JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 83–88

Received July 18, 2000
Accepted September 27, 2000
ISSN 0022-3573

# Synthesis and in-vitro anticancer evaluation of bis-tacrine congeners

Ming-Kuan Hu

**Abstract**

In the search for potential new anticancer drugs, an efficient synthesis of bis-tetrahydroaminoacridine (bis-tacrine) and its congeners was accomplished by bis-amination of 9-chlorotetrahydroacridine and its congeners under heated conditions.

The critical chlorides were efficiently prepared from *o*-aminoaromatic acids and cycloketones in-situ in the presence of phosphorus oxychloride. In-vitro cytotoxic evaluation of the compounds was carried out against a panel of 60 human cancer cell lines. Among them, butyl-linked bis-tacrine (**5b**) exhibited the strongest cytotoxic profile with GI50 (concentration causing 50% growth inhibition) values of approximately 0.04–0.08 $\mu$M against breast, colon, melanoma and non-small lung cancer cells. Congeners bearing a longer alkyl chain were on average 30- to 100-fold less cytotoxic against these cancer cells. Shorter connecting alkyl chains of bis-tacrine or its congeners dramatically decreased the cytotoxic effects.

Compound **5b** has been selected for further biological evaluation of its anticancer profile.

School of Pharmacy, National Defence Medical Center, PO Box 90048-508, Neihu, Taipei 114, Taiwan, Republic of China

Ming-Kuan Hu

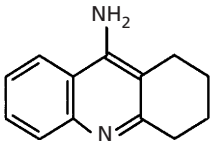
**Correspondence:** Email: hmk@ndmctsgh.edu.tw

**Acknowledgements and funding:** This work was supported in part by the National Science Council of the Republic of China (NSC 88-2314-B-016-048). The author wishes to thank the National Cancer Institute (Bethesda, MD) for testing the compounds in the drug-screening programme. Elemental analyses and high-resolution mass spectra were performed by the National Science Council Regional Instruments Center at National Taiwan University and National Tsing Hua University, and are gratefully appreciated.

## Introduction

Various dimeric compounds have been shown to have broad spectrum activity against a variety of human tumour cells (Wakelin 1986; Spicer et al 2000), and bis-acridines and dimeric naphthalimides have been considered for clinical trials as anticancer candidates (Goldin et al 1981; Brana et al 1997). A positive correlation between DNA bis-intercalation and cytotoxicity was recently investigated (Bailley et al 1996). However, some studies have shown that lipophilic dimeric acridines and naphthalimides still showed significant undesirable toxicity (Segal-Bendirjian et al 1988; Diaz-Rubio et al 1994).

Tetrahydroaminoacridine (**1**, tacrine; Figure 1) is currently one of the major drugs approved for use in Alzheimer's disease (McGeer & McGeer 1995). It has a cyclohexyl-fused quinoline structure and is similar to the planar acridine moiety. Taking advantage of the known ability of the acridine pharmacophore to interact with DNA, a variety of bis-acridines with anticancer activity have been developed (Chen et al 1978; Denny 1989). To my knowledge, there are no reports on the cytotoxic evaluation of the analogous bis-tacrine derivatives. Therefore, a series of bis-tacrine congeners, connected at the amino position of the fused-ring by alkyl chains of varying length, were prepared as potential pharmacophores. Cytotoxic evaluation of the bis-tacrines and their congeners was carried out using a sulforhodamine B (SRB) protein assay against a panel of 60 human cancer cell lines (National Cancer Institute drug-screening programme).




Tacrine (**1**)

**Figure 1** Structure of tacrine (**1**).

## Materials and Methods

### Chemistry

All reagents were commercial materials and were used directly unless otherwise stated. Dimethylformamide was dehydrated over a 4-Å molecular sieve. NMR spectra were recorded on a Varian Gemini at 300 MHz for $^1$H NMR and at 75 MHz for $^{13}$C NMR. Elemental analyses were determined using a Perkin-Elmer 240 EA analyser. Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM; E. Merck). Melting points were recorded on a Thomas Hoover capillary melting-point apparatus in open capillary tubes and are uncorrected.

*9-Chloro-1,2,3,4-tetrahydroacridine (**4a**).*
To a mixture of anthranilic acid (**2a**; 7.4 g, 53.9 mmol) and cyclohexanone (**3a**; 5.36 mL, 51.7 mmol), 30 mL phosphoryl chloride was carefully added in an ice bath. The resulting mixture was heated under reflux for 2 h, then cooled at room temperature, and concentrated to give a viscous slurry. The residue was diluted with EtOAc, neutralized with aqueous $K_2CO_3$, and washed with brine. The organic layer was dried over anhydrous $K_2CO_3$ and concentrated in-vacuo to give a pale brown solid, which was recrystallized with acetone to give the title compound (11.4 g, 94%): mp 68–70°C (lit. mp 66–68°C; Sargent & Samll (1946)); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.13 (d, J = 7.5 Hz, 1H, ArH), 8.00 (d, J = 8.3 Hz, 1H, ArH), 7.63 (dd, J = 9.2, 7.5 Hz, 1H, ArH), 7.51 (dd, J = 9.2, 8.3 Hz, 1H, ArH), 3.10 (t, J = 6.3 Hz, 2H, $CH_2$), 2.97 (t, J = 4.8 Hz, 2H, $CH_2$), 1.91 (s br, 4H, $CH_2$-$CH_2$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 160.1, 147.2, 129.9, 129.5, 129.1, 127.1, 124.3, 34.7, 28.1, 23.2, 23.0; EIMS: 217 ($M^+$, 100), 219 ($M+2^+$, 33); HR-EIMS: exact mass calculated for $C_{13}H_{12}NCl$ $[M]^+$ 217.0659; found 217.0648.

*9-Chloro-1,2,3,4-tetrahydro-cyclohexa[1,2-b]pyrido[2,3-b]pyridine (**4b**).*
According to the above reaction procedure, 2-aminonicotinic acid (4.14 g, 30 mmol) and cyclohexanone (3.11 mL, 30 mmol) were condensed to give the title compound (3.92 g, 86%) as a brown solid: mp 146–149°C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.02 (dd, J = 1.7, 4.2 Hz, 1H, H-6), 8.49 (dd, J = 1.8, 8.4 Hz, 1H, H-8), 7.46 (dd, J = 4.2, 8.3 Hz, 1H, H-7), 3.19 (s br, 2H, $CH_2$), 3.00 (t, J = 6.1 Hz, 2H, $CH_2$), 2.03–1.90 (m, 4H, $CH_2CH_2$); EIMS: m/z 220 $[M+2^+$, 33], 218 [$M^+$, 100].

*10-Chloro-2,3,4,5-tetrahydro-1H-cyclohepta[1,2-b]quinoline (**4c**).*
According to the above procedure, anthranilic acid (2.05 g, 14.9 mmol) and cycloheptanone (1.27 mL, 14.9 mmol) were condensed to give the title compound as a pale brown solid (1.55 g, 45%): mp 87–89°C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.56 (d, J = 8.4 Hz, 1H, ArH), 8.20 (d, J = 8.2 Hz, 1H, ArH), 7.77 (t, J = 6.7 Hz, 1H, ArH), 7.69 (t, J = 7.6 Hz, 1H, ArH), 3.54 (t, J = 4.5 Hz, 2H, $CH_2$), 3.24–3.16 (m, 2H, $CH_2$), 1.90–1.70 (m, 6H, $CH_2CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 232.0, HR-FABMS: exact mass calculated for $C_{14}H_{15}NCl$ $[M+H]^+$ 232.0869; found 232.0888.

*General procedure for the synthesis of alkyl-linked bis-tacrinyl congeners.*
A mixture of 9-chloro-1,2,3,4-tetrahydroacridine (**4a**) or its congeners (**4b**, **c**; 1.0 equiv.), 1,*n*-diaminoalkane (0.5 equiv.), phenol (2.0 equiv.), and NaI (0.025 equiv.) was heated at 180°C in an oil bath for 1.5–5.0 h. The reaction mixture was cooled to room temperature, then diluted with EtOAc and made basic with 10% KOH solution. The organic layer was washed with water, then brine, dried over $MgSO_4$ and concentrated in-vacuo to remove solvent. The resulting residue was purified by flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2$:$CH_3OH$ = 10:1 as eluents) to afford alkyl-linked bis-tacrines in moderate yields.

*Ethyl-linked bis-tacrine (**5a**).*
According to the general procedure, chloride **4a** (0.75 g, 3.50 mmol) and 1,2-diaminoethane (0.12 mL, 1.75 mmol) were condensed under heat for 1.5 h to afford, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2$/$CH_3OH$ = 10:1 as eluents), the title compound (0.40 g, 54%) as an amber solid: mp 123–125°C; $R_f$ 0.27 ($CH_2Cl_2/CH_3OH/NH_4OH$ = 10:1:1); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (d, J = 8.2 Hz, 2H, Ar-H), 7.88 (d, J = 8.2 Hz, 2H, Ar-H), 7.55 (t, J = 7.1 Hz, 2H, Ar-H), 7.33 (t, J = 7.3 Hz, 2H, Ar-H), 4.20 (s br, 2H, 2 NH), 3.71 (s br, 4H, $CH_2CH_2$), 3.04 (t, J = 6.3 Hz, 4H, 2 $CH_2$), 2.59 (t, J = 5.7 Hz, 4H, 2 $CH_2$), 1.95–1.70 (m, 8H, 2 $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 423.2; HR-FABMS: exact mass calculated for $C_{28}H_{31}N_4$ $[M+H]^+$ 423.2584; found 423.2577.

*Butyl-linked bis-tacrine (**5b**).*
According to the general procedure, chloride **4a** (0.75 g, 3.5 mmol) and 1,4-diaminobutane (0.18 mL, 1.75 mmol) were condensed under heat for 2.5 h to afford, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH = 10:1$ as eluents), the title compound (0.40 g, 51%) as an amber glass foam: mp 156–158 °C, $R_f$ 0.34 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (d, J = 4.7 Hz, 2H, Ar-H), 7.90 (d, J = 4.4 Hz, 2H, Ar-H), 7.56 (t, J = 7.4 Hz, 2H, Ar-H), 7.346 (t, J = 7.8 Hz, 2H, Ar-H), 3.51 (s br, 4H, 2 N-$CH_2$), 3.07 (s br, 4H, 2 $CH_2$), 2.67 (s br, 4H, 2 $CH_2$), 1.90 (s br, 8H, 2 $CH_2CH_2$), 1.75 (s br, 4H, $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 451.2; HR-FABMS: exact mass calculated for $C_{30}H_{35}N_4$ $[M+H]^+$ 451.2864; found 451.2840.

*Hexyl-linked bis-tacrine (**5c**).*
Chloride **4a** (0.75 g, 3.5 mmol) and 1,7-diaminoheptane (0.20 g, 1.75 mmol) were condensed under heat for 2 h to afford the title compound (0.47 g, 56%) as an amber glass foam: $R_f$ 0.38 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (d, J = 4.1 Hz, 2H, Ar-H), 7.91 (d, J = 4.1 Hz, 2H, Ar-H), 7.54 (t, J = 7.0 Hz, 2H, Ar-H), 7.32 (t, J = 7.0 Hz, 2H, Ar-H), 3.47 (t, J = 7.1 Hz, 4H, 2 N-$CH_2$), 3.06 (s br, 4H, 2 $CH_2$), 2.68 (s br, 4H, 2 $CH_2$), 1.90–1.86 (m, 8H, 2 $CH_2CH_2$), 1.65 (s br, 4H, $CH_2CH_2$), 1.41 (s br, 4H, $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 479.2; HR-FABMS: exact mass calculated for $C_{32}H_{39}N_4$ $[M+H]^+$ 479.3173; found 479.3183.

*Octyl-linked bis-tacrine (**5d**).*
Chloride **4a** (0.75 g, 3.5 mmol) and 1,8-diaminooctane (0.25 g, 1.75 mmol) were condensed under heat for 4 h to afford the title compound (0.45 g, 51%) as an amber oil: $R_f$ 0.48 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.96 (d, J = 8.7 Hz, 2H, Ar-H), 7.92 (d, J = 8.5 Hz, 2H, Ar-H), 7.55 (t, J = 7.3 Hz, 2H, Ar-H), 7.35 (t, J = 7.0 Hz, 2H, Ar-H), 3.49 (t, J = 7.4 Hz, 4H, 2 N-$CH_2$), 3.07 (s br, 4H, 2 $CH_2$), 2.69 (s br, 4H, 2 $CH_2$), 1.91 (s br, 8H, 2 $CH_2CH_2$), 1.75–1.55 (m, 4H, $CH_2CH_2$), 1.50–1.20 (m, 8H, 2 $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 507.3; HR-FABMS: exact mass calculated for $C_{34}H_{43}N_4$ $[M+H]^+$ 507.3487; found 507.3470.

*N,N′-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2-b]pyrido [2,3-b]pyrid-9-yl)-1,2-diaminoethane (**6a**).*
According to the general procedure, chloride **4b** (0.60 g, 2.75 mmol) and 1,2-diaminoethane (90 $\mu$L, 1.38 mmol) were condensed for 1.5 h to afford, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH = 10:1$ as eluents), the title compound (0.14 g, 24%) as an amber solid: mp 131–133 °C; $R_f$ 0.22 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.79 (s br, 2H, Ar-H), 8.41 (d, J = 8.4 Hz, 2H, Ar-H), 7.26–7.09 (m, 2H, Ar-H), 3.80 (s br, 4H, 2 N-$CH_2$), 2.99 (t, J = 4.5 Hz, 4H, 2 $CH_2$), 2.53 (t, J = 5.4 Hz, 4H, 2 $CH_2$), 1.82–1.74 (m, 8H, 2 $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 425.1; HR-FABMS: exact mass calculated for $C_{26}H_{29}N_6$ $[M+H]^+$ 425.2454; found 423.2454.

*N,N′-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2-b]pyrido-[2,3-b]pyrid-9-yl)-1,4-diaminobutane (**6b**).*
According to the general procedure, chloride **4b** (0.51 g, 2.34 mmol) and 1,4-diaminobutane (0.12 mL, 1.17 mmol) were condensed under heat for 1.5 h to afford, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH = 10:1$ as eluents), the title compound (70 mg, 13%) as an amber solid: mp 121–123 °C; $R_f$ 0.29 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.84 (s br, 2H, Ar-H), 8.28 (d, J = 8.4 Hz, 2H, Ar-H), 7.27 (dd, J = 4.1, 8.4 Hz, 2H, Ar-H), 4.20 (s br, 2H, 2 NH), 3.50 (t, J = 4.2 Hz, 4H, 2 N-$CH_2$), 3.07 (s br, 4H, 2 $CH_2$), 2.58 (s br, 4H, 2 $CH_2$), 1.85 (s br, 8H, 2 $CH_2CH_2$), 1.72–1.60 (m, 4H, $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 453.2; HR-FABMS: exact mass calculated for $C_{28}H_{33}N_6$ $[M+H]^+$ 453.2767; found 453.2768.

*N,N′-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2-b]pyrido [2,3-b]pyrid-9-yl)-1,6-diaminohexane (**6c**).*
Chloride **4b** (0.43 g, 2.0 mmol) and 1,6-diaminohexane (0.12 g, 1.0 mmol) were condensed under heat for 2 h to furnish, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH = 10:1$ as eluents), the title compound (0.12 g, 25%) as an amber glass foam: $R_f$ 0.28 ($CH_2Cl_2/CH_3OH/NH_4OH = 10:1:1$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.86 (d, J = 2.7 Hz, 2H, Ar-H), 8.38 (d, J = 8.2 Hz, 2H, Ar-H), 7.26–7.21 (m, 2H, Ar-H), 4.50 (s br, 2H, 2 NH), 3.60–3.50 (m, 4H, 2 N-$CH_2$), 3.07 (s br, 4H, 2 $CH_2$), 2.70–2.60 (m, 4H, 2 $CH_2$), 1.95–1.80 (m, 8H, 2 $CH_2CH_2$), 1.70–1.60 (m, 4H, 2 $CH_2$), 1.45–1.35 (m, 4H, 2 $CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 481.3; HR-FABMS: exact mass calculated for $C_{30}H_{37}N_6$ $[M+H]^+$ 481.3080; found 481.3057.

*N,N′-Bis-(1,2,3,4-tetrahydro-cyclohexa[1,2-b]pyrido [2,3-b]pyrid-9-yl)-1,8-diaminooctane (**6d**).*
According to the general procedure, chloride **4b** (0.57 g, 2.60 mmol) and 1,8-diaminooctane (0.19 mL, 1.30 mmol) were condensed under heat for 2.5 h to afford,

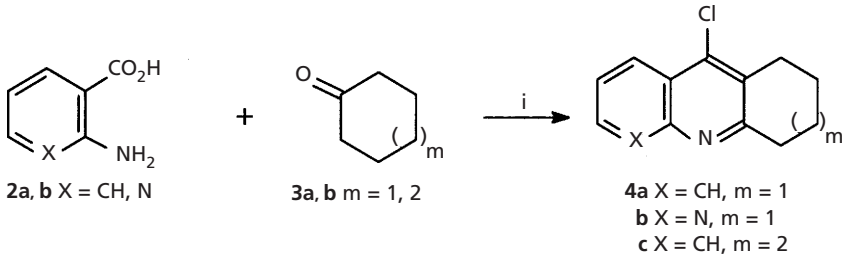



**Figure 2** Synthesis of 9-chlorotetrahydroacridine (**4a**) and its congeners **4b** and **4c**. Reagents: i. $POCL_3$, reflux 2 h.

after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH$ = 10:1 as eluents), the title compound (0.34 g, 51%) as an amber solid: mp 115–117 °C; $R_f$ 0.38 ($CH_2Cl_2/CH_3OH/NH_4OH$ = 10:1:1); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.84 (d, J = 1.8 Hz, 2H, Ar-H), 8.37 (d, J = 8.3 Hz, 2H, Ar-H), 7.19 (dd, J = 4.0, 8.3 Hz, 2H, Ar-H), 4.46 (s br, 2H, 2 NH), 3.50 (s br, 4H, 2 N-$CH_2$), 3.06 (s br, 4H, 2 $CH_2$), 2.62 (s br, 4H, 2 $CH_2$), 1.85 (s br, 8H, 2 $CH_2CH_2$), 1.60 (s br, 4H, $CH_2CH_2$), 1.45–1.20 (s br, 8H, 2 $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 509.3; HR-FABMS: exact mass calculated for $C_{32}H_{41}N_6$ $[M+H]^+$ 509.3393; found 509.3392.

*N,N′-Bis-(2,3,4,5-tetrahydro-1H-cyclohepta[1,2-b] quinolin-10-yl)-1,6-diaminohexane (**7a**).*
According to the general procedure, chloride **4c** (0.25 g, 1.08 mmol) and 1,6-diaminohexane (63 mg, 0.54 mmol) were condensed under heat for 2.5 h to give, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH$ = 10:1 as eluents), the title compound (75 mg, 26%) as an amber glass foam: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.98 (d, J = 7.8 Hz, 2H, Ar-H), 7.88 (d, J = 7.9 Hz, 2H, Ar-H), 7.55 (t, J = 6.9 Hz, 2H, Ar-H), 7.41 (t, J = 7.0 Hz, 2H, Ar-H), 3.30 (t, J = 4.6 Hz, 4H, 2 N-$CH_2$), 3.20–3.10 (m, 4H, 2 $CH_2$), 2.90–2.80 (m, 4H, 2 $CH_2$), 1.95–1.60 (m, 16H, 2 $(CH_2)_3$ & $CH_2CH_2$), 1.50–1.30 (m, 4H, $CH_2CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 507.2; HR-FABMS: exact mass calculated for $C_{34}H_{43}N_4$ $[M+H]^+$ 507.3488; found 507.3483.

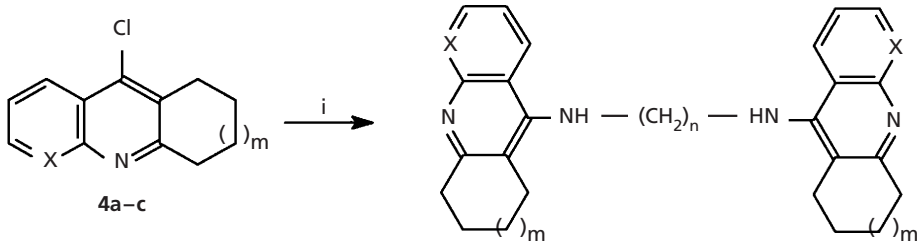
*N,N′-Bis-(2,3,4,5-tetrahydro-1H-cyclohepta[1,2-b] quinolin-10-yl)-1,8-diaminooctane (**7b**).*
According to the general procedure, chloride **4c** (0.29 g, 1.25 mmol) and 1,8-diaminooctane (90 mg, 0.63 mmol) were condensed under heat for 5 h to furnish, after flash chromatography ($CH_2Cl_2$ to $CH_2Cl_2/CH_3OH$ = 10:1 as eluents), the title compound (94 mg, 28%) as an amber glass foam: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.05 (d, J = 8.4 Hz, 2H, Ar-H), 7.94 (d, J = 8.5 Hz, 2H, Ar-H), 7.54 (t, J = 7.3 Hz, 2H, Ar-H), 7.41 (t, J = 8.8 Hz, 2H, Ar-H), 3.37 (t, J = 4.4 Hz, 4H, 2 N-$CH_2$), 3.20 (s br, 4H, 2 $CH_2$), 2.90 (s br, 4H, 2 $CH_2$), 1.95–1.65 (m, 16H, 8 $CH_2$), 1.45–1.20 (m, 8H, 4 $CH_2$); FABMS (NBA as matrix): m/z $[M+H]^+$ 535.1; HR-FABMS: exact mass calculated for $C_{36}H_{47}N_4$ $[M+H]^+$ 535.3801; found 535.3777.

### National Cancer Institute drug-screening programme

These synthetic compounds were selected and evaluated in a drug-screening programme at the National Cancer Institute (Bethesda, MD). Cytotoxicity tests were performed on 60 human cancer cell lines derived from nine different tissues and compounds were tested at a minimum of five concentrations at 10-fold dilutions. A 48-h continuous drug exposure protocol was used, and a SRB protein assay (Skehan et al 1990) was used to estimate cell viability or growth. The GI50 (concentration causing 50% growth inhibition) was determined, which corresponds to the IC50 value defined elsewhere (Alley et al 1988).

## Results and Discussion

The synthesis of 9-chlorotetrahydroacridine (**4a**) and its congeners **4b** and **4c** was accomplished in 84–96% yields by heating the *o*-aminoaromatic acids **2a** and **2b** and cycloketones **3a** and **3b** in the presence of $POCl_3$ as illustrated in Figure 2 (Hu & Lu 2000). Bis-amination of the chloride **4a** was conducted by heating a mixture of **4a** and a variety of 1,*n*-diaminoalkanes (0.5 equiv.) in the presence of phenol and catalytic amounts of sodium iodide at 180°C under an argon atmosphere to furnish the desired bis-tacrines **5a**–**d** (Figure 3). The bis-tacrine congeners **6a**–**d** were then obtained by the same approach with moderate yields. However, the reaction of 1,*n*-diaminoalkanes and heptyl-fused chloride **4c** provided congeners **7a** and **7b** with relatively lower yields even with an extension of the reaction time. These bis-tacrines and their congeners were characterized spectroscopically ($^1H$ NMR, MS, and HR-FABMS).




**5a–d,** X = CH, m = 1, n = 2, 4, 6, 8
**6a–d,** X = N, m = 1, n = 2, 4, 6, 8
**7a–b,** X = CH, m = 2, n = 6, 8

**Figure 3** Synthesis of bis-tacrines **5a**–**d**, by bis-amination of the chloride **4a**. Reagents: i. 1,*n*-diaminoalkane, Phenol, NaI, 180 °C.

**Table 1** Cytotoxicity of bis-tacrine congeners in the National Cancer Institute drug-screening programme.

| Cell line | GI50 (μm)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **5a** | **5b** | **5d** | **6a** | **6b** | **6c** | **6d** | **7a** | **7b** |
| Non-small cell lung cancer | | | | | | | | | |
| NCI-H226 | 31.5 | 0.06 | – | 49.3 | 53.9 | 16.4 | 6.67 | 2.14 | 1.55 |
| NCI-H23 | 22.7 | 0.06 | 1.69 | 59.4 | 70.2 | 13.1 | 4.50 | 1.82 | 1.66 |
| Colon cancer | | | | | | | | | |
| COLO 205 | 10.9 | 0.04 | 1.22 | 16.7 | 18.9 | 5.24 | 0.92 | 0.92 | 0.75 |
| HCT-116 | 11.2 | 1.23 | 0.37 | 14.9 | 20.6 | 2.72 | 0.45 | 0.90 | 0.72 |
| HCT-15 | 1.69 | 0.05 | 1.23 | > 100 | > 100 | 2.75 | 4.36 | 4.54 | 4.13 |
| CNS cancer | | | | | | | | | |
| SF-268 | 17.3 | 0.33 | 1.22 | 16.8 | 59.0 | 9.99 | 10.5 | 1.72 | 1.86 |
| SF-539 | –[b] | 0.03 | – | 25.0 | 26.1 | 7.89 | 0.38 | 1.46 | 1.50 |
| Melanoma | | | | | | | | | |
| LOX IMVI | 15.0 | 0.06 | 1.36 | 17.5 | 18.6 | 11.4 | 5.69 | 1.47 | 1.50 |
| SK-MEL-5 | 18.6 | 0.06 | 1.54 | 14.7 | 18.9 | 11.6 | 5.92 | 1.82 | 1.44 |
| Ovarian cancer | | | | | | | | | |
| OVCAR-8 | 18.0 | 0.07 | 1.36 | 24.5 | 25.1 | 6.73 | 0.61 | 1.34 | 1.34 |
| SK-OV-3 | 34.4 | 18.2 | 1.45 | 35.8 | 53.1 | 10.1 | 10.5 | 1.53 | 1.75 |
| Renal cancer | | | | | | | | | |
| CAKI-1 | 23.6 | 22.6 | 1.88 | 32.8 | 21.8 | 6.19 | 8.64 | 2.69 | 2.21 |
| Breast cancer | | | | | | | | | |
| MCF7 | 16.4 | 3.75 | 1.65 | – | – | 2.25 | 5.45 | 1.18 | 1.17 |
| NCI/ADR RES | 18.6 | 0.04 | 1.86 | 85.1 | > 100 | 19.0 | 1.58 | 2.22 | 2.07 |
| BT-549 | 19.0 | 0.08 | 1.69 | 17.0 | 18.7 | 1.66 | 1.06 | 0.30 | 0.22 |
| mid[c] | 16.84 | 4.66 | 1.32 | 31.47 | 31.67 | 8.80 | 5.36 | 1.52 | 1.65 |

Values are the average of duplicate testing. [a]GI50 = 50% growth inhibition; [b]indicates a default value; [c] the average GI50 value for the drug over the whole cell line panel.

The synthetic bis-tacrine congeners were selected and evaluated against a panel of 60 human cancer cell lines using the standard SRB assay, and the results for fifteen of those cell lines are presented in Table 1 as GI50 values (equivalent to IC50), together with the mid-value (the average GI50 value for the whole cell line). From the representative data shown, bis-tacrine congeners **5b**, **5d**, **7a**, and **7b** exhibited moderate selectivity toward colon and melanoma cells, in which colon 205, HCT-116, LOX IMVI, and SK-MEL-5 were selected as a representative cell line sub panel. Selectivity for colon 205 (GI50 (mid)/GI50 (colon 205)) varied from 1.1 to 116.5, with an average of 18-fold, whereas an average of 4.0-fold selectivity for HCT116 was obtained. In general,

the MCF7 and NCI/ADR RES lines could be used to evaluate the extent to which bis-tacrine congeners are affected by P-glycoprotein-mediated multidrug resistance (Roninson 1992). The extent of resistance indicated by the P-glycoprotein over-producing cell line (measured as GI50 (NCI-ADR)/GI50 (MCF7)] changed from 0.01 (**5b**) to 8.4 (**6c**), with an average value over all the tested compounds of 2.2-fold. These data indicate a less significant degree of resistance for the class. All of the tested compounds had weak cytotoxicity against both the CAKI-1 renal tumour line and SKOV3 ovarian carcinoma, which were selected as representative refractory solid tumours. Interestingly, butyl-linked bis-tacrine (**5b**) exhibited broad and strong cytotoxicity, with GI50 values at sub-micromolar levels particularly against non-small cell lung and colon cancers and melanoma. Its aza congener, **6b,** was almost devoid of activity against these cell lines. These results suggest that the decreased cytotoxic effects might result from the electronic properties of pyridyl nitrogen. The short connecting alkyl chain of bis-tacrine and some congeners (e.g. **5a**, **6a**) induced a dramatic decrease in cytotoxic effects. The cytotoxic profiles of dimers with a longer alkyl chain (e.g. n = 8; congeners **5d**, **6d**, **7b**) were broadly identical, with most GI50 values in the region of 1–10 $\mu$M. The strong cytotoxicity of **5b** suggests that this compound might act as a bifunctional intercalator. This suggestion might explain the moderate potency of congeners **5d**, **6d** and **7b**, in which the longer connecting chains correspond to the intercalating actions on different binding grooves that are also required for anticancer activity. Given the excellent in-vitro results, compound **5b** has been selected for further biological evaluation of its anticancer profile.

It has been reported that the fully aromatic bis-acridines are potent inhibitors of the growth of various tumour cells in culture (Canellakis et al 1976). The bis-tacrine moieties reported here might act as potential bifunctional intercalators, as do the well known bis-acridines. The prominent cytotoxicity of bis-tacrines might reveal an alternative approach toward potent anticancer drugs.

## References


Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., Boyd, M. R. (1988) Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **48**: 589–601

Brana, M. F., Castellano, J. M., Perron, D., Maher, C., Conlon, D., Bousquet, P. F., George, J., Qian, X. -D., Robinson, S. P. (1997) Chromophore-modified bis-naphthalimides: synthesis and antitumor activity of bis-dibenz-[deh]isoquinoline-1,3-diones. *J. Med. Chem.* **40**: 449–454

Canellakis, E. S., Shaw, Y. H., Hanners, W. E. Schwartz, R. A. (1976) Diacridines: bifunctional intercalators. I. Chemistry, physical chemistry and growth inhibitory properties. *Biochim. Biophys. Acta* **418**: 277–289

Chen, T. K., Fico, R., Canellakis, S. E. (1978) Diacridines, bifunctional intercalators. Chemistry and antitumor activity. *J. Med. Chem.* **21**: 868–874

Denny, W. A. (1989) DNA-intercalating agents as antitumour drugs: prospects for future design. *Anticancer Drug Des.* **4**: 241–263

Diaz-Rubio, E., Martin, M., Lopez-Vega, J. M., Casado, A., Benavides, A. (1994) Phase I study of mitonafide with a 3-day administration schedule: early interruption due to severe central nervous system toxicity. *Invest. New Drugs* **10**: 25–26

Goldin, A., Vendetti, M. M., McDonald, J. S., Muggia, F. M., Henney, J. E., DeVita, V. T. (1981) Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. *Eur. J. Cancer* **17**: 129–142

Hu, M. K., Lu, C. F. (2000) A facile synthesis of bis-tacrine isosteres. *Tetrahedron Lett.* **41**: 1815–1818

McGeer, P. L., McGeer, E. G. (1995) Alzheimer's disease: arthritis of the brain? *Drug News Perspect.* **8**: 80–83

Roninson, I. B. (1992) The role of the *MDR*1 (P-glycoprotein) gene in multidrug resistance in vitro and in vivo. *Biochem. Pharmacol.* **43**: 95–102

Sargent, L. J., Samll, L. (1946) The acridine series II. Dialkylaminoalkylamines from 9-chloro-1,2,3,4-tetrahydroacridine. *J. Org. Chem.* **11**: 359–362

Segal-Bendirjian, E., Coulaud, D., Roques, B. P., Le Pecq, J. -B. (1988) Selective loss of mitochondrial DNA after treatment of cells with ditercalinium (NSC 335153), an antitumor bis-intercalating agent. *Cancer Res.* **48**: 4982–4992

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. R., Bokesch, H., Kenney, S., Boyd, M. R. (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl Cancer Inst.* **82**: 1107–1112

Spicer, J. A., Gamage, S. A., Rewcastle, G. W., Finlay, G. J., Bridewell, D. J. A., Baguley, B. C., Denny, W. A. (2000) Bis(phenazine-1-carboxamides): structure-activity relationships for a new class of dual topoisomerase I/II-directed anticancer drugs. *J. Med. Chem.* **43**: 1350–1358

Wakelin, L. P. G. (1986) Polyfunctional DNA intercalating agents. *Med. Res. Rev.* **6**: 277–340