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An overview on dihydrofolate reductase inhibitors

Shinde GH*, Pekamwar SS and Wadher SJ

School of Pharmacy, SRTM University, Nanded, Maharashtra, India.

*Corresponding Author: E-Mail: gajushinde123@gmail.com

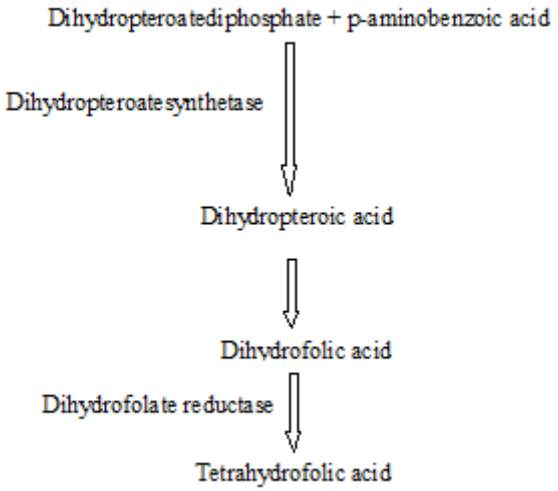
ABSTRACT

Dihydrofolalate reductase (DHFR) inhibitors are of significant interest as potential tool against parasites and other microbial infections. Dihydrofolate reductase is a ubiquitous enzyme present in almost all eukaryotic and prokaryotic cells and has potential importance in biochemistry and medicinal chemistry. It catalyses the various biochemical reactions are of vital importance, inhibition of dihydrofolatereductase plays important role in controlling growth of various parasites. Inhibition of dihydrofolate reductase becomes very useful tool for controlling number of diseases and disorders like cancer, malaria, toxoplasmosis, rheumatoid arthritis and tuberculosis. The various drugs are also available which are based on inhibition of dihydrofolate reductase. This article presents overview on various potential applications of dihydrofolate reductase inhibitors.

Key words: Dihydrofolalate reductase, Malaria, Enzyme, folic acid.

1. INTRODUCTION

DHFR enzyme catalyzing the reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate with $NADP^+$Oxidoreductase being a coenzyme in this process. Tetrahydrofolate is source of methyl group in synthesis of purine, amino acid and thymidylate.Antifolate is oldest antimetabolite class of anticancer drugs due to its biological importance. DHFR has been used as target for antineoplastic, antiprotozoal, antifungal and antimicrobial[1]. DHFR is a medium-sized enzyme with a molecular weight of about 20,000. We chose to study the enzyme DHFR due to its clinical relevance. Its function is to activate the vitamin folic acid by catalyzing the oxidation-reduction reaction where dihydrofolate is an inactive form of folic acid, and tetrahydrofolate is its activated form[2].The Dihydrofolate Reductase inhibitors catalyses the NADPH-dependent reduction of Dihydrofolate to tetrahydrofolate and it play vital role in the synthesis of the several amino acid, thymidylate and purines. Inhibition of DHFR enzymes restricts the growth of cell orDNA synthesisand causes cell death. These enzymes are the targeted by the DHFR inhibitors in the bacterial, protozoal and some neoplastic infections [3]. DHFR is essential enzyme for biosynthesis of DNA and cellular replication. The polypeptide chain of human DHFR is composed of 159 amino acids which is short as compared with Escherichia coli having 229 amino acids. The vertebrate DHFR is slightly larger than bacterial DHFR.It is found in all eukaryotic and prokaryotic organisms. Tetrahydrofolate and its analogue are essential for thymidylate and purine synthesis which is essential for cell growth and cell production[4]. It also play major role in synthesis of nucleic acid precursor[5].DHFR catalyzes the transfer of a hydride from NADPH to dihydrofolate with an associated protonation to produce tetrahydrofolate[4].In the end, dihydrofolate is reduced to tetrahydrofolate and NADPH is oxidized to $NADP^+$. The high flexibility of Met20 and other loops near the active site play a role in support the release of the product, tetrahydrofolate. The Met20 loop helps to stabilize the nicotinamide ring of the NADPH to promote the transfer of the hydride from NADPH to dihydrofolate.



Tetrahydrofolic acid Plasmodium falciparum DHFR-TS (TS-Thymidylatesynthetase) amino acid sequence is differently resistance to cycloguanil and pyrimethamine. Due to alternative mutation at same site pyrimethamine and cycloguanil resistance was developed[6].DHFR inhibitors inhibit or mimic the pteridine ring of the natural analogue of dihydrofolate and complete coils it for the active site of the enzyme[7].The DHFR is essential for the catalysis of NADPH dependent reduction of Dihydrofolateto tetrahydrofolate a necessary co-factor for biosynthesis of purine, thymidylate and certain amino acids[8].Pneumocystis carinii is a vital cause of morbidity and mortality in immunosuppresed patients. The DHFR inhibitors like Pyrimethamine, Trimethoprim, and Diaveridine, has only effect on parasite growth inhibition and sometimes causes side effects in AIDS patients. The in-vitro activities of lytic peptides alone and in combination with macrolide are effective against the inhibition of p. carinii[9].The combination of pyrimethamine and sulfonamide is now effective against toxoplasmosis especially in case of immunosuppresedpatient [10].The drug combination like sulphadoxine-pyrimethamine used against plasmodium falciparum in Haiti due to development of resistance to chloroquine. Evaluation of DHFR and dihydrofolatesynthetase against that combination and found that is alternative treatment against malaria parasite in Haiti[11].Production and charactrisationof the DHFR from human lymphoid cell line having methotrexate resistance. Human lymphoid cells are characterized and sequenced and found methotrexate resistances are different from the normal human cells[12].The effect of pH on inhibition of a partially purified DHFR from Ehrlich ascites cells by amethopterin has studied. They find complete inhibiton of the reduction of DHF at 5.9 and 7.5 pH is responsible for binding both substrate at same or enzyme site[13].[Figure 1]. (PDB code 1hfq)

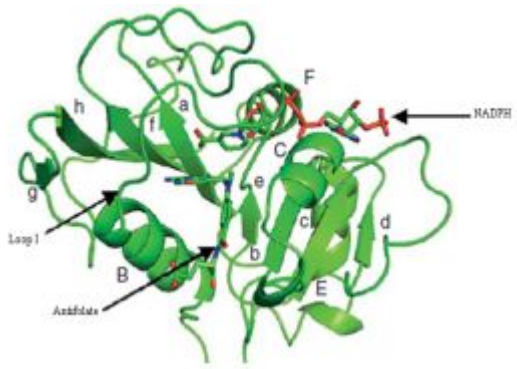


Figure -1: **α**-helices are labeled with upper case letters and **β**-Sheets are labeled with lower case letters

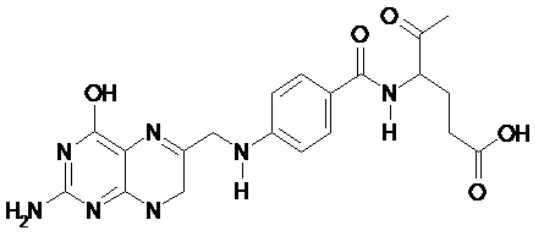


Figure - 2: Structure of Dihydrofolate

1.1. DHFR inhibitors

DHFR inhibitors are classified in to two classes as classical and nonclassicalantifolate respectively. The classical antifolate bears p-aminobenzoylglutamic acid side-chain in their structure. The well known example is methotrexate. The nonclassicalantifolate not having p-aminobenzoylglutamic acid side-chain in their structure but they have a lipophilic side-chain [14-21].

1.2. Classical DHFR Inhibitors

The classical dihydrofolate inhibitors having structural similarity with folic acid. These consist of a pteridines, aryl and a glutamyl group. These substances are readily glutamated by the intracellular enzyme folylpolyglutamylsynthetase and it is known that polyglutamated form is primarily responsible for dihydrofolate reductase inhibition in vivo. The methotrexate is N-{4-[[(2, 4-diamino-6-pteridyl) methyl]-N10-methyl-amino]} benzoylglutamic acid. Now clinically they have more importance together with trimethoprim.

1.3. Non-classical DHFR Inhibitors

Nonclassicalantifolate does not have structural similarity with folic acid. They are not susceptible to glutamation. These generally consist of aryl moiety, diaminopyrimidine moiety, or an aryl moiety and a pteridine moiety. The Non-classical DHFR Inhibitors having lipophilic side-chain.Due to this side chain more potent analoguesare developed to overcome the side effect associated with classical antifolate.

1.4. Study on DHFR inhibitors

1.4.1. Synthesized compound

In successful treatment of the pathologic conditions like toxoplasmosis and malaria it is essential to target DHFR in parasitic protozoa. The trimethoprim-sulfamethoxazole combination is alternative in the treatment of infection due to toxoplasmosis and it also already used in treatment of Pneumocystis pneumonia [22].

M. Graffner-Nordberg et al has performed design, synthesis and computational affinity prediction of analogue of ester soft drugs

prepared by replacing methylamino-bridge of non-classical inhibitors with ester group and found to inhibit dihydrofolatereductasefrom Pneumocystis carinii, the (2,4diaminoquinazone) 6-yl)methyl 1-napthoate was more potent new ester based DHFR inhibitor but less than trimetrexate to suppress the host toxicity[23].

A. Rosowsky et al has studied New 2,4-Diaminopyrido[2,3-d]pyrimidine and 2,4-Diaminopyrrolo[2,3-d]pyrimidine Inhibitors of Pneumocystis carinii, Toxoplasma gondii, and Mycobacterium aviumDihydrofolateReductase and found 2,4-Diaminopyrido[2,3-d]pyrimidine show 10-fold selectivity for Toxoplasma gondii verses rat DHFR[24].

X.Ma, W. K. Chuihas performed study on development of new 6,8,10-triazasprio[4.5] deca-6, 8-dienes and 1,3,5-trazaspiro [5.5] undeca-1,3 dines having antifolate and antiproliferative activity against AS49 cell in human lungs cancer cells with invitro mammalian DHFR inhibitory activity. They identified potent mammalian DHFR inhibitors containing 6,5-spiro bicyclic ring system and 6,6-spiro bicyclic system[25].

M. A. Santos et al has synthesized methotrexate c-hydroxamate derivatives targeting two families of enzyme i.e metalloproteinase and DHFR. The new hydroxamate analogue of methotrexate shows good result in micromolar and nanomolar range against DHFR in cancer cell. This also having good antiproliferative activity against A549 CELL [26].

Senkovich et al studied lipophilic antifolate of trimetrexate is potent against trypanosomacruziin in chagas disease. The trimetrexate is an FDA approved drug used in treatment of Pneumocystis carinii infection in AIDS patient[27].P. Pineda et al has performed study on exceptional resistance to methotrexate but not to trimetrexate DHFR mutant. They found F31A/ F34A mutant is catalytically active and resistant to methotrexate, bis-methotrexate and PT 523 but not trimetrexate[28].

I.O.Donkor et al havesynthesised 7 new 6-aralkyl substituted 2,4-diminothiono (2,3-d) pyrimidine and found that aromatic substitution6-position influences selectivity and potency of compounds[29].The enzymes like DHFR-TS and trypanothionereductase areimportant enzyme for the metabolism of protozoan parasites from the family trypanosomatidae that are now target for novel drug design study. They found most of genetic difference due to the difference among sequence clades from this sequence clade provides information about amount and type of genetic variation of DHFR-TS and TR gene in natural population of this parasite[30].

The drug like epiroprim and dapsonealone or in combination helpful in the treatment of mycobacterium ulcerans infection. The dapsone in combination with epiroprim shows the synergic effects against mycobacterium ulcerans DHFR[31].

1.4.2. CADD Study on DHFR inhibitors

Osvaldo A et al has performed CoMFA/CoMSIA 3D-QSAR study of pyrimidine inhibitors of Pneumocystis cariniidihydrofolatereductase. In their study they used 64 pyridine derivatives from which 51 compound in training set and 13 compoundsas test set.They found 3D QSAR model with good prediction power and is statisticaliyvalid [32].

Saumya K. Patel et al has performed in silico2-D QSAR study. The 2-D QSAR model is based on the certain topological and constitutional descriptors. Methotrexate is a DHFR inhibitor which is given in the treatment of rheumatoid arthritis, due to potential neurotoxicity, the patients has to discontinue the chemotherapy. In present study DHFR inhiditors which were structurally similar to methotrexate had reported biological activity in model organism such as toxoplasma gondii and lactobacillus caseiwereconsidered [33].

In protein evoulation or DHFR evolution is essential to exsisttrade off between robustness and evolvability. The resent research clear that existing phenotype can be robust to the evolution of novel protein function. They assay growth rate and resistance of all 48 combination of 6 DHFR mutation associated with the increased drug resistance. They observe no regular relationship between growth rate and resistance phenotype among the DHFR alleles[34].

N.M. Goodeyhas study on Prediction of residues involved in inhibitor specificity in the dihydrofolate reductase family and gives a novel approach for determining residues involved in the ligand discrimination in a protein family using DHFR as a model system. They found that 18 alignment positions were identified with a strong correlation of similarity in inhibitory specificity out of this 3 lies in active site, 4 lies proximal to active site and 4 clustered together in adenosine binding and 5 on βFβG loop. They also conclude this approach is easier to use. DHFR is a validated drug intention for therapeutics directed against the diseases of the parasitic protozoa: malaria, toxoplasmosis and cryptosporidiosis. Pyrimethamine and cycloguanil are two DHFR inhibitors that have been successful in the fight against malaria like pyrimethamine and trimethoprim have been successful against toxoplasmosis. Despite the progressing success of

these derivatives, however, there is a specific need to develop new DHFR inhibitors for sensitive and resistant strains of Apicomplexan parasitic protozoa. DHFR inhibitors, especially chlorocycloguanil and WR99210, show promising activity against resistant strains of malaria parasites [35].

L. Adane and P. V. Bharatamhas performed in silico study by using computer-aided molecular design approach that involved ab initio molecular orbital and density functional theory calculations, along with molecular electrostatic potential analysis, and molecular docking studies were employed to design 1H-imidazole-2,4-diamine derivatives as potential inhibitors of Pf-DHFR (Pf-plasmodium falciperum) enzyme. Visual inspection of the binding modes of the compounds demonstrated that they all interact, via H-bond interactions, with key amino acid residues (Asp54, Asn/Ser108, Ileu/Leu164 and Ile14) similar to those of WR99210 in the active site of the enzymes used in the study. These interactions are known to be essential for enzyme inhibition.In silico toxicity was performed by using TOPKAT software and found compounds are non-toxic [36].

Senthilraja P et al has performed experiment on potential of mangrove derived compounds against DHFR i.e Insilco study. They used Argus lab software for docking study and studied nine mangrove derived compounds among these five compounds namely tretinoin, stigmasterol, triterpenoid, rubrolide and pyrethrin shows good docking energy scores against DHFR and found could be novel DHFR inhibitors[37].

The construction of ligand-receptor binding models by using free energy force field 3D QSAR analyais for the 18 structurally different compounds like pyrimethamine, cycloguanil, methotrexate, aminopterin, trimethoprim and 13 pyrrolo [2,3-d] pyrimidines from these 3D QSAR analysis or models indicate important descriptors and structural features that relevant to resistance of current antimalarial drug[38].

X. Li et al using Structure-based design of 7-aryl-2,4-diaminoquinazolines new DHFR-based antibacterial agents. Traditionally, the majority of antibacterial DHFR agents are based on the 5-benzyl-2, 4-diaminopyrimidine due their good potency and selectivity for bacterial as well as mammalian DHFR. The most widely used DHFR antibacterial agent is trimethoprim, the use of the trimethoprim from the past as antibacterial but have resistance created by the gram positive and gram negative bacteria. To overcome this problem more potent 5-benzyl-2, 4- diaminopyrimidines have been discovered as exemplified by iclaprim (ICL) a drug that has been clinically investigated through Phase 3 trials [39].

N. M. Goodey et al has performed study on conformational changes associated with inhibitor binding in development of a fluorescently labelled thermostable DHFR. The confirmationally sensitive bacillus starothermophillus DHFR were developed and which is used to determine the kinetic and protein conformational changes with the methotrexate binding and provides a new tool for flurophore[40].

NutanPrakash et al has performed molecular docking study on antimalarial drugs. Due to the common side effects of the marketed drugs it is essential to improve or synthesise new analogue with higher binding properties. In their study they optimise a proguanil using argus lab and Hex software and they found that some of the modified analogues are better than the commercial marketed drugs [41].

W.EdwardMartucci et al have performed in silico study using virtual screen of novel non-active site inhibitors of cryptosporidium hoministhymidylate synthase DHFR. From this screening they identified and characterise noncompetative inhibitors and concludes that novel allosteric pocket amnable to inhibitor targeting and lead compound which helpful in designing novel, potent and selective inhibitor of thymidylateDHFR [42].

VivekSrivastava et al Used molecular modelling in docking of 2, 4-diamino-5-methyl-5-deazapteridine derivatives by using 78 compound 2, 4-diamino-5-methyl-5-deazapteridine derivatives. A new shape-based method, LigandFit, was used for docking study of their derivatives. The result indicates that the molecular docking approach is reliable and produces a good correlation coefficient (r2 = 0.499) for the 73 compounds between docking score and IC50 values. By the use of this approach a novel DHFR analogue of 2, 4-diamino-5-methyl-5-deazapteridine was synthesized [43].

D. Hachet et al hadperformed Quantitative structure–activity relationship. Models were developed for dihydrofolate reductase (DHFR) inhibition by pyrimethamine derivatives using small molecule descriptors derived from MOE and QikProp and linear or nonlinear modeling. During this analysis, the best QSAR models were identified when using MOE descriptors and nonlinear models (artificial neural networks) optimized by evolutionary computation. The resulting models can be used to identify key descriptors for DHFR inhibition and are useful for high-throughput screening of novel drug leads [44].

The in silico study on inhibitors of quadrapole mutant plasmodium falciparum DHFR by using 32 pyrimethamine derivatives in to its active sites by using GOLD. The used x-ray crystal structure 1 J3K.pdb. this approach is used to found the new labraries of their analog in future development of antimalarial drugs discovery. The model generated providing detail about binding mode, molecular interaction, binding affinities for antimalarial drugs [45].

V. M. Popov et al have performed analysis complex of inhibitors with cryptosporidium hominis DHFR. They docked several trimethoprim derivatives having substitution at c7 position and found to interact with cys113. They synthesized and evaluated these C7 trimethoprim derivatives and found that are four time more potent than the parent compound [46].

3D-QSAR model developed by using COMSIA and study 406 structurally different DHFR inhibitors from Pneumocystis carinii and rat liver. They also found that this approach is helpful in the designing potent selective inhibitors and estimating their activity prior to synthesise their analogue [47].

In silico study on 4 - (3H)-Quinazolinone Derivatives which is resembles methotrexate and fit these compounds with active site of human DHFR by using simulation method such as Glide module. Docking of these molecules with individual DHFR target [48].

Rastelli et al has performed docking and data base screnning of new class of plasmodium falciparum DHFR inhibitors. In their study they used specific 3D pharmacophore which is essential for the interaction with enzyme active site [49].

A. I. Suetal has performed molecular docking for reducing variety of compound and suggest novelty of the compound to attempt the variation in the docking list. They docked all derivatives among these only best scoring molecules of high ranking family was allowed in the hit list. Then finally family based docking method was compared with molecule by molecular docking and screen against the structure of thymidylate synthase DHFR and active siteof the mutant T4 lysozome Leu993 Ala this an general approach applicable in every molecular docking study[50].

The technique like molecular dynamics simulation for selective analysis of 5-(arythio)-2,4diaminoquinqzdines. The study was performed on the 10 compounds with difference in structure and activity. These inhibitors are against the candida albicans DHFR. The inhibitory potency and selectivity of compound governed by the favorable binding and specific hydrogen bonding interaction within the active site of the fungal DHFR [51].Enzyme inhibition system is used for identification of potential antimalerials against highly drug resistant mutants of plasmodium falciperumDHFR. The DHFR of different sources used for inhibition studies. This system is used for identifying the new antifolate having high potency and selectivity against drug resistant parasites of malaria [52].The computational approach is used to check affinity and selectivity of 2,4diaminopteridine and 2,4 diaminoquinazoline as inhibitors of DHFR. The binding energy was calculated by using a force field evaluation and thermal sampling by using molecular dynamic simulation. There is no major difference between pteridine and quinazoline binding strength. This approach is helpful in new drug discovery for determination of binding energy of new inhibitors before synthesis [53].

MichelaZolli-Juran et al have performed high-throughput screening tecnique for diverse library of 50,000 small molecules against Escherichia coli dihydrofolate reductase to detect inhibitors. Sixty-two compounds were identified as having significant inhibitory activity against the enzyme. Secondary screening of these revealed twelve molecules that were competitive with dihydrofolate, nine of which have not been previously characterized as inhibitors of dihydrofolate reductase. These novel molecules ranged in potency from 26 nM to 11 mM and may represent fresh starting points for new small molecule therapeutics directed against dihydrofolate reductase [54].

The fragment-based method used in the screening of the dihydrofolate reductase inhibitors compounds like MOLPRINT 2D. Here they used original training set of 50,000 compounds, only marginal enrichment factors could be achieved on the test library. The test and training set structure represents the different structural features associated with activity[55].

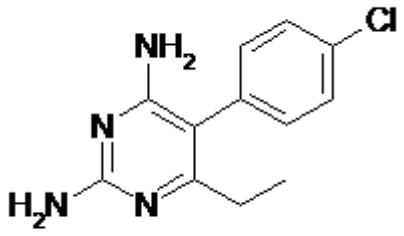
Plasmodium falciparum dihydrofolatereductase three-dimensional structures have now been resolved by a group based in Thailand. This group carried work based on the predictive and crystallographic structures of dihydrofolate reductase protein and on their gene mutagenesis experiments to known the nature and interaction of amino acid residues in the active site of the dihydrofolate reductase by using reference drugs like cycloguanil, WR99210 and pyrimethamine. Based on this basic information the in future various derivatives were synthesised[56].

F.Zucctto et al havesynthesised novel trypanosomacruzi DHFR inhibitor by using homology model of enzyme to search combridge structural database by using dock 3.5 program. They study novel non 2,4 diaminopyrimidine lead structure against T.Cruzi DHFR by using computer aided screening technique[57].The designing of the novel class potent and small peptides inhibitors against mycobacterium tuberculosis DHFR by using in silico structure based approach for antituberculosis drugs discovery. There is a little difference between the human DHFR and mycobacterium DHFR active site. This difference is helpful for designing of new class of Anti-tubercular drugs. Doking study on the designing tripeptide inhibitors indicated that have high potency and selectivity. A set of 2,4- diamino-5-dazapteridine derivative was now proposed. Due to the resent advances in the drug delivary technique, the opputinities for peptide drug development significantly enhanced. The designing of small peptides by using structure based approach with the help of crystal structure of mycobacterium tuberculosis DHFR complex with NADPH and methotrexate and human DHFR complexed NADPH with PT523[58].Interaction studies of DHFR inhibitors with the help of steady state kinetic and high field ^{1}H-NMR spectroscopy and study shows two binding sites for the sulphonamide on enzyme [59].Analysis of DHFR-TS gene sequence in plasmodium vivax among the Korean strains isolates that failed chloroquine treatment and pyrimethamine is an alternative drug choice in the chloroquine resistance vivax malaria in Korea[60].

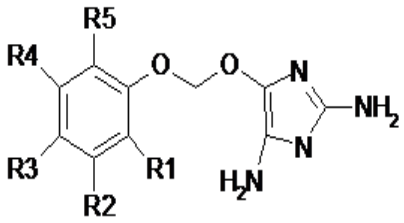
Inhibition of DHFR an enzyme that catalyses 5,6,7.8-tetrahydrofolate synthesis have been used as antimicrobial as well as antimetabolite drugs for a long time. Although structurally belonging to different classes, the majority of DHFR inhibitors cntain 2,4 diamino substitution on pyrimidine ring, with aim to introduce pyrimido-pyrimidine as a novel class of bacterial DHFR inhibitors, 42 compound belong to this class has been tested and compared with 18 pteridines using cell and enzyme models and docking studies. A few pyrimido-pyrimidine compounds show high potency and selectivity as inhibitors of bacterial DHFR[61].

1.4.3. Current DHFR Inhibitor drugs

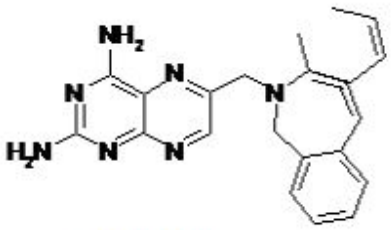
Aminopterin, Iclaprim, Brodimoprim, Trimethoprim, Pralatrexate, Trimethoprim/polymyxin, Proguanil, Methotrexate, Pemetrexed, Tetroxoprim, Pyrimethamine, and Trimetrexate



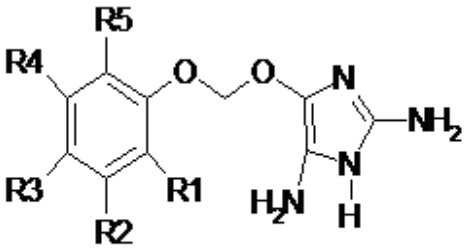
Pyrimethamine



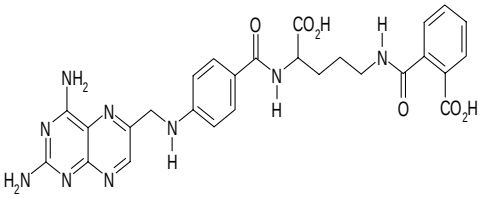
5-methyl,1H- Imidazole 2,4 - diamine



Dibenzazepine



Methyl 1H-imidazole 2,4-diamine



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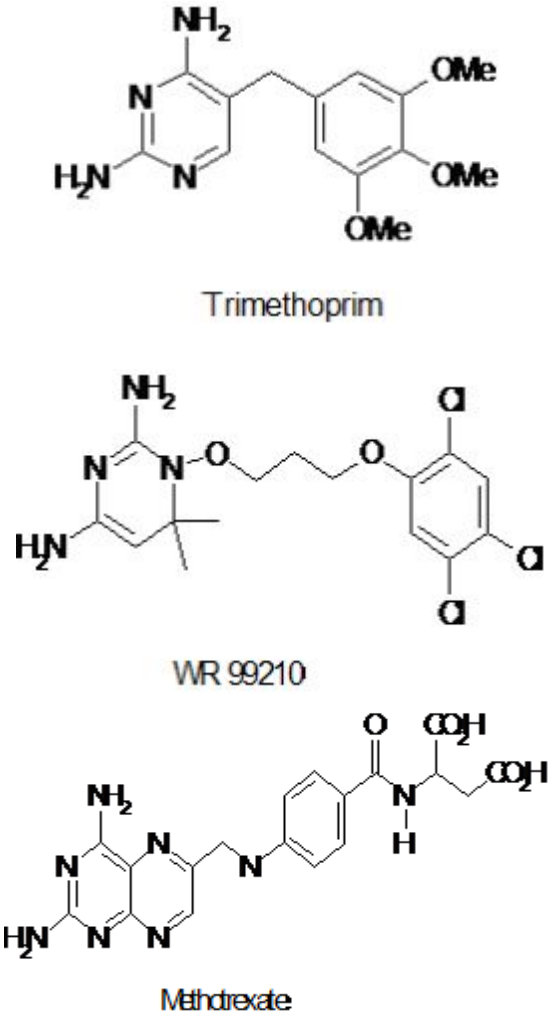

Figure - 3: Structure of DHFR Inhibitors

2. CONCLUSION

Dihydrofolate reductase is a vital constituent of all living organism which catalyses the NADPH-dependent reduction of Dihydrofolate to tetrahydrofolate. They also play important role in the synthesis of the several amino acid, thymidylate and purines. They play important role in various pathologic conditions like cancer, tuberculosis etc.It is concluded that DHFR is a potential target for development of novel analogues of DHFR inhibitors. The crystal structure of various DHFR strain is available which is helpful in designing of the rational inhibitors in future. The various approaches of in silico study also useful in designing of the novel, potent and selective inhibitors of DHFR.

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