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Taylor

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[54] **N-(PYRROLO(2,3-D)PYRIMIDIN-3-YLACYL)-GLUTAMIC ACID DERIVATIVES**

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[21] Appl. No.: **674,541**

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Related U.S. Application Data

[63] Continuation of Ser. No. 448,742, Dec. 11, 1989, abandoned, and Ser. No. 479,655, Feb. 8, 1990, abandoned.

[51] **Int. Cl.**[5] **C07D 487/04**; A61K 31/505

[52] **U.S. Cl.** **544/280**

[58] **Field of Search** 544/280; 514/258

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,889,859	12/1989	Taylor et al.	514/258
4,996,206	2/1991	Taylor et al.	514/258
4,997,838	3/1991	Akimoto et al.	514/258

FOREIGN PATENT DOCUMENTS

334636 9/1989 European Pat. Off. .

Primary Examiner—Emily Bernhard

Attorney, Agent, or Firm—Mathews, Woodbridge & Collins

[57] **ABSTRACT**

N-(Acyl)glutamic acid derivatives in which the acyl group is substituted with 4-hydroxypyrrolo[2,3-d]-pyrimidin-3-yl group are antineoplastic agents. A typical embodiment is N-[4-(2-{4-hydroxy-6-aminopyrrolo-[2,3-d]pyrimidin-3-yl}ethyl)benzoyl]-L-glutamic acid.

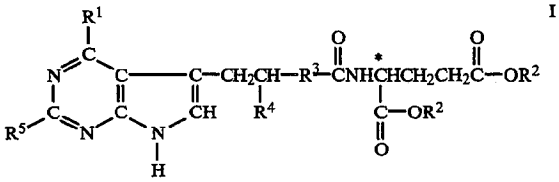
7 Claims, No Drawings

N-(PYRROLO(2,3-D)PYRIMIDIN-3-YLACYL)-GLUTAMIC ACID DERIVATIVES

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of Ser. No. 07/448,742 filed Dec. 11, 1989 and Ser. No. 07/479,655 filed Feb. 8, 1990 both now abandoned.

The present invention pertains to glutamic acid derivatives having the formula:



I

in which:

R^1 is —OH or —NH_2;

R^2 is hydrogen or a pharmaceutically acceptable cation:

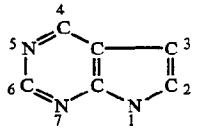
R^3 is 1,4-phenylene or 1,3-phenylene unsubstituted or substituted with chloro, fluoro, methyl, methoxy, or trifluoromethyl; thienediyl or furanediyl each unsubstituted or substituted with chloro, fluoro, methyl, methoxy, or trifluoromethyl; cyclohexanediyl; or alkanediyl;

R^4 is hydrogen, methyl, or hydroxymethyl;

R^5 is hydrogen, alkyl of 1 to 6 carbon atoms, or amino; and

the configuration about the carbon atom designated * is S.

The compounds of this invention are herein described as embodying the pyrrolo[2,3-d]pyrimidine heterocyclic ring system which ring system is numbered as follows:

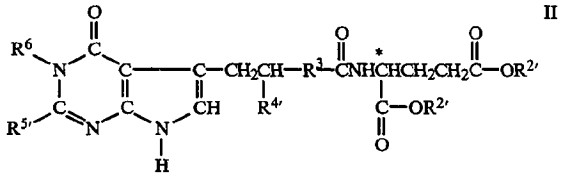


It will be appreciated that the pyrrolo[2,3-d]pyrimidines as depicted by Formula I are the tautomeric equivalent of the corresponding 5-H-6-oxo or 5-H-6-imino structures. Unless otherwise indicated, for simplicity's sake the compounds are depicted herein and named using the 6-hydroxy and 6-amino convention, it being understood the 5-H-6-oxo and 5-H-6-imino structures are fully equivalent.

The compounds of Formula I have an inhibitory effect on one or more enzymes which utilize folic acid, and in particular metabolic derivatives of folic acid, as a substrate. The compounds appear to be particularly active as inhibitors of thymidylate synthetase, which catalyses the methylation of deoxyuridylic acid to deoxythymidylic acid utilizing N^5,N^{10}-methylidenetetrahydrofolate as a coenzyme. The compounds thus can be used, alone or in combination, to inhibit the growth of those neoplasms which otherwise depend upon the inhibited enzyme.

The invention also pertains to the pharmaceutically acceptable salts of the compounds of Formula I, to processes for the preparation of these compounds and their salts, to chemical intermediates useful in preparation of these compounds, to a method of combatting neoplastic growth in a mammal, and to pharmaceutical compositions containing these compounds or their salts.

A first group of useful chemical intermediates, which can be converted directly to the desired final compounds of Formula I through removal of protecting groups, are compounds of the formula:



II

in which:

R^3 is as defined above;

$R^{2'}$ is hydrogen or a carboxy protecting group;

$R^{4'}$ is hydrogen, methyl, hydroxymethyl, or hydroxymethyl carrying a hydroxy protecting group;

$R^{5'}$ is hydrogen, alkyl, amino, or amino carrying a protecting group; and

R^6 is hydrogen or alkanoyoxy;

at least one of $R2'$,$R^{4'}$, and $R^{5'}$ being a carboxy protecting group, a hydroxy protecting group, or an amino protecting group, respectively.

The compounds of Formula I can be employed in the form of the free dicarboxylic acid, in which case both R^2 groups are hydrogen. Alternatively, the compounds often can be employed advantageously in the form of a pharmaceutically acceptable salt, in which case one or both R^2 groups are a pharmaceutically acceptable cation. Such salt forms, including hydrates thereof, are often crystalline and advantageous for forming solutions or formulating pharmaceutical compositions. Pharmaceutically acceptable salts with bases include those formed from the alkali metals, alkaline earth metals, non-toxic metals, ammonium, and mono-, di- and trisubstituted amines, such as for example the sodium, potassium, lithium, calcium, magnesium, aluminum, zinc, ammonium, trimethylammonium, triethanolammonium, pyridinium, and substituted pyridinium salts. The mono and disodium salts, particularly the disodium salt, are advantageous.

The group R^3 is a divalent group having at least two carbon atoms between the carbon atoms carrying the free valence bonds. R^3 for example can be a 1,4-phenylene or 1,3-phenylene ring which is unsubstituted or optionally substituted with chloro, fluoro, methyl, methoxy, or trifluoromethyl.

Alternatively, R^3 can be a thienediyl or furanediyl group, that is, a thiophene or furane ring from which two hydrogen atoms have been removed from two ring carbon atoms, as for example the thiene-2,5-diyl, thiene-3,5-diyl, thiene-2,4-diyl, and thiene-3,4-diyl ring systems and the the furane-2,5-diyl, furane-3,5-diyl, furane-2,4-diyl, and furane-3,4-diyl ring systems, which ring systems can be unsubstituted or substituted with chloro, fluoro, methyl, methoxy, or trifluoromethyl. It will be appreciated that whereas in the abstract the thiene-3,5-diyl system is the equivalent of the thiene-2,4-diyl system, the two terms are utilized herein to denote the two isomeric forms resulting from the orientation of the thiophene ring within the remainder of the molecule:

e.g. the structure in which the depicted carboxy group adjacent to R^3 is in the 2-position of the thiophene ring and that in which the depicted carboxy group adjacent to R^3 is in the 3-position of the thiophene ring. The same conventionas apply to the furane ring.

Alternatively, R^3 can be a cyclohexanediyl group, namely a divalent cycloalkane group of 6 carbon atoms such as cyclohexane-1,3-diyl and cyclohexane-1,4-diyl.

Alternatively, R^3 can be a alkanediyl, namely a straight or branched divalent aliphatic group of from 2 to 4 carbon atoms such as ethano, trimethylene, tetramethylene, propane-1,2-diyl, propane-2,3-diyl, butane-2,3-diyl, butane-1,3-diyl, and butane-2,4-diyl. It again will be appreciated that whereas in the abstract propane-1,2-diyl is the equivalent of propane-2,3-diyl, and butane-1,3-diyl the equivalent of butane-2,4-diyl, the two terms are utilized herein to denote the two isomeric forms resulting from the orientation of an unsymmetrical alkanediyl chain with respect to the remainder of the molecule.

The protecting groups designated by $R^{2'}$, $R^{4'}$ and $R^{5'}$ and utilized herein denote groups which generally are not found in the final therapeutic compounds but which are intentionally introduced at a stage of the synthesis in order to protect groups which otherwise might react in the course of chemical manipulations, thereafter being removed at a later stage of the synthesis. Since compounds bearing such protecting groups thus are of importance primarily as chemical intermediates (although some derivatives also exhibit biological activity), their precise structure is not critical. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works including, for example, "Protective Groups in Organic Chemistry", Plenum Press, London and New York, 1973; Greene, Th. W. "Protective Groups in Organic Synthesis", Wiley, New York, 1981; "The Peptides", Vol. I, Schröder and Lubke, Academic Press, London and New York, 1965; "Methoden der organischen Chemie", Houben-Weyl, 4th Edition, Vol.15/I, Georg Thieme Verlag, Stuttgart 1974, the disclosures of which are incorporated herein by reference.

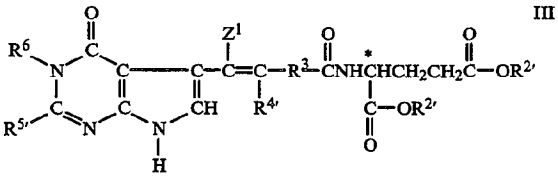
With respect to $R^{2'}$, a carboxy group can be protected as an ester group which is selectively removable under sufficiently mild conditions not to disrupt the desired structure of the molecule, especially a lower alkyl ester of 1 to 12 carbon atoms such as methyl or ethyl and particularly one which is branched at the 1-position such as t.-butyl; and such lower alkyl ester substituted in the 1- or 2-position with (i) lower alkoxy, such as for example, methoxymethyl, 1-methoxyethyl, and ethoxymethyl, (ii) lower alkylthio, such as for example methylthiomethyl and 1-ethylthioethyl; (iii) halogen, such as 2,2,2-trichloroethyl, 2-bromoethyl, and 2-iodoethoxycarbonyl; (iv) one or two phenyl groups each of which can be unsubstituted or mono-, di- or tri-substituted with, for example lower alkyl such as tert.-butyl, lower alkoxy such as methoxy, hydroxy, halo such as chloro, and nitro, such as for example, benzyl, 4-nitrobenzyl, diphenylmethyl, di-(4-methoxyphenyl)methyl; or (v) aroyl, such as phenacyl. A carboxy group also can be protected in the form of an organic silyl group such as trimethylsilylethyl or tri-lower alkylsilyl, as for example trimethylsilyloxycarbonyl.

With respect to $R^{4'}$, a hydroxy group can be protected through the formation of acetals and ketals, as for example through formation of the tetrahydropyr-2-yloxy (THP) derivative.

With respect to $R^{5'}$, an amino group can be protected as an amide utilizing an acyl group which is selectively removable under mild conditions, especially formyl, a lower alkanoyl group which is branched α to the carbonyl group, particularly tertiary alkanoyl such as pivaloyl, or a lower alkanoyl group which is substituted in the position α to the carbonyl group, as for example trifluoroacetyl.

Preferred compounds of Formula I are those wherein R^5 is amino or hydrogen. Within this class, R^1 preferably is hydroxy, R^3 is 1,4-phenylene, and R^4 is hydrogen or hydroxymethyl. Also preferred within this class are the compounds in which R^1 is hydroxy, R^3 is thienediyl, and R^4 is hydrogen or hydroxymethyl.

The compounds of this invention can be prepared according to a first process through catalytic hydrogenation of a compound of the formula:



III

in which:

Z^1 is hydrogen, or Z^1 taken together with $R^{4'}$ is a carbon-carbon bond;

$R^{2'}$ is hydrogen or a carboxy protecting group;

R^3 and R^6 are as defined above;

$R^{4'}$, when taken independently of Z^1, is hydrogen, methyl, hydroxymethyl, or hydroxymethyl substituted with a hydroxy protecting group; and

$R^{5'}$ is hydrogen, alkyl of 1 to 6 carbon atoms, amino, or an amino protecting group.

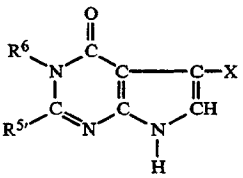
Suitable hydrogenation catalysts include noble metals and noble metal oxides such as palladium or platinum oxide, rhodium oxide, and the foregoing on a support such as carbon or calcium oxide.

When in Formula III, Z^1 taken together with $R^{4'}$ is a carbon-carbon bond, that is, when a triple bond is present between the two carbon atoms to which Z^1 and $R^{4'}$ are bound, $R^{4'}$ in the hydrogenation product will be hydrogen. Absent any chirality in R^3 (or any protecting group encompassed by $R^{2'}$, $R^{4'}$ and/or $R^{5'}$), the hydrogenation product will be a single enantiomer having the S-configuration about the carbon atom designated *. This also is true when Z^1 and $R^{4'}$ are each hydrogen, that is, when a double bond is present between the two carbon atoms to which Z^1 and $R^{4'}$ are bound.

When, on the other hand, $R^{4'}$ is other than hydrogen, a mixture of the R,S and S,S diastereomers is obtained. The diastereomeric mixture can be used therapeutically as such (after removal of the protecting groups) or can be separated mechanically as by chromatography. Alternatively, the individual diastereomers can be separated chemically by forming salts with a chiral acid, such as the individual enantiomers of 10-camphorsulfonic acid, campboric acid, alphabromocamphoric acid, methoxyacetic acid, tartaric acid, diacetyltartaric acid, malic acid, pyrrolidone-5-carboxylic acid, and the like, and then freeing one or both of the individual diastereomeric bases, optionally repeating the process, so as obtain either or both substantially free of the other; i.e., in a form having an optical purity of >95%.

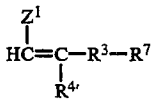
The protecting groups encompassed by $R^{2'}$, $R^{4'}$, $R^{5'}$, and/or R^6 can be removed following hydrogenation through acidic or basic hydrolysis, as for example with hydrogen chloride to cleave an $R^{4'}$ protecting group or with sodium hydroxide to cleave $R^{2'}$ or $R^{5'}$ protecting groups, thereby yielding the compounds of Formula I. Methods of removing the various protective groups are described in the standard references noted above and incorporated herein by reference.

Compounds of Formula III can be prepared utilizing procedures analogous to those described in U.S. Pat. No. 4,818,819, utilizing however the corresponding halogenated pyrrolo[2,3-d]pyrimidine. Thus a pyrrolo[2,3-d]pyrimidine of the formula:



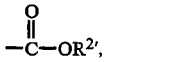
IV

in which X is bromo or iodo, $R^{5'}$, and R^6 are as herein defined, is allowed to react with an unsaturated compound of the formula:

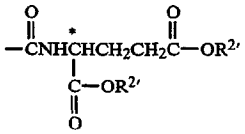


V

in which Z^1, R^3 and $R^{4'}$ are as herein defined, and R^7 is



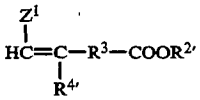
or



in which $R^{2'}$ is as herein defined, in the presence of a palladium/trisubstituted phosphine catalyst of the type described in U.S. Pat. No. 4,818,819, the disclosure of which is incorporated herein by reference.

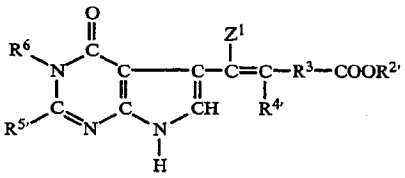
When R^7 is $—CONHCH(COOR^{2'})CH_2CH_2COOR^{2'}$, the product of this coupling reaction is hydrogenated, and any protecting group removed, as described above.

Alternatively, a compound of Formula IV is allowed to react with a compound of the formula:



VI

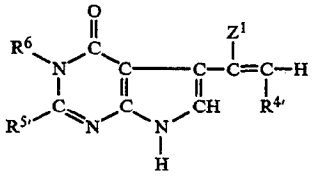
in which Z^1, $R^{2'}$, R^3, and $R^{4'}$ are as herein defined in the presence of a palladium/trisubstituted phosphine catalyst of the type described in U.S. Pat. No. 4,818,819 to yield an intermediate of the formula:



VII

The product of Formula VII then can be hydrogenated, hydrolysed to remove the $R^{2'}$ and R^6 protecting groups, and, optionally with intermediate protection of any amino group encompassed by $R^{5'}$, and coupled with a protected glutamic acid derivative in the manner described in U.S. Pat. No. 4,684,653 using conventional condensation techniques for forming peptide bonds such as DCC or diphenylchlorophosphonate, following which the protecting groups are removed.

In a further variant, compounds of Formula III can be prepared utilizing the procedures described in U.S. Pat. No. 4,818,819. Thus a compound of the formula:



VIII

in which Z^1, $R^{4'}$, $R^{5'}$, and R^6 are as herein defined, is allowed to react with a compound of the formula:

$$X—R^3—R^7 \qquad \text{IX}$$

in which X, R^3, and R^7 are as herein defined, in the presence of a palladium/trisubstituted phosphine catalyst of the type described in U.S. Pat. No. 4,818,819. This variant of the process is particularly suitable for, but is not limited to, preparation of those compounds in which R^4 is hydroxymethyl, in which case $R^{4'}$ in Formula VI is a protected hydroxymethyl group, as for example tetrahydropyran-2-yloxymethyl.

Compounds of Formula VIII also can be obtained by the methods of U.S. Pat. No. 4,818,819 by treating a compound of Formula IV with an unsaturated compound of the formula:

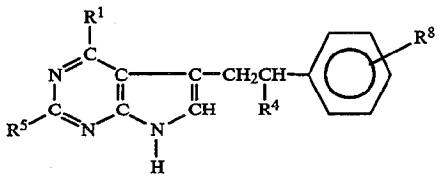
$$H—C\equiv C—R^{4''} \qquad \text{X}$$

in which $R^{4''}$ is methyl, a protected hydroxymethyl, or a trisubstituted silyl group in the presence of a palladium/trisubstituted phosphine catalyst of the type discussed above. This procedure is particularly suitable for, but is not limited to, preparation of those compounds in which R^4 is hydroxymethyl.

Although not always the case, the compounds of Formula IV in which R^6 is hydrogen can tend to be somewhat insoluble in solvents suitable for the reaction described in U.S. Pat. No. 4,818,819. In such instances, the compounds of Formula IV in which R^6 is hydrogen can be first treated with with sodium hydride and a suitable alkyl alkanoate (such as chloromethyl pivalate) to introduce an alkanoyloxy group in the 5-position and increase solubility.

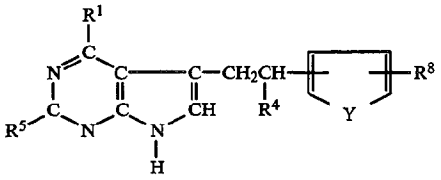
A useful subclass of compounds useful both as intermediates and for their effect on enzymes are derivatives

of Formula XI and XII lacking the glutamic acid side-chain:



XI

and



XII

in which:

R^1 is —OH or —NH_2;

R^4 is hydrogen, methyl, or hydroxymethyl;

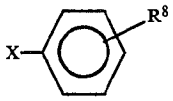
R^5 is hydrogen, alkyl of 1 to 6 carbon atoms, or amino;

R^8 is hydrogen, chloro, fluoro, methyl, methoxy, trifluoromethyl, or carboxy; and

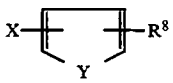
Y is —S— or —O—; and

the pharmaceutically acceptable salts thereof.

Compounds of Formulas XI and XII are obtained by allowing a compound of Formula VII to react with a compound of the formula:

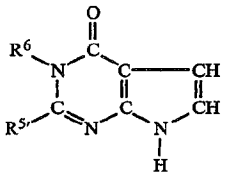


or



in which X, Y, and R^8 are as herein defined, by the methods of U.S. Pat. No. 4,818,819, namely in the presence of a palladium/trisubstituted phosphine catalyst, with the resulting coupled product being hydrogenated and hydrolysed to remove the $R^{2'}$ protecting group. Typical compounds of Formulas XI and XII are 3-(2-phenylethyl)-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(3-fluorophenyl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(4-fluorophenyl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(4-carboxyphenyl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]-pyrimidine, 3-[2-(4-methoxyphenyl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(4-methylphenyl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-(2-phenylethyl)-4-hydroxypyrrolo[2,3-d]pyrimidine, 3-(2-phenylethyl)-4-hydroxy-6-methylpyrrolo[2,3-d]pyrimidine, 3-(2-phenyl-3-hydroxypropyl)-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(thien-2-yl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(thien-2-yl)ethyl]-4-hydroxypyrrolo[2,3-d]pyrimidine, 3-[2 -(thien-2-yl)ethyl]-4-hydroxy-6-methylpyrrolo[2,3-d]-pyrimidine, 3-[2-(thien-3-yl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(thien-3-yl)ethyl]-4-hydroxypyrrolo[2,3-d]pyrimidine, 3-[2-(thien-3-yl)ethyl]-4-hydroxy-6-methylpyrrolo[2,3-d]pyrimidine, 3-[2-(fur-2-yl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(fur-2-yl)ethyl]-4-hydroxypyrrolo[2,3-d]pyrimidine, 3-[2-(fur-2-yl)ethyl]-4-hydroxy-6-methylpyrrolo[2,3-d]pyrimidine, 3-[2-(fur-3-yl)ethyl]-4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidine, 3-[2-(fur-3-yl)ethyl]-4-hydroxypyrrolo[2,3-d]pyrimidine, and 3-[2-(fur-3-yl)ethyl]-4-hydroxy-6-methylpyrrolo[2,3-d]pyrimidine.

As discussed above, the compounds of this invention can be prepared utilizing the palladium catalyzed coupling of various unsaturated compounds described in U.S. Pat. No. 4,818,819 and the glutamic acid coupling reactions described in U.S. Pat. No. 4,684,653, substituting the appropriate pyrrolo[2,3-d]pyrimidine for the pyrido[2,3-d]pyrimidine therein disclosed. The pyrrolo[2,3-d]pyrimidine intermediates of Formula IV above can be obtained by treating a compound of the formula:



XIII

in which $R^{5'}$ and R^6 are as herein defined with N-iodosuccinimide to yield the corresponding 2,3-diiodopyrrolo[2,3-d]pyrimidine which then is treated with zinc and acetic acid to remove selectively the iodine atom in the 2-position, yielding the corresponding 3-iodopyrrolo[2,3-d]pyrimidine of Formula IV.

According to the foregoing processes, compounds of Formula II in which R^1 is —OH are obtained. When a compound of Formula I in which R^1 is —NH_2 is desired, a compound in which R^1 is —OH can be treated with 1,2,4-triazole and (4-chlorophenyl)dichlorophosphate and the product of this reaction then treated with concentrated ammonia.

As noted, the compounds of this invention have an effect on one or more enzymes which utilize folic acid, and in particular metabolic derivatives of folic acid, as a substrate. For example, N-(4-[2-(4-hydroxy-6-aminopyrrolo[2,3-d]pyrimidin-3-yl)ethyl]benzoyl)-L-glutamic acid demonstrates potent inhibitory effects against growth of human T-cell derived lyphoblastic leukemia cells (CCRF-CEM), exhibiting an IC_{50} of 0.004 μ/ml. Cytotoxicity is not reversed by addition of purines such as hypoxanthine or by addition of aminoimidazolecarboxamide but is reversed by addition of thymidine, indicating specific inhibition in the tymidylate cycle and not in de novo purine synthesis. The compounds can be used, under the supervision of qualified professionals, to inhibit the growth of neoplasms including choriocarcinoma, leukemia, adenocarcinoma of the female breast, epidermid cancers of the head and neck, squamous or small-cell lung cancer, and various lymphosarcomas. The compounds can also be used to treat mycosis fungoides and psoriasis.

The compounds can be administered orally but preferably are administered parenterally, alone or in combination with other therapeutic agents including other anti-neoplastic agents, steroids, etc., to a mammal suffering from neoplasm and in need of treatment. Paren-

DOCKET ALARM

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