Introduction
CHAPTER 1
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Asthma is a global health problem causing enormous mortality and morbidity, both in developed and developing countries. Despite remarkable advances in diagnosis and treatment, asthma is still a serious public health problem, particularly due to the off targets and side effects of the commonly available drugs. Asthma has shown a drastic increase in global prevalence, morbidity, mortality and economic burden over the last 40 years, especially in children (Beuther, 2010; Masoli et al., 2004).

Allergic asthma is a common chronic lower airway disease. It is mediated by a dominant T helper 2 (Th2) immune response and its distinctive features include airway hyperresponsiveness (AHR), increased immunoglobulin (IgE) levels, airway inflammation and mucus hypersecretion. An allergic immune response is generated upon stimulation of T cell receptors by antigen ligation which results in differentiation of peripheral CD4+ T cells into effector T cells. These cells are classified as Th1, Th2, Th17 and Tregs (regulatory T-cell) based on their cytokine signature. Th2 lymphocytes producing a type 2 cytokine profile are known to mediate pathophysiology of asthma. These cells are necessary to allergic asthma development in both animals and humans. The amount of type 2 cytokines produced by them is highly elevated in the airway tissues of human asthma subjects and animals in which asthma is induced, using suitable allergens (Gavett et al., 1994; Robinson et al., 1992).

Individually or synergistically, Th2 cytokines mediate vital functions in asthma, e.g. Interleukin-5 (IL-5) is known to play an imperative role in eosinophil maturation, differentiation, recruitment, and survival. IL-4 along with IL-13 is essential in eosinophil accumulation and is a primary factor in IgE production by B cells. IL-13 is however known to play the most important and central role in allergic asthma. This Th2 cytokine alone has been found to be sufficient and necessary for the induction of allergic asthma in mice (Jiang et al., 2000). It contributes in airway fibrosis, airway hyperresponsiveness, mucus production, IgE synthesis and airway inflammation (Wills-Karp and Karp, 2004). Interleukin-4 is responsible for Th2 cell differentiation which is executed after IL-4 binds to its receptor causing phosphorylation and dimerisation of a signaling protein known as signal transducer and activator of transcription 6 (STAT6), a Th2-cell specific transcription factor, through the JAK/STAT cascade. This modulated form of STAT6 translocate from cytoplasm to the nucleus to activate the transcription of cytokine responsive genes, especially GATA3 (Zhu et
al., 2001). GATA3 activates the transcription of IL-5 and IL-13 genes by directly binding to their promoters (Zhou and Ouyang, 2003). Moreover, this transcription factor is also involved in remodeling of chromatin structure and opening of IL-4 locus (Ouyang et al., 2000). Currently available drugs and therapies for asthma involve inhaled corticosteroids, β2-adrenoceptor agonists, leukotriene modifiers, phosphodiesterase inhibitors, cytokine-based immunotherapies and transcription factor modulators. However, none of these drugs or therapies is individually sufficient to treat any form of asthma whether mild, moderate or severe, because of asthma heterogeneity, steroid resistance, and poor biological half life of β2-adrenoceptor agonists. So, the need for newer and novel therapies is warranted (Beuther, 2010; Masoli et al., 2004; Wills-Karp and Karp, 2004; Zhu et al., 2001).

*Adhatoda vasica* is a well-known medicinal plant in Ayurvedic and Unani system of medicine. The juice extracted from *Adhatoda* leaves or their decoction with roots, is primarily used for respiratory disorders like asthma, breathlessness and bronchitis (Gupta and Prajapati, 2010; Kaur et al., 2013). The leaves of this plant contain various alkaloids, mainly Vasicine, N-oxides of Vasicine, Vasicinone, Deoxyvasicine and Maiontone. Vasicine, a heterocyclic alkaloid, has been reported to be responsible for most of the pharmacological activities of *Adhatoda vasica*. This molecule was first isolated in 1924 but faced contradictions in its structural elucidation. Finally its structure was elucidated in 1935, and was established to be a tricyclic heterocycle (Ghose et al., 1932; Lebovitz, 2002; Nepali et al., 2013). However, most of the pharmacological work on this molecule was done between 1950s-1980s (Amin and Mehta, 1959; Atal, 1980; Barry et al., 1955; Chan et al., 1963; Chandhoke et al., 1978; Gupta et al., 1977).

The present study involves the screening of a series of Vasicine semi-synthetic analogues, for their anti-asthmatic effect, evaluated in a preventive ovalbumin induced mouse model of asthma. Through this screening, it was observed that R8, one of analogues, had a significant anti-asthma potential. This was followed by evaluation of therapeutic potential of R8 by employing a therapeutic mouse model of asthma, along with its preventive potential. In this part of the study, R8 was administered before and after the development of asthma symptoms in a preventive and therapeutic model of asthma, respectively. It was observed that R8 significantly reduced asthma features like AHR, airway inflammation, mucus hypersecretion, IgE production and Th2 cytokine production. It also modulated STAT6 phosphorylation and GATA3 expression, which seems to be the reason of significant suppression in intensity of asthma features in therapeutic model, as STAT6 and GATA3 are prime transcription factors for Th2 cell differentiation and hence have a principal role in asthma pathogenesis.
Objectives of the present study

To screen a series of semi-synthetic analogues of Vasicine for their anti-asthmatic activity in a preventive ovalbumin induced murine model of asthma and selection of an active and promising molecule from the series.

To evaluate the therapeutic potential of the active molecule in a murine model of allergic airway inflammation.

To deduce the mechanism of action of the active molecule, for its anti-asthma potential.