Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 41 (2006) 429–434
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Laboratory note

Synthesis and bronchodilator activity of new quinazolin derivative

A. Zabeer *, A. Bhagat, O.P. Gupta, G.D. Singh, M.S. Youssouf, K.L. Dhar, O.P. Suri, K.A. Suri, N.K. Satti, B.D. Gupta, G.N. Qazi

Department of Pharmacology, Regional Research Laboratory (CSIR), Canal Road, Jammu 180 001, India

Received 20 July 2004; received in revised form 1 September 2005; accepted 8 September 2005
Available online 23 January 2006

Abstract

Taking lead from a naturally occurring quinazolin vasicine, a number of compounds were developed and evaluated for bronchodilator and anti-allergic activities. One of these compounds was 2,4-diethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-12-one, hereinafter named 95-4, exhibited marked bronchodilator activity evaluated on contracted trachea or constricted tracheo-bronchial tree. On intestinal smooth muscle too it showed relaxant effect. Tracheal relaxant effect was not found to be mediated through β-adrenoceptors. Cumulative dose–response study with acetylcholine and histamine indicated for its non-specific direct effect on smooth muscles. 95-4 was found to be more potent than theophylline and less to that of salbutamol on dose basis. Tested by a number of experimental models, it was found devoid of anti-allergic activity. It was also found to be free from any adverse effect.

95-4 due to its marked bronchial muscle relaxant effect can find use in conditions associated with spasm of bronchial muscles.

Keywords: Quinazolin; Bronchodilator; Vasicine; Guinea pig tracheal chain; Theophylline; Salbutamol

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) share the common feature of impeded air flow to the lungs leading to wheezing, labored 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 autocoids leading to bronchial constriction, edema and viscid secretions. The expert panel reports of the National Asthma Education and Prevention Program in US [1,2] and similar reports from different countries [3,4] have been proved beneficial for the management of asthma.

Drugs used in the treatment of asthma are sympathomimetics [5], β_2-adrenoceptor agonists [6], methylxanthines [5], antihistaminics [7], anti-cholinergic [8], mast cell stabilizers [9] and corticosteroids [10]. More recent introductions are leukotriene receptor antagonists and zafirlukast [11,12], montelukast [13], leukotriene synthesis inhibitor, zileuton [14] and 5-lipoxygenase inhibitor [15] with the objective to reverse the bronchoconstriction and inflammatory process set in motion due to allergens or other factors. Multiple therapies, including inhaled short- and long-acting beta-2 agonists, inhaled anticholinergics and oral theophylline are the bronchodilators that are used as single agent or in combination to treat patients with COPD, as recommended in most of the guidelines [16]

Vasicine and vasicinone (Fig. 1) [17] obtained from leaves of *Adhatoda vasica* Nees family Acanthaceae, have been reported for moderate degree bronchodilator activity [18]. Hermecz et al. [19] have reported the bronchodilator activity of bis- and tricyclic nitrogen bridgehead derivatives with a pyrimidine-4(3H)-one ring. Vasicine was chemically modified to yield 6,7,8,9,10,12-hexahydro-azepino-[2,1-b]quinozolin-12-

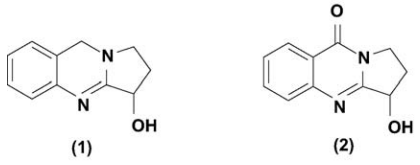

Fig. 1. Vasicine (**1**), vasicinone (**2**).

* Corresponding author. Tel.: +91 191 257 2002, +91 191 257 9117; fax: +91 191 254 3829, +91 191 254 8607.
E-mail address: zabeer@rrljammu.org (A. Zabeer).

0223-5234/$ - see front matter © 2006 Published by Elsevier SAS.
doi:10.1016/j.ejmech.2005.09.010

one (RLX) which is reported to have potent bronchodilator activity than aminophylline [20]. It also possesses anti-inflammatory, anti-arthritic activity [21] and involvement in cell mediated and humoral component of the immune system [22].

On the basis of these observations, the aim of finding a molecule with potent bronchodilator and anti-allergic activity led to the synthesis of vasicine analogue, 95-4, starting with anthranilic acid, which was found to possess manifold more bronchodilator activity.

2. Chemistry

Taking a lead from naturally occurring vasicine and vasicinone (Fig. 1), a similar molecule, 2,4-diethoxy-6,7,8,9,10,12 hexa-hydroazepino [2,1-b] quinazolin-12-one has been synthesized starting from anthranilic acid involving five steps (Fig. 2).

As part of the program, anthranilic acid was subjected to bromination to obtain 3,5-dibromo anthranilic acid (**1**) which was treated with $SOCl_2$ to get an acid chloride, the resulting acid chloride was condensed with caprolactam to yield 2,4-dibromoderivative (**2**) [16,17]. (**2**) was subjected to nucleophilic substitution in p.o. sodium methoxide to obtain 2,4-dimethoxy derivative (**3**), which after demethylation with 49% aq. HBr afforded 2,4-dihydroxy derivative (**4**). (**4**) on alkylation with ethyliodide yielded the desired compound (**5**).

The structures of the synthesized compounds were established with the help of spectral analysis.

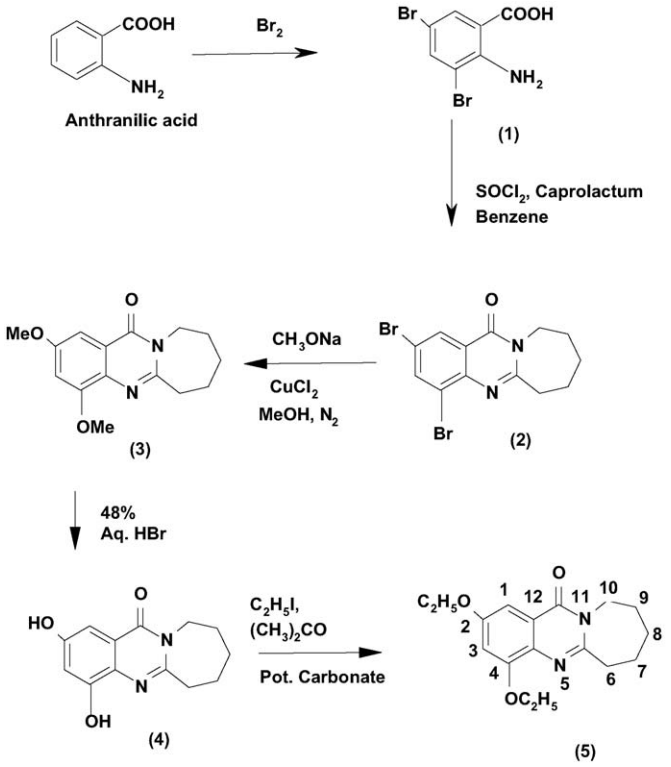

Fig. 2. Synthesis of 2,4-diethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin-12-one.

3. Pharmacology

All the compounds were evaluated for their bronchodilator and antiallergic activity using guinea pig tracheal chain, histamine aerosol induced bronchospasm in guinea pig and protection against systemic anaphylaxis. For antiallergic, the experimental models used were Schultz–Dale reaction, Passive cutaneous anaphylaxis, Mast cells stabilization, Anti-ACh and antihistaminic activities. 95-4, which out of these five compounds was found to be most potent was also studied for its effect on CVS, CNS and for its toxicity.

4. Results and discussion

4.1. Results

4.1.1. Bronchodilatory activity

4.1.1.1. EC_{50} of compound 95-4 observed on guinea pig tracheal chain. Contracted with ACh, histamine and antigen was 6.25, 43.33 and 7.14 times less, respectively, as compared to that of theophylline whereas EC_{50} of salbutamol, a β-adrenergic agonist was far less (Table 1). After propranolol pretreatment, the relaxant effect of 95-4 was found to persist whereas that of salbutamol was blocked.

4.1.1.2. 95-4 in 50 mg/kg p.o. dose afforded 100% protection as compared to protection of 40% and 60% recorded with theophylline and deriphylline, respectively, against histamine aerosol-induced bronchospasm in guinea pigs. This protection was comparable with that of salbutamol (Table 2).

4.1.1.3. In the study of protection against systemic anaphylaxis, 95-4 proved more effective as compared to theophylline against both IgG and IgE induced systemic anaphylaxis. Protection against IgG was more marked as against IgE. As compared to this, salbutamol afforded 100% protection against both IgG and IgE (Table 3)

4.1.2. Antiallergic activity

4.1.2.1. Schultz–Dale reaction. 95-4 checked Schultz–Dale reaction elicited on guinea pig ileum sensitized for either IgG or IgE in 5.13×10^{-5} and 8.16×10^{-5} molar concentration, re-

Table 1
EC_{50} for relaxant effect on guinea pig tracheal chain contracted by Ach, Histamine or antigen

Drug treatment	EC_{50} (M)		
	ACh	Histamine	Antigen Ovalalbumin
95-4	4×10^{-5}	1.2×10^{-5}	7×10^{-5}
Theophylline	2.5×10^{-4}	5.2×10^{-4}	5×10^{-4}
Salbutmaol	0.03×10^{-7}	0.11×10^{-7}	0.02×10^{-7}

Dose of ACh (2×10^{-7}) and histamine (5×10^{-7}) employed caused submaximal contraction and antigen (ovalbumin 100 μg/ml) which caused maximal contraction.

Table 2
Protection against bronchospasm induced by histamine aerosol, 5 ml of 1% histamine solution aerosoled in 2 min

Treatment group	Dose: mg/kg p.o.	Mean time in seconds for onset of bronchospasm Mean ± S.E.M.	Severity of bronchospasm	Survival (%)
1. Control	–	66 ± 4	+ + +	0
2. 95-4	50	132 ± 6	+	100
3. Theophylline	50	90 ± 4	+ +	40
4. Deriphylline	10	102 ± 5	+	60
5. Salbutamol	1	144 ± 4	+	100

$N = 8$, Student's 't' test: $P < 0.05$.

Table 3
Protection against systemic anaphylaxis in guinea pigs

Treatment group	Dose mg/kg	% Survival	
		Actively sensitized for	
		IgG	IgE
1. Control	–	0	0
2. 95-4	12.5 i.p.	60	0
	25 i.p.	100	30
	50 p.o.	100	60
3. Theophylline	25 i.p.	30	10
	50 i.p.	60	30
	100 p.o.	100	40
4. Salbutamol	1.0 p.o.	100	100

$N = 8$.

spectively. The dose of 95-4 required to check histamine-induced contraction in this set up was 3.4×10^{-5} mole (Table 4).

4.1.2.2. Passive cutaneous reaction. In the P.C.A. tests carried out on mice, rat and guinea pigs by different techniques, 95-4 tested upto 100 mg/kg p.o. failed to show any significant effect. As compared to this cromolyn sodium in 20 mg/kg i.v., dose showed complete check of this reaction.

4.1.2.3. Mast cells stabilizing activity. 95-4 failed to show any mast cells stabilizing activity whereas Cromolyn sodium showed complete check against compound 48/80.

4.1.3. Anti ACh and anti-histamine activities

Cumulative dose–response study carried out on guinea pig ileum in the absence and presence of 95-4 with ACh and histamine exhibited shift of curve to the right and reduction of slope and maxima in the presence of compound 95-4 both against ACh and histamine (Fig. 3 and 4).

4.1.4. Effect on cardiovascular system

Isolated guinea pig heart: 95-4 in 10, 30, 100 and 300 μg doses showed no effect on rate rhythm and coronary outflow. 1 and 3 mg doses exhibited same + ve inotropic effect.

Table 4
Effect of 95-4 on Schultz–Dale reaction and histamine induced contraction on guinea pig ileum

Treatment group	Mean effective dose, (M) of 95-4, for 100% check		
	Schultz–Dale reaction		Histamine 0.1 μg/ml
	IgG Sensitized	IgE Sensitized	
1. Control	Contraction	Contraction	Contraction
2. 95-4	5.13×10^{-5}	8.16×10^{-5}	3.4×10^{-5}

$N = 6$.

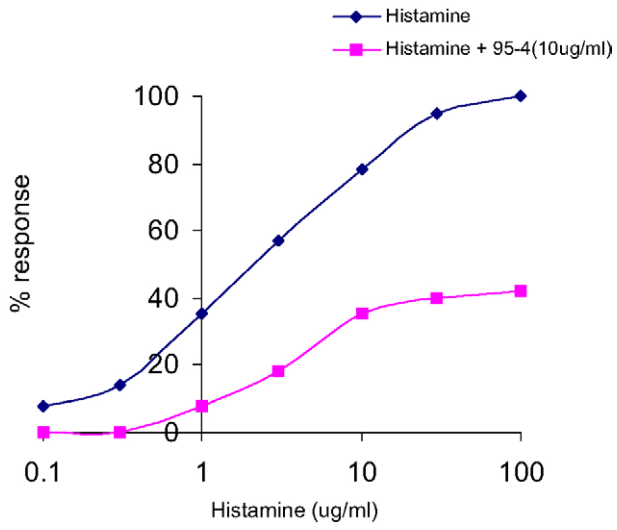

Fig. 3. Cummulative dose–response curve of histamine alone and Hist.+ 95-4 on guinea pig ileum.

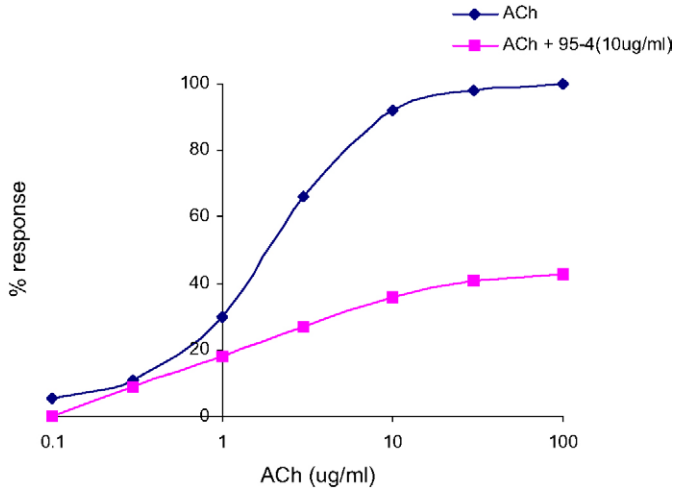

Fig. 4. Cummulative dose–response of ACh alone andACh.+ 95-4 on guinea pig ileum.

Effect on B.P., heart rate and E.C.G.: In 25, 50 and 100 mg/kg intraduodenal doses in normal rats, no appreciable effect was observed.

4.1.5. Effect on CNS and toxicity

95-4 tested in graded doses up to 400 mg/kg i.p. and 800 mg/kg p.o. in mice didn't show any adverse effect in gross

observation models. Sub-acute toxicity carried out in rats with 200 mg/kg p.o. for 21 days also didn't reveal any toxic effect.

4.2. Discussion

Vasicine, the alkaloid of *Adhatoda vasica* has been reported for moderate degree bronchodilator activity. For the possibility that one of its congeners could possess potent bronchodilator activity, the present study was taken up and has proved fruitful. Among the various congeners prepared, 95-4 has shown potent bronchodilator activity as observed by a number of in vitro and in vivo experimental models viz. Relaxant effect on tracheal chain contracted by ACh or histamine or antigen and protection against bronchoconstriction induced by histamine aerosol and systemic anaphylaxis. In terms of efficacy and potency, 95-4 has been found to be more effective as compared to theophylline in the above experimental models. However, β-adrenergic agonist salbutamol was far more effective.

The bronchial relaxant effect exhibited by 95-4 is not being mediated through β-adrenoceptors as propranolol treated trachea too showed relaxant effect. Whether it is acting as inhibitor of phosphodiesterase, blocker of Calcium channels or opener of Potassium channels is being investigated separately. Inhibition of responses of ACh and histamine after 95-4, warranted elucidation whether the reduction in responses was due to anticholinergic and antihistaminic effect or due to relaxant effect of 95-4.

Cumulative dose–response study on guinea pig ileum with ACh and histamine revealed shift of the curves to the right and reduction of the slope and maxima. Evidently, it was not a case of competitive block or surmountable reversal as occurs with an agonist and antagonist acting through the same receptor. Shift of the curve to the right and reduction of the slope and maxima is characteristic of compounds having direct effect non-mediated through receptors.

Studied by a number of experimental models including PCA test, compound is devoid of anti-allergic activity. Protection observed against systemic anaphylaxis and check of Schultz–Dale reaction is attributable to smooth muscle relaxant effect and not due to the effect on any component of immune system since compound failed to show any activity in PCA test, considered specific for anti-allergic activity. 95-4 was also devoid of any mast cells stabilizing activity as tested with compound 48/80.

It has been found to be free from any CNS, CVS and toxicity effects as tested in graded doses in mice and rats.

95-4 has the potential to be used in asthmatic conditions for its bronchodilator activity many times more as compared to that of theophylline. Moreover, theophylline now finds limited therapeutic use primarily because of its modest effect, its narrow therapeutic window and required monitoring of drug level [23].

An ideal anti-asthmatic drug sought today is one possessing bronchodilator and anti-allergic activities as majority of asthma cases are of allergic origin. It is in pursuit of this that 95-4 was subjected to anti-allergic evaluations by a battery of tests but found to be devoid of this activity.

5. Experimental

5.1. Chemistry

All m.p.s were obtained on a BUCHI melting point B-545 apparatus and were uncorrected. Proton (^{1}H) nuclear magnetic resonance spectroscopy was performed using Bruker DPX-200 spectrometer using TMS as internal standard.

5.1.1. 3,5-Dibromoanthranilic acid (*1*)

To a solution of anthranilic acid (137 g, 1 mol) in MeOH (2 l), Br_2 (237 g, 3 mol) diluted with MeOH was added slowly in 3 h with constant stirring at 50 ± 5 °C. Reaction mixture was then cooled to 15 °C, filtered, washed with cold water and the solid obtained was crystallized from EtOH to yield **1** (176 g, 59.66%): m.p. 226–228 °C. ^{1}H-NMR (DMSO-d_6) δ 7.79 (d, 1H,J = 2.4 Hz, CH-3), 7.83 (d, 1H, J = 2.4 Hz, CH-1).

5.1.2. 2,4-Dibromo-6,7,8,9,10,12-hexahydroazepino[2,1-b] quinazolin-12-one (*2*)

3,5-Dibromoanthranilic acid (**1**) was suspended in dry benzene and treated with $SOCl_2$ (177 g, 7.5 mol). Reaction mixture was refluxed on steam bath for 2 h under anhydrous conditions. The resulting clear solution was distilled under reduced pressure to remove excess of $SOCl_2$. Residue was dissolved in dry benzene and treated with caprolactam (66.5 g, 0.5 mol), dissolved in benzene. Reaction mixture was allowed to stand for 12 h, filtered, dissolved in $CHCl_3$ and basified with ammonia solution. The $CHCl_3$ portion was concentrated under reduced pressure and the residue thus obtained was crystallized from acetone to yield (**2**) (137.8 g, 73.65): m.p. 168.3 °C, ^{1}H-NMR ($CDCl_3$) δ 1.82 (bs, 6H, CH_2-7, 8 and 9), 3.07 (d, 2H, J = 6.0 Hz, CH_2-6), 4.32 (d, 2H, J = 6.0 Hz, CH_2-10) , 8.02 (d, 1H, J = 2.2 Hz, CH-3), 8.26 (d, 1H, J = 2.2 Hz, CH-1).

5.1.3. 2,4-Dimethoxy-6,7,8,9,10,12-hexahydroazepino[2,1-b] quinazolin-12-one (*3*)

2,4-Dibromo-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazolin–12–one, CH_3ONa (6 mol, freshly prepared), $CuCl_2$ (20 g) and MeOH (4 l), were put into closed reaction vessel. Reaction was carried out at 120 °C under 82 psi and 203 rpm under nitrogen for 14 h, solvent was removed by distillation under reduced pressure. Residue was triturated with water to remove excess of alkali and then was crystallized from MeOH (52.8 g, 64.23%) m.p. 193 °C. ^{1}H-NMR δ 1.78 (bs, 6H, CH_2-7, 8 and 9), 3.07 (d, 2H, CH_2-6), 3.85 (s, 3H, -OCH_3) 3.93 (s,3H,-OCH_3), 4.34 (bs,2H, CH_2-10),6.72 (d, 1H, J = 2.6 Hz, CH-3),7.15 (d,1H, J = 2.6 Hz, CH-1).

5.1.4. 2,4-Dihydroxy-6,7,8,9,10,12-hexahydroazepino[2,1-b] quinazolin-12-one (*4*)

2,4-Dimethoxy-6,7,8,9,10,12-hexahydroazepino [2,1-b] quinazolin-12-one (41.1g, 0.15 mol) was refluxed with 48% aqu-

eous HBr in a heating mantle for 12 h. Reaction mixture was allowed to cool at room temperature. Separated solid was filtered, dissolved in MeOH, basified with ammonia solution and allowed to stand at 4–8 °C. Separated solid was filtered (17.3 g, 46.88%): m.p. 259 °C. ^{1}H-NMR δ 1.69 (s, 6H, CH_2-7,8 and 9), 2.98 (bs, 2H, CH_2-6), 4.26 (bs, 2H, CH_2-10), 6.62 (d, 1H, J-2.6 Hz, CH-3), 6.80 (d, 1H, $J = 2.6$ Hz, CH-1).

*5.1.5. 2,4-Diethoxy-6,7,8,9,10,12-hexa hydroazepino[2,1-b] quinazolin-12-one (**5**)*

2,4-Dihydroxy-6,7,8,9,10,12-hexahydroazepino [2,1-b] quinazolin –12-one (12.3 g, 0.05 mol), ethyliodide (0.125 mol), anhydrous potassium carbonate 10 g and acetone 2 l were put into reaction vessel. Reaction was carried at 96–99 °C at 34–40 Psi and 205 rpm under nitrogen for 8 h; solvent was removed under reduced pressure. Residue after triturating with water was crystallized from acetone (6.79 g, 75%): m.p. 152–153 °C. ^{1}H-NMR δ 1.49 (dt, 6H, 2x OCH_2 CH_3), 1.82 (s, 6H, CH_2-7, 8 and 9), 3.11 (bs,2H,CH_2-6), 4.17 (dq,4H, 2 xOCH_2CH_3), 4.37 (bs, 2H, CH_2-10), 6.76 (d, 1H, $J = 2.4$ Hz, CH-3), 7.17 (d, 1H, $J = 2.4$ Hz, CH-1).

5.2. Pharmacology

5.2.1. Materials

Compound 95-4 (A quinazolin derivative) prepared from anthranilic acid; cromolyn sodium; theophylline anhydrous; deriphylline; salbutamol; propranolol HCl; compound 48/80; albumin chicken egg; acetylcholine chloride; histamine disphosphate and dexamethasone. Aqueous solutions of these compounds were prepared.

5.2.2. Methods

5.2.2.1. Bronchodilator activity. **Relaxant effect on contracted tracheal chain**. Guinea pig tracheal chain was prepared according to the method of Akcasu [24] and suspended in Kreb–Henseleit solution aerated with carbogen at 37 °C. Tracheal chain was contracted with submaximal dose of ACh or histamine or by antigen on sensitized tissue which led to maximal contraction. Graded doses of 95-4, theophylline and salbutamol for relaxant effect were tested and EC_{50} of each drug determined. Relaxant effect of compound 95-4 and salbutamol was also tested on contracted trachea after propranolol (2 μg/ml) treatment.

Protection against histamine aerosol induced bronchospasm in guinea pigs. Male guinea pigs of 350 ± 25 g were employed. The animals were pretreated with test drugs 95-4, theophylline, deriphylline and salbutamol per oral 1 h before exposure to aerosol. The doses of the known drugs employed were worked out from the human dose. For 95-4, optimum dose was found out by employing graded doses. The animals were kept in the 5 l capacity perspex chamber and exposed to histamine aerosol. 5 ml of 1% solution of histamine was aerosoled in 2 min. The time for onset of bronchospasm, its severity and death of the animals were recorded. The animals remained in the chamber for 5 min after which they were removed. Eight animals employed in each group.

Protection against systemic anaphylaxis induced bronchospasm. Guinea pigs were sensitized for IgG (5 mg of egg albumin on day 1 and 10 mg on day 2, 1% solution i.p.) and for IgE [1 μg of egg albumin + 100 mg $Al(OH)_3$ i.p.] as per the method of Anderson (Chu [25]). After 1 month, the animals were challenged with egg albumin 2 mg in 0.2 ml saline i.v. Test drugs were administered i.p. or p.o., 15 or 45 min, respectively, before being challenged with antigen.

5.2.2.2. Anti-allergic evaluation. **Effect on Schultz–Dale reaction and histamine induced contraction of guinea pig ileum**. Male guinea pigs weighing 210 ± 20 g were employed. The animals were sensitized for IgG or IgE according to the method of Anderson (Chu [25]). Four weeks later, the animals were sacrificed and ileum suspended in organ bath assembly in Tyrode solution aerated with carbogen. The response of the tissue was first checked with ACh or histamine. The tissue was challenged with egg albumin 100 μg/ml of bath fluid. Test drug was allowed to act on tissue for 5 min before the addition of antigen. Dose of the compound 95-4, which caused complete check of histamine induced sub-maximal contraction, was also found out in this set up.

Effect on passive cutaneous anaphylaxis (PCA). *As per the method of Vaz et al. .in mice*(Jindal et al. [26]): Mice were injected with 0.1 μg of ovalbumin in 0.5 ml of $Al(OH)_3$ gel. 4 weeks later, this treatment was repeated. One week later animals were bled with orbital puncture and serum collected. Serial dilution of this serum in 0.03 ml volume were injected i.d. in dorsal skin of mice. 48 hours later, PCA was elicited by injecting 2 mg of ovalbumin and 5 mg Evan blue in 0.5 ml saline into tail vein.30 min later, animals sacrificed, skin on the back reflected and extent of PCA assessed by the area infiltrated by the dye (by multiplying cross section dimensions of the area.)

As per the method of Yue Dai and Jinping in rats (Holgate and Finnerty [27]): Female Wistar rats were injected i.m. with 1.5 mg of egg albumin contained in 0.5 ml of saline and Bordetella pertussis vaccine (1.8×10^9 cells/0.2ml) injected i.p. on day 1 and 2. Twelve days later, blood was collected with syringe from heart of these animals under ether anesthesia. Serum obtained from this blood was injected i.d. at four sites (0.1 ml/site) on the dorsal side of rats clipped of hairs. After 48 hours, these animals were injected i.v. 1 ml of 0.25% Evans blue solution containing 5 mg/ml of egg albumin. Thirty minutes later, rats were sacrificed and dorsal skin reverted. The skin areas infiltrated with dye were dissected out and dye was extracted out with 70% acetone for 12 hours and measured colorimetrically at 610 nm. Three groups of rats with six animals in each group were employed. One group served as a control, second as test drug treated 100 mg/kg p.o. 1 h before challenge with antigen and 3rd group treated with dexamethasone 5 mg/kg p.o. 1 h prior to antigen challenge as a reference standard.

As per the method of Anderson et al. in guinea pigs (Chu [25]): Male guinea pigs 350 ± 25g were used. The antisera was

raised by injecting 1 μg of egg albumin with 100 mg $Al(OH)_3$ in 1 ml saline i.p. Four weeks later blood was drawn from hearts and serum separated. Serial dilutions of sera 1:2, 1:4, 1:8 up to 1:256 were made and 0.1 ml of each dilution injected i.d. in the shaved abdominal skin. One week later, animals were challenged i.v. with 2 mg of egg albumin and 10 mg Evans blue contained in 1 ml normal saline. Two groups with five animals in each group were employed. One group served as control and the animals of other group were treated with test drug, 100 mg/kg p.o. 1 h before the challenge with antigen.

As per the method of Goose and Blair (Rorke and Holgate [28]): Effect of test compound against mast cells degranulation by compound 48/80 was studied. Male Wistar rats of 100 ± 10 g body weight were selected and divided into four groups of five animals each. Abdominal skin of these animals was shaved 1 day before. Group 1 injected with 0.1 ml normal saline i.d. served as the control, group 2 injected with compound 48/80, 5 μg in 0.1 ml saline served as second control and group 3 was treated with test compound 50 mg/kg i.p. 1 h before the injection of compound 48/80 i.d. 4th group was treated with Cromolyn sodium 10 mg/kg i.v. 5 min before the injection of compound 48/80 i.d. These animals were injected 10 min later Evan's blue 10 mg in 1 ml saline. Five minutes later the animals were sacrificed, abdominal skin reflected and the cross section area of the skin infiltrated with dye was measured.

5.2.2.3. Evaluation of anti-ACh and anti-histamine activities. Cumulative dose–response study as per the method of Blatter et al. (Riccioi et al. [29]). Guinea pig ileum suspended in Tyrode solution and aerated with carbogen at 37 °C was employed. Contractions of the ileum with graded doses of Ach and histamine in the absence and presence of compound 95-4 were carried out to know its effect on the slope, maxima and shift of the curve.

5.2.2.4. Effect on other body systems. **Rat uterus**. Uterine horn of rat in estrus stage was suspended in De-Jalon solution at 37 °C aerated with O_2. Different doses of 95-4 were tested to observe any effect on the tone and rhythmic movements of the uterus.

Guinea pig isolated heart. It was put up as per Langendorff technique and perfused with carbogen at 37 °C at a constant pressure of 40 cm of water. Effect on heart rate, rhythm and coronary flow studied.

Rat B.P., heart rate and E.C.G. Rats of 200 ± 10g body weight anesthetized with sodium pentobarbitone were employed. Carotid blood pressure through pressure transducer and E.C.G. were recorded on Grass Polygraph.

Effect on C.N.S. and toxicity study. Groups of Swiss mice of 5 animals in each group were employed. These were administered with compound 95-4, 50, 100, 200 and 400 mg/kg i.p. and 100, 200, 400 and 800 mg/kg p.o. and observed for C.N.S. and acute toxicity (48 h mortality).

Explorative sub-acute toxicity with 200 mg/kg p.o. of 95-4 for 21 days in rats was carried out. Gross observation and body weight were recorded.

Acknowledgement

Authors are thankful to Shree Dharam Raj, Ex-STA, RRL, Jammu for his technical assistance.

References

[1] National Asthma Education and Prevention Program, Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma, National Institutes of Health Pub. No. 91-3642, Bethesda, MD, 1991.
[2] National Asthma Education and Prevention Program, Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma, National Institutes of Health Pub. No. 97-4051, Bethesda, MD, 1997.
[3] British Thoracic Society, National Asthma Campaign, Royal College of Physicians of London et al., The British Guidelines on Asthma Management, 1995 Review and Position Statement, Thorax 52 (Suppl. 1) (1997) S1–S21.
[4] P. Ernst, J.M. FitzGerald, S. Spier, Can. Respir. J. 3 (1996) 89–100.
[5] C.H. Fanta, T.H. Rossing, et al., Am. J. Med. 80 (1) (1986) 5–10.
[6] Current Opinion in Pharmacology 3 (3) (2003) 270–276.
[7] Y. Dai Liu, J. Koch, J. Ethnopharmacol. 55 (2) (1997) 127.
[8] M.J. Moan, C.H. Fanta, Compr. Ther. 21 (8) (1995) 421–427.
[9] J. Goose, A.M.J.N. Blair, J. Immunal. 16 (1969) 749.
[10] L.J. Walsh, C.A. Wong, S. Cooper, A.R. Guhan, M. Pringle, A. E. Tattersfield, Thorax 54 (1999) 296–300.
[11] L. Pescollderungg, Minerva Pediatr. 56 (2) (2004) 151–155.
[12] R.D. Krell, D. Aharony, C.K. Buckner, R.A. Keith, E.J. Kusner, D. W. Snyder, et al., Am. Rev. Resp. Dis. 141 (1990) 978–987.
[13] T.R. Jones, M. Labella, M. Belley, E. Champion, L. Charette, J. Evans, et al., Can. J. Physiol. Pharmacol. 73 (1995) 191–201.
[14] G.W. Carter, P.R. Young, D.H. Albert, J. Bousaka, R. Dyer, R.L. Bell, J. B. Summers, D.W. Brooks, J. Pharmacol. Exp. Ther. 256 (1991) 929–937.
[15] X. Wu, A. Dev, A.B. Leong, Am. J. Haematol. 74 (1) (2003) 23–25.
[16] W. MacNee, P.M.A. Calverley, Thorax 58 (2003) 261–265.
[17] O.P. Gupta, M.L. Sharma, B.J. Ghatak, C.K. Atal, Indian J. Med. Res. 66 (4) (1977) 680–691.
[18] D.P. Jindal, R. Chattopadhaya, M. Minu, Indian Drugs 35 (1998) 606–639.
[19] I. Hermecz, L. Vasvari-Debreczy, A. Horvath, M. Balogh, J. Kakosi, V. C. Dev, L. Rodriguez, J. Med. Chem. 30 (1987) 1543–1549.
[20] C.K. Atal, Chemistry and Pharmacology of Vasicine—A New Oxytocic and Abortifacient, Raj Bandhu Industrial Co, New Delhi, 1980 (148).
[21] G.B. Singh, S. Singh, Indian Drugs 27 (1990) 449–455.
[22] M.L. Sharma, A. Khajuria, A. Kaul, D. Chand, Int. J. Immunopharmac. 14 (4) (1992) 979–986.
[23] S.S. Nasser, P.J. Rees, Drug Saf. 8 (1993) 12–18.
[24] E.O. Vianna, R.J. Martin, Drugs Today 34 (1998) 203–223.
[25] S.S. Chu, Drugs Today 20 (1984) 509–527.
[26] D.P. Jindal, M. Minu, R. Chattopadhaya, Indian Drugs 37 (2000) 1–21.
[27] S.T. Holgate, J.P. Finnerty, J. Allergy Clin. Immunol. 83 (1989) 537–547.
[28] S. Rorke, S.T. Holgate, Am. J. Respir. Med. 1 (2) (2002) 99–105.
[29] G. Riccioni, C.D. Ilio, N. D'Orazio, Expert Opin. Investig. Drugs 13 (7) (2004) 763–776.