It is important to observe that the Cheng–Prusoff equation is only valid for competitive antagonists.

The Schild analysis is often used to determine whether an antagonist is competitive or noncompetitive. In the Schild analysis, the antagonist concentration is kept constant while the agonist concentration is varied. For a competitive antagonist, this will cause a rightward parallel shift of the concentration–response curves without a reduction of the maximal response (Figure 12.12a). The degree of right-shifting is determined as the dose ratio (DR), which is the concentration of agonist giving a particular response in the presence of antagonist divided by the concentration of agonist that gives the same response in the absence of antagonist. Typically, one will chose the EC_{50} values to calculate the DR. In the Schild analysis, the log (DR-1) is depicted as a function of the antagonist concentration (Figure 12.12b). When the slope of the curve equals 1, it is a sign of competitive antagonism and the affinity can then be determined by the intercept of the abscissa. When the slope is significantly different from 1 or the curve is not linear, it is a sign of noncompetitive antagonism, which invalidates the Schild analysis.

As shown in the example in Figure 12.12, five concentration–response curves are generated to obtain one antagonist affinity determination, illustrating that the Schild analysis is rather work-intensive compared to, e.g., the transformation by the Cheng–Prusoff equation where one inhibition curve generates one antagonist affinity determination. However, the latter cannot be used to determine whether an antagonist is competitive or noncompetitive, which is the advantage of the Schild analysis. When testing a series of structurally related antagonists one would thus often determine the nature of antagonism with the Schild analysis for a couple of representative compounds. If these are competitive antagonists, it is reasonable to assume that all compounds in the series are competitive and thus determine the affinity of these by the use of the less work-intensive Cheng–Prusoff equation.

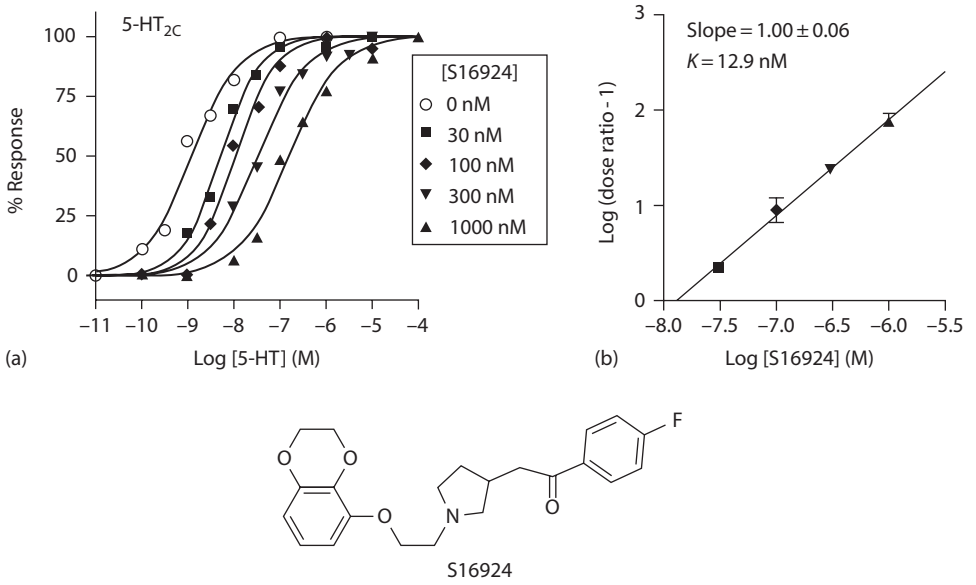

FIGURE 12.12 Schild analysis of the competitive antagonist S16924 on cells expressing the 5-HT_{2C} receptor. (a) Concentration–response curves of the agonist 5-HT were generated in the presence of varying concentrations of S16924. Note the parallel right shift of the curves and the same level of maximum response. (b) Dose ratios are calculated and plotted as a function of the constant antagonist concentration generating a straight line with a slope of 1.00 ± 0.012. These results and the observations from (a) are in agreement with a competitive interaction and the antagonist affinity can thus be determined by the intercept of the abscissa; K = 12.9 nM. (With kind permission from Springer Science+Business Media: *Naunyn Schmiedebergs Arch Pharmacol.*, Antagonist properties of the novel antipsychotic, S16924, at cloned, human serotonin 5-HT_{2C} receptors: A parallel phosphatidylinositol and calcium accumulation comparison with clozapine and haloperidol, 361, 2000, 549, Cussac, J.C., McCormick, D.J., Pang, Y.P. et al.)