CSIRO Publishing

AUSTRALIAN JOURNAL OF CHEMISTRY

AN INTERNATIONAL JOURNAL FOR CHEMICAL SCIENCE

publishing research papers from all fields of chemical science, including synthesis, structure, new materials, macromolecules, supramolecular chemistry, biological chemistry, nanotechnology, surface chemistry, and analytical techniques.

Volume 55, 2002

All enquiries and manuscripts should be directed to:

Dr Alison Green
Australian Journal of Chemistry – an International Journal for Chemical Science
CSIRO PUBLISHING
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7630
Fax: +61 3 9662 7611
E-mail: publishing.ajc@csiro.au

Published by CSIRO PUBLISHING
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/ajc

Aust. J. Chem. **2002**, *55*, iii

Cyclic Voltammetry as an Indicator of Antioxidant Activity

Alexandra Papanikos,[A,B] *John Eklund*,[A] *W. Roy Jackson*,[C,D] *Vijaya B. Kenche*,[A] *Eva M. Campi*,[C] *Alan D. Robertson*,[E] *Bevyn Jarrott*,[F] *Philip M. Beart*,[F] *Fiona E. Munro*[F] *and Jennifer K Callaway*[F]

[A] School of Chemistry, P.O. Box 23, Monash University, Vic. 3800, Australia.
[B] Current address: Carlsberg Laboratory, Department of Chemistry, Copenhagen DK-2500, Denmark.
[C] Centre for Green Chemistry, P.O. Box 23, Monash University, Vic. 3800, Australia.
[D] Author to whom correspondence should be addressed (e-mail: w.r.jackson@sci.monash.edu.au).
[E] AMRAD Operations Pty Ltd, Richmond, Vic. 3121, Australia.
[F] Department of Pharmacology, Monash University, Clayton, Vic. 3800, Australia.

Aust. J. Chem. **2002**, *55*, pp. 205–212

Please note the correct affiliation of authors as indicated above.

0004-9425/02/050iii

Aust. J. Chem. **2002**, *55*, 205–212

Cyclic Voltammetry as an Indicator of Antioxidant Activity

Alexandra Papanikos,[A,B] *John Eklund*,[A] *W. Roy Jackson*,[C,D] *Vijaya B. Kenche*,[A] *Eva M. Campi*,[C] *Alan D. Robertson*,[D] *Bevyn Jarrott*,[E] *Philip M. Beart*,[E] *Fiona E. Munro*[E] *and Jennifer K Callaway*[E]

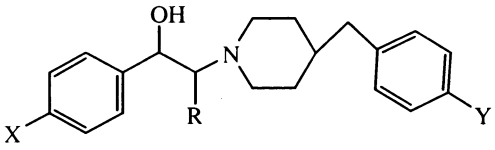
[A] School of Chemistry, P.O. Box 23, Monash University, Vic. 3800, Australia.
[B] Current address: Carlsberg Laboratory, Department of Chemistry, Copenhagen DK-2500, Denmark.
[C] Centre for Green Chemistry, P.O. Box 23, Monash University, Vic. 3800, Australia.
[D] Author to whom correspondence should be addressed (e-mail: w.r.jackson@sci.monash.edu.au).
[E] AMRAD Operations Pty Ltd, Richmond, Vic. 3121, Australia.
[F] Department of Pharmacology, Monash University, Clayton, Vic. 3800, Australia.

A series of compounds based around combining the neuroprotective properties of non-competitive *N*-methyl D-aspartic acid (NMDA) receptor antagonists with antioxidant functionalities have been prepared. The redox chemistry of these compounds has been evaluated using cyclic voltammetry, and the results have been compared with their radical-scavenging properties obtained from two standard biological assays, the inhibition of lipid peroxidation (thiobarbituric acid reacting substances assay) and the Sapphire colorimetric assay. Results from these different methods show general concordance. The most effective antioxidants were substituted phenols, e.g. Trolox®. The antioxidant activity of a series of pyrimidines was shown to be dependent on the presence of three amino substituents.

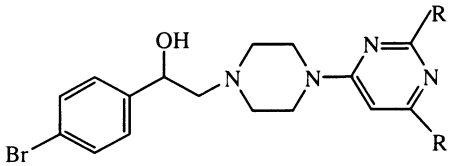
Manuscript received: 5 December 2001.
Final version: 4 February 2002.

Introduction

Novel hybrid molecules based around combining the neuroprotective properties of derivatives of the non-competitive NMDA receptor antagonists ifenprodil (1) and eliprodil[1,2] (2) with different antioxidant functionalities have been prepared.[3,4] The aim of linking two differently acting neuroprotective agents together in a single molecule was to obtain additive or even synergistic effects on the biological activity. Antioxidant groups used for structural modifications of ifenprodil fragments were based on triaminopyrimidines, which have been previously incorporated into steroids e.g. tirilazad mesylate.[5] This strategy has led to compounds (3), (4), and (5). In addition, incorporation of the more commonly used antioxidants,[5], butylated hydroxytoluene and Trolox®, has led to compounds (6) and (7). The antioxidant properties of each of these molecules have been evaluated using cyclic voltammetry, inhibition of lipid peroxidation (thiobarbituric acid reacting substances, TBARS, assay)[6] and a Sapphire colorimetric assay.[6,7] The compounds have shown great promise in the management of ischaemic episodes, such as stroke, and one of them is undergoing Phase 1 trials in a London hospital. A large number of compounds with substituent variation throughout molecules (3)–(7) have been prepared and evaluated. We felt it necessary to establish an independent, non-biological method for the evaluation of antioxidant activity for these molecules. The samples chosen for this study were those with the highest activity.



(1), X = OH, Y = H, R = CH_3
(2), X = Cl, Y = F, R = H

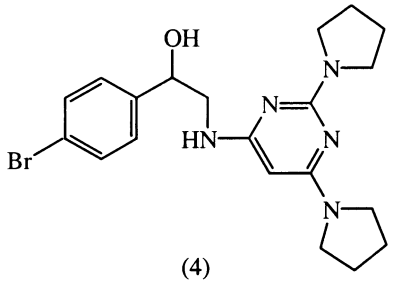


(3a), R = 

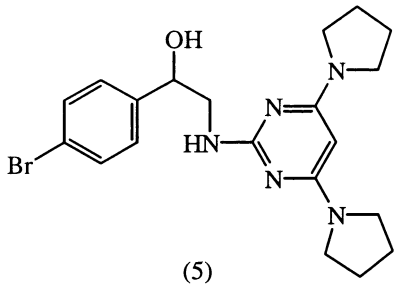
(3b), R = 

(3c), R = 

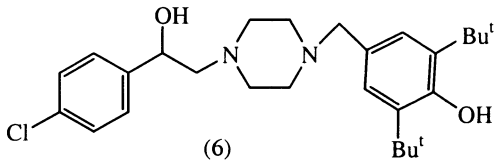
 10.1071/CH01193 0004-9425/02/030205



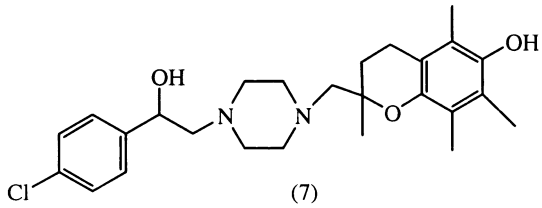
(4)



(5)



(6)



(7)

Results and Discussion

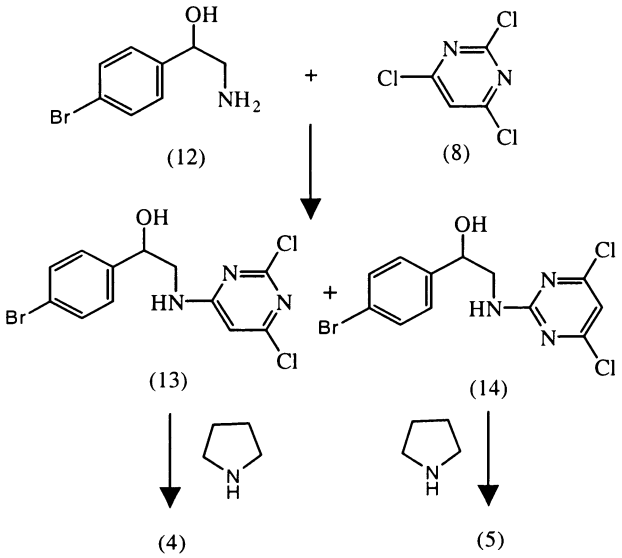
Chemistry

Triaminopyrimidines (3a–c)

These compounds were prepared by the method outlined in Scheme 1. 2,4,6-Trichloropyrimidine (8) was reacted with the appropriate amine, pyrrolidine, diethylamine, or morpholine to give mainly the 2,4-diamino-6-chloropyrimidines (9a–c). Reaction with piperazine at 120°C in a Carius tube using pyridine as a solvent gave (10a–c) in superior yields to those reported using milder reaction conditions.[8]. Reaction with α-bromo-*p*-bromoacetophenone followed by reduction of the intermediate ketones (11) gave the target amino alcohols (3a–c).

Unsymmetrical (4) and Symmetrical (5) Ethanolaminopyrimidines

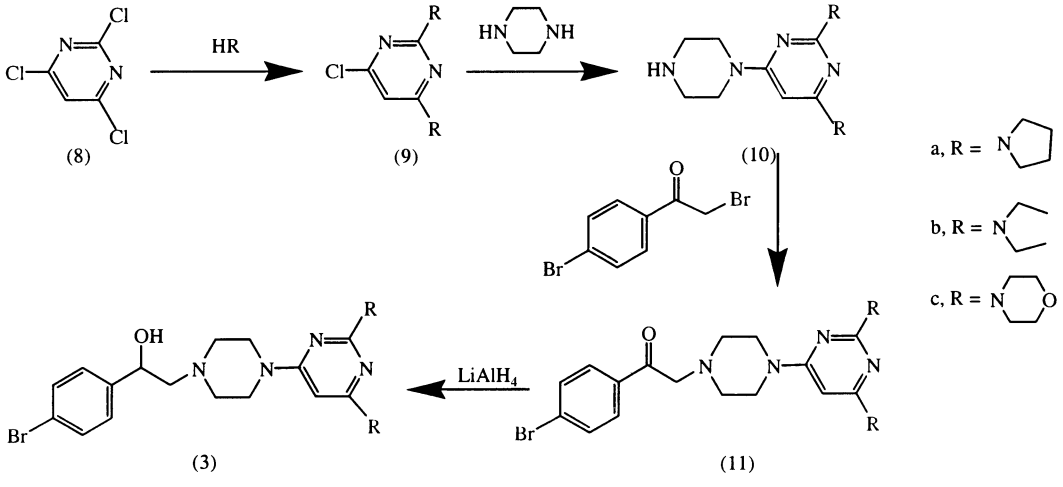
Reaction of the ethanolamine (12) with 2,4,6-trichloropyrimidine (8) gave the readily separated unsymmetrical (13) and symmetrical (14) dichloropyrimidines which, on reaction with pyrrolidine, gave the target compounds (4) and (5) (Scheme 2).



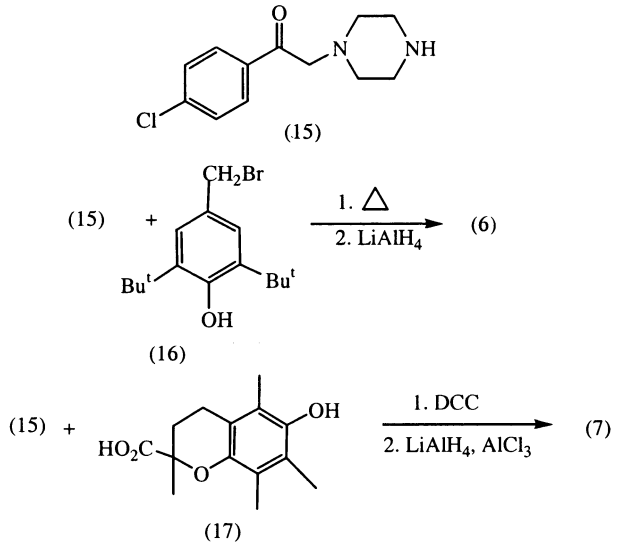
Scheme 2

The t*-Butylhydroxytoluene Derivative (6) and the Trolox® Derivative (7)*

α-Bromo-*p*-chloroacetophenone was reacted with ethylpiperazinecarboxylate and the product decarboxylated to give (15). This compound was coupled with the bromomethylphenol (16) and the product reduced to give (6) (Scheme 3). 1,3-Dicyclohexylcarbodiimide promoted



Scheme 1



Scheme 3

coupling of (15) with the Trolox® acid (17), and reduction of the resulting amide with $LiAlH_4/AlCl_3$ gave (7) (Scheme 3).

The Hydroxyethylpyrimidine (20)

6-Methyl-2,4-bis(pyrrolidin-l-yl)pyrimidine (18) was lithitated by heating under reflux with Bu^{n}Li in the presence of *N,N,N′,N′*-tetramethylethylenediamine to form the lithium salt (19) which was reacted with *p*-bromobenzaldehyde to give (20) (Scheme 4).

Cyclic Voltammetry Studies

Initial attempts to study the cyclic voltammograms of the above compounds involved the use of conditions previously described for recording the cyclic voltammograms of the 21-aminosteroid containing a dipyrrolidinopiperazopyrimidine, Tirilazad mesylate (U74-006F), an antioxidant described by the Upjohn Company.[9] The voltammogram was recorded for solutions in 30% (v/v) aqueous ethanol with 0.2 M sodium acetate as buffer. A peak oxidative potential (E_{pl}^{ox}) of 0.23 V was recorded (versus Ag/AgCl as the reference electrode, scan rate 100 mV s^{-1}) using a glassy carbon working electrode and a Pt auxiliary electrode. The voltammogram of compound (3a), which bears the same antioxidant moiety as U74-006F, was recorded using the conditions described above. The initial voltammogram appeared to show that compound (3a) exhibited identical voltammetric behaviour to that of U74-006F, but it was later revealed that the same voltammogram could be produced even in the absence of compound (3a). It was concluded that the observed electrochemical process was associated with oxidation of the acetate buffer.

Further cyclic-voltammetric studies were thus carried out in either 0.1 M aqueous KCl solution or in CH_3CN with Bu_4NPF_6 as the electrolyte. The latter medium was used for bulk electrolysis experiments. The peak oxidative potentials recorded at a scan rate of 100 mV s^{-1} were similar in both solvent systems.

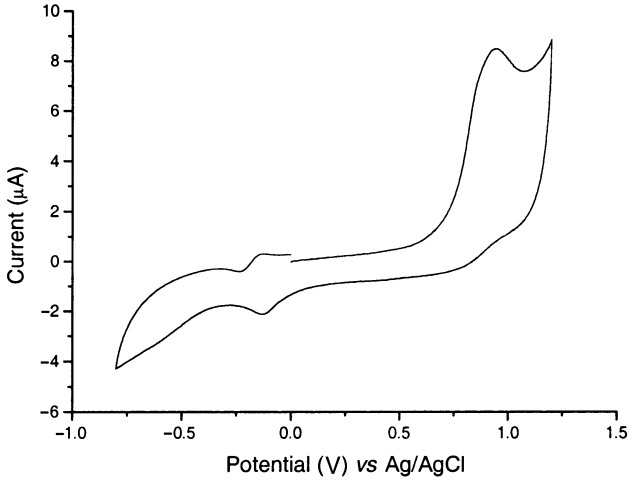
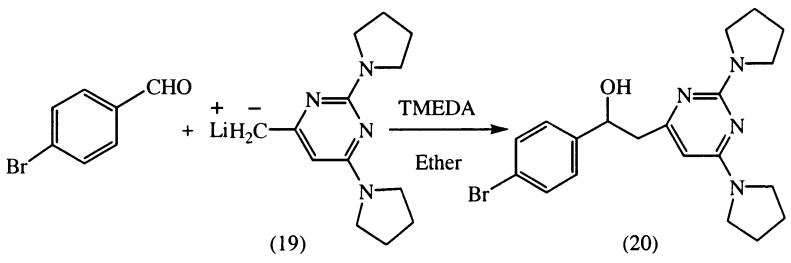


Fig. 1. Cyclic voltammogram of compound (4).

The three closely related compounds (3a–c) exhibited similar voltammetric behaviour and the results are summarized in Table 1. An irreversible anodic oxidation (peak oxidative potential, E_{pl}^{ox}) was evident at scan rates between 10 and 2000 mV s^{-1}, indicating that the cation-radical formed initially decomposed within the time-scale of these voltammetric experiments. The oxidation potential of the three compounds (3a–c) showed some variation, with the pyrrolidinyl compound (3a) being the strongest antioxidant (lowest E_{pl}^{ox} at 0.85 V). The redox process was found to be diffusion controlled as the peak oxidative current (I_p^{ox}) scaled linearly with the square root of the scan rate.[10,11] Evidence for a small amount of absorption onto the electrode surface was found in a few cases at high scan rates due to observed decreases in the peak oxidative current. A voltammogram for the closely related compound (4), which does not contain the piperazine ring of (3a–c), is shown in Figure 1 and closely resembles those for (3a–c). In addition to the irreversible oxidation at 0.94 V, evidence for a reversible reduction at –0.12 V



Scheme 4

Table 1. Comparison of electrochemical and biological evaluations of antioxidant activity

Cpd No.	E_{pl}^{ox} (V)	TBARS assay Fe^{3+} (100 mM)[A] IC_{50} (mM)[B]	max[C]	TBARS assay Fe^{3+} (1000 mM)[A] IC_{50} (mM)[B]	max[C]	Sapphire assay Fe^{3+} (100 mM)[A] IC_{50} (mM)[B]	max[C]
(3a)	0.85	24 (7–44)	90	70[E]	55	123 (87–180)	87
(3b)	0.90	146 (55–443)	63	191 (35–3533)	81	559 (444–864)	76
(3c)	0.98	386 (319–469)	63	542 (463–634)	59	393 (298–528)	68
(4)	0.94	427 (288–718)	49	700 (510–1305)	40	147 (62–528)	39
(5)	0.99	800 (626–1497)	71	839 (559–990)	29	902 (788–1175)	51
(6)	0.78	50 (39–64)	95	37 (22–53)	72	62 (46–82)	81
(7)	0.50	3 (1.5–5)	97	19 (9–40)	91	4 (2.5–5.5)	95
(20)	[D]	> 1000	62	> 1000	70	> 1000	41

[A] Concentration of Fe^{3+} used to stimulate lipid peroxidation. [B] 95% confidence limits in parentheses. [C] Maximum percentage inhibition. [D] No oxidative behaviour. [E] Confidence limits not calculated.

(E_p^{red}), associated with reduction of the pyrimidinium salts, was observed. The mechanism of the electrochemical reduction of pyrimidinium salts has been studied previously.[12]

In order to identify a minimum structural element required for antioxidant activity, the electrochemical behavior of the isolated fragments, 2,4-diamino-6-chloropyrimidine, piperazine, α-bromo-*p*-bromacetophenone, and compound (20) (which lacks the nitrogen substitution at C(6) of the pyrimidine ring compared with (3a)) was investigated. All these compounds showed no oxidation potential. This result strongly suggests that the minimum structural element required for antioxidant activity is a 2-amino-substituted pyrimidine (as in (5)), or a 6-amino-substituted pyrimidine (as in (3) and (4)), but more likely triamino-substitution of the pyrimidine is essential.

The symmetrically substituted compound (5) had a slightly higher oxidation potential (E_{pl}^{ox} 0.99 V) than the unsymmetrical analogue (4) (E_{pl}^{ox} 0.94 V). Bulk electrolysis of (4), followed by liquid chromatography mass spectrometry of the resulting solution, showed an oxidized species with *m/z* $[M-2]^+$ as the major product, suggesting loss of hydrogen from a radical cation initially formed on the N atom at C(6) or on one of the N-atoms in the pyrimidine ring.

The compounds (6) and (7), which contain phenolic antioxidants, both showed irreversible oxidative peak potentials (E_{pl}^{ox} 0.78 and 0.5 V, respectively) indicative of strong and very strong antioxidant activity, respectively. The voltammogram of the Trolox® containing compound (7) showed a second irreversible oxidation which was attributed to oxidation of one of the readily formed decomposition products of the original molecule.

Biological Evaluation of Antioxidant Activity

Compounds (3)–(7) and (20) were examined using the TBARS assay[6] and the Sapphire colorimetric assay.[6,7] The results from the TBARS and Sapphire assays represent 50% inhibition (IC_{50} values) of malondialdehyde (MDA) formation in rat-brain homogenates obtained from concentration response curves. Lipid peroxidation was stimulated using two concentrations of Fe^{3+} (100 and 1000 μM). The results for these assays alongside the voltammetric data described above are summarized in Table 1.

In general there is good concordance between the three sets of results. The biological evaluation of compound (20) with only two nitrogen substituents on the pyrimidine ring gave IC_{50} values > 1000 μM, consistent with the lack of electrochemical behaviour. All the triamino-substituted pyrimidines (3a–c), (4), and (5) showed mild antioxidant activity relative to the phenolic compounds (6) and (7). Surprisingly, the pyrrolidinyl compound (3a) showed higher activity than (3b), (3c), (4), and (5) in both biological and electrochemical evaluations.

This concordance provides an independent, non-biological method for the evaluation of antioxidant activity.

Experimental

Syntheses

Melting points (m.p.) are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 FT-IR spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using Bruker AC-200, AM-300 and DRX-400 spectrometers for solutions in base-washed $CDCl_3$ unless otherwise stated. Electrospray ionization mass spectra (ESI^+) were measured on a Bruker BioApex 47 e– FTMS (Fourier transform mass spectrometer) with a 4.7 Tesla magnet and an Analytica electrospray source. Electron impact mass spectra (EI) were recorded on a VG TRIO-1 Quadrupole Mass Spectrometer at 70 eV with a source temperature of 200°C. Microanalyses were performed by Chemical and Microanalytical Services Pty Ltd, Melbourne. Merck Silica Gel 60 (230–400 mesh, no. 9385) was used for flash chromatography. Light petroleum (b.p. range 60–80°C) was used for chromatography. High-performance liquid chromatography (HPLC) was performed on a Waters Model 6000A (Column Deltapak C18-100 Å, 19 mm by 30 cm, 15 μ or Column HAIsil C18-300 Å, 25 mm by 30 cm, 5 μ), Waters gradient program Model 660 and Waters Model 481 detector. Solvent systems of acetonitrile/water were used.

2,4-Bis-1-amino-6-chloropyrimidines (9a–c)

The three diamines were prepared by the literature method.[13]

2,4-Bis(pyrrolidin-1-yl)-6-chloropyrimidine (9a). (1.23 g, 89% had m.p. 76.8–77.2°C (lit.[13] 77–79°C) and identical spectroscopic data to those recorded previously.

*2,4-Bis(*N,N-*diethylamino)-6-chloropyrimidine (9b).* 2,4,6-Trichloropyrimidine (8) (1.0 g, 5.5 mmol) was reacted with diethylamine (10 mL) and pyridine (10 mL) for 5 days at ambient temperature (23°C). Chromatography (ethyl acetate/light petroleum, 1:19) gave the

title compound (9b) as a colourless *liquid* (1.14 g, 81%), b.p. 85°C (oven)/0.2 mm (Found: C, 56.2; H, 8.4; N, 21.8%. $C_{12}H_{21}ClN_4$ requires C, 56.1; H, 8.2; N, 21.8%). ν_{max} (neat) 2974s, 2932s, 1578s (br), 1509s, 1491s, 1458s, 1431s, 1376s, 1362s, 1329s, 1261s, 1186s, 1130s, 1081s, 966s, 806s, 782s cm^{-1}. ^1H NMR δ (200 MHz) 1.16, t, *J* 7.1 Hz, 12H, NCH_2CH_3; 3.42, q, *J* 7.0 Hz, 4H and 3.55, q, *J* 7.0 Hz, 4H, NCH_2CH_3; 5.70, s, 1H, H5. ^{13}C NMR δ (50 MHz) 13.0; 13.3; 41.6; 42.2; 89.3; 159.6; 160.3; 162.0. Mass spectrum (ESI, CH_3CN) *m/z* 259 $[M(^{37}Cl)+H]^+$, 257 $[M(^{35}Cl)+H]^+$.

2,4-Bis(morpholino)-6-chloropyrimidine (*9c*). The compound was prepared as for (9a) as a white *solid* (1.33 g, 86%), m.p. 145–146°C (Found: C, 50.7; H, 6.1; N, 19.6%. $C_{12}H_{17}ClN_4O_2$ requires C, 50.6; H, 6.0; N, 19.7%). ν_{max} (Nujol) 2930s (br), 2856s, 1579s (br), 1546s (br), 1465s (br), 1378s, 1366s, 1305s, 1242s, 1164s, 1114s, 1006s, 966s, 953s cm^{-1}. ^1H NMR δ (200 MHz) 3.53–3.58, m, 4H, NCH_2CH_2O; 3.64–3.78, m, 12H, NCH_2CH_2O and NCH_2CH_2O; 5.87, s, 1H, H5. ^{13}C NMR δ (50 MHz) 44.2; 44.3; 66.5; 66.8; 91.1; 160.6; 160.8; 163.4. Mass spectrum (ESI, CH_3CN) *m/z* 287 $[M(^{37}Cl)+H]^+$, 285 $[M(^{35}Cl)+H]^+$.

2,4-Bis(diamino)-6-piperazin-1-ylpyrimidines (10a–c)

2,4-Bis(pyrrolidin-l-yl)-6-piperazin-1-ylpyrimidine (10a). This was prepared as described by Jacobsen[13] as a white solid (1.02 g, 85%), m.p. 180°C (dec.) (lit.[13] 177–178°C) with identical spectroscopic values to the literature.

*2,4-Bis(*N,N*-diethylamino)-6-piperazin-1-ylpyrimidine (10b)*. This was prepared as a white solid (2.29 g, 96%), m.p. 184.3–185.5°C (dec.) (Found: $[M+H]^+$ 307.2598. Calc. for $C_{16}H_{31}N_6$: $[M+H]^+$ 307.2619). ν_{max} (neat) 3300s (br), 2964s (br), 2844s (br), 1574s (br), 1538s (br), 1455s (br), 1372s (br), 1322s, 1272s, 1267s, 1238s, 1225s, 1211s, 1190s, 1164s, 1080s (br), 1032s, 998s, 888s (br), 813s, 790s, 772s, 673s cm^{-1}. ^1H NMR δ (400 MHz) 1.12, t, *J* 7.0 Hz, 12H, NCH_2CH_3; 2.90, t, *J* 5.1 Hz, 4H, H3′, H5′; 3.41, q, *J* 7.0 Hz, 4H, NCH_2CH_3; 3.45, t, *J* 5.1 Hz, 4H, H2′, H6′; 3.53, q, *J* 7.0 Hz, 4H, NCH_2CH_3; 4.94, s, 1H, H5. ^{13}C NMR δ (100 MHz) 13.3; 13.6; 41.5; 42.0; 45.6; 45.9; 71.2; 160.5; 162.8; 164.8. Mass spectrum (ESI, CH_3OH) *m/z* 307 $[M+H]^+$. The ^1H NMR data were consistent with literature data.[8]

2,4-Bis(morpholino)-6-piperazin-1-ylpyrimidine (10c).[8] This was prepared as a pale yellow solid (0.96 g, 82%), m.p. 219–220°C (dec.) (Found: $[M+H]^+$ 335.2184. Calc. for $C_{16}H_{26}N_6O_2$: $[M+H]^+$ 335.2195). ν_{max} (CH_2Cl_2) 3054s, 2987s, 1564s (br), 1426s, 1365s (br), 1306s, 1268s (br), 1229s, 1212s, 1195s, 1119s, 1008s, 896s, 790s, 746s (br) cm^{-1}. ^1H NMR δ (200 MHz) 2.92–2.97, m, 4H, H3′, H5′; 3.40–3.56, m, 8H, H2′, H6′ and NCH_2CH_2O; 3.61–3.79, m, 13H, NCH_2CH_2O, NCH_2CH_2O and NH; 5.10, s, 1H, H5. ^{13}C NMR δ (50 MHz) 44.4; 44.5; 44.9; 51.3; 66.8; 67.1; 73.4; 161.3; 164.7; 165.0. Mass spectrum (ESI, CH_3CN) *m/z* 335 $[M+H]^+$.

6-{4-[2-(4-Bromophenyl)-2-hydroxy]ethylpiperazin-1-yl}-2,4-bis(diamino)pyrimidines (3a–c)

A solution of 2,4′-dibromoacetophenone (1.5 mmol), (10a–c) (1.5 mmol), and triethylamine (10 mL) in tetrahydrofuran (20 mL) was heated under reflux for 4 h. The reaction mixture was cooled, filtered and the filtrate concentrated under vacuum. The crude material was redissolved in dichloromethane (30 mL), washed with water (20 mL) followed by saturated NaCl (20 mL) and dried ($MgSO_4$). After evaporation of the solvent, the crude compound was reduced with lithium aluminium hydride (1.5 mmol) in tetrahydrofuran (20 mL) at 0°C under N_2 for 4 h. The mixture was quenched with $Na_2SO_4 \cdot 10H_2O$ until the evolution of hydrogen ceased. The mixture was then filtered and the solvent removed under vacuum. Flash chromatography on silica gel, with ethyl acetate/light petroleum as eluent, gave the pyrimidines (3a–c) as white solids.

6-{4-[2-(4-Bromophenyl)-2-hydroxy]ethylpiperazin-1-yl}-2,4-bis-(pyrrolidin-l-yl)-pyrimidine (3a). (70%) m.p. 168–169°C (Found: $[M+H]^+$ 501.1976. $C_{24}H_{34}{}^{79}BrN_6O$ $[M+H]^+$ requires 501.1978). ^1H NMR δ (300 MHz) 1.79–1.87, m, 8H, NCH_2CH_2; 2.37–2.48, m, 4H, H3′, H5′; 2.66–2.73, m, 2H, $NCH_2CH(OH)$; 3.32–3.61, m, 12H, NCH_2CH_2, H2′, and H6′; 4.65, dd, *J* 10.0, 3.8 Hz, 1H, $NCH_2CH(OH)$; 4.79, s, 1H, H5; 7.19, d, *J* 8.4Hz, 2H and 7.39, d, *J* 8.4 Hz, 2H, ArH. ^{13}C NMR δ (75 MHz) 25.3; 25.5; 44.5; 46.0; 46.2; 52.85; 66.1; 68.2; 72.5; 121.2; 127.5; 131.4; 141.2; 160.2; 162.5; 164.0. Mass spectrum (ESI, CH_3OH) *m/z* 501.2 $[M(^{79}Br)+H]^+$, 503.2 $[M(^{81}Br)+H]^+$.

*6-{4-[2-(4-Bromophenyl)-2-hydroxy]ethylpiperazin-1-yl}-2,4-bis-(*N,N*-diethylamino)pyrimidine (3b).* Chromatography (ethyl acetate/light petroleum, 2:5) followed by semi-preparative HPLC (Waters 6000A HPLC, column HAIsil C18-300 Å, 25 mm by 30 cm, 5 μ) gave (3b) as colourless *crystals* (44%) (Found: $[M+H]^+$ 505.2274. $C_{24}H_{37}{}^{79}BrN_6O$ requires $[M+H]^+$ 505.2290). ν_{max} (CH_2Cl_2) 3054s, 2976s (br), 2931s, 2834s, 1566s (br), 1488s, 1451s, 1425s, 1375s, 1325 s, 1270s, 1239s, 1216s, 1165s, 1141s, 1114s, 1080s, 1010s, 1001s, 896s, 790s, 758s (br) cm^{-1}. ^1H NMR δ (300 MHz) 1.15, t, *J* 7.0 Hz, 12H, NCH_2CH_3; 2.41–2.56, m, 4H, H3′, H5′; 2.74–2.81, m, 2H, $NCH_2CH(OH)$; 3.43, q, *J* 7.1 Hz, 4H, NCH_2CH_3 3.48–3.63, m, 8H, H2′, H6′ and NCH_2CH_3; 4.73, dd, *J* 10.1, 3.7 Hz, 1H, $NCH_2CH(OH)$; 4.97, s, 1H, H5; 7.26, d, *J* 8.5 Hz, 2H and 7.47, d, *J* 8.5 Hz, 2H, ArH. ^{13}C NMR δ (50 MHz) 13.3; 13.6; 41.5; 42.0; 44.5; 52.9; 66.1; 68.1; 71.2; 121.2; 127.5; 131.4; 141.1; 160.5; 162.8; 164.5. Mass spectrum (EI) *m/z* 505 $[(M(^{81}Br)]^+$, (18%), 503 (11), 502 (18), 425 (13), 320 (12), 319 (41), 251 (16), 250 (100), 237 (47), 56 (10).

6-{4-[2-(4-Bromophenyl)-2-hydroxy]ethylpiperazin-1-yl}-2,4-bis-(morpholino)pyrimidine (3c). Chromatography (ethyl acetate/light petroleum, 4:1) followed by recrystallization (ethyl acetate/light petroleum, 1:3) gave (3c) as a white *solid* (64%), m.p. 188.7–189.7°C (Found: C, 54.0; H, 6.2; N, 15.8%. $C_{24}H_{33}BrN_6O_3$ requires C, 54.0; H, 6.2; N, 15.8%). ν_{max} (CH_2Cl_2) 3054s, 2987s, 1564s (br), 1422s, 1266s (br), 1230s (br), 1212s, 1119s, 1010s, 1002s, 896s, 754s, 668s cm^{-1}. ^1H NMR δ (200 MHz) 2.44–2.57, m, 4H, H2′, H6′; 2.74–2.81, m, 2H, $NCH_2CH(OH)$; 3.48–3.51, m, 4H, H3′, H5′; 3.56–3.80, m, 16H, NCH_2CH_2O and NCH_2CH_2O; 3.98, br s, 1H, OH; 4.72, dd, *J* 9.4, 3.9 Hz, 1H, $NCH_2CH(OH)$; 5.10, s, 1H, H5; 7.24, d, *J* 8.4 Hz, 2H and 7.46, d, *J* 8.4 Hz, 2H, ArH. ^{13}C NMR δ (50 MHz) 44.4; 44.8; 52.8; 66.0; 66.7; 67.0; 68.2; 73.3; 121.3; 127.5; 131.5; 141.0; 161.2; 164.5; 164.9. Mass spectrum (ESI, CH_3OH) *m/z* 535 $[M(^{81}Br)+H]^+$, 533 $[M(^{79}Br)+H]^+$.

2-Amino-1-(4-bromophenyl)ethanol (12)

The reaction of 2-(4-bromophenyl)-2-hydroxyacetonitrile (1.84 g, 8.7 mmol) in dry tetrahydrofuran (30 mL) with lithium aluminium hydride in tetrahydrofuran (1 M) (17.4 mL, 17.4 mmol) gave a yellow solid. Trituration with ether gave (12) as a white solid (0.90 g, 77%), m.p. 113.4–114.1°C (lit.[14] 112–113.5°C) (Found: C, 44.4; H, 4.7; N, 6.5%. Calc. for $C_8H_{10}BrNO$: C, 44.5; H, 4.7; N, 6.5%). ν_{max} (Nujol) 2926s (br), 2855s, 1459s (br) cm^{-1}. ^1H NMR δ (400 MHz) 1.82, br s, 3H, OH, NH_2; 2.75, dd, *J* 12.7, 7.8 Hz, 1H and 3.00, dd, *J* 12.7, 3.9 Hz, 1H, H2; 4.58, dd, *J* 7.8, 3.9 Hz, 1H, H1; 7.24, d, *J* 8.3 Hz, 2H and 7.47, d, *J* 8.3 Hz, 2H, ArH. ^{13}C NMR δ (50 MHz) 49.1; 73.4; 121.2; 127.6; 131.5; 141.5. Mass spectrum (ESI, CH_3CN) *m/z* 216 $[M(^{79}Br)+H]^+$, 218 $[M(^{81}Br)+H]^+$.

6-[(4-Bromophenyl)-2-hydroxyethylamino]-2,4-dichloropyrimidine (13) and 2-[(4-Bromophenyl)-2-hydroxyethylamino]-4,6-dichloropyrimidine (14)

A solution of 2,4,6-trichloropyrimidine (0.85 g, 4.63 mmol) in dioxan (10 mL) was added dropwise at ambient temperature to a stirred solution of (12) (1.0 g, 4.63 mmol) in dioxan (20 mL). Once the addition was complete, sodium bicarbonate (2.33 g, 27.8 mmol) was added and the resulting mixture was heated to reflux for 7 h. The mixture was cooled, quenched with water (40 mL) and the pH was adjusted to 12 by addition of a 2 M solution of NaOH. The solution was extracted with dichloromethane (3 × 40 mL) and the combined extracts were dried ($MgSO_4$) and evaporated to give an orange gum (1.87 g). The ^1H NMR spectrum of the crude product indicated that (13) and (14) were present in a ca. 1:1 ratio. Chromatography (diethyl ether/light petroleum, 1:1) gave (14) and (13) as white *solids* (0.87 g, 51%) and (0.81 g, 47%) respectively.

Compound (13). m.p. 130.0–131.4°C (Found: C, 39.6; H 2.8; N, 11.6%. $C_{12}H_{10}BrCl_2N_3O$ requires C, 39.7; H, 2.8; N, 11.6%). ν_{max} (Nujol) 3301s, 3244s (br), 3156s, 3104s, 2925s (br), 2855s, 1604s (br), 1579s, 1508s, 1500s, 1459s (br), 1376s, 1361s, 1288s, 1274s, 1204s, 1125s, 1076s, 1045s, 1010s, 981s, 823s cm^{-1}. ^1H NMR δ (200 MHz, CD_3COCD_3) 2.89, br s, 1H, OH or NH; 3.40–3.59, m, 1H (3.45, dd, *J* 13.6, 7.7 Hz, 1H on D_2O exchange), H1′a; 3.71–3.80, m, 1H (3.64, dd, *J* 13.6, 4.4 Hz, 1H on D_2O exchange), H1′b; 4.83–4.95, m, 2H (4.83, dd, *J* 7.5, 4.4 Hz, 1H on D_2O exchange), H2′ and NH or OH; 6.63, s, 1H, H5; 7.42, d, *J* 8.5 Hz, 2H and 7.51, d, *J* 8.5 Hz, 2H, ArH. [^1H NMR (200 MHz, $CDCl_3$) 6.30, br s, H5.] ^{13}C NMR δ (100 MHz, CD_3COCD_3) 49.2; 72.3; 103.7; 121.5; 129.0; 132.0; 143.2; 158.9; 160.5; 165.6. Mass spectrum (ESI, CH_3OH) *m/z* 362, 364, 366 and 368 $[M+H]^+$.

Compound (14). m.p. 143.1–143.7°C (Found: C, 39.7; H 2.7; N, 11.6%. $C_{12}H_{10}BrCl_2N_3O$ requires C, 39.7; H, 2.8; N, 11.6%). ν_{max} (Nujol) 3474s (br), 3279s, 3122s, 2924s (br), 2854s, 1594s, 1581s, 1568s, 1521s, 1456s (br), 1420s, 1378s, 1068s, 560s cm^{-1}. ^1H NMR δ (200 MHz) 2.97, d, *J* 3.5 Hz, 1H, OH or NH; 3.51, ddd, *J* 14.2, 7.7, 5.3 Hz, 1H (3.49, dd, *J* 14.2, 7.7 Hz, 1H on D_2O exchange), H1′a; 3.83, ddd, *J* 14.2, 7.0, 3.6 Hz, 1H (3.81, dd, *J* 14.2, 3.6 Hz, 1H on D_2O exchange), H1′b; 4.89–4.96, m, 1H, H2′; 5.86, br s, 1H, NH or OH; 6.64, s, 1H, H5; 7.29, d, *J* 8.4 Hz, 2H and 7.50, d, *J* 8.4 Hz, 2H, ArH. ^{13}C NMR δ (100 MHz) 49.0; 72.8; 109.7; 122.0; 127.6; 131.8; 140.5; 161.8; 162.0. Mass spectrum (ESI, CH_3OH) *m/z* 362, 364, 366 and 368 $[M+H]^+$. Note that the ^{13}C NMR spectrum of the symmetrical isomer (14) shows two quaternary carbons for the pyrimidine ring where the unsymmetrical isomer (13) shows three such carbons thus confirming the structural assignment.

6-[2-(4-Bromophenyl)-2-hydroxyethylamino]-2,4-bis(pyrrolidin-1-yl)-pyrimidine (4)

The pyrimidine (13) (0.47 g, 1.3 mmol) was heated to reflux with pyrrolidine (10 mL) for 4 h. The mixture was cooled to ambient temperature, diluted with dichloromethane (20 mL) and washed with saturated $NaHCO_3$ (3x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed under vacuum. Recrystallization (ethyl acetate/light petroleum, 3 : 2) gave (4) as a white *solid* (0.41 g, 73%), m.p. 180°C (dec.) (Found: C, 55.9; H, 5.8; N, 15.9%. $C_{20}H_{26}BrN_5O$ requires C, 55.6; H, 6.1; N, 16.2%). ν_{max} (CH_2Cl_2) 3055s, 2987s, 1593s, 1570s, 1476s, 1458s, 1422s, 1270s (br), 896s, 758s (br) cm^{-1}. ^1H NMR δ (300 MHz) 1.89–1.95, m, 8H, $NCH_2C\mathbf{H}_2$; 3.38–3.47, m, 5H (3.42, dd, *J* 14.7, 6.2 Hz, 1H on D_2O exchange) $NC\mathbf{H}_2CH_2$ and H1′a; 3.72, ddd, *J* 14.6, 6.1, 2.2 Hz, 1H (3.70, dd, *J* 14.6, 2.2 Hz, 1H on D_2O exchange) H1′b; 4.41, t, *J* 5.7 Hz, 1H, NH; 4.71, s, 1H, H5; 4.82, dd, *J* 6.1, 1.9 Hz, 1H (4.81, dd, *J* 6.2, 2.1 Hz, 1H on D_2O exchange) H2′; 7.24, d, *J* 8.4 Hz, 2H and 7.43, d, *J* 8.4 Hz, 2H, ArH; 7.95, br s, 1H, OH. ^{13}C NMR δ (100 MHz) 25.3; 25.5; 46.0; 46.4; 50.6; 72.9; 75.0; 120.6; 127.9; 131.2; 142.7; 159.3; 161.4; 163.4. Mass spectrum (ESI, CH_3OH) *m/z* 432 $[M(^{79}Br)+H]^+$, 434 $[M(^{81}Br)+H]^+$.

2-[2-(4-Bromophenyl)-2-hydroxyethylamino]-4,6-bis(pyrrolidin-1-yl)-pyrimidine (5)

The pyrimidine (14) (0.30 g, 0.8 mmol) was heated to reflux with pyrrolidine (10 mL) for 4 h as described above. Recrystallization (ethyl acetate/light petroleum, 3 : 1) gave (5) as a white *solid* (0.28 g, 79%) m.p. 178.5–179.2° (Found: C, 55.6; H, 6.1; N, 16.2%. $C_{20}H_{26}BrN_5O$ requires C, 55.6; H, 6.1; N, 16.2%). ν_{max} (CH_2Cl_2) 3054s, 2986s, 2522s, 1589s (br), 1557s, 1522s, 1484s, 1471s (br), 1460s, 1422s, 1352s, 1263s (br), 790s, 718s (br) cm^{-1}. ^1H NMR δ (200 MHz) 1.91–1.98, m, 8H, $NCH_2C\mathbf{H}_2$; 3.35–3.42, m, 8H, $NC\mathbf{H}_2CH_2$; 3.48, ddd, *J* 14.8, 6.3, 2.2 Hz, 1H (3.47, dd, *J* 14.8, 6.6 Hz, 1H on D_2O exchange), H1′a; 3.61, ddd, *J* 14.8, 6.3, 2.2 Hz, 1H (3.60, dd, *J* 14.8, 2.2 Hz, 1H on D_2O exchange), H1′b; 4.67, s, 1H, H5; 4.85–4.89, m, 2H, H2′ and NH or OH; 7.28, d, *J* 8.3 Hz, 2H and 7.44, d, *J* 8.3 Hz, 2H, ArH; 7.92, br s, 1H, OH or NH. ^{13}C NMR δ (100 MHz) 25.3; 46.3; 50.9; 73.7; 76.0; 120.6; 128.0; 131.1; 142.8; 161.1; 162.6. Mass spectrum (ESI, CH_3OH) *m/z* 432 $[M(^{79}Br)+H]^+$, 434 $[M(^{81}Br)+H]^+$.

1-[2-(4-Chlorophenyl)-2-hydroxy]ethyl-4-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylpiperazine (6) and 1-[2-(4-Chlorophenyl)-2-hydroxy]ethyl-4-[6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-yl]methylpiperazine (7)

A solution of 2-bromo-4′-chloroacetophenone (0.7 g, 3 mmol), ethyl 1-piperazinecarboxylate (0.47 g, 3 mmol), and triethylamine (0.42 mL, 3 mmol) in diethyl ether (10 mL) was heated under reflux for 3 h (reaction was monitored by thin-layer chromatography, TLC). The reaction mixture was cooled and further diluted with ethyl acetate and washed with water, brine and dried over Na_2SO_4. After evaporation of the solvent under vacuum, the crude viscous liquid was treated with concentrated. HCl (15 mL) at refluxing temperature for 48 h. The cooled reaction mixture was made alkaline by addition of 50% NaOH and extracted with ethyl acetate. The combined extracts were washed with water, brine and dried over Na_2SO_4. Evaporation of the solvent gave (15) as a semisolid, which was reacted without further purification. ^1H NMR δ (300 MHz) 2.54, m, 4H, $NCH_2C\mathbf{H}_2NH$; 2.93, m, 4H, $NC\mathbf{H}_2CH_2NH$; 3.69, s, 2H, CH_2CO; 7.35, d, *J* 8.6 Hz, 2H and 7.88, d, *J* 8.6 Hz, 2H, ArH.

A solution of (15) (0.48 g, 2 mmol) and 4-(bromomethyl)-2,6-bis(1,1-dimethylethyl)phenol (16) (0.55 g, 2 mmol) in tetrahydrofuran (10 mL) was stirred at ambient temperature for 4 h (reaction monitored by TLC). The reaction mixture was concentrated, treated with aqueous sodium bicarbonate and extracted with ethyl acetate. The combined extracts were washed with water, then with brine, and dried with Na_2SO_4. After evaporation of the solvent, the crude compound was reduced with $LiAlH_4$ (1.25 mmol) using diethyl ether (10 mL) as solvent at 0°C under N_2 atmosphere (2 h). The reaction was quenched by the dropwise addition of ice-cold water (2 mL) and Na_2SO_4. The reaction mixture was further diluted with ethyl acetate (50 mL) and filtered. The filtrate was washed with water, then with brine, and dried over Na_2SO_4. After evaporation of the solvent under vacuum, the crude product was subjected to flash chromatography on silica gel. Elution with 70% ethyl acetate/light petroleum gave (6) as a white *solid* (0.83 g 90%), m.p. 149–150°C (Found: C, 70.4; H, 8.6; N, 6.3%. $C_{27}H_{39}ClN_2O_2$ requires C, 70.4; H, 8.6; N, 6.3%). ^1H NMR δ (300 MHz) 1.4, s, 18H, Bu^t; 2.43–2.83, m, 10H, $NC\mathbf{H}_2C\mathbf{H}_2$ and $NC\mathbf{H}_2CH(OH)$; 3.51, s, 2H, $NC\mathbf{H}_2Ar$; 4.71, dd, *J* 10.3, 3.4 Hz, 1H, $NCH_2C\mathbf{H}(OH)$; 5.17, s, 1H, OH; 7.10, s, 2H and 7.30, s, 4H, ArH. Mass spectrum (EI) *m/z* 458, 317, 219 (100%).

The ditartrate salt was prepared by adding a solution of tartaric acid (2 equiv.) in ethanol to a solution of (6) (1 equiv.) in ethyl acetate. The homogenous solution was stirred at 60°C for 15 min. The ditartrate salt precipitated out of the solvent and was filtered off and dried under vacuum, m.p. 170–172°C (^1H NMR 4.16, s, integrated to 4H compared with Bu^t, 1.4, s, 18H).

4-Chloro-2-(piperazin-1-yl)acetophenone (15) was also coupled with 6-hydroxy-2,5,6,8-tetramethylchroman-2-carboxylic acid (17) using 1,3-dicyclohexylcarbodiimide under standard conditions. The resulting oxo amide was reduced as described above using $LiAlH_4$ in tetrahydrofuran in the presence of $AlCl_3$ (ca. 0.3 equiv.) to give (7) (Found: $[M+H]^+$ 459.2410. $C_{26}H_{36}{}^{35}ClN_2O_3$ requires $[M+H]^+$ 459.2414). ^1H NMR δ (300 MHz) 1.23, s, 3H, CH_3CO; 1.65–1.8, m, 1H and 1.9–2.05, m, 1H, CH_2CO; 2.08, s, 3H, 2.11, s, 3H and 2.16, s, 3H, $C\mathbf{H}_3Ar$; 2.4–2.9, m, 14H, $NC\mathbf{H}_2C\mathbf{H}_2$, $NC\mathbf{H}_2CH(OH)$, $NC\mathbf{H}_2CO$ and $C\mathbf{H}_2Ar$; 4.71–4.77, m, 1H, $NCH_2C\mathbf{H}(OH)$; 7.35, s, 4H, ArH. The ditartrate salt was prepared as described above, m.p. 88–90°C (^1H NMR 4.20, s, integrated to 4H compared with 2.1, 3 × CH_3Ar, 9H).

6-Methyl-2,4-bis(pyrrolidin-1-yl)pyrimidine (18)

Pyrrolidine (10 mL) was added dropwise to 6-methyl-2,4-dichloropyrimidine (1.00 g, 6.13 mmol) at 0°C. The reaction mixture was allowed to warm to ambient temperature and was then heated at reflux for 4 h. Once complete, the reaction was diluted with CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 20 mL). The organic phase was collected, dried ($MgSO_4$), filtered and the solvent removed under vacuum. The crude material was recrystallized from ethanol/water (3 : 1) to yield (18) as a white *solid* (1.17 g, 82%) m.p. 77–78°C (Found: C, 67.2; H, 8.7; N, 24.1%. $C_{13}H_{20}N_4$ requires C, 67.2;

H, 8.8; N, 24.2%). ν_{max} (Nujol) 2928s (br), 1580s (br), 1456s, 1420s, 1376s, 1345s, 789s cm^{-1}. ^1H NMR δ (200 MHz) 1.88–1.97, m, 8H, $NCH_2C\mathbf{H_2}$; 2.24, s, 3H, CH_3; 3.23–3.59, m, 8H, $NC\mathbf{H_2}CH_2$; 5.51, s, 1H, H5. ^{13}C NMR δ (50 MHz) 24.5; 25.3; 25.6; 45.9; 46.3; 91.4; 160.7; 161.2; 164.9. Mass spectrum (ESI, CH_3CN) m/z 233 $[M+H]^+$.

6-[2-(4-Bromophenyl)-2-hydroxy]ethyl-2,4-bis(pyrrolidin-1-yl)-pyrimidine (20)

A solution of *n*-butyllithium in hexane (2.05 mL, 1.3 M, 2.6 mmol) was reacted with *N,N,N′,N′*-tetramethylethylenediamine (0.39 mL, 2.6 mmol) and (18) (0.50 g, 2.2 mmol) in diethyl ether (20 mL) under an inert atmosphere. The resulting mixture was heated under reflux for 1 h, cooled to ambient temperature and a solution of 4-bromobenzaldehyde (0.80 g, 4.3 mmol) in diethyl ether (10 mL) was added. The reaction mixture was stirred at ambient temperature for 1 h before it was poured onto ice (20 g) and aqueous sulfuric acid (1 M, 20 mL), and further diluted with diethyl ether (20 mL). The organic layer was separated; the aqueous layer was basified to pH 14 by addition of aqueous sodium hydroxide (4 M) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under vacuum to leave a pale-brown coloured gum. Flash chromatography (ethyl acetate/light petroleum, 1 : 1) gave (20) as a pale yellow solid (0.67 g, 74%). A small amount was recrystallized (diethyl ether/light petroleum, 1 : 1 and charcoal) and gave (20) as a white *solid* m.p. 145.0–146.9°C (Found: C, 57.5; H, 6.0; N, 13.4%. $C_{20}H_{25}BrN_4O$ requires C, 57.6; H, 6.0; N, 13.4%). ν_{max} (Nujol) 2924s (br), 2855s (br), 1589s (br), 1559s, 1495s, 1474s, 1456s, 1416s, 1346s cm^{-1}. ^1H NMR δ (200 MHz) 1.91–1.97, m, 8H, $NC\mathbf{H_2}CH_2$; 2.76, br s, 2H, H1′; 3.43–3.71, m, 8H, $NC\mathbf{H_2}CH_2$; 4.99, dd, *J* 7.1, 4.7 Hz, 1H, H2′; 5.42, s, 1H, H5; 7.33, d, *J* 8.4 Hz, 2H and 7.45, d, *J* 8.4 Hz, 2H, ArH; 7.71, br s, 1H, OH. ^{13}C NMR δ (100 MHz) 25.3; 25.5; 44.4; 46.0; 46.4; 72.7; 91.6; 120.7; 127.7; 131.3; 143.7; 159.1; 161.1; 165.4. Mass spectrum (ESI, CH_3CN) *m/z* 419 $[M(^{81}Br)+H]^+$, 417 $[M(^{79}Br)+H]^+$.

Cyclic Voltammetry

Reagents and Solvents

The potassium chloride (Aldrich) and tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) (BDH) used to prepare the electrolyte solutions were of analytical grade and were used as supplied. The solvents used for cyclic voltammetry were triply distilled water, and acetonitrile (CH_3CN) (Chromatographic 99.99% HPLC grade (Mallinckrodt)), which was passed through activated neutral alumina under nitrogen and stored over 4 Å molecular sieves.

Solutions were prepared just prior to use by dissolution of the compound of interest as either the hydrochloride or tartrate salt in aqueous 0.1 M in KCl using nano-pure water. The solutions were deoxygenated by bubbling nitrogen for ten min prior to each voltammetric experiment. For the bulk electrolysis experiments, the compound of interest was dissolved in acetonitrile, which contained 0.01 M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6).

Methodology

All cyclic and steady-state voltammograms were acquired using a Cypress Systems Model CS-1090 Computer Controlled Electroanalytical system in the cyclic staircase mode (with a 2 mV potential step). The standard three-electrode arrangement was employed. In all cases a Pt wire auxiliary electrode was used. The working electrodes of choice for cyclic voltammetry were either a 3.0 mm diameter glassy carbon disc or a 1.0 mm diameter polished platinum disc. For all aqueous measurements, an aqueous Ag/AgCl (sat. KCl) reference electrode was used, separated from the solution by a salt bridge containing an aqueous 0.1 M NaCl solution. For non-aqueous measurements, the reference electrode was separated from the main solution by a salt bridge containing 0.01M Bu_4NPF_6 in acetonitrile.

Before each voltammetric experiment, the glassy carbon and the platinum electrodes were polished down to 0.3 micron on polishing pads impregnated with deagglomerated alpha alumina. After polishing, the electrodes were rinsed with nano-pure water and then dried with clean tissue paper. All electrochemical experiments were carried out for 5×10^{-4} M concentrations at 20 ± 2°C.

Bulk electrolysis experiments were carried out using a Bioanalytical Systems 100 (BAS 100) electrochemical Analyser with a standard three-electrode arrangement. A glassy carbon working electrode with a large surface area was employed. A larger surface area platinum gauze auxiliary electrode was separated from the solution containing the drug by a salt bridge with a fritted glass disk. The reference electrode was the non-aqueous Ag/Ag^+ (saturated $AgNO_3$). Electrolyses were conducted using approximately 0.01 mmol of the compound in acetonitrile over a period of 3 to 4 h until the current was constant. Nitrogen was purged in the electrochemical cell throughout the entire process. The potential for bulk electrolysis was selected on the basis of the peak oxidative potential of each drug at which potential the working-electrode potential was held 100 mV more positive than the peak potential. Samples of the solution were evaluated using liquid-chromatography mass spectroscopy.

Thiobarbituric Acid Reacting Substances (TBARS) Assay[6]

Rat-brain 10% homogenates and various concentrations of the test compound were incubated for 10 min at 37°C. An aqueous solution of 100 or 1000 μM Fe^{3+} was added to stimulate lipid peroxidation, and the homogenates were further incubated for 30 min at 37°C. The reaction was stopped by the addition of sodium dodecylsulfate and acetic acid. The precipitated proteins were then removed by centrifugation. Aliquots of the clear supernatants were heated with an equal volume of thiobarbituric acid (TBA) solution. Samples were cooled and absorbances were read at 532 nm. Blank values containing no homogenate or drug were subtracted from these values. Each value was expressed as a percentage of that for samples containing homogenate and Fe^{3+} only (i.e. no drug).

Sapphire Assay[6,7]

The procedures outlined in the Sapphire LPO-586 kit were carried out with the following modifications. Briefly, rat-brain 10% homogenates were prepared in aqueous 20 mM tris(hydroxymethyl)aminomethane–HCI buffer, pH 7.4 media using Polytron homogenizer. Assays contained 100 μL of rat-brain homogenate and various concentrations of test compound (10 μL) and were incubated for 10 min at 37°C. Lipid peroxidation was then stimulated by the addition of 10 μL of 100 μM Fe^{3+} solution and the homogenates were incubated for a further 30 min at 37°C. Chromogenic reagent (1-methyl-2-phenylindole, 325 μL) at the concentration of 10.3 mM in acetonitrile was added, and the reaction was initiated by the addition of 75 μL of 37% aqueous HCl to each tube. The reaction mixture was incubated for 60 min at 45°C, followed by being cooled on ice, centrifuged at 5000 g for 7 min. 200 μL of each sample was pipetted into a 96-well microplate and absorbances read at 586 nm using a Ceres UV900C microplate reader. Following subtraction of blank values, analysis was performed as for the TBARS assay.

Acknowledgments

This project has been supported by AMRAD Operations Pty Ltd, including the provision of a post-graduate scholarship (to A.P.). We also thank Dianne Watkins for her assistance in the biological testing program.

References

[1] P. M. Beart, A. Schousboe, A. Frandsen, *Br. J. Pharmacol*. **1995**, *114*, 1359.
[2] C. J. Carter, J. Benavides, P. Legendre, J. D. Vincent, F. Noel, F. Thuret, K. G. Lloyd, S. Arbilla, B. Zivkovic, E. T. MacKenzie, B. Scatton, S. Z. Langer, *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1222.
[3] B. Jarrott, J. K. Callaway, W. R. Jackson, P. M. Beart, *Drug Dev. Res*. **1999**, *46*, 261.

[4] B. Jarrott, P. M. Beart, W. R. Jackson, V. B. Kenche, A. D. Robertson, M. P. Collis, *PCT Int. Appl*. **1997**, WO 97 43259; *Chem. Abstr*. **1998**, *128*, 34784.
[5] D. E. Epps, J. M. McCall, *Handbook of Synthetic Antioxidants* **1997** (Marcel Dekker, Inc.: New York).
[6] J. K. Callaway, P. M. Beart, B. Jarrott, *J. Pharmacol. Toxicol. Methods* **1998**, *39*, 155.
[7] *Sapphire LPO-586* Bioxytech S. A., France.
[8] J. M. McCall, E. J. Jacobsen, F. J. Van Doornik, J. R. Palmer, H. A. Karnes, *PCT Int. Appl*. **1987**, WO 87 01706, *Chem. Abstr*. **1988**, *108*, 6287.
[9] T. P. Ryan, T. W. Petry, *Arch. Biochem Biophys*. **1993**, *300*, 699.
[10] D. A. Fiedler, M. Koppenol, A. M. Bond, *J. Electrochem. Soc*. **1995**, *142*, 862.
[11] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications* **1980** (Wiley: New York).
[12] P. J. Elving, S. J. Pace, J. E. O'Reilly, *J. Am. Chem. Soc*. **1973**, *95*, 647.
[13] E. J. Jacobsen, J. M. McCall, D. E. Ayer, F. J. Van Doornik, J. R. Palmer, K. L. Belonga, J. M. Braughler, E. D. Hall, D. J. Houser, M. A. Krook, *J. Med. Chem*. **1990**, *33*, 1145.
[14] G. I. Poos, J. R. Carson, J. D. Rosenau, A. J. Roszkowski, N. M. Kelley, J. McGowin, *J. Med. Chem*. **1963**, *6*, 266.