JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 653–660

Received November 9, 2002
Accepted February 11, 2003
DOI 10.1211/0022357021053
ISSN 0022-3573

Two lead drug designs based on chloramphenicol as the parent structure, which express alkylation activity with potential for clinical applications

Ronald Bartzatt

Abstract

Chloramphenicol is a bacteriostatic antibiotic which acts primarily as an inhibitor of bacterial protein synthesis. Modification of chloramphenicol structure was accomplished by replacing the two hydroxyl groups (—OH) with a chlorine atom for LEAD-1 or a chloroethyl ether group (—OCH_2CH_2Cl) for LEAD-2. The resulting daughter compounds expressed significant alkylating activity at physiological temperature of 37 °C and at pH 7.4. Alkylation activity was evaluated after reaction with guanosine 5′-diphosphate (GDP), L-serine, L-glutamic acid, and *p*-chloroaniline. The partition coefficient (log P) was determined for chloramphenicol, LEAD-1 and LEAD-2 to be 0.854, 3.409 and 3.10, respectively. The molecular dipole of chloramphenicol, LEAD-1 and LEAD-2 was calculated to be 5.804, 3.961 and 4.097 Debye, respectively. All three structures showed zero violations of the Rule of 5, which indicates good bioavailability. Values for polar surface area (TPSA) indicate an intestinal absorption of 51% and 35% for LEAD-1 and LEAD-2, respectively. The reduction to only one proton donor in LEAD-1 and LEAD-2 structures (chloramphenicol having three proton donors) indicates an improvement of membrane penetration compared to chloramphenicol. ^{13}C NMR analysis of molecular structures was accomplished and fast atom bombardment mass spectrometry analysis of a reaction mixture showed LEAD-1 alkylation of guanosine 5′-diphosphate.

Introduction

Chloramphenicol is an antibiotic with a wide spectrum of activity against Gram-positive and Gram-negative cocci and bacilli (including anaerobes), *Rickettsia, Mycoplasma*, typhoid fever and *Chlamydia*. Its action is considered primarily bacteriostatic in that it binds to the 50S subunit of the ribosome and inhibits bacterial protein synthesis. It is one of the most highly absorbed of antibiotics and penetrates through cell membranes and purulent material. Chloramphenicol is metabolized in the liver to an inactive glucuronide, which is excreted along with non-metabolized chloramphenicol in the urine. Chloramphenicol has shown efficacy as an antimicrobial for the treatment of cancer patients, although its parent structure demonstrates no anticancer activity in itself (Li et al 1977). It has been applied against microbial infections in cancer patients but a significant emergence of resistance is observed in previously susceptible *Escherichia coli* (Kern et al 1994), *Pseudomonas* (Loureiro et al 2002), *Streptococcus pneumoniae* (Maraki et al 2001) and *Staphylococcus aureus* (Al-Haddad et al 2001; Millar et al 2001). Streptococci occurring in cancer patients have been successfully treated with chloramphenicol (Wisplinghoff et al 1999), as has vancomycin-resistant *Enterococcus faecium* (Ricaurte et al 2001).

The structure of chloramphenicol has been modified previously to include a nitrogen mustard substituent (Jen et al 1962), which did show significant inhibitory activity against a sarcoma line. Implacement of a nitrogen mustard substituent gives this drug alkylation activity. In rat hosts, chloramphenicol nitrogen mustard was shown to inhibit development of adrenal necroses (Bogush & Konopleva 1977), lung adenomas (Shabad et al 1977) and carcinogenesis (Blunck 1970).

Anticancer agents that interact with DNA comprise an important portion of all available clinical chemotherapeutics. DNA-interacting agents can be further categorized

University of Nebraska, College of Arts & Sciences, Chemistry Department, Durham Science Center, Dodge 6001, Omaha, Nebraska 68182 USA

Ronald Bartzatt

Correspondence: R. Bartzatt, University of Nebraska, College of Arts & Sciences, Chemistry Department, Durham Science Center, Dodge 6001, Omaha, Nebraska 68182, USA. E-mail: bartzatt@mail.unomaha.edu

Funding: This work was supported by the College of Arts and Science and the Chemistry Department of the University of Nebraska (Omaha, NE).

as DNA strand breakers, DNA alkylators and DNA intercalators (Silverman 1992). DNA alkylators can be subdivided by their major mechanisms of action as follows: nitrogen mustards; ethylenimines; methanesulfonates; and nitrosoureas. The platinum complex cisplatin is a neutral, square planar molecule containing two chlorine atoms in a *cis* configuration. Cisplatin loses its chlorines atoms and becomes a reactive electrophile that binds covalently to DNA. The LEAD drugs introduced in this work are in the DNA-alkylator category. The alkylation mechanism results in the formation of a covalent bond with the target molecule that is not reversible under biological conditions (Gringauz 1997).

Alkylating agents are considered to be irreversible inhibitors of DNA. The alkylation of enzymes, proteins, RNA and DNA can inactivate them, an action that may be detrimental to the cells. However, only the alkylation of DNA is considered irreparable and it is considered to be the actual lethal event leading to cell death (Gringauz 1997). A biological alkylating agent is described as a compound that can replace a hydrogen atom with an alkyl group (Silverman 1992) under physiological conditions of pH 7.4 and 37 °C. The electrophilic alkylating agent will attach itself to a nucleophilic atom such as nitrogen, oxygen, phosphorus or sulfur. For DNA, the most nucleophilic sites are N-7 of guanine > N-3 of adenine > N-1 of adenine > N-1 of cytosine.

Alkylation mechanisms involve either of two types of nucleophilic substitution reactions referred to as unimolecular nucleophilic substitution (SN_1) and bimolecular nucleophilic substitution reaction (SN_2). Alkylating agents that can attach to both strands of DNA effectively prevent strand separation, which is necessary for replication, an action that is considered cytotoxic. Alkylating agents are the oldest class of anticancer drugs. Antitumour alkylating agents are the most widely used anticancer drugs. Most alkylating agents are unstable molecules that must be stored dry. The design and study of novel alkylating agents may provide additional medicinal agents for the clinical treatment of cancer patients.

Materials and Methods

Reagents and computer software

Reagents were from Aldrich (Sigma-Aldrich, St Louis, MO). (LogKo/w) log P values were calculated using the method of Syracuse Research Corporation (Denver, CO). Values of miLogP, polar surface area (TPSA), proton donors and violations of Rule of 5 (MW > 500.0; Clog P > 5.0; no. of H-bond acceptors > 10; no. of H-bond donors > 5) were calculated using Molinspiration methods (Molinspiration Cheminformatics, Bratislava, Slovak Republic). Molecular dipoles and molecular modeling were accomplished utilizing SPARTAN software (Wavefunction, Irvine, CA). Mass spectra were analysed utilizing MASS SPEC software (Trinity Software, Plymouth, NH). A Spectronic 21D UV-Vis spectrometer with 1-cm glass cuvettes was utilized for absorbance measurements.

A Fast Atom Bombardment (FAB) excitation AutoSpec ETOFFPD magnet mass spectrometer was utilized to analyse aqueous samples of alkylation reactions for the presence of high-mass organic and phosphorus-oxygen products.

Synthesis of LEAD-1 and LEAD-2

*LEAD-1 (2,2-dichloro-*N*-[2-chloro-1-(chloromethyl)-2-(4-nitrophenyl) ethyl]acetamide)*

Chloramphenicol (5 mg) was placed into 5 mL of CH_3CN (dried over molecular sieves) and 0.5 mL of pyridine or triethylamine. $SOCl_2$ (0.50 mL) was added and allowed to react at 25 °C for 1.5 h and then 60 °C for 20 min. The product was precipitated over ice-water or −10 °C. The product was filtered out and washed with cold acetone.

*LEAD-2 (2,2-dichloro-*N*-[2-(2-chloroethoxy)-1-[(2-chloroethoxy)methyl]-2-(4-nitrophenyl)ethyl]acetamide)*

Chloramphenicol (15 mg) was placed into dried benzene (dried over molecular sieves), warmed to 50 °C and a slight excess of Na metal (small pieces previously rinsed in dry benzene) was added. After bubbling ceased a sufficient amount of 100% ethanol was added to react with the remaining Na metal. An equal volume of 97% 1,2-dichloroethane and 3% triethylamine was added and the mixture was refluxed for 20 min. The warm liquid supernatant was decanted from any precipitate. Product was precipitated over ice-water or overnight at −10 °C.

The products of LEAD-1 and LEAD-2 synthesis were confirmed as pure by spotting on silica TLC plates and resolving in methylene chloride solvent. Single spots were observed on TLC plates after development in fuming iodine (iodine crystals) chamber, which reveals organic analytes.

Measurement of alkylation efficiency utilizing fluorescamine

A typical reaction was run at 37 °C and in 0.10 M $NaHCO_3$, pH 7.4. Into 700 μL total volume was placed 10 to 15 mg of LEAD compound and up to 500 μg of *p*-chloroaniline or other nucleophile. At known time intervals, 60 μL of reaction solution were withdrawn into suitable vessel and 50 μL of fluorescamine (2 mg mL^{-1} in ethanol) were added. Several minutes were allowed for reaction, 2.5–3.0 mL of water was added and the absorbance at 400 nm was read in 1-cm glass cuvettes.

Results and Discussion

DNA alkylators fall within the three major categories of DNA interactive anticancer agents, which include intercalators, alkylators and strand breakers. DNA alkylators covalently bond to their target and are considered irreversible inhibitors. DNA alkylators are considered to be an

important group of medicinal agents, which express potent cytotoxic effects on the tumour cell target. The parent structure of chloramphenicol shows significant success for the clinical treatment of cancer patients that have microbial infections. Although chloramphenicol itself does not express any antitumour activity, two LEAD structures presented here are shown to express significant alkylation activity, which, together with favourable pharmacological parameters such as log P, TPSA, dipole, solubility and stability, have a potential for clinical applications.

Molecular structures of chloramphenicol and the two derivatives (LEAD-1 and LEAD-2) are presented in Figure 1. The structures shown are dash formulas with carbon atoms numbered for ^{13}C NMR assignment. The replacement of the two hydroxyl groups on chloramphenicol with chlorine atoms (LEAD-1) or chloroethyl ether groups (LEAD-2) did not decrease the stability of the compounds, which remained intact after more than eight weeks storage at $-10\,^{\circ}C$ or one week at $25\,^{\circ}C$. The chlorine atoms increase the lipophilic tendency and lipid bilayer solubility of the compounds.

Synthesis of LEAD-1 and LEAD-2 is not difficult and utilizes the well-characterized chemistry of thionyl chloride ($SOCl_2$). The synthesis is presented stepwise in Figure 2, where LEAD-1 is formed after refluxing chloramphenicol with an excess of $SOCl_2$ and in the presence of a proton sink such as triethylamine or pyridine. The proton sink prevents the hydrolysis of the amide group and promotes the reaction by reacting with the HCl produced by the action of thionyl chloride (Solomons 1994). The general reaction of thionyl chloride with an alcoholic hydroxyl group (—OH) can be represented as follows:

$$R{-}OH + SOCl_2 \rightarrow R{-}Cl + SO_2 + HCl \qquad (1)$$

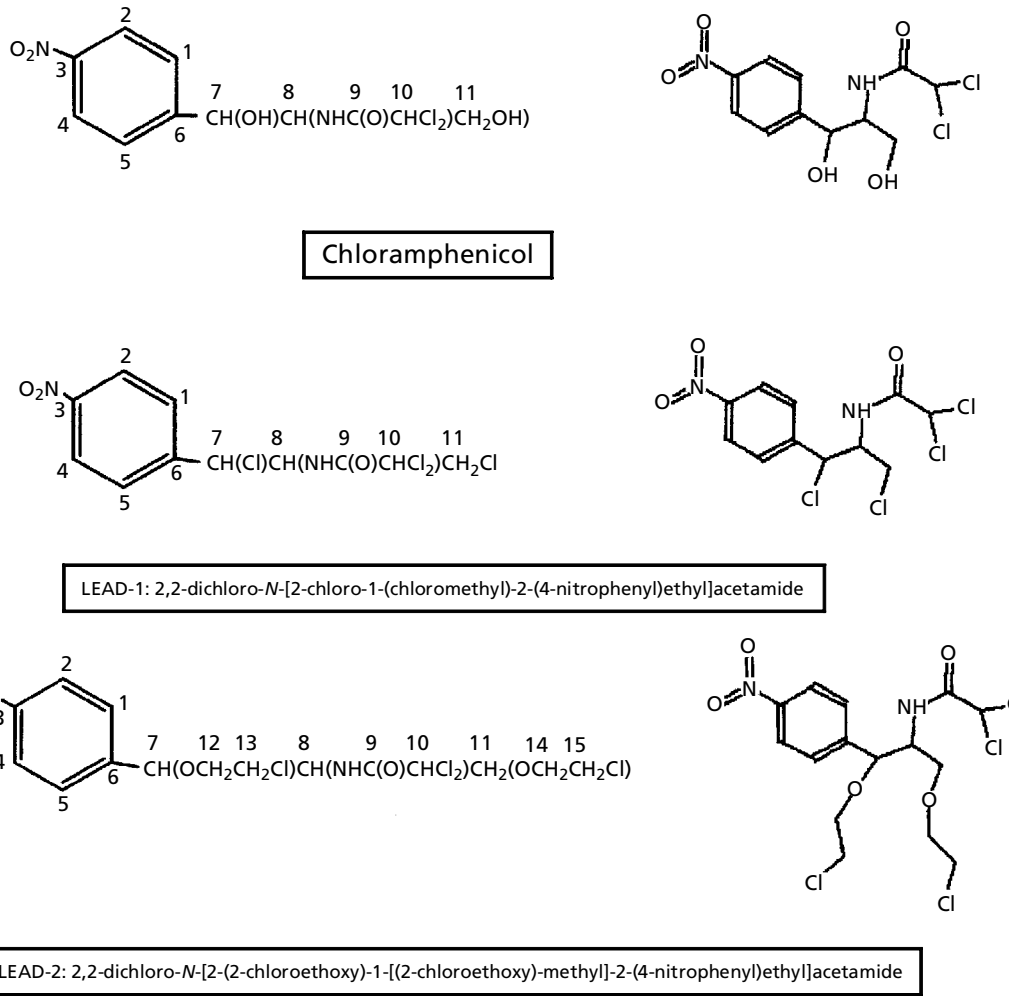


Figure 1 Molecular structures of compounds showing ^{13}C NMR assignments as (carbon no./ppm): Chloramphenicol: 1/129.2, 2/123.7, 3/145.7, 4/123.7, 5/129.2, 6/144.9, 7/74.9, 8/57.3, 9/160.6, 10/74.6, 11/62.5. LEAD-1: 1/129.2, 2/123.7, 3/145.7, 4/123.7, 5/129.2, 6/144.9, 7/58.0, 8/57.1, 9/160.6, 10/74.6, 11/45.6. LEAD-2: 1/129.2, 2/123.7, 3/145.7, 4/123.7, 5/129.2, 6/144.9, 7/77.9, 8/52.9, 9/160.6, 10/74.6, 11/69.2, 12/70.4, 13/46.0, 14/73.0, 15/45.7.

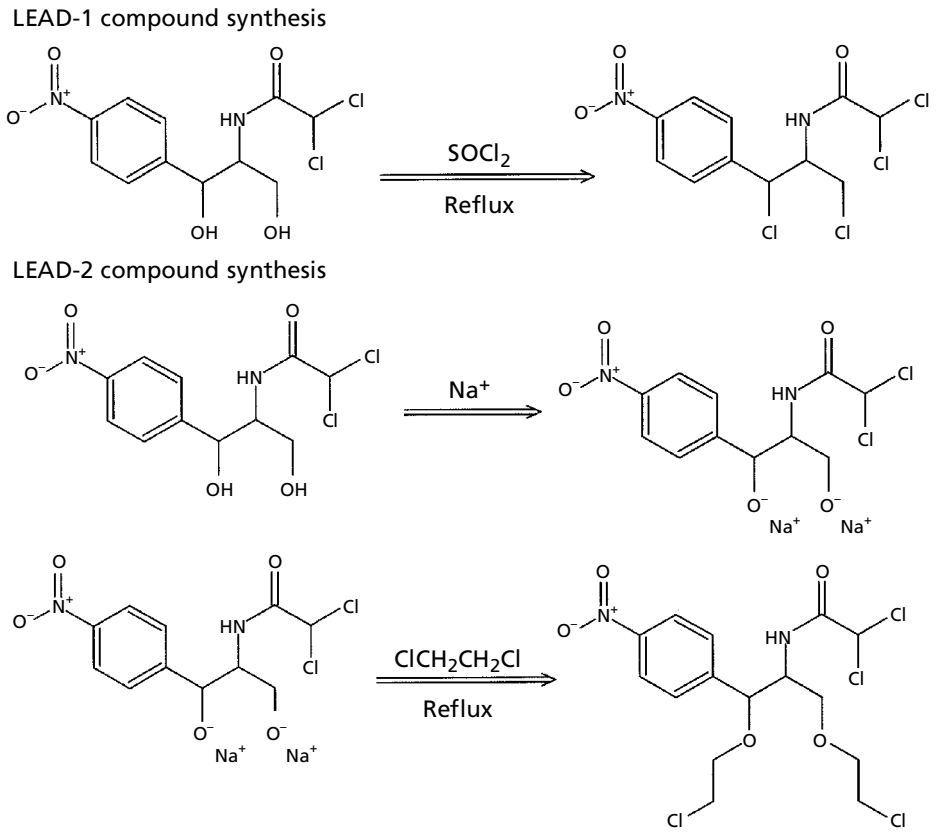


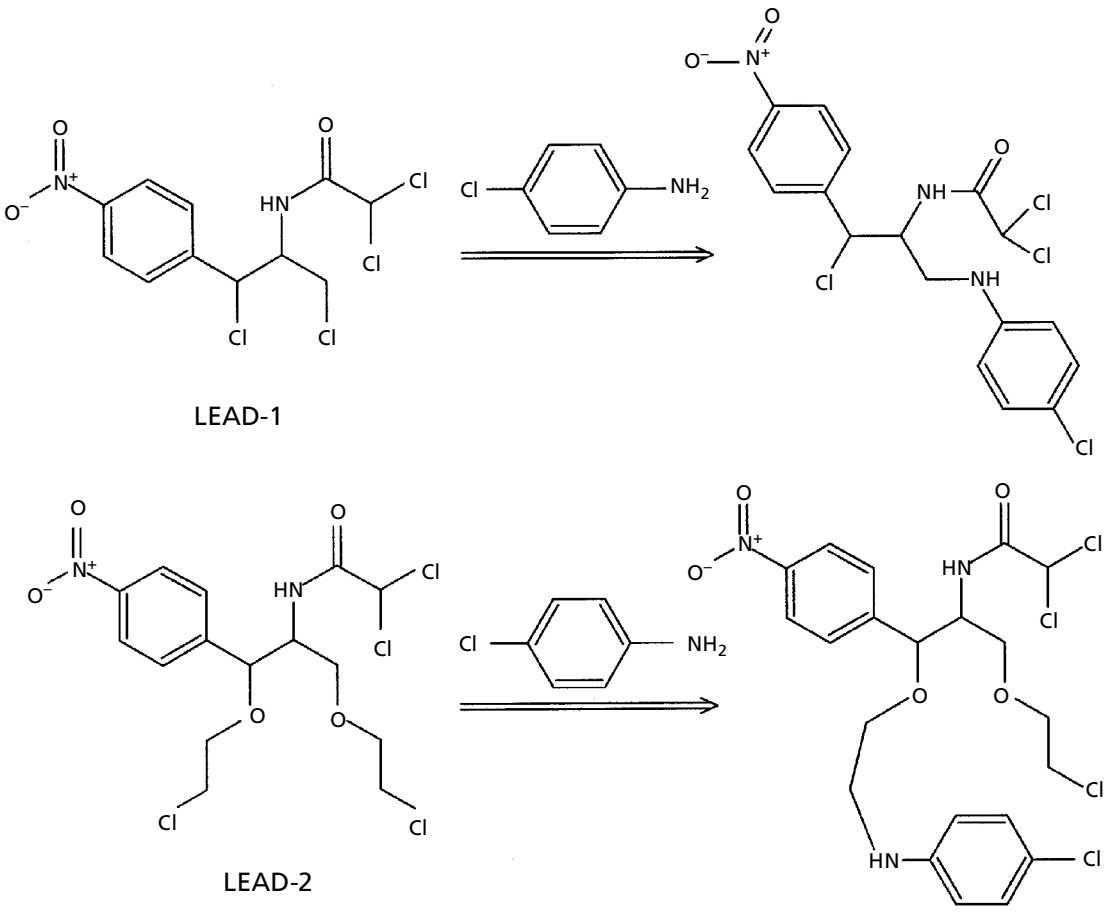
Figure 2 LEAD-1 (2,2-dichloro-*N*-[2-chloro-1-(chloroethyl)-2-(4-nitrophenyl)ethyl]acetamide) synthesis involved a thionyl chloride reaction to replace the hydroxyl groups of chloramphenicol with chlorine atoms. The two-step presentation of LEAD-2 (2,2-dichloro-*N*-[2-(2-chloroethoxy)-1-[(2-chloroethoxy)methyl]-2-(4-nitrophenyl)ethyl]acetamide) synthesis begins: step 1 – replacement of —OH groups with ($—O^- Na^+$); step 2 – reaction with $ClCH_2CH_2Cl$ to form alkyl halide substituents $—OCH_2CH_2Cl$ (Williamson Ether Synthesis).

The synthesis of LEAD-2 is essentially a Williamson Ether Synthesis. Initially, the sodium metal is introduced (Step 1), which produces a moiety that will readily react with an alkyl halide (Step 2). A reflux step in the presence of 1,2-dichloroethane with a proton sink readily produces the product designated LEAD-2.

A sensitive and specific method of monitoring the alkylation reactions of LEAD-1 and LEAD-2 utilizes the fluorescent probe fluorescamine. Fluorescamine forms a tertiary amine derivative with primary amine targets (*p*-chloroaniline here), which fluoresces under ultraviolet light. Fluorescamine reacts specifically with primary amines. The primary amine *p*-chloroaniline was utilized in this study as the target of alkylation reaction with LEAD-1 and LEAD-2 at physiological conditions of pH 7.4 and 37 °C. In addition to the easily seen fluorescence emission under UV light the tertiary amine derivative absorbs in the visible spectrum. The absorbance spectrum is presented in Figure 3, having a strong bell-shaped peak occurring at 400 nm wavelength. The molar absorptivity was calculated to be 18.37 $L\,mol^{-1}\,cm^{-1}$ and may be utilized to quantitate the remaining unreacted primary amine *p*-chloroaniline. After a known time period of reaction at pH 7.4 and 37 °C, a sample of the reaction mixture is withdrawn and, after the addition of fluorescamine (2 $mg\,mL^{-1}$ in ethanol), a measurement of absorbance at 400 nm indicates the extent of the alkylation activity. Figure 3 presents the products of such alkylation reactions in which a secondary amine product is formed from the initial primary amine target compound.

Both LEAD-1 and LEAD-2 potentially may react with two equivalents of the target primary amine *p*-chloroaniline. For LEAD-1, the substituent $—CH_2Cl$ alkylates the primary amine of *p*-chloroaniline to form a secondary amine. For LEAD-2, the substituent $—OCH_2CH_2Cl$ alkylates the primary amine group of *p*-chloroaniline to form a secondary amine. Presumably, both $—OCH_2CH_2Cl$ substituents of LEAD-2 may express alkylation activity.

Important pharmacological parameters of prospective medicinal agents include partition coefficient log P, the lipophilic substituent constant (π) and the molecular dipole. These parameters are presented in Table 1 for all three compounds. Log P values, calculated by the methods of Syracuse Research Corporation and Molinspiration, are presented for comparison. Both LEAD agents have significantly higher log P values than the parent chloramphenicol, which is also observed upon calculation of lipophilic substituent constant, 2.555 and



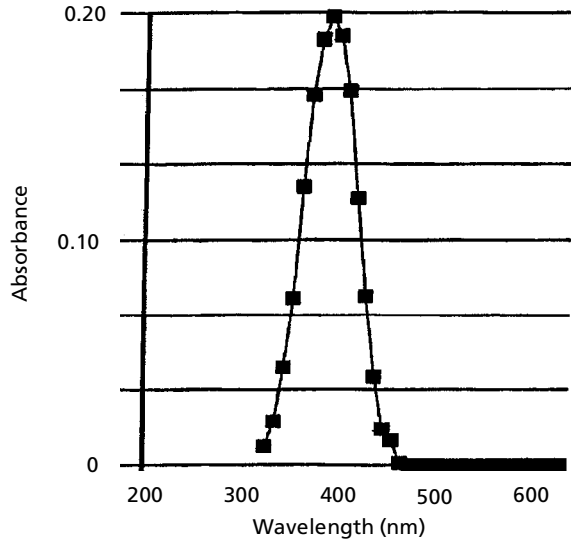


Figure 3 Alkylation reactions of LEAD compounds with nucleophile *p*-chloroaniline. Also presented is the absorbance curve of the fluorescamine–*p*-chloroaniline complex, which is formed upon addition of fluorescamine. The absorbance peak at 400nm was utilized to monitor the extent of alkylation reactions.

Table 1 Comparison of partition coefficient (log P), lipophilic substituent constant (π) and molecular dipole.

Compound	log Ko/w log P[a]	miLog P[b]	(π)[c]	Molecular dipole[d]
Chloramphenicol	0.920	0.854		5.804
LEAD-1	3.244	3.409	2.555	3.961
LEAD-2	3.295	3.100	2.246	4.097

LEAD-1: 2,2-dichloro-*N*-[2-chloro-1-(chloroethyl)-2-(4-nitrophenyl)ethyl]acetamide. LEAD-2: 2,2-dichloro-*N*-[2-(2-chloroethoxy)-1-[(2-chloroethoxy)methyl]-2-(4-nitrophenyl)ethyl]acetamide. [a]Partition coefficient from method of Syracuse Research Corporation. [b]Partition coefficient from method of Molinspiration Corporation. [c]Lipophilic substituent constant utilizing the miLog P values. [d]Units of dipole are Debye, calculated by use of SPARTAN software.

2.246 for LEAD-1 and LEAD-2, respectively (chloramphenicol is considered as parent). The higher positive value of log P for LEAD compounds indicates a greater solubility in lipid bilayers. Molecular dipole of both daughter compounds and chloramphenicol was accomplished utilizing SPARTAN soft-ware. Chloramphenicol has a significantly greater molecular dipole value than both LEAD agents. A strong dipole inhibits the solubility of a medicinal agent in lipid bilayers. The smaller numerical values of molecular dipole for LEAD-1 and LEAD-2 will enhance lipid bilayer solubility (Silverman 1992). The favourable values of log P, π and molecular dipole for both LEAD compounds purports a clinical potential.

Other calculated parameters were found to be favourable, including TPSA, proton donor capacity and Rule of 5 violations. TPSA values were calculated utilizing the method of Molinspiration (Table 2), and determined to be 115.4, 74.92 and 93.4, for chloramphenicol, LEAD-1 and LEAD-2, respectively. TPSA determination has been shown to be a good indicator of drug absorbance in the intestines (Ertl et al 2000). The graph of percent absorption vs TPSA (Figure 4) can be used to predict absorption by utilizing the best trendline drawn. The anticipated intestinal absorption for chloramphenicol, LEAD-1 and LEAD-2 are 18%, 51% and 35%, respectively. LEAD-1 and LEAD-2 agents show an intestinal absorption potential that is greater than the parent compound chloramphenicol and is considered moderate absorption (whereas the absorption for chloramphenicol at 18% is considered low). Also, the reduced proton donor capacity of LEAD-1 and LEAD-2 (see Table 2) compared with chloramphenicol is a parameter that increases lipophilic tendency and reduces aqueous solubility. However, both LEAD agents demonstrated acceptable water solubility at 25 °C and 37 °C. All three compounds showed zero violations of the Rule of 5 (Lipinski et al 1997). Zero violations of the Rule of 5 indicate that the medicinal agent will have good bioavailability. Violations of two or more of the following criteria suggests poor absorption and problems of drug activity due to poor absorption (Rule of 5: MW > 500.0; Clog P > 5.0; no. of H-bond acceptors > 10; no. of H-bond donors > 5).

LEAD-2 compound was placed in aqueous 0.10 M $NaHCO_3$ at pH 7.4 and 37 °C separately with L-serine, L-glutamic acid and *p*-chloroaniline. After 20 h of incubation, samples were withdrawn from reaction mixtures, the absorbances was measured and showed that 35% of the L-serine and 71% of the L-glutamic acid was alkylated. The alkylation of amino acids is considered to be a cytotoxic activity. In a like manner it was found that LEAD-2 will alkylate 20% of nucleophilic primary amine *p*-chloroaniline.

A similar study was accomplished utilizing LEAD-1 in a 0.001 M borate buffer at both 25 °C and 37 °C. After 24 h of incubation, samples were obtained and derivatized by fluorescamine with results indicating that 15.3% of primary amine *p*-chloroaniline was alkylated at 25 °C and 34.2% alkylated at the higher temperature of 37 °C.

LEAD-1 agent was placed into aqueous $NaHCO_3$ (pH 7.4, 37 °C) with 1.0 mg of guanosine 5′-diphosphate for 45 min and the reaction mixture analysed by fast atom bombardment (FAB) mass spectrometry. Peaks at high m/z reveal that LEAD-1 formed an adduct with guanosine 5′-diphosphate at m/z of 754 (loss of CO) and at 611 (loss of C_7NO_2Cl). Subsequent peaks showing fragmentations appear in the lower m/z region and indicate the LEAD-1 species as well: 326 ($C_{11}H_{10}N_2O_3Cl_3$); 290 ($C_{11}H_{10}N_2O_3Cl_2$); and 255 ($C_{11}H_{10}N_2O_3Cl$). Shown in

Table 2 Values of TPSA, no. of —OH & —NH and no. of violations of the Rule of 5 for LEAD-1, LEAD-2 and chloramphenicol.

Compound	TPSA[a]	No. of donors of [H][b]	No. of Rule of 5 violations[c]
Chloramphenicol	115.4	3	0
LEAD-1	74.92	1	0
LEAD-2	93.4	1	0

LEAD-1: 2,2-dichloro-*N*-[2-chloro-1-(chloroethyl)-2-(4-nitrophenyl)ethyl]acetamide. LEAD-2: 2,2-dichloro-*N*-[2-(2-chloroethoxy)- 1-[(2-chloroethoxy)methyl]-2-(4-nitrophenyl)ethyl]acetamide. [a]Molecular polar surface area. [2]Number of oxygens and nitrogens that can donate a hydrogen [H]. [3]Number of violations to the Rule of 5.

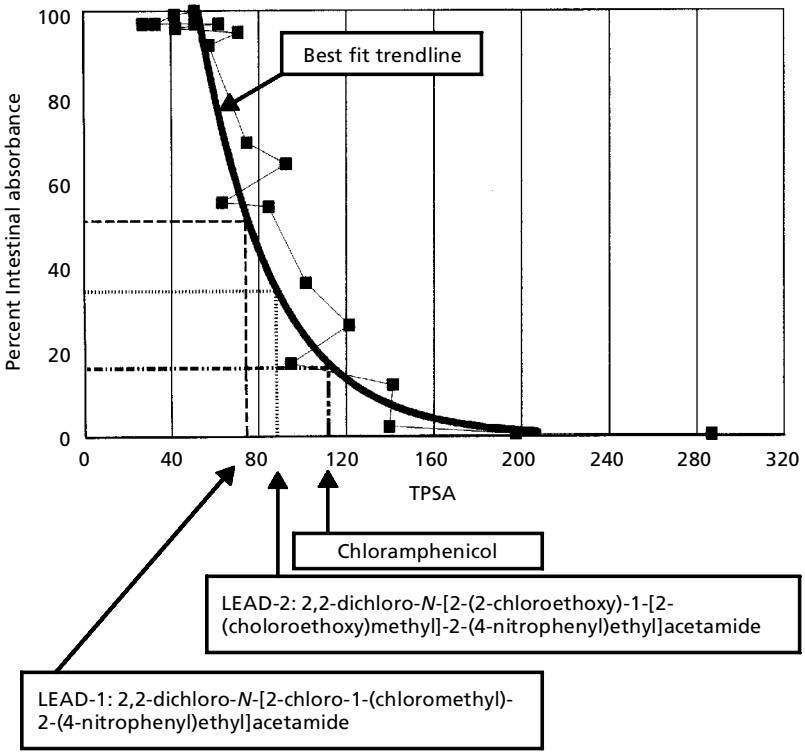
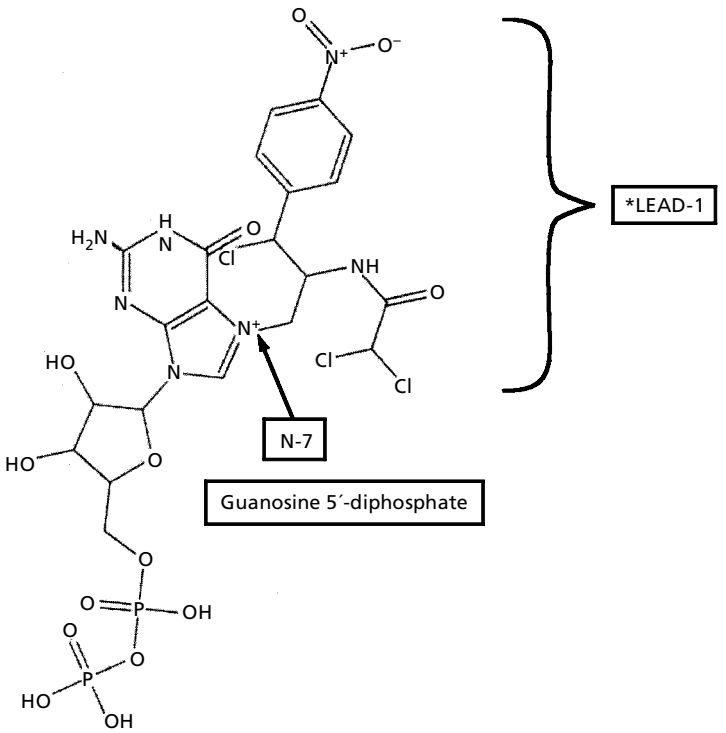


Figure 4 Predicted percent of total amount of drug that will be absorbed in the intestines. TPSA, molecular polar surface area. Those values for chloramphenicol, LEAD-1 and LEAD-2 are 18%, 51% and 35%, respectively.



*2,2-Dichloro-*N*-[2-chloro-1-(chloroethyl)-2-(4-nitrophenyl)ethyl]acetamide

Figure 5 Possible LEAD-1 alkylation of the N-7 position of guanosine 5′-diphosphate.

Figure 5 is a representation of the possible product formed after a LEAD-1 alkylation reaction at the N-7 position of guanosine 5′-diphosphate (GDP).

Conclusions

In summation, two daughter compounds of chloramphenicol were synthesized and studied at physiological conditions. These are considered LEAD compounds as a consequence of the potential for additional substitution reactions placing substituents on the aromatic ring. The LEAD compounds alkylated amino acids, a nucleophilic primary amine compound (*p*-chloroaniline), and guanosine 5′-diphosphate. Products of alkylation reactions were observed by FAB mass spectrometry and fluorescent probing that utilized fluorescamine, which is specific for primary amines. The LEAD compounds showed good stability at room temperature and at −10 °C and were not difficult to synthesize. Pharmacological analysis determined favourable values of parameters including intestinal absorption as shown by TPSA and zero violations of the Rule of 5, a parameter which indicates good bioavailability. Partition coefficients (log P) of LEAD-1 and LEAD-2 indicate greater solubility in lipid bilayers than that of the parent compound chloramphenicol. LEAD-1 and LEAD-2 carry only a single proton donor group which suggests a greater solubility in lipid bilayers.

References

Al-Haddad, A., Udo, E., Mokadas, E., Sanyai, S., Grubb, W. (2001) Persistence of a clone of methicillin-resistant Staphylococcus aureus in a burns unit. *J. Med. Microbiol.* **50**: 558–564

Blunck, J. (1970) Alteration of the RNA/DNA ratio of rat liver associated with the prevention of azo dye carcinogenesis by dietary chloramphenicol. *Life Sci.* **9**: 2

Bogush, T., Konopleva, I. (1977) Protective effect of chloramphenicol and dextramycin on the adenocorticolytic action of 7,12-dimethylbenz(a)anthracine. *Byull. Eksp. Biol. Med.* **83**: 3

Ertl, P., Bernhard, R., Selzer, P. (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport. *J. Med. Chem.* **43**: 3714–3717

Gringauz, A. (1997) *Medicinal chemistry*. Wiley-VCH, New York, pp 93–139

Jen, Y., Chao, S., Kao, I. (1962) Tumor chemotherapy. X. Nitrogen mustards derived from chloramphenicol **28**: 333–340

Kern, W., Andriof, E., Oethinger, M., Kern, P., Hacker, J., Marre, R. (1994) Emergence of fluoroquinolone-resistant Escherichia coli at a cancer center. *Antimicrob. Agents Chemother.* **38**: 681–687

Li, L., Kuentzel, S., Shugars, K., Bhuyan, B. (1977) Cytotoxicity of several marketed antibiotics on mammalian cells in culture. *J. Antibiot. (Tokyo)* **30**: 506–512

Lipinski, C., Lombardo, F., Dominy, B., Feeney, P. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Drug Deliv. Rev.* **23**: 3–25

Loureiro, M., de Moraes, B., Mendonca, V., Quadra, M., Pinheiro, G. Asensi, M. (2002) Pseudomonas aeruginosa: study of antibiotic resistance and molecular typing in hospital infection cases in a neonatal intensive care unit from Rio de Janeiro City, Brazil. *Memorias do Instituto Oswalds Cruz* **97**: 387–394

Maraki, S., Christidou, A., Tselentis, Y. (2001) Antimicrobial resistance and serotype distribution of Streptococcus pneumoniae isolates from Crete, Greece. *Int. J. Antimicrob. Agents* **17**: 465–469

Millar, M., Walsh, T., Linton, C., Zhang, S., Leeming, J., Bennett, P. (2001) Carriage of antibiotic-resistant bacteria by healthy children. *J. Antimicrob. Chemother.* **47**: 605–610

Ricaurte, J., Boucher, H., Turett, G., Moellering, R., Labombardi, V., Kislak, J. (2001) Chloramphenicol treatment for vancomycin-resistant Enterococcus faecium bacteraemia. *Clin. Microbiol. Infect.* **7**: 17–21

Shabad, L., Bogush, T., Konopleva, I., Belitskii, G. (1977) Dextramycine (the dextraisomer of chloramphenicol) as an inhibitor of the induction of lung adenomas in mice. *Neoplasma* **24**: 147–150

Silverman, R. (1992) The organic chemistry of drug design and drug action. Academic Press, New York, pp 220–276

Solomons, T. W. (1994) *Organic chemistry*. John Wiley & Sons, New York, pp 409–420

Wisplinghoff, H., Reinert, R., Cornely, O., Seifert, H. (1999) Molecular relationshisps and antimicrobial susceptibilities of viridans group streptococci isolated from blood of neutropenic cancer patients. *J. Clin. Microbiol.* **37**: 1876–1880