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Synthesis of Neurolysin Modulators for Stroke and Cancer Treatment

Hossam Elaraky
University of Nebraska Medical Center

Aarfa Queen
University of Nebraska Medical Center

MD Shafikur Rahman
University of Nebraska Medical Center

Angie Carmona
University of Nebraska Medical Center

Hamdan Alrefaei
University of Nebraska Medical Center

See next page for additional authors

Author

Hossam Elaraky, Aarfa Queen, MD Shafikur Rahman, Angie Carmona, Hamdan Alrefaei, and Paul C. Trippier

UNMC
UNIVERSITY OF NEBRASKA MEDICAL CENTER

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Hossam Elaraky, Aarfa Queen, Md Shafikur Rahman, Angie Carmona, Hamden Al Refaei
Department of Pharmaceutical Sciences, UNMC, Omaha, NE; UNO, Omaha, NE

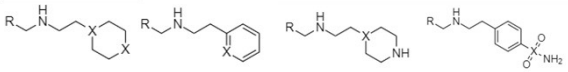
Abstract

Neurolysin (Nln) is a neutral metalloendopeptidase, which is an enzyme that breaks down peptide bonds in amino acids whilst ultilizing a metal catalyst[1]. Nln inactivates several neuro/cerberotoxic neuropeptides such as neurotensin, substance P, and bradykinin[2]. These neuropeptides can cause harm to the brain as they respond to inflammation during an ischemic stroke[3,4]. The peptides generated by Nln are neuro/cerebroprotective which aids in its ability to protect the brain in an ischemic stroke victim[2]. The goal of our lab is to discover a potent neurolysin activator in order to develop a drug that protect victims from brain damage and disability following an ischemic stroke. To begin this process, our lab successfully designed, synthesized, and established the structure-activity relationships of several potential neurolysin activators via a preliminary neurolysin activation assay on several targeted compounds. Secondary amine analogs were created and synthesized to test their neurolysin activation potential. The goals of this experiment are to resynthesize these compounds to gather additional NMR and HPLC data and to optimize the reaction in order to obtain a pure compound with a substantial yield.

Introduction

Stroke is the second-leading cause of death worldwide and the third-leading cause of death and disability[5]. Ischemic stroke comprises 87% of all strokes and occurs when blood flow to the brain is blocked[5]. As a result of decreased blood flow, brain cells begin to die and brain damage can occur. The American Heart Association reports that 75% of victims have dysfunction and 15–30% of stroke survivors have a severe disability[5]. An effective method of reducing or preventing brain damage during a stroke is to protect the brain using a coordinated and diverse mechanism. Neurolysin (Nln) is a peptidase and a potential target for brain protection during stroke. A study conducted on mouse models shows that Nln is upregulated in the post-ischemic brain for at least 7 days[6]. The results of this study suggest that Nln is responsible for modulating the brain's stroke response. Another study shows that the inhibition of Nln in mice brains causes an increase in neurotoxic peptidases[7]. Therefore, Nln is an important target due to its diverse signaling ability and its ability to protect the brain during neurodegenerative disorders such as stoke. If a drug can be synthesized that activates Nln, it can have the potential to reduce or prevent brain damage in ischemic stroke victims. Using structure-activity relationship studies we can create compounds that could potentially activate Nln. Then, using methods of organic synthesis, we can create those compounds and test their potency.

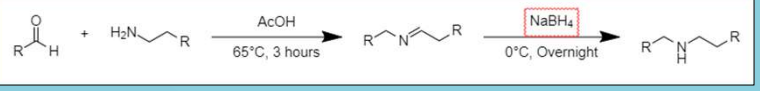
Compounds: Potential Neurolysin Activators



Compound I | Compound II | Compound III | Compound IV

X= Chalcogen or Pnictogen

General Procedure for Reductive Amination



Compound I Results

Compound I Conclusion:

- Compound's polarity required **12%** of MeOH in DCM to elute.
- Reaction was clean and gave **54.5%** yield of a yellow, crystalline oil.
- NMR was conclusive and accurate: product appears pure.

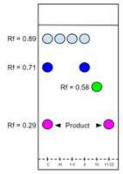


Figure 1: TLC of Compound I

Compound II Results

Compound II Conclusion:

- Compound's polarity required **12%** of MeOH in DCM to elute.
- Reaction was clean and gave **52.6%** yield of a dark, brown oil.
- NMR was conclusive and accurate: product appears pure.

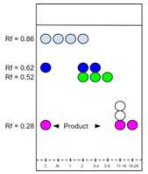


Figure 2: TLC of Compound II

Compound III Results

General Procedure Results (Inconclusive):

- Crude yield was 60 mg of an impure mixture and purification was difficult (Figure 3).
- Aldehyde was impure and contained a carboxylic acid derivative.
- Aldehyde is replenished with a fresh bottle for further reactions.
- A potential side product was also discovered (Figure 4).

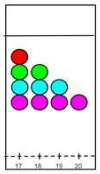


Figure 3: TLC of Compound III Using General Procedure

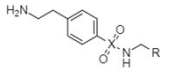


Figure 4: Potential Side Product

Procedure with STAB (Inconclusive):

- 2.5 eq. of Sodium Trioxyborohydride was added to 1 eq. of aldehyde and amine.
- Imine was formed, however, there was too little yield of secondary amine product.
- Imine decomposes on silicon column, so purification is a challenge. Therefore, a better reaction is needed.

General Procedure with Reflux and Additional NaBH4 (Low Yield):

- The reaction mixture was refluxed with the first step for 3 hours followed by 10 eq. of NaBH4.
- After purification, only **7.4 mg** of product remained (**2.54%** yield).

General Procedure with Reflux, Additional NaBH4, and Extra Time (Undetermined):

- I hypothesize that if the first step is left for longer than 3 hours and adequate time is given to form the imine (observed using TLC), then the yield will be much greater.

Compound IV Results

General Procedure Results (Inconclusive):

- Inconclusive since crude yield was approximately 100 mg of an impure mixture.
- Purification is difficult due to another side product which forms more easily than the desired compound (Figure 5).

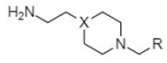


Figure 5: Potential Side Product

General Procedure with Protecting Group and Extra Time Results (Conclusive):

- The amine used contains a BOC group to prevent the side product from forming.
- Step 1 is left for 24 hours to form adequate amine.
- Step 2 is left for 12 hours, and molecular sieves are added to remove water.
- Compound's polarity required **12%** of MeOH in DCM to elute.
- Reaction was clean and gave **64.4%** yield of a light, brown oil.
- Last step is to use TFA to remove the BOC group.
- Final yield not yet determined until deprotection is conducted.

Conclusion

Reductive amination is a relatively simple two step procedure to synthesize secondary amines. The compounds synthesized in lab are potential neurolysin activators and will require further testing in vivo to determine their efficacy. Compound I and II were able to be synthesized using the general procedure with a >50% yield. Compound III required a longer time for the first step to form the imine and reduce the formation of side products. Compound IV required a BOC protecting group in order to prevent side products and the time for the first step was also extended due to steric hinderance of the BOC group. The BOC protected product had the highest yield of 64.4%. All four compound were successfully synthesized with accurate NMR, however, HPLC data is still required. The HPLC data will be acquired as soon as a working machine is available.

Contributions

Thanks to Dr. Trippier for the opportunity to work on his neurolysin project. Thanks to Aarfa, Shafikur, Angie, and Hamden for guiding me through the project and teaching me new methods to try and obtain my product. This project wouldn't be possible without them.

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