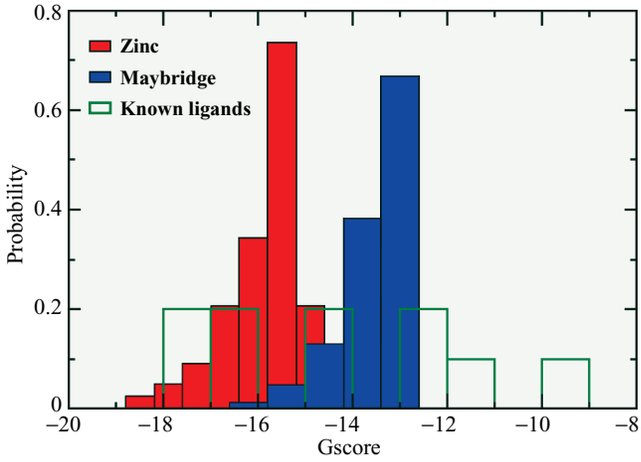


**Figure 1.5.** Distributions of the Glide XP scores for the top-ranked 1,000 ZINC compounds, the top-ranked 1,000 Maybridge compounds, and the 10 known tautomerase inhibitors.

constructive modifications. Specifically, the substituents were removed to yield the anilinylbenzyloxadiazole core. A set of small substituents was reintroduced in place of each hydrogen using BOMB; scoring with BOMB, followed by free-energy perturbation (FEP)-guided optimization, led to synthesis and assaying of several polychloro analogs with $EC_{50}$ values as low as 310 nM in the MT-2 HIV-infected T-cell assay.[5] Further cycles of FEP-guided optimization led to novel, very potent NNRTIs, including the oxazole derivative **4**, as described more below.[24]

A more recent virtual screening exercise was strikingly successful.[25] New protocols had evolved, including use of the much larger ZINC database of approximately 2.1 million commercially available compounds.[26] The goal in this case was to disrupt the binding of macrophage migration inhibitory factor (MIF) to its receptor CD74, an integral membrane protein, and a major histocompatibility complex (MHC) class II chaperone. MIF is a pro-inflammatory cytokine that is released by T-cells and macrophages. It plays a key role in a wide range of inflammatory diseases and is involved in cell proliferation and differentiation and angiogenesis.[27,28] Curiously, MIF is also a keto-enol isomerase. There is evidence that the interaction of MIF with CD74 occurs in the vicinity of the tautomerase active site and that MIF inhibition is directly competitive with MIF/CD74 binding.[29] The docking was performed using Glide 4.0 and the 1ca7 crystal structure of the complex of MIF with *p*-hydroxyphenylpyruvate.[30] In addition to the ZINC collection, the Maybridge HitFinder library was screened, which provided an additional 24,000 compounds. After all structures were processed using SP Glide, the top-ranked 40,000 from ZINC and 1,000 from Maybridge were redocked and rescored using Glide in XP mode.[23] Glide XP scoring was also shown to provide good correlation with experimental data for 10 known inhibitors of MIF's tautomerase activity.

A key observation from the docking is illustrated in Figure 1.5, which shows the distributions of Glide XP scores for the top-ranked 1,000 compounds from ZINC, the top-ranked 1,000 Maybridge compounds, and the ten known MIF inhibitors. Clearly, the large ZINC collection yields many compounds with much more promising XP scores than the Maybridge HitFinder library. The average molecular weights for the two sets of 1,000 compounds are 322 for ZINC and 306 for Maybridge. The variation only amounts to one additional nonhydrogen atom for the ZINC set, so the improved performance with the ZINC collection presumably results from greater structural variety. In view of the sensitivity of activity to structure, as reflected in Figures 1.3 and 1.4, it is highly unlikely that active compounds can be found in small libraries like Maybridge HitFinder unless the assays can be run with the compounds at millimolar or higher concentrations, which is often precluded by solubility limits. Even with a viable core (Figure 1.4), the chance is low that a small library will contain a derivative with a substituent pattern that yields an active in a typical assay.

Finally, the Glide poses for approximately 1,200 of the top-ranked compounds were displayed and 34 compounds were selected by human evaluation of the poses with input from QikProp on predicted properties and structural liabilities. The filtering included rejection of poses where the conformation of the ligand was energetically unlikely or where there were overly short intramolecular contacts and compounds with generally undesirable features such as readily hydrolizable functional groups or substructures such as coumarins, which are promiscuous protein binders. Only 24 of the 34 selected compounds were, in fact, available for purchase, which represents a typical ratio. Ultimately 23 compounds were submitted to a protein-protein binding assay using immobilized CD74 and biotinylated human MIF with streptavidin-conjugated alkaline phosphatase processing *p*-nitrophenyl phosphate as substrate. Remarkably, eleven of the compounds were found to have inhibitory activity in the μM regime including four compounds with $IC_{50}$ values below 5 μM. Inhibition of MIF tautomerase activity was also established for several of the compounds with $IC_{50}$ values as low as 0.5 μM. Representative active compounds are shown in Figure 1.6; optimization of several of the lead series is being pursued. Notably, these are the most potent small-molecule inhibitors of MIF-CD74 binding that have been reported to date.

The first three compounds in Figure 1.6 were ranked in 285th, 696th, and 394th place by the XP scoring, so they were not "high in the deck." However, prior de novo structure building with BOMB had indicated that 6–5 fused bicyclic cores should be promising, so the selections were biased in this direction. The compound ranked first with XP Glide was also purchased and assayed; it turned out to be the 250-μM inhibitor in Figure 1.6. In addition, the compounds ranked 26th and 32nd were purchased and found to be inactive. Overall, it is expected that contributors to the success with the virtual screening in this case were