little or no water (216). To investigate the substrate specificity of a-chymotrypsin in pentanol, a series of X-phenyl esters of N-benzoyl-L-alanine (Fig. 1.5) were synthesized and their binding constants were evaluated in buffer and in pentanol (203). The following QSAR 1.92 and 1.93 were derived in phosphate buffer and pentanol.

5.1.12 Binding of X-Phenyl, *N*-Benzoyl-L-alaninates to α-Chymotrypsin in Phosphate Buffer, pH 7.4 (203)

$$\begin{aligned}\log 1/K_M &= 0.28(\pm0.11)\pi + 0.51(\pm0.24)\sigma^- \qquad (1.92)\\ &\quad + 0.38(\pm0.23)\mathrm{MR} + 3.70(\pm0.24)\\ n &= 16, \quad r^2 = 0.834, \quad s = 0.198\end{aligned}$$

5.1.13 Binding of X-Phenyl, *N*-Benzoyl-L-alaninates to α-Chymotrypsin in Pentanol (203)

$$\begin{aligned}\log 1/K_M &= 0.25(\pm0.09)\pi\\ &\quad + 0.24(\pm0.18)\sigma^- \qquad (1.93)\\ &\quad + 4.10(\pm0.09)\\ n &= 17, \quad r^2 = 0.762, \quad s = 0.156\end{aligned}$$

Outliers in QSAR 1.92 included the 4-t-butyl and 4-OH analogs, whereas the 4-CONH, analog was an outlier in QSAR 1.93. These results were recently reanalyzed by Kim (217,218) with respect to the role of enthalpic and entropic contributions to ligand binding with a-chymotrypsin. Use of the Fujiwara hydrophobic enthalpy parameter *r*, and the hydrophobic entropy parameter π_S led to the development of QSAR 1.94 and 1.95 (219).

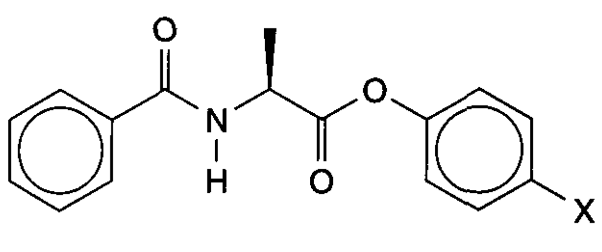

Figure 1.5. X-Phenyl, *N*-benzoyl-L-alaninates.

5.1.14 Binding of X-Phenyl, *N*-Benzoyl-L-alaninates in Aqueous Phosphate Buffer (218)

$$\begin{aligned}\log 1/K_M &= 0.38(\pm0.11)\pi_H + 0.19(\pm0.07)\pi_S\\ &\quad + 0.53(\pm0.11)\sigma^- \qquad (1.94)\\ &\quad + 0.26(\pm0.10)\mathrm{MR} + 3.77(\pm0.11)\\ n &= 15, \quad r^2 = 0.806, \quad s = 0.200\end{aligned}$$

5.1.15 Binding of X-Phenyl, *N*-Benzoyl-L-alaninates in Pentanol (218)

$$\begin{aligned}\log 1/K_M &= 0.21(\pm0.08)\pi_H + 0.31(\pm0.05)\pi_S \qquad (1.95)\\ &\quad + 0.20(\pm0.08)\sigma^- + 4.16(\pm0.04)\\ n &= 15, \quad r^2 = 0.787, \quad s = 0.160\end{aligned}$$

The disappearance of the MR term in QSAR 1.93 and 1.95 is significant. The MR term usually relates to nonspecific, dispersive interactions in polar space. Thus, its presence in QSAR 1.92 and 1.94 suggests that substrates bearing polarizable substituents may displace the ordered-category II water molecules. In pentanol, the substrate may be faced with the task of displacing pentanol, not water, from the enzyme and thus the MR term is no longer of consequence. QSAR 1.94 also indicates that the enthalpy term π_H plays a more critical role in binding than the entropy term π_S. Note that these roles are reversed in QSAR 1.95, suggesting that binding in pentanol is largely an entropic-driven process. Similar results were obtained by Compadre et al. in a study on the hydrolysis of X-phenyl-*N*-benzoyl-glycinates by cathepsin B in aqueous buffer and acetonitrile (220). Kim's analysis provides an excellent example of a study that focuses on mechanistic interpretation and clearly demonstrates that a thermodynamic approach in QSAR can provide pertinent information about the energetics of the ligand binding process.