Studies on Anthracenes. 1. Human Telomerase Inhibition and Lipid Peroxidation of 9-Acyloxy 1,5-Dichloroanthracene Derivatives

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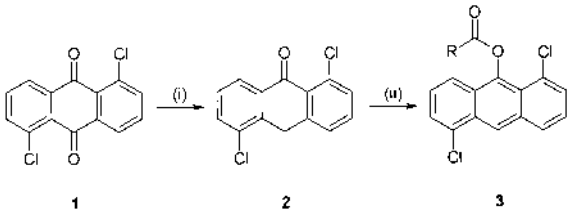
The synthetically useful approaches to 9-acyloxy 1,5-dichloroanthracene derivatives are reported. The system selectively reduces the carbonyl group flanked by the *peri* substituents of the anthracenediones to give the corresponding 1,5-dichloro-9(10*H*)-anthracenone. Simple regioselective acylation of anthracenone is applied with appropriate acyl chlorides in CH_2Cl_2 with catalytic amount of pyridine to give the novel 9-acyloxy 1,5-dichloroanthracene derivatives. Considerable interest has developed in the mechanism of how anthracenone achieves this desirable selectivity. In an attempt to understand the mechanism of this reaction, solid-state structures of anthracene derivatives have been obtained. In addition, the inhibition of lipid peroxidation in model membranes was determined as was their ability to inhibit the telomere-addition function of the human telomerase enzyme together with their inhibition of the *Taq* polymerase enzyme. In contrast to (+)-α-tocopherol, 3b, 3c, 3d, 3g, and 3i do not enhance lipid peroxidation in model membranes. Implications for 9-acyloxy 1,5-dichloroanthracene analogues as potential anticancer agents are discussed.

Key words *peri* substituent; anthracenedione; anthracenone; anthracene; acylation; telomerase; lipid peroxidation

A series of novel 9-acyloxy 1,5-dichloroanthracene derivatives were synthesized by a general synthetic route. Anthraquinones have proved to be important in the development of electrochemically switchable ligands in studies conducted in many laboratories,[1—6] and are increasingly popular in a variety of related applications.[7—12] Anthracene derivatives have been the subject of extensive research mainly due to their well-recognized biological importance and the significant biological applications of their derivatives. Introduction of side chains onto the anthracenone skeleton is usually accomplished by a stepwise procedure *via* the anthracenedione because of the chemical instability of many anthracenones.[13] There is continuing interest in the development of new agents that modify the chromophore anthracenone moiety yet exhibit different potent spectra, together with reduced overall toxicity. Our present research work has been hampered by the lack of synthetic methods for the preparation of substituted anthracene derivatives and more suitable antitumor agents. Reduction of 1,5-dichloroanthraquinone is required as a first step in the synthesis of 9-acyloxy 1,5-dichloroanthracenes. The reaction of anthracenone with an acyl chloride is expected to lead to anthracene. Introduction of the substituted functionality onto the anthrone skeleton was achieved by reaction of the appropriate acyl chloride with anthracenone under weakly basic conditions, where acylation took place at the carbonyl oxygen *via* the isomerization. C-9-Acylated anthracenes were afforded in good yields under base-catalyzed (pyridine). Halide ions from acyl chloride are good leaving groups that elimination from the complex, and take place almost synchronously with addition to the carbonyl group.[14] Analysis of the reaction mixture from the reaction showed, after hydrolysis, the presence of anthracene and the absence of high molecular weight by-products, even after the reaction mixture had been allowed to high temperature. In each experiment the acyl chloride was added directly to the anthracenone, which it may well be that such material is less nucleophilic and hence more selective in its reaction. In particular, substitution with an electron-withdrawing acyl group at the 9-position impairs the reduction ability of anthracenones. We report here a simple and general methodology for the synthesis of 9-acyloxy 1,5-dichloroanthracene derivatives.

Chemistry

The synthesis of the desired anthracenes is shown in Chart 1. 1,5-Dichloro-9(10*H*)-anthracenone **2**, prepared from **1** and $SnCl_2$ in boiling HCl and acetic acid according to the literature,[15] was treated with appropriate acyl chloride in the presence of pyridine to afford corresponding 9-acyloxy 1,5-dichloroanthracenes in 75—90% yield (Table 1). In the ^{1}H-NMR spectrum the 10-H aromatic proton signal was identified while that at δ 8.80 was one single proton signal, explicable downfield chemical shift of acyloxy anthracene 10-H proton signal (δ 8.80) is due to the deshielding effect of aromatic anthracene ring not to C-9 acyloxy group effect. The ^{1}H-NMR spectrum of **2** in $CDCl_3$ lends credence to the structure as it shows evidence for the methylene protons (4.08 ppm) at C-10. Of particular importance are the one 10-H proton chemical shifts between δ 8.69 and 9.23, which are different from the range of the 1,5-dichloro-9(10*H*)-anthracenone possessing two 10-H protons and the IR stretching frequency 1735 cm^{-1}, which indicate the normal ester C=O stretch. Furthermore, the ^{13}C-NMR spectra of these com-



(i) $SnCl_2$, HOAc, HCl, 118°C; (ii) R-COCl, pyridine, CH_2Cl_2, N_2.

Chart 1

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Table 1. Chemical and Physical Data of 9-Acyloxy 1,5-Dichloroanthracene Derivatives

Compd.	R[a]	log *P*	mp (°C)	Yield (%)	*Anal.*[b]
3a	CH_3	4.53	164—166	89	C, H
3b	CH_2Br	4.69	180—181	64	C, H
3c	CH_2Cl	4.50	181—182	66	C, H
3d	CH_2CH_3	4.80	134—135	88	C, H
3e	$CH(CH_3)_2$	4.29	116—118	76	C, H
3f	$CH_3CH(Cl)$	4.99	120—122	86	C, H
3g	$CHCl_2$	4.96	138—140	70	C, H
3h	$(CH_2)_2CH_3$	5.14	132—134	93	C, H
3i	$(CH_2)_3Br$	5.27	118—120	70	C, H
3j	$(CH_2)_3Cl$	5.12	130—132	69	C, H
3k	$(CH_2)_4CH_3$	5.98	120—121	84	C, H
3l	C_6H_5	5.24	166—168	61	C, H
3m	2-$CH_3C_6H_4$	5.69	162—164	81	C, H
3n	3-$CH_3C_6H_4$	5.60	172—173	78	C, H
3o	4-$CH_3C_6H_4$	5.56	204—206	77	C, H
3p	3-ClC_6H_4	5.84	172—174	76	C, H
3q	4-ClC_6H_4	5.67	169—171	90	C, H
3r	3-$NO_2C_6H_4$	5.16	198—200	75	C, H, N
3s	4-$NO_2C_6H_4$	5.38	216—218	83	C, H, N
3t	4-Cl, 2-CH_3O-C_6H_3	3.97	195—196	70	C, H
3u	2,4-$Cl_2C_6H_3$	6.06	190—192	73	C, H
3v	$CH_2C_6H_5$	5.31	152—154	77	C, H
3w	$CH_2CH_2C_6H_5$	5.63	126—128	71	C, H

a) All new compounds displayed ^{1}H-NMR, FTIR, UV and MS spectra consistent with the assigned structure. *b*) Carbon, hydrogen, and nitrogen elemental analyses were obtained for all new targets and were within ±0.4% of the theoretical values except where stated otherwise.

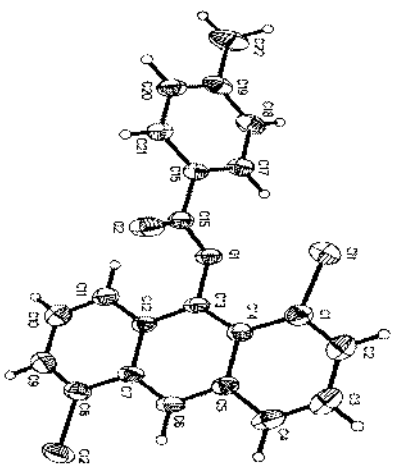

Fig. 1. X-Ray Crystal Structure of Compound **3o**

pounds lack the usual carbonyl chemical shift in the δ 180—190 region that the carbonyl resonance of anthrone, itself occurs at δ 183.7.[2] The new method provides a simple and convenient route to 9-acyloxy 1,5-dichloroanthracene derivatives. Pyridine in CH_2Cl_2 at room temperature for several hours to yield high yield of desired products. The data for all of the direct reactions are presented in Table 1. The structure of **3o** including the side chain was confirmed by X-ray analysis. The ORTEP plot of **3o** is shown in Fig. 1. In the crystal, the toluoyl ring is twisted from the plane of the anthracene ring. However, upon consideration of the intermolecular interaction present in the crystal the reason for this geometry in the crystal became apparent.

Table 2. *Taq* Inhibition and Inhibition of Lipid Peroxidation of 9-Acyloxy 1,5-Dichloroanthracene Derivatives

Compd.	*Taq* Inhibition[a]			Inhibition of Lipid Peroxidation (%)[b]	
	1 mM	0.1 mM	0.01 mM	10 μM	1 μM
3a	ND[c]	ND[c]	ND[c]	34±0.1	26±0.1
3b	+	+	+	100	100
3c	−	−	−	100	100
3d	+	+	+	100	100
3e	ND[c]	ND[c]	ND[c]	29±0.1	2±0.1
3f	ND[c]	ND[c]	ND[c]	61±0.2	10±0.2
3g	+	+	+	100	62±0.4
3h	+	+	+	18±0.4	12±0.2
3i	−	−	−	100	38±0.2
3j	+	+	−	29±0.2	10±0.1
3k	+	+	+	17±0.5	12±0.3
3l	ND[c]	ND[c]	ND[c]	14±0.3	5±0.2
3m	+	−	−	23±0.2	0
3n	+	+	−	22±0.1	11±0.1
3o	ND[c]	ND[c]	ND[c]	26±0.2	3±0.1
3p	+	−	−	31±0.1	9±0.1
3q	+	−	−	ND[c]	ND[c]
3r	−	−	−	ND[c]	ND[c]
3s	−	−	−	36±0.2	6±0.1
3t	ND[c]	ND[c]	ND[c]	10±0.3	0
3u	+	+	−	16±0.1	2±0.1
3v	+	+	−	33±0.2	14±0.1
3w	+	+	+	37±0.2	30±0.4
Control[d]	+	+	+	100	62

a) Key: (+) inhibition, (−) no inhibition. *b*) Relative percentage of inhibition. Inhibition was significantly different with respect to that of the control [(+)-α-tocopherol], $p<0.01$, mean±S.E., $n=4$. Values in parentheses are percent inhibition at the indicated concentration (μM), and standard errors average 10% of the indicated values. *c*) ND=not determined. *d*) Control: *Taq* Inhibition: doxorubicin, mitomycin-C, and methotrexate; Inhibition of lipid peroxidation: (+)-α-tocopherol.

Results and Discussion

9-Acyloxy 1,5-dichloroanthracene derivatives (**3a**—**w**) were synthesized and *Taq* inhibition and lipid peroxidation were evaluated. Prior to the evaluation of compounds in the PCR-based telomerase assay, the agents were tested for their ability to inhibit *Taq* polymerase in order to address the selectivity of polymerase/telomerase inhibition.[10,16—18] Approximately most of the compounds examined completely inhibited *Taq* polymerase activity at concentration of 1 mM, 0.1 mM, and 0.01 mM, respectively, whereas **3b**, **3d**, **3g**, **3h**, **3k**, and **3w**, showed significant inhibitory effect at the three different concentrations. Further, there does not appear to be any correlation between *Taq* polymerase inhibition and cytotoxicity. The anthraquinone-based control drugs doxorubicin, mitomycin-C, and methotrexate showed total inhibition of *Taq* polymerase activity at 1 mM, 0.1 mM, and 0.01 mM, respectively.

The inhibitory effect on lipid peroxidation of these compounds was evaluated with rat brain phospholipid liposomes which provide an ideal model system for lipid peroxidation studies.[19] With respect to the (+)-α-tocopherol, we found better inhibitory effect in 10 μM by compounds **3b**, **3c**, **3d**, **3g**, and **3i**. Furthermore, compounds **3b**, **3c**, and **3d** were significantly more efficient than (+)-α-tocopherol in 1 μM. Although not a potent inhibitor of lipid peroxidation in itself, can provide a useful template for the design of potential anticancer agents. Moreover, the results support our hypothesis that structural modification of 1,5-dichloroanthracenone may

lead to control of the release of active oxygen species. *Taq* inhibitory action of 9-substituted 1,5-dichloroanthracenes was not proportionally related to the lipid peroxidation; compounds which had potent human telomerase inhibition were also effective in lipid peroxidation (**3b**, **3c**, **3d**, **3g**, **3i**). In addition to the redox properties, other factors such as an appropriate geometry of the molecules when bound to the active site of the substrate may be responsible for the human telomerase inhibition activities of the novel anthracene analogs. This is supported by the fact that for these compounds, a definite length of the chain linking the anthracene nucleus and the phenyl rest was optimal for activity. In conclusion, we have described 9-acyloxy 1,5-dichloroanthracene derivatives which show potent *Taq* inhibition and antioxidant activity. To understand whether or not these compounds have potent antitumor and antiproliferative activities, we examined their effects in other cell lines. The results will be reported elsewhere.

Experimental

Melting points were determined with a Büchi 530 melting point apparatus and are uncorrected. All reactions were monitored by TLC, which was performed on precoated sheets on silica gel 60 F_{254}, and flash column chromatography was done in silica gel (E. Merck, 70—230 mesh) with CH_2Cl_2 as eluant. ^{1}H-NMR spectra were recorded with a Varian GEMINI-300 (300 MHz); δ values are in ppm relative to a tetramethylsilane internal standard. Fourier-transform-IR spectra (KBr) were recorded on a Perkin-Elmer 983G spectrometer. Mass spectra (EI, 70 eV, unless otherwise stated) were obtained on a Finnigan MAT TSQ-46 and Finnigan MAT TSQ-700. The X-ray Crystallographic Laboratory of the University of Taiwan performed X-ray structural analysis of compound **3o**.

1,5-Dichloro-9(10*H*)-anthracenone (2) To a solution of 1,5-dichloro anthraquinone (5.56 g, 20 mmol) in glacial acetic acid (200 ml) heated to reflux was added dropwise over 3 h, a solution of $SnCl_2$ (50 g, 220 mmol) in 37% HCl (100 ml). The solution was then cooled, and the resulting crystals were collected by filtration, washed with water, purified by crystallization (acetic acid) and subjected to column chromatography.

General Procedure for the Preparation of 9-Acyloxy 1,5-Dichloro Anthracenes To a solution of **2** (1 mmol) and pyridine (0.1 ml) in dry CH_2Cl_2 (20 ml) was added dropwise a solution of appropriate acyl chlorides (3 mmol) in dry CH_2Cl_2 (10 ml) under N_2. The reaction mixture was stirred at room temperature or refluxed for several hours. The solvent was removed and the residue purified by recrystallization (EtOH) and chromatography.

9-Acetoxy 1,5-Dichloroanthracene (3a) ^{1}H-NMR ($CDCl_3$) δ: 8.79 (H, s), 8.01 (H, d, J=8.4 Hz), 7.90 (H, d, J=8.8 Hz), 7.62—7.58 (2H, m), 7.43—7.34 (2H, m), 2.60 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 170.58, 142.00, 134.51, 132.31, 130.54, 129.87, 129.61, 128.37, 126.97, 126.93, 126.80, 125.97, 123.81, 121.79, 121.67, 22.12. MS *m/z*: 304 (M^+), 262, 227. IR (KBr) cm^{-1}: 1753. *Anal.* Calcd for $C_{16}H_{10}O_2Cl_2$: C, 62.98; H, 3.30. Found: C, 62.98; H, 3.21.

9-Bromoacetoxy 1,5-Dichloroanthracene (3b) ^{1}H-NMR ($CDCl_3$) δ: 8.80 (H, s), 8.03 (H, d, J=8.8 Hz), 7.99 (H, d, J=8.4 Hz), 7.62—7.57 (2H, m), 7.45—7.34 (2H, m), 4.32 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 166.80, 156.93, 134.39, 132.61, 130.83, 129.88, 129.74, 127.96, 127.39, 127.16, 126.99, 126.09, 125.75, 123.44, 121.21, 26.31. MS *m/z*: 384 (M^+), 262. IR (KBr) cm^{-1}: 1759. *Anal.* Calcd for $C_{16}H_9O_2Cl_2Br$: C, 50.04; H, 2.36. Found: C, 50.00; H, 2.36.

9-Chloroacetoxy 1,5-Dichloroanthracene (3c) ^{1}H-NMR: ($CDCl_3$) δ: 8.81 (H, s), 8.00 (H, d, J=8.4 Hz), 7.92 (H, d, J=8.8 Hz), 7.62—7.58 (2H, m), 7.45—7.35 (2H, m), 4.57 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 166.93, 142.53, 134.39, 132.67, 130.87, 129.84, 129.73, 127.92, 127.41, 127.14, 126.81, 126.11, 126.01, 124.47, 121.69, 121.18, 44.88. MS *m/z*: 388 (M^+), 262. IR (KBr) cm^{-1}: 1756. *Anal.* Calcd for $C_{16}H_9O_2Cl_3$: C, 56.59; H, 2.67. Found: C, 56.46; H, 2.67.

9-Propionyloxy 1,5-Dichloroanthracene (3d) ^{1}H-NMR ($CDCl_3$) δ: 8.78 (H, s), 7.99 (H, d, J=8.4 Hz), 7.87 (H, d, J=8.8 Hz), 7.60—7.56 (2H, m), 7.41—7.34 (2H, m), 2.95—2.90 (2H, q), 1.40 (3H, t, J=7.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 173.99, 142.74, 134.51, 132.59, 130.49, 129.87, 129.61, 128.42, 127.09, 126.93, 126.88, 125.95, 123.68, 121.62, 58.96, 28.77, 18.94. MS *m/z*: 318 (M^+), 262. IR (KBr) cm^{-1}: 1757. *Anal.* Calcd for $C_{17}H_{12}O_2Cl_2$: C, 63.97; H, 3.79. Found: C, 63.99; H, 3.88.

9-Isobutyryloxy 1,5-Dichloroanthracene (3e) ^{1}H-NMR ($CDCl_3$) δ: 8.77 (H, s), 7.98 (H, d, J=8.4 Hz), 7.87 (H, d, J=8.8 Hz), 7.60—7.55 (2H, m), 7.40—7.33 (2H, m), 3.21—3.13 (H, m), 1.60—1.39 (6H, m). ^{13}C-NMR ($CDCl_3$) δ: 176.45, 141.64, 134.53, 132.62, 130.47, 129.90, 129.62, 128.51, 127.18, 126.85, 125.93, 123.83, 123.66, 121.45, 35.30, 20.16, 19.07. MS *m/z*: 332 (M^+), 262. IR (KBr) cm^{-1}: 1761. *Anal.* Calcd for $C_{18}H_{14}O_2Cl_2$: C, 65.08; H, 3.94. Found: C, 64.86; H, 4.18.

9-(2-Chloropropionyloxy) 1,5-Dichloroanthracene (3f) ^{1}H-NMR ($CDCl_3$) δ: 8.80 (H, s), 8.11 (H, d, J=11.4 Hz), 7.99 (H, d, J=10.6 Hz), 7.63—7.55 (2H, m), 7.43—7.33 (2H, m), 4.98—4.93 (H, q), 1.86 (3H, d, J=8.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 166.90, 142.76, 133.55, 133.25, 131.58, 130.76, 129.89, 129.74, 128.61, 128.37, 127.36, 127.15, 127.04, 126.02, 124.32, 121.36, 52.53, 31.87, 21.17. MS *m/z*: 352 (M^+), 262. IR (KBr) cm^{-1}: 1766. *Anal.* Calcd for $C_{17}H_{11}O_2Cl_3$: C, 57.74; H, 3.14. Found: C, 57.85; H, 2.99.

9-Dichloroacetoxy 1,5-Dichloroanthracene (3g) ^{1}H-NMR ($CDCl_3$) δ: 8.84 (H, s), 8.05—8.00 (2H, m), 7.62—7.59 (2H, m), 7.47—7.36 (2H, m), 6.51 (H, s). ^{13}C-NMR ($CDCl_3$) δ: 163.31, 140.68, 133.73, 132.12, 130.49, 129.27, 129.15, 127.21, 127.15, 126.67, 126.19, 125.62, 124.39, 120.96, 120.19, 64.59. MS *m/z*: 374 (M^+), 262. IR (KBr) cm^{-1}: 1769. *Anal.* Calcd for $C_{16}H_8O_2Cl_4$: C, 51.38; H, 2.16. Found: C, 51.16; H, 1.99.

9-Butyryloxy 1,5-Dichloroanthracene (3h) ^{1}H-NMR ($CDCl_3$) δ: 8.76 (H, s), 7.97 (H, d, J=8.4 Hz), 7.86 (H, d, J=8.8 Hz), 7.59—7.55 (2H, m), 7.40—7.32 (2H, m), 2.90—2.84 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 173.14, 142.77, 134.51, 132.60, 130.47, 129.61, 129.21, 128.44, 127.12, 126.92, 126.85, 125.94, 123.67, 122.13, 121.68, 37.21, 18.73, 14.53. MS *m/z*: 332 (M^+), 262. IR (KBr) cm^{-1}: 1754. *Anal.* Calcd for $C_{18}H_{14}O_2Cl_2$: C, 64.88; H, 4.23. Found: C, 64.82; H, 4.23.

9-(4-Bromobutyryloxy) 1,5-Dichloroanthracene (3i) ^{1}H-NMR ($CDCl_3$) δ: 8.78 (H, s), 7.99 (H, d, J=8.4 Hz), 7.86 (H, d, J=8.8 Hz), 7.61—7.57 (2H, m), 7.42—7.33 (2H, m), 3.63—3.60 (2H, m), 3.15—3.11 (2H, m), 2.41—2.36 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 172.25, 142.42, 134.48, 132.67, 130.60, 129.87, 129.66, 128.28, 127.06, 127.00, 126.01, 123.91, 122.02, 121.53, 33.33, 33.18, 27.85. MS *m/z*: 412 (M^+), 262. IR (KBr) cm^{-1}: 1747. *Anal.* Calcd for $C_{18}H_{13}O_2Cl_2Br$: C, 52.46; H, 3.18. Found: C, 52.49; H, 3.16.

9-(4-Chlorobutyryloxy) 1,5-Dichloroanthracene (3j) ^{1}H-NMR ($CDCl_3$) δ: 8.74 (H, s), 7.95 (H, d, J=8.4 Hz), 7.84 (H, d, J=8.8 Hz), 7.59—7.55 (2H, m), 7.40—7.30 (2H, m), 3.75 (2H, t, J=6.1 Hz), 2.31 (2H, m), 1.56 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 172.36, 142.41, 134.46, 132.65, 130.59, 129.84, 129.65, 128.27, 127.03, 126.98, 125.99, 123.88, 121.99, 121.51, 44.58, 31.95, 27.79. MS *m/z*: 366 (M^+), 262. IR (KBr) cm^{-1}: 1747. *Anal.* Calcd for $C_{18}H_{13}O_2Cl_3$: C, 58.80; H, 3.56. Found: C, 58.55; H, 3.49.

9-Hexanoyloxy 1,5-Dichloroanthracene (3k) ^{1}H-NMR ($CDCl_3$) δ: 8.69 (H, s), 7.90 (H, d, J=8.4 Hz), 7.79 (H, d, J=8.8 Hz), 7.52—7.48 (2H, m), 7.33—7.26 (2H, m), 2.84—2.79 (2H, m), 1.85—1.79 (2H, m), 1.46—1.32 (4H, m), 0.89 (3H, t, J=7.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 173.34, 142.44, 134.52, 132.61, 130.48, 129.87, 129.62, 128.44, 126.93, 126.85, 125.95, 123.67, 122.13, 121.67, 35.29, 32.03, 24.87, 23.01, 14.55. MS *m/z*: 360 (M^+), 262. IR (KBr) cm^{-1}: 1753. *Anal.* Calcd for $C_{20}H_{18}O_2Cl_2$: C, 66.49; H, 5.02. Found: C, 66.44; H, 5.02.

9-Benzoyloxy 1,5-Dichloroanthracene (3l) ^{1}H-NMR ($CDCl_3$) δ: 8.82 (H, s), 8.40 (2H, d, J=8.1 Hz) 8.01 (H, d, J=8.4 Hz), 7.91 (H, d, J=8.8 Hz), 7.71 (H, t, J=7.4 Hz), 7.61—7.58 (2H, m), 7.56—7.52 (2H, d, J=7.2 Hz), 7.37—7.33 (2H, m). ^{13}C-NMR: ($CDCl_3$) δ: 166.62, 142.41, 134.49, 132.64, 131.38, 130.43, 130.11, 129.98, 129.53, 129.40, 128.65, 127.24, 126.98, 126.00, 125.84, 122.45, 121.81. MS *m/z*: 366 (M^+), 262, 105. IR (KBr) cm^{-1}: 1737. *Anal.* Calcd for $C_{21}H_{12}O_2Cl_2$: C, 68.68; H, 3.29. Found: C, 68.61; H, 3.25.

9-(*o*-Toluoyloxy) 1,5-Dichloroanthracene (3m) ^{1}H-NMR ($CDCl_3$) δ: 8.82 (H, s), 8.59 (H, d, J=7.8 Hz), 8.02 (H, d, J=8.4 Hz), 7.97 (H, d, J=8.8 Hz), 7.61—7.56 (4H, m), 7.45 (H, t, J=7.6 Hz), 7.40—7.35 (2H, m), 2.72 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 166.37, 143.14, 142.98, 134.58, 133.78, 132.83, 132.67, 130.44, 129.95, 129.59, 128.70, 128.52, 127.30, 126.97, 126.80, 126.01, 123.71, 122.49, 121.85, 31.52, 22.78. MS *m/z*: 380 (M^+), 262, 119. IR (KBr) cm^{-1}: 1731. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.11; H, 3.64.

9-(*m*-Toluoyloxy) 1,5-Dichloroanthracene (3n) ^{1}H-NMR ($CDCl_3$) δ: 8.74 (H, s), 8.15 (H, d, J=7.3 Hz) 7.93 (H, d, J=8.4 Hz), 7.85 (H, d, J=8.8 Hz), 7.53—7.39 (4H, m), 7.29—7.25 (2H, m), 2.42 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 166.41, 142.98, 139.25, 135.31, 134.55, 132.58, 131.83, 130.40, 129.94, 129.90, 129.52, 129.31, 128.72, 128.55, 127.19, 126.94, 126.00, 123.79, 122.38, 121.86, 21.97. MS *m/z*: 380 (M^+), 262, 119. IR (KBr) cm^{-1}: 1730. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.05; H,

3.71.

9-(*p*-Toluoyloxy) 1,5-Dichloroanthracene (3o) ^{1}H-NMR ($CDCl_3$) δ: 8.81 (H, s), 8.82 (H, d, J=8.1 Hz) 8.01 (H, d, J=8.4 Hz), 7.91 (H, d, J=8.8 Hz), 7.59—7.54 (2H, m), 7.39 (2H, d, J=8.0 Hz), 7.36—7.32 (2H, m), 2.58 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 166.28, 145.43, 143.03, 134.56, 132.56, 131.43, 130.38, 130.13, 129.91, 129.51, 128.76, 127.28, 127.24, 126.97, 126.91, 125.99, 123.74, 122.49, 121.89, 22.44. MS *m*/*z*: 380 (M^+), 262, 119. IR (KBr) cm^{-1}: 1735. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 68.81; H, 3.65.

X-Ray Crystal Structure of **3o**: $C_{22}H_{14}O_2Cl_2$, Mt=381.25. A needle with approximate dimensions of 0.2×0.25×0.25 mm was mounted a glass fiber along its longest axis. The crystal system was triclinic, space group *P*-1. Cell constants a=8.6360(19), b=9.7774(21), and c=11.662(3) Å; α=85.178 (19)°; β=75.775(18)°; γ=67.100(19)°; V=879.2(3) Å^3; Z=2; μ(Mo$K\alpha$)= 3.810 cm^{-1}; F(000)=393. Cell dimensions were determined using a Nonius CAD4 Kappa Axis XRD & Siemens Smart CCD XRD diffractometer equipped with a graphite monochromator and molybdenum source (λ= 7.1073 Å). Data were collected on the same instrument using ω scans with 2θ varied from 2—50°. A total of 3090 unique reflexions were determined of which 1647 were >2.0σ. The structure was solved using direct methods and was refined using standard techniques. A total of 235 parameters were varied in the final least-squares. The refinement converged at R=0.040 and R_w=0.040. Residual electron density varied from 0.20 to −0.20 e/Å^3.

9-(3-Chlorobenzoyloxy) 1,5-Dichloroanthracene (3p) ^{1}H-NMR ($CDCl_3$) δ: 8.83 (H, s), 8.40—8.39 (H, m), 8.30—8.28 (H, m), 8.02 (H, d, J=8.4 Hz), 7.88 (H, d, J=8.8 Hz), 7.71—7.68 (H, m), 7.62—7.57 (2H, m), 7.55 (H, t, J=8.0 Hz), 7.40—7.36 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.06, 142.49, 135.61, 134.56, 134.52, 132.68, 131.76, 131.29, 130.75, 130.58, 129.89, 129.60, 129.46, 128.47, 127.14, 127.05, 126.08, 124.10, 122.39, 121.58, 112.02. MS *m*/*z*: 400 (M^+), 262, 139. IR (KBr) cm^{-1}: 1740. *Anal.* Calcd for $C_{21}H_{11}O_2Cl_3$: C, 62.79; H, 2.76. Found: C, 62.35; H, 2.76.

9-(4-Chlorobenzoyloxy) 1,5-Dichloroanthracene (3q) ^{1}H-NMR ($CDCl_3$) δ: 8.83 (H, s), 8.34—8.31 (2H, m), 8.02 (H, d, J=8.4 Hz), 7.87 (H, d, J=8.8 Hz), 7.61—7.55 (4H, m), 7.38—7.34 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.06, 148.41, 141.15, 134.56, 132.72, 132.44, 130.55, 129.93, 129.81, 129.60, 128.52, 128.44, 127.11, 127.04, 126.08, 124.04, 121.63, 112.01. MS *m*/*z*: 400 (M^+), 262, 139. IR (KBr) cm^{-1}: 1732. *Anal.* Calcd for $C_{21}H_{11}O_2Cl_3$: C, 62.79; H, 2.76. Found: C, 62.19; H, 2.76.

9-(3-Nitrobenzoyloxy) 1,5-Dichloroanthracene (3r) ^{1}H-NMR ($CDCl_3$) δ: 9.23 (H, s), 8.83 (H, s), 8.70—8.68 (H, m), 8.57—8.53 (H, m), 8.02 (H, d, J=8.4 Hz), 7.85—7.83 (H, m), 7.80 (H, t, J=8.0 Hz), 7.62—7.56 (2H, m), 7.39—7.35 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 164.18, 149.21, 136.87, 136.67, 134.51, 132.79, 131.83, 130.76, 129.90, 129.72, 128.21, 127.34, 127.13, 126.90, 126.25, 126.17, 125.97, 124.34, 121.33. MS *m*/*z*: 411 (M^+), 262, 120. IR (KBr) cm^{-1}: 1744. *Anal.* Calcd for $C_{21}H_{11}NO_4Cl_2$: C, 61.19; H, 2.69; N, 3.40. Found: C, 60.30; H, 2.68; N, 3.69.

9-(4-Nitrobenzoyloxy) 1,5-Dichloroanthracene (3s) ^{1}H-NMR ($CDCl_3$) δ: 8.87 (H, s), 8.57 (2H, d, J=8.8 Hz), 8.43 (2H, d, J=8.8 Hz), 8.05 (H, d, J=8.4 Hz), 7.85 (H, d, J=8.8 Hz), 7.63—7.57 (2H, m), 7.41—7.37 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 165.04, 147.52, 135.35, 134.53, 132.83, 132.48, 130.79, 129.93, 129.74, 127.46, 127.38, 126.90, 126.19, 124.57, 124.47, 121.28, 111.04, 31.52. MS *m*/*z*: 411 (M^+), 262, 120. IR (KBr) cm^{-1}: 1735. *Anal.* Calcd for $C_{21}H_{11}NO_4Cl_2$: C, 61.19; H, 2.69; N, 3.40. Found: C, 60.57; H, 2.71; N, 3.33.

9-(2-Methoxy-4-chlorobenzoyloxy) 1,5-Dichloroanthracene (3t) ^{1}H-NMR ($CDCl_3$) δ: 8.82 (H, s), 8.28 (H, d, J=10.4 Hz), 8.06 (H, d, J=11.0 Hz), 8.01 (H, d, J=10.6 Hz), 7.61—7.54 (2H, m), 7.40—7.33 (2H, m), 7.14—7.09 (2H, m), 3.96 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 161.66, 141.46, 134.89, 134.54, 132.52, 130.41, 129.56, 128.66, 126.96, 125.97, 123.79, 121.12, 121.44, 117.73, 113.68, 56.97. MS *m*/*z*: 430 (M^+), 262, 169. IR (KBr) cm^{-1}: 1738. *Anal.* Calcd for $C_{22}H_{13}O_3Cl_3$: C, 61.21; H, 3.04. Found: C, 61.09; H, 3.02.

9-(2,4-Dichlorobenzoyloxy) 1,5-Dichloroanthracene (3u) ^{1}H-NMR ($CDCl_3$) δ: 8.78 (H, s), 8.38 (H, d, J=8.4 Hz), 7.96 (H, d, J=8.4 Hz), 7.91—7.89 (H, m), 7.57—7.52 (2H, m), 7.52—7.41 (2H, m), 7.35—7.29 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 163.55, 140.40, 137.39, 134.79, 134.50, 132.69, 132.39, 130.07, 129.92, 129.66, 128.32, 128.05, 127.25, 127.05, 126.08, 124.22, 122.19, 121.63. MS *m*/*z*: 436 (M^+), 262, 173. IR (KBr) cm^{-1}: 1741. *Anal.* Calcd for $C_{21}H_{10}O_2Cl_4$: C, 57.84; H, 2.31. Found: C, 57.67; H, 2.32.

9-Phenylacetyloxy 1,5-Dichloroanthracene (3v) ^{1}H-NMR ($CDCl_3$) δ: 8.68 (H, s), 7.90 (H, d, J=8.4 Hz), 7.50—7.45 (4H, m), 7.42 (H, d, J=8.8 Hz), 7.36—7.33 (2H, m), 7.31—7.25 (2H, m), 7.17—7.13 (H, m), 4.15—4.03 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 170.88, 145.32, 134.03, 133.29, 132.33, 130.54, 130.42, 129.63, 129.43, 128.18, 126.88, 125.97, 123.84, 121.41, 42.59. MS *m*/*z*: 380 (M^+), 262. IR (KBr) cm^{-1}: 1750. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.34; H, 3.79.

9-(Phenylpropionyloxy) 1,5-Dichloroanthracene (3w) ^{1}H-NMR ($CDCl_3$) δ: 8.63 (H, s), 7.85 (H, d, J=8.4 Hz), 7.45—7.43 (2H, m), 7.31 (H, d, J=8.8 Hz), 7.26—7.16 (6H, m), 7.13—7.10 (H, m), 3.15—3.07 (4H, m). ^{13}C-NMR ($CDCl_3$) δ: 172.52, 145.17, 134.46, 132.45, 130.51, 129.61, 129.31, 129.24, 127.14, 126.91, 126.86, 125.95, 123.76, 121.72, 37.03, 31.28. MS *m*/*z*: 394 (M^+), 262. IR (KBr) cm^{-1}: 1769. *Anal.* Calcd for $C_{23}H_{16}O_2Cl_2$: C, 69.89; H, 4.06. Found: C, 69.75; H, 4.08.

Log *P* Determination A standard reverse-phase HPLC procedure was used. The method is based on the linear relationship between the capacity factors log k' of the compound and their log P values. The log k' values of five compounds (4-nitrophenol, 4-chlorophenol, benzophenone, naphthalene, and anthracene) with known log P values (1.91, 2.39, 3.18, 3.44, and 4.49, respectively) were determined. A plot of log k' *versus* log P generated from this calibration mixture was used for the calculation of log P values for unknowns from the logarithms of their chromatographic capacity factors (correlation coefficient >0.99; n=3). Methanol/water/acetic acid (77–23–0.1), adjusted to pH 5.5 with concentrated NH_3, was used as eluant. Calibration was performed as described.[20] Log P values as a measure of lipophilicity are given in Table 1.

Assay of Lipid Peroxidation[19] Rat brain homogenate was prepared from the brains of freshly killed Wistar rats and its peroxidation in the presence of iron ions was measured by the thiobarbituric acid (TBA) method as described.[19] Tetramethoxypropane was used as a standard, and the results were expressed as nanomoles of malondialdehyde equivalents per milligram of protein of rat brain homogenates. In brief, whole brain tissue, excluding the cerebellum, was washed and homogenized in 10 volumes of ice-cold Krebs buffer (10 mM *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid (Hepes), 10 mM glucose, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4, 0.7 mM $MgSO_4$, pH 7.4) using a glass Dounce homogenizer. The homogenate was centrifuged at low speed (1000×***g***) for 10 min, and the resulting supernatant (adjusted to 2 mg/ml) was used immediately in lipid peroxidation assays. The reaction mixture with test compounds or vehicle was incubated for 10 min, then stimulated by addition of ferrous ion (200 μM, freshly prepared), and maintained at 37 °C for 30 min. The reactions were terminated by adding 10 μl of ice-cold trichloroacetic acid solution (4% (w/v) in 0.3 N HCl) and 200 μl of thiobarbituric acid-reactive substance reagent (0.5% (w/v) thiobarbituric acid in 50% (v/v) acetic acid). After boiling for 15 min, the samples were cooled and extracted with 1-butanol. The extent of lipid peroxidation was estimated as thiobarbituric acid-reactive substances and was read at 532 nm in a spectrophotometer (Shimadzu UV-160). The results of this assay are provided in Table 2.

***Taq* Polymerase Assay**[10,16—18] Compounds were included at both 10 and 50 μM final concentrations in a PCR 50 μl master mix comprising 10 ng of pCI-neo mammalian expression vector (Promega, Southampton, U.K.), forward (GGAGTTCCGCGT-TACATAAC) and reverse (GTCTGCTC-GAAGCATTAACC) primers (200 nmol), reaction buffer (75 mM Tris–HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% v/v Tween 20), 2.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, and thermostable DNA polymerase ("red hot", Advanced Biotechnologies) 1.25 units. A reaction mix containing water and no drug was used as a positive control, producing a product of approximately 1 kb. Amplification (30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2.5 min) was performed using a thermal cycler (Hybaid, U.K.). PCR products were then separated by electrophoresis on a 2% w/w agarose gel and visualized using ethidium bromide. The results of this assay are provided in Table 2.

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References

1) Barasch D., Zipori O., Ringel I., Ginsburg I., Samuni A., *Eur. J. Med. Chem.*, **34**, 597—615 (1999).
2) Bhawal B. M., Khanapure S. P., Zhang H., Biehl E. R., *J. Org. Chem.*, **56**, 2846—2849 (1991).
3) Johnson M. G., Kiyokawa H., Tani S., Koyama J., Morris-Natschke S. L., *Bioorganic Med. Chem.*, **5**, 1469—1479 (1997).
4) Krohn K., *Tetrahedron*, **46**, 291—318 (1990).
5) Morier-Teissier E., Boitte N., Helbecque N., Bernier J.-L., Pommery N., *J. Med. Chem.*, **36**, 2084—2090 (1993).

6) Wei B. L., Wu S. H., Chung M. I., Won S. J., Lin C. N., *Eur. J. Med. Chem*., **35**, 1089—1098 (2000).
7) Zalupski M. M., Benedetti J., Balcerzak S. P., Hutchins L. F., Belt R. J., *Invest. New Drugs*, **11**, 337—341 (1993).
8) Tam M.-N., Nam N.-H., Jin G.-Z., Song G.-Y., Ahn B.-Z., *Arch. Pharm. Pharm. Med. Chem*., **333**, 189—194 (2000).
9) Scheibler P., *Pharmazie*, **52**, 411—412 (1997).
10) Read M. A., Wood A. A., Harrison J. R., Gowan S. M., Kelland L. R., *J. Med. Chem*., **42**, 4538—4546 (1999).
11) Nordblom G. D., Pachla L. A., Chang T., Whitfield L. R., Showalter H. D., *Cancer Res*., **49**, 5345—5351 (1989).
12) de Isabella P., Palumbo M., Sissi C., Carenini N., Capranico G., *Biochem. Pharmacol*., **53**, 161—169 (1997).
13) Criswell T. R., Klanderman B. H., *J. Org. Chem*., **39**, 770 (1974).
14) Wakefield B. J., "The Chemistry of Organolithium Compounds," Pergamon Press, Oxford, 1974, pp. 21—25.
15) Prinz H., Wiegrebe W., Müller K., *J. Org. Chem*., **61**, 2861—2864 (1996).
16) Perry P. J., Read M. A., Davies R. T., Gowan S. M., Reszka A. P., *J. Med. Chem*., **42**, 2679—2684 (1999).
17) Perry P. J., Gowan S. M., Reszka A. P., Polucci P., Jenkins T. C., *J. Med. Chem*., **41**, 3253—3260 (1998).
18) Perry P. J., Reszka A. P., Wood A. A., Read M. A., Gowan S. M., *J. Med. Chem*., **41**, 4873—4884 (1998).
19) Teng C. M., Hsiao G., Ko F. N., Lin D. T., Lee S. S., *Eur. J. Pharmacol*., **303**, 129—139 (1996).
20) Müller K., Huang H. S., Wiegrebe W., *J. Med. Chem*., **39**, 3132—3138 (1996).