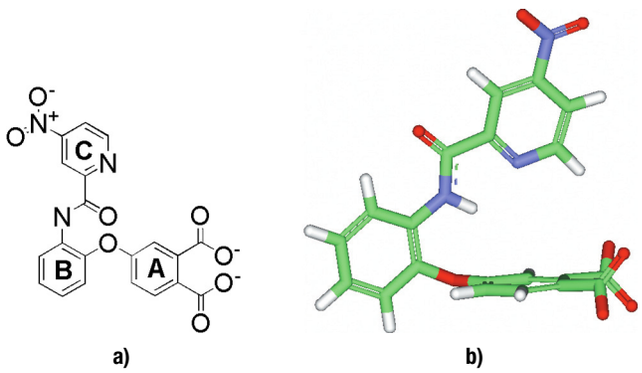

Figure 17.3. Lead compound **1a**. **(a)** Chemical structure of lead compound **1a**. **(b)** The lowest energy conformation of compound **1a** from gas phase calculations. The molecule is shown in stick model with carbons in green and noncarbon atoms in standard colors.

the pyridine, presumably in a hydrogen-bonding fashion, to stabilize the molecule. The two aromatic rings at the distal ends of the molecule are nearly perpendicular to each other with an edge-to-face distance of about 4Å, indicating a potentially favorable π-π stacking interaction within the molecule.

Competitive binding studies were not available to identify the binding site of the inhibitors. Before detailed docking studies could proceed, it was therefore necessary to first identify the most likely binding site of compound **1a**. To this end, 3D similarities between conformers of compound **1a** and three probes derived from known crystal structures were assessed. The glucose analog that acts as an inhibitor at the catalytic site was dismissed from consideration because of the obvious lack of similarity between it and compound **1a**. The 3D similarity/superposition tool SQ[16] was employed to superpose conformers of compound **1a** onto three probes derived from publicly available x-ray crystal structures of known inhibitors in complex with GP: Bayer W1807 at the AMP allosteric site [Protein Data Bank (PDB) entry 3AMV[9]], caffeine at the inhibitor site (PDB entry 1GFZ[11]), and Pfizer CP320626 at the dimer interface site (PDB entry 1C50[13]). Each probe represents a class of inhibitors at a different binding site and their chemical structures are shown in Figure 17.2. The best overlay of compound **1a** onto each of the three probes is depicted in Figure 17.4.

Compound **1a** overlays onto W1807 [Figure 17.4(a)] with the aromatic regions and diacid groups fairly well aligned. W1807 is most similar to compound **1a** among the three probes, so this is not an unexpected result. Like W1807, the diacid moiety of compound **1a** presumably interacts with positively charged arginines at the AMP allosteric site. In contrast, the energetically preferred V shape of compound **1a** precludes it from being able to align well onto the planar structure of caffeine [Figure 4(b)]. Although the superposition of compound **1a** onto CP320626 [Figure 4(c)] is visually appealing, it requires compound **1a** to adopt a conformation energetically disfavored by more than 10 kcal/mol. In this alignment, the diacid groups and the A ring are overlapped onto the 4-hydroxy-piperidyl moiety of CP320626, known to bind in a space filled with water molecules at the dimer interface site.[13] As a result, the diacid group in compound **1a** would not make significant favorable interactions with the enzyme. Based on this analysis of each binding site, it appeared that compound **1a** is likely to bind at the AMP allosteric site.

Docking of compound 1a into AMP allosteric site

Having identified the AMP allosteric site as the most likely binding site for compound **1a** through the SQ overlay, a more extensive docking study was carried out using ICM software.[17] The amino acid sequences of HLGP, HMGP, and RMGP were aligned using Clustal W.[27] HLGP has 80% sequence identity and 90% sequence similarity to HMGP and RMGP. The homology between the two muscle enzymes (HMGP and RMGP) is even higher, with a sequence identity of 97% and sequence similarity of 99%. The residues located within 5Å of W1807 in the AMP allosteric site are conserved among the three enzymes, so it is likely that the binding pocket would be very similar

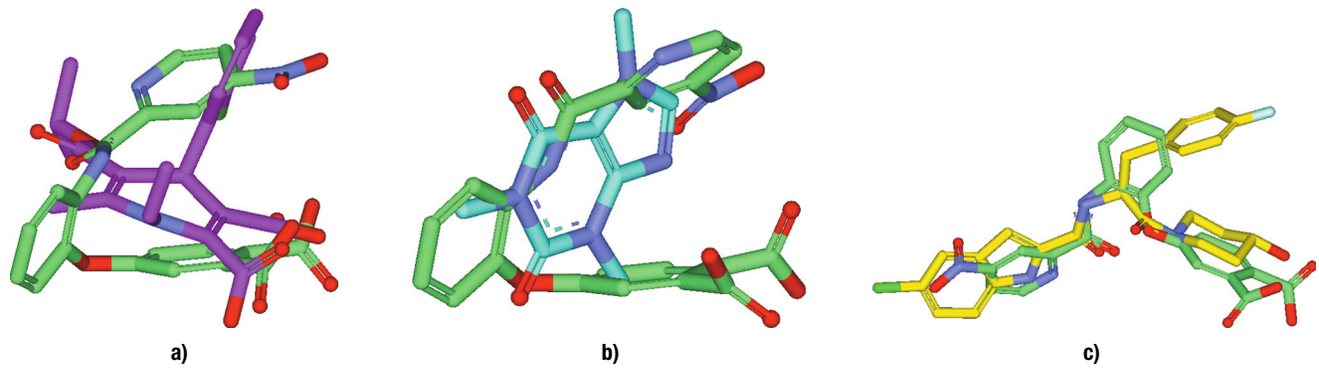

Figure 17.4. Superposition of compound **1a** onto known inhibitors by SQ calculations. Compound **1a** (carbons in green) overlaid onto **(a)** W1807 (carbons in magenta) at the AMP allosteric site, **(b)** caffeine (carbons in cyan) at the inhibitor site, and **(c)** CP320626 (carbons in yellow) at the dimer interface site. Only heavy atoms are shown and noncarbon atoms are in standard colors.