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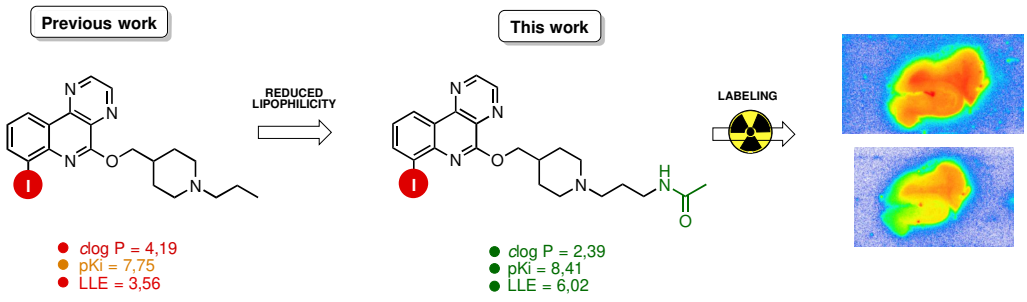
Abstract

A series of iodinated ligands for the SPECT imaging of 5-HT$_4$ receptors was designed starting from the previously reported hit **MR-26132**. We focused on the modulation of the piperidine-containing lateral chain by introducing hydrophilic groups in order to decrease the liphophilicity of the new ligands. All the synthesized compounds were tested for their binding affinities on 5-HT$_4$Rs and based on the Ligand Lipophilicity Efficiency approach, compound **13** was further selected for radioiodination with iodine-125 and imaging experiments. Compound **13** showed its ability to displace the specific signal of the reference compound **[^{125}I]SB-207710** but no significant detection of **[^{125}I]13** was observed *in vivo* in SPECT experiments.



Keywords Serotonin, SPECT Imaging, 5-HT$_4$, Radio-iodination, LLE

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) plays a pivotal role in the control of physiological functions by interacting with seven 5-HT receptors families (5-HT_{1-7}Rs) [1]. Dysregulation of 5-HT neurotransmission results in a number of disorders, and the better knowledge of these pathophysiological ways has led to the development of effective and selective ligands of 5-HTRs to study and modulate serotonin functions [2]. Beyond these pharmacologic and therapeutic strategies, there is a growing interest in using molecular imaging tools such as radiotracers allowing to study *in-vivo* in a non-invasive way the physiological and pathological role of the serotonergic system [3,4].

Among these receptors, the 5-HT_4R, discovered in 1988 [5], is expressed in both peripheral and central nervous system. In Peripheral regions, the 5-HT_4R can be found in the heart, the gastrointestinal tract, the adrenal glands and the urinary bladder [6] and have been related to gastrointestinal disorders [7,8], heart failures [9–11] and hyperaldosteronism [12]. Central 5-HT_4Rs have been found to be involved in psychiatric disorders such as depression [13], anorexia[14] or age associated memory impairment such as in Alzheimer's Disease [15,16]. In this context, design of radioligands able to target the 5-HT_4R can provide effective tools to detect and monitor related dysfunctions using non-invasive techniques such as Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT). To date, three radioligands targeting 5-HT_4Rs were described in the literature (Figure 1). **[^{123}I]SB-207710**, developed for SPECT imaging, was the first radioligand able to label *in vivo* the 5-HT_4R rich areas in monkey but, due to low brain penetration, no further investigations were reported [17]. The PET antagonist radioligand **[^{11}C]SB-207145**, structurally related to **SB-207710**, has been successfully used for *in vivo* studies in human [18,19]. However, the short half-life of the radioisotope ($t_{½}$= 20.4 min) significantly restricted its use for more advanced works in clinic. More recently, **[^{18}F]MNI-698** [20–22], the fluorinated analogue of **SB-207145** also exhibited its abilities to label *in vivo* the 5-HT_4R rich areas in monkey with a suitable brain penetration but its use remains limited due to the short metabolic stability as all compounds of this class caused by esterases.

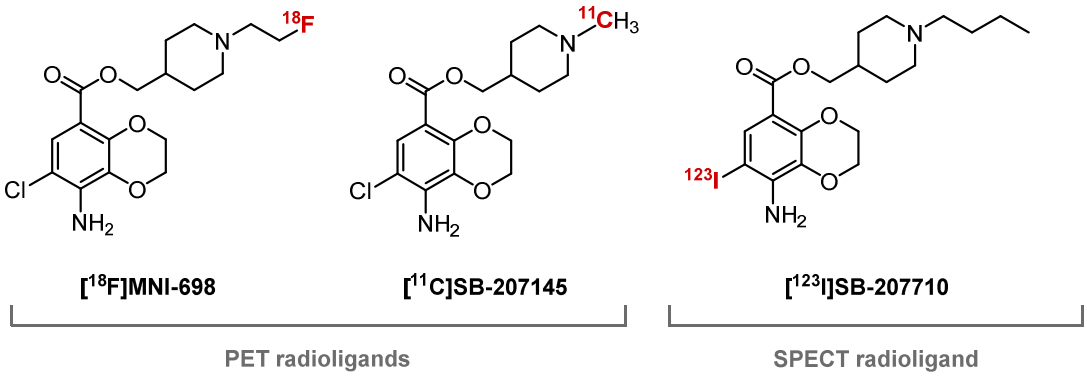


Figure 1. Previously described central 5-HT$_4$R radiotracers

Previously, our group reported the synthesis of radioiodinated ligands of the 5-HT$_4$R based on a iodophenanthridine scaffold [23] (Figure 2). Even if these compounds demonstrated high affinity and selectivity toward the 5-HT$_4$R, none of the synthesized radioligands in this series were able to label 5-HT$_4$R-containing regions in rodent's brain. In a second study [24], our group pointed out that 5-HT$_4$R ligands with reduced lipophilicities in the diazaphenanthridine series could also act as potent and selective 5-HT$_4$R ligands. Among the evaluated radioligands in this work, compound **MR-26132**, demonstrated, in SPECT *in vivo* studies, its ability to label 5-HT$_4$R-containing regions in rat brain along with off-target labelling. Considering the high affinity for the 5-HT$_4$R of **MR-26132** and its selectivity toward the other 5-HTRs, we hypothesize that the lipophilicity of **MR-26132** could be even more finely tuned to abolish the off-target labelling probably due to unspecific interactions. In the present manuscript, a new generation of iodinated diazaphenanthridine ligands with reduced lipophilicity were designed. We chose to introduce nitrogen-, oxygen- and sulfur-based hydrophilic groups on the piperidine moiety of our scaffold to decrease the LogP within a 1-3 range. Here, we report the synthesis and evaluation of twenty-five new iodinated compounds following this strategy. To better address the problem of candidate selection for the imaging experiments, the Ligand Lipophilicity Efficiency (LLE) approach [25] has been used by correlating lipophilicity and 5-HT$_4$R *pKi* for each compound.

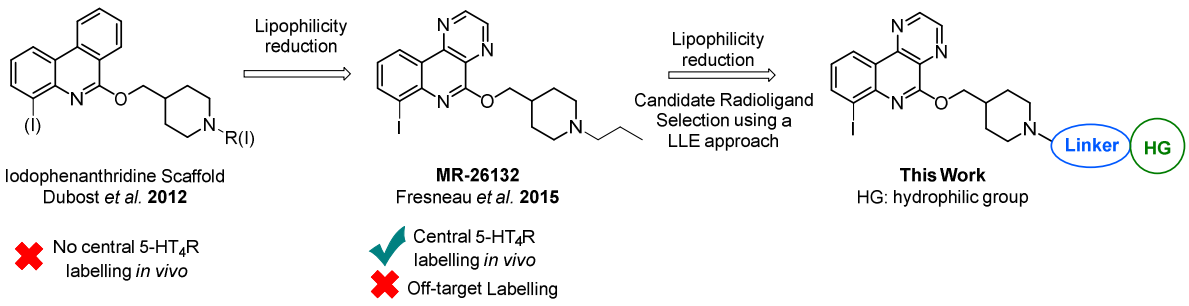
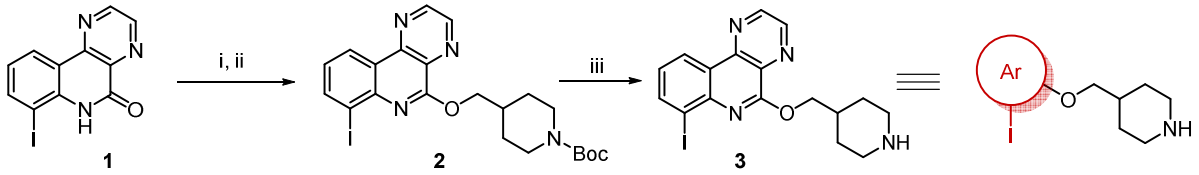


Figure 2. Previous work concerning (diaza)phenanthridines 5-HT$_4$R radioligands and targeted structures

2. Results and Discussion

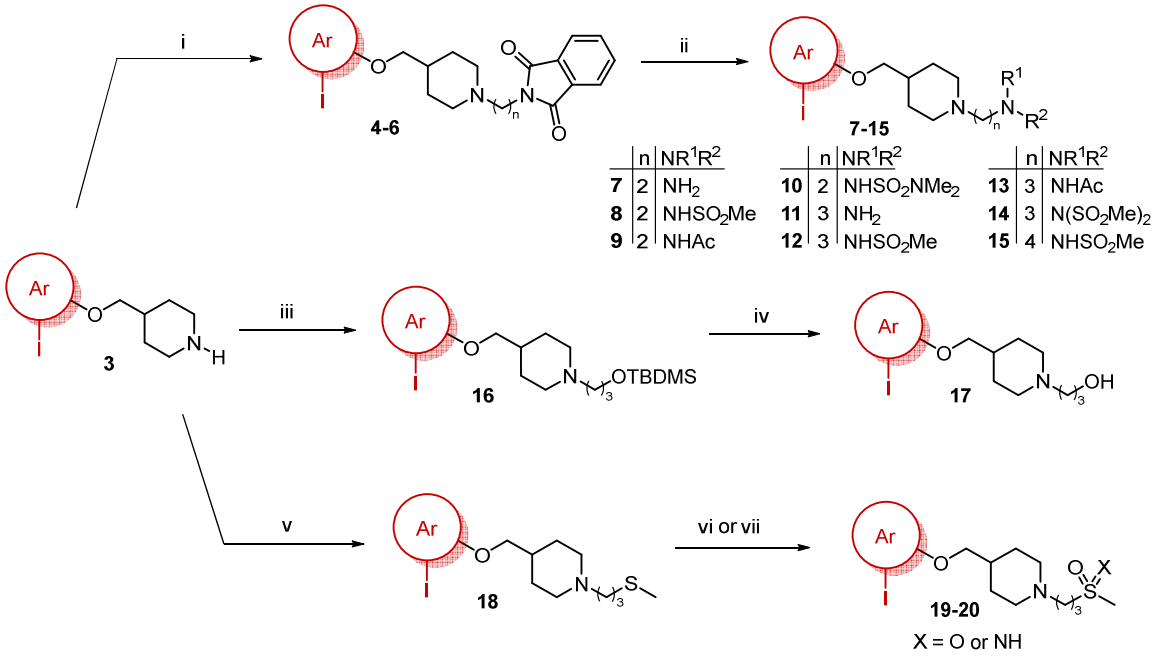
2.1 Chemistry

All the ligands were synthesized from the common precursor **3,** which was obtained following a previously established synthetic route [24]. Starting from 7-iodopyrazino[2,3-*c*]quinolin-5(*6H*)-one **1** [26], the expected compound was isolated after successive chlorodehydroxylation using the Vilsmeier-Haack reagent and nucleophilic substitution with *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (Scheme 1). Boc deprotection with TFA afforded the amine **3**.



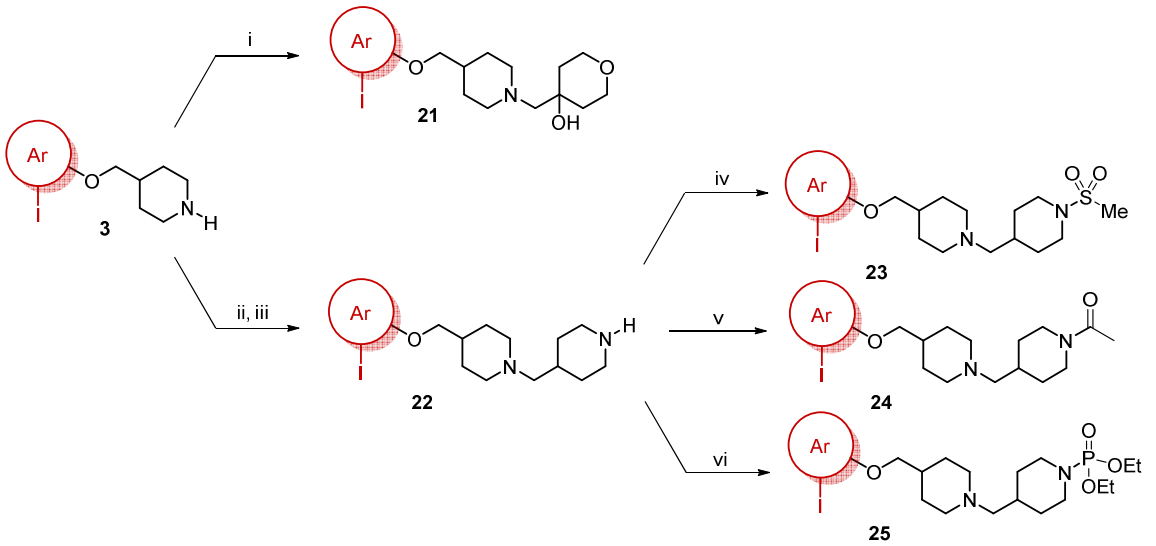
Scheme 1. Synthesis of precursor **3**. Reagents and conditions: (i) $(COCl)_2$, DMF, $CHCl_3$, reflux, 2h; (ii) *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate, NaH, DMF, rt, 2h, 64% over 2 steps; (iii) (a) TFA, DCM, 2h, rt; (b) K_3PO_4, rt, 30min; 98%.

We first designed several ligands where hydrophilic groups were introduced by means of a 2 to 4 carbons alkyl spacer moiety (Scheme 2). Thus, aminoalkyl chains containing 2 to 4 carbons were introduced by reacting the amine **3** and commercial *N*-bromoalkyl-phthalimides. The corresponding phthalimides **4** and **5** were deprotected with hydrazine to give the primary amines **7** and **11**, which were reacted with one equivalent of methanesulfonyl chloride to led to the sulfonamides **8** and **12.** The 4-carbons sulfonamide **15** was obtained from **6** without isolating the corresponding amine. Using two equivalents of methanesulfonyl chloride from **11** gave the disulfonimide **14**. Acetamide ligands **9** and **13** were prepared from **7** and **11** using acetic anhydride whereas the sulfamate ligand **10** was synthesized using *N*,*N*-dimethylsulfamoyl chloride as reactant from **7**. A hydroxypropyl chain was introduced by reacting the commercially available 3-bromopropoxy-*tert*-butyldimethylsilane with **3** to afford compound **16** which was subsequently deprotected by treatment with TBAF to afford the expected alcohol **17**. Finally, sulfur derivatives were investigated. Reaction of 3-(methylthio)propionaldehyde with **3** in the presence of $NaBH(OAc)_3$ gave the thioether **18**. Thus, oxidation of the sulfur atom gave sulfone **19** using *m*-CPBA and sulfoximine **20** using $PhI(OAc)_2$ in the presence of ammonium carbamate [27].



Scheme 2. Synthesis of ligands **4-20**. Reagents and conditions: (i) $Br(CH_2)_nNPhth$, NEt_3, MeCN, reflux, 24 h, 68-85%; (ii) (a) N_2H_4, EtOH, reflux, 2 h; (b) MsCl, NEt_3, DCM, 0 °C to rt or Ac_2O, DCM, 0 °C to rt or Me_2NSO_2Cl, NEt_3, DCM, 0 °C to rt ; for amine **7** and **11**, a fumarate salt was prepared using fumaric acid, iPrOH, 40 °C, 1h; 47-81%; (iii) $Br(CH_2)_3OTBDMS$, NEt_3, MeCN, reflux, 16 h, 63%; (iv) TBAF, THF, rt, 16 h, 49%; (v) 3-(methylthio)propionaldehyde, AcOH, $NaHB(OAc)_3$, DCM, rt, 3 h, 57%; (vi) mCPBA, DCM, rt, 2 h, 66%; (vii) NH_2COONH_4, $PhI(OAc)_2$, MeOH, rt, 16 h, 57%.

Oxane and piperidine groups were then added on the piperidine moiety of **3** (Scheme 3). Ligand **21**, containing an oxane scaffold, was synthesized from **3** using 1,6-dioxaspiro[2.5]octane, previously prepared according to a literature procedure [28]. Reaction of *tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate [29], with amine **3** followed by Boc deprotection provided the bis-piperidyl ligand **22**. The NH terminal piperidine of **22** was then functionalized as sulfonamide **23**, amide **24** and phosphoramidate **25** moieties through reactions with respectively mesyl chloride, acetic anhydride and diethyl chlorophosphate.



Scheme 3. Synthesis of ligands **21-25**. Reagents and conditions: (i) 1,6-dioxaspiro[2.5]octane, NEt_3, MeOH, reflux, 16 h, 81%; (ii) *tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate, NEt_3, MeCN, reflux, 16 h, (iii) (a) TFA, DCM, 2 h, rt; (b) K_3PO_4, rt, 30

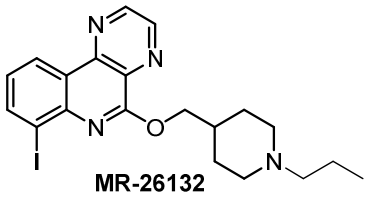
min, 48% over 2 steps; (iv) MsCl, NEt_3, DCM, 0 °C to RT, 10 min, 89%; (v) Ac_2O, DCM, 0 °C to RT, 10 min, 93%; (vi) $ClP(O)(OEt)_2$, NEt_3, DCM, RT, 12 h, 29%.

2.2 Binding and LLE Studies

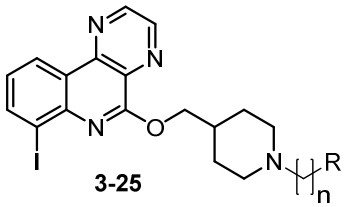
Binding affinities of the synthesized compounds were evaluated toward human 5-HT_4R as inhibition percentages at 10^{-6} M and 10^{-8} M, followed by K_i determination. Lipophilicity (*c*logP) was simulated based on MarvinSketch® software (version 5.2.6) and LLE was calculated for each compound by substracting cLogP to pK_i. Results are summarized in Table 1 and compared to **MR-26132**, the 5-HT_4R control ligand for this study.

In vitro results showed that most of the synthesized ligand displayed a binding affinity toward the 5-HT_4R below 30 nM (pK_i > 7.6) excluding the bulky phthalimide derivatives **4-6**. Among them, eleven compounds such as amine derivatives **8**, **9, 11**, **13**, **15** and **22** as well as sulfur derivatives **19** and **20** and alcohol derivatives **17** and **21** exhibited higher affinities than the previous reference (**MR-26132**, K_i = 17.7 nM). The highest affinity was obtained with an acetamide hydrophilic group linked by a propyl spacer (**13**, K_i = 3.9 nM). Moreover, the length of the carbon chain does not seem to have a crucial effect on binding results. The K*i* of the sulfonamides were rather identical with an ethyl spacer (**8**, K_i = 8.5 nM), a propyl spacer (**12**, K_i = 17.7 nM) or a butyl spacer (**15**, K_i = 11.2 nM). Interestingly, ligands with bulky groups, as described in a recent study [30–32], such as piperidine **22**-**24** or oxane **21** also exhibited high affinity toward 5-HT_4. As expected, lipophilicity simulation showed that introduction of heteroatoms significantly decreases the lipophilicity. Compared to **MR-26132** (*c*logP = 4.19), introduction of a hydroxy group (**17**, *c*logP = 2.68) or amino group (**11**, *c*logP = 2.57) considerably decreases the hydrophobicity of the ligand. Chemical modulations of the amino group lead to the lowest values of clogP with a minimum for the disulfonimide group (**14**, *c*logP = 1.37). Interestingly, sulfur derivatives such as sulfone (**19**, *c*logP = 1.79) turned out to be an attractive function to decrease lipophilicity.

Table 1. Binding Affinities toward human 5-HT_4R and LLE calculation for **MR26132** compound **3** to **25**.

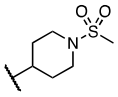
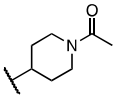
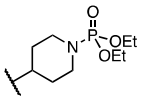


MR-26132



3-25

Ligand	n	R	% inh (10^{-6}M/10^{-8}M)[a]	*Ki* (5-HT_4)[b]	*c*logP[c]	LLE[d]
MR-26132	3	H	100/68	17.7 ± 4.1 nM	4.19	3.56
3	0	H	100/39	36.8 ± 4.7 nM	2.93	4.50

4	2	Nphth	100/37	543 ± 195 nM	4.06	2.21
5	3	Nphth	100/18	312 ± 57 nM	4.12	2.38
6	4	Nphth	100/56	n.m.[e]	4.63	n.m.
7	2	NH_2	100/84	18.3 ± 9.4 nM	2.51	5.23
8	2	$NHSO_2Me$	100/100	8.5 ± 6.8 nM	1.83	6.24
9	2	NHAc	100/95	15.4 ± 2.3 nM	2.33	5.48
10	2	$NHSO_2NMe_2$	100/84	22.6 ± 4.7 nM	1.82	5.83
11	3	NH_2	100/76	9.7 ± 1.7 nM	2.57	5.44
12	3	$NHSO_2Me$	100/68	17.7 ± 2.4 nM	1.89	5.86
13	3	NHAc	100/94	3.9 ± 1.3 nM	2.39	6.02
14	3	$N(SO_2Me)_2$	100/49	56.7 ± 10.8 nM	1.37	5.87
15	4	$NHSO_2Me$	100/96	11.2 ± 4.6 nM	2.40	5.86
16	3	OTBDMS	100/62	21.4 ± 8.8 nM	5.32	2.36
17	3	OH	100/93	8.4 ± 3.9 nM	2.68	5.40
18	3	SMe	100/100	19.7 ± 2.3 nM	4.32	3.39
19	3	SO_2Me	100/93	12.4 ± 10.3 nM	1.79	6.11
20	3	S(O)(NH)Me	100/82	9.5 ± 1.5 nM	2.82	5.20
21	1		100/84	17.0 ± 2.0 nM	2.50	5.27
22	1		100/94	10.5 ± 3.3 nM	3.41	4.57
23	1		100/76	28.0 ± 5.4 nM	2.52	5.03
24	1		100/93	22.6 ± 4.7 nM	3.02	4.42
25	1		100/53	n.m.	3.93	n.m.

[a] Inhibition percentages were measured using human 5-HT_4R, [b] Ki were measured using human 5-HT_4R, [c] Calculated logP were obtained using MarvinSketch® software (version 5.2.6), [d] LLE was obtained by subtracting cLogP to pK_i for each compounds (LLE = pK_i – cLogP), [e] n.m. = not measured

In order to select the best candidate for radioiodination and SPECT imaging experiments, we choose to use the Ligand Lipophiliy Efficiency (LLE), a valuable decision aid tool for medicinal chemistry [33]. *In vitro* binding results toward 5-HT_4R (pK_i), calculated lipophilicity (*c*logP) and ligand lipophilicity efficiency defined as LLE = pKi - cLogP were reported on the plot (Figure 3). The dashed area was defined on the plot to select the more convenient structure for the radiolabeling using

three parameters: an adequate lipophilicity (1 < *c*logP < 3), a suitable binding affinity toward the receptor (pK_i > 8) and an appropriate Ligand Lipophilicity Efficiency (5 < LLE < 7). For comparison, **MR-26132** and **SB-207710** (*p*ki = 8.66 and *c*logP = 3.2 [9]) were also represented on the plot. Among the twenty-two compounds represented on the plot, **8**,**11**,**13**,**17** and **20** are within the three required conditions while fitting in the dashed area. Among these compounds, ligand **13** exhibiting the highest *pKi* value was selected as a candidate for radiolabelling.

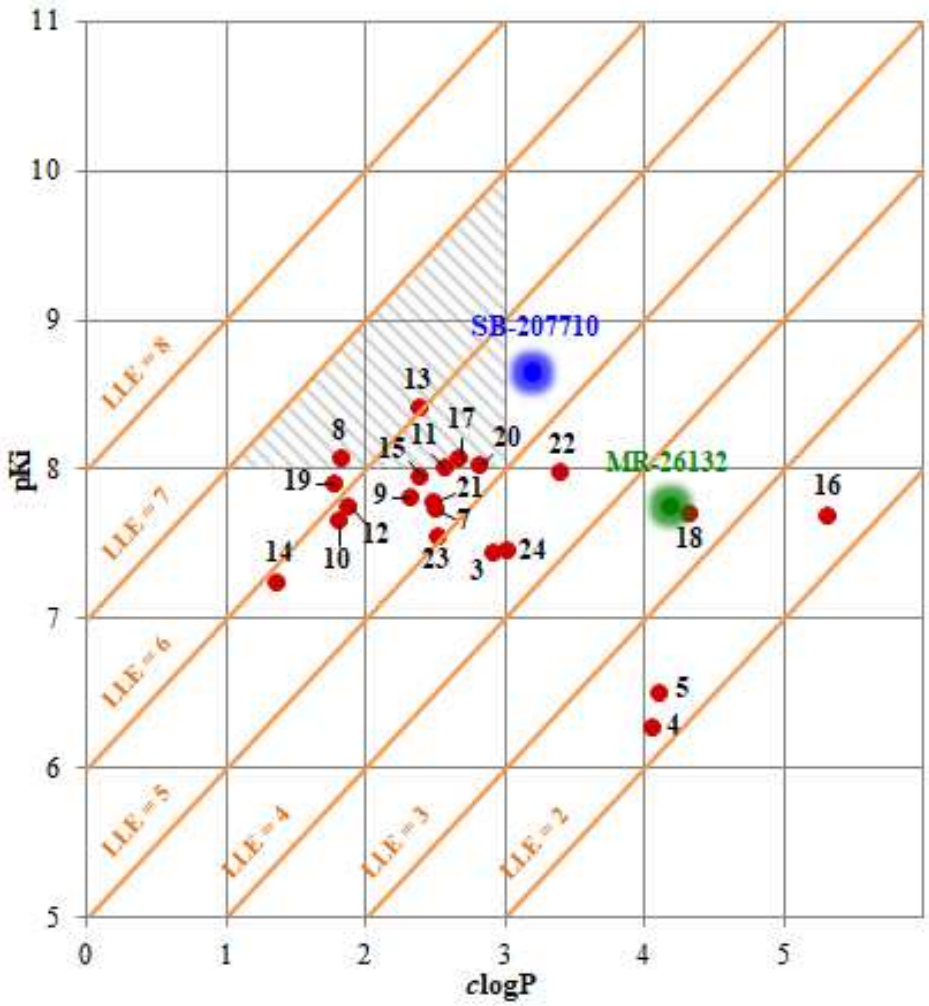


Figure 3. cLogP versus pKi plot (LLE = pKi-cLogP)

Prior to radiolabeling and imaging experiments, selectivity toward other 5-HT receptors and functional profile of compound **13** were assessed (Table 2). Binding affinities of **13** were evaluated toward a panel of 5-HT receptors as inhibition percentages at 10^{-6} M and results show a slight affinity toward 5-HT_{2b} and 5-HT_{2c} receptors. Inhibition percentage of agonist and antagonist response toward 5-HT_4R were also measured and revealed that **13** is 5-HT_4R antagonist.

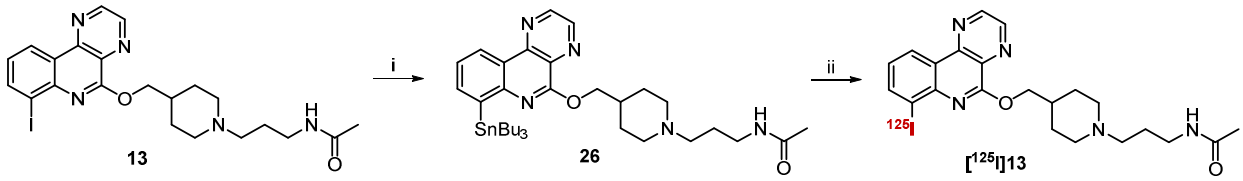
Table 2. Selectivity and functional profile of compound **13**.

5-HTR	% (10^{-6} M of **13**)
5-HT_{1a}[a]	9.9[d]
5-HT_{1b}[b]	6.7[d]
5-HT_{1d}[c]	12.1[d]
5-HT_{2a}[a]	43.4[d]
5-HT_{2b}[a]	75.6[d]
5-HT_{2c}[a]	71.5[d]
5-HT_{3}[a]	16.8[d]
5-HT_{5a}[a]	-3.8[d]
5-HT_{6}[a]	-3.1[d]
5-HT_{7}[a]	10.7[d]
5-HT_{4}[a] agonist effect	-4.1[e]
5-HT_{4}[a] antagonist effect	102[f]

[a]Test performed on human recombinant CHO cells. [b]Test performed on rat cerebral cortex. [c]Test performed on rat recombinant CHO cells. [d]Inhibition percentages were determined at CEREP (n=2). [e]Percentage of agonist response was determined at CEREP (n=2). [f]Inhibition percentage of agonist response was determined at CEREP (n=2).

2.3 Radiochemistry

The preparation of [^{125}I]**13** started from **13**, which was first stannylated using a palladium-catalyzed stannylation procedure to give the tin precursor **26** [34] (Scheme 4).Then, iodine-125 was introduced after a tin-iodine exchange using [^{125}I]NaI as the source of the radioisotope and hydrogen peroxide as the oxidant in acidic medium. Finally, HPLC purification afforded the radio-iodinated compound **[^{125}I]13** with a radiochemical yield of 81% and a molar activity greater than 100 GBq/µmol.



Scheme 4. Radiolabeling of **13**. Reagents and conditions: (i) Pd_2dba_3, $(SnBu_3)_2$, DIPEA, iPrOH, 70 °C, 48 h, 47%; (ii) [^{125}I]NaI, H_2O_2 , AcOH, MeCN, rt, 40 min.

2.4 In vitro Competition Experiments **13** versus **SB-207710**

To determine the specificity of compounds **13** and **MR-26132** to bind to the 5HT4 receptor, a competition experiment with the reference ligand **SB-207710** was performed on human brain hippocampal sections (Figure 4). A displacement experiment was performed and was analyzed by the measurement of the SBR (Specific Binding Ratio) which corresponds to the ratio minus one between the radioactivity measured on a section in the presence of the radioactive compound alone and the

radioactivity measured on a section in the presence of the radioactive compound and the tested compound. An example of displacement of the binding of **[^{125}I]SB-207710** by **13** and **MR-26132** is given in Fig. 4A-C. The SBR in the different areas of the hippocampus shows that compounds **13** and **MR-26132** induce a shift in **[^{125}I]SB-207710** which is even greater than when using **SB-207710**. The mirror experiment using **[^{125}I]13** or **[^{125}I]MR-26132** with **SB-207710** shows a lower capacity of the reference compound to displace the signal due to the compounds tested. Taken together, these results show that both **13** and **MR-26132** bind to the 5-HT$_4$Rs and are more potent than **SB-207710** to displace the specific binding of **[^{125}I]SB-207710.** Nevertheless, the low SBR obtained in the mirror experiment could be correlated to a high unspecific binding of these two compounds which are not displaced by **SB-207710** at high concentration.

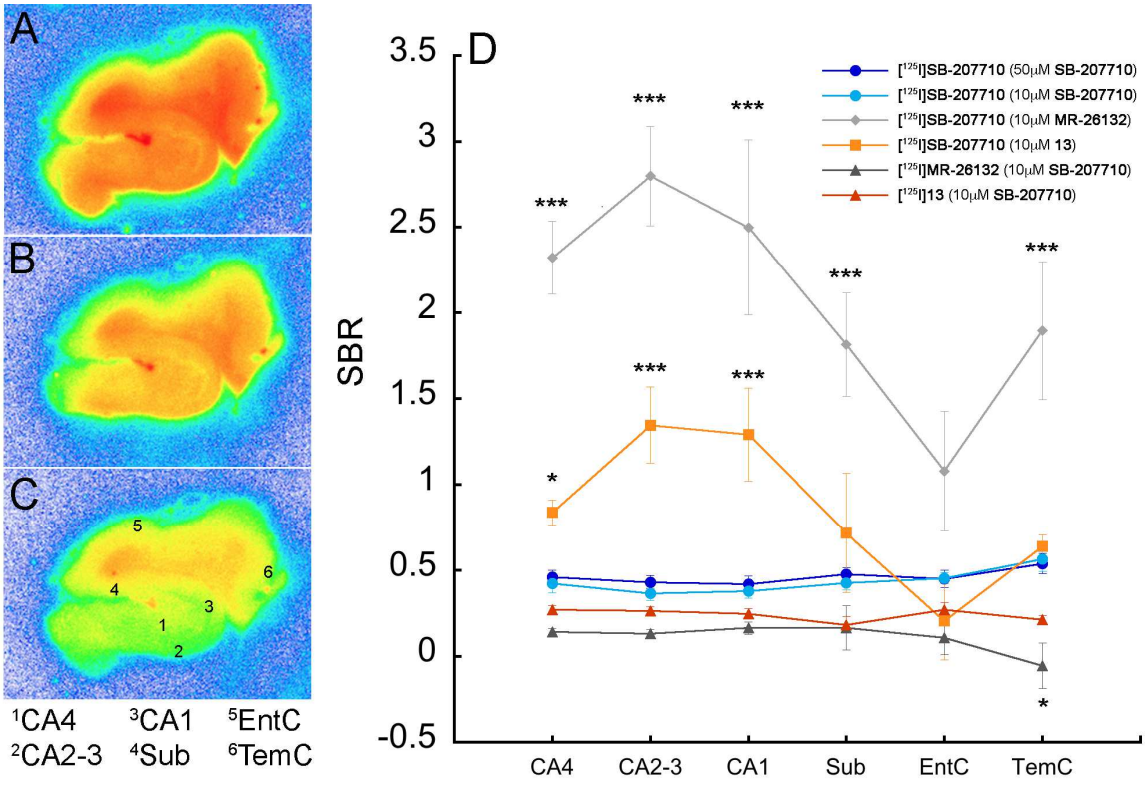


Figure 4. Competition experiment between 13 and MR-26132 and the reference compound SB-207710. Representative examples of binding in human brain slices when exposed to **[^{125}I]SB-207710** alone (A) or in the presence of **13** (B) or **MR-26132** (C). (D) SBR were calculated in different brain areas and using different couples of radioactive / cold molecule as indicated in the figure.

2.5 SPECT in vivo imaging with **[^{125}I]13**

In order to determine the ability of compound **13** to bind *in vivo* to the 5HT$_4$ receptor, a SPECT imaging experiment was performed. After the intravenous injection of **[^{125}I]13**, radioactivity levels were monitored in the hippocampus and striatum, two 5HT$_4$-rich regions (Figure 5). An early peak corresponding to the passage of the blood in the brain is measured. This peak is followed by an

immediate return to residual values, demonstrating the absence of a specific signal in the brain. This result show that compound **13** is either unable to cross the Blood Brain Barrier or strongly bound to plasma proteins leading to a very low concentration in the brain. A quick metabolism cannot be excluded neither a high excretion of the radioligand by Pgp (permeability glycoprotein) [35].

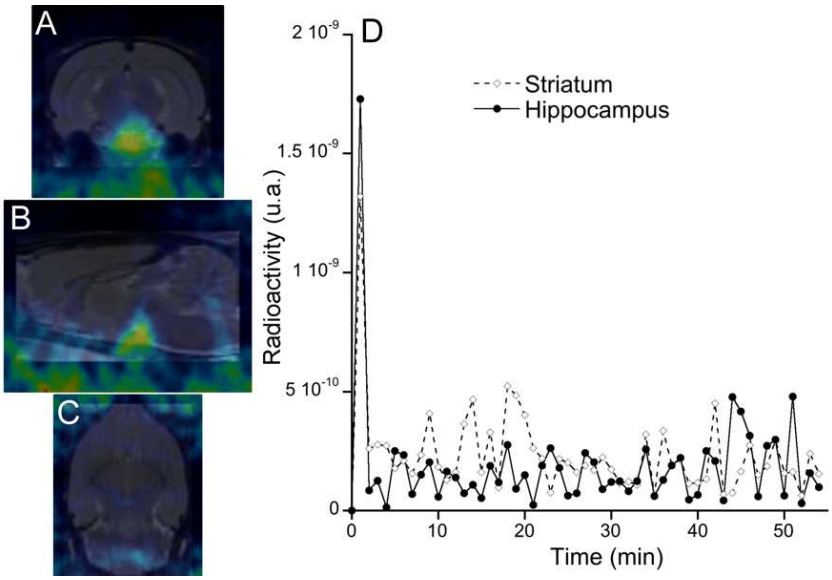


Figure 5. SPECT experiment with [^{125}I]13. Coronal (A), sagittal (B) and axial (C) planes of *in vivo* **[^{125}I]13** SPECT image (10-55 min post-injection) showing the absence and the probable accumulation of **[^{125}I]13** in the brain and the pituitary, respectively. (D) Time-activity-curves for the striatum and the hippocampus.

3. Conclusion

Starting from the previously reported iodinated 5-HT$_4$R ligand **MR-26132** which showed a too high unspecific binding in SPECT imaging experiments, we designed new ligands by adding on the lateral chain of piperidine diverse hydrophilic groups in order to decrease the lipophilicity of **MR-26132**. We were able to obtain new high affinities iodinated 5-HT$_4$R ligand with decreased LogP. Compound **13** has been selected using the Ligand Lipophilicity Efficiency to be radioiodinated. Its ability to displace the specific signal of the reference radioligand **[^{125}I]-SB207710** has been demonstrated *in vitro*, but this compound exhibits a too high off-target unspecific binding as proved by the displacement experiments. Moreover, this compound exhibits a very low signal to noise ratio in *in vivo* SPECT imaging experiments. Overall, even if the LLE has allowed here the selection of a ligand able to displace in vitro a specific radioligand, we think that this approach should be adjusted and/or completed to better suit to the CNS radio-imaging context.

4. Experimental

4.1 Chemistry

4.1.1 General experimental information

All solvents and chemicals were used as purchased unless stated otherwise. All NMR spectra were recorded on Bruker Avance III 400 spectrometer. Proton and carbon-13 NMR spectra are reported as chemical shifts (δ) in parts per million (ppm). Coupling constants (J) are reported in units of hertz (Hz). The following abbreviations are used to describe multiplets: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). High resolution mass spectra (HRMS, *m/z*) were recorded on a Waters Acquity UPLC H-ClassXevo G2-XS spectrometer using positive electrospray ionization (ESI). Infrared spectra were recorded using a Perkin-Elmer Spectrum 65 FT-IR spectrometer. Absorptions are reported in wavenumbers (cm^{-1}) and only peaks of interest are reported. Melting points of solids were measured on a Stuart Automatic Melting point SMP-50 apparatus. Flash column chromatography was performed over silica gel C60 (40-60 µm) or RP-18 (50 µm) using eluent systems as described for each experiment. Unless otherwise specified, all reagents were obtained from commercial suppliers.

4.1.2 General method 1: Removal of phthalimide protection

In a round bottom flask to a suspension of phthalimide derivative (1.00 equiv.) in ethanol (15 mL/mmol), hydrazine monohydrate (15.0 equiv.) was added and the solution was stirred under reflux until the solubilisation of the mixture. The solution was cooled to room temperature and filtered. The precipitate was washed with dichloromethane (10 mL/mmol of phthalimide derivative) and the filtrate was evaporated. The crude mixture was dissolved in dichloromethane (10 mL/mmol of phthalimide derivative), filtered and the filtrate was dried over $MgSO_4$. The resulting solution was filtered and evaporated to give the primary amine. 7-iodopyrazino[2,3-*c*]quinolin-5(6*H*)-one was obtained according to a described procedure [26].

4.1.3 *tert*-Butyl 4-[((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl]piperidine-1-carboxylate **2**

In a round bottom flask, oxalyl chloride (2.2 mL, 25.5 mmol) and DMF (2.00 mL, 26.4 mmol) were added to chloroform (20 mL) at 0 °C and the solution was stirred during 3 hours at room temperature. 7-Iodopyrazino[2,3-*c*]quinolin-5(6*H*)-one **1** (800 mg, 2.47 mmol) was added to the mixture and the solution was refluxed for 2 hours. The resulting mixture was carefully hydrolysed with water and the pH was adjusted to 10 using an aqueous ammonia solution (28%). The resulting

solution was extracted with dichloromethane (3 x 30 mL), dried over $MgSO_4$, filtered and evaporated affording the expected chlorimine. In a round bottom flask, NaH (108 mg, 2.72 mmol) was added to a solution of *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (585 mg, 2.72 mmol) in dry DMF (30 mL) under N_2 at 0 °C. The reaction mixture was stirred for 15 min, warmed to room temperature and was stirred for 15 min. The chlorimine previously obtained above was added and the reaction mixture was stirred at room temperature overnight. Water (30 mL) was carefully added and the resulting mixture was extracted with ethyl acetate (3 x 30 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was then purified by silica gel chromatography using CH_2Cl_2/AcOEt (100/0 to 80/20) as eluent affording the expected product (835 mg, **64%**) as a light orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, ^3J = 2.0 Hz, 1H), 9.01 (d, ^3J = 2.0 Hz, 1H), 8.91 (dd, ^3J = 8.0 Hz, ^4J = 1.4 Hz, 1H), 8.35 (dd, ^3J = 7.6 Hz, ^4J = 1.4 Hz, 1H), 7.32 (t, ^3J = 7.8 Hz, 1H), 4.72 (d, ^3J = 6.8 Hz, 2H), 4.26-4.07 (m, 2H), 2.85-2.70 (m, 2H), 2.49-2.36 (m, 1H), 2.00-1.93 (m, 2H), 1.47 (s, 9H), 1.46-1.34 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 155.0, 147.9, 145.9, 145.5, 143.8, 141.6, 131.1, 126.7, 124.8, 123.4, 100.7, 79.5, 72.4, 43.8 (2C), 35.6, 29.2 (2C), 28.6 (3C). IR (KBr): 2969, 2936, 1692, 1423, 1139, 1047 cm^{-1}. mp 174-176 °C. HRMS/ESI: calculated for $C_{22}H_{25}IN_4NaO_3$ $[M+Na]^+$ 543.0869, found 543.0870.

4.1.4 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidine **3**

In a round bottom flask, **2** (500 mg, 0.96 mmol) was dissolved in dichloromethane (20 mL). Trifluoroacetic acid (1.10 mL, 14.4 mmol) was added and the solution was stirred at room temperature. After 2 hours the solvent was evaporated *in vacuo* and the crude product was dissolved in a saturated solution of K_3PO_4 (20 mL). The solution was stirred 30 min at room temperature and the resulting mixture was extracted with dichloromethane (3 x 15 mL). The combined organics layers were dried over $MgSO_4$, filtered and evaporated to obtain the expected product without further purification as a yellow solid (395 mg, **98%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.04 (d, ^3J = 2.0 Hz, 1H), 8.97 (d, ^3J = 2.0 Hz, 1H), 8.84 (dd, ^3J = 8.0 Hz, ^4J = 1.4 Hz, 1H), 8.30 (dd, ^3J = 7.6 Hz, ^4J = 1.4 Hz, 1H), 7.26 (t, ^3J = 7.8 Hz, 1H), 4.68 (d, ^3J = 6.8 Hz, 2H), 3.18-3.09 (m, 2H), 2.74-2.60 (m, 2H), 2.42-2.30 (m, 1H), 1.99-1.88 (m, 3H), 1.45-1.33 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.8, 145.8, 145.4, 143.7, 141.4, 131.1, 126.5, 124.6, 123.2, 100.7, 73.0, 46.4 (2C), 35.7 (2C), 30.5. IR (KBr): 3435, 3392, 2910, 1592, 1452, 1349, 1178, 1045 cm^{-1}. mp: 178-180 °C. HRMS/ESI: calculated for $C_{17}H_{18}IN_4O$ $[M+H]^+$ 421.0525, found 421.0525.

4.1.5 2-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)ethyl)-2,3-dihydro-*1H*-isoindole-1,3-dione **4**

In a round bottom flask, compound **3** (712 mg, 1.70 mmol) was dissolved in acetonitrile (30 mL) and *N*-(2-bromoethyl)phthalimide (526 mg, 2.08 mmol) and triethylamine (0.45 mL, 3.50 mmol) were added. The solution was stirred at 80 °C for 24 hours and the white precipitate formed was filtered, washed with acetonitrile (2 x 20 mL) affording the expected product without further purification as a white solid (875 mg, **85%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^{3}J = 2.0 Hz, 1H), 9.00 (d, ^{3}J = 2.0 Hz, 1H), 8.90 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.33 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.87-7.81 (m, 2H), 7.73-7.68 (m, 2H), 7.29 (t, ^{3}J = 7.9 Hz, 1H), 4.68 (d, ^{3}J = 6.9 Hz, 2H), 3.84 (t, ^{3}J = 7.0 Hz, 2H), 3.10-3.00 (m, 2H), 2.70-2.59 (m, 2H), 2.32-2.18 (m, 1H), 2.17-2.05 (m, 2H), 2.00-1.89 (m, 2H), 1.54-1.39 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5 (2C), 158.6, 147.8, 145.9, 145.5, 143.9, 141.5, 134.0 (2C), 132.3 (2C), 131.1, 126.6, 124.7, 123.3, 123.3 (2C), 100.7, 72.9, 56.1, 53.5 (2C), 35.6, 35.1, 29.4 (2C). IR (KBr): 2939, 1714, 1592, 1431, 1393, 1329, 1177, 1046 cm^{-1}. mp: 206-208 °C. HRMS/ESI: calculated for $C_{27}H_{25}IN_5O_3$ $[M+H]^+$ 594.1008, found 594.1002.

4.1.6 2-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)propyl)-2,3-dihydro-*1H*-isoindole-1,3-dione **5**

In a round bottom flask, compound **3** (240 mg, 0.57 mmol) was dissolved in acetonitrile (15 mL) and *N*-(3-bromopropyl)phthalimide (185 mg, 0.70 mmol) and triethylamine (0.19 mL, 1.4 mmol) were added. The solution was stirred at 80 °C for 12 hours and the white precipitate formed was filtered, washed with acetonitrile (2 x 20 mL) affording the expected product without further purification as a white solid (284 mg, **81%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^{3}J = 2.0 Hz, 1H), 9.00 (d, ^{3}J = 2.0 Hz, 1H), 8.90 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.34 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.87-7.81 (m, 2H), 7.71-7.67 (m, 2H), 7.31 (t, ^{3}J = 7.8 Hz, 1H), 4.59 (d, ^{3}J = 7.0 Hz, 2H), 3.76 (t, ^{3}J = 6.9 Hz, 2H), 2.95-2.87 (m, 2H), 2.41 (t, ^{3}J = 6.9 Hz, 2H), 2.24-2.11 (m, 1H), 1.95-1.83 (m, 6H), 1.33-1.20 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.6 (2C), 158.6, 147.8, 145.9, 145.5, 143.9, 141.5, 134.0 (2C), 132.5 (2C), 131.1, 126.6, 124.7, 123.4, 123.3 (2C), 100.7, 72.9, 56.7, 53.5 (2C), 37.0, 35.1, 29.4 (2C), 25.6. IR (KBr): 2942, 1716, 1576, 1432, 1382, 1330, 1053 cm^{-1}. mp: 204-205 °C. HRMS/ESI: calculated for $C_{28}H_{27}IN_5O_3$ $[M+H]^+$ 608.1159, found 608.1164.

4.1.7 2-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)butyl)-2,3-dihydro-*1H*-isoindole-1,3-dione **6**

In a round bottom flask, compound **3** (292 mg, 0.70 mmol) was dissolved in acetonitrile (20 mL) and *N*-(4-bromopbutyl)phthalimide (240 mg, 0.85 mmol) and triethylamine (0.19 mL, 1.4 mmol) were

added. The solution was stirred at 80 °C for 24 hours and the white precipitate formed was filtered, washed with acetonitrile (2 x 20 mL) affording the expected product without further purification as a white solid (300 mg, **68%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (d, ^{3}J = 2.0 Hz, 1H), 8.99 (d, ^{3}J = 2.0 Hz, 1H), 8.87 (dd, ^{4}J = 8.0 Hz, ^{3}J = 1.4 Hz, 1H), 8.31 (dd, ^{4}J = 7.6 Hz, ^{3}J = 1.4 Hz, 1H), 7.84-7.78 (m, 2H), 7.72-7.65 (m, 2H), 7.26 (t, ^{3}J = 7.8 Hz, 1H), 4.70 (d, ^{3}J = 6.8 Hz, 2H), 3.70 (t, ^{3}J = 7.1 Hz, 2H), 3.01-2.93 (m, 2H), 2.41-2.33 (m, 2H), 2.30-2.18 (m, 1H), 2.04-1.91 (m, 4H), 1.74-1.64 (m, 2H), 1.60-1.46 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5 (2C), 158.6, 147.8, 145.8, 145.4, 143.8, 141.5, 134.0 (2C), 132.2 (2C), 131.1, 126.6, 124.7, 123.3, 123.3 (2C), 100.7, 72.7, 58.6, 53.6 (2C), 38.0, 35.2, 29.3 (2C), 26.8, 24.5 IR (KBr): 2930, 1708, 1592, 1400, 1351, 1176, 719 cm^{-1}. mp: 154-158 °C. HRMS/ESI: calculated for $C_{29}H_{29}IN_5O_3$ $[M+H]^+$ 622.1323, found 622.1315.

4.1.8 2-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)ethan-1-amine, fumarate salt **7**

Starting from compound **4** (90 mg, 0.15 mmol) and using **General method 1**, the resulting amine was dissolved in propan-2-ol (10 mL) and fumaric acid (17 mg, 0.15 mmol) was added. The solution was stirred at 40 °C for 1 hour and the beige precipitate formed was filtered, washed with propan-2-ol (3 x 5 mL) affording the expected product without further purification as a beige solid (71 mg, **81%**). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.24 (d, ^{3}J = 2.0 Hz, 1H), 9.12 (d, ^{3}J = 2.0 Hz, 1H), 8.80 (dd, ^{4}J = 8.0 Hz, ^{3}J = 1.4 Hz, 1H), 8.36 (dd, ^{4}J = 7.6 Hz, ^{3}J = 1.4 Hz, 1H), 7.37 (t, ^{3}J = 7.8 Hz, 1H), 6.41 (s, 2H), 4.57 (d, ^{3}J = 6.4 Hz, 2H), 2.95-2.85 (m, 4H), 2.52-2.48 (m, 2H), 2.09-1.94 (m, 3H), 1.88-1.78 (m, 2H), 1.51-1.39 (m, 2H) (Signals due to the OH and NH_2 are missing). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.2 (2C), 158.5, 148.8, 146.4, 144.6, 142.9, 141.0, 135.3 (2C), 130.3, 126.8, 124.1, 122.9, 100.9, 71.4, 55.0, 52.8 (2C), 35.9, 34.9, 28.6 (2C). IR (KBr): 3434, 2928, 1594, 1461, 1370, 1310, 1174, 843 cm^{-1}. mp: 173-175 °C. HRMS/ESI: calculated for $C_{19}H_{23}IN_5O$ $[M+H]^+$ 464.0952, found 464.0947.

4.1.9 *N*-(2-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidine-1-yl)ethyl) methanesulfonamide **8**

Starting from compound **4** (280 mg, 0.47 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. Triethylamine (0.13 mL, 0.94 mmol) and methanesulfonyl chloride (0.03 mL, 0.47 mmol) were added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 90/10) as eluent affording the

expected product as a white solid (155 mg, **61%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, ^{3}J = 2.0 Hz, 1H), 9.02 (d, ^{3}J = 2.0 Hz, 1H), 8.90 (dd, ^{4}J = 8.0 Hz, ^{3}J = 1.4 Hz, 1H), 8.34 (dd, ^{4}J = 7.6 Hz, ^{3}J = 1.4 Hz, 1H), 7.31 (t, ^{3}J = 7.8 Hz, 1H), 4.73 (d, ^{3}J = 6.6 Hz, 2H), 3.22-3.19 (m, 2H), 2.97 (s, 3H), 2.95–2.91 (m, 2H), 2.57–2.54 (m, 2H), 2.28–2.17 (m, 1H), 2.14–2.11 (m, 2H), 2.09–1.96 (m, 2H), 1.57–1.49 (m, 2H) (Signal due to the NH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.9, 145.5, 143.8, 141.5, 131.1, 126.7, 124.7, 123.4, 100.7, 72.5, 57.0, 53.2 (2C), 40.2, 39.9, 35.2, 29.4 (2C). IR (KBr): 3265, 2924, 2826, 1596, 1314, 1112, 780 cm^{-1}. mp: 147-149 °C. HRMS/ESI: calculated for $C_{20}H_{25}IN_5O_3S$ $[M+H]^+$ 542.0723, found 542.0723.

4.1.10 (2-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)ethyl)acetamide **9**

Starting from compound **4** (190 mg, 0.32 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. Acetic anhydride (0.03 mL, 0.32 mmol) was added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), extracted twice with dichloromethane (2 x 15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a white solid (100 mg, **71%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (d, ^{3}J = 2.0 Hz, 1H), 8.99 (d, ^{3}J = 2.0 Hz, 1H), 8.86 (dd, ^{4}J = 8.0 Hz, ^{3}J = 1.5 Hz, 1H), 8.31 (dd, ^{4}J = 7.6 Hz, ^{3}J = 1.5 Hz, 1H), 7.28 (t, ^{3}J = 7.8 Hz, 1H), 6.21 (br s, 1H), 4.71 (d, ^{3}J = 6.7 Hz, 2H), 3.38-3.31 (m, 2H), 2.99-2.92 (m, 2H), 2.52-2.45 (m, 2H), 2.34-2.21 (m, 1H), 2.11-2.02 (m, 2H), 1.99 (s, 3H), 1.99-1.94 (m, 2H), 1.59-1.47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 158.5, 147.9, 145.8, 145.4, 143.7, 141.5, 131.0, 126.6, 124.7, 123.3, 100.7, 72.6, 57.0, 53.3 (2C), 36.2, 35.1, 29.2 (2C), 23.5. IR (KBr): 3278, 2909, 1647, 1590, 1455, 1346, 1180 cm^{-1}. mp: 209-211 °C. HRMS/ESI: calculated for $C_{21}H_{25}IN_5O_2$ $[M+H]^+$ 506.1061, found 506.1053.

4.1.11 ((2-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)ethyl)sulfamoyl) dimethylamine **10**

Starting from compound **4** (195 mg, 0.33 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. *N*,*N*-dimethylsulfamoyl chloride (0.03 mL, 0.33 mmol) and triethylamine (0.09 mL, 0.66 mmol) were added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), extracted with dichloromethane (2 x 15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a white solid

(105 mg, **55%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (d, ^{3}J = 2.0 Hz, 1H), 9.01 (d, ^{3}J = 2.0 Hz, 1H), 8.90 (dd, ^{4}J = 8.0 Hz, ^{3}J = 1.4 Hz, 1H), 8.34 (dd, ^{4}J = 7.6 Hz, ^{3}J = 1.4 Hz, 1H), 7.31 (t, ^{3}J = 7.8 Hz, 1H), 4.73 (d, ^{3}J = 6.8 Hz, 2H), 3.15-3.08 (m, 2H), 2.97-2.89 (m, 2H), 2.81 (s, 6H), 2.55-2.50 (m, 2H), 2.33-2.21 (m, 1H), 2.13-2.03 (m, 2H), 2.02-1.93 (m, 2H), 1.57-1.44 (m, 2H) (Signal due to the NH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.9, 145.5, 143.8, 141.6, 131.1, 126.7, 124.8, 123.4, 100.7, 72.5, 56.6, 53.2 (2C), 40.1, 38.2 (2C), 35.3, 29.4 (2C). IR (KBr): 3293, 2937, 2800, 1595, 1427, 1334, 1156, 945 cm^{-1}. mp: 141-142 °C. HRMS/ESI: calculated for $C_{21}H_{28}IN_6O_3$ $[M+H]^+$ 571.0985, found 571.0988.

4.1.12 3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)propan-1-amine, fumarate salt **11**

Starting from compound **5** (75 mg, 0.15 mmol) and using **General method 1**, the resulting amine was dissolved in propan-2-ol (10 mL) and fumaric acid (17 mg, 0.15 mmol) was added. The solution was stirred at 40 °C for 1 hour and the beige precipitate formed was filtered, washed with propan-2-ol (3 x 5 mL) affording the expected product without further purification as a beige solid (54 mg, **74%**). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.24 (d, ^{3}J = 2.1 Hz, 1H), 9.12 (d, ^{3}J = 2.1 Hz, 1H), 8.81 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.37 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.37 (t, ^{3}J = 7.7 Hz, 1H), 6.43 (s, 2H), 4.57 (d, ^{3}J = 6.6 Hz, 2H), 2.97-2.90 (m, 2H), 2.82 (d, ^{3}J = 7.2 Hz, 2H), 2.38 (d, ^{3}J = 6.7 Hz, 2H), 2.10-1.98 (m, 1H), 1.98-1.89 (m, 2H), 1.89-1.81 (m, 2H), 1.77-1.66 (m, 2H), 1.49-1.35 (m, 2H) (Signals due to the OH and the NH_2 are missing). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.0 (2C), 158.5, 148.8, 146.4, 144.6, 142.9, 141.0, 135.3 (2C), 130.3, 126.8, 124.1, 122.9, 100.9, 71.4, 55.2, 52.7 (2C), 37.6, 35.0, 28.6 (2C), 24.1. IR (KBr): 3426, 2934, 1592, 1474, 1461, 1370, 1174 cm^{-1}. mp: 177-180 °C. HRMS/ESI: calculated for $C_{20}H_{25}IN_5O$ $[M+H]^+$ 478.1098, found 478.1104.

4.1.13 *N*-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)propyl) methanesulfonamide **12**

Starting from compound **5** (150 mg, 0.25 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (20 mL) and the solution was cooled down to 0 °C. Triethylamine (0.07 mL, 0.50 mmol) and methanesulfonyl chloride (0.01 mL, 0.25 mmol) were added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a beige solid (83 mg, **61%**). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.19 (d, ^{3}J = 1.9 Hz, 1H), 9.08 (d, ^{3}J = 1.9 Hz, 1H), 8.72 (dd, ^{4}J = 1.3 Hz, ^{3}J = 8.0 Hz, 1H), 8.31 (dd, ^{4}J = 1.3 Hz, ^{3}J = 7.6 Hz,

1H), 7.31 (t, ^{3}J = 7.8 Hz, 1H), 7.02 (br s, 1H), 4.52 (d, ^{3}J = 6.5 Hz, 2H), 3.00-2.91 (m, 4H), 2.89 (s, 3H), 2.42-2.35 (m, 2H), 2.09-2.95 (m, 3H), 1.89-1.81 (m, 2H), 1.69-1.59 (m, 2H), 1.49-1.36 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 158.3, 148.7, 146.3, 144.5, 142.8, 140.9, 130.2, 126.7, 124.0, 122.8, 100.9, 71.3, 55.3, 52.8 (2C), 40.9, 39.2, 34.8, 28.6 (2C), 26.5. IR (KBr): 3243, 2919, 1592, 1456, 1309, 1143, 781 cm^{-1}. mp: 176-178 °C. HRMS/ESI: calculated for $C_{21}H_{27}IN_5O_3S$ $[M+H]^+$ 556.0879, found 556.0881.

4.1.14 *N*-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)propyl)-acetamide **13**

Starting from compound **5** (250 mg, 0.41 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (20 mL) and the solution was cooled down to 0 °C. Acetic anhydride (0.04 mL, 0.41 mmol) was added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), extracted with dichloromethane (2 x 15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 90/10) as eluent affording the expected product as a beige solid (151 mg, **61%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^{3}J = 2.0 Hz, 1H), 8.98 (d, ^{3}J = 2.0 Hz, 1H), 8.88 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.32 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.39 (br s, 1H), 7.30 (t, ^{3}J = 7.8 Hz, 1H), 4.73 (d, ^{3}J = 6.6 Hz, 2H), 3.36-3.30 (m, 2H), 3.07-3.00 (m, 2H), 2.49 (t, ^{3}J = 6.2 Hz, 2H) 2.35-2.22 (m, 1H), 2.07-1.98 (m, 4H), 1.95 (s, 3H), 1.72-1.64 (m, 2H), 1.58-1.46 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 158.5, 147.8, 145.8, 145.4, 143.6, 141.4, 130.9, 126.6, 124.7, 123.3, 100.6, 72.3, 57.9, 53.5 (2C), 39.8, 35.1, 29.5 (2C), 25.1, 23.5. IR (KBr): 3273, 2913, 1642, 1592, 1466, 1346, 1180, 853 cm^{-1}. mp: 135-138 °C. HRMS/ESI: calculated for $C_{22}H_{27}IN_5O_2$ $[M+H]^+$ 520.1212, found 520.1209.

4.1.15 *N*-(3-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)propyl) methanesulfonylmethanesulfonamide **14**

Starting from compound **5** (115 mg, 0.19 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. Methanesulfonyl chloride (0.03 mL, 0.38 mmol) was added dropwise and the mixture was stirred 15 min at 0 °C and 30 min at room temperature. The organic layer was washed with water (15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a white solid (59 mg, **47%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (d, ^{3}J = 2.0 Hz, 1H), 8.99 (d, ^{3}J = 2.0 Hz, 1H), 8.87 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.32 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.29 (t, ^{3}J = 7.8 Hz, 1H), 4.59 (d, ^{3}J = 7.0 Hz, 2H), 3.80 (t, ^{3}J = 6.9 Hz, 2H), 3.29 (s, 6H), 3.05-2.94 (m, 2H), 2.47-2.36 (m, 2H),

2.33-2.20 (m, 1H), 2.07-1.90 (m, 6H), 1.62-1.46 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.9, 147.8, 145.8, 146.6, 143.8, 141.5, 131.1, 126.6, 124.7, 123.3, 100.7, 72.6, 55.4, 53.4 (2C), 47.5, 43.8 (2C), 35.1, 29.2 (2C), 27.8. IR (KBr): 2923, 1594, 1456, 1360, 1323, 1151, 511 cm^{-1}. mp: 176-178 °C. HRMS/ESI: calculated for $C_{22}H_{29}IN_5O_3S_2$ $[M+H]^+$ 634.0655, found 634.0666.

4.1.16 *N*-(4-(4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidine-1-yl)butyl) methanesulfonamide **15**

Starting from compound **6** (170 mg, 0.30 mmol) and using **General method 1**, the resulting amine was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. Triethylamine (0.08 mL, 0.60 mmol) and methanesulfonyl chloride (0.02 mL, 0.30 mmol) were added to the solution and the mixture was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 90/10) as eluent affording the expected product as a beige solid (112 mg, **72%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (d, ^3J = 2.0 Hz, 1H), 9.00 (d, ^3J = 2.0 Hz, 1H), 8.90 (dd, ^4J = 8.0 Hz, ^3J = 1.4 Hz, 1H), 8.34 (dd, ^4J = 7.6 Hz, ^3J = 1.4 Hz, 1H), 7.31 (t, ^3J = 7.8 Hz, 1H), 4.74 (d, ^3J = 6.8 Hz, 2H), 3.13-3.02 (m, 4H), 2.91 (s, 3H), 2.45-2.39 (m, 2H), 2.37-2.25 (m, 1H), 2.13-1.98 (m, 4H), 1.75-1.60 (m, 6H) (Signal to the NH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.9, 145.5, 143.8, 141.6, 131.1, 126.7, 124.7, 123.4, 100.7, 72.5, 58.4, 53.3 (2C), 43.4, 40.2, 35.1, 29.1, 28.7 (2C), 25.0. IR (KBr): 3273, 2921, 2772, 1594, 1313, 1137, 784 cm^{-1}. mp: 139-140 °C. HRMS/ESI: calculated for $C_{22}H_{29}IN_5O_3S$ $[M+H]^+$ 570.1047, found 570.1036.

4.1.17 1-(3-((*Tert*-butyldimethylsilyl)oxy)propyl)-4-(((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidine **16**

Compound **3** (190 mg, 0.45 mmol) was dissolved in acetonitrile (25 mL), (3-bromopropoxy)-*tert*-butyldimethylsilane (88 mg, 0.35 mmol) and triethylamine (0.08 mL, 0.58 mmol) were added. The solution was stirred at reflux for 16 hours. Water was added (20 mL) and the organic layer was extracted with ethyl acetate (3 x 20 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 90/10) as eluent affording the expected product as a white solid (170 mg, **63%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (d, ^3J = 2.0 Hz, 1H), 9.00 (d, ^3J = 2.0 Hz, 1H), 8.90 (dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, 1H), 8.34 (dd, ^4J = 1.4 Hz, ^3J = 7.6 Hz, 1H), 7.31 (t, ^3J = 7.8 Hz, 1H), 4.74 (d, ^3J = 6.8 Hz, 2H), 3.67 (t, ^3J = 6.1 Hz, 2H), 3.29-3.11 (m, 2H), 2.72-2.55 (m, 2H), 2.43-2.19 (m, 3H), 2.14-2.01 (m, 2H), 1.95-1.68 (m, 4H), 0.87 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 147.9, 145.9, 145.5, 143.7, 141.6, 131.0, 126.7, 124.7,

123.4, 100.7, 72.2, 61.3, 55.8, 53.3 (2C), 34.6, 29.3, 28.4 (2C), 26.1 (2C), 18.4, -5.2 (3C). IR (KBr): 2927, 2858, 1591, 1472, 1351, 1110, 835, 778 cm^{-1}. mp: 102-105 °C. HRMS/ESI: calculated for $C_{26}H_{38}IN_4O_2Si$ $[M+H]^+$ 593.1809, found 593.1813.

4.1.18 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)-1-(3-hydroxy)propyl)piperidine, formate salt **17**

In a round bottom flask, compound **16** (190 mg, 0.45 mmol) and TBAF (1 M in THF, 0.75 mL, 0.75 mmol) were added in THF (10 mL). The solution was stirred 16 hours at room temperature and THF was evaporated. The residue was purified by reverse gel chromatography using water/acetonitrile (80/20 to 50/50) and formic acid (2%) as eluent affording the expected product as a white solid (64 mg, **49%**). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.25 (d, ^3J = 2.0 Hz, 1H), 9.13 (d, ^3J = 2.0 Hz, 1H), 8.83 (dd, ^4J = 1.2 Hz, ^3J = 8.0 Hz, 1H), 8.38 (dd, ^4J = 1.2 Hz, ^3J = 7.6 Hz, 1H), 8.33 (br s, 1H), 7.39 (t, ^3J = 7.8 Hz, 1H), 4.58 (d, ^3J = 6.4 Hz, 2H), 3.40 (t, ^3J = 6.3 Hz, 2H), 3.01-2.90 (m, 2H), 2.39 (t, ^3J = 6.3 Hz, 2H), 2.01-1.92 (m, 3H), 1.90-1.81 (m, 2H), 1.64-1.54 (m, 2H), 1.49-1.37 (m, 2H) (Signals due to the OH are missing). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.0, 158,4, 148,8, 146.4, 144,6, 142,8, 141.0, 130.3, 126.8, 124.0, 122.9, 100.9, 71.2, 59.2, 55.0, 52.4 (2C), 34.4, 28.9 (2C), 27.9. IR (KBr): 3430, 2929, 1597, 1353, 1117 cm^{-1}. mp: > 250 °C. HRMS/ESI: calculated for $C_{20}H_{24}IN_4O_2$ $[M+H]^+$ 479.0944, found 479.0944.

4.1.19 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)-1-(3-methylsulfanyl)propyl) piperidine **18**

In a round bottom flask, compound **3** (313 mg, 0.75 mmol) was dissolved in dichloromethane (8 mL), 3-(methylthio)propionaldehyde (0.07 mL, 0.91 mmol) and three drops of acetic acid were added. The solution was stirred for 2 hours at room temperature and sodium triacetoxyborohydride (240 mg, 1.52 mmol) was added. The reaction was stirred at room temperature and monitored by TLC. The solution was carefully hydrolysed with an aqueous solution of NaOH (1 M, 10 mL), extracted with dichloromethane (3 x 15 mL) dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a beige solid (285 mg, **57%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (d, ^3J = 2.0 Hz, 1H), 9.00 (d, ^3J = 2.0 Hz, 1H), 8.90 (dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, 1H), 8.33 (dd, ^4J = 1.4 Hz, ^3J = 7.6 Hz, 1H), 7.30 (t, ^3J = 7.8 Hz, 1H), 4.73 (d, ^3J = 6.8 Hz, 2H), 3.12-3.02 (m, 2H), 2.56-2.46 (m, 4H), 2.36-2.24 (m, 1H), 2.17-2.06 (m, 5H), 2.05-1.98 (m, 2H), 1.91-1.81 (m, 2H), 1.69-1.55 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 147.8, 145.8, 145.4, 143.7, 141.4, 131.0, 126.6, 124.7, 123.3, 100.7, 72.5, 57.8, 53.5 (2C), 35.0, 32.3 (2C), 29.0, 26.3, 15.6. IR (KBr): 2912, 1591, 1454, 1349, 1176, 1142 cm^{-1}. mp: 108-110 °C. HRMS/ESI: calculated for $C_{21}H_{26}IN_4OS$ $[M+H]^+$ 509.0874, found 509.0872.

4.1.20 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)-1-(3-methylsulfonyl)propyl) piperidine **19**

In a round bottom flask, compound **18** (100 mg, 0.20 mmol) was dissolved in dichloromethane (2 mL) at 0 °C. 3-Chloroperoxybenzoic acid (85 mg, 0.49 mmol) was slowly added and the reaction mixture was stirred at room temperature for two hours. The reaction mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (5 mL) and extracted with dichloromethane (2 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a beige solid (71 mg, **66%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, ^3J = 2.0 Hz, 1H), 9.01 (d, ^3J = 2.0 Hz, 1H), 8.91 (dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, 1H), 8.35 (dd, ^4J = 1.7 Hz, ^3J = 7.6 Hz, 1H), 7.31 (t, ^3J = 7.8 Hz, 1H), 4.72 (d, ^3J = 6.8 Hz, 2H), 3.15-3.08 (m, 2H), 3.00-2.93 (m, 2H), 2.92 (s, 3H), 2.50 (t, ^3J = 6.8 Hz, 2H), 2.34-2.20 (m, 1H), 2.10-1.94 (m, 6H), 1.58-1.46 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 147.8, 145.8, 145.4, 143.7, 141.5, 131.0, 126.5, 124.6, 123.2, 100.6, 72.5, 56.4, 53.2 (2C), 52.8, 40.7, 35.1, 29.2 (2C), 20.0. IR (KBr): 2919, 1637, 1595, 1475, 1281, 1136 cm^{-1}. mp: 115-116 °C. HRMS/ESI: calculated for $C_{21}H_{26}IN_4O_3S$ $[M+H]^+$ 541.0768, found 541.0770.

4.1.21 Imino(3-(4-(((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidine-1-yl)propyl) methyl-λ^6-sulfanone **20**

In a round bottom flask, compound **18** (125 mg, 0.25 mmol) was dissolved in methanol (1.5 mL), ammonium carbamate (29 mg, 0.38 mmol) and $PhI(OAc)_2$ (165 mg, 0.51 mmol) were added. The solution was stirred at room temperature for 16 hours. The reaction mixture was evaporated under vacuum then the crude mixture was purified by filtration on silica using dichloromethane/methanol (70/30) as eluent affording the expected product as a brown foam (77 mg, **57%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, ^3J = 2.0 Hz, 1H), 9.01 (d, ^3J = 2.0 Hz, 1H), 8.91 (dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, 1H), 8.35 (dd, ^4J = 1.4 Hz, ^3J = 7.6 Hz, 1H), 7.31 (t, ^3J = 7.8 Hz, 1H), 4.73 (d, ^3J = 6.8 Hz, 2H), 3.21-3.14 (m, 2H), 3.00 (s, 3H), 3.00-2.90 (m, 2H), 2.55-2.47 (m, 2H), 2.35-2.22 (m, 1H), 2.18-1.90 (m, 7H), 1.60-1.45 (m, 2H) (Signal to the NH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.9, 145.5, 143.8, 141.6, 131.1, 126.7, 124.7, 123.4, 100.7, 72.6, 56.7, 55.5, 53.5, 53.3, 43.3, 35.2, 29.4, 29.3, 20.7. IR (KBr): 2912, 1591, 1454, 1349, 1176, 1142 cm^{-1}. HRMS/ESI: calculated for $C_{21}H_{27}IN_5O_2S$ $[M+H]^+$ 540.0931, found 540.0930.

4.1.22 1,6-dioxaspiro[2,5]octane

NaH (0.88 g, 23.26 mmol, 60% dispersion in mineral oil) was added to a suspension of trimethylsulfonoxonium iodide (5.15 g, 23.26 mmol) in THF (40 mL). The reaction mixture was heated

at reflux for 3 h, then tetrahydro-*4H*-pyran-4-one (1.82 mL, 20.0 mmol) was added, and the mixture was held at reflux for a further 2 h. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was redissolved in DCM (40 mL) and any solid material removed by filtration. The filtrate was concentrated in vacuo to give 1,6-dioxaspiro[2,5]octane as a colorless oil (1.60 g, **70%**). Data are consistent with literature [28]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.89-3.76 (m, 4H), 2.68 (s, 2H), 1.90-1.81 (m, 2H), 1.56-1.48 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 66.7 (2C), 56.6, 54.0, 34.0 (2C).

4.1.23 4-((4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)methyl)oxan-4-ol **21**

In a round bottom flask, compound **3** (103 mg, 0.25 mmol) was dissolved in methanol (25 mL), 1,6-dioxaspiro[2,5]octane (60.0 mg, 0.50 mmol) and triethylamine (0.07 mL, 0.50 mmol) were added. The solution was stirred under reflux for 16 hours. Methanol was evaporated and the resulting mixture was dissolved in ethyl acetate (25 mL). The organic layer was washed with brine (25 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a white solid (108 mg, **81%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^{3}J = 2.0 Hz, 1H), 9.00 (d, ^{3}J = 2.0 Hz, 1H), 8.88 (dd, ^{4}J = 1.4 Hz, ^{3}J = 8.0 Hz, 1H), 8.32 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.29 (t, ^{3}J = 7.8 Hz, 1H), 4.70 (d, ^{3}J = 6.8 Hz, 2H), 3.83-3.71 (m, 4H), 2.96-2.88 (m, 2H), 2.46-2.37 (m, 2H), 2.33 (s, 2H), 2.30-2.18 (m, 1H), 1.98-1.89 (m, 2H), 1.62-1.42 (m, 6H) (Signal due to the OH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.9, 145.5, 143.8, 141.5, 131.1, 126.6, 124.7, 123.4, 100.7, 72.6, 69.6, 68.4, 67.4, 64.1, 56.3, 53.9, 37.2, 34.7, 31.9, 29.9, 29.4. IR (KBr): 3442, 2936, 1677, 1591, 1425, 1349, 1177, 1111 cm^{-1}. mp: 169-170 °C. HRMS/ESI: calculated for $C_{23}H_{28}IN_4O_3$ $[M+H]^+$ 535.1206, found 535.1201.

4.1.24 tert-Butyl 4-(iodomethyl)piperidine-1-carboxylate

Under nitrogen at 0 °C, I_2 (2.83 g, 11.16 mmoL) was added to a mixture of triphenylphosphine (2.93 g, 11.16 mmol), imidazole (0.76 g, 11.16 mmol) and tert-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (2.00 g, 9.30 mmol) in anhydrous THF (200 mL). The resulting mixture was allowed to stir for 18 h at room temperature and an aqueous saturated solution of $Na_2S_2O_3$ was added (200 mL). Extraction was performed using EtOAc (3 x 200 mL) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The crude was purified by silica gel chromatography using cyclohexane/EtOAc (95/5) as eluent affording the expected tert-butyl 4-(iodomethyl)piperidine-1-carboxylate as a colorless oil (2.78 g, 92%). Data are consistent with literature [36]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.25-4.00 (m, 2H), 3.10 (d, ^{3}J = 6.9 Hz, 2H), 2.76-2.60 (m, 2H), 1.87-1.78 (m, 2H),

1.67-1.54 (m, 1H), 1.45 (s, 9H), 1.20-1.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.8, 79.7, 43.3 (2C), 38.8, 32.7 (2C), 28.6 (3C), 13.7.

4.1.25 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)-1-((piperidin-4-yl)methyl)piperidine **22**

In a round bottom flask, compound **3** (198 mg, 0.47 mmol) was dissolved in acetonitrile (20 mL), triethylamine (0.14 mL, 0.96 mmol) and *tert*-butyl 4-(iodomethyl)piperidine-1-carboxylate (190 mg, 0.58 mmol) were added. The solution was stirred under reflux for 16 hours and was cooled down to room temperature. The beige precipitate was filtered, washed with acetonitrile (2 x 5 mL) affording the expected intermediate without further purification. In a round bottom flask, *tert*-butyl-4-((4-(((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)methyl)-piperidine-1-carboxylate intermediate was dissolved in dichloromethane (15 mL). Trifluoroacetic acid (0.35 mL, 2.5 mmol) was added and the solution was stirred at room temperature. After 2 hours, the solvent was evaporated *in vacuo* and the crude product was dissolved in a saturated solution of K_3PO_4 (15 mL). The solution was stirred 30 minutes at room temperature and the resulting mixture was extracted with dichloromethane (3 x 20 mL). The combined organics layers were dried over $MgSO_4$, filtered and evaporated affording the expected product without further purification as a beige solid (142 mg, **48%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (d, ^3J = 2.0 Hz, 1H), 9.00 (d, ^3J = 2.0 Hz, 1H), 8.88 (dd, ^4J = 1.4 Hz, ^3J = 8.0 Hz, 1H), 8.32 (dd, ^4J = 1.4 Hz, ^3J = 7.6 Hz, 1H), 7.29 (t, ^3J = 7.8 Hz, 1H), 4.71 (d, ^3J = 6.8 Hz, 2H), 3.16-3.08 (m, 2H), 2.93-2.86 (m, 2H), 2,67-2.58 (m, 2H), 2.29-2.18 (m, 1H), 2.16 (d, ^3J = 7.1 Hz, 2H), 1.99-1.88 (m, 4H), 1.81-1.74 (m, 2H), 1.70-1.58 (m, 1H), 1.57-1.43 (m, 2H), 1.22-1.10 (m, 2H) (Signal due to the NH is missing). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.7, 147.8, 145.9, 145.4, 143.8, 141.5, 131.1, 100.7, 126.6, 124.7, 123.3, 72.9, 65.6, 54.1 (2C), 46.3 (2C), 35.4, 33.8, 31.6 (2C), 29.5 (2C). IR (KBr): 3422, 2927, 2898, 1610, 1354, 1173, 778 cm^{-1}. mp: 140-143°C. HRMS/ESI: calculated for $C_{23}H_{29}IN_5O$ $[M+H]^+$ 518.1419, found 518.1417.

4.1.26 4-(((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)-1-((1-methanesulfonylpiperidin-4-yl)methyl)piperidine **23**

In a round bottom flask, compound **22** (90 mg, 0.17 mmol) was dissolved in dichloromethane (10 mL) and the solution was cooled down to 0 °C. Triethylamine (0.03 mL, 0.25 mmol) and methanesulfonyl chloride (0.01 mL, 0.17 mmol) were added and the solution was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (20 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product as a beige solid (100 mg, **89%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^3J = 2.0 Hz, 1H), 8.99 (d, ^3J = 2.0 Hz, 1H), 8.87 (dd, ^4J = 1.4 Hz, ^3J = 8.0

Hz, 1H), 8.32 (dd, ^{4}J = 1.4 Hz, ^{3}J = 7.6 Hz, 1H), 7.29 (t, ^{3}J = 7.8 Hz, 1H), 4.70 (d, ^{3}J = 6.8 Hz, 2H), 3.81-3.74 (m, 2H), 2.95-2.87 (m, 2H), 2.75 (s, 3H), 2.67-2.59 (m, 2H), 2.30-2.20 (m, 1H), 2.21 (d, ^{3}J = 7.1 Hz, 2H), 2.05-1.91 (m, 4H), 1.91-1.83 (m, 2H), 1.69-1.55 (m, 1H), 1.59-1.46 (m, 2H), 1.34-1.21 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6, 147.9, 145.8, 145.4, 143.8, 141.5, 131.1, 126.6, 124.7, 123.3, 100.7, 72.7, 64.6, 54.1 (2C), 46.3 (2C), 35.2, 34.6, 33.3, 30.5 (2C), 29.3 (2C). IR (KBr): 2918, 2855, 1592, 1318, 1147, 781 cm^{-1}. mp: 173-175 °C. HRMS/ESI: calculated for $C_{24}H_{31}IN_5O_3S$ $[M+H]^+$ 596.1192, found 596.1194.

4.1.27 1-(4-((4-((7-Iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)methyl) piperidin-1-yl)ethan-1-one **24**

In a round bottom flask, compound **22** (100 mg, 0.19 mmol) was dissolved in dichloromethane (10 mL) and the solution was cooled down to 0 °C. Acetic anhydride (0.02 mL, 0.19 mmol) was added and the solution was stirred 5 min at 0 °C and 5 min at room temperature. The organic layer was washed with water (10 mL) and extracted with dichloromethane (2 x 10 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording the expected product (84 mg, **93%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, ^{3}J = 2.0 Hz, 1H), 9.00 (d, ^{3}J = 2.0 Hz, 1H), 8.88 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.4 Hz, 1H), 8.32 (dd, ^{3}J = 7.6 Hz, ^{4}J = 1.4 Hz, 1H), 7.29 (t, ^{3}J = 7.8 Hz, 1H), 4.71 (d, ^{3}J = 6.8 Hz, 2H), 4.62-4.54 (m, 1H), 3.82-3.74 (m, 1H), 3.06-2.96 (m, 1H), 2.96-2.85 (m, 2H), 2.58-2.48 (m, 1H), 2.31-2.20 (m, 1H), 2.20-2.13 (m, 2H), 2.07 (s, 3H), 2.04-1.90 (m, 4H), 1.88-1.80 (m, 1H), 1.79-1.68 (m, 2H), 1.60-1.45 (m, 2H), 1.16-1.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.9, 158.6, 147.8, 145.9, 145.5, 143.8, 141.5, 131.1, 126.6, 124.7, 123.3, 100.7, 72.8, 64.9, 54.4, 53.9, 46.7, 41.8, 35.3, 34.0, 31.6, 30.7, 29.4 (2C), 21.7. IR (KBr): 2937, 1624, 1590, 1454, 1424, 1348, 1178, 1048, 981 cm^{-1}. mp: 165-167 °C. HRMS/ESI: calculated for $C_{25}H_{31}IN_5O_2$ $[M+H]^+$ 560.1517, found 560.1522.

4.1.28 Diethyl (4-((4-(((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)methyl) piperidine-1-yl)phosphonate, fumarate salt **25**

In a round bottom flask, compound **22** (150 mg, 0.29 mmol) was dissolved in dichloromethane (15 mL) and the solution was cooled down to 0 °C. Diethyl chlorophosphate (0.04 mL, 0.29 mmol) and triethylamine (0.08 mL, 0.58 mmol) were added and the solution was stirred 5 min at 0 °C and 12 hours at room temperature. The organic layer was washed with water (15 mL) and extracted with dichloromethane (2 x 15 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 95/5) as eluent affording diethyl (4-((4-(((7-iodopyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl)methyl)piperidine-1-

yl)phosphonate as a beige solid (50 mg, **29%**). In a round bottom flask, the solid (50 mg,) was dissolved in propan-2-ol (15 mL) and fumaric acid (9.0 mg, 0.08 mmol) was added. The solution was stirred at 40 °C for 30 minutes and the beige precipitate formed was filtered, washed with propan-2-ol (3 x 5 mL) affording the expected product without further purification as a beige solid (65 mg). ^{1}H NMR (400 MHz, CD_3OD): δ = 9.16 (d, ^{3}J = 2.0 Hz, 1H), 9.00 (d, ^{3}J = 2.0 Hz, 1H), 8.85 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.4 Hz, 1H), 8.33 (dd, ^{3}J = 7.6 Hz, ^{4}J = 1.4 Hz, 1H), 7.32 (t, ^{3}J = 7.8 Hz, 1H), 6.69 (s, 2H) 4.73 (d, ^{3}J = 6.1 Hz, 2H), 4.07-3.97 (m, 4H), 3.69-3.62 (m, 2H), 3.59-3.49 (m, 2H), 3.09-2.99 (m, 4H), 2.86-2.76 (m, 2H), 2.54-2.42 (m, 1H), 2.28-2.20 (m, 2H), 2.12-2.00 (m, 1H), 1.96-1.86 (m, 2H), 1.85-1.76 (m, 2H), 1.31 (td, $^{3}J_{H\text{-}H}$ = 7.1 Hz, $^{4}J_{H\text{-}P}$ = 0.7 Hz, 6H), 1.30-1.20 (m, 2H) (Signals due to the OH are missing). ^{13}C NMR (100 MHz, CD_3OD): δ = 171.2 (2C), 159.4, 150.0, 146.9, 144.7, 142.7, 136.2 (2C), 131.4, 127.8, 125.6, 124.5, 101.1, 71.9, 63.9 (d, $J_{C\text{-}P}$ = 6.0 Hz, 2C), 63.3, 54.0 (2C), 45.1 (d, $J_{C\text{-}P}$ = 2.6 Hz, 2C), 34.1, 32.6, 31.2 (d, $J_{C\text{-}P}$ = 4.9 Hz, 2C), 27.3 (2C), 16.5 (d, $J_{C\text{-}P}$ = 6.8 Hz, 2C). IR (KBr): 3429, 2944, 1710, 1594, 1458, 1353, 1177, 1137, 1035 cm^{-1}. mp: 133-136 °C. HRMS/ESI: calculated for $C_{27}H_{38}IN_5O_4P$ $[M+H]^+$ 654.1705, found 654.1706.

4.1.29 *N*-(3-(4-(((7-(Tributylstannyl)pyrazino[2,3-*c*]quinolin-5-yl)oxy)methyl)piperidin-1-yl) propyl)acetamide **26**

In a sealed tube under argon, compound **13** (70 mg, 0.13 mmol) and tris(benzylideneacetone)dipalladium(0) (6 mg, 0.007 mmol) were dissolved in propan-2-ol (1 mL). Hexa-*n*-butylditin (0.08 mL, 0.16 mmol) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.33 mmol) were added and the solution was stirred at 70 °C for 48 h. The solution was cooled down to room temperature, diluted with ethyl acetate (2 mL) and filtered through a pad of celite. The organic layer was washed with water (3 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using dichloromethane/methanol (100/0 to 90/10) as eluent affording the expected product as a light brown oil (42 mg, **47%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.05 (d, ^{3}J = 2.0 Hz, 1H), 8.95 (d, ^{3}J = 2.0 Hz, 1H), 8.88 (dd, ^{4}J = 1.6 Hz, ^{3}J = 8.0 Hz, 1H), 7.99-7.84 (m, 1H), 7.61-7.54 (m, 1H), 7.38 (br s, 1H), 4.57 (d, ^{3}J = 6.7 Hz, 2H), 3.39-3.31 (m, 2H), 3.15-3.07 (m, 2H), 2.60-2.52 (m, 2H), 2.22-2.04 (m, 5H), 1.96 (s, 3H), 1.78-1.70 (m, 2H), 1.61-1.48 (m, 8H), 1.37-1.27 (m, 6H), 1.26-1.16 (m, 6H), 0.85 (t, ^{3}J = 7.3 Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.1, 157.2, 149.3, 147.5, 146.6, 144.7, 143.4, 139.8, 131.0, 125.4, 124.4, 122.2, 71.6, 57.7, 53.3 (2C), 39.6, 35.1, 29.4 (3C), 29.3 (2C), 27.6 (3C), 25.0, 23.5, 13.9 (3C), 10.4 (3C). HRMS/ESI: calculated for $C_{24}H_{54}IN_5O_2Sn$ $[M+H]^+$ 680.3308, found 680.3295.

4.2 Binding experiments on 5-HT_4R with MR-26132 and compound **3-25**

For radioligand binding studies, 2.5 µg of proteins (5-HT_{4B} membrane preparations, HTS110M, Eurofins) were incubated in duplicate at 25 °C for 60 min in the absence or the presence of 10^{-6} or 10^{-8} M of each ligands (**MR-26132**, **3-25**) and 0.5 nM **[^{3}H]GR-113808** (NET 1152, Perkin Elmer) in 25 mM Tris buffer (pH 7.4). At the end of the incubation, homogenates were filtered through Whatman GF/C filters (FP-200, Alpha Biotech) presoaked with 0.5% polyethylenimine using a Brandel cell harvester. Filters were subsequently washed three times with 1 mL of ice-cold 25 mM Tris buffer (pH 7.4). Non-specific binding was evaluated in parallel in the presence of 30 µM serotonin. For ligands **MR-26132**, **3-5** and **7-24**, affinity constants were calculated from five-point inhibition curves using the Prism 6 software and expressed as K_i ± SD.

4.3 Selectivity and intrinsic activity of **13**

Compound **13** was evaluated toward other serotonin receptors as well as for intrinsic activity at CEREP. Detailed assay protocols are available at the CEREP web site (http://www.cerep.com) under the following reference numbers. Binding assays: 5-HT1aR (0131), 5-HT1bR (0132), 5-HT1dR (1974), 5-HT2aR (0135), 5-HT2bR (1609), 5-HT2cR (0137), 5-HT3R (0411), 5-HT5aR (0140), 5-HT6R (0142), 5-HT7R (0144). Functional assay: 5-HT4eR (G049).

4.4 Radiochemistry

[^{125}I]SB-207710, **[^{125}I]13** and **[^{125}I]MR-26132** were obtained by incubation (30 min) of their respective tribulytin precursor (50 µg) in acid acetic (2µl) with [^{125}I]NaI (37Mbq) and hydrogen peroxide (1µl). Reactions were injected onto a reversed-phase column (Bondclone C18) and **[^{125}I]SB-207710**, **[^{125}I]13** and **[^{125}I]MR-26132** were isolated by a linear gradient HPLC run (5% to 95% ACN in 7mM H_3PO_4, 10 min). Radiochemical yields were greater than 80%. The molar activities were greater than 100 GBq/µmol, based on the limit of detection of the ultraviolet absorbance and on the calibration curves established with cold reference compounds.

4.5 Evaluation of **13** as SPECT radiotracer

4.5.1 Ethics

All experimental procedures were conducted with the agreement of the Ethics Committee for Animal Experimentation of the Canton of Geneva and the General direction of health of the canton of Geneva, Switzerland.

4.5.2 In vitro competition experiments with **13**

Hippocampal brain sections (26 µm, n = 8) were immersed in 1x PBS (15 min), in radioactive buffer (90 min) and then rinsed twice in 4°C Tris-$MgCl_2$-EtOH buffer (3 min) and briefly washed in cold water. The radioactive buffer consists of Tris-$MgCl_2$-EtOH buffer (50 mM Tris HCl, 50 mM $MgCl_2$, 20% EtOH, pH = 7.4) contains either the radioactive compounds alone or in presence of 10-50 µM of unlabeled compound. Slides were air-dried before exposure onto gamma-sensitive phosphor imaging plates (Fuji BAS-IP MS2325) for 30 min. Brain sections were then treated for Nissl staining in order to delineate the region of interests: cornu ammonis (1-4), entorhinal and temporal cortex and the subiculum. Autoradiograms were analysed with the Fujifilm BAS-1800II phosphorimager using Aida Software V4.06 (Raytest Isotopenmessgerate GmbH) in presence of homemade [^{125}I] calibration curves. Specific binding ratio (SBR) was calculated as follows: (Average radioactivity in ROI / radioactivity in ROI in the presence of unlabeled radiotracer) – 1.

4.5.3 In vivo SPECT imaging with **13**

Animals were anesthetized with 3% isoflurane and placed in the U-SPECT-II imaging system (miLabs, Utrecht, Netherlands). Acquisition (55 frames) was initiated upon intravenous injection of **[^{125}I]13**. SPECT tomograms were reconstructed with an ordered subsets expectation maximization (OSEM) algorithm using the HiSPECT software (SciVis GMBH, Göttingen, Germany). The SPECT images were analysed with PMOD software (PMOD Technologies, Zurich, Switzerland). The tomograms were co-registered to a reference MRI template of the rat brain [37] and the cerebral time activity curve were extracted in the hippocampus and striatum.

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