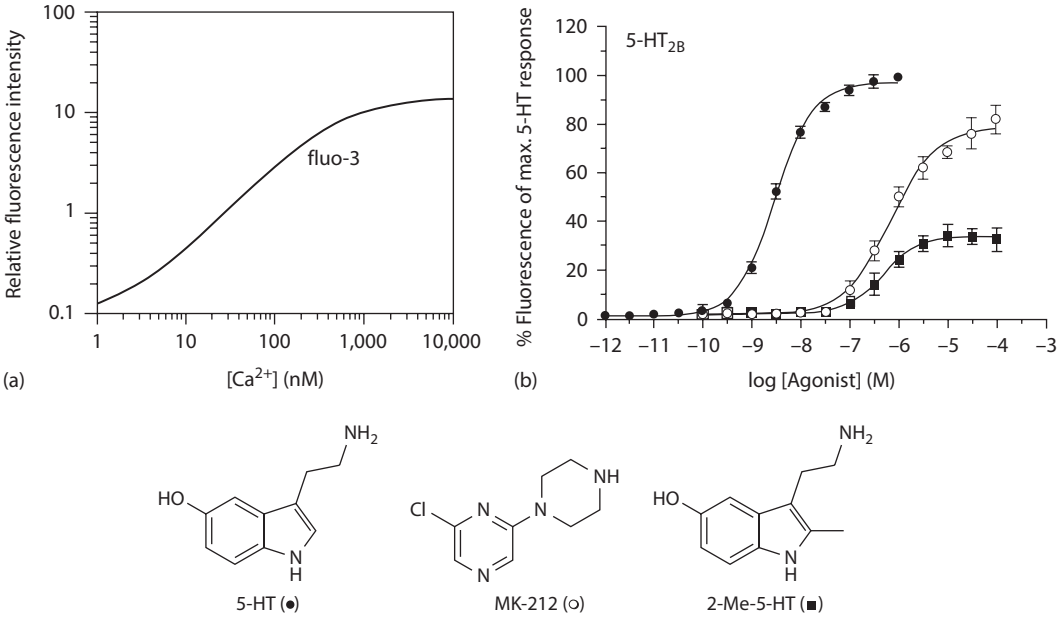

FIGURE 12.11 (a) Relation between Ca^{2+} concentration and relative fluorescence intensity of the fluorescent probe fluo-3. (b) The 5-HT_{2B} receptor subtype belongs to the superfamily of G protein-coupled receptors and is coupled to increase in inositol phosphates and intracellular Ca^{2+}. Cells expressing 5-HT_{2B} receptors were loaded with fluo-3 and the fluorescence was determined upon exposure to the endogenous agonist 5-HT (●) and the partial agonists MK-212 (○) and 2-Me-5-HT (■) on a FLIPR™. (Adapted from Jerman, J.C. et al., *Eur. J. Pharmacol*., 414, 23, 2001. With permission.)

12.3.4 Antagonists

Antagonists do not activate the receptors but block the activity elicited by agonists and accordingly they are only characterized by the parameter affinity. The most common way of characterizing antagonists is by competition with an agonist (functional assay) or a radioactively labeled ligand (binding assay). In both cases, the antagonist concentration is increased and displaces the agonist or radioligand, which are held at a constant concentration. It is then possible to determine the concentration of antagonist, which inhibits the response/binding to 50% (the IC_{50} value). The IC_{50} value can then be transformed to affinity (K) by the Cheng–Prusoff equation:

Functional assay:

$$K = IC_{50}/(1 + [\text{Agonist}]/EC_{50}) \tag{12.1}$$

where

[Agonist] is the agonist concentration

EC_{50} is for the agonist in the particular assay

Binding assay:

$$K = IC_{50}/(1 + [\text{Radioligand}]/K_D) \tag{12.2}$$

where

[Radioligand] is the radioligand concentration

K_D is the affinity of the radioligand