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Physicochemical Prediction of a Brain–Blood Distribution Profile in Polycyclic Amines

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Abstract—Recent investigation into the pharmacological character of the pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecyl and related polycyclic amines has revealed interesting facts regarding their possible use as neuroprotective agents. At this stage however, a clear shortcoming in the quest for further development of this novel class of compounds is the lack of concrete data on their ability to cross the blood-brain barrier (BBB). Working towards the aim of predicting BBB permeability, a series of related N-substituted 8-amino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes were synthesised. Compounds were characterised by both experimental and calculative methods, followed by biological assessment and statistical manipulation of the results obtained. In doing so, a simple biological model was established for the comparative evaluation of brain–blood distribution properties within the class. A highly sensitive ESI-MS.MS analytical procedure was developed for the detection of these compounds in biological tissues, indicating significant drug concentrations in the brain after intraperitoneal administration to C57Bl/6 mice. Stepwise multiple linear regression analysis of all data yielded two meaningful models ($R^2 = 0.9996$ and $R^2 = 0.7749$) depicting lipophilicity (log P_{oct}), solvent accessible molecular volume (SV), molar refractivity (MR) and system energy as the prime determinants of the brain–blood profile for these amines. The inherently high lipophilicity potential within the series is attributed to strong hydrophobic influences dominating hydrogen bonding effects. A possible conformational and energy dependent preference at the site of permeation is also suggested. The proposed estimations allow for the expedient and reliable prediction of brain partitioning behaviour for related polycyclic amines, facilitating the early rejection of unsuitable candidates and enabling research to focus on neuroprotective activity.

Introduction

The chemistry of polycyclic amines has intrigued many a scientist over the past five decades but only recently have researchers commenced probing their pharmacological properties. Based on the premise of structural analogy to the adamantanamines (**1**, **2**), investigation into the antiviral properties of two 4-amino-(D_3)-trishomocubane derivatives (**3**, **4**) revealed in vivo activity against Herpes simplex Type II and Influenza A_2.[1] Shifting the emphasis to central nervous system (CNS) activity, the potential of a series of 8-alkylaminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes (**5**) as possible anti-Parkinsonian agents was also illustrated.[2] Illuminating insights gained on the Ca^{2+} antagonistic effects[3] of related pentacycloundecyl amines (**6**–**8**) signified possible interaction with the ionotropic NMDA receptor and therefore the potential use of these compounds in preventing long-term neurodegeneration (Fig. 1).

Since the postulated target systems for these polycyclic amines are within the brain and CNS, the need exists for generating concrete data and guidelines on their BBB permeability. Despite estimations as to the extent in which these compounds are able to penetrate the brain,[4] no specific BBB permeability data has yet been published. In essence, this project entailed the synthesis and characterisation of a planned series of pentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecyl amines in terms of lipophilicity, minimum energy conformation, molecular size, electronic character, hydrogen bonding behaviour and brain–blood distribution data. Regression analysis was applied in order to examine possible correlations between brain–blood distribution data and physicochemical descriptors. This was done in an attempt to establish a quantitative structure–property relationship (QSPR) model with reliable predictive ability to provide parameter guidelines as to the potential degree of brain penetration for premeditated compounds within the class.

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Results

Physicochemical characterisation

The synthesised compounds were characterised by both experimental and computerised methods. Lipophilicity was quantified at neutral pH as log P_{oct} values. Determinations were done experimentally according to the traditional shake-flask method.[5] Predicted lipophilicity [log P(ACD) and log P(Hyp)] values were calculated algorithmically using Advanced Chemistry Development's ACD/Log$P$® program[6,7] and Hyperchem® modelling software,[8] respectively. Hyperchem® was furthermore employed to calculate parameters providing quantum mechanical expression of minimised system energy [Min.Energy(Hyp)], molar refractivity (MR), polarisability (α), solvent accessible molecular volume (SV) and hydration energy (H) (Table 2).

As an experimental measure of hydrogen bonding potential, a newly explored system to describe *n*-hexane/ethylene glycol partitioning behaviour was employed. This venture was rationalised by the principle difference between two immiscible solvents, one facilitating exclusively lipophilic interaction and the other capable of hydrogen bonding. Equilibrated partitioning between the two solvent phases would therefore allow a single, direct measure of the solute's hydrogen bonding (or desolvation) potential.[9] This parameter was subsequently expressed as the logarithm of the implied *n*-hexane/ethylene glycol partition coefficient and for the purpose of this study denoted as log P_{HB}.

Brain–blood distribution

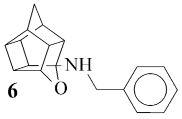
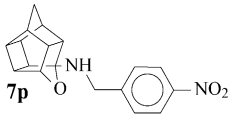
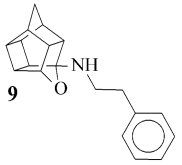
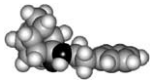
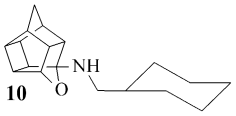
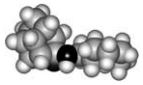
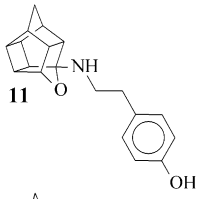
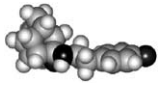
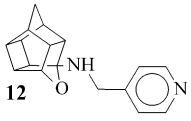
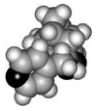
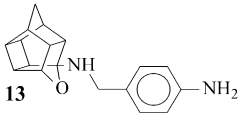
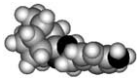
Pilot studies were performed on the lead compound (**6**, Table 1) to evaluate relative brain and blood analyte concentrations (μg g^{-1}) at 30, 60, 90, and 120 min time intervals after intraperitoneal administration to laboratory mice, indicating optimum levels in both brain and blood at approximately 60 min. Although not indicative of true steady-state levels, this observation provided a meaningful centre for comparison within the congeneric series. Brain partitioning data for each test compound was consequentially generated by determining the brain-to-blood concentration ratio [(μg g^{-1}).(μg g^{-1})$^{-1}$] at this arbitrary single time-point (60 min) after administration, and denoted as log BB_{ST}, that is, log([brain]/[blood]) at a single time-point. Log BB_{ST} values for the test compounds ranged between -0.50 ± 0.07 and 0.43 ± 0.05 (Fig. 2), indicating that these compounds significantly crossed the BBB.

QSPR model

Individual correlation trends between brain–blood distribution and physicochemical descriptors were evaluated by means of linear regression analysis as well as polynomial least squares fit procedures. (The nature of the curve was assessed by the linear correlation coefficient R^2_{lin}, and the non-linear correlation coefficient $R^2_{non\text{-}lin}$). Statistical manipulation revealed a parabolic relationship between permeability and log P_{oct} ($R^2_{non\text{-}lin} = 0.91$, Fig. 3, Table 3). Based on poor correlation values, hydrogen bonding capacity within the ranges described for this series of compounds, did not seem to significantly influence BBB permeability. Furthermore, poor correspondence between log P_{oct} and the theoretical parameters log P(ACD) and log P(Hyp) indicated that calculated log P values relate poorly to the lipophilic character of the polycyclic amines.

Stepwise multiple linear regression analysis of all data yielded a four-component model ($R^2 = 0.9996$; $n = 7$, eq 1), depicting lipophilicity (log P_{oct}), total system energy [Min.Energy(Hyp)], solvent accessible molecular volume (SV) and molar refractivity (MR) as the prime determinants of BBB permeability for the polycyclic amines $n = 7$. Omitting the energy parameter Min. Energy(Hyp) from the equation reduced linearity of the subsequent three-component model to $R^2 = 0.7749$ ((Fig. 4) eq 2). No other significant multiple regression correlations were evident (Table 2).

Table 1. Comparison between input and optimised conformations generated by Hyperchem®, with designated energy minima in kcal mol^{-1}

Input structure	Minimised conformation	Min.Energy (Hyp)
 **6**		−4197.6
 **7p**		−4374.4
 **9**		−4478.6
 **10**		−4568.8
 **11**		−4582.4
 **12**		−4072.7
 **13**		−4364.0

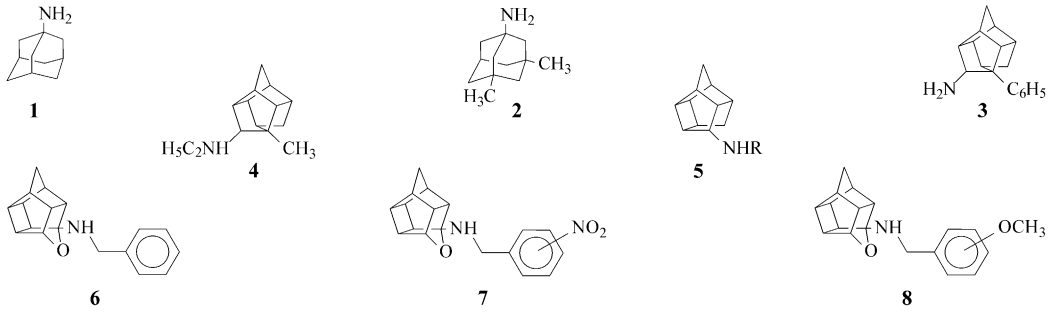


Figure 1. Adamantanamines and substituted polycyclic undecyl amines.

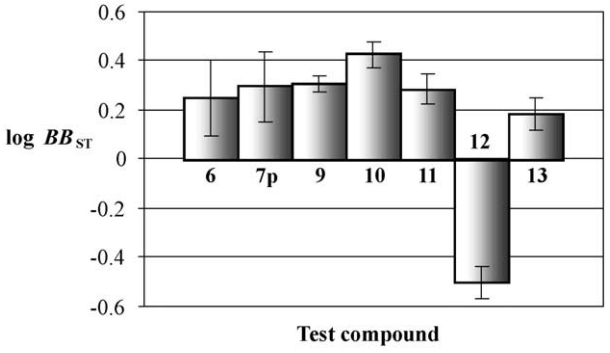


Figure 2. Log BB_{ST} values calculated as the average of six animal experiments within each component group, with SD. All deviations were within acceptable limits.[23]

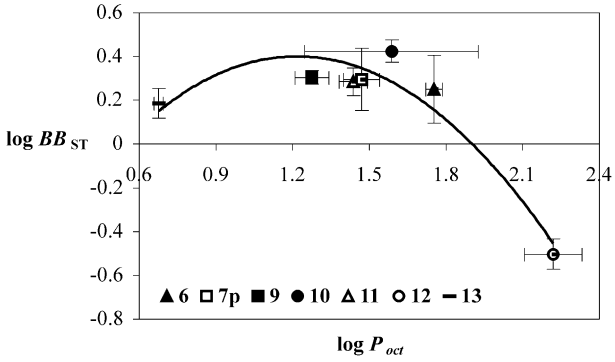


Figure 3. Parabolic correlation trend for log BB_{ST} versus log P_{oct}. Error bars indicate deviation for an average of six respective log BB_{st} assessments, and five log P_{oct} measurements, for each compound.

Table 2. Summary of experimental and computational descriptors

	Log P_{oct}	Log P (ACD)	Log P (Hyp)	Log P_{HB}	MR[a]	α[b]	SA[c]	SV[d]
6	1.75	1.68	2.57	0.57	75.13	29.80	478.66	789.68
7p	1.47	1.41	2.53	−0.29	82.45	31.51	516.24	849.23
9	1.28	1.71	2.83	0.63	79.88	31.63	512.44	841.55
10	1.59	2.22	2.70	0.98	76.26	30.37	499.67	833.13
11	1.44	0.98	2.54	−1.67	81.57	32.27	524.03	862.55
12	2.22	0.18	1.26	−1.6	72.97	29.09	448.80	755.78
13	0.68	0.4	1.79	−2.04	79.83	31.15	496.47	822.71

[a]Molar refractivity (Å^3).[10,11]
[b]Polarisability (Å^3).[12]
[c]Solvent accessible surface area (Å^2).[13,14]
[d]Solvent accessible molecular volume (Å^3).[13,14]

Table 3. Correlation values between log BB_{ST} and respective physicochemical descriptors

	Log P_{oct}	Log P(ACD)	Log P_{HB}	Min.Energy (Hyp)	SV	MR
R^2_{lin}	0.32	0.58	0.27	0.62	0.65	0.36
$R^2_{non\text{-}lin}$	0.91	0.69	0.30	0.82	0.90	0.74

$$\log BB_{ST} = 4.56796 - 0.52226(\log P_{oct}) + 0.00521[\text{Min.Energy(Hyp)}] + 0.05559(\text{SV}) - 0.33873(\text{MR}) \quad (1)$$

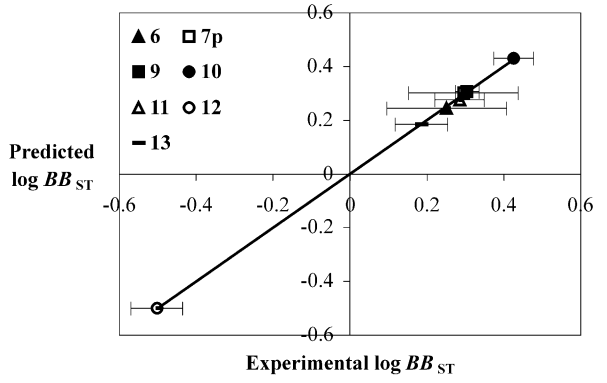


Figure 4. Predicted log BB_{ST} from eq 1 versus experimental log BB_{ST}. Error bars represent variation in experimental values.

$$\log BB_{ST} = -3.13289 - 0.20387(\log P_{oct}) + 0.01133(\text{SV}) - 0.07279(\text{MR}) \quad (2)$$

Discussion

This study unambiguously proves that within this series, the *N*-substituted 8-amino-8,11-oxapentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecanes significantly partition into the brain after systemic administration—independent of the mode of entry or whether specific transport systems or efflux mechanisms are involved. Literature describes log *BB* values ranging between −2.0 and +1.0, suggesting

optimal brain partitioning around 0.3; larger values usually exhibited by highly penetrable drugs, and values <-1.0 indicating poor distribution to the brain.[15] Values for the polycyclic amines ranged between -0.50 and 0.43, with optimal permeability exhibited by compound **10** (log $BB_{ST}=0.43$). Based on these values as crude guidelines, it therefore implies that compound **12**, despite displaying a blood-favoured partitioning ratio, still reached meaningful concentrations in brain parenchyma.

No linear relationships seemed to exist between BBB permeability and the experimentally determined descriptors log P_{oct} and log P_{HB}. Non-linear fit procedures however revealed decidedly significant individual correlation with regard to log P_{oct}, log P(Hyp), Min.Energy(Hyp) and SV. These observations suggest a mainly parabolic correlation between permeability and lipophilicity (log P_{oct}), following the trend described by Hansch,[16] and indicating a local maximum at log $P_{oct}\pm1.5$.

From the results obtained it seems risky to assign specific correlation trends between permeability and the respective semi-empirically derived parameters log P(Hyp), Min.Energy(Hyp) and SV, because they reflect both linear and non-linear tendencies. From this point of view, log P_{oct} seems to be the only reliable guideline for BBB permeability, if one has to evaluate only one physicochemical descriptor. In addition, the parameters as calculated by ACD®, are unsatisfactory predictors for this class of compounds, considering yet again the non-specificity that emerged from such correlations [log P(ACD)] or simply the lack of any (log $D_{7.4}$ and pK_a). Since ACD® values are obtained from a fragmental approach, prediction accuracy may prove inadequate in novel classes of compounds.[17] Atomic-based calculations (Hyperchem®) clearly produce closer estimations to QSPR properties, though still not satisfactory.

Hydrogen bonding (log P_{HB}) does not seem to play a pivotal role in the permeability of these compounds, despite the fact that they differ significantly in their hydrogen bonding capacity. This suggests that BBB permeability here is driven mainly by hydrophobic character. Strangely enough, this reflects the exact opposite situation from that described by Chikhale et al.,[18] where poor correlation with lipophilicity was observed, but hydrogen bonding appeared the significant determinant. A possible explanation might simply lie in the number of hydrogen bonding groups and that there also exists a 'threshold' from which hydrogen bonding capacity starts to elicit meaningful changes in permeability. This seems rational since the current series of polycyclic amines essentially differ by only one group from compound to compound, or even only in the nature of such groups. More work is however required to prove this hypothesis, and to establish whether the log P_{HB} parameter is reliable in its representation of hydrogen bonding capacity. Polar surface area should also be considered in such quantifications.

The multivariate prediction models in eqs 1 and 2 verify the conclusions drawn from evaluating the individual correlation trends between permeability and the various descriptors applied in this investigation. The models establish the significance of lipophilicity, solvent accessible molecular volume, molar refractivity and a energy term with regard to the BBB permeability of the polycyclic amines.

The decrease in linearity resulting from omitting the energy term in eq 1, points toward an energy-influenced affinity at the site of permeation. However, as the energy term can be influenced by the algorithm used and might in some instances not represent the global minimum, estimations of permeability could also be based on eq 2, especially for larger and more flexible congeners.

The negative contribution from log P_{oct} probably reflects on the fact that the measured range of values is mainly distributed around the plateau (optimum) of the established parabolic curve, where slight increases/decreases will reduce permeability. This phenomenon is usually explained in terms of diffusional transport - the inclining leg of the curve simply representing improved lipid-solubility with correspondingly enhanced diffusion across the barrier; the plateau indicative of diffusion through the stagnant layers becoming rate-limiting, while the declining leg represents a trend where diffusion gets inhibited due to membrane association of highly lipophilic compounds.

Unique to this model is the positive contribution to permeability as exercised by the solute size and energy descriptors. Although Abraham et al.[19] reported increased brain penetration with increased solute size, without providing mechanistic understanding of this occurrence, this interesting observation should also be mentioned here. If passive diffusion is regarded as the main route of entry, one should expect the opposite effect from solute size (according to the Stokes–Einstein equation). Close inspection of the minimum energy conformation (Hyperchem®) and volume (SV) of compound **12** reveals that it is the smallest spatially arranged molecule within the series and improved diffusion compared to the rest of the series would thus be expected. Looking at its log P_{oct} value (highest within the series) this is exactly what happens with regard to octanol–water partitioning; yet **12** displays the poorest distribution to the brain. This indicates that there are probably certain other mechanisms, like protein-mediated extrusion transport, involved in the barrier permeability of these compounds and therefore a conformational (energy-dependent) preference at the site of permeation.

The positive y-intercept indicates the inherently high potential of these compounds to reach meaningful concentrations in the brain.

Conclusion

Both regression models establish the significance of lipophilicity, solvent accessible molecular volume and molar refractivity with regard to the BBB permeability

of the polycyclic amines. The good linear regression ($R^2 = 0.9996$, eq 1) obtained by including an energy term also stresses the importance of conformational energy in the transport of these compounds across the BBB. Predicting capabilities are however limited to this class of compounds and within the respective physicochemical descriptor limits investigated. Experimental log P_{oct} determination is required and the model is therefore not purely computational in nature. This is necessary as calculated log P values do not correspond satisfactorily to those determined experimentally. The BBB permeability assessment here is probably not based on passive diffusion alone, but is an integrated estimation of transport mechanisms or preferences that may exist within the range of physicochemically different compounds used to construct it. The experimental log P_{oct} furthermore remains the best lipophilicity descriptor with which these processes can be related, but proves the complexity of BBB transport in that it cannot be regarded as a static lipoid wall, but should rather be recognised as a highly dynamic and selective interface. The derived models do however provide valuable parameter guidelines as to those properties essential for calculating the probability of these amines to significantly reach the brain after systemic administration.

Since the experimental evaluation of permeability potential is difficult, time-consuming and expensive, a rapid yes/no decision concerning brain penetration can be reached with confidence early in the drug development process of these compounds, facilitating rejection of unsuitable candidates.

Experimental

Materials and standard procedures

All reagents and analytical solvents were procured from Sigma-Aldrich, Merck or Fluka and used without intervention. Reaction and elution solvents were purchased from commercial sources. Melting points were determined by means of DSC utilising a Shimadzu DSC-50 instrument while IR spectra were recorded on a Nicolet 470 FT-IR spectrophotometer. Mass spectra and HR-MS were recorded on a VG 7070E mass spectrometer and NMR spectra were obtained on a Varian Gemini 300. ^{1}H spectra were recorded at a frequency of 300.075 MHz and ^{13}C spectra at 75.462 MHz, in a 7 Tesla magnetic field. Tetramethylsilane (TMS) was used as internal standard and a bandwidth of 1000 MHz at 24 kG applied for ^{1}H–^{13}C-decoupling. All chemical shifts are reported in parts per million (ppm) relative to TMS ($\delta = 0$). The following abbreviations are used to indicate multiplicities of respective signals: s, singulet; bs, broad singulet; d, doublet; t, triplet; m, multiplet.

Synthetic procedures

Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione served as the initial structure for all further syntheses and was prepared in bulk according to literature methods.[20] For the synthesis of compounds **6**, **9**, **7p** and **12**, the methods of Malan et al. were used.[21]

8-Benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (6). Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (5.04 g, 28.98 mmol) was dissolved in tetrahydrofuran (THF, 50 mL) and cooled down to 5 °C while stirring on an external ice bath. Benzylamine (3.23 g, 30.21 mmol) was added slowly, with continued stirring of the reaction mixture at lowered temperature. The carbinolamine started precipitating after approximately 10 min, but the reaction was allowed to reach completion for an additional 20 min. This carbinolamine was isolated by filtration. Water was removed azeotropically by refluxing this material in 60 mL sodium-dried benzene (Dean–Stark dehydrating conditions) for 1 h or until no more water was collected in the trap. (The milky suspension turned into a clear solution as the reaction progressed.) The benzene was removed under reduced pressure and the Schiff base (8-benzyliminopentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-11-one, a yellow oil) was dissolved in a mixture of anhydrous methanol (MeOH, 30 mL) and anhydrous THF (150 mL). Reduction was carried out by adding $NaBH_4$ (1.50 g, 39.46 mmol) in excess and stirring the mixture overnight (18 h) at room temperature. The solvents were removed in vacuo, the residue suspended in water (100 mL) and extracted with dichloromethane (DCM, 4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous $MgSO_4$ and evaporated in vacuo to yield a yellowish oil. Purification of the product mixture was accomplished with column chromatography and the desired amine was isolated as a colourless wax. Crystallisation from absolute ethanol rendered the final product as a colourless microcrystalline solid. (Yield: 2.92 g, 11.01 mmol, 38.00%.) $C_{18}H_{19}NO$; mp 78.7 °C; HR-MS: calcd 265.1467, exp. 265.1466; IR (KBr) ν_{max}: 3332, 2970, 1507, 1011 cm^{-1}; MS (EI, 70 eV) m/z: 266 [(M^+) + 1], 265 (M^+), 237, 186, 131, 91, 65, 28; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 7.34–7.15 (m, 5H, H-16,17,18,19,20), 4.62 (t, 1H, $J = 5.2$ Hz, H-11), 3.98 (AB, 1H, $J_{AB} = 13.5$ Hz, H-14a), 3.93 (AB, 1H, $J_{AB} = 13.5$ Hz, H-14b), 2.83–2.47 (2×m, 7H, H-1, 2, 3, 5, 6, H-7/9, H-10), 2.38 (t, 1H, $J = 5.0$ Hz, H-7/9), 2.16 (bs, 1H, H-13), 1.86:1.51 (AB-q, 2H, $J = 10.4$ Hz, H-4a, 4b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 140.96 (s, C-15), 128.40 (d, C-16, 20), 127.92 (d, C-17, 19), 126.85 (d, C-18), 109.62 (s, C-8), 82.48 (d, C-11), 55.22 (d, C-7/9), 54.73 (d, C-7/9), 47.73 (t, C-14), 44.83 (d, 1C), 44.79 (d, 1C), 44.50 (d, 1C), 43.18 (t, C-4), 43.07 (d, 1C), 41.93 (d, 1C), 41.47 (d, 1C).

8 - (4 - Nitrobenzylamino) - 8,11 - oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (7p). A solution of 4-nitrobenzylamine·HCl (5.0 g in 100 mL water) was alkalinised with NaOH (10% w/v) and extracted with DCM (4×50 mL). After in vacuo removal of the solvent at room temperature, the free base (4.00 g, 26.32 mmol) was slowly added to a solution of the pentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (4.40 g, 25.30 mmol) in THF (50 mL) and the stirring mixture cooled down to 5 °C. The carbinolamine started precipitating after approximately 40 min, but the reaction was

allowed to reach completion for an additional 20 min. This carbinolamine, a yellowish cream, was quickly isolated by filtration and then suspended in 60 mL sodium-dried benzene. Molecular water was removed by slowly refluxing this mixture under Dean-Stark dehydrating conditions for 2 h. (The creamy suspension developed into a clear solution as the reaction progressed.) The benzene was removed under reduced pressure and the Schiff base, 8-(4-nitrobenzylimino)-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-11-one, a yellow oil) was dissolved in a mixture of anhydrous MeOH (30 mL) and anhydrous THF (150 mL). $NaBH_4$ (1.52 g, 39.98 mmol) was added in excess and the mixture stirred overnight at ambient temperature. (Decolouration indicated successful reduction of the imine.) The solvents were removed in vacuo, the residue suspended in water (100 mL) and extracted with DCM (4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous $MgSO_4$ and evaporated in vacuo to yield a deep amber-coloured wax with signs of crystallisation. Purification of this product mixture was accomplished with column chromatography and recrystallisation from absolute ethanol rendered the final product as a yellowish microcrystalline solid. (Yield: 3.56 g, 11.50 mmol, 45.44%.) $C_{18}H_{18}N_2O_3$; mp 161.6 °C; HR-MS: calcd 310.1317, exp. 310.1322; IR (KBr) ν_{max}: 3334, 2964, 1507, 1009, 852 cm^{-1}; MS (EI, 70 eV) *m/z*: 311 [(M$^+$)+1], 310 (M$^+$), 231, 131, 91, 28; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 8.12 (d, 2H, *J*=8.9 Hz, H-17, 19), 7.50 (d, 2H, *J*=8.9 Hz, H-16, 20), 4.62 (t, 1H, *J*=5.2 Hz, H-11), 4.10 (AB, 1H, J_{AB}=15.4 Hz, H-14a), 4.04 (AB, 1H, J_{AB}=15.4 Hz, H-14b), 2.83-2.38 (3×m, 8H, H-1, 2, 3, 5, 6, 7, 9, 10), 2.32 (bs, 1H, H-13), 1.86:1.51 (AB-q, 2H, *J*=10.6 Hz, H-4a, 4b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 149.15 (s, C-18), 147.07 (s, C-15), 128.25 (d, C-16, 20), 123.58 (d, C-17, 19), 109.42 (s, C-8), 82.56 (d, C-11), 55.34 (d, C-7/9), 54.71 (d, C-7/9), 47.04 (t, C-14), 44.88 (d, 1C), 44.77 (d, 1C), 44.51 (d, 1C), 43.19 (t, C-4), 43.07 (d, 1C), 41.88 (d, 1C), 41.46 (d, 1C).

8-(2-Phenylethylamino)-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (9). Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (5.02 g, 28.86 mmol) was dissolved in THF (50 mL) and cooled down to 5 °C while stirring on an external ice bath. 2-Phenylethylamine (3.52 g, 29.10 mmol) was added slowly, with continued stirring of the reaction mixture at lowered temperature. The white hydroxylamine started precipitating after approximately 15 min, but the reaction was allowed to reach completion for an additional 30 min. This hydroxylamine was isolated by filtration and washed with cold THF (2×20 mL). Water was removed azeotropically by refluxing this material in 60 mL sodium-dried benzene under dehydrating conditions (Dean–Stark) for 2 h. (The milky suspension developed into a clear solution as the reaction progressed.) The benzene was removed under reduced pressure and the Schiff base [8-(2-phenylethylimino)pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-11-one, a yellowish oil] was dissolved in a mixture of anhydrous MeOH (30 mL) and anhydrous THF (150 mL). $NaBH_4$ (1.62 g, 42.72 mmol) was added in excess and the imine was subsequently reduced by refluxing the mixture for 5 h at 35 °C. The solvents were removed in vacuo, the residue suspended in water (100 mL) and extracted with DCM (4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to yield a yellowish oil. Partial resolution of the product mixture was accomplished with column chromatography and the contaminated amine isolated as a yellow tainted wax. This product was dissolved in acetone (50 mL) and cooled down to −15 °C while stirring on an external ethanol bath. Forced evaporation of the acetone with a mild stream of N_2 gas caused oversaturation and consequent precipitation of a colourless solid. The latter was isolated by decantation and allowed to reach room temperature, affording the purified amine as a colourless wax. (Yield: 2.54 g, 9.10 mmol, 31.52%.) $C_{19}H_{21}NO$; mp 49.7 °C; HR-MS: calcd 279.1534, exp. 279.1538; IR (KBr) ν_{max}: 3312, 2974, 1353, 846, 699 cm^{-1}; MS (EI, 70 eV) *m/z*: 279 (M$^+$), 188, 159, 131, 105, 91, 77; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 7.29-7.13 (m, 5H, H-17, 18, 19, 20, 21), 4.59 (t, 1H, *J*=5.2 Hz, H-11), 3.11–2.99 (m, 2H, H-14a, 14b), 2.82–2.44 (3×m, 9H, H-1, 2, 3, 5, 6, 7/9, 10, 15a, 15b), 2.38 (t, 1H, *J*=4.9 Hz, H-7/9), 1.95 (bs, 1H, H-13), 1.87:1.51 (AB-q, 2H, *J*=10.4 Hz, H-4a, 4b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 139.99 (s, C-16), 128.76 (d, C-17, 21), 128.41 (d, C-18, 20), 126.10 (d, C-19), 109.43 (s, C-8), 82.40 (d, C-11), 55.31 (d, C-7/9), 54.67 (d, C-7/9), 44.87 (t, C-14), 44.72 (d, 1C), 44.54 (d, 1C), 44.40 (d, 1C), 43.18 (t, C-4), 42.96 (d, 1C), 41.84 (d, 1C), 41.43 (d, 1C), 37.21 (t, C-15).

8-Cyclohexylmethylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (10). Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (5.01 g, 28.77 mmol) was dissolved in THF (50 mL) and cooled down to 5 °C. (Aminomethyl)cyclohexane (3.31 g, 29.30 mmol) was added slowly, continuously agitating the reaction mixture at lowered temperature. The precipitated carbinolamine was isolated by filtration and washed with cold THF (2×20 mL). Water was removed azeotropically by refluxing this material in 60 mL sodium-dried benzene (Dean–Stark conditions) for 90 min. The benzene was removed under reduced pressure and the Schiff base [8-(cyclohexylmethylimino)pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-11-one, an amber-coloured oil] was dissolved in a mixture of anhydrous MeOH (30 mL) and anhydrous THF (150 mL). $NaBH_4$ (1.52 g, 39.98 mmol) was added in excess and the mixture refluxed for 5 h at 35 °C. The solvents were removed and the residue suspended in water (100 mL) and extracted with DCM (4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was dissolved in acetone (50 mL) and cooled down to −15 °C on an external ethanol bath. Forced evaporation of the acetone with a mild stream of N_2 gas caused precipitation of a colourless solid, which was quickly isolated by decantation and allowed to reach room temperature, affording the purified amine as a colourless glass. (Yield: 2.22 g, 8.18 mmol, 28.43%.) $C_{18}H_{25}NO$; mp 58.9 °C; HR-MS: calcd 271.1936, exp. 271.1930; IR (KBr) ν_{max}: 3323, 2923, 1489, 1010, 851 cm^{-1}; MS (EI, 70 eV) *m/z*: 272 [(M$^+$)+1], 271 (M$^+$), 188, 176, 159,

131, 91, 55, 28; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 4.57 (t, 1H, $J=5.2$ Hz, H-11), 2.79–2.41 (3×m, 9H, H-1, 2, 3, 5, 6, 7/9,10,14a,14b), 2.36 (t, 1H, $J=5.0$ Hz, H-7/9), 1.88 (bs, 1H, H-13), 1.85:1.49 (AB-q, 2H, $J=10.5$ Hz, H-4a,4b), 1.74–0.81 (4×m, 11H, H-15, 16a, 16b, 17a, 17b, 18a, 18b, 19a, 19b, 20a, 20b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 109.83 (s, C-8), 82.35 (d, C-11), 55.04 (d, C-7/9), 54.69 (d, C-7/9), 50.19 (t, C-14), 44.78 (d, 2C), 44.43 (d, 1C), 43.16 (t, C-4), 42.97 (d, 1C), 41.81 (d, 1C), 41.44 (d, 1C), 38.77 (d, C-15), 31.20 (t, C-16,20), 26.55 (t, C-18), 25.89 (t, C-17,19).

8-[2-(4-Hydroxyphenyl)ethylamino]-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (11). Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (2.59 g, 14.87 mmol) and tyramine (2.08 g, 15.15 mmol) were dissolved separately in dry MeOH (30 mL each). These two solutions were combined and continually stirred at ambient temperature for 50 h. The MeOH was removed in vacuo and 70 mL sodium-dried benzene added to the amber-coloured residue. Water was removed by slowly refluxing this mixture under Dean-Stark dehydrating conditions for 2 h. The benzene was removed under reduced pressure and the Schiff base, 8-[2-(4-hydroxyphenyl)ethylimino]-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-11-one, was dissolved in a mixture of anhydrous MeOH (30 mL) and anhydrous THF (150 mL). Reduction was induced by adding $NaBH_4$ (0.90 g, 23.76 mmol) in excess and slowly refluxing the amber solution for 6 h. The solvents were removed, the residue suspended in water (100 mL) and extracted with DCM (4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous $MgSO_4$ and evaporated to yield an amber-coloured oil. Column chromatography produced an oily substance that was refluxed in acetone with activated charcoal for 15 min. After filtration through Celite® the acetone was removed and the desired amine crystallised from dry MeOH at 5 °C to render colourless grains. (Yield: 1.40 g, 4.75 mmol, 31.91%.) $C_{19}H_{21}NO_2$; mp 160.9 °C; HR-MS: calcd 295.1673, exp. 295.1676; IR (KBr) ν_{max}: 3260, 2970, 1516, 1249, 1016, 827 cm^{-1}; MS (EI, 70 eV) *m/z*: 295 (M^+), 188, 91, 66, 32, 28; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 6.97 (d, 2H, $J=8.5$ Hz, H-18,21), 6.62 (d, 2H, $J=8.5$ Hz, H-17, 22), 4.62 (t, 1H, $J=5.2$ Hz, H-11), 4.01 (bs, 1H, H-20), 3.07–2.97 (m, 2H, H-14a, 14b), 2.83–2.49 (2×m, 9H, H-1, 2, 3, 5, 6, 7/9, 10, 15a, 15b), 2.38 (t, 1H, $J=4.9$ Hz, H-7/9), 1.86–1.50 (AB-q, 2H, $J=10.4$ Hz, H-4a, 4b), (H-13∼no signal); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 154.85 (s, C-19), 130.99 (s, C-16), 129.79 (d, C-17, 22), 115.47 (d, C-18, 21), 109.92 (s, C-8), 82.47 (d, C-11), 55.28 (d, C-7/9), 54.69 (d, C-7/9), 45.00 (t, C-14), 44.76 (d, 1C), 44.50 (d, 1C), 44.42 (d, 1C), 43.16 (t, C-4), 43.02 (d, 1C), 41.86 (d, 1C), 41.44 (d, 1C), 35.81 (t, C-15).

8-(4-Pyridylmethylamino)-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (12). The pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (5.09 g, 29.23 mmol) was dissolved in THF (50 mL) and cooled down to 5 °C while stirring on an external ice bath. 4-(Aminomethyl)pyridine (3.24 g, 30.02 mmol) was added slowly, with continued stirring of the reaction mixture at lowered temperature. The white carbinolamine started precipitating after approximately 3 min, but the reaction was allowed to reach completion for an additional 15 min. The latter product was isolated by filtration and washed with cold THF (2×20 mL). Water was removed azeotropically by refluxing this material in 60 mL sodium-dried benzene (Dean–Stark conditions) for 90 min. (The milky suspension developed into a clear solution as the reaction progressed.) The benzene was removed under reduced pressure and the Schiff base [8-(4-pyridylmethylimino)pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane - 11-one, a yellow oil] was dissolved in a mixture of anhydrous MeOH (30 mL) and anhydrous THF (150 mL). $NaBH_4$ (1.29 g, 33.86 mmol) was added in excess and the mixture stirred overnight at ambient temperature. (Decolouration indicated successful reduction of the imine.) The solvents were removed under reduced pressure, the residue suspended in water (100 mL) and extracted with DCM (4×50 mL). The combined organic fractions were washed with water (2×100 mL), dried over anhydrous MgSO4 and evaporated in vacuo to yield a yellow-green wax with signs of crystallisation. Purification of this product mixture was accomplished with column chromatography and slow recrystallisation from DCM–acetone (1/1) at 5 °C rendered a colourless microcrystalline solid. At room temperature the latter transformed into an amorphous white powder. (Yield: 4.12 g, 15.48 mmol, 52.94%.) $C_{17}H_{18}N_2O$; mp 112.7 °C; HR-MS: calc. 266.1419, exp. 266.1415; IR (KBr) ν_{max}: 3295, 2977, 1374, 1009, 797 cm^{-1}; MS (EI, 70 eV) *m/z*: 266 (M^+), 238, 187, 174, 131, 92, 65, 39; ^{1}H NMR (300 MHz, $CDCL_3$) δ_H: 8.46 (d, 2H, $J=5.7$ Hz, H-17, 19), 7.23 (d, 2H, $J=5.7$ Hz, H-16, 20), 4.59 (t, 1H, $J=5.3$ Hz, H-11), 3.98 (AB, 1H, $J_{AB}=15.5$ Hz, H-14a), 3.92 (AB, 1H, $J_{AB}=15.5$ Hz, H-14b), 2.80–2.35 (3×m, 8H, H-1, 2, 3, 5, 6, 7, 9, 10), 2.29 (bs, 1H, H-13), 1.83:1.48 (AB-q, 2H, $J=10.5$ Hz, H-4a, 4b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 150.39 (s, C-15), 149.76 (d, C-17,19), 122.51 (d, C-16, 20), 109.40 (s, C-8), 82.47 (d, C-11), 55.26 (d, C-7/9), 54.64 (d, C-7/9), 46.44 (t, C-14), 44.78 (d, 1C), 44.70 (d, 1C), 44.44 (d, 1C), 43.12 (t, C-4), 43.01 (d, 1C), 41.82 (d, 1C), 41.39 (d, 1C).

8-(4-Aminobenzylamino)-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane (13). Tin powder (1.32 g, 11.05 mmol) and previously prepared 8-(4-nitrobenzylamino)-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**5**, 2.56 g, 8.27 mmol) were weighed in a 50-mL round-bottomed flask and set up under reflux conditions. HCl (10%, 20 mL) was added slowly down the Liebig condenser, continually stirring the mixture while elevating the reaction temperature to reflux level. Ethanol (3 mL) was added as a solubilisation agent. Further HCl additions (2×30 mL) were made consecutively at 10-min intervals, allowing the reduction of the nitro group to reach completion over a period of 70 min. The clear supernatant was then isolated by decantation, cooled down to room temperature and neutralised past the turning point with NaOH (40%). Extraction was done with DCM (4×50 mL), the combined organic fractions washed with brine (2×50 mL), dried over anhydrous $MgSO_4$ and evaporated in vacuo. Column chromatography afforded the desired 4-aminobenzylamino-derivative as a viscous,

amber-coloured substance. (Yield: 1.02 g, 3.65 mmol, 44.11%.) $C_{18}H_{20}N_2O$; HR-MS: calcd 280.1576, exp. 280.1583; IR (neat) ν_{max}: 3343, 2964, 1616, 1517, 1275, 1009 cm^{-1}; MS (EI, 70 eV) *m/z*: 281 [$(M^+)+1$], 280 (M^+), 175, 131, 106, 91, 77, 28; ^1H NMR (300 MHz, $CDCL_3$) δ_H: 7.12 (d, 2H, $J=8.5$ Hz, H-17, 20), 6.60 (d, 2H, $J=8.5$ Hz, H-16, 21), 4.63 (t, 1H, $J=5.2$ Hz, H-11), 3.87 (AB, 1H, $J_{AB}=12.7$ Hz, H-14a), 3.82 (AB, 1H, $J_{AB}=12.7$ Hz, H-14b), 3.56 (bs, 2H, H-19a, 19b), 2.84–2.48 (2×m, 7H, H-1, 2, 3, 5, 6, 7/9, 10), 2.39 (t, 1H, $J=4.9$ Hz, H-7/9), 2.11 (bs, 1H, H-13), 1.88–1.52 (AB-q, 2H, $J=10.5$ Hz, H-4a, 4b); ^{13}C NMR (75 MHz, $CDCL_3$) δ_C: 145.34 (s, C-18), 130.79 (s, C-15), 129.11 (d, C-16, 21), 115.12 (d, C-17, 20), 109.61 (s, C-8), 82.40 (d, C-11), 55.14 (d, C-7/9), 54.70 (d, C-7/9), 47.29 (t, C-14), 44.76 (d, 2C), 44.45 (d, 1C), 43.14 (t, C-4), 43.03 (d, 1C), 41.90 (d, 1C), 41.43 (d, 1C).

Computer modelling and minimisation

Modelling and structural optimisation were done using the Chemplus™ extension of Hyperchem® modelling software.[8] A previously minimised structure[21] of the lead compound (**6**) served as the initial conformer geometry to which functional group substitutions were made to comprise all components in the series. MM^+ and AM1 were used in sequence to carry out molecular and electronic calculations. Ground state energies were optimised using the Polak–Ribieré procedure.[22] Theoretical physicochemical parameters were computed for all the minimised structures.

Partitioning measurements

Log P_{oct}. Solvent phases of 1-octanol and double-distilled, de-ionised water were thoroughly cross-saturated to equilibration in a separatory funnel for 24 h. Standard solution preparations and concentration determinations were all performed within the 1-octanol phase at room temperature (25 °C). A stock solution of 50.0 μg mL^{-1} was prepared for each test compound and further dilutions performed to render a standard concentration series of 25.0, 20.0, 15.0, 10.0 and 5.0 μg mL^{-1}. 5-mL aliquots of the stock solution were then pipetted into equal volumes (5 mL) of the octanol-saturated water, using Pyrex vials with Teflon-sealed screw caps. The vials were vigorously shaken on a Labotec® reciprocal shaker for 60 min in order to achieve equilibrium partitioning, followed by centrifugation for 30 min at 2500 rpm to effect phase separation. UV absorbance readings for stock, standard and sample solutions were obtained in five-fold on a Shimadzu UV120-02 spectrophotometer at λ_{max}: 211.0 nm (compound **6**), 271.6 nm (**7p**), 211.0 nm (**9**), 207.8 nm (**10**), 224.8 nm (**11**), 208.2 nm (**12**) and 209.6 nm (**13**). Concentrations were calculated from constructed linearly regressed standard curves. The corresponding water-phase concentrations were obtained by the difference between stock and sample solution absorption values.

Log P_{HB}. Equilibrated solvent phases of *n*-hexane and ethylene glycol were used for log P_{HB} determinations. The same procedure was followed as for the establishment of log P_{oct} values. Standard solution preparations and concentration determinations were however performed within the phase of optimal solubility at room temperature (25 °C). Determinations were done in five-fold at λ_{max} −208.4 nm (compound **6** in *n*-hexane), 277.0 nm (**7p** in ethylene glycol), 210.2 nm (**9** in *n*-hexane), 200.0 nm (**10** in *n*-hexane), 224.2 nm (**11** in ethylene glycol), 207.0 nm (**12** in ethylene glycol) and 208.0 nm (**13** in ethylene glycol).

Acquisition of brain–blood distribution data

All animal trials were conducted on adult male C57Bl/6 laboratory mice (25–30 g), with approval and in accordance with the guidelines as laid out by the Medical Ethics Committee of the Potchefstroom University for Christian Higher Education.

Administration and tissue harvesting

Test compounds were dissolved in a 80% (w/v) 2-hydroxypropyl-β-cyclodextrin solution in water for injection, by means of sonification and mild heating. After notation of body mass, this freshly constituted preparation (37.5 mg mL^{-1}) was administered intraperitoneally at an arbitrary dosage of 300 mg kg^{-1} (±0.2 mL per mouse), after which animals were allowed free movement and ad lib access to water and food. (Dosage regimens of 200–400 mg kg^{-1} for each compound were tested beforehand to eliminate the possibility of lethal dosing, but no lethal effects were observed within 120 min post administration.) Animals were promptly sacrificed by decapitation 60 min after administration and whole brain and blood samples collected in pre-weighed open-topped 15 mL Pyrex® vials and 4.5 mL 15% K3E anticoagulant-containing BD Vacutainer™ vials, respectively. Samples were put on ice for immediate further processing.

Standardisation and analyte recovery

Each component analysis comprised three groups of animals, each group consisting of six subjects—an experimental group receiving the compound under investigation, a control group receiving dose-free, but equal volumes (0.2 mL) of the injection medium, and a standard group from which analyte-free brain and blood tissues were harvested for constructing two independent quantitative calibration (standard) curves. Extraction efficiency was not determined beforehand, but rather incorporated into these linear regression curves by introducing both standard and internal standard solutions into equal aliquots of the respective brain and blood standard tissues and submitting both to the same extraction procedure as for the samples and controls. The assumption was made that the quantitative recovery percentage remained constant with regard to small experimental error-variance among the six different standard tissues in each group. A stock solution in $CHCl_3$ (400 μg mL^{-1}) was prepared in each instance and successively diluted into whole blood and brain tissue to render a series of final working standards for ESI-MS.MS detection, ranging between 1.0

and 34.0 μg mL^{-1}, covering the concentrations detected within the sample assay. Compound **10** (35.0 μg mL^{-1}) was uniformly employed as internal standard, while **6** served this purpose in the constituent analysis of **10** itself.

Brain tissue extraction

After careful notation of sample weight, the organs were rinsed with ice-cold water (2×1 mL); HCl (10%; 2 mL) was added to each vial and the tissue homogenised using a Polytron® homogeniser. (The rotator head was thoroughly rinsed with methanolic water between applications, in order to prevent cross-contamination.) The acidic homogenates were then transferred to polyethylene vials (Miniature Pony Vial™), standard and internal standard solutions added and the mixtures vortexed for 50 s. Following centrifugation (15 min at 16,000 rpm; 4 °C), the supernatants were decanted and neutralised with ammonia solution (5%; 2 mL), establishing a final pH of 7–9. $CHCl_3$ (3 mL) was added and the deprotonated amine extracted by vortexing the mixture for 60 s. Solvent phases were separated by centrifugation (5 min at 2800 rpm), the aqueous layer discarded and the organic fraction brought to dryness under a mild stream of nitrogen gas at room temperature (25 °C). The organic residue was re-dissolved in mobile phase (1 mL) composed of 1% formic acid in acetonitrile–water (3/2). A general factor 10 dilution was then performed to render the solutions fit for ESI-MS.MS analysis without significant cross-contamination.

Blood extraction

Sample weight was carefully noted, standard and internal standard solutions introduced and HCl (10%; 2 mL) added to each vial. The mixtures were vortexed for 50 s. Following centrifugation (15 min at 2800 rpm; 4 °C), the supernatants were decanted and neutralised with ammonia solution (5%; 2 mL), establishing a final pH of 7–9. Chloroform (3 mL) was added and the deprotonated amine extracted by vortexing the mixture for 60 s. Solvent phases were separated by centrifugation (5 min at 2800 rpm), the aqueous layer discarded and the organic fraction brought to dryness under a mild stream of nitrogen gas at room temperature (25 °C). The organic residue was re-dissolved in mobile phase (1 mL) composed of 1% formic acid in acetonitrile–water (3/2) by vortexing the vials for 60 s. A general factor 10 dilution was then performed to render the solutions fit for ESI-MS.MS analysis without significant cross-contamination.

Instrumentation and analysis

Electrospray ionisation tandem mass spectrometry (ESI-MS.MS) analysis was performed using a VG Quattro II tandem mass spectrometer (Micromass, Manchester, UK) equipped with a HP1090 liquid chromatograph. Standard samples of each of the seven synthesised compounds were prepared in the mobile phase [1% formic acid in acetonitrile–water (3/2)] and the relevant daughter ion spectra acquired for establishing the selectivity required for direct sample injection. Based on selected daughter ion peaks, conditions for each component's analysis were then optimised. Pertaining to the conditions as for 8 - cyclohexylmethylamino - 8,11 - oxapentacyclo [5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane (**10**), the internal standard, the capillary tip was held at 3.50 kV and the cone voltage maintained at 41 to produce the relevant [(M^+) + 1] parent species (272^+ m/z). Argon-induced fragmentation was instigated at a pressure of 1.4×10^{-3} mBar, with the collision energy setting at 24.7 eV. The resolution of MS2 was held at 13.0 and selective integration values attained using parent ion (PAR) scans in the positive ion mode. Dual function programming allowed for the mutually exclusive quantification of the internal standard and the compound under investigation, both from the same sample. After confirming the absence of analyte in the control samples, calibration curves were employed and sample concentrations were related back to the original tissue mass.

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References and Notes

1. Oliver, D. W.; Dekker, T. G.; Snyckers, F. O. *Arzneim.-Forsch.* **1991**, *41*, 549.
2. Oliver, D. W.; Dekker, T. G.; Snyckers, F. O. *Eur. J. Med. Chem.* **1991**, *26*, 375.
3. Malan, S. F.; Van den Heever, I.; Van der Schyf, C. J. *J. Pharm. Med.* **1996**, *6*, 125.
4. Nguyen, V. H.; Mardon, K.; Kassiou, M.; Christie, M. J. *Nucl. Med. Biol.* **1999**, *26*, 209.
5. Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.
6. *ACD/ChemSketch® 4.54*; Advanced Chemistry Development Inc.: Toronto, Canada, 2000.
7. *ACD/LogP® 4.54*; Advanced Chemistry Development Inc.: Toronto, Canada, 2000.
8. *Hyperchem for Windows®*; Hypercube Inc.: Ontario, Canada, 1994.
9. Burton, P. S.; Conradi, R. A.; Hilgers, A. R.; Ho, N. F. H.; Maggiora, L. L. *J. Control. Release* **1992**, *19*, 87.
10. Ghose, A. K.; Crippen, G. M. *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 21.
11. Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robins, R. K. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163.
12. Miller, K. J. *J. Am. Chem. Soc.* **1990**, *112*, 8533.
13. Bodor, N.; Gabanyi, Z.; Wong, C.-K. *J. Am. Chem. Soc.* **1989**, *111*, 3783.
14. Gavezzotti, A. *J. Am. Chem. Soc.* **1983**, *105*, 5220.
15. Clark, D. E. *J. Pharm. Sci.* **1999**, *88*, 815.
16. Hansch, C. *Acc. Chem. Res.* **1969**, *2*, 232.
17. Feher, M.; Sourial, E.; Schmidt, J. M. *Int. J. Pharm.* **2000**, *201*, 239.
18. Chikhale, E. G.; Ng, K.-Y.; Burton, P. S.; Borchard, R. T. *Pharm. Res.* **1994**, *11*, 412.
19. Abraham, M. H.; Chadha, H. S.; Mitchell, R. C. *J. Pharm. Sci.* **1994**, *83*, 1257.

20. Cookson, R. C.; Grundwell, E.; Hudec, J. *Chem. Ind. (London)* **1958**, 1003.
21. Malan, S. F.; Van der Walt, J. J.; Van der Schyf, C. J. *Arch. Pharm. Pharm. Med. Chem.* **2000**, *333*, 10.
22. Polak, E. *Computational Methods in Optimization, a Unified Approach*; Academic: New York, 1971.
23. Lombardo, F.; Blake, J. F.; Curatolo, W. J. *J. Med. Chem.* **1996**, *39*, 4750.