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SALBUTAMOL IN ASTHMA MANAGEMENT: A COMPARATIVE STUDY WITH BRONCHODIALATORS

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ABSTRACT :

A chronic inflammatory airway illness that affects more than 300 million individuals worldwide, asthma is characterised by hyperresponsiveness and reversible airflow restriction. Its origin is multifaceted, and lung function tests and clinical history are used to make the diagnosis. Short-acting β_2-agonists like salbutamol are important quick-relief drugs because of their great efficacy and quick onset (within 5 minutes). It functions by raising cAMP, triggering bronchodilation, and activating β-receptors. β_2-selectivity is guaranteed structurally by its phenyl ethanolamine core. It comes in a variety of forms (MDI, nebuliser, pills) and is metabolised by the liver and eliminated by the kidneys. Its safety, receptor selectivity, and clinical efficacy—particularly in raising FEV_1 and lowering asthma symptoms—have all been demonstrated by studies.

INTRODUCTION

The hallmark of bronchial asthma is the trachea's bronchial smooth muscle's hyperresponsiveness to various stimuli, which causes the air passages to narrow. This is frequently accompanied by increased secretion, mucosal oedema, and mucus clogging. Dyspnoea, wheezing, coughing, and maybe activity limitation are among the symptoms. It is now known that asthma is largely an inflammatory disease, with inflammation underlying hyperreactivity. Many adult patients and a larger proportion of paediatric patients have an allergic basis.

TYPES

1.Based on the stimulus source, there are three types of asthma: extrinsic, intrinsic, and mixed.

2. According to the length of action, i. Acute Asthma ii. Chronic Asthma

3. By severity

One type of asthma is mild intermittent, while the other is mild persistent.

a) Moderately chronic asthma

b) Prolonged, severe asthma

Ethiology:

A complicated web of poorly understood genetic and environmental factors combine to produce asthma. These affect its intensity as well as how well it responds to therapy. Recent increases in asthma prevalence are thought to be caused by shifting living conditions. Environmental factors are more likely to cause asthma that develops after the age of twelve, but genetic factors are more likely to cause asthma that begins before then.

- Genetic
- Respiratory infections
- Air pollutions
- Smoking

Pathophysiology:

1. Persistent inflammation
2. Hyperresponsiveness of the airways

PERMANENT INDLAMMATION

- It mostly affects the respiratory mucosa in the bronchi, extending from the trachea to the terminal bronchioles.

- The activation of T-helper-2 (Th-2) lymphocytes and eosinophil mass cell infiltration.
- Interleukins 4, 5, and 13 in the Th-2 response.
- ➢ Mass cells are activated by IL-13, IL-4, and IL-9.
- IL-13 promotes the formation of mucus.
- IL-5 promotes the generation of eosinophils.

AIR-WAYHYPERRESPONSIVENESS

The excessive bronchi constriction response to multiple inhaled triggers that would have effects on normal air-ways.

NFLAMMATORY CELL	MEDIATORS	EFFECTS
Mass cells Eosinophils Th-2 cells Basophils Neutrophils Platelets	Histamine Leukotrienes Proteinoids PAF Adenosine Endothelin NO Cytokines Chemokines Growth factor	Bronchospasm Plasma exudation Mucus secretion Structural changes
STRUCTURAL CELLS		
Epithelial cells Smooth muscle cells Endothelial cells Fibroblast Nerves		

Fig 1 Air way hyper responsiveness

MECHANISM

After that, the IgE antibodies attach to basophils and mast cells with high affinity. The mast cells gradually de-granulate after releasing cytokines in response to an inhaled contaminant or risk factor. Mast cells release leukotrienes, prostaglandins, and histamine. The smooth muscle is then contracted by these cells, which tightens the airways. The lymphocytes are essential because they generate GM-CSF and a number of interleukins (IL-4, IL-5, and IL-13) that facilitate cell-to-cell contact and maintain inflammation. Eosinophils and basophils are aided in their survival by IL-3 and IL-5. IL-13 plays a role in hyperplasia, fibrosis, and remodelling. The late phase, which takes place over the course of the following few hours, is characterised by the localisation of eosinophils, basophils, neutrophils, and helper and memory T-cells to the lungs, which results in inflammation and bronchoconstriction.

ASTHMA DIAGNOSIS

- Physical examination
- Spirometry
- Peak Flow Measurement
- Pulmonary Function Tests (PFTs)
- Bronchoprovocation Tests
- Exhaled Nitric Oxide Test
- Allergy Testing

TREATMENT

- Non-Medical Mediation:
- Do not smoke.
- Anticipating presentation to allergens, such as clean, fog, dust, etc.
- Control clear of amazingly chilly temperatures.
- Oxygen treatment ought to be managed in an crisis.
- Due to intense extreme asthma, the understanding ought to be conceded to the clinic.

2.PHARMACOLOGICAL TREATMENT

CLASSIFICATION

1. Bronchodilators
a) Beta sympathomimetics
Eg: Salbutamol, Terbutaline, Salmeterol
b) Methylxanthines
Eg: Theophylline, Aminophylline, Hydroxyethyl theophylline
c) Anticholinergic
Eg: Ipratropium bromide, Tiotropium bromide
2. Leukotriene adversary
Eg: Montelukast, Zafirlukast
3. Pole cell stabilizers
Eg: Sodium cromoglycate, Ketotifen
4. Corticosteroids
a) Systemic
Eg: Hydrocortisone, Prednisolone
b) Inhalational
Eg: Beclomethasone, Fluticasone
5. Anti-Ig E antibody
Eg: Omalizumab

SALBUTAMOL

When inhaled, salbutamol binds to beta-2 adrenergic receptors on the smooth muscle of the bronchi, making it a selective beta-2 agonist. This results in an increase in the intracellular concentration of cyclic AMP and the activation of adenyl cyclase. This results in a decrease in the concentration of ionised calcium, which relaxes the smooth muscle of the bronchi, reduces vascular permeability, and prevents the release of spasmogens by mast cells and the activation of eosinophils. Salbutamol has a half-life of three to six hours and produces a detectable reduction in airway resistance in five to fifteen minutes when inhaled.

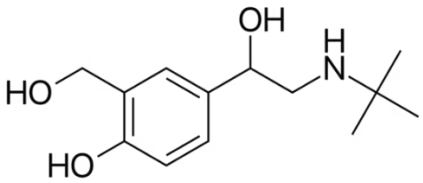


Fig. 2 structure of salbutamol

SYNTHESIS OF SALBUTAMOL

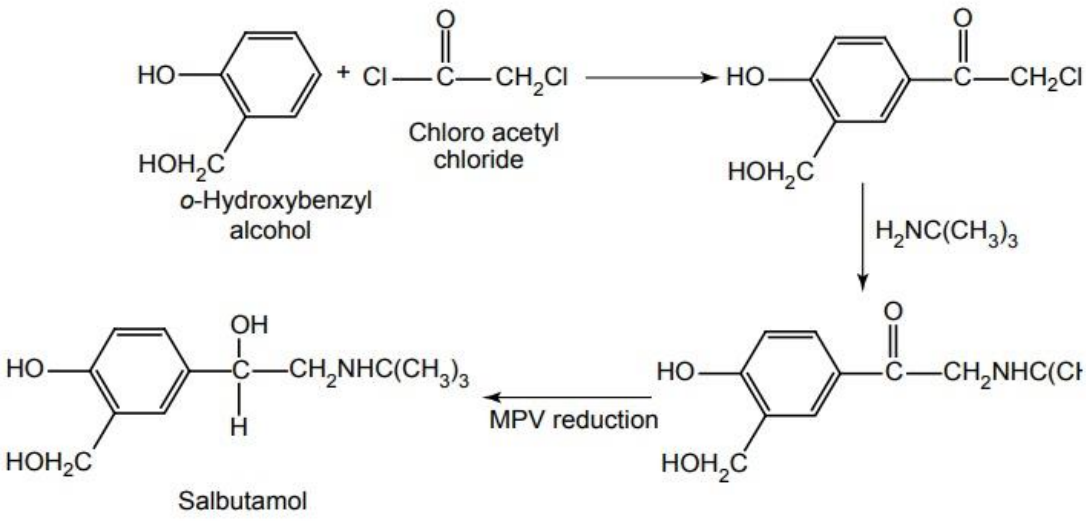


Fig. 3 synthesis of salbutamol

STRUCTURAL ACTIVITY RELATIONSHIP

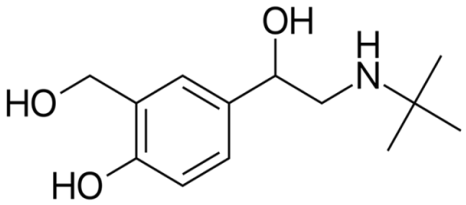


Fig.4 SAR of salbutamol

- **Aromatic Ring:** An essential component of the structure, the benzene ring offers the framework for the attachment of functional groups that engage in receptor-interaction.
- **Hydroxyl Groups:** It is essential that the phenyl ring have two hydroxyl groups (-OH) at the para and meta locations. hence necessary for the binding and activation of receptors.
- **Tertiary Butyl Group:** The selectivity of beta-2 receptors is significantly impacted by the presence of a large tert-butyl group at the nitrogen atom. This group's mass aids in distinguishing between beta-1 and beta-2 receptors.
- **Ethanolamine Side Chain:** The hydroxyl group on an ethyl chain that is joined to an amine is part of the ethanolamine side chain, which is essential for binding to the receptor. The pharmacological characteristics of the medication can be greatly impacted by changes made to this portion of the molecule.

Chemical and physical characteristics

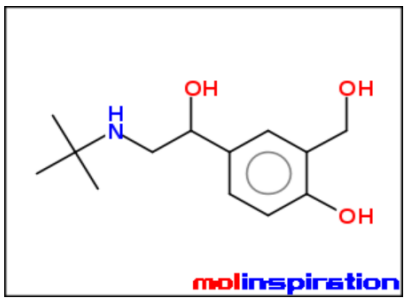
MOLINSPIRATION

In addition to high-quality molecule depiction and cheminformatics software tools that facilitate molecule manipulation and processing, Molinspiration provides molecular database tools that facilitate substructures and similarity searches, normalizes molecules, generates tautomer's, fragments molecules, and computes various molecular properties needed for QSAR, molecular modelling, and drug design.

PHYSICOCHEMICAL PROPERTY	VALUE
Mi logP	1.35
Number of atoms	17
Molecular weight	239.31
Number of hydrogen bond donors	4
Number of hydrogen bond acceptors	4

Fig. 5 Physicochemical properties of salbutamol

miSMILES: CC(C)(C)NCC(O)c1ccc(O)c(CO)c1
Albuterol



Molinspiration property engine v2022.08

miLogP	1.35
TPSA	72.71
natoms	17
MW	239.31
nON	4
nOHNH	4
nviolations	0
nrotb	5
volume	237.12

Get data as text (for copy / paste).

Get 3D geometry BETA

Fig. 6 Molinspiration of salbutamol

LIPINSKI'S RULE OF FIVE

A rule of thumb for assessing drug likeness or figuring out whether a chemical compound with a particular pharmacological or biological activity has chemical and physical characteristics that would likely make it an oral active drug in humans is Lipinski's rule of five, also referred to as Pfizer's rule of five or just the rule of five (RO5). The rule outlines molecular characteristics such as absorption, distribution, metabolism, and excretion ("ADME") that are crucial to a drug's pharmacokinetics in the human body. Nevertheless, a compound's pharmacological activity cannot be predicted by the rule. According to Lipinski's rule, an oral medication often doesn't violate more than one of the following requirements:
Ten hydrogen bond acceptors (all nitrogen or oxygen atoms) and no more than five hydrogen bond providers (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds) are allowed.
A mass of less than 500 Daltons for the molecules
A computed log P (octanol-water partition coefficient) of no more than 5. Salbutamol is seen as complying with Lipinski's Rule of Five since it satisfies all of the aforementioned requirements.

ADMET Prediction:

Swiss ADME makes it possible to predict the pharmacokinetic characteristics and druglike nature of one or more small compounds in addition to computing physicochemical descriptors. The Swiss ADME software was used to evaluate salbutamol's absorption, distribution, metabolism, and excretion.

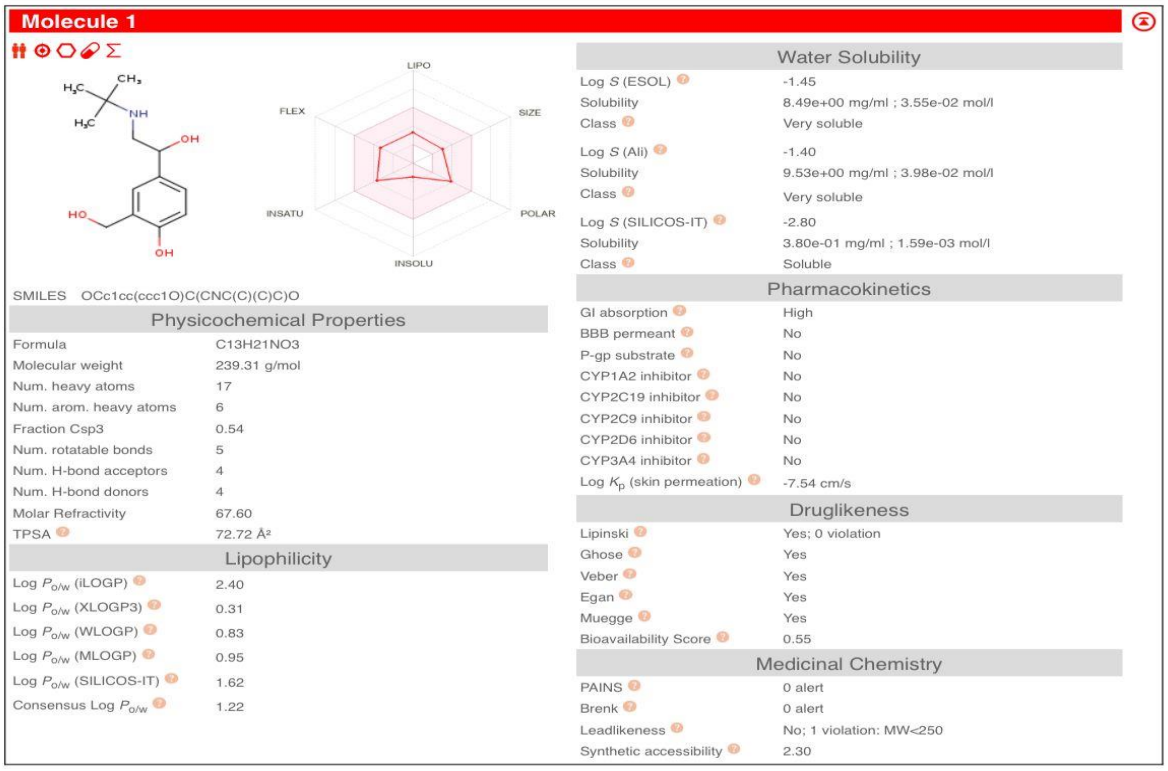


Fig. 7 Swiss ADME of salbutamol

To ascertain bioavailability, use radar.

Radar's bioavailability is displayed for a fast comparison of similar drugs. The six physicochemical properties that are considered include size, polarity, solubility, flexibility, saturation, and lipophilicity. On each axis, a pink zone represented the physicochemical range that the descriptors had indicated. SwissADME was used to analyse the physicochemical characteristics of salbutamol, and the results showed that;

PHYSICOCHEMICAL PROPERTY	VALUE
Molecular weight	225.28g/mol
Number of heavy atoms	16
Number of aromatic heavy atoms	6
Number of hydrogen bond donors	4
Number of hydrogen bond acceptors	4

Fig. 8 Physicochemical properties

Lipophilia

The partition coefficient (log Po/w) between n-octanol and water is the conventional indicator of lipophilicity. This physicochemical characteristic has its own section in SwissADME since it is so important for pharmacokinetics drug development. SwissADME offers the following five freely approximation predictable models: XLOGP3, WLOGP, MLOGP, SILICOS-IT, and Ilogp. SwissADME, a method that evaluates solubility, transport across membranes, and penetration of cell barriers, was used to examine the lipophilicity of salbutamol.

LIPOPHILICITY	VALUE
Log P o/w (ILOGP)	2.33
Log P o/w (XLOGP3)	0.12
Log P o/w (WLOGP)	0.44
Log P o/w (MLOGP)	0.67
Log P o/w (SILICOS-IT)	1.39
Consensus Log Po/w	0.99

Fig. 9 lipophilicity of salbutamol

Many tasks involved in the production of medications, such as formulation and handling, are made easier by water-soluble molecules. To offer an adequate amount of the active component in the small volume of such a pharmaceutical dosage, a drug intended for parenteral administration must also have a high-water solubility. SwissADME uses two topological methods to predict water solubility. The ESOL model36 is used in the first one, while SwissADME is used in the second one, which was modified from Ali et al., to examine salbutamol's water solubility.

Pharmacokinetics

To understand how chemicals interact with cytochromes P450 (CYP), pharmacokinetics is crucial. Through metabolic biotransformation, this superfamily of isoenzymes plays a crucial role in drug clearance. SwissADME was used to investigate the pharmacokinetics of salbutamol. According to research, salbutamol does not affect CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. Due to the drug's or its metabolites' decreased clearance and buildup, the medication does not have any pharmacokinetics-related drug-drug interactions that could cause toxic or other undesirable side effects.

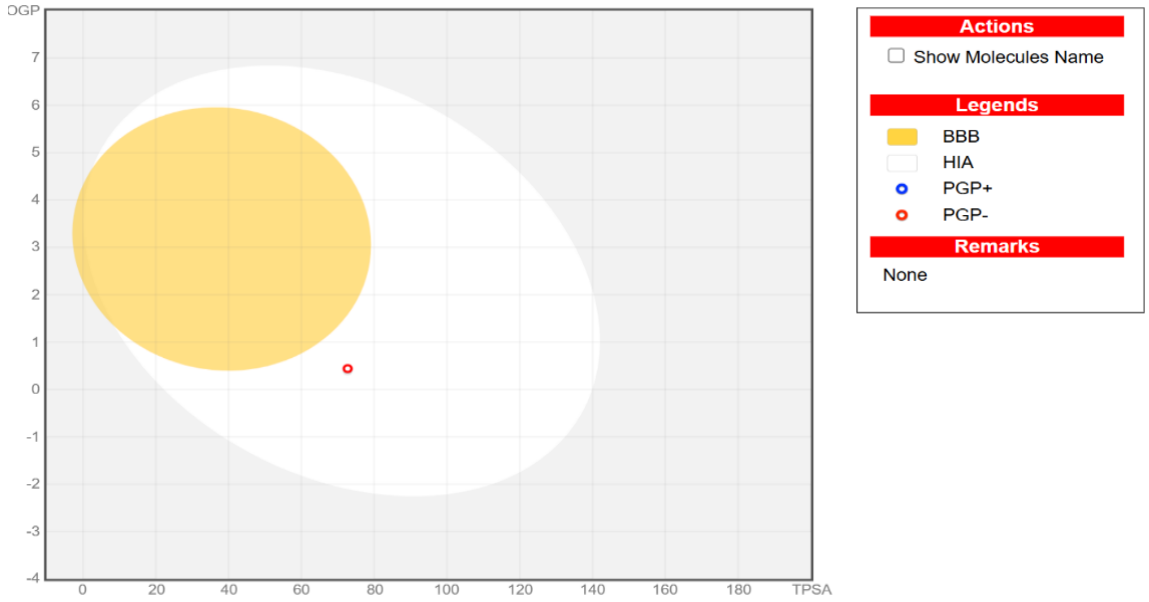


Fig. 10 Pharmacokinetics of salbutamol

The cooked egg allows for an intuitive evaluation of passive gastrointestinal absorption and brain penetration based on the placement of the molecules in the WLOGP-versus-TPSA reference. Moreover, the dots are coloured blue if P-gp is expected to actively efflux them (PGP+) and red if they are considered to be a non-substrate of P-gp (PGP−). The presence of salbutamol in boiled egg whites suggests that it will probably be passively absorbed by the digestive system. Salbutamol is not effluxed by P-gp (PGP+, red dot).

Mechanism of Action

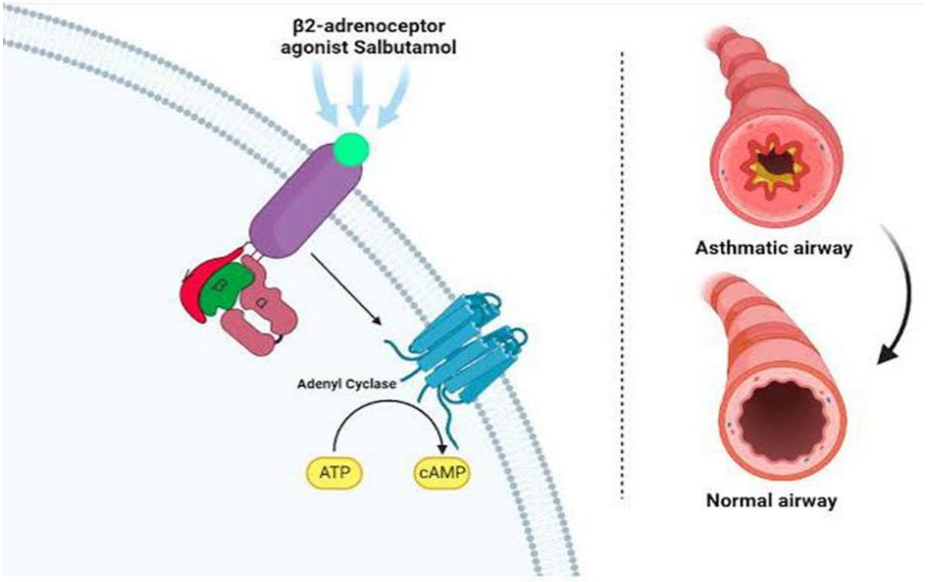


Fig. 11 Mechanism of action of salbutamol

Cyclic AMP levels rise intracellularly when adenyl cyclase is activated by beta2-adrenergic receptors on the smooth muscle of the airways. As a result of this rise in cyclic AMP, protein kinase A is activated, preventing myosin from being phosphorylated and lowering intracellular ionic calcium levels, which leads to relaxation. The smooth muscles that run from the trachea to the terminal bronchioles are relaxed by salbutamol.

ASSAY OF SALBUTAMOL PROCEDURE:

Procedure for Standardization: Standardize the 0.1N perchloric acid using standard solution of potassium hydrogen phthalate (0.1N) and crystal violet indicator. Procedure for Assay: Weigh accurately about 0.9g of salbutamol sulphate and add dissolve in 10ml of glacial acetic acid. Add few drops of crystal violet and titrate with 0.1N perchloric acid. Each ml of 0.1N perchloric acid is equivalent to 0.05767g of salbutamol sulphate.

BETA SYMPATHOMIMETIC COMPARISON STUDY

STRUTURAL ANALYSIS

Beta-2 adrenergic agonists, such as salbutamol, terbutaline, and salmeterol, are frequently used to treat asthma. They cause bronchodilation by activating beta-2 receptors in the airway's smooth muscle. Their structural variations, however, affect their onset, duration of action, and therapeutic applications. The three are compared structurally here:

Albuterol, or SALBUTAMOL

• Synthetic: Salbutamol is indeed a synthetic substance.

• Chemical Structure: Salbutamol consists of a secondary amine group joined to a carbon chain and a hydroxy group (-OH) at the benzene ring.

Salbutamol is a short-acting beta-2 agonist, according to pharmacokinetics. It starts working within five minutes, and its benefits persist for four to six hours.

• Usage: Mostly used during an asthma attack or to provide

TERBUTALINE

• Synthetic: Terbutaline is synthetic as well.

• Chemical Structure: Although the side chain of terbutaline is somewhat altered, it has a structure with salbutamol. On the side chain connected to the beta-amine group, there is a tert-butyl group rather than a methyl group.

• Pharmacokinetics: Although it acts for around six hours longer than salbutamol, terbutaline is also a short-acting beta-2 agonist.

• Usage: Terbutaline has a slightly different pharmacological profile than salbutamol, but it is also utilized as a rescue inhaler for rapid symptom relief.immediate relief from asthma symptoms.

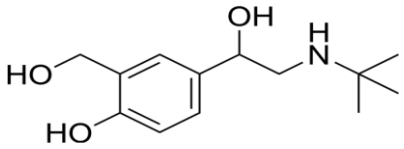
3. SALMETEROL

• Synthetic: Salmeterol is indeed a synthetic medication.

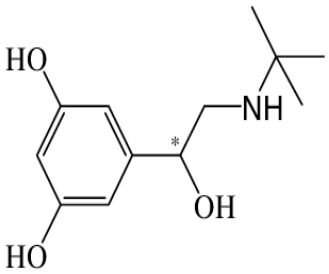
• Chemical Structure: Unlike terbutaline and salbutamol, salmeterol is a long-acting beta-2 agonist with a very different structure. Its prolonged duration of action is attributed to the lengthy alkyl chain (a lipophilic tail) that is joined to its aromatic ring.

• Pharmacokinetics: Although salmeterol takes around 30 minutes to start working, it offers sustained bronchodilation for 12 to 24 hours.

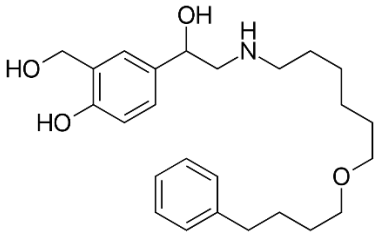
• Usage: Because salmeterol is ineffective at relieving acute symptoms of asthma, it is usually used as maintenance therapy in conjunction with inhaled corticosteroids.



salbutamol



terbutaline



salmeterol

Fig. 12 Structures of salbutamol, terbutaline and salmeterol

The Phenyl Group of the Terbutaline Aromatic Ring and Comparative Structural Activity: A Partnership
• Structure: An ethanolamine side chain is joined to a phenyl (benzene) ring to form terbutaline.
• Function: The beta-2 adrenergic receptor requires the aromatic ring in order to bind. It explains the fundamentals of receptor interaction and the superior selectivity of beta-2 receptors over beta-1 receptors.

Hydroxy Group (-OH) on the Phenyl Ring:

- **Structure**: The phenyl ring in terbutaline has a hydroxy group (-OH) at the para position.
- **Function**: Similar to salbutamol, the hydroxy group enhances the beta-2 receptor selectivity and improves the drug's affinity for the beta-2 receptors. This reduces potential side effects related to beta-1 activation (such as tachycardia).

Ethanolamine Side Chain:

- **Structure**: Terbutaline has an ethanolamine side chain (–CH2–CH2–NH2) attached to the phenyl ring, similar to salbutamol.
- **Function**: The amine group (–NH–) in the ethanolamine side chain is critical for beta-adrenergic activity. It is involved in the interaction with the beta-2 receptors, enhancing the drug's potency and its ability to relax bronchial smooth muscles.

Tert-Butyl Group (–C(CH3)3):

- **Structure**: Terbutaline has a tert-butyl group (–C(CH3)3) attached to the amine group of the ethanolamine side chain.
- **Function**: The tert-butyl group is a key feature in terbutaline's structure, distinguishing it from other beta-2 agonists like salbutamol. The bulky tert-butyl group increases the lipophilicity of the molecule and plays a role in extending its duration of action compared to other short-acting beta-2 agonists

SALMETEROL

Aromatic ring with hydroxyl group: Similar to salbutamol, salmeterol has a catechol-like aromatic ring with hydroxyl (–OH) groups that interact with beta-2 adrenergic receptors, aiding in receptor binding and selectivity.

Hydroxyamino ethanol side chain

- This side chain is vital for binding to the beta-2 receptor and is shared among many beta-agonists. It allows hydrogen bonding and supports beta-2 receptor activation.

Long lipophilic aryloxy alkyl side chain

- Salmeterol's defining feature: a long, lipophilic side chain (a phenylbutoxyhexyl group) attached to the nitrogen. It enables anchoring to an exosite (a secondary binding site) near the beta-2 receptor, prolonging its presence and action. This gives 12+ hours of bronchodilation.

Tert-butyl group (–C(CH₃) ₃)

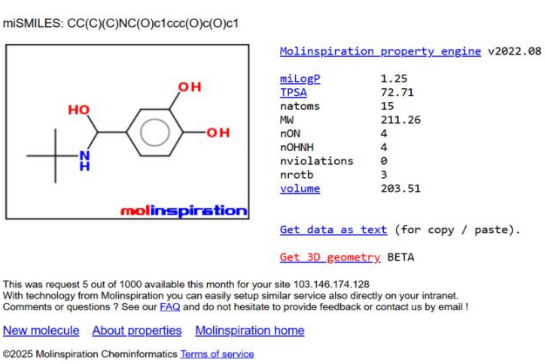
- This bulky group near the amine improves beta-2 selectivity and reduces beta-1 effects, which helps minimize cardiac side effects.

Stereochemistry (chiral centre)

- Salmeterol is chiral, and the R-enantiomer has the active beta-2 agonist properties. The stereochemistry impacts binding affinity and receptor activation.

PHYSICOCHEMICAL PROPERTIES

TERBUTALINE



SALMETEROL

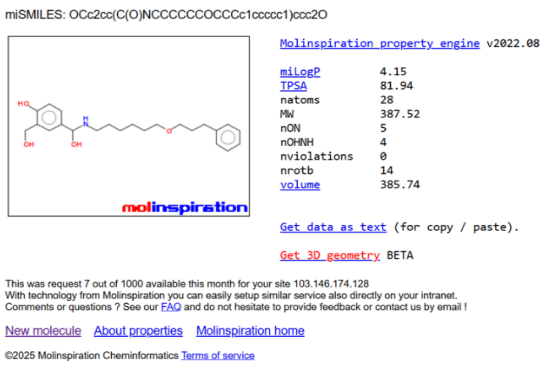


Fig. 13 Molinspiration of terbutaline **Fig. 14 Molinspiration of salmeterol**

DOSAHE FORM V/S MARKETED AVAILABILITY

SALBUTAMOL

Salbutamol (also known as ibuterol in some regions) is widely available in India in various dosage forms.

Inhalers (MDI – Metered Dose Inhaler)

- **Asthalin Inhaler** – Manufactured by Cipla Ltd, available in 100 mcg per puff.
- **Brethol Inhaler** – Produced by Wellona Pharma, also 100 mcg per puff.
- **Sal bucare Inhaler** – Manufactured by AdvoCare Pharma, available in 100 mcg per puff.

Nebulizer Solutions (Respules)

- **Asthalin Respules** – Available in 2.5 mg and 5 mg strengths, manufactured by Cipla Ltd.
- **Salbair Nebulizer Solution** – Produced by Lupin Laboratories Ltd, available in 5 mg/mL concentration.
- **Salbiprime Respules** – Manufactured by Human Biolife India Pvt. Ltd, available in 2.5 mg/2.5 ml.

Oral Tablets

- **Salbetol Tablets** – Available in 2 mg and 4 mg strengths, manufactured by FDC Limited.
- **Asthalin Tablets** – Available in 4 mg strength, manufactured by Cipla Ltd.
- **Sal bucare Tablets** – Manufactured by AdvoCare Pharma, available in 4 mg strength.

Oral Syrups

- **Asthalin Syrup** – Available in 2 mg/5 mL concentration, manufactured by Cipla Ltd.
- **SalbuVac Syrup** – Available in 2.5 mg/5 mL concentration, manufactured by Prevego Healthcare & Research Private Limited.

Injectable Form (Hospital Use)

- **Albuterol Injection** – Available in 5 mg/mL concentration, used in hospital settings for severe asthma attacks.

TERBUTALINE

Inhalers (Metered Dose Inhaler - MDI)

- **Bricanyl Inhaler** – Manufactured by AstraZeneca Pharma India Ltd, delivering 250 mcg per puff.

Nebulizer Solutions (Respules)

- **Bricanyl Respules** – Produced by AstraZeneca Pharma India Ltd, available in 2.5 mg/2.5 mL concentration.

Oral Tablets

- **Bricanyl Tablets** – Available in 2.5 mg and 5 mg strengths, manufactured by AstraZeneca Pharma India Ltd.

Oral Syrups

- **Bricanyl Syrup** – Manufactured by AstraZeneca Pharma India Ltd, available in 1.5 mg/5 mL concentration.

SALMETEROL

Seroflo 125 Inhaler: Contains 25 mcg of Salmeterol and 125 mcg of Fluticasone Propionate per puff. Available in a 120-dose metered dose inhaler (MDI).

Seroflo 500 Ciphaler: Contains 50 mcg of Salmeterol and 500 mcg of Fluticasone Propionate per puff. Available in a 60-dose Ciphaler device.

Seretide Accuhaler: Contains varying strengths of Salmeterol and Fluticasone Propionate. Available in a dry powder inhaler (DPI) format

SUMMERY OF CLINICAL USE BY POTENCY

SALBUTAMOL

Salbutamol is primarily utilized as a *rescue medication* for the immediate relief of asthma symptoms and exacerbations. It is effective in treating:

- *Acute Asthma Attacks*: Provides rapid bronchodilation to alleviate symptoms.
- *Exercise-Induced Bronchoconstriction*: Prevents symptoms when used prior to physical activity.
- *Allergic Reactions*: Helps manage wheezing and breathing difficulties associated with allergies.

TERBUTALINE

- *Acute Asthma Attacks*: Terbutaline provides rapid relief from acute asthma symptoms. The inhaled form typically begins to work within 15 minutes, with effects lasting up to 6 hours.
- *Exercise-Induced Bronchoconstriction*: It can be used prophylactically before exercise to prevent exercise-induced bronchospasm.
- *Chronic Asthma Management*: While effective for short-term relief, terbutaline does not address the underlying inflammation in asthma. Therefore, it is often used in conjunction with inhaled corticosteroids for long-term asthma control.

SALMETEROL

- *Maintenance Therapy*: Salmeterol is used as a maintenance treatment in asthma, especially for individuals whose symptoms are not adequately controlled with ICS alone. It helps in reducing bronchoconstriction and improving lung function over a 12-hour period.
- *Combination Therapy*: It is commonly prescribed in combination with ICS, such as fluticasone, to enhance anti-inflammatory effects and provide sustained bronchodilation. This combination has been shown to improve asthma control and quality of life.
- *Nocturnal Asthma*: Salmeterol has been found effective in reducing nocturnal asthma symptoms, improving sleep quality, and preventing early morning asthma exacerbations.

SCREENING METHODS

IN VITRO METHODS

1.Binding assays

- Histamine receptor assays

2.Cell culture method

- Culture technique
- WST assay

3.Test in isolated organs

- Spasmolytic activity in guinea pig
- Vascular and airway responses lungs to the isolated lung
- Relativity of isolated perfused guinea pig trachea

IN VIVO METHOD

1.Bronchospasmolytic activity in anesthetized guinea pig

2.Arachidonic acid/PAF induced respiratory vascular dysfunction.

3. Anaphylactic micro shock.

4. Serotonin aerosol induced asphyxia

5. Histamine induced bronchoconstriction

6.Pneumotachograph in guinea pig

IN VITRO SCREENING

1. HISTAMINE RECEPTOR ASSAY

To evaluate *binding or functional activity* (agonist or antagonist) of a compound on *histamine receptors* (H_1, H_2, H_3, H_4), which play roles in bronchoconstriction, allergy and inflammation. Measures the *affinity* of a test compound for histamine receptors using *radio labelled ligands***.**

Procedure:

1. Isolate membranes from tissues expressing histamine receptors (e.g., brain, lungs).
2. Incubate with a *radiolabelled histamine receptor ligand* (e.g., [^{3}H]-mepyramine for H_1).
3. Add test compound in varying concentrations.
4. Separate bound from free ligand (via filtration or centrifugation).
5. Measure radioactivity.

Functional (Tissue-Based) Assay: Assesses biological effect (e.g., contraction of smooth muscle) caused by histamine and its inhibition by test drugs.

Example: Guinea pig ileum or tracheal strip (for H_1 response)

Procedure:

1. Mount tissue in an organ bath with physiological saline.
2. Maintain temperature (37°C) and aeration (95% O_2, 5% CO_2).
3. Add histamine to induce contraction.
4. Introduce test compound and observe reduction in contraction.

2. SPACMOLYTIC ACTIVITY IN GUINEA PIG

To assess the *broncho dilatory (spasmolytic)* potential of a drug on *pre-contracted tracheal smooth muscle*, mimicking asthma conditions.

Procedure:

1. Isolate tracheal rings from guinea pigs.
2. Mount in an organ bath filled with Krebs solution (37°C, aerated).
3. Induce contraction using histamine or carbachol.
4. After reaching a plateau, add increasing concentrations of the test compound.
5. Measure relaxation responses via a transducer.

- Degree of relaxation = *spasmolytic activity*. EC_{50} (effective concentration for 50% relaxation) is calculated.

3. VASCULAR AND AIRWAY RESPONSES LUNGS TO ISOLATED LUNG

To study *vascular tone, airway resistance, bronchial reactivity*, and drug effects in a *physiologically intact lung model*. Isolated Perfused Lung (IPL)

Model – Guinea Pig or Rat.

Procedure:

- Anesthetize the animal and exsanguinate.
- Cannulate the *pulmonary artery* and *trachea*.
- Perfuse the lung with *buffer solution* (e.g., Krebs-Henseleit buffer), sometimes with added blood or albumin.
- Ventilate the lung using a *mechanical ventilator*.

4. RELATIVITY OF ISOLATED PERFUCED GUINEA PIG TRACHEA

- Guinea pigs have airway responses that closely mimic human *bronchial reactivity* to agents like *histamine*, *acetylcholine*, and *leukotrienes*.
- Their *airway smooth muscle* shows robust and reproducible contractions in response to *bronchoconstrictors*, unlike rats or mice, which have fewer sensitive airways.
- While "perfusion" typically applies to lungs or organs with vasculature, in some studies, *the inner lumen of the trachea is perfused or exposed to aerosols/drugs* using specialized chambers to mimic in vivo drug exposure.

Procedure:

1. Guinea pig is euthanized humanely.
2. The trachea is isolated and cleaned of surrounding tissues.
3. Tracheal rings (typically 2–3 cartilage rings wide) are cut.
4. Rings are mounted in an *organ bath* filled with *aerated Krebs solution* at 37°C.
5. One end is fixed; the other is connected to an *isometric force transducer*.

5.WST ASSAY (Water-Soluble Tetrazolium Salt Assay)

Materials:

Cells cultured in a 96-well plate, WST-8, Microplate reader (450 nm).

Steps:

- **Cell** *Seeding:*

- Seed cells in a 96-well plate (usually 5,000–10,000 cells/well).
- Incubate 24 hours for attachment (if adherent cells).

- **Treatment***:*

- Add test compounds (e.g., drugs, plant extracts).
- Incubate for the desired exposure time (e.g., 24–72 hours).

- Add *WST Reagent:*

- Add *10 µL of WST-8 solution* to each well (containing 100 µL medium).
- Do not remove the medium.
- Incubate at *37°C for 1–4 hours* (time depends on cell type and density).
- Avoid light exposure.

• Measure *Absorbance:*

- Use a microplate reader to read absorbance at *450 nm*.
- Use 600–650 nm as reference if available (background subtraction).

$$\text{Cell viability (\%)} = \left(\frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \right) \times 100$$

- **OD_sample**: Treated wells
- **OD_control**: Untreated control wells
- **OD_blank**: Medium + WST reagent without cells

IN VIVO SCREENING

1.BRONCHOSPASMOLYTIC ACTIVITY IN ANASTHETIZED GUINEA PIG

Purpose: To assess the efficacy of drugs that relax bronchial smooth muscle and relieve bronchoconstriction (i.e., bronchodilators).

Method:

- Guinea pigs are anesthetized and tracheostomized.
- Bronchospasm is induced (e.g., using histamine, acetylcholine, or serotonin).
- Test drug is administered intravenously or via aerosol.
- Airflow, resistance, and tidal volume are measured to assess bronchodilation.

➢ Reduction in airway resistance or increase in tidal volume after drug administration.

2.ARACHIDONIC ACID/ PLATELET ACTIVATING FACTOR (PAF) INDUCED

RESPIRATORY VASCULAR DYSFUNCTION

Purpose: To model asthma-like inflammation and vascular leakage in the lungs.

***Mechanism*:**

- *Arachidonic acid* is metabolized into leukotrienes and prostaglandins, contributing to bronchoconstriction and oedema.
- *PAF* increases vascular permeability and activates inflammatory cells (eosinophils, neutrophils).

***Procedure*:**

- Intravenous or intratracheal administration of AA or PAF.
- Observe symptoms: respiratory distress, vascular leakage (measured via Evans blue dye or wet-to-dry lung weight).
- Used to assess anti-inflammatory or anti-leukotriene drug

3.ANAPHYLACTIC MICROSHOCK

Purpose: To simulate systemic allergic (IgE-mediated) reactions leading to acute respiratory and cardiovascular collapse.

***Method*:**

- Guinea pigs are sensitized with an allergen (e.g., ovalbumin).
- After 2–3 weeks, challenged with the same allergen to trigger anaphylaxis.
- Observe for micro shock: rapid drop in blood pressure, bronchospasm, cyanosis.
- Drugs (e.g., antihistamines, leukotriene blockers, corticosteroids) tested for protective effects.
- *Endpoint*: Survival time, respiratory rate, histamine/leukotriene levels, lung pathology.

4.SEROTONIN AEROSOL -INDUCED ASPHYXIA

Purpose: To study the role of *5-HT (serotonin)* in bronchoconstriction and test anti-serotonergic agents.

***Mechanism*:**

- Aerosolized *serotonin* induces airway smooth muscle contraction.
- Leads to laboured breathing, reduced airflow, and possible asphyxia if untreated
- Breathing pattern, oxygen saturation, survival time.
- Used to evaluate 5-HT receptor antagonists or bronchodilators.

5.HISTAMINE- INDUCED BRONCHOCONSTRUCTION

Purpose: A classical asthma model to induce bronchospasm and test *antihistamines* or bronchodilators.

***Procedure*:**

- Histamine aerosol administered to guinea pigs.
- Triggers immediate bronchoconstriction via *H1 receptors*.
- Measure time to pre-convulsive dyspnoea (PCD) or use pulmonary function tools.

Drug Evaluation: Compare time to PCD before and after drug treatment.

6.PNEUMATOGRAPH IN GUINEA PIG *Purpose*: A *respiratory function measurement tool* used to record airflow and lung volume changes in real-time. Guinea pig is connected to a pneumotachograph (usually via tracheostomy).

Measures:

- Tidal volume
- Respiratory rate
- Minute ventilation
- Airway resistance

***Use*:**

- Quantify bronchospasm or bronchodilation.
- Monitor changes after challenge with histamine, serotonin, etc.
- Track drug effects dynamically.

CONCLUSION AND SUMMERY

SUMMERY:

Salbutamol is a short-acting β_2-adrenergic agonist widely used as a rescue bronchodilator in asthma. It acts quickly by relaxing airway smooth muscle, offering rapid relief from bronchospasm. Compared to terbutaline, it is more commonly used due to its rapid onset, safety profile, and availability in multiple forms (inhaler, nebulizer, tablet, injection). Unlike long-acting β_2 agonists like salmeterol, salbutamol is for short-term relief and not maintenance. Its β_2-selectivity reduces cardiac side effects. In vitro, it shows high affinity for β_2 receptors, increasing cAMP and relaxing muscles. It also inhibits mast cell mediator release, aiding anti-inflammatory effects. In vivo studies confirm rapid bronchodilation, symptom relief, and effective lung deposition.

CONCLUSION:

Salbutamol remains a key bronchodilator for rapid relief of acute asthma symptoms and exercise-induced bronchospasm, thanks to its quick onset, efficacy, and safety. While not a substitute for maintenance therapies like inhaled corticosteroids, it offers an excellent balance of effectiveness, tolerability, and accessibility. It has a suitable plasma half-life for fast action and consistently shows equal or superior results to other SABAs like terbutaline. For long-term control, it should be used alongside anti-inflammatory agents and possibly long-acting bronchodilators. Future advances aim to improve delivery systems and combine salbutamol with anti-inflammatory therapies for better outcomes

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