Antitumor Agents: Diazomethyl Ketone and Chloromethyl Ketone Analogues Prepared from *N*-Tosyl Amino Acids

Z. Sajadi, M. Kashani,

Division of Medicinal Chemistry, School of Pharmacy, Ferdowsi University, Meshhad, Iran

L. J. Loeffler, and I. H. Hall*

Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
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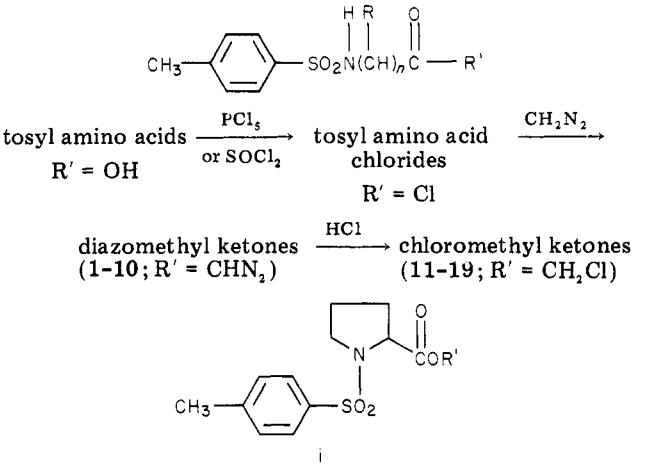
Diazomethyl ketone and chloromethyl ketone analogues prepared from *N*-tosyl amino acids have been synthesized and tested for antitumor activity in Ehrlich ascites carcinoma and P-388 lymphocytic leukemia screens in mice. The *N*-tosyl chloromethyl ketone analogues prepared from glycine, L-alanine, β-alanine, L-valine, and 6-(*N*-tosylamino)caproic acid were the most potent antineoplastic agents in the Ehrlich ascites carcinoma screen. The *N*-tosyl diazomethyl ketone analogues synthesized from glycine, L-leucine, and L-proline were the most active of this series in the Ehrlich ascites screen, along with 5-keto-1-tosyl-2-(diazoacetyl)pyrrolidine and the diazomethyl ketone analogues prepared from 6-(*N*-tosylamino)caproic acid. In the P-388 lymphocytic leukemia screen, the *N*-tosyl chloromethyl ketone prepared from glycine and the compound 5-keto-1-tosyl-2-(diazoacetyl)pyrrolidine were the most active.

The antitumor activity of a number of N-protected amino acids has previously been reported. Schnebli and Burger[1] have reported that tosylphenylalanyl chloromethyl ketone (TPCK) and tosyllysyl chloromethyl ketone (TLCK) inhibit the growth of Swiss SV-3T$_3$ transformed tissue-culture cells. Troll et al.[2] have reported that certain *N*-acyl amino acid derivatives inhibited carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene and promoted by phorbol esters in mouse skin. N-Protected vinyl and 1,2-dibromoethyl and cyanomethyl esters of phenylalanine[3] and other amino acids[4] have been reported as antitumor agents by our laboratory. The *N*-benzoyl derivatives of phenylalanine were also reported to have antimicrobial and antitumor activity.[5] The *N*-benzoyl-protected amino acid cyanomethyl esters were also reported to have antitumor and antiinflammatory activity.[6] Analogues of a number of amino acids having either the diazo ketone group or the chloromethyl ketone group have been shown to have antineoplastic activity.[7,8] Diazo ketone or diazo ester analogues of amino acids include well-known antibiotics, such as 6-diazo-5-oxo-L-norleucine and azaserine. These have been observed to have antineoplastic activity.[9,10] Very recently, antitumor activity of diazomethyl ketone analogues derived from L-alanine have been reported.[11] Contained in this report is a series of diazomethyl ketones and chloromethyl ketones prepared from *N*-tosyl-protected amino acids which were subsequently tested for antitumor activity.

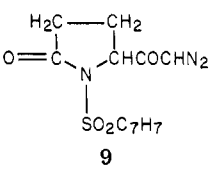
Scheme I. Synthesis of Diazomethyl and Chloromethyl Ketone Analogues Prepared from *N*-Tosyl Amino Acids[a]



[a] R = side chain of natural amino acid when $n = 1$ ($n = 2$ or 4 with R = H). Compounds prepared from L-proline do not conform to this structure but are represented by structure i in the above scheme.

Results and Discussion

Chemistry. The general synthetic procedure for chlorides, diazomethyl ketones (1–10), and chloromethyl ketones (11–20) prepared from *N*-tosyl amino acid is essentially that of Schoellmann and Shaw[12] and is shown in Scheme I. Individual new *N*-tosyl derivatives (1–19) were synthesized by literature techniques in good yield and are listed with their physical data in Tables I and II. In the course of this project, an attempt was made to prepare *N*-tosyl-L-glutamyl bis(diazomethyl) ketone (21) derived



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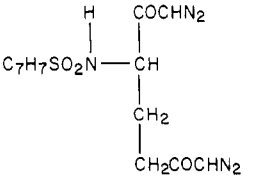

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from *N*-tosyl-L-glutamic acid from its precursor diacid chloride.[13] Addition of an ethereal solution of diazo-

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Table I. Diazomethyl Ketone Analogues of *N*-Tosyl Amino Acids

diazomethyl ketone analogues[d] prepared from	yield, %	mp, °C	solv of crystn	$[\alpha]^{20}_D$, deg[b,c]	formula	anal.[a]
N-Tos-Gly (1)	68	138-140	$CHCl_3$-hexane		$C_{10}H_{11}N_3O_3S$	N
N-Tos-L-Ala (2)	74	75-76	$CHCl_3$-hexane	-12.0	$C_{11}H_{13}N_3O_3S$	N
N-Tos-DL-Ala (3)	80	110-112	$CHCl_3$-hexane		$C_{11}H_{13}N_3O_3S$	N
N-Tos-β-Ala (4)	70	113-115	$CHCl_3$-hexane		$C_{11}H_{13}N_3O_3S$	N
N-Tos-L-Val (5)	68	76-78	$CHCl_3$-hexane	-41.2	$C_{13}H_{17}N_3O_3S$	N
N-Tos-L-Leu (6)	77	80	$CHCl_3$-hexane	-78.0	$C_{14}H_{10}N_3O_3S$	N
N-Tos-DL-Ile (7)	70	64-65	$CHCl_3$-hexane		$C_{14}H_{19}N_3O_3S$	N
N-Tos-L-Pro (8)	51	102-103	$CHCl_3$-hexane	+82.5	$C_{13}H_{17}N_3O_3S$	N
5-keto-1-Tos-2-(ClAc)Pyr (9)	75	154-155	$CHCl_3$-hexane		$C_{19}H_{13}N_3O_4S$	N
6-(*N*-Tos)ACp[e] (10)	68	44-46	$CHCl_3$-hexane		$C_{14}H_{19}N_3O_3S$	N

[a] Analyzed within ±0.4% for N. [b] The optical rotation for the compounds were obtained on a Rudolph Research Co. Autopol III automatic polarimeter. [c] Cl, $CHCl_3$. [d] Abbreviations used: Tos, tosyl; Pyr, pyrrolidine. [e] 6-(*N*-Tos)ACp = 6-(*N*-tosylamino)caproic acid.

Table II. Chloromethyl Ketone Analogues of *N*-Tosyl Amino Acids

chloromethyl ketone analogues[d] prepared from	yield, %	mp, °C	solv of crystn	$[\alpha]^{20}_D$, deg[b,c]	formula	anal.[a]
N-Tos-Gly (11)	64	140-141	$CHCl_3$-ligroin		$C_{10}H_{12}ClNO_3S$	C, H, Cl, N, S
N-Tos-L-Ala (12)	58		$CHCl_3$-ligroin	+18.0	$C_{11}H_{14}ClNO_3S$	C, H, Cl, N, S
N-Tos-DL-Ala (13)	65	91-93	$CHCl_3$-ligroin		$C_{11}H_{14}ClNO_3S$	C, H, Cl, N, S
N-Tos-β-Ala (14)	44	81-83	ether-hexane		$C_{11}H_{14}ClNO_3S$	C, H, Cl, N, S
N-Tos-L-Val (15)	66	115	$CHCl_3$-ligroin	+23.5	$C_{13}H_{18}ClNO_3S$	C, H, Cl, N, S
N-Tos-L-Leu (16)	78	65-66	$CHCl_3$-ligroin	-96.8	$C_{14}H_{20}ClNO_3S$	C, H, Cl, N, S
N-Tos-DL-Ile (17)	20	73-74	$CHCl_3$-ligroin		$C_{14}H_{20}ClNO_3S$	C, H, Cl, N, S
N-Tos-L-Pro (18)	80	93-94	ether-hexane	-173.2	$C_{13}H_{16}ClNO_3S$	C, H, Cl, N, S
6-(*N*-Tos)ACp (19)	53	61-63	$CHCl_3$-ligroin		$C_{14}H_{20}ClNO_3S$	C, H, Cl, N, S

[a] Analyzed within ±0.4% for C, H, Cl, N and S. [b] The optical rotation for the compounds were obtained on a Rudolph Research Co. Autopol III automatic polarimeter. [c] Cl, $CHCl_3$. [d] Abbreviations used: Tos = tosyl. 6-(*N*-Tos)ACp = 6-(*N*-tosylamino)caproic acid.

methane to an ether–chloroform (2:1) solution of *N*-tosyl-L-glutamyl dichloride led to the formation of cyclic compound **9** [5-keto-1-tosyl-2-(diazoacetyl)pyrrolidine] rather than the desired compound **21**. Harington et al.[14] synthesized the chloroacetyl ketone **20** starting from *N*-tosyl-L-glutamic acid, utilizing the mixed anhydride procedure for making 5-keto-1-tosylpyrrolidine-2-carboxylic acid. Subsequent steps were the conversion of this compound to the acid chloride, then to the diazo ketone (**9**), and finally to the known chloroacetyl ketone (**20**) through the action of hydrogen chloride on the diazo ketone: mp 141 °C; $[\alpha]_{5461}$ -18.5° (*c* 5, dioxane).

In our work, cyclization was observed as a result of the addition of diazomethane to the *N*-tosyl-L-glutamyl dichloride. Since fresh acid chloride of *N*-tosyl-L-glutamic acid was prepared,[13] the cyclization had to occur after the addition of the diazomethane to the acid chloride. This was confirmed by ^1H NMR spectroscopy, whereby only a single peak was observed at δ 5.5. Further confirmation was obtained from microanalysis, which demonstrated that 5-keto-1-tosyl-2-(diazoacetyl)pyrrolidine (**9**) was formed instead of the expected *N*-tosyl-L-glutamyl bis(diazomethyl) ketone (**21**).

Biological Testing. Newly synthesized compounds were tested in two pharmacological screens, namely, the Ehrlich ascites carcinoma and P-388 lymphocytic leukemia. All of the *N*-tosyl diazomethyl ketones and chloromethyl ketones tested were shown to be active in the Ehrlich ascites carcinoma screen in mice at 20 (mg/kg)/day ip. In the diazomethyl ketone series, the *N*-tosylglycine analogue (**1**) demonstrated the best inhibitory activity at 99.5%, followed by 5-keto-1-tosyl-2-(diazoacetyl)pyrrolidine (**9**) at 94.4% and the leucine (**6**) and proline (**8**) ketone analogues at 92%. In the chloromethyl ketone series, the *N*-tosylglycine (**11**), the β-alanine (**14**), and the 6-(*N*-tosylamino)caproic acid (**19**) analogues demonstrated 99.9% inhibition against Ehrlich ascites cell proliferation. The L-alanine analogue (**12**) was also active at 98.6%. In the P-388 lymphocytic leukemia screen, of the five compounds tested only two were active. The *N*-tosylglycyl chloromethyl ketone analogue (**11**) was significantly active at 20 (mg/kg)/day with a T/C = 137, and 5-keto-1-tosyl-2-(chloroacetyl)pyrrolidine (**20**) was active with a T/C = 133. *N*-Tosyl-L-leucyl and DL-isoleucyl diazomethyl ketones (**6** and **7**) demonstrated marginal inhibitory activity (T/C ≥ 120 for significant activity).

Experimental Section

Chemical Methods. The general synthetic routes utilized for the preparation of chloromethyl ketone analogues derived from some *N*-tosyl aliphatic and aromatic amino acids are outlined in Scheme I. Details of various steps in these procedures are given below.

Melting points were determined on an Electro-thermal melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in chloroform with a Pey Unicam SP1100 grating spectrophotometer. Nuclear magnetic resonance spectra (NMR) were measured in deuteriochloroform with a Varian T60A spectrometer, and chemical shifts were reported in δ (ppm) units relative to internal tetramethylsilane. The following abbreviations were used in reporting the NMR data: s, singlet; d, doublet; t, triplet; m, multiplet; *J* value in hertz (Hz). Data were consistent with assigned structures for all intermediates and products. Silica gel for thin-layer chromatography (TLC) refers to Merck silica gel G. Compounds were visualized by charring with sulfuric acid (50%). Silica gel used for column chromatography refers to silica AR CC-7, 200–325 mesh. Chloroform or chloroform–ethyl acetate (4:1, 3:1, 2:1) was used as the eluting solvent. Elementary analyses were performed by Dornis U. Kolbe, West Germany. All chemicals were purchased from Merck Chemical Co.

***N*-Tosyl Amino Acids.** These compounds were prepared according to the procedures described by McChesney et al.,[15]

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Table III. Anticarcinoma Activity of Diazomethyl Ketone and Chloromethyl Ketone Analogues Prepared from *N*-Tosyl Amino Acids (Ehrlich Ascites Screen)

no.	compd,[b] 20 (mg/kg)/day	*N*	survival after 10 days	vol of ascrit fluid	ascrit	% inhibn
1	*N*-Tos-Gly N_2Me ketone	6	6/6	0.03 ± 0.01	56.0 ± 3.26	99.5[a]
2	*N*-Tos-L-Ala N_2Me ketone	6	5/6	1.02 ± 0.19	43.5 ± 2.97	87.1[a]
4	*N*-Tos-β-Ala N_2Me ketone	6	6/6	1.66 ± 0.12	44.0 ± 2.74	78.8[a]
5	*N*-Tos-L-Val N_2Me ketone	6	6/6	0.93 ± 0.14	52.7 ± 3.09	85.5[a]
6	*N*-Tos-L-Leu N_2Me ketone	6	6/6	0.63 ± 0.18	42.5 ± 3.16	92.2[a]
7	*N*-Tos-DL-Ile N_2Me ketone	6	5/6	1.92 ± 0.25	45.4 ± 3.63	74.7
8	*N*-Tos-L-Pro N_2Me ketone	6	6/6	0.63 ± 0.09	39.8 ± 3.18	92.7[a]
9	5-keto-1-Tos-2-(diazoacetyl)Pyr	6	6/6	0.60 ± 0.12	32.0 ± 2.76	94.4[a]
10	6-(*N*-Tos)ACp N_2Me ketone	6	5/6	0.50 ± 0.11	42.5 ± 3.12	93.8[a]
11	*N*-Tos-Gly ClMe ketone	6	5/6	0.04 ± 0.01	0.01 ± 0.01	99.9[a]
12	*N*-Tos-L-Ala ClMe ketone	6	5/6	0.25 ± 0.09	18.9 ± 1.24	98.6[a]
13	*N*-Tos-DL-Ala ClMe ketone	8	8/8	1.60 ± 0.26	46.5 ± 3.56	78.4
14	*N*-Tos-β-Ala ClMe ketone	6	5/6	0.02 ± 0.01	0.10 ± 0.03	99.9[a]
15	*N*-Tos-L-Val ClMe ketone	6	6/6	0.63 ± 0.11	46.9 ± 4.16	91.4[a]
16	*N*-Tos-L-Leu ClMe ketone	8	8/8	2.65 ± 1.63	25.5 ± 2.37	80.4[a]
17	*N*-Tos-DL-Ile ClMe ketone	8	5/8	1.34 ± 0.25	34.5 ± 1.97	86.6[a]
18	*N*-Tos-L-Pro ClMe ketone	8	5/8	2.70 ± 0.57	30.0 ± 3.33	76.5
19	6-(*N*-Tos)ACp ClMe ketone	6	5/6	0.02 ± 0.01	0.01 ± 0.01	99.9[a]
20	5-keto-1-Tos-2-(ClAc)Pyr	8	7/8	0.95 ± 0.18	31.0 ± 1.64	91.4[a]
	positive standards					
23	TPCK	6	5/6	0.05 ± 0.02	0.01 ± 0.01	99.9[a]
24	melphalan	6	6/6	3.00 ± 0.73	0.10 ± 0.02	99.9[a]
25	6-mercaptopurine	6	6/6	0.30 ± 0.08	0.70 ± 0.09	99.9[a]
	negative standards					
	N-Tos-L-Phe vinyl ester	6	5/6	7.74 ± 0.68	19.2 ± 1.32	56.8
	N-Tos-L-Tyr vinyl ester	6	6/6	4.92 ± 0.52	17.5 ± 0.94	75.0
	N-Tos-L-Tyr CNMe ester	6	5/6	9.36 ± 0.76	15.8 ± 0.97	56.0
	N-Cbz-L-Tyr CNMe ester	6	6/6	10.82 ± 0.92	19.7 ± 1.58	38.0
	control (1% CMC)	12	12/12	10.62 ± 0.98	32.4 ± 1.69	0

[a] $p = 0.001$. Abbreviations used: Tos, tosyl; N_2Me, diazomethyl; ClMe, chloromethyl; CNMe, cyanomethyl; ACp, aminocaproic acid; Pyr, pyrrolidine; Cbz, carbobenzyloxy.

Holley et al.,[16] and Harris et al.[17]

***N*-Tosyl Amino Acid Chlorides.** All acid chlorides were prepared from the corresponding acids through the action of either thionyl chloride or phosphorus pentachloride in an appropriate solvent according to procedures described by Schallenberg et al.,[18] Beecham,[19] Schoellman et al.,[12] and Stedman.[13]

***N*-Tosyl Diazomethyl Ketone Analogues of Amino Acids.** The general procedure for the preparation of these compounds is as follows. To a solution of tosyl amino acid chloride (10 mmol) in a mixture of dry ether–chloroform (2:1; 30 mL) was added slowly, with stirring, an ice-cold ethereal solution of diazomethane (1.5 g, 35.5 mmol, from 11.0 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide). The reaction mixture was left at room temperature overnight, and excess diazomethane and solvent were removed by passing nitrogen through the reaction mixture. After the yellowish oily residue was dried in vacuo, crystalline material separated. This was filtered and recrystallized from the appropriate solvent (Table I) to give the analytical sample: IR ~2119 (N≡N), ~1720, ~1702, ~1691 (C=O) cm^{-1}; NMR ($CDCl_3$) δ ~5.5 (s, CH).

***N*-Tosyl Chloromethyl Ketone Analogues of Amino Acids.** The general procedure for the preparation of these compounds (Table II) is as follows. A stream of dry hydrogen chloride was passed through an ice-cold solution of the corresponding amino acid diazomethyl ketone for about 15 min. The reaction mixture was allowed to warm to room temperature and then stirred overnight. After evaporation in vacuo, the crystalline residue was taken up in petroleum ether and the mixture filtered. The crystals were washed with petroleum ether and dried. Recrystallization from appropriate solvent gave analytical samples (Table II): IR ~1771, ~1741, ~1705 (keto) cm^{-1}; NMR ($CDCl_3$) δ ~4.2 (s, CH_2).

Table IV. Antileukemic Activity of Diazomethyl Ketones and Chloromethyl Ketone Analogues Prepared from Various *N*-Tosyl Amino Acids (P-388 Lymphocytic Leukemia Screen)

no.	compd	*N*	av days survived	T/C
2	*N*-Tos-L-Ala N_2Me ketone	6	11.6 ± 0.7	120
4	*N*-Tos-β-Ala N_2Me ketone	6	11.5 ± 0.5	119
6	*N*-Tos-L-Leu N_2Me ketone	6	12.1 ± 0.6	125[a]
7	*N*-Tos-DL-Leu N_2Me ketone	6	12.1 ± 0.9	126[a]
10	6-(*N*-Tos)ACp	6	11.8 ± 0.8	122
11	*N*-Tos-Gly ClMe ketone	6	13.2 ± 1.1	137[a]
12	*N*-Tos-L-Ala ClMe ketone	6	11.0 ± 1.0	114
14	*N*-Tos-β-Ala ClMe ketone	6	9.8 ± 0.9	101
17	*N*-Tos-L-Leu ClMe ketone	6	10.5 ± 0.8	109
16	*N*-Tos-DL-Ile ClMe ketone	6	10.3 ± 0.7	107
18	*N*-Tos-L-Pro ClMe ketone	6	10.2 ± 2.1	106
19	6-(*N*-Tos)ACp ClMe ketone	6	11.2 ± 1.1	116
20	5-keto-1-Tos-2-(ClAc)Pyr	6	12.8 ± 0.9	133[a]
22	5-fluorouracil	6	18.0 ± 1.3	186[a]
	1% CMC	6	9.66 ± 0.6	100

[a] T/C ≥ 120 for significant activity. For abbreviations used, see Table III.

Pharmacological Studies. Ehrlich Ascites Screen (Table III). The compounds were tested for antitumor activity using the Ehrlich ascites carcinoma in CF_1 male mice using a procedure described by Piantadosi et al.,[20] with certain modifications. Eight days after tumor transplantation, donor mice were sacrificed and ascites fluid was collected and diluted with isotonic saline. An

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aliquot was placed in a hemocytometer chamber, and the number of cells/cm^3 was calculated. Then, 10^6 cells were injected ip into each test animal using an 18-gauge needle. 6-Mercaptopurine and melphalan were used as internal standards in the test. Test drugs were homogenized in 1% carboxymethylcellulose and administered ip at 20 (mg/kg)/day for 9 days. After 10 days, the inoculated mice were sacrificed, and the ascitic fluid was collected. The volume (mL) of the ascitic fluid was measured, and the total packed ascites cell volume for each group was measured utilizing nonheparinized capillary tubes centrifuged at 3000 rpm for 3–5 min. The control (Tween 80) (C) value for the volume of tumor was 10.62 ± 0.48 (SD) mL and for the ascrit (total packed cell volume) was 32.4 ± 1.69 mL at day 10. Percent inhibition of tumor growth was calculated by the following formula for the treated animals (T):

$$\% \text{ inhibn} = 100 - \left(\frac{\text{vol}_T \times \text{ascrit}_T}{\text{vol}_C \times \text{ascrit}_C}\right)100$$

Any compound that exhibited 80% inhibition of tumor growth was considered significantly active.

Lymphocytic Leukemia P-388 Screen (Table IV). The lymphocytic leukemia P-388 test was carried out in DBA/2 male mice (20 g). In this screen, 10^6 cells were implanted on day 0. The test compounds were administered ip at 20 (mg/kg)/day for 2 weeks. T/C values were calculated according to the NIH protocol.[21] 5-Fluorouracil was used as the internal standard in this test.

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Synthesis and Mutagenicity of A-Ring Reduced Analogues of 7,12-Dimethylbenz[*a*]anthracene[1,2]

Muthiah N. Inbasekaran, Donald T. Witiak,*

Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

Karen Barone, and John C. Loper

Department of Microbiology, College of Medicine, Univeristy of Cincinnati, Cincinnati, Ohio 45267. *Received August 3, 1979*

The synthesis and mutagenicity of two derivatives of 7,12-dimethylbenz[*a*]anthracene (DMBA; 1), i.e., 1,2-H_2DMBA (4) and 1,2,3,4-H_4DMBA (5), are reported. These analogues (4 and 5) represent dihydro and tetrahydro A-ring reduced forms of DMBA, a region in the parent hydrocarbon (1) proposed to be involved in metabolism to the ultimate carcinogen. The synthesis for 4 without isolation of intermediates from the tosylhydrazone of 1,2,3,4-tetrahydrobenz[*a*]anthracene-4,7,12-trione (10) by successive reaction with 8 molar equiv of CH_3Li, HI, and $NaBH_4$ represents a novel approach to this hydrocarbon now available in sufficient quantity for biological studies. Interestingly, both of these reduced analogues 4 and 5 exhibited mutagenic activity in the Ames assay in the presence or absence of microsomal activation for strains TA98 and TA100. In these strains, DMBA was active only in the presence of S-9 fraction. In the plasmid-deficient strain TA1537, only tetrahydro analogue 5 exhibited mutagenic activity both in the absence and presence of S-9 fraction.

7,12-Dimethylbenz[*a*]anthracene (DMBA; 1) is one of the most potent known aromatic carcinogenic hydrocarbons.[3] Recent findings suggest that metabolism in the bay region (A ring in DMBA) plays a critical role in the mutagenicity and carcinogenicity of certain of these polycyclic hydrocarbons.[3-8] We have shown that substitution of F for H in the 2 and 5 positions, but not the 11 position, of DMBA reduces DNA binding in mammalian cultured cells relative to DMBA[9,10] and that such binding is in agreement with their relative oncogenic potential.[11] The recently synthesized dihydrodiol 2, which may serve as a metabolic precursor to epoxides such as 3, has been shown by Slaga et al.[12] to be the most carcinogenic hydrocarbon metabolite thus far tested.

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