Novel compounds to cure *Cryptosporidium parvum* infection in immunosuppressed and immunocompetent people

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Introduction

- *Cryptosporidium* is an important apicomplexan protozoan pathogen that significantly contributes to diarrheal disease in both humans and animals.
- Throughout the world in immunocompetent hosts, infections are generally restricted to intestinal epithelium, causing acute and self-limiting gastroenteritis.
- However, for HIV/AIDS patients and other immunocompromised individuals, such infections can result in life-threatening diarrheal disease.
- Recently, only nitazoxanide (NTZ), a nitrothiazole benzamide, was approved by the Food and Drug Administration (FDA) for the treatment of cryptosporidiosis in immunocompetent adults and children over 1-yr-old. However, this drug is not fully successful for all cases of cryptosporidiosis.
- Paromomycin is one of the compounds used to treat in *Cryptosporidium* in animals and cell culture models.
- *Cryptosporidium* currently causes over half of the reported waterborne disease outbreaks associated with swimming in chlorinated public swimming pools.

Aim of study

The present study investigates several new compounds which may limit *Cryptosporidium* infection.

The full study investigates eleven different chemical compounds, including seven that specifically target *Cryptosporidium parvum* infection in immunosuppressed and immunocompetent people

Materials and Methods

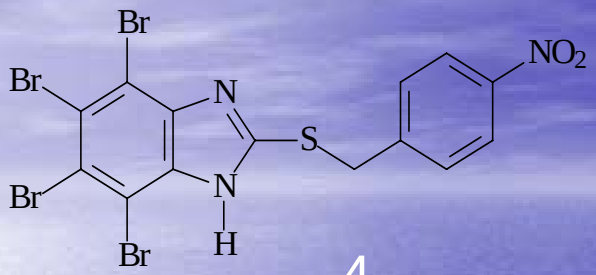
- *Cryptosporidium parvum* oocysts. *C. parvum* (Iowa isolate) oocysts were obtained through experimental infection of female Holstein calves. The oocysts were extracted from the feces using continuous-flow centrifugation. Purified by cesium chloride gradient centrifugation, and stored at 4°C in phosphate-buffered saline(PBS) (pH7,4).

- Drugs. Paromomycin was purchased from MP Biomedicals (Solon, OH) and was diluted in water just prior to use.

- Compound activity in cell culture. HCT-8 cells (CCL-244) were obtained from the American Type Culture Collection(Manassas,VA) and maintained in RPMI 1640 medium supplemented with 10% Opti-MEM (GIBCO-BRL, Grand Island, NY) 2% fetal bovine serum and 2 mM L-Glutamine.

- To determinate *in vitro* compound efficiency, a quantitative alkaline phosphatase immunoassay was used to measure parasite growth inhibition in cell culture.

Chemical Compounds Tested



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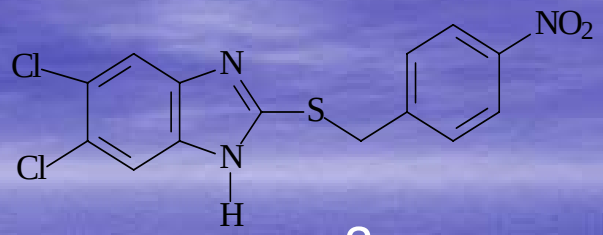

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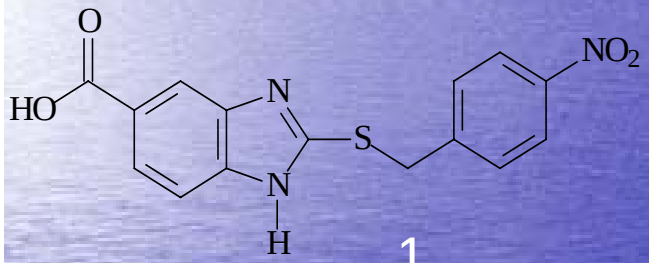

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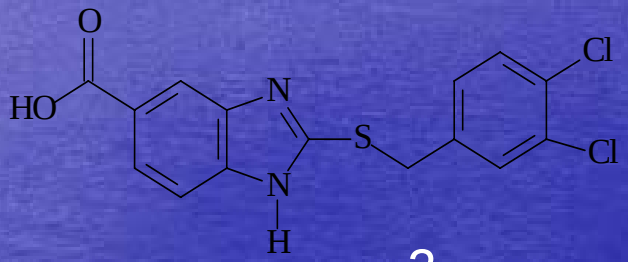

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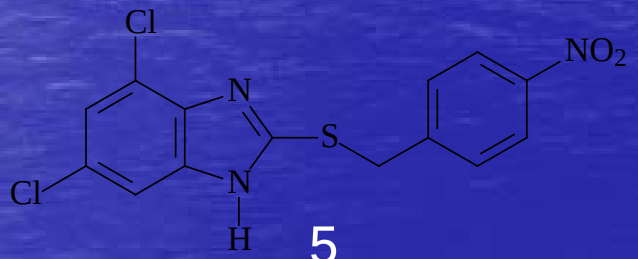

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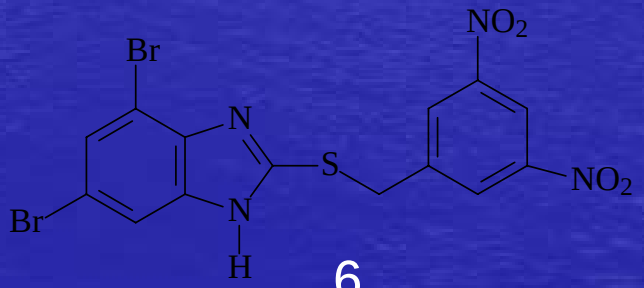

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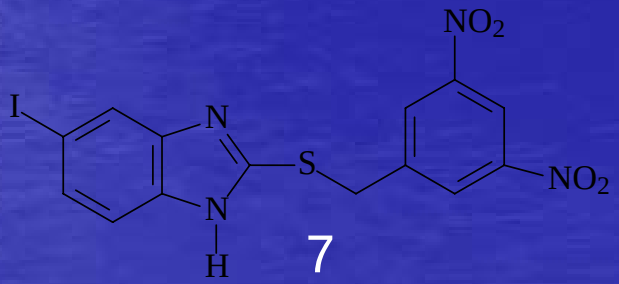

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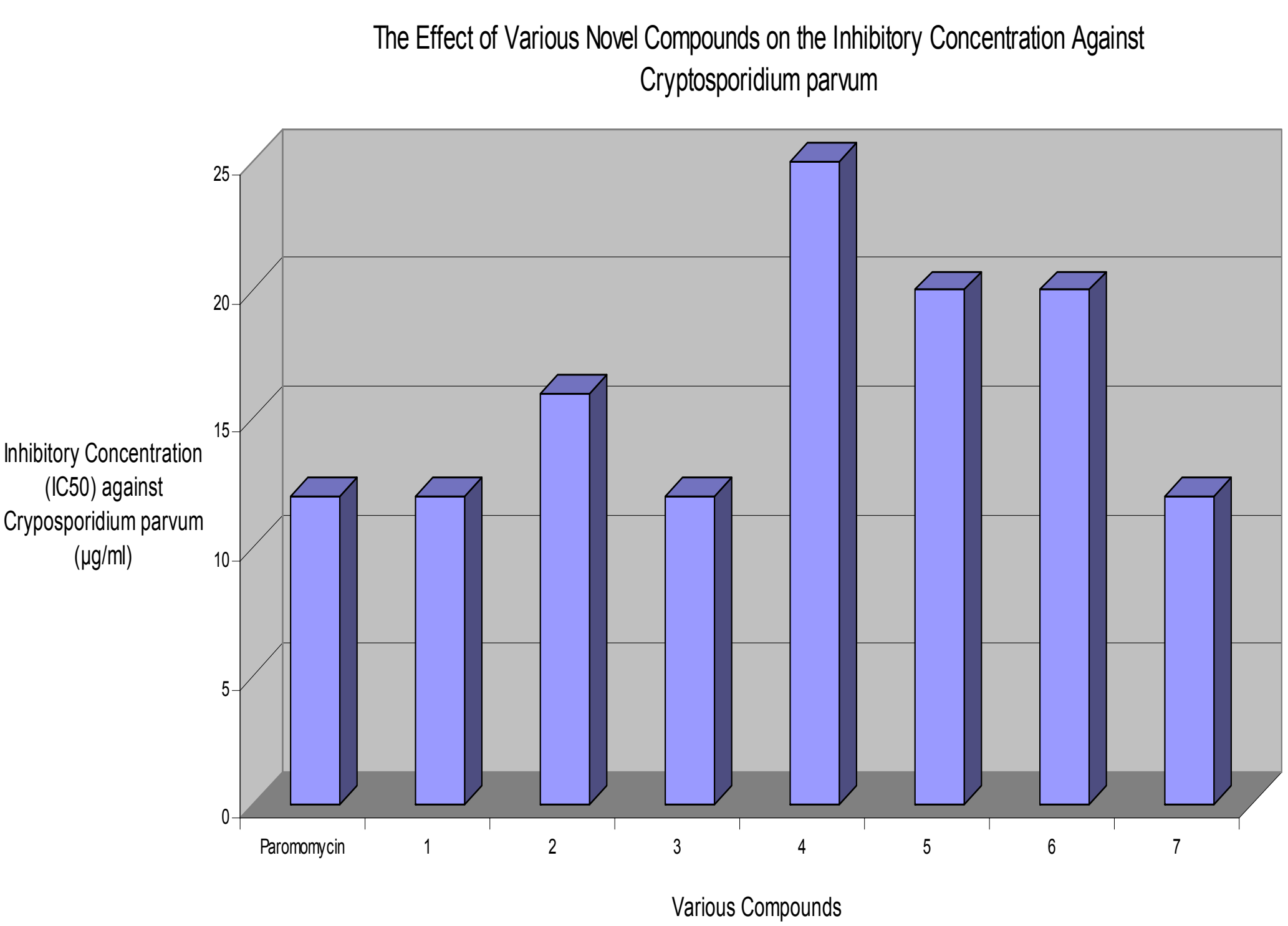
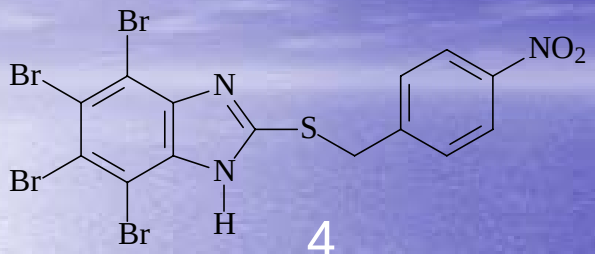
Results

- The effect of seven new compounds and the well known anti-cryptosporidiosis drug – paromomycin were investigated by exposure of growth in cell culture.
- Paromomycin in HCT-8 cells obtained similar levels of *Cryptosporidium* inhibition when compared to tested chemical compounds: 1,3 and 7.
- The ranges of IC_{50} for the new compounds ranged from 12 µg/ml to 24 µg/ml.
- The best compound efficacy indicates that a significant structure is the benzimidazole with thiobenzyl at position 2.


The Effect of Various Novel Compounds on the Inhibitory Concentration Against Cryptosporidium parvum
Inhibitory Concentration (IC50) against Cryposporidium parvum (µg/ml)
25
20
15
10
5
0
Paromomycin
1
2
3
4
5
6
7
Various Compounds

Chemical Compounds Tested



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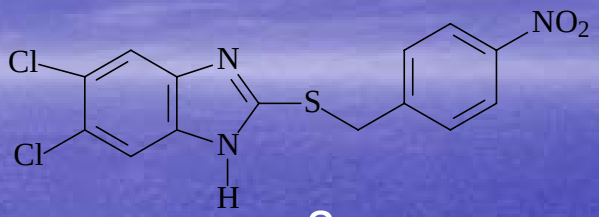

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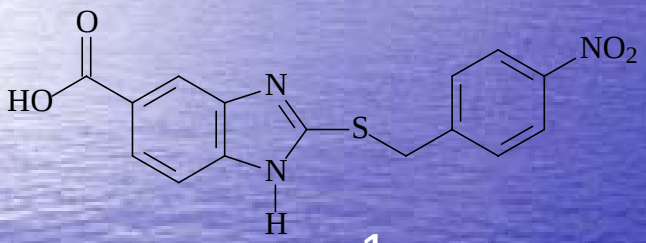

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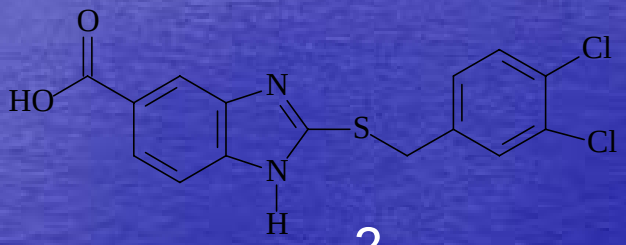

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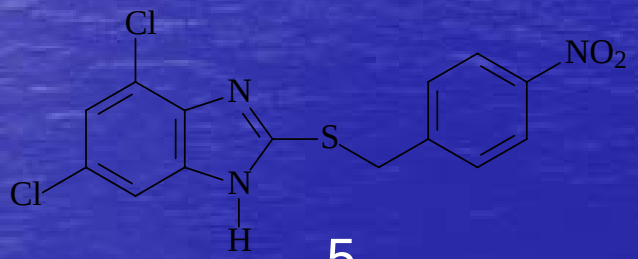

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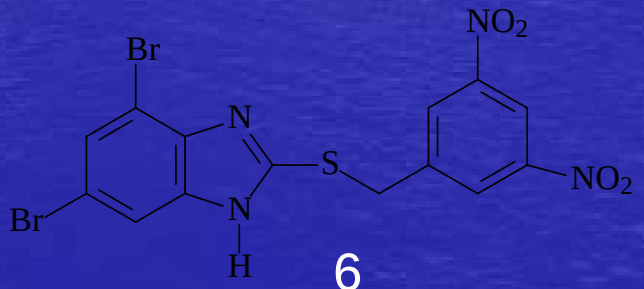

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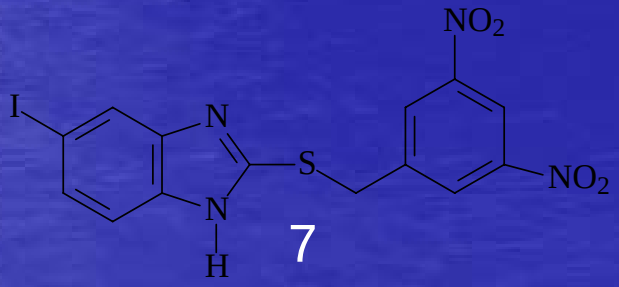

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Discussion

- Because, it is not enough relevant studies for treatment of cryptosporidiosis, our study is very important for immunocompetent and immunocompromised individuals.

- Anti-diarrheal medicine may help remediate diarrhea, but a health care provider should be consulted before such medicine is taken. Nitazoxanide has been FDA-approved for treatment of diarrhea caused by *Cryptosporidium* in people with healthy immune systems and is available by prescription. However, the effectiveness of nitazoxanide in immunosuppressed individuals is unclear.

- HIV-positive individuals who suspect they have cryptosporidiosis should contact their health care provider. For those persons with AIDS, anti-retroviral therapy that improves the immune status will also decrease or eliminate symptoms of cryptosporidiosis. However, even if symptoms disappear, cryptosporidiosis is often not curable and the symptoms may return if the immune status worsens.

Acknowledgments

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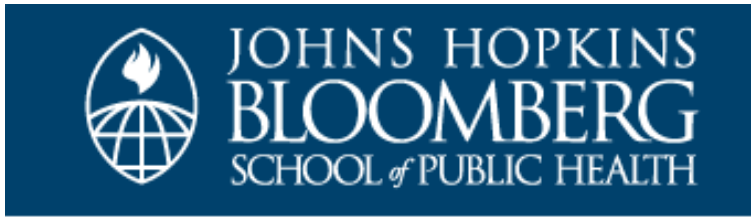
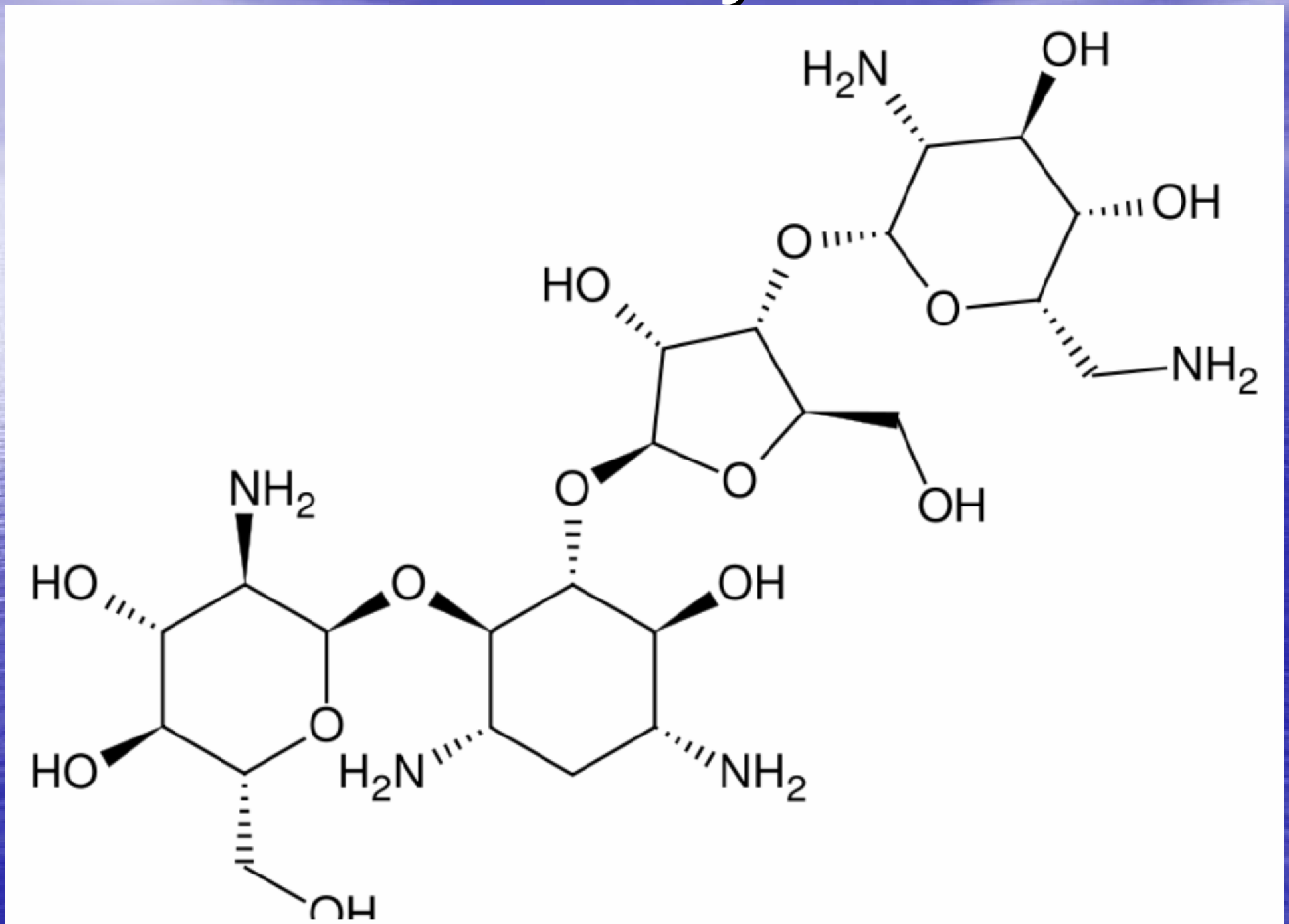
We thank the Foundation for Development Diagnostics and Therapy in Warsaw for providing chemical compounds, and special thanks to the leader of our lab. prof. Lidia Chomicz from Medical University, Department of Medical Biology, Warsaw, Poland.

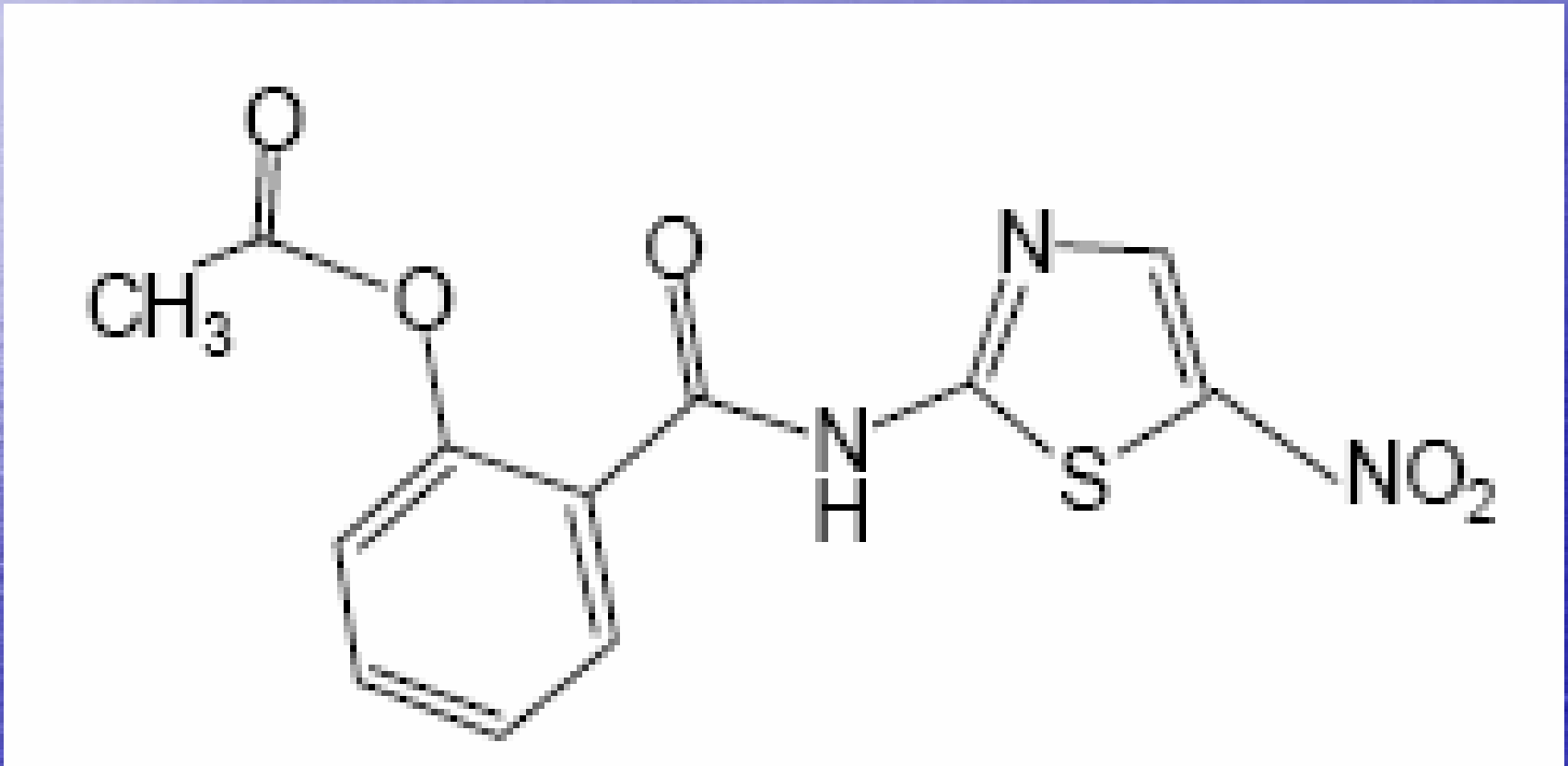
We thank you for your attention

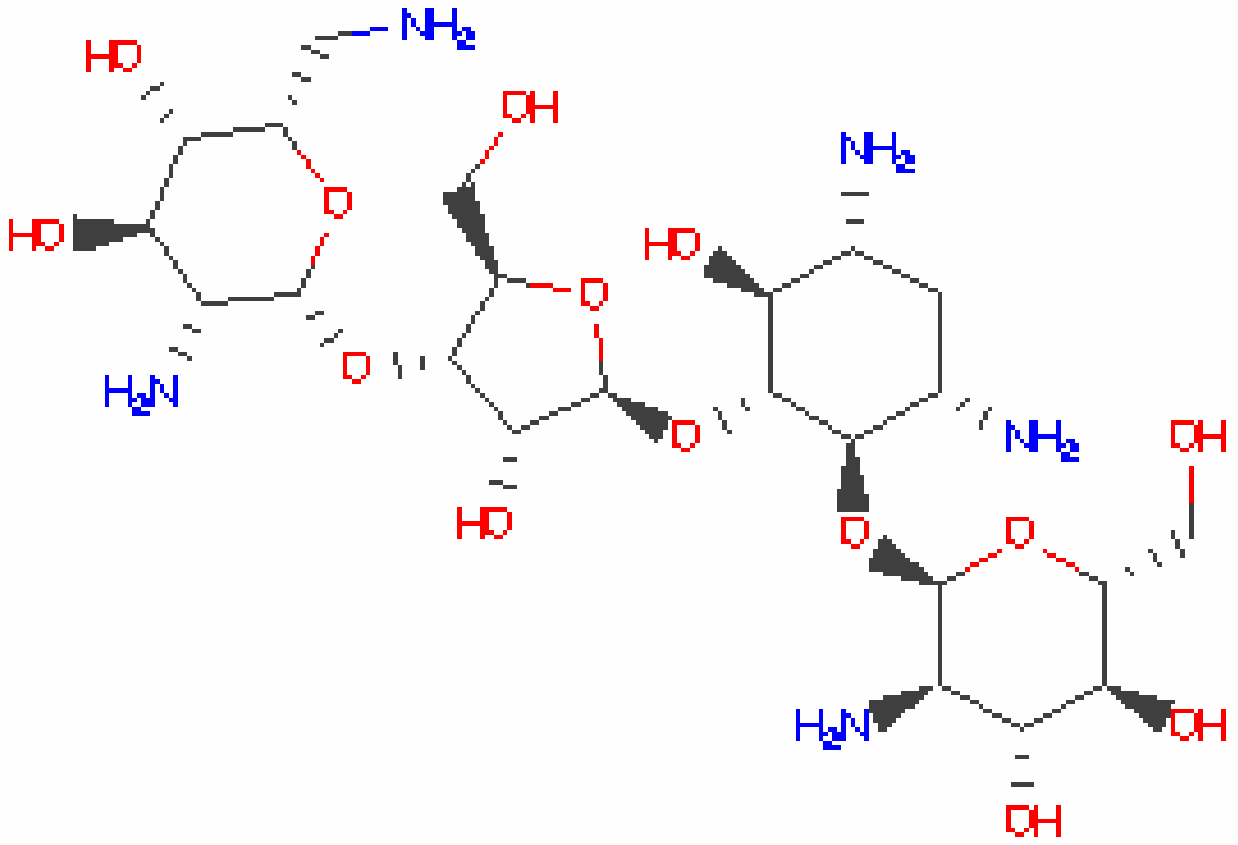


Paromomycin



Nitazoxanide

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