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Synthesis of *N*-phenyl-*N*-(3-(piperidin-1-yl)propyl)benzofuran-2-carboxamides as new selective ligands for sigma receptors

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ABSTRACT

Novel benzofuran-2-carboxamide ligands, which are selective for sigma receptors, have been synthesized via a microwave-assisted Perkin rearrangement reaction and a modified Finkelstein halogen-exchange used to facilitate *N*-alkylation. The ligands synthesized are the 3-methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl)propyl)benzofuran-2-carboxamides (KSCM-1, KSCM-5 and KSCM-11). The benzofuran-2-carboxamide structure was *N*-arylated and *N*-alkylated to include both *N*-phenyl and *N*-(3-(piperidin-1-yl)propyl substituents, respectively. These new carboxamides exhibit high affinity at the sigma-1 receptor with K_i values ranging from 7.8 to 34 nM. Ligand KSCM-1 with two methoxy substituents at C-5 and C-6 of the benzofuran ring, and K_i = 27.5 nM at sigma-1 was found to be more selective for sigma-1 over sigma-2.

1. Introduction

In the mid-70s sigma receptors were mistakenly characterized as a new subtype of opioid receptors.[1] However, cloning of the guinea pig sigma-1 binding site in 1996 revealed this protein to be unique sharing no homology with any other mammalian protein including all types of molecular chaperones.[2] Sigma receptors are classified into two subtypes, sigma-1 and sigma-2. To date, the sigma-2 receptor has not been cloned. Sigma-1 receptors are endoplasmic reticular (ER) proteins consisting of 223-amino acids with a molecular mass of 24-kDa, and two-transmembrane-spanning regions.[3,4] Sigma-1 receptors are widely distributed throughout the body, centrally and peripherally, primarily functioning in a modulatory role on dopamine, acetylcholine, NMDA and opioid receptors.[5] They are known to translocate during signal transduction and have been linked to the modulation or production of various intracellular secondary messengers.[3,6] Sigma-2 receptors are understood to be slightly smaller in size than the sigma-1 receptor. Pharmacological evaluations reveal that sigma-2 receptors may be lipid raft proteins that affect calcium signaling via sphingolipid products and unlike sigma-1 receptors, sigma-2 receptors do not translocate.[7] Both sigma receptors are highly expressed on tumor cell lines from human and rat cancer tissues. The overexpression of sigma-2 receptors in human and murine tumors suggests that these receptors may be a biomarker of tumor cell proliferation.[7]

Various drugs, including antipsychotics (morphine analogs), neuroleptics (haloperidol) and neuroactive steroids (progesterone, DHEA), bind to sigma-1 receptors.[8] Ligands that bind to and modulate the sigma-1 receptor have been proposed to exhibit effects in several therapeutic areas such as neurodegenerative disorders, pain, depression, schizophrenia, amnesia, Alzheimer's disease, Parkinson's disease and stroke.[8] Sigma-1 receptors have also been documented as a target for cocaine and associated with the toxic and stimulant actions of cocaine.[7] In general sigma-1 antagonists may have potential use in the treatment of addiction. The binding site of sigma-1 receptors is reported to consist of an amine binding site flanked on either side by hydrophobic binding pockets that display bulk tolerance and as such, typical pharmacophoric features of sigma-1 receptors include an alkylamine moiety within the general molecular structure.[9] Hence, a common pharmacophoric feature of sigma-1 ligands is an *N*-alkyl, *N,N*-dialkyl or *N*-arylalkyl amine moiety[10] which poses a challenge in the synthesis of these ligands. Interestingly, progesterone which lacks a basic nitrogen, is considered one of the putative endogenous ligands for the sigma-1 receptor.[11] *N,N*-Dimethyltryptamine (DMT) has been identified as a potential endogenous sigma-1 receptor ligand, but, the role of DMT as a sigma-1 receptor modulator is unclear due to low physiological concentrations in brain tissues.[12] Our synthesized ligands have a benzofuran-2-carboxamide structural moiety which has been *N*-arylated and *N*-alkylated to include both *N*-phenyl and *N*-(3-(piperidin-1-yl)propyl substituents, respectively, as summarized structurally in Figure 1. These new carboxamide ligands structurally consist of a basic alkylamine moiety the

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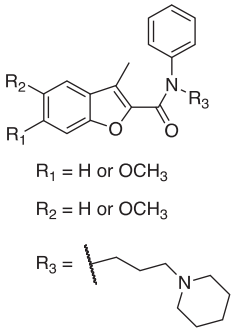


Figure 1. General ligand structure.

N-(3-(piperidin-1-yl)propyl with a protonatable nitrogen and an aromatic phenyl ring and benzofuran moiety as two hydrophobic residues (Fig. 1). Additionally, we introduced structural variations at the benzofuran moiety in the form of methyl ether substituents (R_1 and R_2, Fig. 1) to investigate the effect these substituents have on receptor binding affinity.

2. Results and discussion

2.1. Chemistry

Our microwave-assisted expedited synthetic pathway is straightforward, involving the preparation of 3-methylbenzofuran-2-carboxylic acids (**1a** and **1b**) in quantitative yields via a Perkin rearrangement reaction of mono- and di-methoxy-3-bromocoumarins as previously described.[13] Mono- and di-hydroxycoumarins were methylated and subsequently brominated at position-3 via a microwave-assisted regioselective bromination with *N*-bromosuccinimide (NBS) to yield mono- and di-methoxy-3-bromocoumarins in 85–89% yields.[13]

3-Bromocoumarins traditionally undergo base-catalyzed Perkin rearrangement, which requires 3 hours reflux quantitatively yielding benzofuran-2-carboxylic acids.[14] However under microwave reaction conditions these reactions were completed in 5 min.[13] Mono- and di-methoxy-3-methyl-*N*-phenylbenzofuran-2-carboxamides (**2a** and **2b**) were produced by reacting aniline with the corresponding 3-methylbenzofuran-2-carboxylic acids (**1a** and **1b**) in the presence of DCC and DMAP at room temperature, as outlined in Scheme 1. 3-Methyl-*N*-phenylbenzofuran-2-carboxamide (**2c**) was produced by condensing commercially available 3-methylbenzofuran-2-carboxylic acid (**1c**) with aniline.

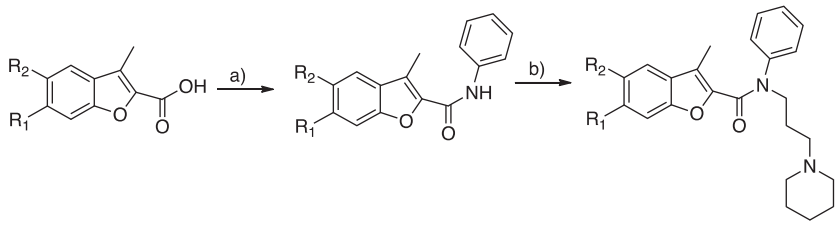
Syntheses of mono- and di-methoxy-3-methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl)propyl)benzofuran-2-carboxamides (KSCM-11 and KSCM-1) were achieved by treating carboxamides (**2a** and **2b**) with NaH followed by *N*-alkylation with 1-(3-iodopropyl)piperidine outlined in Scheme 1. A modified halogen exchange Finkelstein reaction was employed to convert commercially available 1-(3-chloropropyl)piperidine hydrogen chloride salt in the presence of potassium iodide, tetrabutylammonium bromide (TBAB) and potassium carbonate into the more reactive iodopropylpiperidine in situ, which then reacted with anions obtained from treating carboxamides (**2a** and **2b**) with sodium hydride producing KSCM-11 and KSCM-1. 3-Methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl)propyl) benzofuran-2-carboxamide (KSCM-5) was similarly synthesized by *N*-alkylation of carboxamide (**2c**) with 1-(3-iodopropyl)piperidine.

2.2. Binding assay

Compounds were screened by the National Institute of Mental Health-Psychoactive Drug Screening Program (NIMH-PDSP) against a panel of G-protein coupled receptors (GPCRs) and molecular targets due to the fact that many ligands with high affinity at sigma receptors have proven to also exhibit significant binding at one or more other central nervous system (CNS) relevant receptor sites. This evaluation has led to the discovery of three new sigma receptor selective ligands. Primary binding assays were performed at serotonin 5-HTs, adrenergic (Alpha1-A, -B, -D, Alpha2-A, -B,-C, Beta-1, -2, -3), cannabinoid (CB1and CB2), Dopamine (D1–D5), histamine (H1 and H2), opioid (KOR, MOR and DOR), Muscarinic (M1–M5), *N*-methyl-D-aspartate (NMDA), Sigma-1, Sigma-2, metabotropic glutamate (mGluR5 Rat Brain), and benzodiazepine (BZP Rat Brain) receptor sites, as well as, dopamine transporter (DAT), γ-aminobutyric acid type A (GABAA), norepinephrine transporter (NET) and serotonin transporter (SERT) molecular targets. Compounds showing >50% inhibition of radioligand specific binding at the stated GPCRs and molecular targets (Tables 1 and 2) were forwarded for additional screening (secondary binding assays) to determine K_i values at the respective GPCR binding sites using radioligand binding assays. It was determined that compounds KSCM-1, KSCM-5 and KSCM-11 have the desired selectivity for sigma receptors (sigma-1 and sigma-2) over non-sigma receptors.

Secondary binding assays of these compounds showed greater affinity at sigma receptors over non-sigma receptors such as 5-HT2A, 5-HT2B, 5HT3, Alpha2A, Alpha2C, D3, and M4 (Tables 3 and 4). Consistent with documented sigma-1 receptor ligands, the molecular structures of KSCM-1, KSCM-5 and KSCM-11 include a basic alkyl amine group, flanked by two hydrophobic residues (an aromatic phenyl and benzofuran ring).

Based upon the sigma-1 receptor selective ligand pharmacophore profile [10], a sigma-1 selective ligand usually possesses a primary and secondary hydrophobic site separated by an amine. The sigma-1 receptor site displays some bulk tolerance and so this prompted us to explore introducing a benzofuran moiety to produce the general molecular structure summarized in Figure 1. All three compounds have an *N*-arylated benzofuran-2-carboxamide scaffold which was then *N*-alkylated yielding *N*-(3-(piperidin-1-



1a, R_1=OCH_3, R_2=H
1b, R_1=OCH_3, R_2=OCH_3
1c, R_1=H, R_2=H

2a, R_1=OCH_3, R_2=H
2b, R_1=OCH_3, R_2=OCH_3
2c, R_1=H, R_2=H

KSCM-11, R_1=OCH_3, R_2=H
KSCM-1, R=OCH_3, R_2=OCH_3
KSCM-5, R_1=H, R_2=H

Scheme 1. Reagents: (a) DCC, DMAP, aniline, CH_2Cl_2; (b) NaH, 1-(3-chloropropyl)piperidine·HCl, K_2CO_3, KI, TBAB, CH_2Cl_2.

Table 1
Primary binding assay at sigma receptors

Compound	% Inhibition	
	σ-1	σ-2
KSCM-1	99.8	81.4
KSCM-5	100.6	93.5
KSCM-11	97.7	88.5

Primary binding assays performed in sigma binding buffer (50 mM Tris–HCl, pH 8.0). Sigma-1 receptors were labeled with [^{3}H](+)-pentazocine and sigma-2 receptors were labeled with [^{3}H]Ditolylguanidine (DTG) with haloperidol as the reference compound in both cases. Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered >50%. In cases where negative inhibition (−) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 μM.

Table 2
Primary binding assay at nonsigma receptors

Compound	% Inhibition						
	5-HT2A	5-HT2B	5HT3	Alpha2A	Alpha2C	D3	M4
KSCM-1	76.6	43.8	64.2	55.2	78.9	38.8	54.4
KSCM-5	18.1	69.2	2.9	77.7	61.9	60.6	43.1
KSCM-11	61.2	93.9	7.3	81.1	49.0	40.6	68.4

Assays were performed using transiently or stably transfected cell lines (e.g., HEK293, COS, CHO, NIH3T3). Refer to Table 5 for radioligands and reference compounds listing. Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered >50%. In cases where negative inhibition (−) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 μM.

Table 3
Secondary binding assay, K_i determination at sigma receptors

Compound	σ-1 (K_i, nM)	σ-2 (K_i, nM)	σ-2/σ-1
KSCM-1	27.5	528	19
KSCM-5	7.8	16	2
KSCM-11	34	41	1.2
Haloperidol	1.7	13	8

Affinities K_i (nM) were determined in rat brain homogenate (sigma-1), PC12 cells (sigma-2), sigma binding buffer (50 mM Tris–HCl, pH 8.0). Sigma-1 receptors were labeled with [^{3}H](+)-pentazocine and sigma-2 receptors were labeled with [^{3}H]DTG with haloperidol as the reference compound in both cases.

yl)propyl introducing a basic protonatable nitrogen as well as a three-carbon alkyl linker as summarized structurally in Figure 1. Additionally, the benzofuran molecular structure was varied by substitutions at C-5 and C-6 with methyl ether (R_1 and R_2, Fig. 1) to explore the impact that introduction of additional hydrogen bond acceptor (HBA) centers would have on receptor binding affinity and selectivity. We observed that the inclusion of methoxy substituents at both C-5 and C-6 of the benzofuran moiety resulted in both high affinity and selectivity at the sigma-1 receptor over the sigma-2 receptor as observed for KSCM-1 with K_i = 27.5 nM at sigma-1 and 528 nM at sigma-2 (19-fold selectivity for sigma-1 over sigma-2, Table 3). KSCM-1 showed no significant affinity at non-sigma receptors selected for secondary binding assay, K_i determination (Table 4), with K_i values ranging from 945 nM at Alpha2A to 7,612 nM at 5-HT3. The exclusion of a methoxy substituent at C-5 of the benzofuran moiety to produce KSCM-11 resulted in a slightly decreased affinity at sigma-1 (K_i = 34 nM, Table 3), however, a significant increase in affinity at sigma-2 (K_i = 41 nM, Table 3) in comparison to KSCM-1. The mono-methoxy compound KSCM-11 has similar affinity at both sigma receptor binding sites and thus no significant selectivity of sigma-1 over sigma-2 was observed (K_i = 34 nM at sigma-1 and 41 nM at sigma-2, Table 3). KSCM-11 showed no significant affinity at non-sigma receptors selected for secondary binding assay, K_i determination (Table 4), with K_i values ranging from 204 nM at 5-HT2B to >10,000 nM at M4. The exclusion of both methoxy substituents at C-5 and C-6 of the benzofuran moiety to produce KSCM-5 resulted in a significantly increased affinity at both sigma-1 (K_i = 7.8 nM, Table 3) and sigma-2 (K_i = 16 nM, Table 3) in comparison to KSCM-1 and KSCM-11. However, selectivity of KSCM-5 for sigma-1 over the sigma-2 was only twofold. KSCM-5 showed no significant affinity at non-sigma receptors selected for secondary binding assay, K_i determination (Table 4), with K_i values ranging from 249 nM at Alpha2C to 6,739 nM at D3. The sigma-2 receptor secondary binding curves for compounds KSCM-1, KSCM-5, KSCM-11, PDSP control, and haloperidol are shown in Figure 2. The sigma-2 receptor binding curves illustrate the readily noticeable link between affinity at the sigma-2 receptor and the loss of methoxy substituents at C-5 and C-6 of the benzofuran ring. Compound KSCM-5 without methoxy substituents was found to be a potent sigma-2 ligand with comparable potency to haloperidol at sigma-2 as is readily discernible from the binding curve (Fig. 2).

3. Conclusion

In this study, we have identified three new sigma receptor selective ligands based on features of the sigma-1 receptor binding site which consists of an amine binding site flanked on either side by hydrophobic binding pockets. All three ligands have high affinity at sigma-1 with K_i values ranging from 7.8 to 34 nM. KSCM-1 was the most selective with K_i = 27.5nM at sigma-1 and a 19-fold selectivity for sigma-1 over sigma-2. Ligands KSCM-1, KSCM-5 and KSCM-11 all possess basic nitrogen atoms and are structurally composed of benzofuran-2-carboxamide moieties which have been *N*-arylated and *N*-alkylated to include both *N*-phenyl and *N*-(3-(piperidin-1-yl)propyl substituents. The expedited synthetic procedures employed include regioselective microwave-assisted NBS bromination as well as microwave-assisted Perkin rearrangement reactions to prepare benzofuran-2-carboxylic acids (**1a** and **1b**) from the corresponding 3-bromocoumarins in very high yields.

4. Experimental section

^{1}H and ^{13}C NMR spectra were recorded on a JEOL 300 MHz spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR spectra are reported in δ values (parts per million, ppm) relative to an internal standard

Table 4
Secondary binding assay, K_i determination at nonsigma receptors

Compound	K_i (nM)						
	5-HT2A	5-HT2B	5HT3	Alpha2A	Alpha2C	D3	M4
KSCM-1	2564	NT	7612	945	1542	NT	2871
KSCM-5	NT	936	NT	1232	249	6739	NT
KSCM-11	2766	204	NT	653	NT	NT	>10,000

Assays were performed using transiently or stably transfected cell lines (e.g., HEK293, COS, CHO, NIH3T3). Refer to Table 5 for radioligands and reference compounds listing. NT = not tested.

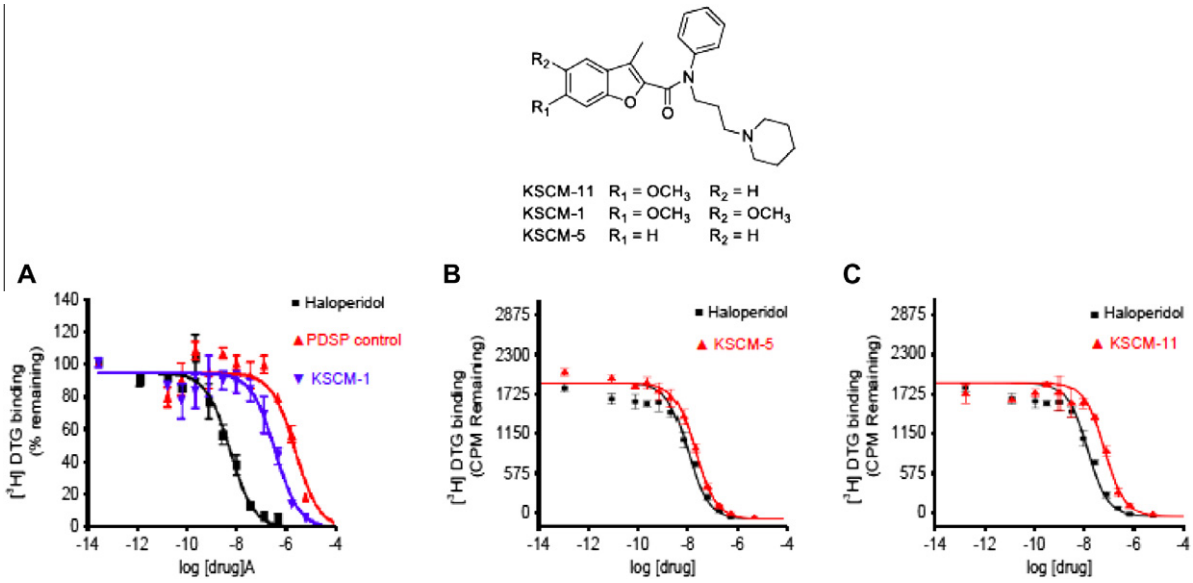


Figure 2. Secondary binding curves for K_i determinations of new ligands at sigma-2. Ligand binding of KSCM-1 (A), KSCM-5 (B) and KSCM-11 (C) at sigma-2 receptors. Sigma-2 receptors were labeled with [^{3}H]DTG and haloperidol as the reference ligand.

of tetramethylsilane (TMS) in $CDCl_3$. Multiplicities are presented as follows: s = singlet, d = doublet, t = triplet, q = quartet, qt = quintuplet, m = multiplet. Microwave syntheses were carried out on a CEM MarsXpress. HRMS data were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. Solvents were purified using standard procedures. TLC analyses were performed on Fluka 200 μm Silica Gel particle size 25 μm F_{254} plates and visualized by quenching of UV fluorescence (λ_{max} = 254 nm). All new compounds were purified on a Biotage Isolera-4 flash purification system using the indicated SNAP cartridges and solvents as eluents.

5. In vitro characterization, primary and secondary binding experiments

Radioligand binding assays using cloned G-protein coupled receptors (GPCRs), ion channels, and transporters were performed by the National Institute of Mental Health-Psychoactive Drug Screening Program (NIMH-PDSP) using membranes from transiently transfected or stable cell lines. Detailed protocols (including cell handling, buffer composition, assay conditions, etc.) for all assays are available online (http://pdsp.med.unc.edu/). Initial primary binding screening assays were performed using a 50 μM (initial) −10 μM (final) assay concentration of reference and test compounds. The percent inhibition of specific binding by the test compound was determined and if the test compound inhibited >50% of radioligand specific binding, then secondary binding assays K_i determinations were performed. Secondary binding assays, K_i determinations were performed by measuring the inhibition of radioligand binding by various concentrations of test and reference compound. In summary, compounds/ligands were prepared as a 1.0 mg/ml stock in Standard Binding Buffer (50 mM Tris HCl pH 8.0) or DMSO according to the solubility of the compound. A similar stock of Haloperidol was also prepared as a reference for a positive control. Dilutions of the test and reference compounds were then prepared in Standard Binding Buffer: 0.05 nM, 0.5 nM, 1.5 nM, 5 nM, 50 nM, 150 nM, 500 nM, 1.5 μM, 5 μM and 50 μM. [^{3}H]Pentazocine (3 nM) was used as the radioligand for sigma-1 receptors and [^{3}H]Ditolylglguanidine (DTG) (1 nM) as the radioligand for sigma-2 receptors. Aliquots (50 μl) of radioligand were dispensed into the wells of a 96-well plate containing 100 μl of Standard Binding Buffer. Then, duplicate 50-μl aliquots of the test and reference compound dilutions were added. Finally, either crude membrane fractions prepared from rat brain homogenate for sigma-1 receptors or PC12 cell homogenates for sigma-2 receptors were added to the wells, and the plates shielded from light to prevent photolysis of light sensitive ligands. For sigma-1 receptors, the reactions were incubated at 37 °C for 2.5 h, and for sigma-2 receptors, the reactions were incubated at room temperature for 2 h. Labeled receptors were harvested by rapid filtration on to Whatman GF/B glass filters pre-soaked with 0.3% polyethyleneimine using 96-well Brandel harvester. Four rapid 500 μl washes are performed with chilled Standard Binding buffer to reduce non-specific binding. Filters were placed in 6-ml scintillation tubes and allowed to dry overnight. EcoScint scintiallation cocktail (National Diagnostics) was added to each tube prior to counting. For higher throughput assays, bound radioactivity was harvested onto 0.3% polyethyleneimine-treated, 96-well filter mats using a 96 well Filtermate harvester. The filter mats were dried, the scintillate melted onto the filters and the radioactivity counted in a Microbeta scintillation counter. Raw data (dpm) representing total radioligand binding (i.e., specific + nonspecific binding) were plotted as a function of the logarithm of the molar concentration of the competitor (i.e., test or reference compound). Non-linear regression of the normalized (i.e., percent radioligand binding compared to that observed in the absence of test or reference compound) raw data was performed in Prism 4.0 (GraphPad Software) using built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites: y = bottom + [(top−bottom)/(1 + 10 × −log IC_{50})] where bottom equals the residual radioligand binding measured in the presence of 10 mM reference (i.e., non-specific binding) and top equals the total radioligand binding observed in the absence of competitor. The log IC_{50} (i.e., the log of the ligand concentration that reduces radioligand binding by 50%) was thus estimated from the data and used to obtain the K_i by applying the Cheng-Prusoff approximation.

5.1. Ligand synthesis

5.1.1. 6-Methoxy-3-methyl-*N*-phenylbenzofuran-2-carboxamide (2a)

To a mixture of aniline (0.689 g, 7.4 mmol) in dichloromethane (25 mL), 3-methyl-6-methoxybenzofuran-2-carboxylic acid (**1a**)

(1.501 g, 7.3 mmol) was added with stirring. Then DMAP (0.094 g, 0.77 mmol) was added, followed by DCC (1.583 g, 7.7 mmol). The reaction was left to stir at room temperature for 23 h. The reaction mixture was then filtered and the filtrate washed with water (20 mL × 2), then 5% acetic acid (20 mL × 2) and again with water (20 mL × 2). The crude product was recrystallized from methanol to yield **2a** as white crystals (1.37 g, 67%), mp 176–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 (s, 3H), 3.88 (s, 3H), 6.93–7.00 (m, 2H), 7.12–7.16 (t, J = 7.41 Hz, 1H) 7.35–7.40 (t, J = 7.65 Hz, 2H), 7.48–7.50 (d, J = 8.6 Hz, 1H), 7.68–7.71 (d, J = 8.5 Hz, 2H), 8.26 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 9.2, 55.8, 95.6, 112.9, 119.9, 121.4, 123.3, 124.4, 129.1, 137.7, 141.8, 154.5, 158.1, 160.5. MS(ESI)$^+$ calcd for $C_{17}H_{16}NO_3$ $[M+H]^+$: 282.1130, found: 282.1124.

5.1.2. 5,6-Dimethoxy-3-methyl-*N*-phenylbenzofuran-2-carboxamide (2b)

To a mixture of aniline (0.398 g, 4.27 mmol) in dichloromethane (15 mL), 3-methyl-5,6-dimethoxybenzofuran-2-carboxylic acid (**1b**) (1.005 g, 4.25 mmol) was added with stirring. Then DMAP (0.090 g, 0.44 mmol) was added, followed by DCC (0.934 g, 4.53 mmol). The reaction was left to stir at room temperature for 23 h. The reaction mixture was then filtered and the filtrate washed with water (20 mL × 2), then 5% acetic acid (20 mL × 2) and again with water (20 mL × 2). The crude product was recrystallized from methanol to yield **2b** as white crystals (0.94 g, 71%), mp 183–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 (s, 3H), 3.95 (s, 6H), 6.97 (s, 1H), 6.99 (s, 1H) 7.12–7.72 (m, 5H), 8.26 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 9.2, 56.3, 56.4, 94.9, 101.2, 119.8, 121.7, 124.3, 129.1, 137.7, 141.8, 147.2, 148.3, 150.8, 158.0. MS(ESI)$^+$ calcd for $C_{18}H_{18}NO_4$ $[M+H]^+$: 312.1236, found: 312.1235.

5.1.3. 3-Methyl-*N*-phenylbenzofuran-2-carboxamide (2c)

To a mixture of aniline (0.690 g, 7.41 mmol) in dichloromethane (25 mL), 3-methylbenzofuran-2-carboxylic acid (**1c**) (1.00 g, 5.7 mmol) was added with stirring. Then DMAP (0.116 g, 0.57 mmol) was added, followed by DCC (1.220 g, 5.9 mmol). The reaction was left to stir at room temperature for 23 h. The reaction mixture was then filtered and the filtrate washed with water (20 mL × 2), then 5% acetic acid (20 mL × 2) and again with water (20 mL × 2). The crude product was recrystallized from methanol to yield **2c** as off-white crystals (0.896 g, 63%), mp 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (s, 3H), 7.13–7.18 (t, J = 7.4 Hz, 1H), 7.30–7.52 (m, 5H), 7.62–7.65 (d, J = 7.57 Hz, 1H) 7.72–7.73 (d, J = 8.57 Hz, 2H), 8.35 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 9.1, 111.6, 120.0, 121.1, 123.4, 123.9, 124.6, 127.5, 129.2, 129.9, 137.5, 142.4, 153.3, 158.1. MS(ESI)$^+$ calcd for $C_{16}H_{14}NO_2$ $[M+H]^+$: 252.1025, found: 252.1027.

5.1.4. 5,6-Dimethoxy-3-methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl) propyl)benzofuran-2-carboxamide (KSCM-1)

Compound **2b** (0.100 g, 0.321 mmol) was added to dry dichloromethane (15 mL) with stirring under nitrogen atmosphere. To this solution NaH (0.130 g, 3.25 mmol) 60% dispersion in mineral oil was added and the reaction heated at reflux for 1 h. The reaction mixture was then cooled in an ice-bath and 1-(3-chloropropyl)piperidine hydrogen chloride salt (0.110 g, 0.555 mmol), potassium carbonate (0.380 g, 2.75 mmol), tetrabutylammoniumbromide (0.046 g, 0.143 mmol) and potassium iodide (0.292 g, 1.76 mmol) added with stirring. The reaction mixture was then heated at reflux for 24 h. The reaction mixture was then cooled and slowly quenched with ethanol. The reaction mixture was washed with water (5 ml × 2) and the organic layer dried over magnesium sulfate. The crude product was purified by high performance flash purification using a Biotage Isolera 4 system, SNAP (SiO_2) KP-NH column, solvent dichloromethane/methanol (9:1) as eluent to give 0.0841 g (60%) of KSCM-1 as a light brown paste. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38–1.56 (m, 6H), 1.81–1.91 (qt, J = 7.58 Hz, 2H), 2.31–2.36 (m, 6H), 2.34 (s, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 3.86-3.93 (m, 2H), 6.52 (s, 1H), 6.81 (s, 1H), 7.10–7.30 (m, 5H).^{13}C NMR (300 MHz, $CDCl_3$) δ 9.9, 10.1, 24.5, 25.3, 26.0, 29.7, 49.0, 54.6, 56.2, 56.3, 56.6 94.7, 100.8, 120.8, 122.4, 126.5, 126.9, 128.9, 143.2, 143.6, 146.7, 148.4, 149.9, 161.4. MS(ESI)$^+$ calcd for $C_{26}H_{33}N_2O_4$ $[M+H]^+$: 437.2440, found: 437.2440.

5.1.5. 3-Methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl)propyl) benzofuran-2-carboxamide (KSCM-5)

Compound **2c** (0.200 g, 0.80 mmol) was added to dry dichloromethane (25 ml) with stirring under nitrogen atmosphere. To this solution NaH (290 mg, 7.25 mmol) 60% dispersion in mineral oil was added and at reflux for 1 h. The reaction mixture was cooled in an ice-bath and 1-(3-chloropropyl)piperidine hydrogen chloride salt (0.238 g, 1.2 mmol), potassium carbonate (0.660 g, 4.8 mmol), tetrabutylammoniumbromide (0.100 g, 0.310 mmol) and potassium iodide (0.299 g, 1.8 mmol) added with stirring. The reaction mixture was reflux for 24 h. The reaction mixture was then cooled and slowly quenched with ethanol. The reaction mixture was washed with water (10 ml × 2) and the organic layer dried over magnesium sulfate. The crude product was purified by high performance flash purification using a Biotage Isolera 4 system, SNAP (SiO_2) KP-NH column, solvent dichloromethane/methanol (9:1) as eluent to give 0.189 g (63%) of KSCM-5 as a light brown paste. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36–1.56 (m, 6H), 1.82–1.92 (qt, J = 7.58 Hz, 2H), 2.31–2.36 (m, 6H), 2.34 (s, 3H), 3.90–3.95 (t, J = 7.64 Hz, 2H), 7.03–7.05 (d, J = 8.03 Hz, 1H), 7.11–7.26 (m, 7H), 7.43–7.45 (d, J = 6.85 Hz, 1H).^{13}C NMR (300 MHz, $CDCl_3$) δ 9.1, 24.5, 25.3, 26.0, 49.0, 54.6, 56.6, 111.4, 120.3, 121.2, 122.6, 126.2, 126.7, 127.0, 128.9, 129.0, 142.8, 144.4, 153.4, 161.5. MS(ESI)$^+$ calculated for $C_{24}H_{29}N_2O_2$ $[M+H]^+$: 377.2229, found: 377.2227.

5.1.6. 6-Methoxy-3-methyl-*N*-phenyl-*N*-(3-(piperidin-1-yl) propyl)benzofuran-2-carboxamide (KSCM-11)

Compound **2a** (0.200 g, 0.71 mmol) was added to dry dichloromethane (25 mL) with stirring under nitrogen atmosphere. To this solution NaH (0.290 g, 7.25 mmol) 60% dispersion in mineral oil was added and the reaction heated at reflux for 1 h. The reaction mixture was then cooled in an ice-bath and 1-(3-chloropropyl)piperidine hydrogen chloride salt (0.238 g, 1.2 mmol), potassium carbonate (0.660 g, 4.8 mmol), tetrabutylammoniumbromide (0.090 g, 0.279 mmol) and potassium iodide (0.357 g, 2.15 mmol) added with stirring. The reaction mixture was then heated at reflux for 24 h. The reaction mixture was then cooled and slowly quenched with ethanol. The reaction mixture was washed with water (10 ml × 2) and the organic layer dried over magnesium sulfate. The crude product was purified by high performance flash purification using a Biotage Isolera 4 system, SNAP (SiO_2) KP-NH column, solvent dichloromethane/methanol (9:1) as eluent to give 0.168 g (58%) of KSCM-11 as a brown paste. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39–1.57 (m, 6H), 1.82–1.92 (qt, J = 7.14 Hz, 2H), 2.33–2.37 (m, 6H), 2.35 (s, 3H), 3.74 (s, 3H), 3.90–3.95 (t, J = 7.57 Hz, 2H), 6.52 (s, 1H), 6.78–6.80 (d, J = 8.61 Hz, 1H), 7.12–7.33 (m, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 9.2, 24.5, 25.3, 26.0, 49.0, 54.6, 55.6, 56.6, 95.3, 112.3, 120.6, 122.1, 122.4, 126.6, 127.0, 129.0, 143.1, 143.6, 154.6, 159.6, 161.4. MS(ESI)$^+$ calcd for $C_{25}H_{31}N_2O_3$ $[M+H]^+$: 407.2335, found: 407.2339.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.044.

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