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Iclaprim, a Novel Diaminopyrimidine with Potent Activity on Trimethoprim Sensitive and Resistant Bacteria

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Abstract—Iclaprim, a new selective dihydrofolate inhibitor was synthesized based on rational drug design. Iclaprim's interaction with a resistant *Staphylococcus aureus dihydrofolate reductase* (DHFR) is outlined in comparison to trimethoprim (TMP). This compound is active against methicillin, TMP and vancomycin resistant strains. Arpida Ltd. is developing Iclaprim for serious hospital infections from Gram-positive pathogens and respiratory tract infections.

Domagk's discovery of sulfonamides in 1935 and the elucidation of their mode of action by Woods[1] in 1940 paved the way for antimetabolites as potential drugs. Hitchings[2] explored the nucleic acid biosynthesis with synthetic analogues of the purine and pyrimidine bases and observations with anti-thymines led to the realization that 2,4-diaminopyrimidines not only acted as 'thymine substitutes' but inhibited folic acid metabolism in *Lactobacilli*. These key observations finally led to the selection of pyrimethamine as an antimalarial and trimethoprim (TMP, Scheme 1: IV) as an antibacterial agent (for reviews see refs 3–6). Sulfonamides and diaminopyrimidines act through inhibition of pteridine reductase (DHPS) and dihydrofolate reductase (DHFR) respectively validating both enzymes as good targets for antibacterial drugs (Scheme 2).

Sulfonamides and diaminopyrimidines have both been clinically used as monotherapies. Sulfonamides by themselves are bacteriostatic and resistance has emerged rapidly, with their use further limited due to allergic reactions in patients. Similarly, diaminopyrimidines, for example TMP, are weakly bactericidal and resistance, albeit limited, has emerged due to the intensive use and misuse.[7] However, exploitation of the synergistic action of these classes of drugs through inhibition of successive steps in the folate metabolism led to the highly successful development of the broad-spectrum, bactericidal drug Co-trimoxazole, (e.g., Bactrim®, TMP and SMX, Scheme 2), in 1968.[8]

The DHFR enzyme is crucial in bacteria to produce tetrahydrofolate initially and to recycle it after reoxidation in the formation of thymidylate from deoxyuridylate. Amino acid sequence analysis of DHFRs showed significant variation of this enzyme in different species[9] and the homology between the bacterial and mammalian enzymes is less than 30%. There are also marked differences in the active sites of the mammalian and bacterial enzymes that have been exploited for developing potent and selective inhibitors.

Since the discovery of TMP in 1965 many companies have initiated programs to synthesize derivatives within the benzyl-diaminopyrimidine series to improve physico-chemical and pharmacological profiles. A representative example of these activities is the comprehensive proton NMR study of the interactions and conformations of rationally designed brodimoprim[10] derivatives (Scheme 1: V R = -carboxypropoxy, -carboxybutoxy) presented by Birsall et al.[11,12] showing an impressive gain in binding properties with *Lactobacillus casei* DHFR. These compounds were designed to interact with Arg57 and His28 in the enzyme (Fig. 1). One of the analogues bound 3 orders of magnitude more tightly than V (Scheme 1, R = CH_3). Kuyper[13] described a similar approach for TMP analogues. During the last decade, many attempts were undertaken to understand the interactions of TMP and related compounds with sensitive and resistant bacterial and mammalian[14]

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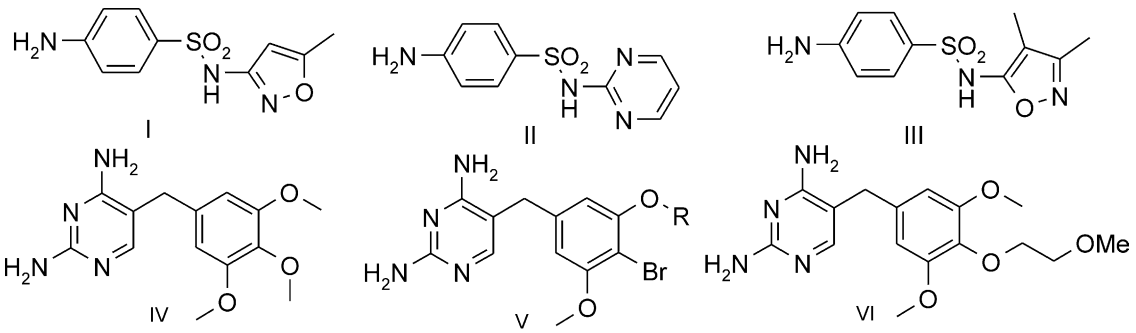
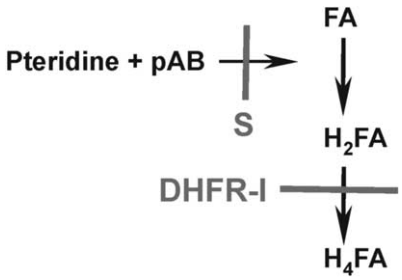


Scheme 1. Sulfamethoxazole (SMX, I), sulfadiazine (II), sulfisoxazole (III), trimethoprim (TMP, IV), brodimoprim (V, R = CH_3), tetroxoprim (VI).



Scheme 2. Folate metabolism: folic acid (FA); dihydrofolic acid (H_2FA); tetrahydrofolate (H_4FA); *p*-aminobenzolic acid (pAB); dihydropteridine synthase inhibitor (S); dihydrofolate reductase inhibitor (DHFR-I).

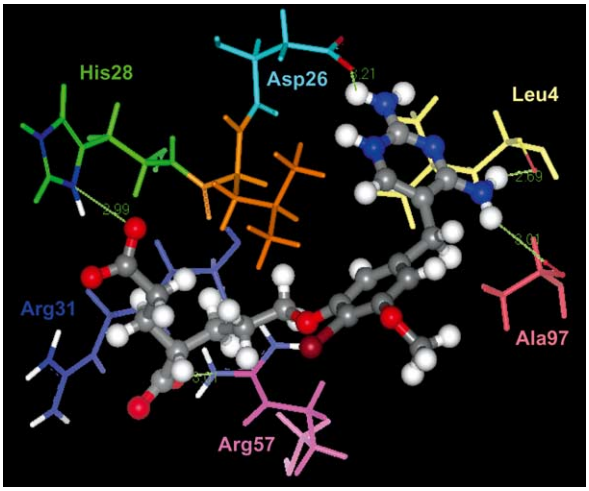


Figure 1. NMR study of *L. casei* DHFR with compounds designed to interact with Arg57 and His28. In addition, the diaminopyrimidine binds to the expected amino acids Asp26, Leu4 and Ala97.[11,12]

DHFRs to improve potency (e.g., QSAR studies[15,16]), selectivity[17] and to overcome resistance. A single amino acid change in the active site, Phe98 to Tyr98 in *Staphylococcus aureus* DHFR (Fig. 2), is responsible for resistance in all *S. aureus* TMP-resistant clinical isolates tested.[18–20] These studies show that the mutation results in the loss of a hydrogen bond between the 4-amino group of the diaminopyrimidine part of TMP and the carbonyl oxygen of Leu5. This important finding of the resistance mechanism at the molecular level could help to design DHFR inhibitors active against multi-resistant *S. aureus*, a serious clinical problem.

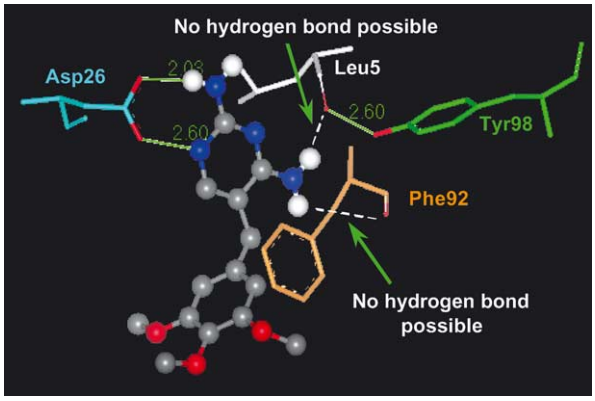


Figure 2. Drawing sketch of resistant *S. aureus* DHFR with TMP to show effect of the mutation of Phe98 to Tyr98 as discussed in ref 18. The carbonyl of Leu5 is occupied by Tyr98 and therefore no longer accessible to bind the 4-amino group of TMP. In addition, the Phe92 carbonyl is moved away and cannot interact with the 4-amino group of TMP.

In vitro, Gram-positive strains like *S. aureus* (incl. methicillin resistant), *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus viridans*, *Enterococcus faecalis* and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp, *Salmonella* spp and *Haemophilus influenzae* are sensitive to TMP (as a representative for other TMP analogues). The following bacteria present a natural resistance to TMP or other benzyl-pyrimidines: *Pseudomonas aeruginosa*, *Acinetobacter*, *Moraxella*, *Neisseria*, *Brucella*, *Campylobacter* spp, *Nocardia*, *Actinomyces*, mycobacteria, *Bacteroides*, *Clostridium* spp.[8] TMP shows a bactericidal action only at multiples of MIC concentrations. In contrast to the sulfonamides TMP has almost no acute toxicity and a superior side-effect profile although at high doses nausea was observed.[8] Although TMP has been used clinically as a monotherapy, it is the combination drug (TMP and sulfamethoxazole) that has been widely used as an antibacterial agent for over three decades. Approximately 60% of the parent compound is excreted in the urine and the high concentration achieved results in an excellent efficacy in urinary tract infections. Further milestones in the application of antimicrobial DHFR inhibitors were the introduction of TMP alone in 1972, the launch of a new combination of tetroxoprim[21] with sulfadiazine (Scheme 1: VI and II) and the successful clinical trials with brodimoprim,[22] which proved clinically efficacious and safe with once-daily low dose monotherapy. Two basic

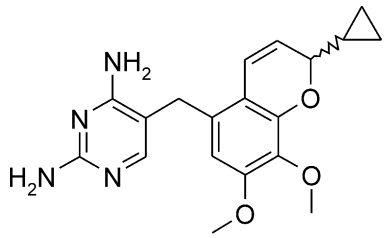
resistance mechanisms have been identified: amino-acid mutations in *S. aureus*, *S. pneumoniae*, *Streptococcus epidermidis*, *E. coli* (several positions were altered[7]) and resistance via a reduction of thymidine dependency.[8]

The target product profiles of new antibacterial DHFR inhibitors could therefore be summarized as improving the in vitro and in vivo activity by enhancing binding properties to both the sensitive and resistant DHFR enzymes. New compounds should either have a broad spectrum of activity, or a specific narrow spectrum profile, for example *Toxoplasma gondii*, *Pneumocystis carinii* (recent publications summarize the results for *Mycobacterium avium*[23] and *P. carinii*[24,25]) and display activity against TMP-, methicillin-, quinolone-, macrolide- and vancomycin-resistant bacteria. In addition, they should be available for monotherapy and combination therapy with differentiated pharmacokinetic properties.

Over the last few decades most major pharmaceuticals have strategically focused almost exclusively on broad-spectrum agents for community infections, and despite intensive efforts (e.g., Burroughs Wellcome and Roche) it has proved difficult to discover DHFR inhibitors with an antibacterial spectrum broader than TMP. Importantly, over the same period, in part due to lack of new therapies, alterations in pathogens and changes in the patient population (increased life span, immunocompromised patients, etc.), there has been an alarming rise in resistance leading to what is today referred to as the 'antibiotic crisis'. In the community *S. pneumoniae* has developed extensive resistance to penicillins, macrolides and quinolones whilst in hospitals *S. aureus* resistant to first and second line therapies is also posing challenges to life-saving drugs such as vancomycin. Several smaller biopharmaceutical companies have focused their attention on the hospital setting.

Roche has used structural information and molecular modeling approaches to design and synthesize diaminopyrimidines that are more active than TMP particularly against Gram-positive pathogens. More importantly, these compounds were designed to gain activity on TMP-resistant strains and in addition showed potent activity against methicillin-, vancomycin-sensitive and -resistant strains. Arpida Ltd., a small biopharmaceutical company, has selected and in-licensed one of these research stage diaminopyrimidines from Roche.[26] Arpida has developed this diaminopyrimidine, INN Iclaprim, which has completed pre-clinical studies as well as Phase I clinical studies. Currently, Iclaprim is in Phase II clinical trials as monotherapy for 'difficult to treat' bacterial infections.

The compound is a racemate (Scheme 3) and Wipf[27] developed a synthesis for one enantiomer. Both enantiomers, after separation by chiral chromatography, exhibit a similar activity against the DHFR enzymes and a similar anti-microbial activity against a broad range of bacteria.[28] Iclaprim specifically and selectively inhibits bacterial DHFR at sub-micromolar concentrations with little or no inhibition of the human enzyme at



Scheme 3. Iclaprim (AR-100, $M_r = 354$).

over 5 orders of magnitude higher concentrations. Iclaprim and TMP potently inhibit DHFR isolated from Gram-negative and Gram-positive pathogens. Against isolated DHFR from the Gram-positive bacteria *S. pneumoniae* and from the fungal pathogen *P. carinii*, Iclaprim is significantly more potent than TMP (Table 1).[29] The mode of action of Iclaprim has also been studied in intact cells using radioactive precursors for the major macromolecular synthesis. Consistent with the selective inhibition of DHFR by Iclaprim and TMP is the observation that both compounds preferentially inhibit DNA and RNA synthesis with little or no effect on cell wall or protein synthesis even at high concentrations.[28]

Microbiological studies against large panels of strains worldwide have demonstrated that Iclaprim is a potent broad spectrum antibacterial agent. Whilst its activity is similar to that of TMP against Gram-negative pathogens, Iclaprim shows a markedly more potent activity against the Gram-positive pathogens, particularly *Staphylococci*. Moreover, Iclaprim shows a potent activity against Gram-positive pathogens resistant not only to TMP but also to methicillin (e.g., methicillin resistant *S. aureus* or MRSA), macrolides, quinolones and glycopeptides (vancomycin/glycopeptide intermediate resistant *S. aureus* or VISA/GISA strains) (Table 2).[29–31] It also exhibits activity against Gram-negative bacteria (*Enterobacter*, *Neisseria*)[32] and important respiratory tract infections (RTI) pathogens[33–35] (*S. pneumoniae*, *H. influenza*, *Moraxella catarrhalis*; Table 2).

Iclaprim, at concentrations close to the minimal inhibitory concentrations, is rapidly bactericidal against Gram-positive pathogens, including resistant *S. aureus* (TMP; MRSA; VISA/GISA, etc.), *S. pneumoniae*, enterococci, as well as against several Gram-negative strains, albeit somewhat slower.[36] As could be expected, Iclaprim also shows strong in vitro synergy with sulfonamides (Sulfamethoxazole, SMX, Scheme 1: I) and very importantly does not exhibit antagonism with any of the major classes of current antibiotics.[33]

Table 1. Inhibition of bacterial and human DHFR enzymes by Iclaprim and TMP[28]

Enzyme	Iclaprim IC_{50} (μM)	Trimethoprim IC_{50} (μM)
Human	**>300**	>300
E. coli	**0.007**	0.007
S. aureus	**0.007**	0.007
S. pneumoniae	**0.008**	0.075
P. carinii	**2.4**	43

Table 2. Antibacterial profile of Iclaprim against Gram-positive and Gram-negative bacteria[a]

Species/antibiotic	Iclaprim	TMP	VAN	LIN	ERY
S. aureus (MSSA)	**0.06**	16	1	8	1
S. aureus (MRSA)	**0.06**	8	1	8	16
S. pyogenes	**0.03**	64	1	2	32
Streptococcus agalactiae	**0.5**	128	0.5	2	8
S. pneumoniae (PEN-R)[c]	**4**	>128	1	2	32
Enterococcus sp.	**0.12**	8	2	4	32
H. influenzae	**0.5**	1	128	64	8
M. catarrhalis	**4**	128	64	8	0.25

[a]MIC_{90} (μg/mL)[b] in comparison to Trimethoprim (TMP), Vancomycin (VAN), Linezolid (LIN) and Erythromycin (ERY).[29–31]
[b]According to NCCLS (2000). National Committee for Clinical Laboratory Standards, 2000, M7-A5, Vol. 20, No. 2.
[c]PEN-R, penicillin resistance.

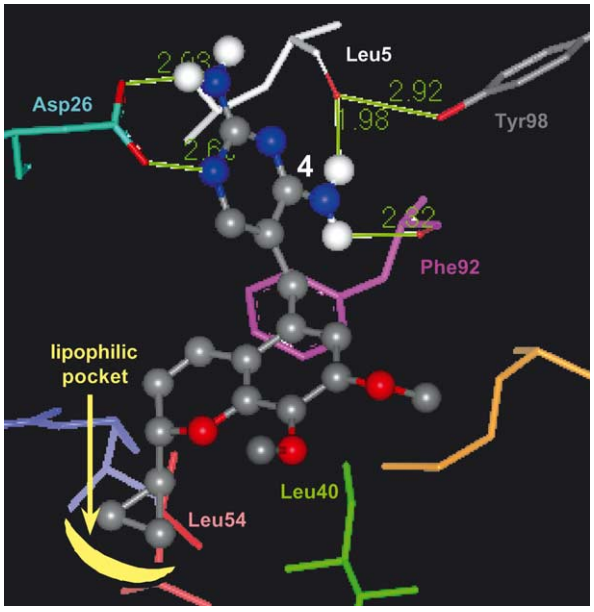


Figure 3. X-ray of co-crystallized Iclaprim in a resistant *S. aureus* DHFR (Phe98 to Tyr98). The distance to Leu5 and Phe92 carbonyls indicate that hydrogen bonds are possible with the 4-amino group. The cyclopropyl group occupies a lipophilic pocket influencing the binding properties of Iclaprim (Roche, unpublished results).

Since the introduction of diaminopyrimidines in clinical use, there has been some development of resistance in bacteria to this class of compounds resulting in reduced susceptibility. However, it is important to note that, compared with other drug classes, even after 3 decades of extensive use of TMP that resistant rates are low (e.g., circa 4% in *S. aureus*). As mentioned above Iclaprim has an impressive in vitro activity against different resistant strains (TMP-, MRSA-, and VISA/GISA resistances). In addition, the propensity for resistance development with Iclaprim is also very low when compared with TMP. Indeed, resistant colonies could not be isolated even with mutagenesis and the calculated frequency of resistance is below 10^{-10}. Similarly, in induction of resistance experiments little or no change in Iclaprim sensitivity was observed even after 15 passages at sub-optimal antibiotic concentration (high level resistance was observed with TMP after 4–5 passages). No differences in induction of resistance was observed between TMP-sensitive and TMP-resistant strains harboring a mutated DHFR.[37]

Table 3. Efficacy of Iclaprim and TMP in murine models of septicaemia and pneumonia[39]

Pathogen	Drug	ED_{50} (mg/kg)
MRSA (septicaemia)	Iclaprim (iv)	4.3
	Iclaprim (po)	17
	Trimethoprim (iv)	15
S. pneumoniae (lung infection)	Iclaprim (sc)	20
	Trimethoprim (sc)	60

In Figure 2, the TMP binding in a resistant *S. aureus* DHFR (mutation of Phe98 to Tyr98) is represented as a sketch based on the reference by Dale et al.[18] A turn of the Phe92 carbonyl and the occupation of the Leu5 carbonyl by Tyr98 disrupt the hydrogen bridges of the 4-amino group of TMP and consequently its binding. Figure 3 represents the active site from an X-ray structure of a resistant *S. aureus* DHFR co-crystallized with Iclaprim. The 4-amino group of Iclaprim is placed in the proximity of the carbonyl groups of Leu5 and Phe92, able to form the hydrogen bridges as observed with the wild-type DHFR enzyme (comparable to Fig. 1). One hypothesis could be that a new interaction of a lipophilic pocket with the cyclopropane group 'pushes' the diaminopyrimidine into the binding site strengthening these hydrogen bridges. These additional binding properties could explain the marked difference in the properties of this novel diaminopyrimidine when compared with TMP. The X-ray also shows that the cyclopropyl ring of the *R*-enantiomer occupies a transoid pseudo-axial position (Fig. 3). One possible explanation for the similar inhibitory and antibacterial activity of the *S*-enantiomer can be based on a lowest energy conformation analysis.[38] A transoid pseudo-equatorial position is seen in this enantiomer. In other words, superimposing the diamino-pyrimidine rings, both enantiomers align part of the cyclopropyl rings close to a similar space. This might be a likely explanation for the comparable binding properties.

Iclaprim has been shown to be efficacious in different animal models of infection (Table 3).[39] Iclaprim is currently in a Phase II clinical trial in complicated skin and soft tissue infections.

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