Supporting Information

Imidazo[2,1-*b*]thiazole System: a Scaffold Endowing Dihydropyridines With Selective Cardiodepressant Activity.

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Details For Chemistry

The substituted 2-aminothiazoles (**1A-E**, **G**), some of the 2-bromo-1-arylethanone (**2**) and aldehyde **4Aj** are commercially available whereas the 2-aminothiazole **1F**[RS1], the bromoacetophenones **2e**[RS2], **2l**[RS3], **2n**[RS4], **2o**[RS5]**,** imidazo[2,1-*b*]thiazoles **3Ac**[RS6] and **3An**[RS7] and aldehydes **4Af**[RS8], **4Ai**[RS8], **4Ak**[RS9], **4Am**[RS10], **4Ap-4Ar**[RS10], **4Ba**[RS11], **4Bb**[RS12], **4Bd**[RS8], **4Bk**[RS13], **4Bp-4Bq**[RS10],**4Ca**[RS14], **4Cb**[RS15], **4Cc**[RS16], **4Ck**[RS13], **4Cp-4Cq**[RS10], **4Db**[RS17], **4Dd**[RS8], **4Eb**[RS17], **4Ed**[RS8], **4Gp**[RS10] were prepared according to the literature method. The synthesis of imidazo[2,1-*b*]thiazoles **3Ae**, **3Ag**, **3Ah, 3Al**, **3Ao**, **3Fk** and aldehydes **4Ac, 4Ae, 4Ag-4Ah, 4Al, 4An, 4Ao, 4Fk** are reported below.

Imidazo[2,1-*b*]thiazoles 3Ae, 3Ag, 3Ah, 3Al, 3Ao, 3Fk.

The 2-aminothiazoles (**1**, 30 mmol) were dissolved in acetone (80 mL) and treated with the appropriate 2-bromo-1-arylethanones (**2**, 30 mmol). The reaction mixture was refluxed for 3 h and after cooling the resulting precipitate was collected, suspended in 100 mL of 2N HCl and refluxed for 2 h. The warm solution, basified with 20 % NH_4OH, after cooling at room temperature yielded the expected imidazo[2,1-*b*]thiazoles which were filtered. For **3Ae** the basified solution was extracted with CH_2Cl_2 (3 x 20mL), the organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. For **3Al** the crude product was purified by flash chromatography (acetone/petroleum ether 55-85 °C, 1:9 v/v). For **3An** the basified solution was extracted with CH_2Cl_2 (3 x 20mL), the organic phase was dried (Na_2SO_4) and evaporated *in vacuo;* the crude product was triturated from ethyl acetate/ petroleum ether 55-85 °C.

Imidazo[2,1-*b*]thiazole-5-carboxaldehydes 4Ac, 4Ae, 4Ag-4Ah, 4Al, 4An, 4Ao, 4Fk.

The Vilsmeier reagent was prepared at 0-5 °C by dropping 32 mmol of $POCl_3$ into a stirred solution of DMF (39 mmol) in $CHCl_3$ (5 mL). The appropriate imidazo[2,1-*b*]thiazoles (**3**, 12 mmol), dissolved in $CHCl_3$ (60 mL), were added dropwise, under stirring at 0-5 °C, to the Vilsmeier reagent. After 3 h at room temperature, the reaction mixture was refluxed for 3-14 h (according to a TLC test acetone/petroleum ether 55-85 °C, 1:9 v/v, 2:8 v/v) and the solvent was

evaporated under reduced pressure. The oily residue was poured into ice and the resulting precipitate was collected and crystallized from ethanol. For **4Al** the mixture was adjusted to pH 8 with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 x 20mL), the organic phase was dried (Na_2SO_4) and evaporated *in vacuo;* the crude product was purified by flash chromatography (acetone/petroleum ether 55-85 °C, 1:9 v/v). For **4An** the mixture was adjusted to pH 8 with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 x 20 mL), the organic phase was dried (Na_2SO_4) and evaporated *in vacuo.*

Table S1. New Starting Compounds.

Comp	R	R_1	R_2	Formula	M W	Yield %	Mp, °C
3Ae	H	H	2-$(CF_3)C_6H_4$	$C_{12}H_7F_3N_2S$	268.2603	51	Oil
3Ag	H	H	2-$(OCF_3)C_6H_4$	$C_{12}H_7F_3N_2OS$	284.2597	58	75-80
3Ah	H	H	3-$(OCF_3)C_6H_4$	$C_{12}H_7F_3N_2OS$	284.2597	50	100-101
3Al	H	H	3,5-$(OCH_3)_2C_6H_3$	$C_{13}H_{12}N_2O_2S$	260.3145	76	>300 dec.
3Ao	H	H	3,4,5-$(OCH_3)_3C_6H_2$	$C_{14}H_{14}N_2O_3S$	290.3406	29	124-126
3Fk	C_2H_5	H	2,5-$(OCH_3)_2C_6H_3$	$C_{15}H_{16}N_2O_2S$	288.3678	35	90-95
4Ac	H	H	CF_3	$C_7H_3F_3N_2OS$	219.9918	66	90-92
4Ae	H	H	2-$(CF_3)C_6H_4$	$C_{13}H_7F_3N_2OS$	296.2705	26	65-70
4Ag	H	H	2-$(OCF_3)C_6H_4$	$C_{13}H_7F_3N_2O_2S$	312.2699	65	105-108
4Ah	H	H	3-$(OCF_3)C_6H_4$	$C_{13}H_7F_3N_2O_2S$	312.2699	92	109-110
4Al	H	H	3,5-$(OCH_3)_2C_6H_3$	$C_{14}H_{12}N_2O_3S$	288.3247	37	130-133
4An	H	H	2,4-$(OCH_3)_2C_6H_3$	$C_{14}H_{12}N_2O_3S$	288.3247	66	196-198
4Ao	H	H	3,4,5-$(OCH_3)_3C_6H_2$	$C_{15}H_{14}N_2O_4S$	318.3508	93	180-182
4Fk	C_2H_5	H	2,5-$(OCH_3)_2C_6H_3$	$C_{16}H_{16}N_2O_3S$	316.3780	29	125-130

Table S2. IR and ^{1}H-NMR of New Compounds.

Comp	IR:[a] ν_{max} cm^{-1}	^{1}H-NMR:[b] δ, ppm in DMSO-d$_6$; J, Hz
3Ae	3105, 1721, 1629, 1555, 1176	7.30 (1H, d, th, J = 6.4), 7.54 (1H, t, ar, J = 7.8), 7.71 (IH, t, ar, J = 7.4), 7.80 (1H, t, ar, J = 8.2), 7.86 (1H,d,ar, J = 7.4), 7.96 (2H, d, th + im, J = 4.8)
3Ag	3165, 3043, 1036, 1260, 1170	7.30 (1H, d, th, J = 4.4), 7.43 (3H, m, ar), 8.00 (1H, d, th, J = 4.6), 8.10 (1H, s, th), 8.24 (1H, m, ar),
3Ah	3139, 1557, 1266, 1150, 718	7.26 (IH, d, ar, J = 8.1), 7.32 (1H, d, th, J = 4.5), 7.54 (1H, t, ar, J = 8.1), 7.82 (1H, s, ar), 7.88 (1H, d, ar, J = 7.8), 7.99 (1H, d, th, J = 4.5), 8.39 (1H, s, im)
3Al	3129, 1608, 1211, 1162, 1068	3.79 (6H, s, OCH_3), 6.40 (1H, t, ar, J = 2.4), 7.01 (2H, d, ar, J = 2.4), 7.27 (1H, d, th, J = 4.4), 7.94 (1H, d, th), 8.27 (1H, s, im)
3Ao	3103, 1643, 1586, 1226, 1130	3.67 (3H, s, OCH_3), 3.83 (6H, s, OCH_3), 7.13 (2H, s, ar), 7.25 (1H, d, th, J = 4.5), 7.93 (1H, d, th, J = 4.8), 8.25 (1H, s, im)
3Fk	3047, 1508, 1495, 1050, 799	1.24 (3H, t, J = 7.5), 2.76 (2H, q, J = 7.5), 3.74 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.79 (1H, dd, ar, J = 3.3, J = 8.7), 6.99 (1H, d, ar, J = 9.00), 7.68 (1H, d, ar, J = 3.00), 7.70 (1H, s, th), 8.09 (1H, s, im)
4Ac	3134, 1659, 1342, 1156, 1107	7.76 (1H, d, th, J = 4.2), 8.43 (1H, d, th, J = 4.5), 9.95 (1H, s, CHO)
4Ae	3134, 3108, 1637, 1313, 1284	7.66 (1H, d, ar, J = 6), 7.78 (4H, m, ar + th), 7.93 (1H, d, ar, J = 6), 8.41 (1H, d, th, J = 3), 9.41 (1H, s, CHO)
4Ag	3139, 3085, 1662, 1488, 1253	7.45 (1H, d, ar, J = 8), 7.56 (1H, d, th, J = 6), 7.62 (1H, t, ar, J = 8), 7.79 (1H, s, ar), 7.88 (1H, d, ar, J = 8), 8.36 (1H, d, th, J = 6), 9.83 (1H, s, CHO)
4Ah	3130, 1651, 1264, 1203, 791	7.45 (1H, d, ar, J = 8), 7.56 (1H, d, th, J = 6), 7.62 (1H, t, ar, J = 8), 7.79 (1H, s, ar), 7.88 (1H, d, ar, J = 8), 8.36 (1H, d, th, J = 6), 9.83 (1H, s, CHO)
4Al	1646, 1275, 1196, 785, 725	3.82 (6H, s, OCH_3), 6.63 (1H, t, ar, J = 2.2), 7.01 (2H, d, ar, J = 2.2), 7.61 (1H, d, th, J = 4.4), 8.41 (1H, d, th, J = 4.4), 9.89 (1H, s, CHO)
4An	3129, 3103, 1611, 1539, 1161	3.78 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.68 (1H,dd, ar, J = 8.4, J = 2.4), 6.71(1H, d, ar, J = 4.8), 7.52 (1H, d, ar, J = 8.4), 7.55 (1H, d, th, J = 4.8), 8.34 (1H, d, th, J = 4.8), 9.62 (1H, s, CHO)
4Ao	3119, 1645, 1588, 1130, 988	3.72 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 7.15 (2H, s, ar), 7.58 (1H, d, th, J = 4.5), 8.40 (1H, d, th, J = 4.2), 9.93 (1H, s, CHO)
4Fk	1642, 1499, 1240, 1042, 804	1.27 (3H, t, J = 7.5), 2.89 (2H, q, J = 7.5, J = 0.9), 3.72 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 7.11 (1H, d, ar, J = 8), 7.13 (1H, d, ar, J =3), 7.42 (1H, dd, ar, J = 9, J = 3.3), 8.19 (1H, t, th, J = 0.9), 9.61 (1H, s, CHO)
5Ac	1698, 1507, 1172,	2.24 (6H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 5.56 (1H, s, py), 7.43 (1H,

	1017	d, J = 3), 7.46 (1H, d, J = 3), 9.15 (1H, s, NH, ex D_2O)
5Ae	1682, 1504, 1223, 1112	1.92 (6H, s, CH_3), 3.41 (6H, s, $COOCH_3$), 5.17 (1H, s, py), 7.25 (1H, d, th, J = 4.5), 7.28 (1H, d, ar, J = 7.2), 7.59 (1H, t, ar, J = 7.2), 7.67 (1H, t, ar, J = 6.3), 7.75 (1H, d, ar, J = 6.6), 7.86 (1H, d, th, J = 4.8), 8.20 (1H, s, NH, ex D_2O)
5Af	1635, 1311, 1214, 717	2.20 (6H, s, CH_3), 3.19 (6H, s, $COOCH_3$), 5,71 (1H, s, py), 7.30 (2H, dd, ar + th, J = 6, J = 9), 7.66 (2H, d, ar + th, J = 6), 8.20-8.12 (2H, m, ar), 9.07 (1H, s, NH, ex D_2O)
5Ag	1671, 1250, 1206, 1015	1.96 (6H, s, CH_3), 3.39 (6H, s, $COOCH_3$), 5.18 (1H, s, py), 7.23 (1H, d, th, J = 6.6), 7.38 (4H, m, ar), 7.79 (1H, d, th, J = 6.6), 8.28 (1H, s, NH, ex D_2O)
5Ah	1690, 1258, 1216, 1097	2.21 (6H, s, CH_3), 3.18 (6H, s, $COOCH_3$), 5.71 (1H, s, py), 7.23 (1H, d, th, J = 6.6), 7.32 (2H, m, ar + th), 7.56 (1H, t, ar, J = 12), 7.91 (2H, m, ar), 9.13 (1H, s, NH, ex D_2O)
5Ai	1702, 1663, 1206, 1116	1.92 (6H, s, CH_3), 3.42 (6H, s, $COOCH_3$), 3,56 (3H, s, OCH_3), 5.11 (1H, s, py), 6.97-6.86 (3H, m, ar), 7.16 (1H, d, th, J = 6), 7.31 (1H, dt, ar, J = 21, J = 9) 7.77 (1H, d, th, J = 6), 8.12 (1H, s, NH, ex D_2O)
5Aj	1701, 1376, 1208, 1106	2.18 (6H, s, CH_3), 3.21 (6H, s, $COOCH_3$), 3.79 (3H, s, OCH_3), 5.69 (1H, s, py), 6.88 (1H, m, ar), 7.43-7.27 (5H, m, 3ar + 2th), 8.98 (1H, s, NH, ex D_2O)
5Ak	1672, 1328, 1328, 1122	1.94 (6H, s, CH_3), 3.42 (6H, s, $COOCH_3$), 3.51 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 5.12 (1H, s, py), 6.55 (1H, t, ar, J = 2), 6.87 (1H, d, ar, J = 2), 7.17 (1H, d, th, J = 4), 7.77 (1H, d, th, J = 4), 8.22 (1H, s, NH, ex D_2O)
5Al	1690, 1598, 1219, 1147	2.19 (6H, s, CH_3), 3.23 (6H, s, $COOCH_3$), 3.77 (6H, s, OCH_3), 5.70 (1H, s, py), 6.46 (1H, t, ar, J = 3), 7.03 (1H, d, ar, J = 3), 7.23 (1H, d, th, J = 3), 7.29 (1H, d, th, J = 3), 9.03 (1H, s, NH, ex D_2O)
5Am	1690, 1219, 1111, 1024	2.18 (6H, s, CH_3), 3.25 (6H, s, $COOCH_3$), 3.79 (6H, s, OCH_3), 5.68 (1H, s, py), 6.98 (1H, d, th, J = 5.2), 7.25 (2H, s, ar), 7.36 (1H, d, th, J = 5.8), 7.42 (1H, s, ar), 8.96 (1H, s, NH, ex D_2O)
5An	1675, 1613, 1214, 1116	1.95 (6H, s, CH_3), 3.42 (6H, s, $COOCH_3$), 3.53 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 5.08 (1H, s, py), 6.47 (2H, dd, ar, J = 9.6, J = 1.5), 6.83 (1H, d, ar, J = 7.8), 7.16 (1H, d, th, J = 4.5), 7.77 (1H, d, th, J = 4.8), 8.22 (1H, s, NH, ex D_2O)
5Ao	1671, 1250, 1206, 1015	2.18 (6H, s, CH_3), 3.24 (6H, s, $COOCH_3$), 3.70 (3H, s, OCH_3), 3.81 (6H, s, OCH_3), 5.73 (1H, s, py), 7.18 (2H, s, ar), 7.23 (1H, d, th, J = 4.8), 7.27 (1H, d, th, J = 4.4), 8.99 (1H, s, NH, ex D_2O)
5Ap	1710, 1680, 1209, 1116	2.08 (6H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 3.60 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.19 (1H, s, py), 7.31 (1H, d, ar, J = 6), 7.47 (1H, d,

		ar, J = 6), 7.57 (1H, d, th, J = 3), 8.34 (1H, d, th, J = 3), 8.39 (1H, s, NH, ex D_2O)
5Aq	1683, 1511, 1277, 1222	1.95 (6H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 3.60 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 5.16 (1H, s, py), 7.04 (1H, s, ar), 7.23 (1H, d, th, J = 6), 7.48 (1H, s, ar), 7.82 (1H, d, th, J = 6), 8.44 (1H, s, NH, ex D_2O)
5Ar	1686, 1647, 1216, 1019	1.97 (6H, s, CH_3), 3.39 (6H, s, $COOCH_3$), 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 5.25 (1H, s, py), 6.97 (1H, s, ar), 7.23 (1H, d, th, J = 6.6), 7.63 (1H, s, ar), 7.72 (1H, d, th, J = 6.6), 8.47 (1H, s, NH, ex D_2O)
5Ba	1702, 1622, 1273, 1098	2.04 (3H, s, H-CH_3), 2.25 (6H, s, CH_3), 3.47 (6H, s, $COOCH_3$), 5.14 (1H, s, py), 7.81 (1H, s, tz), 9.07 (1H, s, NH, ex D_2O)
5Bb	1705, 1608, 1209, 712	2.24 (6H, s, CH_3), 3.45 (6H, s, $COOCH_3$), 5.17 (1H, s, py), 8.26 (1H, s, th), 9.12 (1H, s, NH, ex D_2O)
5Bd	1692, 1275, 1195, 1096	2.14 (6H, s, CH_3), 3.24 (6H, s, $COOCH_3$), 5.57 (1H, s, py), 7.36 (3H, m, ar), 7.59 (1H, s, th), 7.88 (2H, d, ar, J = 6.6), 8.96 (1H, s, NH, ex D_2O)
5Bk	1700, 1660, 1224, 1111	1.81 (6H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 3.49 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 5.02 (1H, s, py), 6.48 (1H, s, ar), 6.87 (2H, s, ar), 8.17 (1H, s, th), 8.22 (1H, s, NH, ex D_2O)
5Bp	1664, 1225, 1127, 1031	1.94 (6H, s, CH_3), 3.47 (6H, s, $COOCH_3$), 3.54 (3H, s, OCH_3), 5.06 (1H, s, py), 7.14 (1H, d, ar, J = 8), 7.30 (1H, d, ar, J = 8), 8.20 (1H, s, th), 8.34 (1H, s, NH, ex D_2O)
5Bq	1695, 1214, 1024, 722	1.93 (6H, s, CH_3), 3.47 (6H, s, $COOCH_3$), 3.59 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 5.10 (1H, s, py), 6.98 (1H, s, ar), 7.48 (1H, s, ar), 8.25 (1H, s, th), 8.38 (1H, s, NH, ex D_2O)
5Ca	1699, 1648, 1205, 1093	2.05 (3H, s, CH_3), 2.25 (6H, s, CH_3), 2.38 (3H, s, CH_3), 3.48 (6H, s, $COOCH_3$), 5.14 (1H, s, py), 9.01 (1H, s, NH, ex D_2O)
5Cb	1701, 1669, 1207, 1119	2.24 (6H, s, CH_3), 2.43 (3H, s, CH_3), 3.46 (6H, s, $COOCH_3$), 5.16 (1H, s, py), 7.51 (1H, s, th), 9.05 (1H, s, NH, ex D_2O)
5Cc	1702, 1654, 1299, 1149	2.25 (6H, s, CH_3), 2.43 (3H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 5.50 (1H, s, py), 7.21 (1H, s, th), 9.10 (1H, s, NH, ex D_2O)
5Ck	1680, 1378, 1285, 1096	1.93 (6H, s, CH_3), 2.44 (3H, d, th-CH_3, J = 3), 3.44 (6H, s, $COOCH_3$), 3.50 (3H, s, OCH_3), 3.70 (3H, s, O CH_3), 6.52 (1H, t, ar, J = 3), 6.85 (2H, d, ar, J = 3), 7.54 (1H, d, th, J = 3), 8.17 (1H, s, NH, ex D_2O)
5Cp	1675, 1306, 1265, 717	1.94 (6H, s, CH_3), 2.44 (3H, s, CH_3), 3.48 (6H, s, $COOCH_3$), 3.54 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 5.06 (1H, s, py), 7.09 (1H, s, ar, J = 15), 7.25 (1H, d, ar, J = 15), 7.69 (1H, s, th), 8.18 (1H, s, NH, ex D_2O)
5Cq	1680, 1334, 1209,	1.94 (6H, s, CH_3), 2.45 (3H, s, CH_3), 3.46 (6H, s, $COOCH_3$), 5.10

	1023	(1H, s, py), 7.03 (1H, s, ar) 7.47 (1H, s, ar), 7.59 (1H, d, th, J = 1.2), 8.41 (1H, s, NH, ex D_2O)
5Db	1704, 1672, 1330, 1212	2.21 (6H, s, CH_3), 2.78 (3H, s, th-CH_3), 3.43 (6H, s, $COOCH_3$), 5.33 (1H, s, py), 6.85 (1H, s, th), 8.98 (1H, s, NH, ex D_2O)
5Dd	1700, 1675, 1209, 1009	1.85 (6H, s, CH_3), 2.80 (3H, s, th-CH_3), 3.44 (6H, s, $COOCH_3$), 5.33 (1H, s, py), 6.77 (1H, s, th), 7.04 (2H, m, ar), 7.31 (3H, m, ar), 7.92 (1H, s, NH, ex D_2O)
5Eb	1705, 1676, 1328, 1204	2.21 (6H, s, CH_3), 2.33 (3H, s, th-CH_3), 2.72 (3H, s, th-CH_3), 3.44 (6H, s, $COOCH_3$), 5.35 (1H, s, py), 8.97 (1H, s, NH, ex D_2O)
5Ed	1672, 1507, 1206, 1116	1.85 (6H, s, CH_3), 2.35 (3H, s, th-CH_3), 2.73 (3H, s, th-CH_3), 3.44 (6H, s, $COOCH_3$), 5.34 (1H, s, py), 7.00 (2H, d, ar, J = 12), 7.29 (3H, m, ar), 7.86 (1H, s, NH, ex D_2O)
5Fk	1700, 1649, 1203, 1116	1.28 (3H, t, J = 9), 1.94 (6H, s, CH_3), 2.82 (2H, q, J = 9), 3.43 (6H, s, $COOCH_3$), 3.50 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 5.07 (1H, s, py), 6.53 (1H, d, ar, J = 3), 6.86 (2H, d, ar, J = 3), 7.56 (1H, s, th), 8.23 (1H, s, NH, ex D_2O)
5Gp	1542, 1268, 1060, 794	0.96 (3H, t, CH_3, J = 10.9), 1.65 (2H, q, CH_2, J = 10.8), 1.92 (6H, s, CH_3), 2.77 (2H, t, CH_2, J = 10.8), 3.45 (6H, s, $COOCH_3$), 3.54 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 5.07 (1H, s, py), 7.10 (1H, d, ar, J = 13.8), 7.26 (1H, d, ar, J = 14.4), 7.70 (1H, s, th), 8.19 (1H, s, NH, ex D_2O)

[a] In all the dihydropyridines the NH groups give broad bands in the range 3400-3100 cm^{-1}.

[b] Abbreviations: th = thiazole, im = imidazole, ar =aromatic, py = pyridine, ex = H linked to N which exchanged with D_2O.

Table S3. **Analytical Data.**

Comp	Calcd C (found)	Calcd H (found)	Calcd N (found)
3Ae	53.73 (53.72)	2.63 (2.62)	10.44 (10.42)
3Ag	50.70 (50.71)	2.48 (2.47)	9.85 (9.84)
3Ah	50.70 (50.67)	2.48 (2.49)	9.85 (9.81)
3Al	59.98 (59.95)	4.65 (4.63)	10.76 (10.75)
3Ao	57.91 (57.90)	4.86 (4.85)	9.65 (9.66)
3Fk	62.48 (62.45)	5.59 (5.58)	9.71 (9.70)
3Ao	57.91 (50.92)	4.86 (4.84)	9.65 (9.68)
4Ac	41.18 (41.17)	1.48 (1.48)	13.72 (13.71)
4Ae	52.70 (52.67)	2.38 (2.37)	9.45 (9.44)
4Ag	50.00 (49.99)	2.26 (2.27)	8.97 (8.98)
4Ah	50.00 (50.01)	2.26 (2.25)	8.97 (8.98)
4Al	58.32 (58.29)	4.19 (4.20)	9.72 (9.71)
4An	58.32 (58.30)	4.19 (4.18)	9.72 (9.73)
4Ao	56.59 (56.61)	4.43 (4.44)	8.80 (8.81)
4Fk	60.74 (60.72)	5.10 (5.11)	8.85 (8.84)
5Ac	50.52 (50.54)	3.77 (3.77)	9.83 (9.83)
5Ae	56.21 (56.23)	4.10 (4.10)	8.55 (8.55)
5Af	56.21 (56.23)	4.10 (4.09)	8.55 (8.54)
5Ag	54.43 (54.45)	3.97 (3.96)	8.28 (8.28)
5Ah	54.43 (54.41)	3.97 (3.97)	8.28 (8.27)
5Ai	60.91 (60.89)	5.11 (5.10)	9.26 (9.26)
5Aj	60.91 (60.90)	5.11 (5.10)	9.26 (9.25)
5Ak	59.61 (59.62)	5.21 (5.20)	8.69 (8.69)

5Al	59.61 (59.60)	5.21 (5.22)	8.69 (8.69)
5Am	59.61 (59.58)	5.21 (5.20)	8.69 (8.68)
5An	59.61 (59.63)	5.21 (5.21)	8.69 (8.69)
5Ao	58.47 (58.49)	5.30 (5.29)	8.18 (8.18)
5Ap	54.54 (54.52)	4.58 (4.57)	10.60 (10.59)
5Aq	54.54 (54.51)	4.58 (4.57)	10.60 (10.60)
5Ar	54.54 (54.56)	4.58 (4.58)	10.60 (10.61)
5Ba	51.58 (51.56)	4.58 (4.57)	10.61 (10.59)
5Bb	46.16 (46.18)	3.63 (3.62)	10.09 (10.10)
5Bd	57.57 (57.59)	4.61 (4.60)	9.16 (9.17)
5Bk	55.65 (55.63)	4.67 (4.67)	8.11 (8.10)
5Bp	51.20 (51.21)	4.12 (4.11)	9.95 (9.95)
5Bq	51.20 (51.18)	4.12 (4.10)	9.95 (9.94)
5Ca	57.58 (57.60)	5.64 (5.62)	11.19 (11.18)
5Cb	51.58 (51.60)	4.58 (4.57)	10.61 (10.62)
5Cc	50.35 (50.33)	4.22 (4.22)	9.78 (9.78)
5Ck	60.35 (60.36)	5.47 (5.46)	8.44 (8.45)
5Cp	55.34 (55.36)	4.83 (4.82)	10.33 (10.32)
5Cq	55.34 (55.32)	4.83 (4.83)	10.33 (10.33)
5Db	51.58 (51.59)	4.58 (4.56)	10.61 (10.61)
5Dd	63.14 (63.16)	5.30 (5.31)	9.60 (9.59)
5Eb	52.74 (52.73)	4.92 (4.92)	10.25 (10.23)
5Ed	62.85 (62.82)	5.73 (5.72)	9.56 (9.56)
5Fk	61.04 (61.06)	5.71 (5.70)	8.21 (8.20)
5Gp	56.83 (56.81)	5.30 (5.29)	9.82 (9.81)

Details for functional assays.

1. Guinea-Pig Atrial Preparations. Guinea-pigs (300-400g female) were sacrificed by cervical dislocation. After thoracotomy the heart was immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution of the following composition (mM): 136.9 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $NaH_2PO_4xH_2O$, 11.9 $NaHCO_3$ and 5.5 glucose. The physiological salt solution (PSS) was buffered at pH 7.4 by saturation with 95% O_2-5% CO_2 gas, and the temperature was maintained at 35 °C. The following isolated guinea-pig heart preparations were used: spontaneously beating right atria and left atria driven at 1 Hz. For each preparation, the entire left and right atria were dissected from the ventricles, cleaned of excess tissue, hung vertically in a 15 mL organ bath containing the PSS continuously bubbled with 95% O_2-5% CO_2 gas at 35 °C, pH 7.4. The contractile activity was recorded isometrically by means of force transducer (FT 0.3, Grass Instruments Corporation, Quincy, MA, USA) using Power Lab® software (AD-Instruments Pty Ltd, Castle Hill, Australia). The left atria were stimulated by rectangular pulses of 0.6-0.8 ms duration and about 50% threshold voltage through two platinum contact electrodes in the lower holding clamp (Grass S88 Stimulator). The right atria were in spontaneous activity. After the tissues were beating for several min, a length-tension curve was determined, and the muscle length was maintained at that which elicited 90% of maximum contractile force observed at the optimal length. A stabilization period of 45-60 min was allowed before the atria were challenged by various agents. During the equilibration period, the bathing solution was changed every 15 min and the threshold voltage was ascertained for the left atria. Atrial muscle preparations were used to examine the inotropic and chronotropic activity of the compounds (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 μM), first dissolved in DMSO and then diluted with PSS. According to this procedure, the concentration of DMSO in the bath solution never exceeded 0.3 %, a concentration which did not produce appreciable inotropic and chronotropic effects. During the generation of cumulative concentration-response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state. All data are presented as mean ± S.E.M.. The EC_{50}, EC_{30} and IC_{50} were calculated from log concentration-response curves.[RS18]

2. Guinea-Pig Aortic Strips and Ileum Longitudinal Smooth Muscle (GPLSM).[RS19] The thoracic aorta and ileum were removed and placed in Tyrode solution of the following composition (mM): 118 NaCl, 4.75 KCl, 2.54 $CaCl_2$, 1.20 $MgSO_4$, 1.19 KH_2PO_4, 25 $NaHCO_3$ and 11 glucose equilibrated with 95% O_2-5% CO_2 gas at pH 7.4. The vessel was cleaned of extraneous connective tissue. Two helicoidal strips (10 mm x 1 mm) were cut from each aorta beginning from the end most proximal to the heart. Vascular strips were then tied with surgical thread (6-0) and suspended

in a jacketed tissue bath (15 mL) containing aerated PSS at 35 °C. Aortic strips were secured at one end to plexiglass hooks and connected via the surgical thread to a force displacement transducer (FT 0.3, Grass Instruments Corporation) for monitoring changes in isometric contraction. Aortic strips were subjected to a resting force of 1 g. The intestine was removed above the ileo-caecal junction. GPILSM segments of 2 cm length were mounted under a resting tension of 300-400 mg. Strips were secured at one end to a force displacement transducer (FT 0.3, Grass Instruments Corporation) for monitoring changes in isometric contraction and washed every 20 min with fresh PSS for 1 h. After the equilibration period, guinea-pig aortic strips were contracted by washing in PSS containing 80 mM KCl (equimolar substitution of K^+ for Na^+). When the contraction reached a plateau (about 45 min) various concentrations of the compounds (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 50 μM) were added cumulatively to the bath allowing for any relaxation to obtain an equilibrated level of force. Addition of the drug vehicle had no appreciable effect on K^+-induced contraction (DMSO for all compounds). All data are presented as mean ± S.E.M.. The IC_{50} were calculated from log concentration-response curves.[RS18]

3. Guinea-pig Isolated Perfused Heart According to Langendorff. Female guinea-pigs (300-400 g), were sacrificed by cervical dislocation. The heart was quickly removed, and rapidly perfused through the aorta at constant flow (11-12 mL x min^{-1} x g^{-1}) with a modified Krebs-Henselait solution with the following composition (mM): 128 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 15 $NaHCO_3$, 1.2 KH_2PO_4, 11.1 glucose and 2 Na-pyruvate, bubbled with a gas mixture (95% O_2 - 5% CO_2) (pH = 7.4 - 7.5) and maintained at 37 °C. A perfusion pressure of 50-60 mmHg was obtained at this flow rate. The addition of Na-pyruvate to the medium has been shown to confer to the isolated heart the same metabolic and functional features of the heart *in situ*.[RS20] A stabilization period of 30 min was given to the heart, under normal electrocardiogram (ECG) conditions, to keep the frequency of spontaneous beating hearts constant at 210 ± 3 beats x min^{-1}. Surface ECG was recorded by means of two electrodes, placed one near the initial portion of the anterior intraventricular artery and the other on the left ventricular free wall. The main ECG intervals (PR = atrio-ventricular (A-V) conduction time; QRS = intra-ventricular conduction time; JT = duration of ventricular depolarization) were measured. The drug-induced changes in conduction velocity of AV node and ventricular myocardium were calculated as changes in the reciprocal of the PR interval and QRS interval, respectively.[RS21] Heart contractility was measured, by means of intraventricular latex balloon, as the positive peak of the first derivative of the left ventricular pressure as a function of time [$+(dP/dt)_{max}$]. Coronary perfusion pressure (CPP) (mmHg) was measured to assess the change in coronary vessel resistance. The compounds were added at increasing concentrations (0.001, 0.01, 0.1, and 1 μM). During the building of concentration-response curves, the next higher

concentration of the compounds was added only after the preparation reached a steady state (about 30 min). All data are presented as mean ± SEM.[RS22] The potency of drugs, expressed as EC_{50} value was evaluated from log concentration-response curves (n = 6-8),[RS22] in the appropriate pharmacological preparations.

Details for Receptor Binding Studies.

Membranes were prepared from guinea pig ventricular myocardium according to Glossmann and Ferry.[RS23] Ventricles were quickly excised from Hartley guinea pigs and all steps of tissue preparation were performed at 4°C. The tissue was homogenized with a glass-Teflon homogenizer, followed by a Polytron (settings 7–8, 15 s×2) in 3 volumes ice-cold buffer solution A containing 20 mM $NaHCO_3$ and 0.1nM phenylmethylsulfonyl fluoride, pH 7.4. After dilution 1:7 in the same buffer, the homogenate was centrifugated at 1500×*g* for 15 min; the supernatant was collected and centrifuged at 45,000×*g* for 15 min. The pellet was resuspended in ice-cold buffer A, centrifuged and washed with the same buffer again. The final pellet was resuspended in 3 volumes of buffer B (Tris–HCl, 50 mM; $CaCl_2$ 1mM; pH 7.4 at 4 °C) and stored at −70 °C. Protein concentration was measured according to the method of Lowry et al.[RS24] with bovine serum albumin as a standard. Binding experiments were performed under conditions of near-darkness. Saturation binding experiments were carried out at concentrations ranging from 0.05 to 5 nM of PN 200-110, (+)-[5-methyl-^{3}H]. Specific binding was determined in the presence of 5 μM nifedipine. Ventricular membranes were incubated at 25 °C for 90 minutes in a final volume of 1mL and incubation was stopped by vacuum filtration over Whatman GF/C filters. Competition binding experiments were performed using 0.5 nM PN200-110, (+)-[5-methyl-^{3}H].

HEK-293 cells were transfected with the Cav1.2a coding plasmid (pcDNA3HK1) or the Cav1.2b coding plasmid (pcDNA3LK1) by using EXGEN 500 (Fermentas) according to manufacturer's instructions; transfected cells were grown in MEM supplemented with 10% FBS and 72h after transfection stable transfectants were selected by adding 400 μg/ml G418 to the growth medium for two weeks (medium was changed every 3rd day) .[RS25]

Experiments were carried out according to Morel et al.:[RS26] briefly, HEK-293 Cav1.2a and HEK293 Cav1.2b were plated into 24-wells microplates for displacement binding assay; cells were incubated at 37 °C for 90 min in MEM, with 5mM KCl and without serum, in the presence of PN200-110, (+)-[5-methyl-^{3}H] (0.1 nM) and various concentration of unlabeled competitors (**Nifedipine**, **5Ac** , **5Al**, **5Cq**). Non-specific binding was determined in the presence of nifedipine 1μM and was subtracted from the total to obtain the specific binding. Reaction was stopped by washing the cells with a solution containing NaCl 0.9 %, BSA 1 %, DMSO 5 %. Cells were solubilized in SDS 0.2 % and radioactivity was counted by scintillation.

Drugs were prepared as stock solutions (10^{-2}M) in DMSO and protected from light. Radioactivity retained on the filters was measured in a Beckman LS 6500 counter and the data were analysed using GraphPad Prism.[RS22] All experiments were performed in triplicate and results are presented as mean ± S.E.

Details for Computational Methods.

Docking Simulations. The AutoDock4 software package,[RS27] as implemented through the graphical user interface called AutoDockTools (ADT), was used to dock **Al$_{21}$**, **Al$_{36}$** and **Al$_{39}$** into the three-dimensional structure of the central pore region of the human LTCC α_{1c} subunit ($Ca_v1.2$).[RS28] Unfortunately, the α_{1c} subunit of guinea pig used for biological evaluation has not been sequenced yet. Nevertheless, it is feasible that the human and guinea pig α_{1c} subunits share a high sequence homology, especially in the DHP binding site. This supports the use of the human α_{1c} subunit model for docking calculations.

The core structures of all ligands were first retrieved from the Cambridge Structural Database (CSD)[RS29] and modified using standard bond lengths and bond angles of the SYBYL fragment library.[RS30] Geometry optimizations were realized with the SYBYL/MAXIMIN2 minimizer by applying the BFGS (Broyden, Fletcher, Goldfarb, and Shannon) algorithm [RS31] and setting a rmsd gradient of the forces acting on each atom of 0.05 kcal/mol Å as the convergence criterion. Then the constructed compounds and the modelled structure of the inner pore of LTCC[RS28] were converted to AutoDock format files using ADT generating automatically all other atom values. The docking area was assigned visually around the active site which was detected in a previous work.[1] A grid of 60 Å × 60 Å × 60 Å with 0.375 Å spacing was calculated around the docking area for the ligand atom types using AutoGrid4. These atom types were sufficient to describe all atoms in the selected ligands. For each ligand, 100 separate docking calculations were performed. Each docking calculation consisted of 15 million energy evaluations using the Lamarckian genetic algorithm local search (GALS) method. The GALS method evaluates a population of possible docking solutions and propagates the most successful individuals from each generation into the subsequent generation of possible solutions. A low-frequency local search according to the method of Solis and Wets is applied to docking trials to ensure that the final solution represents a local minimum. All dockings described in this paper were performed with a population size of 200, and 300 rounds of Solis and Wets local search were applied with a probability of 0.06. A mutation rate of 0.02 and a crossover rate of 0.8 were used to generate new docking trials for subsequent generations, and the best individual from each generation was propagated to the next generation. The docking results from each of the 100 calculations were clustered on the basis of root-mean square deviation (rmsd) between the Cartesian coordinates of the atoms and were ranked on the basis of free energy of binding. The top-ranked compounds were visually inspected for good chemical geometry.

Energy Refinement of DHPs/Channel complexes. Refinement of the predicted DHP/channel complexes was achieved through energy minimizations using the Discover3 module of InsightII.[RS32] Force-field parameters for both ligands and protein residues were taken from the

CVFF force field.[RS33] These geometric optimizations included 5000 steps of a steepest descent minimization reaching a convergence of 10.0 kcal mol-1Å-1, followed by 3000 steps of conjugate gradient minimization reaching a final convergence of 0.01 kcal mol-1 Å-1, keeping the backbone atoms fixed and protein side-chains and the ligand free to move.

One of the major advances incorporated into the new force field implemented in AD4 is the inclusion of intramolecular energies of the ligand and the protein in the estimated free energy of binding. The current method incorporates the intramolecular energy (Internal Energies) of the ligand and of the protein through the use of an unbound structure, so that the difference in energy between the bound and unbound forms is included in the predicted free energy (Estimated Free Energy of Binding). Such a new feature permits to calculated the unbound conformation of the ligand together with its associated intramolecular energy. The energy components calculated for the three inspected ligands are reported in table below.

Ligand	**Ligand Unbound Internal Energy**	**Ligand Bound Internal Energy**	**Estimated Free Energy of Binding**
5Ag	-2.04	-1.36	-7.42
5Cb	-0.92	-0.99	-6.64
5Cq	-2.23	-1.03	-7.43

When looking at the calculated unbound conformations for the inspected ligands in both **5Ag** and **5Cq** the phenyl ring in R_2 parallel to the 1,4-dihydropyridine ring, this might be explained by the presence in such a conformation of the interaction between the π-electron clouds of the phenyl and DHP. Further support to this hypothesis is given by the fact that in **5Cb** no difference are found between the bound and unbound conformations due to the absence of the R_2 aryl ring. On the other hand, the calculated binding conformations for **5Ag** and **5Cq** diverges from the unbound ones, in fact the R_2 phenyl ring is distant from the DHP ring forming alternative interactions with LCC. However, these findings are in accordance with what already postulated by Natale et al. for similar DHPs.[RS34]

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