combined with the failure of many first-generation mAChR drugs in clinical development due to intolerable adverse effects have meant that focus in the field with respect to CNS indications have turned toward selective modulation of M_1, M_4, and M_5 receptors.

M_1 is the most predominant mAChR subtype in the CNS, where it primarily is located at postsynaptic densities. M_1 is expressed in several brain regions important for cognitive processes, and several lines of research have implicated augmentation of M_1 signaling as perhaps the most promising mAChR-based approach to treat the cognitive dysfunctions in AD and schizophrenia. M_4 has also emerged as an interesting target in connection with the psychosis associated with schizophrenia, a potential that has been ascribed to the modulation of dopaminergic signaling exerted by the receptor in striatum, hippocampus, and neocortex. Selective agonists or potentiators of M_4, M_1, or M_1/M_4 are currently being investigated for these indications. The high expression of M_5 receptors on dopaminergic neurons in the ventral tegmental area and regulation of dopamine release in nucleus accumbens exerted by these receptors has prompted an interest in selective M_5 antagonists for the treatment of drug addiction. Finally, despite the aforementioned adverse effects arising from peripheral M_2 receptors, the potential of selective antagonists at this receptor for cognitive enhancement is still being pursued. M_2 is the predominant presynaptic mAChR in the CNS, and antagonism of this autoreceptor attenuates the negative feedback on synaptic ACh release, thereby resulting in increased release of the neurotransmitter and augmented cholinergic signaling (Figure 16.3c).

The medicinal chemistry efforts invested for several decades to exploit some of the potential in mAChRs as drug targets for cognitive disorders have so far not paid off. While mAChR ligands have been and still are used for indications such as glaucoma, peptic ulcer, motion sickness, and asthma, the outcome of these efforts in terms of CNS drugs have been disappointing as compound after compound have been retracted from clinical development due to insignificant efficacy or unacceptable side effects. However, as discouraging as this has been, the recent development of truly subtype-selective allosteric and bitopic ligands for the receptors has sparked a renewed interest in mAChRs as CNS targets.

16.4.1 Orthosteric mAChR Ligands

None of the classical orthosteric mAChR agonists **16.19**–**16.23** in Figure 16.7 display significant selectivity for any of the five receptor subtypes. Muscarine (**16.19**), a constituent of *Amanita muscaria*, has given name to the mAChRs because of its selectivity for these receptors over nAChRs. Replacement of the ester moiety of ACh by a carbamate group yields carbachol (**16.20**) which is not only a nonselective agonist at the five mAChRs but also at the nAChRs. The heterocyclic agonist pilocarpine (**16.21**) from the leaves of South American *Pilocarpus* shrubs is widely used as topical miotic for the control of elevated intraocular pressure associated with glaucoma. Despite being a nonselective mAChR agonist, the low bioavailability of pilocarpine means that it does not induce systemic side effects when administered topically. The potent agonist oxotremorine (**16.22**) has been used extensively as a lead compound for structure–activity studies giving rise to mAChR ligands spanning the entire efficacy range from full agonists over partial agonists to competitive antagonists.

Development of subtype-selective orthosteric mAChR agonists has proven difficult. Arecoline (**16.24**), a constituent of areca nuts (the seeds of *Areca catechu*) and a cyclic "reverse ester" bioisostere of ACh, has constituted the lead for numerous analogs, including xanomeline (**16.25**) in which the metabolically labile ester moiety of **16.24** has been replaced by the more stable thiadiazole ring. Originally proposed to be M_1 selective, subsequent functional characterization of xanomeline has found it to be M_1/M_4-prefering (not selective). Just as several other agonists exhibiting varying degrees of M_1 preference (including **16.26** and **16.27**), xanomeline has been in clinical development for the treatment of AD, and it is the only one among these to have reached phase III trials. Although the clinical development eventually was discontinued, the pro-cognitive and antipsychotic efficacies displayed by xanomeline in these trials have been instrumental for the validation of M_1 and M_4 as putative AD and schizophrenia targets and for the current development of allosteric and bitopic ligands for these receptors (Section 16.4.2).