

Synthesis of Small Molecule Anti-Trypanosomal Drugs: A Brief SAR Study

Samuel Peter Anderson

Department of Chemistry, College of Arts and Sciences, University of Nebraska at Omaha, Omaha, NE 68182

Abstract

Demand for novel chemotherapeutic agents that treat Human African Trypanosomiasis (HAT) persists. A series of compounds sharing a 3,4'-diphenyl ether skeleton were prepared for a structure activity relationship (SAR) study evaluating antitrypanosomal drug candidates. Trypanocidal assays performed *in vitro* by the Swiss Tropical and Public Health Institute identified a lead with an IC_{50} of 1.35 $\mu\text{g/mL}$ analogous to a compound previously made in our laboratory. A slight improvement in activity (10%) was observed when changing the molecular substitution pattern from 4,4' to 3,3'; however, the result is equivocal. Furthermore, *in vitro* test results demonstrated the weakness of amides as side chain functional groups, emphasizing the role of cationic moieties. The methods developed and material produced from this research enables the rapid production of new compounds to further investigate these observations.

Introduction

Human African Trypanosomiasis (HAT) is a debilitating tropical disease caused by the flagellar parasitic protozoa *Trypanosoma Brucei*. Left untreated, trypanosoma parasites invade a patient's brain and induce neurological changes such as sleep disorder, ataxia, psychosis, coma, and ultimately death. Fortunately, aggressive epidemiological intervention by the World Health Organization has limited the spread of the disease in recent years. However, approximately 70 million people distributed over a surface of 1.55 million km^2 are estimated to be at differing levels of risk of contracting HAT. While current drug treatments—suramine, pentamidine, nifurtumox, and melarsoprol—are effective, they are also costly, inconvenient, and toxic. Therefore, there is ongoing demand for novel chemotherapeutic agents that destroy the parasite. Nonetheless, since HAT affects primarily impoverished populations, pharmaceutical firms are disinterested in pursuing new HAT drug compounds. Therefore, academic research is critical to HAT drug discovery.

Contemporary drug design attempts to exploit known biochemical pathways associated with disease. The best therapeutic compounds, potent with limited side effects, often modulate a specific and well understood process. Sadly, the mechanism of action of current HAT treatments is poorly understood. Hence, HAT drug development relies on modifying the chemical structure of compounds with known efficacy. Systematically changing molecular shape, size, rigidity, or functional groups can identify patterns that correlate chemical structure to biological activity. Such experiments are called structure activity relationship (SAR) studies.

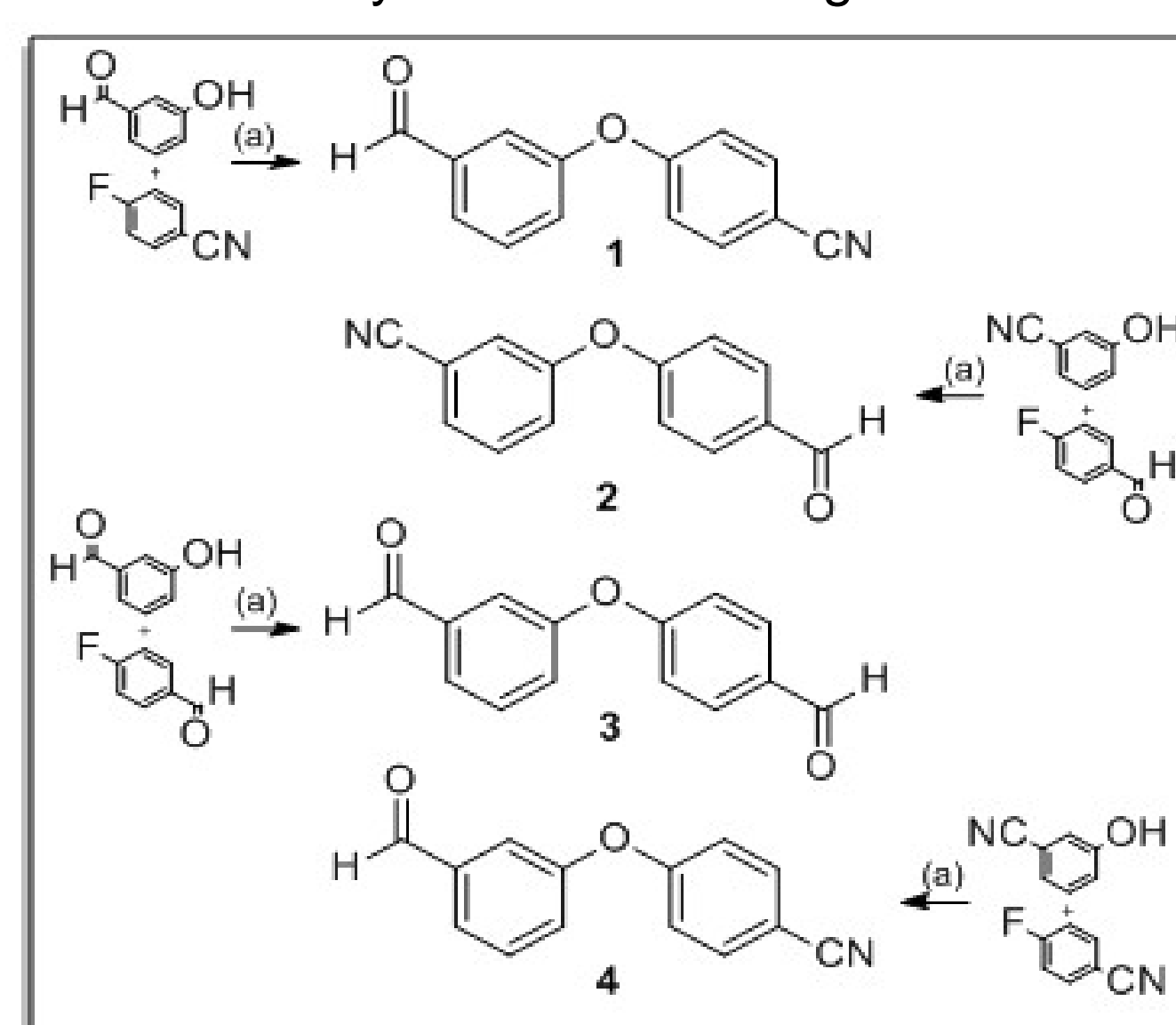
Internationally, the activity of anti-trypanosomal drugs is assayed by the Swiss Tropical and Public Health Institute in Basel, Switzerland. They report the drug concentration necessary to inhibit half of a parasite load, *in vitro* IC_{50} values, measured in triplicate and referenced to melarsoprol ($IC_{50} = 1 \text{ ng/mL}$). A common worldwide assay ensures that compared data is meaningful. Promising preliminary compounds begin with low microgram per milliliter IC_{50} activities; whereas, strong drug candidates require nanogram per milliliter IC_{50} activities.

The diversity of promising HAT drug candidates exhibiting ng/mL IC_{50} is staggering. Many families of compounds have worked, so it's sufficient to merely pick one. This work is based on the lead compound G71ML ($IC_{50} = 0.68 \text{ }\mu\text{g/mL}$), a *para*-substituted diphenyl ether amino acid previously prepared in our laboratory.

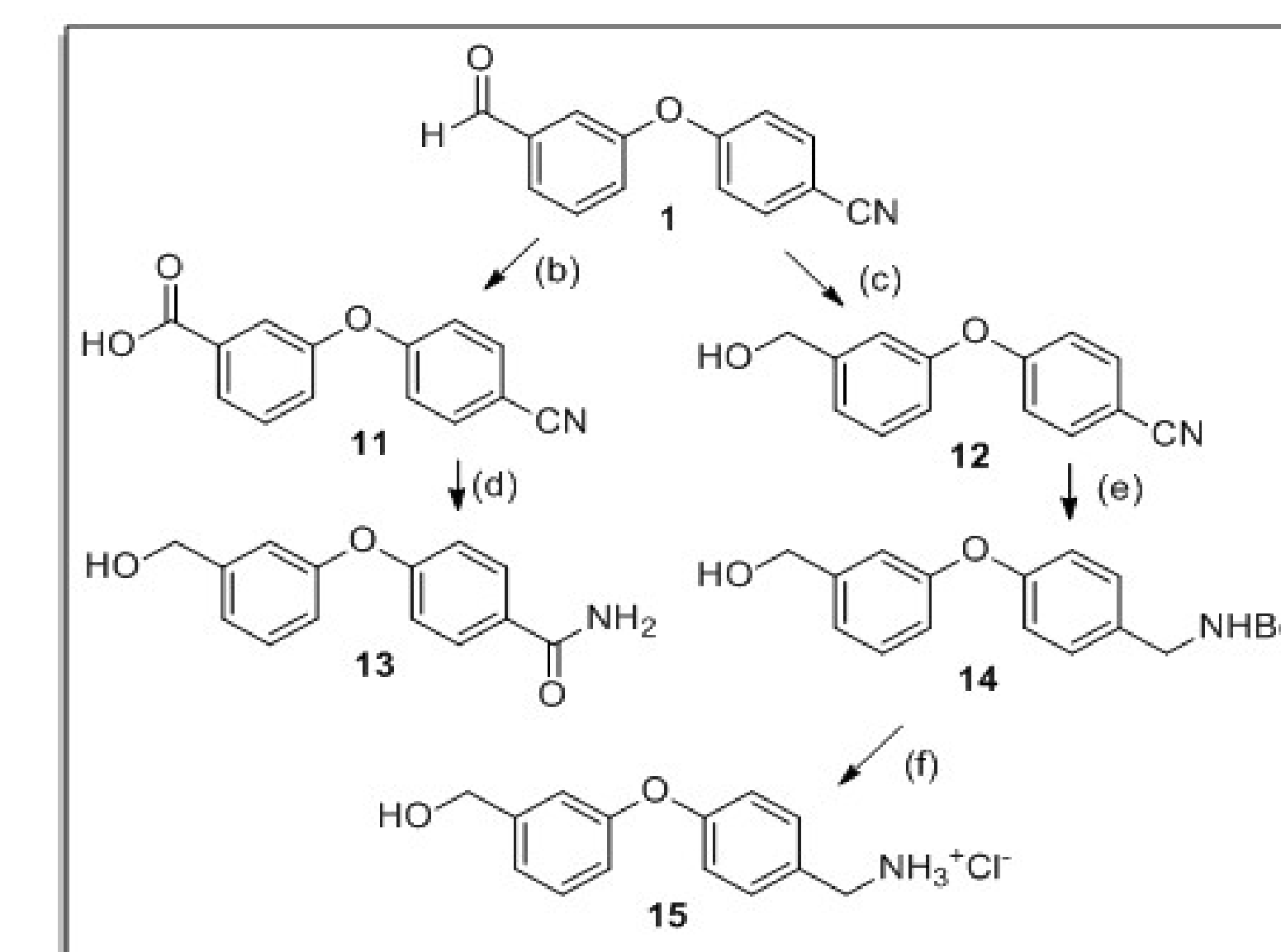
Experimental Methods

3,4'-substituted diphenyl ether starting materials were synthesized from fluoro or hydroxy substituted benzonitriles and benzaldehydes using base-catalyzed nucleophilic aromatic substitution. Then, general oxidative, amidative, and reductive methods derivatized these precursors to testable products. The chemical characterization of each included: melting point, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and elemental analysis.

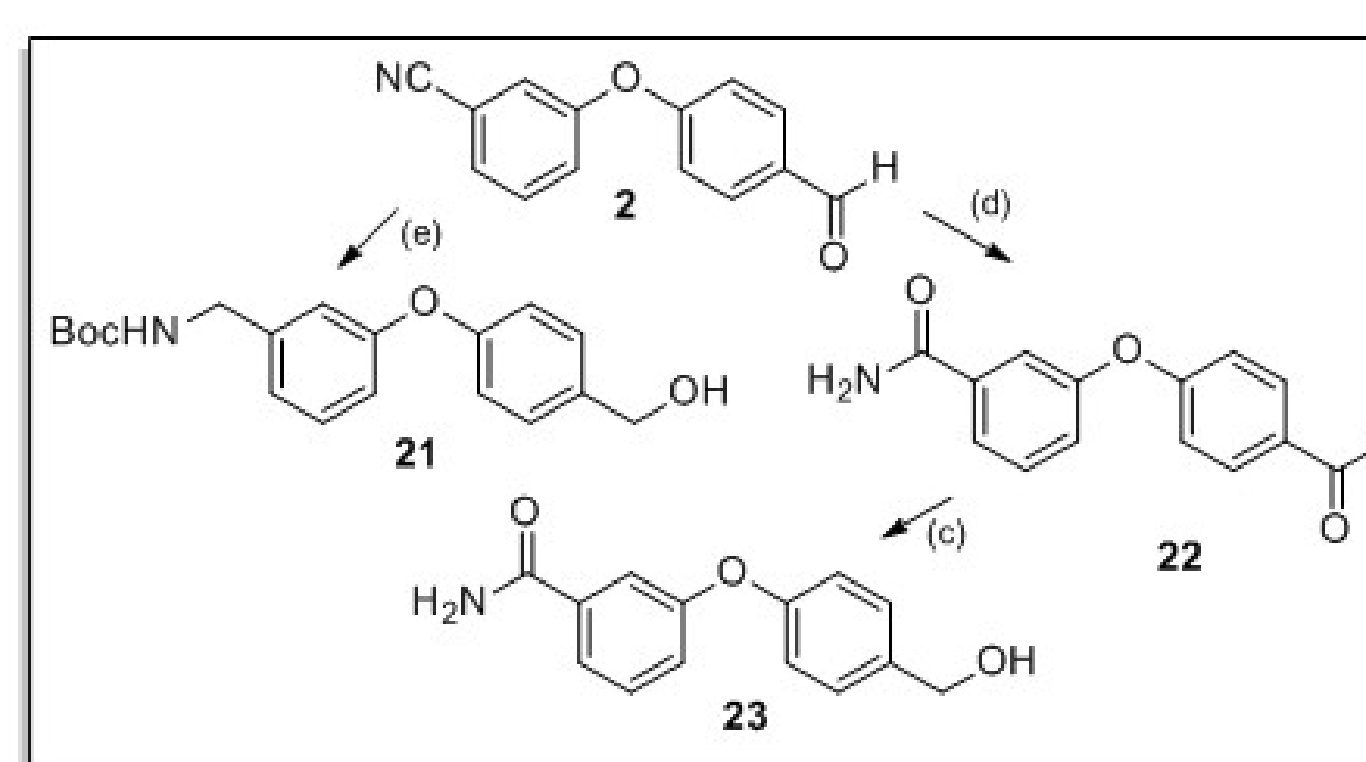
Scheme 1. Synthesis of Starting Materials



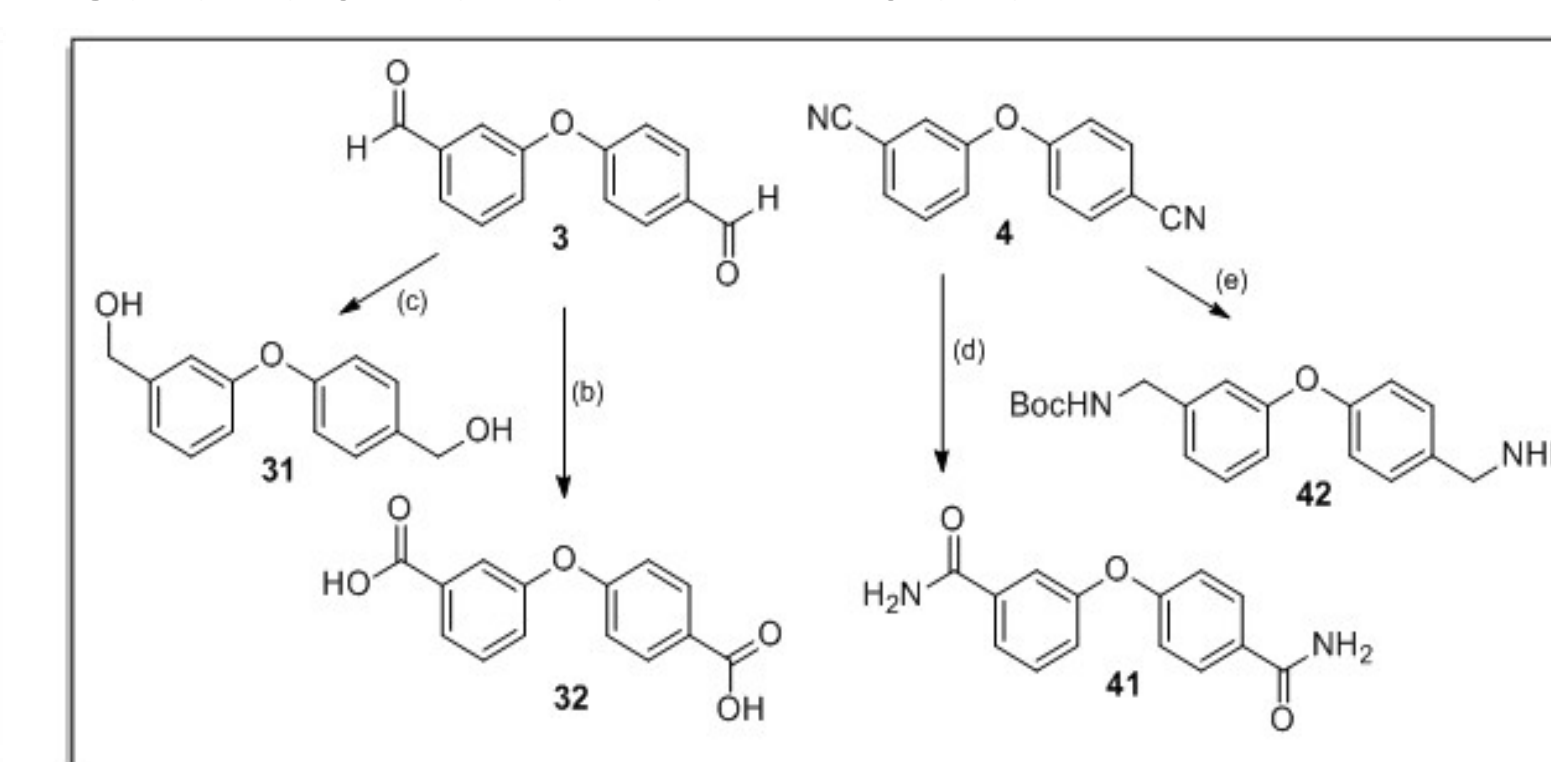
Scheme 2. Derivatization of 1



Scheme 3. Derivatization of 2



Scheme 3. Derivatization of 3 and 4



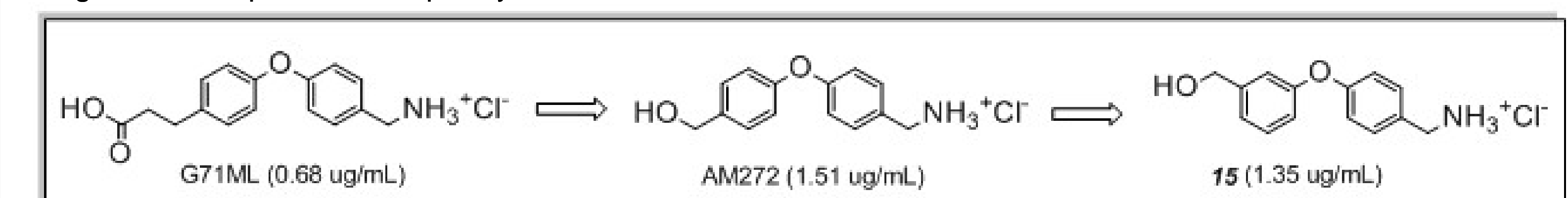
General Methods

- (a) Nucleophilic Aromatic Substitution: K_2CO_3 (2 eq.), anhy. DMF, air free, 130°C , 24 hr
 (b) Cu(II) Bromide Oxidation: 70% TBuOOH (4 eq.), Cu(II)Br_2 (0.1 eq.) acetonitrile, 23°C , 1 hr.
 (c) Sodium Borohydride Reduction: NaBH_4 (3 eq.), methanol, 23°C , 1hr
 (d) Caddick Reduction: NaBH_4 (8 eq.), $\text{NiCl}_2 \cdot 2 \text{H}_2\text{O}$ (0.2 eq.), Boc_2O (2 eq.), 0°C , 15 hr.
 (e) Oxidative Amination: 30% H_2O_2 (1 eq.), K_2CO_3 (0.5 eq.), DMSO, 0°C to 23°C , 1 hr
 (f) Acidic Precipitation: Anhydrous HCl in ethyl acetate, 23°C , 5 min.

Discussion

The 3,4' alcohol amine, **15**, exhibited the highest trypanocidal activity of the compounds tested. This was expected since its chemical functionality is analogous to known compounds made in our laboratory, G71ML and AM272.

Figure 1. Comparison of Diphenyl Ether Derivatives



The prepared derivatives have a 3,4' substitution pattern whereas our previous work focused on 4,4' substituted compounds. The enhanced activity of **15** relative to AM272 suggests that 3,4' substituted compounds may be superior in general. However, at present, the claim is equivocal.

Another important finding is the apparent unsuitability of amides as side chain functional groups. The amide analog of **15**, **13**, is an order of magnitude less active. This emphasizes that cationic groups, such as the protonated amine, are important structural features of active HAT drugs.

Conclusion and Future Work

The preliminary findings of the SAR study support the notion of 3,4' substitution patterns in HAT drugs. Diphenyl ether amide derivatives, however, make poor drug compounds.

Future work will complete the synthesis and assay of the best remaining candidates of the group (3- CH_2NH_3^+ , 4'-OH; 3- CH_2NH_3^+ , 4'- CH_2NH_3^+). Subsequently, the starting materials made during the project will be applied to the rapid synthesis of other promising 3,4' analogs.

Results

Of the sixteen compounds prepared, four were sent to the Swiss TPHI for trypanosomal assay: **13** (3-OH,4'-ONH₂), **15** (3-OH,4'-CH₂NH₃⁺), **31** (3-OH,4'-OH), and **41** (3-ONH₂,4'-ONH₂). The results are summarized in Table 1.

Table 1: Swiss TPHI *in vitro* Test Results

Compound	IC_{50} Activity ($\mu\text{g/mL}$)
13	35.62
15	1.35
31	45.89
41	43.46

References

- (1) Khan, O. *Drug Targets Insights*. **2007**, 2, 129-146.
- (2) Simarro, P. P.; Cecchi, G.; Franco, J. R.; Paone, M.; Diarra, A.; Ruiz-Postigo, J. A.; Fevre, E. M.; Mattioli, R. C.; Jannin, J. G. *PLOS Neglected Tropical Diseases* **2012**, 10, 1-12.
- (3) Montalvo-Quiros, S. Taladriz-Sender, A. Kaiser, M. Dardonville, C. *J. Med. Chem.* **2015**, 58, 1940-1949.
- (4) Caddick, S. Judd, D. B. de K. Lewis, A. K, Reich, M.T. Williams, M.R.V. *Tetrahedron* **2003**, 59, 5417-5423.
- (5) Prinz, H. et. al. *J. Med. Chem.* **2011**, 54, 4227-4263.
- (6) Zhao, H. Vandenbossche, C. P. Koenig, S.G. Singh, S. P. Bakale, R. P. *Organic Letters*. **2008** 3, 505 -507.
- (7) Travis, B.R. Sivakumar, M, Hollist, G.O. Borhan, B. *Org. Lett.* **2003**, 7, 1031 -1034.
- (8) Uyanik, M. Akakura, M. Ishihara, K. *J. Am. Chem. Soc.* **2009**, 131, 251-262.
- (9) Das, R; Chakraborty, D. *Applied Organometallic Chemistry*. **2011**, 25, 437-442.