 30 min. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well and the plate was read to Victor3 (PerkinElmer) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. In these experimental conditions Calcein cell accumulation in the absence and in the presence of tested compounds was

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evaluated and fluorescence basal level was estimated by untreated cells. In treated wells the increase of fluorescence with respect to basal level was measured. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

4. Conclusions

Herein we produced different series of rigid and flexible anilides and anilines linked to the 6,7-dimethoxytetrahydroisoquinoline as basic moiety and we confirmed the preferential binding to σ_2 receptors of ligands that contain a bicyclic or a bicyclic-like moiety in their hydrophobic portion. Except for the flexible amides, all of the novel compounds displayed notable σ_2 affinity and selectivity, with absence of antiproliferative activity and a modest interaction with the P-gp. Two of these compounds, belonging to the bicyclic series - the 3,4-dihydroisoquinolin-(1*H*)2-one (**10a**) and 1,2,3,4-tetrahydroisoquinoline (**11b**) - emerged for the high σ_2 affinity combined to an excellent σ_2 *versus* σ_1 selectivity. Compound **11b** with its low nanomolar σ_2 affinity and a 2807-fold σ_2 *versus* σ_1 selectivity, exceeded the results obtained with the best 3,4-dihydroisoquinolin-(2*H*)1-one compounds developed so far (**1** and **2**). σ Binding profile of **11b** represents also an improvement compared to flexible benzamides RHM-1 and ISO-1, the former being successfully radiolabeled and used to image tumors in preclinical studies and the latter currently under three different clinical trials. Therefore, **10a** and **11b** in particular represent powerful tools for the study of these still enigmatic receptors. In addition, the absence of a cytotoxic effect and the modest interaction with the P-gp, together with an appropriate lipophilicity (cLogP = 3.94 and $cLogD_{7.4}$ = 3.23)[41] suggest that **11b** should be further investigated to furnish a novel possible radiotracer for the imaging of σ_2 receptors via PET in tumors.

Supplemental Information: Synthesis of intermediate compounds 3a-c, 9a-c; table of elemental analyses, table of physical properties of novel compounds.

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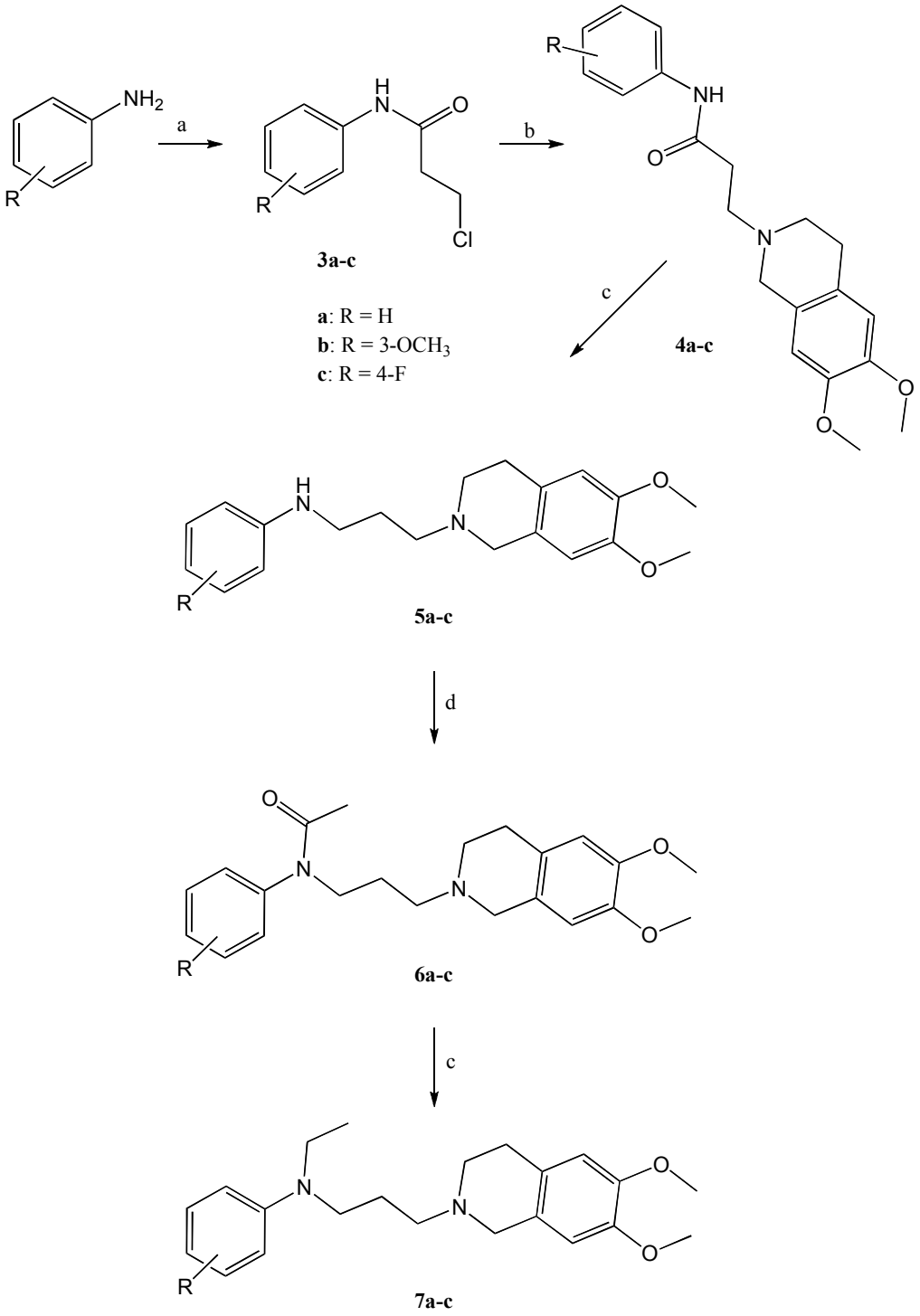
Scheme 1[a]



[a]Reagents: (a) 3-Chloropropionyl chloride, Et_3N, CH_2Cl_2; (b) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, K_2CO_3, CH_3CN; (c) $LiAlH_4$, anhydrous THF; d) CH_3COCl, Et_3N, CH_2Cl_2.

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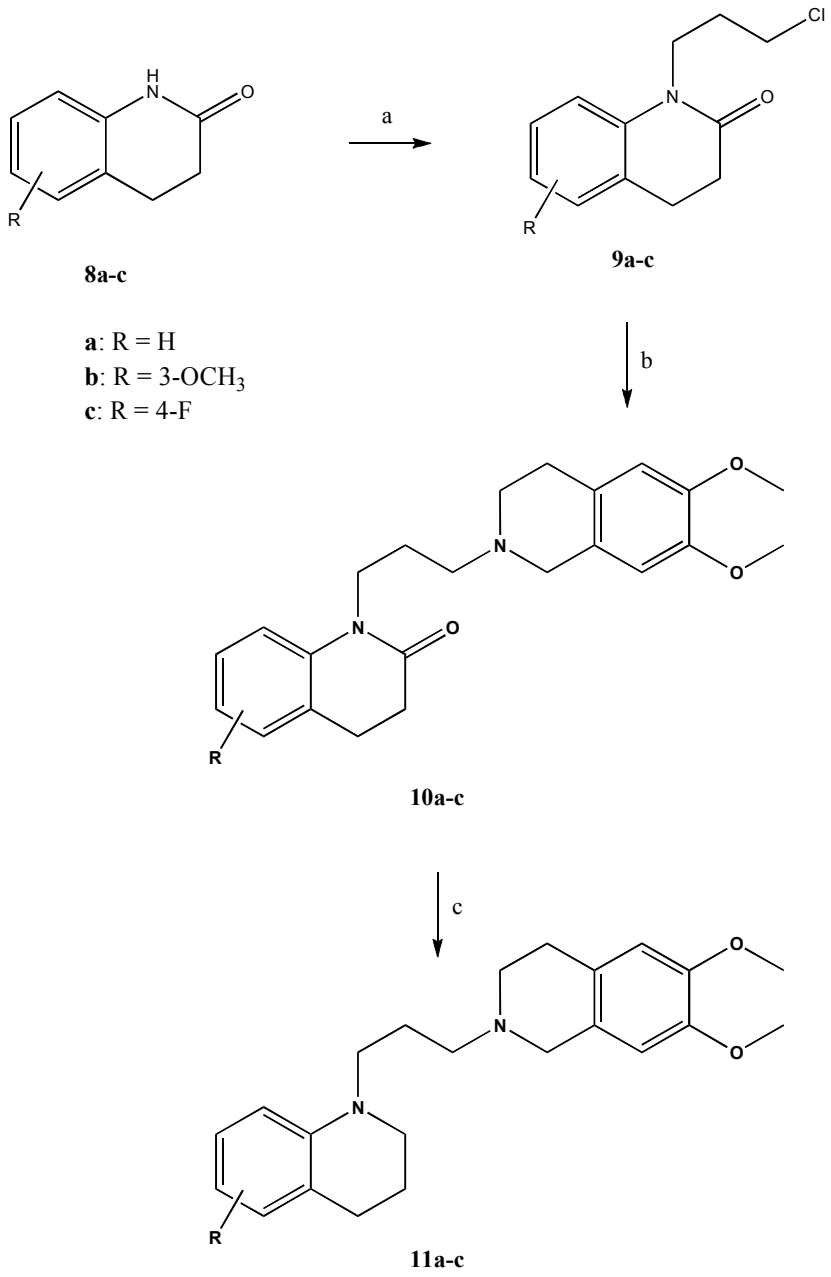
Scheme 2[a]



[a]Reagents: (a) 1-Bromo-3-chloropropane, NaH, DMF; (b) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, K_2CO_3, CH_3CN; (c) $BH_3 \cdot DMS$, anhydrous THF.

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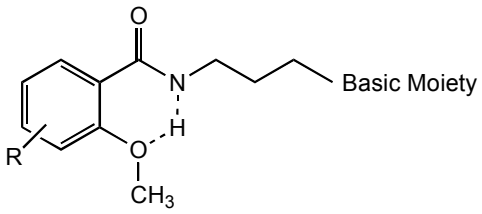
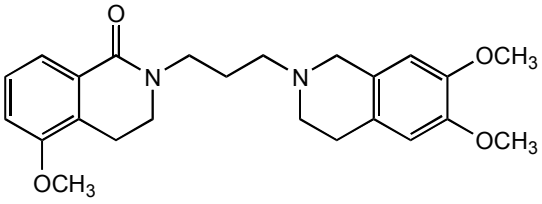
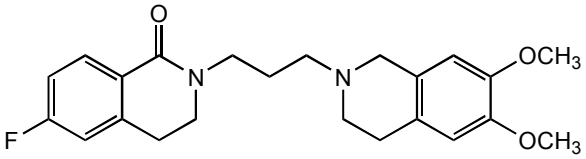
Figure 1.



Hydrogen bond driving the bicyclic-like conformation



1



2

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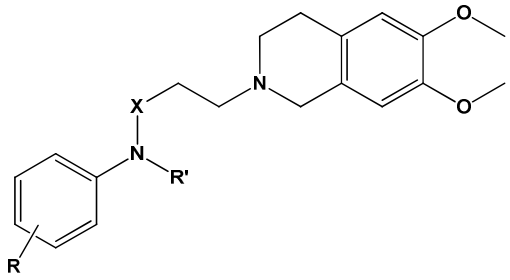
Table 1. Biological Data of Novel Anilide and Aniline Derivatives.



				K_i ± SEM[a] (nM)		EC_{50} ± SEM[a] (μM)	
Comp	R	R'	X	σ_1	σ_2	P-gp	MCF7
4a	H	H	CO	397±24	278±17	9.51±1.1	> 100
4b	3-OCH_3	H	CO	612±31	56.0±7.7	4.04±0.6	> 100
4c	4-F	H	CO	1350±57	50.3±8.3	5.88±0.7	> 100
5a	H	H	CH_2	255±22	3.58±1.5	5.83±0.4	> 100
5b	3-OCH_3	H	CH_2	604±31	9.80±2.7	2.75±0.2	> 100
5c	4-F	H	CH_2	144±17	2.70±1.1	3.70±0.2	> 100
6a	H	$COCH_3$	CH_2	>5000	92.8±2.2	5.52±0.3	> 100
6b	3-OCH_3	$COCH_3$	CH_2	3593±134	40.2±1.9	4.51±0.2	> 100
6c	4-F	$COCH_3$	CH_2	4033±219	83.7±6.9	4.64±0.7	> 100
7a	H	CH_2CH_3	CH_2	1166±67	70%[b]	1.77±0.3	> 100
7b	3-OCH_3	CH_2CH_3	CH_2	1070±69	11.3±0.2	0.86±0.1	> 100
7c	4-F	CH_2CH_3	CH_2	320±42	67%[b]	1.41±0.2	> 100
(+)-pentazocine				3.40±0.7			
DTG					28.5±4.2		
MC18						1.1±0.3	

[a]Values represent the mean of n ≥ 2 separate experiments in triplicate ± SEM. [b]Percent displacement at a concentration of 10^{-11} M was reported as a complete displacement curve was not obtained.

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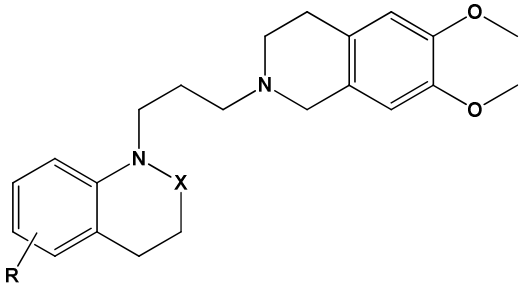
Table 2. Biological Data of Novel 1,2,3,4-Tetrahydroquinoline Derivatives.



			K_i ± SEM[a] (nM)		EC_{50} ± SEM[a] (μM)	
Comp	R	X	σ_1	σ_2	P-gp	MCF7
10a	H	CO	4627±100	7.11±1.4	2.74±0.4	> 100
10b	3-OCH_3	CO	302±29	18.6±2.6	1.97±0.4	> 100
10c	4-F	CO	3024±156	34.2±1.4	2.70±0.1	> 100
11a	H	CH_2	1462±81	80%[b]	1.97±0.2	> 100
11b	3-OCH_3	CH_2	3987±134	1.42 ± 0.33	2.13±0.1	> 100
11c	4-F	CH_2	876±54	73%[b]	1.81±0.3	> 100
(+)-pentazocine			3.40±0.7			
DTG				28.5±4.2		
MC18					1.12±0.3	

[a]Values represent the mean of n ≥ 2 separate experiments in triplicate ± SEM. [b]Percent displacement at a concentration of 10^{-11} M was reported as a complete displacement curve was not obtained.

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Legend of Figure 1

Figure 1. Hydrogen bond driving the bicyclic-like conformation in flexible benzamides, and 3,4-dihydroisoquinolin(2*H*)1-one reference σ_2 ligands.