Award Number: W81XWH-12-1-0436

TITLE: TrkB Activators for the Treatment of Traumatic Vision Loss

PRINCIPAL INVESTIGATOR: P. Michael Iuvone. Ph.D.

CONTRACTING ORGANIZATION: Emory University
Atlanta, GA 30322

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30Sep2014 - 29Sep2015

4. TITLE AND SUBTITLE
TrkB Activators for the Treatment of Traumatic Vision Loss

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0436

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Michael P. Iuvone

E-Mail: miuvone@emory.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Emory University
1365B Clifton Rd NE
Atlanta, GA 30322

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Pressure waves due to explosions can damage the neurons of the eye and visual centers in the brain, leading to functional loss of vision. There are currently few treatments for such injuries that can be deployed rapidly in the field to mitigate such damage. Our research team is developing small molecule activators of TrkB, the cognate receptor for brain-derived neurotrophic factor (BDNF), which can be administered systemically and cross the blood brain/retina barrier (BBB). In the third year of the grant, we tested the effects of the TrkB receptor activator, HIOC, in a blast overpressure model of ocular trauma. HIOC produced a significant preservation of visual function, which in some experiments was nearly complete. We also found that HIOC partially prevented optic nerve degeneration following ocular blast injury. HIOC alone was as effective or more effective than combinations of HIOC and meloxicam, an anti-inflammatory drug, or HIOC and FTY720, a drug that converts microglia from an inflammatory phenotype to a neuroprotective phenotype. We also initiated studies to examine the of blast-induced head injury. In the initial studies, HIOC partially prevented the loss of visual function caused by blast directed at the head.

15. SUBJECT TERMS
trauma, neuroprotection, retina, optic nerve, TrkB, BDNF, brain, TBI

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	28	USAMRMC

Table of Contents

Page

1. Introduction…… 3
2. Keywords…… 3
3. Overall Project Summary……..... 3
4. Key Research Accomplishments……..... 13
5. Conclusion……..... 14
6. Publications, Abstracts, and Presentations……..... 14
7. Inventions, Patents and Licenses……..... 14
8. Reportable Outcomes..……..... 14
9. Other Achievements……..... 14
10. References……..... 14
11. Appendices……..... 16

1. INTRODUCTION

Pressure waves due to explosions can damage the neurons of the eye and visual centers in the brain, leading to functional loss of vision. There are currently few treatments for such injuries that can be deployed rapidly in the field to mitigate such damage. Our research team is developing small molecule activators of TrkB, the cognate receptor for brain-derived neurotrophic factor (BDNF). BDNF has been shown to have neuroprotective effects in a number of degeneration models, including optic nerve crush and bright light-induced retinal degeneration (Gauthier et al., 2005; Weber et al., 2010). However, BDNF must be injected intraocularly or into the brain to be effective, as it does not cross the blood brain/retina barrier (BBB), making it impractical to deploy in the field. In contrast, the compounds we are developing can be administered systemically and readily cross the BBB (Jang et al., 2010a,b,c). Following peripheral injection, the drugs activate TrkB receptors in the retina and the brain, and appear to show no systemic toxicity. In preliminary studies, we have shown that they protect against light-induced retinal degeneration (Shen et al., 2012). The goal of this project is to develop effective treatments for traumatic blast-related retinal and visual system damage that can be delivered on the battlefield. We hypothesize that small molecule activators of TrkB will be useful for this purpose. We proposed 3 specific aims to test this hypothesis, investigating the utility of TrkB activators to prevent retinal ganglion cell death following optic nerve crush, protect retinal cells from blast-induced injury to the eye, and protect central visual pathways from traumatic blast-induced injury.

2. KEYWORDS: trauma, neuroprotection, retina, optic nerve, TrkB, BDNF, brain, TBI

3. OVERALL PROJECT SUMMARY

The statement of work for year 3 was to complete the test of at least two TrkB activators for treatment of blast-induced retinal damage, performing experiments to determine the optimal time window for initiating treatment. It also included the initiation of studies to examine the efficacy of TrkB activators at slowing or preventing traumatic blast-induced neuronal degeneration in visual pathways in the brain. We accomplished these goals and, importantly, demonstrated that HIOC, our lead TrkB activator, significantly reduced loss of visual function following blast injury to the eye and to the brain.

In year 1, experiments were initiated to establish assays for measuring retinal ganglion cell (RGC) loss after optic nerve crush. Three approaches were taken. One was to count Brn3a immunoreactive cells in retinal whole mounts. Brn3a is a specific marker for retinal ganglion cells (Nadal-Nicolas et al., 2009); it is expressed by approximately 90% of ganglion cells. The other approach was to count fluorescent RGCs of Thy1-CFP mice, which express CFP (cyan fluorescent protein) in retinal ganglion cells (Feng et al., 2000), or to measure fluorescence in retinal extracts of these mice. We initiated studies on effects of TrkB agonists on RGC loss following optic nerve crush, but found that systemic injection did not have a consistent neuroprotective effect.

In year 2, we investigated the effects of HIOC, delivered by various routes (i.p. or osmotic minipump), in combination with a variety anti-inflammatory and microglial-modulating drugs on optic nerve crush-induced retinal ganglion cell degeneration. None were effective. We concluded that such severe injury to the optic nerve might be beyond pharmacological intervention, at least with our tools. We built and calibrated the blast cannon, and initiated experiments to test the efficacy of TrkB activators on loss of visual function following blast-induced damage to the eye. In preliminary studies we showed potential efficacy of HIOC in preventing loss of visual function caused by blast-induced damage to the eye.

Year 3

We demonstrated that HIOC was effective in reducing vision loss and optic nerve axon degeneration following blast injury to the eye, that this effect was blocked by a TrkB antagonist, that the drug could be administered up to 3 hours after exposure to blast and still have a beneficial therapeutic effect. We also initiated studies to examine the effects of blast injury to the head on visual function and present the preliminary results of HIOC administered after blast on visual function. The results are detailed below.

Effect of blast injury to the eye on retinal morphology.

We continued to characterize the effect of blast injury to the eye on the retina. Examining retinal flat mounts, we previously saw swelling of retinal ganglion cells (RGCs) of Thy1-CFP mice one week after exposure to blast (~46psi). We re-examined this in retinal cross sections (Figure 1). Consistent with the previous result, CFP positive retinal ganglion cells of the blasted eye appeared swollen compared to control.

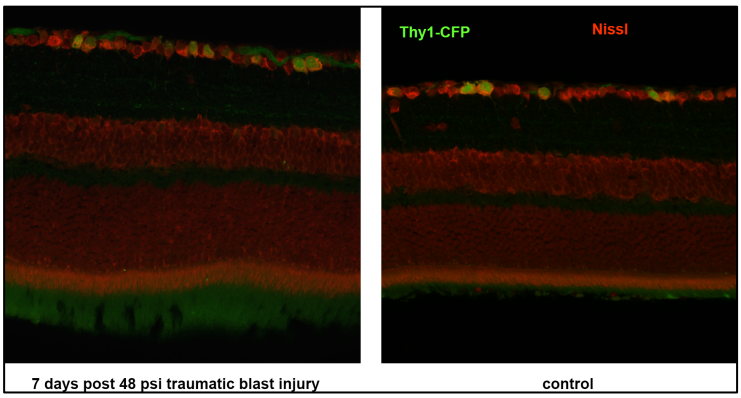


Figure 1. Nissl-stained (red) retinal cross sections stained of Thy1-CFP (green) mice 7 days after exposure to 48psi blast.

Effect of HIOC, with and without anti-inflammatory drugs, on the loss of visual function following blast injury to the eye.

Towards the end of the last year, we began testing the effect of HIOC, our lead TrkB activator, on the loss of visual function due to blast injury to the eye, and we found that it significantly preserves vision. Thy1-CFP mice were treated with 40 mg/kg of HIOC on the day of blast and daily for 1 week. In this experiment, a separate control group that was not exposed to blast was included in order to compare the use of the contralateral eye of the blast mice as control *vs* a naïve control. Exposure to blast caused a 46% decrease in contrast sensitivity in the vehicle treated blast eye group *vs* the naïve control, and only a 14% decrease in the HIOC treated mice (Figure 2A). HIOC significantly protected from blast-induced loss of function ($p<0.001$). A very similar result was observed when using the contralateral eye as control (Figure 19B; Vehicle, 45%; HIOC 17%).

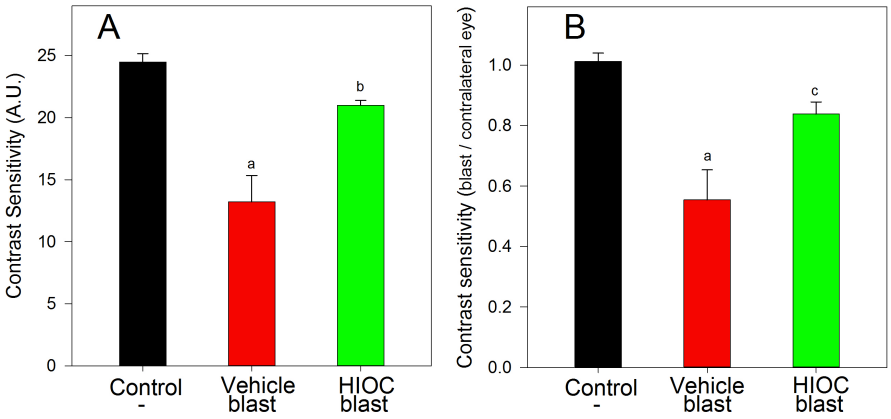


Figure 2. HIOC protects visual function from blast-induced injury. *Mice were treated with HIOC (40 mg/kg, ip) on the day of blast exposure and daily for 1 week. Blast was delivered to the right eye of the Vehicle and HIOC groups. A separate untreated control group, not exposed to blast or drugs, was included. Contrast sensitivity was measured on the 8th day after blast. Comparing left and right eye or blast to untreated control gave comparable results. Blast significantly decreased contrast sensitivity in the vehicle-treated mice ($^{a}P<0.001$). HIOC significantly reduced the blast-induced decrease in contrast sensitivity ($^{b}P<0.01$; $^{c}P<0.05$).*

We also tested LM22A-4, another TrkB activator (Massa et al., 2010), under similar conditions, and found that it also reduced the decrease of contrast sensitivity following blast injury to the eye (data not shown). However, it was not as effective as HIOC at mitigating the effects of blast injury to the eye. Therefore, we have focused our attention on HIOC.

We previously reported that both optic nerve crush and blast injury to the eye were associated with microglial activation and reactive gliosis, indicative of inflammatory reactions. We therefore sought to examine the possible benefit of a combination of HIOC and anti-inflammatory drugs or immune modulators in the treatment of traumatic blast injury to the eye. Thy1-CFP mice were exposed to a single ~48 psi blast directed to the eye and treated with vehicle, the TrkB activator HIOC (40 mg/kg ip), the non-steroidal anti-inflammatory drug meloxicam (5mg/kg sc), or a combination of the two drugs. Drugs were administered for 1 week beginning on the day of blast. Visual function (contrast sensitivity and visual acuity) was assessed by optokinetic tracking (OKT) 1 week and 1 month after blast injury (Figure 3). Exposure to blast reduced contrast sensitivity, measured at a spatial frequency of 0.064 cycles/ degree (c/d), by 35 % and 46 % in the vehicle-treated mice at 1 week and 1 month, respectively (Fig. 3 A, C; $p<0.001$). HIOC partially reversed the loss of contrast sensitivity at 1 week and completely reversed the loss at 1 month ($p<0.001$). Meloxicam alone had a small, but significant protective effect at both time points, but the effect was significantly less than that of HIOC alone ($p<0.01$). The combined effect of HIOC and meloxicam was no more effective that HIOC alone at 1 week, and was significantly less effective than that of HIOC alone at 1 month. Similar trends were observed when measuring visual acuity (Fig. 3B, D), although the effects were smaller in all groups, as observed previously. We conclude that the 40 mg/kg dose of HIOC causes nearly complete recovery of function from the blast injury. Meloxicam alone had a small protective effect against

blast injury, and that meloxicam in combination with HIOC is no more beneficial than HIOC alone under these conditions. It remains to be determined if it might potentiate the effect of a lower dose of HIOC or provide better protection from retinal ganglion cell (RGC) death.

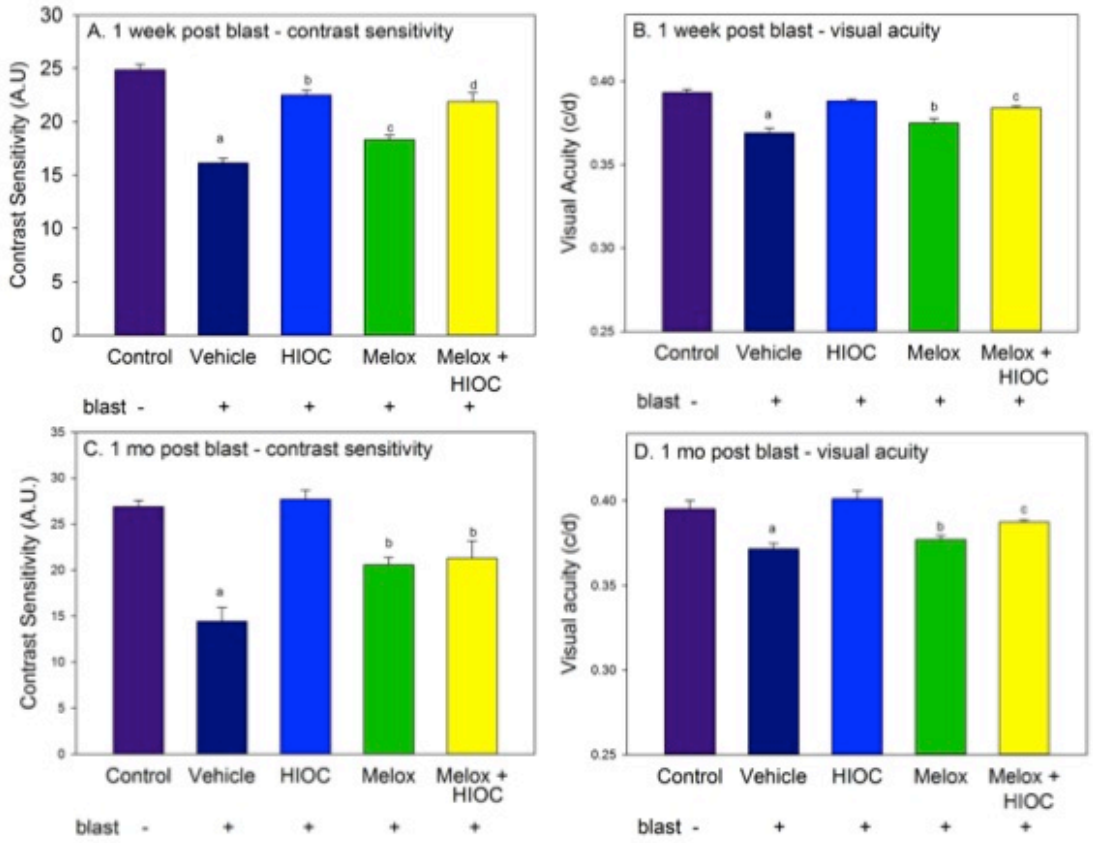


Figure 3. Effect of HIOC and meloxicam (Melox) on loss visual function following blast injury to the eye. *Mice were exposed to single ~48 psi blast directed to the eye. They were treated daily with HIOC (40 mg/kg ip), meloxicam (5 mg/kg sc), the two drugs together, or vehicles for 7 days beginning on the day of blast injury or vehicles. A separate, uninjured group (Control) was included for comparison. Visual function was tested 1 week and 1 month after injury. A. Contrast sensitivity 1 week after injury; N=6-8. a) Control vs Vehicle, p<0.001; b) HIOC vs Vehicle p<0.001, vs Control p<0.05; c) Meloxicam vs Vehicle p<0.05, Meloxicam vs HIOC and vs Control, p<0.001; Meloxicam + HIOC vs Control p<0.01, vs Vehicle p<0.001, vs HIOC not significant (NS), vs Meloxicam p<0.001. B. Visual acuity 1 week after injury; N=6-8. a)Vehicle vs Control p<0.001, vs HIOC p<0.001; b) Meloxicam vs Control p<0.001, vs HIOC p<0.01, vs Vehicle NS; c) Meloxicam + HIOC vs Control p<0.05, vs Vehicle p<0.001, vs HIOC NS, vs Meloxicam NS. C. Contrast sensitivity 1 month after injury; N=6-7. a) Vehicle vs Control p<0.001, vs HIOC p<0.001; Meloxicam or Meloxicam + HIOC vs Control p<0.01, vs Vehicle p<0.01. D. Visual acuity 1 month after injury; N=6-7. a)Vehicle vs Control p<0.001, vs HIOC p<0.001; b) Meloxicam vs Control p<0.001, vs HIOC p<0.01, vs Vehicle NS; c) Meloxicam + HIOC vs Control p<0.05, vs Vehicle p<0.01, vs HIOC NS, vs Meloxicam NS.*

The effect of FTY720 (fingolimod), alone and in combination with HIOC was tested. FTY720 is a sphingosine 1-phosphate receptor agonist that reduces neuroinflammation in part by switching microglia to a neuroprotective phenotype and by inhibiting lymphocyte migration; it has shown clinical efficacy in the treatment of stroke and multiple sclerosis patients (Noda et al., 2013; Yang et al., 2014; Fu et al., 2014). Mice were exposed to a single ~48 psi blast directed to the eye and treated with vehicle, HIOC (40 mg/kg ip), FTY720 (10mg/kg ip), or a combination of the two drugs. Drugs were administered for 1 week beginning on the day of blast. Visual function (contrast sensitivity and visual acuity) was assessed 1 week and 1 month after blast injury (Figure 4). Exposure to blast reduced contrast sensitivity, measured at a spatial frequency of 0.064 cycles/ degree (c/d), by 44 % and 42 % in the vehicle-treated mice at 1 week and 1 month, respectively. FTY720 alone did not significantly improve contrast sensitivity in the mice exposed to blast. HIOC significantly reduced the loss in contrast sensitivity to 15% and 12% at 1 week and 1 month, respectively. HIOC and FTY720 together provided no additional protection over HIOC alone under these conditions. A similar trend was observed when measuring visual acuity, but the effects were not statistically significant.

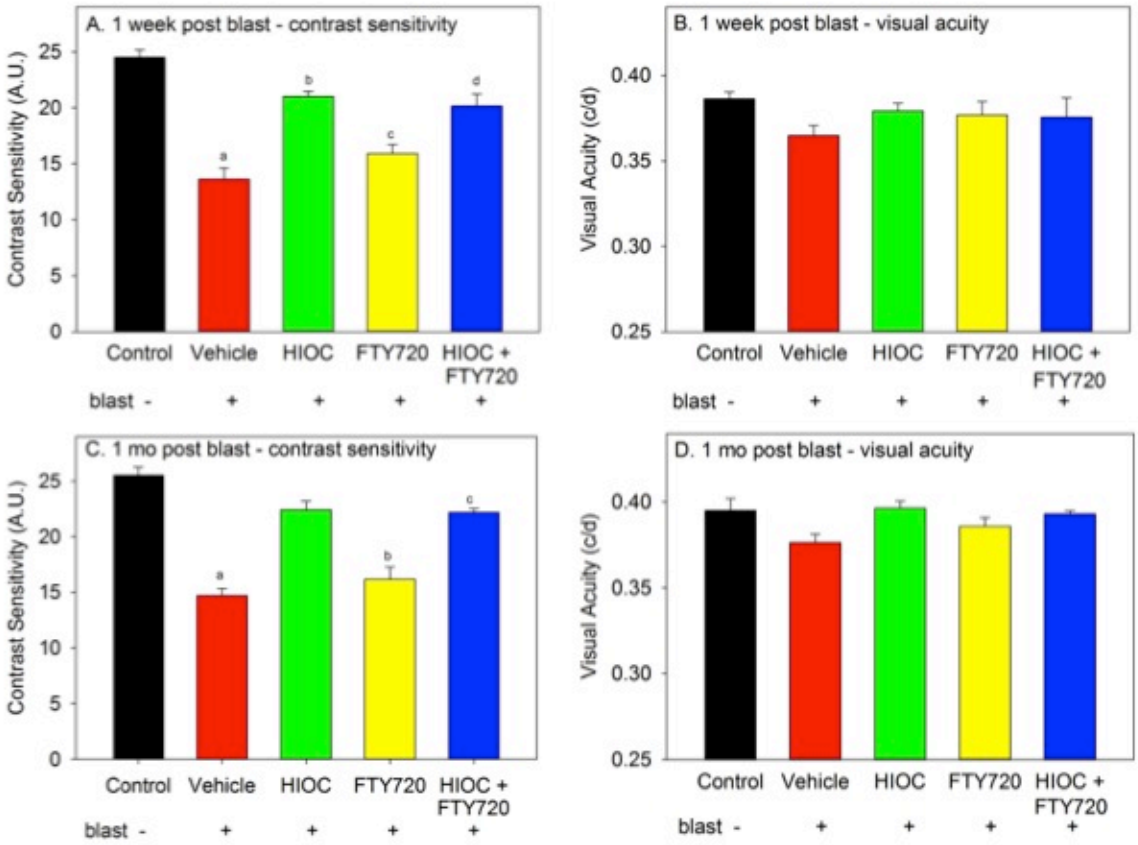


***Figure 4. Effect of HIOC and FTY720 on loss visual function following blast injury to the eye**. Mice were exposed to single ~48 psi blast directed to the eye. They were treated daily with HIOC (40 mg/kg ip), FTY720 (10 mg/kg ip), the two drugs together, or vehicles for 7 days beginning on the day of blast injury or vehicles. A separate, uninjured group (Control) was included for comparison. Visual function was tested 1 week and 1 month after injury.*
A. Contrast sensitivity 1 week after injury; N=6. a) Vehicle vs Control p<0.001; b) HIOC vs Control p<0.01; vs Vehicle p<001; c) FTY720 vs Control p<0.001, vs Vehicle NS, vs HIOC p<0.001; d) HIOC + FTY720 vs Control p=0.001, vs Vehicle p<0.001, vs HIOC NS; vs FTY720 p=0.001. B. Visual acuity 1 week after injury; N=6. No significant differences. C. Contrast sensitivity 1 month after injury; N=6. a) Vehicle vs Control p<0.001, vs HIOC p<0.001; b) FTY720 vs Control p<0.001, vs Vehicle NS, vs HIOC p<0.001; d) HIOC + FTY720 vs Control p<0.05, vs Vehicle p<0.001, vs HIOC NS, vs FTY720 p<0.001. D. Visual acuity 1 month after injury; N=6. No significant differences.

<u>Effect of HIOC on RGC survival following blast injury.</u>

Thy1-CFP mice were exposed to ~48 psi blast and treated with vehicle or HIOC (40 mg/kg) as described above. An untreated (not exposed to blast) group was included as control. Blast injury resulted in a small but significant (p<0.01) decrease in RGCs compared to Control (Fig. 5), similar to that seen previously (see last year's annual report). HIOC showed a trend toward preventing this decrease in RGC number, but this effect was not statistically significant (p=0.095), and RCG number was significantly lower than that of Control (p<0.05). While this needs to be replicated, it raises the possibility that the recovery of visual function with HIOC treatment may be due to reorganization of visual track (e.g., sprouting, preservation of optic nerve axons) rather than prevention of RGC death.

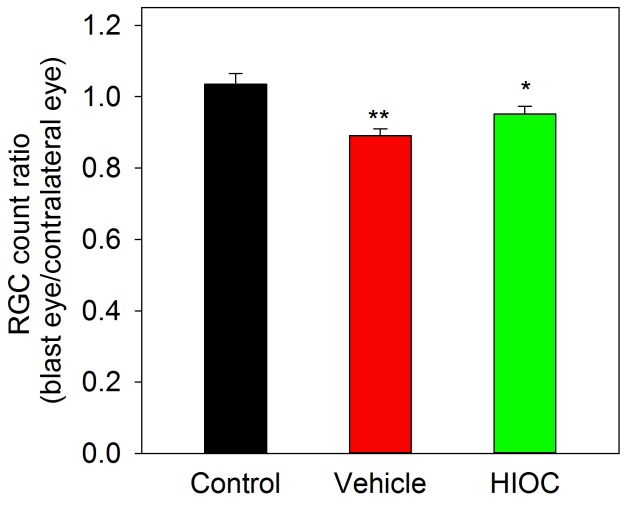


***Figure 5. Effect of blast injury and HIOC on RGCs of Thy1-CFP mice**. Mice were exposed to ~48 psi blast as described above and treated with vehicle or HIOC for 7 days. A separate untreated control group, not exposed to blast or drugs, was included. Fluorescent RGCs were counted in 8 regions of interest in each flat-mounted retina approximately 1 month after blast injury. RGC number in the left eye (exposed to blast in the Vehicle and HIOC) was normalized to RGC number of the contralateral eye. N=5-6 / group; ** Vehicle vs Control p<0.01, vs HIOC p=0.095; * HIOC vs Control p<0.05.*

Defining the optimal time window for initiating HIOC treatment following exposure to a 48psi blast.

In all previous experiments, HIOC was administered 30 minutes prior to exposure to blast. This protocol effectively preserved visual function, as assessed by optokinetic tracking (OKT) measurement of contrast sensitivity. In this experiment, we determined if administration of HIOC 15 minutes to 24 hours after blast would also be effective. HIOC (40 mg/kg ip) or vehicle were administered for 1 week beginning on the day of blast. Visual function (contrast sensitivity and visual acuity) was assessed 1 week and 1 month after blast injury (Figure 6). Exposure to blast reduced contrast sensitivity, measured at a spatial frequency of 0.064 cycles/ degree (c/d), by 51 % and 53 % in the vehicle-treated mice at 1 week and 1 month, respectively. HIOC administered before blast injury partially reversed the loss of contrast sensitivity at 1 week ($P<0.05$) and completely reversed the loss at 1 month ($p<0.001$). HIOC administered 15 minutes, 1 hour, or 3 hours after blast injury was as effective as the drug administered before injury; contrast sensitivity of those treated with HIOC before blast was not statistically different from contrast sensitivity of mice administered HIOC 15, 60 or 180 minutes after blast ($p>0.2$). Visual acuity was not significantly affected one week after blast injury (data not shown). One month after blast, visual acuity was reduced in the blast vehicle group (Figure 7; $p<0.01$). HIOC treatment showed an effect on visual acuity that was similar to that observed on contrast sensitivity.

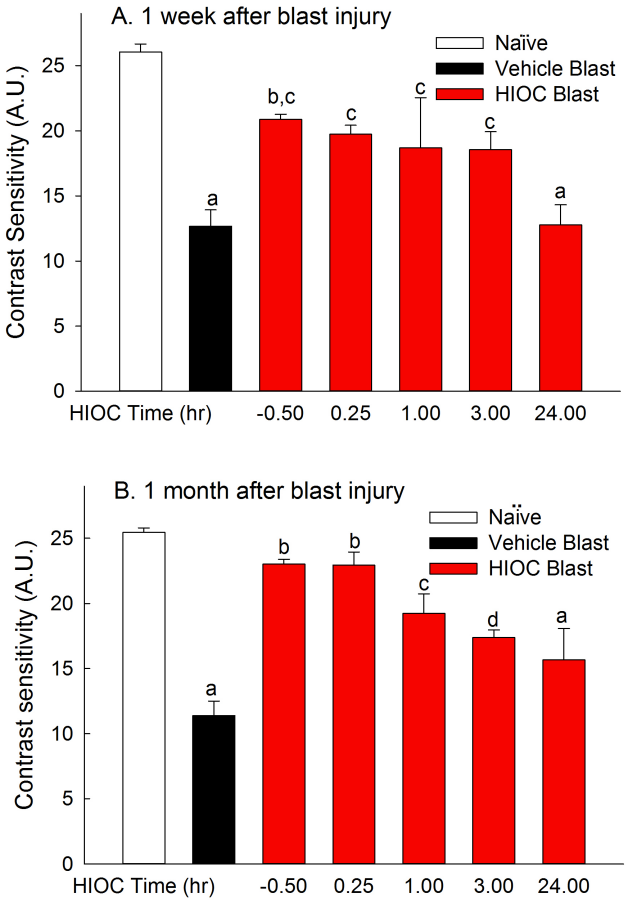


Figure 6. Effect of HIOC, administered before or after exposure to 48psi blast, on visual contrast sensitivity*. Mice were exposed to single ~48 psi blast directed to the eye. They were treated with HIOC (40 mg/kg ip), 0.5 hr before blast or 0.25, 1, 3 or 24 hours after blast. Vehicle was administered 0.5 hours before blast. A separate, uninjured group (Naïve) was included for comparison. HIOC and vehicle were subsequently injected daily for an additional 6 days. Visual function was tested 1 week and 1 month after injury. A. Contrast sensitivity 1 week after injury; N=6. a) $p<0.001$ vs Naïve; b) $p<0.05$ vs Vehicle $p<0.001$, vs Control $p<0.05$; c) NS vs Naïve B. Contrast sensitivity 1 month after injury; N=5-6. a) $p<0.001$ vs Naïve; b) $p<0.001$ vs vehicle; c) $p<0.01$, vs Vehicle $p<0.001$; d) $p<0.05$ vs Vehicle.*

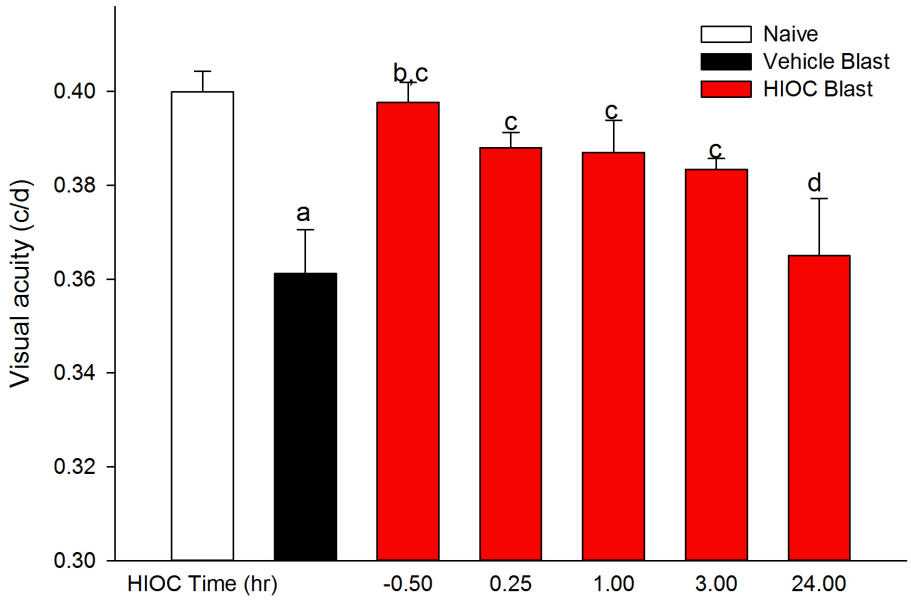


Figure 7. Effect of HIOC, administered before or after exposure to 48psi blast, on visual acuity*. Mice were exposed to single ~48 psi blast directed to the eye. They were treated with HIOC (40 mg/kg ip), 0.5 hr before blast or 0.25, 1, 3 or 24 hours after blast. Vehicle was administered 0.5 hours before blast. A separate, uninjured group (Naïve) was included for comparison. HIOC and vehicle were subsequently injected daily for an additional 6 days. Visual acuity was tested 1 month after injury. N=5-6. a) p<0.01 vs Naïve; b) p<0.02 vs Vehicle; c) NS vs Naïve; d) p<0.05 vs Naïve.*

Mohan et al. (2013) reported progressive loss of visual function and retinal ganglion cells for 4 months after blast injury. Therefore, we followed this cohort of mice and re-assessed visual function and ganglion cell loss at 4 months after injury.

Exposure to blast significantly reduced contrast sensitivity ($p<0.001$), measured at a spatial frequency of 0.064 cycles/ degree (c/d), and visual acuity ($p<0.001$) in the vehicle-treated mice at 4 months (Figure 8). HIOC administered before blast injury completely reversed the loss at 4 months ($p<0.001$). HIOC administered 15 minutes, 1 hour, or 3 hours after blast injury was as effective as the drug administered before blast; contrast sensitivity and visual acuity of mice treated with HIOC before blast was not statistically different from contrast sensitivity and visual acuity of mice administered HIOC 15, 60 or 180 minutes after blast ($p>0.3$). These findings indicate that HIOC provides effective therapy for blast injury if treatment is started within 3 hours of blast exposure, but not if treatment is delayed by 1 day. They also show that one week of HIOC treatment provides protection from vision loss that lasts in mice at least 4 months.

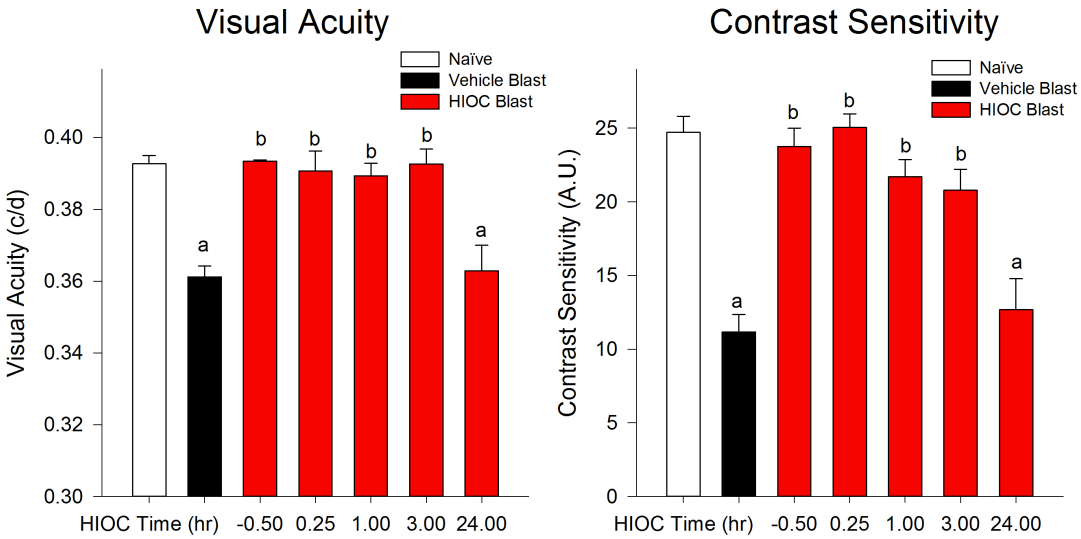


Figure 8. Effect of HIOC, administered before or after exposure to 48psi blast, on visual acuity and contrast sensitivity, assessed 4 months after blast injury*. Mice were exposed to single ~48 psi blast directed to the eye. They were treated with HIOC (40 mg/kg ip), 0.5 hour before blast or 0.25, 1, 3 or 24 hours after blast. Vehicle was administered 0.5 hours before blast. A separate, uninjured group (*Naïve*) was included for comparison. HIOC and vehicle*

were subsequently injected daily for an additional 6 days. Visual function was tested 4 months after injury. a) p<0.001 vs Naïve; b) p<0.001 vs Vehicle and not significantly different from Naïve. N=5-6.

Effect of HIOC, administered after ~48psi blast, on retinal ganglion cell loss.

Thy1-CFP mice were exposed to a single ~48psi blast and were injected with vehicle or HIOC (40 mg/kg ip) 15 minutes later. The mice were then injected with vehicle or HIOC daily for the next 6 days. After 4 months, mice were euthanized and retinal flat mounts were prepared. Fluorescent retinal ganglion cells were counted in eight fields, 4 in central retinal and 4 in peripheral retina. As reported previously, blast caused a small reduction in retinal ganglion cells (RGCs) (Figure 9). The effect was statistically significant only in peripheral retina ($p<0.05$). HIOC administration significantly preserved RGCs in peripheral retina ($p<0.05$). Similar trends were obsserved in central retina, but they were not statistically significant.

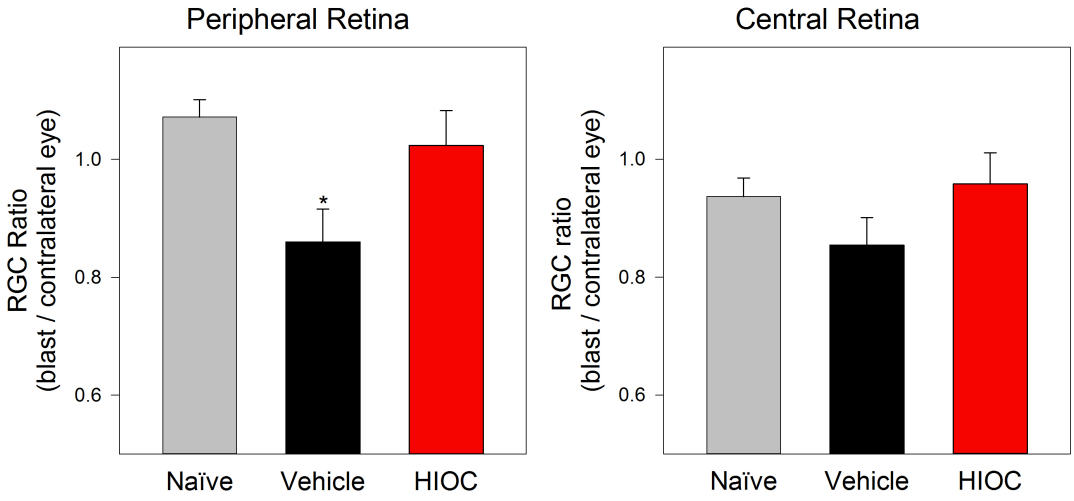


Figure 9. Effect of HIOC, administered after exposure to 48 psi, on retinal ganglion cell loss*. Mice were exposed to single ~48 psi blast directed to the right eye. They were treated with HIOC (40 mg/kg ip) or vehicle 15 min later. A separate, uninjured group (*Naïve*) was included for comparison. HIOC and vehicle were subsequently injected daily for an additional 6 days. Four months after exposure to blast, fluorescent RGCs were counted in eight fields, 4 in central retinal and 4 in peripheral retina. Data are expressed as a ratio of the RGCs in between the right (blast) and left (contralateral) eyes, normalized to account for inter-animal differences in numbers of fluorescent RGCs. *p<0.05 vs Naïve and vs HIOC blast. N=6 / group.*

Effect of blast directed at the eye on optic nerve axon survival; mitigation by HIOC.

Mice were exposed to ~48psi blast to the right eye and treated with HIOC (40 mg/kg, i.p.) or vehicle 15 minutes later; a separate, naïve control group was included for comparison. Treatment continued daily for the next 6 days. Mice were euthanized four months later; optic nerves were dissected, embedded in plastic and sectioned. Optic nerve axons were counted as described in Templeton et al. (2014). Blast caused a 46% reduction in axons in the vehicle-treated mice (**p<0.01), but no significant decrease in axons in the HIOC treated mice (Fig. 10, Fig. 11).

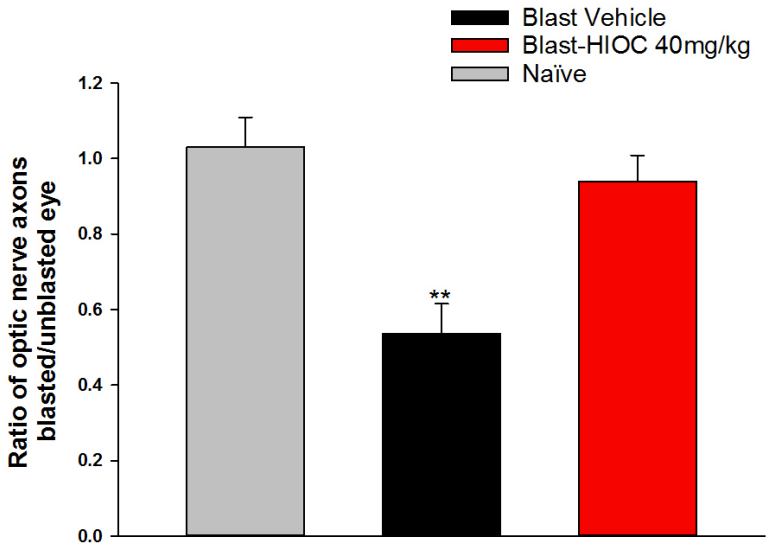


Figure 10. Effect of 48 PSI blast with and without HIOC on optic nerve axon numbers. *Data are expressed as a ratio of the axon numbers in the optic nerve of the right eye, exposed to blast overpressure, divided by the number of axons in the left, contralateral eye of each mouse. N=6 per group.*

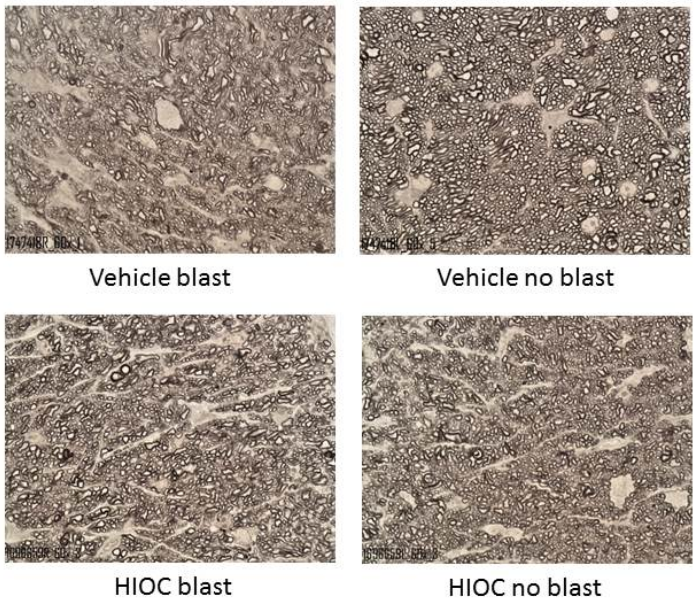


Figure 11. Representative photomicrographs of optic nerves from vehicle-treated or HIOC- treated mice. *On the left are the nerves of eyes exposed to blast; on the right are the nerves from the contralateral eyes of the same mice. Note the loss of axons in the optic nerve of the vehicle treated mouse, but not of the HIOC treated mouse.*

Effect of blast overpressure directed at the side of the head on contrast sensitivity and visual acuity.

Mice were exposed to a single ~68psi blast directed at the side of the head. Visual acuity and constrast sensitivity were measured one week later (Figure 12).

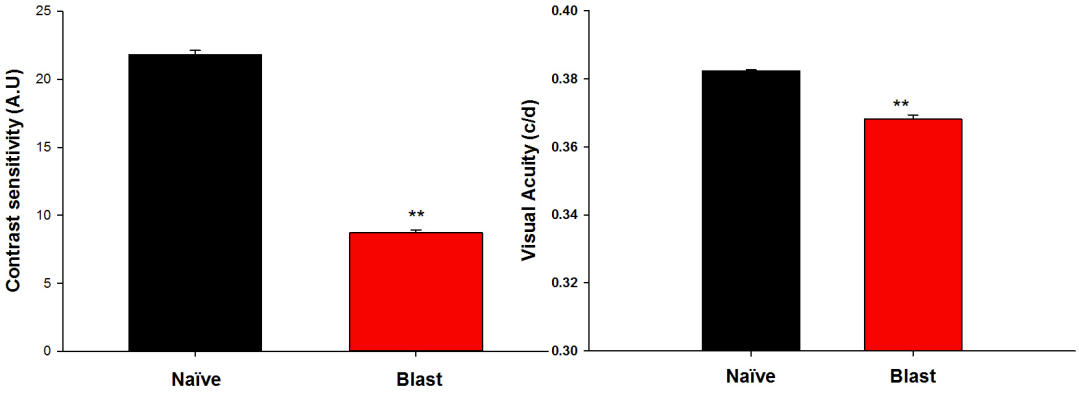


Figure 12. TBI and loss of visual function measured 1 week after blast.
*Exposure to blast significantly reduced both contrast sensitivity and visual acuity (**p<0.001; n=5 per group).*

This decrease in visual function persisted for at least 3 weeks after exposure to blast overpressure (Figure 13).

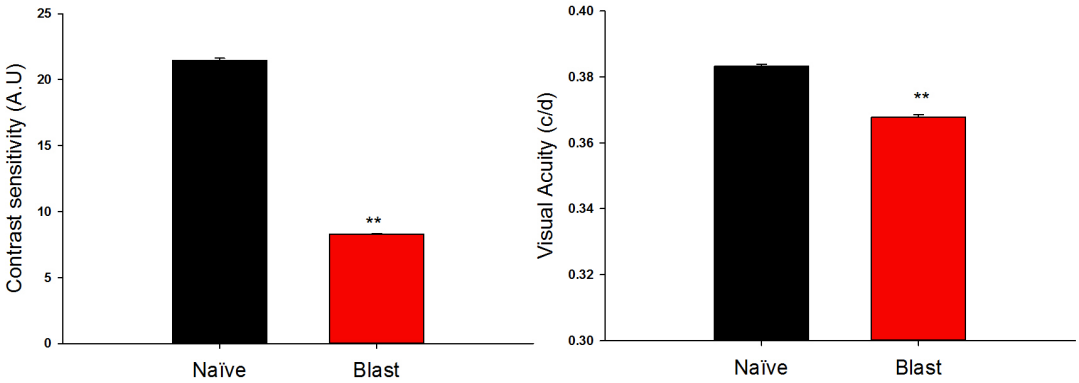


Figure 13. TBI and loss of visual function measured 3 weeks after blast.
*Exposure to blast significantly reduced both contrast sensitivity and visual acuity (**p<0.001; n=5 per group).*

Brains and optic nerves from these mice will be examined for degeneration and inflammatory markers.

We have initiated tests of the efficacy of HIOC in treating the loss of visual function from blast overpressure directed at the side of the head (Figure 14).

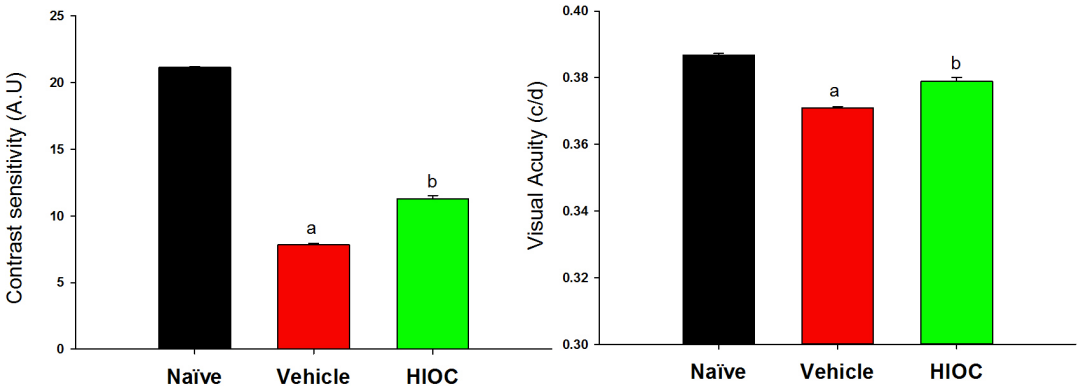


Figure 14. Effect of HIOC on the loss of visual function from head trauma.
Mice were exposed to a single ~68psi blast directed at the side of the head. HIOC (40mg/kg i.p.) or vehicle was administered 15 minutes after exposure to blast, and daily for the next six days. A naïve control group was included for comparison. Visual acuity and contrast sensitivity were tested one week after exposure to blast. Head trauma significantly decreased contrast sensitivity and visual acuity ([a]p<0.001 vs Naïve; n=6/group). HIOC partially prevented this loss of visual function ([b]p<0.001 vs Vehicle; n=6/group).

Does HIOC preserve visual function by stimulating BDNF / TrkB receptors?

We previously showed that HIOC stimulates TrkB, resulting in its phosphorylation and activation of downstream signaling (Shen et al., 2012), but it is unknown if the efficacy of the drug in preventing blast-induced vision loss occurs through this mechanism. We therefore initiated a study to explore the mechanism. ANA-12 is a selective TrkB antagonist that binds to the receptor and inhibits downstream signaling (Cazarola et al., *J Clin Invest* 121:1846–1857, 2011). We tested the ability of ANA-12 to block the protective action of HIOC. Mice were exposed to a single ~48 psi blast directed at the eye and administered HIOC (40 mg/kg ip) or vehcle15 min later. Daily injections continued for 6 days. Mice were pretreated with ANA-12 (0.5 mg/kg ip) or its vehicle 2.5 hours before each HIOC / vehicle injection. One week after exposure to blast, contrast sensitivity was reduced in the vehicle-treated mice ($p<0.001$) (Figure 15). Treatment with HIOC (plus the vehicle for ANA-12) reduced the loss of contrast sensitivity ($p<0.05$). Administration of ANA-12 alone had no effect on the blast-induced loss of contrast sensitivity, but completely blocked the effect of HIOC. Similar results were found when visual function of the mice was re-tested at 1 month after blast exposure (Figure 16). The results support the hypothesis that HIOC mitigates blast-induced vision loss by activating TrkB.

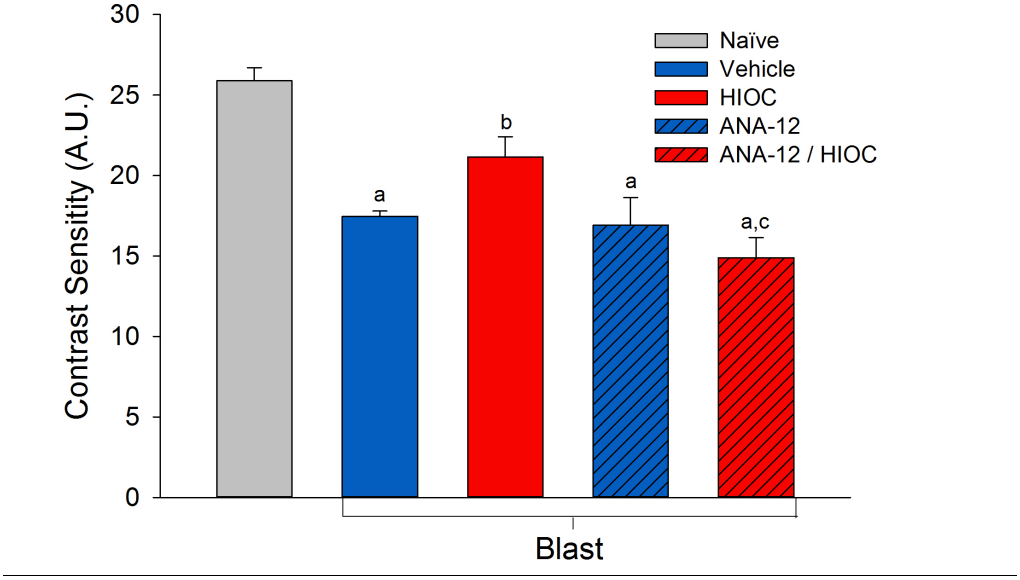


Figure 15. Effect of ANA-12 on the mitigation of blast-induced vision loss by HIOC one week after blast injury. See text for details. N=6 / group. a) p<0.001 vs Naïve; b) p<0.05 vs Vehicle; c) p<0.01 vs HIOC.

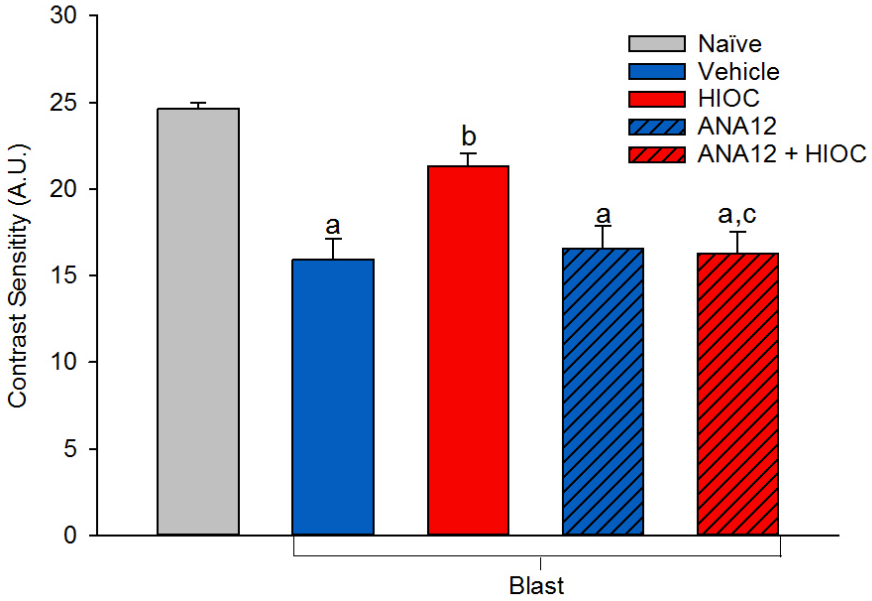


Figure 16. Effect of ANA-12 on the mitigation of blast-induced vision loss by HIOC one month after blast injury. See text for details. N=6 / group. a) p<0.001 vs Naïve; b) p<0.01 vs Vehicle; c) p<0.02 vs HIOC.

Effect of blast injury and HIOC on retinal dopamine.

We showed previously that loss of retinal dopamine is associated with decreased contrast sensitivity and visual acuity (Jackson et al., 2012), similar to that observed following blast injury to the eye. We therefore examined the effect of blast and administration of HIOC on the retinal levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). Mice were exposed to ~48 psi blast and treated with vehicle or HIOC (40 mg/kg) as described above, with the first injection administered immediately after blast. An untreated (not exposed to blast) group was included as control (Naïve). HIOC and vehicle treatments continued daily for 1 week, after which the mice were euthanized and retinas dissected. Blast injury had no significant effect on the retinal levels of dopamine or DOPAC. HIOC also had no significant effect on the levels of dopamine or DOPAC (Table 1).

Table 1

Treatment	dopamine pg / mg protein	DOPAC pg / mg protein
Naïve	1798 ± 43	377 ± 19
Blast vehicle	1974 ± 94	366 ± 11
Blast HIOC	1855 ± 108	350 ± 20

Effect of a soluble TNFα inhibitor on blast-induced loss of visual function.

XPro1595 is a dominant-negative form of TNFα that binds to endogenous soluble TNFα subunits and prevents them from interacting with the TNFα receptor. XPro1595 has been shown to have reduce inflammation and exhibit neuroprotective properties in some models of neurodegeneration (Barnum et al., J Parkinsons Dis 4: 349-60). We tested the effect of daily treatment with XPro1595 for 1 week on the blast-induced loss of visual function. Mice were injected with XPro1595 (5 mg/kg sc) or vehicle 30 min prior to a single exposure to 48 psi blast and then daily for the next six days. Visual contrast sensitivity was assessed 6 weeks after blast. XPro1595 treatment significantly attenuated the loss of contrast sensitivity induced by blast (Figure 17). This finding suggests that soluble TNFα-induced inflammation contributes to the loss of visual function following blast injury, and provides another target for the treatment of soldiers exposed to explosions.

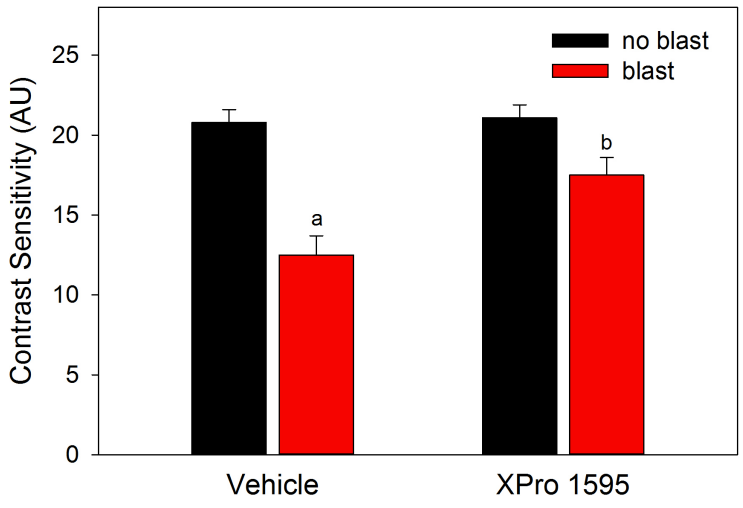


Figure 17. Effect of blast injury and XPro1595 on visual contrast sensitivity. *Mice were exposed to ~48 psi blast as described above and treated with vehicle or XPro1595 for 7 days. N=6 / group; a) p<0.001 vs no blast; b)p<0.001 vs blast vehicle.*

4. KEY RESEARCH ACCOMPLISHMENTS

- HIOC reduces the loss of visual function following blast injury to the eye or the head.
- HIOC has this therapeutic effect when administered before or up to 3 hours after blast injury to the eye.
- HIOC reduces the loss of optic nerve axons following blast injury to the eye.
- The neuroprotective effect of HIOC is not potentiated by anti-inflammatory drugs.
- The neuroprotective effect of HIOC involves activation of BDNF / TrkB receptors.

5. CONCLUSIONS

Our results indicate that HIOC preserves visual function and optic nerve axons following blast injury as long as treatment is begun within three hours of initial exposure to blast. The mechanism of action appears to be activation of BDNF / TrkB receptors. HIOC may be useful for preventing vision loss in soldiers exposed to blast overpressure.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTAIONS

a. Publications

1. Lay press: none

2. Peer-reviewed scientific journals:

Setterholm, N.A., McDonald, F.E., Boatright, J.H., Iuvone, P.M.: Gram scale, chemoselective synthesis of *N*-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC). Tetrahedron Lett., 56: 3413–3415, 2015. http://dx.doi.org/10.1016/j.tetlet.2015.01.167; PMID: 26028783; PMC4445863.

3. Invited articles:

Nothing to report

3. Abstracts:

Sidhu C., Lyuboslavsky P., Chrenek M. Struebing F.L., Sellers J.T., Setterholm N.A., McDonald F.E., Boatright J.H., Geisert E.E., Iuvone P.M.: Traumatic blast-induced injury reduces visual function and retinal ganglion cells of Thy1-CFP mice: Mitigation by a small molecule TrkB activator. ARVO E-Abstract 6032-B0211.

b. Presentations

Sidhu C., Lyuboslavsky P., Chrenek M. Struebing F.L., Sellers J.T., Setterholm N.A., McDonald F.E., Boatright J.H., Geisert E.E., Iuvone P.M.: Traumatic blast-induced injury reduces visual function and retinal ganglion cells of Thy1-CFP mice: Mitigation by a small molecule TrkB activator. Presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology, Denver, May 2015.

7. INVENTIONS, PATENTS AND LICENSES

Nothing to report

8. REPORTABLE OUTCOMES

Published the new method for the synthesis of HIOC (see above).

9. OTHER ACHIEVEMENTS

Nothing to report

10. REFERENCES

Barnum CJ, Chen X, Chung J, Chang J, Williams M, Grigoryan N, Tesi RJ, Tansey MG. (2014) Peripheral administration of the selective inhibitor of soluble tumour necrosis factor (TNF) XPro1595 attenuates nigral cell loss and glial activation in 6-OHDA hemiparkinsonian rats. J Parkinsons Dis 4:349-60.

Cazorla M, Prémont J, Mann A, Girard N, Kellendonk C, Rognan D. (2011) Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. J Clin Invest 121:1846-57.

Feng G., Mellor R.H., Bernstein M., Keller-Peck C., Nguyen Q.T., Wallace M., Nerbonne J.M., Lichtman J.W., and Sanes J.R. (2000) Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP. Neuron 28 (1):41-51.

Fu Y., Zhang N., Ren L., Yan Y., Sun N., Li Y.J., Han W., Xue R., Liu Q., Hao J., Yu C., Shi F.D. (2014) Impact of an immunemodulator fingolimod on acute ischemic stroke. Proc Natl Acad Sci USA 111:18315-20.

Gauthier R., Joly S., Pernet V., Lachapelle P. and Di Polo A. (2005) Brain-Derived Neurotrophic Factor Gene Delivery to Muller Glia Preserves Structure and Function of Light-Damaged Photoreceptors. Investigative Ophthalmology Visual Science 46, 3383-3392.

Jackson CR, Ruan GX, Aseem F, Abey J, Gamble K, Stanwood G, Palmiter RD, Iuvone PM, McMahon DG. (2012) Retinal dopamine mediates multiple dimensions of light-adapted vision. J Neurosci 32: 9359-68.

Jang S. W., Liu X., Pradoldej S., Tosini G., Chang Q., Iuvone P. M. and Ye K. (2010a) N-acetylserotonin activates TrkB receptor in a circadian rhythm. Proc Natl Acad Sci U S A 107, 3876-3881.

Jang S. W., Liu X., Yepes M., Shepherd K. R., Miller G. W., Liu Y., Wilson W. D., Xiao G., Blanchi B., Sun Y. E. and Ye K. (2010b) A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. Proceedings of the National Academy of Sciences 107, 2687-2692.

Jang S. W., Liu X., Chan C. B., France S. A., Sayeed I., Tang W., Lin X., Xiao G., Andero R., Chang Q., Ressler K. J., and Ye K. (2010c) Deoxygedunin, a natural product with potent neurotrophic activity in mice. PLoS.One. 5 (7):e11528.

Massa S.M., Yang T., Xie Y., Shi J., Bilgen M., Joyce J. N., Nehama D., Rajadas J., Longo F. M. (2010) Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest 120 (5):1774-85.

Mohan K, Kecova H, Hernandez-Merino E, Kardon RH, Harper MM. (2013) Retinal ganglion cell damage in an experimental rodent model of blast-mediated traumatic brain injury. Invest Ophthalmol Vis Sci 54: 3440-50.

Nadal-Nicolas F.M., Jiminez-Lopez M., Sobrado-Calvo P., Nieto-Lopez L., Canovas-Martinez I., Salinas-Navarro M. Vidal-Sanz M., and Agudo M. (2009) Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic nerve-injured retinas. Investigative Ophthalmology Visual Science 50 (8):3860-3868.

Noda H., Takeuchi H., Mizuno T., Suzumura A. (2013) Fingolimod phosphate protomotes the neuroprotective effects of microglia. J Neuroimmunol 256: 13-18, 2013.

Shen J., Ghai K., Sompol P., Liu X., Cao X., Iuvone P.M., and Ye K. (2012) N-acetyl serotonin derivatives as potent neuroprotectants for retinas. Proceedings of the National Academy of Sciences 109 (9):3540-3545.

Templeton JP, Struebing FL, Lemmon A, Geisert EE. (2014) ImagePAD, a novel counting application for the Apple iPad, used to quantify axons in the mouse optic nerve. Exp Eye Res 128:102-8.

Weber A. J., Viswaníthan S., Ramanathan C. and Harman C. D. (2010) Combined Application of BDNF to the Eye and Brain Enhances Ganglion Cell Survival and Function in the Cat after Optic Nerve Injury. Investigative Ophthalmology & Visual Science 51, 327-334.

Yang D., Sun Y.Y., Bhaumik S.K., Li Y., Baumann J.M., Lin S.H., Dunn R.S., Liu C.Y., Shie F.S., Lee Y.H., Wills-Karp M., Chougnet C.A., Kallapur S.G., Lewkowich I.P., Lindquist D.M., Murali-Krisna K., Kuan C.Y. (2014) Blocking lymphocyte trafficking with FTY720 prevents inflammation-sensitized hypoxic-ischemic brain injury in newborns. J Neurosci. 34: 16467-81.

Appendix

1. Copy of ARVO abstract: Traumatic blast-induced injury reduces visual function and retinal ganglion cells of Thy1-CFP mice: Mitigation by a small molecule TrkB activator.

2. Reprint of published paper: Setterholm et al., Gram-scale, chemoselective synthesis of **N** -[2-(5hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC), 2015.

Traumatic Blast-Induced Closed Globe Injury Reduces Visual Function and Retinal Ganglion Cells of Thy1-CFP mice: Mitigation by a Small Molecule TrkB Activator

View Session Detail | Print Abstract

Posterboard #: B0211

Abstract Number: 6032 - B0211

Author Block: *Curran Sidhu[1] , Polina Lyuboslavsky[1] , Micah A. Chrenek[1] , Felix L. Struebing[1] , Jana T. Sellers[1] , Noah A. Setterholm[2] , Frank E. McDonald[2] , Jeffrey H. Boatright[1] , Eldon E. Geisert[1] , P. Michael Iuvone[1]*
[1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [1] Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States; [2] Chemistry, Emory University, Atlanta, Georgia, United States; [2] Chemistry, Emory University, Atlanta, Georgia, United States

Disclosure Block: Curran Sidhu, None; Polina Lyuboslavsky, None; Micah A. Chrenek, None; Felix L. Struebing, None; Jana T. Sellers, None; Noah A. Setterholm, None; Frank E. McDonald, None; Jeffrey H. Boatright, None; Eldon E. Geisert, None; P. Michael Iuvone, None

Purpose: Pressure waves due to explosions can damage the neurons of the eye and visual centers in the brain, leading to loss of vision. There are few treatments for such injuries that can be deployed rapidly to mitigate such damage. We hypothesize that activation of the Brain-Derived Neurotrophic Factor (BDNF) receptor TrkB will be useful for this purpose. Here we report initial testing of a putative TrkB agonist in a model of ocular blast injury.

Methods: Traumatic blast-induced ocular injury in mice was produced using a calibrated blast gun that delivers a single blast of ~48psi (range 47-50psi) directed at the front of the eye (Hines-Beard et al., Exp Eye Res. 2012;99:63-70). Transgenic Thy1-CFP (cyan fluorescent protein) mice on a C57BL/6 background were used, allowing us to quantify retinal ganglion cell (RGC) death at various intervals after blast injury by RGC cell counts and by CFP fluorescence measurements in retinal extracts. Retinal morphology, microglial activation, and reactive gliosis were assessed by standard histological and immunocytochemical techniques. Visual and retinal function were assessed by optokinetic tracking (OKT) and electroretinogram (ERG), respectively. Mice were injected with vehicle or N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC; 20 or 40 mg/kg ip) daily for 1 week beginning on the day of blast exposure.

Results: Seven days after exposure to blast, many RGCs were hyper-fluorescent and swollen. These changes were accompanied by an increase in Iba1-immunopositive microglia, suggestive of an inflammatory response to blast, and an increase in GFAP, indicative of reactive gliosis. Exposure to blast suppressed visual contrast sensitivity as early as 1 week ($p<0.001$) and suppression persisted for at least the first 7 weeks ($p<0.001$). Blast caused significant loss of RGCs ($p=0.008$). No decrease in ERG a-wave or b-wave amplitudes was seen after blast, suggesting that damage was primarily in the inner retina. Treatment with HIOC for 1 week significantly decreased the loss of visual function following blast injury at 1 week ($p<0.001$) and at 1 month ($p<0.001$).

Conclusions: Blast injury to the front of the eye results in inner retinal damage and loss of visual function. TrkB activators may be useful in mitigating loss of RGCs and visual function caused by closed-globe blast injury.

Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.:

Tetrahedron Letters 56 (2015) 3413–3415

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Gram-scale, chemoselective synthesis of *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC)

Noah A. Setterholm [a], Frank E. McDonald [a,*], Jeffrey H. Boatright [b,c], P. Michael Iuvone [b,d]

[a] Department of Chemistry, Emory University, 1515 Dickey Drive NE, Atlanta, GA 30322, USA
[b] Department of Ophthalmology, Emory University School of Medicine, 1365B Clifton Road NE, Atlanta, GA 30322, USA
[c] Rehabilitation R&D Center, Atlanta VA Medical Center, Decatur, GA 30033, USA
[d] Department of Pharmacology, Emory University School of Medicine, 1510 Clifton Road NE, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 3 December 2014
Revised 22 January 2015
Accepted 26 January 2015
Available online 31 January 2015

In fond memory of Prof. Harry Wasserman, recognizing his humanity, scholarship, and service

Keywords:
Amide synthesis
Chemoselectivity
Decarboxylation
Kinase activation
Serotonin

ABSTRACT

N-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC) is a potent activator of the TrkB receptor in mammalian neurons and of interest because of its potential therapeutic uses. In the absence of a commercial supply of HIOC, we sought to produce several grams of material. However, a synthesis of HIOC has never been published. Herein we report the preparation of HIOC by the chemoselective *N*-acylation of serotonin, without using blocking groups in the key acylation step.

Introduction

Tropomyosin related kinase B (TrkB) is a neuronal transmembrane receptor protein in humans and other mammals. Small proteins such as brain-derived neurotropic factor (BDNF) bind to the extracellular portion of TrkB, triggering autophosphorylation of tyrosine residues in its intracellular domain. This phosphorylation then initiates cascade-signaling pathways known to promote neuronal differentiation and survival.[1] Several small molecules have been identified as agonists of Trk receptors,[2] including *N*-acetylserotonin (NAS, **2**, Fig. 1) as a TrkB activator.[3] In the course of investigating the TrkB activity of other serotonin derivatives, *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC, **3**) has displayed greater activation of TrkB, and exhibits a longer in vivo half-life than NAS.[4] HIOC has also demonstrated protective activity in an animal model for light-induced retinal degeneration, and can pass the blood-brain and blood-retinal barriers.[4] Thus, HIOC is a compound with high therapeutic potential, provided that this compound can be reliably prepared on a scale suitable for animal studies.

Although HIOC (**3**) is described in patents,[5] a method for its preparation has not been disclosed. Moreover, this compound is not consistently commercially available. Herein we describe a method for the gram-scale synthesis of HIOC.

At the first glance, HIOC would appear to be trivially prepared from the *N*-acylation of serotonin (Fig. 2). In practice, there are a number of plausible challenges:

(1) serotonin can undergo acylation at three sites;
(2) the carboxylic acid synthon **4** may potentially undergo decarboxylation upon standing; and
(3) serotonin-HCl **1** and the carboxylic acid **4** both exhibit poor solubility in most organic solvents.

Results and discussion

Carboxylic acid synthon **4** was prepared by basic hydrolysis of the commercially available ethyl ester **5**.[6,7] However, the literature did not provide a method for isolating this water-soluble

* Corresponding author.
E-mail address: frank.mcdonald@emory.edu (F.E. McDonald).

http://dx.doi.org/10.1016/j.tetlet.2015.01.167
0040-4039/

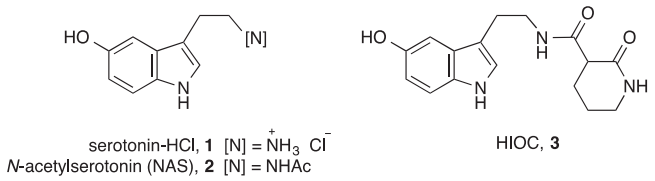


Figure 1. Structures of serotonin and derivatives, including HIOC (**3**).

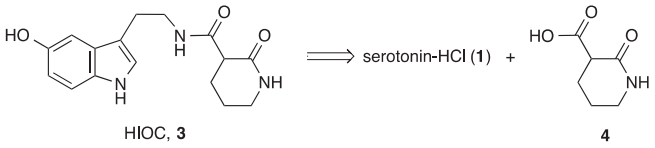
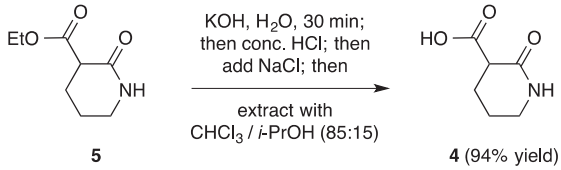
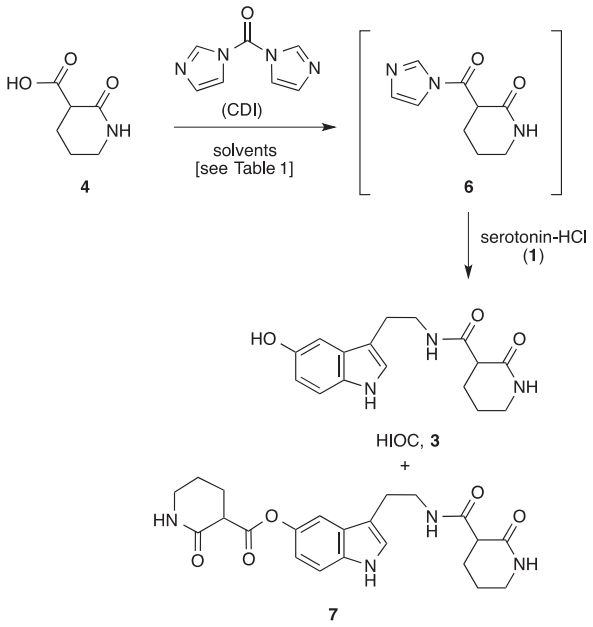


Figure 2. Retrosynthesis of HIOC.



Scheme 1. Preparation and isolation of 2-oxo-3-piperidinecarboxylic acid (**4**).



Scheme 2. Acylation of serotonin with carboxylic acid **4**.

compound. After some optimization we found that treatment of ester **5** with KOH in water, followed by addition of concentrated HCl and saturation of the aqueous layer with solid NaCl allowed for extraction of the desired acid **4** with a solvent mixture of chloroform and isopropanol (Scheme 1).[8]

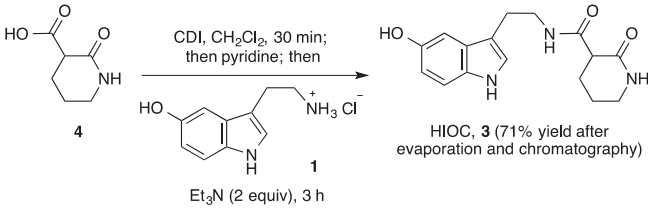
We initially attempted to couple the carboxylic acid **4** with serotonin-HCl (**1**) by activating the acid using carbodiimide reagents[9] as well as through mixed anhydride formation.[10] In the case of the carbodiimide reagents, a small amount of HIOC was detected via LC–MS, but could not be isolated. Attempts to activate **4** with methyl chloroformate furnished the methyl ester of **4** as the only identifiable product. Efforts using pivaloyl chloride were also unsuccessful, resulting only in pivaloylation of the primary amine of serotonin. After screening additional methods we found that the carboxylic acid could be activated with carbonyl diimidazole (CDI).[11] Addition of CDI to an opaque suspension of carboxylic acid **4** in dichloromethane provided complete conversion to the soluble *N*-acyl imidazole derivative **6** (Scheme 2). Addition of serotonin-HCl (**1**) to intermediate **6** gave some conversion to the desired amide **3**. This acylation procedure was plagued by the poor solubility of serotonin-HCl in CH_2Cl_2, and *N*,*O*-bisacylated product **7** was often observed from these heterogeneous reaction conditions, along with some recovered unacylated serotonin. However, dimethylformamide (DMF) solubilized all reaction components, giving 66% conversion to HIOC (**3**) after 3 h, along with only 6% of the bisacylated product **7** (Table 1, entry 1). The product **3** was very difficult to extract from an aqueous acidic workup of the reaction mixture, so we sought an alternative to DMF as the solvent.

Adding an equal volume of triethylamine to the reaction mixture in CH_2Cl_2 prior to the addition of serotonin-HCl (**1**) gave some conversion to product, however the serotonin-HCl was not completely solubilized, leading to a small amount of bisacylated product **7** (entry 2). On the other hand, addition of an equal volume of pyridine (~40 equiv prior to addition of serotonin-HCl **1**) resulted in a completely homogeneous reaction mixture (entry 3). After 3 h, the reaction had proceeded to 66% conversion and high chemoselectivity favoring HIOC (**3**), with only a trace amount of

Table 1
Optimization of reaction conditions

Entry	Solvents	Conversion by ^{1}H NMR		
		Serotonin-HCl (**1**) (%)	HIOC (**3**) (%)	**7** (%)
1	DMF[a]	25	66	6
2	1:1 CH_2Cl_2/Et_3N[b]	68	29	3
3	1:1 CH_2Cl_2/pyridine[b]	33	66	<1

[a] CDI was added to a 0.3 M solution of **4** in DMF. After 30 min, another volume of DMF was added followed by 1 equiv of **1**.
[b] CDI was added to a 0.3 M suspension of **4** in CH_2Cl_2. After aging 30 min, an equal volume of base (triethylamine or pyridine) was added, followed by 1 equiv of **1**.



Scheme 3. Optimized acylation for the synthesis of HIOC (**3**).

N,*O*-bisacylated product detected by NMR.[12] The addition of 2 equiv of triethylamine helped to push the reaction to completion over the course of 3 h, bringing the conversion up to ~85% (Scheme 3). Isolation of the product from the reaction mixture was non-trivial. Concentration of the reaction mixture followed by dry loading of the unwieldy crude onto a silica gel column allowed for chromatographic separation with ethyl acetate/methanol (9:1) as eluent. The resulting solid was washed with hot diethyl ether to remove remaining imidazole impurities to furnish HIOC (**3**) in 71% yield (Scheme 3).

In summary, we have developed a short, scalable, and highly reproducible synthesis of HIOC without the need for protective groups. The key discovery was the utility of the additional volume of pyridine to the acyl-imidazole species, which helped to solubilize the serotonin hydrochloride and also may have behaved as a nucleophilic catalyst.

Acknowledgments

This work was financially supported by DOD W81XWH-12-1-0436, NIH R01EY004864, R01EY014026, P30EY006360, the Abraham J. and Phyllis Katz Foundation, and Research to Prevent Blindness (RPB). PMI is a recipient of the RPB Senior Scientific Investigator Award. We also thank Dr. Brooke Katzman (Emory University) for assistance with LC/MS.

Supplementary data

Supplementary data (experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra for 2-oxopiperidine-3-carboxylic acid (**4**) and HIOC (**3**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.01.167. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Kaplan, D. R.; Miller, F. D. *Curr. Opin. Neurobiol.* **2000**, *10*, 381–391; (b) Huang, E. J.; Reichardt, L. F. *Annu. Rev. Neurosci.* **2001**, *24*, 677–763.
2. (a) Obianyo, O.; Ye, K. *Biochim. Biophys. Acta* **1834**, *2013*, 2213–2218; (b) Webster, N. J. G.; Pirrung, M. C. *BMC Neurosci.* **2008**, *9*, S1.
3. Jang, S.-W.; Liu, X.; Pradoldej, S.; Tosini, G.; Chang, Q.; Iuvone, P. M.; Ye, K. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 3876–3881.
4. Shen, J.; Ghai, K.; Sompol, P.; Liu, X.; Cao, X.; Iuvone, P. M.; Ye, K. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 3540–3545.
5. (a) Tkachenko, S. E.; Okun, I. M.; Rivkis, S. A.; Kravchenko, D. V.; Khvat, A. V.; Ivashchenko, A. V. Russ. Pat. (2007) RU 2303597 C1 (CAN 147:181575). (b) Ran, R. Faming Zhuanli Shenqing (2012) CN 102816103 A (CAN 158:105451).
6. (a) Shapiro, D.; Abramovitch, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 6690–6691; (b) Narayanan, K.; Cook, J. M. *J. Org. Chem.* **1991**, *56*, 5733–5736.
7. Compound **4** (2-oxo-3-piperidinecarboxylic acid) is listed in the Sigma–Aldrich catalog, CBR01770. We determined that a commercial sample (lot# B00037799) was merely valerolactam, corresponding to decarboxylation of compound **4**.
8. Continuous extraction of the salted aqueous layer with dichloromethane was also investigated, but tended to give reduced yields.
9. Basarab, G. S.; Jordan, D. B.; Gehret, T. C.; Schwartz, R. S. *Bioorg. Med. Chem.* **2002**, *10*, 4143–4154.
10. Yamada, K.; Teranishi, S.; Miyashita, A.; Ishikura, M.; Somei, M. *Heterocycles* **2011**, *83*, 2547–2562.
11. Paul, R.; Anderson, G. W. *J. Am. Chem. Soc.* **1960**, *82*, 4596–4600.
12. The diminished yield of bisacylated product **7** relative to HIOC (**3**) may arise from pyridine-promoted transacylation of serotonin-derived esters, although this has not been proven.

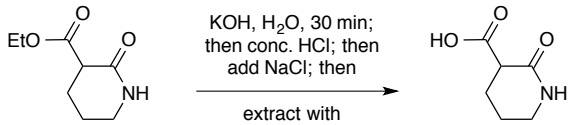
Gram-scale, chemoselective synthesis of *N*-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC)

Noah A. Setterholm, Frank E. McDonald,* Jeffrey H. Boatright, and P. Michael Iuvone

SUPPLEMENTARY MATERIAL

Experimental procedure and characterization data for 2-oxopiperidine-3-carboxylic acid (**4**)... S-2
Experimental procedure and characterization data for HIOC (**3**) S-3
^{1}H NMR spectrum for 2-oxopiperidine-3-carboxylic acid (**4**) S-4
^{13}C NMR spectrum for 2-oxopiperidine-3-carboxylic acid (**4**) S-5
^{1}H NMR spectrum for HIOC (**3**) S-6
^{13}C NMR spectrum for HIOC (**3**) S-7

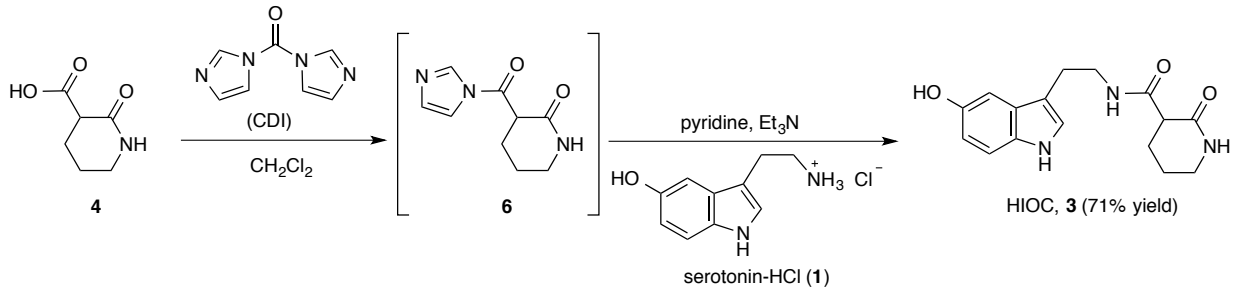
*2-oxopiperidine-3-carboxylic acid (**4**):*



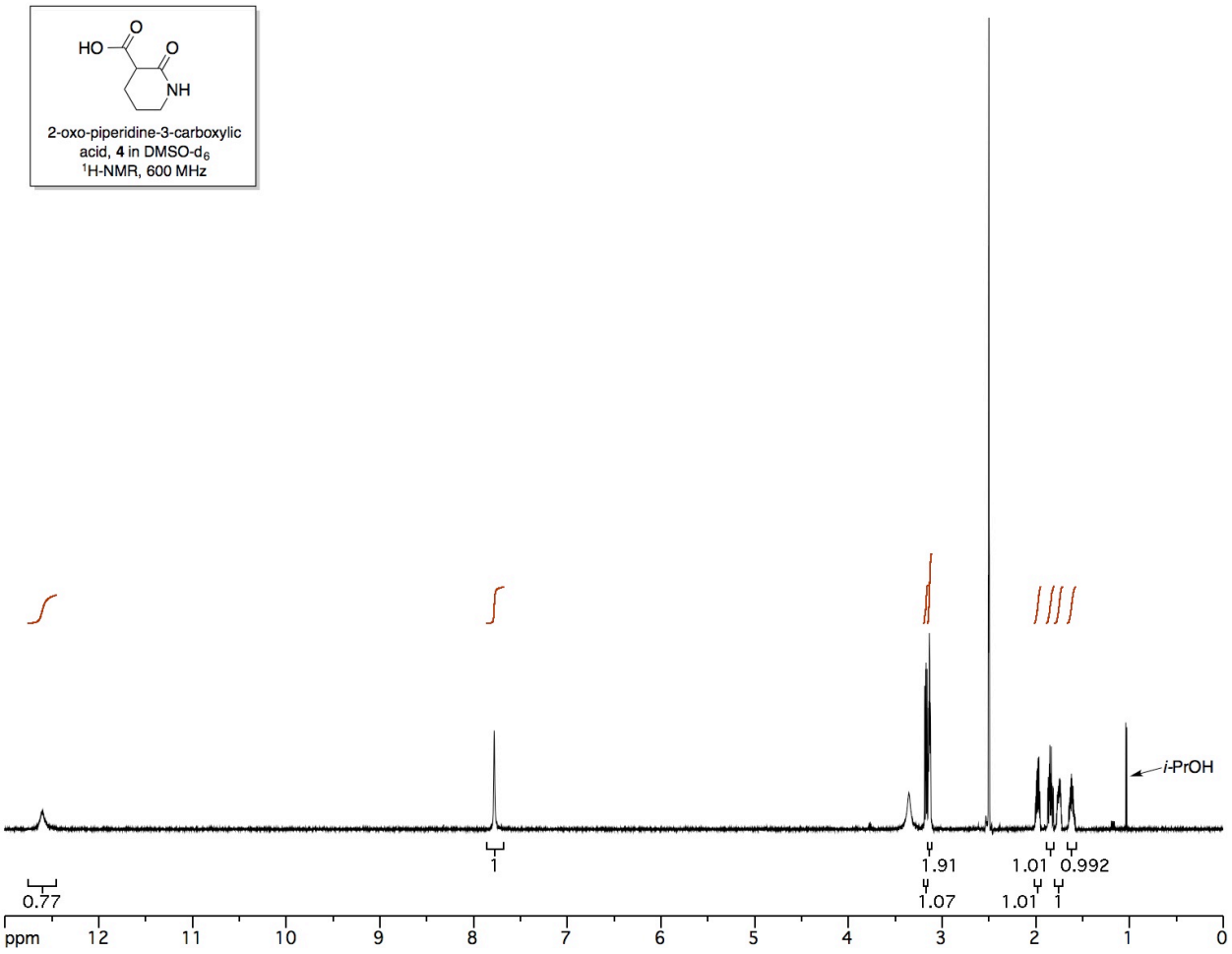
5 **4** (94% yield)

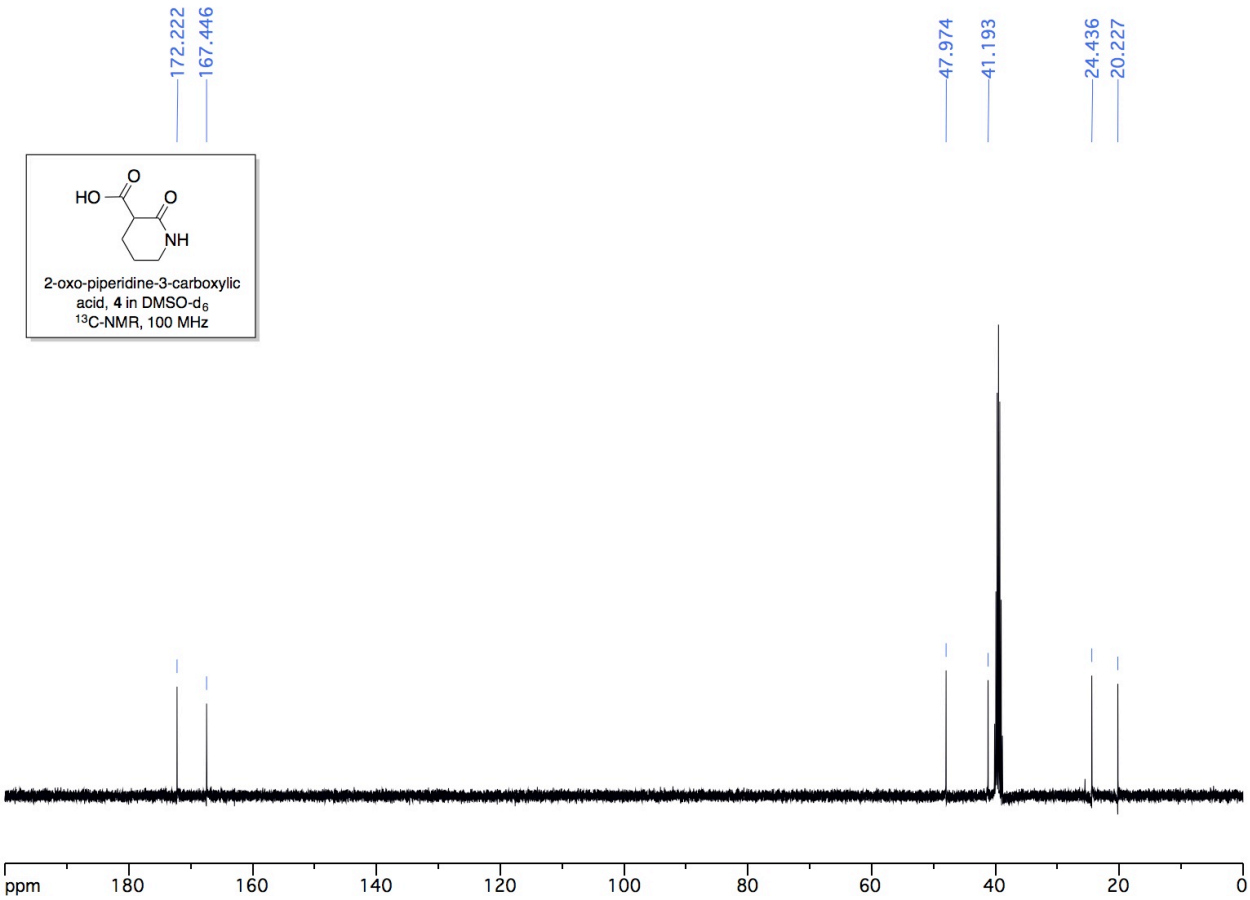
A round-bottomed flask was equipped with a stir bar and charged with 60 mL water, ethyl 2-oxopiperidine-3-carboxylate (**5**, TCI America, catalog # E0742; 4.72 g, 27.6 mmol) and solid KOH (6.45 g, 115 mmol, 4.2 equiv). The mixture was stirred at room temperature under argon for 30 minutes and quenched by the dropwise addition of concentrated HCl (~11 mL, ~130 mmol, ~4.7 equiv). NaCl (20 g) was then added to the solution, and the resulting saturated mixture was extracted with an 85 : 15 solution of $CHCl_3$ / *i*-PrOH (5 x 200 mL; the partition coefficient of the product acid between brine and 85 : 15 $CHCl_3$ / *i*-PrOH is ~0.3). The combined organics were dried over Na_2SO_4 and concentrated *in vacuo* to give 2-oxopiperidine-3-carboxylic acid (**4**, 3.71 g, 25.9 mmol, 94% yield) as a white solid. The product was carried on to the next step without further purification. The isopropyl ester of the acid may occasionally be observed as a trace contaminant in the NMR of the product. Upon storage at room temperature in a desiccator for two months, only a small amount of the decarboxylated material was observed. mp: 115-119 ^{o}C (decomp. with bubbling); ν_{max} (liquid film) 3268, 2961, 2874, 1928, 1681, 1619, 1335, 1212 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 12.62-12.59 (s, 1H), 7.78-7.77 (s, 1H), 3.17 (dd, *J* = 8.9, 6.4 Hz, 1H), 3.14 (ddd, *J* = 7.8, 5.2, 2.3 Hz, 2H), 1.98 (dtd, *J* =13.3, 6.7, 3.3 Hz, 1H), 1.84 (dddd, *J* =13.3, 10.2, 8.9, 3.3 Hz, 1H), 1.78-1.72 (m, 1H), 1.65-1.58 (m, 1H); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 172.2, 167.4, 48.0, 41.2, 24.4, 20.2; HRMS (APCI) calcd for $C_6H_{10}O_3N$ $[M+H]^+$ 144.06552, found 144.06551.

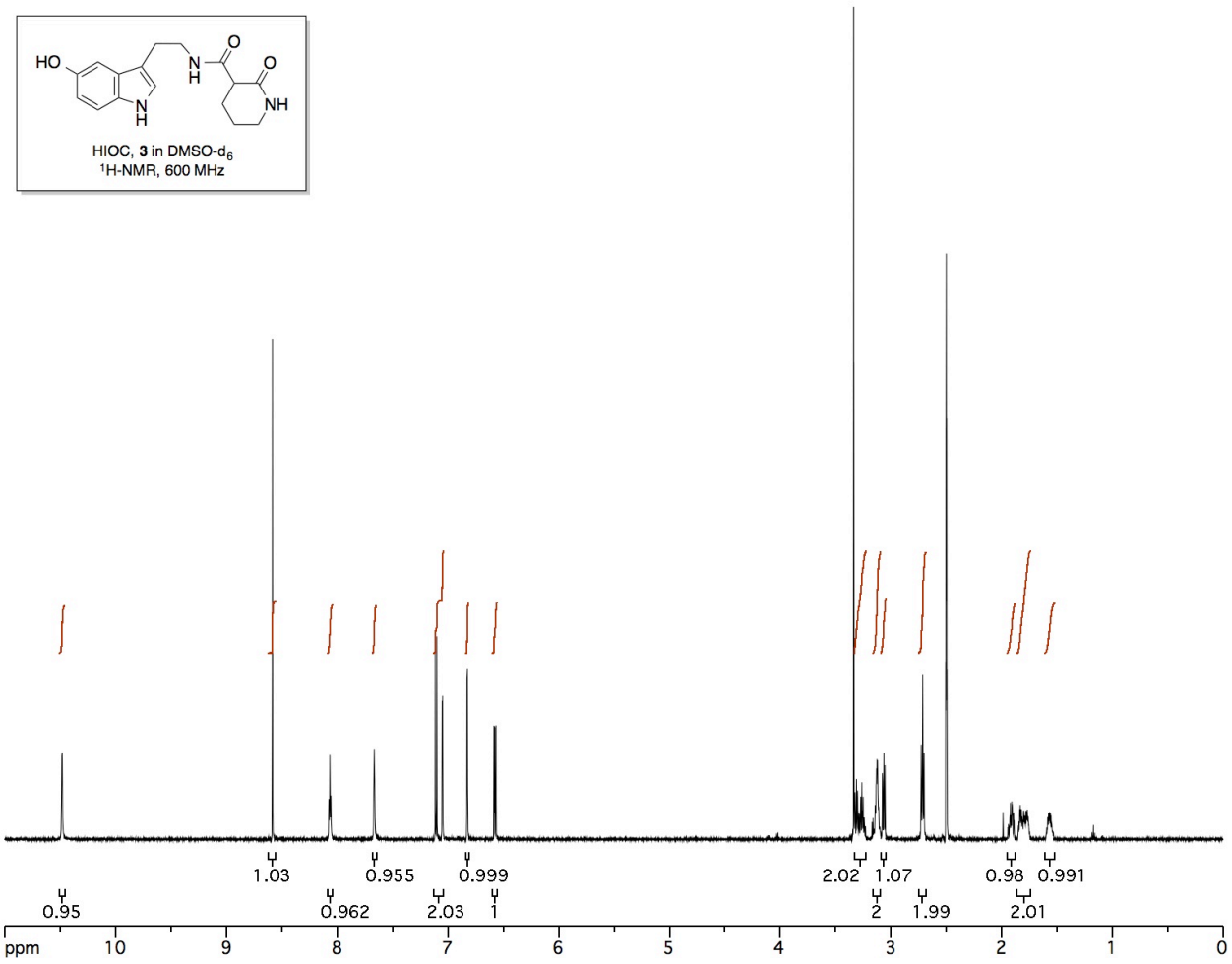
*N-(2-(5-hydroxy-1H-indol-3-yl)ethyl)-2-oxopiperidine-3-carboxamide (HIOC, **3**):*

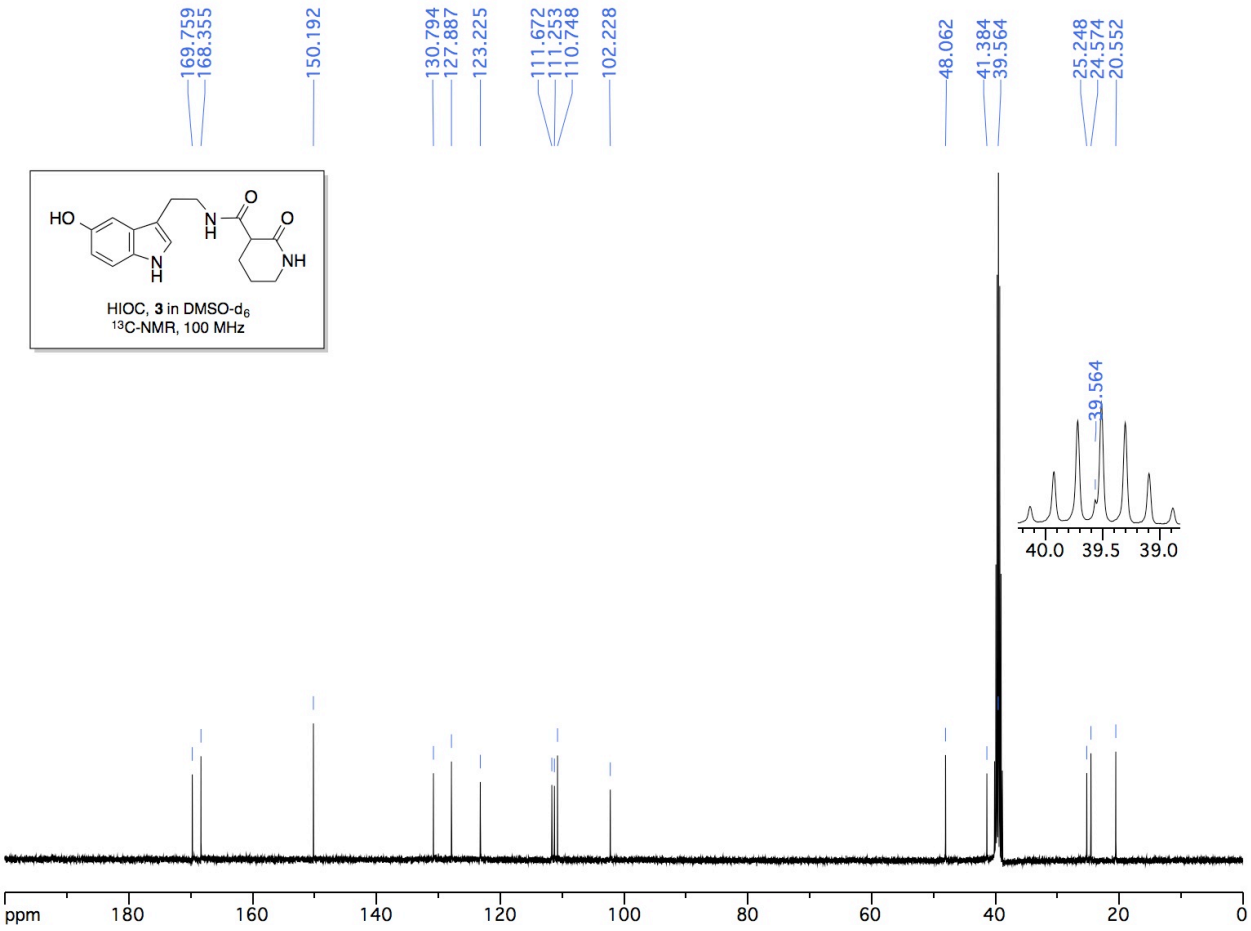


An oven-dried round-bottomed flask was equipped with a stir bar, and charged with 2-oxopiperidine-3-carboxylic acid (**4**, 1.94 g, 13.5 mmol) and CH_2Cl_2 (50 mL). The mixture was purged with argon, and carbonyldiimidazole (2.27 g, 13.7 mmol) was added in one portion. The mixture was stirred at room temperature for 30 min, whereupon pyridine (50 mL) was added, followed by serotonin-HCl (**1**, Alfa Aesar, catalog # B21623; 3.00 g, 13.8 mmol). Upon complete dissolution of the serotonin (~15 minutes), triethylamine (3.8 mL, 27.2 mmol) was added. The reaction mixture was stirred at room temperature for another 3 h and concentrated *in vacuo* (~9 torr), with heating to 45 ^{o}C and azeotroping with several portions of toluene (~100 mL toluene in total) to ensure complete removal of the pyridine. The resulting gum was dissolved in a minimal amount of a 90 : 10 mixture of EtOAc / MeOH, and dry silica gel (~25 g) was added. The mixture was then concentrated via rotary evaporation *in vacuo*, the free flowing silica-adsorbed product was loaded onto a packed chromatography column, and product was eluted with 90 : 10 EtOAc / MeOH. The relevant UV-active fractions (R_f 0.22 in 90 : 10 EtOAc / MeOH; stains purple with anisaldehyde) were combined and concentrated in vacuo. The resulting solid was loaded onto a glass frit and washed with 300 mL of warm (34 ^{o}C) Et_2O, in order to remove any traces of imidazole remaining after chromatography, to furnish *N*-(2-(5-hydroxy-1H-indol-3-yl)ethyl)-2-oxopiperidine-3-carboxamide (HIOC, **3**) as a light violet powder (2.91 g, 9.6 mmol, 71%); mp 205-208 ^{o}C (decomp.); ν_{max} (liquid film) 3245, 2927, 1642, 1584, 1548, 1462, 1374 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.49 (s, 1H), 8.60 (s, 1H), 8.08 (t, *J* = 5.6 Hz, 1H), 7.67 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 7.06 (d, *J* = 2.0 Hz, 1H), 6.84 (d, *J* = 2.0 Hz, 1H), 6.59 (dd, *J* = 8.6, 2.2 Hz, 1H), 3.36-3.24 (m, 2H), 3.17-3.10 (m, 2H), 3.08 (t, *J* = 7.2 Hz, 1H), 2.72 (t, *J* = 7.5 Hz, 2H), 1.95-1.89 (m, 1H), 1.85-1.75 (m, 2H), 1.60-1.53 (m, 1H); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 169.8, 168.3, 150.2, 130.8, 127.9, 123.2, 111.7, 111.2, 110.7, 102.2, 48.1, 41.4, 39.6, 25.2, 24.6, 20.5; HRMS (APCI) calcd for $C_{16}H_{20}O_3N_3$ $[M+H]^+$ 302.15101, found 302.14983.


HO
O
O
NH
2-oxo-piperidine-3-carboxylic acid, 4 in DMSO-d6
1H-NMR, 600 MHz
i-PrOH
0.77
1
1.91
1.07
1.01
1.01
0.992
1
ppm
12
11
10
9
8
7
6
5
4
3
2
1
0


172.222
167.446
47.974
41.193
24.436
20.227
HO
O
O
NH
2-oxo-piperidine-3-carboxylic acid, 4 in DMSO-d6
13C-NMR, 100 MHz
ppm
180
160
140
120
100
80
60
40
20
0


HO
N
H
O
N
H
O
NH
HIOC, 3 in DMSO-d6
1H-NMR, 600 MHz
1.03
0.955
0.999
2.02
1.07
0.98
0.991
0.95
0.962
2.03
1
2
1.99
2.01
ppm
10
9
8
7
6
5
4
3
2
1
0


169.759
168.355
150.192
130.794
127.887
123.225
111.672
111.253
110.748
102.228
48.062
41.384
39.564
25.248
24.574
20.552
HO
N
H
O
N
H
O
NH
HIOC, 3 in DMSO-d6
13C-NMR, 100 MHz
39.564
40.0
39.5
39.0
ppm
180
160
140
120
100
80
60
40
20
0