Sequential Blockade of the Mycobacterial De Novo Folate Pathway

A Review

Norman E. Morrison

The principle of sequential blockade or sequential inhibition through the combined action of two inhibitors on the same metabolic sequence was first proposed by Potter (42) in the field of cancer chemotherapy. Substantiation of the concept (25, 27) provided the basis of elegant work by Hitchings and co-workers (28) for the synthesis of a number of antifolate drugs which proved efficacious in the therapy of plasmodial (29, 31), toxoplasmal (30, 35) and coccidial (26) infections. It was noteworthy that 4:4-diamidinophenyl sulfone (DDS) when combined with pyrimethamine was claimed effective in the treatment of human malaria caused by Plasmodium vivax and P. falciparum (29). A combination of the long-acting sulfonamide, sulfafox, and the 2:4-diaminopyrimidine derivative, trimethoprim, have recently been found markedly efficacious against established infections with multiresistant strains of P. falciparum (31). While against such strains DDS was found to be somewhat slower in action, it was, however, demonstrated to have powerful action in the chemotherapy of drug resistant malarias (27, 39). Likewise, DPD, the diformyl derivative of DDS, is an active antimalarial (12) and has been found to block the multiplication of Mycobacterium leprae in the mouse foot pad (34).

Sequential blockade results in potential synergism of drug action, in contrast to additive synergism, in that very much lower concentrations of drugs in combination, than predicted from additive doses, are effective in suppressing infection. Furthermore, the frequency of the emergence of drug resistant organisms is greatly reduced over that found in monotherapy regimens. The sequential blockade of the de novo folate pathway is brought about by structural analogs of biosynthetic intermediates which, in general, reversibly bind to enzyme active sites resulting in inhibition of two adjacent enzymes (i) the p-aminoenzoate (PABA) utilizing enzyme and (ii) the TPNE-linked dihydrofolate reductase. Enzyme (i) has never been adequately purified, is present only in the parasite and is inhibited by sulfonamides and presumably sulfones, whereas enzyme (ii) has been extensively studied and is present in both parasite and mammalian host. Enzyme (ii) is effectively inhibited by diaminopyrimidines, diaminopteridines, daiminoquinazolines and diaminopteridines, all of which are analogs of the pteridine portion of the folate structure. The exquisite type of molecular selectivity required for drug inhibition of enzyme (ii) is due to marked differences in active site binding between parasite and host. Trimethoprim, for example, is bound 50000 times more strongly by dihydrofolate reductase from some bacteria than by reductases from mammalian tissues (30). Furthermore, the small molecular weight folate analogs possess the important property of being able to rapidly penetrate the parasite by passive diffusion (25) while complete folate structural analogs, such as aminopterin or homofolate acid (14) are unable to readily penetrate unless extremely high external concentrations are present.
It is necessary to point out, however, that the full potential for structural analog inhibition of the mycobacterial de novo folate pathway of the parasite has yet to be attained. It is predictable that the future will see the emergence of structurally analogous analogs selectively blocking the formation of folate peridinol precursors as well as the formation of dihydropteroic acid (1), the immediate precursor of dihydrofolic acid. The significance of differential active site binding to folate enzymes in mammalian tissues is the basis of much investigation to synthesize effective antitumor agents (2, 6, 7). In the case of pathogenic organisms, it is possible to visualize a series of three or four structural analogs functioning in multisequential blockade of the de novo folate pathway found only in the parasite. The antifolate inhibition of the pathogen thus offers greater potential than the tumor cell which requires a peroxynorm source of folate vitamins and thus has less metabolic steps available for structural analog inhibition.

The sequential blockade of the mycobacterial de novo pathway is potentially exploitable in the chemotherapy of human leprosy provided an active inhibitor of dihydrofolate reductase can be found for the leprosy bacillus. The first experimental chemotherapy attempt to sequentially blockade the mycobacterial pathway was made by Shepard (56) who found that trimethoprim did not potentiate DDS activity against the multiplication of M. leprae in the mouse foot pad. Trimethoprim itself, was, in fact, without activity against M. leprae. This observation has been subsequently confirmed by Hibson and coworkers (22).

Experimental chemotherapy involving in vitro mycobacteriologic data. The availability of high level sulfone resistant mutants isolated from Mycobacterium sp. 607 (25) has led to a collaborative research program between laboratories from the Stanford Research Institute, the U.S. Public Health Service Hospital in San Francisco and the Leonard Wood Memorial at Johns Hopkins University. The sulfone resistant mutants were derived by multi-step procedures in liquid medium followed by single colony isolation. The mutants thus have a role in determining the mode of action of DDS and in combination with the mouse foot pad M. leprae screen are being used to determine structure-activity relationships among the sulfones. Furthermore, they are being used to search for effective inhibitors of the dihydrofolate reductase site.

Mode of action of DDS. It was initially found that with the emergence of DDS resistance cross resistance to the sulfonamides, particularly the long-acting sulfonamides, was present (45). A feature of the cross resistance was a remarkable increase in the minimal inhibitory concentration (MIC) of the sulfonamide required to inhibit the sulfone resistant organism. The increase was many times that of DDS required for comparable inhibition (Table 1). It was noteworthy that cross resistance was absent to inhibitors of dihydrofolate reductase, such as, trimethoprim, pyrimethamine or aminopterin thus indicating a different gene locus for synthesis of the enzymes concerned with the two sites of antifolate sequential blockade. The failure of sulfonamide cross resistance was in agreement with the data derived by Rees (46) from mouse foot pad experiments which showed that sulfone resistant M. leprae exhibited cross resistance to sulfadimethoxine and sulfadimethoxine. With

<table>
<thead>
<tr>
<th>Drug</th>
<th>M. lepraee sp. 607</th>
<th>MIC (micrograms/ml)</th>
</tr>
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<tbody>
<tr>
<td>DDS</td>
<td>8.1</td>
<td>3255</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>3.6</td>
<td>1213</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>6.9</td>
<td>8290</td>
</tr>
<tr>
<td>Sulfamethoxine</td>
<td>7.1</td>
<td>10050</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>34</td>
<td>172</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>101</td>
<td>403</td>
</tr>
<tr>
<td>Aminopterin</td>
<td>742</td>
<td>1843</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>B.667</td>
<td>6.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

It was initially found that with the emergence of DDS resistance cross resistance to the sulfonamides, particularly the long-acting sulfonamides, was present (45). A feature of the cross resistance was a remarkable increase in the minimal inhibitory concentration (MIC) of the sulfonamide required to inhibit the sulfone resistant organism. The increase was many times that of DDS required for comparable inhibition (Table 1). It was noteworthy that cross resistance was absent to inhibitors of dihydrofolate reductase, such as, trimethoprim, pyrimethamine or aminopterin thus indicating a different gene locus for synthesis of the enzymes concerned with the two sites of antifolate sequential blockade. The failure of sulfonamide cross resistance was in agreement with the data derived by Rees (46) from mouse foot pad experiments which showed that sulfone resistant M. leprae exhibited cross resistance to sulfadimethoxine and sulfadimethoxine.
respect to the chemotherapy of human leprosy two facts are apparent: (a) the long-acting sulfonamides are of no value in the treatment of sulfone resistant leprosy as has been recently demonstrated (55) and (b) DDS will prove of no value in the treatment of resistance emerging to the clinical use of long-acting sulfonamides. The last point has been investigated using sulfane resistant mutants of Mycobacterium sp. 607 which were found to fully cross resist to DDS.

The second major finding was concerned with reversal studies of DDS inhibition of sulfone sensitive and resistant 607 mutants. It was found (37) that two structural components of the folate molecule, namely, p-aminobenzoic acid or p-aminobenzooylglutamic acid (PABG) would reverse the growth inhibition when simultaneously added along with DDS. Kinetic analyses of growth rates revealed that reversal was strictly competitive with respect to concentration relationships of PABA and PABG with DDS. This finding was made possible by the use of dispersed growth in liquid medium and supports previous data that PABA antagonized DDS inhibition of mycobacterial growth (33, 37, 40, 58). The reversal by PABG was found to take place without prior formation of PABA since cell free extracts from the 607 organism were unable to hydrolytically split out L-glutamic acid and thus form PABA from the compound. It was of particular interest to learn that PABA was re-investigated by Shepard (38, 39) and found to partially reverse growth inhibition of DDS against M. leprae in the mouse foot pad.

The sulfonamide cross resistance and the PABA and PABG competitive reversals thus build a strong case of *prima facie* evidence for the hypothesis that the mode of action of DDS against mycobacteria, and, in particular M. leprae, is that of an antifolate mechanism through inhibition of the p-aminobenzoate utilizing enzyme of the de novo folate pathway. The fact that M. leprae is inhibited in the mouse foot pad by DDS, sulformethoxine, sulfadimethoxine (49) or sulfamethoxypyridazine (28) provides convincing evidence that the de novo folate synthesizing pathway operates in M. leprae and from the cultivation point of view, the organism will not require preformed folic acid of host cell origin in order to carry out cell division. Conversely it can be stated that if M. leprae had growth factor requirements for preformed folic acid then no inhibition of multiplication in the mouse foot pad or the human host would occur with DDS, sulfadimethoxine or sulfamethoxypyridazine.

In many respects DDS is a unique molecule for although it is, like the sulfonamides, a structural analog of PABA, it is excluded from the sulfonamide theory of bacteriostasis proposed by Bell and Roblin (1), since the molecule does not dissociate under physiologic conditions and hence the relative electronegativity of the sulfone group remains unalloyed by dissociation effects. Clearly since the electronic charge on the sulfone group is unable to change without dissociation taking place, the molecule penetrates the mycobacterial cell and binds to the active enzyme site independently of such phenomena used to theorize sulfonamide mechanism of action.

For many years a suspicion has been entertained that DDS interacts physicochemically with biologic membranes in a manner as yet not fully understood. Effects arising from membrane interactions require higher concentrations than those required for antifolate enzyme inhibition and are generally not reversed by PABA. Biologic effects arising from DDS interaction with membranes includes the hemolysis of erythrocytes particularly evident in G6PD deficient individuals (41) and the activation of hydrolytic enzymes found within the lysosome which likewise possesses an extremely reactive membrane (38, 47). It may also be suspected that DDS undergoes interaction with bacterial membranes the exact nature of which requires elucidation.

Interaction with membranes requires that DDS possess a high degree of liposolubility. In support of this fact a recent determination (28) of the partition coefficient of DDS has indicated that the compound is 95 per cent liposoluble when partitioned between ethylene dichloride and water. Clearly the neutral state of the molecule coupled with a high degree of liposolubility are prerequisites necessary for membrane interaction.
It is predictable that DDS interaction with lysosomal membranes results in permeability changes and the subsequent concentration of DDS within the organelle. Concentration of drugs such as chloroquine or quinine into the lysosome have been observed (1). Thus through discharge of lysosomal contents into the phagosome a localized concentration gradient of DDS is available to exert antifolate effects upon plasmamembranous bacteria. Certainly proponents (5, 26, 44) of the theory that DDS exerts its antileprosy action solely through the human host by activation of lysosomal enzymes have failed to take into account the possibility of drug-membrane interaction resulting in drug concentration by this remarkable organelle.

**Sulfone structure-activity data.** A great amount of data on the antituberculosis structure-activity of sulfones is in existence (28). The information was derived from two types of screening programs, an in vitro screen with *M. tuberculosis* H37Rv in liquid medium and (b) an in vivo guinea-pig screen (46, 61). In proposing to screen for antileprosy structure-activity it is not possible to utilize an in vitro *M. leprae* screen, however, the mouse foot pad assay of Shepard (28) offers an animal screening model. It was decided, however, to use the *Mycobacterium sp. 607* sulfone sensitive and resistant mutants as a basis for in vitro screening. The decision was fortunate in that it is now apparent that a considerable degree of correlation exists in structure-activity data obtained from the two screening systems.

<table>
<thead>
<tr>
<th>Sulfone</th>
<th>MIC (µMoles/mL)</th>
<th>M. leprae Mouse foot pad assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:4'-diaminodiphenyl sulfone</td>
<td>8.1</td>
<td>-3210</td>
</tr>
<tr>
<td>4-aminodiphenyl sulfone</td>
<td>1288</td>
<td>2008</td>
</tr>
<tr>
<td>4 amino-4' hydroxydiphenyl sulfone</td>
<td>400</td>
<td>2001</td>
</tr>
</tbody>
</table>

* Sulfones at a dietary concentration of 0.01% (w/w) equivinal with DDS.
* Solubility limit.

Initially the question was asked as to how important to activity was the second amino group in DDS since it was apparent that PABA analogicity was still present in the structure with only one amino group. The data (Table 2) showed that the second amino group was indeed essential to block multiplication of *M. leprae* and exert maximal activity against *Mycobacterium sp. 607*. The data was thus consistent with that obtained from *M. tuberculosis* H37Rv and *M. leprae* murium as to the essential requirements for two amino groups (46, 47). Further investigation led to the hypothesis that the second amino group in DDS played an essential binding role, possibly through H bonding, and that the binding role was essentially different to that of the first amino group which was involved directly in antifolate active site binding (19). It was further reasoned that other groups capable of H bonding, such as -OH, could thus replace at least one of the essential amino groups without significant loss of activity. However, while one amino group was open to replacement, because of differential binding specificity, it appeared unlikely that both amino groups could be replaced with -OH groups and still retain essential antifolate activity in the molecule.

The results (Table 2) were consistent with this argument. It can be seen that 4:4-dihydroxydiphenyl sulfone showed no cross resistance with DDS indicating an inability to act through an antifolate mechanism. It was noteworthy that 4-aminoo-4'-hydroxydiphenyl sulfone showed cross resistance with...
DD and was more active than DDS in inhibiting the growth of DDS resistant 607 organisms while likewise retaining full in vitro activity against M. leprae. The in vitro antileprosy activity was thus consistent with antituberculosis activity found in guinea pig and mouse experimental infections (16). Since 4'-dihydroxyphenyl sulfone has been claimed effective against human leprosy (15) it now becomes important to determine whether similar activity can be found against M. leprae in the mouse foot pad assay in view of the lack of in vitro antifolate activity of the sulfone.

The concept of the importance of a second H bonding group on the sulfone molecule needs extending to the sulfonamide series of drugs. It has not been possible to demonstrate that the long-acting sulfonamides, sulformethoxine and sulfadimethoxine are in any way as efficacious as DDS against M. leprae in the mouse foot pad despite data showing adequate absorption and blood level maintenance (16). Insertion of H bonding groups onto the heterocyclic ring of the sulfonamide may thus increase drug efficiency. Similarly, the possibility exists that insertion of further H bonding groups onto the DDS structure could result in a more efficacious drug.

Second sequential inhibition site. The TPNH-linked dihydrofolate reductase enzyme represents the second inhibition site of the de novo mycobacterial folate pathway (14).

\[
\text{Dihydrofolate} + \text{TPNH} + \text{H}^+ = \text{Tetrahydrofolate} + \text{TPN}^+ 
\]

The enzyme catalyses the addition of 2H to the 5,6-position on the pteridine ring of dihydrofolate to form tetrahydrofolate. Since the properties of the enzyme from the mycobacteria have never been investigated, the inhibitor dissociation constants (Ks) of reductase antifolates are unavailable to use as a basis to select compounds for mouse foot pad testing.

The first report of a mycobacterial folate synthetizing pathway, from M. avium, was made by Katsumata et al. in 1957 (20). This was subsequently confirmed by Brown (4). Recent work in our own laboratory has determined the presence of dihydrofolate reductase in a number of mycobacteria including Mycobacterium sp. 607, M. fortuitum, M. chelonei, M. smegmatis, M. phlei and M. tuberculosis R. Particular attention has been paid to the properties of the enzyme from sulfone sensitive and resistant mutants of Mycobacterium sp. 607. It was found that the Michaelis constant for the substrates, dihydrofolate and TPNH, did not significantly change in the DDS resistant mutant (Table 3). This, providing enzymic evidence that DDS resistance does not involve mutational changes at the dihydrofolate reductase site of the de novo pathway. Furthermore, the data was fully consistent with the fact that DDS does not cross-resist with reductase inhibitors such as trimethoprim, pyrimethamine or aminopterin (Table 1). The availability of purified enzyme will thus provide a useful tool in determining active site binding and inhibitor constants of reductase antifolates with chemotherapeutic potential. The enzyme is inhibited by extremely low concentrations of aminopterin (10^-8 M) whereas high concentrations are required to block cell growth (Table 1) thus this type of folate analog is unable to penetrate the mycobacterial cell with any degree of efficiency to exert inhibition. It was of interest to find that DDS and its monoaacetylated derivative MADDS did not act as inhibitors of the purified reductase enzyme thus providing further circumstantial evidence that their site of action is located at the PABA utilizing enzyme of the de novo folate pathway. It may be noted that the binding of dihydrofolate to the mycobacterial active site is particularly strong in comparison to the Ks's from other bacterial species (Table 3). This fact thus must be taken into account in designing and synthesizing effective antimetabolite inhibitors.

Further characterization of the mycobacterial dihydrofolate reductases have two objectives. On the one hand a study of the range of variation in the Michaelis constants throughout the cultivable species of the genus Mycobacteria will indicate the ability to extrapolate active site binding...
The potentiative synergism of antifolate drugs is one of the most important chemotherapy principles to become recognized since the postulation of the Woods-Fildes theory of sulfonamide action. It is particularly noteworthy that the principle has emerged from a rational approach to the design of analog inhibitors of specific folate enzymes especially since no naturally occurring antibiotics have ever been discovered which block the de novo folate pathway in microorganisms. The sequential blockade of the pathway in *M. leprae* would have two important results in the treatment of human leprosy (1) a faster and more effective drug action at lower doses leading to faster amelioration of clinical symptoms and (2) as already stated, a significant decrease in the frequency of emergence of sulfone resistant organisms. A third feature of sequential blockade effects lies in the recognition that bitherapy regimens can result in strong bactericidal action although the equivalent monotherapy regimens are essentially bacteriostatic.

Data from cultivable mycobacteria to *M. leprae*. Comparative data of this type have emerged from trypanosomal species with the finding that little variation occurs throughout the genus (28). Secondly, structure-activity relationships based on reductase inhibitor binding profiles can be used to map the relationships of the binding regions of the mycobacterial active site. Already data are available indicating unique differences, compared to other bacteria, in the conformation of the hydrophobic binding region (1) adjacent to the enzyme-substrate active site. This information is of importance to the medicinal chemist attempting to synthesize antifolate structures with increased mycobacterial specificity and binding. It also provides an explanation as to the lack of antileprosy drug effects found for the trimethoprim in the mouse (29, 30).

While combined therapy involving drug synergism has long been a standardized regimen in the treatment of tuberculosis only minor consideration has been given to combined therapy in leprosy. It now appears likely that with the emergence of newer drugs experimental chemotherapy of

### TABLE 3. Comparative pH optimum and Michaelis constants for bacterial dihydrofolate reductase.

<table>
<thead>
<tr>
<th>Organism</th>
<th>pH optimum</th>
<th>Dihydrofolate</th>
<th>TPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium</em> sp. 607 (DDS sensitive)</td>
<td>5.8</td>
<td>4.1</td>
<td>20.2</td>
</tr>
<tr>
<td><em>Mycobacterium</em> sp. 607 (DDS resistant)</td>
<td>5.8</td>
<td>4.3</td>
<td>20.4</td>
</tr>
<tr>
<td><em>Diplococcus pneumoniae</em></td>
<td>7.3</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em></td>
<td>6.3</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td><em>Streptococcus</em> parvus</td>
<td>6.2</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td><em>Streptococcus</em> accretum</td>
<td>7.0</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>7.0</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>7.0</td>
<td>25</td>
<td>10</td>
</tr>
</tbody>
</table>

a From Sirotnak, F. M., Donali, G. J. and Hutchison, D. J. J. Biol. Chem. 239 (1964) 2077-2082.

*Organisms expressed in Michaelis constants are not too dissimilar from those of human enzyme (31, 49). This is one of the rare occasions that a drug possesses both effective antileprosy and antituberculosis activity in the human. In the case of sulfone resistance, the 607 mutants have shown that no cross resistance occurs with 8.063 or rifampicin (Table 1) consistent with data reported for DDS-resistant cases (9, 41). It is of interest to note that rifampicin, the semi-synthetic rifamycin SV derivative, has recently been found strongly active in human leprosy (31, 49). This is one of the rare occasions that a drug possesses both effective antileprosy and antituberculosis activity in the human. In the case of sulfone resistance, the 607 mutants have shown that no cross resistance occurs with 8.063 or rifampicin (Table 1) consistent with data reported for DDS-resistant *M. leprae* which likewise show no cross resistance to 8.063 or rifampicin in the mouse foot pad (18, 48).*
leprosy will tend to reflect attempts to establish combined therapy regimens. As Browne (*) has pointed out there is an urgent need for developing more rapidly acting antileprosy drug regimens. While combinations of DDS with B.663 or rifampicin certainly require intensive clinical trials it would appear likely that additive rather than potentiative synergism would result from such combinations since there is no evidence that B.663 or rifampicin act through an antifolate mechanism ('Table 1') thus sequential blockade effects are unlikely to occur.

Epilogue. The experimental chemotherapy of human leprosy offers exciting challenges for the chemical design of newer antimetabolite drugs to effect sequential blockade and synergistic effects from combined therapy. The experimental mouse infection using thymectomized and irradiated animals (25) provides the most advanced model infection for drug screening yet developed. Antileprosy drug development thus follows a Leonard Wood Memorial tradition, espoused by the late Dr. James A. Doull (17), that "...chemical, bacteriologic and pharmacologic research is necessary for development of new drugs of stronger antibacterial action than the sulfones possess."

SUMMARY
The significance of developing drugs that initiate sequential blockade of the de novo folate pathway in mycobacteria has been reviewed. There appears to be no examples of drug combinations available for the therapy of human leprosy which produce potentiative synergism.

Evidence has been presented to indicate that the primary mode of action of DDS against M. leprae is that of an antifolate mechanism. In view of this, drug development work will undoubtedly aim at producing an antimetabolite which in combination with DDS, or the repository sulfone DADDS, can effectively blockade in sequence the enzymic sites along the de novo folate pathway thus resulting in more rapidly acting antileprosy drug effects.

RESUMEN
Se ha hecho una revisión de la importancia que tiene el desarrollo de drogas que inician el bloqueo secuencial de la de novo vía del folato en microbacterias. Parece no haber ejemplos de combinaciones de drogas utilizables en la terapia de la lepra humana que produzcan sinergismo potenciativo.

Se ha presentado evidencia que parece indicar que el principal modo de acción del DDS contra M. leprae es el de un mecanismo anti-folato. En vista de esto, los trabajos relacionados con el desarrollo de drogas están indudablemente diri­gidos a producir un antimetabolito que, en combinación con el DDS o la sulfona de des­pósito DADDS, pueda bloquear secuencialmente, en forma efectiva, las etapas enzimáticas a lo largo de la de novo vía del folato, ob servando así efectos tempraneros de acción más rápidos contra la lepra.

RESUME
On a considéré dans cet article l'importance qu'il y aurait à développer des médicaments qui pourraient déclencher un blocage séquentiel du circuit de novo des folates dans les mycobactéries.

Il semble qu'il n'y ait pas d'exemple de combinaisons médicamenteuses actuellement disponibles dans la thérapeutique de la lepre humaine, qui pourraient produire un synergisme cumulatif de cette espèce.

On a fourni des données qui indiquent que le mécanisme essentiel de l'action de la DDS contre M. leprae est celui d'une action antifolatée. Dès lors, il est indubitabile que les travaux visant à développer de nouveaux médicaments devraient tenir à mettre au point un anti­metabolite qui, en combinaison avec la DDS ou avec la sulfone retard DADDS, pourrait bloquer efficacement, et l'un après l'autre, les sites enzymatiques tout au long du circuit de novo des folates, ce qui aurait pour conséquence une action plus rapide des effets anti-épreux de ce médicament.

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