Structure-Activity Relationships of Polycyclic Aromatic Amines with Calcium Channel Blocking Activity

Sarel F. Malan[a)]*, J. Jurgens Van der Walt[b)], and Cornelius J. Van der Schyf[a,c)]

[a)] Pharmaceutical Chemistry, Potchefstroom University for Christian Higher Education, Private Bag X6001, Potchefstroom, 2520, South Africa

[b)] Department of Physiology, Potchefstroom University for Christian Higher Education, Private Bag X6001, Potchefstroom, 2520, South Africa

[c)] The Harvey W. Peters Center, Departments of Chemistry and of Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212, USA

***Key Words**: Polycyclic aromatic amines; calcium antagonism; structure-activity*

Summary

8-Benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**1**) inhibits the calcium current in L-type calcium channels. A series of nitrobenzylamines (**2**, **3**, **4**), methoxybenzylamines (**5**, **6**, **7**), methylpyridines (**8**, **9**, **10**), and a phenylhydrazine derivative (**11**) of 8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane was synthesized. By substituting the 8,11-oxapentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane skeleton with 3-hydroxyhexacyclo-[6.5.0.0.^{3,7}.0^{4,12}.0^{5,10}.0^{9,13}]tridecane (**12**), 8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecane-9-one (**13**), and pentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**14**), the effect of the polycyclic skeleton could also be investigated. Increased inhibition of calcium current was observed with aromatic substitution (especially *ortho* and *meta* substitution) in the pentacycloundecane series. The calcium channel activities of the methoxy compounds were slightly higher than those of the corresponding nitro compounds while a definite decrease in activity was observed for the phenylhydrazine and aminomethylpyridine derivatives. Increased inhibition of the calcium current was also observed for structures in which the polycyclic 'cages' were enlarged. Structure-activity relationships in this series of compounds therefore appear to be dominated by geometric or steric constraints.

Introduction

Ion channels are a critical component of cellular function and serve to translocate ions across cell membranes in response to a variety of chemical and physical stimuli. Ion channels are therefore a major class of cellular effectors through which information received at receptors is transduced into response [1]. Channels sensitive to membrane potential or voltage-gated ion channels are of particular interest as they are critical to the function of many excitable systems including axonal conduction, neuronal firing, and excitation-contraction coupling [1–4]. The chemical specificity of these ion channels suggests that they may be considered as pharmacological receptors. Therefore, all the properties associated with conventional receptors have been found among the voltage-gated Na^+, K^+, and Ca^{2+} channels [1]. The clinical application especially of the voltage-gated calcium channels has been studied in great depth, particularly following the impact of Ca^{2+} antagonists as cardiovascular agents [5,6].

Calcium channel antagonists are a chemically heterogeneous group of compounds and mostly exhibit activity on the potential dependent L-type calcium channel [6]. 8-Benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (NGP 1-01, **1**)[7,8] (Figure 1) is structurally unrelated to any of the known calcium antagonists but was found to inhibit the calcium current in L-type calcium channels [9,10]. This finding provided a new tool for the study of drug action on ion channels.

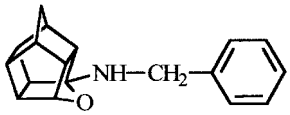

Figure 1. Structure of the lead compound NGP 1-01 (**1**).

As it was postulated that the pentacycloundecane skeleton may serve only as a bulk contributor to the activity of NGP 1-01 [9], we decided to modify the molecule in the aromatic amine side chain to study the structure-activity relationships of this group of calcium channel antagonists. A series of nitrobenzylamines (**2**, **3**, **4**), methoxybenzylamines (**5**, **6**, **7**), methylpyridines (**8**, **9**, **10**), and a phenylhydrazine (**11**) was synthesized for this purpose. By substituting the 8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane skeleton with 3-hydroxyhexacyclo[6.5.0.0.^{3,7}0^{4,12}.0^{5,10}.0^{9,13}]tridecane, 8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecane-9-one, and pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane, the effect of the polycyclic skeleton could also be investigated. These polycyclic 'cages' could be obtained by the boron trifluoride etherate promoted reaction of pentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione with ethyl diazoacetate[11] followed by subsequent decarbalkoxylation[12] (Figure 2), by Baeyer-Villiger oxidation[13] of the diketone ("Cookson's cage"; Figure 3) and by the selective decarboxylation (Huang-Minlon reaction) of the diketone (Figure 4) [14].

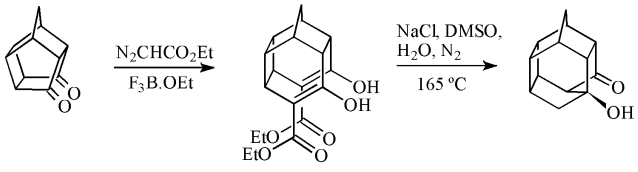

Figure 2. Synthesis of 3-hydroxyhexacyclo[6.5.0.0.^{3,7}0^{4,12}.0^{5,10}.0^{9,13}]tridecan-6-one.

  0365-6233/00/0101/0010 $17.50 +.50/0

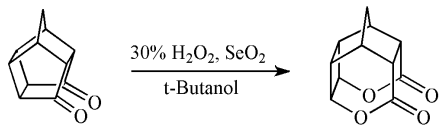

Figure 3. Synthesis of 8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecane-9,12-dione.

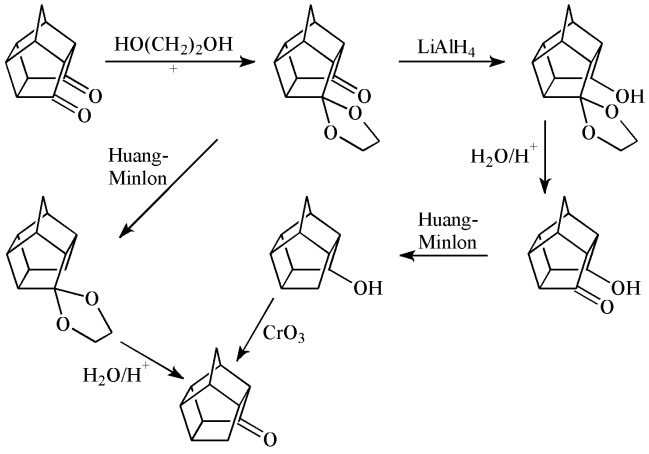

Figure 4. Selective decarboxylation of the diketone to obtain 8-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanone.

Reductive amination of these ketones with benzylamine rendered 6-benzylamino-3-hydroxyhexacyclo-[6.5.0.0^{3,7}.0^{4,12}.0^{5,10}.0^{9,13}]tridecane (**12**), 12-benzylamino-8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecan-9-one (**13**), and 8-benzylaminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**14**).

R	R'
2 – 4 (ortho, meta and para substituted)	**12**
5 – 7 (ortho, meta and para substituted)	**13**
8	**14**
9	—
10	—
11	—

Figure 5. Compounds included in the SAR study.

It is generally accepted that a certain complimentarity exists between the receptor and its ligand in terms of the induced fit theory [15]. This theory implicates a certain selectivity of the receptor macromolecule for the ligand. To ensure effective binding, the drug molecule needs to have specific geometrical, electronic and/or steric characteristics. The physicochemical and electronic properties of any compound will thus determine its biological activity. These properties therefore are indicative of the pharmacodynamic as well as pharmacokinetic properties of any drug.

In the absence of a well-defined receptor structure for the L-type calcium channel, a series of computer-based calculations (Chemplus^{TM}) for the determination of physico-chemical properties (QSAR-data) for the test compounds (Table 1) were performed. Quantum mechanical calculations to determine the minimum energy conformation of each molecule were used. A minimum energy conformation was calculated for each compound because it is assumed to be the most likely conformation for interaction with the receptor [16]. The first order valence connectivity index ($^1\chi^v$), an intermediate non-quantum mechanical calculation was also used to characterize the test compounds [17]. Analysis of these data could then give an indication of the receptor structure or of the properties necessary for drug-receptor interaction within this group of compounds.

Calcium channel antagonism was measured as the inhibition of calcium current after electric stimulation of ventricular myocytes from the hearts of Duncan-Hartley strain guinea pig. Whole cell voltage clamping was done using the chopped clamp method [18].

Results and Discussion

At a test concentration of 10 μM the test compounds exhibited inhibition of calcium current that ranged from 10–45%.

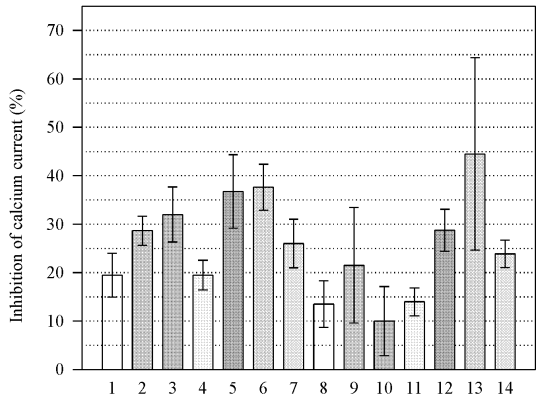

Figure 6. Inhibition of calcium current as measured with whole cell clamping.

The voltage clamping data indicate that aromatic substitution in the pentacycloundecane series results in increased calcium current inhibition (compounds **2**,**3**,**5–7**). This increase in activity is more pronounced when substitution occurs in the *ortho* and *meta* positions (**2**, **3**, **5**, **6**). The activities of *para* substituted compounds (**4**, **7**) correlated with that of the unsubstituted lead compound NGP 1-01 (**1**). The activities of the methoxy compounds (**5–7**) were slightly higher than those of the corresponding nitro compounds (**2–4**) while a definitive decrease in activity was observed when the aro-

matic benzylamine was substituted with phenylhydrazine or aminomethylpyridine (**11**, **8**–**10**).

Changes in the polycyclic structure also had a definitive effect on calcium channel blocking activity. Better inhibition of the calcium current was observed for structures in which the polycyclic 'cages' were enlarged (**12**, **13**). The calcium channel activity of compound **14**, in which the endocyclic ether bond was removed, corresponded to that of the lead compound.

The structure-activity relationships observed in this series of compounds therefore appear to be dominated by geometric or steric constraints rather than by electronic considerations. It is interesting to note that the structures that showed the lowest activity were also those with the lowest calculated energy minima (**8–11**; Figure 7) and that the side chain and aromatic ring were folded back onto the polycyclic structure in these cases. In the calculated minimum energy conformations of the remaining compounds, the side chain and aromatic ring pointed away from the polycyclic structure and better calcium channel blocking activity was observed (**3**, **4**, **6**, **7**, **12**, **13**; Figure 7).

The calculated physico-chemical and connectivity data (log P; MR; α; VA; SA; VV; SV; H; and $^{1}\chi^{v}$; Table 1) correlated well with one another and good linear regression values ($r^2 > 0.9$) between all the variables, with the exception of log *P*, were obtained.

According to the induced fit theory [15] and complementarity between the receptor and ligand a correlation should exist between the semi-empirical and theoretical data and the biological activity. Regression analysis of the calcium channel blocking activity as obtained from the whole cell clamping experiments against these calculated data was performed and a correlation was found between activity and molar refractivity (r^2 = 0.59), polarisability (r^2 = 0.65), Van der Waals surface area (r^2 = 0.7), solvent accessible surface area (r^2 = 0.69), Van der Waals surface bounded volume (r^2 = 0.68),

Compound	Minimum energy conformation	Minimum energy
NH—CH$_2$ O 1		-4198.43
O$_2$N NH—CH$_2$ O 2		-4371.19
NO$_2$ NH—CH$_2$ O 3		-4374.44
NH—CH$_2$ NO$_2$ O 4		-4375.56
CH$_3$O NH—CH$_2$ O 5		-4569.51
OCH$_3$ NH—CH$_2$ O 6		-4569.99
NH—CH$_2$ OCH$_3$ O 7		-4570.31
NH—CH$_2$ N O 8		-4077.02
NH—CH$_2$ N O 9		-4077.75
NH—CH$_2$ N O 10		-4078.52
NH—NH O 11		-4054.87
NH—CH$_2$ OH 12		-4774.16
NH—CH$_2$ O O 13		-4410.39
NH—CH$_2$ 14		-4227.34

Figure 7. Conformations and energy minima (in kcal mol^{-1}) as obtained after structure minimization with Hyperchem®.

Table 1. Physico-chemical and connectivity data of test compounds.

	Log *P*	MR	α	VA	SA	VV	SV	H	$^1\chi^v$
1	2.57	75.13	29.80	275.13	473.55	250.15	782.24	2.02	8.368
2	2.53	82.45	31.64	294.95	510.75	268.82	840.77	6.13	8.874
3	2.53	82.45	31.64	300.60	513.50	269.48	851.83	7.13	8.868
4	2.53	82.45	31.64	298.80	512.38	269.18	841.73	6.91	8.868
5	2.32	81.59	32.27	305.25	517.22	274.90	861.65	2.17	8.897
6	2.32	81.59	32.27	305.87	521.30	274.70	865.34	3.88	8.891
7	2.32	81.59	32.27	306.67	522.43	274.99	866.86	3.99	8.891
8	1.66	72.60	29.09	270.32	471.72	245.91	770.46	3.19	8.228
9	1.26	72.97	29.09	271.57	474.50	245.63	775.05	3.95	8.218
10	1.26	72.97	29.09	270.15	468.76	245.46	768.56	3.70	8.218
11	2.58	75.37	29.31	273.28	478.66	245.37	783.02	4.67	8.161
12	1.78	84.34	33.47	307.34	504.31	280.47	857.97	4.31	9.286
13	1.67	78.24	31.29	293.95	497.73	266.19	834.14	5.10	8.460
14	2.74	75.54	29.93	278.13	477.46	251.03	790.79	1.30	8.206

Where: log P = 1-octanol-water distribution coefficient [23,24]; MR (Å^3) = molar refractivity [23,25]; α (Å^3) = polarisability [26]; VA (Å^2) = Van der Waals surface area ("grid") [27,28]; SA (Å^2) = solvent accessible surface area [25,26]; VV (Å^3) = Van der Waals surface-bounded molecular volume [25,26]; SV (Å^3) = solvent accessible molecular volume [25,26]; H (kcal mol^{-1}) = hydration energy[29], and $^1\chi^v$ = valence first-order connectivity index [17,30].

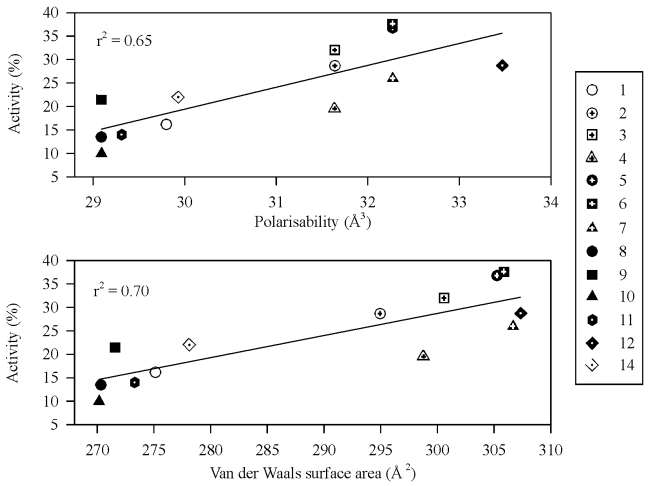

Figure 8. Correlation between calculated data and calcium channel antagonism (Activity = % inhibition of calcium current).

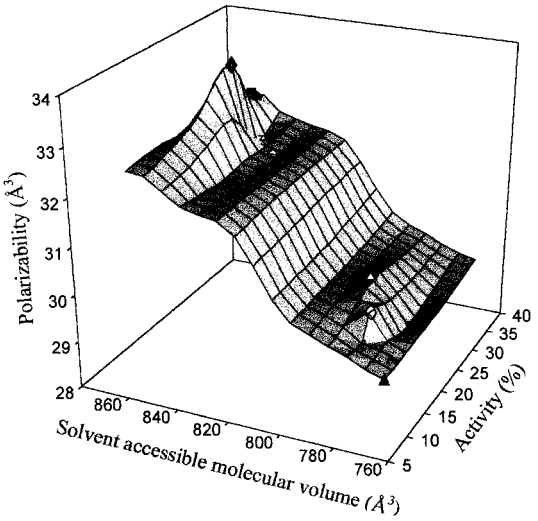

Figure 9. Correlation between calcium channel antagonism, polarisability, and solvent accessible molecular volume graphed three-dimensionally.

solvent accessible molecular volume ($r^2 = 0.73$) as well as for the valence first order connectivity index ($r^2 = 0.57$) (examples shown in Figure 8).

These examples show that an increase in calcium channel blocking activity can be expected as molecular surface area or volume is increased. This observation was also found to be true for an increase in polarisability. Definitive clusters with increased activity were observed at increased volume and/or higher polarisability. In the series of compounds tested the optimum polarisabilities and solvent accessible surface volumes appear to be in the range found for two of the methoxy compounds (compounds **5** and **6**). Further increases in these parameters resulted in diminished activity (Figure 9).

These data confirm the initial observation that the size and geometrical conformation of the test molecules (surface area and volume) are of particular importance for calcium channel blocking activity. Conformations in which the side chain and aromatic ring point away from the polycyclic structure (Figure 7) may therefore be preferred in the binding behavior of these molecules to the channel receptor. The data, however, also indicate that electronic characteristics (molar refractivity and polarisability) influence – albeit to a lesser extent – the affinity and/or activity of these compounds on calcium channel receptors and should always be accounted for.

Experimental

Synthesis

Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu FT IR 4200 spectrophotometer and the MS and high resolution MS on a VG 7070E spectrometer (EI, 70 eV). The ^{1}H and ^{13}C NMR spectra were recorded on a

Varian VXR 300 spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz). C, H and N analyses were all within acceptable limits (± 0.4%).

*Synthesis of Benzylamine (**1**), Nitrobenzylamine (**2–4**), Methoxybenzylamine (**5–7**), Aminomethylpyridine (**8–10**), and Phenylhydrazine (**11**) Derivatives of Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane*

Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (5 g, prepared according to the method of Cookson *et al.*[19,20]) was dissolved in tetrahydrofuran (THF, 50 ml) and cooled to ±5 °C in ice. An equimolar quantity of the desired amine was slowly added under stirring. The white precipitate that formed after about 10 min was filtered and washed with cold THF to render the hydroxylamine. This product was dehydrated in dry benzene under Dean-Stark conditions for 1 h or until no more water collected in the container. Evaporation yielded the Shiff base as a yellowish oil and reduction of this imine was done with sodium borohydride in dry methanol (30 ml) and dry THF (150 ml) for 24 h at room temperature. The solvent was removed under reduced pressure and water (100 ml) was added. The mixture was extracted with dichloromethane (4 × 50 ml) and the combined organic fractions washed with water before being dried over magnesium sulphate. Evaporation yielded the desired products.

*8-Benzylamino-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**1**)*

White needles obtained by recrystallisation from ethanol (yield 43.5%).– HR-MS for $C_{18}H_{19}NO$: calcd, 265.1467, found, 265.1460.– Mp 77 °C.– IR (KBr): ν_{max} = 3300 cm^{-1}, 2950, 1360, 1020.– ^{1}H NMR ($CDCl_3$): δ = 7.25–7.38 (m, 5H), 4.67 (t, 1H, *J* = 5.22 Hz), 4.0 (AB-q, 2H, *J* = 13.25 Hz), 2.5–2.85 (2 × m, 7H), 2.42 (t, 1H, *J* = 4.7 Hz), 2.20 (br. s, 1H), 1.72 (AB-q, 2H, *J* = 10.37 Hz).– ^{13}C NMR ($CDCl_3$): δ = 141.09 (s), 128.62 (d, 2C), 128.15 (d, 2C), 127.08 (d), 109.84 (s), 82.68 (d), 55.36 (d), 54.90 (d), 47.93 (t), 44.98 (d), 44.68 (d), 43.38 (t), 43.25 (d), 42.10 (d), 41.65 (d).– MS (70 eV): *m/z* = 265 [M$^+$], 237, 186, 131, 91, 65, 28.

*8-(2-Nitrobenzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**2**)*

Yellow needles obtained by recrystallisation from ethanol (yield 29.2%).– HR-MS for $C_{18}H_{18}N_2O_3$: calcd, 310.1317, found, 310.1315.– Mp 87 C.– IR (KBr): ν_{max} = 3300 cm^{-1}, 2960, 1525, 1350, 1010.– ^{1}H NMR ($CDCl_3$): δ = 7.92 (dd, 1H, J_{AB} = 8.11, J_{AC} = 1.37), 7.68 (dd, 1H, J_{CD} = 7.76, J_{BD} = 1.09), 7.57 (2dd, 1H, J_{AB} = 8.11, J_{BC} = 7.41, J_{AC} = 1.37), 7.39 (2dd, 1H, J_{AB} = 8.11, J_{BC} = 7.41, J_{AC} = 1.37), 4.64 (t, 1H, *J* = 5.22), 4.28 (AB-q, 2H, *J* = 15.24), 2.40–2.86 (3 × m, 9H), 1.73 (AB-q, 2H, *J* = 10.55).– ^{13}C NMR ($CDCl_3$): δ = 149.13 (s), 136.79 (s), 133.24 (d), 131.09 (d), 128.03 (d), 124.84 (d), 109.45 (s), 82.77 (d), 55.28 (d), 54.78 (d), 45.14 (t), 44.96 (d), 44.61 (d), 43.41 (t), 43.24 (d), 42.0 (d), 41.62 (d).– MS (70 eV): *m/z* = 310 [M$^+$], 292, 276, 231, 159, 131, 91, 78, 44, 32, 28.

*8-(3-Nitrobenzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**3**)*

Yellow needles obtained by recrystallisation from ethanol (yield 40.5%).– HR-MS for $C_{18}H_{18}N_2O_3$: calcd, 310.1317, found, 310.1314.– Mp 154 C.– IR (KBr): ν_{max} = 3320 cm^{-1}, 2970, 1525, 1350, 1010.– ^{1}H NMR ($CDCl_3$): δ = 8.28 (d, 1H, J_{AC} = 1.64), 8.10 (m, 1H), 7.69 (dd, 1H, J_{AB} = 7.83, J_{AC} = 1.64), 7.48 (t, 1H, J_{AB} = 7.83), 4.67 (t, 1H, *J* = 5.27), 4.11 (AB-q, 2H, *J* = 14.87), 2.40–2.88 (4 × m, 8H), 2.36 (br. s, 1H), 1.75 (AB-q, 2H, *J* = 10.47).– ^{13}C NMR ($CDCl_3$): δ = 148.5 (s), 143.75 (s), 134.02 (d), 129.41 (d), 122.76 (d), 122.13 (d), 109.63 (s), 82.77 (d), 55.45 (d), 54.89 (d), 47.01 (t), 45.07 (d), 44.96 (d), 43.41 (t), 43.25 (d), 42.07 (d), 41.64 (d).– MS (70 eV): *m/z* = 310 [M$^+$], 293, 231, 131, 91, 44, 32, 28.

*8-(4-Nitrobenzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**4**)*

Yellow needles obtained by recrystallisation from ethanol (yield 30.9%).– HR-MS for $C_{18}H_{18}N_2O_3$: calcd, 310.1317, found, 310.1313.– Mp 162 C.– IR (KBr): ν_{max} = 3320 cm^{-1}, 2970, 1510, 1340, 1010.– ^{1}H NMR ($CDCl_3$): δ = 8.17 (d, 2H, J_{AB} = 8.90), 7.54 (d, 2H, J_{AB} = 8.90), 4.67 (t, 1H, *J* = 5.22), 4.12 (AB-q, 2H, *J* = 15.31), 2.42–2.88 (4 × m, 8H), 2.34 (br. s, 1H), 1.73 (AB-q, 2H, *J* = 10.59).– ^{13}C NMR ($CDCl_3$): δ = 149.28 (s), 147.20 (s), 128.46 (d, 2C), 123.81 (d, 2C), 109.63 (s), 82.74 (d), 55.45 (d), 54.88 (d), 47.23 (t), 45.01 (d), 44.95 (d), 44.69 (d), 43.39 (t), 43.26 (d), 42.04 (d), 41.64 (d).– MS (70 eV): *m/z* 310 [M$^+$], 282, 231, 131, 106, 91, 32, 28.

*8-(2-Methoxybenzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (**5**)*

Obtained as light yellow oil from column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase (yield 49.5%).– HR-MS for $C_{19}H_{21}NO_2$: calcd, 295.1572, found, 295.1571.– IR (neat): ν_{max} = 3330 cm^{-1}, 2920, 1740, 1600, 1500, 1370, 1240.– ^{1}H NMR ($CDCl_3$): δ = 7.33 (dd, 1H, J_{CD} = 7.42, J_{BD} = 1.76), 7.22 (2dd, 1H, J_{AB} = 8.22, J_{BC} = 7.42 J_{BD} = 1.76), 6.92 (dt, 1H, J_{CD} = 7.42, J_{AC} = 1.07), 6.84 (dd, 1H, J_{AB} = 8.22, J_{AC} = 1.07), 4.67 (t, 1H, *J* = 5.27), 3.99 (AB-q, 2H, *J* = 13.60), 3.82 (s, 3H), 2.40–2.86 (4 × m, 9H), 1.73 (AB-q, 2H, *J* = 10.44).– ^{13}C ($CDCl_3$): δ = 157.71 (s), 129.42 (d and s, 2C), 128.24 (d), 120.70 (d), 110.40 (d), 110.07 (s), 82.74 (d), 55.34 (k), 55.18 (d), 54.91 (d), 45.11 (d), 44.99 (d), 44.65 (d), 43.38 (t), 43.25 (d and t, 2C), 42.10 (d), 41.64 (d). –MS (70 eV): *m/z* = 295 [M$^+$], 266, 216, 136, 121, 91, 77, 65, 39, 28.

*8-(3-Methoxy-benzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (**6**)*

Obtained as light yellow oil from column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase (yield 44.8%).– HR-MS for $C_{19}H_{21}NO_2$: calcd, 295.1572, found, 295.1576.– IR (neat): ν_{max} = 3340 cm^{-1}, 2920, 1700, 1650, 1495, 1387.– ^{1}H NMR ($CDCl_3$): δ = 7.23 (t, 1H, J_{AB} = 8.10), 6.94 (m, 2H), 6.77 (m, 1H), 4.67 (t, 1H, *J* = 5.23), 3.98 (AB-q, 2H, *J* = 13.46), 3.80 (s, 3H), 2.41–2.86 (3 × m, 8H), 2.19 (br. s, 1H), 1.70 (AB-q, 2H, *J* = 10.44).– ^{13}C NMR ($CDCl_3$): δ = 160.01 (s), 142.82 (s), 129.60 (d), 120.37 (d), 113.52 (d), 112.57 (d), 109.89 (s), 82.67 (d), 55.37 (d), 55.28 (k), 54.89 (d), 47.85 (t), 44.97 (d, 2C), 44.67 (d), 43.37 (t), 43.26 (d), 42.11 (d), 41.64 (d).– MS (70 eV): *m/z* = 295 [M$^+$], 267, 136, 121, 91, 77, 28.

*8-(4-Methoxybenzylamino)-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}-]undecane (**7**)*

Obtained as light yellow oil from column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase (yield 35.3%).– HR-MS for $C_{19}H_{21}NO_2$: calcd, 295.1572, found, 295.1570.– IR (neat): ν_{max} = 3330 cm^{-1}, 2970, 1700, 1616, 1517, 1460, 1355, 1242.– ^{1}H NMR ($CDCl_3$): δ = 7.28 (dd, 2H, J_{AB} = 6.62, J_{AC} = 2.16), 6.85 (dd, 2H, J_{AB} = 6.62, J_{AC} = 2.16), 4.67 (t, 1H, *J* = 5.22), 3.93 (AB-q, 2H, *J* = 12.92), 3.79 (s, 3H), 2.41–2.85 (3 × m, 8H), 2.08 (br. s, 1H), 1.72 (AB-q, 2H, *J* = 10.50).– ^{13}C NMR ($CDCl_3$): δ = 158.87 (s), 133.13 (s), 129.38 (d), 113.99 (d), 109.82 (s), 82.66 (d), 55.36 (d), 55.34 (k), 54.90 (d), 47.36 (t), 44.96 (d, 2C), 44.67 (d), 43.37 (t), 43.24 (d), 42.10 (d), 41.64 (d).– MS (70 eV): *m/z* 295 (M$^+$), 267, 136, 121, 91, 77, 28.

*8-[(2-Aminomethyl)pyridine]-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (**8**)*

Obtained as light yellow oil after column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase (yield 31.8%).– HR-MS for $C_{17}H_{18}N_2O$: calcd, 266.1419, found 266.1410.– IR (neat): ν_{max} = 3330 cm^{-1}, 2960, 1725, 1655, 1590, 1470, 1435, 1370.– ^{1}H NMR ($CDCl_3$): δ = 8.55 (d, 1H, J_{AB} = 5.43), 7.63 (m, 1H), 7.32 (d, 1H, J_{CD} = 7.91), 7.14 (t, 1H, J_{AB} = 5.43), 4.66 (t, 1H, *J* = 5.22), 4.15 (AB-q, 2H, *J* = 14.86), 2.41–2.87 (3 × m, 9H), 1.72 (AB-q, 2H, *J* = 10.48).– ^{13}C NMR ($CDCl_3$): δ = 160.17 (s), 149.39 (d), 136.63 (d), 122.16 (d), 121.98 (d), 109.74 (s), 82.67 (d), 55.51 (d), 54.91 (d), 49.15 (t), 44.94 (d, 2C), 44.66 (d), 43.38 (t), 43.24 (d), 42.14 (d), 41.62 (d).– MS (70 eV): *m/z* = 266 [M$^+$], 238, 187, 131, 107, 93, 65, 28.

*8-[(3-Aminomethyl)pyridine]-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (**9**)*

Obtained as light yellow oil after column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase (yield 32.4%).– HR-MS for $C_{17}H_{18}N_2O$: calcd, 266.1419, found 266.1409.– IR (neat): ν_{max} = 3280 cm^{-1}, 2960, 1730, 1490, 1360, 1010.– ^{1}H NMR ($CDCl_3$): δ = 8.60 (s, 1H), 8.49 (d, 1H, J_{AB} = 3.90), 7.70 (d, 1H, J_{BC} = 7.75), 7.25 (m, 1H), 4.67 (t, 1H, *J* = 5.22), 4.02 (AB-q, 2H, *J* = 14.12), 2.42–2.88 (4 × m, 9H), 1.73 (AB-q, 2H, *J* = 10.44).– ^{13}C NMR ($CDCl_3$): δ = 149.74

(d), 148.59 (d), 136.49 (s), 135.81 (d), 123.55 (d), 109.70 (s), 82.73 (d), 55.42 (d), 54.89 (d), 45.35 (t), 44.94 (d, 2C), 44.68 (d), 43.38 (t), 43.21 (d), 42.04 (d), 41.63 (d).– MS (70 eV): *m/z* = 266 [M^+], 238, 187, 131, 107, 92, 65, 28.

*8-[(4-Aminomethyl)pyridine]-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (**10**)*

Off white needles obtained by recrystallisation from ethanol (yield 57.9%).– HR-MS for $C_{17}H_{18}N_2O$: calcd, 266.1419, found, 266.1412.– Mp 110 °C.– IR (KBr): ν_{max} = 3300 cm^{-1}, 2980, 1605, 1560, 1505, 1415, 1375, 1240, 1010, 855.– ^{1}H NMR ($CDCl_3$): δ = 8.53 (d, 2H, J_{AB} = 5.70), 7.31 (d, 2H, J_{AB} = 5.70), 4.66 (t, 1H, *J* = 5.22), 4.02 (AB-q, 2H, *J* = 15.49), 2.38–2.87 (3 × m, 9H), 1.72 (AB-q, 2H, *J* = 10.47).– ^{13}C NMR ($CDCl_3$): δ = 150.63 (s), 150.00 (d, 2C), 122.78 (d, 2C), 109.68 (s), 82.72 (d), 55.44 (d), 54.86 (d), 46.66 (t), 44.98 (d, 2C), 44.66 (d), 43.38 (t), 43.25 (d), 42.05 (d), 41.62 (d).– MS (70 eV): *m/z* = 266 [M^+], 236, 187, 172, 159, 131, 91, 65, 40, 32, 28.

*8-Phenylhydrazine-8,11-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**11**)*

Off white needles obtained by recrystallisation from acetone (yield 62.8%).– HR-MS for $C_{17}H_{18}N_2O$: calcd, 266.1419, found, 266.1418.– Mp 183 °C.– IR (KBr): ν_{max} = 3273 cm^{-1}, 2951, 1603, 1522, 1497, 1352, 1261, 1103, 739, 689.– ^{1}H NMR ($CDCl_3$): δ = 7.17 (m, 2H), 6.90 (d, 2H, J_{AB} = 7.63), 6.76 (t, 1H, J_{BC} = 7.20), 5.68 (br. s, 1H), 4.66 (br. s, 1H), 4.35 (br. s, 1H,), 2.41–2.82 (m, 8H), 1.68 (AB-q, 2H, *J* = 10.44).– ^{13}C NMR ($CDCl_3$): δ = 150.28 (s), 129.23 (d, 2C), 119.02 (d), 112.57 (d, 2C), 110.90 (s), 83.22 (d), 54.56 (d), 53.42 (d), 44.75 (d), 44.48 (d), 43.75 (t), 43.41 (d, 2C), 41.89 (d), 41.55 (d).– MS (70 eV): *m/z* = 266 [M^+], 248, 159, 131, 105, 93, 77, 65, 44, 32, 28.

*Benzylamine Derivatives of 3-Hydroxyhexacyclo-[6.5.0.0.^{3,7}0^{4,12}.0^{5,10}.0^{9,13}]tridecan-6-one (**12**), 8,13-Dioxapentacyclo-[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecane-9,12-dione (**13**), and 8-Pentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanone (**14**), 6-Benzylamino-3-hydroxyhexacyclo-[6.5.0.0^{3,7}.0^{4,12}.0^{5,10}.0^{9,13}]tridecane (**12**)*

3-Hydroxyhexacyclo[6.5.0.0.^{3,7}0^{4,12}.0^{5,10}.0^{9,13}]tridecane-6-one (0.7 g; prepared according to the methods described by Marchand and Arney[11] and Krapcho and Lovey[12]) was dissolved in THF (20 ml) and benzylamine (0.4 g) was slowly added with stirring at room temperature. After 2.5 h the solution turned milky and the solvent was removed under vacuum after a further 30 min to give a fine white precipitate. The hydroxyl amine formed was dehydrated in dry benzene under Dean-Stark conditions. Evaporation of the excess benzene yielded the Schiff base as a light yellow oil. Reduction of this imine was done with sodium borohydride (1 g) in dry THF (50 ml) and dry methanol (10 ml) for 24 h at room temperature. The solvent was removed under reduced pressure and water (50 ml) and dichloromethane (50 ml) was added. The mixture was extracted with dichloromethane (4 × 50 ml) and the combined organic fractions washed with water before being dried over magnesium sulphate. Evaporation yielded the desired product as an off white oil. The white crystalline product (**12**) was obtained by recrystallisation from cyclohexane (Yield, 84.3%).– HR-MS for $C_{20}H_{23}NO$: calcd, 292.1701, found, 292.1701.– Mp 123 C – IR (KBr): ν_{max} = 3300 cm^{-1}, 2950, 1485, 1455, 1330, 1318, 1130, 1063.– ^{1}H NMR ($CDCl_3$): δ = 7.33 (m, 5H), 3.82 (AB-q, 2H, *J* = 12.88), 3.37 (t, 1H, *J* = 3.36), 2.72 (dd, 1H, *J* = 3.02), 2.66 (t, 1H, *J* = 6.04), 2.49 (m, 2H), 2.36 (m, 3H), 2.17 (d, 1H, *J* = 5.43), 1.99 (m, 1H), 1.64 (AB-q, 2H, *J* = 11.33), 1.30 (AB-q, 2H, *J* = 9.61).– ^{13}C NMR ($CDCl_3$): δ = 140.97 (s), 128.61 (d, 2C), 128.44 (d, 2C), 127.11 (d), 85.20 (s), 55.59 (d), 52.85 (t), 51.16 (d), 50.88 (d), 47.64 (d), 42.25 (d,), 40.85 (t), 38.86 (d), 38.52 (t), 37.32 (d), 35.25 (d), 32.92 (d), 31.94 (d).– MS (70 eV): *m/z* = 292 [M^+–1], 202, 186, 149, 106, 91, 69, 57, 43, 32, 28.

*12-Benzylamino-8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecan-9-one (**13**)*

A mixture of 8,13-dioxapentacyclo[6.5.0.0^{2,6}.0^{5,10}.0^{3,11}]tridecane-9,12-dione (2.06 g, prepared according to the method of Surapaneni and Gilardi[13]), benzylamine (1.07 g) and sodium borohydride (0.6 g) was dissolved in dry methanol and heated under reflux for 10 days. The solvent was removed under reduced pressure and water (50 ml) and dichloromethane (50 ml) was added. The mixture was extracted with dichloromethane (4 × 50 ml) and the combined organic fractions washed with water before being dried over magnesium sulphate. Evaporation yielded a yellow oil that contained the product (**13**). Column chromatography with ethyl acetate:ethanol 9:1 gave (**13**) as a crystalline solid (Yield 18.9%).– HR-MS for $C_{18}H_{19}NO_3$: calcd, 297.1365, found, 297.1357.– Mp 178 C.– IR (KBr): ν_{max} = 3550 cm^{-1}, 2950, 1680, 1455, 1370, 1255, 1185, 1100.– ^{1}H NMR ($CDCl_3$): δ = 7.30 (m, 5H), 4.97 (s + d, 2H, *J* = 15.66), 4.63 (m, 1H), 4.51 (dt, 1H, *J* = 7.72), 4.15 (d, 1H, *J* = 15.04), 3.97 (d, 1H, *J* = 4.95), 2.69–2.94 (m, 4H), 2.39–2.53 (2 × m, 2H), 1.74 (AB-q, 2H, *J* = 9.75).– ^{13}C NMR ($CDCl_3$): δ = 176.42 (s), 137.26 (s), 128.87 (d, 2C), 128.75 (d, 2C), 127.48 (d), 80.09 (d), 72.78 (d), 68.84 (d), 50.44 (d), 48.39 (d), 47.06 (t), 43.96 (t), 43.44 (d), 33.41 (d), 33.20 (d), 31.64 (d).– MS (70 eV): *m/z* = 297 [M^+], 269, 228, 199, 173, 106, 91, 65, 40, 32, 28.

*8-Benzylaminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**14**).*

8-Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanone (1 g, prepared according to the method of Oliver *et al.*[14]) was dissolved in a mixture of absolute ethanol (8 ml) and benzylamine (0.9 g) and the reaction mixture was refluxed for 24 h. It was cooled in ice and a solution of sodium borohydride (1.89 g) in cold water (20 ml) was slowly added. After stirring at room temperature for 5 h, the solvent was removed under vacuum. Water (50 ml) and dichloromethane (50 ml) was added and the mixture was extracted with dichloromethane (3 × 50 ml) and diethyl ether (2 × 50 ml). The combined organic fractions were washed with water (2 × 100 ml), dried over magnesium sulphate and evaporated to give a yellow oil. Column chromatography on silica gel with ethyl acetate:dichloromethane:petroleum ether 1:1:1 as mobile phase rendered **14** as a off white oil (Yield 7,6%).– HR-MS for $C_{18}H_{21}N$: calcd, 251.1674, found, 251.1670.– ^{1}H NMR ($CDCl_3$): δ = 7.32 (m, 5H), 3.78 (s, 2H), 2.78 (t, 1H, *J* = 3.42), 2.16–2.68 (5 × m, 10H), 1.41 (AB-q, 2H, *J* = 10.25), 1.02 (dt, 1H, *J* = 3.68).– ^{13}C NMR ($CDCl_3$): δ = 141.25 (s), 128.50 (d, 2C), 128.24 (d, 2C), 126.96 (d), 61.50 (d), 53.45 (t), 47.32 (d), 44.69 (d), 44.15 (d), 42.07 (d), 41.92 (d), 40.91 (d), 37.70 (d), 36.34 (d), 34.74 (t), 28.83 (t).– MS (70 eV): *m/z* = 251 [M^+], 104, 171, 160, 144, 106, 91, 79, 66, 39.

Electrophysiology

Ventricular myocytes from the hearts of Duncan-Hartley strain guinea-pig were isolated by enzymatic dispersion of the cells with collagenase and protease on a Langendorff apparatus [21].

Suction pipettes with resistance values between 2 and 3 MΩ were drawn from borosilicate glass and heat polished. A GΩ seal was formed by light suction. The patch was then ruptured and at least 15 min were allowed for internal dialysis to reach equilibrium. Whole cell voltage clamping was done using the chopped clamp method [18]. A concentration clamp and multibarrel superfusion system that allowed changes in the bath solutions in less than 1 s was used.

Potassium currents were eliminated by adding $CsCl_2$ (20 mM) to the Tyrode solution and TEA-Cl to the pipette solution while the effects of sodium currents were eliminated by pre-clamping at –35 mV. Activation of the calcium channel was done by voltage clamping from a holding potential of –40 mV to a test potential of +10 mV for 400 ms. The pclamp 5.5® program (Axon Instruments, Inc., 1989) was used for the control of the experiment and storage of data. The calcium currents were measured as the maximum negative (inward) current after subtraction of background currents. Test concentrations of 10 μM were used and calcium channel blocking activity was expressed as the percentage inhibition of calcium current as registered before and after inclusion of the test compound.

The composition (in mM) of the Tyrode solution for superfusion was: KCl 5.4; $CaCl_2$ 1.8; CsCl 20; $MgCl_2$ 0.5; NaCl 137.6; glucose 5 and 2-ethanesulphonic acid 11.6. The pipette solution (in mM) was: $MgCl_2$ 5; Na_2ATP 5; HEPES 10; CsCl 125; EGTA 15; TEA-Cl 20 and NaOH to pH = 7.2.

Computer Modeling and Calculation of Physico-Chemical Properties

A minimum energy conformation of each structure was obtained by optimizing the structures with the Hyperchem® program (Release 4.5 for Windows) using MM^+ and AM1 semi empirical calculations and the Polak-Ribiere minimizing procedure [22].

The physico-chemical data (QSAR data) of these minimized structures were calculated using the Chemplus™ extension of the Hyperchem® program. The valence first-order connectivity index ($^1\chi^v$) for each compound was calculated[17] using the following equation symbols used as defined by Kier and Hall [17]):

$$^1\chi^v = \sum_{s=1}^{N_e} (\delta_i^v \delta_j^v)_s^{-1/2}$$

References

[1] Y.W. Kwon, D.J. Triggle, *Chirality* **1991**, *3*, 393–404.

[2] D.J. Triggle, D.A. Langs, *Annu. Rep. Med. Chem.* **1990**, *25*, 225–234.

[3] K.E. Andersson, *Acta Pharmacol. Toxicol.* **1978**, *43* (suppl 1), 5–14.

[4] E. Neher, *Science* **1992**, *256*, 498–502.

[5] S. Hopkins, *Manufacturing Chemist* **1991**, *62*, 28–30.

[6] D.J. Triggle, *Stroke (suppl. IV)* **1990**, *21*, 49–58.

[7] C.J. Van der Schyf, W. Liebenberg, R. Bornman, T.G. Dekker, P.H. Van Rooyen, T.G. Fourie, E. Matthee, F.O. Snyckers, *S. Afr. J. Chem.* **1989**, *42*, 46–48.

[8] A.P. Marchand, B.E. Arney, P.R. Dave, N. Satyanarayana, W.H. Watson, A. Nagel, *J. Org. Chem.* **1988**, *53*, 2644–2647.

[9] C.J. Van der Schyf, G.J. Squier, W.A. Coetzee, *Pharmacol. Res. Commun.* **1986**, *18*, 407–417.

[10] J.J. Van der Walt, C.J. Van der Schyf, J.M. Van Rooyen, J. de Jager, M.N. Van Aarde, *S. Afr. J. Sci.* **1988**, *84*, 448–450.

[11] A.P. Marchand, B.E. Arney Jnr., *J. Org. Chem.* **1987**, *52*, 3455–3457.

[12] A.P. Krapcho, A.J. Lovey, *Tetrahedron Lett.* **1973**, *12*, 957–960.

[13] C.R. Surapaneni, R. Gilardi, *J. Org. Chem.* **1986**, *51*, 2382–2385.

[14] D.W. Oliver, T.G. Dekker, F.O. Snyckers, *Eur. J. Med. Chem.* **1991**, *26*, 375–379.

[15] R.B. Silverman, *The organic chemistry of drug design and drug action*, London, UK, Academic Press. **1992**, 71–72, 422p.

[16] L. Belvisi, S. Brossa, A. Salimbeni, C. Scolastico, R. Todeschini, *J. Comp.-aid. Molec. Des.*, **1991**, *5*, 571–584.

[17] L.B. Kier, L.H. Hall, *Molecular connectivity in chemistry and drug research*, in G. DeStevens (Ed.), *Medicinal chemistry. A series of monographs*, Vol. 14, New York, N.Y. Academic Press, **1976**, 40–80.

[18] O.P. Hamil, A. Marty, E. Neher, B. Sakman, F.J. Sigworth, *Pflüg. Arch.* **1981**, 391, 85–100.

[19] R.C. Cookson, E. Grundwell, J. Hudec, *Chem. Ind.* **1958**, 1003–1004

[20] R.C. Cookson, E. Grundwell, R.R. Hill, J. Hudec, *J. Chem. Soc.* **1964**, 3062–3075.

[21] R. Mitra, M. Morad, *Am.J. Physiol.* **1985**, *249*, H1056–H1060.

[22] E. Polak, *Computational methods in optimization, a unified approach*, New York, N.Y. Academic Press, **1971**, 53–54.

[23] A.K. Ghose, A. Pritchett, G.M. Crippen,. *J. Comput. Chem.* **1988**, *9*, 80–90.

[24] V.N. Viswanhadhan, A.K. Ghose, G.R. Revankar, R.K. Robins, *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163–172.

[25] A.K. Ghose, G.M. Crippen, *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 21–35.

[26] K.J. Miller, *J. Am. Chem. Soc.* **1990**, *112*, 8533–8542.

[27] N. Bodor, Z. Gabanyi, C-K. Wong, *J. Am. Chem. Soc.* **1989**, *111*, 3783–3786.

[28] A. Gavezzotti,. *J. Am. Chem. Soc.* **1983**, *105*, 5220–5225.

[29] C.W. Still, A. Tempczyk, R.C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.

[30] L.B. Kier, *Molecular connectivity as a description of structure for SAR analyses*, in S.H. Yalkowsky, A.A. Sinkula, S.C. Valvani (Eds.), *Physical chemical properties of drugs*, New York, N.Y. Marcel Dekker, **1980**, *227–319.*

Received: June 7, 1999 [FP401]