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Eudistomin derivatives, novel phosphodiesterase inhibitors: synthesis and relative activity

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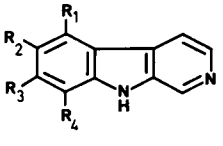
Abstract—The preparations of novel phosphodiesterase inhibitors, 8-acetoxy-5-iodo-6-methoxypyrido[3,4-*b*]indole, 5,7-dibromo-6-hydroxypyrido[3,4-*b*]indole, 5,7-dichloro-6-hydroxypyrido[3,4-b]-indole and 8-acetoxy-5-bromo-6-methoxypyrido[3,4-b]indole, are described together with concentrations giving 50% inhibition against cyclic AMP phosphodiesterase, i.e. 3×10^{-6}, 3×10^{-6}, 7×10^{-6} and 1×10^{-5} M, respectively. The relative potency of these eudistomin derivatives is discussed in terms of the chemical structures compared with those of other inactive eudistomins and derivatives.

The role of cyclic (c) AMP as a modulator of mammalian hormone action makes any compound which alters the phosphodiesterase activity an agent of potential pharmaceutical value. Therefore, many cyclic nucleotide analogues and nitrogen heterocycles have been synthesized to find specific inhibitors of cAMP phosphodiesterase (Miller et al 1984; Hidaka & Endo 1984).

Studies of the pharmacological activities of eudistomins and eudistomidin, which are marine natural products from tunicates, have shown that some have Ca-releasing activity in the sarcoplasmic reticulum (Nakamura et al 1986) or calmodulin-antagonistic activity (Kobayashi et al 1986). The characteristic chemical features of the molecules are a β-carboline skeleton and hydroxy and bromo substituents on the benzenoid ring. We report that introduction of halogeno (Br, Cl or I) and alkyloxy (RO-; R = H, CH_3 or CH_3CO) groups into the benzenoid ring of a β-carboline to afford 8-acetoxy-5-iodo-6-methoxypyrido-[3,4-b]indole (IIIb), 5,7-dibromo-6-hydroxypyrido[3,4-*b*]-indole (IVa), 5,7-dichloro-6-hydroxypyrido[3,4-b]indole (IVb) or 8-acetoxy-5-bromo-6-methoxypyrido[3,4-b]indole (IIIa) leads to the production of novel inhibitors of cAMP phosphodiesterase.



Materials and methods

Compounds. Eudistomins D (Va), N (Vb) and O (Vc) were isolated from extracts of the Caribbean tunicate *Eudistoma olivaceum* (Kobayashi et al 1984). Eudistomin derivatives, IIIa, IIIb, IVa and IVb, were synthesized by the method described in Preparative work. β-Carboline (Ve) and 6-methoxypyrido[3,4-*b*]indole (I) were prepared by the method of Kanaoka et al (1967). 6-Hydroxy-β-carboline (Vd) was prepared by demethylation of (I) with BBr_3. All compounds tested were dissolved in dimethylsulphoxide before application.

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cAMP phosphodiesterase assay. cAMP phosphodiesterase assay was by the method of Butcher & Sutherland (1962) which is both convenient and superior because of the small degree of experimental error. cAMP phosphodiesterase (0·01 unit mL^{-1}) from bovine heart (Sigma) was incubated at 30 °C in 1 mL of 40 mM Tris-HCl buffer (pH 7·4) containing 2 mM $MgCl_2$, 0·2 mM cAMP (Sigma) and 0·7 unit mL^{-1} 5'-nucleotidase (Sigma). After a 5 min preincubation with the test compounds, the reaction was started by the addition of cAMP. The reaction was terminated after 20 min by the addition of one tenth volume of cold 55% trichloroacetic acid, the precipitate was removed by centrifugation, then aliquots of the supernatant fluids were analysed for inorganic phosphate by the method of Martin & Doty (1949).

Results and discussion

Both mono- and dibrominated β-carbolines IIIa and IIa were prepared in a ratio of 1:3 by bromination of 6-methoxy-β-carboline (I) with *N*-bromosuccinimide in acetic acid. The reaction of I with *N*-iodosuccinimide afforded the monoiodinated β-carboline IIIb in a poor yield (31%) with no diiodinated derivative, while only the dichlorinated β-carboline IIb was obtained (76%) by the chlorination of I with *N*-chlorosuccinimide. Demethylation of the methoxy group of IIa or IIb with BBr_3 in methylene chloride gave the phenol IVa or IVb. The reaction with *N*-bromo- or *N*-iodosuccinimide in acetic acid introduced an acetoxy group into the benzenoid ring of β-carboline. These β-carbolines with various halogeno substitutions were expected to alter cAMP phosphodiesterase, since halogenation of cyclic nucleotide had been reported to induce an increase in the inhibitory effect towards the enzyme (Miller et al 1984).

The values of concentrations for 50% inhibition (IC50) of the compounds IIa, IIb, IIIa, IIIb, IVa, and IVb in cAMP phosphodiesterase experiments are shown in Table 1, together with the activity of eudistomins D (Va), N (Vb) and O (Vc), 6-methoxy-β-carboline (I), 6-hydroxy-β-carboline (Vd) and β-carboline itself (Ve). The results show that the four synthetic compounds (IIIa, IIIb, IVa and IVb) inhibit the phosphodiesterase activity more than the natural compound, eudistomin D (Va). The compounds IIIb and IVa were most potent among them and the IC50 value for both was 3×10^{-6} M. Lower activity was observed for I, IIa, IIb, Vb, Vc, Vd and Ve. The 6-hydroxy group and 5- and/or 7-halogen (Br or Cl) on the benzenoid ring of β-carboline (IVa, IVb and Va) are considered to be essential for the inhibitory activity. Despite the lack of a 6-hydroxy group, the addition of an 8-acetoxy group as in IIIa or IIIb seems to increase the activity as a phosphodiesterase inhibitor. It is also suggested that the size of halogens affect the inhibitory activity as magnitude of the diameter (I > Br > Cl) parallels potency.

Preparative work

Melting points are uncorrected. ^1H NMR spectra were recorded on a JEOL JMN FX-270, ^{13}C NMR spectra on a

Table 1. Relationship between phosphodiesterase inhibition and chemical structure.

Compound	IC50 (M) against phospho-diesterase	R_1	R_2	R_3	R_4
IIIb	3×10^{-6}	I	OMe	H	OAc
IVa	3×10^{-6}	Br	OH	Br	H
IVb	7×10^{-6}	Cl	OH	Cl	H
IIIa	1×10^{-5}	Br	OMe	H	OAc
Va	3×10^{-5}	Br	OH	H	H
IIa	3×10^{-5}	Br	OMe	Br	H
IIb	(39%)*	Cl	OMe	Cl	H
Vc	(24%)	H	H	Br	H
Vb	(21%)	H	Br	H	H
I	(13%)	H	OMe	H	H
Ve	(9%)	H	H	H	H
Vd	(7%)	H	OH	H	H

* Percentage of inhibition at 3×10^{-5} M.

JEOL FX-90Q, mass spectra on a Hitachi RMU-7M, and IR spectra on a Hitachi 260-50 infrared spectrophotometer. Assigned structures were established by these spectral data.

*8-Acetoxy-5-bromo-6-methoxypyrido[3,4-*b*]indole (IIIa).* To a solution of 6-methoxypyrido[3,4,-*b*]indole (I) (207 mg) in acetic acid (20 mL) was added *N*-bromosuccinimide (734 mg) and the mixture was stirred at room temperature (25 °C) for 1 h. After removal of the solvent under vacuum, the mixture was diluted with ethyl acetate and washed with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation of the solvent under vacuum followed by silica gel column chromatography (eluting with 1:1 to 1:2 ethyl acetate–hexane) the yield was 251 mg (68%) of IIa, m.p. 222 °C (decomp.) and 76 mg (22%) of IIIa, m.p. 225 °C (decomp.). For IIa, δ ($CDCl_3$/DMSO-d_6 9:1) 7·41 (s, 1H), 8·46 (d, 1H), 8·57 (d, 1H) and 9·03 (s, 1H); m/z 354 (M^+). For IIIa, δ ($CDCl_3$) 2·55 (s, 3H), 7·41 (s, 1H), 7·81 (d, 1H), 8·46 (d, 1H) and 8·91 (s, 1H); *m/z* 334 (M^+); $\nu_{c=o}$ 1760 cm^{-1}.

*5,7-Dibromo-6-hydroxypyrido[3,4-*b*]indole (IVa).* To a solution of IIa (529 mg) in methylene chloride (200 mL) was added BBr_3 (3 mL) and the mixture was refluxed for 2 h. After addition of methanol to decompose excess BBr_3, the solvent was evaporated under vacuum and the residue was diluted with ethyl acetate and washed with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation of the solvent under vacuum followed by silica gel column chromatography (eluting with 1:1 to 2:1 ethyl acetate–hexane) the yield was IVa (409 mg, 84%), m.p. 205 °C (decomp.); δ ($CDCl_3$/DMSO-d_6 9:1) 7·49 (s, 1H), 8·41 (d, 1H), 8·56 (d, 1H) and 9·02 (s, 1H): *m/z* 340 (M^+).

*5,7-Dichloro-6-hydroxypyrido[3,4-*b*]indole (IVb).* To a solution of I (103 mg) in acetic acid (10 mL) was added *N*-chlorosuccinimide (410 mg) at 70 °C for 2 h under stirring. After removal of the solvent under vacuum, the mixture was diluted with ethyl acetate-methylene chloride and washed with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation of the solvent followed by silica gel column chromatography (eluting with 2:1 ethyl acetate–hexane) the yield was IIb (105 mg, 76%), m.p. 198 °C (decomp.); δ ($CDCl_3$/DMSO-d_6 9:1) 7·29 (s, 1H), 8·39 (d, 1H), 8·46 (d, 1H) and 9·02 (s, 1H), *m/z* 266 (M^+). To a solution of IIb (105 mg) in methylene chloride (25 mL) was added BBr_3 (0·5 mL) and the mixture was refluxed for 1·5 h. After addition of methanol, the solvent was evaporated under vacuum and the residue was diluted with ethyl acetate and washed with sat. $NaHCO_3$ and brine. Drying over $MgSO_4$ and evaporation of the solvent under vacuum followed by silica gel column chromatography (eluting with 7% methanol–methylene chloride) yielded IVb (77 mg, 77%), m.p. 185 °C (decomp.); δ ($CDCl_3$/DMSO-d_6 9:1) 7·47 (s, 1H), 8·39 (d, 1H), 8·64 (d, 1H) and 9·17 (s, 1H); *m/z* 252 (M^+).

*8-Acetoxy-5-iodo-6-methoxypyrido[3,4-*b*]indole (IIIb).* To a solution of I (201 mg) in acetic acid (20 mL) was added *N*-iodosuccinimide (1·40 g) and the mixture was stirred at 70 °C for 2 h. After removal of the solvent under vacuum, the residue was diluted with ethyl acetate–methylene chloride and washed with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$ and evaporation of the solvent followed by silica gel column chromatography (eluting with 2:1 ethyl acetate-hexane) the yield was IIIb (120 mg, 31%). m.p. 215 °C (decomp.); δ ($CDCl_3$/DMSO-d_6 9:1), 2·54 (s, 3H), 7·61 (s, 1H), 7·80 (d, 1H), 8·41 (d, 1H) and 9·00 (s, 1H); δ (DMSO-d_6) 20·5 (S,CO*C*H_3), 138·7 (S,*C*-OCOCH_3) and 168·4 (S,*C*OCH_3); *m/z* 382 (M^+); $\nu_{c=o}$ 1760 cm^{-1}.

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