The molecular mode of interaction of cocaine and analogs with DAT has long been the subject of speculation. In particular, it has been debated whether or not the cocaine-binding site in DAT overlaps with that of dopamine. If inhibition of dopamine uptake by cocaine is the result of an allosteric mechanism, it would be possible, at least in theory, to generate a cocaine antagonist for treatment of cocaine addiction that might block cocaine binding without affecting dopamine transport. Unfortunately, a crystal structure of dDAT in complex with cocaine, CFT or RTI-55 shows completely overlapping binding sites with dopamine. The binding is adjacent to both the Na^+ and the Cl^- ions. The tertiary amino group of cocaine forms a salt bridge with Asp48. Subsite B, which accommodates the aromatic moiety of the tropanes, has two nonconserved residues between hDAT and hSERT. This discrepancy could account for the differences in binding affinity of CFT and RTI-55. All tropane-based inhibitors induce an overall outward facing binding conformation, analogous to the dDAT:dopamine complex. This outward conformation as well as the majority of the interacting residues has been verified experimentally in both hDAT and hSERT and with molecular docking models. The major discrepancy between experiments and the crystal structure is that in the structure, the Asp46-Tyr124 hydrogen bond is still present. The experimental data and the docking models suggest that the 2β-methylester substituent of the tropane ring protrudes outward and disrupts this interaction.

For compounds of the tropane class, the tropane ring and the 2β-carbomethoxy group are crucial for their affinity. An exception for this rule is the benztropine class. This group of tropanes lacks the 2β-carbomethoxy group but still bind DAT with high affinity. Instead of the 2β-carbomethoxy group, the benztropines contain a diphenylmethoxy moiety. Recently, there has been increasing focus on benztropine analogs. Several of these compounds possess similar or even higher affinity and greater selectivity for DAT than cocaine. The compounds tested so far readily cross the blood–brain barrier and produce increases in extracellular levels of dopamine even for longer durations than cocaine. Nonetheless, several of these DAT inhibitors are less effective than cocaine as behavioral stimulants in rodent models. Furthermore, one BZT analog, JHW 007, was found to potently antagonize the behavioral effects of cocaine (Figure 14.3). Assuming a correlation between behavioral effects of cocaine in laboratory animals and abuse potential in humans, these findings suggest JHW 007 as a potential lead for development of cocaine abuse pharmacotherapeutics. The reason for this discrepancy in the stimulating effect has been suggested at least in part to be related to different pharmacodynamic properties of the compounds. Interestingly, experimental data suggest that in contrast to the cocaine-like compounds, the benztropine analogs bind and stabilize a more closed conformation of the transporter. It is conceivable that binding to the open and likely more prevalent outward facing conformation of DAT results in a faster on-rate which may facilitate faster inhibition of DAT function and thereby a more rapid rise in extracellular dopamine concentration. In contrast, binding to a more closed and predicted less prevalent conformation of the transporter may result in a slower on-rate of the compound and thereby a slower rise in dopamine levels and a less stimulatory effect. A structure of DAT:benztropine analog complex might clarify this issue.

14.3.2 Amphetamine and Other Nonendogenous Substrates

Several nonendogenous compounds are substrates of the biogenic amine transporters and are used either as medication, drugs of abuse, or biochemical tools. Amphetamine and derivatives thereof, for example, metamphetamine, *p*-chloroamphetamine, and 3,4-methylenedioxymetamphetamine (MDMA or ecstasy) are a class of psychostimulants that are transported by DAT, NET, and SERT (Figure 14.3). Methamphetamine preferentially acts on DAT and NET while *p*-chloroamphetamine and MDMA have higher specificity for SERT. This is supported by analyses of mice deficient in either DAT or SERT, i.e., DAT knock-out mice are hyperactive and do not respond to amphetamine, while SERT-deficient mice display locomotor insensitivity to MDMA. Interestingly, amphetamines do not only increase the synaptic concentration of dopamine by competing with dopamine for