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Parallel synthesis of a series of potentially brain penetrant aminoalkyl benzoimidazoles

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Abstract—Alpha7 agonists were identified via GOLD (CCDC) docking in the putative agonist binding site of an alpha7 homology model and a series of aminoalkyl benzoimidazoles was synthesised to obtain potentially brain penetrant drugs. The array was prepared starting from the reaction of *ortho*-fluoronitrobenzenes with a selection of diamines, followed by reduction of the nitro group to obtain a series of monoalkylated phenylene diamines. *N*,*N*′-Carbonyldiimidazole (CDI) mediated acylation, followed by a parallel automated work-up procedure, afforded the monoacylated phenylenediamines which were cyclised under acidic conditions. Parallel work-up and purification afforded the array products in good yields and purities with a robust parallel methodology which will be useful for other libraries. Screening for alpha7 activity revealed compounds with agonist activity for the receptor.

1. Introduction

An extensive number of studies have demonstrated the important role of nicotinic acetylcholine receptors within the CNS and their role in processes such as memory, cognition, sensory gating and anxiety.[1] Evidence of the association between cholinergic deficit and cognitive impairment in patients with Alzheimer's disease (AD) has led to much research into possible therapies designed to replace loss of function of these receptors.[2] Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels consisting of alpha (2–10) and beta (2–4) subunits in various combinations. Specifically, the alpha7 and alpha4beta2 subtypes are found in abundance in the CNS, suggesting their importance in a variety of biological processes.[3] In the present report, we focus on the challenge to find novel alpha7 nicotinic receptor agonists showing affinity for these brain nAChRs.[4]

A reoccurring issue for medicinal chemists within the pharmaceutical industry is the need for fast identification of novel compounds. One of the key strategies to address this demand has led to the development and use of parallel and automated procedures to not only speed up and simplify the synthesis of potential drugs, but also to reduce company costs. Virtual screening technologies, such as docking and pharmacophore screening, also provide an expeditious route to the identification of molecules ready to enter hit to lead optimisation. Herein we describe how docking experiments of commercially available compounds were carried out utilising GOLD software[5] on an alpha7 homology model. After the initial virtual screening, visual inspection of the results allowed the manual selection of compounds for purchasing. These compounds were assayed in an alpha7 FLIPR assay. The assay displayed three compounds as the most promising (Table 1). Even if compounds **1** and **2** were more active (0.6 and 0.2 μM, respectively), compound **3** was selected as the starting point for Medicinal Chemistry exploration. The choice was dictated by analysis of the literature around the structures of the three compounds. The quinuclidine core of compound **1** and tropane of compound **2** are already widely described for alpha7 nAChR agonists.[6–8] In addition, the same scaffolds often play a key role in the binding to other receptors such as 5-HT_3, muscarinic subtypes and Serotonin Reuptake transporters.[8–10] An initial library was therefore enumerated around compound **3**. The virtual set of compounds so obtained were analysed for drug-like properties using the Accord SDK

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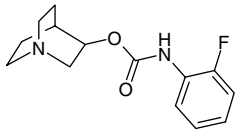
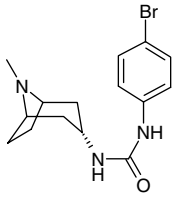
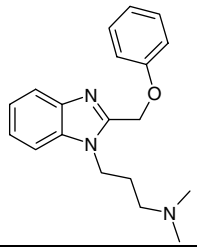
Table 1. Virtual hits tested in concentration-response analysis in Ca-flux assay

Compound	Structure	MW	*A* log *P*	FPSA	Alpha7 nAChR EC_{50} (μM)
1		264	2	42	0.6
2		338	2.1	46	0.2
3		309	3.8	29	2.6

from Accelrys.[11] The criteria for substituent selection were based on modified Lipinski, Veber and Norinder rules (Table 2)[12] and evaluated using Spotfire visualisation.[13] An array of molecules, which fulfilled the selection criteria for about 80% of its components and suitably represented the chemical space covered by the virtual array, was chosen for synthesis. Synthetic validation was undertaken with consideration to library expansion employing automation tools. It was fundamental that the synthetic procedures were optimised with regard to the capabilities of the automation platform to be used, for example, tube size, reaction volumes, block format, etc., to prevent any problems being encountered downstream.

Benzoimidazole derivatives are often exploited in drug discovery programmes[14] and the intention of this study was also to find a robust process for the synthesis of this chemotype, not only for the project in hand but also for future compound library generation.

2. Methods

2.1. Docking calculations

The alpha7 nicotinic acetylcholine receptor (nAChR) is composed of five homologous subunits positioned symmetrically around an axis perpendicular to the cell membrane. Each subunit consists of an extracellular domain and a transmembrane domain.[15] The agonist binding site is found at the interface between two adjacent subunits in the extracellular domain. The alpha7 X-ray crystal structure has not been determined to date, but the research groups of Le Novere[16] and Schapiro[17] have recently developed homology models of alpha7, based on the structure of the highly homologous acetyl choline binding protein (AChBP).[18] For our docking simulations, we used a previously published homology structure of chicken alpha7, containing a docked nicotine molecule in the agonist binding site located at the interface of two subunits.[19] Nicotine was then eliminated from the pdb file. The active site was defined by residues Tyr91, Trp147, Thr148, Tyr186, Cys188, Cys189 and Tyr193 from the alpha subunit and Met36, Trp53, Asn105, Gln115 and Leu117 from the beta subunit. Sdf files for commercially available compounds were directly obtained from Maybridge and Specs.[20] Filters were applied in order to identify hits with a physicochemical property profile compatible with CNS penetration. In particular, all compounds complied with our internal criteria (Table 2). The molecules were then minimised using Corina[21] and protonated at pH 7 using LigPrep.[22] Commercial compounds' databases were docked utilising Gold software.[5] A prerequisite for virtual hit selection was the presence of a protonated nitrogen contributing to binding via a hydrogen bond with the carbonyl side chain of Trp147 or via a cation-pi interaction with the aromatic cage composed of Trp53, Trp147, Tyr186 and Tyr193.[23] Virtual hits were then selected manually based on highest GoldScore fitness values, after visual inspection to ensure optimal geometries for putative binding conformations.

Selected compounds were then purchased from commercially available vendors and screened in an alpha7 FLIPR assay. Given the nature of the FLIPR assay, we identified compound showing both agonist and antagonist properties. The overall hit-rate was

Table 2. Physicochemical properties-criteria for compound selection

Parameter	Selection range
MW	<400
FPSA	<70
*C*log *P*	<4.4
Hydrogen bond donors	0–2
Hydrogen bond acceptors	<7
Carboxylic acids	0

around 3%. Data for selected agonists are reported in Table 1.

Compounds **1** and **2** are analogues of compounds reported to be alpha7 agonists and therefore were deprioritised. To the best our knowledge, benzoimidazole **3** was a novel agonist of alpha7 and was therefore selected for further investigation.

In the putative binding conformation of benzoimidazole **3** (Fig. 1), the protonated nitrogen of the dimethylaminopropyl side chain makes a hydrogen bond with the carbonyl of Trp147. The benzoimidazole ring makes hydrophobic interactions with Trp53 and Tyr193. The oxygen atom of the phenoxy methyl moiety is within H-bond distance from the OH of Tyr193. The phenyl ring experiences a stacking interaction with the same residue. In light of these considerations four main variation points of the hit were identified for scaffold exploration (Fig. 2). The strategy essentially consisted in the modification of four main portions of the hit molecule: different substituents on the benzoimidazole ring (A), various aryl moieties (B), two kinds of linker (C) and a few amino groups (D). Considering the importance of the protonated nitrogen in the binding to nicotinic receptors, several amines with different basicity and steric hindrance were evaluated. An amidic moiety was also introduced in order to confirm the necessity of a basic nitrogen also in this class of molecules.

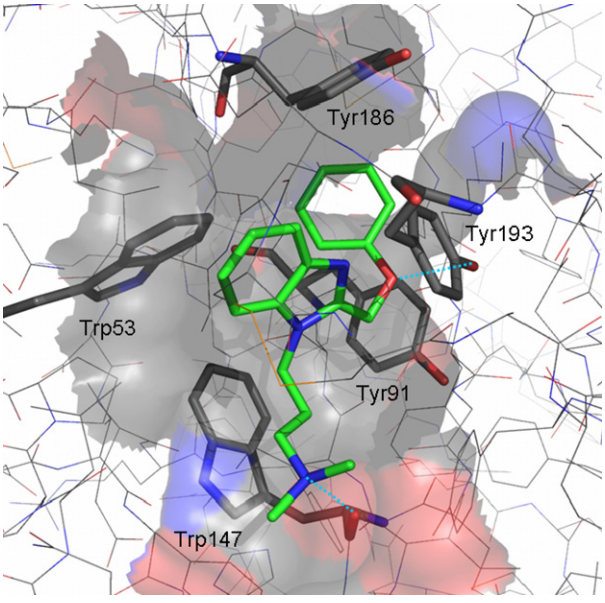


Figure 1. Putative binding conformation of compound 3 with homology structure of chicken alpha7. The protonated nitrogen of the dimethylaminopropyl side chain makes a hydrogen bond with the carbonyl of Trp147. The benzoimidazole ring makes hydrophobic interactions with Trp53 and Tyr193. The oxygen atom of the phenoxy methyl moiety is within H-bond distance from the OH of Tyr193. The phenyl ring experiences a stacking interaction with the same residue.

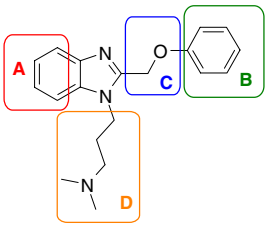


Figure 2. Possible variation points of the hit compound.

2.2. Chemistry

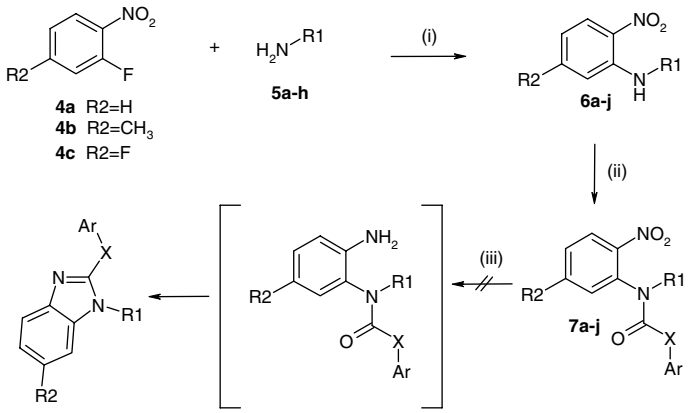
Two synthetic routes to the benzoimidazole series were explored. The first, depicted in Scheme 1, involved the preparation of intermediates **6** following a reported nucleophilic substitution procedure of *ortho*-fluoronitrobenzenes **4a–c** with the designated diamines **5a–h**.[24]

Optimisation of the work-up allowed the synthesis to be carried out using a parallel approach without chromatographic purification and afforded the desired intermediates in medium to high yield (Table 3).

Monoacylation of compounds **6a–j** with in situ generated acid chlorides, followed by cyclisation-reduction of the nitro-derivatives **7**, was attempted in a one-pot approach (Scheme 1). Unfortunately, complete amide hydrolysis was observed, due to the strongly acidic conditions resulting from the use of tin (II) chloride as a reducing agent.

The second route (Scheme 2) employed the tin(II) chloride reduction of the nitro group on intermediates (**6a–j**) to afford the corresponding anilines,[25] resulting in high purity products and quantitative yields (**8a–j**). At this point, small trials could be performed to investigate the cyclisation step further. In the first trial, direct acylation-cyclisation in 37% HCl was tested.[26] A small set of carboxylic acids was used to verify the ring closure conditions' reliability. 4-Bromophenylacetic acid gave complete conversion to the corresponding benzoimidazole, but the conditions did not appear to be suitable for the other substrates (Table 4).

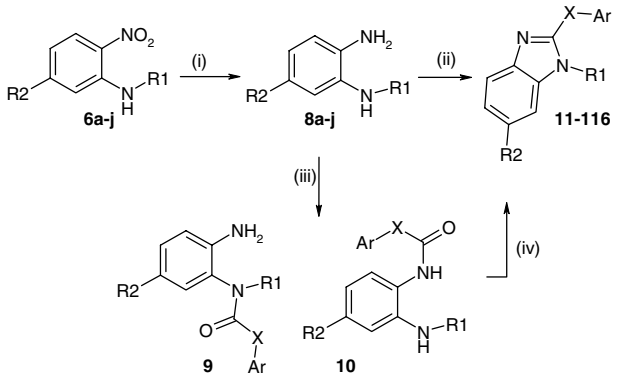
In a further attempt (Scheme 1) a 2-step approach was employed, consisting of monoacylation of phenylenediamine (affording **9** and/or **10**) followed by cyclisation under acidic conditions.[27] Activation of the carboxylic acids using oxalyl chloride was first tested, resulting in an undesired mixture of mono- and diacylated products. Further validation using milder activating agents was therefore required to broaden the scope of the benzoimidazole synthesis to a variety of substitutions on the aromatic ring. Mild coupling agents were explored and it was found that when HATU, PyBOP and CDI were used, complete conversion to a mixture of the two pos-



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, 70 °C; (ii) $(COCl)_2$, ArXCOOH, DCM, rt; (iii) $SnCl_2$, EtOH, reflux.

Table 3. Structure and yields of the intermediates **6a–j**

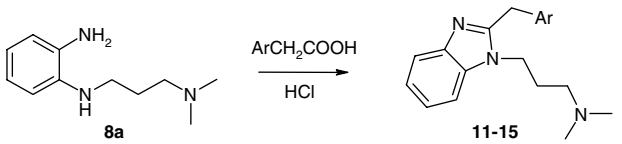
Compound	Fluoronitro benzenes	R_1NH_2	Yield
6a	**4a**	**5a**	78%
6b	**4a**	**5b**	92%
6c	**4a**	**5c**	84%
6d	**4a**	**5d**	89%
6e	**4a**	**5e**	74%
6f	**4a**	**5f**	93%
6g	**4a**	**5g**	57%
6h	**4a**	**5h**	88%
6i	**4b**	**5a**	77%
6j	**4c**	**5a**	65%

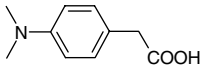
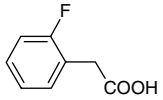
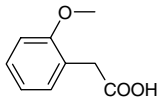
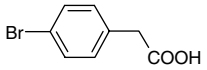
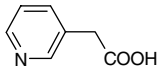


Scheme 2. Reagents and conditions: (i) $SnCl_2$, EtOH, reflux; (ii) ArXCOOH, HCl 37%, reflux; (iii) ArXCOOH, coupling agents; (iv) glacial acetic acid, 80 °C.

sible monoacylated derivatives was achieved. However, this was not the case where EDC was used; in this instance, LC–MS analysis of the reaction suggested the formation of a stable intermediate with the acid, reasoned to be the rearrangement *N*-acylurea product. Overall, it was observed that the reactions using CDI activation procedure gave the cleanest profile by LC–MS analysis. For this reason, for the lower cost of this coupling agent and for the ease of removal of its byproducts, CDI was finally selected for array production. The crude intermediates obtained in the monoacy-

Table 4. Cyclisation conversions using HCl 37%



Compound	$ArCH_2COOH$	Conversion (%)
11		10
12		25
13		10
14		100
15		30

lation trials were then subjected to cyclisation in glacial acetic acid to validate the final step. This proceeded successfully with clean conversion to the desired benzoimidazoles. With the optimised conditions a library of 96 compounds was prepared, exploiting automation technology to assist in the combinatorial amide formation step. In Table 5 structures and reaction yields of the library are reported. A robust work-up procedure using automation was also performed in order to have the most efficient parallel procedure. All the reported automation steps described in this paper were carried out using a Zinsser 'Speedy' liquid handling platform.[28] In the optimised procedure, when amide coupling completion was observed, the reaction blocks were moved to the automation platform for work-up, and a stock solution of sodium hydroxide was dispensed across the vials containing the reaction mixture in dichloromethane. The vials were moved to a Zinsser shaker station for faster mixing and the samples recovered via probe aspiration/dispensing action. Therefore, the samples were aspirated from the bottom of the vials and transferred to phase separators equipped with collection blocks, also located on the platform bed.

During the preproduction phase it became evident that highly hindered phenylenediamines (such as **8g**) were not reactive under the previously validated acylation and cyclisation conditions. The preparation of compounds **83**–**94** required further validation. Acylation difficulties could be related to steric hindrance of the secondary amino group (Scheme 3). This problem was overcome by performing the reaction in DMF at 80 °C with 2 equiv of the designated carboxylic acids as a library 'subset'. The cyclisation step was achievable only under microwave irradiation conditions at 140 °C, again probably due to steric hindrance. Products **83**–**94** were then purified by Si-column or HPLC-prep.

In the case where ring-substituted phenylenediamines were utilised, it was noted that after successful monoacylation, the cyclisation step proved difficult. The major reaction impurity was found to be the acetylated starting material (Scheme 4), suggesting that reaction with solvent was favoured over ring closure. Due to these reactivity problems only a smaller set of compounds was synthesised (Table 6). Where enough product was present by LC–MS crude analysis, Si-column purification was attempted and compounds passing internal purity requirements were submitted for screening.

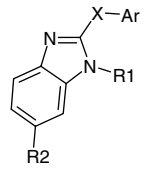
3. Results and conclusions

Ninety-three out of 106 potentially brain penetrant benzoimidazole compounds were successfully synthesised to a yield and purity sufficient for internal compound registration (over 90% purity by LC–MS at 215 nm with no single impurity above 5%). Overall, isolated yields were in the range of 15–60% allowing for sufficient compound to be delivered to both screening and archive. The 93 so-prepared compounds were initially tested at single concentration (5 μM) in a cellular assay based on GH4C1 cells stably expressing the rat alpha7-nAChR using the FLuorometric Imaging Plate Reader (FLIPR).[29] The FLIPR methodology allows the measurements of real time Ca^{2+}-concentration changes in living cells using a Ca^{2+} sensitive fluorescence dye (Fluo-4). Compounds which at 5 μM showed activity greater than 20% of the response given by 10 μM nicotine were further validated in concentration-response curve analysis (Table 7).

Compounds **14** and **50** maintained the same activity of the original hit (**3**), while compound **111** was slightly more active. As the level of activity was only moderate for the majority of compounds synthesised, at this point we could not elaborate a full SAR around the structure. Nevertheless some features appeared to be common in the structure of the active compounds: the presence of a *para* bromine substituent in the aryl moiety (**B** in Fig. 2) and the dimethylamine as ionisable centre (**D** in Fig. 2). These results support the putative binding mode of benzoimidazole **3**, which describes the space around the protonated nitrogen to be limited, with more hindered amines making steric clashes within the aromatic cage. The pyrrolidinone series was inactive confirming the importance of the protonated nitrogen. The insertion of a methyl group in position 5 of the benzoimidazole (compound **111**) improved the interaction with the hydrophobic pocket of the receptor.

The four best compounds were additionally tested for their action against the alpha7-nAChR subtype (Fig. 3). The fluorescence response was evaluated at 10 μM in the absence and in the presence of 1 μM methyllycaconitine (MLA), a selective alpha7 receptor antagonist. The fluorescence induced by the compounds was dramatically reduced when MLA was added to the cells,

Table 5. Structures and yields of compounds **11–106**



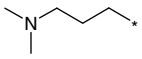
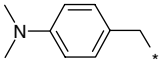
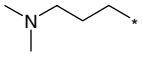
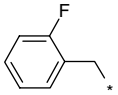
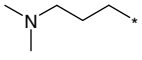
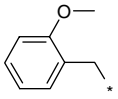
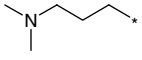
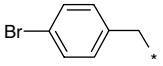
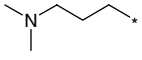
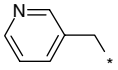
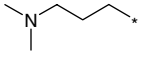
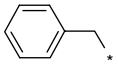
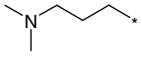
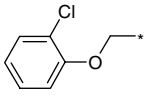
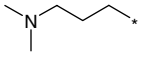
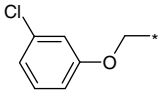
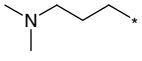
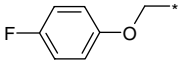
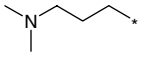
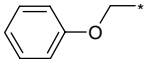
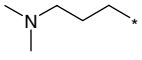
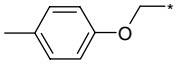
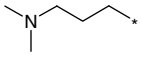
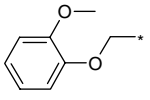
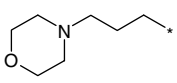
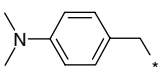
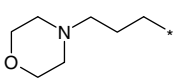
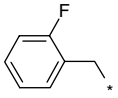
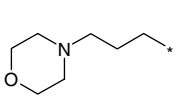
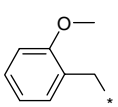
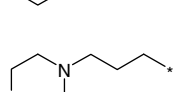
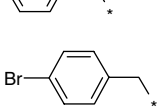
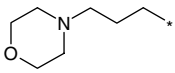
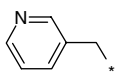
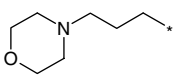
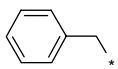
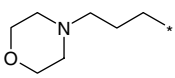
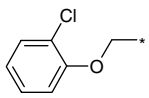
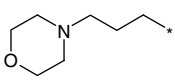
Compound	R_1	Ar-X	Yield (%)
11			—
12			44
13			35
14			29
15			—
16			21
17			30
18			28
19			30
3			26
21			27
22			44
23			35
24			44
25			54
26			23

Table 5 (*continued*)

Compound	R_1	Ar-X	Yield (%)
27			—
28			44
29			60
30			50
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35			11
36			31
37			19
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43			14

Table 5 (*continued*)

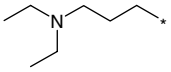
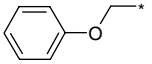
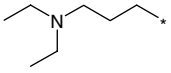
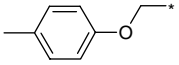
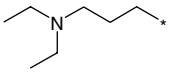
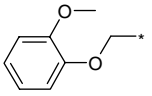
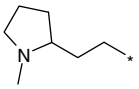
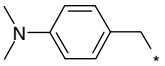
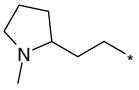
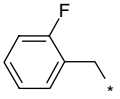
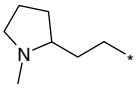
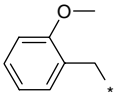
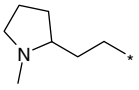
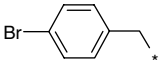
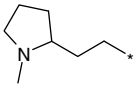
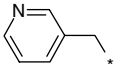
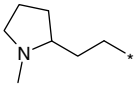
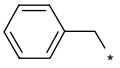
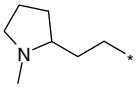
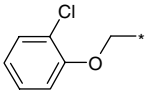
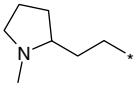
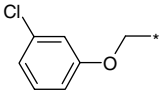
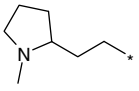
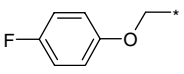
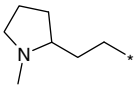
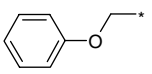
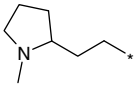
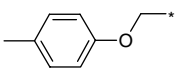
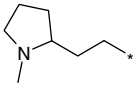
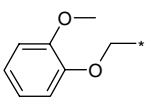
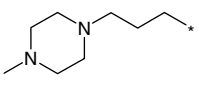
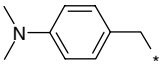
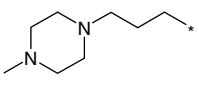
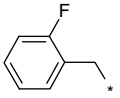
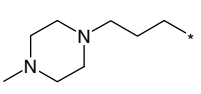
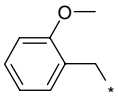
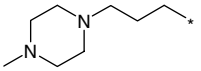
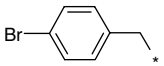
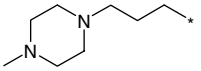
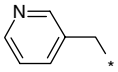
Compound	R_1	Ar-X	Yield (%)
44			27
45			24
46			28
47			—
48			40
49			39
50			43
51			41
52			29
53			32
54			37
55			49
56			27
57			26
58			31
59			—
60			25

Table 5 (*continued*)

Compound	R_1	Ar-X	Yield (%)
61			40
62			40
63			33
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68			18
69			41
70			40
71			36
72			36
73			32
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75			19
76			23

(*continued on next page*)

Table 5 (*continued*)

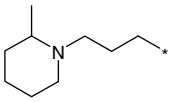
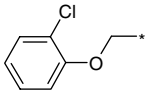
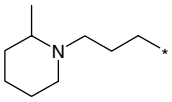
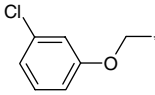
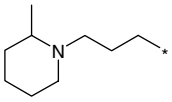
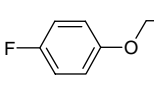
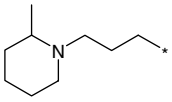
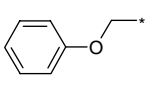
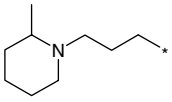
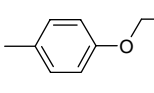
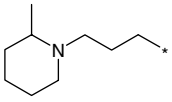
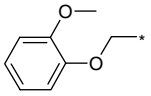
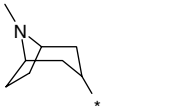
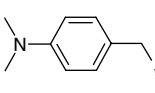
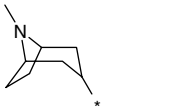
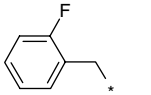
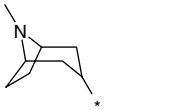
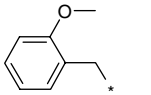
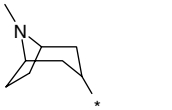
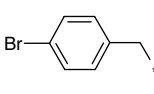
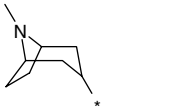
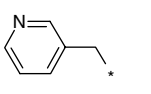
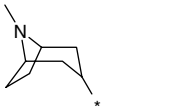
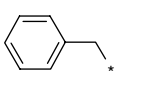
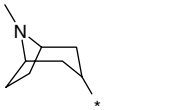
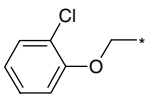
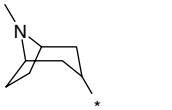
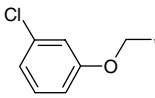
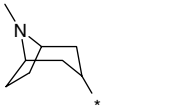
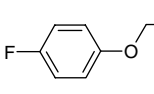
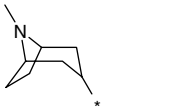
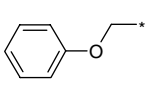
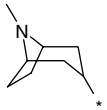
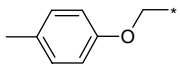
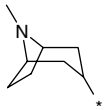
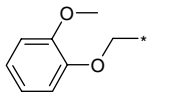
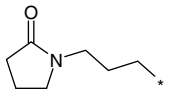
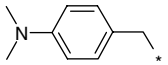
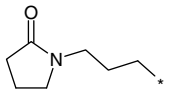
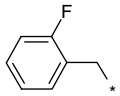
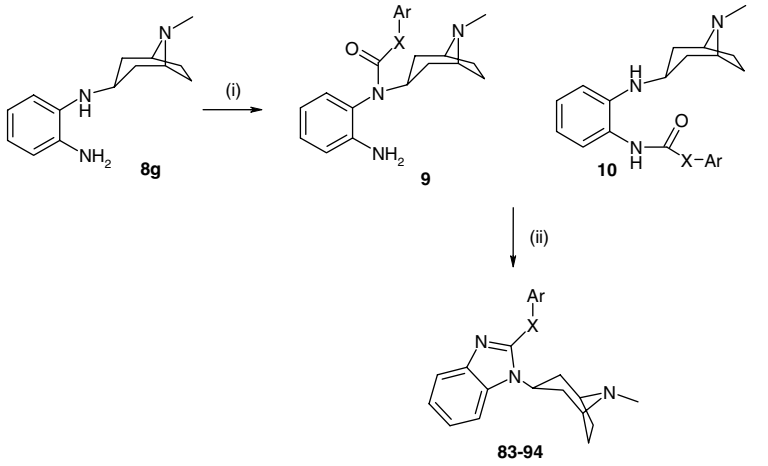
Compound	R_1	Ar-X	Yield (%)
77			41
78			41
79			35
80			39
81			44
82			36
83			—
84			—
85			14
86			17
87			—
88			—
89			15
90			17
91			13
92			—

Table 5 (*continued*)

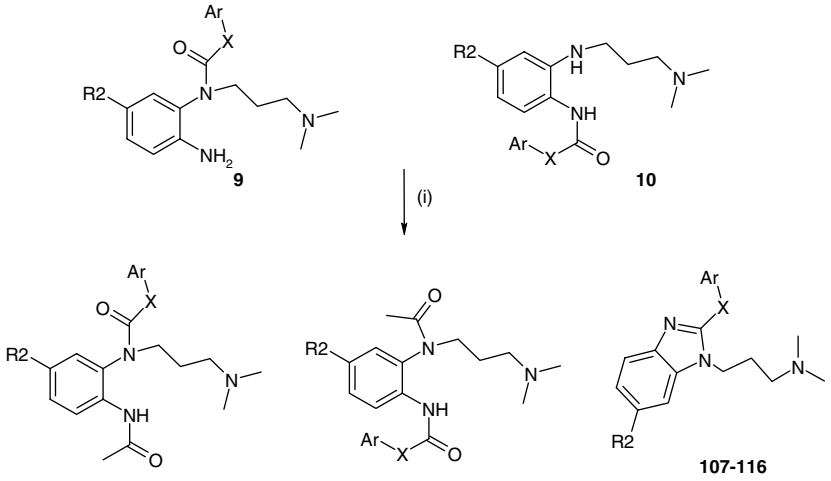
Compound	R_1	Ar-X	Yield (%)
93			20
94			—
95			57
96			55
97			64
98			64
99			20
100			57
101			40
102			56
103			67
104			46
105			40
106			59

proving that activity was indeed related to the alpha7 subtype.

Although we were disappointed to find that only a small fraction of the compounds synthesised showed activity,



Scheme 3. Reagents and conditions: (i) ArXCOOH, CDI, DMF, 80 °C; (ii) glacial acetic acid, 140 °C, microwave irradiation.



Scheme 4. Reagent and condition: (i) glacial acetic acid, 80 °C.

the results obtained suggest that additional future modifications around the structure could improve activity further. This includes varying the **C** and **D** portions, chain lengths and further investigations into substitution on the benzoimidazole ring.

4. Experimental

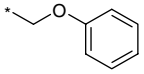
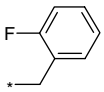
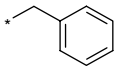
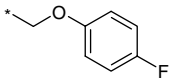
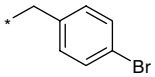
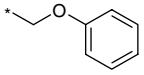
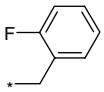
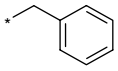
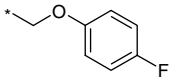
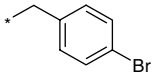
4.1. General information

All solvents and reagents were used as supplied, unless otherwise stated. Reactions using air/moisture sensitive reagents were run in a nitrogen atmosphere. High-performance liquid chromatography (HPLC) analysis was performed using a Waters 2795 module system with Waters Micromass ZQ and Waters PDA 2996 detectors connected to Masslynx 4.0 software. The column used was an XTerra MS C18 (3.5 μm, 2.1×50 mm). ^{1}H NMR spectra were recorded on a NMR Varian Mercury Plus 400 MHz, 5 mm PFG ATB Broadband probe. Chemical shifts are reported in parts per million (ppm) units (s: singlet, d: doublet, t: triplet, dd: doublet doublet, m: multiplet). HRMS analysis was carried out using a Thermo LTQ Orbitrap Mass Spectromer. Analytical thin-layer chromatography (TLC) was carried out using pre-coated plates of silica gel (5×20 cm). Silica gel chromatography was conducted using Isolute prepacked 2 g Flash Si-columns. Microwave reactions were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC). Specs and Maybridge compounds were purchased and screened without preliminary analysis.

4.2. Docking methods

4.2.1. Generation of database for virtual screening. An sdf file of commercially available molecules was obtained directly from SPECS and Maybridge. The sdf file was loaded onto an internal Oracle 10 g database using the Accelrys Chemistry Cartridge. Physicochemical properties calculation was performed utilising the Accelrys Accord SDK. The collections were filtered to exclude undesirable reactive groups by substructure searches. A script was prepared in order to obtain suitable minimised structures and protonation states. The procedure entails addition of hydrogens and structure minimisation by Corina (Corina version 3.2 2005. Molecular

Table 6. Structures and yields of compounds **107–116**

Compound	R_2	Ar-X	Yield (%)
107	Me		20
108	Me		18
109	Me		—
110	Me		13
111	Me		20
112	F		22
113	F		—
114	F		22
115	F		21
116	F		22

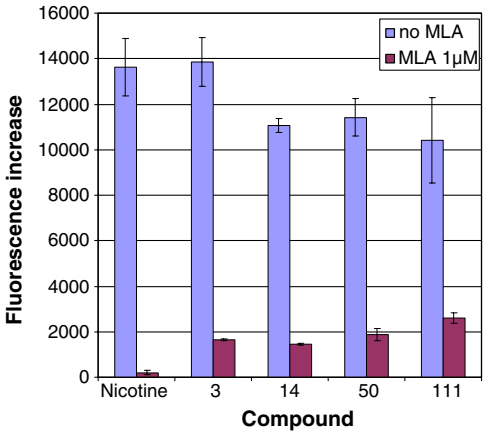
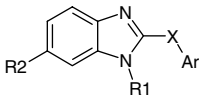


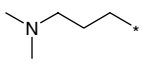
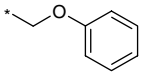
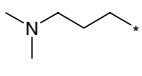
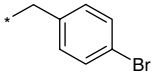
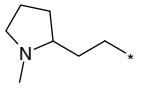
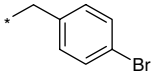
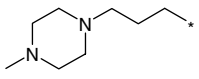
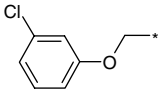
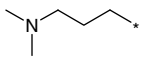
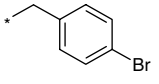
Figure 3. FLIPR/Ca^{2+} influx assay with MLA.

Networks GmbH) followed by protonation at pH 7 and re-minimisation using Ligprep (LigPrep version 1.6 2005. Schrödinger, LLC).

4.2.2. Preparation of homology model for docking. For our docking simulations, we used the homology structure of alpha7 containing a docked nicotine molecule in the agonist binding site located at the interface of two subunits (a7gg.pdb).[19] Nicotine was eliminated from the pdb file. Active sites were selected manually and contained residues Y91, W147, T148, Y186, C188, C189 and Y193 from the alpha subunit and M36, W53, N105, Q115 and L117 from the beta subunit.

Table 7. Results from concentration-response analysis in functional Ca-flux assay for selected compounds



Compound	R_2	R_1	Ar-X	Curve top plateau[a] (%)	Alpha7 EC_{50} (μM)
Nicotine				97	1.5
3	H			52	2.6
14	H			135	2.6
50	H			128	2.7
66	H			[b]	10.0
111	Me			111	1.7

[a] Curve top plateau of concentration-reponse analysis. Normalised to nicotine 10 μM (positive control).
[b] Did not reach the top plateau.

4.2.3. Docking calculation. Docking calculations were performed using GOLD (GOLD version 2.2 2005. CCDC). Default settings were generally used. Thus, ten poses for each ligand were calculated. The command allowing early termination was switched on and used as one of the criteria to select virtual hits, when at least 3 docked conformations for the same ligand were within 1.5 Å rms deviation. Both GoldScore and ChemScore fitness functions were used.

4.3. Chemistry methods

4.3.1. General procedure A (6a–j). To a suspension of potassium carbonate (40 mmol) in DMF (15 mL), 22 mmol of the required *ortho*-fluoronitrobenzenes (**1a–c**) were added, along with 20 mmol of the appropriate aminopropylamine (**2a–j**). The reaction was left mixing at 90 °C for 24 h. On reaction completion, the crude was filtered and the collected solution concentrated. This was then diluted with dichloromethane (25 mL) and extracted twice with water (20 mL per extraction). Products were then extracted from dichloromethane phase using 20 mL of 6 N HCl solution. This aqueous phase was then treated with dichloromethane (20 mL) to remove the excess of *ortho*-fluoronitrobenzenes present. The aqueous phase containing the desired compound was basified with NaOH pellets and the product extracted with dichloromethane (20 mL) which was evaporated to leave the desired products in good yield (60–90%).

4.3.1.1. *N*,*N*-Dimethyl-*N*′-(2-nitro-phenyl)-propane-1,3-diamine (6a). Following general procedure A, 3.48 g was obtained (78% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.84–1.90 (m, 2H), δ_H 2.26 (s, 6H), δ_H 2.41–2.44 (m, 2H), δ_H 3.35–3.41 (m, 2H), δ_H 6.59–6.63 (m, 1H), δ_H 6.86–6.88 (m, 1H), δ_H 7.39–7.45 (m, 1H), δ_H 8.14–8.16 (m, 1H), δ_H 8.45 (s, broad, 1H).

4.3.1.2. (3-Morpholin-4-yl-propyl)-(2-nitro-phenyl)-amine (6b). Following general procedure A, 4.87 g was obtained (92% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.86–1.91 (m, 2H), δ_H 2.42–2.50 (m, 6H), δ_H 3.38 (dd, J = 12.03 Hz, 2H), δ_H 3.70–3.76 (m, 4H), δ_H 6.59–6.63 (m, 1H), δ_H 6.87 (d, J = 8.69 Hz, 1H), δ_H 7.39–7.43 (m, 1H), δ_H 8.14–8.16 (m, 1H), δ_H 8.26 (s, broad, 1H).

4.3.1.3. *N*,*N*-Diethyl-*N*′-(2-nitro-phenyl)-propane-1,3-diamine (6c). Following general procedure A, 4.22 g was obtained (84% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.01–1.05 (t of d, J = 1.6, 5.2, 6H), δ_H 1.82–1.90 (m, 2H), δ_H 2.52–2.58 (m, 6H) δ_H 3.35–3.39 (m, 2H), δ_H 6.60–6.64 (m, 1H), δ_H 6.86–6.98 (d, 1H), δ_H 7.40–7.44 (m, 1H), δ_H 8.16–8.18 (m, 1H), δ_H 8.41 (s, broad 1H).

4.3.1.4. [2-(1-Methyl-pyrrolidin-2-yl)-ethyl]-(2-nitro-phenyl)-amine (6d). Following general procedure A, 4.43 g was obtained (89% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.55–1.63 (m, 1H), δ_H 1.72–1.80 (m, 3H), δ_H 1.94–2.05 (m, 2H), δ_H 2.14–2.19 (m, 1H), δ_H 2.21–2.31 (m, 1H), δ_H 2.36 (s, 3H), δ_H 3.08–3.13 (m, 1H), δ_H 3.28–3.43 (m, 2H), δ_H 6.60–6.64 (m, 1H), δ_H 6.84–6.86 (m, 1H), δ_H 7.41–7.45 (m, 1H), δ_H 8.15–8.17 (m, 1H), δ_H 8.40 (s, broad, 1H).

4.3.1.5. [3-(4-Methyl-piperazin-1-yl)-propyl]-(2-nitro-phenyl)-amine (6e). Following general procedure A, 4.11 g was obtained (74% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.86–1.91 (m, 2H), δ_H 2.30 (s, 3H), δ_H 2.46–2.50 (m, 10H), δ_H 3.36–3.41 (m, 2H), δ_H 6.61–6.65 (m, 1H), δ_H 6.87–6.89 (m, 1H), δ_H 7.40–7.45 (m, 1H), δ_H 8.16–8.18 (m, 1H), δ_H 8.24 (s, broad, 1H).

4.3.1.6. [3-(2-Methyl-piperidin-1-yl)-propyl]-(2-nitro-phenyl)-amine (6f). Following general procedure A, 5.15 g was obtained (93% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.05–1.07 (m, 1H), δ_H 1.25–1.37 (m, 2H), δ_H 1.56–1.69 (m, 4H), δ_H 1.83–1.92 (m, 2H), δ_H 2.10–2.17 (m, 1H), δ_H 2.28–2.36 (m, 1H), δ_H 2.37–2.43 (m, 1H), δ_H 2.81–2.90 (m, 3H), δ_H 3.32–3.37 (m, 2H), δ_H 6.62–6.64 (m, 1H), δ_H 6.86–6.88 (m, 1H), δ_H 7.40–7.45 (m, 1H), δ_H 8.16–8.18 (m, 1H), δ_H 8.24 (s, broad, 1H).

4.3.1.7. (8-Methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-(2-nitro-phenyl)-amine (6g). Following general procedure A, 3.39 g was obtained (65% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.81–1.85 (m, 2H), δ_H 1.96–1.99 (m, 2H), δ_H 2.13–2.17 (m, 2H), δ_H 2.33–2.34 (m, 5H), δ_H 3.22–3.24 (m, 2H), δ_H 3.71–3.95 (m, 1H), δ_H 6.60–6.65 (m, 1H), δ_H 6.73–6.75 (m, 2H), δ_H 7.40–7.44 (m, 1H), δ_H 8.17–8.20 (m, 1H), δ_H 8.69 (s, broad, 1H).

4.3.1.8. 1-[3-(2-Nitro-phenylamino)-propyl]-pyrrolidin-2-one (6h). Following general procedure A, 4.63 g was obtained (88% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.92–1.99 (m, 2H), δ_H 2.04–2.07 (m, 2H), δ_H 2.39–2.43 (m, 2H), δ_H 3.33–3.38 (m, 2H), δ_H 3.41–3.46 (m, 4H), δ_H 6.64–6.67 (m, 1H), δ_H 6.84–6.86 (m, 1H), δ_H 7.42–7.46 (m, 1H), δ_H 8.10 (s, broad, 1H), δ_H 8.16–8.18 (m 1H).

4.3.1.9. *N*,*N*-Dimethyl-*N*′-(4-methyl-2-nitro-phenyl)-propane-1,3-diamine (6i). Following general procedure A, 3.65 g was obtained (77% yield) ^{1}H NMR (400 MHz, MeOD): δ_H 1.87–1.94 (m, 2H), δ_H 2.31 (s, 6H), δ_H 2.35 (s, 3H), δ_H 2.50–2.54 (m, 2H), δ_H 3.39–3.43 (m, 2H), δ_H 6.49–6.51 (m, 1H), δ_H 6.82 (s, 1H), δ_H 7.98–8.00 (m, 2H).

4.3.1.10. *N*′-(4-Fluoro-2-nitro-phenyl)-*N*,*N*-dimethyl-propane-1,3-diamine (6j). Following general procedure A, 3.13 g was obtained (65% yield) ^{1}H NMR (400 MHz, MeOD): δ_H 1.84–1.92 (m, 2H), δ_H 2.29 (s, 6H), δ_H 2.44–2.47 (m, 2H), δ_H 3.35–3.39 (m, 2 H), δ_H 6.39–6.44 (m, 1H), δ_H 6.71–6.75 (m, 1H), δ_H 8.18–8.22 (m, 1H).

4.3.2. General procedure B (8a–j). The nitrodiamine obtained from procedure A (13 mmol) was dissolved in ethanol (30 mL) and $SnCl_2 \cdot H_2O$ was added (13 mmol, 1 equiv). The reaction mixture was stirred at reflux for 16 h. The reaction progress was controlled by LC–MS analysis and if required, a further equivalent of $SnCl_2 \cdot H_2O$ was added. Upon reaction completion, the crude was cooled to room temperature and the solution treated with 10% NaOH to pH 8. The reaction mixture

was then treated with solid sodium tartrate until a clear solution was obtained and the mixture stirred for around 1 h, after which the reaction mixture was filtered and the retained solution treated with NaOH to pH 12. The desired product was extracted using dichloromethane (40 mL), drying the collected organic phase with sodium sulfate before evaporation of the solvent to leave the pure desired product in good yield (~70%).

4.3.2.1. *N*-(3-Dimethylamino-propyl)-benzene-1,2-diamine (8a). Following general procedure B, 3.8 g was obtained (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.82–1.88 (m, 2 H), δ_H 2.27 (s, 6H), δ_H 2.42–2.45 (m, 2H), δ_H 3.17–3.20 (m, 2H), δ_H 3.34–3.35 (s, 2H), δ_H 6.64–6.68 (m, 2H), δ_H 6.70–6.72 (m, 1H), δ_H 6.80–6.84 (m, 1H).

4.3.2.2. *N*-(3-Morpholin-4-yl-propyl)-benzene-1,2-diamine (8b). Following general procedure B, 2.5 g was obtained (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.81–1.87 (m, 2H), δ_H 2.46–2.50 (m, 6H), δ_H 3.15–3.19 (m, 2H), δ_H 3.35 (s, broad, 2H), δ_H 3.72–3.74 (m, 4H), δ_H 6.62–6.97 (m, 3H), δ_H 6.78–6.82 (m, 1H).

4.3.2.3. *N*-(3-Diethylamino-propyl)-benzene-1,2-diamine (8c). Following general procedure B, 2.61 g was obtained (91% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.04 (t, J = 7.14 Hz, 6H), δ_H 1.80–1.86 (m, 2H), δ_H 2.52–2.62 (m, 6H), δ_H 3.15–3.18 (m, 2H), δ_H 3.35 (s, broad, 2H), δ_H 6.62–6.70 (m, 3H), δ_H 6.78–6.82 (m, 1H).

4.3.2.4. *N*-[2-(1-Methyl-pyrrolidin-2-yl)-ethyl]-benzene-1,2-diamine (8d). Following general procedure B, 1.76 g was obtained (62% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.54–1.63 (m, 1H), δ_H 1.68–1.81 (m, 3H), δ_H 1.93–2.02 (m, 2), δ_H 2.12–2.27 (m, 2H), δ_H 2.36 (s, 3H), δ_H 3.06–3.22 (m, 3H), δ_H 3.32 (s, broad, 2H), δ_H 6.64–6.72 (m, 3H), δ_H 6.80–6.84 (m, 1H).

4.3.2.5. *N*-[3-(4-Methyl-piperazin-1-yl)-propyl]-benzene-1,2-diamine (8e). Following general procedure B, 2.77 g was obtained (86% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.83–1.90 (m, 2H), δ_H 2.30 (s, 3H), δ_H 2.41–2.54 (m, 10H), δ_H 3.17–3.20 (m, 2H), δ_H 3.35 (s, broad, 2H), δ_H 6.63–6.71 (m, 3H), δ_H 6.79–6.83 (m, 1H).

4.3.2.6. *N*-[3-(2-Methyl-piperidin-1-yl)-propyl]-benzene-1,2-diamine (8f). Following general procedure B, 2.53 g was obtained (79% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.06 (d, J = 6.24 Hz, 3H), δ_H 1.25–1.35 (m, 2H), δ_H 1.51–1.68 (m, 4H), δ_H 1.77–1.92 (m, 2H), δ_H 2.07–2.14 (m, 1H), δ_H 2.24–2.30 (m, 1H), δ_H 2.35–2.42 (m, 1H), δ_H 2.84–2.96 (m, 2H), δ_H 3.09–3.20 (m, 2H), δ_H 3.34 (s, broad, 2H), δ_H 6.62–6.71 (m, 3H), δ_H 6.78–6.82 (m, 1H).

4.3.2.7. *N*-(8-Methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-benzene-1,2-diamine (8g). Following general procedure B, 1.95 g was obtained (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.77 (d, J = 14.02 Hz, 2H), δ_H 2.02–2.08 (m, 4H), δ_H 2.26–2.34 (m, 5H), δ_H 3.16–3.22 (m, 2H), δ_H 3.35 (s, broad, 2H), δ_H 3.42–3.46 (m, 1H), δ_H 3.64 (s, broad, 1H), δ_H 6.56–6.58 (m, 1H), δ_H 6.66–6.76 (m, 2H), δ_H 6.79–6.84 (m, 1H).

4.3.2.8. 1-[3-(2-Amino-phenylamino)-propyl]-pyrrolidin-2-one (8h). Following general procedure B, 2.21 g was obtained (73% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.82–1.88 (m, 2H), δ_H 2.00–2.06 (m, 2H), δ_H 2.39–2.43 (m, 2H), δ_H 3.14–3.18 (m, 2H), δ_H 3.67–3.43 (m, 4H), δ_H 3.60 (s, broad, 2H), δ_H 6.63–6.71 (m, 3H), δ_H 6.76–6.80 (m, 1H).

4.3.2.9. *N*-(3-Diethylamino-propyl)-4-methylbenzene-1,2-diamine (8i). Following general procedure B, 2.20 g was obtained (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.84–1.88 (m, 2H), δ_H 2.25 (s, 3H), δ_H 2.30 (s, 6H), δ_H 2.47–2.50 (m, 2H), δ_H 3.16–3.19 (m, 2H), 3.35 (s, broad, 2H), δ_H 6.44–6.46 (m 2H), δ_H 6.59–6.61 (m, 1H)

4.3.2.10. *N*-(3-Diethylamino-propyl)-4-fluorobenzene-1,2-diamine (8j). Following general procedure B, 2.14 g was obtained (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.82–1.85 (m, 2H), δ_H 2.26 (s, 6H), δ_H 2.42–2.45 (m, 2H), δ_H 3.13–3.16 (m, 2H), 3.35 (s, broad, 2H), δ_H 6.26–6.35 (m, 2H), δ_H 6.58–6.61 (m, 1H).

4.3.3. General synthesis of 11–116. The required acid was weighed into clean vials (0.39 mmol per reaction) and dissolved in dichloromethane (1 mL per reaction). A solution of *N*,*N*′-carbonyldiimidazole (CDI) in dichloromethane (0.39 mmol per reaction, 1 mL per reaction) was then added as a solution. The vials were then left to shake for 2 h before adding a solution of the desired amine in dichloromethane (0.39 mmol per reaction in 1 mL dichloromethane per reaction). The reactions were then left to mix for 16 h. When LC–MS analysis showed reaction completion, the blocks were moved to the automation platform for work-up. Here, the samples were treated with 10% NaOH solution (2 mL per reaction) and the organic phase collected and dried. (NMR data were not taken at this stage). In the case of highly hindered phenyleneamines, 2 equiv of the required acid was used (0.78 mmol) as well as DMF in place of dichloromethane (same amount as indicated above). The reaction mixtures were heated to 80 °C for 16 h before solvent removal and work-up as with the remainder of the library. The crude samples were dried and treated with glacial acetic acid (3 mL per reaction) and the reaction mixtures left to heat to 80 °C for 4 h. After LC–MS analysis to check for complete cyclisation, the reaction mixtures were concentrated via solvent evaporation. In the case of highly sterically hindered intermediates, the reaction was carried out under microwave irradiation exposure at 140 °C for 15 min and solvent was removed before work-up using the general procedure. The blocks were then moved to the Zinsser platform for redissolution in dichloromethane (2 mL per reaction) followed by an aqueous wash with 15% NaOH (2 mL per reaction) using the procedure described above. The collected organic phases were dried under nitrogen before parallel purification using flash Si-columns. The desired products were found to elute using a dichloromethane/MeOH mix (96:4).

For LC–MS analysis, an automated analytical plate preparation procedure was also utilised to eliminate the need for manual manipulation and hence possible errors in sampling and dilution.

4.3.4. Analytical data of 20 representative samples

4.3.4.1. Dimethyl-[3-(2-phenoxymethyl-benzoimidazol-1-yl)-propyl]-amine(3). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.62 (s, 2H), δ_H 2.02–2.04 (m, 2H), δ_H 2.20 (s, 6H), δ_H 2.28 (t, J = 6.8, 2H), δ_H 5.42 (s, 2H), δ_H 6.97–7.00 (m, 1H), δ_H 7.07–7.09 (m, 1H), δ_H 7.28–7.32 (m, 5H), δ_H 7.42–7.44 (m, 1H), δ_H 7.78–7.80 (m, 1H). $C_{19}H_{23}N_3O$, Mass (calculated) [309.41]; (found) $[M+H]^+$ = 310; LC t_R = 1.16 min, 100% (10 min method). HRMS *m/z* 310.19141 $(M+1)^+$. Calcd mass for $C_{19}H_{24}N_3O$ 310.19139.

4.3.4.2. {3-[2-(2-Chloro-phenoxymethyl)-benzoimidazol-1-yl]-ropyl}-dimethyl-amine (17). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.01–2.08 (m, 2H), δ_H 2.19 (s, 6H), δ_H 2.28–2.31 (t, J = 6.6, 2H), δ_H 4.41–4.45 (t J = 7.4, 2H), δ_H 5.51 (s, 2H), δ_H 6.90–6.94 (m, 1H), δ_H 7.19–7.23 (m, 1H), δ_H 7.27–7.37 (m, 4H), δ_H 7.44–7.46 (m, 1H), δ_H 7.77–7.79 (m, 1H). $C_{19}H_{22}ClN_3O$ Mass (calculated) [343.86]; (found) $[M+H]^+$ = 344; LC t_R = 1.73 min, 100% (10 min method). HRMS *m/z* 344.15252 $(M+1)^+$. Calcd mass for $C_{19}H_{23}ClN_3O$ 344.15242.

4.3.4.3. Dimethyl-{4-[1-(3-morpholin-4-yl-propyl)-1H-benzoimidazol-2-ylmethyl]-phenyl}-amine (23). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.08 (s, 2H), δ_H 2.22 (t, J = 6.8, 2H), δ_H 2.33 (s, 4H), δ_H 2.90 (s, 6H), δ_H 3.71 (t, J = 4.4, 4H), δ_H 4.08 (t, J = 7.2, 2H), δ_H 4.29 (s, 2H), δ_H 6.65 (d, J = 8.4, 2H), δ_H 7.11 (d, J = 8.4, 2H), δ_H 7.23–7.30 (m, 3H), δ_H 7.78–7.80 (m, 1H). $C_{23}H_{30}N_4O$, Mass (calculated) [378.41]; (found) $[M+H]^+$ = 379; LC t_R = 0.35 min, 100% (10 min method). HRMS *m/z* 379.24939 $(M+1)^+$. Calcd mass for $C_{23}H_{31}N_4O$ 379.24924.

4.3.4.4. 2-(2-Chloro-phenoxymethyl)-1-(3-morpholin-4-yl-propyl)-1H-benzoimidazole (29). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.07–2.12 (m, 3H), δ_H 2.37–2.40 (m, 6H), δ_H 3.64–3.67 (t, J = 4.4, 3H), δ_H 4.44–4.47 (t, J = 7.2, 2H), δ_H 5.52 (s, 2H), δ_H 6.92–6.96 (m, 1H), δ_H 7.21–7.25 (m, 1H), δ_H 7.28–7.37 (m, 4H), δ_H 7.43–7.46 (m, 1H), δ_H 7.78–7.81 (m, 1H). $C_{21}H_{24}ClN_3O_2$ Mass (calculated) [385.90]; (found) $[M+H]^+$ = 387; LC t_R = 1.56 min, 100% (10 min method). HRMS *m/z* 386.16301 $(M+1)^+$. Calcd mass for $C_{21}H_{25}ClN_3O_2$ 386.16298.

4.3.4.5. 2-(2-Methoxy-phenoxymethyl)-1-(3-morpholin-4-yl-propyl)-1H-benzoimidazole (34). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.05–2.09 (m, 2H), δ_H 2.33–2.36 (m, 6H), δ_H 3.65 (t, J = 4.4, 4H), δ_H 3.85 (s, 3H), δ_H 4.45 (t, J = 7.2, 2H), δ_H 5.45 (s, 2H), δ_H 6.88–6.91 (m, 2H), δ_H 6.96–6.99 (m, 1H), δ_H 7.17–7.19 (m, 1H), δ_H 7.25–7.31 (m, 2H), δ_H 7.42–7.44 (m, 1H), δ_H 7.76–7.79 (m, 1H). $C_{22}H_{27}N_3O_3$, Mass (calculated) [381.48]; (found) $[M+H]^+$ = 382; LC t_R = 1.24 min, 100% (10 min method). HRMS *m/z* 382.21190 $(M+1)^+$. Calcd mass for $C_{22}H_{28}N_3O_3$ 382.21253.

4.3.4.6. {3-[2-(4-Bromo-benzyl)-benzoimidazol-1-yl]-propyl}-diethyl-amine (38). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.97–1.00 (t, J = 7.2, 6H), δ_H 1.71–1.78 (m, 2H), δ_H 2.33–2.37 (t, J = 6.8, 2H), δ_H 2.45–2.50 (dd, J = 6.8, 7.2, 4H), δ_H 4.02–4.07 (t, J = 7.6, 2H), δ_H 4.32 (s, 2H). $C_{21}H_{26}BrN_3$ Mass (calculated) [400.37]; (found) $[M+H]^+$ = 401; LC t_R = 1.32 min, 96% (10 min method). HRMS *m/z* 400.13744 $(M+1)^+$. Calcd mass $C_{21}H_{27}BrN_3$ 400.13829.

4.3.4.7. Diethyl-[3-(2-*p*-tolyloxymethyl-benzoimidazol-1-yl)-propyl]-amine (45). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.95–0.99 (t, J = 7, 6H), δ_H 1.97–2.04 (m, 2H), δ_H 2.28 (s, 3H), δ_H 2.44–2.52 (m, 6H), δ_H 4.33 (t, J = 7.6, 2H), δ_H 5.37 (s, 2H), δ_H 6.96 (d, J = 8, 2H), δ_H 7.08 (d, J = 8, 2H), δ_H 7.24–7.32 (m, 2H), δ_H 7.40–7.42 (m, 1H), δ_H 7.77–7.79 (m, 1H). $C_{22}H_{29}N_3O$, Mass (calculated) [351.50]; (found) $[M+H]^+$ = 352; LC t_R = 1.62 min, 100% (10 min method). HRMS *m/z* 352.23768 $(M+1)^+$. Calcd mass $C_{22}H_{30}N_3O$ 352.23834.

4.3.4.8. Diethyl-{3-[2-(2-methoxy-phenoxymethyl)-benzoimidazol-1-yl]-propyl}-amine (46). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.95–0.98 (t, J = 7.0, 6H), δ_H 1.98–2.05 (m, 2H), δ_H 2.46–2.51 (m, 6H), δ_H 3.85 (s, 3H), δ_H 4.39–4.43 (t, J = 7.4, 2H), δ_H 5.44 (s, 2H), δ_H 6.86–6.90 (m, 2H), δ_H 6.95–6.99 (m, 1H), δ_H 7.17–7.19 (m, 1H), δ_H 7.25–7.31 (m, 2H), δ_H 7.40–7.43 (m, 1H), δ_H 7.76–7.78 (m, 1H). $C_{22}H_{29}N_3O_2$ Mass (calculated) [367.50]; (found) $[M+H]^+$ = 368; LC t_R = 1.36 min, 100% (10 min method). HRMS *m/z* 368.23258 $(M+1)^+$. Calcd mass for $C_{22}H_{30}N_3O_2$ 368.23325.

4.3.4.9. 2-(4-Fluoro-phenoxymethyl)-1-[2-(1-methyl-pyrrolidin-2-yl)-ethyl]-1H-benzoimidazole (55). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.61 (s, 2H), δ_H 1.68–2.01 (m, 4H), δ_H 2.12–2.2 (m, 2H), δ_H 2.28 (s, 3H), δ_H 3.04–3.09 (m, 1H), δ_H 4.24–4.39 (m, 2H), δ_H 5.36 (s, 2H), δ_H 6.95–7.05 (m, 4H), δ_H 7.28–7.33 (m, 2H), δ_H 7.38–7.40 (m, 1H), δ_H 7.78–7.80 (m, 1H). $C_{21}H_{24}FN_3O$ Mass (calculated) [353.44]; (found) $[M+H]^+$ = 354; LC t_R = 1.50 min, 100% (10 min method). HRMS *m/z* 354.19715 $(M+1)^+$. Calcd mass for $C_{21}H_{25}FN_3O$ 354.19647.

4.3.4.10. 2-(2-Fluoro-benzyl)-1-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-benzoimidazole (60). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.69 (s, 2H), δ_H 1.80–1.87 (m, 2H), δ_H 2.22–2.25 (t, J = 6.8, 2H), δ_H 2.30 (s, 3H), δ_H 2.36–2.46 (m, 6H), δ_H 4.12–4.15 (t, J = 7.2, 2H), δ_H 4.42 (s, 2H), δ_H 7.02–7.10 (m, 2H), δ_H 7.15–7.27 (m, 4H), δ_H 7.35–7.37 (m, 1H), δ_H 7.75–7.77 (m, 1H). $C_{22}H_{27}FN_4$ Mass (calculated) [366.49]; (found) $[M+H]^+$ = 367; LC t_R = 0.53 min, 100% (10 min method). HRMS *m/z* 367.22893 $(M+1)^+$. Calcd mass for $C_{22}H_{28}FN_4$ 367.22925.

4.3.4.11. 2-(3-Chloro-phenoxymethyl)-1-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-benzoimidazole (66). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.03–2.04 (m, 4H), δ_H 2.31 (s, 3H), δ_H 2.33–2.48 (m, 6H + H_2O), δ_H 4.34–4.37 (t, J = 7.0, 4H), δ_H 5.41 (s, 2H), δ_H 6.97–7.00 (m, 2H), δ_H 7.07–7.08 (m, 1H), δ_H 7.21–7.25 (m, 1H), δ_H 7.29–7.32

(m, 2H), δ_H 7.42–7.44 (m, 1H), δ_H 7.79–7.81 (m, 1H). $C_{22}H_{27}ClN_4O$ Mass (calculated) [398.94]; (found) $[M+H]^+ = 400$; LC $t_R = 1.79$ min, 100% (10 min method). HRMS *m/z* 399.19436 $(M+1)^+$. Calcd mass for $C_{22}H_{28}ClN_4O$ 399.19462.

4.3.4.12. 2-(2-Methoxy-phenoxymethyl)-1-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-benzoimidazole (70). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.66 (m, 2H), δ_H 2.02–2.09 (m, 2H), δ_H 2.27 (s, 3H), δ_H 2.33–2.43 (m, 8H), δ_H 3.85 (s, 3H), δ_H 4.42–4.45 (t, $J = 7.2$, 2H), δ_H 5.45 (s, 2H), 6.87–6.91 (m, 2H), δ_H 6.96–6.99 (m, 1H), δ_H 7.18–7.20 (m, 1H), δ_H 7.25–7.31 (m, 2H), δ_H 7.41–7.45 (m, 1H), δ_H 7.76–7.78 (m, 1H). $C_{23}H_{30}N_4O$ Mass (calculated) [394.52]; (found) $[M+H]^+ = 395$; LC $t_R = 1.18$ min, 100% (10 min method). HRMS *m/z* 379.24900 $(M+1)^+$. Calcd mass $C_{23}H_{31}N_4O$ 379.24924.

4.3.4.13. 2-(2-Methoxy-benzyl)-1-[3-(2-methyl-piperidin-1-yl)-propyl]-1H-benzoimidazole (73). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.95–0.96 (d, $J = 6$, 3H), δ_H 1.24–1.30 (m, 2H), δ_H 1.66–1.82 (m, 3H), δ_H 1.96–2.02 (m, 1H), δ_H 2.18–2.27 (m, 2H), δ_H 2.57–2.69 (m, 2H), δ_H 3.88 (s, 3H), δ_H 3.97–4.11 (m, 2H), δ_H 4.30–4.39 (d,d, $J = 16$,6.4, 2H), δ_H 6.82–6.90 (m, 2H), δ_H 7.04–7.06 (m, 1H), δ_H 7.19–7.26 (m, 3H), δ_H 7.30–7.33 (m, 1H), δ_H 7.74–7.77 (m, 1H). $C_{24}H_{31}N_3O$ Mass (calculated) [377.53]; (found) $[M+H]^+ = 378$; LC $t_R = 0.77$ min, 98% (10 min method). HRMS *m/z* 378.25393 $(M+1)^+$. Calcd mass $C_{23}H_{32}N_3O$ 378.25399.

4.3.4.14. 2-Benzyl-1-[3-(2-methyl-piperidin-1-yl)-propyl]-1H-benzoimidazole (76). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.94–0.96 (d, $J = 6.4$, 3H), δ_H 1.29–1.33 (m, 2H), δ_H 1.51–1.80 (m, 5H + H_2O), δ_H 1.94–2.23 (m, 4H), δ_H 2.55–2.68 (m, 2H), δ_H 3.94–4.10 (m, 2H), δ_H 4.32–4.43 (dd $J = 15.6$, 12.8 2H), δ_H 7.22–7.31 (m, 8H), δ_H 7.76–7.78 (m, 1H). $C_{23}H_{29}N_3$ Mass (calculated) [347.51]; (found) $[M+H]^+ = 348$; LC $t_R = 0.71$ min, 100% (10 min method). HRMS *m/z* 348.24328 $(M+1)^+$. Calcd mass $C_{23}H_{30}N_3$ 348.24342.

4.3.4.15. 2-(2-Chloro-phenoxymethyl)-1-[3-(2-methyl-piperidin-1-yl)-propyl]-1H-benzoimidazole (77). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.04–1.05 (d, $J = 6.4$, 3H), δ_H 1.29–1.69 (m, 6H), δ_H 2.10–2.16 (m, 3H), δ_H 2.49–2.56 (m, 2H), δ_H 2.86–2.97 (m, 2H), δ_H 4.33–4.47 (m, 2H), δ_H 5.46–5.54 (d,d, $J = 12.8$, 8, 2H), δ_H 6.92–6.95 (m, 1H), δ_H 7.20–7.25 (m, 1H), δ_H 7.29–7.37 (m, 4H), δ_H 7.46–7.47 (m, 1H). $C_{23}H_{28}ClN_3O$ Mass (calculated) [397.95]; (found) $[M+H]^+ = 399$; LC $t_R = 2.11$ min, 100% (10 min method). HRMS *m/z* 398.19932 $(M+1)^+$. Calcd mass $C_{23}H_{29}ClN_3O$ 398.19937.

4.3.4.16. 1-{3-[2-(2-Methoxy-benzyl)-benzoimidazol-1-yl]-propyl}-pyrrolidin-2-one (97). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.69–1.75 (m, 2H), δ_H 1.92–1.99 (m, 2H), δ_H 2.33–2.37 (m, 2H), δ_H 3.13–3.17 (m, 2H), δ_H 3.23–3.27 (t, $J = 7.2$, 2H), δ_H 3.91 (s, 3H), δ_H 4.04–4.08 (t $J = 8$, 2H), δ_H 4.31 (s, 2H), δ_H 6.83–6.87 (m, 1H), δ_H 6.90–6.92 (d, $J = 8$, 1H), δ_H 7.07–7.09 (m, 1H), δ_H 7.20–7.28 (m, 4H), δ_H 7.75–7.70 (m, 1H). $C_{22}H_{25}N_3O_2$ Mass (calculated) [363.46]; (found) $[M+H]^+ = 364$; LC $t_R = 1.79$ min, 98% (10 min method). HRMS *m/z* 364.20190 $(M+1)^+$. Calcd mass $C_{22}H_{26}N_3O_2$ 364.20195.

4.3.4.17. 1-[3-(2-Pyridin-3-ylmethyl-benzoimidazol-1-yl)-propyl]-pyrrolidin-2-one (99). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.75–1.79 (m, 2H), δ_H 1.96–2.03 (m, 2H), δ_H 2.37–2.41 (m, 2H), δ_H 3.17–3.21 (m, 2H), δ_H 3.28–3.32 (m, 2H), δ_H 4.06–4.10 (m, 2H), δ_H 4.34 (s, 2H), δ_H 7.23–7.29 (m, 4H), δ_H 7.55–7.62 (m, 1H), δ_H 7.75–7.78 (m, 1H), δ_H 8.51–8.52 (m, 1H), 8.58–8.59 (m, 1H). $C_{20}H_{22}N_4O$ Mass (calculated) [334.42]; (found) $[M+H]^+ = 335$; LC $t_R = 0.67$ min, 100% (10 min method). HRMS *m/z* 335.18658 $(M+1)^+$. Calcd mass $C_{20}H_{23}N_4O$ 335.18664.

4.3.4.18. 1-{3-[2-(3-Chloro-phenoxymethyl)-benzoimidazol-1-yl]-propyl}-pyrrolidin-2-one (102). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.88–1.96 (m, 2H), δ_H 2.05–2.12 (m, 2H), δ_H 2.52–2.36 (m, 2H), δ_H 3.27–3.30 (t, $J = 7.0$, 2H), δ_H 3.41–3.44 (t, $J = 7.0$, 2H), δ_H 4.28–4.31 (m, 2H), δ_H 5.36 (s, 2H), δ_H 6.98–7.02 (m, 2H), δ_H 7.06–7.07 (m, 1H), δ_H 7.21–7.25 (m, 1H), δ_H 7.30–7.39 (m, 3H), δ_H 7.79–7.81 (m, 1H). $C_{21}H_{22}ClN_3O_2$ Mass (calculated) [383.88]; (found) $[M+H]^+ = 384$; LC $t_R = 2.69$ min, 100% (10 min method). HRMS *m/z* 384.14752 $(M+1)^+$. Calcd mass $C_{21}H_{23}ClN_3O_2$ 384.14733.

4.3.4.19. {3-[2-(4-Fluoro-phenoxymethyl)-5-methyl-benzoimidazol-1-yl]-propyl}-dimethyl-amine (110). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.84–1.91 (m, 2H), δ_H 2.08 (s, 6H), δ_H 2.15–2.18 (t, $J = 6.4$, 2H), δ_H 2.43 (s, 3H), δ_H 4.23–4.27 (t, $J = 7.2$, 2H), δ_H 5.34 (s, 2H), δ_H 7.01–7.04 (m, 1H), δ_H 7.08–7.16 (m, 4H), δ_H 7.38 (s, 1H), δ_H 7.49–7.51 (d, $J = 8.4$, 1H). $C_{20}H_{24}FN_3O$ Mass (calculated) [341.43]; (found) $[M+H]^+ = 342$; LC $t_R = 1.86$ min, 100% (10 min method). HRMS *m/z* 342.19745 $(M+1)^+$. Calcd mass $C_{20}H_{25}FN_3O$ 342.19762.

4.3.4.20. {3-[2-(4-Bromo-benzyl)-5-fluoro-benzoimidazol-1-yl]-propyl}-dimethyl-amine (116). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.59–1.66 (m, 2H), δ_H 2.04–2.08 (m, 8H), δ_H 4.10–4.13 (t, $J = 7.2$, 2H), δ_H 4.28 (s, 2H), δ_H 6.96–7.03 (m, 1H), δ_H 7.21–7.24 (m, 2H), δ_H 7.39–7.42 (d,d $J = 2.6$,6.8, 1H) δ_H 7.49–7.51 (m, 2H), δ_H 7.52–7.56 (m, 1H). $C_{19}H_{21}BrFN_3$ Mass (calculated) [390.30]; (found) $[M+H]^+ = 392$; LC $t_R = 1.93$ min, 100% (10 min method). HRMS *m/z* 390.09759 $(M+1)^+$. Calcd mass $C_{19}H_{22}BrFN_3$ 390.09757.

LC–MS analysis of all compounds (**12**–**116**) is reported in Supporting information.

4.4. Biological methods

4.4.1. Cell culture. GH4C1-F7 cells stably expressing the rat- alpha7-nAChR were cultivated in HAM's F10 medium supplemented with 2.5% FBS, 15% horse serum, 1% penicillin/streptomycin, 0.5% GLUTAMAX and 0.2 mg/ml Hygromycin at 37 °C and 5% CO_2 and propagated in a Monday–Friday routine. For cellular assays the cells were seeded on poly-D-lysine treated 96-well plates; black/clear bottom; 100,000 cells/well.

4.4.2. FLIPR/Ca^{2+} influx assay. A plate of GH4C1-F7 cells was prepared and the medium was removed. It was then incubated with 100 μL of labelling solution (HBSS-20 mM Hepes supplemented with 4 mM Fluo-4, 0.02% pluronic acid and 5 mM probenecid) at 37 °C for 30 min. After removing the labelling solution 200 μL of HBSS-20 mM Hepes supplemented with 2.5 mM probenecid was added and the plate transferred into the FLIPR. On an additional plate1 the test compounds and controls were prepared as five times concentrated solutions in HBSS-20 mM Hepes. In a second plate2 each of the wells was filled with six times concentrated nicotine solution; except for the assay buffer controls. Both plates were transferred into the FLIPR. During a FLIPR run a baseline was recorded first. After addition from the compound plate (first addition, 5 μM final concentration) the data were collected every second for 1 min, than each 30 s for 10 min. For the second addition nicotine was added (10 μM final concentration) to plate2 followed by an additional data collection every second for 1 min, then each 3 s for 3 min. The height of the peaks for the first and second addition was exported using the MAX–MIN function of the FLIPR software and normalised against the positive control (10 μM nicotine signal). Compounds showing only a signal for the first addition were classified as agonists. Compounds showing no signal at the first addition and a signal for the second addition were classified as inactive, compounds showing no signal for first and second addition were classified as antagonists. Identified hits were further validated in concentration-response curves.

4.4.3. Data analysis. EC_{50} and IC_{50} values were calculated using the IDBS *XLfit4.1* software package employing the sigmoidal concentration-response (variable slope) equation 205:

$$Y = \text{Bottom} + ((\text{Top–Bottom})/(1 + ((EC_{50}/X)^{\text{HillSlope}}))$$

X is the logarithm of the concentration, Y is the response. Bottom is the bottom plateau of the curve, Top the top plateau.

4.4.4. FLIPR/Ca^{2+} influx assay with MLA. A plate of GH4C1-F7 cells was prepared and the medium was removed. It was then incubated with 100 μL of labelling solution (HBSS-20 mM Hepes supplemented with 4 mM Fluo-4, 0.02% pluronic acid and 5 mM probenecid) at 37 °C for 30 min. After removing the labelling solution, 200 μL of washing solution (HBSS-20 mM Hepes supplemented with 2.5 mM probenecid) with or without MLA 1 μM was added. The plate was then transferred into the FLIPR. On an additional plate, test compounds and controls were prepared as five times concentrated solutions in HBSS-20 mM Hepes. Both plates were transferred into the FLIPR. At the beginning of the FLIPR assay the baseline was recorded. After addition of compounds, data were collected every second for 1 min, then each 30 s for 10 min. The peak height was exported using the MAX–MIN function of the FLIPR software. Compounds showing a nicotinic signal that disappeared in the presence of MLA are considered selective alpha7 agonists.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.068.

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