FORM 2
THE PATENT ACT, 1970
(39 of 1970)
&
The Patent Rules, 2003

COMPLETE SPECIFICATION
(See Section 10 and Rule 13)

TITLE OF THE INVENTION: "NOVEL TERPENOIDAL BASED ANALOGUE OF VASICINE OF FORMULA I WITH MULTIPLE THERAPEUTIC ACTIVITIES."

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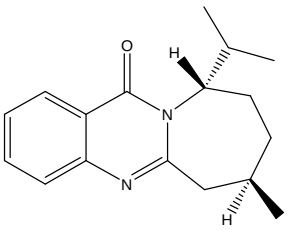
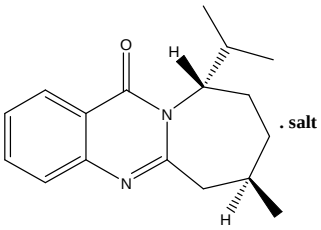
THE FOLLOWING SPECIFICATION PARTICULARLY DESCRIBES THE INVENTION AND THE MANNER IN WHICH IT IS TO BE PERFORMED.

TITLE: Novel compound of formula I and pharmaceutically acceptable salts thereof represented by formula IA with multiple therapeutic activities.



Formula I (KLD)



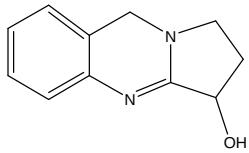
Formula IA

FIELD OF TECHNOLOGY: The present disclosure relates to the novel compound of formula I and pharmaceuticall acceptable salts thereof and use of the said novel compound for the treatment of asthma. The present invention comprises design, synthesis and biological evaluation of a novel terpenoid based compound of formula I chemically known as 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one and pharmaceutical acceptable salts thereof which possess multiple therapeutic effects like bronchodilatory, anti-allergic and anti-tussive effects which are not exhibited by vasicine of formula II. The present invention comprises rational design of novel compound of formula I referred here in above and herein below as KLD and its pharmaceutically acceptable salts comprising incorporation of menthol based azepino skeleton within the vasicine framework imparting to the said designed novel molecule with bronchodilatory, anti-tussive and anti-histaminic activities that graduates the said designated novel molecule as an ideal anti-asthmatic. The said KLD molecule of the instant disclosure is synthesized by condensation of menthol lactam with anthranilic acid. The said molecule KLD and its pharmaceutically acceptable salt has been investigated for pharmaceutical activities such as anti-tussive, anti-histaminic and bronchodilatory activities. The comparisons are made with the

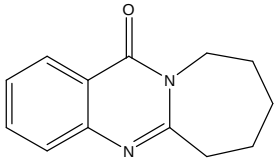
reference standards and previously reported analogue TAZQ of the formula III. The findings of pharmacological investigation indicate that the said KLD and its pharmaceutically acceptable salts possesses significant potential to ameliorate asthma syndrome like bronchi constriction bronchial inflammation and provide anti-tussive effect. The said KLD molecule and its pharmaceutically acceptable salts are found to be more potent than the reference TAZQ of formula III and codeine of formula IV as evidenced by markedly reduced cough responses, hypersensitivity and inflammatory reactions and increased cough latency in KLD and its pharmaceutically acceptable salts treated Guinea pigs.

The term cough frequency mentioned herein above and herein below is referred simply to the number of cough responses in a period of time while the term cough latency is the time elapsed between exposures to a pathogenic organism, or chemical or radiation and when symptoms and signs are first apparent. It signifies the period taken by the multiplying organism to reach a threshold necessary to produce first symptoms in host.

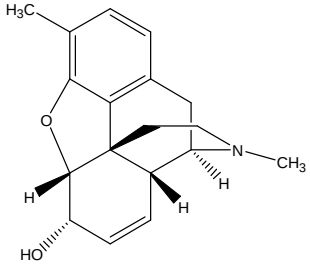
The said novel KLD molecule and its pharmaceutically acceptable salts significantly lower asthmatic abnormalities like dyspnea, anaphylactic shocks and hypersensitivity/allergic reactions and the likes. It decreases the release of pro-inflammatory mediators like NO (nitric oxide) cytokines and chemokines, oxidative reactions involved in progression of chronic asthma and restores antioxidants level in lung tissue and broncho-alveolar lavage fluid (BAL). The effect of said KLD molecule and its pharmaceutically acceptable salts are more potent as compared to Aminophylline (standard anti-asthmatic agent) and TAZQ (potent vasicine analogue). The said novel KLD molecule and its pharmaceutically acceptable salts also reduce histamine induced contractions in trachea showing its bronchodilator effect. Moreover, it prevents hypersensitivity reactions due to ovalbumin (OVA) in presensitized trachea and thereby reduces bronco constriction and contractions *ex-vivo*. Effect of said KLD molecule and its pharmaceutically acceptable salts on lung tissue histology has also been disclosed.



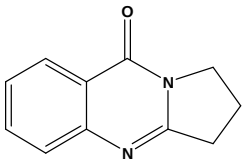
Vasicine (Formula II)



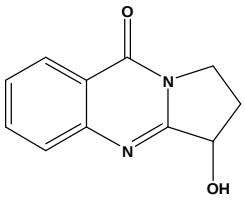
TAZQ (Formula III)



Codeine (Formula IV)



Deoxy vasicinone (Formula V)



Vasicinone (Formula VI)

BACKGROUND OF THE INVENTION: Asthma is an oxidative and inflammatory disease of the airways characterized with bronchi constriction that affects 5–10% of the general population. Epidemiological studies indicate that there is a global increase in the incidence, morbidity, and mortality caused due to asthma despite an expanding repertoire of medications available for the treatment

of this disease (Sears et al., 1991, MMWR. 1996). Asthma affects approximately 53 million people across world mostly in United States, France, Germany, Italy, Spain, United Kingdom, and Japan (Peter et al., 1995). More than 4000 people die every year in India as a result of complications arising from serious asthmatic attacks (Chang et al., 2000). Asthma is characterized as chronic airways inflammation and cellular infiltration into lung and is associated with increased airway hyper-responsiveness leading to recurrent episodes of wheezing and breathlessness (National Asthma Education and Prevention Program, 1991). Asthma and chronic obstructive pulmonary disease (COPD) share the common feature of impeded air flow to the lungs leading to wheezing, laboured breathing or dyspnoea. Asthma attacks are more often allergic in nature caused by exercise, exposure to environmental allergens and chemicals, viral infection, irritants, antigen–antibody reaction, and release of autacoids leading to bronchial constriction, oedema and viscid secretions. The expert panel reports of the National Asthma Education and Prevention Program in US (Bethesda et al., 1997 & The British Guidelines on Asthma Management, 1995) and similar reports from different countries have been beneficial for the management of asthma (Ernst et al., 1996).

The chronic inflammation is associated with airway obstruction and mucus production that leads to increased airway hyper-responsiveness resulting into recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment (Bethesda et al., 1991).

Excessive mucus production has long been considered an important cause of morbidity and mortality in asthma. Epithelial and matrix abnormalities, increased smooth muscle mass and vascularity, thickening of the airway wall and mucus are the factors that contribute to airway remodelling in asthma. Many factors like goblet cell hyperplasia, goblet cell counts and increased area of sub mucosal glands results in mucin hyper production with decreased mucociliary clearance and contribute to chronic airway inflammation, airway obstruction, and asthma exacerbation (Barnes et al., 1990 & Doelman et al., 1990). The inflammatory cells of asthmatics have an increased capability to generate free radicals compared to controls, which further contribute to high concentrations of reactive oxygen species

*(*ROS) (Jarjour et al., 1994). Excess reactive nitrogen species (RNS) may also be produced by asthmatics. Cytokines stimulate increased production of nitric oxide (NO).) (Kharitonov et al., 1994) which reacts with O_2 to form peroxynitrite, a cytotoxic species that has many damaging effects, including lipid oxidation (Radi et al.,1991). NO can also be converted to nitrite, which can oxidise proteins. This may be through nitration of tyrosine, in a reaction catalysed by EPO (Wu et al., 1999). Elevated nitro tyrosine levels have been observed in asthmatics (Hanazawa et al., 2000).

For managing asthmatic attacks, symptomatic relief is a foremost requirement and many combinations of bronchodilator and anti-inflammatory drugs have been studied in search of effective treatment that takes care of both broncho-constriction and inflammation (Connett et al., 1994). Despite the emergence of new effective anti-asthmatic medication, the level of disease control in asthma patients remain unsatisfactory mainly due to insufficient or inappropriate care that may be taken with the use of antioxidant and anti-inflammatory therapies (Rabe et al., 2006).

Vasicine an alkaloid obtained from leaves of Adhatoda vasica Nees, has been reported to possess moderate degree of bronchodilator activity (Gupta et al., 1977). Tetrahydroazepinoquinazoline (TAZQ), a synthetic analogue of Vasicine has been reported to have 6–10 times more potent bronchodilator activity than aminophylline (Atal,1980).TAZQ is reported to inhibit antigen-induced mast cell degranulation and release of histamine from target tissues with an increased outflow of prostaglandin E (lungs) and inhibits 45Ca uptake from peritoneal mast cells (Johri and Zutshi, 2000).

If treatment of the underlying etiology fails to resolve the cough, or if no cause can be identified, then the cough may be treated symptomatically. In most of the cases symptomatic treatment comprises anti-tussive therapy to decrease the frequency and the severity of the cough. Anti-tussive treatment varies in mechanism of action-nonspecific anti-tussive such as dexomethorphan and codeine appear to act in the brain stem to reduce the cough reflex. Other non specific anti-tussive such as benzonatate, act to anesthetise respiratory passages and thus reduce the stimulus to cough.

Other agents aim to decrease the volume of respiratory tract secretions and thus the stimulus and need to cough. These agents are also used to treat certain common underlying etiologies and include antihistamines, Cotigosteroids, antibiotics, decongestants and mast stabilisers. Nonpharmacologic anti-tussive are few but may include, for example honey.

Various drugs from the different class of compounds those are used for the asthama as medication are listed herein below.

DRUGS IN THE TREATMENT OF ASTHMA

A. GLUCOCORTICOIDS:

Pharmacological control of asthma may be obtained in most asthmatics with anti-inflammatory agents (controllers), i.e. inhaled glucocorticoids and theophylline. The most potent controllers are the inhaled glucocorticoids (Salmeron et al., 1989). In most asthmatic patients adequate doses of inhaled glucocorticoids, particularly if combined with long-acting bronchodilators, allow systemic glucocorticoids to be reduced or withdrawn completely Nimmagadda et al., 1998.

Glucocorticoids consistently lessen airway hyper responsiveness in asthma (Barnes et al., 1990). Indeed, long-term treatment with glucocorticoids reduce airway responsiveness to histamine, cholinergic agonists, allergens (affecting both early and late responses), exercise, fog, cold air, bradykinin, adenosine, and irritants such as sulphur dioxide and metabisulfites (van den Berge et al., 2001).The reduction in airway hyper responsiveness may not be maximal until treatment has been given for several months. The magnitude of the reduction varies, and airway responsiveness can remain abnormal (Barnes et al., 1990). When therapy is discontinued airway responsiveness usually returns to pre-treatment levels (Juniper et al., 1991).

B. β_2 –AGONISTS

In addition to their bronchodilator action, in vitro and in vivo long-acting β_2-agonists inhibit mast cell mediator release, plasma exudation and may reduce sensory nerve activation (Johnson et al., 2001). Treatment with a single high dose of salbutamol does not increase exhaled nitric oxide in asthmatics not taking inhaled glucocorticoids and may actually increase exhaled nitric oxide in asthmatics taking inhaled glucocorticoids. In addition, regular use of salmeterol,

either used alone or in combination with inhaled glucocorticoids, does not change exhaled nitric oxide (Yates et al., 1997). The adverse effects of regular long-term treatment of asthma with the highly potent short-acting inhaled β_2-agonist fenoterol have been established in both epidemiological and clinical studies. Also regular treatment with salbutamol may be associated with subtle deterioration in asthma control over time (Yates et al., 1998). Therefore, short-acting β_2-agonists should only be used as required (Yates et al., 1995).

C. XANTHENES & DERIVATIVES

Long-term treatment with sustained-release theophylline alone is effective in controlling asthma symptoms and improving lung function in patients with mild persistent asthma (Yates et al., 1995). The use of theophylline, however, has declined owing to the widespread use of inhaled glucocorticoids, which remain the most effective treatment for asthma. One of the limitations of theophylline in the past has been the side effects observed in many patients at the traditional bronchodilator doses associated with plasma levels of theophylline between 10 and 20 mg/l. However, anti-inflammatory benefits appear to occur at lower plasma theophylline levels (, 10 mg/l), and the incidence of any adverse effects is minimized. Two studies have demonstrated that low-dose theophylline added to inhaled glucocorticoids is equally efficacious when compared with increasing the dose of inhaled glucocorticoids, in symptomatic patients established on inhaled glucocorticoid therapy (Evans et al., 1997). However, in these patients, long-acting inhaled β2-agonists may be more effective than theophylline (Taylor et al., 1998). Theophylline alone, or in combination with inhaled glucocorticoids, may maintain asthma control but in asthmatics regular treatment with sustained-release theophylline does not reduce the severity of bronchial hyper responsiveness (Dutoit et al., 1987).

D. LEUKOTRIENE SYNTHESIS AND LEUKOTRIENE RECEPTOR ANTAGONISTS

The role of LTB4 in the pathogenesis of bronchial asthma, if any, remains controversial (Evans et al., 1996) and the role of cysteinyl leukotrienes in the pathogenesis of asthma is well-established (Borish et al., 2002). The cysteinyl leukotrienes mediate a number of pathways relevant to the pathogenesis of asthma

including smooth muscle contraction, increased vascular permeability and mucus secretion, and decreased mucociliary clearance, recruitment of eosinophils in the airways. Cysteinyl leukotrienes have also been reported to play a role in maintaining the chronic airway inflammatory response in neurogenic inflammation and in airways remodelling (Holgate et al., 1996). Cysteinyl leukotriene receptor antagonists and leukotriene synthesis-inhibitors are clinically effective in some groups of asthmatic patients (Wenzel et al., 1998) and remain a second-choice option in the treatment of mild persistent asthma as low doses of inhaled glucocorticoids are more effective (Wenzel et al., 2002).

E. COMBINATION STRATEGIES IN ASTHMA MANAGEMENT:

The two major conditions in asthma are bronchospasm that leads to breathlessness and inflammation in the lung tissues. Therefore, the mainstream of antiasthmatic therapy aims at to reduce bronchospasm and inflammation of the lung tissue. Many therapies have been studied and suggested including bronchodilators in combination with anti-inflammatory agents. For managing asthma attacks, symptomatic relief is foremost requirement. Thus, bronchodilators and corticosteroids are used simultaneously for the effective treatment of acute and chronic asthma (Kulkarni et al., 2010).

F. NATURAL PRODUCTS IN TREATMENT OF ASTHMA

The molecules isolated from the natural products have diverse pharmacological actions. One of such heterocyclic compounds is vasicine. The alkaloid is derived from the plant *Adhatoda vasica* Nees, family Acanthaceae, its leaves have been used in Indian system medicine for more than 2000 years (Atal et al., 1980). Ayurvedic system of medicine describes the use of this plant for the treatment of respiratory ailments, particularly for the treatment of cough, bronchitis, asthma and tuberculosis. It is also claimed that it causes thinning of sputum and phlegm and asthma. Though the molecule vasicine was investigated in detail as a bronchodilator but its potential as anti-asthmatic, anti-inflammatory and anti-tussive is not explored.

US6676976 discloses the improved process for the production of vasicine by having the improved mode of isolation.

Inventive feature of the present invention lies in the fact that the said novel compound KLD of the formula I and its pharmaceutically acceptable salts have been designed and synthesized and possesses all the three activities viz bronchodilatory, anti-tussive and anti-histaminic; thereby showing synergistic effect and also provides a solution to problems associated with such remedies disclosed therein in the prior art wherein different chemical entities have to be used in a composition as there is no single chemical compound reported therein in the prior art which possesses all the three activities.

PROBLEMS ASSOCIATED WITH THE PRIOR ART:

To meet with the quality requirement of an ideal anti-asthmatic remedy disclosed therein in the prior art wherein different constituents have to be used for the same said purpose. In other words to get an ideal anti-asthmatic treatment different constituents are used in combination in such a way so that one constituent provides bronchodilator activity, second one provides anti-inflammatory, third provides anti-tussive activity and fourth one to satisfy anti-asthmatic activity, thereby requiring four different chemical entities combined into a drug.

TECHNICAL SOLUTION OVER THE EXISTING PRIOR ART:

The said novel compound KLD of the formula I and its pharmaceutically acceptable salts under the subject matter disclosed herein possesses all the three activities viz bronchodilatory, anti-tussive and anti-histaminic; thereby showing synergistic effect and also provides a solution to problems associated with such remedies disclosed therein in the prior art.

OBJECT OF THE INVENTION:

The first aspect of the invention is to design and synthesize a novel compound of the formula I and its pharmaceutically acceptable salts that possess bronchodilatory, anti-tussive and anti-histaminic activities and the likes.

Second aspect of the invention is to evaluate the said compound KLD of formula I and its pharmaceutically acceptable salts for pharmaceutical activities such as bronchodilatory, anti-tussive and anti-histaminic activity and the like.

Third aspect of the invention is to synthesize the compound of the formula I comprising the condensation of menthol lactam (prepared by Beckmann rearrangement of menthol) with anthranilic acid.

Fourth aspect of the invention is to provide spectroscopic details assigning the configuration at the asymmetric centers of the analogue that has been synthesized under the present invention.

Fifth aspect of the invention is to investigate anti-tussive, anti-histaminic and bronchodilatory potential of the said novel compound of formula I and its pharmaceutically acceptable salts.

Sixth aspect of the invention to have significant lowering of asthmatic abnormalities like dyspnea, anaphylactic shocks and hypersensitivity/allergic reactions.

Seventh aspect of the invention is to decrease release of pro-inflammatory mediators like NO, cytokines and chemokines, and also reduced oxidative reactions involved in propagation of chronic asthma and restored antioxidants level in lung tissue.

Eighth aspect of the invention is to reduce histamine induced tracheal contractions thereby enhancing its bronchodilator effect.

Ninth aspect of the invention is to reduce bronco constriction by preventing hypersensitivity reactions due to OVA in presensitized trachea.

Tenth aspect of the invention is to provide the data indicating the superiority of the novel compound of the formula Iand its pharmaceutically acceptable salts over the known compounds disclosed therein in the prior art for the same pharmaceutical activity.

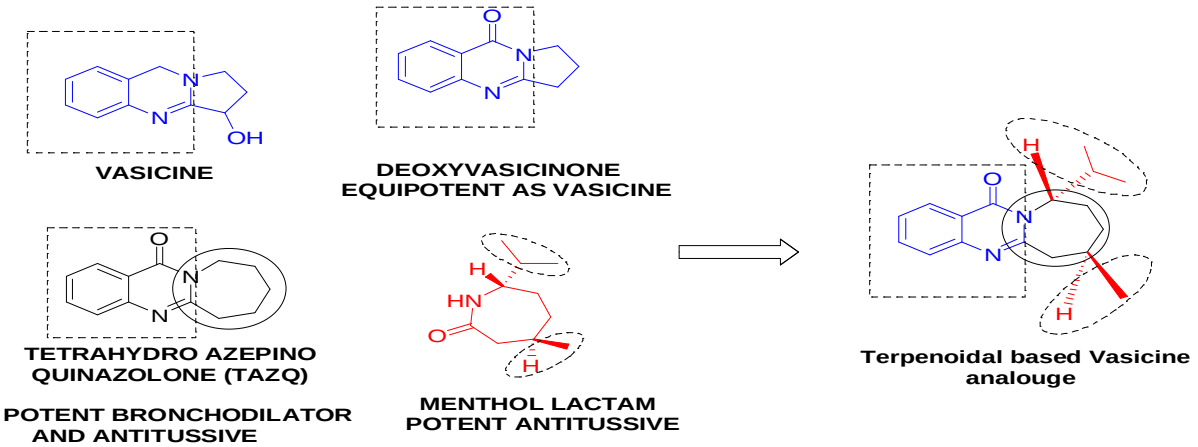
Eleventh aspect of the invention is to observe the effect of the said compound of formula I and its pharmaceutically acceptable salts under the investigation on lung tissue histology.

SUMMARY OF THE INVENTION:

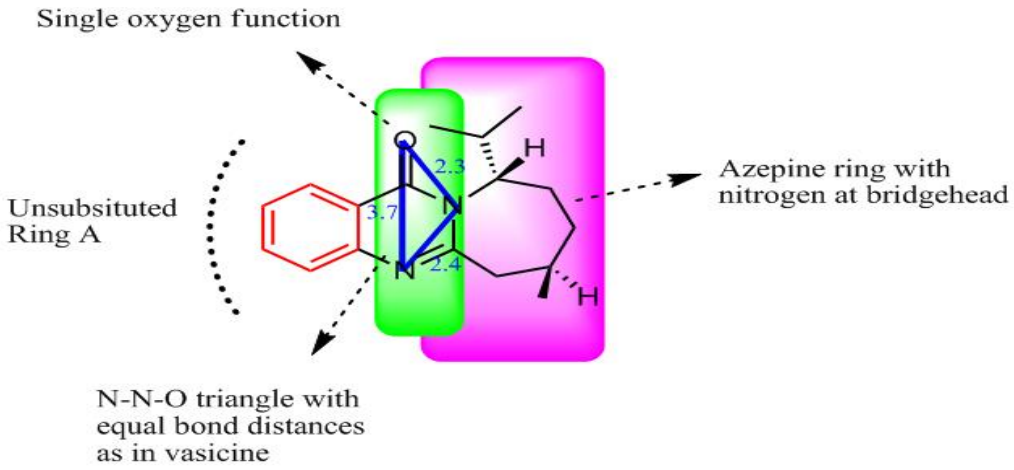
The subject matter disclosed herein relates to the treatment of asthma comprising design, synthesis and biological evaluation of a novel terpenoidal based compound of formula I and its pharmaceutically acceptable salts with multiple therapeutic activities such as bronchodilator, anti-allergic, anti-tussive and the like all together in a single chemical entity. The present invention comprises design of a novel terpenoidal based analogue of formula I and referred herein above and herein below as KLD comprising incorporation of the menthol based azepino skeleton within the vasicine framework imparting the said novel designed molecule bronchodilatory, anti-tussive and anti-histaminic and the like activities that results into the generation of an ideal novel anti asthmatic remedy replacing the remedy disclosed therein in the prior art wherein different constituents have to be used for the same said purpose. The novel said molecule disclosed herein and referred as KLD is synthesized comprising condensation of menthol lactam with anthranilic acid. The said KLD molecule and its pharmaceutically acceptable salts have been investigated for anti-tussive, anti-histaminic and bronchodilatory and the like activities.

DESIGN:

The design strategy based on which the said novel KLD molecule has been designed is given herein below:

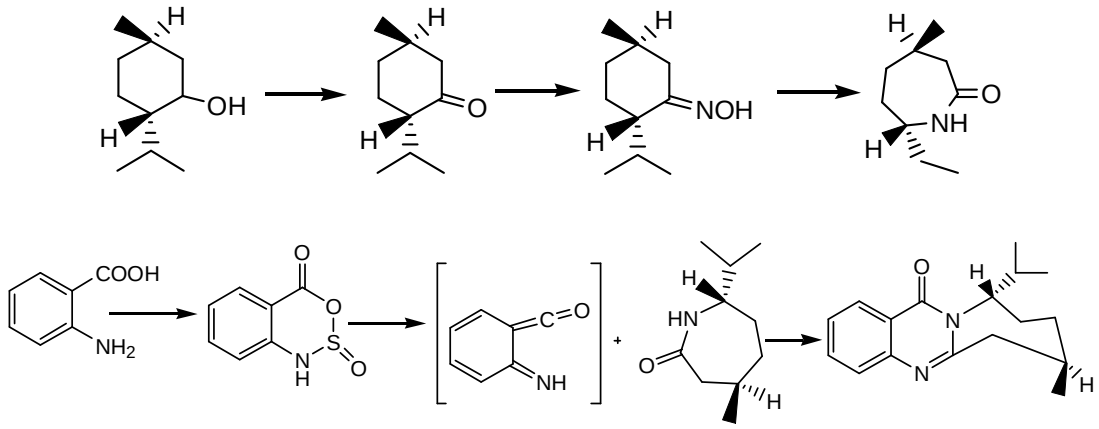


Design Strategy



SYNHESIS:

The processes disclosed herein for the synthesis of said novel KLD molecule are described herein below:



The KLD molecule thus formed is further converted into its corresponding pharmaceutically acceptable salts**.**

DETAILED DESCREPTION OF THE INVENTION:

An ideal anti-asthmatic drug sought today is one possessing bronchodilator and anti-allergic activities (as majority of asthma cases are of allergic origin) together with anti-tussive potential in a single chemical entity. The potential of l-menthol

as an anti-tussive has been disclosed (Laude *et al*, 1994). Lactams prepared by nitrogen insertion in the skeleton of menthol and camphor exhibit anti-tussive potential vis-a-vis the parent mono terpenoids (Kumar *et al*., 2012). Disclosed herein is the rational design of a novel terpenoidal based analogue referred hereinabove and herein below as KLD comprising incorporation of the menthol based azepino skeleton within the nitrogen heterocycle of formula II framework imparting to the designed said novel KLD molecule and its pharmaceutically acceptable salts bronchodilatory, anti-tussive and anti-histaminic pharmacological activities and the like.

The term pharmaceutically acceptable salts refer to salts prepared from pharmaceutically acceptable non toxic acids including inorganic acids or organic acids. Pharmaceutically acceptable salts in general show better solubility in water.

The said novel compound KLD disclosed herein is basic, salts may be prepared from pharmaceutically acceptable non toxic acids. Such acids includes acetic ,benzenesulphonic, benzoic, camphor sulphonic, citric, etanesulphonic, formic, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, malic, maleic, mandelic, metanesulphonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulphuric, tartaric, para toluene sulphonic acid and the likes. Particularly preferred are hydrochloric, hydrobromic acids.

It will be understood that in the discussion of method of treatment which follows, refrences to the compounds of formula I are meant to also include the pharmaceutically acceptable salts represented by formula IA.

Utilities of the invention of the present invention depend upon the ability of the compound of formula I to antagonize the action that will make it useful.

The pharmaceutical composition of present invention comprises a compound of formula I as active pharmaceutical ingredient or a pharmaceutically acceptable salt thereof and may contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The pharmaceutical composition of the present invention suitable for the oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient, as a powder or granules or as a solution or suspension. Such

composition is prepared by any of the method of pharmacy but all the methods include the step of bringing into association the active ingredient with the carrier.

DESIGN STRATEGY ADOPTED FOR THE INVENTION:

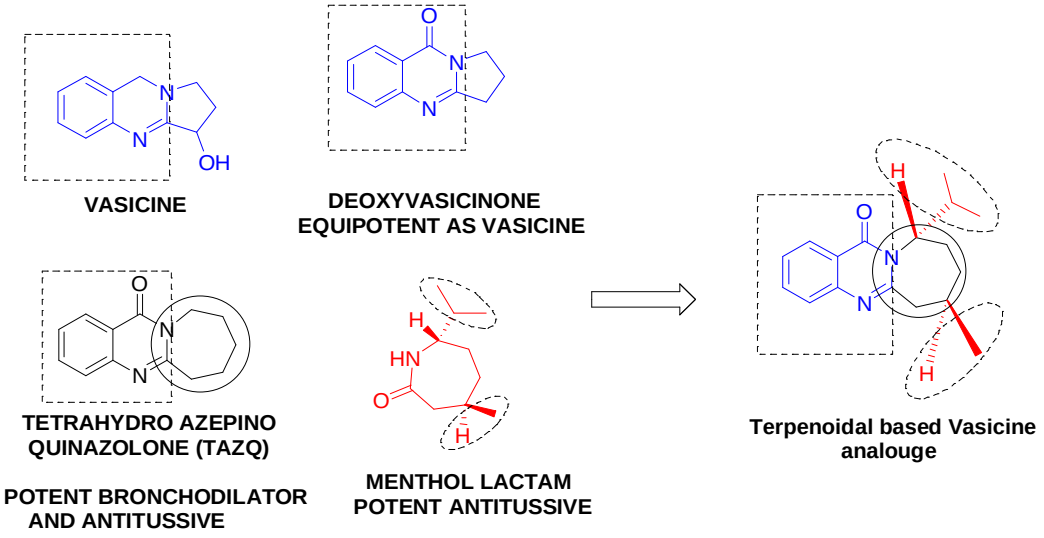
The novel terpenoidal based analogue of formula I is designed as described herein below in Figure 1 keeping in view the pharmacophoric features for bronchodilatory potential of compound of formula II and its analogues as disclosed by Atal et al (Atal et al., 1980). The designed analogue possesses the following structural features.

1) N-N-O Triangle with appropriate bond distances which has been proved as a prerequisite for bronchodilatory potential of vasicine of formula II and deoxyvasicinone of formula V.

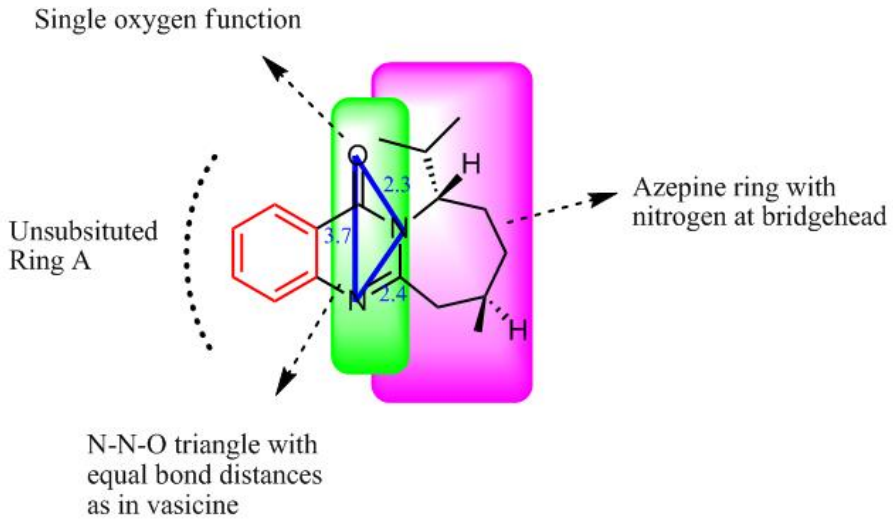
2) only one oxygen function (as in deoxyvasicinone of formula V which is reported to be as potent as vasicine while vasicinone of formula VI appears to be much less potent than vasicine indicating that two oxygen functions in vasicine might be antagonistic to each other).

3) Quinazolone fused azepine with nitrogen at the bridge head 4) unsubstituted quinazolone ring with placement of either electron withdrawing or electron donating groups decreasing the pharmaceutical activity.

4) Fusion of Menthol based azepine ring in place of pyrrolo of vasicine and azepine of TAZQ. The placement of a menthol based azepine ring maintains the stereochemistry of menthol intact and the resultant novel analogues are expected to possess anti-tussive potential as it encompasses dual features of tetrahydro azepino quinazolones as well as menthol azepines.



Design Strategy

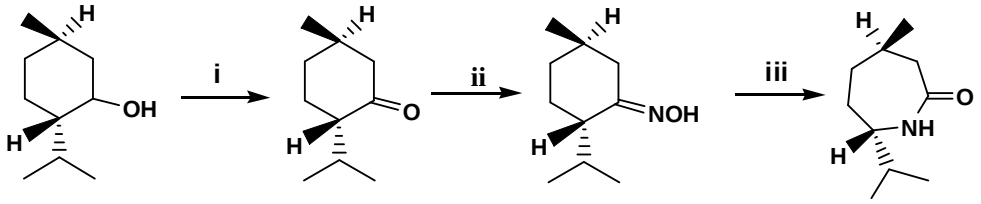


2.1 SYNTHESIS, CHARACTERIZATION AND STEREOCHEMISTRY

(4R,7S)-7-isopropyl-4-methylazepan-2-one has been synthesised as per scheme 1 described herein below. Menthol is oxidized to menthone comprising contacting the said menthol with an oxidizing agent such as chromic acid and the like. The said menthone is converted into corresponding oxime by contacting the said menthone with NH_2OH and the said oxime undergoes Beckmann rearrangement. The nitrogen insertion has not been achieved by the conventional acid catalysed Beckmann rearrangement of menthoneoxime. Instead the tosylated oxime is treated with dilute alkali for the nitrogen inserted lactam. Terpenoidalazepine is

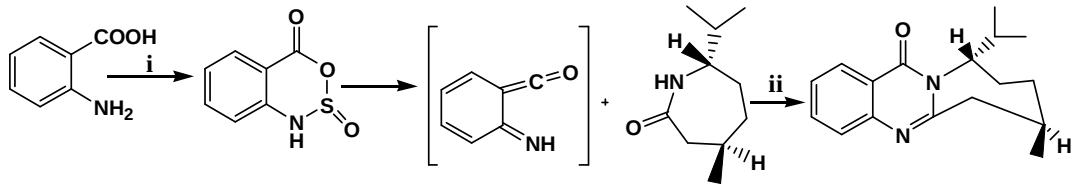
fused with anthranilic acid to get the desired novel compound of formula I (Scheme 2). Anthranilic acid is first converted into sulfonamideanhydride by treatment with thionyl chloride. The said sulfonamide anhydride is treated with menthol lactam as disclosed hereinabove and described in Scheme-I to yield novel compound 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one referred hereinabove and herein below as KLD.

Scheme-1



Reagents and conditions i) Jone's reagent, stirring, 0°C, ii) $NH_2OH.HCl$, NaOH, stirring, 2h iii) tosyl chloride, 5% NaOH, Stirring.

Scheme - 2



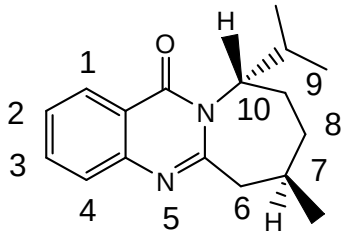
SOCl2, benzene, reflux, 6 h ii) Benzene, stirring, r.t, 12h

The hydrochloride of the analogue was prepared to make it water soluble but the spectroscopic behaviour of the hydrochloride tempted us to explore the molecule by performing COSY, HMQC and HMBC experiments since it clearly indicated the protonation of sp2 nitrogen rather than sp3 nitrogen.

STEREOCHEMISTRY

The three methyl doublets were observed as distinct signals in the proton N.M.R at δ 0.77 (isopropyl shielded methyl), δ 0.93 for methyl at C-7 and δ 1.14 (isopropyl non shielded methyl). The appearance of distinct doublets of the isopropyl methyl is attributed to the restricted rotation of isopropyl group because of methylene at C-9 and shielded doublet due to the methyl facing the pi bond shielding cone by quinazolone moiety. The 7 membered azipene ring takes quasi chair as favoured

conformation (visualised from the molecular models) with the result that isopropyl retains its α configuration with the C-10 H as β and lies in the plane of-C=O at C-12 and so is extremely deshielded due to carbonyl deshielding cone.

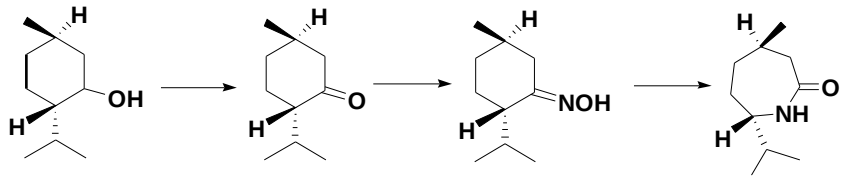


It is interesting to find that the hydrochloride of this molecule indicates the protonation of Sp^2 nitrogen (C=N) bond as the methylene at C-6 get deshielded further due to the influence of $-C=\overset{+}{N}H$ bond and even the methyl at C-7 gets deshielded to get merged with isopropyl methyl. It is also interesting to find that the protonated nitrogen influences the aromatic portion at C-4 which appears as a doublet downshielded to δ 8.22. This is confirmatory proof that Sp2 nitrogen is protonated rather than Sp3 as is directed by I.R. In the I.R spectrum, twin bands (1370 cm-1) were observed for the isopropyl group along with the amide carbonyl (1665 cm-1). The band shift from 1630 (C=N stretch) to 1670 due to the protonated nitrogen in case of hydrochloride was also observed. The detailed characterization of said novel molecule KLD and its hydrochloride is given below:

The proton NMR of the hydrochloride showed a downfield shift of the signals of the aromatic protons as compared to the parent molecule. The two protons of the methylene at C-6 behaved as non equivalent protons (doublet of doublets at δ 3.35 and 2.95 ppm) in the free base form as well as the hydrochloride form. The non equivalent methylene protons are more deshielded as compared to the parent base molecule (not in the salt form) with two multiplets at δ 4.20 and 3.40. However, a slightly shielded carbon (37.2 ppm, C-6) is observed in hydrochloride form as compared to the native form (42 ppm, C-6). The attachment of these non equivalent protons in the hydrochloride form to carbon at (or 3.7) ppm is confirmed by HSQC and HMBC. The signal for the protonated nitrogen in the hydrochloride form at 5 position shows a merged signal with the methine of the isopropyl group at δ 2.5 ppm. The attachment of the proton at C-10 to the methine proton of the isopropyl group is confirmed by COSY experiment. The comparative

deshielding of all the three methyls as well as the protons at C-8 and C-9 clearly demonstrates the effect of protonation on sp2 nitrogen. (See supplementary data)

In the first part of a general embodiment of the instant invention (4R, 7S)-7-isopropyl-4-methylazepan-2-one is synthesised as per scheme 1. Menthol of formula A is oxidized to menthone of formula B using an oxidising agent (optionally in the presence of a solvent), which undergoes oxime formation using an oxime source followed by Beckmann rearrangement. The nitrogen insertion can not be afforded by the conventional acid catalysed Beckmann rearrangement of menthone oxime of formula C rather; the tosylated oxime is exposed to dilute alkali for the nitrogen inserted lactam of formula D.



A **B** **C** D

The oxidising agent used for the purpose of oxidation of menthol to menthone is selected in such a way so that it does not have the adverse impact on the reaction. The oxidising agent is selected from the group comprising chromic acid, sodium dichromate, potassium dichromate, potassium permanganate and the like.

Tosylated oxime used for the purpose is prepared by any conventional method known in the art.

Alkali used for the purpose is selected in such a way so that it does not have ill effect over the reaction.

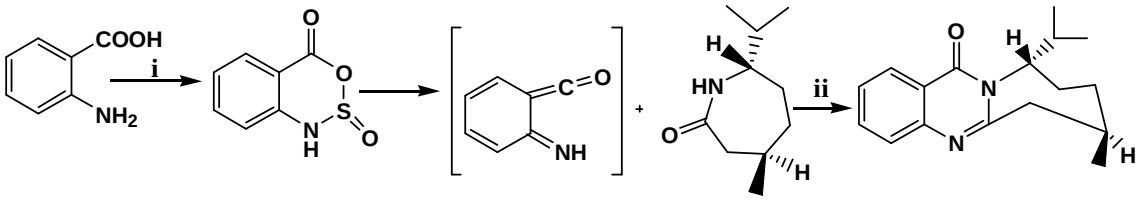
In the second part of a general embodiment Anthranilic acid of formula E is first converted into sulphonamide anhydride of formula F by treatment with thionyl chloride optionally in the presence of a solvent.

Solvent used for the purpose of preparing sulphonamide anhydride is selected in such a way so that it has no adverse impact on the said reaction. The solvent is selected from the group comprising aromatic hydrocarbon.

In the third part of the general embodiment of the invention the said sulfonamide anhydride of formula F is then treated with menthol lactam of formula D to yield said KLD, a novel compound 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one of formula I using a solvent at a suitable temperature.

Solvent used for the purpose of said reaction is selected in such a way so that it does not have any adverse impact on the reaction.

Conditions like temperature, stirring are chosen in such a way so that the reaction produces optimised yield.



E **F** **D** **I**

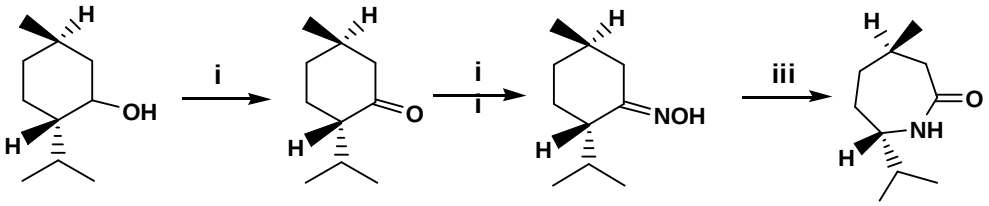
Terpenoidal azepine is condensed with anthranilic acid to get the desired analogue (Scheme 2).

A said analogue is then further converted into its corresponding pharmaceutically acceptable salts with the view of having improved solubility.

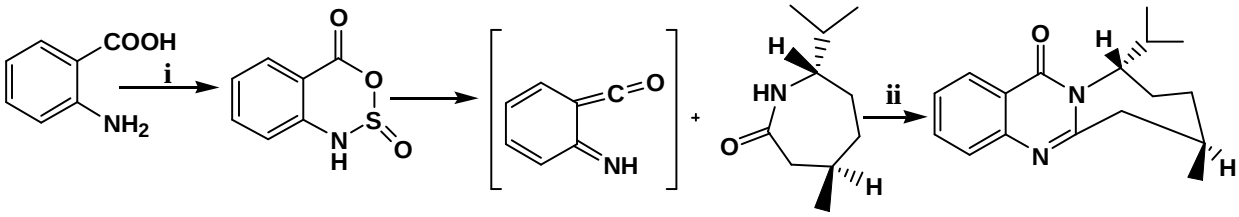
In a specific embodiment of the instant invention (4R,7S)-7-isopropyl-4-methylazepan-2-one is synthesised as per process described herein below. Menthol is oxidized to menthone by contacting with chromic acid and the said menthone undergoes oxime formation by contacting with NH_2OH followed by Beckmann rearrangement of the said oxime. The nitrogen insertion is not afforded by the conventional acid catalysed Beckmann rearrangement of said menthone oxime rather the corresponding tosylated oxime is treated with dilute alkali for the nitrogen inserted lactam. The said terpenoidal azepine is treated with anthranilic acid to get the novel compound of formula I as shown herein below.

Anthranilic acid is first converted into sulphonamide anhydride by treatment with thionyl chloride in anhydrous toluene. The said sulfonamide anhydride prepared is

then condensed with menthol lactam to yield novel 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one herein above and herein below referred as KLD.



Scheme 1 Reagents and conditions i) Jone's reagent, stirring, 0°C, ii) $NH_2OH.HCl$, NaOH, stirring, 2h iii) tosyl chloride, 5% NaOH, Stirring.



E **F** **D** **I**

Scheme 2 Reagents and conditions i) $SOCl_2$, benzene, reflux, 6 h ii) Benzene, stirring, room temperature, 12h

The analogue so formed is dissolved in anhydrous chloroform and HCl gas is passed in the solution using Kipps apparatus to obtain the hydrochloride of the desired analogue.

The synthesis of the compound under the investigation can be best understood by the following illustrative examples:

Synthesis of 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one (KLD)

13.7 g (0.1 mol) of Anthranilic acid was dissolved in dry toluene (200ml) in a 500 ml round bottom flask and 29.5 ml of thionyl chloride (0.25mol) was added to it. The reaction mixture was refluxed for 6 hours on a heating mantle under dry conditions to give sulfinylanthraniloyl chloride. After completion of the reaction excess of thionyl chloride and toluene were distilled off. Benzene (100 ml) was

again added to remove residual thionyl chloride. 16.9 g (0.1mol) of menthol lactam was dissolved in benzene in a 100 ml round bottom flask. The solution was added slowly to the sulfinylanthraniloyl chloride under cold conditions and kept in stirred condition for 12 h. Progress of the reaction was monitored on TLC (toluene : ethylacetate::65:35). The reaction mixture was basified with aq.NH_3 and extracted with chloroform. The chloroform layer was distilled under reduced pressure, which yielded crude product. The crude product was purified by column chromatography on neutral alumina using increasing %age of ethyl acetate in hexane to give **KLD.** The physical data of both the parent compound as well as its hydrochloride form is given below:

7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one (KLD)

Semisolid; I.R. (KBr, cm-1): 2958, 1665, 1630, 1370, 1230, 1140; 1H NMR: (CDCl3, 300 MHz, TMS = 0) : δ = 8.26 (dd, 1H, J=1.2 & 7.2 Hz), 7.13-7.27 (m, 2H) 7.42 (m, 1H), 5.40 (t, 1H, J =5.1 Hz), 3.35 (dd, 1H, J =3.3 and 14.4 Hz), 2.95 (dd, 1H, J =5.1 Hz and 14.4 Hz), 2.45 (m, 1H) 2.25 (m, 1H), 1.95-2.08(m, 4H), 1.14 (d, 3H, J =6.3 Hz), 0.93 (d, 3H, J =7.2 Hz), 0.77 (d, 3H, J =6.3 Hz).; 13C NMR (CDCl3, 75.4 MHzTMS = 0) : δ = 162.98, 156.78, 147.05, 133.95, 127.16, 126.45, 126.25, 119.9, 57.25, 42.40, 29.14, 28.92, 28.67, 24.26, 20.36, 18.97, 16.85.MS (ESI) M+: at m/z 270. Anal.Calcd for C17H22N2O : C, 75.52; H, 8.20; N, 10.36; O, 5.92. Found C, 75.67; H, 8.31; N, 10.21.

7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-onium chloride (KLD)

White coloured powder, m.p. 180 º CI.R. (KBr, cm-1): 3320, 2958, 1670, 1665, 1370, 1230, 1140; mp : 175°C, 1H NMR: (CDCl3, 500 MHz, TMS at δ=0 ppm) δ = 8.51 (1H, d, J =8.0 Hz), 8.29 (1H, d, J =8.0 Hz), 7.93 (t, J =7.5 Hz, 1H), 7.69 (d, J =7.5 Hz, 1H), 5.42 (t, J =5.00 Hz, 1H,), 4.20 (d, J =10.5 Hz, 1H), 3.40 (d, J =14.00 Hz, 1H) 2.55 (bs, 2H), 1.98 – 2.22 (m, 3H), 1.14 (d, J =6.5 Hz, 3H) , 1.11 (d, J =7.0 Hz, 3H), 0.82 (d, J =6.00 Hz, 3H).13C NMR (CDCl3, 125 MHz, TMS at δ=0 ppm) : δ = 162.81. 159.41, 136.96, 136.79, 129.68, 128.24, 120.92, 117.71, 60.53, 37.2, 29.69, 28.76, 28.26, 23.28, 20.26, 19.07, 16.79

PHARMACOLOGICAL EVALUATION:

Effect of the compound under the investigation on the cough responses in guinea pigs in citric acid model.

In citric acid induced cough model, a significant cough response was noted in vehicle control (VC) as evidenced by decreased cough latency and increased cough frequency in the graphical representation. Treatment with Cod.10, TAZQ.20, KLD.10 and KLD.20 produced significant reversal in cough latency and frequency, as compared to VC. The effect produced by KLD.10 & 20 is more significant as compared to standard drug: codeine 10 mg/kg in guinea pigs. Moreover, the effect produced by KLD 20 is also significant in comparison to TAZQ.20 (Figure 1).

VC stands for vehicle control (A control untreated animals)

KLD stands for compound of the formula I under the investigation

Cod stands for Codeine (A standard/ reference compound)

TAZQ is the compound of formula III (A test compound)

Here the meaning of cough latency is considered as reaction time or response time or the latent period.

Looking at the bar diagram involving cough latency; KLD10 and KLD20 meaning 10 and 20 mg/kg compound of the formula I under the investigation has shown gradually higher response over the known ones.

Effect of the compound under the investigation on the antigen induced airway hyper responsiveness in OVA induced asthma model in Guinea pigs:

Challenge with 0.5% ovalbumin aerosol caused significant ($p<0.05$) airway abnormalities in the form of severe cough strokes with the signs of dyspnea that took place 4 min after the onset of aerosolization in OVA control guinea pigs as reflected by decreased cough latency and increased cough frequency, as compared to SC. Treatment with KLD.10 and 20 mg/kg produced significant ($p<0.05$) increase in cough latency and decrease in cough frequency dose dependently, as

compared to OVA control. The effect produced by KLD.20 is more pronounced as compared to standard drug: AMN50, and another analogue TAZQ.20 (FIGURE 2).

Results: mean ± SEM; a: $p<0.05$ vs SC, b: $p<0.05$ vs OVA control, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

Effect of test drugs on antigen induced cell infiltration:

Sensitization with ovalbumin caused a significant ($p<0.05$) increase in cellular infiltrations: TLC, eosinophils, lymphocytes and neutrophils into BAL fluid in OVA control, as compared to SC. Pretreatment with KLD 10 & 20 mg/kg, TAZQ-20 and AMN50 significantly ($p<0.05$) decreased cellular infiltrations, as compared to OVA control. KLD20 shows a significant decrease in cellular infiltrations except neutrophils, as compared to AMN50 (FIGURE 3).

Effect of test drugs on lipid peroxidation (TBARS):

Lipid peroxidation as assessed by TBARS level was significantly ($p<0.05$) higher in OVA sensitized control animals (0.480 ± 0.107 and 4.35 ± 0.209), as compared to SC (0.034 ± 0.004 and 0.621 ± 0.003). The administration of KLD 10 and 20 mg/kg showed significant ($p<0.05$) reduction in TBARS level (0.239 ± 0.13 and 1.09 ± 0.039), as compared to OVA control. This effect of KLD.20 was significant as compared to aminophylline 50 mg/kg and TAZQ 20 mg/kg in BAL and lung tissue respectively (FIGURE 4).

Effect of test drugs on reduced glutathione (GSH):

Ovalbumin sensitization caused significant ($p<0.05$) decrease in antioxidant: GSH level in BAL and lung tissue of OVA control, as compared to SC. The level of reduced GSH was significantly ($p<0.05$) restored on treatment with KLD 10 and 20 mg/kg, AMN50 and TAZQ20, in comparison to OVA control. The effect produced by KLD20 was significant as compared to AMN50 and TAZQ20 (FIGURE 5).

Effect of test drugs on nitrite/nitrate level:

A challenge with OVA sensitization to guinea pigs resulted a significant ($p<0.05$) increase in nitrite/nitrate level as a marker of NOS activity in OVA control animals, as compared to SC. Pretreatment with KLD 10 and 20 mg/kg produced a significant ($p<0.05$) decrease in NO level in BAL and lung tissue (48.277 ± 9.57 µM, 81.777 ± 1.293 µM), as compared to OVA control. The effect produced by KLD20 was more significant as compared to AMN50 (FIGURE 6).

Effect of test drugs on lung tissue histology:

Histological examination of lung tissue from non-sensitized (NC) showed normal lung histological features (Figure 7- A). In contrast, sections of lung tissue from OVA contol guinea pigs exhibited airway inflammation, infiltration of eosinophils, lymphocytes and sub mucosal edema of the lungs, broncho-constriction shown as lumen plugging by mucus and cells (Figure 7 - B). Treatment with KLD 10 and 20mg/kg and TAZQ 20mg/kg prevented the tissue edema, epithelial cell hypertrophy, infiltration of inflammatory cell and airway lumen plugging thereby decreasing inflammation and bronco constriction which leads to normal lumen size (FIGURE 7 - C, 7- D and 7- E).

Ovalbumin Induced Broncho-constriction in Isolated Bronchial Smooth Muscle from Guinea pigs: KLD in 10^{-3} M and 10^{-2} M concentrations inhibited the tracheal contractions induced by OVA and significantly shifted the curves to the right in a dose-dependent manner because 10^{-2} M KLD was significantly more effective than 10^{-3} M. All EC_{50} values of the C-R curves are presented in Table 1 (FIGURE 8).

The table represents the EC_{50} shift values for ovalbumin at various concentrations of the test drugs. The graphed diagram shows that ovalbumine induced contraction was significantly inhibited in the presence of test drugs. The shift of ovalbumin dose response curve towards right values it can be justified that KLD has 5 to 6 times potent bronchodilatory activity when compared to aminophylline on dose basis.

COMPOUNDS	EC_{50} SHIFT VALUE (µM)
Control	0.37
$KLD10^{-4}M$	0.7
$KLD10^{-3}M$	5.35
$KLD10^{-2}M$	15.3
$AMN10^{-3}M$	1.1
$TAZQ10^{-2}M$	6.48

Table 1: Effect of test drugs in ovalbumin induced *In-vitro* tracheal contraction of asthmatic guinea pigs.

ANTI-HISTAMINIC ACTIVITY:

The CRC showed linear concentration response relationship with increasing concentration of histamine within the concentration range of 0.029 to 1.19 mM. Aminophylline at the concentration of 0.39 mM inhibited the contraction response by 49%. 28.84 % contraction response was observed with the incubation of tracheal chain with 0.38 mM TAZQ. KLD at the concentration of 0.12 mM was found to significantly antagonize the histamine induced broncho constriction by 61.97 % suggesting a potential anti-histaminic bronchodilatory activity. (Table 2)

Table 2: Bronchial relaxation against histamine induced bronchial contraction; results expressed as mean ± SEM (n=3)

Drug Concentration	**(% Tracheal Relaxation)**
KLD (0.12mM)	38.03 ± 2.14%
TAZQ (0.39mM)	71.16 ± 1.13 %
AMN (0.38mM)	51 ± 0.93%

Thus from the entire subject matter studied and mentioned herein above present investigation demonstrated the protective effects of KLD, a synthetic analogue of Vasicine against OVA induced asthma and citric acid induced cough in experimental animals and its comparison with another analogue of vasicine: TAZQ and standard medication available today.

Respiratory cough and asthmatic conditions is the chronic lung diseases typically associated with airway obstruction, chronic inflammation, oxidative stress and mucus production (Jeffery,1992). Sensitization of Guinea pigs with ovalbumin produced allergic reactions which are assumed to be the antigen induced pulmonary asthma-like conditions and this was supported by increase deosinophilic accumulation in bronchial tissue and BAL fluid associated with chronic airway inflammation. KLD has been found to be a potent bronchodilator, as observed in present study *in-vitro*, accompanied with notable antioxidant and anti-inflammatory activities at 10 and 20 mg/kg showing significant efficacy in ameliorating respiratory hyper-reactivity, airway eosinophil accumulation and bronchial inflammation induced by an exposure to ovalbumin aerosol in sensitized guinea pigs.

The KLD produces abrogated release of histamine (Johri and Zutshi*et al*.,2000) which may result marked reduction in cough frequency with improvement in resistance to airway abnormalities in present study. The asthma-like symptoms in antigen sensitized guinea pigs prevented by treatment with KLD and this may be due to the induced inhibition of release of histamine and broncho constriction. The airway hyper responsiveness due to inhaled antigen was almost abolished withthe pre-treatment of KLD. Eosinophilic inflammation is known to be a hallmark of bronchial asthma and several studies had reported a significant eosinophilia in BAL fluid of asthmatic patients (Bousquet et al., 1990; DcMonchy et al., 1985) as also observed in sensitized animals with increased eosinophils in lung and BAL fluid, as compared to normal healthy subjects. The present findings showed that the administration of KLD was significantly reduced eosinophil accumulation in the sensitized guinea pigs lungs and BAL fluid and decrease in eosinophilic infiltration indicating its anti-inflammatory effect.
Oxidative stress plays an important role in the pathophysiology of asthma (Barnes,1990) leading to detrimental events like airway dysfunction including

airway smooth muscle contraction, induction of airway hyper-responsiveness (Katsumata et al., 1990), mucus hyper-secretion (Adler et al., 1990), epithelial shedding (Phipps et al., 1986) and vascular exudation (Doelman and Bast, 1990) as evidenced by significant lipid peroxidation reactions and decrease in GSH levels in BAL fluid and lung tissue, as compared to normal control in present study. KLD20 mg/kg significantly prevented the lipid peroxidation reactions in both BAL fluid and lung tissue homogenates. Glutathione is the most abundant intracellular thiol-based antioxidant found in lung cells (Antonicelli et al., 2002). BAL fluid is reported to contain a 100fold concentration ofglutathione compared to blood (Cantinet al., 1987). Decreased concentration of GSH in lung may signify the inflammation and hyper-responsiveness (Rahman and Macnee, 2000) as also observed in sensitized control group with reduced GSH level, in present study. The present study showed that KLD improved the level of GSH in both BAL fluid and lung tissue homogenates. This may signify the ability of KLD as radical scavenger. Various tissues and cells like airway epithelium, vascular endothelium, neurons and immune cells (Ricciardo-lo, 2003) are known to produce and release NO into the circulation, alveoli, and surrounding tissues during allergic asthmatic conditions. In present study, the level of NO in BAL fluid and in lung tissue was significantly elevated in pre-sensitized OVA challenged guinea pigs, as compared to normal control. A significant decrease in NO was observed in animals treated with KLD. KLD at low dose showed a substantial reduction in asthma-like reactions in ovalbumin sensitized asthmatic guinea pigs. This reveals that the compound of the formula under the investigation possesses significant potential to ameliorate asthma, broncho constriction and provide anti-tussive effect in comparison to both the standards employed as well as TAZQ.

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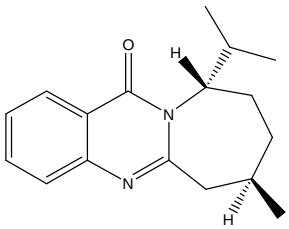
CLAIMS:

We claim,

1. A compound of formula I or a pharmaceutically acceptable salts thereof.

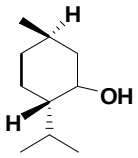


Formula I

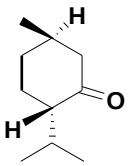
2. A compound of claim 1 with multiple therapeutic activities characterized by bronchodilatory, anti-allergic and anti-tussive effect.

3. A process for the preparation of the compound of formula I comprises two parts; first part comprising:

i) oxidation of menthol of formula A resulting into the formation of menthanone of formula B

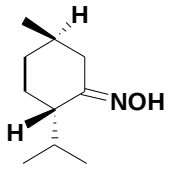


A



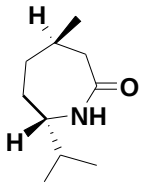
B

ii) conversion of menthone of formula B obtained from the step i) into oxime of formula C



C

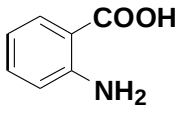
iii) Beckmann rearrangement of menthone oxime of formula C resulting into formation of menthol lactum of formula D



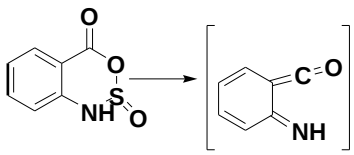
D

Second part of the process comprising:

i) conversion of Anthranilic acid of formula E into sulphonamide of formula F

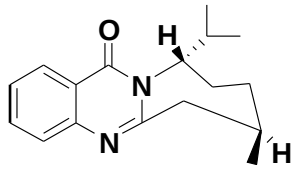


E



F

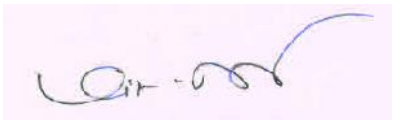
ii) contacting sulphonamide of formula F with menthol lactum of formula D resulting into formula I



FORMULA I

iii) converting the compound of the formula I into pharmaceutically acceptable salts.

4. The use of the compound of claim 1 for the preparation of medicament.



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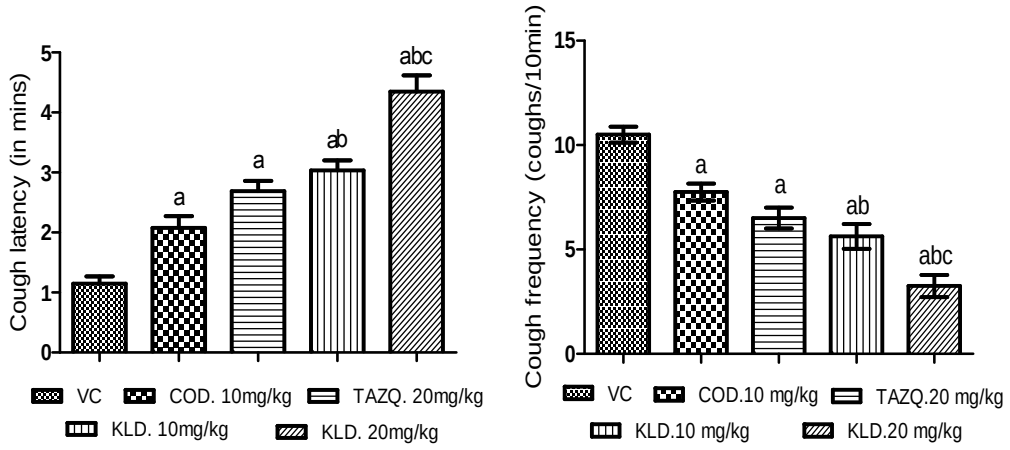

Figure 1: Effect of test drugs on cough latency and frequency induced by citric acid: Results: mean ± SEM; a: $p<0.05$ vs VC, b: $p<0.05$ codeine 10 mg/kg, c: $p<0.05$ vs TAZQ 20 mg/kg.

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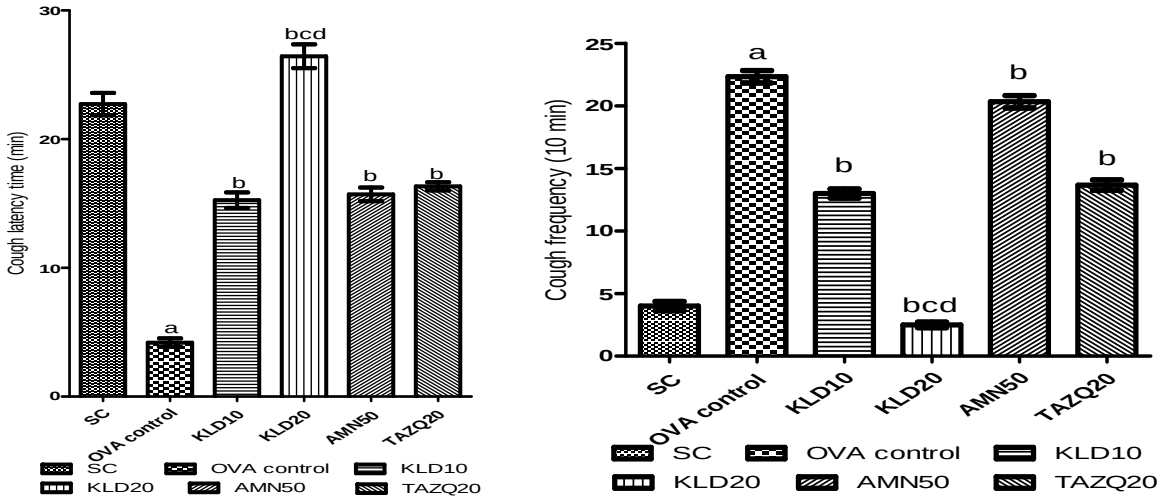

FIGURE 2: Effect of test drugs on antigen induced airway hyper responsiveness:

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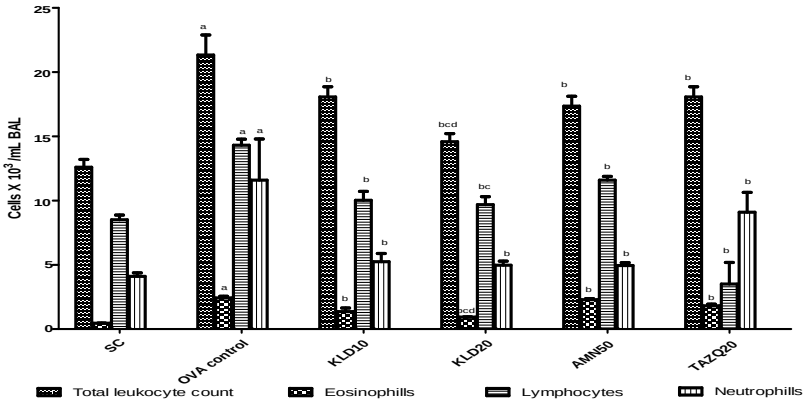

FIGURE 3: Effect of test drugs on antigen induced cell infiltration: Results: mean ± SEM; a: $p<0.05$ vs SC, b: $p<0.05$ vs OVA control, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

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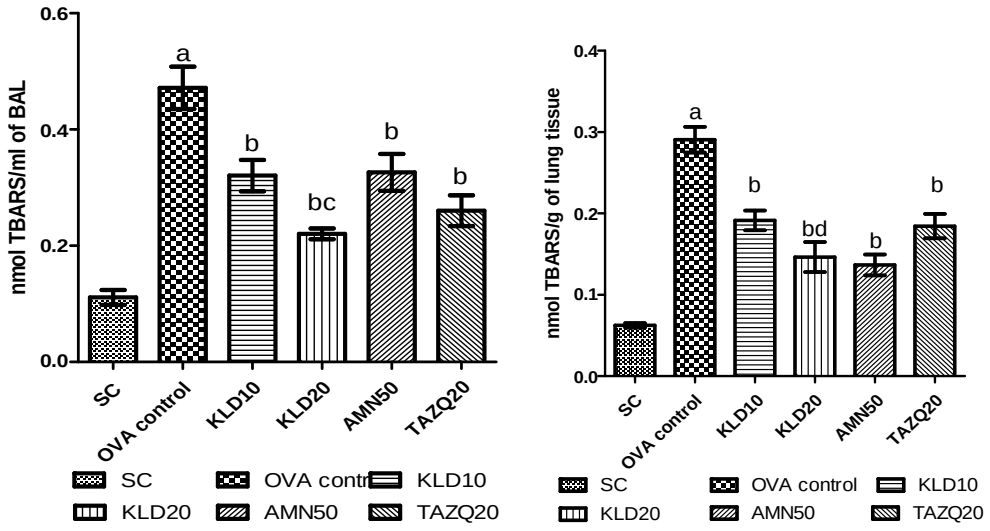

FIGURE 4: Effect of test drugs on lipid peroxidation (TBARS): Results: mean ± SEM; a: $p<0.05$ vs SC, b: $p<0.05$ vs OVA control, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

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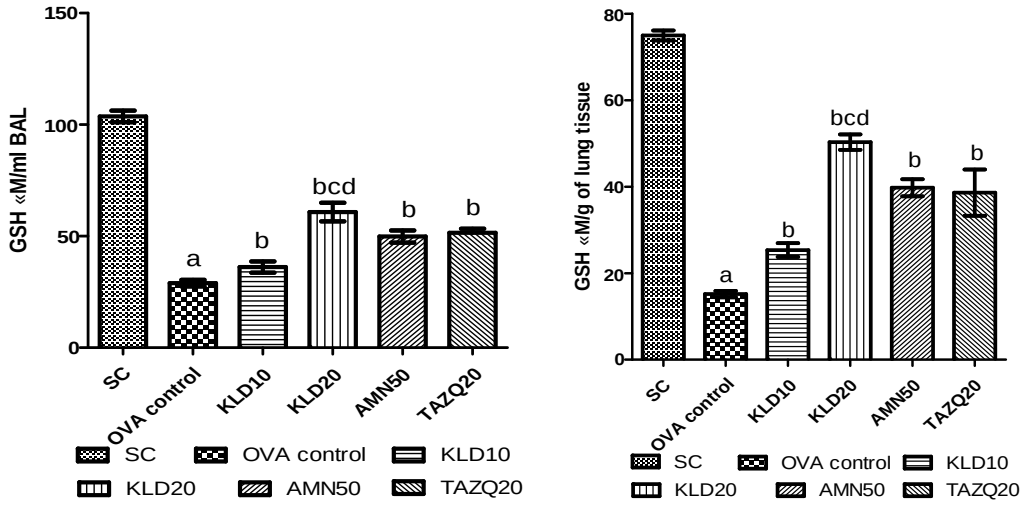

FIGURE 5: Effect of test drugs on reduced glutathione (GSH): Results: mean ± SEM; a: $p<0.05$ vs SC, b: $p<0.05$ vs OVA control, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

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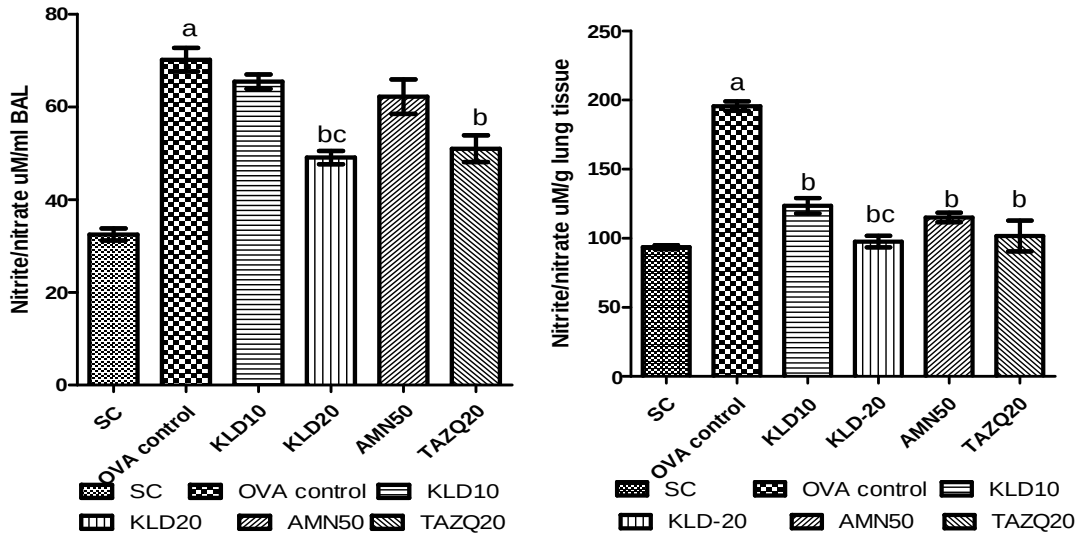

FIGURE 6: Effect of test drugs on nitrite/nitrate level: Results: mean ± SEM; a: $p<0.05$ vs NC, b: $p<0.05$ vs SC, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

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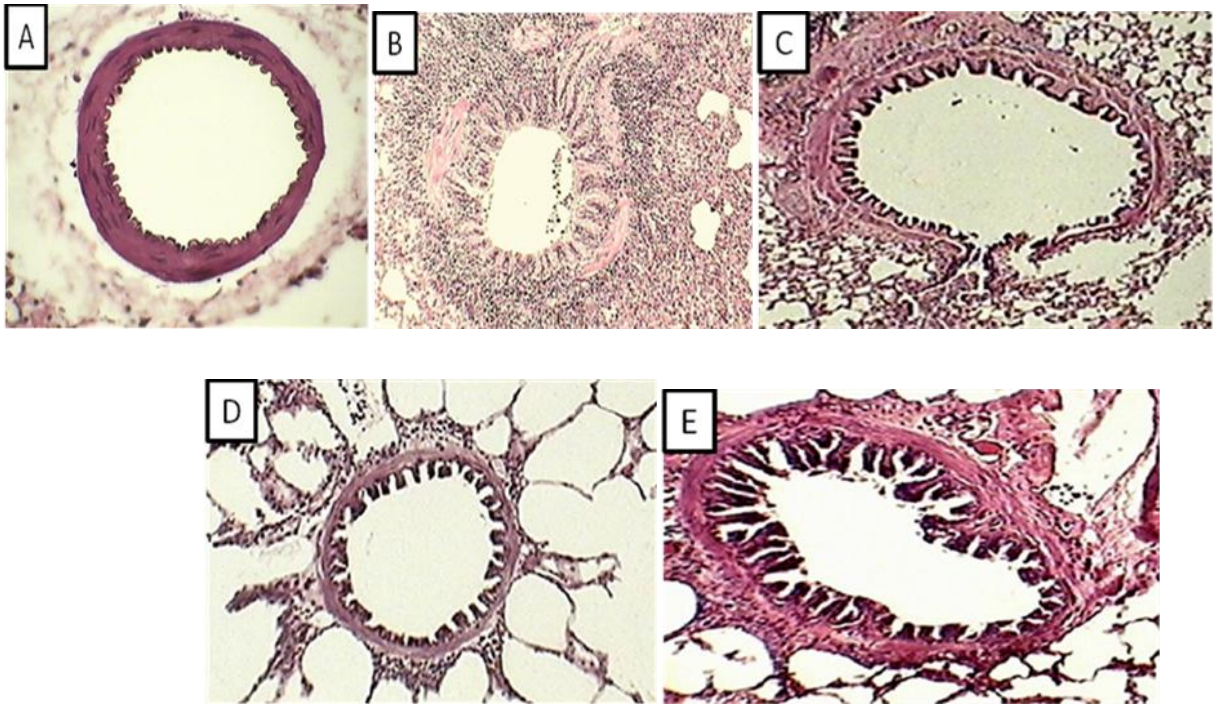

FIGURE 7: Effect of test drugs on nitrite/nitrate level: Results: mean ± SEM; a: $p<0.05$ vs NC, b: $p<0.05$ vs SC, c: $p<0.05$ vs aminophylline 50 mg/kg, d: $p<0.05$ vs TAZQ 20 mg/kg.

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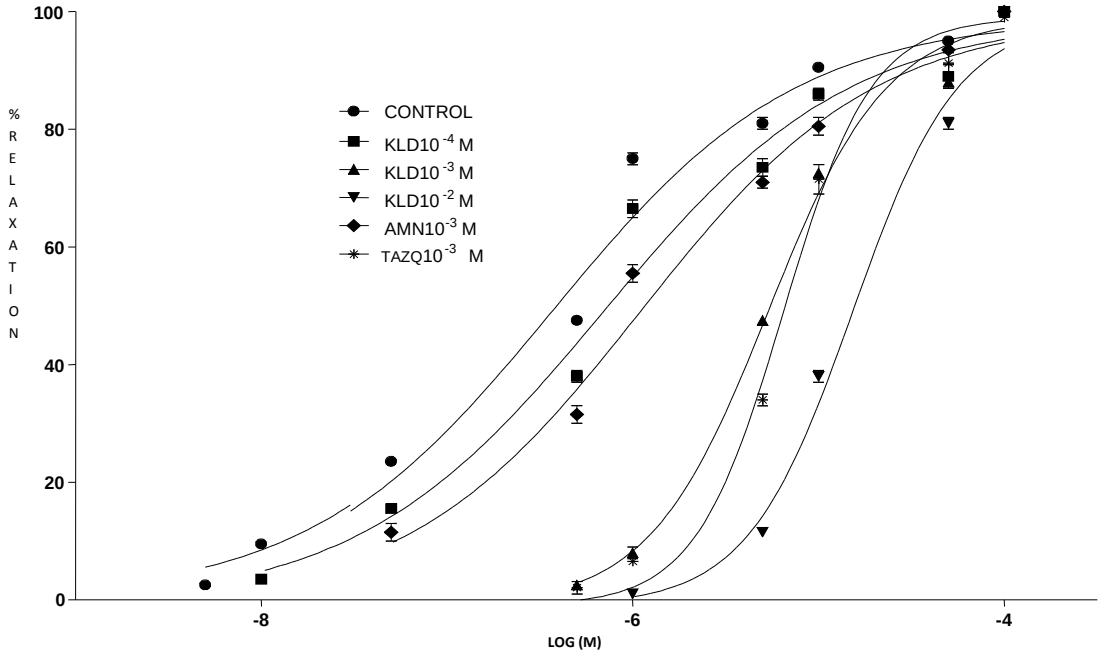

FIGURE 8: Effect of KLD on the concentration-response curves of ovalbumin (OVA). Tensions are expressed as percentages of the peak contractile response, which is taken as 100%.

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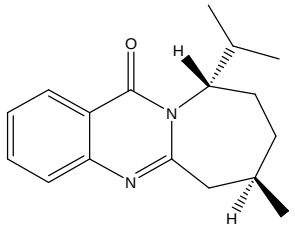
ABSTRACT:

The present invention relates to the treatment of asthma. The present invention comprises design, synthesis and biological evaluation of a novel terpenoid based analogue of vasicine of formula I chemically known as 7,8,9,10-tetrahydro-7-methyl-10-(1-methylethyl)azepino[2,1-b]quinazolin-12(6H)-one which possesses multiple therapeutic activities viz bronchodilator, anti-allergic activities and anti-tussive potential.



Formula I