

Appendix I: Radioligand Binding

Microsomal membranes from guinea pig heart ventricles were prepared as previously described.^{15,16} Binding of the 1,4-dihydropyridine (+) [³H] PN 200 110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridinecarboxylate) and its competition by the 4-isoxazolyl-1,4-dihydropyridines were carried out as previously described. Briefly, membrane protein (40-120 µg) was incubated in 5 mL 50 mM tri [(hydroxymethyl)amino]methane (Tris) buffer at pH 7.2 for 90 min at 25°C with 5×10^{-11} (+) [³H] PN 200 110 and varying concentrations of competing compounds. Duplicate tubes were filtered and washed rapidly with two 5 mL portions of ice-cold Tris buffer in a Brandel cell harvester [Model M-24-R], Brandel Instruments Ltd., Gaithersburg, MD]. Trapped radioactivity was counted by liquid scintillation spectrometry at an efficiency of 40-45%. Competing compounds were prepared in 100% ethanol as 10^{-3} stock solutions. Subsequent dilutions were made in 50% ethanol (10^{-4} M) or distilled water (10^{-5} onwards). All dilutions were conducted on the day of the experiment. Concentrations of ethanol to 0.2% (v:v) did not effect specific binding. Binding data were analyzed by iterative curve fitting programs (BDATA, CDATA, EMF Software, Knoxville, TN). (+) [³H] PN 200 110 with a specific activity of 70Ci/mole [1 Ci = 3.7×10^{10} bq] was purchased from DuPont-New England Nuclear (Boston, MA).

Table 1. Crystallographic data for **1 a**.

empirical formula	$C_{29}H_{32}N_2O_5$
molecular weight	488.57
temperature	173 K
wavelength	0.071073 Å
crystal system	monoclinic
space group	$P2_1/n$
unit cell dimensions	$a = 8.9934 (2) \text{ \AA}$ $\alpha = 90^\circ$ $b = 16.8650 (3) \text{ \AA}$ $\beta = 96.8910 (10)^\circ$ $c = 17.01222 (3) \text{ \AA}$ $\gamma = 90^\circ$
volume, z	$2561.66 (6) \text{ \AA}^3, 4$
absorption coefficient	0.87 mm^{-1}
F(000)	1040
crystal size	0.15 x 0.20 x 0.35 mm
θ range for data collection	1.71 to 24.72°
limiting indices	$-8 \leq h \leq 10, -19 \leq k \leq 19, -19 \leq l \leq 17$
reflections collected	11666
independent reflections	4328 ($R_{int} = 0.0301$)
absorption correction	semi-empirical from psi-scans
max. and min. transmission	0.9651 and 0.8833
refinement method	full-matrix least squares on F^2
data/restraints/parameters	4328/0/454
goodness-of-fit on F^2	1.082
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0462, wR2 = 0.1048$
R indices (all data)	$R1 = 0.0565, wR2 = 0.1106$
extinction coefficient	0.0046 (6)
largest diff. peak and hole	0.240 and -0.171 e\AA^{-3}

Data was collected using a Siemens SMART CCD (charge coupled device) diffractometer equipped with an LT-2 low-temperature apparatus operating at 173 K. A suitable crystal was chosen and mounted on a glass fiber using grease. Data was measured using omega scans of 0.3° per frame for 10 seconds, such that a hemisphere was collected. A total of 1271 frames were collected with a final resolution of 0.85 \AA . The first 50 frames were recollected at the end of data collection to monitor and correct for decay. Cell parameters were retrieved using SMART software and refined using SAINT on all observed reflections. Data reduction was performed using the SAINT software which corrects for L_p and decay. Absorption corrections were applied using XEMP supplied by Siemens in their SHEXTL-PC software.

displacement parameters [$\text{\AA}^2 \times 10^3$] for 1. $U(\text{eq})$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	$U(\text{eq})$
N(1)	2295(2)	-1591(1)	7153(1)	31(1)
N(2)	1121(2)	1864(1)	7451(1)	32(1)
O(1)	2177(2)	1437(1)	7979(1)	32(1)
O(2)	3132(2)	-336(1)	5073(1)	47(1)
O(3)	1480(2)	565(1)	5407(1)	33(1)
O(4)	-1813(2)	-1035(1)	8079(1)	60(1)
O(5)	-2108(1)	-108(1)	7137(1)	30(1)
C(1)	2672(2)	-1189(1)	6494(1)	28(1)
C(2)	1883(2)	-541(1)	6225(1)	25(1)
C(3)	643(2)	-206(1)	6672(1)	23(1)
C(4)	119(2)	-822(1)	7238(1)	24(1)
C(5)	990(2)	-1451(1)	7485(1)	27(1)
C(6)	3968(3)	-1558(1)	6143(2)	42(1)
C(7)	715(3)	-2046(1)	8110(1)	37(1)
C(8)	2255(2)	-123(1)	5516(1)	29(1)
C(9)	1929(2)	1107(1)	4809(1)	33(1)
C(10)	3090(3)	1667(1)	5186(1)	43(1)
C(11)	-1324(2)	-688(1)	7542(1)	30(1)
C(12)	-3587(2)	48(1)	7365(1)	34(1)
C(13)	-4350(3)	630(2)	6778(1)	40(1)
C(14)	1091(2)	560(1)	7115(1)	23(1)
C(15)	2118(2)	661(1)	7760(1)	24(1)
C(16)	495(2)	1337(1)	6949(1)	26(1)
C(17)	-689(3)	1598(1)	6310(1)	35(1)
C(18)	3164(2)	125(1)	8256(1)	27(1)
C(19)	2553(2)	-174(1)	9006(1)	29(1)
C(20)	3577(2)	-758(1)	9488(1)	28(1)
C(21)	4963(2)	-956(1)	9287(1)	33(1)
C(22)	5917(2)	-1499(1)	9744(1)	38(1)
C(23)	5469(2)	-1836(1)	10404(1)	39(1)
C(24)	4047(2)	-1654(1)	10643(1)	33(1)
C(25)	3558(3)	-2005(1)	11323(1)	41(1)
C(26)	2185(3)	-1840(1)	11546(1)	46(1)
C(27)	1229(3)	-1309(1)	11096(1)	45(1)
C(28)	1666(2)	-953(1)	10433(1)	38(1)
C(29)	3086(2)	-1113(1)	10181(1)	30(1)

N(1)-C(5)	1.383 (2)	N(1)-C(1)	1.386 (2)
N(2)-C(16)	1.312 (2)	N(2)-O(1)	1.422 (2)
O(1)-C(15)	1.359 (2)	O(2)-C(8)	1.209 (2)
O(3)-C(8)	1.355 (2)	O(3)-C(9)	1.461 (2)
O(4)-C(11)	1.210 (2)	O(5)-C(11)	1.347 (2)
O(5)-C(12)	1.453 (2)	C(1)-C(2)	1.353 (3)
C(1)-C(6)	1.506 (3)	C(2)-C(8)	1.469 (3)
C(2)-C(3)	1.530 (2)	C(3)-C(14)	1.525 (2)
C(3)-C(4)	1.529 (2)	C(4)-C(5)	1.354 (3)
C(4)-C(11)	1.471 (3)	C(5)-C(7)	1.505 (3)
C(9)-C(10)	1.495 (3)	C(12)-C(13)	1.506 (3)
C(14)-C(15)	1.358 (2)	C(14)-C(16)	1.432 (2)
C(15)-C(18)	1.491 (3)	C(16)-C(17)	1.493 (3)
C(18)-C(19)	1.533 (3)	C(19)-C(20)	1.520 (3)
C(20)-C(21)	1.373 (3)	C(20)-C(29)	1.439 (3)
C(21)-C(22)	1.420 (3)	C(22)-C(23)	1.361 (3)
C(23)-C(24)	1.422 (3)	C(24)-C(25)	1.416 (3)
C(24)-C(29)	1.426 (3)	C(25)-C(26)	1.363 (3)
C(26)-C(27)	1.403 (3)	C(27)-C(28)	1.377 (3)
C(28)-C(29)	1.420 (3)		
C(5)-N(1)-C(1)	123.3 (2)	C(16)-N(2)-O(1)	105.68 (14)
C(15)-O(1)-N(2)	108.26 (13)	C(8)-O(3)-C(9)	116.8 (2)
C(11)-O(5)-C(12)	115.72 (14)	C(2)-C(1)-N(1)	119.9 (2)
C(2)-C(1)-C(6)	126.8 (2)	N(1)-C(1)-C(6)	113.3 (2)
C(1)-C(2)-C(8)	120.2 (2)	C(1)-C(2)-C(3)	120.9 (2)
C(8)-C(2)-C(3)	118.8 (2)	C(14)-C(3)-C(4)	110.34 (14)
C(14)-C(3)-C(2)	113.11 (14)	C(4)-C(3)-C(2)	111.14 (14)
C(5)-C(4)-C(11)	120.9 (2)	C(5)-C(4)-C(3)	121.1 (2)
C(11)-C(4)-C(3)	118.0 (2)	C(4)-C(5)-N(1)	119.7 (2)
C(4)-C(5)-C(7)	127.1 (2)	N(1)-C(5)-C(7)	113.2 (2)
O(2)-C(8)-O(3)	122.1 (2)	O(2)-C(8)-C(2)	126.9 (2)
O(3)-C(8)-C(2)	111.1 (2)	O(3)-C(9)-C(10)	109.5 (2)
O(4)-C(11)-O(5)	121.5 (2)	O(4)-C(11)-C(4)	127.4 (2)
O(5)-C(11)-C(4)	111.2 (2)	O(5)-C(12)-C(13)	107.4 (2)
C(15)-C(14)-C(16)	104.3 (2)	C(15)-C(14)-C(3)	128.3 (2)
C(16)-C(14)-C(3)	127.4 (2)	C(14)-C(15)-O(1)	110.1 (2)
C(14)-C(15)-C(18)	134.6 (2)	O(1)-C(15)-C(18)	115.3 (2)
N(2)-C(16)-C(14)	111.7 (2)	N(2)-C(16)-C(17)	119.2 (2)
C(14)-C(16)-C(17)	129.1 (2)	C(15)-C(18)-C(19)	113.9 (2)
C(20)-C(19)-C(18)	114.1 (2)	C(21)-C(20)-C(29)	118.7 (2)
C(21)-C(20)-C(19)	122.2 (2)	C(29)-C(20)-C(19)	119.1 (2)
C(20)-C(21)-C(22)	121.9 (2)	C(23)-C(22)-C(21)	120.1 (2)
C(22)-C(23)-C(24)	120.7 (2)	C(25)-C(24)-C(23)	121.5 (2)
C(25)-C(24)-C(29)	119.4 (2)	C(23)-C(24)-C(29)	119.2 (2)
C(26)-C(25)-C(24)	121.5 (2)	C(25)-C(26)-C(27)	119.7 (2)
C(28)-C(27)-C(26)	120.6 (2)	C(27)-C(28)-C(29)	121.3 (2)
C(28)-C(29)-C(24)	117.6 (2)	C(28)-C(29)-C(20)	122.9 (2)
C(24)-C(29)-C(20)	119.5 (2)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$\text{\AA}^2 \times 10^3$] for 1.

The anisotropic displacement factor exponent takes the form:

$$-2\pi^2 [(ha^*)^2 U_{11} + \dots + 2hka^* b^* U_{12}]$$

	U11	U22	U33	U23	U13	U12
N(1)	36(1)	21(1)	36(1)	3(1)	5(1)	7(1)
N(2)	38(1)	22(1)	35(1)	1(1)	3(1)	0(1)
O(1)	38(1)	24(1)	32(1)	-3(1)	0(1)	-4(1)
O(2)	57(1)	49(1)	40(1)	6(1)	26(1)	13(1)
O(3)	43(1)	28(1)	29(1)	6(1)	11(1)	1(1)
O(4)	51(1)	69(1)	67(1)	40(1)	31(1)	17(1)
O(5)	26(1)	33(1)	32(1)	6(1)	8(1)	3(1)
C(1)	32(1)	24(1)	29(1)	-4(1)	6(1)	-1(1)
C(2)	26(1)	23(1)	26(1)	-3(1)	3(1)	-1(1)
C(3)	24(1)	20(1)	24(1)	1(1)	2(1)	1(1)
C(4)	28(1)	22(1)	23(1)	-1(1)	2(1)	-3(1)
C(5)	32(1)	23(1)	26(1)	-2(1)	2(1)	-3(1)
C(6)	42(1)	34(1)	51(2)	0(1)	17(1)	11(1)
C(7)	52(1)	26(1)	33(1)	6(1)	5(1)	1(1)
C(8)	31(1)	29(1)	28(1)	-4(1)	4(1)	-2(1)
C(9)	42(1)	33(1)	26(1)	6(1)	6(1)	-3(1)
C(10)	49(1)	39(1)	38(1)	8(1)	1(1)	-11(1)
C(11)	33(1)	28(1)	29(1)	5(1)	5(1)	-3(1)
C(12)	25(1)	41(1)	36(1)	3(1)	10(1)	1(1)
C(13)	29(1)	51(2)	41(1)	8(1)	9(1)	7(1)
C(14)	23(1)	22(1)	24(1)	2(1)	7(1)	-2(1)
C(15)	27(1)	21(1)	26(1)	-2(1)	8(1)	-2(1)
C(16)	29(1)	22(1)	27(1)	1(1)	9(1)	-2(1)
C(17)	38(1)	28(1)	38(1)	5(1)	3(1)	6(1)
C(18)	26(1)	28(1)	27(1)	-2(1)	2(1)	-2(1)
C(19)	31(1)	29(1)	27(1)	-1(1)	1(1)	-1(1)
C(20)	32(1)	26(1)	26(1)	-4(1)	0(1)	-1(1)
C(21)	34(1)	35(1)	30(1)	-4(1)	2(1)	-2(1)
C(22)	33(1)	39(1)	39(1)	-6(1)	-3(1)	4(1)
C(23)	44(1)	34(1)	35(1)	-4(1)	-10(1)	6(1)
C(24)	43(1)	28(1)	26(1)	-4(1)	-5(1)	-1(1)
C(25)	60(2)	35(1)	27(1)	1(1)	-6(1)	1(1)
C(26)	68(2)	43(1)	29(1)	2(1)	9(1)	-4(1)
C(27)	53(2)	48(1)	38(1)	2(1)	14(1)	1(1)
C(28)	42(1)	38(1)	33(1)	2(1)	7(1)	4(1)
C(29)	37(1)	26(1)	26(1)	-3(1)	-2(1)	-2(1)

Table 3. Hydrogen coordinates ($\times 10^3$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for 1.

	x	y	z	U(eq)
H(1)	2838(26)	-1997(14)	7318(14)	46(7)
H(3)	-203(21)	-68(11)	6285(11)	25(5)
H(6C)	4670(29)	-1160(16)	5983(15)	64(8)
H(6B)	4490(30)	-1927(17)	6509(17)	71(8)
H(6A)	3639(33)	-1819(18)	5652(19)	85(10)
H(7C)	1098(30)	-2563(17)	7956(16)	73(8)
H(7B)	-348(36)	-2141(18)	8138(17)	85(10)
H(7A)	1210(34)	-1897(18)	8601(19)	85(10)
H(9B)	2325(23)	789(13)	4390(13)	42(6)
H(9A)	995(25)	1398(13)	4624(13)	43(6)
H(10C)	3364(24)	2075(14)	4801(14)	48(6)
H(10B)	4021(28)	1372(14)	5371(14)	54(7)
H(10A)	2693(30)	1955(16)	5615(17)	70(8)
H(12B)	-3448(22)	266(12)	7913(13)	35(5)
H(12A)	-4121(24)	-475(14)	7368(13)	45(6)
H(13C)	-4348(25)	424(13)	6232(14)	48(6)
H(13B)	-3806(26)	1141(15)	6831(13)	49(7)
H(13A)	-5374(25)	719(12)	6894(12)	37(6)
H(17C)	-278(31)	1696(16)	5802(17)	75(9)
H(17B)	-1120(29)	2101(17)	6451(15)	67(8)
H(17A)	-1415(32)	1204(18)	6170(16)	75(9)
H(18B)	4079(22)	414(11)	8401(11)	27(5)
H(18A)	3383(20)	-328(11)	7925(11)	24(5)
H(19B)	2402(22)	281(13)	9332(12)	33(5)
H(19A)	1555(24)	-418(12)	8854(11)	33(5)
H(21)	5345(22)	-732(12)	8802(12)	34(5)
H(22)	6946(25)	-1604(13)	9563(13)	42(6)
H(23)	6100(26)	-2186(14)	10725(14)	49(6)
H(25)	4248(27)	-2365(14)	11601(14)	52(7)
H(26)	1850(25)	-2109(14)	12003(14)	50(6)
H(27A)	247(29)	-1166(15)	11261(15)	59(7)
H(28)	987(24)	-562(13)	10119(13)	43(6)