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PRINCIPAL INVESTIGATOR: Edward L. Schwartz, Ph.D.

CONTRACTING ORGANIZATION: Montefiore Medical Center
Bronx, New York 10467

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13. ABSTRACT *(Maximum 200*

Tumor angiogenesis is the process by which a growing tumor mass recruits the new blood vessels required for its continued growth, and through which the tumor can spread to distant sites. Indeed the neovascularization process is thought to be one of the rate limiting steps for the growth of primary and metastatic breast tumors. This proposal will focus on the development of inhibitors of a particular angiogenic factor, thymidine phosphorylase/platelet-derived endothelial cell growth factor (TP/PD-ECGF). These inhibitors will target the enzymatic activity of TP/PD-ECGF, since it has been found to be required for angiogenesis. In the past year, several compounds have been synthesized, with one showing good enzyme inhibitory activity *in vitro* (IC_{50} = 30 μM). These compounds will form the basis for further exploration of structure-activity relationships.

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Ed Schmitz 8/11/97

PI - Signature Date

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Introduction. There is a need for alternative approaches to treat metastatic breast cancer. One rapidly developing area of investigation is the study of tumor angiogenesis, the process by which a growing tumor mass recruits the new blood vessels required for its continued growth, and through which the tumor can spread to distant sites. Indeed the neovascularization process is thought to be one of the rate limiting steps for the growth of primary and metastatic tumors.[1-2] Most studies have demonstrated the importance of angiogenesis in the progression of human breast malignancies and found the extent of vascularization to be correlated with tumor size and an indicator of node metastasis.[3-6] Quantitative pathology studies in early breast cancer have shown a worse prognosis for those patients with highly vascular tumors, and similar results were obtained in studies of both node-negative and node-positive breast carcinomas.[5-8] Further, the prognostic influence of tumor angiogenesis was independent of other prognostic indicators.

Several polypeptides and growth factors that are produced by breast cancer epithelial and stromal cells have been identified as having endothelial cell mitogenicity and angiogenic activity: TGFα and β (transforming growth factor), VEGF (vascular endothelial growth factor), PD-ECGF (platelet-derived endothelial cell growth factor), FGF (fibroblast growth factor) and TNFα (tumor necrosis factor).[1,9] Our studies are focusing on the angiogenic factor PD-ECGF, based on recent evidence demonstrating its role in experimental and human cancer, and the finding that PD-ECGF is identical to human thymidine phosphorylase (TP), an enzyme that catalyzes the reversible conversion of thymine to thymidine.[10-12] When transfected into NIH 3T3 cells, TP/PD-ECGF was found to increase the vascularization of tumors growing in nude mice after sc inoculation.[13] Similarly, overexpressing TP/PD-ECGF in MCF7 breast carcinoma cells markedly increased tumor growth *in vivo*, although it had no effect on the growth of the cells *in vitro*.[14] Western blot analysis of primary human breast tissue showed that TP/PD-ECGF expression was elevated in the tumors compared to the normal tissue[14], a finding which provides clinical relevance to the transfection experiments.

Studies suggest that the angiogenic and endothelial cell chemotactic activities of PD-ECGF are dependent upon its enzymatic activity, and this has been confirmed with site-directed mutagenesis studies.[14,15-17] Of the angiogenic factors identified to date, TP/PD-ECGF is the only one in which an enzymatic activity of the factor is required for angiogenic activity. These observations serve as the basis for our hypothesis that inhibition of the catalytic activity of TP/PD-ECGF will also block its angiogenic properties. By synthesizing inhibitors of TP/PD-ECGF, we will be able to test this hypothesis and provide a basis for the development of a novel class of antitumor agents. There are no potent and specific inhibitors of TP/PD-ECGF currently available.

PROPRIETARY

Methods, Results, Discussion

Progress in the Synthesis and Evaluation of Target Compounds:

We had originally proposed the synthesis of four general, structurally distinct classes of pyrimidines or pyrimidine nucleosides to be evaluated as potential TP/PD-ECGF inhibitors. These four classes (I-IV) are illustrated in Fig. 1 (see Appendix). We can now report significant progress in the synthesis and evaluation of two of these classes, namely I and III.

The synthesis of the 2'-deoxypseudouridines of class III was tackled first because of the relative complexicity of the route anticipated. Our choice of this class of C-nucleoside analogues as potential inhibitors of PD-ECGF was based on the stereo electronic similarities that exist between these and thymidine, a natural substrate for the enzyme. The synthetic route originally envisaged had to be modified substantially and the method finally adopted is illustrated in Fig.2. A key modification involved the protection of the two hydroxyl groups in compounds **2-5** by acetylation without which a complex mixture of products (as well as unreacted material) was obtained. The first member of this class, 1-methyl-2'-deoxyuridine (**2-7**), unexpectedly was found to have little inhibitory activity (IC_{50} >4 mM, see table 1). The enzyme, however, can accomodate 2'-deoxyuridines with 5-substituents as large as 2-bromovinyl and propynyloxy groups. It is expected, therefore, that the successful synthetic methodology we have just developed to obtain **2-7** will make it also possible to access 2'-deoxypseudouridines bearing N-1 substituents other than methyl and with greater capacity to inhibit TP/PD-ECGF.

As we had proposed originally, we have synthesized several variously substituted derivatives of 6-aminouracils (Class I inhibitors) to maximize binding to the enzyme and thereby identify compounds with greater potency and specificity. One of our synthetic routes is illustrated in Fig.3 and makes use of the direct nucleophilic displacement of Cl from 6-chlorouracil by a variety of amines. Of the compounds obtained, **3-1a** - **3-1f**, the most active appear to be those where the substitution at C-6 incorporates two phenyl rings connected by a two atom bridge (**3-1e** and **3-1f** with IC_{50s} of 114 μM and 30 μM, respectively). Current synthetic efforts are focusing on the investigation of several new compounds with this same substitution pattern in order to fine tune and further maximize binding to the enzyme. Compounds **3-1g** and **3-1h** (which are now being synthesized in our laboratory) are two examples of this new series of potential inhibitors.

We have also begun the synthesis (see Fig. 4) of a new series of analogs to find out whether structural extension of the aminosubstituents on C-6 of uracil by a short distance might not enhance the inhibitory activity of Class III inhibitors. The new derivatives obtained to date (**4-1c**, **4-1d**, and **4-1e**) differ from their homologues of Fig.3 only by a single methylene group inserted between C-6 and the amino substituent. We had shown that 5-bromo-6-aminouracil is an effective inhibitor of human TP/PD-ECGF, with an Ki of 13 μM and is, therefore, one of the best inhibitor

of this enzyme known to date. It would be of interest to find out therefore whether 6-aminouracil derivatives with 5-substituents other than Br might not also exhibit similar or possibly even better activities. Preliminary synthetic studies in our laboratory would seem to indicate that (as shown in Fig. 5) 6-aminouracil reacts with aromatic isothiocyanates to give the C-5 substituted thioamide product (and not, as might be expected, the C-6 thioureido derivative). If confirmed, this synthetic method might open access to many new analogs of 5-bromo-6-aminouracil with groups other than the halogens and with various steric requirements. These preliminary findings and the activity of the product obtained so far (**5-1a**, **5-1b** and **5-1c**) are now under active investigation.

Conclusions. The synthesis of several 2'-deoxypseudouridines C-nucleosides and derivatives of 6-aminouracil has been completed. The latter have shown good activity, with the 5-bromo-substituted compound and a C6 derivative which incorporates two phenyl rings connected by a two atom bridge being the most potent.

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APPENDIX

Fig. 1

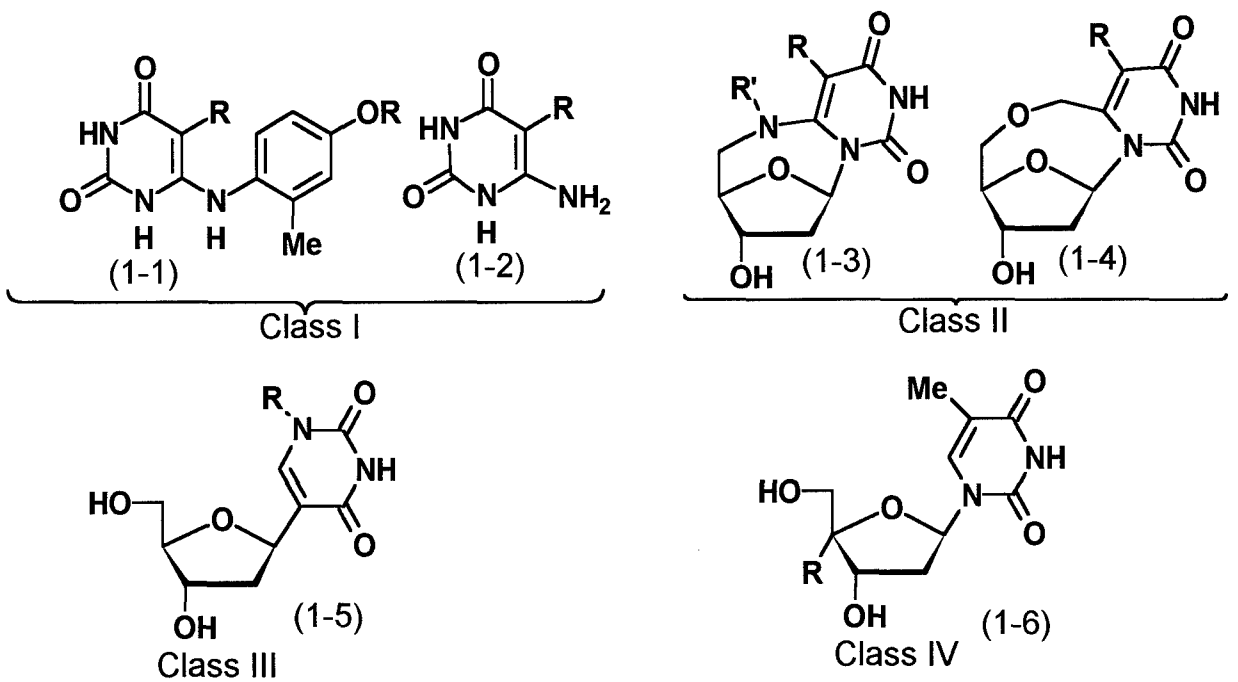

Figure 1: Classes of compounds to be synthesized and tested as inhibitors of TP/PD-ECGF catalytic activity.

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Fig. 2

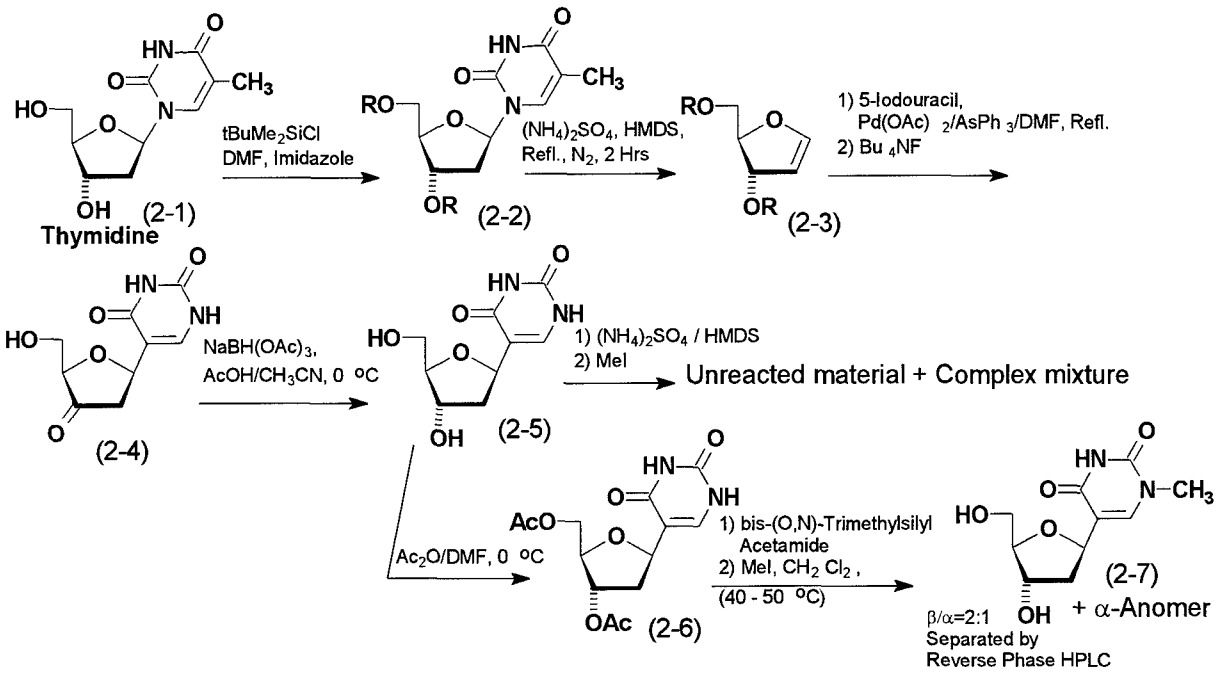

Figure 2: Synthetic route utilized for the synthesis of class III compounds.

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Fig. 3

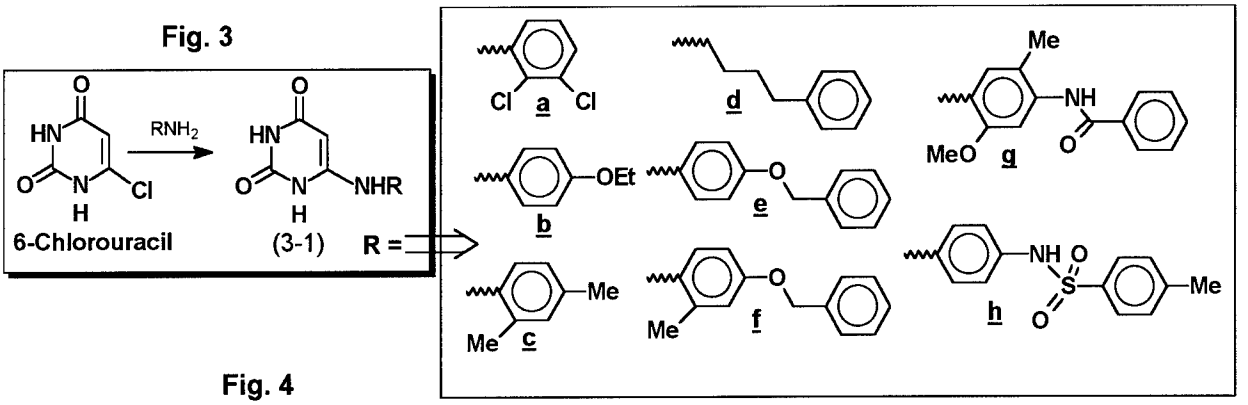

Fig. 4

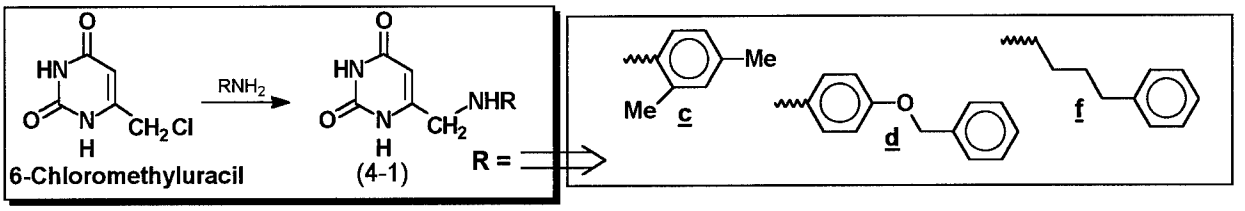

Fig. 5

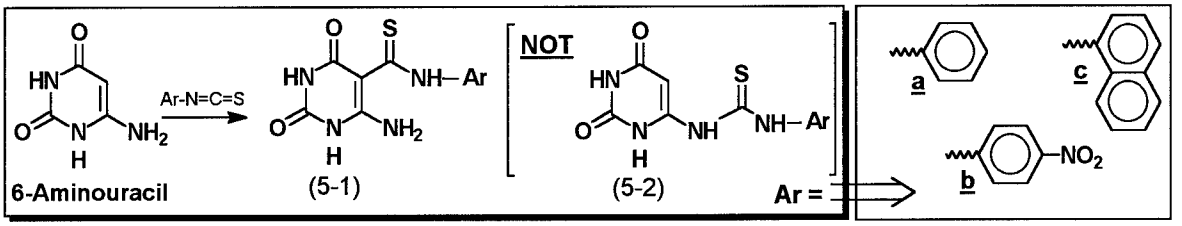

Figure 3: Synthetic route utilized for the synthesis of 6-aminouracil derivatives by direct nucleophilic displacement.

Figure 4: 6-Aminouracil derivatives with a methylene group inserted between C-6 and the amino substituent.

Figure 5: 6-Aminouracil reacts with aromatic isothiocyanates to give the C-5 substituted thioamide product, and not the C-6 thioureido derivative.

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Table 1: Inhibition of TP/PD-ECGF activity.

TP Inhibitory activity of synthesized target compounds (7 / 20 / 97)

Class of TP Inhibitors	Compound (Substituent)	IC50 (Human Recomb. TP)	IC50 (Rabbit Liver TP)
Class III	**1-5** (R=Me), **2-7**	>4 mM (Inactive)	----
Class I	**3-1** (R = **a**)	220 μM	170 μM
	3-1 (R = **b**)	275 μM	170 μM
	3-1 (R = **c**)	160 μM	74 μM
	3-1 (R = **d**)	122 μM	53 μM
	3-1 (R = **e**)	114 μM	----
	3-1 (R = **f**)	30 μM	14 μM

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