Our current knowledge of the isomerase glyoxalase 1 and the thioesterase glyoxalase 2 is based on a variety of prokaryotic and eukaryotic (model) systems with an emphasis on human glyoxalases. During the last decade, important insights on glyoxalase catalysis and structure-function relationships have also been obtained from parasitic protists. These organisms, including apicomplexan and kinetoplastid parasites, are particularly interesting, both because of their relevance as important pathogens, and because of their phylogenetic diversity and host-parasite coevolution which has led to specialized organelar and metabolic adaptations. Accordingly, the glyoxalase repertoire and properties vary significantly among parasitic protists of different major eukaryotic lineages (and even between closely related organisms). For example, several protists have an insular or non-canonical glyoxalase. Furthermore, the structures and the substrate specificities of glyoxalases display significant variation. The aim of this review is to highlight such differences as well as similarities between the glyoxalases of parasitic protists and to emphasize the power of comparative studies for gaining insights into fundamental principles and alternative glyoxalase functions.