Cyst Wall Biosynthesis Inhibitors: Discovery of a New Class of Anti-*giardia* Agents

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Introduction

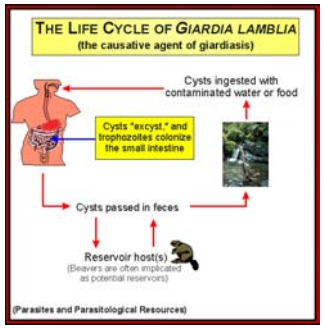
Giardia lamblia

- Unicellar flagellated protozoal parasite
- Spread via contaminated food and water
- *Giardia* is the most commonly diagnosed parasitic cause of diarrhea in North America
- Estimated 100 million cases of giardiasis worldwide
- Promiscuous: a wide variety of vertebrates can be infected
- Contact with companion animals and livestock is a risk factor for *giardiasis* in humans

***Giardia Lamblia* Trophozoite** **_Giardia Lamblia_ Cyst**

Treatment of *Giardiasis*

- Resistance to all anti-parasitics has been reported
  - Failure rates of ~20% reported with currently used meds
- Cysts are highly resistant to chemical treatment
  - 10% bleach or a mixture of 2% sulfuric acid and 2.5% potassium dichromate is needed to fully inactivate cysts
  - Ozone, a new method to treat water supplies is not very effective
- Difficult to detect due to small size

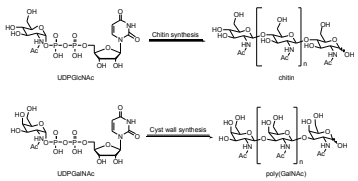


- Encystation is thought to be a response to unfavorable growth conditions and allows survival outside the host
- **Encystation is common to many protozoans**

Cyst Wall Synthesis: a New Target for Anti-Protozoal Therapy

- Many protozoal cyst walls are composed of chitin or a chitin like substance
  - Giardia: β-1-3-linked poly(*N*-acetylgalactosamine) [poly(GalNAc)]
  - *Entamoeba:* chitin
- Enzyme activity in Giardia termed cyst wall synthase has been described[3]
- **An inhibitor of cyst wall synthesis is likely to be a broad spectrum anti-protozoal agent**

Chitin and cyst wall synthesis

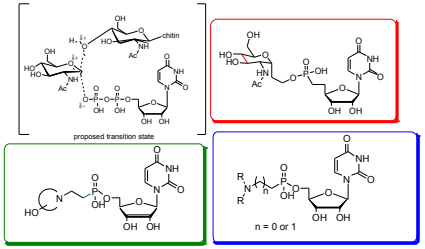


Inhibitor Design

We have designed and synthesized two classes of inhibitors of protozoal cyst wall synthesis

1. A phosphonate C-glycoside UDP-glucosamine analog that is metabolically, chemically stable and is able to penetrate cell membranes (below, red)

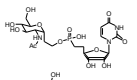
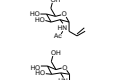
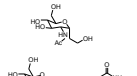
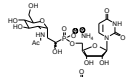
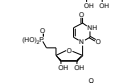
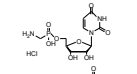
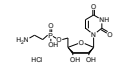
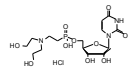
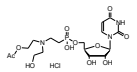
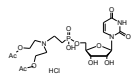
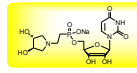
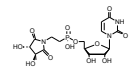
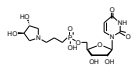
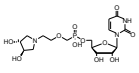
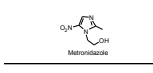
2. Transition-state analogs containing an aza-sugar (green) or aza-sugar analog (blue)



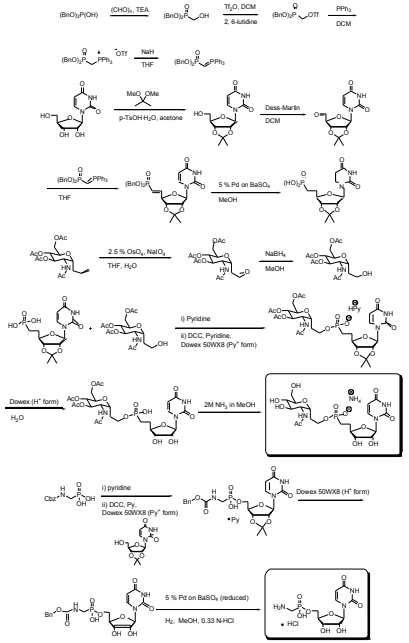
Biological Assays

- **Anti-Giardia**
  - The *G. lamblia* WB-C6 strain was used for the assays.
  - Trophozoite inhibition was determined by culturing the parasites anaerobically for 48 hours in the presence of 2-fold drug dilutions in 96-well plates in duplicate. The XTT reagent was used to measure cell viability.
  - Cyst development was determined in a similar manner except the assay was conducted for 4 days in encysting medium in glass vials followed by counting cysts, washing cells in diH_20 and resuspension in trophozoite culture medium to evaluate cyst viability.
- **Host toxicity**
  - Manine Darbey Bovine Kidney cells (MDBK) were used to evaluate mammalian cell toxicity. 2-fold drug dilutions in RPMI1640 culture medium with 5% FBS were used with cells seeded and grown for 48 hours before performing viability measurements using the XTT reagent.
- **IC_{50} determination**
  - Standard curves were generated as % no drug controls for each drug dilution. At least 3 independent determinations were used to determine the values.

Anti-*Giardia* Activity

Structure	Giardia MIC (μM)	MDBK Cell tox IC50 (μM)	Therapeutic index	Giardia cyst formation percent of control
	3.9	250	64	27.5
	15.6	60	10.7	9.7
	3.9	125	32	58.2
	>20	>250		26.4
	1.9	250	131	10.3
	>20	>250		34.4
	>10	>250		14.9
	>10	>250		72.2
	>10	>250		36.6
	>10	>250		27.5
	0.48	>250	>520	5.73
	>20	>250		28.7
	<20	>250		22.9
	<20	>250		17.2
 Metronidazole	5			13.7

Synthesis of Selected Targets



Conclusion

We have designed and synthesized a new class potent anti-Giardia agents that:

2. Have very low toxicity
3. Have micromolar to sub-micromolar MIC against *Giardia* trophozoite growth

We have discovered an agent that is a highly promising lead for clinical development.

5. Ten times more potent than metronidazde (MIC 0.48 μM vs 5μM)
6. Has very low toxicity
7. Enzyme assays are in progress
8. Assays against other protozoans are in progress
9. Animal model studies will begin in due course

References

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2. http://ryoko.biosci.ohio-state.edu/~parasite/lifecycles/giardia_lifecycle.html
3. Karr, C. D.; Jarroll, E. L. *Microbiology* **2004**, *150*, 1237-1243
4. Yeager, A. R.; Finney, N. S. *J. Org. Chem.* **2004**, *69*, 613