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Chapter 9

# Herbicides as Potential Chemotherapeutic Agents Against Parasitic Protozoa

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## 1. Introduction

Herbicides refer to a large number of compounds widely used to kill plants that interfere with the growth of desired crops, thereby improving the productivity of the crop system. One group of herbicides that includes compounds generally designated as dinitroanilines has been shown to interfere with plant cells by interrupting mitosis and the formation of multinucleated cells. Research has shown that these effects are due to interference with microtubules, i.e., a cytoskeleton structure that is ubiquitous in eukaryotic cells and plays a fundamental role in several biological processes, including the determination and maintenance of cell shape, the motility of several cell types that use flagella and cilia for locomotion, the intracellular transport of organelles, and the movement of chromosomes during cell division. Other processes involving microtubules are not as well characterized. Previous research has shown that dinitroanilines interfere with microtubules by binding to sites on the surface of the longitudinal contacts established between the tubulin subunits that contain lysine and arginine residues, which in turn bind to the nitrile group of dinitroaniline [1,2].

Microtubules are made of $\alpha$-and β-tubulin heterodimers that form long (i.e., several μmeters in length), filamentous, tubular structures when polymerized. The number of tubulin isotypes varies according to the organism species (e.g.,six types of $\alpha$-tubulin and seven types of β-tubulin are found in human cells). They can be very dynamic structures that undergo constant assembly and disassembly in cells. Tubulin molecules may be post-translationally modified by polyglutamylation, polyglycylation, phosphorylation, acetylation, detyrosination/tyrosination, and removal of the penultimate glutamic acid residue found in $\alpha$ tubulins. In addition, an increased number of proteins can interact with microtubules; these proteins are known as microtubule-associated proteins (MAPs)and include dynein, kinesin, etc., all of which interfere

with the stability of the microtubules and their function. Further data on microtubule composition and dynamics can be found in an excellent review by Gardner et al. [3].

## 2. Dinitroanilines

Dinitroanilines correspond to a family of herbicides that were originally discovered through studies evaluating dyes and chemical synthesis intermediates. The most important member of the group is trifluralin, which is widely used in soybean production. The family is divided into the following two subfamilies: the methylanilines, which includes trifluralin, pendimethalin, benefin, dinitramine, fluchloralin, and profluralin, and the sulfonylanilines, which includes oryzalin and nitralin [1,4,5].Initial studies showed that these compounds inhibit cell division by interfering with the assembly of microtubules, thereby interfering with the formation of the plant cell walls and chromosome movement during the mitotic process, which ultimately leads to the appearance of multinucleated cells [6].

One characteristic feature of several pathogenic protozoa is the presence of a large number of structures in which microtubules are a major component. In the case of the Trypanosomatidae family, which includes such important pathogenic species as *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania*, subpellicular microtubules are located immediately below the plasma membrane, establishing connections between them, the plasma membrane, and the profiles of the endoplasmic reticulum. They are seen throughout the protozoan body with exception to the region of the flagellar pocket [7]. This large group of organisms also contains the flagellar microtubules and intranuclear spindle microtubules involved in the process of nuclear division. In the case of Apicomplexa, which includes such pathogens as *Toxoplasma gondii*, *Plasmodium*, *Eimeria*, *Babesia,* etc., researchers have found subpellicular microtubules, i.e., a special type of microtubule that forms the conoid, spindle microtubules, and flagellar microtubules in microgametes [8]. In *Giardia lamblia,* the microtubules are associated with the adhesive disc (i.e., a structure involved in the attachment of the trophozoite to the intestinal epithelial cells) and form the spindle microtubules and flagella. In the case of trichomonads (e.g., *Trichomonas vaginalis* and *Tritrichomonas foetus*) microtubules form the flagella and such structures as the pelta-axostylar system and the spindle microtubules [9, 10].

In the following text, I will review the literature focused on the effects of herbicides on each group of pathogenic protozoa.

## 3. Trypanosomatids

The microtubules that are found in trypanosomatids, especially those that are subpellicular, are considered resistant to several compounds that usually depolymerize microtubules found in eukaryotic cells, including colchicine, vinblastine, and vincristine [11]. However, these organisms show some sensitivity to taxol [12]. Research has shown that trifluralin inhibits cell division in several members of the Trypanosomatidae family, including *Leishmania amazonen-*

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*sis*, *Leishmania mexicana*, *Leishmania infantum, Leishmania major, Leishmania panamensis, T.brucei,* and *T.cruzi* [13-15]. The half maximal inhibitory concentration ($IC_{50}$) for these protozoa ranges from 0.9 to 670 μM. In general, the *Leishmania* species were more sensitive to the herbicides than the *Trypanosoma* species [16]. In general, the amastigotes, which are the predominant and proliferative intracellular form, are more sensitive than the forms that grow in axenic media (i.e., promastigotes and epimastigotes). A microscopic analysis showed that trifluralin induced changes in the shape of *T. cruzi* epimastigotes (i.e., they became more rounded), affected the mitochondrion, interfered with the ingestion of macromolecules through the cytostome, decreased the number of horseradish peroxidase containing reservosomes, induced the appearance of multi-flagellated cells (i.e., probably due to interference with the cell division process), and blocked the process of metacyclogenesis; yet,trifluralin does not disrupt the subpellicular microtubules [17].

Some papers have described attempts to use dinitroanilines in vivo. For instance, promising results were observed when using topical applications of dinitroanilines to treat lesions induced by *L. major* and *L. mexicana* [14] and oral applications of dinitroanilines to treat the chronic phase of Chagas disease in mice. These results are similar to those obtained with benznidazole [18].

## 4. Apicomplexa

More information on the effect of herbicides is available for this group of eukaryotic microorganisms, especially *T.gondii*. Most of the studies on organisms within Apicomplexa were performed by Morrisette and her colleagues. The first paper in 1996 [19] showed that dinitroaniline herbicides inhibited intracellular division in the tachyzoites of *T. gondii*. This classical paper also demonstrated that oryzalin and ethalfluralin inhibited 50% of the protozoan growth at concentrations of 100 nM. In the case of trifluralin, the $IC_{50}$ was 300 nM. These concentrations are very low; most importantly, even at concentrations that were 100 times higher, the drugs did not interfere with the human fibroblasts used to cultivate the protozoa. These compounds blocked the process of endodyogeny, i.e.,a special characteristic of cell division in *T. gondii* trophozoites where two daughter cells are formed inside a mother cell. Oryzalin, not ethalfluralin, disrupted the subpellicular microtubules. None of the compounds interfered with the structure of the conoid, i.e., a structure made of microtubules of a special type [20]. The authors also obtained mutant parasites that were resistant to the herbicides under investigation through chemical mutagenesis. Subsequently, the research showed that in the presence of oryzalin at a concentration of 2.5 μM, the tachyzoites retained the capacity to assemble the spindles and undergo nuclear division. However, due to disintegration of the subpellicular microtubules, the parasites were no longer able to invade new cells. At 2.5 μM, the compound interfered with the spindle microtubules, and the protozoa increased in size [21]. Morrissette and her co-workers further analyzed the obtained mutants and showed that they were localized in or near the M and N loops, i.e., domains that coordinate the lateral interactions between protofilaments [1, 22, 23]. Subsequently, several other oryzalin analogs were synthe-

sized, thereby leading to the acceptance of an antimitotic structure-activity relationship for dinitroanilines.

$N^1$,$N^1$-dipropyl-2,6-dinitro-4-(trifluoromethyl)-1,3benzenediamine is the most potent agent against *T. gondii* [5]. These studies were extended to *Plasmodium falciparum,* and the results indicated that trifluralin and oryzalin inhibited the progression of the protozoa inside erythrocytes by blocking the mitotic division with the accumulation of abnormal microtubular structures [24]. This research also demonstrated that trifluralin is active against the gametocytes of *P. falciparum*, thereby inducing disassembly of the subpellicular microtubules due to the formation of tubular structures containing disassembled microtubules. The researchers used labeled trifluralin and electron microscopy autoradiography to show that the compound binds to the tubular structures [25]. Oryzalin and trifluralin derivatives also showed activity against *Cryptosporidium parvum*. Several derivatives of these compounds were synthesized and, despite their reduced toxicity, showed similar activity [26].

## 5. Anaerobic protozoa

Oryzalin was tested against *Giardia lamblia* trophozoites. The obtained results showed that oryzalin inhibited parasite proliferation in an axenic culture. At 50 and 100 μM, most of the protozoa were killed. Morphological studies showed curling of the flagella in about 60% of the cells, elongation of the median body (i.e., a structure made of microtubules), changes in the shape of the cell, and blockage of cell division (Figures 1-3) [27].

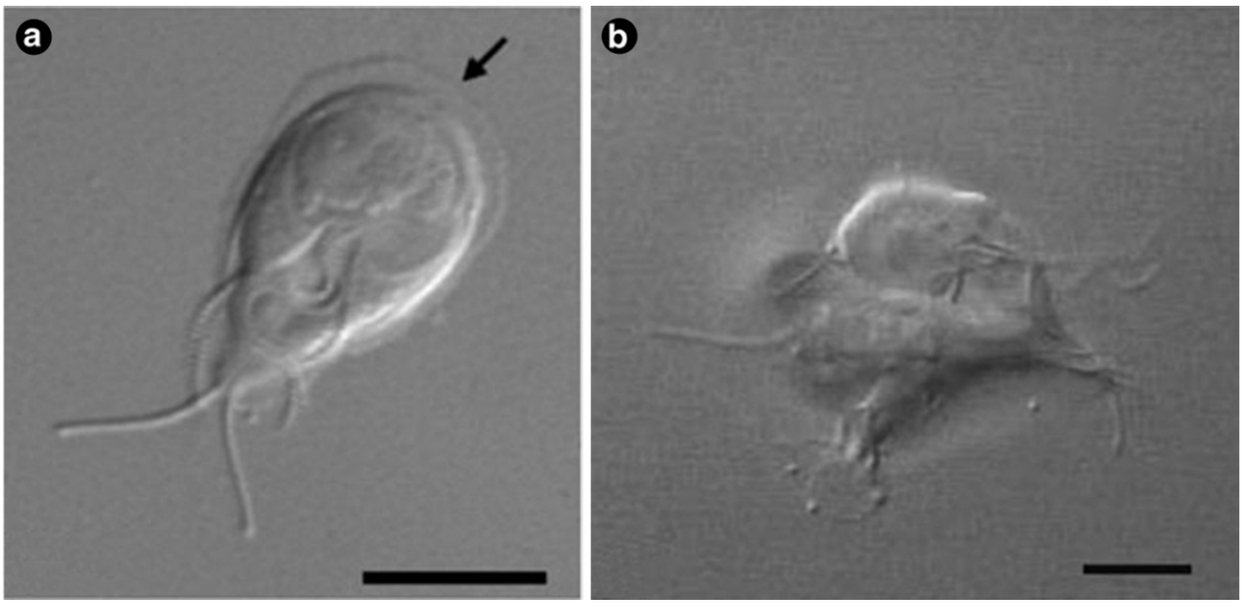


**Figure 1.** Light microscopy of the control (A) and oryzalin-treated (B) trophozoites of *Giardia lamblia*. The control cell displays a pyriform shape with four pairs of clearly identifiable flagella. In the treated cell, the loss of its normal shape is observed. Bar, 3 μm [27].

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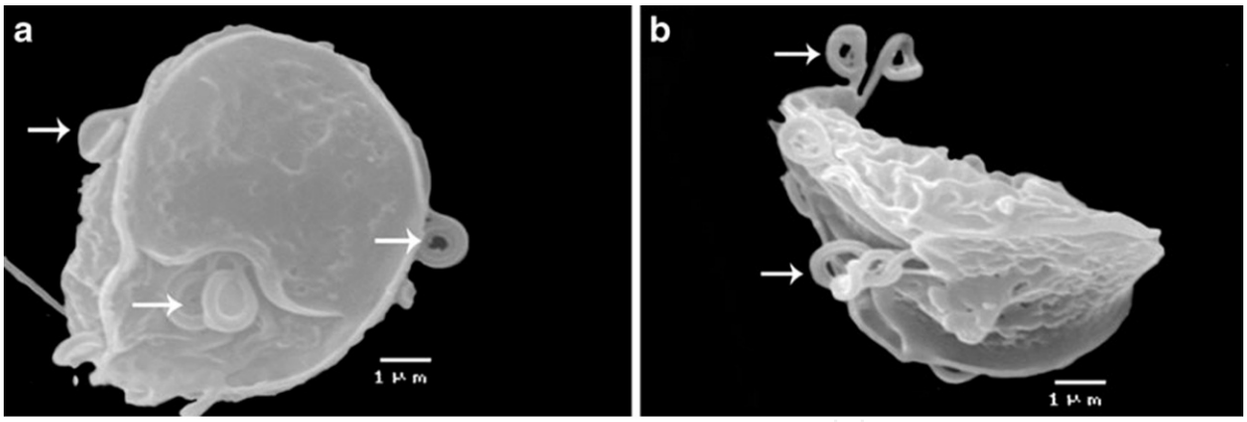


**Figure 2.** Scanning electron microscopy showing several alterations in the organization of the trophozoite form of *G. lamblia,* including shortening and curling of the flagella (arrows in a and b). Bar, 1µm [27].

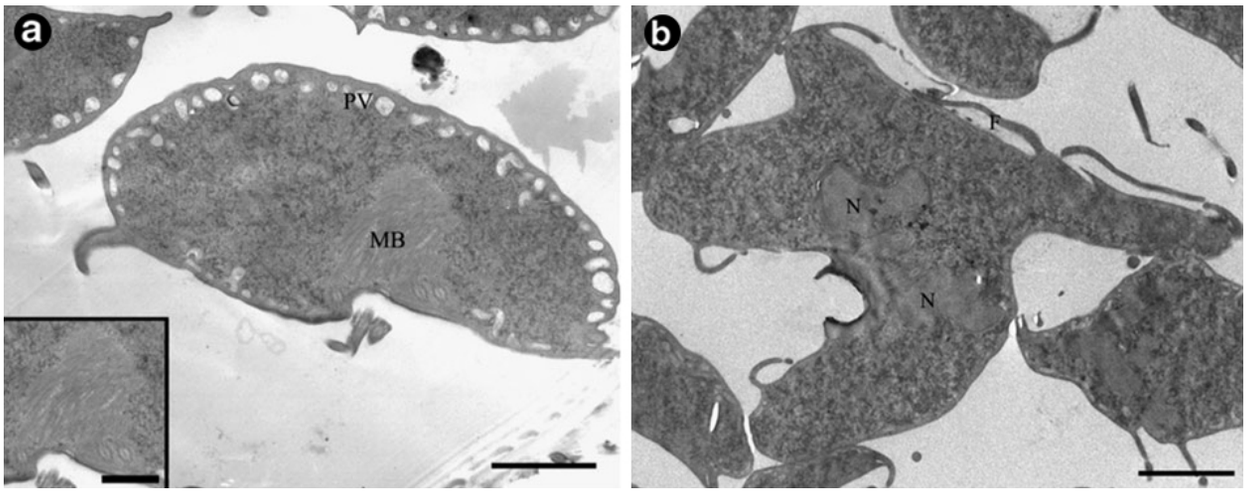


**Figure 3.** Transmission electron microscopy of thin sections of the control (A) and oryzalin-treated (B) trophozoites of *G. lamblia* where inhibition of protozoan division is clearly seen. Bar in A and B, 0.5 and 2 µm, respectively [27].

## 6. Trifluralin associated with phospholipid analogues

Phospholipid analogues, such as miltefosine, have been shown to be very effective against parasitic protozoa, especially *Leishmania donovani,* and are now considered the favorite pharmaceutical treatment for visceral leishmaniasis in India [28]. The association via molecular hybridization combines the pharmacophoric moieties of miltefosine and trifluralin, thereby leading to some compounds that are very active against *T.cruzi* and *L.amazonensis* (submitted for publication). The effects observed on the structural organization of the protozoa seem to also affect the membranes and cytoskeleton structures, thereby offering new possibilities in the treatment of parasitic diseases. Based on the preliminary results obtained with these compounds it seems to me that very soon some of them will be in the phase of clinical trials.

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