Three different approaches have been developed to improve the biostability of 5-FU:

1. The first consists of the co-administration of a large amount of uracil, which saturates the DPD enzyme because uracil is its natural substrate; for instance, the formulation known as UFT uses a 4:1 ratio of uracil and the 5-FU prodrug tegafur.[38] The combination of UFT with leucovorin calcium was called Orzel®.[40]
2. An alternative is the coadministration of 5-FU with DPD inhibitors, such as 5-chloro-2,4-dihydroxypyridine (CDHP; gimeracil, gimestat) and eniluracil (5-ethynyluracil),[41] as well as the use of the UFT combination plus DPD inhibitors.[42] In 2005, eniluracil received FDA orphan drug designation for its use in combination with fluoropyrimidines in the treatment of hepatocellular cancer. Although the eniluracil–5-FU combination (in a 10:1 ratio) tended to produce less antitumor activity than the control therapy in two phase III trials, it was subsequently discovered that the dose and schedule used might not have been optimal because high eniluracil–5-FU ratios decrease antitumor activity.
3. Finally, emitefur (BOF-A2) is an orally active drug that was designed as a mutual prodrug of 5-FU and a DPD inhibitor, namely 5-cyano-6-dihydroxypyridin-2(1*H*)-one. Two consecutive hydrolytic steps liberate the DPD-inhibiting fragment, and a third hydrolysis, followed by an oxidative activation involving the loss of two molecules of acetaldehyde, furnish 5-FU avoiding high peaks of this drug and decreasing the formation of toxic metabolites (Figure 2.26). Emitefur entered clinical trials for colorectal cancer,[43] but later studies showed typical fluorouracil-related toxicities, with some patients experiencing more severe toxicity, and its development was discontinued.

4.4.2 Enhancement of the Inhibition of Thymidylate Synthase by 5-FU

The action of TS requires the presence of 5,10-methylenetetrahydrofolate, and for this reason the coadministration of precursors of this cofactor increases the cytotoxicity of 5-FU in many cancer cell lines. For instance, the combination of 5-FU or tegafur with leucovorin (5-formyl-THF) gave superior response rates compared to those of the single agents, and particularly the use of leucovorin to modulate the uracil–tegafur combination leads to a three-component combination called Orzel® that has been proposed as first-line chemotherapy of colorectal cancer.[44] Another important combination is Folfirinox®, which contains leucovorin and 5-FU together with irinotecan and oxaliplatin and is used to treat metastatic pancreatic cancer.

Leucovorin enters the cell via the reduced folate carrier and is metabolized to 5,10-methylene-THF, without requiring the participation of DHFR, by cyclization to 5,10-methenyl-THF followed by NADP-mediated reduction of the iminium function (Figure 2.27). The International Nonproprietary Names (INN) name for leucovorin is folinic acid, but this may cause some confusion because in biochemistry it is often employed as a collective name, comprising 5-formyl-THF but also other related compounds.

4.4.3 Enhancement of 5-FU Activation

It has been proposed that pretreatment with methotrexate, an antifolate agent, enhances the activity of 5-FU[45] because methotrexate inhibits the biosynthesis of tetrahydrofolic acid (THF), which is necessary for some steps of purine biosynthesis (see Section 6). This leads to accumulation of phosphoribosyl pyrophosphate, essential for the activation of 5-FU, even though the levels of the TS cofactor should also be diminished (Figure 2.28). Clinically, this combination has not always shown increased antitumor activity.[46] On the other hand, several phase II studies have shown a modest clinical benefit of 5-FU modulation utilizing methotrexate and leucovorin in patients with metastatic colorectal cancer.[47]