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Dual acting norepinephrine reuptake inhibitors and 5-HT_{2A} receptor antagonists: Identification, synthesis and activity of novel 4-aminoethyl-3-(phenylsulfonyl)-1*H*-indoles

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ABSTRACT

The discovery of a series of 4-aminoethyl-3-(phenylsulfonyl)-1*H*-indoles, dual acting norepinephrine reuptake inhibitors (NRIs) and 5-HT_{2A} receptor antagonists, is described. The synthesis and structure–activity relationship (SAR) of this novel series of compounds is also presented.

1. Introduction

Drugs that inhibit the reuptake of norepinephrine (NE) selectively, or in combination with serotonin (5-HT), have shown clinical efficacy in a variety of neurological disorders. Selective norepinephrine reuptake inhibitors (NRIs) are used for the treatment of depression (e.g., reboxetine **1**)[1] and attention deficit-hyperactivity disorder (e.g., atomoxetine **2**).[2] Dual 5-HT/NE reuptake inhibitors (SNRIs) are marketed for the treatment of depression (e.g., venlafaxine **3**;[3] desvenlafaxine **4**;[4] duloxetine **5**[5]) and the management of pain associated with diabetic peripheral neuropathy (e.g., duloxetine **5**[6]) (Fig. 1). Neurological disorders such as depression, pain, and vasomotor symptoms are thought to result from dysregulation of the levels of NE and 5-HT in the brain and in particular the cortical, hippocampal, and hypothalamic regions.

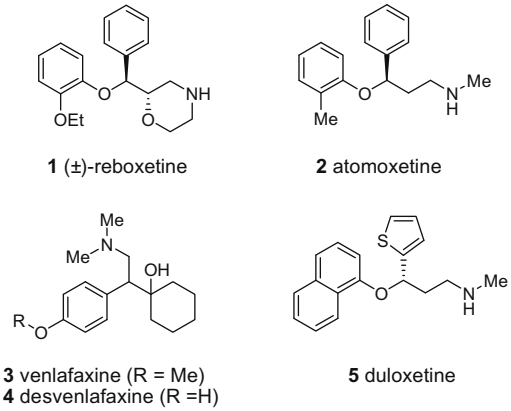

Figure 1. Selective norepinephrine reuptake inhibitors (NRIs) and dual acting serotonin/norepinephrine reuptake inhibitors (SNRIs).

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NRIs exert their effects by binding to the presynaptic norepinephrine transporter (NET), blocking the reuptake of NE from the synaptic cleft. The resulting increase in extracellular levels of the neurotransmitter NE leads to an increase in neurotransmission.

There is a growing interest in developing drugs that act specifically on multiple targets, termed designed multiple ligands (DMLs).[7,8] Many complex CNS disorders, such as schizophrenia and depression, benefit by treatment with drugs with a multi-faceted pharmacological profile.[9] 5-HT_{2A} antagonists have proven beneficial in treating neurological disorders[10] and there is evidence to suggest that 5-HT_{2A} antagonists could have utility in depression,[11] pain,[12,13] and vasomotor disorders.[14] Additionally, Deecher and Merchenthaler[15] reported that the combination of a 5-HT_{2A} antagonist with a NRI achieves greater efficacy in animal models of vasomotor symptoms than attained by the NRI alone.

Given the overlapping potential utilities of NRIs and 5-HT_{2A} receptor antagonists, a drug discovery program was initiated to identify dual acting NRI/5-HT_{2A} receptor antagonists, with selectivity over other amine transporters and 5-HT_2 receptors. The identification of a selective dual acting NRI/5-HT_{2A} antagonist would allow for the determination of the pharmacological effects, and potential therapeutic utility, of dual NET inhibition and 5-HT_{2A} antagonism.

2. Identification of dual NRI/5-HT_{2A} antagonists

One approach to identify dual acting compounds is to develop hybrid molecules where two molecules possessing the desired single activities are fused, or joined by a suitable linker.[7,8] This approach has been used extensively in the synthesis of dual acting D_2/5-HT_{2A} receptor antagonists.[10] A downside to this approach is large molecules with poor physicochemical properties are often the result.

A potentially more attractive approach would be to identify a single template that can lead to both desired activities. Enyedy et al.[16] proposed a simple pharmacophore model for monoamine transporter inhibitors that consists of two aromatic rings and a basic amine in a triangular arrangement.

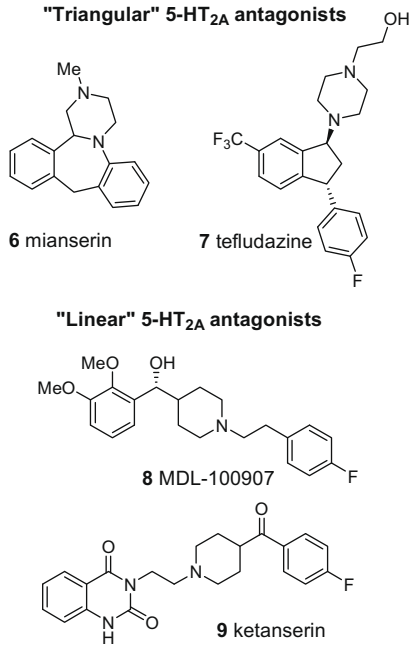


Figure 2. Classes of 5-HT_{2A} receptor antagonists.

Conversely, 5-HT_{2A} antagonists encompass a large degree of structural diversity.[10,17] Rowley et al.[18] suggested that 5-HT_{2A} antagonists can be divided into two pharmacophore classes: (a) those with two aryl rings and a basic amine arranged in a triangular arrangement (e.g., mianserin **6**, tefludazine **7**), and (b) those with a linear arrangement of an aromatic ring, basic amine and a second aryl ring or heteroaromatic group (e.g., MDL-100907 **8**, ketanserin **9**) (Fig. 2). The similarity between the monoamine transporter inhibitor pharmacophore and the 'triangular' 5-HT_{2A} antagonist pharmacophore highlights the intriguing possibility that a single template could deliver both NRIs and 5-HT_{2A} antagonists.[19]

2.1. NRI pharmacophore model

A NRI pharmacophore model was derived from a diverse series of five NRIs ((*S*,*S*)-reboxetine **10**, nisoxetine **11**, maprotiline **12**, desipramine **13**, and indole **14**[20]) (Fig. 3).

Monte Carlo conformational analysis (MMFF94 forcefield, GB/SA solvation model[21]) was conducted on each compound and all conformations within 5 kcal/mol energy from the global minimum were chosen. The average inter-feature distances and tolerances were calculated for each NRI and then assessed for commonalities within the group. This approach identified a simple NRI pharmaco-

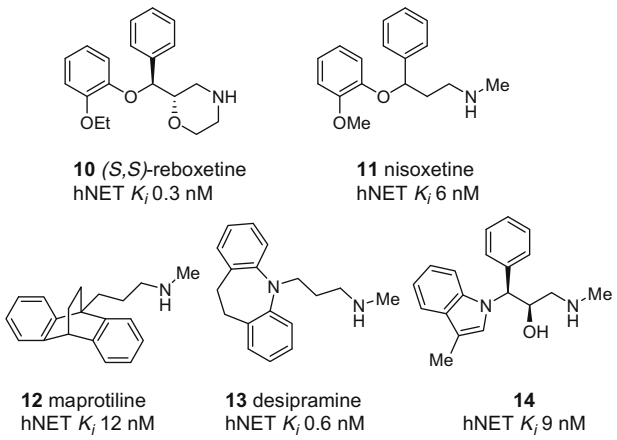


Figure 3. NRIs used to derive the NRI pharmacophore model.

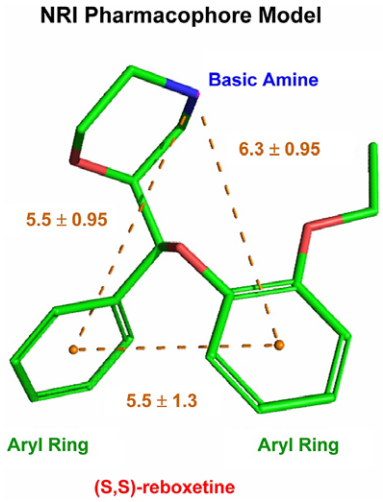
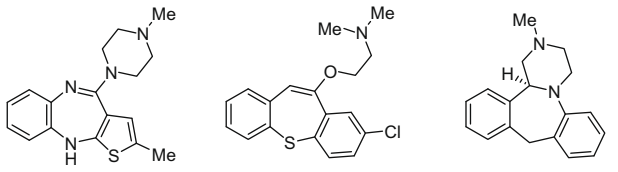


Figure 4. NRI Pharmacophore model with (*S*,*S*)-reboxetine overlaid.



15 olanzapine
h5-HT_{2A} K_i 4 nM

16 zotepine
h5-HT_{2A} K_i 0.5 nM

17 (*S*)-mianserin
h5-HT_{2A} K_i 3 nM

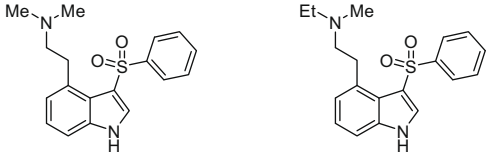
Figure 5. 5-HT_{2A} antagonists that fit the NRI pharmacophore model.

phore model consisting of two aryl rings and a basic amine arranged in a triangular arrangement (Fig. 4), similar to the monoamine transporter inhibitor pharmacophore model reported by Enyedy.[16]

Analysis of a series of 5-HT_{2A} antagonists indicated that olanzapine **15**, zotepine **16** and (*S*)-mianserin **17** fit this NRI pharmacophore model (Fig. 5). This result bears out the similarity of the NRI and 5-HT_{2A} antagonist pharmacophores and supports the use of the NRI pharmacophore model to virtually screen for dual acting NRI/5-HT_{2A} antagonists.

2.2. Virtual screen results

Unity 3-d flex searches were used to search the corporate compound inventory for matches to the NRI pharmacophore model. Additional hit requirements were zero Lipinski violations, polar surface area ⩽120, and no more than 10 rotatable bonds. The virtual screen hits were then further refined to remove highly strained matches and known NRI templates. The refined hits were binned according to ring scaffold and representatives from each scaffold profiled in hNET and h5-HT_{2A} radioligand binding assays. This process successfully identified a number of dual acting compounds including the 4-dialkylaminoethyl-3-(phenylsulfonyl)-1*H*-indoles **18a** and **18b** (Fig. 6) originally prepared as part of a 5-HT_6 program at Wyeth.[22]



18a
hNET K_i 45 nM
h5-HT_{2A} K_i 262 nM

18b
hNET K_i 28 nM
h5-HT_{2A} K_i 222 nM

Figure 6. Dual acting NRI/5-HT_{2A} antagonists identified from the virtual screen. Values reported were generated from radioligand binding assays using the human norepinephrine transporter and human 5-HT_{2A} receptor.

3. Chemistry

To develop the structure–activity relationship (SAR) around the lead molecules, **18a** and **18b**, chemical syntheses were designed to explore the attachment point of the aminoethyl chain to the indole nucleus, the amine portion of the molecule and the length of the alkylamine chain.

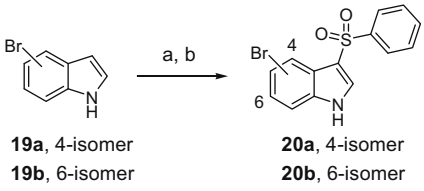
Two methods were utilized to prepare the key bromo-3-(phenylsulfonyl)-1*H*-indole intermediates. The 4-bromo-**20a** and 6-bromo-**20b** analogs were prepared from commercially available 4-bromoindole **19a** and 6-bromoindole **19b**, respectively (Scheme 1).

Electrophilic sulfenylation[23] of the bromoindoles **19a–b** on treatment with thiophenol and potassium triiodide afforded the 3-phenylsulfenyl indoles. Oxidation with OXONE® then gave the bromo-3-(phenylsulfonyl)-1*H*-indoles **20a–b**.

The 5-bromo-**20c** and 7-bromo-**20d** analogs were prepared from the corresponding nitrobenzenes utilizing a vicarious nucleophilic substitution (VNS) approach described by Wojciechowski and Makosza[24] (Scheme 2).

VNS reaction of the bromo-nitrobenzenes **21a–b** with the anion derived from chloromethyl phenyl sulfone gave the 1-nitro-2-[(phenylsulfonyl)methyl]benzenes **22a–b**. Reduction of the nitro group to an amine followed by ethyl formimidate formation and base catalyzed cyclization afforded the 5- and 7-bromo-3-(phenylsulfonyl)-1*H*-indoles **20c** and **20d**, respectively.

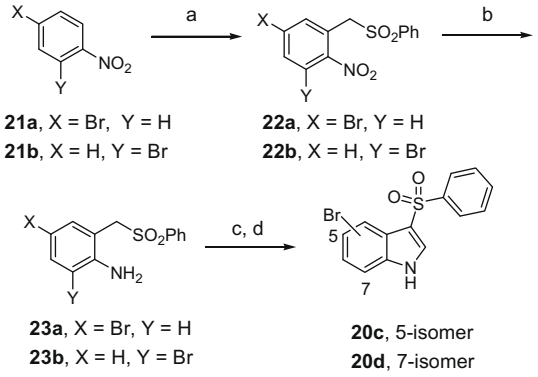
The bromo intermediates were then converted to the desired aminoethyl analogs (Scheme 3). Stille reaction of bromoindoles



19a, 4-isomer
19b, 6-isomer

20a, 4-isomer
20b, 6-isomer

Scheme 1. Reagents and conditions: (a) PhSH, KI_3, EtOH, 92–99% yield; (b) OXONE®, $NaHCO_3$, **aq** acetone, 80–90% yield.



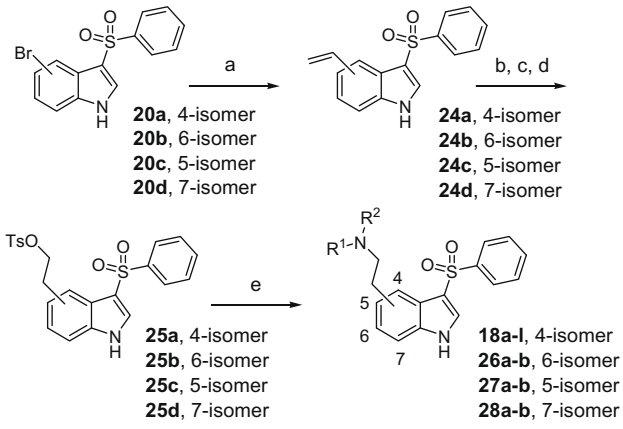
21a, X = Br, Y = H
21b, X = H, Y = Br

22a, X = Br, Y = H
22b, X = H, Y = Br

23a, X = Br, Y = H
23b, X = H, Y = Br

20c, 5-isomer
20d, 7-isomer

Scheme 2. Reagents and conditions: (a) $ClCH_2SO_2Ph$, KO^{t}Bu, −78 °C to rt, 72–73% yield; (b) H_2, Raney-Ni, EtOAc, EtOH, 99% yield; (c) *p*TsOH, $HC(OEt)_3$, DCE, reflux; (d) KO^{t}Bu, THF, rt, 72–95% yield (two steps).



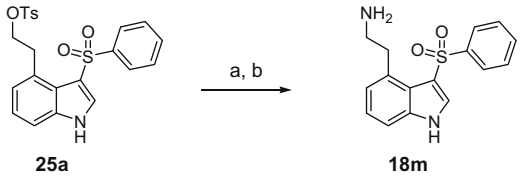
20a, 4-isomer
20b, 6-isomer
20c, 5-isomer
20d, 7-isomer

24a, 4-isomer
24b, 6-isomer
24c, 5-isomer
24d, 7-isomer

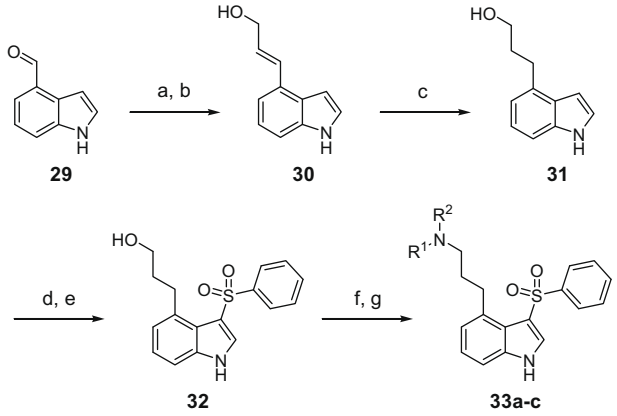
25a, 4-isomer
25b, 6-isomer
25c, 5-isomer
25d, 7-isomer

18a-l, 4-isomer
26a-b, 6-isomer
27a-b, 5-isomer
28a-b, 7-isomer

Scheme 3. Reagents and conditions: (a) tributyl(vinyl)tin, $PdCl_2(PPh_3)_2$, PhMe, reflux, 74–99% yield; (b) BH_3-THF, THF, rt; (c) H_2O_2, aq NaOH, 53–76% yield (two steps); (d) *p*TsCl, pyr, CH_3CN, rt, 86–96% yield; (e) R^1R^2NH, THF, 70 °C, 62–99% yield.



Scheme 4. Reagents and conditions: (a) NaN_3, DMF, 100 °C, 93% yield; (b) H_2, Pd/C, EtOH, 86% yield.



Scheme 5. Reagents and conditions: (a) $EtO_2CCH_2P(O)(OEt)_2$, K_2CO_3, THF, reflux, 95% yield; (b) Dibal-H, THF, −78 °C to rt, 90% yield; (c) H_2, Pd/C, EtOH, EtOAc, 93% yield; (d) PhSH, KI, I2, EtOH, 65 °C, 71% yield; (e) OXONE®, $NaHCO_3$, acetone, H_2O, rt, 90% yield; (f) *p*TsCl, pyr, CH_3CN, rt, 32% yield; (g) R^1R^2NH, THF, 65 °C, 43–69% yield.

20a–d with tributyl(vinyl)tin afforded styrenes **24a–d**, which were hydroborated and oxidized to give the primary alcohols. The alcohols were then converted to the *p*-toluenesulfonates **25a–d** and the tosylate group readily displaced by a variety of basic amines to afford the desired targets **18a–l**, **26a–b**, **27a–b**, and **28a–b**.

The primary amine **18m** was prepared via a standard two step aziridination, reduction procedure (Scheme 4).

The 4-aminopropyl-3-(phenylsulfonyl)-1*H*-indoles **33a–c** were prepared from 4-formylindole **29** (Scheme 5).

Allylic alcohol **30** was prepared from 4-formylindole **29** according to the procedure of Kardos and Genet.[25] Catalytic hydrogenation afforded **31**, which was subjected to the electrophilic sulfenylation/oxidation protocol previously described to give 3-phenylsulfonylindole **32**. Conversion of the primary alcohol to the *p*-toluenesulfonate followed by displacement of the tosylate group with basic amines gave 4-aminopropyl-3-(phenylsulfonyl)-1*H*-indoles **33a–c**.

4. Results and discussion

4.1. In vitro characterization

Compounds **18a–m**, **26a–b**, **27a–b**, **28a–b**, and **33a–c** were evaluated in vitro to determine their affinity for the hNET and ability to inhibit the uptake of NE into MDCK-Net6 cells stably transfected with the human NET. Selected compounds (hNET IC_{50} <1 μM) were then evaluated to determine their affinity for the h5-HT_{2A} receptor and functional h5-HT_{2A} activity.

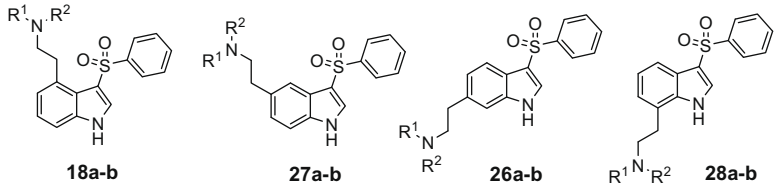
The initial lead compounds, **18a** and **18b**, were potent inhibitors of norepinephrine reuptake (hNET IC_{50} 80 and 49 nM, respectively) and possessed modest h5-HT_{2A} antagonist activity (h5-HT_{2A} IC_{50} 459 and 832 nM, respectively).

A systematic evaluation of attachment point of the aminoethyl chain to the indole nucleus was first explored. It was found that moving the aminoethyl chain from the 4-position to the 5-, 6-, or 7-position on the indole nucleus was clearly detrimental to NRI potency (**18a–b** vs **27a-b, 26a–b,** and **28a–b**) (see Table 1). This is in contrast to the behavior at the 5-HT_6 receptor, where the 5- and 7-aminoethyl-3-(phenylsulfonyl)-1*H*-indoles behave as potent full antagonists and the 4-aminoethyl-3-(phenylsulfonyl)-1*H*-indoles as low affinity, partial antagonists.

Exploration of the amine functionality within the 4-aminoethyl series was then initiated (see Table 2). The isopropylamine **18f** (hNET IC_{50} 51 nM), and piperidine **18i** (hNET IC_{50} 47 nM) analogs possessed similar NRI potency to the lead **18b** (hNET IC_{50} 49 nM). Secondary amines larger than isopropyl led to a reduction in NRI potency (**18f** vs **18g**) as did methyl substitution on the piperidine analog (**18i** vs **18k** and **18l**).

Several analogs possessed improved h5-HT_{2A} receptor antagonist activity over the initial leads (**18a** and **18b**). The cyclopentyl-

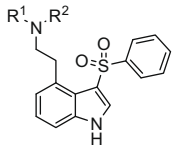
Table 1
Characterization of 4-, 5-, 6- and 7-dialkylaminoethyl-3-(phenylsulfonyl)-1*H*-indoles **18a–b**, **26a–b**, **27a–b** and **28a–b** at the human norepinephrine transporter and human 5-HT_{2A} receptor[a]



Compd	R^1	R^2	hNET K_i, nM[b] (SD)	hNET IC_{50}, nM[c] (SD)	h5-HT_{2A} K_i, nM[d] (SD)	h5-HT_{2A} IC_{50}, nM[e] (SD)
18a	Me	Me	45 (2)	80 (12)	262 (34)	459 (158)
18b	Et	Me	28 (9)	49 (11)	222 (145)	832 (435)
27a	Me	Me		43%[f]		
27b	Et	Me		48%[f]		
26a	Me	Me		12%[f]		
26b	Et	Me		<10%[f]		
28a	Me	Me		<10%[f]		
28b	Et	Me		<10%[f]		

[a] Values are the mean of between 2 and 7 independent runs each in triplicate, unless otherwise indicated.
[b] Inhibition of [^{3}H] nisoxetine binding to MDCK-Net6 cells, stably transfected with the human norepinephrine transporter (hNET).
[c] Inhibition of norepinephrine uptake in MDCK-Net6 cells, stably transfected with the human NET.
[d] Inhibition of [^{3}H] ketanserin binding to membranes from CHO cells, stably transfected with the human 5-HT_{2A} receptor.
[e] Inhibition of DOI induced intracellular calcium mobilization in CHO cells, stably transfected with the human 5-HT_{2A} receptor, measured using FLIPR.
[f] Percentage inhibition measured at a concentration of 1000 nM, data is the average of three triplicate runs.

Table 2
Characterization of 4-aminoethyl-3-(phenylsulfonyl)-1*H*-indoles **18a–m** at the human norepinephrine transporter and human 5-HT_{2A} receptor[a]

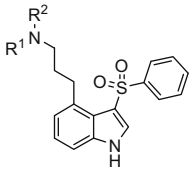


18a-m

Compd	R^1	R^2	hNET K_i, nM (SD)	hNET IC_{50}, nM (SD)	h5-HT_{2A} K_i, nM (SD)	h5-HT_{2A} IC_{50}, nM (SD)
18m	H	H	792 (88)	960 (234)	32%[b]	0%[b]
18c	Me	H	306 (13)	353 (50)	273 (88)	1105[c]
18d	Et	H	78 (11)	146 (27)	389 (37)	1025 (259)
18e	*n*Pr	H	487 (27)	302 (112)	367 (88)	1078[c]
18f	*i*Pr	H	25 (3)	51 (8)	110 (76)	650 (284)
18g	*c*Pent	H	428 (28)	460 (82)	19 (8)	31 (18)
18a	Me	Me	45 (2)	80 (12)	262 (34)	459 (158)
18b	Et	Me	28 (9)	49 (11)	222 (145)	832 (435)
18h	Pyrrolidine		73 (6)	128 (47)	117 (51)	258 (102)
18i	Piperidine		27 (4)	47 (22)	59 (15)	180 (83)
18j	Homopiperidine		208 (62)	221 (67)	33 (18)	69 (63)
18k	2-Me piperidine		125 (30)	116 (32)	89 (20)	92 (29)
18l	3-Me piperidine		1130 (424)	1092 (124)	46 (18)	57 (15)

[a] Values are the mean of between 2 and 7 independent runs each in triplicate, unless otherwise indicated.
[b] Percentage inhibition measured at a concentration of 1000 nM.
[c] Values are for a single experiment run in triplicate.

Table 3
Characterization of 4-dialkylaminopropyl-3-(phenylsulfonyl)-1*H*-indoles **33a–c** at the human norepinephrine transporter and human 5-HT_{2A} receptor[a]



33a-c

Compd	R^1	R^2	hNET K_i, nM (SD)	hNET IC_{50}, nM (SD)	h5-HT_{2A} K_i, nM (SD)	h5-HT_{2A} IC_{50}, nM
33a	Et	Me	21 (9)	29 (1)	713 (174)	477[b]
33b	*i*Pr	H	13 (2)	12 (0.5)	619 (374)	5979[b]
33c	Piperidine		1030 (199)	707 (226)	854 (382)	7972[b]

[a] Values are the mean of between 2 and 3 independent runs each in triplicate, unless otherwise indicated.
[b] Values are for a single experiment run in triplicate.

amine **18g** (h5-HT_{2A} IC_{50} 31 nM), homopiperidine **18j** (h5-HT_{2A} IC_{50} 69 nM), and 3-methyl piperidine **18l** (h5-HT_{2A} IC_{50} 57 nM) analogs were all potent h5-HT_{2A} antagonists.

Finally, increasing the alkyl chain length between the basic amine and the indole nucleus from 2 to 3 carbons was explored (see Table 3). The ethyl methylamine **33a** (hNET IC_{50} 29 nM) and isopropylamine **33b** (hNET IC_{50} 12 nM) analogs were potent NRIs with improved NRI activity over the original leads (**18a** and **18b**). The ethyl methylamine analog **33a** also retained similar h5-HT_{2A} receptor antagonist activity to the leads. In this series, incorporation of a piperidine moiety was detrimental to both NRI and h5-HT_{2A} activity (**33c**, hNET IC_{50} 707 nM, h5-HT_{2A} IC_{50} 7972 nM).

4.2. Selectivity screening

A challenge in designing molecules with activity at more than one target is in achieving selectivity over related biological targets. The piperidine analog **18i** possessed a good balance of NRI (hNET IC_{50} 47 nM) and 5-HT_{2A} antagonist activities (h5-HT_{2A} IC_{50} 180 nM) and was counter-screened against the human serotonin transporter (hSERT) and the human dopamine transporter (hDAT) and against h5-HT_{2B}, h5-HT_{2C} and h5-HT_6 receptors (see Table 4). Piperidine **18i** was highly selective for the NE transporter over the 5-HT and DA transporters, exhibiting poor functional activity at the hSERT (0% inhibition of 5-HT uptake at 1 μM) and weak affinity for the hDAT (3% inhibition of [^{3}H]WIN-35,428 binding at 1 μM). Additionally, **18i** possessed 40-fold greater affinity for the h5-HT_{2A} receptor over the h5-HT_{2C} receptor and little affinity for the 5-HT_{2B} receptor was observed. The 3-phenylsulfonylindole template is a well established 5-HT_6 scaffold,[22,26] however, by incorporating the aminoethyl chain at the 4-position of the indole nucleus, 5-HT_6 affinity was minimized and **18i** possessed sevenfold greater affinity for the 5-HT_{2A} receptor over the 5-HT_6 receptor.

5. In vivo characterization

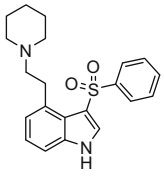
To determine the brain penetration potential for **18i**, plasma, brain, and hypothalamus concentrations were determined following a single sub-cutaneous dose of 30 mg/kg (see Table 5).

Compound **18i** exhibited poor brain to plasma (0.05) and hypothalamus to plasma ratios (0.12), obtained by comparing plasma area-under-the-curve (AUC) to brain and hypothalamus AUCs, respectively. However, the observed C_{max} in the brain (348 ng/g, 0.94 μM) and hypothalamus (782 ng/g, 2.12 μM) was significantly above the hNET and h5-HT_{2A} IC_{50} values.

NE has been shown to stimulate areas of the hypothalamus that are important in temperature regulation[27] and NRIs[28] and SNRIs[29] have proven successful in restoring normal thermoregulation in rat models of vasomotor disorders. Compound **18i** was assessed in a telemetric rat model of ovariectomized (OVX)-induced thermoregulatory dysfunction.[29,30]

Intact cycling rats exhibit a diurnal rhythm during which, over a 24 h period, their tail-skin temperature (TST) decreases during the dark (active) phase and remains elevated during the light (inactive)

Table 4
Selectivity screening of **18i** versus serotonin and dopamine transporters and 5-HT_{2B}, 5-HT_{2C} and 5-HT_6 receptors[a]



18i

Compd	Amine transporters			5-HT_2 receptors			5-HT_6 receptor
	hNET IC_{50}, nM (SD)	hSERT uptake % inhibition at 1 μM[b]	hDAT binding % inhibition at 1 μM[c]	h5-HT_{2A} K_i, nM (SD)	h5-HT_{2B} binding % inhibition at 5 μM[d]	h5-HT_{2C} K_i, nM[f]	h5-HT_6 K_i, nM[g]
18i	47 (22)	0%[e]	3%[e]	59 (15)	28%[e]	2400[e]	434[e]

[a] Values are the mean of between 2 and 6 independent runs each in triplicate, unless otherwise indicated.
[b] Inhibition of serotonin uptake in JAR cells, stably transfected with the human serotonin transporter (hSERT).
[c] Inhibition of [^{3}H] WIN-35,428 binding to membranes from CHO cells, stably transfected with the human dopamine transporter (hDAT).
[d] Inhibition of [^{3}H] 5-HT binding to membranes from CHO cells, stably transfected with the human 5-HT_{2B} receptor.
[e] Values are for a single experiment run in triplicate.
[f] Inhibition of [^{125}I] DOI binding to membranes from CHO cells, stably transfected with the human 5-HT_{2C} receptor.
[g] Inhibition of [^{3}H] LSD binding to membranes from HeLa cells, stably transfected with the human 5-HT_6 receptor.

Table 5
Average pharmacokinetic parameters of **18i** in female rats following a single sc dose of 30 mg/kg[a]

Matrix	C_{max} (ng/mL or ng/g)	T_{max} (h)	AUC_{last} (h ng/mL or h ng/g)	Ratio AUC_{last} tissue/AUC_{last} plasma
Brain	348	2.5	986	0.05
Hypothalamus	782	2.5	2262	0.12
Plasma	6142	1.5	19,096	

[a] 0.5% Methylcellulose/2% Tween-80 in water vehicle.

phase. Reduction in ovarian hormones as a result of ovariectomy causes the TST of ovariectomized (OVX) rats to remain elevated during both the dark and light phases. A drop in TST of 6 °C is observed for intact rats during the dark (active) phase, but the drop in TST is reduced to approximately 1 °C after ovariectomy. Treatment of OVX-rats with estrogen has been reported to restore the diurnal temperature pattern to that seen in an intact rat.[30] At a dose of 30 mg/kg sc compound **18i** significantly reduced the TST (maximum ΔTST – 3.85 °C) of OVX-rats in the active phase with a prolonged duration of action (>12 h) that lasted the entire dark phase (see Fig. 7 and Table 6).

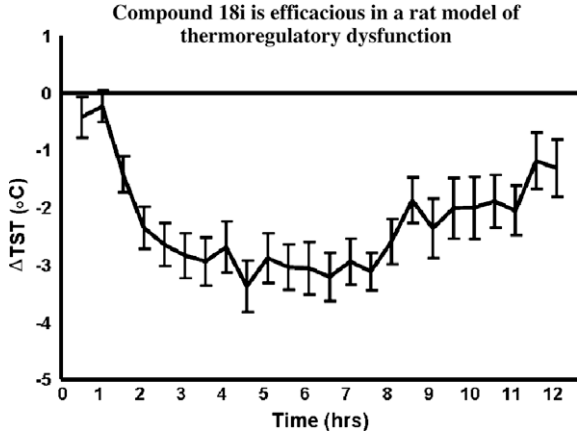


Figure 7. Effect of **18i** (30 mg/kg sc) in a telemetric rat model of ovariectomized (OVX)-induced thermoregulatory dysfunction. Error bars represent standard error of the mean of each data point. Test compound administered 30 min prior to dark phase.

Table 6
Summary of the activity of **18i** in a telemetric rat model of ovariectomized (OVX)-induced thermoregulatory dysfunction[a]

Dose (mg/kg)	Onset of activity (h)	Duration of action (h)	Mean reduction in TST (°C)	Maximum reduction in TST (°C)
30	0.5	>12	−2.40	−3.85

[a] Compound dosed sc (n = 16 rats), 0.5% methylcellulose/2% Tween-80 in water vehicle.

6. Conclusion

A pharmacophore model was derived from a diverse series of five NRIs and was used to virtually screen the corporate compound inventory for dual acting NRI/5-HT_{2A} receptor antagonists. This approach successfully identified the dual acting 4-dialkylaminoethyl-3-(phenylsulfonyl)-1*H*-indoles, **18a** and **18b**, a novel class of NRIs. Exploration of the attachment point of the aminoethyl chain to the indole nucleus, the amine portion of the molecule and the length of the alkylamine chain identified piperidine **18i** (hNET IC_{50} 47 nM, h5-HT_{2A} IC_{50} 180 nM), a balanced NRI and 5-HT_{2A} antagonist. In addition, **18i** exhibited good selectivity for the hNET over the hSERT and hDAT, and for the h5-HT_{2A} receptor over the 5-HT_{2B} and 5-HT_{2C} receptors. Compound **18i** was assessed in a telemetric rat model of ovariectomized (OVX)-induced thermoregulatory dysfunction and produced a significant and long lasting drop in TST at a dose of 30 mg/kg sc.

The 4-aminoalkyl-3-(phenylsulfonyl)-1*H*-indole scaffold could also be utilized to access potent and selective NRIs (e.g., **33b**, hNET IC_{50} 12 nM, >100-fold selective over the h5-HT_{2A} receptor) and h5-HT_{2A} receptor antagonists (e.g., **18g**, h5-HT_{2A} IC_{50} 31 nM, 15-fold selective over the hNET).

7. Experimental

7.1. General methods: chemistry

Solvents were purchased as anhydrous grade and were used without further purification. ^{1}H NMR spectra were recorded on a Varian INOVA 500 instrument, and chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard tetramethylsilane in $CDCl_3$ or DMSO-d_6. Electrospray (ESI) mass

spectra were recorded using a Waters Alliance-ZMD mass spectrometer. Electron impact ionization (EI, EE = 70 eV) mass spectra were recorded on a Finnigan Trace mass spectrometer. Analytical thin layer chromatography (TLC) was performed on pre-coated plates (Silica Gel 60 F-254) and were visualized using UV light and/or staining with a phosphomolybdic acid solution in methanol. In general, compound purity was assessed by ^{1}H NMR and an LC/UV/MS method.[31] Biological results were obtained on compounds of >95% chemical purity as determined by the above methods.

7.1.1. 4-Bromo-3-(phenylthio)-1*H*-indole

To a solution of 4-bromoindole (**19a**) (5.68 g, 0.029 mol) and thiophenol (2.97 mL, 0.029 mol) in ethanol (140 mL) at room temperature under air was added a solution of potassium iodide (4.81 g, 0.029 mol) and iodine (7.35 g, 0.029 mol) in 3:1 v/v water: ethanol (100 mL) and the resulting black reaction mixture stirred at 60 °C for 3 days. The reaction mixture was then diluted with ethyl acetate (1 L), washed with 5% aq $Na_2S_2O_3$ (800 mL), water (1 L) and 1/2 saturated brine (1 L), dried over Na_2SO_4 and concentrated to afford a brown solid. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (90/10 to 80/20) to give 4-bromo-3-(phenylthio)-1*H*-indole (8.1 g, 92%) as a tan crystalline solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.97 (d, J = 7.8 Hz, 2H), 7.02–7.11 (m, 2H), 7.19 (t, J = 7.8 Hz, 2H), 7.24 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.83 (s, 1H), 12.01 (br s, 1H); MS (ESI) m/z 301.9 ([M−H]$^-$).

7.1.2. 6-Bromo-3-(phenylthio)-1*H*-indole

Prepared in essentially the same manner replacing 4-bromoindole (**19a**) with 6-bromoindole (**19b**). Yield 99% of an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 6.97–7.03 (m, 2H), 7.03–7.10 (m, 1H), 7.14–7.24 (m, 3H), 7.31 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 1.8 Hz, 1H), 7.79 (s, 1H), 11.80 (br s, 1H); MS (ESI) m/z 302.1 ([M−H]$^-$).

7.1.3. 4-Bromo-3-(phenylsulfonyl)-1*H*-indole (20a)

To a solution of 4-bromo-3-(phenylthio)-1*H*-indole (8.1 g, 0.0266 mol) in acetone (330 mL) was added 0.2 M aq $NaHCO_3$ (330 mL) followed by OXONE® (40.92 g, 0.0666 mol) added over ~5 min and the reaction mixture stirred at room temperature for 63 h. The acetone was then removed under reduced pressure and the resulting aqueous suspension partitioned between ethyl acetate (750 mL) and water (750 mL). The organic phase was separated, washed with water (750 mL) and 1/2 saturated brine (500 mL), dried over Na_2SO_4 and concentrated to afford a cream solid. The crude product was suspended in 2:1 v/v hexanes:ethyl acetate (600 mL), the suspension stirred vigorously for 12 h then filtered to afford 4-bromo-3-(phenylsulfonyl)-1*H*-indole **20a** (8.08 g, 90%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.13 (dd, J = 8.1 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.48–7.64 (m, 4H), 7.80–7.88 (m, 2H), 8.38 (s, 1H) 12.64 (br s, 1H); MS (ESI) m/z 336.0 ([M+H]$^+$).

7.1.4. 6-Bromo-3-(phenylsulfonyl)-1*H*-indole (20b)

Prepared in essentially the same manner as **20a** replacing 4-bromo-3-(phenylthio)-1*H*-indole with 6-bromo-3-(phenylthio)-1*H*-indole. Yield 80% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.34 (dd, J = 8.5, 1.5 Hz, 1H), 7.51–7.64 (m, 3H), 7.68 (d, J = 1.5 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.91–8.00 (m, 2H), 8.21 (s, 1H), 12.37 (br s, 1H); MS (ESI) m/z 334.1 ([M−H]$^-$).

7.1.5. 5-Bromo-2-nitro-1-(phenylsulfonylmethyl)benzene (22a)

To a stirred solution of 1-bromo-4-nitrobenzene **21a** (5.05 g, 25 mmol) and chloromethylphenylsulfone (4.76 g, 25 mmol) in tetrahydrofuran (50 mL) at −65 °C under nitrogen was added a solution of potassium *tert*-butoxide (1.0 M in tetrahydrofuran, 55 mL, 55 mmol). The deep purple reaction was allowed to warm to 0 °C over 1.5 h and then treated with glacial acetic acid (4 mL). The reaction was diluted with water (100 mL) and saturated aqueous $NaHCO_3$ (100 mL), and then extracted with dichloromethane (2 × 200 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to afford a light orange solid. Trituration with ethyl acetate and hexanes gave 5-bromo-2-nitro-1-(phenylsulfonylmethyl)benzene **22a** (6.45 g, 72%) as a pale yellow solid. ^{1}H NMR (500 MHz, chloroform-d) δ ppm 4.91 (s, 2H), 7.50–7.57 (m, 2H), 7.61 (d, J = 2.0 Hz, 1H), 7.65–7.71 (m, 2H), 7.74 (dd, J = 8.5, 1.1 Hz, 2H), 7.88 (d, J = 8.7 Hz, 1H); MS (ESI) m/z 354.0 [(M−H)$^-$].

7.1.6. 1-Bromo-2-nitro-3-[(phenylsulfonyl)methyl]benzene (22b)

Prepared in essentially the same manner as **22a** replacing 1-bromo-4-nitrobenzene **21a** with 1-bromo-2-nitrobenzene **21b**. Yield 73% of a tan solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.78 (s, 2H), 7.43 (d, J = 7.9 Hz, 1H), 7.53 (t, J = 7.9 Hz, 1H), 7.63 (t, J = 7.8 Hz, 2H), 7.69–7.73 (m, 2H), 7.75–7.80 (m, 1H), 7.91 (dd, J = 8.2, 1.2 Hz, 1H); MS (ESI) m/z 354.0 ([M−H]$^-$).

7.1.7. {4-Bromo-2-[(phenylsulfonyl)methyl]phenyl}amine (23a)

A solution of 5-bromo-2-nitro-1-(phenylsulfonylmethyl)benzene **22a** (0.23 g, 0.64 mmol) in ethyl acetate (30 mL) was hydrogenated in the presence of catalytic Raney nickel and hydrogen (45 psi) for 1 h. The reaction mixture was then filtered through Celite® and concentrated to give {4-bromo-2-[(phenylsulfonyl)methyl]phenyl}amine **23a** (0.21 g, 99%) as a light brown solid. ^{1}H NMR (500 MHz, chloroform-d) δ ppm 4.36 (s, 2H), 5.15 (br s, 2H), 6.71 (d, J = 2.3 Hz, 1H), 6.79 (d, J = 8.5 Hz, 1H), 7.25 (dd, J = 8.5, 2.3 Hz, 1H), 7.56 (dd, J = 8.2, 7.5 Hz, 2H), 7.69 (t, J = 7.5 Hz, 1H), 7.79 (dd, J = 8.2, 1.0 Hz, 2H); MS (ESI) m/z 326.0 [(M+H)$^+$].

7.1.8. {2-Bromo-6-[(phenylsulfonyl)methyl]phenyl}amine (23b)

Prepared in essentially the same manner as **23a** replacing 5-bromo-2-nitro-1-(phenylsulfonylmethyl)benzene **22a** with 1-bromo-2-nitro-3-[(phenylsulfonyl)methyl]benzene **22b**. Yield 99% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.70 (s, 2H), 5.25 (s, 2H), 6.40 (dd, J = 7.9, 7.6 Hz, 1H), 6.80 (dd, J = 7.6, 1.2 Hz, 1H), 7.34 (dd, J = 7.9, 1.2 Hz, 1H), 7.59 (dd, J = 7.5, 7.3 Hz, 2H), 7.72 (t, J = 7.5 Hz, 1H), 7.78 (d, J = 7.3 Hz, 2H); MS (ESI) m/z 326.0 ([M+H]$^+$).

7.1.9. 5-Bromo-3-(phenylsulfonyl)-1*H*-indole (20c)

A stirred solution of {4-bromo-2-[(phenylsulfonyl)methyl]-phenyl}amine **23a** (3.26 g, 10.00 mmol, *para*-toluenesulfonic acid monohydrate (0.20 g) and triethyl orthoformate (8.32 mL, 50 mmol) in 1,2-dichloroethane (70 mL) was heated at reflux under nitrogen for 5 h and then stirred at room temperature for 16 h. The reddish reaction was concentrated under reduced pressure to give a red oil. The resulting crude intermediate iminoether was dissolved in tetrahydrofuran (50 mL) and a solution of potassium *tert*-butoxide (1.0 M in tetrahydrofuran, 13 mL, 13 mmol) added dropwise. After 5 min, a tan precipitate is evident. After 1 h, the reaction is treated with water (30 mL) and NH_4Cl (0.60 g), extracted with dichloromethane (150 mL), dried over $MgSO_4$ and concentrated to afford a light orange solid. Trituration with ethyl acetate and hexanes gave 5-bromo-3-(phenylsulfonyl)-1*H*-indole **20c** (2.78 g, 72%) as a light orange solid. ^{1}H NMR (500 MHz, chloroform-d) δ ppm 7.30 (d, J = 8.7 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.47–7.58 (m, 3H), 7.89 (d, J = 3.1 Hz, 1H), 8.02 (d, J = 8.1 Hz, 2H), 8.09 (s, 1H), 8.95 (br s, 1H); MS (ESI) m/z 334.0 [(M−H)$^-$].

7.1.10. 7-Bromo-3-(phenylsulfonyl)-1*H*-indole (20d)

Prepared in essentially the same manner as **20c** replacing {4-bromo-2-[(phenylsulfonyl)methyl]phenyl}amine **23a** with {2-Bromo-6-[(phenylsulfonyl)methyl]phenyl}amine **23b**. Yield 95% of a yellow foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.15 (dd, J = 8.1, 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.52–7.64 (m, 3H), 7.78 (d, J = 8.1 Hz, 1H), 7.99 (d, J = 7.8 Hz, 2H), 8.21 (d, J = 3.2 Hz, 1H), 12.60 (br s, 1H); MS (ESI) m/z 334.0 ([M−H]$^-$).

7.1.11. 3-(Phenylsulfonyl)-4-vinyl-1*H*-indole (24a)

A solution of 4-bromo-3-(phenylsulfonyl)-1*H*-indole **20a** (8.31 g, 0.0247 mol) and tributyl vinyl tin (10.11 mL, 0.0346 mol) in toluene (210 mL) and dimethoxyethane (70 mL) was purged with nitrogen for 2 h. *trans*-Dichlorobis(triphenylphosphine) palladium(II) (1.73 g, 0.00247 mol) was then added and the reaction mixture heated to reflux for 2 h. Additional *trans*-dichlorobis(triphenylphosphine) palladium(II) (1.73 g, 0.00247 mol) was then added and the mixture heated to reflux for 18 h. 1.0 M aq KF (38.1 mL, 38.1 mmol) was then added to the cooled reaction and mixture stirred at room temperature for 12 h. The mixture was then filtered through silica and the filtrate concentrated under reduced pressure to afford an orange syrup. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (70/30 to 60/40) to give 3-(phenylsulfonyl)-4-vinyl-1*H*-indole **24a** (6.93 g, 99%) as an orange oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.13 (dd, J = 10.8, 1.5 Hz, 1H), 5.56 (dd, J = 17.1, 1.5 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.52 (m, 2H), 7.56–7.61 (m, 1H), 7.64 (dd, J = 17.1, 10.8 Hz, 1H), 7.81 (d, J = 7.6 Hz, 2H), 8.27 (s, 1H), 12.41 (br s, 1H); MS (ESI) m/z 284.1 ([M+H]$^+$).

7.1.12. 3-(Phenylsulfonyl)-6-vinyl-1*H*-indole (24b)

Prepared in essentially the same manner as **24a** replacing 4-bromo-3-(phenylsulfonyl)-1*H*-indole **20a** with 6-bromo-3-(phenylsulfonyl)-1*H*-indole **20b**. Yield 82% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.21 (d, J = 11.0 Hz, 1H), 5.79 (d, J = 17.7 Hz, 1H), 6.80 (dd, J = 17.7, 11.0 Hz, 1H), 7.39 (dd, J = 8.4, 1.1 Hz, 1H), 7.49 (s, 1H), 7.52–7.63 (m, 3H), 7.71 (d, J = 8.4 Hz, 1H), 7.95 (d, J = 7.3 Hz, 2H), 8.17 (s, 1H), 12.29 (br s, 1H); MS (ESI) m/z 284.1 ([M+H]$^+$).

7.1.13. 3-(Phenylsulfonyl)-5-vinyl-1*H*-indole (24c)

Prepared in essentially the same manner as **24a** replacing 4-bromo-3-(phenylsulfonyl)-1*H*-indole **20a** with 5-bromo-3-(phenylsulfonyl)-1*H*-indole **20c**. Yield 74% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.20 (d, J = 11.0 Hz, 1H), 5.75 (d, J = 17.4 Hz, 1H), 6.84 (dd, J = 17.4, 11.0 Hz, 1H), 7.41–7.49 (m, 2H), 7.51–7.64 (m, 3H), 7.76 (s, 1H), 7.98 (d, J = 6.7 Hz, 2H), 8.16 (s, 1H), 12.28 (br s, 1H); MS (ESI) m/z 284.0 ([M+H]$^+$).

7.1.14. 3-(Phenylsulfonyl)-7-vinyl-1*H*-indole (24d)

Prepared in essentially the same manner as **24a** replacing 4-bromo-3-(phenylsulfonyl)-1*H*-indole **20a** with 7-bromo-3-(phenylsulfonyl)-1*H*-indole **20d**. Yield 78% of a yellow oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.42 (d, J = 11.0 Hz, 1H), 5.94 (d, J = 17.4 Hz, 1H), 7.16–7.25 (m, 2H), 7.48 (d, J = 7.3 Hz, 1H), 7.52–7.62 (m, 3H), 7.70 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 6.7 Hz, 2H), 8.21 (d, J = 3.4 Hz, 1H), 12.46 (br s, 1H); MS (ESI) m/z 284.0 ([M+H]$^+$).

7.1.15. 2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethanol

To a solution of 3-(phenylsulfonyl)-4-vinyl-1*H*-indole **24a** (7.05 g, 24.88 mmol) in tetrahydrofuran (140 mL) in a room temperature water bath under nitrogen was added slowly a solution of borane–tetrahydrofuran complex (1.0 M in tetrahydrofuran, 24.88 mL, 24.88 mmol) and the reaction mixture stirred for $2\frac{1}{2}$ h. 10% aq NaOH (11.9 mL, 29.86 mmol) and 30% hydrogen peroxide (3.39 mL, 29.86 mmol) were added sequentially and the mixture stirred at room temperature for $3\frac{1}{2}$ h. The reaction was then quenched by the addition of saturated aq NH_4Cl (140 mL), stirred vigorously for 5 min and then partitioned between ethyl acetate (1 L) and water (1 L). The organic phase was separated, washed with 1/2 saturated brine (1 L), dried over Na_2SO_4 and concentrated to afford an orange foam. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (70/30 to 30/70) to give 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethanol (4.0 g, 53%) as a cream foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.03 (t, J = 7.0 Hz, 2H), 3.22–3.29 (m, 2H), 4.37 (t, J = 5.2 Hz, 1H), 6.97 (d, J = 7.3 Hz, 1H), 7.14 (dd, J = 7.3, 8.2 Hz, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.52–7.65 (m, 3H), 7.82 (d, J = 7.3 Hz, 2H), 8.22 (s, 1H), 12.31 (br s, 1H); MS (ESI) m/z 302.1 ([M+H]$^+$).

7.1.16. 2-[3-(Phenylsulfonyl)-1*H*-indol-6-yl]ethanol

Prepared in essentially the same manner replacing 3-(phenylsulfonyl)-4-vinyl-1*H*-indole **24a** with 3-(phenylsulfonyl)-6-vinyl-1*H*-indole **24b**. Yield 62% of a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.77 (t, J = 7.0 Hz, 2H), 3.53–3.63 (m, 2H), 4.58 (t, J = 5.2 Hz, 1H), 7.05 (dd, J = 8.2, 1.2 Hz, 1H), 7.30 (br. s, 1H), 7.49–7.61 (m, 3H), 7.64 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 7.9, 1.6 Hz, 2H), 8.09 (s, 1H), 12.12 (br s, 1H); MS (ESI) m/z 300.1 ([M−H]$^-$).

7.1.17. 2-[3-(Phenylsulfonyl)-1*H*-indol-5-yl]ethanol

Prepared in essentially the same manner replacing 3-(phenylsulfonyl)-4-vinyl-1*H*-indole **24a** with 3-(phenylsulfonyl)-5-vinyl-1*H*-indole **24c**. Yield 76% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.79 (t, J = 7.0 Hz, 2H), 3.54–3.62 (m, 2H), 4.59 (t, J = 5.3 Hz, 1H), 7.10 (dd, J = 8.2, 1.2 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.50–7.62 (m, 4H), 7.96 (d, J = 8.5 Hz, 2H), 8.11 (d, J = 2.1 Hz, 1H), 12.15 (br s, 1H); MS (ESI) m/z 300.0 ([M−H]$^-$).

7.1.18. 2-[3-(Phenylsulfonyl)-1*H*-indol-7-yl]ethanol

Prepared in essentially the same manner replacing 3-(phenylsulfonyl)-4-vinyl-1*H*-indole **24a** with 3-(phenylsulfonyl)-7-vinyl-1*H*-indole **24d**. Yield 66% of a pink semi-solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.98 (t, J = 6.7 Hz, 2H), 3.62–3.70 (m, 2H), 4.65 (br s, 1H), 7.06 (d, J = 7.5 Hz, 1H), 7.11 (dd, J = 7.5, 7.5 Hz, 1H), 7.50–7.64 (m, 4H), 7.96 (dd, J = 8.1, 1.4 Hz, 2H), 8.13 (s, 1H), 12.24 (br s, 1H); MS (ESI) m/z 302.1 ([M+H]$^+$).

7.1.19. 2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethyl 4-methylbenzenesulfonate (25a)

To a solution of 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethanol (4.0 g, 13.27 mmol) in acetonitrile (100 mL) under nitrogen was added pyridine (2.68 mL, 33.18 mmol) followed by *para*-toluenesulfonyl chloride (3.04 g, 15.93 mmol) and the reaction mixture stirred at room temperature for 2 days. The mixture was then concentrated under reduced pressure to a small volume and partitioned between ethyl acetate (200 mL) and 2.0 M aq HCl (200 mL). The organic phase was separated, washed with 2.0 M aq HCl (200 mL), water (200 mL), and 1/2 saturated brine (200 mL), dried over $MgSO_4$ and concentrated to afford a yellow syrup. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (60/40 to 40/60) to give 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl 4-methylbenzenesulfonate **25a** (5.50 g, 91%) as a white foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.36 (s, 3H), 3.17 (t, J = 6.3 Hz, 2H), 3.87 (t, J = 6.3 Hz, 2H), 6.86 (d, J = 7.3 Hz, 1H), 7.15 (dd, J = 7.3, 8.2 Hz, 1H), 7.29 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 8.2 Hz, 2H), 7.53 (dd, J = 7.6, 7.3 Hz, 2H), 7.58–7.63 (m, 1H),

7.74 (d, J = 7.3 Hz, 2H), 8.19 (s, 1H), 12.39 (br s, 1H); MS (ESI) m/z 455.9 ($[M+H]^+$).

7.1.20. 2-[3-(Phenylsulfonyl)-1*H*-indol-6-yl]ethyl 4-methylbenzenesulfonate (25b)

Prepared in essentially the same manner as **25a** replacing 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethanol with 2-[3-(phenylsulfonyl)-1*H*-indol-6-yl]ethanol. Yield 96% of a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.29 (s, 3H), 2.94 (t, J = 6.3 Hz, 2H), 4.20 (t, J = 6.3 Hz, 2H), 6.98 (dd, J = 8.1, 1.3 Hz, 1H), 7.15 (d, J = 8.2 Hz, 2H), 7.20 (s, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.52–7.61 (m, 3H), 7.64 (d, J = 8.1 Hz, 1H), 7.97 (dd, J = 7.8, 1.7 Hz, 2H), 8.13 (d, J = 3.2 Hz, 1H), 12.14 (br s, 1H); MS (ESI) m/z 454.1 ($[M-H]^-$).

7.1.21. 2-[3-(Phenylsulfonyl)-1*H*-indol-5-yl]ethyl 4-methylbenzenesulfonate (25c)

Prepared in essentially the same manner as **25a** replacing 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethanol with 2-[3-(phenylsulfonyl)-1*H*-indol-5-yl]ethanol. Yield 95% of a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.33 (s, 3H), 2.96 (t, J = 6.3 Hz, 2H), 4.21 (t, J = 6.3 Hz, 2H), 6.99 (dd, J = 8.3, 1.6 Hz, 1H), 7.23 (d, J = 7.8 Hz, 2H), 7.32 (d, J = 8.3 Hz, 1H), 7.46–7.64 (m, 6H), 7.97 (dd, J = 8.3, 1.5 Hz, 2H), 8.13 (d, J = 3.2 Hz, 1H), 12.19 (br s, 1H); MS (ESI) m/z 454.1 ($[M-H]^-$).

7.1.22. 2-[3-(Phenylsulfonyl)-1*H*-indol-7-yl]ethyl 4-methylbenzenesulfonate (25d)

Prepared in essentially the same manner as **25a** replacing 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethanol with 2-[3-(phenylsulfonyl)-1*H*-indol-7-yl]ethanol. Yield 86% of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.33 (s, 3H), 3.16 (t, J = 6.4 Hz, 2H), 4.24 (t, J = 6.4 Hz, 2H), 6.97 (d, J = 7.3 Hz, 1H), 7.07 (dd, J = 7.9, 7.3 Hz, 1H), 7.17 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.52–7.61 (m, 3H), 7.64 (d, J = 7.9 Hz, 1H), 7.97 (dd, J = 8.1, 1.4 Hz, 2H), 8.15 (d, J = 3.4 Hz, 1H), 12.25 (br s, 1H); MS (ESI) m/z 456.2 ($[M+H]^+$).

7.2. General Procedure 1: {2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochlorides (18a–l), {2-[3-(phenylsulfonyl)-1*H*-indol-6-yl]ethyl}amine hydrochlorides (26a–b), {2-[3-(phenylsulfonyl)-1*H*-indol-5-yl]ethyl}amine hydrochlorides (27a–b) and {2-[3-(phenylsulfonyl)-1*H*-indol-7-yl]ethyl}amine hydrochlorides (28a–b)

To a solution of the appropriate 4-methyl-benzenesulfonate **25a**–**25d** (116 mg, 0.25 mmol) in THF (2 mL) was added the appropriate amine (1.25 mmol) and mixture heated to 71 °C for 24 h. The mixture was then partitioned between ethyl acetate (20 mL) and water (20 mL), and the aqueous layer was extracted with ethyl acetate (10 mL). The combined organic layers were washed with 2.0 M aq NaOH, (2 × 15 mL), and saturated brine (30 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel chromatography eluting with 2.0 M ammonia in ethanol solution–dichloromethane (10/90). The product was dissolved in diethyl ether and treated with 1 N HCl in diethyl ether (1.05 equiv) and then filtered to afford the amine hydrochloride.

7.2.1. *N*,*N*-Dimethyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride (18a)

Yield: 85% of a tan solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.63 (br s, 6H), 3.01 (br s, 2H), 3.17 (br m, 2H), 7.03 (d, J = 7.0 Hz, 1H), 7.23 (dd, J = 8.2, 7.0 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.58 (m, 2H), 7.64 (m, 1H), 7.84 (d, J = 7.3 Hz, 2H), 8.21 (d, J = 2.7 Hz, 1H), 9.81 (br s, 1H), 12.49 (br s, 1H); HRMS: m/z calcd for $C_{18}H_{20}N_2O_2S$ + H^+, 329.1317. Found (ESI, $[M+H]^+$), 329.1316.

7.2.2. *N*-Ethyl-*N*-methyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride (18b)

Yield: 97% of a tan solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.24 (t, J = 7.3 Hz, 3H), 2.75 (br s, 3H), 3.01–3.30 (br m, 6H), 7.08 (d, J = 7.3 Hz, 1H), 7.25 (dd, J = 8.1, 7.7 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.59 (m, 2H), 7.65 (m, 1H), 7.87 (m, 2H), 8.20 (d, J = 3.4 Hz, 1H), 10.09 (br s, 1H) 12.55 (br s, 1H); HRMS: m/z calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1474. Found (ESI, $[M+H]^+$), 343.1472.

7.2.3. *N*-Methyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride (18c)

Yield: 94% of a light brown solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.14 (s, 3H), 2.27 (t, J = 7.6 Hz, 2H), 2.97 (t, J = 7.6 Hz, 2H), 6.93 (d, J = 7.3 Hz, 1H), 7.15 (dd, J = 8.2, 7.3 Hz, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.55 (m, 2H), 7.62 (m, 1H), 7.82 (d, J = 7.6 Hz, 2H), 8.23 (s, 1H), 12.31 (br s, 1H); HRMS: m/z calcd for $C_{17}H_{18}N_2O_2S$ + H^+, 315.1161. Found (ESI, $[M+H]^+$), 315.1161.

7.2.4. *N*-Ethyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride (18d)

Yield: 95% of a light brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.19 (t, J = 7.2 Hz, 3H), 2.89 (br s, 2H), 2.99 (br s, 2H), 3.25 (m, 2H), 7.04 (d, J = 7.2 Hz, 1H), 7.23 (dd, J = 7.6, 7.2 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.58 (m, 2H), 7.64 (m, 1H), 7.91 (d, J = 7.0 Hz, 2H), 8.22 (d, J = 3.2 Hz, 1H), 8.70 (br s, 2H), 12.52 (br s, 1H); HRMS: m/z calcd for $C_{18}H_{20}N_2O_2S$ + H^+, 329.1317. Found (ESI, $[M+H]^+$), 329.1316.

7.2.5. *N*-{2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethyl}-*N*-propylamine hydrochloride (18e)

Yield: 96% of a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 0.92 (t, J = 7.3 Hz, 3H), 1.62 (m, 2H), 2.80 (br m, 2H), 3.01 (br m, 2H), 3.24 (m, 2H), 7.03 (d, J = 7.3 Hz, 1H), 7.23 (dd, J = 8.2, 7.3 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.58 (m, 2H), 7.64 (m, 1H), 7.90 (d, J = 7.6 Hz, 2H), 8.22 (d, J = 2.7 Hz, 1H), 8.59 (br s, 2H), 12.50 (br s, 1H); HRMS: m/z calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1474. Found (ESI, $[M+H]^+$), 343.1473.

7.2.6. *N*-{2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethyl}propan-2-amine hydrochloride (18f)

Yield: 95% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.21 (d, J = 6.5 Hz, 6H), 3.00 (br m, 2H), 3.22 (br m, 1H), 3.27 (br m, 2H), 7.06 (d, J = 7.0 Hz, 1H), 7.23 (dd, J = 7.5, 7.0 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.58 (m, 2H), 7.64 (m, 1H), 7.93 (m, 2H), 8.22 (s, 1H), 8.65 (br s, 2H), 12.52 (br s, 1H); HRMS: m/z calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1474. Found (ESI, $[M+H]^+$), 343.1474.

7.2.7. *N*-{2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethyl}cyclopentanamine hydrochloride (18g)

Yield: 99% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.46–1.77 (m, 6H), 1.93 (m, 2H), 3.00 (br m, 2H), 3.29 (br m, 2H), 3.37 (br m, 1H), 7.06 (d, J = 7.3 Hz, 1H), 7.23 (dd, J = 7.5, 7.3 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.58 (m, 2H), 7.64 (m, 1H), 7.93 (m, 2H), 8.21 (d, J = 2.3 Hz, 1H), 8.87 (br s, 2H), 12.53 (br s, 1H); HRMS: m/z calcd for $C_{21}H_{24}N_2O_2S$ + H^+, 369.1630. Found (ESI, $[M+H]^+$), 369.1630.

7.2.8. 3-(Phenylsulfonyl)-4-(2-pyrrolidin-1-ylethyl)-1*H*-indole hydrochloride (18h)

Yield: 98% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.84–2.07 (m, 4H), 3.02 (br s, 2H), 3.27 (br s, 4H), 3.50 (br s, 2H), 7.05 (d, J = 7.2 Hz, 1H), 7.24 (dd, J = 8.1, 7.2 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.56–7.68 (m, 3H), 7.88 (d, J = 7.2 Hz, 2H), 8.21 (d, J = 3.2 Hz, 1H), 10.39 (br s, 1H), 12.53 (br s, 1H); HRMS: m/z calcd for $C_{20}H_{22}N_2O_2S$ + H^+, 355.1474. Found (ESI, $[M+H]^+$), 355.1473.

7.2.9. 3-(Phenylsulfonyl)-4-(2-piperidin-1-ylethyl)-1*H*-indole hydrochloride (18i)

Yield: 95% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (m, 1H), 1.65–1.89 (m, 5H), 2.85 (m, 2H), 3.18 (m, 2H), 3.29 (br m, 2H), 3.39 (br m, 2H), 7.06 (d, J = 7.0 Hz, 1H), 7.25 (dd, J = 7.5, 7.0 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.56–7.69 (m, 3H), 7.86 (d, J = 7.0 Hz, 2H), 8.19 (d, J = 3.1 Hz, 1H), 10.12 (br s, 1H), 12.54 (br s, 1H); HRMS: m/z calcd for $C_{21}H_{24}N_2O_2S$ + H^+, 369.1630. Found (ESI, $[M+H]^+$), 369.1629.

7.2.10. 4-(2-Azepan-1-ylethyl)-3-(phenylsulfonyl)-1*H*-indole hydrochloride (18j)

Yield: 62% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.52–1.73 (m, 4H), 1.77–1.90 (m, 4H), 3.06–3.19 (m, 2H), 3.20–3.48 (m, 6H), 7.09 (d, J = 7.5 Hz, 1H), 7.25 (dd, J = 8.1, 7.5 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.57–7.70 (m, 3H), 7.85 (d, J = 7.5 Hz, 2H), 8.19 (d, J = 3.1 Hz, 1H), 10.05 (br s, 1H), 12.53 (br s, 1H); HRMS: m/z calcd for $C_{22}H_{26}N_2O_2S$ + H^+, 383.1787. Found (ESI, $[M+H]^+$), 383.1787.

7.2.11. 4-[2-(2-Methylpiperidin-1-yl)ethyl]-3-(phenylsulfonyl)-1*H*-indole hydrochloride (18k)

Yield: 69% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.18 (d, J = 6.7 Hz, 1H), 1.28 (d, J = 6.3 Hz, 2H), 1.38–1.91 (m, 6H), 2.78–2.92 (m, 1H), 3.03–3.57 (m, 6H), 7.05 (d, J = 7.0 Hz, 0.66 H), 7.08 (d, J = 7.0 Hz, 0.34 H), 7.22 (m, 1H), 7.44 (m, 1H), 7.52–7.66 (m, 3H), 7.82 (m, 2H), 8.14 (m, 1H), 10.07 (2 $\times$ br s, 1H), 12.50 (br s, 1H); HRMS: m/z calcd for $C_{22}H_{26}N_2O_2S$ + H^+, 383.1787. Found (ESI, $[M+H]^+$), 383.1787.

7.2.12. 4-[2-(3-Methylpiperidin-1-yl)ethyl]-3-(phenylsulfonyl)-1*H*-indole hydrochloride (18l)

Yield: 75% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.92 (d, J = 6.5 Hz, 3H), 1.68–2.02 (m, 4H), 2.75 (m, 1H), 3.08–3.22 (m, 2H), 3.24–3.33 (m, 3H), 3.36–3.48 (m, 3H), 7.06 (d, J = 7.3 Hz, 1H), 7.26 (dd, J = 7.8, 7.3 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.60 (m, 2H), 7.66 (m, 1H), 7.85 (d, J = 7.0 Hz, 2H), 8.20 (d, J = 3.4 Hz, 1H), 10.13 (br s, 1H), 12.54 (br s, 1H); HRMS: m/z calcd for $C_{22}H_{26}N_2O_2S$ + H^+, 383.1787. Found (ESI, $[M+H]^+$), 383.1786.

7.2.13. *N*,*N*-Dimethyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-6-yl]ethyl}amine hydrochloride (26a)

Yield: 80% of a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.78 (s, 6H), 3.05 (m, 2H), 3.26 (m, 2H), 7.12 (dd, J = 8.3, 1.5 Hz, 1H), 7.38 (s, 1H), 7.51–7.62 (m, 3H), 7.73 (d, J = 8.3 Hz, 1H), 7.96 (dd, J = 8.1, 1.5 Hz, 2H), 8.17 (d, J = 2.9 Hz, 1H), 9.84 (br s, 1H), 12.33 (br s, 1H); HRMS: m/z calcd for $C_{18}H_{20}N_2O_2S$ + H^+, 329.1317. Found (ESI, $[M+H]^+$), 329.1317.

7.2.14. *N*-Ethyl-*N*-methyl-2-[3-(phenylsulfonyl)-1*H*-indol-6-yl]ethanamine hydrochloride (26b)

Yield: 90% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.21 (t, J = 7.2 Hz, 3H) 2.75 (br s, 3H) 2.99–3.12 (m, 3H) 3.15–3.30 (m, 3H) 7.13 (d, J = 8.1 Hz, 1H) 7.40 (s, 1H) 7.49–7.63 (m, 3H) 7.72 (d, J = 8.1 Hz, 1H) 7.96 (d, J = 6.7 Hz, 2H) 8.17 (d, J = 3.0 Hz, 1H) 10.03 (br s, 1H) 12.35 (br s, 1H); HRMS: calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1475; found (ESI, $[M+H]^+$), 343.1477.

7.2.15. *N*,*N*-Dimethyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-5-yl]ethyl}amine hydrochloride (27a)

Yield: 77% of a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.82 (s, 6H), 3.06 (m, 2H), 3.28 (m, 2H), 7.16 (dd, J = 8.5, 1.5 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.52–7.63 (m, 3H), 7.71 (s, 1H), 7.98 (d, J = 7.0 Hz, 2H), 8.17 (d, J = 3.0 Hz, 1H), 9.75 (br s, 1H), 12.30 (br s, 1H); HRMS: m/z calcd for $C_{18}H_{20}N_2O_2S$ + H^+, 329.1317. Found (ESI, $[M+H]^+$), 329.1318.

7.2.16. *N*-Ethyl-*N*-methyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-5-yl]ethyl}amine hydrochloride (27b)

Yield: 91% of a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.23 (t, 3H), 2.79 (s, 3H), 3.01–3.16 (br m, 3H), 3.17–3.28 (br m, 3H), 7.18 (dd, J = 8.3, 1.5 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.52–7.63 (m, 3H), 7.72 (s, 1H), 7.98 (d, J = 7.0 Hz, 2H), 8.17 (d, J = 3.4 Hz, 1H), 9.79 (br s, 1H), 12.31 (br s, 1H); HRMS: m/z calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1474. Found (ESI, $[M+H]^+$), 343.1472.

7.2.17. *N*,*N*-Dimethyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-7-yl]ethyl}amine hydrochloride (28a)

Yield: 99% of a yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.80 (s, 6H), 3.27 (s, 4H), 7.11–7.20 (m, 2H), 7.52–7.62 (m, 3H), 7.68 (dd, J = 7.8, 1.1 Hz, 1H), 7.97 (d, J = 6.7 Hz, 2H), 8.26 (s, 1H), 10.16 (br s, 1H), 12.64 (br s, 1H); HRMS: m/z calcd for $C_{18}H_{20}N_2O_2S$ + H^+, 329.1317. Found (ESI, $[M+H]^+$), 329.1316.

7.2.18. *N*-Ethyl-*N*-methyl-*N*-{2-[3-(phenylsulfonyl)-1*H*-indol-7-yl]ethyl}amine hydrochloride (28b)

Yield: 90% of a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.22 (t, 3H), 2.81 (s, 3H), 3.09 (m, 2H), 3.19–3.29 (m, 4H), 7.14–7.21 (m, 2H), 7.52–7.63 (m, 3H), 7.69 (dd, J = 7.2, 1.4 Hz, 1H), 7.97 (d, J = 6.7 Hz, 2H), 8.29 (d, J = 3.0 Hz, 1H), 9.81 (br s, 1H), 12.55 (br s, 1H); HRMS: m/z calcd for $C_{19}H_{22}N_2O_2S$ + H^+, 343.1474. Found (ESI, $[M+H]^+$), 343.1475.

7.2.19. 4-(2-Azidoethyl)-3-(phenylsulfonyl)-1*H*-indole

To a solution of 2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl 4-methyl-benzenesulfonate **25a** (171 mg, 0.30 mmol) in anhydrous DMF (2.5 mL) was added sodium azide (0.14 g, 2.2 mmol) and the mixture heated to 100 °C for 6 h. The cooled reaction mixture was then partitioned between ethyl acetate (20 mL) and water (20 mL), and the aqueous layer extracted with ethyl acetate (10 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, and concentrated to afford the crude product. Purification by silica gel chromatography eluting with hexanes–ethyl acetate (60/40) gave 4-(2-azidoethyl)-3-(phenylsulfonyl)-1*H*-indole (120 mg, 93%) as clear waxy solid. MS (ESI) m/z 327.1 ($[M+H]^+$).

7.2.20. {2-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride (18m)

To a suspension of 10% palladium on carbon (30 mg) in ethanol (10 mL) was added a solution of 4-(2-azidoethyl)-3-(phenylsulfonyl)-1*H*-indole (100 mg, 0.30 mmol) in ethanol (20 mL) and the mixture hydrogenation at 40 psi for 1 h. The reaction mixture was then filtered through Celite® and concentrated in vacuo to give a white solid (0.1 g, 86%). This solid was dissolved in diethyl ether, treated with 1 N HCl in diethyl ether (0.16 mL, 0.16 mmol) and then concentrated to afford {2-[3-(phenylsulfonyl)-1*H*-indol-4-yl]ethyl}amine hydrochloride **18m** as a light brown foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 2.88 (br s, 2H), 3.17 (m, 2H), 7.00 (d, J = 7.3 Hz, 1H), 7.21 (dd, J = 8.2, 7.3 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.57 (m, 2H), 7.63 (m, 1H), 7.90 (br s, 3H), 7.93 (d, J = 7.3 Hz, 2H), 8.24 (d, J = 3.0 Hz, 1H), 12.52 (br s, 1H); HRMS: m/z calcd for $C_{16}H_{16}N_2O_2S$ + H^+, 301.1004. Found (ESI, $[M+H]^+$), 301.1006.

7.2.21. 3-(1*H*-Indol-4-yl)propan-1-ol (31)

To a suspension of 10% palladium on carbon (200 mg) in absolute ethanol (30 mL) was added a solution of (2*E*)-3-(1*H*-indol-4-

yl)prop-2-en-1-ol **30** (prepared according to the procedure of Kardos, N.; Genet, J.-P. *Tetrahedron: Asymmetry* **1994**, *5*, 1525) (0.98 g, 5.66 mmol) in ethyl acetate (30 mL) and the mixture hydrogenated at 50 psi for 15 min. The reaction mixture was filtered through celite and concentrated under reduced pressure to afford a light purple syrup. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (80/20 to 50/50) to give 3-(1*H*-indol-4-yl)propan-1-ol **31** (0.92 g, 93%) as a colorless syrup. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.72–1.87 (m, 2H), 2.82 (t, J = 7.8 Hz, 2H), 3.44 (m, 2H), 4.43 (t, J = 5.2 Hz, 1H), 6.44 (m, 1H), 6.77 (d, J = 7.3 Hz, 1H), 6.96 (dd, J = 8.1, 7.3 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 7.27 (m, 1H), 11.00 (br s, 1H); MS (ESI) m/z 176.2 [(M+H)$^+$].

7.2.22. 3-[3-(Phenylthio)-1*H*-indol-4-yl]propan-1-ol

To a solution of 3-(1*H*-indol-4-yl)propan-1-ol **31** (0.91 g, 5.19 mmol) and thiophenol (0.53 mL, 5.19 mmol) in absolute ethanol (30 mL) was added a solution of potassium iodide (0.862 g, 5.19 mmol) and iodine (1.318 g, 5.19 mmol) in ethanol (7.5 mL) and water (22.5 mL) over 5 min. The reaction was then heated at 65 °C for 5$\frac{1}{2}$ h. The cooled reaction mixture was diluted with ethyl acetate (250 mL), washed with 5% aq $Na_2S_2O_3$ solution (250 mL), water (250 mL) and brine (250 mL), dried over Na_2SO_4, and concentrated to afford an off-white solid. The crude product was purified by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (75/25 to 25/75) to afford a white solid. The product was recrystallized from 3:1 v/v hexanes:ethyl acetate (60 mL) to afford 3-[3-(phenylthio)-1*H*-indol-4-yl]propan-1-ol (1.048 g, 71%) as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.63 (m, 2H), 2.90 (m, 2H), 3.29 (m, 2H), 4.30 (t, J = 5.1 Hz, 1H), 6.80 (d, J = 7.3 Hz, 1H), 6.95 (d, J = 7.3 Hz, 2H), 7.00–7.10 (m, 2H), 7.19 (m, 2H), 7.30 (d, J = 7.3 Hz, 1H), 7.67 (d, J = 2.9 Hz, 1H), 11.67 (br s, 1H); MS (ESI) m/z 284.1 [(M+H)$^+$].

7.2.23. 3-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]propan-1-ol (32)

To a solution of 3-[3-(phenylthio)-1*H*-indol-4-yl]propan-1-ol (1.024 g, 3.61 mmol) in acetone (45 mL) was added a solution of sodium hydrogen carbonate (0.759 g, 9.03 mmol) in water (45 mL) followed by OXONE® (supplied by DuPont, potassium peroxymonosulfate as active ingredient) (5.55 g, 9.03 mol) and the reaction mixture stirred at room temperature for 26 h. The acetone was removed under reduced pressure and the resulting suspension partitioned between ethyl acetate (120 mL) and water (100 mL). The organic phase was separated, washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated to afford a white foam. Ethyl acetate (30 mL) was added to the crude product and the mixture stirred vigorously for 2 h then filtered to afford 3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propan-1-ol **32** (1.027 g, 90%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.39 (m, 2H), 2.82 (m, 2H), 3.28 (m, 2H), 4.33 (t, J = 5.1 Hz, 1H), 6.91 (d, J = 7.3 Hz, 1H), 7.16 (dd, J = 7.5, 7.3 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.49–7.65 (m, 3H), 7.80 (m, 2H), 8.22 (s, 1H), 12.31 (br s, 1H); MS (ESI) m/z 314.0[(M−H)$^-$].

7.2.24. 3-[3-(Phenylsulfonyl)-1*H*-indol-4-yl]propyl 4-methylbenzenesulfonate

To a solution of 3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propan-1-ol **32** (1.006 g, 3.19 mmol) in acetonitrile (25 mL) under nitrogen was added pyridine (0.65 mL, 7.97 mmol) followed by *para*-toluenesulfonyl chloride, and the reaction mixture stirred at room temperature for 11 days. The reaction mixture was then concentrated to a small volume, and the mixture partitioned between ethyl acetate (100 mL) and 1.0 M aq HCl (100 mL). The organic phase was separated, washed with water (2 × 100 mL) and brine (200 mL), dried over Na_2SO_4, and concentrated to afford a yellow syrup. Purification by silica gel chromatography eluting with a hexanes–ethyl acetate gradient (100/0 to 60/40) afforded 3-phenylsulfonyl-4-(3-chloro-propyl)-1*H*-indole (0.51 g, 48%) as a colorless foam. MS (ESI) m/z 334.3 [(M+H)$^+$]. Further elution afforded 3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propyl 4-methylbenzenesulfonate (0.48 g, 32%) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.58 (m, 2H), 2.42 (s, 3H), 2.77 (m, 2H), 3.90 (t, J = 6.3 Hz, 2H), 6.78 (d, J = 7.2 Hz, 1H), 7.11 (dd, J = 7.4, 7.2 Hz, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.44–7.54 (m, 4H), 7.59 (m, 1H), 7.70–7.81 (m, 4H), 8.22 (s, 1H), 12.36 (br s, 1H); MS (ESI) m/z 469.7 [(M+H)$^+$].

7.3. General procedure 2: 3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propan-1-amine hydrochlorides (33a–c)

To a solution of 3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propyl 4-methylbenzenesulfonate (0.21 g, 0.447 mmol) in tetrahydrofuran (5 mL) was added the appropriate amine (8.944 mmol) and the reaction mixture heated to 65 °C in a sealed vessel for 16 h. The cooled reaction mixture was then diluted with 1.0 M aq NaOH (50 mL) and the resulting milky suspension extracted with ethyl acetate (50 mL). The organic phase was separated, washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. The product was dissolved in absolute ethanol, 1.25 M HCl in ethanol (1.3 equiv) added and the resulting suspension filtered to afford the amine hydrochloride.

7.3.1. *N*-Ethyl-*N*-methyl-3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propan-1-amine hydrochloride (33a)

Yield: 69% as white needles; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.18 (t, 3H), 1.80 (m, 2H), 2.64 (s, 3H), 2.82 (m, 2H), 2.89 – 3.08 (br m, 4H), 6.99 (d, J = 6.7 Hz, 1H), 7.20 (dd, J = 7.2, 6.7 Hz, 1H), 7.41 (d, J = 7.2 Hz, 1H), 7.54–7.68 (m, 3H), 7.82 (m, 2H), 8.21 (d, J = 3.3 Hz, 1H), 9.88 (br s, 1H), 12.46 (br s, 1H); HRMS: m/z calcd for $C_{20}H_{24}N_2O_2S + H^+$, 357.1630. Found (ESI, [M+H]$^+$), 357.1628.

7.3.2. *N*-Isopropyl-3-[3-(phenylsulfonyl)-1*H*-indol-4-yl]propan-1-amine hydrochloride (33b)

Yield: 57% of a white crystalline solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.20 (d, J = 6.7 Hz, 6H), 1.78 (m, 2H), 2.78 (m, 2H), 2.87 (m, 2H), 3.22 (m, 1H), 6.98 (d, J = 7.2 Hz, 1H), 7.20 (dd, J = 7.7, 7.2 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.53–7.67 (m, 3H), 7.82 (d, J = 6.9 Hz, 2H), 8.21 (s, 1H), 8.52 (br s, 2H), 12.44 (br s, 1H); HRMS: m/z calcd for $C_{20}H_{24}N_2O_2S + H^+$, 357.1630. Found (ESI, [M+H]$^+$), 357.1629.

7.3.3. 3-(Phenylsulfonyl)-4-(3-piperidin-1-ylpropyl)-1*H*-indole hydrochloride (33c)

Yield: 43% of a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.35 (m, 1H), 1.59–1.89 (m, 7H), 2.72–2.87 (m, 4H), 2.93 (br s, 2H), 3.33 (br s, 2H), 6.99 (d, J = 7.2 Hz, 1H), 7.20 (dd, J = *8.2,* 7.2 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.54–7.69 (m, 3H), 7.82 (d, J = 7.4 Hz, 2H), 8.22 (d, J = 3.1 Hz, 1H), 9.58 (br s, 1H), 12.44 (br s, 1H); HRMS: m/z calcd for $C_{22}H_{26}N_2O_2S + H^+$, 383.1787. Found (ESI, [M+H]$^+$), 383.1789.

7.4. General procedure 3: whole cell radioligand binding assay in cells expressing human norepinephrine transporter (hNET)

Twenty-four hours prior to assay, MDCK-Net6 cells, stably transfected with human NET {Pacholczyk et al. *Nature* **1991**, *6316*, 350} were plated in 96-well plates at 3000–5000 cells/well in growth medium and maintained in a cell incubator (37 °C, 5% CO_2). On day 2, growth medium was replaced with 75 μL of assay buffer (25 mM HEPES; 120 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 2 mg/mL glucose (pH 7.4, 37 °C)) containing 0.2 mg/mL ascorbic acid and 1 μM pargyline. Five microliter aliquots of test compound in assay buffer were added directly

to triplicate wells to yield final test concentrations of 10–10,000 nM. Data from wells containing desipramine (1 μM) were used to define non-specific hNET binding (minimum hNET binding in the presence of an NRI). Total radioligand bound is defined by addition of 5 μL of binding buffer alone in the presence of [^{3}H]nisoxetine (Perkin–Elmer). The radioligand binding reaction was initiated by addition of [^{3}H]nisoxetine in 25 μL of assay buffer to each well for a final concentration of 3 nM. The K_D value estimated for [^{3}H]nisoxetine was 10 nM using intact whole cells. The cells in the assay buffer with test compound and radioligand were incubated for 2 h at 37 °C. The cells were washed twice with 200 μL assay buffer at room temperature to remove unbound radioligand. The plates were inverted and left to dry for 10 min. Microscint20 scintillation fluid (100 μL) (Perkin–Elmer Life and Analytical Sciences, Boston, MA) was added to each well and the plates were sealed. The plates were then placed on an orbital shake table (Bellco) for 10 min to ensure adequate mixing. The plates were counted in the Wallac Microbeta counter (Perkin–Elmer).

7.5. General procedure 4: norepinephrine (NE) uptake assay in cells expressing human norepinephrine transporter (hNET)

On day 1, MDCK-Net6 cells, stably transfected with human NET, {Pacholczyk et al. *Nature* **1991**, *6316*, 350} were plated at 3000 cells/well in a 96-well plate in growth medium and maintained in a cell incubator (37 °C, 5% CO_2). On day 2, growth medium was replaced with 200 μL of assay buffer (25 mM HEPES; 120 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 2 mg/mL glucose [pH 7.4, 37 °C]) containing 0.2 mg/mL ascorbic acid and 10 μM pargyline. Cells were equilibrated in assay buffer for 10 min at 37 °C prior to addition of compounds. A stock solution of desipramine was prepared in DMSO (10 mM) and delivered to triplicate wells containing cells for a final test concentration of 1 μM. Data from these wells were used to define non-specific NE uptake (minimum NE uptake). Test compound stock solutions were prepared in DMSO or DMSO:water (1:1) (10 mM) and diluted in assay buffer according to test range (1–10,000 nM). A 25-μL aliquot of vehicle or various concentrations of test compound were added directly to triplicate wells containing cells in 200 μL of assay buffer. The cells were preincubated with test compound for 10 min at 37 °C. To initiate transport, [^{3}H]NE (DL-[7-^{3}H]norepinephrine) (Perkin–Elmer) was diluted in assay buffer (120 nM final assay concentration) and delivered in 25 μL aliquots to each well and the plates were incubated for 10 min at 37 °C. The cells were washed twice with 200 μL assay buffer to remove unincorporated [^{3}H]NE label. The plates were inverted and left to dry for 10 min. The cells were lysed in 25 μL of 0.25 N NaOH (4 °C), placed on an orbital shake table (Bellco) and vigorously shaken for 5 min. After cell lysis, 75 μL of scintillation cocktail was added to each well and the plates were sealed with film tape. The plates were returned to the shake table and vigorously shaken for a minimum of 10 min to ensure adequate partitioning of organic and aqueous solutions. The plates were counted in a Wallac Microbeta counter (Perkin–Elmer) to collect the raw cpm data.

7.6. General procedure 5: serotonin (hSERT) uptake assay in cells expressing human serotonin transporter

On day 1, JAR cells (human placental choriocarcinoma), purchased from ATCC (Cat. No. HTB-144), were plated at 15,000 cells/well in 96-well plates containing growth medium (RPMI 1640 with 10% FBS) and maintained in a cell incubator (37 °C, 5% CO_2). On day 2, cells were stimulated with staurosporine (40 nM) to increase the expression of the 5-HT transporter {Ramamoorthy et al. *J. Biol. Chem.* **1995**, *270*, 17189}. On day 3, cells were removed from the cell incubator 2 h prior to assay and maintained at room temperature to equilibrate the growth medium to ambient oxygen concentration. Subsequently, the growth medium was replaced with 200 μL of assay buffer (25 mM HEPES; 120 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 2 mg/mL glucose (pH 7.4, 37 °C)) containing 0.2 mg/mL ascorbic acid and 1 μM pargyline and the cells were incubated for 5 min at 37 °C. A stock solution of paroxetine was prepared in DMSO (10 mM) and delivered to triplicate wells containing cells for a final test concentration of 1 μM. Data from these wells were used to define non-specific 5-HT uptake (minimum 5-HT uptake in the presence of a SRI). Test compounds were prepared in DMSO (10 mM) and diluted in assay buffer according to test range (1–1000 nM). Twenty-five microliters of assay buffer (maximum 5-HT uptake) or test compound were added directly to triplicate wells containing cells in 200 μL of assay buffer. The cells were incubated with the compound for 10 min (37 °C). To initiate the reaction, [^{3}H]hydroxytryptamine creatinine sulfate (Perkin–Elmer) diluted in assay buffer was delivered in 25 μL aliquots to each well for a final test concentration of 15 nM. The cells were incubated with the reaction mixture for 9 min at 37 °C. Decanting the supernatant from the plates terminated the reaction. The cells were washed twice with 200 μL assay buffer (37 °C) to remove free radioligand. The plates were inverted and left to dry for 2 min, then re-inverted and air-dried for an additional 10 min. Subsequently, the cells were lysed in 25 μL of 0.25 N NaOH (4 °C) then placed on a shaker table and shaken vigorously for 5 min. After cell lysis, 75 μL of scintillation cocktail was added to the wells, the plates were sealed with film tape and replaced on the shake table for a minimum of 10 min. The plates were counted in a Wallac Microbeta counter (Perkin–Elmer) to collect the raw cpm data.

7.7. General procedure 6: dopamine transporter (hDAT) membrane binding assay

Frozen membrane samples from CHO cells expressing recombinant hDAT, purchased from Perkin–Elmer, Boston, MA (Cat. No. RBHDATM, Lot #2227), were diluted to 7.5 mL in binding buffer (50 mM Tris–HCl; pH 7.4, 100 mM NaCl), homogenized with a tissue-tearer (Polytron PT 1200C, Kinematica AG) and delivered at a volume of 75 μL to each well of a polypropylene 96-well plate. The binding reaction was carried out in polypropylene 96-well plates. Homogenized membrane preparation was delivered at a volume of 75 μL to each well of a reaction plate. A stock solution of mazindol was prepared in DMSO (10 mM) and delivered to triplicate wells containing membranes for a final test concentration of 10 μM. Mazindol has been reported to be a dopamine transporter inhibitor (DRI) {Pristupa et al. *Mol. Pharmacol.* **1994**, *45*, 125} with an IC_{50} value of 20.5 nM in our assays. Data from wells containing mazindol (10 μM) were used to define non-specific (NSB) hDAT binding (minimum hDAT binding in the presence of a DRI). Total radioligand bound is defined by addition of 5 μL of binding buffer alone in the presence of [^{3}H]WIN-35,428. Stock solutions of test compounds were prepared in DMSO at a concentration of 10 mM. On day of assay, the test compound stock solution was diluted in assay buffer according to test range (3000–100,000 nM) ensuring a maximal DMSO concentration of less than 0.5% in the assay reaction wells. Homogenized membranes were preincubated with test compound for 20 min at 4 °C before the initiation of the binding reaction. The binding reaction was initiated by addition of 25 μL of [^{3}H]WIN-35,428 diluted in binding buffer. The final concentration of [^{3}H]WIN-35,428 delivered was 32 nM. The K_D value estimated for [^{3}H]WIN-35,428 (Lot# 2227) in hDAT membranes was 29.7 nM. The plates containing the radioligand binding reactions were incubated for 2 h at 4 °C on a shaking table (Bellco) at

3 rpm. The MultiScreen-FB opaque 96-well filtration plates containing Millipore glass fiber filters were used to terminate the binding reactions and to separate bound from free radioligand. The plates were presoaked with 0.5% polyethylenimine (PEI) in water for a minimum of 2 h at room temperature to reduce non-specific binding of [^{3}H]WIN-35,428 during the harvest procedure. Prior to harvesting the reaction plates, the PEI solution was aspirated from the filter plates using a vacuum manifold. Aliquots of each reaction (90 μL of each 100 μL reaction well) were transferred from the reaction plates to the filter plates using a Zymark Rapid Plate-96 automated pipet station. The binding reaction was terminated by vacuum filtration through the glass fiber filters. The filter plates were aspirated at 5–10 in. Hg, and the wells are washed nine times with 200 μL wash buffer (50 mM Tris–HCl, 0.9% NaCl, pH 7.4; 4 °C) using a 12-channel aspiration/wash system. Plastic bottom supports were removed from the filter plates and the plates were placed in plastic liners. A 100-μL aliquot of scintillation fluid was added to each well and the top of each plate was sealed with adhesive film. The plates were vigorously shaken on an orbital shake table at 5 rpm for 10–15 min to ensure adequate equilibration of aqueous to solvent partitioning. The collection of raw cpm data was done using a Wallac Microbeta counter (Perkin–Elmer).

7.8. General procedure 7: Human Serotonin 2A (5-HT$_{2A}$) membrane binding assay

Frozen cell pellets from CHO cells expressing the human 5-HT$_{2A}$ receptor were provided by Wyeth BioPharma, Andover. Cells are grown in suspension in a 10L bioreactor until they reach a density of 1.5–1.9 × 10^6 cells/mL. Cells are decanted, pelleted in 1 L aliquots, and the pellets are frozen at −80 °C. Each 1 L pellet is re-suspended in 48 mL of binding buffer (50 mM Tris–HCl, 4 mM $CaCl_2$, pH 7.4), aliquotted into 16 × 3 mL samples and homogenized with a tissue-tearer (Polytron PT 1200C) for 10 strokes. The homogenate from all 16 tubes is combined, then aliquoted into eight tubes and centrifuged at 4 °C at low speed (600*g*) for 30 min. The supernatant is collected and re-centrifuged at high speed (40,000*g*) for 30 min, and the resulting pellets are stored at −80 °C. On day of use, pellets are thawed on ice and homogenized in 2–2.5 mL binding buffer for a protein concentration of 0.5 mg/mL (50 μg/100 μL). Protein concentration is determined prior to assay using a BCA Protein Assay Kit (Pierce).

Binding reactions are run in polypropylene 96-well plates using a total reaction volume of 200 μL. Membrane (50 μg) is delivered in a final volume of 160 μL to each well of a reaction plate. A stock solution of mianserin was prepared in DMSO (10 mM) and delivered to triplicate wells containing membranes for a final test concentration of 10 μM. Data from wells containing mianserin (10 μM) were used to define non-specific (NSB) 5-HT$_{2A}$-receptor binding. Total radioligand bound is defined by addition of 5 μL of binding buffer alone in the presence of [^{3}H]ketanserin. Compound solutions at several concentrations are generated by serial dilution of 10 mM stocks made from powder. The resulting solutions are further diluted to delivery concentration (10× assay concentration) in binding buffer. This procedure for compound dilution is followed to ensure that the concentration of solvent (i.e., DMSO) used for the stock solutions is constant across all treatments. Compounds that have been diluted to appropriate delivery concentration are added in 20 μL aliquots to reaction wells containing membrane in binding buffer. The binding reaction is initiated by addition of 25 μL of [^{3}H]ketanserin-HCl (diluted to 4 nM in binding buffer) and incubated 1 h at room temperature. Skatron glass fiber mats (#11731) are incubated in 0.5% polyethylenimine (PEI; Sigma Cat. No. P-3143) in water for 15–30 min at room temperature to reduce non-specific binding of the radioligand prior to harvesting the reaction plates. A Skatron Micro96 cell harvester (Molecular Devices) is used to separate bound from free radioactivity. The drug reaction is aspirated directly from the reaction plate onto a glass fiber mat, and rinsed four times with 200 μL wash buffer (50 mM Tris–HCl, pH 7.4, 4 °C). The individual filter disks are transferred to scintillation vials. Scintillation fluid (Packard Ultima Gold) is added to the vials, and data (dpm) are collected from the Packard 2200CA scintillation counter (Perkin–Elmer).

7.9. General procedure 8: 5-HT$_{2A}$ calcium mobilization FLIPR assay

Chinese hamster ovary cells (CHO-K1) were stably transfected with the human 5-HT$_{2A}$ receptor. The CHO cells were grown in culture medium containing Dulbecco's modified Eagle's Medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 1% penicillin–streptomycin (Invitrogen), 1% non-essential amino acids (Invitrogen), and 1% GlutaMax (Invitrogen). The cells were propagated in selection media containing 800 μg/mL of G418 (Invitrogen) and 500 μg/mL of Zeocin (Invitrogen) and maintained at 37 °C in a 5% CO_2 incubator at 95% humidity. Cells were plated at a density of 0.4 × 10^6 in a tissue culture flask (T75 cm^2) (Falcon) and cultured twice weekly using a final concentration of 0.05% Trypsin–EDTA (Invitrogen). For the calcium mobilization bioassay, cells were plated at 40,000 cells per well in sterile black 96-well plates (Costar) in 200 μL culture medium containing 1.5% FBS for 24 h. The cells were utilized for the functional bioassay between passages 5 and 25.

On day of assay, cells were plated in 96-well plates and visually assessed for cell density and validity. The assay buffer contained Hanks balanced salt solution without phenol red (Invitrogen) and 20 mM HEPES (Invitrogen). The Calcium3 dye (Molecular Devices) was dissolved in 100 mL of assay buffer containing 2.5 mM probenecid (Sigma) prepared fresh on the day of assay. Prior to assay, the cell culture media was decanted and 180 μL of the dye solution was added to cells. The cells were incubated for 60 min at 37 °C in a 5% CO_2 incubator. Two 96-well drug plates were prepared for each cell plate. Plate 1 contained test compounds and 1 μM 5-HT$_{2A/2C}$ receptor agonist, (*R*)-(−)-DOI hydrochloride (Sigma). Plate 2 contained 3 nM DOI that was chosen based on the EC_{80} value of the calcium response. All compounds were diluted in assay buffer from 1 mM stock in DMSO with a final concentration of 1 μM in with a final concentration of 0.1% DMSO present in the wells. The 0.1% DMSO concentration showed no effect alone on cell viability or response (unpublished data). Cell, compound, and DOI plates were loaded into the Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices). The laser intensity was set to a suitable level to obtain basal values of approximately 12,000 fluorescence units. The variation in basal fluorescence units across the plate was usually less than 10%. The baseline fluorescence reading was obtained every second for 1 min and every second for 10 s before and after test compound addition, respectively. Ten microliters of compound were transferred from the compound plate to the cells at a speed of 50 μL per second to ensure rapid equilibrium of compound without dislodging cells. The drug addition was made from a height that would ensure no air bubbles in the wells. To minimize interference with fluorescence measurements, the black tips were not removed at the end of compound addition. The fluorescence signal recorded every 6 s for 2 min to determine if agonist activity was noted. For antagonist determination, 10 μL of 3 nM DOI was transferred from plate 2 to the cells and the fluorescence signal recorded every 6 s for 5 min. The final assay volume was 200 μL per well. Fluorescence measurements were captured by a cooled CCD camera and integrated to an on-line computer. A negative control was defined by assay buffer containing 0.1% DMSO. The maximum fluorescent signal obtained by 1 μM DOI was typically 25,000 fluorescent units and expressed as 100% signal to determine the per-

centage of compound agonist activity. Antagonistic activity of 3 nM DOI stimulation was expressed as a percentage of the response observed with 3 nM DOI alone. A compound would be considered an active antagonist if it inhibited 3 nM DOI mediated calcium stimulation by 60% or greater.

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