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Fluorescent ligands for the histamine H_2 receptor: synthesis and preliminary characterization

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Abstract—3-[3-(Piperidinomethyl)phenoxy]alkyl, *N*-cyano-*N*′-[ω-[3-(1-piperidinylmethyl)phenoxy]alkyl]guanidine and 2-(5-methyl-4-imidazolyl)methyl thioethyl derivatives containing fluorescent functionalities were synthesized and the histamine H_2 receptor affinity was evaluated using the H_2 antagonist [^{125}I]-aminopotentidine. The compounds exhibited weak to potent H_2 receptor affinity with pK_i values ranging from <4 to 8.85. The highest H_2 receptor affinity was observed for *N*-cyano-*N*′-[ω-[3-(1-piperidinylmethyl)phenoxy]alkyl]guanidines substituted with methylanthranilate (**13**), cyanoindolizine (**6**) and cyanoisoindole (**11**) moieties via an ethyl or propyl linker.

1. Introduction

The histamine H_2 receptor is member of the large multigene family of G-protein coupled receptors[1] and has for long been a very useful target for the treatment of gastric ulcers. Although not the first choice treatment any more, the use of H_2 receptor antagonists in the treatment of peptic ulcers and oesophageal reflux is still extensive. In view of the important therapeutic application of the H_2 antagonists a huge number of potent H_2 antagonists with widely diverse structures have been synthesized since the identification of the H_2 receptor in 1972.[2] The 3-[3-(piperidinomethyl)phenoxy]propyl moiety in combination with urea, thiourea or cyanoguanidino structures is well known for high H_2 receptor activity.[3–5] Within this series of potent compounds the radioactive probes [^{125}I]iodoaminopotentidine and [^{125}I]iodoazidopotentidine are well known examples and have proven to be very useful probes to study the localization and molecular properties of the human H_2 receptor (Fig. 1).[3]

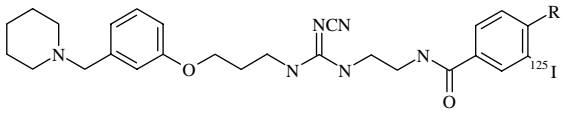

Figure 1. [^{125}I]Iodoaminopotentidine (R = NH_2).

In recent years the use of fluorescent detection methods, that is, confocal laser scanning microscopy and image analysis, in nonradioactive assays have found widespread applicability in receptor pharmacology. Fluorescent ligands are used to determine receptor properties like receptor internalization and sub cellular localization, the thermodynamics and kinetics of ligand binding and to assess the nature of the microenvironment of the ligand binding site. Traditional radioligand binding techniques have at the same time been replaced by more sophisticated techniques like, for example, image analysis and confocal microscopy,[6] fluorescence spectroscopy[7] and flow cytometry.[8,9]

In this study we describe a series of fluorescent analogues of iodoaminopotentidine with high affinity for the histamine H_2 receptor. We used the 3-[3-(piperidinomethyl)phenoxy]propyl, *N*-cyano-*N*′-[ω-[3-(1-piperidinylmethyl)phenoxy]alkyl]guanidine, synthesized as described by Buschauer et al.[10,11] and the well known 2-(5-methyl-4-imidazolyl)-methylthioethylamine[2] moieties as basis

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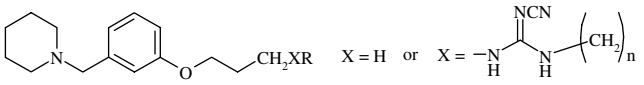
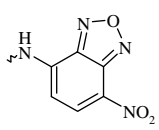
Table 1. Fluorescent derivatives of 3-[3-(piperidinomethyl)phenoxy]-propyl amine and *N*-cyano-*N*′-[ω-[3-(1-piperidinylmethyl)phenoxy]-alkyl]guanidine



where n = 2/3 and R=



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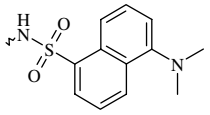

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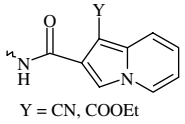
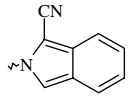


5, 6, 7, 8



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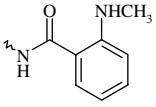

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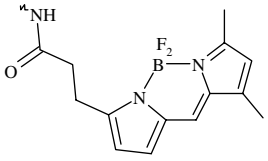

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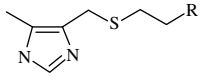
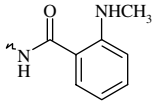
Table 2. Fluorescent derivatives of 2-(5-methyl-4-imidazolyl)-methyl-thioethyl amine



R=



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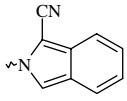

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for synthesis of fluorescent ligands by incorporation of various fluorescent groups (Tables 1 and 2).

The fluorescent groups were selected on the basis of their spectroscopic properties, ease of conjugation with the amine and structure–activity relationship requirements for H_2 affinity. The groups used included dansyl-amido (**1**–**3**),[12] 7-nitrobenzoxadiazoleamino (**4**),[13] 1-cyanoisoindole (**9**–**11**, **18**, **19**),[14,15] *N*-methylanthranil-amido (**12**–**14**),[16] 1-cyano-indolizine-2-carboxamido (**5**–**7**),[17,18] ethyl indolizine-2-carboxylate-1-carboxamido (**8**)[19] and dimethylBodipypropionamido (**15**–**17**).[20]

o-Phtaldialdehyde reacts with primary amines in the presence of potassium cyanide to form the fluorescent isoindole.[14,15] *N*-Methylanthranilic and dansylamido compounds were obtained through the intermediate complex with *N*,*N*′-carbonyldiimidazole and yielded the fluorescent amides on reaction with the primary amines.[3] The indolizine compounds were conjugated to the amines through ammonolysis to give the required amides[21] and 7-nitrobenzoxadiazole chloride was reacted with the primary amine in chloroform to yield the substituted secondary amine.[22] DimethylBODIPY (BODIPY®FL, Molecular Probes Inc.) was reacted with the primary amines via carbodiimide activation chemistry.[3]

2. Results and discussion

All the synthesized compounds were obtained as the free base or as the HCl salt thereof and the structures were confirmed using NMR. The pK_i values, obtained with a [^{125}I]-aminopotentidine ([^{125}I]-APT) binding assay using COS-7 cells transiently expressing the rat H_2 receptor[23] ranged from less than 4 for the imidazolyl compounds to 8.85 for the piperidinylmethylphenoxy (PMP) compounds. Relative fluorescent intensity (RFI; in arbitrary fluorescence units) values in excess of 100% were calculated for some of the test compounds (from 10-fold dilutions; Table 3).

All the pK_i values observed for the piperidinylmethyl-phenoxy (PMP) compounds (5.34–8.85) compared favourably to that of the imidazolyl compounds (<4.0). Changing from the imidazolyl moiety to the PMP moiety leads to a considerable increase in affinity (**9** and **12** vs **18** and **19**). The inclusion of a cyanoguan-

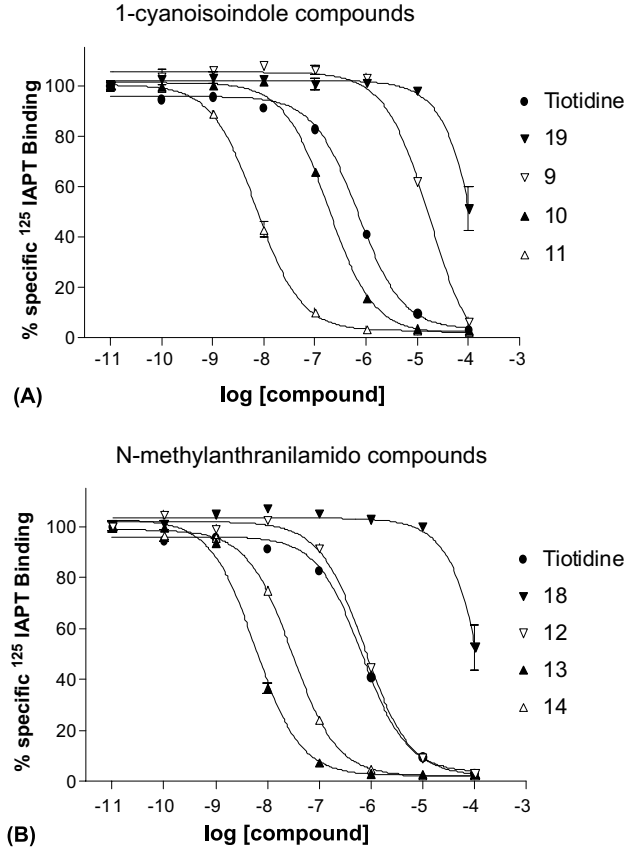
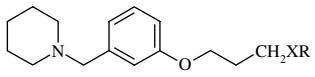
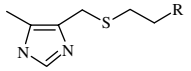


Figure 2. Displacement of [^{125}I]iodoaminopotentidine from the histamine H_2 receptor by the 1-cyanoisoindole (A) and *N*-methylanthranil-amido compounds (B).

Table 3. Histamine H_2 receptor binding data and fluorescent properties of 3-[3-(piperidinomethyl)phenoxy]propyl amine, *N*-cyano-*N*′-[ω-[3-(1-piperidinylmethyl)phenoxy]alkyl]guanidine and 2-(5-methyl-4-imidazolyl)-methylthioethyl amine



Compounds **1**–**17**



Compounds **18**–**19**

	X	R (fluorophore)	Mp (°C)	% Yield	pK_i ± sem	λ_{Ex} (nm)[a]	λ_{Em} (nm)[a]	RFI (%)[a]
1	—	Dansylamido	100	26	7.00 ± 0.15	330	545	34.6
2	$HNC(NCN)NH(CH_2)_2$	Dansylamido	89	38	7.23 ± 0.19	330	545	35.1
3	$HNC(NCN)NH(CH_2)_3$	Dansylamido	126	43	6.63 ± 0.08	330	545	36.4
4	—	7-Nitrobenzoxadiazoleamino	Oil	9	6.77 ± 0.03	460	540	0.8
5	—	1-Cyano-indolizine-2-carboxamido	135	19	7.60 ± 0.22	330	410	>100
6	$HNC(NCN)NH(CH_2)_2$	1-Cyano-indolizine-2-carboxamido	137	8	8.84 ± 0.24	340	400	73.4
7	$HNC(NCN)NH(CH_2)_3$	1-Cyano-indolizine-2-carboxamido	166	35	7.22 ± 0.10	330	410	75.6
8	—	Ethyl indolizine-2-carboxylate-1-carboxamido	Oil	20.5	5.99 ± 0.01	330	410	>100
9	—	1-Cyanoisoindole	Oil	24	5.34 ± 0.24	330	370	1.9
		1-Cyanoisoindole·HCl	76	54.5				
10	$HNC(NCN)NH(CH_2)_2$	1-Cyanoisoindole	89	26.6	7.25 ± 0.15	330	370	5.8
11	$HNC(NCN)NH(CH_2)_3$	1-Cyanoisoindole	114	20	8.31 ± 0.33	330	370	2.7
12	—	*N*-Methylanthranilamido	Oil	20	6.58 ± 0.08	330	440	22.1
13	$HNC(NCN)NH(CH_2)_2$	*N*-Methylanthranilamido	Oil	55	8.85 ± 0.19	340	440	22.1
14	$HNC(NCN)NH(CH_2)_3$	*N*-methylanthranilamido	Oil	73	8.23 ± 0.31	330	440	22.8
15	—	DimethylBodipypropionamido	127	56	6.49 ± 0.17	300	520	154.2
16	$HNC(NCN)NH(CH_2)_2$	DimethylBodipypropionamido	105	27	6.90 ± 0.21	300	520	155.8
						400	520	146.7
17	$HNC(NCN)NH(CH_2)_3$	DimethylBodipypropionamido	156	18	6.32 ± 0.12	300	520	159.1
18	—	*N*-Methylanthranilamido	108	74	<4.00	330	370	5.9
19	—	1-Cyanoisoindole	172	21	<4.00	360	440	22.6

λ_{Ex} = excitation λ; λ_{Em} = emission λ; RFI = relative fluorescent intensity.
[a] At 10^{-6} M in PBS buffer at 22 °C.

idine linker in general increased affinity for the histamine H_2 receptor and the optimum alkyl chain length connecting this group to the fluorophore seems to be an ethyl chain. When this chain is increased to a propyl linker a decrease in affinity is observed. The exception here is the cyanoisoindole (**11**) where a 10-fold increase is observed (Fig. 2).

The preference for groups containing additional cyano groups is clear in comparison between the affinities of **5** and **8**. Although the dansyl compounds exhibited reasonable H_2 affinity, it was clear that the compounds containing sulphonamide groups had lower affinity than those with amide bonds. PMP compounds containing an amide bond, conjugated to an aromatic system, and two carbon atoms removed from the cyanoguanidino moiety seem to be the optimum structure in this series. The introduction of an additional spacer between the amide and aromatic system lead to a decrease in affinity as seen with the BODIPY compounds (**16**, **17**).

The best H_2 receptor affinity in this series was observed for the methylanthranilate (**13**), the cyanoindolizine (**6**) and the cyanoisoindole (**11**). The first two contain the cyanoguanidino moiety as well as an amide conjugated to an aromatic system. The amide is two carbon atoms removed from the cyanoguanidine. The cyanoisoindole also contains the cyanoguanidino moiety but in this case it is three carbons removed from the aromatic system that contains an additional cyanogroup.

The dimethylBodipy compounds exhibited the best fluorescent properties (high relative fluorescent intensity and Stokes shift) but had only moderate H_2 receptor affinity. The high affinity compounds (**6**, **11** and **13**) exhibited low to moderate fluorescent potency and smaller Stokes shifts.

3. Conclusion

We have identified a series of fluorescent structures with high affinity for the histamine H_2 receptor. Although the ideal combination of long wavelength fluorescence, high Stokes shifts and high affinity for the receptor was not found, further experiments to optimize the effective use of some of these compounds in the analysis of receptor binding by fluorescent techniques are in progress. Ultimately, the use of these ligands might provide an attractive alternative to radioligand binding studies and offer an attractive method to directly study the kinetics of ligand receptor interactions.[7]

4. Experimental

4.1. General procedures

Melting points were determined with an 'Electrothermal IA 9200' apparatus and are not corrected. ^{1}H NMR spectra were recorded on a Bruker AC-200 (200 MHz)

spectrometer using the solvent peaks as reference. Chromatographic purifications were performed on silica gel (0.063–0.2 mm, Merck) except when otherwise stated. Exact masses were determined on a Finnigan LCQ_{DECA} ion-trap mass spectrometer under APCI or ESI conditions.

4.2. Synthesis

3-[3-(1-Piperidinylmethyl)phenoxy]propylamine, *N*-cyano-*S*-methyl-*N*′-[3-[3-(1-piperidinylmethyl)phenoxy]-propyl]isothiourea, *N*-(3-aminopropyl)-*N*′-cyano-*N*″-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine and *N*-(2-aminoethyl)-*N*′-cyano-*N*″-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine were obtained by the methods described by Buschauer et al. (1985).[10] Ethyl-1-cyanoindolizine-2-carboxylate and diethylindolizine-1,2-dicarboxylate were obtained by the methods described by Hurst et al. (1965)[18] and Bragg and Wibberley (1963),[19] respectively, and 2-(5-methyl-4-imidazolyl)-methylthioethylamine·2HBr from the synthesis of Horne et al. (1994).[24]

4.2.1. [3-(1-Piperidinylmethyl)phenoxy]alkanamines.

4.2.1.1. 5-Dimethylamino-1-naphthalenesulfonic acid {3-[3-(piperidin-1-ylmethyl)phenoxy]propyl}amide (1). Dansyl chloride (0.94 g, 3.5 mmol) in chloroform (12 mL) was added dropwise to a solution of 3-(piperidin-1-ylmethyl)phenoxypropylamine (1.0 g, 4.0 mmol)[10] in chloroform (5 mL) and stirred for another 20 min. The reaction mixture was washed with 10% $NaHCO_3$ solution (10 mL) and water (10 mL), dried with Na_2SO_4 and concentrated in vacuo. The product was purified by flash chromatography using $CHCl_3$–MeOH–NH_3 (8:1:0.1) (mp 100 °C; 0.439 g, 26%).

$C_{27}H_{35}N_3O_3S$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30–1.63 (m, 6H), 1.88 (qui, 2H, J = 6.4 Hz), 2.25–2.38 (m, 4H), 2.87 (s, 6H), 3.02–3.18 (m, 2H), 3.42 (s, 2H), 3.88 (t, 2H, J = 6.4 Hz), 5.10–5.20 (m, 1H, NH), 6.62 (dd, 1H, J = 7.7, 1.4 Hz), 6.79 (s, 1H), 6.91 (d, 1H, J = 7.7 Hz), 7.13 (d, 1H, J = 7.6 Hz), 7.17 (t, 1H, J = 7.7 Hz), 7.46 (t, 1H, J = 8.7 Hz), 7.52 (t, 1H, J = 8.7 Hz), 8.16–8.27 (m, 2H), 8.53 (d, 1H, J = 8.7 Hz) (Table 4).

4.2.1.2. 5-Dimethylamino-1-naphthalenesulfonic acid (2-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)ethyl]-guanidino}propyl)amide (2). Dansyl chloride (0.65 g, 2.4 mmol) in chloroform (8 mL) was added dropwise to a solution of *N*-aminoethyl-*N*′-cyano-*N*″-[3-(piperidin-1-ylmethyl)phenoxypropyl]-*N*-guanidine (1.0 g, 2.8 mmol)[3] in chloroform (5 mL) and stirred for 60 min. The reaction mixture was washed with 10% $NaHCO_3$ solution (5 mL) and water (5 mL), dried with Na_2SO_4 and concentrated in vacuo. The product was purified by flash chromatography using $CHCl_3$–MeOH–NH_3 (8:1:0.1) (mp 89 °C; 0.540 g, 38%).

$C_{31}H_{41}N_7O_3S$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30–1.60 (m, 6H), 1.93 (qui, 2H, J = 6.2 Hz), 2.33–2.45 (m, 4H), 2.84 (s, 6H), 2.80–2.95 (m, 2H), 3.02–3.31 (m, 4H), 3.46 (s, 2H), 3.92 (t, 2H, J = 6.2 Hz), 5.13–5.26 (m, 1H, NH), 5.26–5.38 (m, 1H, NH), 6.59 (dd, 1H, J = 7.8 Hz, 1.2 Hz), 6.81 (d, 1H, J = 7.4 Hz), 7.02–7.26 (m, 3H+NH), 7.49 (t, 1H, J = 8.5 Hz), 7.53 (t, 1H, J = 8.5 Hz), 8.16 (d, 1H, J = 8.5 Hz), 8.27 (d, 1H, J = 8.5 Hz), 8.51 (d, 1H, J = 8.5 Hz).

4.2.1.3. 5-Dimethylamino-1-naphthalenesulfonic acid (3-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]-guanidino}propyl)amide (3). *N*-3-Aminopropyl-*N*′-cyano-*N*″-[3-3-(1-piperidinylmethyl)phenoxypropyl]guanidine (0.816 g, 2.2 mmol) was dissolved in dichloromethane (15 mL) and TEA (0.61 g, 4.4 mmol) was added and cooled in an ice bath. Next dansyl chloride (0.590 g, 2.2 mmol) in dichloromethane (5 mL) was added and stirred for 1 h in the ice bath and 1 h at room temperature. The mixture was washed with 10% K_2CO_3 (10 mL) and dried with $MgSO_4$. Concentration in vacuo gave 1.28 g of an oil that was purified with flash chromatography ethyl acetate followed by ethyl acetate/MeOH (1:1). Crystallization from $CHCl_3$ yielded the product (mp 126 °C; 0.573 g, 43%).

$C_{32}H_{43}N_7O_3S$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30–1.60 (m, 8H), 1.98 (qui, 2H, J = 6.2 Hz), 2.25–2.40 (m, 4H), 2.85 (s, 6H), 2.70–2.90 (m, 2H), 3.10–3.28 (m, 2H), 3.30–3.45 (m, 2H), 3.46 (s, 2H), 3.94 (t, 2H, J = 6.0 Hz), 5.72–5.85 (m, 1H, NH), 5.85–6.00 (m, 1H, NH), 6.35 (br s, 1H, NH), 6.55–6.75 (m, 1H), 6.84 (d, 1H, J = 7.4 Hz), 6.89 (s, 1H), 7.05–7.20 (m, 2H), 7.45 (t, 1H, J = 7.9 Hz), 7.53 (t, 1H, J = 8.0 Hz), 8.14 (d, 1H, J = 8.0 Hz), 8.27 (d, 1H, J = 8.1 Hz), 8.49 (d, 1H, J = 8.0 Hz).

4.2.1.4. 7-Nitro-4-[3-(3-piperidin-1-ylmethylphenoxy)-propyl]amino-benzo[1,2,5]oxadiazole (4). NBD chloride (0.70 g, 3.5 mmol) in chloroform (12 mL) was added dropwise to a solution of 3-(piperidin-1-ylmethyl)phenoxypropylamine (1.0 g, 4.0 mmol)[10] in chloroform (5 mL) and stirred for 60 min. The reaction mixture was washed with 10% $NaHCO_3$ solution (10 mL) and water (10 mL), dried with Na_2SO_4 and concentrated in vacuo. The product was purified by flash chromatography using $CHCl_3$–MeOH–NH_3 (8:1:0.1) to yield the product as dark yellow oil (0.130 g, 9%).

$C_{21}H_{25}N_5O$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30–1.70 (m, 6H), 2.20–2.45 (m, 6H), 3.46 (s, 3H), 3.71 (t, 2H, J = 6.1 Hz), 4.18 (t, 2H, J = 5.9 Hz), 6.07 (d, 1H, J = 7.8 Hz), 6.78–6.99 (m, 3H), 7.20 (t, 1H, J = 7.6 Hz), 8.43 (d, 1H, J = 7.8 Hz).

4.2.1.5. 1-Cyano-indolizine-2-carboxylic acid 3-[3-(piperidin-1-ylmethyl)phenoxy]propylamide (5). Ethyl-1-cyanoindolizine-2-carboxylate (0.107 g, 0.5 mmol) and 3-[3-(1-piperidinylmethyl)phenoxy]propylamine (0.124 g, 0.5 mmol) were heated together at 150 °C for 6 h and the ethanol formed in the reaction was allowed to evaporate. The dark oily product was added to 50 mL 1 N HCl/dichloromethane, the layers separated and the aqueous layer extracted with dichloromethane (3 × 30 mL). The pH of the aqueous layer was adjusted to 7 by adding K_2CO_3 and extracted with dichloromethane (3 × 30 mL). The latter organic fractions were combined and washed with alkaline water, dried over

Table 4. Exact masses and R_f values of synthesized compounds

	Molecular formula	MH^+ calcd	MH^+ found[a]	R_f[b]	R_f[c]	R_f[d]
1	$C_{27}H_{35}N_3O_3S$	482.25	482.3	0.37	0.89	0.23
2	$C_{31}H_{41}N_7O_3S$	592.31	592.4	0.21	0.05	0.35
3	$C_{32}H_{43}N_7O_3S$	606.32	606.4	0.20	0.08	0.25
4	$C_{21}H_{25}N_5O_4$	412.20	412.3	0.27	0.80	0.26
5	$C_{25}H_{28}N_4O_2$	417.23	417.3	0.14	0.47	0.40
6	$C_{29}H_{34}N_8O_2$	527.29	527.4	0.07	0.00	0.46
7	$C_{30}H_{36}N_8O_2$	541.31	541.4	0.08	0.00	0.43
8	$C_{27}H_{33}N_3O_4$	464.26	464.3	0.23	0.58	0.20
9	$C_{24}H_{27}N_3O$	374.23	374.4	0.32	0.10	0.46
10	$C_{28}H_{33}N_7O$	484.28	484.5	0.20	0.21	0.32
11	$C_{29}H_{35}N_7O$	498.30	498.4	0.20	0.22	0.31
12	$C_{23}H_{31}N_3O_2$	382.25	384.4	0.32	0.93	0.31
13	$C_{27}H_{37}N_7O_2$	492.31	492.4	0.18	0.10	0.40
14	$C_{28}H_{39}N_7O_2$	506.33	506.4	0.21	0.19	0.44
15	$C_{29}H_{37}BF_2N_4O_2$	523.31	523.4	0.23	0.77	0.24
16	$C_{33}H_{43}BF_2N_8O_2$	633.37	633.4	0.12	0.07	0.22
17	$C_{34}H_{45}BF_2N_8O_2$	647.38	647.4	0.13	0.06	0.25
18	$C_{15}H_{20}N_4OS$	305.15	305.2	0.05	0.07	0.62
19	$C_{16}H_{16}N_4S$	297.12	297.1	0.58	0.10	0.57

[a] Exact masses were determined using a Finnigan LCQ_{DECA} ion-trap mass spectrometer under APCI or ESI conditions.
[b] Silicagel 60, ethyl acetate/methanol (3:1).
[c] Alumina 60 neutral (Merck), ethyl acetate.
[d] C18-bonded silicagel for RP-HPTLC (Merck), acetonitrile/water (1:1), 1% formic acid.

$MgSO_4$ and evaporated to yield the product as a light brown crystalline solid (mp 135 °C; 0.039 g, 19%).

$C_{25}H_{28}N_4O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32 (m, 2H), 1.47 (m, 4H), 2.07 (m, 2H), 2.29 (m, 4H), 3.34 (s, 2H), 3.62 (m, 2H), 4.01 (t, 2H, J = 6.13 Hz), 6.70–6.82 (m, 5H), 7.01–7.12 (m, 2H), 7.52 (d, 1H, J = 8.77 Hz), 7.75 (s, 1H), 7.96 (d, 1H, J = 8.72 Hz).

4.2.1.6. 1-Cyano-2-[[*N*-(2-ethylamino)]carbamoyl]indolizine. Ethylenediamine (20 mL) was added to ethyl-1-cyanoindolizine-2-carboxylate (1.122 g, 5.2 mmol) and the reaction mixture was heated under reflux for 5 h. The mixture was cooled and added to water (50 mL). The insoluble solids (mostly unreacted indolizine) were removed by filtration, the water saturated with NaCl and extracted with dichloromethane (5 × 50 mL). The combined organic fraction was dried over $MgSO_4$ and evaporated in vacuo to yield the product as a yellow oil (0.486 g, 41%).

$C_{12}H_{12}N_4O$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.94 (t, 2H, J = 6.76 Hz), 3.51 (q, 2H, J = 6.76 Hz), 6.80 (m, 1H), 6.99 (br s, 1H), 7.12 (m, 1H), 7.61 (d, 1H, J = 8.67 Hz), 7.81 (s, 1H), 7.98 (d, 1H, J = 8.70 Hz).

4.2.1.7. 1-Cyano-indolizine-2-carboxylic acid (2-*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)ethyl]-guanidinopropyl)amide (6). *N*-Cyano-*S*-methyl-*N*′-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]isothiourea (0.346 g, 1 mmol) and 1-cyano-2-[[*N*-(2-ethylamino)]carbamoyl]indolizine (0.228 g, 1 mmol) was heated under reflux in dry pyridine (15 mL) for 3 h. The reaction mixture was poured into brine (50 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic fraction was washed with water (50 mL), dried over $MgSO_4$ and evaporated in vacuo to give a brown oil. Flash chromatography with methanol–dichloromethane (1:4) yielded the pure product as a light yellow wax. The product was recrystallized from dichloromethane/ethyl acetate as white solid (mp 137 °C; 0.004 g, 8%).

$C_{29}H_{34}N_8O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.39 (m, 2H), 1.50 (m, 4H), 2.09 (m, 2H), 2.31 (m, 4H), 3.37 (s, 2H), 3.49 (m, 6H), 4.01 (t, 2H, J = 6.13 Hz), 6.42 (br s, 1H), 6.65 (br s, 1H), 6.71–6.86 (m, 4H), 7.02–7.18 (m, 2H), 7.54 (d, 1H, J = 8.76 Hz), 7.65 (m, 1H), 7.70 (s, 1H), 7.96 (d, 1H, J = 8.74 Hz).

4.2.1.8. 1-Cyano-indolizine-2-carboxylic acid (3-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]-guanidino}propyl)amide (7). 1-Cyanoindolizine-2-carboxylic acid (0.375 g, 2.0 mmol) and hydroxybenzotriazole (0.280 g, 2.0 mmol) in DMF (10 mL) were cooled in an ice bath and dicylohexylcarbodiimide (0.430 g, 2.0 mmol) was added and stirred for 1 h at room temperature. Next *N*-aminoethyl-*N*′-cyano-*N*″-[3-{3-(1-piperidinylmethyl)phenoxy}propyl]guanidine (0.72 g, 2.0 mmol) in DMF (5 mL) was added and stirred at room temperature overnight. Dichloromethane (50 mL) was added and the mixture was washed with 1 M K_2CO_3 (50 mL), water (3 × 50 mL) and dried with $MgSO_4$.

Concentration in vacuo gave 1.03 g of an oil, which was purified by flash chromatography with ethyl acetate–MeOH (9:1–1:1). The product was recrystallized from dichloromethane/ethyl acetate as white solid (mp 166 °C; 0.385 g, 35%).

$C_{30}H_{36}N_8O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30–1.80 (m, 8H), 2.06 (qui, 2H, J = 6.5 Hz), 2.25–2.40 (m, 4H), 3.25–3.60 (m, 6H), 3.38 (s, 2H), 4.07 (t, 2H, J = 6.4 Hz), 5.79 (br s, NH), 6.38 (br s, NH), 6.75–6.95 (m, 4H+NH), 7.10–7.25 (m, 2H), 7.58 (d, 1H, J = 8.6 Hz), 7.74 (s, 1H), 7.96 (d, 1H, J = 8.6 Hz).

4.2.1.9. 1-Carbethoxy-indolizine-2-carboxylic acid {3-[3-(piperidin-1-ylmethyl)phenoxy]propyl}amide (8). Diethylindolizine-1,2-dicarboxylate (0.34 g, 1.3 mmol) and 3-[3-(1-piperidinylmethyl)phenoxy]propylamine (0.3 g, 1.2 mmol) was heated in an open flask at 150 °C for 8 h. The reaction mixture was added to 1 N HCl (30 mL) and extracted with dichloromethane (3 × 30 mL). The pH of the aqueous fraction was adjusted to 8 with Na_2CO_3 and extracted with dichloromethane (3 × 30 mL). The organic fractions were combined and washed with 50 mL Na_2CO_3 water (pH 11). The organic fraction was dried over $MgSO_4$ and evaporated in vacuo to give the crude amine product. Flash chromatography with ethanol–dichloromethane (1:1) yielded the product as a dark brown oil (0.114 g, 20.5%).

$C_{27}H_{33}N_3O_4$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.39 (m, 2H), 1.41 (m, 3H), 1.52 (m, 4H), 2.12 (m, 2H), 2.35 (m, 4H), 3.42 (s, 2H), 3.64 (m, 2H), 4.06 (t, 2H, J = 6.13 Hz), 4.40 (m, 2H), 6.71–6.86 (m, 4H), 7.03–7.20 (m, 2H), 7.98 (d, 1H, J = 8.64 Hz), 8.05 (s, 1H), 8.11 (d, 1H, J = 8.37 Hz), 10.55 (br s, 1H).

4.2.1.10. 1-Cyano-*N*-[3-(3-piperidin-1-ylmethylphenoxy])propyl]isoindole (9). 3-[3-(1-Piperidinylmethyl)-phenoxy]propylamine (1.24 g, 5 mmol) and NaCN (0.245 g, 5 mmol) was dissolved in 20 mL methanol/water. To this was added *o*-phtaldialdehyde (1.34 g, 5 mmol) and the pH was adjusted to 8–9 with acetic acid (glacial). The reaction mixture was protected from light and stirred at room temperature for 2 h. The methanol/water as decanted from the oily precipitate formed and concentrated in vacuo. The oily precipitate was dissolved in 1 N HCl (30 mL) and extracted with dichloromethane (3 × 30 mL). The organic fraction was dried over $MgSO_4$ and evaporated in vacuo to give the HCl salt of the product as a brown foamy solid (mp 75.8 °C; 1.16 g, 54.5%). Extraction of the alkalinized water fraction with dichloromethane (3 × 30 mL) yielded the free base as brown waxy solid (0.41 g, 24%).

$C_{24}H_{27}N_3O$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.40 (m, 2H), 1.53 (m, 4H), 2.32 (m, 6H), 3.41 (s, 2H), 3.88 (t, 2H, J = 6.10 Hz), 4.52 (t, 2H, J = 6.81 Hz), 6.72 (dd, 1H, J = 8.21 Hz), 6.83 (m, 2H), 7.02 (m, 1H), 7.18 (m, 2H), 7.29 (s, 1H), 7.51–7.66 (m, 2H).

4.2.1.11. *N*-Cyano-*N′*-[2-(1-cyano-isoindol-2-yl)-ethyl]-*N″*-[3-(3-piperidin-1-ylmethylphenoxy])propyl]guanidine (10). *N*-(2-Aminoethyl)-*N′*-cyano-*N″*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine (1.69 g 4.47 mmol), NaCN (0.22 g, 4.47 mmol) and *o*-phtaldialdehyde (0.601 g, 4.47 mmol) was dissolved in 30 mL methanol/water and the pH was adjusted to 8–9 with acetic acid (glacial). The reaction mixture was protected from light and stirred at room temperature for 2 h. An oily precipitate forms. The mixture was concentrated in vacuo and extracted with dichloromethane (3 × 50 mL). The organic fraction was dried over $MgSO_4$ and evaporated to give the crude product as a dark foamy solid. Chromatography with ethanol–dichloromethane (1:1) yielded the product as light brown crystalline solid (mp 89 °C; 0.573 g, 26.6%).

$C_{28}H_{33}N_7O$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (m, 2H), 1.55 (m, 4H), 1.95 (m, 2H), 2.37 (m, 4H), 3.30 (m, 2H), 3.41 (s, 2H), 3.61 (t, 2H, J = 6.65 Hz), 3.91 (t, 2H, J = 6.11 Hz), 4.45 (t, 2H, J = 6.74 Hz), 5.73 (t, 1H, J = 5.12 Hz), 5.94 (t, 1H, J = 5.14 Hz), 6.65 (dd, 1H, J = 8.47 Hz), 6.76 (m, 2H), 7.02–7.20 (m, 3H), 7.32 (s, 1H), 7.58 (d, 2H, J = 8.76 Hz).

4.2.1.12. *N*-Cyano-*N′*-[3-(1-cyano-isoindol-2-yl)-propyl]-*N″*-[3-(3-piperidin-1-ylmethylphenoxy])propyl]guanidine (11). *N*-(3-Aminopropyl)-*N′*-cyano-*N″*-[3-[3-(1-piperidinylmethyl)phenoxy]propylguanidine (0.763 g, 2.05 mmol) and NaCN (0.101 g, 2.05 mmol) in methanol/water (20 mL) was stirred at room temperature. *o*-Phtaldialdehyde (0.275 g, 2.05 mmol) was added and the pH was adjusted to 8–9 with acetic acid (glacial). The mixture was protected from light and stirred at room temperature for 2 h. An oily precipitate forms. The mixture was concentrated in vacuo and extracted with dichloromethane (3 × 30 mL). The organic fraction was dried over $MgSO_4$ and evaporated to give the crude product as a dark foamy substance. Upon flash chromatography with ethanol–dichloromethane (1:1) the product precipitated as an off-white solid (mp 114 °C; 0.2 g, 20%).

$C_{29}H_{35}N_7O$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (m, 2H), 1.55 (m, 4H), 1.95 (m, 2H), 2.06 (m, 2H), 2.37 (m, 4H), 3.18 (m, 2H), 3.32 (m, 2H), 3.40 (s, 2H), 3.95 (t, 2H, J = 6.11 Hz), 4.28 (t, 2H, J = 6.79 Hz), 6.14 (m, 2H), 6.71 (m, 3H), 6.99–7.21 (m, 3H), 7.30 (s, 1H), 7.51 (d, 2H, J = 8.72 Hz).

4.2.1.13. 2-Methylamino-*N*-[3-(3-piperidin-1-ylmethylphenoxy])propyl]benzamide (12). *N*-Methylanthranilic acid (0.151 g, 1 mmol) was added to a stirred solution of *N*,*N′*-carbonyldiimidazole (0.162 g, 1 mmol) in anhydrous tetrahydrofuran (20 mL). After 1 h 3-[3-(1-piperidinylmethyl)phenoxy]propylamine (0.248 g, 1 mmol) in tetrahydrofuran (10 mL) was added and the mixture was allowed to react overnight at room temperature. The solution was then poured into a saturated $NaHCO_3$ solution (50 mL) and extracted with dichloromethane (3 × 50 mL), dried over Na_2SO_4 and evaporated in vacuo to give a dark oil (0.381 g). Flash chromatography with ethanol–dichloromethane (1:1) yielded the product as a yellow oil (0.076 g, 20%).

$C_{23}H_{31}N_3O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (m, 2H), 1.54 (m, 4H), 2.10 (m, 2H), 2.38 (m, 4H), 2.83 (d, 3H, J = 5.93 Hz), 3.40 (s, 2H), 3.60 (m, 2H), 4.10 (t, 2H, J = 6.12 Hz), 6.50–6.70 (m, 3H), 6.79 (dd, 1H, J = 8.22 Hz), 6.90 (m, 2H), 7.18–7.36 (m, 3H), 7.48 (br s, 1H).

4.2.1.14. 2-Methylaminobenzoic acid (2-{*N′*-cyano-*N″*-[3-(3-piperidin-1-ylmethylphenoxy)ethyl]-guanidino}propyl)amide (13). *N*-Methylanthranilic acid (0.72 g, 4.75 mmol) was added to a stirred solution of *N*,*N′*-carbonyldiimidazole (0.77 g, 4.75 mmol) in anhydrous tetra-

hydrofuran (30 mL). After 2 h *N*-(2-aminoethyl)-*N*′-cyano-*N*″-[3-[3-(1-piperidinylmethyl)phenoxy]propyl-guanidine (1.7 g, 4.75 mmol) in tetrahydrofuran (10 mL) was added and the mixture was allowed to react overnight at room temperature. After 20 h the reaction mixture was heated to ensure complete reaction. The solvent was evaporated in vacuo and the residue extracted with aqueous Na_2CO_3 and dichloromethane. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated to leave yellow oil. Flash chromatography with ethyl acetate–methanol (9:1) yielded the product as light yellow oil (1.249 g, 55%).

$C_{27}H_{37}N_7O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.39 (m, 2H), 1.50 (m, 4H), 2.00 (m, 2H), 2.32 (m, 4H), 2.73 (d, 3H, J = 5.95 Hz), 3.36 (m, 8H), 3.96 (t, 2H, J = 6.11 Hz), 6.49–6.60 (m, 3H), 6.71–6.88 (m, 4H), 7.10–7.29 (m, 2H), 7.41 (m, 2H), 7.56 (br s, 1H).

4.2.1.15. 2-Methylaminobenzoic acid (3-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]-guanidino}propyl)amide (14). *N*-Methylanthranilic acid (0.55 g, 3.6 mmol) was added to a stirred solution of *N*,*N*′-carbonyldiimidazole (0.58 g, 3.6 mmol) in anhydrous tetrahydrofuran (25 mL). After 2 h *N*-(3-aminopropyl)-*N*′-cyano-*N*″-[3-[3-(1-piperidinylmethyl)phenoxy]propyl-guanidine (1.34 g, 3.6 mmol) in tetrahydrofuran (10 mL) was added and the mixture was allowed to react overnight at room temperature. After 20 h the reaction mixture was heated to ensure complete reaction. The solvent was evaporated in vacuo and the residue extracted with aqueous Na_2CO_3 and dichloromethane. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated to leave yellow oil. Flash chromatography with ethyl acetate–methanol (9:1) yielded the product as light yellow oil (1.34 g, 73%).

$C_{28}H_{39}N_7O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.40 (m, 2H), 1.51 (m, 4H), 1.62 (m, 2H), 2.02 (m, 2H), 2.37 (m, 4H), 2.29 (s, 3H), 3.22–3.41 (m, 8H), 3.99 (t, J = 6.11 Hz, 2H), 6.16 (t, J = 5.08 Hz, 1H), 6.50–6.69 (m, 3H), 6.72–6.89 (m, 3H), 7.1–7.30 (m, 3H), 7.41 (d, J = 8.54 Hz, 2H).

4.2.1.16. 2,2,2-Trichloroethyl 3-(pyrrol-2-yl)propionate. 3-(Pyrrol-2-yl)propionic acid (8.62 g, 61.9 mmol),[25] 2,2,2-trichloroethanol (7.2 mL, 74 mmol) and pyridine (7.5 mL) were dissolved in ethyl acetate (300 mL) and cooled to 0 °C. Dicyclohexylcarbodiimide (12.69 g, 61.5 mmol) in ethyl acetate (100 mL) was added slowly and stirred overnight at room temperature. The solution was filtered and concentrated in vacuo. Flash chromatography with dichloromethane gave 2,2,2-trichloroethyl 3-(pyrrol-2-yl)propionate as a colourless oil (14.6 g, 88.1%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.83 (t, 2H, J = 8.1 Hz), 2.97 (t, 2H, J = 2.81 Hz), 4.75 (s, 2H), 5.87–5.97 (m, 1H), 6.02–6.15 (m, 1H), 6.60–6.72 (m, 2H), 8.35 (br s, 1H).

4.2.1.17. 2,2,2-Trichloroethyl 3-[5-(3,5-dimethylpyrrol-2-yl)methylpyrrol-2-yl)]propionate boron trifluoride adduct. 2,2,2-Trichloroethyl 3-(pyrrol-2-yl)propionate (2.28 g, 8.43 mmol) and 3,5-dimethylpyrrole-2-carbaldehyde (1.20 g, 9.74 mmol)[26] in dichloromethane (10 mL) were cooled to 0 °C. $POCl_3$ (1.42 g, 9.2 mmol) in dichloromethane (5 mL) was added dropwise and stirred an additional 30 min at 0 °C followed by stirring at room temperature. After 6 h the solution was cooled to 0 °C again and freshly distilled borontrifluoride etherate (4.2 mL) was added followed by diisopropylethylamine (6.1 mL, 35 mmol). The reaction mixture was stirred at room temperature overnight. It was washed with water (50 mL), with citric acid dried with Na_2SO_4 (3.0 g) and concentrated in vacuo. The crude product was purified by flash chromatography with dichloromethane to give a dark orange oil with a green fluorescent lustre (2.38 g, 67%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.18 (s, 3H), 2.51 (s, 3H), 2.86 (t, 2H, J = 7.7 Hz), 3.30 (t, 2H, J = 7.7 Hz), 4.72 (s, 2H), 6.03 (s, 1H), 6.22 (d, 1H, J = 3.8 Hz), 6.79 (d, 1H, J = 3.8 Hz), 7.04 (s, 1H).

4.2.1.18. 3-[5-(3,5-Dimethylpyrrol-2-yl)methylpyrrol-2-yl)]propionic acid boron trifluoride adduct[27]. The ester (2.1 g, 5.0 mmol) was dissolved in acetone (75 mL), phosphate buffer (0.1 M, pH = 8.0; 150 mL) was added followed by PLE (21 U/mg) (130 mg) and stirred at room temperature overnight. Citric acid (7.5 g) was added and the mixture was extracted with ethyl acetate (5 × 50 mL). The organic layers were washed with brine and dried with Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (dichloromethane/0–100% ethyl acetate) to give a black solid with a green fluorescent lustre (1.0 g, 68.5%; identical to BODIPY®FL Molecular Probes Inc.).

$C_{14}H_{15}BF_2N_2O_2$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.23 (s, 3H), 2.54 (s, 3H), 2.80 (t, 2H, J = 7.6 Hz), 3.28 (t, 2H, J = 7.6 Hz), 6.09 (s, 1H), 6.26 (d, 1H, 4.0 Hz), 6.86 (d, 1H, J = 4.0 Hz), 7.07 (s, 1H), 12.90 (br s, 1H).

4.2.1.19. 3-(5,7-Dimethyl-4,4-difluoro-3a,4a-diaza-4-bora-*S*-indacen-1-yl)-propionic acid {3-[3-(piperidin-1-ylmethyl)phenoxy]propyl}amide (15). 3-[5-(3,5-Dimethylpyrrol-2-yl)methylpyrrol-2-yl)]propionic acid boron trifluoride adduct (205 mg, 0.70 mmol) and 3-[3-(1-piperidinylmethyl)phenoxy]propaneamine (174 mg, 0.70 mmol) in THF (10 mL) were cooled to −10 °C and dicyclohexylcarbodiimide (155 mg, 0.70 mmol) in THF (5 mL) was added dropwise and allowed to warm to room temperature overnight. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/0–25% methanol) to give a dark orange-red solid with a green fluorescent lustre (mp 127 °C; 205 mg, 56%).

$C_{29}H_{37}BF_2N_4O_2$: ^{1}H NMR (200 MHz, $CDCl_3$ + 2 drops DMSO-d_6): δ 1.40–1.65 (m, 2H), 1.70–1.95 (m, 6H), 2.21 (s, 3H), 2.52 (s, 3H), 2.67 (t, 2H, J = 7.6 Hz), 2.70–3.00 (m, 4H), 3.20–3.40 (m, 4H), 3.75 (t, 2H, J = 6.2 Hz), 3.94 (s, 2H), 6.06 (s, 1H), 6.15 (br s, NH), 6.28 (d, 1H, J = 3.8 Hz), 6.70–6.95 (m, 3H), 6.99 (s, 1H), 7.05–7.35 (m, 2H).

4.2.1.20. 3-(5,7-Dimethyl-4,4-difluoro-3a,4a-diaza-4-bora-*S*-indacen-1-yl)-propionic(2-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)ethyl]-guanidino}propyl)amide (16). 3-[5-(3,5-Dimethylpyrrol-2-yl)methylpyrrol-2-yl)]propionic acid boron trifluoride adduct (0.82 g, 2.80 mmol) and *N*-aminopropyl-*N*′-cyano-*N*″-[3-{3-(1-piperidinylmethyl)phenoxy}propyl]guanidine (1.00 g, 2.79 mmol) in (THF 25 mL) were cooled to 0 °C and dicyclohexylcarbodiimide (0.62 g, 3.00 mmol) in THF (10 mL) was added dropwise and allowed to warm to room temperature overnight. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/0–25% methanol) to give a dark oil with a green fluorescent lustre (105 °C; 0.47 g, 27%).

$C_{33}H_{43}BF_2N_4O_2$: ^{1}H NMR (200 MHz, $CDCl_3$ + 2 drops DMSO-d_6): δ 1.35–1.60 (m, 2H), 1.60–1.85 (m, 6H), 2.17 (s, 3H), 2.46 (s, 3H), 2.55 (t, 2H, J = 7.4 Hz), 2.65–3.00 (m, 4H), 3.15–3.40 (m, 8H), 3.74 (t, 2H, J = 6.2 Hz), 3.83 (s, 2H), 6.04 (s, 1H), 6.21 (d, 1H, J = 3.8 Hz), 6.60 (br s, NH), 6.70–6.95 (m, 3H + 2 × NH), 6.99 (s, 1H), 7.05–7.35 (m, 2H).

4.2.1.21. 3-(5,7-Dimethyl-4,4-difluoro-3a,4a-diaza-4-bora-*S*-indacen-1-yl)-propionic acid (3-{*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]-guanidino}propyl)amide (17). 3-[5-(3,5-Dimethylpyrrol-2-yl)methylpyrrol-2-yl)]propionic acid boron trifluoride adduct (215 mg, 0.74 mmol) and *N*-aminopropyl-*N*′-cyano-*N*″-[3-3-(1-piperidinylmethyl)phenoxypropyl]guanidine (275 mg, 0.74 mmol) in THF (10 mL) were cooled to −10 °C and dicyclohexylcarbodiimide (165 mg, 0.8 mmol) in THF (5 mL) was added dropwise and allowed to warm to room temperature overnight. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/0–25% methanol) to give a dark oil with a green fluorescent lustre (156 °C; 86 mg, 18%).

$C_{34}H_{45}BF_2N_8O_2$: ^{1}H NMR (200 MHz, $CDCl_3$ + 2 drops DMSO-d_6): δ 1.40–1.65 (m, 4H), 1.70–2.05 (m, 6H), 2.20 (s, 3H), 2.48 (s, 3H), 2.61 (t, 2H, J = 7.2 Hz), 2.75–3.05 (m, 4H), 3.05–3.30 (m, 6H), 3.30–3.45 (m, 2H), 3.90–4.10 (m, 4H), 6.07 (s, 1H), 6.17 (br s, NH), 6.20 (d, 1H, J = 4.0 Hz), 6.53 (br s, NH), 6.65–7.00 (m, 3H+NH), 7.05 (s, 1H), 7.10–7.35 (m, 2H).

4.2.2. 2-[(5-Methyl-4-imidazolyl)-methylthio]ethylamines.

4.2.2.1. 2-Methylaminobenzoic acid (2-[5-methylimidazol-4-ylmethylthio]ethyl)amide (18). *N*-Methylanthranilic acid (0.756 g, 5 mmol) was added to a stirred solution of *N*,*N*′-carbonyldiimidazole (0.810 g, 5 mmol) in anhydrous tetrahydrofuran (30 mL). After 1 h 2-(5-methyl-4-imidazolyl)-methylthioethylamine·2HBr (1.665 g, 5 mmol) was added and the mixture was allowed to react overnight at room temperature. After 16 h triethylamine (1.4 mL) was added and the mixture was heated under reflux for a further 2 h to complete the reaction. The reaction mixture was poured into 1 N HCl (30 mL) and extracted with dichloromethane. The pH of the aqueous fraction was adjusted to 7 with Na_2CO_3 and extracted with dichloromethane (3 × 30 mL). The organic fraction was washed with saturated $NaHCO_3$ water (50 mL), dried over $MgSO_4$ and evaporated in vacuo to give the product as a light yellow solid (mp 108 °C; 1.13 g, 74%).

$C_{15}H_{20}N_4OS$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.20 (s, 3H), 2.71 (t, 2H, J = 6.16 Hz), 2.83 (s, 3H), 3.59 (q, 2H, J = 6.16 Hz), 3.71 (s, 2H), 6.51–6.66 (m, 2H), 7.11 (br s, 1H), 723–7.41 (m, 5).

4.2.2.2. 1-Cyano-*N*-(2-[5-methylimidazol-4-ylmethylthio]ethyl)isoindole (19). An aqueous solution of 2-(5-methyl-4-imidazolyl)-methylthioethylamine·2HBr (1.665 g, 5 mmol) and NaCN (0.245 g, 5 mmol) was added to a methanolic solution of *o*-phtaldialdehyde (0.67 g, 5 mmol). The mixture was protected from light and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and extracted with dichloromethane. Subsequent acid–base extraction with dichloromethane yielded the product as a yellowish oil that crystallized on standing (0.31 g, 21%). Recrystallization from ethanol gave light brown plates (mp 172 °C).

$C_{16}H_{16}N_4S$: ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.19 (s, 3H), 2.96 (t, 2H, J = 6.15 Hz), 3.61 (s, 2H), 4.40 (t, 2H, J = 6.15 Hz), 7.06 (t, 1H, J = 8.12 Hz), 7.19 (d, 1H, J = 8.17 Hz), 7.35 (s, 1H), 7.41 (s, 1H), 7.58 (m, 2H).

4.3. Binding assay

The determination of H_2 receptor binding was performed with the H_2 antagonist [^{125}I]-aminopotentidine ([^{125}I]-APT).[23] COS-7 cells transiently expressing the rat H_2 receptor were homogenized in ice cold 50 mM Na/K phosphate buffer (pH = 7.4) with a polytron homogenizer (5 s, max speed) and used for radioligand binding studies. Triplicate assays were performed in polyethylene tubes in 400 μL of 50 mM Na/K phosphate buffer (pH = 7.4) containing gelatine (0.1%), 0.5 nM [^{125}I]-APT, 5–10 μg of membrane proteins in the absence or presence of drug. After 90 min at 30 °C the incubations were stopped by rapid dilution with 3 mL ice cold 20 mM Na/K phosphate buffer (pH = 7.4) supplemented with chicken egg albumin and rapid filtration with a Brandel cell harvester (Semat, UK) through Whatman GF/C glass fibre filters (0.3% polyetheleneimine treated). Filters were washed twice with 3 mL buffer and the radioactivity retained on the filters was counted with a LKB-γ-counter at an efficiency of 63%. Changes in H_2 receptor density were denoted as the percentage of [^{125}I]-APT binding of untreated cells. Protein concentration was determined using bovine serum albumin as a standard.[28] Graphpad Prism® was used to evaluate the data.

4.4. Fluorescence spectrometry

A Shimadzu RF-5001 PC fluorescence spectrometer was used for fluorescence measurements. Stock solutions (10^{-3} M) of the compounds were prepared in ethanol and diluted to 10^{-6} M in PBS buffer (pH = 7.4). Standard fluorescence emission spectra were recorded at the indicated excitation wavelengths using uncalibrated instrument settings. The fluorescence potencies were obtained by comparing the peak heights of the individual spectra, giving a rough indication of the fluorescent

properties of the compounds in physiological conditions.

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