



FIGURE 16.8 Chemical structures of allosteric and bitopic mAChRs ligands.

(see Section 16.4.3). Several equally selective M_1 agonists have followed in the trail of AC-42, including TBPB (16.37) and *N*-desmethylozapine (16.38). Being one of two major *in vivo* metabolites of the prototypic atypical antipsychotic clozapine (Chapter 18), it has been speculated that the M_1 activity of *N*-desmethylozapine may contribute to the clinical efficacy of clozapine in schizophrenic patients where other antipsychotics have limited effects.

In contrast to these M_1 agonists, BQCA (16.39) acts as a classical PAM exhibiting no intrinsic agonist activity at M_1 but potentiating ACh-mediated signaling through the receptor. BQCA is the most explored scaffold of the allosteric M_1 ligands to date, and interestingly BQCA-mediated potentiation of ACh-evoked signaling through M_1 has been shown to induce distinct intracellular effects compared to direct activation of the receptor by AC-42 or TBPB. This suggests that the cellular responses mediated by allosteric agonists and PAMs through M_1 could differ, and this could be important for their respective *in vivo* effects. A considerable number of PAMs exhibiting different degrees of selectivity for the M_4 receptor have also been published. Whereas LY2033298 (16.40) is a highly selective M_4 PAM that has been used as a pharmacological tool to explore the pro-cognitive and antipsychotic potential in the receptor, its analog LY2119620 (16.41) is an equipotent PAM at M_2 and M_4 . Analogously, a series of *N*-methyl isatins exhibit very different selectivity profiles as mAChR PAMs, such as 16.42 that displays comparable EC_{50} values at M_1 , M_2 , M_3 and M_5 and the highly selective M_5 PAM VU0238429 (16.43). These examples illustrate how dramatic implications minor modifications on a modulator scaffold can have on its pharmacological properties. Finally, the selective M_5 receptor NAM ML375 (16.44), developed from a HTP screening hit, exhibits a high nanomolar IC_{50} value at this receptor and >1000-fold selectivity toward the four other receptors. ML375 is currently being applied in rodent studies probing the potential in selective M_5 antagonism for the treatment of drug addiction.

16.4.3 LIGAND BINDING TO AND MODULATION OF THE mAChR

The binding modes of orthosteric and allosteric ligands to mAChRs have been studied extensively in mutagenesis and biochemical studies, and recent high-resolution crystal structures of the M_2 and M_3 receptors have provided detailed insight into these aspects (Figure 16.9). The orthosteric site in