Chagas disease, or American trypanosomiasis, is caused by the flagellate protozoan Trypanosoma cruzi. One century after its discovery, Chagas disease still remains a major health problem in Latin America. Treatment is based on benznidazole that have toxic effects and limited efficacy. Therefore, new chemotherapeutic agents are urgently needed. In this context, the effects of sixteen N-acylhydrazone oxadiazoles were evaluated as potential candidates for anti-T. cruzi agents. The cytotoxicity of compounds was determined by incorporation of [3H]-thymidine in splenocyte cultures obtained from normal mice. The trypanocidal effects of the compounds were evaluated in vitro with the three forms of the parasite (epimastigotes, trypomastigotes and amastigotes of the Y strain T. cruzi). Scanning and transmission electron microscopy were performed to analyze the effect of the most active compounds in the ultrastructural of trypomastigotes. In vivo, trypanocidal activity was evaluated by observing the levels of parasitaemia. All N-acylhydrazone oxadiazoles tested showed no toxicity to mammalian cells. The N-acylhydrazones 6c, 6d, and 6e showed antiproliferative activity for the replicative form epimastigote, as well as cytotoxicity for the infective form trypomastigote and the intracellular form amastigotes of T. cruzi. Oxadiazoles 6c and 6d had an IC50 against trypomastigotes of 3.5 ± 3.1 μM and 11.2 ± 3.1 μM, respectively, and the positive control benznidazole presented an IC50 of 11.3 ± 1.88 μM. Transmission electron microscopy (TEM) revealed that the treatment with hydrazones 6c and 6d, with their respectively IC50 values, resulted in deep ultrastructural changes on the mitochondria, kinetoplast, Golgi apparatus and endoplasmatic reticulum of trypomastigotes. The scanning electron microscopy (MEV) revealed that the treatment with hydrazones 6c and 6d cause the appearance of membrane protusions and descotinuities on the surface of the trypomastigotes after treatment. In vivo, the compounds 6c and 6d were able to reduce significantly the parasitaemia (p < 0.001), as observed in benznidazole-treated controls. Our results showed that the hydrazones were active in vitro and in vivo assays. E-mail: calcio0303@hotmail.com