Bioorganic & Medicinal Chemistry 17 (2009) 5247–5258

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

SAR and biological evaluation of SEN12333/WAY-317538: Novel alpha 7 nicotinic acetylcholine receptor agonist

Simon N. Haydar [a,*,†], Chiara Ghiron [b,†], Laura Bettinetti [b], Hendrick Bothmann [b], Thomas A. Comery [a], John Dunlop [a], Salvatore La Rosa [b], Iolanda Micco [b], Martina Pollastrini [b], Joanna Quinn [b], Renza Roncarati [b], Carla Scali [b], Michela Valacchi [b], Maurizio Varrone [b], Riccardo Zanaletti [b]

[a] Chemical Sciences and Neuroscience Discovery Research, Wyeth Research, CN 8000, Princeton, NJ 08543-8000, USA
[b] Siena Biotech SpA, Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy

ARTICLE INFO

Article history:
Received 3 March 2009
Revised 8 May 2009
Accepted 14 May 2009
Available online 21 May 2009

Keywords:
Neuronal nicotinic acetylcholine receptors
Nicotinic ligands
Cognition
Auditory sensory gating

ABSTRACT

Alpha 7 nicotinic acetylcholine receptor (α_7 nAChR) agonists are promising therapeutic candidates for the treatment of cognitive impairment associated with a variety of disorders including Alzheimer's disease and schizophrenia. Alpha 7 nAChRs are expressed in brain regions associated with cognitive function, regulate cholinergic neurotransmission and have been shown to be down regulated in both schizophrenia and Alzheimer's disease. Herein we report a novel, potent small molecule agonist of the alpha 7 nAChR, SEN12333/WAY-317538. This compound is a selective agonist of the α_7 nAChR with excellent in vitro and in vivo profiles, excellent brain penetration and oral bioavailability, and demonstrates in vivo efficacy in multiple behavioural cognition models. The SAR and biological evaluation of this series of compounds are discussed.

1. Introduction

Cognitive disorders encompass a wide range of diseases, most notably in the psychiatric and neurology therapeutic areas. While the positive symptomatology of schizophrenia (hallucinations, delusions, paranoia) is addressed with current antipsychotic medications that block dopamine D2 receptors, the negative symptomatology (cognitive impairments, reduced affect, social withdrawal, low motivation) is largely unaffected and remains a significant unmet medical need. Among the neurodegenerative diseases, Alzheimer's disease (AD) is the most common form of dementia afflicting 10% of the population in their sixties, and approximately half of all people in their eighties. In AD, the early and predominant disease marker is cognitive dysfunction. Modest symptomatic improvement in cognitive performance is provided by standard of care agents such as cholinesterase inhibitors and NMDA receptor antagonists.

Several lines of experimental evidence support the involvement of the neuronal nicotinic receptors in both schizophrenia and AD.[1,2] These include reduced expression of α_7 nicotinic acetylcholine receptors (nAChR) in brain tissue from schizophrenia and AD patients, and genetic linkage studies in schizophrenia implicating the locus of the α_7 receptor gene promoter.[3] Prototypical α_7 receptor agonists have demonstrated improved cognition in animal models and normalize sensory gating deficits, which are believed to contribute to the cognitive fragmentation in schizophrenia.[4,5] Based on these observations, selective α7 nAChR agonists are predicted to be effective for the improvement of cognition in both schizophrenia and AD.[6–8] In accordance with this hypothesis, the prototypical nAChR agonist nicotine, as well as more selective α7 agonists described recently, have been shown to improve cognitive performance in both animal models and humans clinical trials.[9] Herein we report the synthesis and SAR of a series of novel, potent, small molecule agonists of the α7 nAChR.

As part of a multi-disciplinary approach to the treatment of schizophrenia and Alzheimer's disease, a discovery medicinal chemistry program was initiated to identify selective α_7 agonists. Through the course of a high throughput screen, the advanced hit *N*-{4-[4-(2,4-dimethoxyphenyl)-piperazin-1-yl]butyl}-4-pyridin-2-ylbenzamide **1**, was identified as a weak partial agonist of the α7 nicotinic receptor (EC_{50} = 2.8 μM).[10]

2. Chemistry

Starting from the original hit **1**, initial efforts were made to develop a new series (Fig. 1) with increased potency and improved drug-like properties. In particular, in addition to the initial SAR exploration, we were concerned about the number of rotatable bonds, the molecular weight, and the relatively high number of hydrogen bond acceptors which could impact unfavourably on

* Corresponding author. Tel.: +1 732 274 4518; fax: +1 732 274 4505.
E-mail address: haydars@wyeth.com (S.N. Haydar).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.05.040

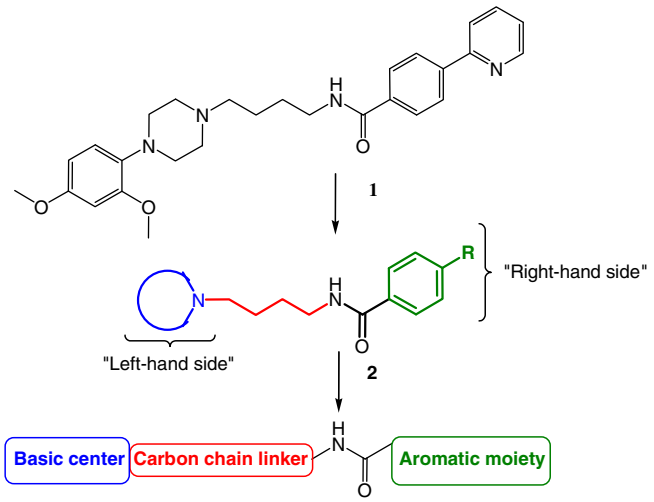


Figure 1. Initially identified pharmacophore.

the polar surface area and consequently brain permeability of the compounds. We were also interested in exploring the possibility of removing the arylpiperazine moiety, which could lead to polypharmacology issues. The basic pharmacophore around the original hit could be described as consisting of three constituents; a basic moiety attached by a flexible length carbon chain bridged to an aromatic moiety. The potential for hydrogen-bond interactions also appear to impart a favourable behaviour.

Two separate synthetic routes were developed to prepare the targeted analogs from readily available starting materials. In the first route (Scheme 1), commercially available *N*-(ω-bromobutyl)phthalimide **3** was reacted with a secondary amine to give the desired amino-phthalimide products **4**. Subsequently, compounds **4** were refluxed in ethanol in the presence of hydrazine hydrate and upon acidic workup the desired mono-substituted amines **5** were obtained as hydrochloride salts. The latter products were then reacted with 4-bromobenzoyl chloride in the presence of Et_3N to give amides **6**. Alternatively, activation of 4-bromobenzoic acid with 1,1-carbonyldiimidazole could be used to prepare these amides **6**. Finally, Suzuki coupling of these advanced intermediates **6** with the appropriate substituted phenyl boronic acid or heterocyclic boronic acid under cross-coupling conditions afforded the final targeted products **7**.

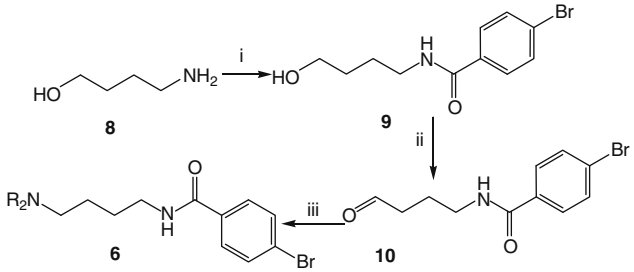
An alternative synthesis of analogs **7**, shown in Scheme 2, is carried out in four steps from commercially available 4-aminobutanol **8**. Hydroxybutyl amide **9** was obtained from the reaction of 4-aminobutanol **8** and 4-bromobenzoic acid in the presence of Hunig's base.

These primary alcohols were then oxidized under Swern conditions to afford the desired aldehydes **10** in good yields. Condensation of these aldehydes **10** with the appropriate secondary amine and reduction of the resultant imine with sodium triacetoxyborohydride gave intermediates **6**.

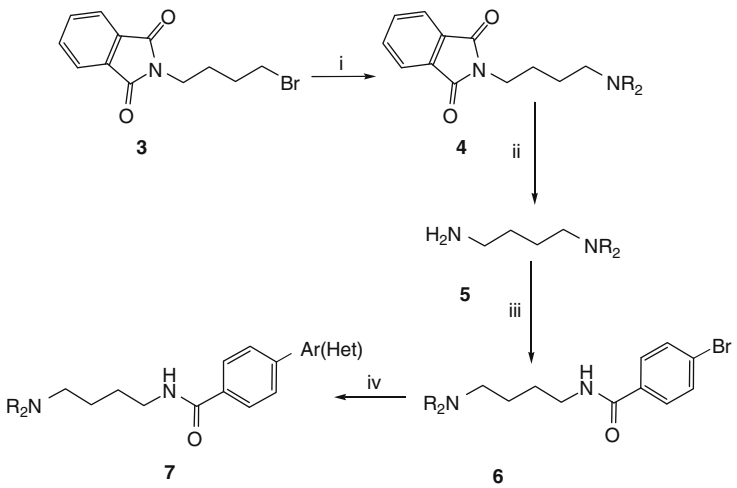
Using a slightly modified route, analogs with a 'reverse' amide linker were efficiently prepared (Scheme 3). To that end, 5-bromovaleryl chloride **11** was reacted with 4-bromoaniline to give amide **12** which was used in the subsequent reaction without purification. Reaction of various secondary amines with compound **12** in the presence of base afforded the penultimate intermediates **13**. Coupling with an aryl boronic acid or heteroaryl boronic acid under a variety of Suzuki conditions afforded the final products **14** in excellent yield.

3. Results and discussion

All functional activities were measured in either calcium flux or membrane potential assays using a fluorescence imaging plate reader (FLIPR™) system (Molecular Devices). Activity at rat α7 nAChRs was determined using a stable recombinant GH4C1 cell line expressing the receptor.[11] For the purpose of SAR discussion, the 'left-hand side' and the 'right-hand side' will be referred to as LHS and RHS respectively as depicted in Figure 1.



Scheme 2. Reagents and conditions: (i) 4-bromobenzoic acid, Hunig's base, CH_2Cl_2; (ii) $(COCl)_2$, DMSO, Et_3N; (iii) $Na(AcO)_3BH$, DCM.



Scheme 1. Reagents and conditions: (i) substituted secondary amine, K_2CO_3, NaI (cat.), 2-butanone, reflux; (ii) hydrazine monohydrate, ethanol, reflux; (iii) 4-bromobenzoyl chloride, Et_3N, or 4-bromobenzoic acid, 1,1-CDI, DMF; (iv) $ArB(OH)_2$ or $HetB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME/EtOH.

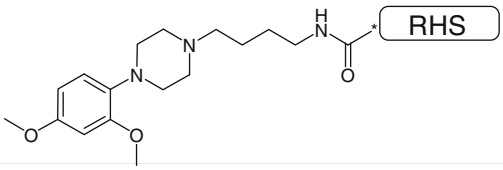
Early work on modifying the RHS of advanced Hit **1** resulted in a somewhat flat SAR, as illustrated in Table 1 Synthesized compounds which retained activity did not show great potency improvement over the initial hit. However, several key qualitative findings were notable from this study. For instance, the activity of this series of compounds at the α7 receptor appeared to require a biaryl moiety in the RHS as demonstrated by the poor activity of compounds **7f** and **7g**. Moreover, substitution of the 2-position of the pendant aryl ring in the biaryl RHS was important to retain potency as illustrated by the poor potency of analog **7e**.

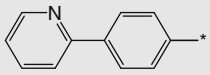
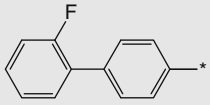
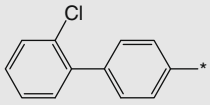
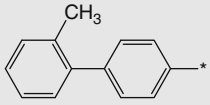
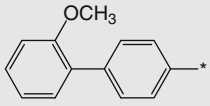
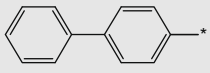
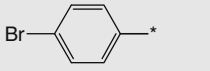
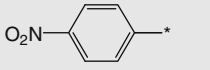
After extensive exploration with a large number of analogs to support the SAR the following conclusions emerged; the amide linker could be 'reversed' while retaining potency at the α_7 nAChR; importantly, the aryl piperazine could be replaced with various cyclic amines such as piperidine (**14a**), and morpholine (**14b**) (Table 2). Acyclic tertiary amine substitution in the LHS, as in compound **14e**, was tolerated. Steric bulk around the basic amine in the LHS was also tolerated, but to a lesser degree. While 6-membered ring **14a** and 7-membered ring **14h** showed similar potency, additional substitution on the ring and on larger heterocycles was less desirable, as illustrated in compounds **14g** and **14i**, respectively.

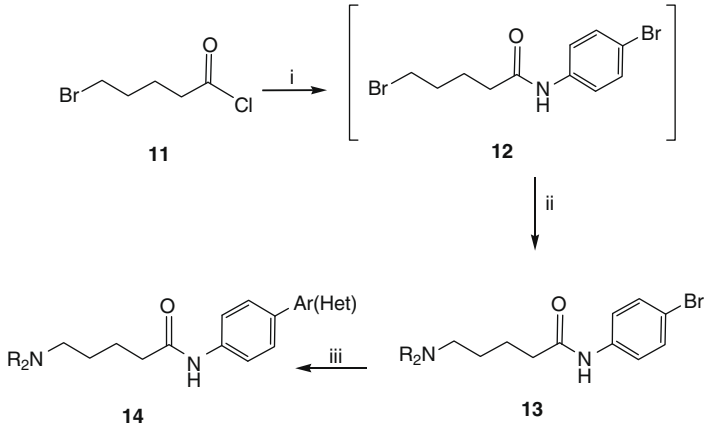
Further efforts to explore the aromatic moiety on the RHS in the 'reverse' amide series show an increased breadth in the SAR of this class of α_7 nAChR ligands (Table 3). In general, biaryl compounds were more potent than mono aryl derivatives as previously observed in the original Hit series. For example, the potency of **14l** is about 10-fold less than that of analog **14j**, although comparison of **14j** with **7f** would indicate that the series is intrinsically more potent. Interestingly, when substitution was moved to the *meta* position as in compound **14o** and **14s**, the potency dropped dramatically. Replacing the biaryl moiety with a fused ring group also led to a drop in activity as shown with compound **14m** and **14n**. Compounds with hydrogen bonding (donor/acceptor) potential on the pendant aryl ring were generally showing increased potency, as exemplified by compounds **14p** and **14r**.

From this emerging SAR, analog **14b** was chosen to be evaluated in in vivo efficacy models based on its generally good in vitro profile, ease of synthesis, selectivity on a related panel of nicotinic (alpha1, alpha3, alpha4beta2) and highly homologous receptors ($5HT_{3A}$). This was confirmed in a panel of ~70 binding sites including all major classes of neurotransmitter, growth factor and peptide receptors whereby no significant activity was observed when the compound was tested at 10 μM concentration except for the histamine H3 receptor where binding was observed leading to receptor antagonism (Novascreen, Caliper Biosciences, Hopkinton MA). Compound **14b** also showed minimal hERG inhibition, desirable drug-like (molecular weight, number of rotatable bonds,

Table 1
Right-hand side exploration of the original Hit **1**

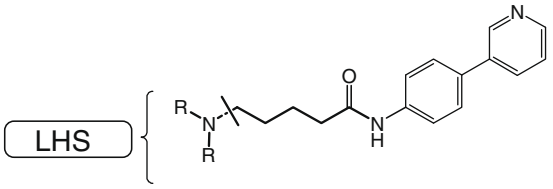


Compound	RHS	EC_{50} ± SEM (*n*) (μM)
1		2.84 ± 0.23 (32)
7a		6.03 ± 3.75 (3)
7b		3.11 ± 0.10 (2)
7c		5.77 ± 2.26 (3)
7d		2.91 ± 0.37 (2)
7e		>30 (1)
7f		>30 (1)
7g		>30 (1)



Scheme 3. Reagents and conditions: (i) Et_3N, 4-bromoaniline, CH_2Cl_2; (ii) secondary amine, Et_2N; (iii) $ArB(OH)_2$ or $HetB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME/EtOH.

Table 2
Left-hand side exploration of the 'reverse' amide series



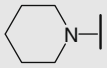
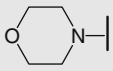
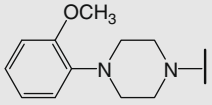
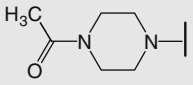
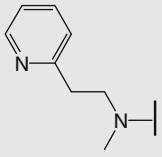
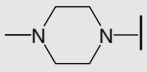
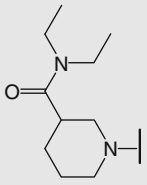
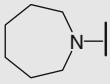
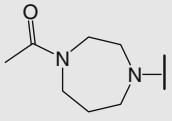
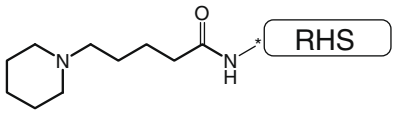
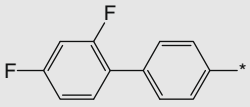
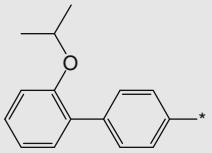
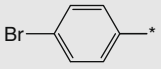
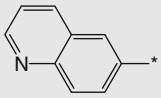
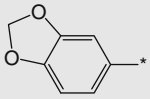
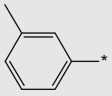
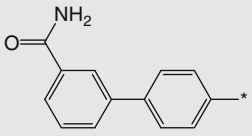
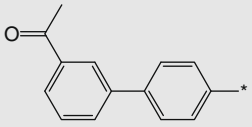
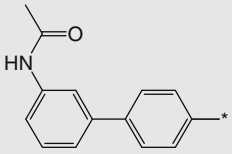
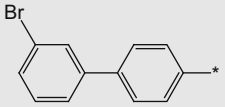
Compound	LHS	EC_{50} ± SEM (*n*) (μM)
14a		0.53 (1)
14b		1.65 ± 0.43 (4)
14c		2.47 ± 0.77 (3)
14d		2.78 ± 0.58 (3)
14e		3.27 (1)
14f		3.56 ± 0.76 (3)
14g		>30 (1)
14h		0.54 (1)
14i		2.66 (1)

Table 3
Right-hand side exploration of the 'reverse' amide series



Compound	RHS	EC_{50} ± SEM (*n*) (μM)
14j		0.16 ± 0.04 (2)
14k		0.24 ± 0.06 (2)
14l		1.35 ± 0.27 (2)
14m		5.07 (1)
14n		10.93 (1)
14o		>30 (1)
14p		0.09 ± 0.01 (2)
14q		0.99 (1)
14r		0.12 ± 0.04 (8)
14s		2.07 (1)

log *P*, polar surface area) and pharmacokinetic properties (Table 4). Compound **14b**, designated SEN12333/WAY-317538, is a full agonist at the α_7 nAChR with an EC_{50} of 1.6 μM as determined on its hydrochloride salt. Further in vitro characterization of SEN12333/WAY-317538 revealed that the compound is selective for the α_7 nAChR over other nAChRs (α_1, α_3) and the closely related 5-HT_3 receptor.

In vivo, SEN12333/WAY-317538 improved performance in rodent behavioural assays of cognitive function and perceptual processing, producing enhancement of normal memory performance.[12] Furthermore, the ability to attenuate pharmacologically induced deficits via either the glutamatergic or cholinergic system (Fig. 2) was demonstrated. For example, treatment with

Table 4
Profile of SEN12333/WAY-317538

α_7			Electrophysiology	
K_i	EC_{50}		EC_{50}	E_{max}
In vitro				
260 nM	1.6 μM		12 μM	88%
α_1	α_3	5-HT_3	α4β2	hERG
Selectivity				
Inactive	5.3-fold	Inactive	45% @10 μM	6% @ 1 μM
C_{max}	$T_{1/2}$	AUClast	B/P	% *F* (po)
Pharmacokinetic (10 mg/kg, ip, male Wistar rat)				
244 ng/mL	0.8–1 h	347 h ng/mL	2.5	57%

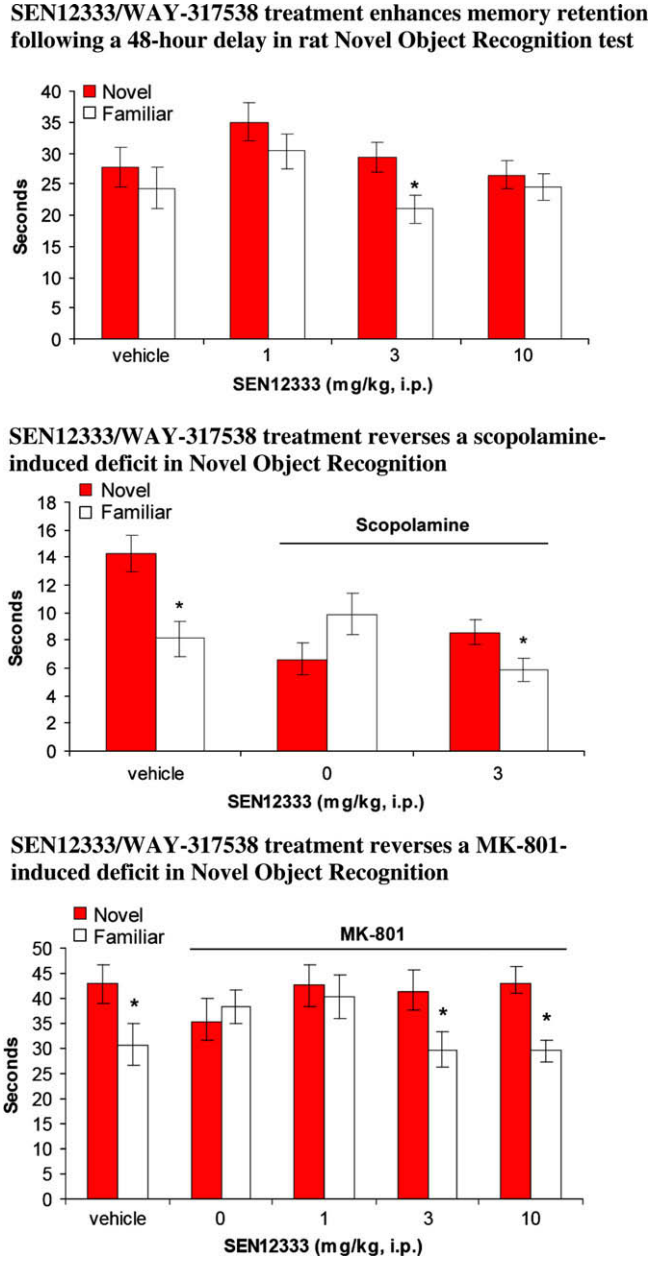


Figure 2. In vivo efficacy of SEN12333/WAY-317538 in novel object recognition.

SEN12333/WAY-317538 (3 mg/kg, ip) minimized spontaneous decay of episodic memory in a novel object recognition task in rats. In addition, the compound was able to reverse both a scopolamine- and an MK-801-induced deficit in the pharmacological models for these recognition memory tests.

4. Conclusion

In summary, we have identified a series of novel, potent small molecule agonists of the α_7 nAChR. One selected example, SEN12333/WAY-317538 showed excellent brain penetrability and a generally favourable pharmacokinetic profile. Pro-cognitive efficacy was demonstrated in multiple behavioural cognition assays with this analog. These data further support the potential utility of α_7 agonists and SEN12333/WAY-317538 for the treatment of neurodegenerative and psychiatric disorders with associated cognitive dysfunction.

5. Experimental

5.1. General methods

Unless otherwise specified, all nuclear magnetic resonance spectra were recorded using a Varian Mercury Plus 400 MHz spectrometer equipped with a PFG ATB Broadband probe.

HPLC–MS analyses (5 and 10 min methods) were performed with a Waters 2795 separation module equipped with a Waters Micromass ZQ (ES ionization) and Waters PDA 2996, using a Waters XTerra MS C18 3.5 μm 2.1 × 50 mm column; alternatively, HPLC–MS analyses were performed with an Agilent 1100 instrument, using a Zorbax Eclipse XDB-C8 4.6 × 150 mm; a Zorbax CN 4.6 × 150 mm column or a Zorbax Extend C18 2.1 × 50 mm column, coupled to API-ES MS for the 2.5 min method.

Preparative HLPC was run using a Waters 2767 system with a binary Gradient Module Waters 2525 pump and coupled to a Waters Micromass ZQ (ES) or Waters 2487 DAD, using a Supelco Discovery HS C18 5.0 μm 10 × 21.2 mm column. Gradients were run using 0.1% formic acid/water and 0.1% formic acid/acetonitrile with gradient 5/95 to 95/5 with a runtime of 10 min unless otherwise stated.

All column chromatography was performed following the method of Still.[13] All TLC analyses were performed on silica gel (Merck 60 F254) and spots revealed by UV visualization at 254 nm and KMnO4 or ninhydrin stain.

When specified for array synthesis, heating was performed on a Buchi Syncore® system.

All microwave reactions were performed in a CEM Discover oven.

5.1.1. General procedure for aminoalkylamine synthesis

To a suspension of the required amine (1 equiv), sodium iodide (0.5 equiv) and potassium carbonate (1.1 equiv) in 2-butanone (4 M solution), *N*-(ω-bromoalkyl)phthalimide (1 equiv) was added. The resulting suspension was stirred for 18 h at 85 °C, then the reaction was filtered and the solvent removed by vacuum distillation. The resulting oil was washed with water and recovered with DCM. The solvent was removed under reduced pressure to yield the amino-phthalimide products pure enough to be used in the following step. If necessary, a further purification was carried out by flash-chromatography. The phthalimides thus obtained (1 equiv) were dissolved in EtOH (2 M solution with respect to phthalimide amount) and hydrazine monohydrate (2 equiv) was added dropwise. The mixture was heated at 80 °C for 4 h, after which the reaction was acidified with 37% HCl and the solid which precipitated was removed by filtration. The solution was concentrated under vacuum and taken up with 1 N HCl. Any residual 2,3-dihydrophthalazine-1,4-dione was removed by filtration. The aqueous

solution was removed under reduced pressure under vacuum to recover the pure product.

In case of acid sensitive derivatives the reaction mixture was filtered and washed with EtOH, concentrated under vacuum and taken up with toluene and DCM to remove excess 2,3-dihydrophthalazine-1,4-dione. Solvent removal under reduced pressure afforded the pure product.

5.1.2. General procedure for acid–amine coupling method using acid chlorides

A mixture of (4-arylpiperazin-1-yl)alkylamine (0.3 mmol), carboxylic acid chloride (0.3 mmol), triethylamine (0.56 mmol) and a catalytic amount of DMAP in CH_2Cl_2 was stirred at 0 °C for 10 min then at room temperature for 4 h. The CH_2Cl_2 layer was washed with water, dried and concentrated. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH 95/5 as eluent to give the title compound.

5.1.3. General procedure A for amide synthesis from ω-haloalkanoyl chlorides

In a round-bottom, 2-neck flask, triethylamine (1 equiv) was added to a solution of aryl or heteroaryl amine (1 equiv) in a volume of DCE such as to obtain a 1.2 M solution of amine; 5-bromovaleryl chloride (0.95 equiv) was then added dropwise as a 1.2 M solution in DCE and the reaction was then stirred at room temperature for 1 h 30 min. A 1.8 M solution of amine (3 equiv) and triethylamine (1 equiv) in DCE were then added and the reaction mixture stirred at 55 °C for a time between 4 and 16 h, until LCMS monitoring showed reaction completion. After this period, the reaction mixture was partitioned between water and DCM; the organic layer was washed with saturated NaCl and dried over Na_2SO_4. The crude ω-aminoalkanamides obtained after solvent evaporation at reduced pressure were purified by trituration from Et_2O/hexane (1/1) or by flash chromatography.

5.1.4. General procedure B for amide synthesis from ω-haloalkanoyl chlorides

A solution of aniline (1 equiv) and triethylamine (1 equiv) in dichloromethane (0.2 mmol/mL) was cooled to 0 °C under nitrogen atmosphere. The ω-bromoalkanoyl chloride (1 equiv) in dichloromethane (0.3 mmol/mL) was slowly added. The mixture was stirred at room temperature for 1.5 h, after which the amine (5 equiv) and triethylamine (1 equiv) were added all at once and the reaction was stirred at room temperature for a further 40 h. If necessary the reaction was heated at 50 °C to push it to completion. The organic solution was washed with brine, dried and the solvent removed under reduced pressure. The product was triturated by hexane/diethyl ether (1/1) or purified by flash chromatography.

5.1.5. General procedure C for amide synthesis from ω-haloalkanoyl chlorides (array synthesis)

To a solution of aniline (1 equiv for each molar equivalent of amine used) and triethylamine (1 equiv for each molar equivalent of amine used) in dichloromethane (0.3 mmol/mL for each amine used) the ω-bromoalkanoyl chloride (1 equiv for each molar equivalent of amine used) was slowly added and the mixture stirred at room temperature for 2 h. The solution was then split over as many as amines used in the array and the each portion added to a vial containing the amine (5 equiv) and triethylamine (1 equiv). The reactions were then shaken at room temperature for 40 h. The organic solutions were washed with saturated brine, dried (Na_2SO_4), and the solvent removed under reduced pressure. The products were purified by preparative HPLC.

5.1.6. General procedure D for amide synthesis from ω-haloalkanoyl chlorides

4-Bromoaniline (6 g, 0.035 mol) and 0.035 mol of Et_3N (4.87 mL) were dissolved in 120 mL of dichloromethane and cooled at 0 °C. To this solution, 0.038 mol of ω-bromoalkanoyl chloride (5.4 mL) were slowly added and the resulting mixture was stirred for 1 h at 0 °C.

When all the starting material was consumed (monitoring by LCMS) the solution was washed with 50 mL of Na_2CO_3 0.4 M, the organic layer was recovered by extraction and drying over Na_2SO_4 and the product used without further purification: 1 equiv of the alkylating agent was dissolved in butanone (5–10 mL/mmol substrate) and to this solution 1 equiv of NaI and 1.1 equiv of the amine were added. The mixture was stirred at 70 °C or 24 h. The mixture was cooled to room temperature. When the products precipitated as salts, they were taken into water, free-based by addition of NaOH 10% to pH 10 and extracted with dichloromethane. In the cases where no product precipitation occurred, the solvent was removed under reduced pressure, the crude was taken into dichloromethane and extracted after adjusting the pH to 10 with NaOH 10%. If necessary, the products were further purified by flash chromatography.

5.1.7. General procedure for cross-coupling reaction with boronic acids (microwave irradiation)

To a degassed mixture of 5-alkylpentanoic acid arylamide (0.1 g, 1 equiv) aryl boronic acid (1.1 equiv) in acetonitrile/sodium carbonate 0.4 M solution 1/1 (4 mL), a catalytic amount of $Pd[(PPh_3)]_4$ (5 mmol %) was added. The reaction mixture was heated at 90 °C for 20 min under microwave irradiation (150 W, pressure max) and then again other 20 min. The organic layer was separated and concentrated, and further purified by SCX column and/or by preparative HPLC. The solvent was removed under reduced pressure to afford the corresponding product.

5.1.8. General procedure for cross-coupling reaction with boronic acids (thermal conditions)

To a degassed mixture of 5-alkylpentanoic acid arylamide (0.1 g, 1 equiv) aryl boronic acid (1.1 equiv) in acetonitrile/sodium carbonate 0.4 M solution 1/1 (4 mL), a catalytic amount of $Pd[(PPh_3)]_4$ (5 mmol %) was added. The reaction mixture was heated at 90 °C for 16 h. The organic layer was separated and concentrated, and further purified by SCX column and/or by preparative HPLC. The solvent was removed under reduced pressure to afford the corresponding products.

5.1.9. *N*-{4-[4-(2,4-Dimethoxyphenyl)-piperazin-1-yl]butyl}-4-(pyridin-2-yl)-benzamide (1)

5.1.9.1. 1-(2,4-Dimethoxyphenyl)piperazine hydrochloride. A solution of 1.48 g (0.0097 mol) of 2,4-dimethoxyaniline, 1.89 g (0.0160 mol) of bis-2-chloroethylamine hydrochloride and 2.00 g of K_2CO_3 in 25 mL of 1-butanol was refluxed for 24 h then filtered hot. The solvent was removed under reduced pressure and the residue triturated with acetone. The resulting powder was filtered and dried to give 1.25 g of the title compound.

^{1}H NMR (DMSO-d_6) δ 9.21 (br s, 1H); 6.82 (d, 1H); 6.52 (s, 1H); 6.42 (d, 1H), 3.74 (s, 3H); 3.68 (s, 3H); 3.12 (s, 4H); 3.07 (s, 4H).

5.1.9.2. 2-{4-[4-(2,4-Dimethoxyphenyl)piperazin-1-yl]-butyl}-isoindole-1,3-dione. A mixture of *N*-(4-bromobutyl)phthalimide (0.00135 mol), 1-(2′,4′-dimethoxyphenyl)-piperazine hydrochloride (0.00135 mol), K_2CO_3 (0.00270 mol), NaI (0.00186 mol) and methyl ethyl ketone (7 mL) was refluxed for 20 h with stirring. After the mixture had cooled, the insoluble materials were removed by filtration and washed with $CHCl_3$. The filtrate and the washings were concentrated to dryness in vacuo. The residue

was purified by chromatography on silica gel with $CHCl_3$/MeOH 95/5 as eluent. Yield: 68%.

^{1}H NMR ($CDCl_3$) δ 7.73 (m, 4H), 6.82 (d, 1H), 6.40 (m, 2H), 3.79 (s, 3H), 3.73 (s, 3H), 3.65 (m, 2H), 2.98 (m, 4H), 2.61 (m, 4H), 2.41 (t, 2H), 1.66 (m, 4H).

5.1.9.3. 4-[4-(2,4-Dimethoxyphenyl)piperazin-1-yl]butylamine. A solution of 2-{4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl} isoindole-1,3-dione (0.236 mmol) and hydrazine hydrate (0.478 mmol) in ethanol (2 mL) was refluxed for 2 h with stirring. After the solution had cooled, any insoluble materials were removed by filtration and washed with EtOH. The filtrate and the washings were concentrated in vacuo to dryness. The residue was taken up with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried and concentrated to give the title amine. Yield: 50%.

^{1}H NMR ($CDCl_3$) δ 6.85 (d, 1H), 6.41 (m, 2H), 3.81 (s, 3H), 3.75 (s, 3H); 3.01 (m, 4H), 2.63 (m, 4H), 2.40 (br t, 2H), 1.35 (m, 6H).

5.1.9.4. *N*-{4-[4-(2,4-Dimethoxyphenyl)piperazin-1-yl]butyl}-4-(pyridin-2-yl)benzamide. A mixture of (4-(2,4-dimethoxyphenyl)piperazin-1-yl)-alkylamine (0.3 mmol), 4-(pyridin-2-yl)-benzoyl chloride (0.3 mmol), triethylamine (0.56 mmol) and a catalytic amount of DMAP in CH_2Cl_2 was stirred at 0 °C for 10 min then at room temperature for 4 h. The CH_2Cl_2 layer was washed with water, dried and concentrated. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH 95/5 as eluent to give the title compound.

Yield: 35%.

Mp 154.5–156 °C (free base), 212–216 °C (HCl salt).

^{1}H NMR ($CDCl_3$) δ 8.66 (d, J = 4.9 Hz 1H), 8.02 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 8.3 Hz, 2H), 7.75 (m, 2H), 7.23 (m, 1H), 6.85 (br t, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.42 (d, J = 2.6 Hz, 1H), 6.33 (dd, J = 8.7, 2.61 Hz, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.47 (m, 2H), 2.97 (m, 4H), 2.62 (m, 4H), 2.47 (br t, 2H), 1.65–1.68 (m, 4H).

Mass (ES) m/z %: 475 (M+1, 100%), 497 (M+Na, 19%).

HPLC: column Zorbax C8 MeOH 80%/H_2O 20%, 1.0 mL/min; 10 min run; t_R 6.54; area = 99%.

HRMS: calcd for $C_{28}H_{34}N_4O_3 + H^+$, 475.27037; found (ESI, $[M+H]^+$ Obsd), 475.2703.

5.1.10. 2′-Fluoro-biphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxy-phenyl)-piperazin-1-yl]-butyl}-amide (7a)

Prepared from 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butylamine and commercially available 2′-fluorobiphenyl-4-carboxylic acid following general procedure 5.1.2.

Yield: 20%.

Mp = 124–125.5 °C.

t_R ($CHCl_3$/MeOH 95/5) 0.21.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.81 (d, J = 8.33 Hz, 2H), 7.56 (d, J = 8.33 Hz, 2H); 7.11–7.41 (m, 4H); 6.99 (br s, 1H); 6.76 (d, J = 8.6 Hz, 1H); 6.43 (d, J = 2.6 Hz, 1H); 6.33 (dd, J = 8.65, 2.6 Hz, 1H); 3.78 (s, 3H); 3.71 (s, 3H); 3.42–3.46 (m, 2H); 2.94 (m, 4H); 2.60 (m, 4H); 2.46 (br t, 2H); 1.65–1.67 (m, 4H).

Mass (ES) m/z %: 492 (M+1, 100%).

HPLC: column Zorbax CN AcCN 50%/H_2O (CF_3COOH pH 2.3) 50%, 0.4 mL/min; 20 min run; t_R = 13.525; area 96%.

HRMS: calcd for $C_{29}H_{34}FN_3O_3 + H^+$, 492.26570; found (ESI, $[M+H]^+$ Obsd), 492.2658.

5.1.11. 2′-Chlorobiphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl}-amide (7b)

The commercially available 2′-chlorobiphenyl-4-carboxylic acid (0.55 mmol) was disolved in dimethylformamide (2 mL) to dissolve, followed by *N,N′*-carbonyldiimidazole (0.55 mmol). The solution was then left to stir for 60 min before adding 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butylamine (0.6 mmol) and the reaction was stirred for a further 16 h. The solvent was removed under reduced pressure and the crude mixture was treated with 5% MeOH in dichloromethane (2 mL) and washed with 10% sodium hydroxide solution (2 mL). This mixture was passed through a column packed with 5 g of diatomaceous earth and the eluting the product with dichloromethane. The collected organic layer, containing the desired compound, was further purified using flash chromatography eluting with 10% MeOH in dichloromethane. Fractions containing the product were combined and the solvent removed under reduced pressure.

HRMS: calcd for $C_{29}H_{34}ClN_3O_3 + H^+$, 508.23615; found (ESI, $[M+H]^+$ Obsd), 508.2362.

HPLC: column Zorbax C8 MeOH 80%/H_2O 20%, 1.0 mL/min; 10 min run; t_R 4.31; area = 98%.

5.1.12. 2′-Methylbiphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl} amide (7c)

5.1.12.1. 2′-Methylbiphenyl-4-carboxylic acid. Prepared with a modification of the procedure outlined in Leadbeater and Marco.[15] In a 10 mL glass tube were placed 4-carboxyphenyl boronic acid (166 mg, 1.0 mmol), 2-bromotoluene (120 μL, 1.0 mmol), Na_2CO_3 (315 mg, 3 mmol), $Pd(OAc)_2$ (1 mg, 0.004 mmol), 2 mL of water and a magnetic stir bar. The vessel was sealed with a septum and placed into the microwave cavity. Microwave irradiation (maximum emitted power 200 W) was used to increase the temperature to 150 °C; the reaction mixture was then kept at this temperature for 5 min.

The mixture was allowed to cool to room temperature, and the reaction mixture was filtered washing with little $CHCl_3$.The aqueous layer was acidified, and the precipitate collected. The product was purified by chromatography on silica gel using petroleum ether/AcOEt 50/50 as eluent to give 67.8 mg of product, yield 32%.

^{1}H NMR (CD_3OD) δ 8.05 (m, 2H, arom); 7.41 (m, 2H, arom); 7.21 (m, 4H, arom); 2.22 (s, 3H, C–CH_3).

Mass (ES) m/z %: 424 (2 M, 100%).

5.1.12.2. 2′-Methylbiphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl} amide. Prepared from 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butylamine and 2′-methylbiphenyl-4-carboxylic acid following general procedure 5.1.2.

Yield: 21%.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80 (d, J = 8.33 Hz, 2H); 7.35 (d, J = 8.33 Hz, 2H); 7.2–7.4 (m, 4H); 6.88 (br s, 1H); 6.79 (d, J = 8.6 Hz, 1H); 6.46 (d, 1H); 6.30–6.40 (m, 1H); 3.82 (s, 3H); 3.76 (s, 3H); 3.50 (m, 2H); 2.98 (m, 4H); 2.66 (m, 4H); 2.47 (m, 2H); 2.25 (s, 3H); 1.70 (m, 4H).

Mass (ES) m/z %: 488 (M+1, 100%).

HPLC: column Zorbax C8 MeCN 40%/H_2O (CF_3COOH pH 2.3) 60%, 1.0 mL/min; 20 min run; t_R = 11.748; area 96%.

HRMS: calcd for $C_{30}H_{37}N_3O_3 + H^+$, 488.29077; found (ESI, $[M+H]^+$ Obsd), 488.2907.

5.1.13. 2′-Methoxybiphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl}amide (7d)

5.1.13.1. 2′-Methoxy-biphenyl-4-carboxylic acid. To a solution of 4-carboxyphenylboronic acid (3.32 g, 20 mmol), Fibrecat®1007 (2 g) and potassium carbonate (3.03 g, 22 mmol) in ethanol/water (20 mL/20 mL), 1-bromo-2-methoxy-benzene was added (4.11 g, 22 mmol). The reaction mixture was heated to reflux for 3 h. After cooling, was filtered and the solution evaporated under reduced pressure. The residue was suspended in aq citric acid (10% w/v), filtered and washed with water and diethyl ether. The resulting solid was dried under vacuum to yield the title compound (4.02 g, 88%).

^{1}H NMR (DMSO-d_6) δ 3.79 (s, 3H), 7.08 (m, 2H), 7.34 (m, 2H), 7.58 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H).

5.1.13.2. 2′-Methoxybiphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl}amide. To a solution of 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]-butylamine hydrochloride (131 mg, 0.4 mmol) in DCM (5 mL) and DMF (5 mL), triethylamine (40 mg, 0.4 mmol) was added. After stirring the resulting mixture for 5 min, CDI (70 mg, 0.44 mmol) was added. The mixture was stirred for a further 1 h at room temperature and then 2′-methoxybiphenyl-4-carboxylic acid (92 mg, 0.4 mmol) was added. The resulting reaction mixture was left stirring for 40 h at room temperature. After evaporation of the solvent, water (5 mL) was added and the suspension sonicated for 20 min. The resulting suspension was filtered and washed with 0.4 M Na_2CO_3 aqueous solution, diethyl ether and dried under vacuum to yield the title compound (67.2 mg, 33%).

^{1}H NMR (DMSO-d_6) δ 1.55 (br s, 4H), 2.75 (br s, 2H), 2.93 (br s, 8H), 3.27 (br t, 2H), 3.64 (s, 3H), 3.68 (s, 3H), 3.69 (s, 3H), 6.41 (dd, J = 8.7, 2.6 Hz, 1H), 6.52 (d, J = 2.7 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.29–7.38 (m, 2H), 7.53 (d, J = 8.3 Hz, 2H), 7.86 (d, J = 8.3 Hz, 2H), 8.53 (s, 1H).

HRMS: calcd for $C_{30}H_{37}N_3O_4 + H^+$, 504.28568; found (ESI, $[M+H]^+$ Obsd), 504.2856.

5.1.14. Biphenyl-4-carboxylic acid {4-[4-(2,4-dimethoxyphenyl)-piperazin-1-yl]-butyl}amide (7e)

To a solution of 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]-butylamine hydrochloride (150 mg, 0.51 mmol) in DCM (10 mL), triethylamine (85 μL, 0.61 mmol), biphenylcarboxilic acid (133 mg, 0.61 mmol) and a catalytic amount of DMAP were added at 0 °C and left stirring for 10 min. The mixture was then stirred for 4 h at room temperature. Water (10 mL) was added, the layers separated and the crude dried over sodium sulphate before solvent removal and purification by flash chromatography ($CHCl_3$/MeOH 95:5) to afford 85 mg of title product.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.55–1.75 (m, 4H), 2.45–2.55 (br t, 2H), 2.60–2.65 (m, 4H), 2.90–3.0 (br s, 4H), 3.45–3.55 (m, 2H), 3.73 (s, 3H), 3.80 (s, 3H), 6.34 (dd, J = 8.6, 2.4 Hz, 1H), 6.45 (d, J = 2.4 Hz, 1H), 6.77 (d, J = 8.6 Hz, 1H), 6.92 (br t, 1H), 7.35–7.46 (m, 2H), 7.53–7.65 (m, 5H), 7.82 (d, J = 8.3 Hz, 2H).

Mass (M+1) e/z 474 (M+1, 100%); 496 ($M+Na^+$, 10%).

HRMS: calcd for $C_{29}H_{35}N_3O_3 + H^+$, 474.27512; found (ESI, $[M+H]^+$ Calcd), 474.2750.

5.1.15. 4-Bromo-N-{4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl}benzamide (7f)

Prepared from 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butylamine and 4-bromobenzoyl chloride according to general procedure 5.1.2. 60% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62–1.66 (br s, 4H), 2.39–2.45 (br t, 2H), 2.60–2.65 (m, 4H), 2.90–3.0 (m, 4H), 3.38–3.44 (m, 2H), 3.74 (s, 3H), 3.79 (s, 3H), 6.34–6.44 (m, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.92 (br t, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H).

HPLC: column Zorbax C8 4.6 × 150 mm; MeOH 80%/H_2O (CF_3COOH pH 6) 20%, 0.9 mL/min; 15 min run; t_R = 4.55; area 96%.

Mass (M+1) e/z 476/478 (M+1, 100%); 498/500 ($M+Na^+$, 25%).

HRMS: calcd for $C_{23}H_{30}BrN_3O_3 + H^+$, 476.15433; found (ESI, $[M+H]^+$ Obsd), 476.1543.

5.1.16. 4-Nitro-*N*-{4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butyl}benzamide (7g)

Prepared from 4-[4-(2,4-dimethoxyphenyl)piperazin-1-yl]butylamine and 4-nitrobenzoyl chloride according to general procedure 5.1.2. in 60% yield.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60–1.75 (br s, 4H), 2.39–2.45 (br t, 2H), 2.58–2.60 (m, 4H), 2.83–2.90 (m, 4H), 3.45–3.55 (m, 2H), 3.73 (s, 3H), 3.78 (s, 3H), 6.36 (dd, J = 8.7, 2.6 Hz, 6.41 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 1H), 7.28 (br t, 1H), 7.88 (d, J = 8.7 Hz, 2H), 8.21 (d, J = 8.7 Hz, 2H).

HPLC: column Zorbax C8 4.6 × 150 mm; MeOH 80%/H_2O (CF_3COOH pH 6) 20%, 1.0 mL/min; 10 min run; t_R = 3.05; area 99%.

Mass (M+1) e/z 444 (M+1, 100%); 465 ($M+Na^+$, 18%).

HRMS: calcd for $C_{23}H_{30}N_4O_5 + H^+$, 443.22890; found (ESI, $[M+H]^+$ Obsd), 443.2288.

5.1.17. 5-Piperidin-1-ylpentanoic acid (4-pyridin-3-ylphenyl)amide (14a)

5.1.17.1. 5-Piperidin-1-yl-pentanoic acid (4-bromophenyl) amide (14l). Obtained following general procedure 5.1.3. starting from 4-bromo-aniline, 5-bromovaleryl chloride and piperidine. The compound was purified by trituration from Et_2O/hexane 1:1 to obtain 5.1 g (yield 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (m, 10H); 2.42 (m, 2H), 2.54 (m, 5H), 2.92 (m, 1H), 7.41 (m, 2H), 7.48 (m, 2H), 8.01 (br s, 1H).

Mass (calculated) [339.27]; (found) [$M+H^+$] = [339/341].

LC t_R = 2.74 (purity 99%, 10 min method).

HRMS: calcd for $C_{16}H_{23}BrN_2O + H^+$, 339.10665; found (ESI, $[M+H]^+$ Obsd), 339.1065.

5.1.17.2. 5-Piperidin-1-ylpentanoic acid (4-pyridin-3-ylphenyl)amide. The title compound was obtained following the general procedure 5.1.8. starting from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide and pyridine-3-boronic acid. The crude was purified by flash-chromatography (DCM/MeOH 100/0 to 95/5), giving 1.04 g of title compound (47% yield).

Mass (calculated) [337]; (found) [$M+H^+$] = 338.

LC t_R = 0.35–1.39 (100%, 10 min method).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (m, 10H), 2.27 (m, 8H), 7.43 (br dd, J = 8.0, 4.8 Hz, 1H), 7.68 (m, 4H), 8.01 (ddd, J = 8.0, 2.4, 2.4 Hz, 1H), 8.50 (dd, J = 4.8, 1.6 Hz, 1H), 8.85 (d, J = 2.4 Hz, 1H), 10.00 (s, 1H).

HRMS: calcd for $C_{21}H_{27}N_3O + H^+$, 338.22269; found (ESI, $[M+H]^+$ Calcd), 338.2227.

5.1.18. 5-Morpholin-4-ylpentanoic acid (4-pyridin-3-ylphenyl) amide (14b)

5.1.18.1. 5-Morpholin-4-ylpentanoic acid (4-bromophenyl) amide. The title compound was obtained following general procedure 5.1.6. starting from morpholine and 5-bromovaleryl chloride. The reaction solution needed to be slightly heated at 50 °C for 3.5 h. The mixture was washed with brine, the organic layer was recovered and dried. The crude material was treated with ether/hexane (1/1) and the desired product was recovered by filtration (6.39 g, 93% yield).

Mass (calculated) [341.25]; (found) [$M+H^+$] = [341–343].

LC t_R = 2.50 (purity 100%, 10 min method).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (m, 2H), 1.59 (m, 2H), 2.31 (m, 8H), 3.55 (m, 4H), 7.44 (d, J = 8.89 Hz, 2H), 7.54 (d, J = 8.90 Hz, 2H), 10.00 (s, 1H).

5.1.18.2. 5-Morpholin-4-ylpentanoic acid (4-pyridin-3-ylphenyl) amide. The title compound was obtained following general procedure 5.1.8. starting from 5-morpholin-4-ylpentanoic acid (4-bromophenyl) amide and pyridine-3-boronic acid. The crude was purified by flash-chromatography (DCM/MeOH 100/0 to 95/5), giving 1.63 g of title compound (53% yield).

Mass (calculated) [339]; (found) [$M+H^+$] = 340.

LC t_R = 0.34 (100%, 10 min method).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (m, 2H), 1.60 (m, 2H), 2.31 (m, 8H), 3.54 (4H), 7.44 (m, 1H), 7.66 (d, J = 8.81 Hz, 2H), 7.71 (d, J = 8.83 Hz, 2H), 8.02 (m, 1H), 8.51 (m, 1H), 8.86 (m, 1H), 10.07 (s, 1H).

HRMS (calculated for $C_{20}H_{25}N_3O_2$) ($M+H^+$) 340.2020, found 340.2017.

5.1.19. 5-[4-(2-Methoxyphenyl)piperazin-1-yl]pentanoic acid (4-pyridin-3-ylphenyl) amide (14c)

Prepared in 0.25 mmol scale from 2-methoxyphenylpiperazine, 5-bromovaleryl chloride, 4-bromoaniline and 3-pyridylboronic acid following general procedures 5.1.3. and 5.1.8. Yield: 40%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.8–1.0 (m, 2H), 1.2–1.3 (m, 2H), 1.55–1.65 (m, 2H), 1.75–1.85 (m, 2H), 2.4–2.5 (m, 4H), 3.00–3.15 (m, 4), 3.85 (s, 3H), 6.8–7.1 (m, 4H), 7.35 (br t, 1H), 7.5–7.6 (m, 2H), 7.6–7.7 (m, 2H), 7.85–7.90 (m, 1H), 8.15–8.20 (m, 1H), 8.55–8.60 (m, 1H), 8.80–8.85 (m, 1H).

Mass (ES) *m*/*z* %: 445 (M+1, 100%).

HPLC: column Zorbax C8 MeOH 80%/H_2O 20%, 1.0 mL/min; 2.5 min run; t_R 1.23; area = 99%.

HRMS: calcd for $C_{27}H_{32}N_4O_2 + H^+$, 445.25980; found (ESI, $[M+H]^+$ Obsd), 445.2595.

5.1.20. 5-(4-Acetylpiperazin-1-yl)pentanoic acid (4-pyridin-3-ylphenyl) amide (14d)

5.1.20.1. 5-(4-Acetylpiperazin-1-yl)pentanoic acid (4-bromophenyl) amide. The title compound was obtained following general procedure 5.1.4. starting from *N*-acetylpiperazine and 5-bromovaleryl chloride. The reaction was stirred at room temperature for 40 h. The product was purified by flash chromatography (DCM/MeOH = 95:5) obtaining 4.0 g (yield 70%) of product.

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (m, 2H); 1.57 (m, 2H), 1.95 (s, 3H), 2.28 (m, 8H), 3.06 (m, 1H), 3.37 (m, 1H), 7.44 (m, 2H), 7.55 (m, 2H), 9.99 (s, 1H).

5.1.20.2. 5-(4-Acetylpiperazin-1-yl)pentanoic acid (4-pyridin-3-ylphenyl) amide. Prepared following general procedure 5.1.8. by reaction of 5-(4-acetylpiperazin-1-yl)pentanoic acid (4-bromophenyl) amide with 3-pyridylboronic acid. Yield: 35%.

Mass (ES) *m*/*z* %: 381 (M+1, 100%).

HPLC: column Zorbax C8 MeOH 80%/H_2O 20%, 1.0 mL/min; 2.5 min run; t_R 0.87; area = 93%.

5.1.21. 5-[Methyl-(2-pyridin-2-ylethyl)amino]pentanoic acid (4-pyridin-3-yl-phenyl) amide (14e)

Prepared in 0.25 mmol scale from methyl-(2-pyridin-2-ylethyl) amine, 5-bromovaleryl chloride, 4-bromoaniline and 3-pyridylboronic acid following general procedures 5.1.4. and 5.1.7 in 27% yield.

Mass (ES) *m*/*z* %: 389 (M+1, 100%).

HPLC: t_R 0.32–0.46; area = 100%.

HRMS: calcd for $C_{24}H_{28}N_4O + H^+$, 389.23359; found (ESI, $[M+H]^+$ Calcd), 389.2335.

5.1.22. 5-(4-Methylpiperazin-1-yl)pentanoic acid (4-pyridin-3-ylphenyl) amide (14f)

5.1.22.1. 5-(4-Methylpiperazin-1-yl)pentanoic acid (4-bromophenyl) amide. The title compound was obtained following general procedure 5.1.6. starting from *N*-methylpiperazine and 5-bromovaleryl chloride. The reaction was stirred at room temperature for 40 h. The product was precipitated by ether/hexane 1:1 obtaining 3.7 g (70% yield) of product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (m, 2H); 1.56 (m, 2H), 2.23 (s, 3H), 2.38 (m, 12H), 7.44 (m, 2H), 7.55 (m, 2H), 10.05 (s, 1H).

5.1.22.2. 5-(4-Methylpiperazin-1-yl)pentanoic acid (4-pyridin-3-ylphenyl) amide. Prepared following general procedure 5.1.8. in 30% yield.

Mass (ES) *m*/*z* %: 353 (M+1, 100%).

HPLC: Zorbax 2.5 min run; t_R 0.92; area = 100%.

HRMS: calcd for $C_{21}H_{28}N_4O + H^+$, 353.23359; found (ESI, $[M+H]^+$ Obsd), 353.2332.

5.1.23. 1-[4-(4-Pyridin-3-ylphenylcarbamoyl)butyl]piperidine-3-carboxylic acid diethylamide (14g)

5.1.23.1. 1-[4-(4-Bromophenylcarbamoyl)butyl]piperidine-3-carboxylic acid diethylamide. The title compound was obtained following general procedure 5.1.6. starting from piperidine-3-carboxylic acid diethylamide and 5-bromovaleryl chloride. Extraction with DCM/H_2O (pH 10) and flash-chromatography (DCM/MeOH 99/1 to 95/5) gave 2.7 g of title compound were obtained as solid by (60% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 1.11 (t, *J* = 7.10 Hz, 3H), 1.23 (t, *J* = 7.13 Hz, 3H), 1.85 (m, 8H), 2.48 (t, *J* = 6.74 Hz, 2H), 3.18 (m, 6H), 3.45 (m, 5H), 7.43 (d, *J* = 8.83 Hz, 2H), 7.52 (d, *J* = 8.87 Hz, 2H).

5.1.23.2. 1-[4-(4-Pyridin-3-ylphenylcarbamoyl)butyl]piperidine-3-carboxylic acid diethylamide. The title compound was obtained following general procedure 5.1.8. starting from 1-[4-(4-bromophenylcarbamoyl)butyl]piperidine-3-carboxylic acid diethylamide and pyridine-3-boronic acid. The crude was purified by SCX column followed by prep-HPLC, giving 56 mg of title compound as formate salt (55% yield).

Mass (calculated) [436]; (found) $[M+H^+]$ = 437; LC t_R = 1.75 (100%, 10 min method).

^{1}H NMR (400 MHz, CD_3OD) δ 1.11 (t, *J* = 7.11 Hz, 3H), 1.23 (t, *J* = 7.13 Hz), 1.91 (m, 9H), 2.51 (m, 4H), 3.14 (m, 7.60), 7.51 (m, 1H), 7.64 (m, 2H), 7.72 (m, 2H), 8.09 (m, 1H), 8.49 (m, 3H), 8.79 (m, 1H).

HRMS: calcd for $C_{26}H_{36}N_4O_2 + H^+$, 437.29110; found (ESI, $[M+H]^+$ Calcd), 437.2910.

5.1.24. 5-Azepan-1-ylpentanoic acid (4-pyridin-3-yl-phenyl) amide (14h)

5.1.24.1. 5-Azepan-1-yl-pentanoic acid (4-bromo-phenyl)-amide. The title compound was obtained following general procedure 5.1.6. starting from azepane and 5-bromovaleryl chloride. The mixture was stirred at 55 °C for further 24 h. The product was washed with Et_2O/hexane 1:1 obtaining 1.64 g (yield 93%) of product.

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (m, 2H); 1.54 (m, 10H), 2.29 (m, 2H), 2.58 (m, 6H), 7.44 (m, 2H), 7.55 (m, 2H), 9.99 (s, 1H).

5.1.24.2. 5-Azepan-1-ylpentanoic acid (4-pyridin-3-yl-phenyl) amide. The title compound was obtained following the general procedure 5.1.8. starting from 5-azepan-1-yl-pentanoic acid (4-bromo-phenyl)-amide and pyridine-3-boronic acid. The crude was purified by SCX column followed by prep-HPLC, giving 41 mg of title compound as formate salt (21% yield). Mass (calculated) [351]; (found) $[M+H^+]$ = 352; LC t_R = 1.52 (100%, 10 min method); ^{1}H NMR (400 MHz, DMSO-d_6): 1.47 (m, 12H), 2.30 (m, 3H), 2.37 (m, 1H), 2.48 (m, 1H), 2.60 (m, 3H), 7.39 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.64 (m, 4H), 7.96 (br d, *J* = 8.0 Hz, 1H), 8.21 (s, 1H), 8.46 (dd, *J* = 4.8, 1.6 Hz,1H), 8.80 (s, 1H), 9.98 (s, 1H).

HRMS: calcd for $C_{22}H_{29}N_3O + H^+$, 352.23834; found (ESI, $[M+H]^+$ Calcd), 352.2383.

5.1.25. 5-(4-Acetyl-[1,4]diazepan-1-yl)pentanoic acid (4-pyridin-3-yl-phenyl) amide (14i)

5.1.25.1. 5-(4-Acetyl-[1,4]diazepan-1-yl)-pentanoic acid (4-bromo-phenyl)-amide. The title compound was obtained following the general procedure 5.1.5. and starting from 5-bromovaleryl chloride and *N*-acetyl-homopiperazine. The reaction was stirred at room temperature for further 22 h and then at 50 °C for 2 h. The product was purified by flash chromatography (DCM/MeOH = 95:5) obtaining 6.0 g (75% yield) of title product.

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.57 (m, 2H); 1.68 (m, 2H), 1.82 (m, 1H), 1.90 (m, 1H), 2.09 (s, 3H), 2.38 (m, 2H), 2.53 (m, 4H), 2.69 (m, 2H), 3.58 (m, 4H), 7.42 (m, 2H), 7.50 (m, 2H).

5.1.25.2. 5-(4-Acetyl-[1,4]diazepan-1-yl)-pentanoic acid(4-pyridin-3-yl-phenyl)-amide formate salt. The title compound was obtained following the general procedure 5.1.7. starting from 5-(4-acetyl-[1,4]diazepan-1-yl)pentanoic acid (4-bromo-phenyl)-amide and pyridine-3-boronic acid. The crude was purified by SCX column followed by prep-HPLC, giving 31 mg of title compound as formate salt (15% yield).

Mass (calculated) [394]; (found) $[M+H^+]$ = 395.

LC t_R = 0.35–0.92 (100%, 10 min method).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54 (m, 8H), 1.91–1.92 (conformer double peak, 3H), 2.27 (m, 2H), 2.39 (m, 2H), 2.47 (m, 1H), 2.52 (m, 2H), 2.60 (m, 1H), 3.38 (m, 2H), 7.39 (ddd, J = 8.0, 4.8, 0.4 Hz, 1H), 7.61 (m, 2H), 7.66 (m, 2H), 7.97 (ddd, J = 8.0, 2.4, 1.6 Hz, 1H), 8.10 (s, 1H), 8.46 (dd, J = 8.0, 1.6 Hz, 1H), 8.80 (d, J = 2.4, 0.8 Hz, 1H), 9.96 (s, 1H). HRMS: calcd for $C_{23}H_{30}N_4O_2 + H^+$, 395.24415; found (ESI, $[M+H]^+$ Calcd), 395.2441.

5.1.26. 5-Piperidin-1-yl-pentanoic acid (2′,4′-difluorobiphenyl-4-yl) amide (14j)

Prepared using general method 5.1.7. from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide (5.1.17.1) and 2,4-difluorophenylboronic acid in 30% yield.

Mass (ES) m/z %: 373 (M+1, 100%).

HPLC: Zorbax 2.5 min run; t_R 1.74; area = 100%.

HRMS: calcd for $C_{22}H_{26}F_2N_2O + H^+$, 373.20859; found (ESI, $[M+H]^+$ Obsd), 373.2087.

5.1.27. 5-Piperidin-1-yl-pentanoic acid (2′-isopropoxybiphenyl-4-yl) amide (14k)

Prepared using general method 5.1.7. from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide (5.1.17.1) and 2-*iso*-propoxyphenylboronic acid in 35% yield.

Mass (ES) m/z %: 395 (M+1, 100%).

HPLC 10 min run; t_R 3.64; area = 100%.

HRMS: calcd for $C_{25}H_{34}N_2O_2 + H^+$, 395.26930; found (ESI, $[M+H]^+$ Calcd), 395.2692.

5.1.28. 5-Piperidin-1-yl-pentanoic acid quinolin-6-ylamide (14m)

6-Aminoquinoline (0.6 mmol, 86 mg) and triethylamine (0.6 mmol, 60 mg) were dissolved in dimethylformamide (0.5 mL) and 5-bromovaleryl chloride (113 mg, 0.57 mmol) in dimethylformamide (0.5 mL) was added drop wise. After 1 h 30 min, piperidine (153 mg, 1.8 mmol) and triethylamine (60 mg, 0.6 mmol) in dimethylformamide (0.5 mL) were added and the reaction mixture heated at 55 °C for 4 h. Work-up followed by preparative HPLC affords the title compound (40 mg, 23% yield) as a white solid as formate salt.

Mass (calculated) [311]; (found) $[M+H^+]$ = 312; 156 (M+2)/2.

LC t_R = 0.38 (100%, 10 min method).

HRMS: calcd for $C_{19}H_{25}N_3O + H^+$, 312.20704; found (ESI, $[M+H]^+$ Calcd), 312.2070.

5.1.29. 5-Piperidin-1-yl-pentanoic acid benzo[1,3]dioxol-5-ylamide (14n)

Benzo[1,3]dioxol-5-ylamine (0.6 mmol, 82 mg) and triethylamine (0.6 mmol, 60 mg) were dissolved in dimethylformamide (0.5 mL) and 5-bromovaleryl chloride (113 mg, 0.57 mmol) in dimethylformamide (0.5 mL) was added drop wise. After 1 h 30 min, piperidine (153 mg, 1.8 mmol) and triethylamine (60 mg, 0.6 mmol) in dimethylformamide (0.5 mL) were added and the reaction mixture heated at 55 °C for 4 h. Work-up followed by preparative HPLC affords the title compound (30 mg, 17% yield) as a white solid as formate salt.

Mass (calculated) [311]; (found) $[M+H^+]$ = 305.

LC t_R = 1.24 (96%, 10 min method).

HRMS: calcd for $C_{17}H_{24}N_2O_3 + H^+$, 305.18597; found (ESI, $[M+H]^+$ Calcd), 305.1859.

5.1.30. 5-Piperidin-1-yl-pentanoic acid *m*-tolylamide (14o)

3-Methylaniline (0.6 mmol, 64 mg) and triethylamine (0.6 mmol, 60 mg) were dissolved in dimethylformamide (0.5 mL) and 5-bromovaleryl chloride (113 mg, 0.57 mmol) in dimethylformamide (0.5 mL) was added drop wise. After 1 h 30 min, piperidine (153 mg, 1.8 mmol) and triethylamine (60 mg, 0.6 mmol) in dimethylformamide (0.5 mL) were added and the reaction mixture heated at 55 °C for 4 h. Work-up followed by preparative HPLC affords the title compound (74 mg, 47% yield) as a white solid as formate salt.

Mass (calculated) [274]; (found) $[M+H^+]$ = 275.

LC t_R = 1.24 (100%, 10 min method).

HRMS: calcd for $C_{17}H_{26}N_2O + H^+$, 275.21179; found (ESI, $[M+H]^+$ Obsd), 275.2118.

5.1.31. 4′-(5-Piperidin-1-yl-pentanoylamino) biphenyl-3-carboxylic acid amide (14p)

Prepared using general method 5.1.7. from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide (5.1.17.1) and 3-(aminocarbonyl)phenylboronic acid in 15% yield.

Mass (ES) m/z %: 380 (M+1, 100%).

LC t_R = 2.41 (99%, 10 min method).

HRMS: calcd for $C_{23}H_{29}N_3O_2 + H^+$, 380.23325; found (ESI, $[M+H]^+$ Obsd), 380.2332.

5.1.32. 5-Piperidin-1-ylpentanoic acid (3′-acetylbiphenyl-4-yl) amide (14q)

Prepared using general method 5.1.7. from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide (5.1.17.1) and 3-acetylphenylboronic acid in 17% yield.

Mass (ES) m/z %: 379 (M+1, 100%).

LC t_R = 2.41 (100%, 10 min method).

HRMS: calcd for $C_{24}H_{30}N_2O_2 + H^+$, 379.23800; found (ESI, $[M+H]^+$ Obsd), 379.2380.

5.1.33. 5-Piperidin-1-ylpentanoic acid (3′-acetylaminobiphenyl-4-yl) amide (14r)

Prepared using general method 5.1.7. from 5-piperidin-1-ylpentanoic acid (4-bromophenyl) amide (5.1.17.1) and 3-(acetylamino)phenylboronic acid in 15% yield.

Mass (ES) m/z %: 394 (M+1, 100%).

LC t_R = 2.63 (92%, 10 min method).

HRMS: calcd for $C_{24}H_{31}N_3O_2 + H^+$, 394.24890; found (ESI, $[M+H]^+$ Obsd), 394.2489.

5.1.34. 5-Piperidin-1-yl-pentanoic acid (3-bromophenyl)amide (14s)

Prepared from 3-bromoaniline and 5-bromovaleryl chloride according to general procedure 5.1.6. to give 1.7 g (33%) of the title compound.

$C_{16}H_{23}N_2OBr$ Mass (calculated) [339.28]; found [M+H+] = 339/341 (Br), LC t_R = 1.86, (98%, 10 min method).

NMR (400 MHz, DMSO-d_6): 1.51–1.64 (m, 10H); 2.34 (m, 2H); 2.23 (m, 2H); 2.76 (m, 4H); 2.97 (m, 2H); 7.12–7.264 (m, 2H); 7.48 (br d, J = 8 Hz, 2H); 7.97 (s, 1H).

5.1.35. Ca^{2+}-flux and membrane potential measurements with a FLIPR™ system

The following recombinant cell lines were used as specific sources of receptors: GH4C1 cells stably transfected with pCEP4/rat α7 nAChR as previously described,[11] HEK293 cell lines stably expressing human 5-HT_{3A} receptors.[11] Native neuroblastoma SH-SY5Y cells were used as source of human ganglionic nAChRs (α3) and TE671 rhabdomyosarcoma cells were used as endogenous source of muscle α1β1δγ receptors. GH4C1 cells expressing α7 and HEK cells expressing 5-HT_{3A} receptors were analyzed by Ca^{2+}-flux measurements employing a Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices) system, whereas the cells expressing the nicotinic receptors subunits α1 and α3 were tested in the FLIPR system with a membrane potential sensitive dye.

For Ca^{2+}-flux analysis, cells were plated in 96-well clear-bottom, poly D-lysine coated black microtiter plates (Costar) at a density of 1×10^5 cells/well for α7 expressing GH4C1 cells or 8×10^4 cells/well for 5-HT_{3A} expressing HEK293 cells and cultured for 24 h prior to experiments. The medium was then replaced with 100 μL of Hank's Balanced Salt Solution-HEPES 20 mM, pH 7.4 (assay buffer) containing 4 μM Fluo-4-AM, 0.02% pluronic acid and 5 mM probenecid. After 40 min of incubation at 37 °C the labeling solution was replaced with 200 μL assay buffer containing 2.5 mM probenecid. Plates were then transferred to the FLIPR system. Compounds to be tested were prepared in assay buffer as 5x-concentrated solutions in a separate 96-well polypropylene plate. Basal fluorescence was recorded for 30 s, followed by addition of 50 μL of test compound (to assess agonist activity; first addition). Measurements were made at 1 s intervals for 1 min, followed by measurements every 30 s for 10 min. Subsequently, for the second addition for alpha 7 expressing GH4C1 cells, nicotine (10 μM final concentration) was added to each well except negative controls (to asses positive modulation and antagonism of nicotine response). For testing 5-HT_{3A} receptors CPBG (1 μM final concentration) was added to each well except negative controls. Measurements were made at 1 s intervals for 1 min after the second addition and at 3 s intervals for the remaining 3 min. Results were exported from the FLIPR raw data as MAX–MIN of fluorescence signal intensity in two intervals corresponding to the first and the second addition of compounds. The responses were normalized to the positive control and EC_{50} and IC_{50} values were calculated using Xlfit version 4.2, with a sigmoidal concentration–response (variable slope) equation: $Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + (EC_{50}/X)^{\text{Hill Slope}})$, where X is the concentration, Y is the response, Bottom is the bottom plateau of the curve and Top the top plateau. Assay performance was robust as reflected by a Z' factor >0.6.

Activity of compounds at the muscle and ganglionic type nAChR receptors was determined using a membrane potential sensitive fluorescent dye. TE671 and SHSY5Y cells were plated at a density of 5×10^4 cells/well 24 h prior to assay. Growth media were removed from the cells by flicking the plates and membrane potential dye (Molecular Devices), reconstituted in HBSS five times more diluted compared to the manufacturer's instructions, was added to the wells. Plates were incubated for 60 min at rt and then directly transferred to the FLIPR system. Compounds to be tested were prepared in assay buffer as 5×-concentrated solutions in a separate 96-well polypropylene plate. Baseline fluorescence was monitored for the first 10 s followed by the addition of compounds. For detection of antagonists or positive allosteric modulator activity, agonist (epibatidine; 1 μM final concentration) was added to every well except negative controls. Signal recordings were performed as above. Results were exported from the FLIPR raw data as SUM of fluorescence signal intensity for the first addition and MAX–MIN for the second addition of compounds. The responses were normalized to the positive control (epibatidine 1 μM final concentration) and EC_{50} values were calculated as described before.

5.1.36. Electrophysiology

GH4C1 cells stably expressing rat α7 nAChR were treated with 0.5 mM Na-butyrate added to the medium for two days before patch clamp recordings. Patch pipettes had resistances of ~7 MΩ when filled with (in mM): 5 EGTA, 120 K-Gluconate, 5 KCl, 10 HEPES, 5 K_2ATP, 5 Na_2-phosphocreatine, 1 $CaCl_2$ and 2 $MgCl_2$. Cells were voltage-clamped at −60 mV with a HEKA EPC-9 amplifier. To measure the fast activation and desensitization of α7 current, the Dynaflow (Cellectricon) fast perfusion system with 16- or 48-well chips was used. Different concentrations of acetylcholine or SEN12333/WAY-317538 were applied to cells in between washes with bath solution (Hanks' Balanced Salt Solution + 10 mM HEPES). Data were acquired at 1 kHz for 2-s episodes (500 ms bath, 500 ms agonist, 1000 ms wash) with a 10-s interval between episodes. Peak current amplitude and total charge (area under the curve) were measured with the HEKA Pulse program. Concentration–response curves and EC_{50} values were plotted and calculated with Origin (MicroCal).

5.1.37. Radioligand binding assay

[^{3}H]-epibatidine binding studies were performed as previously described (Dunlop et al., 2007). Briefly, cell membrane preparations derived from GH4C1 cells stably expressing rat α7 nAChRs were suspended in binding buffer (50 mM HEPES, pH 7.4, 3 mM KCl, 70 mM NaCl, 10 mM $MgCl_2$), 5 nM [^{3}H]-epibatidine (GE Healthcare; S.A. = 53 Ci/mmol) and SEN12333/WAY-317538 to achieve a final volume of 200 μL in a 96-well polypropylene plate. Nicotine at 300 μM was used for determination of nonspecific binding. Following incubation at room temperature (23 °C) for 1 h, samples were rapidly filtered through Unifilter GF/B filters using a Filtermate (Perkin–Elmer) and washed five times with ice-cold binding buffer. Samples were processed and counted for radioactivity using a TopCount NXT (Perkin–Elmer). Competition binding curves were fitted with a four parameter logistic model. K_i values were calculated by the Cheng–Prusoff equation using the GraphPad Prism software package.

5.1.38. Receptor selectivity and hERG activity

Interaction of SEN12333/WAY-317538 with ~70 binding sites including all major classes of neurotransmitter, growth factor and peptide receptors (Novascreen, Caliper Biosciences, Hopkinton MA) was examined at 10 μM concentration. Activity at Histamine-3 receptor was determined in a recombinant HEK293 cell line stably expressing the receptor. Activity at hERG ion channel was determined employing CHO cells stably expressing the channel and an IonWorks recording system.

5.1.39. Pharmacokinetics

Male Wistar rats (age of 6–8 weeks, body weight 200–250 g) were administered a single dose of 5 mg/kg iv or 10 mg/kg ip of SEN12333/WAY-317538 at time 0. Levels in plasma were determined over a time period of 8 h in the iv study and in plasma and brain at 0, 0.5, 1 and 3 h. Concentration of SEN12333/WAY-317538 in rat brain and plasma was measured by high performance liquid chromatography in combination with mass spectrometry (LC–MS/MS) with a limit of detection of 1 ng/mL in plasma and 10 ng/g in brain. Plasma samples were prepared by protein precipitation with acetonitrile containing 250 ng/mL of internal standard, centrifugation and analysis of the supernatant by LC–MS/MS. Brain samples were prepared by homogenization and extraction with methanol. The homogenates were subsequently centrifuged, and the supernatant was analyzed by LC–MS/MS. Quantification was performed in a similar manner to the plasma samples.

5.1.40. Object recognition test

Object recognition was evaluated as described previously[14] employing different amnesic factors. Scopolamine (0.2 mg/kg ip) and MK-801 (0.03 mg/kg, ip) induced amnesia was provoked 30 min before acquisition trials, whereas spontaneous decay of memory was induced by the time delay after first experience (24 h time-delay). SEN12333/WAY-317538 (1–10 mg/kg) was administered 30 min prior to the acquisition trial to determine whether SEN12333/WAY-317538 could reverse the cognitive impairments produced by the amnesic factor. In brief, for scopolamine and time delay protocols, Wistar rats (3 month old, ca. 250 g weight) were placed in a grey polyvinylchloride arena ($60 \times 60 \times 40$ h cm) illuminated by a 50 W lamp suspended 50 cm above the arena. The objects to be discriminated were prisms, pyramids and cylinders made of plastic. The day before testing, rats were habituated to the arena for 2 min. In the scopolamine-induced amnesia, a session of 2 trials separated by an inter-trial interval of 240 min was carried out. In the first trial (acquisition trial, T1) two identical objects were presented in two opposite corners of the arena. The rats were left in the arena until criterion of 20 s of total exploration of the objects was reached. Exploration was defined as directing the nose at a distance <2 cm to the object and/or touching it with the nose. During the second trial (retention trial, T2) one of the objects presented in T1 was replaced by a novel object and rats were left in the arena for 5 min. The time spent exploring the familiar (F) and the novel object (N) were recorded separately and the difference between the two exploration times was taken. From one rat to the next, care was taken to avoid object and place preference by randomly changing the role of the objects (familiar or new object) and their position in the two opposite corners of the box during T2. In the time delay procedure, T2 was performed 24 h after T1 when a spontaneous decay of memory was present in control rats. Student paired *T*-test was used to evaluated differences between exploration time. MK-801 induced novel object recognition deficit was evaluated in a procedure similar to that used for the scopolamine induced deficit. Long Evans rats (3 month old, ca. 250 g weight) were tested in a circular field (diameter ~70 cm, 30 cm high), surrounded by black curtains masking extra-field cues, located in a dimly lit room (~10 lux at the level of the area) in the presence of white noise (~65 dB). Animal performance was tracked by video using Noldus Ethovision XT software. Objects, constructed with Duplo (Lego), were placed on the arena floor in one of four locations spaced evenly around the field approximately 10 cm from the field's edge. Rats were habituated to the arena which contained two identical yellow cubes for a period of 15 min 1 day prior to the T1 and T2 sessions. Following T1 rats were returned to their home cages for a 1 h retention interval and then tested in the T2 trial for recognition memory. T2 consisted of a 5 min exploration of the field containing both a familiar, previously explored, object and a novel object. The location of the objects, counterbalanced across treatment groups, remained constant for each animal during the habituation, T1 and T2 trials. The effect of treatment on object exploration during the retention trial was examined using a one-way ANOVA on total contact time followed by Fisher's LSD group mean pairwise comparisons. The amount of time exploring the novel and familiar objects across treatment groups was analyzed using repeated measures ANOVA followed by Fisher's LSD post hoc comparisons.

Acknowledgements

The authors would like to thank Eva Genesio and Ugo Zanelli for their support in the interpretation of analytical and pharmacokinetic data, respectively.

References and notes

1. Lindstrom, J. *Ion Channels* **1996**, *4*, 377.
2. Newhouse, P.; Singh, A.; Potter, A. *Curr. Top. Med. Chem.* **2004**, *4*, 267.
3. Gotti, C.; Riganti, L.; Vailati, S.; Clementi, F. *Curr. Pharm. Des.* **2006**, *12*, 407.
4. Acker, B. A. et al *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3611.
5. Pichat, P. et al *Neuropsychopharmacology* **2007**, *32*, 17.
6. Lippiello, P. M.; Bencherif, M.; Hauser, T. A.; Jordan, K. G.; Letchworth, S. R.; Mazurov, A. A. *Curr. Top. Med. Chem.* **2004**, *4*, 267.
7. Freedman, R.; Adams, C. E.; Leonard, S. *J. Chem. Neuroanat.* **2000**, *20*, 299.
8. Kem, W. R. *Behav. Brain Res.* **2000**, *113*, 169.
9. Tietje, K. R. et al *CNS Neurosci. Ther.* **2008**, *14*, 65.
10. Gaviraghi, G.; Ghiron, C.; Bothmann, H.; Roncarati, R.; Terstappen, G. C. PCT Int. Appl., WO2006008133A2, 2006.
11. Dunlop, J.; Roncarati, R.; Jow, B.; Bothmann, H.; Lock, T.; Kowal, D.; Bowlby, M.; Terstappen, G. C. *Biochem. Pharmacol.* **2007**, *74*, 1172.
12. Roncarati, R. et al. *J. Pharm. Exp. Ther*. **2009**, *829*, 459.
13. Still, W. C. et al *J. Org. Chem.* **1978**, *14*, 2923.
14. Scali, C.; Giovannini, M. G.; Prosperi, C.; Bartolini, L.; Pepeux, G. *Pharmacol. Res.* **1997**, *36*, 463.
15. Leadbeater, N. E.; Marco, M. *Org. Lett.* **2002**, *4*, 2973.