Methotrexate is often used for the treatment of cancer, but it is also being used at low doses to treat rheumatoid arthritis. It has been thought that by preventing synthesis of purines and pyrimidines needed for DNA and RNA synthesis through the inhibition of dihydrofolate reductase, proliferation of quickly dividing lymphocytes and other cells that cause synovial inflammation can be prevented. Thus, patients could receive relief from symptoms of rheumatoid arthritis. However, when using methotrexate in rheumatoid arthritis patients, it is important to be aware that methotrexate interacts with many other medications.

Dihydrofolate reductase, pictured in Figure 1, reduces dihydrofolate to tetrahydrofolate. Tetrahydrofolate acid is a coenzyme that is important for many reactions, especially in the metabolism of amino acids and nucleic acids. Methotrexate and trimethoprim are both folate analogues that bind to and inhibit dihydrofolate reductase resulting in a decreased concentration of reduced folate cofactors. Figure 2 shows the region of methotrexate that binds to dihydrofolate reductase. These reduced folate cofactors are essential for the synthesis of nucleic acids. Therefore, coadministering methotrexate and trimethoprim results in an additive effect of inhibiting dihydrofolate reductase that can lead to increased toxicity levels.

As shown in Figure 3, both methotrexate and folic acid consist of a pteridine moiety, PABA, and glutamic acid. The main difference is that methotrexate has a 2,4-diaminopyrimidine unit, resulting from replacing the OH group with an amino group at the C-4 site.

Both methotrexate and trimethoprim interact with the same amino acids (Figure 4; shown in pink).
- Glu30A via -Hydrogen bond interactions
- Ile 7A as a -Backbone contact
- Phe 34A via -Pi-Pi interactions

Methotrexate also interacts with two additional amino acids (shown in blue).
- Phe 31A via -Pi-Pi interactions
- Val 115 as a -Backbone contact

Methotrexate on the left and trimethoprim on the right. Blue are nitrogen, red are oxygen, and grey are carbons. Bond lengths between the drugs and dihydrofolate reductase show that methotrexate binds tighter to the receptor. (pdb files 1MV7 and 353V, respectively)