Med Chem Res 14:6 (2005) 347–368

DOI: 10.1007/s00044-006-0141-7

MEDICINAL CHEMISTRY RESEARCH

Synthesis and Structure-Activity Relationships of Vasicine Analogues as Bronchodilatory Agents

Neeraj Mahindroo[a,c*], Zabeer Ahmed[b], Asha Bhagat[b], Kasturi Lal Bedi[b], Ravi Kant Khajuria[a], Vijay Kumar Kapoor[c], Kanaya Lal Dhar[a]

[a]*Natural Products Chemistry Division, Regional Research Laboratory (CSIR), Canal Road, Jammu 180001 India.*

[b]*Pharmacology Division, Regional Research Laboratory (CSIR), Canal Road, Jammu 180001 India.*

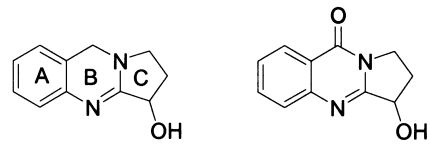
[c]*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014 India*

Abstract – The series of vasicine (**1**) analogues, an alkaloid from *Adhatoda vasica* Nees., were synthesized with changes in A, B or C rings. Compounds **3-19** were evaluated for in vitro bronchodilatory activity using isolated guinea pig tracheal chain. Compounds **3-8** were also synthesized in good yields using microwave-mediated synthesis under solvent free conditions. Compounds **5** and **8** with seven-member C ring were more active than etofylline and caused 100% relaxation of both the histamine and acetylcholine pre-contracted guinea pig tracheal chain. The structure-activity relationship studies showed that the quinazoline and oxo functionalities were essential for activity. The compounds without C ring and instead having aliphatic and phenyl substitutions in B ring showed relaxation against histamine pre-contracted tracheal chain only, 2-methyl substituted analogues, **12** and **13**, being most active with 100% relaxation effect.

* Present address and address for correspondence: Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 7F, 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, Republic of China. Phone: +886-37-246-166 ext. 35748; fax: +886-37-586-456 e-mail: n_mahindroo@yahoo.com

Introduction

Chronic obstructive pulmonary disease (COPD) will become the third leading cause of mortality and fifth leading cause of disability worldwide by year 2020.[1] Bronchodilators are first line agents for the symptomatic management of this disease and have proven to be effective in both stable disease status and exacerbations.[2] *Adhatoda vasica* Nees. leaves have been used in Indian system of medicine for more than 2000 years. The Ayurvedic system of medicine describes the use of this plant for 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 in bronchitis and asthma.[3] The alkaloids from this plant, vasicine (**1**) and vasicinone (**2**) (Figure 1), were reported to have in vitro and in vivo bronchodilatory activity.[3-6] Vasicine causes relaxation of tracheal muscle at low concentrations and contraction at high concentrations. It also exhibited protection against histamine-induced bronchospasm.[5,7]



1 (Vasicine) **2** (Vasicinone)

Figure 1. Bronchodilatory alkaloids from *Adhatoda vasica*.

The structural modifications of vasicine and vasicinone have been carried out to reveal the structure activity relationships.[8-13] Dhar et al.[9] have reported that the presence of only one oxygen function (either at oxo or hydroxyl) is essential for the retention of activity as the presence of oxygen at both the carbons leads to the loss of activity. The deoxyvasicinone was found to possess bronchodilatory activity, thereby suggesting that hydroxyl and carbonyl group in the vasicinone molecule are perhaps competing with each other. RLX (**5**) was reported to be 6-10 fold more potent than aminophylline. The bronchodilatory activity of **5** analogues with oxo group in B ring increases with increase in size of the ring C up to seven-member and gradually decreases thereafter. Reduction of the oxo group resulted in a drop in activity thus indicating that oxygen

function is essential. The 2,3-dialkyl derivatives were found to be devoid of bronchodilatory activity.[10] The diethoxy analogue of **5** was recently shown to have marked bronchial muscle relaxant effect.[14] Jindal et al. have reported the 8,9-dimethoxy derivative of **5** with improved bronchodilatory activity.[13] Bahekar et al. have reported 5-alkyl-2,3-dihydroimidazo[1,2-c]quinazolines and 2,3-dihydroimidazo[1,2-c]quinazolin-5-(6H)-thiones as bronchodilatory agents with longer 5-alkyl substitution showing better activity.[15]

Keeping in view the potential of vasicine analogues as bronchodilatory agents we have further explored the structure-activity relationship by making derivatives with changes in A ring and C ring. The compounds without A ring or C ring were also synthesized. The microwave-mediated synthesis of compounds **3-8** is also reported.

Results and Discussion

Chemistry. Compounds **3-8** were synthesized in a manner similar to that described by Hermecz et al.[11] Anthranilic acids (**20a** and **20b**) were converted to corresponding *N*-sulfinylanthaniloyl chloride by refluxing with thionyl chloride (Figure 2) which was condensed with the appropriate lactam to give the desired quinazoline. 6-Amino-3,4-methylenedioxybenzoic acid **20b** was prepared starting from piperonal **22a** by first nitrating it to 6-nitropiperonal **22b** followed by reduction to 6-aminopiperonal **22c**, the aldehydic group was finally oxidized to give **20b** (Figure 3).

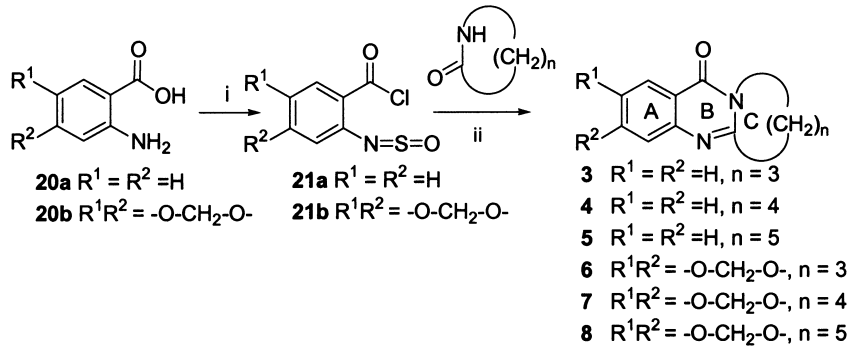


Figure 2. Synthesis of compounds **3-8**. Reagents and conditions: (i) $SOCl_2$, toluene, reflux, 2h; (ii) lactam, toluene, reflux, 2h.

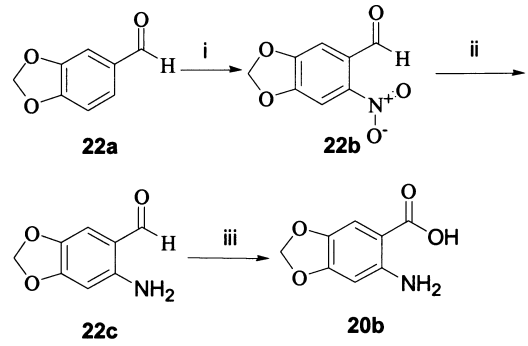


Figure 3. Synthesis of **20b**. Reagents and conditions: (i) HNO_3, 5 °C, 4h; (ii) Fe, NH_4Cl, H_2O, reflux, 2h; (iii) Ag_2O, NaOH, H_2O.

3-Amino-3-phenylpropionic acid **23a** was converted to **23b** by refluxing with thionyl chloride, which was then condensed with various lactams to give compounds **9-11** (Figure 4).

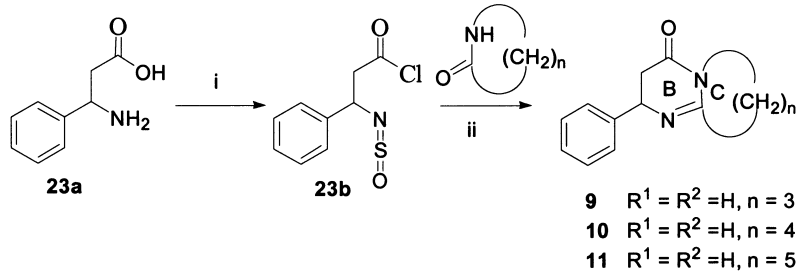


Figure 4. Synthesis of compounds **9-11**. Reagents and conditions: (i) $SOCl_2$, toluene, reflux, 2h; (ii) lactam, toluene, reflux, 2h.

Compounds 12-17 were synthesized by adopting the procedure reported by Errede.[16] 2-Alkylbenzo[*d*][1,3]oxazin-4-ones were synthesized by refluxing anthranilic acid with the appropriate anhydride. 2-Pentylbenzo[*d*][1,3]oxazin-4-one (**24c**) was synthesized

starting from caproic acid **24a** which was reacted with thionyl chloride to give caproyl chloride (**24b**) which was then condensed with anthranilic acid to give **24c**. The 2-alkylbenzo[*d*][1,3]oxazin-4-ones were then reacted with aniline or 4-fluoroaniline to give compounds **12-19** (Figure 5 and 6).

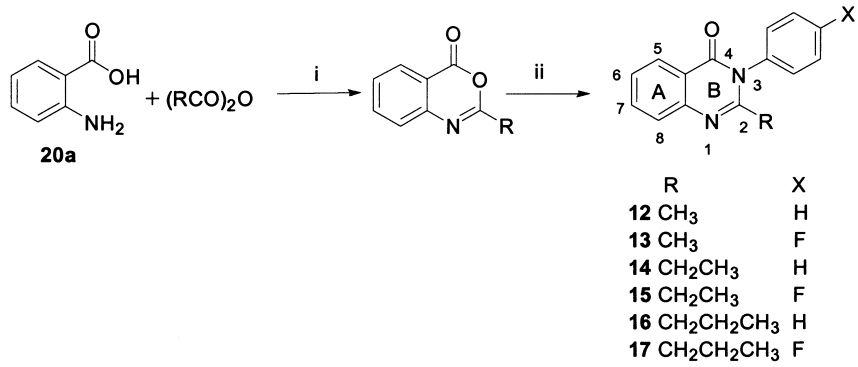


Figure 5. Synthesis of compounds **12-17**. Reagents and conditions: (i) reflux, 4h; (ii) aniline or 4-fluoroaniline, $POCl_3$, CH_2Cl_2.

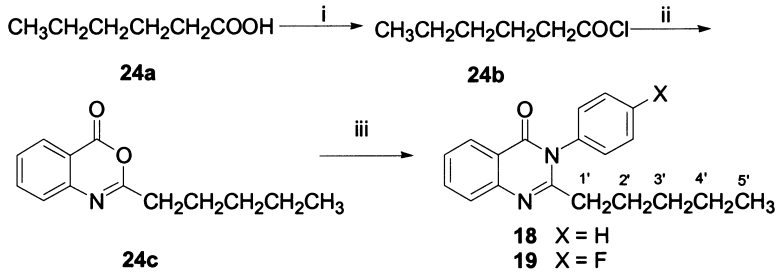


Figure 6. Synthesis of compounds **18-19**. Reagents and conditions: (i) $SOCl_2$, CH_2Cl_2, reflux, 30 min; (ii) **20a**, pyridine, CH_2Cl_2, reflux, 4h; (iii) aniline or 4-fluoroaniline, $POCl_3$, CH_2Cl_2.

The compounds **3-8** were also synthesized by microwave-mediated synthesis under solvent free conditions. The reactants were adsorbed over silica gel and subjected to microwave irradiation. The products were purified by column chromatography. The compounds were obtained in good yields with much cleaner and simpler reaction conditions and processing. The time required for the reaction was also much less as compared to conventional method. Table 1 gives the comparative yields and the reaction times of the compounds prepared by conventional and microwave method. The microwave-mediated synthesis provides a cleaner and faster synthetic route the desired compounds **3-8**.

Table 1. Comparative yields and reaction times by classical and microwaves mediated synthesis

Compound	Yield(%)		Time	
	Classical	MWI	Classical (hrs.)	MWI(min.)
3	64	60	5.30	30
4	64	63	5.30	30
5	70	60	5.30	35
6	44	52	5.30	30
7	47	55	5.30	30
8	58	63	5.30	40

In Vitro Biological Studies. The synthesized compounds were tested for *in vitro* bronchodilatory activity by studying the relaxation effect of the compounds on the isolated guinea pig tracheal chain pre-contracted with histamine diphosphate (1 μg/ml) or acetylcholine (1 μg/ml). The results are summarized in Table 2. Compounds **5** and **8**

with seven-member C ring showed 100% relaxation activity against both histamine and acetylcholine induce tracheal contraction as compared to 40% relaxation shown by etofylline. The decrease in size of the C ring to six-member (**4, 7**) resulted in two fold decrease in activity while further decrease in ring size substantially decreased the relaxation effect. Hermecz et al. had previously reported bronchodilatory activity of compounds **3-5** in Konzett-Rossler test using serotonin, histamine, and acetylcholine as spasmogenic agents with compound **5** being most potent.[11] Compounds without the ring A (**9-11**) showed weak or no relaxation, with only **11** with a seven member ring showing 50% relaxation in histamine pre-contracted tracheal chain thus indicating importance of quinazoline moiety for activity. Sakai and co workers have reported the effect of alkyl substitutions on bronchodilatory activity of xanthines[17]. We also synthesized some vasicine analogues without C ring and instead having aliphatic and phenyl substitution on B ring (**12-19**). Compounds **12-19** could only relax the histamine pre-contracted tracheal chain and showed no relaxation in case of acetylcholine pre-contracted chain. The methyl substituted **12** and **13** showed potent activity with phenyl substituted **12** exhibiting 100% relaxation effect as compared to 80% relaxation shown by the 4-fluorophenyl substituted **13**. The increase in size of the alkyl substitution resulted in gradual decrease in activity with pentyl substituted **18** and **19** showing no relaxation activity.

In conclusion the microwave-mediated synthesis provides a cleaner and faster synthetic route the desired compounds **3-8**. Compounds **5** and **8** with a seven-member C ring showed 100% relaxation activity against both histamine and acetylcholine pre-contracted guinea pig tracheal chain while compounds **12** and **13** without C ring and instead having phenyl and aliphatic substitution in B ring showed relaxation against histamine pre-contracted guinea pig tracheal chain only. The increase in chain length of the aliphatic substituent resulted in decrease in activity thus suggesting that increase in lipophilicity causes decrease in activity. Compounds with 4-fluoro substituted phenyl ring at the 3-position were comparatively less active than compounds without fluoro group. The quinazoline moiety was essential for activity.

Table 2. *In vitro* bronchodilatory activity evaluation of vasicine analogues.

Compound	In vitro guinea pig trachea % relaxation [a]		
	Histamine	Acetylcholine	Conc. (μg/ml)
3	20	-	30
4	40	-	30
5	100	100	30
7	50	40	30
8	100	100	30
9	-	-	30
10	20	-	30
11	50	30	30
12	100	-	30
13	80	-	30
14	100	-	30
15	20	-	30
16	50	-	30
17	10	-	30
18	-	-	30
19	-	-	30
Etofylline	40	40	30

[a]All data within ±15% (n = 3).

Experimental

Chemistry

The spectral data were recorded with the help of instrumental facilities housed at Regional Research Laboratory, Jammu. Infrared spectra were recorded on a Hitachi 270-30 infrared spectrophotometer using potassium bromide (KBr) discs. The UV spectra were recorded in methanol on Shimadzu 1601 PC spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on JNM-PMX 60 NMR Spectrophotometer, Jeol FX 90 Q FT NMR and Brucker 200 Supercon NMR equipment using trimethylsilane (TMS) as internal standard and deuterated trichloromethane ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6) as solvents. Mass spectra were obtained on a Jeol D-300 mass spectrophotometer. Melting points were recorded on Buchi 510 capillary melting point apparatus in silicon oil and are reported uncorrected. Microanalysis was performed on CARLO-ERBA 1106 elemental analyzer. Silica gel 60-120 mesh (Merck) was used for column chromatography. Column fractions were monitored by thin layer chromatography using prepared silica gel G (Merck) plates activated at 110 $^{\circ}C$ for one hour. The thin layer chromatography was also performed on precoated silica gel $60F_{254}$ plates (Merck). Compounds were visualized under UV lamp or by exposure to iodine vapors. Dragendorff's spray and 10% methanolic H_2SO_4 containing 2% ceric ammonium sulphate were also employed as detecting reagents. The solvents used in column chromatography were distilled before use. The solvents used for crystallization were of high purity grade and were distilled prior to use. The solvents were removed *in vacuo* on a Buchi R114 thin film evaporator using Eyela aspirator for vacuum. The microwave-mediated reactions were performed in domestic microwave oven BPL-700T.

General procedure for synthesis of 3-8. (Method A)

A mixture of appropriate anthranilic acid (1.0 eq.) in dry toluene was refluxed under Dean's apparatus for 1.5 h to remove any moisture present. The reaction vessel was cooled to room temperature and thionyl chloride (1.1 eq.) was added and refluxed for two hours. The solvent was distilled off *in vacuo* on a thin film evaporator, so as to completely remove any excess of thionyl chloride to give corresponding *N*-sulfinylanthaniloyl chloride. The residue was redissolved in dry toluene and lactam (1.1

eq.) was added. The reaction mixture was refluxed for two hours, then cooled to room temperature and then washed with 10% w/v potassium carbonate (2 × 50 mL) followed by distilled water (2 × 50 mL), and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* to give the desired product, which was recrystallized from methanol.

General procedure microwave-mediated synthesis of 3-8. (Method B)

The mixture of aromatic amino acid (1.0 eq.) and lactam (1.5 eq.) was dissolved in minimum quantity of dichloromethane. Silica gel 60-120 mesh was added to it and the solvent was removed under vacuum on a rotary evaporator so that compound gets evenly adsorbed on silica gel. It was transferred to Erlenmeyer flask of much larger capacity as compared to quantity of mixture. The flask was covered with a funnel and placed in microwave oven with alumina as heat sink. The flask was subjected to microwave irradiations at 700 watts. The reaction was monitored by TLC. On completion of reaction, the reaction mixture was cooled and extracted with dichloromethane (3 × 15 mL). The solvent was removed *in vacuo* and the product subject to column chromatography over alumina. The purified compounds were identified by spectroscopy and compared with the compounds prepared by the classical procedures by co-TLC and mmp.

7,8-Dihydropyrrolo[2,1-b]quinazolin-9(6H)-one (3)

Compound **3** was synthesized starting from anthranilic acid following the general procedure (Method A) by first preparing *N*-sulfinylanthaniloyl chloride (**21a**) (m pt 32-35 °C, lit mp 32-34 °C[18]) which was condensed with 2-pyrrolidinone to give a yellow colored solid which on crystallization from methanol gave 7,8-dihydropyrrolo[2,1-*b*]quinazolin-9(6*H*)-one **3** (4.25 g, mp 102-104 °C, lit mp 104 °C[9]) in 64% yield. Compound **3** was also synthesized by microwave-mediated synthesis (Method B) in 60% yield. $C_{11}H_{10}N_2O$ (Found C, 71.10; H, 5.32; N, 15.00. $C_{11}H_{10}N_2O$ requires C, 70.95; H, 5.41; N, 15.04.) IR 3060, 2911, 1662, 1630, 1605, 1582, 1499, 1445, 1380 and 766 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.23 (1H, q, J = 2 and 8 Hz), 7.42-7.78 (3H, m), 4.50 (2H, t, J = 8 Hz), 3.18 (2H, t, J = 8 Hz), 2.23-2.37 (2H, m).

7,8,9-Trihydropyrido[2,1-b]quinazolin-11(6H)-one (4)

Compound **4** was synthesized starting from anthranilic acid in a manner similar to compound **3** condensing **21a** with 2-piperdinone to give a yellow colored solid which on crystallization from methanol gave 7,8,9-trihydropyrido[2,1-*b*]quinazolin-11(6*H*)-one **4** (4.6 g, mp 98-100 °C, lit mp 99 °C [9]) in 64% yield. Compound **4** was also synthesized by microwave-mediated synthesis (Method B) in 63% yield. $C_{12}H_{12}N_2O$ (Found C, 71.80; H, 6.25; N, 13.96. $C_{12}H_{12}N_2O$ requires C, 71.98; H, 6.04; N, 13.99.) IR 3058, 2901, 1650, 1632, 1604, 1570, 1495, 1450, 1390 and 756 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.20 (1H, q, J = 2 and 8 Hz), 7.25-7.90 (3H, m), 4.05 (2H, t, J = 10), 3.0 (2H, t, J =10 Hz), 1.70-2.30 (4H, m).

7,8,9,10-tetrahydroazepino[2,1-b]quinazolin-12(6H)-one (5)

Compound **5** was synthesized starting from anthranilic acid in a manner similar to compound **3** condensing **21a** with 2-azepanone to give a yellow colored solid which on crystallization from methanol gave 7,8,9,10-tetrahydroazepino[2,1-*b*]quinazolin-12(6*H*)-one **5** (21.34 g, mp 97-99 °C, lit mp 98-99 °C [9]) in 70% yield. Compound **5** was also synthesized by microwave-mediated synthesis (Method B) in 63% yield. $C_{13}H_{14}N_2O$ (Found C, 73.10; H, 6.45; N, 12.98. $C_{13}H_{14}N_2O$ requires C, 72.87; H, 6.59; N, 13.07), IR 3065, 2910, 1658, 1632, 1600, 1580, 1500, 1450, 1390 and 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.25 (1H, q, J = 2 and 8 Hz), 7.10-7.90 (3H, m), 4.43-4.38 (2H, m), 2.90-3.15 (2H, m) and 1.70-2.20 (6H, m).

Pyrrolo[2,1-b]-1,3-dioxolo[4,5-g]-7,8-dihydroquinazolin-10(6H)-one (6)

Compound **6** was synthesized starting from **20b** following the general procedure (Method A) by first preparing **21b** which was condensed with 2-pyrrolidinone to give a yellow colored solid which on crystallization from methanol gave pyrrolo[2,1-*b*]-1,3-dioxolo[4,5-*g*]-7,8-dihydroquinazolin-10(6*H*)-one **6** (0.280 g, mp 111-113 °C) in 44% yield. Compound **6** was also synthesized by microwave-mediated synthesis (Method B) in 52% yield. $C_{12}H_{10}N_2O_3$ (Found C, 62.74; H, 4.30; N, 12.02. $C_{12}H_{10}N_2O_3$ requires C, 62.59; H, 4.38; N, 12.17.) IR 3290, 3120, 3075, 2907, 2850, 2612, 1658, 1634, 1565, 1460, 1405, 1392, 1338, 1305, 1240, 1180, 1124, 1008, 910, 878, 840 and 775 cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ 7.61 (1H, s), 7.04 (1H, s), 6.13 (2H, s), 4.21 (2H, t, J = 8 Hz), 3.18 (2H, t, J = 8 Hz), 2.09-2.20 (2H, m).

Pyrido[2,1-b]-1,3-dioxolo[4,5-g]-7,8,9-trihydroquinazolin-11(6H)-one (7)

Compound **7** was synthesized starting from **20b** in a manner similar to compound **6** condensing **21b** with 2-piperdinone to give a yellow colored solid which on crystallization from methanol gave pyrido[2,1-*b*]-1,3-dioxolo[4,5-*g*]-7,8,9-trihydroquinazolin-11(6*H*)-one **7** (0.315 g, mp 104-106 °C) in 47% yield. Compound **7** was also synthesized by microwave-mediated synthesis (Method B) in 55% yield. $C_{13}H_{12}N_2O_3$ (Found C, 63.77; H, 5.09; N, 11.25. $C_{13}H_{12}N_2O_3$ requires C, 63.93; H, 4.95; N, 11.47.). IR 3296, 3120, 3068, 2920, 2844, 2616, 1662, 1630, 1562, 1458, 1410, 1388, 1338, 1312, 1244, 1172, 1130, 1008, 900, 880, 844 and 770 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.57 (1H, s), 6.98 (1H, s), 6.10 (2H, s), 4.08 (2H, t, J = 10 Hz), 2.95 (2H, t, J = 10 Hz), 1.91-2.06 (4H, m).

Azepino[2,1-b]-1,3-dioxolo[4,5-g]-7,8,9,10-tetrahydroquinazolin-12(6H)-one (8)

Compound **8** was synthesized starting from **20b** in a manner similar to compound **6** condensing **21b** with 2-azepanone to give a yellow colored solid which on crystallization from methanol gave azepino[2,1-*b*]-1,3-dioxolo[4,5-*g*]-7,8,9,10-tetrahydroquinazolin-12(6*H*)-one **8** (1.48 g, mp 94-96 °C) in 58% yield. Compound **8** was also synthesized by microwave-mediated synthesis (Method B) in 63% yield. $C_{14}H_{14}N_2O_3$ (Found C, 65.32; H, 5.30; N, 10.68. $C_{14}H_{14}N_2O_3$ requires C, 65.11; H, 5.46; N, 10.85.). IR 2904, 1670, 1624, 1576, 1448, 1390, 1348, 1256, 1232, 1192, 1126, 1024, 904, 872, 850 and 776 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.58(1H, s), 7.00 (1H, s), 6.11 (2H, s), 4.40-4.42 (2H, m), 3.02 -3.06 (2H, m), 1.86 (6H, m).

General procedure for synthesis of 9-11. (Method C)

A mixture of 3-amino-3-phenylpropionic acid **23a** (2.250 g, 13.62 mmoles) in dry toluene (50 mL) was refluxed employing Dean-Stark water trap for 1.5 h. The reaction vessel was cooled to room temperature and thionyl chloride (1.5 mL, 20.4 mmoles) was added. The reaction mixture was refluxed for two hours. The solvent was distilled off in vacuo on a thin film evaporator, so as to completely remove any excess of thionyl

chloride to give crude **23b** (1.520 g), which was used for next step without further purification.

Compound **23b** (1.0 eq) was redissolved in dry toluene (25 mL) and lactam (1.1 eq.) was added. The reaction mixture was refluxed for two hours, cooled to room temperature and washed with 10% w/v potassium carbonate (2 × 25 mL) followed by distilled water (2 × 25 mL), then dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and chromatographed over silica gel to give desired product.

2-Phenyl-2,6,7,8-tetrahydro-3H-pyrrolo[1,2-a]pyrimidin-4-one (9)

Compound **9** was synthesized starting from **23b** following the general procedure (Method C) by condensing it with 2-pyrrolidinone to give a yellow colored solid which on column chromatography over alumina gave 2-phenyl-2,6,7,8-tetrahydro-3*H*-pyrrolo[1,2-*a*]pyrimidin-4-one **9** (0.075 g, mp 92-95 °C) in 8% yield. $C_{13}H_{14}N_2O$ (Found C, 73.82; H, 6.95; N, 12.11. $C_{13}H_{14}N_2O$ requires C, 72.87; H, 6.59; N, 13.07.). IR 3100, 2904, 1662, 1630, 1420, 1268, 925 and 725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.39 (5H, m), 4.81-4.89 (1H, m), 4.17-4.21 (2H, t, J = 8 Hz), 3.26 (2H, t, J = 8 Hz), 2.34-2.53 (2H, m), 2.01 (2H, m).

2-Phenyl-2,6,7,8,9-pentahydro-3H-pyrido[1,2-a]pyrimidin-4-one (10)

Compound **10** was synthesized starting from **23b** following the general procedure (Method C) by condensing it with 2-piperdinone to give a yellow colored solid which on column chromatography over alumina gave 2-phenyl-2,6,7,8,9-pentahydro-3*H*-pyrido[1,2-*a*]pyrimidin-4-one **10** (0.090 g, mp 84-85 °C) in 8% yield. $C_{14}H_{16}N_2O$ (Found C, 73.82; H, 6.95; N, 12.11. $C_{14}H_{16}N_2O$ requires C, 73.66; H, 7.06; N, 12.27.). IR 3100, 2904, 1657, 1633, 1420, 1268, 925 and 725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.20 (5H, m), 5.02-5.21 (1H, m), 4.07-4.11 (2H, t, J = 10 Hz), 3.01-3.10 (2H, m), 2.45 (2H, *t*, J = 10 Hz), 1.77-2.05 (4H, m).

2-Phenyl-2,6,7,8,9,10-hexahydro-3H-pyrimido[1,2-a]azepin-4-one (11)

Compound **11** was synthesized starting from **23b** following the general procedure (Method C) by condensing it with 2-azepanone to give a yellow colored solid which on

column chromatography over alumina gave 2-phenyl-2,6,7,8,9,10-hexahydro-3*H*-pyrimido[1,2-*a*]azepin-4-one **11** (0.120 g, mp 72-73 °C) in 11% yield. $C_{15}H_{18}N_2O$ (Found C, 74.20; H, 7.66; N, 11.42. $C_{15}H_{18}N_2O$ requires C, 74.35; H, 7.49; N, 11.56.). IR 3100, 2904, 1657, 1633, 1420, 1268, 925 and 725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.23 (5H, m), 5.02-5.21 (1H, m), 4.27-4.31(2H, m), 3.22-3.30 (2H, m), 2.26-2.69 (2H, m), 1.79 (6H, m).

General procedure for synthesis of 12-17. (Method D)

A mixture of anthranilic acid **20a** (5 g, 36.49 mmoles) and appropriate anhydride (25 mL) was refluxed for 4 h under anhydrous conditions. The excess of acetic anhydride was remove on a rotary evaporator under vacuum to give corresponding 2-alkylbenzo[*d*][1,3]oxazin-4-one (80-85% yield). 2-Alkylbenzo[*d*][1,3]oxazin-4-one (1.0 eq.) and aniline/4-fluoroaniline (1.0 eq.) were suspended in dry dichloromethane (50 mL) and phosphorous oxychloride (1.0 eq.) was added dropwise with constant stirring over a period of 15 minutes. After completion of the addition, the mixture was refluxed for four hours with constant stirring. The solution was cooled to room temperature and the solvent removed *in vacuo*. The residue was digested with 100 mL of 10% (v/v) hydrochloric acid for 2 h. The solution was cooled and neutralized with sodium bicarbonate solution. The resultant solid was filtered, washed with water, dried and crystallized from methanol to give the desired compound.

2-Methyl-3-phenyl-3H-quinazolin-4-one (12)

Compound **12** was synthesized following method D starting from anthranilic acid **20a** (5 g, 36.49 mmoles) and acetic anhydride (25 mL) to give 2-methylbenzo[*d*][1,3]oxazin-4-one (5 g, 85% yield). ^{1}H-NMR ($CDCl_3$) δ 2.61 (s, 3H), 4.56 (bs, 2H, NH_2), 6.63-7.24 (m, 4H). 2-Methylbenzo[*d*][1,3]oxazin-4-one (2 g, 12.42 mmoles) was reacted with aniline (1.155 g, 12.42 mmoles) in presence of phosphorous oxychloride (1.900 g, 12.42 mmoles) to give **12** (mp 146-148 °C, lit mp 147-148 °C [16] 1.5 g, yield 51%). $C_{15}H_{12}N_2O$ (Found C, 76.10; H, 5.25; N, 11.68. $C_{15}H_{12}N_2O$ requires C, 76.25; H, 5.12; N, 11.86.). IR 1692, 1660, 1632, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.35 (1H, d, J = 11 Hz), 7.62-7.85 (3H, m), 7.20 -7.54 (5H, m, Ph), 2.25 (3H, s).

3-(4-Fluorophenyl)-2-methyl-3H-quinazolin-4-one (13)

2-Methylbenzo[d][1,3]oxazin-4-one (2 g, 12.42 mmoles) was reacted with 4-fluoroaniline (1.379 g, 12.42 mmoles) in presence of phosphorous oxychloride (1.900 g, 12.42 mmoles) to give **13** (mp 132-134 °C, 1.65 g, yield 52%). $C_{15}H_{11}N_2OF$ (Found C, 71.09; H, 4.21; N, 10.89. $C_{15}H_{11}N_2OF$ requires C, 70.86; H, 4.36; N, 11.02.). IR 1692, 1660, 1632, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.37 (1H, d, J = 10 Hz), 7.63-7.82 (3H, m), 7.44 (2H, m), 7.25 (2H, m), 2.21(3H, s, CH_3).

2-Ethyl-3-phenyl-3H-quinazolin-4-one (14)

Compound **14** was synthesized following method D starting from anthranilic acid (2 g, 14.49 mmoles) and propionic anhydride (3 mL) to give 2-ethylbenzo[*d*][1,3]oxazin-4-one (2.1 g, 82% yield). ^{1}H-NMR ($CDCl_3$) δ 8.36 (1H, d, J = 11 Hz), 7.51-7.69 (3H, m), 4.35 (bs, 2H), 1.90-2.15 (q, 2H, J = 7 Hz), 1.07 (t, 3H, J = 7 Hz, CH_3). 2-Ethylbenzo[*d*][1,3]oxazin-4-one (1.0 g, 5.71 mmoles) was reacted with aniline (0.531 g, 5.71 mmoles) in dry dichloromethane (25 ml)in presence of phosphorous oxychloride (0.874 g, 5.71 mmoles) to give **14** (mp 124-126°C, 0.6 g, yield 42%). $C_{16}H_{14}N_2O$ (Found C, 76.94; H, 5.72; N, 11.02. $C_{16}H_{14}N_2O$ requires C, 76.78; H, 5.64; N, 11.19.). IR 3100, 2920, 2840, 1658, 1628, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.40 (1H, d, J = 11 Hz), 7.65-7.85 (3H, m), 7.20 -7.54 (5H, m), 2.29-2.64 (2H, q, J = 7 Hz), 1.20 (3H, t, J = 7 Hz).

2-Ethyl-3-(4-fluorophenyl)-3H-quinazolin-4-one (15)

2-Ethylbenzo[*d*][1,3]oxazin-4-one (1.0 g, 5.71 mmoles), was reacted with 4-flouroaniline (0.634 g, 5.71 mmoles) in dry dichloromethane (25 ml) in presence of phosphorous oxychloride (0.874 g, 5.71 mmoles) to give **15** (mp 172 °C, 0.800 g, yield 52%). $C_{16}H_{13}N_2OF$ (Found C, 76.94; H, 5.72; N, 11.02. $C_{16}H_{13}N_2OF$ requires C, 71.63; H, 4.88; N, 10.44.). IR 3100, 2920, 2840, 1658, 1630, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.35 (1H, d, J = 10 Hz), 7.62-7.80 (3H, m), 7.40 (2H, m), 7.28 (2H, m), 2.23-2.60 (2H, q, J = 7 Hz), 1.23 (3H, t, J = 7 Hz).

2-Propyl-3-phenyl-3H-quinazolin-4-one (16)

Compound **16** was synthesized following method D starting from anthranilic acid (2 g, 14.49 mmoles) and butyric anhydride (15 mmoles) to give 2-propylbenzo[*d*][1,3]oxazin-4-one (2.2 g, 80% yield). ^{1}H-NMR ($CDCl_3$) δ 8.39 (1H, d, *J* = 11 Hz), 7.69-7.88 (3H, m), 2.30-2.65 (2H, q, *J* = 7 Hz), 1.48-1.62 (2H, m), 1.02 (3H, t, *J* = 5 Hz). 2-Propylbenzo[*d*][1,3]oxazin-4-one (1.0 g, 5.29 mmoles) was reacted with aniline (0.500 g, 5.31 mmoles) in dry dichloromethane (25 ml)in presence of phosphorous oxychloride (0.815 g, 5.31 mmoles) to give **16** (mp 120-122°C, 0.450 g, yield 45%). $C_{17}H_{16}N_2O$ (Found C, 77.34; H, 6.21; N, 10.43. $C_{17}H_{16}N_2O$ requires C, 77.25; H, 6.10; N, 10.60.). IR 3090, 2925, 2845, 1660, 1632, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.37 (1H, d, *J* = 11 Hz), 7.62-7.85 (3H, m), 7.30 -7.50 (5H, m, Ph), 2.23-2.60 (2H, q, *J* = 7 Hz), 1.45-1.60 (2H, m), 1.05 (3H, t, *J* = 5 Hz).

2-Propyl-3-(4-fluorophenyl)-3H-quinazolin-4-one (17)

2-Propylbenzo[d][1,3]oxazin-4-one (1.0 g, 5.29 mmoles) was reacted with 4-fluoroaniline (0.600 g, 5.34 mmoles) in dry dichloromethane (25 ml)in presence of phosphorous oxychloride (0.815 g, 5.31 mmoles) to give **17** (mp 154 °C, 0.480 g, yield 32%). $C_{17}H_{15}N_2OF$ (Found C, 72.54; H, 5.22; N, 10.02. $C_{17}H_{15}N_2OF$ requires C, 72.33; H, 5.36; N, 9.92.). IR 3100, 2920, 2840, 1670, 1625, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.36 (1H, d, *J* = 11 Hz), 7.54-7.79 (3H, m), 7.42 (2H, m), 7.25 (2H, m), 2.20-2.52 (2H, q, *J* = 7 Hz), 1.43-1.58 (2H, m), 1.10 (3H, t, *J* = 5 Hz).

2-Pentyl-3-phenyl-3H-quinazolin-4-one (18)

Thionyl chloride (2.36 g, 20 mmoles) was added to a solution of caproic acid (**24a**) (2.0 g, 17.2 mmoles) in dichloromethane (25 ml) and the mixture was refluxed for 30 minutes under anhydrous conditions. The solvent and the excess of thionyl chloride were removed *in vacuo* to give caproyl chloride (**24b**), which was redissolved in dichloromethane, and anthranilic acid (2.36 g, 17.2 mmoles) and pyridine (0.2ml) were added. This mixture was refluxed under anhydrous conditions for four hours. The solvent was removed in vacuo to give 2-pentylbenzo[*d*][1,3]oxazin-4-one (**24c**) (3.0 g, yield 80 %).^{1}H-NMR ($CDCl_3$) δ 8.38 (1H, d, *J* = 11 Hz, H at C-5), 7.68-7.85 (3H, m), 2.30-2.65 (2H, q, *J* = 8 Hz), 1.28-1.77 (6H, m), 0.95 (3H, t, *J* = 5 Hz).

2-Pentylbenzo[*d*][1,3]oxazin-4-one (1.7 g, 7.45 mmoles) was reacted with aniline (0.692 g, 7.45 mmoles) in dry dichloromethane (50 ml) in presence of phosphorous oxychloride (1.142 g, 7.45 mmoles) following method D to give **18** (mp 90°C, 0.700 g, yield 30%). $C_{19}H_{20}N_2O$ (Found C, 78.21; H, 6.73; N, 9.52. $C_{19}H_{20}N_2O$ requires C, 78.05; H, 6.89; N, 9.58.) IR 3090, 2930, 2840, 1650, 1624 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.38 (1H, d, J = 11 Hz), 7.57-7.79 (3H, m), 7.18 -7.46 (5H, m), 2.20-2.60 (2H, q, J = 8 Hz), 1.30-1.83 (6H, m), 0.92 (3H, *t*, J = 5 Hz).

2-Pentyl-3-(4-fluorophenyl)-3H-quinazolin-4-one (19)

2-Pentylbenzo[*d*][1,3]oxazin-4-one (1.2 g, 5.53 mmoles) was reacted with 4-fluoroaniline (0.614 g, 5.53 mmoles) in dry dichloromethane (50 ml) in presence of phosphorous oxychloride (0.846 g, 5.53 mmoles) to give **19** (mp 140 °C, 0.500 g, yield 29%). $C_{19}H_{19}N_2OF$ (Found C, 73.69; H, 6.01; N, 9.15. $C_{19}H_{19}N_2OF$ requires C, 73.51; H, 6.17; N, 9.03.) IR 3100, 2920, 2845, 1659, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.39 (1H, d, J = 11 Hz), 7.54-7.80 (3H, m), 7.40 (2H, m), 7.19 (2H, m), 2.15-2.52 (2H, q, J = 8 Hz), 1.32-1.84 (6H, m), 0.93 (3H, t, J = 5 Hz).

6-Nitropiperonal (22b)

To 500 ml of concentrated nitric acid in a conical flask cooled to 2 °C with the help of ice bath was added finely pulverized piperonal **22a** (50 g, 0.33 moles) with constant stirring. The temperature was maintained between 2 and 5 °C during the addition and for three hours thereafter. After this, temperature was allowed to rise to 10°C in order to dissolve all the piperonal. The dark orange solution was poured onto washed and crushed ice where a yellow precipitate was formed. The precipitate was filtered and washed free from HNO_3 with cold water and stirred with a sufficient quantity of 40% $NaHSO_3$ solution to make a thick paste. Two volumes of water were added and the mixture was filtered. The residue was subjected to the same treatment and this was repeated with three succeeding residues. The combined filtrate was made alkaline with NaOH, the precipitates collected, washed thoroughly with water and dried. The product was dissolved in hot ethanol and crystallized to give yellow crystals (mp 98-99 °C, lit

mp 97-98 °C[19], 45.6g, yield 70%). ^{1}H-NMR ($CDCl_3$) δ 10.45 (1H, s, CHO), 7.79 (1H, s, H at C-2), 7.69 (1H, s, H at C-5), 6.23 (2H, s, -O-CH_2-O-).

6-Aminopiperonal (22c)

To a three necked round bottomed flask fitted with a centrally mounted stirrer, carrying a reflux condenser and a dropping funnel were successively introduced iron powder (300 mesh size electrolytic grade, 20 g, 0.357 moles) and ammonium chloride (26.75 g, 0.505 moles) dissolved in 500 ml of distilled water. A methanolic solution of 6-nitropiperonal **22b** (20 g, 0.102 mmoles) was allowed to trickle down into the stirred slurry of iron powder-ammonium chloride solution over duration of ten minutes at room temperature. The resultant mixture was stirred under gentle reflux for 2 h monitoring the progress of the reaction by TLC. The resultant solution was filtered hot followed by methanol (hot) wash. The combined washings were evaporated under reduced pressure. The resultant residue was crystallized from methanol to give 6-aminopiperonal **22c** (13 g, mp 107-109 °C, lit mp 107-108 °C[19], yield 76%). ^{1}H-NMR ($CDCl_3$) δ 9.66 (1H, s, CHO), 6.90 (1H, s, H at C-2), 6.23 (1H, s, H at C-5), 6.00 (2H, s, -O-CH_2-O-).

6-Amino-3,4-methylenedioxybenzoic acid (20b)

A solution of silver nitrate (5.736 g, 0.0339 moles) in 30 ml of water in a 100 ml beaker was treated with stirring with a solution of 1.49 g (0.0372 moles) of 97% sodium hydroxide in 15 ml of water. The mixture was stirred for 5 minutes and the silver oxide was collected in a Buchner funnel with suction and washed free of nitrates. The wet freshly precipitated silver oxide was transferred to 250 ml beaker and covered with 60 ml of water and treated with 7.8 g (0.195 moles) of 97% sodium hydroxide pellets with vigorous stirring. The mixture was warmed to 55-60°C. 6-Aminopiperonal **22c** (5.6 g, 0.0339 moles) was added to the mixture and stirring continued at 55-60°C. The reaction began after a few minutes. The silver oxide was transformed to fluffy metallic silver and a considerable heat was evolved. The stirring was continued for ten minutes, the mixture was filtered, and the precipitated silver was washed with 30 mL of hot water. To the combined filtrate and washings was added sodium metabisulphite (2 g) and the resultant solution was poured into 35 mL of 1:1 solution hydrochloric acid and water

with vigorous stirring. The resultant solution was acidic (as judged by Congo red indicator). The filtrate was cooled to 10 °C with ice and was extracted with ether (3x50 mL), dried over anhydrous sodium sulphate and the solvent removed in vacuo to give the desired product (5.4 g, mp 176-178 °C, lit mp 175-176 °C[20], yield 88%). ^{1}H-NMR ($CDCl_3$) δ 12.10 (1H, s, COOH), 7.72 (1H, s, H at C-2), 7.28 (1H, s, H at C-5), 6.30 (2H, s, -O-CH_2-O-).

Pharmacology

The prepared compounds were tested for in vitro bronchodilatory activity. The isolated guinea pig tracheal chain preparation[21] was used. Inhibition of histamine and acetylcholine induced isotonic contractions in isolated guinea pig tracheal chain preparation were observed.[17] Etofylline was used as standard bronchodilator.

Guinea pigs of either sex, weighing 350-500 g, were sacrificed by head blow and carotid bleeding. The trachea was dissected out and was transferred to a dish containing Krebs-Heneseleit solution (KHS) and cut transversely between the segments of the cartilage, so as to give a number of rings of the trachea. About 7-8 of these were tied to form a chain of approximately 4-5 cm in length. The chain was suspended in 20ml of organ bath containing KHS continuously aerated with 95% oxygen and 5% carbon dioxide and maintained at 37 °C. The composition (mmoles) of KHS was NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9 and (+)-glucose 11.1. The response was recorded isotonically on a kymograph. The tissue was adjusted to an initial tension of 1.5 g and allowed to equilibrate (60-90 min). Relaxation effect of the compounds was studied on the tracheal chain pre-contracted with histamine diphosphate (μg/ml) or acetylcholine (μg/ml). The test compounds were added 8 min after the tonic contraction reached the plateau. The responses were calculated as per cent to relaxing of pre-contracted muscle back to base line tension (100%). If there was relaxation to slightly below the base line it was also taken as 100% relaxation.

Acknowledgements

KLD and NM are thankful to CSIR for awarding the Emeritus Scientist Status and Senior Research Fellowship, respectively. We also thank Ms B. Purnima, Ms Bindu, Mr R. Bhasin and Mr Puroshotam for carrying out the spectral analysis.

References

1. Sin, D. D. Therapeutic options for chronic obstructive pulmonary disease: present and future. *Eur. Rev. Med. Pharmacol. Sci.* **2004**, *8*, 247-258.
2. Weder, M. M.; Donohue, J. F. Role of bronchodilators in chronic obstructive pulmonary disease. *Semin. Respir. Crit. Care. Med.* **2005**, *26*, 221-234.
3. Atal, C. K. *Chemistry and Pharmacology of Vasicine – A New Oxytocic and Abortifacient*. Regional Research Labotatory: Jammu, 1980.
4. Chopra, R. N. Some observations on the pharmacological action and therapeutic properties of *Adhatoda vasica*. *Ind. J. Med. Res.* **1925**, *13*, 205.
5. Cambridge, G. W.; Jansen, A. B.; Jarman, D. A. Bronchodilating action of vasicinone and related compounds. *Nature* **1962**, *196*, 1217.
6. Amin, A. H.; Mehta, D. R. A bronchodilator alkaloid (vasicinone) from Adhatoda vasica Nees. *Nature* **1959**, *184(Suppl 17)*, 1317.
7. Gupta, O. P.; Sharma, M. L.; Ghatak, B. J.; Atal, C. K. Pharmacological investigations of vasicine and vasicinone--the alkaloids of Adhatoda vasica. *Indian. J. Med. Res.* **1977**, *66*, 680-691.
8. Jain, M. P.; Gupta, V. N.; Atal, C. K. Synthesis of Tetrahydropyrido[2,1-b]quinazolin-10(1H)-one and Pentahydroazepino[2,1-b]quinazolin-11(1H)-one Derivatives. *Indian J. Chem.* **1985**, *24B*, 336-337.
9. Malhotra, S.; Koul, S. K.; Sharma, R. L.; Anand, K. K.; Gupta, O. P.; Dhar, K. L. Studies on Some Biologically Active Azepinoquinazolines: Part I - An Approach to Potent Bronchodilatory Compounds. *Indian J. Chem.* **1988**, *27B*, 937-940.
10. Malhotra, S.; Koul, S. K.; Singh, S.; Singh, G. B.; Dhar, K. L. Studies on Some Biologically Active Azepinoquinazolines: Part II - An Approach to Effective Antiinflammatory Drug. *Indian J. Chem.* **1989**, *28B*, 100-104.

11. Hermecz, I.; Vasvari-Debreczy, L.; Horvath, A.; Balogh, M.; Kokosi, J.; DeVos, C.; Rodriguez, L. Nitrogen bridgehead compounds. 66. Bronchodilator nitrogen bridgehead compounds with a pyrimidinone moiety. *J. Med. Chem.* **1987**, *30*, 1543-1549.

12. Kamal, A.; Ramana, K. V.; Rao, M. V. Chemoenzymatic synthesis of pyrrolo[2,1-b]quinazolinones: lipase-catalyzed resolution of vasicinone. *J. Org. Chem.* **2001**, *66*, 997-1001.

13. Jindal, D. P.; Bhatti, R. S.; Ahlawat, S.; Gupta, R. Synthesis and bronchodilatory activity of some nitrogen bridgehead compounds. *Eur. J. Med. Chem.* **2002**, *37*, 419-425.

14. Zabeer, A.; Bhagat, A.; Gupta, O. P.; Singh, G. D.; Youssouf, M. S.; Dhar, K. L.; Suri, O. P.; Suri, K. A.; Satti, N. K.; Gupta, B. D.; Qazi, G. N. Synthesis and bronchodilator activity of new quinazolin derivative. *Eur J Med Chem* **2006**, *41*, 429-434.

15. Bahekar, R. H.; Rao, A. R. Synthesis, evaluation and structure-activity relationships of 5-alkyl-2,3-dihydroimidazo[1,2-c] quinazoline, 2,3-dihydroimidazo[1,2-c]quinazolin-5(6H)-thiones and their oxo-analogues as new potential bronchodilators. *Arzneimittelforschung* **2001**, *51*, 284-292.

16. Errede, L. A. Acylanthranils. I. The pathway of quinazolone formation in the reaction of acylanthranils with anilines. *J. Org. Chem.* **1976**, *41*, 1763-1765.

17. Sakai, R.; Konno, K.; Yamamoto, Y.; Sanae, F.; Takagi, K.; Hasegawa, T.; Iwasaki, N.; Kakiuchi, M.; Kato, H.; Miyamoto, K. Effects of alkyl substitutions of xanthine skeleton on bronchodilation. *J. Med. Chem.* **1992**, *35*, 4039-4044.

18. Garin, J.; Merino, P.; Orduna, J.; Tejero, T.; Uriel, S. On the reaction of anthranilic acid with thionyl chloride: The actual structure of "kametani's sulfinamide anhydride" *Tetrahedron Lett.* **1991**, *32*, 3263-3264.

19. Lin, W. O.; Coutinho, E. d. S. 8,9-Methylenedioxy-3,4-dihydro-1,4,5-benzotriazocin-2(1H)-ones. *Monatsh. Chem.* **1983**, *114*, 1231-1236.

20. Mitscher, L. A.; Gracey, H. E.; Clark, G. W., 3rd; Suzuki, T. Quinolone antimicrobial agents. 1. Versatile new synthesis of 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids. *J. Med. Chem.* **1978**, *21*, 485-489.

21. Akcasu, A. The physiologic and pharmacologic characteristics of the tracheal muscle. *Arch. Int. Pharmacodyn. Ther.* **1959**, *122*, 201-207.

Received: *10-14-05* *Accepted:* *5-15-06*