Moffatt and Khorana[5] for the similar preparation of 5′-ADP and 5′-ATP from 5′-AMP. After separation of the nucleotides by chromatography on a 2.5 × 20 cm column of Dowex 1X8 (chloride) with elution by 2 l. of 0.003 *N* HCl to 2 l. of the same plus 0.5 *N* LiCl, extraneous lithium pyrophosphate was removed from the 8-bromo-5′-ATP and especially the 8-bromo-5′-ADP by dissolving the crude materials in 25 ml of cold H_2O, adjusting the pH to 2.5 with 0.1 *N* HCl, and adsorbing the nucleotides on 1 g of HCl-washed Norit. The pure nucleotides were eluted from the charcoal by twice stirring with 25-ml portions of 50% EtOH–H_2O, adjusting the pH to 7.5 with 0.1 *N* LiOH, and filtering off the charcoal. The filtrates were then evaporated to dryness for 65 mg of 8-bromo-5′-ADP and 46 mg of 8-bromo-5′-ATP, both as the tri- and tetralithium polyhydrates, respectively.

The relative positions of elution from Dowex 1X8 (chloride) and, as given in Table I, R_f values upon ascending paper chromatography identify the 8-bromonucleotides.

TABLE I

CHROMATOGRAPHIC CHARACTERIZATIONS (PAPER) AND YIELDS OBTAINED (COLUMN) OF 8-BROMO NUCLEOTIDES

Compound	R_f value (isobutyric acid–NH_4OH–H_2O, 66:1:33)	Yield, %
8-Bromo-5′-AMP	0.51	27
5′-AMP	0.48	
8-Bromo-5′-ADP	0.31	44
5′-ADP	0.29	
8-Bromo-5′-ATP	0.24	28
5′-ATP	0.22	

With adenylate kinase, 8-bromo-5′-ADP was found not to be phosphorylated by ATP, and 8-bromo-5′-ADP could not form the corresponding bromo derivatives of 5′-AMP and 5′-ATP in the reverse reaction.

Flavin-8-Bromoadenine Dinucleotide.—Condensation of 0.39 g of 8-bromoadenosine 5′-phosphoromorpholidate (0.5 mmole) with 0.81 g of tri-*n*-octylammonium FMN (1 mmole) in 100 ml of anhydrous pyridine plus 5 ml of DMF was done by a modification of the method for FAD synthesis by Moffatt and Khorana.[6] After 1 week at room temperature, the pyridine was evaporated off, the residue was dissolved in 25 ml of H_2O, and the solution was extracted twice with 25 ml of Et_2O. The aqueous phase was carefully neutralized with 1 *N* NH_4OH and the solution was poured over a 2.5 × 40 cm column of DEAE-cellulose (Cl^-). The small amount of riboflavin was washed through with 2 l. of H_2O, and the elution of flavin phosphates with a linear gradient from 2 l. of 0.003 *N* HCl to the same plus 0.1 *M* LiCl was followed by absorbancy measurements at 260 and 450 mμ. The F8-bromoAD which exhibits a 260/450 ratio near 3.7, was eluted after 8-bromo-5′-AMP and FMN, the pH was adjusted to 6 with 0.1 *N* LiOH, and the solution was lyophilized. The residue was stirred in 20 ml of MeOH and the Li_2 salt of F8-bromoAD was precipitated with 200 ml of Me_2CO plus 20 ml of Et_2O. The yellow-orange precipitate was collected by centrifugation and washed twice more to remove all LiCl by resuspending in 3 ml of MeOH and reprecipitating with 30 ml of Me_2CO plus 3 ml of Et_2O. This material was dissolved in 10 ml of H_2O, filtered, and lyophilized for 130 mg; 28% yield based on initial phosphoromorpholidate. After drying at 50° *in vacuo*, a bromine analysis was performed. *Anal.* Calcd for $C_{27}H_{33}BrLi_2N_9O_{15}P_2 \cdot 2H_2O$: Br, 8.6. Found: Br, 8.1. Tlc on MN silica gel S-HR with *n*-BuOH–AcOH–H_2O (2:1:1) as ascending solvent gave R_f values of 0.23 and 0.18 for F8-bromoAD and FAD, respectively (fluorescent uv). The $\lambda_{max}^{neutral}$ values for the F8-bromoAD are the same as for FAD at 450 and 375 mμ, but the bromo compound evidences a slight red shift of 267 mμ from the 264-mμ maximum of FAD.

As shown in Figure 1, F8-bromoAD like FAD has maximal fluorescence between pH 2.5 and 3.0 and decreases toward more nearly neutral pH due to intramolecular complexing with quenching of the isoalloxazine fluorescence by the purine moiety.[7]

The coenzymatic reactivity of F8-bromoAD is being investigated with D-amino acid apooxidase, and an attempt will be made to form crystals of this analog suitable for X-ray studies of the three-dimensional structure.

(6) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 3756 (1958).

(7) D. B. McCormick in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 377.

Synthesis of Potential Antineoplastic Agents. Substituted Phenylcyclohexenes[1]

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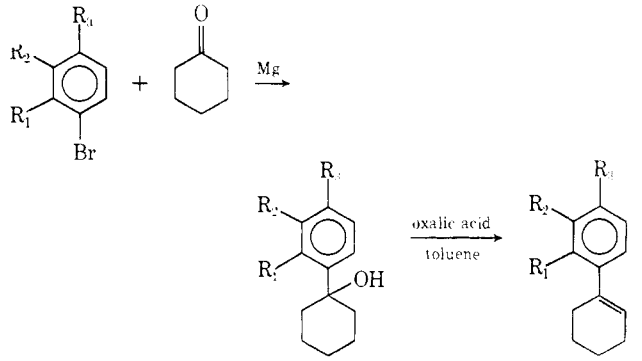
The finding of limited antitumor activity of related intermediates, 1-(2,3-dimethoxyphenyl)cyclohexene and 1-(2-methoxyphenyl)cyclohexene, prompted the attempt to synthesize similar compounds with more potent antitumor activity and relate this activity to chemical activity.

Compounds I–VII were synthesized according to Scheme I. The formation of all Grignard reagents was

SCHEME I

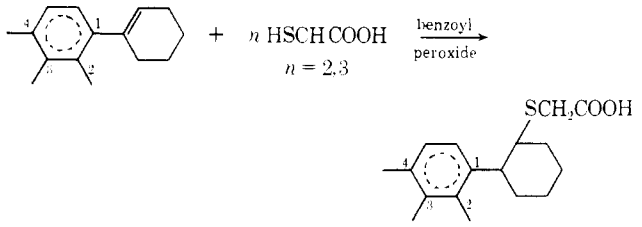


R_1, R_2, R_3 = H, CH_3, OCH_3

spontaneous and vigorous, except that of 1-bromo-2,3,4-trimethoxybenzene which required heating. Condensation of the Grignard reagents with cyclohexanone gave

TABLE I

RELATIVE REACTIVITIES OF SUBSTITUTED PHENYLCYCLOHEXENES TOWARD MERCAPTOACETIC ACID AT 30°



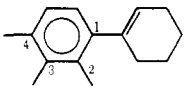
Compd	Ring substituent	Rel reactivity, $n = 2$	No. of runs	Rel reactivity, $n = 3$	No. of runs
I	4-OCH_3	1.33 ± 0.06	2	1.32 ± 0.02	2
III	2-OCH_3	1.26 ± 0.06	2	1.33 ± 0.06	2
II	3-OCH_3	1.00 ± 0.01	2	1.07 ± 0.01	2
IV	4-CH_3	1.12 ± 0.02	2	1.14 ± 0.02	2
V	3-CH_3	1.09 ± 0.05	2	1.03 ± 0.02	2
VIII	None	1.00	2	1.00	
VII	2,3,4-$(OCH_3)_3$	0.95 ± 0.02	2	...	
IX	2,3-$(OCH_3)_2$	0.71 ± 0.00	2	...	
VI	2-CH_3	0.52 ± 0.04	2	0.58 ± 0.06	2

(1) Supported in part by an Institutional General Research Cancer Grant (IN-761D), National Institutes of Health Training Grant 5 T1 GM1171-04, and National Institutes of Health Research Grant 1 R01 CA10270-01.

(2) A portion of this work was abstracted from the Ph.D. dissertation of S. Core.

TABLE II

ANTINEOPLASTIC ACTIVITY[a] OF



Compd[i]	KB cell culture,[b] ED_{50}, μg/ml	T/C (%)/dose (mg/kg) SA[c]	LE[d]	LL[e]	WM[f]
I	0.69 × 10	90/500	94/400		
II			96/50, 93/100, 96/200, 98/400		78/400
III	2.4 × 10	34/500, 48/500,[g] 54/500	100/350	42/400, 31/400, 61/400[h]	
IV			96/50, 96/100, 90/300, 94/400		0/300
V			102/100, 90/200, 96/500		93/200
VI			87/50, 104/100, 96/200, 100/300, 97/400		82/300
VII	2.5 × 10	121/500	101/400	57/400	
VIII		65/500	90/450		
IX	1 × 10^2	81/500	101/400	39/400, 82/400	

[a] Data from CCNSC. [b] ED_{50} = dose that inhibits growth to 50% of control growth. [c] Sarcoma 180. [d] LE 1210 lymphoid leukemia. [e] Lewis lung carcinoma. [f] Walker 256 (intramuscular). [g] Passed stage 2 of sequential screen. [h] Passed stage 3 of sequential screen. [i] See Table I for ring substituents.

good yields of the tertiary alcohols. 1-(2,3-Dimethoxyphenyl)cyclohexanol was prepared by the metallation of dimethoxybenzene with *n*-butyllithium followed by condensation with cyclohexanone.

The tertiary alcohols were isolated by distillation and dehydrated to the olefins with oxalic acid in boiling toluene. Satisfactory purification of the olefins was accomplished by chromatography over neutral Al_2O_3 (Merck) with pentane–ether followed by fractional distillation.

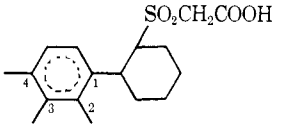
The rate of addition of mercaptoacetic acid to the olefins was studied to compare the reactivities of the different olefins to their antitumor activity. Mercaptoacetic acid was added under free-radical conditions using benzoyl peroxide as the initiator at 30°. Temperatures as high as 90° did not appear to increase the rate. However, when benzoyl peroxide was omitted from the reaction mixture the rate was significantly reduced. The addition of hydroquinone inhibited the reaction.

The reactions were carried out at a 2:1 or 3:1 mercaptoacetic acid:olefin ratio. At a regular time interval (0.5 or 1 hr) the extent of reaction was determined by measuring the unreacted mercaptoacetic acid by I_2 titration. The per cent of reacted compound was then compared to that of phenylcyclohexene and the results are shown in Table I.

The resulting acids were isolated and shown to be the expected *cis* isomers by nmr spectroscopy. Lotspeich and Karickhoff[3] showed *cis*- and *trans*-2-(2,3,4-trimethoxyphenyl)cyclohexanemercaptoacetic acids to be distinguishable by nmr. The *cis* acid showed two broad peaks centered at τ 6.84 and 6.60 for the two tertiary protons and a quartet centered at τ 7.44 representing CH_2 between the sulfur and carboxy group of the side chain. The *trans* acid has an unresolved peak centered at τ 7.85 and a broad peak centered at τ 7.00 for the two tertiary hydrogens and a doublet at τ 6.94 representing the CH_2 of the side chain. The nmr spectra of all the acids of the series in the paper contain the characteristic pattern of the *cis*-(2,3,4-trimethoxyphenyl)cyclohexanemercaptoacetic acid. The acids were converted to the solid sulfones for analysis.

(3) F. J. Lotspeich and S. Karickhoff, *J. Org. Chem.*, **31**, 2183 (1966).

TABLE III



Compd	Mp, °C	Formula[e]
I[a]	92–93	$C_{17}H_{24}O_5S$
II	119–120	$C_{15}H_{20}O_5S$
III	169–170	$C_{15}H_{20}O_5S$
IV	142–144	$C_{15}H_{20}O_4S$
V	125–127	$C_{15}H_{20}O_4S$
VI	140–142	$C_{15}H_{20}O_4S$
VII[b]		
VIII[c]	93–94	$C_{14}H_{18}O_2S$
IX[d]		

[a] Data for the ethyl ester of the sulfone acid. [b] Melting point of sulfide acid 78–79°, lit.[3] mp 77–79°. [c] Data for the sulfide acid. [d] Could not prepare solid derivative. [e] All compounds analyzed correctly for C and H except VII and IX which were not analyzed.

The anticancer screening results for the various substituted phenylcyclohexenes are given in Table II. Compound III passed stage 2 of sequential screen against Sarcoma 180 but failed stage 3. Compound III passed stage 3 of sequential screen against Lewis lung carcinoma. No further results have been received on this screen. Compound IX passed stage 1 against Lewis lung carcinoma but failed all others. No appreciable activity is found for the other compounds either against cell cultures or animal tumors.

Comparing the chemical activity in Table I and the anticancer activity in Table II no correlation between biological and chemical activity can be noted.

Experimental Section[4]

1-(4-Methoxyphenyl)-, 1-(3-methoxyphenyl)-, 1-(2-methoxyphenyl)-, 1-(4-tolyl)-, 1-(3-tolyl)-, 1-(2-tolyl)-, and 1-(2,3,4-trimethoxyphenyl)cyclohexenes were prepared by the treatment of cyclohexanone with the appropriate Grignard in THF according to the modified procedure of Lotspeich and Karickhoff[3] followed

(4) Melting points were taken using a Nalge-Axelrod melting point apparatus and are uncorrected. Nmr spectra were recorded on a Varian HA-60 using tetramethylsilane as an internal standard, and the solvent was $CDCl_3$.

by subsequent dehydration to the corresponding olefin with oxalic acid in boiling toluene.

1-Phenylcyclohexene.—Foote PhLi was condensed with cyclohexanone in Et_2O according to the procedure of Ginsburg and Pappo[5] to give 1-phenylcyclohexanol which was dehydrated with oxalic acid in boiling toluene to yield 1-phenylcyclohexene.

1-(2,3-Dimethoxyphenyl)cyclohexene.—1-(2,3-Dimethoxyphenyl)cyclohexanol was prepared according to Bergmann, *et al.*,[6] by the addition of veratrole to Foote *n*-BuLi followed by condensation with cyclohexanone. The resultant alcohol was dehydrated with oxalic acid.

Rate Measurements.—Olefin, freshly distilled mercaptoacetic acid, and a catalytic amount of Bz_2O were accurately weighed in a 10-ml reaction vessel fitted with a ground-glass stopper, mixed, and placed in a constant-temperature bath (30°). The rates of addition were followed titrimetrically. At appropriate times, aliquots were removed and dissolved in C_6H_6 (Fisher reagent grade), a few drops of pyridine were added, and the unreacted mercaptan was titrated with 0.0500 *N* I_2 in EtOH to a faint yellow color.

(5) D. Ginsburg and R. Pappo, *J. Am. Chem. Soc.*, **73**, 516 (1951).

(6) E. D. Bergmann, R. Paopo, and D. Ginsburg, *J. Chem. Soc.*, 1369 (1950).

Antimalarial Compounds.[1] X.[2] Biguanide and Amidinourea Derivatives of Diphenyl Sulfide, Sulfoxide, and Sulfone

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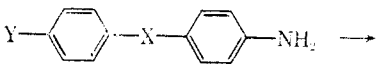
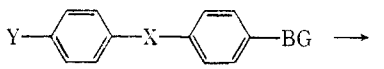
It is known that 4,4′-diaminodiphenyl sulfone (DDS) and some of its derivatives are useful in the treatment of some forms of malaria.[3] As the starting point of the presently described experiments, we obtained a number of biguanide and amidinourea derivatives of diphenyl sulfone, sulfoxide, and sulfide and their mononitro derivatives. The choice of the compounds was based upon our previous findings, that nitroguanil, the amidinourea derivative with a nitro group, was active against malaria.[4]

Chemistry.—The starting substances were commercially available DDS (I) and the intermediates, 4-amino-4′-nitrodiphenyl sulfone (II),[5,6] 4,4′-diaminodiphenyl sulfoxide (III),[5,7] and 4-amino-4′-nitrodiphenyl sulfide (IV).[5] The reactions of I–IV with cyanoguanidine leading to V–XII (Scheme I) are described in the Experimental Section.

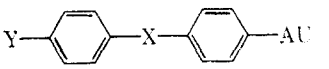
SCHEME I



I, Y = NH_2; X = SO_2
II, Y = NO_2; X = SO_2
III, Y = NH_2; X = SO
IV, Y = NO_2; X = S



V, Y = BG; X = SO_2
VI, Y = NO_2; X = SO_2
VII, Y = BG; X = SO
VIII, Y = NO_2; X = S



IX, Y = AU; X = SO_2
X, Y = NO_2; X = SO_2
XI, Y = AU; X = SO
XII, Y = NO_2; X = S

BG = $NHC(=NH)NHC(=NH)NH_2$
AU = $NHCONHC(=NH)NH_2$

The procedures reported in the literature for the syntheses of 4-nitro-4′-biguanyldiphenyl sulfone (VI)[8,9] and 4,4′-dibiguanyldiphenyl sulfone (V)[8,10] have been modified. In the search for a more convenient method of preparation of the nitrodiphenyl sulfone derivatives VI and X, the oxidation of the corresponding sulfides VIII and XII with peracetic acid was found to give satisfactory results.

Toxicity.[11]—Acute toxicity of V–XII on oral and intraperitoneal administration was tested (Table I).

TABLE I

	Toxicity, mg/kg (mice) LD_{50}			Antimalarial act. rel parasitemia mg/kg/day				
No.	*po*	ip	$LD_{0/4}$[b]	2.5	10	40	160	320
IX	2000	*a*	20				Inactive	
X	2000	800	50			75	32.5	13
VII	1760	77	50				Inactive	
XI	1500	195	112				Inactive	
VIII	1030	48	5				Inactive	
XII	900	640	112	76	101	92	0.224	0.056
V[8,9]	1400	110	20				Inactive	
VI[8,10]	1500	176	112	71	68	58	25	13

[a] Low solubility did not allow the preparation of solutions of an effective concentration. [b] The highest dose, administered on 4 consecutive days, that produced no deaths or weight loss.

Clonic convulsions were observed after administration of V, VII, and VIII. No toxic effects were shown by VI, IX–XI. Considerable differences between the LD_{50} in oral and interperitoneal administration tests of some of the compounds indicate their poor gastrointestinal absorption.

The highest dose which, administered on four consecutive days, produced no death and no decrease of body weight was also determined ($LD_{0/4}$). It ranged

(1) The financial support of this work from the World Health Organization is gratefully acknowledged.

(2) Part IX: T. Urbański, B. Serafin, and J. Żyłowski, *J. Med. Chem.*, **10**, 521 (1967).

(3) L. T. Coggeshall, J. Maier, and C. A. Best, *J. Amer. Med. Ass.*, **177**, 1077 (1941); D. L. Leiker, *Leprosy Rev.*, **27**, 66 (1956); H. M. Archibald and C. M. Ross, *J. Trop. Med. Hyg.*, **63**, 25 (1960).

(4) T. Urbański, B. Serafin, K. Jakimowska, J. Venulet, G. O. Schlütz, J. Sptawinski, T. Potaczek, P. Namirski, and D. F. Clyde, *Tetrahedron*, **20** (Suppl 1), 463 (1964).

(5) G. W. Kaiziss, L. W. Clemence, M. Severac, and J. C. Moetsch, *J. Am. Chem. Soc.*, **61**, 2763 (1939); *Chem. Abstr.*, **34**, 393 (1940).

(6) G. M. Mach and Z. Azarova, *Zh. Prikl. Khim.*, **19**, 580 (1946).

(7) W. Braun, German Patent 964,593 (1957); *Chem. Abstr.*, **53**, 12240 (1959).

(8) Q. Mingoia and P. C. Ferreira, *Anais Fac. Farm. Odontol. Univ. Sao Paulo*, **7**, 43 (1949); *Chem. Abstr.*, **45**, 1972 (1951).

(9) B. C. Jain, B. H. Iyer, and P. C. Guha, *J. Indian Chem. Soc.*, **24**, 225 (1947); *Chem. Abstr.*, **43**, 2593 (1949).

(10) A. Funke and P. Kouman, *Bull. Soc. Sim.*, 1062 (1947).

(11) Tests were carried out at the Institute of Drugs, Warsaw, Poland.