# Antitumor Agents II: Nitrogen Analogs of Mycophenolic Acid

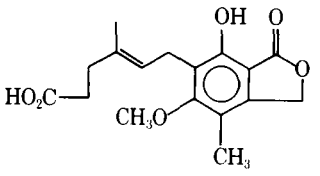
**J. A. BEISLER ˣ and SHARON S. HILLERY**

**Abstract** □ Mycophenolic acid, a novel antibiotic of low toxicity containing no nitrogen atoms in its structure, induces tumor regression in several murine solid tumor assays. It has been reported in extensive structure–activity studies that chemical modifications on the antibiotic itself reduce or eliminate antitumor activity. With the objective of antitumor activity enhancement, nitrogen-containing analogs of mycophenolic acid were synthesized according to a program directed toward the ultimate synthesis of close bioisosteres of the antibiotic. Initial efforts reported here describe the terpenoid side-chain degradation of *N*-geranyl-2(1*H*)-pyridones and *N*-geranylglutarimides, where the terminal isopropylidene is replaced with a carboxyl group as it occurs in mycophenolic acid. The resulting nitrogen-containing analogs of the antitumor antibiotic were inactive in the L-1210 and Walker 256 tumor systems.

**Keyphrases** □ Mycophenolic acid—synthesis and antitumor evaluation of nitrogen analogs □ Antitumor agents—synthesis and antitumor evaluation of nitrogen analogs of mycophenolic acid

Mycophenolic acid (I) was first isolated more than 75 years ago (1) from *Penicillium* culture filtrates and was subsequently shown to possess weak antifungal and antibacterial properties (2–4). Renewed interest in the metabolite was created by communications from three laboratories (5–7) describing its antiviral and antitumor properties.

Virus-induced Rous sarcoma and Friend leukemia were found to be inhibited by I. Although I does not inhibit the growth of L-1210, P-388, and other murine leukemias, it does cause tumor regression in the Walker 256, Sarcoma 180, Lewis lung, and Mecca



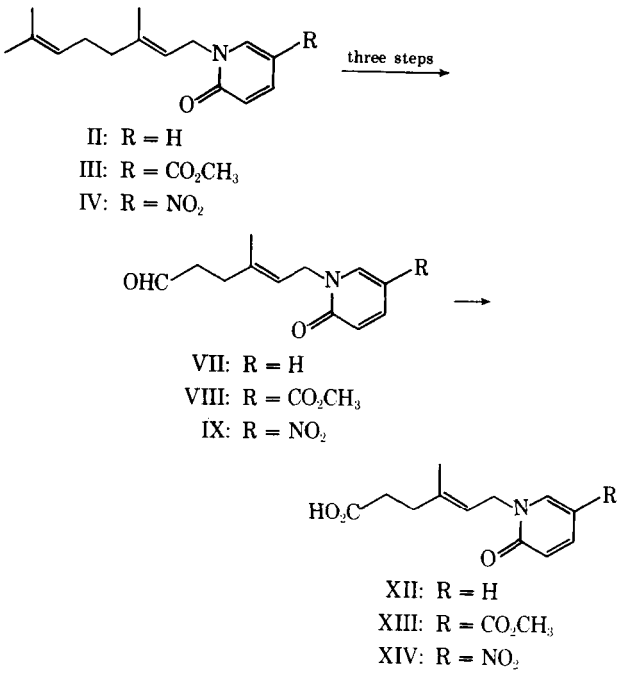
I

lymphosarcoma solid tumor systems. The solid metastasizing tumors can be considered good experimental models of the clinical situation wherein chemotherapy finds its greatest challenge.

Extensive structure–activity relationship studies (8, 9) revealed that even slight structural modifications of I reduced or eliminated antitumor activity. None of the synthetic analogs of I reported to date has shown an activity greater than the parent.

Because of its interesting spectrum of antitumor activity, its unique structure with respect to other antitumor agents, and its low toxicity in rodents (9) and as observed in clinical trials (10, 11), a synthetic study was initiated to develop novel analogs of I as part of a continuing interest in antitumor agents[1].

[1] Part I of series: J. A. Beisler, *J. Med. Chem.*, 14, 1116(1971).

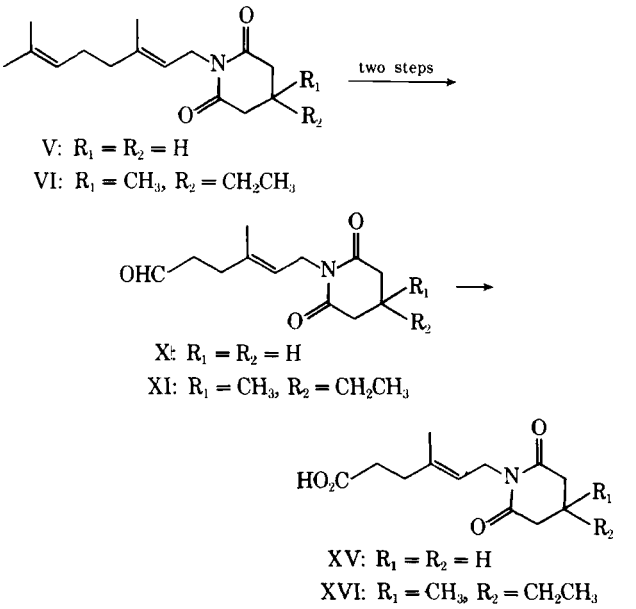



*Scheme I*

Accordingly, this report describes current efforts to prepare nitrogen analogs of mycophenolic acid.

## RESULTS AND DISCUSSION

Tracer experiments have shown that the isoprenoid side chain of mycophenolic acid arises biosynthetically by a selective cleavage of the central double bond in a farnesyl phthalide precursor (12). The




*Scheme II*

**Table I**—Experimental Data

| Compound | Boiling Point (mm Hg) Melting Point (Solvent) | Procedure | Yield, % | Formula | Analysis, % Calc. | Found |
|---|---|---|---|---|---|---|
| II | 126–130°(0.2) | A | 73 | $C_{15}H_{21}NO$ | C 77.88<br>H 9.15<br>N 6.05 | 78.15<br>9.40<br>5.89 |
| III | 169–171°(0.15) | A | 70 | $C_{17}H_{23}NO_3$ | C 70.56<br>H 8.01<br>N 4.84 | 70.36<br>8.03<br>5.01 |
| IV | 38–40°<br>(hexane) | A | 76 | $C_{15}H_{20}N_2O_3$ | C 65.19<br>H 7.30<br>N 10.14 | 64.97<br>7.50<br>9.93 |
| V | 144–148°(0.3) | B | 68 | $C_{15}H_{23}NO_2$ | C 72.25<br>H 9.30<br>N 5.62 | 72.29<br>9.44<br>5.41 |
| VI | 140–144°(0.15) | B | 79 | $C_{18}H_{29}NO_2$ | C 74.18<br>H 10.03<br>N 4.81 | 74.11<br>10.11<br>4.69 |
| VII[a] | 140–141°<br>(ethanol) | A | 43[b] | $C_{18}H_{19}N_5O_5$ | C 56.10<br>H 4.98<br>N 18.17 | 55.97<br>4.91<br>17.96 |
| VIII[c] | 135–136°<br>(water) | A | 62[b] | $C_{15}H_{20}N_4O_4$ | C 56.24<br>H 6.29<br>N 17.49 | 56.15<br>6.21<br>17.32 |
| IX[a] | 185–186°<br>(dimethylformamide–water) | A | 35[b] | $C_{18}H_{18}N_6O_7$ | C 50.23<br>H 4.22<br>N 19.53 | 50.39<br>4.28<br>19.57 |
| X[a] | 119–120°<br>(ethanol) | B | 56[d] | $C_{18}H_{21}N_5O_6$ | C 53.59<br>H 5.25<br>N 17.36 | 53.54<br>5.07<br>17.30 |
| XI[a] | 107–109°<br>(ethanol) | B | 93[d] | $C_{21}H_{27}N_5O_6$ | C 56.62<br>H 6.11<br>N 15.72 | 56.46<br>6.03<br>15.60 |
| XII | Oil | — | 60 | $C_{12}H_{15}NO_3$ | C 65.14<br>H 6.83<br>N 6.33 | 65.12<br>6.84<br>6.24 |
| XII[e] | 112–113°<br>(2-propanol–ether) | — | — | $C_{12}H_{14}NO_3Tl$ | C 33.94<br>H 3.32<br>N 3.30 | 34.08<br>3.38<br>3.28 |
| XIII | 90–91°<br>(ethyl acetate–hexane) | — | 52 | $C_{14}H_{17}NO_5$ | C 60.20<br>H 6.14<br>N 5.02 | 60.34<br>6.17<br>4.96 |
| XIV | 87–88°<br>(ether–cyclohexane) | — | 76 | $C_{12}H_{14}N_2O_3$ | C 54.13<br>H 5.30<br>N 10.52 | 54.27<br>5.35<br>10.48 |
| XV | 95–96°<br>(ether) | — | 63 | $C_{12}H_{17}NO_4$ | C 60.24<br>H 7.16<br>N 5.85 | 60.45<br>6.99<br>5.78 |
| XVI | Oil | — | 74 | $C_{15}H_{23}NO_4$ | C 64.03<br>H 8.24<br>N 4.98 | 63.90<br>8.36<br>5.07 |

[a] Characterized in melting point and analyzed as the 2,4-dinitrophenylhydrazone. [b] Percentage yield over three synthetic steps. [c] Characterized in melting point and analyzed as the semicarbazone. [d] Percentage yield over two synthetic steps. [e] The thallium salt of the carboxylic acid.

cleavage of the 15-carbon atom farnesyl side chain results in the loss of eight atoms while forming the carboxyl function characteristic of the antibiotic.

It was thought that a similar procedure could be adopted from the biosynthesis to develop analogs of I in the necessary multigram amounts required for antitumor testing. However, the $C_{10}$ geranyl group (*i.e.*, having one less isoprene unit than the farnesyl group) seemed more practical for laboratory synthesis and would also afford a double bond in the side chain in the desired position and with a *trans*-disposition as required by I.

It has been demonstrated that one may selectively epoxidize the terminal isopropylidene of the tetrahydropyranyl and acetyl derivatives of geraniol (13). Therefore, it was hoped that the geranyl group bonded to a heterocyclic moiety could also be selectively epoxidized, leading ultimately to the ejection of a three-carbon fragment and formation of a terminal carboxyl group. To that end, geranyl chloride (14) was condensed with 2(1*H*)-pyridone, 5-carbomethoxy-2(1*H*)-pyridone, and 5-nitro-2(1*H*)-pyridone in dimethylformamide solution in the presence of sodium hydride to give the *N*-alkylated products II, III, and IV, respectively.

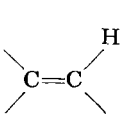
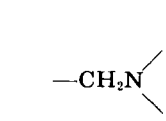
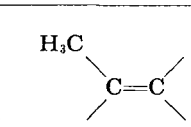
The geranyl pyridones were epoxidized in moderate yield with *m*-chloroperbenzoic acid at the terminal double bond in each instance to the exclusion of any other ether-soluble epoxide products[2]. Following conversion of the epoxides to glycol derivatives with dilute sulfuric acid, the respective aldehydes (VII, VIII, and IX) were formed *via* sodium metaperiodate oxidation (Scheme I).

Glutarimide and bemegride (4-ethyl-4-methylglutarimide) were converted into salts with lithium hydroxide in dimethylformamide solution and condensed with geranyl chloride to provide V and VI, respectively. Epoxidation of V and VI proceeded selectively as already described for the pyridone derivatives (Scheme II). However, through the agency of periodic acid in ether solution, it was possible to cleave the epoxides of V and VI directly into the respective aldehydes (X and XI) without a glycol intermediate.

The aldehydes derived from both the pyridones and the glutarimides were oxidized to their respective carboxylic acids (XII–XVI) with freshly precipitated silver oxide.

[2] In spite of a 50% excess of peracid, it was never quite possible to bring the reaction to completion. TLC always indicated a small amount of starting material.

**Table II**—Spectral Data

| Compound | NMR, ppm, δ | | | | | IR, $cm^{-1}$ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CHO or COOH |  |  |  | | | | | |
| II | | 5.26 (m,2H) | 4.64 (d,2H) | 1.82 (s,3H) | 1.62 (s,3H)<br>1.69 (s,3H) | | 1653 | 1582 | 1529 |
| III | | 5.17 (m,2H) | 4.59 (d,2H) | 1.81 (s,3H) | 1.61 (s,3H)<br>1.66 (s,3H) | 1720 | 1660 | 1610 | 1540 |
| IV | | 5.19 (m,2H) | 4.57 (d,2H) | 1.83 (s,3H) | 1.59 (s,3H)<br>1.67 (s,3H) | | 1670 | 1606 | 1555 |
| V | | 5.05 (m,2H) | 4.23 (d,2H) | 1.75 (s,3H) | 1.58 (s,3H)<br>1.66 (s,3H) | 1714 | 1668 | | |
| VI | | 5.18 (m,2H) | 4.43 (d,2H) | 1.80 (s,3H) | 1.60 (s,3H)<br>1.68 (s,3H) | | | | |
| VII | 9.80 (t,1H) | 5.40 (t,1H) | 4.60 (d,2H) | 1.83 (s,3H) | | 1712 | 1647 | 1572 | |
| VIII | 9.80 (t,1H) | 5.36 (t,1H) | 4.60 (d,2H) | 1.83 (s,3H) | | 1710 | 1650 | 1600 | 1553 |
| IX | 9.84 (t,1H) | 5.41 (t,1H) | 4.66 (d,2H) | 1.87 (s,3H) | | 1716 | 1669 | 1604 | |
| X | 9.66 (t,1H) | 5.21 (t,1H) | 4.42 (d,2H) | 1.82 (s,3H) | | 1711 | 1658 | | |
| XI | 9.75 (t,1H) | 5.18 (t,1H) | 4.47 (d,2H) | 1.81 (s,3H) | | | | | |
| XII | 9.49 (s,1H) | 5.38 (t,1H) | 4.62 (d,2H) | 1.81 (s,3H) | | 1700 | 1646 | 1573 | |
| XIII | 9.81 (s,1H) | 5.40 (t,1H) | 4.62 (d,2H) | 1.84 (s,3H) | | 1705 | 1660 | 1600 | |
| XIV | 9.72 (s,1H) | 5.43 (t,1H) | 4.66 (d,2H) | 1.86 (s,3H) | | | | | |
| XV | 10.03 (s,1H) | 5.22 (t,1H) | 4.41 (d,2H) | 1.82 (s,3H) | | 1715 | 1705 | 1665 | |
| XVI | 9.33 (s,1H) | 5.18 (t,1H) | 4.38 (d,2H) | 1.80 (s,3H) | | 1710 | 1660 | | |

Tables I and II provide analytical and spectral data in support of the assigned chemical structures.

## PHARMACOLOGY

The geranyl derivatives (II–VI) and the carboxylic acids (XII–XVI) were both tested in mice for antitumor activity in the L-1210 leukemia system; the former were suspended in saline with polysorbate 80[3], and the latter carboxylic acids were dissolved in 0.2 *N* NaOH and the pH was adjusted to 8 or 9 prior to administration. In addition, the carboxylic acids were tested in rats using the Walker 256 system.

**L-1210 Leukemia System**—In separate evaluations, according to the standard protocol (15) of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, the compounds were administered on Days 1, 5, and 9 and then daily beginning on Day 1 and continuing to Day 9.

Compounds II–VI and XII–XVI showed no significant activity (Table III), as indicated by the ratio of the mean survival time of the test animals over that of the control animals expressed as a percentage (% T/C). A % T/C value greater than 125 is considered indicative of activity in this test system.

**Walker 256 System**—Since it has been shown (5) that the Walker 256 assay in rats is sensitive to I[4], it was essential that Compounds XII–XVI, within which the exact mycophenolic acid side chain is incorporated, be evaluated in the Walker system. The test was conducted according to the standard Walker 256 protocol (16), where an inoculum of tumor homogenate containing $10^6$ viable cells was implanted intramuscularly in random-bred albino rats. Treatment was begun on Day 3 and continued daily through Day 6.

The ratio of the mean weight of excised tumors of the test animals over that of the control animals was the evaluation parameter expressed as % T/C; a value of 42% or less is considered necessary to demonstrate activity. By that criterion, the I analogs (XII–XVI) showed no activity at the dose levels included.

**Conclusion**—The inactivity of the I analogs (XII–XVI) is probably due to the incomplete functionalization of the heterocyclic portion of the molecules, especially the absence of the lactone group, which perhaps imparts I with an alkylating capacity. Future synthetic goals in this area will concentrate on nitrogen-containing structures which are more rigorously bioisosteric with I.

[3] Tween 80, Sigma Chemical Co.

[4] In this screening laboratory, as a control, mycophenolic acid at 100 mg/kg caused a 90% inhibition of the Walker tumor (*i.e.*, % T/C = 10).

## EXPERIMENTAL[5]

***N*-Alkylation with Geranyl Chloride**—*Procedure A, Pyridones II–IV*—To a suspension of 3.15 g (123 mmoles) of sodium hydride in 300 ml of dry dimethylformamide was added, in small portions, the requisite 2(1*H*)-pyridone (120 mmoles). After stirring the reaction solution, which was protected from moisture with an anhydrous calcium sulfate drying tube, for 1 hr at room temperature, a solution of 20.7 g (120 mmoles) of geranyl chloride (14) in 50 ml of dry dimethylformamide was added, and stirring was continued at room temperature for 24 hr.

The reaction solution was poured into 3 liters of water, and the product was collected by multiple ether extraction. After washing once with water, the extracts were dried and concentrated to an oil under water-pump vacuum. The products were purified by vacuum distillation or, in the instance of IV, by crystallization.

*Procedure B, Glutarimides V and VI*—The appropriate glutarimide (50 mmoles) and lithium hydroxide monohydrate (2.10 g, 50 mmoles) in 100 ml of dimethylformamide were stirred for 1 hr at room temperature while the reaction solution was protected from moisture with a drying tube. Geranyl chloride (8.63 g, 50 mmoles) in 30 ml of dimethylformamide was then added, and the reaction mixture was stirred for 24 hr. The product isolation was identical to that described in Procedure A.

**Side-Chain Degradation**—*Procedure A, Aldehydes VII–IX*—A cold solution of the *N*-geranyl-2(1*H*)-pyridone (35 mmoles) in 180 ml of anhydrous ether was poured into a cold solution of 85% *m*-chloroperbenzoic acid (10.75 g, 53 mmoles) in 180 ml of anhydrous ether. The resulting reaction solution was stored at about −12° for 24 hr. After shaking the ether solution twice with 1 *N* NaOH solution, it was dried and evaporated to give the epoxide in moderate yield as a clear oil. TLC (silica G, chloroform) analysis invariably indicated a small amount of unreacted starting material[2], which was carried through the next two synthetic steps.

The crude epoxide (13 mmoles) was stirred 30–60 min with 100 ml of 10% $H_2SO_4$ solution while cooling the reaction flask in an ice bath. After extracting once with a small portion of ether, which

[5] NMR spectra were determined with a Varian A-60 spectrometer, and chemical shifts are expressed as delta (δ) values downfield from tetramethylsilane as the internal standard. IR spectra were recorded with a Perkin-Elmer model 521 spectrophotometer. Combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Dimethylformamide was dried over a 3A molecular sieve prior to use. Extracts were dried with anhydrous sodium sulfate. Pure geraniol (98%) for the preparation of geranyl chloride was obtained from Bush Boake Allen, Inc., Norwood, N.J. Capillary melting points and boiling points are uncorrected.

**Table III**—Antitumor Evaluation

| Compound | L-1210, % T/C (Dose)[a] | Walker 256, % T/C (Dose)[a] | NSC Number |
|---|---|---|---|
| II | 93(50)[b] | | 160,456 |
| III | 101(400) | | 163,543 |
| IV | 97(400) | | 160,454 |
| V | 93(400) | | 160,455 |
| VI | 97(400) | | 169,795 |
| XII | 96(400) | 106(200) | 173,105 |
| XIII | 98(400) | 102(200) | 173,104 |
| XIV | 97(400) | 71(400) | 173,106 |
| XV | 93(400) | 106(400) | 173,108 |
| XVI | 93(400) | 104(400) | 173,107 |

[a] The specified dose (milligrams per kilogram) was the highest dose level given, producing the indicated % T/C in a dose–response assay. [b] Toxic at 100 mg/kg.

was discarded, the reaction solution was saturated with sodium chloride and the product was collected by exhaustive methylene chloride extraction. After drying the extracts, solvent removal *in vacuo* gave essentially a quantitative yield of the glycol. For solubility reasons, it was necessary to add 20 ml of 1,2-dimethoxyethane to the dilute sulfuric acid in reaction with the epoxide of IV; the ether extraction was then omitted.

In a typical run, the glycol (5 mmoles) was oxidatively cleaved in 50 ml of methanol–water (1:1) by a dropwise addition of sodium metaperiodate (2.14 g, 10 mmoles) in 50 ml of methanol–water (1:1). After the addition, the mixture was stirred in the dark at room temperature for 1.0–1.5 hr. The inorganic precipitate was removed from the reaction mixture by filtration, and the filtrate was concentrated to half under vacuum.

Following saturation of the concentrate with sodium chloride, the solution was thoroughly extracted with methylene chloride. The dried extracts were evaporated to afford the aldehydes in good yield, which were characterized as 2,4-dinitrophenylhydrazone derivatives except for II. Compound II was characterized as a semicarbazone.

*Procedure B, Aldehydes X and XI*—The epoxidation of the *N*-geranylglutarimides (V and VI) was carried out in the same way as in Procedure A. The aldehydes X and XI were formed in good yield directly by treating the corresponding epoxides with ethereal periodic acid. Thus, 10 mmoles of epoxide in 200 ml of ether was treated, while stirring, with 11 mmoles (2.51 g) of periodic acid in 200 ml of ether by dropwise addition at room temperature.

The reaction mixture was stirred for 30 min after the addition and then filtered. The filtrate was washed once with cold, dilute sodium bicarbonate solution and dried. Evaporation of the ether *in vacuo* gave the aldehydes as colorless oils, which were characterized *via* the 2,4-dinitrophenylhydrazones.

**Preparation of the Carboxylic Acids (XII–XVI)**—Silver oxide ($Ag_2O$) was freshly prepared by adding a solution of 5.25 g of sodium hydroxide in 75 ml of water to 10.5 g of silver nitrate in 75 ml of water. The oxide was collected by vacuum filtration, washed several times with water, and suspended in 100 ml of water. The suspension was added to 20 mmoles of aldehyde (VII–XI) slurried in water and stirred at room temperature for 2–6 hr. Aldehydes IX and XI were dissolved in dimethoxyethane and dioxane, respectively, prior to the addition of the silver oxide suspension.

At the end of the reaction time, the reaction mixture was made basic with saturated sodium bicarbonate solution, stirred an additional 30 min, and then vacuum filtered through a pad of diatomaceous silica[6]. After removing neutral materials from the filtrate by ether extraction, the filtrate was acidified with 10% nitric acid solution, saturated with potassium nitrate, and extracted exhaustively with methylene chloride. Evaporation of the extracts gave the carboxylic acids.

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[x] To whom inquiries should be directed.


[6] Celite, Johns-Manville Co.