Preparation of Novel 2-(Benzo[*b*]furan-2-yl)-1*H*-imidazolines for Photoaffinity Labelling and Affinity Isolation of Imidazoline Binding Proteins

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The preparations of 5-aminoefaroxan **8** and 5-azidoefaroxan **9**, which retain the capacity to stimulate insulin secretion *via* the putative I_3 imidazoline receptor and are useful biological tools for affinity chromatography studies and photoaffinity labelling studies of imidazoline binding proteins, are described

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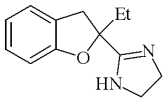
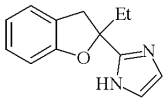
Imidazoline binding sites have been shown to be present in a wide range of tissues and two principal sub-types (I_1 and I_2 imidazoline receptors) are associated with specific responses in both the CNS and peripheral tissues [1,2]. More recently a third sub-type designated I_3, which is found in pancreatic β-cells, has been shown to control insulin secretion [2,3]. This I_3 binding site is believed to regulate K_{ATP} channels in the β-cell membrane and is a potential site of action for new anti-diabetic drugs. Binding of the agonist efaroxan **1** to the I_3 receptor results in closure of the potassium channels, calcium influx, membrane depolarization and insulin secretion [4,5]. We have recently shown that the corresponding imidazole **2** (KU14R) is an I_3 antagonist [6].



1 (efaroxan)

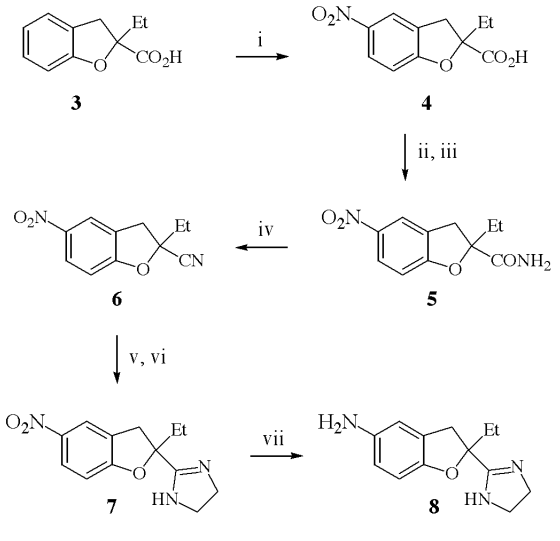


2 (KU14R)

The relationship between the I_3 receptor and the associated K_{ATP} channel is not clear but some pharmacological evidence suggests that the binding site for imidazolines may lie within the ion conducting subunit [7,8]. In order to further study this regulatory system we have prepared specifically functionalised efaroxan derivatives to probe the nature and location of the proteins associated with the I_3 active site. In this paper we describe the preparation and full characterisation of two novel efaroxan derivatives used for photoaffinity labeling studies and affinity isolation of imidazoline binding proteins.

Photoaffinity labeling has been widely applied to the investigation of active sites and requires the incorporation of a photo-activatable substituent without loss of ligand binding. Aryl azides have been used for this purpose [9] and we now describe the azide **9** which was found to have the desired properties. Affinity chromatography requires the covalent attachment of a ligand to a solid support without loss of binding capacity and we have successfully used the 5-aminoefaroxan derivative **8** for this purpose [10].

Scheme 1



Reagents:
i, Ac_2O/HNO_3
ii, $SOCl_2$; iii, NH_3;
iv, $POCl_3$;
v, MeONa/MeOH;
vi, $NH_2CH_2CH_2NH_2$;
vii, H_2/Pd/C;
viii, 4*M* $H_2SO_4/NaNO_2$; ix, NaN_3

The route used to prepare the imidazoline derivatives **8** and **9** is shown in Scheme 1. Nitration of the known carboxylic acid **3** [11] gave predominantly the 5-nitro derivative **4,** which was isolated as a single product upon crystallisation from chloroform. The position of nitration was unambiguously assigned from the ^{1}H nmr spectrum which shows a doublet at 6.99 ppm (*J* 9.8 Hz) corresponding to the aromatic C(7) proton adjacent to the ether substituent. The other two aromatic protons appear as a singlet at 8.12 ppm and a doublet at 8.14 ppm (*J* 9.8 Hz). Using standard methods this acid was then converted to the nitrile **6** *via* the amide **5**.

Treatment of the nitrile **6** with sodium methoxide to form the imidate followed by ethylenediamine gave the crystalline imidazoline **7** in 81% yield. The structure **7** was fully supported by elemental analysis and spectroscopic properties. In both the ^{1}H and ^{13}C nmr spectra the imidazoline CH_2CH_2 fragment gives a single broad signal presumably attributable to rapid tautomerism. Catalytic reduction of compound **7** gave the crystalline amine **8** in 95% yield. Diazotisation of this amine **8** followed by treatment with sodium azide gave the aryl azide **9** as a viscous yellow oil (70%) after purification by chromatography. Samples of the amine **8** and azide **9** prepared in this manner were shown to be pure by elemental analysis and nmr spectroscopy (^{1}H and ^{13}C) and were used for biological studies that have been described elsewhere [10,12]. As in the case of the nitro derivative **7**, the nmr spectra of the imidazolines **8** and **9** showed a single broad signal for the imidazolines protons. In I_3 receptor studies the three 5-substituted derivatives **7**, **8** and **9** showed significant agonist activity. This suggests that substitution at the 5 position does not inhibit binding making them useful biological tools for the purposes for which they were synthesised.

EXPERIMENTAL

^{1}H nmr spectra were recorded on a Bruker Advance DPX300 NMR spectrometer; ir spectra on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer, mass spectra on a Hitachi-Perkin-Elmer MSI 12 spectrometer and microanalyses on a Perkin-Elmer 240 elemental analyzer. Unless otherwise stated, ir spectra were measured as thin films (liquids) or potassium bromide discs (solids) and 300 MHz nmr spectra in deuteriochloroform (tetramethylsilane as internal standard). Only significant bands for the ir spectra are quoted. Melting points were determined on a Kofler block and are uncorrected. Chromatotron chromatography was performed on plates prepared using silica gel 60 PF_{254} containing calcium sulphate.

2,3-Dihydro-2-ethyl-5-nitrobenzo[*b*]furan-2-carboxylic Acid (**4**).

A solution of the carboxylic acid **3** (1.5 g, 7.8 mmol) [11] in acetic anhydride (30 ml) was cooled to -15 °C. This temperature was maintained while concentrated HNO_3 (S.G. 1.43)(5.0 ml) was added slowly with stirring and stirring was continued (15 minutes) after the addition was complete. The mixture was then poured onto ice and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) evaporated to give a brown oil. Crystallisation from $CHCl_3$ gave the desired nitro compound **4** (1.1 g, 60%), colourless needles, mp 172-173 °C; ir: 3088, 2982, 2688, 1710, 1596, 1510, 1472, 1432, 1344, 1304, 1280, 1252, 1186, 1162, 1124, 1106, 1070, 1014, 950, 922, 828, 808, 790, 744 and 668 cm^{-1}: ^{1}H nmr: δ 1.03 (3H, t, *J* 7.3 Hz, CH_2CH_3), 2.11 (2H, m, $CH_aH_bCH_3$), 3.40 (1H, d, *J* 17.1 Hz, $ArCH_aH_b$), 3.70 (1H, d, *J* 17.1 Hz, $ArCH_aH_b$), 6.99 (1H, d, *J* 9.8 Hz, aromatic C(7)*H*), 8.12 (1H, s, aromatic C(4)*H*), 8.14 (1H, d, *J* 9.8 Hz, aromatic C(6)*H*); ^{13}C nmr: δ 7.94 (q), 30.40 (t), 37.26 (t), 92.53 (s), 109.31 (d), 121.21 (d), 125.68 (d), 128.00 (s), 141.59 (s), 164.20 (s), 172.81 (s); ms: m/z 237(35%)(M^+), 192(100%), 146(85%).

2,3-Dihydro-2-ethyl-5-nitrobenzo[*b*]furan-2-carboxamide (**5**).

Thionyl chloride (1.79 g, 15 mmol) was added to a suspension of the acid **4** (1.82 g, 7.7 mmol) in dry toluene (50 ml). The mixture was heated with stirring at 90-100 °C until all the carboxylic acid was in solution (2.5 hours). The solvent and excess thionyl chloride were then removed under diminished pressure to give the acid chloride as an oil. The crude product was dissolved in dry dioxane (6.0 ml) and the solution was added dropwise with stirring to aqueous ammonia (SG 0.88)(9.0 ml) at 0 °C. After the addition was complete, the mixture was allowed to warm to room temperature and water (40 ml) was added. The solid product was recrystallised from $CHCl_3$-CCl_4 and identified as the amide **5** (1.3 g, 72%), colourless crystals, mp 140-141 °C; ir: 3432, 3186, 2976, 2938, 2884, 1678, 1594, 1508, 1476, 1434, 1408, 1328, 1290, 1256, 1194, 1106, 1068, 1016, 950, 924, 904, 874, 824, 746 and 668 cm^{-1}: ^{1}H nmr: δ 1.03 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.98 (1H, m, $CH_aH_bCH_3$), 2.15 (1H, m, $CH_aH_bCH_3$), 3.24 (d, *J* 16.6 Hz, $ArCH_aH_b$), 3.67 (d, *J* 16.6 Hz $ArCH_aH_b$), 6.42 (1H, br.s, $CO.NH_a$), 6.56 (1H, br.s, $CO.NH_b$), 6.90 (1H, d, *J* 8.8 Hz, aromatic C(7)*H*), 8.08-8.14 (2H, m, aromatic C(4)*H* and C(6)*H*); ^{13}C nmr: δ 7.91 (q), 30.83 (t), 37.41 (t), 93.81 (s), 109.97 (d), 121.21 (d), 125.43 (d), 128.10 (s), 141.68 (s), 163.64 (s), 174.00 (s); ms: m/z 236(10%)(M^+), 219(15%), 207(15%), 192(100%), 176(20%), 146(65%), 131(50%).

2-Cyano-2,3-dihydro-2-ethyl-5-nitrobenzo[*b*]furan (**6**).

A solution of the amide **5** (3.3 g, 14 mmol) in dry pyridine (55.0 ml) was cooled to 0 °C and phosphorus oxychloride (6.8 g, 44 mmol) was added with stirring. The solution was then heated under reflux (2.5 hours). The solvent was then removed under reduced pressure and the residue partitioned between CH_2Cl_2 and dilute HCl. The organic phase as washed with 10% aqueous NaCl solution, dried ($MgSO_4$) and evaporated to give the nitrile **6** as a buff solid (2.9 g, 95%) that was shown to be pure by nmr and was used without further purification; ir: 3102, 2980, 2944, 2884, 2684, 2596, 1738, 1598, 1514, 1476, 1434, 1384, 1334, 1252, 1124, 1066, 1020, 980, 942, 922, 874, 828, 806, 748, 702 and 668 cm^{-1}: ^{1}H nmr: δ 1.23 (3H, t, *J* 7.3 Hz, CH_2CH_3), 2.18 (2H, m, $CH_a H_bCH_3$), 3.45 (1H, d, *J* 16.6 Hz, $ArCH_aCH_b$), 3.76 (1H, d, *J* 16.6 Hz, $ArCH_aCH_b$), 6.93 (1H, d, *J* 8.8 Hz, aromatic C(7)*H*), 8.12 (2H, m, aromatic C(4)*H* and C(6)*H*); ^{13}C nmr: δ 8.40 (q), 32.47 (t), 40.10 (t), 85.07 (s), 110.17 (d), 118.69 (s), 121.40 (d), 126.06 (s),126.19 (d), 143.08 (s), 162.48 (s); ms: m/z 218(100%)(M^+), 201(10%), 171(60%), 152(20%), 143(20%).

2-(2,3-Dihydro-2-ethyl-5-nitrobenzo[*b*]furan-2-yl)-1*H*-imidazoline (**7**).

Sodium methoxide (1 M in MeOH)(1.12 ml) was added with stirring to a solution of the nitrile **6** (3.0 g, 14 mmol) in methanol (30.0 ml). After standing at room temperature (18 hours) the solution was cooled to 0 °C and a solution of ethylenediamine (0.83 g, 14 mmol) was added dropwise. After 15 minutes a solution of methanolic hydrochloric acid [concentrated HCl (1.2 ml)/ MeOH (10.0 ml)] was added slowly with cooling. After standing (2 hours), the solvent was removed under diminished pressure and the residue partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The combined organic extracts were washed, dried (Na_2SO_4) and evaporated to give the free base **7** as a buff solid that was recrystallised from ethyl acetate-petroleum ether and identified as compound **7** (2.9 g, 81%), colourless crystals, mp

114-115 °C; ir: 3318, 3112, 2970, 2874, 1614, 1596, 1504, 1432, 1344, 1280, 1264, 1180, 1154, 1112, 1058, 976, 920, 902, 832, 748 and 670 cm^{-1}: ^{1}H nmr: δ 0.99 (3H, t, *J* 7.3 Hz, CH_2CH_3), 2.08 (2H, m, $CH_aH_bCH_3$), 3.26 (1H, d, *J* 16.6 Hz, $ArCH_aH_b$), 3.70 (4H, br.s, CH_2CH_2), 3.83 (1H, d, *J* 16.6 Hz, $ArCH_aH_b$), 5.1 (1H, br.s, N*H*), 6.81 (1H, d, *J* 8.3 Hz, aromatic C(7)*H*), 8.05-8.09 (2H, m, aromatic C(4)*H* and C(6)*H*); ^{13}C nmr: δ 7.93 (q), 32.49 (t), 38.29 (t), 45.0 (br.s), 54.0 (br.s), 92.21 (s), 109.19 (d), 121.36 (d), 125.63 (d), 128.23 (s), 142.32 (s), 163.61 (s), 168.97 (s); ms: m/z 261(55%) (M^+), 244(35%), 232(100%), 198(10%), 186(45%).

Anal. Calcd. for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.67; H, 6.04; N, 16.09.

2-(5-Amino-2,3-dihydro-2-ethylbenzo[*b*]furan-2-yl)-1*H*-imidazoline (**8**).

A solution of the imidazoline **5** (0.25 g, 0.95 mmol) in ethanol was stirred with palladium on charcoal (20 mg) at room temperature under an atmosphere of hydrogen until three equivalents of hydrogen (64.4 ml) had been taken up. The mixture was filtered and evaporated to give a solid that was recrystallised from ethyl acetate and identified as the amine **8** (2.1 g, 95%), pale yellow plates, mp 178-179 °C; ir: 3374, 3184, 2966, 2872, 1610, 1488, 1460, 1382, 1344, 1282, 1252, 1212, 1194, 1118, 1066, 1030, 986, 848 and 800 cm^{-1}: ^{1}H nmr: δ 0.97 (3H, t, *J* 7.3 Hz, CH_2CH_3), 2.01 (2H, m, $CH_aH_bCH_3$), 3.12 (1H, d, *J* 16.1 Hz, $ArCH_aH_b$), 3.40 (6H, br.s, NH_2 + CH_2CH_2), 3.59 (1H, d, *J* 16.1 Hz, $ArCH_aH_b$), 5.0 (1H, v.br.s, N*H*), 6.45 (1H, dd, *J* 2.4 and 8.3 Hz, aromatic C(6)*H*), 6.54 (1H, d, *J* 2.4 Hz, aromatic C(4)*H*), 6.57 (1H, d *J* 8.3 Hz, aromatic C(7)*H*); ^{13}C nmr: δ 8.13 (q), 32.45 (t), 40.02 (t), 88.87 (s), 109.33 (d), 112.68 (d), 114.70 (d), 127.40 (s), 140.57 (s), 151.62 (s), 171.11 (s); ms: m/z 231(55%) (M^+), 214 (100%), 202 (95%), 161 (30%), 146 (40%).

Anal. Calcd. for $C_{13}H_{17}N_3O$: C, 67.51; H, 7.41; N, 18.17. Found: C, 67.83; H, 7.35; N, 18.25.

2-(5-Azido-2,3-dihydro-2-ethylbenzo[*b*]furan-2-yl)-1*H*-imidazoline (**9**).

A solution of the amine **8** (0.22 g, 0.95 mmol) in 4 M sulphuric acid (4.7 ml) and dioxane (4.0 ml) was cooled to -5 °C and diazotised by addition, with stirring, of a solution of sodium nitrite (0.07 g, 0.96 mmol) in water (0.5 ml). A solution of sodium azide (0.075 g, 1.1 mmol) in water (0.5 ml) was added to the diazonium salt and the solution was gently warmed to 30 °C. The cooled solution was treated with aqueous $NaHCO_3$ (20 ml) and extracted with ether. The combined organic layers were dried (Na_2SO_4) and evaporated to give the azide as a yellow oil (crude yield 79%). This was purified by chromatotron chromatography [4:1 petroleum ether (bp 60-80 °C):ethyl acetate as eluent] giving the azide **9** (170 mg, 70%), pale yellow oil (with solidification on refrigeration); ir: 3210, 2938, 2872, 2112, 1618, 1484, 1382, 1288, 1242, 1184, 1106, 982, 944, 848 and 810 cm^{-1}: ^{1}H nmr: δ 0.98 (3H, t, *J* 7.3 Hz, CH_2CH_3), 2.03 (2H, m, $CH_aH_bCH_3$), 3.20 (1H, d, *J* 16.6 Hz, $ArCH_aH_b$), 3.70 (1H, d, *J* 16.6 Hz, $ArCH_aH_b$), 4.82 (1H, br.s, N*H*), 6.71-6.84 (4H, m, aromatic *H*); ^{13}C nmr: δ 8.02 (q), 32.48 (t), 39.57 (t), 49.66 (br.t), 89.77 (s), 110.10 (d), 115.94 (d), 118.75 (d), 128.24 (s), 133.00 (s), 155.80 (s), 170.62(s); ms: m/z 257(15%)(M^+), 229(100%)(M^+-N_2), 200(60%), 186(55%), 149(50%).

Anal. Calcd. for $C_{13}H_{15}N_5O$: C, 60.69; H, 5.88; N, 27.22. Found: C, 60.42; H, 6.29; N, 26.99.

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