Studies on Anthracenes. 2. Synthesis and Cytotoxic Evaluation of 9-Acyloxy 1,8-Dichloroanthracene Derivatives

Hsu-Shan HUANG,*[,a] Hui-Fen CHIU,[d] Jing-Min HWANG,[b] Yee-Min JEN,[b] Chi-Wei TAO,[c] Kung-Yuan LEE,[a] and Yu-Liang LAI[a]

School of Pharmacy,[a] and Department of Radiation Oncology,[b] National Defense Medical Center; Cheng-Hsin Medical Center,[c] Taipei, and Department of Pharmacology,[d] Kaohsiung Medical University, Kaohsiung,Taiwan, R.O.C.
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The synthesis and cytotoxic evaluation of 9-acyloxy 1,8-dichloroanthracene derivatives are described. The system selectively reduces the carbonyl group flanked by the *peri* substituents of the anthracenediones to give the corresponding 1,8-dichloro-9(10*H*)-anthracenone. Simple acylation of anthracenone occurred with appropriate acyl chlorides in CH_2Cl_2 with a catalytic amount of pyridine to give the 9-acyloxy-1,8-dichloroanthracene derivatives. Considerable interest has developed in the mechanism of how anthracenones achieve this desirable selectivity. These compounds were evaluated *in vitro* for their ability to inhibit the growth of human oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM 8401) and Chinese hamster ovary (CHO) cells, respectively, as compared to mitoxantrone. The *in vitro* cytotoxicity evaluation of 9-acyloxy 1,8-dichloroanthracenes against these above cell lines revealed for most of the compounds a cytotoxic potency lower than that of mitoxantrone. The most active compounds were thus selected for further *in vitro* biological evaluation and structural modification.

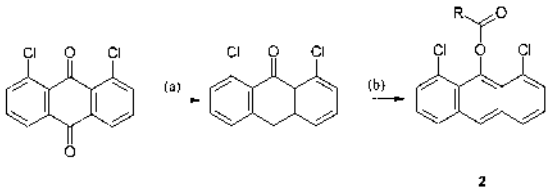
Key words *peri* substituent; anthracenedione; anthracenone; anthracene; acylation

Intercalating agents continue to occupy a prominent position in the treatment of malignant diseases, thus, the antitumor and biochemical effects of these compounds remain as subjects of intensive research.[1—4] DNA intercalating agents interfere with DNA's role as a template in replication and transcription by inserting an intercalator molecule between adjacent base pairs.[5] Of the drug-receptor interactions studied in recent years, the intercalation of planar aromatic molecules between consecutive base pairs of a DNA double helix is one of the most appealing at the molecular level.[6—8] In a previous paper, we described the synthesis and some biological evaluation for 9-acyloxy 1,5-dichloroanthracene derivatives.[9] Detailed structural requirements for the chromophore in this class are still unknown. There is continuing interest in the development of new agents that modify the chromophore anthracenone moiety, yet exhibit different spectra of potency, together with reduced overall toxicity.[10] Non-covalent drug/DNA interactions are difficult to study, and because of this, the significance of such interactions from a safety standpoint and their contribution to positive genetic toxicology test findings is poorly understood.[11] Prompted by the biological results from studies carried out in our laboratories on anthracene and anthracenone, which are chromophore-modified anthracenediones closely related to the anthracenes, we decided to synthesize representative examples of 9-acyloxy 1,8-dichloroanthracene derivatives so that direct cross-series comparisons could be made. Substituted 1,5-dichloroanthracenes with these simple 9-substituents have been reported, and were accessible *via* the chemistry that we developed earlier in Fig. 1. We report in this paper the synthesis and preliminary biological evaluation of a number of 9-substituted 1,8-dichloroanthracenes in which the chromophore was also additionally modified by an acyloxy group at position 9.

Chemistry A series of anthracene derivatives with acyloxy groups sited in the side chain have been synthesized. Synthesis of the target 9-acyloxy 1,8-dichloroanthracenes **2** is given in Chart 1. Key to the successful completion of this target was to utilize a modification of a simple procedure that describes the synthesis of 1,8-dichloro-9(10*H*)-anthracenone with appropriate acyl chloride in the presence of pyridine to afford a corresponding 9-acyloxy 1,8-dichloroanthracene in good yield. The ^{1}H-NMR data for **1** and **2** showed significant differences in chemical shifts of protons for the acyloxy side chain and the proton at C-10. The most striking is the difference of *ca.* 0.45 ppm downfield for signals of the proton on this carbon. In the ^{1}H-NMR spectrum the alkyloxyl alkyl group signal was identified while that at δ 9.25 was one single proton signal, explicable if the alkyloxyl alkyl group at position 10 was sterically compressed between the alkyloxyl at position 9 and the carbonyl. Of particular importance is the one 10-H proton anthracenone chemical shift of δ 9.25, which is different from the range of the anthracenone possessing two 10-H protons. However, in the case of structure **1**, the signal of the non-aromatic proton at position 10 should be shifted downfield in comparison with **2**. The ^{1}H-NMR spectra of the compounds **2a**—**u** show mostly a singlet between δ 9.20 and 9.31, for the 10 position of CH, respectively. This chemistry and related ^{1}H-NMR are discussed in detail in the previous paper.[9]

Results and Discussion

We synthesized 9-acyloxy 1,8-dichloroanthracene derivatives, and tested their biological effects *in vitro* on human



Reagents: (a) $SnCl_2$, HCl, HOAc, 118 °C; (b) RCOCl, pyridine, CH_2Cl_2, N_2.

Chart 1

* To whom correspondence should be addressed. e-mail: huanghs@ndmctsgh.edu.tw

Table 1. Cytotoxic Activity of 9-Acyloxy 1,8-Dichloroanthracene Derivatives on KB, GBM and CHO Tumor Cell Lines.

Compound	R	IC_{50}, μg/ml (S.D.)[a]		
		KB[b]	GBM[c]	CHO[d]
2a	CH_3	1.16±0.68	1.08±0.68	1.13±0.79
2b	CH_2Br	1.23±1.12	1.05±1.03	1.33±1.10
2c	CH_2Cl	0.68±0.35	0.88±0.54	1.56±1.05
2d	CH_2CH_3	1.08±0.55	1.23±1.02	0.56±0.11
2e	$CH(CH_3)_2$	0.95±0.56	1.05±0.75	1.44±1.02
2f	$CH(CH_3)Cl$	0.86±0.44	0.57±0.16	0.76±0.51
2g	$CHCl_2$	0.53±0.01	0.65±0.32	0.38±0.23
2h	$(CH_2)_2CH_3$	0.47±0.02	0.95±0.03	0.88±0.55
2i	$(CH_2)_3Br$	0.13±0.05	0.12±0.05	1.26±0.78
2j	$(CH_2)_3Cl$	0.54±0.02	0.55±0.02	0.25±0.11
2k	$(CH_2)_4CH_3$	0.04±0.01	0.15±0.11	0.11±0.05
2l	C_6H_5	0.14±0.02	4.41±1.25	0.04±0.02
2m	$2\text{-}CH_3C_6H_4$	0.89±0.45	0.21±0.13	1.45±0.58
2n	$3\text{-}CH_3C_6H_4$	0.20±0.06	0.06±0.01	0.23±0.01
2o	$4\text{-}CH_3C_6H_4$	0.09±0.04	0.17±0.11	4.75±2.25
2p	$3\text{-}ClC_6H_4$	1.10±1.01	0.40±0.22	0.17±0.11
2q	$4\text{-}ClC_6H_4$	1.41±0.55	0.14±0.05	4.11±2.46
2r	$3\text{-}NO_2C_6H_4$	0.05±0.02	0.22±0.12	0.41±0.25
2s	$4\text{-}NO_2C_6H_4$	0.12±0.07	0.20±0.15	0.05±0.01
2t	$2,4\text{-}Cl_2C_6H_3$	0.12±0.06	0.71±0.05	0.08±0.03
2u	$CH_2CH_2C_6H_5$	0.50±0.05	0.14±0.03	0.71±0.11
Mitoxantrone		0.009±0.016	0.007±0.012	0.003±0.014

a) IC_{50}, drug concentration inhibiting 50% of cellular growth following 48 h of drug exposure; S.D., standard deviation. *b*) Human oral epidermoid carcinoma cells (KB cell line). *c*) Human cervical carcinoma cells of ME 180 (GBM8401). *d*) Chinese hamster ovary (CHO) cells.

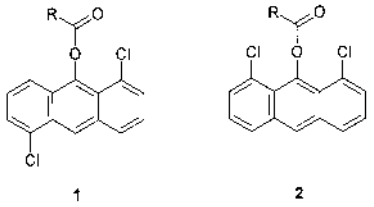


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Fig. 1

oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM 8401), and Chinese hamster ovary (CHO) cells, respectively. Inhibition was found to be statistically significant compared to that of the control (Student's *t*-test; $p=0.05$). IC_{50} values were determined for each compound, derived by interpolation of a log inhibitor concentration *versus* response plot using four or more different concentrations of the compound spanning the 50% inhibition point.[12] The growth inhibitory effect was cell-specific and dose-dependent. The *in vitro* antiproliferative activity values for these derivatives against the KB cell line, GBM8401 and CHO cells, respectively, with comparative data for mitoxantrone are listed in Table 1. On the KB cell line, the derivatives **2** showed cytotoxicity lower than that of mitoxantrone. The most potent compounds are **2k**, **2o**, and **2r**, which retain cytotoxic levels compared to the others. On the GBM cell line, compound **2** showed cytotoxicity lower than that of mitoxantrone; **2n** was the most potent compound. Although evidence indicates that antitumor anthraquinone derivatives (*i.e.* mitoxantrone) bind to nucleic acids by intercalation and the long alkyl side chains bind electrostatically with the anionic exterior of the helix,[13,14] the exact geometry of binding and mode of action has not been conclusively established.[4] The side chain may bestow slow dissociation kinetics on DNA complexes of 9-acyloxy anthracene derivatives, implying that free-radical generation, DNA hydrogen abstraction and sugar fragmentation are rapid compared with DNA-ligand complex lifetimes.[15] The results of the two series of compounds against various cell lines and other SAR evaluation will be reported in a separate paper.

Experimental

General Melting points were determined on a Buchi 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 an eluent. ^{1}H-NMR spectra were recorded with a Varian GEMINI-300 (300 MHz); δ values are in ppm relative to a tetramethylsilane as an 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.

General Procedure for the Preparation of 9-Acyloxy 1,8-Dichloroanthracenes To a solution of 1,8-dichloro-9(10*H*)-anthracenone (1 mmol) and pyridine (0.1 ml) in dry CH_2Cl_2 (20 ml) was added dropwise a solution of an 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 1,8-Dichloroanthracene (**2a**): 89% yield, mp 130—131 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.23 (H, s), 7.87—7.85 (2H, m), 7.64 (2H, t, J=8.0 Hz), 7.45—7.42 (2H, q), 2.62 (3H, s). MS *m/z*: 304 (M^+), 262, 227. IR (KBr) cm^{-1}: 1755. *Anal.* Calcd for $C_{16}H_{10}O_2Cl_2$: C, 62.98; H, 3.30. Found: C, 62.76; H, 3.11.

9-Bromoacetoxy 1,8-Dichloroanthracene (**2b**): 45% yield, mp 192—193 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.24 (H, s), 7.90 (2H, d, J=8.4 Hz), 7.63 (2H, d, J=8.8 Hz), 7.45—7.42 (2H, q), 4.31 (2H, s). MS *m/z*: 384 (M^+), 262, 233, 227. IR (KBr) cm^{-1}: 1750. *Anal.* Calcd for $C_{16}H_9O_2Cl_2Br$: C, 50.04; H, 2.36. Found: C, 50.21; H, 2.43.

9-Chloroacetoxy 1,8-Dichloroanthracene (**2c**): 66% yield, mp 178—180 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.24 (H, s), 7.84 (2H, d, J=8.4 Hz), 7.64 (2H, d, J=8.8 Hz), 7.45—7.42 (2H, q), 4.58 (2H, s). MS *m/z*: 339, 262, 227. IR (KBr) cm^{-1}: 1760. *Anal.* Calcd for $C_{16}H_9O_2Cl_3$: C, 56.59; H, 2.67. Found: C, 56.39; H, 2.51.

9-Propionyloxy 1,8-Dichloroanthracene (**2d**): 88% yield, mp 150—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.22 (H, s), 7.85—7.83 (2H, dd, J=8.8, 6.0 Hz), 7.63 (2H, d, J=8.4 Hz), 7.44—7.41 (2H, dd, J=8.5, 5.8 Hz), 2.98—2.93 (2H, q), 1.56—1.45 (3H, t, J=8.8 Hz). MS *m/z*: 318 (M^+), 262, 227. IR (KBr) cm^{-1}: 1757. *Anal.* Calcd for $C_{17}H_{12}O_2Cl_2$: C, 63.97; H, 3.79. Found: C, 63.79; H, 3.68.

9-Isobutyryloxy 1,8-Dichloroanthracene (**2e**): 76% yield, mp 184—186 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.20 (H, s), 7.80 (2H, d, J=8.4 Hz), 7.61 (2H, d, J=8.8 Hz), 7.41 (2H, t), 3.20—3.15 (H, m), 1.56—1.47 (6H, m). MS *m/z*: 332 (M^+), 262, 227. IR (KBr) cm^{-1}: 1751. *Anal.* Calcd for $C_{18}H_{14}O_2Cl_2$: C, 65.08; H, 3.94. Found: C, 65.29; H, 4.12.

9-(2-Chloropropionyloxy) 1,8-Dichloroanthracene (**2f**): 56% yield, mp 172—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.20 (H, s), 7.88 (2H, d, J=8.4 Hz), 7.64 (2H, dd), 7.44 (2H, dd), 4.96—4.91 (H, q), 2.02 (3H, q). MS *m/z*: 352 (M^+), 262, 227. IR (KBr) cm^{-1}: 1761. *Anal.* Calcd for $C_{17}H_{11}O_2Cl_3$: C, 57.74; H, 3.14. Found: C, 57.85; H, 3.33.

9-Dichloroacetoxy 1,8-Dichloroanthracene (**2g**): 40% yield, mp 178—179 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.32 (H, s), 7.95 (2H, t), 7.69 (2H, d, J=8.5 Hz), 7.53—7.48 (2H, m), 6.45 (H, s). MS *m/z*: 374 (M^+), 262, 227. IR (KBr) cm^{-1}: 1769. *Anal.* Calcd for $C_{16}H_8O_2Cl_4$: C, 51.38; H, 2.16. Found: C, 51.51; H, 2.37.

9-Butyryloxy 1,8-Dichloroanthracene (**2h**): 93% yield, mp 152—153 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.21 (H, s), 7.83 (2H, d, J=8.4 Hz), 7.62 (2H, d, J=8.8 Hz), 7.43—7.39 (2H, dd), 2.90—2.87 (2H, t), 2.01—1.94 (2H, m), 1.18—1.15 (2H, t). MS *m/z*: 333 (M^+), 262, 227. IR (KBr) cm^{-1}: 1754. *Anal.* Calcd for $C_{18}H_{14}O_2Cl_2$: C, 64.88; H, 4.23. Found: C, 64.62; H, 4.11.

9-(4-Bromobutyryloxy) 1,8-Dichloroanthracene (**2i**): 70% yield, mp 155—156 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.25 (H, s), 7.86 (2H, d, J=8.8 Hz), 7.66 (2H, d, J=8.6 Hz), 7.47 (2H, t, J=7.0 Hz), 3.65 (2H, m), 3.19 (2H, t, J=6.6 Hz), 2.45—2.42 (2H, m). MS *m/z*: 412 (M^+), 262, 227. IR (KBr)

cm^{-1}: 1747. *Anal.* Calcd for $C_{18}H_{13}BrO_2Cl_2$: C, 52.46; H, 3.18. Found: C, 52.57; H, 3.21.

9-(4-Chlorobutyryloxy) 1,8-Dichloroanthracene (**2j**): 69% yield, mp 154—155 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.22 (H, s), 7.82 (2H, d, J=8.8 Hz), 7.62 (2H, d, J=8.4 Hz), 7.42 (2H, t, J=7.5 Hz), 3.77—3.75 (2H, m), 3.15 (2H, t, J=7.0 Hz), 2.38—2.32 (2H, m). MS *m/z*: 366 (M^+), 262, 227. IR (KBr) cm^{-1}: 1747. *Anal.* Calcd for $C_{18}H_{13}O_2Cl_3$: C, 58.80; H, 3.56. Found: C, 58.57; H, 3.49.

9-Hexanoyloxy 1,8-Dichloroanthracene (**2k**): 45% yield, mp 136—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.21 (H, s), 7.83 (2H, d, J=8.5 Hz), 7.63 (2H, d, J=8.5 Hz), 7.42 (2H, t, J=6.4 Hz), 2.90 (2H, t, J=6.0 Hz), 1.97—1.91 (2H, m), 1.55—1.41 (2H, m), 0.98—0.96 (3H, t, J=7.3 Hz). MS *m/z*: 360 (M^+), 262, 227. IR (KBr) cm^{-1}: 1750. *Anal.* Calcd for $C_{20}H_{18}O_2Cl_2$: C, 66.49; H, 5.02. Found: C, 66.61; H, 5.16.

9-Benzoyloxy 1,8-Dichloroanthracene (**2l**): 61% yield, mp 183—184 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.26 (H, s), 8.42 (2H, dd, J=8.4, 6.0 Hz), 7.91—7.89 (2H, m), 7.74 (H, dd, J=8.0, 6.0 Hz), 7.61 (4H, dd, J=8.0, 6.0 Hz), 7.38 (2H, dd, J=8.0, 6.0 Hz). MS *m/z*: 366 (M^+), 262, 227, 105. IR (KBr) cm^{-1}: 1735. *Anal.* Calcd for $C_{21}H_{12}O_2Cl_2$: C, 68.68; H, 3.29. Found: C, 68.85; H, 3.46.

9-(2-Toluoyloxy) 1,8-Dichloroanthracene (**2m**): 81% yield, mp 168—169 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.25 (H, s), 8.58—8.56 (2H, dd, J=7.6, 5.8 Hz), 7.94—7.92 (2H, m), 7.64—7.59 (2H, m), 7.45—7.38 (4H, m), 2.70 (3H, s). MS *m/z*: 380 (M^+), 262, 105. IR (KBr) cm^{-1}: 1730. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.51; H, 3.84.

9-(3-Toluoyloxy) 1,8-Dichloroanthracne (**2n**): 58% yield, mp 196—197 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.26 (H, s), 8.22 (2H, dd, J=8.4, 5.4 Hz), 7.91 (2H, d, J=8.5 Hz), 7.63 (2H, d, J=8.5 Hz), 7.56—7.37 (4H, m), 2.50 (3H, s). MS *m/z*: 380 (M^+), 262, 227. IR (KBr) cm^{-1}: 1730. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.55; H, 3.82.

9-(4-Toluoyloxy) 1,8-Dichloroanthracene (**2o**): 77% yield, mp 220—222 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.25 (H, s), 8.30 (2H, dd, J=8.4, 4.2 Hz), 7.91 (2H, d, J=8.5 Hz), 7.63 (2H, dd, J=8.0, 4.0 Hz), 7.42—7.36 (4H, m), 2.51 (3H, s). MS *m/z*: 380 (M^+), 262, 227, 119. IR (KBr) cm^{-1}: 1735. *Anal.* Calcd for $C_{22}H_{14}O_2Cl_2$: C, 69.31; H, 3.70. Found: C, 69.55; H, 3.85.

9-(3-Chlorobenzoyloxy) 1,8-Dichloroanthracene (**2p**): 76% yield, mp 230—232 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.28 (H, s), 8.40 (H, s), 8.29 (H, d, J=8.5 Hz), 7.87 (2H, d, J=8.8 Hz), 7.72 (H, d, J=8.7 Hz), 7.65 (2H, d, J=8.5 Hz), 7.56 (H, t, J=8.0 Hz), 7.40 (2H, t, J=8.0 Hz). 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, 62.95; H, 2.91.

9-(4-Chlorobenzoyloxy) 1,8-Dichloroanthracene (**2q**): 90% yield, mp 178—180 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.27 (H, s), 8.35 (H, s), 8.06—8.03 (2H, m), 7.86 (H, d, J=8.8 Hz), 7.64 (H, d, J=8.7 Hz), 7.60 (2H, d, J=8.5 Hz), 7.50—7.47 (3H, m), 7.40 (H, t, J=8.0 Hz). 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, 62.81; H, 2.94.

9-(3-Nitrobenzoxy) 1,8-Dichloroanthracene (**2r**): 55% yield, mp 258—260 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.20 (H, s), 8.70 (H, dd, J=8.8 Hz), 8.53 (H, m), 8.46 (H, s), 7.93 (2H, d, J=8.8 Hz), 7.86 (H, d, J=8.6 Hz), 7.77 (H, t, J=8.0 Hz), 7.56 (H, d, J=8.8 Hz), 7.42 (H, t, J=8.8 Hz), 7.36 (H, t, J=8.8 Hz). MS *m/z*: 411 (M^+), 261, 150. 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, 61.41; H, 2.78; N, 3.59.

9-(4-Nitrobenzoyloxy) 1,8-Dichloroanthracene (**2s**): 43% yield, mp 234—236 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.31 (H, s), 8.60 (2H, d, J=8.8 Hz), 8.46 (2H, d, J=8.8 Hz), 7.85 (2H, d, J=8.8 Hz), 7.66 (2H, d, J=8.8 Hz), 7.42 (2H, t, J=8.8 Hz). MS *m/z*: 411 (M^+), 261, 150. 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, 61.57; H, 2.71; N, 3.33.

9-(2,4-Dichlorobenzoyloxy) 1,8-Dichloroanthracene (**2t**): 73% yield, mp 213—214 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.31 (H, s), 8.37 (H, d, J=8.8 Hz), 7.96 (H, d, J=8.4 Hz), 7.68 (H, t, J=8.8 Hz), 7.53 (2H, d, J=8.4 Hz), 7.50 (2H, d, J=8.4 Hz), 7.45 (2H, d, J=8.4 Hz). MS *m/z*: 436 (M^+), 261, 227, 173. IR (KBr) cm^{-1}: 1741. *Anal.* Calcd for $C_{21}H_{10}O_2Cl_4$: C, 57.84; H, 2.31. Found: C, 57.97; H, 2.52.

9-(3-Phenylpropionyloxy) 1,8-Dichloroanthracene (**2u**): 71% yield, mp 153—154 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.20 (H, s), 7.61 (2H, d, J=8.4 Hz), 7.53 (2H, m), 7.39—7.30 (7H, m), 4.82—4.81 (2H, m), 3.28—3.26 (2H, m). MS *m/z*: 394 (M^+), 262, 227, 105. IR (KBr) cm^{-1}: 1769. *Anal.* Calcd for $C_{23}H_{16}O_2Cl_2$: C, 69.89; H, 4.06. Found: C, 70.01; H, 4.28.

Cell Culture and Determination of Cell Growth[12,16,17] Human oral epidermoid carcinoma cells (KB cell line), human cervical carcinoma cells of ME 180 (GBM8401) and Chinese hamster ovary (CHO) cells grown in a plateau phase were used in all experiments. Each cell line was further divided into control and experimental groups. Stock solutions of the test compounds were prepared in DMSO and diluted with DMEM to give a final concentration in DMSO of 0.2%. Controls were performed with DMSO or with medium alone. Forty-eight hours after the addition of the test compound to the culture, the medium was removed and each well was rinsed with 100 μl PBS. The cells were then incubated with sterile 0.5% trypsin and 0.2% EDTA in PBS for 20 min at 37 °C. The detached cells from each well were suspended in DMEM and dispersed into single cells by gentle pipetting through an Eppendorf pipette, and cell growth was determined directly by counting the cells in a Neubauer counting chamber using phase contrast microscopy. Inhibition was calculated by comparison of the mean values of the test compound (n=3) with the control (n=6—8) activity: (1−test compound/control) ×100.

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