CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Novel 5-HT7 antagonists, with an unprecedented selectivity profile

Authors: Ali Ates, Pierre Burssens, Olivier Lorthioir, Patrick Lo Brutto, Gwenael Dehon, Jean Keyaerts, Francis Coloretti, Lallemand Bénédicte, Verbois Valérie, Gillard Michel, and Vermeiren Céline

To be cited as: *ChemMedChem* 10.1002/cmdc.201800026

Link to VoR: http://dx.doi.org/10.1002/cmdc.201800026

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

COMMUNICATIONS

DOI: 10.1002/cmdc.200((will be filled in by the editorial staff))

Novel 5-HT$_7$ antagonists, with an unprecedented selectivity profile

Ali Ates,[a]* Burssens Pierre,[a] Lorthioir Olivier,[a] Lo Brutto Patrick,[a] Dehon Gwenael,[a] Keyaerts Jean,[a] Coloretti Francis,[a] Bénédicte Lallemand,[a] Valérie Verbois,[a] Michel Gillard,[a] and Céline Vermeiren[a]

The serotonin 5-HT$_7$ receptor is the last recently identified subtype of the serotonin receptor family. The 5-HT$_7$ receptor was discovered in 1993, and subsequently cloned from a number of species such as rat, mouse or human and many more since then. [1] The discovery that several marketed antipsychotics as well as antidepressant drugs exhibited affinities for this receptor triggered many academic and industrial research projects around the world.

Today, after more than two decades of research, progress has been made in both the understanding of biology and pharmacology of the peripheral and central 5HT$_7$ receptors. Besides depression, potential applications in cognition, migraine as well as modulation of sleep architecture and circadian rhythm have been reported. [2]

In parallel to the progress made in biology and pharmacology, a huge diversity of ligands showing high affinity for this receptor have been reported in the literature. [3] Amongst them, SB-269970 (Fig. 1) reported 17 years ago occupies a cardinal position. [4] Indeed, its high affinity toward the 5-HT$_7$ receptor (5-HT$_7$ pKi = 9.1), coupled to its 50-fold minimal selectivity over the entire serotonin receptor family as well as a range of GPCRs, ion channels and enzymes propelled it to become the reference tool compound in the field (see Table 1 and supporting information). It is noteworthy that despite its poor pharmacokinetic profile (high rodent blood clearance and no oral bioavailability), this compound is still today the only compound used to assess the 5-HT$_7$ pharmacology worldwide. More recently, the structure of JNJ18038683 (Fig. 1), a 5-HT$_7$ antagonist clinical candidate from Johnson & Johnson was disclosed in the literature, together with its selectivity profile and clinical data in major depressive disorder. Its selectivity for 5-HT$_7$ is limited by high affinity toward other receptors such as 5-HT$_6$ (10-fold selectivity) and adrenergic α_1 (15-fold selectivity). [5]

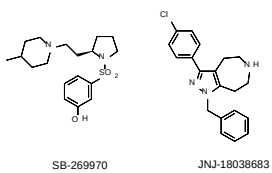


Figure 1: Chemical structure of SB-269970 and JNJ18038683

Table 1: SB-269970 binding affinities (pKi) for the 5-HT$_{7D}$ and off target receptors. Data are the means of separate experiments.

5-HT$_{7D}$	α_{2C}	α_{2A}	α_{1A}	5-HT$_{1A}$	5-HT$_{5A}$	Sigma-1	Selectivity (log)
9.1	5.7	5.7	6.7	6.0	7.5	6.8	1.6

Finally, regarding the large diversity of published 5-HT$_7$ antagonist ligands, only limited data is available regarding the selectivity profile. From the above, it transpires that additional 5-HT$_7$ antagonist ligands exhibiting a high selectivity profile toward the 5-HT$_7$ receptors are needed to explore 5-HT$_7$ receptor pharmacology as well as to progress new compounds to the clinic.

Our medicinal chemistry program was therefore aimed at the identification of novel chemical series, targeting the 5-HT$_7$ receptor and resulted in the identification of tool compounds displaying high selectivity which were suitable for in vivo profiling.

The decahydroisoquinoline (DHIQ) starting hit **1** (Fig. 2), was identified by a High throughput screening campaign of the UCB library (~150000 cpds) on the recombinant human 5-HT$_7$ receptor using a competition binding assay.

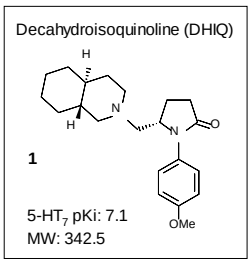
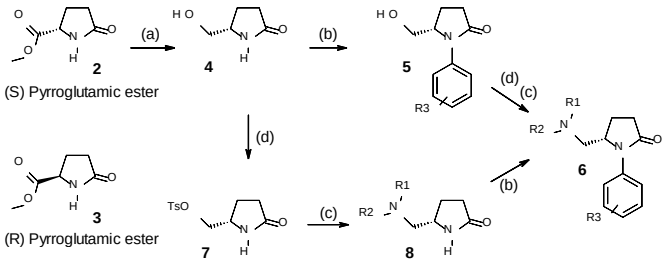


Figure 2: Initial hit

The synthesis of target derivatives **1** started with the reduction of commercially available (S) or (R) pyrroglutamic esters into the corresponding (S) or (R) alcohols **4** (Scheme 1). *N*-arylation using copper catalyst in dioxane with potassium phosphate, followed by the activation of the alcohol moiety into a tosylate intermediate, yielded targeted compounds **6** after substitution with the selected amine (R_1R_2NH) in acetonitrile/potassium carbonate. An alternative synthetic route from alcohol **4** started first with the conversion to the tosylate intermediate **7** in dichloromethane/triethylamine followed by the substitution with the selected amine (R_1R_2NH) in acetonitrile/potassium carbonate. Targeted molecules **6** were then obtained by arylation of the *N*-lactam nitrogen atom of intermediate **8** (Scheme 1).



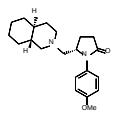
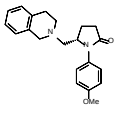
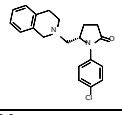
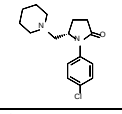
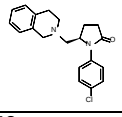
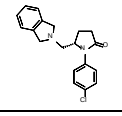
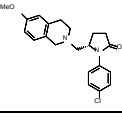
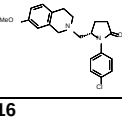
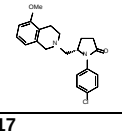
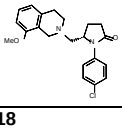
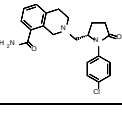
(a) $NaBH_4$, EtOH (99%); (b) Ar-Br, CuI/diamine, K_3PO_4, Dioxane (75-90%); (c) R_1R_2NH, K_2CO_3, MeCN (45-92%); (d) TsCl, TEA, DMAP, CH_2Cl_2 (65-98%); Similar results were obtained with the (R) Pyrroglutamic ester.

Scheme 1

Binding affinities to the 5-HT$_7$ receptor are listed in Table 2. The tetrahydroisoquinoline (THIQ) analogue **9** is a good surrogate of the quite complex *trans*-decahydroisoquinoline (DHIQ) motif present in the starting hit **1**. The replacement of the methoxy group of compound **9** by a chlorine atom was well tolerated (compound **10**).

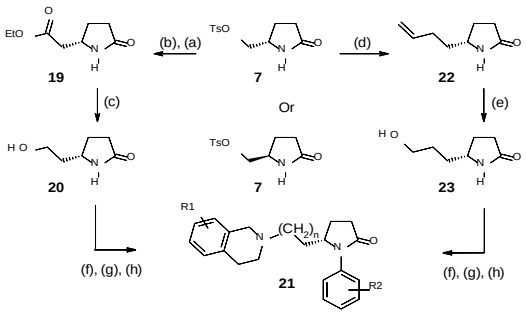
However the removal of the aromatic ring of the THIQ moiety, was not allowed, compound **11** (Table 2). The (S) configuration showed much better activity than the corresponding (R) configuration (compare compounds **10** and **12**). The ring contraction leading to the isoindoline structure **13** was also detrimental to the 5-HT_7 affinity. A methoxy scan around the aromatic ring of the THIQ moiety revealed that a methoxy in position-8 was most favorable for the 5-HT_7 binding affinities (see compounds **14** to **17**). Finally, replacing the methoxy group of compound **17** by an amide group led to compound **18** that displayed also acceptable affinity and selectivity for the 5-HT_7 receptor.

Table 2: Compound binding affinities (pKi) to the 5-HT_{7D} receptor and off target receptor (nt stands for not tested). Data are the means of separate experiments.

Cpd/Structure	5-HT_{7D}	α_{2C}	α_{2A}	α_1	5-HT_{1A}	Selectivity (log)
1 	7.1	6.7	5.8	5.3	5.0	0.4
9 	6.6	nt	<5.0	<5.0	<5.0	1.6
10 	6.8	nt	nt	nt	nt	/
11 	<5.0	nt	nt	nt	nt	/
12 	<5.0	nt	nt	nt	nt	/
13 	<5.0	nt	nt	nt	nt	/
14 	<5.0	nt	nt	nt	nt	/
15 	6.5	nt	nt	nt	nt	/
16 	7.4	*6.8*	*6.3*	*5.5*	*5.3*	0.6
17 	8.0	*7.5*	*7.1*	*6.6*	*7.4*	0.5
18 	7.8	*6.3*	*5.6*	*6.2*	*6.2*	1.5

Unfortunately, the compounds with higher affinity listed in Table 2 displayed a max selectivity of 32-fold (1.5-1.6 log). Their selectivity profiles were limited by residual affinity toward other receptors such as the adrenergic α_{2C} or 5-HT_{1A} receptors.

In order to try to further increase the potency toward the 5-HT_7 receptor, we decided to increase the flexibility of the scaffold by varying the number of methylene units between the nitrogen atom of the THIQ and the pyrrolidone ring system (n = 1 and 2, Scheme 2). Starting from both the optically pure (R) or (S) tosylate intermediates **7** (Scheme 2), the homologated alkyl chain analogues (n=1, Scheme 2) were synthesized by substitution of the tosylate group by potassium cyanide in acetonitrile followed by acid-chloride hydrolysis in ethanol to give the corresponding ethyl esters **19**. Optically pure alcohol **20** was obtained by reduction of ester **19** with sodium borohydride in ethanol. The corresponding three methylene unit alcohols **23** was obtained by reacting the tosylate intermediate **7** with an excess of allyl-magnesium bromide in tetrahydrofurane to yield **22,** followed by ozonolysis and reduction in ethanol. Targeted compounds **21** (n = 1 or 2) were obtained from alcohols **20** or **23** respectively (Scheme 2).



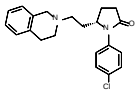
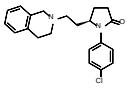
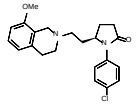
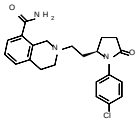
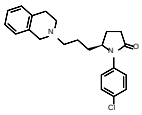
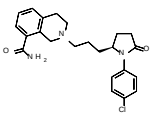
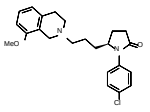
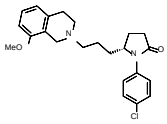
(a) KCN, KI, MeCN, reflux (93%); (b) i. HCl(g), EtOH, 65°C; ii. $NaHCO_3$; iii. Toluene, reflux (77%); (c) $NaBH_4$, EtOH (99%); (d) Allyl-MgBr, THF, -30°C (69%); (e) i O_3, EtOH, -70°C; ii $NaBH_4$, EtOH -70°C (75%); (f) Ar-Br, CuI/diamine, K_3PO_4, Dioxane; (g) TsCl, TEA, DMAP, CH_2Cl_2; (h) THIQ, K_2CO_3, MeCN

Scheme 2

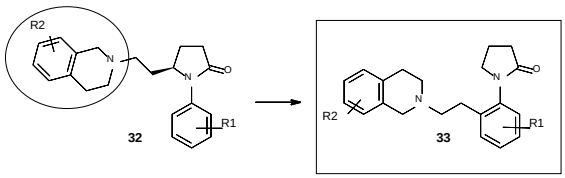
As illustrated in Table 3, the opposite absolute configuration of the pyrrolidone stereochemistry from the compounds in Table 2 gave the best affinities for 5-HT_7 receptor when moving to two or three methylene unit linker (match pair compounds **24**/**25** and **30**/**31** to be compared in Table 3). Importantly, compounds bearing a propyl-linker produced a dramatic increase in 5-HT_7 binding affinity as exemplified by compounds **28-30** (5-HT_7 pKi > 9.0, Table 3).

With the exception of compound **27**, the selectivity profiles of the most potent compounds displayed in Table 3 were rather low and limited by high affinity toward receptors such as the 5-HT_{1A} or the adrenergic α_{2C} receptor. The poor selectivity profile of compounds **28** to **31** (Table 3) could be explained by the high degree of conformational freedom which would allow compounds to sample different low energy conformations thus permitting the binding with various receptors.

Table 3: Compound binding affinities (pKi) to the 5-HT_{7D} receptor and off target receptor (nt stands for not tested). Data are the means of separate experiments.

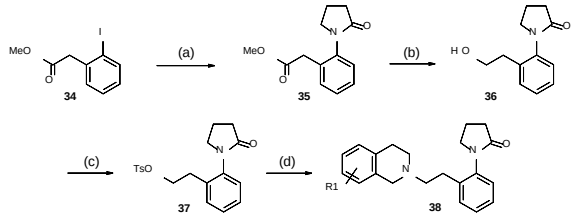
Cpd/Structure	5-HT_{7D}	α_{2C}	α_{2A}	α_1	5-HT_{1A}	Selectivity (Log)
24 	6.7	nt	*6.7*	*5.9*	*5.7*	0
25 	7.4	*7.7*	*7.1*	*6.2*	*6.0*	-0.3
26 	7.6	*7.5*	*7.3*	*6.1*	*7.2*	0.1
27 	8.0	*6.6*	*6.2*	*5.4*	*6.3*	1.4
28 	9.1	*9.1*	*8.2*	*7.1*	*8.4*	0
29 	9.2	*8.6*	*8.0*	*7.3*	*9.7*	-0.5
30 	9.5	*9.4*	*8.7*	*8.3*	*9.7*	-0.2
31 	8.2	*8.5*	*8.4*	*7.7*	*8.1*	-0.3

In an attempt to marry, high affinity (5-HT_7 pKi > 8.0) with good selectivity (> 30-fold), we decided to limit the flexibility of compounds. Thus we limited the linker length to two methylene unit (ethyl) (structure **32**, Scheme 3) whilst simultaneously shifting the alkyl chain bearing the THIQ moiety to the *ortho* position of the aromatic ring linked to the pyrrolidone system (structure **33**, Scheme 3).



Scheme 3

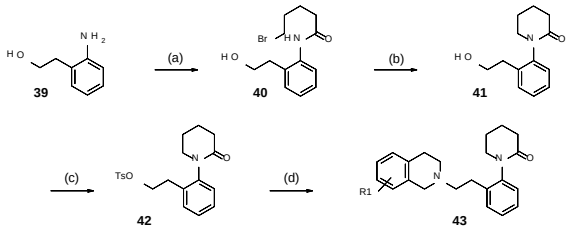
Synthetic access to analogues of **33** started with an Ullman type coupling of the Iodo-aryl-acetic ester **34** with pyrrolidone to yield intermediate **35** (Scheme 4). Reduction of the ester **35** to the corresponding alcohol **36** and subsequent conversion to the tosylate derivative yielded compound **37** as a versatile building block. Finally displacement of the tosyslate group intermediate **37** by THIQ derivatives gave the title compounds **38** (Scheme 4).



(a) CuI, K_3PO_4, Pyrroliodone, 1,2-Cyclohexyl-diamine, Dioxane (55%); (b) $LiBH_4$, THF (94%); (c) NMI, TsCl, DCM (86%); (d) THIQ, MeCN, K_2CO_3, 85°C (55-95%)

Scheme 4

The corresponding piperidone derivatives **43**, were prepared according to Scheme 5. Aniline **39** was amidated using Ʊ-bromo-chloro-pentenoate, followed by a ring closure reaction with potassium tert-butoxyde to furnish intermediate **41** in a good overall yield of 71%. Conversion to the tosylate derivative yielded the building block **42**, precursor of the titled compounds **43** (Scheme 5).



(a) 5-Bromopentanoyl chloride, Et_3N, DCM (100%); (b) tBuOK, THF (71%); TsCl, DMAP, Et_3N, DCM (88%); R_1R_2NH, K_2CO_3, MeCN (55-75%).

Scheme 5

Among the diversity of potential THIQ derivatives, that could be introduced to building blocks **37** and **42** (Scheme 4 and 5), we decided to focus our initial structure activity relationship (SAR) investigation around the derivatives already used in the previous section (Table 3). As can be seen from Table 4, with the pyrrolidone ring system (n = 1), similar SAR was obtained. Highest affinities were obtained with the 8-Methoxy-1,2,3,4-tetrahydroisoquinoline **48** or the 8-amide-1,2,3,4-tetrahydroisoquinoline **49**. However, with the piperidone ring system (n = 2), affinities were higher or equal to the corresponding pyrrolidine ring analogues (n = 1), eg. compare compounds **44** and **50** in Table 4. Again the highest potency was observed for the 8-Methoxy-1,2,3,4-tetrahydroisoquinoline derivative, ie. **51** (n = 2, Table 4).

10.1002/cmdc.201800026

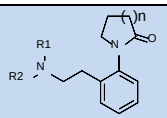
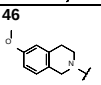
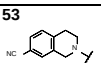


Table 4: Compound binding affinities (pKi) to the 5-HT_{7D} receptor and off target receptor (nt stands for not tested). Data are the means of separate experiments.

R1NR2	n	5-HT_{7D}	α_{2C}	α_{2A}	α_{1A}	5-HT_{1A}	5-HT_{5A}	Selectivity (Log)
44 	1	7.4	6.6	6.8	5.8	<5.0	nt	0.6
45 	1	7.4	6.8	7.2	6.0	6.3	nt	0.2
46 	1	6.1	nt	*nt*	nt	*nt*	nt	/
47 	1	6.9	6.3	5.7	6.2	6.0	nt	0.6
48 	1	8.3	7.2	6.6	6.1	6.3	nt	1.1
49 	1	7.9	5.6	<5	<5	5.8	7.2	0.7
50 	2	8.5	6.0	<5	<5	<5	6.4	2.1
51 	2	9.0	6.8	6.1	5.8	6.5	7.4	1.6
52 	2	8.1	<5	<5	<5	<5	6.5	1.6
53 	2	8.3	<5	<5	<5	<5	5.5	2.8

Much to our delight, the selectivity profile of compounds with best affinities (**48** to **53**) was also very attractive and to the best of our knowledge, unprecedented in the literature. Indeed, the selectivity profile was as high as 630-fold in the case of compound **53** and systematically higher or equal to 40-fold in the case of compounds **50**, **51** and **52** (Table 4). Amongst the GPCRs, enzymes and ion channels screened, the 5-HT_{5A} receptor was systematically the off-target which limited the selectivity profile (compounds **49** to **53**, Table 4). Thus, we describe compound **49** as a dual 5-HT_{7D}/5-HT_{5A} ligand and compounds **50** to **53** as selective 5-HT_7 ligands (for broader profiling see supporting information).

Compounds **50** to **53** demonstrated antagonist properties in a 5-HT_{7D} assay stimulated with 5-CT (Table 5 and experimental part).

Table 5: 5-HT7 antagonism: cell based cAMP functional assay. Data are the means of separate experiments.

Cpd	pIC50 cAMP
50	6.8
51	8.7
52	7.5
53	8.1
SB-269970	9.0
Compounds 50 to 53 were tested up to 10 µM for agonism and no activation of 5-HT7 receptor was observed above 5%.	

In summary, through a rational SAR study based on compound **1** (Figure 2), we have identified a series of novel 5-HT_7 antagonists displaying unprecedented selectivity for 5-HT7 receptor. We believe these selective 5-HT7 antagonists will serve as valuable tools to aid the understanding of the 5-HT7 receptor pharmacology and constitute outstanding starting points for lead optimization.

Acknowledgements

The authors would like to thank Ms. Isabelle Jacques, Mr. Philippe Motte for their technical expertise in in-vitro screening, Ms. Géraldine Longfils, Dr. Benoit Mathieu and Ms. Ariane Descamps for physchem support and Ms. Aurélie Bremaud, Mr. Rudi Jacqmin; Mr. Didier Sénéchal, Mr. Marc Van Thuyne and Dr. Véronique Pinilla for column chromatography support. Dr R. Dieden, Mr A. Fauconnier, Mr J. Claessens, and Mrs C. Derwa for analytical assistance; Dr. B. Barnaby for in vitro PK. This work was financed in part by the Walloon Region (Belgium) under conventions n°5708 & 6272.

Keywords: selective, serotonin, 5-HT_7 receptor, antagonist

Experimental part

5-(R^1R^2aminometyl)-1-aryl-pyrrolidin-2-one **6** and *6-(R^1R^2aminometyl)-1-aryl-piperidin-2-one* **21**.

To a solution of the alcohol (0.26 mol) and halo-aryl (0.26 mol) dissolved in dioxane (750mL) was added K_3PO_4 (0.65 mol) at room temperature. The reaction mixture was heated at 100°C followed by the addition of 1,2-diaminocyclohexane (0.052 mol) and copper(I)-iodide (0.026 mol). The reaction mixture was heated overnight at 100°C. Salts were filtered off, volatiles were removed under vacuo and the crude mixture was purified by column chromatography to afford intermediate compounds *5-(hydroxyalkyl)-1-aryl-pyrrolidin-2-one* or *6-(hydroxyalkyl)-1-aryl-piperidin-2-one* in good yield (75-90%). To a solution of *5-(hydroxyalkyl)-1-aryl-pyrrolidin-2-one* or *6-(hydroxyalkyl)-1-aryl-piperidine-2-one* from above (44 mmol) dissolved in dichloromethane (150 mL) was added N-methylimidazole (255 mmol) at 0°C. The reaction mixture was stirred at 0°C during 1h. 4-methylbenzenesulfonyl chloride (TsCl, 52 mmol) dissolved in dichloromethane (200 mL) was added dropwise at 0°C and the reaction mixture was stirred overnight at room temperature. The organic layer was successively washed with water (200 mL), aqueous HCl (2.5 M, 200 mL) and water (200 mL). The organic layer was dried over $MgSO_4$, the solid was filtered off and volatiles were removed under vacuo. The residue was purified by column chromatography to afford the tosylates intermediates (65-98% yield). The tosylate (1 mmol) and the selected amine derivative (1 mmol) were dissolved in CH_3CN (5 mL) and K_2CO_3 (3 mmol) was added at room temperature. The reaction mixture was stirred overnight at 85°C. Salts were filtered off, volatiles were removed under vacuum and the residue was purified by column chromatography to afford compounds **6** or **21** (45-92%).

(5S)-1-(4-chlorophenyl)-5-[(5-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)methyl]pyrrolidin-2-one **16**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 8.8 Hz, 2 H), 7.44 (d, J = 8.8 Hz, 2 H), 7.12 (t, J = 8.0 Hz, 1 H), 6.79 (d, J = 8.0 Hz, 1 H), 6.65 (d, J = 7.5 Hz, 1 H), 4.68 (t, J = 8.0 Hz, 2 H), 3.75 (m, 6 H), 2.91 (m, 1 H), 2.77 (m, 2 H), 2.65 (m, 4 H), 2.33 (m, 2 H), 2.05 (m, 1 H). LC-MS (97%) MH^+ 371

(5S)-1-(4-chlorophenyl)-5-[(8-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)methyl]pyrrolidin-2-one **17**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (m, 2 H), 7.45 (d, J = 8.8 Hz, 2 H), 7.12 (t, J = 7.8 Hz, 1 H), 6.77 (d, J = 8.0 Hz, 1 H), 6.70 (d, J = 7.5 Hz, 1 H), 3.76 (s, 3 H), 3.62 (s, 2 H), 2.75 (m, 6 H), 2.31 (m, 3 H), 2.07 (m, 1 H). LC-MS (99%) MH^+ 371

2-[[(2S)-1-(4-chlorophenyl)-5-oxo-pyrrolidin-2-yl]methyl]-3,4-dihydro-1H-isoquinoline-8-carboxamide **18**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.60 – 7.53 (m, 2H), 7.46 – 7.38 (m, 2H), 7.30 (s, 1H), 7.25 – 7.09 (m, 3H), 4.54 (tt, J = 7.5, 3.4 Hz, 1H), 3.68 (s, 2H), 2.84 – 2.49 (m, 7H), 2.41 – 2.16 (m, 2H), 2.05 – 1.93 (m, 1H). LC-MS (99%) MH^+ 384

2-[2-[(2R)-1-(4-chlorophenyl)-5-oxo-pyrrolidin-2-yl]ethyl]-3,4-dihydro-1H-isoquinoline-8-carboxamide **27**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (m, 9 H), 4.42 (d, J = 0.8 Hz, 1 H), 4.26 (s, 2 H), 3.18 (m), 3.00 (s, 3 H), 2.60 (m, 3 H), 2.42 (m, 1 H), 2.27 (m, 1 H), 1.98 (d, J = 1.0 Hz, 1 H), 1.81 (m, 2 H). LC-MS (99%) MH^+ 398

2-[3-[(2S)-1-(4-chlorophenyl)-5-oxo-pyrrolidin-2-yl]propyl]-3,4-dihydro-1H-isoquinoline-8-carboxamide **29**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (m, 7 H), 4.36 (m, 2 H), 4.19 (dd, J = 1.3, 0.8 Hz, 3 H), 3.41 (dq, J = 22.8, 7.0 Hz, 2 H), 3.01 (m, 5 H), 2.35 (m, 2 H), 1.81 (m, 1 H), 1.61 (s, 2 H), 1.39 (d, J = 8.3 Hz, 1 H), 1.07 (m, 2 H). LC-MS (100%) MH^+ 412

1-[2-[2-(R1-3,4-dihydro-1H-isoquinolin-2-yl)ethyl]phenyl]pyrrolidin-2-one **38**.

To a solution of pyrrolidine-2-one (1 mmol) and Iodo-aryl **34** (1 eq) dissolved in dioxane (10mL) was added K_3PO_4 (3 eq) at room temperature, followed by the addition of 1,2-diaminocyclohexane (0.2 eq) and copper(I)-iodide (0.1 eq). The reaction mixture was heated overnight at 65°C. Salts were filtered off, volatiles were removed under vacuo and the crude mixture was purified by column chromatography to afford **35** (55%), LC-MS (98%) MH^+ 234
To a solution of **35** (6.27g, 26.88 mmol) dissolved in THF (250mL) was added Lithium borohydride (4 eq, 2.27g) portionwise at 0°C, reaction mixture was then stirred overnight at room temperature. Reaction mixture was poured on crushed ice (250g) followed by extraction with dichloromethane (3x100mL). The organic layer was dried over $MgSO_4$, the solid was filtered off and volatiles were removed under vacuo to afford compound **36** (60%), LC-MS (96%) MH^+ 206
To a solution of **36** from above (1 eq) dissolved in dichloromethane was added N-methylimidazole (5 eq) at 0°C. The reaction mixture was stirred at 0°C during 1 hour. 4-methylbenzenesulfonyl chloride (TsCl, 1.1 eq) dissolved in dichloromethane was added dropwise at 0°C and the reaction mixture was stirred overnight at room temperature. The organic layer was successively washed with water (20 mL), aqueous HCl (2.5 M, 20 mL) and water (20 mL). The organic layer was dried over $MgSO_4$, the solid was filtered off and volatiles were removed under vacuo. The residue was purified by column chromatography to afford **37** (86%), LC-MS (98%) MH^+ 360.
The tosylate **37** (1 mmol) and the selected THIQ-amine derivative (1 mmol) were dissolved in CH_3CN (5 mL) and K_2CO_3 (3 mmol) was added at room temperature. The reaction mixture was stirred overnight at 85°C. Salts were filtered off, volatiles were removed under vacuum and the residue was purified by column chromatography to afford compounds **38** (55-95%).

2-[2-[2-(2-oxopyrrolidin-1-yl)phenyl]ethyl]-3,4-dihydro-1H-isoquinoline-8-carboxamide **49**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (s, 1 H), 7.47 (s, 1 H), 7.41 (dd, J = 9.0, 4.0 Hz, 2 H), 7.32 (m, 4 H), 7.27 (d, J = 3.8 Hz, 1 H), 4.38 (s, 2 H), 3.71 (t, J = 6.8 Hz, 2 H), 3.33 (s, 2 H), 3.21 (m, 2 H), 3.08 (t, J = 5.5 Hz, 2 H), 2.89 (m, 2 H), 2.16 (m, 2 H). LC-MS (100%) MH^+ 364

1-[2-[2-(3,4-dihydro-1H-isoquinolin-2-yl)ethyl]phenyl]piperidin-2-one **43**.

A solution of 2-(2-aminophenyl)ethanol **39** (450 g, 3.28 mol, 1 eq) and triethylamine (332 g, 3.28 mol, 1 eq) in dichloromethane (2 L) was cooled at -5 °C and bromovalerylchloride (654 g, 3.28 mol, 1 eq) in dichloromethane (2 L) was added dropwise without exceeding 0°C. The organic phase was washed with HCl 1N (4x2 L) and brine (1x2 L). The aqueous layer was extracted with dichloromethane (1x2 L). The combined organic layers was dried over $MgSO_4$, filtered and evaporated under vacuum to afford 1008 g of 5-bromo-N-[2-(2-hydroxyethyl)phenyl]pentanamide **40** (100%), LC-MS (96%) MH^+ 300
A solution of 5-bromo-N-[2-(2-hydroxyethyl)phenyl]pentanamide **40** (938 g, 3.28 mol, 1 eq) in THF (6 L) was cooled at -5 °C. Potassium tert-butoxide (552 g, 4.92 mol, 1.5 eq) was added portion-wise, without exceeding 0°C, then the mixture was warmed up to room temperature. The reaction mixture was washed with a saturated aqueous solution of NaCl (3x2 L). The combined aqueous layers

was extracted with dichloromethane (2x2 L), the combined organic layers was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was recrystallized from tert-butyl methyl ether to afford 510 g of 1- [2-(2-hydroxyethyl)phenyl]piperidin-2-one **41** (71 %), LC-MS (100%) MH^+ 220

Dimethylaminopyridine (1.83 g, 15 mmol, 0.05 eq) and triethylamine (63.5 ml, 450 mmol, 1.5 eq) were added to a solution of 1-[2-(2-hydroxyethyl)phenyl]piperidin-2-one **41** (65.7 g, 300 mmol, 1 eq) in dichloromethane (250 mL) at 0°C. The mixture was stirred at 0°C for 15 minutes, then a solution of 4-toluenesulfonyl chloride (63 g, 330 mmol, 1.1 eq) in dichloromethane (250 mL) was added dropwise. The reaction mixture was then warmed up to room temperature and stirred overnight. The reaction mixture was washed with water, 1N HCl, then dried over $MgSO_4$, filtered and evaporated under vacuum. The residue was purified by chromatography over silicagel (gradient: CH2Cl2/MeOH from 100/0 to 98/2) to afford 99 g of 2-[2-(2-oxopiperidin-1-yl)phenyl]ethyl 4-methylbenzenesulfonate **42** (88 %), LC-MS (96%) MH^+ 374

Selected R1-THIQ (2 mmol, 1 eq) and K2CO3 (6 mmol, 3 eq) were added to a solution of 2-[2-(2-oxopiperidin-1- yl)phenyl]ethyl 4-methylbenzenesulfonate **42** (2 mmol, 1 eq) in acetonitrile (10 mL). The reaction mixture was stirred at 85°C overnight, then filtered and the filtrate was condensed under reduced pressure. The residue was purified by basic reverse phase chromatography over silicagel (gradient CH3CN/H2O/NH4OH from 50/50/0.1 to 80/20/0.1) to afford **43** as a free base. Oxalic acid (1 eq) was added to the residue dissolved in diethylether. The precipitate obtained was filtered and dried under vacuum to afford **43** (55 %).

1-[2-[2-(3,4-dihydro-1H-isoquinolin-2-yl)ethyl]phenyl]piperidin-2-one **50**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7,40 (m, 1 H), 7,32 (m, 2 H), 7,22 (m, 5 H), 4,28 (s, 2 H), 3,59 (m, 1 H), 3,35 (m, 3 H), 3,19 (m, 2 H), 3,05 (m, 2 H), 2,89 (m, 2 H), 2,41 (m, 2 H), 1,86 (m, 4 H). LC-MS (100%) MH^+ 335

1-[2-[2-(8-methoxy-3,4-dihydro-1H-isoquinolin-2-yl)ethyl]phenyl]piperidin-2-one, **51**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (dd, J = 6.3, 2.9 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.28 – 7.18 (m, 2H), 6.85 (dd, J = 26.9, 7.9 Hz, 2H), 4.16 – 4.01 (m, 2H), 3.81 (s, 3H), 3.59 (dd, J = 11.8, 6.2 Hz, 1H), 3.32 (dt, J = 27.3, 5.2 Hz, 3H), 3.24 – 3.14 (m, 2H), 3.01 (s, 1H), 2.87 (dt, J = 10.6, 4.9 Hz, 2H), 2.48 – 2.30 (m, 3H), 1.93 – 1.84 (m, 4H). LC-MS (100%) MH^+ 365

2-[2-[2-(2-oxo-1-piperidyl)phenyl]ethyl]-3,4-dihydro-1H-isoquinoline-8-carboxamide **52**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 1 H), 7.37 (m, 1 H), 7.33 (s, 1 H), 7.20 (m, 6 H), 3.72 (d, J = 6.5 Hz, 2 H), 3.59 (m, 1 H), 3.33 (s, 1 H), 2.85 (t, J = 5.0 Hz, 2 H), 2.68 (m, 4 H), 2.59 (m, 2 H), 2.45 (m, 1 H), 2.35 (m, 1 H), 1.86 (m, 4 H). LC-MS (100%) MH^+ 378

2-[2-[2-(2-oxo-1-piperidyl)phenyl]ethyl]-3,4-dihydro-1H-isoquinoline-7-carbonitrile **53**,
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (m, 2 H), 7.39 (m, 2 H), 7.29 (m, 2 H), 7.20 (m, 1 H), 4.05 (s, 2 H), 3.59 (m, 1 H), 3.35 (m, 1 H), 3.13 (s, 2 H), 3.00 (m, 4 H), 2.79 (m, 2 H), 2.38 (m, 2 H), 1.88 (s, 4 H). LC-MS (100%) MH^+ 360

5-HT_7 competitive radioligand binding assay: Compound affinity (pIC_{50}) for 5HT_7 receptor was assessed in duplicate by competition against [^{3}H]5-Carboxyamidotryptamine (5-CT) on HEK293 Flp-In cell membranes expressing human 5-HT_{7D} receptor. [^{3}H]5-CT K_D and radioligand test concentration were of 0.10 ± 0.02 and 0.36 ± 0.03 nM, respectively. pIC_{50} were corrected to pKi according to Cheng and Prusoff (1973) [6]. Reference 5-HT_7 ligand was tested in each experiment for validation.

5-HT_7 cAMP Functional assay: 5-HT_7 receptor is coupled to Gs type G-protein. It's activation by an agonist like serotonin or 5-CT induces an increase in intracellular cAMP concentration. In the presence of 5-HT_7 antagonist, intracellular cAMP concentration induced by the agonist is blocked. In the present study, the agonist *versus* antagonist nature of the test compounds was assessed in each experiment by measuring intracellular cAMP concentration in the absence and presence of 5-CT (EC_{80}), respectively in HEK293 Flp-In cells expressing human recombinant 5-HT_{7D} receptor. GPCR cAMP HTRF assay kit from Cisbio (Codolet, France) was used to measure cAMP intracellular concentration [7]. Reference agonist and antagonist potencies were assessed in each experiment for validation.

Selectivity profiling: Compound selectivity for 5-HT7 receptor was assessed as compared to a broad panel of related and unrelated receptors, enzymes, transporters and ion channels. Selectivity profiling of 10 µM compound was performed in duplicate jointly at UCB BioPharma (Braine-l'Alleud, Belgium) and at CEREP (Celle-l'Evescault, France). For compounds inhibiting off targets above 80% of inhibition at 10 µM, affinity was measured testing compounds at increasing concentrations in competitive binding assays.

References

[1] (a) Ruat, E. Traiffort, R. Leurs, J. Tardivel-Lacombe, J. Diaz, J.M. Arrang, J.C. Schwartz, *Proc Natl Acad Sci U S A*. **1993**, *90,* 8547-51; (b) T. W. Lovenberg, B. M. Baron, L. Lecea, J. D. Miller, R. A. Prosser, M. A. Rea, P. E. Foye, M. Racke, A. L. Slone, B. W. Siegel, P. E. Danielson, J. G. Sutcliffe, M. G. Erlander, *Neuron.* **1993**, 11, 449-458; (c) J. L. Plassat, N. Amlaiky, R. Hen, *Mol. Pharmacol*., **1993**, 44, 229-236; (d) J. A. Bard, J. Zgombick, N. Adham, P. Vaysse, T. A. Branchek, R. L. Weinshank, *J. Biol. Chem.*, **1993**, 268, 23422-23426.

[2] (a) A. Nikiforuk, *CNS Drugs*, **2015**, 29, 265-275; (b) A. J.Roberts; P. B. Hedlund, *Hippocampus*, **2012**, 22(4), 762-771; (c) A. Gasbarri, A. Pompili, *Neurosciences*, **2014**, 25(3), 311-323; (d) M. Leopoldo, E. Lacivita, F. Berard, R. Perrone, P.B. Hedlund, **2011**, *Pharmacol. Ther*. *129*, 120–148; (d) P. B. Hedlund, *Psychopharmacology,* **2009**, 206, 345-354.

[3] (a) V. Pittala, D. Pittala, *Mini-Reviews in Medicinal Chemistry*, **2011**, 11(13), 1108-1121; (b) M. Leopoldo, *Current Medicinal Chemistry*, **2004**, 11(5), 629-661; (c) P. Raubo, M. S. Beer, P. A. Hunt, I. T. Huscroft, C. London, J. A. Stanton, J. Kulagowski, Bioorganic & Medicinal Chemistry Letters **2006**, 16, 1255-1258.

[4] P. J. Lovell, S. M. Bromidge, S. Dabbs, D. M. Duckworth, I. T. Forbes, A. J. Jennings, F. D. King, D. N. Middlemiss, S. K. Rahman, D. V.Saunders, L. L. Collin, J. J. Hagan, J. Riley, D. R. Thomas, Journal of Medicinal Chemistry, **2000**, 43(3), 342-345.

[5] P.Bonaventure, C. Dugovic, M. Kramer, P. Boer, J. Singh, S. Wilson, K. Bertelsen, J. Di, J. Shelton, L. Aluisio, L. Dvorak, I. Fraser, B. Lord, D. Nepomuceno, A. Ahnaou, W. Drinkenburg, W. Chai, C. Dvorak, S. Sands, N. Carruthers, and T. W. Lovenberg; *Journal of Pharmacology and Experimental Therapeutics*, **2012**, 342(2), 429-440.

[6] Cheng Y, Prusoff WH. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol. **1973** Dec 1;22(23):3099-108

[7] Degorce F, Card A, Soh S, Trinquet E, Knapik GP, Xie B HTRF: A technology tailored for drug discovery - a review of

theoretical aspects and recent applications. Curr Chem Genomics. **2009** May 28;3:22-32

[a] UCB Pharma, UCB NewMedicines ; Chemin du Foriest, 1420, Braine-L'Alleud (Belgium)

[a*] UCB Pharma, UCB NewMedicines ; Chemin du Foriest, 1420, Braine-L'Alleud (Belgium), ali.ates@ucb.com

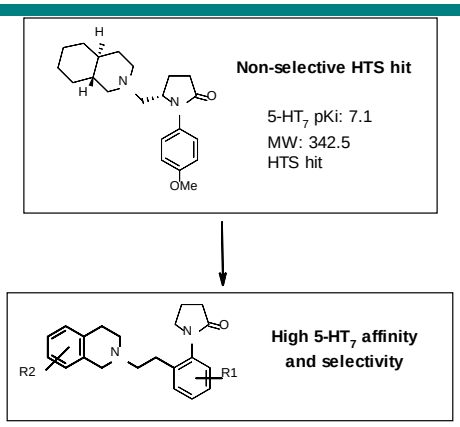
10.1002/cmdc.201800026

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATIONS

In the present study, a novel chemical series of serotonin 5-HT_7 antagonist is described. The synthesis, structure-activity relationships and selectivity profile are reported. This series includes **5-HT7 antagonists with unprecedented good selectivity** for 5-HT7 receptor.



Ali Ates, Burssens Pierre, Lorthioir Olivier, Lo Brutto Patrick, Dehon Gwenael, Keyaerts Jean, Coloretti Francis, Bénédicte Lallemand, Valérie Verbois, Michel Gillard and Céline Vermeiren

Page No. – Page No.
Novel 5-HT_7 antagonists, with an unprecedented selectivity profile