African trypanosomes are sensitive to organometallic drugs such as arsenicals (Friedheim 1949; Loiseau et al. 2000). Although these drugs are responsible for side-effects, their efficacy is of great interest in the chemotherapy of human African trypanosomiasis particularly for their ability to cross the blood brain barrier. Previous studies have confirmed the trypanocidal activity of organometallic complexes both in vitro and in vivo (Dreyfuss et al. 1993, 1995; Loiseau et al. 1992, 1997). Although the mechanism of action of such compounds is still not clear, the trypanocidal activity previously obtained with some compounds and their chemotherapeutic index were cause for hope and justify the pharmacomodulations presented in this paper. We report here an in vitro trypanocidal evaluation of 47 compounds, 18 of them pentamidine derivatives. These metallic structures are derived from iridium, rhodium, ruthenium, platinum and paladium. They were synthesized following a method previously described (Gayral et al. 1992). Ten pentamidine complexes were evaluated in vivo using a Trypanosoma brucei brucei strain responsible for an acute infection, and compared to pentamidine isethionate used as reference compound and kindly supplied by Roger Bellon Laboratories (Paris).

T. b. brucei CMP was obtained from the Institut Pasteur, Paris, in 1973. This strain was kept frozen in liquid nitrogen, and an aliquot was passaged in female CD1 mice before the experiments. The drug incubation infectivity test was used for compound evaluation as previously described (Loiseau et al. 2000). The minimum effective concentration (MEC) was defined as the minimum concentration at which no viable parasite was observed microscopically, and with which naive mice injected intraperitoneally (i.p.) with 150 µl of treated trypanosomes suspension withdrawn from the wells after a 48-h period were aparasitemic 30 days postinfection. Thus, the MEC was assessed both visually using an optical microscope after 1, 24 and 48 h of incubation and in vivo since the mice were checked for parasitemia weekly for 30 days.

Concerning pentamidine derivatives, the results are shown in Table 1. After 1 h of incubation, the most active complex was compound 8 with a MEC of 0.39 µM whereas pentamidine isethionate was inactive. All the other compounds did not have rapid action since they were inactive, except compounds 7 and 18, which exhibited a MEC at 200 µM. After a 24-h incubation period, compounds 7 and 18 were seven times more active than pentamidine isethionate with a MEC at 0.098 µM and 0.78 µM, respectively. The other compounds had a MEC in a range from 0.195 to 12.5 µM. Finally, after a 48-h incubation period, compounds 7 and 18 were the most potent (MEC = 0.049 µM) and remained seven times more active than pentamidine isethionate (MEC = 0.39 µM). Concerning the molecules containing a cyclooctatetraene moiety (COT), rhodium derivatives were four times more active than the iridium ones. Similar data were obtained when cyclooctadiene moiety (COD) replaced COT. In summary, rhodium complexes were four times more active than iridium complexes.

Results of various complexes are shown in Table 2. The complexes had slow action since few were active after 1 h of incubation. After a 24- and 48-h incubation period compound 22, a complex of diminazene, exhibited similar MEC to diminazene aceturate and
pentamidine isethionate. Azidothymidine and its derivative complexes were inactive.

In vivo evaluation was performed with some pentamidine complexes following the protocol previously described (Loiseau et al. 2000). Briefly, female CD1 mice were infected i.p. with 10^4 bloodstream trypanosomes taken from an infected mouse and suspended in 0.1 ml of phosphate-buffered saline, pH 7.2. The infection was allowed to develop for 24 h before treatment was begun. Ten infected mice were used as controls and received only excipient, 1% carboxymethylcellulose by the subcutaneous route in a 0.1 ml volume. The other mice received a single dose of the diluted or suspended compounds in the same manner. Six mice were used per dose. The trypanocidal activity was evaluated by the mean survival time of treated mice for each dose. Treatment was considered to be successful when the mean survival time exceeded 30 days and the mice remained aparasitic. Control mice (infected and untreated) did not survive more than 4 days postinfection. Cure rates were calculated and are expressed as percentages. In vivo results are presented in Table 3. Compounds 5 and 8 were slightly more active than pentamidine isethionate at 6.25 μM/kg. Compound 18, which was one of the most active in vitro (MEC = 0.049 μM), was completely inactive in vivo at 100 μmol/kg. All these compounds were synthesized in a structure–activity relationship study; however, although rhodium complexes were significantly more active in vitro than iridium complexes, no clear-cut correlation emerged between in vitro and in vivo results. Furthermore, the effect of several parameters on in vitro and in vivo trypanocidal activities could not be appreciated clearly, such as the metal nature (iridium or rhodium), counterion X- and the presence of COD or COT. The amphiphilic nature of these molecules could help their uptake through the plasma membrane independently of the presence of the P2 nucleoside transporter able to be recognized by diamidines (Carter et al. 1995). In fact, the diamidine groups need to be free to be recognized by the transporter, which is not the case for the complexes. Therefore, these molecules could accumulate in diamidine-resistant trypanosomes where the P2 transport is altered (Carter et al. 1995). The mechanism of action of these molecules remains to be elucidated. Moreover, compound 5 has been found active against Leishmania donovani in vitro and in vivo (Mbongo et al. 1997, 1998). Furthermore, a synergistic effect was pointed out between compound 5 and pentamidine on promastigote forms demonstrating the absence of competition between both the compounds toward the pentamidine transporter in Leishmania (Mbongo et al. 1998).

This study allowed the selection of two interesting molecules, compounds 5 (Fig. 1) and 8, which have to be further evaluated with regard to the late stage of experimental African trypanosomiasis and diamidine-resistant parasites.