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Original paper

Heterocyclic 1,2-epoxyalkan-3-ones as cytotoxic agents

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Summary — A series of 1-{2-(3-,4-)[1-oxidopyridinyl]}-1,2-epoxybutan-3-ones **13** and 1-[2-(3-,4-)pyridinyl]-1,2-epoxyalkan-3-ones **12**, **16** and **17** was synthesized. *In vitro* screening against L1210 leukemic cells indicated that thes tereochemistry of the oxiranyl substituents and/or size of the R^2 alkyl substituents are determinants of cytotoxic activity. Increasing the length of the R^2 alkyl substituent for **trans-16** decreased activity. The lipophilic effect of the R^2 substituent does not appear to be a significant determinant of activity. Some structure—activity correlations are described.

Résumé — **Epoxy-1,2 alcanones-3 hétérocycliques, agents cytotoxiques.** *Une série d'{[oxidopyridinyl-1](-3,-4)-2}-1-époxy-2-butan-1,2-ones-3* ***13*** *et [pyridinyl(-3,-4)-2]-1-époxyalkan-1,2-ones-3* ***12***, ***16*** *et* ***17*** *a été préparée. L'essai* in vitro *contre les cellules leucémiques L1210 a indiqué que la stéréochimie des substituants oxiranyl et/ou la grosseur des substituants alkyle R^2 sont déterminants pour l'activité cytotoxique. L'accroissement de la longueur du substituant R^2 alkyle pour le dérivé* ***trans-16*** *a diminué l'activité. Il apparaît que l'effet lipophile du substituant R^2 n'est pas déterminant de l'activité. Quelques corrélations de structure—activité sont décrites.*

cytotoxic agents / pyridines / 1,2-epoxyalkan-3-ones

Introduction

Activated alkylating agents that react with cellular nucleophiles, such as L-cysteine, glutathione or sulfhydryl-containing enzymes, exhibit significant cytotoxic activity [1]. Mannich bases **1** [2], α-methylene-γ-lactones **2** [3], *N*-(3-oxoprop-1-enyl)pyrimidines **3** [4] and α,β-epoxysulfoxides **4** react readily with nucleophiles. The β-carbon of **4** is highly reactive to nucleophiles yielding dialkylketones or aldehydes in high yield under mild conditions [5, 6]. A number of compounds, which can be conceived as prodrugs to putative alkylating species, have been developed by Sartorelli and coworkers. Thus, the anti-neoplastic activity of arylsulfonylhydrazones of 2-formylpyridine *N*-oxide **5** [7—9] has been attributed to the potent alkylating species 1-oxidopyridin-2-yldiazomethane **6** [10, 11], that of 1,2-bis-(arylsulfonyl)-1-methylhydrazines **7** has been attributed to the putative alkylating species **8** [12], while that of methylhydrazines **9** may be due to the alkylating species **10** and/or reaction of **9** with tissue nucleophiles [13]. In an earlier study, we described the synthesis of 1-[1-oxido-2-(3-,4-)pyridinyl]-2-methyl oxiranes and their reaction with sulfur, oxygen and nitrogen nucleophiles [14, 15]. It was therefore of interest to extend this study to include activated oxiranes, which may react with cellular thiols, for evaluation as anti-tumor agents. We now describe the synthesis and cytotoxic activity of some 1-(pyridinyl)-1,2-epoxyalkan-3-ones.

Chemistry

The Darzen's reaction of pyridinylcarboxaldehydes **11a—c** with chloroacetone in the presence of potassium *t*-butoxide and benzyltriethylammonium chloride at —60°C afforded *trans*-1-(pyridinyl)-1,2-epoxybutan-3-ones **12a—c** in 25—31% yield. Oxidation of the *trans*-1-(pyridinyl)-1,2-epoxybutan-3-ones **12a—c** with *m*-chloroperbenzoic acid yielded *trans*-1-(1-oxidopyridinyl)-1,2-epoxybutan-3-ones **13a—c** (56—71% yield) as outlined in Scheme 1 and summarized in Table I.

Reaction of a 1:1 mixture of methyl *trans*-3-(2-pyridinyl)-2,3-epoxypropanoate **14** and methyl *cis*-3-(2-pyridinyl)-2,3-epoxypropanoate **15** [16] with *n*-butylmagnesium bromide afforded a mixture of ***trans*-16a** and ***cis*-1**-(2-pyridinyl)-1,2-epoxybutan-3-one **17a**, which were separated by silica gel column chromatography, in 34.5% and 30.5% yields, respectively. Similar reactions of **14** and **15** with *n*-hexyl-, *n*-decyl- and *n*-hexadecylmagnesium bromide yielded the corresponding ***trans*-16b—d** (20—30.5% yield) and ***cis***-

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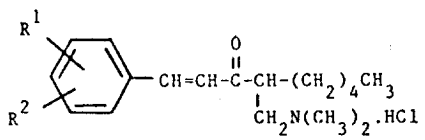
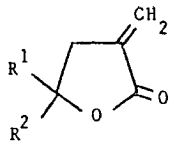


1, $R^1=R^2=H$, Cl



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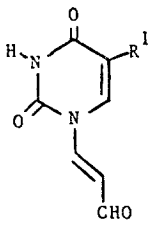
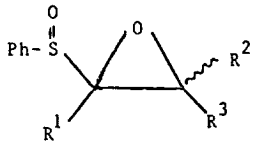
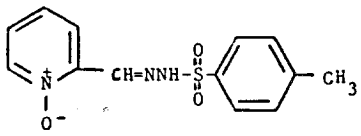


3, R^1 = H, F, CH_3, CF_3



4, R^1 = alkyl, arylalkyl
R^2 = alkyl, aryl
R^3 = H, CH_3



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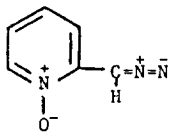

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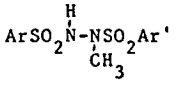

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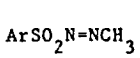

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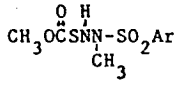

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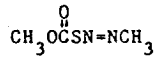

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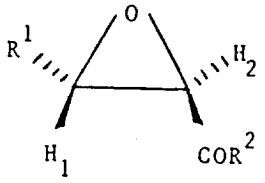
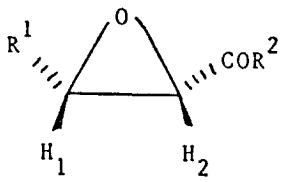
Table I. 1-{2-(3-,4-)[1-Oxidopyridinyl]}-1,2-epoxybutan-3-ones **13** and 1-[2-(3-,4-)pyridinyl]-1,2-epoxyalkan-3-ones **12, 16** and **17.**



12, 13, 16



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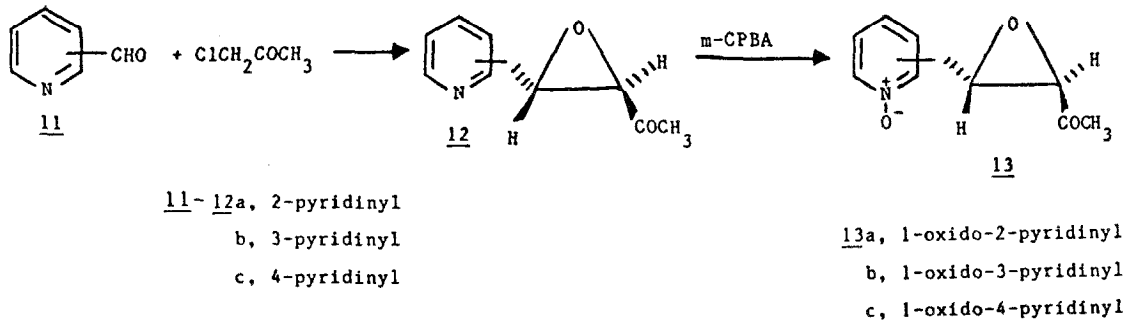
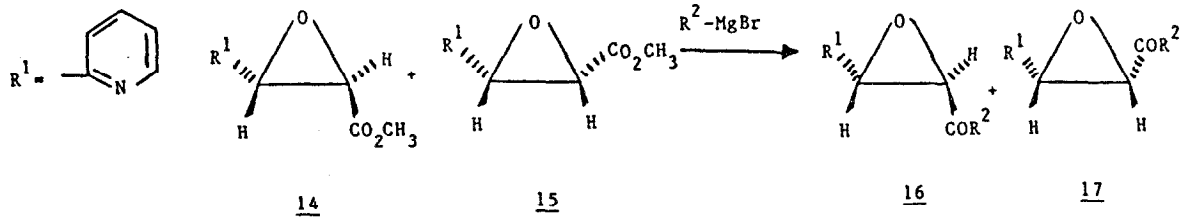
No.	R^1	R^2	Stereo-isomer	Coupling constant $J_{1,2}$ (Hz)	Procedure	Yield (%)	mp (°C)	Formula
12a	2-pyridinyl	CH_3	*trans*	2	A	31	oil	$C_9H_9NO_2$
12b	3-pyridinyl	CH_3	*trans*	2	A	26	oil	$C_9H_9NO_2$
12c	4-pyridinyl	CH_3	*trans*	2	A	25	60	$C_9H_9NO_2$
13a	1-oxido-2-pyridinyl	CH_3	*trans*	2	B	71	134	$C_9H_9NO_3$
13b	1-oxido-3-pyridinyl	CH_3	*trans*	2	B	56	oil	$C_9H_9NO_3$
13c	1-oxido-4-pyridinyl	CH_3	*trans*	2	B	67	oil	$C_9H_9NO_3$
16a	2-pyridinyl	*n*-C_4H_9	*trans*	1.5	C	34.5	oil	$C_{12}H_{15}NO_2$
17a	2-pyridinyl	*n*-C_4H_9	*cis*	4.5	C	30.5	oil	$C_{12}H_{15}NO_2$
16b	2-pyridinyl	*n*-C_6H_{13}	*trans*	1.5	C	30.5	44	$C_{14}H_{19}NO_2$
17b	2-pyridinyl	*n*-C_6H_{13}	*cis*	4.5	C	26.5	oil	$C_{14}H_{19}NO_2$
16c	2-pyridinyl	*n*-$C_{10}H_{21}$	*trans*	1.5	C	25	49	$C_{18}H_{27}NO_2$
17c	2-pyridinyl	*n*-$C_{10}H_{21}$	*cis*	4.5	C	21.5	34	$C_{18}H_{27}NO_2$
16d	2-pyridinyl	*n*-$C_{16}H_{33}$	*trans*	1.5	C	20	69	$C_{24}H_{39}NO_2$
17d	2-pyridinyl	*n*-$C_{16}H_{33}$	*cis*	4.5	C	12.5	59	$C_{24}H_{39}NO_2$



Scheme 1.



Scheme 2.

17b—d (12.5—26.5% yield) products as outlined in Scheme 2 and summarized in Table I. The stereochemistry of the products was readily assigned since the ^{1}H NMR spectra exhibited coupling constants of 1.5 and 4.5 Hz for the ***trans*-16** and ***cis*-17** isomers, respectively [14—16].

Pharmacological Results and Discussion

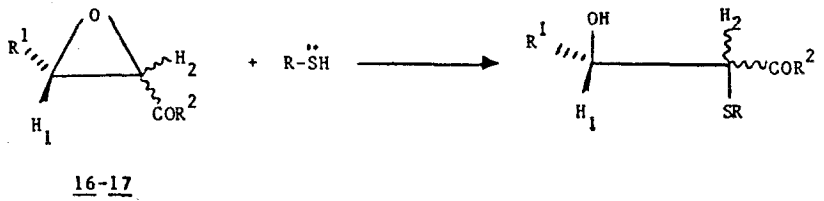
A number of selected compounds were screened against mouse L1210 leukemic cells in culture for which the biological test results are shown in Table II.

The ***trans*-16** and ***cis*-1-(2-pyridinyl)-1,2-epoxyalkan-3-ones 17** were investigated to determine the stereochemical effect of the R^1 2-pyridinyl and R^2 alkyl substituents upon cytotoxic activity. The cytotoxic activity of compounds containing the α,β-unsaturated structural moiety has been attributed to their reaction with cellular nucleophiles [17—20]. In an earlier study, we reported that *cis*- and *trans*-1-(1-oxido-2-pyridinyl)-2-methyloxiranes undergo regiospecific and stereospecific reactions with amine nucleophiles at the C-2 position to yield the respective 1*R*, 2*R* or 1*S*, 2*S* *(threo)* and 1*R*, 2*S* or 1*S*, 2*R* *(erythro)* β-aminoalcohol diastereoisomers in high yield [14]. The relative reaction rates of PhCH=CH—R with thiols is R = COMe > R = CO_2Me > R = $CONH_2$ [20]. It is therefore expected that oxiranes **16—17** having electron-attracting carbonyl and pyridinyl substituents would be highly activated to regiospecific attack by cellular thiol nucleophiles (R—SH) at the C-2 position as illustrated in Scheme 3.

Table II. *In vitro* cytotoxicity of 1-(2-pyridinyl)-1,2-epoxyalkan-3-ones **16** and **17**.

No.	% survival ± SD[a]	
	10 μg/ml	1 μg/ml
16a	34.64 ± 3.83	96.21 ± 1.87
17a	69.32 ± 4.59	99.12 ± 1.45
16b	38.69 ± 4.02	95.33 ± 3.47
17b	35.67 ± 2.70	95.74 ± 6.05
16c	78.82 ± 3.11	93.00 ± 5.48
17c	28.43 ± 1.45	92.54 ± 3.32[b]
16d	96.09 ± 3.57	96.25 ± 2.75
17d	78.47 ± 6.47	98.71 ± 1.14[c]
Melphalan[d]	0.00	2.82 ± 0.68[e]

[a]The result is the mean value ± SD for 3 experiments.
[b]ED_{50} = 5.6 μg/ml (1.93 × 10^{-3} μM).
[c]ED_{50} = 10.2 μg/ml (2.73 × 10^{-2} μM).
[d]4-[*N*-bis-(2-Chloroethyl)amino]phenylalanine.
[e]ED_{50} = 0.15 μg/ml (5.45 × 10^{-4} μM).



Scheme 3.

The test results (Table II) indicate that the stereochemistry of the oxiranyl substituents and/or size of the R^2 alkyl substituents of **16** and **17** are determinants of cytotoxic activity. A comparison of the relative activities of ***trans*-16** with the corresponding ***cis*-17** isomer indicated that *n*-butyl **16a** > **17a***, *n*-hexyl **17b** ≃ **16b****, *n*-decyl **17c** > **16c*** and *n*-hexadecyl **17d** > **16d***. The *trans*-isomer **16a** was more active than the *cis*-isomer **17a** for compounds having a smaller *n*-butyl R^2 substituent, whereas the *cis*-isomers (**17c** and **17d**) were more active than the corresponding *trans*-isomers (**16c** and **16d**), for compounds having larger *n*-decyl and *n*-hexadecyl R^2 substituents. It is plausible that the increased steric effect exhibited by the R^1 and R^2 substituents for compounds possessing the larger *n*-decyl and *n*-hexadecyl substituents is responsible for the greater activity of the *cis*-isomer, relative to the corresponding *trans*-isomer, which would result in a more facile reaction with cellular nucleophiles, as illustrated in Scheme 3. A comparison of the relative activities of the ***trans*-16** products possessing a variety of R^2 alkyl substituents at a concentration of 10 μg/ml indicated *n*-butyl **16a** ≃ *n*-hexyl **16b**** > *n*-decyl **16c*** > *n*-hexadecyl **16d***. These results indicate that increasing the size of the R^2 alkyl substituent for ***trans*-16** decreases activity probably due to increased steric effects. Increasing the size of the R^2 alkyl substituent of ***trans*-16** would be expected to decrease the rate of attack by cellular nucleophiles at the C-2 position of **16**. On the other hand, the relative potency order for the ***cis*-17** compounds was *n*-decyl **17c** > *n*-hexyl **17b***** > *n*-butyl **17a*** > *n*-hexadecyl **17d***. The effect of the R^2 substituent on lipophilicity is not expected to be a significant determinant of activity, since the relative potency sequences for ***trans*-16** and ***cis*-17** would be similar if lipophilicity was the major determinant of activity.

The *trans*-1-oxido-2-pyridinyl compound **13a** exhibited a % *T/C* (treated/control) of 111 for a 240 mg/kg i.p. dose in the P388 lymphocytic screen in mice. The most active compound prepared was *cis*-1-(2-pyridinyl)-1,2-epoxytridecan-3-one **17c** which exhibited an ED_{50} of 5.6 μg/ml in the *in vitro* screen. The remaining compounds were not evaluated in the *in vivo* screen, since compounds exhibiting an $ED_{50} \geqslant 5$ μg/ml in the *in vitro* screen are considered to be inactive cytotoxic agents.

Experimental protocols

Chemistry

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined in deuterochloroform unless otherwise stated with tetramethylsilane (TMS) as the internal standard with a Varian EM-360A, Varian EM-390 or Bruker AM-300 spectrometer. Mass spectra were measured with an AEI MS-50 mass spectrometer and these exact mass measurements are in a few cases used in lieu of elemental analyses. Infrared spectra (potassium bromide unless otherwise noted) were taken on a Perkin—Elmer 267 or Nicolet 5DX spectrometer. All of the products gave rise to a single spot on thin—layer chromatography (TLC), using a solvent system of low, medium and high polarity. Analyses of all compounds prepared in this study were within ± 0.4% of the theoretical values for C, H and N and/or they were analyzed for C, H, N and O, using high resolution mass spectrometry (hrms) (**13b, c**).

trans-*1-(2-Pyridinyl)-1,2-epoxybutan-3-one* ***12a**: Procedure A*
A solution of potassium *t*-butoxide, prepared by dissolution of potassium (2.0 g) in dry *t*-butyl alcohol (45 ml) was added dropwise during 90 min to a mixture of **11a** (5.35 g, 50 mmol), chloroacetone (4.6 g, 50 mmol) and benzyltriethylammonium chloride (0.5 g) in dry tetrahydrofuran (15 ml) with stirring at a rate such that the reaction mixture did not exceed —60°C. The reaction was allowed to proceed for an additional 30 min at —60°C at which time TLC indicated the absence of **11a**. The reaction mixture was allowed to warm slowly to 25°C, was poured onto ice cold water (500 ml), extracted with ether (4 × 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo* to yield **12a**. Purification on a silica gel flash column, using methylene chloride:ether:methanol (50:48:2, v/v/v) afforded **12a** (2.5 g, 31%) as an oil; IR 1712 (CO) cm^{-1}; ^1H NMR δ: 2.18 (s, 3H, CH_3); 3.7 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.18 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.1—7.3 (m, 2H, pyridinyl H-3, H-5); 7.65 (d, $J_{4,5}$ = 8.75 Hz of d, $J_{3,4}$ = 7 Hz of d, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4); 8.68 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_9H_9NO_2$: C: 66.25; H: 5.52; N: 8.58. Found: C: 65.88; H: 5.66; N: 8.39.

The spectrometric and analytical data for compounds **12b, c**, which were prepared in a similar manner, are listed below.

trans-*1-(3-Pyridinyl)-1,2-epoxybutan-3-one* ***12b***
IR (film) 1704 (CO) cm^{-1}; ^1H NMR δ: 2.25 (s, 3H, H-4); 3.68 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.2 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.3—7.8 (m, 2H, pyridinyl H-4, H-5); 8.6—8.88 (m, 2H, pyridinyl H-2, H-6). Anal. Calcd. for $C_9H_9NO_2$: C: 66.25; H: 5.52; N: 8.58. Found: C: 66.65; H: 5.63; N: 8.27.

trans-*1-(4-Pyridinyl)-1,2-epoxybutan-3-one* ***12c***
IR (KBr) 1712 (CO) cm^{-1}; ^1H NMR δ: 2.28 (s, 3H, H-4); 3.0 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.08 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.28 (d, $J_{2,3}$ = $J_{5,6}$ = 6 Hz, 2H, pyridinyl H-3, H-5); 8.7 (d, $J_{2,3}$ = $J_{5,6}$ = 6 Hz, 2H, pyridinyl H-2, H-6). Anal. Calcd. for $C_9H_9NO_2$: C: 66.25; H: 5.52; N: 8.58. Found: C: 66.19; H: 5.60; N: 8.59.

trans-*1-(1-Oxido-2-pyridinyl)-1,2-epoxybutan-3-one* ***13a**: Procedure B*
A solution of *m*-chloroperbenzoic acid (1.66 g of 85%, 82 mmol) in 25 ml of methylene chloride was added dropwise to a solution of **12a** (1.22 g, 75 mmol) in methylene chloride (15 ml) at 0°C with stirring. The reaction mixture was stirred at 0°C for 30 min, 1 h at 25°C followed by heating at reflux for 24 h. The volume was reduced by 50% and the reaction mixture was cooled and filtered. Removal of the solvent from the filtrate gave a residue which was purified by elution from a neutral alumina column with chloroform:methanol (97:3, v/v) as the eluant to yield **13a** (0.95 g, 71%), mp: 134°C; IR 1704 (CO), 1253 (*N*-oxide) cm^{-1}; ^1H NMR δ: 2.3 (s, 3H, CH_3); 3.55 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.8 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.28—7.6 (m, 3H, 1-oxido-2-pyridinyl H-3, H-4, H-5); 8.4 (m, 1H, 1-oxido-2-pyridinyl H-6). Anal. Calcd. for $C_9H_9NO_3$: C: 60.33; H: 5.02; N: 7.82. Found: C: 60.17; H: 5.07; N: 7.73.

The spectrometric data for compounds **13b, c**, which were prepared in a similar manner, are listed below.

trans-*1-(1-Oxido-3-pyridinyl)-1,2-epoxybutan-3-one* ***13b***
IR (film) 1720 (CO) and 1250 (NO) cm^{-1}; ^1H NMR δ: 2.3 (s, 3H, H-4); 3.58 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.15 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.2—7.54 (m, 2H, 1-oxido-3-pyridinyl H-4, H-5); 8.14—8.4 (m, 2H, 1-oxido-3-pyridinyl H-2, H-6). Exact mass calcd. for $C_9H_9NO_3$: 179.0582. Found (hrms): 179.0579.

trans-*1-(1-Oxido-4-pyridinyl)-1,2-epoxybutan-3-one* ***13c***
IR (film) 1713 (CO) and 1245 (NO) cm^{-1}; ^1H NMR δ: 2.25 (s, 3H, H-4); 3.5 (d, $J_{1,2}$ = 2 Hz, 1H, H-2); 4.1 (d, $J_{1,2}$ = 2 Hz, 1H, H-1); 7.28 (d, $J_{5,6}$ = $J_{2,3}$ = 6 Hz, 2H, 1-oxido-4-pyridinyl H-3, H-5); 8.24 (d, $J_{2,3}$ = $J_{5,6}$ = 6 Hz, 2H, 1-oxido-4-pyridinyl H-2, H-6). Exact mass calcd. for $C_9H_9NO_3$: 179.0582. Found (hrms): 179.0579.

*ANOVA (analysis of variants indicated the result is statistically significant ($p < 0.001$).
**ANOVA indicated the result is not statistically significant ($p > 0.05$).
***ANOVA indicated the result is statistically significant ($p < 0.05$).

trans-*1-(2-Pyridinyl)-1,2-epoxyheptan-3-one* ***16a*** *and* cis-***1-****(2-pyridinyl)-1,2-epoxyheptan-3-one* ***17a****: Procedure C*

A solution of *n*-butylmagnesium bromide, prepared by the dropwise addition of 1-bromobutane (1.68 g, 12.3 mmol) in 5 ml of dry tetrahydrofuran to magnesium metal (0.3 g, 12.3 mmol) suspended in 2 ml of dry tetrahydrofuran under a nitrogen atmosphere at 25°C with stirring until all the magnesium metal had reacted, was added to a solution of **14** and **15** (2 g, 11.17 mmol, ratio 1:1) [16] in 60 ml of dry tetrahydrofuran under a nitrogen atmosphere at —78°C. The reaction was allowed to proceed at —78°C, at which time TLC indicated the absence of **14** and **15**, and then water (20 ml) was added. The reaction mixture was allowed to warm to 25°C, the solvent was removed *in vacuo* and the residue was dissolved in 5% aqueous hydrochloric acid (20 ml). Extraction with ether (4 × 40 ml), drying (Na_2SO_4) and removal of the solvent *in vacuo* gave an oil (2 g). Purification by elution from a silica gel column (3 × 40 cm) with ether:hexane (80:20, v/v) as the eluant afforded a mixture consisting of predominately **16a** contaminated with traces of *trans*- and *cis-t*-alcohol products resulting from further reaction of **16a** and **17a**, respectively with *n*-butylmagnesium bromide. Further elution gave **17a** (0.7 g, 30.5%) as an oil; IR (film) 1720 (CO) cm^{-1}; ^1H NMR δ: 0.8 (t, J = 7 Hz, 3H, CH_3); 1.12 (m, 2H, CH_2CH_3); 1.4 (m, 2H, $CH_2CH_2CH_3$); 2.45 (m, 2H, $COCH_2$); 4.0 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-2); 4.4 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-1); 7.24 (d, $J_{4,5}$ = 8 Hz of d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5); 7.46 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3); 7.72 (d, $J_{3,4}$ = 8.0 Hz of d, $J_{4,5}$ = 8 Hz of d, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{12}H_{15}NO_2$: C: 70.24; H: 7.31; N: 6.82. Found: C: 69.89; H: 7.30; N: 6.62. The mixture containing predominately **16a** obtained above was separated on 20 × 20 cm silica gel G plates, 1 mm thick, with ether:hexane (60:40, v/v) as the development solvent, using the multiple development TLC technique. Extraction of the band having R_f = 0.8 with methanol afforded **16a** (0.8 g, 34.5%) as an oil; IR (film) 1712 (CO) cm^{-1}; ^1H NMR δ: 0.9 (t, J = 7 Hz, 3H, CH_3); 1.31 (m, 2H, CH_2CH_3); 1.6 (m, 2H, $CH_2CH_2CH_3$); 2.5 (m, 2H, $COCH_2$); 3.72 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-2); 4.3 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1); 7.28 (m, 2H, pyridinyl H-3, H-5); 7.73 (d, $J_{3,4}$ = 8.0 Hz of d, $J_{4,5}$ = 8 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{12}H_{15}NO_2$: C: 70.24; H: 7.31; N: 6.82. Found: C: 70.37; H: 7.53; N: 6.52.

Compounds **16**, **17b—d** were prepared in a similar manner except for the changes in procedure described below. Products **16b** and **17b** were separated on a silica gel column using ether:hexane (70:30, v/v) as the eluant. The *trans*-product **16b** was purified by preparative multiple development TLC using ether:hexane (1:1, v/v) as the development solvent (R_f = 0.8). The preparation of *n*-decylmagnesium bromide required heating under reflux to complete the reaction and dry tetrahydrofuran (20 ml) was added, prior to reaction with **14** and **15**, to prevent precipitation of the Grignard reagent. Products **16c** and **17c** were separated on a silica gel column using ether:hexane (60:40, v/v) as the eluant. The *trans*-product **16c** was purified by preparative multiple development TLC using ether:hexane (1:1, v/v) as the development solvent. The preparation of *n*-hexadecylmagnesium bromide required the addition of a crystal of iodine as catalyst, heating under reflux to complete the reaction and the addition of dry tetrahydrofuran (30 ml), prior to reaction with **14** and **15**, to prevent precipitation of the Grignard reagent. The reaction of the Grignard reagent with **14** and **15** was allowed to proceed for 8 h at —78°C. Products **16d** and **17d** were separated on a silica gel column with ether:hexane (1:1, v/v) as the eluant. The *trans*-product **16d** was purified by preparative multiple development TLC using ether:hexane (30:70, v/v) as the development solvent (R_f = 0.8).

The spectroscopic and analytical data for **16**, **17b—d** are listed below.

trans-*1-(2-Pyridinyl)-1,2-epoxynonan-3-one* ***16b***

IR (KBr) 1712 (CO) cm^{-1}; ^1H NMR δ: 0.9 (t, J = 7 Hz, 3H, H-9); 1.25 (m, 6H, H-6 to H-8); 1.62 (m, 2H, H-5); 2.5 (m, 2H, H-4); 3.72 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-2); 4.16 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1); 7.25 (m, 2H, pyridinyl H-3, H-5); 7.76 (d, $J_{4,5}$ = 8 Hz of d, $J_{3,4}$ = 8 Hz of d, $J_{4,6}$ = 1.5 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.5 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{14}H_{19}NO_2$: C: 72.07; H: 8.20; N: 6.00. Found: C: 71.73; H: 8.20; N: 5.83.

cis-*1-(2-Pyridinyl)-1,2-epoxynonan-3-one* ***17b***

IR (film) 1720 (CO) cm^{-1}; ^1H NMR δ: 0.8 (t, J = 7 Hz, 3H, H-9); 1.20 (m, 6H, H-6 to H-8); 1.3 (m, 2H, H-5); 2.4 (m, 2H, H-4); 4.0 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-2); 4.4 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-1); 7.25 (d, $J_{4,5}$ = 8 Hz of d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5); 7.46 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3); 7.72 (d, $J_{3,4}$ = 8 Hz of d, $J_{4,5}$ = 8 Hz of d, $J_{4,6}$ = 1.5 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.5 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{14}H_{19}NO_2$: C: 72.07; H: 8.20; N: 6.00. Found: C: 72.21; H: 8.26; N: 5.86.

trans-*1-(2-Pyridinyl)-1,2-epoxytridecan-3-one* ***16c***

IR (KBr) 1712 (CO) cm^{-1}; ^1H NMR δ: 0.9 (t, J = 7 Hz, 3H, H-13); 1.3 (m, 14H, H-6 to H-12); 1.65 (m, 2H, H-5); 2.5 (m, 2H, H-4); 3.72 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-2); 4.16 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1); 7.28 (m, 2H, pyridinyl H-3, H-5); 7.73 (d, $J_{4,5}$ = 8 Hz of d, $J_{3,4}$ = 8 Hz of d, $J_{4,6}$ = 1.5 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{18}H_{27}NO_2$: C: 74.70; H: 9.40; N: 4.83. Found: C: 74.69; H: 9.10; N: 4.56.

cis-*1-(2-Pyridinyl)-1,2-epoxytridecan-3-one* ***17c***

IR (KBr) 1721 (CO) cm^{-1}; ^1H NMR δ: 0.96 (t, J = 7 Hz, 3H, H-13); 1.0—1.4 (m, 16H, H-5 to H-12); 2.4 (m, 2H, H-4); 4.0 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-2); 4.4 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-1); 7.24 (d, $J_{4,5}$ = 8 Hz of d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5); 7.46 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3); 7.72 (d, $J_{3,4}$ = 8 Hz of d, $J_{4,5}$ = 8 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{18}H_{27}NO_2$: C: 74.70; H: 9.40; N: 4.83. Found: C: 74.62; H: 9.32; N: 4.62.

trans-*1-(2-Pyridinyl)-1,2-epoxynonadecan-3-one* ***16d***

IR (KBr) 1712 (CO) cm^{-1}; ^1H NMR δ: 0.84 (t, J = 7 Hz, 3H, H-19); 1.25 (m, 26H, H-6 to H-18); 1.6 (m, 2H, H-5); 2.5 (m, 2H, H-4); 3.7 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-2); 4.14 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1); 7.24 (d, $J_{4,5}$ = 8 Hz of d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5); 7.46 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3); 7.72 (d, $J_{3,4}$ = 8 Hz of d, $J_{4,5}$ = 8 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{24}H_{39}NO_2$: C: 77.16; H: 10.52; N: 3.74. Found: C: 77.00; H: 10.64; N: 3.51.

cis-*1-(2-Pyridinyl)-1,2-epoxynonadecan-3-one* ***17d***

IR (KBr) 1720 (CO) cm^{-1}; ^1H NMR δ: 0.91 (t, J = 7 Hz, 3H, H-19); 1.0—1.5 (m, 28H, H-5 to H-18); 2.5 (m, 2H, H-4); 4.0 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-2); 4.4 (d, $J_{1,2}$ = 4.5 Hz, 1H, H-1); 7.24 (d, $J_{4,5}$ = 8 Hz of d, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5); 7.46 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3); 7.72 (d, $J_{3,4}$ = 8 Hz of d, $J_{4,5}$ = 8 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-4); 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 1.6 Hz, 1H, pyridinyl H-6). Anal. Calcd. for $C_{24}H_{39}NO_2$: C: 77.16; H: 10.52; N: 3.74. Found: C: 76.83; H: 10.45; N: 3.44.

Pharmacology

In vitro *L1210 cytotoxic activity*

Mouse L1210 leukemia cells were cultivated as a suspension in Fischer's medium supplemented with 10% heat-inactivated horse serum and incubated at 37°C in a humidified 5% CO_2 atmosphere to prepare a cell stock solution. The number of cells/ml of medium was determined using a Model ZF Coulter Counter 48 h after incubation. The test compound was dissolved in saline:ethanol (1:1, v/v) and 20 µl of this solution was added to test wells containing 2 ml of suspended L1210 cells (10^5 cells/ml) such that 2 ml of the cell suspension had a test compound concentration of 50, 10 and 1 µg/ml of medium, respectively. Control wells were identical, except that the test compound was absent. Compounds for which ED_{50} values were obtained had the following test compound concentration (µg/ml of medium): **17c** and **17d** (50, 25, 10, 5, 2.5 and 1.25), and melphalan (10, 1, 0.5, 0.25, 0.1 and 0.05). All tests and controls were grown in triplicate. The % cell survival was calculated using the formula: % survival = $(T_{48} - T_0)/(C_{48} - C_0) \times 100$; where T_{48} is the mean number of living cells/ml for each test drug concentration at 48 h, T_0 is the mean number for the test wells at time zero (normally 10^5), C_{48} is the mean number for the control at 48 h and C_0 is the mean number for the control at time zero (normally, $T_0 = C_0 = 10^5$ cells/ml).

Anti-neoplastic activity (P388 lymphocytic screen)
Compound **13a** was screened under the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, U.S.A., using their standard protocols [21]. A once daily maximum non-toxic dose (60—240 mg/kg range) in saline was administered by i.p. injection into mice for a total of nine doses. Compound **13a** (240 mg/kg) provided a % *T/C* value (% treated/control) of 111 and is therefore considered to be inactive. In this screen, the reference compound, 5-fluorouracil, exhibited a % *T/C* value of > 135 at a once daily dose of 20 mg/kg i.p. for nine days [22]. A % *T/C* > 127 is considered to be active in this screen.

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