Study Title: Effect of novel compounds on a model of Asthma

Study Objectives
The goal of the study was to test the potential of test article as a treatment for asthma.

METHODS AND RECORDS
After 7 days of environmental acclimation, the mice began study (study day 1). On study days 1 and 14, mice were injected IP with a mixture of 20mg Ovalbumin and 2mg alum (volume not exceeding IACUC guidelines). On study days 28, 29 and 30, mice were treated with 1% aerosolized OVA for 20 minutes each day. On day 32, the dosing began with the following groups:
Vehicle
Positive control
Test compound single injection 1 hour prior to BUXCO
Test compound, two injections total; 24 hours and 1 hour prior to BUXCO
Test compound, two injections total; up to 12 hours and 1 hour prior to injections

On study day 32, mice were injected via IV with test compound and subjected to a rising methacholine challenge within BUXCO plethysmographs to ascertain Airway Hyper Responsiveness. The BUXCO plethysmographs measure respiratory rate and tidal volume in conscious, unrestrained mice. The chambers allow for application of nebulized methacholine for analysis of enhanced pause (Penh) in experimental asthma models. The increase in Penh is a measure of increasing airway resistance, and in conjunction with an increasing methacholine dose, this provides a measure of airway responsiveness.

Mice were then sacrificed and Broncho alveolar lavage fluid (BALF) and lung were collected. Lavage fluid was split. A portion of the lavage fluid and the lung tissue were analyzed for concentration of test compound and GSNOR activity. The other portion of lavage fluid was sent for assessment of cytokine levels utilizing the LINCOplex cytokine assay for the following cytokines:
IL-4
IL-5  
IL-9  
IL-10  
IL-12  
IL-13  
IFN\text{gamma}

**Procedural Timeline:**

<table>
<thead>
<tr>
<th>Date</th>
<th>Receive mice</th>
<th>IP injections - OVA</th>
<th>IP injections - OVA</th>
<th>IV injection of N319</th>
<th>IV injection - novel compound prior to BUXCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/3/07</td>
<td>12/10/07</td>
<td>12/24/07</td>
<td>1/7/08</td>
<td>1/8/08</td>
<td>1/10/08</td>
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<tr>
<td>Acclimation</td>
<td>1% OVA for 20 minutes</td>
<td></td>
<td></td>
<td></td>
<td>BUXCO methacholine challenge</td>
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<td>Necropsy (BALF and lung tissue)</td>
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</tbody>
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Key:  
- BALF: Broncho Alveolar Lavage Fluid  
- IP: Intraperitoneal  
- IV: Intravenous  
- OVA: Ovalbumin