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2-(4,5-Dihydroimidazol-2-yl)benzimidazoles as highly selective imidazoline I_2/adrenergic α_2 receptor ligands

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Abstract—2-(4,5-Dihydroimidazol-2-yl)benzimidazoles have been identified as selective imidazoline I_2/α_2-adrenoceptor ligands. 4-Methyl (**2**) and 4-chloro (**4**) derivatives display I_2 affinity at nanomolar concentration (K_i = 4.4 and 17.7 nM, respectively) and high I_2/α_2 selectivity ratio = 4226 and 5649, respectively. An evidence has been obtained that pK_a value influences considerably the I_2/α_2-selectivity ratio of this class of imidazoline I_2 receptor ligands.

1. Introduction

It has been well known that selective imidazoline I_2 receptor ligands exhibit several important effects in vivo such as elevation of brain monoamines, efficacy in the Porsolt swim test[1] and increased feeding behaviour in rats.[2] Moreover, these receptors have been implicated in a variety of disease states such as psychiatric disorders, opiate withdrawal, Parkinson's and Alzheimer's diseases as well as Huntington's chorea.[3–9]

Compounds possessing selective activity at imidazoline I_2 versus α_2-adrenoceptors are likely to have enhanced efficacy while minimizing the side effects often encountered with non-selective agents and could serve as valuable tools in defining the roles of imidazoline receptors.

The past investigations on I_2-ligands led to the discoveries of selective imidazoline derivatives such as 2-BFI,[10] BU-224,[11] tracizoline,[12] 4-Cl-indazim,[13] benazoline[14] and other 2-arylimidazolines.[15] As shown in Figure 1, one of the structural features which may contribute to I_2 activity is the presence of CH group which might participate in hydrogen bonding or stacking interactions with an aromatic ring of the receptor protein, and a basic nitrogen atom separated by a two-atom bridge. However, SAR and QSAR studies performed for the above compounds were clearly not successful in predicting the biological consequences of the chemical structure changes, apparently due to different ways of interaction with the I_2 receptor.

Recently, some of us have reported the syntheses and structure – binding affinity relationships of several series of pyrazino[1,2-*a*]indoles (**I**),[16] 1,2,3,4-tetrahydrocarboline derivatives (**II**),[17] imidazo[1′,2′:1,2]pyrido[3,4-*b*]indoles (**III**)[18] and pyrazino[1,2-*a*]benzimidazoles (**IV**)[16] (Fig. 2). Analysis of the binding affinities described for these compounds seemed to confirm the above hypothesis that the presence of either *HC*═C–C–N (**I**) or *HN*–C–C–N (**II**, **III**) moiety might be a necessary prerequisite for selective action at imidazoline I_2 receptors since the compound **IV** with pyridine-type nitrogen (–*N*═C–C–N) showed a considerably reduced affinity (Fig. 2).

On the other hand, based on binding data obtained for 2-(imidazolin-2-yl)indazoles we have hypothesized[13] that the affinity of imidazoline compounds such as 4-Cl-Indazim for α_2-adrenoceptors is considerably reduced due to their low basicity compared to other standard ligands. It means that good I_2 ligands of this class with pK_a values ~6–7 exhibit high I_2/α_2 selectivity, mainly due to the reduced affinity at α_2-adrenoceptors.

Keywords: 2-(4,5-Dihydroimidazol-2-yl)benzimidazoles; Selective imidazoline I_2 receptor ligands; Synthesis; Radioligand binding studies.
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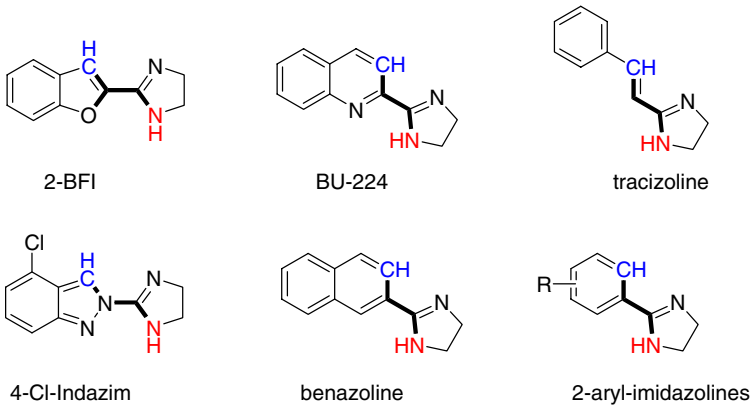

Figure 1. Structure of selective I_2/α_2 ligands showing common structural features: a CH group (blue) and basic nitrogen atom (red) connected by a two-atom C–C or N–C bridge.

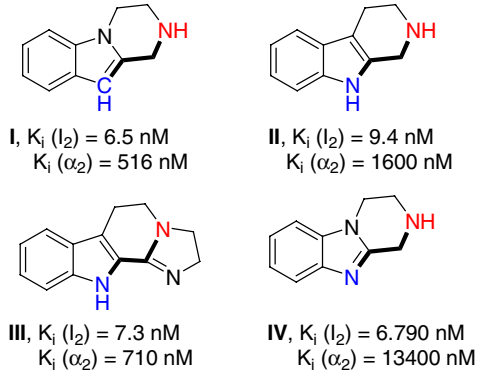

Figure 2. Structure of I_2/α_2 ligands showing common structural features: a weakly acidic proton of the pyrrole-type NH group (blue) and basic nitrogen atom (red) connected by a two-atom C–C bridge.

On the basis of the above considerations, we reasoned that the placement of benzimidazol-2-yl substituent with electron-withdrawing properties at position 2 of imidazoline ring could result in compounds that retain affinity at I_2 receptors and show either a very low or no affinity at α_2-adrenoceptors.

2. Results and discussion

We prepared four series of benzimidazole derivatives shown in Figure 3: series 1 with variations at position 4 and 5 of the phenyl ring; series 2 in which the two heterocycles are interconnected with polymethylene moiety, series 3 resulting from replacement of the imidazoline ring for oxazoline ring system and series 4 in which benzimidazole NH group is replaced with oxygen or sulfur atom.

The known free bases of benzimidazole compounds **1**, **3**, **7**, **10**, **11** and benzothiazole **13**, as well as novel derivatives **2** and **4**–**6**, were prepared according to the previously described procedure by reacting corresponding 2-trichloromethylbenzimidazole with appropriate diamine or aminoalcohol.[19] Benzoxazole **12** was synthesized as previously described[20] from 2-cyano-benzoxazole and ethylenediamine. Novel 2,3,5,6-tetrahydrobenzo-[4,5]imidazo[1,2-*a*][2,1-*c*]pyrazine (**8**) and 2,3,6,7-tetrahydro-5*H*-benzo[4,5]imidazo[1,2-*a*]imidazo[2,1-*c*]diazepine (**9**) were prepared by reacting benzimidazole **1** with 1-bromo-2-chloroethane or 1,3-dibromopropane in DMF solution in the presence of 35% aqueous NaOH.

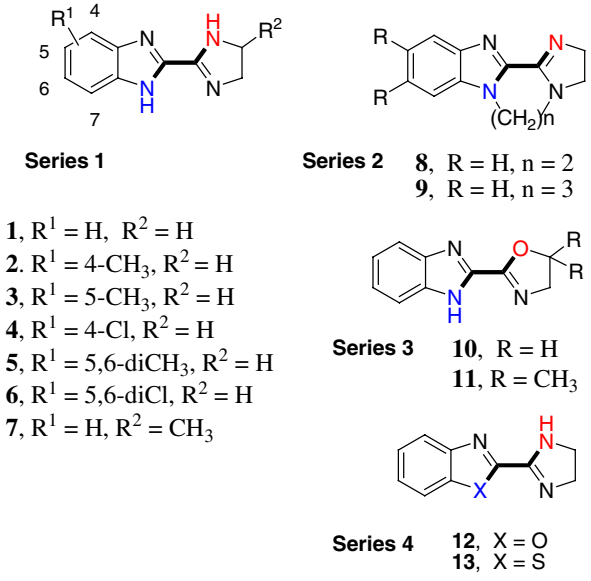

Figure 3. Benzimidazoles **1**–**11**, benzoxazole **12** and benzothiazole **13**.

Radioligand binding experiments were conducted using whole rat brain for both α_2-adrenoceptors and imidazoline I_2 receptors. The equilibrium dissociation constant (K_i) was determined by the method of Cheng and Prusoff[21] and the resulting values are given as means ± SD for 3–4 separate experiments. The results are shown in Table 1.

As shown in Table 1 and Figure 4, among the compounds of series 1 incorporating *HN*–C–C–*NH* grouping, the 4-CH_3-substituted benzimidazole **2** displayed a high I_2 affinity (K_i = 4.4 nM) and high selectivity with

Table 1. Binding affinities, I_2/α_2 selectivity and pK_a data for benzimidazoles **1**–**13**[a]

Compound	K_i (nM) I_2	K_i (nM) α_2	Selectivity I_2/α_2	pK_a
1[b]	48.2 ± 24.9	>100,000	2074	7.05
2	4.4 ± 2.4	18596 ± 2220	4226	7.25
3[b]	40.6 ± 19.5	17895 ± 1888	440	7.27
4	17.7 ± 11.9	>100,000	>5649	6.30
5	90.0 ± 9.0	>10,000	>111	6.79
6	239.0 ± 201.0	>100,000	>418	6.82
7[b]	778.4 ± 582.3	>100,000	>128	7.38
8	169.7 ± 58.3	>100,000	>591	7.64
9[c]	418 ± 177.7	>10,000	>24	7.69
10[b]	>100,000	>100,000	—	8.12
11[b]	>100,000	>100,000	—	8.30
12[b]	114.5 ± 32.9	69616 ± 8092	608	6.79
13[b]	23.1 ± 1.0	19053 ± 4243	824	6.82

[a] Compounds obtained and tested in form of hydrochlorides.
[b] Free bases prepared according to the procedures described previously in Refs. 19,20.
[c] Compound obtained and tested in form of hydrobromide.

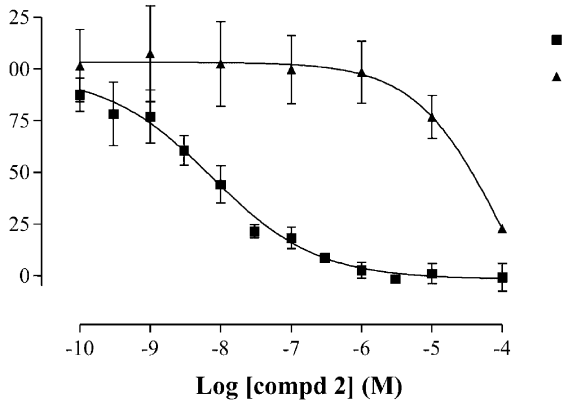

Figure 4. Competition binding curve of **2** on I_2 (filled squares) and α_2 (filled triangles) on whole brain membranes.

respect to α_2-adrenoceptors ($I_2/\alpha_2 = 4226$). The 5-CH_3-substituted analogue **3** and 5,6-disubstituted compounds **5** and **6** showed affinity about 10–55 times lower at I_2 receptors and retained low α_2 activity. Substitution at imidazoline C4(5) with methyl group (entry **7**) proved to be detrimental for activity at both I_2 and α_2 receptors.

We then investigated the effect of polymethylene bridge on I_2 affinity. Comparison between compounds of series 2 with compound **1** indicates that introduction of ethylene or propylene bridge to afford compounds **8** and **9** reduced affinity by 3- to 5-fold (K_i = 169.7 and 418 vs 48.2 nM). These results suggest that unsubstituted NH group of imidazoline ring may be involved in the binding process with I_2 receptors. This is further confirmed by the fact that oxazoline analogues of series 3 with *HN*–C–C–*O* structural motif (entries **10** and **11**) failed to elicit any I_2 activity at concentrations up to 1×10^{-4} M.

On the other hand, the importance of a weakly acidic benzimidazole NH group is not confirmed by binding data obtained for benzoxazole **12** and benzothiazole **13** (series 4). Thus, replacement of the NH function in benzimidazole derivative **1** with oxygen or sulfur atom gives compounds with moderate or good affinity to I_2

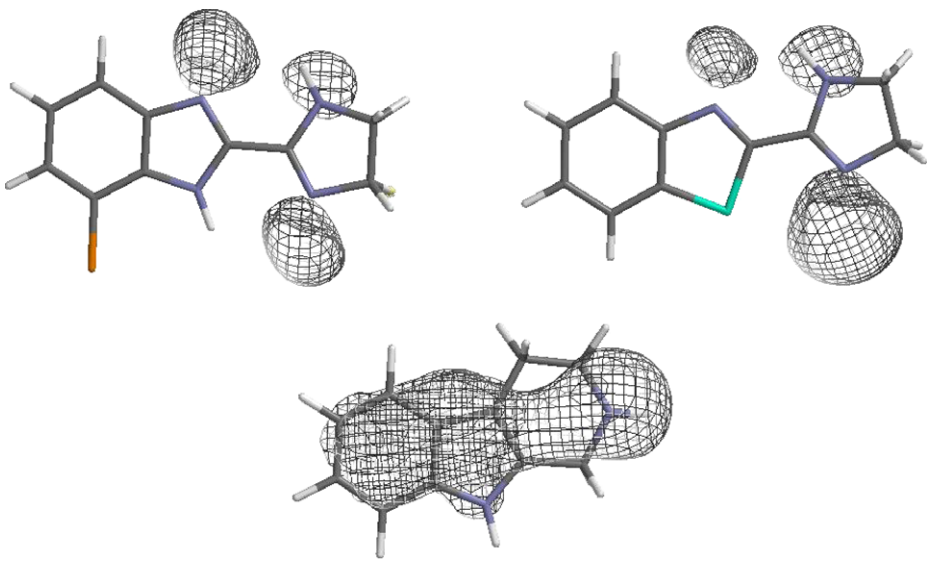

Figure 5. Comparison of the electrostatic potential maps of imidazoline derivative **4** (top left), **13** (top right) and pyrazino[1,2-*a*]indole **I** (bottom).

receptors (114.5 and 23.1 nM, respectively) and a relatively high I_2/α_2 selectivity ratio.

Interestingly, for benzimidazole series 1, which is analogous to 4-Cl-Indazim,[13] the hypothesis that imidazolines of this type with reduced basicity (i.e. pK_a value around 6–7)[22] exhibit very low or no affinity to α_2-adrenoceptors seems to be confirmed. Thus, of the ligands **2** and **4** (pK_a = 7.25 and 6.30, respectively) the 4-Cl-benzimidazole **4** displays much lower affinity to α_2-adrenoceptors (K_i > 100,000 vs 18,595). Although activity of **4** at I_2 receptors compared to 4-CH_3 analogue **2** is also reduced (K_i = 17 vs 4.4 nM), the I_2/α_2 selectivity ratio is considerably enhanced (>5649 vs 4226).

The I_2 activity of the compounds **4** (most selective I_2/α_2 agent), benzothiazole **13** and pyrazino[1,2-*a*]indole (**I**) was studied by comparison of their 3D electrostatic potential maps.[23] The comparison between electrostatic potential maps of **4** and **13** shows that three superimposable negative wells appear around three heteroatoms, while in compound **I** electrostatic region is positioned around the aromatic indole ring and the nitrogen atom of pyrazine moiety (see Fig. 5). Therefore, a different way of interaction with the I_2 receptor may be assumed by imidazoline-containing and non-imidazoline ligands.

Summing up, we have obtained 2-(4,5-dihydroimidazol-2-yl)-4-methyl- and 4-chloro-benzimidazole (**2** and **4**) which exhibit a high affinity at imidazoline I_2 receptors and high I_2/α_2 selectivity. Moreover, for this type of imidazoline derivatives an evidence has been provided that the I_2/α_2 selectivity ratio may depend upon pK_a value, the factor which is often neglected during the design process of selective I_2 ligands.

3. Experimental

Melting points (mp) were determined on Büchi SMP-20 apparatus without correction. Infrared (IR) spectra (KBr pellet) were recorded on a Perkin-Elmer FT-IR 1600 spectrophotometer. NMR spectra were obtained at ambient temperature on a Varian Unity 500 Plus or Varian Gemini 200 apparatus using solvent signal as the reference standard. Mass spectra were recorded on Finnigan MAT95 spectrometer at 70 eV.

2-Trichloromethylbenzimidazoles reacted with appropriate diamine or aminoalcohol gave 2-(4,5-dihydro-1*H*-imidazol-2-yl)- or 2-(4,5-dihydroloxazol-2-yl)benzimidazoles, respectively, as described in Ref. 19.

According to the above methods the following compounds were prepared:

3.1. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)-1*H*-benzimidazole (1)

Yield 84%; mp 261–263 °C (MeOH), Ref. 19 280 °C (acetone). IR λ_{max} (KBr) cm^{-1}: 3166, 2948, 2798, 2749, 1630, 1612, 1546, 1493, 1462, 1446, 1421, 1327, 1278, 1182, 1032, 1003. ^1H NMR (200 MHz, DMSO–d_6) δ: 3.70 (s, 4H, CH_2), 7.23 (m, 2H, aromat), 7.59 (m, 2H, aromat).

Compound **1** × HCl, yield 83%; mp 328–331 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3314, 3092, 3006, 2814, 2643, 2575, 2476, 1657, 1638, 1610, 1478, 1280, 1228, 1202, 1148, 1017. ^1H NMR (200 MHz, DMSO–d_6) δ: 4.07 (s, 4H, CH_2), 6.6–7.0 (bs, NH), 7.44 (m, 2H, aromat), 7.79 (m, 2H, aromat), 11.15 (s, 2H, NH).

3.2. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)-4-methyl-1*H*-benzimidazole (2)

Yield 70%; mp 250–252 °C ($CHCl_3$). IR λ_{max} (KBr) cm^{-1}: 3289, 3072, 2966, 2884, 1638, 1619, 1548, 1429, 1403, 1344, 1327, 1292, 1008. ^1H NMR (200 MHz, DMSO–d_6) δ: 2.55 (s, 3H, CH_3), 3.70 (s, 4H, CH_2), 7.00 (m, 1H, aromat), 7.12 (m, 1H, aromat), 7.37 (m, 1H, aromat).

Compound **2** × HCl, yield 79%; mp 283–285 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3272, 3141, 2988, 2895, 2810, 2678, 2642, 2531, 2463, 1654, 1637, 1621, 1541, 1481, 1442, 1283, 1245, 1206, 1178. ^1H NMR (200 MHz, DMSO–d_6) δ: 2.60 (s, 3H, CH_3), 4.07 (s, 4H, CH_2), 6.0–6.6 (bs, NH), 7.23 (m, 1H, aromat), 7.32 (m, 1H, aromat), 7.60 (m, 1H, aromat), 11.18 (s, 2H, NH).

3.3. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)-5-methyl-1*H*-benzimidazole (3)

Yield 92%; mp 231–233 °C (benzene), Ref. 19 240 °C (benzene).

Compound **3** × HCl, yield 89%; mp 262–266 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3385, 3244, 3093, 2999, 2937, 2812, 2597, 2470, 1656, 1633, 1608, 1540, 1485, 1284, 1235, 1203, 1180, 1137, 1034, 1017. ^1H NMR (500 MHz, DMSO–d_6) δ: 2.47 (s, 3H, CH_3), 4.05 (s, 4H, CH_2), 7.26 (d, J = 8.1 Hz, 1H, aromat), 7.56 (s, 1H, aromat), 7.68 (d, J = 8.1 Hz, 1H, aromat), 11.22 (bs, 2H, NH).

3.4. 4-Chloro-2-(4,5-Dihydro-1*H*-imidazol-2-yl)-1*H*-benzimidazole (4)

Yield 98%; mp 271–272 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3223, 3073, 2936, 2794, 2641, 1625, 1583, 1549, 1479, 1407, 1378, 1337, 1286, 1192, 1138, 1038, 1007. ^1H NMR (200 MHz, DMSO–d_6) δ: 3.95 (s, 4H, CH_2), 3.9–4.2 (bs, NH), 7.04 (m, 1H, aromat), 7.12 (m, 1H, aromat), 7.49 (m, 1H, aromat).

Compound **4** × HCl, yield 93%; mp 302–305 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3264, 3089, 2988, 2855, 2782, 1638, 1607, 1479, 1465, 1433, 1282, 1249, 1199, 1175, 1145, 1032, 1012. ^1H NMR (500 MHz, DMSO–d_6) δ: 4.08 (s, 4H, CH_2), 7.44 (m, 1H, aromat), 7.52 (d, J = 5.9 Hz, 1H, aromat), 7.76 (d, J = 7.8 Hz, 1H, aromat), 10.8–11.4 (bs, NH).

3.5. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)-5,6-dimethyl-1*H*-benzimidazole (5)

Yield 66%; mp 242–243 °C (benzene). IR λ_{max} (KBr) cm^{-1}: 3295, 3454, 3045, 2965, 2938, 2898, 1627, 1553, 1436, 1378, 1288. ^1H NMR (200 MHz, DMSO–d_6) δ: 2.30 (s, 6H, CH_3), 3.66 (s, 4H, CH_2), 7.34 (s, 2H, aromat). MS (EI) *m*/*z*: 214 (M^+).

Compound **5** × HCl, yield 65%; mp 298–301 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3242, 3105, 3066, 2975, 2861, 2779, 1635, 1599, 1441, 1288, 1259, 1180, 1013. ^1H NMR (500 MHz, DMSO–d_6) δ: 2.36 (s, 6H, CH_3), 4.04 (s, 4H, CH_2), 7.55 (s, 2H, aromat), 11.06 (bs, 2H, NH), 14.20 (bs, 1H, NH).

3.6. 5,6-Dichloro-2-(4,5-Dihydro-1*H*-imidazol-2-yl)-1*H*-benzimidazole (6)

Yield 87%; mp 285–287 °C (toluene). IR λ_{max} (KBr) cm^{-1}: 3424, 3082, 2938, 2786, 2606, 1628, 1585, 1382, 1322, 1286, 1087. ^1H NMR (200 MHz, DMSO–d_6) δ: 3.88 (s, 4H, CH_2), 7.77 (s, 2H, aromat).

Compound **6** × HCl, yield 61%; mp 178–181 °C (*i*-PrOH). IR λ_{max} (KBr) cm^{-1}: 3405, 3071, 2976, 2897, 2840, 2796, 1643, 1607, 1402, 1317, 1290, 1094. ^1H NMR (200 MHz, DMSO–d_6) δ: 4.07 (s, 4H, CH_2), 8.13 (s, 2H, aromat).

3.7. 2-(4,5-Dihydro-4-methyl-1*H*-imidazol-2-yl)-1*H*-benzimidazole (7)

Yield 80%; mp 244–246 °C ($CHCl_3$), Ref. 19 253 °C ($CHCl_3$).

Compound **7** × HCl, yield 97%; mp 292–293 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3358, 3137, 3092, 2980, 2818, 2740, 2564, 2475, 1654, 1636, 1610, 1478, 1443, 1325, 1274, 1004. ^1H NMR (200 MHz, DMSO–d_6) δ: 1.38 (d, J = 6.4 Hz, 3H, CH_3), 3.66 (dd, J = 8.1 Hz, J = 11.4 Hz, 1H, CH_2), 4.20 (m, 1H, CH_2), 4.55 (m, 1H, CH), 7.44 (m, 2H, aromat), 7.78 (m, 2H, aromat), 8.6–9.0 (bs, NH), 11.21 (s, 1H, NH), 11.40 (s, 1H, NH).

3.8. 2-(4,5-Dihydrooxazol-2-yl)-1*H*-benzimidazole (10)

Yield 86%; mp 263–265 °C (MeOH), Ref. 19 268 °C (MeOH). IR λ_{max} (KBr) cm^{-1}: 3068, 2976, 2935, 2867, 2750, 2639, 1663, 1621, 1585, 1529, 1489, 1434, 1395, 1323, 1299, 1258, 1153, 1016.

Compound **10** × HCl, yield 90%; mp 296–299 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3420, 3090, 2988, 2478, 1750, 1610, 1551, 1522, 1499, 1360, 1313, 1255. ^1H NMR (200 MHz, DMSO–d_6) δ: 3.30 (m, 2H, CH_2), 4.60 (m, 2H, CH_2), 7.45 (m, 2H, aromat), 7.75 (m, 2H, aromat).

3.9. 2-(4,5-Dihydro-4,4-dimethyloxazol-2-yl)-1*H*-benzimidazole (11)

Yield 85%; mp 249–251 °C (benzene), Ref. 19 250 °C (benzene). IR λ_{max} (KBr) cm^{-1}: 3066, 2972, 2892, 1665, 1618, 1587, 1522, 1489, 1436, 1384, 1365, 1346, 1318, 1282, 1269, 1213, 1189, 1146, 1134, 1014.

Compound **11** × HCl, yield 70%; mp 282–285 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3419, 2983, 2901, 2714, 2581, 1748, 1603, 1552, 1524, 1508, 1484, 1365, 1317, 1255. ^1H NMR (200 MHz, DMSO–d_6) δ: 1.41 (s, 6H, CH_3), 4.42 (s, 2H, CH_2), 7.47 (m, 2H, aromat), 7.77 (m, 2H, aromat).

3.10. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)benzothiazole (13)

Yield 20%; mp 147–150 °C (cyclohexane), Ref. 19 151–152 °C (cyclohexane).

Compound **13** × HCl, yield 75%; mp 264–266 °C (*i*-PrOH). IR λ_{max} (KBr) cm^{-1}: 3395, 3213, 3090, 3024, 2923, 2719, 1610, 1586, 1478, 1351, 1285, 1258, 1027. ^1H NMR (200 MHz, DMSO–d_6) δ: 4.08 (s, 4H, CH_2), 7.54 (m, 2H, aromat), 8.27 (m, 1H, aromat), 8.44 (m, 1H, aromat), 11.55 (bs, 2H, NH).

3.11. Preparation of 2,3,5,6-tetrahydrobenzo[4,5]imidazo[1,2-*a*]imidazo[2,1-*c*]pyrazine (8)

Thirty-five percent aqueous NaOH was slowly added to stirred suspension of **1** 1.86 g (10 mmol) in 10 ml DMF and then 2.5 g (1.7 equiv) of 1-bromo-2-chloroethane was added. The temperature was maintained below 30 °C. After 12 h of stirring, the solid was filtered and washed with DMF. Filtrate and washings were combined and evaporated to dryness (rotary evaporator, bath temperature ca. 100–110 °C). The yellow residue was extracted with boiling MeCN. After evaporation of the solvent, the residue was subjected to purification by means of flash chromatography (silica gel, $CHCl_3$/MeOH/H_2O, 5:0.2:0.02), yielded 0.15 g, 7%; mp 220–223 °C ($CHCl_3$). IR λ_{max} (KBr) cm^{-1}: 3404, 3057, 3042, 2930, 2863, 1636, 1474, 1426, 1348, 1336, 1266, 1194, 1182, 1128, 1044. ^1H NMR (200 MHz, DMSO–d_6) δ: 3.38 (m, 2 H, CH_2), 3.51 (m, 2H, CH_2), 3.82 (m, 2H, CH_2), 4.47 (m, 2H, CH_2), 7.34 (m, 2H, aromat), 7.66 (m, 1H, aromat), 7.75 (m, 1H, aromat). ^{13}C NMR (50 MHz, DMSO–d_6) δ: 41.4, 44.9, 51.8, 54.0, 111.3, 120.4, 123.2, 124.0, 134.0, 141.2, 143.1, 155.5.

Compound **8** × HCl, yield 0.10 g, 90%; mp 262–265 °C (EtOH). IR λ_{max} (KBr) cm^{-1}: 3345, 2965, 2923, 1653, 1631, 1478, 1346, 1316, 1286, 1200, 1133. ^1H NMR (500 MHz, DMSO–d_6) δ: 4.02 (m, 2H, CH_2), 4.08 (m, 2H, CH_2), 4.12 (m, 2H, CH_2), 4.67 (m, 2H, CH_2), 7.46 (m, 1H, aromat), 7.56 (m, 1H, aromat), 7.83 (d, J = 8.3 Hz, 1H, aromat), 7.90 (d, J = 7.8 Hz, 1H, aromat), 11.60 (bs, 1H, NH).

3.12. Preparation of 2,3,6,7-tetrahydro-5*H*-benzo[4,5]imidazo[1,2-*a*]imidazo[2,1-*c*][1,4]diazepine hydrobromide (9)

Thirty-five percent aqueous NaOH was slowly added to stirred suspension of **1** 4.00 g (21.5 mmol) in 20 ml DMF and then 7.6 g (1.7 equiv) of 1,3-dibromopropane was added. The temperature was maintained below 30 °C. After 48 h of stirring, the solid was filtered and washed

with DMF. Filtrate and washings were combined and evaporated to dryness (rotary evaporator, bath temperature ca. 100–110 °C). The residue was extracted with $CHCl_3$/MeOH (5:0.2). After standing, white crystals precipitated from the extract, yielded 0.30 g, 5% of **9 × HBr**; mp 259–261 °C ($CHCl_3$). IR λ_{max} (KBr) cm^{-1}: 3519, 3430, 3342, 3006, 2900, 1626, 1594, 1478, 1444, 1428, 1386, 1348, 1291. ^1H NMR (200, DMSO–d_6) δ: 2.45 (m, 2H, CH_2), 3.85 (m, 2H, CH_2), 3.93 (m, 2H, CH_2), 4.21 (m, 2H, CH_2), 4.58 (m, 2H, CH_2), 7.49 (m, 2H, aromat), 7.85 (m, 2H, aromat), 10.65 (bs, 1H, NH).

Free base **9** was obtained by treatment of an aqueous solution of **9** (0.20 g) with 10% NaOH at 5 °C. Extraction ($CHCl_3$) and recrystallization (toluene) yield 0.11 g, 69% of **9**; mp 195–197 °C (toluene). IR λ_{max} (KBr) cm^{-1}: 3420, 3060, 2962, 2843, 1568, 1511, 1448, 1282, 1255, 1098, 1040. ^1H NMR (200, DMSO–d_6) δ: 2.15 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 4.45 (m, 2H, CH_2), 7.30 (m, 2H, aromat), 7.70 (m, 2H, aromat).

The reaction of 2-cyanobenzoxazole with ethylenediamine carried out as described previously[20] afforded.

3.13. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)benzoxazole (12)

Yield 18%; mp 157–158 °C (benzene), Ref. 20 152–156 °C (benzene/petroleum ether).

Compound **12** × HCl, yield 80%; mp 263–265 °C (*i*-PrOH). IR λ_{max} (KBr) cm^{-1}: 3406, 3021, 2965, 2868, 2737, 2643, 2574, 1647, 1622, 1526, 1474, 1443, 1380, 1363, 1292, 1160, 1028. ^1H NMR (500 MHz, DMSO–d_6) δ: 4.08 (s, 4H, CH_2), 7.96 (m, 1H, aromat), 7.98 (m, 1H, aromat), 8.03 (d, J = 8.3 Hz, 1H, aromat), 8.05 (d, J = 8.3 Hz, 1H, aromat).

3.14. Radioligand binding assays

Crude P_2 brain membranes were prepared as follows, all procedures were carried out at 4 °C unless otherwise stated, rat brains (male, Wistar, 250–300 g) were taken and homogenised in 10 vols of ice-cold buffer (50 mM Tris–HCl, 1 mM $MgCl_2$ and 320 mM sucrose, pH 7.4). The homogenate was centrifuged (1000 *g* for 10 min) and the precipitate discarded. The supernatant was centrifuged (32,000*g* for 20 min) and the supernatant discarded, with the remaining precipitate making up the crude P_2 membrane preparation. This was washed twice in excess buffer (50 mM Tris–HCl, 1 mM $MgCl_2$) at room temperature, 30 ml was added, the precipitate resuspended and centrifuged (32,000*g* for 20 min). The washed membrane preparations were stored at −70 °C until use. Prior to use they were thawed and washed (as above) a further two times. Membrane aliquots (400 μl, 0.2–0.3 mg protein) were incubated with 11 concentrations of the test compound over the range 0.01 nM–100 μM in the presence of the selective I_2 binding site ligand [^{3}H]2BFI (1 nM) or the α_2-adrenoceptor antagonist [^{3}H]RX821001 (1 nM), to a final volume of 500 μl. Non-specific binding was determined using 10 μM BU224, I_2 binding, and 10 μM rauwolscine, α_2-adrenoceptor binding. Each incubation was performed in triplicate, at room temperature and allowed to reach equilibrium (45 min). Bound and free radioactivities were separated by rapid filtration through pre-soaked (0.5% polyethyleneimine) glass-fibre filters (Whatman GF/B). Filters were then washed twice with 5 ml of ice-cold buffer and membrane-bound radioactivity remaining on the filters was determined by liquid scintillation counting. Data were analysed by iterative non-linear regression curve fitting procedures in GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, California, USA). Each experiment was analysed individually and the equilibrium dissociation constant (K_i), determined by the method of Cheng and Prusoff [Ref. 21] and the resulting values are given as means ± S.D. for 3–4 separate experiments.

References and notes

1. Hudson, A. L.; Nutt, D. J.; Husbands, S. M. *Pharm. News* **2002**, *8*, 18.
2. Edwards, M. M. et al. *Br. J. Pharmacol.* **2002**, *135*, 110P.
3. Meana, J. J.; Barturen, F.; Martin, I.; Garcia-Sevilla, J. A. *Biol. Psychiatry* **1993**, *34*, 498.
4. Garcia-Sevilla, J. A.; Escriba, P. V.; Sastre, M.; Walzer, C.; Busquets, X.; Jaquet, G.; Reis, D. J.; Guiman, J. *Arch. Gen. Psychiatry* **1996**, *53*, 803.
5. Garcia-Sevilla, J. A.; Escriba, P. V.; Walzer, C.; Bouras, C.; Guimon, J. *Neurosci. Lett* **1998**, *247*, 95.
6. Sastre, M.; Ventayol, P.; Garcia-Sevilla, J. A. *NeuroReport* **1996**, *7*, 509.
7. Hudson, A. L.; Nutt, D. J.; Husbands, S. M. *Pharm. News* **2001**, *8*, 26–32.
8. Reynolds, G. P.; Boulton, R. M.; Pearson, S. J.; Hudson, A. L.; Nutt, D. J. *Eur. J. Pharmacol.* **1996**, *301*, R19.
9. Ruiz, J.; Martin, I.; Callado, I. F.; Meana, J. J.; Barturen, F.; Garcia-Sevilla, J. A. *Neurosci. Lett.* **1993**, *160*, 109.
10. Nutt, D. J.; French, N.; Handley, S.; Hudson, A. L.; Husbands, S.; Jackson, H.; Jordan, S.; Lalies, M. D.; Lewis, J.; Lione, L.; Mallard, N.; Prattm, J. *Ann. NY Acad. Sci.* **1995**, *763*, 125.
11. Hudson, A. L.; Gough, R.; Tyacke, R. J.; Liona, L.; Lalies, M.; Lewis, J.; Husbands, S.; Knight, P.; Murray, F.; Hutson, P.; Nutt, D. J. *Ann. NY Acad. Sci.* **1999**, *881*, 81.
12. Pigini, M.; Bousquet, P.; Carotti, A.; Dontenwill, M.; Giannella, M.; Mariconi, R.; Piergentilli, A.; Quaglia, W.; Tayebati, S. K.; Brasili, L. *Bioorg. Med. Chem.* **1997**, *5*, 833.
13. Sączewski, F.; Hudson, A. L.; Tyacke, R. J.; Nutt, D. J.; Man, J.; Tabin, P.; Sączewski, J. *Eur. J. Pharm. Sci.* **2003**, *20*, 201.
14. Pigini, M.; Bousquet, P.; Brasili, L.; Carrieri, A.; Cavagna, R.; Dontenwill, M.; Gentilli, F.; Giannella, M.; Leonetti, F.; Piergentilli, A.; Quaglia, W.; Carotti, A. *Bioorg. Med. Chem.* **1998**, *6*, 2254.
15. Anastassiadou, M.; Daunon, S.; Crane, L.; Baziraq-Mouysset, G.; Payard, M.; Caignard, D.-H.; Rettori, M.-C.; Renard, P. *Bioorg. Med. Chem.* **2001**, *9*, 585.
16. Chang-Fong, J.; Tyacke, R. J.; Lau, A.; Westaway, J.; Hudson, A. L.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1003.
17. Glennon, R. A.; Grella, B.; Tyacke, R. J.; Lau, A.; Westaway, J.; Hudson, A. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 999.

18. Glennon, R. A.; Grella, B.; Tyacke, R. J.; Lau, A.; Westaway, J.; Hudson, A. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 527.
19. Ennis, B. C.; Holan, G.; Samuel, E. L. *J. Chem. Soc. C* **1967**, 33.
20. Möller, H.; Gloxhuber, Ch.; Patent DE 2436279, **1976**.
21. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
22. pK_a values were determined at 25 °C by potentiometric titration using Metrohm 794 Titrino apparatus with the TiNet 2.5 software.
23. The geometry of the compounds **4**, **13** and **I** was fully optimized using a molecular orbital ab initio method at the Hartree-Fock level of theory with the 6-31G** basis set. The calculations were carried out using the PCSpartan program distributed by Wavefunction Inc. and installed on a PC Pentium IV computer.