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Synthesis and in vitro binding of *N*,*N*-dialkyl-2-phenylindol-3-yl-glyoxylamides for the peripheral benzodiazepine binding sites

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Abstract—A series of *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides bearing the halogens iodine and bromine were synthesised and their binding affinity for the peripheral benzodiazepine binding sites (PBBS) in rat kidney mitochondrial membranes was evaluated using [^{3}H]PK11195. Central benzodiazepine receptor (CBR) affinities were also evaluated in rat cortices using [^{3}H]flumazenil to determine their selectivity for PBBS over CBR. The tested compounds had PBBS binding affinities (IC_{50}) ranging from 7.86 to 618 nM, with all compounds showing high selectivity over the CBR (CBR $IC_{50} > 5000$ nM). Among the 12 compounds tested, those with a diethylamide group were the most potent. The highest affinity iodinated PBBS ligand, *N*,*N*-diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide (**4c**), was radiolabelled with iodine-123. This high affinity and selective radioligand may be useful for imaging neurodegeneration, inflammation and tumours using single photon emission computed tomography.

1. Introduction

The peripheral benzodiazepine binding sites (PBBS) (also termed the peripheral benzodiazepine receptors or PBR) are multimeric protein complexes localised mainly on the outer mitochondrial membranes of cells in peripheral organs such as the kidney, heart and steroid producing cells of the adrenals, testes and ovaries.[1,2] The PBBS are pharmacologically and anatomically different to the central benzodiazepine receptors (CBR). Within the normal central nervous system, PBBS are expressed at low levels mainly on the choroid plexus, ependymal lining of the ventricles and the olfactory bulb.[3] Dramatic upregulation of PBBS has been reported in such diverse neuropathologic states including Huntington's and Alzheimer's diseases and multiple sclerosis.[4,5] Significantly enhanced PBBS expression has also been observed in melanoma, breast, prostate, ovarian and glial tumours.[6–9]

The development of specific radiotracers for the PBBS has not only contributed to the elucidation of the receptor's biochemical functions, but when imaged with positron emission tomography (PET) and single photon emission computed tomography (SPECT) provides a means by which these changes can be monitored and correlated to disease.[10–13] A diverse range of chemical structures have been shown to bind to various components of the PBBS. The isoquinoline carboxamide ligand PK 11195, the classical 1,4-benzodiazepine, Ro 5-4864 and the imidazopyridine Alpidem (Fig. 1) have been shown to bind to various binding domains of the PBR. [^{11}C]PK 11195 has been used to study the PBBS in vivo using PET to detect brain inflammation in humans and to image early Alzheimer's disease.[14]

More recently, imidazopyridines[15] and pyridazines[16] and indoles such FGIN-1-27[17,18] (Fig. 1) have all been reported to bind to the PBBS. Several of these ligands have been radiolabelled with iodine-123, for potential imaging using SPECT.[16,19,20]

N,*N*-Dialkyl-2-phenylindolyl-3-glyoxylamides (Fig. 2) represent a new class of potent PBBS ligands, whose development was based on conformationally constrained analogues of 2-phenylindole-3-acetamides.[21] These compounds offer the potential for radiolabelling

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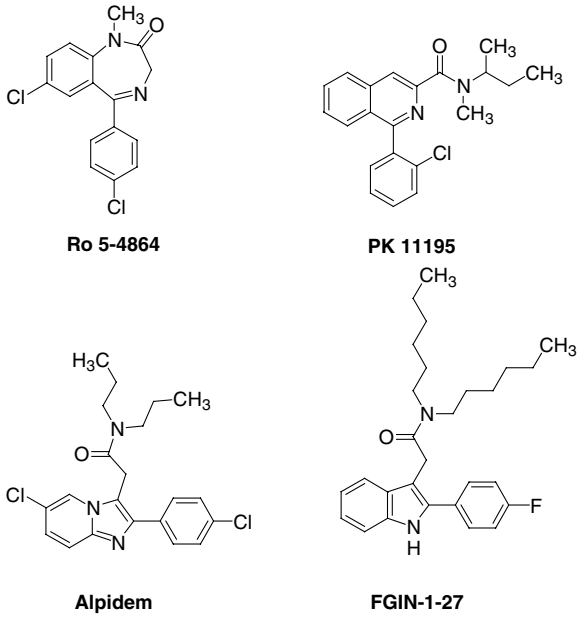


Figure 1. Structures of PBBS ligands.

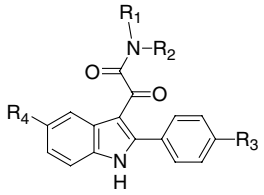
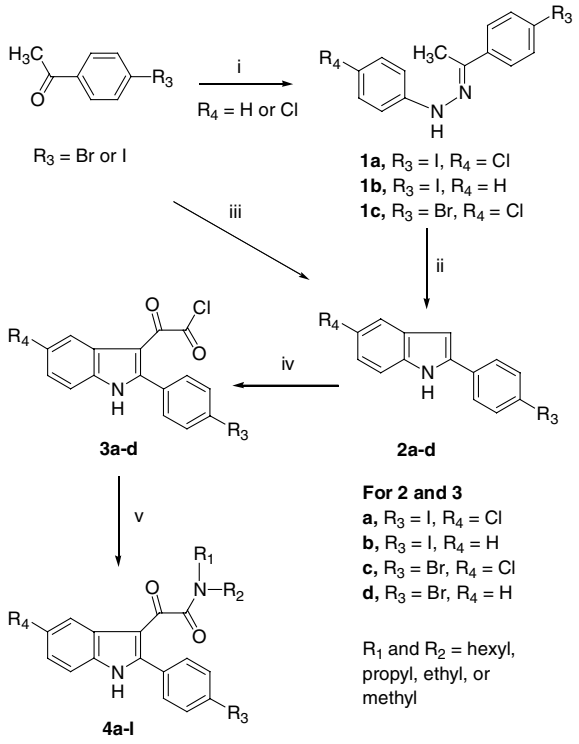


Figure 2. Structure of *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides.

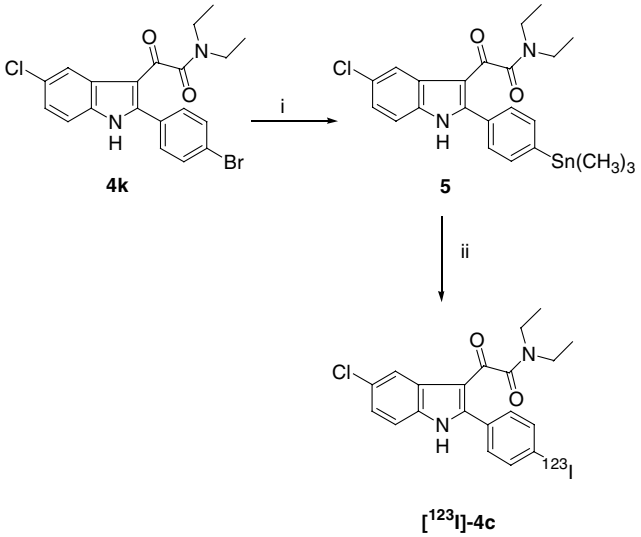
with radionuclides for both PET and SPECT imaging. In this investigation, we synthesised and tested several of halogenated derivatives of these systems with the aim of radiolabelling with iodine-123 for studying PBBS using SPECT.

2. Chemistry

The *N*,*N*-dialkyl-2-phenylindol-3-ylglyoxylamides **4a**–**l** were synthesised following literature methods[21] for similar compounds and is outlined in Scheme 1. Briefly, 2-(4-bromophenyl)indole **2d** was synthesised in one step, from 4-bromoacetophenone and phenylhydrazine with polyphosphoric acid,[22] while indoles **2a**–**c** were synthesised in two steps via their phenylhydrazone derivatives **1a**–**c**.[23] The phenylhydrazones were formed by reacting 4-bromo or 4-iodo acetophenone with phenylhydrazine or 4-chlorophenylhydrazine hydrochloride, followed by cyclisation by the Fischer indole synthesis using polyphosphoric acid. Subsequent acylation using oxalyl chloride gave the indolylglyoxylyl chlorides **3a**–**d**, which were aminated using the appropriate amines, for example, dihexylamine, di-*n*-propylamine, diethylamine, or dimethylamine giving the final *N*,*N*-dialkyl-2-phenylindolyl-3-glyoxamides **4a**–**l** in good overall yields.



Scheme 1. Reagents: (i) Ph-$NHNH_2$ or 4-Cl-Ph$NHNH_2$·HCl, ethanol, CH_3CO_2H; (ii) to synthesis **2a**–**c**, polyphosphoric acid; (iii) to synthesise **2d**, Ph-$NHNH_2$, polyphosphoric acid; (iv) ClCOCOCl, anhydrous ether; (v) $NH(alkyl)_2$, triethylamine, anhyd toluene.



Scheme 2. Preparation of [^{123}I]*N*,*N*-diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide. Reagents and conditions: (i) $(Sn(CH_3)_3)_2$, $Pd(PPh_3)_4$, anhydrous toluene, reflux, 8 h; (ii) $Na^{123}I$, per-acetic acid, ethanol, acetic acid.

To prepare the [^{123}I]-radiolabelled analogue of **4c**, the trimethylstannyl precursor **5** was prepared from its corresponding bromo derivative **4k**, using hexamethylditin in the presence of a catalytic amount of tetrakis(triphenylphoshine)palladium(0) in anhydrous toluene. The radioiodination was achieved by an oxidative iododestannylation reaction of **5** and $Na^{123}I$ using per-acetic

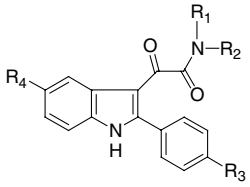
acid as an oxidising agent (Scheme 2). The radioligand, [^{123}I]*N*,*N*-diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]-glyoxylamide was purified and characterised by reverse-phase HPLC. The radiochemical yield was in the range of 55–60% ($n = 3$).

3. Results and discussion

The binding affinities (IC_{50}) of the 12 new compounds for the PBBS were determined by measuring the displacement of [^{3}H]PK11195 bound to rat kidney mitochondrial membranes. To determine the selectivity of the compounds for PBBS versus CBR, CBR binding affinities were determined using [^{3}H]flumazenil on rat cortical membranes. The results of the binding affinities of compounds **4a**–**l** for the PBBS and the CBR are shown in Table 1. The compounds examined displayed a medium to high affinity for the PBBS, ranging from 7.86 to 618 nM. All compounds had high selectivity for PBBS over CBR, with all CBR $IC_{50} > 5000$ nM. The effects of chemical modification to groups R_1 to R_4 on the binding to PBBS were studied, including the length of the alkyl chains (R_1 and R_2), the presence of a chloro substituent on position 5 of the indole (R_4) and halogen substitution (bromine or iodine) on the phenyl ring (R_3). The most potent ligands for the PBBS were compounds **4c** and **4k**, both displaying IC_{50} of <10 nM. Decreasing the length of the alkyl chains from hexyl to propyl to ethyl increased the binding affinity for the PBBS. However, compounds with R_1 and R_2 as methyl groups had a lower affinity than the compounds with ethyl groups. The compounds with a bromo substituent on R_3, **4i**–**l**, had higher PBBS affinities than their respective iodo analogues **4a**–**c**, and **4e**. This suggests that the smaller the halogen on R_3, the better the binding affinity. Although no direct comparison can be made, compounds reported previously[21] with smaller halogen (fluorine and chlorine) substituents on R_3 appeared to have a higher PBBS binding affinity than the compounds presented here. It was also reported[21] that the optimum alkyl chain lengths (R_1 and R_2) for compounds with no chloro substituent on R_4 were hexyl groups, whereas with the chloro substituent, the optimum length was propyl. In this work, it was found that compounds with hexyl groups had the lowest affinities, with and without a chloro substituent. It was also found that affinities increased with the addition of a chloro substituent in compounds containing propyl, ethyl or methyl groups.[21] Compounds **4e** and **4l** containing hexyl groups showed higher PBBS affinity than compounds **4a** and **4i** with a chloro group in the R_4. This suggests that it is more difficult to accommodate the sterically more demanding hexyl groups on molecules with $R_4 = Cl$ than in compounds with $R_4 = H$, suggesting different molecular orientations are possible.

To perform radiolabelling, the corresponding trimethyl stannane **5** was prepared by treatment of the bromo derivative **4k** with hexamethylditin and palladium tetrakistriphenylphosphine in refluxing toluene. [^{123}I]**4c** was prepared by electrophilic iododestannylation in the presence of per-acetic acid as the oxidant in 55–60% radiochemical yield and above 98% radiochemical purity.

Table 1. Binding affinities for PBBS and CBR, measured and calculated Log *P* of compounds **4a**–**l**



Compound	R_1	R_2	R_3	R_4	Log *P*	Calcd log *P*[a]	PBBS IC_{50}[b] (nM)	CBR IC_{50}[c] (nM)
4a	Hexyl	Hexyl	I	Cl	> 6	8.29	169 ± 35	18786 ± 1634
4b	Propyl	Propyl	I	Cl	4.71 ± 0.19	5.78	37.4 ± 6.1	16156 ± 579
4c	Ethyl	Ethyl	I	Cl	4.00 ± 0.16	4.81	8.23 ± 2.2	15652 ± 45
4d	Methyl	Methyl	I	Cl	3.36 ± 0.13	4.13	17.5 ± 4.3	10146 ± 1967
4e	Hexyl	Hexyl	I	H	> 6	7.73	115 ± 22	13803 ± 137
4f	Propyl	Propyl	I	H	4.00 ± 0.16	5.22	43.6 ± 1.8	14662 ± 127
4g	Ethyl	Ethyl	I	H	3.27 ± 0.13	4.25	19.1 ± 2.5	13197 ± 2975
4h	Methyl	Methyl	I	H	2.69 ± 0.11	3.58	618 ± 39	5939 ± 815
4i	Hexyl	Hexyl	Br	Cl	> 6	7.76	138 ± 35	13115 ± 3212
4j	Propyl	Propyl	Br	Cl	4.56 ± 0.18	5.25	16.7 ± 4.7	10463 ± 2817
4k	Ethyl	Ethyl	Br	Cl	3.89 ± 0.15	4.28	7.86 ± 1.2	11045 ± 1909
4l	Hexyl	Hexyl	Br	H	> 6	7.20	54.7 ± 22.6	16348 ± 716
PK 11195						5.30	3.7 ± 1.2	>1000

[a] Estimated by Chem Draw Ultra.

[b] The concentration of tested compounds that inhibited [^{3}H]PK11195 binding to rat kidney mitochondrial membranes (IC_{50}) by 50% was determined with 6 concentrations of the test compounds, each performed in triplicate. IC_{50} values are means ± SEM derived from at least 2 independent experiments.

[c] The concentration of tested compounds that inhibited [^{3}H]flumazenil binding to rat cortex membranes (IC_{50}) by 50% was determined with 6 concentrations of the test compounds, each performed in triplicate. IC_{50} values are means ± SEM derived from at least 2 independent experiments.

Lipophilicity measurements of all compounds were assessed in order to explain their in vivo properties. As expected the presence of bulky alkyl groups (R_1 and R_2) greatly influenced the overall lipophilicity of the molecules resulting in compounds **4a**, **4e**, **4i** and **4l** having log *P* values greater than 6 and molecules with propyl groups **4b**, **4f** and **4j** greater than 4. In addition, incorporating a chloro substituent on R_4 increased the log *P* values from 3.27 in **4g** and 2.69 in **4h** to 4.0 in **4c** and 3.36 in **4d**, respectively. However, the presence of small alkyl groups such as ethyl and methyl in the R_1 and R_2 positions and hydrogen in the R_4 positions can yield compounds with log *P* values between 2 and 4.

4. Conclusion

A series of *N*,*N*-dialkyl-[2-(4′-iodo- and 4′-bromo-phenyl-indol-3-yl]glyoxylamides) **4a**–**l** were synthesised and their binding affinity for the PBBS and CBR was determined using [^{3}H]PK11195 and [^{3}H]flumazenil on rat kidney mitochondrial and cortical membranes, respectively. The brominated and iodinated compounds **4c** and **4k** bearing a chloro substituent on R_4 and ethyl groups at R_1 and R_2 displayed the highest affinity for the PBBS. All compounds displayed low affinity for the CBR (IC_{50} > 5000 nM). Compound **4c** was radiolabelled with iodine-123 by electrophilic iododestannylation using per-acetic acid as the oxidant in 55–60% radiochemical yield and >98% radiochemical purity.

5. Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 Elemental analyser at the Campbell Microanalytical Laboratory, University of Otago (Dunedin, New Zealand), and their results are within ±0.4% of theoretical values. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 400 MHz spectrometer. Chemical shifts (δ) are expressed in ppm, and the coupling constants (*J*) in Hz. Signals are recorded as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Mass spectra (MS) were obtained using either a VG Quattro triple quadrupole system or an Autospec high-resolution mass spectrometer. Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} precoated polyester plates with a thickness of 250 μm. Column chromatography was performed on Merck Keiselgel 60 (220–440 mesh). Reagents and solvents were purchased from commercial sources and were used without further purification. Petroleum ether of boiling point range 30–60 °C was used. Yields refer to purified products and are not optimised. Chromatographic separation of radiolabelled product was carried out on a Phenomenex bondclone semi-preparative RP C-18 column (10 μ, 7.8 × 300 mm) using a Waters 510 pump, an Activon Linear UV detector set at 254 nm, and an in-line NaI-Berthold radioactivity detector. Carrier free $Na^{123}I$ in 0.1 M NaOH was obtained from Australian Radioisotopes and Industrials (ARI), Australia. [^{3}H]PK11195 and [^{3}H]flumazenil were purchased from Perkin-Elmer Life Sciences (Boston, MA, USA). PK11195 and flumazenil were purchased from Sigma-RBI. For binding studies, male Sprague–Dawley rats were purchased from Animal Resources Centre (Perth, WA, Australia). All procedures were carried out in compliance with Australian laws governing animal experimentation.

5.1. 4-Iodoacetophenone 4-chlorophenylhydrazone (1a)

A mixture of 4-iodoacetophenone (4.15 g, 16.9 mmol), 4-chlorophenylhydrazine hydrochloride (3.00 g, 16.8 mmol) and a few drops of glacial acetic acid in ethanol (40 mL) was stirred and heated at reflux for 45 min. A precipitate that formed was filtered and washed with dilute HCl followed by cold 95% ethanol (10 mL). The product was recrystallised in ethanol to yield **1a** (3.34 g, 54%) as yellow crystals: mp 130–132 °C. ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H, CH_3), 7.11 (d, 2H, *J* = 8.9, 2ArH), 7.24 (d, 2H, *J* = 8.9, 2ArH), 7.34 (bs, 1H, NH), 7.51 (d, 2H, *J* = 8.6, 2ArH), 7.71 (d, 2H, *J* = 8.6, 2ArH). ^{13}C NMR ($CDCl_3$) δ 12.0, 94.2, 114.8, 125.4, 127.7, 129.6, 137.8, 138.8, 141.0, 144.0. MS (EI) *m*/*z* 370 (M^+, 33%). Anal. Calcd for $C_{14}H_{12}ClIN_2$: C, 45.37; H, 3.26; N, 7.56. Found: C, 45.67; H, 3.37; N, 7.47.

5.2. 4-Iodoacetophenone phenylhydrazone (1b)

In similar manner, 4-iodoacetophenone (5.00 g, 20.3 mmol) and phenylhydrazine (2.0 mL, 20.3 mmol) in ethanol (30 mL) gave **1b** (4.38 g, 64%) as orange crystals.^{1}H NMR ($CDCl_3$) δ 2.19 (s, 3H, CH_3), 6.89 (t, 1H, *J* = 7.2, ArH4), 7.16 (d, 2H, *J* = 7.8, 2ArH), 7.28 (m, 2H, 2ArH), 7.52 (d, 2H, *J* = 8.6, 2ArH), 7.68 (d, 2H, *J* = 8.6, 2ArH). MS (EI) *m*/*z* 336 (M^+, 100%).

5.3. 4-Bromoacetophenone 4-chlorophenylhydrazone (1c)

In a similar manner, 4-bromoacetophenone (6.89 g, 34.6 mmol) and 4-chlorophenylhydrazine hydrochloride (6.20 g, 34.6 mmol) in ethanol (60 mL) gave **1c** (8.38 g, 75%) as pale yellow crystals: mp 132–134 °C. ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, CH_3), 7.09 (d, 2H, *J* = 8.9, 2ArH), 7.23 (d, 2H, *J* = 8.9, 2ArH), 7.33 (bs, 1H, NH), 7.49 (d, 2H, *J* = 8.7, 2ArH), 7.64 (d, 2H, *J* = 8.7, 2ArH). ^{13}C NMR ($CDCl_3$) δ 12.1, 114.8, 122.6, 125.4, 127.5, 129.6, 130.2, 131.8, 138.1, 144.0. MS (EI) *m*/*z* 324 (M^+, 74%), 126 (M^+−198, 100%). Anal. Calcd for $C_{14}H_{12}N_2ClBr$: C, 51.96; H, 3.74; N, 8.66. Found: C, 52.07; H, 3.59; N, 8.88.

5.4. 5-Chloro-2-(4-iodophenyl)indole (2a)

A mixture of **1a** (3.10 g, 8.4 mmol) and polyphosphoric acid (20 g) was stirred for 30 min at 110 °C. Ice water (250 mL) was added and the mixture was stirred. The precipitate was filtered, washed with water and recrystallised from 95% ethanol, to yield **2a** (1.66 g, 56%) as a white solid: mp 210–211 °C. ^{1}H NMR (DMSO-d_6) δ 6.92 (s, 1H, H3), 7.10 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.0, H6), 7.39 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.57, (d, 1H,

$J_{4,6}$ = 1.9, H4), 7.66 (d, 2H, J = 8.4, 2ArH), 7.83 (d, 2H, J = 8.4, 2ArH), 11.77 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 95.4, 100.6, 114.5, 120.9, 123.5, 125.7, 128.8, 131.3, 132.9, 137.3, 139.4, 139.9. MS (CI) m/z 354 (M^+, 34%), 228 (M^+-126, 100%). Anal. Calcd for $C_{14}H_9ClIN$: C, 47.56; H, 2.57; N, 3.96. Found: C, 47.56; H, 2.36; N, 4.18.

5.5. 2-(4-Iodophenyl)indole (2b)[23]

Treatment of a mixture of **1b** (3.09 g, 9.19 mmol) and polyphosphoric acid (15 g) at 60–70 °C as above gave **2b** (1.70 g, 58%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, H3), 7.12 (t, 1H, H5 or H6), 7.21 (t, 1H, H6 or H5), 7.38 (d, 3H, 2ArH and H7), 7.62 (d, 2H, J = 7.8, H4), 7.76 (d, 2H, J = 10.8, 2ArH), 8.27 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 94.1, 100.5, 112.5, 120.7, 121.4, 123.1, 128.1, 129.7, 132.9, 137.7, 138.4, 138.8. MS (EI) m/z 319 (M^+, 100%).

5.6. 2-(4-Bromophenyl)-5-chloroindole (2c)

Treatment of a mixture of **1c** (5.12 g, 15.8 mmol) and polyphosphoric acid (30 g) as above gave **2c** (3.35 g, 69%) as a pale yellow solid: mp. 206–207 °C. ^{1}H NMR (DMSO-d_6) δ 6.91 (s, 1H, H3), 7.11 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.0, H6), 7.40 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.57 (d, 1H, $J_{4,6}$ = 1.9, H4), 7.66 (d, 2H, J = 8.5, 2ArH), 7.80 (d, 2H, J = 8.5, 2ArH), 11.79 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 99.0, 112.8, 119.2, 120.8, 121.8, 124.1, 127.1, 129.7, 130.9, 131.9, 135.7, 138.1. MS (CI) m/z 308 (M^+,79%), 228 (M^+-79, 100%). Anal. Calcd for $C_{14}H_9BrClN$: C, 54.85; H, 2.96; N, 4.57. Found: C, 54.93; H, 3.11; N, 4.50.

5.7. 2-(4-Bromophenyl)indole (2d)[22]

To a mixture of 4-bromoacetophenone (6.89 g, 34.6 mmol) and phenylhydrazine (3.4 mL, 34.6 mmol) was added polyphosphoric acid (40 g). The reaction mixture was heated at 110 °C, stirring occasionally until the mixture turned a deep reddish brown colour. The reaction mixture was worked up the same as **2a** to yield **2d** (5.41 g, 58%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, H3), 7.13 (t, 1H, J = 7.9, H5 or H6), 7.21 (t, 1H, J = 8.2, H6 or H5), 7.39 (d, 1H, J = 8.0, H7), 7.51 (d, 2H, J = 8.7, 2ArH), 7.56 (d, 2H, J = 8.7, 2ArH), 7.62 (d, 1H, J = 8.0, H4), 8.27 (bs, 1H, NH). MS (EI) m/z 271 ($^{79}BrM^+$, 100%), 273 ($^{81}BrM^+$, 98%). HRMS-EI$^+$ calculated for $C_{14}H_{10}NBr$: 270.9997, found 270.9995.

5.8. [5-Chloro-2-(4-iodophenyl)indol-3-yl]glyoxylyl chloride (3a)

To a partially dissolved solution of indole **2a** (0.65 g, 1.84 mmol) in anhydrous diethyl ether at 0 °C was added dropwise oxalyl chloride (0.22 mL, 2.57 mmol) and the mixture was stirred for 6 h at room temperature. The precipitate was collected by filtration and washed with a small portion of diethyl ether to yield **3a** (0.72 g, 88%), which was directly used to synthesise **4a**–**d**. ^{1}H NMR (DMSO-d_6) δ 7.33 (d, 1H, $J_{6,7}$ = 8.6, H6), 7.38 (d, 2H, J = 8.1, 2× ArH), 7.54 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.89 (d, 2H, J = 8.1, 2× ArH), 8.08 (s, 1H, H4).

5.9. [2-(4-Iodophenyl)indol-3-yl]glyoxylyl chloride (3b)

Treatment of **2b** (0.39 g, 1.22 mmol) and oxalyl chloride (0.15 mL, 1.70 mmol) in diethyl ether (6.5 mL) for 1 h as above gave **3b** (0.34 g, 68%). ^{1}H NMR (DMSO-d_6) δ 7.25–7.35 (m, 2H, H5 and H6), 7.39 (d, 2H, J = 8.2, 2× ArH), 7.52 (d, 1H, $J_{7,6}$ = 7.6, H7), 7.89 (d, 2H, J = 8.2, 2× ArH), 8.09 (d, 1H, $J_{4,5}$ = 7.4, H4), 12.83 (s, 1H, NH).

5.10. [2-(4-Bromophenyl)-5-chloroindol-3-yl]glyoxylyl chloride (3c)

Reaction of **2c** (1.55 g, 5.06 mmol) and oxalyl chloride (0.62 mL, 7.08 mmol) in diethyl ether (14 mL) for 4 h gave **3c** (1.96 g, 98%). ^{1}H NMR (DMSO-d_6) δ 7.34 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.1, H6), 7.38 (d, 2H, J = 8.3, 2× ArH), 7.54 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.90 (d, 2H, J = 8.3, 2× ArH), 8.08 (d, 1H, $J_{4,6}$ = 2.0, H4), 12.84 (s, 1H, NH).

5.11. [2-(4-Bromophenyl)indol-3-yl]glyoxylyl chloride (3d)

Reaction of **2d** (2.00 g, 7.35 mmol) and oxalyl chloride (0.90 mL, 10.3 mmol) in diethyl ether (20 mL) for 1 h gave **3d** (1.75 g, 66%). ^{1}H NMR ($CDCl_3$) δ 7.37–7.41 (m, 2H, H5 and H6), 7.42 (d, 2H, J = 8.5, 2× ArH), 7.44–7.49 (m, 1H, H7), 7.66 (d, 2H, J = 8.5, 2× ArH), 8.24–8.29 (m, 1H, H4), 8.92 (bs, 1H, NH).

5.12. *N*,*N*-Dihexyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide (4a)

To a solution of the chloride **3a** (0.30 g, 0.67 mmol) and triethylamine (0.11 mL, 0.81 mmol) in anhydrous toluene (30 mL) at 0 °C was added dropwise a solution of dihexylamine (0.17 mL, 0.74 mmol) in anhydrous toluene (20 mL). The solution was stirred at room temperature for 24 h. The solution was filtered and the filtrate was washed sequentially with 0.5 M HCl, saturated $NaHCO_3$ and water. The combined organic extracts were dried with $MgSO_4$, filtered, and the solvent was evaporated. The crude product was purified by column chromatography (methanol/chloroform, 1:19 v/v) and then recrystallised (ethyl acetate/petroleum ether, 1:9 v/v) to give **4a** (0.12 g, 30%) as a white solid: mp 108–110 °C. ^{1}H NMR (DMSO-d_6) δ 0.75 (t, 3H, CH_3), 0.88 (t, 3H, CH_3), 1.00–1.20 (m, 10H, $5CH_2$), 1.21–1.35 (m, 4H, $2CH_2$), 1.36–1.46 (m, 2H, CH_2), 2.94–3.08 (m, 4H, $2NCH_2$), 7.32 (dd, 1H, $J_{6,7}$ = 8.7, $J_{6,4}$ = 1.8, H6), 7.37 (d, 2H, J = 8.1, 2× ArH), 7.50 (d, 1H, $J_{7,6}$ = 8.7, H7), 7.88 (d, 2H, J = 8.1, 2× ArH), 8.04 (s, 1H, H4), 12.68 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 15.4, 15.6, 23.5, 23.7, 27.2, 27.9, 28.1, 29.3, 32.3, 32.8, 45.4, 48.9, 98.7, 111.0, 115.5, 121.7, 125.3, 128.9, 129.5, 131.4, 133.5, 136.0, 138.6, 148.9, 168.6, 188.6. MS (EI) m/z 592 (M^+, 3%), 380 (M^+-212, 100%). HRMS-ES calculated for $C_{28}H_{35}ClIN_2O_2$: 593.1432, found 593.1470. Anal. Calcd for $C_{28}H_{34}ClIN_2O_2$: C,

56.72; H, 5.78; N, 4.72. Found: C, 56.49; H, 5.75; N, 4.72.

5.13. *N*,*N*-Di-*n*-propyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide (4b)

Reaction of **3a** (0.72 g, 1.62 mmol) and di-*n*-propylamine (0.25 mL, 1.78 mmol) for 7 h as above gave after trituration with petroleum ether, **4b** (0.47 g, 57%) as an off-white solid: mp 148–150 °C. ^{1}H NMR (DMSO-d_6) δ 0.69 (t, 3H, J = 7.4, CH_3), 0.78 (t, 3H, J = 7.4, CH_3), 1.12–1.28 (m, 2H, CH_2), 1.40–1.50 (m, 2H, CH_2), 2.95 (t, 2H, J = 7.8, NCH_2), 3.01 (t, 2H, J = 7.7, NCH_2), 7.31 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.1, H6), 7.35 (d, 2H, J = 8.3, 2× ArH), 7.51 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.87 (d, 2H, J = 8.3, 2× ArH), 8.02 (d, 1H, $J_{4,6}$ = 1.9, H4). ^{13}C NMR (DMSO-d_6) δ 12.5, 13.1, 21.6, 22.8, 47.2, 50.8, 98.6, 111.1, 115.5, 121.6, 125.3, 128.9, 129.5, 131.4, 133.4, 136.0, 138.6, 149.0, 168.8, 188.5. MS (ES) *m*/*z* 509 (M+1, 88%), 106 (100%). HRMS-ES calculated for $C_{22}H_{23}ClIN_2O_2$: 509.0493, found 509.0524. Anal. Calcd for $C_{22}H_{22}ClIN_2O_2$: C, 51.94; H, 4.36; N, 5.51. Found: C, 52.14; H, 4.58; N, 5.53.

5.14. *N*,*N*-Diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl-glyoxylamide (4c)

Reaction of **3a** (0.36 g, 0.80 mmol) and diethylamine (0.09 mL, 0.88 mmol) for 19 h yielded after column chromatography (ethyl acetate/petroleum ether, 1:1 v/v) **4c** (0.14 g, 36%) as colourless crystals: mp 221–222 °C. ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, J = 7.1, CH_3), 1.02 (t, 3H, J = 7.0, CH_3), 3.07 (q, 2H, J = 7.1, CH_2), 3.15 (q, 2H, J = 7.0, CH_2), 7.32 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.1, H6), 7.37 (d, 2H, J = 8.3, 2× ArH), 7.51 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.88 (d, 2H, J = 8.3, 2× ArH), 8.05 (d, 1H, $J_{4,6}$ = 2.0, H4). ^{13}C NMR (DMSO-d_6) δ 12.5, 14.1, 38.4, 42.2, 97.4, 109.8, 114.3, 120.5, 124.1, 127.7, 128.4, 130.3, 132.3, 134.8, 137.3, 147.9, 167.1, 187.4. MS (ES) *m*/*z* 478 (M−1, 100%). HRMS-ES calculated for $C_{20}H_{19}ClIN_2O_2$: 481.0180, found 481.0180. Anal. Calcd for $C_{20}H_{18}ClIN_2O_2$: C, 49.97; H, 3.77; N, 5.83. Found: C, 50.27; H, 4.05; N, 5.85.

5.15. *N*,*N*-Dimethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide (4d)

Dimethylamine (30 mL, 40% wt/vol in water) was gently heated and under constant N_2 pressure bubbled through NaOH pellets into a solution of **3a** (0.65 g, 1.46 mmol) in toluene. After purification by column chromatography (ethyl acetate/petroleum ether, 1:1 v/v) and trituration with ethyl acetate/petroleum ether, 3:7 v/v, **4d** (65 mg, 10%) was obtained as a solid: mp 238 °C (decomposed). ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H, NCH_3), 2.79 (s, 3H, NCH_3), 7.32 (d, 2H, J = 8.3, 2× ArH), 7.33 (dd, 1H, $J_{6,7}$ = 8.6, H6), 7.52 (d, 1H, $J_{7,6}$ = 8.6, H7), 7.91 (d, 2H, J = 8.3, 2× ArH), 8.11 (d, 1H, J = 2.0, H4). ^{13}C NMR (DMSO-d_6) δ 33.7, 37.4, 97.9, 110.6, 114.8, 121.1, 124.7, 128.3, 128.8, 130.6, 132.5, 135.3, 137.7, 148.8, 167.5, 188.1. MS (ES) *m*/*z* 453 (M+1, 21%), 338 (M−114, 100%). HRMS-ES calculated for $C_{18}H_{15}ClIN_2O_2$: 452.9867, found 452.9872. Anal. Calcd for $C_{18}H_{14}ClIN_2O_2$: C, 47.76; H, 3.12; N, 6.19. Found: C, 47.54; H, 3.39; N, 5.89.

5.16. *N*,*N*-Dihexyl-[2-(4-iodophenyl)indol-3-yl]glyoxylamide (4e)

Reaction of **3b** (0.23 g, 0.56 mmol) and dihexylamine (0.15 mL, 0.62 mmol) for 18 h followed by column chromatography (ethyl acetate/petroleum ether, 2:3 v/v) and recrystallisation (ethanol/water) gave **4e** (0.10 g, 32%) as a white solid: mp 120–122 °C. ^{1}H NMR (DMSO-d_6) δ 0.74 (t, 3H, J = 7.0, CH_3), 0.84 (t, 3H, J = 6.9, CH_3), 1.00–1.45 (m, 16H, 8CH_2), 2.95–3.07 (m, 4H, 2NCH_2), 7.21–7.31 (m, 2H, H5 and H6), 7.37 (d, 2H, J = 8.3, 2× ArH), 7.48 (d, 1H, J = 7.7, H7), 7.87 (d, 2H, J = 8.3, 2× ArH), 8.04 (d, 1H, J = 7.7, H4). ^{13}C NMR (DMSO-d_6) δ 13.8, 14.0, 21.8, 22.1, 25.6, 26.2, 26.4, 27.5, 30.6, 31.1, 43.7, 47.3, 96.7, 109.8, 112.1, 120.9, 122.5, 123.6, 126.7, 130.2, 131.9, 135.8, 136.8, 146.0, 167.3, 187.1. MS (EI) *m*/*z* 558 (M^+, 4%), 346 (M^+−212, 100%). HRMS-EI calculated for $C_{28}H_{35}N_2O_2I$: 558.1743, found 558.1747.

5.17. *N*,*N*-Di-*n*-propyl-[2-(4-iodophenyl)indol-3-yl]glyoxylamide (4f)

Reaction of **3b** (0.23 g, 0.56 mmol) and di-*n*-propylamine (0.09 mL, 0.62 mmol) for 20 h, followed by column chromatography (ethyl acetate/petroleum ether, 1:1 v/v) and trituration with ethyl acetate/petroleum ether, 3:7, v/v, gave **4f** (58.2 mg, 22%) as an off-white solid: mp 159–161 °C. ^{1}H NMR (DMSO-d_6) δ 0.69 (t, 3H, J = 7.4, CH_3), 0.79 (t, 3H, J = 7.4, CH_3), 1.13–1.25 (m, 2H, CH_2), 1.41–1.52 (m, 2H, CH_2), 2.96 (t, 2H, J = 7.9, NCH_2), 3.03 (t, 2H, J = 7.7, NCH_2), 7.21–7.33 (m, 2H, H5 and H6), 7.37 (d, 2H, J = 8.4, 2× ArH), 7.48 (d, 1H, J = 7.3, H7), 7.88 (d, 2H, J = 8.4, 2× ArH), 8.03 (d, 1H, J = 7.3, H4). ^{13}C NMR (DMSO-d_6) δ 11.9, 12.4, 20.9, 22.2, 46.5, 50.2, 97.6, 110.8, 113.1, 121.8, 123.5, 124.5, 127.6, 131.3, 132.9, 136.8, 137.8, 147.0, 168.4, 188.0. MS (EI) *m*/*z* 474 (M^+, 6%), 346 (M^+−128, 100%). HRMS-EI calculated for $C_{22}H_{23}N_2O_2I$: 474.0804, found 474.0795.

5.18. *N*,*N*-Diethyl-[2-(4-iodophenyl)indol-3-yl]glyoxylamide (4g)

Reaction of **3b** (0.14 g, 0.34 mmol) and diethylamine (0.05 mL, 0.47 mmol) for 3 h gave after recrystallisation (ethyl acetate/petroleum ether, 1:1 v/v) **4g** (60 mg, 40%) as off-white crystals: mp 169–171 °C. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.1, CH_3), 1.02 (t, 3H, J = 7.0, CH_3), 3.07 (q, 2H, J = 7.1, NCH_2), 3.15 (q, 2H, J = 7.0, NCH_2), 7.22–7.32 (m, 2H, H5 and H6), 7.37 (d, 2H, J = 8.3, 2× ArH), 7.49 (d, 1H, J = 7.3, H7), 7.87 (d, 2H, J = 8.3, 2× ArH), 8.06 (d, 1H, J = 7.2, H4). ^{13}C NMR (DMSO-d_6) δ 13.8, 15.3, 39.5, 43.5, 98.3, 111.5, 113.8, 122.5, 124.2, 125.3, 128.4, 131.9, 133.6, 137.3, 138.4, 147.7, 168.7, 188.8. MS (ES) *m*/*z* 445 (M−1, 100%). HRMS-EI calculated for $C_{20}H_{19}IN_2O_2$: 446.0491, found 446.0489. Anal. Calcd

for $C_{20}H_{19}IN_2O_2$: C, 53.83; H, 4.29; N, 6.28. Found: C, 53.50; H, 4.41; N, 6.11.

5.19. *N*,*N*-Dimethyl-[2-(4-iodophenyl)indol-3-yl]glyoxylamide (4h)

Dimethylamine (30 mL, 40% wt/vol in water) was gently heated and under constant N_2 pressure bubbled through NaOH pellets into a solution of **3b** (0.30 g, 0.73 mmol) in toluene. After column chromatography (ethyl acetate/petroleum ether, 1:1 v/v), **4h** was obtained (14 mg, 5%) as an off-white solid: mp 231–232 °C. ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, NCH_3), 2.79 (s, 3H, NCH_3), 7.20–7.30 (m, 2H, H5 and H6), 7.34 (d, 2H, J = 8.1, 2× ArH), 7.48 (d, 1H, J = 7.0, H7), 7.89 (d, 2H, J = 8.3, 2× ArH), 8.07 (d, 1H, H4). ^{13}C NMR (DMSO-d_6) δ 34.4, 38.1, 98.0, 111.3, 114.0, 122.5, 124.3, 125.2, 128.2, 130.5, 133.3, 138.2, 168.8, 187.2. MS (EI) *m*/*z* 418 (M^+, 6%), 346 (M^+-72, 100%). HRMS-EI calculated for $C_{18}H_{15}N_2O_2I$: 418.0178, found 418.0161. Anal. Calcd for $C_{18}H_{15}IN_2O$. H_2O: C, 49.56; H, 3.93; N, 6.42. Found: C, 49.70; H, 3.46; N, 6.29

5.20. *N*,*N*-Dihexyl-[2-(4-bromophenyl)-5-chloroindol-3-yl]glyoxylamide (4i)

Reaction of **3c** (0.46 g, 1.16 mmol) and dihexylamine (0.30 mL, 1.27 mmol) for 7 h, followed by purification by column chromatography (ethyl acetate/petroleum ether, 2:3 v/v) and recrystallisation (ethyl acetate/petroleum ether, 1:9 v/v), gave **4i** (0.20 g, 32%) as a white solid: mp 103–105 °C. ^{1}H NMR (DMSO-d_6) δ 0.77 (t, 3H, J = 7.0, CH_3), 0.87 (t, 3H, J = 6.8, CH_3), 1.00–1.46 (m, 16H, $8CH_2$), 2.95–3.09 (m, 4H, $2NCH_2$), 7.32 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.0, H6), 7.51 (d, 1H, $J_{7,6}$ = 8.7, H7), 7.54 (d, 2H, J = 8.4, 2× ArH) 7.72 (d, 2H, J = 8.4, 2× ArH), 8.03 (s, 1H, H4). ^{13}C NMR (DMSO-d_6) δ 14.7, 14.9, 22.8, 23.0, 26.5, 27.1, 27.4, 28.6, 31.6, 32.0, 44.7, 48.2, 110.4, 114.8, 121.0, 124.5, 124.6, 128.1, 128.7, 130.4, 132.0, 132.9, 135.3, 148.0, 167.9, 187.8. MS (EI) *m*/*z* 546 (M^+, 1%), 334 (M^+-212, 100%). HRMS-EI calculated for $C_{28}H_{34}{}^{81}Br^{37}ClN_2O_2$: 548.1442, found 548.1442. Anal. Calcd for $C_{28}H_{34}BrClN_2O_2$: C, 61.60; H, 6.28; N, 5.13. Found: C, 61.35; H, 6.02; N, 5.06.

5.21. *N*,*N*-Di-n-propyl-[2-(4-bromophenyl)-5-chloroindol-3-yl]glyoxylamide (4j)

Reaction of **3c** (0.41 g, 1.03 mmol) and di-*n*-propylamine (0.16 mL, 1.13 mmol) for 24 h, followed by purification by column chromatography (ethyl acetate/petroleum ether, 1:1 v/v) and recrystallisation (diethyl ether/petroleum ether), gave **4j** (0.25 g, 53%) as a white solid: mp 128–129 °C. ^{1}H NMR (DMSO-d_6) δ 0.69 (t, 3H, J = 7.4, CH_3), 0.77 (t, 3H, J = 7.4, CH_3), 1.15–1.25 (q, 2H, J = 7.8, CH_2), 1.40–1.50 (q, 2H, J = 7.6, CH_2), 2.96 (t, 2H, J = 7.8, NCH_2), 3.02 (t, 2H, J = 7.7, NCH_2), 7.31 (dd, 1H, $J_{6,7}$ = 8.6, $J_{6,4}$ = 2.1, H6), 7.51 (d, 1H, $J_{7,6}$ = 8.7, H7), 7.52 (d, 2H, J = 8.5, 2× ArH), 7.70 (d, 2H, J = 8.5, 2× ArH), 8.00 (dd, 1H, $J_{4,6}$ = 1.74, H4). ^{13}C NMR (DMSO-d_6) δ 11.5, 12.0, 20.6, 21.8, 46.2, 49.9, 110.1, 114.6, 120.6, 124.2, 124.3, 127.9, 128.4, 130.2, 131.7, 132.5, 135.0, 147.8, 167.8, 187.4. MS (ES) *m*/*z* 463 (^{81}Br M+1, 61%), 106 (M−357, 100%). HRMS-ES calculated for $C_{22}H_{23}N_2O_2ClBr$ 461.0631, found 461.0649. Anal. Calcd for $C_{22}H_{22}BrClN_2O_2$: C, 57.22; H, 4.80; N, 6.07. Found: C, 57.33; H, 4.89; N, 6.01.

5.22. *N*,*N*-Diethyl-[2-(4-bromophenyl)-5-chloroindol-3-ylglyoxylamide (4k)

To a solution **3c** (0.72 g, 1.81 mmol) in anhydrous toluene (40 mL) at 0 °C was added dropwise a solution of triethylamine (0.30 mL, 2.18 mmol) and diethylamine (0.21 mL, 1.99 mmol) in anhydrous toluene (10 mL). The reaction mixture was stirred at room temperature for 18 h. A white precipitate that formed was filtered, washed with water (25 mL) and extracted into dichloromethane (2× 40 mL). The combined organic extracts were dried with $MgSO_4$, filtered, and the solvent was evaporated in vacuo. After recrystallisation (ethyl acetate/petroleum ether, 1:1 v/v), **4k** (0.462 g, 59%) was obtained as a white solid: mp 216–217 °C. ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.1, CH_3), 1.02 (t, 3H, J = 7.0, CH_3), 3.07 (q, 2H, J = 6.9, NCH_2), 3.15 (q, 2H, J = 6.8, NCH_2), 7.32 (d, 1H, J = 8.6, H6), 7.47–7.56 (m, 3H, 2× ArH and H7), 7.72 (d, 2H, 2× ArH), 8.05 (s, 1H, H4). ^{13}C NMR (DMSO-d_6) δ 13.0, 14.6, 38.9, 42.7, 110.4, 114.9, 121.0, 124.5, 124.6, 128.1, 128.9, 130.1, 132.0, 132.9, 135.3, 148.5, 167.7, 188.0. MS (EI) *m*/*z* 434 (M^+, 4%), 334 (M^+-100, 100%). Anal. Calcd for $C_{20}H_{18}BrClN_2O_2$: C, 55.38; H, 4.18; N, 6.46. Found: C, 55.37; H, 4.36; N, 6.40.

5.23. *N*,*N*-Dihexyl-[2-(4-bromophenyl)indol-3-yl]glyoxylamide (4l)

Reaction of **3d** (0.96 g, 2.65 mmol) and dihexylamine (0.68 mL, 2.91 mmol) for 24 h gave after purification by column chromatography (methanol/dichloromethane, 1:19 v/v) and recrystallisation (ethanol/water), **4l** (0.62 g, 46%) as a white solid: mp 106–110 °C. ^{1}H NMR (DMSO-d_6) δ 0.74 (t, 3 H, J = 7.0, CH_3), 0.88 (t, 3H, J = 6.9, CH_3), 1.00–1.20 (m, 10H, $5CH_2$), 1.20–1.30 (m, 4H, $2CH_2$), 1.35–1.47 (m, 2H, CH_2), 2.95–3.08 (m, 4H, $2NCH_2$), 7.21–7.32 (m, 2H, H5 and H6), 7.48 (d, 1H, J = 7.6, H7), 7.53 (d, 2H, J = 8.4, 2× ArH), 7.70 (d, 2H, J = 8.4, 2× ArH), 8.05 (d, 1H, J = 7.8, H4), 12.49 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 15.4, 15.6, 23.5, 23.7, 27.2, 27.8, 28.1, 29.4, 32.3, 32.7, 45.4, 49.0, 111.5, 113.8, 122.6, 124.2, 124.9, 125.3, 128.3, 131.6, 132.6, 133.7, 137.4, 147.4, 168.9, 188.8. MS (CI) *m*/*z* 511 (^{79}Br M^+, 4%), 513 (^{81}Br M^+, 3%), 298 (^{79}Br M^+-213, 100%), 300 (^{81}Br M^+-213, 80%). HRMS-EI calculated for $C_{28}H_{35}BrN_2O_2$: 510.1882, found 510.1874. Anal. Calcd for $C_{28}H_{35}BrN_2O_2$: C, 65.75; H, 6.90; N, 5.48. Found: C, 65.64; H, 6.78; N, 5.49.

5.24. *N*,*N*-Diethyl-5-chloro-[2-(4-trimethylstannylphenyl)indol-3-yl]glyoxylamide (5)

To a solution of **4k** (112 mg, 0.259 mmol) in anhydrous toluene (8 mL) were added hexamethylditin (230 μg,

0.702 mmol) and a catalytic amount of $Pd(0)(PPh_3)_4$. The reaction mixture was heated at reflux under a nitrogen atmosphere for 8 h. The reaction mixture was passed through Celite and the solvent evaporated. The product was purified by column chromatography (ethyl acetate/petroleum ether, 1:1 v/v) to yield [^{123}I]**4c** as a white solid (42.7 mg, 32%). ^{1}H NMR (CD_3OD) δ 0.36 (s, 9H, $Sn(CH_3)_3$), 0.79 (t, 3H, $J = 7.2$, CH_3), 1.15 (t, 3H, $J = 7.2$, CH_3), 3.07 (q, 2H, $J = 7.2$, NCH_2), 3.24 (q, 2H, $J = 7.2$, NCH_2), 7.32 (dd, 1H, $J_{6,7} = 8.4$, $J_{6,4} = 2.0$, H6), 7.47 (d, 1H, $J_{6,7} = 8.4$, H7), 7.54 (d, 2H, $J = 8.0$, 2ArH), 7.65 (d, 2H, $J = 8.0$, 2ArH), 8.27 (d, 1H, $J_{4,6} = 2.0$, H4). MS-ES *m/z* 517 (M−1, 70%). HRMS-ES calculated for $C_{23}H_{28}N_2O_2Cl^{116}Sn$: 515.0857, found 515.0903.

5.25. [^{123}I]*N*,*N*-Diethyl-[5-chloro-2-(4-iodophenyl)indol-3-yl]glyoxylamide

To a solution of stannane, **5** (125 μg in ethanol (50 μL) and acetic acid (200 μL)) were added Na^{123}I (311–544 MBq) and per-acetic acid (30%, 25 μL). After 5 min at room temperature, the reaction was quenched with $Na_2S_2O_5$ (51 mg/mL, 100 μL) and $NaHCO_3$ (48–51 mg/mL, 100 μL). Mobile phase (acetonitrile/0.01 M ammonium acetate, 3:2 v/v, 350 μL) was added and the solution was injected onto a semi-preparative C-18 RP HPLC column. The retention time at a flow rate of 3 mL/min was 16 min. The radiochemical purity was >96% after formulation in saline.

5.26. Lipophilicity measurements

Samples were analysed using a C18 column (X-Terra, 5μ, 4.6 × 250 mm) and a mobile phase of methanol and 0.1 M phosphate buffer (65:35 v/v, pH 7.5) with a flow rate of 1 mL/min.[24] The log *P* values were estimated by comparing HPLC retention times (RT) of test compounds with retention times of standards having known log *P* values. The standards used were aniline, benzene, bromobenzene, ethylbenzene and trimethylbenzene. A calibration curve of log *P* versus ln RT was generated. The equation was linear with an r^2 of 0.9969. Calculated log *P* values were found using Chem Draw Ultra.

5.27. In vitro binding studies

5.27.1. Peripheral benzodiazepine binding sites. Mitochondrial membranes were prepared from kidney extracts sourced from male Sprague–Dawley rats. The kidneys were removed, rinsed with ice-cold 50 mM Tris–HCl buffer (pH 7.4), weighed and finely cut. After the addition of 20 volumes of ice-cold buffer, the preparation was homogenised and the suspension was centrifuged at 49,000*g* for 15 min at 4 °C. The pellets were resuspended in buffer to achieve a protein concentration of 4 mg/mL. Membranes were stored at −80 °C until required.[25,26] Protein content in the membrane suspensions was measured according to a colorimetric method.[27] For determination of the inhibition constant (IC_{50}), assays were run in triplicate with concentrations of test compounds ranging from 10^{-5} to 10^{-10} M in 50 mM Tris–HCl at pH 7.4 in the presence of [^{3}H]PK11195 (2 nM) at a final protein concentration of 250 μg/mL in a final volume of 0.5 mL. Non-specific binding was determined using PK11195 (10 μM), a selective PBBS ligand. Samples were incubated for 1 h at 4 °C and then filtered under reduced pressure on glass fibre filters (Whatman GF/B) pre-soaked in 50 mM Tris–HCl. After incubation, filters were immediately washed 4 times with 4 mL ice-cold Tris–HCl buffer and the radioactivity remaining on the filters was measured in a β-scintillation counter (Packard). IC_{50} values were calculated using an iterative non-linear least-squared curve fitting program (Kell Radioligand).

5.27.2. Central benzodiazepine receptor. Cortical membranes were extracted from male Sprague–Dawley rats. The cortex was removed, rinsed with ice-cold 50 mM Tris–HCl buffer (pH 7.4), weighed and finely cut. After the addition of 20 volumes of ice-cold buffer, the preparation was homogenised and the suspension was centrifuged at 20,000*g* for 20 min at 4 °C. The pellets were resuspended in buffer and centrifuged as above. The pellet was resuspended in 50 mM Tris–HCl, 0.32 M sucrose buffer (pH 7.4) to achieve a protein concentration of 4 mg/mL, and the membranes were stored at −80 °C until required. For determination of the inhibition constant (IC_{50}), assays were run as for PBBS binding but using [^{3}H]flumazenil (2 nM) and cortex membranes at a final concentration of 250 μg/mL. Samples were incubated for 45 min at 25 °C. Non-specific binding was determined using flumazenil (20 μM), a selective CBR ligand.

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