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New Bronchodilators - 2 : Synthesis of 6-alkylbenzimidazo [1,2-c]quinazolines

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Synthesis of 6-alkylbenzimidazo[1,2-c]quinazolines (4) has been investigated based on the cyclization of the 2-(2-aminophenyl) benzimidazoles (3) by refluxing with appropriate aliphatic acid anhydrides. The intermediates (3) were in turn obtained from two routes; a dehydrocyclization of different anthranilic acids (1) (Method A) and isatoic anhydrides (2) (Method B) with o-phenylenediamine. Attempts were also made to synthesize the title compounds using 2-alkyl 3, 1-benzoxazin-4-one as starting materials. Since most of the 2-alkylbenzoxazinones were unstable, yields obtained by this route were low. The title compounds (4) were characterized on the basis of their elemental analyses and spectral (IR, 1H-NMR and Mass) data. The bronchodilatory activity of compounds 4a and 4b is reported using *in vitro* and *in vivo* animals models.

Bronchial asthama is a chronic debilitating disease which in its severe forms can even be life threatening. It is, in general, characterized by both bronchoconstriction and airway inflammation which leads to a bronchial hyperresponsiveness to various stimuli[1]. At present, different classes of drugs have been employed to combat the symptoms of this disease like bronchodilators, antiallergic agents and corticosteroids. Broncho-constriction can be effectively inhibited by bronchodilators such as β-agonists and xanthine derivatives while airway inflammation and bronchial hyperresponsiveness are well-controlled by corticosteroids. Methylxanthines are a major class of bronchodilators employed in the treatment of asthma despite a narrow therapeutic index. Their pharmacological actions are attributed to be based on multiple biochemical pathways, which include inhibition of phosphodiesterase (PDE) enzyme there by increasing intracellular c-AMP levels, direct and indirect effect on intracellular calcium concentration, increase in uncoupling of intracellular calcium with contractile elements and antagonism towards adenosine receptors[2].

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Currently new heterocyclic compounds designed on the basis of xanthine skeleton are being investigated as possible bronchodilators with a wider margin of safety. A literature survey revealed that imidazo[2,1-b]quinazolin-5 (10H)-ones, imidazo[4,5-c]quinolin-4(5H)-one derivatives exhibited potent bronchodilatory activities[3,4]. Based on these observations, we have recently made attempts to synthesize fused imidazo[1,2-c]quinazoline derivatives as a new class of bronchodilators[5,6].

In the present investigation, we report the synthesis and bronchodilatory activity of 6-alkylbenzimidazo[1,2-c]quinazolines (4), using 2-(2-aminophenyl) benzimidazoles (3), which were in turn obtained by two different methods (A and B). Attempts were also made to synthesize the title compounds using 2-alkyl-3, 1-benzoxazin-4-ones as starting materials. Since most of the 2-alkylbenzoxazinones were unstable, the overall yields obtained by this route were low. The bronchodilatory activity was carried out for some of the title compounds (4a and 4b) in both *in vivo* and *in vitro* methods using

standard animal models and the results are presented in this communication.

MATERIALS AND METHODS

Melting points were determined in open capillaries using a Thermonik Precision Melting Point cum Boiling Point Apparatus, model C-PMB-2 and are uncorrected. Purity of the compounds was checked by precoated tlc plates (E. Merck Kieselgel 60 F_{254}). IR spectra were recorded using KBr pellets on a Perkin-Elmer 337 Spectrophotometer (V_{max} in cm^{-1}) and ^1H NMR spectra on a Varian EM-390 90 MHz Spectrometer using TMS as internal standard (chemical shifts expressed in δ ppm), EI-MS spectra at 70 eV on a VG Micromass 7070H Mass Spectrometer. Elemental analyses was carried out using Heraus Carlo Erba 1108 CHN analyser. Isatoic anhydride and all the aliphatic acid anhydrides were procured from Otto Chemie, Bombay.

The intermediate 2-(2-aminophenyl)benzimidazole (3) was synthesized by two different methods as shown in Scheme I.

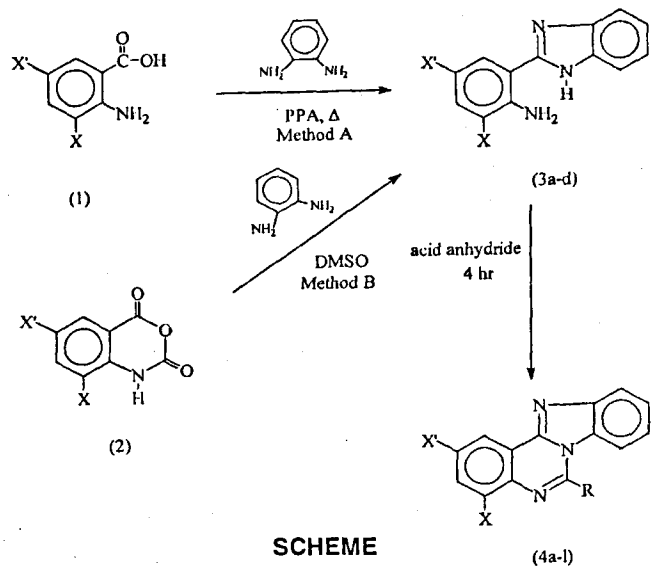
Method A :

To a clear solution of polyphosphoric acid (15 ml), anthranilic acid (1) (0.01 mol) and o-phenylenediamine (0.02 mol) was added and heating for 4 at 240±5° with constant stirring. On cooling, followed by pouring onto crushed ice and bringing the pH of the resultant solution to 7 using 10% sodium hydroxide solution resulted in a solid product which on recrystallization from aqueous alcohol (70%) yielded the product (3).

Method B :

A mixture of isatoic anhydride (2) (0.01 mol) and o-phenylenediamine (0.015 mol) was refluxed for 30 min in dimethyl sulfoxide (DMSO). On cooling, followed by pouring onto crushed ice resulted in a solid product which on recrystallization from aqueous alcohol (70%) yielded a pure product (3). Four such compounds (3a-d) were synthesized and characterized (Table-1).

Following the above procedures 2 (5-iodo-2-aminophenyl) benzimidazole (3d) was obtained as white crystals Rf: 0.73 (ethyl acetate) IR (KBr): 3400 (b), 3200 (w), 1620 (m), 1390 (m) cm^{-1}; PMR ($CDCl_3$) : 3.6-3.8 (br, s, 2H, NH_2), 8.46-8.58 (m, 6H, Ar), 8.82 (s, 1H, 6-H), 11.1 (br, s, 1H, NH) δ ppm; MS (m/e%) : 336 (M+1, 18.5), 335 (M^+, 100), 319 ($M+NH_2$, 7.2), 227 (319-PhNH, 70.2),



SCHEME

201 (227-CN, 30.2), 74 (201-I, 53.2); (Found : C 46.7; H 2.99; N 12.57, $C_{13}H_{10}N_3I$, requires : C 46.5; H 2.98; N 12.53%).

6-alkylbenzimidazo[1,2-c]quinazoline (4):

Compound 3 (0.01 mol) was heated under reflux in 15 ml of anhydrous acid anhydride for 4 h. The excess of acid anhydride was distilled off under reduced pressure and the reaction mixture was poured onto crushed ice to get a solid mass. The product so obtained was recrystallized from aqueous alcohol (70%) so as to obtain a pure product. Twelve such compounds (4a-l) were synthesized and characterized (Table-1).

Following the above procedure 10-iodo-6-methyl benzimidazo[1,2-c]quinazoline (4d) was obtained as white crystals Rf : 0.66 (Chloroform) IR (KBr) : 3200 (b), 1580 (s), 1420 (s), 1380 (w), 1180 (b), 815 (w) cm^{-1}; PMR ($CDCl_3$): 1.83 (s, 3H, $-CH_3$) and at 6.8 - 8.4 (m, 7H, aromatic) δ ppm; MS (m/e%) : 359 (M^+, 100%), 232 (M^{+-1}, 30%), 116 (232-$C_7N_2H_4$, 20%), 75 (116-(N=C-CH_3), 10%); (Found : C 50.27; H 2.79; N 11.60; $C_{15}H_{10}N_3I$, requires : C 50.13 H 2.78; N 11.69%).

Bronchodilatory activity :

Inhibition of histamine-induced isotonic contraction in isolated guinea pig tracheal chain preparation (*in vitro*):[9]

Guinea pigs of either sex, weighing 250-300 g were sacrificed by a head blow and exsanguination. The trachea was excised and transferred to a dish containing

TABLE 1 : PHYSICAL DATA OF COMPOUNDS 3 AND 4

Compd.	X	X'	R	m.p.* (°C) Yield@(%)	Rf$	Mol. Formula** (mol.wt.)
3a	H	H	-	213[a] 70 (40)	0.73	$C_{13}H_{11}N_3$ [209]
3b	H	Br	-	190 68 (38)	0.72	$C_{13}H_{10}N_3Br$ [288]
3c	Br	Br	-	194[b] 68 (40)	0.71	$C_{13}H_9N_3Br_2$ [367]
3d	H	I	-	198 65 (41)	0.72	$C_{13}H_{10}N_3I$ [335]
4a	H	H	CH_3	176[c] 68	0.65	$C_{15}H_{11}N_3$ [233]
4b	H	Br	CH_3	182 65	0.66	$C_{15}H_{10}N_3Br$ [312]
4c	Br	Br	CH_3	274[d] 69	0.65	$C_{15}H_9N_3Br_2$ [391]
4d	H	I	CH_3	280 60	0.66	$C_{15}H_{10}N_3I$ [359]
4e	H	H	C_2H_5	189 56	0.67	$C_{16}H_{13}N_3$ [247]
4f	H	Br	C_2H_5	185 58	0.68	$C_{16}H_{12}N_3Br$ [326]
4g	Br	Br	C_2H_5	270 52	0.66	$C_{16}H_{11}N_3Br_2$ [405]
4h	H	I	C_2H_5	275 50	0.66	$C_{16}H_{12}N_3I$ [373]
4i	H	H	C_2H_5	179 51	0.67	$C_{17}H_{15}N_3$ [261]
4j	H	Br	C_3H_7	186 53	0.68	$C_{17}H_{14}N_3Br$ [340]
4k	BR	Br	C_3H_7	268 52	0.66	$C_{17}H_{13}N_3Br_2$ [419]
4l	H	I	C_3H_7	275 49	0.67	$C_{17}H_{14}N_3I$ [387]

* Recrystallized from aq. alcohol (70%) ** CHN analysis indicated that the calculated and observed-values were within the acceptable limit (+ 0.4%); $Mobile phase (chloroform : ethylacetate; 9:1) [a]Lit. m.p. 213°(11), [b] Lit. m.p. 194° (11), [c] Lit. m.p. 176° (11), [d] Lit. m.p. 274° (11)

@For compounds 3 a-d, the yields given in parentheses indicate those of obtained by method-B

Krebs-Hanseleit solution (KHS) and cut transversely between the segments of the cartilage, so as to give a number of rings of the trachea. About 5-6 rings were tied to form a chain of approximately 4-5 cm length. The chain was suspended in 20 ml of tissue bath containing KHS continuously aerated with carbogen (95% of oxygen and 5% of carbondioxide) and maintained at 37±1°. The composition (mH) 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 responses were recorded isotonically on a kymograph. Relaxation effects of the compounds was studied on tracheal chain precontracted with histamine acid phosphate (3.2×10^{-5} M, sample obtained from Hi-media, Bombay). Aminophylline (obtained from Unichem, Bombay) was used as standard bronchodilator. The bronchodilatory activity of the test compounds (dissolved in propylene glycol) is expressed as IC_{50} values (in molar concentration). The solvent propylene glycol did not show any significant inhibition of histamine induced contractions.

Protection against histamine-induced bronchospasm on conscious guinea pig (*in vivo*) :

A modification of the technique of van Arman[10] was used. Hartley guinea pigs of either sex (250-300 g) were fasted for 24 h. The test compound, dosed intraperitonially at the dose of 10 mg/kg and challenged with histamine aerosol (0.2% aqueous solution of histamine acid phosphate in a Vaponephrin Pocket Nebulizer) sprayed into a closed transparent cage. The respiratory status reflecting the increasing degree of bronchoconstriction was recorded. The time for the onset of convulsions was recorded. Animals remaining stable (appearing normal, without increased respiratory rate and convulsions) for more than 6 min were considered protected against histamine-induced bronchospasm. An intraperitoneal injection of chlorpheniramine maleate I.P. (Avil®; Hoechst) at a dose of 25 mg/kg was given for the recovery of the test animals.

RESULTS AND DISCUSSION

The tile compounds (4) were obtained by a cyclocondensation reaction of 2-(2-aminophenyl) benzimidazoles (3) with different aliphatic acid anhydrides. The intermediate 3, was obtained from two different methods (A and B). In the method A, a direct heating of different substituted anthranilic acids (1) with o-phenylenediamine (o-PDA) in the presence of a strong dehydrating agent, polyphosphoric acid (PPA) at 240±5° for 4 h, with constant stirring yielded the product-3[7]. Analogously (method B) they (3) were obtained by a cyclocondensation of isatoic anhydrides (2) with o-PDA by refluxing for 30 min in DMSO[8]. Further the compound 3 on reacting with an appropriate acid anhydride led to the formation of the title compounds, 6-alkyl benzimidazo[1,2-c]quinazolines (4) (Scheme-I) in quantitative yields (Table-1).

Method A was found to be more facile as it was easy to work out and yields obtained by this route were comparatively high. The lower yields in method B may be due to the formation of other biproducts like benzimidazo[1,2-c]quinazoline-4(5H)-ones and dimer[8]. Using intermediate 3, twelve title compounds have been synthesized and characterized basing on their spectral and analytical data (Table-1).

The comparative overall per cent yields of 2-(2-aminophenyl) benzimidazole (3) and 6-alkylbenzimidazo[1,2-c]quinazoline (4) were calculated based on the starting material (1) and are presented in Table-1.

Compounds 4a and 4b were screened for bronchodilatory activity using both *in vitro* and *in vivo* methods employing standard animal models. Both these compounds were found to exhibit moderate bronchodilatory activity *in vitro* (IC_{50} for 4a and 4b, 1.6×10^3 and 1.7×10^3 in comparison to standard aminophylline IC_{50} 1.0×10^3) but could show good protection against histamine challenge in guinea pigs when tested *in vivo* (recorded as time of onset of convulsions in sec) and found to offer good protection (272±9.57 and 321.5±9.57 sec. respectively as against control 88.75±4.78 sec). Screening for bronchodilatory potency of the other compounds of the series and other pharmacological studies are in progress and will be published elsewhere.

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